J|A|C|S COMMUNICATIONS

Published on Web 01/30/2007

Highly Efficient G-Quadruplex Recognition by Bisquinolinium Compounds

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Trying to control and regulate the expression of genes is emerging as a very appealing anticancer strategy. Indeed, chemotherapy aiming at designing molecules able to interact with unusual structures of nucleic acids is currently subjected to a close examination.[1] In that sense, quadruplex−DNA is a particularly attractive high-order structure since it appears to be putatively present in pivotal genomic regions such as telomeres,[2] promoters of oncogenes, and most growth control genes.[3] Convincing reports on the efficiency of quadruplex interacting molecules as therapeutically active agents are beginning to appear in the literature.[4]

Thus, intensive investigations are currently oriented toward the design and development of new G-quadruplex ligands. Of particular interest are molecules with high quadruplex affinity that exhibit the ability to discriminate quadruplex−DNA from nucleus predominant duplex-DNA. Up to now, the leading G-quadruplex binder has been the natural product telomestatin.[5] Nevertheless, its total synthesis was achieved only very recently, and the complexity of the process renders its convenient exploitation difficult.[6] Recently, some of us reported on bisquinolinium compounds that exhibit exceptional affinity and selectivity for quadruplex-forming oligonucleotides.[4c,7] The anti-proliferative activity of these compounds has been demonstrated, as well as their preferential binding to telomeric regions of human chromosomes,[7b] thus providing new insights on quadruplex existence in vivo. These exciting results prompted us to develop the bisquinolinium family of compounds which was furthermore facilitated by a convenient and rapid synthetic access.

In the initial series, the two quinolinium moieties are connected through a 2,6-pyridodicarboxamide unit.[4c,7] This motif, well-known to adopt an internally organized H-bonded *syn*−*syn* conformation (Figure 1A),[8] was shown to be critical for quadruplex binding. Indeed, inversion of the amide connectivity leads to a loss of affinity (Supporting Information).[4c] This suggests that the central pyridodicarboxamide unit locks the ligand in a crescent-shaped conformation highly favorable for G-quartet overlap. On this basis, we reasoned that expanding the aromaticity of the central core without disrupting either the H-bonds network or modifying the cationic side-arm nature could result in improved recognition properties.

To this end, 6,6′-disubstituted-2,2′-bipyridine and 2,9-disubstituted-1,10-phenanthroline units that are also susceptible to be conformationally locked via H-bonding were considered as good candidates for replacing the pyridine central core. Ligands **1a**/**b** and **2a**/**b** (Figure 1B) were synthesized via straightforward three- and four-step procedures respectively, from inexpensive commercially available material (Supporting Information).

The ligand's ability to stabilize a quadruplex structure was evaluated by FRET experiments using the quadruplex-forming engineered oligonucleotide F21T (*FAM*-$G_3[T_2AG_3]_3$-*Tamra*), which

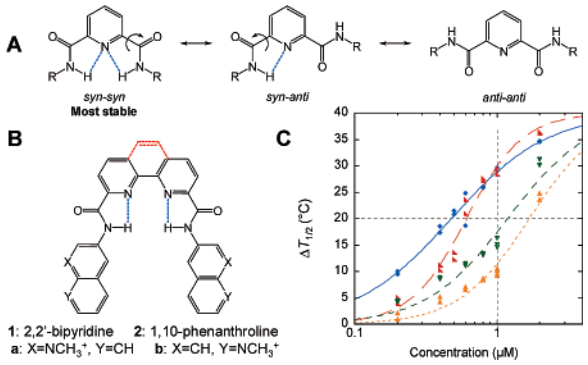

Figure 1. (A) Alternative conformations of pyridodicarboxamide unit (H-bonds appear as dotted lines). (B) Structure of **1a**/**b** and **2a**/**b** (with triflate as counterions). (C) Dose−response curves for FRET results ($\Delta T_{1/2}$) in function of ligand concentration for **1a** (green), **1b** (orange), **2a** (blue), and **2b** (red). The baseline ($\Delta T_{1/2}$ = 0 °C) was set at the melting temperature of the structure without ligand (48 °C).

mimics the human telomeric repeat (Supporting Information).[9] As shown in Figure 1C, compounds **2a**/**b** (blue/red curves) appear as remarkably strong quadruplex stabilizers, while more modest effects are obtained with **1a**/**b** (green/orange curves). Indeed, $\Delta T_{1/2}$ values at 1 μM dose of ligand (vertical gray line, Figure 1C) are significantly higher with **2a**/**b** ($\Delta T_{1/2(1\mu M)}$ = 29.7 and 28.5 °C, respectively) than with **1a**/**b** ($\Delta T_{1/2(1\mu M)}$ = 15.2 and 9.6 °C, respectively). Accordingly, the concentration required to achieve a $\Delta T_{1/2}$ value of 20 °C (horizontal gray line, Figure 1C) is also significantly lower with **2a**/**b** ($[conc]_{(\Delta T_{1/2}=20°C)}$ = 0.48 and 0.61 μM, respectively) than with **1a**/**b** ($[conc]_{(\Delta T_{1/2}=20°C)}$ = 1.19 and 1.69 μM, respectively).[10] Altogether, these data reflect a very high level of quadruplex stabilization for ligands **2** and represent a significant improvement as compared to the pyridine series. Interestingly, these results make this new series fully competitive with the high-affinity G-quadruplex binders such as telomestatin, extended acridines, and organometallic complexes all exhibiting $\Delta T_{1/2(1\mu M)} > 20$ °C.[10] Most important, the differences between the two series highlight that structural rigidity is a key parameter for quadruplex recognition, the free rotation around the biaryl axis of ligands **1** being responsible for the lower performance of this series.

To gain further insights into the intrinsic qualities of these ligands, competitive FRET experiments were performed in the presence of various amounts of 26 bp duplex-DNA (ds26, from 0 to 10 μM, Supporting Information).[11] Remarkably, the thermal stabilization induced by **2a**/**b** is only poorly affected (∼10% loss) by the presence of 10 molar equiv of ds26 (Figure 2A,B). This indicates that ligands **2** exhibit an exquisite quadruplex versus duplex selectivity, thus behaving similarly to telomestatin in the same conditions (Supporting Information).[12] Again, bipyridine derivatives **1** appear less competent since they are more sensitive

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10.1021/ja067352b CCC: $37.00

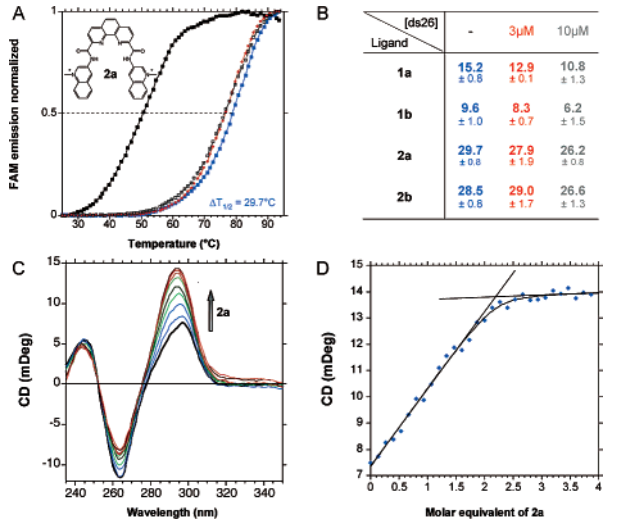

Figure 2. (A) FRET experiments carried out with **2a** and F21T without ligand (black) and with 1 μM **2a** in absence (blue) or presence of competitive duplex (ds26, 3 equiv (red) or 10 equiv (gray)). (B) FRET results ($\Delta T_{1/2}$, °C) for **1a**/**b** and **2a**/**b** (1 μM) in absence (blue) or presence of competitive ds26 (3 equiv (red) or 10 equiv (gray)). (C and D) CD titration of 22AG (3 μM in 10 mM lithium cacodylate, pH 7.2, 100 mM NaCl buffer) by increasing amounts of **2a**: (C) some CD spectra at 296 nm from the titration experiment; the arrow indicates the increasing amounts of ligand (from black to red curves: 0, 1.2, 2.4, 3.6, 4.7, 5.9, 7.1, 8.2, 9.4, and 10.9 μM); (D) CD signal as a function of **2a** molar equivalents (0.4 μM increments).

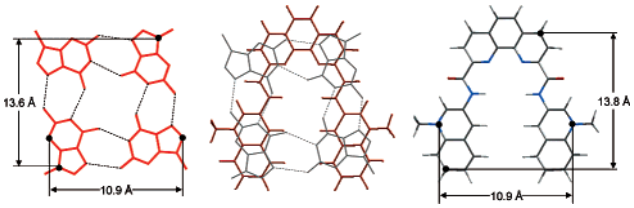

Figure 3. Selected dimensions of a G-quartet (left, determined from X-ray structure) and of **2a** (right, after molecular mechanics (MM2) calculations (Chem3D Ultra 8.0, CambridgeSoft Corp., MA)).

to the duplex competition (29 and 35% loss in stabilization for **1a**/**b**, respectively (Figure 2B).

To determine the stoichiometry of association of **2a** and G-quadruplexes, CD titrations were carried out using the 22AG sequence ($AG_3[T_2AG_3]_3$, in Na^+ buffer, Figure 2C and Supporting Information).[13] Analysis of the data indicates that the curve inflection occurs at ~2:1 ligand/quadruplex ratio (Figure 2D). This 2:1 stoichiometry is consistent with a binding mode based on the stacking of the ligand onto the two external G-quartets of the quadruplex (Supporting Information).

A close examination of the crystal structure of 22AG (1KF1, RCSB Protein Data Bank)[14] shows that a G-quartet can be considered as a square aromatic surface whose dimensions are closely related to that of **2a** (Figure 3 and Supporting Information). The strong stabilization properties of ligands **2** could thus originate in this accurate geometrical complementarity. Subsequently, the molecular size may be unfavorable for interaction with a classical base pair in duplex DNA,[1] resulting in the high preference for the quadruplex. Consequently, the molecular organization of the central core (internal H-bonds) and electronic/electrostatic properties (two quinolinium side arms) make phenanthroline bisquinolinium derivatives **2** perfectly fitted for the recognition of the quadruplex target.

Finally, the inhibitory properties of these ligands were evaluated via a classical TRAP assay (Supporting Information). However, preliminary data were obtained which suggest that inhibition measured by TRAP does not actually reflect telomerase inhibition but may result from an inhibition of the PCR amplification on quadruplex-prone motifs even though the internal PCR control (ITAS) is not affected (Supporting Information). Detailed explanations will be reported elsewhere. Nevertheless, the obtained results, among the best reported to date, represent a ~20-fold improvement as compared to the parent pyridine series[4c,7] and may reflect somehow the very good affinity of those ligands for quadruplex-prone motifs, confirming FRET melting results.

In conclusion, the present paper describes the quadruplex-binding properties of new members of the bisquinolinium family. Their easy synthetic access combined with exceptional quadruplex affinities and selectivities place these ligands among the most potent ones reported so far. Further in vitro evaluations are currently underway.

Acknowledgment. This work was supported by ARC (#3365) and E.U. FP6 "MolCancerMed" (LSHC-CT-2004-502943) grants. The authors gratefully thank Dr. P. Mailliet for helpful discussions.

Supporting Information Available: Synthesis and characterization of **1a**−**2b**; experimental procedures and additional FRET and CD data. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA067352B