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Synthesis and DNA binding profile of *N*-mono- and *N*,*N*′-disubstituted indolo[3,2-*b*]carbazoles†

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A series of *N*-monosubstituted and *N*,*N*′-disubstituted derivatives of the indolo[3,2-*b*]carbazole chromophore have been prepared, and their binding affinity for duplex DNA has been evaluated by ultraviolet and fluorescence spectroscopies. It has been found that indolo[3,2-*b*]carbazoles bearing basic *N*-alkyl substituents are intercalators that bind DNA with affinities in the micromolar and submicromolar range and a preference for associating with sequences of mixed composition and purine-pyrimidine steps.

Over the past decade, numerous derivatives of the indolo[3,2-*b*]-carbazole chromophore have been prepared in order to explore their photophysical properties and their application as materials for organic electroluminescent devices.[1] The precursor to these substances, the chromophore indolo[3,2-*b*]carbazole, contains two nitrogen atoms on opposing sides of the aromatic scaffold which can be readily substituted with a variety of electrophiles using standard synthetic methods.[2]

In analogy to napthalenediimide[3a–h] and acridine-3- and 4-carboxamide[3i–k] heterocycles, we envisioned that the extended planar π surface of the indolo[3,2-*b*]carbazole chromophore could efficiently stack between the nucleobases of duplex DNA *via* an intercalative mode of binding,[4a] and thus derivatives of indolo[3,2-*b*]carbazole may function as high affinity nucleic acid ligands.[4b] Depending on the orientation of the planar chromophore in the intercalation complex, the two substituents on the nitrogen atoms could be positioned in either the same groove or in both the major and minor grooves of DNA, where non-covalent interactions may afford a level of sequence specificity. To test this hypothesis, we have synthesized a series of *N*-substituted derivatives of indolo[3,2-*b*]carbazole (Fig. 1) and evaluated their affinity for duplex DNA *via* ultraviolet and fluorescence spectroscopies.

It is well established that ligands containing positively charged residues have a high affinity for duplex nucleic acids due to favourable electrostatic interactions with the negatively charged phosphate backbone and electronegative atoms in the major and minor grooves developed upon initial association with the biopolymer.[5] Thus the primary series of indolocarbazole derivatives targeted for synthesis (**2a–e** and **3a–e**) contain basic nitrogen atoms.[6]

Both Waring[7] and McGee[8] have previously demonstrated that the presence of a carbohydrate moiety on an intercalating chromophore contributes positively and significantly to the binding association with DNA. The bis-*C*-aryl glycoside natural product altromycin B has been shown by NMR studies to associate with DNA *via* a helix-threading mode of binding, with carbohydrate moieties positioned in opposite grooves of the duplex,[9] where non-covalent interactions between functional groups present on the sugar and residues in the major and minor grooves are established. Given the facile substi-

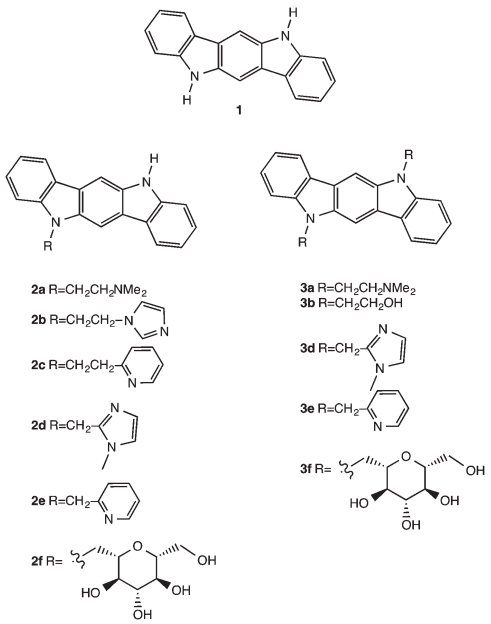

Fig. 1 Indolo[3,2-*b*]carbazole (**1**) and synthetic derivatives studied (**2**, **3**).

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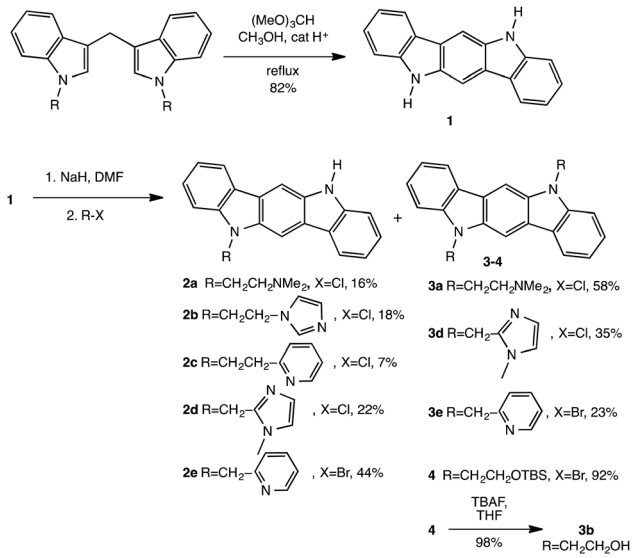
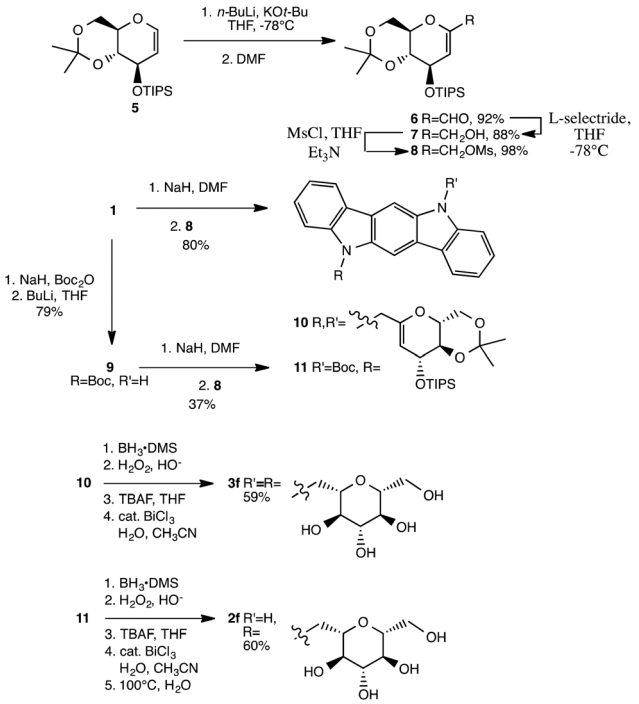


Scheme 1 Synthesis of indolo[3,2-*b*]carbazoles **2a–e** and **3a–3e**.

tution of the opposing nitrogen atoms on the indolo[3,2-*b*]-carbazole chromophore, we considered that mono- and bis-carbon glucoside derivatives (**2f** and **3f**) could also easily be prepared, allowing a direct comparison of the DNA-binding affinity of ligands containing one or two attached glycosyl moieties.

The synthesis of the parent chromophore **1** was accomplished from 3,3′-diindolylmethane in 82% yield *via* an acid-promoted cyclization as described by Pindur and Mueller (Scheme 1).[1] Alkylation of **1** under basic conditions (NaH, DMF) with 2-dimethylamino-1-chloroethane·HCl provided **3a** (71%) when more than 2 equivalents of alkylating agent were used, and separable mixtures of **2a** (16%) and **3a** (58%) when less than 2 equivalents of alkylating agent were employed.[2] In an analogous fashion, substrates **2d** (22%), **3d** (35%) and **2e** (44%), **3e** (23%) were prepared by reaction of **1** with *N*-methyl-2-chloromethyl imidazole hydrochloride and 2-bromomethyl-pyridine, respectively, in the presence of sodium hydride. Reaction of the monosodium salt of **1** in DMF with 2-chloroethylimidazole hydrochloride or 2-chloroethylpyridine-hydrochloride provided substrates **2b** and **2c** in 18% and 7% yields, respectively. Low yields were encountered in these alkylation reactions due to elimination side reactions, and as a result double alkylation of **1** under basic conditions could not be achieved with these reagents. However, **1**·2Na reacted with excess TBS-protected 2-bromoethanol to provide bis-silyl ether **4** in 92% yield, which was then treated with TBAF to provide alcohol **3b** in 98% yield.

The synthesis of indolo[3,2-*b*]carbazole derivatives **2f** and **3f** required preparation of an appropriate carbohydrate alkylating agent (Scheme 2). Glucal acetonide **5**[10] was deprotonated with Schlosser's base in THF at −78 °C for 1 hour[11] and then DMF was added to furnish the corresponding aldehyde **6** in 92% yield. Reduction of the aldehyde to the allylic alcohol was accomplished in 88% yield by treatment with L-selectride in



Scheme 2 Synthesis of indolo[3,2-*b*]carbazole derivatives **2f** and **3f**.

THF at −78 °C. Mesylation of the derived alcohol (MsCl, THF, Et_3N, 0 °C) then provided a reactive alkylating agent which smoothly coupled with the sodium salt of **1** to provide **10** in 80% yield. Glycoside **10** was then subjected to hydroboration/oxidation, silyl ether deprotection, and acetonide hydrolysis to furnish the bis glycoside **3f** in 59% overall yield.

The synthesis of monoglycoside **2f** required preparation of the mono-Boc indolo[3,2-*b*]carbazole **9**, and this was accomplished in two steps (79%) according to the protocol of Bergman.[2] Combination of the sodium salt of **9** with **8** furnished glycoside **11** in 37% yield. Hydroboration/oxidation, silyl ether deprotection with TBAF, and acetonide hydrolysis gave the penultimate Boc-protected glycoside, which upon carbamate thermolysis in water gave **2f** in 60% overall yield from **11**.

The binding of the indolo[3,2-*b*]carbazole derivatives to duplex DNA was explored by fluorescence and UV spectroscopies. Excitation of the most basic derivative **3a** (0.064 μM in 10 mM Tris-EDTA, pH = 5.48) at 320 nm in the presence of increasing concentrations of calf thymus (CT) DNA resulted in a corresponding decrease of the emission intensity at 396 nm (Fig. 2), indicative of the quenching of the fluorescence of **3a** upon binding to DNA.[12] Monitoring the UV absorbance of **3a** at 336 nm upon titration with CT-DNA in Tris-EDTA buffer revealed a distinct hypochromic shift along with a red shift to 342 nm (isosbestic point = 340 nm), observations suggestive of an intercalative mode of binding (see below).

To compare the strengths of binding of compounds **2a–e** and **3a–e** to CT DNA, the competitive ethidium displacement technique was employed to obtain apparent association constants (K_{app}).[13] From this analysis (Table 1) the tightest binding indolo[3,2-*b*]carbazole was bisimidazole derivative **3d**

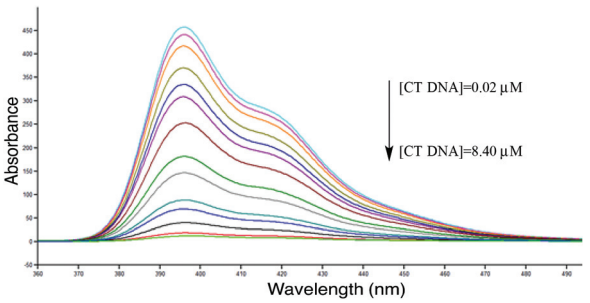


Fig. 2 Fluorescence spectra of **3a** in the presence of varying concentrations of CT-DNA at pH 5.48, [**3a**] = 6.4 × 10^{-8} M, [CT-DNA] = 0.02, 0.03, 0.05, 0.08, 0.13, 0.20, 0.30, 0.40, 0.60, 0.90, 1.20, 2.4, 5.4, and 8.40 × 10^{-6} M, respectively.

Table 1 Comparison of the binding of **2a–2f** and **3a–3f** to CT DNA[a] by ethidium displacement[a] and thermal denaturation techniques[b]

Ligand	K_{app} [a] (×10^6 M^{-1})	ΔT_M [b] (°C)
2a	1.47 (±0.11)	1
2b	6.26 (±0.63)	—[d]
2c	2.06 (±0.14)	(−47)[d,20]
2d	3.49 (±0.65)	(−50)[d,20]
2e	0.70 (±0.45)	—[d]
2f	<0.01	—[c]
3a	4.52 (±0.51)	6
3b	<0.01	—[c]
3d	12.3 (±0.13)	(−18)[d,20]
3e	3.77 (±0.39)	—[d]
3f	<0.01	0

[a] K_{app} values obtained by the competitive ethidium displacement method (2 mM NaOAc, 9.3 mM NaCl, 0.1 mM Na_2EDTA, pH = 5.0), where $K_{app} = K_e \times 1.26/C_{50}$ and $K_e = 2.1 \times 10^6$ M^{-1}.[13] [b] T_M values obtained (10 mM Tris-EDTA, 0.1 M NaCl, pH = 5.0) from first derivative analysis ($\Delta A/\Delta T$ *vs.* ΔT) of the sigmoidal melting curves (*A vs. T*); $\Delta T_M = T_M$(ST DNA + ligand)-T_M(ST DNA).[14] [c] Compounds **2f** and **3b** display poor solubility in the buffer used for the thermal denaturation studies. [d] Overlap of the UV absorbances of pyridine- and imidazole-substituted derivatives **2b–2e** and **3d,e** with DNA in the 260–280 nm region prevented accurate T_M determinations.[20]

with K_{app} = 1.23 × 10^7 M^{-1}, a value which is more than threefold higher than that obtained for monoimidazole derivative **2d** (K_{app} = 3.49 × 10^6 M^{-1}). Similarly, the apparent binding constant for bis(dimethylaminoethyl) derivative **3a** (4.52 × 10^6 M^{-1}) is approximately threefold greater than that obtained for the mono(dimethylaminoethyl) derivative **2a** (1.47 × 10^6 M^{-1}). Interestingly, compounds **2d** and **2e** have lower apparent binding constants for CT DNA than their homologs **2b** and **2c**, respectively, indicating the importance of the additional methylene unit between the indolocarbazole chromophore and the imidazole or pyridine moiety for tighter DNA binding. Finally, all derivatives lacking basic amine groups (**3b**, **2f**, and **3f**) display relatively poor binding to CT DNA, suggesting that positively charged groups on the indolocarbazole side chains significantly enhance the association with DNA.

Thermal denaturation studies[14] were also performed with compounds **2a–e** and **3a–e** and calf thymus (CT) DNA (10 mM Tris-EDTA, 0.1 M NaCl, pH = 5.0). Poor solubility in 0.1 M NaCl buffer hampered T_M determinations for compounds **2f** and **3b** in the presence of CT DNA; furthermore, overlap of absorbances in the 260–280 nm region of the UV spectra of imidazole- and pyridine-substituted derivatives **2b–2d** and **3d,e** with CT DNA complicated T_m analysis for these compounds.[20] Reliable data obtained for compounds **2a**, **3a**, and **3f** confirmed the strong binding of **3a** to CT DNA.

To investigate the importance of electrostatic interactions[5] in the binding of the basic indolocarbazole derivatives to DNA, we evaluated the association of compound **3a** with CT DNA under high- and low-salt conditions (Fig. 3).[4] Increasing NaCl concentrations from 0–0.01 M results in a modest two-fold drop in **3a**'s DNA binding affinity; however, at a salt concentration of 0.1 M $a > 10$ fold drop in affinity is observed. These data suggest that the positively charged protonated nitrogen atoms may be involved both in polar hydrogen bonding interactions in the grooves of DNA and in ionic interactions with the phosphate backbone.

To further probe the DNA binding mode of the indolocarbazole derivatives, we assayed for ligand intercalation using the technique of viscometry.[15] Increasing concentrations of **3a** (Fig. 4) in the presence of CT-DNA resulted in an increase in solution viscosity comparable to that obtained with reference compound ethidium bromide (EB), indicative of the helix lengthening and rigidification that occurs upon ligand intercalation.

Depending on the orientation of the chromophore upon intercalation, the nitrogen substituents may be positioned in either the minor or major grooves or both. To check for major groove occupancy, a fixed concentration of **3a** and CT DNA was titrated with increasing concentrations of the non-intercalating major-groove binding dye methyl green,[16] and displacement of **3a** from DNA was observed, as evidenced by fluorescence enhancement at 410 nm (Fig. 5). In contrast, increasing concentrations of methyl green in the presence of **3a** but in the absence of CT DNA showed no significant enhancement of

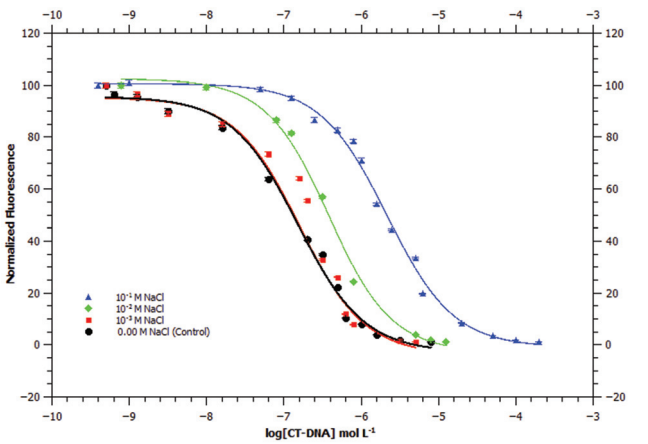


Fig. 3 Binding isotherms for the titration of **3a** with CT DNA in the presence of varying concentrations of NaCl. [a]Black circle: [NaCl] = 0.0 M, K_a = 6.82(±0.86) × 10^6 M^{-1}; [b]red square: [NaCl] = 1.0 × 10^{-3} M, K_a = 6.69(±0.98) × 10^6 M^{-1}; [c]green diamond: [NaCl] = 1.0 × 10^{-2} M, K_a = 3.02(±0.37) × 10^6 M^{-1}; [d]blue triangle: [NaCl] = 1.0 × 10^{-1} M, K_a = 4.69(±0.36) × 10^5 M^{-1}.

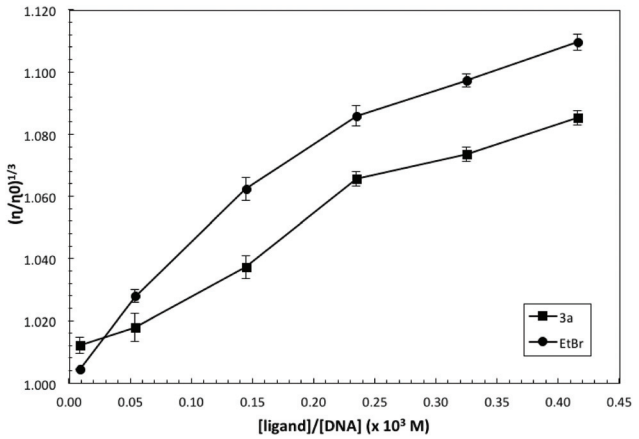


Fig. 4 Effect of increasing amount of EB (●) and **3a** (■) on the relative viscosity of CT-DNA. EB (●): [CT-DNA] = 4.81 × 10^{-4} M, [EB] = 0.44, 2.61, 6.96, 11.3, 15.7, and 20.0 × 10^{-5} M; **3a** (■): [CT-DNA] = 2.00 × 10^{-4} M, [**3a**] = 0.78, 2.72, 6.60, 10.5, 14.4, and 18.2 × 10^{-5} M.

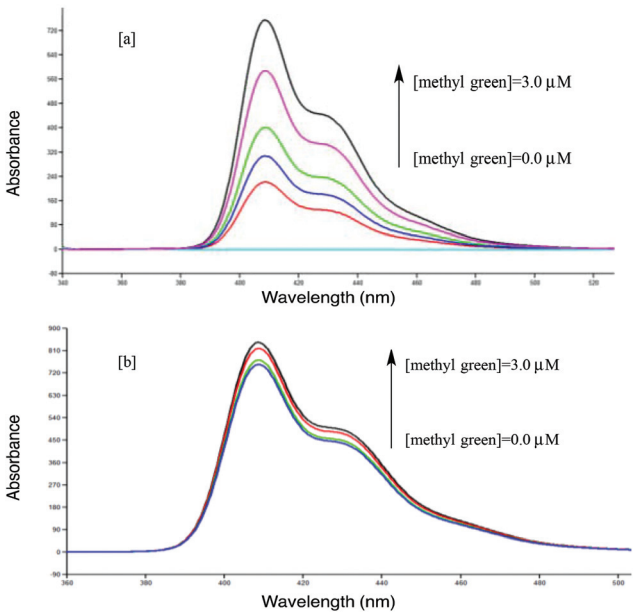


Fig. 5 [a] Fluorescence spectra of **3a** (0.12 × 10^{-6} M) in the presence of CT DNA (0.37 × 10^{-6} M) and varying concentrations of methyl green: 0.00, 0.76, 1.52, 3.04 × 10^{-6} M. [b] Fluorescence spectra of **3a** (0.12 × 10^{-6} M; no CT DNA) in the presence of varying concentrations of methyl green: 0.00, 0.76, 1.52, 3.04 × 10^{-6} M.

fluorescence. The UV absorption spectra of **3a** in the presence of polydA·2polydT or polydA·polydT show similar shifts in the 335 nm (λ_{max}) region as compared to compound **3a** in the absence of DNA, indicative of binding to both duplex and triplex forms. Since the third strand of polydA·2polydT occupies the major groove, this result implies that **3a** can also associate with the minor groove of DNA. Indeed, titration of **3a** and CT DNA with increasing concentrations of the minor groove-binding drug Hoechst 33342 gave rise to fluorescence enhancement, which is suggestive of minor groove contacts by the ligand.

We examined the sequence selectivity of **3a** by measuring its binding to a series of sequence-diverse DNA hairpins 5′-CGXXXXXC-AAAAA-GXXXXXCG-3′ (Table 2).[17] A plot of the change in fluorescence *versus* molar equivalents of ligand for a given hairpin provides a titration curve from which the stoichiometry of binding may be derived; performing this analysis for **3a** in the presence of hairpins 5′-AATCT-3′ (5′-TTAGA-3′) and 5′-TTTTT-3′ (5′-AAAAA-3′) gave stoichiometries of 1.10 and 0.85, respectively, indicating ~1 : 1 binding. Titration of a fixed concentration of hairpin with **3a** yields a sigmoidal binding curve, fitting analysis of which provides K_a. As can be seen in Table 2, tightest binding was observed to the hairpins containing the mixed sequences 5′-AGAGA-3′, 5′-GGTAG-3′, 5′-AACGG-3′ and 5′-TATAT-3′, with K_a values two- to three-fold higher than those for the hairpins containing continuous AT and GC tracts. With the exception of 5′-AGAGA-3′, all of the tightest bound hairpins contain purine-pyrimidine steps. Purine-pyrimidine steps are more weakly π-stacked than purine-purine/pyrimidine-pyrimidine steps and thus intercalators typically display a preference for binding these sequences.[19,22]

Finally a preliminary assessment of the cell permeability and cytotoxicity of the indolo[3,2-*b*]carbazoles was performed with compound **3a**. Treatment of liver carcinoma (Hep2G) cells with **3a** (at 1.38 and 55.2 μM concentrations) resulted in significant cellular uptake within 4.5 hours, as evidenced by residual fluorescence (at 410 nm) of the medium after extensive buffer washing (including heparin, low pH, high salt, and trypsin treatment) and cell lysis (see ESI†) as compared to controls lacking **3a**. Furthermore, the viability of acute leukemia monocytes in the presence and absence of **3a** was assayed using the trypan blue exclusion test.[18] It was found that within 20 hours <10% of cells treated with **3a** (1.3 × 10^{-5} M) were still viable. These early results suggest a generalized cytotoxicity of

Table 2 Binding of **3a** to DNA hairpins

5′-CG**XXXXX**C A A
3′-GC**XXXXX**G A A

Hairpin	TGTGT ACACA	TATAT ATATA	AATCT TTAGA	GGGGG CCCCC	AAAAA T TTT T
K_a (×10^7 M^{-1})	29.2 (±2.6)	36.5 (±5.5)	23.5 (±3.2)	15.7 (±1.6)	17.3 (±1.5)
Hairpin	GCGCG CGCGC	AACGG TTGCC	GGTAG CCATC	AGCCT TCGGA	AGAGA TCTCT
K_a (×10^6 M^{-1})	23.9 (±3.2)	41.3 (±4.7)	47.6 (±3.9)	21.8 (±1.8)	48.1 (±5.2)

cell-permeable indolo[3,2-*b*]carbazole derivatives likely associated with tight DNA binding. Detailed cytotoxicity studies (including MTT/MTS assays)[21] are currently in progress and will be reported in due course.

Conclusions

A variety of *N*-monosubstituted and *N*,*N*-disubstituted indolo[3,2-*b*]carbazoles have been prepared and evaluated for their binding to duplex DNA. It has been found that derivatives possessing one or two basic *N*-substituted groups bind tightly to DNA, with bis-imidazole compound **3d** displaying the highest apparent affinity. Evidence for an intercalative mode of DNA binding has been established, and it appears likely that the *N*-alkyl substituents project into the minor and/or major grooves of the double helix. Indolo[3,2-*b*]carbazole **3a** prefers to bind sequences of mixed composition and those containing purine-pyrimidine steps, perhaps due to the greater ease of intercalation at these sites. The cell permeability and cytotoxicity of these compounds have been preliminarily investigated, and the results may be of interest for the materials industry seeking to utilize such substituted photoactive chromophores in the design of organic electronics.

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Notes and references

1 U. Pindur and J. Mueller, *Arch. Pharm.*, 1987, **320**, 280.
2 L. N. Yudina and J. Bergman, *Tetrahedron*, 2003, **59**, 1265.
3 Naphthalenediimides: (*a*) R. E. McKnight, E. Reisenauer, M. V. Pintado, S. R. Polasani and D. W. Dixon, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 4288; (*b*) S. M. Hampel, A. Sidibe, M. Gunaratnam, J. F. Riou and S. Neidle, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 6459; (*c*) J. Lee, V. Guelev, S. Sorey, D. W. Hoffman and B. L. Iverson, *J. Am. Chem. Soc.*, 2004, **12**, 14036; (*d*) D. A. Gianolio, J. M. Segismundo and L. W. McLaughlin, *Nucleic Acids Res.*, 2000, **28**, 2128; (*e*) R. E. McKnight, A. B. Gleason, J. A. Keyes and S. Sahabi, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 1013; (*f*) K. Ohtsuka, K. Komizo and S. Takenaka, *J. Organomet. Chem.*, 2010, **695**, 1281; (*g*) C. L. Mazzitelli, Y. Chu, J. J. Reczek, B. L. Iverson and J. S. Brodbelt, *J. Am. Soc. Mass Spectrom.*, 2007, **18**, 311; (*h*) V. Guelev, J. Lee, J. Ward, S. Sorey, D. W. Hoffman and B. L. Iverson, *Chem. Biol.*, 2001, **8**, 415. Acridine-3- and 4-carboxamides: (*i*) L. A. Howell, R. Gulam, A. Mueller, M. A. O'Connell and M. Searcey, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 6596; (*j*) B. D. Gooch and P. A. Beal, *J. Am. Chem. Soc.*, 2004, **126**, 10603; (*k*) C. Bourdouxhe-Housiaux, P. Colson, C. Houssier, M. J. Waring and C. Bailly, *Biochemistry*, 1996, **35**, 4251.
4 (*a*) W. Sajewicz and A. Dlugosz, *J. Appl. Toxicol.*, 2000, **20**, 305; (*b*) T. Jia, J. Xiang, J. Wang, P. Guo and J. Yu, *Org. Biomol. Chem.*, 2013, **11**, 5512.
5 V. K. Misra and B. Honig, *Proc. Natl. Acad. Sci. U. S. A.*, 1995, **92**, 4691.
6 Approximate pK_b values for pyridine (8.77), imidazole (6.90) and trimethylamine (4.20) are derived from the corresponding conjugate acid pK_a values in water: E. V. Anslyn and D. A. Dougherty, *Modern Physical Organic Chemistry*, University Science Books, Sausalito, 2006, p. 281.
7 C. Bailly, P. Colson, C. Houssier, E. Rodrigues-Periera, M. Prudhomme and M. J. Waring, *Mol. Pharmacol.*, 1998, **53**, 77.
8 Y. C. Tse-Dinh and L. R. McGee, *Biochem. Biophys. Res. Commun.*, 1987, **143**, 808.
9 M. R. Hansen and L. Hurley, *J. Am. Chem. Soc.*, 1995, **117**, 2421.
10 K. H. Dotz, F. Otto and M. Nieger, *J. Organomet. Chem.*, 2001, **621**, 77.
11 (*a*) L. A. Paquette and J. A. Oplinger, *Tetrahedron*, 1989, **45**, 107; (*b*) R. W. Friesen and C. F. Sturino, *Sci. Synth.*, 2006, **8a**, 841.
12 Analysis of the fluorescence data by least squares fitting analysis gave $K_a = 3.8(\pm0.4) \times 10^6$ M^{-1}. For non-linear regression techniques, see: J. McGhee and P. von Hippel, *J. Mol. Biol.*, 1974, **86**, 469.
13 A. R. Morgan, J. S. Lee, D. E. Pulleyblank, N. L. Murray and D. H. Evans, *Nucleic Acids Res.*, 1979, **7**, 547.
14 W. D. Wilson, F. A. Tanious, M. Fernandez-Saiz and C. T. Rigl, *Methods Mol. Biol. Drug-DNA Interact. Protoc.*, 1997, **90**, 219.
15 P. C. Dedon, In *Curr. Protoc. Nucleic Acid. Chem*, John Wiley and Sons, New York, 2000, 8.1.1.
16 S. K. Kim and B. Norden, *FEBS Lett.*, 1993, **315**, 61.
17 (*a*) W. C. Tse and D. L. Boger, *Acc. Chem. Res.*, 2004, **37**, 61; (*b*) D. L. Boger and W. C. Tse, *Bioorg. Med. Chem.*, 2001, **9**, 2511.
18 W. Strober, *Curr. Protoc. Immunol.*, 2001, Appendix 3, Appendix 3b, p. 1.
19 (*a*) W. C. Tse and D. L. Boger, *Chem. Biol.*, 2004, **11**, 1607; (*b*) D. S. Johnson and D. L. Boger, *"DNA binding agents" In Comprehensive Supramolecular Chemistry*, ed. Y. Murakami, Elsevier, Oxford, 1996, p. 73.
20 The apparent T_M's of CT DNA in the presence of compounds **2c**, **2d**, and **3d** were lower than in their absence, suggestive of preferential binding to single-stranded DNA. See ref. 14.
21 (*a*) T. Mossmann, *J. Immunol. Methods*, 1983, **65**, 55; (*b*) A. H. Cory, T. C. Owen, J. A. Barltrop and J. G. Cory, *Cancer Commun.*, 1991, **3**, 207.
22 Hairpin melting data in the presence and absence of **3a** roughly corroborate the trends observed in Table 2 (see ESI†).