

Synthesis and DNA binding of 6-(alkylamino)indolo[1,2-*b*][2,7]naphthyridine-5,12-quinones

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This paper is to celebrate George A. Kraus for his many outstanding contributions to organic chemistry

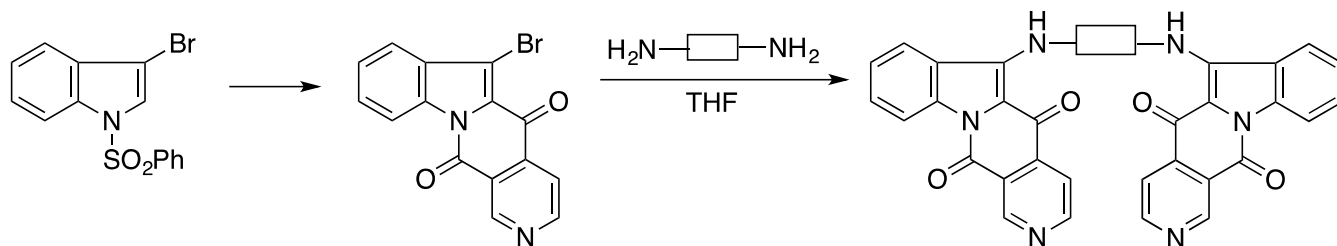
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Abstract

We describe the synthesis of eight novel putative mono- and bis-DNA intercalators from a common precursor, 6-bromoindolo[1,2-*b*][2,7]naphthyridine-5,12-dione. Of these new indoloquinones, our data indicate that two are most likely DNA mono-intercalators, but weaker than ethidium bromide, and two others are DNA bis-intercalators. Our indoloquinones are inactive against mammalian topoisomerase II.



Keywords: Indole, DNA intercalation, amination, 6-bromoindolo[1,2-*b*][2,7]naphthyridine-5,12-dione, indoloquinone

Introduction

The antitumor activity and DNA affinity of amino-substituted quinones is well established,¹⁻⁴ and several drugs in this category have seen utility in the cancer clinic; for example, mitomycin (**1**),^{5,6} ametantrone (**2**),⁷ mitoxantrone (**3**),^{8,9} pixantrone (**4**),¹⁰⁻¹² and WEHI-150 (**5**)^{13,14} (Figure 1).

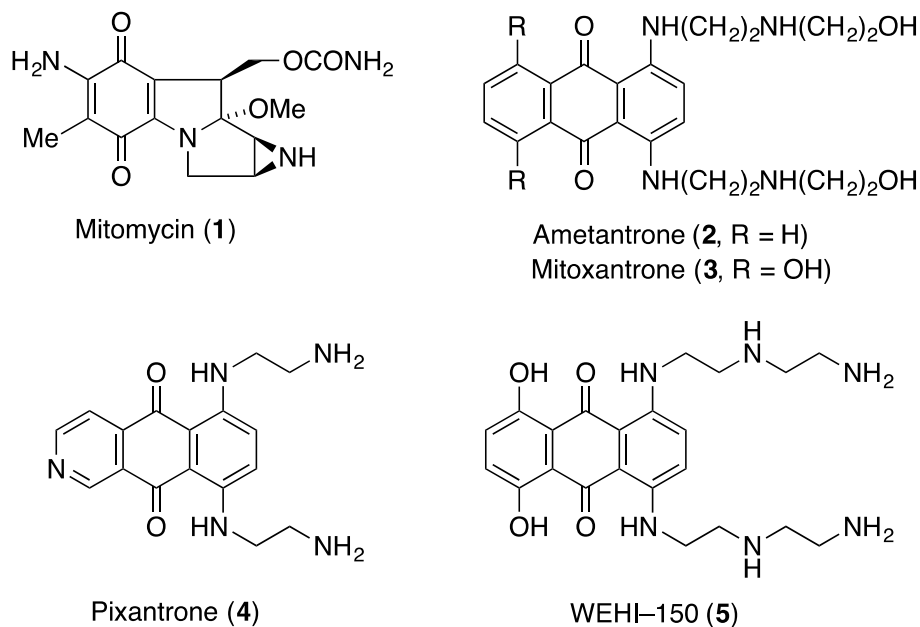


Figure 1

We have previously synthesized and utilized the indolo[1,2-*b*][2,7]naphthyridine-5,12-dione (**6**) ring system to forge several members of the 6*H*-pyrido[4,3-*b*]carbazole family of antitumor alkaloids, including ellipticine (**7**), 9-methoxyellipticine (**8**), olivacine (**9**), 13-oxoellipticine (**10**), 7,8,9,10-tetrafluoroellipticine (**11**), and ellipticine quinone (**12**)¹⁵⁻²⁰ (Figure 2). A variation of our method allowed for the synthesis of 10*H*-pyrido[2,3-*b*]carbazoles (**13**)²¹ and 6,11-disubstituted-benzo[*b*]carbazoles (**14**).²² The shape similarity of quinone (**6**) with that of ellipticine quinone (**12**) and calothrixin B (**15**), both of which display antitumor activity,²³⁻²⁵ suggested that it would be fruitful to examine indoloquinone **6** and its amino-substituted derivatives for DNA binding, given the known enhancement of DNA binding and resulting biological activity imparted by the alkylamino side chains in antitumor quinones **2–5**.

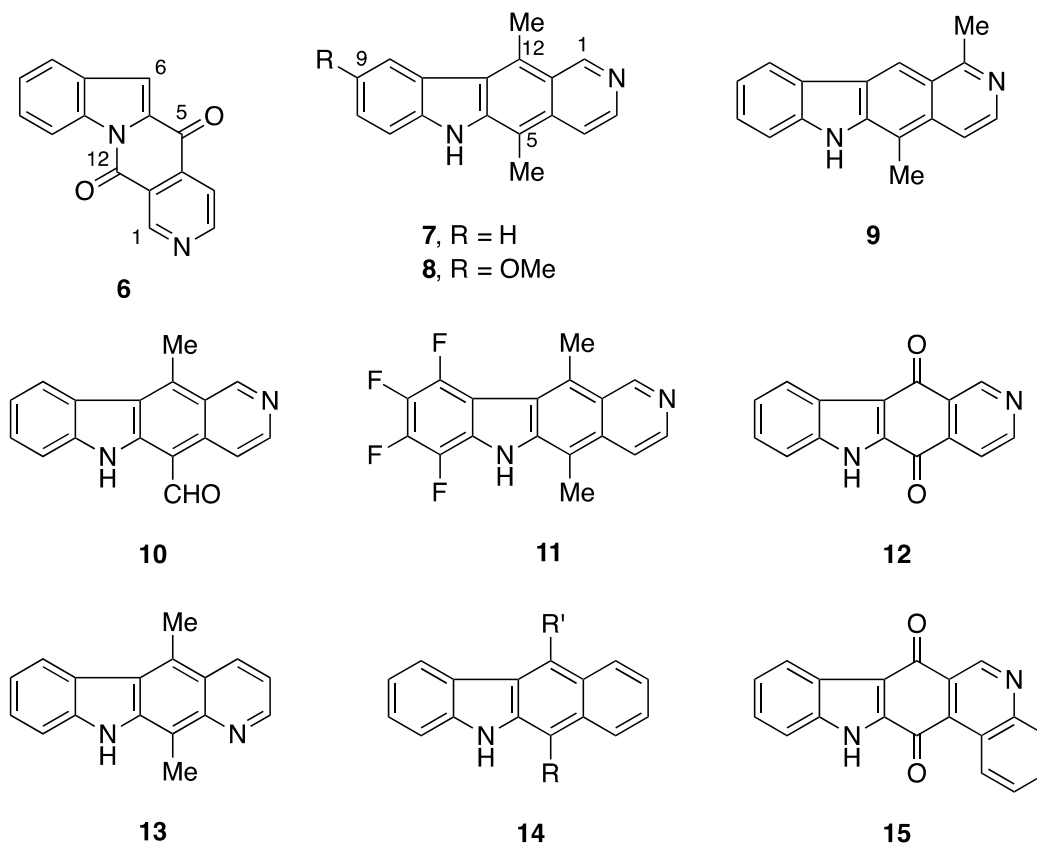
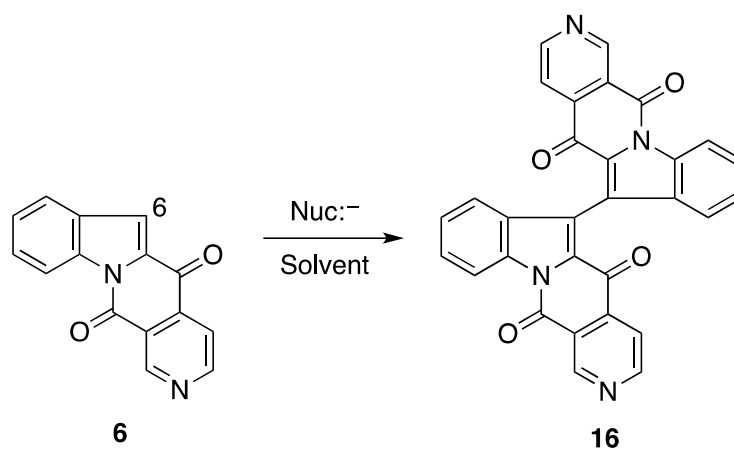


Figure 2

Fatefully, during attempts to isomerize indoloquinone **6** to ellipticine quinone (**12**) with various nucleophiles, we discovered the propensity for **6** to undergo oxidative dimerization at C-6 to give **16**²⁶ (Scheme 1).



Nuc: = NaCN, KF, NaCl, Mg(OMe)₂, NaSPh
Solvent = THF, DMF, DMSO, HMPA

Scheme 1

This observation fortuitously provided a convenient synthesis of the C-6 alkylamino derivatives **17a-d** and the bis-compounds **18a-d**, which we describe herein. Furthermore, it was conceivable that compounds of type **17** (or **18**) might irreversibly acylate DNA, given the tendency of *N*-acylindoles to undergo facile nucleophilic cleavage of the nitrogen-carbonyl bond^{27,28} (i.e., **17** → **19**) (Figure 3).

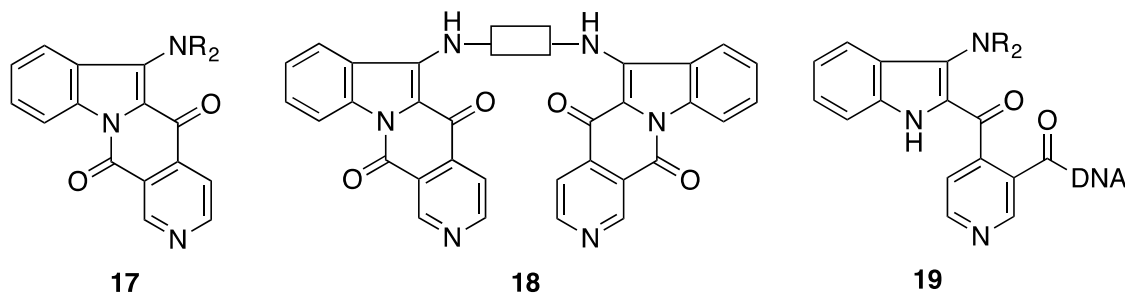
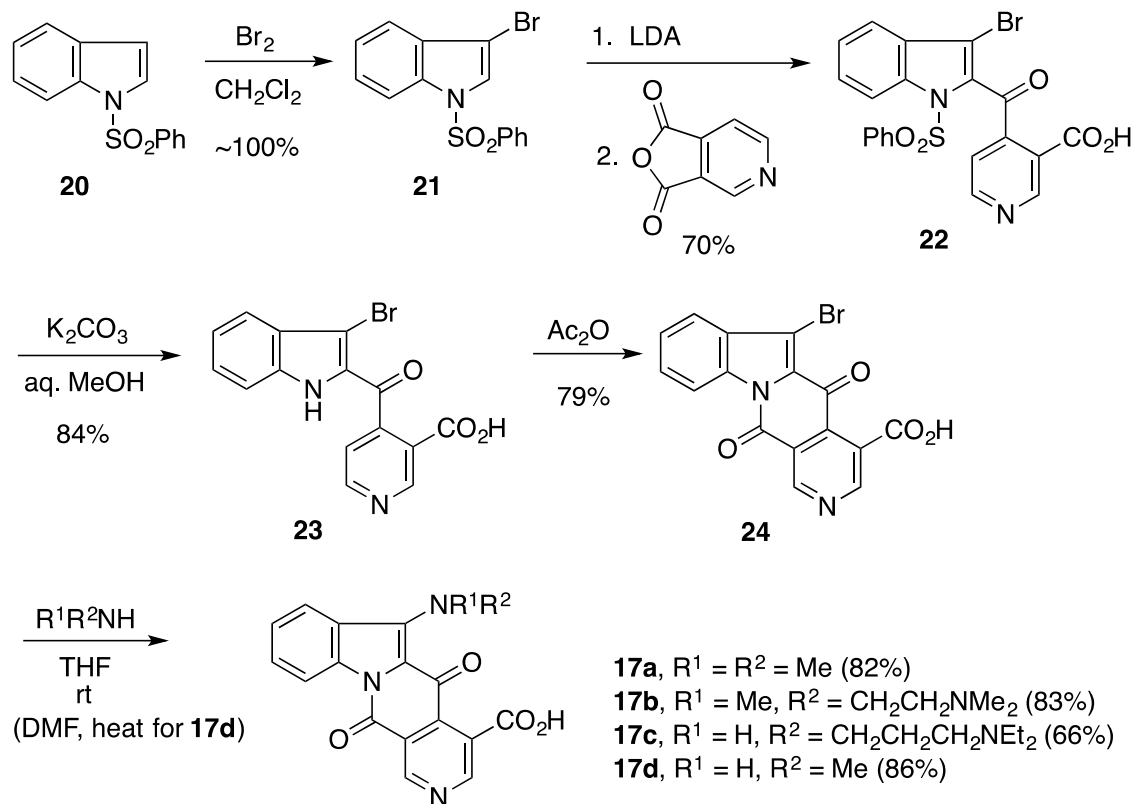


Figure 3

Results and Discussion

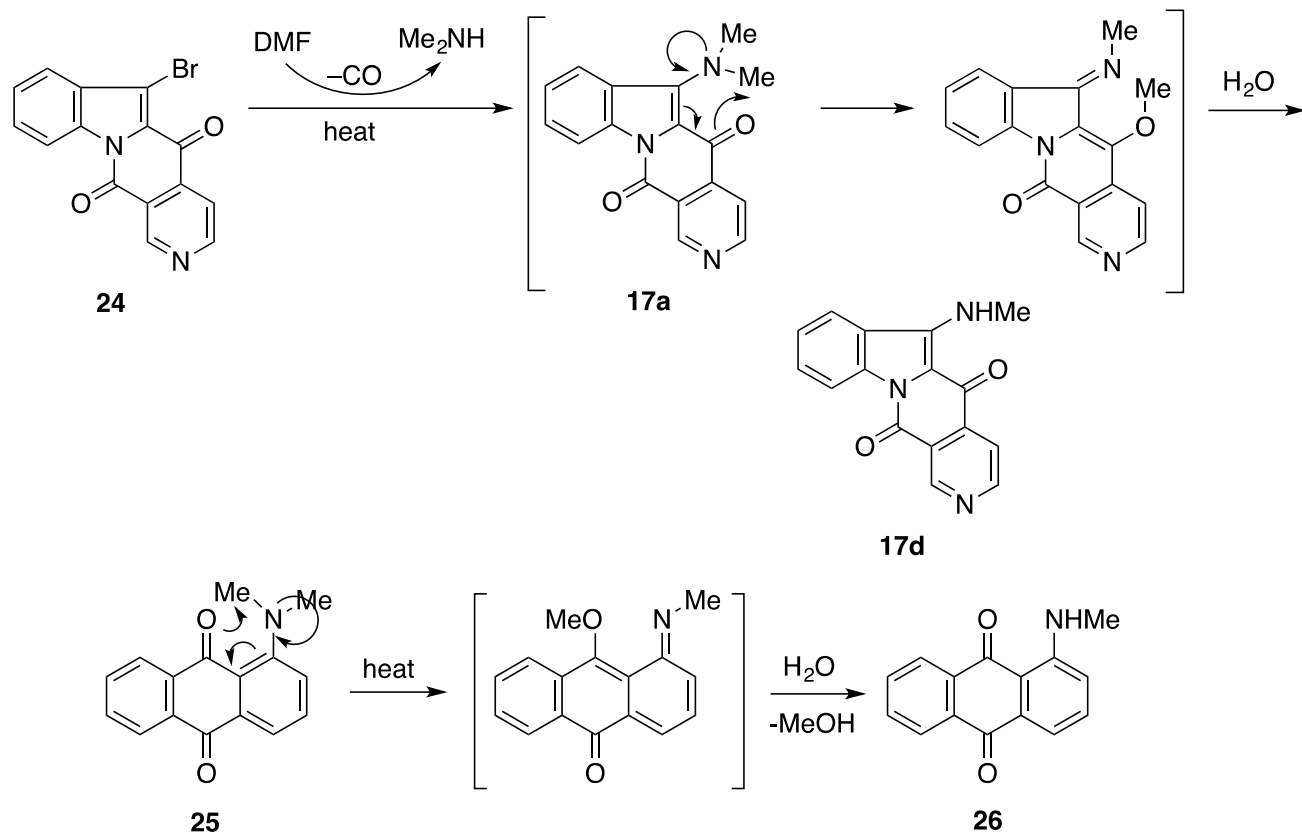
The targeted 6-(alkylamino)indolo[1,2-*b*][2,7]naphthyridine-5,12-diones (**17a-d**) and bis[6-aminoindolo[1,2-*b*][2,7]naphthyridine-5,12-diones (**18a-d**) were synthesized from 1-(phenylsulfonyl)indole (**20**) as shown in Scheme 2. The synthesis of bromo indoloquinone (**24**) parallels our earlier synthesis of indoloquinone **6**.¹⁵ The known 3-bromo-1-(phenylsulfonyl)indole (**21**), prepared quantitatively from **20** and bromine in methylene chloride, was treated with lithium di-isopropyl amide (LDA) at -78 °C, stirred at that temperature for one hour, and quenched with 3,4-pyridinedicarboxylic anhydride at -100 °C to give the protected keto-acid (**22**) in 70% yield, after recrystallization from acetone to remove the minor regioisomer (< 2%). The phenylsulfonyl group was removed with K_2CO_3 in aqueous MeOH-water (84%), and the resulting deprotected keto acid (**23**) was cyclized to indoloquinone **24** in hot acetic anhydride (79%).

As noted earlier, our motivation to explore the reaction of **24** with various amines, and examine their DNA affinity, was that the aminoanthraquinones ametantrone (**2**) and mitoxantrone (**3**) show strong DNA affinity and good antineoplastic activity in clinical trials.⁷ In actuality, treatment of **24** with an excess of dimethylamine (25% aqueous solution) in THF at room temperature provided 5-dimethylaminoindolo[1,2-*b*][2,7]naphthyridine-6,11-dione (**17a**) cleanly in high yield. Similarly, compounds **17b** and **17c** were synthesized by allowing solutions of **24** in THF to react with excess *N,N,N'*-trimethylethylenediamine and 3-diethylaminopropylamine, respectively. These reactions doubtlessly proceed by a typical nucleophilic addition-elimination mechanism.²⁹



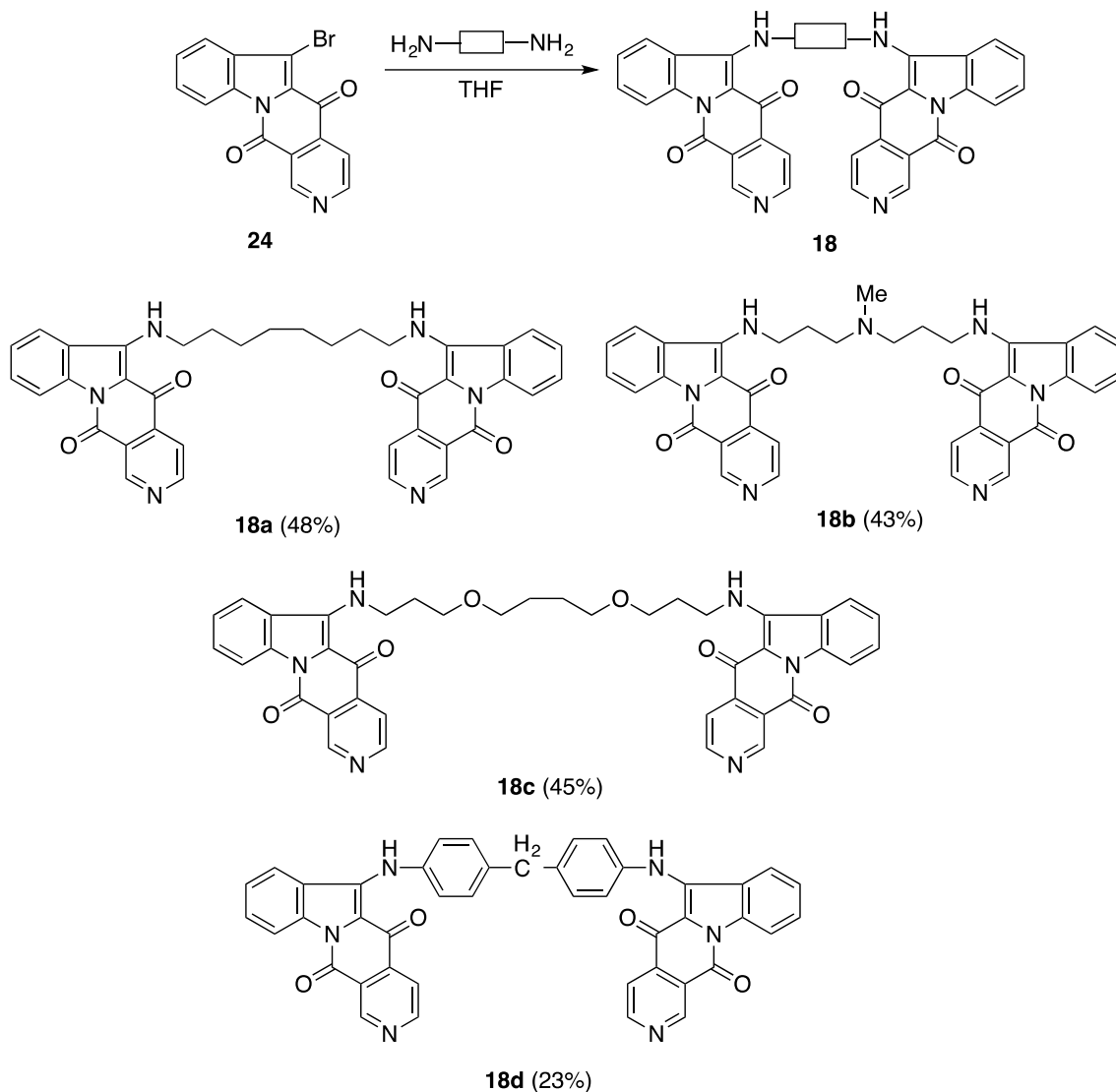
Scheme 2

Interestingly, the reaction of **24** with hot dimethylformamide (DMF) produced 5-(methylamino)indolo[1,2-*b*][2,7]naphthyridine-6,11-dione (**17d**) in 86% yield (Scheme 2). This peculiar reaction was discovered during attempts to displace bromide from **24** with 2-amino-2-methyl-1,3-propanediol³⁰ in hot DMF, since milder conditions (THF, rt) were unproductive. To confirm that the methylamino group had come from DMF rather than via the decomposition of 2-amino-2-methyl-1,3-propanediol, a solution of **24** was heated in DMF for 24 hours; once, again, there was a clean conversion to **17d**. This phenomenon had been observed twenty years earlier by Lord and Peters,³¹ who observed that heating 1-chloroanthraquinone for 32 hours in DMF at reflux afforded a mixture of 1-dimethylaminoanthraquinone (**25**) and 1-methylaminoanthraquinone (**26**). Moreover, **25** was demethylated to **26** by additional heating in DMF for 73 hours. In contrast, 2-chloroanthraquinone gave only 2-dimethylaminoanthraquinone in refluxing DMF. These observations are wholly consistent with a demethylation mechanism involving assistance by the neighboring carbonyl group as shown for the formation of **17d** from **24** (Scheme 3).



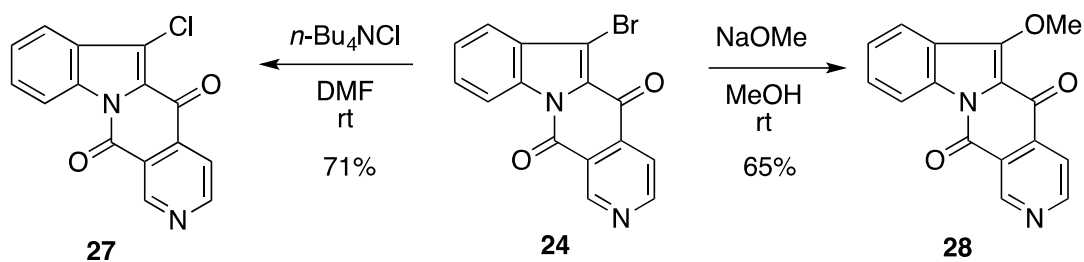
Scheme 3

Our motivation for preparing the bis-indoloquinones **18a-d** was to attain novel DNA bis-intercalators, and we were encouraged by the results from our previous studies with bis-acridines.³² Indeed, as we found with bis-acridines, adjustment of the length and flexibility of the diamino-linkage (tether) can dramatically alter the DNA-binding of these compounds. Therefore, we prepared bis-indoloquinones **18a-d** using a method similar to the method used to synthesize compounds **17a** (Scheme 4). The reactions were run in the presence of excess **24** so as to encourage the formation of the bis-indoloquinones. Nonetheless, some mono-indoloquinones were still present in the reaction mixture after 24 hours of stirring at room temperature.



Scheme 4

The bromine in indoloquinone **24** is also susceptible to displacement by other nucleophiles (Scheme 5). Thus, treatment of **24** with $(n\text{-Bu})_4\text{NCl}$ in DMF gave an orange compound that was identical to an authentic sample of 6-chloroindolo[1,2-*b*][2,7]naphthyridine-5,12-dione (**27**).¹⁷ Similarly, **24** was treated with sodium methoxide in methanol to give a product identical to an authentic sample of 6-methoxyindolo[1,2-*b*][2,7]naphthyridine-5,12-dione (**28**).¹⁷ Such behavior is not unexpected as β -haloenones are known to act as Michael acceptors for halogens, amines, alkoxides, and thiolates as well as carbon-based nucleophiles.³³



Scheme 5

Our results of DNA affinity studies for **17a-d** and **18a-d** are summarized in Table 1. The change in DNA melting, ΔT_m , is the change in the thermal denaturation of DNA by a drug as a result of intercalation.³⁴ From these results, it can be seen that compounds **17b** and **17c** are intercalators, although less effective when compared to a powerful intercalator such as the aminoacridine, quinacrine, which has a ΔT_m value of 24.9.³⁴ The other experiment was to determine the slope of the calf thymus viscometric titration. When a drug intercalates into DNA, the DNA helix lengthens. Thus, the line of concern here is the ratio of the increase in DNA contour length versus the drug/nucleotide ratio.³⁴ Unfortunately, in further tests, neither of these compounds exhibited any activity against mammalian topoisomerase II, which are enzymes that cause double-strand breaks in DNA, cause the DNA to unwind, and reseal the breaks.³⁵ They are believed to be necessary for replication, since the helical winding of the DNA must be released to allow strand splitting in replication. Thus, it is currently believed that topoisomerase activity is crucial for useful anticancer activity.³⁵ We find that **17a-d** have no activity against mammalian topoisomerase II at a drug concentration of 100 μ M.

Table 1. DNA affinity results for **17a-d** and **18 b,c**

Compound	CT DNA ^a	ΔT_m °C		Slope of Calf Thymus viscosity titration
		Poly AT ^b	Poly GC ^c	
17a	0.3 ± 0.2	—	—	—
17b	8.7 ± 0.05	7.6 ± 0.03	3.7 ± 0.65	1.7 ± 0.2
17c	8.5 ± 0.02	7.2 ± 1.0	4.5 ± 1.8	0.5 ± 0.1
17d	0.56 ± 0.41	—	—	—
18b	21.7 ± 7.4	—	—	—
18c	7.8 ± 6.8	0.4 ± 0.2	-0.2 ± 1.2	—
24	-0.4 ± 0.2	—	—	—
Ethidium bromide	11.9 ± 1.1	9.9 ± 0.4	5.3 ± 0.7	—

^aCT is sonicated calf thymus DNA.

^bPoly AT is sonicated poly(dA)•poly(dT) homopolymer.

^cPoly GC is sonicated poly(dG-dC) with alternating GCGCGC on both strands.

Conclusions

We have synthesized eight novel indoloquinones, **17a-d** and **18a-d** for DNA binding studies. Of these, **17a** and **17d** do not bind to DNA as evidenced by the calf thymus DNA assay, suggesting that the pyridine ring system alone is insufficiently basic to become protonated in the presence of the polyphosphates of DNA. Moreover, as might be expected, the exocyclic amino group is rendered less basic by the C-5 carbonyl group (vinylogous amide). In contrast, our data indicate that **17b** and **17c** are probably mono-intercalators. These two quinones show a binding profile similar to other DNA intercalators, and they exhibit a GC preference. By comparison to ethidium bromide, these two compounds are somewhat weaker intercalators. In contrast, indoloquinones **18b** and **18c** are likely bis-intercalators, since the high CT for **18b** and its biphasic nature suggests a double intercalator. The lesser DNA affinity of **18c** is probably imparted by the less electronegative oxygen atoms in the tether. Our eight new compounds, **17a-d** and **18a-d**, will serve in future biological studies.

Experimental Section

General. Melting points were determined on a Büchi 510 apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Perkin-Elmer 599 spectrometer and are referenced to the 1601 cm^{-1} band of polystyrene. ^1H NMR (300 MHz) and ^{13}C NMR (75 MHz) were recorded on a Varian XL-300 multinuclear Fourier transform spectrometer. Unitary resolution mass spectra (MS) were obtained on a Finnigan 4023 GC/MS system. High resolution mass spectra (HRMS) were recorded at the National Institutes of Health regional facility at the Massachusetts Institute of Technology. Ultraviolet (UV) spectra were recorded on a Hewlett Packard 8451A diode array spectrophotometer. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA. Analytical thin-layer chromatography (TLC) was performed on precoated Silica Gel 60 F₂₅₄ plates from EM Reagents. Visualization was accomplished with 254 and 365 nm UV light, iodine vapor, ceric ammonium sulfate spray (3% in 10% sulfuric acid) or “van Urk’s reagent” spray (*p*-dimethylaminobenzaldehyde in ethanolic sulfuric acid). Flash chromatography was performed with EM Reagents Silica Gel 60 (230-400 mesh). All reactions were performed under a static head of predried (CaSO₄ tower) nitrogen or argon in glassware that had been dried for at least 1 h at 135 °C. Benzenesulfonyl chloride and 3,4-pyridinedicarboxylic anhydride were distilled prior to use.

3-Bromo-1-(phenylsulfonyl)indole (21). To a stirred solution of 1-(phenylsulfonyl)indole³⁶ (**20**) (20.0 g, 77.8 mmol) in CH₂Cl₂ (125 mL) was added bromine (4.5 mL, 86 mmol) in CH₂Cl₂ (100 mL) dropwise with stirring over 1 h at rt, during which time HBr gas was evolved. After stirring for an additional 2 h, saturated aqueous sodium thiosulfate solution (250 mL) was added and the biphasic mixture was stirred for 15 min. The layers were separated and the aqueous phase was extracted with CH₂Cl₂ (3 x 50 mL). The combined organic phase was washed with saturated aqueous NaHCO₃ (2 x 200 mL), H₂O (2 x 200 mL), brine (200 mL), dried (Na₂SO₄), and concentrated in vacuo to yield 26.1 g (100%) of **21** as a white solid: mp 116–118 °C (lit.,³⁶ mp 119–120 °C); IR (CHCl₃) 1605, 1585, 1445, 1370, 1265 cm⁻¹; ^1H NMR (CDCl₃) δ 7.15–8.15 (m, 9H), 7.63 (s, 1H); ^{13}C NMR (CDCl₃) δ 137.7, 134.2, 134.1, 129.4, 129.3, 126.8, 125.8, 124.7, 123.9, 120.0, 113.5, 99.8.

3-Bromo-1-(phenylsulfonyl)indol-2-yl 3-carboxy-4-pyridyl ketone (22). To a solution of lithium diisopropylamide (7.14 mmol) prepared from diisopropylamine (1.00 mL, 7.14 mmol) and *n*-butyllithium (1.22 M in hexane; 6.00 mL, 7.32 mmol) in dry THF (40 mL) under nitrogen at –78 °C was added dropwise with stirring over 20 min a solution of **21** (2.00 g, 5.95 mmol) in dry THF (40 mL). The mixture was stirred at –78 °C for 1 h then cooled to –100 °C and treated as rapidly as possible with a solution of 3,4-pyridinedicarboxylic anhydride (1.21 g, 8.11 mmol) in dry THF (40 mL) while maintaining efficient cooling and stirring. The mixture was allowed to warm slowly to rt with stirring over 18 h and then concentrated in vacuo. The resulting brown viscous oil was dissolved in H₂O (250 mL), cooled to 0 °C, and slowly acidified with dilute HCl. The resulting white precipitate was collected and dried in vacuo to give 2.53 g (88%) of crude product. Recrystallization from acetone yielded 2.01 g (70%) of **22** as a white powder: mp 234–236 °C (dec); IR (KBr) 1715, 1675, 1450, 1365, 1260, 1175, 1070, 950, 860, 755, 735, 675 cm⁻¹; ^1H NMR (DMSO-*d*₆) δ 9.07 (s, 1H), 8.97 (d, 1H, *J* 4.8 Hz), 8.14 (d, 1H, *J* 8.0 Hz), 7.98 (d, 1H, *J* 8.0 Hz), 7.79–7.56 (m, 7H), 7.50 (m, 1H); ^{13}C NMR (DMSO-*d*₆) δ 184.7, 166.5, 153.0, 150.1, 144.9, 136.6, 136.4, 135.1, 133.5, 129.72, 129.67, 128.6, 127.1, 126.7, 125.7, 123.0, 121.8, 115.7, 110.9; MS *m/e* 486 (M⁺+2), 484 (M⁺), 328, 326, 264, 220, 164, 141, 77 (100%). Anal. Calcd for C₂₁H₁₃BrN₂O₅S + C₃H₆O: C, 53.05; H, 3.52; Br, 14.71; N, 5.15; S, 5.90. Found: C, 52.86; H, 3.30; Br, 14.95; N, 5.25; S, 6.05.

3-Bromoindol-2-yl 3-Carboxy-4-pyridyl ketone (23). A magnetically stirred solution of keto acid **22** (1.00 g, 2.06 mmol), K₂CO₃ (1.2 g, 8.7 mmol), H₂O (8 mL), and MeOH (25 mL) was heated under reflux for 45 min. The mixture was cooled and the MeOH was removed in vacuo. The dark, oily residue was dissolved in H₂O (100

mL), cooled to 0 °C, and slowly acidified with dilute HCl with stirring. The yellow precipitate was collected by filtration and dried in vacuo to yield 0.49 g of **23**. An additional 0.11 g of product was obtained by continuous extraction of the filtrate with CH₂Cl₂ to give a total of 0.60 g (84%) of **23**: mp 194–195 °C; IR (KBr) 1710, 1625, 1505, 1335, 1260, 1230, 740 cm⁻¹; ¹H NMR (DMSO-*d*₆) δ 12.38 (s, 1H), 9.22 (s, 1H), 8.94 (d, *J* 4.3 Hz, 1H), 7.61 (d, *J* 4.3 Hz, 1H), 7.50 (m, 2H), 7.41 (m, 1H), 7.20 (m, 1H); ¹³C NMR (DMSO-*d*₆) δ 183.0, 165.6, 153.6, 151.8, 150.9, 148.6, 136.5, 131.0, 127.1, 124.6, 123.0, 121.6, 121.5, 120.6, 113.2. A satisfactory analysis could not be obtained for this product so it was used directly in the next step.

6-Bromoindolo[1,2-*b*][2,7]naphthyridine-5,12-dione (24). A solution of **23** (0.60 g, 1.7 mmol) in Ac₂O (25 mL) was heated at 80 °C for 4 h. After cooling, most of the solvent was removed in vacuo, then H₂O (50 mL) and CH₂Cl₂ (50 mL) were added. The layers were separated and the aqueous phase was further extracted with CH₂Cl₂ (2 x 50 mL). The combined organic phase was washed with H₂O (2 x 100 mL) and brine (100 mL), dried (Na₂SO₄), and adsorbed onto silica gel. Flash chromatography with EtOAc gave 0.45 g (79%) of **24** as a yellow-green powder. Recrystallization from CH₂Cl₂/hexane gave the analytical sample as fine yellow needles: mp 230–232 °C (dec); IR (CHCl₃) 2980, 2920, 1705, 1680, 1600, 1535, 1450, 1365, 1335, 1255, 1220 cm⁻¹; ¹H NMR (DMSO-*d*₆) δ 9.35 (s, 1H), 9.16 (d, *J* 4.7 Hz, 1H), 8.54 (d, *J* 7.9 Hz, 1H), 8.18 (d, *J* 4.7 Hz, 1H), 7.75 (m, 2H), 7.56 (m, 1H); ¹³C NMR (DMSO-*d*₆) δ 174.1, 155.4, 150.0, 147.9, 134.7, 131.4, 131.2, 126.2, 126.0, 122.0, 120.8, 119.0, 118.3, 116.6, 108.7; MS *m/e* 328 (M⁺+2, 100%), 326 (M⁺, 100%), 300, 298, 272, 270, 247, 219, 191, 164, 114. Anal. Calcd for C₁₅H₇BrN₂O₂: C, 55.07; H, 2.16; Br, 24.43; N, 8.56; O, 9.78. Found: C, 54.82; H, 2.18; Br, 24.53; N, 8.49; O, 9.88.

6-Dimethylaminoindolo[1,2-*b*][2,7]naphthyridine-5,12-dione (17a). To a solution of **24** (100 mg, 0.31 mmol) in THF (25 mL) was added excess Me₂NH (25% aqueous solution, 20 mL). The mixture was stirred at rt for 6 h, during which time the solution became bright orange. The solution was poured into EtOAc (100 mL) and washed with saturated aqueous NaHCO₃ (2 x 50 mL), H₂O (2 x 50 mL) and brine (50 mL), then dried (Na₂SO₄) and concentrated to give an orange powder. Recrystallization from THF-hexane gave 73 mg (82%) of **17a** as small orange needles: mp 240–242 °C; IR (CHCl₃) 3050, 1680, 1635, 1575, 1545, 1495, 1410, 1370 cm⁻¹; ¹H NMR (CDCl₃) δ 9.62 (s, 1H), 8.98 (d, 1H, *J* 4.0 Hz), 8.86 (d, 1H, *J* 9.6 Hz), 8.06 (d, 1H, *J* 4.0 Hz), 8.00 (d, 1H, *J* 9.6 Hz), 7.62 (m, 1H), 7.33 (m, 1H), 3.49 (s, 6H); MS *m/e* 291 (M⁺), 276 (100%), 262, 249, 233, 220, 192, 164, 102, 77, 44; Anal. Calcd for C₁₇H₁₃N₃O₂: C, 70.08; H, 4.50; N, 14.42. Found: C, 69.87; H, 4.39; N, 14.41.

6-[*N*-[2-(Dimethylamino)ethyl]methylamino]indolo[1,2-*b*][2,7]naphthyridine-5,12-dione (17b). To a solution of **24** (65 mg, 0.20 mmol) in THF (5 mL) was added excess *N,N,N'*-trimethylethylenediamine (0.10 mL, 0.78 mmol). The mixture was refluxed for 1 h, during which time the solution became dark orange. The solution was cooled and poured into EtOAc (50 mL) and washed with saturated aqueous NaHCO₃ (2 x 25 mL), H₂O (2 x 25 mL) and brine (25 mL), then dried (Na₂SO₄) and concentrated to give a red powder. Recrystallization from CH₂Cl₂-hexane gave 57 mg (83%) of **17b** as short red needles: mp 129–131 °C; IR (CHCl₃) 2950, 2800, 1675, 1630, 1570, 1530, 1495, 1460, 1410, 1370, 1330 cm⁻¹; ¹H NMR (CDCl₃) δ 9.62 (s, 1H), 8.99 (d, 1H, *J* 5.1 Hz), 8.87 (d, 1H, *J* 8.1 Hz), 8.08 (d, 1H, *J* 5.1 Hz), 8.01 (d, 1H, *J* 8.1 Hz), 7.64 (m, 1H), 7.35 (m, 1H), 3.95 (t, 2H), 3.50 (s, 3H), 2.68 (t, 2H), 2.22 (s, 6H); ¹³C NMR (CDCl₃) δ 174.7, 168.0, 157.9, 153.9, 151.0, 150.9, 141.4, 137.5, 131.6, 124.9, 124.5, 124.3, 124.0, 118.5, 118.3, 57.6, 54.8, 45.7, 43.1; MS *m/e* 348 (M⁺), 303, 290, 277, 262, 248, 233, 80, 58 (100%); Anal. Calcd for C₂₀H₂₀N₄O₂ + 0.5 H₂O: C, 67.21; H, 5.92; N, 15.67. Found: C, 67.78; H, 5.76; N, 15.23.

6-[3-(Diethylamino)propylamino]indolo[1,2-*b*][2,7]naphthyridine-5,12-dione (17c). To a solution of **24** (56 mg, 0.17 mmol) in THF (5 mL) was added excess 3-diethylaminopropylamine (0.10 mL, 0.63 mmol). The mixture was refluxed for 1 h, during which time the solution became dark orange. The solution was cooled and poured into EtOAc (50 mL) and washed with saturated aqueous NaHCO₃ (2 x 25 mL), H₂O (2 x 25 mL) and brine

(25 mL), and then dried (Na_2SO_4) and concentrated to give an orange semi-solid. Recrystallization from CH_2Cl_2 -hexane gave 42 mg (66%) of **17c** as an orange powder: mp 102–103 °C; IR (CHCl_3) 3450, 2980, 2940, 2820, 1675, 1635, 1575, 1550, 1450, 1350 cm^{-1} ; ^1H NMR (CDCl_3) δ 9.58 (s, 1H), 8.95 (d, 1H, J 4.3 Hz), 8.68 (d, 1H, J 8.6 Hz), 8.02 (d, 1H, J 4.3 Hz), 7.96 (d, 1H, J 8.6 Hz), 7.62 (m, 1H), 7.29 (m, 1H), 3.96 (m, 1H), 3.90 (m, 2H), 2.63–2.46 (m, 6H), 1.34–0.87 (m, 8H); ^{13}C NMR (CDCl_3) δ 172.6, 168.4, 156.6, 153.5, 153.0, 151.2, 141.2, 138.4, 132.7, 124.9, 124.7, 124.6, 120.5, 118.0, 117.6, 50.0, 46.9, 44.8, 28.0, 11.6; MS m/e 376 (M^+), 290, 263, 248, 100, 86 (100%), 72, 58. Anal. Calcd for $\text{C}_{22}\text{H}_{24}\text{N}_4\text{O}_2 + 0.5 \text{H}_2\text{O}$: C, 68.55; H, 6.54; N, 14.54. Found: C, 68.67; H, 6.41; N, 14.40.

6-Methylaminoindolo[1,2-*b*][2,7]naphthyridine-5,12-dione (17d). A solution of **24** (0.102 g, 0.31 mmol) in DMF (20 mL) was heated at 130 °C for 24 h. The dark orange red solution was cooled and poured into saturated aqueous NaHCO_3 (50 mL) and extracted with CH_2Cl_2 (3 x 25 mL). The combined organic phase was washed with H_2O (2 x 50 mL) and brine, dried (Na_2SO_4), concentrated in vacuo, and dried for 12 h at 60 °C in vacuo to give a brick-red solid. Recrystallization from CH_2Cl_2 -hexane gave 0.073 g (86%) of **17d** as a dark red powder: mp 278–280 °C; IR (CHCl_3) 3400, 2930, 2860, 1720, 1675, 1635, 1535, 1450, 1360, 1330 cm^{-1} ; ^1H NMR (CDCl_3) δ 9.52 (s, 1H), 8.99 (d, 1H, J 4.5 Hz), 8.74 (d, 1H, J 8.7 Hz), 8.07 (d, 1H, J 4.5 Hz), 8.01 (d, 1H, J 8.7 Hz), 7.68 (m, 1H), 7.35 (m, 1H), 3.70–3.45 (bs, 1H), 3.56 (s, 3H); MS m/e 277 (M^+ , 100%), 248, 220, 192, 164, 104, 91, 77. Anal. Calcd for $\text{C}_{16}\text{H}_{11}\text{N}_3\text{O}_2 + 0.25 \text{H}_2\text{O}$: C, 68.20; H, 4.11; N, 14.91. Found: C, 68.47; H, 3.97; N, 14.60.

6-Chloroindolo[1,2-*b*][2,7]naphthyridine-5,12-dione (27). To a solution of **24** (50 mg, 0.15 mmol) in DMF (5 mL) was added (*n*-Bu) $_4$ NCl (50 mg, 0.18 mmol). The mixture was stirred at rt for 12 h and then poured into H_2O (25 mL). The precipitate was collected by filtration, dried and recrystallized from CH_2Cl_2 -hexane to yield 29 mg (71%) of **27** that was identical with an authentic sample by TLC, mp, and MS: mp 220–221 °C (lit.¹⁷ mp 219–220 °C); MS m/e 282 (M^+ , 100%), 254, 247, 226, 219, 191, 164, 114.

6-Methoxyindolo[1,2-*b*][2,7]naphthyridine-5,12-dione (28). A solution of **24** (0.0579 g, 0.177 mmol) in 2:1 THF-MeOH (10 mL) was added dropwise to a solution of NaOMe (from 0.012 g, 0.52 mmol, of Na) in MeOH (10 mL) at 0 °C. The mixture was allowed to warm to rt over 2 h and then poured in H_2O (50 mL) and extracted with EtOAc (3 x 25 mL). The combined organic phase was washed with H_2O (2 x 50 mL) and brine (50 mL), then dried (Na_2SO_4) and concentrated in vacuo to give an orange solid. Recrystallization from CH_2Cl_2 -hexane gave 0.0321 g (65%) of **28** that was identical with an authentic sample by TLC and mixed melting point: mp 212–215 °C (lit.¹⁷ mp 214–219 °C); MS m/e 278 (M^+), 277 (100%), 199, 183, 152, 129, 77, 57.

***N,N'*-Octylenebis[6-aminoindolo[1,2-*b*][2,7]naphthyridine-5,12-dione] (18a)**. To a solution of **24** (0.50 g, 1.5 mmol) in THF (25 mL) was added 1,8-diaminooctane (0.10 g, 0.70 mmol). The mixture was refluxed for 6 h, then cooled, poured into saturated aqueous sodium bicarbonate solution (100 mL) and extracted with CH_2Cl_2 (3 x 50 mL). The combined organic phase was washed with saturated aqueous sodium bicarbonate solution (50 mL), water (50 mL), and saturated aqueous sodium chloride solution (50 mL), then dried (Na_2SO_4) and adsorbed onto silica gel. Elution first with EtOAc, then acetone, then 16:3:1 acetone-MeOH-TEA yielded 0.27 g (48%) of the di-HBr salt of **18a** as an orange powder: mp 140–145 °C (dec); IR (CHCl_3) 3460, 3020, 2940, 2880, 1700, 1680, 1630, 1575, 1545, 1455, 1360 cm^{-1} ; ^1H NMR ($\text{DMSO}-d_6$) δ 11.93 (bs), 9.38–8.52 (m, 6H), 8.03–7.04 (m, 8H), 3.02 (m, 4H), 1.64–0.80 (m, 12H); Anal. Calcd for $\text{C}_{38}\text{H}_{32}\text{N}_6\text{O}_4 + 2\text{HBr}$: C, 57.16; H, 4.29; N, 10.52. Found: C, 58.69; H, 4.67; N, 10.57.

***N,N'*-(*N*-Methyl-4-aza-heptylene)bis[6-aminoindolo[1,2-*b*][2,7]naphthyridine-5,12-dione] (18b)**. To a solution of **24** (0.50 g, 1.5 mmol) in THF (25 mL) was added 3,3'-diamino-*N*-methyldipropylamine (0.10 g, 0.70 mmol). The mixture was refluxed for 6 h, then cooled, poured into saturated aqueous sodium bicarbonate solution (100 mL) and extracted with CH_2Cl_2 (3 x 50 mL). The combined organic phase was washed with saturated aqueous sodium bicarbonate solution (50 mL), water (50 mL), and saturated aqueous sodium

chloride solution (50 mL), then dried (Na_2SO_4) and adsorbed onto silica gel. Elution first with EtOAc then acetone then 16:3:1 acetone-MeOH-TEA yielded 0.19 g (43%) of **18b** as an orange-red powder: mp 170–175 °C (dec); ^1H NMR (CDCl_3) δ 9.41 (s, 2H), 8.85 (m, 2H), 8.44 (d, 2H, J 8.4 Hz), 7.85 (d, 2H, J 5.5 Hz), 7.52 (d, 2H, J 8.2 Hz), 7.01 (m, 2H), 6.69 (m, 2H), 3.95 (m, 4H), 2.80 (m, 4H), 2.48 (s, 3H), 2.07 (m, 4H); MS m/e 638 (M^+). Anal. Calcd for $\text{C}_{37}\text{H}_{31}\text{N}_7\text{O}_4 + 2\text{H}_2\text{O}$: C, 65.97; H, 5.23; N, 14.55. Found: C, 66.31; H, 4.90; N, 14.86.

***N,N'*-(4,9-Dioxa-dodecylene)bis[6-aminoindolo[1,2-*b*][2,7]naphthyridine-5,12-dione] (18c)**. To a solution of **24** (0.50 g, 1.5 mmol) in THF (25 mL) was added 4,9-dioxa-1,12-dodecanediamine (0.15 mL, 0.70 mmol). The mixture was refluxed for 6 h, then cooled, poured into saturated aqueous sodium bicarbonate solution (100 mL) and extracted with CH_2Cl_2 (3 x 50 mL). The combined organic phase was washed with saturated aqueous sodium bicarbonate solution (50 mL), water (50 mL), and saturated aqueous sodium chloride solution (50 mL), then dried (Na_2SO_4) and adsorbed onto silica gel. Elution first with EtOAc, then acetone, then 16:3:1 acetone-MeOH-TEA yielded 0.22 g (45%) of **18c** as an orange powder: mp 165–170 °C (dec); ^1H NMR (CDCl_3) δ 9.23 (s, 2H), 8.98 (m, 2H), 8.62 (d, 2H, J 8.0 Hz), 8.02 (m, 2H), 7.94 (d, 2H, J 8.1 Hz), 7.60 (m, 2H), 7.28 (m, 2H), 3.92 (m, 4H), 3.63 (m, 4H), 3.54 (m, 4H), 2.07 (m, 4H), 1.78 (m, 4H); MS m/e 697 (M^+). Anal. Calcd for $\text{C}_{40}\text{H}_{36}\text{N}_6\text{O}_6 + 1.75 \text{H}_2\text{O}$: C, 66.04; H, 5.13; N, 11.55. Found: C, 66.16; H, 5.13; N, 11.83.

***N,N'*-(Methylenedi-4,1-phenylenebis[6-aminoindolo[1,2-*b*][2,7]naphthyridine-5,12-dione] (18d)**. To a solution of **24** (0.73 g, 2.22 mmol) in THF (25 mL) was added 4,4'-methylenedianiline (0.20 g, 1.0 mmol). The mixture was refluxed for 6 h, then cooled, poured into saturated aqueous sodium bicarbonate solution (100 mL) and extracted with CH_2Cl_2 (3 x 50 mL). The combined organic phase was washed with saturated aqueous sodium bicarbonate solution (50 mL), water (50 mL), and saturated aqueous sodium chloride solution (50 mL), then dried (Na_2SO_4) and adsorbed onto silica gel. Elution first with EtOAc, then acetone, then 16:3:1 acetone-MeOH-TEA yielded 0.16 g (23%) of **18d** as a red powder: mp 238–239 °C (dec); IR (CHCl_3) 3420, 3020, 2965, 1685, 1630, 1615, 1595, 1570, 1550, 1515, 1365, 1210 cm^{-1} ; ^1H NMR (CDCl_3) δ 9.70 (s, 2H), 9.04 (d, 2H, J 5.1 Hz), 8.72 (d, 2H, J 8.5 Hz), 8.11 (d, 2H, J 5.1 Hz), 7.62 (m, 2H), 7.29 (m, 4H), 7.03 (d, 4H, J 8.4 Hz), 6.68 (d, 4H, J 8.4 Hz), 3.96 (s, 2H), 3.63 (bs, 2H); Anal. Calcd for $\text{C}_{43}\text{H}_{26}\text{N}_6\text{O}_4$: C, 74.78; H, 3.79; N, 12.17. Found: C, 75.02; H, 4.81; N, 12.04.

Procedure for determination of ΔT_m values. The thermal denaturation studies were done by the method of Cory³⁴ on a Varian 2290 UV-visible spectrophotometer with a heating rate of 18 °C/h. The five cuvettes were on a 2 min cycle time with a 5 sec dwell time. ΔT_m values were calculated from the printout using the difference between the compound T_m and the DNA. The calf thymus DNA had a T_m of 56.8 °C.

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References

1. Polyakov, N.; Leshina, T.; Fedenok, L.; Slepneva, I.; Kirilyuk, I.; Furso, J.; Olchawa, M.; Sarna, T.; Elas, M.; Bilkis, I.; Weiner, L. *Antioxidants Redox Signal.* **2018**, *28*, 1394.
<https://doi.org/10.1089/ars.2017.7406>
2. Tišler, M. *Adv. Heterocycl. Chem.* **1989**, *45*, 37.
[https://doi.org/10.1016/S0065-2725\(08\)60329-3](https://doi.org/10.1016/S0065-2725(08)60329-3)

3. Franck, R. W.; Tomasz, M. Wilman, D. E. V., Ed. Blackie: London, 1990. *Chemistry of Antitumour Agents*; Chap. 15, The Chemistry of Mitomycins; p379.
4. Delgado, J. L.; Hsieh, C-M.; Chan, N-L.; Hiasa, H. *Biochem. J.* **2018**, *475*, 373.
<https://doi.org/10.1042/BCJ20160583>
5. Remers, W. A.; Cragg, G. M.; Kingston, D. G. I.; Newman, D. J., Eds. CRC Press: Boca Raton, FL, 2012. *Anticancer Agents from Natural Products; The Mitomycins*; 2nd Ed. p623.
6. Han, I.; Russell, D. J.; Kohn, H. *J. Org. Chem.* **1992**, *57*, 1799.
<https://doi.org/10.1021/jo00032a037>
7. Martelli, S.; Dzieduszycka, M.; Stefanska, B.; Bontemps-Gracz, M.; Borowski, E. *J. Med. Chem.* **1988**, *31*, 1956.
<https://doi.org/10.1021/jm00118a015>
8. Enache, M.; Toader, A. M.; Enache, M. I. *Molecules* **2016**, *21*, 1356.
<https://doi.org/10.3390/molecules21101356>
9. Green, A. K.; Corty, R. W.; Wood, W. A.; Meenaghan, M.; Reeder-Hayes, K. E.; Basch, E.; Milowsky, M. I.; Dusetzina, S. B. *The Oncologist* **2015**, *20*, 516.
<https://doi.org/10.1634/theoncologist.2014-0432>
10. Krapcho, A. P. *Curr. Org. Chem.* **2015**, *19*, 886.
<https://doi.org/10.2174/1385272819666150119224450>
11. Huebel, K. *Exp. Opin. Pharmacother.* **2018**, *19*, 1829.
<https://doi.org/10.1080/14656566.2018.1528232>.
12. Minotti, G.; Han, H.; Cattani, V.; Egorov, A.; Bertoni, F. *Exp. Rev. Hematol.* **2018**, *11*, 587.
<https://doi.org/10.1080/17474086.2018.1476848>
13. Konda, S. K.; Kelso, C.; Medan, J.; Sleebs, B. E.; Phillips, D. R.; Cutts, S. M.; Collins, J. G. *Org. Biomol. Chem.* **2016**, *14*, 10217.
<https://doi.org/10.1039/C6OB02100J>
14. Konda, S. K.; Kelso, C.; Pumuye, P. P.; Medan, J.; Sleebs, B. E.; Cutts, S. M.; Phillips, D. R.; Collins, J. G. *Org. Biomol. Chem.* **2016**, *14*, 4728.
<https://doi.org/10.1039/C6OB00561F>
15. Saulnier, M. G.; Gribble, G. W. *J. Org. Chem.* **1982**, *47*, 2810.
<https://doi.org/10.1021/jo00135a034>
16. Saulnier, M. G.; Gribble, G. W. *Tetrahedron Lett.* **1983**, *24*, 3831.
[https://doi.org/10.1016/S0040-4039\(00\)94287-4](https://doi.org/10.1016/S0040-4039(00)94287-4)
17. Ketcha, D. M.; Gribble, G. W. *J. Org. Chem.* **1985**, *50*, 5451.
<https://doi.org/10.1021/jo00350a001>
18. Obaza-Nutaitis, J. A.; Gribble, G. W. *J. Nat. Prod.* **1986**, *49*, 449.
<https://doi.org/10.1021/np50045a011>
19. Gribble, G. W.; Saulnier, M. G.; Obaza-Nutaitis, J. A.; Ketcha, D. M. *J. Org. Chem.* **1992**, *57*, 5891.
<https://doi.org/10.1021/jo00048a022>
20. Gruver, E. J.; Onyango, E. O.; Gribble, G. W. *Arkivoc.* **2018**, *iii*, 144.
<https://doi.org/10.24820/ark.5550190.p010.414>
21. Gribble, G. W.; Fletcher, G. L.; Ketcha, D. M.; Rajopadhye, M. *J. Org. Chem.* **1989**, *54*, 3264.
<https://doi.org/10.1021/jo00275a008>
22. Fraser, H. L.; Gribble, G. W. *Can. J. Chem.* **2001**, *79*, 1515.
<https://doi.org/10.1139/v01-127>

23. Nishiyama, T.; Hatae, N.; Mizutani, M.; Yoshimura, T.; Kitamura, T.; Miyano, M.; Fujii, M.; Satsuki, N.; Ishikura, M.; Hibino, S.; Choshi, T. *Eur. J. Med. Chem.* **2017**, *136*, 1.
<https://doi.org/10.1016/j.ejmech.2017.04.071>
24. Bernardo, P. H.; Chai, C. L. L.; Heath, G. A.; Mahon, P. J.; Smith, G. D.; Waring, P.; Wilkes, B. A. *J. Med. Chem.* **2004**, *47*, 4958.
<https://doi.org/10.1021/jm049625o>
25. Parvatkar, P. T. *Curr. Org. Chem.* **2016**, *20*, 839.
<https://doi.org/10.2174/1385272819666150803235103>
26. Davis, D. A.; Gribble, G. W. *Heterocycles* **1992**, *34*, 1613.
<https://doi.org/10.3987/COM-92-6087>
27. Saxton, J. E. *J. Chem. Soc.* **1952**, 3592.
<https://doi.org/10.1039/jr9520003592>
28. Sundberg, R. J. Academic: New York, 1970. *The Chemistry of Indoles*, p34.
29. Ashcroft, W. R.; Dalton, L.; Beal, M. G.; Humphrey, G. L.; Joule, J. A. *J. Chem. Soc., Perkin Trans. 1.* **1983**, 2409.
<https://doi.org/10.1039/P19830002409>
30. Bair, K. W.; Andrews, C. W.; Tuttle, R. L.; Knick, V. C.; Cory, M.; McKee, D. D. *J. Med. Chem.* **1991**, *34*, 1983.
<https://doi.org/10.1021/jm00111a010>
31. Lord, W. M.; Peters, A. T. *J. Chem. Soc. (C)*. **1968**, 783.
<https://doi.org/10.1039/j39680000783>
32. Gribble, G. W.; Mosher, M. D.; Jaycox, G. D.; Cory, M.; Fairley, T. A. *Heterocycles* **2014**, *88*, 535.
[https://doi.org/10.3987/COM-13-S\(S\)77](https://doi.org/10.3987/COM-13-S(S)77)
33. Pohland, A. E.; Benson, W. R. *Chem. Rev.* **1966**, *66*, 161.
<https://doi.org/10.1021/cr60240a003>
34. Cory, M.; McKee, D. D.; Kagan, J.; Henry, D. W.; Miller, J. A. *J. Amer. Chem. Soc.* **1985**, *107*, 2528.
<https://doi.org/10.1021/ja00294a054>
35. Ross, W. E. *Biochem. Pharmacol.* **1985**, *34*, 4191.
[https://doi.org/10.1016/0006-2952\(85\)90273-4](https://doi.org/10.1016/0006-2952(85)90273-4)
36. Conway, S. C.; Gribble, G. W. *Heterocycles* **1990**, *30*, 627.
<https://doi.org/10.3987/COM-89-S79>