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Conformationally Restricted and *Bis*-Aryl Guanidinium Derivatives: Towards Improved Alpha$_2$-Adrenergic Affinity and Antagonism for the Treatment of Depression

Declaration

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Acknowledgments

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Abstract

The evident necessity to develop novel therapeutics to treat depression has been recently highlighted by the World Health Organisation who has listed depression to be the second largest global health burden by the year 2020. The heterogeneity of depression implies that multiple neuronal substrates and mechanisms contribute to its aetiology, making therapeutic research advancements in this area extremely difficult. Pharmacological manipulation of monoamine transmission constitutes the mechanism of all clinical antidepressants through the normalisation of chemical levels and hence the alleviation of depressive symptoms.

Noradrenergic transmission plays a central role in the mediation of stress where alterations in its signalling as well as changes in physical features of its plasticity are heavily implicated in stress-related disorders. Dysfunction of noradrenergic transmission in depression has been hypothesised to be directly linked to the α_2-adrenergic receptors (α_2-AR), as an increase in receptor density in patients with depression has been demonstrated. Clinical α_2-AR antagonists have been shown to desensitize α_2-AR density in the prefrontal cortex and stimulate neurogenesis in the hippocampus affording more potent and faster behavioural antidepressant responses than other clinical antidepressant agents.

In this project, thirty six novel guanidine derivatives (divided in five families) have been prepared as α_2-AR ligands (34 of them being α_2-AR antagonists) for potential use as antidepressants. These molecules were designed using combinations of computational studies and pharmacological results previously obtained in the group, along with rational ligand-based design for certain compounds that displayed novel and more complex structures. Their affinity toward the α_2-AR was measured *in vitro* using human brain frontal cortex tissue by competition binding assays against the selective α_2-AR radioligand [^{3}H]RX821002. Those compounds that displayed good affinity ($pK_i > 6.5$) were subjected to further *in vitro* functional [^{35}S]GTPγS binding experiments to determine their nature as antagonists or agonists.

A comprehensive structural activity relationship has been developed for all families prepared highlighting some evident structural features that should be explored for future work to obtain more potent antagonist ligands of the α_2-AR. Encouragingly, two compounds in particular displayed competitive antagonist activity and obtained the highest α_2-AR affinity to date in the past 15 years of research of our laboratory in this area. These compounds are now seen as our two new leads for future design.

Abbreviations

5HIAA:	5-Hydroxyindoleacetic acid
5-HTTP:	5-Hydroxytryptophan
Ach:	Acetylcholine
AchE:	Acetylcholinesterase
ADH:	Aldehyde Dehydrogenase
Akt:	Protein Kinase B
AMP:	Adenosine Monophosphate
AMPA:	α-Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic Acid
AR:	Adrenoceptor
Arc:	Activity-Regulated Cytoskeleton-Associated Protein
BDNF:	Brain-Derived Neurotrophic Factor
BINAP:	2,2'-Bis(diphenylphosphino)-1,1'-binaphthyl
CaMKII:	Calmodulin-Dependent Protein Kinase II
cAMP:	Cyclic Adenosine Monophosphate
CNG:	Cyclic-Nucleotide Gated
CoMFA:	Comparative Molecular Field Analysis
COMT:	Catechol *O*-Methyltransferase
CRE:	cAMP Response Element
CREB:	cAMP Response Element Binding Protein
CRF:	Corticotropin Releasing Factor
CUS:	Chronic Unpredictable Stress
DAT:	Dopamine Transporter
DCC:	*N,N'*-Dicyclohexylcarbodiimide
DMAP:	4-Dimethylaminopyridine
DOMA:	3,4-Dihydroxymandelic acid

DOPEG:	3,4-Dihydroxyphenylglycol
DSM V:	Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
EAS:	Electrophilic Aromatic Substitution
ECT:	Electro Convulsion Therapy
EDCI:	Ethyl-3-aminopropyl carbodiimide
ERK:	Extracellular Signal-Regulated Kinases
FST:	Forced Swim Test
GABA:	Gamma-aminobutyric acid
GCR:	Glucocorticoid Receptor
GDP:	Guanosine Diphosphate
GPCR:	G-Protein Coupled Receptor
GTP:	Guanosine Triphosphate
HPA:	Hypothalamic Pituitary Adrenal
HPLC:	High Performance Liquid Chromatography
iGluR:	Ionotropic Glutamate Receptors
IMHB:	Intramolecular Hydrogen Bond
LTP:	Long Term Potentiation
mAChR:	Muscarinic Acetylcholine Receptors
MAO:	Monoamine Oxidase
MAOI:	Monoamine Oxidase Inhibitors
MAPK:	Mitogen-Activated Protein Kinases
MDD:	Major Depressive Disorder
mGluR:	Metabotropic Glutamate Receptor
MOPEG:	3-Methoxy-4-hydroxyphenylethylene glycol
mTOR:	Mammalian Target of Rapamycin
nAChR:	Nicotinic Acetylcholine Receptors
NARI:	Selective Noradrenalin Reuptake Inhibitors

NAT:	Noradrenaline Transporter
NMDA:	*N*-Methyl-D-Aspartate
NMJ:	Neuromuscular Junction
NMR:	Nuclear Magnetic Resonance
NT:	Neurotransmitter
PDE4I:	Phosphodiesterase type 4 Inhibitors
PFC:	Prefrontal Cortex
PI3K:	Phosphatidylinositide 3-Kinases
PKA:	Protein Kinase A
QSAR:	Quantitative Structure–Activity Relationship
RIMA:	Reversible and Selective Inhibitors of Monoamine Oxidase
RX821002:	2-Methoxyidazoxan
SAR:	Structural Activity Relationship
SCN:	Superchiasmic Nucleus
SDM:	Site Directed Mutagenesis
SDT:	Sleep Deprivation Therapy
SERT:	Serotonin Transporter
SNRI:	Serotonin and Noradrenaline Reuptake Inhibitors
SNS:	Sympathetic Nervous System
SSRI:	Selective Serotonin Reuptake Inhibitors
TCA:	Tricyclic Antidepressants
TFAA:	Trifloroacetic Anhydride
THF:	Tetrahydrofuran
TLC:	Thin Layer Chromatography
TMEDA:	Tetra-methylethylenediamine
TMS:	Trimethylsilane
Trk B:	Tropomyosin Receptor B

TST: Tail Suspension Test

VMA: Vanillylmandelic acid

VMAT: Vesicular Monoamine Transporter

WHO: World Health Organisation

Contents

Chapter 1- Introduction

1.1. Depression

Depression, in simplistic terms, is an affective or mood disorder that causes feelings of sadness and loss of interest; it affects how you think and behave, and can lead to a variety of emotional and physical problems, with the intensity and persistency of these feelings encompassing everything from mild anxiety to major depressive disorder (MDD). The term MDD came about in the 1960s when the treatment of depression was revolutionized by the discovery of the first medical agents to treat it, and is now is identified by the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM V). This diagnosis is based solely on signs and symptoms of the patients, where a certain number of symptoms, such as suicidal thoughts, hopelessness, changes in appetite, decreased interest in pleasurable stimuli etc., are felt for a period over two weeks and disrupt normal social and occupational functions.[1] Diseases such as cancer are of course diagnosed through objective diagnosis tests: biopsy or imaging; however, in the case of depression diagnosis is based on a changeable series of subjective symptoms, and therefore depression can be seen as a heterogeneous disease comprising of numerous disorders.[2]

The prevalence of depression is becoming an alarming issue as the WHO in 2012 listed 300 million people to be affected worldwide; additionally, neuropsychiatric disorders as a whole, with depression having the highest numbers, are now the second largest global health burden.[3] In 2012, from the National Survey on Drug Use and Health (NSDUH) in the U.S. an estimated 16 million adults aged 18 or older had at least one major depressive episode in the past year (Figure 1.1.1). This represented 6.9% of all U.S. adults, with higher levels in both women and younger age groups.[4] On seeing these statistics it is not surprising that the economic burden plays an enormous part in affective disorders in terms of both patient and government expenditure. An estimation from the International Labour Organization was that 3-4% of gross national product in EU states was paid towards care costs for psychiatric illnesses.[5]

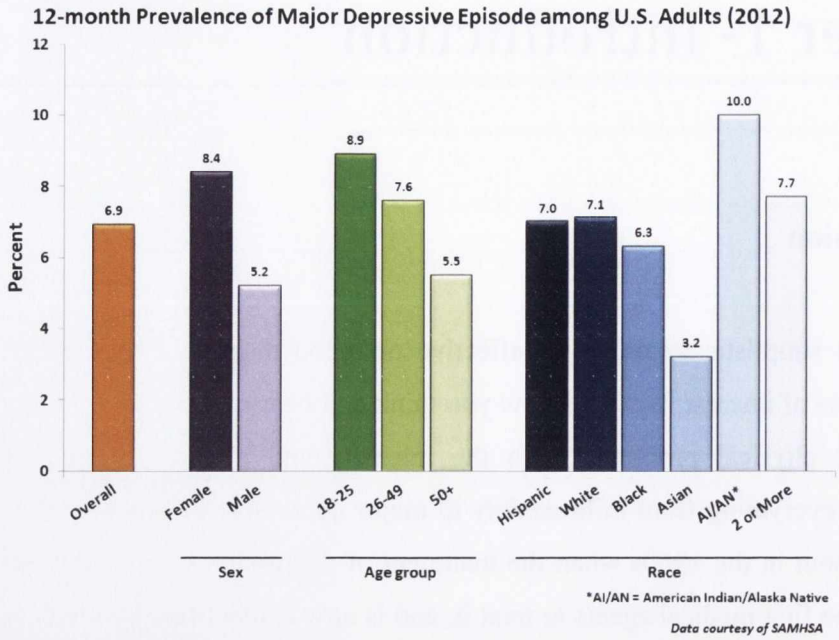


Fig. 1.1.1. Bar chart illustrating the percentage of depressive episodes in the U.S.[4]

Moreover, an individual attending a GP with feelings of depression may not have the finance or medical insurance to back up psychotherapy. There are many studies on the number of sessions needed to affect change in a person's life, and these journeys are extremely complex and relationally intricate. With little public resource it is understandable why the cheaper medical option is often presented, and of course this continuously feeds the issue of over-prescribing antidepressants.[6]

In terms of symptoms, there are two major subcategories of unipolar depression: reactive and physical. Formerly known as exogenous depression, meaning depression from the environment, reactive depression is a response to a particularly stressful or emotionally traumatic event, the death of a loved one, being rejected, divorce, or serious illness can bring about its onset. Physical or endogenous depression, meaning depression from within, is thought to be the result of deficiencies in neuron communications in the brain. In the world of evidence-based medicine, researches are increasingly trying to move beyond just 'what works', to 'what works for whom', as depressive states do share some genetic and neurobiological features, but otherwise differ in symptoms and aetiology.

Hence, when patients present with feelings of depression it is very important to take a full history of the person's life. Where reactive depression is triggered, the natural response is grief, and this is a highly important emotion that allows the mind to adjust to the loss that has occurred. It is important in these cases, whilst highly uncomfortable and disruptive psychologically, that the automatic response from a medical practitioner is not just a prescription. Conversely, as mentioned, physical depression will not be situational or circumstantial, it is born, and manifests in the individual; this is where combinations of medical and psychological therapy are necessary.

Physical depression invokes/suggests questions of a specific genetic cause to the disorder. Indeed, genetic factors play an important role in depression, as seen from the prevalence amongst family and twins studies; however, no genetic mutation or gene has been identified to increase risk factor for MDD.[7] This is mainly due to the complex genetic heterogeneity associated with the disorder, and that each potential gene involved contributes so little to the total genetic factor that gene localisation and identification is very difficult.[8]

In terms of medical treatment, the most problematic issue is that all antidepressants on the market today, although are much improved in terms of adverse effects from those developed in the 1950s, work solely by the same mechanism, indicating sub-optimal advances in the past 60 years. Moreover, current antidepressants display a delayed onset of therapeutic action (4-8 weeks), resulting in non-compliance and a dramatic increased risk for suicidal behaviour. And this itself is becoming an increasing issue, as now approx. 15% of clinically depressed patients commit suicide, with 90% of all suicide related deaths being linked to depression or another diagnosable mental or substance abuse disorder.[9]

This demonstrates an undeniable need of transformation in the research and development of medical treatments for psychiatric disorders. Recently, a growing number of neurobiological theories have emerged to explain the pathology of depression, with many containing consistent associated signalling pathways involved in the protection of synaptic plasticity. Further advancement and expansion into these theories may facilitate a more integrated biological conceptualisation of MDD, and thus give additional opportunities to develop effective and sustainable antidepressants for the treatment of depression.

1.2. Synaptic neurotransmission

The first two clinical antidepressants were discovered over fifty years ago, both of which act by increasing neurotransmitter (NT) availability within the central nervous system (CNS) to elicit an antidepressant response. Due to the basis of their discovery, which involves ideas from the 'chemical hypothesis' of depression, an understanding of the communication between chemical signalling networks and associated receptors is essential for both the design of new drugs and the understanding of the neurological foundation of depression.

The key component within the CNS is the neuron, a cell which is capable of processing and coordinating electrical and chemical stimuli. Neural networks, formed between neurons, are therefore responsible for all major communication of information thought out the brain (Figure 1.2.1).

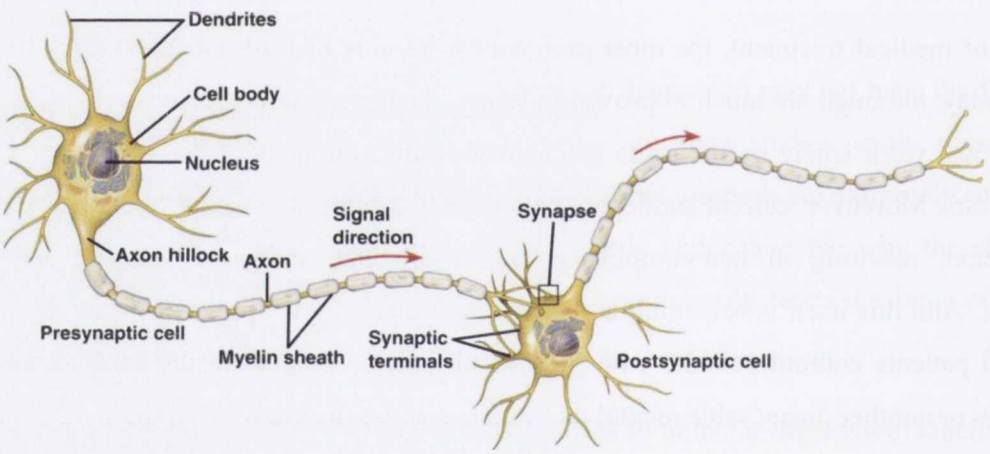


Fig. 1.2.1. Representation of communication between two neurons at the synapse.[10]

The nucleus of the neuron is found in the cell body from which dendrites project, these are the vital arms that are responsible for receiving information from other neurons, and they may receive messages from multiple neurons at a time, which can act to either stimulate or destimulate the neuron. In depression dendrite destabilisation can occur after prolonged periods of stress, which cause alterations in customary information signalling leading to depressive symptoms (see Section 1.3).[11] The cell body processes the stimuli from the

dendrites and transmits it down the axon to the synaptic buttons, which are connected to other dendrites of neighbouring neurons to transport the signals downstream.

All neurons in the CNS are electrically polarized, at resting potential (-70 mV) the polarization is maintained at a constant until the arrival of a synaptic input which will cause depolarization or hyperpolarisation. If depolarisation persists it can cause the membrane potential to rise above the threshold (-55 mV) and trigger an action potential, inducing a consistent depolarization across the neuron body *via* the axon, whereby voltage-gated ion channels regulate repolarization of the membrane.[12] The action potential will propagate along the axon to the terminus (synaptic button) where this will trigger the opening of voltage-dependant Ca^{2+} channels, inducing vesicles containing the NT to fuse transiently with the synaptic membrane and be released into the synaptic cleft *via* exocytosis (Figure 1.2.2)

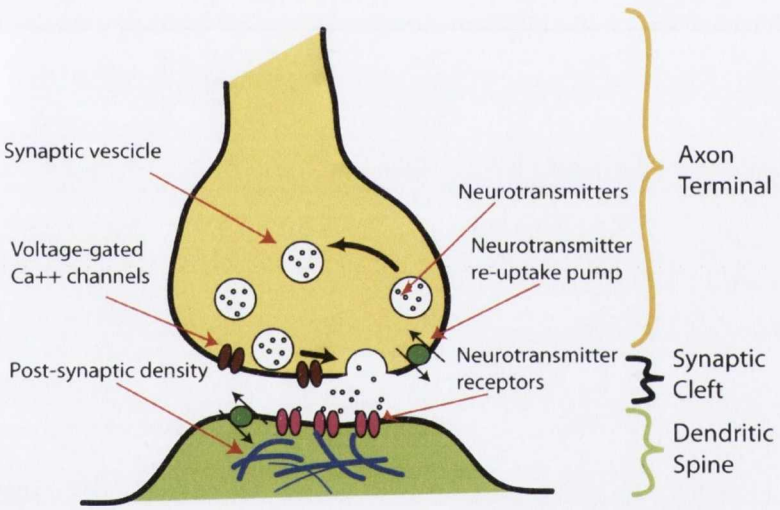


Fig. 1.2.2. Synaptic cleft between two communicating neural dendrites.[13]

Once released, the NT can bind to various post-synaptic receptors on a neighbouring dendrite to further transmit the signal. This propagation can be in the form of an excitatory postsynaptic potential, causing the influx of positive ions to depolarise the neuron, or inhibitory postsynaptic potential, which will generate a more negative potential *via* influx of negative ions. These receptors can be classified into two types, ionotropic (ligand gated ion channels), which open to allow Na^+, K^+, $Ca^{2+,}$ or Cl^- to pass through the membrane once

activated, and metabotropic (G-protein coupled receptors -GPCR-), which activate downstream effectors and ion channels through signal transduction cascades.

Aside from binding to postsynaptic receptors, there are several regulatory ways that remove NTs from the synapse to allow repolarisation of the presynaptic neuron back to the resting potential. Firstly, the NT can interact with autoreceptors, which are located at the presynaptic membrane, and act as a regulatory feedback mechanism for the neuron.[14] Upon NT binding an inhibitory G-protein (G_i) will be activated to stop release of the NT into the synapse. Similarly, heteroceptors have the same effect of switching off NT release but are activated by a different NT than the one being released. Additionally, there are NT transporter proteins, also located on presynaptic membranes, that directly transport specific NT across the neuronal membrane from the synapse.[15] This transport is usually performed by electrochemical gradient diffusion, where co-transportation of both Na^+ ions and NT simultaneously occur by the transporter *via* symporter mechanism. Lastly, degradation enzymes such as catechol *O*-methyltransferase (COMT), monoamine oxidase (MAO), and acetylcholinesterase (AchE) are present to ensure metabolism of the NT within the synaptic cleft.

1.2.1. Neurotransmitters in central nervous system

Before the early 20th century it was believed that all communication throughout the brain was in the form of electrical circuits which occurred in a very narrow cavity known as the gap junction (3.5 nm) between pre- and postsynaptic neurons. At these electric synapses the gap junction allows for communication *via* ion transport between neurons after an initial change in membrane potential. Therefore, these transmissions conduct extremely fast nerve impulses, such as defence reflexes but lack overall complexity compared to chemical messengers.[16] Later, after further examination and histology studies of the distances, it was found that gaps of 20-40 nm also existed between neurons (synaptic cleft), which suggested that communication in these regions occur through chemical neurotransmission.

As seen previously NTs are released from a presynaptic neuron, and are therefore responsible for relaying signals to postsynaptic networks whereby each NT can be excitatory or inhibitory, i.e. to increase or decrease the likelihood of producing an action potential. Over

100 different chemical transmitters have been identified, and all must meet certain criteria to be termed a "neurotransmitter": (i) they must be synthesised in or otherwise available in the presynaptic neuron; (ii) there must be a mechanism of degradation; and (iii) it must produce a response when bound to target receptors on a post synaptic neuron. The major NTs families are: (a) the amino acids, such as glycine, γ-aminobutyric acid (GABA), and glutamate; (b) the monoamines such as dopamine (DA), noradrenaline (NA), adrenaline, serotonin (5-HT) and melatonin; (c) the peptides such as substance P; and (d) others, for example acetylcholine. In the next sections the NTs that may have specific involvement in the pathology of depression will be discussed in detail, with particular relevance to their associations with stress-induced alterations in emotionality and behavioural circuits.

1.2.1.1. Acetylcholine

Acetylcholine (Ach), abundant predominantly at neuromuscular junctions (NMJ), is a very important NT due to its varying effects on neuronal strengthening and muscular function. It is synthesised from acetyl-CoA and choline, and has two main classes of receptors, the nicotinic acetylcholine receptors (nAChR) and the muscarinic acetylcholine receptors (mAChR), which are ionotropic and metabotropic, respectively.

In relation to neuropsychiatric disorders, such as depression, its role is limited as it is less prominent in the brain regions associated with these illnesses; therefore, it will not be discussed in great detail. Nonetheless, the acetylcholine theory of depression has recently emerged due to the observation that hypercholinergic transmission is associated with depressed mood states, possibly through nicotinic receptor activation. In fact, the nAChR antagonist mecamylamine has shown to reduce symptoms of unipolar and bipolar depression (Figure 1.2.3.).[17] Validity for this theory may come from experimental evidence proving an association between hyperactivity of cholinergic signalling and deregulation of stress-regulatory hormones.[18] Moreover, there is some indication of an imbalance between monoamine transmission (noradrenergic in particular) and cholinergic transmission in MDD,[19] where further expansion into these findings may offer additional information on the role of Ach in stress disorders.

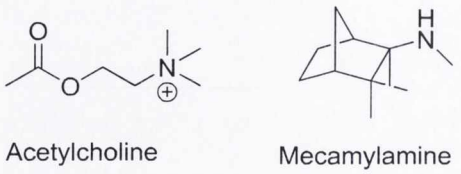


Fig. 1.2.3. Chemical structures of acetylcholine and a nAChR antagonist.

1.2.1.2. Glutamate

Glutamate is the main excitatory NT in the CNS and plays the principal role in neural activation. It is classified under the subset of amino acid NTs including aspartate, glycine and γ-aminobutyric acid (GABA). Glutamate is produced from glutamine or in the citric acid cycle upon transamination of α-ketoglutarate, and is metabolised back to glutamine, by the enzyme glutamine synthetase, or decarboxylated to GABA (Figure 1.2.4)

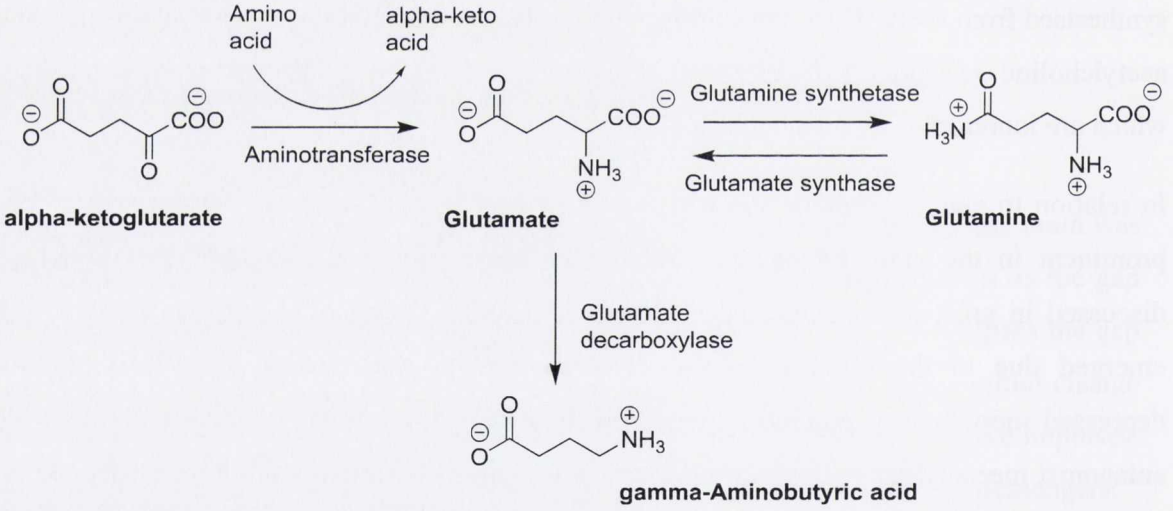


Fig. 1.2.4. Biosynthesis and metabolism of amino acid glutamate.

Once Glutamate is released it can bind to two different groups of receptors: ionotropic and metabotropic glutamate receptors. The ionotropic glutamate receptors (iGluRs) include *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainate receptors. These are all permeable to Na^+ ions allowing for

depolarisation on post synaptic membranes; however, importantly the NMDAR is also porous to Ca^{2+} ions. The metabotropic glutamate receptors (mGluRs), in which there are three groups, indirectly activate ion channels on the plasma membrane through signalling cascades that initially involve G-protein stimulation.

Quite recently, there has been a marked shift from the classic monoamine based theory of depression to a neuroplasticity hypothesis in which glutamate holds a key role.[20] Briefly, long term potentiation (LTP) is the basis of synaptic plasticity which involves the long lasting enhancement in signal transmission between two neurons after repeated stimulation, and importantly has shown to be destabilised in depression.[21] Most central to the initiation of LTP is Ca^{2+} influx, which is, in particular, facilitated by the postsynaptic NMDA receptor and indirectly through the AMPA receptor. This allows for further changes in signal transduction cascades that are necessary for maintenance of homeostatic mechanisms involved in synaptic protection (Figure 1.2.5). The activation of these multiple pathways and other surface level signalling systems, and their involvement in depression will be discussed in Section 1.3.2.[22]

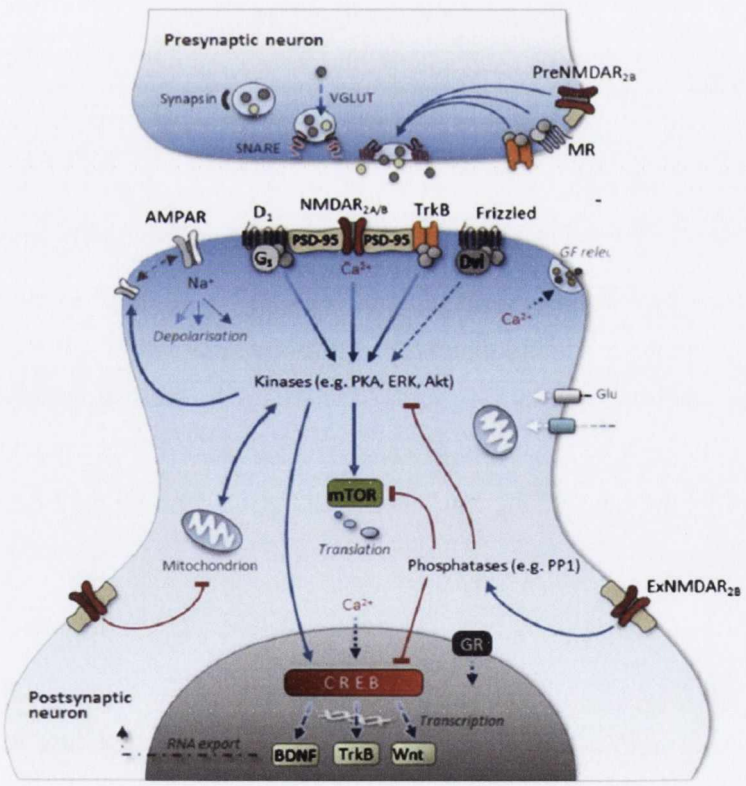


Fig. 1.2.5. Signalling pathways involved in antidepressant response initiated by glutamate NMDAR and AMPAR.[22]

The glutamate hypothesis of depression is effectively illustrated by the rapid antidepressant actions of the non-selective NMDA receptor antagonist ketamine.[23] A single dose of ketamine alleviates depressive symptoms within hours in patients who have failed to respond to two or more conventional antidepressants, and the effects are sustained for 7 to 10 days.[24] Unfortunately, ketamine's psychotomimetic and analgesic effects makes it unsuitable for therapeutic use; however, NMDA-2B receptor antagonists are being investigated as antidepressants which generate similar and more selective responses.[25]

1.2.1.3. Dopamine

Dopamine (DA) plays a crucial role in many different brain regions due to its involvement in responses ranging from reward circuits to voluntary movements. Since its discovery, over 50 years ago, four major dopaminergic pathways have been identified: the nigrostriatal, mesolimbic, mesocortical and tuberoinfundibular systems. All of these systems transmit DA to various brain regions such as the substantia nigra, ventral tegmented area, prefrontal cortex and nucleus accumbens.[26] Many critical brain signalling cascades stem from these areas, which is why it is not surprising that DA dysfunction is implicated in so many neurological diseases.[27]

The DA receptors are classified into two groups D_1-like, containing D_1 and D_5, and D_2-like, containing D_2, D_3 and D_4. They are all GPCRs, and although both subfamilies contain high levels of homology they have distinct pharmacological properties that defines them.[28] The D_1 family activates the G_s subunit of the G-protein which will facilitate activation of downstream cAMP signalling pathway through adenylyl cyclase, whereas the D_2 family activates the G_i protein which has an inhibitory effect on adenylyl cyclase thereby dampening cAMP signalling.

The biosynthesis of DA begins from the amino acid L-tyrosine (Figure 1.2.6). The enzyme tyrosine hydroxylase introduces a second hydroxyl group to form the core catechol ring system producing L-DOPA, which is then decarboxylated by dopa decarboxylase to produce DA. This NT itself is a precursor to other monoamines as it can be hydroxylated by DA β-hydroxylase in the cytosol to form NA, and in the adrenal medulla NA is *N*-methylated to yield adrenaline. After its synthesis, DA is transported into vesicles, by the vesicular

monoamine transporter 2 (VMAT2), where is stored until the arrival of an action potential, which induces its release into the synapse. Once in the synapse, DA can bind to its respective receptors, it can be successively metabolised to homovanillic acid by COMT and MAO, or can also be taken back by the DA transporter (DAT) for its reuse. As mentioned, DA is the precursor of NA; therefore, its structural similarity allows it to be taken up into adrenergic neurons by the NA transporter (NAT) to be recycled into NA.

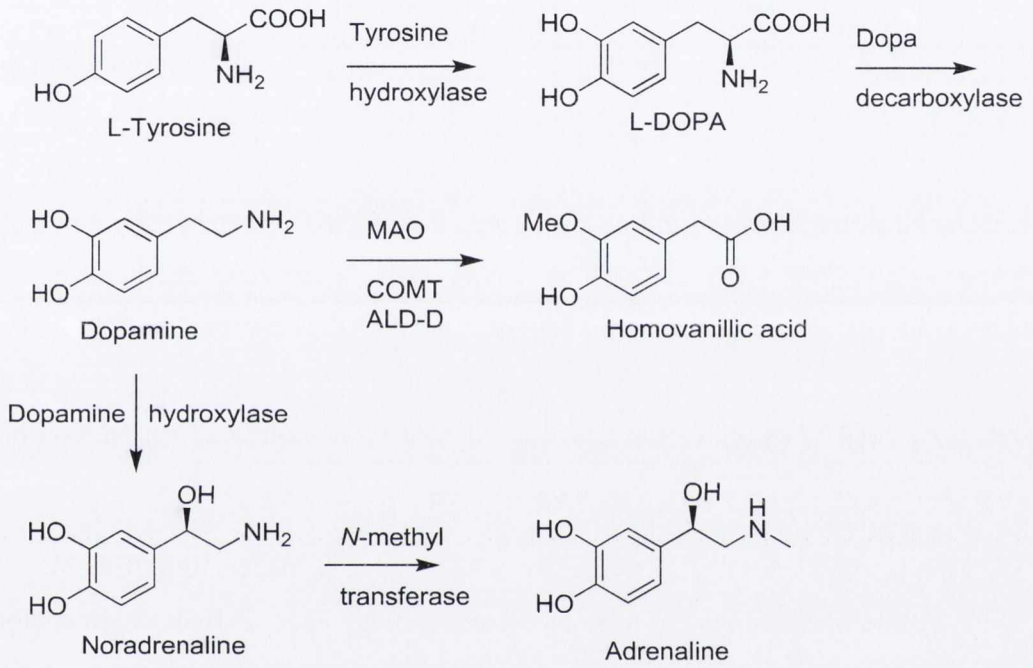


Fig. 1.2.6. Biosynthesis and metabolism of dopamine, and biosynthesis of noradrenaline and adrenaline.

An increased understanding of the dopaminergic system has led to surge of interest in its involvement in neurobiological diseases. In relation to neurodegenerative conditions, the work of Avrid Carlsson in 1950s showed that treatment with L-DOPA, which will be converted into DA once passed the BBB, reduces the symptoms of Parkinson's disease, and is still the most widely used treatment today.[29] Dopamine also has involvement in neuropsychiatric disorders, as many antipsychotic drugs, to treat schizophrenia, display antagonist activity at DA receptors. Moreover, in anxiety disorders such as depression, dopaminergic transmission is decreased either through reduction in its release or impairment

in its signalling.[30] The elevated therapeutic interest in this area at present will undoubtedly further evolve our knowledge of dopaminergic signalling and, hence, aid our understanding of a variety of associated brain regions.

1.2.1.4. Noradrenaline

Noradrenaline, another monoamine NT, plays crucial roles in many associated pathways in the CNS as it is involved in various systems in the brain such as the cerebal cortex, hypothalamus, limbic system, cerebellum and the spinal cord. The most prominent cluster of noradrenergic neurons is situated in the locus coeruleus (LC), which acts as the pivotal source of noradrenergic transmission, sending signals bilaterally to the brain regions mentioned before. Its localisation in the LC and its projections within the CNS demonstrate its important role in many cogitative responses such as mood, sleep or arousal, which have particular relevance in the regulation of stress and anxiety, and therefore depression.[31] Noradrenaline also plays a major role in the sympathetic nervous system (SNS), having high concentrations of receptors in the lungs and heart to affect dilation of bronchiole and constriction of blood vessels, respectively.

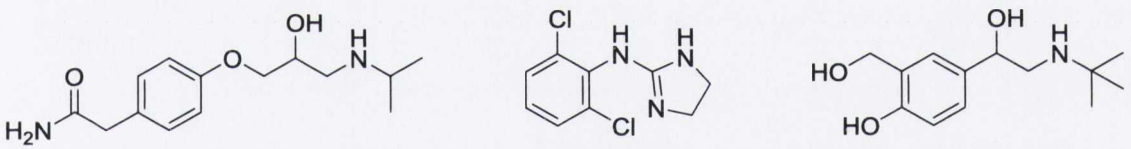
There are two families of adrenergic receptors (AR), α and β, which were discovered in the mid-1940s, by the observation that certain catecholamines gave distinct pharmacologically effects depending on the receptor subtype.[32] Later, a more detailed analysis led to the discovery of nine total subtypes within the two families, α_{1A}, α_{1B}, and α_{1C}, α_{2A}, α_{2B}, and α_{2C}, and β_1, β_2, and β_3, all of which have characteristic structural and functional differences.[33] Both families are GPCR, with the α_1 activating the G_q protein, stimulating the inositol-phospholipid dependant pathway (PLC-IP_3), and the α_2 and β being negatively and positively coupled to adenylyl cyclase induced cAMP tone *via* the G_i and G_s proteins respectively.[34] It is evident from NA's role in many regulatory body functions that the adrenergic receptors can be exploited for potential drugs targets.

Cardiovascular regulation by the SNS is predominately controlled by the adrenergic system, namely the β_1 and α_2-AR. β_1-AR antagonists, such as atenolol (Figure 1.2.7), comprise of an important category of drugs used to treat hypertension, angina, and cardiac dysrhythmias. Noradrenaline's activation of the β_1-AR in the heart has positive effects on the force of

muscle contractions and heart rate, while blocking this activation will induce a decrease in physical exertion of rate and force of the heart muscles to consequently treat cardiovascular related problems.[35]

Conversely, activation of the presynaptic α_2-AR causes a decrease in plasma NA by cAMP-dependant pathway inhibition, producing a decrease in blood pressure. Thus, selective ligands that act as functional agonists of this receptor subtype also have application as antihypertensives.[36] Clonidine is an example of a sympatholytic drug that centrally activates the α_2-AR to treat hypertension. Antagonists of the α_2-AR, which act to increase synaptic NA concentration have shown clinical efficacy in the treatment of depression, due to the relevance in this research this class of drugs will be discussed in detail in Section 1.4.

The β_2-ARs, localised in the lungs, are responsible for the dilation of bronchial smooth muscles. Its stimulation causes muscle relaxation by the activation of kinase associated signal transduction pathways initiated by an increase in intracellular cAMP.[35] Accordingly, short acting agonists, such as salbutamol, are used as bronchodilators in respiratory conditions such as asthma and chronic obstructive pulmonary disease (Figure 1.2.7).



Atenolol Clondine Salbutamol

Fig. 1.2.7. Chemical structures of adrenoceptor ligands for the β_1, α_2 and β_2, respectively.

Noradrenaline is synthesised from DA by the enzyme DA-β-hydroxylase in the synaptic vesicles (Figure 1.2.6), once released into the synaptic cleft it can be taken back up by the NAT for reuse by the presynaptic neuron; this is a principal target for antidepressant treatment (see Section 1.3). Noradrenaline's degradation pathway is similar to that of DA, through the enzymes COMT and MAO. Further metabolic steps in the catabolism of NA lead to a more complex pathway, involving the enzymes aldehyde reductase (AR) and aldehyde dehydrogenase (ADH), to give the easily execrable products: vanillylmandelic acid (VMA),

3,4-dihydroxymandelic acid (DOMA), 3-methoxy-4-hydroxyphenylethylene glycol (MOPEG), and 3, 4-dihydroxyphenylglycol (DOPEG) (Figure 1.2.8).

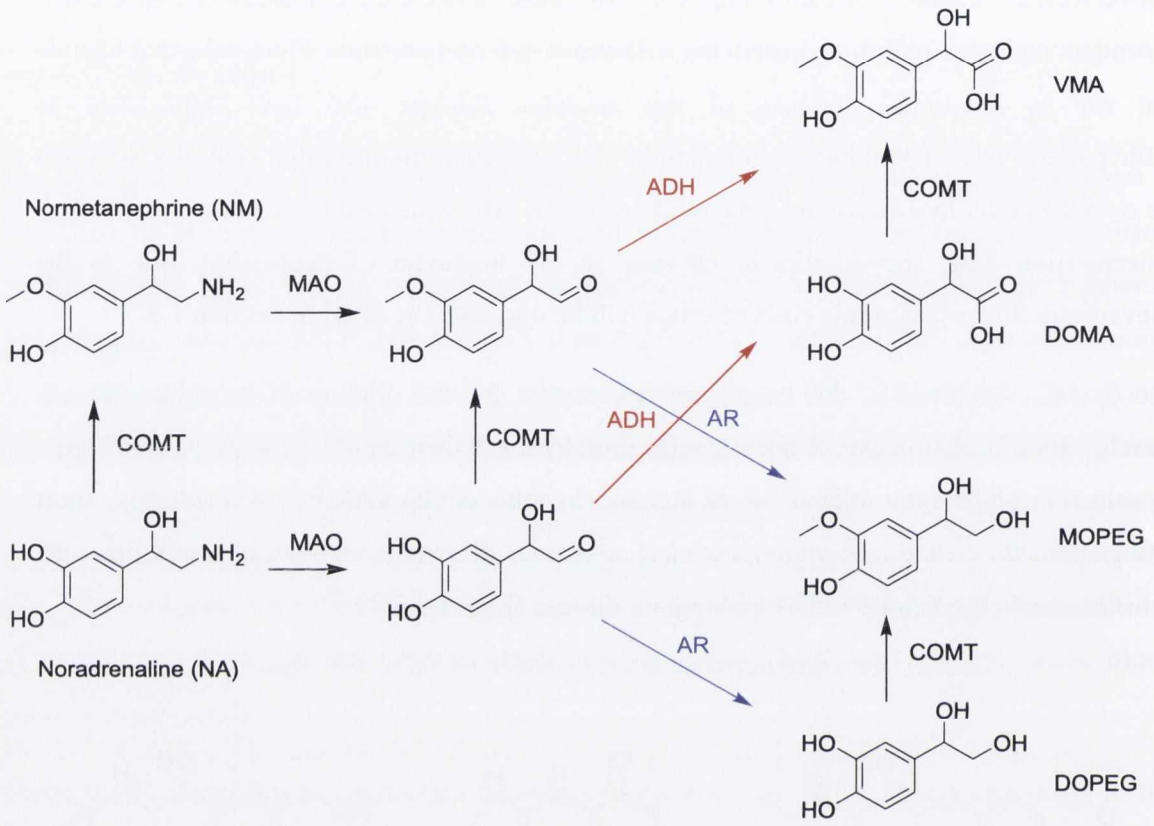


Fig. 1.2.8. Metabolic pathways of noradrenaline.

As mentioned, the link connecting NA to stress and subsequently depressive episodes is due to the neuroanatomical structure of the noradrenergic system from the LC to the surrounding brain areas. This has been somewhat exemplified using animal models of chronic stress. Here, induced stress causes significant changes in both β and α adrenoceptors' density, as well as a decrease in cAMP signalling, which is mediated by these receptors to some extent. These changes are also observed in the brains of suicide patients that had been diagnosed with depression, effectively illustrating the association of adrenergic transmission to stress and anxiety.[37] The influence and potential targeting of the adrenergic system in relation to

treating depression is of great interest in this research and will be discussed in more detail in Section 1.4.

1.2.1.5. Serotonin

Serotonin, or 5-hydroxytryptamine (5-HT), is a key NT being present in many human tissues including lung, kidney, platelets and the gastrointestinal tract. However, it has been elucidated that it is largely prevalent at nerve endings of neurons in the CNS, as modulation of 5-HT is required for the regulation of sleep, mood and appetite, all of which are affected in patients suffering with depression.[38]

Serotonin is a member of the tryptamine family of monoamines, and, like DA and NA, is a biogenic molecule with similar biosynthetic and degradation pathways. Serotonin biosynthesis begins with the amino acid tryptophan which is hydroxylated to 5-hydroxytryptophan (5-HTP) by tryptophan hydroxylase. This is then decarboxylated by L-amino acid decarboxylase to 5-HT (Figure 1.2.9). Pharmacological studies show that both enzymatic transformations occur instantly in the presence of tryptophan, with tryptophan hydroxylase being the rate limiting enzyme.[39]

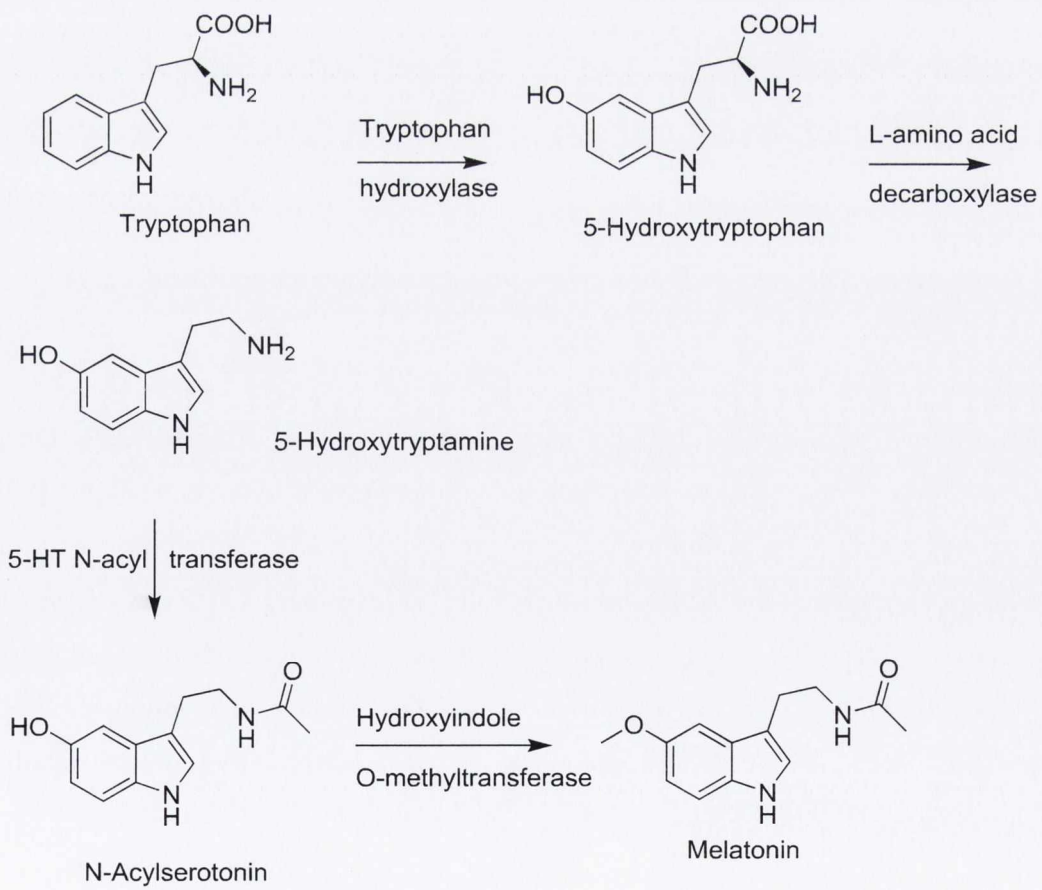


Fig. 1.2.9. Biosynthesis of monoamines serotonin and melatonin.

Melatonin is synthesised from 5-HT in the dark phase of the circadian rhythm cycle in the pineal gland by two enzymes, 5-HT *N*-acyl transferase and hydroxyindol *O*-methyltransferase (Figure 1.2.9). Melatonin is a neurohormone also implicated in depression having two receptors, M_{T1} and M_{T2}, both GPCRs, which activate the G_i protein to inhibit adenylyl cyclase. Its role in mood disorders comes from the high concentration of its receptors in the suprachiasmic nucleus (SCN), which is the vital hub that is responsible for maintaining 24 hr cycles of regulatory sleep *via* circadian rhythmicity. This cycle has seen to be dysregulated in depression and will be further discussed in Section 1.3.4.[40]

Metabolism by MAO is the principal degradation pathway of 5-HT, similarly to the other monoamine NTs, with aldehyde dehydrogenase (ALD-D) completing the transformation to give 5-hydroxyindoleacetic acid (5HIAA), which is excreted primarily in the urine (Figure 1.2.10).

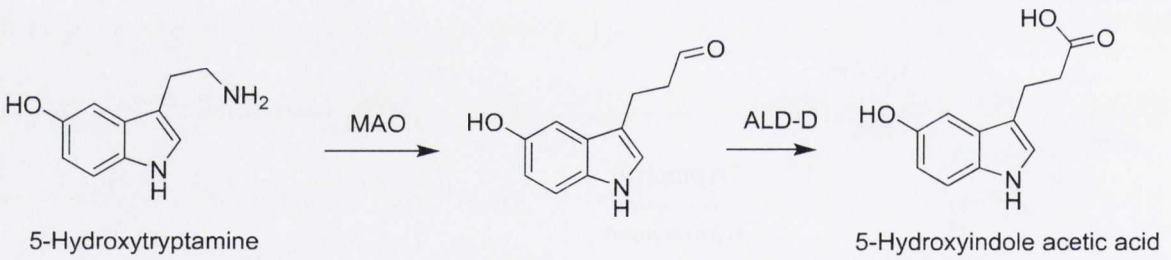


Fig. 1.2.10. Degradation pathway of serotonin to 5-hydroxyindoleacetic acid.

Melatonin, however, follows a different catabolic pathway than other NTs. In the pineal gland, cytochrome P450 either hydroxylates melatonin to give 6-hydroxymelatonin (which is susceptible to sulfation by sulfotransferase enzymes, followed by excretion) or it converts melatonin to *N*-acylserotonin *via* *O*-demethylation.[41] Interestingly sub forms of cytochrome P450 have been identified in the brain, which illustrates its bio-degradation once passed the blood brain barrier. It can also be broken down into similar metabolites as 5-HT (5-methoxyindole acetic acid) through melatonin deacetylase followed by further metabolism by MAO and ALD-D (Figure 1.2.11).

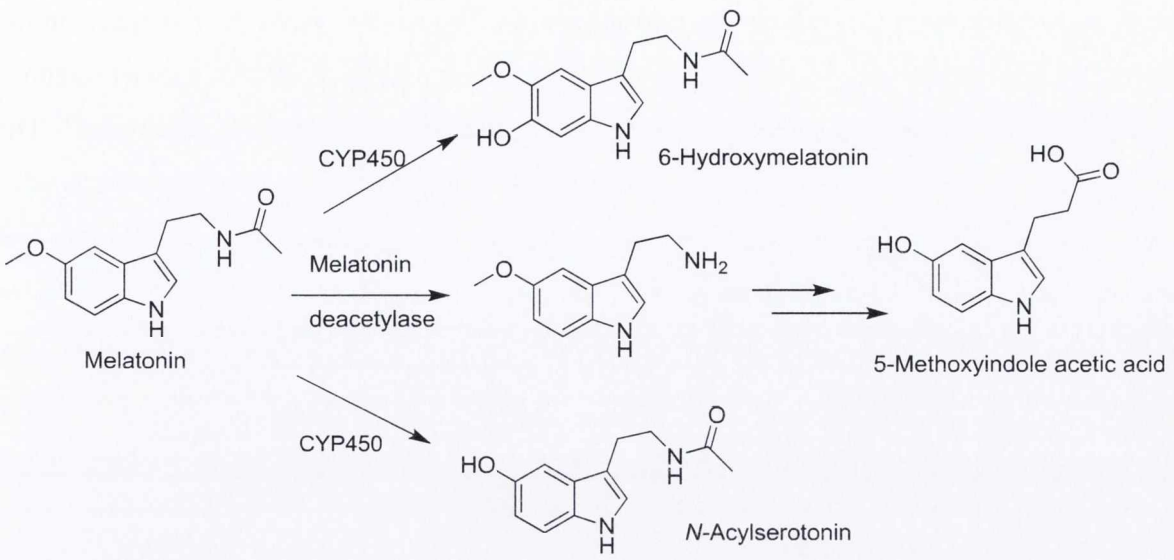


Fig. 1.2.11. Metabolic pathways of melatonin.

Serotonin has a crucial role in the modulation of several behavioural and neuropsychological processes including mood, appetite, sexuality, reward and attention, many of which are altered in depressive episodes, but also by 5-HT based antidepressants. The behavioural processes mentioned above are regulated by multiple 5-HT receptors which are expressed in various brain regions. The major three subtypes, 5-HT_1, 5-HT_2 and 5-HT_3, have only 25% homology which illustrates the vast level of variety in function throughout the family of receptors.[42]

Due to the fact that the earliest antidepressants modulate the level of monoamines in the brain, with changes in 5-HT concentration being the most effective, it is not surprising that the serotoninergic system has been the primary target for monoamine based antidepressant development in recent years. The 5-HT transporter (SERT) is the presynaptic monoamine transport protein which takes 5-HT back into the presynaptic neuron for metabolism or reuse. This is one of the main ways in which the brain controls the levels of 5-HT in the synapse, and also the primary target for new-age antidepressant therapies: the selective 5-HT reuptake inhibitors (SSRI).[43] These agents inhibit the SERT to ensure high levels of 5-HT in the synapse with fluoxetine (Prozac) being the first SSRI to come to market almost 20 years ago (see Section 1.4.4).[44]

Certainly, these therapies give the desired antidepressant response through an increase in the levels of 5-HT; however, excess 5-HT can lead to severe undesired effects such as seizures, tremors, hyperthermia and sometimes death, in a condition known as 5-HT syndrome.[45] The newer generation of more efficient antidepressants have illustrated increases in incidents of 5-HT toxicity amongst patients, and these dangers must be recognised when developing and prescribing doses of 5-HT based drugs.[46]

1.3. The pathology of depression

The heterogeneity of depression implies that multiple neuronal substrates and mechanisms contribute to its etiology. Although in recent years there has been a huge advancement in understanding neural regions and circuits implicated in stress responses of affective disorders, there is still limited novel treatments to show for it. Regionally, the brain is intricately complex, with areas from the limbic system to the cerebral cortex, as well as their associated signalling networks, being directly involved in the cause and treatment of depression. However, due to the heterogeneity of this disorder, different regions and different networks may be affected differently depending on the individual.

As mentioned, depression is a highly heritable disorder (40-50% heritability), yet no specific gene has been identified to increase the risk of its pathology; non genetic factors such as stress, emotional trauma or even alterations in the brain during early development must also be seen as links towards understanding its etiology.[47] Thus, depression must be seen as a disease of multiple disorders caused by perhaps a genetic predisposition or environmental factors leading to persistent damage over time.[48] Learning how these stimuli cause changes in brain chemistry and plasticity is becoming not only an important topic but also a way of finding new targets for treatment; an overview on the most significant theories to understand and explain this will now be discussed.

1.3.1. The chemical hypothesis

The chemical hypothesis describes the pathology depression in terms of stress induced alterations in chemical concentrations in the brain leading to depressive-like symptoms.

These are explained in particular in terms of changes in monoamine availability and neuro-hormones (glucocorticoid) concentration.

1.3.1.1. Monoamine theory

The monoamine hypothesis was the first theory evolved to explain the aetiology of depression, which proposes that its underlying source stems from a decrease in the availability of brain monoamines, namely NA, 5-HT, and DA.[49] This theory came from the acute mechanism exhibited by the first agents that elicited an antidepressant response, the tricyclic antidepressants (TCA) and the monoamine oxidase inhibitors (MAOI), which both act to increase the synaptic concentration of NA and 5-HT.[50] In the 1950s, it was initially believed that NA was the main NT involved; however, it was quickly discovered that an increase in 5-HT had a principal role in alleviating the signs and symptoms of depression. Despite these observations, attempts to obtain more direct evidence of this theory regarding biochemical changes in monoamine metabolites or changes in monoamine concentration in post-mortem studies gave inconsistent results. Moreover, experiments with pharmacological agents that cause depletion of monoamines do not produce a pro-depressant effect,[51] and if antidepressants work solely by increasing chemical concentration, then, their therapeutic effect should be instant, not the delayed response of 4-8 weeks observed.[48] Thus, although pharmacological manipulation of monoamine transmission remains the most successful therapeutic approach, it is now believed that these drugs indirectly affect downstream signalling pathways to exert their response (Figure 1.3.1). This is hypothesised to occur through synaptic remodelling or neurogenesis, as these changes exhibit a similar time period of current antidepressant onset; the intricacies of this model will be discussed in Section 1.3.2.

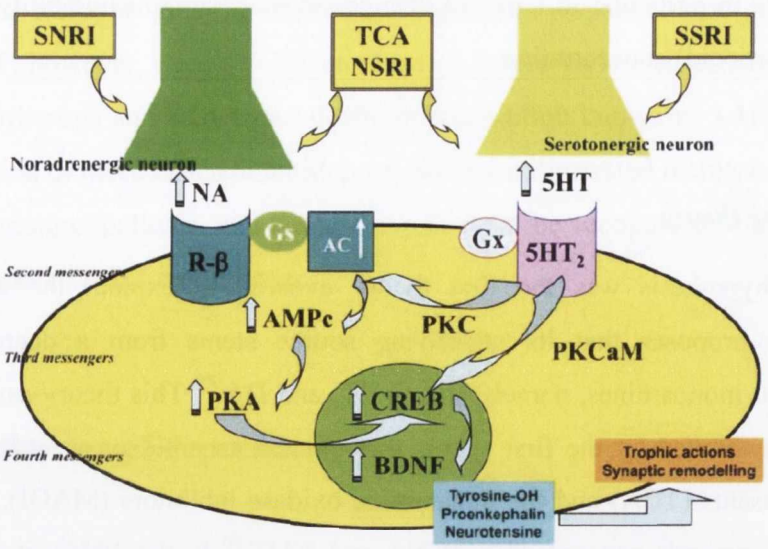


Fig. 1.3.1. Proposed activation of downstream signalling cascades to induce synaptic remodelling from monoamine based antidepressants (Selective NA reuptake inhibitors (NARI), TCA, SSRI, NA and 5-HT reuptake inhibitors (SNRI)).[52]

1.3.1.2. The hypothalamic pituitary adrenal (HPA) axis

The hypothalamic pituitary adrenal (HPA) axis is a major part of the neuroendocrine system encompassing the hypothalamus, anterior pituitary and adrenal cortex, and controls reactions to stress by directly influencing the communication of neurohormones between these brain regions. In particular, the HPA axis is controlled by the release of corticotropin releasing factor (CRF) from the hypothalamus, which incidentally leads to the glucocorticoid (cortisol) release from the adrenal cortex. Due to its localisation in the brain and involvement in stress reactions, it is not surprising that a strong relationship exists between the HPA axis and affective disorders.

Aside from usual regulatory functions, glucocorticoids also act as a negative feedback on the axis by inhibiting CRF release *via* glucocorticoid receptor (GCR), with the hippocampus mediating these events.[53] Dysregulation or hyperactivity of the HPA axis, which occurs though CRF overexpression, and consequently leads to glucocorticoid hyper-secretion (hypercortisolaemia) is a key neurobiological feature of depression.[54] The toxicity induced by excessive cortisol release has shown to have detrimental effects on hippocampal plasticity though the reduction essential of growth factors such as brain-derived neurotrophic factor (BDNF), and this may in turn limit the ability of the hippocampus to regulate HPA axis

homeostasis.[55] Additionally, high levels of glucocorticoids lessen the level of LTP amplification, another damaging effect, as this is required for normal synaptic strengthening.[56]

Although antidepressants have shown to reverse stress-induced changes in CRF expression,[57] CRF antagonists are now under development to reduce the downstream secretion of cortisol. Physiological actions of CRF are mediated through two types of receptor, CRF_1 and CRF_2, both of which are GPCR coupled to the G_s protein to activate PKA-cAMP dependant pathway. CRF_1 is highly expressed throughout the limbic system in the brain, where its blockade has illustrated anxiolytic and antidepressant efficacy in animal models.[47]

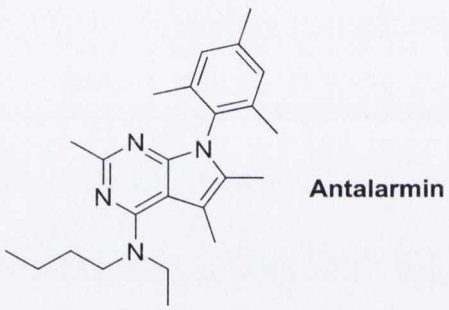


Fig. 1.3.2. Structure of a selective CRF_1 antagonist (antalarmin).

Antalarmin is an example of a selective CRF_1 antagonist (Figure 1.3.2) which has encouragingly demonstrated a reduction in animal behavioural responses to stressful stimuli.[58] Aside from this however, the development of CRF_1 antagonists has recently decelerated as their potency does not match that of conventional antidepressants.[59]

1.3.2. Neural networking theory- alterations in synaptic plasticity

Neuroplasticity is the fundamental mechanism of neural adaptation, and at the synaptic level it refers to the modification, either weakening or strengthening, of connectivity between synapses, neuronal circuits and NT receptor density in response to environmental changes in their activity.

As mentioned above, the chemical or monoamine theory explains depression to be a disorder caused by a decrease in availability of monoamines in response to particular stressors, where antidepressants work to normalise chemical levels and alleviate depressive symptoms. It is yet to be identified whether stress precipitates or exacerbates depression; nonetheless, it is known to cause major disruptions in neuroplasticity which are modelled in this theory. Antidepressants directly work by increasing chemical NTs in the brain, but have now shown to indirectly induce the gradual strengthening of synapses over the course of treatment.[60] The idea of a complementary chemical and network hypothesis is now evolving; where information is transferred by chemical means, and stored through the complex interactions of neural networking circuits within the brain.[61]

Before discussing the synaptic signalling systems that are involved and disrupted in MDD, an introduction on the basic mechanisms of synaptic plasticity will be presented in the form of long term potentiation (LTP), which is the best studied form of synaptic plasticity (see Section 1.2.1.2). In essence LTP is the persistent strengthening of signal transmissions between synaptic circuits, and is thought to be the cellular and molecular event underpinning learning and memory.[62]

In this model, the glutamate receptor signalling initialises events through a high frequency action potential from presynaptic neuron leading to a high concentration of glutamate at the synapse. Binding of glutamate to AMPARs induces depolarisation of the postsynaptic neuron to trigger the opening of NMDAR allowing Ca^{2+} influx, which acts as an important secondary messenger in most of the signalling systems involved in synaptogenesis. In early phase LTP, Ca^{2+} activates multiple kinases pathways: the cAMP-PKA-dependant, the Ca^{2+}/calmodulin-dependent protein kinase, and the Ras-MAPK pathways, which stimulate the generation of new AMPAR for future depolarisation events. Late phase LTP is induced by changes in gene expression and protein synthesis brought about by the persistent activation of the mentioned protein kinases activated during early LTP. This is mediated through the transcription factor cAMP response element binding protein (CREB), which modulates the gene expression of essential growth factors, such as brain derived neurotropic factor (BDNF), and synaptic proteins required for the maintenance of homeostatic plasticity.

Recognising and understanding theses pathways encompasses the basis of synaptic plasticity, and with particular relevance to the transcription factor CREB and the neurotrophin BDNF,

will now be discussed in detail with regards to their involvement in stress induced neuronal atrophy seen in depression.

1.3.2.1. Transcription factor cAMP response element binding protein (CREB)

As mentioned above, CREB acts as the central mediator of synaptic induced events involved in long term neuronal survival and protection. Its function is modulated *via* phosphorylation at Ser^{133} which allows binding to cAMP response element (CRE); inducing the transcription of synaptic protein and growth factor target genes that are vital in neural adaptations.[63]

CREB activation is a multistep process involving initial Ca^{2+} signalling to mediate the intricate and diverse kinase pathways mentioned in LTP (Figure 1.3.3).[64] The cAMP-PKA pathway is activated *via* GPCR mediated increase in cAMP concentration which in turn activates protein kinase A (PKA) to stimulate CREB phosphorylation. The binding of Ca^{2+} to the calmodulin-dependent protein kinase II (CaMKII) induces AMPAR trafficking which increases the sensitivity of synapses to presynaptic depolarization in LTP; persistent activation of binding leads to downstream activation of CREB.[65] Finally, activation of the Ras-MAPK (Ras-Raf-MEK-ERK) pathway leads to the direct phosphorylation of neighbouring kinases in a signalling cascade that ultimately phosphorylate CREB.[66]

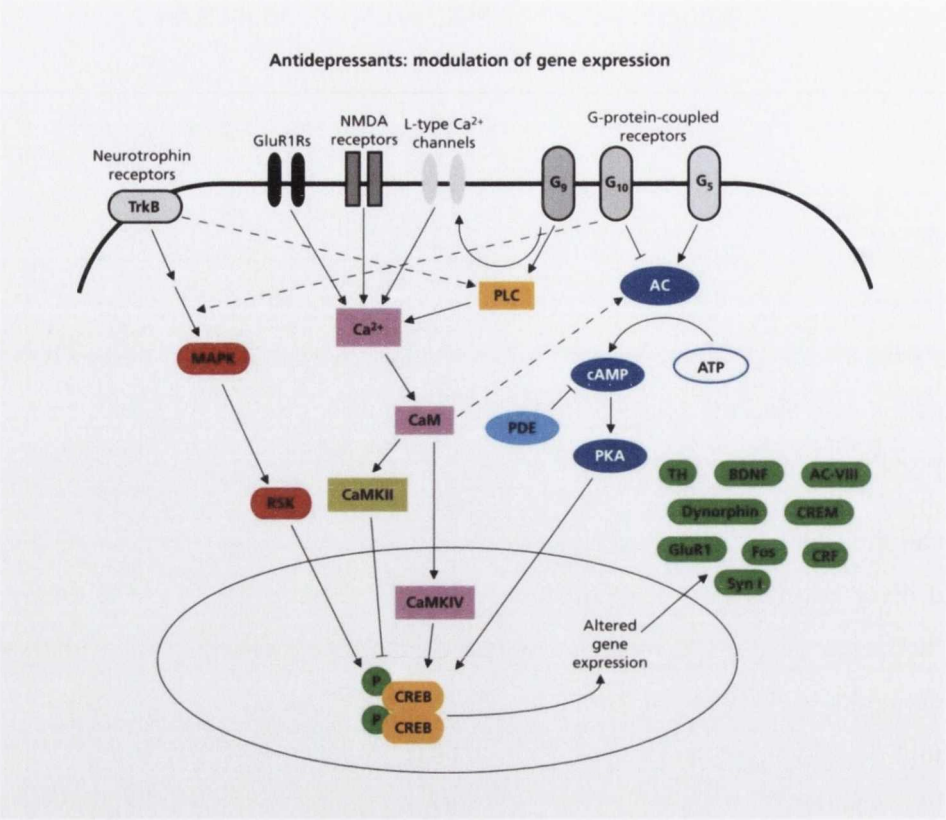


Fig. 1.3.3. Schematic of pathways (Ras-MAPK, PKA-cAMP and CaMKII) involved in the downstream phosphorylation of CREB.[64]

The evolved understanding of CREB and CREB-related gene activation has stimulated elaborate investigations on how this signalling system is perturbed in depression. The most compelling arguments come from the indication that there is a decrease in cAMP tone in depressed patients, demonstrated by a decrease in both CREB mRNA expression in the temporal cortex and adenylyl cyclase in the cerebral cortex in depressed patients.[67,68]

It is now hypothesised that monoamine antidepressants may indirectly activate CREB signalling which results in the therapeutic molecular and cellular changes observed after treatment. This is based on the findings that numerous antidepressant therapies as well as electro convulsion therapy (ECT) increase CREB gene expression.[69] Monoamine distinct antidepressants may activate CREB through cAMP-PKA pathway by NA and 5-HT acting on postsynaptic the β-AR or $5\text{-HT}_{4,6,7}$ receptors to increase cAMP *via* G_s protein.[70] Moreover,

monoamine NTs acting on α-AR or 5-$HT_{1A,7}$ have also shown to activate the Ras–MAPK cascade to mediate their effects on CREB, and hence on synaptic plasticity.[71]

Direct drug targeting of the CREB system has been achieved by the use of phosphodiesterase type 4 inhibitors (PDE4I), which block the enzyme that acts predominantly in the CNS to hydrolyse cAMP to adenosine monophosphate (AMP). Rolipram was the first example of this class of inhibitors to be used as a potential antidepressant; however, it was discontinued due to its severe vomiting adverse effects during early clinical trials.[47] Interestingly, a combination of rolipram with imipramine gives significantly better responses in animal behavioural models compared to either drug alone, suggesting an association between CREB up-regulation and behavioural antidepressant responses.[72]

1.3.2.2. Neurotrophic Hypothesis

One of the principal genes expressed by CREB is the *BDNF* gene which codes for the neurotrophic growth factor BDNF, where its release is dependent on the kinase cascades that mediate CREB phosphorylation. This protein functions primarily in the brain to promote survival, growth and differentiation of new neurons and synapses; hence, it is seen as an essential regulator of synaptic transmission.[73]

BDNFs effects are mediated though the tropomyosin receptor B (Trk B), another target gene of CREB, whose postsynaptic signalling is coupled to the activation of the Ras-MAPK and PI3K-Akt pathways to have an overall protective effect on neuroplasticity.[22] These pathways themselves have seen to be altered in depression as post mortem studies on suicide victims show a decrease in Raf-ERK signalling in the PFC and hippocampus, which parallels with a decrease in activity of Akt signalling in similar regions.[74,75]

In addition to their activation of CREB, the Ras-MAPK and PI3K-Akt pathways act as upstream regulators of mTOR, another neural localised protein with critical involvement in cell growth, proliferation and survival, as well as synaptic protein synthesis, and transcription (Figure 1.3.4).[76]

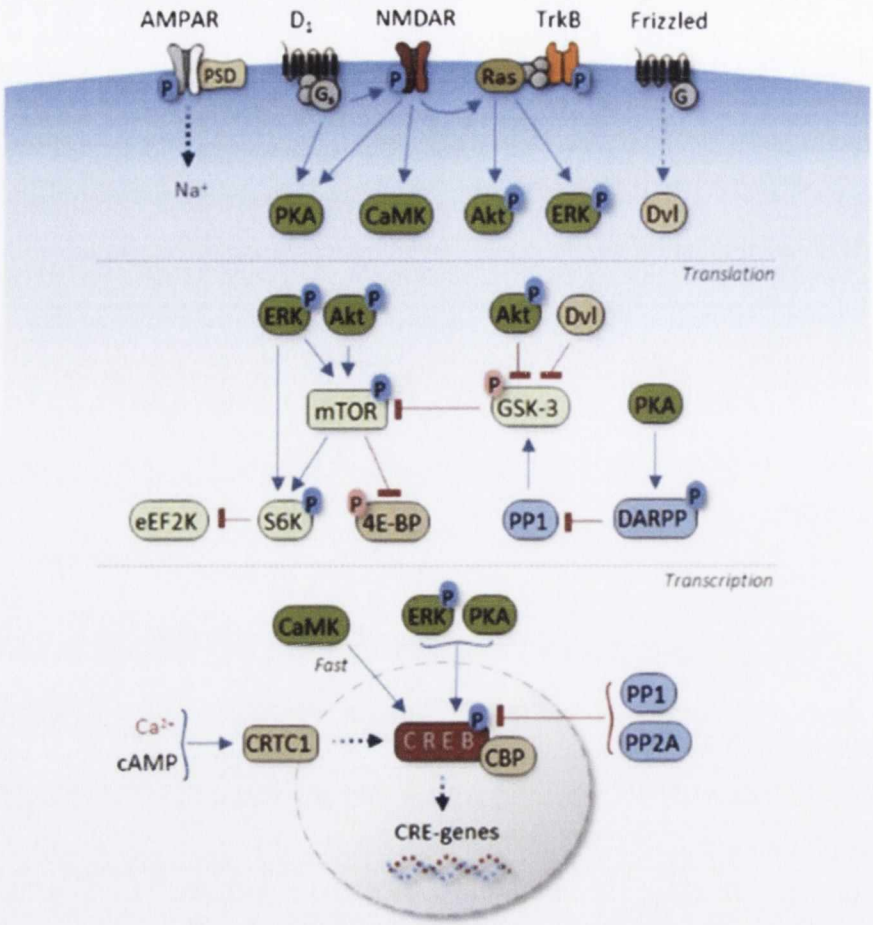


Fig. 1.3.4. Signalling pathways mediated through BDNF-TrkB complexation- in particular mTOR and CREB phosphorylation.[22]

The importance of mTOR signalling has recently emerged due to the expanded research geared towards understanding the rapid antidepressant response (within hours) induced by the NMDAR antagonist ketamine.[24] Ketamine's fast-activation of mTOR signalling, *via* initial upstream BDNF release, results in sustained elevation of synapse associated proteins and spines in the PFC,[77] and has led to the hypothesis that defects in the mTOR-dependant translocation pathway may contribute to the molecular pathology observed in depression.[78]

Aside from alterations in the downstream signalling cascades mediated by BDNF-TrkB activity, changes in BDNF expression are also observed in depression and antidepressant response. Firstly, chronic stress is shown to induce down-regulation of BDNF in the hippocampus, whereas multiple antidepressant treatments reverse this by increasing BDNF expression.[79] This implies that long term antidepressant therapy may block or slow the stress-

induced alterations in BDNF levels, as well as its associated signalling pathways. Furthermore, transgenic *BDNF* knockout mice attenuate the behavioural responses of antidepressants and also reveal a decrease in limbic plasticity and LTP,[80] while direct infusion of BDNF into the midbrain displays a rapid antidepressant response.[81] From these observations it seems very plausible that stress related disorders cause an imbalance in homeostatic BDNF levels leading to detrimental effects on overall plasticity, where antidepressant therapies act by restoring regular BDNF function through CREB.

1.3.2.3. Hippocampal neurogenesis

While somewhat related to neuroplasticity, neurogenesis involves the making of new neurons from neural stem and progenitor cells, and is also heavily implicated and affected in stress responses associated with depression. Neurogenesis occurs predominantly in the hippocampus and is most active in pre-natal development; however, in the recent years it has been shown that this process also occurs to a lesser extent in adult human brain. This was proved by infusion of a thymine analogue biomarker (bromodeoxyuridine) into the adult brain, where post-mortem studies showed new neurons with incorporation of the unnatural base in the DNA.[82]

The association of neurogenesis and depression surfaced when MRI imaging of patients that had persistent depression episodes showed a significant reduction in hippocampal volume,[83] further supported from the same observation of reduced neurogenesis in animal models of chronic stress.[84] This of course relates to the fact that hippocampal function is vital in the regulation of stress response *via* the HPA axis, where stress-induced decrease in hippocampal plasticity and volume will lead to a reduction in inhibition of HPA function, thus causing excessive cortisol release and stress-stimulated neuronal atrophy.[85]

In relation to antidepressant effects, the most compelling evidence to support the 'hippocampal theory' comes from the fact that the behavioural responses of antidepressant therapy are completely blocked when neurogenesis is inhibited by X-ray irradiation in the hippocampus.[86] This strongly suggests a requirement of neurogenesis in reducing emotional and behavioural signs and symptoms of depression, possibly due to its influence in activity in the prefrontal cortex, amygdala and nucleus accumbens, structures that are associated with emotionality.[87] However, although antidepressant therapies do stimulate an increase in

hippocampal progenitor cell proliferation, this is most likely accompanied by changes in network activity or plasticity. Complications with this theory have begun to arise based on the counterargument that disruptions in antidepressant behavioural effects induced by X-ray irradiation experiments are actually caused by negative alterations in hippocampal plasticity, not neurogenesis.[88] This is further supported by the fact that antidepressants have shown to retain their efficacy in the presence of methylazoxymethanol, a cytostatic agent used to arrest neurogenesis.[89]

Nonetheless, neurogenesis is regulated by stress and, in somewhat, antidepressant response; however, it may just be that this regulation is only one the many aspects that constitute the complex machinery encompassing hippocampal plasticity and neurogenesis in depression.

1.3.3. Lipid raft microdomains- towards a novel monoamine mechanism?

The changes in chemical balance induced by monoamine based antidepressants do not explain the slow onset of efficacy of these compounds. The behavioural responses of these agents is hypothesised to come from their activation of the cAMP system, initiated by the activation of adenylyl cyclase *via* the $G_{s\alpha}$ subunit of a GPCR.[90] As mentioned above cAMP has been implicated both in depression and antidepressant response.[70] The involvement of lipid raft microdomains, which are areas within the plasma membrane that contain high concentrations of cholesterol, tubulin and other glycosphingolipids, have been recently linked to various neurological and psychiatric disorders.[91]

Many components which required for cAMP signalling (the G protein -$G_{s\alpha}$-, its receptor – GPCR-, and the effector -adenylyl cyclase-), have been reported to be localized, independently one of another, in lipid rafts and these associations differentially alter (facilitate or dampen) NT signalling (Figure 1.3.5).[92]

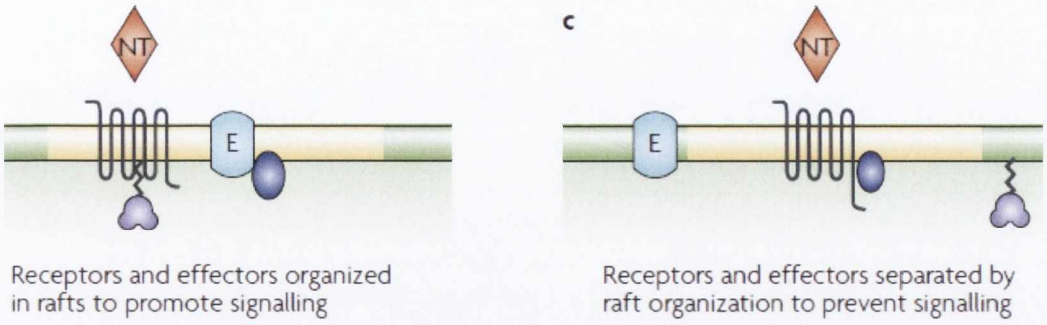


Fig. 1.3.5. (*left*) GPCR, G-Protein, and effector within lipid raft (yellow), (*right*) only GPCR localised in raft.[91]

Recently, it has been shown that in the brain of suicide/depressed patients there is an increase of $G_{s\alpha}$ within lipid rafts, where it is less likely to activate adenylyl cyclase and facilitate cAMP signalling.[93] However, monoamine distinct antidepressants, which have shown to accumulate in lipid rafts, induce the movement of $G_{\alpha s}$ (but not other G proteins) out of lipid rafts and into a closer association with adenylyl cyclase.[94] This is observed for all clinical monoamine antidepressants which elicit behavioural responses; however, other psychotropic drugs such as D-amphetamine (which also increases the levels of monoamines) does not alter $G_{s\alpha}$ diffusion. This suggests that this process has no association with monoamine concentration, and that these antidepressants may have an alternate site of action within or involving lipid rafts which facilitate increased cAMP tone and the accompanying synaptic changes.

1.3.4. Circadian rhythm network

The circadian rhythm clock is an intricate multi-oscillating network of genes containing positive and negative transitional loops, where these elements perform together as a monitoring response mechanism allowing for oscillating cycles of approximately 24 h. The genetic network that maintains this system consists of clock genes (CLOCK, BMAL-1, PER, CRY) making up an auto-regulatory feedback loop (Figure 1.3.6).

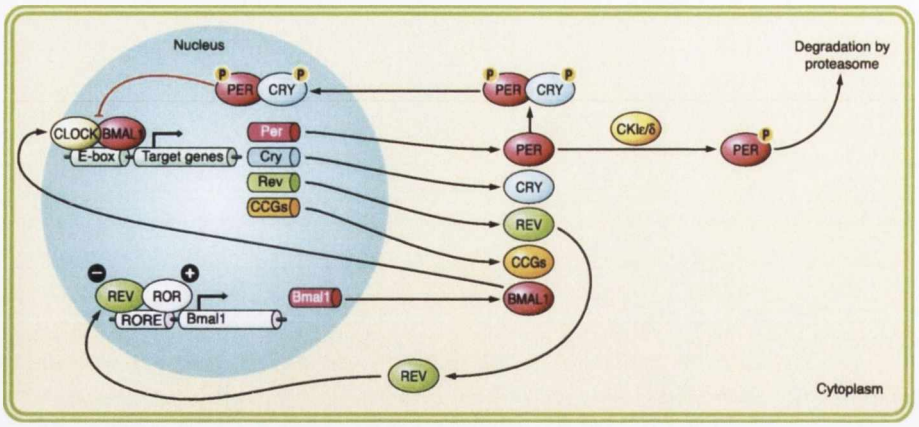


Fig. 1.3.6. Genetic clock gene network that regulate circadian rhythms.[95]

In the central loop the positive transcriptional activators BMAL 1 and CLOCK form a heterodimer within the nucleus where they bind to the circadian E-box to promote the downstream phosphorylation of the negative regulators PER and CRY in the cytoplasm.[96] These proteins interact to inhibit the activity of BMAL 1-CLOCK complexes, whereby the time between activation and repression leads to the 24 hr oscillations within the circadian rhythm clock. The mammalian circadian clock is composed of a hierarchy of multiple components, where the superchiasmic nucleus (SCN) is the central cog in the wheel. It receives photo inputs from the retina and acts as an internal pacemaker within the multi oscillating network to coordinate these light and dark cycles to control the physiological output rhythms (Figure 1.3.7).[40]

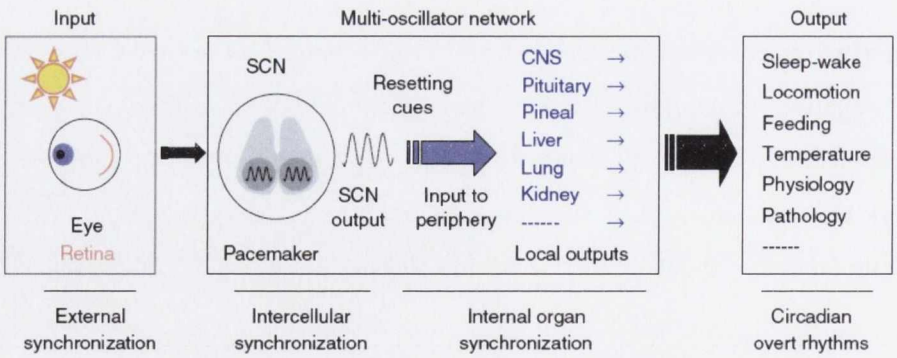


Fig. 1.3.7. Components of the circadian cycle on mammalian level.[40]

There has been compelling clinical evidence that affective disorders such as depression may be circadian-rhythm-related illnesses. This was initially based on the observation that MDD causes major disruptions in sleeping patterns, but now it is understood that there is an actual affiliation between depressive symptoms and de-synchronisation of circadian rhythms. Bunney *et al.* have recently investigated disruptions in circadian clock genes, using microarray analysis, in brain regions implicated in depression. Their results showed the first direct evidence of circadian dysregulation of clock gene expression in patients with depression compared to control subjects.[97]

The most significant finding, regarding the understanding of the relationship between circadian dysfunction and depressive episodes, is that sleep deprivation therapy (SDT) elicits a rapid antidepressant response, comparable to low dose ketamine. It is hypothesised that ketamine and SDT reset abnormal clock gene rhythmicity as they share additional mechanisms of action (Figure 1.3.8). As explained previously, the rapid antidepressant response of ketamine is owed to its induction of synaptogenesis *via* activation of the mTOR signalling pathway *via* glutamate signalling (NMDAR and AMPAR). These pathways are all also modulated by clock genes, as mTOR displays strong rhythmicity in the SCN, and is directly associated with PER expression. Furthermore SDT elevates both AMPA and NMDA receptor density in cerebral cortex, with AMPAR also directly influencing PER expression.[98]

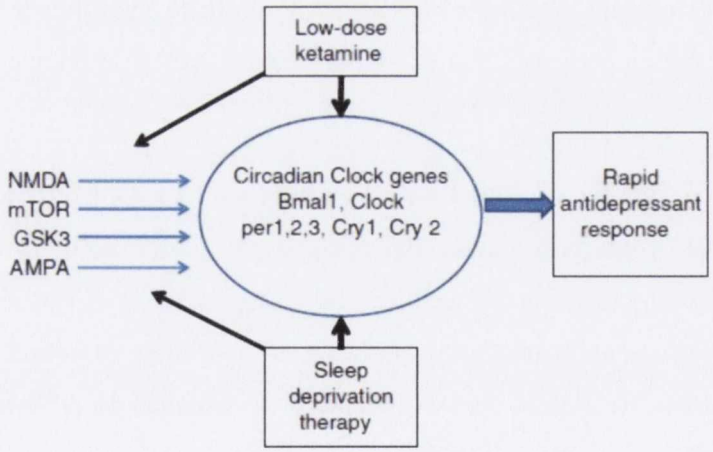


Fig. 1.3.8. Chart illustrating the possible association of SDT to antidepressant response.[98]

It is still under investigation as to whether monoamine antidepressants have a similar effect on the pathways involved in the clock circuits mentioned. Moreover, these genes, or control elements within the circadian network could, themselves, be exploited as novel targets for future antidepressants therapy, stepping away from solely monoamine enhancing molecules.

Regarding this, researchers have started looking at the melatonin system as this is the key neuro-hormone involved in the circadian rhythmic cycle. When secreted, in the dark phase, it is charged into the SCN where there is a high concentration of melatonin receptors and, hence, can be seen as a crucial modulator of events within the SCN with potential antidepressant activity. Agonists of the melatonin M_{T1} and M_{T2} receptors illustrate a unique pharmacological antidepressant profile *via* the resynchronization of circadian rhythms with minimal adverse effects. Agomelatine (Valdoxan®) is a clinically available example of this, which acts as an agonist to melatonin receptors yet also displays antagonist activity at the 5-HT_{2C} receptor, due to their high concentration in the SCN. Regardless of its behavioural antidepressant efficacy, this drug has been tested in several animal models to detect phase advances of circadian rhythms and to fully validate its site of action in the SCN.[99]

1.3.5. Thoughts on theories

The main problem in the development of novel antidepressants is the lack of understanding of the real mechanism by which they exert their therapeutic response. It is clear, of course, that they initially increase monoamine concentrations in the brain to then induce their response upon activation of signalling pathways that will have a protective effect on neuronal plasticity and neurogenesis, yet this is still not well understood.

The evidence in favour of the neuroplasticity theory is undeniable with causing triggers (stress) and treatments (drugs) both having direct effects on the plasticity of multiple brain regions as shown in both post-mortem studies and brain imaging.[11] The neurobiological theories, both chemical and network, have implications regarding cognition and emotions whatever the stressor may be. Figure 1.3.9 represents a hierarchical model where alterations in the function of neurons and neural pathways gives rise to changes in maladapted brain circuitry which associate with persistent shifts in cognition and emotion observed in depression.[22]

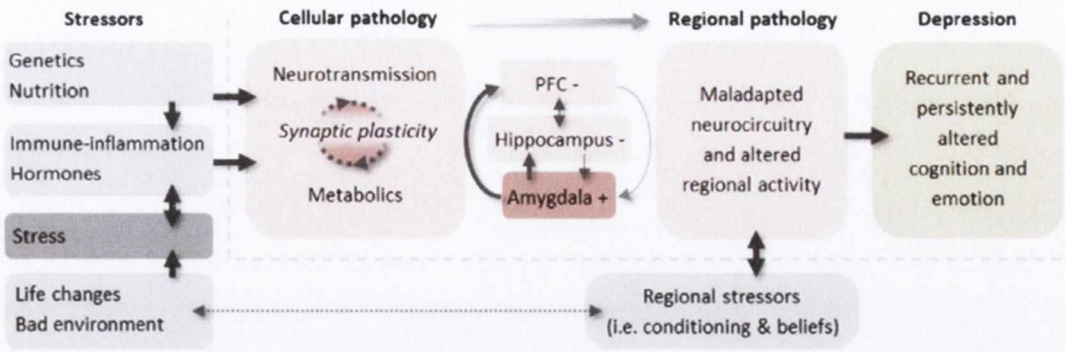


Fig. 1.3.9. Hierarchical model of the association of depressive symptoms to neural pathways induced by various stressors.[22]

So far research has been geared towards region-specific changes in synaptic form in the PFC and hippocampus; however, the pathology is less studied in other brain regions directly linked to emotional and behavioural circuitry such as the nucleus accumbens and the amygdala. Perhaps a more integrated understanding of full brain (depression specific) alterations in neuro-circuitry may give us the additional depth needed to treat the multiple disorders of depression as a whole, as more than likely there is large variations in the underlying biology across depressed patients.

1.4. Treating depression

1.4.1. Non-medical treatments

1.4.1.1. Psychotherapy

The premise of psychotherapy is that people have potential for understanding themselves and resolving their own problems. As human beings we are capable of self-awareness and the greater our awareness the greater the possibilities are for self-directed growth. Most schools of psychotherapy, behaviourists and more traditional analytical schools, would regard therapy as the discovery in a therapeutic relationship of a person's character traits, drives and value system. These have been informed and shaped by their history and the development of complex defence systems that often progress in response to the threat or perceived threat of a person's environment. In the safety of a good therapeutic relationship these defences can be explored and their effect understood to be internalized with a new mature perspective.

Aaron Beck developed a cognitive theory of depression; he found the cognitions of depressed individuals to be characterized by errors in logic that he called 'cognitive distortions'. For Beck, negative thoughts reflect underlying dysfunctional beliefs and assumptions, and when these beliefs are triggered by situational events a depressive pattern is put in motion.[100] He believed people could gain relief by modifying their dysfunctional thinking by taking an active role both during and outside of the therapy sessions, drawing from a variety of cognitive and behavioural strategies to bring about change. The analytical model of Freud and Jung linked the state of melancholia to mourning. They theorized that objective loss such as the loss of a relationship, results in subjective loss as well; the depressed individual has identified himself with the object and not only is the outside world viewed negatively but the ego itself is compromised.[101] The patient's decline of self-perception is revealed in his belief of his own blame inferiority and unworthiness.

Whatever the theorists aim to explain, most see depression as 'a messenger', the current system of mental processing is not working and the severity of the psychological disturbance will direct patients to different solutions. It has to be observed that a healthy reaction to life's events is sometimes a depressed state. To come to terms with loss we have to grieve, it is a time of low mood and introspection but also of growth and adjustment, this low mood should not be automatically labelled as a pathological condition. The subject is highly complex, the measurement of human suffering is subjective, and ultimately the ego strength and developmental stage of the person plays a huge role in how to most empathically treat them.

1.4.1.2. Electro convulsion therapy (ECT)

Electro convulsion therapy was first introduced in the 1930s as a method for alleviating the symptoms of affective disorders through the induction of seizures. This was initially progressed through the observation that electrical impulses to the brain triggered an anesthetized state, and was hence applied to medicinal uses.

Nowadays, it is only used as a last resort treatment when patients have shown non-compliance or resistance to multiple drugs, and when psychotherapy is not relieving any emotional symptoms. It remains quite a controversial topic, as stimulations of seizures are usually above the necessary threshold and can lead to irreversible memory loss.[102] Indeed many practitioners believe ECT is ineffective when weighed against its possible damaging

effects on the brain, while some think it safe and effectual.[103] Nonetheless, its efficacy in psychiatric treatment is indisputable; as 50% of either unipolar or bipolar patients show significant improvement after only one session.[104]

Despite the therapeutic success encompassing ECT, the exact mechanism of action still remains elusive, even with numerous theories recently presented to explain its response. The most significant finding comes from its influence on limbic system plasticity, where an increase of neurogenesis and thus hippocampal volume is seen 3-5 days after treatment.[105] Furthermore, correspondingly with clinical antidepressants, an increase in 5-HT and NA receptor density is observed in certain brain regions; however, no change in synaptic availability of NT is ascertained.[106] These observations, while acceptable, do not explain how ECT truly works and what components (seizures, recovery, amnesia, anaesthesia) are required to alleviate signs and symptoms of depression.

1.4.2. History and development of antidepressants

Most of the major symptoms associated with depression date back to almost prehistorical times. Even with recognizable similarities to the description of depression from then until modern times, it was not until the late 19th and early 20th century that psychoactive agents such as barbiturates, opioid derivatives and amphetamines were used to treat certain mental illnesses. The use of these agents as treatments for symptoms associated with melancholia however came about centuries earlier. In the early 16th century elixirs were prepared containing the active ingredient of the poppy plant which produced analgesic and antidepressant effects. It was later discovered that opium, containing alkaloids with narcotic properties, was responsible for these effects and was hence introduced as a treatment for depression.[107] In the 17th century Johannes Purkinje, who was famous for his numerous discoveries in cellular biology, found that nutmeg induced elated moods. Although nutmeg contains no psychoactive compounds, it has been suggested that its metabolites are molecules structurally related to amphetamines.[108] Thus, although their pharmacology was unknown at the time these psychoactive molecules, which are now recognised today, were the first discoveries of antidepressant-like compounds.

1.4.3. First generation antidepressants

In the early 1950s through a series of serendipitous discoveries the first generation of antidepressants were unearthed: the monoamine oxidase inhibitors (MAOI) and tricyclic antidepressants (TCA). Although both types of drugs are still used today, their greatest contribution was that they paved the way for further improvements in the pharmacological profile of the more widely used clinical drugs.

The MAOI were discovered first in 1952, where it was found that the anti-tubercular agent iproniazid elevated depressive moods in patients (Figure 1.4.1).[109] Later, it was experimentally confirmed that this drug increased the concentration of 5-HT in rat models, and was found to be an inhibitor of MAO.[110] This initial discovery lead to further agents that had better efficacy as inhibitors of MAO such as phenelzine;[111] however, these drugs gave rise to unwanted interactions with primary amine containing food sources, such as tyramine, and lead to hypertension and in some cases strokes.[112] It was later found that the associated toxicity was due to the existence of two functional forms of MAO,[113] MAO-A which is associated with metabolism of NA, and 5-HT, and MAO-B which metabolises benzylamine, tyramine and β-phenylethylamine. This discovery, along with the later findings that the classic MAOIs in fact bind irreversibly to both iosenzymes, encouraged the development of the reversible and selective inhibitors of MAO (RIMA). The first of these drugs was moclobemide (Figure 1.4.1) which was selective for MAO-A (avoiding the harmful side reactions induced by MAO-B inhibition) and bound competitively yet reversibly.[114] MAO-B inhibitors are used in combination with Levodopa and peripheral inhibitors of dopa-decarboxylase in the treatment of Parkinson's disease.

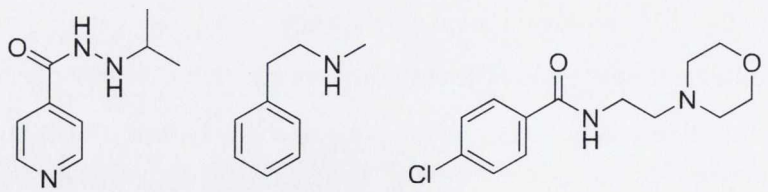
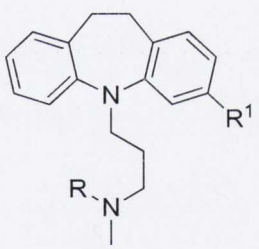


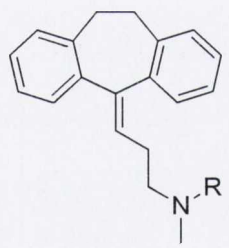
Fig. 1.4.1. Structures of classic MAOIs: iproniazid, phenelzine and the selective MAO-A inhibitor moclobemide.

The rise of the TCAs, which act primarily as 5-HT and NA reuptake inhibitors (SNRIs), started shortly after the initial finding of the MAOIs, when Roland Kuhn observed that imipramine, which was originally developed as an anti-histamine agent, improved the moods of patients illustrating signs of depressive psychosis.[115] The first imipramine-placebo controlled clinical trial was carried out in 1959 which truly demonstrated the height of its efficacy, with a 65% improvement rate amongst depressed patients,[116] and indeed it has maintained its status as one of the most effective antidepressants used today.

Due to the timeline of discovery between these two diverse pharmacological families an escalation of intense competition arose between the drug types. The TCA continued to rise with the introduction of many other drugs in the 1960s (Figure 1.4.2), some of which were later found to be selective noradrenalin reuptake inhibitors (NARI): desipramine, and nortriptyline. This rise was accompanied by the decline of the MAOI, with many, including iproniazid, being withdrawn from the market due to hypertension issues. Even with the introduction of the safer RIMAs they were only used in cases of intolerance or no response to TCA.[107]



R= CH_3 R^1= H Imipramine
R= H R^1 = H Desipramine
R= CH_3 R^1= Cl Clomipramine



R= CH_3 Amitryptyline
R= H Nortriptyline

Fig. 1.4.2. Chemical structures of tricyclic antidepressants.

The first generation of antidepressants formed the idea that correcting the 'chemical imbalance' observed in depressed individuals will relieve the signs and symptoms of depression, which gave rise to the initial evolution of understanding and theories of depression.

1.4.4. Second generation antidepressants and beyond

As mentioned the MAOI and TCA highlighted the mechanism required for antidepressant response at the synaptic level, which brought about the birth of the monoamine theory of depression. This surged research in the development of selective agents to increase monoamine levels as the TCAs, like MAOIs, had adverse effects including irreversible cardiotoxicity which was thought to be due to their non-selective nature. They also exhibited problems of death on overdose, an adverse effect that is extremely problematic when the prescription is given to a patient who is at risk of suicide.

The SSRIs were the first class of antidepressant created with rational and direct design. Their development came from early studies of anti-histamine agents during the boom of the TCAs, since imipramine was initially developed as a histamine antagonist. It was found that diphenylhydramine, a first generation partial agonist of the histamine H_1 receptor, inhibited monoamine transporters.[117] Many analogues of this compound were synthesised and it was found that small chemical modifications to the core structure, now a phenoxyphenylpropylamine, gave selective inhibition of the SET over the DAT and NAT in brain synaptosomes (Figure 1.4.3).

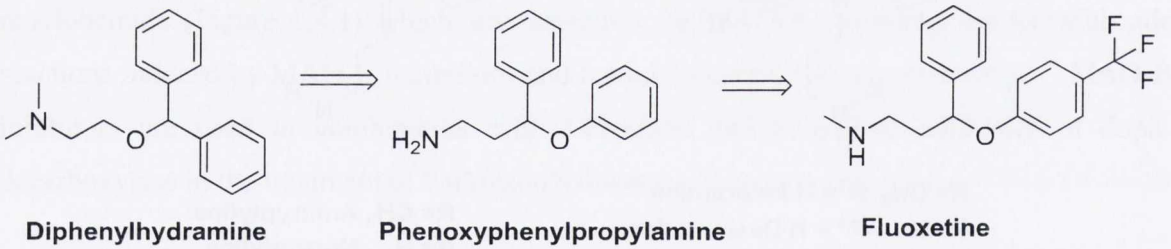


Fig. 1.4.3. Development of SSRIs from first generation histamine antagonists.

Fluoxetine (Prozac) displayed the greatest antidepressant efficacy in the series; furthermore, it showed 100-fold selectivity for SERT over the NAT with little or no affinity to other neuro-receptors, and this was attributed to its CF_3 moiety.[118,119] Fluoxetine's rise was the most rapid growth of a psychotropic drug in history, and within its first three years it was the second most prescribed drug (after Xanax) in the USA.[120] The discovery of fluoxetine

escalated the rise of the second generation antidepressants, with many other analogues launching into the market within a small time frame (Figure 1.4.4).

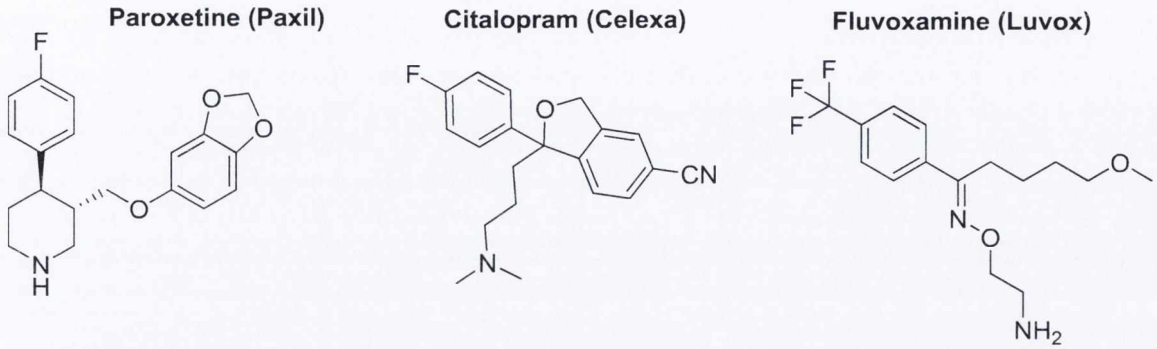


Fig. 1.4.4. Examples of SSRI development in 1990s.

Of course the attraction for these compounds was based on the safer profile compared to their non-selective precursors. However, they still have side effects such as anorexia, impotence, insomnia, decreased libido, nausea, and in particular 5-HT syndrome, as previously mentioned, which can cause seizures, tremors, hyperthermia and sometimes death (see Section 1.2.1.5). This guided research back to molecules that could modify both NA and 5-HT (similarly to the TCAs mechanism of action), but without antagonist effects on histamine H_1, adrenergic α_1 or muscarinic Ach receptors. These new systems were named serotonin and noradrenaline reuptake inhibitors (SNRIs).

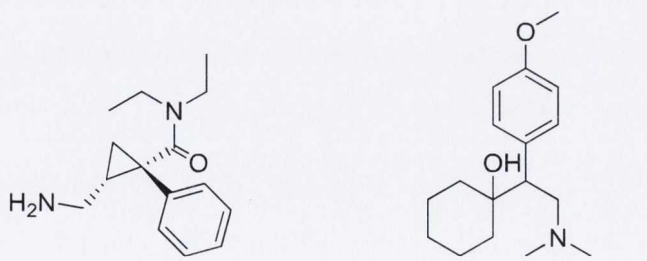


Fig. 1.4.5. Examples of clinical SNRIs Savella and Effexor.

Examples of this SNRI family are venlafaxine and milnacipran (Figure 1.4.5), both of which have differential effects on reuptake of NA and 5-HT. Milnacipran is approximately twice as effective in inhibiting NAT whereas venlafaxine is a more effective SERT inhibitor.[121,122] Due to their relative potencies venlafaxine and milnacipran can give dose-dependent side effects including nausea and vomiting, and dysuria, respectively. The second category of novel antidepressants that modify both the noradrenergic and serotonergic systems include the tetracyclic antidepressants mianserin and mirtazapine, which act primarily as antagonists of the α_2-AR, but also block the 5-HT_{2A} receptor. Due to their relevance in this research this class of compounds will be discussed more in depth in Section 1.5.3.

1.4.5. Thoughts on antidepressant development

It seems evident, as previously discussed, that there is a lack of understanding on how monoamine based antidepressants exert their therapeutic response. This raises the question of how we can really design and develop therapeutic agents for psychiatric illnesses if we do not understand the complexity of the disease itself, let alone the mechanism by which clinical agents act. Aside from this impossible difficulty there are many more problems associated with the progression of antidepressants agents, from the preliminary experiments to the clinical trials and subsequently their prescription.

At the preclinical level, the first difficulty in envisioning substantial advancement in the therapeutic effectiveness of novel compounds is the lack of valid animal models. Depression is diagnosed using Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-V), in which diagnosis are based purely on emotional and cogitative signs and symptoms of the patient. These symptoms cannot be truly correlated to the disturbances induced in animal models, therefore the use or association of animal models to a mental illness can only be a very rough approximate.[123] Further development in genomes sequencing, and neuroimaging are essential to facilitate viable and effective animals models.

The effectiveness of human clinical trials for psychiatric disorders is another ascending concern in neuropsychiatric drug development. Firstly, a significant proportion of individuals in the placebo sub-group within clinical trials show improvement in symptoms. Moreover, the use of active placebos, which will mimic the adverse effects of the drugs to convince the individual that they are taking the active drug, show extremely small differences in mood

improvement compared to the active subgroup.[124] The robust placebo response makes interpretation of data difficult and leads to higher trial failure rates, and hence, delays the delivery of new treatments to market. There is clearly a need to reconstruct the methods behind the current placebo trials, such as duration of trail, lead-in periods, and limiting patient expectancy and intensity of therapeutic contact, in an attempt to receive lower responses in placebo subjects.

Once on the market, the over prescribing of antidepressants and, as a result, the reduction in the amount of patients seeking psychotherapy is a growing concern. As mentioned previously, two of the most prescribed drugs in the U.S. were fluoxetine (Prozac) and alprazolam (Xanax) for many years, both of which are used to aid symptoms of depression and anxiety. The National Centre for Health Statistics (NCHS) reported that from 1988-1994 through 2005-2008, the rate of antidepressant use in the United States among persons of all ages increased by nearly 400%.[125] Notwithstanding the level of increase in depression worldwide in recent years, these statistics imply that antidepressant medications are now being given as a first choice treatment to reactive depression, with psychotherapy only being used in extreme cases of physical depression in combination with medical treatment.

1.5. Noradrenaline transmission and the α_2-adrenoceptor

Noradrenaline has multiple roles in the CNS as both a NT and a hormone, and therefore plays a central role in the mediation of stress and predispositions to stress (see Section 1.2.4). Alterations in NA signalling as well as changes in physical features of its system are heavily implicated in stress-related disorders such as depression. One of the main targets to modulate regular noradrenergic function is through the α_2-AR whose structure, function and implication in relation to depression will now be discussed.

1.5.1. The α_2-adrenoceptor

The α_2-ARs are members of the rhodopsin-like family (Class A) of G-protein coupled receptors which represent the largest family of membrane proteins in the human genome.[126] They consist of seven helical hydrophobic transmembrane domains with an extracellular

amino and intracellular carboxyl terminus (Figure 1.5.1). As mentioned in Section 1.2.4, three homologous subtypes of the α_2-AR have been identified: α_{2A}, α_{2B}, and α_{2C}. The function of each of these receptors was largely unknown due to the lack of subtype-selective drugs. However, recent identification of the tissue distribution has given some insight into the role of each receptor subtypes, with α_{2A} having the highest density in the locus coeruleus, and is thus linked to NA transmission in the CNS.[127]

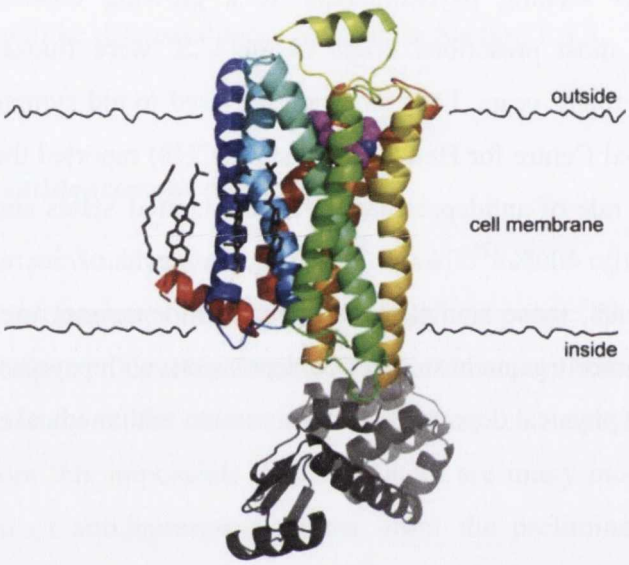


Fig. 1.5.1. Crystal structure of the β2-adrenergic receptor.[128]

GPCRs are bound to a heterotrimeric G-protein complex consisting of G_α, G_β and G_γ subunits, where the G_α subunit, possessing enzymatic activity, binds to guanine nucleotides. In the resting state G_α is reversibly bound to guanosine diphosphate (GDP), upon receptor activation by ligand binding (NA in this case) the G-protein is allostericlly activated by facilitating the exchange of GDP to guanosine triphosphate (GTP) at G_α (Figure 1.5.2). Consequently, the heterotrimer G-protein dissociate from the GPCR and from each other yielding a G_α-GTP monomer and a $G_{\beta\gamma}$ dimer, both independently modulating the activity of their specific effectors to promote downstream signalling cascades leading to cellular response. Next, the enzymatic ability of GTPases facilitates hydrolysis of GTP to GDP allowing the G_α-GDP inactive form to be regenerated and re-associate with $G_{\beta\gamma}$ to bring the G-protein bound GPCR back to its resting state.

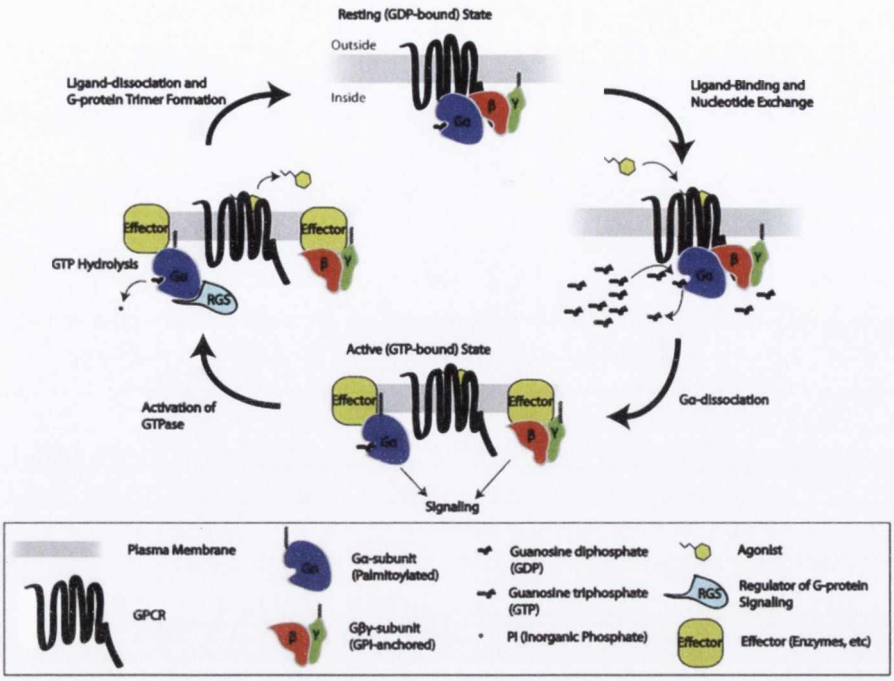


Fig. 1.5.2. Activation of GPCR on agonist binding, and deactivation back to resting state.

More specifically, the α_2-AR is associated with the G_i protein, which acts as an inhibitory regulator of cAMP dependant-PKA signalling (Figure 1.5.3). When the α_2-AR is activated on the presynaptic neuron by NA it causes the $G_{i\alpha}$ subunit to initiate a negative feedback loop on NA transmission by repressing the production of cAMP upon inhibition of adenylyl cyclase. In normal neural function, cAMP is required to open cyclic-nucleotide gated (CNG) ion channels to allow Ca^{2+} influx into the presynaptic neuron, here Ca^{2+} can interact with calcium-binding proteins such as calmodulin, which in turn mediate synaptic vesicle and membrane interactions to facilitate NA release into the synapse.[129] Thus, in essence NA binding to α_2-AR induces an auto-regulatory event through decreasing cAMP availability to prevent further NA release, and thus, competitive antagonists may block this event so the NA is continuously released.

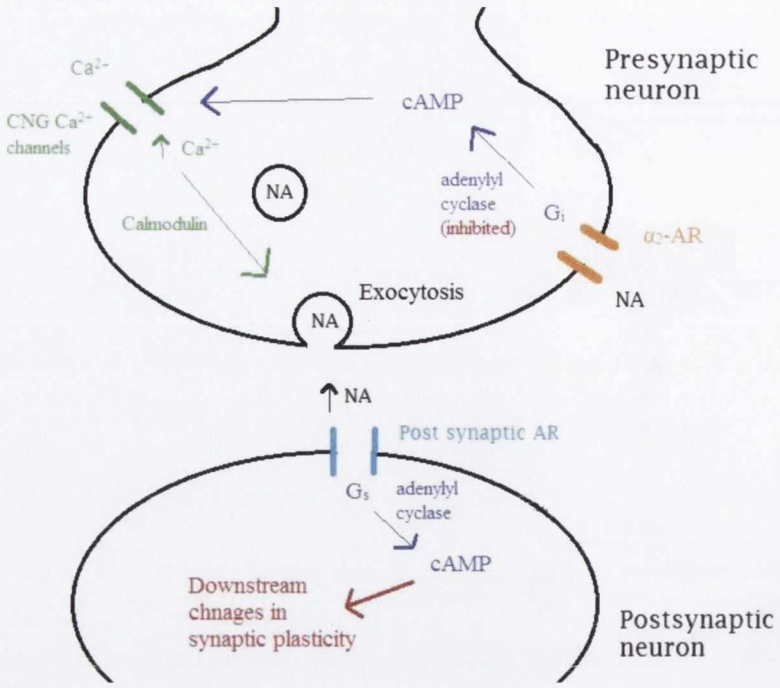


Fig. 1.5.3. Mechanism of NA binding to α_2-AR to 'switch off' further NA release.

1.5.2. Implication of α_2-adrenoceptors in depression

Dysfunction of noradrenergic transmission in depression has been hypothesised to be directly linked to the α_2-AR, albeit in former years conflicting findings have led to a slow progression of this theory. Recently, a number of experimental studies have formed a foundation of the idea that alterations in α_2-AR expression and activity may be very much associated with depressive states. The most compelling evidence, through both biochemical assays and patient models, is the upregulation or increase in receptor density of the α_2-AR in patients with depression (Figure 1.5.4).[130]

Evidence is primarily based on studies of platelet α_2-ARs, which have the advantage of monitoring changes in living patients with active depression. Advancement of accurate results in this model were hindered by the initial use of radiolabelled antagonists to measure the receptor density, as no major change was observed.[131] This is because GPCRs can exist in both active and inactive states, where antagonists have equal affinity for the high and low affinity conformational state, and therefore binding represents the total density of all receptors in any conformation. However, the use of radiolabelled agonists in these experiments lead to a marked increase in α_2-AR density, which is hypothesised to result from

an upregulation of only the high-affinity (agonist binding active form) conformational state in MDD.[132] These results were further confirmed by *in vitro* studies as a similar increase in radiolabelled agonist binding was observed in post-mortem brain tissue of depressed patients.[133] Furthermore, the α_2-AR can be directly modelled in humans through physiological response tests, as an increase in density of the high-affinity receptor state would cause an elevation in responsiveness and sensitivity to agonists. Indeed this has been observed as patients with depression exhibit heightened responses to clonidine, where this effect is reversed by antidepressant treatment (Figure 1.5.4).[134]

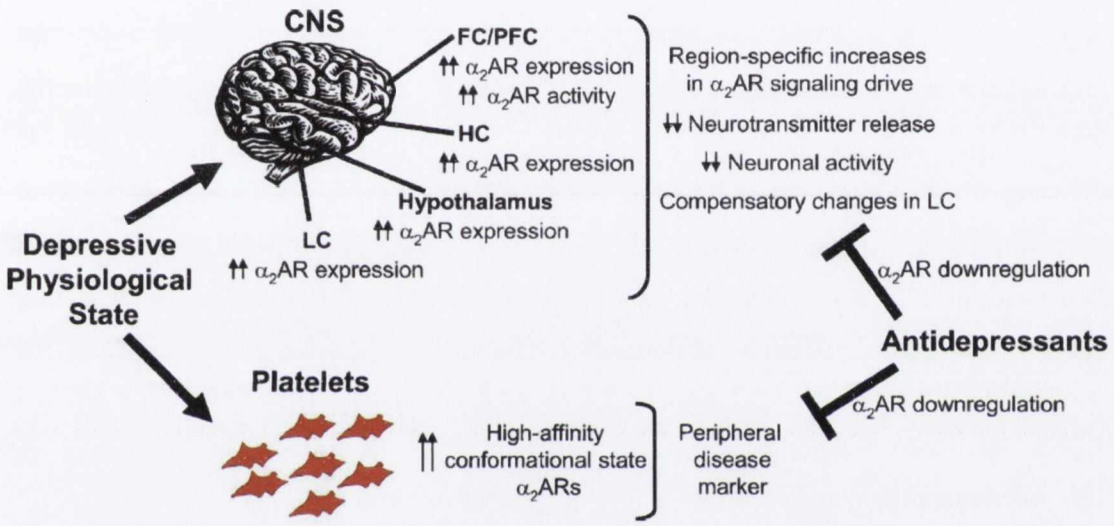


Fig. 1.5.4. Model of α_2-AR dysfunction in depression.[130]

The effects of antidepressant therapy further supports the role of the α_2-AR in depression, as both clinical and experimental models have shown a decrease in expression or downregulation of α_2-AR density after normal treatment.[135] Moreover, the use of selective α_2-AR antagonists have shown to enhance the efficacy of SSRI and NARI, at specifically increasing 5-HT and NA respectively.[136] This demonstrates that desensitising the α_2-AR, though antagonism, can contribute to the therapeutic effect of clinical antidepressants and when used in combination with other agents may give improved responses.[137]

The increase in α_2-AR expression and regulation has a knock on effect by initially decreasing NA transmission which may contribute to the downstream decrease in plasticity in brain

regions associated with noradrenergic signalling (Figure 1.5.4). Regarding plasticity, it has been recently shown that α_2-AR antagonists induce activity-regulated cytoskeleton-associated protein (Arc) gene expression through a monoamine-glutamate interaction involving the recruitment of AMPAR, NMDAR and mGluR5.[138] Arc is an early immediate protein that facilitates the expansion and stabilisation of dendritic spines, and thus has critical role in learning and memory plasticity processes that have now been shown to be implicated in depression.[24]

The α_2-AR also regulates key adaptive neurogenic processes, as adult hippocampal progenitor cells express α_2-heteroceptor subtypes, where neurogenesis in the hippocampus is an important mediator of the behavioural response in depression (see Section 1.3.2.3). Using both *in vitro* and *in vivo* approaches it has been shown that the α_2-AR decreases progenitor cell proliferation. On the contrary, blockade (antagonism) stimulates neurogenesis in the hippocampus through the proliferation and differentiation of new-born neurons and increase of the expression of hippocampal BDNF.[139] Moreover, combining α_2-AR antagonists with clinical antidepressants robustly increase the behavioural responses times in animal models, and hence illustrates their importance as an adjunct agents for potential faster acting antidepressants.[140]

1.5.3. α_2-Adrenoceptors' antagonists

The β_2-AR crystal structure has recently been elucidated with a 3.7 Å resolution;[128] however it shares less than 50% homology with the α_2-AR, whose structure is yet to be determined due to GPCRs inherent flexibility and instability in detergent solutions making them notoriously difficult to crystallise.

Site directed mutagenesis (SDM) also illustrates subtle differences in molecular interactions between ARs, but encouragingly has given valuable information on the amino acids present in the binding pocket of the α_2-AR that are necessary to form interactions with certain ligands (Figure 1.5.5).[141]

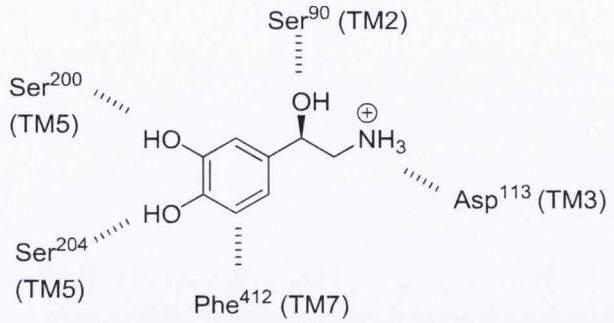
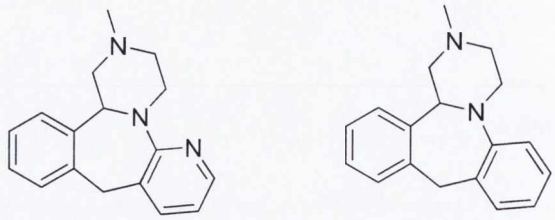


Fig. 1.5.5. Assumed interactions of amino acid residues with NA at α_2-AR.

In particular, the negatively charged Asp^{113} residue on TM3 is hypothesised to form a salt bridge with the amino moiety of NA, as no binding is exhibited when this residue is mutated. Serine residues play a crucial role in hydrogen bonding as replacement of Ser^{200} and Ser^{204} on TM5 with alanine results in a dramatic loss of affinity for NA. An interaction through π-π stacking is thought to occur through the aromatic residue, Phe^{412} on TM7, which is further supported by identification of a hydrophobic pocket in this region through homology modelling.[142,143] The information gained from SDM, homology modelling and docking studies is predominantly used today in the rational development of novel ligands for the α_2-AR.

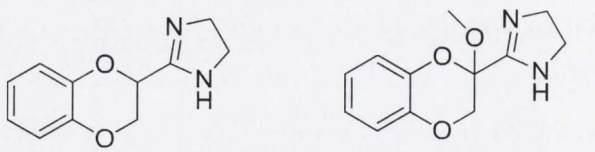
The discovery of α_2-AR antagonists as antidepressant agents happened serendipitously when pharmaceutical development started to gear towards more selective and safer derivatives of the potent TCAs, and this lead to the discovery of the tetracyclic antidepressants. The first of this class, mianserin (Figure 1.5.6), exhibited a unique antidepressant profile which was later attributed to its effect on the blockade of the inhibitory α_2-AR to enhance NA transmission.[144] Although it had no effect on monoamine reuptake sites, mianserin strongly inhibited both the histamine H_1 receptor and the α_1-AR causing side effects of sedation and hypotension respectively. Thus, its potential therapeutic value was quickly surpassed by its 6-aza derivative, mirtazapine, which illustrated a significantly safer profile regarding its effects on blood pressure levels due to its enhanced selectivity for α_2 over α_1-AR. Mirtazapine has shown to be more effective after two weeks than both SSRIs and NARIs, suggesting that it has a faster behavioural response than the typical reuptake inhibitors,[145] which is thought to be owed to its dual action by enhancing both noradrenergic and serotonergic function within the brain, as it also acts on postsynaptic 5-HT_2 and 5-HT_3 receptors.



Mirtazapine (Remeron) **Mianserin (Norval)**

Fig. 1.5.6. Structures of clinical α_2-AR antagonists.

It is quite surprising that with their sound efficacy, fast response, and reasonable side effect profile that mirtazapine and mianserin are the only α_2-AR antagonists on the market today. Other compounds that have been investigated are imidazoline derivatives such as idazoxan, which took structural inspiration from the marketed α_2-AR agonist clonidine. Idazoxan (Figure 1.5.7) has been investigated for the treatment of depression but without any advancement; however, it has shown to have antipsychotic effects by enhancing DA transmission through antagonism of the α_{2C}-AR in striatum and hippocampus.[146] Its closely related derivative 2-methoxyidazoxan, although not a clinical molecule, is used in scientific research as a radiolabelled competition ligand due to its high binding affinity (pK_i 9.10) and selectivity for α_2-AR (see Section 1.5.4).



Idazoxan **2-Methoxyidazoxan**

Fig. 1.5.7. Structures of imidazoline based α_2-AR antagonists.

1.5.4. Pharmacological studies of α_2-adrenoceptor ligands

Herein, a description of the specific pharmacological protocol followed to evaluate potential antidepressants as performed by Rozas group in collaboration with Callado's laboratory (University of the Basque Country, School of Medicine, Leioa, Spain) is presented. Potential antagonists of the α_2-AR must be primarily evaluated *in vitro* using pharmacological assays to determine firstly if the ligand binds with a reasonable affinity to the target receptor, and secondly if it display the correct functional activity when bound, in this case antagonism. After those assays, microdialysis experiments are carried out to assess the antagonistic potential *in vivo* by measuring the direct increase in NT concentration in the brain of alert rats. Lastly, compounds that have met the necessary criteria in these experiments will be subjected to animal models of depression to evaluate their potential behavioural antidepressant response.

1.5.4.1. Binding affinity

The affinity toward the α_2-AR is measured *in vitro* using human brain frontal cortex tissue by competition assay against the selective radioligand [^{3}H]RX821002 (2-methoxyidazoxan), which has a known affinity (pK_i = 9.10). In this assay the prepared receptor proteins are incubated with the radioligand, which is used at a constant concentration of 1 nM, and then with varying concentration of the testing compound with an unknown affinity. A plot of the percentage bound radioligand against the negative logarithm of the unknown ligand concentration can be produced (Figure 1.5.8) to determine the degree at which the radioligand is displaced using scintillation spectrometry. This graph can be used to measure the IC_{50}, which is the concentration of unknown ligand when half of the radioligand is displaced.

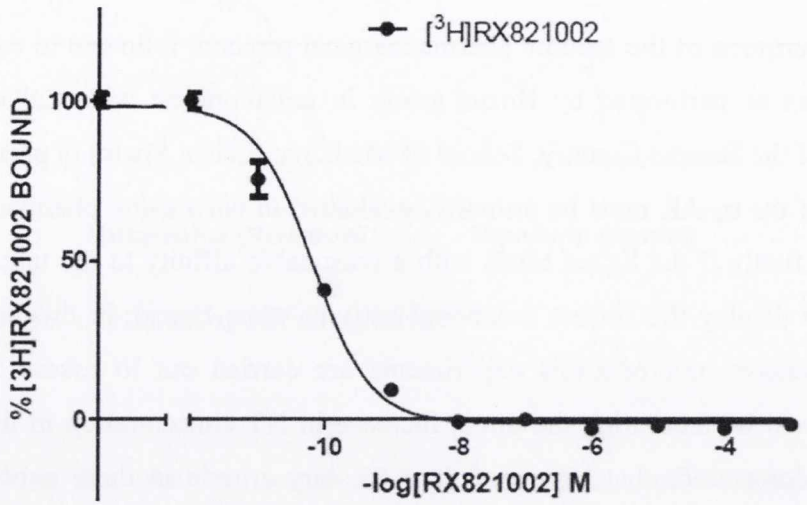


Fig. 1.5.8. Standard binding curve of RX821002.

The IC_{50} itself is not a direct measure of binding affinity as it depends on the concentration of membrane used; however, it can be directly related to the dissociation constant of affinity (K_i) using the Cheng-Prusoff equation when the concentration ([S]) and affinity (K_m) of the radioligand are known.[147] To maintain consistency with antagonist pharmacology the pK_i, the negative logarithm of K_i, is used to define binding affinities in this work.

$$K_i = \frac{IC_{50}}{1 + \frac{[S]}{K_m}}$$

Equation 1. The Cheng-Prusoff equation relating IC_{50} to K_i.

1.5.4.2. Functional activity

Compounds which displayed an affinity less than 1 μM (pK_i = 6) are subjected to *in vitro* [^{35}S]GTPγS binding experiments in human prefrontal cortex tissue to determine their nature as agonists or antagonists. Activation of the α_2-AR leads to the dissociation of GDP-G_α, followed by GDP-GTP exchange and subsequent modulation of downstream effectors. GTP-G_α hydrolysis is facilitated by GTPases to reform the inactive heterotrimeric G-protein with

G_α bound to GDP (see Section 1.5.1). GTPγS is a G-protein analogue of GTP in which one of the oxygens on the γ-phosphate is substituted for a sulphur atom (Figure 1.5.9). Here the phosphodiester bond that links the γ-phosphate to the rest of the nucleotide cannot be hydrolysed to reform GDP, and hence prevents the GTP binding protein from being inactivated, allowing for facile scintillation experiments.[148] Thus, direct evaluation of the degree of G-protein activation upon ligand binding can be made by determining guanine nucleotide exchange using radiolabelled GTPγS ([^{35}S]GTPγS), to observe agonist, antagonist of inverse agonist activity.

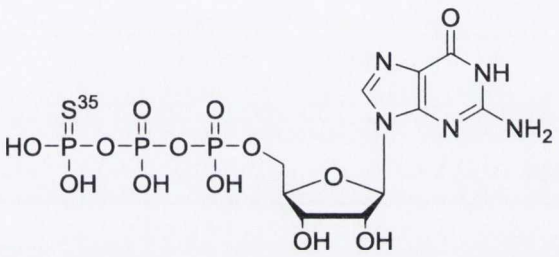


Fig. 1.5.9. Structure of radiolabelled [^{35}S]GTPγS.

The assay is carried out at a range of ligand concentration to obtain a plot of percentage bound of [^{35}S]GTPγS *versus* the negative logarithm of ligand concentration, which can be used to determine the functional activity of the ligand, as well as its EC_{50} (Figure 1.5.10). Agonists will generally stimulate binding, as they activate the G-protein to allow for GDP-[^{35}S]GTPγS exchange, and inverse agonists will display the opposite effect by decreasing the activity below the basal level.

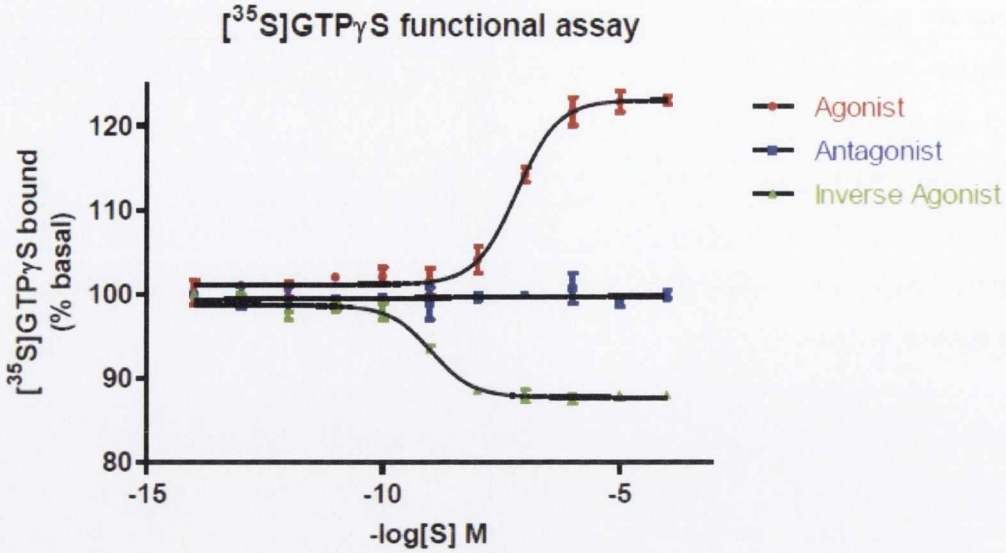


Fig. 1.5.10. Plot of a typical [^{35}S]GTPγS experiment showing agonist, antagonist and inverse agonist binding curves.

Antagonists do not stimulate any activity, thus their potency in this case is only an indirect measure. To obtain definite results regarding their efficacy as antagonists a competition assay against a selective α_2-AR agonist (UK14304) is performed. Here a decrease in bound [^{35}S]GTPγS, illustrated by a rightward curve shift or increase in EC_{50} of the known agonist indicates that the unknown ligand is functioning as a competitive antagonist, as a higher concentration of agonist is needed to give elicit its activation of the G-protein.

1.5.4.3. Microdialysis

Microdialysis has revolutionised *in vivo* testing for antagonistic activity through a minimally-invasive procedure that allows for the continuous measurement of NT levels in a certain brain region. The technique uses a small probe that consists of a semipermeable fibre membrane; this is inserted into the tissue of interest where small solutes can pass through this membrane by passive diffusion and the increase (or decrease) in concentration of solute collected can then be monitored and analysed. This technique is highly important to determine the effectiveness as antagonists agents that act on the brain, as it gages the local release increase

of many different chemicals other than NTs such as neuromodulators (cAMP), amino acids and energy substrates (glucose), but also verifies that the drug being tested has the ability to cross the blood brain barrier to exert its effect if administered systemically.[149]

1.5.4.4. Animal behavioural tests

The heterogeneity and complexity of the clinical states that encompass depression make valid animal models extremely difficult to predict, as they can only really focus on the physiological and neurobiological changes rather than the emotional and cogitative states observed in humans.[150] Many of the signs and symptoms of depression may be related to neurological mechanisms, but some may cut across various categorical diagnosis schemes, and only a subset of these symptoms or alterations may be modelled in animals.[151] The two most classical and widely used behavioural paradigms for evaluating antidepressant-like behaviour are the forced swim test (FST) and the tail suspension test (TST). These tests act as screening examinations whereby the animal is subjected to stressful scenarios in the attempt to reproduces aspects of anxiety related human pathology.

While both tests are respected in preclinical trials their results can be somewhat arbitrary in relation to reproducibility in human clinical trials.[152,153] As already seen, hyperactivity of the HPA axis in humans expressing signs of depression and anxiety is an evident characteristic of depression. This is the main method that can be exploited in the attempt to produce somewhat valid animal models.[123] Chronic unpredictable stress (CUS) models are performed by subjecting rodents to repeated periods of physical stress over a period of weeks; the resulting symptoms that hold some common ground to those observed in humans, which can then attempted to be reversed by antidepressant treatment.[154]

1.6. Previous work in Rozas group

In the past 15 years the Rozas group have worked on the preparation and pharmacological evaluation of a series ligands targeting the α_2-AR.[155,156,157] Over 100 compounds have been developed, all sharing the common structural feature of an aromatic guanidinium or 2-aminoimidazolinium with varying functionalization of electron donating or withdrawing groups *para* or *meta* substituted on the aromatic ring.

From preliminary studies hit compound **1** was developed,[158,159] which shares the diaromatic motif observed in mianserin and mirtazepine, and showed excellent binding affinity (pK_i 8.80) for the α_2-AR; however with un-favoured agonist activity (Figure 1.6.1). From this initial discovery many structurally related *bis*-guanidine and *bis*-2-aminoimidazoline analogues were prepared, along with shorter monomeric analogues, bearing some resemblance to the known α_2-AR antagonist 2-methoxyidazoxan.

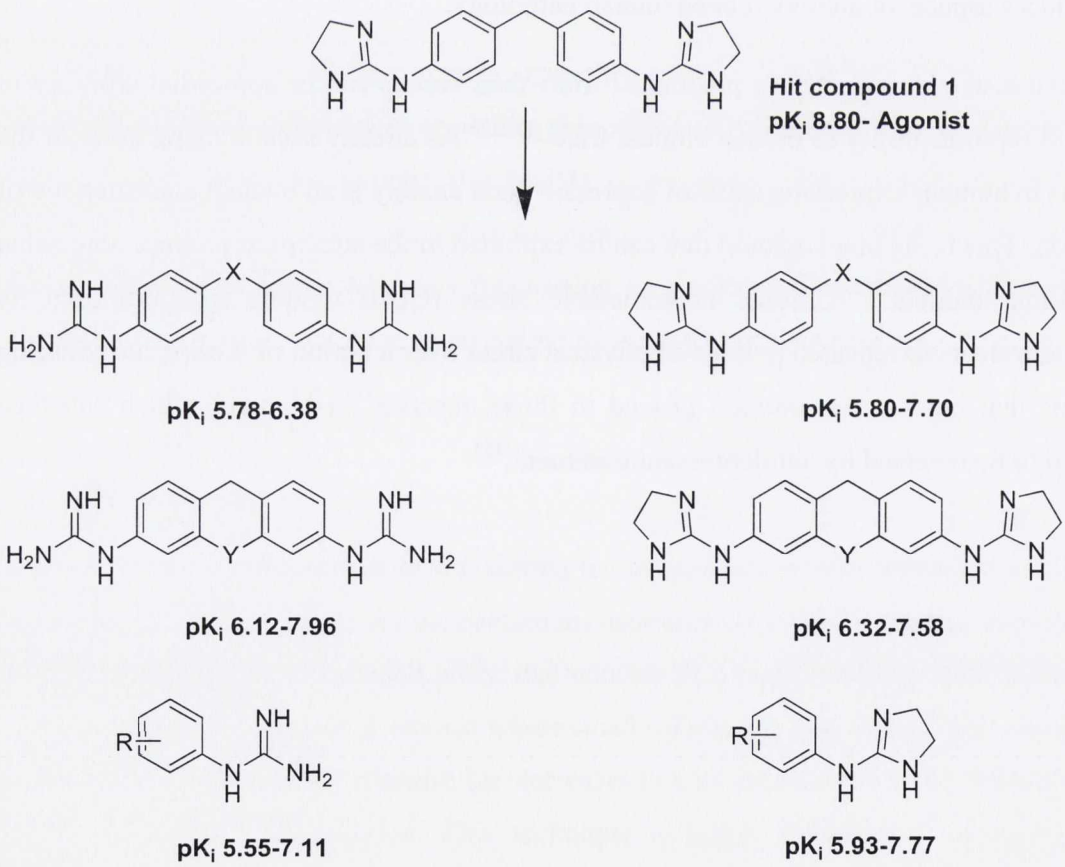


Fig. 1.6.1. Structures of hit compound **1** with *mono*- and *bis*-guanidine-like derivatives.

The affinity of these compounds for the α_2-AR was evaluated *in vitro* using prefrontal human brain tissue by competition assays against radiolabelled 2-methoxyidazoxan ([^{3}H]RX821002). This radioligand binds very selectively and competitively to the α_2-AR, thus the measure of displacement by the tested compound will give an indication of whether it binds to the active site of the receptor. Compounds that show reasonable affinity, with a pK_i over 6 (binding less than 1 μM) were then assessed by [^{35}S]GTPγS assays in prefrontal human brain tissue to determine their functional activity (see Section 1.5.4).

Five novel compounds were thus selected (Figure 1.6.2), illustrating good binding affinity with antagonist activity, and were consequently assessed *in vivo* in mouse models using microdialysis experiments to test their antidepressant efficacy. This determined whether there was a significant change in NT concentration in the extracellular area where the probe was implanted,[149] but also the use of systemic administration helped to confirm their ability to cross the blood brain barrier.

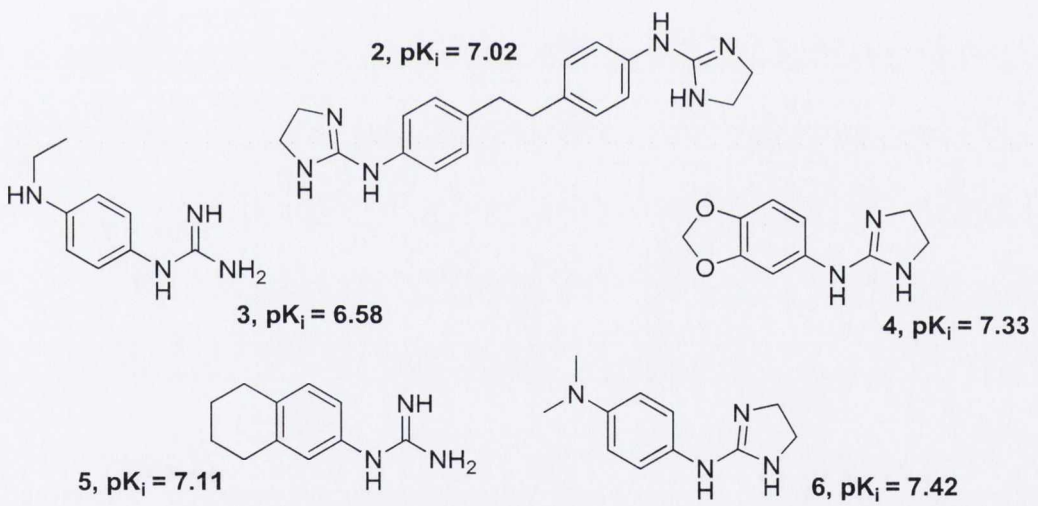


Fig. 1.6.2. Structures of lead novel antagonist ligands of α_2-AR.

Three of the compounds (**2, 3,** and **5**) that gave the most promising results as antagonists *in vivo* were selected to be investigated in animal behavioural models to evaluate their pharmacological antidepressant profile in relation to emotional and stressful disturbances.

Encouragingly, the two guanidine derivatives **3** and **5**, displayed better antidepressant efficacy than both fluoxetine and mirtazapine in the TST and FST.[160]

One of the main difficulties in the development of new compounds is the conflicting functional activity, agonist or antagonist, amongst very similar structural derivatives, thus making a rational structural activity relationship (SAR) difficult to develop. The 2-aminoimidazoline derivatives tend to give higher binding affinities; however, none have shown a sufficient antidepressant response. For example, considering the 2-aminoimidazoline analogues of the lead compounds **3** and **5**, compounds **7** and **8** respectively, these both bind with higher affinity but have agonist activity (Figure 1.6.3).

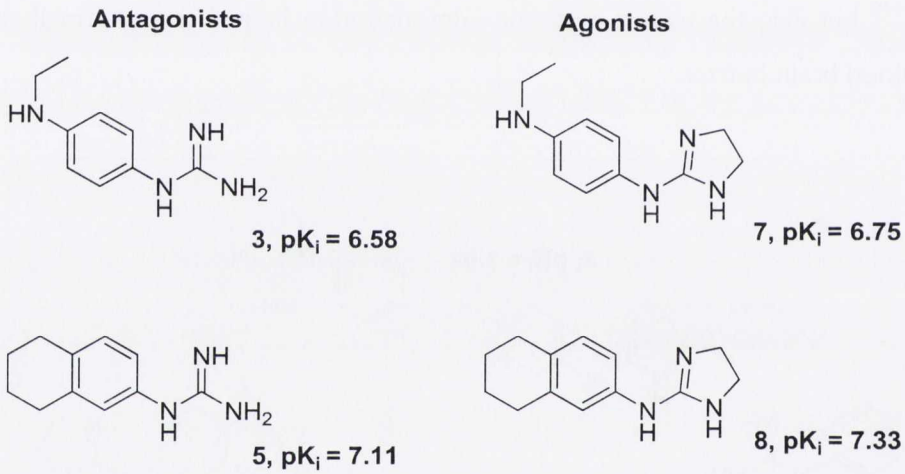


Fig. 1.6.3. Differences in functional activity between similar structured ligands.

Activity at the receptor is of paramount importance in this research. Therefore, a 3D pharmacophore (see Section 3.2.1.1) was developed within the group, incorporating a wide range of antagonist ligands of the α_2-AR, in an attempt to underline what structural elements are necessary for α_2-AR antagonism, and subsequently use this information to design a new generation of compounds. This study displayed the requirements of R_2 substitution at the cationic moiety in the form of *N,N*-di-substituted arylguanidines and 4-substituted-2-aryliminoimidazolidines (Figure 1.6.4, structures A and B respectively).[161] This alteration gave consistent antagonist activity compared to the mono substituted analogues; however, had detrimental effects on the overall affinity (best $pK_i = 6.67$).

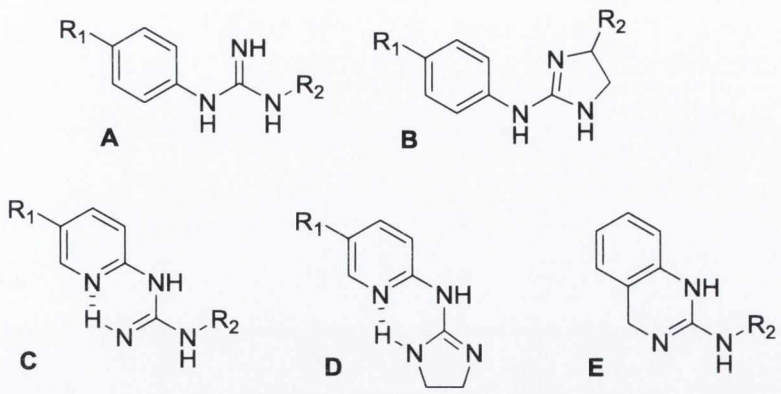


Fig. 1.6.4. Alterations in chemical structure searching for high affinity and consistent antagonist activity.

Recently, we have published a series of guanidine and 2-aminoimidazoline derivatives of pyridine as well as 1,4-dihydroquinazolin-2-amines (Figure 1.6.4, structures C, D, and E, respectively), as confomationally controlled analogues of our previous lead compounds, allowing investigation of the importance of molecular conformation and rigidity on binding to, and antagonist activity at, the α_2-AR.[162] These derivatives markedly improved affinity (best pK_i = 7.02) with consistent antagonist/inverse agonist activity, and also demonstrated excellent efficacy *in vivo* in rat brain as shown by microdialysis experiments.

Now that difficulties in relation to activity have been overcome it is evident that an improvement in binding affinity is required, as a pK_i of 8.80 (Figure 1.6.1, compound **1**) has yet to be surpassed. This could potentially create a novel class of high affinity α_2-AR antagonists with an enhanced antidepressants efficacy profile.

Chapter 2 - Objectives

In the past ten years, over 100 structures that target the α_2-AR have been developed within the Rozas group. [155,156,163] The most significant problem throughout the course of these studies was the unpredictable pharmacological results regarding functional activity at the α_2-AR between structurally similar agonists and antagonists. Hence, in more recent times, research has been particularly geared towards understanding what structural features are required for compounds to elicit the functional activity of interest for our group, antagonism.[161,162] Indeed, this problem has now been partially solved (as discussed in Section 1.6) and, thus, this project focuses on the development of antagonist ligands with enhanced binding affinity compared to our previously developed leads. Here we will use rational design to modify the core structure of our preceding families of α_2-AR ligands in the hope to not only create high binding antagonist ligands, but also to develop a comprehensive structural activity relationship (SAR) to understand all functional elements required to progress research in this area for the future. Figure 2.1 illustrates the five key families that were investigated in the present research with their progression from previous leads. A brief explanation of the goals behind each target is also shown in Figure 2.1, while the in depth rational behind the design of each family will be discussed in detail in Chapter 3.

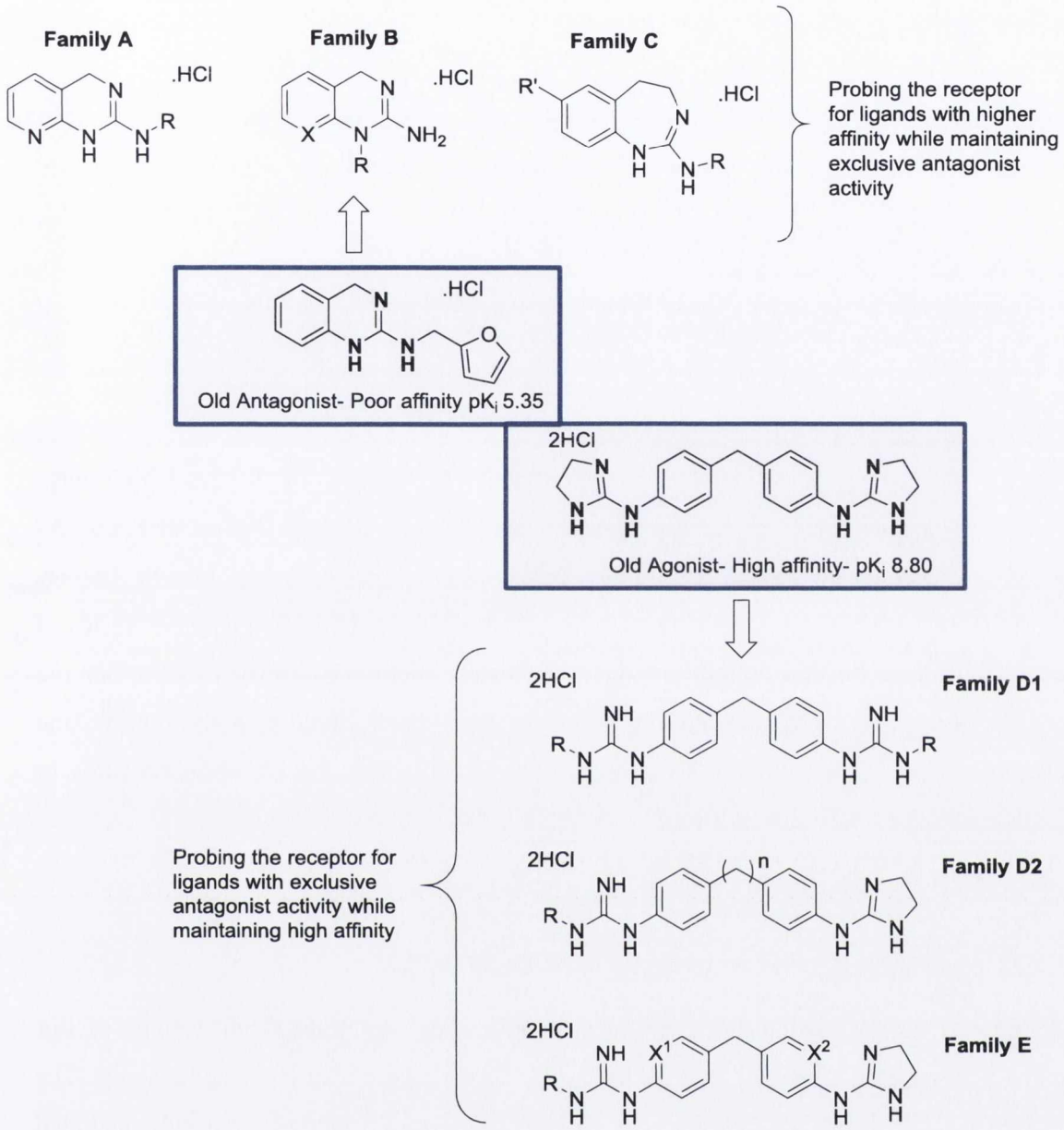


Fig. 2.1. Structures of all families investigated in this project as antagonist ligands of the α_2-AR.

2.1. Synthetic Objectives

We aim to prepare the compounds in Families A to E as shown in Figure 2.1 by using a combination of novel synthetic pathways as well as synthetic methodologies previously established within our group to produce several new generation high binding α_2-AR antagonists.

2.1.1. The synthesis of cyclic guanidine derivatives

Previously in our group, research has been exclusively focused on the synthetic routes towards acyclic guanidine derivatives even though, recently, a small family of cyclic *N*-substituted 1,4-dihydroquinazolin-2-amines have been prepared using known literary procedures. However, the structural features included in the novel cyclic guanidine series of this project required entirely new synthetic pathways including ample variation in the methods between the different families. The synthesis will predominantly involve the preparation of novel substituted diamines, where modifications of known procedures to establish cyclisation will be employed.

2.2.2. The synthesis of *bis*-aryl guanidine derivatives

According to our rational design, families encompassing structural modifications of our previous lead *bis*-aryl aminoimidazoline have appeared to be promising synthetic targets. The synthesis of these families will utilise the commonly employed mercuric chloride mediated guanidylation of *bis*-aryl amines with thiourea derivatives, which has been developed in our group for *mono*- and di-guanidylation as well as to install the 2-aminoimidazoline moiety. In some cases, however, before the guanidylation step several new synthetic routes had to be developed to obtain the non-commercially available starting *bis*-aryl amines.

2.2. Pharmacological Objectives

All final compounds prepared throughout this research will be pharmacologically evaluated to first determine their binding affinity for the α_2-AR. Their affinity toward the α_2-AR will be measured *in vitro* using human brain frontal cortex tissue by competition binding assays against the selective α_2-AR radioligand [^{3}H]RX821002 (2-methoxyidazoxan). Those compounds that show good affinity ($pK_i > 6.5$) will be further subjected to *in vitro* [^{35}S]GTPγS functional experiments to determine their nature as antagonists or agonists.

Those compounds that encompass high binding properties with antagonist activity will then be examined *in vivo,* using the minimally invasive microdialysis technique, to measure the extracellular increase in noradrenaline concentration *via* both local and systemic administration of the compound. Further *in vivo* studies may include animal behavioural models such as the TST and FST, or more meticulously, animal models of chronic stress such as CUS models.

Chapter 3 - Results and Discussion

3.1. Conformationally restricted guanidines- Families A, B, C

3.1.1. Family A: *N*-substituted 2-amino-1,4-dihydropyrido[2,3-*d*]pyrimidines

Guanidine's ability to form multiple interactions in the body, such as ionic, hydrogen bonding and π-cation stacking, make it an ideal functionality of small-molecule drug structures. Guanidines high basicity, pK_{aH} of 13.6 in water,[164] means it will be protonated as the guanidinium cation at physiological pH (7.4). This cation can exhibit a number of resonance structures through conjugation of the nitrogen lone pairs where there is an abundance of protons that are available for hydrogen bonding interactions with various acceptors (Figure 3.1.1.1); this resonance make the guanidinium cation a very stable one facilitating its ionic interaction with negatively charged biological systems (i.e. glutamate residues or phosphate groups).

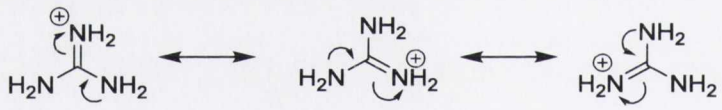


Fig. 3.1.1.1. Resonance forms of the guanidininium cation.

Guanidine acting as a hydrogen bond donor source has been studied in the Rozas group through the investigation of conformational control induced by intramolecular hydrogen-bonding (IMHB) interactions in 2-pyridoguanidine systems (see Section 3.2.2.3).[165] Guanidines ability to form π-cation interactions has also been extensively studied within our group due to its 6π electrons existing in the bonding orbitals.[166] It hence adopts a type of Y-aromaticity (delocalisation energy of 26.4 kcal mol^{-1}) which has been shown to be sufficient to weakly interact with not only numerous planar electron rich ring system in biological systems,[167,168,169] but also with anionic moieties.[170]

With all these interactions in mind it is not surprising that there are many examples of guanidine functionalities in biologically active molecules that exhibit a wide range of diverse medicinal applications.[171]

3.1.1.1. Design

As seen in Section 1.6 the development of pyridinoguanidines and pyridino-2-aminoimidazolines resolved previous difficulties regarding functional activity at the α_2-AR. In these molecules, intramolecular hydrogen bonds (IMHBs) are formed between the pyridine nitrogen lone-pair of electrons and a proton from either the guanidinium or 2 aminoimidazolinium cations, creating pseudo bicyclic systems with restricted rotatable bonds affording ligands with exclusive antagonist activity.[165] Indeed it is well known that constrained conformation is favoured for antagonism;[172] additionally, it was also postulated that further probing alterations in conformation may also give positive effects regarding affinity. This lead to the design of fused bicyclic aryl guanidinium derivatives, 2-amino-1,4-dihydroquinazolines (Family O), as conformationally restricted analogues of pyridin-2-yl-guanidines (Figure 3.1.1.2).[162]

Unfortunately, these *N*-substituted 2-amino-1,4-dihydroquinazolines gave poorer results, in terms of affinity, than expected. It was hypothesised that the rigidity induced by the 6 membered ring caused the molecules to be too constrained to orient substituents correctly for optimal interactions in the binding pocket of the receptor.

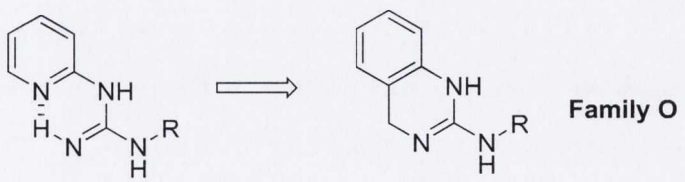
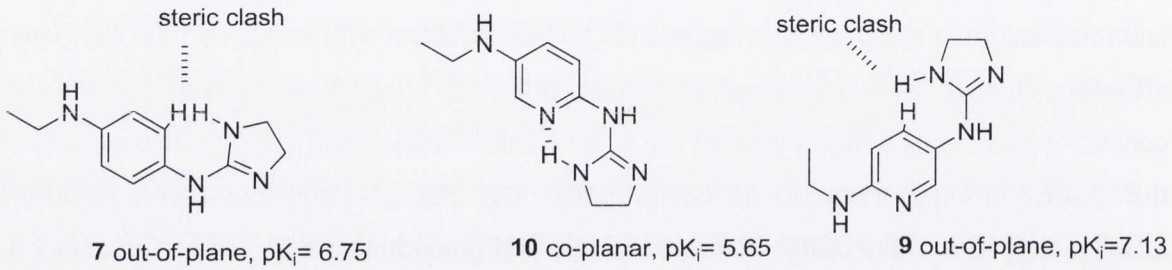


Fig. 3.1.1.2. Conformationally restricted pyridin-2-yl-guanidines (*left*) and 2-amino-1,4-dihydroquinazolines (*right*).

It has been shown that substituting the phenyl ring by a pyridine system has not only resulted in antagonist activity, but also a marked improvement regarding binding affinity for certain

compounds. Indeed, pyridin-2-ylguanidines have shown to give higher affinities for the α_2-AR than the corresponding pyridin-3-yl derivatives, and this is thought to result from their ability to form IMHBs. However, it must be noted that the opposite is observed for the pyrido-2-aminoimidazolines; here, the best pyridine analogue, **9**, is a pyridin-3-yl derivative (Figure 3.1.1.3), and cannot interact through IMHB in this manner. Like the phenyl-2-aminoimidazoline **7**, it is forced into an out of plane arrangement due to avoidance of repulsion between aromatic protons from the pyridine ring and NH protons from the cationic groups.



7 out-of-plane, pK_i= 6.75 **10** co-planar, pK_i= 5.65 **9** out-of-plane, pK_i=7.13

Fig. 3.1.1.3. Differences in affinity (expressed as the pK_i values) across 2/3-aminoimidazoline series: phenyl-2-aminoimidazoline and (pyridin-3-yl)-2-aminoimidazoline adopt an out-of-plane conformation (due to steric clash) and (pyridin-2-yl)-2-aminoimidazoline shows a co-planar arrangement (due to IMHB interactions).

It is thought that the pyridine ring in this case may adopt additional interactions through its hydrogen bonding accepting ability, which may account for the observed improvements in affinity with respect to the phenyl analogue **7**. Regarding this, we have designed Family A (2-amino-1,4-dihydropyrido[2,3-*d*]pyrimidines) as a series of pyridine analogues of 2-amino-1,4-dihydroquinazolines, to probe if reintroduction of the pyridine ring may improve the affinity of the conformationally restricted guanidine series (Figure 3.1.1.4).

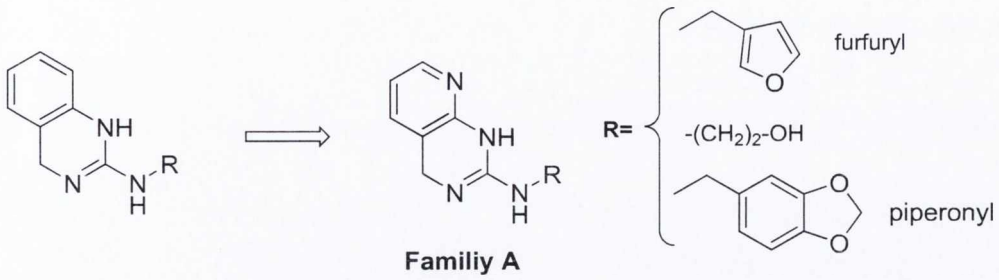


Fig. 3.1.1.4. Structure of *N*-substituted 2-amino-1,4-dihydropyrido[2,3-*d*]pyrimidines, Family A.

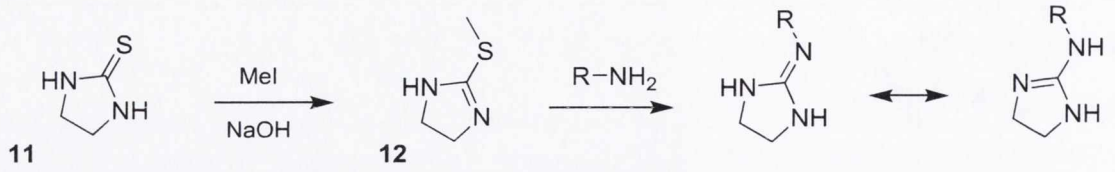
These molecules will incorporate substituents in the *N*-position which had previously given the best results in the analogous family, such as hydroxyethyl and furanyl. We have also considered the introduction of the piperonyl substituent, which has not previously been used in our group, since it was thought to be beneficial due to its flexibility, aromaticity and hydrogen bond accepting ability.

3.1.1.2. Literature methods for the preparation of cyclic guanidines

Five and six membered monocyclic guanidines

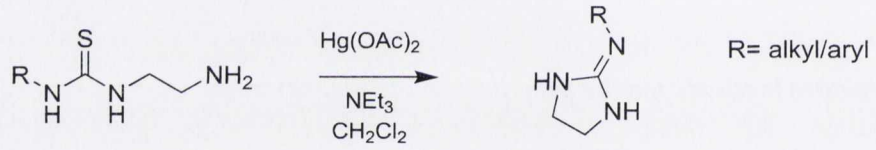
A novel synthesis towards 5 membered cyclic guanidines has been studied in detail in our group *via* the preparation of *bis*-Boc protected 2-iminoimidazolidine intermediates using mercury (II) chloride; this route will be used in the development of Families D and E, and hence it will be discussed in Section 3.2. A similar pathway involves the trapping of imidazoline-2-thione **11** with methyl iodide creating the electrophilic *S*-methyl intermediate **12** which can easily undergo nucleophilic addition of an amine (Scheme 3.1.1.1).[173] This methodology has been applied to alkyl and aryl amines, and is one of the pathways used in the synthesis of the antihypertensive drug clonidine.

Scheme 3.1.1.1.



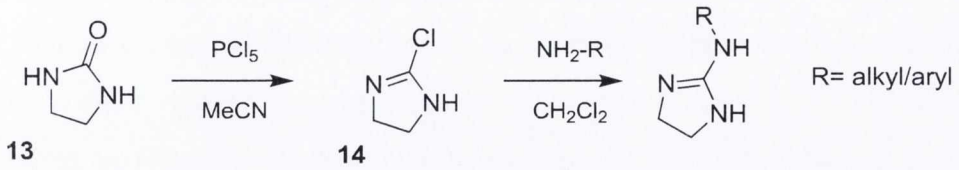
The use of mercury (II) salts as Lewis-acid promoters in the synthesis of cyclic guanidines is also applicable to non-cyclic starting materials through intramolecular cyclisation using *N*-(2-aminoethyl)-*N*-substituted thioureas (Scheme 3.1.1.2).[174] In a similar manner, the development of 6 membered cyclic guanidines with altered substitution positions are currently being investigated in our group.[175]

Scheme 3.1.1.2.



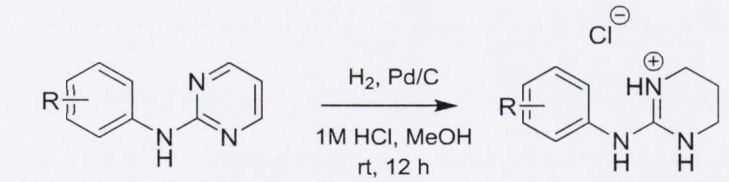
Although mercury mediated guanidylation reactions are extremely reliable and give excellent yields, the use of these reagents in term of their toxicity can be disconcerting. The urea analogue of imidazolidine-2-thione has also shown value in the synthesis of 2-aminoimidazolines and avoids the use of mercury reagents. Imidazolidin-2-one **13** can be reacted with phosphorus pentachloride to yield 2-chlorodihydroimidazole **14** *in situ*, after which the appropriate amine can be added to displace the chloride to afford the cyclic guanidine in reasonable yield (Scheme 3.1.1.3).[176] Other chlorinating agents such as phosphorus trichloride and phosphorus oxychloride have been employed in this method; however, gave sluggish reactions with complex mixtures of products.

Scheme 3.1.1.3.



There is little precedence in the literature for the preparation of 6 membered cyclic guanidines that parallels with the classic methods mentioned above. However, a novel synthesis of 2-(arylamino)-tetrahydro-1,4,5,6-pyrimidines has recently been published in our group *via* Pd catalysed hydrogenation of *N*-aryl-2-aminopyrimidines. This method utilises dilute protic acid as a driving force for the reduction through initial protonation of the aryl-2-aminopyrimidine (pK_{aH} = 3) coupled with hydrogenation to afford the aryl guanidinium hydrochlorides in high purity and excellent yield (Scheme 3.1.1.4).[177]

Scheme 3.1.1.4.



R= EW or ED substituent

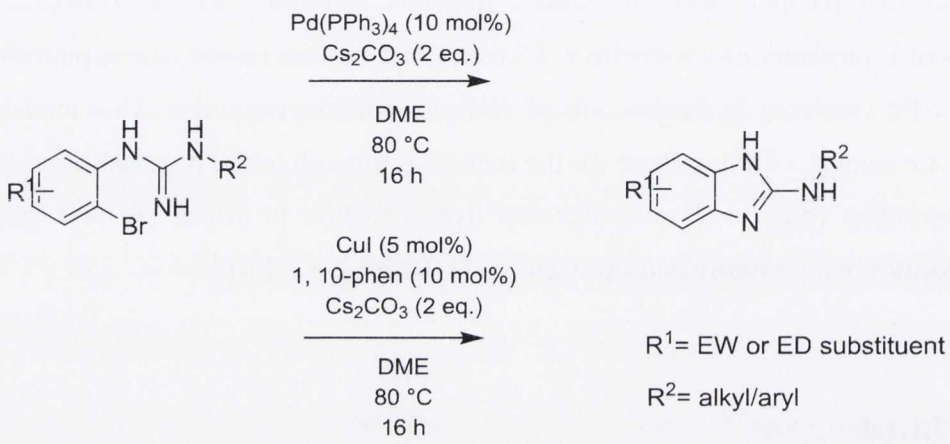
Five and six membered bicyclic guanidines

Our research towards the development of cycloguanidines is solely focused on the preparation of fused bicyclic guanidines, namely 2-amino-1,4-dihydroquinazolines and their related heterocycles. These are synthesised in an alternate manner to those previously described and some relevant literature examples will now be discussed.

Buchwald-Hartwig/Ullman type chemistry can be used to promote *N*-arylation with transition metal catalysts to cyclise guanidines that are bonded to an aromatic system.[178] Evindar and Batey reported the use of tetrakis(triphenylphosphine)palladium(0) as a catalyst for the Pd-

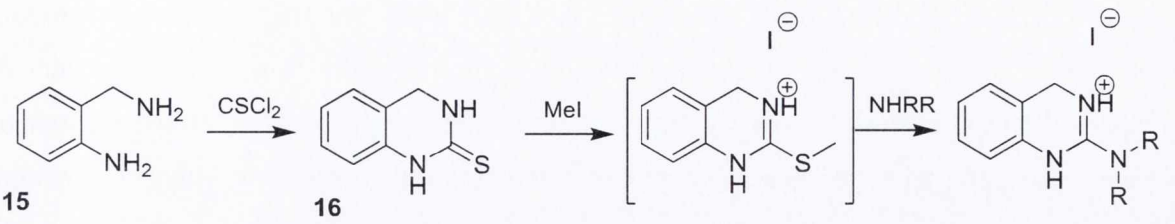
catalysed intramolecular *N*-arylation of *N*-(*o*-bromophenyl)guanidines using Cs_2CO_3 as base (Scheme 3.1.1.5).[179] These reactions were applicable to a variety of guanidine derivatives and gave good to excellent yields of the 2-aminobenzimidazoles. However, high catalyst loading was required in the case of Pd; thus, a Cu-catalysed variant for these transformations was developed which gave essentially the same results employing a lower catalyst loading.[179]

Scheme 3.1.1.5.



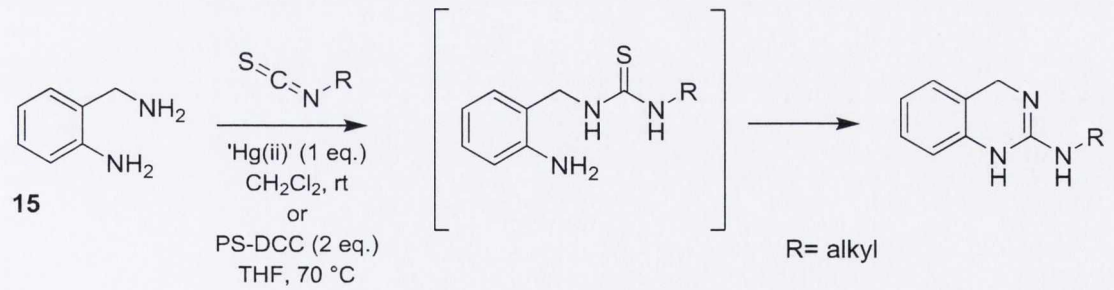
The most common starting point for the synthesis of related heterocycles of *N*-substituted 2-amino-1,4-dihydroquinazolines is from the appropriate aryl diamine. One of the older methods involves the condensation of thiophosgene with *o*-aminobenzylamine **15** to form an intermediate thione **16**, which can be readily alkylated with methyl iodide to facilitate the displacement of methanethiol *in situ* with the appropriate primary or secondary alkyl amine (Scheme 3.1.1.6).[180]

Scheme 3.1.1.6.



Due to the insidious toxicity of thiophosgene many safer synthetic methodologies towards associated heterocycles have recently emerged using similar diamine starting materials. Most of these involve initial thiourea formation using functionalised isothiocyanates followed by agents that promote desulphurization such as mercury oxide and mercury chloride to cyclise the thiourea as seen similarly before (Scheme 3.1.1.7).[181,182] Carbodiimides such as *N,N'*-dicyclohexylcarbodiimide (DCC) can also be employed instead of metal-mediated reagents to facilitate cyclodesulphurisation.[183] Cee *et al.* have described the synthesis of 2-aminobenzimidazoles using PS-carbodiimide as a solid support to simplify product isolation (Scheme 3.1.1.7). The reaction gives modest yields with wide range of structurally diverse diamines; unfortunately, for our purposes the yields for the synthesis of 2-amino-1,4-dihydroquinazolie derivatives were distinctly lower.[184]

Scheme 3.1.1.7.

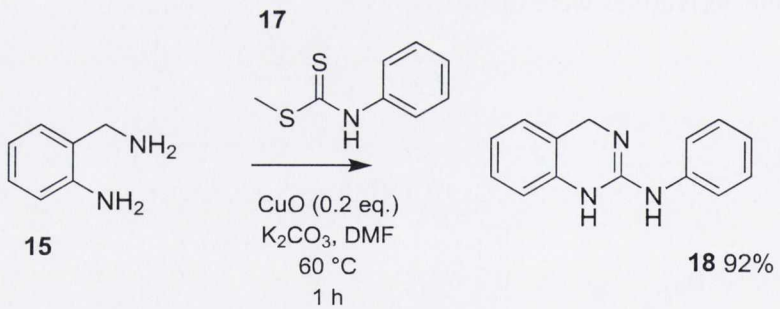


3.1.1.3. Synthesis of compounds in Family A

Although the methods described are efficient they have many associated problems including the expense and toxicity of certain reagents required for cyclisation, but in particular they all require the use of functionalised isothiocyanates. In general, there is a limited number of diverse isothiocyanates commercially available; moreover, their synthesis either requires the use of highly toxic thiophosgene or long reaction pathways, and they are inherently unstable when stored for longer periods of time.

Das *et al.* have since reported an efficient one-pot synthesis of 2-aminobenzimidazoles *via* copper (II) oxide mediated cyclisation.[185] The procedure uses dithiocarbamates as the electrophilic source whereby a large library can be easily synthesised using carbon disulphide and methyl iodide with various amines. The reaction is applicable to a variety of diamines which gives good yields regardless of electron withdrawing or donating substituents, and encouragingly the yields do not greatly differ for the synthesis of six membered systems, namely 2-amino-1,4-dihydroquinazolines (Scheme 3.1.1.8).

Scheme 3.1.1.8.

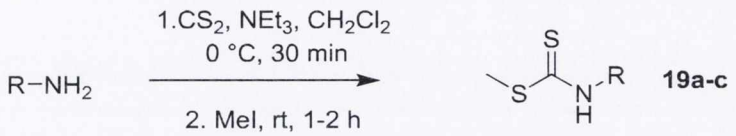


Synthesis of N-substituted dithiocarbamates

The synthesis of dithiocarbamates has been described in various synthetic pathways towards the preparation of phytoalexin brassinin whose biological activity is dependent on its diothiocarbamate core. The synthesis was initially described under solvent free conditions where the appropriate amine is reacted at 0 °C with carbon disulphide and triethylamine for 15 min. After which, methyl iodide is added to alkylate the thiol intermediate affording the

product.[186] However, it has been observed that the reaction is difficult to control in the absence of solvent for more reactive amines. The reaction has since been modified and now is performed in dichloromethane at 1M concentration illustrating slight changes in reaction time but no overall changes in yield (Table 3.1.1.1).[187]

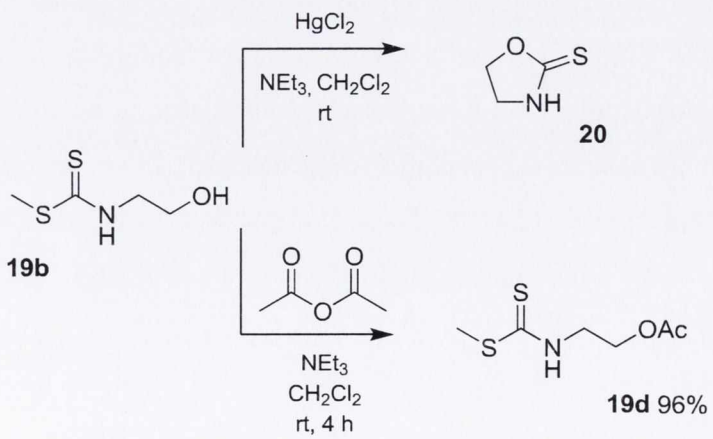
Table 3.1.1.1. Preparation of *N*-substituted dithiocarbamates.



Compound	R	Yield (%)
19a	furfuryl	78
19b	-$(CH_2)_2$-OH	66
19c	piperonyl	72

N-Hydroxyethyl-*S*-methyldithiocarbamate **19b** was protected as the acetyl derivative since it has been observed previously that, in the presence of mercury (II) chloride, similar thiourea derivatives can cyclise to give oxazolidine-2-thione **20**.[188] It was thought that a similar side reaction may take place in the presence of thiophilic copper; thus, this protection step was performed by reacting the free alcohol with excess acetic anhydride and triethylamine to give *N*-acetoxyethyl-*S*-methyldithiocarbamate **19d** in excellent yield (Scheme 3.1.1.9).

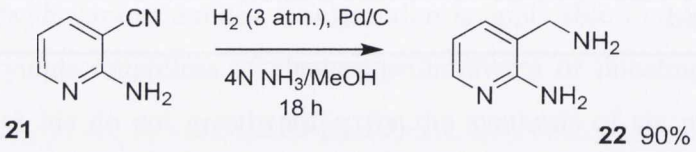
Scheme 3.1.1.9.



Synthesis of 2-amino-3-aminomethylpyridine

In this particular case, the starting diamine for cyclisation was not commercially available though it could be readily synthesised from 2-amino-3-cyanopyridine **21** by Pd-catalysed hydrogenation. This reduction has been described in a number of patents preparing prokineticin 1 receptor antagonists where the nitrile is hydrogenated at 3 atm. in a 4N solution of NH_3:MeOH for 18 h to afford the corresponding diamine **22** in near quantitative yield (Scheme 3.1.1.10).[189]

Scheme 3.1.1.10.

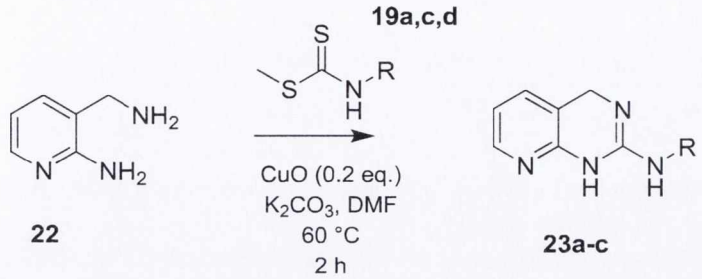


21 **22** 90%

Cyclisation to the N-substituted 2-amino-1,4-dihydropyrido[2,3-d]pyrimidines

Coupling of *N*-substituted dithiocarbamates with **22** following the procedure of Das *et al.* gave significantly lower yields than reported, which was thought to be attributed to the inherent poor nucleophilicity of 2-aminopyridines (Table 3.1.1.2). Anilines' poor reactivity comes from a decrease in availability of the lone pair of the amino group to react with electrophiles since the amine is adjacent to the electron withdrawing phenyl ring. For 2-aminopyridines the amine can delocalise its lone pairs into the pyridine ring and the resulting negative charge is stabilised by the pyridine nitrogen (illustrating amino:imino tautomeric forms) hence reducing its reactivity compared to aniline. Alterations in reaction time and temperature as well as increasing the amount of catalyst loading up to 1 eq. did not show noticeable improvement in product conversion. Another issue to be considered is that these *N*-substituted 2-amino-1,4-dihydropyrido[2,3-*d*]pyrimidines **23a-c** are a lot more polar that their corresponding phenyl derivatives. Thus, multiple water and brine washes on work up are needed to remove the dimethylformamide and this caused a further reduction in yield due to extraction of product into the aqueous phase.

Table 3.1.1.2. Synthesis of the *N*-substituted 2-amino-1,4-dihydropyrido[2,3-*d*]pyrimidines **23a-c**.



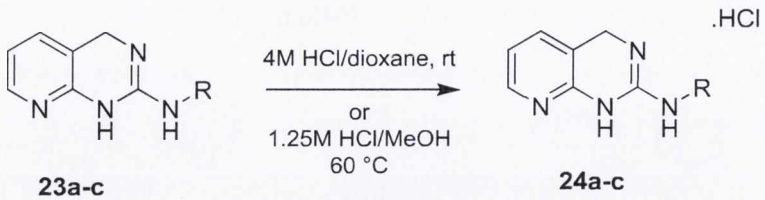
Compound	R	Yield (%)
23a	-furfuryl	57
23b	-$(CH_2)_2$-OAc	65
23c	-piperonyl	78

Although not discussed, the reaction is likely to follow a similar mechanism to those described using isothiocyanates *via* thiourea formation upon initial loss of methanethiol. Indeed it has been observed in our group that reaction of *o*-aminobenzylamine **15** with such dithiocarbamates in the absence of CuO affords the thiourea intermediate.[190] Intramolecular cyclisation by the aryl amine is then facilitated by CuO to afford the *N*-substituted 2-amino-1,4-dihydropyrido[2,3-*d*]pyrimidines.

Protonation to guanidinium hydrochlorides of Family A

Although these molecules are highly polar they are only partially water soluble and are therefore protonated to the hydrochloride salts for pharmacological testing using a 4M solution of HCl/dioxane.[162] However, for the acetoxy derivative a 1.25M solution of HCl/MeOH is used for simultaneous protonation and acetyl deprotection (Table 3.1.1.3). Protonation of these compounds was extremely fast, as seen by TLC, which agrees with their calculated pK_{aH} to be around 9-11. Encouragingly, their basicity is analogous to our previously tested lead acyclic arylguanidines (pK_{aH} = ~10), which is a good indication that these cyclic guanidine derivatives will behave, in pharmacokinetic terms, similarly to the acyclic analogues at physiological pH, and more importantly in the central nervous system.

Table 3.1.1.3. Protonation of the *N*-substituted 2-amino-1,4-dihydropyrido[2,3-*d*]pyrimidines.



23a-c **24a-c**

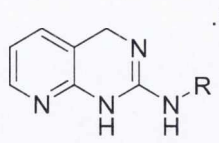
Compound	R	Yield (%)
24a	furfuryl	70
24b	$-(CH_2)_2-OH$	84
24c	piperonyl	87

The crude hydrochloride salts were purified using small scale reverse phase chromatography with silica C-8 using 100% water as mobile phase. Their purity was then assessed using reverse phase HPLC with a diode-array detector scanning wavelengths from 200-950 nm. A minimum purity of 95% was set for compounds to be tested pharmacologically.

3.1.1.4. Pharmacological evaluation of Family A

The affinity of the three *N*-substituted 2-amino-1,4-dihydropyrido[2,3-*d*]pyrimidines toward the α_2-AR was measured *in vitro* using human brain frontal cortex tissue by competition assay against the selective radioligand [^{3}H]RX821002. The α_2-AR affinities are shown in Table 3.1.1.4 and are expressed as pK_i values with RX821002 acting as the control ligand.

Table 3.1.1.4. Binding affinity to α_2-AR of compounds **24a-c** in Family A expressed as pK_i values.



Family A, 24a-c

Compound	R	pK_i
RX821002	-	9.10
24a	furfuryl	4.50
24b	$-(CH_2)_2-OH$	4.49
24c	piperonyl	5.30

Unfortunately, the α_2-AR binding affinity results for Family A did not show the expected improvement from the original *N*-substituted 2-amino-1,4-dihydroquinazolines (Family O). It had been anticipated that introduction of the pyridine ring may lead to additional hydrogen bonding interactions to enhance the molecules binding properties; however, compounds **24a** and **24b** show significantly lower pK_i values, 4.50 and 4.49, when compared with the corresponding phenyl analogues (pK_i = 5.35 and 6.26, respectively).

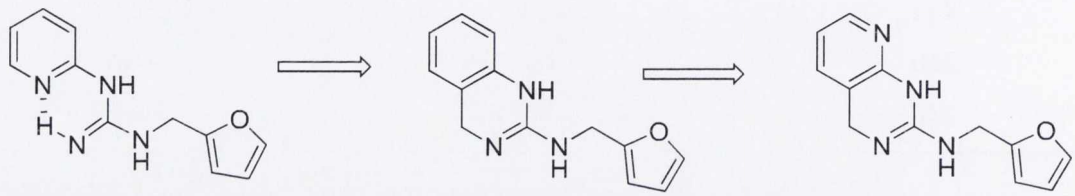
The somewhat uniform results in low affinity amongst the two families supports the idea that the constrained conformation of these molecules prevents optimal orientation of the substituents and possibly a certain degree of flexibility is necessary for effective binding to the α_2-AR. Encouragingly, however, the newly introduced piperonyl substituent gave significantly higher affinities than previously chosen R functionalities, and will therefore be further used in other cyclic series of compounds (see Sections 3.1.2 and 3.1.3).

Since no compound in this series had pK_i values $\geq$ 6.50, the functional activity at the α_2-AR receptor was not evaluated following our pharmacological protocol. However, it is expected that they will act as antagonists as their constrained cyclic phenyl counterparts as well as all the pyridinoguanidines (2 or 3 substituted) displayed exclusive antagonist/inverse agonist activity.

3.1.2. Family B: *N*-substituted 2-amino-1,4-dihydroquinazolines and *N*-substituted 2-amino-1,4-dihydropyrido[2,3-*d*]pyrimidines

3.1.2.1. Design

As previously observed in Family A, the *N*-substituted 2-amino-1,4-dihydropyrido[2,3-*d*]pyrimidines and their original phenyl analogues showed significantly lower affinity values compared to the corresponding pyridin-2-yl guanidines (Figure 3.1.2.1).



25 $pK_i = 6.32$ **26** $pK_i = 5.35$ **24a** $pK_i = 4.50$

Fig. 3.1.2.1. Comparison of pK_i values across the conformationally restricted guanidine series.

It was postulated that the rigidity induced by the methylene linkage in compounds **26** and **24a** was preventing optimum alignment or positioning of substituents R within the binding pocket of the receptor, leading to a notable drop in affinity. Hence, Family B will encompass the phenyl and pyridyl derivatives with R substitution altered from the exocyclic amino group to the interior nitrogen of the cyclic guanidine. This change will allow the probing of the receptor's active site hopefully improving the interactions with the receptors amino acid residues (Figure 3.1.2.2). The synthetic pathway towards compounds in Family B involves the preparation of *N*-benzyl-*N*-substituted cycloguanidines (Family B'); due to their availability, these derivatives were also tested as potential ligands for the α_2-AR.

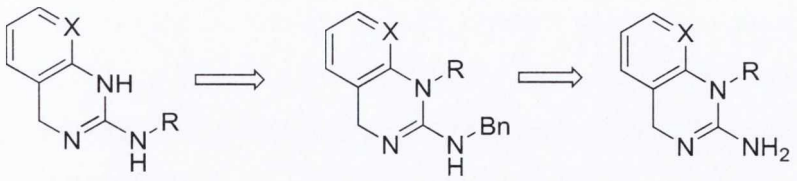


Fig. 3.1.2.2. Proposed general structures for Family B and B' series of compounds.

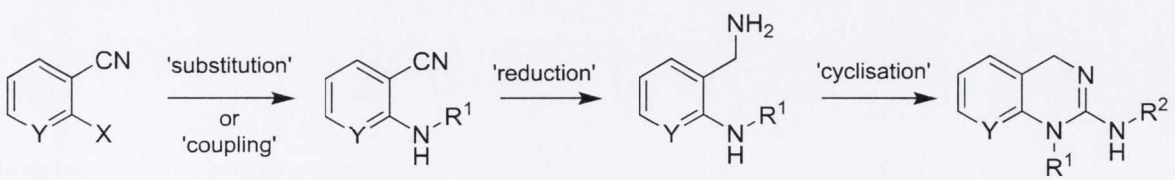
As in the previous series, these molecules were functionalised with groups that gave the best results in analogous families (hydroxyethyl and piperonyl), as well as phenyl and propyl. The previously used furfuryl substituent was avoided since it gave many problems regarding acid stability in other cyclic families (see Section 3.1.3).

3.1.2.2. Synthesis of compounds in Families B and B'

Synthesis of N-substituted aryldiamines

The synthesis of Families B and B' required the initial preparation of a library of *N*-substituted diamines to be used in the subsequent cyclisation reactions to the appropriate 2-amino-1,4-dihydroquinazolines and 2-amino-1,4-dihydropyrido[2.3-*d*]pyrimidines. The synthetic pathway towards these diamines involves an initial substitution or coupling reaction from the available functionalised aryl halides followed by the reduction of the nitrile group (Scheme 3.1.2.1).

Scheme 3.1.2.1.

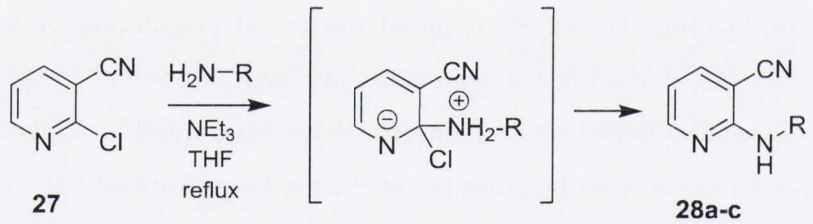


Y = CH, N
X = Cl or Br

In the case of the *N*-subtituted-2-aminopyridines (Scheme 3.1.2.1, Y= N), the nitrogen atom of pyridine influences its chemical properties to accommodate nucleophilic substitution reactions. Pyridine itself has relatively low electron density at the 2 and 4 position, as seen by its resonance forms. Thus, if these electrophilic positions are substituted with a good leaving group the pyridine ring becomes more reactive towards nucleophilic aromatic substitution reactions (NAS) since the resulting negative charge is effectively delocalised to the pyridine nitrogen.

Hence, pyridine reactivity was used in the synthesis of the *N*-subtituted-2-aminopyridines starting from 2-chloro-3-cyanopyridine **27**. In this case, the resulting negative charge can also be conjugated to the carbon bonded to the electron withdrawing nitrile to further facilitate nucleophilic substitution.[191] The pyridyl halide was refluxed in anhydrous THF using a slight excess of the appropriate amine and triethylamine, affording the substituted 2-aminopyridine derivatives **28a-c** in good yield (Table 3.1.2.1).[192]

Table 3.1.2.1. NAS of 2-chloro-3-cyanopyridine **27** with the appropriate amines.



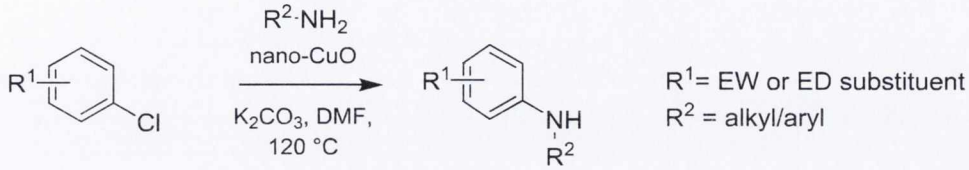
27 **28a-c**

Compound	R	Yield (%)
28a	-$(CH_2)_2$-CH_3	70
28b	-$(CH_2)_2$-OH	62
28c	-piperonyl	63

In the case of *N*-substituted anilines (Scheme 3.1.2.1, Y= CH), transition metal catalysed cross coupling reactions for the formation of C-N bonds *via* amination of aryl halides was found to be an extremely efficient method for their preparation. One of the most frequently used classic strategies in C-N bond formation is through copper mediated Ullman

condensation of aryl halides with amines.[193] Kantam *et al.* have recently described the *N*-arylation of chloroarenes in excellent yield using nanocrystalline copper oxide (7-9 nm particle size) and potassium carbonate at high temperatures (Scheme 3.1.2.2).[194]

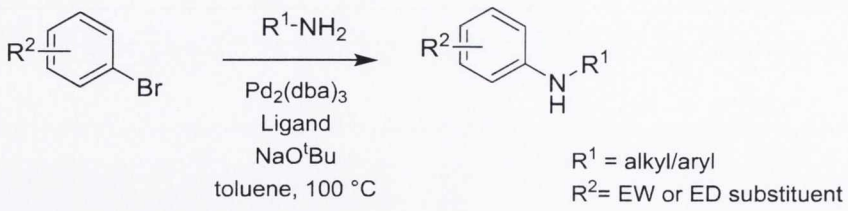
Scheme 3.1.2.2.



This methodology was attempted using 2-chlorobenzonitrile as the starting halide; unfortunately, the reaction produced a mixture of products. A similar Ullman type reaction using aryl iodides with CuO nanoparticles (33 nm particle size) and potassium hydroxide gave comparably mixed results.[195] Looking at similar reactions reported in the literature, the formation of these mixtures was thought to be attributed to the high volatility of our amines used under these reaction temperatures. Since C-N cross coupling chemistry relies on complexes with copper as well as palladium, we decided to employ Buchwald-Hartwig amination in the synthesis of the *N*-substituted anilines.[196]

The first palladium catalysed formation of aryl C-N bonds was reported by T. Migita *et al.* in 1983 through the use of tributyltin amides (aminotin compounds) and aryl bromides as coupling partners.[197] In 1995 S. Buchwald and J. Hartwig concurrently discovered that the aminotin compound in this reaction could be replaced by the free amine if a strong base was used.[198,199] A typical procedure involves the reaction of aryl bromides with an amine in the presence of a strong base (e.g. sodium *tert*-butoxide), a phosphine type ligand and a catalytic amount of a palladium precatalyst (e.g. tris(dibenzylideneacetone)dipalladium, $Pd_2(dba)_3$) (Scheme 3.1.2.3).

Scheme 3.1.2.3.



In the catalytic cycle, the activated $Pd^{(0)}$ catalyst is formed through initial ligand dissociation followed by the binding of the phosphine ligand to the free Pd coordination sites (Figure 3.1.2.3). The steric bulk and strong electron donor ability of the phosphine ligands increases the electron density around the metal centre to encourage oxidative addition of the aryl halide. Once the stable $Pd^{(II)}$ species is formed, the amine coordinates to the metal centre leading to an increase in acidity of the amine protons which triggers deprotonation by the base. The bulkiness of the phosphine ligands and the now reduced electron density around the metal centre facilitates reductive elimination to give the arylamine product and regenerated $Pd^{(0)}$ catalyst.[200]

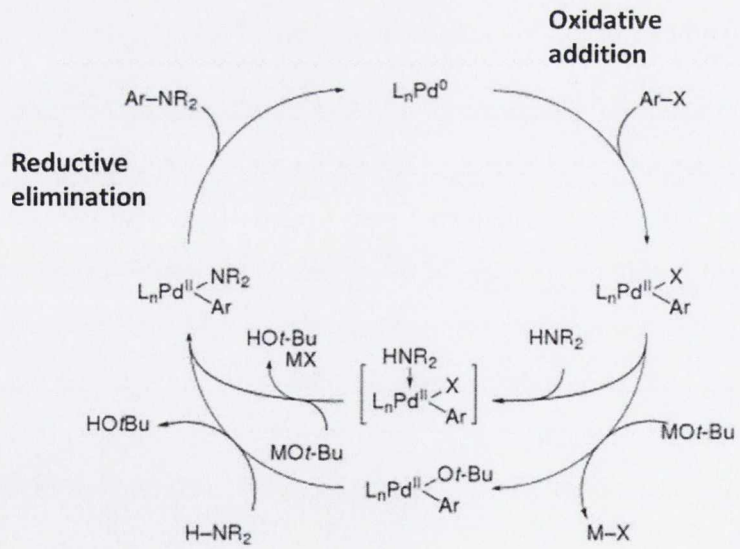
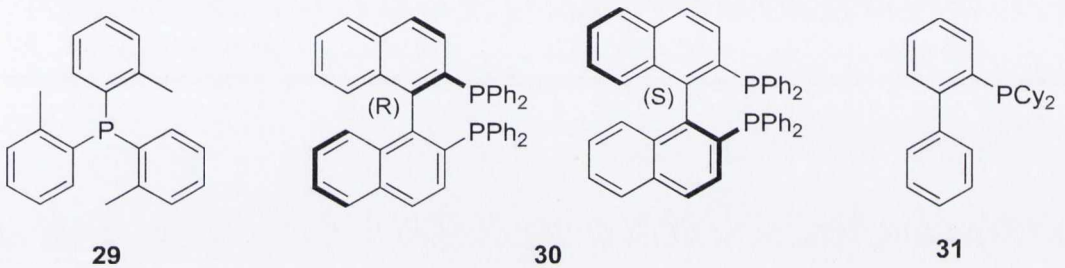


Fig. 3.1.2.3. Pd catalytic cycle for *N*-arylation of arylhalides.[201]

The first generation catalytic systems displayed a competitive side reaction to reductive elimination, β-hydride elimination yielding the hydrodehalogenated arene and imine products due to the use of the modestly bulky tris(o-tolyl)phosphine ligand **29** (Figure 3.1.2.4). Thus, the second generation catalytic systems employed chelating bidentate ligands such as 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl (BINAP) **30**, which controlled two coordination sites of the metal centre to effectively supress the inner sphere hydride elimination.[202] The third generation of electron-rich and bulky monodentate ligands, such as (2-biphenyl)dicyclohexylphosphine **31**, led to an even broader scope regarding the use of less electrophilic aryl halides, less nucleophilic amines and gave enhanced selectivity for monoarylation *versus* diarylation of primary amines.[203]

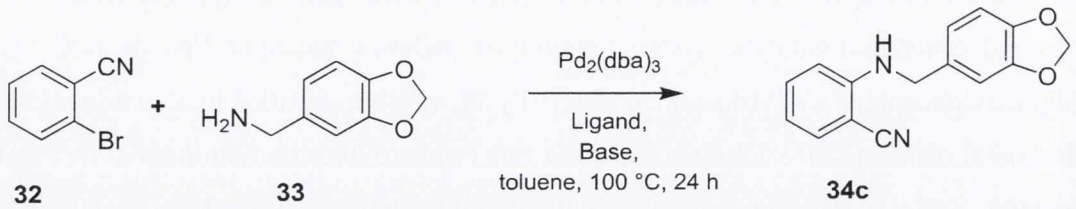


29 **30** **31**

Fig. 3.1.2.4. Structures of 1st, 2nd, and 3rd generation ligands: tris(o-tolyl)phosphine (**29**), BINAP (**30**), (2-biphenyl)dicyclohexylphosphine **(31)**, respectively.

The choice of ligand, base and solvent are all vital to the success of the reaction as well as the amount of catalyst loading and the choice of substrates. In our case, the reaction was performed using 2-bromobenzonitrile **32** and the conditions were optimised by using piperonylamine **33** with systematic alterations of the base, ligand, solvent and catalyst loading. Fortunately, only a small optimisation was needed avoiding the tedious modifications to reaction time and temperature. The ideal conditions for our coupling reaction were: $Pd_2(dba)_3$ (5 mol%), BINAP (3 mol%), and NaO^tBu in toluene at 90 °C for 24 h. (Table 3.1.2.2).

Table 3.1.2.2. Optimisation conditions for the *N*-arylation of 2-bromobenzonitrile with piperonylamine.



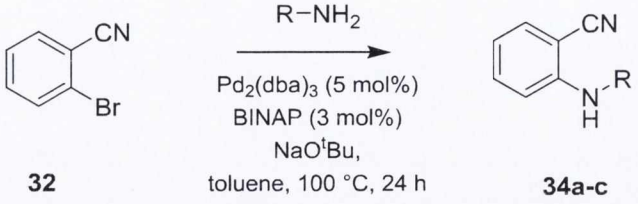
32 **33** **34c**

Entry	Pd (mol%)	Ligand	Base	Solvent	Yield (%)
1	3	31	K_3PO_4	DME	56
2	3	31	NaO^tBu	DME	51
3	3	31	NaO^tBu	Toluene	63
4	3	30	NaO^tBu	Toluene	77
5	5	30	NaO^tBu	Toluene	89

All reactions were carried out using 3 mol% of ligand at 24 h at 90 °C under argon.
31: (2-biphenyl)dicyclohexylphosphine, **30**: BINAP (Fig. 3.1.2.4).
Solvent concentration was set using 1ml per mmol of arylhalide.

In cross coupling reactions weaker bases are often used when the substrates possess electron withdrawing substituents, thus K_3PO_4 was initially used because of the nitrile functionality. However, the combination of NaOtBu with toluene showed a marked increase in yield compared to that of K_3PO_4 in DME. Furthermore, the selection of the ligand seemed most important for the success of the reaction and thus, the use of BINAP was optimal due to its facilitation of oxidative addition and reductive elimination by its bidentate binding influencing the catalyst both electronically and sterically. These optimised conditions were applied to all substrates and gave excellent yields for aliphatic amines with a substantial decrease when aniline was employed (Table 3.1.2.3).

Table 3.1.2.3. Preparation of 2-*N*-substituted benzonitriles.

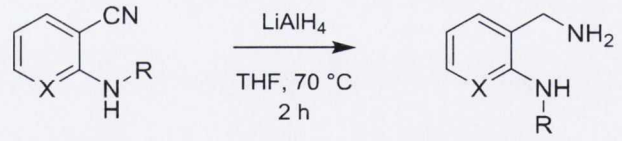


32 **34a-c**

Compound	R	Yield (%)
34a	-$(CH_2)_2$-CH_3	95
34b	-C_6H_5	54
34c	-piperonyl	89

Reduction of the nitrile derivatives **28a-c** and **34a-c** was successfully performed by refluxing them in THF in a suspension of lithium aluminium hydride ($LiAlH_4$) for 2 h affording the corresponding *N*-substituted aryl diamines **35a-f** in excellent yield (Table 3.1.2.4).[204] Due to the high solubility in water of these diamines, aqueous work-up was avoided by using the Fieser procedure, where excess $LiAlH_4$ (n grams) was quenched by the careful successive dropwise addition of n mL of water, n mL of 15% NaOH solution, and 3n mL of water. This method provided a granular inorganic precipitate that was solely removed by filtration with no further work-up required.[205]

Table 3.1.2.4. Conditions for nitrile reduction using $LiAlH_4$.



X= N **28a-c**
X=CH **34a-c**

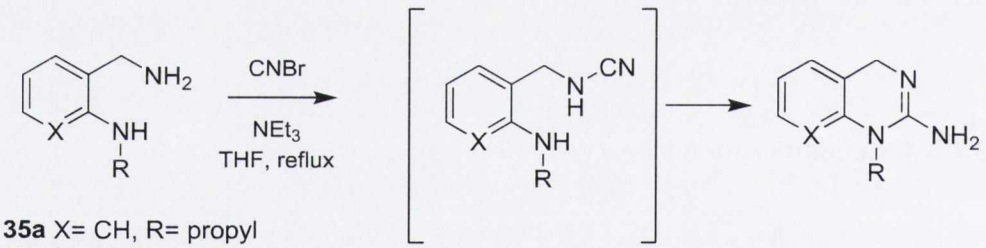
35a-f

Compound	X	R	Yield (%)
35a	N	-$(CH_2)_2$-CH_3	84
35b	N	-$(CH_2)_2$-OH	79
35c	N	-piperonyl	63
35d	CH	-$(CH_2)_2$-CH_3	70
35e	CH	-C_6H_5	77
35f	CH	-piperonyl	69

Cyclisation to the cyclic intermediates of Family B

The first attempt towards cyclisation of the *N*-substituted aryl diamines was performed by using cyanogen bromide. It is well documented that various amines can react with cyanogen bromide to give *mono-* or dialkylcyanamides,[206] and these are known to undergo further reactions with aliphatic amines or hydroxylamines to give guanidine or hydroxyguanidine products respectively (see Section 3.2.1).[207] Unfortunately, there is less precedence for the reaction of aromatic amines with alkylcyanamides since they require harsh conditions and give poor product conversion.[208] Nonetheless, it was thought that in our system the reaction may be supported as it is an intramolecular cyclisation from cyanamide to guanidine which should be favoured according to Baldwin's rules, as the CN antibonding π^*orbital is in the same plane as the aryl amine lone pair in a 6-exo-dig cyclisation. In this manner the amine lone pair can easily attack from the required transition state trajectory angle of 120° to overlap with the CN antibonding orbital yielding the cyclised product (Scheme 3.1.2.4).

Scheme 3.1.2.4.



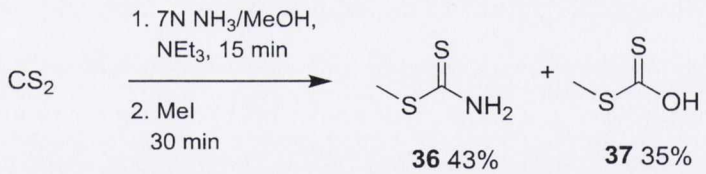
35a X= CH, R= propyl
35d X= N, R= propyl

After initial optimisation 35% conversion to guanidine was achieved by refluxing the diamine with excess cyanogen bromide and triethylamine in THF. Although initial formation of the cyanamide was fast, as observed by both TLC and ^{13}C-NMR analysis, there was never full conversion of the starting diamine, with no improvements seen when using extra equivalents of cyanogen bromide or longer reaction times. This became problematic as the starting diamine, the cyanamide intermediate and the guanidine product are all highly polar with similar Rf values. Purification using silica gel chromatography required a solvent system with a higher polarity than 30% MeOH in CH_3Cl which caused the silica to dissolve in all

fractions and, unfortunately, separation was unsuccessful despite many attempts. Small scale reverse phase chromatography was also attempted using silica C-8, yet purification remained ineffective. Due to the difficulties regarding purification and set up of the reaction with highly toxic cyanogen bromide this synthetic pathway was abandoned for a safer and more facile route.

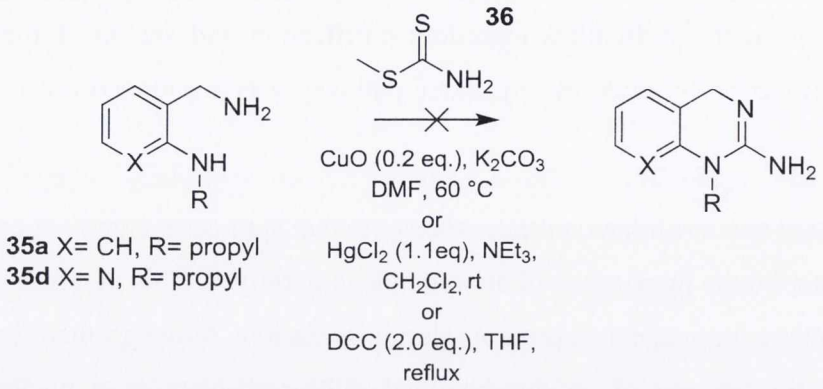
It was postulated that a similar synthetic strategy to that applied in Family A could be utilised for the final step in the preparation of compounds in Family B. This involves using ammonia dithiocarbamate and a reagent to promote desulphurisation. Although there is precedence in the literature for the use of an unsubstituted dithiocarbamate as a nucleophile in both condensation and elimination reactions,[209,210,211] there are no examples of this functioning as an electrophilic component. Nonetheless, due to the ease of the procedure, this synthetic approach was attempted. Ammonia dithiocarbamate **36** was synthesised in reasonable yield through the reaction of carbon disulphide with a 7N solution of NH_3/MeOH and triethylamine followed by subsequent addition of methyl iodide to trap the thiol intermediates (Scheme 3.1.2.5).

Scheme 3.1.2.5.



Cyclisation was attempted using several reagents that have been previously described to activate thiols towards nucleophilic attack (see Section 3.1.1), including copper (II) oxide, mercury (II) chloride and *N,N'*-dicyclohexylcarbodiimide (DCC). Unfortunately, no reaction was observed in any case, suggesting that substitution on the dithiocarbamate amino group is essential for the electronic properties of the molecules to allow for nucleophilic addition (Scheme 3.1.2.6).

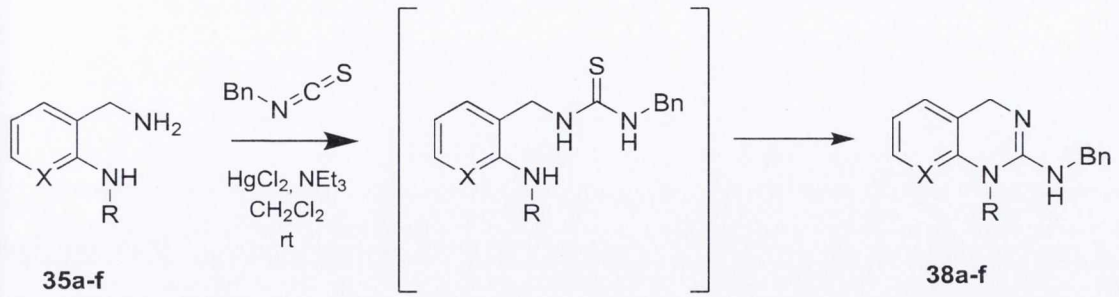
Scheme 3.1.2.6.



Since R substitution was required on the dithiocarbamate core (see Scheme 3.1.2.6), it was thought the amino moiety could be functionalised with a protecting group that could be removed later. In a similar manner, a shorter synthetic route was accessible by the reaction of the *N*-substituted diamines with commercially available benzyl isothiocyanate, using a Lewis acid in tandem to promote cyclisation.

The reaction was tested using various thiophilic promotors, all of which gave reasonably low yields, yet Mercury (II) chloride gave the best results and, hence, was the chosen promotor (Table 3.1.2.5). As previously mentioned, the use of toxic mercury-containing reagents is undesirable yet its effectiveness compared to other reagents in promoting nucleophilic addition of deactivated aryl amines cannot be denied. Mercury by-products are easily and safely removed by filtering through a pad of Celite with any traces being completely removed after silica gel chromatography.

Table 3.1.2.5. Cyclisation of *N*-substituted aryl diamines **35a-f** using Mercury (II) chloride as a promotor.



35a-f **38a-f**

Compound	X	R	Yield (%)
38a	N	$-(CH_2)_2-CH_3$	43
38b	N	$-(CH_2)_2-OH$	41
38c	N	-piperonyl	35
38d	CH	$-(CH_2)_2-CH_3$	35
38e	CH	$-C_6H_5$	58
38f	CH	-piperonyl	64

The low yields obtained for the cyclisation are thought to be due to the poor reactivity of both the nucleophilic and electrophilic components. The aryl amines themselves are very deactivated: electronically by the adjacent π-system and sterically by R substitution. Additionally, the thiourea, formed from the isothiocyanate, is very unreactive. The original mercury (II) chloride mediated guanidylation developed by Kim and Qian requires a further increase in electrophilicity of the thiourea carbon by the use of electron withdrawing Boc protecting groups, which are not available in this procedure.[212] This method will be prominently used in the synthesis of Families D and E, and, therefore, the background and mechanisms involved in this guanidylation procedure will be discussed in Section 3.2.

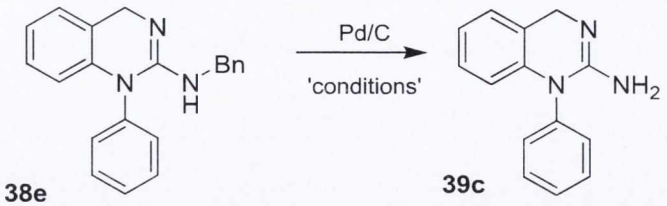
Since these cyclic guanidines have a higher basicity (pK_{aH} = ~ 9-11) than triethylamine (pK_{aH} = ~ 9) it is expected that they will exist as the corresponding guanidinium hydrochlorides after the reaction. Yet, an aqueous work up is required to remove the remaining triethylamine, which would cause a further reduction in yield *via* product extraction into the aqueous phase. To overcome this issue, the organic phase was washed

with a 50% NaOH (pK_{aH} = ~15) solution, which effectively removed the triethylamine and afforded the less polar cyclic guanidine free bases that were subsequently extracted using mixtures of isopropanol and chloroform.

N-Debenzylation of intermediate compounds of Family B'

Removal of the benzyl protecting group was a more difficult process than previously anticipated. It is well known that in some cases debenzylation of amino groups can be notoriously difficult depending on the amine substituent(s). Moreover, *N*-debenzylation of guanidines or guanidine-like compounds, such as amidines, is unprecedented in the literature with the only exception of one example in the synthesis of guanosine nucleotides.[213] In this procedure cyclohexene is used as a hydrogen transfer source with palladium oxide refluxing in MeOH affording the desired product in 20% yield. This procedure was viewed with some scepticism due to the poor yield achieved and the significant variation in substituents off the guanidine core, which will evidently affect the overall reactivity. The reaction was optimised using 1-phenyl-2-benzylamino-1,4-dihydroquinazoline **38e** under varying conditions of hydrogenation as shown in Table 3.1.2.6.

Table 3.1.2.6. Optimisation of the *N*-benzyl deprotection of benzylguanidine derivative **38e**[a].



38e **39c**

Entry	H_2 (pressure/source)	Temp (°C)	Time (h)	Solvent (0.1M)	Result
1	1 atm	RT	24	MeOH	No Rxn
2	1 atm	60	24	MeOH	No Rxn
3	3 atm	RT	24	MeOH	No Rxn
4	3 atm	RT	24	MeOH:AcOH (4:1)	Degradation
5	1 atm	RT	24	MeOH:AcOH (4:1)	No Rxn
6	1 atm	60	72	MeOH:AcOH (4:1)	~10%
7	12 atm	RT	24	MeOH	No Rxn
8	NH_4HCO_2	reflux	72	MeOH	50%
9	NH_4HCO_2	RT	24	MeOH	No Rxn

[a]Reactions were performed using Pd/C, 50 wt.% and 10 eq. of NH_4HCO_2.

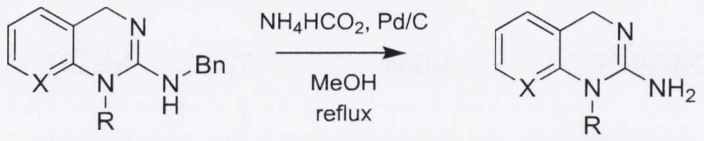
From Table 3.1.2.6 it is evident that temperature is a critical component towards the success of the reaction, with acetic acid aiding catalysis when used in synergy with higher temperatures (entry 6). It is important to note that the reaction was inert to catalytic hydrogenation at very high pressures (entry 7), which agrees with the reported literature,[213] as staggering pressures (up to 34 atm) have been attempted towards the *N*-debenzylation of guanidines with no successful result to date. The use of ammonium formate as a hydrogen

transfer source guided the success of the reaction with the added advantage of no hydrogen storage requirement, delivery system, gas measurement or pressure equipment. Moreover, this method allows for higher temperatures with continuous *in situ* generation of hydrogen.

Taking all this into consideration and despite the relatively long reaction times, the optimised conditions for benzyl cleavage were: refluxing the appropriate benzyl guanidine with excess NH_4HCO_2 in MeOH for 3 days, giving modest yields of the corresponding *N*-substituted 2-amino-1,4-dihydroquinazolines and 2-amino-1,4-dihydropyrido[2,3-d]pyrimidines (Table 3.1.2.7).

Unfortunately, under these conditions the benzyl-like piperonyl substituent in compounds **38c** and **38f** was also cleaved. Attempts to slightly alter the reaction conditions for milder hydrogenation only resulted in starting material, and, thus, finding a balance between cleavage of *N*-benzyl over *N*-piperonyl was unsuccessful. More dishearteningly, the deprotection of the hydroxyethyl derivative **38b** gave a range of highly polar side products under these conditions. Purification in this case was not possible and no identification of the desired product from the crude was found by ^{1}H-NMR, ^{13}C-NMR or mass spectrometry. It is still not clear why degradation was observed for this compound.

Table 3.1.2.7. Conditions for *N*-debenzylation of benzylguanidines **38a-f**.



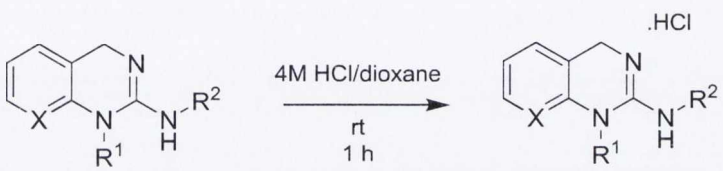
38a-f **39a-c**

Compound	X	R	Yield (%)
39a	N	$-(CH_2)_2-CH_3$	50
-	N	$-(CH_2)_2-OH$	-
-	N	-piperonyl	-
39b	CH	$-(CH_2)_2-CH_3$	58
39c	CH	$-C_6H_5$	53
-	CH	-piperonyl	-

Protonation to guanidinium hydrochlorides of Family B' and B

Protonation of Families B and B' followed the same procedure as in Family A using a 4M solution of HCl/dioxane to yield the corresponding guanidinium hydrochloride salts **40a-i** in good yield (Table 3.1.2.8). Purification was achieved similarly using small scale reverse phase chromatography with C-8 silica using 100% water as mobile phase, and their purity was assessed using reverse phase HPLC.

Table 3.1.2.8. Synthesis of the guanidinium hydrochlorides **40a-i** using 4M HCl/dioxane.



38a-f
39a-c

40a-i

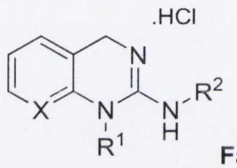
Compound	X	R^1	R^2	Yield (%)
40a	N	$-(CH_2)_2-CH_3$	$-CH_2-(C_6H_5)$	87
40b	N	$-(CH_2)_2-OH$	$-CH_2-(C_6H_5)$	74
40c	N	-piperonyl	$-CH_2-(C_6H_5)$	77
40d	N	$-(CH_2)_2-CH_3$	H	74
40e	CH	$-(CH_2)_2-CH_3$	$-CH_2-(C_6H_5)$	86
40f	CH	$-C_6H_5$	$-CH_2-(C_6H_5)$	85
40g	CH	-piperonyl	$-CH_2-(C_6H_5)$	88
40h	CH	$-(CH_2)_2-CH_3$	H	85
40i	CH	$-C_6H_5$	H	91

3.1.2.3. Pharmacological evaluation of compounds in Family B and B'

Binding Affinity to α_2-AR

The affinity of the substituted 2-amino-1,4-dihydroquinazoline and 2-amino-1,4-dihydropyrido[2,3-d]pyrimidine hydrochlorides (**40a-i**) toward the α_2-AR was measured, as in Family A, by *in vitro* competition assays using human brain frontal cortex tissue. The α_2-AR affinities are shown in Table 3.1.2.9 and are expressed in terms of pK_i values with RX821002 acting as the control ligand.

Table 3.1.2.9. Binding affinity to α_2-AR for compounds **40a-i**, expressed in terms of pK_i values.

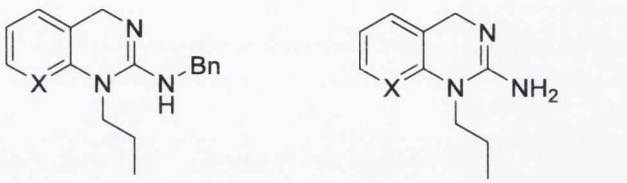


Family B, 40a-i

Compound	X	R^1	R^2	pK_i
RX821002	-	-	-	9.10
40a	N	$-(CH_2)_2-CH_3$	$-CH_2-(C_6H_5)$	5.05
40b	N	$-(CH_2)_2-OH$	$-CH_2-(C_6H_5)$	5.07
40c	N	-piperonyl	$-CH_2-(C_6H_5)$	5.15
40d	N	$-(CH_2)_2-CH_3$	H	4.99
40e	CH	$-(CH_2)_2-CH_3$	$-CH_2-(C_6H_5)$	5.29
40f	CH	$-C_6H_5$	$-CH_2-(C_6H_5)$	4.92
40g	CH	-piperonyl	$-CH_2-(C_6H_5)$	6.64
40h	CH	$-(CH_2)_2-CH_3$	H	5.11
40i	CH	$-C_6H_5$	H	5.85

Although it is primarily noted that the binding affinities for Families B and B' are not extremely high, these values illustrate some interesting features which are important for developing a concise structural activity relationship (SAR) for this series. The first notable

observation is the difference in α_2-AR binding between the phenyl and pyridine derivatives, with the pyridine analogues giving lower affinities in all cases. This is in complete agreement with the results obtained for Family A (Section 3.1.1.4). Secondly and surprisingly, the benzyl analogues (Family B'), which were tested only as intermediates to the real drug, gave higher binding affinities that their corresponding *mono*-substituted counterparts (Family B), for both the phenyl and pyridine derivatives (Figure 3.1.2.5).



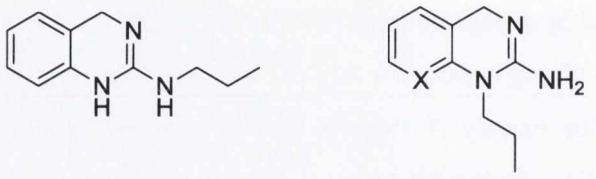
40a X = N, pK_i = 5.05
40e X = CH, pK_i = 5.29

40d X = N, pK_i = 4.99
40h X = CH, pK_i = 5.11

Fig. 3.1.2.5. Comparison of α_2-AR pK_i values between benzylguanidines **40a** and **40e** and de-benzylated derivatives **40d** and **40h**.

In the case of compound **40g**, the piperonyl substituent has again prominently given the highest α_2-AR binding affinity. Encouragingly, compound **40g**'s affinity (pK_i = 6.64) was shown to not only surpass all previous cyclic derivatives but also all previously developed acyclic di-substituted phenyl guanidines and on average most molecules of the acyclic *mono*-/di-substituted pyridoguanidine series.[162]

Lastly and most satisfactory, was to find that the derivatives synthesised for Family B gave significantly enhanced α_2-AR binding affinities compared to the previously developed original family of *N*-substituted 2-amino-1,4-dihydroquinazolines (Family O, **41**), even in the case of the pyridine analogues of 1,4-dihydropyrido[2,3-d]pyrimidines (Figure 3.1.2.6). Thus, the original design of Family B, that consisted in changing the R substitution from the exocyclic amino to the interior one of the cyclic guanidine for an optimal interaction with the α_2-AR receptor is adequately confirmed by the pharmacological results. This structural modification is now an important feature to be taken into account in the design of novel guanidine-type α_2-AR ligands (cyclic or acyclic).



41 $pK_i = 4.33$

40d X=N, $pK_i = 4.99$
40h X=CH, $pK_i = 5.11$

Fig. 3.1.2.6. Comparison of α_2-AR binding affinities between previously prepared 2-amino-1,4-dihydroquinazoline (**41** from Family O) and Family B (**40d** and **40h**).

Functional activity

Compound **40g** displayed a α_2-AR affinity greater than $pK_i = 6.5$ and, thus, was subjected to *in vitro* [^{35}S]GTPγS binding experiments to determine its nature as agonist or antagonist. Compound **40g** showed no stimulation of [^{35}S]GTPγS binding which confirms the predicted antagonist activity of this conformationally restricted molecule (Figure 3.1.2.7).

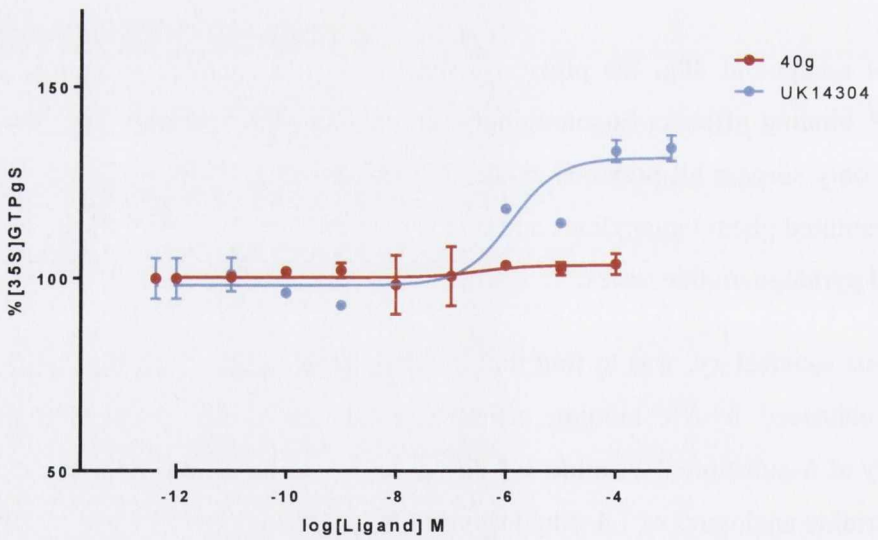


Fig. 3.1.2.7. [^{35}S]GTPγS binding assay curve of agonist UK14304 and antagonist **40g**.

3.1.3. Family C: *N*-substituted-2-amino-4,5-dihydro-1,3-benzodiazepines

3.1.3.1. Design

As previously mentioned, the low α_2-AR affinity results obtained for the methylene linked cyclic guanidine systems (Family O, Family A and Family B) is thought to be attributed to the conformational constriction, preventing substituents to be positioned correctly for optimal interactions within the receptor. While altering the substitution pattern in Family B was one possible approach to consider, it was also thought that a certain degree of movement of substituents R could be necessary to achieve higher binding affinities. Thus, Family C was designed to increase the ring size by adding a CH_2 in order to recover some flexibility allowing freedom of movement of the substituents within the binding pocket while still retaining some conformational constrain (Figure 3.1.3.1). These 2-amino-4,5-dihydro-1,3-benzodiazepines will include *N*-substituents on the exocyclic amino group of the guanidine (R^1), as in Family A, as well as substituents in the aryl ring in *para* position to the cationic moiety (R^2), which have augmented α_2-AR affinity and activity in previous compounds (Figure 3.1.3.1).

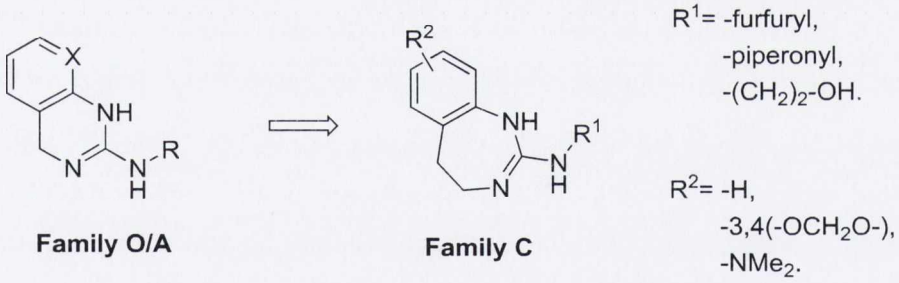
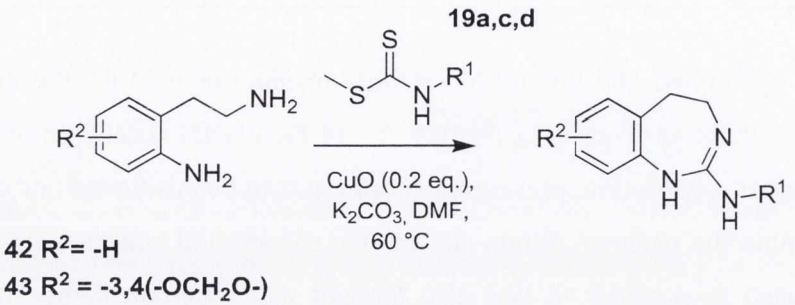


Fig. 3.1.3.1. Proposed designed structure for Family C compounds.

3.1.3.2. Synthesis of compounds in Family C

The synthesis towards the *N*-substituted-2-amino-4,5-dihydro-1,3-benzodiazepines followed an analogous procedure to that of Family A, described by Das *et al.*[185] This method was only performed using the unsubstituted phenyl and the piperonyl diamines **42** and **43** with the appropriate dithiocarbamates **19a,c,d** (Scheme 3.1.3.1), as the dimethylamino derivatives were synthesised in an alternate manner which will be described later in this section.

Scheme 3.1.3.1.



42 R^2= -H

43 R^2 = -3,4(-OCH_2O-)

Similarly to previous families the diamines had to be prepared prior to cyclisation from available starting materials.

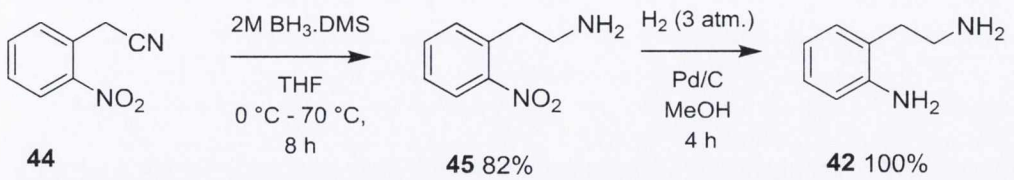
*Synthesis of substituted aryl diamines **42** and **43***

The preparation of diamine **42** is described using a variety of quite dated but efficient procedures starting from *o*-nitrophenylacetonitrile **44**. The first involves an attractive dual reduction of nitrile and nitro from a modification of the Kornblum and Iffland procedure,[214] *via* catalytic hydrogenation using platinum oxide to give 37% yield of product.[215] Towards a more effective method without the use of highly expensive PtO, Jen *et al.*'s procedure involves a selective reduction of nitrile using a 1M BH_3-THF solution followed by catalytic hydrogenation of the crude mixture to give 43% overall yield.[216] More recently, Hah *et al.* have improved this synthesis by similarly reducing the nitrile with borane followed by nitro reduction using tin (II) chloride to give the product in 53% overall yield.[217]

Dual reduction was primarily attempted with Pd/C using high pressures of hydrogen (up to 12 atm), yet poor yields and mixtures of polar products were obtained. Thus, a sequential approach was taken by reducing one functional group at a time using optimised conditions of the methods previously explained. We found that the best conditions for nitrile reduction were to reflux *o*-nitrophenylacetonitrile **44** in a 2M THF solution of BH_3.DMS for 8 h affording compound **45** in 82% after purification (Scheme 3.1.3.2).[218] Due to the polarity of **42**, reduction with tin (II) chloride was avoided due to the requirement of an aqueous work-up, thus catalytic hydrogenation was employed using Pd/C at 3 atm, which gave the desired

product **42** in quantitative yield. This represents a significant improvement in over two steps compared to the methods described in the literature.

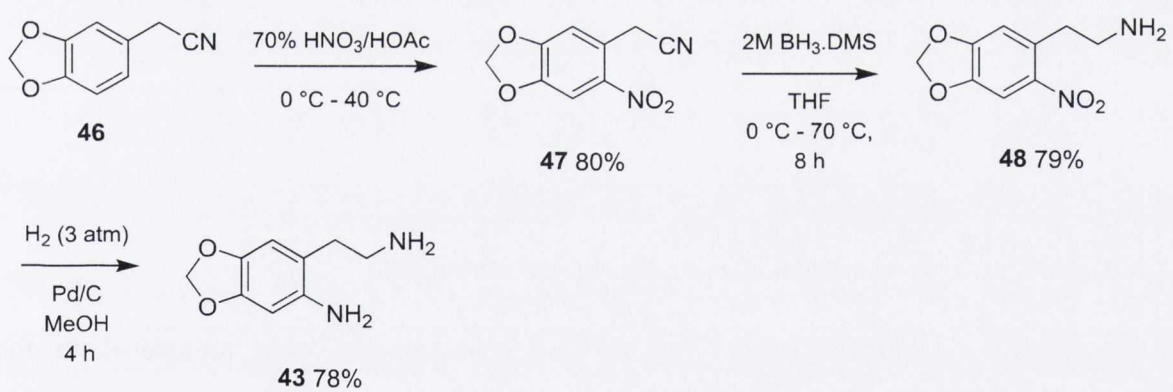
Scheme 3.1.3.2.



44 **45** 82% **42** 100%

The synthetic pathway towards diamine **43** followed the same procedure described in the synthesis of **42** after initial nitration of cyanopiperonyl **46** using 70% nitric acid in AcOH (where the moderately activating $-OCH_2O-$ directed nitration to the *para* position) to give **47** exclusively in 80% yield (Scheme 3.1.3.3).[219]

Scheme 3.1.3.3.



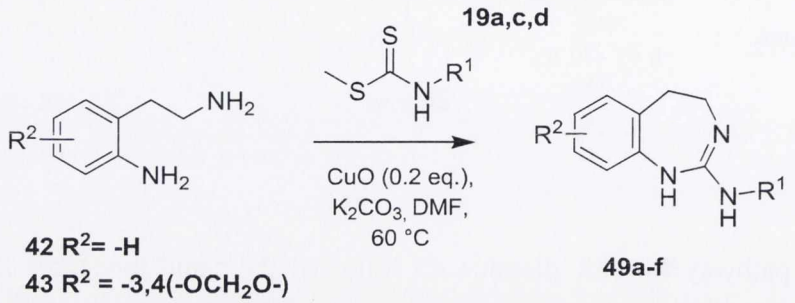
46 **47** 80% **48** 79% **43** 78%

Cyclisation to the N-substituted 2-amino-4,5-dihydro-1,3-benzodiazepines

As mentioned, cyclisation followed the copper (II) oxide mediated cyclodesulphurisation as seen in the preparation of Family A, which gave modest yields of the substituted 2-amino-4,5-dihydro-1,3-benzodiazepines as presented in Table 3.1.3.1. It is important to note that the low yields shown for compounds **49d-f** are due to their highly insoluble nature what made

separation extremely difficult from the similarly insoluble diamine and dithiocarbamate. Hence, the overall low yields of these derivatives were the result of difficulties in the purification process not issues with the reaction itself.

Table 3.1.3.1. Synthesis of the *N*-substituted-2-amino-4,5-dihydro-1,3-benzodiazepines.



42 R^2= -H
43 R^2 = -3,4(-OCH_2O-)

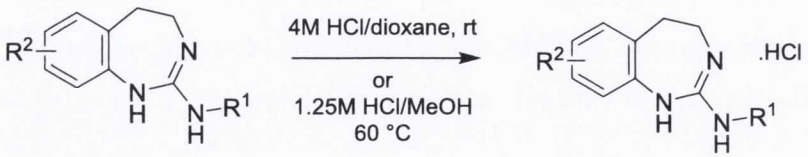
49a-f

Compound	R^1	R^2	Yield (%)
49a	furfuryl	H	52
49b	-$(CH_2)_2$-OAc	H	60
49c	-piperonyl	H	69
49d	furfuryl	-3,4(-OCH_2O-)	23
49e	-$(CH_2)_2$-OAc	-3,4(-OCH_2O-)	19
49f	-piperonyl	-3,4(-OCH_2O-)	26

Protonation to the guanidinium hydrochlorides of Family C

Most of the neutral 2-amino-4,5-dihydro-1,3-benzodiazepines were protonated to the hydrochloride salts without any difficulty and in good yield following the procedure described in Section 3.1.1.3 (Table 3.1.3.2). However, attempts at protonating the highly problematic compound **49f** lead to a residue that was insoluble in all solvents, including DMF and DMSO. It was thought that the two piperonyl moieties of the molecule were creating an extremely unorganised structure that could not be characterised, purified or pharmacologically tested due to the poor solubility induced by its complex nature.

Table 3.1.3.2. Synthesis of the 2-amino-4,5-dihydro-1,3-benzodiazepines hydrochlorides.

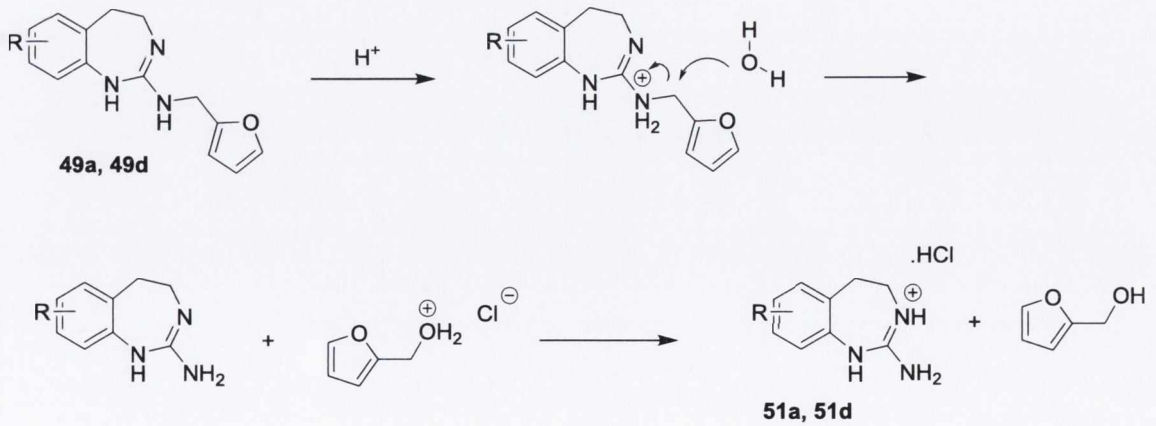


49a-f **50a-e**

Compound	R^1	R^2	Yield (%)
50a	furfuryl	H	-
50b	$-(CH_2)_2-OH$	H	92
50c	piperonyl	H	80
50d	furfuryl	$-3,4(-OCH_2O-)$	-
50e	$-(CH_2)_2-OH$	$-3,4(-OCH_2O-)$	96
-	piperonyl	$-3,4(-OCH_2O-)$	-

Moreover, on exposure to acidic conditions degradation was observed for the furfuryl derivatives (**49a** and **49d**). In this case it was thought that the substituted exocyclic amino group was being protonated facilitating nucleophilic addition to the now highly electrophilic methylene group affording the cleaved product and possibly furfuryl alcohol (Scheme 3.1.3.4).

Scheme 3.1.3.4.



49a, 49d **51a, 51d**

The cleaved salt **51a** precipitated out of solution and both ^{1}H-NMR and HRMS spectra confirmed the cleavage product (Figure 3.1.3.2). In an attempt to identify the other moiety resulting from the cleavage, the ^{1}H-NMR of the filtrate was recorded suggesting a polymeric product; this could be due to furfuryl alcohol being known to polymerise under acidic conditions.

Despite many optimisation reactions being performed (altering solvent, acid concentration and temperature) and even under anhydrous conditions, these degradation products were always formed. It was found that diluting the starting guanidine in anhydrous ether (0.1M) at 0 °C and adding 1 eq. of a 1M HCl/ether solution dropwise gave predominately starting material with ~5% conversion of the desired product. If higher concentrations of acid and elevated temperatures were applied, formation of the cleavage product was observed. Disappointingly, after obtaining the desired products **50a** and **50d**, degradation was observed in water over time, and hence these derivatives had to be excluded from pharmacological testing. Nonetheless, these experiments gave us a clear ^{1}H-NMR spectrum of the desired product **50a** for comparison and proof of mechanism against the cleaved guanidine **51a** (Figure 3.1.3.2).

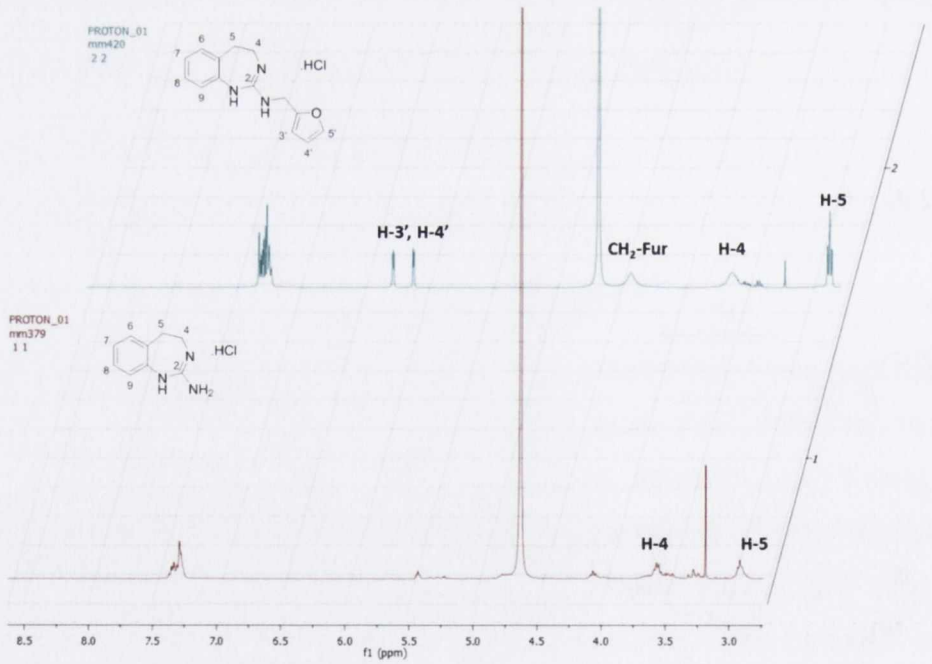
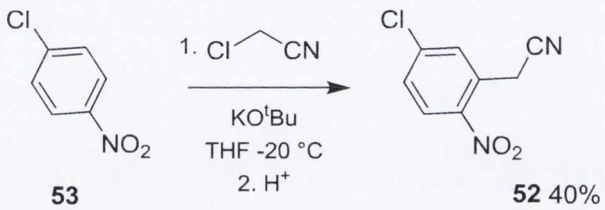


Fig. 3.1.3.2. ^{1}H-NMR spectra in D_2O comparing the desired product **50a** (*top*) and degradation product **51a** (*bottom*).

Synthesis of N-substituted-7-dimethylamino-4,5-dihydro-1,3-benzodiazepines

In the first step towards the synthesis of the *N*-substituted-7-dimethylamino-4,5-dihydro-1,3-benzodiazepines, the phenylacetonitile **52** had to be prepared by the reaction of 1-chloro-4-nitrobenzene **53** with chloroacetonitile.[220] The reaction was performed at -20 °C under basic conditions with subsequent quenching with dilute sulphuric acid (Scheme 3.1.3.5).

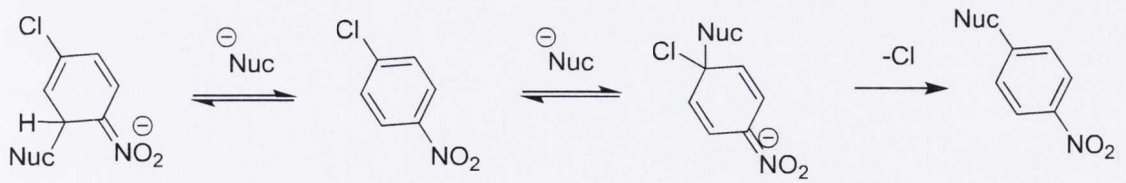
Scheme 3.1.3.5.



53 **52** 40%

In similar reactions with halo *mono*-nitroarenes, nucleophilic addition to the electron deficient *ortho* or *para* positions to give the corresponding sigma complexes I and II would be expected (Scheme 3.1.3.6). Upon attack in the *para* position (complex I), the chlorine anion can subsequently leave to give the substitution product (*right*), while for the *ortho* addition the hydride anion is a poor leaving group and therefore the sigma complex II dissociates back to the starting molecule.[221]

Scheme 3.1.3.6.

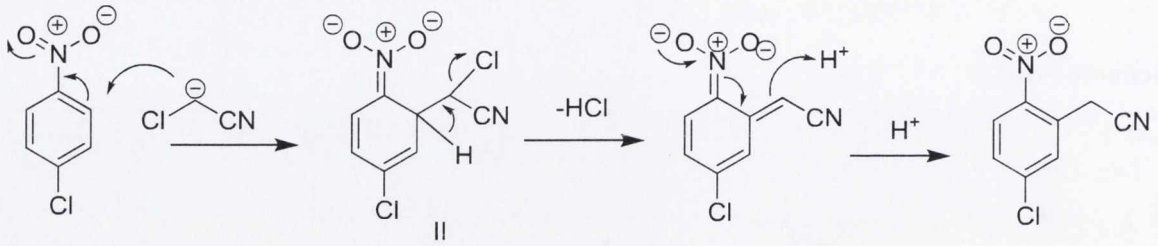


II I

However, in the case of the 1-chloro-4-nitrobenzene reaction with chloroacetonitile the attacking nucleophile contains a substituent that is able to leave as an anion. Thus, the sigma

complex II reacts further *via* the migration of the hydride anion from the aromatic carbon to the central carbon atom of the nucleophile eliminating HCl with subsequent product formation by acid-catalysed re-aromatisation (Scheme 3.1.3.7).[222]

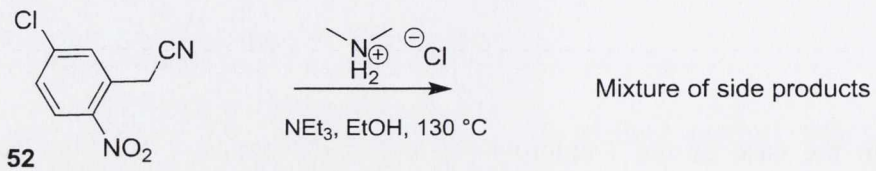
Scheme 3.1.3.7.



This nucleophilic replacement of hydrogen with such carbanion proceeds faster than the substitution of halogens *para* to the nitro group, and hence the reaction is quenched immediately after the addition of base since longer reaction times led to a mixture of substitution products.

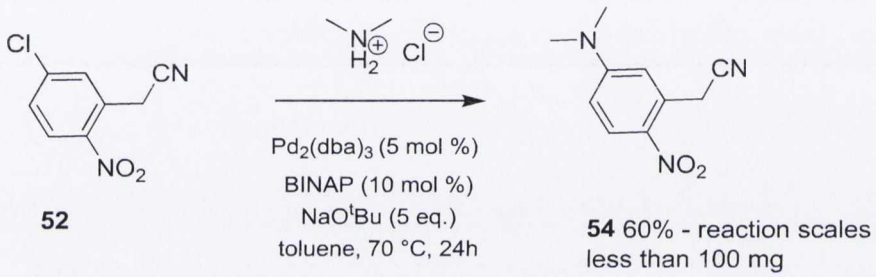
After the synthesis of 2-(5-chloro-2-nitrophenyl)acetonitrile our attention turned to installing the required dimethylamino moiety *via* nucleophilic aromatic substitution with dimethylammonium chloride and excess of base at high temperatures.[223] Unfortunately, under these conditions many side reactions occurred due to nucleophilic addition of the amine to the nitrile affording amidine side products (Scheme 3.1.3.8); this has been described in the literature for such nitriles using hydroxylamine hydrochloride as the nucleophilic component.[224]

Scheme 3.1.3.8.



Thus, our synthetic efforts moved to the use of the formerly reliable Buchwald-Hartwig chemistry. Li *et al.* have recently developed a methodology for the palladium catalysed amination of arylbromines with highly volatile amines, such as dimethylamine (bp 7 °C).[225] This procedure works in good yields for a variety of volatile amines due to the use of sealed pressure tubes, and has been utilised using dimethylammonium chloride with arylbromides using excess NaO^tBu and bidentate phosphine ligands. We decided to extend this methodology to arylchlorines like **52**, and found that the best conditions were using $Pd_2(dba)_3$ (5 mol%), BINAP (10 mol%), and NaO^tBu (5 eq.) in dry toluene at 70 °C in a pressure tube for 24 h (Scheme 3.1.3.9).

Scheme 3.1.3.9.

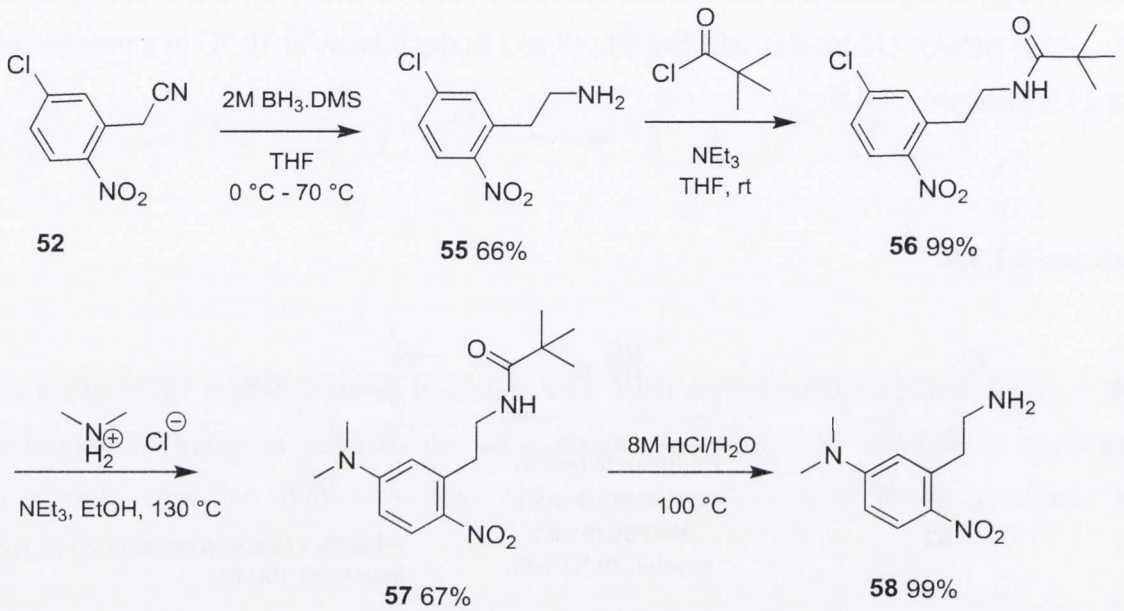


These conditions gave good yields of compound **54** (~60%) on small scales up to 100 mg; however, reaction scales above this amount lead to disastrously sluggish reactions with only degradation products observed. Optimisation of the scale up was attempted but without any successful result. It was thought that the amount of solid base required was affecting the reaction as it was somewhat obstructing and congesting the solution from stirring in the pressure tube. Yet, when a lesser amount of base was used extremely poor yields were obtained, thus this strategy was abandoned for a more reliable route.

The nitrile was introduced to act as an amino precursor, hence it was decided to reduce it using borane as described in the literature,[216] with subsequent protection as the pivaloyl amide.[226] The reduction was carried out gave the desired amine **55** in good yield. The protection step, using pivaloyl chloride, gave the amide **56** in quantitate yield, and lead to no side reactions when next subjected to nucleophilic substitution with dimethylammonium chloride to give compound **57**. This was expected as these amides are known to be stable

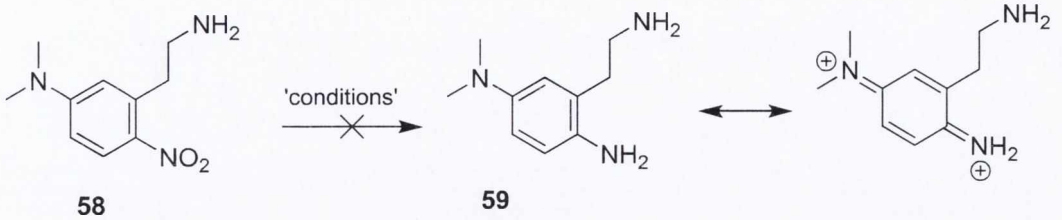
under weakly basic conditions. Subsequently, after a small optimisation, acid catalysed amide hydrolysis using 8M HCl/H_2O gave the appropriate amine **58** in quantitative yield (Scheme 3.1.3.10).[227]

Scheme 3.1.3.10.



With compound **58** now prepared, the reduction of the nitro functionality was attempted; however, attempts to prepare triamine **59** under catalytic hydrogenation gave a mixture of highly polar products impossible to characterise. Moreover, various attempts using metal reductions resulted in unsuccessful separation of **59** from the metal salts in the aqueous phase. The high polarity of compound **59** is thought to be due to a combination of both the aliphatic amine and amino-iminium resonance species (Scheme 3.1.3.11).

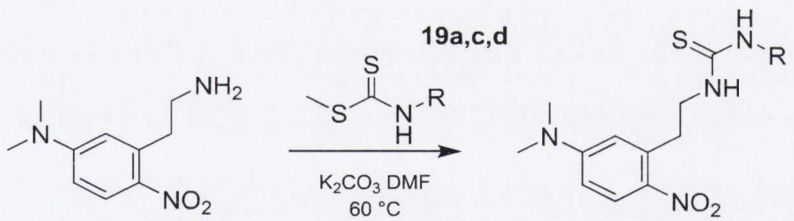
Scheme 3.1.3.11.



58 **59**

Since the polarity of triamine **59** was problematic, we decided to create a better synthetic intermediate by first reacting amine **58** with the *N*-substituted dithiocarbamates **19a, c, d** (Table 3.1.3.3). This would form the thiourea intermediates prior to nitro reduction, thus increasing the lipophilicity of the molecules to better control the issues regarding reactivity, solubility, and purity.

Table 3.1.3.3. Synthesis of thiourea derivatives **60a-c** from the appropriate *N*-substituted dithiocarbamates.

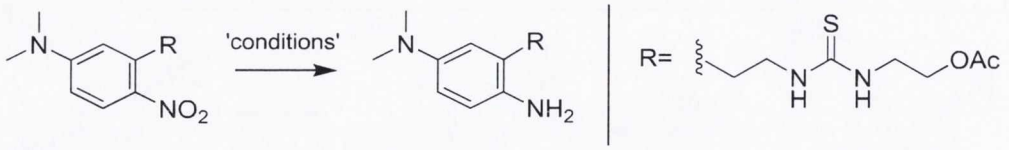


58 **60a-c**

Compounds	R	Yield (%)
60a	furfuryl	55
60b	-$(CH_2)_2$-OAc	59
60c	piperonyl	75

Next, reduction of the nitro group was tested under a variety of single electron transfer metal reductions as catalytic hydrogenation could not be employed due to the sensitivity of certain functional groups present in these molecules (Table 3.1.3.4).

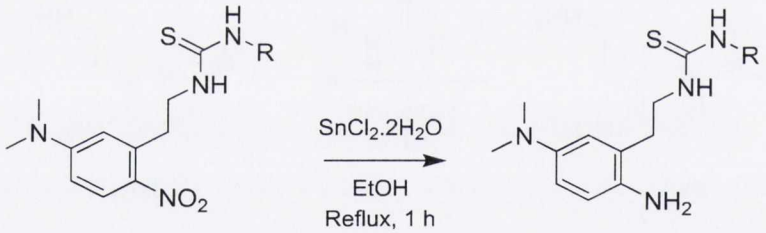
Table 3.1.3.4. Conditions screened towards arylnitro reduction.



Entry	Conditions	Yield (%)
1	Zn	56
2	Fe, $CaCl_2$	13
3	$Na_2S.9H_2O$	58
4	$SnCl_2.2H_2O$	92

It was found that refluxing the appropriate nitro derivative in ethanol with excess tin (II) chloride dihydrate for 1 h gave excellent yields (entry 4, Table 3.1.3.4), and thus these conditions were used with the rest of the compounds without further need for purification by column chromatography (Table 3.1.3.5).[228]

Table 3.1.3.5. Reduction conditions for the synthesis of anilines **61a-c.**



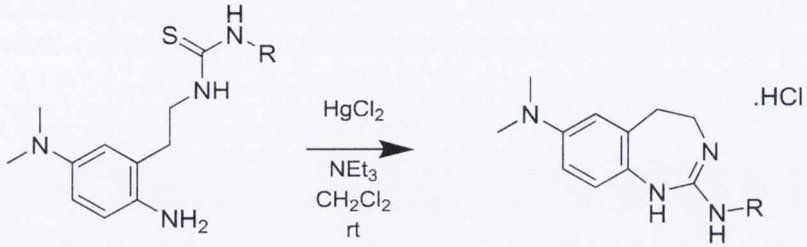
60a-c **61a-c**

Compounds	R	Yield (%)
61a	furfuryl	97
61b	$-(CH_2)_2$-OAc	92
61c	piperonyl	98

Cyclisation was facilitated again by using mercury (II) chloride, resulting in good yields of the *N*-substituted-7-dimethylamine-4,5-dihydro-1,3-benzodiazepines (Table 3.1.3.6).

Similarly to Family B', the guanidines were obtained as the corresponding hydrochloride salts *in situ* due to their high basicity. Thankfully, the extra CH_2 present in the 7-membered ring of these molecules added some lipophilicity, and therefore these compounds could be extracted from the aqueous layer as the guanidinium salts using mixtures of isopropanol and chloroform, without the need for a base wash and re-protonation.

Table 3.1.3.6. Synthesis of the 2-amino-4,5-dihydro-1,3-benzodiazepines hydrochlorides

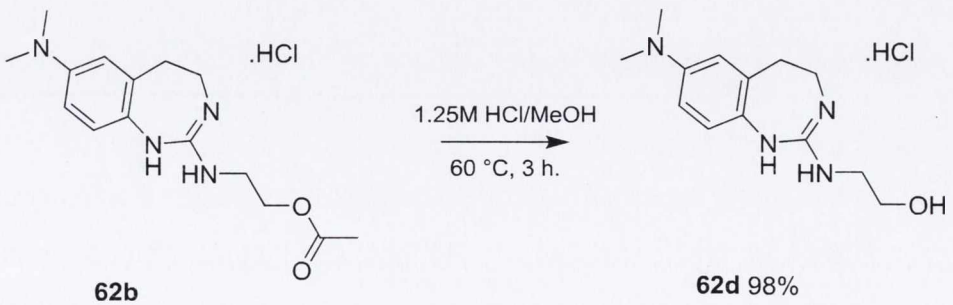


61a-c **62a-c**

Compounds	R	Yield (%)
62a	furfuryl	90
62b	-$(CH_2)_2$-OAc	79
62c	piperonyl	66

Compound **62b** was deprotected in an analogous manner to compound **23b** under acidic conditions using a 1.25M solution of HCl/MeOH yielding **62d** in 98% (Scheme 3.1.3.12).

Scheme 3.1.3.12.



62b **62d** 98%

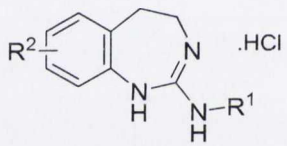
Purification of all *N*-substituted-2-amino-4,5-dihydro-1,3-benzodiazepines was achieved using small scale reverse phase chromatography with C-8 silica using 100% water as mobile phase, and their purity was assessed using reverse phase HPLC.

3.1.3.3. Pharmacological evaluation of compounds in Family C

Binding Affinity to α_2-AR

The α_2-AR binding affinities for compounds in Family C were measured, in the same manner previously described for all other families, by *in vitro* competition assays in human prefrontal cortex tissue and the results are shown in Table 3.1.3.7.

Table 3.1.3.7. Binding affinity to α_2-AR for compounds in Family C, expressed as pK_i values.



Family C, 50b-c, 50e, 62a, 62c-d

Compound	R^1	R^2	pK_i
RX821002	-	-	9.10
50b	$-(CH_2)_2$-OH	H	5.81
50c	-piperonyl	H	5.57-6.45
50e	$-(CH_2)_2$-OH	-4,5-(-OCH_2O-)	5.90
62d	$-(CH_2)_2$-OH	-4-NMe_2	5.32
62c	piperonyl	-4-NMe_2	6.14
62a	furfuryl	-4-NMe_2	5.36

The α_2-AR binding affinity results for Family C show no major trends; it is important to note however that, in general, these compounds do exhibit higher binding affinities throughout the entire series than the 6-membered cyclic guanidine analogues (Families O, A and B). Most of

the previously developed 2-amino-1,4-dihydroquinazolines (Family O) and Families A-B show affinities in the area of pK_i = ~5 (except for one exception), whilst most of the 7-membered cyclic guanidine derivatives (Family C) achieve affinities near pK_i = ~6. This shows that the extra CH_2 within the ring system is allowing some alleviation of constrain for these derivatives, and potentially gives the system extra flexibility within the α_2-AR binding pocket to attain optimum interactions. Interestingly, in the case of compound **50c**, the data obtained from the binding assay fitted best to a biphasic curve, where two pK_i values were calculated from the high and low affinity binding curves (Figure 3.1.3.3). It is thought that compounds displaying a biphasic binding curve are illustrating subtype selectivity. The α_{2A}-AR and α_{2C}-AR subtypes are located predominantly in the CNS where compound **50c** may have a higher affinity for one subtype over the other leading to high and low affinity binding curves.

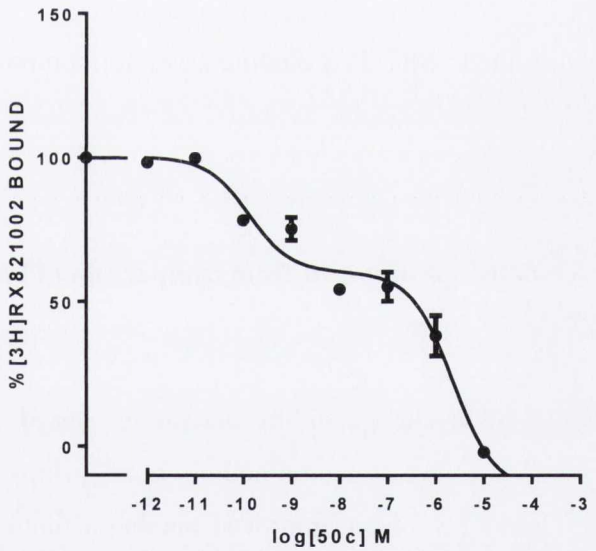


Fig. 3.1.3.3. Biphasic α_2-AR binding curve obtained for compound **50c**.

Functional activity

Only the biphasic binding compound **50c** displayed an α_2-AR affinity greater than pK_i = 6, and was hence subjected to *in vitro* [^{35}S]GTPγS binding assays. As predicted with all cyclic

conformationally restricted derivatives, **50c** showed no stimulation of [^{35}S]GTPγS binding, confirming desired antagonist or inverse agonist activity (Figure 3.1.3.4).

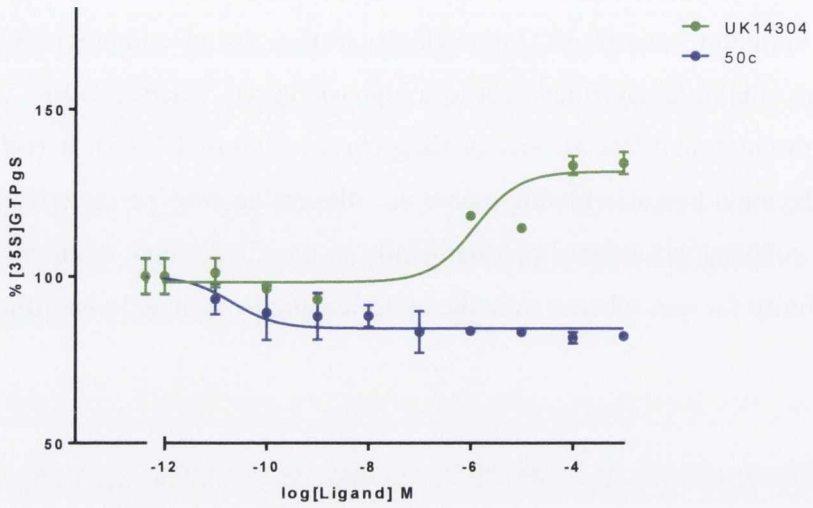


Fig. 3.1.3.4. Results obtained in the [^{35}S]GTPγS binding assay for compound **50c** indicating antagonist activity.

3.1.4. Structure-Activity Relationships obtained from comparison of biological results across Families O, A, B, and C

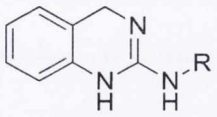
Since, as previously predicted, all cyclic guanidine derivatives tested were found to be antagonists, we developed comprehensive structural activity relationships to determine what exact structural features are essential to obtain improved binding affinities compared to the previously developed 2-amino-1,4-dihydroquinazolines (Family O).

From the results obtained for Families A and B it is evident that introduction of the pyridine ring has detrimental effects on the overall affinity compared to the corresponding phenyl analogues. It was initially thought that the pyridine ring may facilitate additional interactions at the receptor through its hydrogen bonding accepting ability, as many of the acyclic pyridoguanidine derivatives gave improved affinities compare to their phenyl counterparts. Nonetheless, in the case of the cyclic series this is not observed, and for future development

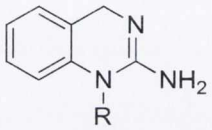
and design of similar compounds a bioisosteric change from benzene to pyridine should avoided since it is not required to improve affinity or activity.

The variation in binding affinities across the set of *N*-substituted 2-amino-1,4-dihydroquinazoline hydrochlorides can be explained in terms of the alteration in substitution positions. Table 3.1.4.1 summarises a few examples of the binding affinities across the three families of *N*-substituted 2-amino-1,4-dihydroquinazolines.

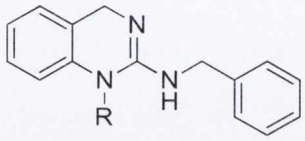
Table 3.1.4.1. Comparison of the pK_i values across Families O, B and B'.



Family O



Family B



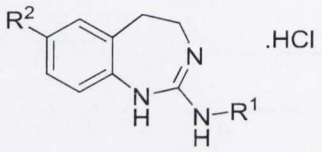
Family B'

Family	R	pK_i
O	$-(CH_2)_2-CH_3$	4.33
O	$-C_6H_5$	4.40
B	$-(CH_2)_2-CH_3$	5.11
B	$-C_6H_5$	5.85
B'	$-(CH_2)_2-CH_3$	5.29
B'	$-C_6H_5$	4.99

Significantly, from Table 3.1.4.1 it is clear that when the R substituent is on the intracyclic amine of the guanidine (Family B) as opposed to the exocyclic amine (Family O), higher binding affinities are obtained. This supports our original hypothesis that the substituents of the initial conformationally restricted guanidines (Family O) are incorrectly orientated to give optimum interactions. The di-substituted cyclic derivatives (Family B') also give enhanced affinities compared to Families O and B, suggesting that the aromatic moiety of the benzyl substituent may be positioned in an alternate hydrophobic pocket to give additional interactions. This implies that di-substitution of the guanidine core may be optimal for higher binding to the α_2-AR.

The 2-amino-4,5-dihydro-1,3-benzodiazepines (Family C), as previously mentioned (Section 3.1.3.3), gave overall higher binding affinities compared to their 6-membered cyclic analogues, which is attributed to their added flexibility. It is notable that the R^2 substitution, that was introduced in the *para* position to the cationic moiety, and that in previous acyclic compounds gave improved results, did not show any significant difference in α_2-AR binding affinities compared to the unsubstituted derivatives (Table 3.1.4.2). Due to the added synthetic steps to prepare such intermediates this structural feature should be exempt from future design of cyclic guanidines.

Table 3.1.4.2. Comparing the differences in α_2-AR pK_i values caused by substituent R^2 within Family C.



Compound	R^1	R^2	pK_i
50b	$-(CH_2)_2$-OH	H	5.81
62d	$-(CH_2)_2$-OH	$-NMe_2$	5.22
50e	$-(CH_2)_2$-OH	-4,5-(-OCH_2O-)	5.90
50c	-piperonyl	H	6.57
62c	-piperonyl	$-NMe_2$	6.14

All the information gained from the pharmacological studies performed on the cyclic guanidines prepared, regarding the structural requirements to improve the α_2-AR affinity can be summarised in the scheme shown in Figure 3.1.4.1.

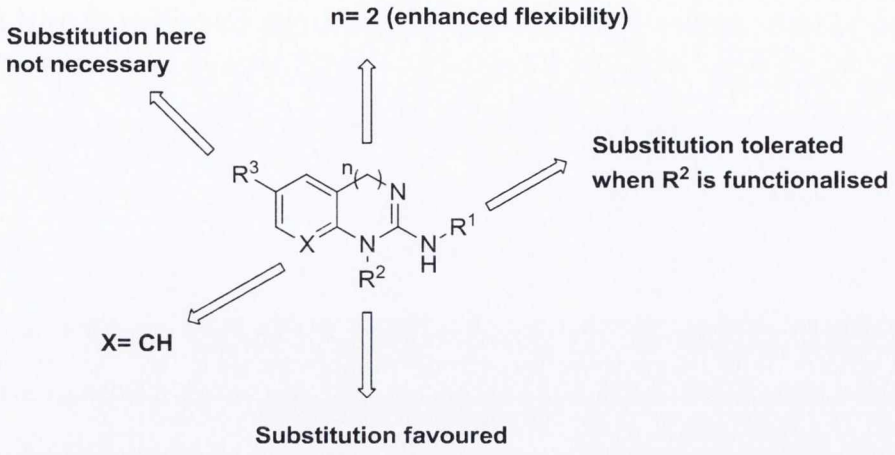


Fig. 3.1.4.1. Structure-activity relationships obtained for the conformationally restricted guanidine series of α_2-AR antagonists.

3.2. *Bis*-Aryl guanidine and 2-aminoimidazolines: Families D and E

3.2.1. Families D1 and D2: *bis*-(*N,N'*-di-substituted guanidine) and *bis*-[*N,N'*-di-(2-aminoimidazoline)] diaryl derivatives

3.2.1.1. Design

To date no ligand of our design has surpassed the α_2-AR binding affinity of our initial lead *bis*-2-aminoimidazoline **1** (pK_i = 8.80) and considering that one of the objectives of this work was to prepare high affinity antagonists of the α_2-AR, we focused our attention in *bis*-aryl guanidine-like derivatives to improve their α_2-AR affinity. As seen in Section 1.6 many structurally related dimeric *bis*-aryl guanidine and *bis*-aryl 2-aminoimidazoline analogues of **1** had been developed in Rozas' laboratory with varying linkers (NH, CO, O, CH_2, SO_2); however, none exceeded the affinity of lead **1** and again most displayed disfavoured agonist activity.

In recent years, we have gained further insight into what structural features are important for α_2-AR antagonism through a comparative molecular field analysis (CoMFA) study. CoMFA is a 3D QSAR method used to derive correlations between the biological activity of a set of molecules and their 3D shape, electrostatic and hydrogen bonding characteristics; these correlations are derived from a series of superimposed conformations, one for each molecule in the set. In Rozas' laboratory a CoMFA study was carried out using a diverse range of α_2-AR antagonists both from the literature as well as over several compounds previously developed within our laboratory (Figure 3.2.1.1). Molecular fields are calculated around each molecule and its conformation providing a three-dimensional picture of the electrostatic and steric features which correlate to favoured and disfavoured activity. This 3D pharmacophore highlighted that hydrophobic steric extension at the cationic moiety (HY1), creating *N,N'*-di-substituted guanidines, should display favourable α_2-AR antagonist activity (Figure 3.2.1.1).[161]

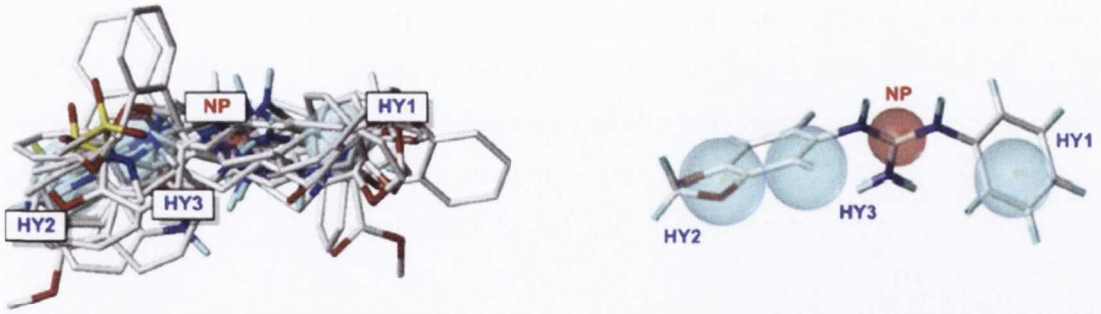
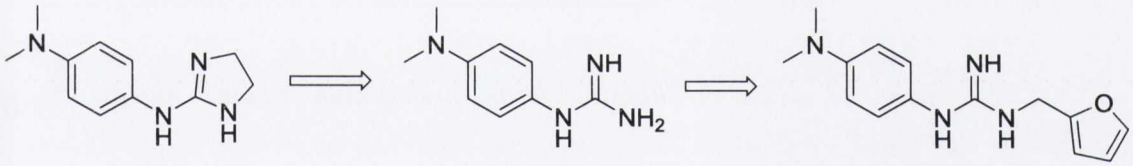


Fig. 3.2.1.1. Superimposed conformations of α_2-AR antagonists (*left*) and 3D pharmacophore obtained for *N,N'*-di-substituted guanidine analogues (*right*).

Indeed, these *N,N'*-di-substituted guanidines showed preference for antagonism, however, it was notable that the α_2-AR binding affinity of these molecules was significantly less than their *mono*-substituted guanidine and 2-aminoimidazoline analogues (Figure 3.2.1.2)



6 pK_i 7.42 **63** pK_i 7.02 **64** pK_i 6.67

Fig. 3.2.1.2. Comparison of α_2-AR pK_i values between agonist compounds **6** and **63**, and antagonist compound **64**.

Accordingly, we chose to incorporate this functional modification into the higher affinity 'twin' molecules to develop a new series of *bis*-aryl *N,N'*-di-substituted guanidines, Family D1 (Figure 3.2.1.3), that will hopefully show α_2-AR antagonist activity with high α_2-AR affinity. Moreover, since it has been previously established that the 2-aminoimidazoline derivatives exhibit higher α_2-AR binding affinities (Section 1.6), and since we know now that the *N,N'*-di-substituted guanidines favour α_2-AR antagonist activity, we decided to combine these two structural features to create asymmetric *bis*-aryl guanidine and 2-

aminoimidazolines, Family D2 (Figure 3.2.1.3). It is predicted that these structural modifications may inverse the functional activity of lead compound **1** while retaining high α_2-AR binding affinity. These molecules will be synthesised with both methylene and ethylene linkers to probe if flexibility in this position is important, as α_2-AR antagonist activity has been obtained for previously prepared ethylene linked *bis*-aryl molecules.[155]

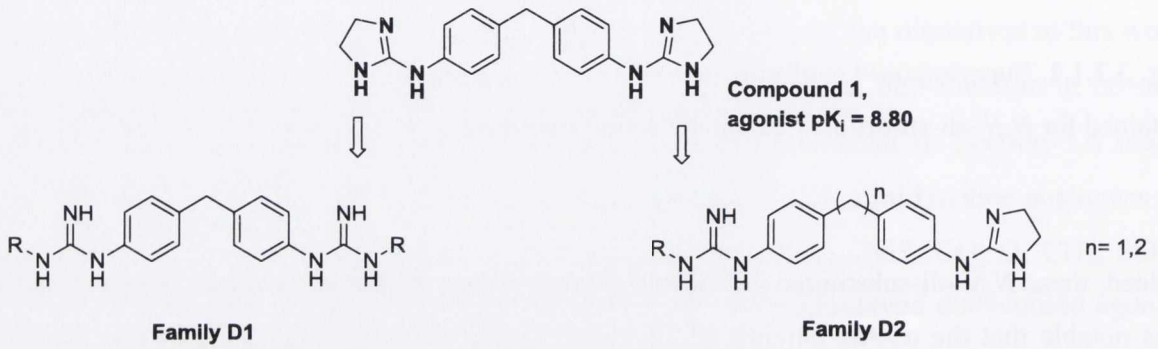


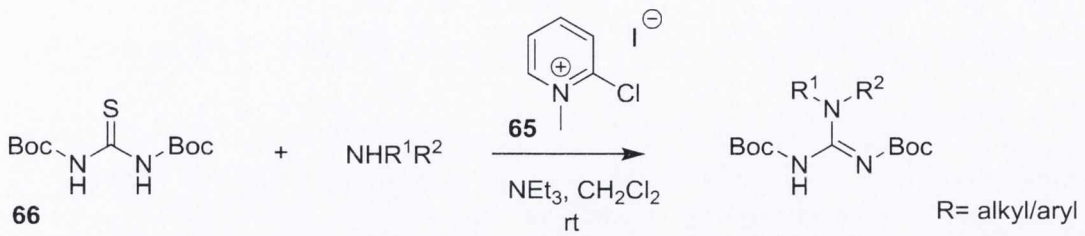
Fig. 3.2.1.3. Proposed structures of Families D1 and D2 from modification of the existing high α_2-AR affinity and agonist lead compound **1**.

3.2.1.2. Synthetic methods found in the literature for the preparation of mono- and di-substituted guanidines

Thioureas and isothioureas are the most common reagents for the synthesis of guanidines through the use of specific sulphur activating agents. In particular the use of mercury (II) chloride as a promotor for guanidylation is the most frequently employed reagent and will be discussed in detail in Section 3.2.1.3 due to its relevance to this research.

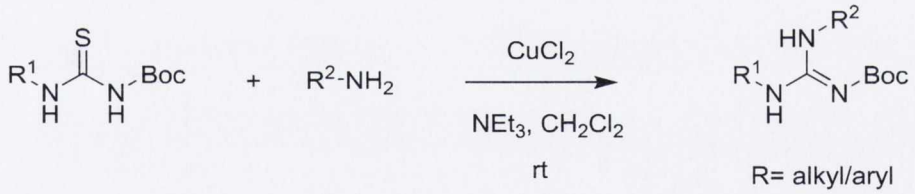
For the synthesis of *mono*-substituted guanidines, 1-methyl-2-chloropyridinium iodide **65** (Mukaiyama's reagent) is regularly used as a promotor for nucleophilic addition of unreactive and hindered aryl amines to thioureas (Scheme 3.2.1.1).[229] In this case the thiourea is often substituted with electron withdrawing Boc groups (compound **66**) to accelerate the reaction through initial formation of a highly electrophilic and shortly lived *bis*-Boc carbodiimide intermediate. The resulting product can then be easily deprotected under acidic conditions.

Scheme 3.2.1.1.



Unfortunately, this reaction is restricted to *mono*-substituted guanidines since reasonable yields require the use of *bis*-Boc protected thioureas. Recently, our group has published a concise and broadly applicable method for the synthesis of *N,N'*-di-substituted guanidines through the coupling of aryl amines with *N*-(*tert*-butxoycarbonyl)-*N'*-alkyl/aryl substituted thioureas in the presences of copper (II) chloride (Scheme 3.2.1.2).[230] This method was developed to reduce the use of the commonly employed mercury (II) chloride mediated guanidylation reactions. Indeed, yields were almost on a par with typical promotors of desulphurisation, and encouragingly avoided the use of more toxic and expensive reagents.

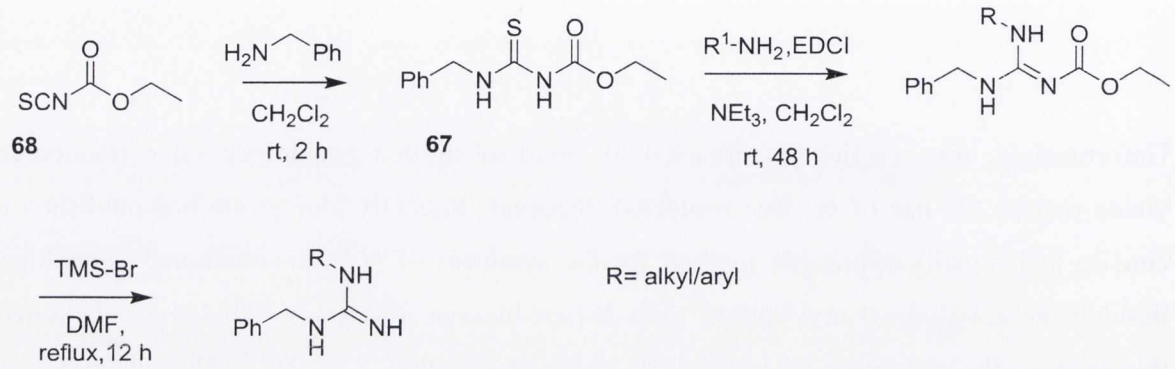
Scheme 3.2.1.2.



The utilisation of carbodiimide coupling reagents (see Section 3.1.1.2) is well known in the synthesis of amides from the corresponding carboxylic acids and is also applicable towards the synthesis of *mono*- or di-substituted guanidines. A number of literature examples illustrate the use of EDCI (1-ethyl-3dimethlaminopropyl carbodiimide) as a guanidylation promotor for certain thiourea derivatives. Manimala *et al.* use EDCI in the coupling of ethoxycarbonyl substituted thioureas **67** (prepared from the corresponding isothiocyanates **68**) with a wide range of amines giving excellent yields (< 80%) of the *N*-ethoxycarbonyl

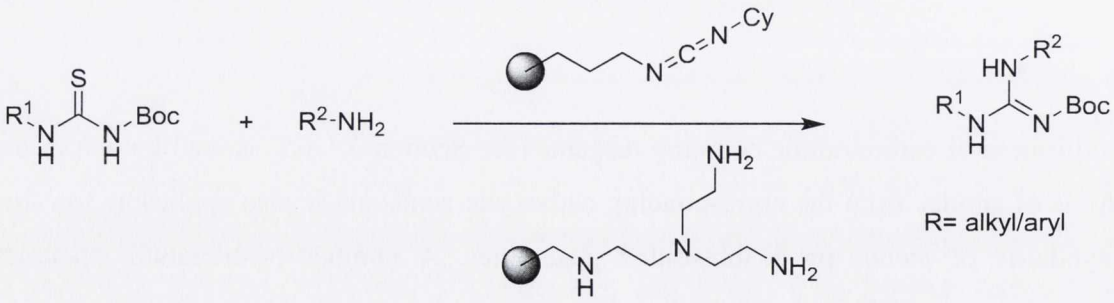
substituted guanidines. These can subsequently be deprotected, with quantitative yields, using TMS bromide (Scheme 3.2.1.3).[231]

Scheme 3.2.1.3.



In more recent years, further improvements have been made through the use of solid-phase synthesis, which has allowed the production of large libraries of guanidines *via* high throughput methodologies using polymer-supported reagents.[232] Pastor *et al.* reported the first use of a polymer-supported carbodiimide as a thiourea activating agent; this is used with a polymer-supported trisamine as a scavenger to give the appropriate guanidines in solution phase with no additional purification steps required (Scheme 3.2.1.4).[233]

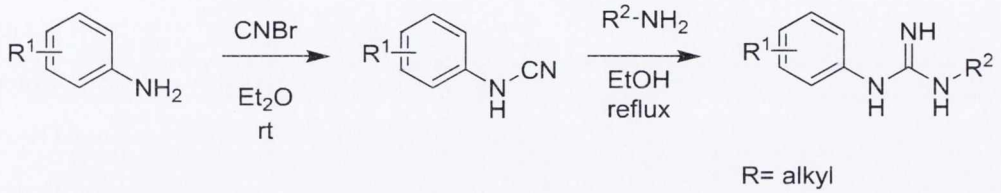
Scheme 3.2.1.4.



Guanidine synthesis is not limited to the use and preparation of thioureas and their analogues. Cyanamides, synthesised from the corresponding amine using cyanogen bromide, serve as

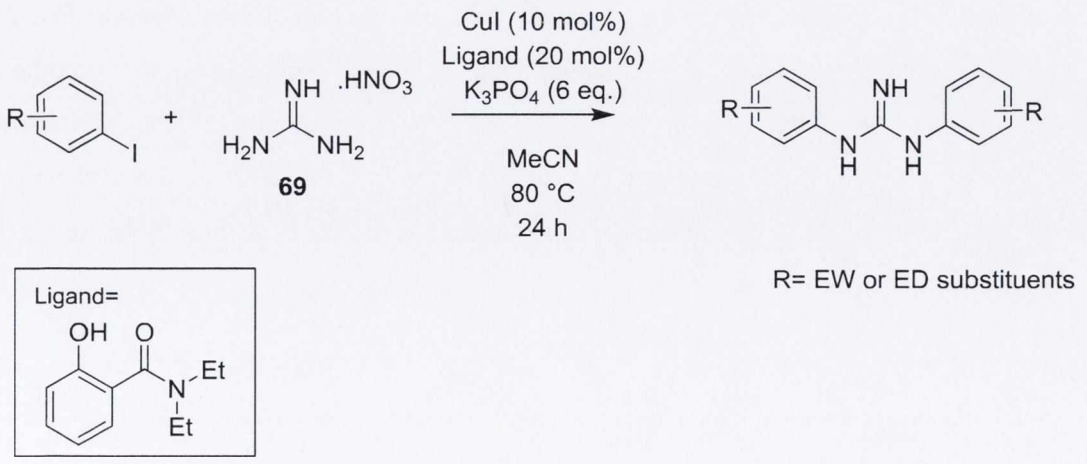
versatile starting materials for the preparation of *N,N'*-di-substituted guanidines (Scheme 3.2.1.5). A recent example describes the synthesis of a variety of cyanamide intermediates with subsequent nucleophilic addition of various alkyl amines affording the guanidine products in reasonable yield (40-60%).[234]

Scheme 3.2.1.5.



As seen in Section 3.1.1.2, metal catalysed C-N bond formation can be used to synthesise cyclic guanidines *via* intramolecular cyclisation with *N*-(*o*-bromophenyl)guanidines.[178] A similar strategy can be applied in the preparation of substituted acyclic guanidines through Cu-catalysed intermolecular *N*-arylations of guanidines, which have been much more investigated than the Pd-catalysed syntheses. In 2010, it was described the coupling of aryl iodides with guanidinium nitrate **69** using CuI/*N,N*-di-ethylsalicylamide in the presence of K_3PO_4 (Scheme 3.2.1.6) giving symmetrical *N,N'*-di-arylated guanidines in good to excellent yield (19-93%).[235]

Scheme 3.2.1.6.



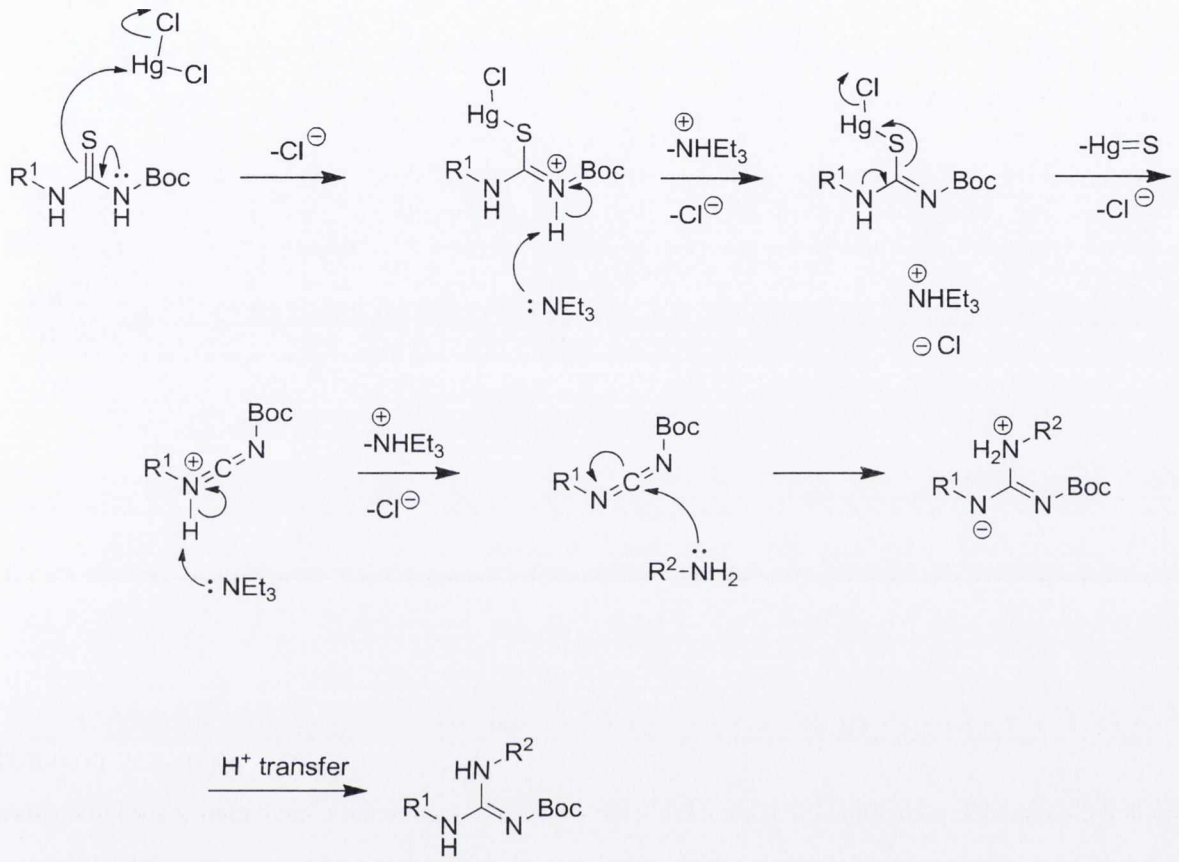
3.2.1.3. Synthesis of 4,4'-bis-(N,N'-di-substituted guanidines) diaryl derivatives: Family D1

The methods described in the previous section towards the synthesis of guanidines are just a few relevant examples but there are countless more.[236] Although they are efficient, the scope of many of these reaction methodologies is limited to the preparation of *mono*-substituted guanidines or to the use of aliphatic amines as the nucleophilic component.

The copper (II) chloride method seems the most effective towards our desired products within the Family D1 series. Although its usefulness in the synthesis of *mono*-substituted guanidines from the more electrophilic *N,N'*-di-(*tert*-butoxycarbonyl)thiourea **66** is analogous to that of the mercury (II) chloride mediated guanidylation, initial test reactions towards our *N,N'*-di-substituted guanidines showed significantly better results when mercury (II) chloride was employed. This is possibly due to the fact that Hg^{2+} salts are more thiophilic than Cu^{2+} as they have a low energy LUMO to allow antibonding interactions to sulphur's high energy HOMO, as well as having a full d electron count in outer orbitals to facilitate π-back bonding. Thus, in the synthesis of the diaryl 4,4'-*bis*-(*N,N'*-di-substituted guanidines), mercury (II) chloride was used, and although the use of toxic reagents is undesirable, the removal of mercury salt by-products by filtration through Celite is extremely efficient and safe.

This guanidylation method was originally developed by Kim and Qian towards an improved synthesis of *bis*-Boc protected *mono*-substituted guanidines from *N,N'*-di-(*tert*-butoxycarbonyl)thiourea **66** using amino compounds which are highly deactivated either sterically or electronically.[212] Although not discussed by the authors, as per our experience *mono*-Boc substitution on the thiourea is sufficient to increase the electrophilicity of the thiourea carbon giving a slightly lower range of yields for the preparation of *mono*-Boc *N,N'*-di-substituted guanidines. Based on Kim and Qian proposed mechanism, we hypothesised that the reaction goes through the initial formation of a highly reactive *N*-Boc-*N'*-substituted carbodiimide, which can subsequently undergo nucleophilic addition of the aryl amine to give the Boc protected *N,N'*-di-substituted guanidine after proton exchange (Scheme 3.2.1.7).

Scheme 3.2.1.7.



This method requires the preparation of the *N*-Boc-*N'*-substituted thioureas, which have been previously described by Yin *et al.* This two pot procedure involves initial di-Boc protection of thiourea **70**, followed by treatment with sodium hydride and trifluoroacetic anhydride in the presence of an amine.[237] Recently, we have developed a simpler one-pot procedure for the preparation of these *N*-Boc-*N'*-alkyl/aryl thioureas through initial *in situ* generation of *N,N'*-di-(*tert*-butoxycarbonyl)thiourea **66** using sodium hydride and di-*tert*-butyl dicarbonate.[238] In the same pot, a second aliquot of sodium hydride is added to facilitate *N*-acylation with trifluoroacetic anhydride affording a *N*-Boc-*N*-trifloroacetyl intermediate that can undergo facile nucleophilic attack by the amine to give the desired product (Scheme 3.2.1.8).[239]

Scheme 3.2.1.8.

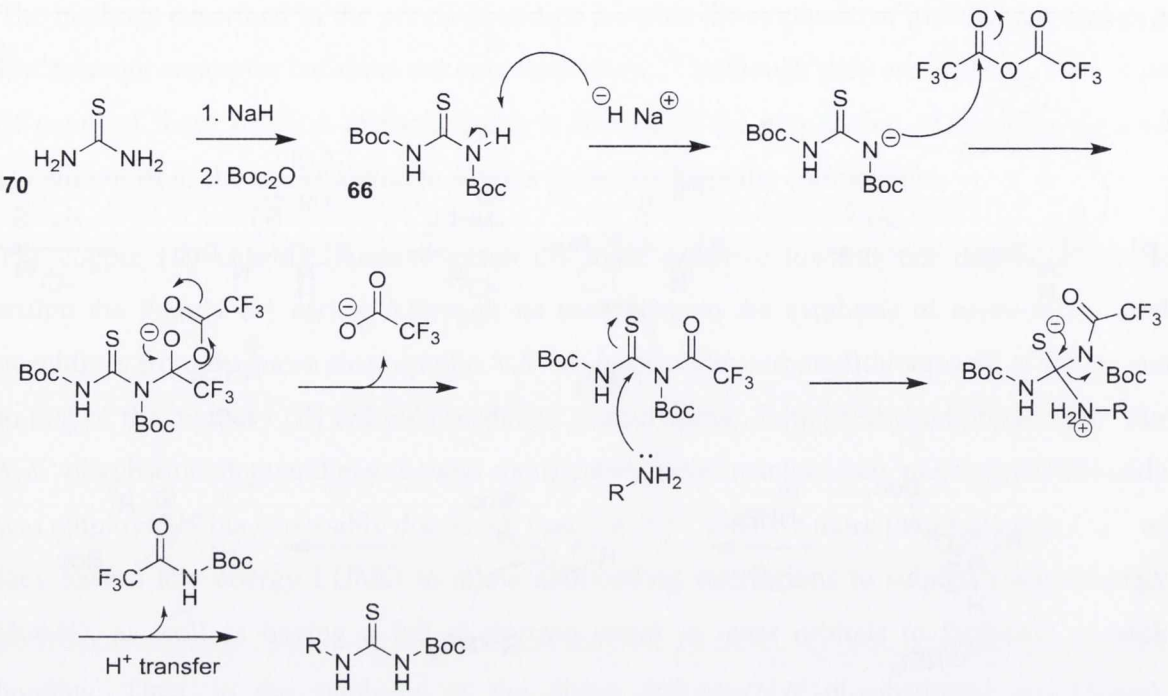
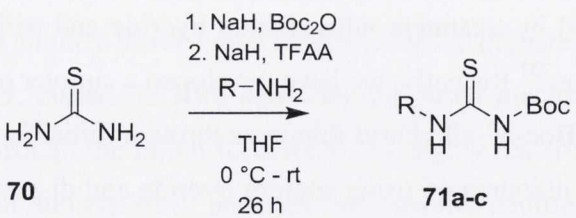


Table 3.2.1.1 shows the yields obtained for the *N*-Boc-*N'*-substituted thioureas prepared using the one pot procedure. It is notable that Yin's two-pot method was also tested and gave significantly lower yields (-20%) over two steps than those obtained using our methodology.

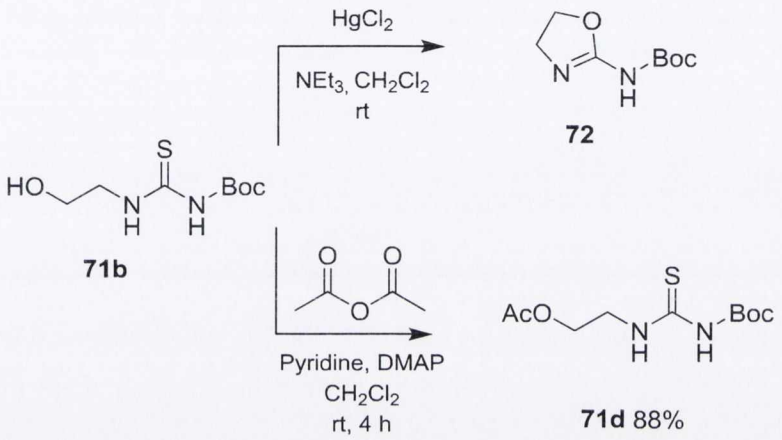
Table 3.2.1.1. Preparation of the *N*-Boc-*N'*-substituted thioureas



Compound	R	Yield (%)
71a	furfuryl	67
71b	-$(CH_2)_2$-OH	58
71c	-$(CH_2)_2$-CH_3	60

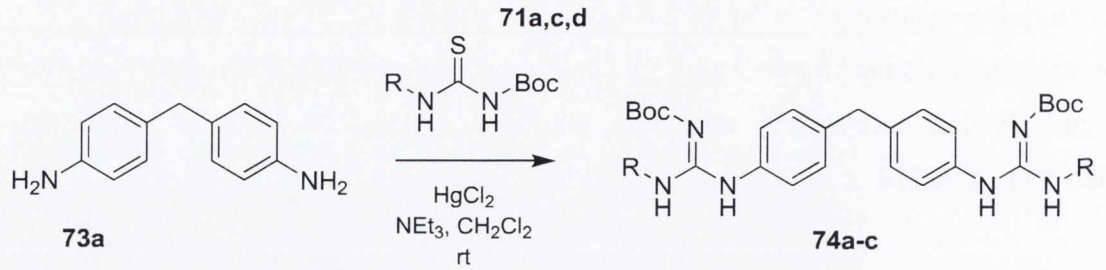
As seen in Section 3.1.1.3, exposure of the thiourea-like hydroxyethyl derivatives to desulfurizing agents can lead to intramolecular cyclisation yielding (*E*)-oxazolidin-2-(*N-tert*-butoxycarbonyl)imine **72** in this case. Accordingly, compound **71b** was acetyl protected prior to guanidylation using acetic anhydride, employing pyridine and DMAP as nucleophilic catalysts to give **71d** in excellent yield (Scheme 3.2.1.9).

Scheme 3.2.1.9.



The *N*-Boc-*N'*-substituted thiourea derivatives were coupled with commercially available 4,4'-diaminodiphenylmethane **73a** in the presence of mercury (II) chloride, using triethylamine as base to give the Boc protected *N,N'*-di-substituted guanidines **74a-c** in good yield (Table 3.2.1.2). As expected, these yields are slightly lower than those reported by Kim *et al.* as in our case the thioureas carbon centre is less susceptible to nucleophilic attack by the aryl amine due to the loss of one electron withdrawing Boc substituent.

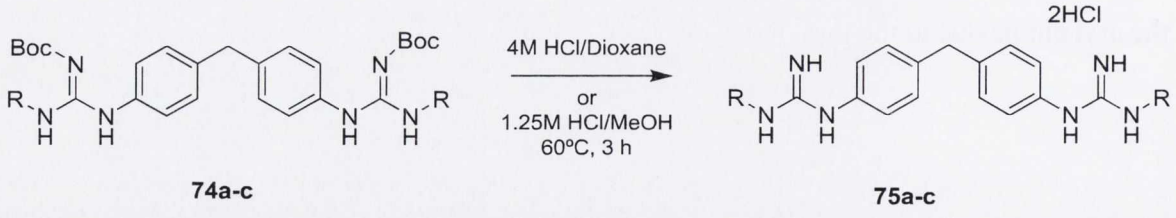
Table 3.2.1.2. Synthesis of the Boc protected guanidine derivatives **74a-c.**



Compound	R	Yield (%)
74a	furfuryl	61
74b	-$(CH_2)_2$-OAc	67
74c	-$(CH_2)_2$-CH_3	65

The removal of the Boc protecting groups was achieved by stirring the appropriate compound in 4M HCl/dioxane (6 eq. per Boc group) at 60 °C for 3 h, which afforded the corresponding *N,N'*-di-substituted guanidinium hydrochlorides **75a-c** in excellent yield (Table 3.2.1.3).[240] For the acetoxy derivative the same procedure was performed except that now 1.25M HCl/MeOH was used to remove the acetyl and Boc groups in one step. The crude hydrochloride salts were purified using small scale reverse phase chromatography with C-8 silica, and their purity assessed by reverse phase HPLC.

Table 3.2.1.3. Preparation of the *N,N'*-di-substituted guanidinium hydrochlorides.



Compound	R	Yield (%)
75a	furfuryl	91
75b	-$(CH_2)_2$-OH	88
75c	-$(CH_2)_2$-CH_3	89

3.2.1.4. Conformational considerations of Boc protected N,N'-di-substituted guanidines

Novel Boc protected *N,N'*-di-substituted guanidines, previously developed in our group, illustrated considerable structural complexity due to the presence of different tautomers and conformational isomers. It is known that substituted guanidines may exist in three possible tautomeric forms, with the double bond conjugating with either of the three substituents (Figure 3.2.1.4).[241]

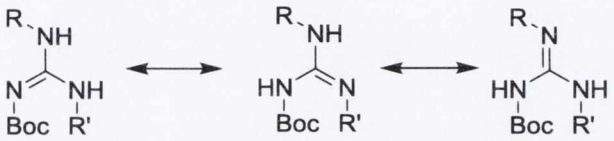


Fig. 3.2.1.4. Tautomeric forms of Boc protected *N,N'*-di-substituted guanidines.

The most stable tautomer will predominate, with localisation of the imine bond to the nitrogen bearing the most electron withdrawing substituent, usually the *N*-Boc, where experimental evidence for the existence of such tautomeric forms has been supported by ^{1}H-NMR, ^{15}N-NMR and X-ray crystallography.[242] Further complications regarding structural character may arise due to the existence of conformational or geometrical isomers about the imine bond, adopting either *E* or *Z* geometry (Fig 3.2.1.5).[188]

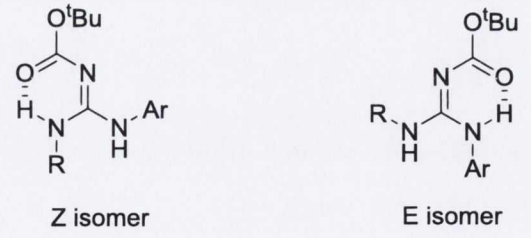


Fig. 3.2.1.5. Example of two conformational isomers of Boc protected *N,N'*-di-substituted guanidines with the imine bond localised to the N atom bearing the Boc group.

Taking all that into account, the characterisation of the Boc protected 4,4'-*bis*-(*N,N'*-di-substituted guanidine) diaryl derivatives (**74a-c**) proved rather difficult. The ^{1}H-NMR and ^{14}C-NMR presented spectra with broad, poorly resolved multiplets within the aromatic and aliphatic regions (Figure 3.2.1.6). This is probably due to the restricted rotation of the aromatic substituents attached to the central guanidine core, yielding shorter relaxation times of the aromatic signals, paired with the inter-conversion between different tautomers. Furthermore, there is the possibility of equilibration between *E* and *Z* isomers as the imine bond may localise not only to the nitrogen bearing the Boc group but also to the aryl substituent. Thus, in theory, a total number of four isomers are possible: two tautomers with *E* or *Z* configuration each, causing additional difficulties concerning structural identification.

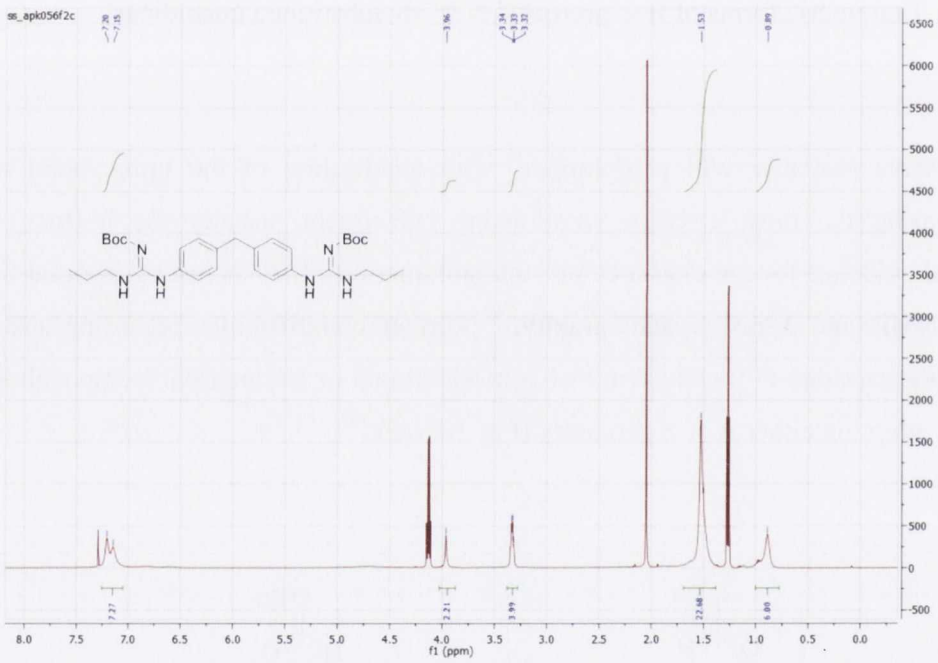


Fig. 3.2.1.6. ^{1}H-NMR for Boc protected *N,N'*-di-substituted guanidine **74c** illustrating poorly resolved broad multiplets in the aromatic region.

Deprotection of the Boc groups afforded the guanidinium salts with sharper, well resolved signals in the aromatic region. This was due to the imine bond now being delocalised around the guanidinium centre, removing the possibility of tautomerisation, coupled with the

removal of the bulky Boc groups alleviating the steric congestion adjacent to the aromatic rings to return relaxation times to normal levels (Figure 3.2.1.7).

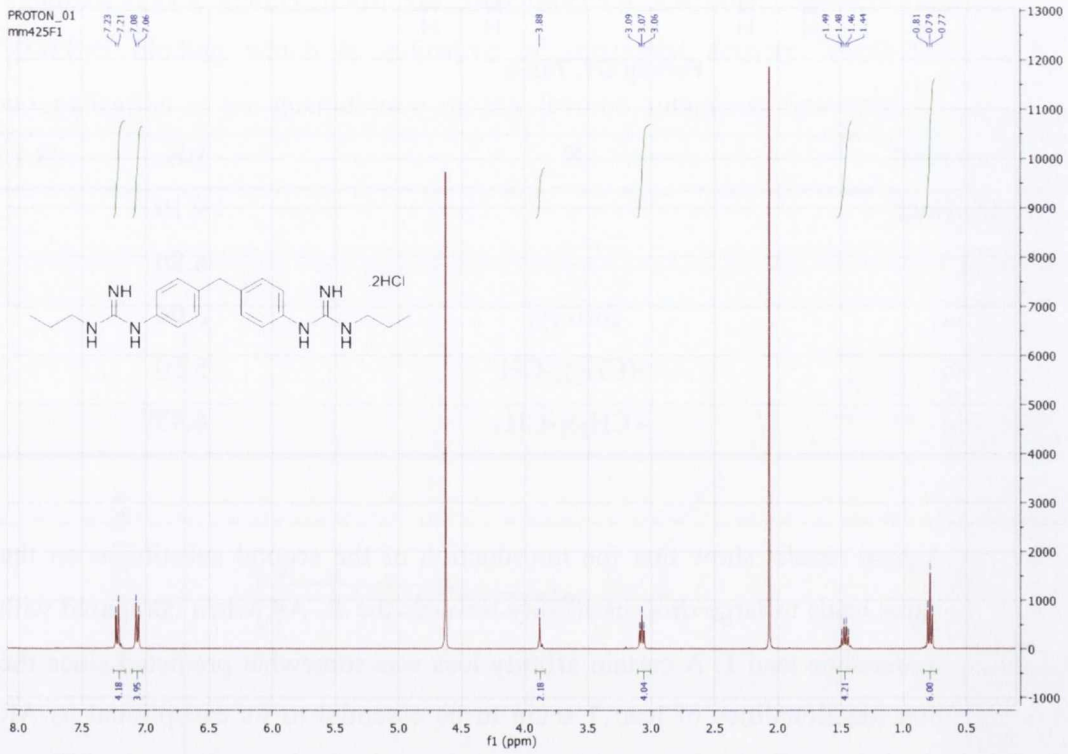


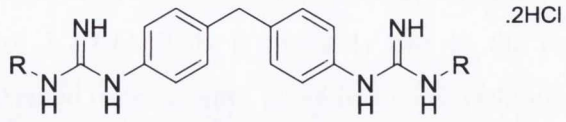
Fig 3.2.1.7. ^{1}H-NMR for *N,N'*-di-substituted guanidinium hydrochloride **75c** showing sharp, well resolved signals in aromatic region.

3.2.1.5. Pharmacological evaluation of compounds from Family D1

Binding Affinity to α_2-AR

The binding affinity of the *bis*-aryl *N,N'*-di-substituted guanidinium hydrochlorides **75a-c** toward the α_2-AR was measured, as with previous families of compounds, by *in vitro* competition assays using human brain frontal cortex tissue. The α_2-AR affinities are shown in Table 3.2.1.4 and are expressed in terms of pK_i values with RX821002 (2-methoxyidazoxan) acting as the control ligand.

Table 3.2.1.4. Binding affinities for the α_2-AR of compounds in Family D1 expressed as pK_i values.



Family D1, 75a-c

Compound	R	pK_i
RX821002	-	9.10
1	-	8.80
75a	furfuryl	6.04
75b	$-(CH_2)_2-OH$	5.50
75c	$-(CH_2)_2-CH_3$	6.45

These pharmacological results show that the introduction of the second substituent on the guanidinium moieties leads to large drop in affinity towards the α_2-AR when compared with the *bis*-2-aminoimidazoline lead **1**. A certain affinity loss was somewhat predicted since the 2-aminoimidazoline functionalities of lead **1** seem to be essential to its exceptional α_2-AR binding properties. It is predicted that both cations independently interact at two different sites within the receptor pocket to contribute to its binding ability, as loss of one of these functional groups leads to a large drop in affinity.[155] Thus, if both of these groups are replaced with the inherently lower binding guanidine this will lead to an initial reduction in affinity. However, the significant loss in α_2-AR affinity observed for compounds **75a-c** was not expected or formerly observed.

This large decrease could be explained because the guanidine derivatives are now di-substituted and this drop in affinity has been observed in the case of the *mono*-cationic examples previously discussed. In the present sub-family of compounds there are two cationic *N,N'*-di-substituted groups contributing even further to the lower affinity. Indeed this is observed as there is roughly a twofold loss in affinity for these di-cationic *N,N'*-di-substituted guanidines and only a one unit drop for the *mono*-cationic *N,N'*-di-substituted guanidines when compared to their corresponding 2-aminoimidazoline analogues (Section 3.2.1.1).

Functional Activity

Compounds **75a** and **75c** displayed an α_2-AR affinity less than 1 μM ($pK_i = 6$) and were thus subjected to *in vitro* [^{35}S]GTPγS binding experiments to determine their nature as agonists or antagonists. Encouragingly, these *N,N'*-di-substituted derivatives showed no stimulation of [^{35}S]GTPγS binding which is indicative of antagonist activity; confirming that steric functionalization at the guanidinium moiety favours antagonist functional activity (Figure 3.2.1.8).

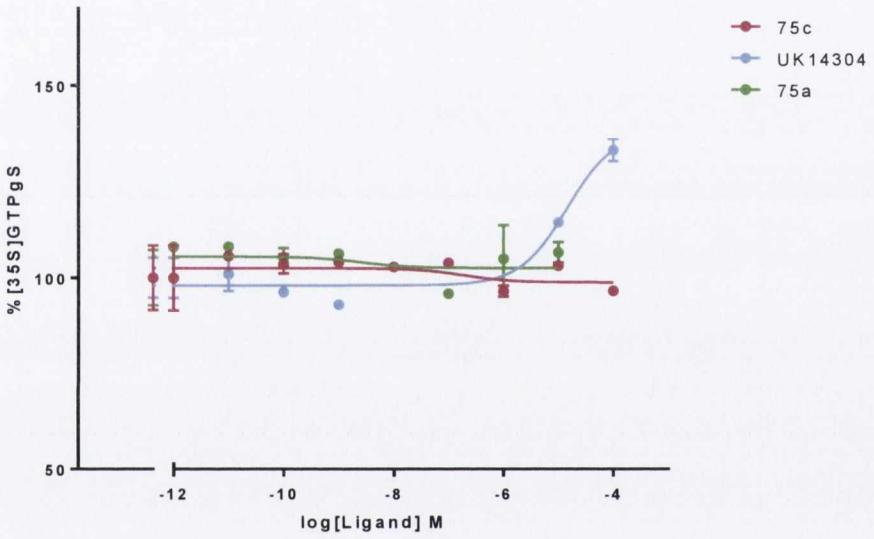
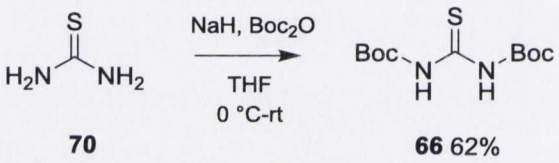


Fig. 3.2.1.8. Functional activity results for compounds **75a** and **75c** indicating antagonist activity *versus* known agonist UK14304.

3.2.1.6. Synthesis of the asymmetric diaryl N,N'-di-substituted guanidine and 2-aminoimidazolidine derivatives: Family D2

The preliminary step for the synthesis of compounds in Family D2 is analogous to the diaromatic 4,4'-*bis*-(*N,N'*-di-substituted guanidines) (Family D1), the preparation of the *N*-Boc-*N'*-substituted thioureas. Next, the use of mercury (II) chloride as an activating agent will facilitate the guanidylation process. However, since the compounds in Family D2, which are asymmetric-type di-cationic derivatives, have not been previously studied it was decided to also prepare a *mono*-substituted guanidine in the series which may alleviate the drop in affinity induced by the di-substituted guanidines. The synthesis towards the *mono*-substituted guanidine derivative required first the preparation of compound **66**, from the treatment of thiourea with excess sodium hydride and di-*tert*-butyl dicarbonate (Scheme 3.2.1.10).

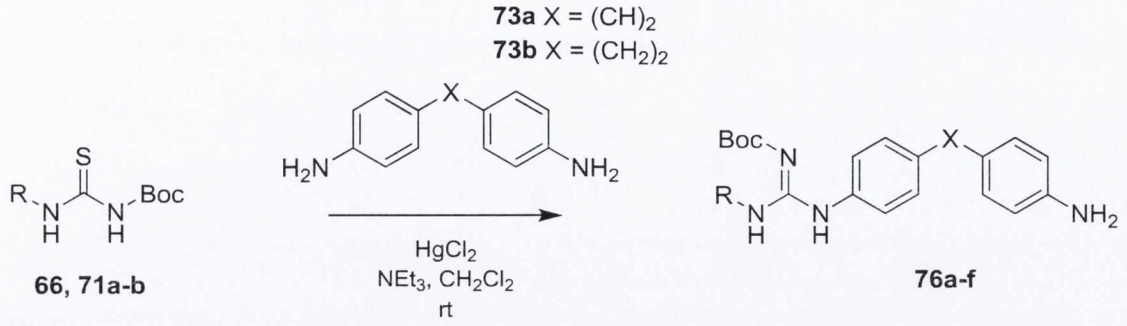
Scheme 3.2.1.10.



70 **66** 62%

For all the compounds in Family D2, *mono*-guanidylation was required first to create the asymmetric core. This was achieved by using a 3 fold excess of the amine **73a** or **73b** to the thiourea affording the desired *mono*-coupled products exclusively. As expected, the more electrophilic di-Boc protected thiourea **66** gave significantly better yields than the *N*-Boc-*N'*-substituted thioureas **71a-b** (Table 3.2.1.5).

Table 3.2.1.5. *Mono*-guanidylation reaction towards compounds **76a-c**.

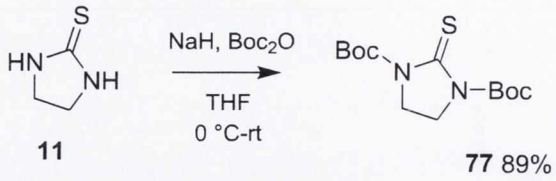


Compound	X	R	Yield (%)
76a	CH_2	-Boc	82
76b	CH_2	-furfuryl	50
76c	CH_2	$-(CH_2)_2-CH_3$	59
76d	$(CH_2)_2$	-Boc	84
76e	$(CH_2)_2$	-furfuryl	58
76f	$(CH_2)_2$	$-(CH_2)_2-CH_3$	69

The next step in the preparation of compounds in Family D2 involved installing the 2-aminoimidazoline moiety to the di-aryl framework to give the Boc-protected 4-(*N*-substituted guanidine)-4'-(2-aminoimidazoline)diphenylmethane/-1,2-diphenylethane derivatives. As seen in Section 3.1.1.2 there are many methods available for the preparation of 5 membered cyclic guanidines; unfortunately, in most cases purification may be tedious since these examples afford the highly polar salt or free base of the 2-aminoimidazoline.

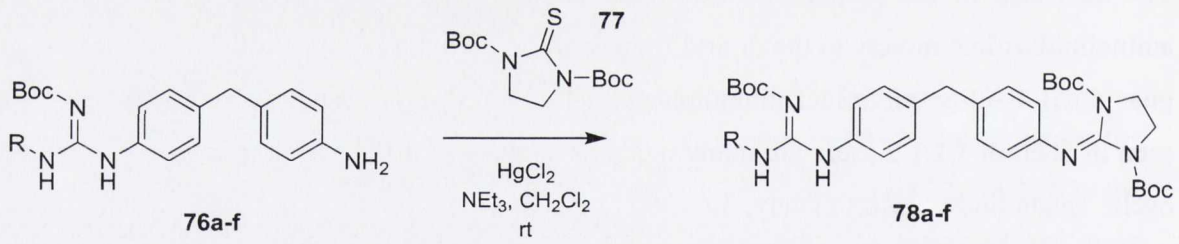
As an extension to the Kim and Qian guanidylation method Daradonville and Rozas developed a novel synthesis of 2-aminoimidazolines using a cyclic thiourea derivative which parallels with compound **66**, the *N,N'*-di-(*tert*-butoxycarbonyl)imidazolidin-2-thione **77**.[159] This can be synthesised in a similar manner to **66** by treating imidazolin-2-thione **11** with excess sodium hydride and di-*tert*-butyl dicarbonate (Scheme 3.2.1.11).

Scheme 3.2.1.11.



Compound **77** can be subjected to the usual guanidylation conditions with the appropriate amine in the presence of mercury (II) chloride and triethylamine to give the *bis*-Boc protected 2-iminoimidazolidine derivatives **78a-f** in excellent yields (Table 3.2.1.6). The Boc protecting groups present make these notoriously polar compounds easier to handle and purify, which is one of the main advantages of this method over the several other routes previously described, coupled with the reactions effectiveness in preparing aryl 2-iminoimidazolidines from unreactive aryl amines.

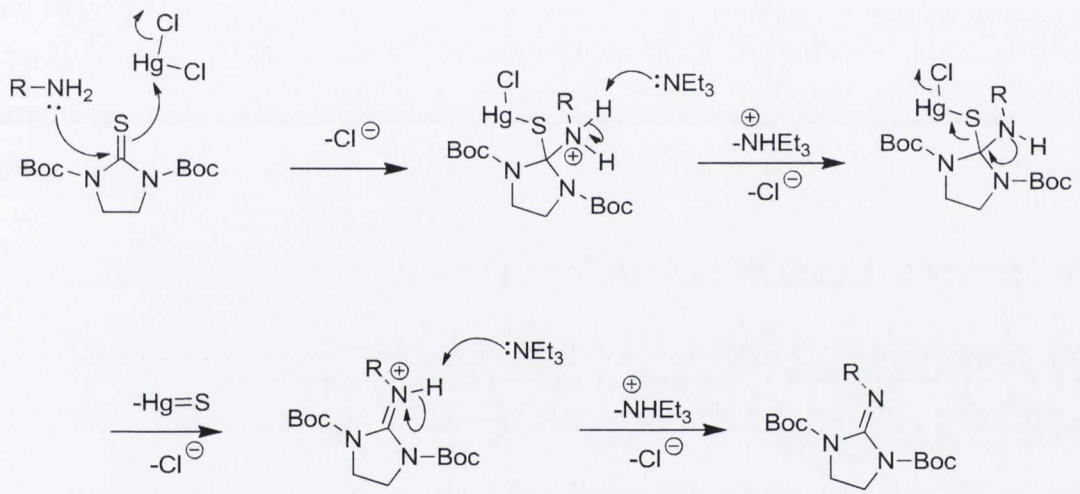
Table 3.2.1.6. Synthesis of the *bis*-Boc protected 2-iminoimidazolidine derivatives **78a-f**.



Compound	X	R	Yield (%)
78a	CH_2	-Boc	78
78b	CH_2	furfuryl	91
78c	CH_2	$-(CH_2)_2-CH_3$	74
78d	$(CH_2)_2$	-Boc	72
78e	$(CH_2)_2$	furfuryl	96
78f	$(CH_2)_2$	$-(CH_2)_2-CH_3$	93

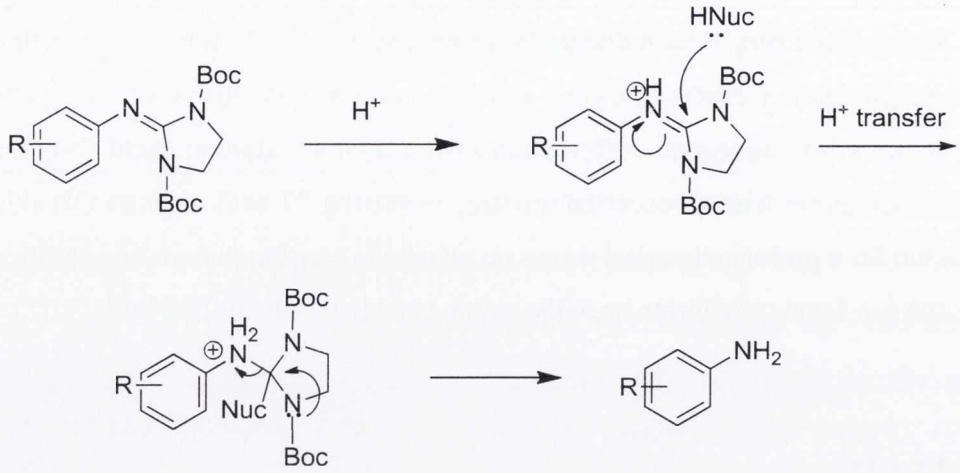
The mechanism of guanidylation with the 2-iminoimidazolidine system differs from that seen for the non-cyclic thioureas, as the formation of the carbodiimide would not be possible within a 5-membered ring system due to the geometrical strain. In this case, it is thought that an addition-elimination process occurs, through nucleophilic attack of the amine to the thiourea centre with simultaneous formation of the mercury-sulphur bond. Investigation of the mechanism agrees with a concerted process, as stirring **77** with mercury (II) chloride and triethylamine for a prolonged period shows no change in starting material, suggesting that the mercury-sulphur bond only forms once the amine is added (Scheme 3.2.1.12).

Scheme 3.2.1.12.



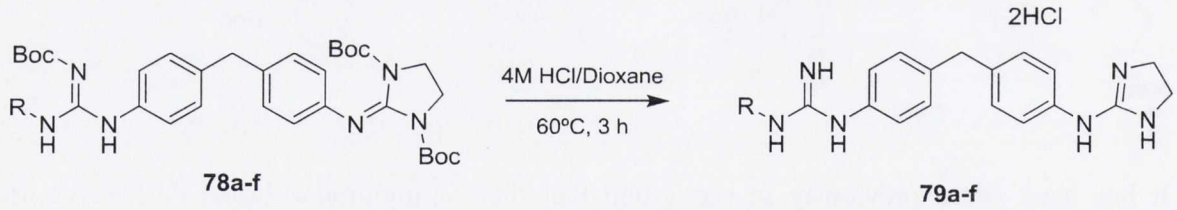
It has been noted previously in our group that the 2-iminoimidazolidine derivatives are highly-acid sensitive. Protonation of the imine nitrogen can facilitate hydrolysis of the bond upon nucleophilic attack cleaving the molecule to the resulting aniline starting material (Scheme 3.2.1.13). This degradation is observed following silica gel chromatography under normal conditions. High speed alumina chromatography has been used, and although cleavage is prevented, this method leads to poor separation and loss of product. It was found that pre-treatment of the silica gel with triethylamine (3 mL per 100 mL of hexane) basifies the stationary phase sufficiently to avoid degradation and encouragingly gives good separation with no loss of material

Scheme 3.2.1.13.



Removal of the Boc protecting groups was performed in a similar manner as that followed for compounds in Family D1 by using 4M HCl/dioxane. This yielded the appropriate 4-(*N*-substituted guanidine)-4'-(2-aminoimidazoline)diphenylmethane and 4-(*N*-substituted guanidine)-4'-(2-aminoimidazoline)-1,2-diphenylethane hydrochlorides in excellent yield (Table 3.2.1.7).

Table 3.2.1.7. Preparation of the guanidinium hydrochlorides of Family D2.



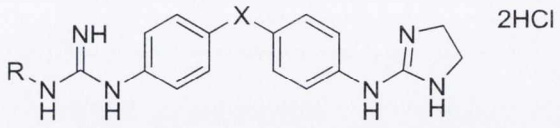
Compound	X	R	Yield (%)
79a	CH_2	-H	98
79b	CH_2	furfuryl	80
79c	CH_2	$-(CH_2)_2-CH_3$	96
79d	$(CH_2)_2$	-H	86
79e	$(CH_2)_2$	furfuryl	82
79f	$(CH_2)_2$	$-(CH_2)_2-CH_3$	91

3.2.1.7. Pharmacological evaluation of Family D2

Binding Affinity to α_2-AR

The binding affinities of the asymmetric compounds in Family D2 towards the α_2-AR were measured in the same manner as for the compounds in Family D1 (in human prefrontal cortex tissue) and the corresponding pK_i values are shown in Table 3.2.1.8.

Table 3.2.1.8. Binding affinities of compounds **79a-f** for the α_2-AR, expressed in pK_i values.



Family D2, 79a-f

Compound	X	R	pK_i
RX821002	-	-	9.10
1	-	-	8.80
79a	CH_2	-H	7.91
79b	CH_2	furfuryl	8.78
79c	CH_2	$-(CH_2)_2-CH_3$	9.10
79d	$(CH_2)_2$	-H	6.67
79e	$(CH_2)_2$	furfuryl	5.76
79f	$(CH_2)_2$	$-(CH_2)_2-CH_3$	6.38

As can be observed in Table 3.2.1.8 the α_2-AR binding affinities of the methylene linked derivatives of Family D2 **79a-c** are on par to that of *bis*-2-aminoimidazoline lead compound **1** (pK_i = 8.80). This illustrates that reintroduction of the 2-aminoimidazoline moiety does contribute to significantly higher binding affinities, regardless of the presence of the di-substituted guanidine. Encouragingly, the propyl derivative **79c** actually gave a notable increase in α_2-AR pK_i value compared to **1**, suggesting that two 2-aminoimidazoline groups are not necessary for high affinity at the α_2-AR in the case of the *bis*-aryl series. Thus, it is

postulated that the di-substituted cationic functional groups of these derivatives are occupying an alternate pocket to give optimum interactions which contribute to its binding affinity compared to **1**. It is notable that the ethylene linked derivatives of Family D2 **79d-f** gave significantly lower pK_i values than the methylene series, which signifies that flexibility in this position is not tolerated for high binding to the α_2-AR.

Functional Activity

Although the ethylene linked derivatives showed $pK_i > 6$, they did not compare against the higher α_2-AR binding of the methylene linked derivatives and, thus, were not subjected to the [^{35}S]GTPγS functional binding assays. Compounds **79b** and **79c** showed unusual results which had not been previously seen in our group. In the [^{35}S]GTPγS assay both compounds displayed agonist activity through the stimulation of GTP, but only at extremely high concentrations. This evidently does not correspond with the high binding properties observed for these compounds because if they strongly bind to the α_2-AR, a small dose should be sufficient to exert the corresponding activity.

For that reason, compounds **79b** and **79c** were subjected to a different [^{35}S]GTPγS experiment with co-administration of the known α_2-AR agonist UK14304, where an increase in agonist EC_{50} is indicative of antagonistic competitive binding. Indeed, both compounds **79b** and **79c** increased the EC_{50} of UK14304 (Figure 3.2.1.9); however, this increase was blocked in the presence of the selective α_2-AR antagonist, RX821002.

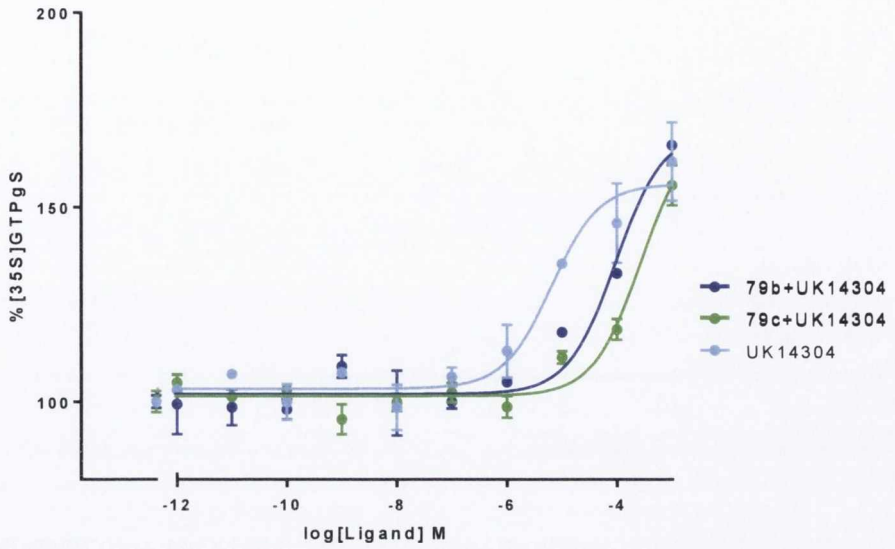


Fig. 3.2.1.9. [^{35}S]GTPγS experiment of compounds **79b** and **79c** with co-administration of the α_2-AR agonist UK14304. Note: the block of increase in EC_{50} by α_2-AR antagonist RX821002 is not illustrated in this figure.

It is hypothesised that these compounds are acting as α_2-AR partial agonists, which explain the competitive binding *versus* an agonist, but not in front of an antagonist, and only give receptor activation at high concentrations since they have only partial efficacy compared to a full α_2-AR agonist. This result indicates that the combination of the 2-aminoimidazoline and di-substituted guanidine groups into the diaryl core does alter the α_2-AR activity in the right direction, as partial agonists do display some antagonist-like properties, yet certain structural changes are still required to achieve full α_2-AR antagonism.

Moreover, the *mono*-substituted guanidine derivative, compound **79a**, exhibited no stimulation of [^{35}S]GTPγS and was further tested against UK14304, which confirmed competitive α_2-AR antagonism by an increase in agonist EC_{50} (Figure 3.2.1.10). Thus, compound **79a** has now become our highest binding α_2-AR antagonist ligand to date in 15 years of research and will now be viewed as our new lead compound.

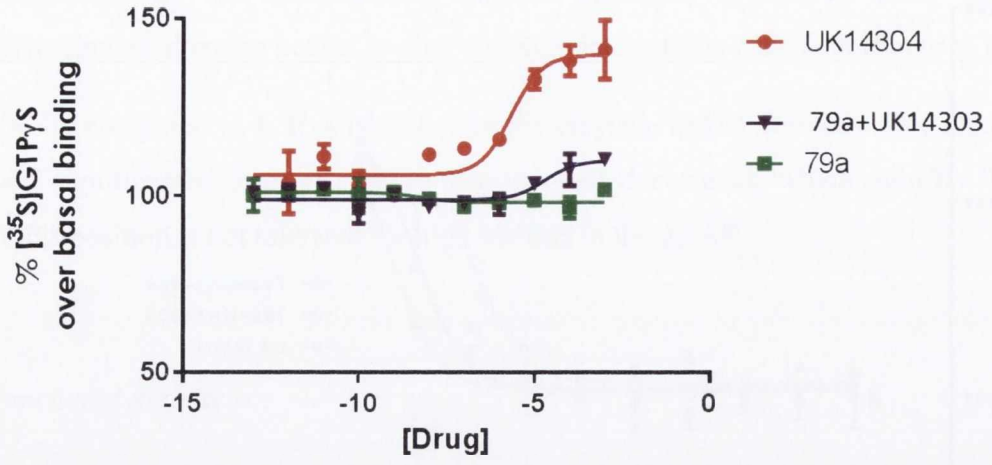


Fig. 3.2.1.10. [^{35}S]GTPγS binding curve of compound **79a**, UK14304 and **79a**+UK14304, showing competitive α_2-AR antagonist activity.

3.2.2. Family E: Diaryl pyridinyl containing guanidine and 2-aminoimidazoline derivatives

3.2.2.1. Design

The pharmacological results obtained for Family D2 were extremely encouraging, with very high affinities obtained, one compound showing competitive antagonist activity and the others interestingly illustrating partial agonism. Thus, considering also the good outcomes obtained with the pyridine *mono*-cations, we designed a new set of compounds (Family E) comprising of the corresponding pyridine analogues of Family D2. These compounds would incorporate this heterocycle to the core di-aromatic framework with the combination of the guanidine and 2-aminoimidazoline moieties on either side of *bis/mono*-pyridin-2-yl system (Figure 3.2.2.1).

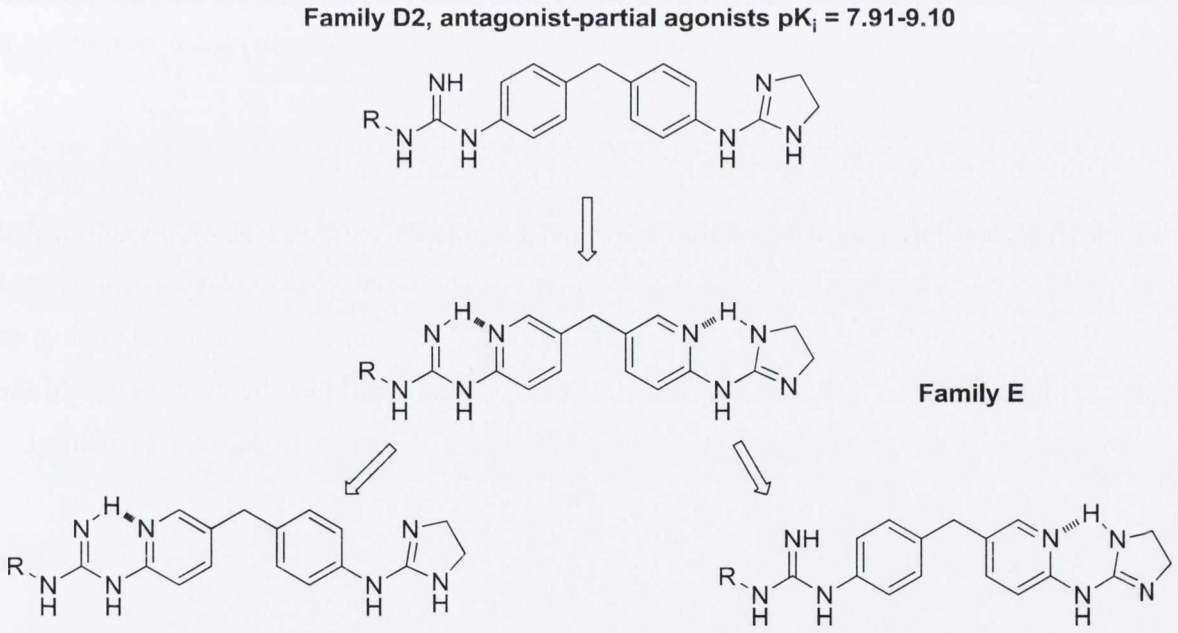
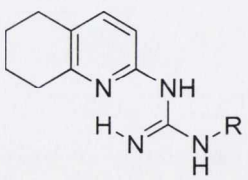


Fig. 3.2.2.1. Proposed structures of pyridinyl containing derivatives of Family E (E1: *top*, E2: *right*, E3: *left*).

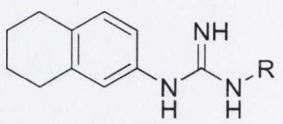
As mentioned in Sections 1.6 and 3.1.1.1, the pyridin-2-yl guanidines have shown to give exclusive antagonist activity for the α_2-AR as they can adopt a co-planar arrangement through

IMHB to create conformationally controlled pseudo bicyclic systems. Adding a degree of conformational restriction is now a known strategy for preventing receptor activation and amplifying antagonist activity, as seen for compounds in Families A-C. Accordingly, Family E will encompass three subgroups with altering pyridinyl positioning in an attempt to exhibit exclusive antagonist activity across all high affinity *bis*-aryl analogues. The first subgroup (Family E1) will be the di-pyridin-2-yl analogue of Family D2 (asymmetric *N*-substituted guanidine/2-aminoimidazoline). The *mono*-pyridin-2-yl analogues of the di-aryl core will include the E2 subgroup where the pyridine forms an IMHB with the 2-aminoimidazoline and the E3 subgroup where the IMHB is formed with the guanidine. These altering structures are considered as large differences in α_2-AR affinity have been observed between the 2-guanidinopyridines and the 2-(2-aminoimidazolino)pyridines in previous work in our laboratory.[162] Usually, the 2-aminoimidazoline moiety gives higher binding affinities; however, when it is involved in IMHBs a decrease in affinity has been observed for the monomeric derivatives. Hence, we wanted to probe the difference in α_2-AR affinity between these two di-aryl pyridinyl subfamilies (E2 and E3) concerning conformational restriction at the cationic moiety.

Regarding α_2-AR affinity, previously developed phenyl *mono*-substituted guanidines usually exhibited higher binding affinities than the corresponding pyridin-2-yl *mono*-substituted derivatives.[156,162] However, recently we have shown that the pyridin-2-yl di-substituted guanidines **80a-c** in fact exhibit higher pK_i values than their phenyl di-substituted analogues **81a-c** (Figure 3.2.2.2).[161] Thus, from these results, it was expected that the introduction of the pyridine ring into the di-aryl series would not grant a marked change or decrease in affinity.



80a R = -furfuryl) **pK_i = 6.67**
80b R = -$(CH_2)_2$-OH **pK_i = 5.83**
80c R = -C_6H_5 **pK_i = 6.75**

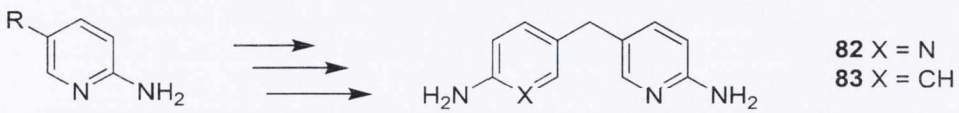


81a R = -furfuryl **pK_i = 6.29**
81b R = -$(CH_2)_2$-OH **pK_i = 5.76**
81c R = -C_6H_5 **pK_i = 6.58**

Fig. 3.2.2.2. Comparison of pK_i values between di-substituted pyridin-2-ylguanidines (*left*) and di-substituted phenylguanidines (*right*).

Unlike Family D, the synthetic route towards pyridinyl derivatives carrying guanidine and 2-aminoimidazoline primarily involves the preparation of the appropriate diamines **82** and **83** from available 2-aminopyridine derivatives (Scheme 3.2.2.1). Once synthesised, these amines can be subjected to the previously described guanidylation chemistry using mercury (II) chloride.

Scheme 3.2.2.1

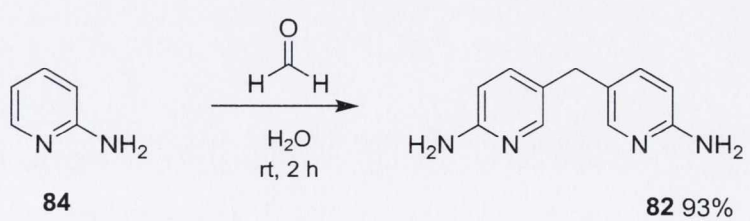


3.2.2.2. Synthesis of bis-amino di-pyridin-2-yl starting compounds

*Synthesis of bis-amino dipyridinyl derivative **82***

The synthesis of diamine **82** has recently been described by Pradeep *et al. via* the condensation of aromatic amines with formaldehyde affording the corresponding diphenyl methane diamines through electrophilic aromatic substitution (EAS) (Scheme 3.2.2.2).[243] In this procedure the appropriate amine is dissolved in water and a solution of formaldehyde (37% in water) is added dropwise. After 2 h they report the desired product precipitating out of water as a white solid in near quantitative yield.

Scheme 3.2.2.2

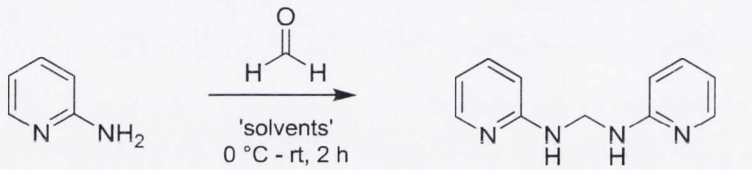


84 **82** 93%

On our first attempt at this procedure we observed a white precipitate after exactly 2 h as expected which was similarly obtained in quantitative yield. However, after further analysis

we found the white solid to be *N,N'*-di-(2-pyridyl)methylnediamine, compound **85**, whereby the amine of the 2-aminopyridine **84** is primarily condensing with formaldehyde with loss of water to give this undesired product (Scheme 3.2.2.3). This was confirmed by ^{1}H and ^{13}C NMR, mass spectrometry and by its melting point. Moreover, in the literature compound **85** is synthesised similarly to this by the reaction of 2-aminopyridine with paraformaldehyde using benzene as solvent.[244]

Scheme 3.2.2.3



84 **85** <90%

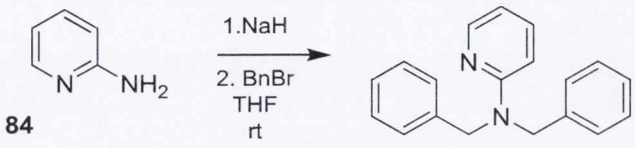
The reaction was repeated several times making certain alterations; recrystallizing the starting material, adding the formaldehyde slower, cooling the reaction, changing solvent types, yet all reactions gave quantitative conversion to **85**. We now looked at the procedure of Pradeep *et al.* with some scepticism. Firstly, the authors gave no characterisation on the final product, which lead us to believe that perhaps they also produced **85** due to the similarities in appearance and formation. They described this procedure for the synthesis of a variety of di-amino diphenyl methanes; thus, we repeated the reaction with benzylamine (which they had also performed) and we similarly observed condensation *via* the amine, as for **85**, where they apparently observed again condensation *via* electrophilic aromatic substitution.

Only one example in the literature has illustrated reproduction of this procedure for the synthesis of **82**; however, their NMR data was evidently incorrect as they reported observing NH_2 peaks in a D_2O ^{1}H-NMR as well as the aromatic pyridine signals not matching the expected product at all.[245] This was again looked at with some disbelief.

It was decided to alter this route and di-protect the amine to prevent condensation occurring at this position, hence forcing the reaction towards electrophilic aromatic substitution. The benzyl protecting group was used to somewhat retain the electron donating properties of the

amino moiety of 2-aminopyridine. The starting material **84** was reacted first with excess sodium hydride (60% in mineral oil) and subsequently the anion was trapped with benzylbromide giving the dibenzyl protected product **86** in excellent yield (Scheme 3.2.2.4).[246]

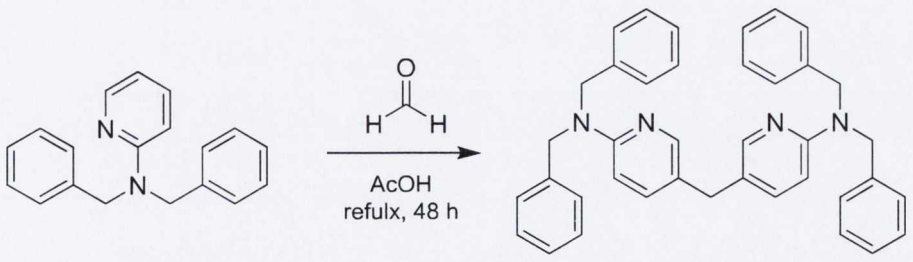
Scheme 3.2.2.4.



84 **86** 93%

Since the pyridine ring was now less susceptible towards EAS, it was found, after several reaction attempts, that a more forcing environment was required to achieve condensation. A rather comparable example in the literature used acetic acid to excel EAS reactions with substituted aminopyridines.[247] Thus, it was found that refluxing **86** in neat acetic acid with formaldehyde for 48 h gave the desired benzyl protected product **87** in good yield (Scheme 3.2.2.5).

Scheme 3.2.2.5.

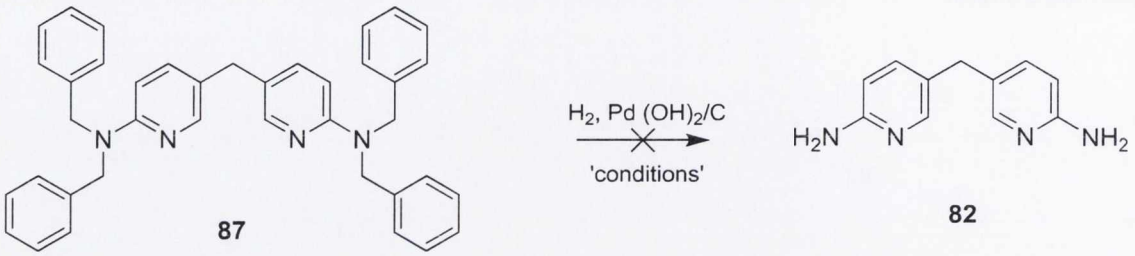


86 **87** 60%

Removal of the benzyl protecting groups *via* catalytic hydrogenation was a difficult process, as similarly seen with the benzylguanidine derivatives in Section 3.1.2.2. A large

optimisation protocol was followed in an attempt to achieve cleavage by systematically altering the pressure, temperature, time and solvent of the hydrogenation reaction and some key examples are summarised in Table 3.2.2.1.

Table 3.2.2.1. Table of key optimisation steps for *N*-debenzylation of **87** by hydrogenation.



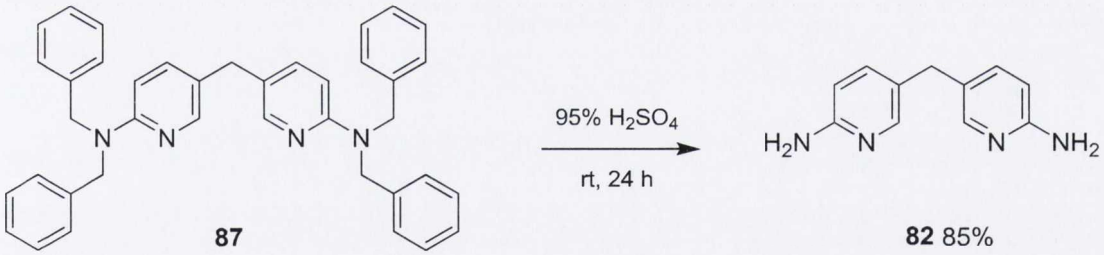
Entry	Pressure (atm)	Temperature (°C)	Time (h)	Solvent (0.1 M)	Result
1	1	rt	18	MeOH (20% AcOH)	No Rxn
2	3	rt	18	MeOH (20% AcOH)	No Rxn
3	6	rt	18	MeOH (20% AcOH)	Decomposition
4	6	rt	12	MeOH (10% AcOH)	Decomposition
5	6	rt	18	MeOH	No Rxn
6	6	rt	6	MeOH (10% AcOH)	Decomposition
7	1	50	18	MeOH	No Rxn
8	1	50	18	MeOH (20% AcOH)	No Rxn
9	NH_4HCO_2	50	72	MeOH	No Rxn
10	12	rt	72	MeOH	No Rxn

In particular, Pearlman's catalyst ($Pd(OH)_2/C$) was used in the optimisation as this is one of the most robust deprotection catalysts and is a well-established standard for *N*-debenzylation.[248] The starting material **87** was found only to be soluble in THF; however, initial attempts at hydrogenation in this solvent were abandoned as THF causes a premature cessation of H_2 uptake, preventing the reaction from progressing.[249] Hence, the reaction was

performed in MeOH at low concentrations to aid solvation, with heating used when required. As seen from Table 3.2.2.1 no conditions for conclusive *N*-debenzylation were found. High pressures (entry 5 and 10) of hydrogen as well as the use of *in situ* generation of hydrogen using ammonium formate, which proved successful in previous *N*-debenzylations (Section 3.1.2.2), gave no conversion to product. It is seen that when high pressures are combined with acidic conditions, which are known to catalyse *N*-debenzylations, decomposition of the product occurs.[250] In these examples, the ^{1}H-NMR and ^{13}C-NMR analysis of the crude mixture were absent of pyridine peaks; unfortunately identification of these degradation products was unsuccessful.

At this point an article was found detailing the *N*-debenzylation of 2-aminopyridines using acidic conditions alone. In this procedure the appropriate benzyl protected 2-aminopyridine is stirred in 95% sulphuric acid for 24 h affording the debenzylated product in excellent yield.[251] This exact method was applied to our *tetra*-benzyl protected 2-aminopyridine **87**, and encouragingly gave our desired product **82** in good yield (Scheme 3.2.2.6).

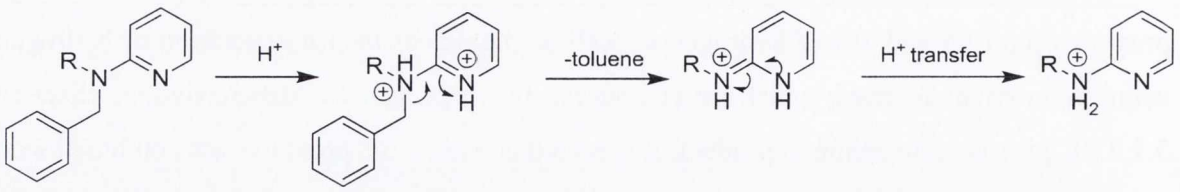
Scheme 3.2.2.6.



87 **82** 85%

Although not described in the article, the mechanism is likely to go through initial protonation of the amine and pyridine nitrogen, creating a highly electrophilic carbon at the 2 position of the pyridine ring to facilitate loss of toluene with subsequent re-aromatisation to the pyridine aromatic system (Scheme 3.2.2.7).

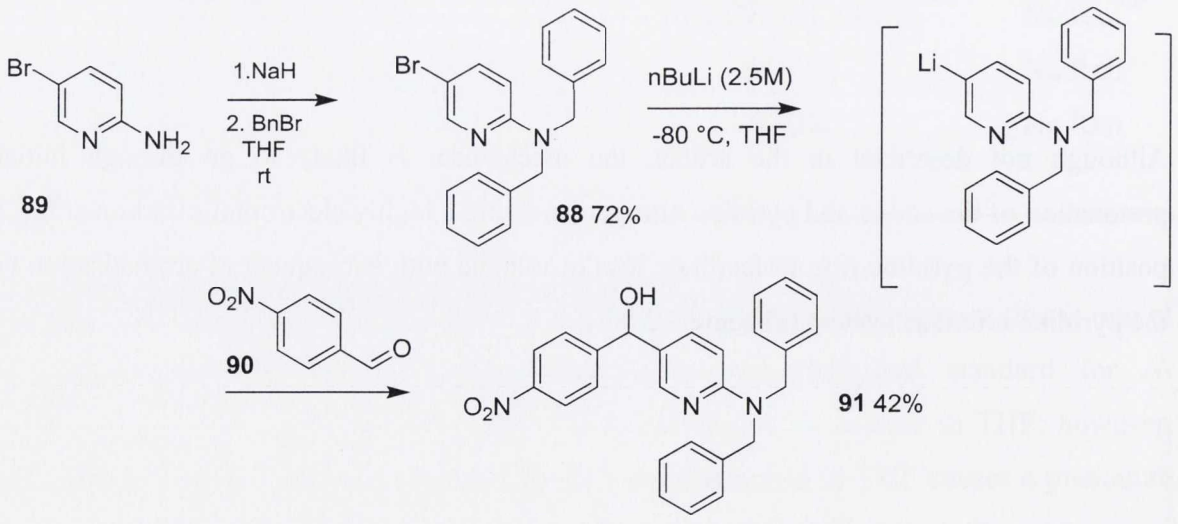
Scheme 3.2.2.7.



*Synthesis of phenylpyridinylmethane diamine **83***

First, the synthesis of the core di-aryl framework of diamine **83** was facilitated by lithium halogen exchange using *n*BuLi and dibenzyl protected 2-amino-5-bromopyridine **88**, which was synthesised in an analogous manner to compound **86** from 2-amino-5-bromopyridine **89**. Once lithium halogen exchange was observed by TLC analysis, the anion was reacted *in situ* with 4-nitrobenzaldehyde **90** to give compound **91** in reasonable yield (Scheme 3.2.2.8). Optimisation of this reaction was not carried out as most methods use similar conditions for anion formation and successive reactions; any alterations to the reaction conditions did not show much difference in yield.[252,253] It is notable that the reaction was found to be undesirably unpredictable most of the time, which may be attributed to issues with the reaction scale used, the actual concentration of *n*BuLi or dryness of solvent (as the dehydrodehalogenated starting material was observed).

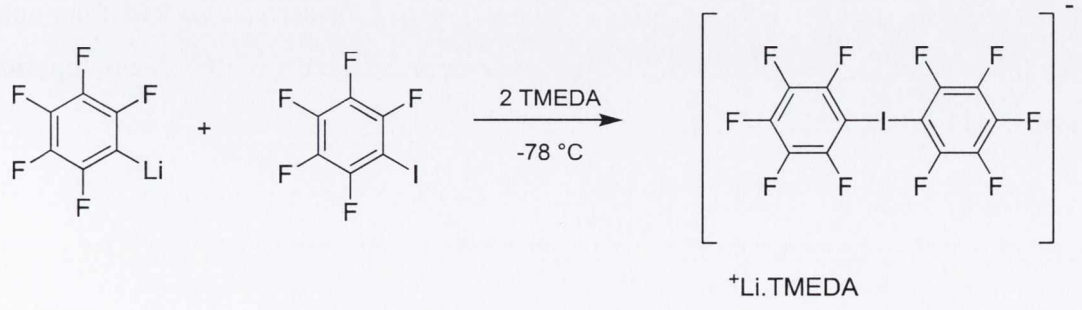
Scheme 3.2.2.8.



The reaction of halogen lithium exchange involves an equilibrium between the starting and product organolithium species, and the equilibrium will favour that organolithium system that can best stabilise the partial negative charge (i.e. the most acidic). Thus, in the above example the sp^2 hybridised pyridinyllithium product (pK_a = 41) will be more stable and, hence, favoured against the starting sp^3 hybridised butyllithium (pK_a = 50). In particular, for these reactions aryl iodides react much faster than aryl bromides while aryl chlorides are essentially inert.[254]

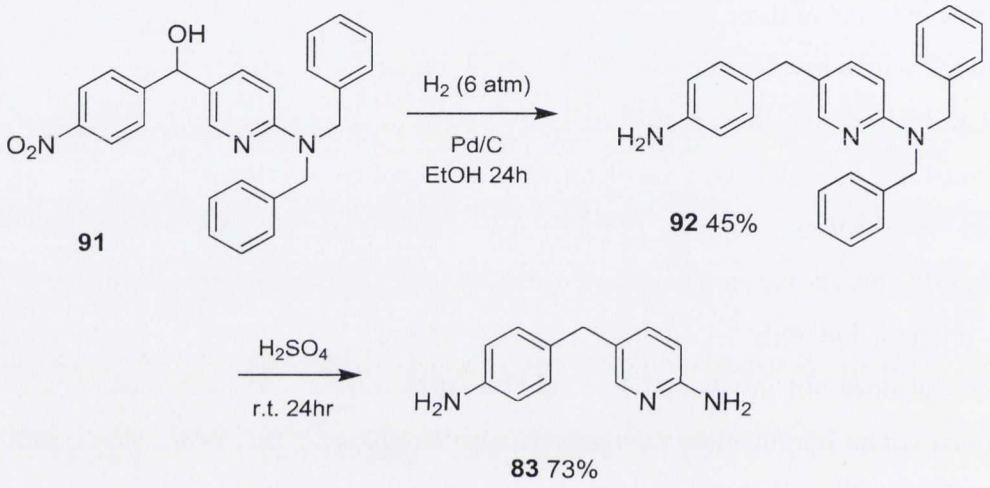
The mechanism of lithium-halogen exchange remains 'something of an enigma' as described by Bailey.[255] The results of experimental studies to understand this reaction have been interpreted in terms of three principal mechanisms. The first one is the four-centred transition state model, which involves concerted bond breaking and making between organolithium and organohalide; however, there is little, if any, direct evidence that supports a concerted process but it must be acknowledged as theoretical possiblility.[256] The next one is the radical-mediated mechanism, which describes the formation of radical organolithium and organohalide species *via* single electron transfer (SET). Experimental evidence supports a radical process, but only as a minor pathway as there still must exist a metal-exchange pathway that does not involve SET.[257] The last and most accepted one is the nucleophilic mechanism *via* an intermediate 'halogenate' type complex by way of the nucleophilic attack of the carbanion-like alkyl lithium onto the halogen of the aryl halide, where the intermediate "ate-complex" can undergo further reactions with electrophiles.[258] Furthermore, this pathway was proved by Farnham and Calabrese who obtained the X-ray crystal structure of a hypervalent (10-I-2) iodine compound having the exact structure of the "ate-complex" using tetra-methylethylenediamine (TMEDA) as a complexing agent (Scheme 3.2.2.9).[259]

Scheme 3.2.2.9.



Once compound **91** was obtained, a dual reduction of the nitro and alcohol groups was carried out under hydrogenation conditions (6 atm) using Pd/C and yielding almost 50:50 ratio of the desired product **92** and the intermediate amino alcohol **93** (Scheme 3.2.2.10). Attempts at employing acidic systems to catalyse alcohol reduction lead to degradation of the pyridine ring as seen before. As expected, the benzyl groups were not affected under these conditions and they were removed, similarly to compound **87**, using concentrated H_2SO_4, to give diamine **83** (Scheme 3.2.2.10).

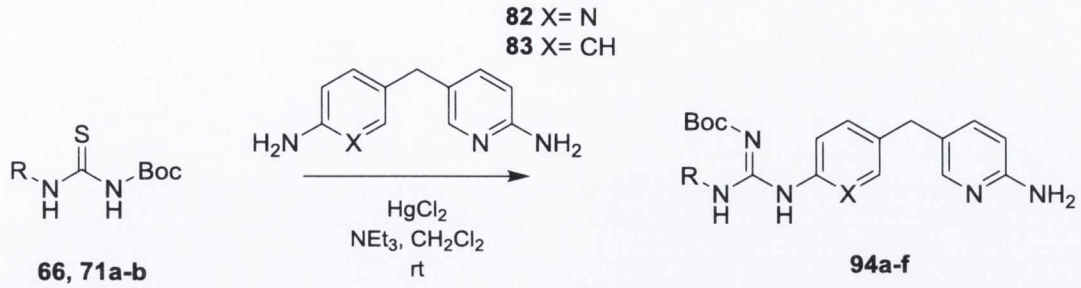
Scheme 3.2.2.10.



3.2.2.3 Synthesis of the hydrochlorides of the pyridinyl guanidine and 2-aminoimidazoline di-aryl derivatives: Families E1, E2 and E3

In the case of compounds from Families E1 and E2, *mono*-guanidylation of diamines **82** and **83** was first carried out by the standard mercury (II) chloride method, using excess of the amine in the presence of the appropriate Boc protected thiourea (**66** and **71a-b)** and triethylamine. As seen in Table 3.2.2.2, the yields obtained for derivatives **94d-f** are notably higher than **94a-c**, as expected, due to the poor nucleophilicity of the 2-aminopyridines compared with aniline.

Table 3.2.2.2. *Mono*-guanidylation reactions, using Mercury (II) chloride, to yield derivatives **94a-f**.



66, 71a-b **94a-f**

Compound	X	R	Yield (%)
94a	N	-Boc	42
94b	N	furfuryl	43
94c	N	$-(CH_2)_2-CH_3$	38
94d	CH	-Boc	83
94e	CH	furfuryl	64
94f	CH	$-(CH_2)_2-CH_3$	63

When analysing the data obtained for these guanidines, it was noted that the ^{1}H-NMR spectrum for the *bis*-Boc-pyridin-2-ylguanidine **94a** showed a significant difference for the H-3 peak compared to the *N*-Boc-*N'*-substituted pyridin-2-ylguanidines **94b-c** where a broadening and high field shift of the signal was observed (Figure 3.2.2.3).

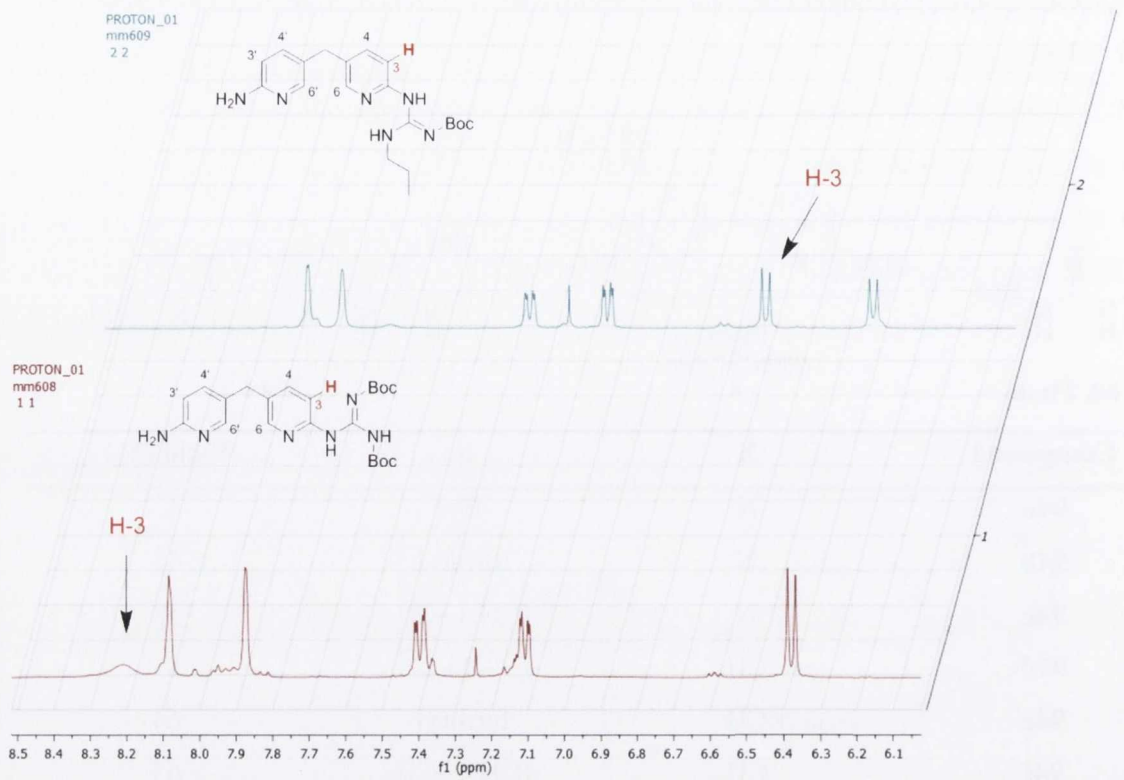


Fig. 3.2.2.3. ^{1}H-NMR comparing the H-3 signal of compounds **94c** (*top*) and **94a** (*bottom*).

Our group has recently published an article discussing the conformational control induced by intramolecular hydrogen-bonding (IMHB) interactions in 2-pyridoguanidine systems by performing a systematic conformational analysis at the B3LYP/6-31+G(d,p) computational level.[165] This research showed that the conformational preference of the *N,N'*-di-Boc substituted 2-aminopyridines is for the *anti*-configuration as this is the most stable conformer by a significant energetic margin. The H-3 of the pyridine ring will hence be involved in IMHB to the guanidine nitrogen which will induce the distortion observed in the ^{1}H-NMR spectra (Figure 3.2.2.4). The *N*-Boc-*N'*-substituted pyridin-2-ylguanidines, however, will adopt the more stable *syn* conformation, which will not affect H-3, having two possible tautomeric forms with the imine bond localised to the *N*-aminopyridine or the *N*-Boc (Figure 3.2.2.4). It is notable that for compounds **94b-c** two *syn* isomers were not observed in the ^{1}H-NMR, yet are seen for similar compounds discussed later.

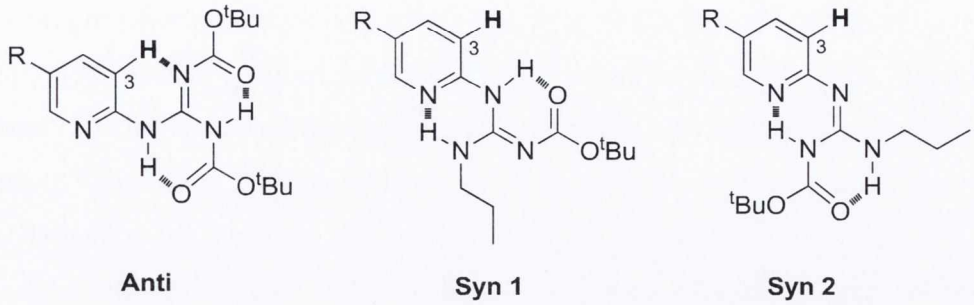
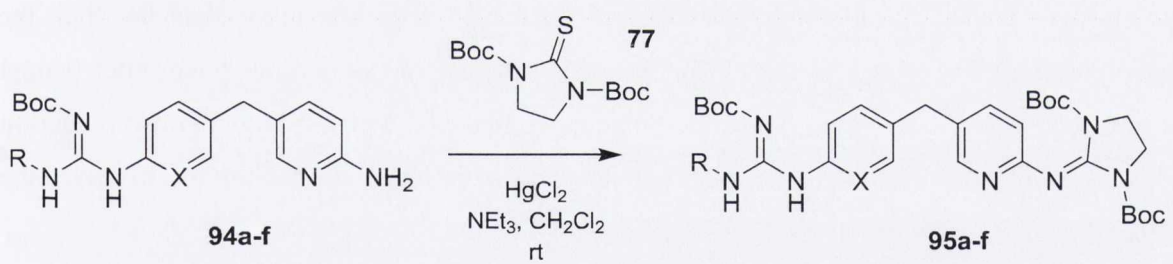


Fig. 3.2.2.4. Conformational preferences of the *N,N'*-di-Boc substituted pyridin-2-ylguanidines (*anti*), *N*-Boc-*N'*-substituted pyridin-2-ylguanidines (*syn*).

Next, the *bis*-Boc 2-iminoimidazolidine moiety was installed using *N,N'*-di-(*tert*-butoxycarbonyl)imidazolidine-2-thione **77** in the presence of mercury (II) chloride and triethylamine to give the Boc protected products **95a-f** (Table 3.2.2.3).

Table 3.2.2.3. Preparation of the Boc protected *N*-substituted guanidine/2-iminoimidazolidines **95a-f** (Families E1 and E2).

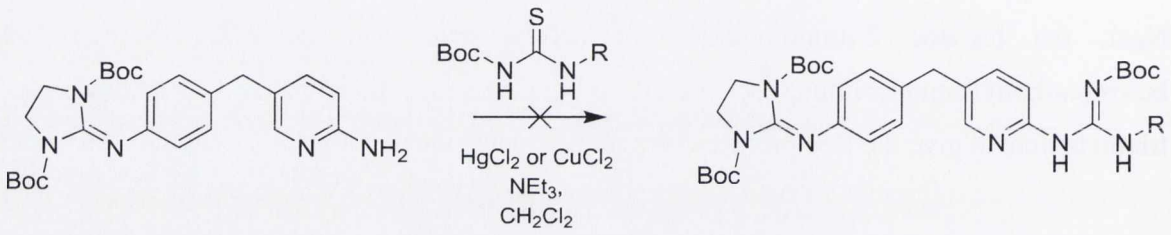


Compound	X	R	Yield (%)
95a	N	-Boc	62
95b	N	-furfuryl	63
95c	N	$-(CH_2)_2-CH_3$	66
95d	CH	-Boc	89[a]
95e	CH	-furfuryl	97[a]
95f	CH	$-(CH_2)_2-CH_3$	99[a]

[a]Isolated yield without purification due to acid sensitivity (see Section 3.2.1.6).

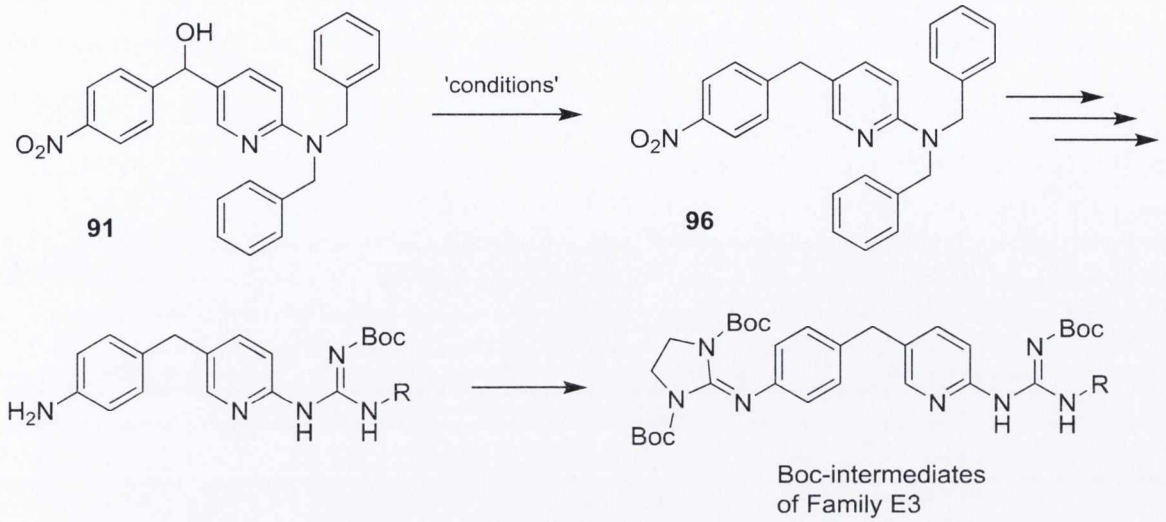
For the synthesis of the Family E3 of pyridin-2-ylguanidines from the asymmetric amine **83**, the *bis*-Boc 2-iminoimidazolidine functionality must be introduced first as the aniline amino group is more reactive than the 2-aminopyridine amine. Unfortunately in this case, the 2-iminoimidazolidine moiety is not stable under the conditions of the next step of guanidylation and leads to degradation products under both mercury (II) chloride and copper (II) chloride conditions (Scheme 3.2.2.11).

Scheme 3.2.2.11.



To overcome this problem, it was thought that if selective reduction of the alcohol of previously synthesised compound **91** could be achieved affording compound **96**, then the nitro group could act as a precursor for the amino moiety of the aniline. Then, after benzyl deprotection, the 2-aminopyridine could be guanidylated first with subsequent nitro reduction and guanidylation with *N,N'*-di-(*tert*-butoxycarbonyl)imidazolidine-2-thione to give the desired intermediates of Family E3 (Scheme 3.2.2.12).

Scheme 3.2.2.12.

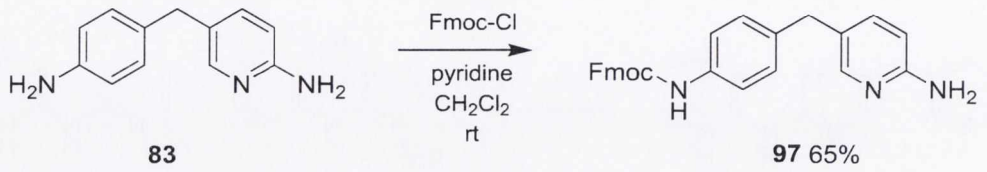


The traditional way of accomplishing the transformation of alcohol or carbonyl to alkane is *via* the Clemmensen reduction[260] (using zinc and mercury) or the Wolff-Kishner reduction[261] (using hydrazine). However, both of these methodologies have their limitations in our particular case, as they will not be selective for the alcohol functionality over the nitro group. In recent years, sodium borohydride has become the reductant of choice for the conversion of alcohols to alkanes, and this was preferable in our case as it would not affect the nitro functionality. Aryl substituted alcohols, like **91**, can be deoxygenated by using sodium borohydride when electrophilic assistance (e.g. combinations with trifluoroacetic acid) is employed.[262] This was attempted with our starting alcohol, yet no result was obtained after numerous efforts. It was thought that, under these conditions, the nitro functionality was preventing the SN_1 mechanism of deoxygenation through its strong electron withdrawing effect, although it was thought that the 2-aminopyridine would aid the reduction *via* electron donation. We thus tried first activating the alcohol by acetylation, mesylation and tosylation to make it a better leaving group and facilitate reduction. However, none of the listed methods gave any result when sodium borohydride was employed. Combinations of aluminium trichloride with sodium borohydride, which have shown to be effective for the deoxygenation of diaryl alcohols, similarly gave no result for our system.[263]

The lack of literature precedent for the reduction of alcohols substituted with *para*-nitroaryl groups or 2-aminopyridines lead us to abandon this route. Alternatively, it was decided to add

a facile orthogonal protection step to the original asymmetric amine **83**. This was achieved using fluorenylmethyloxycarbonyl chloride (Fmoc-Cl) and pyridine, which gave the Fmoc protected product **97** in good yield (Scheme 3.2.2.13).

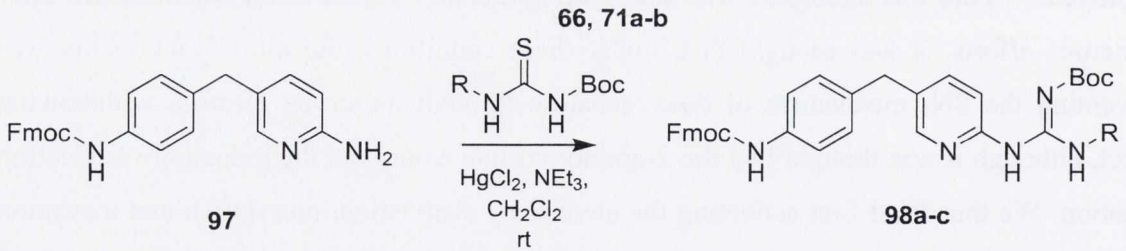
Scheme 3.2.2.13.



83 **97** 65%

With the aniline amino group now protected, guanidylation with the appropriate thiourea was performed to give derivatives **98a-c** in reasonable yield (Table 3.2.2.4). In this case, the *N*-Boc-*N'*-substituted pyridin-2-ylguanidines **98b-c** show existence in the ^{1}H-NMR as two *syn* conformational isomers with the imine bond localised to the *N*-pyridine or the *N*-Boc in the ratio of 1:1.2; which is known to occur as previously discussed (see Experimental section).

Table 3.2.2.4. Synthesis of the Fmoc and Boc protected *mono-* and di-substituted guanidine diaryl derivatives **98a-c**.

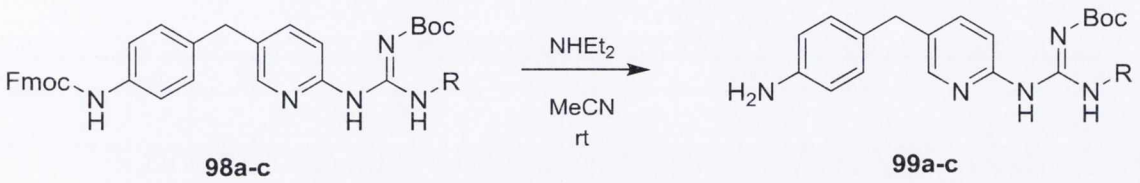


97 **98a-c**

Compound	R	Yield (%)
98a	-Boc	40
98b	furfuryl	48
98c	$-(CH_2)_2-CH_3$	48

Deprotection of the Fmoc group was easily achieved by stirring compounds **98a-c** in a large excess of diethylamine for 1 h, which effectively removed the Fmoc system without affecting the Boc protecting group, affording the desired anilines **99a-c** in excellent yield (Table 3.2.2.5).[264]

Table 3.2.2.5. Conditions for deprotection of Fmoc protecting group.

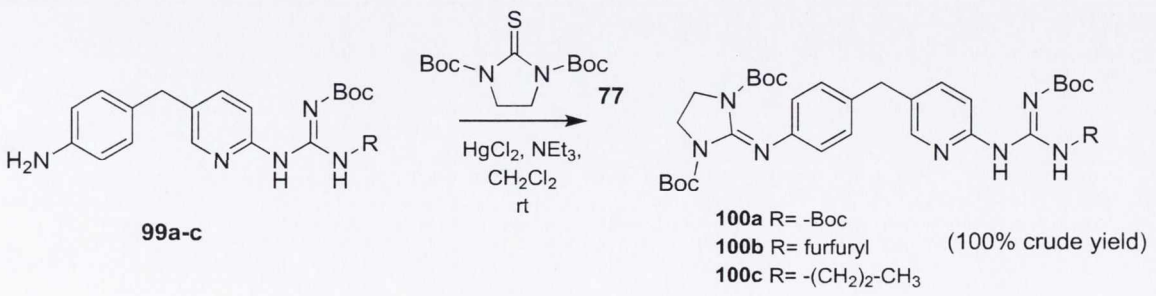


98a-c **99a-c**

Compound	R	Yield (%)
99a	-Boc	98
99b	furfuryl	97
99c	$-(CH_2)_2-CH_3$	90

Lastly, the now free aniline amino groups of **99a-c** were guanidylated with *N,N'*-di-*(tert*-butoxycarbonyl)imidazolidine-2-thione **77** in the presence of mercury (II) chloride and triethylamine to give products **100a-c**. It needs to be noted that Scheme 3.2.2.14 displays the crude yields obtained, as these derivatives showed extremely high acid sensitivity, even using base treated silica gel chromatography, thus further purification was not carried out.

Scheme 3.2.2.14



99a-c

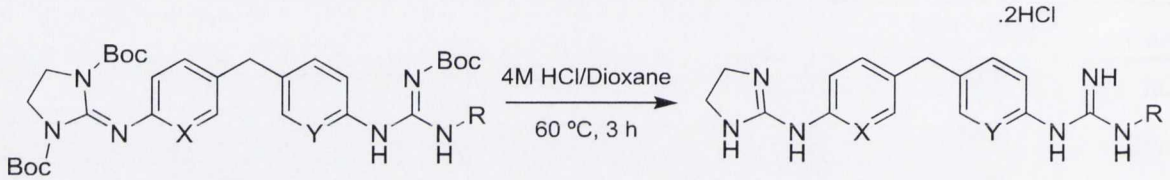
100a R= -Boc
100b R= furfuryl
100c R= $-(CH_2)_2-CH_3$

(100% crude yield)

Removal of the Boc groups of all pyridin-2-ylguanidine derivatives was performed in an analogous manner to Family D using 4M HCl/dioxane to give the corresponding hydrochlorides **101a-i** in excellent yield (Table 3.2.2.6).

These pyridin-2-yl guanidiniums exist in the *syn* conformation, due to the already mentioned IMHB interactions; thus, compounds that had displayed changes of the pyridine H-3 through the *anti*-conformational effects, returned to normal ^{1}H-NMR shifts. Additionally, compound **100b-c** no longer existed as two *syn* isomers as the double bond is now effectively delocalised around the guanidinium centre (see Experimental section).

Table 3.2.2.6. Preparation of the hydrochlorides **101a-i**: Families E1, E2 and E3.



95a-f, 100a-c **101a-i**

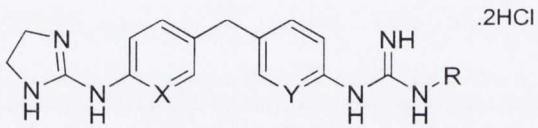
Compound	X	Y	R	Yield (%)
101a	N	N	H	92
101b	N	N	furfuryl	82
101c	N	N	$-(CH_2)_2-CH_3$	85
101d	N	CH	H	98
101e	N	CH	furfuryl	80
101f	N	CH	$-(CH_2)_2-CH_3$	87
101g	CH	N	H	82
101h	CH	N	furfuryl	90
101i	CH	N	$-(CH_2)_2-CH_3$	98

3.2.2.4 Pharmacological evaluation of Families E

Binding Affinity towards α_2-AR

The affinity towards the α_2-AR of compounds in Families E was measured by competition assays *in vitro* using human brain frontal cortex tissue. The α_2-AR affinities obtained are shown in Table 3.2.2.7 and are expressed in terms of pK_i values using RX821002 acting as the control ligand.

Table 3.2.2.7. Binding affinities for the α_2-AR of all derivatives of Families E, expressed in pK_i values.



Family E, 101a-i

Compound	Family	X	Y	R	pK_i
RX821002	-	-	-	-	9.10
1	-	-	-	-	8.80
101a	E1	N	N	H	6.86
101b	E1	N	N	furfuryl	6.58
101c	E1	N	N	$-(CH_2)_2-CH_3$	7.05
101d	E2	N	CH	H	6.17
101e	E2	N	CH	furfuryl	6.36
101f	E2	N	CH	$-(CH_2)_2-CH_3$	6.92
101g	E3	CH	N	H	7.06
101h	E3	CH	N	furfuryl	7.47
101i	E3	CH	N	$-(CH_2)_2-CH_3$	7.17

An evident trend is seen across the pK_i values in Table 3.2.2.7 for the pyridine-2-yl guanidine series. It is notable that compounds **101g-h** (Family E3), where IMHB is occurring only through the pyrindin-2-yl guanidine moiety, exhibited very good affinity for the α_2-AR. These affinity values were substantially larger than those of the *bis*-pyridin-2-yl derivatives

101a-c (Family E1) and the pyridinyl 2-aminoimidazolines **101d-f** (Family E2), which are compounds that include conformational restriction of the 2-aminoimidazoline. This was expected as it has been demonstrated previously for monomeric analogues that the pyridinyl 2-aminoimidazolines give lower binding affinities.[162] Thus, when this moiety is non-restricted (i.e. not interacting in IMHB), it is beneficial for the pharmacological profile of the molecule. This is in agreement with our previous work indicating that the 2-aminoimidazoline group is required alone for higher binding to the α_2-AR. Nonetheless, compounds **101c** and **101f**, with the 2-aminoimidazoline constrained, still display good affinity for the α_2-AR with pK_i values in the range of those previously shown by lead monomeric phenylguanidines (see Section 1.6).

Discouragingly, a significant drop in affinity is observed across Families E compared to Family D2, which suggests that, for the entire di-aryl series, rigidity of the cationic moiety is unfavourable, unlike the monomeric di-substituted pyridine-2-ylguanidines. Nevertheless, it must be emphasised that the principal reasoning behind the introduction of the pyridine series is for desired α_2-AR functional activity not for the affinity.

Functional activity

Compounds **101c**, **101f-i** all exhibited pK_i values > 6.9 and, thus, were subjected to [^{35}S]GTPγS binding assays to determine their nature as α_2-AR agonists or antagonists. Encouragingly, as predicted within the pyridinyl series, compounds **101c**, **101f**, **101g** and **101i** showed no stimulation of [^{35}S]GTPγS indicating antagonist activity (Figure 3.2.2.5). Interestingly, compound **101h**, which was the pyridin-2-ylguanidine with best α_2-AR affinity, stimulated [^{35}S]GTPγS binding at high concentrations, suggesting that it exhibits agonist or, like compounds in Family D2, partial agonist functional activity.

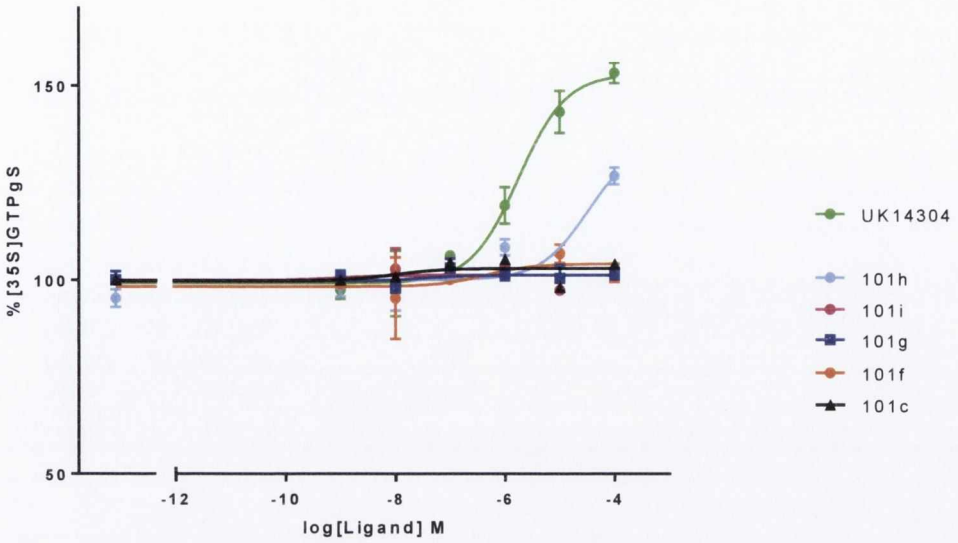


Fig. 3.2.2.5. [^{35}S]GTPγS binding assay of compounds **101c**, **101f**, **101g**, **101i,** and **101h.**

All six compounds were subsequently exposed to the second [^{35}S]GTPγS binding assay against the selective α_2-AR agonist UK14304 (as seen in Section 3.2.1.7). Here, co-administration of the testing compound and the agonist (UK14303) should result in an increase in the EC_{50} of the agonist, confirming competitive antagonist binding properties. Indeed, compounds **101c**, **101f**, **101g** and **101i** significantly induced a rightward shift (increase EC_{50}) in the binding curve of UK14304, which promisingly signified that they all act as competitive antagonists (Figure 3.2.2.6). Surprisingly, compound **101h** also showed antagonist properties by significantly blocking the binding of the agonist UK14304, which would suggest that it may be acting as a partial agonist similarly to compounds **79a** and **79b** of Family D2 (see Section 3.2.1.7).

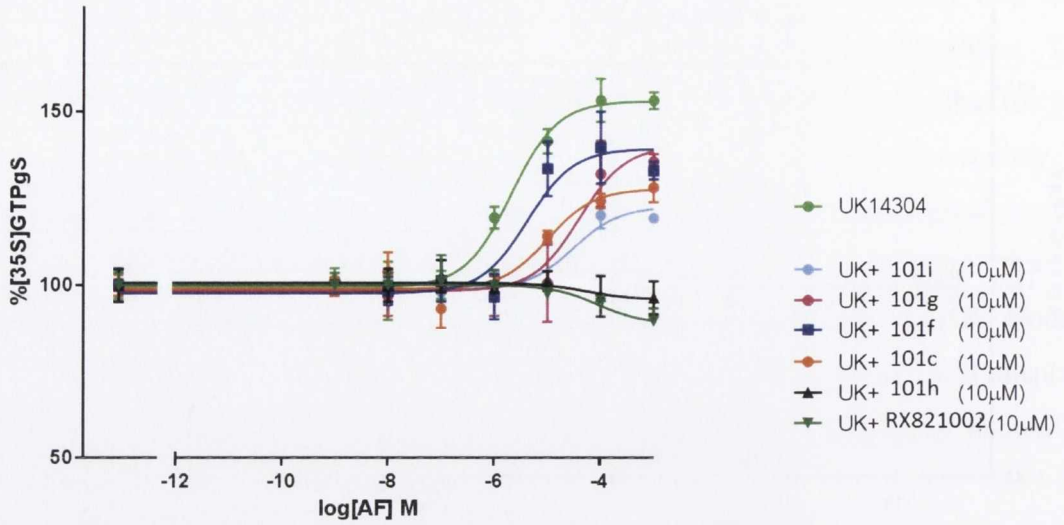


Fig. 3.2.2.6. [^{35}S]GTPγS binding assay of compounds **101i**, **101g, 101f, 101c, 101h** and RX821002 with co-administration of α_2-AR agonist UK14304- all demonstrating competitive binding properties.

Compound **101h** was then subjected to a third [^{35}S]GTPγS binding assay to observe if its stimulation of [^{35}S]GTPγS would be blocked by the selective α_2-AR antagonist, RX821002, which would confirm its functional activity as a partial agonist. Figure 3.2.2.7 illustrates the binding curve of compound **101h** alone and with co-administration of RX821002, where it is evident that RX821002 does not block compound **101h**'s stimulation of [^{35}S]GTPγS.

These results strongly suggests that compound **101h** is acting as a potent α_2-AR antagonist, as it blocks the agonist activity of UK14304 and is not antagonised by RX821002, and that its stimulation of [^{35}S]GTPγS is perhaps the result of it exhibiting agonist properties at an alternative GPCRs such as metabotropic dopamine, serotonin or glutamate receptors.

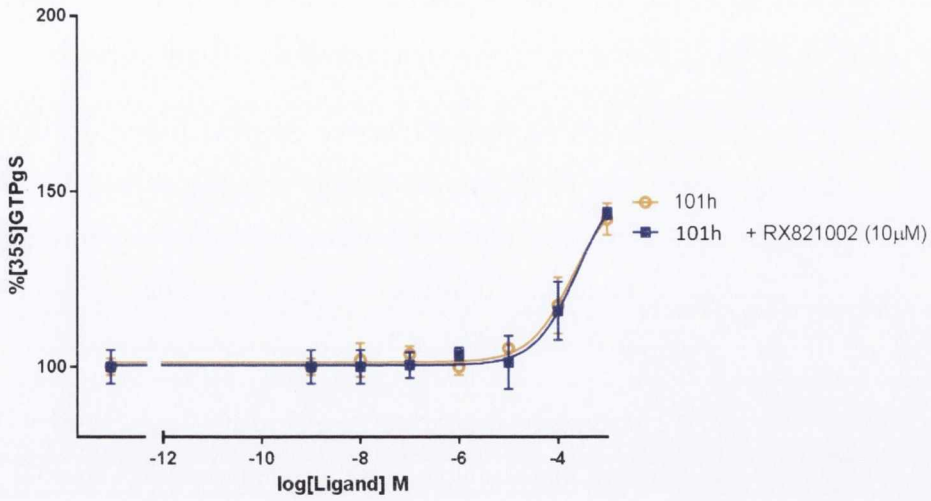


Fig.3.2.2.7. [^{35}S]GTPγS binding assay of compound **101h** with co-administration of the selective α_2-AR antagonist RX821002.

3.2.3. Structure-Activity Relationship obtained from comparison of biological results across Families D1-2 and E1-3

The di-aryl series comprising of Families D and E were originally developed with the intention of preparing novel molecules that while maintaining high binding properties will exhibit antagonist activity. Interesting results in terms of both affinity and activity have been obtained which has allowed the formulation of a thorough structural activity relationship. This SAR has been developed to determine the key functional requirements within the core di-aryl framework to develop and improve future α_2-AR ligands possessing both the desired affinity and activity.

3.2.3.1. Binding affinity

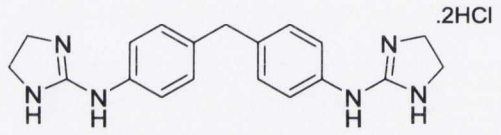
The most evident essential structural feature in terms of affinity, which is highlighted in both Families D and E, is the requirement for the 2-aminoimidazoline moiety on at least one half of the molecule. If this functionality is completely removed from the core structure (i.e. Family D1 where it is replaced for a di-substituted guanidine) or is involved in conformationally restriction (i.e. Family E1 and E2 through its IMHB to the pyridine) a drop

in affinity is observed. Thus, one free 2-aminoimidazoline moiety should be included on one side of the molecule in all future *bis*-aryl systems to maintain higher affinity ligands.

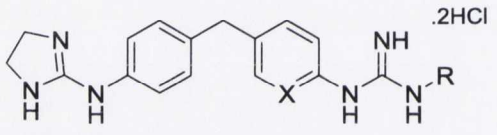
In general, the methylene linker should be retained, as the ethylene linked derivatives in Family D2 induced a significant drop in affinity, indicating that larger flexibility in this position is not necessary for strong binding. Moreover, previous results have illustrated that other linkers (NH, CO, O) also yield inferior results than the methylene linked present in lead compound **1**.[155]

The two subfamilies that gave the best results in terms of α_2-AR affinity are Families D2 and E3, and the corresponding pK_i values of these compounds are summarised in Table 3.2.3.1.

Table 3.2.3.1. Comparison of pK_i values across highest binding subfamilies D2 and E3.



1



Family D2- **79a-c**
Family E3- **101g-i**

Compound	Family	X	R	pK_i
Compound 1	-	-	-	8.80
79a	D2	CH	-H	7.91
79b	D2	CH	-furfuryl	8.78
79c	D2	CH	$-(CH_2)_2-CH_3$	9.10
101g	E3	N	-H	7.06
101h	E3	N	-furfuryl	7.47
101i	E3	N	$-(CH_2)_2-CH_3$	7.17

As seen from Table 3.2.3.1 there is no evident trend for pK_i values across differing R substituents. It was originally thought that steric extension (di-substitution) at the guanidine was tolerated within the pyridine-2-yl systems, as the previously developed monomeric di-substituted pyridoguanidines gave improved α_2-AR binding affinities compared to their corresponding di-substituted phenylguanidine counterparts. Interestingly, within the *bis*-aryl

series the opposite is seen, as Family D2 gave significantly stronger affinities for the α_2-AR than Family E3. Moreover, di-substitution on previously developed *mono*-cationic analogues usually results in a large drop in affinity compared to *mono*-substitution. Yet the opposite is observed in Family D2, as the mono-substituted analogue **79a** has a lower affinity compared to the di-substituted guanidines **79b-c**. These unexpected observations in Family D2 and E3 may be attributed to the presence of the two cationic moieties which are able to interact at alternate sites within the receptor pocket. It is hypothesised, through computational experiments, that our guanidines can interact within the α_2-AR pocket either electrostatically, *via* a negatively charged Asp^{113} residue on TM3, or through hydrogen bonding interactions to two $Ser^{200/204}$ residues on TM5, yet these compounds cannot interact with both sites due to distance restrictions. Thus, the two cationic functionalities of Families D2 and E3 may occupy either 'interaction site' within the receptor, and this will convey differing characteristics to the molecules in terms of their affinity and activity.

3.2.3.2. Functional activity

The incorporation of two structural modifications taken from previous studies: (i) di-substituted guanidines, and (ii) 2-guanidinopyridines and the 2-(2-aminoimidazolino)pyridines, have led to α_2-AR antagonists. Indeed, these structural changes have led to molecules with exclusive antagonist activity within Family D1 and all subfamilies of Family E. More interesting are the results within the Family D2. Here the *mono*-substituted guanidine derivative **79a** displays favoured antagonist activity while the di-substituted guanidine derivatives were found to be partial agonists. This was an unexpected result because previously developed mono-cationic di-substituted guanidines have exhibited exclusive antagonist activity, while the opposite is observed for *bis*-cationic Family D2. This unpredicted result again may be attributed to differences in each cationic binding to either interaction site within the α_2-AR pocket, and future docking studies will need to be performed to understand this effect in more detail.

Nonetheless, from all the information gained a comprehensive SAR has now been developed for the entire di-aryl series towards the development of novel strong binding α_2-AR antagonist and is summarised in Figure 3.2.3.1.

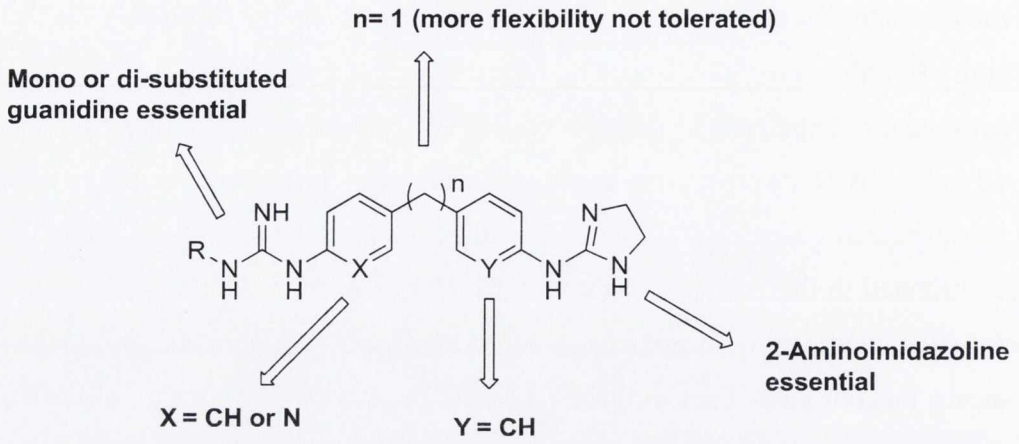


Fig. 3.2.3.1. SAR of essential features for strong binding to, and antagonist activity at the α_2-AR.

Chapter 4 - Conclusions and Future Work

4.1. Conclusions

In this project, thirty six novel guanidine derivatives have been prepared as α_2-AR ligands (34 of them are α_2-AR antagonists) for potential use as antidepressants and high-affinity binders. These compounds are categorised into five new families including *mono*-cationic:

(i) Cyclic guanidine derivatives of *N*-substituted 1,4-dihydropyridopyrimidin-2-amines, *N*-substituted 1,4-dihydroquinazolin-2-amines and *N*-substituted-2-amino-4,5-dihydro-1,3-benzodiazepines (Families A, B and C);

as well as di-cationic:

(ii) *Bis*-aryl guanidine derivatives of *N,N'*-(di)(*mono*)-substituted guanidine and 2-aminoimidazolines (Family D) and *N,N'*-(di)(*mono*)-substituted pyridin-2-ylguanidines and pyridinyl 2-aminoimidazolines (Family E).

These molecules were carefully designed using combinations of computational predictions and pharmacological results previously obtained in the group, along with rational ligand based design for certain compounds that displayed new and more complex structures.

For the cyclic guanidine series, Family A was prepared using known literature procedures already established in our group; however, for Families B and C completely new synthetic pathways had to be developed to prepare the necessary guanidinium hydrochlorides. These routes predominantly involved preparing the corresponding aryl diamines from available starting materials and subsequently modifying known methods to promote cyclisation with a given thiourea-type derivative. Due to the copious variation between the structures of the cyclic series no complete pathway was the same between families and often sub-families, as presented in Chapter 3. Thus, a large number of novel substituted aryl diamine derivatives and guanidine intermediates have been prepared within this project which have not been

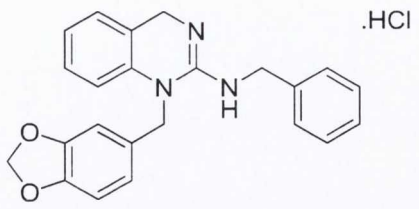
reported in the literature to date. Many of these compounds could be used as useful building blocks towards alternate drug structures due to their versatile functional cores.

In the case of the *bis*-aryl Families D and E, all compounds were synthesised using the mercury(II) chloride meditated coupling of an aryl amine with the appropriate thiourea derivative. In particular, for Family E the necessary amines that were not commercially available were synthesised using novel synthetic pathways yielding new pyridin-2-yl amines that had not been previously reported in the literature.

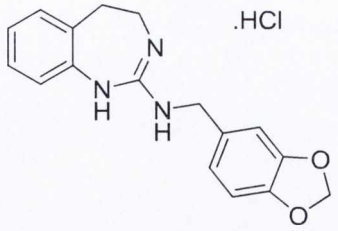
The binding affinities of all 36 compounds for the α_2-AR were assessed using competition binding assays with the selective α_2-AR radioligand [^{3}H]RX821002. For the cyclic series of Families B, B' and C, although in most of the cases the range of α_2-AR affinity was relatively low, all compounds gave higher pK_i values than their original cyclic analogues of *N*-substituted 2-amino-1,4-dihydroquinazolines (Family O). Additionally, all these derivatives display antagonist activity as assessed by functional [^{35}S]GTPγS binding experiments. A number of important structural features were hence highlighted by this research:

1) When an R substituent is on the intracyclic guanidine amino group attached to the aromatic ring (Family B) as opposed to the exocyclic amino group (Family O), higher binding affinities are obtained.
2) When the cyclic guanidine derivatives are di-substituted (Family B') enhanced affinities are obtained compared to Families O and B.
3) When added flexibility is incorporated into cyclic guanidine system, like the 7 membered ring derivatives of Family C, overall higher binding affinities are observed compared to their 6-membered cyclic analogues (Families O and B).

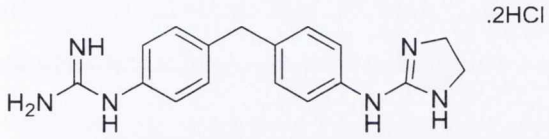
Furthermore, two cyclic derivatives (**40g** and **50c**, Figure 4.1.1), which are α_2-AR antagonists, showed extremely promising pK_i values (6.64 and 6.57). The α_2-AR affinity values for these compounds were in fact larger than all corresponding acyclic *mono*-cationic *N,N*-di-substituted guanidines previously developed.



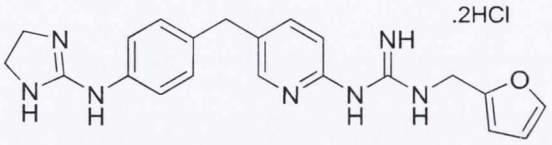
40g, pK_i 6.64 (Family B')



50c, pK_i 6.57 (Family C)



79a, pK_i 7.91 (Family D2)



101h, pK_i 7.47 (Family E3)

Fig. 4.1.1. Structures of new lead antagonists of cyclic (*top*) and *bis*-aryl (*bottom*) guanidine derivatives prepared in this research.

For the *bis*-aryl series (Families D and E), the α_2-AR binding affinities were much higher having pK_i values in the range of 6.04-9.10. The two strongest α_2-AR binding ligands (**79b** and **79c** with pK_i = 8.78 and 9.10, respectively) were interestingly found to be partial agonists of the α_2-AR, which is the first time this functional activity has been observed in our laboratory. Moreover, two compounds in particular (**79a** and **101h**, Figure 4.1.1) gave excellent affinities (pK_i = 7.91 and 7.47) and were also found to be α_2-AR competitive antagonists. These two compounds are the highest binding antagonist ligands in this research and to date in our group. They are now seen as the two new lead compounds for future design.

The promising results obtained across Families D and E, in terms of both affinity and activity, highlight the importance of the *bis*-aryl core and the requirement of at least one 2-aminoimidazoline moiety on one side of the molecule in order to obtain ligands with excellent binding affinities and potent antagonist activity at the α_2-AR.

4.2. Future work

4.2.1. Pharmacology

Five compounds from the *bis*-aryl series (from Families D2 and E) demonstrated competitive antagonist activity and exhibited excellent binding affinities with pK_i values ranging from 7.05 to 7.91. The efficacy of these derivatives will thus be measured *in vivo* using microdialysis to illustrate their effectiveness at increasing the synaptic extracellular concentration of NA through systemic and local administration in rats.

The pharmacological antidepressant profile of those compounds that demonstrate promising results at increasing NA through microdialysis will be further tested *in vivo* through animal behavioural models. Specifically our collaborators use the Forced Swimming Test (FST) and Tail Suspension Test (TST) as these are the two more widely-used behavioural paradigms for the evaluation of antidepressant-like activity. The molecules efficiency in these models of stress will be measured against the widely-known SSRI fluoxetine (Prozac) and the clinically available α_2-AR antagonist mirtazapine (Remeron).

4.2.2. Docking studies

In parallel with this research, Dr. Brendan Kelly has constructed two homology models of the human α_{2A}AR and α_{2C}AR subtypes (which are the most prevalent subtypes in the human CNS and seem to be involved in depression) based on three template crystallographic structures of the homologous β_2-adrenoceptor.[162] Examples of all previous acyclic and cyclic *mono*-cationic guanidines (including Family A from this research) have been docked into this model to gain insight towards a comprehensive SAR for these derivatives; however, the *bis*-aryl systems have not yet been docked into these models. Based on the interesting and differing pharmacological results found for these di-cationic analogues it seems extremely important for future work to obtain docking information of these systems. This will allow a complete understanding of the interactions of each cationic moiety of the di-cationic system with the different amino acid residues in the receptor binding pocket to aid future design in the progression of the *bis*-aryl series.

4.2.3. Future α_2-AR antagonists within the guanidine-like family

As seen in Sections 3.1.4 and 3.2.3, a thorough SAR has been developed for both the cyclic and *bis*-aryl guanidine families, highlighting some evident features that should be explored for future work to obtain more potent antagonist ligands of the α_2-AR.

1) For the cyclic analogues (Families A to C), steadily increasing α_2-AR binding affinities have been obtained by rationally modifying the functional positioning and flexibility of the system to gain optimal interactions of the substituents within the α_2-AR binding pocket. Thus, this information gained from the SAR should be combined to create a novel family of cyclic guanidines (Figure 4.2.1). These derivatives should incorporate the extra CH_2 within the ring system (like compounds in Family C) for enhanced flexibility and include di-substitution on the guanidine (like Family B') which has also shown to augment α_2-AR affinity.

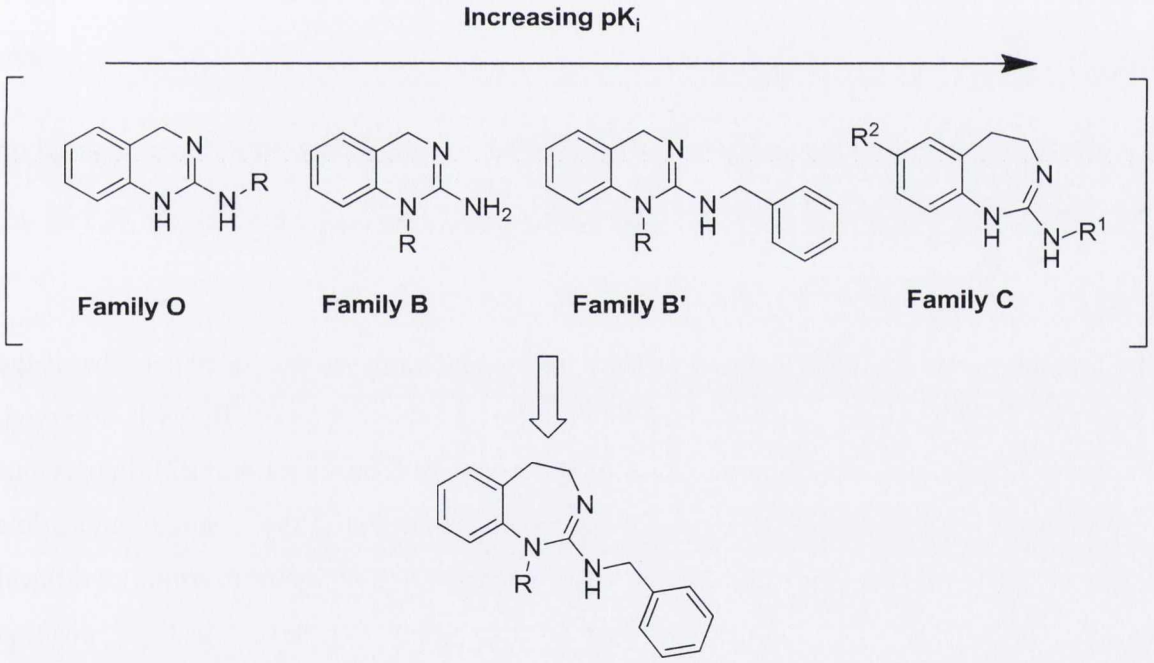


Fig. 4.2.1. Combination of the essential features of all cyclic guanidines to create a novel high binding cyclic guanidine family of α_2-AR ligands.

2) From the results obtained in the cyclic guanidine series, it has become evident that altering the positions of substituents on the guanidine moiety significantly enhances the molecules' binding properties, in particular, if the amino group attached to the aromatic system is functionalised over the exocyclic amino group. Altering the substitution pattern has not yet been attempted for any acyclic derivative, *mono*- or di-cationic. We hypothesise that encompassing this modification of 'interior' guanidine substitution onto the core *bis*-aryl framework may lead to a further enhancement in α_2-AR affinity and also help to gain additional information on the SAR of these acyclic systems (Figure 4.2.2).

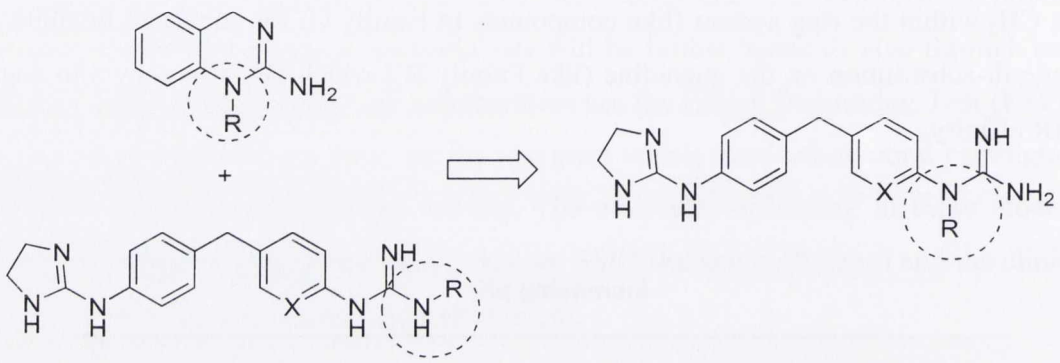


Fig. 4.2.2. Probing for enhanced α_2-AR affinity of *bis*-aryl ligands with R functionalised on the amino guanidine attached to the aromatic ring.

3) Lastly, from the SAR deduced of the pyridinoguanidine series it is evident that if the 2-aminoimidazoline moiety is constrained through IMHB a large drop in affinity is observed, as seen for the pyridin-2-yl(2-aminoimidazoline) derivatives (Family E1 and E2). In previous work with the *mono*-cationic analogues, it has been shown that if the 2-aminoimidazoline moiety is located in the 3 position of the pyridine ring [3-(2-aminoimidazolino)pyridines] significantly better α_2-AR affinities are obtained than when it is present in the 2 position (Figure 4.2.3). Thus, for future pyridoguanidine series, this positioning alteration should be included to the *bis*-aryl core of Family E, creating novel 3-(2-aminoimidazolino)pyridines, where this modification is predicted to significantly amplify the pharmacological profile of our newly found leads (Figure 4.2.3).

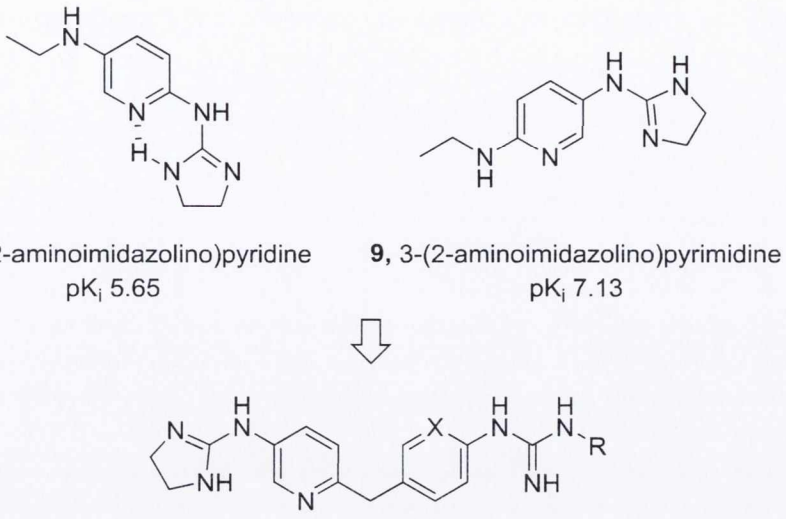


Fig. 4.2.3. Incorporation of 3-(2-aminoimidazolino)pyridine into the core framework of Family E.

4.2.4. Investigation into the agonist properties of compound 101h at alternate GPCRs

As seen in Section 3.2.2.4 compound **101h** (Family E3, Figure 4.1.1) was shown to be a potent competitive antagonist of the α_2-AR; however the [^{35}S]GTPγS binding experiments strongly suggested that **101h** has agonist properties at alternate GPCRs. No other compounds in Family E demonstrated these properties, and since they all display strong structural similarities, for future work it would be interesting to screen compound **101h** in terms of affinity and activity, against other GPCRs localised in the CNS. In particular the serotonin 5-$HT_{1,2,4,5,6,7}$ receptors, the dopamine $D_{1\text{-}5}$ receptors and the glutamate $mGluR_{1\text{-}8}$ receptors should be screened to obtain additional information about the physiological properties of this molecule.

4.2.5. Crystallization of α_2-AR

Considering that a number of high affinity α_2-AR antagonists have been produced in the present research, the already started collaboration with Prof. Martin Caffrey from Trinity College Dublin, aiming to the crystallization the α_2-AR, should be continued. Since current drug design in this area is based purely on theoretical calculations, as well as information gained from previous pharmacological results, to obtain a crystal structure of α_2-AR would

highly benefit the design of new therapeutic agents for the treatment of a large number of diseases.

Chapter 5 - Experimental

5.1. Synthetic Chemistry

5.1.1. Materials and Methods

All commercial chemicals were obtained from Sigma-Aldrich or Fluka and used without further purification. Deuterated solvents for NMR use were purchased from Apollo. Dry solvents were prepared using standard procedures, according to Vogel,[265] with distillation prior to use. Solvents for synthesis purposes were used at GPR grade. Analytical TLC was performed using Merck Kieselgel 60 F254 silica gel plates or Polygram Alox N/UV254 aluminium oxide plates. Visualisation was by UV light (254 nm). NMR spectra were recorded on Bruker DPX–400 Avance spectrometers, operating at 400.13 MHz and 600.1 MHz for ^{1}H NMR; 100.6 MHz and 150.9 MHz for ^{13}C-NMR. Shifts are referenced to the internal solvent signals.[266] NMR data were processed using Bruker TOPSPIN software. HRMS spectra were measured on a Micromass LCT electrospray TOF instrument with a WATERS 2690 autosampler and methanol/acetonitrile as carrier solvent. Melting points were determined using a Stuart Scientific Melting Point SMP1 apparatus and are uncorrected. Infrared spectra were recorded on a Perkin Elmer Spectrum One FT-IR Spectrometer equipped with a Universal ATR sampling accessory.

5.1.2. Purity Assessment of Hydrochloride Salts

HPLC purity analysis was carried out using a Varian ProStar system equipped with a Varian Prostar 335 diode array detector and a manual injector (20 µL). For purity assessment, UV detection was performed at 245 nm and peak purity was confirmed using a purity channel. The stationary phase consisted of an ACE 5 C18-AR column (150 × 4.6 mm), and the mobile phase used the following gradient system, eluting at 1 mL/min: aqueous formate buffer (30 mM, pH 3.0) for 10 min, linear ramp to 85% methanol buffered with the same system over 25 minutes, hold at 85% buffered methanol for 10 min. Minimum requirement for purity was set at 95.0%.

5.1.3. General procedures

Method A: Preparation of *S*-methyl-*N*-substituted dithiocarbamates.

To a solution of CS_2 (1.1 eq., 4.52 mmol) and NEt_3 (1.05 eq., 4.31 mmol) in CH_2Cl_2 (4 mL) at 0 °C was added starting amine (1.0 eq., 4.11 mmol). After 15 min MeI (1.1 eq., 4.52 mmol) was added dropwise. The reaction was warmed to RT and stirred for 2 h. It was then diluted with EtOAc (30 mL) and added to a 1M solution of H_2SO_4 (30 mL). The aqueous layer was extracted with EtOAc (2 × 30 mL) and the combined organic layers were washed with H_2O (2 × 20 mL) and dried over Na_2SO_4. Filtration and removal of solvents yielded a residue which was purified by silica gel column chromatography, eluting in the appropriate hexane:EtOAc mixture.

Method B: Preparation of *N*-substituted cyclic guanidines and *N,N'*-di-substituted thioureas.

To a suspension of the amine (1.0 eq., 0.57 mmol) and the appropriate *S*-methyl-*N*-substituted dithiocarbamate (1.1 eq., 0.63 mmol) in DMF (2 mL) at RT were added Cu(II)O (0.2 eq., 0.11 mmol) and K_2CO_3 (2.0 eq., 1.14 mmol). The resulting mixture was heated to 60 °C and kept at this temperature for 2-3 h. It was then cooled to RT, diluted with EtOAc (60 mL) and filtered through Celite. The filtrate was washed with brine (1 × 30 mL) and H_2O (4 × 30 mL) to remove traces of DMF, dried over Na_2SO_4, filtered and concentrated under vacuum. The resulting residue was purified using silica gel column chromatography, eluting with the appropriate hexane:EtOAc mixture.

Method C: Protonation of *N*-substituted and *N,N*-di-substituted cyclic guanidines using hydrochloric acid in 1,4-dioxane.

To a solution of the appropriate cyclic guanidine (1.0 eq., 0.71 mmol) in CH_3OH (0.1 mL) was added excess 4M HCl/dioxane (3.0 eq., 2.13 mmol). Stirring was continued for 1 h after which solvent and excess HCl were removed under vacuum. The crude salt was then purified using reverse phase chromatography (C-8 silica) eluting in 100% H_2O.

Method D: Preparation of *N*-substituted 3-cyanopyridines.

To a solution of 2-chloro-3-cyanopyridine (1.0 eq., 7.38 mmol) and NEt_3 (3.0 eq., 22.14 mmol) in anhydrous THF (7 mL) at 0 °C was added the appropriate amine (1.5 eq., 11.07 mmol). The resulting mixture was then stirred at 70 °C for 24 h. The solution was cooled and diluted with EtOAc (60 mL). The organic layer was washed with H_2O (2 × 15 mL), dried over Na_2SO_4, filtered, and concentrated under vacuum. The resulting residue was purified using silica gel column chromatography, eluting with the appropriate hexane:EtOAc mixture.

Method E: Preparation of *N*-substituted 3-benzonitriles.

To a Schlenk tube charged with $Pd_2(dba)_3$ (0.11 mmol, 5 mol%) and BINAP (0.16 mmol, 3 mol%) was added 2-bromobenzonitrile (1.0 eq., 2.2 mmol) and NaOtBu (1.4 eq., 3.08 mmol). The tube was evacuated and backfilled with argon 3 times. Dry toluene (1 mL per mmol of arylhalide) was then added *via* syringe followed by the appropriate amine (1.2 eq., 2.64 mmol). The mixture was heated to 90 °C for 24 h. The reaction mixture was cooled, filtered through a pad of Celite, diluted with H_2O (50 mL), and then extracted with EtOAc (3 × 30 mL). The organic layers were then combined, washed with brine (1 × 50 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The product was then purified by silica gel chromatography, eluting in hexane:EtOAc.

Method F: Preparation of *N*-substituted aryl diamines.

A solution of the appropriate nitrile (1.0 eq., 1.19 mmol) in anhydrous THF (3 mL) under argon was added dropwise to a stirred suspension of $LiAlH_4$ (2.0 eq., 2.38 mmol) in anhydrous THF (3 mL) at 0 °C. The resulting mixture was stirred at 70 °C under argon for 2 h. The mixture was quenched by the careful successive dropwise addition of the same number of mL of H_2O as grams of $LiAlH_4$ used, same volume (mL) of 15% NaOH solution, and three times the volume (mL) of H_2O.[205] The resulting slurry was filtered through a pad of Celite and concentrated under reduced pressure. The product was then purified by silica gel chromatography, eluting in CH_2Cl_2:CH_3OH mixture.

Method G: Synthesis of the benzyl protected *N*-substituted 2-amino 1,4-dihydropyridopyrimidines and 2-amino-1,4-dihydroquinazolines.

To a stirred solution of starting diamine (1.0 eq., 3.7 mmol) and NEt_3 (3.0 eq., 11.1 mmol) in DMF (4 mL) at 0 °C was added benzyl isothiocyanate (1.1 eq., 4.07 mmol) dropwise. The resulting solution was stirred at RT until complete disappearance of the starting material was observed by TLC (1-2 h). Then $HgCl_2$ (1.2 eq., 4.44 mmol) was added and the reaction was left to stir at RT for 2 h. The reaction was diluted with $CHCl_3$:^{i}PrOH (8:2) (25 mL), and filtered through a bed of Celite to remove mercuric by-products. The filtrate was washed with 50% NaOH (3 × 20 mL) and brine (1 × 20 mL), to remove any traces of DMF. The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography, eluting with the appropriate CH_2Cl_2:CH_3OH mixture.

Method H: Synthesis of *N*-substituted 2-amino-1,4-dihydropyridopyrimidines and 2-amino-1,4-dihydroquinazolines.

To a solution of the appropriate cyclic benzyl guanidine (1.0 eq., 0.18 mmol) in CH_3OH (2 mL) was added 10% Pd/C (50 wt. %) and NH_4HCO_2 (10.0 eq., 1.8 mmol). The mixture was stirred at 50 °C for 48 h. It was then diluted with CH_3OH (20 mL) filtered and concentrated under reduced pressure. The resulting residue was diluted with $CHCl_3$:^{i}PrOH (8:2) (30 mL) and washed with 20% NaOH solution (2 × 20 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under vacuum. The resulting residue was purified by silica gel chromatography using gradient elution of CH_2Cl_2:CH_3OH mixture.

Method I: Preparation of the *N*-(2-amino-5-dimethylaminophenethyl)-*N'*-substituted thioureas.

To a solution of appropriate nitro (1.0 eq., 0.7 mmol) in EtOH (2 mL) was added $SnCl_2.2H_2O$ (10.0 eq., 7.0 mmol). The mixture was refluxed for 1 h, after which all starting material was consumed as adjudged by TLC analysis. The reaction was then diluted with EtOAc (30 mL), washed with H_2O (2 × 20 mL) and brine (1 × 20 mL). The organic phase was dried over Na_2SO_4, filtered and concentrated under vacuum. No further purification was required.

Method J: Preparation of the *N*-substituted-*N,N*-dimethyl-4,5-dihydro-1*H*-benzo[*d*][1,3]diazepine hydrochlorides.

To a stirred solution of starting thiourea (1.0 eq., 0.43 mmol) and NEt_3 (3.0 eq., 1.29 mmol) in DMF (2 mL) at 0 °C was added $HgCl_2$ (1.0 eq., 0.43 mmol). The solution was stirred at RT for 3 h. The reaction was diluted with $CHCl_3$:^{i}PrOH (8:2, 25 mL), and filtered through a bed of Celite to remove mercuric by-products. The filtrate was washed with H_2O (3 × 20 mL) and brine (1 × 20 mL), to remove any traces of DMF. The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography, eluting in CH_2Cl_2:CH_3OH.

Method K: Preparation of *N*-(*tert*-butoxycarbonyl)-*N'*-substituted thioureas.

Thiourea (1.0 eq., 6.58 mmol) was dissolved in dry THF (70 mL) under argon at 0 °C, to which NaH (60% suspension in mineral oil, 4.5 eq., 29.61 mmol) was added. The reaction was stirred at RT for 45 min to complete formation of the anion and re-cooled to 0 °C prior to the addition of di-*tert*-butyl dicarbonate (2.2 eq., 14.48 mmol). After stirring at RT for 8 h, the reaction was re-cooled to 0 °C and NaH (60% suspension in mineral oil, 1.7 eq., 11.19 mmol) was added. After 1 h TFAA (1.54 eq., 10.13 mmol) was added and the reaction was stirred for 1 h before the appropriate amine (1.54 eq., 10.13 mmol) was added and the reaction was allowed to come to RT overnight. The reaction was then cooled to 0 °C and dropwise H_2O (20 mL) was added to quench the reaction. The product was extracted with EtOAc (3 × 20 mL) and the combined organic phases were washed with brine (1 × 30 mL) and H_2O (1 × 30 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The resulting residue was purified by silica gel chromatography using gradient elution of hexane:EtOAc mixture.

Method L: Synthesis of the *N*-(*tert*-butoxycarbonyl)-*N'*-substituted (arylamino)guanidines.

To a solution of starting diamine (3.0 eq., 4.89 mmol), the appropriate thiourea derivative (1.0 eq., 1.63 mmol) and NEt_3 (3.0 eq., 4.89 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added $HgCl_2$ (1.1 eq., 1.79 mmol). The reaction was then left to stir at RT for 16 h. When the reaction was complete, the solution was diluted with EtOAc (60 mL) and then filtered

through a bed of Celite, to remove any mercury sulphide by-products. The filter cake was rinsed with EtOAc (20 mL) and the organic phase was washed with H_2O (2 × 20 mL) and brine (1 × 30 mL). It was then dried over Na_2SO_4, filtered and concentrated under vacuum. The product was purified using silica gel chromatography eluting with the appropriate hexane:EtOAc mixture.

Method M: Synthesis of *N,N'*-di(*tert*-butoxycarbonyl)-2-iminoimidazolidines and *N*-(*tert*-butoxycarbonyl)-*N'*-substituted aryl guanidines.

To a solution of starting amine (1.0 eq., 0.68 mmol), the appropriate thiourea derivative (1.0 eq., 0.68 mmol) and NEt_3 (3.0 eq., 2.04 mmol) in CH_2Cl_2 (5.5 mL) at 0 °C was added $HgCl_2$ (1.1 eq., 0.75 mmol). The reaction was then left to stir at RT for 16 h. When the reaction was complete, the solution was diluted with EtOAc (60 mL) and then filtered through a bed of Celite, to remove any mercury sulphide by-products. The filter cake was rinsed with EtOAc (20 mL) and the organic phase was washed with H_2O (2 × 20 mL) and brine (1 × 30 mL). It was then dried over Na_2SO_4, filtered and concentrated under vacuum. The product was purified using silica gel chromatography eluting with the appropriate hexane:EtOAc mixture.

Method N: Synthesis of *bis-N*-(*tert*-butoxycarbonyl)-*N'*-substituted aryl guanidines

To a solution of starting amine (1.0 eq., 2.3 mmol), the appropriate thiourea derivative (2.0 eq., 4.6 mmol) and NEt_3 (6.0 eq., 13.8 mmol) in CH_2Cl_2 (5.5 mL) at 0 °C was added $HgCl_2$ (2.2 eq., 5.06 mmol). The reaction was then left to stir at RT for 16 h. When the reaction was complete, the solution was diluted with EtOAc (60 mL) and then filtered through a bed of Celite, to remove any mercury sulphide products. The filter cake was rinsed with EtOAc (20 mL) and the organic phase was washed with H_2O (2 × 20 mL) and brine (1 × 30 mL). It was then dried over Na_2SO_4, filtered and concentrated under vacuum. The product was purified using silica gel chromatography eluting with the appropriate hexane:EtOAc mixture.

Method O: Generation of guanidine hydrochlorides from *N*-(*tert*-butoxycarbonyl) protected guanidine and iminoimidazolidine derivatives using hydrochloric acid in 1,4-dioxane.

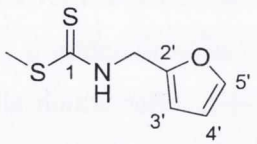
To the starting protected guanidine was added 4M HCl/dioxane (6.0 eq. per Boc group) and a 1:1 solution of ^{i}PrOH:CH_2Cl_2, such as to maintain a reaction concentration of 0.2 M. The mixture was stirred at 55 °C for 3-4 h, after which all starting material was consumed as adjudged by TLC analysis. Solvent and excess HCl were then removed under vacuum and the crude salt was dissolved in a minimum volume of H_2O. It was washed with CH_2Cl_2 (2 × 5 mL) and then purified using reverse phase chromatography (C-8 silica) using 100% H_2O as mobile phase.

Method P: Preparation of the *N,N'*-(2-(*tert*-butoxycarbonyl)-3-substituted)-*N*-(5-(4-aminobenzyl)pyridin-2-yl)guanidines

To a solution of the FMOC-protected amine (1.0 eq., 0.12 mmol) in MeCN (4 mL) was added $NHEt_2$ (115.0 eq., 13.8 mmol). The resulting mixture was stirred at RT for 45 min, after which it was diluted with EtOAc (30 mL) and washed with H_2O (3 × 20 mL). The organic phases were dried over Na_2SO_4, filtered, concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography eluting with the appropriate hexane:EtOAc mixture.

5.1.4. Synthesis and Characterisation

_N_-Furanyl-2-methyl-_S_-methyldithiocarbamate 19a



Following Method A, furfurylamine (1.0 eq., 363 μL, 4.11 mmol) was added to a solution of CS_2 (1.1 eq., 273 μL, 4.52 mmol) and NEt_3 (1.05 eq., 602 μL, 4.31 mmol) in CH_2Cl_2 (4 mL) at 0 °C. After 15 min MeI (1.1 eq., 281 μL, 4.52 mmol) was added dropwise and the reaction was stirred at RT for 2 h. Usual workup and purification afforded **19a** (598 mg, 78%) as a brown solid.

Mp: 47-50 °C.

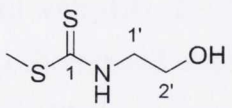
δ_H (400 MHz, $CDCl_3$): 2.63 (s, 3H, CH_3), 4.90 (d, 2H, J 4.1, CH_2), 6.33 (m, 2H, H-3', H-4'), 7.12 (br s, 1H, NH), 7.37 (br s, 1H, H-5').

δ_C (100 MHz, $CDCl_3$): 18.3 (CH_3), 43.8 (CH_2), 106.1 (CH, Ar-3'), 110.6 (CH, Ar-4'), 142.7 (CH, Ar-5'), 149.2 (q, Ar-2'), 199.1 (q, 1).

ν_{max} (ATR)/cm^{-1}: 3233 (NH), 2989, 2917, 1667, 1497, 1422, 1368, 1323, 1303, 1271, 1224, 1189, 1145 (C=S), 1084, 1063, 1011, 929, 815.

HRMS (m/z ESI^-): Found: 186.0056 (M^+ - H, $C_7H_9NOS_2$ Requires: 186.0047).

_N_-Hydroxyethyl-_S_-methyldithiocarbamate 19b



Following Method A, ethanolamine (1.0 eq., 771 μL, 11.46 mmol) was added to a solution of CS_2 (1.1 eq., 761 μL, 12.60 mmol) and NEt_3 (1.05 eq., 1676 μL, 12.03 mmol) in CH_2Cl_2 (6 mL) at 0 °C. After 15 min MeI (1.1 eq., 784 μL, 12.60 mmol) was added dropwise and the reaction was stirred at RT for 2 h. Usual workup and purification afforded **19b** (1.145 g, 66%) as a clear oil.

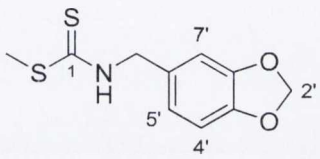
δ_H (400 MHz, $CDCl_3$): 2.59 (s, 3H, CH_3), 2.97 (br s, 1H, OH), 3.86 (m, 4H, CH_2-1', CH_2-2'), 7.89 (br s, 1H, NH).

δ_C (100 MHz, $CDCl_3$): 18.2 (CH_3), 49.8 (CH_2-1'), 59.9 (CH_2-2'), 199.9 (q, 1).

ν_{max} (ATR)/cm^{-1}: 3256 (OH), 3129 (NH), 2919, 2880, 1504, 1423, 1382, 1326, 1305, 1266, 1213, 1113 (C=S), 1081, 1043, 870, 789.

HRMS (m/z ESI^-): Found: 150.0046 (M^+ - H, $C_4H_9NOS_2$ Requires: 150.0084).

***N*-(1,3-Benzodioxolyl-5-methyl)-*S*-methyldithiocarbamate 19c**



Following Method A, piperonylamine (1.0 eq., 411 μL, 4.34 mmol) was added to a solution of CS_2 (1.1 eq., 288 μL, 4.77 mmol) and NEt_3 (1.05 eq., 635 μL, 4.55 mmol) in CH_2Cl_2 (4 mL) at 0 °C. After 15 min MeI (1.1 eq., 297 μL, 4.77 mmol) was added dropwise and the reaction was stirred at RT for 2 h. Usual workup and purification afforded **19c** (751 mg, 72%) as a yellow solid.

Mp: 95-100 °C.

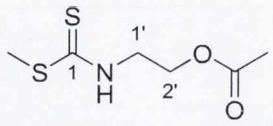
δ_H (400 MHz, $CDCl_3$): 2.55 (s, 3H, CH_3), 4.73 (d, 2H, J 4.1, CH_2), 5.85 (s, 2H, CH_2-2'), 6.69-6.71 (m, 3H, H-4', H-5', H-7'), 7.65 (br s, 1H, NH).

δ_C (100 MHz, $CDCl_3$): 18.1 (CH_3), 50.8 (CH_2-Bn), 101.1 (CH_2-2'), 106.3 (CH, Ar-4'), 108.7 (CH, Ar-7'), 121.7 (CH, Ar-5'), 130.2 (q, Ar-6'), 147.4 (q, Ar-1'a or Ar-3'a), 147.8 (q, Ar-1'a or Ar-3'a), 198.9 (q, 1).

ν_{max} (ATR)/cm^{-1}: 3180 (NH), 2995, 2959, 2902, 1651, 1520, 1497, 1482, 1424, 1376, 1346, 1310, 1272, 1240, 1191, 1132 (C=S), 1101, 960, 935, 908, 856, 768, 729, 698.

HRMS (m/z ESI^-): Found: 240.0153 (M^+ - H, $C_{10}H_{10}NO_2S_2$ Requires: 240.0148).

_N_-Acetoxyethyl-_S_-methyldithiocarbamate 19d



_N_-Hydroxyethyl-_S_-methyldithiocarbamate **19b** (1.0 eq., 1.5 g, 9.92 mmol), NEt_3 (3.0 eq., 4.15 mL, 29.76 mmol) and acetic anhydride (1.1 eq., 1.03 mL, 10.91 mmol) were added to CH_2Cl_2 (20 mL) under stirring at 0 °C. The reaction was warmed to RT and stirred for 4 h. At this point TLC analysis showed no starting material and the reaction was diluted with CH_2Cl_2 (30 mL). The organic layer was separated, washed with brine (1 × 30 mL) and H_2O (2 × 30 mL), dried over Na_2SO_4 and filtered. Removal of solvents under reduced pressure yielded a residue which was purified using silica-gel chromatography (hexane:EtOAc), affording **19d** (1.841 g, 96%) as a yellow oil.

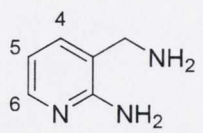
δ_H (400 MHz, $CDCl_3$): 2.06 (s, 3H, CH_3-Ac), 2.59 (s, 3H, CH_3), 3.98 (t, 2H, J 7.1, CH_2-1'), 4.28 (t, 2H, J 7.1, CH_2-2') 7.48 (br s, 1H, NH).

δ_C (100 MHz, $CDCl_3$): 18.2 (CH_3), 20.9 (CH_3-Ac), 46.4 (CH_2-1'), 62.2 (CH_2-2'), 171.5 (q, C=O), 199.9 (q, 1).

ν_{max} (ATR)/cm^{-1}: 3287 (NH), 3043 (NH), 2956, 2919, 1719 (C=O), 1504, 1427, 1278, 1333, 1131, 1044 (C=S), 984, 875, 809, 726, 672.

HRMS (m/z ESI$^-$): Found: 192.0304 (M^- - H. $C_6H_{10}NO_2S_2$ Requires: 192.0142).

2-Amino-3-methylenaminopyridine 22



To a solution of 2-amino-3-cyanopyridine (300 mg, 2.52 mmol) in NH_3/CH_3OH (4N, 7 mL) was added 10% Pd/C (30 mg, 10 wt. %). The mixture was stirred under an atmosphere of hydrogen (3 atm) for 18 h. It was then diluted with CH_3OH (30 mL), filtered and concentrated under reduced pressure. The resulting residue was purified by silica gel

chromatography using gradient elution of CH_2Cl_2:CH_3OH giving title compound **22** as a white solid (284 mg, 90%).

Mp: 120-124 °C.

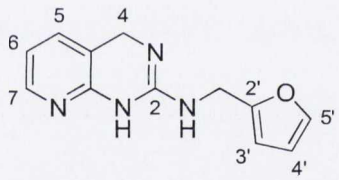
δ_H (400 MHz, $CDCl_3$): 1.92 (br s, 2H, NH_2), 3.95 (s, 2H, CH_2), 5.21 (br s, 2H, NH_2), 6.53 (m, 1H, H-5), 7.31 (d, 1H, J 7.2, H-4), 7.89 (br s, 1H, H-6).

δ_C (100 MHz, $CDCl_3$): 42.0 (CH_2), 112.9 (CH, Ar-5), 114.0 (q, Ar-3), 133.1 (CH, Ar-4), 146.4 (CH, Ar-6), 154.5 (q, Ar-2).

ν_{max} (ATR)/cm^{-1}: 3349 (NH), 3282 (NH), 3011, 2862, 2636, 1627, 1600, 1574, 1439, 1381, 1323, 1289, 1274, 1239, 1132, 1071, 1024, 975, 928, 815, 771, 760, 754, 697.

HRMS (m/z ESI^+): Found: 124.0875 ($M^+ + H$, $C_6H_{10}N_3$ Requires: 124.0875).

2-(Furanyl-2-methylamino)-1,4-dihydropyrido[2,3-*d*]pyrimidine 23a



Following Method B, Cu(II)O (0.2 eq., 9.06 mg, 0.11 mmol) and K_2CO_3 (2.0 eq., 157 mg, 1.14 mmol) were added to a solution of **22** (1.0 eq., 70 mg, 0.57 mmol) and **19a** (1.1 eq., 117 mg, 0.63 mmol) in DMF (2 mL) at RT. The resulting mixture was heated to 60 °C and kept at this temperature for 2 h. Usual workup and purification afforded **23a** (75 mg, 57%) as a yellow solid.

Mp: 185-190 °C.

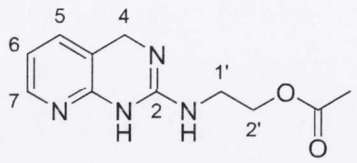
δ_H (400 MHz, $CDCl_3$): 4.63 (s, 2H, CH_2-4), 4.81 (s, 2H, CH_2), 6.23 (d, 1H, J 7.1, H-3'), 6.26 (d, 1H, J 7.1, H-4'), 6.50 (app. t, 1H, J 7.2, H-6), 6.59 (br s, 1H, NH), 6.90 (br s, 1H, NH), 7.25 (m, 2H, H-5, H-5'), 7.83 (d, 1H, J 7.2, H-7).

δ_C (100 MHz, $CDCl_3$): 41.4 (CH_2-4), 43.1 (CH_2), 107.1 (CH, Ar-3'), 108.1 (CH, Ar-4'), 113.1 (CH, Ar-6), 114.2 (q, Ar-4a), 136.6 (CH, Ar-5), 142.1 (CH, Ar-5'), 147.5 (CH, Ar-7), 150.8 (q, Ar-2'), 159.0 (q, Ar-8a), 182.7 (q, 2).

ν_{max} (ATR)/cm^{-1}: 3235 (NH), 2921, 1650, 1598, 1534, 1501, 1488, 1443, 1336, 1328, 1245, 1189, 1098, 1035, 926, 883, 807, 770.

HRMS (m/z ESI$^-$): Found: 229.1090 (M^+ + H, $C_{12}H_{13}N_4O$ Requires: 229.1089).

2-*N*-(Acetylethylamino)-1,4-dihydropyrido[2,3-*d*]pyrimidine 23b



Following Method B, Cu(II)O (0.2 eq., 20.6 mg, 0.26 mmol) and K_2CO_3 (2.0 eq., 359 mg, 2.60 mmol) were added to a solution of **22** (1.0 eq., 160 mg, 1.29 mmol) and **19d** (1.1 eq., 276 mg, 1.43 mmol) in DMF (4 mL) at RT. The resulting mixture was heated to 60 °C and kept at this temperature for 2 h. Usual workup and purification afforded **23b** (195 mg, 65%) as a yellow oil.

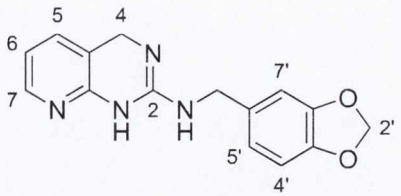
δ_H (400 MHz, $CDCl_3$): 1.92 (s, 3H, CH_3), 3.70 (br s, 2H, CH_2-1'), 4.09 (m, 2H, CH_2-2') 4.62 (s, 2H, CH_2-4), 6.50 (app. t, 1H, J 7.5, H-6), 6.81 (br s, 1H, NH), 7.00 (br s, 1H, NH), 7.29 (d, 1H, J 7.5, H-5), 7.80 (d, 1H, J 7.5, H-7).

δ_C (100 MHz, $CDCl_3$): 20.8 (CH_3), 43.4 (CH_2-1'), 45.6 (CH_2-4), 59.8 (CH_2-2'), 113.6 (CH, Ar-6), 116.2 (q, Ar-4a), 138.4 (CH, Ar-5), 147.0 (CH, Ar-7), 151.3 (q, Ar-8a), 171.4 (q, C=O), 182.7 (q, 2).

ν_{max} (ATR)/cm^{-1}: 3314 (NH), 2926, 1725 (C=O), 1622, 1596, 1543, 1446, 1367, 1224, 1977, 1040, 952, 773.

HRMS (m/z ESI$^+$): Found: 235.1199 (M^+ + H, $C_{11}H_{15}N_4O_2$ Requires: 235.1195).

2-(1,3-Benzodioxolyl-5-methylamino)-1,4-dihydropyrido[2,3-*d*]pyrimidine 23c



Following Method B, Cu(II)O (0.2 eq., 12.9 mg, 0.16 mmol) and K_2CO_3 (2.0 eq., 224 mg, 1.62 mmol) were added to a solution of **22** (1.0 eq., 100 mg, 0.82 mmol) and **19c** (1.1 eq., 215 mg, 0.89 mmol) in DMF (3 mL) at RT. The resulting mixture was heated to 60 °C and kept at this temperature for 2 h. Usual workup and purification afforded **23c** (182 mg, 78%) as a yellow oil.

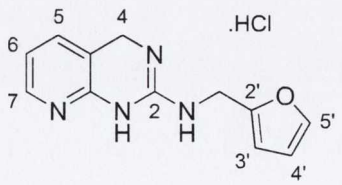
δ_H (400 MHz, $CDCl_3$): 4.46 (br s, 2H, CH_2-4), 4.57 (d, 2H, J 5.1, CH_2), 5.82 (s, 2H, CH_2-2'), 6.44 (app. t, 1H, J 7.1, H-6), 6.62 (m, 2H, H-4', H-5'), 6.67 (s, 1H, H-7'), 6.80 (br s, 1H, NH), 7.05 (br s, 1H, NH), 7.20 (d, 1H, J 7.1, H-5), 7.73 (d, 1H, J 7.1, H-7).

δ_C (100 MHz, $CDCl_3$): 45.6 (CH_2), 48.0 (CH_2-4), 101.0 (CH_2-2'), 108.2 (CH, Ar-4'), 108.9 (CH, Ar-7'), 113.4 (CH, Ar-6), 116.4 (q, Ar-4a), 120.8 (CH, Ar-5'), 131.3 (q, Ar-6'), 138.4 (CH, Ar-5), 146.8 (CH, Ar-7), 146.9 (q, Ar-1'a or Ar-3'a), 147.8 (q, Ar-1'a or Ar-3'a), 157.1 (q, Ar-8a), 182.2 (q, 2).

ν_{max} (ATR)/cm^{-1}: 3229 (NH), 2921, 2852, 1783, 1653, 1543, 1500, 1488, 1443, 1377, 1327, 1245, 1097, 1037, 924, 887, 805, 769.

HRMS (m/z ESI^+): Found: 283.1195 (M^+ + H, $C_{15}H_{15}N_4O_2$ Requires: 283.1191).

2-(Furanyl-2-methylamino)-1,4-dihydropyrido[2,3-*d*]pyrimidine hydrochloride 24a



Following Method C, 4M HCl/dioxane (3.0 eq., 400 µL, 1.53 mmol) was added to a solution of **23a** (1.0 eq., 116 mg, 0.51 mmol) in CH_3OH (0.5 mL). The solution was stirred at RT for 2 h. After usual workup and purification **24a** was obtained as a yellow solid (95 mg, 70%).

Mp: 181-186°C.

δ_H (400 MHz, DMSO): 4.53 (s, 2H, CH_2-4), 4.59 (s, 2H, CH_2), 6.25 (d, 1H, J 6.8, H-3'), 6.34 (m, 1H, H-4'), 6.85 (app. t, 1H, J 7.3, H-6), 7.53 (d, 1H, J 6.8, H-5'), 7.79 (d, 1H, J 7.3, H-5), 7.87 (d, 1H J 7.3, H-7), 8.11 (br s, 1H, NH), 8.39 (br s, 1H, NH), 8.48 (br s, 1H, NH).

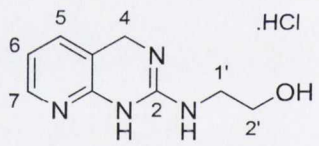
δ_C (100 MHz, DMSO): 41.1 (CH_2), 42.9 (CH_2-4), 107.8 (CH-3'), 110.9 (CH-4'), 112.5 (CH, Ar-6), 123.5 (q, Ar-4a), 134.5 (CH, Ar-5), 141.4 (CH, Ar-5'), 142.7 (CH, Ar-7), 151.7 (q, Ar-2'), 152.5 (q, Ar-8a), 172.5 (q, 2).

ν_{max} (ATR)/cm^{-1}: 3328 (NH), 2987, 2956, 1753 (C=O), 1599, 1573, 1509, 1478, 1426, 1390, 1277, 1093, 1001, 987.

HRMS (m/z ESI^-): Found: 229.1098 ($M^+ + H$, $C_{12}H_{13}N_4O$ Requires: 229.1098).

Purity by HPLC: 96.2% (t_R 23.27 min).

2-*N*-Hydroxyethylamino-1,4-dihydropyrido[2,3-*d*]pyrimidine hydrochloride 24b



Following Method C, to a solution of 1.25M HCl/CH_3OH (6.0 eq., 3.9 mL, 4.86 mmol) was added **23b** (1.0 eq., 190 mg, 0.81 mmol). The solution was stirred at 60 °C for 4 h. After usual workup and purification, **24b** was obtained as a yellow solid (154 mg, 84%).

Mp: 235-239 °C, decomposed.

δ_H (400 MHz, DMSO): 3.52 (br s, 2H, CH_2-1'), 3.86 (br s, 2H, CH_2-2'), 4.49 (br s, 2H, CH_2-4), 6.86 (app. t, 1H, J 7.1, H-6), 7.88 (d, 1H, J 7.1, H-5), 7.94 (d, 1H, J 7.1, H-7), 8.18 (br s, 1H, NH), 8.25 (br s, 1H, NH), 10.61 (br s, 1H, NH).

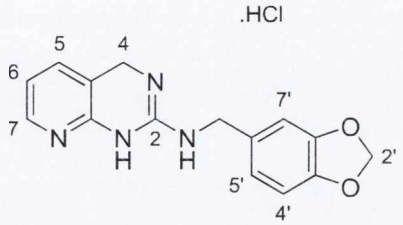
δ_C (100 MHz, DMSO): 31.0 (CH_2-1'), 43.5 (CH_2-4), 49.2 (CH_2-2'), 112.9 (CH, Ar-6), 134.0 (q, Ar-4a), 135.4 (CH, Ar-5), 142.6 (CH, Ar-7), 151.8 (q, Ar-8a), 182.4 (q, 2).

ν_{max} (ATR)/cm^{-1}: 3289 (NH), 2987, 1736, 1678, 1578, 1501, 1367, 1302, 1297, 1267, 1109, 1067, 876, 712.

HRMS (m/z ESI$^+$): Found: 193.1083 (M^+ + H, $C_9H_{13}N_4O$ Requires: 193.1089).

Purity by HPLC: 97.7% (t_R 2.55 min).

2-(1,3-Benzodioxolyl-5-methylamino)-1,4-dihydropyrido[2,3-*d*]pyrimidine hydrochloride 24c



Following Method C, 4M HCl/dioxane (3.0 eq., 550 µL, 2.13 mmol) was added to a solution of **23c** (1.0 eq., 200 mg, 0.71 mmol) in CH_3OH (0.5 mL). The solution was stirred at RT for 2 h. After usual workup and purification **24c** was obtained as a yellow solid (197 mg, 87%).

Mp: 210-123 °C decomposed.

δ_H (400 MHz, DMSO): 4.59 (br s, 2H, CH_2-4), 4.62 (s, 2H, CH_2), 5.98 (s, 2H, CH_2-2'), 6.76 (app. t, 1H, J 7.1, H-6), 6.87 (m, 2H, H-4', H-5'), 6.87 (s, 1H, H-7'), 7.74 (d, 1H, J 7.1, H-5), 7.92 (d, 1H, J 7.1, H-7), 8.07 (br s, 1H, NH), 8.33 (br s, 1H, NH), 8.49 (br s, 1H, NH).

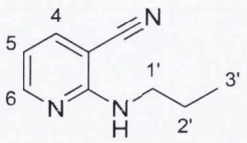
δ_C (100 MHz, D_2O): 40.8 (CH_2), 44.3 (CH_2-4), 101.1 (CH_2-2'), 107.6 (CH, Ar-4'), 108.5 (CH, Ar-7'), 120.1 (q, Ar-4a), 120.6 (CH, Ar-6), 120.8 (CH, Ar-5'), 129.4 (q, Ar-6'), 136.3 (CH, Ar-5), 146.1 (CH, Ar-7), 146.9 (q, Ar-1'a or Ar-3'a), 147.5 (q, Ar-1'a or Ar-3'a), 155.1 (q, Ar-8a), 181.3 (q, 2).

ν_{max} (ATR)/cm^{-1}: 3378 (NH), 2986, 2935, 1768, 1634, 1601, 1598, 1489, 1423, 1309, 1147, 1099, 1037, 985, 735.

HRMS (m/z ESI$^+$): Found: 283.1190 (M^+ + H, $C_{15}H_{15}N_4O_2$ Requires: 283.1195).

Purity by HPLC: 99.8% (t_R 24.40 min).

2-Propylamino-3-cyanopyridine 28a



Following Method D, to a solution of 2-chloro-3-cyanopyridine (1.0 eq., 1.0 g, 7.38 mmol) and NEt_3 (3.0 eq., 4.21 mL, 22.14 mmol) in anhydrous THF (7 mL) at 0 °C was added propylamine (1.5 eq., 910 µL, 11.07 mmol). The resulting mixture was heated to 70 °C and stirred until conversion of starting material was observed by TLC. Usual workup and purification afforded **28a** (836 mg, 70%) as white crystals.

Mp: 53-57°C.

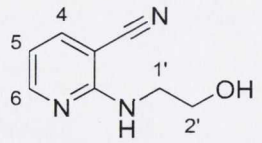
δ_H (400 MHz, $CDCl_3$): 0.95 (t, 3H, J 7.0, CH_3-3'), 1.61 (m, 2H, CH_2-2'), 3.43 (t, 2H, J 7.0, CH_2-1'), 5.18 (br s, 1H, NH), 6.52 (app. t, 1H, J 7.1, H-5), 7.59 (d, 1H, J 7.1, H-4), 8.23 (d, 1H, J 7.1, H-6).

δ_C (100 MHz, $CDCl_3$): 11.3 (CH_3-3'), 22.6 (CH_2-2'), 43.1 (CH_2-1'), 92.2 (q, Ar-3), 115.2 (CH, Ar-5), 116.8 (q, CN), 141.2 (CH, Ar-4), 152.6 (CH, Ar-6), 158.5 (q, Ar-2).

ν_{max} (ATR)/cm^{-1}: 3364 (NH), 3345 (NH), 2933, 2848, 2226 (CN), 1943, 1592, 1578, 1511, 1478, 1426, 1362, 1343, 1297, 1195, 1189, 1073,906, 802, 767, 751.

HRMS (m/z ESI^+): Found: 162.1026 ($M^+ + H$, $C_9H_{12}N_3$ Requires: 162.1030).

2-Hydroxyethylamino-3-cyanopyridine 28b



Following Method D, to a solution of 2-chloro-3-cyanopyridine (1.0 eq., 1.0 g, 7.38 mmol) and NEt_3 (3.0 eq., 4.21 mL, 22.14 mmol) in anhydrous THF (7 mL) at 0 °C was added 2-aminoethanol (1.5 eq., 668 µL, 11.07 mmol). The resulting mixture was heated to 70 °C and stirred until conversion of starting material was observed by TLC. Usual workup and purification afforded **28b** (742 mg, 62%) as a white solid.

Mp: 71-73 °C.

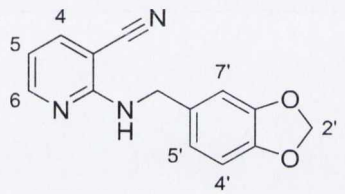
δ_H (400 MHz, $CDCl_3$): 3.65 (t, 2H, J 7.4, CH_2-1'), 3.81 (t, 2H, J 7.4, CH_2-2'), 5.68 (br s, 1H, NH), 6.60 (app. t, 1H, J 7.1, H-5), 7.65 (d, 1H, J 7.1, H-4), 8.21 (d, 1H, J 7.1, H-6).

δ_C (100 MHz, $CDCl_3$): 44.4 (CH_2-1'), 62.5 (CH_2-2'), 91.9 (q, Ar-3), 112.4 (CH, Ar-5), 116.8 (q, CN), 141.7 (CH, Ar-4), 152.2 (CH, Ar-6), 158.7 (q, Ar-2).

ν_{max} (ATR)/cm^{-1}: 3390 (OH), 3319 (NH), 2874, 2212 (CN), 1603, 1571, 1513, 1467, 1400, 1310, 1210, 1179, 1021, 943, 785, 760, 728.

HRMS (m/z ESI^-): Found: 162.0661 (M^+ - H, $C_8H_8N_3O$ Requires: 162.0667).

2-(1,3-Benzodioxolyl-5-methylamino)-3-cyanopyridine 28c



Following Method D, to a solution of 2-chloro-3-cyanopyridine (1.0 eq., 1.0 g, 7.38 mmol) and NEt_3 (3.0 eq., 4.21 mL, 22.14 mmol) in anhydrous THF (7 mL) at 0 °C was added piperonylamine (1.5 eq., 1.38 mL, 11.07 mmol). The resulting mixture was heated to 70 °C and stirred until conversion of starting material was observed by TLC. Usual workup and purification afforded **28c** (1.19 g, 63%) as an off-white solid.

Mp: 92-95°C.

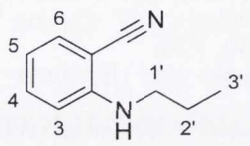
δ_H (400 MHz, $CDCl_3$): 4.58 (s, 2H, CH_2), 5.45 (br s, 1H, NH), 5.91 (s, 2H, CH_2-2'), 6.59 (m, 1H, H-5), 6.73 (d, 1H, J 7.1, H-4'), 6.78 (d, 1H, J 7.1, H-5'), 6.80 (s, 1H, H-7'), 7.63 (d, 1H, J 7.1, H-4), 8.28 (d, 1H, J 7.1, H-6).

δ_C (100 MHz, $CDCl_3$): 44.6 (CH_2), 91.3 (q, Ar-3), 100.6 (CH_2-2'), 107.8 (CH, Ar-4', Ar-7'), 111.7 (CH, Ar-5), 116.5 (q, CN), 120.6 (CH, Ar-5'), 131.7 (q, Ar-6'), 140.9 (CH, Ar-4), 146.5 (q, Ar-1'a or Ar-3'a), 147.4 (q, Ar-1'a or Ar-3'a), 152.3 (CH, Ar-6), 157.2 (q, Ar-2).

ν_{max} (ATR)/cm^{-1}: 3366 (NH), 2899, 2852, 2780, 2217 (CN), 1973, 1887, 1593, 1581, 1522, 1488, 1444, 1372, 1347, 1321, 1288, 1182, 1132, 1093, 1038, 940, 811, 804, 763, 712, 673.

HRMS (m/z ESI$^+$): Found: 254.0919 (M^+ + H, $C_{14}H_{12}N_3O_2$ Requires: 254.0930).

2-Propylaminobenzonitrile 34a



Following Method E, to a Schlenk tube charged with $Pd_2(dba)_3$ (100.7 mg, 0.11 mmol, 5 mol%) and BINAP (41.1 mg, 0.07 mmol, 3 mol%), was added 2-bromobenzonitrile (1.0 eq., 400 mg, 2.2 mmol) and NaOtBu (1.4 eq., 398 mg, 3.08 mmol). The tube was evacuated and backfilled with argon 3 times. Dry toluene (4 mL) was then added *via* syringe followed by propylamine (1.2 eq., 217 µL, 2.64 mmol) by syringe. The mixture was heated to 90 °C for 24 h. Usual workup and purification afforded **34a** (335 mg, 95%) as a clear oil.

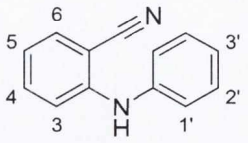
δ_H (400 MHz, $CDCl_3$): 0.96 (t, 3H, J 6.9, CH_3-3'), 1.62 (m, 2H, CH_2-2'), 3.08 (t, 2H, J 6.9, CH_2-1'), 4.60 (br s, 1H, NH), 6.58 (m, 2H, H-3, H-5), 7.31 (m, 2H, H-4, H-6).

δ_C (100 MHz, $CDCl_3$): 11.4 (CH_3-3'), 22.3 (CH_2-2'), 44.9 (CH_2-1'), 95.3 (q, Ar-1), 110.5 (CH, Ar-3), 116.08 (CH, Ar-5), 117.3 (q, CN), 132.6 (CH, Ar-4), 134.1 (CH, Ar-6), 150.4 (q, Ar-2).

ν_{max} (ATR)/cm^{-1} : 3375 (NH), 2962, 2932, 2875, 2210 (CN), 1605, 1576, 1514, 1496, 1382, 1324, 1287, 1165, 1071, 1041, 989, 744.

HRMS (m/z ESI$^-$): Found: 159.0926 (M^+ - H, $C_{10}H_{11}N_2$ Requires: 159.0922).

2-Phenylaminobenzonitrile 34b



Following Method E, to a Schlenk tube charged with $Pd_2(dba)_3$ (100.7 mg, 0.11 mmol, 5 mol%) and BINAP (41.1 mg, 0.07 mmol, 3 mol%), was added 2-bromobenzonitrile (1.0 eq., 400 mg, 2.2 mmol) and NaOtBu (1.4 eq., 398 mg, 3.08 mmol). The tube was evacuated and backfilled with argon 3 times. Dry toluene (4 mL) was then added *via* syringe followed by aniline (1.2 eq., 241 μL, 2.64 mmol) by syringe. The mixture was heated to 90 °C for 24 h. Usual workup and purification afforded **34b** (230 mg, 54%) as a brown oil.

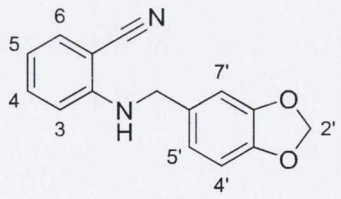
δ_H (400 MHz, $CDCl_3$): 6.82 (d, 1H, J 7.2, H-3), 7.12 (app. t, 1H, J 7.2, H-5), 7.19 (m, 3H, H-1', H-3'), 7.35 (m, 3H, H-4, H-2'), 7.48 (app. t, 1H, J 7.2, H-6).

δ_C (100 MHz, $CDCl_3$): 98.5 (q, Ar-1), 114.1 (CH, Ar-1'), 117.6 (q, CN), 119.1 (CH, Ar-3), 121.6 (CH, Ar-3'), 124.2 (CH, Ar-5), 129.5 (CH, Ar-2'), 132.9 (CH, Ar-4), 133.8 (CH, Ar-6), 139.9 (q, Ar-Ph), 147.2 (q, Ar-2).

ν_{max} (ATR)/cm^{-1} : 3210 (NH), 2930, 2891, 2211 (CN), 1604, 1597, 1567, 1432, 1398, 1324, 1299, 1207, 1189, 1107, 1045, 1040, 918, 897, 750, 742.

HRMS (m/z ESI^-): Found: 193.0771 (M^+ - H, $C_{13}H_9N_2$ Requires: 193.0766).

2-(1,3-Benzodioxolyl-5-methylamino)benzonitrile 34c



Following Method E, to a Schlenk tube charged with $Pd_2(dba)_3$ (100.7 mg, 0.11 mmol, 5 mol%) and BINAP (41.1 mg, 0.07 mmol, 3 mol%), was added 2-bromobenzonitrile (1.0 eq., 400 mg, 2.2 mmol) and NaOtBu (1.4 eq., 398 mg, 3.08 mmol). The tube was evacuated and backfilled with argon 3 times. Dry toluene (4 mL) was then added *via* syringe followed by

piperonylamine (1.2 eq., 326 μL, 2.64 mmol) by syringe. The mixture was heated to 90 °C for 24 h. Usual workup and purification afforded **34c** (495 mg, 89%) as a white solid.

Mp: 79-81 °C.

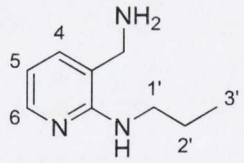
δ_H (400 MHz, $CDCl_3$): 4.30 (s, 2H, CH_2), 5.04 (br s, 1H, NH), 5.91 (s, 2H, CH_2-2'), 6.62-6.67 (m, 2H, H-4', H-5'), 6.74 (s, 1H, H-7'), 6.77 (d, 1H, J 7.1, H-3), 6.80 (m, 1H, H-5), 7.31 (app. t, 1H, J 7.1, H-4), 7.37 (d, 1H, J 7.1, H-6).

δ_C (100 MHz, $CDCl_3$): 47.2 (CH_2), 95.9 (q, Ar-1), 101.1 (CH_2-2'), 107.6 (CH, Ar-3), 108.4 (CH, Ar-5), 111.0 (CH, Ar-4'), 116.7 (CH, Ar-7'), 117.8 (q, CN), 120.3 (CH, Ar-5'), 131.6 (q, Ar-6'), 132.7 (CH, Ar-4), 134.2 (CH, Ar-6), 147.0 (q, Ar-1'a or Ar-3'a), 148.1 (q, Ar-1'a or Ar-3'a), 150.0 (q, Ar-2).

ν_{max} (ATR)/cm^{-1}: 3391 (NH), 2998, 2904, 2782, 2216 (CN), 1687, 1607, 1587, 1496, 1486, 1439, 1374, 1351, 1316, 1301, 1222, 1170, 1099, 1084, 955, 896, 807, 769.

HRMS (m/z ESI^-): Found: 251.0816 (M^+ - H, $C_{15}H_{11}N_2O_2$ Requires: 251.0821).

3-Aminomethyl-2-propylaminopyridine 35a



Following Method F, to a suspension of $LiAlH_4$ (2.0 eq., 271.7 mg, 7.16 mmol) in anhydrous THF (3 mL) at 0 °C was added a solution of compound **28a** (1.0 eq., 580 mg, 3.58 mmol) in anhydrous THF (3 mL) dropwise. The resulting mixture was heated to 70 °C and stirred until full conversion of starting material was observed by TLC. Usual workup and purification afforded **35a** (498 mg, 84%) as a clear oil.

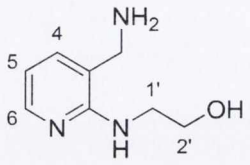
δ_H (400 MHz, $CDCl_3$): 0.97 (t, 3H, J 7.0, CH_3-3'), 1.20 (br s, 2H, NH_2), 1.65 (m, 2H, CH_2-2'), 3.39 (t, 2H, J 7.0, CH_2-1'), 3.81 (s, 1H, CH_2), 6.40 (br s, 1H, NH), 6.42 (m, 1H, H-5), 7.15 (d, 1H, J 7.0, H-4), 8.02 (dd, 1H, ^{3}J 7.0, ^{4}J 2.1, H-6).

δ_C (100 MHz, $CDCl_3$): 11.7 (CH_3-3'), 22.8 (CH_2-2'), 43.1 (CH_2), 53.4 (CH_2-1'), 111.3 (CH, Ar-5), 120.1 (q, Ar-3), 135.4 (CH, Ar-4), 146.6 (CH, Ar-6), 158.2 (q, Ar-2).

ν_{max} (ATR)/cm^{-1}: 3286 (NH), 2959, 2928, 2870, 1599, 1586, 1509, 1463, 1415, 1378, 1336, 1295, 1252, 1076, 1021, 969, 849, 760.

HRMS (m/z ESI^+): Found: 166.1337 ($M^+ + H$, $C_9H_{16}N_3$ Requires: 166.1344).

3-Aminomethyl-2-hydroxyethylaminopyridine 35b



Following Method F, to a suspension of $LiAlH_4$ (2.0 eq., 325.6 mg, 8.58 mmol) in anhydrous THF (10 mL) at 0 °C was added a solution of compound **28b** (1.0 eq., 700 mg, 4.29 mmol) in anhydrous THF (10 mL) dropwise. The resulting mixture was heated to 70 °C and stirred until full conversion of starting material was observed by TLC. Usual workup and purification afforded **35b** (643 mg, 79%) as a clear oil.

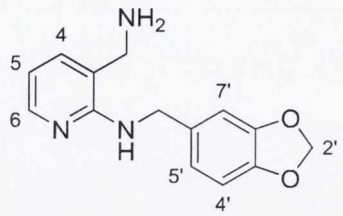
δ_H (400 MHz, $CDCl_3$): 3.47 (s, 2H, CH_2), 3.68 (t, 4H, J 6.9, CH_2-1', CH_2-2'), 6.39 (app. t, 1H, J 7.1, H-5), 6.65 (br s, 1H, NH), 7.09 (m, 1H, H-4), 7.84 (dd, 1H, ^{3}J 7.1, ^{4}J 2.1, H-6).

δ_C (100 MHz, $CDCl_3$): 44.1 (CH_2), 44.9 (CH_2-1'), 63.7 (CH_2-2'), 112.2 (CH, Ar-5), 120.3 (q, Ar-3), 136.3 (CH, Ar-4), 145.6 (CH, Ar-6), 158.1 (q, Ar-2).

ν_{max} (ATR)/cm^{-1}: 3301 (NH), 3209 (OH), 1679, 1612, 1509, 1309, 1300, 1278, 1236, 1067, 978, 725.

HRMS (m/z ESI^+): Found: 190.0956 ($M^+ + Na$, $C_8H_{13}N_3NaO$ Requires: 190.0951).

3-Aminomethyl-2-(1,3-benzodioxolyl-5-methylamino)pyridine 35c



Following Method F, to a suspension of $LiAlH_4$ (2.0 eq., 93.3 mg, 2.46 mmol) in anhydrous THF (3 mL) at 0 °C was added a solution of compound **28c** (1.0 eq., 310 mg, 1.23 mmol) in anhydrous THF (3 mL) dropwise. The resulting mixture was heated to 70 °C and stirred until full conversion of starting material was observed by TLC. Usual workup and purification afforded **35c** (199 mg, 63%) as a clear oil.

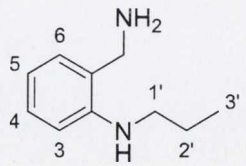
δ_H (400 MHz, $CDCl_3$): 1.49 (br s, 2H, NH_2), 3.74 (s, 2H, CH_2), 4.54 (s, 2H CH_2), 5.83 (s, 2H, CH_2-2'), 6.45 (m, 1H, H-5), 6.71 (d, 1H, J 7.2, H-4'), 6.79 (d, 1H, J 7.2, H-5'), 6.86 (s, 1H, H-7'), 7.12 (d, 1H, J 7.2, H-4), 8.01 (d, 1H, J 7.2, H-6).

δ_C (100 MHz, $CDCl_3$): 44.6 (CH_2), 45.0 (CH_2), 100.8 (CH_2-2'), 108.0 (CH, Ar-4'), 108.1 (CH, Ar-7'), 111.9 (CH-5), 119.8 (q, 3), 120.5 (CH, Ar-5'), 134.3 (q, Ar-6'), 135.6 (CH-4), 146.3 (CH-6), 146.3 (q, Ar-1'a or Ar-3'a), 147.2 (q, Ar-1'a or Ar-3'a), 157.8 (q, Ar-2).

ν_{max} (ATR)/cm^{-1}: 3295 (NH), 2891, 1600, 1501, 1488, 1463, 1442, 1415, 1370, 1326, 1247, 1114, 1038, 927, 809.

HRMS (m/z ESI^+): Found: 258.1237 ($M^+ + H$, $C_{14}H_{16}N_3O_2$ Requires: 258.1243).

2-Aminomethyl-*N*-propylaniline 35d



Following Method F, to a suspension of $LiAlH_4$ (2.0 eq., 142.6 mg, 3.76 mmol) in anhydrous THF (3 mL) at 0 °C was added a solution of compound **34a** (1.0 eq., 300 mg, 1.88 mmol) in anhydrous THF (3 mL) dropwise. The resulting mixture was heated to 70 °C and stirred until

full conversion of starting material was observed by TLC. Usual workup and purification afforded **35d** (217 mg, 70%) as a yellow oil.

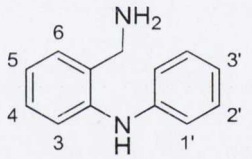
δ_H (400 MHz, $CDCl_3$): 1.19 (t, 3H, J 7.0, CH_3-3'), 1.87 (m, 2H, CH_2-2'), 3.25 (t, 2H, J 7.0, CH_2-1'), 4.00 (s, 2H, CH_2), 6.79 (m, 2H, H-3, H-5), 7.17 (d, 1H, J 7.3, H-6), 7.33 (app. t, 1H, J 7.3, H-4).

δ_C (100 MHz, $CDCl_3$): 11.8 (CH_3-3'), 22.0 (CH_2-2'), 45.5 (CH_2), 49.9 (CH_2-1'), 110.5 (CH, Ar-3), 116.3 (CH, Ar-5), 123.6 (q, Ar-1), 128.5 (CH, Ar-6), 128.7 (CH, Ar-4), 147.6 (q, Ar-2).

ν_{max} (ATR)/cm^{-1}: 3330 (NH), 2958, 2928, 2871, 1605, 1587, 1517, 1456, 1379, 1313, 1258, 1195, 1150, 1076, 1050, 1017, 969, 926, 850, 777, 728.

HRMS (m/z ESI^+): Found: 165.1390 ($M^+ + H$, $C_{10}H_{17}N_2$ Requires: 165.1392).

2-Aminomethyl-*N*-phenylaniline 35e



Following Method F, to a suspension of $LiAlH_4$ (2.0 eq., 78.2 mg, 2.06 mmol) in anhydrous THF (3 mL) at 0 °C was added a solution of compound **34b** (1.0 eq., 200 mg, 1.03 mmol) in anhydrous THF (3 mL) dropwise. The resulting mixture was heated to 70 °C and stirred until full conversion of starting material was observed by TLC. Usual workup and purification afforded **35e** (157 mg, 77%) as a dark yellow oil.

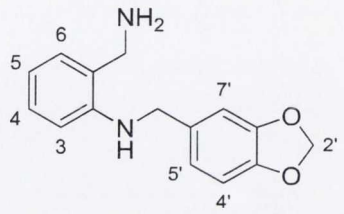
δ_H (400 MHz, $CDCl_3$): 4.04 (s, 2H, CH_2), 6.94 (d, 1H, J 7.3, H-3), 7.00 (app. t, 1H, J 7.3, H-5), 7.24 (m, 3H, H-1', H-3'), 7.31 (app. t, 1H, J 7.2, H-6), 7.37 (app. t, 2H, J 7.2, H-2'), 7.51 (app. t, 1H, J 7.3, H-4).

δ_C (100 MHz, $CDCl_3$): 53.6 (CH_2), 116.2 (CH, Ar-3'), 117.8 (CH, Ar-1'), 119.9 (CH, Ar-3), 120.3 (CH, Ar-5), 128.2 (CH, Ar-6), 129.1 (q, Ar-1), 129.4 (CH, Ar-4), 129.7 (CH, Ar-2'), 143.5 (q, Ar-2 or Ar-Ph), 143.6 (q, Ar-2 or Ar-Ph).

ν_{max} (ATR)/cm^{-1}: 3260 (NH), 3042 (NH), 2861, 1590, 1576, 1516, 1495, 1458, 1324, 1304, 1249, 1175, 1154, 1108, 1047, 1018, 970, 937, 865, 840, 790, 742, 698.

HRMS (m/z APCI$^+$): Found: 197.1057 (M^+ - H, $C_{13}H_{13}N_2$ Requires: 197.1084).

2-Aminomethyl-*N*-(1,3-benzodioxolyl-5-methylen)aniline 35f



Following Method F, to a suspension of $LiAlH_4$ (2.0 eq., 90.3 mg, 2.38 mmol) in anhydrous THF (3 mL) at 0 °C was added a solution of compound **34c** (1.0 eq., 300 mg, 1.19 mmol) in anhydrous THF (3 mL) dropwise. The resulting mixture was heated to 70 °C and stirred until full conversion of starting material was observed by TLC. Usual workup and purification afforded **35f** (213 mg, 69%) as a yellow oil.

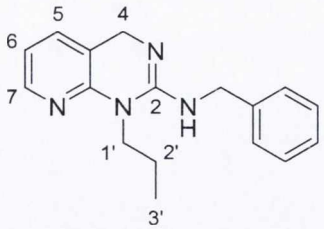
δ_H (400 MHz, $CDCl_3$): 4.01 (s, 2H, CH_2), 5.48 (s, 1H, CH_2), 5.98 (s, 2H, CH_2-2'), 6.75 (m, 2H, H-3, H-5), 6.88 (br s, 1H, H-4'), 6.96 (d, 1H, J 7.0, H-5'), 7.01 (s, 1H, H-7'), 7.13 (d, 1H, J 7.1, H-6), 7.25 (m, 1H, H-4).

δ_C (100 MHz, $CDCl_3$): 45.6 (CH_2), 47.5 (CH_2), 101.1 (CH_2-2'), 107.9 (CH, Ar-4'), 108.2 (CH, Ar-7'), 110.7 (CH, Ar-3), 116.5 (CH, Ar-5), 120.3 (CH, Ar-5'), 125.9 (q, Ar-1), 128.5 (CH, Ar-6), 129.0 (CH, Ar-4), 133.8 (q, Ar-6'), 146.5 (q, Ar-2), 147.8 (q, Ar-1'a or Ar-3'a), 147.9 (q, Ar-1'a or Ar-3'a).

ν_{max} (ATR)/cm^{-1}: 3296 (NH), 2889, 1604, 1586, 1500, 1440, 1309, 1234, 1193, 1160, 1094, 1035, 971, 924, 856, 807, 746, 728 .

HRMS (m/z ESI$^+$): Found: 257.1289 (M^+ + H, $C_{15}H_{17}N_2O_2$ Requires: 257.1290).

2-Benzylamino-1-propyl-1,4-dihydropyrido[2,3-*d*]pyrimidine 38a



Following Method G, to a stirred solution of compound **35a** (1.0 eq., 775 mg, 4.7 mmol) and NEt_3 (3.0 eq., 1.96 mL, 14.1 mmol) in DMF (5 mL) at 0 °C was added benzyl isothiocyanate (1.0 eq., 622 µL, 4.7 mmol) dropwise. After 2 h $HgCl_2$ (1.0 eq., 1.27 g, 4.7 mmol) was added and the reaction was stirred at RT for 2 h. Usual workup and purification afforded compound **38a** (560 mg, 43%) as a clear oil.

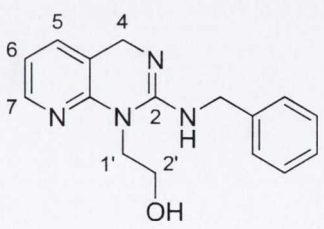
δ_H (400 MHz, $CDCl_3$): 0.90 (t, 3H, J 7.1, CH_3-3'), 1.67 (m, 2H, CH_2-2'), 4.00 (t, 2H, J 7.1, CH_2-1'), 4.40 (s, 2H, CH_2-4), 4.54 (s, 2H, CH_2), 6.86 (app. t, 1H, J 7.3, H-6), 7.21 (d, 2H, J 7.2, H-Ar Bn), 7.27 (t, 2H, J 7.4, H-Ar Bn), 7.35 (m, 2H, H-5, H-Ar Bn), 8.10 (dd, 1H, ^{3}J 7.3, ^{4}J 1.3, H-7).

δ_C (100 MHz, $CDCl_3$): 10.3 (CH_3-3'), 20.6 (CH_2-2'), 42.9 (CH_2-4), 43.9 (CH_2-1'), 45.2 (CH_2), 116.1 (q, Ar-4a), 119.1 (CH, Ar-6), 126.8 (CH, Ar-*p*-Bn), 126.9 (CH, Ar-*o*-Bn), 127.0 (CH, Ar-*m*-Bn), 128.7 (CH, Ar-5), 146.2 (CH, Ar-7), 148.4 (q, Ar), 159.8 (q, Ar-8a), 168.9 (q, 2).

ν_{max} (ATR)/cm^{-1}: 3227 (NH), 3029 (NH), 2961, 2932, 1624, 1594, 1551, 1430, 1396, 1320, 1299, 1231, 1139, 1162, 1105, 1028, 856, 782, 734, 696.

HRMS (m/z ESI$^+$): Found: 281.1760 (M^+ + H, $C_{17}H_{21}N_4$ Requires: 281.1766).

2-Benzylamino-1-hydroxyethyl-1,4-dihydropyrido[2,3-*d*]pyrimidine 38b



Following Method G, to a stirred solution of compound **35b** (1.0 eq., 500 mg, 2.9 mmol) and NEt_3 (3.0 eq., 1.21 mL, 8.7 mmol) in DMF (5 mL) at 0 °C was added benzyl isothiocyanate (1.0 eq., 384 µL, 2.9 mmol) dropwise. After 2 h $HgCl_2$ (1.0 eq., 785 mg, 2.9 mmol) was added and the reaction was stirred at RT for 2 h. Usual workup and purification afforded compound **38b** (178 mg, 21%) as a clear gum.

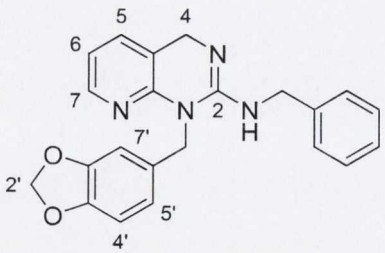
δ_H (400 MHz, $CDCl_3$): 3.96 (t, 2H, J 7.2, CH_2-1'), 4.11 (t, 2H, J 7.2, CH_2-2'), 4.56 (s, 2H, CH_2-4), 4.62 (s, 2H, CH_2), 6.94 (app. t, 1H, J 7.0, H-6), 7.10 (d, 1H, J 7.3, H-Ar Bn), 7.17 (t, 2H, J 7.0, H-Ar Bn), 7.26 (d, 2H, J 7.1, H-Ar Bn), 7.36 (d, 1H, J 7.0, H-5), 8.11 (dd, 1H, ^{3}J 7.0, ^{4}J 2.9, H-7).

δ_C (100 MHz, $CDCl_3$): 40.4 (CH_2-4), 46.3 (CH_2), 48.1 (CH_2-1'), 50.1 (CH_2-2'), 115.7 (CH, Ar-6), 120.5 (q, Ar-4a), 127.8-128.7 (5 CH, Ar-Bn), 134.3 (q, Ar), 135.3 (CH, Ar-5), 147.7 (CH, Ar-7), 148.1 (q, Ar-8a), 153.5 (q, 2).

ν_{max} (ATR)/cm^{-1}: 3369 (OH), 3190 (NH) 2947, 2951, 1609, 1472, 1423, 1401, 1395, 1327, 1109, 1101, 1075, 978, 879, 778, 734, 654.

HRMS (m/z ESI^+): Found: 283.1560 (M^+ + H, $C_{16}19_2N_4O$ Requires: 283.1553).

1-(1,3-Benzodioxolyl-5-methyl)-2-benzylamino-1,4-dihydropyrido[2,3-*d*]pyrimidine 38c



Following Method G, to a stirred solution of compound **35c** (1.0 eq., 950 mg, 3.7 mmol) and NEt_3 (3.0 eq., 1.54 mL, 11.1 mmol) in DMF (5 mL) at 0 °C was added benzyl isothiocyanate (1.0 eq., 490 µL, 3.7 mmol) dropwise. After 2 h $HgCl_2$ (1.0 eq., 1.001 g, 3.7 mmol) was added and the reaction was stirred at RT for 2 h. Usual workup and purification afforded compound **38c** (480 mg, 35%) as a yellow oil.

δ_H (400 MHz, $CDCl_3$): 4.43 (d, 2H, J 5.0, CH_2), 4.57 (s, 2H, CH_2-4), 5.27 (s, 2H, CH_2), 5.95 (s, 2H, CH_2-2'), 6.65 (d, 2H, J 7.9, H-4', H-5'), 6.72 (m, 1H, H-Ar Bn), 6.92 (app. t, 1H, J

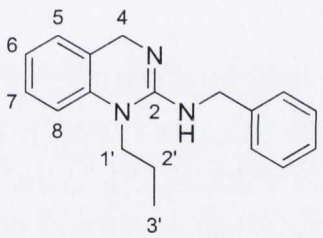
7.3, H-6), 7.11 (m, 3H, H-7', H-Ar Bn), 7.14-7.21 (m, 2H, H-Ar Bn), 7.36 (d, 1H, J 7.3, H-5), 8.13 (dd, 1H, ^{3}J 7.3, ^{4}J 1.4, H-7).

δ_C (100 MHz, $CDCl_3$): 44.5 (CH_2), 44.9 (CH_2), 45.9 (CH_2-4), 100.1 (CH_2-2'), 107.1 (CH, Ar-6), 107.9 (CH, Ar-4'), 117.3 (q, Ar-4a), 118.1 (CH, Ar-7'), 119.5 (CH, Ar-5'), 126.9 (CH, Ar-*p*-Bn), 128.4 (CH, Ar-*o*-Bn), 129.4 (CH, Ar-*m*-Bn), 133.2 (CH, Ar-5), 145.6 (CH, Ar-7), 146.6 (q, Ar-1'a or Ar-3'a), 147.8 (q, Ar-1'a or Ar-3'a), 150.3 (q, Ar), 151.9 (q, Ar-6'), 154.6 (q, Ar-8a), 165.9 (q, 2).

ν_{max} (ATR)/cm^{-1}: 3262 (NH), 3027 (NH), 2935, 1637, 1564, 1500, 1497, 1345, 1234, 1821, 1094, 1035, 921, 892, 864, 759, 745, 697.

HRMS (m/z ESI$^+$): Found: 373.1666 (M^+ + H, $C_{22}H_{21}N_4O_2$ Requires: 373.1665).

2-Benzylamino-1-propyl-1,4-dihydroquinazoline 38d



Following Method G, to a stirred solution of compound **35d** (1.0 eq., 260 mg, 1.59 mmol) and NEt_3 (3.0 eq., 664 µL, 4.77 mmol) in DMF (5 mL) at 0 °C was added benzylisothiocyanate (1.0 eq., 211 µL, 1.59 mmol) dropwise. After 2 h $HgCl_2$ (1.0 eq., 430 mg, 1.59 mmol) was added and the reaction was stirred at RT for 2 h. Usual workup and purification afforded compound **38d** (300 mg, 35%) as a yellow gum.

δ_H (400 MHz, $CDCl_3$): 0.71 (t, 3H, J 7.0, CH_3-3'), 1.50 (m, 2H, CH_2-2'), 3.80 (t, 2H, J 7.0, CH_2-1'), 4.12 (s, 2H, CH_2-4), 4.46 (s, 2H, CH_2), 6.78 (d, 1H, J 7.2, H-8), 6.90 (m, 3H, H-5, H-6, H-7), 7.07 (t, 1H, J 7.1, H-Ar Bn), 7.15 (t, 2H, J 7.3, H-Ar Bn), 7.27 (d, 2H, J 7.0, H-Ar Bn).

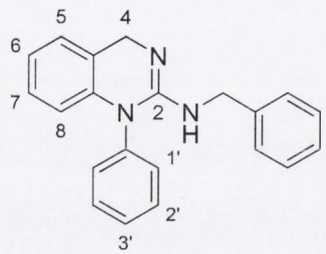
δ_C (100 MHz, $CDCl_3$): 10.5 (CH_3-3'), 20.4 (CH_2-2'), 41.7 (CH_2-4), 47.2 (CH_2-1'), 49.6 (CH_2), 115.4 (CH, Ar-8), 123.9 (CH, Ar-6), 124.3 (CH, Ar-7), 125.6 (CH, Ar-5), 127.5 (CH,

Ar-*o*-*p*-Bn), 128.0 (CH, Ar-*m*-Bn), 128.4 (q, Ar-4a), 137.2 (q, Ar), 153.5 (q, Ar-8a), 178.5 (q, 2).

ν_{max} (ATR)/cm^{-1}: 3253 (NH), 2963, 1636, 1601, 1565, 1479, 1429, 1295, 1235, 1266, 1160, 1094, 1033, 920, 808, 786, 751, 696.

HRMS (m/z ESI$^+$): Found: 280.1828 ($M^+ + H$, $C_{18}H_{22}N_3$ Requires: 280.1814).

2-Benzylamino-1-phenyl-1,4-dihydroquinazoline 38e



Following Method G, to a stirred solution of compound **35e** (1.0 eq., 300 mg, 1.41 mmol) and NEt_3 (3.0 eq., 589 µL, 4.23 mmol) in DMF (5 mL) at 0 °C was added benzyl isothiocyanate (1.0 eq., 186 µL, 1.41 mmol) dropwise. After 2 h $HgCl_2$ (1.0 eq., 382 mg, 1.41 mmol) was added and the reaction was stirred at RT for 2 h. Usual workup and purification afforded compound **38e** (260 mg, 58%) as an orange oil.

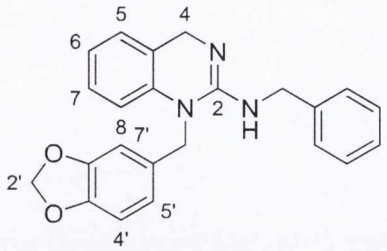
δ_H (400 MHz, $CDCl_3$): 4.49 (s, 2H, CH_2-4), 4.65 (s, 2H, CH_2), 6.09 (t, 1H, J 7.1, H-8), 6.94 (t, 2H, J 7.1, H-7, H-6), 7.01 (t, 1H, J 7.1, H-5), 7.17 (m, 3H, H-1', H-3'), 7.22 (m, 2H, H-2'), 7.31 (d, 2H, J 7.3, H-Ar *o*-Bn), 7.48 (d, 1H, J 7.3, H-Ar *p*-Bn), 7.53 (t, 2H, J 7.3, H-Ar *m*-Bn).

δ_C (100 MHz, $CDCl_3$): 45.2 (CH_2-4), 46.0 (CH_2), 113.9 (CH, Ar-8), 121.0, (CH, Ar-6), 123.0 (CH, Ar-7), 125.7 (CH, Ar-3'), 127.2 (CH, Ar-*p*-Bn), 127.3 (CH, Ar-*o*-Bn), 128.5 (CH, Ar-1', Ar-*m*-Bn), 129.7 (q, Ar-4a), 130.3 (CH, Ar-2'), 130.9 (CH, Ar-5), 136.5 (q, Ar), 138.2 (q, Ar-8a), 139.4 (q, Ar), 159.4 (q, 2).

ν_{max} (ATR)/cm^{-1}: 3270 (NH), 2965, 1637, 1602, 1435, 1307, 1237, 1117, 1096, 1037, 922, 882, 808, 694.

HRMS (m/z ESI$^+$): Found: 314.1659 ($M^+ + H$, $C_{21}H_{20}N_3$ Requires: 314.1657).

1-(1,3-Benzodioxolyl-5-methyl)-2-benzylamino-1,4-dihydroquinazoline 38f



Following Method G, to a stirred solution of compound **35f** (1.0 eq., 400 mg, 1.56 mmol) and NEt_3 (3.0 eq., 652 μL, 4.68 mmol) in DMF (5 mL) at 0 °C was added benzyl isothiocyanate (1.0 eq., 206 μL, 1.56 mmol) dropwise. After 2 h $HgCl_2$ (1.0 eq., 422 mg, 1.56 mmol) was added and the reaction was stirred at RT for 2 h. Usual workup and purification afforded compound **38f** (375 mg, 64%) as a clear oil.

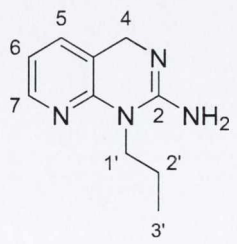
δ_H (400 MHz, $CDCl_3$): 4.33 (s, 2H, CH_2-4), 4.45 (s, 2H, CH_2), 5.01 (s, 2H, CH_2), 5.85 (s, 2H, CH_2-2'), 6.63 (m, 3H, H-6, H-7, H-8), 6.75 (d, 1H, J 7.0, H-5), 6.94 (m, 2H, H-4', H-5'), 7.06 (m, 1H, H-7'), 7.22 (m, 5H, H-Ar Bn).

δ_C (100 MHz, $CDCl_3$): 43.4 (CH_2-4), 46.7 (CH_2), 48.9 (CH_2), 101.1 (CH_2-2'), 106.8 (CH, Ar-4'), 108.4 (CH, Ar-7'), 114.9 (CH, Ar-8), 119.8 (CH, Ar-6), 123.6 (CH, Ar-5'), 124.0 (CH, Ar-7), 125.5 (CH, Ar-*p*-Bn), 127.1 (CH, Ar-*o*-Bn), 127.7 (CH, Ar-*m*-Bn), 128.4 (CH, Ar-5), 129.3 (q, Ar-4a), 138.0 (q, Ar), 138.2 (q, Ar), 139.4 (q, Ar-8a), 147.0 (q, Ar-1'a or Ar-3'a), 148.1 (q, Ar-1'a or Ar-3'a), 152.7 (q, 2).

ν_{max} (ATR)/cm^{-1}: 2920 (NH), 1636, 1603, 1561, 1487, 1441, 1466, 1355, 1234, 1121, 1068, 921, 807, 731, 695.

HRMS: (m/z ESI^+): Found: 372.1709 ($M^+ + H$, $C_{23}H_{22}N_3O_2$ Requires: 372.1712).

2-Amino-1-propyl-1,4-dihydropyrido[2,3-*d*]pyrimidine 39a



Following Method H, to a solution of compound **38a** (1.0 eq., 50 mg, 0.18 mmol) in CH_3OH (2 mL) was added 10% Pd/C (25 mg, 50 wt. %) and NH_4HCO_2 (10.0 eq., 112 mg, 1.8 mmol). The mixture was stirred at 50 °C for 48 h. Usual workup and purification yielded title compound **39a** as a clear gum (17 mg, 50%).

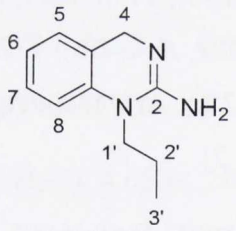
δ_H (400 MHz, D_2O): 0.84 (t, 3H, J 6.7, CH_3-3'), 1.65 (m, 2H, CH_2-2'), 4.23 (t, 2H, J 6.7, CH_2-1'), 4.71 (s, 2H, CH_2-4), 7.40 (app. d, 1H, J 7.1, H-6), 8.37 (app. t, 1H, J 7.1, H-5), 8.71 (m, 1H, H-7).

δ_C (100 MHz, D_2O): 9.7 (CH_3-3'), 20.0 (CH_2-2'), 39.9 (CH_2-4), 55.7 (CH_2-1'), 120.9 (CH, Ar-6), 122.6 (q, Ar-4a), 135.7 (CH, Ar-5), 146.5 (CH, Ar-7), 154.2 (q, 2), 157.0 (q, Ar-8a).

ν_{max} (ATR)/cm^{-1}: 3200 (NH), 3047 (NH), 2961, 2873, 1642, 1596, 1566, 1375, 1353, 1331, 1288, 1235, 1137, 1102, 1095, 958, 904, 758, 733.

HRMS (m/z ESI^+): Found: 191.1290 ($M^+ + H$, $C_{10}H_{15}N_4$ Requires: 191.1291).

2-Amino-1-propyl-1,4-dihydroquinazoline 39b



Following Method H, to a solution of compound **38d** (1.0 eq., 50 mg, 0.18 mmol) in CH_3OH (2 mL) was added 10% Pd/C (25 mg, 50 wt. %) and NH_4HCO_2 (10.0 eq., 112 mg, 1.8 mmol). The mixture was stirred at 50 °C for 48 h. Usual workup and purification yielded title compound **39b** as a clear gum (20 mg, 58%).

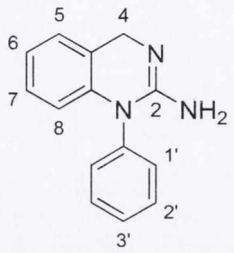
δ_H (400 MHz, $CDCl_3$): 1.03 (t, 3H, J 7.1, CH_3-3'), 1.80 (m, 2H, CH_2-2'), 3.93 (t, 2H, J 7.1, CH_2-1'), 4.43 (s, 2H, CH_2-4), 7.07 (d, 1H, J 7.0, H-8), 7.10 (app. t, 1H, J 7.0, H-6), 7.12 (app. t, 1H, J 7.0, H-7), 7.29 (app. t, 1H, J 7.0, H-5), 8.30 (br s, 1H, NH), 9.04 (br s, 1H, NH).

δ_C (100 MHz, $CDCl_3$): 10.7 (CH_3-3'), 20.3 (CH_2-2'), 41.3 (CH_2-4), 46.5 (CH_2-1'), 114.8 (CH, Ar-8), 121.0 (q, Ar-4a), 124.9 (CH, Ar-6), 126.4 (CH, Ar-7), 128.4 (CH, Ar-5), 134.8 (q, Ar-8a), 154.2 (q, 2).

ν_{max} (ATR)/cm^{-1}: 3035 (NH), 2924, 1693, 1654, 1603, 1543, 1495, 1398, 1275, 1237, 1087, 1043, 895, 869, 824, 750, 728.

HRMS (m/z ESI$^+$): Found: 190.1339 (M^+ + H, $C_{11}H_{16}N_3$ Requires: 190.1333).

2-Amino-1-phenyl-1,4-dihydroquinazoline 39c



Following Method H, to a solution of compound **38e** (1.0 eq., 50 mg, 0.16 mmol) in CH_3OH (2 mL) was added 10% Pd/C (25 mg, 50 wt. %) and NH_4HCO_2 (10.0 eq., 100 mg, 1.6 mmol). The mixture was stirred at 50 °C for 48 h. Usual workup and purification yielded title compound **39c** as a yellow gum (19 mg, 53%).

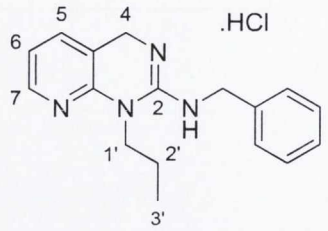
δ_H (400 MHz, $CDCl_3$): 4.71 (s, 2H, CH_2), 7.12 (m, 2H, H-6, H-8), 7.34 (m, 2H, H-5, H-7), 7.67 (m, 5H, H-Ar).

δ_C (100 MHz, $CDCl_3$): 41.1 (CH_2-4), 115.7 (CH, Ar-8), 118.2 (CH, Ar-6), 125.4 (CH, Ar-3'), 126.5 (CH, Ar-1'), 128.4 (q, Ar), 128.6 (CH, Ar-2'), 129.2 (CH, Ar-7), 129.5 (CH, Ar-5), 133.1 (q, Ar), 135.7 (q, Ar), 153.3 (q, 2).

ν_{max} (ATR)/cm^{-1}: 3229 (NH), 3073, 2925, 1604, 1560, 1491, 1342, 1283, 1204, 1188, 1073, 1045, 1000, 948, 921, 758.

HRMS: (m/z ESI$^+$): Found: 224.1182 (M^+ + H, $C_{14}H_{14}N_3$ Requires: 284.1189).

2-Benzylamino-1-propyl-1,4-dihydropyrido[2,3-*d*]pyrimidine hydrochloride 40a



Following Method C, 4M HCl/dioxane (3.0 eq., 133 μL, 0.53 mmol) was added to a solution of **38a** (1.0 eq., 50 mg, 0.17 mmol) in CH_3OH (0.1 mL). The solution was stirred at RT for 2 h. Usual workup and purification yielding **40a** as a yellow solid (47 mg, 87%).

Mp: 113-117 °C.

δ_H (400 MHz, $CDCl_3$): 0.91 (t, 3H, J 7.0, CH_3-3'), 1.65 (m, 2H, CH_2-2'), 4.31 (t, 2H, J 7.1, CH_2-1'), 4.49 (s, 2H, CH_2-4), 4.86 (s, 2H, CH_2), 6.96 (app. t, 1H, J 7.0, H-6), 7.10 (d, 1H, J 7.1, H-Ar Bn), 7.16 (t, 2H, J 7.1, H-Ar Bn), 7.26 (d, 2H, J 7.1, H-Ar Bn), 7.59 (d, 1H, J 7.3, H-5), 8.18 (d, 1H, J 7.3, H-7), 9.27 (br s, 2H, NH).

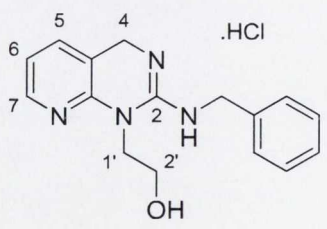
δ_C (100 MHz, $CDCl_3$): 10.8 (CH_3-3'), 21.6 (CH_2-2'), 40.2 (CH_2-4), 44.0 (CH_2), 45.2 (CH_2-1'), 115.5 (q, Ar-4a), 120.1 (CH, Ar-6), 127.6-128.4 (5 CH, Ar-Bn), 134.2 (q, Ar), 136.2 (CH, Ar-5), 147.2 (CH, Ar-7), 147.4 (q, Ar-8a), 152.0 (q, 2).

ν_{max} (ATR)/cm^{-1}: 3044 (NH), 2923, 1719, 1653, 1556, 1549, 1423, 1361, 1330, 1257, 1116, 1050, 1018, 897, 763.

HRMS (m/z ESI^+): Found: 281.17588 ($M^+ + H$, $C_{17}H_{21}N_4$ Requires: 281.1760).

Purity by HPLC: 95.5% (t_R 27.29 min).

2-Benzylamino-1-hydroxyethyl-1,4-dihydropyrido[2,3-*d*]pyrimidine hydrochloride 40b



Following Method C, 4M HCl/dioxane (3.0 eq., 80 μL, 0.31 mmol) was added to a solution of **38b** (1.0 eq., 30 mg, 0.11 mmol) in CH_3OH (0.5 mL). The solution was stirred at RT for 2 h. Usual workup and purification yielded **40b** as a white solid (26 mg, 74%).

Mp: 148-151 °C.

δ_H (400 MHz, $CDCl_3$): 4.02 (t, 2H, J 6.9, CH_2-1'), 4.32 (t, 2H, J 6.9, CH_2-2'), 4.44 (s, 2H, CH_2-4), 4.71 (s, 2H, CH_2), 6.97 (app. t, 1H, J 7.0, H-6), 7.13 (t, 1H, J 7.1, H-Ar Bn), 7.20 (t, 2H, J 7.1, H-Ar Bn), 7.27 (d, 2H, J 7.0, H-Ar Bn), 7.38 (d, 1H, J 7.1, H-5), 8.13 (d, 1H, J 7.1, H-7), 9.33 (br s, 1H, NH), 9.62 (br s, 1H, NH).

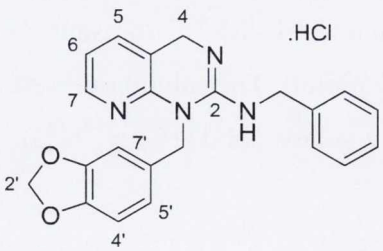
δ_C (100 MHz, $CDCl_3$): 40.4 (CH_2-1'), 46.3 (CH_2-4), 48.1 (CH_2), 60.9 (CH_2-2'), 115.7 (CH, Ar-6), 120.4 (q, Ar-4a), 127.8, (CH, Ar-*p*-Bn), 127.9 (CH, Ar-*o*-Bn), 128.7 (CH, Ar-*m*-Bn), 134.2 (q, Ar), 135.3 (CH, Ar-5), 146.1 (CH, Ar-7), 148.8 (q, Ar-8a), 153.5 (q, 2).

ν_{max} (ATR)/cm^{-1}: 3229 (NH), 2947, 1673, 1598, 1567, 1434, 1497, 1358, 1311, 1079, 1050, 1001, 911, 857, 793, 741.

HRMS (m/z ESI^+): Found: 283.1566 ($M^+ + H$, $C_{16}H_{19}N_4O$ Requires: 283.1553).

Purity by HPLC: 97.1% (t_R 25.15 min).

1-(1,3-Benzodioxolyl-5-methyl)-2-benzylamino-1,4-dihydropyrido[2,3-*d*]pyrimidine hydrochloride 40c



Following Method C, 4M HCl/dioxane (3.0 eq., 172 μL, 0.69 mmol) was added to a solution of **38c** (1.0 eq., 80 mg, 0.23 mmol) in CH_3OH (0.5 mL). The solution was stirred at RT for 2 h. Usual workup and purification yielding **40c** as a yellow solid (71 mg, 77%).

Mp: 91-95 °C.

δ_H (400 MHz, $CDCl_3$): 4.66 (s, 2H, CH_2), 4.82 (s, 2H, CH_2), 5.42 (s, 2H, CH_2-4), 5.95 (s, 2H, CH_2-2'), 6.67 (m, 2H, H-4', H-5'), 6.73 (s, 1H, H-7'), 6.84 (dd, 1H, J 7.3, 5.0, H-6), 7.14 (m, 3H, H-Ar Bn), 7.24 (m, 2H, H-Ar Bn), 7.36 (d, 1H, J 7.3, H-5), 8.27 (d, 1H, J 7.3, H-7).

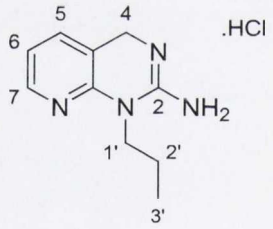
δ_C (150 MHz, $CDCl_3$): 40.3 (CH_2), 46.3 (CH_2), 47.9 (CH_2-4), 101.5 (CH_2-2'), 107.5 (CH, Ar-6), 108.7 (CH, Ar-4'), 108.9 (CH, Ar-7'), 115.3 (q, Ar-4a), 120.1 (CH, Ar-5'), 120.5 (CH, Ar-*p*-Bn), 127.1 (CH, Ar-*o*-Bn), 128.3 (CH, Ar-*m*-Bn), 134.9 (CH, Ar-5), 135.0 (q, Ar), 135.2 (q, Ar), 147.1 (CH, Ar-7), 148.0 (q, Ar-1'a or Ar-3'a), 148.8 (q, Ar-1'a or Ar-3'a), 152.0 (q, Ar-8a), 152.5 (q, 2).

ν_{max} (ATR)/cm^{-1}: 3121 (NH), 2922, 2852, 1640, 1599, 1547, 1488, 14235, 1337, 1310, 1247, 1167, 1095, 922, 848, 769.

HRMS (m/z ESI^+): Found: 373.1672 ($M^+ + H$, $C_{22}H_{21}N_4O_2$ Requires: 373.1659).

Purity by HPLC: 95.5% (t_R 29.20 min).

2-Amino-1-propyl-1,4-dihydropyrido[2,3-*d*]pyrimidine hydrochloride 40d



Following Method C, to a solution of 4M HCl/dioxane (3.0 eq., 66 μL, 0.26 mmol) was added **39a** (1.0 eq., 17 mg, 0.09 mmol). The solution was stirred at RT for 2 h. Usual workup and purification yielded **40d** as a yellow solid (15 mg, 74%).

Mp: 211-220 °C, decomposed.

δ_H (400 MHz, D_2O): 0.80 (t, 3H, J 6.7, CH_3-3'), 1.58 (m, 2H, CH_2-2'), 3.85 (t, 2H, J 6.7, CH_2-1'), 4.40 (s, 2H, CH_2-4), 7.09 (dd, 1H, J 7.1, 4.8, H-6), 7.49 (app. t, 1H, J 7.1, H-5), 8.10 (m, 1H, H-7).

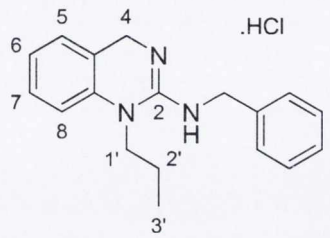
δ_C (100 MHz, CH_3OH): 9.5 (CH_3-3'), 20.5 (CH_2-2'), 39.9 (CH_2-4), 43.5 (CH_2-1'), 115.1 (q, 4a), 120.6 (CH, Ar-6), 134.8 (CH, Ar-5), 145.9 (CH, Ar-7), 154.2 (q, Ar-8a), 159.5 (q, 2).

ν_{max} (ATR)/cm^{-1}: 3268 (NH), 2962, 2933, 2917, 1624, 1595, 1335, 1234, 1155, 1138, 1002, 957, 903, 863, 757, 789, 732.

HRMS (m/z ESI$^+$): Found: 191.1289 (M^+ + H, $C_{10}H_{15}N_4$ Requires: 191.1291).

Purity by HPLC: 95.3% (t_R 22.84 min).

2-Benzylamino-1-propyl-1,4-dihydroquinazoline hydrochloride 40e



Following Method C, 4M HCl/dioxane (3.0 eq., 375 µL, 1.5 mmol) was added to a solution of **38d** (1.0 eq., 140 mg, 0.5 mmol) in CH_3OH (0.5 mL). The solution was stirred at RT for 2 h. Usual workup and purification yielded **40e** as a yellow solid (137 mg, 86%).

Mp: 124-130 °C.

δ_H (400 MHz, $CDCl_3$): 0.75 (t, 3H, J 7.3, CH_3-3'), 1.62 (m, 2H, CH_2-2'), 4.04 (t, 2H, J 7.3, CH_2-1'), 4.31 (s, 2H, CH_2-4), 4.72 (d, 2H, J 5.2, CH_2), 6.82 (d, 1H, J 7.4, H-8), 6.99 (app. t, 1H, 7.4, H-6), 7.05 (app. t, 1H, J 7.4, H-7), 7.10 (m, 1H, H-5), 7.20 (t, 3H, J 7.3, H-Ar Bn), 7.46 (d, 2H, J 7.3, H-Ar Bn), 9.10 (br s, 1H, NH), 9.27 (br s, 1H, NH).

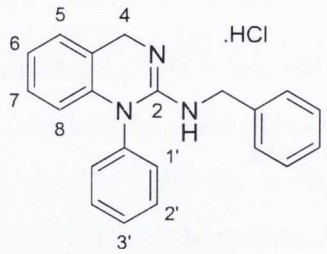
δ_C (100 MHz, $CDCl_3$): 10.5 (CH_3-3'), 20.4 (CH_2-2'), 41.7 (CH_2-4), 47.2 (CH_2-1'), 49.6 (CH_2), 115.4 (CH, Ar-8), 123.9 (CH, Ar-6), 124.3 (CH, Ar-7), 125.6 (CH, Ar-5), 127.5 (CH, Ar-*o*-*p*-Bn), 128.0 (CH, Ar-*m*-Bn), 128.4 (q, Ar-4a), 137.2 (q, Ar), 152.6 (q, Ar-8a), 153.3 (q, 2).

ν_{max} (ATR)/cm^{-1}: 3100 (NH), 2959, 1657, 1602, 1566, 1482, 1404, 1395, 1278, 1253, 1176, 1063, 1029, 795.

HRMS (m/z ESI$^+$): Found: 280.1805 (M^+ + H, $C_{18}H_{22}N_3$ Requires: 280.1808).

Purity by HPLC: 99.1% (t_R 28.39 min).

2-Benzylamino-1-phenyl-1,4-dihydroquinazoline hydrochloride 40f



Following Method C, 4M HCl/dioxane (3.0 eq., 59 μL, 0.23 mmol) was added to a solution of **38e** (1.0 eq., 25 mg, 0.08 mmol) in CH_3OH (0.1 mL). The solution was stirred at RT for 2 h. Usual workup and purification yielded **40f** as an off-white solid (24 mg, 85%).

Mp: 115-120 °C.

δ_H (400 MHz, $CDCl_3$): 4.86 (s, 4H, CH_2-4, CH_2 Bn), 6.16 (app. t, 1H, J 7.2, H-8), 7.10 (m, 3H, H-5, H-7, H-6), 7.25 (m, 5H, J 7.3, H-1', H-2', H-3'), 7.37 (d, 2H, J 7.2, H-Ar Bn), 7.63 (m, 3H, H-Ar Bn).

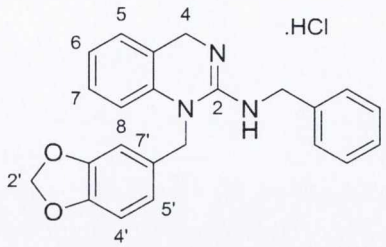
δ_C (100 MHz, $CDCl_3$): 41.5 (CH_2-4), 47.1 (CH_2-Bn), 115.5 (CH, Ar-8), 119.3 (CH, Ar-6), 125.2 (CH, Ar-7), 125.9 (CH, Ar-3'), 126.4 (CH, Ar-*p*-Bn), 127.8 (CH, Ar-*o*-Bn), 128.1 (CH, Ar-1'), 128.8 (CH, Ar-*m*-Bn), 129.9 (q, Ar-4a), 131.2 (CH, Ar-2'), 131.7 (CH, Ar-5), 133.4 (q, Ar), 135.4 (q, Ar-8a), 136.2 (q, Ar), 151.1 (q, 2).

ν_{max} (ATR)/cm^{-1}: 3290 (NH), 2925, 1641, 1602, 1558, 1490, 1357, 13,26, 1244, 1207, 1072, 1028, 752.

HRMS (m/z ESI^+): Found: 314.1659 (M^+ + H, $C_{21}H_{20}N_3$ Requires: 314.1651).

Purity by HPLC: 99.3% (t_R 29.17 min).

1-(1,3-Benzodioxolyl-5-methyl)-2-benzylamino-1,4-dihydroquinazoline hydrochloride 40g



Following Method C, 4M HCl/dioxane (3.0 eq., 90 μL, 0.36 mmol) was added to a solution of **38f** (1.0 eq., 45 mg, 0.12 mmol) in CH_3OH (0.5 mL). The solution was stirred at RT for 2 h. Usual workup and purification yielded **40g** as a yellow solid (43 mg, 88%).

Mp: 98-103 °C.

δ_H (400 MHz, $CDCl_3$): 4.44 (d, 2H, J 5.1, CH_2), 4.70 (d, 2H, J 4.9, CH_2), 5.21 (s, 2H, CH_2-4), 5.90 (s, 2H, CH_2-2'), 6.58 (m, 2H, H-4', H-5'), 6.66 (br s, 1H, H-8), 6.94 (m, 1H, H-6), 7.07 (m, 2H, H-7, H-5), 7.17 (m, 6H, H-7', H-Ar Bn), 8.09 (br s, 1H, NH), 10.01 (br s, 1H, NH).

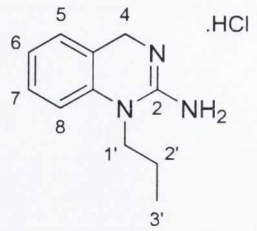
δ_C (100 MHz, $CDCl_3$): 43.4 (CH_2-4), 46.7 (CH_2), 48.9 (CH_2), 101.1 (CH_2-2'), 106.8 (CH, Ar-4'), 108.4 (CH, Ar-7'), 114.9 (CH, Ar-8), 119.8 (CH, Ar-6), 123.6 (CH, Ar-5'), 124.0 (CH, Ar-7), 125.5 (CH, Ar-*p*-Bn), 126.8 (CH, Ar-*o*-Bn), 127.7 (CH, Ar-*m*-Bn), 128.4 (CH, Ar-5), 129.3 (q, Ar-4a), 138.0 (q, Ar), 138.2 (q, Ar), 141.2 (q, Ar-8a), 147.0 (q, Ar-1'a or Ar-3'a), 148.1 (q, Ar-1'a or Ar-3'a), 151.2 (q, 2).

ν_{max} (ATR)/cm^{-1}: 3211 (NH), 2957, 1632, 1600, 1598, 1488, 1444, 1358, 1236, 1170, 1095, 1053, 921, 908, 751.

HRMS (m/z ESI^+): Found: 372.1714 (M^+ + H, $C_{23}H_{22}N_3O_2$ Requires: 372.1706).

Purity by HPLC: 97.3% (t_R 28.64 min).

2-Amino-1-propyl-1,4-dihydroquinazoline hydrochloride 40h



Following Method C, 4M HCl/dioxane (3.0 eq., 79 μL, 0.32 mmol) was added to a solution of **39b** (1.0 eq., 20 mg, 0.11 mmol) in CH_3OH (0.5 mL). The solution was stirred at RT for 2 h. Usual workup and purification yielded **40h** as a yellow solid (21 mg, 85%).

Mp: 210-214 °C, decomposed.

δ_H (400 MHz, D_2O): (t, 3H, J 7.4, CH_3-3'), 1.60 (m, 2H, CH_2-2'), 3.65 (m, 2H, CH_2-1'), 4.27 (s, 2H, CH_2-4), 7.09 (m, 3H, H-6, H-7, H-8), 7.29-7.21 (m, 1H, H-5).

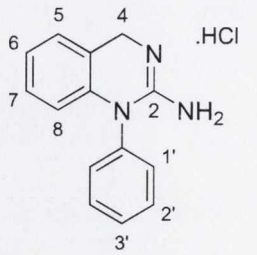
δ_C (100 MHz, D_2O): 9.64 (CH_3-3'), 19.6 (CH_2-2'), 40.4 (CH_2-4), 46.2 (CH_2-1'), 115.4 (CH, Ar-8), 121.8 (q, Ar-4a), 125.1 (CH, Ar-6), 126.1 (CH, Ar-7), 128.5 (CH, Ar-5), 133.9 (q, Ar-8a), 149.1 (q, 2).

ν_{max} (ATR)/cm^{-1}: 3276 (NH), 2941, 1648, 1601, 1575, 1512, 1392, 1243, 1149, 1020, 795, 691.

HRMS (m/z ESI^+): Found: 190.1359 ($M^+ + H$, $C_{11}H_{16}N_3$ Requires: 190.1338).

Purity by HPLC: 97.6% (t_R 24.19 min).

2-Amino-1-phenyl-1,4-dihydroquinazoline hydrochloride 40i



Following Method C, to a solution of 4M HCl/dioxane (3.0 eq., 63 μL, 0.25 mmol) was added **39c** (1.0 eq., 19 mg, 0.08 mmol). The solution was stirred at RT for 2 h. Usual workup and purification yielded **40i** as a yellow solid (19 mg, 91%).

Mp: 240-250 °C, decomposed.

δ_H (400 MHz, D_2O): 4.73 (s, 2H, CH_2-4), 6.20 (m, 1H, H-6), 7.05-7.16 (m, 3H, H-5, H-7, H-8), 7.36 (d, 2H, J 7.7, H-1'), 7.67 (m, 3H, H-2', H-3').

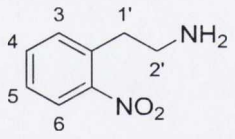
δ_C (100 MHz, CH_3OH): 40.6 (CH_2-4), 115.9 (CH, Ar-8), 118.9 (CH, Ar-6), 125.0 (CH, Ar-3'), 126.1 (CH, Ar-1'), 128.5 (q, Ar-4a), 129.5 (CH, Ar-2'), 130.8 (CH, Ar-7), 131.1 (CH, Ar-5), 133.9 (q, Ar), 136.0 (q, Ar-8a), 153.0 (q, 2).

ν_{max} (ATR)/cm^{-1}: 3337 (NH), 3249 (NH), 2934, 2858, 1643, 1600, 1492, 1421, 1345, 1286, 1143, 1026, 761, 705.

HRMS (m/z ESI$^+$): Found: 224.1188 (M^+ + H, $C_{14}H_{14}N_3$ Requires: 224.1182).

Purity by HPLC: 98.7% (t_R 24.16 min).

2-Aminoethylnitrobenzene 45[217]

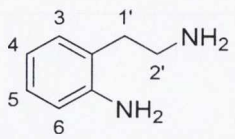


To a solution of 2-cyanomethylnitrobenzene (1.5 g, 9.26 mmol) in anhydrous THF (25 mL) under argon was added BH_3.DMS solution (2M in THF, 10.6 mL, 21.3 mmol) dropwise at 0° C. After addition, the mixture was refluxed for 8 h. The solution was cooled to 0 °C and quenched with CH_3OH (15 mL). The solvent was removed under reduced pressure and the residue purified by silica gel chromatography, eluting in CH_2Cl_2:CH_3OH, affording compound **45** (1.26 mg, 82%) as a brown oil.[217]

δ_H (400 MHz, $CDCl_3$): 1.67 (br s, 2H, NH_2), 2.90 (br s, 4H, CH_2-1', CH_2-2'), 7.25 (m, 2H, H-3, H-5), 7.43 (app. t, 1H, J 7.3, H-4), 7.80 (d, 1H, J 7.3, H-6).

HRMS (m/z ESI$^+$): Found: 167.0820 (M^+ + H, $C_8H_{11}N_2O_2$ Requires: 167.0821).

2-Aminoethylaniline 42[217]

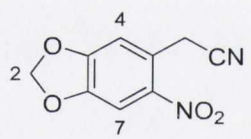


To a solution of compound **45** (400 mg, 2.41 mmol) in EtOH (4 mL) was added 10% Pd/C (40 mg, 10 wt. %). The mixture was stirred under an atmosphere of hydrogen (3 atm) for 12 h at RT. It was diluted with CH_3OH (30 mL), filtered and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography using gradient elution of CH_2Cl_2:CH_3OH to give title compound **42** (327 mg, 99%) as a brown oil.[217]

δ_H (400 MHz, $CDCl_3$): 2.64 (t, 2H, J 7.0, CH_2-1'), 2.95 (app. t, 2H, J 7.0, CH_2-2'), 6.68 (m, 2H, H-4, H-6), 7.02 (m, 2H, H-3, H-5).

HRMS (m/z ESI^+): Found: 137.1074 ($M^+ + H$, $C_8H_{13}N_2$ Requires: 137.1079).

5-Cyanomethyl-6-nitro-1,3-benzodioxole 47



Homopiperonylnitrile (2.0 g, 12.4 mmol) was added to a mixture of HNO_3 (65% in H_2O, 8 mL, 70% in AcOH, 2 mL) at 0 °C. The solution was stirred at RT for 30 min, and then at 40 °C for 30 min. The mixture was cooled to RT, after which ice was added to the reaction mixture and the resulting precipitate was filtered to give **47** (1.8 g, 70% yield) as a bright yellow powder.

Mp: 115 °C.

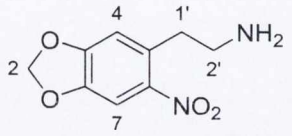
δ_H (400 MHz, $CDCl_3$): 4.09 (s, 2H, CH_2), 6.14 (s, 2H, CH_2-2), 7.03 (s, 1H, H-4), 7.61 (s, 1H, H-7).

δ_C (100 MHz, $CDCl_3$): 23.3 (CH_2), 103.6 (CH_2-2), 111.1 (q, Ar 5), 106.2 (CH, Ar-4), 109.7 (CH, Ar-7), 116.5 (q, CN), 141.7 (q, Ar-6), 148.1 (q, Ar-1a or Ar-3a), 152.6 (q, Ar-1a or Ar-3a).

ν_{max} (ATR)/cm^{-1}: 3091 (NH), 2991, 2257 (CN), 1734, 1618, 1504 (NO_2), 1478, 1402, 1319, 1258, 1237, 1163, 1141, 1103, 1032, 1019, 1006, 946, 918, 888, 846, 775.

HRMS (m/z ESI$^-$): Found: 205.0254 (M - H, $C_9H_5N_2O_4$ Requires: 205.0249).

5-Aminoethyl-6-nitro-1,3-benzodioxole 48



To a solution of compound **47** (5.2 g, 25.2 mmol) in anhydrous THF (70 mL) under argon was added BH_3.DMS solution (2M in THF, 29.0 mL, 58.0 mmol) dropwise at 0 °C. The reaction was refluxed for 8 h, after which it was cooled to 0 °C and quenched with methanol (50 mL). Solvent was removed under reduced pressure and the residue purified by silica gel chromatography, eluting in CH_2Cl_2:CH_3OH, affording compound **48** (3.97 g, 75%) as a brown oil.

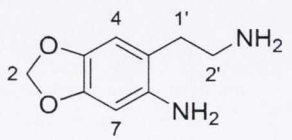
δ_H (400 MHz, CDCl3): 2.89 (br s, 4H, CH_2-1', CH_2-2'), 5.98 (s, 2H, CH_2-2), 6.66 (s, 1H, H-4), 7.37 (s, 1H, H-7).

δ_C (100 MHz, $CDCl_3$): 42.3 (CH_2-1'), 49.8 (CH_2-2'), 102.8 (CH_2-2), 110.7 (CH, Ar-4), 110.7 (CH, Ar-7), 131.5 (q, Ar-5), 142.6 (q, Ar-6), 151.7 (q, Ar-1a or Ar-3a), 156.3 (q, Ar-1a or Ar-3a).

ν_{max} (ATR)/cm^{-1}: 3377 (NH), 3133 (NH), 2985, 2872, 2836, 1733, 1618, 1578 (NO_2), 1409, 1397, 1381, 1087, 1031, 925, 737, 718.

HRMS (m/z ESI$^+$): Found: 211.0711 (M^+ + H, $C_9H_{11}N_2O_4$ Requires: 211.0719).

5-Amino-6-aminoethyl-1,3-benzodioxole 43



To a solution of compound **48** (2.7 g, 15.0 mmol) in EtOH (25 mL) was added 10% Pd/C (270 mg, 10 wt. %). The mixture was stirred under an atmosphere of hydrogen (3 atm) for 18 h. It was then diluted with CH_3OH (50 mL), filtered and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography using gradient elution of CH_2Cl_2:CH_3OH giving title compound **43** (2.1 g, 78%) as a red oil.

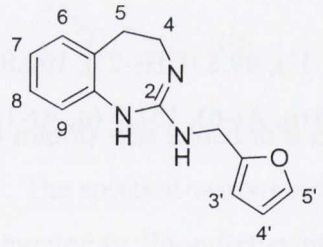
δ_H (400 MHz, $CDCl_3$): 2.54 (t, 2H, J 7.1, CH_2-1'), 2.89 (t, 2H, J 7.1, CH_2-2'), 5.79 (s, 2H, CH_2-2), 6.25 (s, 1H, H-7), 6.50 (s, 1H, H-4).

δ_C (100 MHz, $CDCl_3$): 34.9 (CH_2-1'), 41.8 (CH_2-2'), 98.2 (CH_2-2), 100.5 (CH, Ar-4), 109.9 (CH, Ar-7), 116.9 (q, Ar-5), 139.1 (q, Ar-6), 140.3 (q, Ar-1a or Ar-3a), 146.5 (q, Ar-1a or Ar-3a).

ν_{max} (ATR)/cm^{-1}: 3320 (NH), 2967, 1701, 1678, 1567, 1501, 1409, 1378, 1234, 1200, 1172, 976, 873, 724.

HRMS (m/z ESI^+): Found: 181.0971 (M^+ + H, $C_9H_{13}N_2O_2$ Requires: 181.0977).

2-(Furanyl-2-methylamino)-4,5-dihydro-1,3-benzodiazepine 49a



Following Method B, Cu(II)O (0.2 eq., 20.3 mg, 0.17 mmol) and K_2CO_3 (2.0 eq., 234 mg, 1.7 mmol) were added to a solution of compound **42** (1.0 eq., 116 mg, 0.85 mmol) and **19a** (1.1 eq., 174 mg, 0.93 mmol) in DMF (2 mL) at RT. The resulting mixture was heated to 60 °C for 2 h. Usual workup and purification yielded **49a** (109 mg, 52%) as a yellow gum.

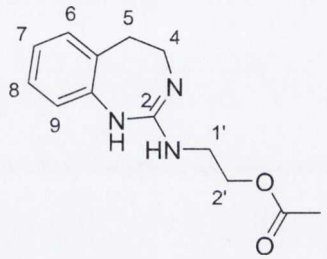
δ_H (400 MHz, $CDCl_3$): 2.77 (t, 2H, J 7.0, CH_2-5), 3.61 (t, 2H, J 7.0, CH_2-4), 4.56 (s, 2H, CH_2), 6.21 (d, 1H, J 7.4, H-3'), 6.28 (t, 1H, J 7.4, H-4'), 6.63 (d, 1H, J 7.1, H-9), 6.69 (app. t, 1H, J 7.1, H-7), 6.96 (d, 1H, J 7.1, H-6), 7.00 (app. t, 1H, J 7.1, H-8), 7.30 (br s, 1H, H-5').

δ_C (100 MHz, $CDCl_3$): 31.5 (CH_2-5), 41.5 (CH_2), 44.2 (CH_2-4), 107.9 (CH, Ar-3'), 111.2 (CH, Ar-4'), 116.1 (CH, Ar-9), 118.9 (CH, Ar-7), 123.2 (q, Ar-5a), 127.8 (CH, Ar-8), 130.3 (CH, Ar-6), 142.1 (CH, Ar-5'), 144.5 (q, Ar-2'), 150.1 (q, Ar-9a), 181.9 (q, 2).

ν_{max} (ATR)/cm^{-1}: 3394 (NH), 3177 (NH), 2921, 2851, 1624, 1531, 1450, 1417, 1431, 1339, 1288, 1163, 1140, 1018, 891, 860, 773, 709.

HRMS (m/z ESI^+): Found: 242.1283 (M^+ + H, $C_{14}H_{16}N_3O$ Requires: 242.1293).

2-Acetylethylamino-4,5-dihydro-1,3-benzodiazepine 49b



Following Method B, Cu(II)O (0.2 eq., 28.1 mg, 0.23 mmol) and K_2CO_3 (2.0 eq., 326 mg, 2.36 mmol) were added to a solution of compound **42** (1.0 eq., 160 mg, 1.18 mmol) and **19d** (1.1 eq., 250 mg, 1.31 mmol) in DMF (2.5 mL) at RT. The resulting mixture was heated to 60 °C for 2 h. Usual workup and purification, yielded **49b** (175 mg, 60%) as an off-white solid.

Mp: 98-102 °C.

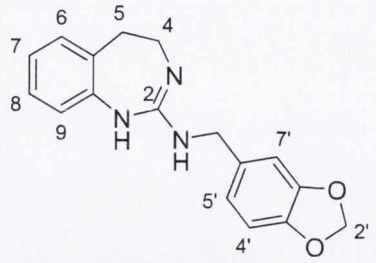
δ_H (400 MHz, $CDCl_3$): 1.99 (s, 3H, CH_3), 2.75 (t, 2H, J 7.0, CH_2-5), 3.57 (t, 2H, J 7.0, CH_2-4), 3.66 (t, 2H, J 7.0, CH_2-1'), 4.12 (t, 2H, J 7.0, CH_2-2'), 6.62 (d, 1H, J 7.2, H-9), 6.83 (app. t, 1H, J 7.2, H-7), 6.93 (d, 1H, J 7.2, H-6), 6.99 (app. t, 1H, J 7.2, H-8).

δ_C (100 MHz, $CDCl_3$): 20.9 (CH_3), 31.5 (CH_2-5), 43.2 (CH_2-1'), 44.2 (CH_2-4), 62.8 (CH_2-2'), 116.0 (CH, Ar-9), 118.9 (CH, Ar-7), 123.1 (q, Ar-5a), 127.8 (CH, Ar-8), 130.3 (CH, Ar-6), 144.9 (q, Ar-9a), 171.5 (q, C=O), 182.3 (q, 2).

ν_{max} (ATR)/cm^{-1}: 3455 (NH), 3229, 2988, 2745, 1732 (C=O), 1628, 1557, 1514, 1490, 1407, 1313, 1293, 1222, 1047, 1019, 846, 807, 776.

HRMS (m/z ESI^+): Found: 248.1392 (M^+ + H, $C_{13}H_{18}N_3O_2$ Requires: 248.1399).

2-(1,3-Benzodioxolyl-5-methylamino)-4,5-dihydro-1,3-benzodiazepine 49c



Following Method B, Cu(II)O (0.2 eq., 25.5 mg, 0.22 mmol) and K_2CO_3 (2.0 eq., 296 mg, 2.14 mmol) were added to a solution of compound **42** (1.0 eq., 145 mg, 1.07 mmol) and **19c** (1.1 eq., 283 mg, 1.18 mmol) in DMF (2.5 mL) at RT. The resulting mixture was heated to 60 °C for 2 h. Usual workup and purification yielded **49c** (215 mg, 69%) as a yellow solid.

Mp: 162-165 °C.

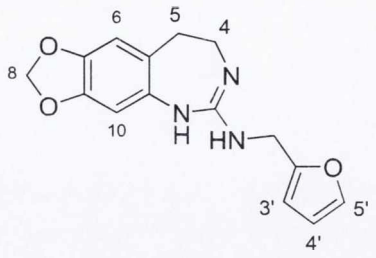
δ_H (400 MHz, $CDCl_3$): 2.74 (t, 2H, J 7.1, CH_2-5), 3.60 (t, 2H, J 7.1, CH_2-4), 4.43 (s, 2H, CH_2), 5.86 (s, 2H, CH_2-2'), 6.59 (d, 1H, J 7.2, H-9), 6.63 (m, 1H, H-7), 6.66 (s, 1H, H-7'), 6.71 (d, 1H, J 7.0, H-4'), 6.90 (d, 1H, J 7.0, H-5'), 6.97 (d, 1H, J 7.2, H-6), 7.18 (app. t, 1H, J 7.2, H-8).

δ_C (100 MHz, $CDCl_3$): 31.1 (CH_2-5), 44.6 (CH_2-4), 44.7 (CH_2), 100.9 (CH_2-2'), 107.4 (CH, Ar-4'), 108.3 (CH, Ar-7'), 118.7 (CH, Ar-9), 120.8 (CH, Ar-5'), 122.9 (q, Ar-5a), 128.9 (CH, Ar-7), 129.2 (CH, Ar-8), 130.7 (CH, Ar-6), 135.9 (q, Ar-6'), 144.8 (q, Ar-9a), 145.7 (q, Ar-1'a or Ar-3'a), 147.8 (q, Ar-1'a or Ar-3'a), 181.5 (q, 2).

ν_{max} (ATR)/cm^{-1}: 3374 (NH), 2322, 2992, 2866, 2773, 1623, 1535, 1460, 1403, 1315, 1283, 1222, 1141, 1001, 931, 892, 809, 773.

HRMS (m/z ESI^+): Found: 296.1391 ($M^+ + H$, $C_{17}H_{18}N_3O_2$ Requires: 296.1399).

2-(Furanyl-2-methylamino)-8,9-dihydro-5*H*-[1,3]dioxolo[4,5]benzo[1,2-*d*][1,3]diazepine 49d



Following Method B, Cu(II)O (0.2 eq., 47 mg, 0.39 mmol) and K_2CO_3 (2.0 eq., 538 mg, 3.9 mmol) were added to a solution of compound **43** (1.0 eq., 350 mg, 1.95 mmol) and **19a** (1.1 eq., 400 mg, 2.15 mmol) in DMF (3 mL) at RT. The resulting mixture was heated to 60 °C for 2 h. Usual workup and purification yielded **49d** (123 mg, 23% isolated yield) as a white gum.

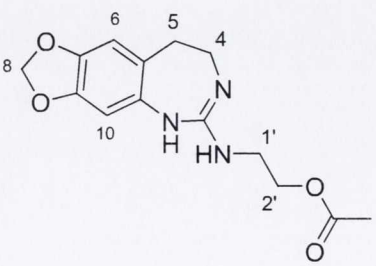
δ_H (400 MHz, $CDCl_3$): 2.73 (t, 2H, J 6.2, CH_2-5), 3.62 (br s, 2H, CH_2-4), 4.50 (s, 2H, CH_2), 5.83 (s, 2H, CH_2-8), 6.13 (br s, 1H, NH), 6.26 (br s, 2H, H-3', H-4'), 6.32 (s, 1H, H-10), 6.40 (s, 1H, NH), 6.50 (s, 1H, H-6), 7.35 (br s, 1H, H-5').

δ_C (100 MHz, $CDCl_3$): 22.5 (CH_2-5), 30.8 (CH_2-4), 59.7 (CH_2), 97.1 (CH_2-8), 99.8 (CH, Ar-10), 107.2 (CH, Ar-6), 109.8 (CH, Ar-3'), 110.8 (CH, Ar-4'), 114.1 (q, Ar-5a), 141.5 (q, Ar-2'), 142.4 (CH, Ar-5'), 146.0 (q, Ar-6a, Ar-9a), 151.7 (q, Ar-10a), 181.2 (q, 2).

ν_{max} (ATR)/cm^{-1}: 3210 (NH), 2979, 1636, 1551, 1459, 1376, 1309, 1270, 1244, 1213, 1185, 1127, 1033, 1009, 980, 925, 883, 766, 675.

HRMS (m/z ESI^+): Found: 286.1196 ($M^+ + H$, $C_{15}H_{16}N_3O_3$ Requires: 286.1186).

2-(Acetylethylamino)-8,9-dihydro-5*H*-[1,3]dioxolo[4,5]benzo[1,2-*d*][1,3]diazepine 49e



Following Method B, Cu(II)O (0.2 eq., 47 mg, 0.39 mmol) and K_2CO_3 (2.0 eq., 538 mg, 3.9 mmol) were added to a solution of compound **43** (1.0 eq., 350 mg, 1.95 mmol) and **19d** (1.1 eq., 413 mg, 2.15 mmol) in DMF (3 mL) at RT. The resulting mixture was heated to 60 °C for 2 h. Usual workup and purification, yielded **49e** (111 mg, 19% isolated yield) as a white gum.

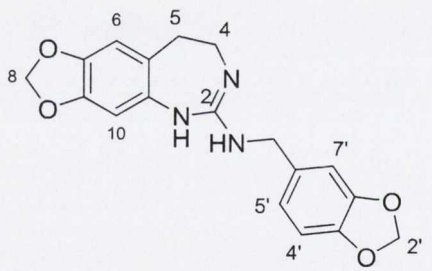
δ_H (400 MHz, $CDCl_3$): 2.03 (s, 3H, CH_3), 3.42 (t, 2H, J 7.1, CH_2-1'), 3.64 (br s, 2H, CH_2-5), 4.12 (t, 2H, J 7.1, CH_2-4), 4.76 (br s, 2H, CH_2-2'), 5.80 (s, 2H, CH_2-8), 6.30 (s, 1H, H-10), 6.54 (s, 1H, H-6), 7.54 (br s, 1H, NH), 7.62 (br s, 1H, NH).

δ_C (100 MHz, $CDCl_3$): 20.8 (CH_3), 31.1 (CH_2-5), 43.1 (CH_2-1'), 43.6 (CH_2-4), 62.8 (CH_2-2'), 97.1 (CH, Ar-6), 100.0 (CH_2-8), 109.9 (CH, Ar-10), 114.0 (q, Ar-5a), 138.4 (q, Ar-6a or Ar-9a), 141.5 (q, Ar-6a or Ar-9a), 146.3 (q, Ar-10a), 170.5 (q, C=O), 182.6 (q, 2).

ν_{max} (ATR)/cm^{-1}: 3208 (NH), 3008, 1734 (C=O), 1653, 1552, 1458, 1501, 1445, 1332, 1298, 1246, 1213, 1170, 1032, 957, 867, 763, 681.

HRMS (m/z ESI^+): Found: 292.1299 ($M^+ + H$, $C_{14}H_{18}N_3O_4$ Requires: 292.1297).

2-(1,3-Benzodioxolyl-5-methylamino)-8,9-dihydro-5*H*-[1,3]dioxolo[4,5]benzo[1,2-*d*][1,3]diazepine 49f



Following Method B, Cu(II)O (0.2 eq., 47 mg, 0.39 mmol) and K_2CO_3 (2.0 eq., 538 mg, 3.9 mmol) were added to a solution of compound **43** (1.0 eq., 350 mg, 1.95 mmol) and **19c** (1.1 eq., 516 mg, 2.15 mmol) in DMF (3 mL) at RT. The resulting mixture was heated to 60 °C for 2 h. Usual workup and purification yielded **49f** (175 mg, 26% isolated yield) as a white gum.

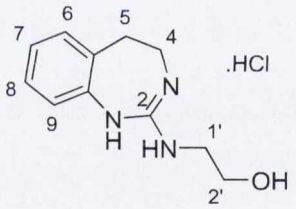
δ_H (600 MHz, DMSO): 2.60 (t, 2H, J 7.1, CH_2-5), 3.47 (br s, 2H, CH_2-4), 4.71 (s, 2H, CH_2), 5.75 (s, 2H, CH_2-2' or CH_2-8), 5.94 (s, 2H, CH_2-2' or CH_2-8), 6.25 (s, 1H, H-10), 6.49 (s, 1H, H-6), 6.74 (d, 1H, J 7.1, H-4'), 6.83 (m, 2H, H-5', H-7'), 7.44 (br s, 1H, NH), 7.84 (br s, 1H, NH).

δ_C (125 MHz, DMSO): 29.1 (CH_2-5), 31.1 (CH_2-4), 60.0 (CH_2), 97.1 (CH, Ar-6), 100.1 (CH_2-2'or CH_2-8), 101.1 (CH_2-2' or CH_2-8), 108.2 (CH, Ar-4'), 108.3 (CH, Ar-7'), 109.9 (CH, Ar-10), 120.5 (CH, Ar-5'), 128.5 (q, Ar-5a), 138.5 (q, Ar-6'), 141.7 (q, Ar), 146.3 (q, Ar), 146.4 (q, Ar), 147.4 (q, Ar), 155.4 (q, Ar-10a) 182.4 (q, 2).

ν_{max} (ATR)/cm^{-1}: 3038 (NH), 2986, 1553, 1487, 1410, 1368, 1349, 1331, 1269, 1211, 1169, 1033, 954, 923, 857, 834, 803, 767, 678.

HRMS (m/z ESI^+): Found: 340.1292 (M^+ + H, $C_{18}H_{18}N_3O_4$ Requires: 340.1297).

2-Hydroxyethylamino-4,5-dihydro-1,3-benzodiazepine hydrochloride 50b



Following Method C, to a solution of 1.25M HCl/CH_3OH (6.0 eq., 1.92 mL, 2.42 mmol) was added **49b** (1.0 eq., 100 mg, 0.40 mmol). The solution was stirred at 60 °C for 4 h. After usual workup and purification **50b** was obtained as a purple solid (92 mg, 95%).

Mp: 235-240 °C.

δ_H (400 MHz, D_2O): 2.87 (t, 2H, J 8.0, CH_2-5), 3.34 (m, 2H, CH_2-1'), 3.52 (m, 2H, CH_2-2'), 3.73 (m, 2H, CH_2-4), 7.22-7.30 (m, 4H, H-Ar).

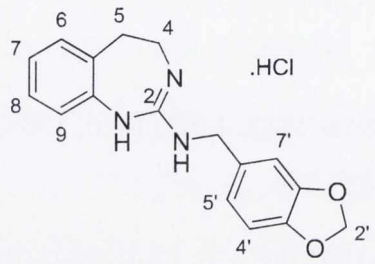
δ_C (100 MHz, D_2O): 28.2 (CH_2-4), 30.6 (CH_2-5), 44.2 (CH_2-1'), 48.9 (CH_2-2'), 123.7 (CH, Ar-9), 128.6 (CH, Ar-7), 128.8 (q, Ar-5a), 129.2 (CH, Ar-8), 130.7 (CH, Ar-6), 131.4 (q, Ar-9a), 170.9 (q, 2).

ν_{max} (ATR)/cm^{-1}: 3142 (NH), 2962, 2895, 1640, 1589, 1559, 1496, 1228, 1009, 950, 896, 875, 748.

HRMS (m/z ESI$^+$): Found: 206.1289 (M^+ + H, $C_{11}H_{16}N_3O$ Requires: 206.1287).

Purity by HPLC: 95.7% (t_R 17.51 min).

2-(1,3-Benzodioxolyl-5-methylamino)-4,5-dihydro-1,3-benzodiazepine hydrochloride 50c



Following Method C, 4M HCl/dioxane (3.0 eq., 127 μL, 0.50 mmol) was added to a solution of **49c** (1.0 eq., 50 mg, 0.17 mmol) in CH_3OH (0.5 mL). The solution was stirred at RT for 2 h. After usual workup and purification **50c** was obtained as a yellow solid (45 mg, 80%).

Mp: 196-199 °C.

δ_H (400 MHz, $CDCl_3$): 2.94 (br s, 2H, CH_2-5), 3.59 (br s, 2H, CH_2-4), 4.43 (s, 2H, CH_2), 5.75 (s, 2H, CH_2-2'), 6.60 (d, 1H, J 7.2, H-9), 6.63 (m, 2H, H-7, H-4'), 7.00 (m, 2H, H-5', H-7'), 7.11 (d, 1H, J 7.4, H-6), 7.20 (app. t, 1H, J 7.3, H-8), 8.46 (br s, 2H, NH), 10.11 (br s, 1H, NH).

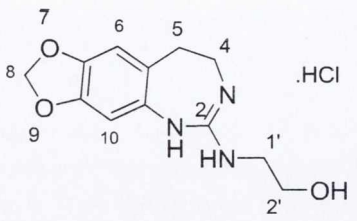
δ_C (100 MHz, $CDCl_3$): 34.6 (CH_2-5), 44.3.(CH_2-4), 46.3 (CH_2), 101.2 (CH_2-2'), 108.1 (CH, Ar-4'), 108.3 (CH, Ar-7'), 120.8 (q, Ar-5a), 121.1 (CH, Ar-5'), 124.2 (CH, Ar-9), 127.7 (CH, Ar-7), 129.0 (CH, Ar-8), 130.2 (CH, Ar-6), 136.0 (q, Ar-6'), 147.9 (q, Ar-1'a or Ar-3'a), 148.0 (q, Ar-1'a or Ar-3'a), 154.3 (q, Ar-9a), 169.8 (q, 2).

ν_{max} (ATR)/cm^{-1}: 2923 (NH), 2853, 1682, 1610, 1586, 1489, 1448, 1364, 1240, 1098, 924, 859, 805, 756.

HRMS (m/z ESI$^+$): Found: 296.1391 (M^+ + H, $C_{17}H_{18}N_3O_2$ Requires: 296.1393).

Purity by HPLC: 96.7% (t_R 27.49 min).

2-(Hydroxyethylamino)-8,9-dihydro-5*H*-[1,3]dioxolo[4,5]benzo[1,2-*d*][1,3]diazepine hydrochloride 50e



Following Method C, to a solution of 1.25M HCl/CH_3OH (6.0 eq., 3.28 mL, 4.10 mmol) was added **49e** (1.0 eq., 200 mg, 0.68 mmol). The solution was stirred at 60 °C for 4 h. After usual workup and purification **50e** was obtained as a red solid (187 mg, 96%).

Mp: 230-233 °C.

δ_H (600 MHz, D_2O): 2.78 (t, 2H, J 8.0, CH_2-5), 3.37 (m, 2H, CH_2-1'), 3.48 (m, 2H, CH_2-2'), 3.78 (m, 2H, CH_2-4), 5.89 (s, 2H, CH_2-8), 6.74 (s, 1H, H-10), 6.78 (s, 1H, H-6).

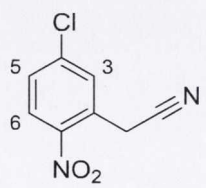
δ_C (125 MHz, DMSO): 28.1 (CH_2-4), 30.6 (CH_2-5), 44.5 (CH_2-1'), 48.9 (CH_2-2'), 102.0 (CH_2-8), 104.5 (CH, Ar-6), 109.5 (CH, Ar-10), 121.6 (q, Ar-5a), 147.8 (q, Ar-6a, Ar-9a), 147.8 (q, Ar-10a), 171.3 (q, 2).

ν_{max} (ATR)/cm^{-1}: 3141 (NH), 2819, 2533, 1639, 1569, 1437, 1362, 1204, 1066, 1078, 1027, 926, 873.

HRMS: (m/z ESI^+): Found: 250.1198 (M^+ + H, $C_{12}H_{16}N_3O_3$ Requires: 250.1186).

Purity by HPLC: 98.5% (t_R 19.09 min).

4-Chloro-2-cyanomethylnitrobenzene 52[220]



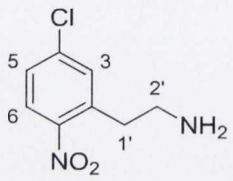
A mixture of 4-chloronitrobenzene (1.0 eq., 10.0 g, 63.5 mmol) and chloroacetonitrile (1.0 eq., 4.0 ml, 63.5 mmol) was dissolved in 120 mL of anhydrous THF. The solution was added

dropwise to a suspension of KOtBu (2.0 eq., 14.2 g, 127 mmol) in anhydrous THF (120 mL) at –20 °C. The reaction was quenched immediately by the addition of 150 mL of aqueous H_2SO_4 (10%, v/v), and extracted with CH_2Cl_2 (3 × 60 mL). The combined organic layers were washed with H_2O (2 × 20 mL), dried with Na_2SO_4, filtered and evaporated under reduced pressure. The crude oil was purified by silica gel chromatography, eluting in hexane:EtOAc, affording compound **52** (4.9 g, 40%) as a pale yellow solid.

Mp: 86-87 °C.[220]

δ_H (400 MHz, $CDCl_3$): 4.21 (s, 2H, CH_2), 7.52 (d, 1H, J 7.0, H-5), 7.73 (s, 1H, H-3), 8.17 (d, 1H, J 7.0, H-6).

2-Aminoethyl-4-chloronitrobenzene 55[267]

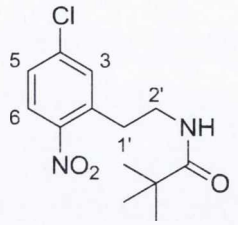


To a solution of compound **52** (4.9 g, 25 mmol) in anhydrous THF (70 mL) under argon was added BH_3.DMS solution (2M in THF, 29.0 mL, 57.5 mmol) dropwise at 0 °C. After addition, the mixture was refluxed for 8 h. The solution was cooled to 0 °C and quenched with CH_3OH (30 mL). The solvent was removed under reduced pressure and the residue purified by silica gel chromatography, eluting in hexane:EtOAc, affording compound **55** (3.1 g, 62%) as a brown oil.[267]

δ_H (400 MHz, $CDCl_3$): 1.45 (br s, 2H, NH_2), 2.99 (s, 4H, CH_2-1', CH_2-2'), 7.30 (d, 1H, J 7.1, H-5), 7.35 (s, 1H, H-3), 7.87 (d, 1H, J 7.1, H-6).

HRMS (m/z ESI^+): Found: 201.0421 ($M^+ + H$, $C_8H_{10}N_2O_2Cl$ Requires: 201.0431).

2-Pivalaminoethyl-4-chloronitrobenzene 56



To a solution of compound **55** (1.0 eq., 3.0 g, 15 mmol) and NEt_3 (2.5 eq., 5.2 mL, 37.5 mmol) in anhydrous THF (120 mL) was added a solution of pivaloyl chloride (1.03 eq., 1.9 mL, 15.5 mmol) in anhydrous THF (50 mL) dropwise. The reaction was stirred at RT for 2 h, then diluted with H_2O (80 mL), and extracted with EtOAc (3 × 30 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under vacuum. The resulting residue was recrystallised from hexane to afford compound **56** (4.26 g, 99%) as a crystalline brown solid.

Mp: 85-87 °C.

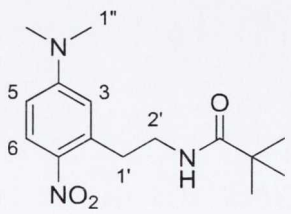
δ_H (400 MHz, $CDCl_3$): 1.11 (s, 9H, CH_3), 2.97 (t, 2H, J 7.1, CH_2-1'), 3.41 (t, 2H, J 7.1, CH_2-2'), 6.32 (br s, 1H, NH), 7.20 (d, 1H, J 7.3, H-5), 7.23 (s, 1H, H-3), 7.76 (d, 1H, J 7.3, H-6).

δ_C (100 MHz, $CDCl_3$): 26.5 (CH_3-Piv), 32,6 (CH_2-1'), 38.4 (q, C Piv), 39.5 (CH_2-2'), 126.1 (CH, Ar-6), 127.5 (CH, Ar-5), 132.6 (CH, Ar-3), 136.4 (q, Ar-2), 139.0 (q, Ar-4), 147.5 (q, Ar-1), 178.9 (q, C=O).

ν_{max} (ATR)/cm^{-1}: 3285 (NH), 2982, 2892, 1692 (C=O), 1513 (NO_2), 1335, 831, 743, 701, 680.

HRMS (m/z ESI$^+$): Found: 285.0999 (M^+ + H, $C_{13}H_{18}N_2O_3Cl$ Requires: 285.1006).

2-Pivalamidoethyl-4-dimethylaminonitrobenzene 57



To a solution of compound **56** (1.0 eq., 1.0 g, 3.52 mmol) and NEt_3 (7.0 eq., 3.5 mL, 24.6 mmol) in EtOH (14 mL) was added dimethyammonium chloride (5.5 eq., 1.5 mL, 19.4 mmol). The reaction was stirred at 120 °C in a pressure tube for 48 h. The mixture was cooled, diluted EtOAc (50 mL) and washed with H_2O (3 × 20 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under vacuum. The crude oil was purified by silica gel chromatography, eluting in hexane:EtOAc, affording compound **57** (715 mg, 70%) as a brown solid.

Mp: 136-137 °C.

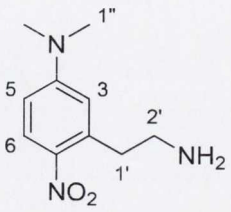
δ_H (400 MHz, $CDCl_3$): 1.12 (s, 9H, CH_3-Piv), 3.06 (s, 6H, CH_3-1"), 3.17 (t, 2H, J 7.2, CH_2-1'), 3.56 (t, 2H, J 7.2, CH_2-2'), 6.13 (br s, 1H, NH), 6.42 (s, 1H, H-3), 6.48 (d, 1H, J 7.3, H-5), 8.06 (d, 1H, J 7.3, H-6).

δ_C (100 MHz, $CDCl_3$): 27.5 (CH_3-Piv), 34.3 (CH_2-1'), 38.9 (CH_2-2'), 40.4 (CH_3-1"), 40.8 (q, C Piv), 109.5 (CH, Ar-5), 113.7 (CH, Ar-3), 128.9 (CH, Ar-6), 136.9 (q, Ar-2), 137.9 (q, Ar-1), 153.3 (q, Ar-4), 178.6 (q, C=O).

ν_{max} (ATR)/cm^{-1}: 3364 (NH), 3150, 2976, 2879, 2692, 1743 (C=O), 1630, 1557 (NO_2), 1528, 1477, 1432, 1401, 1340, 1260, 1228, 1238, 1132, 1018, 944, 808, 777.

HRMS (m/z ESI^+): Found: 294.1808 ($M^+ + H$, $C_{15}H_{24}N_3O_3$ Requires: 294.1818).

2-Aminoethyl)-4-dimethylaminonitrobenzene 58



To an aqueous solution of 8M HCl (22.3 mL, 178 mmol) was added **57** (1.5 g, 5.11 mmol), and resulting mixture was refluxed for 24 h. The solution was cooled, neutralised to pH 10 with a 10% NaOH solution and extracted with EtOAc (3 × 30 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under vacuum to give a crude oil that was purified by silica gel chromatography, eluting in CH_2Cl_2:CH_3OH, to afford compound **58** (1.02 g, 95%) as a yellow gum.

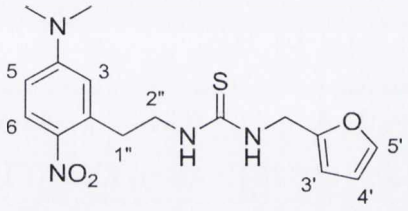
δ_H (400 MHz, $CDCl_3$): 3.08 (s, 6H, CH_3-1"), 3.27 (t, 2H, J 7.0, CH_2-1'), 3.88 (t, 2H, J 7.0, CH_2-2'), 6.51 (m, 2H, H-3, H-5), 8.10 (d, 1H, J 7.4, H-6).

δ_C (100 MHz, $CDCl_3$): 29.6 (CH_2-1'), 40.6 (CH_3-1"), 42.6 (CH_2-2'), 109.2 (CH, Ar-5), 113.6 (CH, Ar-3), 128.4 (CH, Ar-6), 136.9 (q, Ar-2), 138.2 (q, Ar-1), 153.2 (q, Ar-4).

ν_{max} (ATR)/cm^{-1}: 3299 (NH), 3152 (NH), 2990, 1536 (NO_2), 1417, 1403, 1339, 1314, 1293, 1284, 1220, 1163, 1140, 1038, 1018, 944, 892, 859, 777, 705.

HRMS (m/z ESI^+): Found: 210.1245 (M^+ + H, $C_{10}H_{16}N_3O_2$ Requires: 210.1243).

***N*-(5-Dimethylamino-2-nitrophenylethyl)-*N'*-(furanyl-2-methyl)thiourea 60a**



Following a modification of Method B, K_2CO_3 (2.0 eq., 347 mg, 3.36 mmol) was added to a solution of compound **58** (1.0 eq., 350 mg, 1.68 mmol) and **19a** (1.1 eq., 345 mg, 1.84 mmol) in DMF (3 mL) at RT. The resulting mixture was heated to 60 °C for 2 h. Usual workup and purification yielded **60a** (314 mg, 55%) as a yellow solid.

Mp: 105-107 °C.

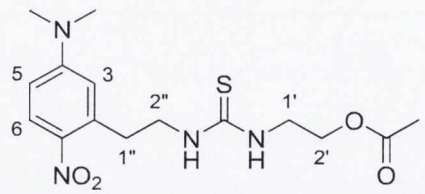
δ_H (400 MHz, $CDCl_3$): 3.04 (s, 6H, CH_3), 3.21 (t, 2H, J 7.1, CH_2-1"), 3.74 (br s, 2H, CH_2-2"), 4.65 (s, 2H, CH_2), 6.24 (m, 2H, H-3', H-4'), 6.45 (m, 2H, H-3, H-5), 6.61 (s, 1H, NH), 6.79 (s, 1H, NH), 7.27 (br s, 1H, H-5'), 8.02 (d, 1H, J 7.3, H-6).

δ_C (100 MHz, $CDCl_3$): 31.8 (CH_2), 34.4 (CH_2-1"), 40.1 (CH_3), 41.0 (CH_2-2"), 107.8 (CH, Ar-3'), 109.5 (CH, Ar-4'), 110.4 (CH, Ar-5), 113.9 (CH, Ar-3), 128.6 (CH, Ar-6), 136.6 (q, Ar-1), 137.6 (q, Ar-2), 142.1 (CH, Ar-5'), 150.6 (q, Ar-2'), 153.5 (q, Ar-4), 182.0 (q, C=S).

ν_{max} (ATR)/cm^{-1}: 3073 (NH), 2945, 1539 (NO_2), 1548, 1522, 1466, 1341, 1309, 1258, 1169, 1032, 960, 890, 809, 730, 695, 635.

HRMS (m/z ESI^+): Found: 349.1331 (M^+ + H, $C_{16}H_{21}N_4O_3S$ Requires: 349.1329).

2-[3-(5-Dimethylamino-2-nitrophenethyl)thioureido]ethyl acetate 60b



Following a modification of Method B, K_2CO_3 (2.0 eq., 347 mg, 3.36 mmol) was added to a solution of compound **58** (1.0 eq., 350 mg, 1.68 mmol) and **19d** (1.1 eq., 356 mg, 1.84 mmol) in DMF (3 mL) at RT. The resulting mixture was heated to 60 °C for 2 h. Usual workup and purification yielded **60b** (347 mg, 59%) as a yellow oil.

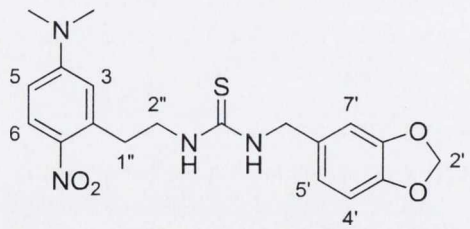
δ_H (400 MHz, $CDCl_3$): 1.93 (s, 3H, CH_3-Ac), 2.96 (s, 6H, CH_3), 3.13 (t, 2H, J 7.1, CH_2-1"), 3.71 (br s, 4H, CH_2-2", CH_2-1'), 4.10 (br s, 2H, CH_2-2'), 6.38 (m, 2H, H-3, H-5), 6.74 (s, 1H, NH), 6.97 (s, 1H, NH), 7.92 (d, 1H, J 7.4, H-6).

δ_C (100 MHz, $CDCl_3$): 20.8 (CH_3-Ac), 27.3 (CH_2-1"), 34.6 (CH_2-2", CH_2-1'), 40.1 (CH_3), 50.1 (CH_2-2'), 109.5 (CH, Ar-5), 113.8 (CH, Ar-3), 128.0 (CH, Ar-6), 135.6 (q, Ar-2), 137.9 (q, Ar-1), 151.5 (q, Ar-4), 170.9 (q, C=O), 181.5 (q, C=S).

ν_{max} (ATR)/cm^{-1}: 3329 (NH), 3074 (NH), 2922, 1600 (C=O), 1545 (NO_2), 1439, 1414, 1261, 1196, 1075, 1032, 1008, 941, 890, 809, 731, 663, 654.

HRMS (m/z ESI^+): Found: 355.1443 ($M^+ + H$, $C_{15}H_{23}N_4O_4S$ Requires: 355.1435).

***N*-(1,3-Benzodioxolyl-5-methyl)-*N'*-(5-dimethylamino-2-nitrophenethyl)thiourea 60c**



Following a modification of Method B, K_2CO_3 (2.0 eq., 199 mg, 1.92 mmol) was added to a solution of compound **58** (1.0 eq., 200 mg, 0.96 mmol) and **19c** (1.1 eq., 254 mg, 1.01 mmol) in DMF (3 mL) at RT. The resulting mixture was heated to 60 °C for 2 h. Usual workup and purification yielded **60c** (289 mg, 75%) as a yellow solid.

Mp: 146-149 °C.

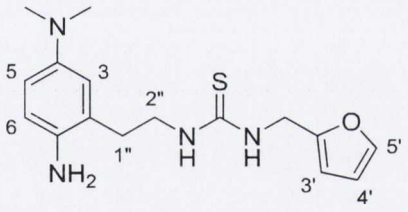
δ_H (400 MHz, $CDCl_3$): 3.04 (s, 6H, CH_3), 3.19 (t, 2H, J 7.1, H-1"), 3.72 (br s, 2H, CH_2-2"), 4.52 (s, 2H, CH_2), 5.86 (s, 2H, CH_2-2'), 6.45 (s, 1H, H-3), 6.60 (m, 1H, H-5), 6.68 (m, 4H, H-4', H-5', NH, NH), 6.74 (s, 1H, H-7'), 7.99 (d, 1H, J 7.4, H-6).

δ_C (100 MHz, $CDCl_3$): 31.8 (CH_2), 34.1 (CH_2-1"), 40.3 (CH_3), 40.5 (CH_2-2"), 101.4 (CH_2-2'), 108.7 (CH, Ar-7'), 109.5 (CH, Ar-4', Ar-5), 113.8 (CH, Ar-3), 120.9 (CH, Ar-5'), 128.6 (CH, Ar-6), 136.6 (q, Ar-2), 137.6 (q, Ar-1, Ar-6'), 147.0 (q, Ar-1'a or Ar-3'a), 147.8 (q, Ar-1'a or Ar-3'a), 153.5 (q, Ar-4), 181.9 (q, C=S).

ν_{max} (ATR)/cm^{-1}: 3334 (NH), 3073 (NH), 2922, 1599, 1544, 1488, 1414, 1378, 1302, 1247, 1145, 1008, 941, 809, 731, 693, 654.

HRMS (m/z ESI^+): Found: 403.1452 (M^+ + H, $C_{19}H_{23}N_4O_4S$ Requires: 403.1440).

***N*-(2-Amino-5-dimethylaminophenylethyl)-*N'*-(furanyl-2-methyl)thiourea 61a**



Following Method I, to a solution of compound **60a** (1.0 eq., 280 mg, 0.8 mmol) in EtOH (2 mL) was added $SnCl_2.2H_2O$ (10.0 eq., 1.8 g, 8.0 mmol). The mixture was refluxed for 1 h, after which all starting material was consumed, as judged by TLC. General work up as described afforded compound **61a** (254 mg, 98%) as a white solid.

Mp: 83-85 °C.

δ_H (400 MHz, $CDCl_3$): 2.67 (t, 2H, J 7.2, CH_2-1"), 2.74 (s, 6H, CH_3), 3.52 (br s, 2H, CH_2-2"), 4.56 (s, 2H, CH_2), 6.16 (app. t, 1H, J 7.1, H-3'), 6.23 (d, 1H, J 7.1, H-4'), 6.47-6.56 (m, 3H, H-3, H-5, H-6), 6.84 (s, 1H, NH), 7.00 (s, 1H, NH), 7.21 (br s, 1H, H-5').

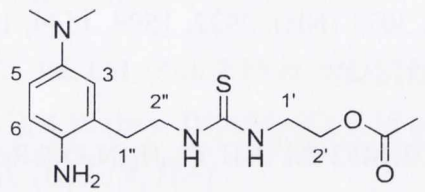
δ_C (100 MHz, $CDCl_3$): 31.3 (CH_2-1"), 40.9 (CH_2), 41.8 (CH_3), 44.7 (CH_2-2"), 106.7 (CH, Ar-3'), 110.4 (CH, Ar-4'), 113.8 (CH, Ar-5), 116.5 (CH, Ar-3), 117.8 (CH, Ar-6), 125.4 (q,

Ar-2), 135.8 (q, Ar-1 or Ar-4), 136.1 (q, Ar-1 or Ar-4), 145.1 (CH, Ar-5'), 152.4 (q, Ar-2'), 181.9 (q, C=S).

ν_{max} (ATR)/cm^{-1}: 3378 (NH), 3227 (NH), 2926, 2797, 1565, 1483, 1438, 1333, 1295, 1219, 1186, 1094, 1068, 963, 732, 660, 502.

HRMS (m/z ESI$^+$): Found: 319.1599 (M^+ + H, $C_{16}H_{23}N_4OS$ Requires: 319.1587).

2-[3-(2-Amino-5-dimethylaminophenethyl)thioureido]ethyl acetate 61b



Following Method I, to a solution of compound **60b** (1.0 eq., 240 mg, 0.7 mmol) in EtOH (2 mL) was added $SnCl_2.2H_2O$ (10.0 eq., 1.57 g, 7.0 mmol). The mixture was refluxed for 1 h, after which all starting material was consumed, as adjudged by TLC. General work up as described afforded compound **61b** (222 mg, 92%) as a white solid.

Mp: 74-75 °C.

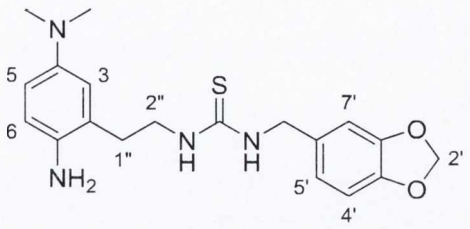
δ_H (400 MHz, $CDCl_3$): 2.01 (s, 3H, CH_3-Ac), 2.76 (br s, 2H, CH_2-1"), 2.80 (s, 6H, CH_3), 3.65 (br s, 4H, CH_2-2", CH_2-1'), 4.15 (br s, 2H, CH_2-2'), 6.38 (s, 1H, NH), 6.54 (m, 2H, H-3, H-5), 6.65 (d, 1H, J 7.1, H-6), 7.00 (s, 1H, NH).

δ_C (100 MHz, $CDCl_3$): 20.8 (CH_3-Ac), 31.6 (CH_2-1"), 41.7 (CH_2-1'), 43.3 (CH_2-2"), 45.3 (CH_3), 61.7 (CH_2-2'), 113.7 (CH, Ar-5), 116.2 (CH, Ar-3), 118,4 (CH, Ar-6), 125.8 (q, Ar-2), 135.2 (q, Ar-1), 145.5 (q, Ar-4), 171.2 (q, C=O), 182.1 (q, C=S).

ν_{max} (ATR)/cm^{-1}: 3382 (NH), 3330 (NH), 3224 (NH), 2935, 1729, 1616, 1509, 1378, 1323, 1239, 1225, 1076, 956, 893.

HRMS (m/z ESI$^+$): Found: 347.1509 (M^+ + Na, $C_{15}H_{24}N_4NaO_2S$ Requires: 347.1512).

_N_-(2-Amino-5-dimethylaminophenethyl)-_N'_-(1,3-benzodioxolyl-5-methyl)thiourea 61c



Following Method I, to a solution of compound **60c** (1.0 eq., 220 mg, 0.55 mmol) in EtOH (2 mL) was added $SnCl_2.2H_2O$ (10.0 eq., 1.2 g, 5.5 mmol). The mixture was refluxed for 1 h, after which all starting material was consumed, as adjudged by TLC. General work up as described afforded compound **61c** (200 mg, 98%) as a white solid.

Mp: 127-129 °C.

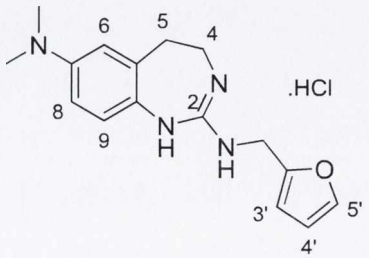
δ_H (400 MHz, $CDCl_3$): 2.75 (t, 2H, J 7.0, CH_2-1"), 2.80 (s, 6H, CH_3), 3.63 (br s, 2H, CH_2-2"), 4.43 (s, 2H, CH_2), 5.92 (s, 2H, CH_2-2'), 6.36 (s, 1H, NH), 6.48-6.60 (m, 3H, H-Ar), 6.67-6.73 (m, 3H, H-Ar).

δ_C (100 MHz, $CDCl_3$): 27.5 (CH_2-1"), 31.6 (CH_2-2"), 41.7 (CH_3), 48.0 (CH_2), 101.1 (CH_2-2'), 108.1 (CH, Ar-4'), 108.3 (CH, Ar-7'), 113.6 (CH, Ar-5), 116.1 (CH, Ar-3), 120.9 (CH, Ar-5'), 125.6 (CH, Ar-6), 130.4 (q, Ar-2), 135.6-135.9 (q, Ar-1, Ar-4, Ar-6'), 147.1 (q, Ar-1'a or Ar-3'a), 147.9 (q, Ar-1'a or Ar-3'a), 182.2 (q, C=S).

ν_{max} ATR)/cm^{-1}: 3376 (NH), 3221 (NH), 2929, 2871, 1570, 1487, 1438, 1380, 1359, 1296, 1219, 1129, 1094, 1037, 1009, 962, 809, 660, 539.

HRMS (m/z ESI$^-$): Found: 371.1530 (M^+ - H, $C_{19}H_{23}N_4O_2S$ Requires: 371.1542).

7-(Dimethylamino)-2-(furanyl-2-methylamino)-4,5-dihydro-1*H*-benzo[*d*][1,3]diazepine hydrochloride 62a



Following Method J, to a stirred solution of **61a** (1.0 eq., 250 mg, 0.77 mmol) and NEt_3 (3.0 eq., 320 µL, 2.31 mmol) in DMF (2 mL) at 0 °C was added $HgCl_2$ (1.0 eq., 208 mg, 0.77 mmol). The reaction was stirred at RT until reaction was complete as adjudged by TLC analysis. Usual work up and purification afforded compound **62a** (224 mg, 90%) as a brown solid.

Mp: 244-250 °C, decomposed.

δ_H (400 MHz, $CDCl_3$): 2.82 (s, 6H, CH_3), 2.87 (s, 2H, CH_2-4), 3.57 (s, 2H, CH_2-5), 4.57 (s, 2H, CH_2), 6.16 (d, 1H, J 7.2, H-3'), 6.30 (t, 1H, J 7.2, H-4'), 6.42 (m, 2H, H-6, H-8), 7.08 (d, 1H, J 7.1, H-9), 7.21 (br s, 1H, H-5'), 7.96 (br s, 1H, NH), 8.60 (br s, 1H, NH), 9.94 (br s, 1H, NH).

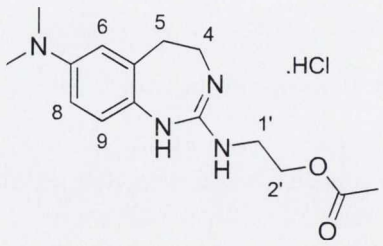
δ_C (100 MHz, $CDCl_3$): 34.6 (CH_2-5), 39.5 (CH_2-4), 40.6 (CH_3), 43.7 (CH_2), 108.5 (CH, Ar-3'), 110.4 (CH, Ar-4'), 111.7 (CH, Ar-6), 113.1 (CH, Ar-8), 122.0 (CH, Ar-9), 125.3 (q, Ar-5a), 142.5 (CH, Ar-5'), 147.6 (q, Ar-7), 149.3 (q, Ar-9a), 151.6 (q, Ar-2'), 177.4 (q, 2).

ν_{max} (ATR)/cm^{-1}: 3378 (NH), 3221 (NH), 2996, 2793, 1569, 1505, 1438, 1379, 1333, 1249, 1219, 1124, 1073, 1021, 952, 848, 794.

HRMS (m/z ESI^+): Found: 285.1714 ($M^+ + H$, $C_{16}H_{21}N_4O$ Requires: 285.1710).

Purity by HPLC: 94.8% (t_R 23.12 min).

2-(7-Dimethylamino-4,5-dihydro-1*H*-benzo[*d*][1,3]diazepinyl)aminoethyl acetate hydrochloride 62b



Following Method J, to a stirred solution of **61b** (1.0 eq., 140 mg, 0.43 mmol) and NEt_3 (3.0 eq., 180 µL, 1.29 mmol) in DMF (2 mL) at 0 °C was added $HgCl_2$ (1.0 eq., 120 mg, 0.43 mmol). The reaction was stirred at RT until reaction was complete as adjudged by TLC

analysis. Usual work up and purification afforded compound **62b** (111 mg, 79%) as a brown gum.

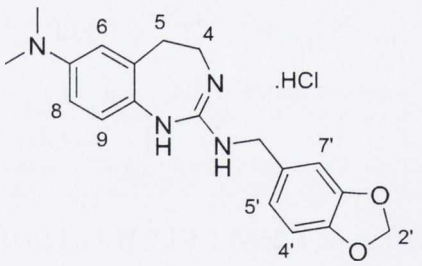
δ_H (400 MHz, $CDCl_3$): 2.07 (s, 3H, CH_3-Ac), 2.86 (s, 6H, CH_3), 3.03 (t, 2H, J 7.1, CH_2-5), 3.67 (br s, 4H, CH_2-4, CH_2-1'), 4.19 (t, 2H, J 7.1, CH_2-2'), 6.35 (s, 1H, H-6), 6.50 (d, 1H, J 7.4, H-8), 7.13 (d, 1H, J 7.4, H-9), 8.07 (br s, 1H, NH), 8.45 (br s, 1H, NH), 9,89 (br s, 1H, NH).

δ_C (100 MHz, $CDCl_3$): 20.7 (CH_3-Ac), 34.7 (CH_2-5), 40.5 (CH_3), 41.2 (CH_2-1'), 43.9 (CH_2-4), 62.1 (CH_2-2'), 111.8 (CH, Ar-6), 113.1 (CH, Ar-8), 122.0 (CH, Ar-9), 130.2 (q, Ar-5a), 147.5 (q, Ar-7), 154.1 (q, Ar-9a), 171.2 (q, C=O), 176.8 (q, 2).

ν_{max} (ATR)/cm^{-1}: 3158 (NH), 2995, 2919, 2849, 2797, 1744, 1675, 1593, 1549, 1456, 1431, 1326, 1269, 1216, 1167, 1111, 1044, 945, 862.

HRMS (m/z ESI^+): Found: 291.1819 (M^+ + H, $C_{15}H_{23}N_4O_2$ Requires: 291.1816).

2-(1,3-Benzodioxolyl-5-methylamino)-7-dimethylamino-4,5-dihydro-1*H*-benzo[*d*][1,3]diazepine hydrochloride 62c



Following Method J, to a stirred solution of **61c** (1.0 eq., 200 mg, 0.54 mmol) and NEt_3 (3.0 eq., 146 µL, 1.62 mmol) in DMF (2 mL) at 0 °C was added $HgCl_2$ (1.0 eq., 146 mg, 0.54 mmol). The reaction was stirred at RT until reaction was complete as adjudged by TLC analysis. Usual work up and purification afforded compound **62c** (121 mg, 66%) as a yellow solid.

Mp: 205-209 °C, decomposed.

δ_H (400 MHz, $CDCl_3$): 2.82 (s, 8H, CH_3, CH_2-5), 3.54 (s, 2H, CH_2-4), 4.43 (s, 2H, CH_2), 5.74 (s, 2H, CH_2-2'), 6.29 (s, 1H, H-6), 6.39 (d, 1H, J 7.1, H-8), 6.56 (d, 1H, J 7.1, H-9), 6.80 (m, 2H, H-4', H-5'), 7.01 (s, 1H, H-7'), 7.90 (br s, 1H, NH), 8.30 (br s, 1H, NH), 9.61 (br s, 1H, NH).

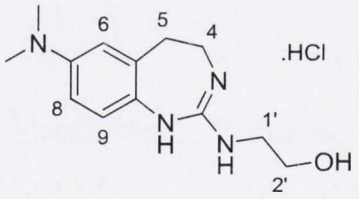
δ_C (100 MHz, $CDCl_3$): 34.7 (CH_2-5), 40.6 (CH_3), 43.6 (CH_2-4), 46.1 (CH_2), 101.0 (CH_2-2'), 108.2 (CH, Ar-4'), 108.3 (CH, Ar-7'), 111.7 (CH, Ar-6), 113.1 (CH, Ar-8), 121.2 (CH, Ar-5'), 121.8 (CH, Ar-9), 129.9 (q, Ar-5a), 129.6 (q, Ar-7), 130.3 (q, Ar-6'), 147.1 (q, Ar-1'a or Ar-3'a), 147.5 (q, Ar-1'a or Ar-3'a), 153.5 (q, Ar-9a), 168.1 (q, 2).

ν_{max} (ATR)/cm^{-1}: 3161 (NH), 2962, 2904, 1673, 1619, 1541, 1517, 1492, 1468, 1401, 1346, 1326, 1246, 1198, 1094, 999, 961, 834, 635.

HRMS (m/z ESI^+): Found: 339.1818 (M^+ + H, $C_{19}H_{23}N_4O_2$ Requires: 339.1816).

Purity by HPLC: 96.1% (t_R 23.97 min).

2-Hydroxyethylamino-7-dimethylamino-4,5-dihydro-1*H*-benzo[*d*][1,3]diazepine hydrochloride 62d



Following Method C, to a solution of 1.25M HCl/CH_3OH (6.0 eq., 1.98 mL, 2.48 mmol) was added **62b** (1.0 eq., 120 mg, 0.41 mmol). The solution was stirred at 60 °C for 3 h. Usual workup and purification afforded **62d** as a yellow solid (112 mg, 98%).

Mp: 237-241 °C.

δH (400 MHz, D_2O): 2.86 (s, 6H, CH_3), 2.97 (m, 2H, CH_2-5), 3.30 (m, 2H, CH_2-1'), 3.48 (m, 2H, CH_2-4), 3.63 (t, 2H, J 7.1, CH_2-2'), 6.70 (s, 1H, H-6), 6.75 (d, 1H, J 7.4, H-8), 6.87 (d, 1H, J 7.4, H-9).

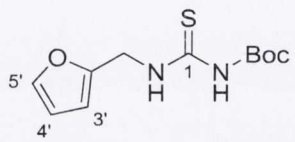
δ_C (100 MHz, D_2O): 33.1 (CH_2-5), 41.1 (CH_2-1'), 43.5 (CH_2-4), 48.7 (CH_3), 62.1 (CH_2-2'), 114.8 (CH, Ar-6), 116.6 (CH, Ar-8), 121.8 (CH, Ar-9), 127.9 (q, Ar-5a), 131.2 (q, Ar-7), 150.1 (q, Ar-9a), 161.9 (q, 2).

ν_{max} (ATR)/cm^{-1}: 3287 (NH), 2923, 1619, 1516, 1406, 1332, 1062, 972, 784.

HRMS (m/z ESI^+): Found: 249.1704 ($M^+ + H$, $C_{13}H_{21}N_4O$ Requires: 249.1709).

Purity by HPLC: 94.9% (t_R 16.55 min).

***N*-(*tert*-Butoxycarbonyl)-*N*'-(furanyl-2-methyl)thiourea 71a**[238]

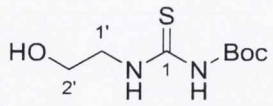


Following Method K, thiourea (1.0 eq., 700 mg, 9.21 mmol) was dissolved in dry THF (70 ml) under argon at 0 °C, to which NaH (60% suspension in mineral oil, 4.5 eq., 994 mg, 41.5 mmol) was added. The reaction was stirred at RT for 45 min to complete formation of the anion and re-cooled to 0 °C prior to the addition of di-*tert*-butyl dicarbonate (2.2 eq., 4.4 g, 20.3 mmol). After stirring at RT for 8 h, the reaction was re-cooled to 0 °C and NaH (60% suspension in mineral oil, 1.7 eq., 373 mg, 15.6 mmol) was added. After 1 h TFAA (1.54 eq., 1.99 mL, 14.1 mmol) was added and the reaction was stirred for 1 h before furfurylamine (1.54 eq., 1.25 mL, 14.1 mmol) was added and the reaction was allowed to come to RT overnight. Usual work up and purification afforded compound **71a** (1.6 g, 67%) as white crystals.

Mp: 97-98 °C.[238]

δ_H (400 MHz, $CDCl_3$): 1.51 (s, 9H, CH_3-Boc), 4.87 (d, 2H, J 4.2, CH_2), 6.37 (m, 2H, H-3', H-4'), 7.43 (d, 1H, J 7.1, H-5'), 7.95 (br s, 1H, NH), 9.96 (br s, 1H, NH).

_N_-(*tert*-Butoxycarbonyl)-_N'_-(hydroxyethyl)thiourea 71b[238]

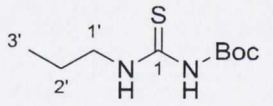


Following Method K, thiourea (1.0 eq., 1.0 g, 13.1 mmol) was dissolved in dry THF (80 mL) under argon at 0 °C, to which NaH (60% suspension in mineral oil, 4.5 eq., 1.4 g, 58.9 mmol) was added. The reaction was stirred at RT for 45 min to complete formation of the anion and re-cooled to 0 °C prior to the addition of di-*tert*-butyl dicarbonate (2.2 eq., 6.2 g, 28.2 mmol). After stirring at RT for 8 h, the reaction was re-cooled to 0 °C and NaH (60% suspension in mineral oil, 1.7 eq., 628 mg, 22.2 mmol) was added. After 1 h TFAA (1.54 eq., 2.80 mL, 20.1 mmol) was added and the reaction was stirred for 1 h before ethanolamine (1.54 eq., 1.2 mL, 20.1 mmol) was added and the reaction was allowed to come to RT overnight. Usual work up and purification afforded compound **71b** (1.66 g, 58%) as yellow crystals.

Mp: 100-102 °C.[238]

δ_H (400 MHz, $CDCl_3$): 1.43 (s, 9H, CH_3-Boc), 3.80 (m, 4H, CH_2-1', CH_2-2'), 5.15 (br s, 1H, OH), 7.48 (br s, 1H, NH), 9.97 (br s, 1H, NH).

_N_-(*tert*-Butoxycarbonyl)-_N'_-(propyl)thiourea 71c[238]

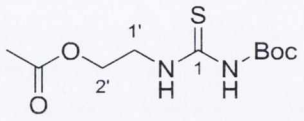


Following Method K, thiourea (1.0 eq., 1.0 g, 13.1 mmol) was dissolved in dry THF (80 mL) under argon at 0 °C, to which NaH (60% suspension in mineral oil, 4.5 eq., 1.4 g, 58.9 mmol) was added. The reaction was stirred at RT for 45 min to complete formation of the anion and re-cooled to 0 °C prior to the addition of di-*tert*-butyl dicarbonate (2.2 eq., 6.2 g, 28.2 mmol). After stirring at RT for 8 h, the reaction was re-cooled to 0 °C and NaH (60% suspension in mineral oil, 1.7 eq., 628 mg, 22.2 mmol) was added. After 1 h TFAA (1.54 eq., 2.80 mL, 20.1 mmol) was added and the reaction was stirred for 1 h before propylamine (1.54 eq., 1.62 mL, 20.1 mmol) was added and the reaction was allowed to come to RT overnight. Usual work up and purification afforded compound **71c** (1.7 g, 60%) as white crystals.

Mp: 60-62 °C.[238]

δ_H (400 MHz, $CDCl_3$): 1.48 (m, 3H, CH_3-3'), 1.52 (m, 11H, CH_3-Boc, CH_2-2'), 3.91 (m, 2H, CH_2-1'), 8.0 (br s, 1H, NH), 10.03 (br s, 1H, NH).

2-[3-(*tert*-Butoxycarbonyl)thioureido]ethyl acetate 71d[238]

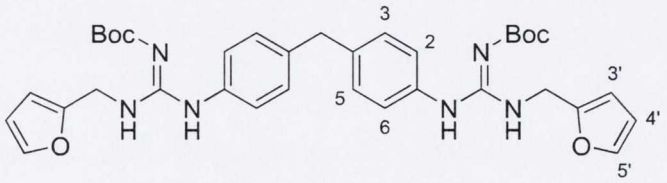


To a solution of Compound **71b** (1 eq., 2.62 g, 11.95 mmol), pyridine (3 eq., 2.88 mL, 35.88 mmol) and acetic anhydride (1.5 eq., 1.7 mL, 17.94 mmol) was added 4-(dimethylamino)pyridine (0.02 eq., 29 mg, 0.23 mmol) at 0 °C. The reaction was stirred at RT for 4 h. The reaction was then quenched with saturated $NaHCO_3$ solution (15 mL) and extracted with Et_2O (2 × 20 mL). The combined organic phase was washed with H_2O (1 × 15 mL) and brine (1 × 15 mL). The organic phased was then dried over Na_2SO_4, filtered and the solvent removed under vacuum. Purification using silica gel chromatography, eluting in hexane:EtOAc, afforded compound **71d** as an off-white crystalline solid (88%, 2.78 g).

Mp: 78-81 °C.[238]

δ_H (400 MHz, $CDCl_3$): 1.52 (s, 9H, CH_3-Boc), 2.12 (s, 3H, CH_3), 3.97 (m, 2H, CH_2-1'), 4.32 (t, 2H, J 7.1, CH_2-2'), 8.03 (br s, 1H, NH), 9.94 (br s, 1H, NH).

4,4'-Di[2-(*tert*-butoxycarbonyl)-3-(furanyl-2-methyl)guanidino]diphenylmethane 74a



Following Method N, to a solution 4,4'-diaminodiphenylmethane (1.0 eq., 455 mg, 2.3 mmol), compound **71a** (2.0 eq., 1.3 g, 4.6 mmol) and NEt_3 (6.0 eq., 1.92 mL, 13.8 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added $HgCl_2$ (2.2 eq., 1.3 g, 5.06 mmol). The reaction was then

left to stir at RT overnight. Usual work up and purification afforded compound **74a** (911 mg, 61%) as an thick orange gum.

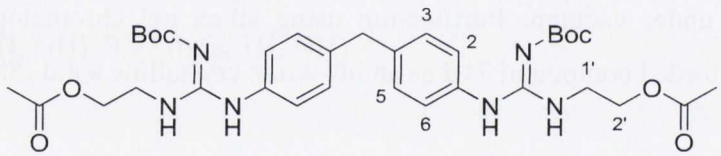
δ_H (400 MHz, $CDCl_3$): 1.56 (s, 18H, CH_3-Boc), 3.96 (s, 2H, CH_2) , 4.60 (s, 4H, CH_2), 6.21 (m, 2H, H-3') , 6.32 (m, 2H, H-4'), 7.16-7.20 (m, 8H, H-Ar), 7.34 (br s, 2H, H-5'), 10.72 (br s, 2H, NH).

δ_C (125 MHz, $CDCl_3$): 28.4 (CH_3-Boc), 38.3 (CH_2), 40.7 (CH_2), 78.7 (q, Boc), 107.4 (CH, Ar-3'), 110.3 (CH, Ar-4'), 125.6 (CH, Ar-2, Ar-6), 130.3 (CH, Ar-3, Ar-5), 133.7 (q, Ar-4), 138.7 (q, Ar-1), 142.2 (CH, Ar-5'), 151.0 (q, Ar 2'), 158.2 (q, C=O), 164.2 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3006 (NH), 2928 (NH), 1773 (C=O), 1718 (C=O), 1591, 1544, 1507, 1456, 1390, 1244, 1169, 1147, 1118, 1085, 1066, 937, 871, 884, 918 ,825, 803, 733.

HRMS: (m/z ESI$^+$) Found: 643.3264 (M^+- H, $C_{35}H_{43}N_6O_6$ Requires: 643.3244).

4,4'-Di[2-(*tert*-butoxycarbonyl)-3-(ethylacetate)guanidino]diphenylmethane 74b



Following Method N, to a solution 4,4'-diaminodiphenylmethane (1.0 eq., 340 mg, 1.72 mmol), compound **71d** (2.0 eq., 900 mg, 3.44 mmol) and NEt_3 (6.0 eq., 1.43 mL, 10.3 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added $HgCl_2$ (2.2 eq., 1.03 g, 3.8 mmol). The reaction was then left to stir at RT overnight. Usual work up and purification afforded compound **74b** (640 mg, 67%) as a pale yellow solid.

Mp: 56-60 °C.

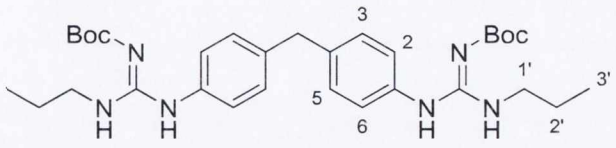
δ_H (400 MHz, $CDCl_3$): 1.55 (s, 18H, CH_3-Boc), 2.00 (s, 6H, CH_3), 3.64 (m, 4H, CH_2-1') , 4.01 (s, 2H, CH_2), 4.17 (m, 4H, CH_2-2'), 7.17 (m, 4H, H-2, H-6), 7.23 (m, 4H, H-3, H-5).

δ_C (125 MHz, $CDCl_3$): 20.8 (CH_3), 28.7 (CH_3-Boc), 40.19 (CH_2-1'), 40.49 (CH_2), 63.35 (CH_2-2'), 79.1 (q, Boc), 115.3 (CH, Ar-2, Ar-6), 124.04 (CH, Ar-3, Ar-5), 134.1 (q, Ar-4), 139.7 (q, Ar-1), 153.5 (q, C=O), 159.0 (q, C=O), 164.3 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3371 (NH), 2977 (NH), 1733 (C=O), 1692, 1592, 1549, 1512, 1512, 1456, 1385, 1336, 1227, 1167, 1136, 1043, 867, 735, 635.

HRMS: (m/z ESI$^+$) Found: 655.3440 (M$^+$- H, $C_{33}H_{47}N_6O_8$ Requires: 655.3455).

4,4'-Di[2-(*tert*-butoxycarbonyl)-3-(propyl)guanidino]diphenylmethane 74c



Following Method N, to a solution 4,4'-diaminodiphenylmethane (1.0 eq., 181 mg, 0.92 mmol), compound **71c** (2.0 eq., 398 mg, 1.83 mmol) and NEt_3 (6.0 eq., 768 µL, 5.52 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added $HgCl_2$ (2.2 eq., 552 mg, 2.0 mmol). The reaction was then left to stir at RT overnight. Usual work up and purification afforded compound **74c** (265 mg, 65%) as an orange solid.

Mp: 85-87 °C.

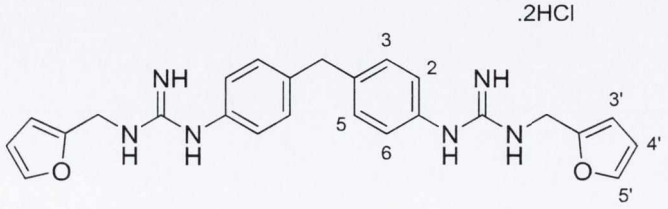
δ_H (400 MHz, $CDCl_3$): 0.89 (m, 6H, CH_3-3'), 1.52 (s, 18H, CH_3-Boc), 3.33 (m, 4H, CH_2-2'), 3.96 (s, 2H, CH_2), 4.15 (m, 4H, CH_2-1'), 7.15-7.20 (m, 8H, H-Ar), 10.68 (br s, 2H, NH).

δ_C (125 MHz, $CDCl_3$): 11.3 (CH_3-3'), 22.6 (CH_2-2'), 28.3 (CH_3-Boc), 40.7 (CH_2), 42.7 (CH_2-1'), 78.1 (q, Boc), 123.8 (q, Ar-4), 125.4 (CH, Ar-2, Ar-6), 130.1 (CH, Ar-3, Ar-5), 134.5 (q, Ar-1), 158.5 (q, C=O), 164.2 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 2985 (NH), 2254, 1729 (C=O), 1593,1459, 1511, 1340, 1247, 1170, 1133, 1047, 906, 806, 728.

HRMS: (m/z ESI$^+$) Found: 567.3653 (M$^+$- H, $C_{31}H_{47}N_6O_4$ Requires: 567.3659).

4,4'-Di[3-(furanyl-2-methyl)guanidino]diphenylmethane dihydrochloride 75a



Following Method O, to a solution of 4M HCl/1,4-dioxane (12.0 eq., 600 µL, 2.28 mmol) was added compound **74a** (1.0 eq., 120 mg, 0.19 mmol). The mixture was stirred at 55 °C until completion, as adjudged by disappearance of starting material in TLC. Usual work up and purification afforded compound **75a** (89 mg, 91%) as yellow solid.

Mp: 135-139 °C.

δ_H (400 MHz, D_2O): 3.90 (s, 2H, CH_2), 4.34 (s, 4H, CH_2), 6.21 (app. t, 2H, J 4.0, H-3') , 6.32 (app. t, 2H, J 4.0, H-4'), 7.08 (d, 4H, J 8.2, H-2, H-6), 7.23 (d, 4H, J 8.2, H-3, H-5), 7.38 (app. t, 2H, J 4.0, H-5').

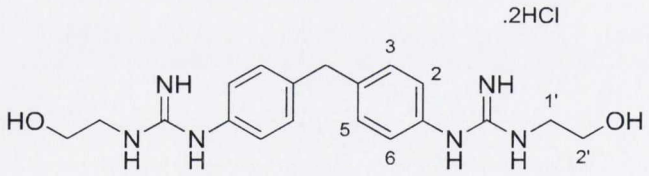
δ_C (125 MHz, D_2O): 38.1 (CH_2), 48.7 (CH_2), 107.1 (CH, Ar-3'), 110.3 (CH, Ar-4'), 126.3 (CH, Ar-2, Ar-6), 130.1 (CH, Ar-3, Ar-5), 132.1 (q, Ar-4), 141.1 (q, Ar-1), 143.3 (CH, Ar-5'), 149.1 (q, Ar-2'), 155.3 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3134 (NH), 2929, 1626, 1609, 1437, 1343, 1073, 1017, 975.

HRMS: (m/z ESI^+): Found: 443.2190 ($M^+ + H$, $C_{25}H_{27}N_6O_2$ Requires: 443.2193).

Purity by HPLC: 98.4% (t_R 23.63 min).

4,4'-Di[3-(hydroxyethyl)guanidino]diphenylmethane dihydrochloride 75b



Following Method O, to a solution of 1.25M HCl/CH_3OH (15.0 eq., 4.56 mL, 5.7 mmol) was added **74b** (1.0 eq., 250 mg, 0.38 mmol). The solution was stirred at 60 °C for 5 h. After usual workup and purification compound **75b** was obtained as a yellow gum (148 mg, 88%).

δ_H (400 MHz, D_2O): 3.25 (t, 4H, J 8.0, CH_2-1') , 3.57 (t, 4H, J 8.0, CH_2-2'), 3.83 (s, 2H, CH_2), 7.05 (d, 4H, J 8.0, H-2, H-6), 7.17 (d, 4H, J 8.0, H-3, H-5).

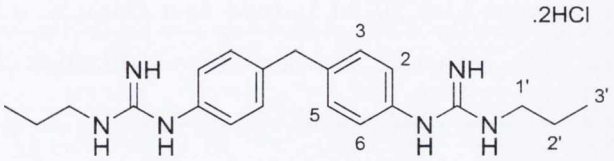
δ_C (125 MHz, D_2O): 40.1 (CH_2-1'), 42.6 (CH_2), 59.8 (CH_2-2'), 125.9 (CH, Ar-2, Ar-6), 130.1 (CH, Ar-3, Ar-5), 132.2 (q, Ar-4), 140.9 (q, Ar-1), 155.7 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3319 (NH), 3161 (NH), 2963, 1600, 1622, 1514, 1351, 1248, 1061, 1019, 920.

HRMS: (m/z ESI$^+$): Found: 371.2190 (M^+ + H, $C_{19}H_{27}N_6O_2$ Requires: 371.2199).

Purity by HPLC: 95.5% (t_R 19.95 min).

4,4'-Di[3-(propy)guanidino]diphenylmethane dihydrochloride 75c



Following Method O, to a solution of 4M HCl/1,4-dioxane (12.0 eq., 1.55 mL, 6.12 mmol) was added compound **74c** (1.0 eq., 290 mg, 0.51 mmol). The mixture was stirred at 55 °C until completion, as adjudged by disappearance of starting material in TLC. Usual work up and purification afforded compound **75c** (199 mg, 89%) as yellow solid.

Mp: 144-150 °C.

δ_H (400 MHz, D_2O): 0.79 (t, 6H, J 8.0, CH_3-3'), 1.46 (d, 4H, J 8.0, CH_2-2'), 3.07 (t, 4H, J 8.0, CH_2-1'), 3.83 (s, 2H, CH_2), 7.08 (d, 4H, J 8.1, H-2, H-6), 7.21 (d, 4H, J 8.1, H-3, H-5).

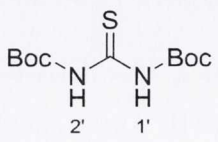
δ_C (125 MHz, D_2O): 10.2 (CH_3-3'), 21.2 (CH_2-2'), 39.8 (CH_2), 43.1 (CH_2-1'), 126.1 (CH, Ar-2, Ar-6), 130.1 (CH, Ar-3, Ar-5), 132.3 (q, Ar-4), 140.9 (q, Ar-1), 155.7 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3129 (NH), 2934, 1625, 1599, 1511, 1296, 1246, 1147, 1048, 805.

HRMS: (m/z ESI$^+$): Found: 367.2605 (M^+ + H, $C_{21}H_{31}N_6$ Requires: 367.2605).

Purity by HPLC: 98.3% (t_R 24.60 min).

***N,N'*-di(*tert*-Butoxycarbonyl)thiourea 66**[161]

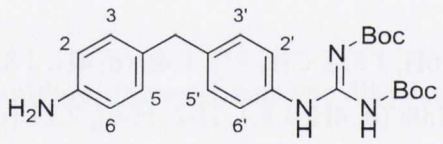


To a suspension of NaH (5.68 g, 236.47 mmol) in anhydrous THF (250 mL) was added a solution of thiourea (4 g, 52.55 mmol) in anhydrous THF (250 mL) at 0 °C under argon. The reaction was stirred on ice for 5 min at 0 °C before being allowed to warm and stir for 15 min at RT. The reaction mixture was again cooled to 0 °C in an ice bath before di-*tert*-butyl dicarbonate (25.23 g, 115.61 mmol) was added neat. After 30 min, the ice-bath was removed and the reaction mixture was stirred overnight at RT. The reaction mixture was quenched dropwise with a saturated $NaHCO_3$ solution (30 mL). The mixture was poured into H_2O (200 mL) and the aqueous layer was extracted with EtOAc (2 × 75 mL). The combined organic phases were washed with brine (2 × 50 mL), dried over Na_2SO_4, filtered and concentrated under vacuum affording an off-white powder. Recrystallization from hexane afforded compound **66** (9.025 g, 62%) as a white crystalline powder.

Mp: 132-135 °C.[161]

δ_H (400 MHz, $CDCl_3$): 1.52 (s, 18H, CH_3-Boc), 10.20 (br s, 2H, NH-1', NH-2').

4-Amino-4'-[2,3-di*(tert*-butoxycarbonyl)guanidino]diphenylmethane 76a



Following Method L, to a solution of 4,4'-diaminodiphenylmethane (3.0 eq., 968 mg, 4.89 mmol), compound **66** (1.0 eq., 450 mg, 1.63 mmol) and NEt_3 (3.0 eq., 750 µL, 4.89 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added $HgCl_2$ (1.1 eq., 485 mg, 1.79 mmol). The reaction was then left to stir at RT overnight. Usual work up and purification afforded compound **76a** (596 mg, 71%) as an off-white solid.

Mp: 130-133 °C.

δ_H (400 MHz, $CDCl_3$): 1.49 (s, 9H, CH_3-Boc), 1.51 (s, 9H, CH_3-Boc), 3.81 (s, 2H, CH_2), 6.59 (d, 2H, J 8.4, H-2, H-6), 6.93 (d, 2H, J 8.4, H-2', H-6'), 7.10 (d, 2H, J 8.4, H-3, H-5), 7.47 (d, 2H, J 8.4, H-3', H-5'), 10.23 (br s, 1H, NH), 11.60 (br s, 1H, NH).

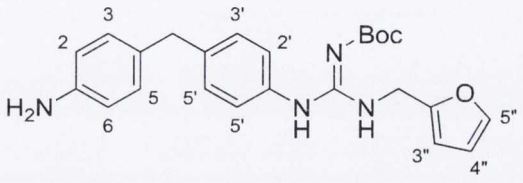
δ_C (125 MHz, $CDCl_3$): 28.41 (CH_3-Boc), 28.2 (CH_3-Boc), 40.4 (CH_2), 79.1 (q, Boc), 83.4 (q, Boc), 115.2 (CH, Ar-2, Ar-6), 122.2 (CH, Ar-2', Ar-6'), 129.1 (CH, Ar-3, Ar-5), 129.7 (CH,

Ar-3', Ar-5'), 131.0 (q, Ar-4'), 134.5 (q, Ar-4), 138.5 (q, Ar-1), 144.4 (q, Ar-1'), 153.3 (q, C=O), 153.5 (q, C=O), 163.5 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3388 (NH), 2979, 1715 (C=O), 1637, 1602, 1515, 1364, 1336, 1296, 1147, 1114, 1095, 852, 802, 758.

HRMS: (m/z ESI^+): Found: 441.2501 (M^+ + H, $C_{24}H_{33}N_4O_4$ Requires: 441.2496).

4-Amino-4'-[2-(*tert*-butoxycarbonyl)-3-(furanyl-2-methyl)guanidino]diphenylmethane 76b



Following Method L, to a solution of 4,4'-diaminodiphenylmethane (3.0 eq., 2.75 g, 13.8 mmol), compound **71a** (1.0 eq., 1.3 g, 4.63 mmol) and NEt_3 (3.0 eq., 1.93 mL, 13.8 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added $HgCl_2$ (1.1 eq., 1.36 g, 5.09 mmol). The reaction was then left to stir at RT overnight. Usual work up and purification afforded compound **76b** (967 mg, 50%) as an off-white solid.

Mp: 150-154 °C.

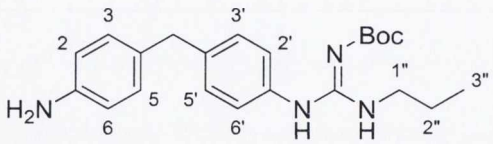
δ_H (400 MHz, $CDCl_3$): 1.57 (s, 9H, CH_3-Boc), 3.62 (s, 2H, CH_2), 4.61 (d, 2H, J 4.1, CH_2), 6.20 (m, 1H, H-3"), 6.31 (m, 1H, H-4") , 6.65 (d, 2H, J 7.4, H-2, H-6), 6.99 (d, 2H, J 7.4, H-2', H-6'), 7.12 (d, 2H, J 7.4, H-3, H-5), 7.19 (d, 2H, J 7.4, H-3', H-5'), 7.34 (br s, 1H, H-5"), 10.72 (br s, 2H, NH).

δ_C (125 MHz, $CDCl_3$): 28.4 (CH_3-Boc), 38.3 (CH_2), 40.53 (CH_2), 78.6 (q, Boc), 107.4 (CH, Ar-3"), 110.3 (CH, Ar-4"), 115.3 (CH, Ar-2, Ar-6), 125.6 (CH, Ar-2', Ar-6'), 129.7 (CH, Ar-3, Ar-5), 130.2 (CH, Ar-3', Ar-5'), 130.3 (q, Ar-4'), 133.8 (q, Ar-4), 140.9 (q, Ar-1), 142.2 (CH, Ar-5"), 144.7 (q, Ar-1'), 151.1 (q, Ar-2"), 158.3 (q, C=O), 164.29 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3369 (NH), 2920 (NH), 1644 (C=N), 1707, 1613 (C=O), 1599, 1545, 1506, 1492, 1366, 1308, 1268, 1151, 1016, 1104, 870, 831, 796, 775, 708.

HRMS: (m/z ESI^+) Found: 421.2256 (M^+- H, $C_{24}H_{29}N_4O_3$ Requires: 421.2240).

4-Amino-4'-[2-(*tert*-butoxycarbonyl)-3-propyl)guanidino]diphenylmethane 76c



Following Method L, to a solution of 4,4'-diaminodiphenylmethane (3.0 eq., 1.01 g, 5.59 mmol), compound **71c** (1.0 eq., 400 mg, 1.83 mmol) and NEt_3 (3.0 eq., 769 µL, 5.59 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added $HgCl_2$ (1.1 eq., 549 mg, 2.01 mmol). The reaction was then left to stir at RT overnight. Usual work up and purification afforded compound **76c** (412 mg, 59%) as an off-white solid.

Mp: 114-116 °C.

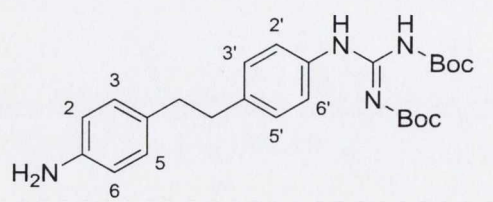
δ_H (400 MHz, $CDCl_3$): 0.82 (m, 3H, CH_3-3"), 1.43 (m, 2H, CH_2-2"), 1.48 (s, 9H, CH_3-Boc), 3.27 (m, 2H, CH_2-1"), 3.79 (s, 2H, CH_2), 6.55 (d, 2H, J 7.4, H-2, H-6), 6.90 (d, 2H, J 7.3, H-2', H-6'), 7.05 (m, 2H, H-3, H-5), 7.13 (m, 2H, H-3', H-5'), 10.59 (br s, 2H, NH).

δ_C (125 MHz, $CDCl_3$): 11.1 (CH_3-3"), 22.4 (CH_2-2"), 28.3 (CH_3-Boc), 40.3 (CH_2), 42.5 (CH_2-1"), 78.9 (q, Boc), 115.0 (CH, Ar-2, Ar-6), 125.3 (CH, Ar-2', Ar-6'), 129.4 (CH, Ar-3, Ar-5), 129.8 (CH, Ar-3', Ar-5'), 134.1 (q, Ar-4, Ar-4'), 140.2 (q, Ar-1), 144.8 (q, Ar-1'), 158.6 (q, C=O), 164.1 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3369 (NH), 2987, 1707 (C=O), 1644 (C=N), 1615, 1599, 1545, 1492, 1506, 1366, 1268, 1246, 1151, 1016, 1104, 870, 831, 796, 775, 708.

HRMS: (m/z ESI^+) Found: 383.2446 (M^+- H, $C_{22}H_{31}N_4O_2$ Requires: 383.2447).

{4-Amino-4'-[2,3-di(*tert*-butoxycarbonyl)guanidino]}-1,2-diphenylethane 76d



Following Method L, to a solution of 4,4'-ethylenedianiline (3.0 eq., 1.6 g, 7.59 mmol), compound **66** (1.0 eq., 700 mg, 2.53 mmol) and NEt_3 (3.0 eq., 1.16 mL, 7.59 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added $HgCl_2$ (1.1 eq., 754 mg, 2.78 mmol). The reaction was then

left to stir at RT overnight. Usual work up and purification afforded compound **76d** (856 mg, 84%) as a white solid.

Mp: 234-237 °C decomposed.

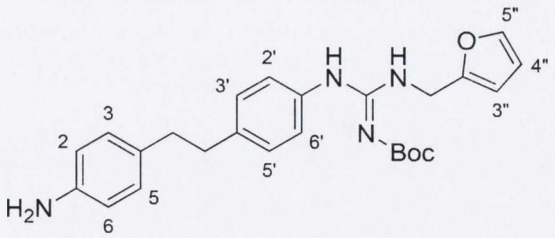
δ_H (400 MHz, $CDCl_3$): 1.50 (s, 18H, CH_3-Boc), 2.78 (m, 4H, CH_2), 6.62 (d, 2H, J 8.2, H-2, H-6), 6.94 (d, 2H, J 8.0, H-2', H-6'), 7.09 (d, 2H, J 8.2, H-3, H-5), 7.47 (d, 2H, J 8.0, H-3', H-5'), 10.25 (br s, 1H, NH), 11.61 (br s, 1H, NH).

δ_C (125 MHz, $CDCl_3$): 28.4 (CH_3-Boc), 28.2 (CH_3-Boc), 37.0 (CH_2), 37.6 (CH_2), 79.5 (q, Boc), 83.5 (q, Boc), 115.4 (CH, Ar-2, Ar-6), 122.1 (CH, Ar-2', Ar-6'), 128.8 (CH, Ar-3, Ar-5), 129.2 (CH, Ar-3', Ar-5'), 132.0 (q, Ar-4 or Ar-4'), 134.5 (q, Ar-4 or Ar-4'), 138.5 (q , Ar-1), 141.8 (q, Ar-1'), 153.4 (q, C=O), 163.5 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3447 (NH), 3365 (NH), 3260 (NH), 2973, 2923, 1718, 1551, 1514, 1367, 1290, 1237, 1053, 1031, 961, 881, 850, 771.

HRMS: (m/z ESI^+): Found: 477.2452 (M^+ + Na, $C_{25}H_{34}N_4NaO_4$ Requires: 477.2472).

{4-Amino-4'-[2-(*tert*-butoxycarbonyl)-3-(furanyl-2-methyl)guanidine]}-1,2-diphenylethane 76e



Following Method L, to a solution of 4,4'-ethylenedianiline (3.0 eq., 992 mg, 4.68 mmol), compound **71a** (1.0 eq., 400 mg, 1.56 mmol) and NEt_3 (3.0 eq., 710 µL, 4.68 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added $HgCl_2$ (1.1 eq., 465 mg, 1.72 mmol). The reaction was then left to stir at RT overnight. Usual work up and purification afforded compound **76e** (467 mg, 69%) as a yellow gum.

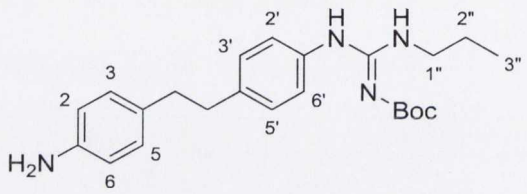
δ_H (400 MHz, $CDCl_3$): 1.51 (s, 9H, CH_3-Boc), 2.71-2.88 (m, 4H, CH_2), 4.55 (br s, 2H, CH_2), 6.18 (br s, 1H, H-3"), 6.29 (br s, 1H, H-4"), 6.58 (d, 2H, J 8.2, H-2, H-6), 6.89 (d, 2H, J 8.0, H-2', H-6'), 7.09 (m, 2H, H-3', H-5'), 7.08-7.16 (m, 2H, H-3, H-5), 7.36 (m, 1H, H-5").

δ_C (125 MHz, $CDCl_3$): 28.3 (CH_3-Boc), 36.8 (CH_2), 37.6 (CH_2), 38.3 (CH_2), 80.5 (q, Boc), 107.3 (CH, Ar-3"), 110.3 (CH, Ar-4"), 115.1 (CH, Ar-2', Ar-6', Ar-2, Ar-6), 129.1 (CH, Ar-3, Ar-5), 129.9 (CH, Ar-3', Ar-5'), 131.3 (q, Ar-4 or Ar-4'), 134.6 (q, Ar-4 or Ar-4'), 142.1 (CH, Ar-5"), 144.3 (q, Ar-1), 147.3 (q, Ar-1'), 151.6 (q, Ar-2"), 154.7 (q, C=O), 158.9 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3365 (NH), 2979, 2927, 1729, 1544, 1389, 1185, 1087, 1045, 1010, 913, 884, 804.

HRMS: (m/z ESI^+): Found: 435.2410 (M^+ + H, $C_{25}H_{31}N_4O_3$ Requires: 435.2390).

{4-Amino-4'-[(2-(*tert*-butoxycarbonyl)-3-(propyl)guanidino]}-1,2-diphenylethane 76f



Following Method L, to a solution of 4,4′-ethylenedianiline (3.0 eq., 1.4 g, 6.87 mmol), compound **71c** (1.0 eq., 500 mg, 2.29 mmol) and NEt_3 (3.0 eq., 1.05 mL, 6.87 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added $HgCl_2$ (1.1 eq., 682 mg, 2.52 mmol). The reaction was then left to stir at RT overnight. Usual work up and purification afforded compound **76f** (520 mg, 58%) as an orange gum.

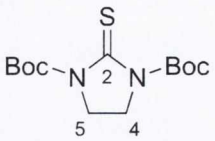
δ_H (400 MHz, $CDCl_3$): 0.83 (br s, 3H, CH_3-3"), 1.48 (br s, 11H, CH_3-Boc, CH_2-2"), 2.76 (m, 2H, CH_2), 2.81 (m, 2H, CH_2), 3.27 (br s, 2H, CH_2-1"), 6.55 (d, 2H, J 8.2, H-2, H-6), 6.88 (d, 2H, J 8.0, H-2', H-6'), 7.09 (br s, 2H, H-3', H-5'), 7.10 (d, 2H, J 8.2, H-3, H-5).

δ_C (125 MHz, $CDCl_3$): 11.3 (CH_3-3"), 22.7 (CH_2-2"), 28.4 (CH_3-Boc), 36.8 (CH_2), 37.6 (CH_2), 42.7 (CH_2-1"), 78.5 (q, Boc), 115.1 (CH, Ar-2, Ar-6), 120.5 (CH, Ar-2', Ar-6'), 129.1 (CH, Ar-3, Ar-5), 129.9 (CH, Ar-3', Ar-5'), 131.1 (q, Ar-4 or Ar-4'), 133.8 (q, Ar-4 or Ar-4'), 139.5 (q, Ar-1), 144.2 (q, Ar-1'), 158.0 (q, C=O), 164.1 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3321 (NH), 2966, 2931, 1725, 1590, 1574, 1389, 1278, 1168, 1131, 1065, 821, 803, 758, 698.

HRMS: (m/z ESI^+): Found: 397.2615 (M^+ + H, $C_{23}H_{33}N_4O_2$ Requires: 397.2598).

_N,N'_-di(*tert*-Butoxycarbonyl)imidazolidine-2-thione 77[159]

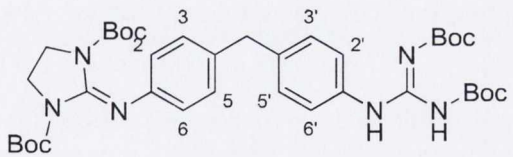


To a cooled solution of imidazolidine-2-thione (1.0 eq., 4.21 g, 41.21 mmol) in dry THF (40 mL) under argon was added a 60% suspension of NaH in mineral oil (4.5 eq., 7.42 g, 185.44 mmol). After 5 min, the ice-bath was removed and the reaction was stirred for 10 min at RT. The mixture was re-cooled to 0 °C and di-*tert*-butyldicarbonate (2.2 eq., 19.79 g, 90.66 mmol) was added. After 30 min, the reaction mixture was warmed to RT and stirred for 16 h. The reaction was quenched by the dropwise addition of saturated $NaHCO_3$ solution (25 mL) and extracted with EtOAc (3 × 40 mL). The combined organic phases were washed with brine (1 × 20 mL) and H_2O (1 × 20 mL), dried over Na_2SO_4, filtered and concentrated under vacuum. Recrystallization from hexane afforded compound **77** (11.09 g, 89%) as bright yellow crystalline needles.

Mp: 117-119 °C.[159]

δ_H (400 MHz, $CDCl_3$): 1.56 (s, 18H, CH_3-Boc), 3.92 (m, 4H, CH_2-4, 5).

4-[Di(2,3-*tert*-butoxycarbonyl)guanidino]-4'-[di(*tert*-butoxycarbonyl)-2-iminoimidazolidino]diphenylmethane 78a



Following Method M, to a solution of compound **76a** (1.0 eq., 300 mg, 0.68 mmol), compound **77** (1.0 eq., 135 mg, 0.68 mmol) and NEt_3 (3.0 eq., 290 µL, 2.04 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added $HgCl_2$ (1.1 eq., 202 mg, 0.75 mmol). The reaction was then left to stir at RT overnight. Usual work up and purification afforded compound **78a** (378 mg, 78%) as an off white powder.

Mp: 114-116 °C.

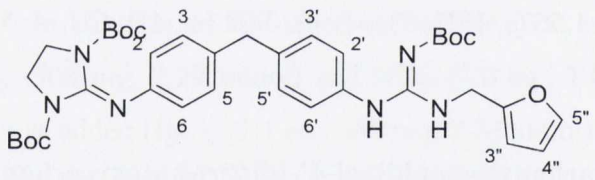
δ_H (400 MHz, $CDCl_3$): 1.27 (s, 18H, CH_3-Boc), 1.46 (s, 9H, CH_3-Boc), 1.50 (s, 9H, CH_3-Boc), 3.77 (s, 4H, CH_2), 3.83 (s, 2H, CH_2), 6.88 (d, 2H, J 8.4, H-2', H-6'), 6.93 (d, 2H, J 8.2, H-2, H-6), 7.10 (d, 2H, J 8.4, H-3', H-5'), 7.47 (d, 2H, J 8.2, H-3, H-5), 10.20 (br s, 1H, NH), 11.59 (br s, 1H, NH).

δ_C (125 MHz, $CDCl_3$): 27.8 (CH_3-Boc), 28.0 (CH_3-Boc), 28.1 (2 CH_3-Boc), 40.4 (CH_2), 43.0 (CH_2-Imid), 79.4 (q, Boc), 82.6 (q, Boc), 83.5 (2 q, Boc), 121.5 (CH, Ar-2', Ar-6'), 122.0 (CH, Ar-2, Ar-6), 129.1 (CH, Ar-3, Ar-5, Ar-3', Ar-5'), 134.5 (q, Ar-4 or Ar-4'), 138.5 (q, Ar-4' or Ar-4), 146.2 (q, Ar-1'), 150.1 (q, Ar-1), 153.2 (q, C=O), 153.4 (q, C=O), 163.5 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3201 (NH), 3014, 2942, 1701, 1630, 1365, 1299, 1233, 1078, 973, 865.

HRMS: (m/z ESI^+): Found: 709.3941 ($M^+ + H$, $C_{37}H_{53}N_6O_8$ Requires: 709.3919).

4-[2-(*tert*-Butoxycarbonyl)-3-(furanyl-2-methyl)guanidino]-4'-[1,3-(*tert*-butoxycarbonyl)-2-iminoimidazolidino]diphenylmethane 78b



Following Method M, to a solution of compound **76b** (1.0 eq., 350 mg, 0.83 mmol), compound **77** (1.0 eq., 249 mg, 0.83 mmol) and NEt_3 (3.0 eq., 343 µL, 2.49 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added $HgCl_2$ (1.1 eq., 249 mg, 0.91 mmol). The reaction was then left to stir at RT overnight. Usual work up and purification afforded compound **78b** (536 mg, 93%) as a dark orange gum.

δ_H (400 MHz, $CDCl_3$): 1.32 (s, 18H, CH_3-Boc),1.56 (s, 9H, CH_3-Boc), 3.83 (br s, 4H, CH_2), 3.90 (s, 2H, CH_2), 4.60 (d, 2H, J 4.2, CH_2), 6.20 (m, 1H, H-3"), 6.32 (m, 1H, H-4"), 6.95 (d, 2H, J 8.2, H-2' H-6'), 7.06 (d, 2H, J 8.3, H-2, H-6), 7.09 (d, 2H, J 8.2, H-3', H-5'), 7.18 (d, 2H, J 8.3, H-3, H-5), 7.34 (m, 1H, H-5").

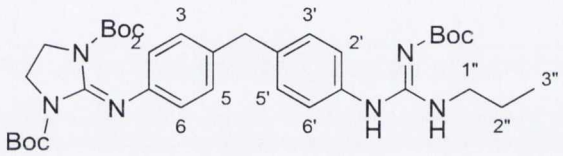
δ_C (125 MHz, $CDCl_3$): 27.8 (CH_3-Boc), 28.4 (2 CH_3-Boc), 38.3 (CH_2), 40.9 (CH_2), 43.1 (CH_2-Imid), 77.25 (q, Boc), 82.1 (2 q, Boc), 107.3 (CH, Ar-3"), 109.9 (CH, Ar-4"), 124.3 (CH, Ar-2', Ar-6'), 125.4 (CH, Ar-2, Ar-6), 128.6 (CH, Ar-3', Ar-5'), 129.1 (CH, Ar-3, Ar-

5), 134.2 (q, Ar-4 or Ar-4'), 130.1 (q, Ar-4 or Ar-4'), 140.3 (q, Ar-1 or Ar-1'), 142.4 (q, Ar-1 or Ar-1'), 147.8 (CH, Ar-5"), 150.2 (q, Ar-2"), 153.2 (q, C=O), 158.2 (q, C=O), 163.8 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3363 (NH), 2930, 2986, 1754 (C=O), 1636, 1597, 1547, 1506, 1457 ,1479, 1365, 1301, 1348, 1271, 1247, 1078, 1148, 975, 917, 847, 767,730.

HRMS: (m/z ESI^+) Found: 689.3675 ($M^+ + H$, $C_{37}H_{49}N_6O_7$ Requires: 689.3681).

4-[2-(*tert*-Butoxycarbonyl)-3-(propyl)guanidino]-4'-[di(*tert*-butoxycarbonyl)-2-iminoimidazolidino)diphenylmethane 78c



Following Method M, to a solution of compound **76c** (1.0 eq., 317 mg, 0.83 mmol), compound **77** (1.0 eq., 249 mg, 0.83 mmol) and NEt_3 (3.0 eq., 343 µL, 2.49 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added $HgCl_2$ (1.1 eq., 249 mg, 0.91 mmol). The reaction was then left to stir at RT overnight. Usual work up and purification afforded compound **78c** (398 mg, 74%) as a pale yellow solid.

Mp: 80-83 °C.

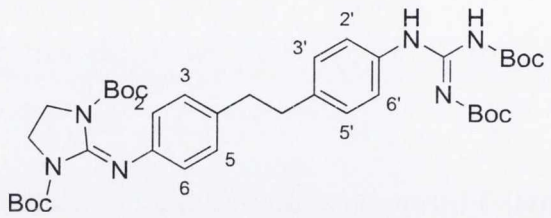
δ_H (400 MHz, $CDCl_3$): 0.87 (m, 3H, CH_3-3"), 1.30 (s, 18H, CH_3-Boc), 1.48 (m, 2H, CH_2-2"), 1.53 (s, 9H, CH_3-Boc), 3.32 (m, 2H, CH_2-1"), 3.81 (s, 4H, CH_2), 3.89 (s, 2H, CH_2), 6.93 (d, 2H, J 8.1, H-2', H-6'), 7.05 (d, 2H, J 8.2, H-2, H-6), 7.08 (m, 2H, H-3', H-5'), 7.18 (m, 2H, H-3, H-5), 10.63 (br s, 2H, NH).

δ_C (100 MHz, $CDCl_3$): 11.2 (CH_3-3"), 22.7 (CH_2-2"), 27.8 (CH_3-Boc), 28.4 (2 CH_3-Boc), 40.9 (CH_2), 42.7 (CH_2-1"), 43.1 (CH_2-Imid), 79.9 (q, Boc), 82.2 (2 q, Boc), 121.5 (CH, Ar-2', Ar-6'), 125.5 (CH, Ar-2, Ar-6), 129.1 (CH, Ar-3', Ar-5'), 129.8 (CH, Ar-3, Ar-5), 134.4 (q, Ar-4 or Ar-4'), 139.0 (q, Ar-4 or Ar-4'), 146.5 (q, Ar-1 or Ar-1'), 150.1 (q, Ar-1 or Ar-1'), 153.3 (q, C=O), 158.5 (q, C=O), 164.5 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3373 (NH), 2977, 2133, 1755 (C=O), 1691 (C=O), 1592, 1542, 1506, 1454, 1437, 1366, 1300, 1242, 1145, 1017, 975, 942, 849, 787, 744, 724.

HRMS: (m/z ESI$^+$)**:** Found: 651.3829 (M^+ + H, $C_{35}H_{51}N_6O_6$ Requires: 651.3831).

{4-[Di(2,3-*tert*-butoxycarbonyl)guanidino]-4'-[di(*tert*-butoxycarbonyl)-2-iminoimidazolidino]}-1,2-diphenylethane 78d



Following Method M, to a solution of compound **76d** (1.0 eq., 350 mg, 0.77 mmol), compound **77** (1.0 eq., 231 mg, 0.77 mmol) and NEt_3 (3.0 eq., 330 µL, 2.31 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added $HgCl_2$ (1.1 eq., 229 mg, 0.85 mmol). The reaction was then left to stir at RT overnight. Usual work up and purification afforded compound **78d** (401 mg, 72%) as a white solid.

Mp: 101-105 °C.

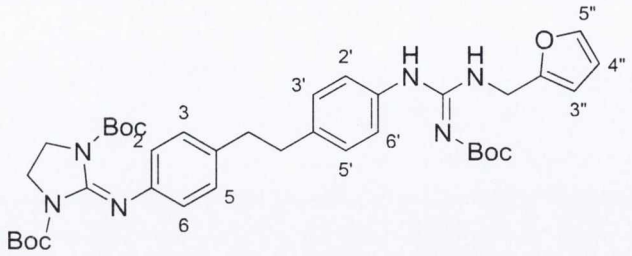
δ_H (400 MHz, $CDCl_3$): 1.29 (s, 9H, CH_3-Boc), 1.48 (s, 9H, CH_3-Boc), 1.51 (s, 18H, CH_3-Boc), 2.79 (m, 4H, CH_2), 3.70 (br s, 2H, CH_2), 3.79 (br s, 2H, CH_2), 6.89 (d, 2H, J 8.1, H-2', H-6'), 7.02 (d, 2H, J 8.0, H-2, H-6), 7.15 (d, 2H, J 8.1, H-3', H-5'), 7.47 (d, 2H, J 8.0, H-3, H-5), 10.23 (br s, 1H, NH), 11.61 (br s, 1H, NH).

δ_C (100 MHz, $CDCl_3$): 27.8 (CH_3-Boc), 27.9 (CH_3-Boc), 28.0 (CH_3-Boc), 28.1 (CH_3-Boc), 37.3 (CH_2), 37.6 (CH_2), 43.0 (CH_2-Imid) 79.3 (q, Boc), 82.5 (q, Boc), 83.1 (q, Boc), 83.5 (q, Boc), 121.4 (CH, Ar-2', Ar-6'), 122.1 (CH, Ar-2, Ar-6), 128.5 (CH, Ar-3', Ar-5'), 128.7 (CH, Ar-3, Ar-5), 135.8 (q, Ar-4 or Ar-4'), 138.4 (q, Ar-4 or Ar-4'), 146.1 (q, Ar-1 or Ar-1'), 150.3 (q, Ar-1 or Ar-1'), 153.2 (q, C=O), 153.4 (q, C=O), 163.5 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3275 (NH), 2978, 1705, 1629 (C=O), 1513, 1409, 1366, 1234, 1089, 973, 811, 765.

HRMS: (m/z ESI$^+$): Found: 723.4096 (M^+ + H, $C_{38}H_{55}N_6O_8$ Requires: 723.4076).

{4-[2-(*tert*-Butoxycarbonyl)-3-(furanyl-2-methyl)guanidino]-4'-[1,3-(*tert*-butoxycarbonyl)-2-iminoimidazolidino]}-1,2-diphenylethane 78e



Following Method M, to a solution of compound **76e** (1.0 eq., 200 mg, 0.46 mmol), compound **77** (1.0 eq., 138 mg, 0.46 mmol) and NEt_3 (3.0 eq., 210 μL, 1.38 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added $HgCl_2$ (1.1 eq., 137 mg, 0.51 mmol). The reaction was then left to stir at RT overnight. Usual work up (without further purification) afforded compound **78e** (301 mg, 93%) as an orange gum.

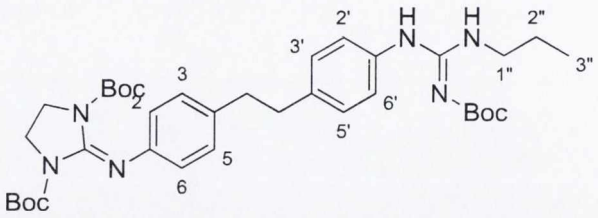
δ_H (400 MHz, $CDCl_3$): 1.31 (s, 9H, CH_3-Boc), 1.54 (s, 18H, CH_3-Boc), 2.84 (br s, 4H, CH_2), 3.79 (br s, 2H, CH_2), 3.82 (br s, 2H, CH_2), 4.59 (s, 2H, CH_2), 6.20 (br s, 1H, H-3"), 6.31 (br s, 1H, H-4"), 6.92 (m, 2H, H-2', H-6'), 7.02 (m, 2H, H-2, H-6), 7.08 (m, 2H, H-3', H-5'), 7.18 (m, 2H, H-3, H-5), 7.35 (m, 1H, H-5").

δ_C (125 MHz, $CDCl_3$): 27.4 (CH_3-Boc), 27.9 (2 CH_3-Boc), 36.9 (CH_2), 37.1 (CH_2), 39.9 (CH_2), 42.6 (CH_2-Imid) 82.2 (q, Boc), 82.8 (q, Boc), 83.8 (q, Boc), 106.8 (CH, Ar-3"), 109.9 (CH, Ar-4"), 121.0 (CH, Ar-2', Ar-6'), 125.1 (CH, Ar-2, Ar-6), 128.1 (CH, Ar-3', Ar-5'), 129.7 (CH, Ar-3, Ar-5), 138.6 (q, Ar-4 or Ar-4''), 141.7 (q, Ar-4 or Ar-4'), 145.6 (q, Ar-1 or Ar-1'), 146.9 (q, Ar-1 or Ar-1'), 149.9 (CH, Ar-5"), 150.8 (q, Ar-2"), 154.7 (q, C=O), 155.5 (q, C=O), 163.8 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3123 (NH), 2976, 2930, 1711, 1634, 1594, 1512, 1457, 1417, 1366, 1300, 1242, 1044, 1018, 973, 846, 767.

HRMS: (m/z ESI^+): Found: 703.3828 ($M^+ + H$, $C_{38}H_{51}N_4O_7$ Requires: 703.3814).

{4-[2-(*tert*-Butoxycarbonyl)-3-(propyl)guanidino]-4'-[di(*tert*-butoxycarbonyl)-2-iminoimidazolidino]}-1,2-diphenylethane 78f



Following Method M, to a solution of compound **76f** (1.0 eq., 200 mg, 0.5 mmol), compound **77** (1.0 eq., 151 mg, 0.5 mmol) and NEt_3 (3.0 eq., 230 µL, 1.5 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added $HgCl_2$ (1.1 eq., 149 mg, 0.55 mmol). The reaction was then left to stir at RT overnight. Usual work up (without further purification) afforded compound **78f** (321 mg, 96%) as a yellow gum.

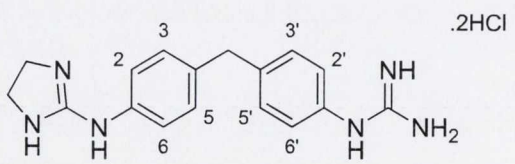
δ_H (400 MHz, $CDCl_3$): 0.84 (m, 3H, CH_3-3"), 1.31 (s, 18H, Boc), 1.52 (s, 11H, CH_3-Boc, CH_2-2"), 2.83 (br s, 4H, CH_2), 3.30 (m, 2H, CH_2-1"), 3.79 (br s, 4H, CH_2), 6.89 (d, 2H, J 8.0, H-2', H-6'), 7.00 (d, 2H, J 8.3, H-2, H-6), 7.11 (m, 2H, H-3', H-5'), 7.15 (m, 2H, H-3, H-5).

δ_C (125 MHz, $CDCl_3$): 13.7 (CH_3-3"), 21.9 (CH_2-2"), 27.4 (CH_3-Boc), 27.9 (2 CH_3-Boc), 36.3 (CH_2), 37.1 (CH_2), 42.3 (CH_2-Im), 42.6 (CH_2-Im), 44.1 (CH_2-1"), 82.1 (q, Boc), 83.4 (2 q, Boc), 121.0 (CH, Ar-2', Ar-6'), 125.1 (CH, Ar-2, Ar-6), 128.1 (CH, Ar-3', Ar-5'), 129.5 (CH, Ar-3, Ar-5), 135.5 (q, Ar-4 or Ar-4'), 138.5 (q, Ar-4 or Ar-4'), 145.6 (q, Ar-1 or Ar-1'), 149.9 (q, Ar-1 or Ar-1'), 158.3 (q, C=O), 163.5 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3266 (NH), 2979, 2931, 1738, 1710, 1591, 1504, 1476, 1366, 1301, 1239, 1145, 1046, 975, 804, 751.

HRMS: (m/z ESI^+): Found: 665.4030 ($M^+ + H$, $C_{36}H_{53}N_6O_6$ Requires: 665.4027).

4-Guanidino-4'-(2-aminoimidazoline)diphenylmethane dihydrochloride 79a



Following Method O, to a solution of 4M HCl/1,4-dioxane (24.0 eq., 850 µL, 3.36 mmol) was added compound **78a** (1.0 eq., 100 mg, 0.14 mmol). The mixture was stirred at 55 °C

until completion, as adjudged by disappearance of starting material in TLC. Usual work up and purification afforded compound **79a** (52 mg, 98%) as yellow solid.

Mp: 219-221 °C decomposed.

δ_H (400 MHz, D_2O): 3.59 (s, 4H, CH_2), 3.88 (s, 2H, CH_2), 7.08 (m, 4H, H-2', H-6', H-2, H-6), 7.22 (m, 2H, H-3', H-5', H-3, H-5).

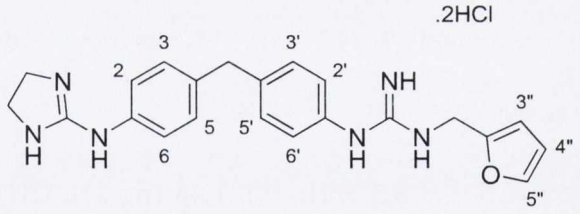
δ_C (100 MHz, D_2O): 40.4 (CH_2), 42.6 (CH_2-Imid), 124.6 (CH, Ar-2', Ar-6'), 126.3 (CH, Ar-2, Ar-6), 130.0 (CH, Ar-3', Ar-5'), 130.1 (CH, Ar-3, Ar-5), 132.1 (q, Ar-4 or Ar-4'), 133.5 (q, Ar-4 or Ar-4'), 140.6 (q, Ar-1 or Ar-1'), 144.6 (q, Ar-1 or Ar-1'), 156.3 (q, C=N), 158.7 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3384 (NH), 3270 (NH), 2993, 1602, 1518, 1406, 1232, 1147, 801.

HRMS: (m/z ESI^+): Found: 309.1826 ($M^+ + H$, $C_{17}H_{21}N_6$ Requires: 309.1822).

Purity by HPLC: 97.6% (t_R 19.91 min).

4-(2-Aminoimidazolino)-4'-[3-(furanyl-2-methyl)guanidino)diphenylmethane dihydrochloride 79b



Following Method O, to a solution of 4M HCl/1,4-dioxane (18.0 eq., 1.30 mL, 5.4 mmol) was added compound **78b** (1.0 eq., 200 mg, 0.30 mmol). The mixture was stirred at 55 °C until completion, as adjudged by disappearance of starting material in TLC. Usual work up and purification afforded compound **79b** (111 mg, 80%) as white solid.

Mp: 201-206 °C decomposed.

δ_H (400 MHz, D_2O): 3.75 (s, 4H, CH_2) 4.03 (s, 2H, CH_2), 4.50 (s, 2H, CH_2), 6.44 (d, 1H, J 4.0, H-3"), 6.47 (t, 1H, J 4.0, H-4"), 7.22 (d, 4H, J 8.3, H-2, H-6, H-2', H-6'), 7.36 (d, 4H, J 8.3, H-3, H-5, H-3', H-5'), 7.34 (br s, 1H, H-5").

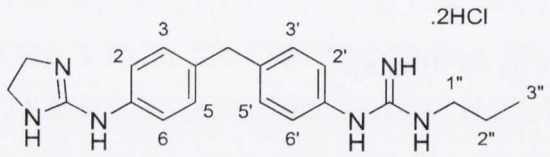
δ_C (100 MHz, D_2O): 38.0 (CH_2), 40.0 (CH_2), 42.6 (CH_2-Imid), 108.7 (CH, Ar-3"), 110.7 (CH, Ar-4"), 124.8 (CH, Ar-2', Ar-6'), 126.4 (CH, Ar-2, Ar-6), 130.2 (CH, Ar-3', Ar-5'), 130.5 (CH, Ar-3, Ar-5), 132.3 (q, Ar-4 or Ar-4'), 133.3 (q, Ar 4 or Ar-4'), 140.8 (q, Ar-1 or Ar-1'), 141.4 (q, Ar-1 or Ar-1'), 143.3 (CH, Ar-5"), 149.2 (q, Ar-2"), 155.5 (q, C=N), 158.6 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3140 (NH), 2981, 1647, 1570, 1387, 1244, 1096, 1022, 975.

HRMS: (m/z ESI^+): Found: 389.2084 (M^+ + H, $C_{22}H_{25}N_6O$ Requires: 389.2094).

Purity by HPLC: 95.1% (t_R 23.44 min).

4-[3-(propyl)guanidino]-4'-(2-aminoimidazolino)diphenylmethane dihydrochloride 79c



Following Method O, to a solution of 4M HCl/1,4-dioxane (18.0 eq., 1.40 mL, 5.58 mmol) was added compound **78c** (1.0 eq., 200 mg, 0.31 mmol). The mixture was stirred at 55 °C until completion, as adjudged by disappearance of starting material in TLC. Usual work up and purification afforded compound **79c** (126 mg, 96%) as brown solid.

Mp: 221-225 °C decomposed.

δ_H (400 MHz, D_2O): 0.78 (t, 3H, J 8.3, CH_3-3"), 1.45 (m, 2H, CH_2-2"), 3.07 (t, 2H, J 8.3, CH_2-1"), 3.59 (s, 4H, CH_2), 3.87 (s, 2H, CH_2), 7.06 (d, 4H, J 8.1, H-2, H-6, H-2', H-6'), 7.2 (d, 4H, J 8.1, H-3, H-5, H-3', H-5').

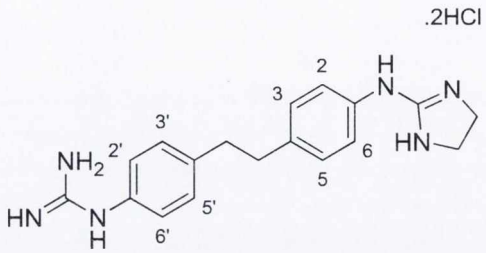
δ_C (100 MHz, D_2O): 10.3 (CH_3-3"), 21.4 (CH_2-2"), 40.0 (CH_2), 42.6 (CH_2-Imid), 43.0 (CH_2-1"), 124.6 (CH, Ar-2', Ar-6'), 126.2 (CH, Ar-2, Ar-6), 130.0 (CH, Ar-3', Ar-5'), 130.1 (CH, Ar-3, Ar-5), 132.3 (q, Ar-4 or Ar-4'), 133.0 (q, Ar-4 or Ar-4'), 140.7 (q, Ar-1 or Ar-1'), 141.0 (q, Ar-1 or Ar-1'), 155.2 (q, C=N), 158.7 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3159 (NH), 2968, 1717, 1650, 1592, 1485, 1468, 1284, 1240, 1088, 1018, 820, 758.

HRMS: (m/z ESI^+): Found: 351.2292 (M^+ + H, $C_{20}H_{27}N_6$ Requires: 351.2292).

Purity by HPLC: 99.7% (t_R 22.49 min).

[4-Guanidino-4'-(2-aminoimidazolino)]-1,2-diphenylethane dihydrochloride 79d



Following Method O, to a solution of 4M HCl/1,4-dioxane (24.0 eq., 1.24 mL, 5.04 mmol) was added compound **78d** (1.0 eq., 150 mg, 0.21 mmol). The mixture was stirred at 55 °C until completion, as adjudged by disappearance of starting material in TLC. Usual work up and purification afforded compound **79d** (71 mg, 86%) as yellow solid.

Mp: 210-215 °C decomposed.

δ_H (400 MHz, D_2O): 2.83 (s, 4H, CH_2), 3.58 (s, 4H, CH_2), 7.03 (m, 4H, H-2', H-6', H-2, H-6), 7.18 (m, 4H, H-3', H-5', H-3, H-5).

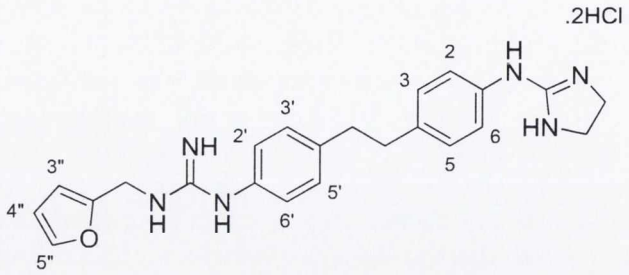
δ_C (125 MHz, D_2O): 35.8 (CH_2), 35.8 (CH_2), 42.6 (CH_2-Imid), 124.1 (CH, Ar-2', Ar-6'), 125.7 (CH, Ar-2, Ar-6), 129.6 (CH, Ar-3', Ar-5'), 129.9 (CH, Ar-3, Ar-5), 131.7 (q, Ar-4 or Ar-4'), 132.7 (q, Ar-4 or Ar-4'), 140.5 (q, Ar-1 or Ar-1''), 141.5 (q, Ar-1 or Ar-1'), 156.2 (q, C=N), 158.6 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3135 (NH), 2986, 1648, 1608, 1578, 1512, 149, 1255, 1019, 827.

HRMS: (m/z ESI^+): Found: 323.1977 ($M^+ + H$, $C_{18}H_{23}N_6$ Requires: 323.1987).

Purity by HPLC: 97.9% (t_R 21.29 min).

{4-(2-Aminoimidazolino)-4'-[3-(furanyl-2-methyl)guanidino]}-1,2-diphenylethane dihydrochloride 79e



Following Method O, to a solution of 4M HCl/1,4-dioxane (18.0 eq., 540 µL, 2.16 mmol) was added compound **78e** (1.0 eq., 80 mg, 0.12 mmol). The mixture was stirred at 55 °C until completion, as adjudged by disappearance of starting material in TLC. Usual work up and purification afforded compound **79e** (57 mg, 82%) as yellow solid.

Mp: 237-240 °C decomposed.

δ_H (400 MHz, D_2O): 2.86 (br s, 4H, CH_2), 3.59 (br s, 4H, CH_2), 4.34 (s, 2H, CH_2), 6.28 (m, 1H, H-3"), 6.32 (m, 1H, H-4"), 7.04 (m, 4H, H-2', H-6', H-2, H-6'), 7.39 (m, 4H, H-3', H-5', H-3, H-5'), 7.39 (br s, 1H, H-5").

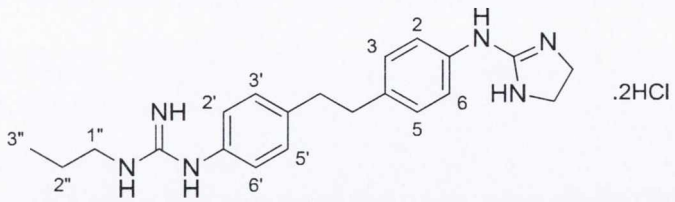
δ_C (100 MHz, D_2O): 35.8 (2 CH_2), 38.0 (CH_2), 42.6 (CH_2-Imid), 108.1 (CH, Ar-3"), 110.9 (CH, Ar-4"), 124.3 (CH, Ar-2', Ar-6'), 125.9 (CH, Ar-2, Ar-6), 129.8 (CH, Ar-3', Ar-5'), 130.0 (CH, Ar-3, Ar-5), 131.7 (q, Ar-4 or Ar-4'), 132.2 (q, Ar-4 or Ar-4'), 141.0 (q, Ar-1 or Ar-1'), 141.5 (q, Ar-1 or Ar-1'), 143.2 (CH, Ar-5"), 149.1 (q, Ar-2"), 155.4 (q, C=N), 158.7 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3143 (NH), 2892, 1648, 1602, 1612, 1461, 1381, 1345, 1240, 1148, 1084, 1016, 825

HRMS: (m/z ESI^+): Found: 403.2240 (M^+ + H, $C_{23}H_{27}N_6O$ Requires: 403.2240).

Purity by HPLC: 96.6% (t_R 24.16 min).

{4-[3-(propyl)guanidino]-4'-(2-aminoimidazolino)}-1,2-diphenylethane dihydrochloride 79f



Following Method O, to a solution of 4M HCl/1,4-dioxane (18.0 eq., 890 µL, 3.6 mmol) was added compound **78f** (1.0 eq., 130 mg, 0.20 mmol). The mixture was stirred at 55 °C until completion, as adjudged by disappearance of starting material in TLC. Usual work up and purification afforded compound **79f** (80 mg, 91%) as yellow solid.

Mp: 271-275 °C decomposed.

δ_H (400 MHz, D_2O): 0.79 (t, 3H, J 7.4, CH_3-3"), 1.46 (m, 2H, CH_2-2"), 2.82 (br s, 4H, CH_2), 3.07 (t, 2H, J 7.4, CH_2-1"), 3.59 (br s, 4H, CH_2), 7.02 (d, 4H, J 8.3, H-2', H-6', H-2, H-6), 7.11 (d, 4H, J 8.3, H-3', H-5', H-3, H-5).

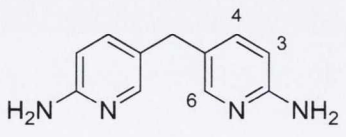
δ_C (100 MHz, D_2O): 10.2 (CH_3-3"), 21.4 (CH_2-2"), 35.8 (2 CH_2), 42.6 (CH_2-Imid), 43.0 (CH_2-1"), 124.1 (CH, Ar-2', Ar-6'), 125.8 (CH, Ar-2, Ar-6), 129.8 (CH, Ar-3', Ar-5'), 129.9 (CH, Ar-3, Ar-5), 132.0 (q, Ar-4 or Ar-4'), 132.7 (q, Ar-4 or Ar-4'), 140.9 (q, Ar-1 or Ar-1'), 141.3 (q, Ar-1 or Ar-1'), 155.1 (q, C=N), 158.6 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3018 (NH), 2849, 1593, 1508, 1355, 1234, 1144, 1081, 1017, 826.

HRMS: (m/z ESI^+): Found: 365.2305 ($M^+ + H$, $C_{21}H_{29}N_6$ Requires: 365.2296).

Purity by HPLC: 95.1% (t_R 23.01 min).

6,6'-(Di-amino)dipyridinyl-3-methane 82



To a solution of sulphuric acid (95-97%, 17 mL) was added compound **87** (3.6 g, 6.4 mmol), and the resulting dark residue was left stirring for 24 h at RT. The mixture was then poured

into H_2O (25 mL) and neutralized to pH 4 using 15% NaOH at 0 °C. The precipitate was filtered off and the pH value of the filtrate was adjusted to 12 with 15% NaOH. The resulting solution was extracted with $CHCl_3$:^{i}PrOH (8:2, 4 × 30 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. After which the residue was purified using silica gel chromatography, eluting in CH_2Cl_2:CH_3OH, to afford compound **82** (1.1 g, 85%) as an off-white solid.

Mp: 96-98 °C.

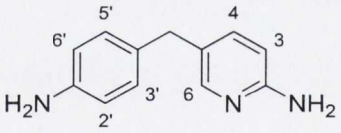
δ_H (400 MHz, $CDCl_3$) : 3.66 (s, 2H, CH_2), 4.36 (br s, 4H, NH_2), 6.42 (d, 2H, J 8.4, H-3), 7.20 (dd, 2H, ^{3}J 8.4, ^{4}J 1.7, H-4), 8.08 (s, 2H, H-6).

δ_C (100 MHz, $CDCl_3$): 34.0 (CH_2), 108.7 (CH, Ar-3), 124.2 (q, Ar-5), 138.0 (CH, Ar-4), 147.0 (CH, Ar-6), 156.7 (q, Ar-2).

ν_{max} (ATR)/cm^{-1}:3306 (NH), 3145 (NH), 2980, 2890, 1629, 1606, 1561, 1500, 1427, 1250, 1141, 1016, 945, 820.

HRMS: (m/z ESI$^+$): Found: 201.1142 (M^+ + H, $C_{11}H_{13}N_4$ Requires: 201.1140).

5-(4-Aminobenzyl)pyridin-2-amine 83



To a solution of sulphuric acid (95-97%, 9 mL) was added compound **92** (1.2 g, 3.15 mmol), and the resulting dark residue was left stirring for 24 h at RT. The mixture was then poured into H_2O (25 mL), and neutralized to pH 4 using 15% NaOH at 0 °C. The precipitate was filtered off and the pH value of the filtrate was adjusted to 12 with 15% NaOH. The resulting solution was extracted with $CHCl_3$:^{i}PrOH (8:2, 3 × 30 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. After which the residue was purified using silica gel chromatography, eluting in CH_2Cl_2:CH_3OH, to afford compound **83** (450 g, 72%) as a brown gum.

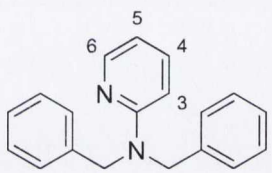
δ_H (400 MHz, $CDCl_3$) : 3.72 (s, 2H, CH_2), 6.42 (d, 1H, J 8.4, H-3), 6.62 (d, 2H, J 8.3, H-2', H-6'), 6.92 (d, 2H, J 8.3, H-3', H-5'), 7.19 (dd, 1H, ^{3}J 8.4, ^{4}J 2.4, H-4), 7.94 (d, 1H, ^{4}J 2.4, H-6).

δ_C (100 MHz, $CDCl_3$): 37.2 (CH_2), 108.6 (CH, Ar-3), 115.2 (CH, Ar-2', Ar-6'), 127.1 (q, Ar-5), 129.4 (CH, Ar-3', Ar-5'), 130.8 (q, Ar-4'), 138.5 (CH, Ar-4), 144.5 (q, Ar-1'), 147.4 (CH, Ar-6), 156.6 (q, Ar-2).

ν_{max} (ATR)/cm^{-1}: 3316 (NH), 3024 NH), 2938, 1616, 1571, 1498, 1403, 1246, 1142, 1021, 946, 821, 675.

HRMS: (m/z ESI$^+$): Found: 200.1198 (M^+ + H, $C_{12}H_{14}N_3$ Requires: 200.1188).

2-[*N,N*-(Dibenzyl)amino]pyridine 86[246]

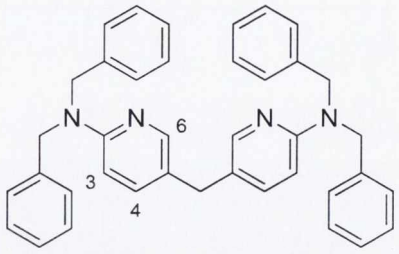


A solution of 2-aminopyridine (1.0 eq., 10.0 g, 106 mmol) in anhydrous THF (100 mL) under argon was added dropwise to a stirred suspension of sodium hydride (60% in mineral oil, 3.0 eq., 7.63 g, 318 mmol) in anhydrous THF (100 mL) at 0 °C. Benzylbromide (2.0 eq., 25.0 mL, 212 mmol) was added and the resulting mixture stirred at RT under argon for 8 h. The mixture was cooled to 0 °C, quenched with H_2O (30 mL), and extracted with EtOAc (3 × 30 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting residue was then purified by silica gel chromatography, eluting in hexane:EtOAc, affording compound **86** (27.4 g, 93%) as a yellow oil.[246]

δ_H (400 MHz, $CDCl_3$): 4.81 (s, 4H, CH_2), 6.47 (d, 1H, J 8.6, H-3), 6.59 (m, 1H, H-5), 7.25 (m, 5H, H-Ar Bn), 7.35 (m, 5H, H-Ar Bn), 7.39 (m, 1H, H-4), 8.23 (m, 1H, H-6).

HRMS: (m/z ESI$^+$): Found: 275.1540 (M^+ + H, $C_{19}H_{19}N_2$ Requires: 275.1542).

6,6'-Di(*N,N*-dibenzylamino)dipyridinyl-3-methane 87



To a solution of compound **86** (8.0 g, 29.1 mmol) in AcOH (6 mL) was added formaldehyde solution (37 wt. % in H_2O, 600 µL, 19.5 mmol) dropwise over 15 min. The reaction was refluxed for 48 hr. It was cooled to 0 °C, neutralised to pH 7 with a 10% NaOH solution and extracted with EtOAc (3 × 30 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting residue was then purified by silica gel chromatography, eluting in hexane:EtOAc, affording compound **87** (9.65 g, 60%) as a light-brown powder.

Mp: 129-133 °C.

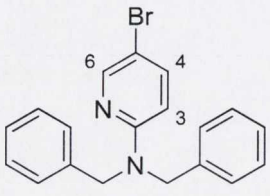
δ_H (400 MHz, $CDCl_3$): 3.70 (s, 2H, CH_2), 4.80 (s, 8H, CH_2-Bn), 6.41 (d, 2H, J 8.7, H-3), 7.20-7.26 (m, 14H, Ar-H Bn), 7.29-7.33 (m, 8H, Ar-H Bn, H-4), 8.08 (s, 2H, H-6).

δ_C (100 MHz, $CDCl_3$): 34.3 (CH_2), 50.9 (CH_2-Bn), 105.7 (CH, Ar-3), 124.4 (q, Ar-5), 126.9 (CH, Ar-*p*-Bn), 127.0 (CH, Ar-*o*-Bn), 128.5 (CH, Ar-*m*-Bn), 138.0 (q, Ar-Bn), 138.5 (CH, Ar-4), 147.6 (CH, Ar-6), 157.3 (q, Ar-2).

ν_{max} (ATR)/cm^{-1}: 2990, 1603, 1551, 1488, 1450, 1359, 1236, 1202,, 1154, 1075, 940, 898, 728, 700.

HRMS: (m/z ESI^+): Found: 561.3019 (M^+ + H, $C_{39}H_{37}N_4$ Requires: 561.3018).

2-[(*N,N*-Dibenzyl)amino]-5-bromopyridine 88[252]

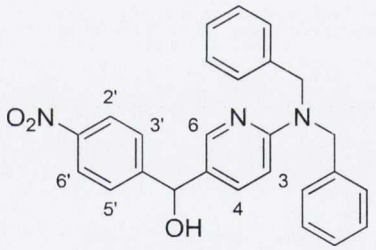


A solution of 2-amino-5-bromopyridine (1.0 eq., 5.0 g, 27.2 mmol) in anhydrous THF (50 mL) under argon was added dropwise to a stirred suspension of sodium hydride (60% in mineral oil, 3.0 eq., 1.958 g, 81.6 mmol) in anhydrous THF (50 mL) at 0 °C. Benzylbromide (2.0 eq., 6.46 mL, 54.4 mmol) was added and the resulting mixture stirred at RT under argon for 9 h. The mixture was cooled to 0 °C, quenched with H_2O (25 mL), and extracted with EtOAc (3 × 30 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting residue was then purified by silica gel chromatography, eluting in hexane:EtOAc, affording compound **88** (6.87g, 72%) as a white powder.

Mp: 61-63 °C.[268]

δ_H (400 MHz, $CDCl_3$): 4.79 (s, 4H, CH_2), 6.38 (d, 1H, J 8.0, H-3), 7.25 (m, 3H, Ar-H Bn), 7.29-7.34 (m, 7H, Ar-H Bn), 7.39 (dd, 1H, ^{3}J 8.0, ^{4}J 2.4 H-4), 8.24 (d, 1H, ^{4}J 2.4, H-6).

5-[(4-Nitrophenyl)hydroxymethyl)]-2-[(*N*,*N*-dibenzyl)amino]pyridine 91



To a solution of compound **88** (1.0 eq., 4.1 g, 11.65 mmol) in anhydrous THF (80 mL) at -80 °C under argon was added n-BuLi solution (2.5M in hexanes, 1.0 eq., 5.0 mL, 11.65 mmol). The reaction was stirred at -80 °C for 1 h to allow complete formation of the anion, adjudged by TLC analysis. A solution of 4-nitrobenzaldehye (1.1 eq., 1.93 g, 12.8 mmol) in anhydrous THF (30 mL) was then added dropwise over 30 min at -80 °C. After which it was left to slowly warm to RT over 6 h. The reaction was quenched with a saturated solution of NH_4Cl (30 mL), and extracted with EtOAc (3 × 20 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting residue was then purified by silica gel chromatography, eluting in hexane:EtOAc, to afford compound **91** (2.11 g, 42%) as a bright yellow gum.

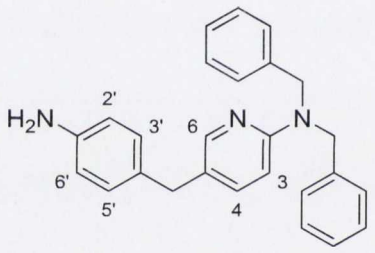
δ_H (400 MHz, $CDCl_3$): 4.79 (s, 4H, CH_2-Bn), 5.75 (s, 1H, CH), 6.42 (d, 1H, J 8.9, H-3), 7.19-7.31 (m, 11H, Ar-H Bn, H-4), 7.58 (d, 2H, J 8.1, H-3', H-5'), 8.14 (s, 1H, H-6), 8.17 (d, 2H, J 8.1, H-2', H-6').

δ_C (100 MHz, $CDCl_3$): 51.0 (CH_2-Bn), 73.1 (CH), 106.2 (CH, Ar-3), 123.6 (q, Ar-5), 126.8 (CH, Ar-3', Ar-5'), 126.9 (CH, Ar-*p*-Bn), 127.0 (CH, Ar-*o*-Bn), 127.1 (CH, Ar-2', Ar-6'), 128.6 (CH, Ar-*m*-Bn), 136.3 (q, Ar-Bn), 137.9 (CH, Ar-4), 146.9 (CH, Ar-6), 147.0 (q, Ar-4'), 150.7 (q, Ar-1'), 158.7 (q, Ar-2).

ν_{max} (ATR)/cm^{-1}: 3381 (OH), 3028, 2903, 1733, 1558, 1594, 1451, 1343, 1341, 1202, 1072, 924, 841, 725.

HRMS: (m/z ESI^+): Found: 426.1815 ($M^+ + H$, $C_{26}H_{24}N_3O_3$ Requires: 426.1818).

5-[(4-Amino)benzyl]-2-[(*N,N*-dibenzyl)amino]pyridine 92



To a solution of compound **91** (3.0 g, 7.05 mmol) in EtOH (25 mL) was added 10% Pd/C (900 mg, 30 wt. %). The mixture was stirred under an atmosphere of hydrogen (6 atm) for 24 h. It was then diluted with CH_3OH (30 mL), filtered and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography using gradient elution of CH_2Cl_2:CH_3OH giving title compound **92** (1.2 g, 45%) as an orange oil.

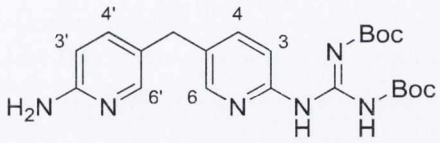
δ_H (400 MHz, $CDCl_3$): 3.70 (s, 2H, CH_2), 4.76 (s, 4H, CH_2-Bn), 6.39 (d, 1H, J 8.4, H-3), 6.61 (d, 2H, J 9.0, H-2',H-6'), 7.00 (d, 2H, J 9.0, H-3', H-5'), 7.13-7.23 (m, 6H, H-Ar Bn, H-4), 7.23-7.32 (m, 5H, H-Ar Bn), 8.05 (d, 1H, ^{4}J 2.8, H-6).

δ_C (100 MHz, $CDCl_3$): 37.2 (CH_2), 51.0 (CH_2-Bn), 105.8 (CH, Ar-3), 115.4 (CH, Ar-2', Ar-6'), 125.3 (q, Ar-5), 126.9 (CH, Ar-*p*-Bn), 127.1 (CH, Ar-*o*-Bn), 128.5 (CH, Ar-*m*-Bn), 129.5 (CH, Ar-3', Ar-5'), 131.9 (q, Ar-4'), 138.0 (q, Ar-Bn), 138.6 (CH, Ar-4), 147.4 (CH, Ar-6), 148.7 (q, Ar-1'), 157.1 (q, Ar-2).

ν_{max} (ATR)/cm^{-1}: 3026 (NH), 2916, 2891, 1670, 1557, 1451, 1359, 1238, 1202, 1169, 1027, 951, 807, 771.

HRMS: (m/z ESI$^+$): Found: 380.2125 (M^+ + H, $C_{26}H_{26}N_3$ Requires: 380.2127).

6-Amino-6'-[2,3-Di(*tert*-butoxycarbonyl)guanidino]dipyridinyl-3-methane 94a



Following Method L, to a solution of compound **82** (3.0 eq., 324 mg, 1.62 mmol), compound **66** (1.0 eq., 150 mg, 0.54 mmol) and NEt_3 (3.0 eq., 250 µL, 1.62 mmol) in DMF:CH_2Cl_2 (1:1, 6 mL) at 0 °C was added $HgCl_2$ (1.1 eq., 161 mg, 0.59 mmol). The reaction was then left to stir at RT overnight until completion, as adjudged by TLC analysis. Usual work up and purification afforded compound **94a** (101 mg, 42%) as a white crystalline solid.

Mp: 103-109 °C.

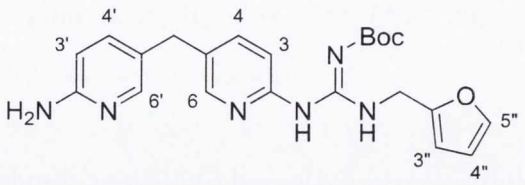
δ_H (400 MHz, $CDCl_3$): 1.46 (s, 18H, CH_3-Boc), 3.65 (s, 2H, CH_2), 4.47 (br s, 4H, NH_2), 6.43 (d, 1H, J 8.4, H-3'), 7.10 (m, 1H, H-4'), 7.47 (m, 1H, H-4), 7.89 (s, 1H, H-6'), 8.09 (s, 1H, H-6), 8.21 (br s, 1H, H-3), 10.78 (br s, 1H, NH), 11.47 (br s, 1H, NH).

δ_C (100 MHz, $CDCl_3$): 28.2 (CH_3-Boc), 34.8 (CH_2), 83.2 (q, Boc), 109.2 (CH, Ar-3'), 115.6 (CH, Ar-3), 124.4 (q, Ar-5, Ar-5'), 138.7 (CH, Ar-4'), 138.8 (CH, Ar-4), 147.2 (CH, Ar-6'), 147.6 (CH, Ar-6), 152.1 (q, C=O), 153.4 (q, C=O), 156.7 (q, Ar-2, Ar-2'), 163.4 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3350 (NH), 2979, 1720, 1624, 1553, 1500, 1391, 1369, 1303, 1230, 1105, 1026, 810, 770.

HRMS: (m/z ESI$^+$): Found: 443.2411 (M^+ + H, $C_{22}H_{31}N_6O_4$ Requires: 443.2407).

6-Amino-6'-[2-(*tert*-butoxycarbonyl)-3-(furanyl-2-methyl)guanidino]dipirydinyl-3-methane 94b



Following Method L, to a solution of compound **82** (3.0 eq., 375 mg, 1.86 mmol), compound **71a** (1.0 eq., 160 mg, 0.62 mmol) and NEt_3 (3.0 eq., 300 μL, 1.86 mmol) in DMF:CH_2Cl_2 (1:1, 6 mL) at 0 °C was added $HgCl_2$ (1.1 eq., 186 mg, 0.68 mmol). The reaction was then left to stir at RT overnight until completion, as adjudged by TLC analysis. Usual work up and purification afforded compound **94b** (112 mg, 43%) as a clear gum.

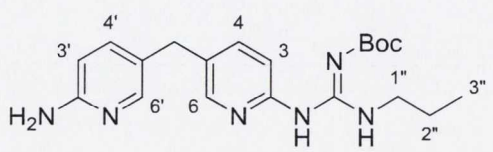
δ_H (400 MHz, $CDCl_3$): 1.49 (s, 9H, CH_3-Boc), 3.72 (s, 2H, CH_2), 4.67 (d, 2H, J 5.1, CH_2), 6.24 (d, 1H, J 7.1, H-3"), 6.30 (app. d, 1H, J 7.1, H-4"), 6.43 (d, 1H, J 8.0, H-3'), 6.74 (d, 1H, J 8.0, H-3), 7.15 (d, 1H, J 8.0, H-4'), 7.40 (m, 2H, H-4, H-5"), 7.89 (s, 1H, H-6'), 7.97 (s, 1H, H-6), 10.45 (br s, 1H, NH), 12.03 (br s, 1H, NH).

δ_C (100 MHz, $CDCl_3$): 28.1 (CH_3-Boc), 34.5 (CH_2), 38.1 (CH_2), 78.7 (q, Boc), 107.0 (CH, Ar-3"), 108.7 (CH, Ar-4"), 110.3 (CH, Ar-3'), 113.1 (CH, Ar-3), 130.3 (q, Ar-5, Ar-5'), 138.2 (CH, Ar-4'), 138.9 (CH, Ar-4), 142.0 (CH, Ar-5"), 145.0 (CH, Ar-6'), 145.4 (CH, Ar-6), 148.1 (q, Ar-2"), 151.4 (q, C=O), 157.1 (q, Ar-2 or Ar-2'), 157.3 (q, Ar-2 or Ar-2'), 164.3 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3345 (NH), 2976, 2929, 1712, 1560, 1475, 1444, 1378, 1134, 1058, 917, 871, 806, 753, 700.

HRMS: (m/z ESI^+): Found: 423.2149 (M^+ + H, $C_{22}H_{27}N_6O_3$ Requires: 423.2145).

6-Amino-6'-[2-(*tert*-butoxycarbonyl)-3-(propyl)guanidino]dipyridinyl-3-methane 94c



Following Method L, to a solution of compound **82** (3.0 eq., 330 mg, 1.65 mmol), compound **71c** (1.0 eq., 120 mg, 0.55 mmol) and NEt_3 (3.0 eq., 250 µL, 1.65 mmol) in DMF:CH_2Cl_2 (1:1, 6 mL) at 0 °C was added $HgCl_2$ (1.1 eq., 163 mg, 0.61 mmol). The reaction was then left to stir at RT overnight until completion, as adjudged by TLC analysis. Usual work up and purification afforded compound **94c** (79 mg, 38%) as a yellow gum.

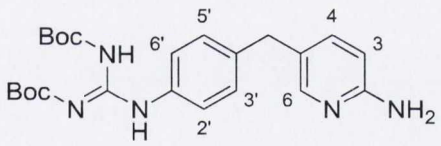
δ_H (400 MHz, $CDCl_3$): 1.00 (m, 3H, CH_3-3"), 1.51 (s, 9H, CH_3-Boc), 1.58 (m, 2H, CH_2-2"), 3.39 (m, 2H, CH_2-1"), 3.68 (s, 2H, CH_2), 6.41 (d, 1H, J 8.4, H-3'), 6.71 (d, 1H, J 8.5, H-3), 7.14 (m, 1H, H-4'), 7.34 (m, 1H, H-4), 7.87 (s, 1H, H-6'), 7.95 (s, 1H, H-6), 10.09 (br s, 1H, NH), 11.99 (br s, 1H, NH).

δ_C (100 MHz, $CDCl_3$): 11.2 (CH_3-3"), 21.0 (CH_2-2"), 28.1 (CH_3-Boc), 34.5 (CH_2), 42.5 (CH_2-1"), 78.5 (q, Boc), 108.7 (CH, Ar-3'), 113.1 (CH, Ar-3), 130.1 (q, Ar 5, Ar-5'), 138.2 (CH, Ar-4'), 138.8 (CH, Ar-4), 145.6 (CH, Ar-6'), 147.5 (CH, Ar-6), 151.5 (q, C=O), 157.2 (q, Ar-2 or Ar-2'), 157.4 (q, Ar-2 or Ar-2'), 164.1 (q, C=N).

ν_{max} (ATR)/cm^{-1}:3349 (NH), 3197 (NH), 2963, 2930, 1636, 1591, 1493, 1473, 1347, 1245, 1232, 1105, 1024, 944, 805, 736.

HRMS: (m/z ESI^+): Found: 385.2354 (M^+ + H, $C_{20}H_{29}N_6O_2$ Requires: 385.2352).

2-Amino-5-{4-[2,3-(di-*tert*-butoxycarbonyl)guanidino]benzyl} pyridine 94d



Following Method L, to a solution of compound **83** (3.0 eq., 120 mg, 0.60 mmol), compound **66** (1.0 eq., 55 mg, 0.20 mmol) and NEt_3 (3.0 eq., 111 µL, 0.60 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added $HgCl_2$ (1.1 eq., 90 mg, 0.22 mmol). The reaction was then left to stir at RT overnight until completion, as adjudged by TLC analysis. Usual work up and purification afforded compound **94d** (74 mg, 83%) as a thick clear gum.

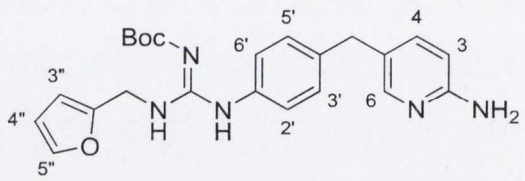
δ_H (400 MHz, $CDCl_3$): 1.43 (s, 9H, CH_3-Boc), 1.51 (s, 9H, CH_3-Boc), 3.70 (s, 2H, CH_2), 6.43 (d, 1H, J 8.4, H-3), 7.00 (d, 2H, J 8.3, H-2', H-6'), 7.14 (d, 2H, J 8.3, H-3', H-5'), 7.17 (dd, 1H, ^{3}J 8.4, ^{4}J 2.4, H-4), 7.66 (d, 1H, ^{4}J 2.4, H-6), 10.21 (br s, 1H, NH), 11.62 (br s, 1H, NH).

δ_C (100 MHz, $CDCl_3$): 28.1 (CH_3-Boc), 37.4 (CH_2), 79.6 (q, Boc), 83.7 (q, Boc), 110.0 (CH, Ar-3), 123.0 (CH, Ar-2', Ar-6'), 126.0 (q, Ar-5), 129.2 (CH, Ar-3', Ar-5'), 134.7 (q, Ar-4'), 136.9 (q, Ar-1'), 139.2 (CH, Ar-4), 146.4 (CH, Ar-6), 153.2 (q, C=O), 153.8 (q, C=O), 157.1 (q, Ar-2), 163.4 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3289 (NH), 2980, 1717, 1625, 1592, 1507, 1402, 1368, 1345, 1027, 958, 808, 767.

HRMS: (m/z ESI^+): Found: 442.2456 (M^+ + H, $C_{23}H_{32}N_5O_4$ Requires: 442.2454).

2-Amino-5-{4-[2-(*tert*-butoxycarbonyl)-3-(furanyl-2-methyl)guanidino]benzyl}pyridine 94e



Following Method L, to a solution of compound **83** (3.0 eq., 133 mg, 0.66 mmol), compound **71a** (1.0 eq., 56 mg, 0.22 mmol) and NEt_3 (3.0 eq., 120 µL, 0.66 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added $HgCl_2$ (1.1 eq., 71 mg, 0.24 mmol). The reaction was then left to stir at RT overnight until completion, as adjudged by TLC analysis. Usual work up and purification afforded compound **94e** (59 mg, 64%) as a yellow gum.

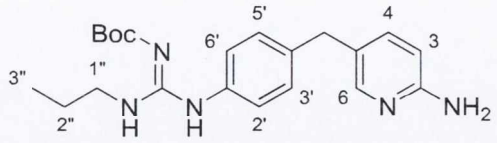
δ_H (400 MHz, $CDCl_3$): 1.47 (s, 9H, CH_3-Boc), 3.72 (s, 2H, CH_2), 4.53 (br s, 4H, CH_2, NH_2), 6.16 (br s, 1H, H-3"), 6.26 (br s, 1H, H-4"), 6.42 (d, 1H, J 8.4, H-3), 6.98-7.15 (m, 4H, H-2', H-3', H-5', H-6'), 7.19 (dd, 1H, ^{3}J 8.4, ^{4}J 2.4, H-4), 7.29 (br s, 1H, H-5"), 7.88 (d, 1H, ^{4}J 2.4, H-6).

δ_C (100 MHz, $CDCl_3$): 28.1 (CH_3-Boc), 37.5 (CH_2), 38.2 (CH_2) 79.6 (q, Boc), 106.9 (CH, Ar-3"), 107.3 (CH, Ar-4"), 110.3 (CH, Ar-3), 122.8 (q, Ar-5), 125.6 (q, Ar-4'), 130.0 (CH, Ar-2', Ar-6', Ar-3', Ar-5'), 134.7 (q, Ar-1'), 138.7 (CH, Ar-4), 142.1 (CH, Ar-5"), 145.1 (q, Ar-2"), 147.4 (CH, Ar-6), 157.0 (q, C=O), 158.4 (q, Ar-2), 164.3 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3359 (NH), 3202 (NH), 2975, 1594, 1497, 1365, 1282, 1259, 1147, 1101, 934, 853, 805, 734.

HRMS: (m/z ESI$^+$): Found: 422.2203 (M^+ + H, $C_{23}H_{28}N_5O_3$ Requires: 422.2192).

2-Amino-5-{4-[2-(*tert*-butoxycarbonyl)-3-(propyl)guanidino]benzyl}pyridine 94f



Following Method L, to a solution of compound **83** (3.0 eq., 150 mg, 0.69 mmol), compound **71c** (1.0 eq., 55 mg, 0.23 mmol) and NEt_3 (3.0 eq., 110 µL, 0.69 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added $HgCl_2$ (1.1 eq., 82 mg, 0.25 mmol). The reaction was then left to stir at RT overnight until completion, as adjudged by TLC analysis. Usual work up and purification afforded compound **94f** (56 mg, 63%) as a yellow gum.

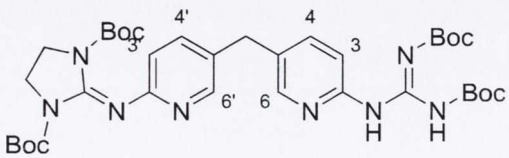
δ_H (400 MHz, $CDCl_3$): 0.88 (m, 3H, CH_3-3"), 1.20 (m, 2H, CH_2-2"), 1.51 (s, 9H, CH_3), 3.31 (m, 2H, CH_2-1"), 3.70 (s, 2H, CH_2), 4.52 (br s, 2H, NH_2), 6.40 (d, 1H, J 8.4, H-3), 7.01 (br s, 2H, H-2', H-6'), 7.11 (m, 2H, H-3', H-5'), 7.18 (dd, 1H, ^{3}J 8.4, ^{4}J 2.4, H-4), 7.88 (d, 1H, ^{4}J 2.4, H-6).

δ_C (100 MHz, $CDCl_3$): 11.8 (CH_3-3"), 22.6 (CH_2-2"), 28.1 (CH_3-Boc), 37.4 (CH_2), 42.7 (CH_2-1"), 81.7 (q, Boc), 108.8 (CH, Ar-3), 125.7 (CH, Ar-2', Ar-6'), 129.0 (q, Ar-5), 129.9 (CH, Ar-3', Ar-5'), 132.9 (q, Ar-4'), 138.5 (CH, Ar-4), 144.0 (q, Ar-1'), 147.5 (CH, Ar-6), 157.1 (q, Ar-2), 158.3 (q, C=O), 165.1 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3360 (NH), 2999, 2975, 1607, 1500, 1476, 1365, 1282, 1259, 1189, 1134, 934, 853, 810, 734.

HRMS: (m/z ESI$^+$): Found: 384.2404 (M^+ + H, $C_{21}H_{30}N_5O_2$ Requires: 384.2400).

6-(Di(*tert*-butoxycarbonyl)-2-iminoimidazolidine)-6'-[2,3-di(*tert*-butoxycarbonyl)guanidino]dipirydinyl-3-methane 95a



Following Method M, to a solution of compound **94a** (1.0 eq., 100 mg, 0.23 mmol), compound **77** (1.0 eq., 69 mg, 0.23 mmol) and NEt_3 (3.0 eq., 130 µL, 0.69 mmol) in CH_2Cl_2 (2 mL) at 0 °C was added $HgCl_2$ (1.1 eq., 81 mg, 0.25 mmol). The reaction was then left to stir at RT overnight. Usual work up and purification afforded compound **95a** (101 mg, 62%) as an off-white crystalline solid.

Mp: 86-90 °C.

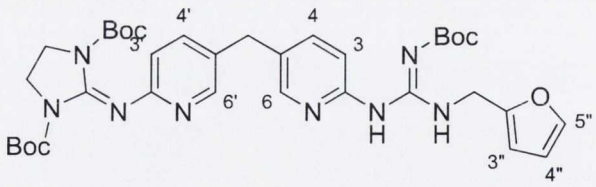
δ_H (400 MHz, $CDCl_3$): 1.29 (s, 18H, CH_3-Boc),1.47 (s, 18H, CH_3-Boc), 3.78 (br s, 6H, CH_2), 6.89 (d, 1H, J 8.3, H-3'), 7.32 (m, 1H, H-4'), 7.48 (m, 1H, H-4), 7.99 (s, 1H, H-6'), 8.07 (s, 1H, H-6), 8.21 (br s, 1H, H-3), 10.76 (br s, 1H, NH), 11.46 (br s, 1H, NH).

δ_C (100 MHz, $CDCl_3$): 27.9 (CH_3-Boc), 34.9 (CH_2), 42.7 (CH_2-Imid), 82.5 (q, Boc), 108.8 (CH, Ar-3'), 118.4 (CH, Ar-3), 128.7 (q, Ar-5'), 131.1 (q, Ar-5), 137.8 (CH, Ar-4'), 138.3 (CH, Ar-4), 147.2 (CH, Ar-6'), 147.7 (CH, Ar-6), 149.9 (q, Ar-2'), 152.9 (q, C=O), 159.2 (q, Ar-2), 163.4 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3400 (NH), 2980, 1719, 1626, 1545, 1474, , 1367, 1288, 1232, 1104, 1058, 953, 806, 766.

HRMS: (m/z ESI^+): Found: 711.3828 ($M^+ + H$, $C_{35}H_{51}N_8O_8$ Requires: 711.3830).

6-[1,3-Di(*tert*-butoxycarbonyl)-2-iminoimidazolidino]-6'-[2-(*tert*-butoxycarbonyl)-3-(furanyl-2-methyl)guanidino]dipirydinyl-3-methane 95b



Following Method M, to a solution of compound **94b** (1.0 eq., 75 mg, 0.17 mmol), compound **77** (1.0 eq., 59 mg, 0.17 mmol) and NEt_3 (3.0 eq., 100 µL, 0.53 mmol) in CH_2Cl_2 (2 mL) at 0 °C was added $HgCl_2$ (1.1 eq., 59 mg, 0.19 mmol). The reaction was then left to stir at RT overnight. Usual work up and purification afforded compound **95b** (77 mg, 66%) as a white crystalline solid.

Mp: 81-84 °C.

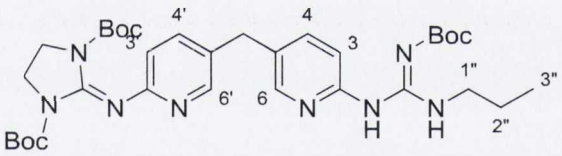
δ_H (400 MHz, $CDCl_3$): 1.31 (s, 9H, CH_3-Boc), 1.51 (s, 18H, CH_3-Boc), 3.75 (s, 2H, CH_2), 3.80 (s, 4H, CH_2), 4.69 (d, 2H, J 5.0, CH_2), 6.24 (d, 1H, J 7.1, H-3"), 6.30 (d, 1H, J 7.1, H-4"), 6.77 (d, 1H, J 8.0, H-3'), 6.95 (d, 1H, J 8.0, H-3), 7.36 (m, 3H, H-4', H-4, H-5"), 7.95 (s, 1H, H-6'), 8.01 (s, 1H, H-6), 10.45 (br s, 1H, NH), 12.02 (br s, 1H, NH).

δ_C (100 MHz, $CDCl_3$): 27.8 (CH_3-Boc), 28.1 (2 CH_3-Boc), 34.6 (CH_2), 38.1 (CH_2), 42.8 (CH_2-Imid), 78.7 (q, Boc), 82.6 (2 q, Boc), 107.0 (CH, Ar-3"), 110.3 (CH, Ar-4"), 112.9 (CH, Ar-3'), 118.4 (CH, Ar-3), 128.4 (q, Ar-5'), 130.3 (q, Ar-5), 138.0 (CH, Ar-4'), 138.8 (CH, Ar-4), 142.0 (CH, Ar-5"), 145.4 (CH, Ar-6'), 147.2 (CH, Ar-6), 148.0 (q, Ar-2"), 151.2 (q, C=O), 151.4 (q, C=O), 157.1 (q, Ar-2 or 2'), 159.3 (q, Ar-2 or 2'), 164.3 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3600 (NH), 2980, 2900, 1712 (C=O), 1637, 1593, 1474, 1366, 1288, 1233, 1146, 972, 804, 732.

HRMS: (m/z ESI^+): Found: 691.3573 (M^+ + H, $C_{35}H_{47}N_8O_7$ Requires: 691.3568).

6-[Di(*tert*-butoxycarbonyl)-2-iminoimidazolidino]-6'-[2-(*tert*-butoxycarbonyl)-3-(propyl)guanidino]dipirydinyl-3-methane 95c



Following Method M, to a solution of compound **94c** (1.0 eq., 80 mg, 0.21 mmol), compound **77** (1.0 eq., 65 mg, 0.21 mmol) and NEt_3 (3.0 eq., 110 µL, 0.62 mmol) in CH_2Cl_2 (2 mL) at 0 °C was added $HgCl_2$ (1.1 eq., 70 mg, 0.23 mmol). The reaction was then left to stir at RT overnight. Usual work up and purification afforded compound **95c** (87 mg, 63%) as an off-white crystalline solid.

Mp: 93-97 °C.

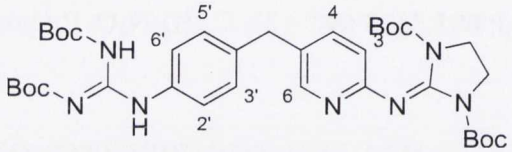
δ_H (400 MHz, $CDCl_3$): 0.95 (m, 3H, CH_3-3"), 1.31 (s, 18H, CH_3-Boc), 1.51 (s, 9H, CH_3-Boc), 1.59 (m, 2H, CH_2-2"), 3.40 (m, 2H, CH_2-1"), 3.73 (s, 2H, CH_2), 3.80 (s, 4H, CH_2), 6.70 (d, 1H, J 8.4, H-3'), 6.91 (d, 1H, J 8.5, H-3), 7.36 (m, 2H, H-4', H-4), 7.96 (s, 1H, H-6'), 7.99 (s, 1H, H-6), 10.10 (br s, 1H, NH), 12.00 (br s, 1H, NH).

δ_C (100 MHz, $CDCl_3$): 11.5 (CH_3-3"), 22.5 (CH_2-2"), 27.8 (CH_3-Boc), 28.1 (2 CH_3-Boc), 34.9 (CH_2), 42.6 (CH_2-1"), 42.8 (CH_2-Imid), 78.2 (q, Boc), 82.1 (2 q, Boc), 113.0 (CH, Ar-3'), 118.5 (CH, Ar-3), 128.4 (q, Ar-5'), 130.0 (q, Ar 5), 137.9 (CH, Ar-4'), 138.7 (CH, Ar-4), 145.3 (CH, Ar-6'), 147.2 (CH, Ar-6), 149.6 (q, C=O), 151.5 (q, C=O), 157.4 (q, Ar-2 or Ar-2'), 159.3 (q, Ar-2 or Ar-2'), 164.3 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3102 (NH), 2950, 2896, 1739, 1379, 1231, 1151, 955.

HRMS: (m/z ESI^+): Found: 653.3376 (M^+ + H, $C_{33}H_{49}N_8O_6$ Requires: 653.3775).

2-[Di(*tert*-butoxycarbonyl)-2-iminoimidazolidino]-5-{4-[2,3-di(*tert*-butoxycarbonyl)guanidino]benzyl}pyridine 95d



Following Method M, to a solution compound **94d** (1.0 eq., 120 mg, 0.27 mmol), compound **77** (1.0 eq., 81 mg, 0.27 mmol) and NEt_3 (3.0 eq., 122 µL, 0.81 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added $HgCl_2$ (1.1 eq., 80 mg, 0.29 mmol). The reaction was then left to stir at RT overnight. Usual work up (without further purification) afforded compound **95d** (171 mg, 89%) as a yellow gum.

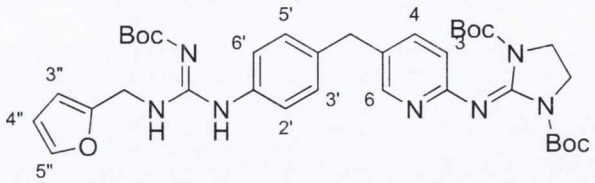
δ_H (400 MHz, $CDCl_3$): 1.46 (s, 18H, CH_3-Boc), 1.50 (s, 18H, CH_3-Boc), 3.70 (s, 2H, CH_2), 3.81 (s, 4H, CH_2), 6.91 (d, 1H, J 8.4, H-3), 7.07 (d, 2H, J 8.3, H-2', H-6'), 7.36 (dd, 1H, ^{3}J 8.4, ^{4}J 2.4, H-4), 7.47 (d, 2H, J 8.3, H-3', H-5'), 8.02 (d, 1H, ^{4}J 2.4, H-6), 10.22 (br s, 1H, NH), 11.60 (br s, 1H, NH).

δ_C (100 MHz, $CDCl_3$): 27.9 (CH_3-Boc), 28.0 (CH_3-Boc), 39.2 (CH_2), 47.5 (CH_2-Imid), 82.2 (q, Boc), 83.1 (q, Boc), 118.3 (CH, Ar-3), 122.2 (CH, Ar-2', Ar-6'), 126.0 (q, Ar-5), 129.7 (CH, Ar-3', Ar-5'), 134.9 (q, Ar-4'), 137.1 (q, Ar-1'), 138.1 (CH, Ar-4), 149.7 (CH, Ar-6), 153.2 (q, C=O), 153.3 (q, C=O), 158.9 (q, Ar-2), 163.4 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3292 (NH), 2980, 2929, 1737, 1601, 1476, 1453, 1392, 1340, 1029, 977, 880, 810, 767.

HRMS: (m/z ESI^+): Found: 710.3890 (M^+ + H, $C_{36}H_{52}N_7O_8$ Requires: 710.3877).

2-[1,3-(*tert*-butoxycarbonyl)-2-iminoimidazolidino]-5-{4-[2-(*tert*-butoxycarbonyl)-3-(furanyl-2-methyl)guanidino]benzyl}pyridine 95e



Following Method M, to a solution compound **94e** (1.0 eq., 38 mg, 0.09 mmol), compound **77** (1.0 eq., 27 mg, 0.09 mmol) and NEt_3 (3.0 eq., 50 µL, 0.27 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added $HgCl_2$ (1.1 eq., 270 mg, 0.99 mmol). The reaction was then left to stir at RT overnight. Usual work up (without further purification) afforded compound **95e** (62 mg, 99%) as a yellow gum.

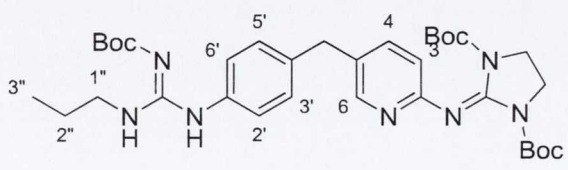
δ_H (400 MHz, $CDCl_3$): 1.47 (s, 9H, CH_3-Boc), 1.50 (s, 18H, CH_3-Boc), 3.81 (br s, 6H, CH_2), 4.53 (br s, 2H, CH_2), 6.15 (br s, 1H, H-3"), 6.26 (br s, 1H, H-4"), 6.90 (d, 1H, J 8.4, H-3), 7.00-7.15 (m, 4H, H-2', H-3', H-5', H-6'), 7.31 (dd, 1H, ^{3}J 8.4, ^{4}J 2.4, H-4), 7.34 (br s, 1H, H-5"), 7.99 (d, 1H, ^{4}J 2.4, H-6).

δ_C (100 MHz, $CDCl_3$): 27.1 (CH_3-Boc), 28.1 (2 CH_3-Boc), 37.9 (CH_2), 42.7 (CH_2-Imid), 82.9 (q, Boc), 107.4 (CH, Ar-3"), 110.1 (CH, Ar-4"), 118.1 (CH, Ar-3), 125.2 (q, Ar-5), 128.9 (q, Ar-4'), 130.2 (CH, Ar-2', Ar-3', Ar-5', Ar-6'), 135.5 (q, Ar-1'), 138.1 (CH, Ar-4), 142.0 (CH, Ar-5"), 147.3 (q, Ar-2"), 149.4 (CH, Ar-6), 156.4 (q, C=O), 158.2 (q, Ar-2), 164.6 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3026 (NH), 2920, 1603, 1556, 1451, 1416, 1393, 1239, 1201, 1133, 1074, 1000, 950, 808.

HRMS: (m/z ESI$^+$): Found: 690.3583 (M^+ + H, $C_{36}H_{48}N_7O_7$ Requires: 690.3615).

2-[Di(*tert*-butoxycarbonyl)-2-iminoimidazolidino]-5-{4-[2-(*tert*-butoxycarbonyl)-3-(propyl)guanidino]benzyl}pyridine 95f



Following Method M, to a solution compound **94f** (1.0 eq., 45 mg, 0.12 mmol), compound **77** (1.0 eq., 36 mg, 0.12 mmol) and NEt_3 (3.0 eq., 72 μL, 0.36 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added $HgCl_2$ (1.1 eq., 76 mg, 0.13 mmol). The reaction was then left to stir at RT overnight. Usual work up (without further purification) afforded compound **95f** (76 mg, 97%) as a yellow-orange gum.

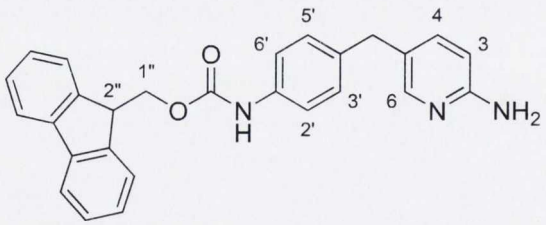
δ_H (400 MHz, $CDCl_3$): 0.86 (m, 3H, CH_3-3"), 1.29 (s, 9H, CH_3-Boc), 1.51 (s, 20H, CH_3-Boc, CH_2-2"), 3.27 (m, 2H, CH_2-1"), 3.68 (s, 2H, CH_2), 3.75 (m, 2H, CH_2), 3.80 (m, 2H, CH_2), 6.87 (m, 1H, H-3), 6.96 (m, 2H, H-2', H-6'), 7.07 (m, 2H, H-3', H-5'), 7.40 (m, 1H, H-4), 8.03 (br s, 1H, H-6).

δ_C (100 MHz, $CDCl_3$): 11.3 (CH_3-3"), 22.4 (CH_2-2"), 27.9 (CH_3-Boc), 28.1 (2 CH_3-Boc), 37.5 (CH_2), 39.5 (CH_2-Imid), 42.7 (CH_2-1"), 82.4 (q, Boc), 83.1 (2 q, Boc), 113.3 (CH, Ar-3), 125.3 (CH, Ar-2', Ar-6'), 129.0 (q, Ar-5), 130.1 (CH, Ar-3', Ar-5'), 134.1 (q, Ar-4'), 138.1 (CH, Ar-4), 145.4 (q, Ar-1'), 149.7 (CH, Ar-6), 157.1 (q, Ar-2), 155.7 (q, C=O), 156.0 (q, C=O), 165.8 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3110 (NH), 2985, 2920, 1605, 1556, 1445, 1419, 1393, 1239, 1201, 1133, 1074, 969, 871.

HRMS: (m/z ESI$^+$): Found: 652.3846 (M^+ + H, $C_{34}H_{50}N_7O_6$ Requires: 652.3823).

2-Amino-5-[4-(*N*-fluorenylmethyloxycarbonyl)aminobenzyl]pyridine 97



Pyridine (2.0 eq., 130 μL, 1.6 mmol) was added to a solution of compound **83** (1.0 eq., 160 mg, 0.8 mmol) and fluorenylmethyloxycarbonyl chloride (1.0 eq., 206 mg, 0.8 mmol) in anhydrous THF (4 mL) under argon. The resulting mixture was stirred under argon for 3 h. The reaction was concentrated under reduced pressure, and purified by silica gel chromatography, eluting in CH_2Cl_2:CH_3OH, affording compound **97** (220 mg, 65%) as a white solid.

Mp: 121-123 °C.

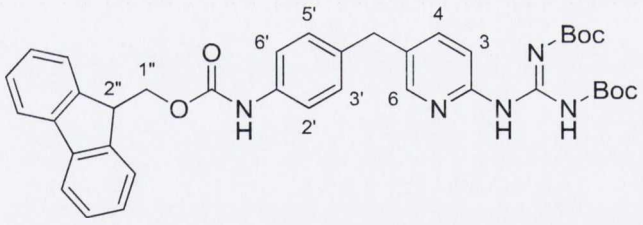
δ_H (400 MHz, $CDCl_3$): 3.70 (s, 2H, CH_2), 4.23 (t, 1H, J 6.6, CH-2"), 4.51 (d, 2H, J 6.6, CH_2-1"), 6.40 (d, 1H, J 8.4, H-3), 7.04 (d, 2H, J 8.3, H-3', H-5'), 7.14 (d, 2H, J 7.4, H-Ar), 7.19 (dd, 1H, ^{3}J 8.4, ^{4}J 2.4, H-4), 7.27 (t, 2H, J 7.4, H-Ar), 7.38 (t, 2H, J 7.4, H-Ar), 7.58 (t, 2H, J 7.4, H-Ar), 7.75 (d, 2H, J 8.3, H-2', H-6'), 7.91 (d, 1H, ^{4}J 2.4, H-6).

δ_C (100 MHz, $CDCl_3$): 34.7 (CH_2), 47.1 (CH_2-1"), 66.6 (CH-2"), 108.8 (CH, Ar-3), 119.9 (CH, Ar-3', Ar-5'), 124.9 (CH, 2 Ar), 126.4 (q, Ar-5), 127.4 (CH, 4 Ar), 127.7 (CH, 2 Ar), 129.4 (CH, Ar-2', Ar-6'), 131.2 (q, Ar-4'), 136.3 (q, Ar-1'), 138.5 (CH, Ar-4), 141.4 (q, 2 Ar), 143.7 (q, 2 Ar), 147.2 (CH, Ar-6), 153.3 (q, C=O), 156.9 (q, Ar-2).

ν_{max} (ATR)/cm^{-1}: 3367 (NH), 2981, 1708 (C=O), 1616, 1525, 1498, 1313, 1105, 1050, 819, 757.

HRMS (m/z ESI^+): Found: 422.1866 (M^+ + H, $C_{27}H_{24}N_3O_2$ Requires: 422.1869).

2-[2,3-di(*tert*-butoxycarbonyl)guanidine]-5-[4-(*N*-fluorenylmethyloxycarbonyl)aminobenzyl]pyridine 98a



Following Method M, to a solution compound **97** (1.0 eq., 90 mg, 0.22 mmol), compound **66** (1.0 eq., 63 mg, 0.22 mmol) and NEt_3 (3.0 eq., 90 µL, 0.66 mmol) in CH_2Cl_2 (4 mL) at 0 °C was added $HgCl_2$ (1.1 eq., 67 mg, 0.24 mmol). The reaction was then left to stir at RT overnight. Usual work up and purification afforded compound **98a** (58 mg, 40%) as a yellow gum.

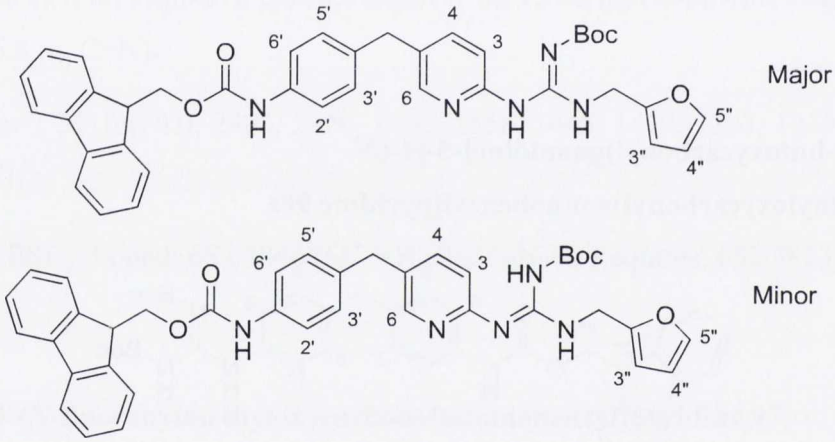
δ_H (400 MHz, $CDCl_3$) : 1.47 (s, 18H, CH_3), 3.81 (s, 2H, CH_2), 4.27 (t, 1H, J 6.6, CH-2"), 4.45 (d, 2H, J 6.6, CH_2-1"), 7.01 (d, 2H, J 8.3, H-3', H-5'), 7.25 (m, 4H, H-Ar), 7.35 (m, 2H, H-Ar), 7.39 (dd, 1H, ^{3}J 8.4, ^{4}J2.4, H-4), 7.55 (m, 2H, H-Ar), 7.71 (d, 2H, J 8.3, H-2', H-6'), 8.01 (d, 1H, ^{4}J 2.4, H-6), 8.10 (br s, 1H, H-3), 10.78 (br s, 1H, NH), 11.49 (br s, 1H, NH).

δ_C (100 MHz, $CDCl_3$): 28.3 (CH_3-Boc), 37.7 (CH_2), 46.8 (CH_2-1"), 66.4 (CH-2"), 79.5 (q, Boc), 119.0 (CH, Ar-3), 119.9 (CH, Ar-3', Ar-5'), 124.9 (CH, 2 Ar), 127.5 (CH, 4 Ar), 127.5 (CH, Ar-2', Ar-6'), 129.0 (q, Ar-5), 129.3 (CH, 2 Ar), 132.4 (q, Ar-4'), 136.1 (q, Ar-1'), 138.4 (CH, Ar-4), 141.1 (q, 2 Ar), 143.7 (q, 2 Ar), 147.7 (CH, Ar-6), 149.9 (q, Ar-2), 152.8 (q, C=O), 153.4 (q, C=O), 160.1 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3257 (NH), 2965, 1721 (C=O), 1625, 1391, 1257, 1225, 1149, 1055, 1023, 758, 738.

HRMS (m/z ESI^+): Found: 664.3143 ($M^+ + H$, $C_{38}H_{42}N_5O_6$ Requires: 664.3135).

2-[2-(*tert*-Butoxycarbonyl)-3-(furanyl-2-methyl)guanidino]-5-{4-[(9*H*-fluoren-9-yl)methylcarbamoyl]benzyl}pyridine 98b



Following Method M, to a solution compound **97** (1.0 eq., 100 mg, 0.24 mmol), compound **71a** (1.0 eq., 62 mg, 0.24 mmol) and NEt_3 (3.0 eq., 60 µL, 0.72 mmol) in CH_2Cl_2 (4 mL) at 0 °C was added $HgCl_2$ (1.1 eq., 71 mg, 0.26 mmol). The reaction was then left to stir at RT overnight. Usual work up and purification afforded compound **98b** (75 mg, 48%) as a light orange gum.

δ_H (400 MHz, $CDCl_3$): *Major isomer:* 1.51 (s, 9H, CH_3-Boc), 3.83 (s, 2H, CH_2), 4.23 (t, 1H, J 6.6, CH), 4.49 (d, 2H, J 6.6, CH_2), 4.70 (d, 2H, J 6.7, CH_2-Fur), 6.29 (m, 2H, H-3", H-4") 6.72 (d, 1H, J 8.4, H-3), 7.04 (d, 2H, J 8.3, H-3', H-5'), 7.29 (m, 4H, H-Ar), 7.37 (m, 4H, 2H-Ar, H-4, H-5"), 7.59 (m, 2H, H-Ar), 7.74 (d, 2H, J 8.3, H-2', H-6'), 7.99 (d, 1H, ^4J 2.4, H-6), 8.05 (br s, 1H, NH), 10.49 (br s, 1H, NH).

δ_H (400 MHz, $CDCl_3$): *Minor isomer:* 1.46 (s, 9H, CH_3-Boc), 3.83 (s, 2H, CH_2), 4.23 (t, 1H, J 6.6, CH), 4.46 (d, 2H, J 6.7, CH_2-Fur), 4.49 (d, 2H, J 6.6, CH_2), 6.23 (m, 2H, H-3", H-4"), 6.72 (d, 1H, J 8.4, H-3), 7.04 (m, 3H, H-3', H-5', NH), 7.29 (m, 4H, H-Ar), 7.37 (m, 4H, 2H-Ar, H-4, H-5"), 7.59 (m, 2H, H-Ar), 7.74 (d, 2H, J 8.3, H-2', H-6'), 7.99 (d, 1H, ^{4}J 2.4, H-6), 12.04 (br s, 1H, NH).

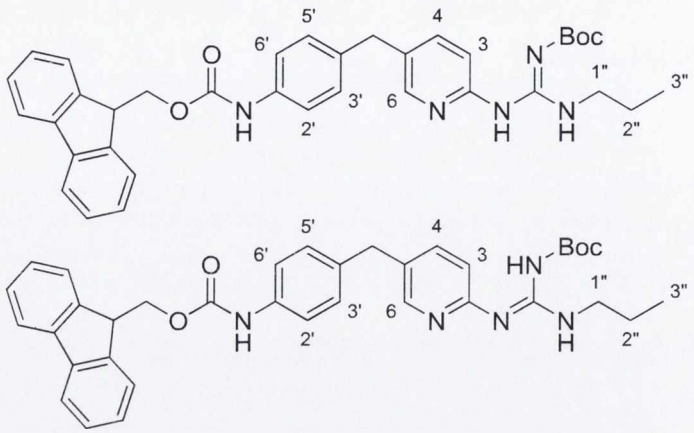
δ_C (100 MHz, $CDCl_3$): *Major isomer:* 28.4 (CH_3-Boc), 37.5 (CH_2), 38.0 (CH_2), 47.0 (CH_2-Fmoc), 66.8 (CH-Fmoc), 82.7 (q, Boc), 107.3 (CH, Ar-3"), 110.3 (CH, Ar-4"), 113.0 (CH, Ar-3), 119.3 (q, Ar-5), 119.9 (CH, Ar-3', Ar-5'), 124.9 (CH, 2 Ar), 127.0 (CH, 2 Ar), 127.3 (CH, 4 Ar), 129.1 (CH, Ar-2', Ar-6'), 130.6 (q, Ar-4'), 139.0 (CH, Ar-4), 141.3 (q, 2 Ar), 142.1 (CH, Ar-5"), 143.7 (q, 2 Ar), 145.5 (CH, Ar-6), 151.0 (q, Ar-5"), 151.4 (q, Ar-1'), 153.5 (q, C=O), 157.3 (q, Ar-2), 164.0 (q, C=N).

δ_C (100 MHz, $CDCl_3$): *Minor isomer:* 27.8 (CH_3-Boc), 37.5 (CH_2), 36.7 (CH_2), 47.0 (CH_2-Fmoc), 66.6 (CH-Fmoc), 78.6 (q, Boc), 107.3 (CH, Ar-3"), 110.3 (CH, Ar-4"), 113.0 (CH, Ar-3), 119.3 (q, Ar-5), 119.9 (CH, Ar-3', Ar-5'), 124.9 (CH, 2 Ar), 127.0 (CH, 2 Ar), 127.3 (CH, 4 Ar), 129.1 (CH, Ar-2', Ar-6'), 130.6 (q, Ar-4'), 139.0 (CH, Ar-4), 141.3 (q, 2 Ar), 142.1 (CH, Ar-5"), 143.7 (q, 2 Ar), 145.5 (CH, Ar-6), 151.0 (q, Ar-2"), 151.4 (q, Ar-1'), 153.5 (q, C=O), 157.3 (q, Ar-2), 164.0 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 2975 (NH), 2905, 1712, 1596, 1534, 1476, 1449, 1348, 1219, 1147 1113, 1095, 806, 736.

HRMS (m/z ESI$^+$): Found: 644.2875 (M^+ + H, $C_{38}H_{38}N_5O_5$ Requires: 644.2867).

2-[2-(*tert*-Butoxycarbonyl)-3-(propyl)guanidino]-5-{4-[(9*H*-fluoren-9-yl)methylcarbamoyl]benzyl}pyridine 98c



Following Method M, to a solution compound **97** (1.0 eq., 100 mg, 0.24 mmol), compound **71c** (1.0 eq., 52 mg, 0.24 mmol) and NEt_3 (3.0 eq., 60 µL, 0.72 mmol) in CH_2Cl_2 (4 mL) at 0 °C was added $HgCl_2$ (1.1 eq., 71 mg, 0.26 mmol). The reaction was then left to stir at RT overnight. Usual work up and purification afforded compound **98c** (70 mg, 48%) as a yellow gum.

δ_H (400 MHz, $CDCl_3$): *Major isomer:* 0.96 (m, 3H, CH_3-3"), 1.49 (s, 9H, CH_3-Boc), 1.60 (m, 2H, CH_2-2"), 3.44 (t, 2H, J 7.1, CH_2-1"), 3.83 (s, 2H, CH_2), 4.24 (t, 1H, J 6.6, CH), 4.49 (d, 2H, J 6.6, CH_2), 6.72 (d, 1H, J 8.4, H-3), 7.04 (d, 2H, J 8.3, H-3', H-5'), 7.28 (m, 4H, H-Ar), 7.36 (m, 3H, H-Ar, H-4), 7.56 (m, 2H, H-Ar), 7.74 (d, 2H, J 8.3, H-2', H-6'), 7.78 (br s, 1H, NH), 7.99 (d, 1H, ^{4}J 2.4, H-6), 10.78 (br s, 1H, NH).

δ_H (400 MHz, $CDCl_3$): *Minor isomer:* 0.91 (m, 3H, CH_3-3"), 1.43 (s, 9H, CH_3-Boc), 1.54 (m, 2H, CH_2-2"), 3.22 (t, 2H, J 7.1, CH_2-1"), 3.83 (s, 2H, CH_2), 4.24 (t, 1H, J 6.6, CH), 4.49 (d, 2H, J 6.6, CH_2), 6.72 (d, 1H, J 8.4, H-3), 7.04 (d, 2H, J 8.3, H-3', H-5'), 7.12 (br s, 1H, NH), 7.28 (m, 4H, H-Ar), 7.36 (m, 3H, H-Ar, H-4), 7.56 (m, 2H, H-Ar), 7.74 (d, 2H, J 8.3, H-2', H-6'), 7.99 (d, 1H, ^{4}J 2.4, H-6), 12.05 (br s, 1H, NH).

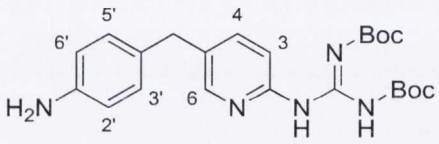
δ_C (100 MHz, $CDCl_3$): *Major isomer:* 11.4 (CH_3-3"), 22.7 (CH_2-2"), 28.3 (CH_3-Boc), 37.4 (CH_2), 42.4 (CH_2-1"), 46.9 (CH_2-Fmoc), 66.6 (CH-Fmoc), 82.4 (q, Boc), 112.9 (CH, Ar-3), 119.8 (CH, Ar-3', Ar-5'), 120.1 (q, Ar-5), 124.8 (CH, 2 Ar), 126.9 (CH, 2 Ar), 127.6 (CH, 4 Ar), 129.2 (CH, Ar-2', Ar-6'), 130.3 (q, Ar-4'), 138.8 (CH, Ar-4), 141.1 (q, 2 Ar), 143.7 (q, 2 Ar), 145.3 (CH, Ar-6), 151.3 (q, Ar-1'), 153.1 (q, C=O), 157.4 (q, Ar-2), 164.2 (q, C=N).

δ_C (100 MHz, $CDCl_3$): *Minor isomer:* 11.2 (CH_3-3"), 22.4 (CH_2-2"), 27.9 (CH_3-Boc), 37.4 (CH_2), 41.2 (CH_2-1"), 46.9 (CH_2-Fmoc), 66.6 (CH-Fmoc), 78.4 (q, Boc), 112.9 (CH, Ar-3), 119.8 (CH, Ar-3', Ar-5'), 120.1 (q, Ar-5), 124.8 (CH, 2 Ar), 126.9 (CH, 2 Ar), 127.6 (CH, 4 Ar), 129.2 (CH, Ar-2', Ar-6'), 130.3 (q, Ar-4'), 138.8 (CH, Ar-4), 141.1 (q, 2 Ar), 143.7 (q, 2 Ar), 145.3 (CH, Ar-6), 151.3 (q, Ar-1'), 153.1 (q, C=O), 157.4 (q, Ar-2), 164.2 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3124 (NH), 2989, 2784, 1714 (C=O), 1635, 1611, 1534, 1398, 1145, 1001, 972, 725.

HRMS (m/z ESI^+): Found: 606.3078 ($M^+ + H$, $C_{36}H_{40}N_5O_4$ Requires: 606.3080).

2-[2,3-Di(*tert*-butoxycarbonyl)guanidino]-5-(4-aminobenzyl)pyridine 99a



Following Method P, to a solution of compound **98a** (1.0 eq., 58 mg, 0.09 mmol) in MeCN (3 mL) was added $NHEt_2$ (115 eq., 1.1 mL, 10.3 mmol). The resulting mixture was stirred at RT for 45 min. Usual work up and purification afforded compound **99a** (39 mg, 98%) as an orange solid.

Mp: 76-80 °C.

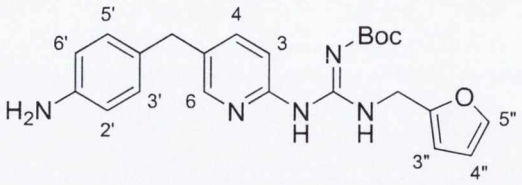
δ_H (400 MHz, $CDCl_3$): 1.50 (s, 18H, CH_3), 3.78 (s, 2H, CH_2), 6.59 (d, 2H, J 8.3, H-2', H-6'), 6.92 (d, 2H, J 8.3, H-3', H-5'), 7.42 (dd, 1H, ^{3}J 8.4, ^{4}J 2.4, H-4), 8.12 (d, 1H, ^{4}J 2.4, H-6), 8.22 (br s, 1H, H-3), 10.78 (br s, 1H, NH), 11.49 (br s, 1H, NH).

δ_C (100 MHz, $CDCl_3$): 27.9 (CH_3-Boc), 37.9 (CH_2), 79.3 (q, Boc), 115.3 (CH, Ar-2', Ar-6'), 115.8 (CH, Ar-3), 129.5 (q, Ar-5), 129.9 (CH, Ar-3', Ar-5'), 132.9 (q, Ar-4'), 138.5 (CH, Ar-4), 145.7 (CH, Ar-6), 147.4 (q, Ar-1'), 148.4 (q, Ar-2), 153.0 (q, C=O), 164.0 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3285 (NH), 2978, 2942, 1712 (C=O), 1624, 1515, 1368, 1325, 1229, 1056, 1026, 805.

HRMS (m/z ESI^+): Found: 464.2277 ($M^+ + Na$, $C_{23}H_{31}N_5Na\ O_4$ Requires: 464.2274).

2-[2-(*tert*-Butoxycarbonyl)-3-(furanyl-2-methyl)guanidino]-5-(4-aminobenzyl)pyridine 99b



Following Method P, to a solution of compound **98b** (1.0 eq., 75 mg, 0.11 mmol) in MeCN (3 mL) was added $NHEt_2$ (115 eq., 1.3 mL, 12.6 mmol). The resulting mixture was stirred at RT for 45 min. Usual work up and purification afforded compound **99b** (42 mg, 90%) as a yellow solid.

Mp: 113-117 °C.

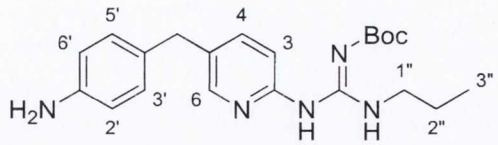
δ_H (400 MHz, $CDCl_3$): 1.51 (s, 9H, CH_3-Boc), 3.60 (br s, 2H, NH_2), 3.76 (s, 2H, CH_2), 4.69 (d, 2H, J 6.7, CH_2), 6.24 (m, 2H, H-3"), 6.30 (m, 1H, H-4"), 6.61 (d, 2H, J 8.3, H-2', H-6'), 6.73 (d, 1H, J 8.4, H-3), 6.91 (d, 2H, J 8.3, H-3', H-5'), 7.35 (m, 1H, H-5"), 7.39 (dd, 1H, ^{3}J 8.4, ^{4}J 2.4, H-4), 7.99 (d, 1H, ^{4}J 2.4, H-6), 10.51 (br s, 1H, NH), 12.00 (br s, 1H, NH).

δ_C (100 MHz, $CDCl_3$): 28.4 (CH_3-Boc), 37.4 (CH_2), 38.1 (CH_2), 78.5 (q, Boc), 107.0 (CH, Ar-3"), 110.3 (CH, Ar-4"), 112.9 (CH, Ar-3), 115.3 (CH, Ar-2', Ar-6'), 129.5 (CH, Ar-3', Ar-5'), 129.6 (q, Ar-5), 131.3 (q, Ar-4'), 139.0 (CH, Ar-4), 142.0 (CH, Ar-5"), 144..8 (q, Ar-1'), 145.4 (CH, Ar-6), 151.0 (q, Ar-2"), 151.5 (q, C=O), 157.3 (q, Ar-2), 164.1 (q, C=N).

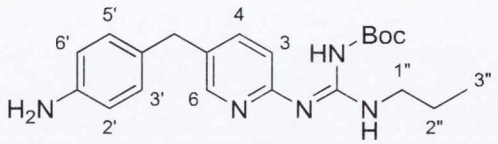
ν_{max} (ATR)/cm^{-1}: 3352 (NH), 2979, 1715 (C=O), 1639, 1599, 1551, 1479, 1351, 1249, 1152, 1113, 1053, 736.

HRMS (m/z ESI$^+$): Found: 422.2181 (M^+ + H, $C_{23}H_{28}N_5O_3$ Requires: 422.2186).

2-[2-(*tert*-Butoxycarbonyl)-3-(propyl)guanidino]-5-(4-aminobenzyl)pyridine 99c



Major



Minor

Following Method P, to a solution of compound **98c** (1.0 eq., 70 mg, 0.12 mmol) in MeCN (3 mL) was added $NHEt_2$ (115 eq., 1.4 mL, 13.8 mmol). The resulting mixture was stirred at RT for 45 min. Usual work up and purification afforded compound **99c** (45 mg, 97%) as a thick yellow gum.

δ_H (400 MHz, $CDCl_3$): *Major isomer*: 0.96 (m, 3H, CH_3-3"), 1.48 (s, 9H, CH_3-Boc), 1.62 (m, 2H, CH_2-2"), 3.45 (m, 2H, CH_2-1"), 3.61 (br s, 2H, NH_2), 3.78 (s, 2H, CH_2), 6.61 (d, 2H, J 8.2, H-2', H-6'), 6.72 (d, 1H, J 8.4, H-3), 6.90 (d, 2H, J 8.2, H-3', H-5'), 7.39 (dd, 1H, ^{3}J 8.4, ^{4}J 2.1, H-4), 7.73 (br s, 1H, NH),7.99 (d, 1H, ^{4}J 2.1, H-6), 10.71 (br s, 1H, NH).

δ_H (400 MHz, $CDCl_3$): *Minor isomer:* 0.91 (m, 3H, CH_3-3"), 1.45 (s, 9H, CH_3-Boc), 1.55 (m, 2H, CH_2-2"), 3.22 (m, 2H, CH_2-1"), 3.61 (br s, 2H, NH_2), 3.78 (s, 2H, CH_2), 6.61 (d, 2H, J 8.2, H-2', H-6'), 6.72 (d, 1H, J 8.4, H-3), 6.83 (br s, 1H, NH), 6.90 (d, 2H, J 8.3, H-3', H-5'), 7.39 (dd, 1H, ^{3}J 8.4, ^{4}J 2.1, H-4), 7.99 (d, 1H, ^{4}J 2.1, H-6), 11.98 (br s, 1H, NH).

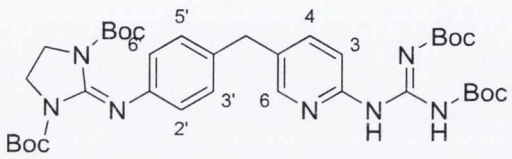
δ_C (100 MHz, $CDCl_3$): *Major isomer:* 11.5 (CH_3-3"), 22.8 (CH_2-2"), 28.4 (CH_3-Boc), 37.4 (CH_2), 42.5 (CH_2-1"), 82.3 (q, Boc), 113.0 (CH, Ar-3), 115.3 (CH, Ar-2', Ar-6'), 129.5 (CH, Ar-3', Ar-5'), 129.7 (q, Ar-5), 131.3 (q, Ar-4'), 138.9 (CH, Ar-4), 145.3 (CH, Ar-6), 151.3 (q, Ar-1'), 153.5 (q, C=O), 157.5 (q, Ar-2), 164.2 (q, C=N).

δ_C (100 MHz, $CDCl_3$): *Minor isomer:* 11.4 (CH_3-3"), 22.5 (CH_2-2"), 28.0 (CH_3-Boc), 37.4 (CH_2), 41.5 (CH_2-1"), 78.4 (q, Boc), 113.0 (CH, Ar-3), 115.3 (CH, Ar-2', Ar-6'), 129.5 (CH, Ar-3', Ar-5'), 129.7 (q, Ar-5), 131.3 (q, Ar-4'), 138.9 (CH, Ar-4), 145.3 (CH, Ar-6), 151.3 (q, Ar-1'), 153.5 (q, C=O), 157.5 (q, Ar-2), 164.2 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3320 (NH), 2926, 2850, 1593, 1501, 1463, 1425, 1337, 1249, 1165, 1131, 1043, 668.

HRMS (m/z ESI$^+$): Found: 384.2396 (M^+ + H, $C_{21}H_{30}N_5O_2$ Requires: 384.2394).

2-[2,3-Di(*tert*-butoxycarbonyl)guanidino]-5-{4-[di(tert-botoxycarbonyl)-2-aminoimidazolino]benzyl}pyridine 100a



Following Method M, to a solution compound **99a** (1.0 eq., 30 mg, 0.07 mmol), compound **66** (1.0 eq., 21 mg, 0.07 mmol) and NEt_3 (3.0 eq., 30 µL, 0.21 mmol) in CH_2Cl_2 (3 mL) at 0 °C was added $HgCl_2$ (1.1 eq., 21 mg, 0.08 mmol). The reaction was then left to stir at RT overnight. Usual work up (without further purification) afforded compound **100a** (53 mg, 100%) as a yellow gum.

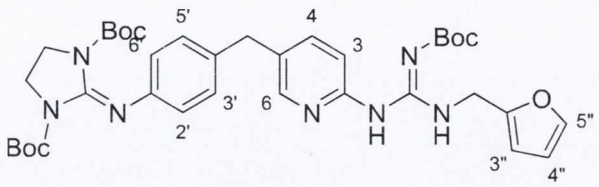
δ_H (400 MHz, $CDCl_3$): 1.46 (s, 9H, CH_3-Boc), 1.49 (s, 9H, CH_3-Boc), 1.51 (s, 18H, CH_3-Boc), 3.77 (s, 4H, CH_2), 3.80 (s, 2H, CH_2), 6.88 (d, 2H, J 8.3, H-2',H- 6'), 6.98 (d, 2H, J 8.3, H-3', H-5'), 7.41 (dd, 1H, ^{3}J 8.4, ^{4}J 2.4, H-4), 8.09 (d, 1H, ^{4}J 2.4, H-6), 8.20 (br s, 1H, H-3), 10.76 (br s, 1H, NH), 11.47 (br s, 1H, NH).

δ_C (100 MHz, $CDCl_3$): 27.8 (CH_3-Boc), 27.9 (CH_3-Boc), 28.1 (2 CH_3-Boc), 37.8 (CH_2), 44.2 (CH_2-Imid), 79.7 (q, Boc), 82.4 (q, Boc), 83.7 (2 q, Boc), 115.7 (CH, Ar-3), 121.6 (CH, Ar-2', Ar-6'), 127.6 (q, Ar-5), 128.9 (CH, Ar-3', Ar-5'), 133.1 (q, Ar-4'), 138.4 (CH, Ar-4), 146.6 (CH, Ar-6), 147.8 (q, Ar-1'), 150.3 (q, Ar-2), 156.0 (q, C=O), 156.7 (q, C=O), 162.2 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3320 (NH), 2947, 2837, 1597, 1459, 1497, 1393, 1337, 1240, 1157, 1043, 937, 790.

HRMS: (m/z ESI$^+$) Found: 710.3877 (M^+ + H, $C_{36}H_{52}N_7O_8$ Requires: 710.3877).

2-[2-(*tert*-butoxycarbonyl)-3-(furanyl-2-methyl)guanidino]-5-{[1,3-(*tert*-butoxycarbonyl)-2-iminoimidazolidino]benzyl}pyridine 99b



Following Method M, to a solution compound **99b** (1.0 eq., 29 mg, 0.07 mmol), compound **71a** (1.0 eq., 21 mg, 0.07 mmol) and NEt_3 (3.0 eq., 30 µL, 0.21 mmol) in CH_2Cl_2 (3 mL) at 0 °C was added $HgCl_2$ (1.1 eq., 21 mg, 0.08 mmol). The reaction was then left to stir at RT overnight. Usual work up (without further purification) afforded compound **100b** (47 mg, 97%) as a yellow gum.

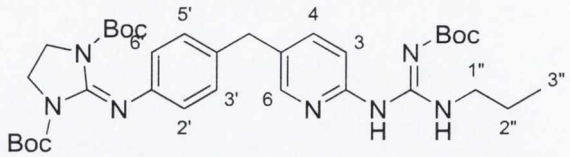
δ_H (400 MHz, $CDCl_3$): 1.25 (s, 9H, CH_3-Boc), 1.50 (s, 18H, CH_3-Boc), 3.78 (s, 4H, CH_2), 3.87 (s, 2H, CH_2), 4.69 (d, 2H, J 4.9, CH_2), 6.23 (m, 2H, H-3"), 6.30 (m, 1H, H-4"), 6.70 (d, 1H, J 8.4, H-3), 6.89 (d, 2H, J 8.3, H-2', H-6'), 6.98 (d, 2H, J 8.3, H-3', H-5'), 7.36 (m, 2H, H-5", H-4), 7.96 (d, 1H, ^{4}J 2.4, H-6), 10.49 (br s, 1H, NH), 11.98 (br s, 1H, NH).

δ_C (100 MHz, $CDCl_3$): 27.8 (CH_3-Boc), 28.4 (2 CH_3-Boc), 37.9 (CH_2), 43.1 (CH_2-Imid), 44.4 (CH_2), 78.9 (q, Boc), 82.6 (2 q, Boc), 107.0 (CH, Ar-3"), 110.3 (CH, Ar-4"), 112.8 (CH, Ar-3), 121.7 (CH, Ar-2', Ar-6'), 128.3 (q, Ar-5), 128.9 (CH, Ar-3', Ar-5'), 131.3 (q, Ar-4'), 133.7 (q, Ar-1'), 139.0 (CH, Ar-4), 142.0 (CH, Ar-5"), 145.4 (CH, Ar-6), 150.1 (q, Ar-5"), 150.9 (q, C=O), 151.5 (q, C=O), 157.3 (q, Ar-2), 164.0 (q, C=N).

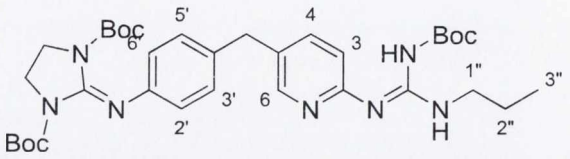
ν_{max} (ATR)/cm^{-1}: 2923 (NH), 2850, 1711 (C=O), 1639, 1599, 1475, 1367, 1303, 977.

HRMS (m/z ESI$^+$): Found: 690.3604 (M^+ + H, $C_{36}H_{48}N_7O_7$ Requires: 690.3609).

2-[2-(*tert*-Butoxycarbonyl)-3-(propyl)guanidino]-5-{[1,3-(*tert*-butoxycarbonyl)-2-iminoimidazolidino]benzyl}pyridine 100c



Major



Minor

Following Method M, to a solution compound **99c** (1.0 eq., 25 mg, 0.07 mmol), compound **71c** (1.0 eq., 21 mg, 0.07 mmol) and NEt_3 (3.0 eq., 30 µL, 0.21 mmol) in CH_2Cl_2 (3 mL) at 0 °C was added $HgCl_2$ (1.1 eq., 21 mg, 0.08 mmol). The reaction was then left to stir at RT overnight. Usual work up (without further purification) afforded compound **100c** (47 mg, 100%) as a yellow gum.

δ_H (400 MHz, $CDCl_3$): *Major isomer:* 0.95 (m, 3H, CH_3-3"), 1.36 (s, 9H, CH_3-Boc), 1.50 (s, 18H, CH_3-Boc), 1.61 (m, 2H, CH_2-2"), 3.43 (m, 2H, CH_2-1"), 3.78 (s, 4H, CH_2), 3.87 (s, 2H, CH_2), 6.68 (d, 1H, J 8.4, H-3), 6.89 (d, 2H, J 8.2, H-2', H-6'), 6.97 (d, 2H, J 8.2, H-3', H-5'), 7.36 (dd, 1H, ^{3}J 8.4, ^{4}J 2.1, H-4), 7.72 (br s, 1H, NH),7.96 (d, 1H, ^{4}J 2.1, H-6), 10.15 (br s, 1H, NH).

δ_H (400 MHz, $CDCl_3$): *Minor isomer:* 0.88 (m, 3H, CH_3-3"), 1.36 (s, 9H, CH_3-Boc), 1.49 (s, 18H, CH_3-Boc), 1.55 (m, 2H, CH_2-2"), 3.20 (m, 2H, CH_2-1"), 3.78 (s, 4H, CH_2), 3.87 (s, 2H, CH_2), 6.68 (d, 1H, J 8.4, H-3), 6.79 (br s, 1H, NH), 6.89 (d, 2H, J 8.2, H-2', H-6'), 6.97 (d, 2H, J 8.3, H-3', H-5'), 7.36 (dd, 1H, ^{3}J 8.4, ^{4}J 2.1, H-4), 7.96 (d, 1H, ^{4}J 2.1, H-6), 11.96 (br s, 1H, NH).

δ_C (100 MHz, $CDCl_3$): *Major isomer*: 11.6 (CH_3-3"), 22.8 (CH_2-2"), 27.8 (CH_3-Boc), 28.0 (2 CH_3-Boc), 37.7 (CH_2), 42.5 (CH_2-1"), 43.1 (CH_2-Imid), 82.6 (q, Boc), 83.0 (2 q, Boc), 112.7 (CH, Ar-3), 121.6 (CH, Ar-2', Ar-6'), 128.9 (CH, Ar-3', Ar-5'), 131.0 (q, Ar-5), 133.7 (q, Ar-4'), 138.9 (CH, Ar-4), 145.3 (CH, Ar-6), 146.8 (q, Ar-1'), 150.1 (q, C=O), 151.2 (q, C=O), 157.6 (q, 2), 164.1 (q, C=N).

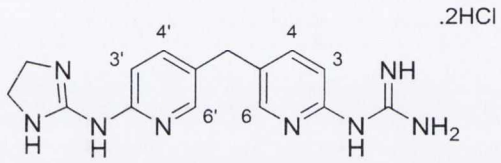
δ_C (100 MHz, $CDCl_3$): *Minor isomer:* 11.5 (CH_3-3"), 22.5 (CH_2-2"), 27.8 (CH_3-Boc), 27.9 (2 CH_3-Boc), 37.7 (CH_2), 41.4 (CH_2-1"), 43.1 (CH_2-Imid), 78.4 (q, Boc), 82.6 (2 q, Boc), 112.7

(CH, Ar-3), 121.6 (CH, Ar-2', Ar-6'), 128.9 (CH, Ar-3', Ar-5'), 131.0 (q, Ar-5), 133.7 (q, Ar-4'), 138.9 (CH, Ar-4), 145.3 (CH, Ar-6), 146.8 (q, Ar-1'), 150.1 (q, C=O), 151.2 (q, C=O), 157.6 (q, 2), 164.1 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3100 (NH), 2965, 2925, 1725 (C=O), 1705, 1638, 1596, 1365, 1299, 1245, 1057, 974, 806, 766

HRMS (m/z ESI$^+$): Found: 652.3818 (M^+ + H, $C_{34}H_{50}N_7O_6$ Requires: 652.3817).

6-(2-Aminoimidazolino)-6'-(guanidino)dipyridinyl-3-methane dihydrochloride 101a



Following Method O, to a solution of 4M HCl/1,4-dioxane (24.0 eq., 840 µL, 3.36 mmol) was added compound **95a** (1.0 eq., 100 mg, 0.14 mmol). The mixture was stirred at 55 °C until completion, as adjudged by disappearance of starting material in TLC. Usual work up and purification afforded compound **101a** (49 mg, 92%) as a yellow solid.

Mp: 149-151 °C decomposed.

δ_H (400 MHz, D_2O) : 3.64 (s, 2H, CH_2), 3.67 (s, 4H, CH_2), 6.74 (d, 2H, J 8.1, H-3',H-3), 7.45 (dd, 2H, ^{3}J 8.1, ^{4}J 2.2, H-4', H-4), 7.99 (d, 2H, ^{4}J 2.1, H-6', H-6).

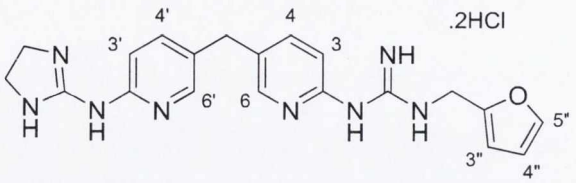
δ_C (100 MHz, D_2O): 33.4 (CH_2), 42.0 (CH_2-Imid), 112.6 (CH, Ar-3'), 113.3 (CH, Ar-3), 131.9 (q, Ar-5, Ar-5'), 139.7 (CH, Ar-4, Ar-4'), 146.0 (CH, Ar-6'), 146.5 (CH, Ar-6), 148.8 (q, Ar-2' or Ar-2), 149.8 (q, Ar-2 or Ar-2'), 154.6 (q, C=N), 156.3 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3185 (NH), 2980, 1624, 1600, 1481, 1355, 1242, 1027, 965.

HRMS: (m/z ESI$^+$): Found: 311.1728 (M^+ + H, $C_{15}H_{19}N_8$ Requires: 311.1733).

Purity by HPLC: 98.4% (t_R 21.75 min).

6-(2-Aminoimidazolino)-6'-[2-(furanyl-2-methyl)guanidino]dipirydinyl-3-methane dihydrochloride 101b



Following Method O, to a solution of 4M HCl/1,4-dioxane (18.0 eq., 400 μL, 1.62 mmol) was added compound **95b** (1.0 eq., 64 mg, 0.09 mmol). The mixture was stirred at 55 °C until completion, as adjudged by disappearance of starting material in TLC. Usual work up and purification afforded compound **101b** (34 mg, 82%) as a yellow solid.

Mp: 164-167 °C decomposed.

δ_H (400 MHz, D_2O): 3.76 (s, 2H, CH_2), 3.79 (s, 4H, CH_2), 4.54 (s, 2H, CH_2), 6.44 (br s, 2H, H-3", H-4"), 6.91 (m, 2H, H-3', H-3), 7.51 (br s, 1H, H-5"), 7.51 (dd, 1H, ^3J 8.1, ^4J 2.4, H-4'), 7.61 (dd, 1H, ^3J 8.1, ^4J 2.4, H-4), 7.96 (s, 1H, H-6'), 8.11 (s, 1H, H-6).

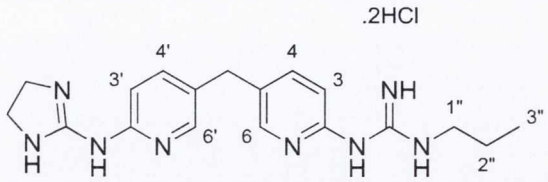
δ_C (100 MHz, D_2O): 33.7 (CH_2), 37.8 (CH_2-Imid), 42.2 (CH_2), 108.5 (CH, Ar-3"), 110.6 (CH, Ar-4"), 112.5 (CH, Ar-3'), 113.2 (CH, Ar-3), 128.7 (q, Ar-5 or Ar-5'), 130.6 (q, Ar-5 or Ar-5'), 139.6 (CH, Ar-4'), 139.8 (CH, Ar-4), 143.3 (CH, Ar-5"), 145.5 (CH, Ar-6'), 146.5 (CH, Ar-6), 148.5 (q, Ar-2"), 149.5 (q, Ar-2 or Ar-2'), 149.5 (q, Ar-2 or Ar-2'), 153.7 (q, C=N), 156.0 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3127 (NH), 2918, 2894, 1629, 1601, 1574, 1388, 1281, 1234, 1045, 1025, 828, 741.

HRMS: (m/z ESI^+): Found: 391.1993 ($M^+ + H$, $C_{20}H_{23}N_8O$ Requires: 391.1995).

Purity by HPLC: 98.5% (t_R 24.03 min).

6-(2-Aminoimidazolino)-6'-[2-(propyl)guanidino]dipirydinyl-3-methane dihydrochloride 101c



Following Method O, to a solution of 4M HCl/1,4-dioxane (18.0 eq., 378 µL, 1.52 mmol) was added compound **95c** (1.0 eq., 55 mg, 0.084 mmol). The mixture was stirred at 55 °C until completion, as adjudged by disappearance of starting material in TLC. Usual work up and purification afforded compound **101c** (30 mg, 85%) as a yellow solid.

Mp: 148-151 °C decomposed.

δ_H (400 MHz, D_2O) : 0.81 (t, 3H, J 7.4, CH_3-3"), 1.50 (m, 2H, CH_2-2"), 3.11 (t, 2H, J 7.4, CH_2-1"), 3.62 (s, 2H, CH_2), 3.66 (s, 4H, CH_2), 6.75 (d, 2H, J 8.4, H-3', H-3), 7.45 (m, 2H, H-4', H-4), 7.94 (d, 1H, ^{4}J 2.2, H-6'), 8.00 (d, 1H, ^{4}J 2.2, H-6).

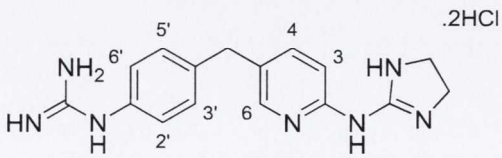
δ_C (100 MHz, D_2O): 10.3 (CH_3-3"), 21.1 (CH_2-2"), 30.1 (CH_2), 33.7 (CH_2-1"), 42.7 (CH_2-Imid), 111.0 (CH, Ar-3'), 112.6 (CH, Ar-3), 130.2 (q, Ar-5 or Ar-5'), 132.0 (q, Ar 5 or Ar-5'), 139.7 (CH, Ar-4'), 139.8 (CH, Ar-4), 145.7 (CH, Ar-6'), 148.8 (CH, Ar-6), 148.8 (q, Ar-2 or Ar-2'), 151.6 (q, Ar-2 or Ar-2'), 156.0 (q, C=N), 156.5 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3280 (NH), 2918, 1629, 1601, 1483, 1348, 1281, 1243, 1044, 1027, 1025, 926, 829, 741.

HRMS: (m/z ESI^+): Found: 353.2206 (M^+ + H, $C_{18}H_{25}N_8$ Requires: 353.2202).

Purity by HPLC: 98.5% (t_R 23.93 min).

2-(2-Aminoimidazolino)-5-(4-guanidino)benzyl]pyridine dihydrochloride 101d



Following Method O, to a solution of 4M HCl/1,4-dioxane (24.0 eq., 660 µL, 2.64 mmol) was added compound **95d** (1.0 eq., 80 mg, 0.11 mmol). The mixture was stirred at 55 °C until completion, as adjudged by disappearance of starting material in TLC. Usual work up and purification afforded compound **101d** (41 mg, 98%) as an off-white solid.

Mp: 167-170 °C decomposed.

δ_H (400 MHz, D_2O): 3.67 (s, 4H, CH_2), 3.84 (s, 2H, CH_2), 6.79 (d, 1H, J 8.4, H-3), 7.10 (d, 2H, J 8.3, H-2', H-6'), 7.19 (d, 2H, J 8.3, H-3', H-5'), 7.52 (dd, 1H, ^{3}J 8.4, ^{4}J 2.2, H-4), 8.07 (d, 1H, ^{4}J 2.2, H-6).

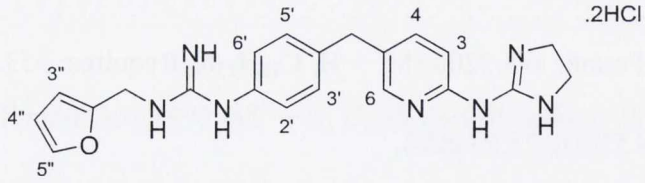
δ_C (100 MHz, D_2O): 36.4 (CH_2), 42.2 (CH_2-Imid), 112.7 (CH, Ar-3), 126.1 (CH, Ar-2', Ar-6'), 130.1 (CH, Ar-3', Ar-5'), 132.3 (q, Ar-5), 132.8 (q, Ar-4'), 139.8 (CH, Ar-4), 140.4 (q, Ar-1'), 146.6 (CH, Ar-6), 148.7 (q, Ar-2), 155.7 (q, C=N), 156.2 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3136 (NH), 2923, 2853, 1575, 1629, 1603, 1485, 1391, 1242, 1144, 1079, 1023, 926.

HRMS: (m/z ESI$^+$): Found: 310.1781 (M^+ + H, $C_{16}H_{20}N_7$ Requires: 310.1780).

Purity by HPLC: 96.1% (t_R 21.05 min).

2-[2-Aminoimidazolino]-5-{4-[3-(furanyl-2-methyl)guanidino]benzyl}pyridine dihydrochloride 101e



Following Method O, to a solution of 4M HCl/1,4-dioxane (18.0 eq., 320 µL, 1.26 mmol) was added compound **95e** (1.0 eq., 44 mg, 0.07 mmol). The mixture was stirred at 55 °C until completion, as adjudged by disappearance of starting material in TLC. Usual work up and purification afforded compound **101e** (26 mg, 80%) as a yellow solid.

Mp: 122-130 °C decomposed.

δ_H (400 MHz, D_2O): 3.64 (s, 4H, CH_2), 3.79 (s, 2H, CH_2), 4.30 (s, 2H, CH_2), 6.24 (d, 1H, J 2.8, H-3"), 6.28 (m, 1H, H-4"), 6.77 (d, 1H, J 8.4, H-3), 7.06 (d, 2H, J 9.0, H-2', H-6'), 7.17 (d, 2H, J 9.0, H-3', H-5'), 7.34 (br s, 1H, H-5"), 7.50 (dd, 1H, ^{3}J 8.4, ^{4}J 2.3, H-4), 8.05 (d, 1H, ^{4}J 2.3, H-6).

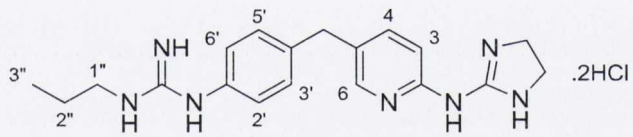
δ_C (100 MHz, D_2O): 36.7 (CH_2), 42.6 (CH_2-Imid), 47.6 (CH_2), 108.1 (CH, Ar-3"), 110.5 (CH, Ar-4"), 112.6 (CH, Ar-3), 126.1 (CH, Ar-2', Ar-6'), 130.1 (CH, Ar-3', Ar-5'), 132.2 (q, Ar-5), 132.8 (q, Ar-4'), 139.8 (CH, Ar-4), 140.3 (q, Ar-2"), 143.1 (CH, Ar-5"), 146.5 (CH, Ar-6), 148.1 (q, Ar-1'), 149.0 (q, Ar-2), 155.0 (q, C=N), 156.3 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3199 (NH), 2923, 29854, 1636, 1599, 1511, 1242, 1147, 1076, 1016, 925, 742, 678.

HRMS: (m/z ESI$^+$): Found: 390.2038 (M^+ + H, $C_{21}H_{24}N_7O$ Requires: 390.2042).

Purity by HPLC: 96.4% (t_R 23.95 min).

2-(2-Aminoimidazolino)-5-[(4-(2-propylguanidino)benzyl]pyridine dihydrochloride 101f



Following Method O, to a solution of 4M HCl/1,4-dioxane (18.0 eq., 360 µL, 1.44 mmol) was added compound **95f** (1.0 eq., 53 mg, 0.08 mmol). The mixture was stirred at 55 °C until completion, as adjudged by disappearance of starting material in TLC. Usual work up and purification afforded compound **101f** (30 mg, 87%) as a light yellow solid.

Mp: 175-179 °C decomposed.

δ_H (400 MHz, D_2O): 0.78 (t, 3H, J 7.4, CH_3-3"), 1.47 (m, 2H, CH_2-2"), 3.08 (m, 2H, CH_2-1"), 3.67 (br s, 4H, CH_2), 3.87 (s, 2H, CH_2), 6.80 (d, 1H, J 8.4, H-3), 7.08 (d, 2H, J 8.4, H-2', H-6'), 7.18 (d, 2H, J 8.4, H-3', H-5'), 7.53 (dd, 1H, ^{3}J 8.4, ^{4}J 2.2, H-4), 8.03 (d, 1H, ^{4}J 2.2, H-6).

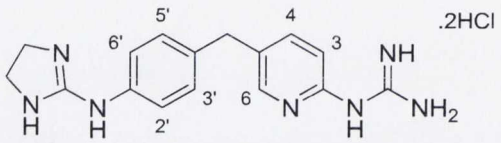
δ_C (100 MHz, D_2O): 9.9 (CH_3-3"), 21.2 (CH_2-2"), 36.8 (CH_2), 42.0 (CH_2-Imid), 43.0 (CH_2-1"), 112.0 (CH, Ar-3), 126.2 (q, Ar-5), 130.1 (CH, Ar-2', Ar-3', Ar-5', Ar-6'), 132.5 (q, Ar-4'), 133.1 (q, Ar-1'), 139.1 (CH, Ar-4), 146.6 (CH, Ar-6), 148.7 (q, Ar-2), 155.7 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3324 (NH), 2963, 2833, 1655, 1572, 1395, 1202, 1164, 1097, 1020, 774.

HRMS: (m/z ESI$^+$): Found: 352.2254 (M^+ + H, $C_{19}H_{26}N_7$ Requires: 352.2250).

Purity by HPLC: 97.0% (t_R 22.64 min).

2-Guanidino-5-[(2-aminoimidazolino)benzyl]pyridine dihydrochloride 101g



Following Method O, to a solution of 4M HCl/1,4-dioxane (24.0 eq., 480 µL, 1.92 mmol) was added compound **100a** (1.0 eq., 53 mg, 0.08 mmol). The mixture was stirred at 55 °C until completion, as adjudged by disappearance of starting material in TLC. Usual work up and purification afforded compound **101g** (25 mg, 82%) as an off-white solid.

Mp: 155-159 °C decomposed.

δ_H (400 MHz, D_2O): 3.58 (s, 4H, CH_2), 3.78 (s, 2H, CH_2), 6.78 (d, 1H, J 8.4, H-3), 7.07 (d, 2H, J 8.3, H-2', H-6'), 7.18 (d, 2H, J 8.3, H-3', H-5'), 7.50 (dd, 1H, ^{3}J 8.4, ^{4}J 2.2, H-4), 8.03 (d, 1H, ^{4}J 2.2, H-6).

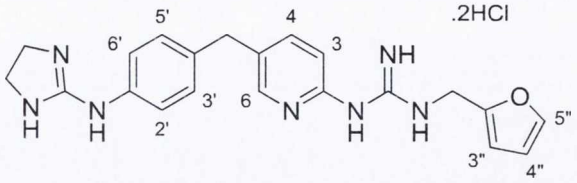
δ_C (100 MHz, D_2O): 36.8 (CH_2), 42.6 (CH_2-Imid), 113.5 (CH, Ar-3), 124.5 (CH, Ar-2', Ar-6'), 130.0 (CH, Ar-3', Ar-5'), 132.9 (q, Ar-5), 133.4 (q, Ar-4'), 139.5 (q, Ar-1'), 140.0 (CH, Ar-4), 146.0 (CH, Ar-6), 149.2 (q, Ar-2), 158.0 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3338 (NH), 2934, 1652, 1606, 1492, 1391, 1240, 1068, 1025, 845, 757.

HRMS (m/z ESI$^+$): Found: 310.1783 (M^+ + H, $C_{16}H_{20}N_7$ Requires: 310.1775).

Purity by HPLC: 98.8% (t_R 21.47 min).

2-[2-(Furanyl-2-methyl)guanidino]-5-[4-(2-aminoimidazolino)benzyl]pyridine dihydrochloride 101h



Following Method O, to a solution of 4M HCl/1,4-dioxane (18.0 eq., 315 µL, 1.26 mmol) was added compound **100b** (1.0 eq., 47 mg, 0.07 mmol). The mixture was stirred at 55 °C until completion, as adjudged by disappearance of starting material in TLC. Usual work up and purification afforded compound **101h** (29 mg, 90%) as an off-white solid.

Mp: 134-137 °C, decomposed.

δ_H (400 MHz, D_2O): 3.75 (s, 4H, CH_2), 3.91 (s, 2H, CH_2), 4.55 (s, 2H, CH_2), 6.47 (br s, 2H, H-3", H-4"), 6.90 (d, 1H, J 8.5, H-3), 7.20 (d, 2H, J 8.1, H-2', H-6'), 7.29 (d, 2H, J 8.1, H-3', H-5'), 7.53 (br s, 1H, H-5"), 7.61 (dd, 1H, ^{3}J 8.4, ^{4}J 1.6, H-4), 8.07 (d, 1H, ^{4}J 1.6, H-6).

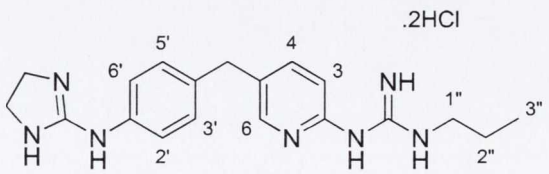
δ_C (100 MHz, D_2O): 36.8 (CH_2), 37.8 (CH_2), 42.6 (CH_2-Imid), 108.4 (CH, Ar-3"), 110.6 (CH, Ar-4"), 113.2 (CH, Ar-3), 124.3 (CH, Ar-2', Ar-6'), 129.9 (CH, Ar-3', Ar-5'), 132.7 (q, Ar-5), 133.3 (q, Ar-4'), 139.6 (q, Ar-2"), 139.9 (CH, Ar-4), 143.3 (CH, Ar-5"), 145.7 (CH, Ar-6), 148.7 (q, Ar-1'), 149.6 (q, Ar-2), 153.9 (q, C=N), 158.4 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3337 (NH), 2945, 2834, 1655, 1449, 1019, 1113, 1045, 956, 729.

HRMS (m/z ESI$^+$): Found: 390.2051 (M^+ + H, $C_{21}H_{24}N_7O$ Requires: 390.2037).

Purity by HPLC: 98.6% (t_R 24.91 min).

2-[2-(Propyl)guanidino)]-5-[4-(2-aminoimidazolino)benzyl]pyridine dihydrochloride 101i



Following Method O, to a solution of 4M HCl/1,4-dioxane (18.0 eq., 315 µL, 1.26 mmol) was added compound **100c** (1.0 eq., 47 mg, 0.07 mmol). The mixture was stirred at 55 °C until completion, as adjudged by disappearance of starting material in TLC. Usual work up and purification afforded compound **101i** (27 mg, 98%) as a yellow solid.

Mp: 164-168 °C, decomposed.

δ_H (400 MHz, D_2O): 0.78 (t, 3H, J 7.4, CH_3-3"), 1.47 (m, 2H, CH_2-2"), 3.08 (m, 2H, CH_2-1"), 3.67 (m, 4H, CH_2), 3.87 (s, 2H, CH_2), 6.80 (d, 1H, J 8.4, H-3), 7.08 (d, 2H, J 8.4, H-2', H-6'), 7.18 (d, 2H, J 8.4, H-3', H-5'), 7.53 (dd, 1H, ^{3}J 8.4, ^{4}J 2.2, H-4), 8.03 (d, 1H, ^{4}J 2.2, H-6).

δ_C (100 MHz, D_2O): 9.9 (CH_3-3"), 21.3 (CH_2-2"), 36.8 (CH_2), 42.6 (CH_2-Imid), 42.7 (CH_2-1"), 112.9 (CH, Ar-3), 124.3 (CH, Ar-2', Ar-6'), 127.5 (q, Ar-5), 129.8 (CH, Ar-3', Ar-5'), 133.4 (q, Ar-4'), 139.5 (CH, Ar-4), 139.7 (q, Ar-1'), 145.5 (CH, Ar-6), 149.5 (q, Ar-2), 153.3 (q, C=N), 158.6 (q, C=N).

ν_{max} (ATR)/cm^{-1}: 3350 (NH), 2955, 2921, 1650, 1606, 1509, 1492, 1391, 1278, 1034, 837, 736.

HRMS (m/z ESI^+): Found: 352.2243 (M^+ + H, $C_{19}H_{26}N_7$ Requires: 352.2244).

Purity by HPLC: 98.8% (t_R 23.23 min).

5.2. Pharmacological Methods

5.2.1. Preparation of Membranes

Neural membranes (P2 fractions) were prepared from the PFC of human brains obtained at autopsy in the Instituto Vasco de Medicina Legal, Bilbao, Spain. Post-mortem human brain samples of each subject (~1 g) were homogenized using a Teflon-glass grinder (10 up-and-down strokes at 1500 rpm) in 30 volumes of homogenization buffer (1 mM $MgCl_2$ and 5 mM Tris-HCl, pH 7.4) supplemented with 0.25M sucrose. The crude homogenate was centrifuged for 5 min at 1,000 g (4 °C), and the supernatant was centrifuged again for 10 min at 40,000 g (4 °C). The resultant pellet was washed twice in 20 volumes of homogenization buffer and re-centrifuged in similar conditions. Aliquots of 1 mg protein were stored at -70 °C until assay. Protein concentration was measured according to the Bradford method,[269] using bovine serum albumin as standard and was similar in the different brain samples.

5.2.2. [^{3}H]RX821002 Binding Assays

Specific [^{3}H]RX821002 binding was measured in 0.55 mL-aliquots (50 mM Tris HCl, pH 7.5) of the neural membranes which were incubated with [^{3}H]RX821002 (1 nM) for 30 min at 25 °C in the absence or presence of the competing compounds (10^{-12} to 10^{-3} M, 10 concentrations). Incubations were terminated by diluting the samples with 5 mL of ice-cold Tris incubation buffer (4 °C). Membrane bound [^{3}H]RX821002 was separated by vacuum filtration through Whatman GF/C glass fibre filters. Then the filters were rinsed twice with5 mL of incubation buffer and transferred to minivials containing 3 mL of OptiPhase "HiSafe" II cocktail and counted for radioactivity by liquid scintillation spectrometry. Specific binding was determined and plotted as a function of the compound concentration. Nonspecific binding was determined in the presence of adrenaline (10^{-5} M). Analysis of competition experiments to obtain the inhibition constant (K_i) were performed by non-linear regression using the Graph Pad Prism 5 program. All experiments were analysed assuming a one-site model of radioligand binding, K_i values were normalized to pK_i values.

5.2.3. [^{35}S]GTPγS Binding Assays

The incubation buffer for measuring [^{35}S]GTPγS binding to brain membranes contained, in a total volume of 500 μL, 1 mM ethylene glycol tetraacetic acid (EGTA), 3 mM $MgCl_2$, 100 mM NaCl, 50 mM GDP, 50 mM Tris-HCl at pH 7.4, and 0.5 nM [^{35}S]GTPγS. Protein aliquots were thawed and re-suspended in the same buffer. The incubation was started by addition of the membrane suspension (40 μg of membrane proteins) to the previous mixture and was performed at 30 °C for 120 min with shaking. To evaluate the influence of the compounds on [^{35}S]GTPγS binding, 8 concentrations (10^{-10} to 10^{-3} M) of each compound were added to the assay. Incubations were terminated by adding 3 mL of ice-cold re-suspension buffer followed by rapid filtration through Whatman GF/C filters pre-soaked in the same buffer. The filters were rinsed twice with 3 mL of ice-cold re-suspension buffer, transferred to vials containing 5 mL of OptiPhase HiSafe II cocktail (Wallac, UK), and the radioactivity trapped was determined by liquid scintillation spectrometry (Packard 2200CA). The [^{35}S]GTPγS bound was about 7-14% of the total [^{35}S]GTPγS added. Nonspecific binding of the radioligand was defined as the remaining [^{35}S]GTPγS binding in the presence of 10 μM unlabelled GTPγS.

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