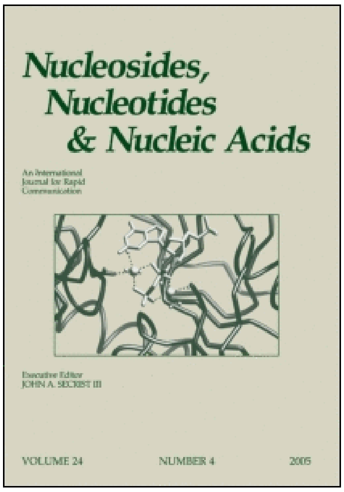

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THERMODYNAMIC ANALYSIS OF QUADRUPLEX DNA-DRUG INTERACTION

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□ *This work studies the binding properties of distamycin and its carbamoyl analog, containing four pyrrole units, with the [d(TGGGGT)]$_4$ quadruplex by means of isothermal titration calorimetry (ITC). Analysis of the ITC data reveals that drug/quadruplex binding stoichiometry is 1:1 for both interactions and that distamycin analog gives approximately a 10-fold increase in the quadruplex affinity.*

Keywords G-quadruplex; distamycin; isothermal titration calorimetry (ITC)

Telomeric DNA found at the ends of chromosomes contains continuous repeats of guanine-rich sequences that protect its ends from degradation and recombination. Sequence repeats are simple motifs that vary depending on the organism; examples are TTAGGG, TTTTGGGG, and TTGGGG, which are found in telomeres of *Homo sapiens*, *Oxytricha*, and *Tetrahymena*, respectively.[1] Telomeric sequences have the ability to form G-quadruplex structures. The basic unit of the G-quadruplex structures is the G-tetrad, which consists of a planar association of four guanines in a cyclic Hoogsteen hydrogen-bonding arrangement.[2] The truncated sequence of *Oxytricha* and *Tetrahymena* telomeric DNA, d(TGGGGT), forms a tetramolecular quadruplex in presence of cations, with a parallel-stranded, right-handed helical structure containing four equivalent grooves (Figure 1).[3]

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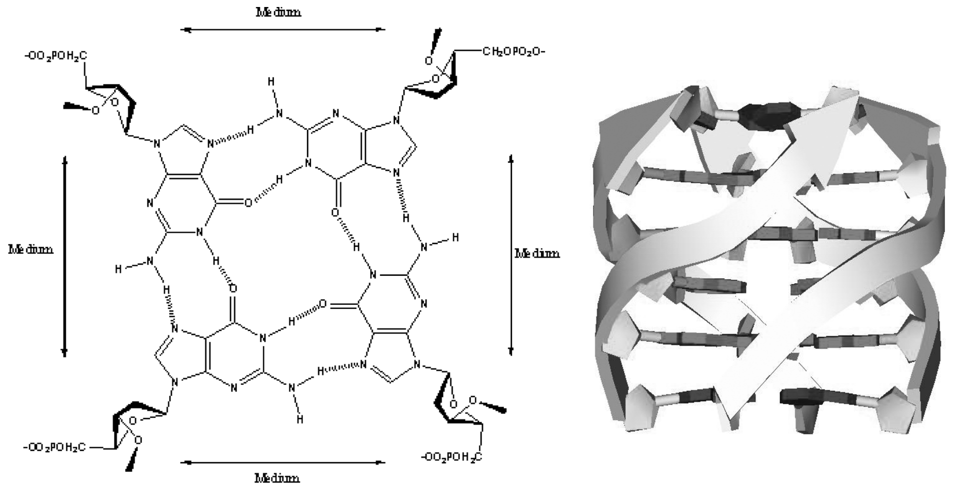
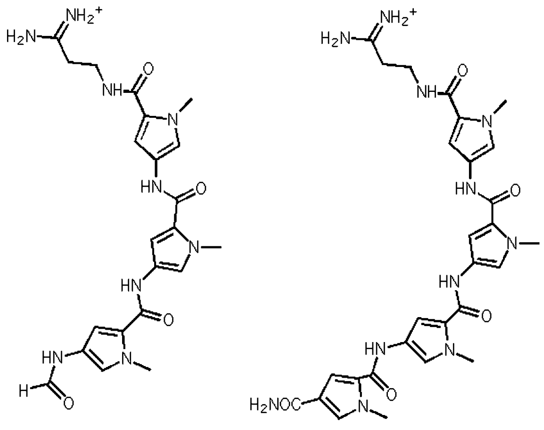


FIGURE 1 Molecular representation of a G-tetrad with four grooves of identical medium width (left) and [d(TGGGGT)]$_4$ right-handed helical structure with parallel strands (right).

The formation of G-quadruplex structures plays a key role in interfering with telomerase action, suggesting it as a possible therapeutic target for telomerase inhibition in cancer therapy. Agents that bind and stabilize G-quadruplexes have, then, the potential to act as anticancer agents.[4]

A large number of such small quadruplex-binding molecules has been studied and some show cellular effects consistent with G-quadruplex formation and telomere targeting.[5]

Distamycin A (Figure 2) is a duplex DNA binding ligand but, recently, has also been shown able to interact with quadruplex DNA.[6,7] Additionally,



Distamycin **1**

FIGURE 2 Chemical structures of the quadruplex-interactive compounds.

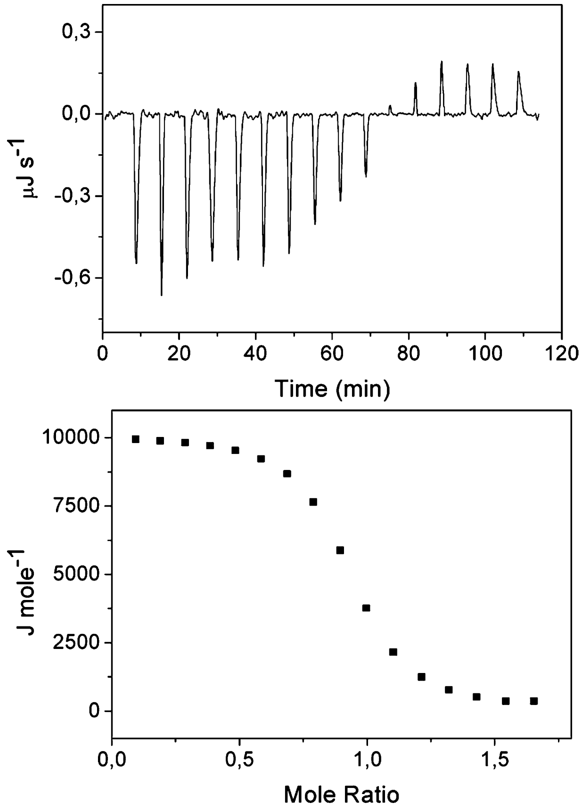


FIGURE 3 ITC experiments for the binding of **1** to [d(TGGGGT)]$_4$. Calorimetric response (top panel) and the plot of heat versus mole ratio (bottom panel) are shown.

derivatives of distamycin A have been reported to be inhibitors of the human telomerase enzyme.[8]

The aim of this work was to investigate the interaction of distamycin A and its analog (**1**) with the [d(TGGGGT)]$_4$ quadruplex by means of isothermal titration calorimetry (ITC).

ITC is the only technique that directly measures the binding enthalpy and is a valuable tool for investigating the energetics of macromolecule-ligand interactions since allows dissection of the free energy of binding into enthalpic and entropic components in order to reveal the overall nature of the forces that drive the binding reaction.[9]

Compound **1**, a carbamoyl analog of distamycin A containing four pyrrole units (Figure 2), was synthesized as previously described.[10]

DNA quadruplex was formed by dissolving solid lyophilized oligonucleotide in the appropriate buffer and heating the solution at 90°C for 5 min. The solution was then cooled slowly to room temperature and equilibrated for 1 day at 4°C. The buffer used was 10 mM sodium phosphate,

TABLE 1 Thermodynamic parameters for Binding to [d(TGGGGT)]$_4$ in Na$^+$ containing buffer solution at 25°C

Ligand	n	$K_b(M^{-1})$	$\Delta_bH°$ (kJ mol^{-1})	$\Delta_bG°$ (kJ mol^{-1})	$T\Delta_bS°$ (kJ mol^{-1})
Distamycin	1.0 ± 0.1	$2.0 \cdot 10^5 \pm 1 \cdot 10^5$	−14.0 ± 0.5	−30 ± 1	16 ± 1
1	0.9 ± 0.1	$2.3 \cdot 10^6 \pm 1 \cdot 10^6$	10.0 ± 0.5	−36 ± 1	46 ± 1

70 mM NaCl, 0.2 mM EDTA at pH = 7.0. Figure 3 shows the results for the calorimetric titration of compound **1** into [d(TGGGGT)]$_4$ solution.

Calorimetric titrations were typically carried out by injecting 15 μL aliquots of a 300–900 μM ligand solution into a 30–50 μM quadruplex solution at 400 second intervals at 25°C for a total of 16 injections. Titration curves were corrected for heat of dilution determinated by injecting the ligands into the buffer.

The binding isotherms resulting from titrations, after the appropriate correction, were fitted to give the binding enthalpy ($\Delta H°$), equilibrium binding constant (K_b), and stoichiometry (n). The remaining thermodynamic parameters, $\Delta G°$ and $T \cdot \Delta S°$, were derived using the standard relationships.

The thermodynamic parameters determined are collected in Table 1. Although the binding stoichiometry is 1:1 for both interactions, the binding behavior was found to be considerably different. In fact, examination of the data reveals that: 1) the binding is enthalpy driven for the distamycin and entropy driven for the compound **1**; 2) Compound **1** gives approximately a 10-fold increase in quadruplex affinity, particularly, K_b values of 2.3×10^6 M^{-1} and 2×10^5 M^{-1} have been found for **1** and for distamycin, respectively.

In summary, this ITC study shows that the structural modifications of the compound **1** influence the energetic of interaction with [d(TGGGGT)]$_4$.

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