Terms and Conditions of Use of Digitised Theses from Trinity College Library Dublin

Copyright statement

All material supplied by Trinity College Library is protected by copyright (under the Copyright and Related Rights Act, 2000 as amended) and other relevant Intellectual Property Rights. By accessing and using a Digitised Thesis from Trinity College Library you acknowledge that all Intellectual Property Rights in any Works supplied are the sole and exclusive property of the copyright and/or other IPR holder. Specific copyright holders may not be explicitly identified. Use of materials from other sources within a thesis should not be construed as a claim over them.

A non-exclusive, non-transferable licence is hereby granted to those using or reproducing, in whole or in part, the material for valid purposes, providing the copyright owners are acknowledged using the normal conventions. Where specific permission to use material is required, this is identified and such permission must be sought from the copyright holder or agency cited.

Liability statement

By using a Digitised Thesis, I accept that Trinity College Dublin bears no legal responsibility for the accuracy, legality or comprehensiveness of materials contained within the thesis, and that Trinity College Dublin accepts no liability for indirect, consequential, or incidental, damages or losses arising from use of the thesis for whatever reason. Information located in a thesis may be subject to specific use constraints, details of which may not be explicitly described. It is the responsibility of potential and actual users to be aware of such constraints and to abide by them. By making use of material from a digitised thesis, you accept these copyright and disclaimer provisions. Where it is brought to the attention of Trinity College Library that there may be a breach of copyright or other restraint, it is the policy to withdraw or take down access to a thesis while the issue is being resolved.

Access Agreement

By using a Digitised Thesis from Trinity College Library you are bound by the following Terms & Conditions. Please read them carefully.

I have read and I understand the following statement: All material supplied via a Digitised Thesis from Trinity College Library is protected by copyright and other intellectual property rights, and duplication or sale of all or part of any of a thesis is not permitted, except that material may be duplicated by you for your research use or for educational purposes in electronic or print form providing the copyright owners are acknowledged using the normal conventions. You must obtain permission for any other use. Electronic or print copies may not be offered, whether for sale or otherwise to anyone. This copy has been supplied on the understanding that it is copyright material and that no quotation from the thesis may be published without proper acknowledgement.

THE DESIGN, SYNTHESIS AND BIOCHEMICAL EVALUATION OF NOVEL FLEXIBLE AND CONFORMATIONALLY RESTRAINED ANTIESTROGENS

by

Rosario B. Hughes

A thesis presented to the University of Dublin for the degree of Doctor of Philosophy in Pharmaceutical Chemistry.

Based on research carried out under the supervision of

Mary J. Meegan B.Sc., Ph.D. (N.U.I.), M.R.S.C., C. Chem.

At

The Department of Pharmaceutical Chemistry

The School of Pharmacy,

Trinity College,

February 2000.

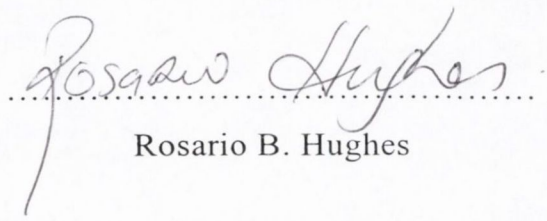
The Library may copy or lend this Thesis when appropriate.

Rosario B. Hughes

Rosario B. Hughes

DECLARATION

This thesis has not been submitted as an exercise for a degree at any other University. The work described is entirely my own work except where duly acknowledged.



Rosario B. Hughes

"As long as you live, keep learning how to live,
Alone we can do so little, together we can do so much."

"Destiny is no matter of chance.
It is a matter of choice.
It is not a thing to be waited for,
it is a thing to be achieved."

" A real friend is one who walks in,
when the rest of the world walks out."

Abstract

This thesis is presented in four different sections. In the first section the synthesis of a number of structural (novel flexible) analogues of the antiestrogen drug tamoxifen is described. Preparation of novel conformationally restrained analogues using benzoxepins as precursors is investigated in the second section. In the third section the prepared compounds are biochemically analysed for their binding, antiproliferative and cytotoxicity affinity for human MCF-7 breast adenocarcinoma cancer cells. In the final section highly resolved computational docking procedures are used to ascertain the degree of interaction of these novel compounds with specific activity-related residues in a model of the estrogen receptor.

Interest in the use of triphenylethylenes as anti-cancer agents has increased due to the clinical success of tamoxifen in breast cancer. The structure and pharmacological activity of tamoxifen and its clinical analogues are iterated in Chapter 1.

In Chapter 2, a series of triphenylethylene flexible analogues possessing an extra benzylic group were prepared using a seven step synthetic route and via the McMurry coupling reaction.

A series of novel conformationally restrained benzoxepins were synthesised in Chapter 3, some possessing an extra benzylic group, others a nitro group and further benzoxepin analogues having substituted aryl and hetrocyclic groups. These compounds were mainly prepared via the Suzuki coupling reaction.

In Chapter 4, the biochemical analysis of these compounds is described, detailing their antiproliferative activity, binding affinity and cytoxicity profiles. Chapter 5 depicts highly resolved crystal structural studies of the estrogen receptor and exhibits computational docking procedures which were used to ascertain the degree of interaction of the novel synthesised compounds with the specific activity-related residues in a model of the estrogen receptor.

Acknowledgements

I wish to express my sincere gratitude to Dr. Mary J. Meegan whose guidance, encouragement and kindness throughout this research shall always be remembered and appreciated.

A special thanks to Dr. John O'Brien for all the NMR spectra, together with his helpfulness and advice. I would also like to thank Dr. Pierce Kavanagh for the low resolution spectra and Mick O'Shea, University College Cork, for the high resolution mass spectra.

Grateful thanks to the staff of the School of Pharmacy for all their assistance, in particular Ray and Rhona. I would also like to express my thanks for the help and support, which I received from the staff and postgrads of the Biochemistry Department, especially from Dr. Daniela Zisterer.

I would also like to extend my gratitude to BioResearch Ireland for their financial contributions to this project.

A very special thank you to 'Mary's group' Sandra, JJ, Catherine, Dave, Mary and Miriam for those special moments that made life in and out of the lab memorable, together with their friendship which I bring with me. Also many thanks to my fellow post-graduate students, my life long friends and house mates.

A special thank you to my brother Eamonn, sisters Niamh and Christina for their invaluable help and kindness. To my Mam and Dad, words cannot express my thanks to them for all they have done for me throughout the years. Thank you Granny for your inspiration.

Finally, Declan, my fiancé, 'the bestest person in the world', for his endless patience and kindness, to whom I will always be indebted and gracious in helping me through each step of the way!

Abbreviations

anhyd	Anhydrous
Ar	Aryl
AF	Activated Functions
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
$Ba(OH)_2$	Barium hydroxide
BBr_3	Boron tribromide
Bmax	Maximum binding capacity
BSA	Bovine serum albumin
cAMP	Cyclic Adenosine Monophosphate
cdks	Cyclin-Dependent Kinases
CH3CN	Acetonitrile
CNS	Central Nervous System
COSY	Correlation spectroscopy NMR experiment
DUB	Dysfunctional uterine bleeding
DNA	Deoxyribonucleic acid
DMBA	Dimethylbutyric acid
DIBAH	Diisobutylaluminium hydride
DMSO	Dimethylsulphoxide
DEPT	Distortionless Enhancment by Polarisation Transfer
δ	Delta (chemical shift, ppm)
DMF	Dimethylformamide
DCC	Dextran coated charcoal
ER	Estrogen Receptor
ERE	Estrogen Response Element
E1/E2	Elimination reactions
EDTA	Ethylenediaminetetraacetic acid
EMEM	Eagles Modified Essential Medium
EBDA	Equilibrium Binding Data Analysis
EIMS (LR)	Electron Impact Low Resolution Mass Spectroscopy
EIMS (HR)	Electron Impact High Resolution Mass Spectroscopy
EtOH	Ethanol
FSH	Follicle Stimulating Hormone
FCS	Fetal Calf Serum
FACS	Fluoresence Activated Cell Sorting
GnRH	Gonadrotropin-Releasing Hormone

GH	Growth Hormone
HSP	Hormone Secretion Protein
HMPT	Hexamethylphosphorustriamide
Hg	Mercury
[3H] estradiol	tritiated estradiol
HPLC	High Liquid Performance Chromatography
hr	Hours
IR	Infra Red Spectroscopy
IGH	Insulin-like Growth Hormone
IC_{50}	Concentration of displacer inhibiting 50% of specific binding
J	Absolute value of the coupling constant in Hz
KOH	Potassium hydroxide
$KHSO_4$	Potassium hydrogen sulphate
K_2CO_3	Potassium carbonate
K	Potassium
Ki	Inhibitor constant
KBr	Potassium bromide
KD	Equilibrium or dissociation constant
LH	Lutenising Hormone
LPC	Ligand Protein Contact
Li	Lithium
LDH	Lactate dehydrogenase
lit.	Literature
LVT	Low valent titanium
MAP	Mitogen Activated Protein
MDR	Multi-Drug Resistance
MXT	Mammary tumours
MeOH	Methanol
MCF-7	Breast adenocarcinoma cells
MTT	3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide
ml	Millilitres
M	Molarity in moles per litre
M^+	Molecular ion
m.p.	Melting point
m/z	Mass of an ion divided by its charge
Ni	Nickel

NMR	Nuclear Magnetic Resonance
NEAAM	Non Essential Amino Acid Medium
n-BuLi	*n*-Butyllithium
OHT	4-Hydroxytamoxifen
OAc	Acetate
PKC	Protein Kinase C
PET	Positron Emission Tomography
P_2O_5	Phosphorus pentoxide
PhLi	Phenyllithium
$Pd(PPh_3)_4$	Tetrakis(triphenylphosphine)palladium (0)
PPh_3	Triphenylphosphine
PBS	Phosphate Buffered Saline
PDB	Protein structure file (Brookhaven format)
RNA	Ribonucleic Acid
R_f	In TLC, ratio of distance travelled by compound to distance travelled by solvent front
RBA	Relative Binding Affinity
SERM	Selective Estrogen Receptor Modulator
SN	Substitution reactions
SEM	Standard Error of the Mean
SAR	Structure Activity Relationship
TPE	Triphenylethylene
TGF	Tamoxifen-like Growth Factors
Ti	Titanium
$TiCl_3$	Titanium trichloride
$TiCl_4$	Titanium tetrachloride
TLC	Thin Layer Chromatography
THF	Tetrahydrofuran
νmax	Frequency maximum of IR absorption bands
WHO	World Health Organisation
Zn-Cu	Zinc Copper couple
Zn	Zinc
$ZnCl_2$	Zinc chloride

Table of Contents

Table of Figures

Table of Tables

Table of Schemes

1. Development of clinical antiestrogens

1.1. Introduction

Cancer is a condition in which certain body cells multiply without any apparent control and destroy healthy tissue and organs. The medical term for "cancer" or "tumour" is neoplasm, which means a relatively autononous growth of tissue. Tumour is a general term indicating any abnormal mass or growth of tissue, not necessarily life-threatening.[1] [Figure 1]

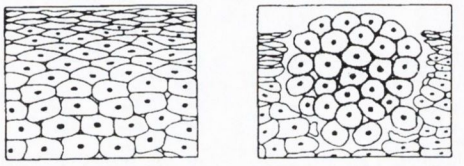


Figure 1: Normal cells and tumour cells

A "cancerous tumour" is a malignant neoplasm with potential danger. The critical difference between benign and malignant neoplasms, is that benign tumours do not metastasize, whereas malignant tumours (or cancers) do. In a benign tumour, if the cells continue to grow at the original site, they may cause problems by pressing on the surrounding organs or tissues.[2] A malignant tumour however, consists of cancer cells which have the ability to spread beyond the original site and if left untreated may invade and destroy surrounding tissues. A metastasis is a secondary growth originating from the primary tumour and growing elsewhere in the body. Cancerous cells have the ability to spread to the other organs in the body via the bloodstream or lymphatic system.

1.2. Breast cancer

Breast cancer is the biggest single fatal illness among women aged between 35-54 years and is responsible for one in five of all female cancer deaths in the western world.[2] The disease claims the lives of around six hundred and sixty women in Ireland each year, and The Irish Cancer Society suggests that one in eleven Irish women will develop breast cancer at some stage during their lifetime. The Department of Health acknowledges that the Irish incidence of breast cancer of 1,200 new cases diagnosed annually is one of Europe's highest.[3]

1.2.1. Treatment of breast cancer

Surgery (i.e. mastectomy or lumpectomy), radiotherapy, hormone therapy, chemotherapy or a combination may be used to treat breast cancer. The treatment depends on the stage of tumour development, the type and size of the tumour and the general health and age of the patient suffering the disease.[3] More recent approaches involve treatment with high doses of chemotherapy followed by a bone marrow or stem cell transplant.[2]

1.3. Estrogen dependent breast cancer

The dependence of some human breast cancers on ovarian hormones has been recognised since the 14th century. As far back as 1836, a correlation was observed between tumour growth and the menstrual cycle[4] (Cooper 1836). Many human breast tumours grow as estrogen dependent tumours and much research has tried to elucidate the mechanism for estrogen stimulated cell proliferation of human breast cancer.[5] However, not all breast cancers are estrogen derived, some are estrogen independent e.g. due to a genetic mutation (heritable factors). The ability of cancer cells to produce and secrete proteins with growth factor activity was shown already in 1978,[6] and Sirbasku[7] proposed in 1981 that estradiol (1) [Figure 2] stimulates all the proliferation observed in estrogen receptor positive human breast cancer cells, by inducing synthesis and secretion of proteins with mitogenic activity.[5]

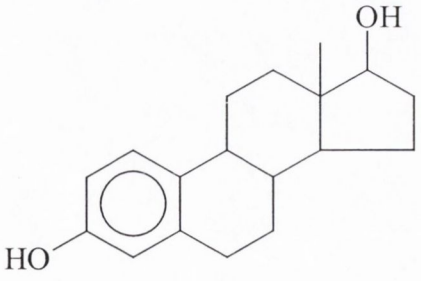


Figure 2: 17β-Estradiol (1)

Although estrogens do not cause transformation of breast tissue from a state of normoplasia to one of neoplasia, a striking association between estrogens and breast cancer is supported by the following observations.[8]

1. the rapid growth of the malignant clone when estrogens are added *in vitro* to human breast cancer cell lines
2. diminution of tumour mass after oophorectomy in premenopausal women with hormone sensitive breast carcinoma, and

3. the small but significant increase in breast cancer risk amongst postmenopausal women after 10 or more years of estrogen replacement therapy

Estrogens are thought to be the primary mitogen for hormone-dependent breast cancer. Thus estrogen deprivation is fundamental to the treatment of many benign and malignant diseases of the breast and reproductive tract. Also, decreasing ovarian and extraovarian production of these hormones by procedural or pharmacological means can produce measurable reduction of tumour mass or delay disease progression.[4]

Approximately 60% of all breast cancer patients have hormone-dependent breast cancer, with these cancers characterised as containing estrogen receptors and requiring estrogen for tumour growth.[9,10] The hormone plays an important role in the initiation and maintenance of the cancerous state, by stimulating the escalation of the uncontrolled cell proliferation.

Estrogens are involved in numerous physiological processes,[4,10] including the development and maintenance of female sexual organs (uterus and vagina), the reproductive cycle and various neuroendocrine functions in centres such as the anterior pituitary and hypothalamus.[11] They have been implicated in the pathology of benign gynaecological conditions such as endometriosis, uterine fibroids and dysfunctional uterine bleeding (DUB), each of which is responsible for annual morbidity.

1.4. The Estrogen Receptor

The biochemical roles of estrogens in the development of breast cancer remain to be fully elucidated.[10] A proposed mechanism for estrogen stimulation involves the binding of estradiol to estrogen receptors. Bound estrogen receptors interact with estrogen-responsive elements on cell chromatin and induce alterations in specific gene transcription and protein synthesis.[5]

1.4.1. Structure of Estrogen Receptor

The estrogen receptor (ER) belongs to a family of transcription factors called the nuclear hormone receptor superfamily, that can initiate or enhance the transcription of genes containing specific hormone responsive elements.[12] This ligand-inducible transcription factor has a molecular organisation which has distinct regions associated with DNA binding, hormone binding, receptor

dimerisation and gene activation. Binding of the natural hormone, estradiol, to the ligand-binding domain (LBD) of the ER triggers dimerisation and nuclear translocation of the receptor, with the assembly of a functional transcription complex through recruitment of various coactivators. Studies in a number of laboratories are beginning to provide an insight into detailed structural mechanisms and functions.[13,14]

Two ER isoforms, termed α and β, have been described and are shown to have distinct tissue distribution profiles. The ERα regulates the differentiation and maintenance of neural, skeletal, cardiovascular and reproductive tissues. Compounds that modulate ERα transcriptional activity are currently being used to treat osteoporosis, cardiovascular disease and breast cancer.[15] ERβ was recently identified in the epithelial cells of the rat prostate and in the granulosa cells of the ovary. This novel estrogen receptor is highly specific for estradiol, but has different ligand-binding specificities from the classic estrogen receptor.[16] Of the two receptors, only ERα is found in breast tissues.

The ERα protein consists of 595 amino acids with a molecular weight of 66kDa and is separated into six different functional domains.[17] It is located on chromosome 6q-sub band 25.1. All ERα ligands bind exclusively to the C-terminal ligand-binding domain (LBD). The LBD recognises a variety of compounds diverse in shape, size and chemical properties. Some of these ligands include the endogenous estrogen 17β-estradiol (E_2) and the synthetic nonsteroidal estrogen diethylstilbestrol, which function as pure agonists. Synthetic ligands such as ICI 164,384, tamoxifen and raloxifene function as antagonists.[14,15]

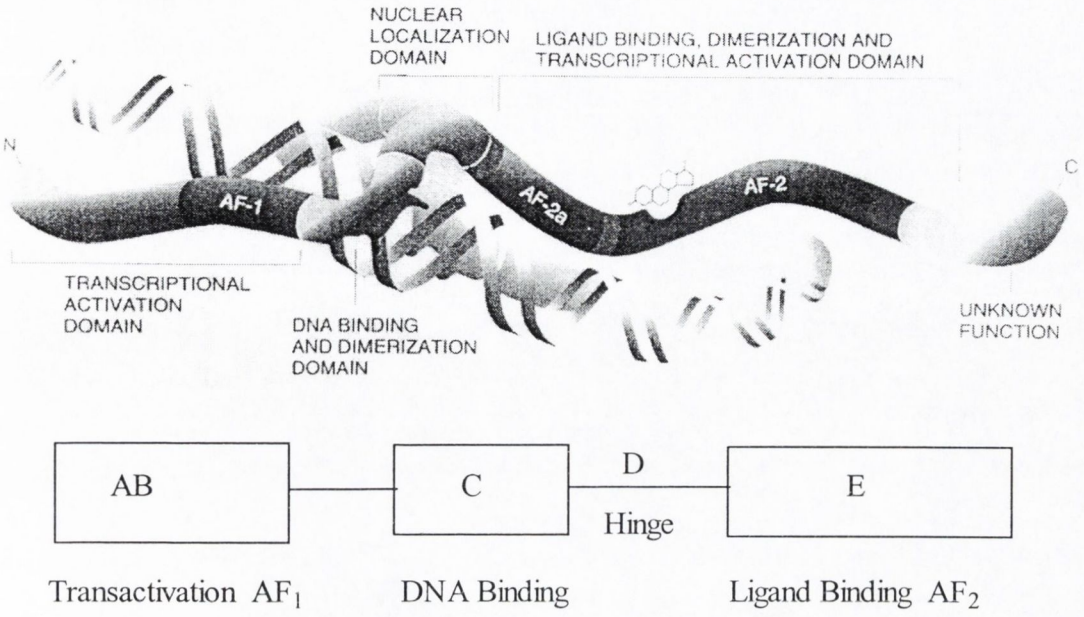


Figure 3: Structure of the ERα complex[13,14]

Transcription activation by ERα is mediated by at least two separate activation functions (AFs), AF-1 in the *N*-terminus and AF-2 in the LBD (Figure 3). The activity of AF-1 is regulated by growth factors acting through a mitogen activated protein (MAP) kinase pathway, while AF-2 activity is responsive to ligand binding. Functionality which occurs at AF-2 involves dimerisation, nuclear localisation and HSP interaction, and it is ligand dependent. The binding of agonists triggers AF-2 activity, whereas the binding of antagonists does not. A third site involved in transcriptional activation was recently located and is termed AF-2a. Recent structural studies suggest that ligands regulate AF-2 activity by directly affecting the structure of the LBD.[13,14]

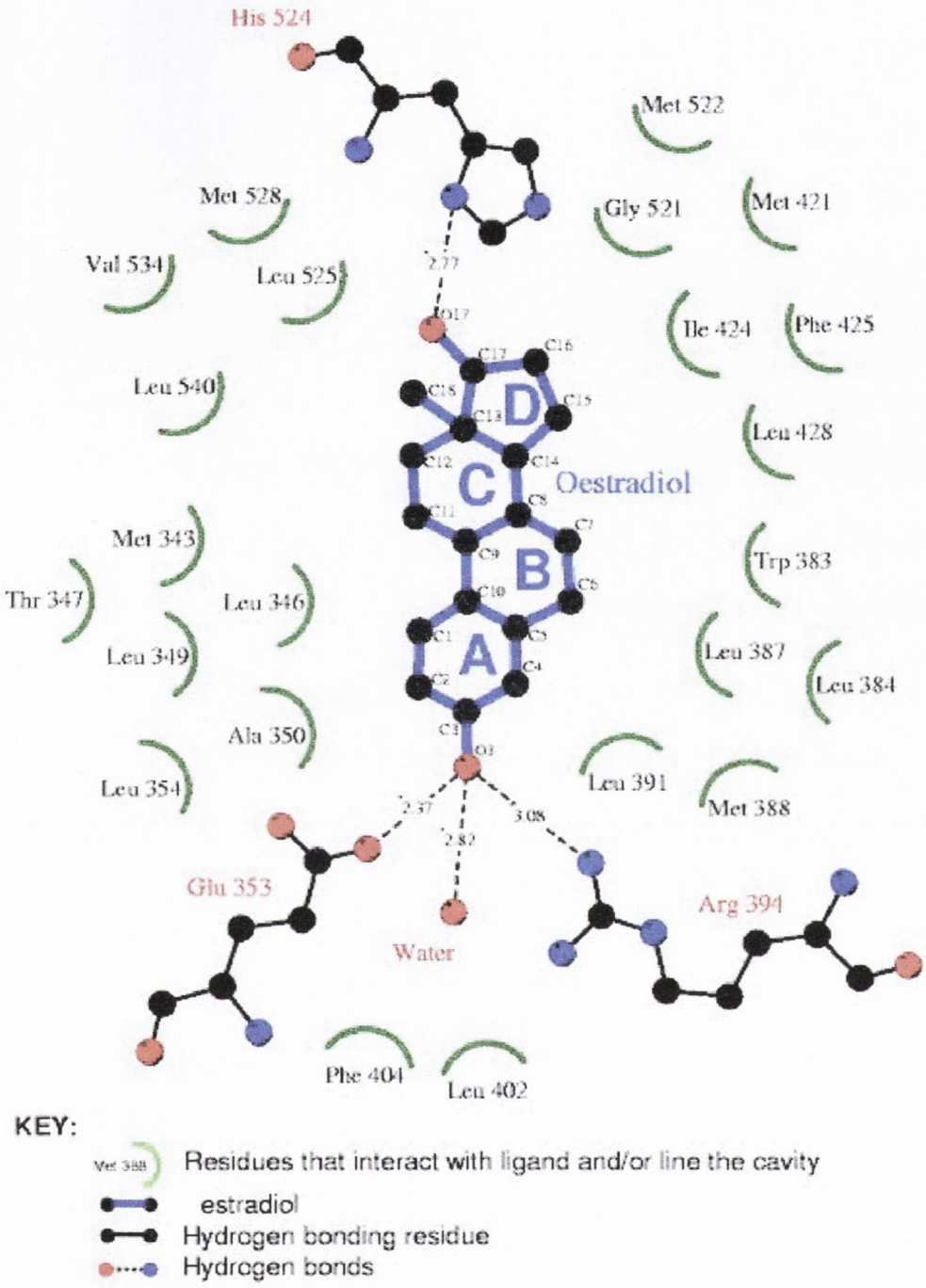


Figure 4: Schematic representation of ligand-binding cavity in the 17β-estradiol complex[12]

Hubbard and co-workers have recently determined the crystal structure of the LBD of the α-isoform of the ER in complex with 17β-estradiol (3.1Å) and the selective estrogen receptor raloxifene i.e. antagonist (SERM; a selective estrogen receptor modulator) (2.6 Å).[12]

A representation of the residue interactions of both estradiol (1) and raloxifene (28) within the binding cavity are depicted in Figure 4 and Figure 5 respectively. The binding cavity is completely partitioned from the external environment and occupies a relatively large portion of the ER LBD's hydrophobic core. Residues that interact with the ligand and/or the cavity are shown in their approximate positions. Those that make direct hydrogen bonds are depicted in ball-and-stick style with broken lines between the interacting atoms. The hydrogen-bond distances shown are averaged between the six (estradiol) or two (raloxifene) monomers. The atom names and ring nomenclature are also given. An in-depth LPC analysis for the specific residues is discussed in Chapter 5.

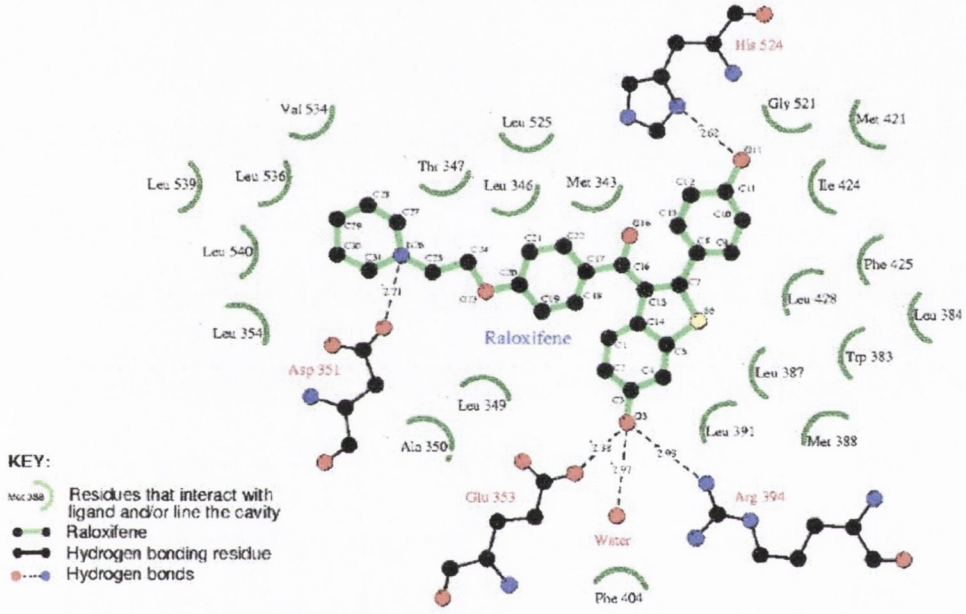


Figure 5: Schematic representation of ligand-binding cavity in the raloxifene complex

The structures provide both an insight into the binding of different ligands to the receptor and a possible structural mechanism for antagonist activity in the nuclear receptor superfamily. The overall ER-LBD structure comprises of twelve helices (H1-H12) and is folded into a three-layered antiparallel α-helical sandwich comprising a central core layer of three helices (H5/6, H9 and H10) which in turn is sandwiched between two additional layers of helices (H1-4 and H7, H8 and H11). This helical arrangement creates a 'wedge-shaped' molecular scaffold that maintains a sizeable ligand-binding cavity at the narrower end of the domain. Estrogen binds to the LBD in this large hydrophobic pocket that is formed by the proteins α-helical scaffold.[12,14] Hormone recognition can be achieved through a combination of specific hydrogen bonds and the

complementary characteristics of the binding cavity and the hormones non-polar character. The remaining secondary structural elements consist of three small, two-stranded, anti-parallel β-sheet (S1 and S2) and H12, are located at this ligand binding protein of the molecule. The ER has a very distinctive pharmacophore, where the phenolic group can bind to the amino acid residue of the receptor, for example the 17β hydroxyl (O-17) of the D-ring makes a single hydrogen bond with His 524 in H11 and the OH group at H3 has contact with Glu 353 and Arg 394. The remainder of the 17β-estradiol molecule participates in a number of hydrophobic contacts.[12,14]

1.4.2. Dimerisation of ER

The ER normally resides in the nucleus in an inactivated state. When estradiol diffuses into cells, it recognises the receptor protein and binds to it with high affinity. Hormone binding activates the receptor and facilitates the formation of dimers as depicted in Figure 6.

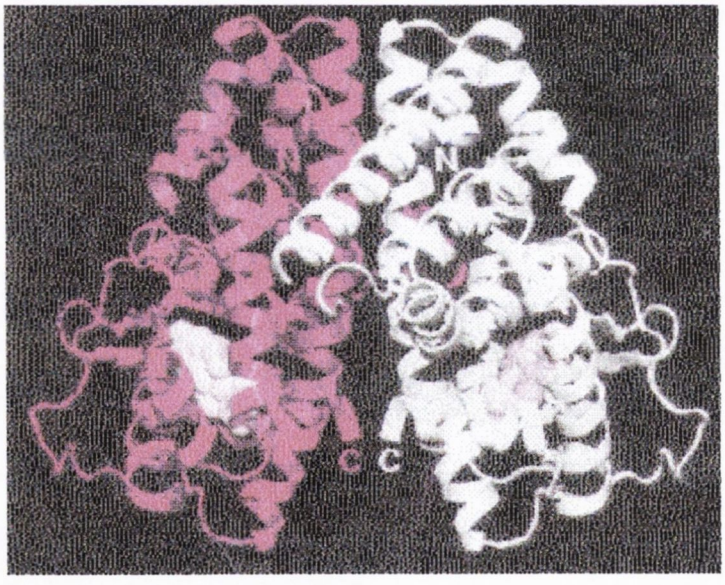


Figure 6: Dimerisation of ER

In ER-estradiol and ER-raloxifene complexes, the LBD crystallises as a homodimer. Phosphorylation of the receptor on serine, threonine and tyrosine residues may be important for its activation and biological function. Receptor dimers bind to an estrogen response element (ERE) of DNA adjacent to target genes to initiate gene transcription.[13] The dimer axis coincides with the longest dimension of the LBD with a molecule tilted approximately 10° away from the twofold layers. This symmetric 'head-to-head' arrangement locates the *N*-

termini of each monomer on opposite sides of the dimer and the carboxy termini projecting towards the two fold axis.[12,14,15]

1.4.3. Agonist and antagonist action at ER

Agonists and antagonists bind at the same site of the receptor, but demonstrate different binding modes at the 'D-ring end' of the cavity. The non-steroidal antagonist raloxifene possesses a long sidechain, a pyrrolidinylethoxy group, that prevents the alignment of Helix 12 (H12) over the ligand-binding cavity. The sidechain makes extensive hydrophobic contacts with H3 and H5/H6, H11 and the loop between H11 and H12. However, as the sidechain is over 11Å in length, it is too long to be contained within the confines of the binding cavity and displaces H12 and protrudes from the pocket between H3 and H11. The hydroxyl group of the benzothiophene moiety binds in the polar pocket between H3 and H6.[12,14]

This observation of helix displacement is anticipated as a general mechanism for both steroidal and nonsteroidal antiestrogens that possess bulky substituents. The ligand-dependent transcription activation function (AF-2) of ER is located on H12. This helix is essential for transactivation, as either loss or mutation in this region results in a receptor that is unresponsive to the ligand.

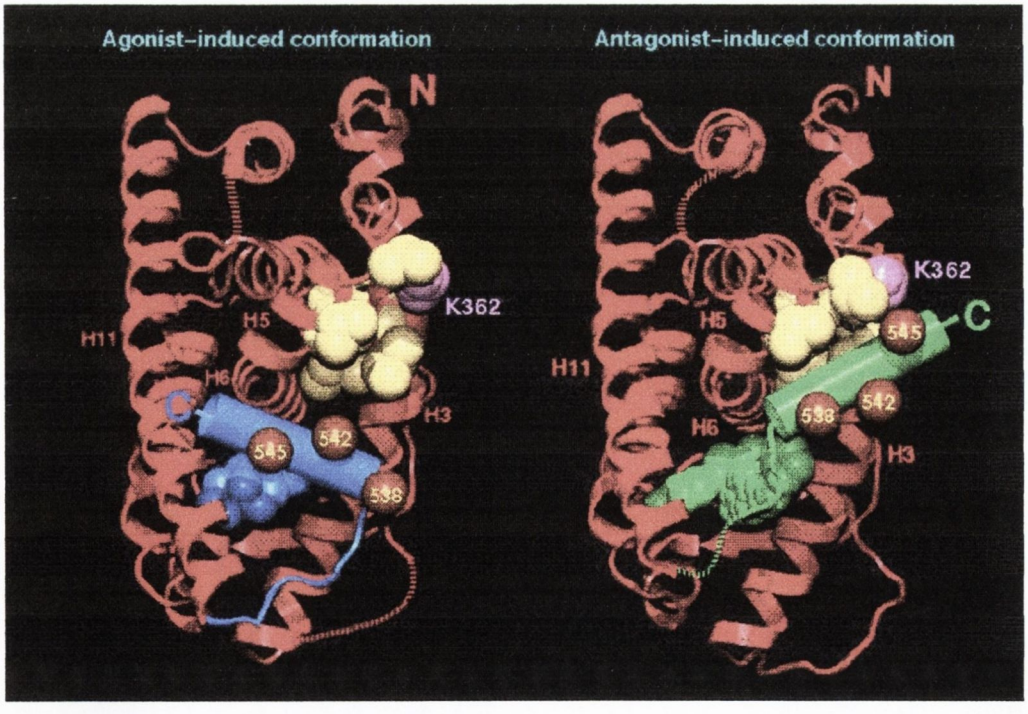


Figure 7: Hubbard's depiction of agonist and antagonist induced conformation of the ER

In the agonist estradiol (E_2)-ligand complex (Figure 7), H12 sits snugly over the hormone-binding cavity, with its charged surface exposed and is packed against H3, H5/6 and H11. The precise positioning of H12 has been observed in all liganded forms of nuclear receptor LBD's, and appears to be a prerequisite for transcription activation as it forms the "lid" (seal) of the binding cavity and projects its inner hydrophobic surface towards the bound hormone. In doing so it generates a competent AF-2 which is capable of recruiting transcription coregular proteins and interacting co-activators.[12,14,15]

In contrast the alignment of H12 over the cavity is prevented by raloxifene (Figure 7) and instead the helix lies in a groove formed by H5 and the carboxy-terminal H3. This antagonist induced repositioning of H12 involves a rotation of 130° combined with a 10Å rigid-body shift towards the amino terminus of the LBD when compared with the agonist induced conformation. A consequence of this movement is to mask a highly conserved lysine residue (Lys 362 i.e. K362) which is required for efficient estradiol-dependant recruitment of certain co-activators.[18] This is relocated at one end of this hydrophobic groove and is partly buried by the reoriented helix. Taken together, these observations provide compelling evidence that the antagonistic properties of drugs such as raloxifene are based on their ability to prevent the formation of a transcriptionally 'competent' AF-2 conformation. The movement of H12 clearly disrupts the overall structural topography of AF-2.[14]

Hubbard's research has given valuable insights into the binding of ligands to this ERα receptor and provides the basis for the structure-based design of improved agonists and antagonists for the treatment of estrogen-related diseases.

1.5. The role of estrogen in tumour growth

The role of endogenous estrogen in binding to the ER is outlined as follows:

Estrogen is a lipid soluble steroid hormone,[13,19] that diffuses freely across the plasma cell membrane into the interior of the cell where it binds to the estrogen receptor forming a complex that binds to specific DNA sequences in promoter regions of responsive genes. For many years it was generally thought that estrogen bound to the ER in the cytoplasm and translocated into the nucleus, but it is now known that the ER is a nuclear transcription factor which initially interacts with estrogen in the nucleus (King and Greene, 1984; Welshons *et al.*, 1984).

The ER is a potent transcription factor or co-activator for a variety of genes termed estrogen response elements (ERE). These include genes encoding the progesterone receptor and growth regulating proteins.[20,21,22,23]

Some of the regulatory protein molecules,[13] when secreted by a tumour cell stimulate tumour growth by binding to receptors on the same cell (autocrine stimulator) or on neighbouring tumour cells (paracrine stimulation). Others stimulate growth of stromal tissues, such as fibroblasts and blood vessels. Stromal cells complete the loop by releasing their own growth factors, which in turn stimulate the cancer cells, or release proteolytic enzymes that promote invasion and metastasis. This is why biochemical observations have noted that once the estrogen binds to the ligand-binding domain (LBD) of the ER, heat shock proteins[14] dissociate and cause a change in conformation and homodimerization occurs.[19]

Tumour growth is also dependent on polypeptide hormones and growth factors in serum. Growth factors such as TGV-A, IGI-I, IGF-II, TGF-B and the cathespin D have been found to be regulated by estradiol in breast cancer cells,[5,9,24,25,26] but the mitogenic effects of each individual factor seem to be dependent on growth conditions.[9,27, 28, 29]

Inhibition of these pathways by reducing hormone levels or by interfering with binding of hormones to receptors is the basis for a variety of experimental breast cancer treatment strategies.

1.6. Alternative functions of estrogen and the Estrogen Receptor

Estrogen elicits actions specific to various cells and target tissues, although the estrogen receptor proteins (ERα or ERβ) present in all tissues appear identical.[30] For example, estrogen stimulates a variety of uterine effects such as increased RNA synthesis, DNA synthesis, protein synthesis, mitosis and hyperplasia (Quarmby and Korach, 1984b), i.e. the proliferation of stromal, endometrial, and glandular tissue in the uterus, whereas in the anterior pituitary gland estrogen acts to suppress production of FSH and LH and increase synthesis and secretion of prolactin (Rosenfeld *et al.,* 1987). Estradiol activation[20] of the estrogen receptor has several consequences. In human female secondary sex organs, there is proliferation of stromal and ductal tissue in the breast (as previously outlined). In addition to these classical target tissues, further studies have revealed non-traditional target sites for estrogen action, such as bone (Ettinger *et al.*, 1985), brain centres that maintain body temperature and the cardiovascular system (Barrett-Connor and Bush, 1991).[30]

Estrogen is believed to preserve bone density[31] helping to keep a balance between its creation and degradation. It also safeguards the heart, largely by limiting the build up of atherosclerotic plaque in the coronary arteries. The arteries are protected in part by estrogens ability to modulate the manufacture of cholesterol in the liver. A complete understanding of the effects of estrogen in these tissues has been difficult due to the absence of an appropriate physiological model.

The action of estrogen has been thought of as a binary system i.e. ligand + receptor → product. This hypothesis implies that simple stoichiometry exists which determines estrogen action. Several lines of evidence exist which show that the action of estrogen through the estrogen receptor is regulated by a much more complex series of reactions in all tissues. Katzenellenbogen *et al.,*[20,32] refer to the complex as a tripartive system: ligand + receptor + effectors → products. The effectors bestow on the ligand-receptor complex the tissue and cell specificity.

Estradiol, diethylstilbestrol and hexestrol binds with high affinity to the estrogen receptor, whereas other endogenous and exogenous compounds, for example a myriad of other polycyclic chemicals such as OP-DDT, methoxychlor, several phytoestrogens and some alkylphenols bind with much less affinity. The estrogen receptor is a relatively promiscuous receptor that binds to several other

substituted stilbenes, including clomiphene, tamoxifen and toremifene due to its large LBD cavity.[20,33]

The actions of estrogens through the receptor are tissue and cell specific. For the most part 17β-estradiol is a stimulatory agent, including its effects on gene expression and growth in target cells,[20,34] but other ligands may result in varying responses.

1.7. Development of antiestrogens for use in breast cancer

Treatment options for hormone responsive metastatic breast cancer focus principally on interfering with the endocrine system, in an attempt to modify some of the effects of estrogen. Ablation of estrogen production can be accomplished surgically (by oophorectomy, adrenalectomy or hypophysectomy) or radiologically.[8,35] In addition there are three pharmacological approaches for depriving tumour cells of estrogen:

1. Agents that block estrogen receptors (e.g. antiestrogens)
2. Agents that inhibit the release of gonadotrophic hormones[36] (e.g. gonadotropin- releasing hormone [GnRH] agonists) and
3. Agents that decrease circulatory estrogens, by suppressing their biosynthesis[37, 38] (e.g. aromatase inhibitors).

Progestins and androgens are also used in the management of hormone-sensitive breast carcinoma. Although the antitumour mechanisms of these agents are still not clearly understood, inhibition of tumour cell proliferation may be partly related to the reduction in circulating estrogen levels as a result of the agents negative feedback effect on the hypothalamic-pituitary-adrenal axis.[39,40]

Antiestrogens have proven to be effective in controlling the growth of hormone responsive breast cancers.[41] The presence of such receptors provides the rationale for treatment of the disease with antiestrogens or estrogen blockers.[42,43]

Antiestrogens can be classified into two major groups: those that have mixed estrogenic / antiestrogen actions e.g. tamoxifen and its metabolites (Type I) and those that are pure antiestrogens (Type II) which possess no estrogen like properties.[19] Their classification may be based on different mechanisms of action. A number of selective estrogen receptors modulators (SERMs) can

replicate estrogen's crucial benefits for bones and the heart, but will act as antiestrogens (estrogen blockers) in the breast and uterus. These so-called designer estrogens e.g. raloxifene will mimic (agonist) estrogen effects in some tissues but will combat harmful effects.[31]

1.8. Antiestrogenic action in breast cancer cells

Antiestrogens are therapeutic agents used as 'steroid blockers' or 'anti-steroids' which bind to the cytosolic steroid receptor (antagonistic action). These agents act at several sites in the chain of events of estrogen action: competitive and non-competitive binding to the receptor, binding to nuclear transport proteins, disruption of binding of the estrogen receptor-estrogen response elements complex, disruption of nuclear transport of the estrogen receptor, post-translational modification of 17β-estradiol responses and functional alteration of the target tissue.[20,44,45,46,47] The most important feature of these therapeutic agents is that at the concentration of antiestrogens achieved in the blood of breast cancer patients taking antiestrogens (up to $2x10^{-6}M$), antiestrogens selectively inhibit the proliferation of estrogen receptors containing breast cancer cells, and this inhibition is reversible by estradiol. Antiestrogens also inhibit estrogen-stimulation of several specific protein synthetic activities in breast cancer cells, including increases in plasminogen activator activity, progesterone receptor levels and production of several secreted glycoproteins and intracellular proteins.[41]

Many antiestrogens undergo bioactivation and metabolism *in vivo* and hydroxylated forms of the antiestrogen have markedly enhanced affinities for the estrogen receptor. Detailed studies indicate that antiestrogens induce important conformational changes in the receptor that are reflected in the estrogen receptor complex, reduced interaction with DNA and dissociation kinetics of the antiestrogen-estrogen receptor complex. These conformational changes effected by antiestrogens are likely to result in different interactions with chromatin, causing altered cell proliferation and protein synthesis.[41]

Present day antihormonal treatment of metastatic breast cancer relies heavily on the use of the single nonsteroidal antiestrogen tamoxifen, which belongs to the class of the triphenylethylene (TPE) derivatives.[48,49,50,51] Tamoxifen, its metabolites and analogues have been reported to have many molecular targets including the estrogen receptor[52,53] membrane receptors (possibly the histamine, dopamine and muscarinic receptors), a primarily microsomal antiestrogen

binding protein (calmodulin),[54,55,56] several enzymes (prostaglandin synthase,[57] glutamate dehydrogenase[58]) and at least two Ca^{2+}-dependent kinases (calmodulin kinase[59] and protein kinase C [60,61,62](PKC)). The relevance of interaction with these targets to growth-promotion or inhibition is not yet fully understood. [Figure 8]

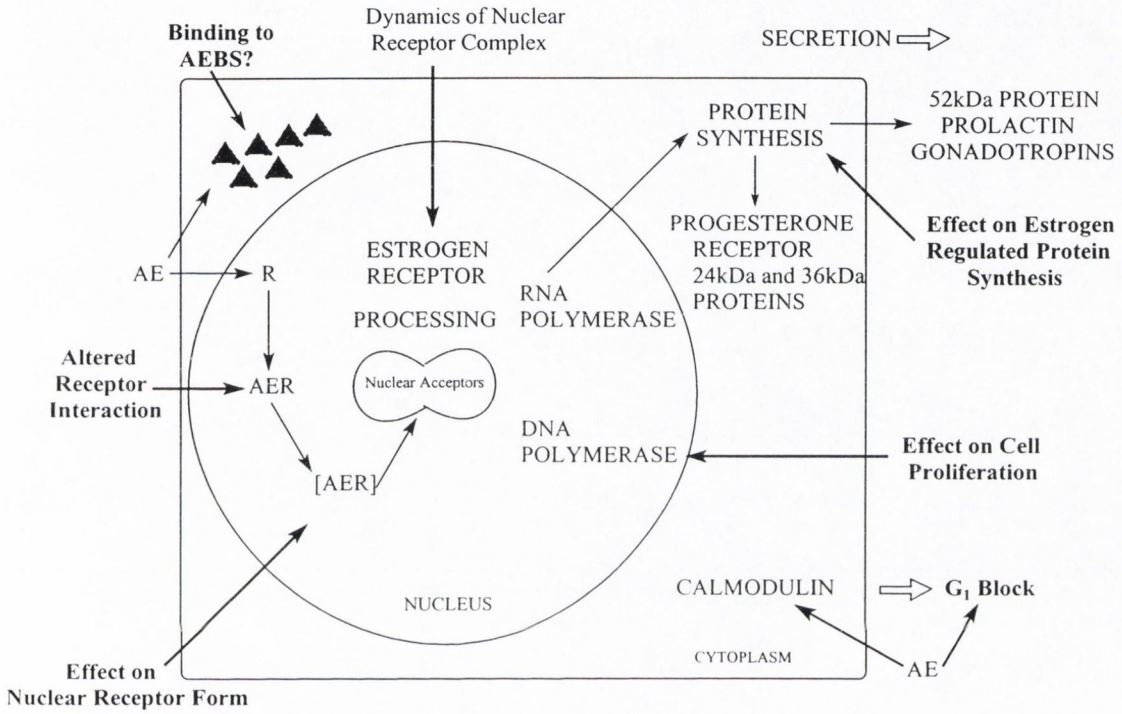


Figure 8: Potential mechanistic action of antiestrogens (AE)

Tamoxifen, like estrogen, binds to ER and induces dimerisation and DNA binding, but it fails to induce transcription of certain genes that are critical for breast cancer.

Several potential antiestrogens and SERMs have been designed and synthesised, some of which are in clinical development. These will be discussed in a later section.

1.9. Tamoxifen

1.9.1. Introduction

Tamoxifen, a synthetic nonsteroidal antiestrogen, was initially developed by ICI Pharmaceuticals (now Zeneca) in 1969 as a possible oral contraceptive. Although effective in rodents, it had the opposite effect of stimulating ovulation in humans and was subsequently developed as a fertility agent.[63,64] However, it was found in clinical testing to be an effective treatment for advanced metastatic breast cancer in postmenopausal women[36,65] and shown was to control and inhibit the growth and spread of hormone responsive breast cancers.[49,50,66] With this potent triphenylethylene compound, it appears possible to achieve non-invasively the same hormonal effects and tumour suppression[41], which normally follows the more devastating endocrine ablative surgeries (ovariectomy, adrenalectomy, and hypophysectomy). Tamoxifen has been widely accepted as the drug used for treatment and prevention of both early and advanced stage breast cancer.[67] [Figure 9]

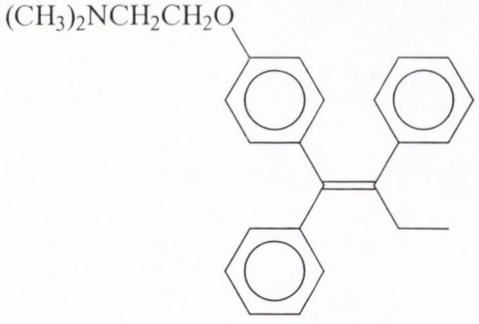


Figure 9: Tamoxifen (2)

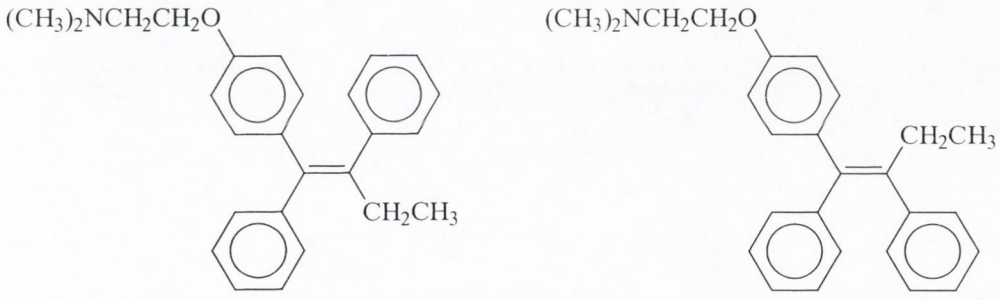
This estrogen receptor antagonist is extensively used in the hormonal treatment of breast cancer in postmenopausal women with metastatic disease and in first time treatment for premenopausal women. It is believed that tamoxifen (2) is cytostatic and exhibits competitive inhibitory activity by binding to cell nuclei where it prevents cell proliferation.[5,68]

Tamoxifen (2) exerts both antiestrogenic and estrogenic effects, depending upon the ambient estrogen concentration, the tissue and the species.[21,69] In humans tamoxifen seems to be mainly an antagonist in the brain and the breast and an agonist in the bone, uterus and cardiovascular tissues.[70,71,72] The side-effects of tamoxifen (2), when compared to those of other chemotherapeutic agents are

relatively mild and has increased the overall survival rates of its recipients.[73,74,75,76]

1.9.2. Structural elucidation of Tamoxifen

Tamoxifen (2) chemically defined as 1-[4-(2-dimethylaminoethoxy)phenyl]-1,2-diphenyl-1-butene),[77] exists in both the E and Z isomer[67,78] [Figure 10], the unsubstituted phenyl groups being respectively *cis* and *trans* relative to the ethylenic double bond.[79] Although the Z-isomer (*trans* tamoxifen) has the ability to antagonise estradiol, and the E-isomer (*cis* tamoxifen) has the properties of an agonist, both isomers are substrates for microsomal conversion to phenolic forms which have a high affinity for estrogen receptor.[23,80,81] The Z-isomer when converted to a high affinity ligand (*trans*-4-OH-tamoxifen) has a relative binding equal to or greater than estradiol.[80,81,82,83] Isomer interconversions have been observed which explain functional reversals where *cis*-4-OH-tamoxifen, an agonist acquires properties of an antagonist.[77]



Z - Tamoxifen
ICI 47,699

E - Tamoxifen
ICI 46,474

Figure 10: E and Z isomers of Tamoxifen

The configuration of the isomers was established in early work by a combination of proton magnetic resonance spectroscopy (^{1}H NMR) and dipole moment measurement.[78]

The protons of the basic side chain of Z- tamoxifen resonated at somewhat higher fields and the effect on the aromatic protons was much more pronounced, with the centre A_2B_2 system of the disubstituted ring moving to a higher field by 0.4ppm when compared with the E-isomer. It was also observed that only in the Z-isomer do the OCH_2 protons of the side chain have a chemical shift (δ) of less than 4.0ppm owing to the through space shielding influence of the vicinal Z-phenyl substituents.[84,85] These findings were rationalised by considering that the

aromatic ring and the sidechain of the Z-isomer were sandwiched between two other rings and experience a double shielding effect from their ring currents.[78]

X- ray crystallography[86] also demonstrates that the phenyl rings in tamoxifen (2) are twisted out of the plane of the double bond by more than 50°, thus indicating

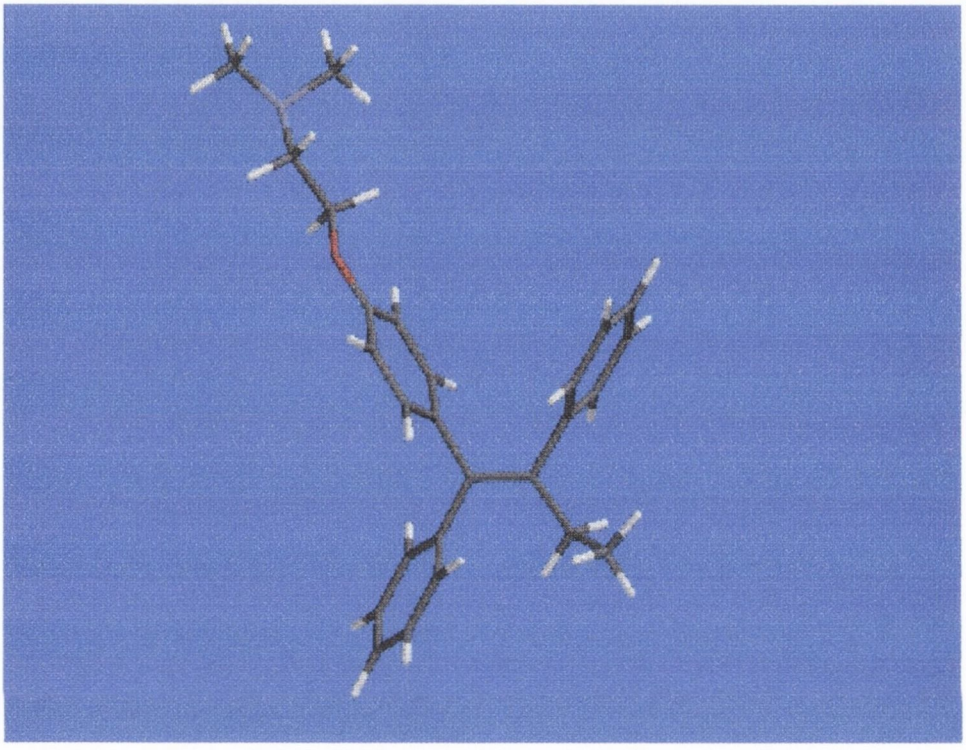


Figure 11: 3D image of Tamoxifen (Macromodel)

that Z-tamoxifen has a propeller like conformation.[87] [Figure 11] This "propeller" conformation is observed for the three rings in all triphenylethylenes. The 3D structure of tamoxifen and novel tamoxifen analogues will be discussed in Chapter 5.

1.9.3. Metabolites of Tamoxifen

As an endocrine agent for the treatment of breast cancer, tamoxifen (2) functions by competitively antagonising the mitogenic signal, which results from the interaction of estradiol with estrogen receptors (ER), in ER positive cells. There are several pharmacological factors, which may determine its ability to achieve this *in vivo*. These include the bioavailability and pharmacokinetics of tamoxifen, together with the formation of various metabolites which may have different agonist-antagonist profiles and the ability of these compounds to interact with ER and form inappropriate complexes which are no longer able to

regulate the estrogen-responsive genes involved in the growth pathway. It is as a consequence of the latter, namely tamoxifen interaction with ER, that the majority of pharmacological effects occur both *in vivo* and *in vitro*.[88]

The predominant metabolism of tamoxifen (2) occurs in the liver via cytochrome P-450 enzymes located in the microsomes.[89] The two major pathways of tamoxifen (2) metabolism in humans are shown in Figure 12 and involved demethylation, deamination and hydroxylation of the key position on the phenyl groups of tamoxifen. Demethylation of the tertiary amine results in the major metabolite found in human serum, *N*-desmethyltamoxifen.[90,91,92,93,94,95] Further demethylation produces *N,N*-didesmethyltamoxifen[96] and subsequent deamination results in the polar compound metabolite Y.[97] The alternative route of metabolism involves hydroxylation of tamoxifen at the 4-position to form 4-hydroxytamoxifen, a potent antiestrogen.[81,98]

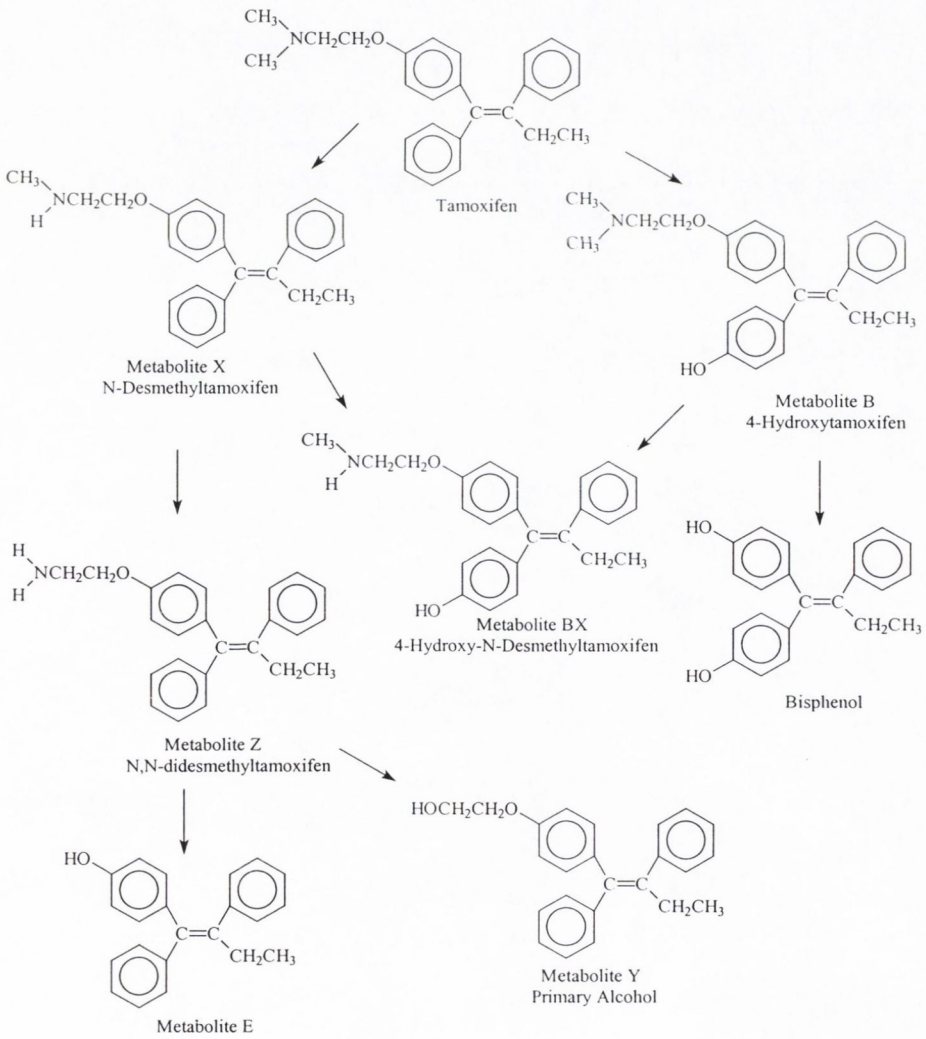


Figure 12: The metabolic pathway for Tamoxifen

All major metabolites of tamoxifen will bind to the estrogen receptor *in vitro* and competitively inhibit estradiol–stimulated growth of MCF-7 cells. However there is a considerable range in the relative binding affinity (RBA) of the different metabolites to the ER. In particular metabolites which are hydroxylated in the 4-position e.g. 4-hydroxy-*N*-desmethyltamoxifen, have a high RBA which is comparable to that of estradiol,[99] and they are approximately 100 times more potent than the parent compound tamoxifen (2) in inhibiting MCF-7 growth *in vitro*.[100] However, some of the minor metabolites, namely metabolite E and bisphenol, appear to have significantly more agonist than antagonist properties in a variety of bioassay systems.[101]

Metabolites of tamoxifen which are hydroxylated on the phenyl ring, namely 4-hydroxytamoxifen and metabolite E, are capable of under going time-dependent and temperature isomerisation from the Z (*trans*) isomer to the E (*cis*) isomer.[102] The electron withdrawing hydroxyl group weakens the ethylene bond and permits the substituents attached to the carbon atom to rotate around the double bond. This is referred as push-pull isomerisation, where the electron withdrawing group decreases the double-bond character and allows easier rotation for captodative ethylenes.[103] These configurations are important when discussing the relative estrogenic and antiestrogenic activities of these metabolites [Figure 13]. The Z (*trans*) 4-hydroxy tamoxifen has a high RBA similar to that of estradiol and is a potent antiestrogen, where as E (*cis*) 4-hydroxytamoxifen has a low RBA and is a weaker antiestrogen.[83,88]

The amine side chain is thought to be essential for antiestrogenic activity of tamoxifen.[104] In metabolite E this side chain is replaced by a hydroxyl group and upon isomerisation to the E-isomer the hydroxyl group occupies the same phenyl position as in Z-4-hydroxytamoxifen and consequently exhibits high binding affinity for the estrogen receptor [Figure 13]. However without the amine side chain, the E isomer of metabolite E behaves as a potent estrogen.[105] In contrast the Z isomer of metabolite E has much lower binding affinity and is only a weak estrogen.

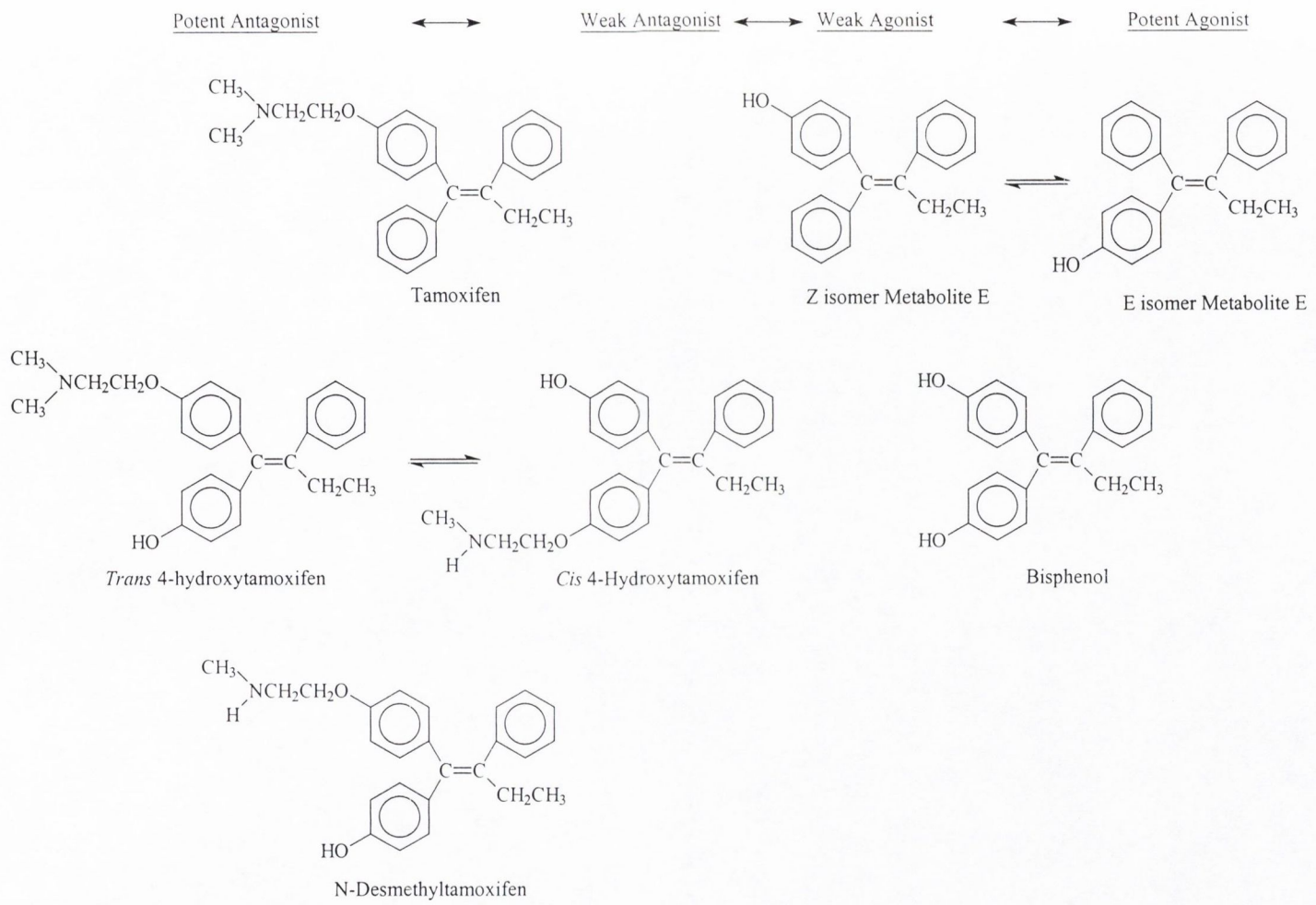


Figure 13: The structural forms of tamoxifen which may have either a predominantly agonistic or antagonistic effect upon interaction with ER

Tamoxifen (2) exerts other effects that cannot be reversed by estrogen,[106] including the inhibition of protein kinase C, antagonism of the calcium binding protein calmodulin and stimulation/suppression of growth factors and other enzymes which effect cell growth and activity.

The apparently independent actions of tamoxifen (2) and its metabolites on tumours and drug resistance, can be categorised as follows:

1. Facilitation for the chemotherapeutical agent to reach and enter the target cell by influx and efflux modification and / or possible competition (for example reversal of MDR phenotype).

2. Re-establishment of normal apoptotic pathways by interference with inhibitors of apoptosis (programmed cell death)[85]

3. Direct or indirect action on tumour cell multiplication including their accumulation in vulnerable phases of cell cycle

4. Enhancement of cytostatic action(s) and toxicity of drugs

5. Synergistic effects with interferon(s) and immune response.

This enumeration is tentative and certainly not complete, but it illustrates a variety of tamoxifen's actions.[88]

1.9.4. Other pharmacological actions of Tamoxifen.

Further studies have illustrated alternative ways in which tamoxifen (2) is believed to inhibit the growth of tumour cells. These are outlined below.

Insulin-like growth factor I (IGF-I) has been shown to be a potent mitogen for breast cancer cells *in vitro*, and IGF-I receptors have been located on human primary breast neoplasms. In a randomised, placebo-controlled study it has been found that administration of tamoxifen (2) to patients with breast cancer was associated with a statistically significant ($p=0.002$) reduction in the serum level of IGF-I and this reduction may contribute to the therapeutic effect of the drug.[107]

Calmodulin, a calcium-dependent regulatory protein of numerous cellular processes, including proliferation, interacts directly with tamoxifen (2), which results in the inhibition of cAMP phosphodiesterase action. Since this is a key component of the secondary messenger system and regulates the metabolism of

cyclic nucleotides, the inhibition of breast cancer cell growth may be due to the antagonism of calmodulin activity by tamoxifen.[106,108,109,110]

Production of the enzyme protein kinase C, which plays a key role in tumour promotion, is reported to be inhibited by tamoxifen (2), and this is thought to contribute to the antitumour action of the drug.[111,112,113]

Tamoxifen (2) induces the production of the negative growth factor TGF-B, which has been proven effective in decreasing the growth of estrogen tumours.[114,115]

It appears that at least one other anti-tumour effect of tamoxifen (2) is mediated through induction of polyamine depletion. This action has been recorded both *in vitro* and *in vivo*[116,117] and may be produced by tamoxifen (2) inhibiting the rise of ornithine decarboxylase which leads in MCF-7 human breast tumours to a dose related decrease of the polyamines putrescine and spermidine.[118]

In Ishikawa (endometrial carcinoma) cells, 4-hydroxytamoxifen significantly inhibits cell proliferation and simultaneously decreases both TGF-AmRNA and TGF-A secretion.[119]

Other estrogenic and non-estrogenic related methods by which tamoxifen (2) may exert its action in inhibiting tumour cell growth are widely documented.[50] New evidence suggests some patients suffering from estrogen independent breast cancer are giving a positive clinical response when treated with the drug.[66,67,73]

1.9.5. Beneficial aspects of Tamoxifen

By the mid 1980's, tamoxifen was listed by the World Health Organisation as an essential drug for cancer chemotherapy (WHO, 1985). It represented one of the most strongly established treatment modalities i.e. treatment of advanced breast carcinoma and prophylaxis against recurrence.[120,121,122] Recent data suggests that tamoxifen may be used for the treatment of other malignancies that are neither estrogen receptor positive nor related to breast cancer.[123,124]

There were genuine concerns that treating women with an antiestrogen would affect their lipid profile adversely and lead to an increased risk of heart disease. However analysis of nine separate studies revealed an average decrease in total cholesterol of 13% and an average decrease in low-density lipoprotein (LDL) of 19%.[125,126] A study carried out by McDonald and Stewart concluded that the risk of coronary heart disease is significantly lowered for those taking of tamoxifen (2).[19]

Furthermore toxicity studies indicate that tamoxifen (2) is estrogenic on other important tissues in the body, i.e. bone tissues.[19,66,127] As tamoxifen is an antiestrogen it might be expected to cause osteoporosis,[128,129] due to a lack of estrogen. However, tamoxifen (2) reduces bone loss in these patients[130] possibly because it can act as an estrogen agonist.[131,132] Currently the biological mechanisms underlying bone-sparing effects of tamoxifen are unclear.[131] Notably tamoxifen (2) abrogates the effects of exogenous glucocorticoids *in vivo,*[133] anti-inflammatory agents that are potent inducers of bone loss *in vivo.*[134] It has been demonstrated that tamoxifen attenuates most glucocorticoid effects on osteogensis *in vitro*. Several studies on postmenopausal patients treated with tamoxifen (2) confirm that bone mineral density is preserved or increased, for example, noted preservation of trabecular bone at the femoral neck, a common site of post menopausal osteoporotic fractures.

1.9.6. Carcinogenicity and toxicity of Tamoxifen

Tamoxifen has been described as having a relative lack of severe side effects. At normal dosage, less than 3% of patients are withdrawn from therapy due to intolerance. In general the side effects of tamoxifen are similar to symptoms experienced during the menopause.[135] Less common effects are hypercalcaemia, oedema, anorexia, pruritus vulvae, depression, dizziness, light-headedness and headache.[21,136] Recent studies have however suggested more serious adverse reactions to the drug. In particular, concerns have been raised with regard to the potential carcinogenicity of this product.[88]

Toxicological studies have been carried out to assess the carcinogenicity of tamoxifen in rats.[137] Tamoxifen (2) was found to be carcinogenic in the liver of male and female[138] rats, i.e. hepatocellular carcinoma.[139] It also acted as a promoting agent in a two-stage model of carcinogenesis in rat liver (Williams *et al.*, and Hirsimaki *et al.*,).[140,141] In the rat, tamoxifen is metabolised to α-hydroxytamoxifen,[142] where it is thought to be further activated to a product that binds principally to the exocyclic amino group of deoxyguanosine in DNA. Available data clearly indicates major differences between women and rats with respect to the activation of tamoxifen and formation of DNA adducts, and brings into question the validity of direct extrapolation of data generated in the single susceptible species, the rat, to women in assessing potential risks attendant to tamoxifen (2) administration.[137]

1.10. Clinical and experimental antiestrogens in breast cancer treatment

Antiestrogens are classified into several major groups:

(a) analogues of tamoxifen (2) or structural derivatives of the triphenylethylene type of drug which have mixed estrogenic / antiestrogenic actions – used for cancer chemotherapy

(b) antiestrogens that represent a departure from the standard triphenylethylene structure

(c) pure antiestrogens that have no estrogen-like properties

(d) designer estrogens, known as selective estrogen receptor modulators (SERMs), which behave like estrogen in some tissues but block the action in others e.g. used for estrogen activity as anti-osteoporotic agents.

1.10.1. Structural derivatives of the triphenylethylene structure of Tamoxifen

The triphenylethylene structure of tamoxifen (2) has provided the basis for several new analogues that are being investigated clinically. The finding that tamoxifen (2) is metabolised to a potent antiestrogen (4-hydroxytamoxifen)[99] has also provided a central theme for drug development.

1.10.1.1 Toremifene

Toremifene (3) or chlorotamoxifen (Fareston®)[19,143] is the first antiestrogen to be approved for the treatment of stage IV postmenopausal breast cancer since the introduction of tamoxifen (2) [Figure 14]. The drug is a chlorinated derivative of tamoxifen (2) having a high affinity for the estrogen receptor with similar clinical efficacy to tamoxifen.[50] The compound is of interest because it does not produce DNA adducts in rat liver[144] and as a result has a lower carcinogenic potential than tamoxifen (2). The drug is active against DMBA-induced rat mammary tumours[145,146,147] and exhibits properties of a tumouristic agent[147] and may be effective against hormone-dependent mouse uterine sarcoma.[146]

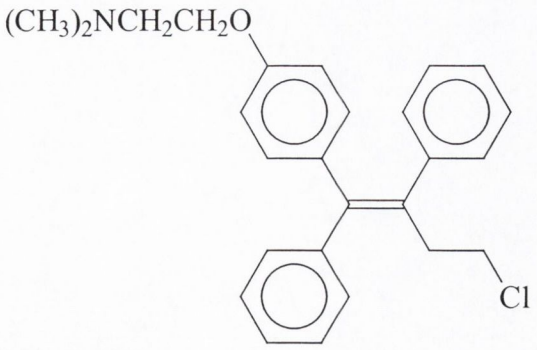


Figure 14: Toremifene (3)

Its partial estrogenic activity includes altering lipid levels, which is associated with a reduced risk of coronary heart disease and prevention of bone loss in the lumbar spine and the femoral neck.[148]

1.10.1.2 Droloxifene

Droloxifene (4) or 3-hydroxytamoxifen[19,50] is an antiestrogen with well-documented antitumour activity in laboratory models.[149] [Figure 15] This drug was initially designed to treat atherosclerosis.[150] Extensive clinical treatment has shown it to be effective (at large doses) in the treatment of advanced breast cancer in postmenopausal patients[151] and it has been observed to have antiestrogenic activity.

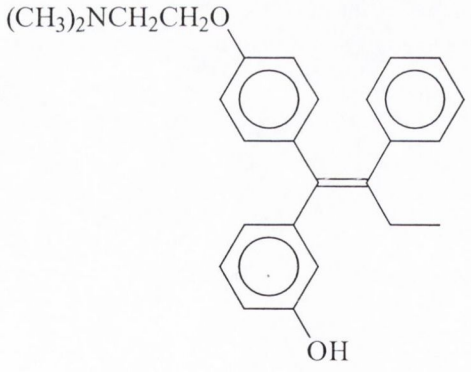


Figure 15: Droloxifene (4)

Droloxifene subsequently been developed as a result of the finding that hydroxylation of tamoxifen (2) to produce 4-hydroxytamoxifen dramatically increases its antiestrogenic properties and binding affinity for the estrogen receptor.[99,152,153,154] However, the binding affinity of droloxifene (4) is not as high as that of 4-hydroxytamoxifen.[50] The hydroxyl group of droloxifene (4) is believed to be important as it is entrusted into the lipophillic zone of the molecule which is involved in the binding to ER.[155,156] Hydroxylation increases

drug clearance, therefore a considerably shorter half-life is expected *in vivo* for droloxifene compared with tamoxifen (2).

1.10.1.3 Clomiphene

Clomiphene (5) is an antiestrogen[157] used for chemotherapy of infertility (ovulation inducement) and estrogen dependent breast cancer.[158] [Figure 16]

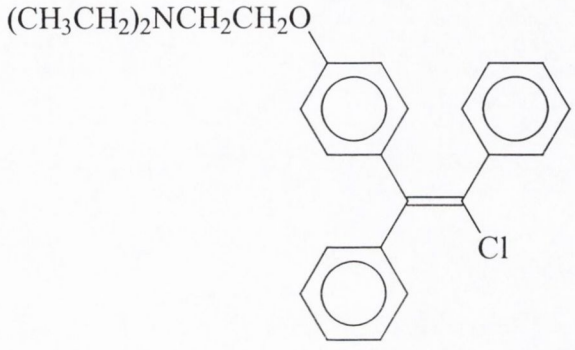


Figure 16: Clomifene (5)

The parent compound does not undergo isomerisation, but the highly active 4-hydroxy metabolite formed *in vivo* isomerises readily. The Z-isomer, zuclomiphene is estrogenic in rat uterine weight tests, whereas the E-isomer enclomiphene is a partial agonist with antiestrogen properties.[1] The most prominent use of clomiphene (5) is in the treatment of infertility, where it produces an increase in estrogen and gonadotrophin secretion which induces ovulation. This effect is as a result of the binding of clomiphene to estrogen receptors in the hypothalamus leading to a blockade to the feedback inhibition exhibited by estrogens.[1,136] Clomiphene's (5) role as a therapeutic agent for breast cancer are currently under study.

1.10.1.4 Halogenated analogues of Tamoxifen

N,*N*-diethyl halogenated analogues[159,160,161] were prepared for preliminary evaluation as agents for imaging estrogen receptors using positron emission tomography (PET) or single photon emission computed tomography (SPECT). Such agents may predict the efficiency of tamoxifen (2) therapy for breast tumours.[162] Substitution of *N*,*N*-diethyl for the *N*,*N*-dimethyl portion of tamoxifen has been established and are believed to increase binding to the estrogen receptor by a factor of four.[159,163]

1.10.1.4.1 *N,N*-Diethylfluoromethyltamoxifen

[1-(4-(2-Diethylaminoethoxy)phenyl)1,2-diphenyl-5-fluoro-1-pentene] i.e. *N,N*-diethylfluoromethyltamoxifen (6) can be prepared via a 3-step synthetic procedure from clomiphene (5) [Figure 17]. Like tamoxifen, it exists as both Z and E isomers. The Z isomer has a greater affinity for the estrogen receptor than the E isomer, however the E isomer has been found to be the more potent.[159] The presence of fluorine in the aliphatic side chain preserves the major portion of the molecule for binding with a minimum of alteration. *N,N*-diethylfluoromethyltamoxifen (6) chelates to the tumour cell phospholipid membrane and this change in membrane permeability may cause cell death,[85,156] as reported by McCague and Leclercq following cell culture experiments. *N,N*-diethylfluoromethyltamoxifen (6) binds to the estrogen receptor with a 30-fold greater affinity than tamoxifen.

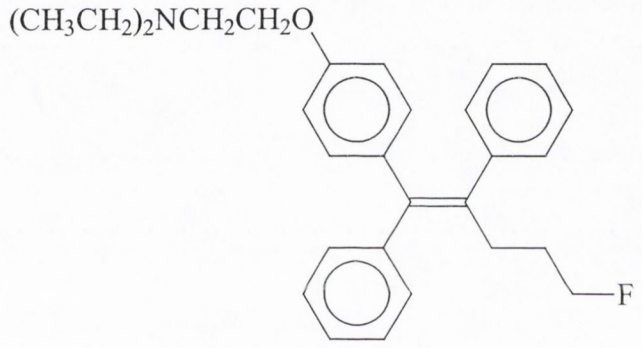


Figure 17: N,N-diethylfluoromethyltamoxifen (6)

The data obtained from *in vitro* receptor assays suggested that *N,N*-diethylfluoromethyltamoxifen (6) is a potential ligand for mapping the estrogen receptor by PET (Positron Emission Tomography).[161]

1.10.1.4.2 *N,N*-Diethyliodomethyltamoxifen

This iodo-analogue (7), like the fluorotamoxifen analogue (6), is a useful diagnostic compound for predicting the response of estrogen-receptor-positive breast tumours to tamoxifen analogues used in chemotherapy.[160] [Figure 18]

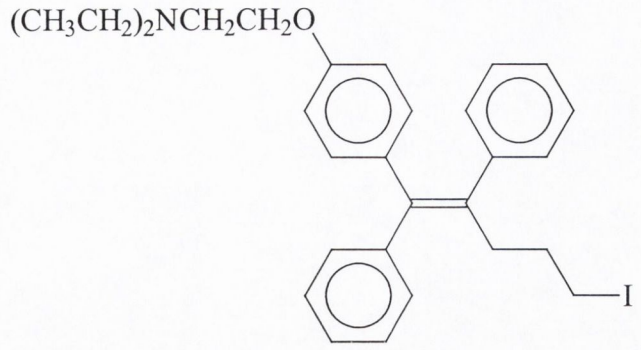


Figure 18: N,N-Diethyliodomethyltamoxifen (7)

The iodo analogue binds to the estrogen receptor fifteen times greater than tamoxifen but half that of the fluoro analogue. However, it is more potent and exerts a greater cytostatic effect (in the E-isomeric form) than both tamoxifen and its fluorinated counterpart. The lipophilic character of the iodo analogue seems to have an important role in cell growth inhibition and like the fluoro analogue causes tumour cell death due to phospholipid chelation.

1.10.1.5 C-ring and rigid aza-analogues of Tamoxifen

Replacing the C-ring[164] by an isoteric heterocyclic aromatic ring results in the possibility of hydrogen bond formation. This kind of heterocyclic replacement has so far only been studied for the A-ring of tamoxifen.[165] [Figure 19]

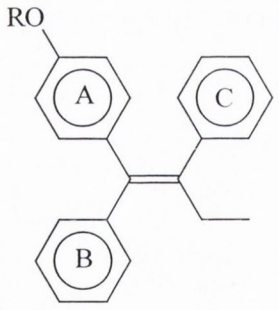
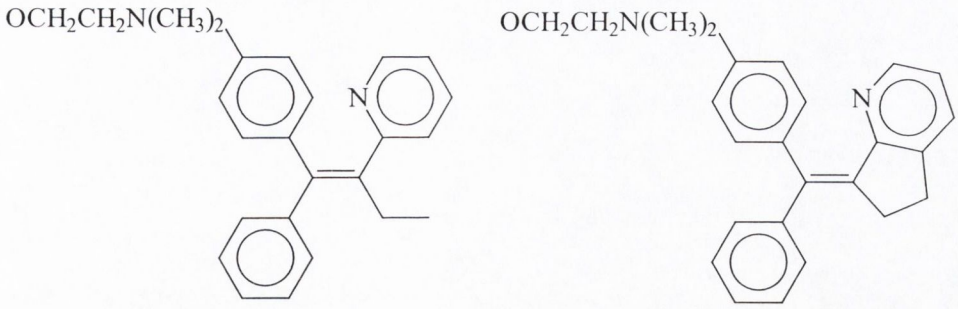


Figure 19: A,B,C-aromatic rings of Tamoxifen (8)

Two such analogues exhibit promising preliminary affinity for the estrogen receptor in breast cancer tissues, and include the 2-pyridyl C-ring (9)[166] and rigid aza-analogues[167] (10) [Figure 20]. Antiproliferative and preliminary binding studies of these 1,2-diphenylpyridyl-but-1-enes show these to be potential antiestrogens. However they are believed to be cytotoxic to various breast cancer cell lines in *in vitro* laboratory tests.



2-Pyridyl C-ring aza analogue (9)

2-Pyridyl rigid aza analogue derivative (10)

Figure 20: C-ring (9) and rigid (10) Aza-analogues of Tamoxifen

1.10.1.6 Tat-59

Tat-59 (11) [Figure 21] is a prodrug that is being developed for the treatment of breast cancer. The hydroxyphosphate group was introduced as it was believed to increase the binding affinity of the molecule for the estrogen receptor, with the hydroxy groups binding to the polar pocket (Section 1.4.3). Tat-59 (11) is activated metabolically to a dephosphorylated form that binds to the estrogen receptor.[168] It has been shown to inhibit growth of the ER-positive, DMBA-induced rat mammary carcinomas[169] (Toko *et al.*, 1990). The drug inhibits the growth of estrogen-stimulated, ER-positive breast cancer cells which were transplanted into athymic mice.[170,171] The results of clinical studies using Tat-59 for the treatment of advanced breast cancer have yet to be published.

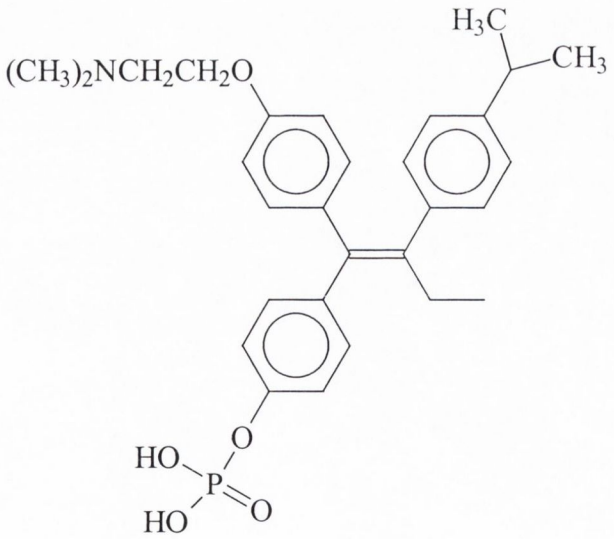


Figure 21: Tat-59 (11)

1.10.2. Antiestrogens that represent a departure from the standard triphenylethylene structure

These antiestrogenic compounds illustrate an alteration in the positioning of the alkylaminoethane side chain while retaining potent antiestrogenic activity. The side chain is clearly able to interact with areas of the ER outside the plane of the ligand-binding site.

1.10.2.1 Zindoxifene

Zindoxifene (12) [Figure 22] was discovered as a result of extensive SAR studies of 1,2-bis phenolic ethane,[172,173] triphenylbutene,[174,175] indene[175] and indole[176,177] derivatives at the University of Regensburg, Germany.

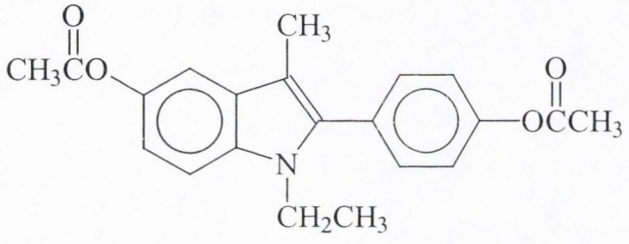


Figure 22: Zindoxifine (12)

Some non-isomerizable analogues [(13)-(15)][87,178,179,180,181] [Figure 23] of tamoxifen are known and the endocrinological properties of these cyclic analogues paralleled that of tamoxifen, although in the MCF-7 breast tumour cell they were slightly less effective than 4-hydroxytamoxifen.

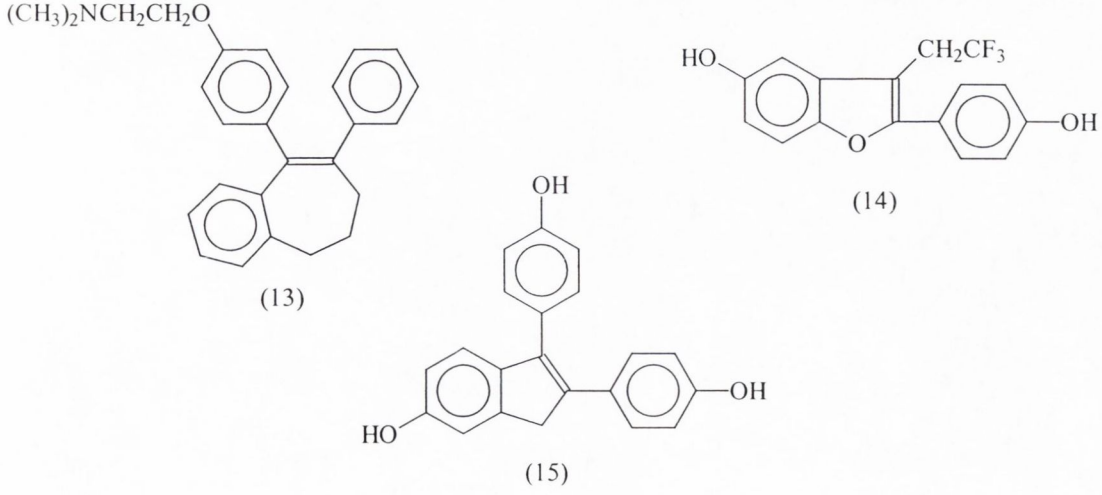


Figure 23: Non-isomerisable analogues of tamoxifen (13)-(15)

Zindoxifene (12) is an acetylated indole derivative[50] which is hydrolytically cleaved to produce a dihydroxy-indole (D15414) with a high affinity for the

estrogen receptor.[176] Zindoxifene (12) has mixed agonist / antagonist properties that inhibit growth of DMBA-induced rat mammary carcinoma.[176,182,183] However, clinical results of phase I/II studies of Zindoxifene (12) in advanced breast cancer have been disappointing as a large range of oral doses failed to produce any objective responses.[184] It is possible that the lack of bioavailability and metabolic transformation precludes significant levels of the drug from locating at the target site. However, the estrogenic potency of Zindoxifene (12) *in vivo* is much less than that of diethylstilbestrol.

1.10.3. Aromatase inhibitors

Anastrozole (16)[8] (Arimidex®) is a new orally active nonsteriodal selective aromatase inhibitor used in treatment of hormone-responsive metastatic breast cancer. Aromatization of adrenal androgens to estrogen in peripheral tissue (adipose, muscle and liver) after the menopause, may contribute to tumour growth and progression in patients with hormone-dependent breast cancer. Therefore pharmacological inhibition of extracellular aromatase (estrogen synthetase) decreases extraglandular estrogen biosynthesis. Anastrozole (16), (2,2-[5-(1H-1,2,4-triazol-l-ylmethyl)-1,3-phenylene]-di-[2-methyl propinonitrile]), an analogue of the antifungal triazole drugs, prevents the conversion of androgens to estrogen by aromatase inhibition. [Figure 24]

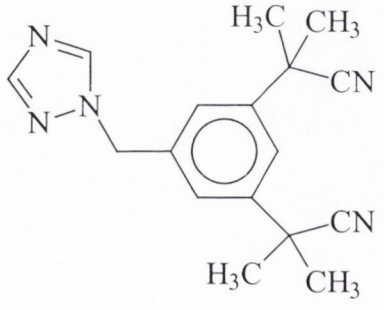


Figure 24: Anastrozole (16)

Molecular modelling studies indicate that the 3-dimensional structure of this nonsteroidal aromatase inhibitor is similar to that of androstenedione.[185] The high binding affinity of the aromatase inhibitors for estrogen synthetase is thought to reside in the *N*-4 nitrogen of the triazole ring that co-ordinates with the heme iron of the aromatase enzyme complex. Anastrozole (16) inhibits the last step of the metabolic pathway: the conversion of androgens to estrogens by aromatase. Aromatization is a unique reaction that offers a highly restrictive target for inhibition of estrogen synthesis. Anastrozole (16) is cleared from the

systemic circulation primarily by the liver, which produces three major and several minor metabolites. Triazole is the only metabolite that is believed to be devoid of pharmacological activity.[186]

Preliminary clinical trials[187,188] show that anastrozole therapy can be well tolerated with little side effects. The total clinical impact of selective aromatase inhibitors remains to be fully elucidated, but there are a number of exciting potential applications for these agents that merit continual testing, including their use as first-line therapy in hormone-responsive metastatic disease, in adjuvant therapy and in combination chemohormonal therapy.

1.10.4. Pure antiestrogens

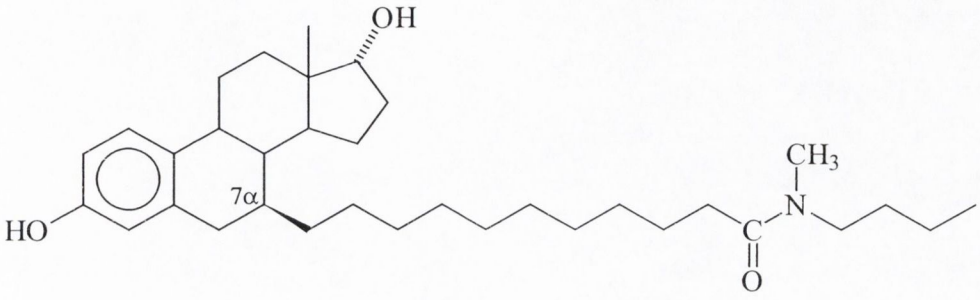
It is believed that pure antiestrogens[189] (devoid of estrogenic activity)[190,191,192,193,194] may be more effective than partial agonists in reducing the mitogenic action of estrogen on the growth of breast cancer cells, but they may exhibit antiproliferative effects more readily where the growth is estrogen dependent. Tumours that lose hormone dependency usually become resistant to Tamoxifen. Recently, a new breast cancer cell line, which expresses ER but grows independently of estrogen, was reported.[195] This prompted the search for antibreast cancer agents, which are effective against both estrogen dependent and independent growth.

Pure antiestrogens can be divided into several groups, estradiol derived, flavonoids and cyclopropanes to mention but a few. Wakeling and colleagues discovered the first pure antiestrogens.[196]

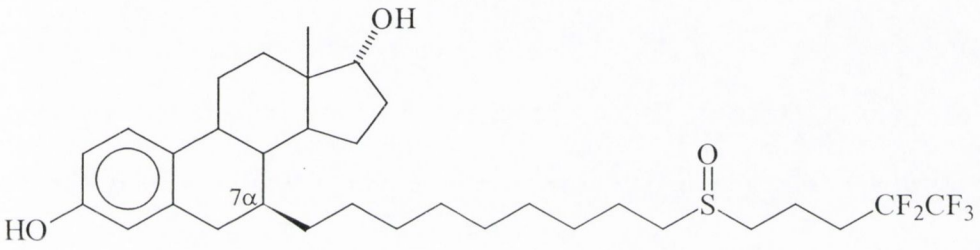
1.10.4.1 Estradiol derived pure antiestrogens

ICI 164,384 (17), the 7α-alkylamine derivative[50,197] of 17β-estradiol is a steroidal antiestrogen specifically designed to eliminate partial agonist activity to reduce the risk of toxicity associated with the estrogenic action of tamoxifen. This compound was the lead compound in the discovery of pure antiestrogens. ICI 164,384 (17) binds to the ER with high affinity[198,199] and has all the characteristics of a pure antiestrogen. It completely inhibits estrogen of tamoxifen-induced uterine growth in immature rats and mice almost equivalent to the effects observed with ovariectomy.[196,200,201] The structure-activity relationships are well established: 7β- substitution is ineffective at producing antiestrogenic activity and the length of the carbon chain determines optimal activity.[202]

The compound ICI 182,780 (18) (clinically available) is more potent than ICI 164,384 (17)[203] [Figure 25] and is being evaluated as a clinically useful agent after failure of tamoxifen. *In vitro* findings and early clinical experience with ICI 182,780 have prompted interest in the development of the drug as a therapeutic agent for estrogen-dependent indications such as breast cancer and certain benign gynaecological conditions.



ICI 164,384



ICI 182,780

Figure 25: ICI 164,384 (17) and ICI 182,780 (18)

The discovery of ICI 164,384 and ICI 182,780[19] has stimulated others to improve on bioavailability and the biological activity profile. Both ICI 164,384 and ICI 182,780 are poorly soluble and have low oral activity.[203] This resulted in consideration of depot injections for clinical applications.

The compound RU 58,668 (19)[19] is substituted in the 11β-position with a long hydrophobic side chain.[204] This produces the same spatial arrangement for a side chain as the 7α-substitution in relation to the plane of the steroid nucleus. Studies *in vivo* and *in vitro* have demonstrated that the RU 58,668 (19) has the properties of a pure antiestrogen. [Figure 26]

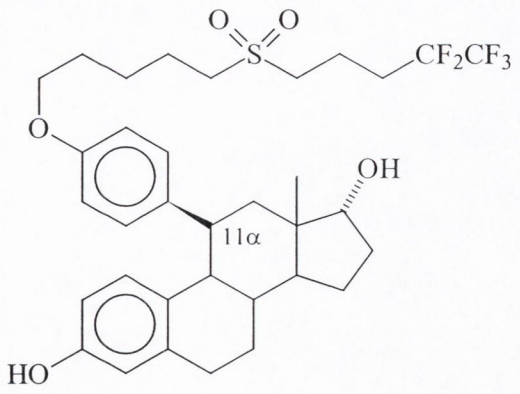


Figure 26: RU 58,668 (19)

The compound EM-139 (20) [Figure 27] is both an inhibitor of hydroxysteroid dehydrogenase and a pure antiestrogen.[205]

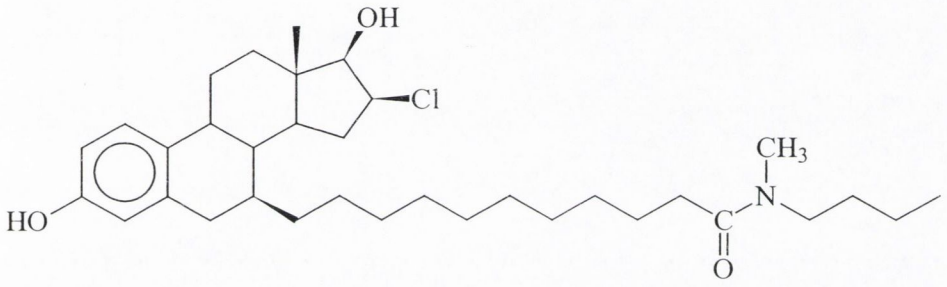
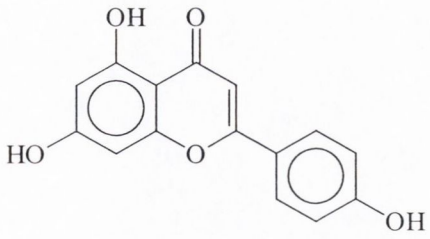


Figure 27: EM-139 (20)

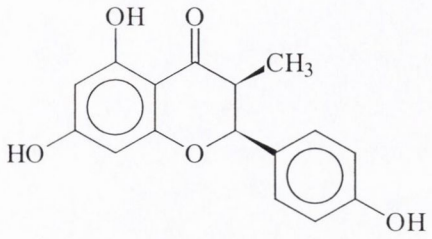
The role of these pure antiestrogens is not only to block the receptor but also to reduce the conversion of estrone to the more potent estrogen, estradiol (E_2), in the postmenopausal patients.

1.10.4.2 Flavonoids

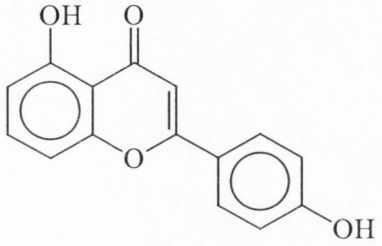
Flavonoids, either natural or synthetic, are well known to exhibit various biological activities.[206] There is a profound relationship between the flavonoids and anti-cancer activity. A large number of flavonoids are known to exhibit antiproliferative effects against breast cancer cells and binding affinities for the ER. Novel amino-substituted flavone derivatives are now known as potential antitumour agents in breast cancer.[189] [Figure 28]



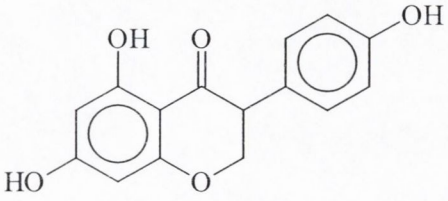
Apigenin (21)



BE-14348B (22)



6,4'-Dihydroxyflavone (23)



Daizein (R=H) (24)
Genistein (R=OH) (25)

Figure 28: Structures of Apigenin (21), BE-14348B (22), 6,4'-Dihydroxyflavone (23), Daizein (24) and Genistein (25)

Apigenin (21) and some of its congeners are reported to possess antiproliferative activity against the human breast cancer cell line ZR-75-1.[207] 6,4'-Dihydroxy-flavone (23) has a binding affinity for the estrogen receptor[208] and the flavone derivative BE-14348B (22)[209] exhibits both estrogenic and antiproliferative activity. Investigation of the ER binding affinity and the estrogenic activity of isoflavone derivatives daizein and geinstein[210] has also shown favourable results. Recent reviews report that L86-8275 (26) [Figure 29] exhibits antitumour activity against several types of human breast cancer cell lines.[211]

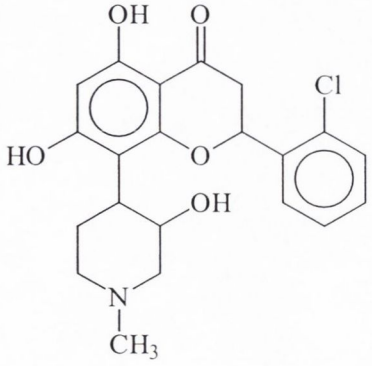


Figure 29: L86-8275 (26)

Further research is being carried out on flavone derivatives as potential pure antiestrogens. It has been hypothesised that the flavone derivatives substituted

with an amino group can function as hydrogen bond donors or acceptors, and may exhibit antitumour activity in breast cancer.[189]

1.10.4.3 Diarylcyclopropanes as pure antiestrogens

Z-1,1-dichloro-2,3-diphenylcyclopropane (27)[68,212,213] is an effective anti-breast cancer agent in rodents and in cell culture due to its pure antiestrogen activity [Figure 30]. It differs pharmacologically from tamoxifen (2) in being devoid of partial agonist (estrogen-like)[214] activity in the mouse.[215] 1,1-Dichloro-2,3-diphenylcycloprone (27) was first tested for biological activity during a study of rigid functionality minimised analogues of known olefinic estrogens, synthesised in an effort to reduce estrogenic activity.[216]

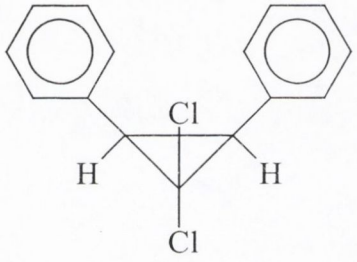


Figure 30: Z-1,1-dichloro-2,3-diphenylcyclopropane (27)

It is effective in delaying the development of secondary tumours and, following cessation of treatment, it prevents metastatic tumour development longer than tamoxifen, e.g. metastatic rat mammary tumours.[217]

1.10.5. Selective Estrogen Receptor Modulators (SERMs)

SERMs are designer estrogens, which behave like estrogen in some tissues but block its action in others. Researchers hope that these selective estrogen receptor modulators will mimic the effect of estrogens in the liver, heart, and bones but will combat its harmful effects in the breast and uterus.[31,218]

1.10.5.1 Raloxifene

The 2-arylbenzothiophene raloxifene (28)[219] [Figure 31] is a selective estrogen receptor modulator, which is currently in clinical use for the prevention and treatment of postmenopausal osteoporosis.[220,221]

Raloxifene (28) (LY 156,758, Evista®)[19,222] is the first SERM shown to prevent bone loss in postmenopausal women. The drug also reduces levels of fibrinogen and cholesterol, a potential benefit in postmenopausal women who are at increased risk of cardiovascular disease. In addition it appears to prevent the

development of breast and endometrial cancer in women. These benefits are suggested by findings from the Multiple Outcome of Raloxifene Evaluation[223] (MORE) trial and supported by the results of a large meta-analysis. However these findings require confirmation by current clinical trials.[220,222]

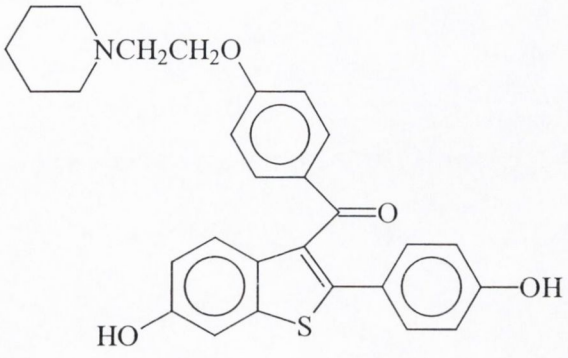


Figure 31: Raloxifene (28)

Raloxifene (28), possessing high binding affinity for the estrogen receptor,[220] exhibits potent antiestrogen activity but has little uterotrophic activity in rodents.[224,225] R.E. Hubbard *et al.*,[31] compared the structure of a receptor bound by estrogen with one bound by raloxifene (discussed in Section 1.4.3).

Further studies indicate that raloxifene displays antitumour activity against breast cancer cells *in vitro*[226] and prevents rat mammary carcinogensis.[227,228] Overall raloxifene (28) displays the profile of a selective ER modulator that could be utilised as a potential prophylactic for osteoporosis but with the additional benefit of preventing coronary heart disease[19] and endometrial cancer in postmenopausal women.

Preliminary results from clinical trials report that raloxifene has a short biological half-life, which may impair its ability to maintain a complete blockade of ER's at relevant sites, although further studies are being carried out.

1.10.5.2 Idoxifene

Idoxifene (29) [Figure 32] is a novel SERM[229] that is currently in clinical development for treatment of breast cancer. Compared to tamoxifen, idoxifene (29) is metabolically more stable, with a higher relative binding affinity for ER and reduced agonist activity in breast and uterine cells. Idoxifene (29) also inhibits calmodulin, a calcium-binding protein which is also involved in cell signal transduction pathways. It was synthesised to avoid the reported toxicity associated with tamoxifen in the rat liver.[85]

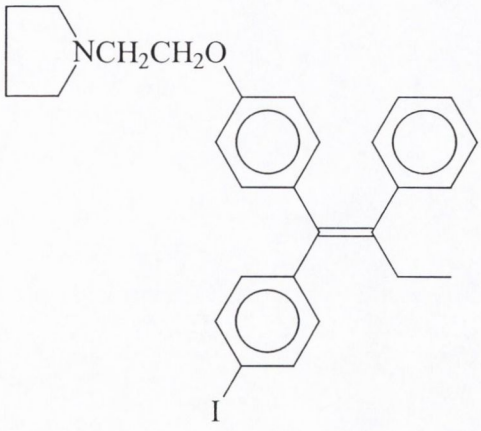


Figure 32: Iodoxifene (29)

Substitution of halogens in the 4-position of tamoxifen is known to reduce antiestrogen potency by preventing conversion to 4-hydroxytamoxifen (4-OHT).[230] while the pyrrolidine group prevents side chain metabolism. Recent reviews suggest that reduced demethylation of the side chain would also avoid the formation of formaldehyde in the liver.[85] Initially the goal was to develop a drug with efficacy both in the prevention of osteoporosis and in the treatment of breast cancer. However, published reports focus on the potential of idoxifene as an antiestrogen-anticancer agent.[231] Idoxifene inhibits hormone-dependent breast cancer growth and is more effective than tamoxifen in inhibiting both MCF-7 growth *in vitro* and carcinogen-induced rat mammary tumour growth *in vivo*. Recent studies show that idoxifene induces apoptosis as well as impairing cell proliferation and further research is presently being carried out on the apoptotic effect of idoxifene.[229,232]

1.10.5.3 Trioxifene

Trioxifene (30) [Figure 33] deviated from the triphenylethylene structure by the introduction of a ketone bridging group that links the phenyl ring containing the pyrrolindyl side chain with the rest of the molecule[233] and it has been found to possess potent antiestrogenic activity in the rat.[234]

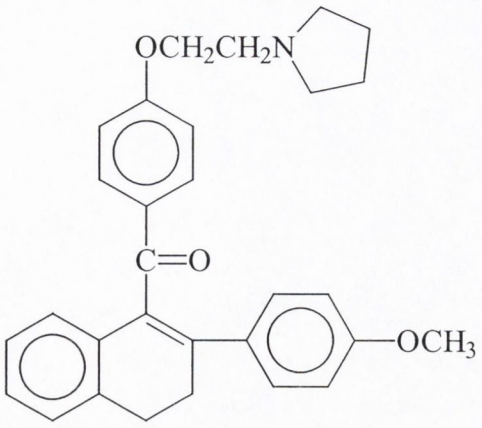


Figure 33: Trioxifene (30)

Trioxifene mesylate was found to markedly suppress serum growth hormone (GH) levels in the rat. Clinical studies[235,236] with trioxifene (30) have demonstrated its efficacy in the treatment of advanced (stage IV) breast cancer where it suppresses arginine-stimulated growth hormone secretion. The response correlated with positive ER status, long disease-free interval and low tumour burden. Trioxifene (30) appears to exhibit more estrogenic activity than tamoxifen (2). Further clinical trials are needed to evaluate the effectiveness of trioxifene as an anti tumour agent in women with hormone-responsive breast cancer.[234]

1.11. Objectives of thesis

In this thesis the main objective was to prepare a series of novel structural analogues of tamoxifen. The objectives are summarised as follows:

1) To develop novel antiestrogenic compounds with activity similar to tamoxifen with reduced side effects i.e. toxicity and carcinogenicity.

2) To synthesise flexible analogues that possess a similar carbon skeleton to that of tamoxifen but contain an additional benzylic methylene group adjacent to the ethylene function.

3) To prepare conformationally restrained cyclic analogues of tamoxifen, which possess the basic triphenylethylene structure.

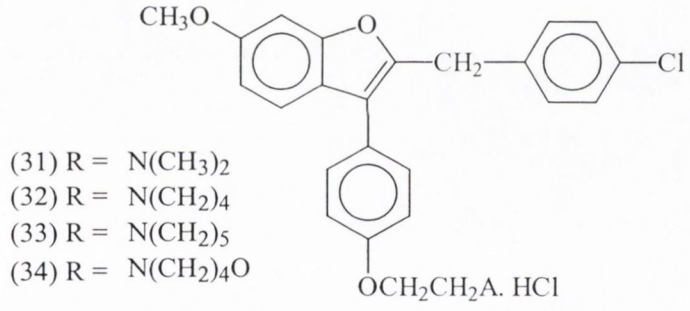
4) To carry out biochemical studies on the above compounds, to investigate their binding ability, cytoxicity and inhibition of proliferation of the estrogen receptor on MCF-7 breast adenocarcinoma cells.

5) To use computational methods to rationalise the biological activity of the novel compounds.

2. Synthesis of flexible Tamoxifen analogues

2.1. Introduction

In this thesis a series of novel non-steroidal flexible tamoxifen analogues are to be prepared deviating from the 'traditional' triarylethylene structure through the introduction of specific spacing methylene groups between the aryl and vinylic systems associated with 'classical' analogues of tamoxifen. Traditionally, research into novel antiestrogens has favoured the approach of simply modifying the tamoxifen structure [69,84,237] by altering the ethyl side chain or introducing substituents onto the aryl rings. It had been suggested that building flexibility into the rigid backbone of antiestrogens could enhance their activity and binding affinity for the estrogen receptor.[238] Removal of one of the aryl groups from the vinyl carbon structure of tamoxifen may alter the overall shape and size of the molecule thus enabling easier access to the estrogen receptor. Teo *et al.*, synthesised a number of basic ethers 2-(*p*-chlorobenzyl)-3-aryl-6-methoxybenzofurans [Figure 34] possessing a variety of alkylaminoethoxy side chains at the 4'-position of the phenyl ring and an additional methylene group at the C-2 position of the benzofuran ring.[239]



(31) R = $N(CH_3)_2$
(32) R = $N(CH_2)_4$
(33) R = $N(CH_2)_5$
(34) R = $N(CH_2)_4O$

Figure 34: 2-(p-Chlorobenzyl)-3-aryl-6-methoxybenzofurans

Preliminary screening of these compounds in the human breast cancer-derived MCF-7 cell line indicated that they display antiproliferative activity. These findings led to the synthesis of several basic ethers of 2-(4-halogenobenzyl)-3-arylbenzo[b]thiophenes and a 2-(4-fluorobenzyl)-3-arylbenzo[b]selenophene, each compound possessing the additional methylene group. Raloxifene[276] (28), a 2-arylbenzothiophene compound, is a well known antiestrogen possessing a high binding affinity for the estrogen receptor and exhibiting anti-carcinogenic properties in numerous animal tumour model systems.[240] This compound's dimethylaminoethoxyphenyl substituent is distanced from the thiophene ring by a carbonyl group which gives the molecule flexibility such that it can which adopt a favourable conformation for interaction with the estrogen receptor.

In this research novel flexible antiestrogens were synthesised which possessed a similar carbon skeleton to tamoxifen but contained an additional benzylic methylene group adjacent to the ethylene function. Four different structural types were prepared and biochemically tested to investigate the binding ability, cytotoxicity and inhibition of proliferation of these compounds with the estrogen receptor on human MCF-7 breast adenocarcinoma cells. These compound series are depicted in Figure 35.

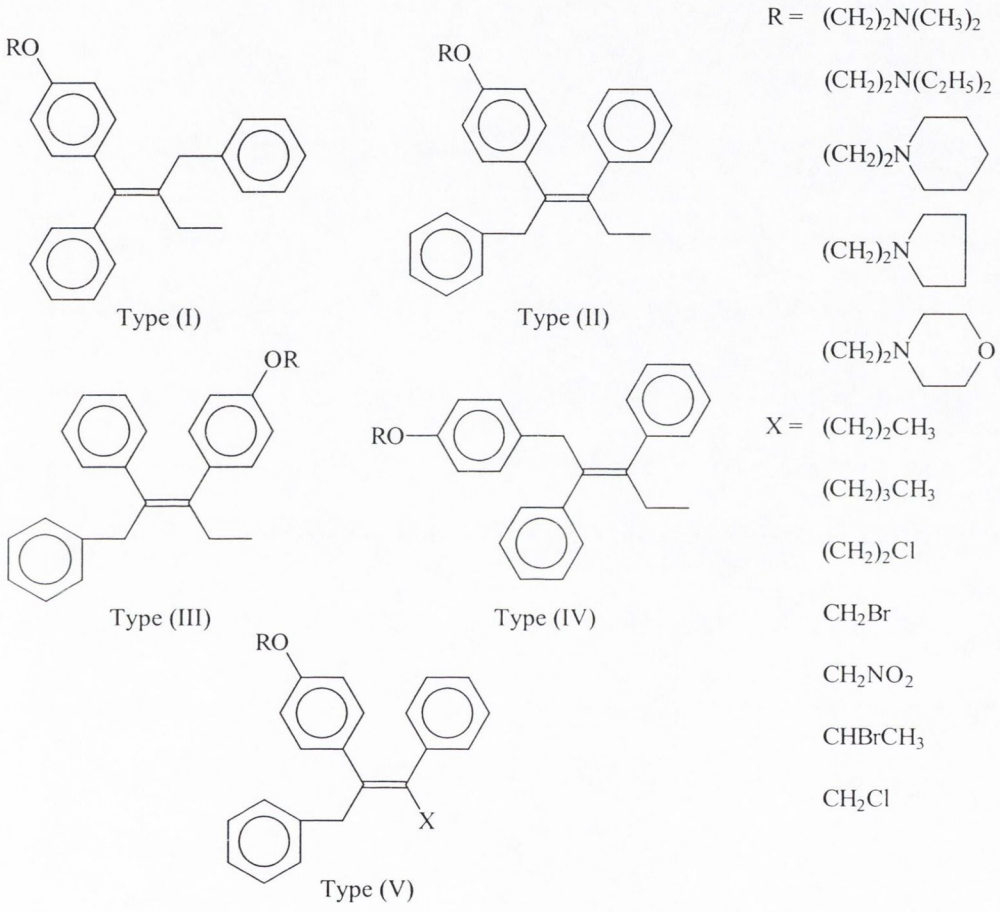


Figure 35: Series of Flexible Tamoxifen Analogues

Novel flexible analogues (Type I) were prepared via two different synthetic routes; a seven step Grignard type route from chalcones and a two step McMurry coupling reaction of aryl ketones. Analogues of Types II-IV were prepared using only the titanium-based McMurry reaction.

2.1.1. Synthetic routes to Tamoxifen

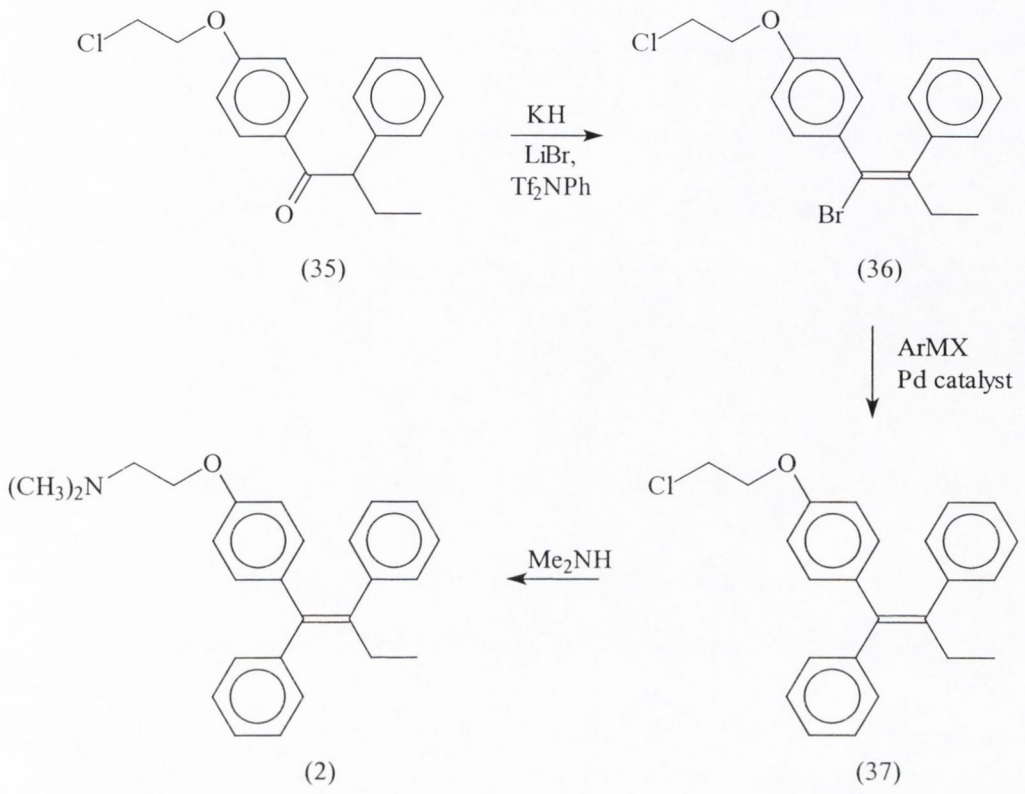
Bedford and Richardson[78] first described the synthesis of tamoxifen in 1966, where a mixture of E and Z tamoxifen isomers were obtained from the dehydration of 1-(4-β-dimethylaminoethoxyphenyl)-1,2-diphenylbutanol using

ethanolic hydrochloric acid. Each isomer was isolated by fractional crystallisation from petroleum ether.

Jarman and McCague[241] prepared perfluorotolyl derivatives of tamoxifen from 1,2-diphenyl-1-(4-methoxyphenyl)but-1-one. Removal of the perfluorotolyl group (by treatment with sodium methoxide in DMF), followed by treatment with dimethylaminoethoxy chloride under basic conditions yielded the pure E and Z isomers of tamoxifen, usually without detectable isomerisation.

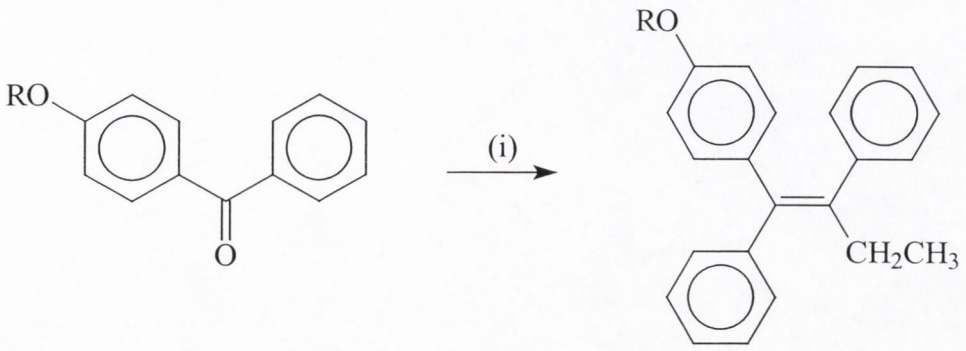
An analogous preparation by McCague[242] reports the stereoselective dehydration of 1-(4-alkoxyphenyl)-1,2-diphenylbutan-1-ols to derivatives of tamoxifen. The tertiary alcohol was prepared by reaction of 1-(4-methoxyphenyl)-1,2-diphenylbutan-1-one with phenylmagnesium bromide. Dehydration using HCl of this tertiary alcohol gave a 2:1 ratio of olefin products.

Potter and McCague[243] applied an alternative approach to the synthesis of tamoxifen, utilising vinylbromides or triflates as intermediates, since the bromide and triflate functions are easily replaced by an aryl group via a palladium complex catalysed coupling reaction, with retention of configuration [Scheme 1]. The conversion of the readily prepared ketones (35) resulted in high stereoselectivity (E: Z; 20: 1) and the pure E isomer (36) was obtained after recrystallisation. Reaction of this E-vinyl bromide (36) with phenylzinc chloride, catalysed by tetrakis(triphenylphosphine)palladium (0) in toluene gave a 99% yield of (37). Conversion of (37) to Z-tamoxifen (2) was accomplished with ease in 93% yield.



Scheme 1: Potter and McCagues synthetic route for tamoxifen[243]

Coe and Scriven[244] used cross coupling of functionalised ketones by low valent titanium to synthese tamoxifen. It was found that 4-substituted benzophenones and propiophenones were suitable for the coupling, resulting in high yields with a marked preponderance of the Z-isomer. Initial attempts using $TiCl_3$/Li, 4-[2-[(*N*,*N*-dimethylamino)ethoxy]benzophenone and propiophenone to prepare tamoxifen in one step failed. However (37) and (41) were synthesised using this method with appropriate benzophenones. A 7:1 mixture of the Z and E isomers was obtained for both but-1-enes (37) and (41). According to Coe and Scriven[244] the advantage of the $TiCl_3$/Li approach is that (37) can be converted to Z-tamoxifen (2) on reaction with dimethylamine. [Scheme 2]



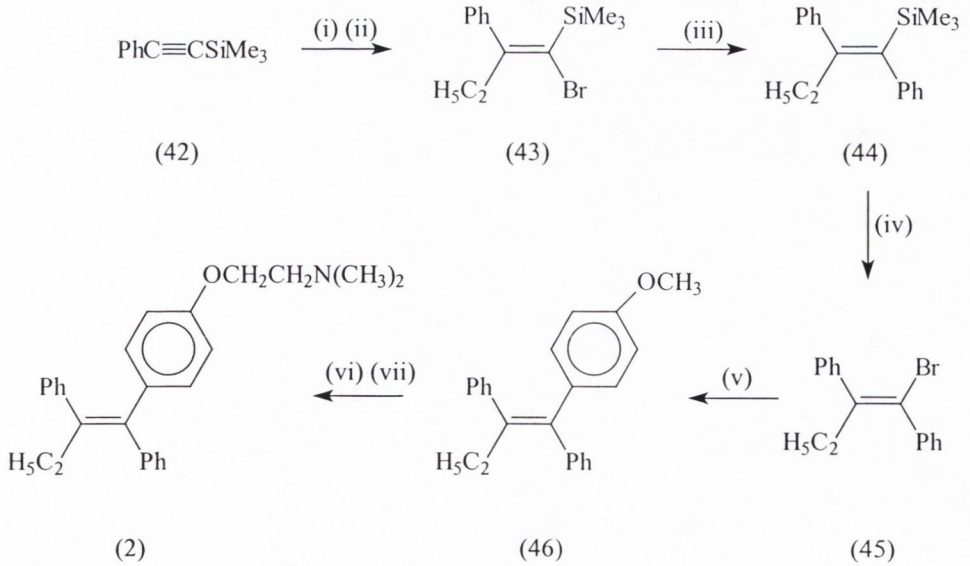
(38) R = $CH_2CH_2NMe_2$
(39) R = CH_2CH_2Cl
(40) R = CH_2CH_2Br

(2) R = $CH_2CH_2NMe_2$
(37) R = CH_2CH_2Cl
(41) R = CH_2CH_2Br

Scheme 2: (i) PhCOEt –$TiCl_3$ -Li or $TiCl_4$ -Zn

An alternative method using $TiCl_4/Zn$[244] to produce tamoxifen using 4-[2-*N*,*N*-diethylaminoethoxy]benzophenone and propiophenone proved successful resulting in an 88% yield with a Z:E isomeric ratio of 3 :1.

To date most synthetic approaches to tamoxifen have been nonstereospecific, producing mixtures of both Z and E isomers, which have been separated by recrystallisation. However, a stereospecific synthesis of Z-tamoxifen using the carbometallation of an alkylnylsilane as the key step was carried out by Miller and Al-Hassan[245] [Scheme 3].

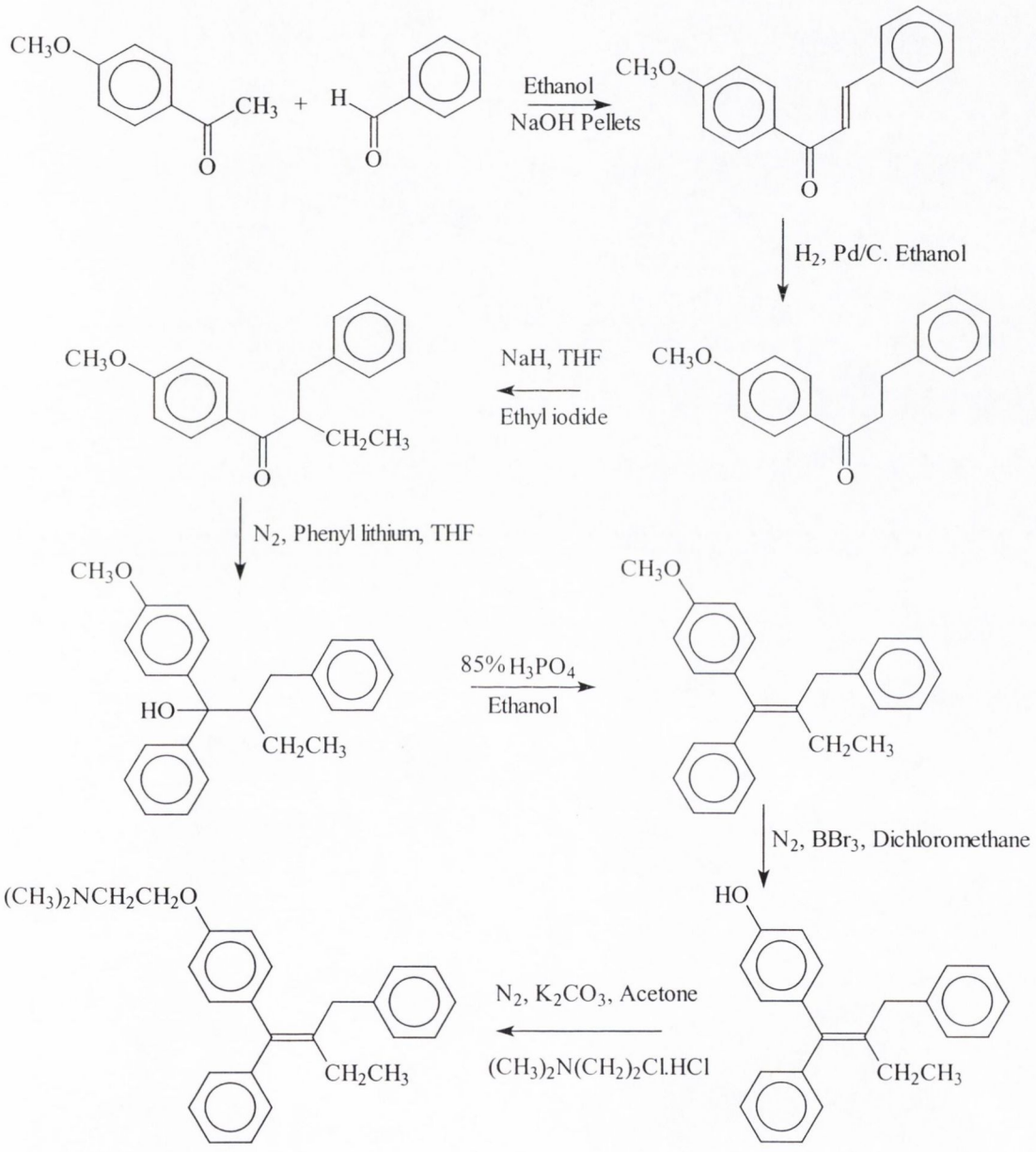


Scheme 3: (i) Et_2AlCl, Cp_2TiCl_2 (ii) NBS (iii) PhZnCl, $Pd(PPh_3)_4$ (iv) Br_2, NaOMe/MeOH (v) 4-$MeOC_6H_4ZnCl$, $Pd(PPh_3)_4$ (vii) NaSEt (vii) $Cl(CH_2)_2NMe_2$.HCl, NaOEt

The first step in the synthesis establishes the stereochemistry around the double bond. Phenyl(trimethylsilyl)acetylene (42) was carbometallated with diethylaluminium chloride-titanocene dichloride to give an organometallic intermediate which was cleaved by *N*-bromosuccinimide. This product (43) was assigned E-stereochemistry. The bromine group of (43) was stereospecifically replaced with a phenyl group by palladium catalysed coupling with phenylzinc chloride to give (44), a vinylsilane. Treatment of (44) with bromine sodium methoxide afforded a vinyl bromide (45), which was coupled with (4-methoxyphenyl)zinc chloride to give (46), an ethyl triaryl olefin. This was transformed into tamoxifen (2) by demethylation with sodium ethylthiolate, followed by reaction with 2-(dimethylamino)ethyl chloride.

2.2. Synthesis of Type I analogues

In the present work Type I flexible analogues were prepared via two different synthetic routes, a seven step synthesis and a McMurry coupling reaction (which will be discussed in Section 2.3). The synthetic plan for Type I analogues using the seven step synthetic route is depicted in Scheme 4.

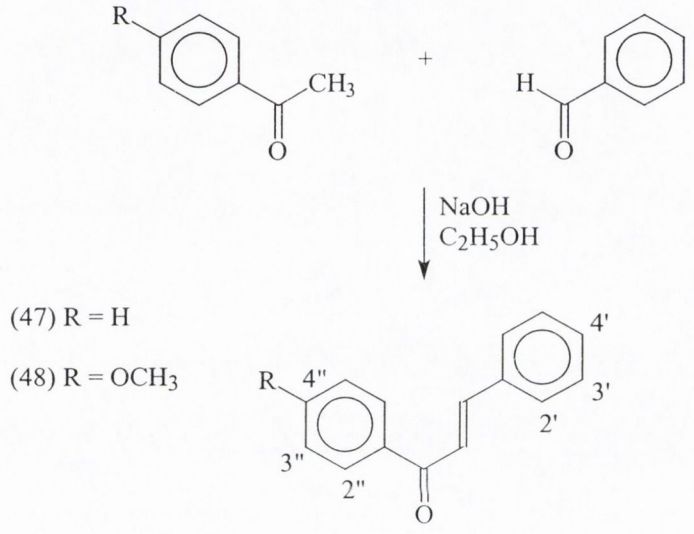


Scheme 4: 7 Step synthesis of 2-Benzyl-1-[(4-dimethylaminoethoxy)phenyl]-1-phenylbut-1-ene [Z-isomer illustrated]

2.2.1. α,β-Unsaturated ketones

Chalcones such as (47) and (48) were prepared as shown in Scheme 5 using a base catalysed condensation reaction.

Numerous methods, including the use of acidic[246] and basic catalysts,[246,247] have been employed to condense aryl aldehydes and ketones.[248] Ellern[249] claimed the "last word" on the preparation of benzalacetophenone, however Wattansin and Murphy[250] have reported a simple base catalysed (NaOH) method which results in pure easily isolated products.

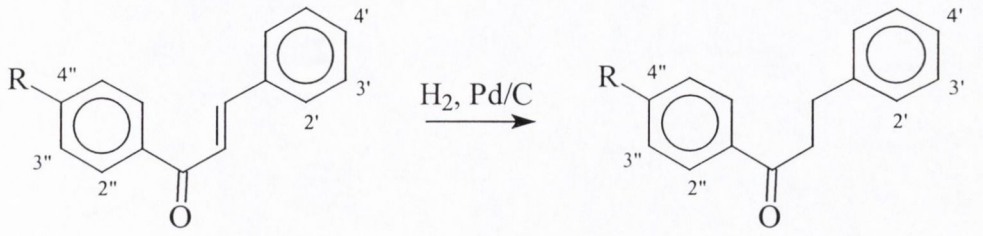


Scheme 5: 1,3-Diarylpropen-1-ones

The 1,3-diarylpropen-1-ones (47), (48) were obtained as crystalline solids in good yields. Positive identification of the products was obtained from spectroscopic data.

2.2.2. Dihydrochalcones

In this work the required series of dihydrochalcones was obtained by the hydrogenation of the corresponding chalcones (47), (48), using palladium on carbon as a catalyst with ethanol as the solvent. These reactions were carefully controlled by frequent TLC monitoring, to avoid the reduction of the carbonyl function [Scheme 6]. Numerous reagents have been employed for reduction of chalcones to dihydrochalcones, including DIBAH (diisobutylaluminium hydride),[251] sodium dithionite,[252] sodium formate[253] and rhodium or platinum catalysts.[254]



(49) R = H
(50) R = OCH_3

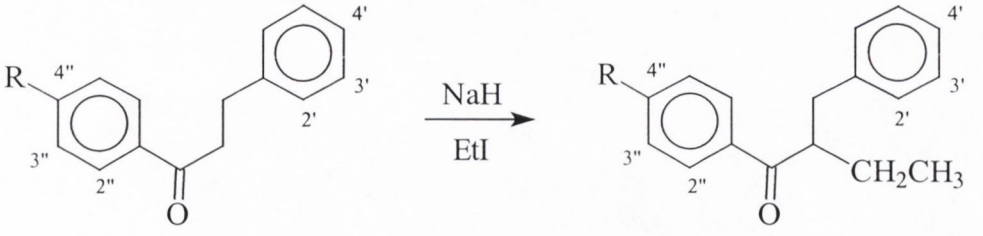
Scheme 6: Hydrogenation of chalcones to afford 1,3-diphenylpropan-1-ones

This hydrogenation method proved successful and the hydrogenated products obtained provided a convenient route to precursors for the corresponding 1,2-diarylbutan-1-ones.

These compounds (49), (50) were characterised using infrared, ^{1}H NMR and ^{13}C NMR. The infrared spectra of compounds (49) and (50) show the carbonyl stretching between ν1670-1680cm^{-1}. In the ^{1}H NMR of 1-(4-methoxyphenyl)-3-phenylpropan-1-one, the H-3 protons are observed as a triplet (J=8.3Hz) at δ3.05, whereas the H-2 protons occur further downfield at δ3.28 due to the deshielding effects of the carbonyl moiety, as a triplet (J=8.3Hz). In the ^{13}C NMR spectrum the C-2 and C-3 signals which are inverted in the DEPT 135 spectrum, are observed at 40.04ppm and 30.28ppm respectively. The carbonyl C-1 quaternary signal occurs downfield at 198.01ppm.

2.2.3. Ethylated dihydrochalcones

The dihydrochalcones (49), (50) were ethylated to the corresponding 1,2-diarylbutan-1-ones (51), (52) by treatment with ethyl iodide, using sodium hydride in ethanol at room temperature[255] [Scheme 7].



(49) R = H
(50) R = OCH_3

(51) R = H [96%]
(52) R = OCH_3 [79%]

Scheme 7: Synthesis of 1,2-diarylbutan-1-ones

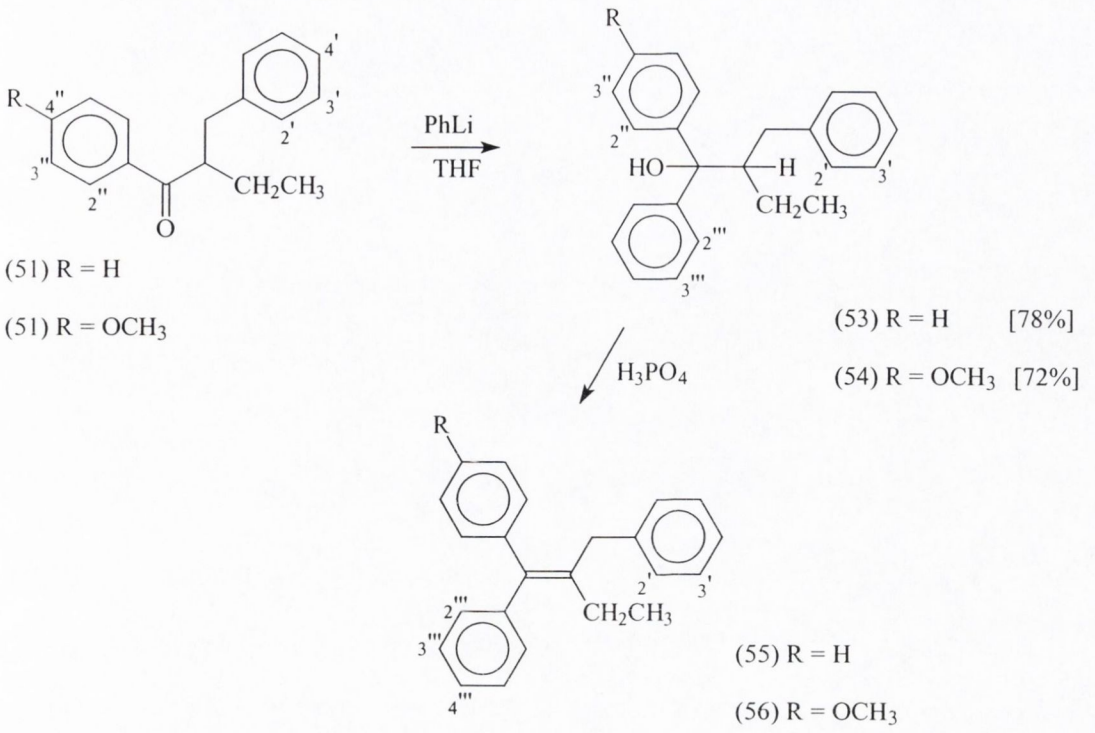
The compounds (51) and (52) were afforded in good yields as oils. The infrared spectra of these compounds show the carbonyl stretching in the range ν1673-1680 cm^{-1}.

In the ^1H NMR spectrum of 2-benzyl-1-(4-methoxyphenyl)butan-1-one (52), the H-4 methyl protons are observed at δ0.89 as a triplet (J=7.4Hz). These are found to couple to a complex multiplet in the range δ1.51-1.80, which are assigned to the adjoining H-3 methylene protons. A multiplet integrating for one proton between δ3.54-3.58 is assigned to the H-2. The H-2 is coupled to a pair of double doublet signals, which are assigned to the benzylic methylene protons. These methylene protons consists of two non-equivalent hydrogens H_a and H_b, each represented by a double of doublets. The H_a signal appears upfield at δ2.79 (J=13.6 and 6.6Hz), while the H_b which is slightly deshielded occurs at δ3.03 (J=13.9 and 7.5Hz).

2.2.4. 1,1,3-Triarylbut-1-enes

Arylation of 1,2-diarylbutan-1-ones (51), (52) was investigated as a route to the required tamoxifen analogues. The resulting 1,1,3-triarylbut-1-enes possess a similar aromatic skeleton to that of tamoxifen but have an additional aliphatic methylene group connecting the double bond to one of the aromatic rings.

Previous attempts[256] to phenylate 1,2-diarylbutan-1-one (51), (52) using the Grignard reagent phenylmagnesium bromide, had proven unsuccessful. Further investigations using the phenylating reagent phenyllithium however proved favourable. Thus the 1,1,2-triarylbut-1-enes (55) and (56) were prepared using phenyllithium in THF, followed by dehydration with 85% phosphoric acid which was carried out after the isolation of the intermediate alcohol product or *in situ* [Scheme 8]. Previous research indicated difficulties when dehydration was attempted with sulphuric acid or hydrochloric acid.[256]



(51) R = H

(51) R = OCH_3

(53) R = H [78%]

(54) R = OCH_3 [72%]

(55) R = H

(56) R = OCH_3

Scheme 8: Synthetic route involved in the phenylation of 1,1,2-triarylbutan-1-ones (Z isomer illustrated)

The 1,1,3-triarylbutan-1-ols (53), (54) were obtained as oils in moderate to good yields. Positive identification of the products was obtained from spectroscopic data.

The characteristic broad stretching for the hydroxy group in (53) and (54) is observed between ν3460-3580 cm^{-1} in the infrared spectrum. In the ^1H NMR spectrum of 2-benzyl-1-(4-methoxyphenyl)-1-phenylbutan-1-ol (54), [Figure 36], the methyl protons are observed at δ0.65 as a triplet (J=7.5Hz), while the methylene H-3 protons occur as a complex multiplet in the range δ1.29-1.65. The H-2 proton is found as a complex multiplet between δ2.50-2.57 while the methylene (CH_2) protons occur at δ2.79. The hydroxy proton is observed as a broad singlet at δ2.20. This assignment was confirmed by deuteration. The 4''-methoxy protons are found characteristically as a singlet at δ3.81.

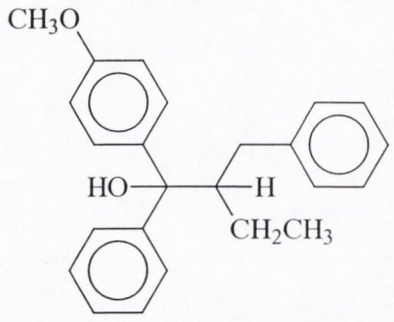
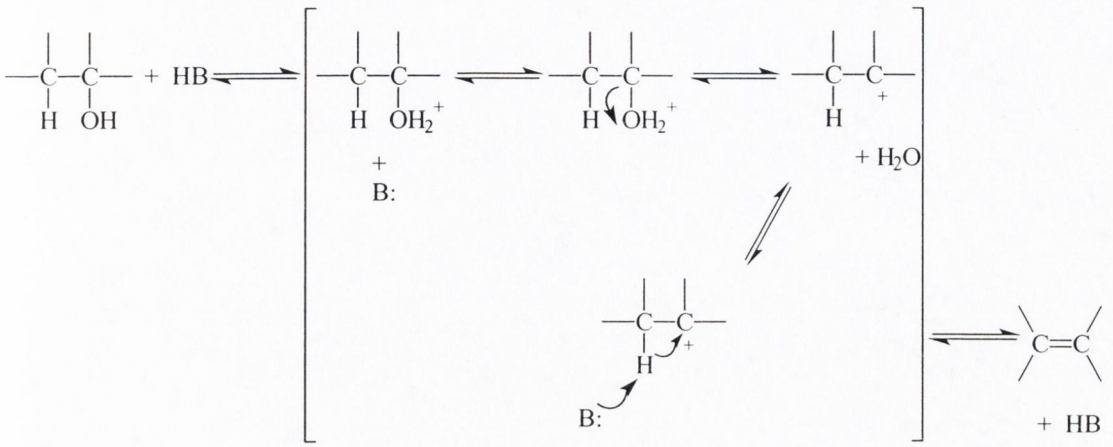


Figure 36: 2-Benzyl-1-(4-methoxyphenyl)-1-phenylbutan-1-ol (54)

In the ^{13}C NMR spectrum of (54), the methyl C-4 is characteristically observed upfield at 13.34ppm, with the C-2 signal occurring at 49.70ppm. Two inverted signals are observed in the DEPT 135 spectrum at 23.24ppm and 36.31ppm corresponding to the methylene C-3 and the somewhat deshielded benzyl CH_2 respectively. The 4''-methoxy signal appears at 55.13ppm. The shielded C-3'' and C-5'' are observed at 113.47ppm. The quaternary C-1'' and C-1''' signals are observed at 146.96 and 147.87ppm. Low resolution mass spectrometry afford the molecular ion as M^+346 ($C_{24}H_{26}O_2$).

2.2.5. Dehydration of tertiary alcohols

Dehydration of alcohols[257,258,259] results from a 1,2-elimination of water, which is acid catalysised and may require the application of heat. The various classes of alcohols differ widely in ease of dehydration, with the tertiary alcohols being more readily dehydrated than the primary and secondary alcohols.[103,257,259,260] The dehydration of secondary and tertiary alcohols involves a carbocation mechanism [E1], where a fast acid-base reaction between the alcohol and the catalysing acid results in a protonated alcohol and a conjugate base of the acid.[103,260] The protonated alcohol formed undergoes hydrolysis to form water and a carbocation that loses a β-proton to the base to afford an alkene [Scheme 9].

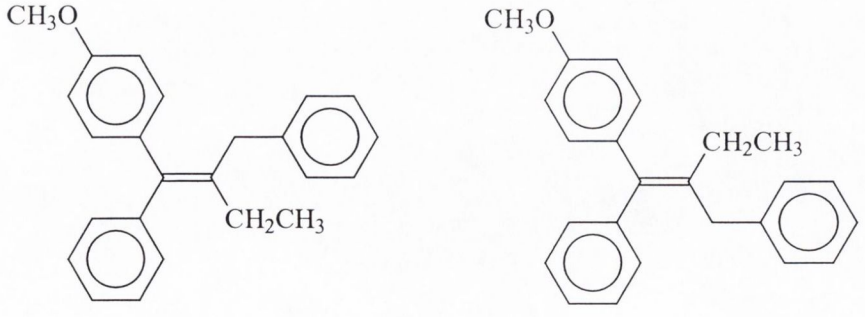


Scheme 9: Carbocation mechanism involved in the dehydration of secondary and tertiary alcohols.

Dehydration can be accomplished in several ways,[257,258,259] the most common reagents utilised being sulphuric[261] and phosphoric acids,[262] which effectively protonate the alcohol to afford a carbocation, however the use of such reagents may lead to rearrangement products[103,257,260]. Other reagents used on occasion to dehydrate alcohols include P_2O_5,[263,264] I_2,[265] DMSO,[266,267] HMPT, KOH,[268] $KHSO_4$ and anhydrous $CuSO_4$.[269,270]

Dehydration of 1,1,2-triarylbutan-1-ols (53), (54) using 85% polyphosphoric acid resulted in the formation of 1,1,2-triarylbutan-1-ene (55), (56) as oils in good yield. The but-1-ene skeleton of these compounds is similar to that of tamoxifen, however (55) and (56) possess a benzyl substituent rather than a phenyl group at C-2. In the infrared spectrum the C=C stretching was found in ν1601-1605cm^{-1} region with no hydroxy stretching as expected.

The identity of compounds (55), (56) was confirmed using ^{1}H and ^{13}C NMR along with high resolution mass spectrometry.



Z isomer E isomer

Figure 37: Z and E isomers of compound (56)

In the ^{1}H NMR spectrum of 2-benzyl-1-(4-methoxyphenyl)-1-phenylbutan-1-ene (56), the methyl H-4 protons resonate as a multiplet in the range δ0.98-1.03, while the methylene H-3 protons are observed as a multiplet between δ2.05-2.14. The methylene protons of the benzyl group at C-2 occur at δ3.62 and δ3.58 as singlets integrating for two protons, representing the Z and E isomer respectively. The relative peak integrals of the methylene benzyl group were used to assign the isomeric ratio of the compound (56) [Table 1]. The 4''-methoxy protons are observed as singlest at δ3.80 and δ3.83, integrating for three protons and representing the Z and E isomer respectively [Figure 37].

^{1}H NMR spectroscopy of (56) showed the expected resonances for the ethyl and aryl protons and confirmed from the integral ratios the 1.2:1 isomer distribution. The stereochemistry of tamoxifen has been discussed in Section 1.9.2, and establishes that the protons of the basic side chain and those of the ethyl group of Z-tamoxifen resonate at a lower frequency when compared to E-tamoxifen. A distinctive pattern is also observed for protons constituting the A_2B_2 system of the 4-substituted aryl ring of tamoxifen. These results were rationalised by considering that the aromatic ring and the side chain of the Z-isomer were sandwiched between two other aryl rings and experience a double shielding effect from their ring currents. These observations have been established as the diagnostic tool for the determination of the E/Z isomer ratios of tamoxifen related compounds.[244]

In compound (56), the methoxy aryl group is sandwiched between the other aryl and benzyl groups and thus the substituted aryl protons are found well separated from the remaining aryl protons. In the Z-isomer the aryl protons lie in the region δ6.10-6.80, whereas the corresponding aryl protons in the E-isomer coincide with the remaining aryl protons at δ6.85-7.42. This effect is presumed to be due to the different shielding effects of the phenyl and the ethyl groups on the 4-substituted ring in the different isomers. The geometrical E/Z isomer assignments of (56) are based on those for tamoxifen (2) and similarities can be seen when they are spectroscopically compared,[271] with the more shielded methyl H-4 (CH_3) group of tamoxifen resonating as a triplet at δ0.90, and the less shielded H-3 (CH_2) group being found as a quartet at δ2.40. However, the aromatic A_2B_2 system observed in tamoxifen is not present for 2-benzyl-1-(4-methoxyphenyl)-1-phenylbutan-1-ene (56), as the aryl protons are found as a multiplet. This multiplet depicts the aromatic protons of the E-isomer down field compared to the Z-isomeric aryl protons, which are found at a lower frequency, these observations equate to those described for tamoxifen. The peak

heights of the methylene benzylic group allows calculation of the isomeric ratio for compound (56), while the aromatic protons indicates the positioning of the E and Z isomer. However the isomeric ratio of (56) has been established from both the methoxy and benzylic methylene groups in the compound.

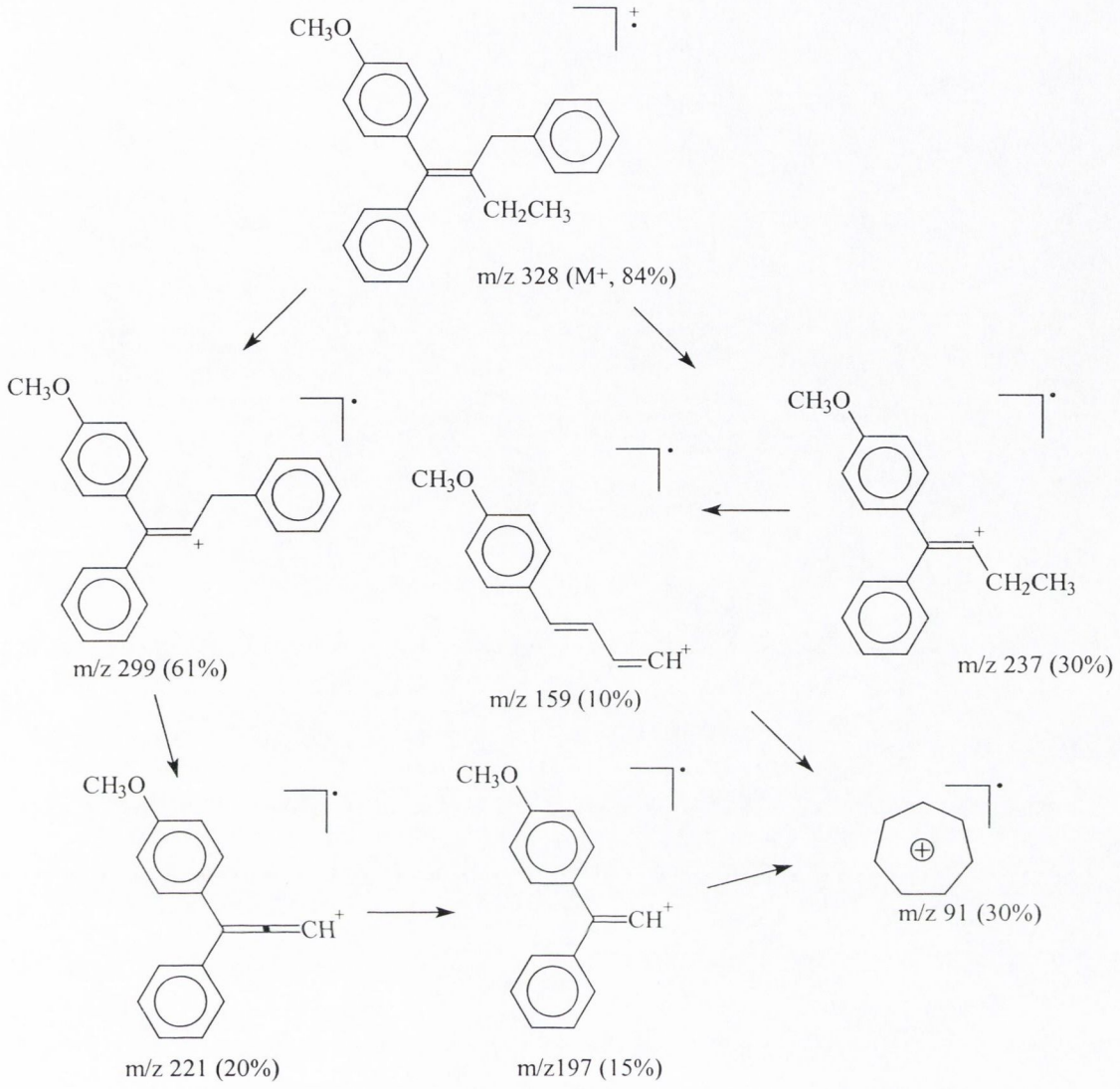
Compound	Yield %	IRυ_{max} (film cm^{-1})	Isomeric Ratio[a]
(55)	83	1601 (C=C)	-
(56)	79	1605 (C=C)	1.2:1 (6:5)

[a] Ratio determined as major : minor present Z isomer : E isomer, based on ^{1}H NMR assignment and theoretical reaction predictions

Table 1: Yield, infrared and isomeric data for compound (55) and (56)

In the ^{13}C NMR spectrum of 2-benzyl-1-(4-methoxyphenyl)-1-phenylbutan-1-ene (56), the C-4 methyl signal is observed at 13.22ppm and the C-3 signal is found inverted in the DEPT 135 at 24.65ppm. The benzyl methylene CH_2 signal is also found inverted in the DEPT 135 spectrum at 37.11 and 37.17ppm, corresponding to the Z and E isomers respectively. The 4"-methoxy carbon signal was observed at 55.13ppm. The quaternary C-1' is found at 140.25ppm and C-1"and C-1'" are observed at 143.05 and 144.36ppm. The shielded C-3" and C-5" are found at 113.50ppm, while the remaining aromatic signals occur between 125.55-129.18ppm, with the exception of C-4' found at 125.73ppm and C-4", C-4'" observed at 126.11ppm.

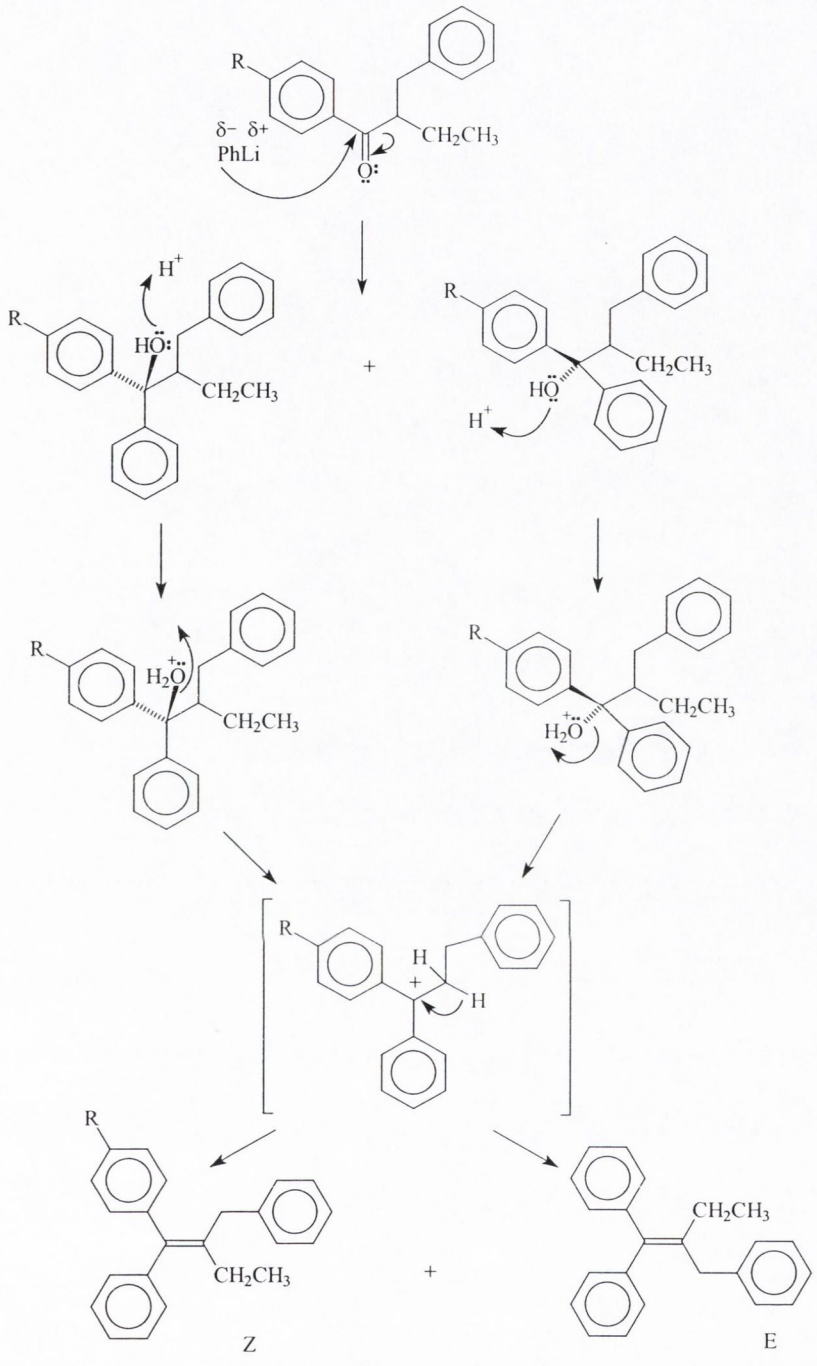
The proposed mass spectral fragmentation pattern of (56) is detailed in Scheme 10. The molecular ion is observed at m/z 328.1861 ($C_{24}H_{24}$) in 84% abundance with High Resolution Mass Spectrometry. Cleavage from the molecular ion of C_2H_5 and then C_6H_6 affords the m/z 299 (61%) and m/z 221 (20%) fragments. Further cleavage results in fragments m/z 197 (15%) and finally the tropylium ion m/z 91 (30%). The m/z 237 (30%) fragment is afforded from the molecular ion by cleavage of C_7H_7.



Scheme 10: Postulated mass spectral fragmentation pattern for compound (56).

2.2.6. Addition of PhLi to compounds (51), (52) and subsequent dehydration

The proposed mechanism of the reaction of compounds (51) and (52) is depicted in Scheme 11.[272,273] Initially the phenyl lithium reagent reacts with the carbonyl via nucleophilic addition to the carbon of the carbon-oxygen double bond. Water or dilute acid is added to the reaction mixture once aryl lithium addition is complete, an acid-base reaction takes place to produce the corresponding enantiomeric alcohols.



Scheme 11: Proposed mechanism for addition of the PhLi to compound (51), (52) and subsequent dehydration

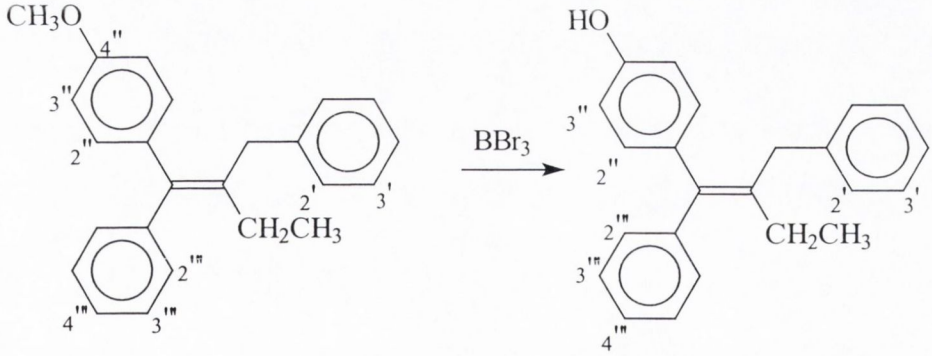
Dehydration of these enantiomeric alcohol products was carried out using concentrated sulphuric acid or 85% phosphoric acid and is proposed to occur by an E1 mechanism [Scheme 11]. Initially a proton is transferred from the acid to one of the unshared electron pairs of the alcohol, representing a simple acid-base reaction. The presence of the positive charge on the oxygen of the protonated alcohol weakens all bonds from oxygen, including the carbon-oxygen bond, which is cleaved heterolytically. The bonding electrons depart with the leaving

group, a water molecule, leaving behind a carbocation. Thus the carbocation is highly reactive and as a result stabilises itself by transferring a proton to a molecule of water which, results in the formation of an hydronium ion and 1,1,2-triarylbut-1-ene products (55), (56). Dehydration results in the Z/E isomeric mixture, observed for compound (56).

2.2.7. Demethylation of 2-benzyl-1-(4-methoxyphenyl)-1-phenylbutan-1-ene

Demethylation of 2-benzyl-1-(4-methoxyphenyl)-1-phenylbutan-1-ene (56) was then carried out in order to form the phenolic derivative, which may be used in future alkylation reactions to form flexible tamoxifen analogues. The cleavage of the methyl ether function from structurally analogous compounds has been selectively performed with hydrobromic acid, pyridine hydrochloride[87,178,179] and boron tribromide.[166,274]

Boron tribromide was used to demethylate (56) [Scheme 12] affording 2-benzyl-1-(4-hydroxyphenyl)-1-phenylbut-1-ene (57) as an oil in 87% yield. Due to the success of boron tribromide in removing the methyl ether function from the methoxy group, no other demethylation procedures were investigated.



(56) (57)

Scheme 12: Demethylation of 2-benzyl-1-(4-hydroxyphenyl)-1-phenylbut-1-ene (57) [Z isomer illustrated]

The infrared spectrum of compound (57) showed the aromatic and aliphatic C=C stretching at υ3572-3157cm^{-1}. In the ^{1}H NMR spectrum of 2-benzyl-1-(4-hydroxyphenyl)-1-phenylbut-1-ene (57), the methyl H-4 protons are observed as a multiplet at δ0.99, while the methylene H-3 protons resonate at δ2.10 as a multiplet. The methylene protons of the benzyl group occur at δ3.57 and δ3.61 as singlets, integrating for two protons. The relative peak heights of the benzyl methylene groups are used to assign the isomeric ratio [Z:E] of the compound (57) and its assignment is based on those for tamoxifen, where the aryl protons

for the Z-isomer are observed at a lower frequency than that of the corresponding E-isomer. The compound is obtained in a 1:1 isomeric ratio, which is similar to that of the methoxy compound (56).

The hydroxy proton is observed as a doublet at δ4.77. This assignment was confirmed by deuteration. The 4''-methoxy group of (56) is not observed in (57), thus confirming the removal of the methyl ether group. The shielded aromatic H-3''', H-5''' are observed as a multiplet in the range δ6.77-6.81. The remaining aromatic protons occur as a compact multiplet between δ7.11-7.31.

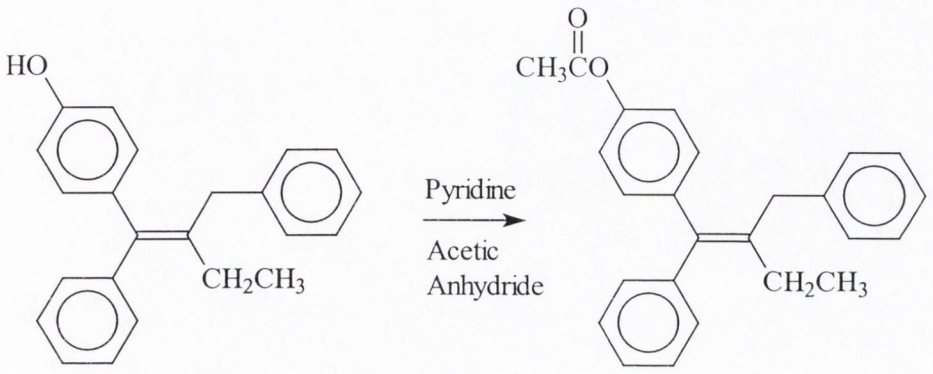
In the ^{13}C NMR spectrum of (57), the methyl C-4 is characteristically observed upfield at 12.78ppm. Two inverted signals are observed in the DEPT 135 spectrum at 24.17 and 36.71ppm. A C-H COSY spectrum allowed for clear identification of those signals, correlating the H-3 multiplet at δ2.10 in the ^{1}H NMR spectrum with the ^{13}C NMR signal at 24.17 and 24.29ppm, therefore confirming the presence of two isomers. The singlets at δ3.57, δ3.61 were found to couple with the signals at 36.71 and 36.83ppm (due to the E/Z isomers) which as a result was assigned to the benzyl methylene CH_2. The quaternary C-1 and C-2 signals are observed at 138.12 and 138.43ppm, while the C-1' was found at 140.21ppm and C-1'' and C-1''' are observed at 142.98ppm. The remaining aromatic signals occur between 127.99-129.17ppm, with the exception of the C-3'', C-5'' signals found at 114.53 and 114.49ppm. Correlation of the C-3'', C-5'' with the H-3'', H-5'' signals was evident from the C-H COSY spectrum. The C-4' signal was found at 125.73ppm and C-4'', C-4''' were observed at 126.13ppm.

High resolution mass spectrometry afforded the molecular ion as 314.1692 ($C_{23}H_{22}$) in 88% abundance. The fragmentation pattern of (57) is similar to that of its preceding methoxy compound (56).

2.2.8. Acylation of 2-benzyl-1-(4-hydroxyphenyl)-1-phenylbut-1-ene

It was proposed by Schneider[175] that a 4-acetoxy group in ring A of tamoxifen may be essential for good estradiol receptor affinity. They also postulated that the introduction of a second 4-acetoxy substituent on one of the other aromatic rings may enhance the binding affinity and that para-substitution generally results in compounds of higher affinity than meta-substitution.[275] These observations have led to some new antiestrogenic compounds that possess strong antitumour activity on a mammary tumour model and on the immature mouse[175] without estrogenic side effects.

Acylation of 2-benzyl-1-(4-hydroxyphenyl)-1-phenylbut-1-ene (57) which was carried out using acetic anhydride and pyridine (base) to give the acetoxy substituted 1,1,2-triarybut-1-ene product (58). [Scheme 13]



(57) (58)

Scheme 13: Synthesis of 1-(4-acetoxyphenyl)-2-benzyl-1-phenylbut-1-ene (58) [Z-isomer illustrated]

The infrared spectrum of compound (58) showed the aromatic and aliphatic C=C stretching at $\upsilon 1603 cm^{-1}$. An hydroxy group stretch is not observed, thus confirming that acetylation has occured. A peak at $\upsilon 1738 cm^{-1}$ confirms the presence of a C=O, i.e. [CH_3COO] stretch.

In the ^1H NMR of 1-(4-acetoxyphenyl)-2-benzyl-1-phenylbut-1-ene (58), the methyl H-4 protons are observed as a multiplet at $\delta 0.97$, while the methylene H-3 protons resonate a $\delta 2.05$ as a multiplet. The methylene (CH_2) protons occur at $\delta 3.56$ and $\delta 3.58$, as singlets, integrating for two protons. The relative peak heights of the benzyl CH_2 group were used to assign the isomeric ratio [Z:E] of the compound (58). The isomeric ratio of (58) is identical to that of its hydroxy starting material (57) i.e. a 1:1 ratio. The $CH_3C=O$ group appears as a multiplet at $\delta 2.36$. The aromatic peaks are observed as a multiplet between $\delta 7.01$-7.28. The molecular ion is observed at m/z 328.1861 ($C_{25}H_{24}O_2$) in 100% abundance, using High Resolution Mass Spectrometry.

2.2.9. Alkylation of 2-benzyl-1-(4-hydroxyphenyl)-1-phenylbut-1-ene

The majority of triphenylethylene antiestrogens contain an alkylamino side-chain, which appears to be essential for their activity.[276,277,278] Research has shown that triphenylethylene derivatives of tamoxifen that lack the side chain are pure estrogen agonists.[279] The composition of this side chain was found to be critical for antagonist activity.[280,281]

Substitution of the aminoethoxy side chain with an allyl side chain produces a compound with partial agonist activity, as do compounds containing bulky aryl or large alkyl groups, which are believed to decrease the affinity for the receptor to an imperceptible degree.[179] Therefore, the antiestrogenic activity of tamoxifen is not solely due to the length or bulk of the constituents in the side chain region. Recent studies suggest that side chain constituents containing a lone pair of electrons (such as oxygen or nitrogen) are necessary for antiestrogenic activity.[282] In this research a selection of alkylamino side-chains i.e. dimethyl, diethyl, piperidinyl, pyrrolidinyl and morpholinyl were introduced onto the parent structure (57) (Figure 38).

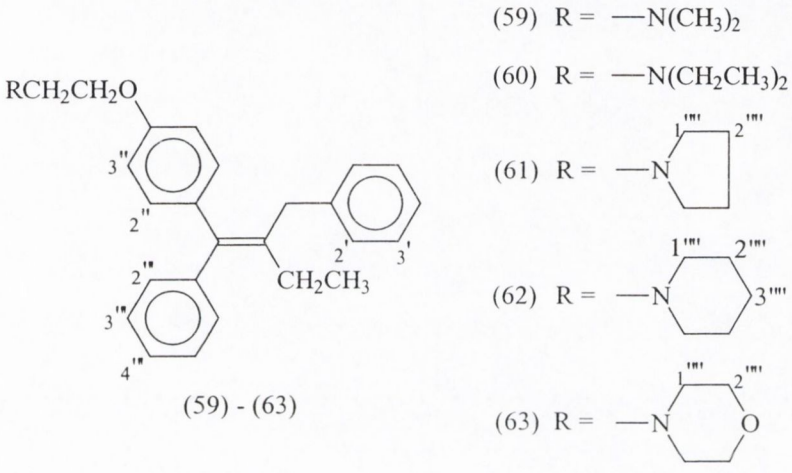
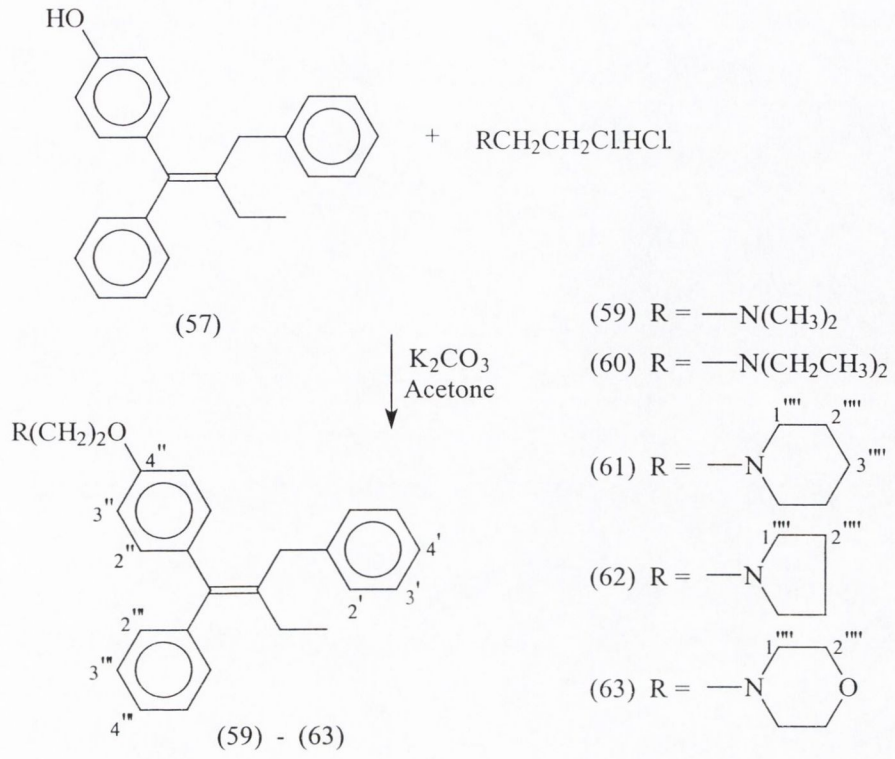


Figure 38: 2-Benzyl-1-[(4-alkylaminoethoxy)phenyl]-1-phenylbut-1-ene (59)-(63)

A number of reagents have been employed to introduce alkylamino groups onto hydroxyphenyl rings, including DMF with sodium hydride[179,283] or anhydrous toluene with sodium methoxide.[158]

In this research alkylation of (57) with a variety of alkylamino halides were carried out using a procedure outlined by Jones *et al.*,[191] and Mittal *et al.*[284] This consisted of refluxing 2-benzyl-1-(4-hydroxyphenyl)-1-phenylbut-1-ene (57), with anhydrous K_2CO_3, in dry acetone under a nitrogen atmosphere with the corresponding alkylamino derivative. A series of compounds were prepared using this procedure [Scheme 14].

The 2-benzyl-1-[4-(alkylaminoethoxy)phenyl]-1-phenylbut-1-enes (59)-(63) were obtained as oils in moderate yields. Positive identification of the products was obtained from spectroscopic data. The yield, isomeric ratio and infrared data are given in Table 2.



Scheme 14: Preparation of 2-benzyl-1-[4-(alkylaminoethoxy)phenyl]-1-phenylbut-1-enes [Z-isomer illustrated]

The infrared spectra of (58)-(63) show the C=C alkene stretch between 1604-1608cm^{-1}.

Compound	Yield %	IRυ_{max} (film cm^{-1})	Isomeric Ratio[a]
(58)	68	1603 (C=C)	1:1
(59)	80	1606 (C=C)	1.1:1
(60)	59	1608 (C=C)	1:1
(61)	83	1605, 1575 (C=C)	1.1:1
(62)	86	1604 (C=C)	1.1:1
(63)	69	1606 (C=C)	-

[a] Ratio determined as major : minor present Z isomer : E isomer, based on ^{1}H NMR assignment and theoretical reaction predictions

Table 2: Yield, infrared and isomeric data for compound (58) to (63)

Analysis of the integration in the ^{1}H NMR spectrums of (59)-(63), verified the stereoisomeric nature of these compounds, the E/Z isomers being afforded in almost equal amounts. The benzyl methylene (CH_2) protons appear as two singlets, each integrating for two protons, the relative peaks heights of the benzyl CH_2 group in compounds (59)–(62), being used to estimate the isomeric ratio

[Z:E]. The E and Z isomers were again assigned using aromatic proton assignment used for tamoxifen, where the Z-isomeric protons were found at a lower frequency when compared to the E-isomeric aryl protons. The 2-benzyl-1-[4-(morpholinylethoxy)phenyl]-1-phenylbut-1-ene (63) appears to be isolated as the Z isomer only, verification of this can be seen from the ^{1}H NMR spectrum of the compound.

In the ^{1}H NMR spectrum of 2-benzyl-1-[4-(dimethylaminoethoxy)phenyl]-1-phenylbut-1-ene (59), the methyl H-4 protons are observed as a multiplet at δ0.98, and the methylene H-3 protons occur as a multiplet at δ2.06. The $(CH_3)_2$ protons appear as a doublet (J=7.3Hz) at δ2.40. Both the $N(CH_2)$ and $O(CH_2)$ protons are observed as complex multiplets, at δ2.78 and δ4.08 respectively, integrates for two protons. The shielded aromatic H-3", H-5" protons appear as a double doublet (J=9.2Hz), in the range δ6.84-6.88. The benzyl CH_2 group exists as two singlets, at δ3.55, δ3.59, their peak heights indicating a 1:1 E/Z isomeric ratio. The remaining aromatic protons occur as a compact multiplet between δ7.13-7.31, the Z isomeric aryl protons were observed between δ7.13-7.24, while the aromatic protons of the E-isomer were found at δ7.28-7.35. The ^{1}H NMR spectrum of compound (59) is depicted in Figure 39.

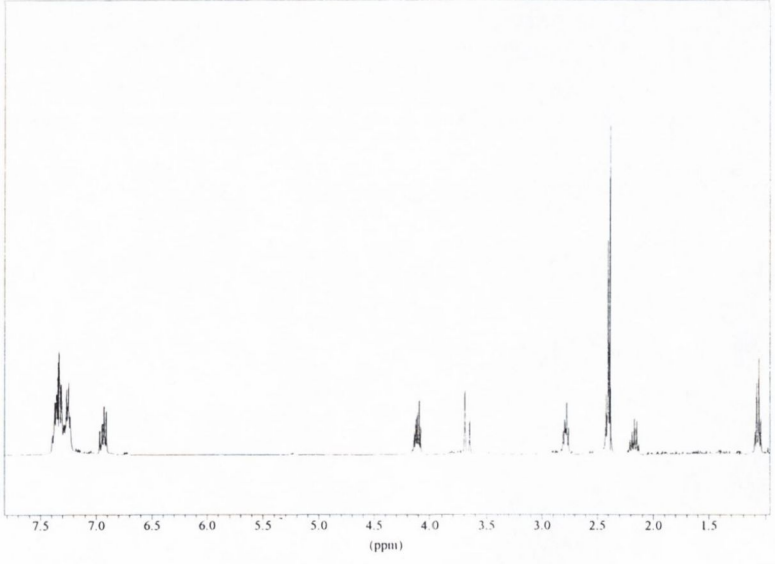
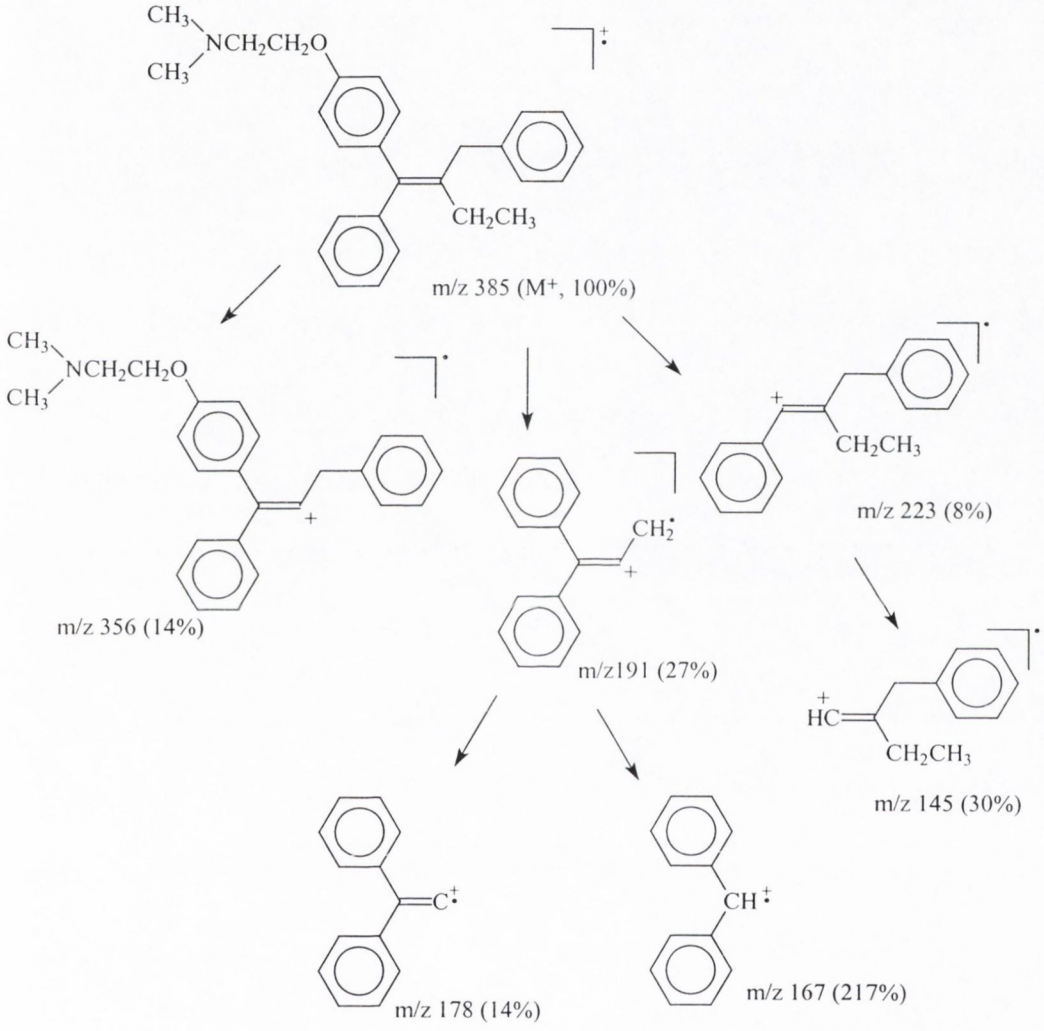


Figure 39: ^{1}H NMR Spectrum of compound (59)

In the ^{13}C NMR of 2-benzyl-1-[4-(dimethylaminoethoxy)phenyl]-1-phenylbut-1-ene (59), the methyl C-4 is characteristically observed upfield at 13.20ppm, with the $N(CH_3)_2$ signal occurring at 45.63ppm. In the DEPT 135, there are four inverted signals observed, corresponding to the methylene C-3 at 24.62ppm, the somewhat deshielded benzyl CH_2 at 37.17ppm, and the remaining signals

represent the NCH_2 and OCH_2 groups. The inverted signal at 65.59ppm is assigned to the OCH_2. The partially shielded C-3", C-5" are observed at 114.04 and 114.12ppm. The remaining aromatic signals occur between 125.70-140.22ppm.

The suggested mass fragmentation for (59) is detailed in Scheme 15. The molecular ion m/z 385.2405 ($C_{27}H_{31}NO$) is observed in 100% abundance.



Scheme 15: Proposed mass fragmentation pattern for compound (59).

Cleavage of the dimethylaminoethoxyphenyl substituent affords the m/z 223 (8%), and further cleavage of a phenyl group results in the fragment m/z 145 (30%). Cleavage from the molecular ion of C_2H_5 affords the m/z 356 (14%) fragment. The m/z 191 (27%) fragment is afforded from the molecular ion by cleavage of the C_2H_5 and dimethylaminoethoxy substituents.

2.3. McMurry coupling reactions

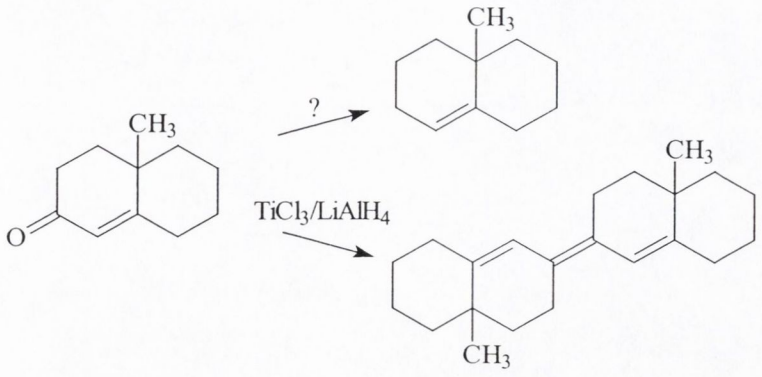
2.3.1. Introduction

As seen in section 2.2, the synthetic route for 2-benzyl-[(4-alkylamino ethoxy)phenyl]-1-phenylbut-1-enes (Type I) is long and results in only moderate overall yields. This inefficient synthesis prompts the need for an alternative improved synthetic method. Hence, the McMurry reaction was investigated as a convenient two step procedure for the target alkenes, i.e. Type I-IV flexible analogues.

The reductive coupling of carbonyl compounds using low valent transition metals (e.g. low valent titanium salts) to produce alkenes constitutes an important method for the formation of carbon-carbon double bonds [C=C].[285] Low valent titanium-mediated coupling was first described by Mukaiyama[286] but greatly extended by McMurry.[287] There have been a number of reviews of the scope and limitations of the reaction by McMurry,[288] Yee-Hing Lai[289] and Finocchiaro *et al.*[290]

McMurry and his co-workers found it necessary to carry out a high-yield transformation of an α,β-unsaturated ketone into the corresponding olefin without migration of the double bond [Scheme 16]. They found that this kind of transformation often fails with classic methods of carbonyl deoxygenation such as the Wolff-Kishner reaction or dithioacetal desulfurization.

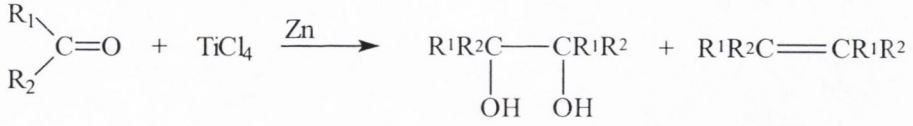
McMurry and his researchers[288] therefore sought to devise a new method: a one-pot reaction in which a good hydride donor such as $LiAlH_4$ might be used in conjunction with an appropriate transition-metal salt and due to the great strength of the titanium-oxygen bond, $TiCl_3$ was chosen. They hoped that if initial hydride reduction of the carbonyl group was to be followed by strong co-ordination of the alkoxide anion with the metal, a second hydride delivery might occur in an S_N2-type fashion, leading to the desired product. The product was obtained by $TiCl_3/LiAlH_4$ treatment of the enone in THF and was indeed a hydrocarbon, but not the required compound.



Scheme 16: McMurry trials (1974)

The transformation that had occurred – reductive dimerization of a ketone to yield an alkene – was an unknown reaction at the time, although two other groups, led by Mukaiyama[286,291] and Tyrlik,[292] made similar discoveries almost simultaneously. McMurry and co-workers explored the scope of this new reaction and they soon learned that the reductive coupling reaction was not limited to α,β-unsaturated ketones, but was general for all types of ketones and aldehydes. They also discovered that many reducing agents besides $LiAlH_4$ could be used with $TiCl_3$ in the reaction, for example the zinc-copper couple.[288] This method developed by McMurry,[293] and its modifications, has greatly facilitated attempts to prepare various classes of hindered olefins such as: symmetrical tetra-alkylsubstituted ethylenes e.g. tetra*neo*pentylethylene,[294] fused bicyclic *trans*-cycloalkenes,[295,296,297] α-carotene,[293] isorenieratene,[298] geometric isomers of C_{20} analogues of phytoene e.g. (poly-Z)-carotenoids[299] and complex natural products including Taxol.[300]

Mukaiyama [286,301] also explored this McMurry reaction with the reagents; $TiCl_4$ and Zn.



pinacol olefin

Mukaiyama discovered that olefins were produced at elevated temperatures, using these reagents, for example stilbene was obtained in 98% yield. Mukaiyama also noted:

1. A combination of $TiCl_4$ and Zn is essential for this reduction. When magnesium or butyllithium was used instead of Zn in the reduction of benzophenone, tetraphenylethylene was produced in much lower yields.[286]

2. The reaction occurs successfully when using a molar ratio of 1:1 or 1:2[302,303] for $TiCl_4$ and carbonyl compounds in their molecular complexes.

Tyrlik and Wolochowicz[292] examined the use of $TiCl_3$/Mg-THF, however this reagent combination was reported to be effective only for aryl ketones, with limited success for alkyl ketones. McMurry's $TiCl_3/LiAlH_4$ reagent system was seen to give excellent yields in both aryl and alkyl ketone reduction. McMurry also found that the carbonyl reaction can be reproducibly carried out using titanium metal prepared by reducing $TiCl_3$ with 3 equiv. of potassium, i.e. Ti(0)-induced coupling reaction.[291] Since the discovery of the McMurry reaction, the usefulness of this reaction has expanded to obtain pinacolic coupling,[290] in fact Corey *et al.*,[304] have obtained key intermediates in the total synthesis of gibberellic acid.

The McMurry reaction also has limitations: the reagent does not react cleanly with substrates which contain other functional groups like alcohols, ethers and olefins. However Castedo *et al.*,[305] have recently reported a study on the selective reductive coupling of aromatic aldehydes and ketones containing carboxylate or tosylate ester functionalities to the corresponding stilbenes, thus widening the use of the McMurry synthesis. Recently, due to limitations encountered when using the $TiCl_3/ LiAlH_4$ reagent system, the $TiCl_4$/Zn system initially proposed by Mukaiyama has become widely used.

2.3.2. Reductive coupling of carbonyl compounds

McMurry coupling reactions are used for aliphatic, aromatic and heterocyclic carbonyl compounds and involve intramolecular reductive coupling.[290]

(a) Aliphatic carbonyl compounds

The reductive reactions of the aliphatic carbonyl compounds occur readily, but by increasing the steric hindrance of the alkyl groups, the olefinic products are isolated in lower yields. For example, the methyl *tert*-butyl ketone can be coupled to form the corresponding olefin while the ethyl *tert*-butyl ketone fails to give the coupling product under any conditions.[306]

(b) Aromatic carbonyl compounds

Again it has been noted that the reductive coupling reactions of the aromatic ketones can be carried out successfully, but the yield of the olefins decreases with increasing steric congestion around the carbonyl group.

(c) Dicarbonyl compounds

The McMurry reagent is also used for intramolecular coupling of dialdehydes, ketoaldehydes and diketones to give the cycloalkenes. When the $TiCl_3/LiAlH_4$[307] reagent is used, yields of 50-60% are obtained. Yields can be improved by using the reducing agent copper-zinc couple.[291]

(d) Heterocyclic compounds

For heterocyclic ketones, olefins are formed when the carbonyl group is distal to the heteroatom. The McMurry reaction has been useful in the synthesis of heterocyclic compounds e.g. tetraphenylfuran. Various carbonyl derivatives were used as starting materials and acyl chloride was reported to give the highest yield of tetraphenylfuran.[308]

2.3.3. Proposed mechanism for McMurry reaction

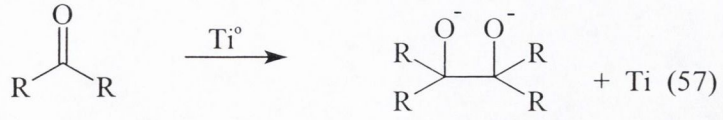
The mechanism of this reaction has been extensively studied.[308,309,310] Recent reports from Dams *et al.*,[311] suggest a mechanism, which has been widely accepted by many researchers, however it is not fully conclusive[244] [Scheme 17].

Mechanistic studies[312] of this coupling reaction suggest it occur in a heterogeneous process on the surface of an active titanium particle.[290] The reaction proceeds by initial electron transfer followed by coupling to form a pinacolate that on deoxygenation liberates the olefin. The coupling of unsymmetrical ketones leads to the formation of stereoisomeric alkenes, the ratio depending on the steric demand of substituents.

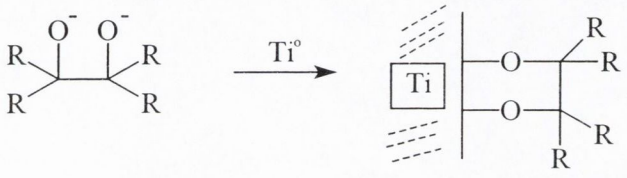
In the first step of the coupling reaction the ketone becomes attached to [M] (the active coupling species), and one electron is transferred from the titanium to the ketone, yielding the organic anion radical and an oxidised titanium.[313,314,315] This radical dimerizes to a titanium pinocolate i.e. a pinacolate-like intermediate that is co-ordinated to titanium, in the second step. It is speculated that this step is the rate-determining step in the reaction, although conclusive evidence is not available to confirm this. However, it has been detected that the amount of hydrogenation products of the anion radicals is higher than that of any other side product of the reaction, suggesting that the anion radical is a longer living intermediate, therefore corroborating with the assumptions made. In subsequent steps, the titanium then withdraws two oxygen atoms from the pinacolate and the formed olefin is kept pi (π) bonded to Ti. Cleavage of the C-O bonds via a

deoxygenation reaction leads to the formation of an alkene and the by-product titanium dioxide.[292,312] The mechanism is depicted in Scheme 17.

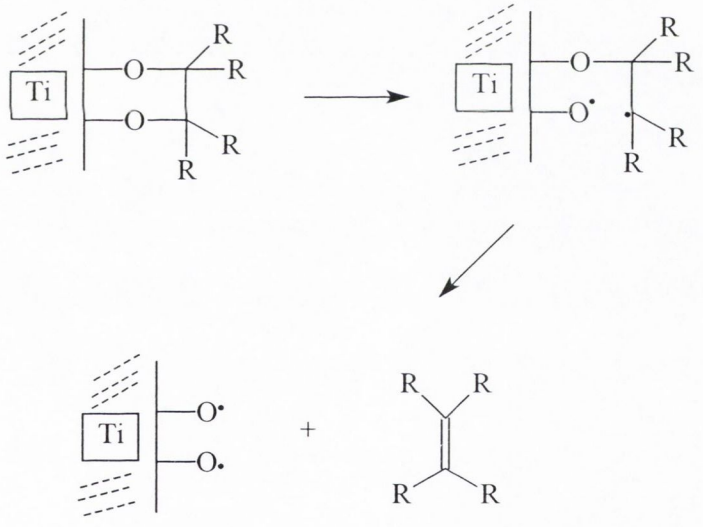
STEP 1: Electron transfer to the carbonyl generates a dianion via a ketyl-like intermediate



STEP 2: The dianion binds to the titanium particle



STEP 3: Sequential homolytic cleavage liberates the alkene and the titanium dioxide



Alkene from coupled ketone/aldehyde

Scheme 17: Proposed McMurry Coupling Mechanism

The pinacolisation can be carried out with a variety of reductants, but the deoxygenation is unique to low-valent titanium (LVT) reagents. Although titanium is known for its high oxophilicity, the extrusion of oxygen from the pinacolates necessitates the use of solvent–reflux temperatures and prolonged reaction times. At lower temperatures (less than room temperature), the McMurry reaction primarily furnishes pinacols,[314,315,316,317,318,319,320] while the

olefins are obtained only at higher temperatures i.e. after refluxing the reaction mixture in dioxane or THF.[300]

McMurry's experiments discredited alternative mechanisms such as formation of a discrete cyclic (or acyclic) titanium dialkoxy species.

2.3.4. Reaction conditions necessary for the McMurry coupling

Mukaiyama[286] has described some conditions, which are necessary for the coupling reaction [Section 2.3.1]. The optimum conditions were proposed by Dams *et al.*,[320] after much research. With the use of ESR studies the nature of the active coupling species [M], the formal valence state of Ti in [M] and its successor [M,O] have been elucidated.

$$\text{Ti} + \text{reducing agent} + \text{solvent} \xrightarrow{\text{Ar}} [\text{M}]$$

$$[\text{M}] + \text{ketone} \xrightarrow[\text{solvent}]{\text{Ar}} [\text{M,O}] + \text{coupling products}$$

(a) Choice of reducing agent

Three classes of reducing agents are commonly employed to reduce $TiCl_3/TiCl_4$ to [M] : alkali metals e.g. Li or K,[291,321] group II metals e.g. Mg or Zn-Cu couple[291,322] and metal hydrides e.g. $LiAlH_4$.[309, 323]

(b) Choice of solvent

The reactivity of [M] restricts the choice of solvent largely to hydrocarbons and ethers. Solvents such as benzene, cyclopentadienes, hexane, furan, thiophene, pyridine, anisole, tetrahydrofuran, dioxane, glyme, diglyme and diethyl ether have been investigated. Researchers have found that the combination of reducing agent-solvent is critical for the reaction. Of all the solvents tested, THF and dioxane performed best, producing high yielding compounds. To obtain olefins in high yields, the solvent dioxane is used at elevated temperatures. Diols are obtained when using THF at low temperatures.[286,311]

(c) Choice of metals

Titanium salts were chosen for this reaction primarily due to the affinity of the metal ion for the oxygen atom and the stability of the counter ion.[288,301]

$TiCl_3$ has been the transition-metal salt of choice for numerous years and has been reported to deoxygenate sulfoxides,[324] nitroalkanes[325] and oximes.[326]

Recently $TiCl_4$ and Zn[327] have become the agents of preference for both aldehydes and ketones.

(d) Molar ratio of $TiCl_3/TiCl_4$ to reducing agents

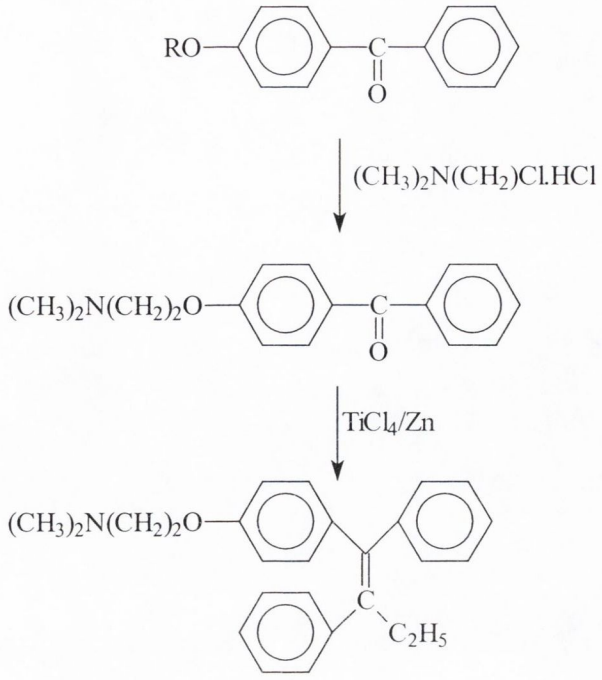
Results of Tyrlik *et al.*,[292] on the $TiCl_3$/Mg system have indicated that the yield of olefin depends upon the ratio of $TiCl_3$ to Mg, while for the $TiCl_3$/K system ratios larger than 1:3, resulted in inferior yields of the olefin.[291] No systematic optimisation of this reaction parameter seems to have been performed to date.

(e) Molar Ratio of $TiCl_3$ / $TiCl_4$ to ketone.

Tyrlik *et al.*,[291] gave the first indications that Ti-salt/ketone ratio was important. At low titanium salt / ketone ratios, small amounts of pinacol are formed. It was discovered[286] that the most efficient molar ratio of $TiCl_3$ or $TiCl_4$/carbonyl compounds is 1:1 or 1:2. At lower ratios the additional amount of ketone is left unreacted. Per titanium atom only one ketone molecule can be coupled. This provides evidence that the coupling does not proceed on a single Ti atom, but rather takes place on the surface of a Ti microcrystallite.[286]

2.3.5. Synthesis of 2-Benzyl-1-[4-(alkylaminoethoxy)phenyl]-1-phenylbut-1-ene Type 1 using the McMurry coupling reaction.

The 2-benzyl-1-[4-(alkylaminoethoxy)phenyl]-1-phenylbut-1-enes (59)-(63) [Type I] have already been prepared via a seven step route in Section 2.2.9. In this section, the preparation of these flexible tamoxifen analogues is described via the McMurry coupling reaction.[328] The McMurry reaction has been used for the synthesis of tamoxifen (2), Z-4-hydroxytamoxifen[329] and Z-4-hydroxytoremifene due to its steroselectivity.[245,330] In some cases the reductive-coupling reaction is known to avoid isomerisation in compounds.[331] [Scheme 18]

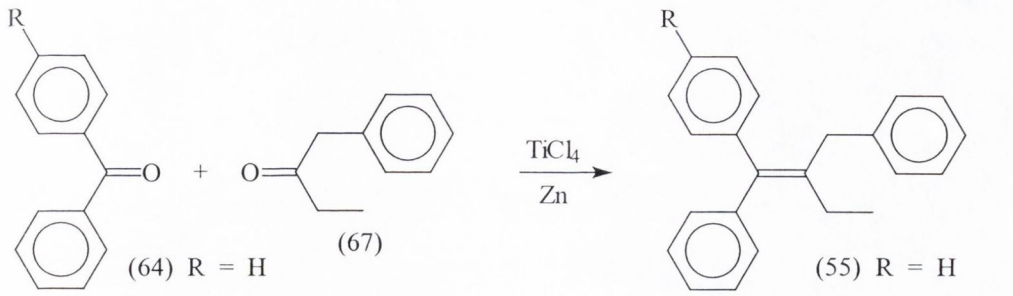


(2)

Scheme 18: Reductive Coupling Reaction for Tamoxifen (2) [Z-isomer illustrated]

To investigate the efficiency of this method, a series of substituted 1,1,2-triarylbut-1-enes[328] were prepared, [Scheme 19] using the arylketone 1-benzyl-2-butanone with benzophenone, 4-hydroxybenzophenone and 4-methoxybenzophenone. The reaction conditions use $TiCL_4$, dry dioxane and zinc dust. The reaction mixture was initially stirred at –78°C under a nitrogen atmosphere and then refluxed for 3-4hours.

The 1,1,2-trialkylbut-1-enes (55)-(57) [Method 2] were afforded as oils in 89-94% yield. The spectroscopic data of compounds (55)-(57) are similar and yields are considerably increased compared to those obtained from the longer route [Method 1] in Section 2.2.6 and 2.2.8. The infrared spectrum of compounds (55)-(57) showed the aromatic and aliphatic C=C stretching at ν1574-1610cm^{-1}.



(64) R = H

(65) R = OCH_3

(66) R = OH

(55) R = H

(56) R = OCH_3

(57) R = OH

Scheme 19: Preparation of 1,1,2-triakylbut-1-enes (55)-(57), [Z isomer illustrated]

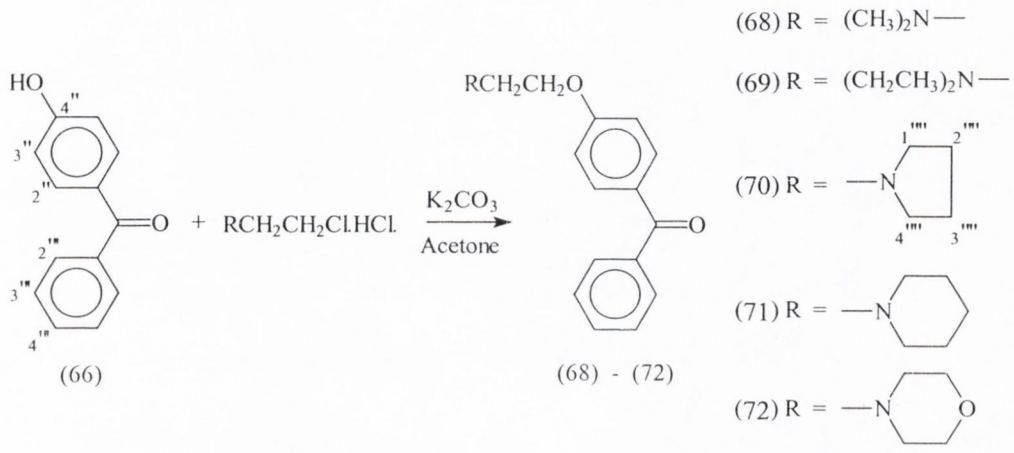
However, the isomeric Z/E ratio of the compounds (55)-(57) differs from that of the previously synthesised analogues. According to present and previous work,[244] variations in the stereoselectivity depend upon the nature of the substituents on benzophenone. It has been postulated that the phenol functionality is always on the opposite side of the ethyl chain in the McMurry product, thus providing an important element when considering the stereochemical assignment of these products.[329] Therefore the stereochemistry of the compounds is based on the positioning of the oxygen group, which clarifies the existence of (56) and (57) in an isomeric ratio of 2.5:1 and 2:1 (Z:E) respectively, which shows greater stereoselectivity than that previously obtained (1:1). It has also been suggested that for McMurry coupling of alkylaryl ketones, Z-isomers predominate for sterically undemanding alkyl groups, whereas bulky alkyl groups favour the formation of E-isomer. These assignments have been based on the analysis of ^{1}H NMR.[330]

The ^{1}H NMR of the 2-benzyl-1-(4-hydroxyphenyl)-1-phenylbut-1-ene (57), shows the methylene protons (CH_2) of the benzyl group at δ3.55 and δ3.59, as singlets. The relative peak heights of the methylene benzyl group were used to assign the isomeric ratio of the compounds (56) and (57). The aromatic protons were assigned using the rational proposed for aryl proton assignment of tamoxifen, as discussed earlier in the chapter. The aromatic protons are observed at δ7.13-7.24 representing the Z-isomeric form while the aryl protons of the E-isomer are found between δ7.28-7.39.

Due to the success of this McMurry reaction, this method was used for the direct preparation of the target structures 2-benzyl-1-[4-(alkylaminoethoxy)phenyl]-1-phenylbut-1-enes (59)-(63) [Method 2]. This involves a two step procedure, the

first step is the alkylation of 4-hydroxybenzophenone and the second step involves the reductive coupling reaction.

Initially a series of alkylated benzophenones[328] were prepared, by reaction of 4-hydroxybenzophenone and the alkylating agent of choice with anhydrous potassium carbonate, in dry acetone followed by reflux for 5-6hr. [Scheme 20]



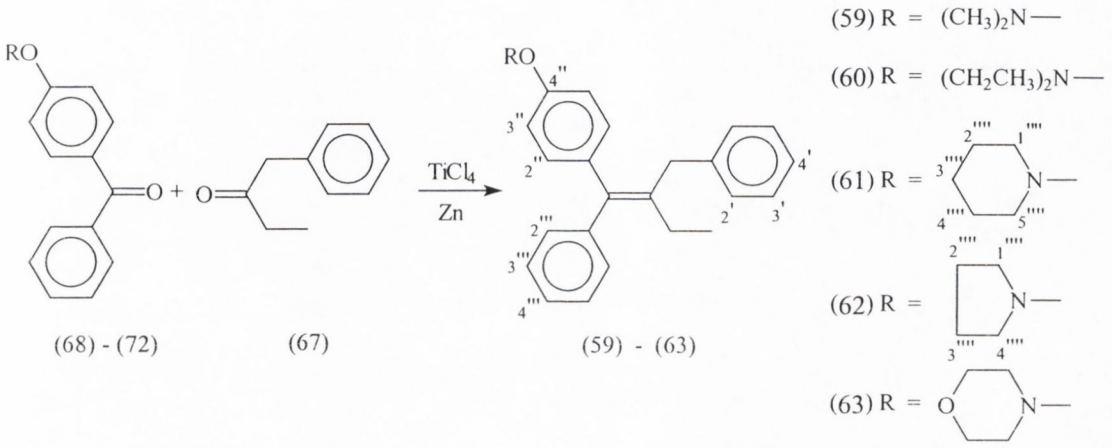
Scheme 20: Synthesis of alkylated benzophenones (68)-(72)

The infrared spectrum of compounds (68)-(72) show the presence of the carbonyl (C=O) stretch between ν1651-1658cm^{-1} and the aromatic (C=C) stretch in the range ν1601-1605cm^{-1}.

In the ^{1}H NMR of 4-(pyrrolindylethoxy)benzophenone (70) the NCH_2 protons are observed as a triplet (J=6.0Hz) at δ2.94, while the corresponding OCH_2 protons are observed at δ4.20 as a triplet (J=6.0Hz). The pyrrolidinyl protons are observed as multiplets. The H-2'''', H-3'''' protons are found as a multiplet at δ1.82, while the corresponding H-1'''', H-4'''' also occur as a multiplet at δ2.57 as a multiplet also. The shielded H-3", H-5" are observed as double doublets (J=8.5, 2.0Hz) at δ6.97-7.00; all remaining aromatic protons are found in the range δ7.45-7.90.

The ^{13}C NMR of (70) indicates the presence of four inverted signals, corresponding to the signals of C-2'''', C-3'''', C-1'''', C-4'''', NCH_2, and OCH_2 at 23.47, 54.83, 57.86 and 67.37ppm respectively. The C-3", C-5" which experience the shielding influence of the pyrrolidinylethoxy substituent occur upfield at 113.67ppm. The remaining aromatic signals occur between 127.68-137.88ppm and the C-4" signal is found at 162.08ppm. The C=O signal is characteristically observed at 195.03ppm.

These 4-(alkylamino)benzophenones were then coupled with 1-phenyl-2-butanone (67), in the same manner as that described for compounds (55)-(57), producing a series of 2-benzyl-1-[(4-alkylaminoethoxy)phenyl]-1-phenylbut-1-enes[328] (68)-(72) identical to those prepared via the longer ArLi type route. [Scheme 21].



Scheme 21: Preparation of 2-benzyl-1-[(4-alkylaminoethoxy)phenyl]-1-phenylbut-1-ene (59)-(63) [Z-isomer illustrated]

Previous work on the stereospecificity of tamoxifen and related analogues using this McMurry coupling reaction has only identified the formation of that product which has a *trans* arrangement of the ethyl side chain relative to the original phenolic system across the double bond.[244,329] This literature data in conjunction with NMR peak assignment/analysis allowed the prediction of the major species in Type I flexible analogues (59)-(63) as Z-isomers. The results were rationalised by considering that the phenolic ring and the side chain of the Z-isomer were sandwiched between the other two rings and thus experience a double shielding effect from their ring currents. Spectral isomeric assignment interpretations were based on the relative positions of those aryl proton signals arising from the A_2B_2 para-system of the 4-substituted phenyl ring together with the position of the benzyl methylene signal.

Positive identification of the products was obtained from spectroscopic data demonstrating that compounds (59)-(63) are identical to those prepared via Method 1. However a difference between the two sets of compounds is apparent regarding their isomeric ratios. The yield, isomeric ratio and infrared data are detailed in [Table 3].

Compound	Yield %	IRυ_{max} (film cm^{-1})	Isomeric Ratio[a]
(59)	69	1610, 1572 (C=C)	2:1
(60)	72	1607, 1574 (C=C)	2:1
(61)	67	1605, 1573 (C=C)	2:1
(62)	68	1606, 1573 (C=C)	2:1
(63)	65	1605, 1574 (C=C)	2:1

[a] Ratio determined as major : minor present Z isomer : E isomer, based on ^1H NMR assignment and theoretical reaction predictions

Table 3: Yield, infrared and isomeric data for compound (59)-(63)

In the ^1H NMR spectrum of 2-benzyl-1-phenyl-1-[4-(piperidinylethoxy)phenyl] but-1-ene (61), the methyl H-4 protons are observed as a multiplet at δ0.98, and the methylene H-3 protons also occur as a multiplet at δ2.09. Both the NCH_2 and OCH_2 protons are observed as complex multiplets at δ2.81 and δ4.11 respectively, each integrating for two protons. The piperidinyl protons are observed as multiplets H-3"" is found at δ1.48, H-2"", H-4"" are observed at δ1.65 and the protons of H-1"", H-5"" are evident at δ2.55. The benzyl CH_2 group exists as two singlets and are found at δ3.57 and δ3.61. It is the peak heights of these CH_2 groups that determine the isomeric ratios (E:Z) of the compound. The partially shielded H-3", H-5" protons are found as a doublet (J=8.5Hz) between δ6.82-6.88. The remaining aromatic protons are observed as a multiplet in the range δ7.15-7.39. The aryl protons of the E-isomer are observed up field between δ7.29-7.39, while the aromatic protons of the Z-isomer are found at a slightly lower field δ7.15-7.24.

In the ^{13}C NMR of (61), the methyl C-4 is characteristically observed upfield at 12.81ppm. The DEPT 135 shows six inverted carbons, five of which are assigned to the piperidinyl carbons. C-3"" signal is observed at 24.28ppm, C-2"", C-4"" is found at 25.36ppm and C-1"", C-5"" are observed a little more downfield at 54.50ppm. The remaining inverted carbons correspond to the methylene C-3 at 23.65ppm and the NCH_2 and OCH_2, which are found at 57.42 and 65.30ppm respectively. The somewhat deshielded benzyl CH_2 has double signals at 36.75 and 36.85ppm, illustrating the presence of the geometric Z and E-isomers. The C-3" and C-5" are observed at 113.69 and 113.77ppm. The quaternary C-1 and C-2 signals are found at 138.05 and 138.13ppm, while the C-1' signal occurs at 143.04ppm. The remaining aromatic signals are observed between 125.32 and 129.84ppm. Low resolution mass spectrometry for (62) affords the molecular ion m/z 425 ($C_{30}H_{35}NO$) in 100% abundance.

The isomeric ratio of these novel compounds (59)-(63) via the McMurry coupling reaction [2:1], produces a greater amount of the Z-isomer, compared to the 1:1 ratio obtained using the long seven step method. The E and Z isomers could not be separated using either TLC or crystallisation methods. Current knowledge of the mechanisms of the McMurry mixed carbonyl-coupling reaction does not provide an explanation for the stereoselectivity of Z-isomer formatiom.

This series of compounds (59)-(63) were biochemically tested for their binding affinity, cytotoxicity and potency in binding and antiproliferative assays in a human MCF-7 breast cancer cell line. (Chapter 4)

2.4. Preparation of novel Tamoxifen analogues (Type II-IV)

Due to the success of the McMurry reaction in preparing 2-benzyl-[4-(alkylaminoethoxy)phenyl]-1-phenybut-1-enes (59)-(63) [Method 2], it was envisaged that this coupling reaction could be used to prepare further novel tamoxifen analogues. Each of these compounds are derived from the traditional triphenylethylene backbone associated with tamoxifen and other antiestrogens, through the introduction of a methylene (benzylic) spacing group between one of the aryl rings and the alkene group and through variations in the basic side chain.

2.4.1. Synthesis of 1-benzyl-1-(4-alkylaminoethoxyphenyl)-2-phenylbut-1-enes [Type II]

A series of 1-benzyl-1-[(4-alkylaminoethoxy)phenyl]-2-phenylbutenes[328] (81)-(85) were prepared, using the facile McMurry Coupling reaction. [Figure 40]

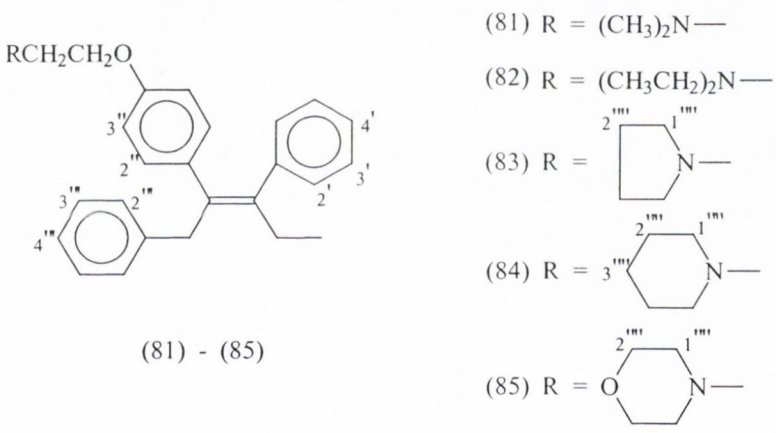
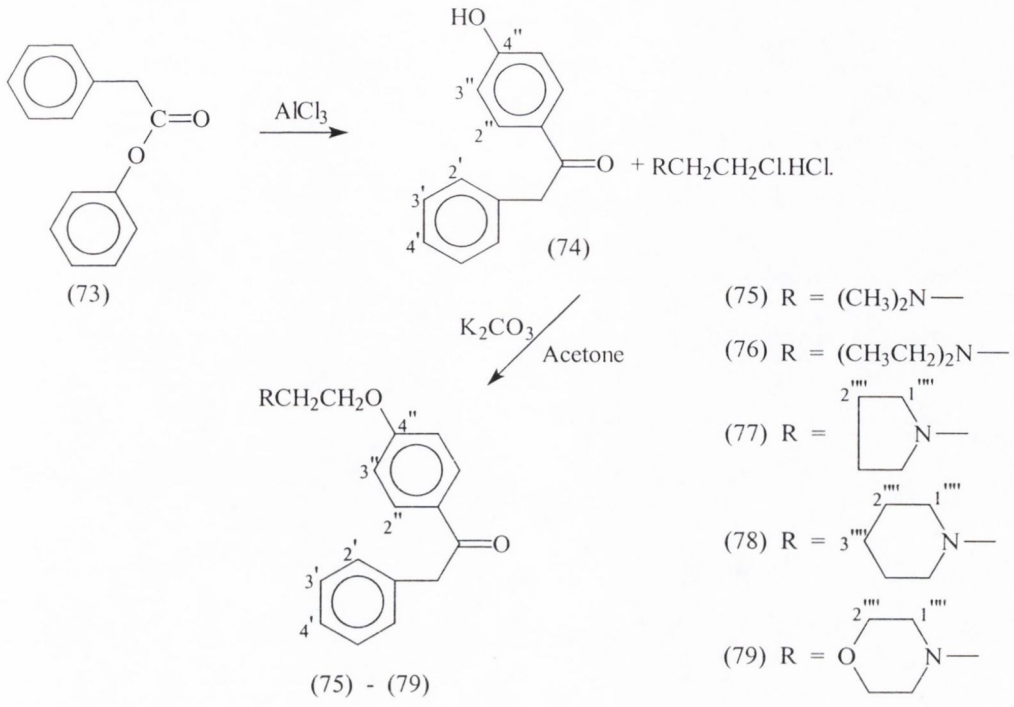


Figure 40: 1-benzyl-1-[(4-alkylaminoethoxy)phenyl]-2-phenylbutenes [Z-isomer only illustrated]

Initially the basic side chain was introduced to the appropriate phenol ketones. In this case 1-(4-hydroxyophenyl)-2-phenylethan-1-one (74) was prepared from phenylphenyl acetate (73) and aluminium chloride using a Fries Rearrangement. This is a synthetically useful reaction, which allows the rearrangement of phenolic esters by heating with Friedel Craft catalyst. Compound (74) was then alkylated, affording a series of 1-(4-alkylaminoethoxyphenyl)-2-phenylethan-1-ones[328] (75)-(79) [Scheme 22].



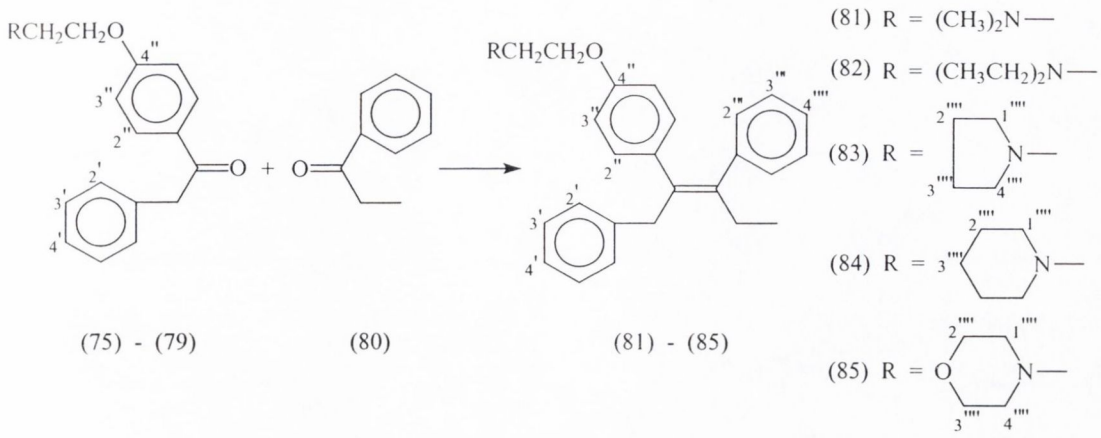
Scheme 22: Preparation of 1-(4-alkylaminoethoxyphenyl)-2-phenylethan-1-ones

The 1-(4-alkylaminoethoxyphenyl)-2-phenylethan-1-ones (75)-(79) were afforded as oils in good yield (65-99%). The characteristic carbonyl (C=O) stretch is found at ν1665-1674 cm^{-1} for compounds (75)-(79) in the infrared spectrum.

In the ^1H NMR spectrum of 1-(4-diethylaminoethoxyphenyl)-2-phenylethan-1-one (76), the $(CH_3)_2$ group of the ethyl side chain appears as a multiplet at δ1.10, and the corresponding $(CH_2)_2$ protons are observed as a multiplet at δ2.66. The NCH_2 signal is found as a triplet (J=6.3Hz) at δ2.91 and its corresponding OCH_2 protons are found as a triplet (J=6.3Hz) at δ4.12. The methylene protons of the benzyl group occur at δ4.24. The remaining aromatic protons occur as a multiplet between δ6.92-7.33.

2.4.2. McMurry coupling of 1-(alkylaminoethoxyphenyl)-2-phenylethan-1-ones

The 1-benzyl-1-[(4-alkylaminoethoxy)phenyl]-2-phenylbut-1-ene (81)-(85)[328] target compounds were prepared via implementation of the titanium tetrachloride / zinc mediated McMurry coupling reaction as depicted in [Scheme 23].



Scheme 23: Preparation of 1-benzyl-1-[(4-alkylaminoethoxy)phenyl]-2-phenylbut-1-ene (81)-(85) [Z-isomer illustrated]

The coupling reaction involves the reductive reaction of two carbonyl compounds directly to produce the required alkenes. The 1-(alkylaminoethoxy phenyl)-2-phenylethan-1-ones (75)-(79) are reacted with propiophenone (80) in a 1:1 molar ratio, using the $TiCl_4$/Zn reagent. A series of 1-benzyl-1-(4-alkylaminoethoxyphenyl)-2-phenylbut-1-enes (81)-(85) were afforded in moderate to good yields as oils. The stereospecificity of the McMurry coupling reaction predicts the formation of products as having a *trans* arrangement, with respect to the ethyl side chain and the original phenolic system across the double bond.[244,329] These results were rationalised by considering that the aromatic ring and side chain of the Z-isomer are sandwiched between two other rings and experience a double shielding effect from their ring currents. The NMR peak assignment/ analysis allowed the prediction of the major species in Type II flexible analogues (81)-(85) as Z-isomers. Spectral isomeric assignment ratios were based on relative peak heights of the benzyl signal in the ^1H NMR spectra, whereas isomeric assignment was based on the positioning of the aromatic signals.

Details of yield, infrared and isomeric data are listed in Table 4. High resolution mass spectrometry results positively confirm the identity of these novel compounds (75)-(85).

Compound	Yield %	IRυ_{max} (film cm^{-1})	Isomeric Ratio[a]
(81)	69	1605, 1574 (C=C)	4:1
(82)	43	1601, 1575 (C=C)	6:5
(83)	75	1602, 1576 (C=C)	2:1
(84)	80	1600, 1575 (C=C)	2:1
(85)	41	1600 (C=C)	2:1

[a] Ratio determined as major : minor present Z isomer : E isomer, based on ^{1}H NMR assignment and theoretical reaction predictions

Table 4: Yield, infrared and isomeric data for compound (81)-(85)

In the ^{1}H NMR of 1-benzyl-1-[(4-morpholinylethoxy)phenyl]-2-phenylbut-1-ene (85), the methyl H-4 protons are observed as a triplet (J=7.5Hz) at δ0.98, while the corresponding methylene H-3 protons are observed as a quartet (J=7.5Hz), at δ1.73. Both the NCH_2 and OCH_2 protons are found as multiplets at δ2.84 and δ4.12 respectively. The benzyl CH_2 group exists as at δ4.25 and δ4.27, the peak heights of which gave the Z and E isomeric ratio of the compound. The morpholinyl protons appear as multiplets at δ3.02 and δ3.61, corresponding to H-1"", H-4"" and H-2"", H-3"" respectively, each multiplet integrating for four protons. The partially shielded H-3", H-5" appear as double doublets (J=8.5, 1.5Hz) at δ6.83-6.86 and the corresponding H-2", H-6" are found as double doublets (J=8.5, 1.4Hz) at δ7.97-7.99. All remaining aromatic protons are observed in the range δ6.93-7.58, the Z-isomeric protons lying between δ6.93-7.20 whereas the E-isomer signals are observed between δ7.25-7.58.

In the ^{13}C NMR of (85), the methyl C-4 is observed upfield at 7.05ppm, while the corresponding methylene C-3 is found inverted in the DEPT 135 at 27.23ppm. The aminoethoxy NCH_2 and OCH_2 signals, which were also found inverted in the DEPT 135, occurred at 57.25 and 65.61ppm respectively. The benzyl methylene CH_2 signal is observed inverted in the DEPT 135 at 44.24 and 43.95ppm. The morpholinyl signals appear inverted at 53.62ppm, corresponding to the C-1"", C-4"" and at 66.43ppm representing the C-2"", C-3"". The aromatic signals are observed, between 113.89-130.49ppm.

In the high resolution mass spectrum of (85), the molecular ion is observed at m/z 427.2499 ($C_{29}H_{33}NO_2$) in 73% abundance.

2.4.3. Preparation of Type III flexible analogues

A series of 1-benzyl-1,2-diphenylbut-1-enes (Type III) [Figure 41] were prepared using the McMurry coupling reaction, possessing an additional methylene group on one of the phenyl rings in an alternative position to those already prepared. The alklyamino group was therefore found in a different position.

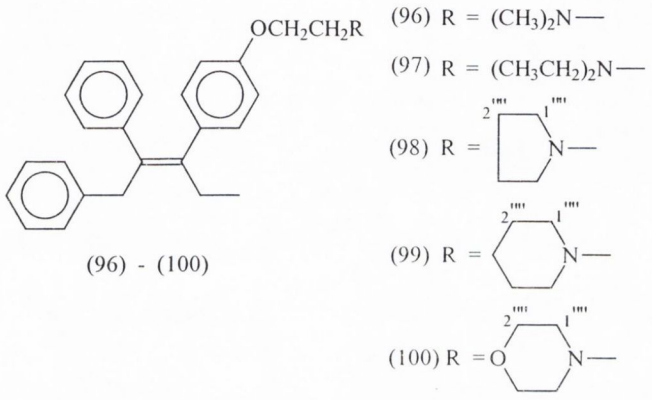
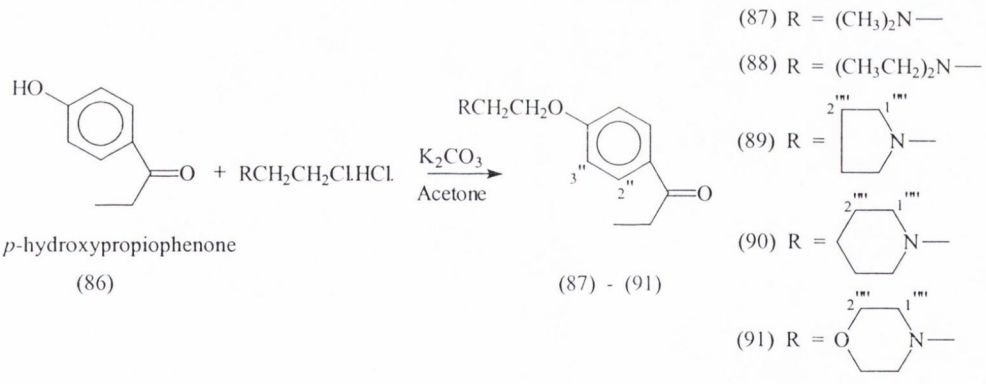


Figure 41: 1-Benzyl-2-[(4-alkylaminoethoxy)phenyl]-1-phenylbut-1-enes

The synthesis of the Type III compounds involved firstly the preparation of *p*-alkylaminoethoxy propiophenones,[328] which were synthesised from *p*-hydroxy propiophenone and the corresponding alkylating agent under basic conditions to yield compounds (87)-(91). [Scheme 24]



Scheme 24: Synthesis of p-alkylaminoethoxy propiophenones

Positive identification of the products was obtained from spectroscopic data. The *p*-alkylaminoethoxypropiophenones were obtained as oily gels in moderate to good yields. The yield and infrared data of these compounds (87)-(91) are detailed in Table 5.

Compound	Yield %	IRν_{max} (film cm^{-1})
(87)	83	1679 (C=O)
(88)	93	1661 (C=O)
(89)	91	1680 (C=O)
(90)	64	1675 (C=O)
(91)	85	1679 (C=O)

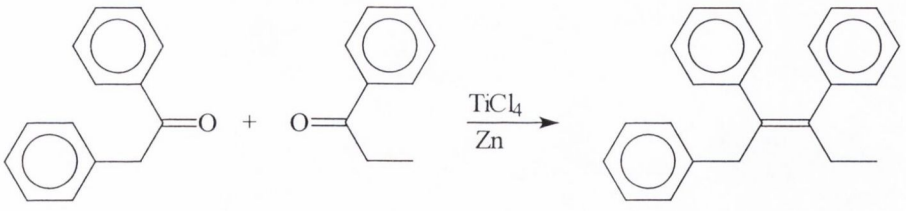
Table 5: Yield and infrared data for compounds (87)-(91)

In the ^1H NMR spectrum of *p*-pyrrolidinylethoxy propiophenone (89) the methyl CH_3 protons are observed as a triplet (J=7.3Hz) at δ1.21 and the corresponding methylene CH_2 protons are observed in the same region as NCH_2 as a multiplet between δ2.91-2.98. The OCH_2 proton signals occur as a triplet (J=5.8Hz) at δ4.20. The pyrrolidinyl protons, are observed as multiplets at δ1.84 and δ2.08, corresponding to H-2"", H-3"" and H-1"", H-4"" respectively. The H-3", H-5" protons are shielded by the substituent at the 4"-position and occur as a double doublet (J=8.5, 2.0Hz), at δ7.92-7.94.

The high resolution mass spectrum of (89) showed a molecular ion M^+ at 247.1572 ($C_{15}H_{21}NO_2$) in 85% abundance.

2.4.4. McMurry coupling reaction yielding symmetric products [Type III]

Prior to coupling the alkylsubstituted propiophenones with desoxybenzoin to afford 1-benzyl-2[(4-alklaminoethoxy)phenyl]-1-phenylbut-1-enes (96)-(100),[328] propiophenone (80) itself was reacted with desoxybenzoin (92) in a 1:1 ratio via the McMurry reaction. [Scheme 25]



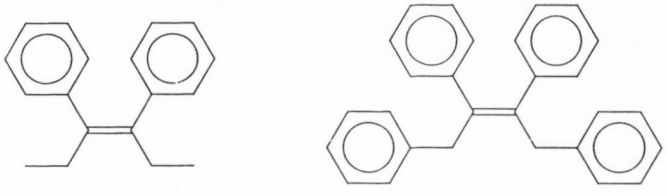
Desoxybenzoin (92) | *p*-propiophenone (80) | (93)

Scheme 25: Preparation of 1-benzyl-1,2-diphenylbut-1-ene (93) [Z-isomer illustrated]

The resulting 2-benzyl-1,2-diphenylbut-1-ene (93) was afforded as colourless oil in 75% yield. The characteristic aromatic and aliphatic C=C stretches were observed at υ1599, 1576 cm^{-1} in the infrared spectrum.

In the ^{1}H NMR the methyl H-4 is observed as a triplet (J=7.4Hz) at δ1.06 and the corresponding methylene H-3 is found as a quartet (J=7.5Hz) at δ2.74. The benzyl methylene CH_2 protons occur as a singlet, at δ4.03 and 4.11 and the isomeric ratio of compound (93) is obtained from the peak heights of this benzyl group. The chemical shifts of the phenyl rings of tamoxifen indicate that Z-tamoxifen aryl protons are found up-field in comparison to the E-tamoxifen aromatic signals which are observed further down field. Using this rational the aryl protons of compound (93) were assigned. The aromatic protons are observed as a complex multiplet between δ6.96-7.31 with the Z-isomeric signals observed between δ6.96-7.10 and the E-isomeric protons between δ7.14-7.31. The ^{1}H NMR spectrum indicate the presence of one main isomer, the isomeric ratio being recorded as 13:1, the Z-isomer being the major product. Using high resolution mass spectrometry, the molecular ion for (93) was obtained at 298.1751 ($C_{23}H_{22}$).

Together with the main product being produced in the reaction, two other symmetric by-products (94) and (95) were isolated in an isomer ratio of 5:1, the Z-isomer being the major product. [Figure 42]. This was confirmed by the use of spectroscopic data from 3,4-diphenyl-hex-3-ene, which was prepared by Besancon *et al.*[332] They noted that the Z-isomer is the major product from the ^{1}H NMR spectra, where the aryl protons for the Z-isomer were found at δ6.99 and those of the E-isomer at δ7.28. Similar trends were observed for compound (95).



(94) (95)

Figure 42: Compounds (94), (95) [Z-isomer illustrated]

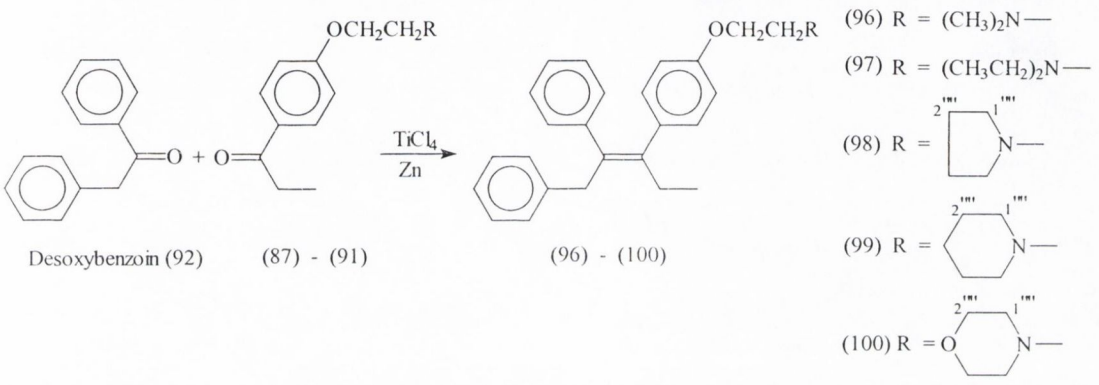
The 3,4-diphenylhex-3-ene (94) and 1,2-dibenzyl-1,2-diphenylethene (95) by-products were afforded in 14% and 5% yield respectively. These products arose from self-coupling of desoxybenzoin (92) and self-coupling of propiophenone (80). The identity of these compounds was confirmed using ^{1}H and ^{13}C NMR along with high resolution mass spectrometry.

The ^{1}H NMR spectrum of (95) shows the benzylic methylene protons (CH_2) as a singlet at δ4.08, integrating for four protons. The remaining aromatic protons are found as a complex multiplet in the range δ7.00-7.28. The high resolution

mass spectrometry of compound (95) showed the molecular ion m/z at 360.1878 ($C_{28}H_{24}$).

2.4.5. *Preparation 1-benzyl-2-[(4-alkylaminoethoxy)phenyl]-1-phenylbut-1-enes [Type III]*

The *p*-(alkylamino)propiophenones (87)-(91) prepared in Section 2.4.2, were coupled with desoxybenzoin (92), under suitable conditions [$TiCL_4$/Zn in a 1:1 ratio] affording the novel synthesised compounds (96)-(100)[328] as oils in only moderate yields [Scheme 26].



Scheme 26: Preparation of 1-benzyl-2-[(4-alkylaminoethoxy)phenyl]-1-phenyl but-1-enes [Z-isomer illustrated]

These products were analysed using spectroscopic methods. The infrared spectra of (96)-(100) show a C=C alkene stretch in the region ν1606-1603cm^{-1} and ν1581-1574cm^{-1}, corresponding to the aromatic and aliphatic C=C.

The isomeric ratio of these compounds (96)-(100) could not be determined from the benzyl methylene (CH_2) group as this appears as a singlet in the ^{1}H NMR spectrum. Compound (96) appears to be produced as a single isomer the Z form, evidence for this is found from NMR data, where the methylene CH_2 group is observed as a singlet and the aromatic protons are found as a compact multiplet. The remaining compounds (97)-(100) exists in both the Z and E isomeric form and exhibit a 67% (Z) over (E) excess. Previous research has identified the propensity of phenolic ketones used in the coupling reaction to favour the formation of that product which has a *trans* assignment of the ethyl side chain relative to the original phenolic system across the double bond .[244,329]

The yield, infrared and isomeric data of compounds (96)-(100) are detailed in Table 6.

Compound	Yield %	IRυ_{max} (film cm^{-1})	Isomeric Ratio[a]
(96)	25	1605, 1576 (C=C)	Z-isomer apparent
(97)	35	1606, 1574 (C=C)	5:1
(98)	25	1603, 1580 (C=C)	5:1
(99)	40	1605, 1581 (C=C)	5:1
(100)	29	1606, 1581 (C=C)	5:1

[a] Ratio determined as major : minor present Z isomer : E isomer, based on ^{1}H NMR assignment and theoretical reaction predictions

Table 6: Yield, infrared and isomeric data for compound (96)-(100)

In the ^{1}H NMR spectrum of (97) [Figure 43], the methyl H-4 protons are observed as a triplet (J=7.5Hz), and the corresponding H-3 protons are found as a multiplet in the same range as the $N(CH_2)_2$ protons, at δ2.68. This signal integrates for six protons, equivalent to the two protons from C-3 and the four protons from $N(CH_2)_2$. The $N(CH_3)_2$ protons of the diethyl group occur as a multiplet at δ1.14. The NCH_2 protons are observed as a triplet (J=6.3Hz) at δ2.90 and the corresponding OCH_2 protons are found at δ4.03 as a triplet (J=6.0Hz). The benzyl CH_2 protons exist as a singlet at δ3.98, integrating for two protons. The aromatic protons occur as a complex multiplet between δ6.66-7.31, the Z-isomeric aryl protons are found at δ6.93-7.05, while the E-isomeric aromatic protons are observed between δ7.16-7.31, (using the rational proposed for tamoxifens aryl protons).

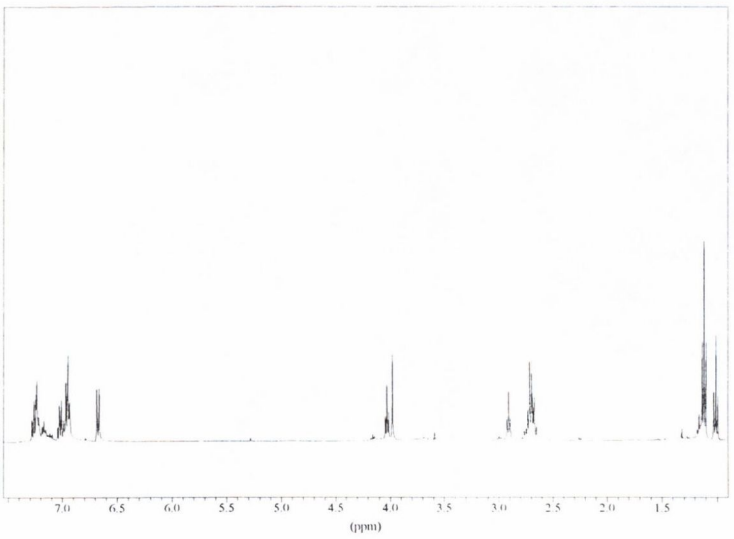


Figure 43: ^{1}H NMR Spectrum of compound (97)

In the ^{13}C NMR of (97), the methyl C-4 signal is characteristically observed upfield at 12.54ppm, and the $(CH_3)_2$ signal of the ethyl group is also found upfield at 11.06ppm. In the DEPT 135 spectrum, the benzyl CH_2 signal is found at 39.62 and 40.02ppm (representing the E and Z isomers) and the $N(CH_2)_2$ signal is observed at 47.31ppm. Two further inverted signals are observed in the DEPT 135 spectrum at 51.12 and 65.49ppm, and these are assigned to the NCH_2 and OCH_2 respectively. The shielded C-3'' and C-5'' are found at 113.18 and 113.79ppm. The remaining aromatic signals occur between 124.10-139.62ppm, with the exception C-4''' which is observed at 156.27ppm.

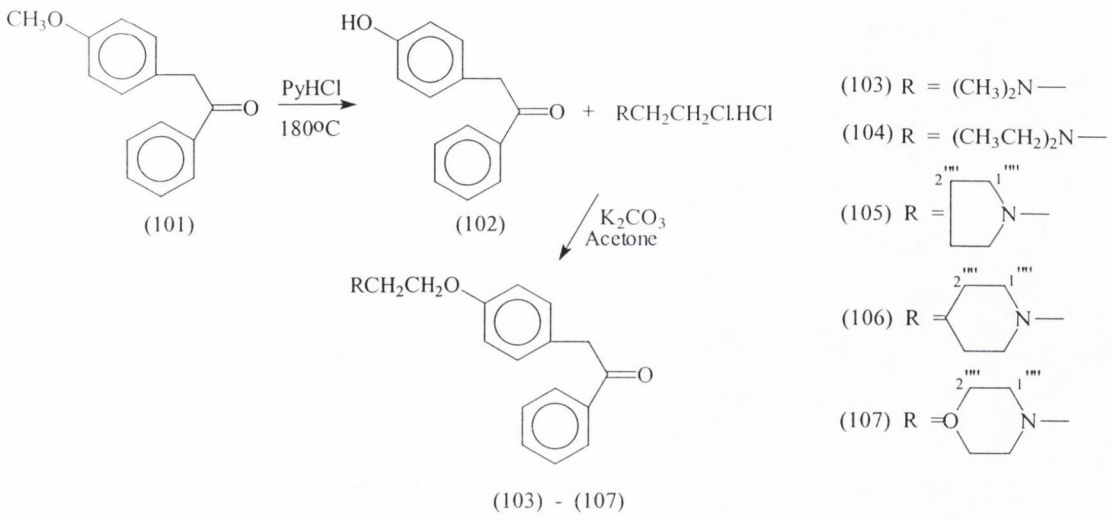
In the high resolution mass spectrum of (99), the molecular ion is observed at m/z 425.2703 ($C_{30}H_{35}NO$) in 72% abundance.

2.4.6. Preparation of 2-[4-(alkylaminoethoxy)benzyl]-1,2-diphenylbut-1-ene [Type IV]

The fourth series of compounds which were prepared possessed the alkylaminoethoxy substituent on the benzyl ring. These compounds were obtained by firstly preparing the appropriate 2-(alkylaminoethoxyphenyl)-1-phenylethanones,[328] which were subsequently coupled to propiophenone via implementation of the titanium tetrachloride / zinc (1:1 ratio) mediated McMurry coupling reaction.

2.4.6.1 Preparation of phenylethanones

The novel ketones (103)-(107)[328] were synthesised from previously prepared 2-(4-hydroxyphenyl)-1-phenylethanone (102). 2-(4-methoxyphenyl)-1-phenyl ethanone (101) was demethylated using pyridinium hydrochloride to form 2-(4-hydroxyphenyl)-1-phenylethanone (102). The synthetic route used for the introduction of the alkyl basic side chain to the hydroxy ketone is outlined in Scheme 27.



Scheme 27: Preparation of phenylethanones

The 2-(4-alkylaminoethoxyphenyl)-1-phenylethanones (103)-(107) were afforded as oils, in good yield (85-92%). The characteristic carbonyl (C=O), stretch is found at ν1670-1654cm^{-1}. The yield and infrared data of (103)-(107) are listed in Table 7.

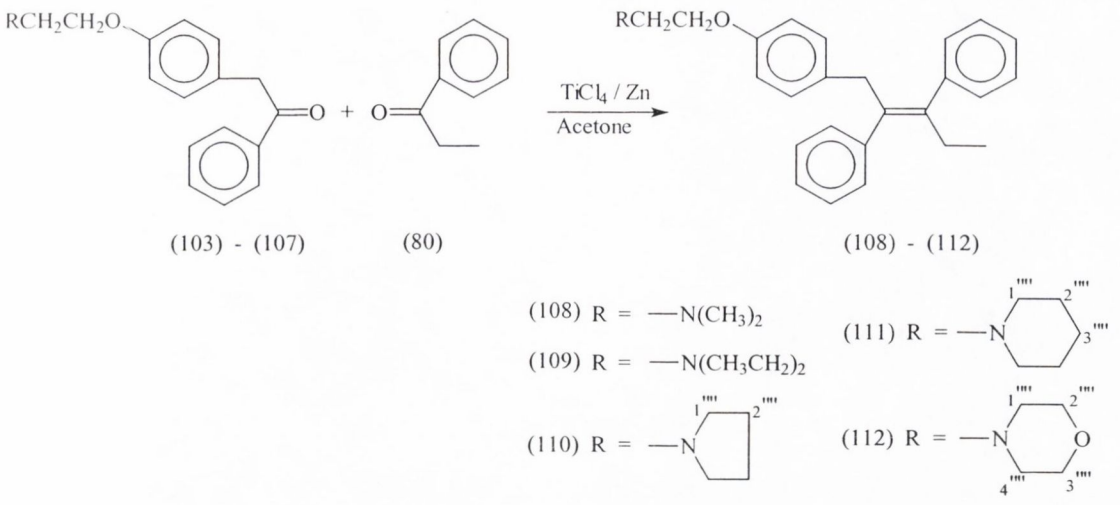
In the ^{1}H NMR spectrum of 1-phenyl-2-(pyrrolidinylethoxyphenyl)ethanone (105), the benzyl methylene (CH_2) protons are observed as a singlet at δ3.64. The NCH_2 protons are found as a triplet (J=6.0Hz) at δ2.73 while the OCH_2 protons are found as a triplet (J=6.2Hz) at δ4.24. The pyrrolidinyl protons are found as multiplets at δ1.76 and δ2.53, corresponding to H-2"", H-3"" and H-1"", H-4"" respectively. The aromatic protons were found between δ6.93-7.99. The identity of compounds (103)-(107) were confirmed using High Resolution Mass Spectroscopy.

Compound	Yield %	IRυ_{max} (film cm^{-1})
(103)	90	1670 (C=O)
(104)	90	1654 (C=O)
(105)	91	1670 (C=O)
(106)	92	1664 (C=O)
(107)	85	1654 (C=O)

Table 7: Yield and infrared data for compounds (103)-(107)

2.4.6.2 McMurry coupling reaction of phenylethanones

The novel 2-[(4-alkylaminoethoxy)benzyl]-1,2-diphenylbut-1-enes (108)-(112)[328] compounds were prepared by coupling the phenylethanones (103)-(107) with propiophenone using the McMurry reaction [Scheme 28].



Scheme 28: Preparation of 2-[(4-alkylaminoethoxy)benzyl]-1,2-diphenylbut-1-ene (108)-(112) [E-isomer illustrated]

The novel compounds (108)-(112) were obtained in only moderate yields as oils. The characteristic aromatic and aliphatic C=C stretching is observed in the range 1606-1574cm^{-1}. The stereospecificity of this McMurry reaction for synthesis of tamoxifen and related analogues identifies the propensity of phenolic ketones used in the coupling reaction to favour the formation of that product which has a *trans* arrangement across the double bond of the ethyl side chain relative to the original phenolic system.[244,329] This literature data in conjunction with NMR peak assignment/analysis allowed the prediction of the major species in type IV flexible analogues (108)-(112) as E-isomers due to the positioning of the ethyl side chain relative to the oxygen group. The isomeric ratios are determined either from the relative peak heights of the benzyl CH_2 or from the relative positions of those aryl proton signals arising from A_2B_2 *para* system of the 4-substituted phenyl ring.[329] In this series the E-isomeric excess ranges from 9-80%. Details of yields, infrared and isomeric data are listed in Table 8.

Compound	Yield %	IRν_{max} (film cm^{-1})	Isometric Ratio[a]
(108)	21	1605, 1576 (C=C)	6:5
(109)	40	1605 (C=C)	9:1
(110)	19	1602, 1576 (C=C)	6:5
(111)	39	1601, 1586 (C=C)	6:1
(112)	29	1600 (C=C)	3:1

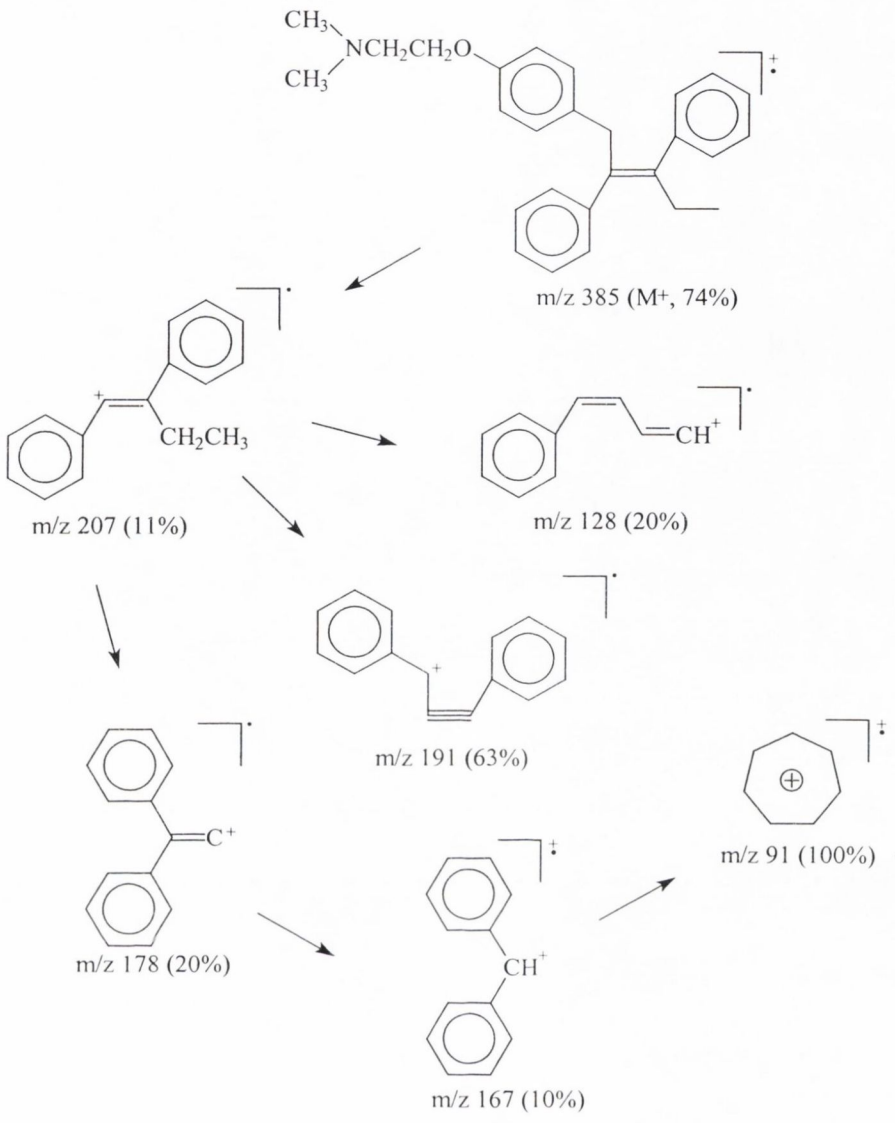
[a] Ratio determined as major minor present E isomer : Z isomer, based on ^{1}H NMR assignment and theoretical reaction predictions

Table 8: Yield, infrared and isomeric data for compound (108)-(112)

In the ^{1}H NMR spectrum of 1,2-diphenyl-2-[(4-piperidinylethoxy)benzyl]but-1-ene (111), the methyl H-4 protons are observed at δ1.01 as a triplet (J=7.5Hz), while the adjoining methylene H-3 protons are found as a quartet (J=7.5Hz) at δ2.58. The benzyl CH_2 protons occur as a singlet at δ3.63 and δ3.67, corresponding to the E/Z isomers, while the NCH_2 and OCH_2 are found as multiplets at δ3.03 and δ4.30 respectively. The piperidinyl protons are all observed as multiplets the H-3"" at δ2.07, the H-2"", H-4"" protons at δ2.31 and H-1"", H-6"" protons as a doublet (J=9.0Hz) at δ7.92-8.00, while the remaining aromatic protons are found in the range δ6.79-7.18. The rational used for predicting the E/Z isomeric ratio of tamoxifen was used for compound (111), where the aromatic protons for the Z-isomer were observed between δ6.96-7.04, while those for the E-isomer were found at δ7.10-7.18.

In the ^{13}C NMR of (111) the C-4 methyl signal is characteristically observed upfield at 13.61ppm, while the adjoining C-3 is found inverted in the DEPT 135 at 31.45ppm. The methylene benzyl group is found at 40.91 and 41.31ppm, providing evidence for the presence of both isomers. The aminoethoxy NCH_2 and OCH_2 signals are also found inverted in the DEPT 135 at 56.08 and 61.07ppm respectively. The remaining inverted signals from the DEPT 135 spectrum are assigned to the piperidinyl carbons; C-3"" is found at 23.11ppm, C-2"", C-4"" are observed at 26.81 and 27.22ppm, while the signal at 53.66ppm is assigned to C-1"", C-5"". The remaining aromatic signals are observed between 124.94-129.30ppm, with the exception of the shielded C-3", C-5" signals which are observed at 115.10 and 115.24ppm.

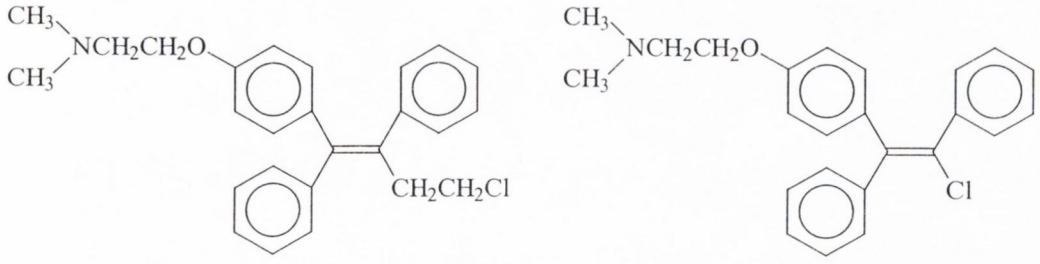
In the high resolution mass spectrometry a molecular ion (M^+) at 385.2409 ($C_{27}H_{31}NO$) was obtained in 74% abundance for compound (108). The base peak corresponds to the tropylium ion at m/z 91. The mass fragmentation pattern of (108) is seen in Scheme 29. Cleavage from the molecular ion of the alkylsubstituted benzyl group affords m/z 207 (11%). Further cleavage results in the fragments m/z 178 (20%) and m/z 167 (10%).



Scheme 29: Postulated mass spectral fragmentation pattern for compound (108)

2.5. Preparation of 1-benzyl-1-[(4-dimethylaminoethoxy)phenyl]-2-phenylbut-1-enes (Type V)

Toremifene (3) and Clomiphene (5) are two well-known antiestrogens, with structures only deviating slightly from that of tamoxifen. Toremifene[333] is a chlorinated derivative (2-chloroethyl chain) of tamoxifen and Clomiphene[158] posses a chlorine atom in place of the ethyl group of tamoxifen. [Figure 44]. Toremifene has also been synthesised via the McMurry coupling reaction and Clomiphene is used as the starting material for the preparation of *N,N*-diethylfluoroethyl tamoxifen.[159]

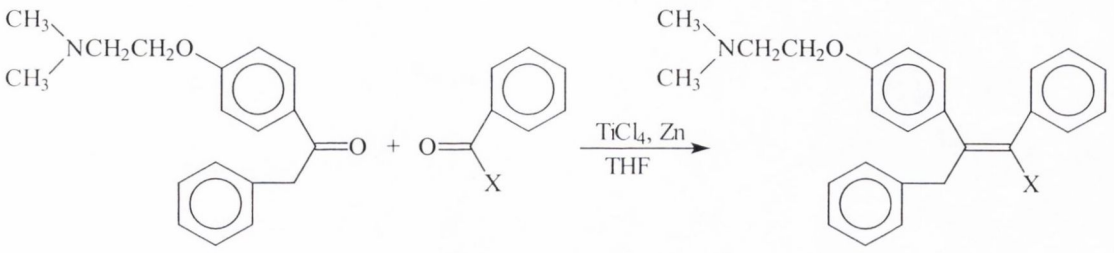


(3) (5)

Figure 44: Structure of Toremifene (3) and Clomiphene (5)

Due to the success of tamoxifen compounds modified at the ethyl group in the treatment of breast cancer, the introduction of halogens or other modifications on the ethyl moiety might increase antiestrogenic activity. Much work has been done on preparing a variety of ethyl side chain derivatives of tamoxifen analogues. Hydroxylation of the ethyl group of tamoxifen was carried out by Fromson *et al.*,[81] while a variety of chloro[158,333] bromo,[283] fluoro and iodo[161] groups have been introduced into the ethyl side chain. The relative binding affinities and the inhibitory profiles of these compounds have been studied and are shown to have an antiestrogenic effect similar to tamoxifen.

In order to modify the structures of the novel compounds (81)-(85), i.e. 1-benzyl-1-[(4-alkylaminoethoxy)phenyl]-2-phenylbut-1-enes (Type II) the products (120)-(126) were prepared, having the same structure as Type II [(81)-(85)] analogues, but differ in the ethyl function [Scheme 30] The functional groups included halo-, nitro- and extended ethyl side chains.



(75) (113) - (119) (120) - (126)

(120) X = $CH_2CH_2CH_2CH_3$ [Z]
(121) X = CH_2CH_2Cl [Z]
(122) X = CH_2Br [E]
(123) X = $CHBrCH_3$ [E]
(124) X = CH_2NO_2 [Z]
(125) X = $CH_2CH_2CH_3$ [Z]
(126) X = CH_2Cl [Z]

Scheme 30: Preparation of Compounds (120)-(126) [Z and E-isomer illustrated]

These compounds were prepared using 1-(4-dimethylaminoethoxy)phenyl-2-phenylethanone (75) and various substituted propiophenones (113)-(119), via the McMurry coupling reaction. Some functionalised ketones have previously been coupled via the McMurry coupling reaction, including chlorinated derivatives i.e. toremifene and long alkylated side chains. Additional ketones which have been used in coupling reactions include; dimesityl ketones, diferrocenyl ketones, methyl ferrocenyl ketones, cholestanones and cyclooctanones. Reductive coupling of these compounds have produced products with various isomer ratios. The Type V novel compounds prepared in this work possess some functionalised side chains which have not yet been researched, these include the nitro- and brominated ketones.

These compounds (120)-(126) were afforded as oily gels in only moderate yield. The characteristic C=C stretch was observed in the range υ1607-1574cm^{-1}. Using ^{1}H NMR, ^{13}C NMR and high resolution mass spectrometry, the identity of these compounds was confirmed. The effects of the various substituents on the ethyl side chain were observed and it was found that compounds (122) and (123) are prepared in mainly the E-isomer. The remaining compounds are mainly present in the Z-isomeric form. These isomeric preditions were confirmed by ^{1}H NMR and HPLC data where tamoxifen was used as a reference. Details of yields, infrared and isomeric data are listed in Table 9.

Compound	Yield %	IRυ_{max} (film cm^{-1})	Isomeric Ratio[a]
(120)	48	1605, 1573 (C=C)	2:1
(121)	41	1605, 1574 (C=C)	1:1
(122)	39	1605 (C=C)	1:9
(123)	34	1605 (C=C)	1:6
(124)	42	1605 (C=C)	11:1
(125)	46	1603,1582 (C=C)	2:1
(126)	44	1607,1581 (C=C)	2:1

[a] Ratio determined as major : minor present Z isomer : E isomer, based on ^{1}H NMR and HPLC assignment and theoretical reaction predictions

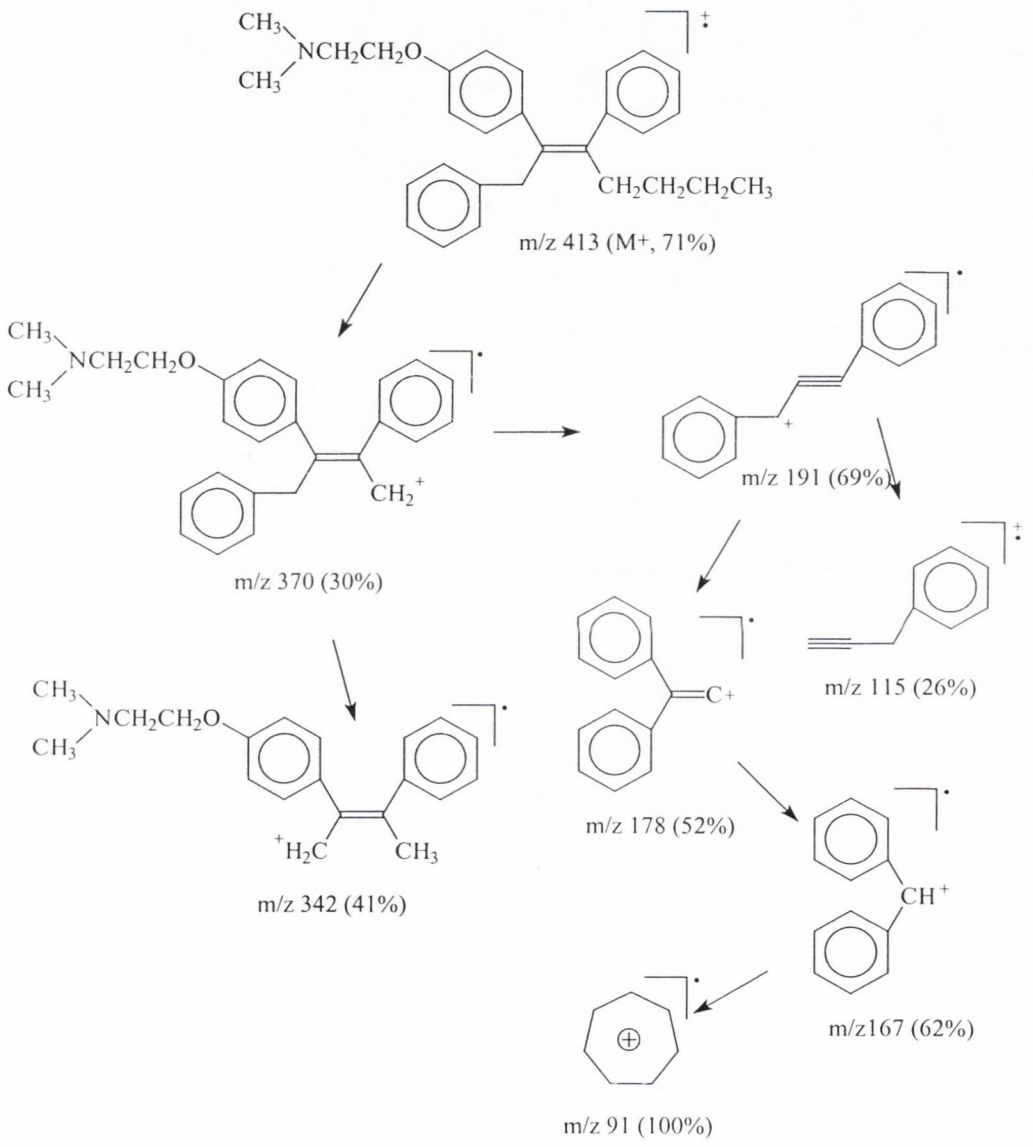
Table 9: Yield, infrared and isomeric data for compound (120)-(126)

Known tamoxifen analogues which possess modified chlorinated ethyl side chains have been prepared by the McMurry coupling reaction, and the stereospecificity of this method has been researched for other related tamoxifen analogues. Therefore this literature data in conjunction with the NMR and HPLC peak assignment/analysis allowed the prediction of the major species in Type V as Z-isomers as illustrated, when compared to the isomeric peaks of tamoxifen.

In the ^{1}H NMR of 1-benzyl-3-bromo-1-[(4-dimethylaminoethoxy)phenyl)]-2 phenylbut-1-ene (123), the methyl CH_3 protons are found as a doublet (J=5.0Hz), at δ2.35, while the adjoining CH(Br) proton is observed as a multiplet at δ2.79. The dimethyl $N(CH_3)_2$ protons occur as a singlet at δ2.40, while the NCH_2 and OCH_2 occur at δ2.70 and δ4.11, as triplets (J=6.0Hz and J=6.0Hz) respectively. The benzyl methylene (CH_2) protons are found as a singlet at δ3.97 and a small peak is observed at δ3.95, indicating the presence of the minor Z-isomer. This therefore confirms the isomeric ratio of 9:1. The substituted aromatic ring and the side chain of the Z-isomer are sandwiched between two other rings and experience a double shielding effect from their ring currents. The aromatic protons are observed as a multiplet in the range δ6.57-7.34, where the Z-isomeric signals are found between δ6.57-6.87 and the E-isomer protons are present at δ7.05-7.34.

In the ^{13}C NMR, the CH signal is observed upfield at 9.47ppm, with the corresponding CH_3 signal found at 12.94ppm. A signal at 47.72ppm was assigned to the $(CH_3)_2$ group and NCH_2 was found at 58.18ppm. The OCH_2 signal is observed at 65.55 and 65.58ppm confirming the presence of the E and Z isomers. The benzyl CH_2 signal occurs at 39.60 and 39.99ppm signifying the presence of both the E and Z isomers. The shielded C-3", C-5" signals are

observed at 113.45 and 114.05ppm, while the corresponding C-2", C-6" are found at 128.19ppm. The quaternary C-1' is found at 130.79ppm, and the C-1" and C-1'" are observed at 134.19 and 139.69ppm respectively. The remaining aromatic signals are observed in the range 127.52-129.76ppm, with the exception of C-4', C-4" which occur at 125.59ppm and the C-4'" signal which is found at 127.19ppm.



Scheme 31: Postulated mass fragmentation pattern for (120)

In the low resolution mass spectrum of (120), ($C_{29}H_{35}NO$) the molecular ion is observed at m/z 413 in 73% abundance. The fragmentation pattern of (120) is outlined in Scheme 31. Cleavage of the benzyl ring results in m/z 342 (41%) and also cleavage of C_3H_7 affords the fragment m/z 370 (30%). Further cleavages results in fragments m/z 115 and the tropylium ion m/z 91 (100%).

The isomeric ratios of these novel compounds were confirmed using HPLC (High Performance Liquid Chromatography). The chromatographic conditions used were those outlined in the British Pharmacopoeia 1998 for tamoxifen. The mobile phase consisted of a mixture of acetonitrile (300mL), water (125mL), THF (75mL) and 18M ammonia (2mL), with a flow rate of 1.5mL per minute and a detection wavelength of 240nm. The product samples (120)-(126) [25mg] were dissolved in the mobile phase [10mL]. The stationary phase was packed with octadecylsilyl silica gel C (5μm).

The results found using HPLC analysis, i.e. percentage of each isomer present are compared to the isomeric ratios obtained from the ^{1}H NMR.

Further compounds may be prepared via the McMurry coupling reaction, due to the success of the synthesis of Type V analogues. These compounds are predicted to have a high affinity for the estrogen receptor, as the halogenated and alkylated side chains are believed to chelate to a tumour cell causing cell death.[159,161]

2.6. Summary of Type I – V compounds

In summary, a series of novel antiestrogens structurally derived from the known antiestrogen tamoxifen were prepared via two different synthetic routes a seven step route and a two step McMurry reaction. Each of these compounds deviated from the traditional triphenylethylene backbone associated with common antiestrogens through the introduction of a methylene (benzylic) spacing group between one of the aryl rings and the ethylene group, and through variations in the basic side chain moiety. Thus, four various types of novel compounds were prepared, each possessing five different side chain moieties on one of the aryl rings.

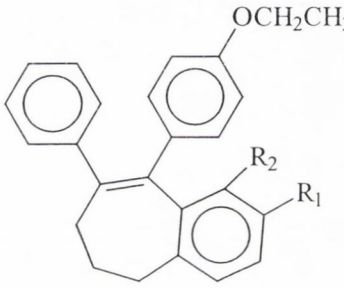
The antiproliferative effects of Type I – Type V compounds on human MCF-7 breast cancer tumour cells were investigated, along with their binding affinity for the estrogen receptor, the results of which will be discussed in Chapter 4. The biochemical evaluation of Type II compounds (81)-(85) indicated that these were the most active. This therefore initiated the preparation a series of (Type V) compounds where the ethylene function was altered.

3. Synthesis of non-isomerisable analogues of Tamoxifen

3.1. Introduction.

The synthesis of a number of tamoxifen-like analogues possessing a similar carbon skeleton to tamoxifen but contain an additional benzylic methylene group adjacent to the ethylene function is described in Chapter 2. These compounds existed however as a mixture of geometric isomers which could not be readily separated into the E or Z form. Thus it was decided to investigate the synthesis of a number of related, conformationally restricted cyclic analogues of tamoxifen.

A number of non-isomerizable analogues of tamoxifen with antiestrogenic activity have been reported. McCague *et al.*,[87,179] have prepared a series of non-isomerizable analogues (127)-(129) utilising benzosuberone as the template. These compounds mimic the structure of Z-tamoxifen. [Figure 45].



(127) $R_1 = H$, $R_2 = H$

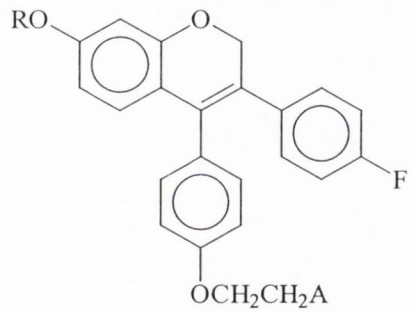
(128) $R_1 = OH$, $R_2 = H$

(129) $R_1 = OH$, $R_2 = Me$

Figure 45: Structure of substituted diarylbenzocycloheptenes (127)-(129)

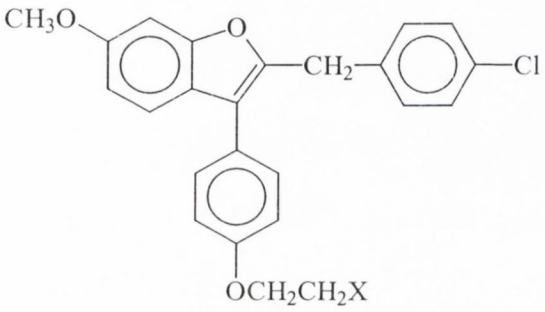
X-Ray crystallography studies revealed that the seven membered rings provide these compounds with stereochemical features very similar to those of tamoxifen, with the aryl rings having a propellor-like conformation. McCague reported that these compounds have similar biological properties to tamoxifen, indicating that they would be a good substitute for tamoxifen in studies where possible isomerism, would cause complications.[179] Other five[334,335,336] and six membered[87,337] ring restricted derivatives have been synthesised, but their effectiveness is limited, possessing much reduced activity compared to the seven-membered analogues.

Teo *et al.*,[239] synthesised a number of arylchrom-3-ene compounds (130)-(134).



(130) R = H, A = $N(CH_2CH_2)_2$
(131) R = CH_3, A = $N(CH_2CH_2)_2$
(132) R = CH_3, A = $N(CH_2CH_3)_2$
(133) R = CH_3, A = $N(CH_2CH_2)_2CH_2$
(134) R = CH_3, A = $N(CH_2CH_2)_2O$

Pharmacological studies showed that these compounds displayed interesting antiestrogenic properties, where it was concluded that the chromene nucleus caused a profound decrease in binding to the estrogen receptor sites. A series of 2-(4-chlorobenzyl)-3-aryl-6-methoxybenzofurans (135)-(138)[338] were also investigated.



(135) X = $N(CH_3)_2$
(136) X = $N(CH_2CH_2)_2$
(137) X = $N(CH_2CH_2)_2CH_2$
(138) X = $N(CH_2CH_2)_2O$

These compounds were shown to possess a higher binding affinity for the estrogen binding sites than the corresponding tamoxifen derivatives.

Raloxifene[226] (28) and similar 2-(4-halogenobenzyl)-3-arylbenzo[b]-thiophenes have been prepared as selective ligands for antiestrogen-binding sites. Zhu *et al.*,[219] synthesised a 2-(4-fluorobenzyl)-3-arylbenzo[b]selenophene derived from chalcone as a part of a project to prepare non-isomerizable antiestrogens for structure-activity studies.

In this research a series of conformationally restricted non-isomerizable analogues were prepared displaying the general structure 2,3,4,5-tetrahydro-1H-benzoxepin-5-one [Figure 46]. These compounds are related to the overall tamoxifen triaryl structure, however the problems with E and Z isomeric mixtures are eliminated.

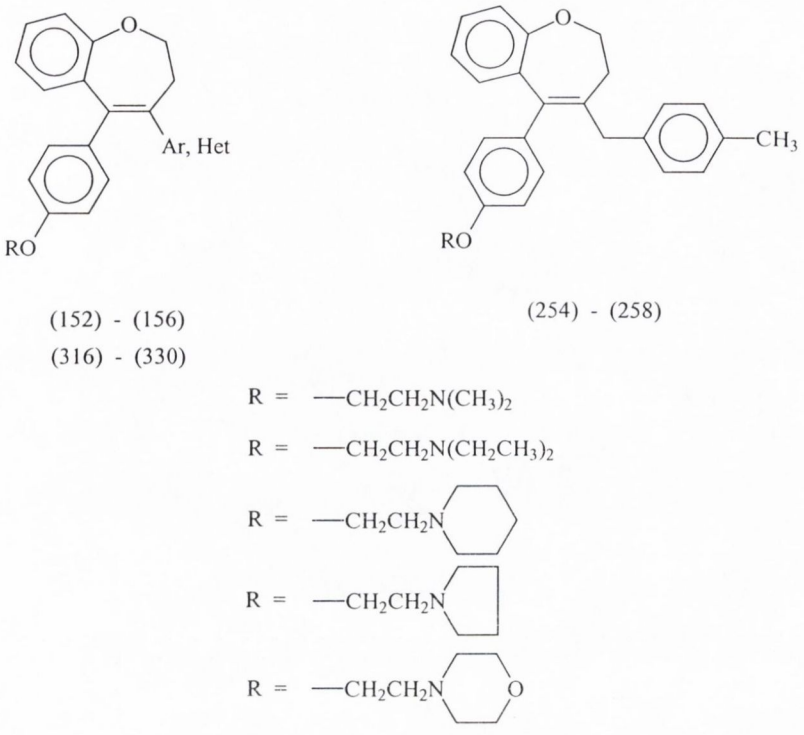


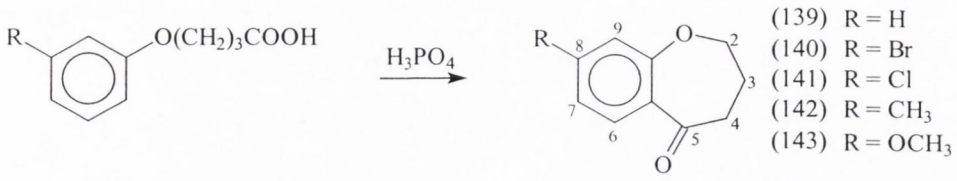
Figure 46: Proposed benzoxepin structure

These contain an oxygen atom within the seven membered ring system, which may afford an additional site for binding to the estrogen receptor. A series of compounds were synthesised, each possessing the benzo-fused seven membered ring system, some of which attain a similar structure to tamoxifen. Others incorporated an additional methylene substituent, these structures being related to the tamoxifen analogues synthesised by Teo and others.[219,239,338,339] The synthesis of these non-isomerisable compounds depends on a viable preparation of the 2,3,4,5-tetrahydro-1H-benzoxepin-5-one.

3.1.1. Synthesis of 2,3,4,5-tetrahydro-1-benzoxepin-5-one.

2,3,4,5-Tetrahydro-1-benzoxepin-5-one (139) was first synthesised by Powell and Anderson[340] in 1931, in poor yield, via cyclisation of 4-phenoxybutyric acid. Dann and Ardt[341] re-examined this approach in 1954 and found that (139) could be prepared quite satisfactorily by using polyphosphoric acid. The effectiveness of added xylene in polyphosphoric acid cyclisation of 4-phenoxybutyric acid and the conversion of 4-phenoxybutyric chloride into 2,3,4,5-tetrahydro-1-benzoxepin-5-one (139) under the influence of stannic chloride, were reported by Fontaine and Maitte[342,343] and Buckle.[344] Dann and Arndt[341] investigated the use of hydrogen fluoride as a reaction medium for the preparation of (139) and related compounds. The synthesis of several substituted 2,3,4,5-tetrahydro-1-

benzoxepin-5-ones have been reported, using the method proposed by Fontaine.[341,342,345] [Scheme 32]



Scheme 32: Preparation of 2,3,4,5-tetrahydro-1-benzoxepin-5-one

The method proposed by Fontaine however, had some limitations, one of which included the difficult work up of the benzoxepin, which in turn led to low yielding products. Attempts to improve earlier reported yields of (139), included using phosphoric oxide in methanesulphonic acid, polyphosphoric acid or sulphuric acid. Attempts to improve this method identified phosphoric oxide-celite (1:2)[346] as the optimal reaction conditions for cyclisation of 4-phenoxybutyric acid and allowed the formation of compound (139) in 75% yield.

In this research the method proposed by Tandon *et al.*, was used to prepare (139)[347,256] as mustard coloured syrup (oily gel) in 76% yield.

Positive identification of this product was obtained from spectroscopic data. The infrared spectrum gave the carbonyl ketone at $\nu 1689 cm^{-1}$, and the C-O-C ether stretch appeared at $\nu 1289 cm^{-1}$.

In the ^1H NMR spectrum of 2,3,4,5-tetrahydro-1-benzoxepin-5-one (139), the methylene protons at H-3 were observed as a multiplet at $\delta 2.56$, while the methylene protons at H-4 and H-2 were found as triplets (J=6.6Hz and J=7.0Hz), at $\delta 4.27$ and $\delta 2.93$ respectively. The aromatic protons occur in the region $\delta 6.95$-7.14 mainly as a multiplet.

3.2. Synthesis of benzoxepin analogues of tamoxifen.

3.2.1. Introduction

McCague[179,348] prepared an analogue of tamoxifen into which had been introduced sufficient rigidity to allow observation of individual enantiomeric atropisomers. The benzocycloheptene's fused ring is prevented from rotating completely out of plane due to the olefinic bond. The benzocycloheptenes attain the overall tamoxifen structure with the triphenyl groups and the ethylene function fused into the ring. [Figure 47].

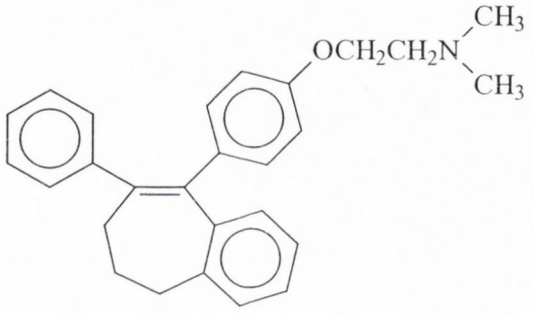
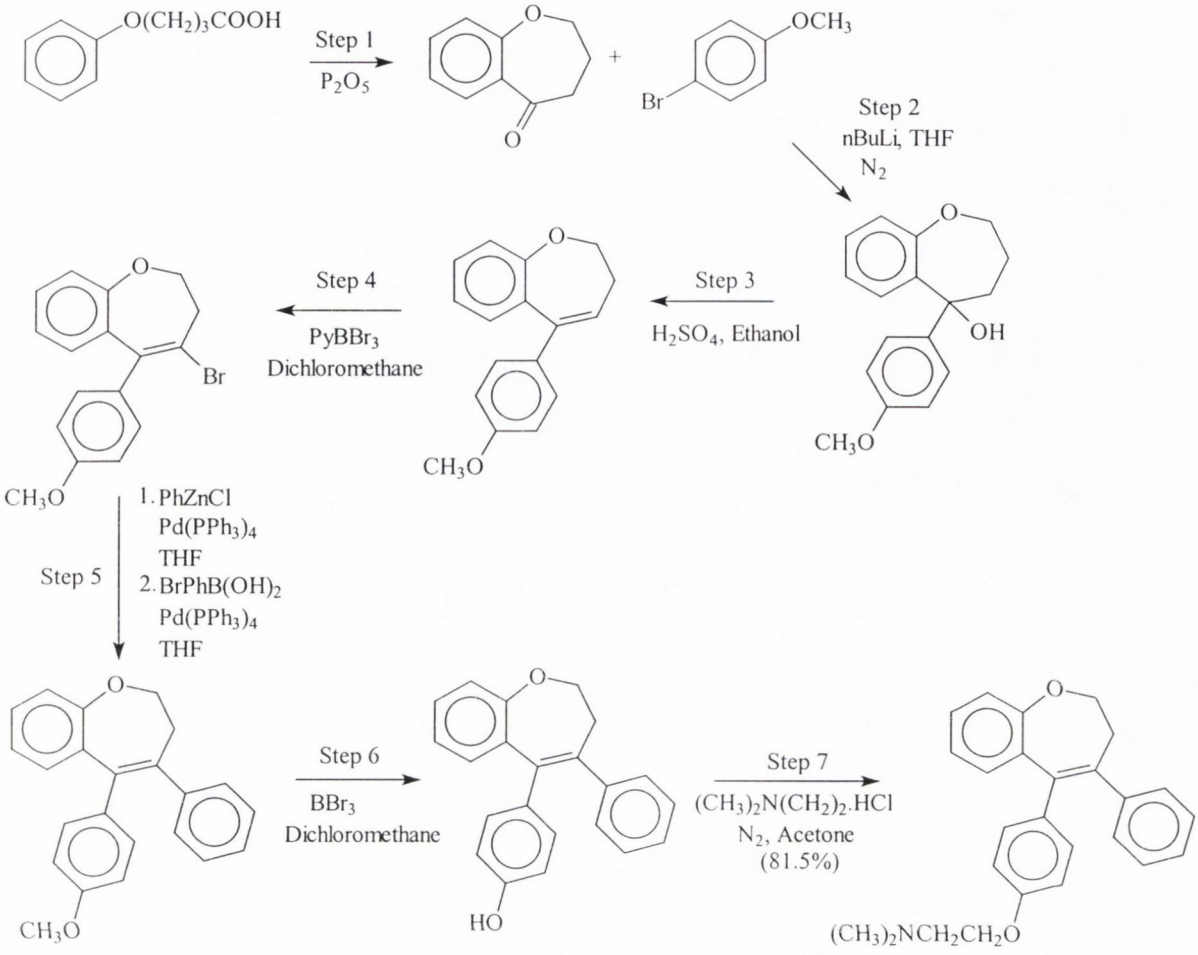


Figure 47: 1-methyl-8-phenyl-9-[4-(2-dimethylamino)ethoxy]phenyl-6,7-dihydro-5H-benzocycloheptene (144)

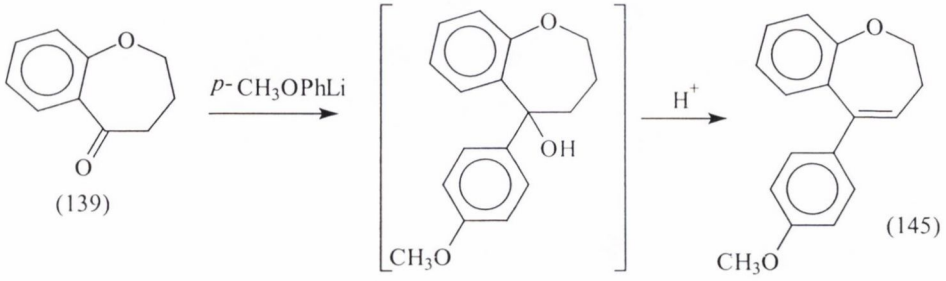
It was envisaged that similar compounds could be prepared with the 2,3,4,5-tetrahydro-1H-benzoxepin-5-one framework. The synthetic route for these benzoxepin analogues is outlined in Scheme 33 and involves seven steps.



Scheme 33: Synthetic route for 5-(4-dimethylaminoethoxyphenyl)-4-phenyl-2,3-dihydro-1-benzoxepin

3.2.2. Synthesis of 5-aryl-2,3-dihydro-1-benzoxepin.

5-(4-Methoxyphenyl)-2,3-dihydro-1-benzoxepin (145)[347] was synthesised from 2,3,4,5-tetrahydro-1-benzoxepin-5-one (139) with the use of the reagents *n*-butyllithium and 4-bromoanisole, under conditions described in the previous sections. The resultant tertiary alcohol product was dehydrated *in situ* using 85% polyphosphoric acid and was not isolated in this case. [Scheme 34].



Scheme 34: Synthetic route from (139) to compound (145)

5-(4-Methoxypheny)-2,3-dihydro-1-benzoxepin (145) was afforded as a colourless crystalline solid (m.p. 79-81°C) in 55% yield. The compound was positively identified using infrared, ^{1}H NMR, ^{13}C NMR spectroscopy and elemental analysis.

In the infrared spectrum of (145), the medium intensity alkene stretch occurs at ν1599cm^{-1} together with the aromatic C=C stretching at ν1571 cm^{-1}. The C-O-C ether stretch occurs at ν1287 and 1219cm^{-1}.

In the ^{1}H NMR spectrum of 2,3-dihydro-5-(4-methoxyphenyl)-1-benzoxepin (145), the H-3 protons resonated at δ2.50 as a multiplet (J=6.0Hz), being split by H-2 and H-4. H-2 is observed as a triplet (J=6.0Hz), being split by H-3, integrating for two protons at δ4.52, while H-4 is found somewhat downfield at δ6.28 as a triplet (J=6.3Hz) being split by the H-3 methylene protons. The aromatic H-3' and H-5' are found as a multiplet between δ6.88-6.90, somewhat upfield due to the shielding effects of the 4'-methoxy substituent which is found to resonate as a singlet at δ3.85. The H-6 and H-7 protons are observed as a multiplet between δ7.01-7.03, while the remaining aromatic protons resonate as a multiplet at δ7.11 -7.28.

In the ^{13}C NMR spectrum the 4'-methoxy signal is observed at 54.82ppm. Two signals appear inverted in the DEPT 135, at 29.45 and 77.28ppm, corresponding to C-3 and C-2 respectively. The shielded C-3', C-5' signal occurs at 113.10ppm, while the adjoining C-2', C-6' signal is found at 129.20ppm. The aromatic C-9 and C-7 signals are found at 121.37 and 122.66ppm, while those of C-8 and C-6 occur at 127.90 and 130.66ppm respectively. The quaternary C-5 and C-9a are observed at 132.49 and 157.46ppm, while the C-1' quaternary is found at 134.87ppm. Elemental analysis verified the molecular formula assignments of the compound (145).

3.2.3. Synthesis of 5-aryl-4-bromo-2,3-dihydro-1-benzoxepin.

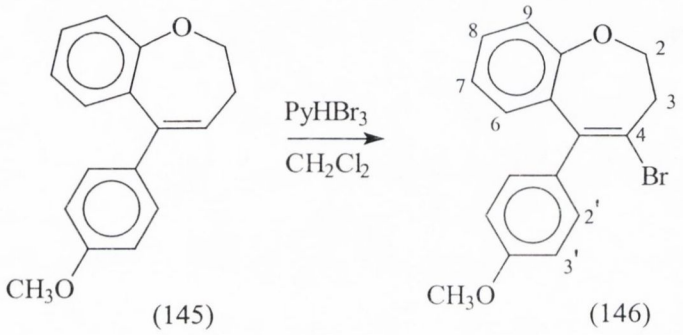
4-Bromo-5-(4-methoxyphenyl)-2,3-dihydro-1-benzoxepin (146) was synthesised via bromination of the accessible vinylic carbon at C-4.

3.2.3.1 4-Bromo-5-(4-methoxyphenyl)-2,3-dihydro-1-benzoxepin (146)

4-Bromo-5-(4-methoxyphenyl)-2,3-dihydro-1-benzoxepin (146)[347] was prepared from (145) using pyridine hydrobromide perbromide in dichloromethane at 0°C affording needle-like colourless crystals in 92% yield. [Scheme 35]

The substitution of an alkene hydrogen with a halogen has been reported using a series of reagents.[87,349,350,351,352] Pyridine hydrobromide perbromide is a convenient reagent for the addition of bromine to a double bond.[352,353,354]

Bromination studies of *cis* and *trans*-stilbene with bromine and pyridine bromide perbromide have shown that the latter reagent possesses a greater stereoselectivity than bromine.[353,355] Freedman and Dooakian[356] have reported a case for the use of pyridine bromide perbromide as a mild brominating reagent particularly suited to the tetraphenylchlorobutadiene, whereas nickel bromide complex and other compounds are prone to decomposition.



(145) (146)

Scheme 35: Synthesis of 4-bromo-5-(4-methoxyphenyl)-2,3-dihydro-1-benzoxepin (146)

The identity of this compound was confirmed using infrared, ^{1}H NMR and ^{13}C NMR spectroscopy. The elemental composition of compound (146) was positively confirmed using elemental analysis. In the infrared spectrum of (146), the broad aliphatic and aromatic C=C stretches are observed at ν1599 and ν1568cm^{-1}, while the ether C-O-C stretch is found at ν1286 and ν1202cm^{-1}.

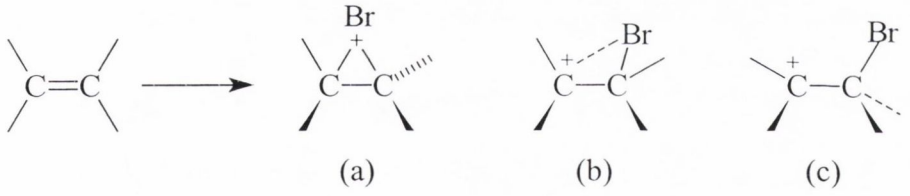
In the ^{1}H NMR spectrum of 4-bromo-5-(4-methoxyphenyl)-2,3-dihydro-1-benzoxepin (146) the 4'-methoxy substituent is observed as a singlet at δ3.86. The methylene H-3 protons are found as a triplet (J=5.9Hz), at δ3.04, being split by the adjoining methylene H-2 protons, which are found as a triplet (J=5.9Hz) at δ4.61. A double doublet (J=7.8, 1.7Hz) is observed at δ6.80-6.82, corresponding to H-6 while the deshielded H-9 is found as a doublet (J=8.3Hz) at δ7.09. The aromatic H-3', H-5' protons are found along with H-7 as a multiplet in the range δ6.91-6.98, while the remaining aromatic protons occur as a multiplet between δ7.18-7.22. There is no proton peak observed at C-4, thus confirming that displacemant with bromine has occurred.

In the ^{13}C NMR spectrum of (146) the 4'-methoxy substituent signal occurs at 54.73ppm. The signals at 40.74ppm and 77.24ppm, which were found inverted

in the DEPT 135 spectrum, correspond to C-3 and C-2. The shielded C-3', C-5' signal occurs at 113.01ppm while the corresponding C-2', C-6' signal is seen at 130.69ppm. The aliphatic C-4 and C-5 quaternary signals are observed at 121.51 and 133.06ppm respectively, while C-1' is found at 134.35ppm. C-9 and C-7 are observed somewhat upfield at 122.92ppm and the C-6 and C-8 signals appeared at 126.36 and 130.96ppm respectively. The C-5a quaternary signal is found at 139.01ppm. Elemental analysis verified the molecular formula assignment for the compound (146).

3.2.3.2 Postulated mechanism for the bromination of (146)

The mechanism of the electrophilic addition of bromine to alkenes has been widely investigated both from the kinetic and the stereochemical point of view.[357,358,359] Apart from the relative importance of the various kinetically significant processes involved.[357,360,361,362] it has been proven that the nature of the intermediates of the addition depends on the structure of the substrate and on the reaction medium. These intermediates [Scheme 36] range from the strongly bridged bromium ions (a), originally postulated by Roberts & Kimball[363] to a weakly bridged cation species,[357,364] (b) or an unbridged / open cation (c)[357,364] species.



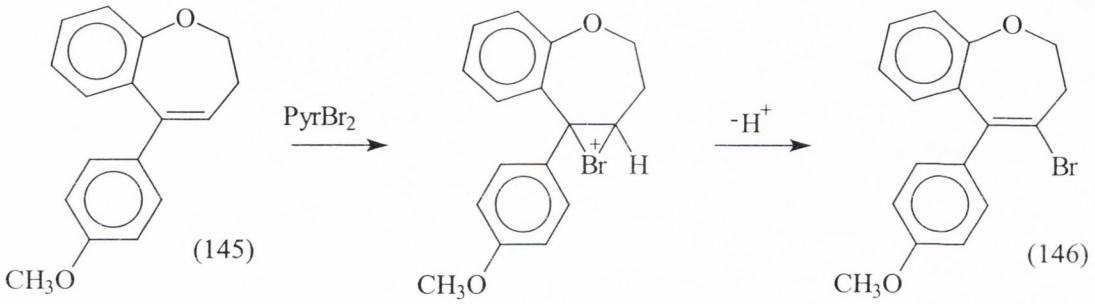
Scheme 36: Intermediates of bromination proposed by Roberts & Kimball[363]

McCague *et al.*,[87] utilised the reagent pyridinium bromide perbromide ($PyHBr_3$) to selectively prepare a vinyl brominated precursor to novel non-isomerisable antiestrogens (benzocycloheptenes). The precise mechanism for bromination using the reagent $PyH^+Br_3^-$ is controversial, however it is believed to be electrophilic and complies with the general mechanism involving Br_2. Dubois and Huynh[365] suggested, that the tribromide Br_3^- reacts via electrophilic attack on reactive alkenes compared to nucleophilic attack by Br^- on the Br_2 charge transfer complex when the alkene is of low reactivity.

Rolston and Yates[357] and Barili *et al.*,[362,366] suggested that $PyHBr_3$ is converted to $PyBr_2$ and therefore $PyBr^+Br^-$ can act as the electrophilic agent for both reagents. Heasley *et al.*,[367] confirmed that the differences in reaction of bromine and halogen complexes result from the fact that with molecular bromine, two or

more halogen molecules participate in the transition state (second order), whereas reactions with the halogen complexes limit the availability of halogen and impose a first order mechanism.

The mechanism postulated for the bromination of 5-(4-methoxyphenyl)-2,3-dihydro-1-benzoxepin (146) is depicted in Scheme 37



Scheme 37: Postulated mechanism of bromination

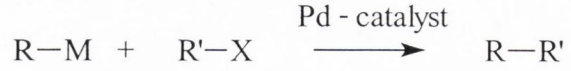
It is proposed that from $PyHBr_3$ is generated the electrophile, Br^+, which adds to the benzoxepin (145) substrate, resulting in a bridged intermediate. This then undergoes H^+ elimination, with regeneration of the double bond resulting in the mono-substituted product (146). The next reaction step involves arylation at C-4, hence a short discussion of arylation reactions.

3.2.4. Aryl-Aryl coupling reactions.

3.2.4.1 Introduction

Biaryls (Ar^1-Ar^2) and their homologues such as tetraryls, oligoaryls and polyaryls are an important class of organic compound. The biaryl unit is represented in several types of compounds, including natural products, polymers, liquid crystals, ligands and molecules of medicinal interest.[368] A number of catalytic methods for synthesising these biphenyls from monoaryl precursors in cross-coupling reactions have been developed over the last two decades by Kharasch,[368] Negishi,[369] Stille[368] and more recently Suzuki.[370]

Cross-coupling reactions are now accessible via a variety of organometallic reagents and provide a fundamentally common synthetic methodology.[369] [Equation 1]



R—M = organometallic reagent

R, R' = aryl

Equation 1: Cross-coupling reactions using organometallic reagents

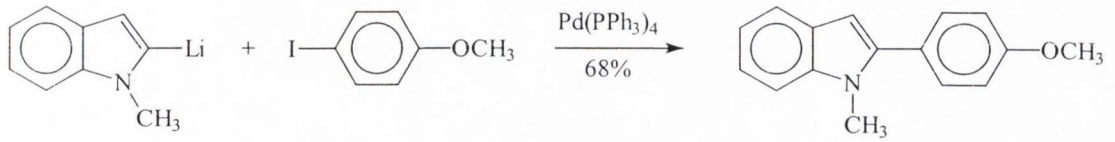
In the early 1970's Kumada and Tamao[371] and Corriu[372] reported independently that the reaction of organomagnesium reagents with alkenyl or aryl halides could be catalysed by Ni (II) complex. Tamura[373] found Fe(III) catalyst efficient for the cross coupling of Grignard reagents with 1-halo-1-alkenes, while Li_2-$CuCl_4$ was found suitable for haloalkanes. The palladium catalysed reaction of Grignard reagents was first reported by Yamamura,[374] the synthetic utility of which was then amply demonstrated by Negishi[375] on the reaction of organoaluminium, zinc, and zirconium reagents. Further discoveries have shown that many other organometallic reagents are highly useful as nucleophiles for the cross-coupling reaction, e.g. organolithiums by Murahashi,[376] organostannanes by Kosugi[377] and Stille,[378] 1-alkenylcopper(I) by Alexakis[379] and organosilicon compounds by Hatanaka.[380]

The four most commonly used catalytic methods in biaryl synthesis are the Kharasch, Negishi (Heck-type reaction), Stille and Suzuki reactions. These reactions enable the preparation of both symmetrical and asymmetrical biaryls in a cross-coupling reaction and invariably proceed using Group III transition metals,[381] such as nickel or palladium catalysts.

The Kharasch reaction began to achieve importance as a method for biaryl synthesis in the mid to late 1970's. In this reaction an aryl Grignard reagent (Ar^1MgX, X=halogen) is generally reacted with an aryl halide (Ar^2X) in the presence of an appropriate catalyst to yield the biaryl (Ar^1-Ar^2). Other functionalised aryls can also partner the Grignard reagent in the reactions, for example, phenolic derivatives such as triflates, mesylates, ethers as well as thiophenolic derivatives such as sulphides and sulphones have been used. However the disadvantage of the Kharasch reaction is that the polar nature of the Grignard reagent (strong nucleophile) percludes the use of several types of functional groups in the coupling partner such as aldehydes, ketones, esters and nitro groups.[368]

The Negishi reaction utilises arylzinc reagents (Ar^1ZnX, X=halogen) and aryl halides or triflates (Ar^2X, X=halogen or triflate) and began to assume importance in the mid 1970's.[368] In 1978, Negishi reported that iodobenzene selectively

coupled with the 1-alkynyl group on lithium 1-hexynyl (tributyl) borate through a palladium-catalysed addition elimination sequence (Heck-type reaction).[369] Unlike the Kharasch reaction, functional groups such as aldehydes, ketones, esters, amines and nitro groups etc., are tolerated in the coupling partner of the arylzinc reagent. Although arylmagnesium and arylzinc reagents are precursors to biaryls in the Kharasch and Negishi reactions respectively, aryllithiums are not generally used due to their highly polar and basic nature. There are, however, a few isolated examples where aryllithiums have been used successfully in biaryl synthesis, as illustrated in Scheme 38.



(147) (148) (149)

Scheme 38: Synthesis of compound (149) via the Negishi Reactions

Arylation of olefins via the Heck (Negishi) reaction can be achieved by treatment with an arylpalladium reagent that can be generated *in situ* by several methods.[382]

(1) By treatment of an arylzincbromide with a palladium-triarylphosphine complex (ArZnBr-ArPdBr),[383,384] which is used in this research.

(2) By treatment of an aryl iodide[385] with palladium acetate[386] in the presence of a base such as tributylamine or potassium acetate (ArI-ArPdI).[387]

(3) By treatment of an arylmercury compound (either Ar_2Hg or ArHgX) with $LiPdCl_3$ (ArHgX-ArPdX).[388]

(4) By the reaction of an aromatic compound with palladium acetate or palladium metal and silver acetate in acetic acid (ArH-ArPdOAc).[389]

The Heck reaction can also be extended to other functional groups, such as ether, carboxyl, phenolic or cyano groups.[390]

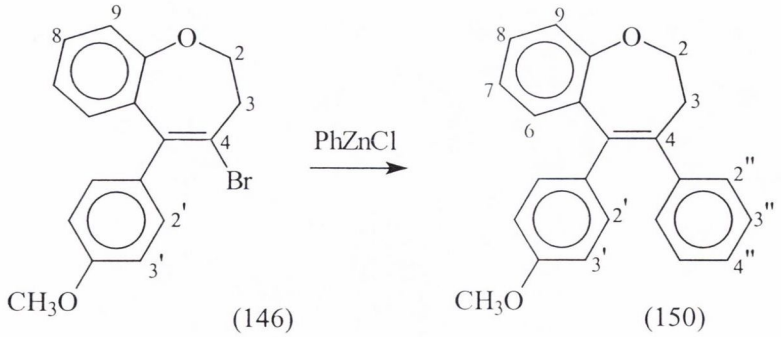
In the late 1970's, the Stille reaction started to be used in biaryl synthesis, using arylstannanes (Ar^1SnR_3, R=Me, Bu) and aryl halides or triflates (Ar^2X, X=halogen or triflate) as the coupling partners. This reaction is extremely versatile, proceeds under neutral conditions and can tolerate a wide range of substituents on both coupling partners. Thus, substituents which are not compatible with the Kharasch and Negishi reactions are often tolerated in the Stille reaction. The major disadvantage of the Stille reaction is the toxicity of the organotin reagents and byproducts.[368]

The 1980's saw the advent of the Suzuki reaction, which like the Stille reaction has proved extremely versatile and has found extensive use in natural product synthesis. Boronic acids [$Ar^1B(OH)_2$] are the usual substrates in this reaction together with aryl halides or triflates (Ar^2X, X=halogen or triflate), although esters or arylboranes are frequently used.[391] Palladium-catalysed cross coupling reactions of aryl halides or triflates with boronic acids, the Suzuki reaction has now become the most powerful and popular tool for selective construction of carbon-carbon bonds.[392,393] The palladium catalysed coupling of aryl bromides with aryl boronic acids (the Suzuki reaction) has been shown to have great utility in the synthesis of biaryl compounds[370] and will be discussed later in this Chapter [Section 3.4]. This widely employed reaction protocol utilises aqueous organic solvents in the presence of an inorganic base (typically carbonate, bicarbonate or hydroxide) and triphenylphosphine as ligand.[381] Wallow[393] showed that phosphine ligand limits the catalytic efficiency of palladium. Ligandless palladium catalytic species give fast coupling reactions[394] and the phosphine-related side reactions can be suppressed.[395]

3.2.4.2 Synthesis of 5-(4-methoxyphenyl)-4-phenyl-2,3-dihydro-1-benzoxepin (150) via Heck coupling reaction.

In this section the preparation of 5-(4-methoxyphenyl)-4-phenyl-2,3-dihydro-1-benzoxepin (150) using the Heck reaction will be discussed.

Phenylation of 4-bromo-5-(4-methoxyphenyl)-2,3-dihydro-1-benzoxepin (146) using phenylzinc chloride and tetrakis(triphenylphosphine)palladium(0) in THF afforded 5-(4-methoxyphenyl)-4-phenyl-2,3-dihydro-1-benzoxepin (150) as colourless crystals in 89% yield. [Scheme 39]



(146) (150)

Scheme 39: Arylation of 4-bromo-5-(4-methoxyphenyl)-2,3-dihydro-1-benzoxepin to form compound (150)

The compound (150) was positively identified using spectroscopic data and elemental analysis.

In the ^{1}H NMR of 5-(4-methoxyphenyl)-4-phenyl-2,3-dihydro-1-benzoxepin (150), H-3 protons are observed as a triplet (J=6.3Hz) at δ2.74, while the adjoining H-2 protons are found at δ4.55 as a triplet (J=6.0Hz). The 4'-methoxy protons are found as a singlet at δ3.76. The shielded H-3', H-5' protons are observed at δ6.66-6.68 as a double doublet (J=6.5, 2.0Hz), while the corresponding H-2', H-6' protons occur as a double doublet (J=6.5, 2.0Hz) at δ6.90-6.92. The aromatic H-9 proton is slightly deshielded and occurs as a double triplet (J=8.3, 1.5Hz) between δ6.98-7.02. The remaining aromatic protons are found in the range δ7.08-7.24.

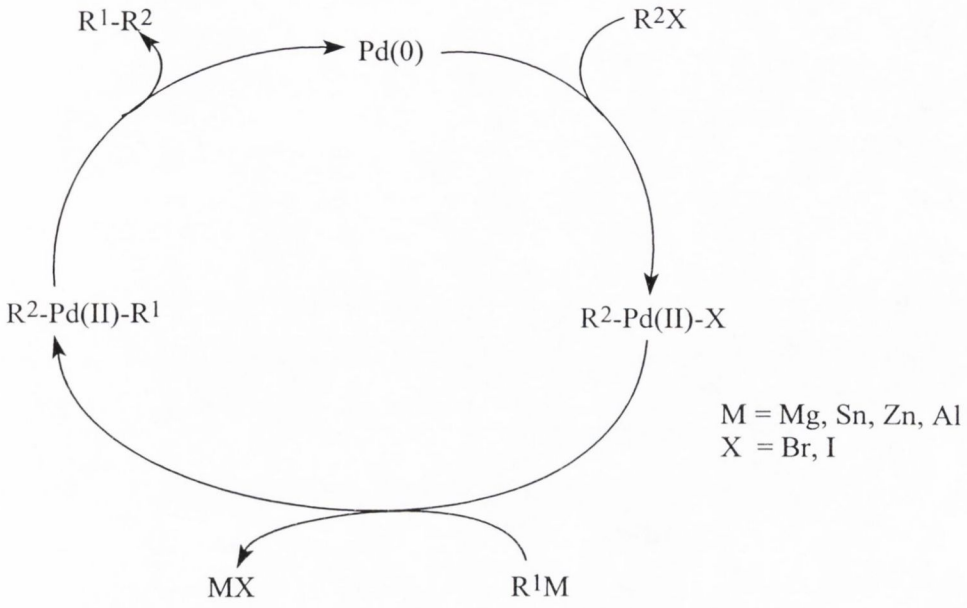
In the ^{13}C NMR, the 4'-methoxy signal is characteristically observed at 55.07ppm. Two inverted signals are observed in the DEPT 135 spectrum, at 35.72 and 80.44ppm, corresponding to C-3 and C-2 respectively. The shielded C-3', C-5' are found at 113.07ppm, while the corresponding C-2', C-6' signal occurs at 131.02ppm. The C-4' quaternary signal is found downfield at 158.16ppm. The aromatic C-9 and C-7 signals are found at 122.06 and 123.49ppm, while those of C-8 and C-6 occur at 132.52 and 126.28ppm, respectively. The quaternary C-5 and C-9a signals are observed at 133.81 and 156.71ppm, while the remaining aromatic signals are found between 128.39-129.50ppm.

The elemental analysis results for compound (150) proved satisfactory.

3.2.4.3 Proposed arylation mechanism.

The cross-coupling reaction of organic eletrophiles with organometallic reagents in the presence of a transition metal, has proven to be a very mild and straight forward method of forming C-C bonds.[396,397,398] Negishi and co-workers[399,400,401,402,403] demonstrated that organometallics containing zinc can enter into cross-coupling reactions when used in conjunction with palladium (or nickel) catalysts. The presence of the co-catalyst $ZnCl_2$ was also found to enhance the rate of cross coupling[404] with Pd and Ni catalysts. The various palladium assisted coupling reactions occur by several closely related mechanisms.[367,383]

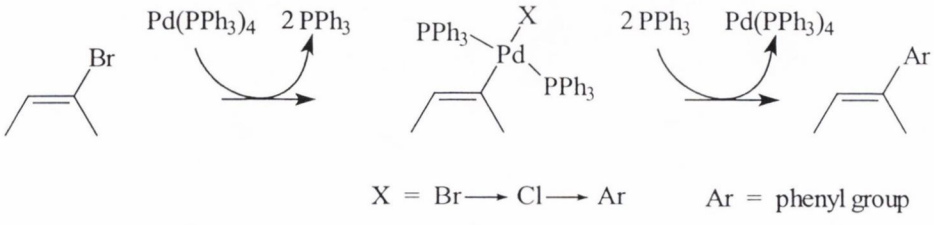
Numerous mechanistic studies of the biaryl coupling reaction have been performed,[369,381,405,406,407] resulting in a general catalytic cycle for the cross-coupling of organometallics with organic halides, catalysed by transition metals [usually nickel (0) or palladium (0) species], which is depicted in Scheme 40.



Scheme 40: A general catalytic cycle for cross coupling

These coupling reactions involve the oxidative addition of organic halides to the palladium (0) complex to form organopalladium halides (R^2-Pd(II)-X). This is followed by transmetalation with main-group organometallics (R^1M), to provide the diorganopalladium complex (R^2-Pd(II)-R^1), which can undergo a reductive elimination, leading to carbon-carbon bond formation (R^1-R^2) and regeneration of the catalyst.[408] Although each step involves further complicated processes including ligand exchanges, research has verified the presence of the intermediates (R^2-Pd(II)X and R^2-Pd(II)-R^1) via isolation and spectroscopic analysis.[384,409]

In this case the bromide group [R^2X] of 4-bromo-5-(4-methoxyphenyl)-2,3-dihydro-1-benzoxepin (146) is stereospecifically replaced by a phenyl group, via palladium catalysed coupling of the bromide with phenyl zinc chloride to afford[410] 5-(4-methoxyphenyl)-4-phenyl-2,3-dihydro-1-benzoxepin (150). McCague[348] proposed that this reaction type might also proceed via a catalytically active chloro-palladium intermediate. [Scheme 41]



Scheme 41: Proposed mechanism for arylation of bromo alkenes

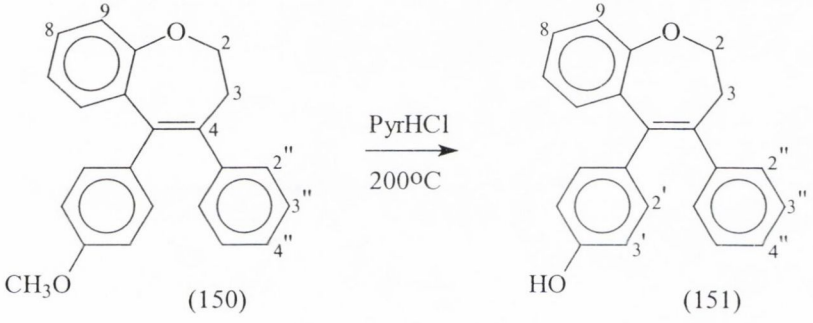
The initial step of a coupling reaction is believed to involve the formation of an organopalladium salt which involves the oxidative addition of (146) to the Pd(0)

species.[348,404,411,412] The catalyst $Pd(PPh_3)_4$ used, is considered to generate the catalytically active $Pd(PPh_3)_2$[410] species explaining the reference to $2PPh_3$ in Scheme 41. The palladium complex formed with the organometallic reagent (PhZnCl) can then undergo reductively elimination to afford (150).[348,383,412,413]

Further investigations have led scientists to suggest that the mechanism of the metathesis step could be susceptible to steric retardation.

3.2.5. 5-(4-Hydroxyphenyl)-4-phenyl-2,3-dihydro-1-benzoxepin (151)

Demethylation of 5-(4-methoxyphenyl)-4-phenyl-2,3-dihydro-1-benzoxepin (150) was carried out with the demethylating agent pyridine hydrochloride to afford 5-(4-hydroxyphenyl)-4-phenyl-2,3-dihydro-1-benzoxepin (151) as an oil in moderate yield. [Scheme 42] Demethylation using boron tribromide was found to be fruitless.



(150) (151)

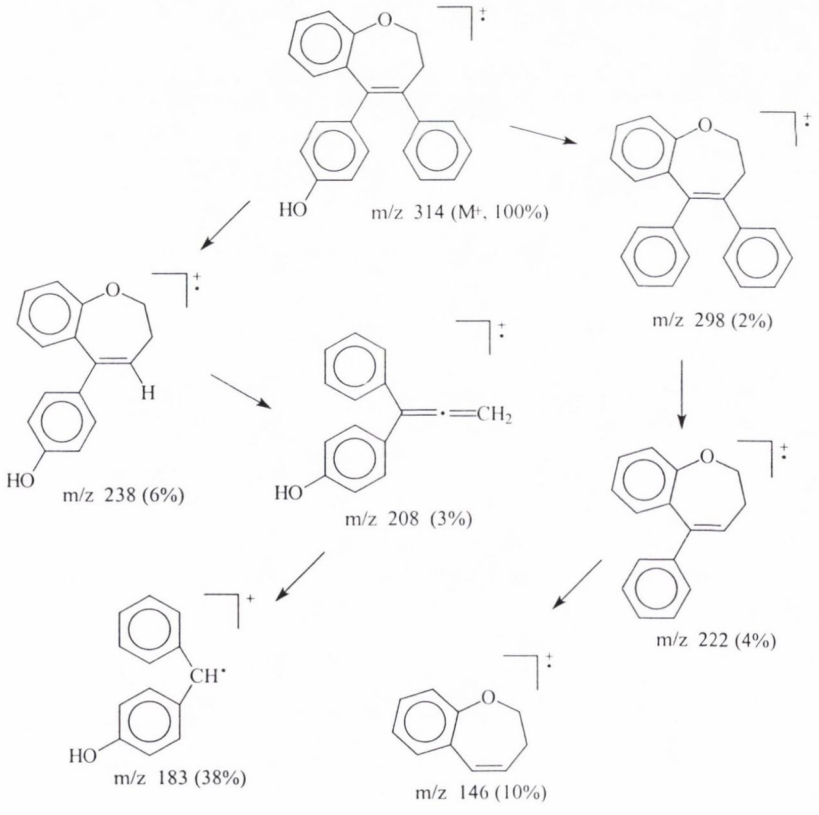
Scheme 42: Demethylation of 5-(4-methoxyphenyl)-4-phenyl-2,3-dihydro-1-benzoxepin

The characteristic broad hydroxy stretching of (151) is observed at $\nu 3339 cm^{-1}$ in the infrared spectrum. The aromatic and aliphatic C=C stretching is found at $\nu 1609 cm^{-1}$, while the ether C-O-C stretch is found at $\nu 1255$ and $1217 cm^{-1}$.

In the ^1H NMR spectrum of 5-(4-hydroxyphenyl)-4-phenyl-2,3-dihydro-1-benzoxepin (151), no methoxy protons are observed, verifying the loss of a methyl group. The H-3 protons are found as a triplet (J=6.0Hz), at δ2.74, being split by the methylene H-2 protons, which are found at δ4.64 as a triplet. The H-3', H-5' protons occur as a double doublet (J=8.5, 2.0Hz) at δ6.68-6.70 while the corresponding H-2', H-6' protons are found at δ6.93-6.95 as a double doublet (J=8.5, 2.0Hz) also. The proton at H-9 is observed as a double triplet (J=7.5, 1.3Hz) at δ6.98-7.02, while the remaining aromatic protons are found as an unresolved complex multiplet between δ7.14-7.28.

In the DEPT 135 spectrum of (151), two inverted signals are apparent at 35.23 and 79.97ppm, corresponding to C-3 and C-2 respectively. The shielded C-3', C-5' are found at 114.13ppm, while the corresponding C-2', C-6' signal occurs at 130.53ppm. The aromatic C-9 and C-7 signals are observed at 121.60 and 123.07ppm, while those of C-8 and C-6 occur at 132.25 and 125.84ppm respectively. The remaining aromatic signals are found between 126.50-129.03ppm.

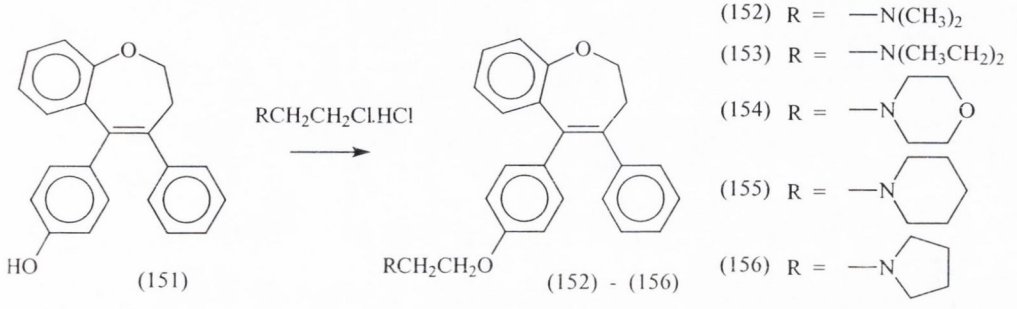
In the low resolution mass spectrum of 5-(4-hydroxyphenyl)-4-phenyl-2,3-dihydro-1-benzoxepin (151), the molecular ion is observed with a m/z 314 ($C_{22}H_{18}O_2$), as the base peak. The proposed fragmentation pattern is displayed in Scheme 43. Loss of the molecular ion of OH gives m/z 298 (2%), followed by cleavage of the phenyl substituents results in the m/z 222 (4%) and further cleavage of a phenyl group afforded the m/z 146 (1%) fragment. Alternatively cleavage from the molecular ion of OCH_2 and the phenyl substituents gives the m/z 208 (3%) fragment; further loss of C_2H_2 affording the m/z 183 (38%) fragment.



Scheme 43: Postulated mass spectral fragmentation pattern of 5-(4-hydroxyphenyl)-4-phenyl-2,3-dihydro-1-benzoxepin (151)

3.2.6. Preparation of 5-(4-alkylaminoethoxyphenyl)-4-phenyl-2,3-dihydro-1-benzoxepin.

Alkylation of 5-(4-hydroxyphenyl)-4-phenyl-2,3-dihydro-1-benzoxepin (151) was carried out to afford a series of 5-(4-alkylaminoethoxyphenyl)-4-phenyl-2,3-dihydro-1-benzoxepins (152)-(156) [Scheme 44], possessing the basic triphenyl structure of tamoxifen, with the ethylene function contained in a seven membered ring.



Scheme 44: Alkylation of 5-(4-hydroxyphenyl)-4-phenyl-2,3-dihydro-1-benzoxepin

These novel compounds (152)-(156) were positively identified using ^{1}H NMR, ^{13}C NMR and low resolution mass spectrometry. The yield, and infrared data of compounds (152)-(156) are listed in Table 10.

Compound	Yield %	$IR_{\upsilon max}$ cm^{-1}
(152)	44	1608, 1591 (C=C)
(153)	66	1606, 1572 (C=C)
(154)	78	1607, 1579 (C=C)
(155)	52	1607, 1569 (C=C)
(156)	38	1599, 1577 (C=C)

Table 10: Yield and infrared data of compound (152)-(156)

In the ^{1}H NMR spectrum [Figure 48] of 5-[(4-dimethylaminoethoxy)phenyl]-4-phenyl-2,3-dihydro-1-benzoxepin (152), the dimethyl ($N(CH_3)_2$) substituent was observed as a singlet, integrating for six protons at δ2.35. The methylene H-3 protons are found as a multiplet, along with the NCH_2 protons in the range δ2.71-2.75, integrating for four protons. The H-2 protons are observed as a triplet (J=6.0Hz) at δ4.64, while the OCH_2 protons occur at δ4.02, as a triplet (J=5.8Hz). The shielded H-3', H-5' protons are found as a double doublet (J=9.0Hz), at δ6.67-6.69. The aromatic H-2', H-6' and H-9 protons are observed as a multiplet between δ6.88-7.02, while the remaining aromatic protons are found at δ7.04-7.08.

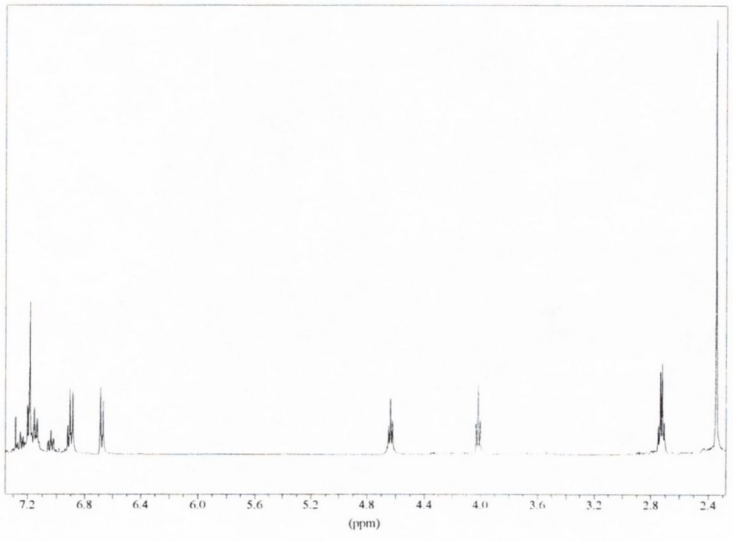


Figure 48: ^{1}H NMR Spectrum of compound (152)

In the DEPT 135 spectrum of (152), the characteristic inverted signals at 35.25 and 79.97ppm, correspond to C-3 and C-2 respectively, while the signals at 57.82 and 65.37ppm are assigned to NCH_2 and OCH_2. In the ^{13}C NMR spectrum, the dimethyl $N(CH_3)_2$ signal is found at 45.42ppm. The shielded C-3', C-5' signals occur at 113.24, 113.32ppm, while the corresponding C-2', C-6' signal is found at 130.57ppm. The aromatic C-9 and C-7 signals are observed 121.60 and 123.03ppm, while those of C-8 and C-6 occur at 125.82 and 132.05ppm. The quaternary C-4 signal is observed at 121.60ppm, interchangeable with C-9. The remaining aromatic signals are found between 127.51-129.04ppm.

Low resolution mass spectrometry showed the molecular ion of 5-[(4-dimethylaminoethoxy)phenyl]-4-phenyl-2,3-dihydro-1-benzoxepin (152) at m/z 385 ($C_{26}H_{27}NO_2$), as the base peak.

3.3. Palladium catalysed cross-coupling reactions

In the previous section 3.2.4.2, 5-(4-methoxyphenyl)-4-phenyl-2,3-dihydro-1-benzoxepin (150) was synthesised via the Heck coupling reaction. In this section a series of 4-aryl-5-(4-methoxyphenyl)-2,3-dihydro-1-benzoxepins were prepared by a palladium catalysed cross-coupling reaction using organoboronic acids the Suzuki coupling reaction.

3.3.1. Organoboronic acids.

3.3.1.1 Introduction

The popularity of the palladium-mediated Suzuki reaction, which combines arylboronic acids and aryl halides or triflates to give biaryl compounds, is largely responsible for the explosive growth in the chemistry of arylboronic acids.

Organoboron compounds[414] are highly electrophilic, but the organic groups on boron are weakly nucleophilic, thus limiting the use of organoboron reagents for ionic reactions. The co-ordination of a negatively charged base to the boron atom has been recognised as an efficient method of increasing its nucleophilicity, and so aids transfer of the organic group on boron to the adjacent positive centre (1,2-migration reaction).[415] However, intermolecular transfer reactions such as Grignard-like reactions are relatively rare. Fortunately, organoboron compounds, like organoboronic acids and esters, have sufficient reactivity for transmetalation to other metals. Transmetalations to silver (I),[416] magnesium, zinc (II), aluminium, tin,[417] copper[418] and mercury (II) halides have been extensively studied. The cross-coupling reaction of organoboron compounds, which involves the transmetalation to palladium (II) halides as a key step, has been found to proceed smoothly to form a wide range of selective carbon-carbon bonds when it is activated with suitable bases.[419] Organoboronic acids are more often than not, the agents of choice.

The chemical stability of boronic acids [$R^1B(OH)_2$] and their esters [$R^1B(OR^2)_2$] and the favourable steric properties of the latter, make them outstanding reagents for asymmetric synthesis as well as for geometrically controlled olefin synthesis.

The useful properties of boron derive from its close relationship to carbon. Boron can fit into organic compounds as a heteroatom as easily as can nitrogen and oxygen, and the first compounds containing carbon boron bonds date from

1860.[420] These include triethylborane [$(C_2H_5)_3B$], a spontaneously flammable liquid and ethylboronic acid [$C_2H_5B(OH)_2$] a stable solid, having a sweet taste.

Boronic acids are convenient reagents, generally thermally stable, inert to water and oxygen, easy to manipulate (they can be handled without special precautions) and are not particularly toxic. They are highly reactive compounds in a variety of replacement reactions.[369,425]

3.3.1.2 Synthesis of boronic acids.

Several syntheses of boronic acids were reported in the subsequent century.[421] The classical synthesis[422] of aryl- and 1-alkenylboronic acids or their esters from Grignard reagents and trialkylborates[421,423,424] is an efficient method for making relatively simple boron compounds in large quantities [Equation 2].[425,426]

$$ArMgX + B(OMe)_3 \longrightarrow \xrightarrow{H_3O^+} ArB(OH)_2 \qquad (2)$$

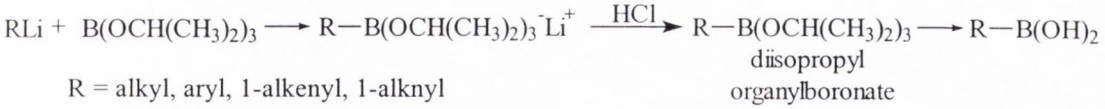
X = Cl, Br, I

Equation 2: Synthesis of boronic acid

However, this Grignard reaction[427] produces low yielding compounds and is regarded as a very tedious procedure. The classical procedure also suffers from contamination with small amounts of the opposite stereoisomers, or bis-alkylation leading to boronic acid derivatives and the formation of trialkylboranes.

Other well established methods for organoboronic acid preparations include partial oxidation of the corresponding triorganoboranes,[428] halogenation of triorganoboranes followed by hydrolysis of the resulting halides and hydroboration[429] with excess diborane, followed by alcoholysis of the BH_2 group,[430] yielding the acids. For example, alkeneboronic [$HO\sim\sim\sim\sim\sim\sim=B(OH)_2$] acids, can easily be prepared by hydroboration of the appropriate acetylenic precursor with catecholborane.[431]

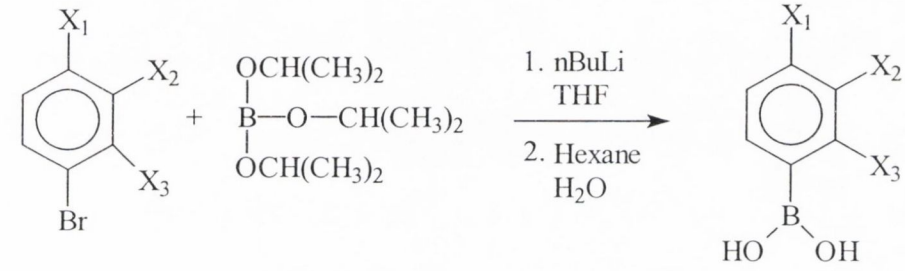
A recent useful variant of the classical approach utilises organolithium reagents and triisopropyl borate, followed by acidification with anhydrous hydrogen chloride to give directly alkyl-, aryl-, 1-alkynyl- and 1-alkenylboronic acids and esters in high yields. [Equation 3].[432] Triisopropyl borate is shown to be the best of available alkyl borates to avoid multiple alkylation of the borates.



Equation 3: Preparation of diisopropyl organoboronate

3.3.1.3 Preparation of substituted boronic acids (168)-(177).

In this present work, a series of substituted boronic acids were prepared, using *n*-butyllithium, substituted aryl halides and triisopropyl borate, under the conditions described by Morgan *et al.*[433] [Scheme 45]. Once synthesised the boronic acids were vacuum dried at less than 30°C, as these compounds are sensitive to heat and may decompose readily to the anhydride form once heated above this temperature.



(157) - (166) (167) (168) - (177)

(168)	$X_1 = OCH_3$,	$X_2 = H$,	$X_3 = H$
(169)	$X_1 = H$,	$X_2 = OCH_3$,	$X_3 = H$
(170)	$X_1 = H$,	$X_2 = H$,	$X_3 = OCH_3$
(171)	$X_1 = CH_3$,	$X_2 = H$,	$X_3 = H$
(172)	$X_1 = H$,	$X_2 = CH_3$,	$X_3 = H$
(173)	$X_1 = H$,	$X_2 = H$,	$X_3 = CH_3$
(174)	$X_1 = Cl$,	$X_2 = H$,	$X_3 = H$
(175)	$X_1 = F$,	$X_2 = H$,	$X_3 = H$
(176)	$X_1 = CN$,	$X_2 = H$,	$X_3 = H$
(177)	X_3 = naphthyl		

Scheme 45: Synthesis of boronic acids (168) – (177)

The substituted boronic acids were afforded as crystalline solids in moderate to good yields. Positive identification of the product was obtained from spectroscopic data. The yield, melting point and infrared data of these compounds are detailed in Table 11. In the infrared spectrum the characteristic broad hydroxy stretching was found in the ν3733-3016cm^{-1} region. The aromatic C=C stretch for these compounds (168)-(177) is observed at ν1605-1593cm^{-1}, while the B-OH stretch occurs at ν1165 and 1105cm^{-1}.

Compound	Yield %	m.p. °C (lit m.p. °C)	IRυ_{max} (KBr) cm^{-1}
(168)	91	159 – 164 (160 – 163)	3401 (OH), 1604 (C=C)
(169)	29	158 – 162 (160 – 163)	3278 (OH), 1593 (C=C)
(170)	69	106 – 108 (105 – 107)	3354 (OH), 1604 (C=C)
(171)	52	249 – 260 (256 – 263)	3250 (OH), 1615 (C=C)
(172)	57	74 – 76 (69 – 71)	3272 (OH), 1605 (C=C)
(173)	57	162 – 164 (162 – 164)	3440 (OH), 1600 (C=C)
(174)	34	278 – 280 (284 – 289)	3262 (OH), 1595 (C=C)
(175)	17	260 – 261 (263 – 265)	3434 (OH), 1599 (C=C)
(176)	6	> 270 (285 – 292)	3427 (OH), 1610 (C=C)
(177)	36	202 – 208 (210 – 211)	3264 (OH), 1574 (C=C)

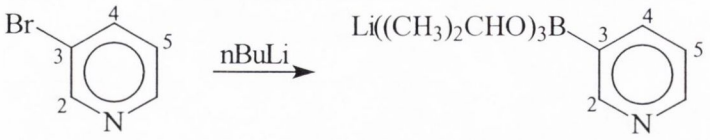
Table 11: Yield, m.p. and infrared data of compounds (168)-(177)

In the ^{1}H NMR spectrum of 4-methoxyphenylboronic acid (168), the 4-methoxy protons are observed at δ3.76 as a singlet. The H-3 and H-5 protons are shielded by the methoxy group and are found as a doublet (J=8.0Hz) at δ6.86, while the corresponding H-2 and H-6 protons occur as a doublet (J=7.5Hz) at δ7.69.

In the ^{13}C NMR spectrum of (168) the 4-methoxy carbon signal is found characteristically at 55.13ppm. The shielded C-3, C-5 signal is observed at 113.20ppm, with the corresponding C-2, C-6 signal occurring at 132.22ppm. The C-4 quaternary carbon is observed at 159.10ppm while the C-1 quaternary signal is found at 172.71ppm.

A ^{11}B NMR spectrum of compounds (168)-(177) also verified the identity of these products, showing the presence of a Boron signal in the region 380-370ppm, referenced from boron trifluoroetherate.

This method could not be used to prepare 3-pyridinylboronic acid directly. Black and co-workers[434] found that the isolation of pyridine-3-boronic acid was problematic. As a result, it was found that the Suzuki coupling could be carried out very efficiently using the crude trialkylboronate. Thus 3-bromopyridine was lithiated (*n*-BuLi, ether, and -78°C), and then quenched with triisopropylborate. Removal of solvent provided the lithium trialkylborate [Scheme 46], which was used directly in the Suzuki coupling without the use of additional base. This method obviated the problem of isolating the water-soluble pyridinylboronic acid. 3-Pyridyltriisopropyl borate (179) was afforded in 97% yield.



(178) (179)

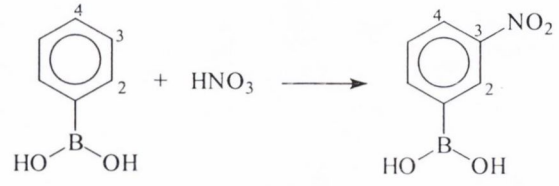
Scheme 46: Synthesis of compound (179)

In the infrared spectrum of compound (179), the pyridine peaks are observed at 1658 and 1583 cm^{-1}.

In the ^{1}H NMR, a singlet is observed at δ1.18, which is assigned to the methyl protons $(CH_3)_6$ and integrates for 18 protons. The $(CH)_3$ protons are found as a multiplet at δ7.95. The H-5 proton occurs as a multiplet at δ7.21, while the H-4 and H-2 protons are observed at δ7.92 and δ8.20 respectively. The H-6 proton is found at δ8.59 as singlet.

The ^{13}C NMR spectrum of (179) shows signals at 23.42 and 62.88ppm, corresponding to $(CH_3)_6$ and $(CH)_3$ respectively. The pyridine signals are found at 121.99ppm for C-5, 141.32ppm for C-4 and 144.15ppm for C-6. The C-2 signal is observed at 152.48ppm.

The boronic acid *m*-nitrophenylboronic acid (182) was prepared in 28% yield according to the method of Seamen and Johnson,[435] modified to employ 2.0 equiv. of fuming nitric acid.[436] This procedure involves the addition of dry phenylboronic acid to an ice cool mixture of fuming nitric acid and a little urea. [Scheme 47]



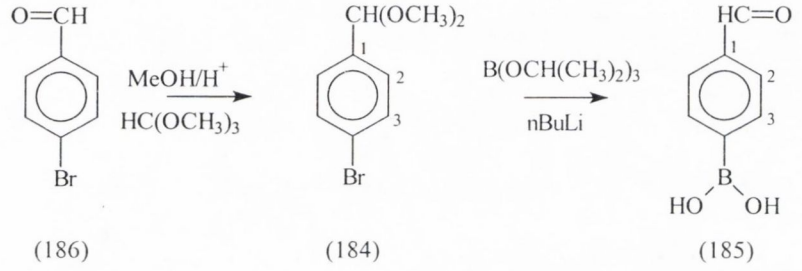
(180) (181) (182)

Scheme 47: Preparation of m-nitrophenylboronic acid (182)

The procedure outlined by Morgan *et al.*, in preparing 4-formylbenzeneboronic acid and 4-acetophenylboronic acid was unsuccessful, as the *n*-butyllithium recycled with the carbonyl group of the aldehyde and ketone in the reaction. Therefore, it was necessary to protect[437,438] the carbonyl (C=O) group as an acetal[439] prior to preparation of the boronic acid derivative. Acetals are stable to attack by nucleophiles and therefore are inert toward hydrides as reducing

agents. Deprotection to the aldehyde once the boronic acid is prepared can be carried out by mild hydrolysis.

Thus 4-bromobenzaldehyde dimethyl acetal[428] (184) was prepared using trimethyl orthoformate and *p*-toluenesulphonic acid with 4-bromobenzaldehyde. Once the protected compound (184) was isolated, the 4-formylbenzene boronic acid[440] (185) was synthesised in the usual manner [Scheme 48]. The dimethyl acetal protecting group was removed during the work up. In the ^{1}H NMR spectrum of 4-bromobenzaldehyde dimethyl acetal (184), the methoxy $(OCH_3)_2$ protons were characteristically observed at δ3.24 as a singlet, while the CH proton is found as a singlet at δ5.30. The shielded H-3 and H-5 protons are found as a doublet (J = 7.0 Hz) at δ7.26 - 7.29, with the corresponding H-2 and H-6 aromatic protons at δ7.42-7.45 as double doublet (J=7.0Hz, 1.5Hz).

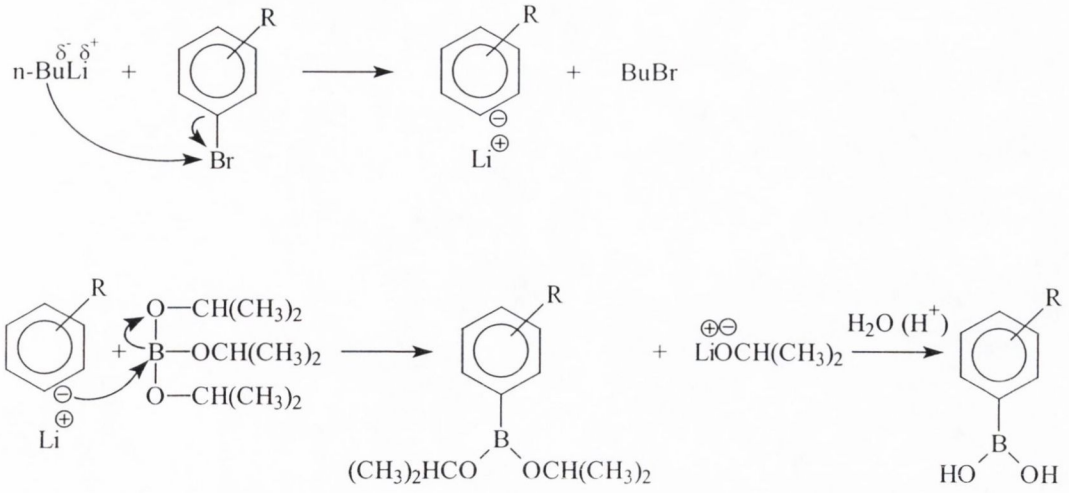


(186) (184) (185)

Scheme 48: Synthesis of 4-formylbenzeneboronic acid (185)

In the ^{13}C NMR spectrum of (185), the methoxy signal is found at 51.73ppm, while a signal at 101.57ppm is assigned to CH. A single signal is observed for C-3, C-5 and C-2, C-6 at 128.11 and 130.76ppm respectively. The quaternary C-1 and C-4 signals are found at 121.90 and 136.86ppm.

The proposed mechanism for boronic acid preparation[441] involves firstly the formation of the aryllithium (carbanion) nucleophile followed by nucleophillic attack on triisopropylborate and finally hydrolysis to form the boronic acid [Scheme 49].



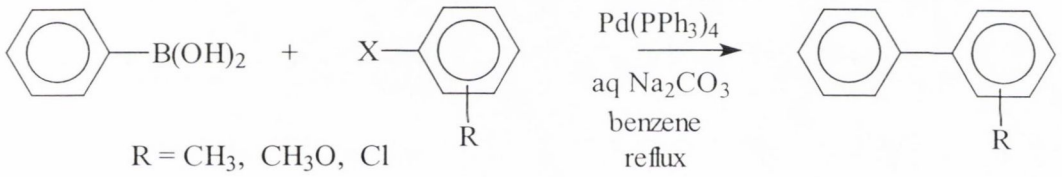
Scheme 49: Postulated boronic acid preparation

The first step involves the formation of a carbanion nucleophile, which is conveniently prepared with the arylbromide and *n*-butyllithium. This nucleophile attacks the vacant orbital at the tri-coordinate site of triisopropyl borate. Once the borate intermediate is formed, hydrolysis yields the required boronic acid.

3.4. Suzuki coupling reaction.

3.4.1. Introduction

Palladium-catalysed cross coupling between arylboronic acids and arylhalides or aryl triflates has been shown to be a versatile method for the preparation of biaryls.[381,442] The original procedure developed by Suzuki[443,444] has achieved widespread popularity in organic synthesis since it is compatible with a large variety of functional groups, and the conditions tolerate aqueous reaction media. The various functional groups that can survive the reaction include cyano, carbonyl, nitro, alcohol, acetal and amino groups as well as an ester function. Moreover, the inorganic by-product of the Suzuki reaction is non-toxic and easily removed from the reaction mixture, thereby making the Suzuki protocol suitable for industrial processes. In 1981 Suzuki and his co-workers first reported that benzeneboronic acid (186) could be coupled with aryl halides, catalysed by a palladium phosphine complex in the presence of sodium carbonate.[369,408] [Scheme 50]



(186) (187) - (189) (190) - (192)

Scheme 50: Suzuki coupling reaction

This reaction is catalysed by 3% of tetrakis(triphenyl)phosphinepalladium (0), with benzene or toluene as the solvent and requires 2 equivalents of aqueous sodium carbonate solution. Since the discovery, various modifications have been made to the reaction conditions.

3.4.2. Reaction conditions necessary for Suzuki coupling

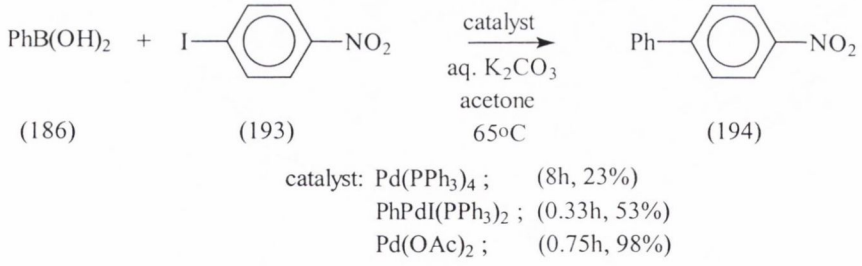
The Suzuki Coupling reaction usually gives high yields under mild conditions and employs reagents which are stable to air and moisture, which are generally of low toxicity. Modifications to the initial coupling reaction conditions have enabled synthesis of a number of functionalised biaryls and heterobiaryls. A variety of reaction conditions are listed below:

(a) Aryl halides.

The most frequently employed halides in boronic acid reactions are aryl bromides, but the reactivity of halides shows the order Ar-I>Ar-Br>Ar-Cl.[408,445] However, research has shown that aryl iodides do not give complete conversion in simple displacement reactions (S_N2) as reactions ceases after one hour, and therefore aryl bromides are found to be more useful. Recent research shows that some electron-deficient heteroaryl chlorides and aryl chlorides are reactive enough to participate in the cross coupling. Recent studies by Mitchell and Wallbank show that nitrogen-containing electron-deficient heteroaryl chlorides can be coupled with areneboronic acid to give biaryls in good yields, by using [1,4-bis(diphenyl-phosphine)butane]palladium(II)chloride [$Pd(dppb)Cl_2$] as the catalyst.[446]

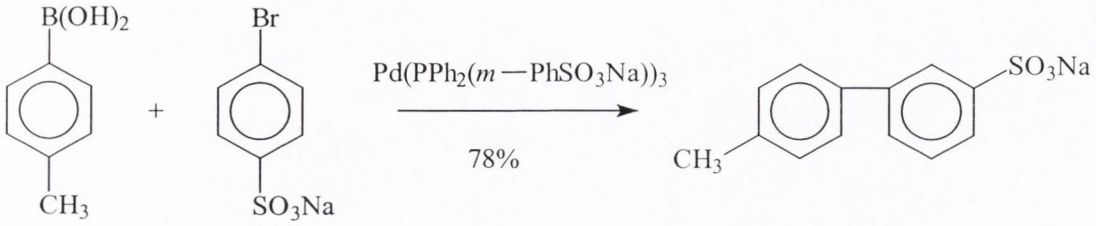
(b) Palladium Catalysts.

Phosphine-based palladium catalysts for example $Pd(dppb)Cl_2$[446] and $Pd(dppf)(OAc)_2$ are generally used, since they are stable on prolonged heating, although, extremely high coupling reaction rates can sometimes be achieved by using palladium catalysts without a phosphine ligand[369] such as $Pd(OAc)_2$[447] and $PdCl_2$.[448] Phosphine-free palladiums are approximately one order of magnitude more active that $ArPd^{II}I(PPh_3)_2$, both of which are in turn markedly more active that $Pd(PPh_3)_4$.[369] [Scheme 51]



Scheme 51: Suzuki coupling using palladium catalysts

Recent reports by Casalnuovo and Calabrese[449] reported that the water soluble Pd(0) complex $Pd[Ph_2(m\text{-}PhSO_3M)]_3$ (M = Na, K) catalysed the cross-coupling of highly lipophobic sodium *p*-bromobenzenesulfonate with *p*-methylbenzene boronic acid to give the coupling product in the yield of 78% compared with a yield of 36% catalysed by $Pd(PPh_3)_4$. [Scheme 52]

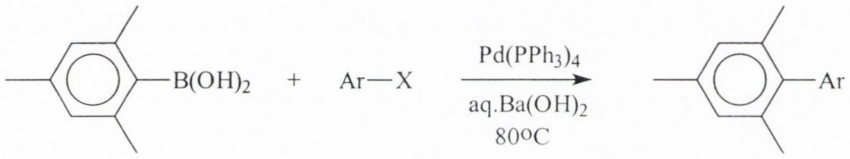


(171) (195) (196)

Scheme 52: Coupled product of p-methylboronic acid and sodium p-bromobenzenesulfonate

(c) Base

In contrast with the cross-coupling reactions of tin or zinc reagents that do not require the presence of a base, the boronic acid coupling reaction does require a base.[408] The addition of strong bases, e.g. aqueous NaOH or $Ba(OH)_2$ both in benzene and DME exerts a remarkable effect on the acceleration of the coupling rate.[450,451,452] Although weak bases give better results for less hindered arylboronic acids.[Scheme 53]



(197) (201)

(198) ArX = 2 - $MeOC_6H_4I$ (80%)
(199) ArX = 2 - ClC_6H_4I (94%)
(200) ArX = 2 - bromonaphthalene (86%)

Scheme 53: Coupling reactions using $Ba(OH)_2$ base

However weak bases give better results for less hindered arylboronic acids, the order of reactivity for mesitylboronic acids corresponding to the basic strength: $Ba(OH)_2$ > NaOH > K_3PO_4 > Na_2CO_3 > $NaHCO_3$.[450,453] Although sodium carbonate is the most frequently used base, other bases are also effective. For instance sodium hydrogen carbonate,[454,455] triethylamine,[456,457] and thallium hydroxide[458,459] have been applied for this particular purpose. Thompson and co-workers[456] first reported the use of a non-aqueous system consisting of triethylamine in DMF for boronic acid coupling and this system has proved to be suitable for the synthesis of fluoroquinoline.[460]

(d) Reaction medium

Although research has found that the solvent does not play an important role in the reaction, a comparison of the reaction rates at 50°C revealed that the reaction apparently accelerates in polar solvents e.g. DMF > CH_3CN > THF > toluene.[461]

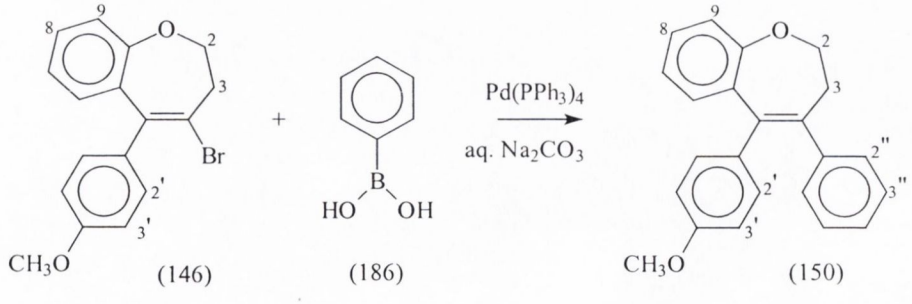
The reaction conditions used in this research employed the use of palladium tetrakis(triphenyl)phosphine, aqueous sodium carbonate, THF and arylbromides.

Andersen and co-workers[443] have recently developed an *in-situ* Suzuki coupling method for synthesising C_2-symmetric biaryls, which obviates the need for boronic acid isolation. This was carried out by treating the starting haloarene with only 0.5 equivalents of *n*-butyllithium followed by an excess of trimethoxyborate. Under these conditions it was then possible to generate the required 1:1 molar ratio of haloarene and arylboronic acid *in situ* which was then subsequently coupled under modified Suzuki conditions. However, the reaction conditions for this *in situ* procedure have yet to be optimised.

3.4.3. Suzuki coupling reactions of 4-bromo benzoxepins.

3.4.3.1 Preparation of 5-(4-methoxphenyl)-4-phenyl-2,3-dihydro-1-benzoxepin.

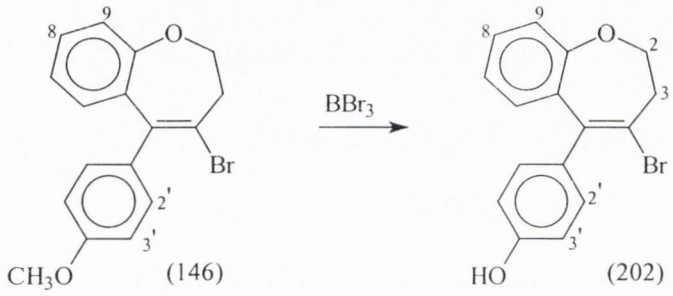
As discussed in Section 3.2.4.2, 4-bromo-5-(4-methoxyphenyl)-2,3-dihydro-1-benzoxepin (146) was arylated using the Heck coupling reaction. However, it was found that this route of synthesis of 5-(4-methoxyphenyl)-4-phenyl-2,3-dihydro-1-benzoxepin (150) is not very efficient, as yields are low. Thus the Suzuki coupling reaction was employed, using phenylboronic acid (186) and (146) under mild conditions, to produce 5-(4-methoxyphenyl)-4-phenyl-2,3-dihydro-1-benzoxepin (150) [method 2]. The reaction was successful, affording (150), an oil in 96% yield. [Scheme 54] The spectroscopic data of (150), via the Suzuki coupling method was identical to that of 5-(4-methoxyphenyl)-4-phenyl-2,3-dihydro-1-benzoxepin (150) which was prepared via the Heck coupling method.



Scheme 54: Synthesis of compound (150) via the Suzuki coupling method

3.4.3.2 Synthesis of 4-bromo-5-(4-hydroxyphenyl)-2,3-dihydro-1-benzoxepin (202).

Due to the success of the Suzuki coupling reaction using 4-bromo-5-(4-methoxyphenyl)-2,3-dihydro-1-benzoxepin (146) and phenylboronic acid (186), it was envisaged that a number of arylated benzoxepins could be prepared with various substituted aryl and heterocyclic boronic acids using this synthetic route. Thus a series of 5-(4-hydroxyphenyl)-4-(aryl)-2,3-dihydro-1-benzoxepins (203)-(213) and 5-(4-hydroxyphenyl)-4-(heterocyclic)-2,3-dihydro-1-benzoxepins (217)-(220) were prepared. Prior to synthesis of these Suzuki coupled benzoxepins, 4-bromo-5-(4-hydroxyphenyl)-2,3-dihydro-1-benzoxepin (202) was prepared from 4-bromo-5-(4-methoxyphenyl)-2,3-dihydro-1-benzoxepin (146) with boron tribromide as the demethylating agent [Scheme 55].



Scheme 55: Demethylation of compound (146) to form 4-bromo-5-(4-hydroxyphenyl)-2,3-dihydro-1-benzoxepin (202)

4-Bromo-5-(4-hydroxyphenyl)-2,3-dihydro-1-benzoxepin (202) was afforded as an oil in moderate yield. In the infrared spectrum of (202), the characteristic broad hydroxy stretch is observed at $\nu 3402 cm^{-1}$.

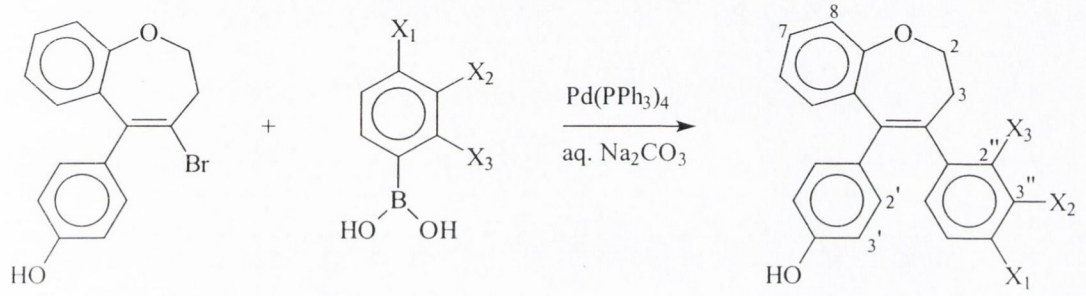
In the ^{1}H NMR spectrum of compound (202), the H-3 protons are observed as a triplet (J=5.8Hz), at δ3.03, being split by the adjoining H-2 protons which are found as a triplet (J=6.0Hz) at δ4.60. The aromatic protons are found as a multiplet between δ6.81-6.88 and δ7.07-7.24. The H-9 aromatic proton occurs as a multiplet at δ6.97.

In the ^{13}C NMR spectrum of 4-bromo-5-(4-hydroxyphenyl)-2,3-dihydro-1-benzoxepin (202), the 4-methoxy signal is not observed, thus verifying the removal of the CH_3 groups. The C-3 and C-2 signals are observed inverted in the DEPT 135 at 41.07 and 77.35ppm. The shielded C-3', C-5' signals are found at 114.97, 115.21ppm, while the corresponding C-2', C-6' signals occur at 129.01ppm. The aromatic C-9 and C-7 signals are found at 121.92 and 123.42ppm, while those of C-8 and C-6 occur at 131.21 and 128.80ppm,

respectively. The quaternary C-1' and C-5a signals are observed at 134.08 and 138.99ppm.

3.4.3.3 Synthesis of 5-(4-hydroxyphenyl)-4-(aryl/heteroaryl)-2,3-dihydro-1-benzoxepin

Initially, Suzuki coupling was carried out on 4-bromo-5-(4-hydroxyphenyl)-2,3-dihydro-1-benzoxepin (202) with aryl substituted boronic acids (168)-(177), (182) and (185). The reaction was carried out under mild conditions, with catalytic amounts of tetrakis(triphenyl)phosphinepalladium (0) and aqueous sodium carbonate. A series of 5-(4-hydroxyphenyl)-4-(aryl)-2,3-dihydro-1-benzoxepins (203)-(213) were synthesised and afforded as oils in good yield. [Scheme 56]



(202) (168) - (177) (203) - (213)

(203)	$X_1 = OCH_3$,	$X_2 = H$,	$X_3 = H$	(209)	$X_1 = Cl$,	$X_2 = H$,	$X_3 = H$
(204)	$X_1 = H$,	$X_2 = OCH_3$,	$X_3 = H$	(210)	ArX_1, X_2, X_3 =	naphthyl	
(205)	$X_1 = H$,	$X_2 = H$,	$X_3 = OCH_3$	(211)	$X_1 = H$,	$X_2 = NO_3$,	$X_3 = H$
(206)	$X_1 = CH_3$,	$X_2 = H$,	$X_3 = H$	(212)	$X_1 = CN$,	$X_2 = H$,	$X_3 = H$
(207)	$X_1 = H$,	$X_2 = CH_3$,	$X_3 = H$	(213)	$X_1 = CHO$,	$X_2 = H$,	$X_3 = H$
(208)	$X_1 = H$,	$X_2 = H$,	$X_3 = CH_3$				

Scheme 56: Suzuki Coupling reaction of 4-bromo-5(4-hydroxyphenyl)-2,3-dihydro-1-benzoxepins

Table 12 details the yields and infrared data of compounds (203)-(213). The characteristic hydroxy stretch for these compounds is found in the range ν3612-3007cm^{-1}. The aliphatic and aromatic alkene stretch occurs between ν1614-1574cm^{-1}, while the C-O-C ether stretch at ν1247 and 1195cm^{-1}.

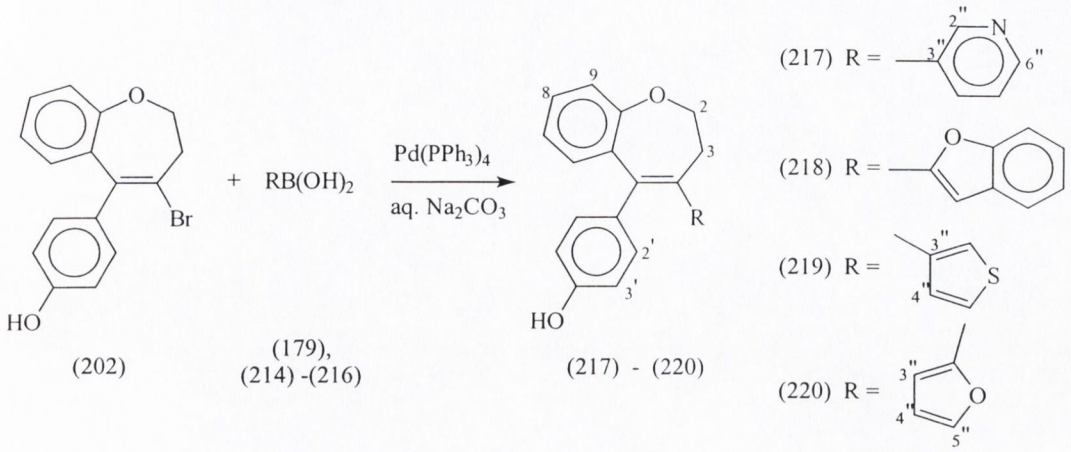
Compound	Yield %	$IR_{\upsilon max}$ (KBr) cm^{-1}
(203)	80	3402 (OH), 1608 (C=C)
(204)	85	3268 (OH), 1597, 1574 (C=C)
(205)	48	3421 (OH), 1614 (C=C)
(206)	93	3310 (OH), 1610 (C=C)
(207)	54	3389 (OH)
(208)	62	3382 (OH), 1607 (C=C)
(209)	70	3401 (OH), 1603 (C=C)
(210)	50	3397 (OH), 1606 (C=C)
(211)	47	3488 (OH), 1604, 1574 (C=C)
(212)	34	3401 (OH), 1607 (C=C)
(213)	73	3325 (OH), 1735 (C=O)

Table 12: Yields and infrared data of compounds (203)-(213)

In the ^{1}H NMR of 4-(4-cyanophenyl)-5-(4-hydroxyphenyl)-2,3-dihydro-1-benzoxepin (212), the H-3 protons are observed as a triplet (J= 6.0Hz) at δ2.73, while the adjoining H-2 protons are found at δ4.62, as a triplet (J=6.0Hz), both integrating for two protons. The aromatic protons are observed as a complex unresolved multiplet between δ6.16-7.55.

The ^{13}C NMR of (212) shows the C≡N signal at 104.19ppm. The DEPT 135 shows two inverted signals at 34.76 and 79.80ppm, which are assigned to C-3 and C-2 respectively. The shielded C-3', C-5' signals are found at 114.14 and 115.90ppm, while the corresponding C-2', C-6' signal occurs at 129.79ppm. The aromatic C-7 and C-6 signals are observed at 123.42 and 128.72ppm, with the C-8 signal occurring at 131.40ppm. The remaining aromatic signals are observed between 130.67 and 133.02ppm, with exception of the quaternary C-1' signal which is found at 133.81ppm. In the low resolution mass spectrum, of (310) the molecular ion ($C_{23}H_{17}NO_2$) is observed as the base peak at m/z 339.

As an extension of this work, the biaryl coupling reaction was carried out with heterocyclic boronic acids. Four different heterocyclic boronic acids were employed in the coupling reaction with the benzoxepin (202); these included 3-pyridinyltriisopropylborate (179), benzo[b]furan-2-boronic acid (214), thiophene-3-boronic acid (215) and furan-2-boronic acid (216) [Scheme 57].



Scheme 57: Synthesis of compound (217)-(220) via the Suzuki Coupling method

These products (217)-(220) were afforded as oils in moderate to good yields. In the infrared spectra of compounds (217)-(220), the characteristic broad hydroxy stretch is found between ν3429-3397cm^{-1}. The yield and infrared data of compounds (217)-(220) are listed in Table 13.

Compound	Yield (%)	IR$_{\upsilon max}$ (KBr) cm^{-1}
(217)	83	3430 (OH), 1658, 1590 (pyridyl peaks)
(218)	46	3408 (OH), 1603 (C=C)
(219)	64	3397 (OH), 2227 (thienyl group)
(220)	81	3429 (OH), 1601, 1584 (C=C)

Table 13: Yield and infrared data of compounds (217)-(220)

In the ^{1}H NMR spectrum of 5-(4-hydroxyphenyl)-4-(3-thienyl)-2,3-dihydro-1-benzoxepin (219), the H-3 protons are observed as a triplet (J=6.0Hz) at δ2.73, while the adjoining H-2 protons are also found as a triplet (J=6.2Hz) at δ4.65. The aromatic protons occur as a complex multiplet between δ6.66-7.24.

In the DEPT 135 spectrum of (219), two signals are found inverted at 34.80 and 80.26ppm, corresponding to C-3 and C-2 respectively. The C-3', C-5' signals, which are shielded from the hydroxy group are found at 114.42ppm, with the C-2', C-6' signals occurring at 129.77 and 130.45ppm. The remaining aromatic signals are found between 123.86-130.45ppm, with the exception of the C-1' and C-3'' quaternary signals at 136.80 and 128.28ppm respectively. The quaternary C-4'' signal is observed at 127.93ppm, while the C-5 and C-5a signals are found at 132.02 and 142.11ppm.

The molecular ions of compounds (203)-(220) were confirmed by low resolution mass spectroscopy.

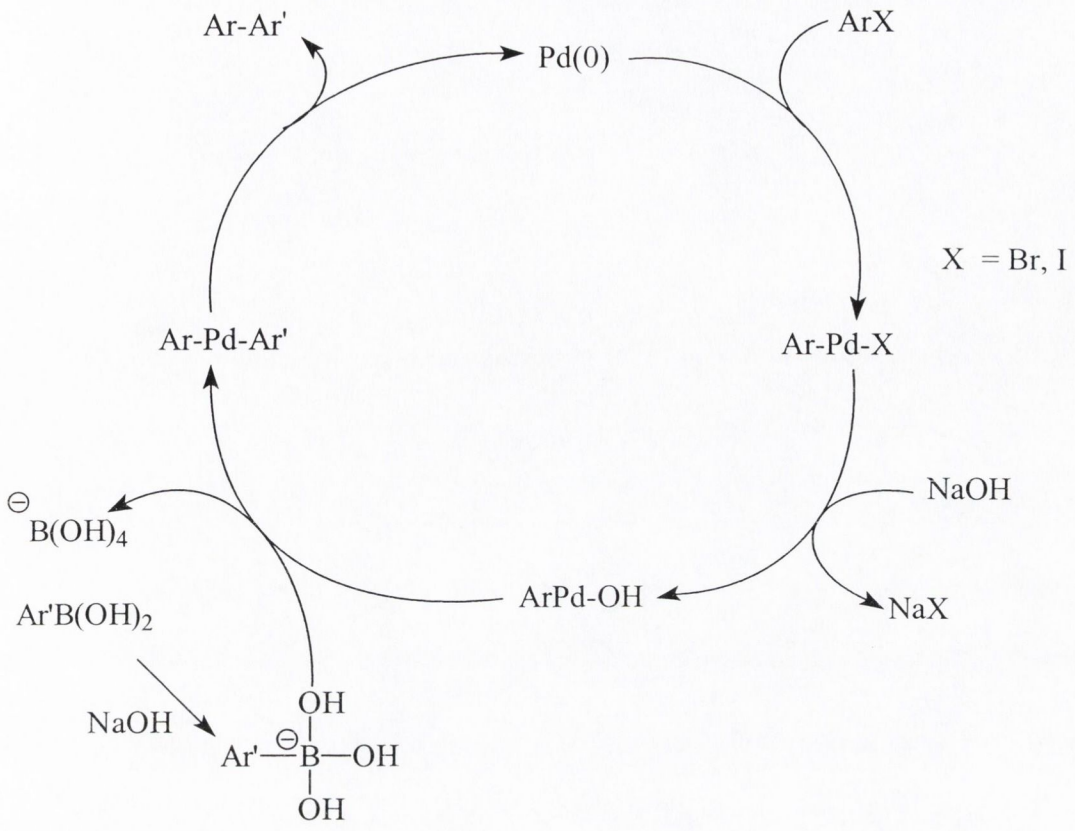
The Suzuki coupling reactions produced high yielding aryl and heterocyclic benzoxepin compounds (203)-(220), although, the reaction was unsuccessful when 4-fluoroboronic acid (175) was reacted with 4-bromo-5-(4-hydroxyphenyl)-2,3-dihydro-1-benzoxepin (202)

Thompson and co-workers[456] found that 4-fluorophenylboronic acid did not couple successfully to a variety of 2-amino-6-halopyrazinoate esters, when using the tetrakis(triphenylphosphine)palladium catalyst. They found that by substituting the $Pd(PPh_3)_4$ catalyst with the binuclear catalyst, 1,1-bis(diphenylphosphine)ferrocene-ligated palladium $[Pd(dppf)(OAc)_2]$,[462,463] the desired coupling reaction was promoted. The postulated reason for this may be that, the decreased steric hinderance enforced by the rigid ferrocene backbone may stretch the Pd-P bond distance from its usual length, thus accounting for the greater efficiency of the $Pd(dppf)_2(OAc)_2$ catalyst. Further work may incorporate the use of this catalyst when coupling 4-fluorophenyl boronic acid (175) to 4-bromo-5-(4-hydroxyphenyl)-2,3-dihydro-1-benzoxepin (202).

3.4.4. Proposed mechanism for Suzuki coupling

Miyaura and Suzuki[464] has suggested a catalytic cycle for the cross coupling of organoboranes with organic halides. The crucial difference noted between organoborane cross coupling and the general catalytic cycle is that, in the former, the oxidative addition is followed by the metathetical displacement of the halide ion from R-Pd-X by a basic species to give an organopalladium alkoxide (R-Pd-OR) or organopalladium hydroxide (R-Pd-OH), depending on the base used.[408] These organopalladium alkoxides and organopalladium hydroxides are believed to be more reactive than the organopalladium halide *(vide infra)* [Scheme 58].

Further mechanistic studies demonstrate that water and base are required to activate the boronic acid and that the rate-determining step depends on the identity of the aryl halide.[405]



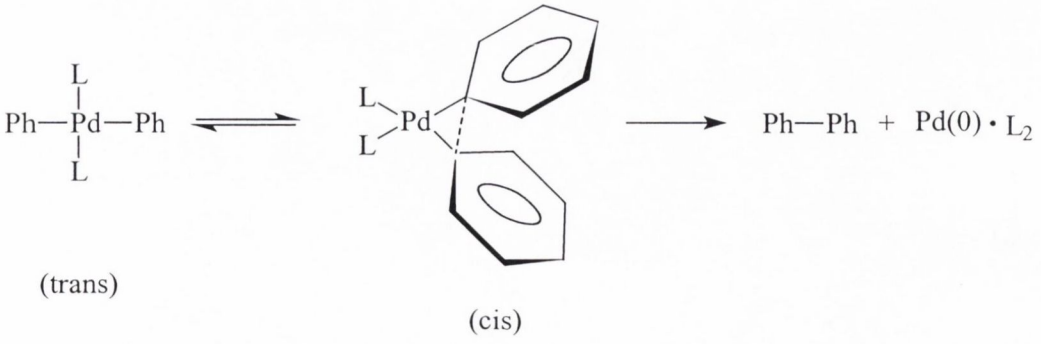
Scheme 58: Proposed catalytic-cycle mechanism for Suzuki coupling

As shown in Scheme 58, two equivalents of base are required in this catalytic cycle. One equivalent is utilised in the formation of boronate, which is consistent with the fact that boronic acids act as Lewis acids, with the formation of a tetravalent boron atom.[465,466] The anionic nature of the organic group in organoboronic acids is enhanced by the formation of the organoboronate.[380] The second equivalent of base is consumed in the metathetical displacement of the halide to form organopalladium hydroxide or alkoxide. The organopalladium hydroxide/alkoxide is more reactive than the organopalladium halide, since the Pd-O bond is more polar than the Pd-Br bond, owing to the greater electronegativity of oxygen relative to bromine. As a result, the electrophilicity of the organopalladium hydroxide is stronger than that of the organopalladium bromide and the electrophilic transmetalation reaction is facilitated. Thus, the transmetalation reaction is favoured by the formation of both the arylboronate and the organopalladium hydroxide. This catalytic cycle clearly explains the failure of attempts to couple organoboronic acids in the absence of a base.[444] It also implies the possibility of selectively coupling organometallics bearing boron and other metal functionalities such as tin with electrophiles, since it is known that the tin coupling reaction (Stille reaction) does not require the presence of a base.

The mechanisms of the oxidative addition and reductive elimination sequences have been intensively studied and are reasonably well understood.[467,468] They are proposed to be fundamentally common processes for all cross-coupling reactions of organometallics. However, much less is known about the transmetalation step because the mechanism is highly dependent on organometallics or the reaction conditions used for the couplings. Stille has inferred that the transmetalation reaction takes place by an electrophilic substitution mechanism, with the organopalladium halide acting as an electrophile.[378]

The oxidative addition is often the rate-determining step in a catalytic cycle. Electron-withdrawing substituents enhance the rate of the coupling, permitting the use of bromides.[407] The relative reactivity decreases in the order of I>OTf>Br>>Cl. Aryl and 1-alkenyl halides activated by the proximity of electron-withdrawing groups are more reactive to oxidative addition, than those with a donating group, thus allowing the use of chlorides such as 3-chloroenone for the cross-coupling reaction. A wide range of palladium (0) catalysts or precursors can be used for the cross-coupling reaction. $Pd(PPh_3)_4$ is most commonly used, but $PdCl_2(PPh_3)_2$ and $Pd(OAc)_2$ plus PPh_3 or other phosphine ligands are also efficient, since they are stable to air and readily reduced to the active Pd(0) complexes with organometallics or phosphines which are used for cross-coupling.[469] Palladium complexes that contain fewer than four phosphine ligands or bulky phosphines such as tris(2,4,6-trimethoxyphenyl) phosphine are in general highly reactive for the oxidative addition because of the ready formation of co-ordinate unsaturated palladium species[369,470].

Reductive elimination of the organic partners from Ar-Pd-Ar' reproduces the palladium (0) complex.[468,471] The reaction takes place directly from *cis*-Ar-Pd-Ar' and the *trans*-Ar-Pd-Ar' reacts after its isomerisation to the corresponding *cis*-complex. [Equation 4]



Equation 4: Reductive elimination to reproduce Pd (0) complex

The order of reactivity is diaryl->(alkyl)aryl->dipropyl->diethyl->dimethyl palladium (II), suggesting participation of the π-orbital of the aryl group during the bond formation.[472] Similar effects are observed in the reductive elimination of related platinum (II) complexes.[473]

The transmetalation between 1-hexenylboronic acid and palladium (II) acetate was first reported by Dieck and Heck.[474] The *in situ* preparation of (E)- or (Z)-1-alkenylpalladium (II) species and its addition to ethyl acrylate readily proceeds at room temperature while retaining their original configurations (Equation 5).

$$BuCH{=}CHB(OH)_2 + Pd(OAc)_2 + CH_2{=}CHCO_2Et \longrightarrow BuCH{=}CHCH{=}CHCO_2Et$$

Equation 5

There is some experimental evidence for transmetalation to transition metals. The reaction of organoboranes with organomercurials proceeds under neutral conditions when $Hg(OAc_2)$, $Hg(OR)_2$ or HgO is used.[475] It has also been reported that the addition of sodium hydroxide or other base exerts a remarkable effect on the transmetalation rate of organoboron reagents with metallic halides, such as mercuric,[475,476] silver,[416] auric[477] and platinic halides.[477] Thus transmetalation with transition-metal complexes proceeds very well, but the choice of suitable bases and ligands on transition-metal complexes is essential.[369]

In summary the aryl-aryl Suzuki coupling reaction involves:

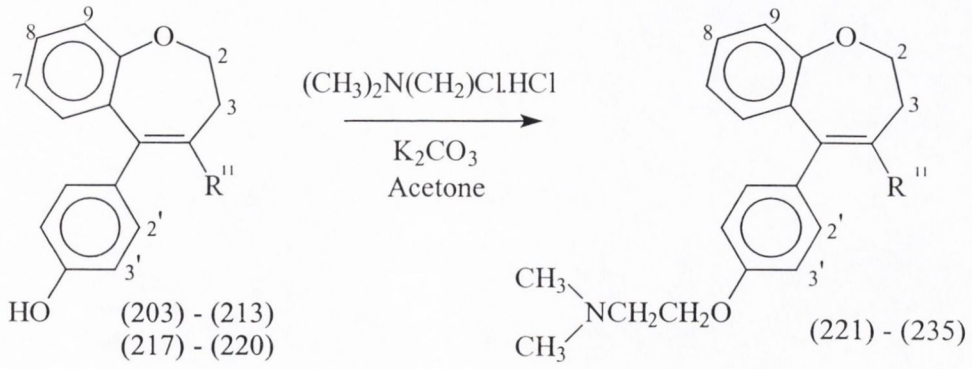
(1) oxidative addition of Pd(0) to aryl halides

(2) transmetalation of the ArPdBr with $Ar'B(OH_3)^-Na^+$, and finally

(3) reductive elimination to give Ar-Ar'.[87,381]

3.4.5. Alkylation of Suzuki coupled benzoxepins

In the present work alkylation of 5-(4-hydroxyphenyl)-4-(aryl/heteroaryl)-2,3-dihydro-1-benzoxepins (203)-(213) / (217)-(220) was carried out using 2-dimethylaminoethylchloride hydrogen chloride to produce the tamoxifen side chain benzoxepin derivatives (221)-(235). [Scheme 59]



(221) R = 4-methoxyphenyl	(226) R = 2-methylphenyl	(231) R = 4-formylphenyl
(222) R = 2-methoxyphenyl	(227) R = 4-chlorophenyl	(232) R = 3-pyridilyl
(223) R = 3-methoxyphenyl	(228) R = 1-naphthyl	(233) R = benzo(b)furyl
(224) R = 4-methylphenyl	(229) R = 3-nitrophenyl	(234) R = 3-thienyl
(225) R = 3-methylphenyl	(230) R = 4-cyanophenyl	(235) R = 2-furyl

Scheme 59: Alkylation reactions to produce 5-[(4-dimethylaminoethoxy)phenyl]-4-(aryl or heterocyclic)-2,3-dihydro-1-benzoxepins (221)-(235)

The compounds were prepared using the alkylation procedure described in Section 2.7 and were afforded as oils in moderate yields. Table 14 outlines the yield and infrared spectroscopic data for compounds (221)-(235).

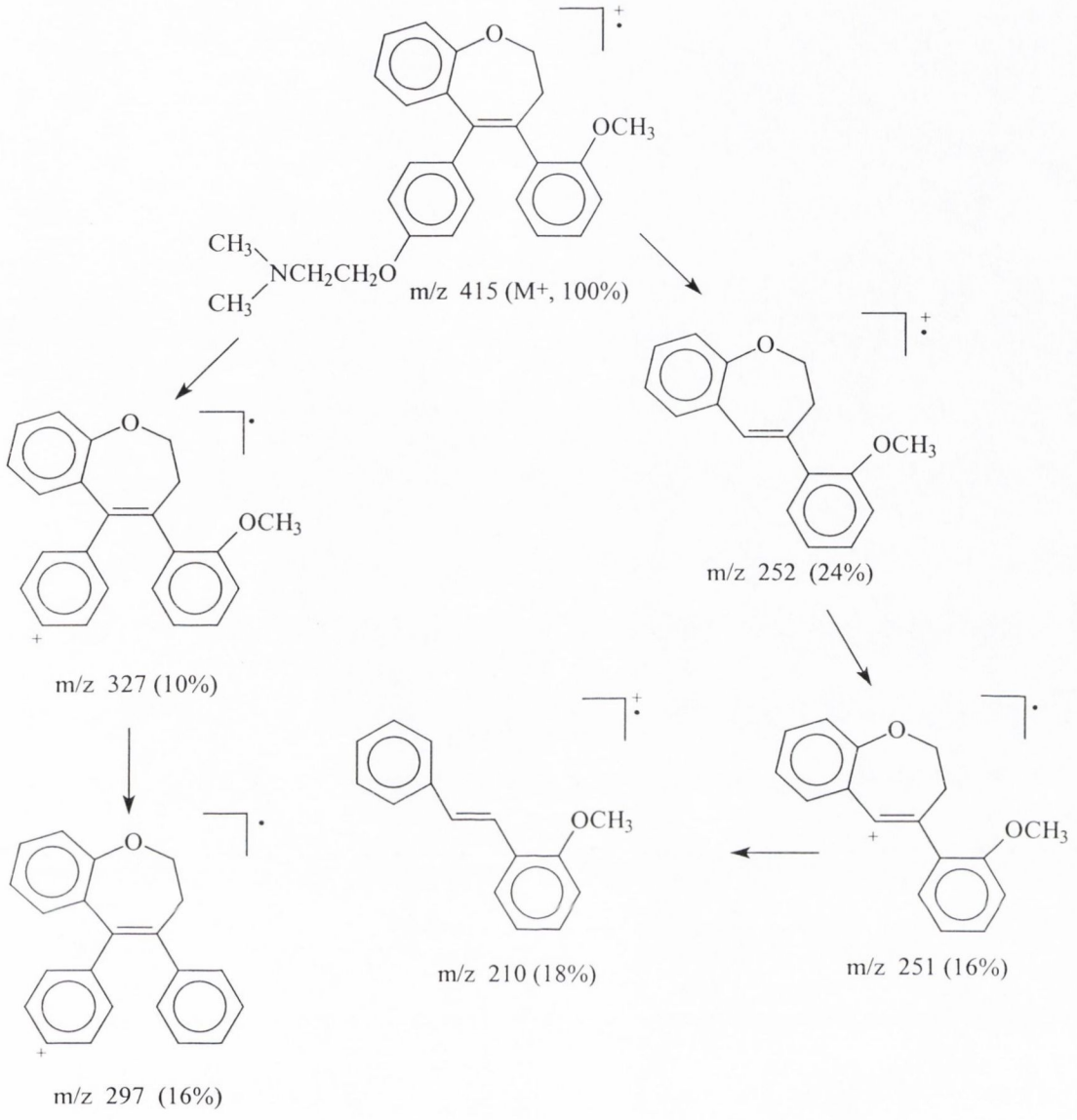
Compound	Yield %	$IR_{\upsilon max}$ (KBr) cm^{-1}	M^+
(221)	60	1607 (C=C)	415
(222)	50	1606, 1576 (C=C)	415
(223)	54	1605 (C=C)	415
(224)	64	1606, 1575 (C=C)	399
(225)	41	1606 (C=C)	399
(226)	45	1603 (C=C)	399
(227)	95	1602 (C=C)	419
(228)	46	1606 (C=C)	435
(229)	76	1605 (C=C)	430
(230)	83	1608 (C=C)	410
(231)	74	1724 (C=C)	413
(232)	49	1607 (C=C)	386
(233)	68	1606 (C=C)	425
(234)	71	1606, 1570 (C=C)	391
(235)	38	1606 (C=C)	375

Table 14: Yield and infrared data of compounds (221)-(235)

In the ^{1}H NMR of 5-[(4-dimethylaminoethoxy)phenyl]-4-(3-pyridinyl)-2,3-dihydro-1-benzoxepin (232), the methyl (CH_3) protons are found as a singlet at δ2.36. The methylene H-3 protons are observed as a multiplet along with the NCH_2 protons in the range δ2.72-2.75, integrating for four protons. The H-2 methylene protons are found as a triplet at δ4.63 (J=6.0Hz). The aromatic protons are found between δ6.68-7.61.

In the ^{13}C NMR spectrum of (232), the methyl ($(CH_3)_2$) signal is found at 45.23ppm. The signals for C-3 and C-2 are found inverted in the DEPT 135 at 34.65 and 79.96ppm. Two further inverted signals are observed at 57.65 and 65.22ppm and are assigned to NCH_2 and OCH_2. The shielded C-3' and C-5' are observed at 113.64ppm, while the C-2' and C-6' signals occur at 129.32ppm. The C-8 signal is observed at 130.60ppm, while C-6 and C-7 signals are found at 128.41 and 123.20ppm respectively. The pyridinyl signals are found at 121.68 and 139.58ppm corresponding to C-3" and C-4". The signals for C-2" and C-6" are observed at 149.83 and 146.78ppm respectively.

Table 14 depicts the molecular ions for compounds (221)-(235), which were obtained via low resolution mass spectra. The proposed fragmentation pattern for (222) is displayed in Scheme 60. Loss of the dimethylaminoethoxy substituent gives m/z 327 (10%), while further cleavage of OCH_3 gives m/z 297 (16%) fragment. Cleavage of $C_{10}H_{14}NO$ afforded the m/z 252 (24%) fragment; further loss of H afforded the m/z 251 (16%) fragment. The m/z 210 (18%) fragment was afforded from the molecular ion by loss of $C_{12}H_{10}$.



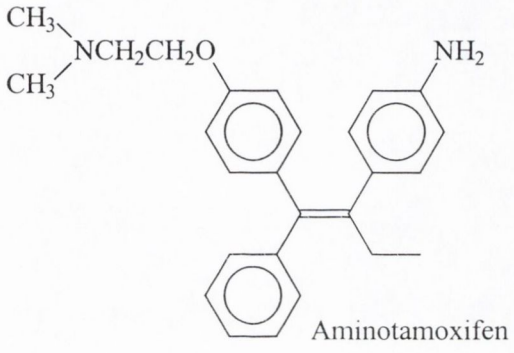
Scheme 60: Postulated mass spectral fragmentation pattern for 5-[(4-dimethylaminoethoxy)phenyl)]-4-(4-methoxyphenyl)-2,3-dihydro-1-benzoxepin (222)

3.4.6. Summary of tamoxifen-like structures

The Suzuki coupling reactions for synthesis of these benzoxepin compounds was very successful in producing a wide variety of novel products. These compounds were biochemically tested for their inhibition of proliferation and binding ability for breast MCF-7 cancer cells (discussed in Chapter 4).

3.5. Nitro-benzoxepins

Aminotamoxifen (236) [Figure 49] has relative binding affinity to estradiol similar to that of tamoxifen.[42] The amino portion of the molecule is known to exhibit a remarkable antineoplastic activity on a wide spectrum of tumours by acting on the guanine residue of the DNA molecule.[478] It is believed that multiple cellular effects of amino-derivatives may include effects on prostaglandin synthetase, on Ca^{2+}-calmodulin-dependent enzymes, on protein kinase C or an interaction with membrane phospholipids.[479]



Aminotamoxifen

Figure 49: Structure of Aminotamoxifen (236)

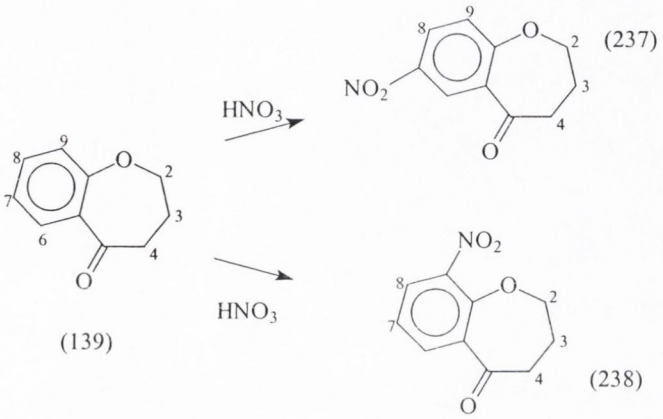
It was envisaged that nitration, followed by conversion to amino derivatives of 2,3,4,5-tetrahydro-1-benzoxepin-5-one (139) could afford conformationally restricted analogues similar to iodotamoxifen, but containing an oxygen atom within the seven membered ring. It is thought that the combination of the nitro or amino group and the oxygen atom in the benzoxepin ring, may afford an extra site for binding to the estrogen receptor. The amino group could also be converted to a number of other functional groups including cyano or halide derivatives, which in turn may be useful in the preparation of new antiestrogens.

In this research both 7- and 9-nitro-2,3-dihydro-1-benzoxepin-5-one were prepared. Subsequent arylation with bromoanisole, bromination at C-4, followed by a Suzuki coupling with phenylboronic acid was carried out to form 5-(4-methoxyphenyl)-7-nitro-4-phenyl-2,3-dihydro-1-benzoxepin (242).

3.5.1. Synthesis of 7- and 9-nitro-2,3-dihydro-1-benzoxepin-5-one.

Buckle and co-workers[344] prepared both 7- and 9-nitro-2,3-dihydro-1-benzoxepin-5-one on route to synthesis of 7-cyanobenzoxepin, which is believed to be a smooth muscle relaxant and a potassium-channel activator. White fuming nitric acid was added to a solution of 2,3,4,5-tetrahydro-1-benzoxepin-5-one (139) in concentrated nitric acid at -30°C. Buckle found that when the reaction

was carried out between –50 and -70°C, the 7,9-dinitro derivative was formed. However, under the milder reaction conditions (-10 to -20°C), it is possible to limit nitration to the formation of monoderivatives, and a 5:1 mixture of 7- and 9-nitro compounds respectively can be produced. [Scheme 61]. In the present work suitable conditions were not found for the selective formation of one or other mono nitro derivative, but pure isomers were isolatable by chromatography.[344]



Scheme 61: Synthetic route to 7- and 9-nitro-2,3-dihydro-1-benzoxepin

The 7-nitro isomer (237) was isolated as oily crystals in 41% yield, while the 9-nitro isomer (238) was afforded as an oil in only 9% yield. Table 15 details the yield and infrared spectral data of compounds (237) and (238).

Compound	Yield %	$IR_{\upsilon max}$ (KBr) cm^{-1}
(237)	41	1692 (C=O), 1534, 1360 (Ar-NO_2)
(238)	9	1687 (C=O), 1531, 1328 (Ar-NO_2)

Table 15: Yield and infrared data of compounds (237) and (238)

In the ^{1}H NMR of 7-nitro-2,3-dihydro-1-benzoxepin-5-one (237), the methylene H-3 protons are observed as a triplet (J=6.8Hz) at δ2.32, while the adjoining H-2 protons, being split by H-3 are found at δ2.95 as a triplet (J=7.3Hz). The H-4 methylene protons are observed downfield due to the proximity of the C=O group as a triplet (J=6.5Hz) at δ4.36. The aromatic proton H-9 was found as a doublet (J=9.0Hz) at δ7.18, while H-8 occurs at δ8.25 as a double doublet (J=9.0, 3.0Hz). The remaining aromatic proton H-6 was found further downfield at δ8.63 due to the deshielding effect of the 7-NO_2 group, as a doublet (J=3.0Hz).

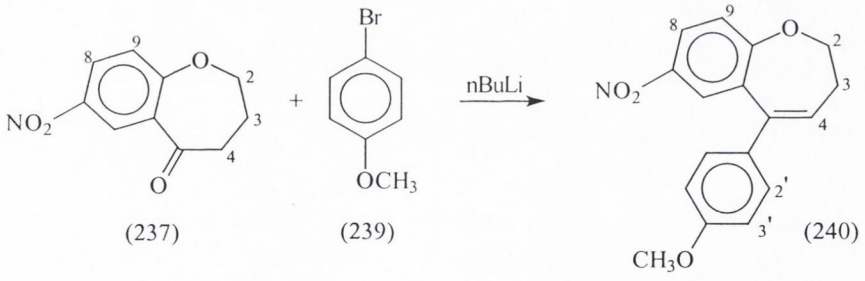
Table 16 details the ^{13}C NMR spectral data of the 7-nitro (237) and 9-nitro (238)-2,3-dihydro-1-benzoxepin-5-ones.

Carbon	($CDCl_3$)ppm (237)	($CDCl_3$)ppm (238)
C-2	73.40	75.23
C-3	26.05	26.25
C-4	40.23	40.51
C-5a	133.39	135.60
C-6	125.70	126.32
C-7	128.13	122.01
C-8	122.33	122.45
C-9	121.17	129.12
C=0	207.91	204.11

Table 16: ^{13}C NMR spectral details of (237) and (238)

3.5.2. Arylation of 7-nitro-2,3-dihydro-1-benzoxepin.

5-(4-Methoxyphenyl)-7-nitro-2,3-dihydro-1-benzoxepin (240) was afforded from 7-nitro-2,3-dihydro-1-benzoxepin-5-one (237) with 4-methoxyphenyllithium as a pair of rotamers in a 59% yield. [Scheme 62]. The infrared spectrum of 5-(4-methoxyphenyl)-7-nitro-2,3-dihydro-1-benzoxepin (240) shows the aliphatic and aromatic alkene stretching at υ1605 and 1572 cm^{-1}, while the C-O-C ether stretch occurs at υ1248 and 1179cm^{-1}. The aryl-nitro medium intensity stretches are found at ν1492 and 1342cm^{-1}.



Scheme 62: Synthesis of 5-(4-methoxyphenyl)-7-nitro-2,3-dihydro-1-benzoxepin (240)

The ^1H NMR spectrum of (240) at ambient temperature shows chemical non-equivalence of the geminally related methylene protons on the benzoxepin ring. McCague[179] observed similar features when 1-methyl-8-phenyl-9-(4-methoxyphenyl)-6,7-dihydro-5-benzocycloheptene was prepared. The protons of compound (240) at C-2 and C-3 give distinct separate multiplets. At ambient temperature the rotamers do not interconvert on the NMR time scale. At elevated temperatures the signals broaden and coalesce at 137°C which from the

Eyring equation corresponds to $\Delta G\pm = 20.5$ kcal mol^{-1} for the racemisation and an energy barrier of this magnitude should allow the separation of enantiomers at around 0°C.[179]

The nitro bearing ring suffers hindered rotation and the barrier against chemical equivalence of its diastereotopic aryl-hydrogen atoms has a specific ΔG, estimated around 13kcal mol^{-1}. Therefore the fused phenyl ring of compound (240) is prevented from rotating completely out of plane of the olefinic bond and the presumed mechanism for conformer inversion is by flipping of the two remaining rings depicted in Figure 50. The nitro group brings the unfused ring out of the plane with the olefinic bond more readily, allowing passage to the racemised transition state of (240). A further consequence of the unfused ring being out of plane is that this ring no longer activates the olefinic bond to electrophilic attack.

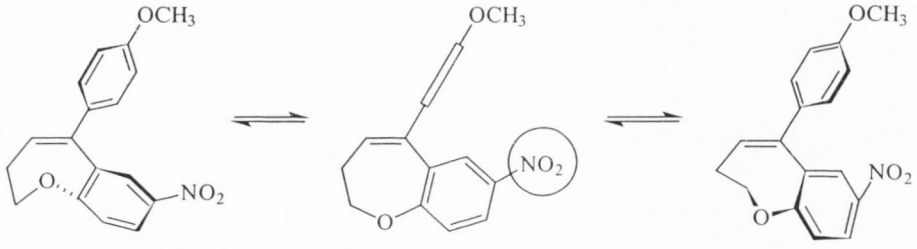


Figure 50: Presumed mechanism for racemisation in compound (240)

Thus the rotamers of compound (240) are due to the two different conformations of the 7-membered ring. Okamoto *et al*[480] and Gunther[481] have demonstrated the efficient separation of enantiomeric conformers differing by the helicity generated by the orientation of aromatic rings by analytical HPLC. This system separates the enantiomers of compounds completely at -5°C, but the isolated enantiomers rapidly racemise if allowed to heat up to room temperature. Similarily enantiomers may be seperated using NMR at elevated temperatures, however due to the poor dissolution of (240) in DMSO, a ^{1}H NMR spectrum at the elevated temperature (approx. 137°C) was not obtained. Also dynamic NMR studies can reveal an interaction between the aryl π-face and the nitro group, however no evidence was available from the NMR of compound (240).

Table 17 details the ^{1}H NMR, rotamers (A) and (B) corresponding to the AB system of 5-(4-methoxyphenyl)-7-nitro-2,3-dihydro-1-benzoxepin (240).

Proton	240(A)	240(B)
OCH_3	3.84 (3H, s)	3.87 (3H, s)
H-2	4.46 (2H, m)	4.48 (2H, m)
H-3	2.47 (2H, m)	2.62 (2H, m)
H-4	6.28 (1H, m)	6.37 (1H, m)
ArH	6.78-8.09 (7H, m)	6.78-8.09 (7H, m)

Table 17: ^{1}H NMR details of AB system of compound (240)

Figure 51 shows the A and B rotamer of compound (240).

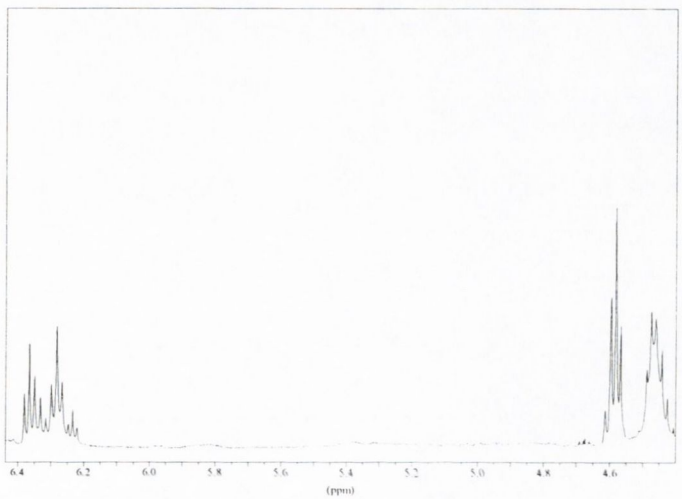
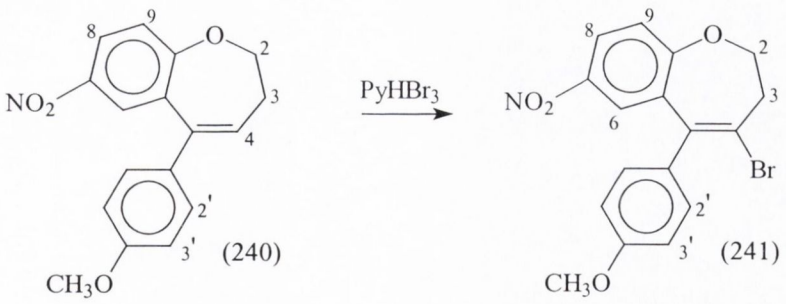


Figure 51: ^{1}H NMR Spectrum of compound (240)

In the ^{13}C NMR spectrum of (240), distinct signals are observed for C-2, C-3 and the OCH_3 group. In the DEPT 135, the C-3 signal is found inverted at 31.86(A) and 32.39(B)ppm, while the adjoining C-2 occurs at 77.21(A) and 77.96(B)ppm. The 4'-methoxy signal is observed at 55.21(A) and 55.40(B)ppm, while the C-4 signal is found at 158.47ppm. The aromatic C-3', C-5' signals are observed at 112.44-114.89. The aromatic C-8 and C-6 signals are found at 122.12(A), 122.59(B)ppm and 123.14(A), 123.50(B)ppm respectively. The C-9 signal occurs at 121.31ppm, while C-7 is observed at 126.35(A) and 126.85(B)ppm.

3.5.3. 4-Bromo-5-(4-methoxyphenyl)-7-nitro-2,3-dihydro-1-benzoxepin (241).

4-Bromo-5-(4-methoxyphenyl)-7-nitro-2,3-dihydro-1-benzoxepin (241) was prepared using pyridinium bromide perbromide mediated bromination of the corresponding 5-(4-methoxyphenyl)-7-nitro-2,3-dihydro-1-benzoxepin (240) [Scheme 63].



(240) (241)

Scheme 63: Synthesis of 4-bromo-5-(4-methoxyphenyl)-7-nitro-2,3-dihydro-1-benzoxepin (241)

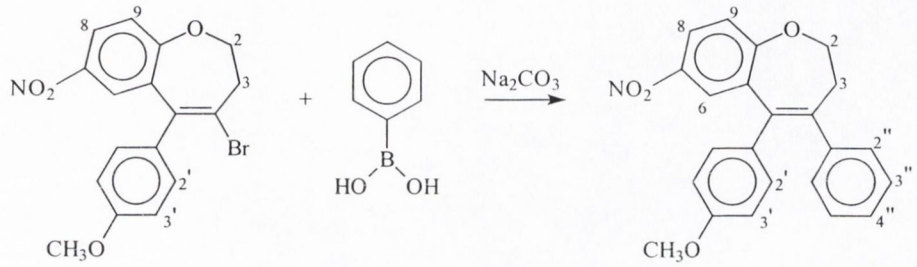
Compound (241) was synthesised with a view to subsequent arylation and investigation as a conformationally restricted tamoxifen like structure. 4-Bromo-5-(4-methoxyphenyl)-7-nitro-2,3-dihydro-1-benzoxepin was obtained as an oil in only a 22% yield.

In the ^{1}H NMR spectrum of (241) the methylene H-3 protons are observed as a triplet (J=5.8Hz) at δ3.13, with the H-2 protons occurring at δ4.69, also as a triplet (J=5.5Hz). The 4'-methoxy protons are observed as a singlet at δ3.89. The aromatic H-8 and H-9 protons appear as a doublet (J=8.5Hz), while the H-6 proton is observed at δ8.06 as a double doublet (J=9.0, 3.0Hz). The remaining aromatic protons are found between δ7.15-7.74. There was no evidence of rotamers present in this compound.

In the ^{13}C NMR spectrum of (241), the 4'-methoxy signal is observed at 54.78ppm. The C-2 and C-3 signals are found inverted in the DEPT 135 spectrum at 76.71 and 40.96ppm respectively. The shielded C-3' and C-5' signals occur at 113.59, 115.22ppm, while the corresponding C-2', C-6' are found at 130.45ppm. The aromatic C-9 and C-8 signals are observed at 122.34 and 123.50ppm, with the C-6 and C-7 signals occurring at 124.61 and 127.12ppm. The quaternary signals of C-5a and C-9a are found at 132.43ppm. Verification of the identity of the compound (241) was obtained from low resolution mass spectrometry, as the molecular ion is observed at m/z 375 and m/z 377 for $C_{17}H_{14}BrNO_4$ corresponding to the two isotopes of bromine.

3.5.4. Suzuki coupling of 4-bromo-5-(4-methoxyphenyl)-7-nitro-2,3-dihydro-1-benzoxepin.

5-(4-Methoxyphenyl)-7-nitro-4-phenyl-2,3-dihydro-1-benzoxepin (242) was prepared via Suzuki coupling with phenylboronic acid, using the conditions described in Section 3.4.3 [Scheme 64].



(241) (186) (242)

Scheme 64: Synthesis of 5-(4-methoxyphenyl)-7-nitro-4-phenyl-2,3-dihydro-1-benzoxepin (242)

In the infrared spectrum of compound (242), the aliphatic and aromatic alkene stretch is observed at υ1606, 1571 cm^{-1}, while the ether C-O-C stretch is found at 1247, 1171cm^{-1}.

In the ^{1}H NMR spectrum of 5-(4-methoxyphenyl)-7-nitro-4-phenyl-2,3-dihydro-1-benzoxepin (242), the C-3 protons are observed as a triplet (J=5.8Hz) at δ2.79, while the H-2 protons are found at δ4.75 as a triplet (J=5.8Hz). The 4'-methoxy protons occur as a singlet at δ3.78. The aromatic protons are found between δ6.68-7.82. No rotamers observed for compound (242).

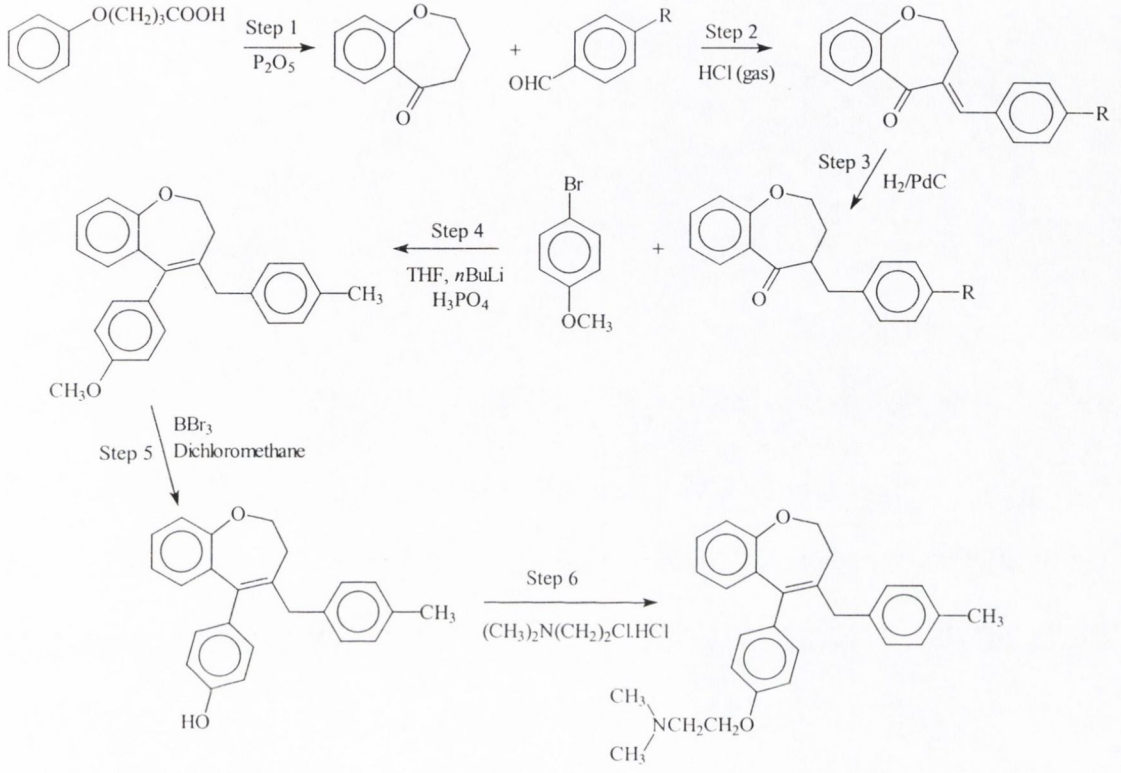
In the ^{13}C NMR of compound (242), the 4'-methoxy signal is found at 54.66ppm. The C-3 and C-2 signals are found inverted in the DEPT 135 spectrum at 35.14 and 80.82ppm respectively. The shielded C-3', C-5' signals occur at 113.13, 114.82ppm, while the corresponding C-2', C-6' signals are observed at 130.42 and 130.45ppm. The aromatic C-9 and C-8 signals are found at 122.58 and 123.19ppm respectively, with the C-6 and C-7 signals occurring at 126.38 and 127.69ppm. The remaining aromatic signals are found between 128.33 and 130.45ppm.

In the low resolution mass spectrum of 5-(4-methoxyphenyl)-7-nitro-4-phenyl-2,3-dihydro-1-benzoxepin (242), the molecular ion is observed at m/z373 ($C_{23}H_{19}NO_4$).

In conclusion, these nitro compounds may be the precursors for future work. The nitro group could undergo catalytic hydrogenation to give the corresponding amino benzoxepin. Once the methoxy group is converted to an alkylamino phenyl group it would contain the overall structure of aminotamoxifen and therefore may obtain comparable antiestrogenic activity.

3.6. Synthesis of benzoxepin analogues possessing an extra benzylic methylene group

The overall synthetic route for these compounds which contain an extra benzylic methylene group is described in Scheme 65 and involves six steps.

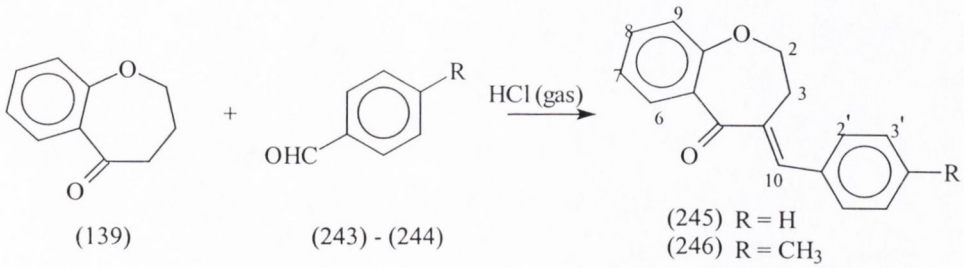


Scheme 65: Synthetic route to 5-[(4-dimethylaminoethoxy)phenyl]-4-(4-methylphenyl)methyl-2,3-dihydro-1-benzoxepin.

The novel-flexible tamoxifen analogues, the preparation of which was described in Chapter 2, possessed an extra methylene benzyl group. These compounds possessed good binding and antiproliferative activities, however they were produced as both the E- and Z-isomers. It was proposed that because the benzoxepins are non-isomerisable, the presence of the extra methylene benzyl group in these compounds may enhance their affinity for the estrogen receptor.

3.6.1. Synthesis of 4-arylmethine-2,3,4,5-tetrahydro-1-benzoxepin-5-ones.

4-Arylmethine-2,3,4,5-tetrahydro-1-benzoxepin-5-ones (245), (246) were obtained by acid catalysed (HCl) condensation[482] of 2,3,4,5-tetrahydro-1-benzoxepin-5-one (139) with the appropriately substituted benzaldehyde at room temperature as outlined in Scheme 66.



Scheme 66: Synthesis of 4-arylmethine-2,3,4,5-tetrahydro-1-benzoxepin-5-ones

The 4-arylmethine-2,3,4,5-tetrahydro-1-benzoxepin-5-ones (245), (246) were obtained in moderate yields. Positive identification of the products was obtained from spectroscopic data. Table 18 details the yield, melting point and infrared data for compounds (245) and (246).

Compound	Yield %	m.p. °C	$IR_{\upsilon max}$ cm^{-1}
(245)	38	oil	1657 (C=O), 1605, 1574(C=C)[69]
(246)	81	116.5 – 117.5	1665 (C=O), 113 (C=C)[347]

Table 18: Yield, m.p. and infrared data of compound (245)-(246)

It is proposed that the 4-arylmethine-2,3,4,5-tetrahydro-1-benzoxepin-5-ones (245), (246) are obtained in E-configuration or as the *trans* isomer, where the aryl ring lies *trans* to the carbonyl group, which is in keeping with related assignments proposed by Brady *et al.*[483] Brady and co-workers suggested that the olefinic proton of the *trans* [E] isomer of 2,3-dihydro-2-phenylmethylene-1H-indan-1-one occurs at higher chemical shift values, being masked by the aromatic protons, than the corresponding [Z] isomer, due to the deshielding effects resulting from the diamagnetic anisotropy of the carbonyl group. As a result of these studies, it was concluded that the *trans* derivative of arylmethylene cyclohexanones, indanones and tetralones are usually produced by acid / base catalysed aldol condensation reactions, except when a high degree of steric overcrowding renders the *trans* isomer unstable. Hence, the compounds synthesised here are conventionally assigned as the *trans* isomeric form.

The carbonyl stretching in the infrared spectrum of (245) and (246) is observed at ν1665 -1676cm^{-1} and the alkene C=C stretching is found at ν1603-1605cm^{-1}.

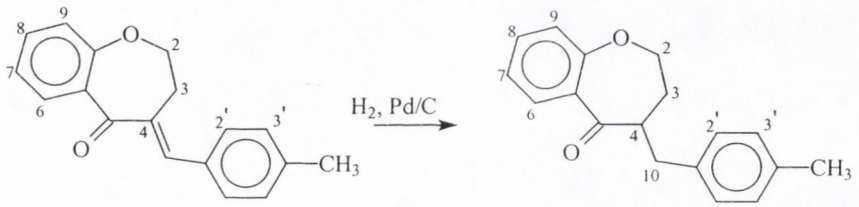
In the ^{1}H NMR spectrum of (246), the methyl protons resonate as a singlet at δ2.36 and the H-3 protons occur at δ2.97 as a triplet (J=5.6Hz). The deshielded H-2 protons are observed as a triplet (J=5.6Hz) at δ4.34. The vinylic H-10 proton is found downfield as a singlet at δ7.78. The aromatic protons are

observed in the range δ7.07 - 7.42, with the exception of the H-6 proton which is found as a double doublet (J=7.7, 1.8Hz) at δ7.97.

The ^{13}C NMR spectrum of (246) shows a signal at 31.13ppm, which is assigned to the methyl (CH_3) group. In the DEPT 135, two inverted signals are observed at 32.25 and 72.28ppm, corresponding to C-3 and C-2 respectively. The C-4 signal is found at 56.76ppm. The aromatic signals are found within the range 119.93-135.36 cm^{-1}.

3.6.2. Synthesis of 4-arylmethylene-2,3,4,5-tetrahydro-1-benzoxepin-5-one.

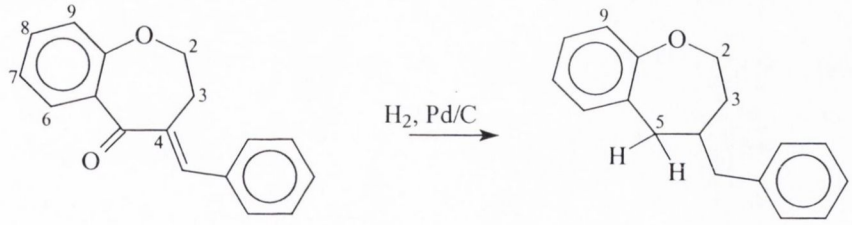
4-(4-Methylphenyl)-methylene-2,3,4,5-tetrahydro-1-benzoxepin-5-one (248)[347] was obtained from the hydrogenation of 4-(4-methylpheny)methyine-2,3,4,5-tetrahydro-1-benzoxepin-5-one (245) over palladium/carbon in good yield. The reaction was monitored by TLC analysis [Scheme 67].



(246) (248)

Scheme 67: Hydrogenation of (246) to obtain 4-(4-methylphenyl)methylene-2,3,4,5-tetrahydro-1-benzoxepin-5-one (248)

Hydrogenation of 4-phenylmethine-2,3,4,5-tetrahydro-1-benzoxepin-5-one (245) resulted in conversion of the carbonyl (C=O) group to a CH_2 [Scheme 68].



(245) (247)

Scheme 68: Over-hydrogenation to compound (247)

The reaction was repeated several times and the reaction conditions for isolation of the desired product were determined.

In the infrared spectrum of (248) the carbonyl stretch is observed at ν1687cm^{-1}, while the aromatic alkene stretch is observed at ν1600cm^{-1} and ν1573cm^{-1}, with

no aliphatic alkene stretching being observed. The alkene stretching is present at $\nu 1208 cm^{-1}$.[484]

In the ^1H NMR spectrum of (248)[347] the methyl protons occur as a singlet at δ2.35 and the H-4 proton is found to resonate as a multiplet at δ3.40. Two multiplets in the ranges δ1.78-1.80 and δ2.37-2.49 are assigned to H-3 protons, which consist of two non-equivalent hydrogens Ha and Hb, one being slightly more shielded than the other and therefore resonating slightly more downfield. Each set of multiplets for H-3 integrates for one proton. These latter signals also couple with the H-4 multiplet and an apparent double triplet (J=12.1, J=5.0Hz) at δ3.92-3.99 and a double quartet (J=12.4, J=6.9Hz) at δ4.49 and are assigned to the non-equivalent H-2 methylene protons (Hc and Hd). A pair of double doublets at δ2.79 and δ3.29 was found to couple to each other and to the H-4 signal and thus were assigned to the non-equivalent methylene protons at H-10 [He and Hf]. The protons He and Hf are represented by double doublets, due to geminal coupling and H-4. Hf appears downfield at δ3.29 (J=14.0, 6.3Hz), while He which is more shielded, appears at δ2.79 (J=13.9, 8.1Hz). The aromatic H-7 proton is observed as a double triplet (J=7.9, 2.0Hz) at δ7.42, while H-6 is found at δ7.74 as a double doublet (J=7.8, 1.7Hz) having been split by H-7 and H-8. The remaining six aromatic protons resonate as an unresolved multiplet in the range δ6.94-7.12.

3.6.3. Synthesis of 4-(4-methylphenyl)methyl-5-phenyl-2,3-dihydro-1-benzoxepin (252).

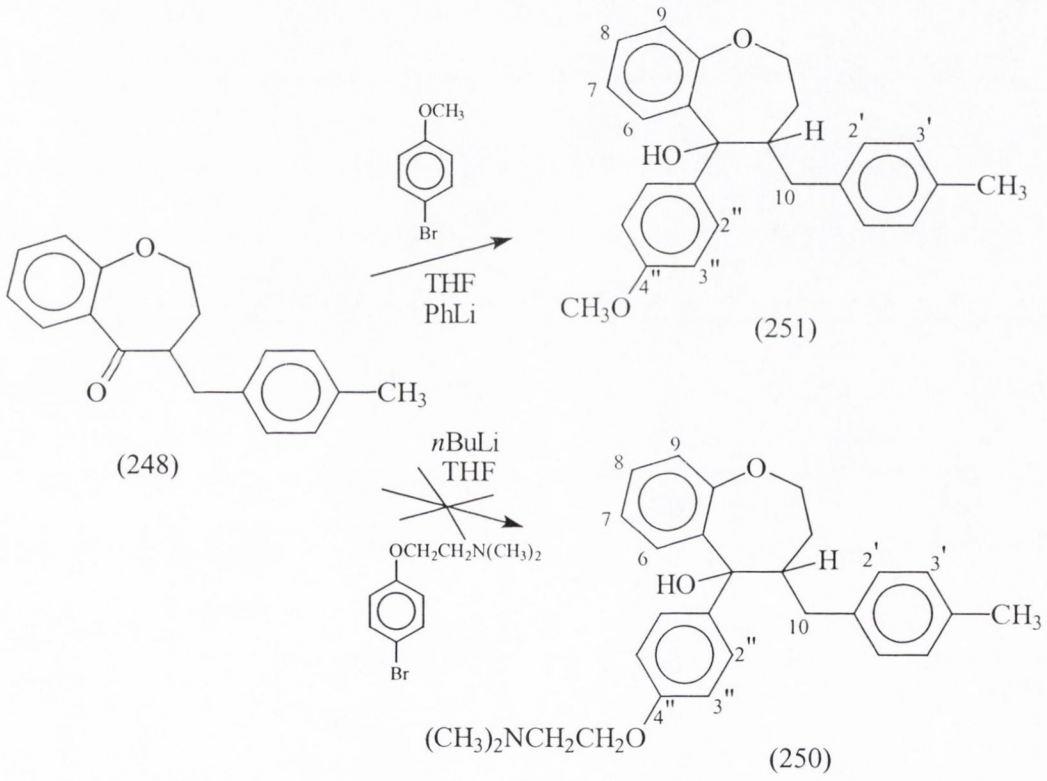
This compound was prepared from (248) using the method described in Section 3.2.2, with the reagent *n*-butyllithium and 4-bromoanisole. The resulting alcohol was initially isolated and characterised, after which it was dehydrated to the corresponding alkene.

3.6.3.1 Preparation of 4-arylmethyl-5-phenylbenzoxepin-5-o1.

4-(4-Methylphenyl)methyl-2,3,4,5-tetrahydro-1-benzoxepin-5-one (248) was phenylated using two different reagents. Initially 4-[(2-dimethylamino)ethoxy] phenylbromide (249) was reacted with (248), using *n*-butyllithium in dry THF to form 5-[(4-dimethyaminoethoxy)phenyl]-4-(4-methylphenyl)methyl-2,3-dihydro-1-benzoxepin-5-ol (250), but the reaction afforded only the starting material. 4-[(2-Dimethylamino)ethoxy] phenylbromide (249) was prepared by refluxing 4-bromophenol and 2-dimethylaminoethylchloride hydrogen chloride in acetone.

Compound (249) was afforded as an oily gel in 98% yield. Using this reagent it had been anticipated to reduce the number of steps in the overall synthetic scheme. Due to the failure of this method, a longer route of synthesis was devised, using 4-bromoanisole and *n*-butyllithium to obtain 5-(4-methoxyphenyl)-4-(4-methylpheny)methyl-2,3,4,5-tetrahydro-1-benzoxepin-5-ol (251). Dehydration of (251) can be carried out *in situ* with concentrated HCI or on the isolated product (251) with 85% H_3PO_4.

The compound (251) was isolated as an oil in 74% yield, prior to dehydration to the corresponding 5-(4-methoxyphenyl)-4-(4-methylphenyl)methyl-2,3-dihydro-1-benzoxepin (252). This compound was positively identified using infrared, ^1H NMR, ^{13}C NMR and mass spectrometry. [Scheme 69].



Scheme 69: Synthesis of 4-arylmethyl-5-phenylbenzoxepin-5-ol (251)

In the infrared spectrum of (251) the hydrogen bonded (OH) stretch is observed in the range ν3602-3175cm^{-1}, while the aromatic alkene stretches occur at ν3050cm^{-1}.

The ^1H NMR spectral assignment of 5-(4-methoxyphenyl)-4-(4-methylphenyl) methyl-2,3,4,5-tetrahydro-1-benzoxepin-5-o1 (251), was aided by a H-H COSY spectrum which provided information on coupling between individual protons of this system. The methyl and methoxy protons occur as singlets at δ2.32 and δ3.81 respectively, showing no coupling in the H-H COSY spectrum as

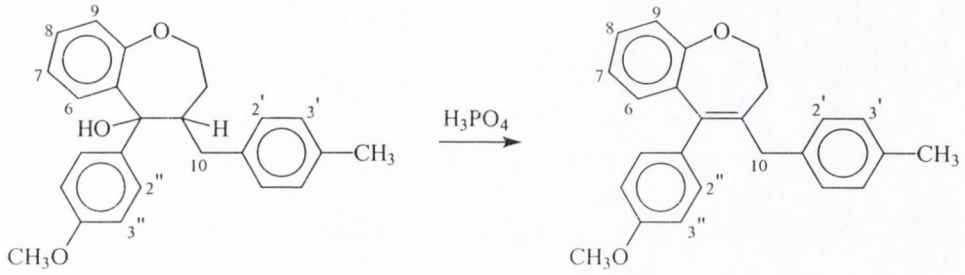
expected. The methylene protons were all found to be non-equivalent (as in (248)). The two multiplets in the ranges δ1.80-1.89 and δ2.49-2.53 were found to be coupled in the H-H COSY spectrum and were assigned to H-3, which consists of two non-equivalent hydrogens (discussed section 3.2.3). The protons of H-10 are observed as a double doublet (J=10.6, 3.5Hz) at δ3.04 and are found to be coupled to H-4 which is found as a multiplet at δ2.90. The non-equivalent H-2 methylene protons appear as two pairs of multiplets at δ3.80 and δ4.01, representing the two protons Hc and Hd. The ^{1}H NMR spectrum also shows the OH proton at δ2.08 as a broad singlet, which exchanges with D_2O. The H-3'', H-5'' protons are shielded by the methoxy substituent at C-4'', and thus they appear as a doublet (J=8.52Hz) at δ6.85. The remaining aromatic protons are observed as a complex multiplet between δ7.04-7.43, with the exception of H-6, which appears down field at δ7.62 as a double doublet (J=8.0, 1.5Hz).

The ^{13}C NMR spectral assignment of compound (251) was aided by a C-H COSY spectrum, which effectively displays the coupling that occurs between the proton and the carbon signals in the compound, along with the knowledge of the spectral details from compound (248). The methyl (CH_3) singlet at δ2.32 is found to couple with a signal at 20.50ppm, while the methoxy signal is assigned to 54.75ppm as it correlates with the singlet at δ3.81. The inverted signals which appear in the DEPT 135 spectrum and are identified using the C-H COSY spectrum, show that the double doublet signal at δ3.04 couple with the ^{13}C NMR signal at 29.23ppm, and are, as a result, assigned to C-10. The signal at 28.05ppm is found to correlate with the multiplets at δ1.89 and δ2.51 and is assigned to C-3, while the ^{1}H NMR signals at δ3.80 and δ4.01 are found to couple to the ^{13}C NMR signal at 67.59ppm, and this was therefore assigned to C-2 which is effectively deshielded by the adjacent oxygen. The signal at 49.95ppm is found to correspond to C-4. The shielded aromatic C-3'', C-5'' signals, which appear at δ6.85 in the ^{1}H NMR are found to couple in the ^{13}C NMR to the signals at 112.99, 113.21ppm. The aromatic double doublet at δ7.62 is found to correlate to the signal at 128.59ppm and is assigned to C-6. The C-8, C-9 signals occur upfield at 121.82 and 123.70ppm, while the quaternary C-9a signal was observed at 158.43ppm. The aromatic signals are observed in the range 126.57-136.66ppm, while the quaternary C-1' is found at 136.99ppm.

Low resolution mass spectrometry afforded the molecular ion as 374 ($C_{25}H_{26}O_3$).

3.6.3.2 Dehydration of 4-arylmethyl-5-phenyl-1-benzoxepin-5-ol (251).

The dehydration reaction was carried out by refluxing (251) in 85% polyphosphoric acid for 2-3 hours to obtain 5-(4-methoxyphenyl)-4-(4-methylphenyl)methyl-2,3-dihydro-1-benzoxepin (252) as an oil in 79% yield [Scheme 70]. The infrared spectrum of compound (252) showed the aliphatic and aromatic C=C stretching at ν1605 and 1573 cm^{-1} respectively, with no hydroxy stretching as expected. The identity of this compound was confirmed using ^{1}H NMR and ^{13}C NMR, along with low resolution mass spectrometry.



(251) (252)

Scheme 70: Dehydration of compound (251)

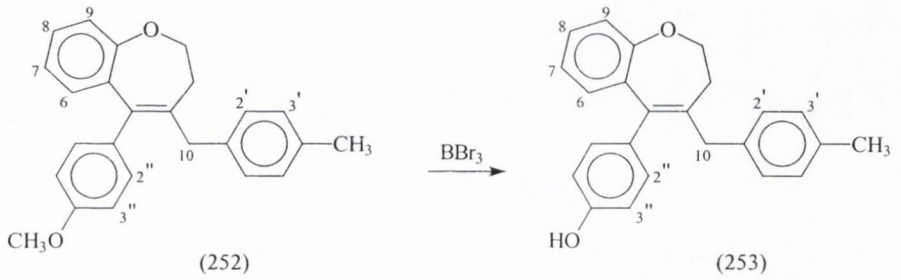
In the ^{1}H NMR of (252), the methylene protons at H-3 are observed as a triplet (J=6.3Hz) at δ2.30, while the adjoining H-2 protons are found at δ4.34 also as a triplet (J=6.3Hz). The methyl and methoxy protons were observed as singlets, integrating for three protons each at δ2.35 and δ3.83 respectively. The methylene protons at H-10 occur as a singlet at δ3.64. The shielded H-3'', H-5'' are observed as a double doublet at δ6.83-6.87. The remaining aromatic protons are found as a complex unresolved multiplet between δ6.98-7.28.

The ^{13}C NMR spectrum of (252) shows signals at 20.54ppm and 54.73ppm, corresponding to the methyl and methoxy carbons. The C-2 and C-3 signals are found at 79.97 and 31.99ppm respectively, while the methylene C-10 signal occurs at 40.04ppm. The aromatic C-8 and C-9 signals are observed at 121.44 and 122.79ppm, while C-7 and C-6 are found at 130.49 and 128.31ppm respectively. The C-2' and C-6' signal is found at 128.64ppm, while the corresponding C-3' and C-5' signal occurs at 130.50ppm. The shielded C-3'', C-5'' are observed downfield at 113.06ppm from the corresponding C-2'', C-6'' signal at 128.31ppm. The C-4'' signal is observed at 127.35ppm. The quaternary signals are observed between 136.08-136.44ppm, with the exception of C-9a found at 155.31ppm.

In the low resolution mass spectrum of 5-(4-methoxyphenyl)-4-(4-methylphenyl)methyl-2,3-dihydro-1-benzoxepin (252), the molecular ion is observed as the base peak at m/z356 ($C_{25}H_{24}O_2$).

3.6.4. Synthesis of 5-(4-hydroxyphenyl)-4-(4-methylphenyl)methyl-2,3-dihydro-1-benzoxepin (253).

Demethylation of (252) was carried out using boron tribromide as previously described. [Scheme 71] Alternative demethylating agents[87,178,274] include aluminium chloride, concentrated hydrobromic and hydrochloric acids. The phenolic compound (253) was afforded in 98% yield as an oily gel.



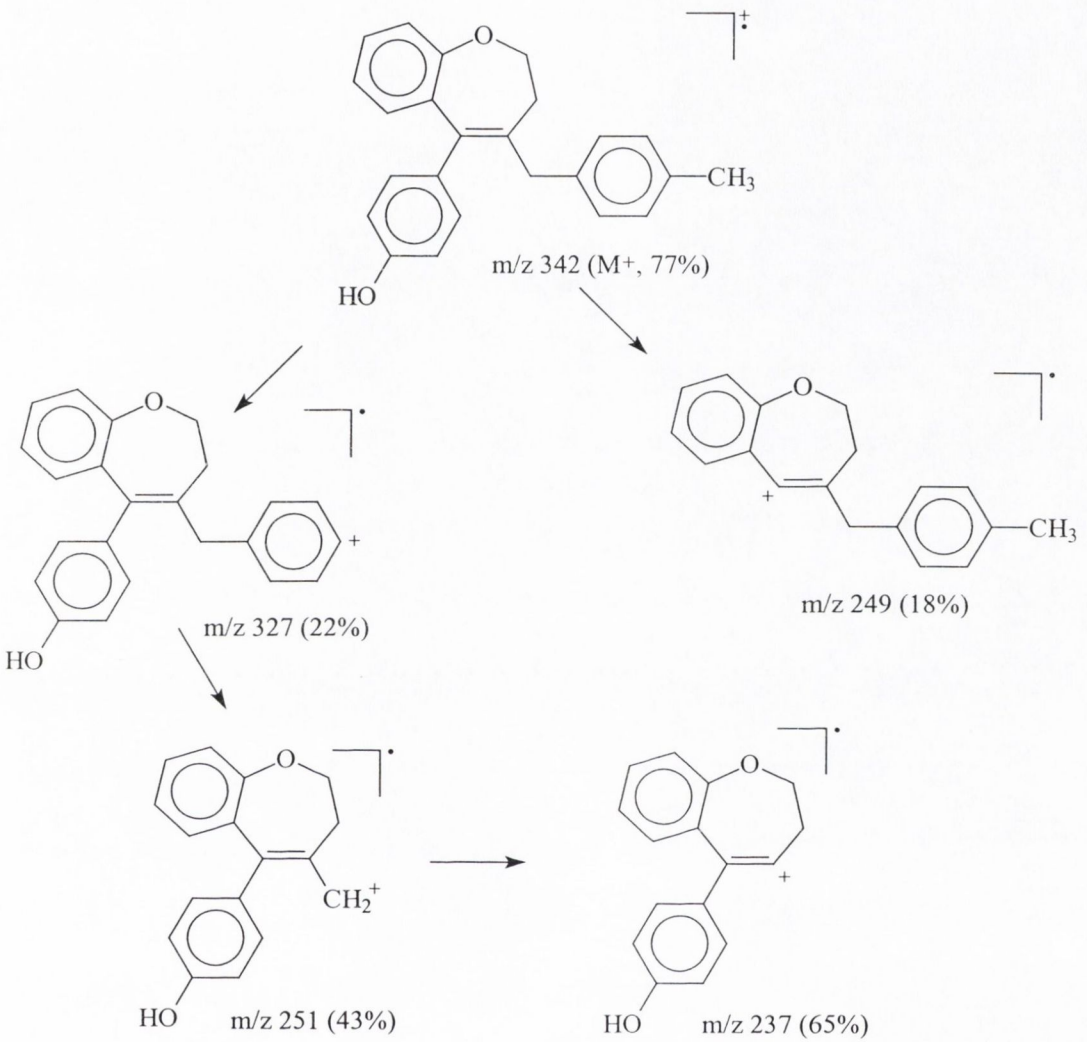
Scheme 71: Preparation of 5-(4-hydroxyphenyl)-4-(4-methylphenyl)methyl-2,3-dihydro-1-benzoxepin (253)

The infrared spectrum of (253) shows a broad hydroxy stretch between ν3678-3202cm^{-1}, while the aliphatic and aromatic (C=C) stretches are found at ν1608 and 1579cm^{-1} respectively.

In the ^{1}H NMR spectrum the methyl (CH_3) protons are observed as a singlet at δ2.45, while the methylene H-10 protons are found at δ3.63 also as a singlet. The H-3 protons occur as a triplet (J=6.3Hz) at δ2.28, with the adjacent H-2 protons being found at δ4.33 as a triplet (J=6.3Hz). The aromatic protons are observed as a multiplet in the range δ6.79-7.12.

In the ^{13}C NMR spectrum of (253), the methyl signal is found at 21.00ppm. In the DEPT 135 spectrum, the inverted peaks at 32.44, 40.48 and 80.45ppm were assigned to C-3, C-10 and C-2 respectively. The C-3'', C-5'' are shielded by the hydroxy group and are found at 114.98ppm, while the corresponding C-2'', C-6'' signals occur at 128.79ppm. The C-8 and C-9 signals are observed characteristically at 121.94 and 123.29ppm while the C-6 and C-7 signals are found at 127.85 and 130.93ppm. The quaternary C-4'' signal is observed at 154.47ppm and C-9a at 155.77ppm.

In the low resolution mass spectrum of 5-(4-hydroxyphenyl)-4-(4-methylphenyl)methyl-2,3-dihydro-1-benzoxepin (253), the molecular ion is observed at 342 ($C_{24}H_{22}O_2$) in 77% abundance. The proposed fragmentation pattern of (253) is outlined in Scheme 72.



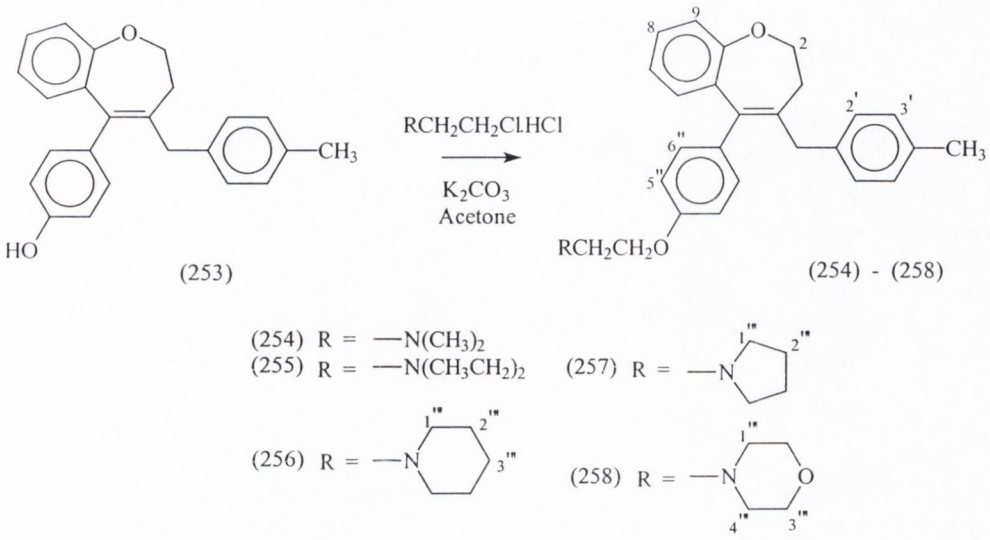
Scheme 72: Mass fragmentation of compound (253)

Cleavage of the methyl group affords the m/z 327 (22%), fragment and further loss of C_6H_4 and a methylene group afford the m/z 251 (43%) and m/z 237 (65%) fragments respectively. Cleavage of a phenyl substituent from the molecular ion affords the m/z 249 (18%) fragment.

3.6.5. Alkylation reactions of 5-(4-hydroxyphenyl)-4-(4-methylphenyl)-2,3-dihydro-1-benzoxepin.

A series of 5-[(4-alkylaminoethoxy)phenyl]-4-(4-methylphenyl)methyl-2,3-dihydro-1-benzoxepins (254)-(258) were prepared in the usual manner, by

refluxing 5-(4-hydroxyphenyl)-4-(4-methylphenyl)methyl-2,3-dihydro-1-benzoxepin (253) with the required alkylating agent in the presence of potasium carbonate and dry acetone. [Scheme 73]



Scheme 73: 5[(4-Alkylaminoethoxy)phenyl]-4-(4-methylphenyl)methyl-2,3-dihydro-1-benzoxepins (254)-(258)

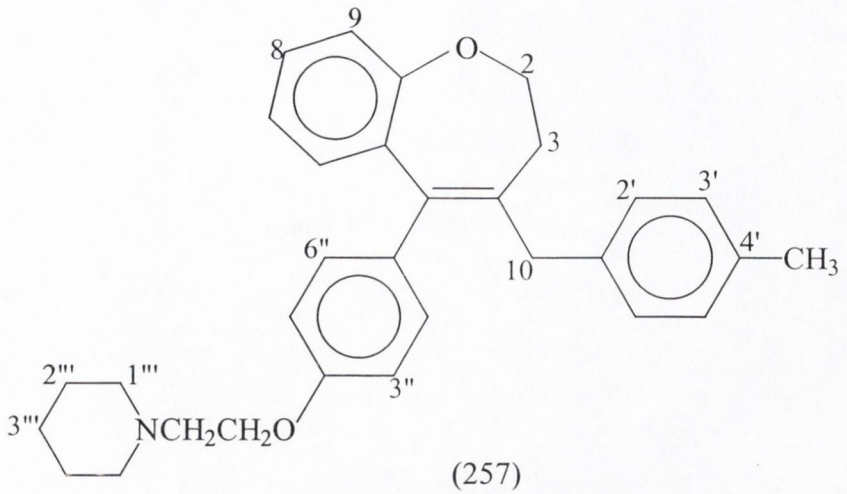
The alkylated benzoxepins (254)-(258) were afforded as oils in poor yields (starting material was recovered in the reaction). These compounds were positively identified from spectroscopic data and confirmed with the use of both low and high resolution mass spectrometry.

The infrared spectrum of (254)-(258) shows the aliphatic and aromatic alkene (C=C) stretching in the range ν1608-1604cm^{-1} and ν1581-1570cm^{-1} respectively. The yield and infrared spectral data are detailed in Table 19.

Compound	Yield %	$IR_{\upsilon max}$ cm^{-1}
(254)	20	1605, 1581 (C=C)
(255)	27	1608, 1573 (C=C)
(256)	21	1575 (C=C)
(257)	31	1607, 1570 (C=C)
(258)	19	1604, 1575 (C=C)

Table 19: Yield and infrared data of compound (254)-(258)

In the ^{1}H NMR spectrum of 4-(4-methylphenyl)methyl-5-[(4-piperidinyl ethoxy)phenyl]-2,3-dihydro-1-benzoxepin (257) [Figure 52], the methyl protons are observed as a singlet at δ2.34, integrating for three protons.



(257)

Figure 52: Structure of 4-(4-methylphenyl)methyl-5-[(4-piperidinyl ethoxy)phenyl]-2,3-dihydro-1-benzoxepin (257)

The methylene H-3 protons are found as a triplet (J=6.3Hz) at δ2.29, while the H-2 protons are observed as a triplet (J=6.0Hz) at δ4.32. The CH_2 protons at C-10 are found as a singlet at δ3.63. The NCH_2 and OCH_2 protons are observed at δ2.80 and δ4.13 respectively, where both occur as triplets (J=6.0Hz) and the OCH_2 occurs as a triplet (J=6.04Hz) also. The piperidinyl protons are observed as multiplets, where H-2''', H-3''', H-4''' are found at δ1.62 and H-1''', H-5''' at δ2.54. The H-3'' and H-5'' protons are shielded by the alkyl substituent and are observed as a double doublet (J=10.6, 2.0Hz) at δ6.84-δ6.87. The remaining aromatic protons are found as a complex multiplet between δ6.97-7.25. The ^1H NMR is depicted in Figure 53.

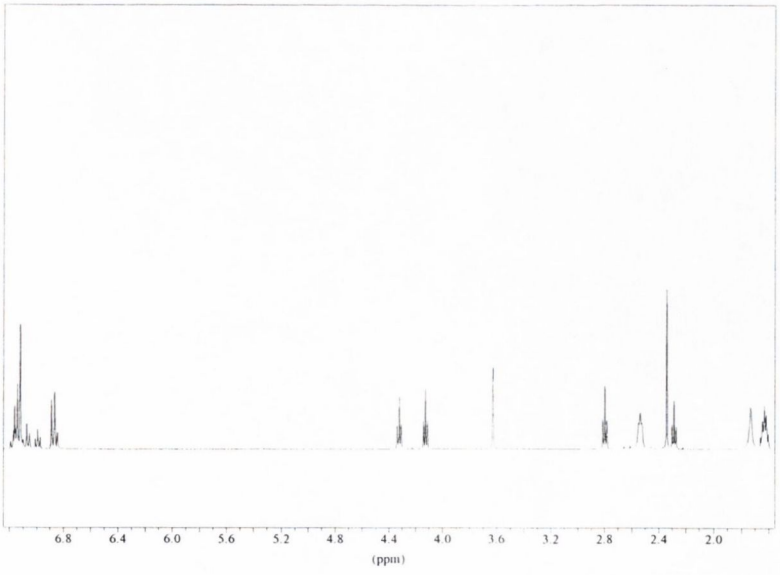


Figure 53: ^1H NMR Spectrum of compound (257)

In the ^{13}C NMR spectrum of (257) the methyl signal is found at 22.54ppm. The carbons are all observed, inverted in the DEPT 135 spectrum. The C-3 and C-2 signals occur at 31.97 and 80.72ppm, while the amino ethoxy methylene signals of NCH_2 and OCH_2 are found at 57.51 and 70.77ppm respectively. A signal at 40.02ppm is assigned to C-10. The piperidinyl signals are observed at 25.44 and 54.55ppm corresponding to C-2''', C-3''', C-4''' and C-1''', C-5''' respectively. The aromatic C-8, C-9 signals occur at 121.44 and 122.80ppm while the C-6 and C-7 signals are found at 127.34 and 130.85ppm respectively. The shielded C-3'', C-5'' are observed at 113.68ppm. The remaining aromatic signals are found between 128.31-130.49ppm, with the exception of C-1' at 136.06ppm and the signal at 135.18ppm is assigned to C-4.

In the low resolution mass spectrum of 4-(4-methylphenyl)methyl-5-[(4-piperidinylethoxy)phenyl]-2,3-dihydro-1-benzoxepin (257), the molecular ion is observed at 453 ($C_{31}H_{35}NO_2$).

The products obtained represent a novel series of benzoxepins containing an extended tamoxifen structure in a conformationally restricted 7-membered oxygen containing ring template.

3.7. Conclusion

A number of benzoxepin products were prepared which represent novel restricted tamoxifen analogues containing a seven membered ring skeleton with aryl substituents, one of which is joined to the skeleton by a methylene group at C-4. The remaining novel benzoxepins synthesised possess a triaryl structure similar to that of tamoxifen. Due to the success of the Suzuki reaction, this allowed the coupling of a wide variety of aryl and heterocyclic compounds onto the benzoxepin ring. Nitro-benzoxepins were also prepared with a view to modifying the nitro group and the methoxy-aryl substituent. The preparation of these benzoxepins via the Suzuki coupling reaction produced high yielding compounds, whereas the 4-methylene benzoxepins were synthesised by a six step synthetic route, which proved more difficult.

The series of benzoxepin compounds prepared in this research were biochemically analysed for their binding affinity and inhibition of proliferation for MCF-7 breast cancer cells. These results will be discussed in Chapter 4.

4. Biochemical studies on novel flexible and conformationally restrained antiestrogens

4.1. Introduction

Breast cancer is the biggest single fatal illness to affect women. The Irish Cancer Society suggests that one in eleven women will develop breast cancer in their lifetime. Estrogens are thought to be the primary mitogen for hormone-dependent breast cancer and therefore deprivation of estrogen is fundamental to treatment of many benign and malignant breast tumours.[30] The growth of estrogen dependent tumours is described in Chapter 1.

Ablation of estrogen production i.e. chemical castration can be accomplished surgically or radiologically. The most widely used pharmacological approach for depriving tumour cells of estrogen stimulation involves the use of antiestrogens. These agents have been discussed in detail in Chapter 1. Antiestrogens have proven to be effective in controlling the growth of hormone-responsive breast cancer for decades.[41] Tamoxifen, a potent nonsteroidal antiestrogen, has been widely used in the treatment of human breast tumours. It is cytostatic and exerts competitive inhibitory activity with estrogen at the receptor level. Its effects include stimulation or inhibition of enzymes, synthesis, activation or repression. It binds to cytoplasmic estrogen receptors and is translocated to cell nuclei, where cell proliferation[161] is prevented.

The effects of tamoxifen on the proliferation of various human breast cancer cell lines that differ in their estrogen receptor content have been examined. It has been shown that the cell line sensitivity to growth suppression by tamoxifen correlates with their estrogen receptor content. The MCF-7 cells, which contain high levels of estrogen receptors have their growth markedly inhibited by tamoxifen, whereas T47D cells, which contain low levels of estrogen receptor, have their growth inhibited weakly by tamoxifen and MDA-MB-231 cells, which contain no detectable estrogen receptors (estrogen independent cells), have their growth unaffected by tamoxifen.[41] The AL-1 cell line is also resistant to Tamoxifen, perhaps due to its agonist effect in some tissues[5]. Therefore, *in vitro* tests for tamoxifen and other antiestrogenic compounds have been successfully carried out on MCF-7 breast tumour cells.

In the present work biochemical studies were carried out on the novel synthesised analogues i.e. the flexible antiestrogenic compounds and the conformationally restrained tamoxifen derivatives. These studies firstly examined the ability of these compounds to bind to the estrogen receptor. Rat uteri cytosol was used as a source of ER as it has previously been shown to be rich in this receptor.[161] The binding affinity of these compounds was then

compared with that of tamoxifen. These studies were extended to determine the effects of these compounds on cell proliferation using the MCF-7 cell line as a model system. Once again, the potency with which these compounds exhibited antiproliferative/cytotoxic effects was compared with that of tamoxifen.

4.2. Materials and methods.

4.2.1. Materials

The full names and addresses of the sources listed below are given at the end of the list.

Material	Supplier
BSA	Sigma
DCC	Sigma
DMSO	Sigma
Eagles minimum essential medium	Sigma
Ecoscint Scintillation fluid	National Diagnostics
EDTA	Sigma
β-Estradiol	Sigma
[^{3}H] Estradiol	Amersham International
FCS	Grenier
Gentamycin	Sigma
LDH cytotoxicity kit	Promega Corporation
L-glutamine	Sigma
MCF-7 cells	E.C.A.C.C.
MTT	Sigma
Non Essential amino acid medium	Sigma
Norit (Fischer) charcoal	Sigma
Pipettes (sterile)	Sterilin
Rats (Sprague Dawley)	Biochemistry Department TCD
Sodium azide	Riedel de Haen
Tamoxifen	Sigma
Tissue culture flasks and plates	Grenier
Tris	Sigma
Trypsin	Sigma

All compounds used in this analysis are described in Chapter 2 and 3 i.e. tamoxifen-like (flexible) analogues and benzoxepin (conformationally restrained) derivatives and were prepared in the Pharmaceutical Chemistry Department, TCD. All other reagents were of analytical grade, where possible, and were obtained from BDH, Riedel de Haen or Sigma.

4.2.2. Addresses of suppliers

Amersham International Plc., Amersham Place, Little Chalfont, Buchinghamshire, HP79NA, UK.

British Drugs House (BDH) Chemicals Ltd., c/o Lennox Chemicals, J.F. Kennedy Drive, Dublin 12, Ireland.

European Collection of Animal Cell Cultures (E.C.A.C.C.), PHLS, Centre for Applied Microbiology and Research, Porton Down, Salisbury, SP40JG, UK.

Grenier, Gmbtt, Maybachtrasse 2, P.O. Box 1162, D-7443-Frickenhausen, Germany.

National Diagnostics, 305 Patton Drive, Atlanta Georgia 30336, USA.

Promega Corporation, 2800 Woods Hollow Road, Madison, W153711-5399, USA.

Riedel de Haen AG, c/o R.B. Chemicals Ltd, Hoecht House, Cookstown Industrial Estate, Tallaght, Co., Dublin.

Sigma Chemical Co. Ltd., Fancy Road, Pool, Dorset, UK.

Sterilin, Bibby Sterilin Ltd., Stone, Staffs, UK.

4.2.3. Growth and maintenance of cells.

4.2.3.1 Growth and maintenance of MCF-7 cells.

MCF-7 cells, a human breast adenocarcinoma cell line, cloned from a 69 year old female caucasian, (Soule *et al.*,)[485] were grown as monolayer cultures at 37°C, under a humidified atmosphere of 95% O_2, 5% CO_2 in 75cm^2 flasks containing Eagles Modified Essential Medium [EMEM], with 10% (v/v) foetal calf serum (FCS), 1% (v/v) Non Essential Amino Acid Medium [NEAAM], 2mM L-glutamine and supplemented with 100μg/mL gentamycin (complete medium).

Cells were harvested and re-seeded after reaching confluence (once weekly) by first washing with serum-free EMEM (10mL). They were then removed from the surface of the flask by a 5min exposure to 2.5% (v/v) trypsin (1mL). The cells were then sedimented by centrifugation at 600 x g for 5min and the pellet was resuspended in 1mL of complete medium. Cell numbers were counted using a haemocytometer. An aliquot of cells (1.5 x 10^6 cells) was seeded in 75cm^2 flasks in 20mL complete medium.

4.2.3.2 Cryopreservation of cells.

MCF-7 cells grown to a state of subconfluency, were harvested and counted as described (section 4.2.3.1). The cells were pelleted and resuspended in 9mL of fetal calf serum plus 10% dimethylsulphoxide (DMSO). Aliquots of 1mL were transferred into 1.5mL cryotubes and placed at –20°C for 4hr. The cryotubes were then transferred to –70°C for 2hr, before storage in a liquid nitrogen vessel.

When required, an aliquot of cells was removed from the liquid nitrogen vessel rapidly thawed and resuspended in 10mL of complete medium. This cell suspension was centrifuged at 600 x g for 5min, the medium (containing DMSO) discarded and the pellet resuspended in complete medium. Cells were then seeded in tissue culture flasks as previously described (Sections 4.2.3.1)

4.2.4. Bradford protein assay.

Protein concentration measurements of cell lysates were carried out using a modification of the method of Bradford.[486] [Figure 54]

To a 96-well microtitre plate was added 200μL of reagent A, (0.01% (w/v) Coomassie Brilliant Blue G-250, 4.7% (v/v) ethanol and 8.5% (w/v) orthophosphoric acid), 18μL of H_2O and 2μL of the protein sample and this was then left at room temperature for 5min. The absorbance at a wavelength of 595nm was measured using a Dynatech MR5000 plate reader and the results were compared to the standard curve prepared using known concentrations of the standard protein bovine serum albumin (BSA). The protein determination was performed in triplicate.

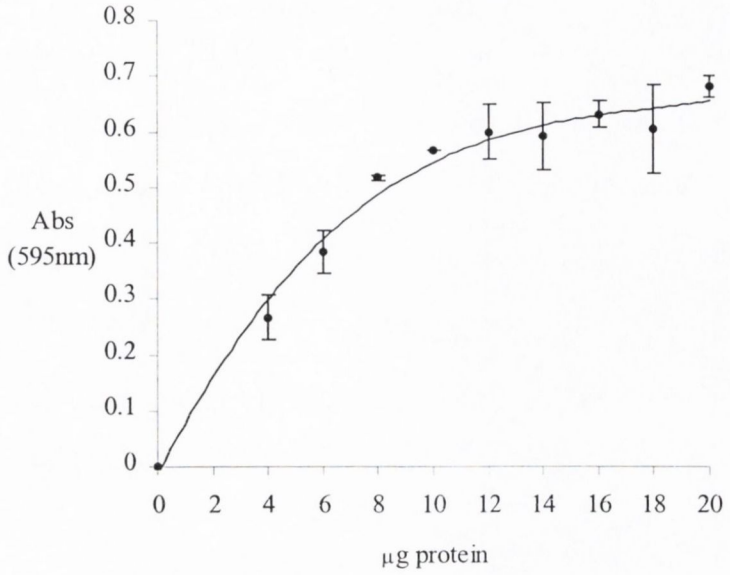


Figure 54: Bradford standard curve for protein assay

4.2.5. Assessment of cytotoxic / antiproliferative effects of novel compounds

4.2.5.1 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide (MTT) assay

MTT assay was performed by a modification of the method of Mosmann.[487] 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), a tetrazolium salt, is taken up only by metabolically active cells and cleaved to form a formazan dye by mitchondrial dehydrogenases. This formazan dye is initially localised within the mitochondria as small purple crystals, which are solubilised in DMSO and the resulting purple solution is measured spectrophotometrically. An increase or decrease in cell number results in a concomitant change in the amount of formazan formed, indicating the degree of growth or cytotoxicity caused by the different compounds tested.

MCF-7 cells were cultured as described in 4.2.3.1, and seeded down at 1.5×10^4 cells per well with 100µL of complete medium in a 96-well plate at 37°C. After 24hr the cells were then exposed to varying concentrations of the synthesised analogues and left for a further 72hr. Following the final incubation period, 50µL aliquot samples were removed to a fresh 96-well plate and set aside.

To assay for cell damage, the remaining medium was removed from cell chambers and the cells were washed with 100µL PBS and then 50µL of MTT solution (final concentration 1mg/mL) was added to each well. The cells were

then incubated in the dark at 37°C for 2-3hr before addition of 200μL of DMSO. The plates were then wrapped in aluminium foil and left for 20min to ensure solubilisation of the blue/purple formazan crystals. The absorbance was read at 570nm in a Dynatech MR5000 plate reader and cell viability expressed as a percent of control.

4.2.5.2 Lactate dehydrogenase (LDH) assay

The release of the cytoplasmic enzyme lactate dehydrogenase (LDH), used as a measure of cell death, was performed following a modification of a method by Youkin *et al.*[488]

MCF-7 cells were cultured as described in Section 4.2.3.1 and seeded down at a density of 1.5 $x10^4$ cells per well with 100μL complete medium in a 96-well plate at 37°C. After 24hr the cells were then treated with varying concentrations of the novel synthesised analogues and incubated for a further 72hr, before determination of LDH activity.

To assay for LDH activity an aliquot (50μL) of each sample was transferred in triplicate to a 96-well plate. To this, 50μL of substrate mix from the LDH assay kit (lyophilised diaphorase, lactate and NAD^+) was added and the plate was left covered at room temperature for 30min. After this period, 50μL of stop solution (0.1M NaOH) was added to each well before reading the plate at an absorbance of 490nm.

A 100% lysis control was a set of untreated cells, which were lysed by adding 10μL lysis solution (detergent) to the media 45min prior to harvesting. This was necessary each time an assay was undertaken in order to calculate the percentage cytotoxicity. As a result, the data could be represented as percentage cell lysis compared to control versus concentration of compound.

4.2.6. Preparation of rat uteri cytosol

The uteri-cytosols was prepared following the method of Fishman[489] and Yang *et al.*[161] The uteri were excised from immature Sprague-Dawley rats (150-200g) and placed in Tris buffer (10M Tris, 1.5M EDTA, 1M sodium azide, pH 7.4), 1 uterus/2mL buffer at 4°C.

The fat tissue and muscle were removed from the tissues and the uteri were placed in fresh ice-cold Tris buffer (20mL). The uteri-cytosol tissue was then homogenised in buffer using a Potter-Elvehjem glass homogeniser with tight

fitting Teflon pestles. The homogenate was centrifuged at 100,000 x g at 4°C for 1hr. The resulting supernatants contained the uteri cytosol and a solid pellet of fat tissue was observed at the bottom of the tubes which was discarded. The uteri cytosol was then pretreated with dextran-coated charcoal [DCC] (5g Norit (Fisher) charcoal, 0.5g Dextran T70 in 100mL distilled water). In pretreatment, the DCC dispersion was centrifuged at 1200 x g for 10min, the supernatant discarded, uteri cytosol added, the DCC pellet redispersed and the system incubated on ice for a further 2hr. The system was then centrifuged at 1200 x g in the cold, the supernatant decanted and centrifuged again to ensure removal of any residual charcoal properties. One mL of the original DCC dispersion was used in preparing a pellet for treating 2mL of cytosol. The protein concentration of the uteri cytosol was measured by the method of Bradford as described in Section 4.2.4.

4.2.7. Measurement of [^{3}H] ligand binding to rat uteri-cytosolic homogenates (estrogen receptor)

This procedure was carried out as described by Yang *et al.*,[490] and Fishman.[489] Uteri cytosol homogenates (50-250μg) were incubated with 0.1-100nM [^{3}H] estradiol (157Ci/mmol), in a total volume of 140μL Tris buffer at 4°C. For other assays, where the homogenate protein concentration was varied a fixed concentration of [^{3}H] estradiol (5nM) was used. Total and non-specific binding was determined in the absence and presence of 0.2mM unlabelled estradiol respectively. All samples were incubated at 4°C for 16hr. The incubated samples were then DCC treated (0.07mL) as described in Section 4.2.6 and the treated samples were incubated on ice for 15min. The incubation mixture was then centrifuged at 1800 x g for 10min, to remove unbound radioligand. The centrifuged aliquots (170μL) were transferred to plastic vials containing 10mL of scintillation fluid and were counted for radioactivity via liquid scintillation counting. When testing the potency of a compound with respect to inhibition of [^{3}H] estradiol binding, samples were incubated with a stated concentration of [^{3}H] estradiol and varying concentrations of compound (100nM-100μM), and subsequently treated as above.

4.2.8. Liquid scintillation counting

The [^{3}H] radioactivity was measured using a Packard 1500 scintillation counter or a Packard Top-Count. The scintillant cocktail used was the commercial scintillant microscint 20. The average counting efficiency for [^{3}H] counting in

the Parkard 1500 scintillation counter and in the Packard Top-Count was calculated to be 45%, based on a quench-correction curve relating counting efficiency to channels ratio.

4.2.9. Analysis of binding data

Binding values were obtained as counts per minute (cpm) and converted to pmol of bound radioligand using counting efficiency and specific radioactivity values. Specific binding was calculated from the difference between total and non-specific binding. The equilibrium constant (K_D) and the maximal binding capacity (B_{max}), describing the saturable binding of [^{3}H] ligand to a single class of binding sites were obtained by fitting mean values for specific binding and free ligand concentration using the method by Wilkinson[491] and the program MacCurvefit. Also single-site displacement curves, describing the competition of [^{3}H] estradiol binding by novel flexible and conformationally restrained tamoxifen analogue compounds, were generated by use of the computer program EBDA (Equilibrium Binding Data Analysis). EBDA uses a sigmodial curve-fitting program to fit the displacement curve. Logit-log transformation of the data were performed (i.e. linear regression of log [% bound/100 - % bound] against log [concentration of displacer]) to obtain initial estimates of certain parameters including IC_{50} (concentration of displacer inhibiting 50% of specific binding). These are then fed to the iterative portion of the program to obtain final estimates of the parameters. The equation used to fit the competitive displacement curve is:

$$\text{Bound (dpm)} = \frac{B_{max}\text{-BG}}{(1 + [\text{Displacers concentration} / IC_{50}])^P}$$

where: B_{max} = the total amount of radioligand bound (dpm) in the absence of any displacer.

BG = the estimate of non-specific binding (dpm)

P = the slope factor (Hill coefficient)

The calculated IC_{50} value is then used to determine K_i (inhibition constant which describes potency of inhibition), assuming simple competitive behaviour, by applying the equation: -

$$IC_{50} = K_i (1 + [L]/K_D)$$

Where [L] is the concentration of [^{3}H]-ligand and K_D is the dissociation constant for the binding site.

4.3. Results and discussion

4.3.1. Introduction

The mode of action of tamoxifen has been explained previously in terms of its binding to cytoplasmic estrogen receptors and this tamoxifen-estrogen receptor complex is translocated to cell nuclei where cell proliferation is prevented.[492] Tamoxifen is a competitive inhibitor, competing directly with estrogenic ligands for the binding domain active site. The ability of the novel flexible and conformationally restrained tamoxifen analogues synthesised in this work to inhibit the proliferation of a human breast tumour MCF-7 cell line was determined. The ability of selected compounds (those exhibiting significantly antiproliferative effects on MCF-7 cell growth i.e. low IC_{50} values) to bind with high affinity to the estrogen receptor was also examined.

4.3.2. Selected Tamoxifen analogues inhibit the proliferation of MCF-7 cells

The effect of tamoxifen (as reference control) and the novel synthesised analogues on MCF-7 cells were studied.

A partially confluent flask of MCF-7 cells maintained and grown as described in Section 4.2.3.1 are depicted in Figure 56. MCF-7 cells were seeded in a 96-well plate at a density of 1.5 x 10^4 cells/well and maintained in complete medium at 37°C for 24hr, before adding varying concentrations (1nM-100μM) of the compounds. The ability of the compounds to inhibit proliferation of MCF-7 cells was assessed using the enzymatic colourimetric MTT assay as described in Section 4.2.5.1. The change in colour from yellow to purple during the assay was monitored at 570nm and served to indicate the degree of cell proliferation for the cell line in the presence of the test compound. Cytotoxicity of the compounds was determined through the use of a Promega LDH assay as described in Section 4.2.5.2. These studies allowed an evaluation of the extent of cytostasis induced by the novel compounds and in combination with the MTT assay, facilitated graphical distinction between the cytostatic and cytotoxic activities of these compounds. Figure 57-64 displays antiproliferative activity and cytotoxicity profiles for compounds (59), (81), (99), (112), (122), (152), (224), (255) and the tamoxifen reference control. Each of these compounds are representative of a series. The individual IC_{50} values of all the flexible tamoxifen analogues tested are recorded in Table 20 and the conformationally restrained benzoxepin analogues are found in Table 21.

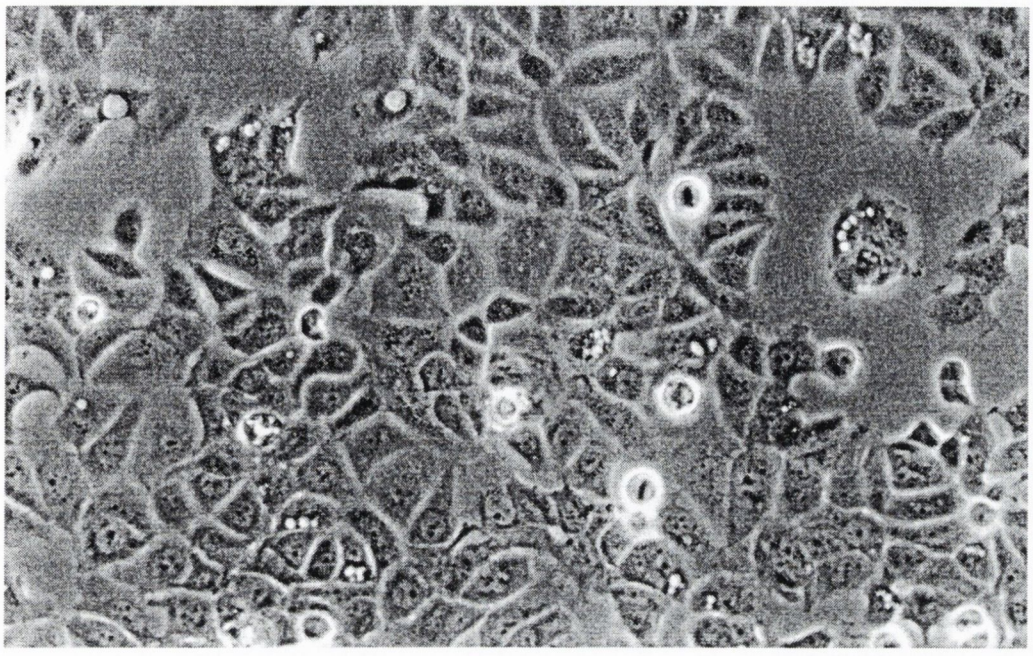


Figure 55: MCF-7 human breast cancer cells.

MCF-7 cells were routinely grown as a monolayer (1.5 x 10^6 cells) at 37°C under a humidified atmosphere of 95% O_2, 5% CO_2 in 75cm^2 flasks, containing complete medium (20mL) as described in Section 4.2.3.1 Cells were harvested and reseeded after reaching confluence. The photograph above [Figure 55] depicts healthy MCF-7 cells prior to confluency as seen under a 115 x magnification.

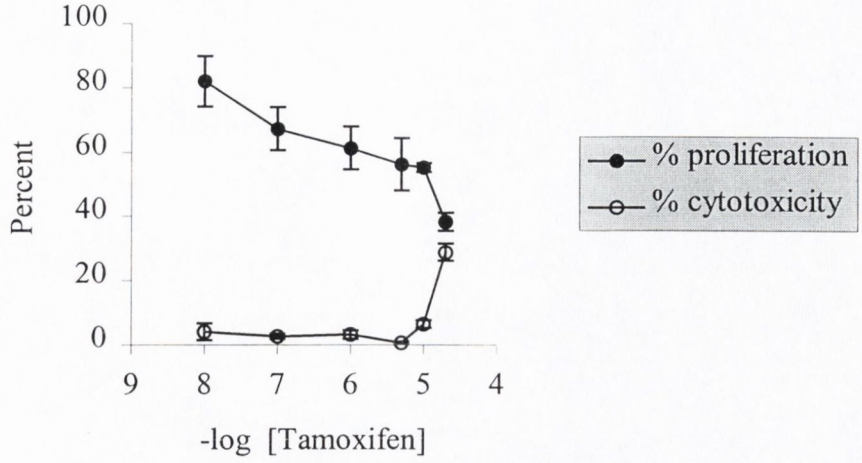


Figure 56: Tamoxifen inhibits the proliferation and induces some cytotoxicity of MCF-7 cells

MCF-7 cells were seeded at a density of 1.5 x 10^4 cells per well in 96 multiwell plates and allowed attach to the surface of the walls for 24hr. After this period varying concentrations (10nM-20μM) of tamoxifen were added and the cells were left for a further 72hr. Determination of cell proliferation was then carried out using the MTT assay described in Section 4.2.5.1 and the cytotoxicity was evaluated using the LDH assay as described in Section 4.2.5.2. Each point represents the mean ± S.E.M. of triplicates. The absence of error bars indicates the error was smaller than the size of the symbol. [Figure 56]

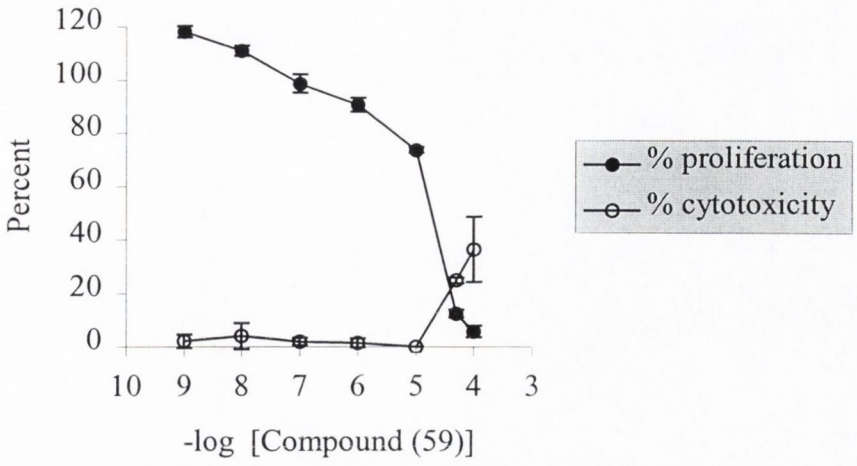


Figure 57: Compound (59) inhibits the proliferation and induces some cytotoxicity of MCF-7 cells

MCF-7 cells were seeded at a density of 1.5 x 10^4 cells per well in 96 multiwell plates and allowed attach to the surface of the walls for 24hr. After this period varying concentrations (1nM-100μM) of compound (59), 2-benzyl-1-[4-(dimethylaminoethoxy)phenyl]-1-phenylbut-1-ene, [Type I analogue] were added and the cells were left for a further 72hr. Determination of cell proliferation was then carried out using the MTT assay described in Section 4.2.5.1 and the cytotoxicity was evaluated using the LDH assay as described in Section 4.2.5.2. Each point represents the mean ± S.E.M. of triplicates. The absence of error bars indicates the error was smaller than the size of the symbol.

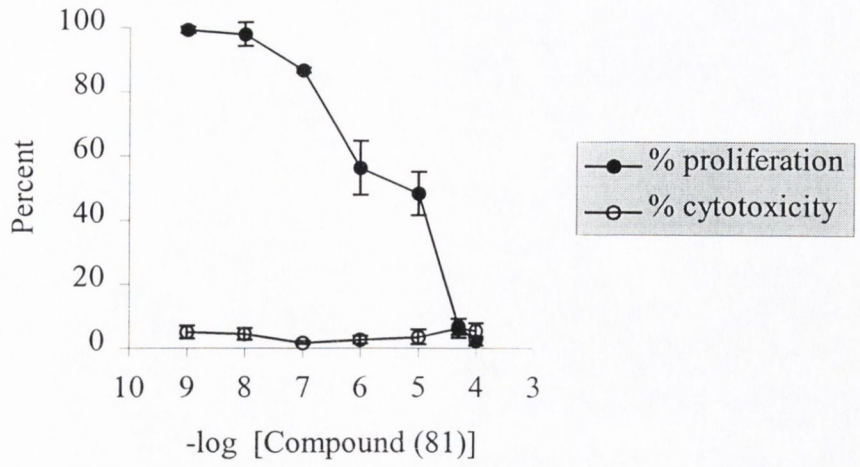


Figure 58: Compound (81) inhibits the proliferation of MCF-7 cells

MCF-7 cells were seeded at a density of 1.5 x 10^4 cells per well in 96 multiwell plates and allowed attach to the surface of the walls for 24hr. After this period varying concentrations (1nM-100μM) of compound (81), 1-benzyl-1-[4-(dimethylaminoethoxy)phenyl]-2-phenylbut-1-ene, [Type II analogue] were added and the cells were left for a further 72hr. Determination of cell proliferation was then carried out using the MTT assay described in Section 4.2.5.1 and the cytotoxicity was evaluated using the LDH assay as described in Section 4.2.5.2. Each point represents the mean ± S.E.M. of triplicates. The absence of error bars indicates the error was smaller than the size of the symbol.

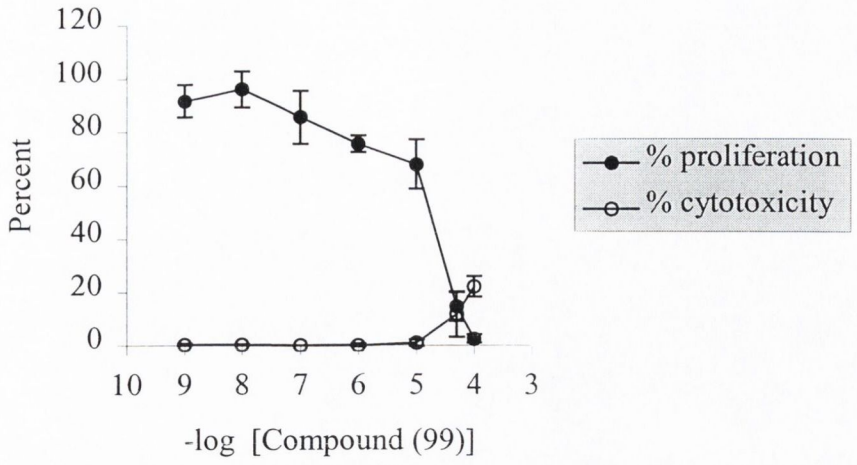


Figure 59: Compound (99) inhibits the proliferation and induces some cytotoxicity of MCF-7 cells

MCF-7 cells were seeded at a density of 1.5 x 10^4 cells per well in 96 multiwell plates and allowed attach to the surface of the walls for 24hr. After this period varying concentrations (1nM-100μM) of compound (99), 1-benzyl-1-phenyl-2-[4-(piperidinylethoxy)phenyl]but-1-ene, [Type III analogue] was added and the cells were left for a further 72hr. Determination of cell proliferation was then carried out using the MTT assay described in Section 4.2.5.1 and the cytotoxicity was evaluated using the LDH assay as described in Section 4.2.5.2. Each point represents the mean ± S.E.M. of triplicates. The absence of error bars indicates the error was smaller than the size of the symbol.

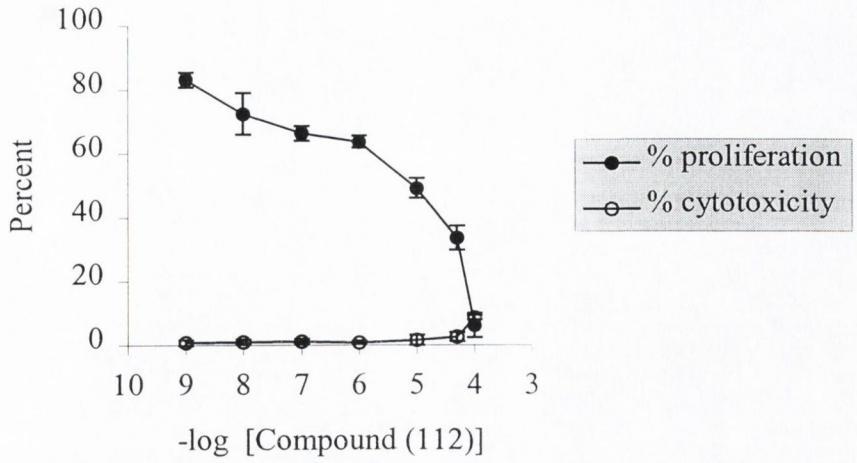


Figure 60: Compound (112) inhibits the proliferation of MCF-7 cells

MCF-7 cells were seeded at a density of 1.5 x 10^4 cells per well in 96 multiwell plates and allowed attach to the surface of the walls for 24hr. After this period varying concentrations (1nM-100μM) of compound (112), 1,2-diphenyl-2-[(4-morpholinylethoxy)benzyl]but-1-ene, [Type IV analogue] was added and the cells were left for a further 72hr. Determination of cell proliferation were then carried out using the MTT assay described in Section 4.2.5.1 and the cytotoxicity was evaluated using the LDH assay as described in Section 4.2.5.2. Each point represents the mean ± S.E.M. of triplicates. The absence of error bars indicates the error was smaller than the size of the symbol.

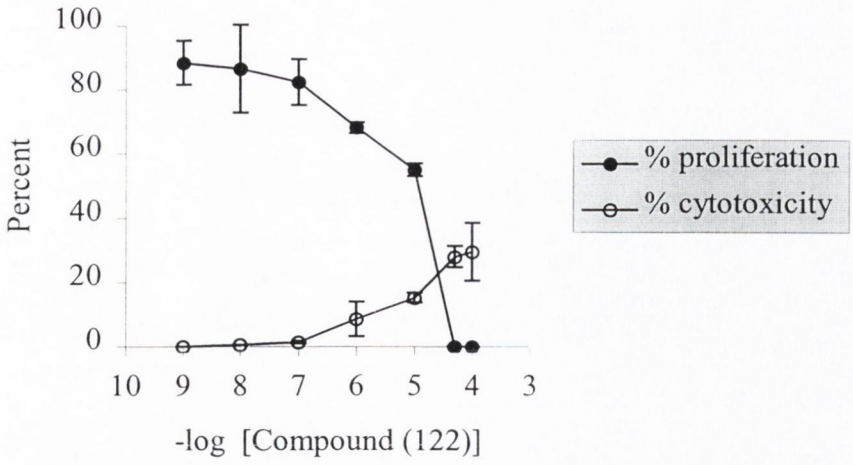


Figure 61: Compound (122) inhibits the proliferation and induces some cytotoxicity of MCF-7 cells

MCF-7 cells were seeded at a density of 1.5 x 10^4 cells per well in 96 multiwell plates and allowed attach to the surface of the walls for 24hr. After this period varying concentrations (1nM-100μM) of compound (122), 1-benzyl-3-bromo-1-[4-(dimethylaminoethoxy)phenyl]-2-phenylprop-1-ene, [Type V analogue] were added and the cells were left for a further 72hr. Determination of cell proliferation was then carried out using the MTT assay described in Section 4.2.5.1 and the cytotoxicity was evaluated using the LDH assay as described in Section 4.2.5.2. Each point represents the mean ± S.E.M. of triplicates. The absence of error bars indicates the error was smaller than the size of the symbol.

Compound		IC_{50} (μM)
Tamoxifen		11.3 ± 0.01
Type I	(59)	14.9 ± 3.7
	(60)	12.8 ± 3.9
	(61)	3.6 ± 0.4
	(62)	4.6 ± 0.4
	(63)	45.6 ±13.3
Type II	(81)	21.7 ± 5.3
	(82)	19.0 ± 5.2
	(83)	10.3 ± 2.4
	(84)	76.6 ± 10.5
	(85)	2.5 ± 0.3
Type III	(96)	117.2 ± 10.3
	(97)	20.7 ± 0.002
	(98)	22.4 ± 6.2
	(99)	19.1 ± 0.002
	(100)	51.1 ± 8.5
Type IV	(108)	25.1 ± 5.6
	(109)	58.0 ± 4.3
	(110)	142.2 ± 8.2
	(111)	68.8 ± 7.1
	(112)	16.6 ± 7.7
Type V	(120)	9.2 ± 3.3
	(121)	7.9 ± 3.1
	(122)	14.9 ± 2.6
	(123)	17.9 ± 2.1
	(124)	12.1 ± 3.9
	(125)	40.9 ± 4.9
	(126)	12.9 ± 0.2

Table 20: IC_{50} values of flexible tamoxifen analogues (59)-(126), for their antiproliferative effects on a human MCF-7 breast cancer cell line. IC_{50} value: the concentration required to inhibit 50% of MCF-7 cell growth. Values represent mean ± S.E.M. of triplicates.

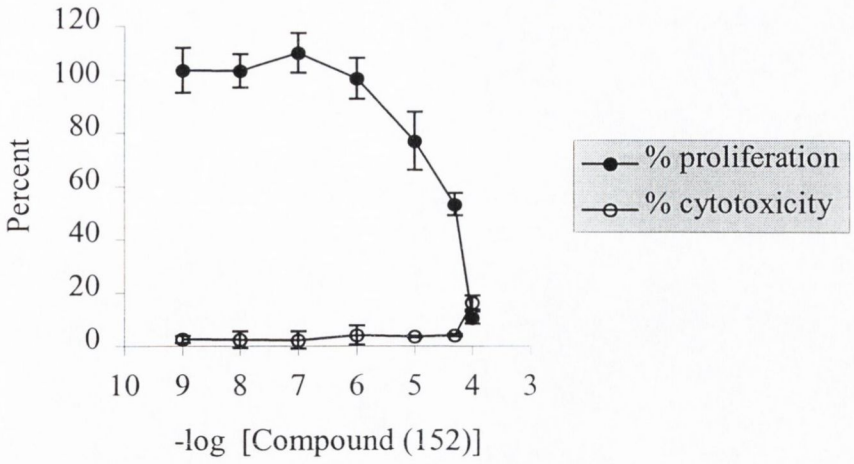


Figure 62: Compound (152) inhibits the proliferation of MCF-7 cells

MCF-7 cells were seeded at a density of 1.5 x 10^4 cells per well in 96 multiwell plates and allowed attach to the surface of the walls for 24hr. After this period varying concentrations (1nM-100μM) of compound (152), 5-[(4-dimethyl aminoethoxy)phenyl]-4-phenyl-2,3-dihydro-1-benzoxepin, were added and the cells were left for a further 72hr. Determination of cell proliferation was then carried out using the MTT assay described in Section 4.2.5.1 and the cytotoxicity was evaluated using the LDH assay as described in Section 4.2.5.2. Each point represents the mean ± S.E.M. of triplicates. The absence of error bars indicates the error was smaller than the size of the symbol.

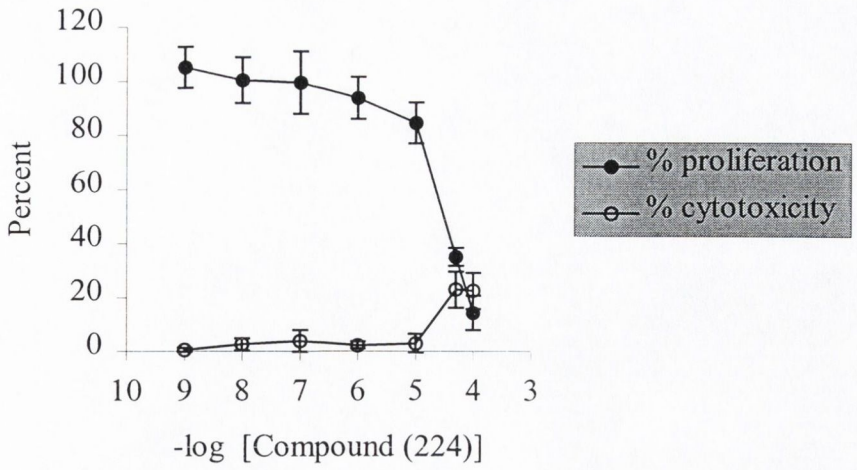


Figure 63: Compound (224) inhibits the proliferation and induces some cytotoxicity of MCF-7

MCF-7 cells were seeded at a density of 1.5 x 10^4 cells per well in 96 multiwell plates and allowed attach to the surface of the walls for 24hr. After this period varying concentrations (1nM-100μM) of compound (224), 5-[(4-dimethyl aminoethoxy)phenyl]-4-(4-methylphenyl)-2,3-dihydro-1-benzoxepin, were added and the cells were left for a further 72hr. Determination of cell proliferation was then carried out using the MTT assay described in Section 4.2.5.1 and the cytotoxicity was evaluated using the LDH assay as described in Section 4.2.5.2. Each point represents the mean ± S.E.M. of triplicates. The absence of error bars indicates the error was smaller than the size of the symbol.

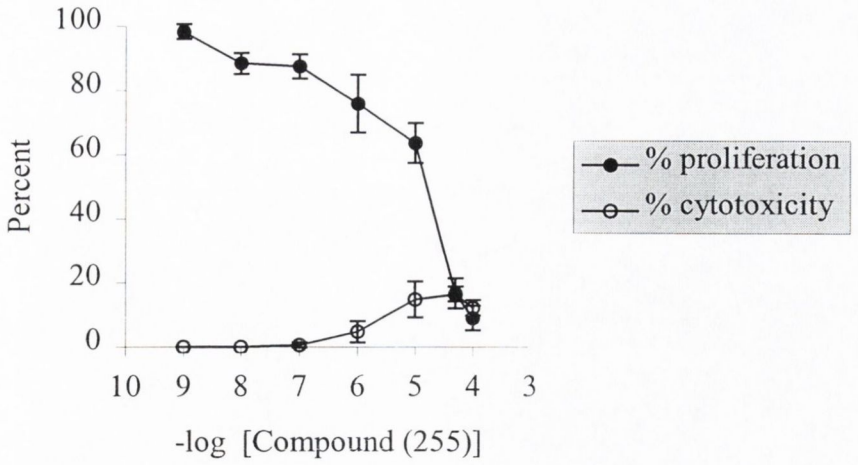


Figure 64: Compound (255) inhibits the proliferation and induces some cytotoxicity of MCF-7 cells

MCF-7 cells were seeded at a density of 1.5 x 10^4 cells per well in 96 multiwell plates and allowed attach to the surface of the walls for 24hr. After this period varying concentrations (1nM-100μM) of compound (255), 5-[(4-diethyl aminoethoxy)phenyl]-4-(4-methylphenyl)methyl-2,3-dihydro-1-benzoxepin, were added and the cells were left for a further 72hr. Determination of cell proliferation was then carried out using the MTT assay described in Section 4.2.5.1 and the cytotoxicity was evaluated using the LDH assay as described in Section 4.2.5.2. Each point represents the mean ± S.E.M. of triplicates. The absence of error bars indicates the error was smaller than the size of the symbol.

Compound	IC_{50} (μM)
Tamoxifen	11.3 ± 0.01
(152)	26.2 ± 6.8
(153)	14.2 ± 1.6
(154)	7.6 ± 0.6
(155)	10.6 ± 0.6
(156)	6.1 ± 2.6
(221)	11.1 ± 4.8
(222)	11.7 ± 2.1
(223)	56.6 ± 6.5
(224)	15.7 ± 4.7
(225)	33.6 ± 3.5
(226)	79.4
(227)	18.3 ± 1.7
(228)	20.4 ± 0.4
(229)	64.6 ± 1.5
(230)	74.3 ± 5.1
(231)	—
(232)	59.7 ± 3.4
(233)	19.9
(234)	16.7 ± 2.1
(235)	17.8 ± 1.2
(254)	199.5
(255)	56.2
(256)	39.5 ± 5.9
(257)	10.4 ± 2.7
(257)	35.0 ± 7.5

Table 21: IC_{50} values of benzoxepin analogues (152)-(156) and (221)-(235), for their antiproliferative effects on a human MCF-7 breast cancer cell line. IC_{50} value: the concentration required to inhibit 50% of MCF-7 cell growth. Values represent mean ± S.E.M. of triplicates.

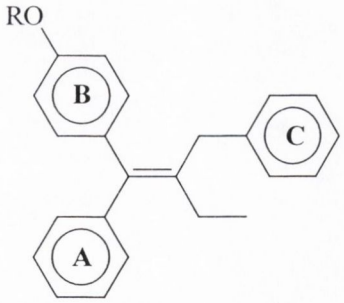
4.3.3. Discussion: antiproliferative and cytotoxicity profiles

4.3.3.1 Flexible tamoxifen type analogues

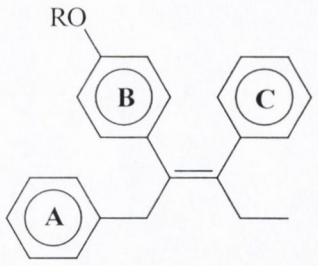
The data obtained were examined in two ways: by grouping the compounds into structural series (Type I-Type V) and by further grouping the compounds within each series by the presence of different basic side-chains (i.e. dimethyl, diethyl, piperidinyl, pyrrolidinyl, morpholinyl).[328]

On comparing the antiproliferative activities of four of the structural series of flexible tamoxifen-like analogues (Type I, II, III, IV), the Type II series is the most active therefore initiated the synthesis of Type V analogues. The Type V analogues are similar to Type II analogues, but they possess extended and halogenated ethylene functions. The IC_{50} values obtained from this series (Type V) were also similar to tamoxifen.

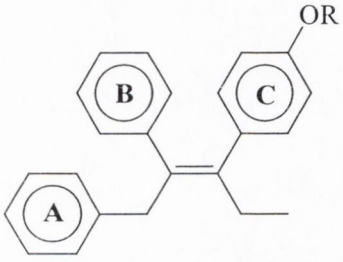
The side chain groupings from each series shows that the diethyl containing basic side chain compounds are, on average, the most active of the five units studied. If the structural diversification from tamoxifen of series I, II, III, IV is examined in conjunction with the average IC_{50} values obtained for the series as a whole, a 'ring order' for the compounds, based on the arbitrary 'ring order' for tamoxifen can be allocated. This is illustrated in Figure 65. In general terms, it is possible to determine the overall effect of the inclusion of a methylene (benzylic) spacing moiety into these compounds. Table 20 shows the average activity (± standard error of the mean) for each series type, with reference to tamoxifen, and Figure 65 highlights the area in which the additional flexibility was introduced to the structure. It can be seen from this data the result of introduction of a spacing group which removes the aryl ring from the vinylic system by one carbon (and the resulting flexibility this group confers to the molecule).



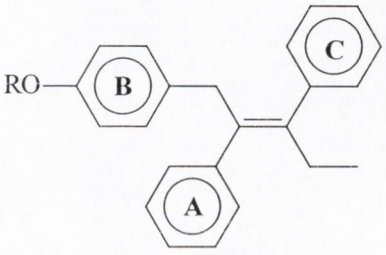
Type I (59) - (63)



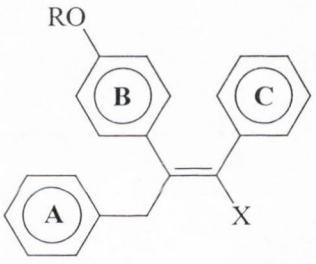
Type II (81) - (85)



Type III (96) - (100)



Type IV (108) - (112)



Type V (120) - (126)

R = —$(CH_2)_2N(CH_3)_2$

R = —$(CH_2)_2N(CH_2CH_3)_2$

R = —$(CH_2)_2$N

R = —$(CH_2)_2$N

R = —CH_2N O

X = $CH_2CH_2CH_3$

X = $CH_2CH_2CH_2CH_3$

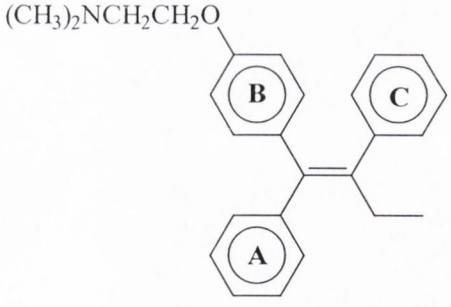
X = CH_2CH_2Cl

X = CH_2Br

X = CH_2NO_2

X = $CHBrCH_3$

X = CH_2Cl



Tamoxifen

Figure 65: Flexible tamoxifen-like analogues

For Type I series where the C-ring is displaced from the vinylic system, compounds (61) and (62) have IC_{50} values which, are 69% and 59% lower than that recorded for tamoxifen. The IC_{50} values for compounds (59) and (60) are only marginally higher than tamoxifen, but compound (63) shows a weak

antiproliferative effect. When the A ring is displaced, through the introduction of a methylene group as in Type II and III series, only a moderate reduction in efficacy is found, although it is noted that the most active compound (85) (IC_{50}, 78% lower than that of tamoxifen), stems from this series.

Within these series of compounds, it can be seen that the translocation of the basic side chain from ring B to C has no benefit with respect to antiproliferative activity. This investigation has shown that the least beneficial ring displacement arises when a methylene group is introduced to space ring B from the vinyl system.

All members of the Type IV series demonstrated antiproliferative activity, but the compounds were on average the least active of the compounds investigated.

From these observations, it can be concluded that the added flexibility between the vinyl system and the ring bearing the basic side chain in these compounds is detrimental to antiestrogenic activity. A direct comparison of the activity of those analogues with the *N*-dimethyl basic side chain to that recorded for tamoxifen can be made. Compounds (59), (81) and (108) are all directly derived structurally from tamoxifen, insofar as the overall distribution of side chains is conserved. As can be seen from the data, the increased flexibility bestowed on those compounds does not detract from their potency as antiestrogenic inhibitors of proliferation.

In terms of therapeutic potential, the potency of any given compound in terms of its inhibition of cellular proliferation should not arise from its propensity for cytotoxicity. With the design of novel antiestrogenic compounds, the achievement of cytostasis is the primary objective. Previous research has indicated that the antiproliferative effects manifested by tamoxifen are due in part to its inherent cytotoxicity.[493] Therefore, all compounds assayed for antiproliferative effects were concurrently tested for cytotoxicity. Several compounds possessed extremely good cytotoxicity profiles, but in most cases the compound's cytotoxicity did not exceed 10% and remained almost constant across the concentration range studied. This observation thus indicated that the mode of action of these flexible tamoxifen-like analogues is cytostatic rather than cytotoxic. The toxicity profiles are recorded in Figure 56 - 64 and clearly show that at the concentrations studied, the activity of the compounds is not due to cytotoxic effects but rather due to its antiproliferative effect. The cytotoxicity of compound (59) rises only slightly at a concentration of about 50μM and for compound (112) a small increase at 100μM. Contrary to this, a sharp (and

significantly larger) increase in the cytoxicity of tamoxifen can be seen above a concentration of 20μM. The level of cytotoxic-induced antiproliferative effect observed for tamoxifen at the 20μM concentration is approximately 29%, whereas when compounds (59) and (112) are examined at the same concentration, the cytotoxic contributions are less than 4%.

Therefore it can be concluded that the flexible tamoxifen analogues inhibit MCF-7 cell growth (i.e. inhibit cell proliferation) with a similar potency to that of tamoxifen, but are less cytotoxic than tamoxifen. The IC_{50} values of these synthesised analogues therefore suggest that they are efficient cytostatic agents.

4.3.3.2 Conformationally restrained benzoxepin analogues

The data obtained from Table 21 suggests that these conformationally restrained analogues do not exhibit antiproliferative effects as potent as those of the flexible tamoxifen-like analogues.

When reviewed, the benzoxepins with the extra methylene group do not have enhanced antiproliferative effects. In fact compounds (224) and (255) exhibit very poor antiproliferative activity on the MCF-7 breast cancer cells. This therefore suggests that displacing one of the aryl groups in the conformationally restrained analogues has no benefit for the overall activity of the compounds, but reduces antiproliferative activity.

The benzoxepin compounds (152)-(156), which possess the basic triphenylethylene tamoxifen structure, exhibit favourable IC_{50} values similar to that of tamoxifen, if not lower in some cases. This is perhaps due to the fact that these compounds maintain the overall tamoxifen triphenylethylene structure.

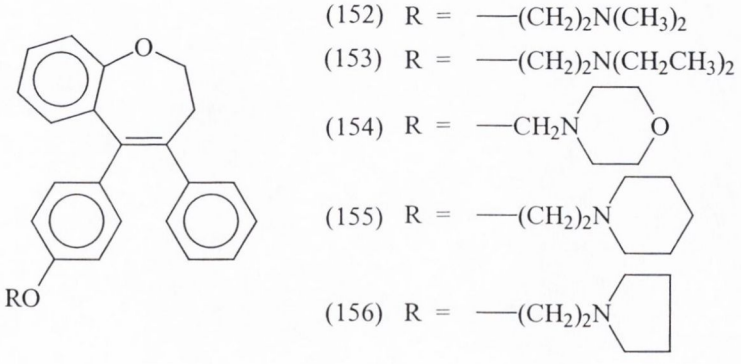


Figure 66: Structure of 5-(4-alkylaminoethoxyphenyl)-4-phenyl-2,3-dihydro-1-benzoxepin (152)-(156)

These antiproliferative results initiated the synthesis of a variety of benzoxepin compounds with the basic triphenylethylene tamoxifen structure, with a view to substituting the unsubstituted phenyl ring with a variety of methoxy-, methyl-, halogenated-, nitro- and hetero-aryl groups, thus estimating the benefit of substituted and unsubstituted benzoxepin analogues. It can be seen from the data that compounds (221) and (222), which possessed a methoxyphenyl group, gave the best IC_{50} values, which were similar to that of tamoxifen.

It can therefore be concluded that the conformationally restrained benzoxepin analogues are less active than the flexible tamoxifen-like compounds, except for the unsubstituted aryl benzoxepins. In particular, compound (156) has an IC_{50} value 46% better than that of tamoxifen.

The cytotoxic effect of these compounds is of prime concern, as discussed in Section 4.3.3.1. Each compounds cytotoxic effect was evaluated using the LDH assay. Most of the conformationally restrained analogues possess very good cytotoxic profiles and like the flexible analogues, few exceed the 10% cytotoxic effect values. This indicates that these benzoxepin type analogues are cytostatic rather than cytotoxic. The graphs indicate that the activity of these compounds is clearly due to their antiproliferative effects rather than their cytotoxicity. The cytotoxicity of compound (152) rises only slightly at a concentration of about 100μM and for compound (224), an increase is observed in cytotoxicity at the 20μM concentration.

It is therefore concluded, that the benzoxepin analogues, although being less active than their flexible-type counterparts, possess good antiproliferative effects, with little or no cytotoxicity observed for MCF-7 human breast adenocarcinoma cells.

4.3.4. Use of rat uteri cytosol as a model system for binding affinity

Preliminary experiments were carried out to characterise the binding of estradiol to the estrogen receptor in the cytosol of rat uteri. The dependence of binding on cytosol homogenate protein concentration was firstly determined. Specific binding of [^{3}H] estradiol (0.03nM) to uteri cytosol homogenate was proportional to the amount of protein present up to 150μg, after which the binding reached a plateau (Figure 67). For subsequent binding studies, protein amounts were selected to lie within the linear range.

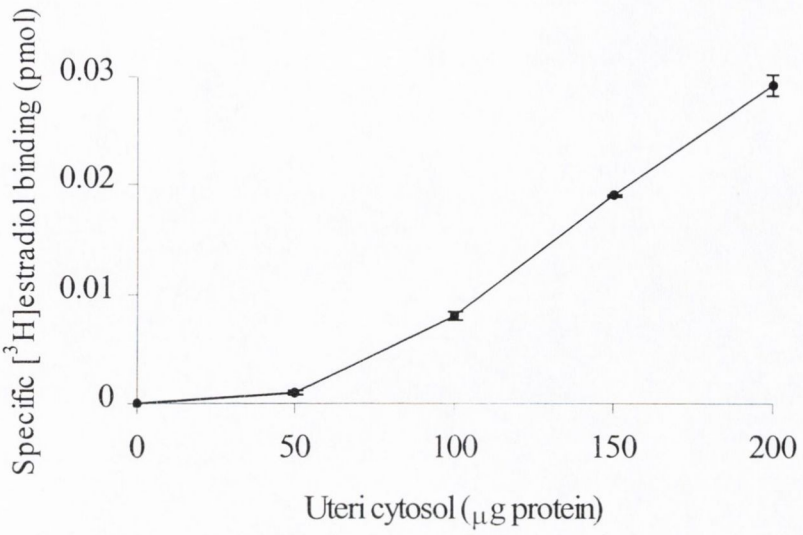


Figure 67: Dependence of specific [^{3}H] estradiol binding on uteri cytosol concentration

Aliquots of uteri cytosol homogenate ranging from 1-200μg protein were incubated with [^{3}H] estradiol (5nM, 157Ci/mmol) for 16hr at 4°C in the presence (non-specific binding) and the absence (total binding) of unlabelled estradiol (20μM), as described in Section 4.2.7. Specific binding was calculated from the difference between total and non-specific binding. Each point represents the mean ± S.E.M. of incubations performed in triplicate.

The high affinity binding of [^{3}H] estradiol to uteri cytosol homogenate was then determined. Binding was found to be saturable over the range 0-140nM, to a single class of binding sites, with a dissociation constant of 4.3 ± 1.9nM at a density of 0.70 ± 0.07 pmol/mg (Figure 68). This is in agreement with previous reports which demonstrate that, uteri cytosol cells exhibit a B_{max} of 0.90pmol/mg protein.

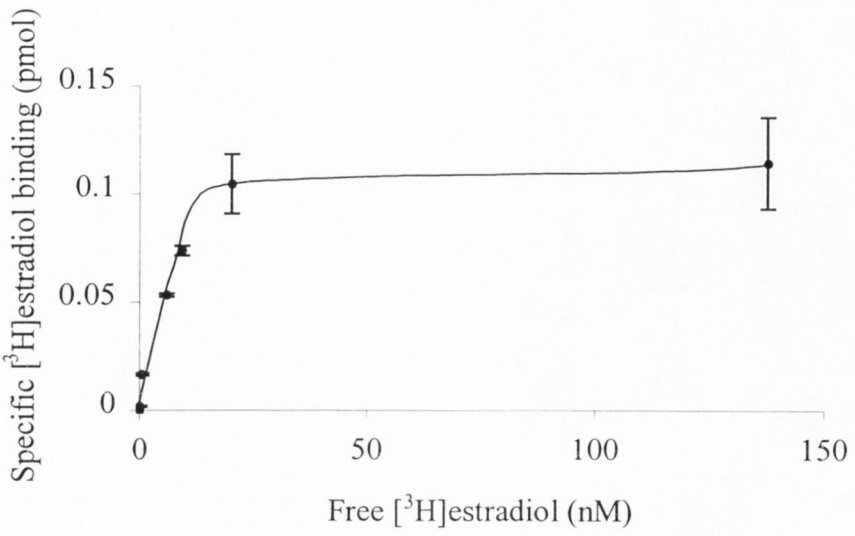


Figure 68: Saturable binding of [^{3}H] estradiol to homogenates of uteri cytosol

Uteri cytosol homogenate (150μg protein/assay) was incubated with [^{3}H] estradiol (0-140nM, 157Ci/mmol) for 16hr at 4°C in the presence (non-specific binding) and absence (total binding) of unlabelled estradiol (20μM), as described in Section 4.2.7. Specific binding was calculated from the difference between the total and non-specific binding. Each point is the mean of triplicate determinations and the error bars represent the S.E.M. Absence of error bars indicates the error was smaller than the size of the symbol. The data were fitted by the method of Wilkinson, with the use of the computer program MacCurvefit, yielding K_D and B_{max} values of 4.3 ± 1.9nM and 0.70 ± 0.07 pmol/mg protein respectively.

4.3.5. Binding studies on the cytosol of rats uteri

Binding assays were performed on selected potent antiproliferative compounds from the flexible and conformationally restrained analogue series. These studies determined the affinity of the tested compounds for the estrogen receptor based on their ability to compete with or replace bound radiolabelled estradiol. Such displacement studies have previously been used in determining the affinity of compounds for the ER.[161,490]

Binding studies were performed as described in Section 4.2.7. The amount of radioactivity bound in the presence of displacer was expressed as a percentage of control binding. Control binding is defined as specific binding in the absence of

unlabelled estradiol. The displacement curves generated for some of the synthesised compounds (Figure 69-77) are found in Table 22, which represents the affinity of the compounds for the ER (K_i value ± S.E.M.) obtained from the computer programs EBDA and LIGAND, as described in Section 4.2.9, for selected compounds.

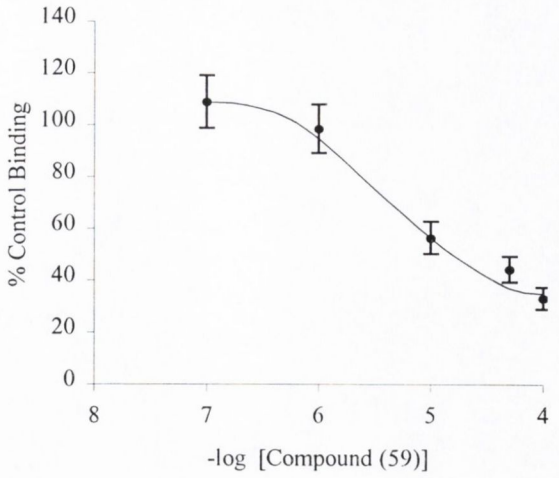


Figure 69: Compound (59) inhibits the specific binding of [^{3}H] estradiol to the estrogen receptor in rat uteri cytosol (ER).

Uteri cytosol (150μg) were incubated in triplicate with 5nM [^{3}H] estradiol (157Ci/mmol) and varying concentrations of compound (59) (100nM-100μM) for 16hr at 4°C as described in Section 4.2.7. Control binding is defined as specific binding in the absence of unlabelled estradiol. The amount of radioactivity bound in the presence of the displacer was expressed as a percentage of control binding and each point represents the mean value ± S.E.M. of incubations performed in triplicate. The best fit was obtained with the computer programs EBDA and LIGAND, yielding a K_i value of 459 ± 230nM.

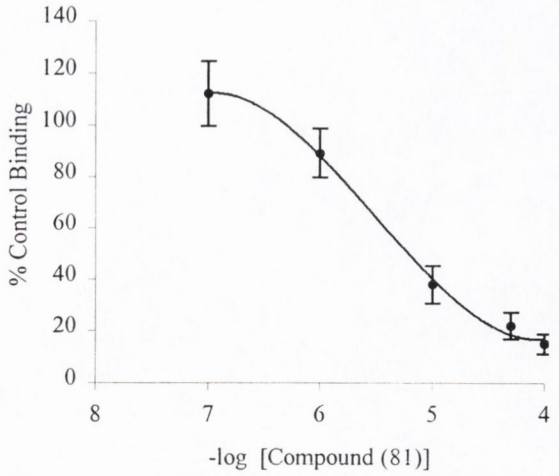


Figure 70: Compound (81) inhibits the specific binding of [^{3}H] estradiol to the estrogen receptor in rat uteri cytosol (ER).

Uteri cytosol (150μg) were incubated in triplicate with 5nM [^{3}H] estradiol (157Ci/mmol) and varying concentrations of compound (81) (100nM-100μM) for 16hr at 4°C as described in Section 4.2.7. Control binding is defined as specific binding in the absence of unlabelled estradiol. The amount of radioactivity bound in the presence of the displacer was expressed as a percentage of control binding and each point represents the mean value ± S.E.M. of incubations performed in triplicate. The best fit was obtained with the computer programs EBDA and LIGAND, yielding a K_i value of 503 ± 98nM.

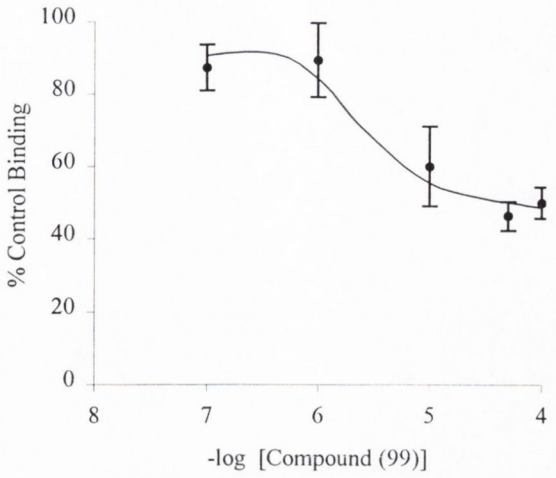


Figure 71: Compound (99) inhibits the specific binding of [^{3}H] estradiol to the estrogen receptor in rat uteri cytosol (ER).

Uteri cytosol (150μg) were incubated in triplicate with 5nM [^{3}H] estradiol (157Ci/mmol) and varying concentrations of compound (99) (100nM-100μM) for 16hr at 4°C as described in Section 4.2.7. Control binding is defined as specific binding in the absence of unlabelled estradiol. The amount of radioactivity bound in the presence of the displacer was expressed as a percentage of control binding and each point represents the mean value ± S.E.M. of incubations performed in triplicate. The best fit was obtained with the computer programs EBDA and LIGAND, yielding a K_i value of 2.39 ± 0.35μM.

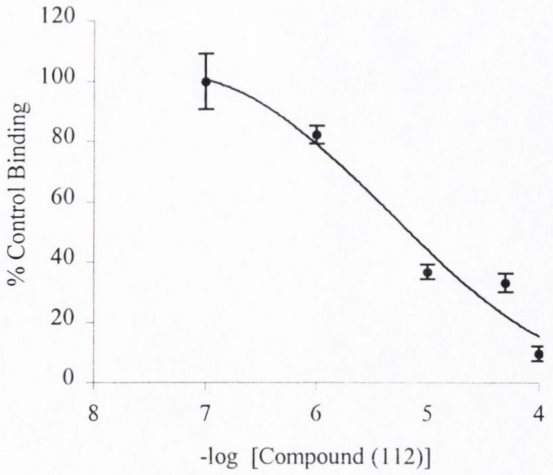


Figure 72: Compound (112) inhibits the specific binding of [^{3}H] estradiol to the estrogen receptor in rat uteri cytosol (ER).

Uteri cytosol (150μg) were incubated in triplicate with 5nM [^{3}H] estradiol (157Ci/mmol) and varying concentrations of compound (112) (100nM-100μM) for 16hr at 4°C as described in Section 4.2.7. Control binding is defined as specific binding in the absence of unlabelled estradiol. The amount of radioactivity bound in the presence of the displacer was expressed as a percentage of control binding and each point represents the mean value ± S.E.M. of incubations performed in triplicate. The best fit was obtained with the computer programs EBDA and LIGAND, yielding a K_i value of 2.48 ± 0.45μM.

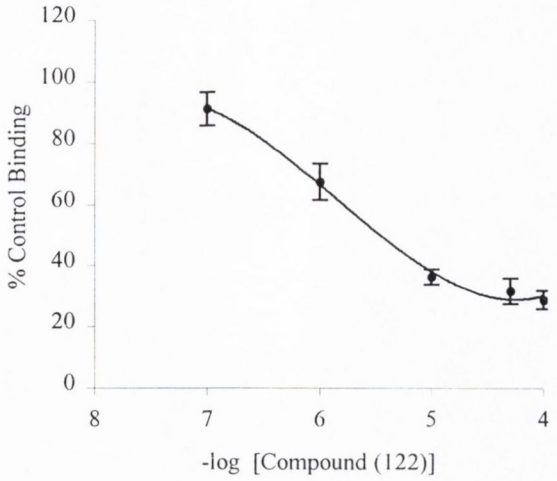


Figure 73: Compound (122) inhibits the specific binding of [^{3}H] estradiol to the estrogen receptor in rat uteri cytosol (ER).

Uteri cytosol (150μg) were incubated in triplicate with 5nM [^{3}H] estradiol (157Ci/mmol) and varying concentrations of compound (122) (100nM-100μM) for 16hr at 4°C as described in Section 4.2.7. Control binding is defined as specific binding in the absence of unlabelled estradiol. The amount of radioactivity bound in the presence of the displacer was expressed as a percentage of control binding and each point represents the mean value ± S.E.M. of incubations performed in triplicate. The best fit was obtained with the computer programs EBDA and LIGAND, yielding a K_i value of 496 ± 0.4μM.

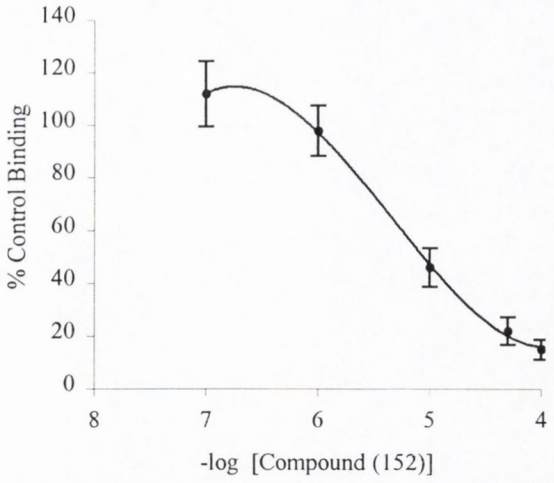


Figure 74: Compound (152) inhibits the specific binding of [^{3}H] estradiol to the estrogen receptor in rat uteri cytosol (ER).

Uteri cytosol (150μg) were incubated in triplicate with 5nM [^{3}H] estradiol (157Ci/mmol) and varying concentrations of compound (152) (100nM-100μM) for 16hr at 4°C as described in Section 4.2.7. Control binding was defined as specific binding in the absence of unlabelled estradiol. The amount of radioactivity bound in the presence of the displacer was expressed as a percentage of control binding and each point represents the mean value ± S.E.M. of incubations performed in triplicate. The best fit was obtained with the computer programs EBDA and LIGAND, yielding a K_i value of 3.21 ± 2.06μM.

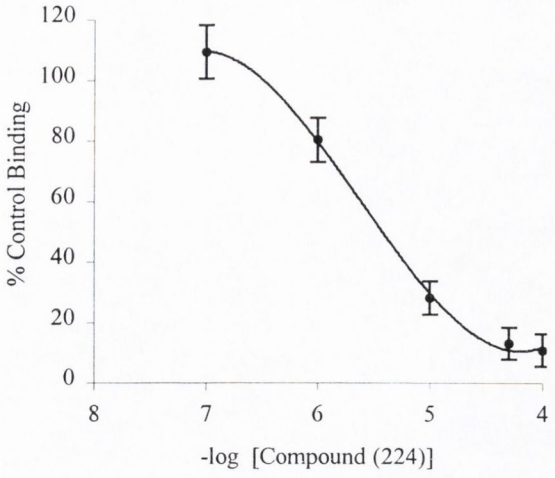


Figure 75: Compound (224) inhibits the specific binding of [^{3}H] estradiol to the estrogen receptor in rat uteri cytosol (ER).

Uteri cytosol (150μg) were incubated in triplicate with 5nM [^{3}H] estradiol (157Ci/mmol) and varying concentrations of compound (224) (100nM-100μM) for 16hr at 4°C as described in Section 4.2.7. Control binding is defined as specific binding in the absence of unlabelled estradiol. The amount of radioactivity bound in the presence of the displacer was expressed as a percentage of control binding and each point represents the mean value ± S.E.M. of incubations performed in triplicate. The best fit was obtained with the computer programs EBDA and LIGAND, yielding a K_i value of 828 ± 106nM.

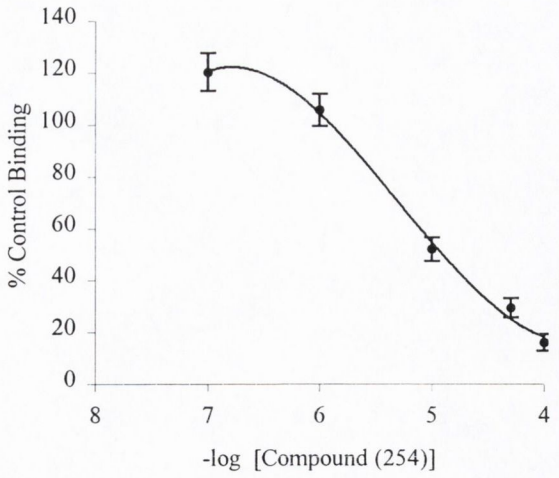


Figure 76: Compound (254) inhibits the specific binding of [^{3}H] estradiol to the estrogen receptor in rat uteri cytosol (ER).

Uteri cytosol (150μg) were incubated in triplicate with 5nM [^{3}H] estradiol (157Ci/mmol) and varying concentrations of compound (254) (100nM-100μM) for 16hr at 4°C as described in Section 4.2.7. Control binding is defined as specific binding in the absence of unlabelled estradiol. The amount of radioactivity bound in the presence of the displacer was expressed as a percentage of control binding and each point represents the mean value ± S.E.M. of incubations performed in triplicate. The best fit was obtained with the computer programs EBDA and LIGAND, yielding a K_i value of 14.18 ± 7.9μM.

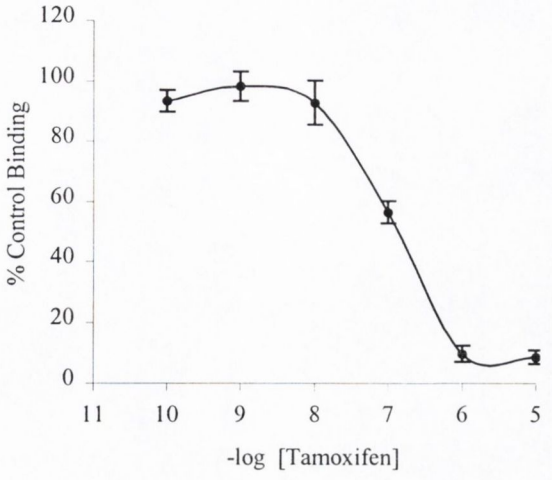


Figure 77: Tamoxifen inhibits the specific binding of [^{3}H] estradiol to the estrogen receptor in rat uteri cytosol (ER).

Uteri cytosol (150μg) were incubated in triplicate with 5nM [^{3}H] estradiol (157Ci/mmol) and varying concentrations of tamoxifen (100nM-100μM) for 16hr at 4°C as described in Section 4.2.7. Control binding is defined as specific binding in the absence of unlabelled estradiol. The amount of radioactivity bound in the presence of the displacer was expressed as a percentage of control binding and each point represents the mean value ± S.E.M. of incubations performed in triplicate. The best fit was obtained with the computer programs EBDA and LIGAND, yielding a K_i value of 157 ± 24nM.

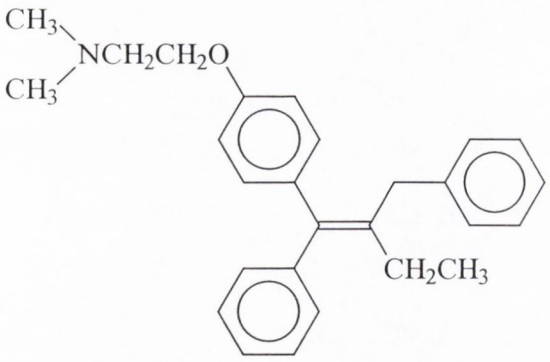
Binding affinity, determined by the K_i value was measured for compounds (59), (81), (99), (112), (122), (152), (224), (254) and are tabulated with reference to tamoxifen in Table 22

Compound	K_i Values
Tamoxifen	157 ± 24nM
Type I (59)	459 ± 23nM
Type I (81)	503 ± 98nM
Type III (99)	2.4 ± 0.4μM
Type IV (112)	2.5 ± 0.5μM
Type V (122)	496 ± 0.4μM
(152)	3.2 ± 2.1μM
(224)	828 ± 106nM
(254)	14.2 ± 7.9μM

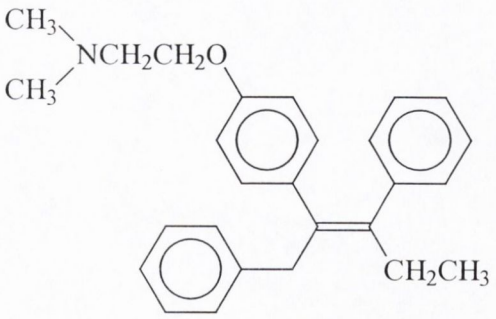
Table 22: Binding affinities of selected compounds for ER: comparison with tamoxifen. Values are expressed as the mean ± S.E.M for triplicate determinations.

4.3.6. Discussion: Binding affinities of compounds for ER

Binding assays were carried out on selected compounds from each series of flexible and conformationally restrained analogues that possessed high antiproliferative activity. The binding affinities (K_i values) were determined for each of the selected compounds and compared to that of tamoxifen as illustrated in Table 22. The binding affinity (K_i value) of tamoxifen for the ER in rat uteri cytosol is similar to previous findings[161]. Each of the active compounds assayed demonstrated good binding affinity for the estrogen receptor (i.e. cytosol of rat uteri), comparable to that recorded for tamoxifen. It is noted however that the binding affinities of the flexible tamoxifen-like analogues are better than the conformationally restrained benzoxepin analogues. When the data were correlated with the inhibitory activity of the compounds, it is perceived that compounds (59) and (81) [from Type I and II] possess the highest binding affinity for the estrogen receptor and are comparable to that of tamoxifen in the nanomolar range. These synthesised compounds are shown in Figure 78 and were observed as the having best activity of all the synthesised analogues as regards inhibitory activity and binding affinity for the estrogen receptor in breast cancer cells.



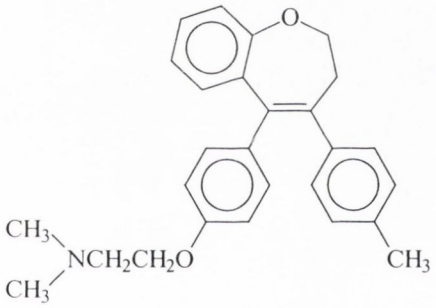
(59)



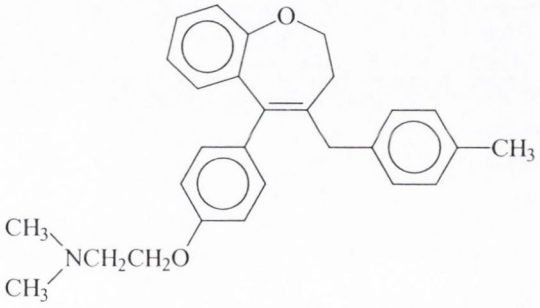
(81)

Figure 78: Structure of compounds (59) and (81)

Compound (122), which has an extended ethylene function, possesses a reasonable binding affinity as well as favourable antiproliferative effects. A similar correlation can be observed for Type III and IV series i.e. representative compounds (99) and (112) display only moderate binding affinities together with poor antiproliferative effects, which were discussed in Section 4.3.3.1.



(224)



(254)

Figure 79: Structure of compounds (224) and (254)

When the antiproliferative effects and binding affinities of the conformationally restrained benzoxepins are compared, it is noted that compound (254), the benzoxepin with the extra methylene moiety, possesses a poor binding affinity for the estrogen receptor along with a poor ability to inhibit proliferation of human breast cancer cells. Compound (224) [containing a methylaryl group], is noted to possess a favourable binding affinity for the ER in the nanomolar range, however, it is not as good as tamoxifen or the tamoxifen-like analogues (59) and (81).

In conclusion, there appears to be a correlation between binding affinity and antiproliferative potency for the series of compounds prepared. None of the analogues tested to date exhibit better binding affinities for the ER than tamoxifen, but some of the compounds exhibit more potent antiproliferative

activity on MCF-7 cell growth. The present study may thus be useful in helping to design further novel antiestrogens which exhibit more potent antiproliferative effects on breast tumour growth and which display higher binding affinities for the estrogen receptor.

5. Structure activity investigation on Tamoxifen and related-novel flexible analogues

5.1. Introduction

The estrogen receptor is responsible for the mediation of physiological effects of estrogen steroid hormones,[494] when hormone-binding to the ligand-binding domain of the receptor takes place. This hormone ligand then initiates a cascade of molecular and biochemical events, which ultimately can express themselves in the growth or modulation of certain tissues through the activation or inactivation of particular genes.[495] This has been discussed in detail in Chapter 1. In Chapter 2 the preparation of a series of novel compounds which, while related structurally to tamoxifen, deviate from the traditional approach of triphenylethylene analogues as antiestrogens through the introduction of a methylene group between the aryl and vinylic systems were described.[69,496,497] It has been suggested, that building flexibility into the rigid backbone of antiestrogens may enhance their antiproliferative activity and binding affinity for the estrogen receptor.[238]

The availability of highly resolved studies of crystal structures of the estrogen receptor has allowed the investigation of both actual and theoretical interactions of estrogenic and antiestrogenic materials in the ligand binding domain.[14,498] This data were used in conjunction with computational methods to provide rationalised S.A.R. (structure activity relationships) for the biological activity observed for the novel flexible (Type I-IV) compounds [the most active series], as described in Chapter 4.

These studies were carried out by firstly dividing the novel flexible analogues into their individual series Types I-IV. The objectives of these studies included:

a) attaining global minimised structures of each series Type, using SPARTAN,[499]

b) comparison of receptor bound and globally minimised 4-hydroxytamoxifen,

c) a docking study on the most active compound from the series Types,

d) identifying the critical residue interactions and compare these with those of 4-hydroxytamoxifen.

5.2. Structural images of Tamoxifen and novel-flexible analogues I-IV

The triarylethylene moiety of tamoxifen uniformly exists in a propellor conformation where the three rings are twisted in the same way although to a different extent.[500] It has been argued that the dihedral angles of the propellor blades (rings) are related to the relative binding affinity (RBA) of the triarylethylene system to estrogen receptors: the smaller the torsion angles, the lower the binding affinity.[87,501] The three aryl rings in all conformers exist in a propellor conformation in which the torsion angles (α, β and γ) are in the 52-74° range.[502]

The images detailed in Figures 81 - 85 using the Spartan programme depicts the 3D image of the flexible analogues (Type I-IV) compared to that of tamoxifen. The Type II analogues, having already been observed to possess favourable binding affinities and antiproliferative effects, show the benzylic group in the same plane as the corresponding aryl group of tamoxifen. Type II compounds have already been shown to possess the best biochemical activity when compared to the remaining novel flexible analogue Types and therefore it is interesting to note that Type II analogues have a similar 3D structure to that of tamoxifen. In Type I, III and IV analogues, the benzyl group is observed out of the plane, thus correlating to the biochemical activities of these compounds, where the antiproliferative and binding affinities are not as good as those of Type II analogues. The geometric, structural and electronic similarities between tamoxifen and the novel tamoxifen analogues are depicted in the following images.

In the 3D energy-minimised structures, the electron density is depicted using various colours. In the scale the blue colour suggests a high electron density whereas the lower electron density is represented by the red colour.

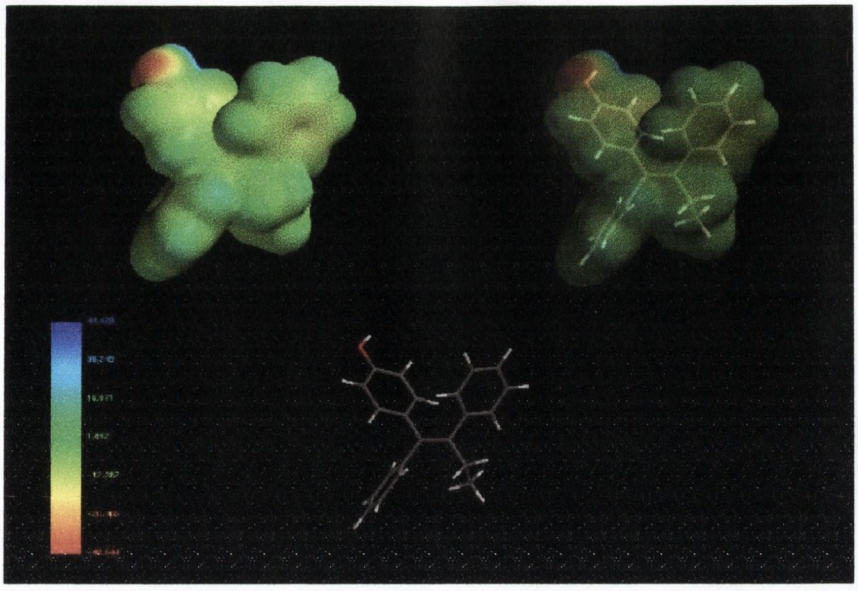


Figure 80: 3D Energy minimised structure of Tamoxifen (2)

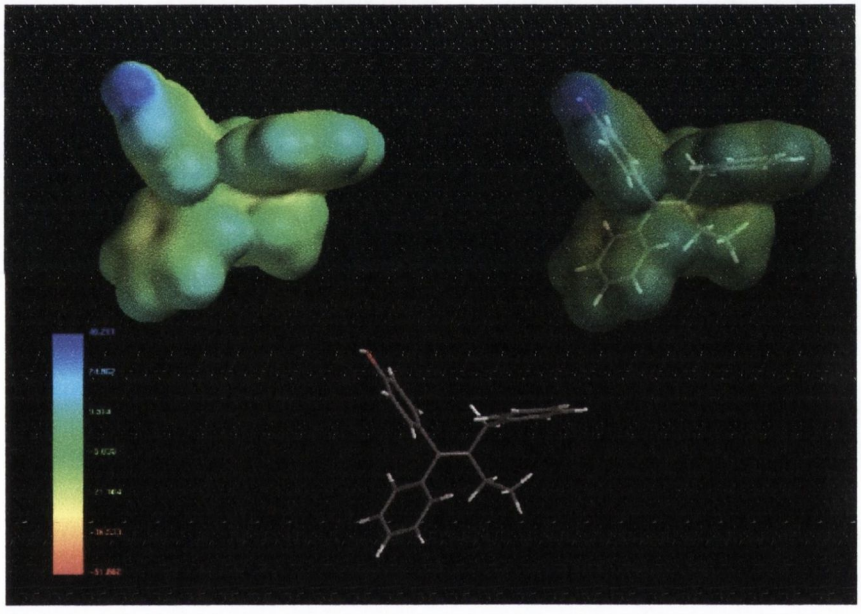


Figure 81: 3D Energy minimised structure of flexible analogues Type I

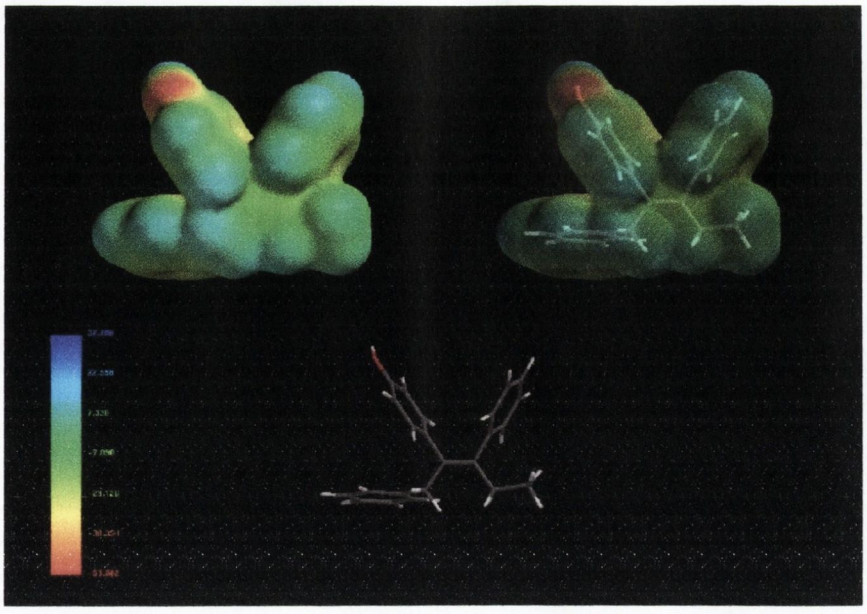


Figure 82: 3D Energy minimised structure of flexible analogues Type II

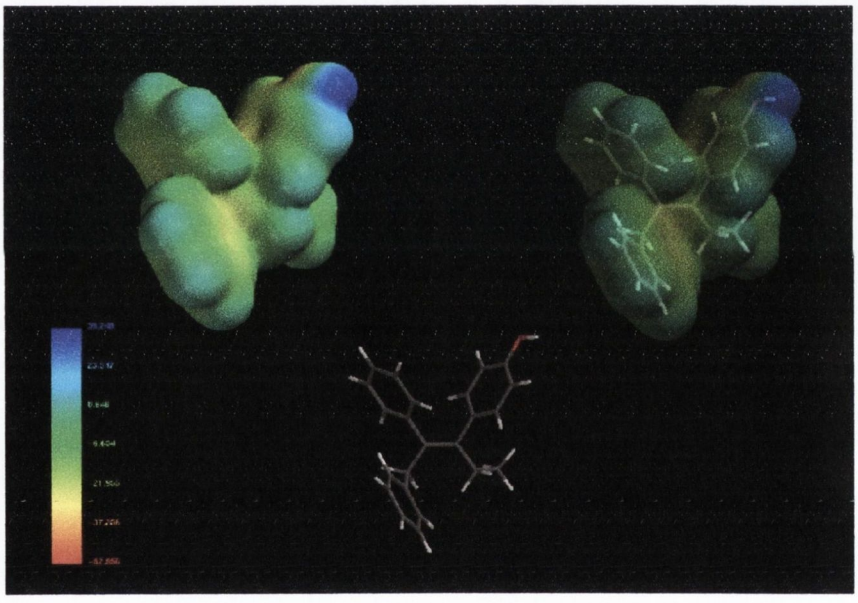


Figure 83: 3D Energy minimised structure of flexible analogues Type III

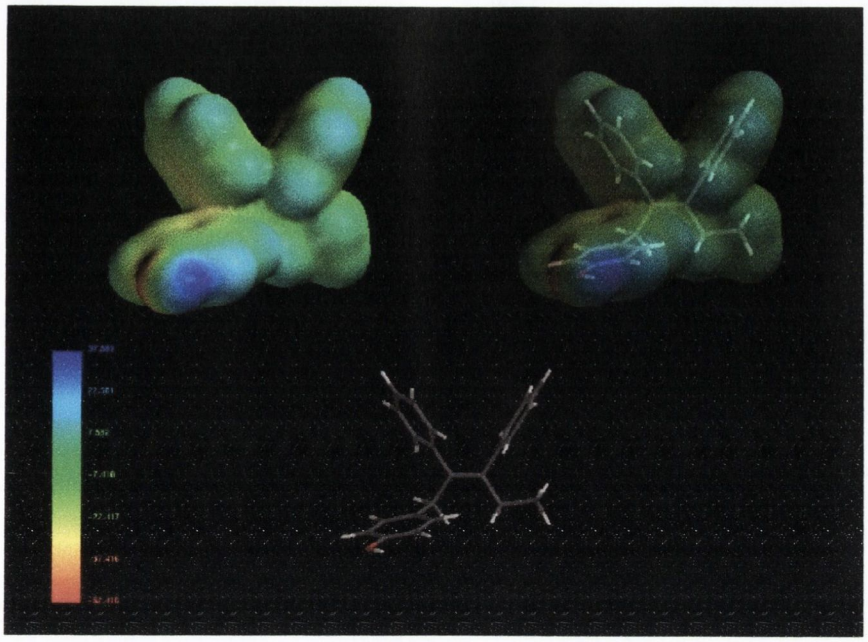


Figure 84: 3D Energy minimised structure of flexible analogues Type IV

With the use of these global minimised structures, the 3D structures of Type I-IV analogues are illustrated and compared to tamoxifen (2). Type II analogues are shown to possess a propellor like-structure similar to tamoxifen. This data correlates with the favourable biochemical activities observed for the same analogues, as depicted in Chapter 4.

Further computational methods were used to rationalise the biochemical results obtained after a clear relationship was observed between binding and antiproliferative activities. This comprised of initial modelling and minimisation of the novel flexible analogues. The minimisation procedure furnished detailed three-dimensional representations of the novel flexible analogues (Type I-IV) and tamoxifen.

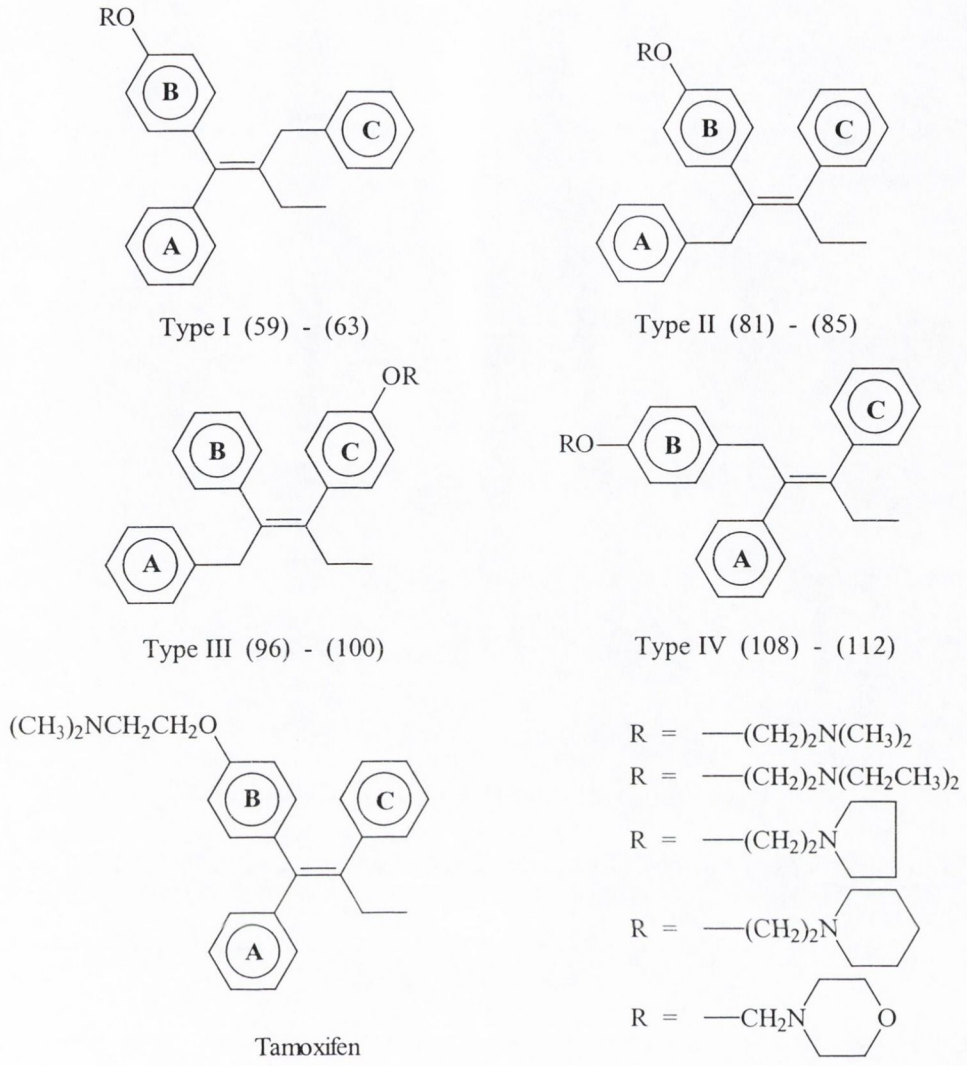


Figure 85: Structure of novel flexible analogues

Figure 86 illustrates a direct structural comparison of the most active compounds (62), (85), (99) and (112) (representing each of the four series Types I-IV) superimposed on a minimised tamoxifen (2) and the receptor-bound conformation of 4-hydroxytamoxifen.

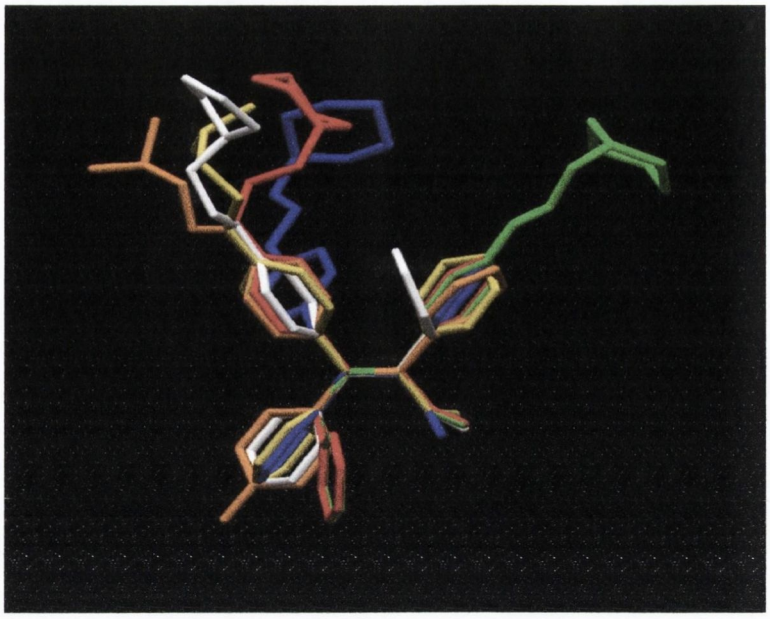


Figure 86: MacroModel superimposition of selected minimised compounds with tamoxifen and 4-hydroxytamoxifen

Yellow	:	Tamoxifen (2)
White	:	(62)
Red	:	(85)
Green	:	(99)
Blue	:	(112)
Orange	:	4-Hydroxytamoxifen (4)

It can be seen clearly from Figure 86 where the incorporated methylene spacing groups bestow additional flexibility on the novel flexible compounds. In each case, the overlap of the compounds with 4-hydroxytamoxifen is high, deviating significantly only where the methylene spacing group is introduced. While the basic side chain for (99) i.e. piperidine, is translocated to ring **C** of the structure (Figure 85), it can be seen for compounds (62), (85) and (112) that their respective basic side chains are orientated in a similar manner to that observed for 4-hydroxytamoxifen (4), with a slight deviation noticed for 1,2-diphenyl-2-[(4-morpholinylethoxy)benzyl]but-1-ene (112), (blue molecule) as the benzylic spacer between the vinylic system and ring **B** imparts additional flexibility in this region.

5.3. Docking studies

High-quality crystal structures of agonists and antagonists co-crystallised in the ligand-binding domain of the estrogen receptor are now available.[503,504] The information attained from these structures has shed much light on those residues responsible for the mediation of estrogenic and antiestrogenic responses arising from ligand binding. The availability of a good representation estrogen receptor binding the potent antiestrogen 4-hydroxytamoxifen (OHT)[503] enabled docking studies to be carried out with the novel flexible analogues, with a view to rationalising the antiestrogenic activity through analysing ligand-receptor interactions.

Work by Klinge *et al.*, described the similarities in the ligand-binding behaviours of 4-hydroxytamoxifen and tamoxifen, validating the choice of the 4-hydroxytamoxifen model.[505] The Brookhaven PDB file was manipulated on a Silicon Graphics O2 workstation using a combination of text editors[506] and the computational chemistry package, MacroModel.[507] To illustrate proof of concept, a comparison between the configuration of receptor bound 4-hydroxytamoxifen and energy minimised[508] 4-hydroxytamoxifen was carried out and is visually represented in Figure 87. This clearly shows the conformational correlation between the receptor bound and minimised structures. The calculated R.M.S. structural deviation between the geometries presented is 0.61Å.[509]

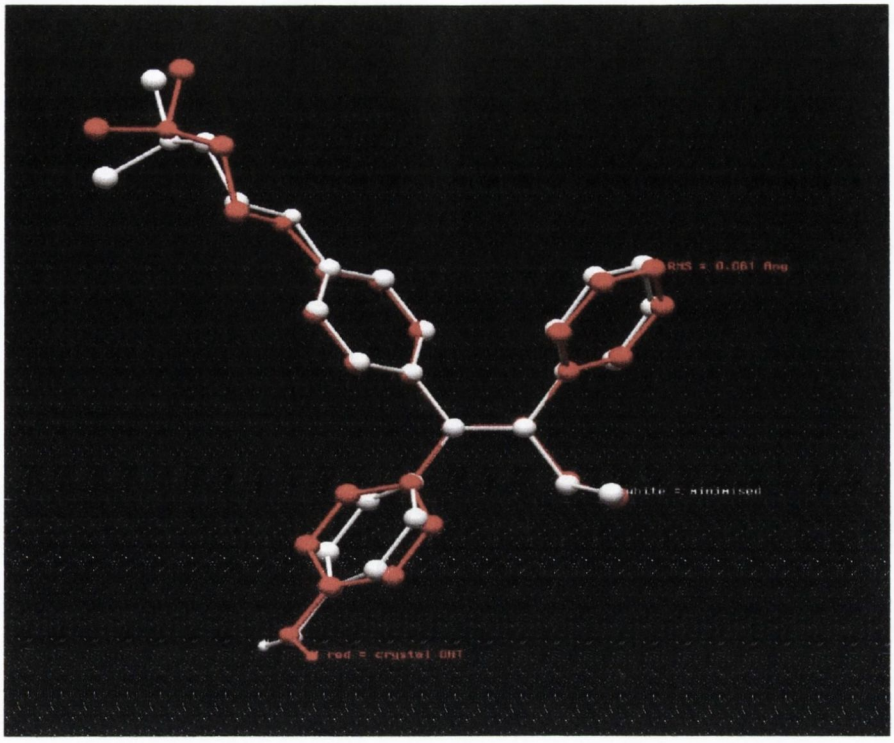


Figure 87: Comparison between the receptor-bound (red) and globally minimised (white) conformations of 4-hydroxytamoxifen. RMS structural deviation = 0.61Å. Minimisation was performed using a Monte-Carlo conformational search under the MM3 forcefield from MacroModel. Bound conformation taken from 3ERT PDB entry.

This energy minimisation procedure was applied to the most active compounds in each series chosen i.e. (62), (85), (99), (112) (Type I-IV) so as to prepare a set of globally minimised ligands for docking. Once the globally minimised ligand of interest had been manually positioned in the same location and orientation as 4-hydroxytamoxifen, the OHT was removed and a docking analysis performed using LIGIN.[510] This program was evaluated on its ability to re-insert the ligand OHT in the 3ERT[504] structure of the estrogen receptor. The accuracy of the program is reflected in Figure 88 where the LIGIN-docked OHT is shown in relation to its location and orientation in the original crystal structure.

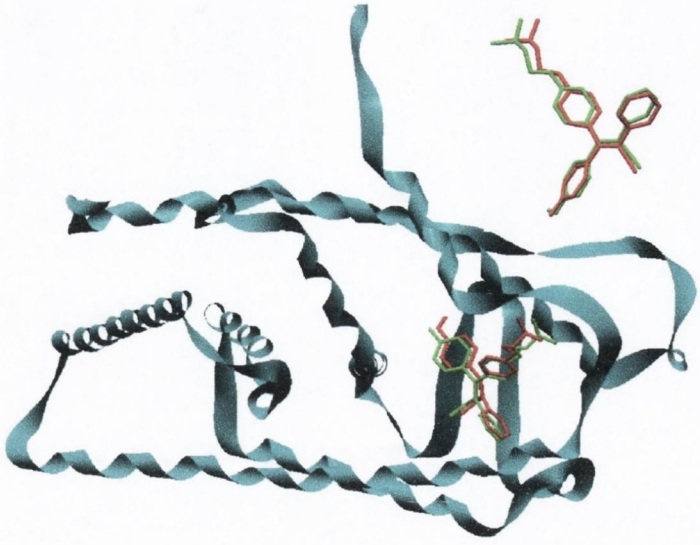


Figure 88: Comparison of the LIGIN-docked (green) and crystal structure [bound] (red) positions of 4-hydroxytamoxifen in the estrogen receptor, visualised using MacroModel. Top right inset shows enlarged rendering of the respective ligands.

The program output when manipulated through MacroModel allowed the generation of a set of 'model structures' for the novel ligands docked in the estrogen receptor. The data from these docking operations provided a set of ligand-protein contact (LPC)[511] information which was compared to that available for the antiestrogen- containing PDB entries 3ERT and 1ERR.[503,504]

The in-depth LPC analysis was concentrated primarily on the following specific residues: Glu 353 and Arg 394 (which are involved primarily in anchoring ligands in the active site), His 524 (identified as an important estrogenic residue from studies with diethylstilbestrol[504] and estradiol[503]), Asp 351 (identified by Brzozowski *et al.*,[503] as an important antiestrogenic residue) and finally with Thr 347, which, through analysis of the differing interactions from antiestrogens and estrogens, is also deemed a significant residue for antiestrogenic activity.

The LPC data details all close contacts between the docked molecules and specific residues in the estrogen receptor. The program LIGPLOT[512] was utilised to deconvolute the three-dimensional information present in the LIGIN / MacroModel generated 'model structures'[513] for the compound 2-benzyl-1-phenyl-1-[(4-pyrrolidinylethoxy)phenyl]but-1-ene (62), into more easily referenced two dimensional plots as shown in Figure 89.

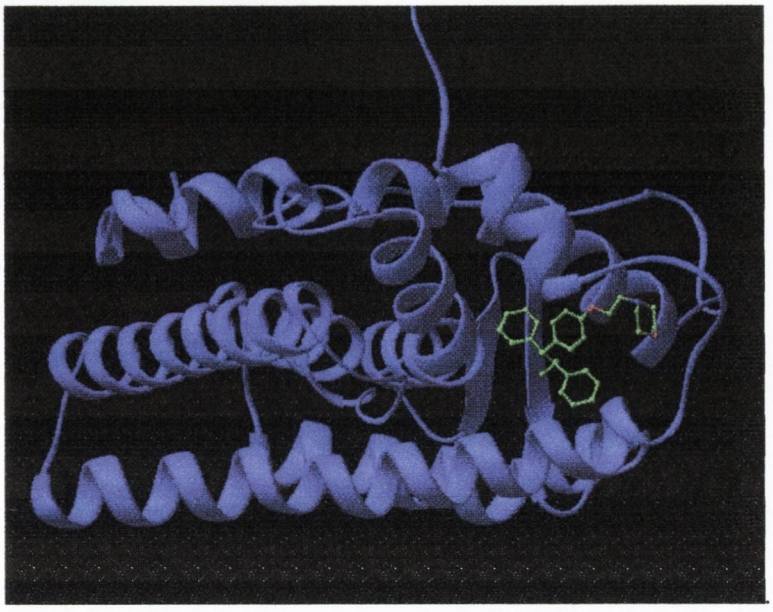


Figure 89a: Model structure generated for compound (62), docked into the human estrogen receptor site, using the LIGIN program in the docking mode. Visualisation rendered from the program Ribbons.

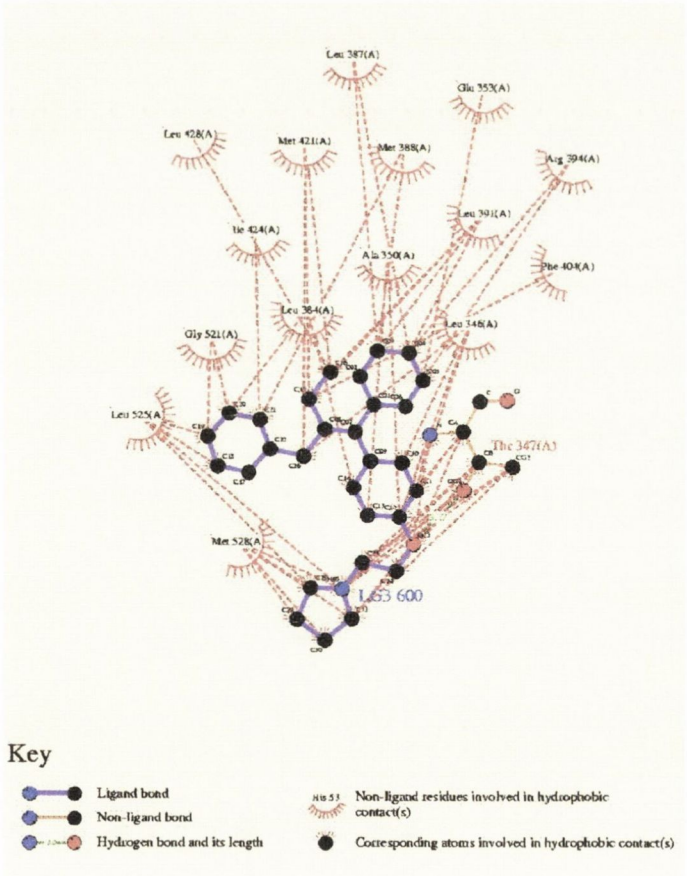


Figure 89b: Possible interaction of compound (62) with residues in the active site.

The docked orientation of each compound within the estrogen receptor ligand-binding domain could be readily visualised and similarities to existing receptor-bound crystal structures appreciated. The LPC program written by Sobolev *et al.*,[511] operates based on calculated complementarity values for differing types of atoms, e.g. hydrophobic contacts, hydrophilic interactions, hydrogen bond donor or acceptor atoms. The program classifies and generates a list of possible interactions for the docked ligand within the receptor, based on the interaction types detailed and the co-ordinate file input. For each LPC trial the types of atoms present in subject compounds were manually explicitly defined. Analysis of the LPC output allowed the determination of the degree of interaction between synthesised compounds and key residues, which had been previously identified as being of significance for estrogenic or antiestrogenic activity as well as other residues that are deemed to be important from published crystal structures.

The basis for this assignment stems from the much higher interactions seen between antiestrogenic compounds and this residue than those observed for estrogenic compounds.[328] Presently, no crystal structure exists for tamoxifen complexed with the human estrogen receptor. The computational protocol detailed above was followed and a 'model structure' for tamoxifen was generated and a set of LPC data for this theoretical docking amassed. Table 23 illustrates the LPC data for one of the most active compound (62) while Table 24 depicts the specific residues of choice for each compound (62), (85), (99) and (112) representatives of Type I – IV respectively with reference to those contacts calculated from existing crystal structures and the docked tamoxifen 'model structure' data.

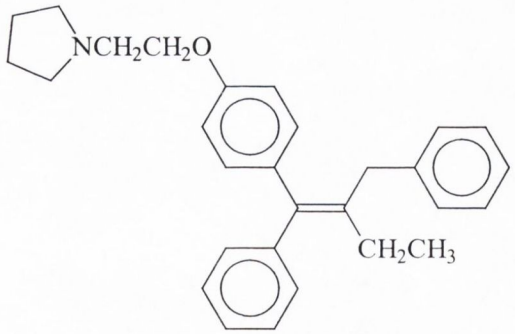


Figure 90: Structure of compound (62)

Residue	Dist	Surf	Specific contacts			
			HB	Arom	Phob	DC
343A MET*	3.9	20.3	-	-	+	-
346A LEU*	2.7	73.8	-	-	+	-
347A THR*	2.9	50.8	+	-	+	-
349A LEU*	4.4	7.6	-	-	+	-
350A ALA*	3.8	18.4	-	-	+	-
351A ASP*	4.7	7.2	-	-	-	+
353A GLU*	3.4	22.9	-	-	-	-
383A TRP*	4.6	10.3	-	+	-	-
384A LEU*	3.6	26.7	-	-	+	-
387A LEU*	2.9	57.9	-	-	+	-
388A MET*	3.4	24.9	-	-	+	-
391A LEU*	3.5	16.4	-	-	+	-
394A ARG*	3.7	23.8	-	-	-	-
404A PHE*	3.2	35.4	-	+	+	-
419A GLU	5.4	0.4	-	-	-	-
420A GLY	5.8	1.8	-	-	-	-
421A MET*	3.3	21.1	-	-	+	-
424A ILE*	3.5	20.4	-	-	+	-
428A LEU*	2.8	39.5	-	-	+	-
521A GLY*	3.2	33.7	-	-	-	-
524A HIS*	4.7	5.6	-	-	+	-
525A LEU*	2.8	113.3	-	-	+	+
528A MET*	3.1	30.7	-	-	+	+
529A LYS*	5.7	2.9	-	-	-	-
530A CYS*	5.5	13.0	-	-	+	+

Dist : nearest distance (Angstroms) between atoms of the ligand and the residue
Surf : contact surface area (Angstroms^2) between the ligand and the residue
HB : hydrophilic-hydrophilic contact (probable hydrogen bond)
Arom : aromatic-aromatic contact
Phob : hydrophobic-hydrophobic contact
DC : hydrophobic-hydrophilic contact (destabilizing contact)
+/- : indicates presence/absence of a specific contact
* : indicates residues contacting ligand by their side chain

Table 23: Data from LPC for compound (62)

These tables, in conjunction with the LIGPLOT renderings such as that illustrated in Figure 89b, give some insight into the possible interactions occurring at the active site. In Table 24, a comparison is made between the novel flexible ligands and known antiestrogens. Their theoretical dockings show that they are anchored to much the same extent as antiestrogenic and estrogenic compounds via residues Arg 394 and Glu 353. Contact with the 'estrogenic' residue His 524 is low and is comparable to that of the known antiestrogen OHT, whereas raloxifene[503] is a special case, insofar as it is a selective estrogen

receptor modulator (SERM) and its mode of action can best be described as an 'estrogen-like' antiestrogen.[514] Contact with the 'antiestrogenic' residue Asp 351 is lower than that recorded for known antiestrogens and that predicted for tamoxifen itself, whereas contact with the Thr 347 residue reflects the results observed for both known antiestrogenic compounds and the predicted value for tamoxifen, which far outweigh the contacts recorded for estrogenic compounds.

Compound	Thr 347	Asp 351	His 524	Arg 394	Glu 353
(62)	50.82	7.18	5.61	23.78	22.88
(85)	37.02	9.80	13.70	21.99	10.77
(99)	49.60	12.79	3.59	24.23	8.52
(112)	38.80	19.30	19.30	13.00	28.70
OHT	40.50	28.95	14.36	22.18	34.18
RAL	39.30	30.90	32.80	22.00	32.70
TAM	50.70	40.80	11.00	22.00	29.20
EST	7.20	0.00	40.40	17.80	35.90
DES	8.30	0.00	38.80	15.80	39.00

OHT: 4-Hydroxytamoxifen from PDB entry 3ERT.
RAL: Raloxifene from PDB entry 1ERR.
TAM: Tamoxifen– LPC contacts generated from docked file.
EST: Estradiol from PDB entry 1ERE.
DES: Diethylstilbestrol from PDB entry 3ERD.

Table 24: LPC results for specific residues with comparison to known estrogens and antiestrogens-data given as overlap (Angstroms ^{2}) with residue

5.4. Conclusion

To rationalise the biological data for the flexible analogues, in the absence of a crystal structure, computational docking and ligand-protein contact studies were carried out indicating that these molecules (model structures) dock in the same region of the estrogen receptor and interact with the same key residues as known antiestrogenic compounds. These computational studies correlate with the biological data obtained and confirm that Type II analogues are in fact the most active novel compounds synthesised.

Although the docking and resulting LPC data are theoretical, until such time as a crystal structure is obtained for one of these novel compounds in the ligand binding domain of the estrogen receptor, such tools will have to serve to furnish some measure of explanation for the biological results obtained because no direct correlation can be made between the LPC data and activity demonstrated. Further studies utilising this computational protocol in the pre-synthetic modification of selected compounds and in the design of novel flexible ligands, with a view to increasing specific residue interactions and antiestrogenic activity are to be carried out in the future.

6. Future work

6.1. Proposed Developments

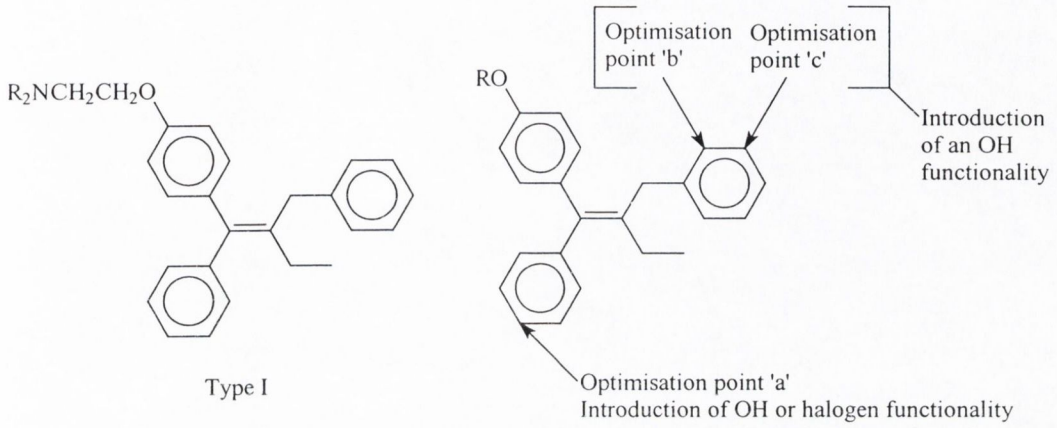
Many studies focusing on antiestrogens, such as tamoxifen, have indicated that these compounds, apart from preventing estrogen binding to its receptor, act in both a cytostatic and cytotoxic manner. In the present study a novel series of tamoxifen analogues were developed and it was demonstrated that some of these analogues prevent estrogen binding to its receptor with a potency similar to that of tamoxifen.[328] In addition these compounds inhibited the proliferation of a human MCF-7 breast carcinoma cell line, again with similar potency to tamoxifen. Furthermore, it has been shown that some of these analogues induce a cytotoxic effect on this cell line although the nature of this cytotoxic effect (necrosis/apoptosis) has not as yet been examined. These novel tamoxifen analogues may have potential as anticancer therapeutics in the treatment of breast cancer.

The validity of any new therapy and/or therapeutic agent is more easily established if a sound mechanistic basis can be shown. In order to understand the mechanism underlying the antiproliferative/cytotoxic action of these novel tamoxifen derivatives, it is necessary to answer important questions about the initial biochemical target events for the compounds and to further identify the steps in the cellular response pathway(s) leading to cell cycle arrest and/or apoptosis.[515]

The ability of the novel tamoxifen derivatives to induce apoptosis in MCF-7 cells could be assessed and the potency of action compared to tamoxifen. Apoptosis is a "cell suicide" mechanism, in which the cell programs itself to die. It is characterised by shrinkage of the cell, condensation of the chromatin, DNA fragmentation and the packaging of DNA into small apoptotic bodies. Apoptosis is quite distinct from another type of uncontrolled cell death called necrosis where the cell bursts and release its contents. The mechanism by which many chemotherapeutic drugs kill cancerous cells remains unclear but the drugs ultimately induce apoptosis in these cells.[516] Indeed, tamoxifen has previously been shown to induce apoptosis of MCF-7 cells and this has been suggested to contribute to its anti-tumour action. Therefore, the design of tamoxifen analogues which induce apoptosis of MCF-7 cells may be advantageous.

Tamoxifen has also been reported to have effects on other important components of intracellular signalling pathways, such as antagonism of calmodulin action and inhibition of the enzyme protein kinase C (PKC). These activities are thought to be significant in the antitumour action of the drug.[517]

The general structural features of the novel flexible analogues Type I (lead compound), in the context of those interactions required for antiestrogenic activity in the model of the estrogen receptor (ER), direct the development of the following proposed optimisation targets.



6.1.1. Substitution at position 'a' indicated above by hydroxy or halide functionalities to yield compounds with predicted higher activity than the parent compound.

This is evidenced by the high activities of 4-hydroxytamoxifen (4) and idoxifene (29). This higher activity is due to increased favorable interactions of such substituents with two specific residues within the ER - Glu 353 and Arg 394. These residues play an anchoring role in the binding of ligands to the ER.

6.1.2. Hydroxy substitution of the lead compound in either the 'b' and/or 'c' positions to favourably increase ligand interaction with His 524 in the ER binding site.

The 'b' and 'c' positions in the lead compound have been chosen for modification due to the docking orientation of this parent ligand in the ER.

6.1.3. SAR studies for compound optimisation.

Detailed SAR and QSAR analyses will be routinely performed correlating the data from *in vitro* assays with molecular properties, so as to continually optimise the molecular design.

6.1.4. The preparation of active pro-drugs for in vivo *assay to counteract the rapid bioelimination of phenolic optimised leads.*

Phenolic drugs are often administered as esters, which are metabolically cleaved *in vivo* to furnish the desired species. Preliminary molecular modelling studies of phosphate or formate ester prodrugs of the optimised leads discussed above have indicated that these prodrug species themselves inherently exhibit favourable residue interactions with the model of the estrogen receptor.

6.1.5. Computational simulation and subsequent synthesis of metabolites of the pro-drugs and optimised lead compounds.

Studies of metabolites of known antiestrogens have found that the major metabolites of these compounds are themselves antiestrogens - this will be investigated for these novel ligands.

7. Experimental section

7.1. General experimental details

Spectroscopic and physical data

Melting points (m.p.) were determined on a Gallenkamp apparatus and are uncorrected.

Spectra were obtained on the following instruments:

Infra red (IR): Nicolet 205 FT-IR and band positions are given in cm^{-1}. Solid samples were analysed by potassium bromide disc (KBr); oils were analysed as films on NaCl plates.

Nuclear magnetic resonance (NMR): ^{1}H NMR, Bruker DPX 400 (400.14 MHz); ^{13}C NMR, Bruker DPX 400 (100.625MHz); ^{14}C DEPT 135, Bruker DPX 400. NMR spectra were analysed with Bruker WIN-NMR software. The NMR spectra were recorded in deuterated chloroform with tetramethylsilane (TMS) as internal standard except where indicated. Peak positions were assigned relative to the $CDCl_3$ resonances at 7.26ppm for ^{1}H NMR and 77.00ppm for ^{13}C NMR. Chemical shift values are reported on the δ scale relative to TMS and coupling constants are reported in Hertz. Chemical shifts are reported values (number of protons, description of resonance, coupling constant(s) where applicable and assignment).

Abbreviations used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet.

Mass spectrometry:

Low resolution mass spectrometry (EIMS-LR) was carried out in the Department of Pharmacology and Therapeutics, Trinity College Dublin by Dr. Pierce Kavanagh. Low resolution spectra were run on a Hewlett-Packard 5973 MSD GC-MS system.

High resolution mass spectrometry (EIMS-HR) was carried out in the Department of Chemistry, University College Cork. High resolution accurate mass determinations were made on a Kratos Prohile HV-4 mass spectrometer using the direct insertion probe and electron impact ionisation techniques.

Chromatographic methods

R_f values are quoted for thin layer chromatography on Merck F-254 plates. Column chromatography was carried out with Merck Kiesegel 60 (particle size 0.040-0.063 mm). Preparative plate chromatography was carried using 15PSC Kiesegel 60F254 (Platter 20x20cm, 2mm). Compounds were visually detected with UV at 254nm.

HPLC (High Performance Liquid Chromatography)

The chromatographic conditions used were those outlined in the British Pharmacopoeia 1998 for tamoxifen. The mobile phase consisted of a mixture of acetonitrile (300mL), water (125mL), THF (75mL) and 18M ammonia (2mL), with a flow rate of 1.5mL per minute and a detection wavelength of 240nm. The stationary phase was packed with octadecylsilyl silica gel C_{18} column (5μm).

Purification of solvents

All solvents were distilled prior to use. Anhydrous solvents were prepared according to literature methods.[518]

Acetone, the analytical reagent generally contains less than 0.1% organic impurities but may have up to about 1% water. It was dried with anhydrous potassium carbonate, distilled and used directly. Dichloromethane was predried with calcium chloride and then over phosphorus pentoxide. It is then distilled and used. Dioxane usually contains impurities such as acetaldehyde, ethylene acetal, acetic acid, water and peroxides. This solvent was predried over calcium chloride granules and then over sodium and benzophenone until a purple colour was observed. Dioxane was distilled and used immediately. THF was initially dried over calcium hydride, then further dried by refluxing over sodium and benzophenone. When a blue/purple colour was observed, the THF was distilled and used immediately.

Pet. ether is defined as petroleum ether with a boiling range between 40-60°C

7.2. Experimental details

7.2.1. Chalcone synthesis

General Method 2.1

To a solution of benzaldehyde (0.05M) and an appropriately substituted acetophenone (0.05M) in ethanol (50mL) at ambient temperature was added a catalytic quantity of ground NaOH (5-6 pellets). The mixture was stirred for 1-6hr. The precipitated product was filtered, washed with ethanol, water, dried under vacuum and recrystallised from ethanol.

1,3-Diphenylpropen-1-one (47)

This product was prepared using the general method 2.1 with benzaldehyde and acetophenone, as a colourless solid post recrystallisation from ethanol in 96% yield; m.p. 56°C [lit. m.p. 55-57°C] [519]

IR ν_{max} (KBr) 1658 (C=O), 1601 (C=C) cm^{-1}

^{1}H NMR $\delta(CDCl_3)$ 6.78-7.72 (12H, m, H-2, H-3, 10ArH)

^{13}C NMR $\delta(CDCl_3)$ 120.23 (C-2), 124.77 (C-4'), 127.32, 127.49 (C-3'', C-5''), 127.60, 128.65 (C-2', C-3', C-5', C-6'), 128.11, 128.30 (C-2'', C-6''), 131.68 (C-4''), 132.57 (C-1''), 145.12 (C-3), 202.15 (C-1)

1-(4-Methoxyphenyl)-3-phenylpropen-1-one (48)

This product was obtained using the method above with benzaldehyde and 4-methoxyacetophenone, as a colourless / lemon crystalline solid after recrystallisation with ethanol in 94% yield; m.p. 102-104°C [lit. m.p. 104°C][246]

IR ν_{max} (KBr) 1653 (C=O), 1603 (C=C) cm^{-1}

^{1}H NMR $\delta(CDCl_3)$ 3.90 (3H, s, OCH_3), 7.00 (2H, d, J = 10.52Hz, H-3', H-5'), 7.42-7.44 (3H, m, H-3'', H-4'', H-5''), 7.56 (2H, d, 15.56Hz, H-2), 7.65-7.67 (2H, m, H-2'', H-6''), 7.82 (1H, d, 15.52Hz, H-3), 8.06 (2H, d, J = 9.04Hz, H-2', H-6')

^{13}C NMR δ($CDCl_3$) 55.41 (OCH_3), 113.65 (C-3'', C-5''), 121.70 (C-2), 128.28 (C-2', C-6'), 128.92 (C-3', C-5'), 130.35 (C-4'), 130.69 (C-2'', C-6''), 130.96 (C-1'), 134.96 (C-1''), 143.49 (C-3), 163.34 (C-4''), 189.70 (C-1)

7.2.2. Dihydrochalcone synthesis

General Method 2.2

A suspension of the appropriately substituted chalcone (0.05M) in ethanol (50mL) was stirred under heat, until complete dissolution occurred. 10% Pd (0.035M) on activated charcoal was added and the reaction mixture was stirred at room temperature under an atmosphere of H_2. Stirring was maintained until TLC analysis verified that hydrogenation of the starting material was complete. The catalyst was then removed via filtration, washed with ethanol and the solvent was evaporated under reduced pressure. The crude product was purified by recrystallisation from ethanol.

1,3-Diphenylpropan-1-one (49)

The general method 2.2 was employed using 1,3-diphenylpropen-1-one (0.024M), 10% Pd on activated charcoal (0.017M) and ethanol (35mL). The crude product was recrystallised from ethanol and produced a colourless crystalline solid in 95% yield, m.p. 70-72°C [lit. m.p. 70-71°C][260]

IR ν_{max} (KBr) 1676(C=O), 1450 (CH_2) cm^{-1}

^{1}H NMR δ($CDCl_3$) 3.15 (2H, t, J = 8.01Hz, H-3), 3.38 (2H, t, J=8.00Hz, H-2), 7.44-8.23 (10H, m, ArH)

^{13}C NMR δ($CDCl_3$) 30.10 (C-3), 40.44 (C-2), 126.16 (C-4'), 128.01 (C-3'', C-5''), 128.39, 128.52 (C-2', C-6', C-3', C-5'), 128.58 (C-2'', C-6''), 132.97 (C-4''), 136.80 (C-1''), 140.11 (C-1'), 199.21 (C-1)

1-(4-Methoxyphenyl)-3-phenylpropan-1-one (50)

The previous procedure 2.2 was employed using 1-(4-methoxyphenyl)-3-phenylpropen-one (0.018M), 10% Pd on activated charcoal and ethanol (30ml). The product was recrystallised from ethanol as a colourless crystalline solid in 95% yield; m.p. 95-97°C [lit. m.p. 96-97°C][520]

IR ν_{max} (KBr) 1672 (C=O) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 3.05 (2H, t, J = 8.30Hz, H-3), 3.28 (2H, t, J=8.32Hz, H-2), 3.88 (3H, s, 4''-OCH_3), 6.90-7.05 (2H, m, H-3'', H-5''), 7.27-7.35 (5H, m, H-2', H-6, H-4', H-3', H-5'), 7.91-8.09 (2H, m, H-2'', H-6'')

^{13}C NMR $\delta(CDCl_3)$ 30.28 (C-3), 40.04 (C-2), 55.38 (4''-OCH_3), 113.59 (C-3'', C-5''), 126.05 (C-4'), 128.37, 128.49 (C-2', C-6', C-3', C-5'), 129.95 (C-1''), 130.28 (C-2'', C-6''), 140.41 (C-1'), 198.01 (C-1)

7.2.3. Alkylation of dihydrochalcones

General method 2.3

A suspension of NaH (60% dispersion in oil) in dry THF (30mL) was prepared at 0°C. To this was added dropwise the appropriately substituted 1,3-diarylpropan-1-one in dry THF (30mL) at 0°C. This solution was stirred at ambient temperature for 1 hour. Post this period it was cooled to 0°C and ethyl iodide was added in one portion. The reaction mixture was allowed to stir for 16-18hr at room temp. The reaction was quenched with slow addition of HCl (5% v/v, 15mL) poured onto H_2O and extracted with diethyl ether (3 × 25mL). The ether extracts were combined, washed with saturated NaCl solution and dried over $MgSO_4$. The product was purified using flash column chromatography.

2-Benzyl-1-phenylbutan-1-one (51)

The general method 2.3 outlined was employed using NaH [60% dispersion] (0.042M), 1,3-diphenylpropan-1-one (0.01M) and ethyl iodide (0.034M). The work up was carried out as previously detailed above. Flash column chromatography [eluant petroleum ether : ethyl acetate; 96:4] afforded a mustard coloured oil[256] in 96% yield, possessing the following physical properties.

IR ν_{max} (film) 1673 (C=O) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 0.97-1.02 (3H, t, J = 7.47Hz, H-4), 1.56-1.92 (2H, m, H-3), 2.82 (1H, dd, J = 13.73, 6.66Hz, CH_2), 3.15 (1H, dd, J = 13.71, 7.51Hz, CH_2), 7.11-7.45 (8H, m, ArH), 7.84-7.89 (2H, m, H-2'', H-6'')

^{13}C NMR δ($CDCl_3$)	11.63 (C-4), 25.23 (C-3), 37.66 (CH_2), 49.67 (C-2), 126.08 (C-4'), 128.15 (C-2', C-6'), 128.32 (C-3', C-5'), 128.53 (C-3'', C-5''), 128.98 (C-2'', C-6''), 132.79 (C-4''), 137.48 (C-1''), 140.00 (C-1'), 203.77 (C-1)

2-Benzyl-1-(4-methoxyphenyl)butan-1-one (52)

The general method 2.3 was employed using NaH [60% dispersion] (0.03M), 1-(4-methoxyphenyl)-3-phenylpropan-1-one (0.012M), and ethyl iodide (0.036M). Column chromatography (eluant petroleum ether : ethyl acetate; 100:0.6), presented the pure product as a yellow coloured oil in 79% yield.[256]

IR ν_{max} (film)	1679 (C=O) cm^{-1}
^{1}H NMR δ($CDCl_3$)	0.89 (3H, t, J = 7.40Hz, H-4), 1.51-1.80 (2H, m, H-3), 2.79 ($1H_{(a)}$, dd, J = 13.64, 6.59Hz, CH_2), 3.03 ($1H_{(b)}$, dd, J = 13.87, 7.51Hz, CH_2), 3.54- 3.58 (1H, m, H-2), 3.80 (3H, s, 4''-OCH_3), 6.82-6.87 (2H, m, H-3'', H-5''), 7.13-7.18 (5H, m, H-2', H-3', H-4', H-5, H-6'), 7.75-7.92 (2H, m, H-2'', H-6'')
^{13}C NMR δ($CDCl_3$)	11.68 (C-4), 25.43 (C-3), 37.92 (CH_2), 49.27 (C-2), 55.37 (4''-OCH_3), 113.67 (C-3'', C-5''), 126.01 (C-4'), 128.26, 128.96 (C-2', C-3', C-5', C-6'), 130.26 (C-2'', C-6''), 130.40 (C-1''), 140.20 (C-1'), 163.31 (C-4'), 202.20 (C-1)

7.2.4. Synthesis of 2-benzyl-1,1-diphenylbutan-1-ols

General method 2.4

To dry THF (15mL) at –78°C under nitrogen was added PhLi (0.01M, 1.8 M in hexane). This solution was allowed to stir for 30min under these conditions after which the appropriately substituted butan-1-one (0.005M) in dry THF (10mL) was added slowly at -78°C. This reaction mixture was maintained for 1 hour at –78°C and then stirred at ambient temperature for 12hr. Post this period the reaction was extracted with ethyl acetate (3x20mL), washed with NaCl (10% w/v, 20mL), dried over Na_2SO_4 and concentrated. Product purification was carried out using flash column chromatography.

2-Benzyl-1,1-diphenylbutan-1-ol (53)

The general method 2.4 was employed using PhLi (0.01M/5.56mL), 2-benzyl-1-phenylbutan-1-one (0.005M) in dry THF (20mL). Column chromatography (eluant petroleum ether : ethyl acetate; 95:5), presented a mustard coloured oil in 78% yield with the following physical properties.[256]

IR ν_{max} (film) 3573 (OH), 3100-3040, 2970-2880 (CHs), 1510 (CH_2), 1455 (CH_3) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 0.65 (3H, t, J = 7.50Hz, H-4), 1.33-1.39 (2H, m, H-3), 2.17 (1H, s, OH ex. D_2O), 2.50-2.54 (1H, m, H-2), 2.80-2.87 (2H, m, CH_2), 7.08-7.31 (11H, m, ArH), 7.53-7.57 (4H, m, H-3'', H-5'', H-3''', H-5''')

^{13}C NMR $\delta(CDCl_3)$ 13.35 (C-4), 23.43 (C-3), 36.73 (CH_2), 49.42 (C-2), 125.60 (C-2'', C-6'', C-2''', C-6'''), 125.69 (C-4'), 126.37 (C-4''', C-4''), 128.16 (C-3''', C-5''', C-3'', C-5''), 128.36, 129.02 (C-2', C-6', C-3', C-5'), 141.44 (C-1'), 145.99 (C-1''', C-1'')

2-Benzyl-1-(4-methoxyphenyl)-1-phenylbutan-1-ol (54)

The general method 2.4 was applied using PhLi (0.014M / 7.8 mL), 2-benzyl-1-(4-methoxyphenyl)-1-phenylbutan-1-one (0.0071M) in dry THF (30mL). Flash column chromatography (eluant petroleum ether : ethyl acetate; 93:7), afforded a lemon coloured oil in 72% yield.

IR ν_{max} (film) 3468 (OH), 3175-3021, 2981-2870 (CHs), 1521 (CH_2), 1475 (CH_3) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 0.65 (3H, t, J = 7.49Hz, H-4), 1.29-1.65 (2H, m, H-3), 2.21 (1H, s, OH ex. D_2O), 2.50-2.57 (1H, m, H-2), 2.79 (2H, m, CH_2), 3.81 (3H, s, OCH_3), 7.12-7.49 (11H, m, ArH), 7.55-7.59 (2H, m, H-3''', H-5''')

^{13}C NMR $\delta(CDCl_3)$ 13.34 (C-4), 23.24 (C-3), 36.31 (CH_2), 49.70 (C-2), 55.13 (4''-OCH_3), 113.47 (C-3'', C-5''), 125.55 (C-2'', C-6'', C-2''', C-6'''), 125.66 (C-4'), 126.32 (C-4'''), 126.83 (C-3''', C-5'''), 128.15, 129.01 (C-2',

C-6', C-3', C-5'), 141.70 (C-1'), 146.96, 147.87 (C-1'', C-1''')

EIMS (HR) $C_{24}H_{26}O_2$: calculated M^+ 346.1926

observed M^+ 346.1933

7.2.5. Synthesis of 2-benzyl-1,1-diphenylbut-1-enes

2-Benzyl-1,1-diphenylbut-1-ene (55)

Method 1: To a solution of 2-benzyl-1,1-diphenylbutan-1-ol (0.012M) in EtOH (30 mL) was added concentrated H_2SO_4 (8mL) and the mixture was refluxed gently for 24hr. The resulting solution was concentrated, neutralised with NaOH (10% w/v), extracted with ethyl acetate (2 × 20mL), washed with saturated NaCl solution, dried over Na_2SO_4 and concentrated. Flash column chromatography (eluant petroleum ether : ethyl acetate; 99:1), afforded the product as a mustard-coloured oil in 83% yield.

IR ν_{max} (film) 3084-3026, 2968-2867 (CHs),1601,1570 (C=C) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 1.02 (3H, t, J = 7.47Hz, H-4), 2.11 (2H, q, J = 7.48Hz, H-3), 3.59 (2H, s, CH_2), 7.23-7.32 (15H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 12.79 (C-4), 24.19 (C-3), 36.76 (CH_2), 125.36 (C-4'), 125.80, 125.86 (C-4'', C-4'''), 127.63, 127.70 (C-2'', C-6'', C-2''', C-6'''), 127.84, 128.25 (C-2', C-6', C-3', C-5'), 128.77 (C-3'', C-5'', C-3''', C-5'''), 138.38, 139.04 (C-1, C-2), 140.14 (C-1'), 142.65, 142.72 (C-1'', C-1''')

2-Benzyl-1-(4-methoxyphenyl)-1-phenylbut-1-ene (56)

Method 1: A solution 2-benzyl-1-(4-methoxyphenyl)-1-phenylbutan-1-ol (0.0016M) in EtOH (20mL) was allowed to reflux at 90-95°C for 30min prior to addition of 85% polyphosphoric acid (0.08M) and the mixture was refluxed gently for 2-4hr. The resulting solution was neutralised with NaOH (10% w/v), poured onto H_2O, extracted with ethyl acetate (3 × 25mL), washed with brine, dried over Na_2SO_4 and concentrated. Column chromatography (eluant petroleum

ether: ethyl acetate, 96:4), afforded a pure product as cream coloured oil in 79% yield.

IR ν_{max} (film)	3095-3018, 2985-2828 (CHs), 1605 (C=C) cm^{-1}
^1H NMR $\delta(CDCl_3)$	0.98-1.03 (3H, m, H-4), 2.05-2.14 (2H, m, H-3), 3.58, 3.62 (2H, 2 x s, CH_2), 3.80, 3.83 (3H, 2 x s, OCH_3), 6.83-6.89 (2H, m, H-3'', H-5''), 7.18-7.32 (12H, m, ArH)
^{13}C NMR $\delta(CDCl_3)$	13.22 (C-4), 24.65 (C-3), 37.17 (CH_2), 55.13 (4''-OCH_3), 113.50 (C-3'', C-5''), 125.55 (C-2'', C-6'', C-2''', C-6'''), 125.73 (C-4'), 126.11 (C-4'', C-4'''), 127.99, 128.06 (C-2'', C-6'', C-2''', C-6'''), 128.25, 128.63 (C-2', C-6', C-3', C-5'), 129.18 (C-3''', C-5'''), 140.25 (C-1'), 143.05, 144.36 (C-1'', C-1''')
EIMS (HR)	$C_{24}H_{24}O$: calculated M^+ 328.1827 observed M^+ 328.1861
Mass Spectrum (m/z)	328 (M^+, 84%), 299 (M^+-29, 61%), 237 (M^+-91, 30%), 221 (M^+-107, 20%), 213 (M^+-115, 100%), 197 (M^+-131, 15%), 191 (M^+-137, 19%)

7.2.6. Demethylation of 2-benzyl-1-(4-methoxyphenyl)-1-phenylbut-1-ene

2-Benzyl-1-(4-hydroxyphenyl)-1-phenylbut-1-ene (57)

Method 1: A solution of 2-benzyl-1-(4-methoxyphenyl)-1-phenylbut-1-ene (56)(0.027M) in dry dichloromethane (35 mL) was stirred at –78°C for 30min under nitrogen. Post this period boron tribromide (0.025M) was added dropwise and the mixture was allowed to stir for a further 1-2hr at –78°C under N_2. The product mixture was then allowed to stir overnight at ambient temperature. The resulting solution was neutralised with NaOH (20% w/v), extracted with dichloromethane (3x30mL), washed with brine, dried over $MgSO_4$ and concentrated. The product was purified using flash column chromatography (eluant dichloromethane : petroleum ether, 60:40), which afforded a light brown coloured oil in 87% yield.

IR ν_{max} (film)	3572-3157 (OH), 3086-3019, 2985-2890 (CHs), 1607 (C=C), 1516 (CH_2), 1460 (CH_3) cm^{-1}

^{1}H NMR δ($CDCl_3$)	0.99 (3H, m, H-4), 2.10 (2H, m, H-3), 3.57, 3.61 (2H, 2 x s, CH_2), 4.77 (1H, d, J = 7.86Hz, OH ex. D_2O), 6.77-6.81 (2H, m, H-3'', H-5''), 7.11-7.32 (12H, m, ArH)
^{13}C NMR δ($CDCl_3$)	12.78 (C-4), 24.17, 24.29 (C-3), 36.71, 36.83 (CH_2), 114.53, 114.49 (C-3'', C-5''), 125.73 (C-4'), 126.13 (C-4'', C-4'''), 127.99, 128.06 (C-2'', C-6'', C-2''', C-6'''), 128.23, 128.62 (C-2', C-6', C-3', C-5'), 129.17 (C-3''', C-5'''), 138.12, 138.43 (C-1, C-2), 140.21 (C-1'), 142.98 (C-1'', C-1'''), 153.52 (C-OH)
EIMS (HR)	$C_{23}H_{22}O$: calculated M^+ 314.1671 observed M^+ 314.1692
Mass Spectrum (m/z)	314 (M^+, 88%), 285 (M^+-29, 65%), 223 (M^+-91, 31%), 207 (M^+-107, 46%), 191 (M^+-123, 27%)

7.2.7. Acylation of 2-benzyl-1-(4-hydroxyphenyl)-1-phenylbut-1-ene

1-(4-Acetoxyphenyl)-2-benzyl-1-phenylbut-1-ene (58)

To 2-benzyl-1-(4-hydroxyphenyl)-1-phenylbut-1-ene (57) (0.0004M) was added acetic anhydride (1.2mL), and anhydrous pyridine (1.2mL). The mixture was shaken gently for 5min and then placed in darkness at room temperature overnight. The resulting solution was poured onto ice water (50mL) to quench the reaction and extracted with diethyl ether (2 × 25mL). The ethereal extracts were washed with HCl (0.1N, 40mL), H_2O, saturated $NaHCO_3$ solution, dried over Na_2SO_4 and concentrated under reduced pressure. Flash column chromatography (eluant dichloromethane : petroleum ether; 50:50), afforded the product as an amber coloured oil in 68% yield with the following physical properties.

IR ν_{max} (film)	3064-3004, 2965-2842 (CHs), 1738 (CH_3C=OO) 1603 (C=C) cm^{-1}
^{1}H NMR δ($CDCl_3$)	0.97 (3H, m, H-4), 2.05 (2H, m, H-3), 2.36 (3H, m, CH_3C=O), 3.56, 3.58 (2H, 2 x s, CH_2), 7.01-7.03 (2H, m, H-3'', H-5''), 7.21-7.28 (12H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$	13.63 (C-4), 24.14 (C-3), 31.46 ($CH_3C=O$), 36.71 (CH_2), 113.72 (C-3'', C-5''), 125.39 (C-2'', C-6'', C-2''', C-6'''), 125.96 (C-4'), 127.66 (C-3''', C-5'''), 128.20, 128.80 (C-2', C-6', C-3', C-5'), 142.43 (C-1')
EIMS (HR)	$C_{25}H_{24}O_2$: calculated M^+ 356.1776 observed M^+ 356.1765
Mass Spectrum (m/z)	356 (M^+, 100%), 327 (M^+-29, 13%), 314 (M^+-42, 47%), 297 (M^+-59, 16%), 285 (M^+-71, 94%), 271 (M^+-85, 12%), 236 (M^+-120, 14%), 223 (M^+-133, 40%), 207 (M^+-149, 31%), 165 (M^+-191, 17%)

7.2.8. Alkylation of 2-benzyl-1-(4-hydroxyphenyl)-1-phenylbut-1-ene

General Method 2.8

To 2-benzyl-1-(4-hydroxyphenyl)-1-phenylbut-1-ene (57) (0.00045M) in dry acetone was added anhydrous potassium carbonate (0.0072M) and stirred gently for 5-10min under N_2. Post this period was added 2-(alkylamino)ethyl chloride hydrogen chloride and the resulting solution was refluxed for 5-6hr after which TLC analysis showed full consumption of starting material. The resulting reaction mixture was vacuum filtered, washed with acetone and concentrated under reduced pressure. The product was purified using flash column chromatography.

2-Benzyl-1-[4-(dimethylaminoethoxy)phenyl]-1-phenylbut-1-ene (59)

Method 1: The general method 2.8 outlined was employed using 2-benzyl-1-(4-hydroxyphenyl)-1-phenylbut-1-ene (0.00045M) in dry acetone (8mL), anhydrous K_2CO_3 (0.0072M) and 2-(dimethylamino)ethyl chloride hydrogen chloride (0.0018M). Flash chromatography (eluant methanol : dichloromethane; 60:40) afforded a brown coloured oil in 80% yield.

IR ν_{max} (film)	3077-2950, 2919-2741 (CHs), 1606 (C=C), 1509 (NCH_2), 1458 (NCH_3, CH_2), 1374 (CH_3), 1277, 1242 (CN) cm^{-1}
^1H NMR $\delta(CDCl_3)$	0.98 (3H, m, H-4), 2.06 (2H, m, H-3), 2.40 (6H, d, J = 7.28Hz, $N(CH_3)_2$), 2.78 (2H, m, NCH_2), 3.55, 3.59

(2H, 2 x s, CH_2), 4.08 (2H, m, CH_2O), 6.84-6.88 (2H, dd, J = 9.18Hz, H-3'', H-5''), 7.13- 7.31 (12H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 13.20 (C-4), 24.62 (C-3), 37.17 (CH_2), 45.63 ($N(CH_3)_2$), 58.15 (NCH_2), 65.59 (CH_2O), 114.04, 114.12 (C-3'', C-5''), 125.70 (C-4'), 126.10 (C-4'', C-4'''), 127.97, 128.04 (C-2'', C-6'', C-2''', C-6'''), 128.21, 128.62 (C-2', C-6', C-3', C-5'), 129.16 (C-3''', C-5'''), 138.07, 138.14 (C-1, C-2), 140.22 (C-1')

EIMS (HR) $C_{27}H_{31}NO$: calculated M^+ 385.2405

observed M^+ 385.2405

Mass Spectrum (m/z) 385 (M^+, 100%), 356 (M^+-29, 14%), 314 (M^+-71, 15%), 285 (M^+-100, 21%), 223 (M^+-162, 8%), 207 (M^+-178, 11%), 191 (M^+-194, 27%), 178 (M^+-207, 14%), 165 (M^+-220, 25%), 149 (M^+-236, 37%)

2-Benzyl-1-[4-(diethylaminoethoxy)phenyl]-1-phenylbut-1-ene (60)

Method 1: The general method 2.8 outlined was employed using 2-benzyl-1-(4-hydroxyphenyl)-1-phenylbut-1-ene (0.00064M) in dry acetone (10mL), anhydrous K_2CO_3 (0.0145M) and 2-(diethylamino)ethyl chloride hydrogen chloride (0.0023M). Flash chromatography (eluant methanol : dichloromethane; 40:60) afforded a mustard coloured oil in 59% yield.

IR ν_{max} (film) 3111-3010, 2979-2805 (CHs), 1608 (C=C), 1577, 1508 (NCH_2), 1460 (NCH_3, CH_2), 1382 (CH_3) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 0.96 (3H, m, H-4), 2.06 (2H, m, H-3), 2.19 (5H, s, NCH_2CH_3), 2.64 (5H, s, NCH_2CH_3), 2.86 (2H, m, NCH_2), 3.54, 3.58 (2H, 2 x s, CH_2), 4.04 (2H, m, CH_2O), 6.82-6.88 (2H, dd, J = 9.84Hz, H-3'', H-5''), 7.12- 7.42 (12H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 11.36 (CH_3), 13.18 (C-4), 24.59 (C-3), 37.22 (CH_2), 47.75 ($N(CH_2CH_3)_2$), 53.80 (NCH_2), 66.35 (CH_2O), 113.99 (C-3'', C-5''), 125.69 (C-4'), 126.07 (C-4'', C-4'''), 127.95, 128.01 (C-2'', C-6'', C-2''', C-6'''),

128.19, 128.61 (C-2', C-6', C-3', C-5'), 129.15 (C-3''', C-5'''), 137.99, 138.53 (C-1, C-2), 140.21 (C-1')

EIMS (HR) $C_{29}H_{35}NO$: calculated M^+ 413.2725

observed M^+ 413.2704

Mass Spectrum (m/z) 413 (M^+, 100%), 398 (M^+-15, 23%), 314 (M^+-99, 19%), 285 (M^+-128, 12%), 191 (M^+-222, 46%), 178 (M^+-235, 35%), 165 (M^+-248, 25%)

2-Benzyl-1-phenyl-1-[4-(piperidinylethoxy)phenyl]but-1-ene (61)

Method 1: The general method 2.8 was applied using 2-benzyl-1-(4-hydroxyphenyl)-1-phenylbut-1-ene (0.00057M) in dry acetone (6mL), anhydrous K_2CO_3 (0.013M) and 1-(chloroethyl)piperidine monohydrochloride (0.0021M). Flash chromatography (eluant methanol : dichloromethane; 35:65) afforded a light brown / mustard coloured oil in 83% yield.

IR ν_{max} (film) 3099-2996, 2981-2756 (CHs), 1605,1575 (C=C), 1554,1506 (NCH_2), 1452 (CH_2), 1369 (CH_3) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 0.94 (3H, m, H-4), 1.56 (6H, m, H-2'''', H-3'''', H-4'''') 2.02 (2H, m, H-3), 2.59 (2H, m, H-1'''', H-5''''), 2.73 (2H, m, NCH_2), 3.49, 3.53 (2H, 2 x s, CH_2), 4.04 (2H, m, CH_2O), 6.82-6.88 (2H, dd, J = 9.73Hz, H-3'', H-5''), 7.12- 7.42 (12H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 13.15 (C-4), 23.85 (C-3), 25.81 (C-3''''), 27.80 (C-2'''', C-4''''), 35.14 (CH_2), 47.93 (C-1'''', C-5''''), 58.58 (NCH_2), 65.76 (CH_2O), 113.96, 114.03 (C-3'', C-5''), 125.67 (C-4'), 126.05, 126.10 (C-4'', C-4'''), 127.93, 128.02 (C-2'', C-6'', C-2''', C-6'''), 128.17, 128.56 (C-2', C-6', C-3', C-5'), 129.10 (C-3''', C-5'''), 138.05, (C-1, C-2), 142.94 (C-1')

EIMS (HR) $C_{30}H_{35}NO$: calculated M^+ 425.2718

observed M^+ 425.2743

Mass Spectrum (m/z) 425 (M^+, 77%), 381 (M^+-44, 4%), 326 (M^+-99, 3%), 253 (M^+-172, 10%), 252 (M^+-173, 9%), 191 (M^+-234, 2%), 178 (M^+-247, 12%), 165 (M^+-260, 15%), 129 (M^+-296, 23%), 112 (M^+-313, 65%)

2-Benzyl-1-phenyl-1-[4-(pyrrolidinylethoxy)phenyl]but-1-ene (62)

Method 1: The general method 2.8 was employed using 2-benzyl-1-(4-hydroxyphenyl)-1-phenylbut-1-ene (0.00056M) in dry acetone (6mL), anhydrous K_2CO_3 (0.013M) and 1-(2-chloroethyl)pyrrolidine hydrochloride(0.0020M). Flash chromatography (eluant methanol : dichloromethane; 35:65) afforded a light brown coloured oil in 86% yield.

IR ν_{max} (film) 3086-2901, 2871-2765 (CHs), 1604 (C=C), 1509 (NCH_2), 1489 (CH_2), 1364 (CH_3) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 0.98 (3H, m, H-4), 1.82 (4H, m, H-2'''', H-3''''), 2.05 (2H, m, H-3), 2.85 (4H, m, H-1'''', H-4''''), 2.93 (2H, m, NCH_2), 3.54, 3.62 (2H, 2 x s, CH_2), 4.12 (2H, m, CH_2O), 6.84-6.87 (2H, dd, J = 8.97Hz, H-3'', H-5''), 7.13- 7.31 (12H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 13.14 (C-4), 23.39, 23.52 (C-3), 24.53, 24.63 (C-2'''', C-3''''), 37.07, 37.18 (CH_2), 53.39, 53.85 (C-1'''', C-4''''), 57.80 (NCH_2), 66.63 (CH_2O), 114.03, 114.12 (C-3'', C-5''), 125.63 (C-4'), 125.84 (C-4'', C-4'''), 127.95, 128.00 (C-2'', C-6'', C-2''', C-6'''), 128.10, 128.57 (C-2', C-6', C-3', C-5'), 129.11 (C-3''', C-5'''), 138.01, 138.09 (C-1, C-2), 142.96 (C-1')

EIMS (HR) $C_{29}H_{33}NO$: calculated M^+ 411.2573

observed M^+ 411.2541

Mass Spectrum (m/z) 411 (M^+, 82%), 351 (M^+-60, 9%), 327 (M^+-84, 5%), 297 (M^+-114, 4%), 267 (M^+-144, 19%), 253 (M^+-158, 21%), 191 (M^+-220, 50%), 178 (M^+-233, 35%), 165 (M^+-246, 48%)

2-Benzyl-1-[4-(morpholinylethoxy)phenyl]-1-phenylbut-1-ene (63)

Method 1: The general method 2.8 was employed using 2-benzyl-1-(4-hydroxyphenyl)-1-phenylbut-1-ene (0.00057M) in dry acetone (7mL), anhydrous K_2CO_3 (0.013M) and 4-(2-chloroethyl)morpholine hydrogen chloride (0.0021M). Flash chromatography (eluant methanol : dichloromethane; 20:80) presented a light brown coloured oil in 69% yield.

IR ν_{max} (film)	3083-2902, 2881-2671 (CHs), 1606 (C=C), 1513 (NCH_2), 1454 (CH_2), 1380 (CH_3) cm^{-1}
^1H NMR $\delta(CDCl_3)$	0.97 (3H, m, H-4), 2.08 (2H, m, H-3), 2.74 (2H, m, NCH_2), 2.79 (4H, m, H-1'''', H-4''''), 3.60 (4H, m, H-2'''', H-3''''), 3.72 (2H, s, CH_2), 4.10 (2H, m, CH_2O), 6.82-6.86 (2H, d, J = 8.49Hz, H-3'', H-5''), 7.12- 7.28 (12H, m, ArH)
^{13}C NMR $\delta(CDCl_3)$	13.16 (C-4), 24.52 (C-3), 29.42 (CH_3), 37.08 (CH_2), 53.34, 53.98 (C-1'''', C-4''''), 57.55, 57.60 (NCH_2), 65.62 (CH_2O), 66.72, 66.86 (C-2'''', C-3''''), 114.04, 114.46 (C-3'', C-5''), 125.69 (C-4'), 126.12 (C-4'', C-4'''), 127.94, 128.01 (C-2'', C-6'', C-2''', C-6'''), 128.17, 128.55 (C-2', C-6', C-3', C-5'), 129.10 (C-3''', C-5'''), 138.11, (C-1, C-2), 142.91 (C-1')
EIMS (HR)	$C_{29}H_{33}NO_2$: calculated M^+ 427.2517 observed M^+ 427.2541
Mass Spectrum (m/z)	427 (M^+, 100%), 381 (M^+-46, 23%), 322 (M^+-105, 21%), 253 (M^+-174, 18%), 183 (M^+-244, 22%), 114 (M^+-313, 100%)

7.2.9. McMurry coupling reactions

General method 2.9

An appropriately substituted benzophenone (0.0047M) and 1-phenyl-2-butanone (0.0047M) were dissolved in dry dioxane (25mL) and allowed to stir in an ice-cool bath (0-5°C) under argon for 10min. Titanium tetrachloride (0.0095M) was added dropwise over 15min [exothermic reaction] and the reaction mixture was

allowed to stir for a further 30min at 0-5°C under Ar, prior to addition of zinc dust [<10 micron] (0.03M). The reaction mixture was allowed to heat up to room temperature, before refluxing for 3-4hr. TLC analysis post overnight cooling to ambient temperature indicated complete product formation. The product was washed with 10% K_2CO_3 (50mL), water, brine and extracted with diethyl ether (3x25mL). The organic layer was then washed with 3N HCl (3x10mL) and further washing with water was carried out. The coupled product was dried over $MgSO_4$ and concentrated down. Flash column chromatography was carried out to purify the crude product.

2-Benzyl-1,1-diphenyl-but-1-ene (55)

Method 2: The general method 2.9 was applied using benzophenone (0.0047M), 1-benzyl-2-butanone (0.0047M), dry dioxane (25mL), titanium (IV) chloride (0.0095M) and zinc dust (0.03M). The reaction mixture was refluxed for 4hr after which full consumption of the starting material was observed. Column chromatography (eluant petroleum ether : dichloromethane, 85:15) was carried out affording a lemon coloured oil in 89% yield.

IR ν_{max} (film) 3111-3023, 2995-2876 (CHs), 1600, 1574 (C=C), 1465 (CH_2), 1443 (CH_3) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 1.70 (3H, t, J = 7.44Hz, H-4), 2.26 (2H, q, J = 7.38Hz, H-3), 3.77 (2H, s, CH_2), 7.36-7.47 (15H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 13.09 (C-4), 24.40 (C-3), 36.97 (CH_2), 125.57 (C-4'), 126.00, 126.05 (C-4'', C-4'''), 127.82, 127.88 (C-2'', C-6'', C-2''', C-6'''), 128.04, 128.42 (C-2', C-6', C-3', C-5'), 128.96 (C-3'', C-5'', C-3''', C-5'''), 138.54, 139.25 (C-1, C-2), 140.28 (C-1'), 142.81, 142.87 (C-1'', C-1''')

EIMS (LR) $C_{23}H_{22}$: calculated M^+ 298

observed M^+ 298

2-Benzyl-1-(4-methoxyphenyl)-1-phenylbut-1-ene (56)

Method 2: The general method 2.9 was applied using 4-methoxybenzophenone (0.0047M), 1-benzyl-2-butanone (0.0047M), dry dioxane (25mL), titanium (IV) chloride (0.0095M) and zinc dust (0.03M) stirred under argon. The reaction

mixture was refluxed for 5hr after which full consumption of the starting material was observed. Vacuum filtration was carried out to remove the zinc, washing with ethyl acetate. Work up was carried out as per method 2.9, except the product was extracted with ethyl acetate. Column chromatography (eluant petroleum ether : ethyl acetate; 94:6) afforded a yellow coloured oil in 90% yield.

IR ν_{max} (film) 3095-2997, 2986-2836 (CHs), 1606 (C=C) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 0.99 (3H, m, H-4), 2.08 (2H, m, H-3), 3.57, 3.61 (2H, d, 2 x s, CH_2), 3.79, 3.82 (3H, d, 2 x s, OCH_3), 6.82-6.85 (2H, dd, J = 8.68Hz, H-3'', H-5''), 7.16-7.33 (12H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 13.21 (C-4), 24.59, 24.70 (C-3), 37.15, 37.26 (CH_2), 55.11 (OCH_3), 113.43, 113.51 (C-3'', C-5''), 125.73 (C-4'), 126.11, 126.16 (C-4'', C-4'''), 127.98, 128.06 (C-2'', C-6'', C-2''', C-6'''), 128.24, 128.63 (C-2', C-6', C-3', C-5'), 129.17 (C-3''', C-5'''), 138.14 (C-1, C-2), 140.26 (C-1'), 143.05 (C-1'', C-1''')

EIMS (LR) $C_{23}H_{22}O$: calculated M^+ 328

observed M^+ 328

2-Benzyl-1-(4-hydroxyphenyl)-1-phenylbut-1-ene (57)

Method 2: The general method 2.9 was applied using 4-hydroxybenzophenone (0.0047M), 1-benzyl-2-butanone (0.0047M), dry dioxane (25mL), titanium (IV) chloricde (0.0095M) and zinc dust (0.03M) stirred under argon. The reaction mixture was refluxed for 6hr after which full consumption of the starting material was observed. Product was extracted with ethyl acetate. Column chromatography (eluant petroleum ether : dichloromethane, 40:60) was carried out presenting a light mustard coloured oil in 94% yield.

IR ν_{max} (film) 3586-3114 (OH), 3089-3001, 2965-2829 (CHs), 1610 (C=C) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 0.97 (3H, m, H-4), 2.06 (2H, m, H-3), 3.57 (2H, d, J = 14.56Hz, CH_2), 4.69 (1H, d, J = 10.14Hz, OH ex. D_2O), 6.73-6.77 (2H, dd, J = 8.67Hz, H-3'', H-5''),

7.09-7.11 (2H, dd, J = 8.64Hz, H-2'', H-6'') 7.13-7.30 (10H, m, ArH)

^{13}C NMR δ($CDCl_3$) 13.18 (C-4), 24.69 (C-3), 37.12 (CH_2), 114.93 (C-3'', C-5''), 125.33 (C-4'), 126.58 (C-4'', C-4'''), 127.83, 127.98 (C-2'', C-6'', C-2''', C-6'''), 128.23, 128.60 (C-2', C-6', C-3', C-5'), 129.15 (C-3''', C-5'''), 138.13, 139.45 (C-1, C-2), 140.20 (C-1'), 142.98 (C-1'', C-1'''), 153.50 (C-4''OH)

EIMS (LR) $C_{23}H_{22}O$: calculated M^+ 314

observed M^+ 314

7.2.10. Preparations of substituted alkylated benzophenones

General Method 2.10

To a solution of hydroxybenzophenone (0.005M) in dry acetone was added anhydrous K_2CO_3 (0.05M) with continual stirring. Stirring was maintained for 15min under a nitrogen atmosphere, post this period alkylaminoethyl chloride hydrogen chloride was added. The reaction mixture was stirred for a further ten minutes and then heated to reflux for 5-6hr. The reaction was monitored via TLC, once all the starting material was consumed it was then cooled to room temperature, vacuum filtered, washed with acetone and concentrated under reduced pressure. The crude product was purified via column chromatography.

4-(Dimethylaminoethoxy)benzophenone (68)

The general method 2.10 was applied using 4-hydroxybenzophenone (0.005M) in dry acetone (20mL), anhydrous K_2CO_3 (0.05M) and 2-dimethylaminoethyl chloride hydrogen chloride (0.0115M) under nitrogen. The crude product was purified via column chromatography (eluant methanol : dichloromethane; 60:40) to afford an orange coloured oil[521] in 98%.

IR ν_{max} (film) 3096-2928, 2901-2760 (CHs), 1652 (C=O), 1601 (C=C), 1507 (CH_2), 1445 (NCH_3), 1281, 1258 (C–N) cm^{-1}

^{1}H NMR δ($CDCl_3$) 2.32 (6H, s, $N(CH_3)_2$), 2.74 (2H, t, J = 5.64Hz, CH_2N), 4.15 (2H, t, J = 5.66Hz, CH_2O), 6.90-6.91

(2H, dd, J = 7.00, 1.97Hz, H-3'', H-5''), 7.36-7.75 (7H, m, ArH)

^{13}C NMR δ($CDCl_3$) 45.61 ($N(CH_3)_2$), 57.85 (NCH_2), 65.99 (OCH_2), 114.02 (C-3'', C-5''), 125.53 (C-4'', C-4'''), 128.07 (C-2'', C-6'', C-2''', C-6'''), 129.54 (C-3''', C-5'''), 137.76 (C-1)

4-(Diethylaminoethoxy)benzophenone (69)

The general method 2.10 outlined above was applied, using 4-hydroxybenzophenone (0.005M), dry acetone (28mL), anhydrous K_2CO_3 (0.05M) and 2-diethylaminoethoxychloride hydrochloride (0.0115M). The product was isolated using flash column chromatography (eluant methanol : dichloromethane; 60:40) as a lemon oil[522] in 98% yield.

IR ν_{max} (film) 3030, 2969-2934 (CHs), 1668 (C=O), 1601 (C=C), 1508 (CH_2), 1446 (NCH_3), 1281, 1256 (C–N) cm^{-1}

^{1}H NMR δ($CDCl_3$) 2.64-2.76 (10H, m, $N(CH_2CH_3)_2$), 2.97 (2H, t, J = 6.02Hz, CH_2N), 4.21 (2H, t, J = 6.02Hz, CH_2O), 6.97-6.99 (2H, dd, J = 5.04, 2.00Hz, H-3'', H-5''), 7.45-7.84 (7H, m, ArH)

^{13}C NMR δ($CDCl_3$) 11.01 (CH_3), 47.34 (CH_2), 51.15 (NCH_2), 66.05 (OCH_2), 113.65 (C-3'', C-5''), 127.70 (C-4'''), 129.23 (C-2'', C-6''), 129.84 (C-2''', C-6'''), 131.39 (C-3''', C-5'''), 132.06 (C-1''), 137.84 (C-1'''), 161.89 (C-4''), 195.04 (C=O)

4-(Pyrrolidinylethoxy)benzophenone (70)

This product was prepared via the general method 2.10 from hydroxybenzophenone (0.005M), dry acetone (25mL), anhydrous K_2CO_3 (0.05M) and 1-(2-cholorethyl)pyrrolidine hydrochloride (0.012M). Column chromatography (eluant methanol : dichloromethane; 50:50) afforded a light brown oil[523] in 95% yield.

IR ν_{max} (film) 3059, 2963 (CHs), 1651 (C=O), 1604 (C=C), 1508 (CH_2), 1282, 1256, (C–N), 1173 (C-O) cm^{-1}

^{1}H NMR δ($CDCl_3$) 1.82 (4H, m, H-2'''', H-3''''), 2.57 (4H, m, H-1'''', H-4''''), 2.94 (2H, t, J = 5.96Hz, CH_2N), 4.20 (2H, t, J = 6.02Hz, CH_2O), 6.97-7.00 (2H, dd, J = 8.52, 1.98Hz, H-3'', H-5''), 7.45-7.90 (7H, m, ArH)

^{13}C NMR δ($CDCl_3$) 23.47 (C-2'''', C-3''''), 54.83 (C-1'''', C-4''''), 57.86 (NCH_2), 67.37 (OCH_2), 113.67 (C-3'', C-5''), 127.68 (C-4'''), 129.21 (C-2''', C-6'''), 132.03 (C-3''', C-5'''), 137.88 (C-1'''), 162.08 (C-4''), 195.03 (C=O)

4-(Piperidinylethoxy)benzophenone (71)

The general method 2.10 was applied using hydroxybenzophenone (0.005M), dry acetone (25mL), N_2, anhydrous K_2CO_3 (0.05M) and 1-(2-cholorethyl)piperidine monohydrochloride (0.012M). The product was isolated as a mustard coloured oil[524] in 89% yield following column chromatography (eluant methanol : dichloromethane; 40:60), with the following physical properties.

IR ν_{max} (film) 3058, 2935-2853 (CHs), 1652 (C=O), 1604 (C=C), 1508 (CH_2), 1281, 1255, (C–N) cm^{-1}

^{1}H NMR δ($CDCl_3$) 2.51 (2H, m, H-3''''), 2.60 (4H, m, H-2'''', H-4''''), 2.71 (4H, m, H-1'''', H-5''''), 2.84 (2H, t, J = 5.78Hz, CH_2N), 4.19 (2H, t, J = 5.76Hz, CH_2O), 6.95-6.98 (2H, dd, J = 8.52, 2.00Hz, H-3'', H-5''), 7.45-7.82 (7H, m, ArH)

^{13}C NMR δ($CDCl_3$) 28.82 (C-3''''), 40.21 (C-2'''', C-4''''), 53.43 (C-1'''', C-5''''), 56.98 (NCH_2), 66.35 (OCH_2), 113.67 (C-3'', C-5''), 127.72 (C-4'), 129.22, 129.88 (C-2''', C-6'''), 131.40 (C-3''', C-5'''), 132.04 (C-1''), 137.81 (C-1'''), 161.89 (C-4''), 194.96 (C=O)

4-(Morpholinylethoxy)benzophenone (72)

The general method 2.10 was applied using 4-(2-cholorethyl)morpholine hydrochloride (0.0115M) as the alkylating agent. Flash column chromatography (eluant methanol : dichloromethane; 40:60) purified the crude product as a cream oil in 89% yield.[523]

IR ν_{max} (film) 3060, 2962-2857 (CHs), 1652 (C=O), 1508 (CH_2), 1282, 1255 (C–N), 1173 (C-O) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 2.65 (4H, m, H-1'''', H-4''''), 2.81 (2H, t, J = 6.02Hz, CH_2N), 3.60 (4H, m, H-2'''', H-3''''), 4.18 (2H, t, J = 6.02Hz, CH_2O), 6.96-6.98 (2H, dd, J = 8.52, 2.00Hz, H-3'', H-5''), 7.45-7.83 (7H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 54.56 (C-1'''', C-4''''), 57.71 (NCH_2), 56.98 (NCH_2), 60.66 (C-2'''', C-3''''), 66.32 (OCH_2), 114.11 (C-3'', C-5''), 128.11 (C-4'''), 129.64, 130.14 (C-2''', C-6'''), 131.77 (C-3''', C-5'''), 132.46 (C-1''), 138.31 (C-1'''), 162.50 (C-4''), 195.43 (C=O)

7.2.11. Preparation of 2-Benzyl-1-[4-(alkylaminoethoxy)phenyl]-1-phenylbut-1-ene using the McMurry coupling reaction

2-Benzyl-1-[4-(dimethylaminoethoxy)phenyl]-1-phenylbut-1-ene (59)

Method 2: The general method outlined 2.9 was applied in the preparation of this compound, using 4-(dimethylaminoethoxy)benzophenone (0.0045M), dry dioxane (25mL), 1-phenyl-2-butanone (0.0045M), titanium tetrachloride (0.0091M) and zinc dust (0.028M) under argon. The crude product was purified using column chromatography (eluant dichloromethane : methanol; 40: 60) to yield an amber coloured oil in 69% yield.

IR ν_{max} (film) 3118-2986, 2971-2756 (CHs), 1610, 1572 (C=C), 1508 (NCH_2), 1455 (NCH_3, CH_2), 1371 (CH_3), 1281, 1244 (CN) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 0.99 (3H, m, H-4), 2.07 (2H, m, H-3), 2.48 (6H, d, J = 7.95Hz, $N(CH_3)_2$), 2.90 (2H, m, NCH_2), 3.56, 3.59 (2H, d, 2 x s, CH_2), 4.16 (2H, m, CH_2O), 6.82-6.84 (2H, dd, J = 8.74Hz, H-3'', H-5''), 7.14- 7.33 (12H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 12.81 (C-4), 24.20 (C-3), 36.72 (CH_2), 44.75 ($N(CH_3)_2$), 57.34 (NCH_2), 64.67 (CH_2O), 113.67, 113.75 (C-3'', C-5''), 125.34 (C-4'), 126.78 (C-4'',

C-4'''), 127.60, 127.84 (C-2'', C-6'', C-2''', C-6'''), 128.21, 128.76 (C-2', C-6', C-3', C-5'), 129.35 (C-3''', C-5'''), 138.15, 138.47 (C-1, C-2), 140.19 (C-1')

EIMS (HR) $C_{27}H_{31}NO$: calculated M^+ 385.2405

observed M^+ 385.2406

2-Benzyl-1-[4-(diethylaminoethoxy)phenyl]-1-phenylbut-1-ene (60)

Method 2: The general method 2.9 was employed using 4-(diethylaminoethoxy)benzophenone (0.0044M) and 1-phenyl-2-butanone (0.0044M). The product was isolated using column chromatography (eluant dichloromethane : methanol; 75:25) to afford a brown oil in 72% yield.

IR ν_{max} (film) 3060-2969, 2933-2874 (CHs), 1607, 1574 (C=C), 1508 (NCH_2), 1454 (NCH_3, CH_2), 1375 (CH_3) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 0.96 (3H, m, H-4), 2.08 (2H, m, H-3), 2.51 (5H, m, NCH_2CH_3), 2.72 (5H, m, NCH_2CH_3), 2.93 (2H, m, NCH_2), 3.60, 3.64 (2H, d, 2 x s, CH_2), 4.13 (2H, m, CH_2O), 6.80-6.86 (2H, dd, J = 8.34Hz, H-3'', H-5''), 7.18-7.42 (12H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 11.15 (CH_3), 12.81 (C-4), 24.29 (C-3), 36.80 (CH_2), 47.29 ($N(CH_2CH_3)_2$), 51.18 (NCH_2), 65.64 (CH_2O), 113.60, 113.71 (C-3'', C-5''), 125.73(C-4'), 126.10, 126.15 (C-4'', C-4'''), 126.89, 128.05 (C-2'', C-6'', C-2''', C-6'''), 128.23, 128.47 (C-2', C-6', C-3', C-5'), 129.55 (C-3''', C-5'''), 135.20 (C-1, C-2)

EIMS (HR) $C_{29}H_{35}NO$: calculated M^+ 413.2725

observed M^+ 413.2719

2-Benzyl-1-phenyl-1-[4-(piperidinylethoxy)phenyl]but-1-ene (61)

Method 2: The general method 2.9 was applied using 4-(piperidinylethoxy)benzophenone (0.007M) and 1-phenyl-2-butanone (0.007M). The pure product was isolated using column chromatography (eluant methanol : dichloromethane; 30:70) which afforded a mustard coloured oil in 67% yield.

IR ν_{max} (film) 3059-2932, 2854-2785 (CHs), 1605, 1573 (C=C), 1506 (NCH_2s), 1453 (CH_2), 1372 (CH_3) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 0.98 (3H, m, H-4), 1.48 (2H, m, H-3''''), 1.65 (4H, m, H-2'''', H-4'''') 2.09 (2H, m, H-3), 2.55 (2H, m, H-1'''', H-5''''), 2.81 (2H, m, NCH_2), 3.57, 3.60 (2H, d, 2 x s, CH_2), 4.11 (2H, m, CH_2O), 6.82-6.88 (2H, d, J = 8.52Hz, H-3'', H-5''), 7.15-7.49 (12H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 12.81 (C-4), 23.65 (C-3), 24.28 (C-3''''), 25.36 (C-2'''', C-4''''), 36.75, 36.85 (CH_2), 54.50 (C-1'''', C-5''''), 57.42, 57.47 (NCH_2), 65.30 (CH_2O), 113.69, 113.77 (C-3'', C-5''), 125.32 (C-4'), 125.70, 125.75 (C-4'', C-4'''), 127.39, 127.81 (C-2'', C-6'', C-2''', C-6'''), 128.23, 128.77 (C-2', C-6', C-3', C-5'), 129.84 (C-3''', C-5'''), 138.05, 138.13 (C-1, C-2), 143.04 (C-1')

EIMS (HR) $C_{30}H_{35}NO$: calculated M^+ 425.2718

observed M^+ 425.2719

2-Benzyl-1-phenyl-1-[4-(pyrrolidinylethoxy)phenyl]but-1-ene (62).

Method 2: The general method 2.9 was applied using 4-(pyrrolidinylethoxy)benzophenone (0.007M) and 1-phenyl-2-butanone (0.007M), after which the crude product was isolated using flash chromatography (eluant methanol : dichloromethane; 10:90) which produced a light brown coloured oil in 68% yield.

IR ν_{max} (film) 3059-2931, 2874-2784 (CHs), 1606, 1573 (C=C), 1507 (NCH_2), 1454 (CH_2), 1373 (CH_3) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 1.05 (3H, m, H-4), 1.86 (4H, m, H-2'''', H-3''''), 2.14 (2H, m, H-3), 2.71 (4H, m, H-1'''', H-4''''), 2.98 (2H, m, NCH_2), 3.63, 3.67 (2H, d, 2 x s, CH_2), 4.16 (2H, m, CH_2O), 6.89- 6.95 (2H, d, J = 8.04Hz, H-3'', H-5''), 7.21-7.38 (12H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 12.93 (C-4), 23.50 (C-3), 24.79 (C-2'''', C-3''''), 37.25 (CH_2), 54.60 (C-1'''', C-4''''), 55.01 (NCH_2),

66.77 (CH_2O), 113.84 (C-3'', C-5''), 125.42 (C-4'), 125.80, 125.87 (C-4'', C-4'''), 127.56, 127.92 (C-2'', C-6'', C-2''', C-6'''), 128.03, 128.88 (C-2', C-6', C-3', C-5'), 129.39 (C-3''', C-5'''), 138.09, 138.18 (C-1, C-2)

EIMS (HR) $C_{29}H_{33}NO$: calculated M^+ 411.2573

observed M^+ 411.2562

2-Benzyl-1-[4-(morpholinylethoxy)phenyl]-1-phenylbut-1-ene (63)

Method 2: The general method 2.9 was employed using 4-(morpholinylethoxy)benzophenone (0.0044M) and 1-phenyl-2-butanone (0.0044M). Column chromatography (eluant methanol : dichloromethane; 10:90) presented a light mustard coloured oil in 65% yield.

IR ν_{max} (film) 3058-2963, 2881-2692 (CHs), 1605, 1574 (C=C), 1507 (NCH_2), 1453 (CH_2), 1373 (CH_3) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 0.98 (3H, m, H-4), 2.06 (2H, m, H-3), 2.61 (2H, m, NCH_2), 2.81 (4H, m, H-1'''', H-4''''), 3.56, 3.59 (2H, d, 2 x s, CH_2), 3.74 (4H, m, H-2'''', H-3''''), 4.13 (2H, m, CH_2O), 6.82-6.87 (2H, d, J = 8.52Hz, H-3'', H-5''), 7.15-7.29 (12H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 13.20 (C-4), 24.69 (C-3), 37.14 (CH_2), 54.03 (C-1'''', C-4''''), 57.65 (NCH_2), 65.66 (CH_2O), 66.87 (C-2'''' , C-3''''), 114.16 (C-3'', C-5''), 125.73 (C-4'), 126.12, 126.17 (C-4'', C-4'''), 127.94, 127.99 (C-2'', C-6'', C-2''', C-6'''), 128.05, 128.61 (C-2', C-6', C-3', C-5'), 129.15 (C-3''', C-5''')

EIMS (HR) $C_{29}H_{33}NO_2$: calculated M^+ 427.2517

observed M^+ 427.2511

7.2.12. Preparation of substituted 2-phenylethanones

Phenylphenyl acetate (73)

Phenylacetyl chloride (0.022M) and phenol (0.011M) were added together and heated to 150°C for 8hours, after which the reaction was cooled to room temperature and stirred overnight. Post this period the compound was washed with water (3x25mL), extracted with diethyl ether (3x15mL), dried over sodium sulphate and concentrated down. The product was purified using column chromatography (eluant petroleum ether : dichloromethane; 90:10), presenting a cloudy coloured oil in 79% yield; m.p. 48-50°C [lit. m.p. 50°C].[525]

IR ν_{max} (KBr)	3125-2834 (CHs), 1756 (C=O), 1493, 1453 (CH_2), 1213, 1195 (C=C-O) cm^{-1}
^1H NMR $\delta(CDCl_3)$	3.92 (2H, s, CH_2), 7.12-7.48 (10H, m, ArH)
^{13}C NMR $\delta(CDCl_3)$	41.37 (CH_2), 125.76 (C-4'), 127.27 (C-2', C-6'), 128.65 (C-3', C-5'), 129.16, 129.23 (C-3'', C-5''), 129.30 (C-2'', C-6''), 133.45 (C-1''), 150.73 (C-4''), 169.86 (C=O)

1-(4-Hydroxyphenyl)-2-phenylethan-1-one (74)

Phenylphenyl acetate (0.0047M) was heated in a silicon oil bath to 100°C prior to addition of aluminium chloride (0.0097M). This solid mixture was heated for a further hour at 110-115°C and then stirred at 120°C for 4hr. Post this period the reaction was cooled with ice and to the resulting solution was added conc. HCl (30mL). The ketone was extracted with diethyl ether, washed with 0.5% NaOH (15mL), water (4x20mL), dried over magnesium sulphate and concentrated to yield a crude product. The product was purified using column chromatography (eluant dichloromethane) to afford, after recrystallisation from ethanol / petroleum ether an orange coloured crystalline material in 54% yield; m.p. 152°C [lit. m.p. 151°C].[525]

IR ν_{max} (KBr)	3723-2992 (-OH), 2961-2841 (CHs), 1658 (C=O), 1453 (CH_2) cm^{-1}
^1H NMR $\delta(CDCl_3)$	4.25 (2H, s, CH_2), 6.86-6.93 (2H, d, J = 8.56Hz, H-3'', H-5''), 7.17-7.32 (5H, m, ArH), 7.81-8.03 (2H, d, J = 8.58Hz, H-2'', H-6'')

^{13}C NMR δ($CDCl_3$) 44.79 (CH_2), 114.98 (C-3'', C-5''), 126.38 (C-4'', C-4'), 128.21 (C-2'', C-6''), 128.92 (C-2', C-6'), 129.19 (C-3', C-5'), 132.91 (C-1'')

General Method 2.12

To a solution of 1-(4-hydroxyphenyl)-2-phenylethan-1-one (0.005M) in dry acetone was added anhydrous K_2CO_3 (0.05M) with continual stirring. Stirring was maintained for 15min under a nitrogen atmosphere. Alkylaminoethyl chloride hydrogen chloride (0.011M) was then added. The reaction mixture was stirred for a further 10min and then heated to reflux for 5-6hr. The reaction was monitored via TLC; once all the starting material was consumed it was then cooled to room temperature, vacuum filtered, washed with acetone and concentrated under reduced pressure. The crude product was purified via column chromatography.

1-(4-Dimethylaminoethoxyphenyl)-2-phenylethan-1-one (75)

The general method 2.12 was applied using 1-(4-hydroxyphenyl)-2-phenylethan-1-one (0.001M), anhydrous K_2CO_3 (0.01M), dry acetone (20mL) and 2-dimethylaminoethylchloride hydrogen chloride (0.0022M). The mixture was refluxed for 6-8hr under nitrogen. Column chromatography (eluant methanol : dichloromethane; 50:50) was carried out and afforded a light brown coloured oil[526] in 89% yield with the following physical properties.

IR ν_{max} (film) 3097-3016, 2957-2703 (CHs), 1674 (C=O), 1601 (C=C), 1511, 1497 (CH_2), 1456 (CH_3) cm^{-1}

^{1}H NMR δ($CDCl_3$) 2.37 (6H, s, $N(CH_3)_2$), 2.77 (2H, t, J = 5.04Hz, CH_2N), 4.14 (2H, t, J = 5.52Hz, CH_2O), 4.23 (2H, s, CH_2), 6.94-6.96 (2H, d, J = 9.04Hz, H-3'', H-5''), 7.24-7.32 (5H, m, ArH), 7.98-8.00 (2H, d, J = 9.04Hz, H-2'', H-6'')

^{13}C NMR δ($CDCl_3$) 44.77 (CH_2), 45.33 ($N(CH_3)_2$), 57.55 (CH_2N), 65.72 (CH_2O), 113.90 (C-3'', C-5''), 126.28 (C-4'), 128.15 (C-2'', C-6''), 128.93 (C-2', C-6'), 129.30 (C-3', C-5'), 130.43 (C-1'), 132.91 (C-1''), 162.28 (C-4''), 195.70 (C=O)

1-(4-Diethylaminoethoxyphenyl)-2-phenylethan-1-one (76)

The general method 2.12 was used to prepare this compound from 1-(4-hydroxyphenyl)-2-phenylethan-1-one (0.00083M), anhydrous K_2CO_3 (0.008M) and 2-diethylaminoethoxychloride hydrochloride (0.0021M). The product was isolated as a mustard coloured oil[526] in 65% yield following flash column chromatography (eluant methanol : dichloromethane; 40:60).

IR ν_{max} (film) 2939-2850 (CHs), 1668 (C=O), 1599, 1574 (C=C), 1509, 1496, 1454 (CH_2), 1377 (CH_3) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 1.10 (6H, m, $(CH_3)_2$), 2.66 (4H, m, $(CH_2)_2$), 2.91 (2H, t, J = 6.26Hz, CH_2N), 4.12 (2H, t, J = 6.26Hz, CH_2O), 4.24 (2H, s, CH_2), 6.92-6.96 (2H, d, J = 9.52Hz, H-3'', H-5''), 7.23-7.33 (5H, m, ArH), 8.01-8.16 (2H, d, J = 9.54Hz, H-2'', H-6'')

^{13}C NMR $\delta(CDCl_3)$ 11.33 ($(CH_3)_2$), 44.78 (CH_2), 47.41 ($N(CH_2)_2$), 51.15 (CH_2N), 66.42 (CH_2O), 113.88 (C-3'', C-5''), 126.27 (C-4'), 128.15 (C-2'', C-6''), 128.92 (C-2', C-6'), 129.22 (C-3', C-5'), 130.44 (C-1'), 134.55 (C-1''), 162.39 (C-4'')

2-Phenyl-1-(4-pyrrolidinylethoxyphenyl)ethan-1-one (77)

The general method 2.12 was applied using 1-(4-hydroxyphenyl)-2-phenylethan-1-one (0.002M), anhydrous K_2CO_3 (0.02M) and 1-(2-chloroethyl)pyrrolidine hydrochloride (0.0035M). A pure product was directly isolated as a light brown oil[526] in 94% yield.

IR ν_{max} (film) 2959-2925, 2858-2804 (CHs), 1667 (C=O), 1598 (C=C), 1510, 1490, 1454 (CH_2) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 1.86 (4H, m, H-2'''', H-3''''), 2.63 (4H, m, H-1'''', H-4''''), 2.98 (2H, m, CH_2N), 4.17 (2H, m, CH_2O), 4.24 (2H, s, CH_2), 6.92-7.02 (2H, m, H-3'', H-5''), 7.32-7.54 (5H, m, ArH), 7.99-8.08 (2H, m, H-2'', H-6'')

^{13}C NMR $\delta(CDCl_3)$ sample too insoluble for C^{13} NMR

EIMS (LR) $C_{20}H_{23}NO_2$: calculated M^+ 309

observed M^+ 309

1-(4-Piperidinylethoxyphenyl)-2-phenylethan-1-one (78)

The general method 2.12 outlined above was applied using 1-(4-hydroxyphenyl)-2-phenylethan-1-one (0.0012M), anhydrous K_2CO_3 (0.012M), 1-(2-chloroethyl)-piperidine monohydrochloride (0.0035M). The product was isolated directly as a rusty coloured oily gel [526] in 99% yield, with the following physical properties.

IR ν_{max} (film) 3062-2934,2853-2786 (CHs), 1672 (C=O), 1600, 1575 (C=C), 1509, 1496, 1450, 1419 (CH_2) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 1.58 (6H, m, H-2'''', H-3'''', H-4''''), 2.66 (4H, m, H-1'''', H-5''''), 2.79 (2H, t, J = 6.02Hz, CH_2N), 4.15 (2H, t, J = 6.02Hz, CH_2O), 4.24 (2H, s, CH_2), 6.91-6.95 (2H, d, J = 15.08Hz, H-3'', H-5''), 7.23-7.41 (5H, m, ArH), 7..98-8.12 (2H, d, J = 15.08Hz, H-2'', H-6'')

^{13}C NMR $\delta(CDCl_3)$ 24.01 (C-3''''), 25.89 (C-2'''', C-4''''), 44.78 (CH_2), 54.95 (C-1'''', C-5''''), 57.26 (CH_2N), 65.88 (CH_2O), 113.91 (C-3'', C-5''), 126.27 (C-4'), 128.14 (C-2', C-6', C-2'', C-6''), 128.91, 129.31 (C-3', C-5'), 130.44 (C-1'), 156.58 (C-4'')

1-(4-Morpholinylethoxyphenyl)-2-phenylethan-1-one (79)

The general method 2.12 outlined above was applied using 1-(4-hydroxyphenyl)-2-phenylethan-1-one (0.0012M), anhydrous K_2CO_3 (0.012M) and 4-(2-chloroethyl)morpholine hydrochloride (0.00354M). The product was isolated using column chomatography (eluant dichloromethane : methanol; 50:50), as a brown coloured oily gel in 98% yield.

IR ν_{max} (film) 2943-2850 (CHs), 1665 (C=O), 1600 (C=C), 1509, 1497, 1453, 1420 (CH_2) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 2.74 (2H, t, J = 6.84Hz, CH_2N), 2.84 (4H, m, H-1'''', H-4''''), 3.61 (4H, m, H-2'''', H-3''''), 4.18 (2H, t, J = 6.52Hz, CH_2O), 4.25 (2H, s, CH_2), 6.93-6.95 (2H, d, J = 8.56Hz, H-3'', H-5''), 7.25-7.99 (5H, m, ArH), 8.00-8.02 (2H, d, J = 8.52Hz, H-2'', H-6'')

^{13}C NMR δ($CDCl_3$) 44.80 (CH_2), 53.11, 53.62 (C-1'''', C-4''''), 59.73 (CH_2N), 65.78 (CH_2O), 66.38, 66.42 (C-2'''', C-3''''), 113.89 (C-3'', C-5''), 126.30 (C-4', C-4''), 128.04 (C-2'', C-6''), 128.17 (C-2', C-6'), 129.39 (C-3', C-5'), 130.48 (C-1'), 195.48 (C=O)

EIMS (HR) $C_{20}H_{21}NO_3$: calculated M^+ 325.1682

observed M^+ 325.1678

Mass Spectrum (m/z) 325 (M^+, 80%), 234 (M^+-91, 36%), 176 (M^+-144, 45%), 165 (M^+-160, 62%), 147 (M^+-178, 71%), 114 (M^+-211, 61%), 91 (M^+-234, 89%)

7.2.13. McMurry coupling of 1-(4-alkylaminoethoxyphenyl)-2-phenylethan-1-one

1-Benzyl-1-[(4-dimethylaminoethoxy)phenyl]-2-phenylbut-1-ene (81)

The general method outlined 2.9 was applied in the preparation of this compound, using 1-(4-dimethylaminoethoxyphenyl)-2-phenylethan-1-one (0.0063M), dry dioxane (25mL), propiophenone (0.0063M), titanium tetrachloride (0.013M) and zinc dust (0.041M) under nitrogen. The crude product was purified using column chromatography (eluant dichloromethane : methanol; 60: 40) to yield a mustard coloured oil in 69% yield.

IR ν_{max} (film) 3057-2921, 2871-2771 (CHs), 1605, 1574 (C=C), 1508 (NCH_2), 1493, 1453 (NCH_3, CH_2), 1372 (CH_3), 1283, 1242 (CN) cm^{-1}

^{1}H NMR δ($CDCl_3$) 1.01 (3H, m, H-4), 2.34 (6H, s, $N(CH_3)_2$), 2.39 (2H, m, H-3), 2.64 (2H, m, NCH_2), 2.70 (2H, m, CH_2O), 3.96, 3.97 (2H, d, 2 x s, CH_2), 6.55 (2H, d, J = 8.52Hz, H-3'', H-5''), 6.81-7.43 (12H, m, ArH)

^{13}C NMR δ($CDCl_3$) 12.81 (C-4), 27.51 (C-3), 39.55 (CH_2), 45.25 ($N(CH_3)_2$), 57.72 (NCH_2), 65.07 (CH_2O), 113.02 (C-3'', C-5''), 125.14, 125.29 (C-4', C-4''), 126.86 (C-4'''), 127.06, 127.75 (C-3''', C-5''', C-2''', C-6'''), 128.01 (C-2'', C-6''), 129.31, 129.33 (C-2', C-6', C-

3', C-5'), 130.37 (C-1'), 134.14 (C-1''), 138.74 (C-1, C-2), 139.66 (C-1'), 156.06 (C-4''')

EIMS (HR) $C_{27}H_{31}NO$: calculated M^+ 385.2404

observed M^+ 385.2406

Mass Spectrum (m/z) 385 (M^+, 74%), 314 (M^+-71, 4%), 267 (M^+-118, 7%), 252 (M^+-133, 13%), 239 (M^+-146, 7%), 202 (M^+-183, 19%), 191 (M^+-194, 48%), 178 (M^+-207, 41%), 165 (M^+-220, 44%), 115 (M^+-270, 30%), 91 (M^+-294, 100%)

1-Benzyl-1-[(4-diethylaminoethoxy)phenyl]-2-phenylbut-1-ene (82)

The general method 2.9 was employed using 1-(4-diethylaminoethoxyphenyl)-2-phenylethan-1-one (0.0048M), dry dioxane (15mL), propiophenone (0.0048M), titanium tetrachloride (0.0098M) and zinc dust (0.031M) under nitrogen. The crude product was isolated using column chromatography (eluant dichloromethane : ethyl acetate; 95:5) to afford a brown oil in 43% yield.

IR ν_{max} (film) 3058-2926, 2874-2854 (CHs), 1601, 1575 (C=C), 1511, 1495 (NCH_2), 1454 (NCH_3, CH_2), 1378 (CH_3) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 0.99 (3H, m, H-4), 1.21 (6H, m, $N(CH_3)_2$), 1.73 (2H, m, H-3), 2.10 (4H, m, $N(CH_2)_2$), 2.84 (2H, m, NCH_2), 3.66 (2H, m, CH_2O), 4.24, 4.25 (2H, 2 x s, CH_2), 6.90-6.94 (2H, dd, J = 8.78Hz, H-3'', H-5''), 7.08-7.62 (10H, m, ArH), 7.91-8.08 (2H, dd, J = 8.56Hz, H-2'', H-6'')

^{13}C NMR $\delta(CDCl_3)$ 9.81($(CH_3)_2$), 10.76 (C-4), 27.56 (C-3), 45.17 (CH_2), 47.47 ($N(CH_2)_2$), 51.25 (NCH_2), 65.49 (CH_2O), 113.86, 115.15 (C-3'', C-5''), 124.10, 125.40 (C-4', C-4''), 126.19 (C-4'''), 126.35, 127.84 (C-2''', C-6''', C-3''', C-5'''), 128.08, 128.17 (C-2'', C-6''), 128.48, 128.91 (C-2', C-6'), 130.51 (C-3', C-5'), 130.69 (C-1'), 132.39 (C-1''), 139.93 (C-1''')

EIMS (HR) $C_{29}H_{35}NO$: calculated M^+ 413.2722

observed M^+ 413.2719

Mass Spectrum (m/z) 413 (M^+, 73%), 398 (M^+-15, 18%), 322 (M^+-91, 33%), 253 (M^+-160, 32%), 236 (M^+-177, 16%), 221 (M^+-192, 55%), 207 (M^+-206, 41%), 191 (M^+-222, 80%), 178 (M^+-235, 100%), 165 (M^+-248, 100%), 115 (M^+-298, 100%), 91 (M^+-322, 100%)

1-Benzyl-2-phenyl-1-[(4-pyrrolidinylethoxy)phenyl]but-1-ene (83)

The general method 2.9 was applied using 2-phenyl-1-(4-pyrrolidinylethoxyphenyl)ethan-1-one (0.0011M), dry dioxane (15mL), propiophenone (0.0011M), titanium tetrachloride (0.0023M) and zinc dust (0.0072M) under nitrogen. The product was isolated using column chromatography (eluant dichloromethane : methanol; 90:10) to afford a brown oil in 75% yield.

IR ν_{max} (film) 3058-2928, 2875-2810 (CHs), 1602, 1576 (C=C), 1510 (NCH_2), 1494, 1453 (CH_2), 1375 (CH_3) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 0.93 (3H, t, J = 7.52Hz, H-4), 1.83 (4H, m, H-2'''', H-3''''), 2.08 (2H, q, J = 7.54Hz, H-3), 2.67 (4H, m, H-1'''', H-4''''), 3.02 (2H, m, NCH_2), 4.11 (2H, m, CH_2O), 4.25 (2H, s, CH_2), 6.53-6.55 (2H, d, J = 8.56Hz, H-3'', H-5''), 6.99-7.25 (10H, m, ArH), 8.01-8.04 (2H, d, J = 8.84Hz, H-2'', H-6'')

^{13}C NMR $\delta(CDCl_3)$ 8.02 (C-4), 23.96 (C-3), 28.25, 28.42 (C-2'''', C-3''''), 40.68 (CH_2), 55.36 (C-1'''', C-4''''), 55.66 (NCH_2), 66.25 (CH_2O), 114.26, 115.27 (C-3'', C-5''), 126.58 (C-4'''), 127.21, 128.21 (C-3''', C-5''', C-2''', C-6'''), 128.45 (C-2'', C-6''), 129.05 (C-2', C-6'), 129.63, 129.91 (C-3', C-5'), 138.88 (C-1, C-2), 141.09 (C-1''), 142.47 (C-1'), 144.09 (C-1''')

EIMS (HR) $C_{29}H_{33}NO$: calculated M^+ 411.2569

observed M^+ 411.2562

Mass Spectrum (m/z) 411 (M^+, 73%), 327 (M^+-84, 3%), 267 (M^+-144, 18%), 252 (M^+-159, 20%), 239 (M^+-172, 12%), 215

(M^+-196, 8%), 202 (M^+-209, 29%), 191, (M^+-220, 85%), 178 (M^+-233, 65%), 165 (M^+-246, 66%), 152 (M^+-259, 29%), 91 (M^+-320, 100%)

1-Benzyl-2-phenyl-1-[(4-piperidinylethoxy)phenyl]but-1-ene (84)

The general method 2.9 was employed using 1-(4-piperidinylethoxyphenyl)-2-phenylethan-1-one (0.0017M), dry dioxane (20mL), propiophenone (0.0017M), titanium tetrachloride (0.0024M) and zinc dust (0.0076M) under nitrogen. The product was purified using column chromatography (eluant dichloromethane : methanol; 90:10) to afford a light brown oil in 80% yield.

IR ν_{max} (film) 3058-2932, 2853-2787 (CHs), 1600,1575 (C=C), 1509 (NCH_2s), 1453 (CH_2), 1372 (CH_3) cm^{-1}

^{1}H NMR $\delta(CDCl_3)$ 0.95 (3H, m, H-4), 1.48 (6H, m, H-3'''', H-2'''', H-4'''') 2.05 (2H, m, H-3), 2.70 (4H, m, H-1'''', H-5''''), 2.78 (2H, m, NCH_2), 4.13 (2H, m, CH_2O), 4.20 (2H, s, CH_2), 6.49-6.51 (2H, d, J = 8.52Hz, H-3'', H-5''), 6.89-7.49 (10H, m, ArH), 7.91-7.93 (2H, d, J = 8.56Hz, H-2'', H-6'')

^{13}C NMR $\delta(CDCl_3)$ 7.83 (C-4), 24.40 (C-3''''), 25.89, 26.10 (C-2'''', C-4''''), 27.67 (C-3), 45.63 (CH_2), 55.35 (C-1'''', C-5''''), 58.10 (NCH_2), 65.68 (CH_2O), 113.89, 114.77 (C-3'', C-5''), 126.01 (C-4'''), 127.18, 127.94 (C-3''', C-5''', C-2''', C-6'''), 128.36 (C-2'', C-6''), 128.61 (C-2', C-6'), 129.01 (C-3', C-5'), 130.19 (C-1')

EIMS (HR) $C_{30}H_{35}NO$: calculated M^+ 425.2712

observed M^+ 425.2719

Mass Spectrum (m/z) 425 (M^+, 72%), 327 (M^+-98, 3%), 268 (M^+-157, 10%), 252 (M^+-173, 26%), 239 (M^+-186, 18%), 228 (M^+-197, 7%), 215 (M^+-210, 15%), 191 (M^+-234, 100%), 178 (M^+-247, 100%), 165 (M^+-260, 100%), 152 (M^+-273, 46%), 128 (M^+-297, 46%), 98 (M^+-327, 100%), 91 (M^+-334, 100%)

1-Benzyl-1-[(4-morpholinylethoxy)phenyl] 2-phenylbut-1-ene (85)

The procedure 2.9 outlined was applied using 1-(4-morpholinylethoxyphenyl)-2-phenylethan-1-one (0.0012M), dry dioxane (20mL), propiophenone (0.0012M), titanium tetrachloride (0.0024M) and zinc dust (0.0075M) under nitrogen. The product was isolated using column chromatography (eluant dichloromethane : methanol; 70:30) to afford a dark brown oil in 41% yield.

IR ν_{max} (film) 3059-2933, 2856-2801 (CHs), 1600 (C=C), 1510 (NCH_2), 1453 (CH_2), 1358 (CH_3) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 0.98 (3H, t, J = 7.52Hz, H-4), 1.73 (2H, q, J = 7.54Hz, H-3), 2.84 (2H, m, NCH_2), 3.02 (4H, m, H-1'''', H-4''''), 3.61 (4H, m, H-2'''', H-3''''), 4.12 (2H, m, CH_2O), 4.25 (2H,s, CH_2), 6.83-6.86 (2H, dd, J = 8.52, 1.48Hz, H-3'', H-5''), 6.93-7.58 (10H, m, ArH), 7.97-7.99 (2H, dd, J = 8.52, 1.43Hz, H-2'', H-6'')

^{13}C NMR $\delta(CDCl_3)$ 7.05 (C-4), 27.23 (C-3), 44.24 (CH_2), 53.11, 53.62 (C-1'''', C-4''''), 57.25 (NCH_2), 65.61 (CH_2O), 66.43 (C-2'''', C-3''''), 113.89, 114.06 (C-3'', C-5''), 124.47, 125.29 (C-4', C-4''), 126.37 (C-4'''), 127.22, 127.99 (C-3''', C-5''', C-2''', C-6'''), 128.08, 128.17 (C-2'', C-6''), 128.88, 129.14 (C-2', C-6', C-3', C-5'), 130.39 (C-1'), 130.49 (C-1'')

EIMS (HR) $C_{29}H_{33}NO_2$: calculated M^+ 427.2499

observed M^+ 427.2511

Mass Spectrum (m/z) 427 (M^+, 73%), 268 (M^+-159, 14%), 252 (M^+-175, 14%), 191 (M^+-236, 55%), 178 (M^+-249, 41%), 165 (M^+-262, 36%), 152 (M^+-259, 29%), 129 (M^+-298, 20%), 114 (M^+-313, 100%), 91 (M^+-336, 100%)

7.2.14. Preparation of p–substituted propiophenone

p-(Dimethylaminoethoxy)propiophenone (87)

The general method 2.12 was applied in the preparation of this compound, using *p*–hydroxypropiophenone (0.0021M), anhydrous K_2CO_3 (0.02M), dry acetone (25mL) and 2-dimethylaminoethylchloride hydrogenchloride (0.0046M). The reaction mixture was refluxed for 6-8hr under nitrogen. The product was isolated using flash column chromatography (eluant dichloromethane : methanol, 90:10) as a lemon coloured oily gel[527] in 83% yield.

IR ν_{max} (film) 3067-2939, 2878-2772 (CHs), 1679 (C=O), 1610, 1575 (C=C), 1509, 1458 (CH_2), 1419, 1350 (CH_3) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 1.20 (3H, t, J = 7.28Hz, CH_3), 2.34 (6H, s, $N(CH_3)_2$), 2.75 (2H, t, J = 5.28Hz, CH_2N), 2.93 (2H, q, J = 7.24Hz, CH_2), 4.12 (2H, t, J = 5.24Hz, CH_2O), 6.93-6.96 (2H, dd, J = 8.52, 1.52Hz, H-3'', H-5''), 7.92-7.94 (2H, dd, J = 9.00, 2.00Hz, H-2'', H-6'')

^{13}C NMR $\delta(CDCl_3)$ 10.16 (CH_2), 33.10 (CH_2), 47.64 ($N(CH_3)_2$), 59.87 (CH_2N), 68.03 (CH_2O), 115.97 (C-3'', C-5''), 131.88 (C-2'', C-6''), 164.32 (C-4''), 201.14 (C=O)

p-(Diethylaminoethoxy)propiophenone (88)

The general procedure 2.12 was employed using *p*–hydroxypropiophenone (0.002M), anhydrous K_2CO_3 (0.02M), dry acetone (25mL) and 2-diethylaminoethylchloride hydrogenchloride (0.0046M). The product was isolated by column chromatography (eluant dichloromethane : methanol; 80:20) as a light brown oily gel[527] in 93% yield.

IR ν_{max} (film) 2971-2936, 2878-2811 (CHs), 1661 (C=O), 1602, 1575 (C=C), 1458, 1419 (CH_2), 1375, 1350 (CH_3) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 1.07 (6H, m, $(CH_3)_2$), 1.20 (3H, t, J = 5.52Hz, CH_3), 2.64 (4H, m, $(CH_2)_2$), 2.91 (4H, m, CH_2, CH_2N), 4.11 (2H, t, J = 6.02Hz, CH_2O), 6.91-6.94 (2H, dd, J

= 9.00, 2.00Hz, H-3'', H-5''), 7.92-7.94 (2H, dd, J = 8.56, 1.78Hz, H-2'', H-6'')

^{13}C NMR δ($CDCl_3$) 8.81 (CH_3), 12.22 ((CH_3)$_2$), 31.78 (CH_2), 48.27 (N(CH_2)$_2$), 52.02 (CH_2N), 67.28 (CH_2O), 114.68 (C-3'', C-5''), 130.54 (C-2'', C-6''), 163.01 (C-4''), 199.77 (C=O)

_p_-Pyrrolidinylethoxypropiophenone (89)

The general procedure 2.12 was employed using _p_–hydroxypropiophenone (0.002M), anhydrous K_2CO_3 (0.02M), dry acetone (25mL) and 1-(2-chloroethyl)pyrrolidine hydrogenchloride (0.0046M) under nitrogen. The product was isolated as a brown oil in 91% yield following column chromatography (eluant dichloromethane : methanol; 80:20).

IR ν_{max} (film) 3061-2933, 2880-2779 (CHs), 1680 (C=O), 1601, 1575 (C=C), 1509, 1459, 1419 (CH_2), 1351 (CH_3) cm^{-1}

^{1}H NMR δ($CDCl_3$) 1.21 (3H, t, J = 7.28Hz, CH_3), 1.84 (4H, m, H-2'''', H-3''''), 2.08 (4H, m, H-1'''', H-4''''), 2.91-2.98 (2H, m, CH_2N, CH_2), 4.20 (2H, t, J = 5.76Hz, CH_2O), 6.93-6.95 (2H, dd, J = 8.52, 2.00Hz, H-3'', H-5''), 7.92-7.94 (2H, dd, J = 8.52, 2.00Hz, H-2'', H-6'')

^{13}C NMR δ($CDCl_3$) 9.80 (CH_3), 24.87 (C-2'''', C-3''''), 32.76 (CH_2), 56.07, 56.18 (C-1'''', C-4'''', N(CH_2)), 68.46 (OCH_2), 115.03 (C-3'', C-5''), 131.56 (C-2'', C-6''), 163.85 (C-4''), 200.81 (C=O)

EIMS (HR) $C_{15}H_{21}NO_2$: calculated M^+ 247.1572

observed M^+ 247.1572

Mass Spectrum (m/z) 247 (M^+, 87%), 147 (M^+-100,100%), 133 (M^+-114, 100%), 120 (M^+-127, 91%), 104 (M^+-143, 100%), 98 (M^+-149,100%), 92 (M^+-155, 100%), 84 (M^+-163, 100%)

p-(Piperidinylethoxy)propiophenone (90)

The general procedure 2.12 was employed using *p*-hydroxypropiophenone (0.002M), anhydrous K_2CO_3 (0.02M), dry acetone (25mL) and 1-(2-chloroethyl)piperidine monohydrochloride (0.0046M) under nitrogen. The product was purified as a mustard oily gel[527] in 64% yield following column chromatography (eluant dichloromethane : methanol; 85:15).

IR ν_{max} (film) 2984-2848, 27863-2748(CHs), 1675 (C=O), 1600, 1575 (C=C), 1558, 1540, 1506, 1455 (CH_2), 1351 (CH_3) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 1.21 (3H, t, J = 7.52Hz, CH_3), 1.45 (2H, m, H-3''''), 1.61 (4H, m, H-2'''', H-4''''), 2.51 (4H, m, H-1'''', H-5''''), 2.78 (2H, t, J = 6.02Hz, CH_2N), 2.95 (2H, q, J = 7.49Hz, CH_2), 4.16 (2H, t, J = 6.02Hz, CH_2O), 6.92-6.94 (2H, d, J = 9.04Hz, H-3'', H-5''), 7.92-7.94 (2H, d, J = 8.52Hz, H-2'', H-6'')

^{13}C NMR $\delta(CDCl_3)$ 9.06 (CH_3), 24.77 (C-3''''), 26.53, 26.56 (C-2'''', C-4''''), 32.00 (CH_2), 54.71 (C-1'''', C-5''''), 58.36 (CH_2N), 66.91 (CH_2O), 114.88 (C-3'', C-5''), 130.78 (C-2'', C-6'')

p-(Morpholinylethoxy)propiophenone (91)

The general procedure 2.12 was employed using *p*–hydroxypropiophenone (0.002M), anhydrous K_2CO_3 (0.02M), dry acetone (20mL) and 4-(2-chloroethyl)morpholine hydrochloride (0.0046M) under nitrogen. The product was isolated as a light brown oil[527] in 85% yield following column chromatography (eluant dichloromethane : methanol; 85:15) .

IR ν_{max} (KBr) 3018-2937, 2855-2809 (CHs), 1679 (C=O), 1601, 1575 (C=C), 1509, 1455, 1419 (CH_2), 1356 (CH_3) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 1.22 (3H, t, J = 7.28Hz, CH_3), 2.61 (4H, m, H-1'''', H-4''''), 2.83 (2H, t, J = 5.52Hz, CH_2N), 2.96 (2H, q, J = 7.69Hz, CH_2), 3.73 (4H, m, H-2'''', H-3''''), 4.18 (2H, t, J = 5.56Hz, CH_2O), 6.93-6.95 (2H, dd, J

= 8.56, 2.04Hz, H-3'', H-5''), 7.93-7.96 (2H, dd, J = 9.00, 2.00Hz, H-2'', H-6'')

^{13}C NMR δ($CDCl_3$) 8.36 (CH_3), 31.33 (CH_2), 53.50 (C-1'''', C-4''''), 57.38 (CH_2N), 65.38 (CH_2O), 66.76, 66.82 (C-2'''', C-3''''), 114.17 (C-3'', C-5''), 130.12 (C-2'', C-6''), 196.45 (C=O)

1-Benzyl-1,2-diphenylbut-1-ene (93)

The general method 2.9 was applied using desoxybenzoin (0.0047M) and propiophenone (0.0047M) in dry dioxane. Titanium (IV) chloride (0.0095M) and zinc dust (0.03M), were added as previously outlined. The organic layer was extracted with diethyl ether and post washing with 3N HCl (pH 6) was recorded. Column chromatography (eluant petroleum ether : dichloromethane; 95:5), produced a colourless oil in 75% yield with the following physical properties.

IR ν_{max} (film) 3102-3012, 2988-2855 (CHs), 1599, 1576 (C=C), 1443 (CH_2), 1372 (CH_3) cm^{-1}

^{1}H NMR δ($CDCl_3$) 1.06 (3H, t, J = 7.44Hz, H-4), 2.74 (2H, q, J = 7.45Hz, H-3), 4.03 (2H, s, CH_2), 6.96-7.31 (15H, m, ArH)

^{13}C NMR δ($CDCl_3$) 12.94 (C-4), 27.93 (C-3), 40.01 (CH_2), 125.53 (C-4'), 125.73, 125.80 (C-4'', C-4'''), 127.30, 127.47 (C-2'', C-6'', C-2''', C-6'''), 128.22, 128.48 (C-2', C-6', C-3', C-5'), 128.96 (C-3'', C-5'', C-3''', C-5'''), 139.55 (C-1, C-2), 141.52 (C-1'), 142.32, 142.73 (C-1'', C-1''')

EIMS (HR) $C_{23}H_{22}$: calculated M^+ 298.1722

observed M^+ 298.1751

Mass Spectrum (m/z) 298 (M^+, 81%), 269 (M^+-29, 63%), 236 (M^+-62, 100%), 207 (M^+-91, 100%), 191 (M^+-107, 66%), 178 (M^+-120, 49%), 129 (M^+-169, 100%), 91 (M^+-207, 100%)

Symmetric by-products of 1-Benzyl-1,2-diphenylbut-1-ene (93)

3,4-Diphenylhex-3-ene (94)

This product was recovered from flash column chromatography (eluant petroleum ether : dichloromethane; 98:2), as a colourless oil[332] in 14% yield with the following physical properties.

IR ν_{max} (film) 3091-3017, 2986-2835 (CHs), 1600, 1575 (C=C), 1441 (CH_2), 1371 (CH_3) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 1.02 (6H, t, J = 7.46Hz, H-4), 2.61 (4H, q, J = 7.48Hz, H-3), 6.96-7.11 (10H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 13.23 (CH_3), 27.23 (CH_2), 125.37 (C-4'', C-4'''), 127.93, (C-2'', C-6'', C-2', C-6'), 128.71, 129.72 (C-3'', C-5'', C-3', C-5'), 138.75 (C-3, C-4), 142.78 (C-1', C-1'')

1,2-Dibenzyl-1,2-diphenyl-ethene (95)

This product was recovered from flash column chromatography (eluant petroleum ether : dichloromethane; 90:10), as a colourless crystalline solid in 5% yield.

IR ν_{max} (KBr) 3010-3095, 2987-2833 (CHs), 1597, 1574 (C=C), 1451 (CH_2) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 4.08 (4H, s, CH_2), 7.00-7.28 (20H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 40.61 (CH_2), 125.42, 125.52 (C-4'', C-4'''), 126.87 (C-4', C-4''''), 127.01, 127.79 (C-2'''', C-6'''', C-2', C-6'), 127.87, 128.08 (C-2'', C-3'', C-5'', C-6'', C-2''', C-3''', C-5''', C-6'''), 129.34, 129.41 (C-3'''', C-5'''', C-3', C-5'), 137.62 (C-1, C-2), 139.02 (C-1''), 142.38 (C-1'''')

EIMS (HR) $C_{28}H_{24}$: calculated M^+ 360.1878

observed M^+ 360.1903

7.2.15. McMurry coupling of p-(alkylamino)propiophenones

1-Benzyl-2-[(4-dimethylaminoethoxy)phenyl]-1-phenylbut-1-ene (96)

The general method 2.9 was employed using *p*-(dimethylaminoethoxy)propiophenone (0.0016M), dry dioxane (25mL), desoxybenzoin (0.0016M), titanium tetrachloride (0.0032M) and zinc dust (0.01M) under nitrogen. The product was isolated using flash column chromatography (eluant dichloromethane : methanol; 90:10) to afford a brown oil in 25% yield.

IR ν_{max} (film) 3083-2920, 2871-2775 (CHs), 1605, 1576 (C=C), 1508 (NCH_2), 1494, 1453 (NCH_3, CH_2), 1381, 1338 (CH_3), 1283, 1242 (CN) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 0.99 (3H, t, J = 7.54Hz, H-4), 2.45 (6H, s, $N(CH_3)_2$), 2.66 (2H, q, J = 7.52Hz, H-3), 2.86 (2H, m, NCH_2), 3.96 (2H, s, CH_2) 4.07 (2H, m, CH_2O), 6.65-6.67 (2H, d, J = 8.52Hz, H-3'', H-5''), 6.94-7.56 (10H, m, ArH), 8.07-8.09 (2H, d, J = 9.04Hz, H-2'', H-6'')

^{13}C NMR $\delta(CDCl_3)$ 12.52 (C-4), 27.47 (C-3), 39.59 (CH_2), 44.66 ($N(CH_3)_2$), 57.21 (NCH_2), 64.62 (CH_2O), 113.18, 113.81 (C-3'', C-5''), 124.98, 125.31 (C-4', C-4''), 126.83, 127.39 (C-3''', C-5''', C-2''', C-6'''), 128.01, 128.21 (C-2'', C-6''), 129.39, 129.55 (C-2', C-6'), 130.27 (C-3', C-5'), 134.43 (C-1'), 134.90 (C-1''), 139.60 (C-1'''), 156.04 (C-4''')

EIMS (HR) $C_{27}H_{31}NO$: calculated M^+ 385.2407

observed M^+ 385.2406

Mass Spectrum (m/z) 385 (M^+, 74%), 267 (M^+-118, 98%), 252 (M^+-133, 12%), 215 (M^+-170, 6%), 202 (M^+-183, 23%), 191 (M^+-194, 55%) 178 (M^+-207, 62%), 165 (M^+-220, 49%), 128 (M^+-257, 16%), 91 (M^+-294, 100%)

1-Benzyl-2-[(4-diethylaminoethoxy)phenyl]-1-phenylbut-1-ene (97)

The general method 2.9 was employed using *p*-(diethylaminoethoxy)propiophenone (0.0017M), dry dioxane (20mL),

desoxybenzoin (0.0017M), titanium tetrachloride (0.0033M) and zinc dust (0.01M) under nitrogen. The crude product was isolated using column chromatography (eluant dichloromethane : methanol; 90:10) to afford a brown oily gel in 35% yield.

IR ν_{max} (film) 3080-2968, 2931-2724 (CHs), 1606, 1574 (C=C), 1508, 1493 (NCH_2), 1453 (NCH_3, CH_2), 1372 (CH_3) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 1.01 (3H, t, J = 7.54Hz, H-4), 1.14 (6H, m, $N(CH_3)_2$), 2.68 (6H, m, H-3, $N(CH_2)_2$), 2.90 (2H, t, J = 6.26Hz, NCH_2), 3.98 (2H, s, CH_2), 4.03 (2H, t, J = 6.04Hz, CH_2O), 6.66-6.68 (2H, d, J = 8.52Hz, H-3'', H-5''), 6.93-7.31 (12H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 11.06 ($(CH_3)_2$), 12.54 (C-4), 27.49 (C-3), 39.62 (CH_2), 47.31 ($N(CH_2)_2$), 51.12 (NCH_2), 65.49 (CH_2O), 113.18, 113.79 (C-3'', C-5''), 124.10, 125.40 (C-4', C-4''), 127.76, 127.93 (C-2''', C-6''', C-3''', C-5'''), 128.02 (C-2'', C-6''), 129.41 (C-2', C-6'), 130.34 (C-3', C-5'), 134.36 (C-1'), 134.67 (C-1''), 139.62 (C-1'''), 156.27 (C-4''')

EIMS (HR) $C_{29}H_{35}NO$: calculated M^+ 413.2723

observed M^+ 413.2719

Mass Spectrum (m/z) 413 (M^+, 73%), 398 (M^+-115, 23%), 267 (M^+-146, 49%), 252 (M^+-161, 28%), 239 (M^+-174, 18%), 228 (M^+-185, 7%), 203 (M^+-210, 37%), 191 (M^+-222, 100%) 178 (M^+-235, 100%), 165 (M^+-248, 91%), 128 (M^+-285, 37%), 91 (M^+-322, 100%)

1-Benzyl-1-phenyl-2-[(4-pyrrolidinylethoxy)phenyl]but-1-ene (98)

The general method 2.9 was employed using *p*-(pyrrolidinylethoxy)propiophenone (0.0017M), dry dioxane (20mL), desoxybenzoin (0.0017M), titanium tetrachloride (0.0034M) and zinc dust (0.011M) under nitrogen. The product was isolated using column chromatography (eluant dichloromethane : methanol; 85:15) to afford a light brown oil in 25% yield.

IR ν_{max} (film)	3059-2961, 2928-2873 (CHs), 1603, 1580 (C=C), 1509 (NCH_2), 1494, 1451 (CH_2), 1377 (CH_3) cm^{-1}
^1H NMR $\delta(CDCl_3)$	0.99 (3H, t, J = 7.00Hz, H-4), 1.95 (4H, m, H-2'''', H-3''''), 2.65 (2H, q, J = 7.01Hz, H-3), 3.01 (4H, m, H-1'''', H-4 ''''), 3.14 (2H, t, J = 5.04Hz, NCH_2), 3.96 (2H, s, CH_2), 4.17 (2H, t, J = 5.26Hz, CH_2O), 6.64-6.66 (2H, d, J = 8.52Hz, H-3'', H-5''), 6.91-7.58 (10H, m, ArH), 8.03-8.05 (2H, d, J = 8.52Hz, H-2'', H-6'')
^{13}C NMR $\delta(CDCl_3)$	12.50 (C-4), 22.86, 22.92 (C-2'''', C-3'''') 27.46 (C-3), 39.60 (CH_2), 45.05 (NCH_2), 53.92, 53.93 (C-1'''', C-4''''), 64.75 (CH_2O), 113.59, 114.08 (C-3'', C-5''), 125.01, 125.31 (C-4', C-4''), 126.41, 127.74 (C-3''', C-5''', C-2''', C-6'''), 128.00, 128.15 (C-2'', C-6''), 129.39 (C-2', C-6'), 130.42 (C-3', C-5'), 132.66 (C-1'), 134.51 (C-1''), 139.56 (C-1'''), 155.70 (C-4''')
EIMS (HR)	$C_{29}H_{33}NO$: calculated M^+ 411.2567 observed M^+ 411.2562
Mass Spectrum (m/z)	411 (M^+, 73%), 341 (M^+-70, 4%), 314 (M^+-97, 5%), 298 (M^+-113, 5%), 267 (M^+-144, 33%), 252 (M^+-159, 25%), 239 (M^+-172, 17%), 215 (M^+-196, 21%) 191 (M^+-220, 100%), 178 (M^+-233, 100%), 165 (M^+-246, 83%), 129 (M^+-282, 25%), 91 (M^+-320, 100%)

1-Benzyl-1-phenyl-2-[(4-piperidinylethoxy)phenyl]but-1-ene (99)

The general method 2.9 was employed using *p*-(piperidinylethoxy)propiophenone (0.0009M), dry dioxane (15mL), desoxybenzoin (0.0009M), titanium tetrachloride (0.0018M) and zinc dust (0.0057M) under nitrogen. The product was purified using column chromatography (eluant dichloromethane : methanol; 90:10) to afford a light brown oil in 40% yield.

IR ν_{max} (film) 3060-2932, 2856 (CHs), 1605,1581 (C=C), 1509, 1494 (NCH_2s), 1452 (CH_2), 1380 (CH_3) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 0.98 (3H, t, J = 7.52Hz, H-4), 1.52 (2H, m, H-3''''), 1.75 (4H, m, H-2'''', H-4 '''') 2.66 (2H, q, J = 7.54Hz, H-3), 2.75 (4H, m, H-1'''', H-5''''), 2.98 (2H, m, NCH_2), 3.95 (2H, s, CH_2), 4.14 (2H, m, CH_2O), 6.63-6.65 (2H, d, J = 8.56Hz, H-3'', H-5''), 6.75-7.56 (10H, m, ArH), 8.02-8.10 (2H, d, J = 8.64Hz, H-2'', H-6'')

^{13}C NMR $\delta(CDCl_3)$ 12.90 (C-4), 23.40 (C-3''''), 24.72 (C-2'''', C-4''''), 27.86 (C-3), 39.99 (CH_2), 54.39 (C-1'''', C-5''''), 57.13 (NCH_2), 66.65 (CH_2O), 113.59 (C-3'', C-5''), 124.86, 124.98 (C-4', C-4''), 126.42-127.74 (C-3''', C-5''', C-2''', C-6'''), 128.03, 128.15 (C-2'', C-6''), 129.14, 129.39 (C-2', C-6'), 130.38 (C-3', C-5'), 134.46 (C-1''), 156.19 (C-4''')

EIMS (HR) $C_{30}H_{35}NO$: calculated M^+ 425.2703

observed M^+ 425.2719

Mass Spectrum (m/z) 425 (M^+, 72%), 314 (M^+-111, 4%), 297 (M^+-128, 4%), 285 (M^+-140, 6%), 267 (M^+-158, 42%), 239 (M^+-186, 23%), 203 (M^+-222, 50%), 191 (M^+-234, 100%) 178 (M^+-247, 100%), 165 (M^+-260, 100%), 128 (M^+-297, 51%), 98 (M^+-327, 100%), 91 (M^+-334, 100%)

1-Benzyl-2-[(4-morpholinylethoxy)phenyl]-1-phenyl-but-1-ene (109)

The general method 2.9 was employed using *p*-(morpholinylethoxy)propiophenone (0.0013M), dry dioxane (20mL), desoxybenzoin (0.0013M), titanium tetrachloride (0.0027M) and zinc dust (0.0085M) under nitrogen. The product was isolated using column chromatography (eluant dichloromethane : methanol; 90:10) to afford a dark brown oil in 29% yield.

IR ν_{max} (film) 3059-2960, 2929-2856 (CHs), 1606, 1581 (C=C), 1509, 1494 (NCH_2), 1453 (CH_2), 1370 (CH_3) cm^{-1}

^{1}H NMR δ($CDCl_3$)	0.99 (3H, t, J = 7.54Hz, H-4), 2.65 (4H, m, H-1'''', H-4 ''''), 2.83 (2H, t, J = 5.52Hz, NCH_2), 2.90 (2H, q, J = 7.03Hz, H-3), 3.77 (4H, m, H-2'''', H-3''''), 3.96 (2H, s, CH_2), 4.06 (2H, t, J = 5.52Hz, CH_2O), 6.65-6.67 (2H, d, J = 8.56Hz, H-3'', H-5''), 6.92-7.56 (12H, m, ArH)
^{13}C NMR δ($CDCl_3$)	12.52 (C-4), 27.48 (C-3), 39.62 (CH_2), 55.45 (C-1'''', C-4 ''''), 57.09 (NCH_2), 64.93 (CH_2O), 66.23 (C-2'''', C-3''''), 113.22, 113.86 (C-3'', C-5''), 124.97, 125.32 (C-4', C-4''), 126.92-127.75 (C-3''', C-5''', C-2''', C-6'''), 128.01, 128.53 (C-2'', C-6''), 129.34, 129.40 (C-2', C-6'), 130.35 (C-3', C-5'), 131.95 (C-1'), 134.82 (C-1''), 139.59 (C-1'''), 156.18 (C-4'')
EIMS (HR)	$C_{29}H_{33}NO_2$: calculated M^+ 427.2499 observed M^+ 427.2511
Mass Spectrum (m/z)	427 (M^+, 73%), 311 (M^+-116, 2%), 267 (M^+-160, 15%), 252 (M^+-175, 16%), 239 (M^+-188, 9%), 228 (M^+-199, 3%), 215 (M^+-212, 6%), 203 (M^+-224, 19%) 191 (M^+-236, 49%), 178 (M^+-249, 57%), 165 (M^+-262, 39%), 114 (M^+-313, 100%), 91 (M^+-336, 100%)

7.2.16. Preparation of substituted 1-phenylethanones

2-(4-Methoxyphenyl)-1-phenylethanone (101)

Phenylacetyl chloride (0.16M), anisole (0.19M) and carbon disulphide (1.35M) were mixed together for 10-15min. To this mixture was added aluminium chloride (0.015M) and the mixture was stirred at 0-5°C for about 2hr. Post this period a red coloured solution was observed and the reaction mixture was allowed to heat up to room temperature. The reaction product was washed with ice-water, 20% NaOH (40mL), extracted with ethyl acetate (3x25mL), dried over sodium sulphate and concentrated down. Column chromatography (eluant petroleum ether : ethyl acetate; 95:5) was carried out to purify the compound providing a lemon crystalline solid[528] in 67% yield; m.p. 68-75°C [lit. m.p. 76°C]

IR ν_{max} (KBr) 2997-2829 (CHs), 1677 (C=O), 1596 (C=C), 1509 (CH_2), 1455 (OCH_3), 1261 (C-O) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 3.88 (3H, s, OCH_3), 4.26 (2H, s, CH_2), 6.93-6.96 (2H, d, J = 8.52Hz, H-3'', H-5''), 7.28-7.34 (5H, m, ArH), 8.00-8.03 (2H, d, J = 9.04 H-2'', H-6'')

^{13}C NMR $\delta(CDCl_3)$ 44.81 (CH_2), 54.99 (OCH_3), 113.35 (C-3'', C-5''), 126.30 (C-4'', C-4'), 128.17, 128.92 (C-2', C-6', C-2'', C-6''), 130.49 (C-1''), 163.08 (C-4''), 195.73 (C=O)

2-(4-Hydroxyphenyl)-1-phenylethanone (102)

To 2-(4-methoxyphenyl)-1-phenylethanone (0.0044M) was added pyridine hydrochloride (0.025M) and stirred at 180°C for 3hr. Post this period the reaction mixture was cooled to room temperature and dissolved in dichloromethane (15mL) and stirred for a further 15min. The product solution was then washed with dilute HCl (1N, 20mL), water, extracted with diethyl ether (3 x 20mL) and dried over Na_2SO_4 and concentrated down. Flash column chromatography (eluant petroleum ether : ethyl acetate; 75:25) was carried out to purify the compound, affording a light brown gel[529] in 89% yield, with the following physical properties.

IR ν_{max} (film) 3434 (OH), 3241-3116, 3101-2943 (CHs), 1706 (C=O), 1598 (C=C), 1451 (CH_2) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 4.26 (2H, s, CH_2), 6.90-6.92 (2H, d, J = 8.56Hz, H-3'', H-5''), 7.32-7.54 (5H, m, ArH), 7.89-8.00 (2H, d, J = 8.52, H-2'', H-6'')

^{13}C NMR $\delta(CDCl_3)$ 40.58 (CH_2), 115.60 (C-3'', C-5''), 126.89 (C-4'', C-4'), 128.19, 128.53 (C-2'', C-6''), 128.91, 129.48 (C-2', C-6'), 136.27 (C-1''), 161.83 (C-4'')

2-(4-Dimethylaminoethoxyphenyl)-1-phenylethanone (103)

The general method 2.12 was applied in the preparation of this compound, using 2-(4-hydroxyphenyl)-1-phenylethanone (0.0004M), anhydrous K_2CO_3 (0.004M), dry acetone (20mL) and 2-dimethylaminoethylchloride hydrogenchloride (0.00095M). The reaction mixture was refluxed for 6-8hr under nitrogen. The

product was isolated using flash column chromatography (eluant dichloromethane : methanol; 90:10) as a brown oil in 90% yield.

IR ν_{max} (film) 3061-3029, 2931 (CHs), 1670 (C=O), 1599 (C=C), 1495, 1453 (CH_2), 1374 (CH_3) 1251 (C-N) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 2.34 (6H, s, $N(CH_3)_2$), 2.56 (2H, t, J = 5.76Hz, CH_2N), 3.69 (2H, s, CH_2), 4.13 (2H, t, J = 5.78Hz, CH_2O), 6.93-6.96 (2H, d, J = 9.04Hz, H-3'', H-5''), 7.26-7.73 (5H, m ArH), 7.96-7.99 (2H, d, J = 8.58Hz, H-2'', H-6'')

^{13}C NMR $\delta(CDCl_3)$ 40.79 (CH_2), 45.11 ($N(CH_3)_2$), 57.19, 57.55 (CH_2N), 65.76 (CH_2O), 113.61, 114.28 (C-3'', C-5''), 126.27, 126.53 (C-4', C-4''), 128.05, 128.29 (C-2'', C-6''), 128.47, 128.76 (C-2', C-6'), 128.81, 128.91 (C-3', C-5'), 131.87 (C-1''), 132.83 (C-1'), 162.32 (C-4"), 171.13 (C=O)

EIMS (HR) $C_{18}H_{21}NO_2$: calculated M^+ 283.1576

observed M^+ 283.1572

Mass Spectrum (m/z) 283 (M^+, 82%), 250 (M^+-33, 7%), 225 (M^+-58, 5%), 192 (M^+-91, 28%), 178 (M^+-105, 29%), 165 (M^+-118, 78%), 147 (M^+-136, 5%), 121 (M^+-162, 100%), 104 (M^+-179, 100%)

2-(4-Diethylaminoethoxyphenyl)-1-phenylethanone (104)

The general procedure 2.12 was employed using 2-(4-hydroxyphenyl)-1-phenylethanone (0.00047M), anhydrous K_2CO_3 (0.0047M), dry acetone (25mL) and 2-diethylaminoethylchloride hydrogenchloride (0.0012M). The product was isolated by column chromatography (eluant dichloromethane : methanol; 90:10) as a brown oil[530] in 90% yield.

IR ν_{max} (film) 3146-2941, 2930-2812 (CHs), 1654 (C=O), 1600 (C=C), 1496, 1452 (CH_2), 1377 (CH_3) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 1.02 (6H, m, $(CH_3)_2$), 2.55 (4H, m, $(CH_2)_2$), 2.71 (2H, t, J = 6.28Hz, CH_2N), 3.65 (2H, s, CH_2), 4.19 (2H, t, J = 6.26Hz, CH_2O), 4.24 (2H, s, CH_2), 6.76-

6.96 (2H, d, J = 9.04Hz, H-3'', H-5''), 7.28-7.54 (5H, m, ArH), 7.96-7.99 (2H, d, J = 9.04Hz, H-2'', H-6'')

^{13}C NMR $\delta(CDCl_3)$ 11.39 ($(CH_3)_2$), 40.93 (CH_2), 47.93 ($N(CH_2)_2$), 50.56 (CH_2N), 62.72 (CH_2O), 113.21, 114.43 (C-3'', C-5''), 126.55, 127.21 (C-4', C-4''), 128.09, 128.29 (C-2'', C-6''), 128.46, 128.77 (C-2', C-6'), 128.82, 129.42 (C-3', C-5'), 131.88 (C-1'), 171.06 (C=O)

1-Phenyl-2-(4-pyrrolidinylethoxyphenyl)ethanone (105)

The general method 2.12 was applied using 2-(4-hydroxyphenyl)-1-phenylethanone (0.0007M), anhydrous K_2CO_3 (0.007M), dry acetone (25mL) and 1-(2-chloroethyl)-pyrrolidine hydrochloride (0.0019M). A pure product was isolated using column chromatography (eluant dichloromethane : methanol; 95:5) as a mustard coloured oil in 91% yield.

IR ν_{max} (film) 3063-2963, 2878-2800 (CHs), 1670 (C=O), 1599, 1576 (C=C), 1509, 1497, 1455 (CH_2) cm^{-1}

^{1}H NMR $\delta(CDCl_3)$ 1.76 (4H, m, H-2^{iv}, H-3^{iv}), 2.53 (4H, m, H-1'''', H-4''''), 2.73 (2H, t, J = 6.04Hz, CH_2N), 3.64 (2H, s, CH_2), 4.24 (2H, t, J = 6.18Hz, CH_2O), 6.93-6.95 (2H, d, J = 9.02Hz, H-3'', H-5''), 7.24-7.34 (5H, m, ArH), 7.97-7.99 (2H, d, J = 8.52Hz, H-2'', H-6'')

^{13}C NMR $\delta(CDCl_3)$ 23.03 (C-2'''', C-3''''), 40.88 (CH_2), 53.89 (C-1'''', C-4''''), 53.98 (NCH_2), 63.32 (OCH_2), 113.89 (C-3'', C-5''), 125.43, 126.26 (C-4', C-4''), 126.55, 128.03 (C-2'', C-6''), 128.08, 128.13 (C-2', C-6'), 128.82, 128.91 (C-3', C-5'), 130.44 (C-1''), 171.03 (C=O)

EIMS (HR) $C_{20}H_{23}NO_2$: calculated M^+ 309.1741

observed M^+ 309.1729

Mass Spectrum (m/z) 309 (M^+, 80%), 267 (M^+-42, 85%), 239 (M^+-70, 66%), 225 (M^+-84, 50%), 214 (M^+-95, 48%), 148 (M^+-161, 51%) 133 (M^+-176, 95%), 119 (M^+-190, 80%), 98 (M^+-211, 100%)

1-Phenyl-2-(4-piperidinylethoxyphenyl)ethanone (106)

The general method 2.12 outlined above was applied using 2-(4-hydroxyphenyl)-1-phenylethanone (0.001M), anhydrous K_2CO_3 (0.01M), dry acetone (25mL) and 1-(2-chloroethyl)piperidine monohydrochloride (0.0026M). The product was isolated using column chromatography (eluant dichloromethane : methanol; 90:10), as a dark mustard coloured oil in 92% yield, with the following physical properties.

IR ν_{max} (film)	2939, 2854 (CHs), 1664 (C=O), 1600 (C=C), 1509, 1496, 1454 (CH_2) cm^{-1}
^1H NMR $\delta(CDCl_3)$	1.53 (6H, m, H-2'''', H-3'''', H-4''''), 2.40 (4H, m, H-1'''', H-5''''), 2.60 (2H, t, J = 5.76Hz, CH_2N), 3.62 (2H, d, J = 4.04Hz, CH_2), 4.20 (2H, t, J = 5.78Hz, CH_2O), 6.88-6.98 (2H, d, J = 8.52Hz, H-3'', H-5''), 7.19-7.26 (5H, m, ArH), 7.90-7.92 (2H, d, J = 8.52Hz, H-2'', H-6'')
^{13}C NMR $\delta(CDCl_3)$	23.31 (C-3''''), 24.93, 25.29 (C-2'''', C-4''''), 40.63 (CH_2), 53.99, 54.20 (C-1'''', C-5''''), 56.45 (CH_2N), 61.53 (CH_2O), 113.90, 115.35 (C-3'', C-5''), 126.53, 126.92 (C-4', C-4''), 127.85, 128.07 (C-2'', C-6''), 128.70 (C-2', C-6'), 128.92 (C-3'', C-5'')132.75 (C-1''), 170.15 (C=O)
EIMS (HR)	$C_{21}H_{25}NO_2$: calculated M^+ 323.1881 observed M^+ 323.1885
Mass Spectrum (m/z)	323 (M^+, 79%), 299 (M^+-24, 70%), 267 (M^+-56, 68%), 232 (M^+-91, 75%), 192 (M^+-131, 68%), 165 (M^+-158, 82%) 147 (M^+-176, 85%), 133 (M^+-190, 80%), 121 (M^+-202, 95%), 112 (M^+-211, 100%), 98 (M^+-225, 100%)

2-(4-Morpholinylethoxyphenyl)-1-phenylethanone (107)

The general procedure 2.12 was employed using 2-(4-hydroxyphenyl)-1-phenylethanone (0.00067M), anhydrous K_2CO_3 (0.0067M), dry acetone (20mL) and 4-(2-chloroethyl) morpholine hydrochloride (0.0017M) under nitrogen. The

product was isolated as an orange coloured oil in 85% yield following column chromatography (eluant petroleum ether : ethyl acetate; 85:15) .

IR ν_{max} (film) 2926 (CHs), 1654 (C=O), 1508, 1458, 1422 (CH_2) cm^{-1}

^1H NMR δ(CD3OD) 1.24 (4H, m, H-1'''', H-4''''), 1.43 (2H, t, J = 6.52Hz, CH_2N), 2.26 (2H, t, J = 6.52Hz, CH_2O), 2.30 (4H, m, H-2 '''', H-3''''), 2.80 (2H, s, CH_2), 5.39-5.48 (2H, d, J = 8.76Hz, H-3'', H-5''), 5.80-6.14 (5H, m, ArH), 6.38-6.52 (2H, dd, J = 8.78Hz, H-2'', H-6'')

^{13}C NMR δ($CDCl_3$) 37.30 (CH_2), 42.54 (CH_2N), 51.07 (C-1'''', C-4''''), 57.58 (CH_2O), 63.83 (C-2'''', C-3''''), 113.00, 113.73 (C-3'', C-5''), 124.67, 124.97 (C-4', C-4''), 126.22, 126.38 (C-2'', C-6''), 126.51-129.13 (C-2', C-3', C-5', C-6'), 160.59 (C=O)

7.2.17. McMurry coupling of substituted 1-phenylethanones

2-[(4-Dimethylaminoethoxy)benzyl]-1,2-diphenylbut-1-ene (108)

The general method outlined 2.9 was applied in the preparation of this compound, using 2-(4-dimethylaminoethoxyphenyl)-1-phenylethanone (0.00032M), dry dioxane (15mL), propiophenone (0.00032M), titanium tetrachloride (0.00065M) and zinc dust (0.0021M) under nitrogen. The crude product was purified using preparative layer chromatography (developing solvent dichloromethane : ethyl acetate : methanol; 50:40:10) to yield a light brown coloured oil in 21% yield.

IR ν_{max} (film) 3120-2919, 2886-2712 (CHs), 1605, 1576 (C=C), 1496, 1451 (NCH_3, CH_2), 1382, 1369 (CH_3), 1282, 1241 (CN) cm^{-1}

^1H NMR δ($CDCl_3$) 1.46 (3H, t, J = 6.86Hz, H-4), 2.07 (6H, s, $N(CH_3)_2$), 2.74 (2H, m, NCH_2), 3.76 (2H, q, J = 6.85Hz, H-3), 3.94 (2H, s, CH_2), 4.14 (2H, m, CH_2O), 6.52-6.56 (2H, m, H-3'', H-5''), 6.83-7.54 (12H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$	14.19 (C-4), 29.68 (C-3), 38.68 (CH_2), 47.43, 47.54 ($N(CH_3)_2$), 58.48 (NCH_2), 60.38 (CH_2O), 113.02, 114.84 (C-3'', C-5''), 125.82, 126.16 (C-4', C-4''), 128.22, 128.43 (C-2'', C-6''), 128.84 (C-2', C-6'), 128.98 (C-3''', C-6''', C-3''', C-5'''), 129.44, 129.76 (C-3', C-5'), 130.87 (C-1''), 130.95 (C-1')
EIMS (HR)	$C_{27}H_{31}NO$: calculated M^+ 385.2409
	observed M^+ 385.2405
Mass Spectrum (m/z)	385 (M^+, 78%), 314 (M^+-71, 5%), 267 (M^+-118, 10%), 252 (M^+-133, 15%), 239 (M^+-146, 11%), 215 (M^+-170, 5%), 191 (M^+-194, 63%) 178 (M^+-207, 45%), 165 (M^+-220, 46%), 128 (M^+-111, 20%), 115 (M^+-270, 35%), 91 (M^+-294, 100%)

2-[(4-Diethylaminoethoxy)benzyl]-1,2-diphenylbut-1-ene (109)

The general method 2.9 was employed using 2-(4-diethylaminoethoxyphenyl)-1-phenylethanone (0.0004M), dry dioxane (15mL), propiophenone (0.0004M), titanium tetrachloride (0.00079M) and zinc dust (0.0025M) under nitrogen. The crude product was isolated using preparative layer chromatography (developing solvent dichloromethane : ethyl acetate : methanol; 50:40:10) to afford a light brown oil in 40% yield.

IR ν_{max} (film)	3059-2924, 2851-2774 (CHs), 1605 (C=C), 1494, 1454 (CH_2), 1374 (CH_3) cm^{-1}
^1H NMR $\delta(CDCl_3)$	0.86 (6H, m, $N(CH_3)_2$), 0.98 (3H, t, J = 7.52Hz, H-4), 2.36 (4H, m, $N(CH_2)_2$), 2.66 (2H, q, J = 7.52Hz, H-3), 2.76 (2H, m, NCH_2), 3.92 (2H, s, CH_2), 4.08 (2H, m, CH_2O), 6.54-6.56 (2H, d, J = 9.00Hz, H-3'', H-5''), 6.80-7.35 (12H, m, ArH)
^{13}C NMR $\delta(CDCl_3)$	sample too insoluble for C^{13} NMR
EIMS (HR)	$C_{29}H_{35}NO$: calculated M^+ 413.2697
	observed M^+ 413.2719
Mass Spectrum (m/z)	413 (M^+, 73%), 385 (M^+-28, 33%), 362 (M^+-51, 17%), 314 (M^+-99, 50%), 283 (M^+-130, 100%), 207

(M^+-206, 53%) 192 (M^+-221, 54%), 178 (M^+-235, 40%), 165 (M^+-248, 78%), 128 (M^+-285, 75%), 91 (M^+-322, 100%)

1,2-Diphenyl-2-[(4-pyrrolidinylethoxy)benzyl]but-1-ene (110)

The general method 2.9 was applied using 2-(4-pyrrolidinylethoxyphenyl)-1-phenylethanone (0.00065M), dry dioxane (20mL), propiophenone (0.00065M), titanium tetrachloride (0.00132M) and zinc dust (0.0042M) under nitrogen. The product was isolated using preparative layer chromatography (developing solvent dichloromethane : ethyl acetate : methanol; 60:40:8) to afford a brown oil in 19% yield.

IR ν_{max} (film) 3058-2928, 2875-2810 (CHs), 1602, 1576 (C=C), 1510 (NCH_2), 1494, 1453 (CH_2), 1375 (CH_3) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 0.90 (3H, t, J = 7.28Hz, H-4), 2.63 (4H, m, H-2'''', H-3''''), 2.82 (2H, q, J =7.04Hz, H-3), 3.68 (4H, m, H-1'''', H-4''''), 3.78 (2H, m, NCH_2), 4.15, 4.17 (2H, d, 2 x s, CH_2), 4.35 (2H, m, CH_2O), 7.25-7.47 (14H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 17.58 (C-4), 24.17 (C-2'''', C-3''''), 29.21 (C-3), 39.50 (CH_2), 47.07 (NCH_2), 63.28 (C-1'''', C-4''''), 66.36 (CH_2O), 116.39 (C-3'', C-5''), 123.69, 124.37 (C-4', C-4''), 125.75, 126.01 (C-2'', C-6''), 126.23, 127.18 (C-3''', C-5''', C-2''', C-6'''), 126.80, 127.18 (C-2', C-6'), 127.45, 128.18 (C-3', C-5')

EIMS (HR) $C_{29}H_{33}NO$: calculated M^+ 411.2525

observed M^+ 411.2562

Mass Spectrum (m/z) 411 (M^+, 73%), 368 (M^+-43, 18%), 326 (M^+-85, 33%), 285 (M^+-126, 40%), 236 (M^+-175, 55%), 191 (M^+-220, 68%), 163 (M^+-248, 75%), 98 (M^+-313, 100%)

1,2-Diphenyl-2-[(4-piperidinylethoxy)benzyl]but-1-ene (111)

The general method 2.9 was employed using 2-(4-piperidinylethoxyphenyl)-1-phenylethanone (0.00062M), dry dioxane (20mL), propiophenone (0.00062M),

titanium tetrachloride (0.0013M) and zinc dust (0.0021M) under nitrogen. The product was purified using column chromatography (eluant dichloromethane : methanol; 97:3) to afford a brown oily gel in 39% yield.

IR ν_{max} (film) 3060-3020, 2927-2853 (CHs), 1601,1586 (C=C), 1511 (NCH_2s), 1494, 1451 (CH_2), 1378 (CH_3) cm^{-1}

^{1}H NMR $\delta(CDCl_3)$ 1.01 (3H, t, J = 7.54Hz, H-4), 2.07 (2H, m, H-3''''), 2.31 (4H, m, H-2'''', H-4''''), 2.58 (2H, q, J = 7.52Hz, H-3), 2.74 (4H, m, H-1'''', H-5''''), 3.03 (2H, m, NCH_2), 3.63 (2H, s, CH_2), 4.30 (2H, m, CH_2O), 6.79-7.18 (12H, m, ArH), 7.92-8.00 (2H, d, J = 9.04Hz, H-2'', H-6'')

^{13}C NMR $\delta(CDCl_3)$ 13.61 (C-4), 23.11 (C-3''''), 26.81, 27.22 (C-2'''', C-4''''), 31.45 (C-3), 40.91 (CH_2), 53.66 (C-1'''', C-5''''), 56.08 (NCH_2), 61.07 (CH_2O), 115.10, 115.24 (C-3'', C-5''), 124.94 (C-4'), 126.37, 126.63 (C-2", C-6"), 128.08, 128.28 (C-3''', C-5''', C-2''', C-6'''), 129.30 (C-3', C-5'), 130.49 (C-1''), 130.74 (C-1')

EIMS (HR) $C_{30}H_{35}NO$: calculated M^+ 425.2732

observed M^+ 425.2719

Mass Spectrum (m/z) 425 (M^+, 72%), 357 (M^+-68, 10%), 326 (M^+-99, 15%), 267 (M^+-158, 19%), 252 (M^+-173, 28%), 191 (M^+-234, 100%), 178 (M^+-247, 98%), 165 (M^+-260, 96%), 112 (M^+-313, 100%), 98 (M^+-327, 100%)

1,2-Diphenyl-2-[(4-morpholinylethoxy)benzyl]but-1-ene (112)

The procedure 2.9 outlined using 2-(4-morpholinylethoxyphenyl)-1-phenylethanone (0.00015M), dry dioxane (15mL), propiophenone (0.00015M), titanium tetrachloride (0.00032M) and zinc dust (0.0008M) under nitrogen. The product was isolated using preparative layer chromatography (developing solvent petroleum ether : ethyl acetate; 90:10) to afford a brown / mustard oil in 29% yield.

IR ν_{max} (film) 3085-3024, 2974-2874 (CHs), 1600 (C=C), 1510, 1492, 1462 (CH_2), 1370 (CH_3) cm^{-1}

^{1}H NMR δ($CDCl_3$)	0.99 (3H, t, J = 7.52Hz, H-4), 2.27 (4H, m, H-1'''', H-4''''), 2.68 (2H, q, J = 7.48Hz, H-3), 2.74 (2H, t, J = 6.26Hz, NCH_2), 3.89 (4H, m, H-2'''', H-3''''), 4.64 (2H, t, J = 6.26Hz, CH_2O), 4.72 (2H,s, CH_2), 6.46-6.48 (2H, d, J = 8.52Hz, H-3'', H-5''), 6.66-7.32 (10H, m, ArH), 7.38-7.40 (2H, d, J = 8.52Hz, H-2'', H-6'')
^{13}C NMR δ($CDCl_3$)	13.34 (C-4), 29.63 (C-3), 39.90 (CH_2), 41.45 (NCH_2), 55.45 (C-1'''', C-4'''', CH_2O), 63.71 (C-2'''', C-3''''), 114.29, 114.86 (C-3'', C-5''), 127.37 (C-2'', C-6''), 127.49, 127.80 (C-3''', C-5''', C-2''', C-6''), 128.05, 128.86 (C-3', C-5')
EIMS (HR)	$C_{29}H_{33}NO_2$: calculated M^+ 427.2517 observed M^+ 427.2511
Mass Spectrum (m/z)	427 (M^+, 73%), 425 (M^+-2, 70%), 386 (M^+-141, 29%), 342 (M^+-85, 15%), 326 (M^+-101, 60%), 314 (M^+-113, 23%), 273 (M^+-154, 23%), 191 (M^+-236, 29%), 165 (M^+-262, 35%), 98 (M^+-329, 100%)

7.2.18. McMurry coupling of 1-(4-alkylylethoxyphenyl)-2-phenylethan-1-one with substituted propiophenone

1-Benzyl-1-[(4-dimethylaminoethoxy)phenyl]-2-phenylhex-1-ene (120)

The general method outlined 2.9 was applied in the preparation of this compound, using 1-(4-dimethylaminoethoxyphenyl)-2-phenylethan-1-one (0.0036M), dry dioxane (30mL), valerophenone (0.0036M), titanium tetrachloride (0.0073M) and zinc dust (0.23M) under nitrogen. The crude product was purified using column chromatography (eluant dichloromethane : methanol; 85: 15) to yield a mustard / brown coloured oily gel in 48% yield.

IR ν_{max} (film)	3029-2950, 2817-2774 (CHs), 1605, 1573 (C=C), 1508, 1453 (CH_2), 1370 (CH_3) cm^{-1}
^{1}H NMR δ($CDCl_3$)	0.91 (3H, m, CH_3), 2.34 (6H, s, $N(CH_3)_2$), 2.40 (2H, t, J = 7.52Hz, CH_2), 2.61 (2H, t, J = 7.54Hz, CH_2), 2.69 (4H, m , CH_2, NCH_2), 3.96 (2H, t, J = 6.02Hz,

CH_2O), 3.99 (2H, s, CH_2), 6.57-6.60 (2H, d, J = 8.52Hz, H-3'', H-5''), 6.85-7.43 (12H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 13.97 (CH_3), 22.75 (C-4), 30.54, (C-2), 34.68 (C-3), 40.16 (CH_2), 45.73 ($N(CH_3)_2$), 58.19 (NCH_2), 65.55 (CH_2O), 113.46 (C-3'', C-5''), 125.14, 125.32 (C-4', C-4''), 126.88 (C-4'''), 127.09, 127.77 (C-3''', C-5''', C-2''', C-6'''), 128.07 (C-2'', C-6''), 129.33 (C-2', C-6', C-3', C-5'), 130.47 (C-1'), 134.76 (C-1''), 137.88 (C-1, C-2), 139.68 (C-1'''),

EIMS (HR) $C_{29}H_{35}NO$: calculated M^+ 413.2719

observed M^+ 413.2709

Mass Spectrum (m/z) 413 (M^+, 73%), 370 (M^+-43, 30%), 342 (M^+-71, 41%), 297 (M^+-1144, 39%), 252 (M^+-161, 46%), 233 (M^+-180, 43%), 215 (M^+-198, 50%), 191 (M^+-222, 69%), 165 (M^+-248, 62%), 139 (M^+-274, 50%), 91 (M^+-322, 100%)

1-Benzyl-4-chloro-1-[(4-dimethylaminoethoxy)phenyl]-2-phenylbut-1-ene (121)

The general method 2.9 was employed using 1-(4-dimethylaminoethoxyphenyl)-2-phenylethan-1-one (0.0036M), dry dioxane (20mL), 3-chloropropiophenone (0.0036M), titanium tetrachloride (0.0073M) and zinc dust (0.023M) under nitrogen. The crude product was isolated using column chromatography (eluant dichloromethane : methanol; 85:15) to afford a light brown oil in 41% yield.

IR ν_{max} (film) 3025-2941, 2930-2821 (CHs), 1605, 1574 (C=C), 1508, 1453 (CH_2), 1368 (CH_3), 699 (C-Cl) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 2.34 (6H, m, $N(CH_3)_2$), 2.78 (2H, t, J = 6.04Hz, NCH_2), 3.18 (2H, t, J = 7.53Hz, CH_2), 3.49 (2H, t, J = 7.28Hz, CH_2Cl), 3.95 (2H, t, J = 5.80Hz, CH_2O), 4.05 (2H, s, CH_2), 6.60-6.62 (2H, d, J = 8.52Hz, H-3'', H-5''), 6.88-7.45 (12H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 37.43 (CH_2), 39.99 (CH_2Cl), 42.16 (CH_2), 45.42 (($CH_3)_2$), 57.83, 57.89 (NCH_2), 65.27, 65.44 (CH_2O), 113.21, 113.69 (C-3'', C-5''), 125.57,

125.78 (C-4', C-4''), 127.48 (C-4'''), 127.53, 127.93 (C-2''', C-6''', C-3''', C-5'''), 128.03, 128.11 (C-2'', C-6''), 128.46, 128.52 (C-2', C-6'), 129.31, 129.64 (C-3', C-5'), 130.37 (C-1'), 133.18 (C-1''), 139.93 (C-1'''),

EIMS (HR) $C_{27}H_{30}ClNO$: calculated M^+ 419.1998

observed M^+ 419.2016

Mass Spectrum (m/z) 419 (M^+, 56%), 383 (M^+-36, 15%), 279 (M^+-40, 29%), 252 (M^+-167, 47%), 215 (M^+-204, 44%), 202 (M^+-217, 79%), 191 (M^+-228, 80%), 165 (M^+-254, 78%), 91 (M^+-328, 100%)

1-Benzyl-3-bromo-1-[(4-dimethylaminoethoxy)phenyl]-2-phenylprop-1-ene (122)

The general method 2.9 was employed using 1-(4-dimethylaminoethoxyphenyl)-2-phenylethan-1-one (0.0036M), dry dioxane (20mL), 2-bromoacetophenone (0.0036M), titanium tetrachloride (0.0073M) and zinc dust (0.023M) under nitrogen. The product was purified using column chromatography (eluant dichloromethane : methanol, 85:15) to afford a brown gel in 39% yield.

IR ν_{max} (film) 3028-2968, 2821-2770 (CHs), 1605 (C=C), 1453 (CH_2), 1370 (CH_3), 700 (C-Br) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 2.36 (6H, s, $N(CH_3)_2$), 2.74 (2H, m, NCH_2), 3.99 (2H, s, CH_2), 4.04 (2H, m, CH_2O), 4.11 (2H, m, CH_2Br), 6.62-6.68 (2H, d, J = 16.00Hz, H-3'', H-5''), 6.70-7.45 (12H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 35.72 (CH_2Br), 40.45 (CH_2), 45.33 ($(CH_3)_2$), 57.78 (NCH_2), 65.19, 65.35 (CH_2O), 113.20, 113.67 (C-3'', C-5''), 125.26, 126.40 (C-4', C-4''), 126.16 (C-4'''), 127.25-127.84 (C-2''', C-6''', C-3''', C-5'''), 128.11 (C-2'', C-6''), 128.51 (C-2', C-6'), 128.78, 129.23 (C-3', C-5'), 130.27 (C-1'), 134.40 (C-1"), 139.53 (C-1''')

EIMS (HR) $C_{26}H_{28}BrNO$: calculated M^+ 449.1510

observed M^+ 449.1354

Mass Spectrum (m/z) 449 (M^+, 38%), 371 (M^+-78, 95%), 269 (M^+-180, 25%), 252 (M^+-197, 38%), 221 (M^+-228, 28%), 191 (M^+-258, 92%), 165 (M^+-284, 91%), 115 (M^+-334, 84%), 91 (M^+-358, 100%) 79 (M^+-370, 100%)

1-Benzyl-3-bromo-1-[(4-dimethylaminoethoxy)phenyl]-2-phenylbut-1-ene (123)

The general method 2.9 was employed using 1-(4-dimethylaminoethoxyphenyl)-2-phenylethan-1-one (0.0036M), dry dioxane (20mL), 2-bromopropiophenone (0.0036M), titanium tetrachloride (0.0073M) and zinc dust (0.023M) under nitrogen. The product was purified using column chromatography (eluant dichloromethane : methanol; 90:10) to afford a mustard coloured gel in 34% yield.

IR ν_{max} (film) 3028-2941, 2822-2771 (CHs), 1605 (C=C), 1509, 1447 (CH_2), 1369 (CH_3), 700 (C-Br) cm^{-1}

^{1}H NMR $\delta(CDCl_3)$ 2.35 (3H, d, J = 5.00Hz, CH_3), 2.40 (6H, s, $N(CH_3)_2$), 2.70 (2H, t, J = 5.94Hz, NCH_2), 2.79 (2H, m, CHBr), 3.95 (2H, s, CH_2), 4.11 (2H, t, J = 6.02Hz, CH_2O), 6.57-7.34 (14H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 9.47 (CHBr), 12.94 (CH_3), 39.99 (CH_2), 45.72, 45.81 ($(CH_3)_2$), 58.18 (NCH_2), 65.55 (CH_2O), 113.45, 114.05 (C-3'', C-5''), 125.59 (C-4', C-4''), 127.19 (C-4'''), 127.52, 127.92 (C-2''', C-6''', C-3''', C-5'''), 128.19 (C-2'', C-6''), 128.44 (C-2', C-6'), 129.31, 129.76 (C-3', C-5'), 130.79 (C-1'), 134.19 (C-1''), 139.69 (C-1''')

EIMS (HR) $C_{27}H_{30}NOBr$: calculated M^+ 463.1999

observed M^+ 463.1511

Mass Spectrum (m/z) 463 (M^+, 38%), 385 (M^+-78, 92%), 314 (M^+-149, 65%), 297 (M^+-166, 12%), 269 (M^+-194, 27%), 252 (M^+-211, 34%), 235 (M^+-228, 22%), 215 (M^+-248, 23%), 191 (M^+-272, 91%), 165 (M^+-298, 80%), 115

(M^+-348, 81%), 91 (M^+-372, 100%), 79 (M^+-384, 100%)

1-Benzyl-1-[(4-dimethylaminoethoxy)phenyl]-3-nitro-2-phenylprop-1-ene (124)

The general method outlined 2.9 was employed using 1-(4-dimethylaminoethoxyphenyl)-2-phenylethan-1-one (0.0036M), dry dioxane (30mL), benzoylnitromethane (0.0036M), titanium tetrachloride (0.0073M) and zinc dust (0.23M) under nitrogen. The product was isolated using column chromatography (eluant dichloromethane : methanol; 85: 15) to yield a yellow oily gel in 42% yield.

IR ν_{max} (film) 3010-2930, 2811-2770 (CHs), 1605 (C=C), 1511, 1454 (CH_2), 1368 (CH_3), 1244 (C-NO_2) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 2.30 (6H, s, $N(CH_3)_2$), 2.69 (4H, t, J = 5.84Hz, NCH_2), 2.83 (2H, s, CH_2NO_2), 4.00 (2H, t, J = 5.86Hz, CH_2O), 4.04 (2H, s, CH_2), 6.77-6.80 (2H, d, J = 7.80Hz, H-3'', H-5''), 6.85-7.18 (12H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 36.98 (CH_2NO_2), 37.71 (CH_2), 45.35 ($N(CH_3)_2$), 57.85 (NCH_2), 65.51 (CH_2O), 114.06, 114.41 (C-3'', C-5''), 125.42 (C-4', C-4''), 125.84 (C-4'''), 127.27, 127.78 (C-3''', C-5''', C-2''', C-6'''), 128.81, 127.86 (C-2'', C-6''), 128.04, 128.20 (C-2', C-6'), 128.88 (C-3', C-5'), 130.47 (C-1'), 133.59 (C-1''), 141.40 (C-1''')

EIMS (HR) $C_{26}H_{28}N_2O_3$: calculated M^+ 416.2010

observed M^+ 416.1999

Mass Spectrum (m/z) 416 (M^+, 71%), 397 (M^+-19, 84%), 374 (M^+-42, 85%), 344 (M^+-72, 100%), 324 (M^+-92, 68%), 269 (M^+-147, 100%), 252 (M^+-164, 65%), 197 (M^+-219, 54%), 178 (M^+-238, 100%), 165 (M^+-251, 100%), 149 (M^+-267, 91%), 91 (M^+-325, 100%)

1-Benzyl-1-[(4-dimethylaminoethoxy)phenyl]-2-phenylpent-1-ene (125)

The general method 2.9 outlined was applied in the preparation of this compound, using 1-(4-dimethylaminoethoxyphenyl)-2-phenylethan-1-one (0.0036M), dry dioxane (30mL), butyrophenone (0.0036M), titanium tetrachloride (0.0073M) and zinc dust (0.23M) under nitrogen. The crude product was isolated using column chromatography (eluant dichloromethane : methanol; 90:10) to yield a brown gel in 46% yield.

IR ν_{max} (film)	3026-2957, 2830-2782 (CHs), 1603, 1582 (C=C), 1511, 1454 (CH_2), 1377 (CH_3) cm^{-1}
^1H NMR $\delta(CDCl_3)$	1.00 (3H, m, CH_3), 2.32 (6H, s, $N(CH_3)_2$), 2.74 (2H, t, J = 6.50Hz, CH_2), 2.95 (4H, t, J = 5.52Hz, NCH_2), 3.96 (2H, t, J = 5.52Hz, CH_2O), 4.22 (2H, s, CH_2), 4.68 (2H, t, J = 6.48Hz, CH_2), 6.81-7.54 (14H, m, ArH)
^{13}C NMR $\delta(CDCl_3)$	13.68 (CH_3), 36.62 (CH_2[3]), 37.75 (CH_2[2]), 41.05 (CH_2), 45.06 ($N(CH_3)_2$), 57.56 (NCH_2), 65.24 (CH_2O), 114.10, 115.41 (C-3'', C-5''), 125.63 (C-4', C-4''), 126.79 (C-4'''), 127.87, 127.92 (C-3''', C-5''', C-2''', C-6'''), 128.10, 128.26 (C-2'', C-6''), 128.82, 128.96 (C-2', C-6'), 129.03, 129.41 (C-3', C-5'), 130.49 (C-1'),
EIMS (HR)	$C_{28}H_{33}NO$: calculated M^+ 399.2568 observed M^+ 399.2562
Mass Spectrum (m/z)	399 (M^+, 73%), 354 (M^+-45, 39%), 328 (M^+-71, 11%), 273 (M^+-126, 58%), 253 (M^+-146, 50%), 239 (M^+-160, 19%), 203 (M^+-196, 49%), 191 (M^+-208, 81%) 178 (M^+-221, 79%), 165 (M^+-234, 58%), 128 (M^+-271, 30%), 91 (M^+-308, 100%)

1-Benzyl-3-chloro-1-[(4-dimethylaminoethoxy)phenyl]-2-phenylprop-1-ene (126)

The general method 2.9 was employed using 1-(4-dimethylaminoethoxyphenyl)-2-phenylethan-1-one (0.0035M), dry dioxane (20mL), 2-chloroacetophenone (0.0035M), titanium tetrachloride (0.0072M) and zinc dust (0.023M) under

nitrogen. The product was purified using column chromatography (eluant dichloromethane : ethyl acetate : methanol, 70:24:6) to afford a light brown oily gel in 44% yield.

IR ν_{max} (film)	3058-2940, 2866-2771 (CHs), 1607, 1581 (C=C), 1510, 1494, 1454 (CH_2), 1371 (CH_3), 1352 (CH_2Cl) cm^{-1}
^1H NMR $\delta(CDCl_3)$	2.39 (6H, s, $N(CH_3)_2$), 2.79 (2H, m, NCH_2), 3.68 (2H, m, CH_2Cl), 4.11-4.18 (4H, m, CH_2O, CH_2), 6.59-6.68 (2H, d, J = 8.52Hz, H-3'', H-5''), 6.72-7.38 (12H, m, ArH)
^{13}C NMR $\delta(CDCl_3)$	40.45 (CH_2Cl), 43.26 (CH_2), 45.38 ($(CH_3)_2$), 57.83 (NCH_2), 65.55 (CH_2O), 113.18, 114.04 (C-3'', C-5''), 125.25, 125.39 (C-4', C-4''), 126.51 (C-4'''), 126.85-127.81 (C-2''', C-6''', C-3''', C-5'''), 127.91, 127.94 (C-2'', C-6''), 128.08, 128.76 (C-2', C-6'), 129.08, 129.34 (C-3', C-5'), 130.25 (C-1'), 134.50 (C-1''), 140.23 (C-1''')
EIMS (HR)	$C_{26}H_{28}ClNO$: calculated M^+ 405.1876 observed M^+ 405.1859
Mass Spectrum (m/z)	405 (M^+, 57%), 371 (M^+-34, 100%), 355 (M^+-40, 72%), 300 (M^+-105, 97%), 285 (M^+-120, 35%), 267 (M^+-138, 89%), 252 (M^+-153, 100%), 191 (M^+-214, 100%) 178 (M^+-227, 98%), 165 (M^+-240, 100%), 139 (M^+-266, 91%), 91 (M^+-314, 100%)

7.3. Experimental details - benzoxepins conformationally restrained analogues

7.3.1. Preparation of benzoxepins

2,3,4,5-Tetrahydro-1-benzoxepin-5-one (139)

Method 1

A solution of xylene (180mL), phosphorus pentoxide (0.2M), celite (24g) and 85% phosphoric acid (0.06M) were heated to 95-100°C for 3hr. Post this period a solution of 4-phenoxybutyric acid (0.05M) in xylene was added dropwise over 1 hour at 95-100°C. Once the addition was complete the solution was stirred at 95-100°C for a further 6hr. The reaction mixture was then cooled to room temperature and poured into crushed ice (100g). When the ice melts two layers were apparent. The xylene layer was separated, washed with water, saturated NaCl, extracted with diethyl ether and dried over Na_2SO_4. The aqueous layer was washed, extracted with ethyl acetate and dried over Na_2SO_4. The xylene and ethyl acetate layers were combined and the solvents were evaporated under reduced pressure. Column chromatography (eluant petroleum ether : ethyl acetate; 84:16), was carried out to purify the compound as a mustard coloured oily gel[342] in 49% yield.

Method 2

A mixture of polyphosphoric acid (0.84M) and 4-phenoxybutyric acid (0.11M) were heated on a steam bath for 2-3hr with occasional shaking. Post this period the wine coloured syrup was poured onto crushed ice, and the organic layer was extracted with ethyl acetate, washed with water, 3N NaOH (2x20mL), dried over Na_2SO_4 and concentrated down. The product was isolated from column chromatography (eluant dichloromethane : petroleum ether; 50:50) as a mustard / brown oily gel[347] in 76% yield

IR ν_{max} (KBr)	3080-2943, 2939-2834 (CHs), 1689 (C=O), 1604 (C=C), 1480, 1449 (CH_2), 1289 (C-O-C) cm^{-1}
^1H NMR $\delta(CDCl_3)$	2.56 (2H, m, H-3), 2.93 (2H, t, J = 6.99Hz, H-2), 4.27 (2H, t, J = 6.55Hz, H-4), 6.95-7.14 (2H, m, H-7, H-9), 7.45 (1H, m, H-8), 7.79-8.81 (2H, dd, J = 7.76, 1.78Hz, H-6)

^{13}C NMR δ($CDCl_3$) 26.33 (C-3), 40.61(C-4), 72.80 (C-2), 121.75, 122.74 (C-7, C-8), 129.33 (C-9a), 129.42 (C-6), 133.69 (C-9), 161.62 (C-5a), 200.21 (C-5)

7.3.2. Synthesis of 5-aryl-2,3-dihydro-1-benzoxepins

5-(4-Methoxyphenyl)-2,3-dihydro-1-benzoxepin (145)

The general method 3.2 was carried out using *n*-BuLi (0.03M), 4-bromoanisole (0.03M), dry THF (40mL) and 2,3,4,5-tetrahydro-1-benzoxepin-5-one (0.01M) under nitrogen at -78°C. The compound was dehydrated with H_2SO_4 (4mL) in ethanol (20mL). The product was then purified using column chromatography (eluant petroleum ether : ethyl acetate; 85:15) and recrystallised from ethanol to afford colourless crystals[6] in 55% yield; m.p. 79-81°C

IR ν_{max} (film) 3068-2963, 2878 (CHs), 1599, 1571 (C=C), 1464 (CH_2), 1448 (OCH_3), 1287, 1219 (C-O-C) cm^{-1}

^{1}H NMR δ($CDCl_3$) 2.50 (2H, q, J = 6.01Hz, H-3), 3.85 (3H, s, OCH_3), 4.52 (2H, t, J = 6.02Hz, H-2), 6.28 (1H, t, J = 6.28Hz, H-4), 6.88-6.90 (2H,m, H-3', H-5'), 7.01-7.03 (2H, m, H-6, H-7), 7.11-7.14 (1H, m, H-9), 7.19-7.28 (3H, m, H-2', H-6', H-8)

^{13}C NMR δ($CDCl_3$) 29.45 (C-3), 54.82 (OCH_3), 77.28 (C-2), 113.10 (C-3', C-5'), 121.37 (C-9), 122.66 (C-7), 126.58 (C-4), 127.90 (C-8), 129.20 (C-2', C-6'), 130.66 (C-6), 132.49 (C-5), 134.87 (C-1'), 157.46 (C-9a)

Elemental Analysis $C_{17}H_{16}O_2$: requires C, 80.92; H, 6.40

found C, 80.90; H, 6.46

7.3.3. Synthesis of 5-aryl-4-bromo-2,3-dihydro-1-benzoxepin

4-Bromo-5-(4-methoxyphenyl)-2,3-dihydro-1-benzoxepin (146)

5-(4-Methoxyphenyl)-2,3-dihydro-1-benzoxepin (0.004M) was dissolved in dry dichloromethane (30mL) and allowed to stir at 0°C for 30minutes. Pyridinium bromide perbromide (0.0057M) was added in portions over 15minutes. The solution was allowed to stir for 12hr after which TLC monitoring indicated the

complete consumption of the starting material. The solution was washed with $NaHCO_3$ (10% w/v, 2x20mL), water (30mL), extracted with dichloromethane (2x25mL), dried over Na_2SO_4 and concentrated down. Column chromatography (eluant petroleum ether : ethyl acetate; 97:3) was carried out to purify the product followed by recrystallisation with ethanol to produce needle-like colourless crystals[256] in 92% yield.

IR ν_{max} (KBr)	3067-2970, 2878 (CHs), 1599, 1568 (C=C), 1476 (CH_2), 1448 (OCH_3), 1286, 1202 (C-O-C) cm^{-1}
^1H NMR $\delta(CDCl_3)$	3.04 (2H, t, J = 5.86Hz, H-3), 3.86 (3H, s, OCH_3), 4.61 (2H, t, J = 5.88Hz, H-2), 6.80-6.82 (1H,dd, J = 7.80, 1.70Hz, H-6), 6.91-6.98 (3H, m, H-7, H-3', H-5'), 7.08-7.10 (2H, d, J = 8.32Hz, H-9,), 7.18-7.22 (3H, m, H-2', H-6', H-8)
^{13}C NMR $\delta(CDCl_3)$	40.74 (C-3), 54.73 (OCH_3), 77.24 (C-2), 113.01 (C-3', C-5'), 121.51 (C-4), 122.92 (C-7, C-9), 126.36 (C-6), 130.69 (C-2', C-6'), 130.96 (C-8), 133.06 (C-5), 134.35 (C-1'), 139.01 (C-5a)
Elemental Analysis	$C_{17}H_{15}O_2Br$: requires C, 61.65; H, 4.56 found C, 61.20; H, 4.56

7.3.4. Preparation of 5-aryl-4-phenyl-2,3-dihydro-1-benzoxepin

5-(4-Methoxyphenyl)-4-phenyl-2,3-dihydro-1-benzoxepin (150)

Method 1

A solution of phenyl zinc chloride was prepared by addition of phenyl lithium (1.1mL of a 1.8M in hexane / ether, 0.002M) to a stirred solution of zinc chloride (0.002M), dry THF (4mL) under nitrogen at 0°C. This was allowed to stir for 1 hour at room temperature. To this was added a solution of 4-bromo-5-(4-methoxyphenyl)-2,3-dihydro-1-benzoxepin (0.0007M) in dry THF (6mL) containing tetrakis(triphenylphosphine)palladium (40mgs). The mixture was then heated to reflux and maintained at same for 4-5hours. After this period it was cooled to room temperature and let stir overnight. The reaction mixture was washed with 1N HCl (30mL), water, extracted with diethyl ether (3x20mL), dried over sodium sulphate and concentrated. Column chromatography (eluant petroleum ether : dichloromethane; 80:20) was necessary to purify the product

followed by recrystallisation from ethanol to afford colourless crystals in 89% yield.

IR ν_{max} (KBr) 3115-2930, 2810-2723 (CHs), 1608, 1580 (C=C), 1482, 1458 (CH_2), 1442 (OCH_3), 1246, 1217 (C-O-C) cm^{-1}

^1H NMR $\delta(CD_3OD)$ 2.74 (2H, t, J = 6.28Hz, H-3), 3.76 (3H, s, OCH_3), 4.55 (2H, t, J = 6.04Hz, H-2), 6.66-6.68 (2H, dd, J = 6.52, 2.04Hz, H-3', H-5'), 6.90-6.92 (2H, dd, J = 6.50, 2.02Hz, H-2', H-6'), 6.98-7.02 (1H, dt, J = 8.28, 1.50Hz, H-9), 7.08-7.24 (8H, m, ArH)

^{13}C NMR $\delta(CD_3OD)$ 35.72 (C-3), 55.07 (OCH_3), 80.44 (C-2), 113.07 (C-3', C-5'), 122.06 (C-4, C-9), 123.49 (C-7), 126.28 (C-6), 127.97 (C-4''), 128.39 (C-2'', C-6''), 129.50 (C-3'', C-5''), 131.02 (C-2', C-6'), 132.52 (C-8), 133.81 (C-5), 156.17 (C-9a), 158.16 (C-4')

Elemental Analysis $C_{23}H_{20}O_2$: requires C, 84.11; H, 6.14

found C, 83.89; H, 6.27

Method 2

To a stirred solution of 4-bromo-5-(4-methoxyphenyl)-2,3-dihydro-1-benzoxepin (0.006M) in dry THF (10mL) was added $Pd(PPh_3)_4$ (40mgs) and let stir for 10-15min prior to addition of phenylboronic acid (0.0021M) followed by aqueous sodium carbonate (2M, 0.0024M). The mixture was heated by an oil bath at 80°C for 4hr and then cooled to room temp and let stir for 10-12hr. Post this period the mixture was poured onto water (40mL), washed with brine, extracted with dichloromethane and dried over $MgSO_4$ and concentrated. The product was isolated using column chromatography (eluant dichloromethane : petroleum ether; 30:70) as a lemon coloured oil in 96% yield with the following physical properties.

IR ν_{max} (film) 2990-2920, 2897-2850 (CHs), 1610 (C=C), 1483, 1465 (CH_2), 1441 (OCH_3), 1245, 1217 (C-O-C) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 2.76 (2H, t, J = 6.02Hz, H-3), 3.77 (3H, s, OCH_3), 4.66 (2H, t, J = 6.02Hz, H-2), 6.68-6.70 (2H,dd, J = 6.52, 2.00Hz, H-3', H-5'), 6.93-6.95 (2H, dd, J =

6.52, 2.00Hz, H-2', H-6'), 6.98-7.02 (1H, dt, J = 7.54, 1.50Hz, H-9), 7.16-7.50 (8H, m, ArH)

^{13}C NMR δ($CDCl_3$) 35.30 (C-3), 55.61 (OCH_3), 79.99 (C-2), 112.64 (C-3', C-5'), 121.63 (C-4, C-9), 123.06 (C-7), 125.85 (C-6), 126.71 (C-4''), 127.53, 127.96 (C-2'', C-6''), 128.30 (C-3'', C-5''), 130.59 (C-2', C-6'), 132.08 (C-8), 133.37 (C-5), 155.75 (C-9a), 157.74 (C-4')

EIMS (LR) $C_{23}H_{20}O_2$: calculated M^+ 328

observed M^+ 328

7.3.5. Demethylation of 5-(4-methoxyphenyl)-4-phenyl-2,3-dihydro-1-benzoxepin

5-(4-Hydroxyphenyl)-4-phenyl-2,3-dihydro-1-benzoxepin (151)

A mixture of 5-(4-methoxyphenyl)-4-phenyl-2,3-dihydro-1-benzoxepin (0.0017M) and pyridine hydrochloride (0.0096M) were stirred and heated by means of an oil bath maintained at 200°C. After 4hr, the mixture was cooled to ambient temperature and dissolved in dichloromethane (10mL). This solution was diluted with diethyl ether (2x20mL), washed with dilute HCl (1M, 15mL), water (2x25mL), dried with magnesium sulphate and concentrated. The product was isolated using column chromatography (eluant dichloromethane : ethyl acetate; 98:2) to afford brown oil in 61% yield.

IR ν_{max} (film) 3339 (OH), 3056-2961, 2921-2855 (CHs), 1609 (C=C), 1484, 1443 (CH_2), 1255, 1217 (C-O-C) cm^{-1}

^{1}H NMR δ($CDCl_3$) 2.74 (2H, t, J = 6.02Hz, H-3), 4.64 (2H, t, J = 6.26Hz, H-2), 6.68-6.70 (2H, dd, J =8.52, 2.00Hz, H-3', H-5'), 6.93-6.95 (2H, dd, J = 8.52, 2.00Hz, H-2', H-6'), 6.98-7.02 (1H, dt, J = 7.54, 1.25Hz, H-9), 7.14-7.28 (8H, m, ArH)

^{13}C NMR δ($CDCl_3$) 35.23 (C-3), 79.97 (C-2), 114.13 (C-3', C-5'), 121.60 (C-4, C-9), 123.07 (C-7), 125.84 (C-6), 126.50 (C-4''), 127.96 (C-2'', C-6''), 129.03 (C-3'', C-5''), 130.53 (C-2', C-6'), 132.25 (C-8)

EIMS (LR)	$C_{22}H_{18}O_2$: calculated M^+ 314
	observed M^+ 314
Mass Spectrum (m/z)	314 (M^+, 100%), 298 (M^+-16, 2%), 286 (M^+-28, 19%), 238 (M^+-76, 6%), 221 (M^+-93, 4%), 211 (M^+-103, 52%), 183 (M^+-131, 38%) 165 (M^+-149, 16%), 152 (M^+-162, 8%), 119 (M^+-195, 9%)

7.3.6. Alkylation of 5-(4-hydroxyphenyl)-4-phenyl-2,3-dihydro-1-benzoxepin

5-[(4-Dimethylaminoethoxy)phenyl]-4-phenyl-2,3-dihydro-1-benzoxepin (152)

The general method 2.12 was applied in the preparation of this compound using 5-(4-hydroxyphenyl)-2,3-dihydro-4-phenyl-1-benzoxepin (0.00048M), dry acetone (20mL), anhydrous K_2CO_3 (0.0048M) and 2-dimethylaminoethylchloride hydrogenchloride (0.0013M) under nitrogen. The reaction mixture was refluxed for 6hr. The product was purified using column chromatography (eluant dichloromethane : ethyl acetate : methanol; 70:30:2), to afford a light brown / mustard coloured oil in 44% yield.

IR ν_{max} (film)	3003-2926, 2863-2774 (CHs), 1608, 1591 (C=C), 1483, 1462 (CH_2), 1441 (CH_3), 1172 (C-O-C) cm^{-1}
^{1}H NMR $\delta(CDCl_3)$	2.35 (6H, s, $N(CH_3)_2$), 2.72 (4H, m, CH_2N, H-3), 4.02 (2H, t, J = 5.78Hz, CH_2O), 4.64 (2H, t, J = 6.02Hz, H-2), 6.67-6.69 (2H, dd, J = 9.04Hz, H-3', H-5'), 6.88-7.02 (3H, m, H-2', H-6', H-9), 7.04-7.28 (8H, m, ArH)
^{13}C NMR $\delta(CDCl_3)$	35.25 (C-3), 45.42 ($N(CH_3)_2$), 57.85 (NCH_2), 65.37 (CH_2O), 79.97 (C-2), 113.24, 113.32 (C-3', C-5'), 121.59 (C-4, C-9), 123.02 (C-7), 125.81 (C-6), 127.49 (C-4"), 127.92, 128.14 (C-2'', C-6''), 129.04 (C-3'', C-5''), 130.57 (C-2', C-6'), 132.01 (C-8)
EIMS (HR)	$C_{26}H_{27}NO_2$: calculated M^+ 385.2050
	observed M^+ 385.2042

Mass Spectrum (m/z) 385 (M^+, 100%), 327 (M^+-58, 3%), 314 (M^+-71, 7%), 298 (M^+-87, 3%), 281 (M^+-104, 19%), 265 (M^+-120, 16%), 252 (M^+-133, 31%), 239 (M^+-146, 41%) 202 (M^+-183, 22%), 165 (M^+-220, 34%)

5-[(4-Diethylaminoethoxy)phenyl]-4-phenyl-2,3-dihydro-1-benzoxepin (153)

The general method 2.12 was employed using 5-(4-hydroxyphenyl)-4-phenyl-2,3-dihydro-1-benzoxepin (0.00049M), dry acetone (25mL), anhydrous K_2CO_3 (0.0049M) and 2-diethylaminoethyl chloride hydrogenchloride (0.0013M) under nitrogen. The product was isolated using column chromatography (eluant dichloromethane : ethyl acetate : methanol; 60:40:10), to afford a light brown coloured oil in 66% yield, with the following physical properties.

IR ν_{max} (film) 3058-3026, 2967-2813 (CHs), 1606, 1572 (C=C), 1508, 1481 (CH_2), 1442 (CH_3), 1175 (C-O-C) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 1.10 (3H, t, J = 7.28Hz, $(CH_3)_2$), 2.66-2.75 (6H, m, $(CH_2)_2$, CH_2N), 2.90 (2H, t, J = 6.26Hz, H-3), 4.02 (2H, t, J = 6.02Hz, CH_2O), 4.64 (2H, t, J = 6.02Hz, H-2), 6.66-6.68 (2H,d, J = 9.04Hz, H-3', H-5'), 6.89-7.05 (3H, m, H-2', H-6', H-9), 7.13-7.29 (8H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 11.20 ($(CH_3)_2$), 35.26 (C-3), 47.36 ($(CH_2)_2$), 51.19 (NCH_2), 65.69 (CH_2O), 79.96 (C-2), 113.23 (C-3', C-5'), 121.60 (C-4, C-9), 123.03 (C-7), 125.82 (C-6), 127.51 (C-4''), 127.94 (C-2'', C-6''), 129.04 (C-3'', C-5''), 130.57 (C-2', C-6'), 132.05 (C-8)

EIMS (HR) $C_{28}H_{31}NO_2$: calculated M^+ 413.2355

observed M^+ 413.2355

Mass Spectrum (m/z) 413 (M^+, 38%), 398 (M^+-15, 23%), 340 (M^+-73, 2%), 326 (M^+-87, 2%), 281 (M^+-132, 10%), 265 (M^+-148, 9%), 252 (M^+-161, 16%), 239 (M^+-174, 23%) 226 (M^+-187, 10%), 165 (M^+-248, 26%), 100 (M^+-313, 100%)

5-[(4-Morpholinylethoxy)phenyl]-4-phenyl-2,3-dihydro-1-benzoxepin (154)

The general method 2.12 was employed using 5-(4-hydroxyphenyl)-4-phenyl-2,3-dihydro-1-benzoxepin (0.00016M), dry acetone (20mL), anhydrous K_2CO_3 (0.0016M) and 4-(2-chloroethyl)morpholine hydrogenchloride (0.00041M) under nitrogen. The product was isolated using column chromatography (eluant dichloromethane : ethyl acetate; 80:20), to afford a light brown coloured oil in 78% yield, with the following physical properties.

IR ν_{max} (film)	3059-2926, 2856 (CHs), 1607, 1592 (C=C), 1509, 1483, 1453 (CH_2), 1442 (CH_3), 1144 (C-O-C) cm^{-1}
^1H NMR $\delta(CDCl_3)$	2.55 (2H, t, J = 5.50Hz, CH_2N), 2.74 (6H, m, H-1'''', H-4'''', H-3), 3.60 (4H, m, H-2'''', H-3''''), 4.05 (2H, t, J = 5.52Hz, CH_2O), 4.63 (2H, t, J = 6.02Hz, H-2), 6.64-6.67 (2H, d, J = 8.52Hz, H-3', H-5'), 6.87-7.03 (3H, m, H-2', H-6', H-9), 7.12-7.27 (8H, m, ArH)
^{13}C NMR $\delta(CDCl_3)$	35.26 (C-3), 53.11, 53.65 (C-1'''', C-4''''), 57.25 (NCH_2), 65.22 (CH_2O), 66.38, 66.46 (C-2'''', C-3''''), 79.95 (C-2), 113.26 (C-3', C-5'), 121.60 (C-4, C-9), 123.00 (C-7), 125.81 (C-6), 127.49 (C-4''), 127.94 (C-2'', C-6''), 129.02 (C-3'', C-5''), 130.53 (C-2', C-6'), 132.04 (C-8)
EIMS (HR)	$C_{28}H_{29}NO_3$: calculated M^+ 427.2158 observed M^+ 427.2147
Mass Spectrum (m/z)	427 (M^+, 42%), 369 (M^+-58, 1%), 321 (M^+-106, 2%), 297 (M^+-130, 2%), 281 (M^+-146, 6%), 252 (M^+-175, 10%), 239 (M^+-188, 11%), 215 (M^+-212, 3%) 178 (M^+-249, 5%), 165 (M^+-262, 14%), 114 (M^+-313, 100%)

4-Phenyl-5-[(4-piperidinylethoxy)phenyl]-2,3-dihydro-1-benzoxepin (155)

The general method 2.12 was applied using 5-(4-hydroxyphenyl)-4-phenyl-2,3-dihydro-1-benzoxepin (0.00019M), dry acetone (25mL), anhydrous K_2CO_3 (0.0019M) and 1-(2-chloroethyl)piperidine monohydrochloride (0.0005M) under nitrogen. The product was purified using flash column chromatography (eluant

dichloromethane : ethyl acetate :methanol; 60:40:2), to afford a light brown coloured oil in 52% yield.

IR ν_{max} (film) 3063-2923, 2848-2782 (CHs), 1607, 1569 (C=C), 1484, 1462, 1440 (CH_2), 1172 (C-O-C) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 1.47 (2H, m, H-3''''), 1.63 (4H, m, H-2'''', H-4''''), 2.53 (4H, m, H-1'''', H-5''''), 2.73 (2H, t, J = 6.02Hz, CH_2N), 2.77 (2H, t, J = 6.02Hz, H-3), 4.06 (2H, t, J = 6.02Hz, CH_2O), 4.63 (2H, t, J = 6.28Hz, H-2), 6.64-6.67 (2H, d, J = 9.04Hz, H-3', H-5'), 6.87-7.04 (3H, m, H-2', H-6', H-9), 7.13-7.28 (8H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 23.48 (C-3''''), 25.09 (C-2'''', C-4''''), 35.25 (C-3), 54.44 (C-1'''', C-5''''), 57.31 (NCH_2), 65.00 (CH_2O), 79.96 (C-2), 113.26 (C-3', C-5'), 121.59 (C-4, C-9), 123.01 (C-7), 125.82 (C-6), 127.50 (C-4''), 127.92 (C-2'', C-6''), 129.02 (C-3'', C-5''), 130.55, 130.69 (C-2', C-6'), 132.04 (C-8)

EIMS (HR) $C_{29}H_{31}NO_2$: calculated M^+ 425.2341

observed M^+ 425.2355

Mass Spectrum (m/z) 425 (M^+, 50%), 281 (M^+-144, 20%), 265 (M^+-160, 10%), 252 (M^+-173, 40%), 239 (M^+-186, 40%), 226 (M^+-199, 10%), 189 (M^+-236, 30%) 165 (M^+-260, 30%), 112 (M^+-313, 100%)

4-Phenyl-5-[(4-pyrrolidinylethoxy)phenyl]-2,3-dihydro-1-benzoxepin (156)

The general method 2.12 was employed using 5-(4-hydroxyphenyl)-4-phenyl-2,3-dihydro-1-benzoxepin (0.00023M), dry acetone (25mL), anhydrous K_2CO_3 (0.0023M) and 1-(2-chloroethyl)pyrrolidine hydrochloride (0.00059M) under nitrogen. Column chromatography (eluant dichloromethane : ethyl acetate :methanol; 90:10:2), was used to isolate the product as a light brown / mustard coloured oil in 38% yield.

IR ν_{max} (film) 3062-2927, 2868-2846 (CHs), 1599, 1577 (C=C), 1484, 1458, 1443 (CH_2), 1175 (C-O-C) cm^{-1}

^{1}H NMR $\delta(CDCl_3)$	1.84-1.87 (4H, m, H-2'''', H-3''''), 2.71-2.75 (4H, m, H-1'''', H-4''''), 2.84 (2H, t, J = 5.52Hz, CH_2N), 2.97 (2H, t, J = 5.78Hz, H-3), 4.10 (2H, t, J = 5.52Hz, CH_2O), 4.63 (2H, t, J = 6.26Hz, H-2), 6.52-6.54 (2H, dd, J = 9.04Hz, H-3', H-5'), 6.66-7.28 (11H, m, ArH)
^{13}C NMR $\delta(CDCl_3)$	23.30, 23.39 (C-2'''', C-3''''), 35.65 (C-3), 54.39, 54.45 (C-1'''', C-4''''), 54.83 (NCH_2), 66.22, 66.36 (CH_2O), 80.36 (C-2), 112.92, 113.65 (C-3', C-5'), 120.88, 121.99 (C-4, C-9), 123.43 (C-7), 126.24 (C-6), 127.25 (C-4''), 127.91, 127.87 (C-2'', C-6''), 128.03, 128.34 (C-3'', C-5''), 129.43, 130.53 (C-2', C-6'), 131.24 (C-8)
EIMS (HR)	$C_{28}H_{29}NO_2$: calculated M^+ 411.2184 observed M^+ 411.2198

7.3.7. Preparation of substituted boronic acids

General Method 3.7

n-Butyllithium (16.8mL, 2.5M in hexane) was added over 5min to a stirred suspension of an appropriately substituted bromoarylcompound (0.042M) in dry THF (120mL) at -78°C under nitrogen (vacuum dried apparatus). The suspension was allowed to warm up (*ca.* -10°C) until all solid material is dissolved, then recooled to -78°C, and stirred for 1 hour. Post this period was added triisopropyl borate (0.042M) dropwise over 5-10min and the mixture was stirred for a further hour at -78°C under N_2. This solution was warmed up to room temperature and water (40mL) was added slowly with stirring until precipitation occurs. The product reaction mixture was stirred vigorously at room temperature for 10-15min and then vacuum filtered, washed with water (20mL), and then with hexane (50mL). If precipitation does not take place post addition of water, the mixture is partitioned between diethyl ether (3x25mL), saturated NH_4Cl (10mL) and water (40mL). The diethyl ether layer was washed with saturated sodium sulphate, dried over Na_2SO_4 and the solvent is extracted <35°C. The resulting boronic acid is vacuum dried for 1-2 days at temperature

less than ambient.[433] Column chromatography may be necessary to remove any traces of impurities.

4-Methoxyphenylboronic acid (168)

The general method 3.7 was employed using 4-bromoanisole (0.042M), dry THF (120mL), *n*-BuLi (0.042M) and trisopropyl borate (0.042M) under nitrogen. The product precipitated out post addition of water. The compound was vacuum dried (<30°C) affording a colourless powder in 91% yield; m.p. 159-164°C [lit. m.p. 160-163°C][456]

IR ν_{max}(KBr)	3733-3016 (OH), 2955-2833, 2567 (CHs), 1604 (C=C), 1512 (OCH_3), 1169, 1112 (B-OH) cm^{-1}
^1H NMR $\delta(CD_3OD)$	3.76 (3H, s, OCH_3), 6.85-6.87 (2H, d, J = 8.04Hz, H-3, H-5), 7.68-7.70 (2H, d, J = 7.52Hz, H-2, H-6)
^{13}C NMR $\delta(CD_3OD)$	55.13 (OCH_3), 113.20 (C-3, C-5), 132.22 (C-2, C-6), 159.10 (C-4), 172.71 (C-1)
^{11}B NMR $\delta(CD_3OD)$	Boron triflouroetherate (ref)
	Isopropyl borate 380
	Product (boron) 375

m-Methoxyphenylboronic acid (169)

The general method 3.7 was employed using 3-bromoanisole (0.042M), dry THF (120mL), *n*-BuLi (0.042M) and trisopropyl borate (0.042M) under nitrogen. The product was extracted with diethyl ether. The compound was purified with column chromatography (eluant dichloromethane : ethyl acetate; 90:10) and vacuum dried (<30°C) affording a colourless powder in 29% yield; m.p. 158-162°C [lit. m.p. 160-163°C][531]

IR ν_{max}(KBr)	3278 (OH), 3014-2937 (CHs), 1593, 1583 (C=C), 14196 (OCH_3) cm^{-1}
^1H NMR $\delta(CD_3OD)$	3.80 (3H, s, OCH_3), 6.94-6.97 (1H, dd, J = 2.00, 8.04Hz, H-2), 7.15 (1H, m, H-4), 7.24-7.33 (2H, m, H-5, H-6)
^{13}C NMR $\delta(CD_3OD)$	54.07 (OCH_3), 114.77 (C-2), 118.24 (C-4), 125.10 (C-5), 128.15 (C-6), 158.65 (C-3)

^{11}B NMR δ(CD_3OD)	Boron triflouroetherate (ref)
	Isopropyl borate 380
	Product (boron) 379

_o_-Methoxyphenylboronic acid (170)

The general method 3.7 was employed using 2-bromoanisole (0.042M), dry THF (120mL), *n*-BuLi (0.042M) and trisopropyl borate (0.042M) under nitrogen. The product was extracted with diethyl ether. The compound was isolated with column chromatography (eluant dichloromethane : ethyl acetate; 95:5) and vacuum dried (<30°C) affording a colourless crystalline solid in 69% yield; m.p. 106-108°C [lit. m.p. 105-107°C][532]

IR ν_{max}(KBr)	3354 (OH), 2971-2820 (CHs), 1604, 1575 (C=C), 1486 (OCH_3), 1164, 1105 (B-OH) cm^{-1}
^{1}H NMR δ(CD_3OD)	3.81 (3H, s, OCH_3), 6.89-6.95 (2H, m, H-4, H-5), 7.36 (1H, t, J = 7.78Hz, H-3), 7.67-7.68 (1H, d, J = 7.00Hz, H-6)
^{13}C NMR δ(CD_3OD)	54.85 (OCH_3), 110.05 (C-4), 120.74 (C-5), 132.20 (C-3), 135.92 (C-6), 164.54 (C-2)
^{11}B NMR δ(CD_3OD)	Boron triflouroetherate (ref)
	Isopropyl borate 380
	Product (boron) 377

4-Tolyboronic acid (171)

The general method 3.7 was employed using 4-bromotoluene (0.042M), dry THF (120mL), *n*-BuLi (0.042M) and trisopropyl borate (0.042M) under nitrogen. The product was extracted using diethyl ether and concentrated down. The product was isolated using column chromatography (eluant dichloromethane : ethyl acetate; 98:2) and vacuum dried (<30°C) affording a cream solid in 52% yield; m.p. 249-260°C [lit. m.p. 256-263°C][533]

IR ν_{max}(KBr)	3250 (OH), 3098-2987 (CHs), 1615 (C=C), 1363 (CH_3), 1164, 1105 (B-OH) cm^{-1}

^{1}H NMR δ(CD_3OD)	2.21 (3H, s, CH_3), 7.12-7.14 (2H, d, J = 7.04Hz, H-3, H-5), 7.48-7.50 (1H, d, J = 6.52Hz, H-2), 7.65-7.67 (1H, d, J = 7.04Hz, H-6)
^{13}C NMR δ(CD_3OD)	19.81 (CH_3), 127.41, 127.51 (C-3, C-5), 132.84, 133.15 (C-2, C-6), 139.42 (C-4)
^{11}B NMR δ(CD_3OD)	Boron triflouroetherate (ref)
	Isopropyl borate 380
	Product (boron) 415

_m_-Methylphenyl boronic acid (172)

The general method 3.7 was applied using 3-bromotoluene (0.042M), dry THF (120mL), *n*-BuLi (0.042M) and trisopropyl borate (0.042M) under nitrogen. The product was extracted using diethyl ether and concentrated under reduced pressure. The product was purified using column chromatography (eluant dichloromethane : ethyl acetate; 98:2) and vacuum dried (<30°C) affording a colourless solid in 57% yield; m.p. 74-76°C [lit. m.p. 69-71°C][534]

IR ν_{max}(KBr)	3272 (OH), 3033-2917 (CHs), 1605, 1582 (C=C), 1348 (CH_3) cm^{-1}
^{1}H NMR δ(CD_3OD)	2.33 (3H, s, CH_3), 7.20-7.24 (2H, m, H-2, H-4), 7.37-7.38 (1H, m, H-5), 7.54-7.58 (1H, m, H-6)
^{13}C NMR δ(CD_3OD)	19.66 (CH_3), 126.72 (C-2), 129.52 (C-4), 133.62 (C-5), 136.21 (C-6)
^{11}B NMR δ(CD_3OD)	Boron triflouroetherate (ref)
	Isopropyl borate 380
	Product (boron) 377

_o_-Methylphenyl boronic acid (173)

The general method 3.7 was applied using 2-bromotoluene (0.042M), dry THF (120mL), *n*-BuLi (0.042M) and trisopropyl borate (0.042M) under nitrogen. The product was extracted using diethyl ether and concentrated down. The product was purified using column chromatography (eluant dichloromethane : ethyl

acetate; 95:5) and vacuum dried (<30°C) affording a colourless solid[535] in 57% yield; m.p. 162-164°C [lit. m.p. 162-164°C]

IR ν_{max}(KBr)	3440 (OH), 3092-2923 (CHs), 1600, 1567 (C=C), 1340 (CH_3) cm^{-1}
^{1}H NMR $\delta(CD_3OD)$	2.33 (3H, s, CH_3), 7.13-7.19 (2H, m, H-4, H-5), 7.23-7.26 (2H, m, H-3, H-6)
^{13}C NMR $\delta(CD_3OD)$	20.17 (CH_3), 124.01 (C-3), 127.89 (C-4), 128.26 (C-5), 130.26 (C-6), 139.08 (C-2)
^{11}B NMR $\delta(CD_3OD)$	Boron triflouroetherate (ref)
	Isopropyl borate 380
	Product (boron) 377

4-Chlorophenylboronic acid (174)

The general method 3.7 was employed using 4-chlorobromobenzene (0.042M), dry THF (120mL), *n*-BuLi (0.042M) and trisopropyl borate (0.042M) under nitrogen. The product was extracted using diethyl ether and concentrated down. The product was purified using column chromatography (eluant dichloromethane : ethyl acetate; 95:5) and vacuum dried (<30°C) affording a colourless crystalline solid in 34% yield; m.p. 278-280°C [lit. m.p. 284-289°C][536]

IR ν_{max}(KBr)	3262 (OH), 2980-2954 (CHs), 1595, 1563 (C=C), ~1257 (C-Cl) cm^{-1}
^{1}H NMR $\delta(CD_3OD)$	7.34 (2H, m, H-3, H-5), 7.60-7.71 (2H, m, H-2, H-6)
^{13}C NMR $\delta(CD_3OD)$	126.86 (C-3, C-5), 134.32 (C-2, C-6)
^{11}B NMR $\delta(CD_3OD)$	Boron triflouroetherate (ref)
	Isopropyl borate 380
	Product (boron) 373

4-Fluorophenylboronic acid (175)

The general method 3.7 was employed using 1-bromo-fluorobenzene (0.042M), dry THF (120mL), *n*-BuLi (0.042M) and trisopropyl borate (0.042M) under nitrogen. The product was extracted using diethyl ether and concentrated down.

The product was isolated using column chromatography (eluant dichloromethane : ethyl acetate; 95:5) and vacuum dried (<30°C) affording a colourless powder in 17% yield; m.p. 260-261°C [lit. m.p. 263-265°C][537]

IR ν_{max}(KBr)	3434 (OH), 3050 (CHs), 1599 (C=C) cm^{-1}
^{1}H NMR $\delta(CD_3OD)$	7.01-7.11 (2H, m, H-3, H-5), 7.65-7.78 (2H, m, H-2, H-6)
^{13}C NMR $\delta(CD_3OD)$	113.26-113.72 (C-3, C-5), 135.06-135.32 (C-2, C-6)
^{19}F NMR $\delta(CD_3OD)$	-113.61 (C-F)
^{11}B NMR $\delta(CD_3OD)$	Boron triflouroetherate (ref)
	Isopropyl borate 380
	Product (boron) 375

Benzonitrileboronic acid (176)

The general method 3.7 was employed using 4-bromobenzonitrile (0.042M), dry THF (120mL), *n*-BuLi (0.042M) and trisopropyl borate (0.042M) under nitrogen. The product was extracted using diethyl ether and concentrated down. The product was isolated using column chromatography (eluant dichloromethane : ethyl acetate; 75:25) was vacuum dried (<30°C) affording a colourless solid in 6% yield; m.p. >270°C [lit. m.p. 285-292°C][538]

IR ν_{max}(KBr)	3511-3342 (OH), 3068-3025 (CHs), 2229 (C≡N), 1610, 1506 (C=C) cm^{-1}
^{1}H NMR $\delta(CD_3OD)$	7.43-7.51 (4H, m, H-2, H-3, H-7, H-8), 7.78-7.85 (3H, m, H-4, H-5, H-6)
^{13}C NMR $\delta(CD_3OD)$	112.43 (C≡N), 118.10 (C-B), 130.22 (C-3, C-5), 133.54 (C-2, C-6)
^{11}B NMR $\delta(CD_3OD)$	Boron triflouroetherate (ref)
	Isopropyl borate 380
	Product (boron) 376

1-Naphthaleneboronic acid (177)

The general method 3.7 was employed using 1-bromonaphthalene (0.042M), dry THF (120mL), *n*-BuLi (0.042M) and trisopropyl borate (0.042M) under nitrogen. The product was extracted using diethyl ether and concentrated down. Column chromatography (eluant dichloromethane : ethyl acetate; 95:5) and was carried out to purify the product and was then vacuum dried (<30°C) affording a creamy white solid in 36% yield; m.p. 202-208°C [lit. m.p. 210-211°C][539]

IR ν_{max}(KBr)	3264 (OH), 3044 (CHs), 1574, 1508 (C=C) cm^{-1}
^{1}H NMR $\delta(CD_3OD)$	7.43-7.51 (4H, m, H-2, H-3, H-7, H-8), 7.78-7.85 (3H, m, H-4, H-5, H-6)
^{13}C NMR $\delta(CD_3OD)$	124.18-125.22 (C-3, C-6, C-7), 127.18-129.24 (C-2, C-4, C-5, C-8), 132.75 (C-9), 134.45 (C-10)
^{11}B NMR $\delta(CD_3OD)$	Boron triflouroetherate (ref)
	Isopropyl borate 380
	Product (boron) 380

3-Pyridinyltriisopropylborate (179)

The general method 3.7 was employed using 4-bromopyridine (0.042M), dry diethyl ether (120mL), *n*-BuLi (0.042M) and triisopropyl borate (0.042M) under nitrogen. The product was concentrated down until an orange crystalline solid was obtained. *n*-Hexane (32mL) was added with stirring to give a sticky gum and the remaining solvent was removed and the residue was vacuum dried (<30°C) affording a cream coloured solid[540] in 97% yield.

IR ν_{max}(KBr)	2940-2824 (CHs), 1658, 1583 (pyridine peaks), 1401-1328 (C-N) cm^{-1}
^{1}H NMR $\delta(CD_3OD)$	1.18 (18H, s, $(CH_3)_6$), 3.95 (3H, m, $(CH)_3$), 7.21 (1H, m, H-5), 7.92 (1H, m, H-4), 8.20 (1H, m, H-2), 8.59 (1H, s, H-6)
^{13}C NMR $\delta(CD_3OD)$	23.42 ($(CH_3)_6$), 62.88 ($(CH)_3$), 121.99 (C-5), 141.32 (C-4), 144.15 (C-6) , 152.48 (C-2)

m-Nitrophenylboronic acid (182)

In an ice-salt freezing mixture colourless fuming nitric acid (1.20M), to which a little urea had a little had been added, was cooled to –15°C. Phenyl boronic acid (0.074M) was added slowly over a period of 1-2hr, such that the temperature did not rise above –9°C. The mixture was then stirred for a further 15-30min, after which it was poured onto ice and the product precipitates out. The precipitated nitrophenyl boronic acid was vacuum filtered, recrystallised from a small volume of water, with the addition of decolorising charcoal. Hot vacuum filtration was then carried out to discard charcoal, washed initially with water and then methanol. The original filtrate was cooled in an ice-bath, neutralised to an orange / red solution with strong NaOH solution and then acidulated with nitric acid. The solution was extracted with diethyl ether, washed with water and concentrated down at room temperature. Both fractions from the work up were combined and column chromatography (eluant petroleum ether : dichloromethane; 80:20), removed all traces of impurities to afford lemon prismatic crystals in 28% yield; m.p. 282-286°C (char), [lit. m.p. 284-285°C (char.)][435]

IR ν_{max}(KBr)	3440 (OH), 2927-2855 (CHs), 1616, 1583 (C=C), 1529, 1349 (C-NO_2) cm^{-1}
^{1}H NMR $\delta(CD_3COCD_3)$	7.68 (1H, t, J = 7.78Hz, H-6), 8.27-8.32 (2H, m, H-4, H-5), 8.70 (1H, s, H-2)
^{13}C NMR $\delta(CD_3COCD_3)$	125.43 (C-2), 128.89 (C-4), 129.50 (C-5), 140.88 (C-6)
^{11}B NMR $\delta(CD_3OD)$	Boron triflouroetherate (ref)
	Isopropyl borate 380
	Product (boron) 378

4-Bromobenzaldehyde dimethyl acetal (184)

A solution of 4-bromobenzaldehyde (0.011M), trimethyl orthoformate (0.022M), methanol (10mL) was treated with *p*-toluenesulphonic acid and the mixture was stirred at room temperature for 24hr. Post this period the reaction mixture was refluxed gently for 4hr. The acid was neutralised with saturated sodium bicarbonate (2x40mL), washed with water, brine, extracted with dichloromethane and dried over sodium sulphate. Vacuum distillation was

carried out to purify the product, presenting a colourless oily solution[438] in 82% yield, with the following physical properties;

IR ν_{max}(film)	2991-2935, 2904-2828 (CHs), 1592 (C=C), 1485 (OCH_3), 1351, 1205 (C-Br) cm^{-1}
^{1}H NMR $\delta(CDCl_3)$	3.24 (6H, s, $(OCH_3)_2$), 5.30 (1H, s, CH), 7.26-7.29 (2H, d, J = 7.00Hz, H-3, H-5), 7.42-7.45 (2H, dd, J = 7.04, 1.48Hz, H-2, H-6)
^{13}C NMR $\delta(CDCl_3)$	51.73 ($(OCH_3)_2$), 101.57 (CH), 121.90 (C-1), 128.11 (C-3, C-5), 130.76 (C-2, C-6), 136.86 (C-4)

4-Formylbenzene boronic acid (185)

The general method 3.7 was applied using 4-bromobenzaldehyde dimethyl acetal (0.043M), dry THF (120mL), *n*-BuLi (0.043M) and trisopropyl borate (0.043M) under nitrogen. During the procedure the dimethyl acetal protecting group was removed to yield the original formyl group. The product was extracted using diethyl ether, concentrated down and purified using column chromatography (eluant dichloromethane : ethyl acetate : methanol; 90:10:10) and vacuum dried (<30°C) affording a beige coloured oily gel[437] in 5% yield

IR ν_{max}(film)	3400 (OH), 3051-2930, 2865-2736 (CHs), 1604, 1511 (C=C), 1444 (OCH_3) cm^{-1}
^{1}H NMR $\delta(CD_3OD)$	7.27-7.93 (4H, m, ArH), 9.90 (1H, s, CHO)
^{13}C NMR $\delta(CD_3OD)$	103.62 (CH), 125.44-126.28 (C-3, C-5), 128.54-129.38 (C-2, C-6), 133.23 (C-4), 186.65 (C=O)
^{11}B NMR $\delta(CD_3OD)$	Boron triflouroetherate (ref)
	Isopropyl borate 380
	Product (boron) 378

7.3.8. Demethylation of 4-bromo-5-(4-methoxyphenyl)- 2,3-dihydro-1-benzoxepin

4-Bromo-5-(4-hydroxyphenyl)-2,3-dihydro-1-benzoxepin (202)

The procedure incorporated in 6.2.6 was employed to prepare this compound, using 4-bromo-5-(4-methoxyphenyl)-2,3-dihydro-1-benzoxepin (0.0061M) in dry

dichloromethane (25mL) and boron tribromide (0.035M). This solution was stirred at –78°C for 1 hour under nitrogen. The product was isolated using column chromatography (eluant petroleum ether : ethyl acetate; 80:20) affording a light brown coloured oil in 48% yield.

IR ν_{max} (film)	3402 (OH), 2945-2929, 2871 (CHs), 1610, 1574 (C=C), 1481 (CH_2), 1234, 1171 (C-O-C) cm^{-1}
^1H NMR $\delta(CDCl_3)$	3.03 (2H, t, J = 5.78Hz, H-3), 4.60 (2H, t, J = 6.02Hz, H-2), 6.81-6.88 (4H, m, H-3', H-5', H-6, H-7), 6.96-6.98 (1H, m, H-9), 7.07-7.24 (3H, m, H-2', H-6', H-8)
^{13}C NMR $\delta(CDCl_3)$	41.07 (C-3), 77.35 (C-2), 114.97, 115.21 (C-3', C-5'), 121.92 (C-4, C-9), 123.42 (C-7), 128.80 (C-6), 129.01 (C-2', C-6'), 131.21 (C-8), 133.73 (C-5), 134.08 (C-1'), 138.99 (C-5a)
EIMS (LR)	$C_{22}H_{18}O_2$: calculated M^+ 317
	observed M^+ 317

7.3.9. Suzuki coupling reactions

General Method 3.9

To a stirred solution of 4-bromo-5-(4-hydroxyphenyl)- 2,3-dihydro-1-benzoxepin (0.0002M) in dry THF (20mL) was added $Pd(PPh_3)_4$ (10mg) and stirred for 10-15min prior to addition of the appropriately substituted arylboronic acid or hetrocyclic boronic acid, followed by 2M sodium carbonate (0.0014M). The mixture was heated by an oil bath to 80°C for 6-8hr and then cooled to room temp and let stir for 10-12hr. Post this period the mixture was poured onto water (35mL), washed with brine, extracted with dichloromethane and dried over Na_2SO_4 and concentrated. The product was purified using column chromatography.

5-(4-Hydroxyphenyl)-4-methoxyphenyl-2,3-dihydro-1-benzoxepin (203)

The general method 3.9 was employed using 4-bromo-2,3-dihydro-5-(4-hydroxyphenyl)-1-benzoxepin (0.00018M), dry THF (20mL) and $Pd(PPh_3)_4$ (10mg). To this 4-methoxyphenyl boronic acid (0.00072M) and 2M sodium

carbonate (0.0014M) were added and refluxed at 80°C. The product was isolated using flash column chromatography (eluant petroleum ether : ethyl acetate : dichloromethane; 80:10:10) to afford a light brown coloured oil in 80% yield.

IR ν_{max} (film) 3402 (OH), 3061-2960, 2896-2732 (CHs), 1608 (C=C), 1462 (CH_2), 1432 (OCH_3), 1283 (C-OH), 1247, 1195 (C-O-C) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 2.71 (2H, t, J = 5.78Hz, H-3), 3.79 (3H, s, OCH_3), 4.64 (2H, t, J = 6.02Hz, H-2), 6.59-6.61 (2H, d, J = 8.04Hz, H-3'', H-5''), 6.73-7.15 (10H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 35.18 (C-3), 54.72 (OCH_3), 80.17 (C-2), 113.00, 114.22 (C-3'', C-5''), 114.55, 115.61 (C-3', C-5'), 121.60 (C-4, C-9), 123.15 (C-7), 127.45 (C-6), 127.80 (C-4''), 130.16, 130.21 (C-2'', C-6''), 130.48, 130.90 (C-2', C-6'), 132.17 (C-3), 132.23 (C-8)

EIMS (LR) $C_{23}H_{20}O_3$: calculated M^+ 344

observed M^+ 344

5-(4-Hydroxyphenyl)-4-(2-methoxyphenyl)-2,3-dihydro-1-benzoxepin (204)

The general method 3.9 was employed using 4-bromo-5-(4-hydroxyphenyl)-2,3-dihydro-1-benzoxepin (0.00022M), dry THF (20mL), $Pd(PPh_3)_4$ (10mg), *o*-methoxyphenyl boronic acid (0.00072M) and 2M sodium carbonate (0.0014M) and the reaction was refluxed at 80°C. The product was purified using flash column chromatography (eluant petroleum ether : ethyl acetate : dichloromethane; 50:5:50) to afford a brown coloured gel in 85% yield.

IR ν_{max} (film) 3421-3114 (OH), 2951-2914, 2872 (CHs), 1597, 1574 (C=C), 1484, 1460 (CH_2), 1434 (OCH_3), 1281 (C-OH), 1235, 1198 (C-O-C) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 2.66 (2H, t, J = 6.78Hz, H-3), 3.83 (3H, s, OCH_3), 4.61 (2H, t, J = 6.02Hz, H-2), 6.56-6.59 (2H,dd, J = 2.04, 6.52Hz, H-4'', H-5''), 6.73-7.24 (8H, m, ArH), 7.46-7.48 (1H, dt, J = 2.00, 7.04Hz, H-3''), 7.86-7.88 (1H, dd, J = 1.52, 7.35Hz, H-6'')

^{13}C NMR $\delta(CDCl_3)$	34.64 (C-3), 55.41 (OCH_3), 79.95 (C-2), 110.45 (C-4''), 114.31 (C-3', C-5'), 120.35 (C-5''), 121.19 (C-4, C-9), 123.18 (C-7), 127.83 (C-6), 131.49 (C-5), 131.94 (C-8), 132.79 (C-3''), 136.77 (C-6'')
EIMS (LR)	$C_{23}H_{20}O_3$: calculated M^+ 344
	observed M^+ 344

5-(4-Hydroxyphenyl)-4-(3-methoxyphenyl)-2,3-dihydro-1-benzoxepin (205)

The general method 3.9 was applied using 4-bromo-5-(4-hydroxyphenyl)-2,3-dihydro-1-benzoxepin (0.00025M), dry THF (20mL), $Pd(PPh_3)_4$ (10mg), *m*-methoxyphenyl boronic acid (0.001M) and 2M sodium carbonate (0.0016M) and the reaction was refluxed at 80°C. The product was purified using flash column chromatography (eluant petroleum ether : ethyl acetate : dichloromethane; 40:1:40) to afford a mustard coloured oil in 48% yield, with the following physical properties;

IR ν_{max} (film)	3421 (OH), 2955-2924, 2850-2732 (CHs), 1614 (C=C), 1484, 1464 (CH_2), 1377 (OCH_3), 1284 (C-OH), 1248, 1168 (C-O-C) cm^{-1}
^1H NMR $\delta(CDCl_3)$	2.73 (2H, t, J = 6.02Hz, H-3), 3.80 (3H, s, OCH_3), 4.63 (2H, m, H-2), 6.56-6.59 (2H, dd, J = 2.04, 6.52Hz, H-4'', H-5''), 6.44-7.17 (10H, m, ArH)
^{13}C NMR $\delta(CDCl_3)$	35.12 (C-3), 54.79 (OCH_3), 80.04 (C-2), 114.17-115.71 (C-3', C-5'), 115.85, 115.95 (C-2''), 118.63 (C-4''), 121.29, 121.59 (C-4, C-9), 123.12, 123.36 (C-7), 124.04 (C-5''), 128.39 (C-6''), 128.47 (C-6), 129.16, 129.63 (C-2', C-5'), 132.04 (C-5), 132.12 (C-8), 158.67 (C-3'')
EIMS (LR)	$C_{23}H_{20}O_3$: calculated M^+ 344
	observed M^+ 344

5-(4-Hydroxyphenyl)-4-(4-methylphenyl)-2,3-dihydro-1-benzoxepin (206)

The gereral method 3.9 was applied using 4-bromo-5-(4-hydroxyphenyl)-2,3-dihydro-1-benzoxepin (0.00022M), dry THF (20mL), $Pd(PPh_3)_4$ (10mg), 4-

methylphenyl boronic acid (0.0007M) and 2M sodium carbonate (0.0014M) and the reaction was refluxed at 80°C. The product was isolated using flash column chromatography (eluant petroleum ether : dichloromethane; 70:30) to afford an orange / mustard coloured oil in 93% yield, with the following physical properties:

IR ν_{max} (film)	3612-3007 (OH), 2924, 2876 (CHs), 1610 (C=C), 1478 (CH_2), 1458 (CH_3), 1262 (C-O-C) cm^{-1}
^1H NMR $\delta(CDCl_3)$	2.31 (3H, s, CH_3), 2.72 (2H, m, H-3), 4.63 (2H, m, H-2), 6.59-6.61 (2H, d, J = 8.04Hz, H-3'', H-5''), 6.83-7.28 (10H, m, ArH)
^{13}C NMR $\delta(CDCl_3)$	20.65 (CH_3), 35.24 (C-3), 80.04 (C-2), 114.37, 114.53 (C-3', C-5'), 120.14, 121.52 (C-4, C-9), 122.96, 123.05 (C-7), 127.83 (C-6), 128.23 (C-4''), 128.29, 128.40 (C-2'', C-6''), 128.83, 128.91 (C-3'', C-5''), 130.50 (C-2', C-6'), 131.30 (C-5), 132.55 (C-8)
EIMS (LR)	$C_{23}H_{20}O_2$: calculated M^+ 328
	observed M^+ 328

5-(4-Hydroxyphenyl)-4-(3-methylphenyl)-2,3-dihydro-1-benzoxepin (207)

The general method 3.9 was applied using 4-bromo-5-(4-hydroxyphenyl)-2,3-dihydro-1-benzoxepin (0.00016M), dry THF (20mL), $Pd(PPh_3)_4$ (10mg), *m*-methylphenyl boronic acid (0.0005M) and 2M sodium carbonate (0.001M) and the reaction was refluxed at 80°C. The product was purified using flash column chromatography (eluant petroleum ether : dichloromethane : ethyl acetate; 40:60:2) to afford a light brown coloured oil in 54% yield, with the following physical properties:

IR ν_{max} (film)	3389 (OH), 2917, 2848 (CHs), 1462 (CH_2), 1432 (CH_3), 1282 (C-OH), 1196 (C-O-C) cm^{-1}
^1H NMR $\delta(CDCl_3)$	2.25 (3H, s, CH_3), 2.72 (2H, t, J = 6.26, H-3), 4.63 (2H, m, H-2), 6.58-6.60 (2H, d, J = 8.52Hz, H-2'', H-4''), 6.84-7.15 (10H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 20.88 (CH_3), 35.23 (C-3), 79.97 (C-2), 114.06, 114.53 (C-3', C-5'), 121.52, 121.59 (C-4, C-9), 122.94 (C-7), 126.61 (C-2''), 127.33 (C-6), 127.89 (C-4'), 129.61 (C-4''), 130.52, 130.92 (C-2', C-6'), 132.22 (C-5, C-8), 133.68 (C-5''), 136.47 (C-6'')

EIMS (LR) $C_{23}H_{20}O_2$: calculated M^+ 328

observed M^+ 328

5-(4-Hydroxyphenyl)-4-(2-methylphenyl)-2,3-dihydro-1-benzoxepin (208)

The general method 3.9 was applied using 4-bromo-5-(4-hydroxyphenyl)-2,3-dihydro-1-benzoxepin (0.00016M), dry THF (20mL), $Pd(PPh_3)_4$ (10mg), *o*-methylphenyl boronic acid (0.0005M) and 2M sodium carbonate (0.001M) and the reaction was refluxed at 80°C. The product was isolated using column chromatography (eluant petroleum ether : dichloromethane : ethyl acetate; 30:70:1) to afford a mustard coloured oil in 62% yield.

IR ν_{max} (film) 3382 (OH), 2951-2919, 2849 (CHs), 1607 (C=C), 1461 (CH_2), 1431 (CH_3), 1282 (C-OH), 1195 (C-O-C) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 2.08 (3H, s, CH_3), 2.63 (2H, m, H-3), 4.63 (2H, m, H-2), 6.53-6.55 (2H, m, H-4'', H-5''), 6.79-6.81 (2H, m, H-3'', H-6''), 7.07-7.29 (8H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 19.71 (CH_3), 35.91 (C-3), 79.79 (C-2), 114.32, 114.55 (C-3', C-5'), 121.96 (C-4, C-9), 123.44 (C-7), 125.45 (C-3''), 126.56 (C-6), 127.06 (C-4''), 128.35 (C-5''), 129.60 (C-4'), 129.60, 129.72 (C-2', C-6'), 130.97 (C-6'') 131.79 (C-8), 133.50 (C-5), 141.51 (C-2'')

EIMS (LR) $C_{23}H_{20}O_2$: calculated M^+ 328

observed M^+ 328

4-(4-Chlorophenyl)-5-(4-hydroxyphenyl)-2,3-dihydro-1-benzoxepin (209)

The general method 3.9 was applied using 4-bromo-5-(4-hydroxyphenyl)-2,3-dihydro-1-benzoxepin (0.00016M), dry THF (20mL), $Pd(PPh_3)_4$ (10mg), 4-

chlorophenylboronic acid (0.0005M) and 2M sodium carbonate (0.0014M) and the reaction was refluxed at 80°C. The product was isolated using flash column chromatography (eluant petroleum ether : dichloromethane : ethyl acetate; 60:40:1) to afford a wine coloured oil in 70% yield, with the following physical properties.

IR ν_{max} (film) 3401 (OH), 2955-2919, 2880-2849 (CHs), 1603 (C=C), 1481, 1463 (CH_2), ~1259 (C-Cl)), 1161 (C-O-C) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 2.70 (2H, t, J = 6.02Hz, H-3), 4.62 (2H, m, H-2), 6.61-6.63 (2H,d, J = 8.56Hz, H-3'', H-5''), 6.78-7.23 (10H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 35.04 (C-3), 79.10 (C-2), 114.34, 116.22 (C-3', C-5'), 121.53, 121.63 (C-4, C-9), 123.07 (C-7), 125.76 (C-3'', C-5''), 128.24 (C-6), 128.99, 129.09 (C-2', C-6'), 130.36, 130.93 (C-2'', C-6''), 132.22 (C-4'', C-5, C-8)

EIMS (LR) $C_{22}H_{17}O_2Cl$: calculated M^+ 348

observed M^+ 348

5-(4-Hydroxyphenyl)-4-(1-naphthyl)-2,3-dihydro-1-benzoxepin (210)

The general method 3.9 was employed using 4-bromo-5-(4-hydroxyphenyl)-2,3-dihydro-1-benzoxepin (0.00022M), dry THF (20mL), $Pd(PPh_3)_4$ (10mg), 1-naphthylboronic acid (0.0009M) and aqueous sodium carbonate (2M, 0.0014M) and the reaction was refluxed at 80°C. The product was isolated using column chromatography (eluant petroleum ether : dichloromethane : ethyl acetate; 60:40:2) to present a dark green coloured oil in 50% yield.

IR ν_{max} (film) 3397 (OH), 3058-2924, 2852 (CHs), 1606 (C=C), 1465 (CH_2), 1261, 1190 (C-O-C) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 2.64 (2H, m, H-3), 4.64 (2H, m, H-2), 6.40-6.42 (2H, d, J = 8.56Hz, H-3', H-5'), 6.80-7.71 (13H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 35.70 (C-3), 79.70 (C-2), 113.91, 114.67 (C-3', C-5'), 121.66 (C-4, C-9), 123.14 (C-7), 124.90, 125.56

(C-3'', C-6'', C-7''), 126.10, 127.99 (C-2'', C-4'', C-5'', C-8''), 128.13 (C-6), 131.01 (C-8), 132.21 (C-9''), 133.32 (C-1'', C-5)

EIMS (LR) $C_{26}H_{20}O_2$: calculated M^+ 364

observed M^+ 364

5-(4-Hydroxyphenyl)-4-(3-nitrophenyl)-2,3-dihydro-1-benzoxepin (211)

The general method 3.9 was employed using 4-bromo-5-(4-hydroxyphenyl)-2,3-dihydro-1-benzoxepin (0.00022M), dry THF (20mL), $Pd(PPh_3)_4$ (10mg), *m*-nitrophenylboronic acid (0.00087M) and 2M sodium carbonate (0.0014M) and the reaction was refluxed at 80°C. The product was purified using column chromatography (eluant petroleum ether : dichloromethane : ethyl acetate; 40:55:5) to present a brown coloured oil in 47% yield.

IR ν_{max} (film) 3488 (OH), 3108-2929, 2872-2776 (CHs), 1604, 1574 (C=C), 1486, 1443 (CH_2), 1538, 1349 (C-NO_2), 1250, 1184 (C-O-C) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 2.69 (2H, m, H-3), 4.62 (2H, m, H-2), 6.60-6.62 (2H, d, J = 8.52Hz, H-3', H-5'), 6.77-7.19 (10H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 35.21 (C-3), 79.96 (C-2), 114.26, 115.80 (C-3', C-5'), 121.44, 121.61 (C-4, C-9), 123.21 (C-7), 124.93 (C-2''), 128.08 (C-6), 128.37 (C-2', C-6'), 128.41 (C-4''), 129.71 (C-5''), 130.91 (C-8), 132.21 (C-5), 140.57 (C-6'')

EIMS (LR) $C_{22}H_{17}NO_2$: product decomposes upon analysis

4-(4-Cyanophenyl)-5-(4-hydroxyphenyl)-2,3-dihydro-1-benzoxepin (212)

The general method 3.9 was applied using 4-bromo-5-(4-hydroxyphenyl)-2,3-dihydro-1-benzoxepin (0.00022M), dry THF (20mL), $Pd(PPh_3)_4$ (10mg), benzonitrileboronic acid (0.00087M) and 2M sodium carbonate (0.0014M) and the reaction was refluxed at 80°C. The product was isolated using flash column chromatography (eluant petroleum ether : dichloromethane : ethyl acetate; 50:50:8) to afford a brown / mustard coloured oil in 34% yield, with the following physical properties:

IR ν_{max} (film) 3401 (OH), 2923, 2849 (CHs), 2251 (C≡N), 1607 (C=C), 1481, 1448 (CH_2), 1248, 1166 (C-O-C) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 2.73 (2H, t, J = 6.04Hz, H-3), 4.62 (2H, t, J = 6.02Hz, H-2), 6.16-7.55 (12H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 34.76 (C-3), 79.80 (C-2), 104.19 (C≡N), 114.49-115.90 (C-3', C-5'), 121.71 (C-4, C-9), 123.42 (C-7), 128.72 (C-6), 129.79 (C-2', C-6'), 130.67 (C-3'', C-5''), 131.40 (C-8), 132.23 (C-5), 133.02 (C-2'', C-6''), 133.81 (C-1')

EIMS (LR) $C_{23}H_{17}NO_2$: calculated M^+ 339

observed M^+ 339

4-(4-Formylphenyl)-5-(4-hydroxyphenyl)-2,3-dihydro-1-benzoxepin (213)

The general method 3.9 was employed using 4-bromo-5-(4-hydroxyphenyl)-2,3-dihydro-1-benzoxepin (0.00016M), dry THF (20mL), $Pd(PPh_3)_4$ (10mg), 4-formylbenzeneboronic acid (0.00087M) and 2M sodium carbonate (0.001M) and the reaction was refluxed at 80°C. The product was isolated using column chromatography (eluant petroleum ether : dichloromethane : ethyl acetate; 60:40:5) to afford a light brown coloured oil in 73% yield.

IR ν_{max} (film) 3325 (OH), 2925, 2853 (CHs), 1735 (C=O), 1450 (CH_2), 1248, 1108 (C-O-C) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 2.76 (2H, t, J = 6.28Hz, H-3), 4.63 (2H, t, J = 6.02Hz, H-2), 6.60-7.35 (8H, m, ArH), 7.71-7.73 (2H , d, J = 8.04Hz, H-2'', H-6''), 7.82-7.84 (2H, d, J = 8.52Hz, H-3'', H-5''), 9.90 (1H, s, CHO)

^{13}C NMR $\delta(CDCl_3)$ 34.89 (C-3), 79.83 (C-2), 106.61 (CH), 113.25-115.46 (C-3', C-5'), 121.25, 121.71 (C-4, C-9), 123.27 (C-7), 127.48 (C-6), 128.55, 128.92 (C-2', C-6'), 129.07, 129.92 (C-2'', C-6'', C-3'', C-5''), 131.65 (C-8), 133.83 (C-5), 134.75 (C-1''), 136.20 (C-5a), 139.02 (C-4''), 190.25 (C=O)

EIMS (LR) $C_{23}H_{18}O_3$: product decomposes upon analysis

5-(4-Hydroxyphenyl)-4-(3-pyridinyl)-2,3-dihydro-1-benzoxepin (217)

The general method 3.9 was applied using 4-bromo-5-(4-hydroxyphenyl)-2,3-dihydro-1-benzoxepin (0.00013M), dry THF (20mL), $Pd(PPh_3)_4$ (10mg), 3-pyridinyltriisopropyl borate (0.0004M) and 2M sodium carbonate (0.0008M) and the reaction was refluxed at 80°C. The product was isolated using column chromatography (eluant petroleum ether : dichloromethane : ethyl acetate; 40:50:10) to afford a deep yellow solid in 83% yield, with the following physical properties.

IR ν_{max} (film) 3430 (OH), 3068-2970, 2926 (CHs), 1658, 1590 (pyridinyl peaks), 1484, 1437 (CH_2), 1378, 1311 (C-N), 1180 (C-O-C) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 2.71 (2H, t, J = 6.04Hz, H-3), 4.61 (2H, t, J = 6.02Hz, H-2), 6.66-7.71 (12H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 34.79 (C-3), 79.81 (C-2), 114.78 (C-3', C-5'), 121.60 (C-5'', C-9), 123.14 (C-7), 128.05 (C-6), 130.77, 130.92 (C-2', C-6'), 131.51 (C-8), 132.92 (C-5), 136.39 (C-1'), 139.67 (C-4''), 146.45 (C-6''), 149.65 (C-2'')

EIMS (LR) $C_{21}H_{17}NO_2$: calculated M^+ 315

observed M^+ 315

4-(Benzo[b]furyl)-5-(4-hydroxyphenyl)-2,3-dihydro-1-benzoxepin (218)

The general method 3.9 was employed using 4-bromo-5-(4-hydroxyphenyl)-2,3-dihydro-1-benzoxepin (0.00022M), dry THF (20mL), $Pd(PPh_3)_4$ (10mg), benzo(b)furan-2-boronic acid (0.0009M) and 2M sodium carbonate (0.0014M) and the reaction was refluxed at 80°C. Column chromatography (eluant petroleum ether : dichloromethane : ethyl acetate; 55:45:5) was used to remove any impurities to proffer a dark brown coloured oily gel in 46% yield.

IR ν_{max} (film) 3408 (OH), 2926, 2853 (CHs), 1603 (C=C), 1480, 1463 (CH_2), 1249, 1170 (C-O-C) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 2.93 (2H, t, J = 6.02Hz, H-3), 4.72 (2H, t, J = 6.26Hz, H-2), 6.16 (1H, s, CH), 6.79-7.50 (12H, m, ArH)

^{13}C NMR δ($CDCl_3$) 41.03 (C-3), 81.14 (C-2), 110.77 (CH), 114.91-115.69 (C-3', C-5'), 120.71, 121.60 (C-4, C-9), 123.14 (C-7), 124.01-125.63 (C-3'', C-4''), 128.53-129.23 (C-2', C-6'), 131.02 (C-8), 132.05 (C-9''), 155.14, 155.77 (C-1'', C-6'')

EIMS (LR) $C_{24}H_{18}O_3$: calculated M^+ 354

observed M^+ 354

5-(4-Hydroxyphenyl)-4-(3-thienyl)-2,3-dihydro-1-benzoxepin (219)

The general method 3.9 was employed using 4-bromo-5-(4-hydroxyphenyl)-2,3-dihydro-1-benzoxepin (0.00013M), dry THF (20mL), $Pd(PPh_3)_4$ (10mg), thiophene-3-boronic acid (0.0004M) and 2M sodium carbonate (0.0008M) and the reaction was refluxed at 80°C. The compound was isolated by column chromatography (eluant petroleum ether : dichloromethane : ethyl acetate; 30:60:10) to afford a brown coloured oily gel in 64% yield.

IR ν_{max} (film) 3397 (OH), 2926, 2882-2854 (CHs), 2227 (thienyl group), 1617 (C=C), 1482, 1457 (CH_2), 1260, 1171 (C-O-C) cm^{-1}

^{1}H NMR δ($CDCl_3$) 2.73 (2H, t, J = 6.02Hz, H-3), 4.65 (2H, t, J = 6.26Hz, H-2), 6.66-7.24 (11H, m, ArH)

^{13}C NMR δ($CDCl_3$) 34.80 (C-3), 80.26 (C-2), 114.42 (C-3', C-5'), 121.38, 121.63 (C-4, C-9), 123.86 (C-7), 125.43 (C-2'', C-5''), 127.93 (C-4''), 128.28 (C-3''), 128.62 (C-6), 129.77, 130.45 (C-2', C-6'), 131.38 (C-8), 132.02 (C-5), 136.80 (C-1'), 142.11 (C-5a)

EIMS (LR) $C_{20}H_{16}SO_2$: calculated M^+ 320

observed M^+ 320

4-(2-Furyl)- 5-(4-hydroxyphenyl)-2,3-dihydro-1-benzoxepin (220)

The general method 3.9 was employed using 4-bromo-5-(4-hydroxyphenyl)-2,3-dihydro-1-benzoxepin (0.00022M), dry THF (20mL), $Pd(PPh_3)_4$ (10mg), furan-2-boronic acid (0.00072M) and 2M sodium carbonate (0.0014M) and the reaction was refluxed at 80°C. Column chromatography (eluant petroleum ether :

dichloromethane : ethyl acetate; 55:45:5) was used to remove any impurities to present a light brown coloured oil in 81% yield.

IR ν_{max} (film) 3429 (OH), 2953-2929, 2853 (CHs), 1601, 1584 (C=C), 1461, 1445 (CH_2), 1245, 1169 (C-O-C) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 2.86 (2H, t, J = 6.04Hz, H-3), 4.70 (2H, t, J = 6.02Hz, H-2), 5.74 (1H, m, H-4''), 6.25 (1H, m, H-3''), 6.70-7.24 (9H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 35.70 (C-3), 80.55 (C-2), 109.28 (C-4''), 110.59 (C-3''), 113.09, 114.94 (C-3', C-5'), 120.05, 121.72 (C-4, C-9), 123.68 (C-7), 127.97 (C-6), 128.03, 129.98 (C-2', C-6'), 131.15 (C-8), 133.93 (C-5), 136.65 (C-1'), 140.35 (C-5a), 141.42 (C-5'')

EIMS (LR) $C_{20}H_{16}O_3$: calculated M^+ 304

observed M^+ 304

7.3.10. Alkylation of Suzuki coupled reaction products

5-[(4-Dimethylaminoethoxy)phenyl]-4-(4-methoxyphenyl)-2,3-dihydro-1-benzoxepin (221)

The general method 2.12 was applied in the preparation of this compound using 5-(4-hydroxyphenyl)-4-(4-methoxyphenyl)-2,3-dihydro-1-benzoxepin (0.00013M), dry acetone (20mL), anhydrous K_2CO_3 (0.0013M) and 2-dimethylaminoethylchloride hydrogenchloride (0.00032M) under nitrogen. The reaction mixture was refluxed for 6hr. The product was isolated directly to afford a light brown / mustard coloured oily gel in 60% yield.

IR ν_{max} (film) 3032-2928, 2868-2772 (CHs), 1607 (C=C), 1483, 1464 (CH_2), 1442 (OCH_3), 1234, 1175 (C-O-C) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 2.38 (6H, s, $N(CH_3)_2$), 2.70 (4H, m, NCH_2, H-3), 3.77 (3H, s, OCH_3), 4.03 (2H, m, CH_2O), 4.61 (2H, t, J = 6.02Hz, H-2), 6.66-7.21 (12H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 31.23 (C-3), 45.41, 45.45 ($N(CH_3)_2$), 54.66 (OCH_3), 57.89, 57.95 (NCH_2), 65.42 (CH_2O), 80.09 (C-2), 113.20, 113.29 (C-3'', C-5''), 114.17, 115.12 (C-3',

C-5'), 121.55 (C-4), 122.99 (C-7), 127.71 (C-4''), 128.75 (C-6), 130.18 (C-2'', C-6'), 130.47 (C-2', C-6'), 131.98 (C-3)

EIMS (HR) $C_{27}H_{29}NO_3$: calculated M^+ 415.2137

observed M^+ 415.2147

Mass Spectrum (m/z) 415 (M^+, 100%), 339 (M^+-76, 17%), 327 (M^+-88, 22%), 313 (M^+-102, 24%), 282 (M^+-133, 17%), 269 (M^+-146, 17%), 227 (M^+-188, 33%) 215 (M^+-200, 20%), 189 (M^+-226, 11%), 165 (M^+-250, 33%)

5-[(4-Dimethylaminoethoxy)phenyl]-4-(2-methoxyphenyl)-2,3-dihydro-1-benzoxepin (222)

The general method 2.12 was employed using 5-(4-hydroxyphenyl)-4-(2-methoxyphenyl)-2,3-dihydro-1-benzoxepin (0.00012M), dry acetone (20mL), anhydrous K_2CO_3 (0.0012M) and 2-dimethylaminoethylchloride hydrogenchloride (0.00032M) under nitrogen. The product was purified using flash column chromatography (eluant dichloromethane : ethyl acetate : methanol; 90:5:5) to afford a mustard coloured gel in 50% yield.

IR ν_{max} (film) 3057-2932, 2867-2770 (CHs), 1606, 1576 (C=C), 1484, 1462 (CH_2), 1440 (OCH_3), 1372 (CH_3), 1240, 1174 (C-O-C) cm^{-1}

^{1}H NMR $\delta(CDCl_3)$ 2.33 (6H, s, $N(CH_3)_2$), 2.65-2.72 (4H, m, NCH_2, H-3), 3.83 (3H, s, OCH_3), 3.99 (2H, t, J = 5.76Hz, CH_2O), 4.61 (2H, t, J = 6.02Hz, H-2), 6.63-7.16 (12H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 34.64 (C-3), 45.78 ($N(CH_3)_2$), 55.23 (OCH_3), 58.23 (NCH_2), 65.72 (CH_2O), 79.96 (C-2), 110.45 (C-4''), 113.42, 113.63 (C-3', C-5'), 120.37 (C-5''), 121.87 (C-4, C-9), 123.13 (C-7), 127.80 (C-6), 128.12 (C-4'), 131.15, 131.52 (C-2', C-6'), 131.63 (C-5), 131.71 (C-8), 131.80 (C-3''), 136.15 (C-6'')

EIMS (HR) $C_{27}H_{29}NO_3$: calculated M^+ 415.2131

observed M^+ 415.2147

Mass Spectrum (m/z)	415 (M^+, 100%), 327 (M^+-88, 10%), 297 (M^+-118, 16%), 255 (M^+-160, 24%), 239 (M^+-176, 30%) 226 (M^+-189, 22%), 189 (M^+-226, 30%), 165 (M^+-250, 60%)

5-[(4-Dimethylaminoethoxy)phenyl]-4-(3-methoxyphenyl)-2,3-dihydro-1-benzoxepin (223)

The general method 2.12 was applied using 5-(4-hydroxyphenyl)-4-(3-methoxyphenyl)- 2,3-dihydro-1-benzoxepin (0.00006M), dry acetone (20mL), anhydrous K_2CO_3 (0.0006M) and 2-dimethylaminoethylchloride hydrogenchloride (0.0002M) under nitrogen. The product was isolated using column chromatography (eluant dichloromethane : ethyl acetate : methanol; 80:20:2) to afford a brown / mustard coloured in 54% yield.

IR ν_{max} (film)	3050-2927, 2854-2777 (CHs), 1605 (C=C), 1508, 1485 (CH_2), 1465 (OCH_3), 1241, 1174 (C-O-C) cm^{-1}
^1H NMR $\delta(CDCl_3)$	2.56 (6H, s, $N(CH_3)_2$), 2.87 (2H, m, NCH_2), 3.03 (2H, m, H-3), 3.80 (3H, s, OCH_3), 4.23 (2H, m, CH_2O), 4.60 (2H, m, H-2), 6.50-7.21 (12H, m, ArH)
^{13}C NMR $\delta(CDCl_3)$	34.18 (C-3), 45.20 ($N(CH_3)_2$), 55.25 (OCH_3), 57.54 (NCH_2), 64.67 (CH_2O), 80.43 (C-2), 113.74, 113.95 (C-3', C-5'), 115.76 (C-2''), 120.74 (C-4''), 121.92, 122.73 (C-4, C-9), 123.36 (C-7), 123.74 (C-5''), 128.13 (C-6''), 128.13 (C-6), 129.20, 129.47 (C-2', C-6'), 131.97 (C-8), 132.38 (C-5)
EIMS (HR)	$C_{27}H_{29}NO_3$: calculated M^+ 415.2116 observed M^+ 415.2147
Mass Spectrum (m/z)	415 (M^+, 100%), 357 (M^+-58, 10%), 328 (M^+-87, 10%), 311 (M^+-104, 10%), 269 (M^+-146, 12%), 252 (M^+-163, 33%), 239 (M^+-176, 40%), 226 (M^+-189, 35%) 189 (M^+-226, 21%), 165 (M^+-250, 67%)

5-[(4-Dimethylaminoethoxy)phenyl]-4-(4-methylphenyl)-2,3-dihydro-1-benzoxepin (224)

The general method 2.12 was applied using 5-(4-hydroxyphenyl)-4-(4-methylphenyl)-2,3-dihydro-1-benzoxepin (0.00012M), dry acetone (20mL), anhydrous K_2CO_3 (0.0012M) and 2-dimethylaminoethylchloride hydrogenchloride (0.00031M) under argon. The reaction mixture was refluxed for 4-6hr. The product was isolated using column chromatography (eluant dichloromethane : ethyl acetate : methanol; 90:5:5) to afford a light brown coloured gel in 64% yield, with the following physical properties.

IR ν_{max} (film)	2924, 2856-2772 (CHs), 1606, 1575 (C=C), 1477, 1461 (CH_2), 1376 (CH_3), 1239, 1174 (C-O-C) cm^{-1}
^1H NMR $\delta(CDCl_3)$	2.30 (3H, s, CH_3), 2.36 (6H, s, $N(CH_3)_2$), 2.69-2.80 (4H, m, NCH_2, H-3), 4.13 (2H, m, CH_2O), 4.62 (2H, m, H-2), 6.67-6.69 (2H, m, H-3'', H-5''), 6.86-7.19 (10H, m, ArH)
^{13}C NMR $\delta(CDCl_3)$	20.64 (CH_3), 35.24 (C-3), 45.34 ($N(CH_3)_2$), 57.79 (NCH_2), 65.28 (CH_2O), 80.03 (C-2), 113.24, 113.61 (C-3'', C-5''), 121.50, 121.57 (C-4, C-9), 122.92 (C-7), 127.79 (C-6), 128.23 (C-4''), 128.28, 128.36 (C-2'', C-6''), 128.83, 128.92 (C-3', C-5'), 130.52, 130.67 (C-2', C-6'), 131.35 (C-5), 131.99 (C-8)
EIMS (HR)	$C_{27}H_{29}NO_2$: calculated M^+ 399.2195 observed M^+ 399.2198
Mass Spectrum (m/z)	399 (M^+, 100%), 341 (M^+-58, 8%), 295 (M^+-104, 15%), 265 (M^+-134, 15%), 252 (M^+-147, 31%), 239 (M^+-160, 43%), 226 (M^+-173, 42%) 189 (M^+-210, 15%), 165 (M^+-234, 62%)

5-[(4-Dimethylaminoethoxy)phenyl]-4-(3-methylphenyl)-2,3-dihydro-1-benzoxepin (225)

The general method 2.12 was employed using 5-(4-hydroxyphenyl)-4-(3-methylphenyl)-2,3-dihydro-1-benzoxepin (0.00006M), dry acetone (15mL), anhydrous K_2CO_3 (0.0006M) and 2-dimethylaminoethylchloride

hydrogenchloride (0.00017M) under nitrogen. The product was isolated directly to present a light brown coloured oil in 41% yield.

IR ν_{max} (film)	3051-2925, 2854-2775 (CHs), 1606 (C=C), 1482, 1462 (CH_2), 1376 (CH_3), 1242, 1174 (C-O-C) cm^{-1}
^1H NMR $\delta(CDCl_3)$	2.37 (3H, s, CH_3), 2.65 (6H, s, $N(CH_3)_2$), 2.69-2.73 (4H, m, NCH_2, H-3), 4.01 (2H, t, J = 5.78Hz, CH_2O), 4.61 (2H, m, H-2), 6.63-6.68 (2H, d, J = 8.52Hz, H-2'', H-4''), 6.88-7.26 (10H, m, ArH)
^{13}C NMR $\delta(CDCl_3)$	22.64 (CH_3), 35.57 (C-3), 45.20, 45.79 ($N(CH_3)_2$), 58.19 (NCH_2), 65.61 (CH_2O), 80.48 (C-2), 113.54, 117.04 (C-3', C-5'), 121.98 (C-4, C-9), 123.45 (C-7), 126.68 (C-2''), 126.82 (C-6), 126.99 (C-4'), 127.73 (C-4''), 129.99, 130.94 (C-2', C-6'), 132.38 (C-5, C-8)
EIMS (HR)	$C_{27}H_{29}NO_2$: calculated M^+ 399.2195 observed M^+ 399.2198
Mass Spectrum (m/z)	399 (M^+, 100%), 341 (M^+-58, 8%), 295 (M^+-104, 20%), 281 (M^+-118, 25%), 265 (M^+-134, 20%), 252 (M^+-147, 38%), 239 (M^+-160, 45%), 226 (M^+-173, 20%) 202 (M^+-197, 20%), 189 (M^+-210, 21%), 165 (M^+-234, 50%)

5-[(4-Dimethylaminoethoxy)phenyl]-4-(2-methylphenyl)-2,3-dihydro-1-benzoxepin (226)

The general method 2.12 was applied with the use of 5-(4-hydroxyphenyl)-4-(2-methylphenyl)-2,3-dihydro-1-benzoxepin (0.00006M), dry acetone (15mL), anhydrous K_2CO_3 (0.0006M) and 2-dimethylaminoethyl chloride hydrogenchloride (0.00017M) under nitrogen. Column chromatography (eluant dichloromethane : ethyl acetate : methanol; 90:5:5) was used to purify the product to present a light brown coloured oil in 45% yield.

IR ν_{max} (film)	3053-2926, 2853 (CHs), 1603 (C=C), 1482, 1464 (CH_2), 1366 (CH_3), 1264, 1173 (C-O-C) cm^{-1}
^1H NMR $\delta(CDCl_3)$	2.32 (3H, s, CH_3), 2.36 (6H, s, $N(CH_3)_2$, 2.51-2.63 (4H, m, NCH_2, H-3), 4.51 (2H, t, J = 6.02Hz,

CH_2O), 4.64 (2H, m, H-2), 6.61-6.63 (2H, m, H-4'', H-5''), 6.79-7.29 (10H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 19.60 (CH_3), 37.56 (C-3), 45.12, 45.20 ($N(CH_3)_2$), 56.57 (NCH_2), 66.50 (CH_2O), 79.39 (C-2), 113.01, 114.63 (C-3', C-5'), 121.01, 121.36 (C-4, C-9), 123.01 (C-7), 125.18 (C-3'') 126.12 (C-6), 127.94 (C-4''), 130.60 (C-6'') 131.17, 131.24 (C-8)

EIMS (HR) $C_{27}H_{29}NO_2$: calculated M^+ 399.2182

observed M^+ 399.2198

4-(4-Chlorophenyl)-5-[(4-dimethylaminoethoxy)phenyl]-2,3-dihydro-1-benzoxepin (227)

The general method 2.12 was employed using 4-(4-chlorophenyl)-5-(4-hydroxyphenyl)-2,3-dihydro-1-benzoxepin (0.000062M), dry acetone (20mL), anhydrous K_2CO_3 (0.0006M) and 2-dimethylaminoethylchloride hydrogenchloride (0.00016M) under argon. The product was isolated using column chromatography (eluant dichloromethane : ethyl acetate : methanol; 70:28:2) to afford a light brown / mustard coloured oil in 95% yield, with the following physical properties.

IR ν_{max} (film) 3058-2926, 2870-2774 (CHs), 1602 (C=C), 1491, 1465 (CH_2), ~1284 (C-Cl), 1243, 1173 (C-O-C) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 2.36 (6H, s, $N(CH_3)_2$), 2.74 (2H, m, NCH_2), 2.92 (2H, t, J = 6.78Hz, H-3), 4.05 (2H, m, CH_2O), 4.62 (2H, m, H-2), 6.69-7.45 (12H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 35.45 (C-3), 45.79 ($N(CH_3)_2$), 58.20 (NCH_2), 65.65 (CH_2O), 80.38 (C-2), 113.74, 115.77 (C-3', C-5'), 120.74, 121.92 (C-4, C-9), 123.36 (C-7), 128.13, 128.53 (C-3'', C-5''), 128.77 (C-6), 129.20, 129.47 (C-2', C-6'), 130.79, 130.95 (C-2'', C-6''), 131.36, 134.97 (C-5, C-8) 132.38 (C-4'')

EIMS (HR) $C_{26}H_{26}NClO_2$: calculated M^+ 419.1645

observed M^+ 419.1652

5-[(4-Dimethylaminoethoxy)phenyl]-4-(1-naphthyl)-2,3-dihydro-1-benzoxepin (228)

The general method 2.12 was applied using 5-(4-hydroxyphenyl)-4-(1-naphthyl)-2,3-dihydro-1-benzoxepin (0.0001M), dry acetone (15mL), anhydrous K_2CO_3 (0.001M) and 2-dimethylaminoethylchloride hydrogenchloride (0.00034M) under nitrogen. The reaction mixture was refluxed for 6-8hours. The product was purified using flash column chromatography (eluant dichloromethane : ethyl acetate : methanol; 70:20:5) to afford a light brown coloured oily gel in 48% yield, with the following physical properties;

IR ν_{max} (film)	3056-2928, 2864-2771 (CHs), 1606 (C=C), 1482, 1464 (CH_2), 1394 (CH_3), 1243, 1174 (C-O-C) cm^{-1}
^1H NMR $\delta(CDCl_3)$	2.90 (6H, s, $N(CH_3)_2$), 2.64 (2H, t, J = 5.76Hz, NCH_2), 2.89-2.96 (2H, m, H-3), 3.91 (2H, t, J = 5.78Hz, CH_2O), 4.61-4.68 (2H, m, H-2), 6.49-8.03 (15H, m, ArH)
^{13}C NMR $\delta(CDCl_3)$	35.71 (C-3), 45.32 ($N(CH_3)_2$), 57.73 (NCH_2), 65.20 (CH_2O), 79.68 (C-2), 113.00, 113.35 (C-3', C-5'), 121.63 (C-4, C-9), 123.05 (C-7), 125.09-125.58 (C-3'', C-6'', C-7''), 126.44-127.97 (C-2'', C-4'', C-5'', C-8''), 128.07 (C-6), 131.97 (C-8), 133.27 (C-9''), 133.31 (C-10'', C-5)
EIMS (HR)	$C_{30}H_{29}NO_2$: calculated M^+ 435.2208 observed M^+ 435.2198
Mass Spectrum (m/z)	435 (M^+, 100%), 378 (M^+-57, 4%), 364 (M^+-71, 5%), 347 (M^+-88, 4%), 331 (M^+-104, 15%), 313 (M^+-122, 16%), 289 (M^+-146, 27%), 263 (M^+-172, 12%), 239 (M^+-196, 22%) 215 (M^+-220, 11%), 189 (M^+-246, 10%), 165 (M^+-270, 20%)

5-[(4-Dimethylaminoethoxy)phenyl]-4-(3-nitrophenyl)-2,3-dihydro-1-benzoxepin (229)

The general method 2.12 was applied using 5-(4-hydroxyphenyl)-4-(3-nitrophenyl)- 2,3-dihydro-1-benzoxepin (0.00008M), dry acetone (15mL), anhydrous K_2CO_3 (0.0008M) and 2-dimethylaminoethylchloride

hydrogenchloride (0.0003M) under nitrogen. The product was isolated using column chromatography (eluant dichloromethane : ethyl acetate : methanol; 60:25:15) to afford a mustard coloured oil in 78% yield.

IR ν_{max} (film) 3035-2926, 2855-2707 (CHs), 1605 (C=C), 1530 (C-NO_2), 1482, 1442 (CH_2), 1351 (CH_3, Ar-NO_2), 1243, 1176 (C-O-C) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 3.04 (6H, s, $N(CH_3)_2$), 3.48-3.63 (4H, m, NCH_2, H-3), 4.55-4.64 (4H, m, CH_2O, H-2), 6.66-7.92 (12H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 35.68 (C-3), 44.11 ($N(CH_3)_2$), 58.64 (NCH_2), 62.54 (CNO_2), 63.32 (CH_2O), 80.38 (C-2), 113.64, 115.13 (C-3', C-5'), 121.19, 121.99 (C-4, C-9), 123.46 (C-7), 123.62 (C-2''), 128.60 (C-6), 128.94 (C-2', C-6', C-4''), 130.79 (C-5''), 130.95 (C-8), 132.72 (C-5), 137.29 (C-6'')

EIMS (HR) $C_{26}H_{26}N_2O_4$: calculated M^+ 430.1987

observed M^+ 430.1893

4-(4-Cyanophenyl)-5-[(4-dimethylaminoethoxy)phenyl]-2,3-dihydro-1-benzoxepin (230)

The general method 2.12 was employed using 4-(4-cyanophenyl)-5-(4-hydroxyphenyl)-2,3-dihydro-1-benzoxepin (0.000062M), dry acetone (20mL), anhydrous K_2CO_3 (0.0006M) and 2-dimethylaminoethylchloride hydrogenchloride (0.00016M) under nitrogen. The product was purified using flash column chromatography (eluant dichloromethane : ethyl acetate : methanol; 40:60:10) to afford a light brown coloured oil in 83% yield.

IR ν_{max} (film) 2923, 2880-2850 (CHs), 2225 (C≡N), 1608 (C=C), 1467, 1434 (CH_2), 1372 (CH_3), 1259, 1156 (C-O-C) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 2.19 (6H, s, $N(CH_3)_2$), 2.37 (2H, m, NCH_2), 2.83 (2H, m, H-3), 3.37 (2H, m, CH_2O), 4.35 (2H, m, H-2), 6.68-7.88 (12H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 33.99 (C-3), 44.50 ($N(CH_3)_2$), 56.94 (NCH_2), 65.41 (CH_2O), 113.87 (C-3', C-5'), 122.20 (C-4, C-9), 124.94 (C-7), 128.98 (C-6), 129.92 (C-2', C-6'), 130.17 (C-3'', C-5''), 131.85 (C-8), 132.66 (C-5), 135.14 (C-2'', C-6'')

EIMS (HR) $C_{27}H_{26}N_2O_2$: calculated M^+ 410.2003

observed M^+ 410.1994

5-[(4-Dimethylaminoethoxy)phenyl]-4-(4-formylphenyl)-2,3-dihydro- 1-benzoxepin (231)

The general method 2.12 was employed using 4-(4-formylphenyl)-5-(4-hydroxyphenyl)-2,3-dihydro-1-benzoxepin (0.00006M), dry acetone (20mL), anhydrous K_2CO_3 (0.0006M) and 2-dimethylaminoethylchloride hydrogenchloride (0.00019M) under nitrogen. The product was isolated using column chromatography (eluant dichloromethane : ethyl acetate : methanol; 70:25:5) to afford a light brown coloured oil in 74% yield.

IR ν_{max} (film) 3053-2926, 2853 (CHs), 1724 (C=O), 1464, 1429 (CH_2), 1372 (CH_3), 1376 (CH_3), 1264, 1108 (C-O-C) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 2.42 (6H, s, $N(CH_3)_2$), 2.98-3.07 (4H, m, NCH_2, H-3), 4.16 (2H, m, CH_2O), 4.51 (2H, m, H-2), 7.02-8.06 (12H, m, ArH), 10.77 (1H, s, CHO)

^{13}C NMR $\delta(CDCl_3)$ 33.40 (C-3), 43.65 ($N(CH_3)_2$), 57.03, 57.39 (NCH_2), 63.91 (CH_2O), 79.94 (C-2), 114.34 (C-3', C-5'), 119.57 (C-9), 120.91 (C-4), 123.61 (C-7), 126.00 (C-6), 127.02, 128.07 (C-2', C-6'), 129.21, 129.59 (C-2'', C-3'', C-5'', C-6''), 131.64 (C-8), 134.67 (C-1'', C-5), 172.64 (C=O)

EIMS (HR) $C_{27}H_{27}NO_3$: calculated M^+ 413.2025

observed M^+ 413.1991

5-[(4-Dimethylaminoethoxy)phenyl]-4-(3-pyridinyl)-2,3-dihydro-1-benzoxepin (232)

The general method 2.12 was applied using 5-(4-hydroxyphenyl)-4-(3-pyridinyl)-2,3-dihydro-1-benzoxepin (0.00011M), dry acetone (20mL), anhydrous K_2CO_3 (0.0011M) and 2-dimethylaminoethylchloride hydrogenchloride (0.00037M) under nitrogen. The product was purified using column chromatography (eluant dichloromethane : ethyl acetate : methanol; 70:25:5) to afford a brown / mustard coloured oil in 49% yield, with the following physical properties.

IR ν_{max} (film) 3047-2919, 2850 (CHs), 1662, 1570 (pyrindyl peaks), 1607 (C=C), 1483, 1442 (CH_2), 1265, 1174 (C-O-C) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 2.36 (6H, s, $N(CH_3)_2$), 2.72-2.75 (4H, m, NCH_2, H-3), 4.02 (2H, t, J = 5.76Hz, CH_2O), 4.63 (2H, t, J = 6.02Hz, H-2), 6.68-7.61 (12H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 34.88 (C-3), 45.23 ($N(CH_3)_2$), 57.65 (NCH_2), 65.22 (CH_2O), 79.76 (C-2), 113.64 (C-3', C-5'), 121.68 (C-3''), 123.20 (C-7), 128.41 (C-6), 129.32 (C-2', C-6'), 130.60 (C-8), 132.07 (C-6), 136.36 (C-1'), 146.78 (C-6''), 139.58 (C-4''), 149.83 (C-2''),

EIMS (HR) $C_{25}H_{26}N_2O_2$: calculated M^+ 386.1998

observed M^+ 386.1994

4-(Benzo[b]furyl)-5-[(4-dimethylaminoethoxy)phenyl]-2,3-dihydro-1-benzoxepin (233)

The general method 2.12 was applied with the use of 4-(benzo[b]furyl)- 5-(4-hydroxyphenyl)-2,3-dihydro-1-benzoxepin (0.000062M), dry acetone (15mL), anhydrous K_2CO_3 (0.0006M) and 2-dimethylaminoethylchloride hydrogenchloride (0.00022M) under nitrogen. Column chromatography (eluant dichloromethane : ethyl acetate : methanol; 60:40:2) was used to purify the product to present a light brown coloured oil in 68% yield.

IR ν_{max} (film) 2924, 2853-2772 (CHs), 1606 (C=C), 1509, 1482, 1463 (CH_2), 1363 (CH_3), 1239, 1173 (C-O-C) cm^{-1}

^{1}H NMR δ($CDCl_3$) 2.45 (6H, s, $N(CH_3)_2$), 2.80 (2H, m, NCH_2), 2.94 (2H, t, J = 5.76Hz, H-3), 4.40 (2H, t, J = 5.52Hz, CH_2O), 4.61 (2H, t, J = 5.78Hz, H-2), 6.82-7.89 (12H, m, ArH)

^{13}C NMR δ($CDCl_3$) 41.07 (C-3), 45.64 ($N(CH_3)_2$), 58.05 (NCH_2), 65.98 (CH_2O), 78.36 (C-2), 111.71 (CH), 114.01, 116.25 (C-3', C-5'), 121.38, 121.92 (C-4, C-9), 123.36 (C-7), 124.09, 125.92 (C-3'', C-4''), 128.22 (C-6), 131.07 (C-8), 132.07 (C-5), 155.22, 156.37 (C-1'', C-6'')

EIMS (HR) $C_{28}H_{27}NO_3$: calculated M^+ 425.1957

observed M^+ 425.1991

5-[(4-Dimethylaminoethoxy)phenyl]-4-(3-thienyl)-2,3-dihydro-1-benzoxepin (234)

The general method 2.12 was employed using 5-(4-hydroxyphenyl)-4-(3-thienyl)- 2,3-dihydro-1-benzoxepin (0.00007M), dry acetone (20mL), anhydrous K_2CO_3 (0.0007M) and 2-dimethylaminoethylchloride hydrogenchloride (0.00022M) under nitrogen. The product was purified using flash column chromatography (eluant dichloromethane : ethyl acetate : methanol; 50:40:10) to afford a light brown coloured oil in 71% yield.

IR ν_{max} (film) 2927-2869, 2821-2772 (CHs), 1606, 1570 (C=C), 1482, 1465 (CH_2), 1243, 1172 (C-O-C) cm^{-1}

^{1}H NMR δ($CDCl_3$) 2.41 (6H, s, $N(CH_3)_2$), 2.73 (2H, t, J = 6.04Hz, NCH_2), 2.81(2H, t, J = 5.78Hz, H-3), 4.09 (2H, t, J = 5.78Hz, CH_2O), 4.65 (2H, t, J = 6.02Hz, H-2), 6.73-7.28 (11H, m, ArH)

^{13}C NMR δ($CDCl_3$) 34.82 (C-3), 45.16 ($N(CH_3)_2$), 57.64 (NCH_2), 65.11 (CH_2O), 80.23 (H-2), 113.51 (C-3', C-5'), 121.62, 122.55 (C-4, C-9), 123.07 (C-7), 124.24 (C-2'', C-3''), 127.89 (C-4''), 128.62 (C-6, C-1''), 130.45 (C-2', C-6'), 131.46 (C-8), 132.02 (C-5)

EIMS (HR) $C_{24}H_{25}NSO_2$: calculated M^+ 391.1609

observed M^+ 391.1606

Mass Spectrum (m/z) 391 (M^+, 100%), 320 (M^+-71, 5%), 287 (M^+-104, 29%), 269 (M^+-122, 26%), 245 (M^+-146, 29%), 213 (M^+-178, 17%) 189 (M^+-202, 24%), 165 (M^+-226, 36%)

5-[(4-Dimethylaminoethoxy)phenyl]-4-(2-furyl)-2,3-dihydro-1-benzoxepin (235)

The general method 2.12 was applied with the use of 4-(2-furyl)-5-(4-hydroxyphenyl)-2,3-dihydro-1-benzoxepin (0.00015M), dry acetone (15mL), anhydrous K_2CO_3 (0.0015M) and 2-dimethylaminoethyl chloride hydrogenchloride (0.00048M) under nitrogen. Column chromatography (eluant dichloromethane : ethyl acetate : methanol; 60:40:2) was used to purify the product to afford a dark brown coloured oil in 38% yield.

IR ν_{max} (film) 3050-2916, 2848-2775 (CHs), 1606 (C=C), 1509, 1464 (CH_2), 1365 (CH_3), 1244, 1174 (C-O-C) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 2.40 (6H, s, $N(CH_3)_2$), 2.76 (4H, m, NCH_2, H-3), 4.11 (2H, m, CH_2O), 4.68 (2H, m, H-2), 5.71-5.75 (1H, m, H-4''), 6.23-6.26 (1H, m, H-3''), 6.77-7.25 (9H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 32.37 (C-3), 45.75 ($N(CH_3)_2$), 58.19 (NCH_2), 65.65 (CH_2O), 80.02 (C-2), 109.70 (C-4''), 111.57 (C-3''), 113.43-114.37 (C-3', C-5'), 120.40, 121.14 (C-4, C-9), 123.51 (C-7), 128.36 (C-6), 128.55, 129.29 (C-2', C-6'), 131.66 (C-8), 132.34 (C-5), 140.56 (C-5a)

EIMS (HR) $C_{24}H_{25}NO_3$: calculated M^+ 375.1845

observed M^+ 375.1834

Mass Spectrum (m/z) 375 (M^+, 100%), 316 (M^+-59, 4%), 287 (M^+-88, 3%), 255 (M^+-120, 6%), 231 (M^+-144, 13%), 202 (M^+-173, 16%) 165 (M^+-210, 10%), 139 (M^+-236, 6%)

7.3.11. Preparation of nitrobenzoxepins

7- and 9-Nitro-2,3-dihydro-1-benzoxepin-5-one (236)

White fuming nitric acid (14mL) was added to a stirred solution of 2,3,4,5-tetrahydro-1-benzoxepin-5-one (0.0092M) in conc. nitric acid (12.5mL) at –30°C, at a rate such that the temperature was maintained between –5 and -10°C. The solution was stirred for 0.5-1 hour at -10°C, then poured into ice-water (100mL), extracted with dichloromethane (3x20mL) and the organic layer was washed with water. Ethyl acetate was used to extract the aqueous layer, washed with water and brine. The organic layer and the extracts from the aqueous layer were combined and concentrated under reduced pressure. Column chromatography (eluant petroleum ether : ethyl acetate : methanol; 90:5:5) was carried out to separate the 7-nitro and 9-nitro compounds.[344] Recrystallisation from ethanol was used to purify the products further. A small quantity of mixed isomers were recovered in 22% yield as a mustard coloured oily gel.

7-Nitro-2,3-dihydro-1-benzoxepin-5-one (237)

The pure 7-nitro isomer was isolated in 41% yield as lemon coloured oily crystals.

IR ν_{max} (film) 3103-3023, 2886-2779 (CHs), 1692 (C=O), 1577 (C=C), 1534, 1360 (Ar-NO_2), 1493, 1450 (CH_2), 1277 (C-O-C) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 2.32 (2H, t, J = 6.78Hz, H-3), 2.95 (2H, t, J = 7.28Hz, H-2), 4.36 (2H, t, J = 6.54Hz, H-4), 7.18 (1H, d, J = 9.02Hz, H-9), 8.23-8.26 (1H, dd, J = 9.00, 3.00Hz, H-8), 8.63 (1H, d, J = 3.04Hz, H-6)

^{13}C NMR $\delta(CDCl_3)$ 26.05 (C-3), 40.23 (C-4), 73.40 (C-2), 121.17 (C-9), 122.33 (C-8), 125.70 (C-6), 128.13 (C-7), 133.39 (C-5a), 207.91 (C=O)

9-Nitro-2,3-dihydro-1-benzoxepin-5-one (238)

The pure 9-nitro isomer was isolated as an orange coloured oil in 9% yield.

IR ν_{max} (film) 3102-2995, 2888-2830 (CHs), 1687 (C=O), 1608,1575 (C=C), 1531, 1328 (Ar-NO_2), 1485, 1460 (CH_2), 1280 (C-O-C) cm^{-1}

^{1}H NMR δ($CDCl_3$) 2.27 (2H, t, J = 7.02Hz, H-3), 2.95 (2H, t, J = 6.78Hz, H-2), 4.43 (2H, t, J = 6.76Hz, H-4), 7.22 (1H, t, J = 8.52Hz, H-7), 7.89-8.00 (2H, dd, J = 8.54, 2.00Hz, H-8, H-6)

^{13}C NMR δ($CDCl_3$) 26.25 (C-3), 40.51 (C-4), 75.23 (C-2), 122.01 (C-7), 122.45 (C-8), 126.32 (C-6), 129.12 (C-9), 135.60 (C-5a), 204.11 (C=O)

7.3.12. Preparation of 5-aryl-7-nitro-benzoxepin

5-(4-Methoxyphenyl)-7-nitro-2,3-dihydro-1-benzoxepin (240)

To a solution of *n*-BuLi (0.0038M), 4-bromoanisole (0.0038M) and dry THF (30mL) was added 7-nitro-2,3-dihydro-1-benzoxepin-5-one (0.0015M) dropwise under nitrogen at -78°C. [For further details review the general method 3.18] The compound was dehydrated with H_2SO_4 (3mL) in ethanol (20mL). The product was then purified using column chromatography (eluant petroleum ether : ethyl acetate : dichloromethane; 90:5:5) to afford a dark brown coloured oily gel in 59% yield (rotamers; ^{A,B}).

IR ν_{max} (film) 2955-2924, 2851 (CHs), 1605, 1572 (C=C), 1510 (CH_2), 1464 (OCH_3), 1492, 1342 (Ar-NO_2), 1248, 1179 (C-O-C) cm^{-1}

^{1}H NMR δ($CDCl_3$) 2.47^{A}, 2.62^{B} (2H, m, H-3), 3.84^{A}, 3.87^{B} (3H, s, OCH_3), 4.46^{A}, 4.58^{B} (2H, m, H-2), 6.28^{A}, 6.37^{B} (1H, m, H-4), 6.78-8.09 (7H x 2, m, ArH)

^{13}C NMR δ($CDCl_3$) 31.86^{A}, 32.39^{B} (C-3), 55.21^{A}, 55.40^{B} (OCH_3), 77.21^{A}, 77.96^{B} (C-2), 112.44, 114.89 (C-3', C-5'), 121.31 (C-9), 122.12^{A}, 122.59^{B} (C-8), 123.14^{A}, 123.50^{B} (C-6), 126.35^{A}, 126.85^{B} (C-7), 131.75 (C-5), 133.67 (C-5a, C-9a), 158.47 (C-4)

EIMS (LR) $C_{17}H_{15}NO_4$: calculated M^+ 297

observed M^+ 297

Mass Spectrum (m/z) 297 (M^+, 100%), 282 (M^+-15, 7%), 266 (M^+-31, 9%), 251 (M^+-46, 8%), 222 (M^+-75, 5%), 192 (M^+-105, 4%) 190 (M^+-107, 10%), 178 (M^+-119, 13%)

7.3.13. Synthesis of 4-bromo-7-nitro-benzoxepin

4-Bromo-5-(4-methoxyphenyl)-7-nitro-2,3-dihydro-1-benzoxepin (241)

The procedure 3.3 was incorporated using 5-(4-methoxyphenyl)-7-nitro-2,3-dihydro-1-benzoxepin (0.0005M) in dry dichloromethane (20mL) and pyridinium bromide perbromide (0.00076M). The solution was allowed to stir at room temperature for 12hr. Column chromatography (eluant petroleum ether : ethyl acetate : dichloromethane; 95:5:5) was carried out to purify the product as a wine coloured oil in 22% yield.

IR ν_{max} (film) 2955-2916, 2847 (CHs), 1609, 1567 (C=C), 1548, 1341 (Ar-NO_2), 1508, 1479 (CH_2), 1459 (OCH_3), 1246 (C-O-C) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 3.13 (2H, t, J = 5.78Hz, H-3), 3.89 (3H, s, OCH_3), 4.69 (2H, t, J = 5.52Hz, H-2), 6.95-6.98 (2H,d, J = 8.52Hz, H-8, H-9), 7.15-7.74 (4H, m, ArH), 8.05-8.08 (1H, dd, J = 9.04, 3.00Hz, H-6)

^{13}C NMR $\delta(CDCl_3)$ 40.96 (C-3), 54.78 (OCH_3), 76.71 (C-2), 113.59, 115.22 (C-3', C-5'), 122.34 (C-9), 123.50 (C-8), 124.61 (C-6), 127.12 (C-7), 130.45 (C-2', C-6'), 132.43 (C-5a, C-9a), 158.87 (C-4)

EIMS (LR) $C_{17}H_{14}BrNO_4$: calculated M^+ 376

observed M^+ 377 and 375

Mass Spectrum (m/z) 377 (M^+, 100%), 377 (M^+-2, 88%), 347 (M^+-30, 5%), 296 (M^+-80, 47%), 282 (M^+-95, 7%), 270 (M^+-107, 37%), 250 (M^+-127, 25%), 235 (M^+-122, 19%), 224 (M^+-153, 15%), 205 (M^+-172, 21%), 189 (M^+-282, 32%) 176 (M^+-201, 57%), 152 (M^+-225, 41%)

7.3.14.Suzuki coupling of 7-nitro benzoxepin

5-(4-Methoxyphenyl)-7-nitro-4-phenyl-2,3-dihydro-1-benzoxepin (242)

The general method 3.9 was employed using 4-bromo-5-(4-hydroxyphenyl)-7-nitro-2,3-dihydro-1-benzoxepin (0.00005M), dry THF (20mL) and $Pd(PPh_3)_4$ (10mg). To this was added phenylboronic acid (0.00018M) and 2M sodium carbonate (0.0004M) and the resulting solution was refluxed at 80°C for 6-8hr. The product was isolated using flash column chromatography (eluant petroleum ether : ethyl acetate; 95:5) to afford a light brown coloured oil in 41% yield.

IR ν_{max} (film)	3051-2926, 2850 (CHs), 1606, 1571 (C=C), 1519, 1343 (Ar-NO_2), 1462 (CH_2), 1375 (OCH_3), 1247, 1171 (C-O-C) cm^{-1}
^1H NMR $\delta(CDCl_3)$	2.79 (2H, t, J = 5.76Hz, H-3), 3.78 (3H, s, OCH_3), 4.75 (2H, t, J = 5.76Hz, H-2), 6.68-7.82 (12H, m, ArH)
^{13}C NMR $\delta(CDCl_3)$	35.14 (C-3), 54.66 (OCH_3), 80.82 (C-2), 113.13, 114.82 (C-3', C-5'), 120.31 (C-4), 122.58 (C-9), 123.19 (C-8), 126.38 (C-6), 126.59 (C-4''), 127.69 (C-7), 128.38, 128.82 (C-2'', C-6''), 129.18 (C-3'', C-5''), 130.42, 130.45 (C-2', C-6'), 131.87 (C-5a, C-9a)
EIMS (LR)	$C_{23}H_{19}NO_4$: calculated M^+ 373 observed M^+ 373
Mass Spectrum (m/z)	373 (M^+, 100%), 345 (M^+-28, 9%), 282 (M^+-91, 5%), 270 (M^+-103, 64%), 253 (M^+-120, 15%), 239 (M^+-134, 19%), 224 (M^+-149, 27%)

7.3.15.Synthesis of 4-arylmethylene benzoxepin-5-one

General Method 3.15

2,3,4,5-tetrahydro-1-benzoxepin-5-one (0.004M) and the appropriately substituted benzaldehyde (0.004M) were dissolved in ethanol (15mL) and stirred gently. HCl gas was bubbled through the solution, until the reaction was observed to be complete via TLC monitoring. The solution was stirred in an ice

cool bath for a further 12hr, after which a solid precipitate is observed. The product is filtered, washed with water and sodium metabisulphite (40%). Column chromatography and / or recrystallisation were carried out to purify the compound.

4-Phenylmethylene-2,3,4,5-tetrahydro-1-benzoxepin-5-one (245)

The general method 3.15 was employed using 2,3,4,5-tetrahydro-1-benzoxepin-5-one (0.003M) and benzaldehyde (0.003M). Column chromatography (eluant dichloromethane : petroleum ether; 75:15) was used to purify the product as a lemon coloured oil[482] in 38% yield.

IR ν_{max} (film) 3107-3016, 2993-2797 (CHs), 1676 (C=O), 1605, 1574 (C=C), 1456 (CH_2), 1105 (C-O-C) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 3.07 (2H, t, J = 5.68Hz, H-3), 4.38 (2H, t, J = 5.68Hz, H-2), 7.08-7.75 (10H, m, 9ArH, 1vinyl H)

^{13}C NMR $\delta(CDCl_3)$ 28.71 (C-3), 57.65 (C-4), 72.27 (C-2), 119.55 (C-8), 122.46 (C-9), 128.86 (C-5a), 128.86 (C-2', C-6'), 129.34 (C-6), 129.56, 130.94 (C-3', C-4', C-5'), 133.39 (C-7), 136.78 (C-1'), 161.78 (C-9a), 199.13 (C-5)

4-(4-Methylphenyl)methylene-2,3,4,5-tetrahydro-1-benzoxepin-5-one (246)

The general method 3.15 was applied using 2,3,4,5-tetrahydro-1-benzoxepin-5-one (0.012M) and *p*–tolualdehyde (0.012M). The product was isolated from column chromatography (eluant dichloromethane : petroleum ether; 50:50), followed by recrystallisation from ethanol as a cream coloured crystalline material in 81% yield; m.p. 116.5-117.5°C [lit. m.p. 117-118°C][256]

IR ν_{max} (KBr) 3082-2999, 2976-2785 (CHs), 1665 (C=O), 1603 (C=C), 1451 (CH_2), 1376 (CH_3) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 2.36 (3H, s, CH_3), 2.97 (2H, t, J = 5.64Hz, H-3), 4.37 (2H, t, J = 5.64Hz, H-2), 7.07 (1H, d, J = 7.98Hz, H-9), 7.12 (1H, d, J = 7.56Hz H-8), 7.19-7.21 (2H, m, H-3', H-5'), 7.30-7.36 (1H, m, H-2', H-

6'), 7.42 (1H, t, J =7.76Hz, H-7), 7.78 (1H, s, H-10), 7.97 (1H, dd, J = 7.71, 1.76Hz, H-6)

^{13}C NMR $\delta(CDCl_3)$ 31.13 (CH_3), 32.25 (C-3), 56.76 (C-4), 72.28 (C-2), 119.93, 122.40 (C-8, C-9), 128.74 (C-5a), 128.98 (C-2', C-6'), 129.14 (C-3', C-5'), 129.38 (C-6), 133.30 (C-7), 135.36 (C-4'), 137.11 (C-1'), 161.04, 161.70 (C-9a), 199.12 (C-5)

7.3.16. Preparation of 4-arylmethylbenzoxepin-5-one

General Method 3.16

A suspension of the appropriately substituted 4-arylmethylenebenzoxepin (0.018M) in ethanol (40mL) was stirred under heat until complete dissolution occurred. 10% Pd (0.017M) on activated charcoal was added and the reaction mixture was stirred at room temperature under an atmosphere of H_2. Stirring was maintained until TLC analysis verified that hydrogenation of the starting material was complete. The catalyst was then removed via filtration, washed with ethanol and the solvent was evaporated under reduced pressure. The crude product was purified by recrystallisation from ethanol.

4-Phenylmethyl-2,3,4,5-tetrahydro-1-benzoxepin (247)

The general method 3.16 was applied using 4-phenylmethylene-2,3,4,5-tetrahydro-1-benzoxepin-5-one (0.0016M) in ethanol (25mL) and 10% Pd catalyst. The product was isolated directly as lemon coloured oil in 98% yield. Upon analysis an alternative product was produced where the carbonyl group was also hydrogenated to a CH_2.

IR ν_{max} (film) 3086-2995, 2983-2814 (CHs), 1602, 1579 (C=C), 1492, 1456 (CH_2), 1218 (C-O-C) cm^{-1}

^{1}H NMR $\delta(CDCl_3)$ 1.80 (1H, m, H-3), 1.95 (2H, m, H-5), 2.63 (1H, m, H-3), 2.75 (1H, m, H-10), 12.80 (1H, m, H-10), 3.76 (1H, dt, J = 12.23, 2.37Hz, H-2), (1H, m, H-2), 6.99-7.31 (7H, m, ArH), 7.19-7.21 (1H, d, J = 7.22Hz, H-7), 7.31-7.33 (1H, d, J = 7.55Hz, H-6)

^{13}C NMR $\delta(CDCl_3)$	37.44 (C-3), 37.85 (C-4), 39.47 (C-10), 42.24 (C-5), 71.42 (C-2), 12.59, 122.92 (C-8, C-9), 128.70 (C-2', C-6'), 129.11, 130.85 (C-6, C-3', C-5'), 133.01 (C-7), 140.05 (C-1', C-4')
EIMS (LR)	$C_{17}H_{18}O$: calculated M^+ 252.3122
	observed M^+ 238 (Product over-hydrogenated)

4-(4-Methylphenyl)methyl-2,3,4,5-tetrahydro-1-benzoxepin-5-one (248)

The procedure 3.16 outlined was employed using 4-(4-methylphenyl)methylene-2,3,4,5-terahydro-1-benzoxepin-5-one (0.018M) in ethanol (50mL) and 10% Pd catalyst. The product was isolated using column chromatography (eluant petroleum ether : ethyl acetate; 90:10) as yellow coloured oil[256] in 83% yield, with the following physical properties.

IR ν_{max} (film)	3080-2996, 2977-2827 (CHs), 1687 (C=O), 1600, 1573 (C=C), 1506, 1477, 1450 (CH_2), 1377 (CH_3), 1208 (C-O-C) cm^{-1}
^1H NMR $\delta(CDCl_3)$	1.79 (1H, m, H-3), 2.35 (3H, s, CH_3), 2.37-2.49 (1H, m, H-3), 2.79 (1H, dd, J = 13.89, 8.06Hz, H-10), 3.29 (1H, dd, J = 13.98, 6.31Hz, H-10), 3.40 (1H, m, H-4), 3.92-3.99 (1H, dt, J = 12.10, 4.99Hz, H-2), 4.49 (1H, ddd, J = 12.42, 6.95, 2.59Hz, H-2), 6.94-7.12 (6H, m, ArH), 7.42 (1H, dt, J = 7.88, 2.00Hz, H-7), 7.74 (1H, dd, J = 7.79, 1.67Hz, H-6)
^{13}C NMR $\delta(CDCl_3)$	20.94 (CH_3), 34.36 (C-3), 34.78 (C-10), 51.26 (C-4), 72.34 (C-2), 119.97, 122.24 (C-8, C-9), 128.86 (C-5a), 128.98 (C-2', C-6'), 129.03 (C-3', C-5', C-6), 132.74 (C-7), 135.18 (C-4'), 136.12 (C-1'), 161.91 (C-9a), 201.57 (C-5)

7.3.17. Preparation of substituted phenyl bromide

4-[2-(Dimethylamino)ethoxy]phenyl bromide (249)

The title compound was prepared using the general method 2.12 with 4-bromophenol (0.0059M), anhydrous K_2CO_3 (0.059M), dry acetone (25mL) and

2-dimethylaminoethylchloride hydrogenchloride (0.00095M). The reaction mixture was refluxed for 4-6hr under nitrogen. The product was isolated using flash column chromatography (eluant dichloromethane : methanol; 90:10) as a rusty / brown coloured oil[541] in 98% yield.

IR ν_{max} (film)	3104-3020, 3000-2912 (CHs), 1592 (C=C), 1488 (CH_2), 1376 (NCH_3), 1288, 1244 (C–N), 1100-1076 (C-Br) cm^{-1}
^{1}H NMR $\delta(CDCl_3)$	2.20 (6H, s, $N(CH_3)_2$), 2.58 (2H, t, J = 5.72Hz, CH_2N), 3.88 (2H, t, J = 5.71Hz, CH_2O), 6.65-6.69 (2H, dd, J = 5.50, 1.79Hz, H-3'', H-5''), 7.20-7.24 (2H, dd, J = 5.59, 2.16Hz, H-2'', H-6'')
^{13}C NMR $\delta(CDCl_3)$	45.29 ($N(CH_3)_2$), 57.58 (NCH_2), 65.76 (OCH_2), 112.27 (C-1''), 115.87 (C-3'', C-5''), 131.61 (C-2'',C-6''), 157.43 (C-4'')

7.3.18. Preparation of 4-arylmethyl-5-phenylbenzoxepin-5-ol

General Method 3.18

n-BuLi (0.011M, 2.5M in hexane) was added dropwise to a stirred solution of a substituted phenyl bromide (0.0041M) in dry THF (25mL) at –78°C under an atmosphere of nitrogen. This solution was allowed to stir for 1 hour under these conditions after which a solution of 4-(4-methylphenyl)methyl-2,3,4,5-tetrahydro-1-benzoxepin-5-one (0.0021M) in dry THF (20mL) was added slowly at -78°C. This reaction mixture for 1 hour at –78°C and then stirred at ambient temperature for 12hr. Post this period the reaction was extracted with diethyl ether (3x20mL), washed with water, saturated NaCl (20mL), dried over $MgSO_4$ and concentrated. Product purification was carried out using flash column chromatography.

Attempted preparation of 5-[(4-dimethylaminoethoxy)phenyl]-4-(4-methylphenyl)methyl-2,3-dihydro-1-benzoxepin-5-ol (250)

The general method 3.18 was employed using *n*-BuLi (3.2mL, 2.5M in Hexane), 4-[2-(dimethylamino)ethoxy]phenyl bromide (0.0023M) in dry THF (25mL) and 4-(4-methylphenyl)methyl-2,3,4,5-tetrahydro-1-benzoxepin-5-one (0.00075M) under nitrogen. Column chromatography (eluant dichloromethane : ethyl acetate

: petroleum ether : triethylamine; 85:10:5:0.2), was carried out to purify this product affording the starting material in 100% yield.

5-(4-Methoxyphenyl)-4-(4-methylphenyl)methyl-2,3,4,5-tetrahydro-1-benzoxepin-5-ol (251)

The general procedure 3.18 was applied using *n*-BuLi (7.0mL, 2.5M in Hexane), 4-bromoanisole (0.0047M) in dry THF (25mL) and 4-(4-methylphenyl)methyl-2,3,4,5-tetrahydro-1-benzoxepin-5-one (0.0023M) under nitrogen. The product was isolated using column chromatography (eluant dichloromethane : ethyl acetate; 98:2), as a light brown coloured oil[256] in 74% yield.

IR ν_{max} (film) 3602-3175 (OH), 3169-3034, 2991-2896 (CHs), 1610, (C=C), 1511, 1485 (CH_2), 1376 (CH_3) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 1.89 (1H, m, H-3), 2.08 (1H, s, OH ex. D_2O), 2.32 (3H, s, CH_3), 2.49-2.53 (1H, m, H-3), 2.90 (1H, m, H-4), 3.04 (2H, dd, J = 10.56, 3.48Hz, H-10), 3.80 (1H, m, H-2), 3.81 (3H, s, OCH_3), 4.01 (1H, m, H-2), 6.83-6.87 (2H, d, J = 8.52Hz, H-3'', H-5''), 7.04-7.43 (9H, m, ArH), 7.61-7.63 (1H, dd, J = 8.00, 1.48Hz, H-6)

^{13}C NMR $\delta(CDCl_3)$ 20.50 (CH_3), 28.05 (C-3), 29.23 (C-10), 49.95 (C-4), 54.75 (OCH_3), 67.59 (C-2), 112.99, 113.21 (C-3'', C-5''), 121.82, 123.70 (C-8, C-9), 126.57 (C-4''), 127.46 (C-5a), 128.43 (C-2'', C-6''), 128.53 (C-2', C-6'), 128.59 (C-6, C-3', C-5'), 134.81 (C-7), 136.66 (C-4'), 136.99 (C-1'), 156.31 (C-5), 158.43 (C-9a)

EIMS (LR) $C_{25}H_{26}O_3$: calculated M^+ 374

observed M^+ 374

7.3.19. Dehydration of 4-arylmethyl-5-phenyl-1-benzoxepin-5-ol

5-(4-Methoxyphenyl)-4-(4-methylphenyl)methyl-2,3-dihydro-1-benzoxepin (252)

A solution of 5-(4-methoxyphenyl)-4-(4-methylphenyl)methyl-2,3,4,5-tetrahydro-1-benzoxepin-5-ol (0.00088M) in EtOH (30mL) and 85%

polyphosphoric acid (0.12M) was refluxed for 2-3hr. The resulting solution was neutralised with NaOH (20% w/v, 20mL), washed with water (70mL), brine extracted with ethyl acetate (3x25mL), dried over Na_2SO_4 and concentrated. Column chromatography (eluant dichloromethane : petroleum ether; 40:60) to afford a cream coloured oil in 79% yield, with the following physical properties.

IR ν_{max} (film) 2984-2881, 2872-2809 (CHs), 1605, 1573 (C=C), 1483, 1462 (CH_2), 1444 (OCH_3), 1379 (CH_3) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 2.30 (2H, t, J = 6.28Hz, H-3), 2.35 (3H, s, CH_3), 3.64 (2H, s, H-10), 3.83 (3H, s, OCH_3), 4.34 (1H, t, J = 6.26Hz, H-2), 6.83-6.87 (2H,dd, J = 9.04, 1.70Hz, H-3'', H-5''), 6.98-7.28 (11H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ 20.54 (CH_3), 31.99 (C-3), 40.04 (C-10), 54.73 (OCH_3), 79.97 (C-2), 113.06 (C-3'', C-5''), 121.44, 122.79 (C-8, C-9), 127.35 (C-4''), 128.31 (C-6, C-2'', C-6''), 128.64 (C-2', C-6'), 130.50 (C-3', C-5'), 130.49 (C-7), 133.37 (C-4), 136.08, 136.12, 136.35 (C-5a, C-1', C-5), 136.44 (C-4'), 155.31 (C-9a)

EIMS (LR) $C_{25}H_{24}O_2$: calculated M^+ 350

observed M^+ 350

7.3.20. Demethylation of 4-arylmethyl-5-(4-methoxyphenyl)-1-benzoxepin

5-(4-Hydroxyphenyl)-4-(4-methylphenyl)methyl-2,3-dihydro-1-benzoxepin (253)

The procedure incorporated in 4.2.6 was carried out to prepare this compound, using 5-(4-methoxyphenyl)-4-(4-methylphenyl)methyl-2,3-dihydro-1-benzoxepin (0.0004M) in dry dichloromethane (15mL) and boron tribromide (0.0082M). This solution was stirred at –78°C for 1 hour. The product was purified using column chromatography (eluant dichloromethane : ethyl acetate; 99:1), as a brown oily gel in 98% yield.

IR ν_{max} (film) 3678-3202 (OH), 3078-2924, 2914-2839 (CHs), 1608, 1579 (C=C), 1479, 1454 (CH_2), 1379 (CH_3), 1251, 1216 (C-O-C), 1181 (C-OH) cm^{-1}

^{1}H NMR δ($CDCl_3$) 2.28 (2H, t, J = 6.28Hz, H-3), 2.45 (3H, s, CH_3), 3.63 (2H, s, H-10), 4.33 (2H, t, J = 6.28Hz, H-2), 6.79-7.12 (11H, m, ArH)

^{13}C NMR δ($CDCl_3$) 21.00 (CH_3), 32.44 (C-3), 40.48 (C-10), 80.45 (C-2), 114.98 (C-3'', C-5''), 121.94, 123.29 (C-8, C-9), 127.85 (C-6), 128.79 (C-2'', C-6'', C-2', C-6'), 129.12 (C-3', C-5'), 130.93 (C-7), 154.47 (C-4''), 155.77 (C-9a)

EIMS (HR) $C_{28}H_{31}O_2$: calculated M^+ 342.1638

observed M^+ 342.1620

7.3.21. Alkylation Reactions of benzoxepins

5-[(4-Dimethylaminoethoxy)phenyl]-4-(4-methylphenyl)methyl-2,3-dihydro-1-benzoxepin (254)

The general method 2.8 outlined was employed using 5(4-hydroxyphenyl)-4-(4-methylphenyl)methyl-2,3-dihydro-1-benzoxepin (0.00013M) in dry acetone (6mL), anhydrous K_2CO_3 (0.003M) and 2-(dimethylamino)ethylchloride hydrogenchloride (0.00045M). Preparative layer chromatography (developing solvent dichloromethane : ethyl acetate : methanol; 50:50:10) afforded an orange coloured oil in 20% yield, with the following characteristics.

IR ν_{max} (film) 3002-2889, 2873-2791 (CHs), 1605, 1581 (C=C), 1462 (CH_2), 1380 (CH_3), 1284, 1242 (CN), 1175 (C-O-C) cm^{-1}

^{1}H NMR δ($CDCl_3$) 2.28 (2H, t, J = 8.48Hz, H-3), 2.32 (6H, s, $N(CH_3)_2$), 2.34 (3H, s, CH_3), 2.75 (2H, m, NCH_2), 3.60 (2H, s, H-10), 4.05 (2H, m, CH_2O), 4.30 (2H, t, J = 8.34Hz, H-2), 6.78-6.83 (2H, d, J = 9.04Hz, H-3'', H-5''), 6.86-7.15 (10H, m, ArH)

^{13}C NMR δ($CDCl_3$) 20.39 (CH_3), 29.62 (C-3), 40.39 (C-10), 45.67 ($N(CH_3)_2$), 58.10 (NCH_2), 65.82 (CH_2O), 80.45 (C-2), 114.07 (C-3'', C-5''), 120.48, 120.91(C-8, C-9), 127.75 (C-6), 127.99-128.32 (C-2', C-6', C-2'',C-6''), 130.20, 130.31 (C-3', C-5'), 130.74 (C-7),

134.51 (C-4), 138.15 (C-5a, C-1', C-5), 158.35 (C-4''), 159.67 (C-9a)

EIMS (HR) $C_{28}H_{31}NO_2$: calculated M^+ 413.2709

observed M^+ 413.2355

Mass Spectrum (m/z) 413 (M^+, 73%), 355 (M^+-58, 68%), 342 (M^+-71, 72%), 325 (M^+-88, 59%), 295 (M^+-118, 56%), 281 (M^+-132, 18%), 264 (M^+-149, 22%), 252 (M^+-161, 45%), 239 (M^+-174, 23%), 215 (M^+-198, 94%), 202 (M^+-211, 83%), 189 (M^+-224, 89%), 178 (M^+-235, 100%) 165 (M^+-248, 100%), 152 (M^+-261, 100%), 115 (M^+-298, 97%), 105 (M^+-308, 100%),

5-[(4-Diethylaminoethoxy)phenyl]-4-(4-Methylphenyl)methyl-2,3-dihydro-1-benzoxepin (255)

The general method 2.8 was applied using 5-(4-hydroxyphenyl)-4-(4-methylphenyl)methyl-2,3-dihydro-1-benzoxepin (0.0001M) in dry acetone (5mL), anhydrous K_2CO_3 (0.003M) and 2-(diethylamino)ethylchloride hydrogenchloride (0.0004M). The product was isolated as a colourless oil using preparative layer chromatography (developing solvent dichloromethane : ethyl acetate : methanol; 50:50:10) in 27% yield.

IR ν_{max} (film) 3063-2895, 2881-2807 (CHs), 1608, 1573 (C=C), 1485, 1465 (CH_2), 1381 (CH_3), 1278, 1244 (CN), 1174 (C-O-C) cm^{-1}

^1H NMR $\delta(CDCl_3)$ 2.19 (5H, s, $N(CH_3CH_2)$), 2.29 (2H, t, J = 6.28Hz, H-3), 2.34 (8H, s, $N(CH_3CH_2)$, CH_3), 2.73 (2H, m, NCH_2), 3.62 (2H, s, H-10), 4.11 (2H, m, CH_2O), 4.32 (2H, t, 6.82Hz, H-2), 6.86-6.89 (2H, dd, J = 9.04, 2.48Hz, H-3'', H-5''), 6.86-7.15 (10H, m, ArH),

^{13}C NMR $\delta(CDCl_3)$ 22.69 (CH_3), 29.69 ($N(CH_3)_2$), 32.35 (C-3), 40.40 (C-10), 47.59 ($N(CH_2CH_3)_2$), 53.42 (NCH_2), 70.77 (CH_2O), 80.72 (C-2), 113.38, 114.00 (C-3'', C-5''), 121.92, 123.29 (C-8, C-9), 127.81 (C-6), 128.74, 129.21 (C-2', C-6', C-2', C-6''), 130.74 (C-3', C-5'),

130.92 (C-7), 135.65 (C-4), 136.49, 136.85 (C-5a, C-1', C-5), 155.70 (C-4''), 157.67 (C-9a)

EIMS (HR) $C_{30}H_{35}NO_2$: calculated M^+ 441.2669

observed M^+ 441.2668

Mass Spectrum (m/z) 441 (M^+, 72%), 368 (M^+-73, 65%), 295 (M^+-146, 53%), 281 (M^+-160, 49%), 264 (M^+-177, 48%), 252 (M^+-189, 35%), 239 (M^+-202, 36%), 221 (M^+-220, 21%), 189 (M^+-252, 19%), 178 (M^+-263, 55%) 165 (M^+-276, 100%), 128 (M^+-313, 97%). 105 (M^+-336, 90%), 86 (M^+-355, 100%),

4-(4-Methylphenyl)methyl-5-[(4-pyrrolidinylethoxy)phenyl]-2,3-dihydro-1-benzoxepin (256)

The general method 2.8 was applied using 5-(4-hydroxyphenyl)-4-(4-methylphenyl)methyl-2,3-dihydro-1-benzoxepin ($8.3x10^{-5}$M) in dry acetone (5mL), anhydrous K_2CO_3 (0.0024M) and 4-(2-chloroethyl)pyrrolidine hydrochloride (0.00025M). The product was isolated as a light brown coloured oil using preparative layer chromatography (developing solvent dichloromethane : ethyl acetate : methanol; 55:44:6) in 21% yield.

IR ν_{max} (film) 2955-2917, 2849 (CHs), 1575 (C=C), 1514, 1463, 1458 (CH_2), 1376 (CH_3) cm^{-1}

^{1}H NMR $\delta(CDCl_3)$ 1.73 (4H, m, H-2'''', H-3''''), 2.08 (2H, m, H-3), 2.19 (3H, s, CH_3), 2.82 (4H, m, H-1'''', H-4''''), 2.89 (2H, m, NCH_2), 3.67 (2H, s, H-10), 4.12 (2H, m, CH_2O), 4.19 (2H, m, H-2), 6.54-6.55 (2H, d, J = 4.52Hz, H-3'', H-5''), 6.81-7.56 (10H, m, ArH)

^{13}C NMR $\delta(CDCl_3)$ sample too insoluble for C^{13} NMR

EIMS (HR) $C_{30}H_{33}NO_2$: calculated M^+ 439.2542

observed M^+ 439.2511

4-(4-Methylphenyl)methyl-5-[(4-piperidinylethoxy)phenyl]-2,3-dihydro-1-benzoxepin (257)

The general method 2.8 was applied using 5(4-hydroxyphenyl)-4-(4-methyl phenyl)methyl-2,3-dihydro-1-benzoxepin (0.00025M) in dry acetone (5mL), anhydrous K_2CO_3 (0.0072M) and 1-(2-chloroethylpiperidine)monohydrochloride (0.0015M). The product was purified as a lemon coloured oil using preparative layer chromatography (developing solvent dichloromethane : ethyl acetate : methanol; 50:60:8) in 31% yield.

IR ν_{max} (film)	3093-2993, 2976-2828 (CHs), 1607, 1570 (C=C), 1485, 1462, 1442 (CH_2), 1352 (CH_3) cm^{-1}
^1H NMR $\delta(CDCl_3)$	1.62 (6H, m, H-2''', H-3''', H-4'''), 2.29 (2H, t, J = 6.26Hz, H-3), 2.34 (3H, s, CH_3), 2.54 (4H, m, H-1''', H-5'''), 2.80 (2H, t, J = 6.02Hz, NCH_2), 3.63 (2H, s, H-10), 4.13 (2H, t, J = 6.04Hz, CH_2O), 4.32 (2H, t, J = 6.02Hz, H-2), 6.84-6.87 (2H, dd, J = 10.56, 2.00Hz, H-3'', H-5''), 6.97-7.25 (10H, m, ArH)
^{13}C NMR $\delta(CDCl_3)$	22.54 (CH_3), 25.44 (C-2''', C-3''', C-4'''), 31.97 (C-3), 40.02 (C-10), 54.55 (C-1''', C-5'''), 57.51 (NCH_2), 70.77 (CH_2O), 80.72 (C-2), 113.68 (C-3'', C-5''), 121.44, 122.80 (C-8, C-9), 127.34 (C-6), 128.31, 128.71 (C-2', C-6', C-2'', C-6''), 130.49 (C-3', C-5'), 130.85 (C-7), 135.18 (C-4), 136.06, 136.13 (C-5a, C-1', C-5), 157.28 (C-9a)
EIMS (HR)	$C_{31}H_{35}NO_2$: calculated M^+ 453.2660 observed M^+ 453.2668
Mass Spectrum (m/z)	453 (M^+, 71%), 423 (M^+-30, 51%), 368 (M^+-85, 49%), 325 (M^+-128, 63%), 264 (M^+-189, 15%), 252 (M^+-201, 10%), 239 (M^+-214, 29%), 219 (M^+-234, 51%), 202 (M^+-251, 63%), 189 (M^+-264, 45%). 178 (M^+-275, 28%) 165 (M^+-288, 13%), 152 (M^+-301, 75%). 112 (M^+-341, 98%) 98 (M^+-355, 100%)

4-(4-Methylphenyl)methyl-5-[(4-morpholinylethoxy)phenyl]-2,3-dihydro-1-benzoxepin (258)

The general method 2.8 was applied using 5-(4-hydroxyphenyl)-4-(4-methyl phenyl)methyl-2,3-dihydro-1-benzoxepin (8.25×10^{-5}M) in dry acetone (8mL), anhydrous K_2CO_3 (0.0024M) and 4-(2-chloroethylmorpholine)hydrochloride (0.00025M). The product was purified as a light brown coloured oil using preparative layer chromatography (developing solvent dichloromethane : ethyl acetate : methanol; 50:44:6) in 19% yield.

IR ν_{max} (film) 2955-2917, 2849 (CHs), 1604, 1575 (C=C), 1514, 1463, 1454 (CH_2), 1377 (CH_3) cm^{-1}

^{1}H NMR $\delta(CDCl_3)$ 2.03 (2H, m, H-3), 2.34 (3H, s, CH_3), 2.64 (2H, m, NCH_2), 2.86 (4H, m, H-1''', H-4'''), 3.67 (2H, s, H-10), 3.76 (4H, m, H-2''', H-3'''), 4.18 (2H, m, CH_2O), 4.34 (2H, m, H-2), 6.63-6.66 (2H, dd, J = 7.52, 1.50Hz, H-3'', H-5''), 6.73-7.11 (10H, m, ArH),

^{13}C NMR $\delta(CDCl_3)$ 22.69 (CH_3), 31.93 (C-3), 39.51 (C-10), 54.11 (C-1''', C-4'''), 57.65 (NCH_2), 67.00 (CH_2O, C-3''', C-2'''), 69.02 (C-2), 113.30 (C-3'', C-5''), 121.67 (C-8, C-9), 128.82 (C-2', C-6', C-2'', C-6''), 130.92 (C-3', C-5'), 132.43 (C-7), 136.40 (C-4), 137.52 (C-5a, C-1', C-5)

EIMS (HR) $C_{30}H_{33}NO_3$: calculated M^+ 455.2464

observed M^+ 455.2460

8. References

[1] Foye, W., Lemke, T.L., Williams, D. A., *Principles of Medicinal Chemistry*, 4th Edition, **1985**, Lee & Febiger

[2] *Understanding Cancer of the Breast*, **1994**, Backup, London

[3] *The Irish Breast Cancer Society Booklet*, **1998-1999**

[4] DeFriend, D.J., Howell, A., Nicholson, R.I., Anderson, E., Dowsett, M., Mansel, R.E., Blamey, R.W., Bundred, N.J., Robertson, J.F., Saunders, C., *Cancer Research*, **1994**, 54, 408-414

[5] Lykkesfeldt, A.E., Sorensen, E.K., *Acta. Oncologica*, **1992**, 31, No. 2, 131-138

[6] DeLarco, J.E., Todaro, G.J., *Proc. Nat. I Acid. Sci.*, USA, **1978**, 75, 4001-4005

[7] Sirbasku, D.A., *Hormones and Cancer*, Banbury Report **1981**, 8, 425-443

[8] Higa, G.M., al Khouri, N., *Am. J. Health-Syst. Pharm.*, **1998**, 55, 445-452

[9] American Cancer Society, *Cancer Facts and Figures*, **1996**

[10] Lovely, C.J., Bhat, A.S., Coughenour, H.D., Gilbert, N.E., Brueggemeier, R.W., *J. Med. Chem*, **1997**, 40, 3756-3764

[11] Ekena, K., Wis, K.E., Katzenellenbogen, J.A., Katzenellenbogen, B.S., *The Journal of Biological Chemistry*, **1996**, 271, No. 33, 20053-20059

[12] Hubbard, R.E., *Structural insights into Agonism, antagonism and Activation in Estrogen Receptors*, Sept., **1999**, 10TH RSC-SCI Medicinal Symposium. (http://www.yorvic.york.ac.uk/gallery/er/index.htm)

[13] Osbourne, C.K., Ellendege, R.M., Fugua, S.A.W., *Scientific American Science and Medicine*, **1996**, 32-41

[14] Brzozowski, A.M., Pike, A.C.W., Dauter, Z., Hubbard, R.E., Bonn, T., Engstrom, O., Ohman, L., Greene, G.L., Gustafsson, J.A., Carlquist, M. *Nature,* **1997**, 389, 753-758

[15] Shiau, A.K., Barstal, D., Loria, P.M., Cheng, L., Kushner, P.J., Agard, D.A., Greene, G.L., *Cell*, **1998**, 95, 927-937

[16] Kuiper, G., Enmark, E., Peltro-Huikko, M., Nilsson, S., Gustaffsson, J.A., *Proc. Natl. Acad. Sci.*, USA, **1996**, 93, 5925-5930

[17] Carson-Jurica, M.A., Schrader, W.T., O'Malley, B.W., *Endocrin. Rev.*, **1990**, 11, 201-220

[18] Hentu, P.M., Kalkhoven, B., Parker, M.G., *Mol. Cell Biol.*, **1997**, 17, 1832-1839

[19] MacGregor, J.I., Jordan, V.C., *Pharmacological Reviews*, **1998**, 50, No. 2, 151-196

[20] Kaufmann, D., Gallo, M.A., *Semin Oncol*, **1997**, 24, No. 1, (Suppl 1), S1-71→S1-80

[21] Evans, R.M., *Science*, **1988**, 240, 889-895

[22] Green, S., Chambon, P., *Trends Genet.*, **1988**, 4, 309-314

[23] Parker, M.G., Baker, O., *Nuclear Hormones Receptors*, London UK, Academic Press, **1991**, 377-395

[24] Dickson, R.B., Thompson, Lippman, M.E., *J. Steroid Biochem Mol. Biol*, **1990**, 37, 305-316

[25] Osbourne C.K. Arteaga, C. L., *Breast Cancer Res. Treat*, **1990**, 15, 3-11

[26] Vignon, F., Capony F., Chambon, M., Freiss, G., Garcia, M., Rochefort, H., *Endocrinol.*, **1986**, 118,1537-1545

[27] Karey, K.P., Sirbasku, D.A., *Cancer Res.*, **1988**, 48, 4083-4092

[28] van der Burg, B., Isbrucker, L., van Selm-Miltenburg, A.J., de Laat, S.W., van Zoeleon, E.J., *Cancer Res.*, **1990**, 50, 7770-7774

[29] Arteaga, C.L., Osbourne, C.K., *Cancer Res.*, **1989**, 49, 6237-6241

[30] Korach, K.E., Couse, J.F., Curtis, S.W., Washburn, T.F., Lindzey, J., Kmbro, K.S., Eddy, E.M., Migliaccio, S., Snedeker, S.M., Lubahn, D.B., Schomberg, D.W., Smith, E.P., *Recent Progress in Hormone Research*, **1996**, 151, 159-188

[31] Jordan, V.C., *Scientific American*, **1998**, 36-43

[32] Katzenellenboben, J.A., O'Malley, B.w., Katzenellenbogen, B.S., *Mol. Endocrinol.* **1996**, 10, 119-131

[33] McLachlan, J.A., *Estrogens in the Environment II*, New York, NY Elsevier, **1985**

[34] Bocquel, M.T., Kumar, V., Stricker, C., Chambon, P., Gronemeyer, H., *Nucleic Acid Res.*, **1989**, 17, 2581-2595

[35] Ratzkowski, E., Aider, B., Hochman, A., *Oncology*, **1973**, 28, 385-397

[36] Cole, M.P., Jones, C.T., Todd, I.D., *Br. J. Cancer*, **1971**, 25, 270-275

[37] Conn, P.M., Browley, W.F., *N. Engl. J. Med.*, **1991**, 324, 93-103

[38] Thompson, E.A., Siiteri, P.K., *J. Biol. Chem.*, **1974**, 249, 5364-5372

[39] Lundgren, S., Lonning, P.E., Utaaker, E., Aakvag, A., Kvinnsland, S., *J. Steroid Biochem.*, **1990**, 36, 99-104

[40] Blossey, H.C., Wander, H.E., Koebberling, J., Nagel, G.A., *Cancer*, **1984**, 54, 1208-1215

[41] Katzenellenbogen, B.S., Miller, M.A., Mullick, A., Sheen, Y.Y., *Breast Cancer Research and Treatment*, **1985**, 5, 231-243

[42] Strickland, L.A., Ponce, Y.Z., Hunter, D.H., Zabel, P.L., Powe, J.E., Morrissey, G., Driedger, A.A., Chamberlain, M.J., Tustanoff, E.R., *Drug Design and Delivery*, **1990**, 6, 195-212

[43] Leclerq, G., Toma, S., Paridaens, R., Heuson, J.C., *Clinical Int. of Steroid Hormone Receptors in Breast Cancer*, **1984**

[44] Groom, G.V., Griffiths, K., *J. Endocrinol.*, **1976**, 70, 421-428

[45] Gibbons, W.E., O'Brien, J.J., *Prog. Cancer Res. Ther.*, **1983**, 24, 99-100

[46] Dauvois, S., Parker, M.G., *Steroid Hormone Action*, **1993**, 166-185

[47] Dauvois, S., Parker, M.G., *Hormonal Carcinogenesis II*, **1996**

[48] Dore, J.C., Gilbert, J., Bignon, E., dePaulet, A.C., Ojasoo, T., Pons, M., Raynaud, J.P., Miquel, J.F., *J. Med. Chem.*, **1992**, 35, 573-583

[49] Buckley M.M., Goa, K.L., *Drugs*, **1989**, 37, 451-490

[50] Jordan, V.C., Murphy, C.S., *Endocrine Reviews*, **1990**, 11(4), 578-610

[51] Miquel, J.F., Gilbert, J., *J. Steroid Biochem.*, **1988**, 31, 525

[52] Brandes, L.J., Bogdanovic, R.P., *Biochem. Biophys. Res. Commun.*, 1986, 134, 601

[53] Borgna, J., Rochefort, H., *Cancer Res.*, **1982**, 42, 317

[54] Lazier, C. B., Bapat, B.V., *J. Steroid. Biochem.*, **1988**, 31, 665

[55] Tang, B.L., Teo, C.C., Sim, K.Y., Ng, M.L., Kon, O.L., *Biochim. Biophys. Acta*, **1989**, 1014, 162

[56] Poirot, M., Garnier, M., Bayard, F., Riviere, I., Traore, M., Wilson, M., Fargin, A., Faye, J.C., *Biochem. Pharmacol.*, **1990**, 40, 425-429

[57] Gilbert, J., Miquel, J.F., Precigoux, G., *J. Med. Chem.*, **1983**, 26, 693

[58] Pons, M., Michel, F., Descomps, B., dePaulet, C.A., *Eur. J. Biochem*, **1978**, 84, 257

[59] Barrera, G., Screpanti, I., Paradis, L., Parola, M., *Biochem. Pharmacol.*, **1986**, 35, 2984

[60] Liskamp, R.M., Solomon, D.H., Weinstein, I, *Cancer Res.*, **1985**, 45, 2462

[61] O'Brian, C.A., Liskamp, R.M., Solomon, D.H., Weinstein, I., *J. Natl. Cancer Inst.*, **1986**, 76, 1243

[62] Soho, Mazzei, G.J., Vogler, W.R., Kuo, J.F., *Biochem. Pharmacol.*, **1985**, 34, 3649

[63] Powles, *Semin Oncology*, **1997**, 24, No.1, (Suppl 1), S1-48→S1-54

[64] Klopper, A., Hall, M., *Preliminary Trial in Women*, BMJ, **1971**, 2,152-154

[65] Mansi, J.L., Smith, I.E., *Cancer Topic*, **1989**, 7, 57-59

[66] Powles, T.J., Hardy, J.R., Ashley, S.E., Farrington, G.M., Cosgrove, D., Davey, J.B., Dowsett, M., McKinna, J.A., Nash, A.G., Sinnett, H.D., *Br. J. Cancer*, **1989**, 60, 126-131

[67] Wiseman, H., *Tamoxifen, Molecular Basis of Use in Cancer Treatment and Prevention*, Wiley & Sons, Chichester, New York, **1994**

[68] Magaeian, R.A., Avor, K.S., Overacre, L.B., Kunchandy, J., Parton, T.R., *Anti-Cancer Drug Design*, **1995**, 10, 311-331

[69] Lerner, L.J., Jordan, V.C., *Cancer Res.*, **1990**, 50, 4177-4189

[70] Jordan, V.C., *Br. J. Pharmaccol.*, **1993**, 110, 507-517

[71] Love, R.R., Wiebe, D.A., Newcomb, P.A., Cameron, L., Leventhal, H., Jordan, V.C., Feyzi, J., DeMets, D.L., *Ann. Intern. Med.*, **1991**, 115, 860-864

[72] Love, R.R., Wiebe, D.A., Newcomb, P.A., *N. Eng. J. Med.*, **1992**, 326, 852-856

[73] Henderson, I.C., Mourdisen, H., *New Eng. J. Med.*, **1988**, 319, 1681-1689

[74] Nayfield, S.G., Karp, J.E., Ford, L.G., Dorr, F.A., Kramer, B.S., *J. Natl. Cancer Inst.*, **1991**, 83, 1450-1459

[75] Henderson, I.C., Collins, R., Gray, R., *Lancet*, Trial Results, **1992**

[76] Jonat, W., Meass, H., *J. Steroid Biochem.*, **1987**, 27, 499-502

[77] DeGregorio, M.W., Wiebe, V.J., *Tamoxifen and Breast Cancer*, Yale University Press, New Haven, London, **1994**

[78] Bedford, G.R., Richardson, D.N., *Nature*, **1966**, 121,733-734

[79] Harper, M.J.K., Walpole, A.L., *Nature*, **1966**, 212, 87

[80] Borgna, J.L., Rochefort, H., *Cr. Acad., Sci. (Paris)*, **1979**, 289, 1141,

J. Biol. Chem., **1981**, 256, 859

[81] Fromson, J.M., Pearson, S., Bramah, S., *Xenobiotica*, **1973**, 3, 693-709 and 711

[82] Robertson, D.W., Katzenellenbogen, J.A., Long, D.J., Rorke, E.A., *J. Steriod. Biochem.*, **1982**, 16,1

[83] Katzenellenbogen, B.S., Norman, M.J., Eckert, R.L., Peltz, S.W., Mengel, W.F., *Cancer Res.*, **1984**, 44, 112-119

[84] Shani, J., Gazit, A., Livshitz, T., Biran, S., *J. Med. Chem.*, **1985**, 28, 1504-1511

[85] McCague, R., Leclercq, G., Legros, N., Goodman, J., Blackburn, G.M., Jarman, M., Foster, A., *J. Med. Chem.*, **1989**, 32, 2527-2533

[86] Richardson, D.N., *Drug Design & Delivery*, **1988**, 3, 1-14

[87] McCague, R., Kuroda, R., Leclercq, G., Stoessel, S., *J. Med., Chem.*, **1986**, 29, 2053-2059

[88] Kellen, J.A., *Tamoxifen, Beyond the Antiestrogen*, **1996**, 2-25, Birkhauser Boston

[89] Jaolot, F., Simon, I., Dreano Y., Beaune, P., Riche, P., Berthou, Y., *Biochem. Pharmacol.*, **1991**, 41, 1911-1919

[90] Adam, H.K., Douglas, E.J., Kemp, J.V., *Biochem. Pharmacol.*, **1979**, 27, 145-147

[91] Jordan, V.C., *Pharmacological Review*, **1984**, 36, 4, 245-276

[92] Balen, D.J., Foster, B.B., Griggs, L.T., Larma, N.M., Leclercq, G., Deukeschouwer, N., *Biochem. Pharmacol.*, **1982**, 31, 2823-2828

[93] Jordan, V.C., Bain, R.R., Brown, R.R., Gosden, B., Santos, M.A., *Cancer Res.*, **1983**, 43,1146-1148

[94] Reddel, R.R., Murphy, L.C., Sutherland, R.L., *Cancer Res.*, **1983**, 43, 4618-4622

[95] Daniel, C.P., Gaskel, S.J., Bishop, H., Cambell, C., Nickelson, R.I., *Eur. J. Cancer Clin. Encol.*, **1981**, 17, 1183-1187

[96] Kemp, J.V., Adam, H.K., Wakeling, A.E., Slater, R., *Biohem. Pharmaol.*, **1983**, 32, 2045-2052

[97] Bain, R.R., Jordan, V.C., *Biochem. Pharmacol.*, **1983**, 32, 373-375

[98] Daniel, C.P., Gaskel, S.J., Bishop, H., Cambell, C., Nickelson, R.I., *J. Endocrinol.*, **1979**, 83, 401-408

[99] Jordan, V.C., Collins, M.M., Rowsby, L., Prestwich, G., *J. Endocrinology*, **1977**, 75, 305-316

[100] Coezy, E., Borgna, J-L., Rochefort, H., *Cancer Res.*, **1982**, 42, 317-323

[101] Lyman, S.D., Jordan, V.C., *Biochem. Pharmacol.*, **1985**, 34, 2787-2794

[102] Jordan, V.C., Haldemann, B., Allen, K.E., *Endocrinology*, **1981**, 108, 1353-1361

[103] March, J., *Advanced Organic Chemistry, Reactions, Mechanisms and Structure*, Wiley & Sons, New York, 4th Edition, **1996**

[104] Jordan, V.C., Gosden, B., *Molec. Cell Endocrinology*, **1982**, 27, 291-306

[105] Murphy, .S., Langan, F.S., McCague, R., Jordan, V.C., *Mol. Pharmacol.*, **1990**, 38 (5), 737-743

[106] Korack, K.S., Levy, L.A., Sarver, P.J., *J. Steroid Biochem.*, **1987**, 27, 281-290

[107] Pollak, M., Costantino, J., Polychronakos, C., Blauer, S.A., Guyda, H., Redmond, C., Fisher, B., Margolese, R., *J. Natl. Cancer, Inst.*, **1990**, 82, 1693-1697

[108] Lopes, M.C.F., Vale, M.G.P., Carvalio, A.T., *Cancer Res.*, 1990, 50, 2753-2758

[109] Lam, L.Y.P., *Biochem. Biophys. Rescommun.*, **1984**, 118, 27-32

[110] Gulino, A., Barrea, G., Vacea, A., Farina, A.I., Ferretti, C., Screpanti, I., Dianzani, M.U., Frati, L., *Cancer Res.*, **1986**, 46, 6274-6278

[111] Sartor, P., Vancher, P., Mollard, P., Duffy, B., *Endocrinol.*, **1988**, 123, 534-540

[112] Bowman, W.R., Rand, M.C., *Textbook of Pharmacology*, Blackwell Scientific Publications, Oxford, **1980**

[113] Nishizuki, Y., *Nature*, **1988**, 334, 661-665

[114] Knabbe, C., Zugmaier, G., Schmahl, M., Dietel, M., Lippman, M.E., Dickson, R.B., *Am. J. Clin. Oncol.*, **1991**, 14, S15-20

[115] Butta, A., Maclennon, K., Flanders, K.C., Sachs, N.P., Smith, I., McKinna, A., Dowsett, M., Wakefield, L.M., Sporn, M.B., Baum, M., *Cancer Res.*, **1992**, 52, 4261-4264

[116] Manni, A., Badger, B., Kuk, G., Wright, C., Caplan, R., Rochette, H., Ahmed, S.R., Bartholomew, M., *Breast Cancer Res.Treat*, **1988**, 11, 231-240

[117] Manne, A., *Crit. Rev. Oncogenesis*, **1989**, 1, 163-174

[118] Cohen, F.J., Manni, A., Glikman, P., Bartholonew, M., Demers, L., *Cancer Res.*, **1988**, 48, 6819-6825

[119] Gong, Y., Ballejo, G., Murphy, L.K., Murphy, L.C., *Cancer Res.*, **1992**, 52, 1702-1709

[120] Sukhu, R., Rotenberg, B., Binkert, C., Kohno, T., Zohar, R.A.G., McCullach, Tenenbaum, H.C., *Endocrinology*, **1997**, 138, No. 8, 3269-3275

[121] Heis, H.P., *Jr, Int. Surg*, **1993**, 78, 176-182

[122] Love, R.R., *Oncology*, **1992**, 6, 33-40, 43

[123] Pollak, M.N., Huynh, H.F., Lefebure, *Breast Cancer Res. Treat*, **1992**, 22, 91-100

[124] Wada, T., Nishiyana, K., Meada, M., Hara, S., Tanaka, N., Yasutomi, M., Kurita, T., *Anticancer Res.*, **1995**, 15, 1581-1584

[125] Bilimona, M.M., Assiki, V.J., Jordan, V.C., *Cancer J. Sci. Am.*, **1996**, 2, 140-150

[126] Gylling, H., Pyrhonen, S., Mantyla, E., Maenpaa, H., Kangas, L., Miettinei, T.A., *J. Clin. Oncol.*, **1995**, 13, 2900-2905

[127] Fentiman, I.S., Caleffi, M., Murby, B., Fogelman, I., *Br. J. Clin. Prac.*, **1988**, 52, Supp 156, 18

[128] Ryan, W.G., Wolter, J., Bagdade, J.D., *Osteoporsis Int.*, **1991**, 2, 39-41

[129] Feldmann, S., Minne, H.W., Parvizi, F., Pfeifer, M., Lamper, U.G., Bauss, F., Zigegler, R., *Bone Miner*, **1989**, 7, 245-254

[130] Ward, R.L., Morgan, G., Dalley, D., Kelly, P.J., *Bone Miner*, **1993**, 22, 87-94

[131] Wright, C.O.P., Comptson, J.E., *J. Med. Chem.*, **1995**, 88, 307-310

[132] Wiliams, D.C., Paul, D.C., Black, L.J., *Bone Miner*, **1991**, 14, 205-220

[133] Fentinan, I.S., Saad, Z., Caleffi, M., Chaudury, M.A., Fogelman, I., *Eur. J. Cancer*, **1992**, 28, 684-685

[134] Lukert, B.F., Johnson, B.E., Robinson, R.G., *J. Bone Miner Res.*, **1992**, 2, 7, 1063-1069

[135] Sutherland, R.L., Jordan, V.C., *Nonsteroidal Antiestrogens (Molecular Pharmacology and Antitumour Activity)*, **1981**, Academic Press Australia

[136] Goodman and Gillman, *The Pharmacological Basis of Therapeutics*, **1996**, 9TH Edition, McGrawHill Company, USA

[137] Wogan, G.W., *Semin Oncol.*, **1997**, 24, Suppl 1, S1-87→S1-97

[138] Tucker, M.J., Adam, H.K., Patterson, J.S., *Safety Testing of New Drugs*, **1984**, McClean AGM:WeatherHall, M. (Eds), Orlanda, Fl, Academic, 125-161

[139] Dragan, Y.P., Fahey, S., Street, K., Vaughan, J., Jordan, V.C., Pitot, H.C., *Breast Cancer Res. Treat.*, **1994**, 31, 11-25

[140] Williams, G.M., Iatropoulos, M.J., Djordjevic, M.V., Kaltenberg, O.P., *Carcinogensis*, **1993**, 14, 315-317

[141] Hirsimaki, P., Hirsimaki, Y, Nieminen, L., Payne, B.J., *Arch. Toxicol.*, **1993**, 67, 49-54

[142] Phillips, D.H., Carmichael, P.L., Hewer, A., Cole, K.J., Hardcastle, I.R., Poon, G.K., Keogh, A., Strain, A.J., *Carcinogensis*, **1996**, 17, 89-94

[143] Adis International Ltd., *Drugs and Perspectives*, **1997**, 10. No.11

[144] Hard, G.C., Iatropoulos, M.J., Jordan, V.C., Radi, L., Kaltenberg, O.P., Imondi, R., Williams, G.M., *Cancer Res.*, **1993**, 53, 4534-4541

[145] Kangas, L., Nieminen, A.L., Blaco, G., Grontroos, M., Kallico, S., Perilla, M., Sonderval, M., Toivola, T., Karjallanen, M., *Cancer Chemother. Pharmacol.*, **1986**, 17, 109

[146] Robinsons, P., Meul, D.A., Jordan, V.C., *Eur. J. Cancer Clin. Oncol.*, **1988**, 24, 1817

[147] Kangas, L., Haapananta, M., Paul, R., Roeda, D., Spilila, H., *Pharmacol. Toxicol.*, **1989**, 64, 373

[148] Plotkin, D., Lechner, J.J., Jung, W.E., Rosen, P.J., *J. American Medical Association*, **1978**, 240, 2644-2646

[149] Eppenberger, U., Wosikowki, K., Kung, W., *Am. J. Clin. Oncol.*, **1991**, 14, S5-S14

[150] Patients, *Chemistry & Industry*, **1998**, 478

[151] Rausching, W., Pritchard, K.I., *Breast Cancer Res. Treat.*, **1994**, 31, 83-94

[152] Lieberman, M.E., Gorski, J., Jordan, V.C., *J. Biol. Chem.*, **1983**, 258, 4741

[153] Reunitz, P.C., Bagley, J.R., Molker, C.M., *J. Med., Chem.*, **1983**, 25, 1056

[154] Roos, W., Oeze, L., Loser, R., Eppenburger, U., *J. Natl. Cancer Inst.*, **1983**, 71, 55

[155] Leung, O-T., McCague, R., Leclercq, G., Devleeschouwer, N., *J. Med. Chem.*, **1985**, 28, 1491-1497

[156] McCague, R., Leclercq, G., *J. Med. Chem.*, **1987**, 30, 1761-1767

[157] Edashige, K., Sato, E.F., Akimaru, K., Yoshioka, T., Utsumi, K., *Cell Structure and Function*, **1991**, 16, 273-281

[158] Stanciuc, O., Niculescu-Duvaz, I., Stanciuc, G., Balaban, A.T., *Revue Roumaine de Chime*, **1997**, 42 (9), 733-741

[159] Yang, D.J., Cherif, A., Tansey, W., Kuang, L.R, Li, C., Wright, K.C., Kim, E.E., Wallace, S., Eur. *J. Med. Chem.*, **1992**, 27, 919-924

[160] Yang, D.J., Tewson, T., Tansey, W., Kuang, L.R., Regger, G., Cherif, A., Wright, K.C., Moult, R.G., Tilbury, R.S., Chu, K., Kim, E.E., Wallace, S., *J. Pharmaceutical Science*, **1992**, 81, 622-625

[161] Yang, D.J., Wallace, S., Tansey, W., Wright, K.C., Kuang, L-R., Tilbury, R.S., Diego, I., Lim, J-L., Emran, A.M., Kim, E.E., *Pharmaceutical Research*, **1991**, 8, No.2, 174-177

[162] Mintum, M.A., Weich, M.J., Siegel. B.A., Mathias, C.J., Brodack, J.W., McGuire, A.H., Katzenellenbogen, J.A., *Radiology*, **1988**, 169, 45-48

[163] Foster, A.B., McCague, R., Seago, A., Leclercq, G., Stoessel, S., Roy, F., *Anticancer Drug Design*, **1986**, 1, 245-247

[164] Olier-Reuchet, C., Aitken, D.J., Husson, H-P., *Heterocyclic Communications*, **1998**, 14, No.1, 59-62

[165] Scheinman, L., Berrodin, K.W., Dice, J.R., *J. Med. Chem.*, **1965**, 9, 176

[166] Schwartz, W., Hartmann, R.W., Schonenberger, H., *Arch. Pharm. (Weinheim)*, **1991**, 324, 223-229

[167] Olier-Reuchet, C., Aitken, D.J., Bucourt, R., Husson, H-P., *Tetrahedron Lett.*, **1995**, 36, 8221

[168]Toko, T., Maksuo, K.I., Shibata, J., Wierzba, K., Nutatsuka, M., Takeda, S., Yamada, Y., Asao, T., Hirose, T., Sato, B., *J. Steroid. Biochem. Mol. Biol.*, **1992**, 43, 507-514

[169] Toko, T., Sigimoto, Y., Matsuo, K.I., Yamasaki, R., Takeda, S., Wierzba, K., Asa. Y., Yamada, Y., *Eur. J. Cancer*, **1990**, 26, 397-404

[170] Koh, J.I., Kubota, T., Asanuma, F., Yamada, U., Kawamura, E., Hosoda, Y., Hashimoto, M., Yamamoto, O., Sakai, S., Maeda, K., *J. Surg. Oncol*, **1992**, 51, 254-258

[171] Iino, Y., Morishita, Y., Takai, Y., Ando, T., Chwada, S., Houghchi, J., Yokoe, T., Sugamata, N., Takei, H., Iijaki, *Cancer Chemother. Pharmacol.*, **1994**, 34, 372-376

[172] Hartman, R.W., Buchborn, H., Kranzfelder, G., Schonenberger, H., *J. Med. Chem.*, **1984**, 27, 577

[173] Hartman, R.W., Kranzfelder, G., von Angere, E., Schonenberger H., *J. Med. Chem.*, **1980**, 23,841

[174] Schneider, M.R., *J. Cancer Res. Clin. Oncol.*, **1986**, 112, 119

[175] Schneider, M.R., Ball, H., Schiller, C-D., *J. Med. Chem.*, **1986**, 29, 1355-1362

[176] Von Angerer, E., *Cancer Treat.*, Rev II (Suppl 1), A, **1988**, 147

[177] Von Angerer, E., Strohmeiren, J., *J. Med. Chem.*, **1987**, 30, 131

[178] McCague, R., Leclerq, G., Jordan, V.C., *J. Med. Chem.*, **1988**, 31, 1285-1290

[179] McCague, R., *Tetrahedron Asymmetry*, **1990**, 11, No.2, 97-110

[180] Erber, S., Ringshandl, R., Von Angerer, E., *Anti-Cancer Drug Design*, **1991**, 6, 417

[181] Von Angerer, E., Prekajae, J., Strohmeiren, J., *J. Med. Chem.*, **1984**, 27, 1439

[182] Von Angerer, E., Prekajae, J., Berger, M., *Eur. J. Cancer Clin. Oncol.*, **1985**, 21, 531

[183] Hilgard, P., Stekar, J., Wutlke, W., Elliger, M., Holtei, *Progress in Cancer Research and Therapy*, **1988**, Vol.35, Hormone and Cancer 3, New York, Raven Press, 369

[184] Stein, R.C., Dowsetti, M., Cunningham, D.C., Davenport, J., Ford, H.T., Gazet, J-C., Von Angerer, E., *Br. J. Cancer*, **1990**, 61, 431

[185] Schieweck, K., Bhatnagar, A.S., Batzl, C.H., Lang, M., *J. Steroid. Biochem. Mol. Biol.*, **1993**, 44, 633-636

[186] Arimidex professional information brochure., **1995**, Zeneca Pharmaceuticals

[187] Jonat, W., Howell, A., Blomqvist, C., Eiermann, W., Winbald, G., Tyrrell, C., Mauriac, L., Roche, H., Lundgren, S., Hellmund, R., Azab, M., *Eur. J. Cancer*, **1996**, 32A, 404-412

[188] Buzder, A.V., Jones, S.E., Plourde, P., *Cancer*, **1996**, 79, 730-739

[189] Akama, T., Shida, Y., Sugaya, T., Ishida, HG., Gomi, K., Kasai, M., *J. Med Chem.*, **1996**, 39, 3461-3469

[190] Thompson, E.W., Katz, D., Shima, T.B., Wakeling, A.E., Lippman, M.E., Dickson, R.B., *Cancer Res.*, **1989**, 49, 6929-6934

[191] Jones, C.D., Blaszczak, L.C., Goettel, M.E., Suarez, T., Crowell, T.A., Mabry, T.E., Ruenitz, P.C., Srivatsan, V., *J. Med. Chem.*, **1992**, 35, 931-938

[192] Angerer, E.V., Knebel, N., Ganss, B., *J. Med. Chem.*, **1990**, 33, 2635-2640

[193] Veide, P.V., Nique, F., Bremaud, J., Hameau, M.C., Lucas, D., Moratille, C., Viet, D., Teutsch, G., *J. Steroid. Biochem. Mol. Biol.*, **1994**, 48 (213), 187-196

[194] Poirier, D., Auger, S., Merand, Y., Simard, J., Labne, F., *J. Med Chem.*, **1994**, 37, 1115-1125

[195] Kurebayashi, J., Kurosumi, M., Sonoo, H., *Br. J. Cancer*, **1995**, 71, 841-853

[196] Wakeling, A.E., Bowler, J., *J. Endocrinol.*, **1987**, 112, R7-R10

[197] Wakeling, A.E., *Long-Term Tamoxifen Treatment for Breast Cancer*, **1994**, 219-234,University of Wisconsin Press, Madison

[198] Wakeling, A.E., Bowler, J., *J. Steroid. Biochem.*, **1988**, 30, 141-147

[199] Weatherhill, P.J., Wilson, A.P.M., Nicholson, R.I., Davis, P., Wakeling, A.E., *J. Steroid. Biochem.*, **1988**, 30, 263

[200] Wakeling, A.E., Bowler, J., *J. Steroid. Biochem.*, **1988**, 31, 645

[201] Wakeling, A.E., *Horm. Res.*, **1989**, 32, Suppl 1, 257

[202] Bowler, J., Lilley, T.J., Pitman, J.D., Wakeling, A.E., *Steroids*, **1989**, 54, 71-99

[203] Wakeling, A.E., Dukes, M., Bowler, J., *Cancer Res.*, **1991**, 51, 3867-3873

[204] Van de Velde, P., Nique, F., Bouchoux, F., Bremaud, J., Hameau, M.C., Lucas, D., Moratille, C., Viet, S., Philibert, D., Teutsch, G., *J. Steroid. Biochem. Mol. Biol.*, **1994**, 48, 187-196

Van de Velde, P., Nique, F., Planchon, P., Prevost, G., Bremaud, J., Hameau, M.C., Magnien, V., Philibert, D., Teutsch, G., *J. Steroid. Biochem. Mol. Biol.*, **1996**, 59, 449-457

[205] Li, S., Levesque, C., Geng, C-S., Yan, X., Labrie, F., *Breast Cancer Res. Treat.*, **1995**, 34, 147-159

[206] Harbourne, J.B., Ed., *The Flavonoids Advances in Research since 1986*, **1994**, 619-652, Chap & Hall, London

[207] Hirano, T., Oka, K., Akiba, M., *Res. Commun. Chem. Pathol. Pharmacol.*, **1989**, 64 (1), 69-78

[208] Ibrahim, A-R., Abul-Haji, Y.J., *J. Steroid. Biochem. Mol. Biol.*, **1990**, 37 (2), 257-260

[209] Konda, h., Nakajima, S., Yamamoto, N., Okura, A., Satoh, F., Suda, H., Okanishi, M., Tanaka, N., *J. Antibiot.*, **1990**, 43 (12), 1533-1542

[210] Cheng, E., Yoder, L., Stoery, C.D., Burrough, S.W., *Science*, **1954**, 120, 575-576

[211] Kaur, G., Sebers, S., Stetler-Stevenson, M., Worland, P., Sedlacek, H., Myres, C., Czech, J., Naik, R., Sausville, E., *J. Natl., Cancer Inst.*, **1992**, 84, 1736-1740

[212] Day, B.W., Magarian, R.A., Jain, P.T., Pento, J.T., Mousissian, G.K., Meyer, K.L., *J. Med. Chem.*, **1991**, 34, 842-851

[213] Jonnalagadda, S.S., Haar, E.E., Hamel, E., Lin, C.M., Magarian, R.A., Day, D.W., *Biorganic & Medicinal Chem.*, **1997**, 5, No.4, 715-722

[214] Pento, J.T., Magarian, R.A., King, M.M., *Cancer Lett.*, **1982**, 15, 261

[215] Terenius, L., *Acta. Endocrinologica*, **1971**, 66 (Suppl 1), 431

[216] Magarian, R.A., Benjamin, E.J., *J. Pharm. Sci.*, **1975**, 64, 1626

[217] King, M.M., Pento, J.T., Magarian, R.A., Brueggemann, G.L., *J. Natl. Cancer Inst.*, **1985**, 74, 447

[218] Jones, M.G., Jevnikar, M.G., Pike, A.J., Peters, M.K., Black, L.J., Thompson, A.R., Falcone, J.F., Clemens, J.A., *J. Med. Chem.*, **1984**, 27, 1057-1066

[219] Zhu, J., Srikanth, N., Ng., S-C., Kon, O-L., Sim, K-Y., *J. Chem. Research (S)*, **1994**, 98-99

[220] Grese, T.A., Cho, S., Finly, D.R., Godfry, A.G., Jones, C.D., Lugar, III, Martin, M.J., Matsumnto, K., Pennington, L.D., Winter, M., *J. Med. Chem.*, **1997**, 40, 146-167

[221] Grese, T.A., Pennington, L.D., Sluka, J.P., Adrian, M.D., Cole, H.W., Fuson, T.R., Magee, D.E., Phillips, D.L., Rowley, E.R., Shelter, P.K., Short, L.L., Venugopalan, M., Yang, N.N., Sato, M., Glasebrook, A.L., Byrant, H.U., *J. Med. Chem.*, **1998**, 41, 1272-1283

[222] Adis International Ltd., *Drugs and Therapy Perspectives*, **1998**, 12, No. 7, 1-5

[223] Carlson, R., *Inpharma*, **1998**, 1141, 13-14

[224] Black, L.J., Jones, C.D., Falcone, J.F., *Life Sci.*, **1983**, 32, 1031-1036

[225] Black, L.J., Goode, R.L., *Life Sci*, **1980**, 26, 1453-1458,

Endocrinology, **1981**, 109, 987-989

[226] Scholl, M., Huff, K.K., Lippman, M.E., *Endocrinology*, **1983**, 113, 611-617

[227] Gottardis, M.M., Jordan, V.C., *Cancer Res.*, **1987**, 47, 4020-4024

[228] Anzano, M.A., Peer, C.W., Smith, J.M., Mullen, L.T., Shrader, M.W., Logsdon, D.L., Driver, C.L., Brown, C.C., Roberts, A.B., Sporn, M.A., *Cancer*, **1996**, 88, 123-125

[229] Johnston, S.R.D., Boeddinghaus, I.M., Riddler, S., Haynes, B.P., Hardcastle, I.R., Rowlands, M., Grimshaw, R., Jarman, M., Dowsett, M., *Cancer Research*, **1999**, 58, (15), 3646-3651

[230] Allen, K.E., Clark, E.R., Jorrdan, V.C., *Br. J. Pharmacol*, **1980**, 71, 87-91

[231] Hardcastle, I.R., Rowlands, M.G., Grimshaw, R.W., Houghton, J., Jarman, M., Sharrff, A., Neidle, S., *J. Med. Chem.*, **1996**, 39, (4), 999-1004

[232] Johnston, S.R.D., Rideller, S., Haynes, B.P., A'Hern, R., Smith, I.E., Jarman, M., Bowsett, M., *Br. J. Cancer*, **1997**, 75, 804-809

[233] Jones, C.D., Jevnikar, M.G., Pike, A.J., Peters, M.K., Black, L.J., Thompson, A.R., Falcone, J.F., Clemens, J.A., *J. Med. Chem.*, **1984**, 27, 1057-1066

[234] Arafah, B., Manni, A., Pearson, O.H., *Breast Cancer, Experimental and Clinical Aspects*, **1980**, 281-285, Pergamon Press

[235] Witte, R.S., Pruitt, B., Carbone, P.P., Tormey, D.C., Noss, S., Rose, D.P., Falkson, G., Ramirez, G., Falkon, H., Pretorius, F.J., *Cancer*, **1986**, 57, 34

[236] Lee, R.W., Rubdar, A.U., Blumenschein, G.R., Hortobagyi, G.N., *Cancer*, **1986**, 57, 40

[237] Leichtl, S., Von Angerer, E., *Arch. Pharm.*, **1998**, 331, 283-289

[238] Bradbury, S.P., Mekenyan, O.G., Ankley, G.T., *Environ. Toxicol. Chem.*, **1998**, 17, 15-25

[239] Teo, C.C., Kon, O.L., Sim, K.Y., Ng, S-C., *J. Med. Chem.*, **1992**, 35, 1330-1339

[240] Schrauzer, G.N., *Selenium in Medicine and Biology*, Ed., Neve, J., Favier, A., de Gruyter, W., Berlin, **1988**, 251-261

[241] Jarman, M., McCague, R., *J. Chem. Res. (S)*, **1985**, 116-117

[242] McCague, R., *J. Chem. Soc. Perkin Trans 1.*, **1987**, 1011-1015

[243] Potter, G. A., McCague, R., *J. Org. Chem.*, **1990**, 55, 6184-6187

[244] Coe, P. L., Scriven, C. E., *J. Chem. Soc. Perkin Trans. 1*, **1986**, 475-477

[245] Miller, R. B., Al-Hassan, M. I., *J. Org. Chem.*, **1985**, 50, 2121-2123

[246] Davey, W., Tivey, D.J., *J. Chem. Soc.*, **1958**, 1230-1236

[247] Kohler, E.P., Caldwell, H.M., *Org. Synth. Coll. Vol. 1*, **1941**, 2nd Edition, 1, 78

[248] Neilson, A.T., Houlihan, W.J., *Org. React.*, **1968**, 16, 1

[249] Ellern, J.B., *J. Chem. Ed.*, **1979**, 56, 418-421

[250] Wattansin, S., Murphy, W.S., *Synth.*, **1980**, 647-650

[251] Antus, S., Gottsegen, A., Nogradi, M., *Synth*, **1981**, 574-576

[252] Makrandi, J., Grover, S., *Ind. J. Chem.*, **1980**, 19B, 732-734

[253] Krishnamurty, H.G., Ghost, S., Sathyanaraya, N.S., *Ind. J. Chem.*, **1986**, 26B. 1253-12536

[254] Carson, J.R., Carmosin, R.J., Vaught, J.L., Gargochi, J.F., Costanzo, M.J., Raffa, R.B., Almond, H.R., *J. Med. Chem.*, **1992**, 35, 28555-2563

[255] Robertson, D.W., Katzenellenbogen, *J. Org. Chem.*, **1982**, 47, 2387-2393

[256] Manigo, O., *Studies in the Synthesis of Tamoxifen Analogues and Pharmacologically active pyridines,* **1996**

[257] Saunders, B., Cockerill, G., *Mechanism of Elimination Reactions, for review of Dehydration Mechanism*, Wiley & Sons, New York, **1973**

[258] Banthorpe, A., *Elimination Reactions*, American Elsevier, New York, **1963**

[259] Knozinger, H., *Patai Chemistry of the Hydroxyl Group*, Part 2, Interscience, New York, **1971**

[260] Smith, M.B., *Organic Synthesis*, McGraw-Hill Inc., New York, 1ST Edition, **1994**

[261] Grummit, O., Becker, E.J., *Org. Synth. Coll.*, **1963**, 4, 771-793

[262] Brown, H.C., Vanderjagt, D.L., Rothberg, I., Hammar, W.J., Kawakami, J.H., *J. Org. Chem.*, **1985**, 50, 2179-2188

[263] Monson, R.S., *Tetrahedron Lett.*, **1971**, 567-570

[264] Lomas, J.S., Saggatys, D.S., Dubois, J.E., *Tetrahedron Lett.*, **1972**, 165-167

[265] Dixon, S., Wiggins, L.F., *J. Chem. Soc.*, **1954**, 594-597

[266] Traynelis, V.J., Hergenrother, W.L., Livingston, J.R., Valicenti, J.A., *J. Org. Chem.*, **1962**, 27, 2377-2379

[267] Ichihara, A., Miki, M., Tazaki, H., Sakamura, S., *Tetrahedron Lett.*, **1987**, 28, 1175-1178

[268] Cavalieri, L.F., Wolfrom, M.L., *J. Am. Chem. Soc*, **1947**, 69, 2022-2025

[269] Hoffman, R.F., Bishop, R.D., Fitch, P.M., Hardenstein, N., *J. Org. Chem.*, **1980**, 45, 917-921

[270] Pourahmady, N., Vickery, E.H., Eisenbraun, E.J., *J. Org. Chem.*, **1982**, 47, 2590-2593

[271] Miller, R.B., Leclercq, G., Jordan, V.C., *J. Med. Chem.*, **1988**, 31, 1285-1290

[272] Sykes, P., *Mechanisms of Organic Reactions*, 2ND Edition, Longmans, London

[273] Solomons, T.W.G. *Fundamentals of Organic Chemistry*, 3RD Edition, Wiley & Sons, New York, **1982**

[274] Carter, R.H., Garson, M.J., Hill, R.A., Stauntan, J., Sunter, D.C., *J.Chem. Soc. Perkin 1*, **1981**, 471-479

[275] Schneider, M.R., Von Angerer, E., Schonenberger, H., Michel. R-T., Fortmeyer, H.P., *J. Med. Chem.*, **1982**, 25, 1070

[276] Supran, C.T., Manole, G., Andruh, M., *J. Inorg. Biochem.*, **1993**, 49, 97-103

Supran, C.T., Andruh, M., *Rev. Roum. Chim.*, **1994**, 39, 1229-1234

[277] Luca, C., Barboiu, M., Supran, C.T., *Rev. Roum. Chim.*, **1991**, 36, 1169-1173

[278] Supran, C.T., Puscas, J., Carbonic Anhydrase and Modulation of Physiologic and Pathologic Process in the Organism, Ed., Helicon, Timisoara, **1994**, 113-146

[279] Jordan, V.C., Koch, R., Mittal, S., Schneider, M.R., *Br. J. Pharmacol*, **1986**, 87, 217

Jordan, V.C., Gosden, B., *Mol. Cell Endocrinol.*, **1982**, 27, 291

[280] Jarman, M., Leung, O-T., LeClercq, G., Devleeschouwer, N., Stoessel, S., Coombes, R.C., Skilton, R.A., *Anti-Cancer Drug Design*, **1986**, 1, 259

[281] Murphy, C.S., Jordan, V.C., *J. Steroid., Biochem.*, **1989**, 34, 407

[282] Robertson, D.W., Katzenellenbogen, J.A., Hayes, J.R., Katzenellenbogen, B.S., *J. Med. Chem.*, **1982**, 25, 167

[283] Foster, A.B., Jarman, M., Leung, O-T., McCague, R., Leclercq, G., Devleeschouwer, N., *J. Med. Chem.*, **1985**, 28, 1491-1497

[284] Mittal, S., Durani, S., Kapil, R.S., *J. Med. Chem.*, **1985**, 28, 492-497

[285] Robertson, G.M., *Comprehensive Organic Synthesis*, Trost, B.M., Fleming, I., Eds., Pergamon Press; Oxford, **1991**, 2, 563

[286] Mukaiyama, T., Sata, T., Hauna, J., *Chemistry Letters*, **1973**, 1041-1044

[287] McMurry, J.E., *Acc. Chem. Soc.*, **1974**, 96, 1708

[288] McMurry, J.E., *Acc. Chem. Res.*, **1983**, 16, 405-411

[289] Lai, Y-H., *Org. Prep. and Proceed. Int.*, **1980**, 12 (6), 361-391

[290] Finocchiaro, P., Libertini, E., Recca, A, *La Chimica E L'industria*, **1982**, 64, 10

[291] McMurry, J.E., Fleming, M.P., Kees, K.L., Krepski, L. R., *J. Org. Chem.*, **1978**, 48, No. 17, 3255-3266

[292] Tyrlik, S., Wolochowicz, I., *Bull. Soc. Chim. Fr.*, **1973**, 2147

[293] McMurry, J.E., *Acc. Chem. Res.*, **1974**, 7, 281

[294] Olah, G.A., Surya, G.K., Prakash, *J. Org. Chem.*, **1977**, 42, 50

[295] Marshall, J.A., *Acc. Chem. Res.*, **1980**, 13, 213

[296] Marshall, J.A., Black, T.H., J. *Am. Chem. Soc.*, **1980**, 102, 7583

[297] Marshall, J.A., Cotantino, M., Black, T.H., *Synth. Comm.*, **1980**, 10, 689

[298] Akiyama, S., Nakatsuji, S., Eda, S., *Tetrahedron Lett.*, **1979**, 30, 2813

[299] Barlow, L., Pattenden, G., *J. Chem. Soc. Perkin I*, **1976**, 1029

[300] Talukdar, S., Nayak, S.K., Banerji, A., *J. Org. Chem.*,**1998**, 63, 4925-4929

[301] Mukaiyama, T., *Challenges in Synthetic Organic Chemistry*, Ch 20, 95-111, Translation, Eds. Baldwin, J.E., (FRS), Oxford

[302] Bradley, D.C., Hancock, D.C., Wardlaw, W., *J. Chem. Soc*, **1952**, 2773

[303] Furstner, A., *Pure & Appl. Chem.*, **1998**, 70, No. 5, 1071-1076

[304] Corey, E.J., Danheiser, R.L., Chandrasekaran, S., *J. Org. Chem.*, **1976**, 41, 260

[305] Castedo, L., Saa, J.M., Suan, R., Tojo, G., *J. Org. Chem.*, **1981**, 46, 4292

[306] Lenoir, D., Burghard, H., *J. Chem. Res.*, **1980**, 396

[307] Baumstark, A.L., McMleoskey, C.J., Witt, K.E., *J. Org. Chem.*, **1978**, 43, 3609

[308] Newkome, G.R., Roper, J.M., *J. Org. Chem.*, **1979**, 44, 502

[309] Blaumstark, A.L., McCloskey, C.J., Westdrop, I., Geise, H.Y., *J. Org. Chem.*, **1982**, 42, 248-259

[310] Walborsky, H.M., Murari, M.P., *J. Am. Chem., Soc.*, **1980**, 102, 426

[311] Dams, R., Malinowski, M., Westdrop, I., Geise, H.Y., *J. Org. Chem.*, **1982**, 47, 248-259

[312] Smith, M.B., *Organic Synthesis, Theory, Reactions and Methods*, **1996**, 13, 1446-1447

[313] Furstner, A., *Angew Chem.*, **1993**, 105, 171; *Angew Chem. Int. Ed. Engl.*, **1993**, 32, 164

[314] McMurry, J.E., *Chem. Rev.*, **1989**, 89, 1513-1524

[315] Furstner, A., Bogdanovic, B., *Angew Chem. Int., Ed. Engl.*, **1996**, 35, 2442

[316] McMurry, J.E., Rico, J.G., *Tetrahedron Lett.*, **1989**, 30, 116

[317] McMurry, J.E., Dushin, R.G., *J. Am. Chem. Soc.*, **1990**, 112, 694

[318] Furstner, A., Weidmann, H., *Synthesis*, **1987**, 1071

[319] Furstner, A., Csuk, R., Rohrer, C., Weidmann, H., *J. Chem. Soc. Perkin Trans. I*, **1988**, 1729

[320] Furstner, A., Hupperts, A., *J. Am. Chem. Soc.*, **1995**, 117, 4468

[321] Nishida, S., Kataoka, F., *J. Org. Chem.*, 1978, 43, 1612

[322] McMurry, J.E., Kees, K.L. *J. Org. Chem.*, 1977, 42, 2655

[323] McMurry, J.E., Fleming, M.P., *J. Am. Chem. Soc.*, **1974**, 96, 4708

[324] Ho, T-L, Wong, C.M., *Synthetic Communications*, **1973**, 337

[325] McMurry, J.E., Melton, J.J., *Amer. Chem. Soc.*, **1971**, 5309

[326] Timms, G.H., Wildsmith, *Tetrahedron Lett.*, **1971**, 195

[327] Sharpless, K.B., Umbreit, M.A., Neith, M.T., Folld, T.C, *J. Amer. Chem. Soc.*, **1972**, 94, 6538

[328] Meegan, M.J., Hughes, R.B., Lloyd, D.G., Wiliams, D.C., Zisterer, D.M., *Eur. J. Med. Chem.*, **1999**, Submitted

[329] Gauthier, S., Maihot, J., Labrie, F., *J. Org. Chem.*, **1996**, 61, 3890-3983

[330] Daik, R., Feast, W.J., Batsanov, A.S., Howard, J. A. K., *New J. Chem.*, **1998**, 1047-1049

[331] Katzenellenbogen, J.A., Carlson, K.E., Katzenellenbogen, B.S., *J.Steroid. Biochem.*, **1985**, 22, 589

[332] Besancon, J., Szynoniak, J., Moise, C., *J. of Organometallic Chemistry*, **1992**, 426, 325-330

[333] Kangas, L.,*J. Steroid. Biochem.*, **1990**, 36, 191

[334] Crenshaw, R.R., Jefferies, A.T., Luke, G.M., Cheney, L.C., Bialy, G., *J. Med. Chem.*, **1971**, 14, 1185-1187

[335] Lednicer, D., Babcock, J.C., Lyster, S.C., Duncan, G.W., *Chem. Ind. (London)*, **1961**, 2098-2099

[336] Lednicer, D., Babcock, J.C., Marlett, P.E., Lyster, S.C., Duncan, G.W., *J. Med. Chem.*, **1965**, 8, 52-55

[337] Anstead, G.M., Altenbach, R.J., Wilson, S.R., Katzenellenbogen, J.A., *J. Med. Chem.*, **1988**, 31, 1316-1326

[338] Teo, C.C., Kon, O-L., Sim, K-Y., *J. Chem. Res.*, **1990**, 4-5

[339] Ng, S-C., Kon, O-L., Sim, K-Y., Srikanth, N., *Synt. Commun.*, **1993**, 23, 1843-1853

[340] Powell, S.G., Anderson, L., *J. Amer. Chem. Soc.*, **1931**, 53, 811-813

[341] Dann, O., Arndt, W.D., *Justus Liebigs., Ann. Chem.*, **1954**, 587, 38-42

[342] Fontaine, S.G., *Ann. Chem. (Paris)* **1968**, 14, 179-187

[343] Fontaine, S.G., Maitte, P.C.R., *Acad. Sci. Paris*, **1964**, 258, 4583-4587

[344] Buckle, D.R., Eggleston, D.S., Houge-Frydrych, C.S.V., Pinto, I.L., Readshaw, S.A., Smith, D.G., Webster, R.A.B., *J. Chem. Soc. Perkin Trans1*, **1991**, 2763-2771

[345] German Patent, 2, 436, 075 (*Chem. Abstr.*, 9834w, **1975**)

[346] Kametani, T., Fukumoto, K., *J. Chem. Soc. Perkin I.*, **1972**, 394-396

[347] Tandon, V.K., Khanna, J.M., Arand, N., Srimal, R.C., Prasad, C.R., *Indian J. Chem.* **1975**, 13, 1-8

[348] McCague, R., *Tetrahedron Letters*, **1987**, 28, No.6, 701-702

[349] Eckes, L., Hanack, M., *Synth.*, **1978**, 217-221

[350] Engman, L., *Tetrahedron Lett.*, **1987**, 28, 1463, J. Org. Chem., 1987, 52, 4086-4094

[351] Raucher, S., *Tetrahedron Lett.*, **1977**, 3909-3912

[352] Fieser, L.F. and Fieser, H., *Reagents for Organic Chemistry*, Vol. 1, Wiley & Sons, London, **1987**, 967-970

[353] Straus, F., *Annalende Chemie*, **1905**, 342, 262-264

[354] Lansinger, P., Ronand, M., *Synth. Commun.*, **1979**, 9, 341-343

[355] Fieser, L.F., *J. Chem.* Ed., **1954**, 31, 291-293

[356] Freedman, H.H., Dooakian, G.A., *Tetrahedron*, **1964**, 20, 2181-2185

[357] Rolston, J.H., Yates, K., *J. Am. Chem. Soc.*, **1969**, 91, 1469-1491

[358] Dubois, J.E., Goetz, E., *J. Chem. Phys.*, **1966**, 63, 780-785

[359] de la Mare, P.B.D., Bolton, R., *Electrophilic Additions to Unsaturated Systems*, Elsevier, New York, **1966**

[360] Fahey, C., Schneider, H.J., *J. Am. Chem. Soc.*, **1968**, 90, 4429-4434

[361] Buckles, R.E., Miller, J.L., Thurmaier, R.J., *J. Org. Chem.*, **1967**, 32, 888-892

[362] Bellucci, G., Barili, P.L., Marioni, F., Morelli, I., Scartoni, V., *J. Org. Chem.*, **1972**, 37, 4353 – 4357, 947-951

[363] Roberts, R.E., Kimball, G.E., *J. Am. Chem. Soc.*, **1937**, 59, 947-951

[364] Wilson, M.A., Woodgate, P.D., *J. Chem. Soc. Perkin. Trans. II*, **1976**, 141 - 147

[365] Dubois, J.E., Huynh, X.Q., *Tetrahedron Lett.*, **1971**, 3369-3372

[366] Barili, P.L., Bellucci, G., Marioni, F., Morelli, I. Scartoni, V., *J. Org. Chem.*, **1972**, 37, 4353-4357

[367] Heasley, V.L., Arnold, S., Gipe, A., McKee, D., Orr, R., Rogers, S.L., Shellamer, D.F., *J. Org. Chem.*, **1978**, 14, 2793-2799

[368] Stanforth, S.P., *Tetrahedron*, **1998**, 54, 263-303

[369] Miyaura, N., Suzuki, A., *Chem. Rev.*, **1995**, 95, 2457-2483

[370] Brown, A.G., Crimmin, M.J., Edwards, P.D., **1992**, *J. Chem. Soc. Perkin Trans. 1*, 1992, 123-130

[371] Tamao, K., Sumitani, K., Kumada, M., *J. Am. Chem. Soc.*, **1972**, 94, 2374
Kumada, M., *Pure Appl. Chem.*, **1980**, 52, 669

[372] Corriu, R.J.P., Masse, J.P., *J. Chem. Soc. Chem. Commun.*, **1972**, 144

[373] Tamura, M., Kochi, J.K., *J. Am. Chem. Soc.*, **1971**, 93, 1487

Kochi, J.K., *Acc. Chem. Res.*, **1974**, 7, 351

[374] Yamamura, M., Moritani, I., Murahashi, S., *J. Organomet. Chem.*, **1975**, 91, C39

[375] Negishi, E., Baba, S., *J. Chem. Soc., Chem. Commun.*, **1976**, 596

[376] Murahashi, S., Yamamura, M., Yanagisawa, K., Mita, N., Kondo, K., *J. Org. Chem.*, **1979**, 44, 2408

[377] Kosugi, M., Simizu, Y., Migita, T., *Chem. Lett.*, **1977**, 1423

[378] Stille, J.K., *Angew. Chem. Int. Ed. Engl*, **1986**, 25, 508-524

[379] Alexakis, N.J.A., Normant, J.F., *Tetrahedron Lett.*, **1981**, 22, 959

[380] Hatanaka, Y., Hiyama, T., *J. Org. Chem.*, **1988**, 53, 918

[381] Badone, D., Baroni, M., Cardamone, R., Jelmini, A., Guzzi, U., *J. Org. Chem.*, **1997**, 62, 7170-7172

[382] Heck, R.F., *Palladium in Organic Synthesis, Academic Press*, 2nd Edition, London, **1987**

[383] Tsuji, T., *Organic Synthesis with Palladium Compounds*, Springer-Verlag, Berlin, **1980**

[384] Kochi, J.K., *Organometallic Mechanism and Catalysis*, Academic Press, New York, **1978**

[385] Bozell, Vogt., *J. Am. Chem. Soc.*, **1988**, 110, 2655

[386] Anderson, Karabelas, Hallberg, *J. Org. Chem.*, **1986**, 50, 3891

[387] Mizoroki, Mori, Ozaki, *Bull. Chem. Soc.* Jpn., **1971**, 44, 581, 1973, 46, 1505

[388] Heck, *J. Am. Chem. Soc.*, **1968**, 90, 5518, 5526, 5535

[389] Kozhevnikov, *Russ. Chem. Rev.*, **1983**, 52, 138-151

[390] Daves, Hallberg, *Chem. Rev.*, **1989**, 89, 1433-1445

[391] Galland, J-C., Savignac, M., Genet, J-P., *Tetrahedron Letters*, **1999**, 40, 2323-2326

[392] Suzuki, A., *Pure Appl. Chem.*, **1991**, 63, 419,

Oh-e, T. Miyaura, N., Suzuki, A. *J. Org. Chem.*, **1993**, 58, 2201

[393] Wallow, T.I., Norak, B.M., *J. Org. Chem.*, **1994**, 59, 5034

[394] Marck, G., Villiger, A., Buchecker, R., *Tetrahedron Lett.*, **1994**, 35, 3277

[395] Migita, T., Nagai, T., Kiuchi, K., Kosugi, M., *Bull Chem. Soc.* Jpn., **1983**, 59, 2869

Hunt, A.R., Stewart, S.K., Whiting, A., *Tetrahedron Lett.*, **1993**, 34, 3599

[396] Miyaura, N., Ishigama, T., Sasaki, H., Ishikawa, A.M., Sotoh, M., Suzuki, A., *J. Am. Chem. Soc.*, **1989**, 111, 314-321

[397] Spencer, R., J. *Organomet. Chem.*, **1983**, 258, 101-104

[398] Bender, P., Staken, N., Heck, R.F., *J. Org. Chem.*, **1982**, 47, 1278-1281

[399] Negishi, E., Valent, L.F., Kobayashi, M., *J. Am. Chem. Soc.*, **1980**, 102, 3298-3301

[400] Kobayashi, M., Negishi, E., *J. Org. Chem.*, **1978**, 45, 5223-5226

[401] Negishi, E., *Acc. Chem. Res.*, **1982**, 15, 340 -350

[402] Negishi, E., King, A.O., Okukado, N., *J. Org. Chem.*, **1977**, 42, 1821-1822

[403] King, A.O., Negishi, E., *J. Org. Chem.*, **1978**, 43, 358-361

[404] Hayashi, T., Konishi, M., Kobori, Y., Kumadd, M., Higuchi, T., Hirotsu, K., *J. Am. Chem. Soc.*, **1984**, 101, 158-163

[405] Smith, G.B., Dezeny, G.C., Hughes, D.L., King, A.O., Verhoeven, T.R., *J. Org. Chem.*, **1994**, 59, 8156-8159

[406] Negishi, E-I., Takahashi, T., Baba, S., Van Hora, D.E., Okukado, N., *J. Am. Chem. Soc.*, **1987**, 109, 2393-2401

[407] Ishiyama, T., Murata, M., Miyaura, N., *J. Org. Chem.*, **1995**, 60, 7508-7510

[408] Martin, A.R., Yang, Y., *Acta. Chemica Scandinavica*, **1993**, 47, 221-230

[409] Aliprantis, A.O., Canary, J.W., *J. Am. Chem. Soc.*, **1994**, 116, 6985

[410] Ng, J.S., Katzenellenbogen, J.A., Kilbourn, M.R., *J. Org. Chem.*, **1981**, 46, 2520-2522

[411] Elnagdi, M.H., Negm, A.M., Sudek, K.V., *Synth.*, **1994**, 27-31

[412] Cabri, W., Candiani, F., *Acc. Chem. Res.*, 1995, 28, 2 -7

[413] Trost, B.M., *Tetrahedron (report)*, **1977**, 33, 2615-2649

[414] Matteson, D.S., *Boron Chemistry, Proceedings of 6th Int. Meeting on Boron Chemistry*, **1987**, Hervmauek, S., World Scientific

[415] Mikhailov, B.M., Bubnov, Yu., N., *Organoboron Compounds in Organic Synthesis*, Harwood Academic Pub., Amsterdam, **1983**

[416] Gardner, J.H., Borgstrom, P., *J. Am. Chem. Soc.*, **1929**, 51, 3375

[417] George, T.A., Lappert, M.F., *J. Chem. Soc. Chem. Commun.*, **1966**, 43

[418] Brown, H.C., Molander, G.A., *J. Org. Chem.*, **1981**, 46, 645

[419] Suzuki, A., *Pure Appl. Chem.*, **1994**, 66, 213 and **1985**, 57, 1749

Miyaura, N., Suzuki, A., *J. Synth. Org. Chem.* Jpn., **1993**, 51, 1043

[420] Frankland, E., Duppa, B.F., Justus, *Liebigs Ann: Chem.*, **1960**, 115 319

[421] Matteson, D.S., *The Chemistry of the Metal-Carbon bond*, Vol. 4, Hartley, Patai, S., Eds. J. Wiley & Sons, **1987**, 307

[422] Washburn, R.M., Levens, E. Albright, C.F., Billig, F.A., *Organic Syntheses*, Coll. Vol. 4, Wiley, New York, **1963**, 68-72

[423] Knotinsky, E., Melamed, M., *Chem. Ber.*, **1909**, 43, 3090

[424] Snyder, H.R., Kuck, J.A., Johnson, J.R., *J. Am. Chem. Soc.*, **1938**, 60, 105

[425] Matteson, D.S., *Tetrahedron*, 45, No. 7, **1989**, 1859-1885

[426] Matteson, D.S., Liedtke, J.D., *J. Am. Chem. Soc.*, **1965**, 87, 1526

[427] Kabalka, G.W., Sastry, U., Sastry, K.A.R., *J. Organometallic Chem.*, **1983**, 259, 269-274

[428] Frankland, E., Duppa, D.F., *Ann. Chem.*, **1862**, 124, 129

[429] Johnson, J.R., Snyder, H.R., Van Campen, M.G., *J. Am. Chem. Soc.*, **1938**, 60, 115

[430] Matteson, D.S., Shdo, J.G., *J. Ann. Chem. Soc.*, **1963**, 85, *2684*
J. Org. Chem., **1964**, 2742, 29

[431] Miyaura, N., Suginome, H., Suzuki, A., *Tetrahedron*, **1983**, 39, 3271
Satoh, M., Nomato, Y., Miyaura, N., Suzuki, A., *Tetrahedron Lett.*, **1989**, 30, No. 29, 3789-3792

[432] Brown, H.C., Cole, T.E., *Organometallics*, **1983**, 2, 1316

Brown, H.C., Rangaishenvi, M.V., *Tetrahedron Lett.*, **1990**, 49, 7113-7115

[433] Morgan, J., Hambley, T.W., Pinley, J.T., *J. Chem. Soc., Perkin Trans. 1*, **1996**, 2173-2176

[434] Black, W.C., Brideau, C., Chan, C-C., Charleson, S., Chauret, N., Claveau, D., Ethier, D., Gordon, R., Greig, G., Guay, J., Hughes, G., Jolicoeur, P., Leblanc, Y., Nicoll-Griffith, D., Ouimet, N., Riendeau, D., Visco, D., Wang, Z., Xu, L., Prasit, P., *J. Med. Chem.*, **1999**, 42, 1274-1281

[435] Seamen, W., Johnson, J.R., *J. Am. Chem. Soc.*, **1931**, 53, 711-723

[436] Groziak, M.P., Ganguly, A.D., Robinson, P.D., **1994**, *J. Am. Chem. Soc.*, 116, 7597-7605

[437] Wong, M.S., Nicoud, J-F., *Tetrahedron Letters*, **1993**, 34, No. 51, 8237-8240

[438] Creary, X., Aldridge, T., *J. Org. Chem.*, **1991**, 56, 4280-4285

[439] Greene,T.W., "*Protective Groups in Organiuc Chemisrtry*", **1981**, Wiley & Sons

[440] Kirihara, M., Morimoto, T., Ichimoto, I., *Biosci. Biotech. Biochem.*, **1993**, 57(11), 1940 – 1941
Snyder, H.R., Reedy, A.J., Lennarz, W.J., *J. Am. Chem. Soc.*, **1958**, 80, 835-838

[441] Vollhardt, K.P.C., *Organic Chemistry*, **1987**, Freeman & Company, New York

[442] Moreno-Manas, M., Perez, M., Pleixats, R., *J. Org. Chem.*, **1996**, 61, 2346

[443] Anderson, N.C., Maddaford, S.P., Keay, B.A., *J. Org. Chem.*, **1996**, 61, 9556-9559

[444] Miyaura, N., Yanogi, T., Suzuki, A., *Synth. Common.*, **1981**, 11, 513,
Hoshino, Y., Miyaura, N., Suzuki, A., *Bull. Chem. Soc.* Jpn., **1988**, 61, 3008

[445] Potter, G.A., McCague, R., *J. Org. Chem.*, **1990**, 55, (25), 6184-6187

[446] Mitchell, M.B., Wallbank, P.J., *Tetrahedron Lett.*, **1991**, 32, 2273

[447] Bumagin, N.A., Bykov, V.V., Belestkaya, I.P., Izv, Akad., Naud, SSSR., *Ser. Khim*, **1989**, 10, 2394

[448] Bumagin, N.A., Bykov, V.V., Belestkaya, I.P., Dokl. Akad., Naud, SSSR., *Ser. Khim*, **1990**, 315, 113

[449] Casalnuovo, A.L., Calabrese, J.C., *J. Am. Chem. Soc.*, **1990**, 112, 4324

[450] Watanabe, T., Miyaura, N., Suzuki, A. *Synlett.*, **1992**, 207

[451] Rocca, P., Marsais, F., Godard, A., Queguiner, G., *Tetrahedron Lett.*, **1993**, 34, 2937

[452] Kelly, T.R., Garcia, A., Long, F., Walsh, J.J., Bhaskar, K.V., Boyd, M.R., Gotz, R., Keller, P.A., Walter, J.J., Bringmann, G., *Tetrahedron Lett.*, **1994**, 35, 7621

[453] Zhang, H., Kwong, F.Y., Tian, Y., Chan, K.S., *J. Org. Chem.*, **1998**, 63, 6886-6890

[454] Gronowitz, S., Bobosik, V., Lawitz, K., *Chem. Scr.*, **1984**, 23, 20

[455] Yang, Y., Hornfeldt, A-B., Gronowitz, S., *J. Heterocycl. Chem.*, **1989**, 26, 865

[456] Thompson, W.J., Jones, J.H., Lyle, P.A., Thies, J.E., *J. Org. Chem.*, **1988**, 53, 2052-2055

[457] Thompson, W.J., Gaudino, J., *J. Org. Chem.*, **1984**, 49, 5237

[458] Utenishi, J-I., Beau, J-M., Armstrong, R.W., Kishi, Y., *J. Am. Chem. Soc.*, **1987**, 109, 4756

[459] Roush, W.R., Brown, B.B., Drozitz, S.E., *Tetrahedron Lett.*, **1988**, 29, 3541

[460] Yang, Y., Martin, A.R., *Heterocycles*, **1992**, 34, 1395

[461] Ishiyama, T., Matsuda, N., Miyaura, N., Suzuki, A., *J. Am. Chem. Soc.*, **1993**, 115, 11018-11019

[462] Ishikura, M., Kamada, M., Terashima, M., *Synthesis*, **1984**, 936

[463] Hayashi, T., Konishi, M., Kumada, M., *Tetrahedron Lett.*, **1979**, 1871

[464] Miyaura, N., Yamada, K., Suginome, H., Suzuki, A., *J. Am. Chem. Soc.*, **1985**, 107, 972

[465] Lorand, J.P., Edwards, J.O., *J. Org. Chem.*, **1959**, 24, 769

[466] Torssell, K. Ark. *Kemi.*, **1957**, 10, 541

[467] Stille, J.K., Lau, K.S., Y., *Acc. Chem. Res.*, **1977**, 10, 434

[468] Gille, A., Stille, J.K., *J. Am. Chem. Soc.*, **1980**, 102, 4933

[469] McCrindle, R., Ferguson, G., Arsenault, G.J., McAlees, A.J., Stephanson, D.K., *J. Chem. Res.* (S), **1984**, 360

[470] Farina, V., Krishnan, B., *J. Am. Chem. Soc.*, **1991**, 113, 9585

[471] Yamamoto, A., *Organotransition Metal Chemistry – Fundamental Concepts and Applications*, Wiley, New York, **1986**

[472] Ozawa, F., Yamamoto, A., *Chem. Soc.* Jpn., **1987**, 773

[473] Stang, P.J., Kowalski, M.H., *J. Am. Chem. Soc.*, **1989**, 111, 3356

[474] Dieck, H.A., Heck, R.F., *J. Org. Chem.*, **1975**, 40, 1083

[475] Honeycutt, J.B., Riddle, J.M., *J. Am. Chem. Soc.*, **1959**, 81, 2593

[476] Ainley, A.D., Challenger, F., *J. Chem. Soc.*, **1930**, 2171

[477] Brown, H.C., Herbert, N.C., Snyder, C.H., *J. Am. Chem. Soc.*, **1961**, 83, 1001

[478] Hydes, P.C., Russell, M.J.H., *Cancer and Metastasis Rev.*, **1988**, 7, 67-89

Lemaire, P.C., Fouchert, M.H., Koxelka, J., *J. Inorg. Biochem.*, **1994**, 53, 261-271

[479] Grenier, G., Berube, G., Gicquaud, C., *Chem. Pharm. Bull.*, **1998**, 46, 9, 1480-1483

[480] Okamoto, Y., Hatada, K., *J. Liq. Chromatogr.*, **1986**, 9, 369

[481] Gunther, H., *NMR Spectroscopy, Basic Concepts, principles and applications in Chemistry*, **1995**, 2nd Edition

[482] Campbell, R., *Synthesis of cyclohepta[b]pyridines, benzocyclohepta[1,2-b]pyridines, and [1]benzoxepin* [5,4-b]pyridines, **1994**

[483] Brady, B.A., Kennedy, J.A., O'Sullivan, W.I., *Tetrahedron*, **1973**, 29, 359-362

[484] Silverstein, R.M., Bassler, G.C., Morrill, T.C., *Spectroscopic Identification of Organic Compounds*, 5th Edition, Wiley & Sons, New York, **1991**

[485] Soule, H.D., Vazque, Z.J., Long, A., Albert, S., Brennan, M., *J. Nat. Cancer Inst.*, **1973**, 51, 1409-1413

[486] Bradford, M.M., *Anal. Biochem.*, **1976**, 72, 248-254

[487] Mosmann, T., *J. Immunol. Methods*, **1983**, 65, 55-63

[488] Younkin, D.P., Tang, C.M., Hardy, M., Reddy, V.R., Shi, Q.Y., Pleasure, S.J., Lee, V. M-Y., Pleasure, D., *Proc. Natl. Acad. Sci.*, **1993**, USA, 90, 2174-2178

[489] Fishman, J.H., *Biochem. and Biophys. Research. Comm.*, **1983**, 110, No. 3, 713-718

[490] Yang, D.J., Tewson, T., Tansey, W., Kuang, L-R., Reger, G., Cherif, A., Wright, K.C., Moult, R.G., Tilbury, R.S., Chu, K., Kim, E.E., Wallace, S., *J. Pharm. Sci.*, **1992**, 80, No. 7, 622-625

[491] Wilkinson, G.N., *J. Biochem.*, **1961**, 80, 324-332

[492] Yang, D.J., Tewson, T., Tansey, W., *J. Pharm. Sciences*, **1997**, 81, No. 7, 622-625

[493] Lamberts, S.W., Verleun, T., *Eur. J. Cancer Clin. Oncol*, **1987**, 8, 1117-1123

[494] Katzenellenbogen, B.S., *Biol. Reprod.* **1996**, 54, 287-293

[495] Beato M., Sanchez-Pacheco, A., *Endocr. Rev.* **1996**, 17, 587-609.

[496] Wolf D.M., Langanfahey S.M., Parker C.J., *J. Natl. Cancer I*, **1993**, 85 806-812.

[497] Leichtl, S., Von Angerer, E., *Arch. Pharm.*, **1998**, 331, 283-289.

[498] Wurtz, J.M., Egner, U., Heinrich, N., Moras, D., Mueller-Fahrnow, A. *J. Med Chem.*, **1998**, 41, 1803-1814.

[499] Spartan,Version 5.1, Wavefunction Inc., California

[500] Bali, S.E., Kaftory, M., Rappoport, Z., *J. Org. Chem.*,**1989**, 54 No. 20, 4959-4962

[501] Hossain, M.B., van der Helm., D., Schmitz, F.J., Podesimo, E. O., *J. Med. Chem.*, **1994**, 37, 1670-1683

[502] Kuramochi, H., *J. Med. Chem.*, **1996**, 39, 2877-2886

[503] Brzozowski, A.M., Pike, A.C.W. *Brookhaven Protein Database entry 1ERR and 1ERE*, **1998**

[504] Shiau, A.K., Barstad, D., Loria, P.M., Cheng, L., Kushner, P.J., Agard, D.A., Greene, G.L., *Brookhaven database entry 3ERT and 3ERD*, **1999**.

[505] Klinge, C.M., Studinski-Jones, A.L., Kulakosky, P.C., Bambara, R.A., Hilf, R. *Mol. Cell. Endocrinol.*, **1998,** 143, 79–90.

[506] Mohamadi, F., Richards, N.G.J., Guida, W.C., Liskamp, R., Lipton, L., Caufield, C., Chang G., Hendrickson, T., Still, W.C., *J. Comput. Chem.*, **1990**, 11, 440.

[507] Macromodel, Version 6.5, Schrodinger Inc., Portland, New Jersey

[508] Alinger, N.L., Yuh, Y.H., Lii, J.-H., *J. Am. Chem. Soc.*, **1989**, 111, 8551.

[509] Kabsch, W., *Acta Crystallogr.*, **1976**, A. 32, 922, **1978,** 34, 827.

[510] Sobolev, V., Wade, R.C., Vriend, G., *Proteins*, **1996**, 25, 120-129

[511] Sobolev, V., Sorokine, A., Prilusky, J., Abola, E.A., Edelman, M., *Bioinformatics*, **1999**, 15, 327-332.

[512] Wallace, A.C., Laskowski, R.A., Thornton, J.M., *Prot. Eng.*, **1995**, 8, 127-134.

[513] Carson, M., *Enzymol.*, **1997**, 277, 493-505

[514] Fuchs-Young, R., Glasebrook, A.L., Short, L.L., Draper, M.W., Rippy, M.K., Cole, H.W., Magee, D.E., Termine, J.D., Bryant, H.U., *Ann. N.Y. Acad. Sci.*, **1995**, 761, 355-360

[515] Chen, H.M., Tritton, T.R., Kenny, M., Absher, M., Chiu, J.F., *J. Cell. Biochem.*, **1996**, 61, 9-17

[516] Osborn, M.T., Chambers, T.C., *J. Biol. Chem*, **1996**, 271, 30950-30955

[517] Rowlands, M.G., Budworth, J., Jarman, M., Hardcastle, I.R., McCague, R., Gescher, A., *Biochem. Pharmacol.*, **1995**, 50, 723-726

[518] Perrin, D.D., Armarego, W.L.F., Perrin, D.R., *Purification of Laboratory Chemicals*, 2[ND] Edition, Pergamon Press, **1985**

[519] Hurd, Roe, *J. Am. Chem. Soc.*, **1939**, 61, 3355

[520] Weir, J.R., Patel, B.A., Ghosh, S., Sathyanaraya, N.S. *J. Org. Chem.*, **1980**, 45, 4920-4923

[521] Tikalova, J., *Czeck 183,524, C1 C07C97/065*, **1980**, 95, P6825F

[522] Pribeck, F. et al, *Teljes, Hung 17321, C1 C07C*, **1978**, 92, P180820Y

[523] Allen, R.E., Palopoli, F.P., Schumunn, E.L., *US Patent US 2914529*, **1959**

[524] Brown, T.H., Cooper, D.G., *Int Patent Apl. WO 9413291, GB Patent GB 922611*, **1994**, 255651

[525] Chara, T.C., Mahal, H.S., Venkataraman, K., *J. of Chemical Society*, **1933**, 2, 1459-1462

[526] Schickander, H., Roland, I., *US Patent US 4,*764,533, **1988**

[527] Da Re, P., Verlicchi, L., Setnikar, I., *J. Med. Chem*, **1967**, 10 (2), 266-270

[528] Ney, E., *Diese Berichte* (Beilstein), **1888**, 21, 2445

[529] *Appl. Environ., Microbiol.*, **1996**, 62(8), 2811-2817

[530] Collins, D.J., Hobbs, J.J., Emmens, C.W., *J. Med. Chem*, **1971**, 14(10), 952-957

[531] Huth, A., Beetz, I., Schumann, I., *Tetrahedron*, **1989**, 45, No. 21, 6679-6682

[532] Ward, P., Armour, D.R., Bays, D.E., Evans, B., Giblin, G.M.P., Heron, N., Hubbard, T., Liang, K., Middlemiss, D., Mordaunt, J., Naylon, A., Pegg, N.A., Vinader, V., Watson, S.P., Bountra, C., Evans, D.C., *J. Med. Chem*, **1995**, 38, 4985-4992

[533] Juneja, R.K., Robinsonn, K.D., Johnson, C.P., Alwood, J.L., *J. Am. Chem. Soc.*, **1993**, 115, 3818-3819

[534] Ian, Y., Vogt, A, Sebti, S.M., Hamilton, A.D., *J. Med. Chem*, **1996**, 39, 217-223

[535] Lancaster Catalogue, **1997-1999**

[536] Muller, W., Lowe, D.A., Neijt, H., Urwyler, S., Herrling, P.L., *Helv. Chim. Acta.*, **1992**, 75, 855-864

[537] Koen, M.J., Morgan, J., Pinley, J.T., Sherry, C.J., *J. Chem, Soc. Perkin Trans 1*, **1997**, 487-498

[538] Post Doctorate work: Dr. Richard Werner, TCD., *Private communication*, unpublished work

[539] Shieh, W-C., Carlson, J.A., *J. Org. Chem.*, **1992**, 57, 379-381

[540] Black, W.C., Brideau, C., Chan, C.C., Charleson, S., Chauret, N., Claveau, D., Ethier, D., Gordon, R., Greig, G., Guay, J. Hughes, G., Jolicoeur, P. Leblanc, Y., Nicoll-Griffith, D., Ouimet, N., Riendeau, D., Visco, D., Wangg, Z., Xu, L. Prasit, P., *J. Med. Chem.*, **1999**, 42, 1274-1281

[541] El-Wakil, Hoda, A., *Org. Prep. Proceed.*, **1991**, 25(6), 754-757