Bis(benzimidazole)pyridine derivative as a new class of G-quadruplex inducing and stabilizing ligand†

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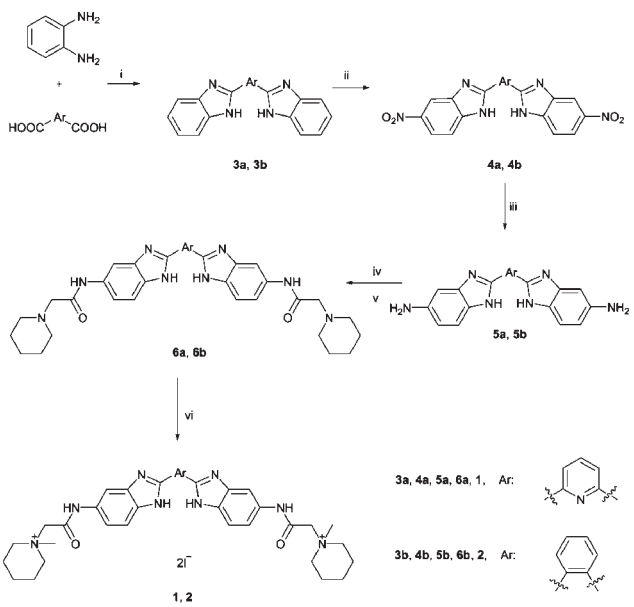
Received (in Cambridge, UK) 9th May 2008, Accepted 9th June 2008
First published as an Advance Article on the web 4th August 2008
DOI: 10.1039/b807916a

Two new bis(benzimidazole)aryl derivatives have been prepared and one of them has been shown to induce and stabilize formation of a G-quadruplex.

It has been reported that guanine-rich oligonucleotides could form G-quadruplexes *via* Hoogsteen hydrogen bonding.[1] In recent years G-quadruplexes have attracted significant attention because of their potential biological application. A large number of putative quadruplex forming sequences have been identified in many chromosomal locations, such as the telomeric region[2] and some gene promoters.[3–6] The human telomeric quadruplex has been extensively studied and formation of G-quadruplexes will lead to inhibition of telomere extension.[7] Further reports indicated that quadruplex motifs exist in the promoter regions of genes which suggests they are involved in the regulation of gene expression.[5,8,9] So the development of G-quadruplex binding ligands has been a focus of interest and a wide range of such ligands have been found in many groups over the past few years.[10–12]

The benzimidazole moiety is structurally related to purine bases[13] and is found in several biologically relevant natural compounds such as vitamin B12.[14] Benzimidazole derivatives have been studied and displayed a wide range of biological activity.[15] In addition, bis-benzimidazoles have been extensively studied as minor groove binding agents.[16] However, as far as we know, there are only few reports that applied them for G-quadruplex binding.[17] These facts have prompted us to develop benzimidazole derivatives and evaluate their abilities for G-quadruplex binding. It was reported that bisquinolinium linked with pyridine could form H bonds, which leads to a molecular V planar shape that is crucial for quadruplex affinity.[18] In order to evaluate the effect of a planar core on G-quadruplex binding, we synthesized the benzimidazole derivatives **1**, **2**. The conformation optimization results, using DFT method, showed that **1** exhibits a planar central core, while **2** does not (Fig. S1, see ESI†).

The compounds **1**, **2** were synthesized *via* the route shown in Scheme 1. The bis(benzimidazole)aryls **3a**, **3b** were prepared by condensation of aromatic diamines with aromatic dicarboxylic acids.[19] The dinitro compounds **4a**, **4b** were obtained by direct nitration of the unsubstituted **3a**, **3b**.[20] The dinitro compounds **4a**, **4b** were reduced by $SnCl_2$ to give the diamino compounds **5a**, **5b**. Then they were reacted with chloroacetyl chloride and then piperidine to yield the piperidineacetamide benzimidazole derivatives **6a**, **6b**.[21] In order to increase the water solubility, they were methylated by CH_3I in $CHCl_3$ to yield the desired bis(benzimidazole) derivatives **1**, **2**. All the new compounds were fully characterized by NMR, HRMS (see ESI†).



Scheme 1 Synthesis of compounds **1**, **2**. *Reagents and conditions*: (i) polyphosphoric acid, 210 °C for 6 h; (ii) concentrated H_2SO_4, fuming HNO_3, 0 °C for 4 h; (iii) $SnCl_2{\cdot}2H_2O$, glacial acetic acid, concentrated HCl, reflux for 6 h; (iv) $ClCH_2COCl$, DMF, pyridine, −10 °C for 0.5 h; (v) piperidine, CH_3OH, 30 °C, overnight; (vi) MeI, $CHCl_3$, rt, overnight.

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† Electronic supplementary information (ESI) available: Synthesis of compounds **1**, **2**, molecular modeling, Exonuclease I hydrolysis assay, SPR binding constants, CD, PCR stop assay and UV-Vis titration experiments. See DOI: 10.1039/b807916a

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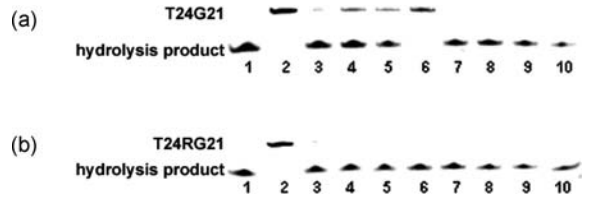

Fig. 1 Quadruplex formation by T24G21 and its resistance to hydrolysis by exonuclease I. (a) Hydrolysis of T24G21 by exonuclease I as a function of compounds concentration. Lane 1: T24G21 treated with exonuclease I; lane 2: T24G21 control; lanes 3–6: T24G21 treated with 1, 5, 10, 25 μM of compound **1** before the addition of exonuclease I; lanes 7–10: T24G21 treated with 1, 5, 10, 25 μM of compound **2** before the addition of exonuclease I; (b) Hydrolysis of T24RG21 by exonuclease I as a function of compounds concentration. Lane 1: T24RG21 treated with exonuclease I; lane 2: T24RG21 control; lanes 3–6: T24RG21 treated with 1, 5, 10, 25 μM of compound **1** before the addition of exonuclease I; lanes 7–10: T24RG21 treated with 1, 5, 10, 25 μM of compound **2** before the addition of exonuclease I.

To identify whether **1** and **2** could induce the formation of the G-quadruplex, the exonuclease I hydrolysis assay was used. This method was introduced by Tan's group for evaluating G-quadruplex stabilization by small molecules.[22] It is based on the fact that oligonucleotide T24G21 ($(T_{24}(G_3T_2A)_3G_3)$) can be hydrolyzed by exonuclease I, while the formation of a G-quadruplex in the oligomer inhibits its hydrolysis. The results are shown in Fig. 1 and the experiment was carried out in the absence of K^+, Na^+ cations. With the increase of the concentration of compound **1**, the hydrolysis product decreased, while compound **2** did not resist the hydrolysis (Fig. 1(a)). A non-quadruplex-forming oligomer T24RG21 (T_{24}GTGTGAGTGGAGGTGTGAGGT) was used to discriminate the inhibitory effect from different sources. As shown in Fig. 1(b), the hydrolysis of T24RG21 was not affected by compound **1**. The compound **1** induced resistance to hydrolysis is explained by quadruplex formation of the T24G21. The hydrolysis of the T24RG21 was not inhibited because the oligomer T24RG21 does not form a quadruplex.

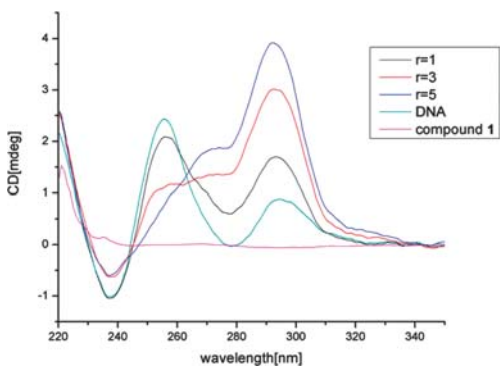

Fig. 2 CD titration of $d[T_2AG_3]_4$ (12.5 μM) in 10 mM Tris-HCl, 1 mM EDTA buffer at pH 7.4. (r = compound **1**/DNA strand concentration) CD spectra were recorded on a Jasco-810 spectropolarimeter (Jasco, Easton, MD), scanning speed of 100 nm min^{-1} with a response time of 1 s, and over a wavelength range of 220–350 nm at room temperature.

To confirm the results and determine the conformation of G-quadruplex induced by the compounds, circular dichroism (CD) spectroscopy was carried out.[12c] Without any metal cations, the CD spectra of the human telomeric $d[T_2AG_3]_4$ sequence exhibited a negative peak near 237 nm, a major positive peak near 256 nm, and a small positive peak around 292 nm. As shown in Fig. 2, when compound **1** was added, the peak at 256 nm was gradually suppressed, and the peak at 292 nm increased dramatically with increase of the concentration of compound **1**. At the same time, a small positive peak at about 270 nm started to appear. The results showed compound **1** induced the formation of the hybrid G-quadruplex.[12c] Consistent with the results of the exonuclease I hydrolysis assay, compound **2** could not induce the formation of the G-quadruplex (data not shown).

These results have prompted us to evaluate their abilities of binding and stabilizing the G-quadruplex. The binding abilities of the compounds to duplex and quadruplex DNA were tested by surface plasmon resonance (SPR).[23] The chosen quadruplex-forming sequence was the human telomeric sequence. The steady-state equilibrium binding constants and kinetic constants of compound **1**, **2** with duplex and quadruplex DNA were both measured under previously described experimental conditions.[12f,23] (Table S2 and Fig. S2, see ESI†). The results showed compound **1** had a high binding constant and showed an obvious selectivity with more than one order of magnitude in favour of quadruplex DNA. The results also revealed compound **1** stabilized the quadruplex by a factor of one order of magnitude relative to compound **2** (Table S2, see ESI†). During the experiment, compound **2** was observed to adhere to the Biacore sensor chip only at high concentrations, which indirectly proved it as a weak binding ligand. Further experimental data from the CD melting method also supported that compound **1** can stabilize the G-quadruplex more strongly than compound **2** (Table S1, see ESI†). Compared with the well-estabilished G-quadruplex stabilizer TMPyP4,[12a] although compound **1** had lower affinity, the selectivity was much better.[12b]

To get quantitative estimates of G-quadruplex stabilization by the two compounds, PCR stop assay was performed.[24] Since all the above experiments was performed using the human telomeric sequence, 21G (5′-GGGTTAGGGT-TAGGGTTAGGG-3′) was chosen as the test oligonucleotide. The formation of the G-quadruplex in 21G will block its hybridization with a complementary strand and lead to the

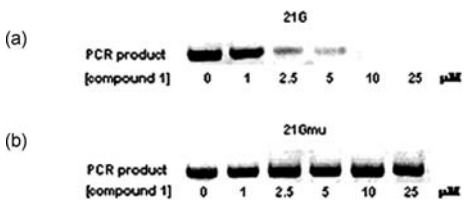

Fig. 3 Effect of compound **1** on the formation of the PCR-stop assay with G-quadruplex forming 21G oligomer (a) or with control mutated 21 Gmu oligomer (b). Increasing concentrations of compound **1** (1–25 μM) were added to G-quadruplex forming 21G oligomer or mutated 21 Gmu oligomers. With the mutated 21 Gmu oligomer the double-stranded PCR product was formed.

double-stranded DNA PCR product being undetectable. As shown in Fig. 3(a), the PCR product was inhibited in a concentration-dependent manner by compound **1**. The IC_{50} value, which indicates the concentration of compound **1** required to achieve 50% inhibition of the reaction, was found to be 1.8 μM. To discriminate inhibitory effects from different sources, a parallel experiment using a mutated oligomer 21 Gmu (5′-GGGTTAGAATTAGGGTTAGGG-3′) which could not form G-quadruplex was performed. In that case, no inhibition was observed even at the highest concentration of 25 μM (see Fig. 3(b)). Compound **2** was also tested in this assay, and not surprisingly, it showed weak inhibition ability (Fig. S3, see ESI†). The IC_{50} was about 100 μM.

All the above results have shown compound **1** is a good G-quadruplex inducing and stabilizing ligand, while **2** is not. We suggested the tremendous difference between the two compounds was due to the structural difference of the cores. Most recently bis-indole carboxamides linked with a pyridine ring have been studied for G-quadruplex recognition.[25] The H-bonds between NH of the pyridine ring and benzimidazole may play an important role in the planar structure, and a planar central core may be vital for the binding of this kind of ligand with the G-quadruplex. UV-Vis titration was used to evaluate the binding mode of compound **1** with the G-quadruplex. Compound **1** demonstrated 9 nm red shifts and significant hypochromicity of 42% (Fig. S4, see ESI†). We assumed the benzimidazole group may be ideally suited for π–π stacking interactions with guanine, considering their structural similarity, and compound **1** could be bound to the ends of the G-quadruplex through π–π stacking.[26]

In summary, two new bis(benzimidazole)aryl derivatives have been synthesized and evaluated as a new class of G-quadruplex inducing and stabilizing ligands. All the experimental results have shown that the bis(benzimidazole)pyridine derivative **1** can induce and stabilize the formation of a G-quadruplex. SPR sensorgrams showed the strong binding and obvious selectivity of compound **1** for G-quadruplex DNA *vs.* ds DNA. The difference of activities between **1** and **2** convinced us that, for this kind of compounds, the planar conformation of cores is vital for their binding with G-quadruplex.

This work was partially supported by the National Science of Foundation of China (No. 20672084, 20621502), National Science Fund for Distinguished Young Scholars (No. 20425206), the Cultivation Fund of the Key Scientific and Technical Innovation Project, the Ministry of Education of China (No. 706040).

Notes and references

1 J. T. Davis, *Angew. Chem., Int. Ed.*, 2004, **43**, 668.
2 E. H. Blackburn, *Nature*, 1991, **350**, 569.
3 T. Simonsson, P. Pecinka and M. Kubista, *Nucleic Acids Res.*, 1998, **26**, 1167.
4 D. Sun, K. Guo, J. J. Rusche and L. H. Hurley, *Nucleic Acids Res.*, 2005, **33**, 6070.
5 S. Cogoi, F. Quadrifoglio and L. E. Xodo, *Biochemistry*, 2004, **43**, 2512.
6 S. Rankin, A. P. Reszka, J. Huppert, M. Zloh, G. N. Parkinson, A. K. Todd, S. Ladame, S. Balasubramanian and S. Neidle, *J. Am. Chem. Soc.*, 2005, **127**, 10584.
7 (*a*) J. F. Riou, L. Guittat, P. Mailliet, A. Laoui, E. Renou, O. Petitgenet, F. Mégnin-Chanet, C. Hélène and J. L. Mergny, *Proc. Natl. Acad. Sci. USA*, 2002, **99**, 2672; (*b*) Y. Mikami-Terao, M. Akiyama, Y. Yuza, T. Yanagisawa, O. Yamada and H. Yamada, *Cancer Lett.*, 2008, **261**, 226–234.
8 (*a*) A. K. Todd, M. Johnston and S. Neidle, *Nucleic Acids Res.*, 2005, **33**, 2901; (*b*) J. L. Huppert and S. Balasubramanian, *Nucleic Acids Res.*, 2005, **33**, 2908; (*c*) J. L. Huppert and S. Balasubramanian, *Nucleic Acids Res.*, 2007, **35**, 406.
9 (*a*) A. Siddiqui-Jain, C. L. Grand, D. J. Bearss and L. H. Hurley, *Proc. Natl. Acad. Sci. USA*, 2002, **99**, 11593; (*b*) T.-M. Ou, Y.-J. Lu, C. Zhang, Z.-S. Huang, X.-D. Wang, J.-H. Tan, Y. Chen, D.-L. Ma, K.-Y. Wong, J. C.-O. Tang, A. S.-C. Chan and L.-Q. Gu, *J. Med. Chem.*, 2007, **50**, 1465.
10 J. Cuesta, M. A. Read and S. Neidle, *Mini-Rev. Med. Chem.*, 2003, **3**, 11.
11 (*a*) J. E. Reed, A. A. Arnal, S. Neidle and R. Vilar, *J. Am. Chem. Soc.*, 2006, **128**, 5992; (*b*) K. Jantos, R. Rodriguez, S. Ladame, P. S. Shirude and S. Balasubramanian, *J. Am. Chem. Soc.*, 2006, **128**, 13662; (*c*) A. D. Moorhouse, A. M. Santos, M. Gunaratnam, M. Moore, S. Neidle and J. E. Moses, *J. Am. Chem. Soc.*, 2006, **128**, 15972; (*d*) A. D. Cian, E. DeLemos, J.-L. Mergny, M.-P. Teulade-Fichou and D. Monchaud, *J. Am. Chem. Soc.*, 2007, **129**, 1856; (*e*) P. S. Shirude, E. R. Gillies, S. Ladame, F. Godde, K. Shin-ya, I. Huc and S. Balasubramanian, *J. Am. Chem. Soc.*, 2007, **129**, 11890; (*f*) M. Bejugam, S. Sewitz, P. S. Shirude, R. Rodriguez, R. Shahid and S. Balasubramanian, *J. Am. Chem. Soc.*, 2007, **129**, 12926.
12 (*a*) R. T. Wheelhouse, D. Sun, H. Han, F. X. Han and L. H. Hurley, *J. Am. Chem. Soc.*, 1998, **120**, 3261; (*b*) P. Wang, L.-G. Ren, H.-P. He, F. Liang and X. Zhou, *ChemBioChem*, 2006, **7**, 1155; (*c*) E. M. Rezler, J. Seenisamy, S. Bashyam, M.-Y. Kim, E. White, W. D. Wilson and L. H. Hurley, *J. Am. Chem. Soc.*, 2005, **127**, 9439; (*d*) E. S. Baker, J. T. Lee, J. L. Sessler and M. T. Bowers, *J. Am. Chem. Soc.*, 2006, **128**, 2641; (*e*) D. P. N. Gonçalves, R. Rodriguez, S. Balasubramanian and J. K. M. Sanders, *Chem. Commun.*, 2006, 4685; (*f*) L.-G. Ren, A.-M. Zhang, J. Huang, P. Wang, X.-C. Weng, L.-X. Zhang, F. Liang, Z. Tan and X. Zhou, *ChemBioChem*, 2007, **8**, 775; (*g*) I. M. Dixon, F. Lopez, A. M. Tejera, J.-P. Estève, M. A. Blasco, G. Pratviel and Bernard Meunier, *J. Am. Chem. Soc.*, 2007, **129**, 1502; (*h*) B.-Q. Fu, J. Huang, L.-G. Ren, X.-C. Weng, Y.-Y. Zhou, Y.-H. Du, X.-J. Wu, X. Zhou and G.-F. Yang, *Chem. Commun.*, 2007, 3264.
13 B. M. O'Neill, J. E. Ratto, K. L. Good, D. C. Tahmassebi, S. A. Helquist, J. C. Morales and E. T. Kool, *J. Org. Chem.*, 2002, **67**, 5869.
14 A. R. Batteraby, *J. Nat. Prod.*, 1988, **51**, 643.
15 (*a*) K. S. Gudmundsson, G. A. Freeman, J. C. Drach and L. B. Townsend, *J. Med. Chem.*, 2000, **43**, 2473; (*b*) D. J. Skalitzky, J. T. Marakovits, K. A. Maegley, A. Ekker, X.-H. Yu, Z. Hostomsky, S. E. Webber, B. W. Eastman, R. Almassy and J. Li, *J. Med. Chem.*, 2003, **46**, 210; (*c*) Y. He, J. Yang, B.-G. Wu, L. Risen and E. E. Swayze, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 1217; (*d*) P. P. Seth, A. Miyaji, E. A. Jefferson, K. A. Sannes-Lowery, S. A. Osgood, S. S. Propp, R. Ranken, C. Massire, R. Sampath, D. J. Ecker, E. E. Swayze and R. H. Griffey, *J. Med. Chem.*, 2005, **48**, 7099.
16 (*a*) F. A. Tanious, D. Hamelberg, C. Bailly, A. Czarny, D. W. Boykin and W. D. Wilson, *J. Am. Chem. Soc.*, 2004, **126**, 143; (*b*) C. Bailly, G. Chessari, C. Carrasco, A. Joubert, J. Mann, W. D. Wilson and S. Neidle, *Nucleic Acids Res.*, 2003, **31**, 1514.
17 S. Maiti, N. K. Chaudhury and S. Chowdhury, *Biochem. Biophys. Res. Commun.*, 2003, **310**, 505.
18 A. D. Cian and J. L. Mergny, *Nucleic Acids Res.*, 2007, **35**, 2483.
19 (*a*) A. W. Addison and P. J. Burke, *J. Heterocycl. Chem.*, 1981, **18**, 803; (*b*) H. Vogel and C. S. Marvel, *J. Polym. Sci.*, 1961, **50**, 511.
20 M. Berrada, F. Carriere, Y. Abboud, A. Abourriche, A. Benamara, N. Lajrhed, M. Kabbajc and M. Berrada, *J. Mater. Chem.*, 2002, **12**, 3551.
21 C. L. Sann, A. Baron, J. Mann, H. van den Berg, M. Gunaratnamc and S. Neidle, *Org. Biomol. Chem.*, 2006, **4**, 1305.
22 Y. Yao, Q. Wang, Y.-H. Hao and Z. Tan, *Nucleic Acids Res.*, 2007, **35**, e68.
23 I. M. Dixon, F. Lopez, J.-P. Estève, A. M. Tejera, M. Blasco, G. Pratviel and B. Meunier, *ChemBioChem*, 2005, **6**, 123.
24 T. Lemarteleura, D. Gomeza, R. Paterskia, E. Mandineb, P. Mailliet and J.-F. Riou, *Biochem. Biophys. Res. Commun.*, 2004, **323**, 802.
25 J. Dash, P. S. Shirude and S. Balasubramanian, *Chem. Commun.*, 2008, 3055.
26 R. Kieltyka, J. Fakhoury, N. Moitessier and H. F. Sleiman, *Chem. Eur. J.*, 2008, **14**, 1145.

