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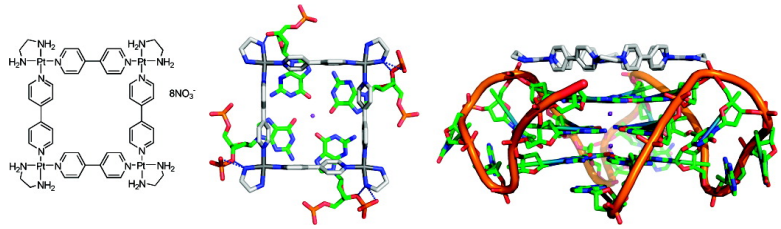
J. Am. Chem. Soc., **2008**, 130 (31), 10040-10041 • DOI: 10.1021/ja8014023 • Publication Date (Web): 11 July 2008



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Published on Web 07/11/2008

A Platinum Supramolecular Square as an Effective G-Quadruplex Binder and Telomerase Inhibitor

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In recent years, significant attention has been devoted to the role of G-quadruplexes in cancer. These structures can arise from the folding of the single-stranded G-rich 3′-end of the telomere into a planar tetrad of guanine bases, held together by a Hoogsteen hydrogen-bonded array.[1−3] Small molecules that bind and stabilize this structure have been demonstrated to inhibit the enzyme telomerase, which is active in 85−90% of cancer cells and inactive in somatic cells.[4] In addition, stabilization of G-quadruplexes by small molecules has recently been shown to inhibit the transcriptional activity of some oncogenes.[5−7] Thus, the G-quadruplex motif has emerged as a promising target for the design of selective antitumor therapeutics.[8,9]

Conventional G-quadruplex binders are organic molecules that possess large π-aromatic surfaces and positive charges near the center of the molecule or on side arms to increase affinity to the grooves of the quadruplex.[10−14] These features have been designed considering the G-quadruplex structure, but also what can be realistically achieved using traditional multistep synthesis. A particularly attractive strategy would be to construct G-quadruplex binders using *supramolecular self-assembly* rather than covalent synthesis. This approach can create complex structures from simple building blocks, in a single operation and often quantitatively. It also readily lends itself to the generation of synthetic libraries to optimize binding affinity and specificity. Importantly, this method would result in molecules with distinct structural features, which are unattainable using conventional covalent synthesis, and are particularly useful for targeting higher-order biological motifs such as the G-quadruplex.[17−19]

As a molecular recognition target, the G-quadruplex presents four guanine residues oriented in a square-like arrangement, with an electron-rich π-surface. One supramolecular structure that is potentially compatible with these features is the platinum "square" complex, which has been extensively explored by the groups of Fujita[15] and Stang.[16] Herein we report that the self-assembled platinum molecular square $[Pt(en)(4,4'\text{-dipyridyl})]_4$ (**1**) is an efficient G-quadruplex binder and telomerase inhibitor. Molecular modeling studies show that the square arrangement of the four bipyridyl ligands, the highly electropositive nature of the overall complex, as well as hydrogen bonding interactions between the ethylenediamine ligands and phosphates of the DNA backbone all contribute to the observed strong binding affinity to the G-quadruplex. Through thermal denaturation studies with duplex and quadruplex FRET probes and enzymatic assays, we demonstrate that platinum square **1** strongly binds to G-quadruplexes and can act as an inhibitor of telomerase. This study thus underlines the potential of supramolecular self-assembly to readily generate scaffolds of unique geometries for effective targeting of G-quadruplexes and for the ultimate development of selective antitumor therapies.[17−20]

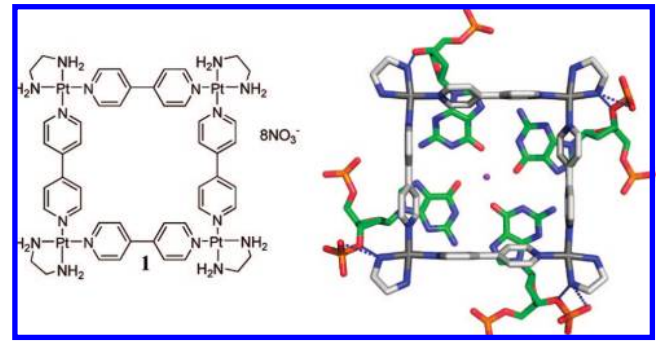

Figure 1. Left: Structure of the platinum molecular square **1**. Right: Average structure of the complex between **1** and 22-mer DNA G-quadruplex. Complex **1** is in gray and blue, and hydrogen bonding to phosphates is shown by blue dotted lines.

Molecular modeling studies were conducted to understand the mode of binding and the fit of complex **1** within the G-quadruplex structure. For this, an approach that combines automated docking, molecular dynamics (MD) simulations, and evaluation of binding affinity was examined. Our recently developed docking program FITTED,[21a] previously found to accurately predict binding modes of protein ligands,[21b] was modified for nucleic acids. With this tool, we docked **1** to an X-ray crystal structure of a G-quadruplex 22-mer.[2] The predicted most favorable binding mode was one where the platinum square **1** is parallel to the plane of the terminal G-quartet (Figure 1). In this mode, short Pt−P distances are consistent with electrostatic interaction of each of the Pt atoms of **1** with the backbone phosphates. Moreover, we observed that the NH_2 groups of each of the ethylenediamine ligands are hydrogen bonded to the phosphate oxygens.[22] As well, one of the aromatic rings in each of the 4,4′-bipyridyl ligands interacts with a guanine base in a distorted T-shape geometry.

This binding mode, along with other representative docked complexes, was further evaluated by running 4 ns MD simulations.[23] Relative free energies of binding show a stabilization of ~10 kcal/mol for the parallel mode over all other structures, consistent with end-stacking of previously reported G-quadruplex binders. Thus, molecular modeling studies confirmed the excellent complementarity in size and interactions between square **1** and the quadruplex, in a parallel end-stacked mode.

On the basis of these promising predictions, we started the synthesis and biological evaluation of **1**. Platinum molecular square **1** was readily obtained in a single step from two simple building blocks, commercially available 4,4′-bipyridine and $enPt(NO_3)_2$, which can be readily accessed from $enPtCl_2$.[24] The binding ability of square **1** to the G-quadruplex[25] was first evaluated using a FRET

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10.1021/ja8014023 CCC: $40.75

Table 1. Stabilization Temperatures Obtained for **1** Using FRET

	ΔT_m(°C) at 0.75 μM ligand concentration		[lig] for ΔT_m = 20 °C (μM)
	G4DNA	dsDNA	G4DNA
1	34.5	12.1	0.40

melting assay (Table 1). Results show large stabilization of the G-quadruplex, with an increase of 34.5 °C in the thermal denaturation temperature with 0.75 μM of **1**. This significant increase is competitive with many of the best reported quadruplex binders, such as telomestatin (30.3 °C),[13] a nickel salen complex (33.2 °C),[10] a macrocyclic oligoamide (33.8 °C),[13] a bisquinolinium (29.7 °C),[14] and BRACO-19 (27.5 °C),[10] which required 1 μM of the ligand to achieve these values. As well, the concentration of **1** necessary to achieve a ΔT_m of 20 °C (0.40 μM) is comparable or lower than that of most potent G-quadruplex binders, such as telomestatin (0.65 μM, ΔT_m = 15 °C),[13] a nickel salen complex (0.70 μM, ΔT_m = 20 °C),[10] a macrocyclic oligoamide (0.38 μM, ΔT_m = 15 °C),[13] and BRACO-19 (0.20 μM, ΔT_m = 20 °C).[10] Thus, platinum square **1** is an excellent stabilizer of the G-quadruplex motif. A subsequent FRET assay was performed to evaluate the selectivity of **1** for G-quadruplex versus duplex structures.[26] **1** does stabilize duplex DNA, undoubtedly because of its high positive charge; however, far greater stabilization for G-quadruplex was observed (Table 1 and Figure 2). These results compare well with ΔT_m values of quadruplex (27.5 °C) and duplex DNA (14.5 °C) such as for BRACO-19,[10] which inhibits telomerase in vivo.[27] Therefore, complex **1** strongly binds to G-quadruplex DNA, and this simple first generation structure already shows significant selectivity for G-quadruplexes over duplex DNA. Due to their facile construction, less positively charged derivatives of **1** can be readily accessed and may further reduce B-DNA binding affinity.

We were then interested in the potential of complex **1** to inhibit the enzyme telomerase. For this, a modified version of the telomeric repeat amplification protocol (TRAP) assay was performed (Figure 2).[23] Inhibition of telomerase by complex **1** was found to be one of the strongest of reported G-quadruplex binders, with an IC_{50} value of 0.197 $\pm$ 0.056 μM. Thus, complex **1** shows significant telomerase inhibition.[28]

In summary, we have shown that self-assembled metallosupramolecular square **1** displays high binding affinity to G-quadruplexes and efficient telomerase inhibition, which are competitive with the most potent reported binders of this motif. These data correlate well with the excellent match in size and complementarity in the interactions between this square and the G-quadruplex, as predicted by molecular modeling. This study has thus shown the potential of supramolecular self-assembly to generate scaffolds that can effectively target biological structures. Considering the wealth of readily obtainable ligands and the exceedingly simple methods to introduce synthetic modifications into these platinum squares, this allows rapid optimization of their already sizable binding to G-quadruplexes for use in selective antitumor therapy.

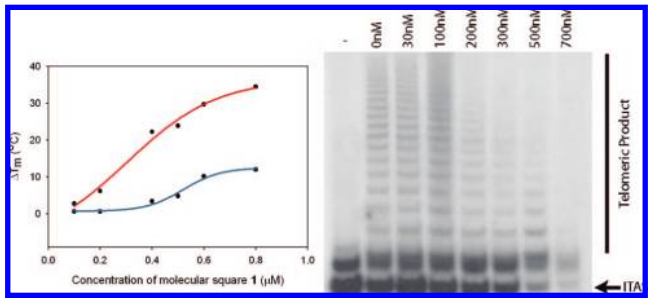

Figure 2. Left: FRET stabilization curve of square **1** with quadruplex (red) and duplex DNA (blue). Right: TRAP assay of complex **1**, showing ladders generated by the action of telomerase on a TS primer (PCR amplified). The lower band is an internal control primer (ITAS).[23]

Acknowledgment. This work was supported by NSERC, CIHR, CFI, and CSACS. C.A. is a chercheur-boursier of FRSQ. J.F. holds a CIHR Cancer Training Grant Award. P.E. holds a McConnell Fellowship. We would like to thank CLUMEQ for allocation of computer resources, and McGill and Génome Québec Innovation Centre for use of the ABI 7900HT. H.S. is a Cottrell Scholar of the Research Corporation.

Supporting Information Available: Synthesis, FRET experiments, and molecular modeling details. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA8014023