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Stabilization of G-Quadruplex DNA by Highly Selective Ligands via Click Chemistry

Adam D. Moorhouse,[†] Ana Mafalda Santos,[†] Mekala Gunaratnam,[‡] Michael Moore,[‡] Stephen Neidle,*[,‡] and John E. Moses*[,†]

Department of Pharmaceutical and Biological Chemistry and CRUK Biomolecular Structure Group, The School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX, United Kingdom

Received September 1, 2006; E-mail: john.moses@pharmacy.ac.uk

The telomeres of eukaryotic species comprise specialized protein−DNA complexes that protect chromosome ends, ensuring that end-to-end fusions, recombination, or damage do not occur.[1] These involve tandem repeats of guanine-rich DNA sequences, typified by [TTAGGG] in vertebrates. There are fundamental differences between telomere maintenance in somatic compared to cancer cells, which may be exploitable therapeutically.[2] The progressive shortening of telomeres in the former eventually leads to senescence and apoptosis. Telomeres in cancer cells, by contrast, are stabilized in length, in the overwhelming majority of tumors by the activation of the telomerase enzyme complex, which has reverse transcriptase activity such that it is able to catalyze the synthesis of further telomere repeats onto the 3′ single-stranded end of telomeres.[3] Telomerase is expressed in 80−85% of tumor cells and primary tumors[4] and plays a major role in cellular immortalization and thus in tumorigenesis itself. An approach to telomerase inhibition that has received particular attention utilizes telomeric DNA sequences themselves, which have been shown to fold into guanine-rich quadruplex structures,[5] as detailed by crystallographic[6] and NMR studies.[7] Since the enzyme's endogenous RNA template has an absolute requirement for the 3′ terminus of the DNA telomere primer strand to be single-stranded in order for effective hybridization with it to occur, stabilization of these quadruplex structures by small molecules can lead to the inhibition of telomerase,[8] thereby selectively interfering with telomeric maintenance in tumor cells. A large number of such small molecule ligands have been reported,[9] and experimental in vivo activity has been shown for the synthetic molecule BRACO-19[10] and the natural product telomestatin.[11] Both compounds involve multistep syntheses.[10b,12]

Through molecular modeling and structural data, it has been possible to develop a rational approach to the design and optimization of quadruplex stabilizing ligands.[13] Most G-quadruplex ligands have a central planar pharmacophore capable of binding to guanine tetrads by means of $\pi-\pi$ interactions. The most selective synthetic binders possess at least two side chains which are directed toward the quadruplex grooves. Typically, tertiary amine functionality, which can be protonated at physiological pH, is positioned at the termini of these side chains.[10]

We report here a new class of synthetically highly accessible and selective G-quadruplex stabilizing ligands. These small molecule ligands were prepared using a "click chemistry"[14] approach, taking advantage of the powerful Cu(I)-catalyzed Huisgen cycloaddition reaction.[15] The 1,4-triazole product, itself a planar heteroaromatic functionality, was found to be ideally suited for stabilizing $\pi-\pi$ stacking interactions when forming part of a pharmacophore.

In the design of our preliminary triazole-derived ligands, we selected readily accessible azide building blocks, resembling the side chains in BRACO-19[12] and other fused-ring G-quadruplex ligands.[13] Using the copper-catalyzed process,[15] each of the azide molecules was linked to a 1,3-diethynylbenzene core to yield several candidate bistriazole ligands (Scheme 1).

The ability of these compounds to stabilize G-quadruplex DNA was investigated using a high-throughput FRET (fluorescence resonance energy transfer) assay.[16] Table 1 shows the effect of different concentrations of the most strongly binding compounds **32**−**34** on the melting temperature (ΔT_m) of two labeled oligomers in 60 mM potassium cacodylate buffer at pH 7.4 (see Supporting Information for compounds **27**−**31**). F21T represents the human telomeric sequence (*5′-FAM*-d(GGG[TTAGGG]$_3$)-*TAMRA-3′*), whereas F10T (ds) is a hairpin double helix forming labeled oligomer (*5′-FAM*-dTATAGCTATA-HEG-TATAGCTATA-*TAMRA-3′*), with an internal hexaethyleneglycol (HEG) linker to form a hairpin loop. Data for the lead G-quadruplex binders BRACO-19 and telomestatin are shown for comparison.

This study revealed that compounds **32**−**34** stabilized the quadruplex with greater magnitude than **27**−**31**, perhaps due to the increased basicity of the amine side chains or to preferred interactions with the sugar−phosphate loops. **33** was shown to be the superior binder over the concentration range tested. Its effect on the melting temperature of the F21T oligomer is comparable to the potent telomerase inhibitors, telomestatin and BRACO-19.

Most significant, however, was the observed selectivity for G-quadruplex DNA versus duplex DNA displayed by all ligands over the given concentration range. None of the compounds increased the melting temperature of the duplex forming oligomer F10T (ds). We suggest that this is a consequence of the steric requirements of these compounds that do not allow intercalative binding to duplex DNA to take place. Furthermore, competition FRET experiments of F21T versus calf thymus dDNA revealed negligible effect on the binding of ligands **33**−**34** to F21T, compared to BRACO-19 (Figure 1a).

Qualitative molecular modeling using manual docking and molecular mechanics energy minimizations (Figure 1b) showed good overlap between the ligands **32**−**34** and guanine quartets.[6]

These encouraging results prompted us to investigate whether these compounds would also show telomerase inhibition in the two-step TRAP assay (Figure 2). This assay has been widely used to provide qualitative and quantitative estimates of telomerase inhibition. Using this assay, compounds **32**, **33**, and **34** showed high activity with $^{tel}EC_{50}$ values of 13.3, 17.1, and 23.5 μM, respectively. Although ultra-potent telomestatin has an $^{tel}EC_{50}$ of 0.66 μM in the same assay, the values for **32**−**34** are significant and form the basis for development of a new class of telomerase inhibitors that are readily synthesized.

[†] Department of Pharmaceutical and Biological Chemistry.
[‡] CRUK Biomolecular Structure Group.

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Scheme 1. Synthesis of Bistriazole Ligands

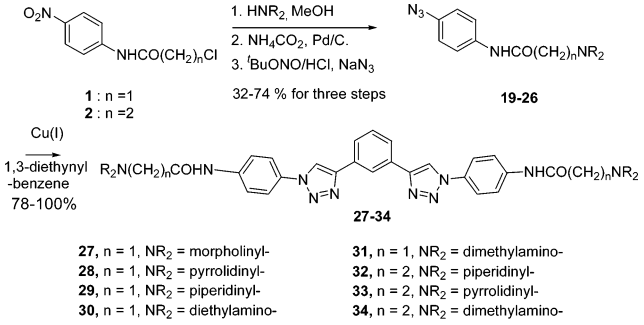

Table 1. Stabilization Temperatures Determined by FRET

	ΔT_m (°C)			
	F21T			F10T (ds)
compound	0.5 μM	1 μM	2 μM	1 μM
32	3.9	14.4	20.5	0.0
33	8.3	17.8	24.2	0.0
34	12.5	18.7	23.8	0.0
telomestatin	N/A	27.4	30.1	0.0
BRACO-19	21.6	27.5	31.2	14.5

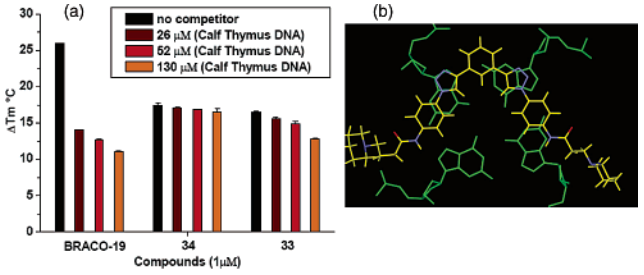

Figure 1. (a) A qualitative molecular model of compound **33** (with C, H atoms colored yellow), interacting with the G-quartet at the end of a unimolecular quadruplex structure. Only the terminal G-quartet is shown, colored green. (b) Competition FRET experiment showing melting temperature (ΔT_m) of F21T in the presence of 1 μM of ligand(s) with various concentration of calf thymus dDNA (μM (phosphate)).

In summary, we have designed a new class of G-quadruplex stabilizing ligands and demonstrated that "clicked" triazole linkers form part of a pharmacophore capable of π-stacking interactions with G-tetrads. We have synthesized a first generation of compounds which show excellent affinity and selectivity for the G-quadruplex. Moreover, we have demonstrated that click chemistry, and specifically the Cu(I)-catalyzed Huisgen cycloaddition reaction, has applicability in the field of G-quadruplex ligand discovery. These discoveries should enable a new modular approach to the identification and synthetic attainment of lead structures, analogues, and decoration of existing quadruplex binders, leading to increased diversity and, in turn, selectivity and potency.

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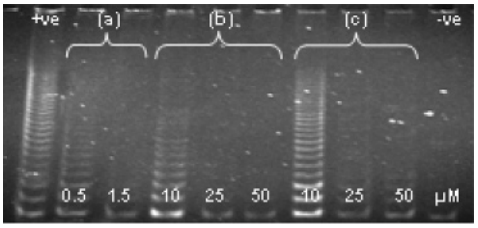

Figure 2. TRAP gel for (a) telomestatin, (b) **32**, and (c) **34**, showing characteristic ladders produced by PCR amplification of the oligonucleotides generated by the activity of telomerase on a TS primer.

Supporting Information Available: Synthetic procedures for all compounds. Details for FRET and TRAP assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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