Design of Cancer-Specific Antitumor Agents Based on Aziridinylcyclopent[*b*]indoloquinones

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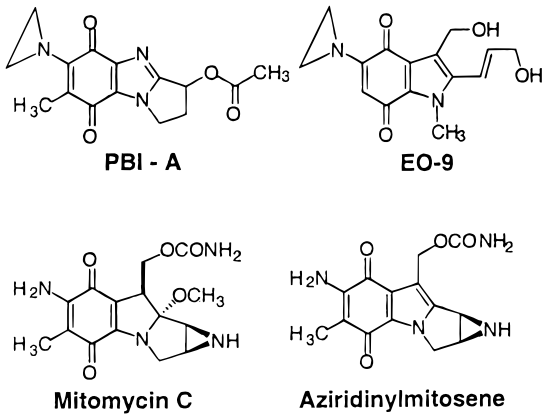
The merits of *N*-unsubstituted indoles and cyclopent[*b*]indoles as DNA-directed reductive alkylating agents are described. These systems represent a departure from *N*-substituted and pyrrolo[1,2-*a*]-fused systems such as the mitomycins and mitosenes. The cyclopent[*b*]indole-based aziridinylquinone system, when bearing an acetate leaving group with or without an *N*-acetyl group, was cytotoxic and displayed significant in vivo activity against syngeneic tumor implants. These analogues were superior to the others studied in terms of both high specificity for the activating enzyme DT-diaphorase and high percent DNA alkylation. Alkylation by a quinone methide intermediate as well as by the aziridinyl group could lead to cross-linking. The possible metabolites of the most active indole species were prepared and found to retain cytotoxicity, suggesting that in vivo activity could be sustained. The indole systems in the present study display selectivity for melanoma and, depending on the substituents present, selectivity for non-small-cell lung, colon, renal, and prostate cancers. The cancer specificities observed are believed to pertain to differential substrate specificities for DT-diaphorase.

Introduction

Previous findings from this laboratory showed that small molecule reductive alkylating agents called pyrrolobenzimidazoles or PBIs (Chart 1) show a high degree of specific cytotoxicity for some cancers.[1] This specificity is very likely due to the presence of the two-electron reducing enzyme DT-diaphorase, which reduces the PBI in these cells.[2,3] Reduction of the PBI to the corresponding hydroquinone causes the aziridine nitrogen to become more basic resulting in protonation and nucleophilic attack (alkylation). Similarly, indoloquinones such as mitomycin C[4] and EO9[5] (Chart 1) require two-electron reductive activation by DT-diaphorase.

The present article discusses the design of selective cytotoxic agents based on the aziridinylindoloquinones shown in Chart 2. Although indoloquinone-based antitumor agents are well-known, the compounds in Chart 2 possess one distinguishing feature: the *N*-unsubstituted indole system. Typically, the indoloquinone-based antitumor agents (mitomycins and mitosenes) have an alkyl substituent at this position. We were interested in a possible hydrogen bond donor role in the DNA major groove for the indole NH moiety. Previously, we documented DNA major groove hydrogen-bonding interactions with *N*-protonated reduced PBI antitumor agents.[6] Also, the quinone methide species derived from the pyrrolo[1,2-*a*]-fused and the cyclopent[*b*]-fused systems, shown in the inset of Chart 2, was expected to possess different stability and reactivity patterns. Finally, the differential DT-diaphorase substrate specificity of these novel indole analogues could lead to cancer specificity. Cyclopent[*b*]indole systems, **3c** and its *N*-acetyl analogue **14**, possessed high in vivo activity and high cancer specificity making them suitable compounds for further development.

Chart 1



Results and Discussion

Synthesis. The preparation of the indole series **1** was carried out as described in Scheme 1. The nitration and reduction of the previously prepared indole **4**[7] afforded **5**, which was converted to quinone derivatives by reduction/Fremy oxidation.[8,9]

The indole series **2**, which have the methyl and aziridinyl groups of **1** interchanged, were prepared by starting with **7** as outlined in Scheme 2. Access to **7** was possible employing the Japp-Klingmann/Fischer indole reactions starting with *p*-toluidine. This procedure is analogous to a reported indole synthesis starting with *p*-anisidine.[10]

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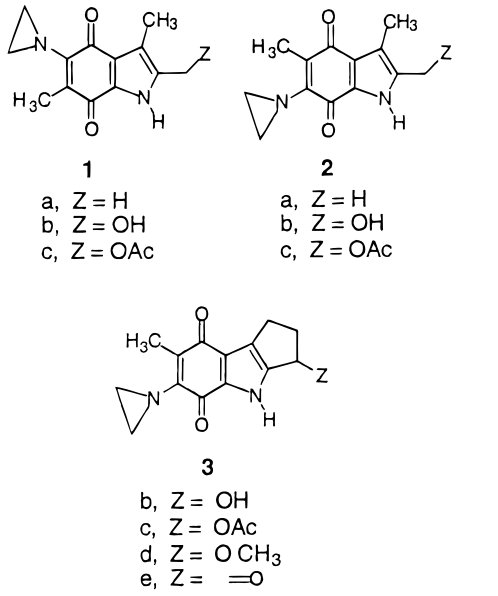
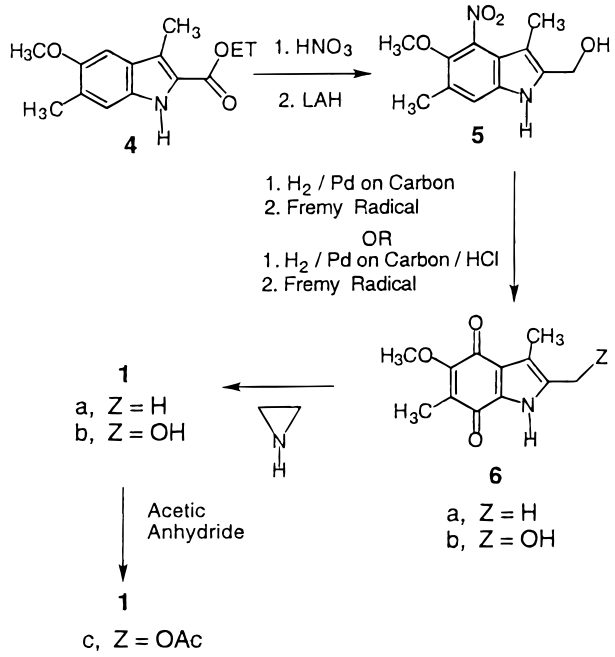
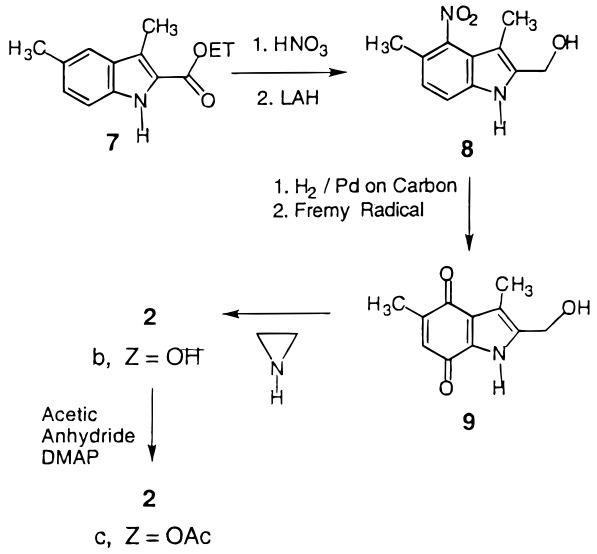
Chart 2



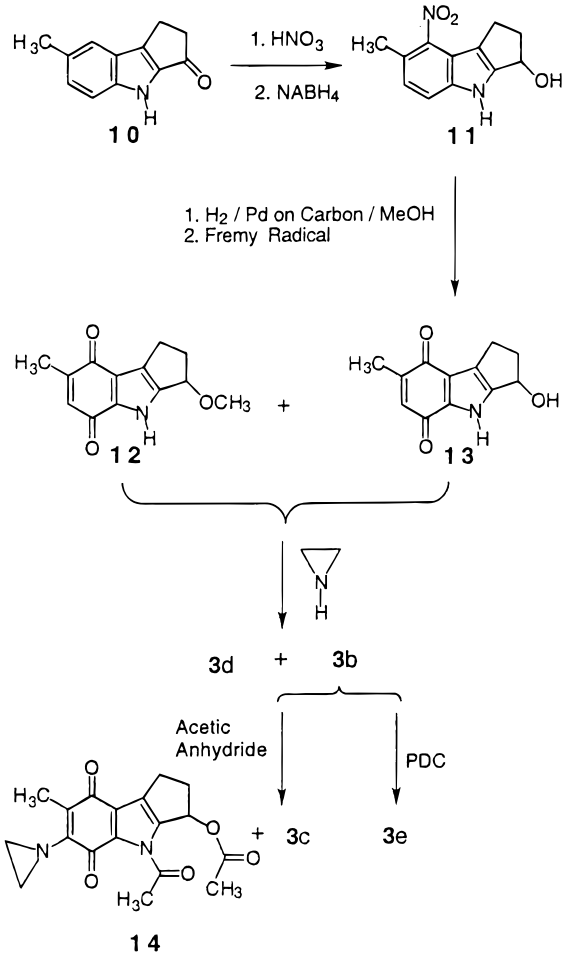
Scheme 1



Scheme 2



Scheme 3



The preparation of the cyclopentane-fused indole series **3** was carried out as outlined in Scheme 3 starting with **10**. The preparation of **10** was possible by employing the Fischer indole reaction starting with 1,2-cyclopentanedione[11] and *p*-toluidine. The nitration of **10** afforded only the 8-nitro derivative, identifiable by an AB quartet in the aromatic region with a J value = 8.7 Hz. The carbonyl and nitro groups were reduced by borohydride and H_2/Pd on carbon, respectively, followed by Fremy oxidation to afford **12** and **13**. The formation of **12** is believed to arise from the following three-step process: elimination of water from **11** resulting in a quinone methide, addition of methanol solvent to this species, and finally Fremy oxidation to the quinone product. Cyclopent[*b*]indole **12** was converted to cyclopent[*b*]indole **3d**. The alcohol derivative **3b** was then converted to **3c**, **3e**, and **14**.

Table 1. Log LC_{50} (concentration in mol/L causing 50% lethality) Values and Histological Cancer Type for the Indole Series **1a**−**c**[a]

cancer	1a	1b	1c
leukemia	> −4	> −4	> −4
non-small-cell lung	−4.29	−4.37	−4.48
colon	> −4	−4.32	−5.08
CNS	> −4	> −4	−4.83
melanoma	−4.42	−5.39	−5.64
ovarian	> −4	> −4	−4.46
renal	> −4	−4.53	−4.54
prostate	> −4	−4.78	−5.01
breast	> −4	−4.56	−4.57

[a] Each cancer type represents the arithmetic average of six to eight different cancer cell lines. Melanomas are the most sensitive cancer to this series of indoles, followed by prostate and colon for the more active indole analogues **1b** and **1c.**

Table 2. Log LC_{50} (concentration in mol/L causing 50% lethality) Values and Histological Cancer Type for the Indole Series **2a**−**c**[a]

cancer	2a	2b	2c
leukemia	> −4	> −4	> −4
non-small-cell lung	−4.3	−4.1	−4.1
colon	−4.05	−4.2	> −4
CNS	> −4	−4.05	> −4
melanoma	−4.15	−4.4	−4.15
ovarian	> −4	> −4	> −4
renal	> −4	−4.23	−4.14
prostate	> −4	−4.2	> −4
breast	> −4	−4.05	> −4

[a] Each cancer type represents the arithmetic average of six to eight different cancer cell lines. The large log LC_{50} values in the acetate-substituted indoles **2c** and to a lesser extent **2b**, compared to **1c** and **1b,** respectively, in Table 1, reflect the substantial loss of cytotoxicity observed when the aziridinyl group is moved from the 5- to the 6-position.

Table 3. Log LC_{50} (concentration in mol/L causing 50% lethality) Values and Histological Cancer Type for the Cyclopent[*b*]indole Series **3b**−**e** and **14**[a]

cancer	3e	3d	3c	3b	14
leukemia	> −4	−4.06	−4.3	−4.06	> −4
non-small-cell lung	−5.37	−4.2	−4.65	−4.22	−4.42
colon	−5.44	−4.32	−4.85	−4.45	−4.77
CNS	−5.13	−4.17	−4.7	−4.54	−4.75
melanoma	−5.99	−4.92	−4.99	−4.85	−5.06
ovarian	−4.86	> −4	−4.4	−4.05	−4.18
renal	−5.34	−4.58	−4.72	−4.73	−4.78
prostate	−4.8	−4.46	−4.63	−4.5	−4.71
breast	−4.9	−4.33	−4.53	−4.47	−4.5

[a] Each cancer type represents the average of six to eight different cancer cell lines. The lower log LC_{50} values show the increase of cytotoxicity with the addition of the fused cyclopentane ring. Melanomas are again the most sensitive cancer to this series of indoles followed by renal, colon, and non-small-cell lung cancers for the more active cyclopent[*b*]indole analogues **3c**, **3e**, and **14.**

In Vitro Screening Results. The cytotoxicities of the indole series **1**−**3** against a variety of cancer cell lines are compared in Tables 1−3. These data were *derived* from National Cancer Institute mean graph data and are not direct reproductions thereof. For each histologic cancer type, the average −log LC_{50} value was determined from an NCI panel consisting of six to eight human cancer cell lines.[12,13]

Comparison of the data in Tables 1 and 2 reveals the importance of the position of the aziridinyl group: when this group was moved from the 5- to the 6-position cytotoxic activity was substantially decreased. The importance of the position of the aziridinyl ring in cytotoxicity was also apparent in the PBIs.[14] The most active indoles shown in Table 1, **1b** and **1c**, possess a high specificity for lung, melanoma, and prostrate cancers. Studies described in the following section reveal that the position of the aziridinyl ring did not influence either reduction by rat liver DT-diaphorase or the percent alkylation of DNA.

When the relatively inactive analogues **2** are functionalized with the fused cyclopentane ring to afford cyclopent[*b*]indoles **3**, cytotoxic activity is substantially restored despite the position of the aziridinyl group, Table 3.

Our studies with rat liver DT-diaphorase described below revealed that the addition of a fused cyclopentane ring to the indole system can increase the substrate specificity for this enzyme greatly. Furthermore, this fused ring increases the percent alkylation of DNA. Consequently, the addition of the fused cyclopentane ring enhances both cytotoxicity and in vivo activity. This phenomenon is analogous to that seen when a fused pyrrole ring is added to the benzimidazole system.[14]

Table 4. Results of Indoles **1c** and **2c** and Cyclopent[*b*]indole **3c** in the B-16 Melanoma Model in C57/bl Mice (run at University of Arizona Cancer Center)[a]

dose	1c	2c	3c
control	11.39	11.39	11.39
1 mg/kg	10.94	13.83	6.67
3 mg/kg	15.82	8.59	6.92

[a] These compounds were studied at two dose levels: 1 and 3 mg/kg ip on days 1, 5, and 9 after tumor implantation into the front flank muscle. Shown in the table are the values for the area under the tumor growth curve (AUC) for indoles **1c** and **2c** and cyclopent[*b*]indole **3c** administered at 1 and 3 mg/kg doses. The control was obtained with drug-free animals.

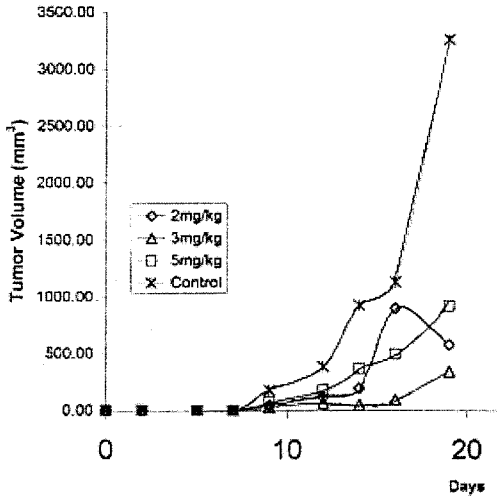
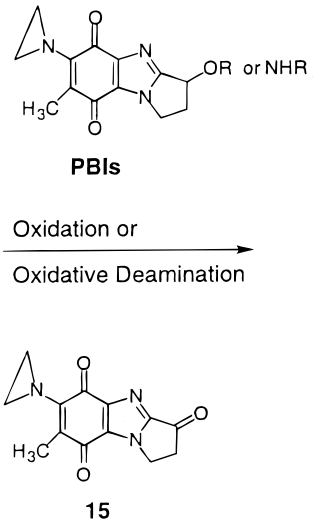


Figure 1. Results of cyclopent[*b*]indole **14** in the B-16 melanoma model in C57/b1 mice (run at University of Arizona Cancer Center). This compound was studied at three dose levels: 2, 3, and 5 mg/kg ip on days 1, 5, and 9 after tumor implantation into the front flank muscle. Shown in the plot are the AUC values as a function of time for each dosage. The control was obtained with drug-free animals.

In Vivo Screening Results. Shown in Table 4 and Figure 1 are B16 melanoma syngraft assays[15] which confirm the in vivo activity of the cyclo[*b*]indoles. Thus cyclopent[*b*]indole **3c** (1 and 3 mg/kg on days 1, 5, and 9 after subcutaneous implantation of 10^5 cells in the front flank on day 0) was able to reduce the tumor mass substantially. In contrast, indole **2c** decreases tumor

Scheme 4



mass only at the 3 mg/kg dose, and **1c** is inactive at both concentrations.

Unexpectedly, the most cytotoxic compound of Table 3 (**3e**) was completely inactive in the B16 syngraft assays. This finding suggests that oxidative metabolism will deactivate the cyclopent[*b*]indole series **3**. Similarly, the PBIs are completely noncytotoxic (50% lethal concentration or $LC_{50} > 10^{-4}$ M) upon oxidation to the ketone at the 3-position, Scheme 4. However, metabolism by acyl transfer to the indole NH group to afford cyclopent[*b*]indole **14** will serve to enhance cytotoxicity. Conversely, deacetylation reactions in the cyclopent[*b*]-indole series will result in sustained activity.

The results enumerated above indicate that the development of cancer-specific antitumor agents is still possible, although the selection of active analogues is still carried out in a random manner. This suggests that a combinatorial synthesis/testing approach would be required to identify the best compounds. For example, our cytotoxicity studies show that **3c** and **14** display in vitro potency with high selectivity as well as in vivo selectivity against melanoma, renal, and colon cancers.

DT-Diaphorase Substrate Activity and Percent DNA Reductive Alkylation. Although DT-diaphorase is present in both normal and cancerous tissues,[16] this enzyme may be the key to developing selective antitumor agents. Depending on the organ and species source, the substrate and inhibition properties of the enzyme can vary widely.[17,18] The structure of the enzyme even varies by ethnic origin, which may be a factor in the success of cancer chemotherapy in some patients.[19] The differential cytotoxicity of the indole series **1** and **3** discussed in the last section could originate from differential DT-diaphorase specificity, as well as differential concentrations of this enzyme.

In this section, we compare the V_{max}/K_M for reduction of the indoles with purified rat liver DT-diaphorase as well as the percent reductive alkylation of 600-bp calf thymus DNA, Figure 2. The data in Figure 2 shows that **3c** not only has the highest V_{max}/K_M (26.21×10^{-4} s^{-1}) but also has the highest percent alkylation of DNA. These observations provide a clear rationale for the observed in vivo activity of **3c**. Likewise the active compound cyclpent[*b*]indole **14** is an excellent substrate for DT-diaphorase and readily alkylates DNA.

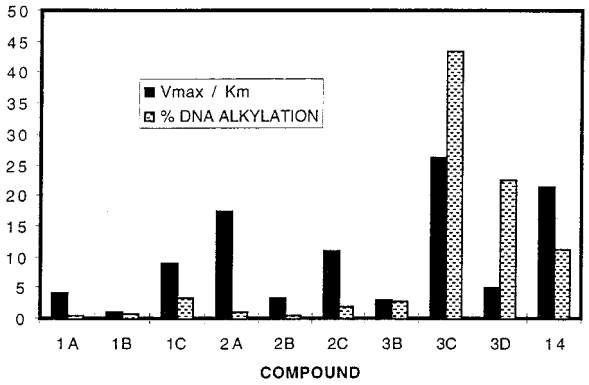


Figure 2. Bar graph of the specificity (V_{max}/K_M) for rat liver DT-diaphorase and the percent DNA base pair reductive alkylation of 600-bp calf thymus DNA for the indole series **1**−**3**.

Inspection of the data in Figure 2 reveals that the position of the aziridinyl group influences the DT-diaphorase reductase activity somewhat (compare **1a** with **2a**) but has little effect on the reductive alkylation of DNA. In fact, for the indole series **1** and **2**, neither the position of the aziridinyl group nor the substituent promote DNA alkylation. For the three indole series, the acetate substituent substantially increases the specificity for DT-diaphorase compared to the hydroxyl substituent. Inspection of the components of the specificity (V_{max}/K_M) for the acetate-substituted indoles reveals that the acetate substituent influences the K_M much like the other substituents ($10^{-14} \times 10^{-4}$ M), but also provides up to a 10-fold higher V_{max} value (up to 263×10^{-9} M/s).

Addition of the fused cyclopentane ring increases both the specificity for DT-diaphorase reduction as well as the percent alkylation of DNA for all analogues. Comparison of the acetate derivatives **2c** and **3c** reveals a large increase in substrate specificity for DT-diaphorase as well as a large increase in the percent DNA alkylation. The V_{max} rather than the K_M is largely responsible for this specificity difference. Addition of a fused ring, either fused pyrrole or fused cyclopentane, appears to be important for DT-diaphorase activity in some systems. Examples from this laboratory even show that two fused pyrrolo rings are better than one, Chart 3. This is not a general phenomenon since reductive alkylating compounds such as DZQ[20] and EO9[21] do not require fused rings for activation by DT-diaphorase.

The importance of the fused ring in reductive alkylation is very likely related to quinone methide formation upon elimination of acetate from the hydroquinone species, Scheme 5. Shown in this scheme are the two possible alkylation reactions of reduced **3c** starting with the nucleophile-mediated opening of the protonated aziridinyl group followed by quinone methide formation and nucleophile trapping of this species. The presence of the fused ring would promote elimination because the more substituted methide double bond is the product (in contrast to the terminal quinone methide resulting from reduced **1c** and **2c)**. The indole shown in the inset of Scheme 5 was prepared in this laboratory and found to be noncytotoxic in the NCI's 60-cancer cell line panel. This result was surprising since this indole could form a quinone methide species upon reduction and also was substituted with a reactive aziridinyl ring. Apparently,

Scheme 5

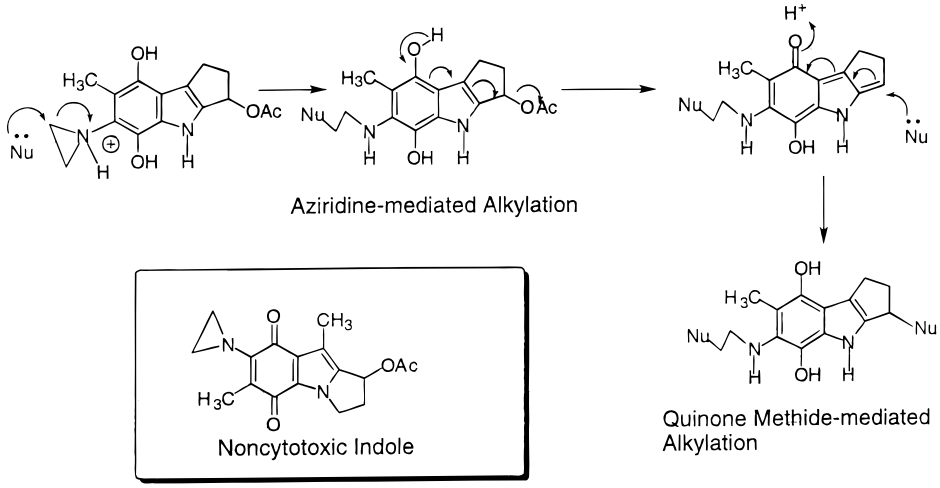
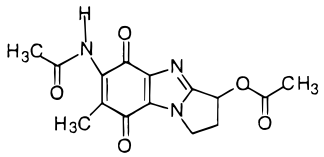
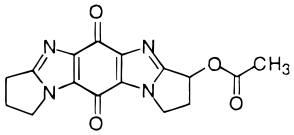


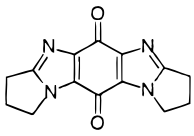
Chart 3



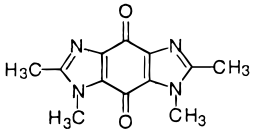
APBI - A $V_{max} / K_M = 2.6 \times 10^{-4}$ sec^{-1}



16 $V_{max} / K_M = 6.24 \times 10^{-4}$ sec^{-1}



17 $V_{max} / K_M = 7.1 \times 10^{-4}$ sec^{-1}



18 No substrate activity

ring fusion and the aziridinyl substituent location must be important components of cytotoxicity.

The role of ring fusion in cytotoxicity may be related to ring strain. Models shown in Figure 3 reveal that the quinone methide derived from cyclopent[*b*]indole is more stable (based on a lower positive heat of formation) than that derived from the pyrrolo[1,2-*a*]indole. The origin of the difference in stability is the change in geometry of the indole nitrogen when the quinone methide is formed. While in the aromatic indole form, this nitrogen has sp^2 geometry but becomes tetrahedral when the system becomes quinonoid. Strain results if this nitrogen is part of a five-membered ring. Thus the quinone methide derived from the cyclopent[*b*]indole system may form faster and have a low enough reactivity to trap nucleophiles selectively.

COMPARE Analysis. COMPARE was developed at the NCI to compare the patterns of cytotoxicity in their 60-cell line cancer panel.[12] Antitumor agents with identical mechanisms of action possess identical or nearly identical cytotoxicity patterns (correlation coefficient > 0.8). For example anthracycline analogues (doxorubicin, rubidazone, daunamycin) have a high correlation (>0.9) with each other as do the DNA alkylating agents (chlorambucil, thiotepa, triethylenemelamine). The cytotoxicity (LC_{50}) and growth inhibition (GI_{50}) profiles of **3c** were compared with those of known antitumor agents in the NCI archives. We observed a high correlation of the GI_{50} profile of **3c** with the concentration of cellular DT-diaphorase (correlation coefficient = 0.76). The cytotoxicity profile of **3c** correlated well with that of mitomycin C (correlation coefficient = 0.7). These correlations are consistent with the diaphorase-mediated activation and the possible DNA cross-linking reaction of reduced **3c**.

Conclusions

The above data show the merits of the cyclopent[*b*]-indole ring system in the design of new DNA-directed reductive alkylating agents. The combination of cyclopent[*b*] fusion and the indole NH (cyclopent[*b*]indole **3c**) results in a system exhibiting both cytotoxicity and antitumor activity. However, the addition of the *N*-acetyl group (cyclopent[*b*]indole **14**) enhances antitumor activity greatly.

The role of the fused cyclopentane ring in cytotoxicity is as yet unclear, and studies in this area are underway. We postulate that decreased ring strain may play a role in cyclopent[*b*]-fused quinone methide stability. The cyclopent[*b*]indole system represents a departure from the pyrrolo[1,2-*a*] fusion moieties typically found in the mitomycins and mitosenes as well as the many synthetic analogues thereof such as the PBI antitumor agents developed in this laboratory.[22−27]

Compound series **1** and **3** show a high degree of cancer selectivity with melanoma being the common target cancer of both series. Similarly, the PBI compounds also target this type of cancer, probably because of its high DT-diaphorase concentrations. Agents in series **1** also target colon and prostate cancers, while agents in series **3** target colon along with renal cancers. The mechanism

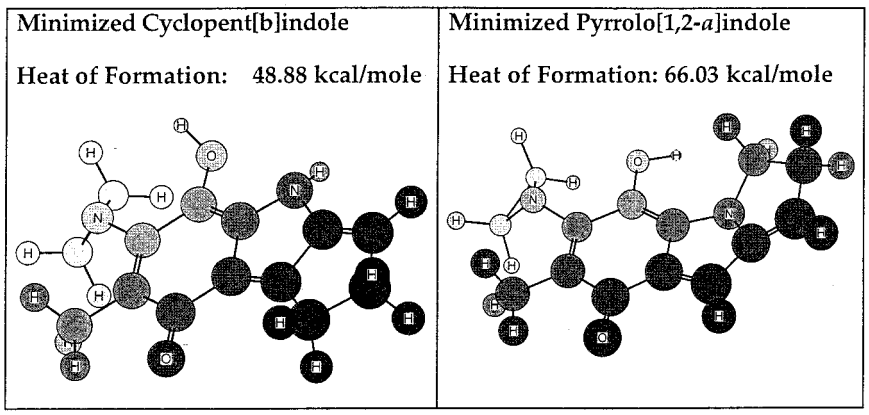


Figure 3. Minimized structures (Austin model I, closed shell wave function) of the cyclopent[*b*]indole and pyrrolo[1,2-*a*]indole quinone methides.

of **3c** cytotoxicity must closely resemble that of mitomycin C,[22,28,29] based on the high COMPARE correlation. These studies show that it is possible to design cancer-specific agents based on small molecules.

Experimental Section

All solutions and buffers for kinetic, DNA, and electrophoresis studies used doubly distilled water. All analytically pure compounds were dried under high vacuum in a drying pistol over refluxing toluene. Elemental analyses were run at Atlantic Microlab, Inc., Norcross, GA. All TLCs were performed on silica gel plates using a variety of solvents and a fluorescent indicator for visualization. IR spectra were taken as thin films and the strongest absorbances are reported. ^{1}H NMR spectra were obtained from a 300-MHz spectrometer. All chemical shifts are reported relative to TMS.

The syntheses of new compounds are outlined below.

2-(Hydroxymethyl)-3,6-dimethyl-5-methoxy-4-nitroindole (5) was prepared from **4** by the following two-step reaction. To a mixture consisting of 500 mg (2.02 mmol) of **4** and 15 mL of glacial acetic acid, cooled to 15 °C with a ice bath, was added 0.8 mL of nitric acid (69−71%). The reaction mixture was stirred for 15−20 min at room temperature and then poured into 100 mL of ice water and stirred for 10 min. The solids were filtered off and were washed with cold water. The wet solid was dissolved in chloroform, dried over Na_2SO_4, and concentrated to a residue, which was recrystallized from chloroform/hexane to afford nitrated **4** as yellow needles: 423 mg (70%) yield; mp 100−102 °C; TLC (chloroform/methanol, 90:10) $R_f = 0.72$; IR (KBr pellet) 3447, 3319, 2926, 1676, 1529, 1282, 1207, 1155, 1026, 869, 781 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.76 (bs, 1H, indole), 7.29 (1H, s, H-7), 4.42 (2H, d, $J = 7.2$ Hz, methylene of ethyl), 3.87 (3H, s, 5-methoxy), 2.44 (6H, s, 3,6-dimethyl), 1.42 (3H, t, $J = 7.2$ Hz, methyl of ethyl); MS (EI mode) *m*/*z* 292 (M^+), 275 ($M^+ - OH$), 263 ($M^+ - CH_2CH_3$), 246, 229, 215. Anal. ($C_{14}H_{16}N_2O_5$) C, H, N.

To a solution of 200 mg of lithium aluminum hydride and 5 mL of dried THF, chilled to 0 °C, was added 200 mg (0.68 mmol) of nitrated **4** in 10 mL of THF. The reaction was stirred at 0 °C for 15 min and then slowly combined with 5 mL of ethyl acetate and stirred for 5 min more. The solid residue was filtered off utilizing Celite and the filtrate was concentrated to dark red oil, which was dissolved in chloroform and washed with water. The chloroform was dried over Na_2SO_4 and concentrated to a solid residue, which was recrystallized from chloroform/hexane to afford **5a** as a yellow powder: 148 mg (87%) yield; mp 152−153 °C; TLC (chloroform/methanol, 90:10) $R_f = 0.41$; IR (KBr pellet) 3421, 3284, 2924, 1637, 1523, 1460, 1365, 1213, 1112, 1012, 866, 781 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.33 (1H, bs, indole), 7.23 (1H, s, H-7), 4.83 (2H, d, $J = 5.7$ Hz, 2-methylene), 3.87 (3H, s, 5-methoxy), 2.42 and 2.09 (6H, 2s, 3,6-dimethyl), 1.73 (1H, t, $J = 5.7$ Hz, hydroxy); MS (EI mode) *m*/*z* 250 (M^+), 249 ($M^+ - H$), 235 ($M^+ - CH_3$) 233 ($M^+ - OH$), 215, 202, 189, 174. Anal. ($C_{12}H_{14}N_2O_4$) C, H, N.

2,3,6-Trimethyl-5-methoxyindole-4,7-dione (6a) was prepared from **5** by the following two-step procedure. A suspension of 500 mg (1.71 mmol) of **5** in 20 mL of methanol, containing 250 mg of (5%) Pd on charcoal and a few drops of 2 N hydrochloride, was shaken under 50 psi of H_2 for 8 h. The reaction mixture was filtered through Celite, and the filtered cake was washed with methanol. The solvent was evaporated off to afford the amine as a residue, which was used in the next step without further purification.

The residue was dissolved in a solution of 1.5 g of Fremy salt and 0.75 g of monobasic potassium phosphate in 150 mL of water. The reaction was stirred at room temperature for 4 h and then extracted three times with 50 mL of chloroform. The dried extracts (Na_2SO_4) were concentrated to an oil and then chromatographed employing silica gel with chloroform as the eluant. The product was recrystallized from chloroform/hexane to afford pure **6a**: 92 mg (24%) yield; mp 235 °C; TLC (chloroform/methanol, 90:10) $R_f = 0.69$; IR (KBr pellet) 3242, 2926, 2854, 1639, 1498, 1460, 1367, 1305, 1222, 1111, 999, 949, 763 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.62 (1H, bs, indole proton), 4.00 (3H, s, 5-methoxyl) 2.25, 2.23 and 1.95 (9H, 3s, 2,3,6-trimethyl); MS (EI mode) *m*/*z* 219 (M^+), 204 ($M^+ - CH_3$), 190, 176, 160, 148. Anal. ($C_{12}H_{13}NO_3$) C, H, N.

2-(Hydroxymethyl)-3,6-dimethyl-5-methoxyindole-4,7-dione (6b) was prepared from **5b** by the following two-step procedure. A suspension of 100 mg (0.4 mmol) of **5b** in 15 mL of methanol, containing of 95 mg of 5% Pd on charcoal, was shaken under 50 psi of H_2 for 45 min. The reaction mixture was filtered through Celite, and the filtered cake was washed with methanol. The solvent was evaporated off to afford the amine as a greenish oil, which was used without further purification.

The amine was dissolved in 5 mL of acetone and then combined with 5 mL of 0.055 M KH_2PO_4 buffer. This mixture was combined with a solution of 800 mg of Fremy salt in 40 mL of 0.055 M KH_2PO_4 buffer and the reaction mixture was stirred at room temperature for 45 min. The completed reaction was extracted five times with 20-mL portions of chloroform. The dried extracts (Na_2SO_4) were concentrated to an oil and then chromatographed on silica gel with chloroform as the eluant. The product was recrystallized from chloroform/hexane to afford **6b** as a yellow solid: 45 mg (47%) yield; mp 196−198 °C; TLC (chloroform/methanol, 90:10) $R_f = 0.43$; IR (KBr pellet) 3445, 3240, 2951, 1638, 1508, 1465, 1307, 1111, 949, 746; ^{1}H NMR ($CDCl_3$) δ 9.67 (1H, bs, indole proton), 4.72 (2H, d, $J = 5.4$ Hz, 2 methylene), 4.02 (3H, s, 5-methoxy), 2.31 (1H, t, $J = 5.4$ Hz, hydroxy), 2.26 and 1.96 (6H, 2s, 3.6-dimethyl); MS (EI mode) *m*/*z* 235 (M^+), 220 ($M^+ - CH_3$), 218 ($M^+ - OH$), 202, 189, 174. Anal. ($C_{12}H_{13}NO_4$) C, H, N.

5-Aziridinyl-2,3,6-trimethylindole-4,7-dione (1a). To a solution of 42 mg (0.19 mmol) of **6a** in 8 mL of methanol was added 0.87 mL of ethylenimine. The reaction was stirred at room temperature for 2.5 h and then the reaction mixture was placed directly on a silica gel chromatography column employing chloroform as the eluant. The product was recrystallized

from chloroform/hexane to afford **1a** as a red solid: 27 mg (60%) yield; mp 251−253 °C; TLC (chloroform/methanol, 90:10) $R_f = 0.63$; IR (KBr pellet) 3213, 2922, 1664, 1626, 1587, 1498, 1460, 1373, 1346, 1248, 1153, 1101, 960, 767 cm^{-1}; ^1H NMR (DMSO-d_6) δ 12.18 (1H, bs, indole proton), 2.19 (4H, s ethylene) 2.09, 2.08 and 1.89 (9H, 3s, 2,3,6-trimethyl); MS (EI mode) 230 (M^+), 215 ($M^+ - CH_3$), 201, 185, 174. Anal. ($C_{13}H_{14}N_2O_2$) C, H, N.

5-Aziridinyl-2-(hydroxymethyl)-3,6-dimethylindole-4,7-dione (1b). To a solution of 15 mg (0.06 mmol) of **6b** in 4 mL of methanol was added 0.35 mL (0.48 mmol) of ethylenimine. The reaction mixture was stirred at room temperature for 2.5 h and then concentrated to a residue, which was recrystallized from chloroform/hexane to afford **1b** as a red solid: 15 mg (93%) yield; mp 224−225 °C; TLC (chloroform/methanol, 90:10) $R_f = 0.42$; IR (KBr pellet) 3493, 3246, 2924, 1685, 1618, 1502, 1377, 1350, 1253, 1151, 1020, 828, 752 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.39 (1H, bs, indole proton), 4.69 (2H, d, J = 5.7 Hz, 2-methylene), 2.32 (4H, s, ethylene) 2.26 and 2.05 (6H, 2s, 3,6-dimethyl); MS (EI mode) m/z 246 (M^+), 229 ($M^+ - OH$), 213, 201, 190, 172. Anal. ($C_{13}H_{14}N_2O_3$) C, H, N.

2-(Acetoxymethyl)-5-aziridinyl-3,6-dimethylindole-4,7-dione (1c). To a mixture of 10 mg of **1b** (0.04 mmol) and 5 mg (0.04 mmol) of 4-(dimethylamino)pyridine (DMAP) in 5 mL of chloroform was added 100 mg of acetic anhydride. The reaction was stirred at room temperature for 25 min and then added directly to a silica gel chromatography column employing chloroform as the eluant. The purified **1c** was recrystallized from chloroform/hexane: 11 mg (95%) yield; mp 210−212 °C; TLC (chloroform/methanol, 90:10) $R_f = 0.68$; ^1H NMR ($CDCl_3$) δ 9.38 (1H, bs, indole proton), 5.03 (2H, s, 2-methylene), 2.32 (4H, s, ethylene), 2.31 (3H, s, 3-methyl) 2.08 and 2.05 (6H, 2s, methyls); IR (KBr pellet) 3207, 2924, 1734, 1664, 1624, 1566, 1500, 1361, 1350, 1242, 1209, 1155, 1020, 962, 817 781 cm^{-1}; MS (EI mode) m/z 288 (M^+), 245 ($M^+ - CH_3{-}C{=}O$), 228, 213, 201, 185. Anal. ($C_{15}H_{16}N_2O_4$) C, H, N.

Ethyl 3,5-Dimethylindole-2-carboxylate (7). To a solution of 5.6 g (0.052 mol) of *p*-toluidine in 15 mL of concentrated HCl and 25 mL of H_2O was added dropwise a solution of 3.9 g (0.057 mol) of $NaNO_2$ in 5 mL of H_2O at −5 °C. After complete addition, the mixture was stirred at 0 °C for 15 min and brought to pH 3−4 by addition of 5 g of sodium acetate. In a separate flask, a solution of 9 g (0.055 mol) of ethyl α-ethylacetoacetate in 40 mL of EtOH was cooled to 0 °C and combined with 3.5 g of KOH (0.064 mol) in 10 mL of H_2O. To this solution was added 70 g of ice followed by addition of the diazonium salt prepared above. The mixture was then adjusted to pH 5−6 and stirred at 0 °C for 15 h. The completed reaction was extracted five times with 50-mL portions of CH_2Cl_2 and the combined extracts were washed with brine and dried over Na_2SO_4. Most of the solvent was removed under reduced pressure, and the liquid residue was added dropwise to a solution of 14.5% ethanolic HCl at reflux. After refluxing this mixture for 2 h, the solvent was removed under reduced pressure and the residue was combined with a mixture of 50 mL of water and 100 mL of CH_2Cl_2. The CH_2Cl_2 layer was removed and the aqueous layer was extracted three times with 50-mL portions of CH_2Cl_2. The combined extracts were dried over Na_2SO_4 and concentrated to a residue, which was applied to a silica gel column prepared with CH_2Cl_2. Product fractions were evaporated to afford a white solid: 5.74 g (51%) yield; mp 131−133 °C; TLC ($CHCl_3$) $R_f = 0.25$; IR (KBr pellet) 3306, 2924, 2854, 1680, 1548, 1475, 1384, 1332, 1263, 798 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.56 (1H, bs, indole proton), 7.43 (1H, s, 4-proton), 7.26 (1H, d, J = 8.4 Hz, 7-proton), 7.15 (1H, d, J = 8.4 Hz, 6-proton), 4.40 (2H, q, J = 7.2 Hz, methylene) 2.59 and 2.46 (6H, 2s, 3,5- dimethyl), 1.42 (3H, t, J = 7.2 Hz, methyl of ethyl); MS (EI mode) m/z 217(M^+), 188 ($M^+ - CH_2CH_3$), 171, 142, 115. Anal. ($C_{13}H_{15}NO_2$) C, H, N.

2-(Hydroxymethyl)-3,5-dimethyl-4-nitroindole (8) was prepared from **7** by the following two-step procedure. To a solution of 219 mg (1.01 mmol) of **7** in 10 mL of acetic acid, cooled in an ice/salt bath, was added dropwise a solution of 0.5 mL of nitric acid (69−71%) in 2 mL of acetic acid. After complete addition, the ice bath was removed and the reaction stirred for 4 h at room temperature. The solution was vacuum-dried and purified by a silica gel flash column using $CHCl_3$ as eluant. The nitrated derivative of **7** was recrystallized from $CHCl_3$ and hexane as a yellow solid: 43.3 mg (16%) yield; mp 163−164 °C; TLC ($CHCl_3$) $R_f = 0.28$; ^1H NMR ($CDCl_3$) δ 8.87 (1H, bs, indole proton), 7.40 (1H, d, J = 8.4 Hz, 6-proton) 7.18 (1H, d, J = 8.4 Hz, 7-proton), 4.43 (2H, q, J = 7.2 Mz, methylene of ethyl), 2.47 and 2.40 (6H, s, 3,5-methyls) 1.43 (3H, t, J = 7.2 Hz, methyl of ethyl); IR (KBr pellet) 3337, 2926, 1687, 1516, 1363, 1344, 1259, 1201, 1016, 775 cm^{-1}, MS (EI mode) 262 (M^+), 245 ($M^+ - OH$), 216, 199, 185, 169, Anal. ($C_{13}H_{14}N_2O_4$) C, H, N.

The ester reduction step was the same as that employed for the preparation of **6b**: 92% yield; mp 120 °C; TLC (chloroform/methanol, 90:10) $R_f = 0.30$; ^1H NMR (DMSO-d_6) δ 11.38 (1H, bs, indole proton), 7.42 (1H, d, J = 8.1 Hz, 6-H), 7.00 (1H, d, J = 8.1 Hz, 7-H), 5.25 (1H, t, J = 5.7 Hz, hydroxy proton), 4.58 (2H, d, J = 5.7 Hz, methylene), 2.29 (3H, s, 3-methyl) 1.98 (3H, s, 5-methyl); IR (KBr pellet) 3420, 3256, 2925, 1628, 1516, 1354, 1323, 1188, 999, 806 cm^{-1}; MS (EI mode) 220 (M^+), 203 ($M^+ - OH$), 185, 173, 156, 144, 130, 115. Anal. ($C_{11}H_{12}N_2O_3$) C, H, N.

2-(Hydroxymethyl)-3,5-dimethylindole-4,7-dione (9) was prepared from **8** by the same procedure employed for the preparation of **6b**: 57% yield; mp 203−205 °C; TLC (chloroform/methanol, 90:10) $R_f = 0.2$; ^1H NMR (DMSO-d_6) δ 12.38 (1H, bs, indole proton), 6.42 (1H, q, J = 1 Hz, 6-proton), 5.02 (1H, t, J = 5.4 Hz, hydroxy proton), 4.38 (2H, d, J = 5.4 Hz, methylene), 2.20 (3H, s, 3-methyl), 1.94 (3H, d, J = 1 Hz, 5-methyl); IR (KBr pellet) 3356, 3209, 2958, 1639, 1602, 1491, 1375, 1271, 1195, 1118, 993, 889, 781 cm^{-1}; MS (EI mode) m/z 205 (M^+), 188 ($M^+ - OH$), 176, 160, 148, 131, 119. Anal. ($C_{11}H_{11}NO_3$) C, H, N.

6-Aziridinyl-2-(hydroxymethyl)-3,5-dimethylindole-4,7-dione (2b). To a solution of 31.5 mg (0.15 mmol) of **9** in 10 mL of methanol was added 1 mL of ethylenimine and the resulting mixture stirred at room temperature for 2 days. The solution was concentrated to dryness and the solid residue was purified by flash chromatography on silica gel using 1% methanol in chloroform as the eluant. The red product fraction was dried and recrystallized from chloroform and hexane: 8.1 mg (21%) yield; mp 227−231 °C; TLC (chloroform/methanol, 80:20) $R_f = 0.62$; IR (KBr pellet) 3429, 3238, 2926, 1637, 1587, 1506, 1378, 1336, 1271, 1155, 1058, 950, 827, 748 cm^{-1}; ^1H NMR (DMSO-d_6) δ 12.21 (1H, bs, indole proton), 4.96 (1H, t, J = 4.8 Hz, hydroxyl proton), 4.36 (2H, d, J = 4.8 Hz, methylene), 2.20 (4H, s, ethylene), 2.17 & 1.90 (6H, 2s, 3,5-dimethyl); MS (EI mode) m/z 246 (M^+), 229 ($M^+ - OH$) 213, 201, 190, 172. Anal. ($C_{13}H_{14}N_2O_3$) C, H, N.

2-(Acetoxymethyl)-6-aziridinyl-3,5-dimethylindole-4,7-dione (2c). To a solution of 13 mg (0.052 mmol) of **2b** and 12 mg of DMAP in 4 mL of dried methylene chloride was added 80 mg of acetic anhydride. The reaction mixture was stirred for 1 min and then placed directly on a silica gel column with chloroform/acetone (98:2) as the eluant. The product was recrystallized from chloroform/hexane: 13 mg (89%) yield; mp 218−220 °C; TLC (chloroform/methanol, 90:10) $R_f = 0.78$; IR (KBr) 3464, 3240, 2926, 1751, 1637, 1571, 1334, 1251, 1334, 1251, 1057 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.28 (1H, bs, indole NH), 5.04 (2H, s, methylene), 2.32, 2.09, 2.07 (9H, 3s, methyls), 2.27 (4H, s, aziridine); MS(EI mode) m/z 288 (M^+), 245 ($M^+ - CH_3CO$), 228, 213, 201, 185. Anal. ($C_{15}H_{16}N_2O_4$) C, H, N.

7-Methyl-1,4-dihydrocyclopent[*b*]indol-3(2*H*)-one (10). To a solution of 290 mg (1.83 mmol) of *p*-tolylhydrazine hydrochloride in 10 mL of ethanol was added a solution of 356 mg (3.62 mmol) of 1,2-cyclopentanedione in 10 mL of ethanol and 10 mL of concentrated hydrochloric acid. This mixture was refluxed at 110 °C for 3 h and then concentrated to a residue under vacuum. This residue was purified by flash chromatography on silica gel using 1% methanol in chloroform as the eluant: 55 mg (16%) yield; mp 223 °C; TLC (chloroform/methanol, 95:5) $R_f = 0.50$; ^1H NMR ($CDCl_3$) δ 8.57 (1H, bs, indole proton), 7.50 (1H, s, 8-proton), 7.35 & 7.24 (2H, AB

quartet, $J = 8.1$ Hz, 5 & 6 aromatic protons), 3.07 (2H, m, 1-methylene), 2.99 (2H, m, 2-methylene) 2.46 (3H, s, 5-methyl); IR (KBr pellet) 3414, 3232, 2992, 1655, 1602, 1309, 1091, 802 cm^{-1}; MS (EI mode) *m/z* 185 (M^+), 170 ($M^+ - CH_3$), 157 ($M^+ -$ CO), 128, 115. Anal. ($C_{12}H_{11}NO$) C, H, N.

1,4-Dihydro-3-hydroxy-7-methyl-8-nitro-(2*H*)-cyclopent-[*b*]indole (11) was prepared from **10** by the following two-step procedure. To a solution of 367 mg (1.98 mmol) of **10** in 20 mL of concentrated sulfuric acid was added dropwise over 15 min a solution of 199 mg (2.34 mmol) of sodium nitrate in 5 mL of concentrated sulfuric acid. The resulting solution was stirred for another 15 min at 0 °C and then was poured over 200 g of crushed ice. The aqueous solution was extracted four times with 100-mL portions of chloroform. The combined extracts were washed with saturated sodium bicarbonate solution, dried over sodium sulfate, and vacuum-dried. The solid residue was recrystallized from chloroform/hexane to afford the 8-nitro derivative of **10** as a yellow solid: 317 mg (70%) yield; mp 268 °C dec; TLC (chloroform/methanol, 95:5) $R_f = 0.62$; ^{1}H NMR (DMSO-d_6) δ 12.29 (1H, s, indole proton), 7.66 & 7.57 (1H, AB quartet, $J = 8.7$ Hz, 5 and 6 protons), 2.98 (m, 2H, 1-methylene), 2.87 (m, 2H, 2-methylene), 2.51 (3H, s, 7-methyl); IR (KBr pellet) 3414, 3196, 2926, 1678, 1618, 1520, 1365, 1259, 1074, 835 cm^{-1}; MS (EI mode) *m/z* 230 (M^+), 213 ($M^+ -$ OH), 195, 183 ($M^+ - HNO_2$), 171, 154, 143. Anal. ($C_{12}H_{10}N_2O_3 \cdot 0.2H_2O$) C, H, N.

To a solution of 251 mg (1.09 mmol) of the product obtained above in 40 mL of methanol was added a solution of 462 mg (12.8 mmol) of sodium borohydride in 20 mL of methanol. This solution was stirred at room temperature for 15 min followed by the addition of 50 mL of water. The aqueous solution was extracted four times with 50-mL portions of chloroform. The extracts was dried over sodium sulfate and then vacuum-dried to afford a solid residue, which was recrystallized from chloroform/hexane: 213 mg (84%) yield; mp 142−144 °C; TLC (chloroform/methanol, 90:10) $R_f = 0.50$; ^{1}H NMR ($CDCl_3$) δ 8.42 (1H, bs, indole proton), 7.40 & 7.04 (2H, AB quartet, $J = 8.1$ Hz, 5- and 6-protons), 5.36 (1H, m, 3-methine proton), 3.00 & 2.35 (4H, 2m, 1 & 2-methylenes protons), 2.59 (3H, s, 7-methyl); IR (KBr pellet) 3508, 3237, 2970, 1631, 1500, 1354, 1074, 804 cm^{-1}; MS (EI mode) *m/z* 232 (M^+), 215 ($M^+ -$ OH), 185 ($M^+ - HNO_2$), 173, 156, 145. Anal. ($C_{12}H_{12}N_2O_3$) C, H, N.

1,4-Dihydro-3-methoxy-7-methyl-(2*H*)-cyclopent[*b*]indole-5,8-dione (12) and 1,4-dihydro-3-hydroxy-7-methyl-(2*H*)-cyclopent[*b*]indole-5,8-dione (13) were both prepared from **11** by the following two-step procedure. A mixture of 101 mg (0.435 mmol) of **11** in 40 mL of methanol with 100 mg of 5% Pd on carbon was reduced under 50 psi H_2 for 30 min. The reaction mixture was filtered through Celite and the filtrate concentrated to a residue, which was then dissolved in 5 mL of acetone. To this solution was added a solution consisting of 390 mg (2.87 mmol) of monobasic potassium phosphate and 790 mg (2.94 mmol) of Fremy salt in 80 mL of water. The resulting solution was stirred for 4.5 h and then extracted four times with 50-mL portions of chloroform. The extracts were dried (Na_2SO_4) and then concentrated to a residue. This residue was purified by flash chromatography on silica gel using 1% methanol in chloroform as the eluant and the products were recrystallized from chloroform/hexane.

12: 16 mg (16%) yield; mp 143−145 °C; TLC (chloroform/methanol, 80:20) $R_f = 0.84$; ^{1}H NMR ($CDCl_3$) δ 9.78 (1H, bs, indole NH), 6.41(1H, q, $J = 1.5$ Hz, 6-proton), 4.81 (1H, m, 3-methine proton), 3.36 (3H, s, methoxy), 2.92, 2.77 and 2.42 (4H, 3m, 1 & 2-methylene protons), 2.07 (3H, d, $J = 1.5$ Hz, 7-methyl); IR (KBr pellet) 3425, 3246, 2943, 1655, 1587, 1465, 1165, 1100, 800, 669 cm^{-1}; MS (EI mode) *m/z* 231 (M^+), 216 ($M^+ - CH_3$), 200 ($M^+ - OCH_3$), 188, 174, 160, 132, 115. Anal. ($C_{13}H_{13}NO_3$) C, H, N.

13: 20.5 mg (22%) yield; mp 199−201 °C; TLC (chloroform/methanol, 80:20) $R_f = 0.60$; ^{1}H NMR ($CDCl_3$) δ 9.93 (1H, bs, indole NH), 6.38 (1H, q, $J = 1.5$ Hz, 6-proton), 5.23 (1H, m, 3-methine proton), 2.92, 2.75 & 2.38 (4H, 3m, 1 & 2-methylene protons), 2.06 (3H, d, $J = 1.5$ Hz, 7-methyl); IR (KBr pellet) 3475, 3414, 3232, 2930, 1637, 1479, 1406, 1294, 1230, 1087, 1039, 952, 912 cm^{-1}; MS (EI mode) *m/z* 217 (M^+), 200 ($M^+ -$ OH), 189 ($M^+ -$ CO), 174, 160, 146, 132. Anal. ($C_{12}H_{11}NO_3$) C, H, N.

6-Aziridinyl-1,4-dihydro-3-methoxy-7-methyl-(2*H*)-cyclopent[*b*]indole-5,8-dione (3d) and 6-Aziridinyl-1,4-dihydro-3-hydroxy-7-methyl-(2*H*)-cyclopent[*b*]indole-5,8-dione (3b). To a solution of 0.2 mmol of **12** or **13** in 20 mL of methanol was added 0.5 mL of ethylenimine and the solution was stirred at room temperature for 4 days. The solvent was removed under vacuum and the residue was purified by flash chromatography on silica gel using 5% acetone in chloroform as the eluant, and either product was recrystallized from chloroform/hexane as red prisms.

3b: 74% yield; mp 193−195 °C; TLC (chloroform/methanol, 90:10) $R_f = 0.25$; ^{1}H NMR ($CDCl_3$) δ 9.24 (1H, bs, indole NH), 5.20 (1H, m, 3-methine proton). 3.51 (1H, d, $J = 4.5$ Hz, hydroxy proton), 2.91, 2.73 and 2.30 (4H, 3m, 1 & 2-methylene protons) 2.29 (4H, s, aziridine), 2.07 (3H, s, 7-methyl); IR (KBr pellet) 3448, 3160, 2960, 2926, 1655, 1325, 1041, 821, 748, 601 cm^{-1}; MS (EI mode) *m/z* 258 (M^+), 240 ($M^+ - H_2O$), 225 ($M^+ - H_2O$, CH_3), 213, 184, 170, 158, 131. Anal. ($C_{14}H_{14}N_2O_3 \cdot H_2O$) C, H, N.

3d: 81% yield; mp 186−188 °C; TLC (chloroform/methanol, 90:10) $R_f = 0.51$; ^{1}H NMR ($CDCl_3$) δ 9.03 (1H, bs, indole NH), 4.79 (1H, m, 3-methine proton), 3.35 (3H, s, methoxy protons), 2.90, 2.76 and 2.42 (4H, 3m, 1 & 2-methylene protons), 2.29 (4H, s, aziridine), 2.07 (3H, s, 7-methyl protons); IR (KBr pellet) 3277, 2924, 2859, 1655, 1643, 1591, 1340, 1097, 1026, 908, 825 cm^{-1}; MS (EI mode) 272 (M^+) 257 ($M^+ - CH_3$), 241 ($M^+ - CH_3O$), 225, 213, 200, 184, 172, 158, 132. Anal. ($C_{15}H_{16}N_2O_3$) C, H, N.

3-Acetoxy-6-aziridinyl-1,4-dihydro-7-methyl-(2*H*)-cyclopent[*b*]indole-5,8-dione (3c) and 3-Acetoxy-4-acetyl-6-aziridinyl-1,4-dihydro-7-methyl-(2*H*)-cyclopent[*b*]indole-5,8-dione (14). To a solution of 21 mg (0.08 mmol) of **3b** in 3 mL of methylene chloride with 22 mg of DMAP was added 32 mg (0.32 mmol) of acetic anhydride and the solution was stirred at room temperature for 10 min. The reaction mixture was then placed on a silica gel column employing methylene chloride as the eluant.

3c: 8.3 mg (34%) yield; mp 166−168 °C; TLC (chloroform/methanol, 90:10) $R_f = 0.68$; IR (KBr pellet) 3267, 3065, 2926, 2856, 1736, 1637, 1377, 1329, 1251, 1089, 601 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.26 (1H, bs, indole proton), 5.62 (1H, m, 3-methine); 2.96, 2.80 and 2.59 (4H, 3m, 1 & 2-methylene protons) 2.28 (4H, s, aziridine); 2.06 (6H, 2s, dimethyl); MS (EI mode) *m/z* 300 (M^+), 257 ($M^+ - CH_3CO$), 240 ($M^+ - CH_3COOH$), 225, 213, 199, 184. Anal. ($C_{16}H_{16}N_2O_4 \cdot 0.5H_2O$) C, H, N.

14: 7 mg (25%) yield; mp 135−137 °C; TLC (chloroform/methanol, 90:10) $R_f = 0.75$; ^{1}H NMR ($CDCl_3$) δ 6.23 (1H, m, 3-methine), 2.95, 2.84 (4H, 2m, 1 & 2 methylene), 2.73 (3H, s, 4-acetyl), 2.33 (4H, s, aziridine), 2.08 and 2.03 (6H, s, dimethyl); IR (KBr pellet) 3211, 2924, 2854, 1741, 1655, 1591, 1373, 1336, 1251, 1224, 1030, 891 cm^{-1}; MS (EI mode) 342 (M^+), 300 ($M^+ - CH_2CO$), 283 ($M^+ - CH_3COO$), 258, 240, 225, 213, 189, 184. Anal. ($C_{18}H_{18}N_2O_4$) C, H, N.

6-Aziridinyl-1,4-dihydro-7-methyl-(2*H*)-cyclopent[*b*]indole-3,5,8-trione (3e). To a solution of 10.2 mg (0.04 mmol) of **3b** in 2 mL of dried methylene chloride was added 60 mg of pyridinium dichromate. The reaction mixture was stirred at room temperature for 2 h and then flash chromatographed on a silica gel column with chloroform as the eluant. The product was recrystallized from chloroform/hexane: 2.7 mg (26%yield); IR (KBr pellet) 3257, 3159, 2928, 1699, 1637, 1577, 1518, 1340, 1255, 1147, 1051, 985 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.38 (1H, bs, indole NH), 3.14 & 2.96 (4H, 2m, 1 & 2-methylenes), 2.33 (4H, s, aziridine), 2.13 (3H, s, 7-methyl); MS (EI mode) *m/z* 256 (M^+), 241 ($M^+ - CH_3$), 227, 213, 199, 174. Anal. ($C_{14}H_{12}N_2O_3$) C, H, N.

6-Aziridinyl-2,3-dihydro-7-methyl-1*H*-pyrrolo[1,2-*a*]-benzimidazole-3,5,8-trione (15). To a solution of 200 mg (0.77 mmol) of the 3-hydroxy derivative[30] in 60 mL of methylene chloride was added 1 g of pyridinium dichromate and the reaction stirred for 15 h at room temperature. The solvent

was then evaporated and the residue chromatographed on a silica gel column using chloroform and methanol (97:3). The dark red product band was collected, concentrated to a residue, and recrystallized from chloroform/hexane: 45 mg (23%) yield; ^{1}H NMR (DMSO-d_6) δ 4.42 and 3.2 (4H, 2t, ethylene bridge), 2.48 (4H, s, aziridinyl), 2.35 (3H, s, methyl). Anal. ($C_{13}H_{11}N_3O_3$) C, H, N.

Alkylation of DNA by Reduced Indoloquinones. To a mixture of 1−2 mg of sonicated (600 bp) calf thymus DNA in 2.0 mL of 0.05 M pH 7.4 Tris buffer and 2 mg of Pd on carbon was added a five-to-one base pair equivalent amount of the indoloquinone dissolved in 0.5 mL of dimethyl sulfoxide (DMSO). The resulting solution was degassed under argon for 30 min, after which the mixture was purged with H_2 for 10 min. The solution was then purged with argon for 10 min and placed in a 30 °C bath for 24 h. The reaction was opened to the air, and the catalyst was removed with a Millex-PF 0.8-μm syringe filter. The filtrate was adjusted to 0.3 M acetate with a 3 M stock solution of pH 5.1 acetate and then diluted with two volumes of ethanol. The mixture was chilled at −20 °C for 12 h and the DNA pellet collected by centrifuging at 12000*g* for 20 min. The pellet was redissolved in water and then precipitated and centrifuged again. The resulting blue or red pellet was suspended in ethanol, centrifuged, and dried. The dried pellet was weighed and dissolved in 1 mL of double distilled water resulting in a clear-colored solution with $\lambda_{max} \sim 550$ nm, $\epsilon \sim 750$ M^{-1} cm^{-1} . This is the chromophore of the aminoquinone resulting from nucleophile-mediated opening of the aziridine ring. Model 2′-chloroethylaminoquinones for extinction coefficient determination were prepared by treatment of the indoloquinone with HCl.[31]

DT-Diaphorase Reduction Kinetics Studies. Rat liver DT-diaphorase was isolated as previously described.[3,32] Kinetic studies were carried out in 0.05 M pH 7.4 Tris·HCl buffer, under anaerobic conditions, employing Thunberg cuvettes. A 2 mM stock solution of each indoloquinone was prepared in DMSO. To the top port was added the quinone stock and to the bottom port were added DT-diaphorase and NADH in the Tris buffer. The top and bottom ports were purged with argon for 20 min and equilibrated to 30 °C. The ports were then mixed, and the reaction was followed at 296 nm for 25 min in order to obtain initial rates. The concentrations obtained after mixing were: 0.3 mM NADH, 1−20 × 10^{-5} M quinone, and 14.5 nM (based on flavin) enzyme active sites. The value of $\Delta\epsilon$ was calculated from the initial and final absorbance values for complete quinone reduction; usual value for ϵ is 6000−8000 M^{-1} cm^{-1}. The value of $\Delta\epsilon$ was used to calculate V_{max} in M s^{-1}. The results were fitted to a Lineweaver−Burke plot from which V_{max}/K_m values were calculated.

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