Brief Articles

Synthesis, DNA Binding, and *Leishmania* Topoisomerase Inhibition Activities of a Novel Series of Anthra[1,2-*d*]imidazole-6,11-dione Derivatives

Padmaparna Chaudhuri,[†] Hemanta K. Majumder,[‡] and Santanu Bhattacharya*[,†,§]

Department of Organic Chemistry, Indian Institute of Science, Bangalore 560012, India, Molecular Parasitology Department, Indian Institute of Chemical Biology, 4 Raja S. C. Mullick Road, Kolkata 700032, India, and Chemical Biology Unit of JNCASR, Bangalore 560012, India

Received September 6, 2006

Nine novel anthra[1,2-*d*]imidazole-6,11-diones, differing in their side chain, were synthesized. UV−vis spectroscopy and viscometric titrations of these molecules with duplex DNA were used to assess their binding with DNA. Five of the nine compounds showed high inhibition activity against topoisomerase I of *Leishmania donovani*, with the one bearing the tetrazole side chain exhibiting an $IC_{50} \sim 1\ \mu M$. The inhibition activities were not related with their DNA binding affinity and depended on the nature of the side chain.

Introduction

Leishmania donovani, a unicellular kinetoplastid protozoan parasite, is the causative agent for human visceral leishmaniasis, which is a fatal disease affecting the liver and spleen. It has currently been listed by the World Health Organization as the second most important protozoan disease after malaria in terms of patient mortalities and is prevalent in many tropical countries.[1] Treatment of leishmaniasis depends entirely upon chemotherapy, yet the available range of effective drugs is highly limited.[1] In an attempt to identify new molecular targets for antiparasitic drugs, attention has been focused on DNA topoisomerases,[2] a class of enzymes playing critical roles in virtually every aspect of DNA metabolism.[3,4] Herein, we report a series of new small molecules, some of which have emerged as potent inhibitors of topoisomerase I (topo I) of parasitic pathogen *Leishmania donovani*.[5]

As both benzimidazole[4] and anthraquinone derivatives[4] are known topoisomerase inhibitors, we have synthesized nine imidazole-fused anthraquinone derivatives. The side chain appended to the aromatic core was varied to examine if the pK_a and electronegativity of the side-chain nitrogen influenced the duplex (*ds*-) DNA complexing or topoisomerase inhibiting properties of these compounds. The interactions of the molecules (**1**−**9**) with *ds*-DNA and their inhibitory effect on the catalytic activity of *L. donovani* and human topo I were examined. Compounds with tetrazole, imidazole, or morpholine side chains exhibited potent inhibition activity at the μM level against the parasitic enzyme, with activity significantly greater than that shown by camptothecin (cpt), a well-known topo I poison.[4,5]

Experimental Section

Syntheses. Compound 4-(3-bromopropyloxy)benzaldehyde (**1a**) was prepared according to the literature.[6] 4-[(3-Dimethylamino)-propyloxy]benzaldehyde (**1b**) was synthesized by passing dry $HNMe_2$ gas through a solution of **1a** in toluene. The removal of solvent and excess amine, followed by aqueous workup, yielded pure **1b** in 90% yield.

4-[(3-Dimethylamino)propyloxy]phenyl-1*H*-anthra[1,2-*d*]imidazole-6,11-dione (1). 1,2-Diaminoanthraquinone (0.5 g, 2.1 mmol) and **1b** (0.45 g, 2.1 mmol) were dissolved in nitrobenzene (15 mL) and stirred at 130 °C for 16 h. Once the starting materials were consumed, as indicated by TLC, the reaction mixture was cooled to room temperature. Petroleum ether was added to precipitate out the crude product. The supernatant organic layer was decanted off, and the procedure was repeated several times until most of the nitrobenzene had been removed. The product was finally purified by column chromatography over silica gel to yield the pure compound as a dark brown, hygroscopic solid in ∼50% yield. The pure compound was eluted out with 8−10% MeOH in $CHCl_3$. ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 2.07 (quint., 2H), 2.35 (s, 6H), 2.6 (t, 2H), 4.14 (t, 2H), 7.08 (d, J = 8.7 Hz, 2H), 7.80−7.84 (m, 2H), 8.09−8.11 (m, 3H), 8.22−8.24 (m, 1H), 8.29−8.30 (m, 1H), 8.35−8.36 (m, 1H), 11.2 (br s, 1H). ^{13}C NMR (100.5 MHz, CD_3-OD): δ ppm 25.3, 43.0, 55.5, 65.3, 114.5, 120.8, 121.2, 126.0, 126.5, 128.0, 129.1, 133.0, 133.3, 133.5, 133.8, 157.4, 161.2, 182.3, 183.5. HR-MS $(M + 1)^+$ calcd, 426.1817; found, 426.1808. Anal. ($C_{26}H_{23}N_3O_3 \cdot 3.5H_2O$) for C, H, N.

4-Hydroxyphenyl-1*H*-anthra[1,2-*d*]imidazole-6,11-dione (2a). 1,2-Diaminoanthraquinone (0.39 g, 1.64 mmol) and 4-hydroxybenzaldehyde (0.2 g, 1.64 mmol) were dissolved in dry nitrobenzene (10 mL) and heated with stirring at 120 °C for 20 h. Nitrobenzene was removed by washing the precipitated solid with petroleum ether. The process was repeated several times until TLC indicated the product as pure (R_f = 0.4 at 8% MeOH in $CHCl_3$). The solid material was isolated in 60% yield. FT-IR (KBr; ν cm^{-1}) 3400 (O−H str.). ^{1}H NMR (300 MHz, $CDCl_3$ + CD_3OD): δ ppm 7.08 (d, J = 8.7 Hz, 2H), 7.80−7.83 (m, 2H), 8.09−8.11 (m, 3H), 8.22−8.36 (m, 3H). HR-MS $(M + 1)^+$ calcd, 341.0926; found, 341.0939.

4-[3-Bromopropyloxy]phenyl-1*H*-anthra[1,2-*d*]imidazole-6,-11-dione (2b). To a solution of **2a** (0.04 g, 0.12 mmol) in acetone (15 mL), anhydrous K_2CO_3 (0.1 g, 0.6 mmol) was added. The mixture was stirred for 30 min before the addition of 1,3-dibromopropane (0.07 g, 0.35 mmol), after which the reaction mixture was refluxed for 12 h. Once **2a** was consumed, acetone was removed. This was followed by a water workup, and the product was extracted into chloroform. After drying the organic layer over anhydrous Na_2SO_4, solvent was removed and the residue was purified by column chromatography using silica gel. The pure product was eluted out in chloroform and was obtained as a yellow solid in ∼60% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 2.4

* To whom correspondence should be addressed. Phone: (91)-80-2293-2664. Fax: (91)-80-2360-0529. E-mail: sb@orgchem.iisc.ernet.in.
[†] Indian Institute of Science.
[‡] Indian Institute of Chemical Biology.
[§] Chemical Biology Unit of JNCASR.

10.1021/jm0610604 CCC: $37.00
Published on Web 04/20/2007

(quint., 2H), 3.65 (t, 2H), 4.23 (t, 2H), 7.1 (d, $J = 8.7$ Hz, 2H), 7.80−7.83 (m, 2H), 8.08−8.13 (m, 3H), 8.24 (d, $J = 8.4$ Hz, 1H), 8.27−8.30 (m, 1H), 8.34−8.37 (m, 1H), 11.23 (s, 1H).

General Procedure for the Synthesis of Compounds 2−7. Compound **2b** (1 equiv) was added to a stirred solution of appropriate amine (2 equiv) in dry acetone containing excess (5 equiv) anhydrous K_2CO_3. The reaction was continued under refluxing condition for 12−15 h until TLC indicated complete disappearance of **2b**. After removal of acetone, the reaction mixture was washed with water and the crude product was extracted in chloroform. The organic layer was collected and dried over anhydrous Na_2SO_4, and then the solvent was evaporated. Column chromatography on silica gel yielded the pure product as a hygroscopic solid upon elution with 2−5% MeOH in $CHCl_3$. The yields of all the compounds ranged from 50 to 70%.

4-[(3-Diethylamino)propyloxy]phenyl-1*H*-anthra[1,2-*d*]imidazole-6,11-dione (2). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 1.41 (t, 6H), 2.2 (quint., 2H), 3.2 (quart., 4H), 3.26 (t, 2H), 4.15 (t, 2H), 7.05 (d, $J = 8.7$ Hz, 2H), 7.81−7.83 (m, 2H), 8.09 (d, $J = 8.6$ Hz, 1H), 8.12 (d, $J = 8.7$ Hz, 2H), 8.29 (d, $J = 8.7$ Hz, 1H), 8.30−8.31 (m, 1H), 8.34−8.36 (m, 1H), 11.26 (br s, 1H). ^{13}C NMR (100.5 MHz, $CD_3OD + CDCl_3$): δ ppm 25.7, 32.5, 43.0, 55.5, 65.3, 114.4, 120.8, 121.2, 125.0, 126.5, 128.0, 129.1, 133.0, 133.3, 133.5, 133.8, 157.4, 161.2, 182.3, 183.5. HR-MS $(M + 1)^+$ calcd, 454.2130; found, 454.2137. Anal. ($C_{28}H_{27}N_3O_3 \cdot 3H_2O$) for C, H, N.

4-[(3-(4-(2-Hydroxyethyl)-1-piperazinyl))propyloxy]phenyl-1*H*-anthra[1,2-*d*]imidazole-6,11-dione (3). ^{1}H NMR (400 MHz, CD_3OD): δ ppm 2.05 (quint., 2H), 2.54−2.66 (m, 10H), 3.32 (t, 2H), 3.72 (t, 2H), 4.19 (t, 2H), 7.05 (d, $J = 8.8$ Hz, 2H), 7.7−7.84 (m, 2H), 7.92 (d, $J = 8.4$ Hz, 1H), 8.10 (d, $J = 8.8$ Hz, 2H), 8.20−8.30 (m, 3H). ^{13}C NMR (100.5 MHz, CD_3OD): δ ppm 25.4, 51.7, 52.0, 54.2, 57.5, 59.0, 65.4 114.0, 120.0, 120.9, 125.6, 126.2, 127.6, 128.6, 132.5, 132.9, 133.0, 133.4, 157.0, 169.9, 182.0, 183.3. HR-MS $(M + 1)^+$ calcd 511.2345; found, 511.2351. Anal. ($C_{30}H_{30}N_4O_4 \cdot 3.5H_2O$) for C, H, N.

4-[(3-(4-Methyl-1-piperazinyl))propyloxy]phenyl-1*H*-anthra[1,2-*d*]imidazole-6,11-dione (4). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 2.05 (quint., 2H), 2.46 (s, 3H), 2.64−2.71 (m, 10H), 4.12 (t, 2H), 7.07 (d, $J = 8.8$ Hz, 2H), 7.81−7.83 (m, 2H), 8.09 (d, $J = 8.6$, 1H), 8.11 (d, $J = 8.8$ Hz, 2H), 8.23 (d, $J = 8.6$ Hz, 1H), 8.28−8.30 (m, 1H), 8.35−8.37 (m, 1H), 11.23 (s, 1H). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ ppm 26.4, 45.3, 52.1, 54.5, 54.7, 66.2, 115.2, 117.8, 121.2, 122.0, 125.2, 126.5, 127.6, 128.3, 128.7, 133.5, 133.7, 134.1, 134.4, 149.8, 156.7, 161.6, 182.6, 185.3. HR-MS $(M + 1)^+$ calcd, 481.2239; found, 481.2242. Anal. ($C_{29}H_{28}N_4O_3 \cdot 3H_2O$) for C, H, N.

4-[(3-(4-Morpholinyl))propyloxy]phenyl-1*H*-anthra[1,2-*d*]imidazole-6,11-dione (5). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 2.06 (quint., 2H), 2.54−2.62 (m, 6H), 3.77 (t, 4H), 4.15 (t, 2H), 7.09 (d, $J = 8.8$ Hz, 2H), 7.8−7.84 (m, 2H), 8.10 (d, $J = 8.4$ Hz, 1H), 8.12 (d, $J = 8.8$ Hz, 2H), 8.24 (d, $J = 8.4$ Hz, 1H), 8.29−8.31 (m, 1H), 8.35−8.37 (m, 1H), 11.23 (s, 1H). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ ppm 53.6, 55.5, 80.7, 115.4, 117.8, 122.0, 125.3, 126.5, 127.6, 128.3, 128.8, 133.3, 133.8, 134.1, 134.5, 149.8, 156.0, 161.6, 182.7, 185.4. HR-MS $(M + 1)^+$ calcd, 468.1946; found, 468.1923. Anal. ($C_{28}H_{25}N_3O_4 \cdot H_2O$) for C, H, N.

4-[(3-(1-Imidazolyl))propyloxy]phenyl-1*H*-anthra[1,2-*d*]imidazole-6,11-dione (6). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 2.3 (quint., 2H), 4.01 (t, 2H), 4.24 (t, 2H), 6.95 (s, 1H), 7.06 (d, $J = 8.8$ Hz, 2H), 7.09 (s, 1H), 7.52 (s, 2H), 7.80−7.83 (m, 2H), 8.09 (d, $J = 8.4$ Hz, 1H), 8.12 (d, $J = 8.8$ Hz, 2H), 8.24 (d, $J = 8.4$ Hz, 1H), 8.28−8.30 (m, 1H), 8.35−8.37 (m, 1H), 11.23 (s, 1H). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ ppm 30.7, 43.4, 64.0, 115.1, 117.8, 118.9, 121.7, 122.0, 125.3, 126.5, 127.6, 128.3, 128.9, 129.8, 131.3, 133.3, 133.5, 133.7, 134.1, 134.4, 137.3, 149.7, 156.6, 161.0, 182.7, 185.3. HR-MS $(M + 1)^+$ calcd, 449.1613; found, 449.1611. Anal. ($C_{27}H_{20}N_4O_3 \cdot 2H_2O$) for C, H, N.

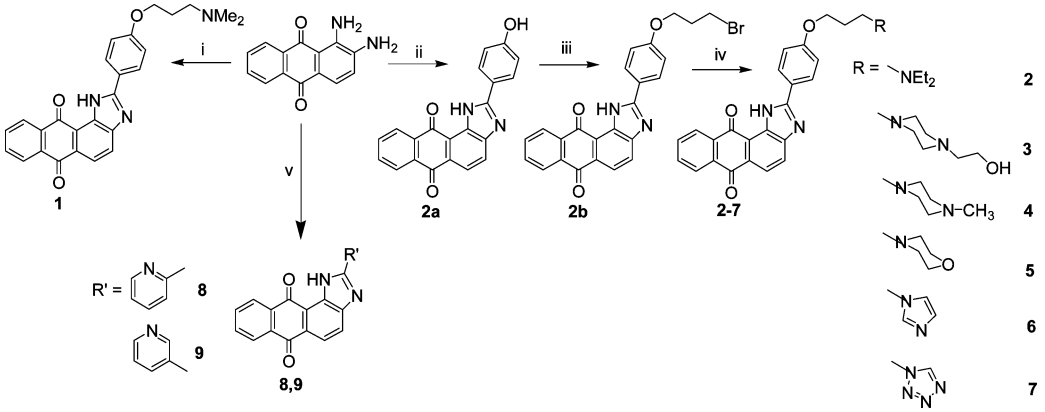
4-[(3-(1-Tetrazolyl))propoxy]phenyl-1*H*-anthra[1,2-*d*]imidazole-6,11-dione (7). ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 2.6 (quint., 2H), 4.15 (t, 2H), 4.95 (t, 2H), 7.06 (d, $J = 8.7$ Hz, 2H), 7.80−7.83 (m, 2H), 8.09 (d, $J = 8.4$ Hz, 1H), 8.12 (d, $J = 8.7$ Hz, 2H), 8.23 (d, $J = 8.4$ Hz, 1H), 8.28−8.30 (m, 1H), 8.34−8.36 (m, 1H), 8.55 (s, 1H), 11.23 (s, 1H). ^{13}C NMR (100.5 MHz, $CDCl_3$): δ ppm 29.0, 49.9, 64.4, 115.2, 117.9, 121.7, 122.0, 125.3, 126.5, 127.6, 128.4, 128.8, 133.3, 133.5, 133.7, 134.1, 134.4, 149.7, 152.9, 156.6, 161.0, 182.6, 185.3. ESI-MS $(M + 1)^+$ calcd, 451.2; found, 451.2. Anal. ($C_{25}H_{18}N_6O_3 \cdot 1.25H_2O$) for C, H, N.

For the synthesis of compounds **8** and **9**, 1,2-diminoanthraquinone and 2- or 3-pyridinecarboxaldehyde were taken in a 1:1 ratio in dry $PhNO_2$ and heated at 120 °C for 15−17 h until TLC indicated the completion of the reaction. Precipitation of the product with petroleum ether and repeated washing with the same removed nitrobenzene completely to yield the pure products in almost 65% yield.

2-Pyridyl-1*H*-anthra[1,2-*d*]imidazole-6,11-dione (8). ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 7.45 (t, 1H), 7.80−7.83 (m, 2H), 7.9 (t, 1H), 8.17 (d, $J = 8.4$ Hz, 1H), 8.26 (d, $J = 8.4$ Hz, 1H), 8.30−8.37 (m, 2H), 8.46 (d, 1H), 8.76 (m, 1H). ^{13}C NMR (100.5 MHz, $CDCl_3$): 118.8, 121.9, 122.4, 125.6, 126.5, 127.2, 127.4, 133.2, 133.6, 133.9, 134.3, 137.4, 137.5, 146.7, 149.0, 149.5, 155.8, 182.9, 184.4. HR-MS $(M + Na)^+$ calcd, 348.0749; found, 348.0700. Anal. ($C_{20}H_{11}N_3O_2 \cdot H_2O$) for C, H, N.

3-Pyridyl-1*H*-anthra[1,2-*d*]imidazole-6,11-dione (9). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 7.56−7.57 (m, 1H), 7.84−7.85 (m, 2H), 8.19 (d, $J = 8.6$ Hz, 1H), 8.28−8.38 (m, 3H), 8.53 (d, 1H), 8.81 (d, 1H), 9.43 (br s, 1H). ^{13}C NMR (100.5 MHz, $CDCl_3$): 118.9, 122.0, 124.1, 125.1, 125.4, 126.4, 127.2, 129.1, 133.0, 133.4, 133.8, 134.3, 135.5, 147.8, 148.5, 150.8, 154.4, 182.8, 184.3. HR-MS $(M + 1)^+$ calcd, 326.0929; found, 326.0934. Anal. ($C_{20}H_{11}N_3O_2 \cdot H_2O$) for C, H, N.

Scheme 1[a]



[a] Reagents, conditions, and yields: (i) 4-($Me_2N(CH_2)_3O$)-C_6H_5CHO (**1b**), dry $PhNO_2$, 130 °C, 16 h, 50%; (ii) 4-OH−C_6H_5CHO, dry $PhNO_2$, 120 °C, 20 h, 60%; (iii) $Br(CH_2)_3Br$, K_2CO_3, dry acetone, reflux, 12 h, 60%; (iv) amine (RH), K_2CO_3, dry acetone, reflux, 12−15 h, 50−70%; (v) 2- or 3-pyridine carboxaldehyde (R′CHO), dry $PhNO_2$, 120 °C, 15−17 h, 65%.

Table 1. Association Constants (*K*) for Drug DNA Binding[a]

drug	K^b (10^5 M^{-1})		
	CT DNA	poly[d(A−T)]$_2$	poly[d(G−C)]$_2$
1	2.1 ± 0.5	4.1 ± 0.2	3.0 ± 0.1
2	1.9 ± 0.6	3.2 ± 0.3	2.3 ± 0.5
3	1.9 ± 0.3	3.0 ± 0.1	2.2 ± 0.4
4	1.0 ± 0.4	2.8 ± 0.5	1.9 ± 0.2
5	0.6 ± 0.2	1.1 ± 0.1	0.5 ± 0.2
6	1.1 ± 0.1	1.8 ± 0.4	0.6 ± 0.4
7	0.5 ± 0.1	0.5 ± 0.2	0.3 ± 0.2
8 and **9**	[c]	[c]	[c]

[a] See Supporting Information for details. [b] The results are the average of two trials. [c] For compounds **8** and **9**, binding was too low to be measured accurately.

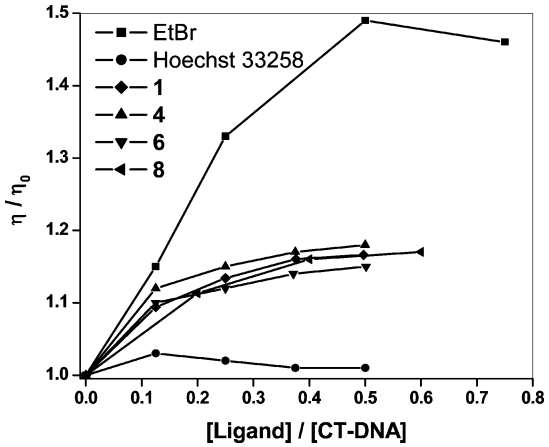


Figure 1. Viscometric titration of CT-DNA with compounds ethidium bromide (EtBr), Hoechst 33258, **1**, **4**, **6**, and **8**. The data are the average of several readings within ±0.04 s of each other.

Results and Discussion

Chemistry. Oxidative condensation between **1b** and 1,2-diaminoanthraquinone in nitrobenzene furnished **1** in 50% yield. For the syntheses of compounds **2**−**7**, a common synthetic route was followed. Compound **2a** was synthesized by oxidative condensation between 1,2-diaminoanthraquinone and 4-hydroxybenzaldehyde in nitrobenzene at 120 °C. Compound **2a** was converted to **2b** using 1,3-dibromopropane. In the final step, *N*-alkylation of the respective amines were achieved using compound **2b** to produce the compounds **2**−**7** in 50−70% yields. Coupling involving 1,2-diaminoanthraquinone and the requisite 2- or 3-pyridinecarboxaldehyde in nitrobenzene at 120 °C afforded compounds **8** and **9** in ∼65% yield. All the synthetic steps have been shown in Scheme 1.

DNA Binding. DNA binding studies were performed using UV−vis spectroscopy, where incremental concentrations of *ds*-DNA (CT-DNA, poly [d(A-T)]$_2$, or poly [d(G-C)]$_2$) were titrated into the ligand solution in buffer, and changes in the ligand absorbance were noted. DNA binding was marked by hypochromicity and red-shift of the long wavelength absorption band of the ligands (Figures S10−S15). The binding constants, determined by half-reciprocal plot method,[7] listed in Table 1, show strong DNA binding for **1**−**4** and **6** and weak binding for **5** and **7**. The hypochromicity observed on DNA addition for the compounds **8** and **9** were too low for determination of binding constants. To probe further into the nature of binding, viscometric titrations were performed. The molecules were shown to cause helix lengthening, as evident from increased viscosities of the solutions (Figure 1).[8] However, the increase was much less than that observed with intercalator EtBr but higher than that of minor groove binder Hoechst 33258. These

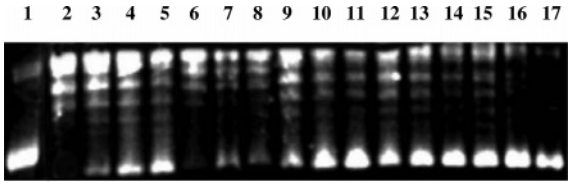


Figure 2. Inhibition of topo I relaxation activity. Lane 1, supercoiled plasmid DNA; Lane 2, DNA + topo I; Lanes 3−5, DNA + topo I + cpt (10, 20, 50 μM); Lanes 6−8, DNA + topo I + **4** (10, 20, 50 μM); Lanes 9−11, DNA + topo I + **5** (10, 20, 50 μM); Lanes 12−14, DNA + topo I + **6** (10, 20, 50 μM); Lanes 15−17, DNA + topo I + **7** (10, 20, 50 μM).

data for the new conjugates thus provide convincing evidence that there is neither classical intercalation here nor a minor groove binding.[9] Binding constants were also determined using the McGhee−von Hippel site exclusion model (Table S2).[10] The low values of the excluded site size ($n < 2$; Table S2) present a possibility that these agents stack and bind as dimers. Benzimidazole-based conjugates have indeed been shown earlier to bind to *ds*-DNA by stacked dimer formation.[11]

Biology. Inhibition Assays. To investigate the inhibition activity of these molecules against *L. donovani* topo I, the enzyme was purified following the procedure given in literature.[5] A relaxation assay[12] was performed by simultaneous incubation of supercoiled plasmid DNA, enzyme, and drug (Supporting Information). The inhibition activity of the drugs was compared to that of the standard topo I poison cpt (Figure 2, lanes 3, 4, 5). The inhibition activity of cpt is evident from the reduced topoisomer bands and ∼60% recovery of the supercoiled DNA band at 50 μM inhibitor concentration. The dialkylamine bearing drugs **1**, **2** (Figure S16), and piperazine derivatized **3** (Figure S16) and **4** (Figure 2, lanes 6−8) showed poor enzyme inhibition. Morpholine-based **5**, imidazole bearing **6**, and tetrazole derivatized **7**, however, showed effective inhibition of the enzyme activity. This was evident from the recovery of the supercoiled DNA band with increasing drug concentration (Figure 2, lanes 9−17). Compound **7** was found to be the most potent inhibitor of the relaxation activity induced by topo I, showing nearly full inhibition at 20 μM (Figure 2, lanes 15−17). Compound **6** (Figure 2, lanes 12−14) was nearly as potent, closely followed by **5** (Figure 2, lanes 9−11), which showed >75% inhibition at 20 μM and nearly full inhibition at 50 μM. Compounds **5**−**7** were, thus, more potent than cpt in inhibiting the activity of *L. donovani* topo I. The pyridine-based derivatives, **8** and **9**, showed moderate inhibition under simultaneous incubation condition (Figure S17).

Next, as a control, experiment was performed using human topo I,[13] which is a well characterized type IB topoisomerase.[14] The same trend in activity of the drugs was observed also with this enzyme. Compound **1**−**4** (data not shown) showed very poor activity against the enzyme whereas compounds **5**−**7** exhibited modest inhibitory activities against human topo I (Figure S18). However, cpt was a better inhibitor of human topo I than our drugs (Figure S18, lanes 3−5), while the reverse was true for *L.donovani* topo I. These observations provide significant leads toward the development of less cytotoxic drugs exhibiting selectivity for parasitic enzyme over host enzyme.

Next, to investigate if the drugs had any independent interaction with the parasitic topo I, they were preincubated with the enzyme at 37 °C for 5 min prior to the addition of DNA. The inhibition profile of **5**−**7** (Figure S19) was only marginally improved by enzyme preincubation. For **1**−**4**, preincubation with the enzyme did not significantly enhance inhibition (Figure S20).

Table 2. Activity of Drugs against *L. donovani* Topoisomerase I[a] and the Correlation with the Nature of Side-Chain Heteroatom

	recovery of supercoiled DNA[b] (%)			nature of side-chain *N*	
drug	10 μM[c]	20 μM[c]	50 μM[c]	Hyb[d]	pK_a
1	[e]	[e]	5	sp3	9.8
2	[e]	2	10	sp3	10.7
3	[e]	[e]	5	sp3	7.8
4	[e]	[e]	10	sp3	7.8
5	46	77	92	sp3	7.5
6	62	77	91	sp2	7.0
7	80	92	95	sp2	−2.4
8	9	20	75	sp2	5.2
9	9	15	80	sp2	5.2
cpt	39	55	65		

[a] The assay was done under simultaneous incubation (of plasmid DNA, enzyme and drug). [b] The amount of supercoiled monomer DNA band fluorescence after EtBr staining was quantitated. Data are the averages of two independent experiments and are within 8−10% error limit. [c] The concentration of the drug is expressed in μM. [d] Nature of hybridization. [e] Negligible inhibition.

For **8** and **9**, however, a significant enhancement of inhibition was observed on preincubation (Figure S17).

Careful scrutiny of the above results sheds light on some possible structure−activity relationships of the drugs. The pK_a of the side-chain nitrogen was found to be an important determinant of the efficacies of the drugs against the topo I enzyme. Strong basicity of the side-chain *N* in **1**−**4** rendered them significantly charged at physiological pH. Earlier studies on indenoisoquinoline-based topo I inhibitors have shown that the presence of H-bond acceptors on the side-chain amine enhances inhibition,[15] possibly by facilitating H-bond formation with the positively charged amino acid residues in the ternary cleavage complex.[14,15] But **1**−**4** being protonated at neutral pH may only serve as H-bond donors. However, **6,** despite having a protonation pK_a ∼7 for the imidazole *N*, showed good inhibition. Probably aromatic group on the side-chain facilitated inhibition, which made imidazole and tetrazole based **6** and **7** better inhibitors than all others. Again in **7,** the weakly basic tetrazole side-chain (protonation pK_a ∼-2) could also function as hydrogen bond acceptor, thus explaining the high inhibition activity of **7**. The same was true for **8** and **9,** in which the pyridine *N* had protonation pK_a ∼5. In fact, for **8** and **9**, inhibition was greatly improved on preincubation with the enzyme suggesting significant drug-enzyme interactions. Morpholine side-chain in **5** had *N* protonation pK_a ∼7.5, but the distal side-chain heteroatom was oxygen, whose ability to act as H-bond acceptor probably led to enhanced inhibition activity of **5**. The results of the above are summarized in Table 2. The activities of the drugs have been expressed in terms of their ability to prevent the enzyme mediated relaxation of supercoiled DNA. This inhibition activity has been correlated with the hybridization state and pK_a of the side-chain *N*.

Next we attempted to analyze if drug−DNA interaction affected the enzyme inhibition activity. Compounds **1**-**4,** which showed strong interaction with DNA, were found to be poor inhibitors. However, compound **7**, which only had weak interaction with DNA, was the best inhibitor. Again compounds **8** and **9** despite having negligible DNA interaction showed moderate topo I inhibition activity. Thus DNA binding appears to have little or no bearing on the topo I enzyme inhibition activity of these molecules.

While the anthra[1,2-d]imidazole-6,11,diones insert partially between the DNA base pairs, their side-chains possibly project outward, interacting with the polyanionic phosphodiester DNA backbone. The amino acid residues in the core catalytic domain of the topoisomerase are positively charged and are conserved for both *L.donovani* (R314, K352, R410, H453) and human topo I (R488, K532, R590, H632).[14] A H-bond acceptor in the side-chain may form favorable H bonding interactions with the above amino acid residues in the active site, stabilizing the ternary cleavable complex. In several inhibition studies reported previously, it has been observed that side-chain possessing a heteroatom capable of serving as a H-bond acceptor at physiological pH, generally provide the best results regarding topo I inhibition.[15] Our results are in keeping with those observations. However, the likely interpretation of structure−activity relationship is less obvious for compounds **8** and **9**. Probably the flat aromatic π surface enables the molecules to form favorable stacking interaction with certain residues of the enzyme at the active site.

Thus, we have successfully developed a series of imidazole-linked, anthraquinone-based topoisomerase inhibitors by systematically varying the side chain appended to the central aromatic moiety. Five of the nine drugs studied have emerged as very potent and selective inhibitors of topo I of *L. donovani*. As the compounds are also synthetically facile and chemically stable, the observations made in the present study provide useful insights toward developing potent inhibitors of the parasitic enzyme. Indeed, the nature of the amine-based side chain and its pK_a would hold the key in such design.

Acknowledgment. This work was supported by a grant from the Department of Biotechnology, Government of India.

Supporting Information Available: NMR characterization of **1**−**9**, elemental analyses, details of the DNA binding, and enzyme inhibition assays. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Jean-Moreno, V.; Rojas, R.; Goyeneche, D.; Coombs, G. H.; Walker, J. *Leishmania donovani*: Differential activities of classical topoisomerase inhibitors and antileishmanials against parasite and host cells at the level of DNA topoisomerase I and in cytotoxicity assays. *Exp. Parasitol.* **2006**, *112*, 21−30.

(2) Cheesman, S. J. DNA Topoisomerases as targets for antiprotozoal therapy. *Parasitol. Today* **2000**, *16*, 277−281.

(3) Nitiss, J. L. Investigating the biological functions of DNA topoisomerases in eukaryotic cells. *Biochim. Biophys. Acta* **1998**, *1400*, 63−81.

(4) Pommier, Y.; Pourquier, P.; Fan, Y.; Strumberg, D. Mechanism of action of eukaryotic DNA topoisomerase I and drugs targeted to the enzyme. *Biochim. Biophys. Acta* **1998**, *1400*, 83−106.

(5) Das, B. B.; Sen, N.; Ganguly, A.; Majumder, H. K. Reconstitution and functional characterization of the unusual bi-subunit type I topoisomerase from *Leishmania donovani*. *FEBS Lett.* **2004**, *565*, 81−88.

(6) Mandal, S. S.; Kadirvelraj, R.; GuruRow, T. N.; Bhattacharya, S. Exceptionally long crystal formation from 4-(3-bromopropyloxy)-salicylaldehyde, X-ray crystallographic investigation. *Chem. Commun.* **1996**, 2725−2726.

(7) Bhattacharya, S.; Mandal, S. S. DNA cleavage by intercalatable cobalt−bispicolylamine complexes activated by visible light. *Chem. Commun.* **1996**, 1515−1516.

(8) Bordelon, J. A.; Feieraband, K. J.; Siddiqui, S. A.; Wright, L. L.; Petty, J. T. Viscometry and atomic force microscopy studies of the interactions of a dimeric cyanine dye with DNA. *J. Phys. Chem. B.* **2002**, *106*, 4838−4843.

(9) Satyanarayan, S.; Dabrowiak, J. C.; Chaires, J. C. Tris(phenanthroline)ruthenium(II) enantiomer interaction with DNA: Mode and specificity of binding. *Biochemistry* **1993**, *32*, 2573−2584.

(10) McGhee, J. D.; von Hippel, P. H. Theoretical aspects of DNA−protein interactions: Cooperative and noncooperative binding of large ligands to a one-dimensional homogenous lattice. *J. Mol. Biol.* **1974**, *86*, 469−489.

(11) Wang, L.; Carrasco, C.; Kumar, A.; Stephens, C. E.; Bailly, C.; Boykin, D. W.; Wilson, W. D. Evaluation of the influence of compound structure on stacked-dimer formation in the DNA minor groove. *Biochemistry* **2001**, *40*, 2511−2521.

(12) Chowdhury, A. R.; Sharma, S.; Mandal, S.; Goswami, A.; Mukhopadhyay, S.; Majumder H. K. Luteolin, an emerging anticancer flavenoid, poisons eukaryotic DNA topoisomerase I. *Biochem. J.* **2002**, *366*, 653−661.
(13) Stewart, L.; Ireton, G. C.; Parker, L. H.; Madden, K. R.; Champoux, J. J. Biochemical and biophysical analyses of recombinant forms of human topoisomerase I. *J. Biol. Chem.* **1996**, *271*, 7593−7601.
(14) Davies, D. R.; Mushtaq, A.; Interthal, H.; Champoux, J. J.; Hol, W. G. J. The structure of the transition state of the heterodimeric topoisomerase I of *Leishmania donovani* as a vanadate complex with nicked DNA. *J. Mol. Biol*. **2006**, *357*, 1202−1210.
(15) Nagarajan, M.; Morrell, A.; Ioanoviciu, A.; Anthony, S.; Kohlhagen, G.; Agama, K.; Hollingshead, M.; Pommier, Y.; Cushman, M. Synthesis and evaluation of indenoisoquinoline topoisomerase I inhibitors substituted with nitrogen heterocycles. *J. Med. Chem*. **2006**, *49*, 6283−6289.

JM0610604