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A two-pronged attack on DNA – targeting guanine quadruplexes with nonplanar porphyrins and DNA binding small-molecules

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Core quote "A combination of DNA binding small-molecules with fluorescent, nonplanar porphyrins within one system can offer potential as theranostic agents for nucleic acid targeting."

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Cancer therapy requires the identification and selective targeting of cancer-specific effectors. DNA is considered to be a chemotherapeutic target for cancer because of its involvement in cellular proliferation. In addition to the well-known double-stranded structures (A, B, Z-DNA), DNA can form a number of multi-stranded structures (triplexes, quadruplexes, i-motifs) that have distinct biological relevance. In particular, guanine quadruplexes (GQs) are four-stranded nucleic acid structures formed by guanine-rich sequences that fold into non-canonical secondary structures [1]. They consist of two or more G-tetrads which form when four guanine residues connected by hydrogen bonds are held in a planar arrangement and stabilized further by cations such as Na^+ and K^+. Although GQs can take numerous conformations, there are common features among the GQ structures that may be harnessed to develop small-molecules that bind to them [2]. GQs are present in biologically important regions, such as at the end of telomeres as well as in the regulatory regions of oncogenes (c-MYC, c-KIT, RET, KRAS) [3]. Formation of GQs in gene promoters results in the suppression of transcription; therefore, ligands that stabilize GQs in such gene promoters will inhibit transcriptional activation. GQs are stable under physiological conditions and form *in vivo* in the genome of mammalian cells, which corroborates the utility of stabilizing ligands to target GQs and interfere with their function [4]. Hence, GQs are considered as an emerging oncology target for the development of

novel anti-cancer drugs [5]. However, targeting GQs involves a higher degree of selectivity, as the drugs should bind to GQs and not the double stranded DNA (dsDNA), and GQs are much less prominent in the cell. Therefore, selectivity and detection are the key factors in designing novel molecules targeting GQs [6].

Non-covalent binding of small-molecules to GQs can be achieved by different interactions, thus these compounds: a) can modify DNA by stacking with terminal G-tetrads (up or down), b) may bind into the groove, or c) could intercalate between two consecutive G-tetrads. A variety of small-molecules (such as diamidoanthraquinones, disubstituted acridines, trisubstituted isoalloxazines, quindolines, pentacyclic acridinium analogues, dibenzophenanthrolines, or fluoroquinolones) have been reported which bind effectively to GQs. Many of these probes suffer from low selectivity for GQ *versus* dsDNA or toward a specific GQ geometry [7]. Common features present in all of these structures include a flat aromatic system and cationic residues [8]. For example, two lead compounds, 307A and 360A [8,9], which are very selective for GQs *versus* dsDNA and inhibit cell proliferation, characteristically contain a 2,6-pyridine dicarboxamide core and two quinolinium cations. Similarly, compound 12459 [10], which displayed antitelomerase activity in the nanomolar range, presents a triazine core as well as two quinoliniums [8]. Recently, it has been suggested that a high GQ affinity benzothiazolyl piperazinyl chromenone, which also has an aromatic core and a cationic system, can bind to the groove of a GQ [11]. These common structural features are related to those exhibited by DNA minor-groove binders (MGBs) such as netropsin, bisamidines (*e.g.,* furamidine) or bisguanidine-like derivatives [12], all of which share a semi-flat core and two cation-like functionalities at opposite sites of this core, facilitating the fitting and ionic interaction within the narrow and positively charged groove.

Moreover, the large planar surface of a terminal GQ has led to the development of drug candidate families based on heteroaromatic systems which complement the GQ platform [2]. As mentioned previously, a common feature of GQ DNA-binding ligands (such as BQQ1, telomestatin, oxazoles, porphyrins, etc.) is the presence of an extended aromatic ring system that allows binding through π-π overlap of terminal G-tetrads. Some examples of GQ-stacking ligands are the alkylamine substituted acridines developed by Neidle (*e.g.,* BRACO-19, with anti-cancer activity by stabilizing the telomer of human uterus carcinoma) [3,8], the perylene diimide (PIPER, stacks on the terminal G-tetrad of the human telomer) and the fluoroquinolone (quarfloxin, in phase-II clinical trials for treatment of neuro-endocrine tumors by targeting GQ-cMyc gene promoter) derivatives prepared by Hurley and coworkers [13], the naphtalenediimides reported by Neidle and Hartley (MM_{41}, with anti-tumor activity against pancreatic cancer) [3,8], or the dibenzophenanthrolines (*e.g.,* MMQ_3) and bisquinolinium derivatives (Phen-DC6, high affinity GQ ligand) developed by Teulade-Fichou and Mergny [3,14]. Although generally flat aromatic molecules stacking on G-tetrads show higher binding constants than non-planar systems, planarity by itself is insufficient for high-affinity and unlikely to confer selectivity.

Another class of compounds which has gained wide interest with regards to DNA binding is porphyrins. Initially, planar compounds, such as 5,10,15,20-tetrakis(*N*-methyl-

pyridyl)porphyrin, were found to bind to GQs through π-π-stacking with external GQs [15]. However, their affinity for dsDNA and GQ is quite similar. Selectivity for the latter could be improved through the addition of bulky cationic substituents at the meso position of the porphyrin or tetrapyrrole core manipulations, although only the former resulted in increased telomerase inhibition. Generally speaking, the present state-of-the-art of structure-activity relationships indicates that porphyrin-DNA binding is dependent on a subtle interplay of base-stacking and charge-charge interactions [16].

Another intriguing approach relates to the use of non-planar porphyrins [17]. At a first glance this might seem counter-intuitive, as distorted porphyrins cannot π-stack with bases. Nevertheless, work has indicated that non-planar porphyrins can also be GQ specific. Classic non-planar porphyrins are *N*-substituted derivatives, wherein core substitution results in macrocycle distortion. Historically, these were developed as inhibitors of ferrochelatase and now are under scrutiny with regard to their DNA interactions [18]. The free-base (core neutral) 21-methyl mesoporphyrin has excellent GQ specificity but relatively poor GQ affinity due to the two propionate side chains [19]. Modulation of its fluorescence can be used to detect GQ *in vitro*, the porphyrin up-regulates genes with promoters showing a high potential for GQ formation, and it also suppresses rDNA activity in yeast. Intriguingly, it is specific for parallel-stranded GQ structures and does not bind to anti-parallel ones. A recent crystal structure now shows that the *N*-methyl group fits into the center of the parallel GQ core increasing complementarity with the G-tetrad, sitting at the end of a chain of bridging potassium ions and identifies design principles for optimized binding [20].

The recent approval of the DNA binder *Trabectidin* (Yondelis, Johnson & Johnson) for the treatment of some cancers has renewed the interest in DNA MGBs [21]. Following on from this, a combination of groove binding and stacking interactions traditionally used in GQs ligands will result in agents with enhanced efficacy and selectivity. As such, taking these recent developments together, a new approach to use *N*-substituted porphyrin based ligands to target GQs comes to mind. For example, the preparation of *N*-alkylated porphyrins with peripheral amino groups would allow their linkage to other DNA binding small-molecules (*e.g.*, bisguanidine-like systems) to yield molecules with dual modality. Such conjugates are hypothesized to target GQs well by binding to the grooves through their DNA binding part or by intercalating between or stacking with the G-tetrads *via* the porphyrin moiety. Additionally, this will enhance detection of GQs by means of the porphyrin dyes. This would be a new concept to achieve dual-modality binders, having features of high-affinity small-molecules with the added benefits of porphyrins as fluorescent imaging agents. Furthermore, it would circumvent the current use of 21-methyl mesoporphyrin as a mixture of stereoisomers by employing chemically pure compounds in a more rational fashion.

At present, and considering the serious side-effects of existing drugs, DNA specific recognition is the critical factor for the development of DNA-targeting small-molecule drugs. Current efforts concentrate on drug specific interaction with DNA recognition points (*i.e.,* mismatches or bulge recognition), specific sequence recognition and secondary structure

recognition. Fluorescent probes capable of the structure-specific reporting of GQ structures *in vivo* are needed as tools for basic biological research and the exploration of GQ as a potential drug target. Hence, the development of structure-selective fluorescent probes capable of detecting GQs in living cells is mandatory. As outlined, the semi-planar arrangement of the aromatic rings in *N*-substituted porphyrins provides the potential for specific binding to GQs *via* stacking on the G-tetrad and locking into the central space. In addition, porphyrins are fluorescent (*i.e.,* suitable for diagnostics) and also can produce reactive oxygen species, thus photochemically inducing strand breaks (although nonplanar porphyrins have lower triplet quantum yields and thus require molecular engineering [18]). Therefore, they offer potential as theranostic agents for nucleic acid targeting.

Clearly both individual approaches – small DNA binding molecules and porphyrin-based intercalators – suffer some drawbacks, but a combination thereof can be envisaged to yield new dual-modality portmanteau binders to achieve selective GQ stabilization and enhanced detection. Even though challenging, GQ *versus* dsDNA selectivity is the key for efficient targeted anti-cancer therapy since more selective drugs may avoid secondary effects on cancer patients, assuring their cure.

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