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Tetrasubstituted naphthalene diimide ligands with selectivity for telomeric G-quadruplexes and cancer cells

Sonja M. Hampel [a], Assitan Sidibe [b], Mekala Gunaratnam [a], Jean-François Riou [b], Stephen Neidle [a,*]

[a] CRUK Biomolecular Structure Group, The School of Pharmacy, University of London, London WC1N 1AX, UK
[b] Laboratoire de Biophysique, Muséum National d'Histoire Naturelle, INSERM U655, CNRS UMR 7196, Paris, France

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ABSTRACT

A series of tetrasubstituted naphthalene diimide compounds with *N*-methylpiperazine end groups has been synthesized and evaluated as G-quadruplex ligands. They have high affinity and selectivity for telomeric G-quadruplex DNA over duplex DNA. CD studies show that they induce formation of a parallel G-quadruplex topology. They inhibit the binding of hPOT1 and topoisomerase IIIα to telomeric DNA and inhibit telomerase activity in MCF7 cells. The compounds have potent activity in a panel of cancer cell lines, with typical IC_{50} values of ~0.1 μM, and up to 100-fold lower toxicity in a normal human fibroblast cell line.

The immortality of cancer cells is at least in part dependent on the maintenance of telomeres, the nucleoprotein complexes at the ends of chromosomes.[1] Telomeres are shortened in somatic cells, leading to their limited life span, whereas they are stabilized in cancer cells. The function of telomerase, which is over-expressed in >80% of cancer cells, is to both physically protect and maintain telomere length.[2] As telomeric DNA comprises repeated short G-tracts, it is able to fold into G-quadruplex structures, in which three planes of hydrogen-bonded G-quartets are held together by π–π interactions. Depending on the coordinating ion, the presence of small-molecule ligands, the DNA sequence and other factors such as molecular crowding, telomeric DNA G-quadruplexes can have a diversity of topologies.[3]

Quadruplex formation can affect a wide range of cellular processes. The stabilization of G-quadruplex structures in the single-stranded 3′ telomeric DNA overhang by small-molecule ligands has been shown to indirectly inhibit telomerase and telomere maintenance in cancer cells.[4] Quadruplex formation along the overhang can also displace the single-stranded binding protein hPOT1.[5] The function of telomere-associated Holliday junction decatenases such as topoisomerase IIIα (Topo IIIα), (essential for the maintenance of telomeres in the ALT (alternative lengthening of telomeres) pathway,[6] can be affected by quadruplex-binding small molecules.[21c] Promoter regions of oncogenes such as *c-myc* and *c-kit* contain sequences that may fold into G-quadruplexes,

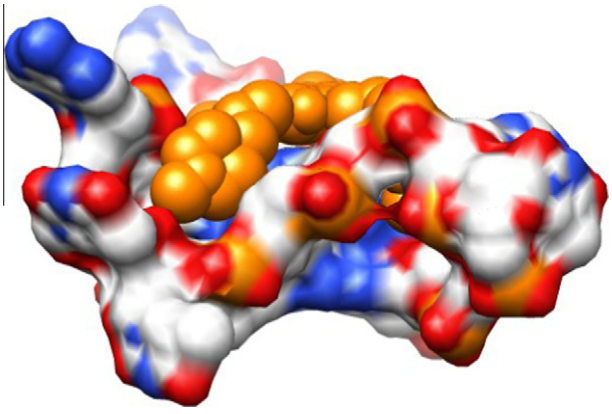

Figure 1. Modeled structure of compound **1** (orange) docked on the 3′ face of the parallel human telomeric G-quadruplex 22-mer DNA (side view) using the crystal structure of a known ND complex as a starting-point.[11] Positive and negative charge regions on the solvent-accessible surface of the DNA quadruplex are marked in blue and red, respectively.

and their stabilization can down-regulate oncogene expression.[7] The multiple pathways caused by the stabilization of telomeric G-quadruplexes can lead to uncapping and end-to-end fusions of chromosomes, followed by cell cycle arrest, senescence and growth inhibition, as well as DNA damage response and apoptosis in cancer cells.[5b,c]

A large number of G-quadruplex binding small molecules have been reported.[8] Tetrasubstituted naphthalene diimides (NDs) are

* Corresponding author. Tel.: +44 207 7535969; fax: +44 207 7535970.
E-mail address: stephen.neidle@pharmacy.ac.uk (S. Neidle).

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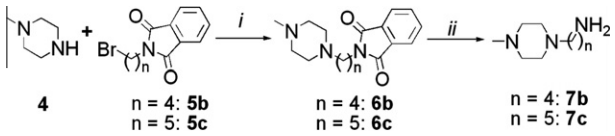
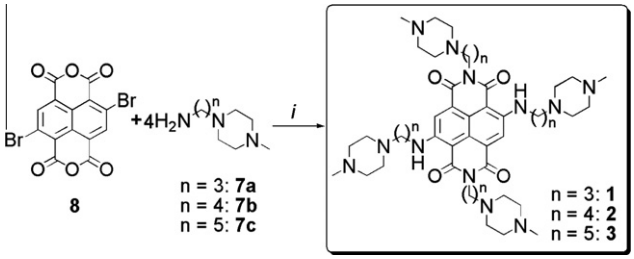


Scheme 1. Synthesis of the side chain amines **7b** and **7c**. Reagents and conditions: (i) Na_2CO_3, toluene, reflux, 16–18 h, 58–85%. (ii) (1) N_2H_4, EtOH, reflux, 3 h; (2) HCl, reflux, 30 min, 46–61%. The amine **7a** ($n = 3$) was commercially available.



Scheme 2. Synthesis of the NDs **1**–**3**. Reagents and conditions: (i) microwave, 150 °C, 20 min–2 h, 11–19%. The starting material **8** was synthesized according to the established procedure.[14]

Table 1
G-quadruplex stabilization of compounds **1**–**3** in the FRET melting temperature assay. Esds in ΔT_m are ±0.1 K.

DNA type	FRET ΔT_m [K], $c = 0.5$ μM		
	1	2	3
F21T G4	28.3	24.7	23.8
c-kit1 G4	1.8	4.9	1.5
c-kit2 G4	15.2	16.7	7.7
T-loop	1.3	0.1	0.2

very potent G-quadruplex ligands with high cellular toxicity.[9] NDs have been of broader interest because of their tunable electro- and photo-chemical properties and their applications in supramolecular chemistry.[10] They have a delocalized electron system, which is able to effectively stabilize the terminal G-quartets of a G-quadruplex by stacking interactions. Four substituents with amino end groups can fit into the four grooves at the sides of the G-quadruplex and interact electrostatically, as indicated by molecular modeling (Fig. 1), and demonstrated by X-ray crystallography.[11] One compound from these series, which has high affinity for the G-quadruplexes found in the promoter region of the *c-kit* gene, has been studied for its effects on gastrointestinal stromal tumor cell lines.[12] We report here on a novel subset of NDs with *N*-methylpiperazine end groups, which have enhanced telomeric quadruplex and cellular selectivity.

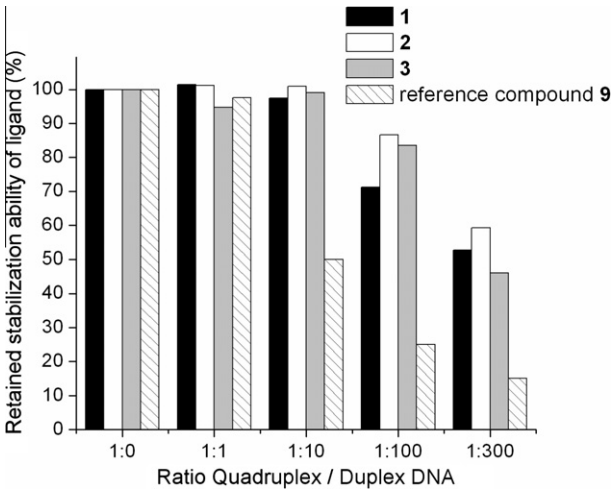


Figure 2. Competition FRET experiment showing the selectivity of **1**–**3** for the F21T G-quadruplex DNA sequence over duplex DNA. Different ratios of excess duplex DNA were added (concentrations of G-quartets vs nucleotide phosphates of duplex DNA). The reference compound **9** is a derivative of compound **1** with dimethylamine end groups (see Supplementary data).

Initially, novel ND derivatives with enhanced selectivity for telomeric G-quadruplexes were conceived by qualitative molecular modeling using a parallel G-quadruplex from a ND co-crystal structure.[11] Side-chains of 3–5 carbon atoms with bulky methylpiperazine end groups gave optimal groove interactions. Overlay of **1** onto the known position of the related ND compound (Fig. 1) indicated that the *N*-methylpiperazine groups, which are bulky and can be protonated easily, reach deep into the grooves at the sides of the G-quadruplex and interact strongly with phosphate moieties.

The amines **7b** and **7c**, which were used as side-chains, were obtained via Gabriel synthesis (Scheme 1).[13] For this, *N*-methylpiperazine was added to the bromoalkylphthalimides **5b** or **5c** in

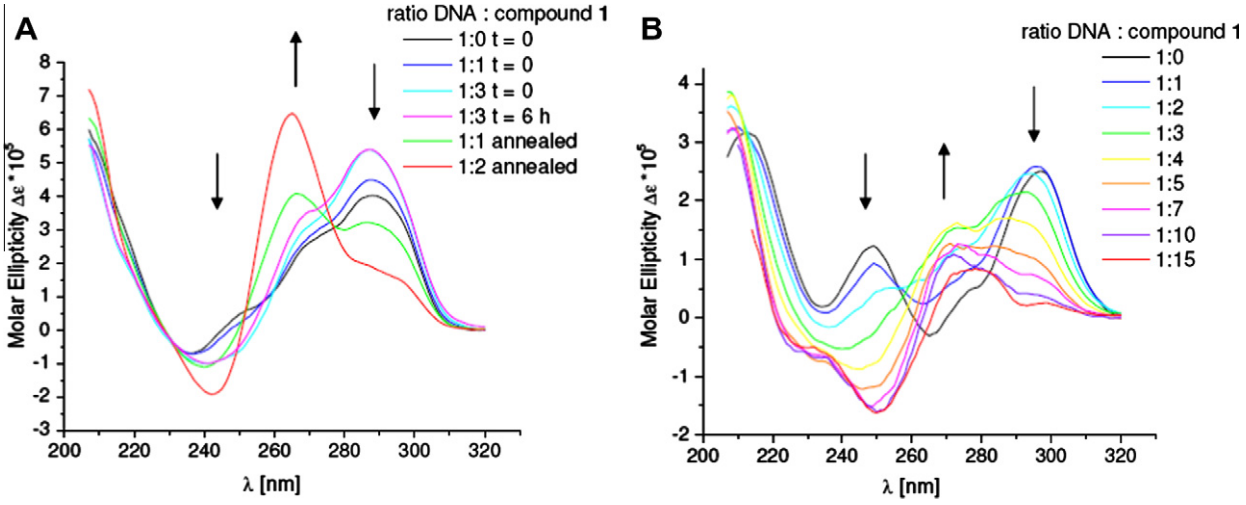


Figure 3. Circular dichroism (CD) spectra of a human telomeric 23-mer DNA with various ratios of compound **1**, at pH 7.4. (A) CD spectrum, 100 mM K^+. (B) CD spectrum, 100 mM Na^+. Compounds **2** and **3** gave similar results (see Supplementary data).