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Stabilization of G-Quadruplex DNA and Inhibition of Telomerase Activity by Square-Planar Nickel(II) Complexes

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The enzyme telomerase has an elevated activity in 85−90% of human cancer cells in comparison to normal somatic cells.[1] Telomerase inhibition has thus been identified as an attractive target for cancer chemotherapy with the potential for selective toxicity for cancer cells over normal ones.[2] Telomerase maintains telomeric DNA integrity and prevents critical shortening of the telomere so that cells cannot reach crisis points of senescence and apoptosis. Human telomeric DNA consists of the tandem repeat sequence TTAGGG and is in cancer cells typically 3−6 KB in length. The 3′-terminal 100−200 bases are single-stranded. Crystallographic[3] and NMR studies[4] have shown that repeats of this sequence can fold into guanine-rich quadruplex structures.[5] Since the substrate of telomerase is the 3′-single-stranded overhang of telomeric DNA, the stabilization of these quadruplex structures by small molecules can lead to the inhibition of telomerase[6] thereby selectively interfering with telomere maintenance in tumor cells.[7] A considerable number of organic molecules, mostly with heteroaromatic groups, have been evaluated for their ability to stabilize the formation of these quadruplex DNA structures and inhibit telomerase.[8] A few have been evaluated for anticancer activity.[9]

A detailed picture of quadruplex structure is emerging from crystallographic and NMR studies, and together with computer modeling, it has been possible to develop a rational approach to the design and optimization of quadruplex stabilizing compounds.[10] The desirable features of these stabilizing molecules are (1) a π-delocalized system that is able to stack on the face of a guanine quartet; (2) a partial positive charge that is able to lie in the center of the quartet, increasing stabilization by substituting the cationic charge of the potassium or sodium that would normally occupy that site; (3) positively charged substituents to interact with the grooves and loops of the quadruplex and the negatively charged backbone phosphates.

We have utilized these principles to design a series of nickel(II)−salphen complexes. We report here the remarkable ability of the two complexes **3** and **4** (see Scheme 1) to selectively stabilize G-quadruplex DNA.

Despite the unique electronic, structural, and optical properties of metal complexes, their ability as quadruplex DNA stabilizers remains practically unexplored.[11] Qualitative computer modeling studies (Figure 1) showed good overlap between the complexes **1**−**4** and guanine quartets. Although metal−salen and metal−salphen complexes have previously been shown to interact with duplex DNA,[12] to the best of our knowledge, they have not been reported to stabilize quadruplex structures. To increase the solubility of **1** and **2** (which were practically insoluble in water and hence their quadruplex stabilization properties could not be evaluated),

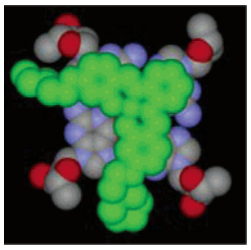
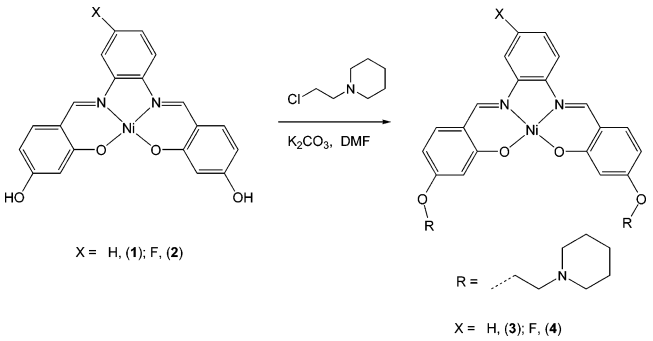


Figure 1. Docking of the nickel(II) complex **3** with the human parallel intramolecular quadruplex formed from four repeats of telomeric DNA.[3] The model shows very good stacking between the rings of the metal complex and three of the guanine rings of the quadruplex DNA.

Scheme 1. Schematic Representation of the Compounds under Study



they were functionalized with piperidine (as shown in Scheme 1) to yield the substituted derivatives **3** and **4**.

Under slightly acidic conditions (pH range 5−6), these complexes showed increased water solubility (due to protonation of the piperidine groups; see Supporting Information for measurements of their pK_a), enabling their ability to stabilize quadruplex DNA and inhibit telomerase from being investigated. Besides increasing their solubility in water, the piperidine group on the alkylamine was chosen due to the increase in binding observed when such groups have been previously attached to 3,6-disubstituted acridines.

To study whether changes in the electronic distribution of the phenyl ring on the backbone of the ligand would modify the ability of the nickel(II) complex to stack onto the G-quadruplex, a fluorine substituent was introduced in **4** (see Scheme 1). The electron-withdrawing effect of the fluorine will lower the electron density in the π-system, which should favor a stronger interaction with the electron-rich π-system of the guanine quartet. Furthermore, the presence of fluorine in the complex provides an extra spectroscopic handle (e.g., ^{19}F NMR spectroscopy) to study the interaction of this species with DNA.

The ability of **3** and **4** to stabilize G-quadruplex DNA (sequence: 5′-*FAM*-d(GGG[TTAGGG]3)-*TAMRA*-3′) was first investigated by a FRET (Fluorescence Resonance Energy Transfer)

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Table 1. Stabilization Temperatures Determined by FRET

	ΔT_m 1 μM (°C)		[conc] $\Delta T_m = 20$ °C	[conc] $\Delta T_m = 2$ °C
compound	G4 DNA	dsDNA	G4 DNA	dsDNA
3	33.2	0	0.2 μM	4.1 μM
4	32.8	0	0.1 μM	5.3 μM
BRACO-19	27.5	14.5	0.7 μM	0.1 μM

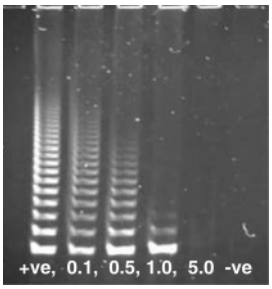

Figure 2. TRAP gel for compound **3** showing the characteristic ladders produced by PCR amplification of the oligonucleotides generated by the activity of telomerase on a TS primer. With increasing concentration of **3**, a decrease in the intensity of the ladder is observed (i.e., increase in telomerase inhibition). The negative control was run under identical conditions but omitting the protein extract to ensure absence of PCR artifacts. The intensity of the ladders was normalized with respect to the positive and negative controls, and a dose−response curve was fitted to calculate the concentration for 50% enzyme inhibition (EC_{50} value).

melting assay.[13] This study showed that both nickel(II) complexes induce a very high degree of stabilization for quadruplex DNA (T_m = 59 °C in the absence of complex; see Table 1), while high DNA concentrations are required for even a very low level of duplex DNA stabilization (sequence: 5′-*FAM*-dTATAGCTATA-**HEG**-TATAGCTATA-*TAMRA*-3′; T_m = 60 °C in the absence of complex), suggesting a selectivity of >50-fold. Data for the trisubstituted acridine compound BRACO-19 are shown for comparison.

These encouraging results prompted us to investigate if 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, both complexes showed high activity with $^{tel}EC_{50}$ values in the region of 0.1 μM ($^{tel}EC_{50} = 0.14 \pm 0.01$ and 0.12 ± 0.01 μM for **3** and **4**, respectively). This is comparable to results from the lead compound BRACO-19. A separate Taq inhibition assay was performed to measure nonspecific inhibition of Taq polymerase. Results showed inhibition of the Taq assay occurred at ca. 50-fold greater concentration (5.69 ± 0.15 and 4.78 ± 1.08 μM for **3** and **4**) than that needed for TRAP inhibition. The presence of fluorine in **4** does not appear to have a significant effect either on quadruplex binding or biological activity.

The results presented here show that the planar nickel(II) complexes **3** and **4** are excellent G-quadruplex DNA stabilizers. This is probably due to a combination of structural and electrostatic factors. The planar arrangement of the salphen rings (forced planar by coordination to the metal center) and their appropriate spacing make the complexes ideal to stack on top of the guanine tetrads. In addition, the protonated piperidine substituents are likely to interact with the grooves and loops of the quadruplex. The Ni^{2+} ion, on the other hand, is possibly playing an important role in replacing one of the metal ions that is normally coordinated in and at the ends of the central ion channel of the quadruplex. The computer modeling (Figure 1) predicts that the Ni^{2+} lies directly above the channel. The surprisingly high selectivity for quadruplex versus duplex DNA is a consequence of the particular characteristics of these compounds, which we are currently exploring further. Previous reports[12] on metal−salphen complexes have shown binding to duplex DNA, but in no case has a salphen complex been reported with the specific pendant side chains as in **3** and **4**. We therefore speculate that these are the origin of the quadruplex selectivity shown by **3** and **4**.

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Supporting Information Available: Synthetic procedures for **1**−**4**. Details for FRET, and TRAP and Taq assays; pK_a determination of **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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