# *Articles*

## Aziridinyl Quinone Antitumor Agents Based on Indoles and Cyclopent[*b*]indoles: Structure−Activity Relationships for Cytotoxicity and Antitumor Activity

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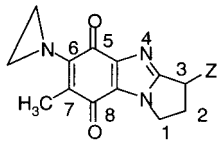
A large number of aziridinyl quinones represented by series **1**−**9** were studied with respect to their DT-diaphorase substrate activity, DNA reductive alkylation, cytostatic/cytotoxic activity, and in vivo activity. As a result, generalizations have been made with respect with respect to the following: DT-diaphorase substrate design, DT-diaphorase−cytotoxicity quantitative structure−activity relationship (QSAR), and DNA reductive alkylating agent design. A saturating relationship exists between the substrate specificity for human recombinant DT-diaphorase and the cytotoxicity in the human H460 non-small-cell lung cancer cell line. The interpretation of this relationship is that reductive activation is no longer rate-limiting for substrates with high DT-diaphorase substrate specificities. High DT-diaphorase substrate specificity is not desirable in the indole and cylopent[*b*]indole systems because of the result is the loss of cancer selectivity along with increased toxicity. We conclude that aziridinyl quinones of this type should possess a substrate specificity ($V_{max}/K_M$) < $10 \times 10^{-4}$ $s^{-1}$ for DT-diaphorase in order not to be too toxic or nonselective. While some DNA alkylation was required for cytostatic and cytotoxic activity by series **1**−**9**, too much alkylation results in loss of cancer selectivity as well as increased in vivo toxicity. Indeed, the most lethal compounds are the indole systems with a leaving group in the 3α-position (like the antitumor agent EO9). We conclude that relatively poor DNA alkylating agents (according to our assay) show the lowest toxicity with the highest antitumor activity.

Many of the clinically used antitumor agents that have withstood the test of time are DNA-directed alkylating agents utilizing the aziridine alkylating center. Noteworthy examples include the nitrogen mustards,[1] nitrosoureas,[2] thiotepa,[3] AZQ,[3] triethylenemelamine,[3] and mitomycin C.[4] These compounds have been thoroughly studied and have been subjected to intense analogue development for over 40 years. Examples of aziridinyl quinone antitumor agents developed recently are shown in Chart 1.

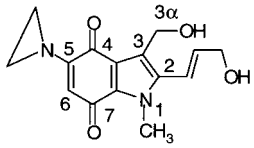
The pyrrolo[1,2-*a*]benzimidazoles (PBIs) were developed in this laboratory and were found to possess cytotoxicity with minimal antitumor activity.[5,6] The indoloquinone EO9 was considered to be a promising antitumor agent,[7,8] but phase I clinical trials revealed short plasma half-lives as well as toxicity.[9−11] The cyclopent[*b*]indoles, reported in the past year,[12] possess promising antitumor activity.

This report describes a comprehensive structure−activity study of cyclopent[*b*]indole-based (series **1**−**5**) and indole-based (series **6**−**9**) aziridinyl quinones represented by the nine general structures shown in Chart 2. Diverse structures were generated by varying the

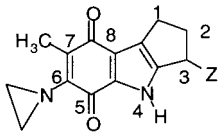
**Chart 1**



Pyrrolobenzimidazole (PBI)



EO-9



Cyclopent [ b] indole

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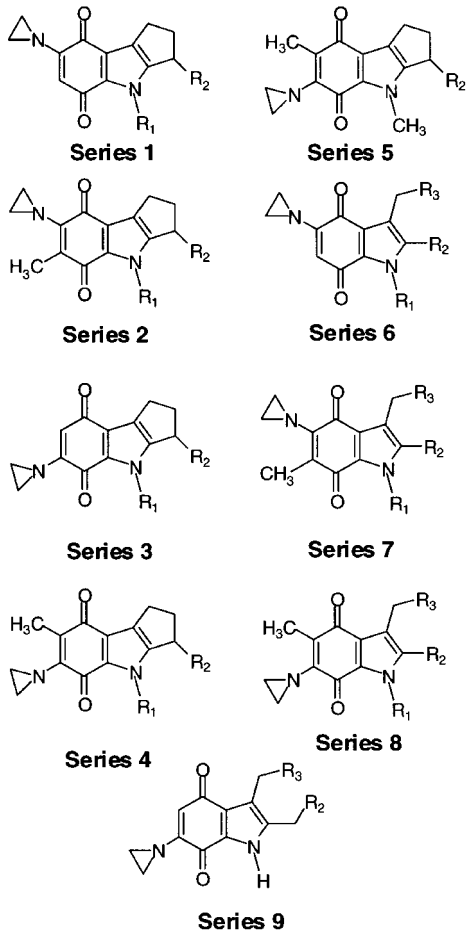
**Chart 2**




position of the aziridinyl and quinone methyl groups (if present) as well as the indole N-substituent. These structural features can influence the interaction of quinone antitumor agents with DNA,[12,13] and this study would clarify the structural requirements for such binding. The structure of EO9[14−17] inspired the synthesis of indole-based diol derivatives and their acetylated analogues.

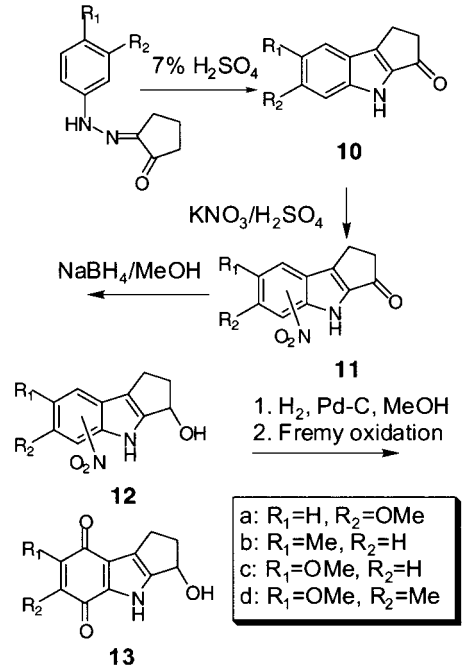
The study of a large number of compounds required use of a chemical/biochemical prescreen that could predict biological activity. We successfully used the quinone substrate specificity ($V_{max}/K_M$) for human DT-diaphorase and the percent DNA alkylation to assess cytotoxic and antitumor capability. From this study, we were able to elucidate structure activity relationships valuable in future antitumor design.

## Results and Discussion

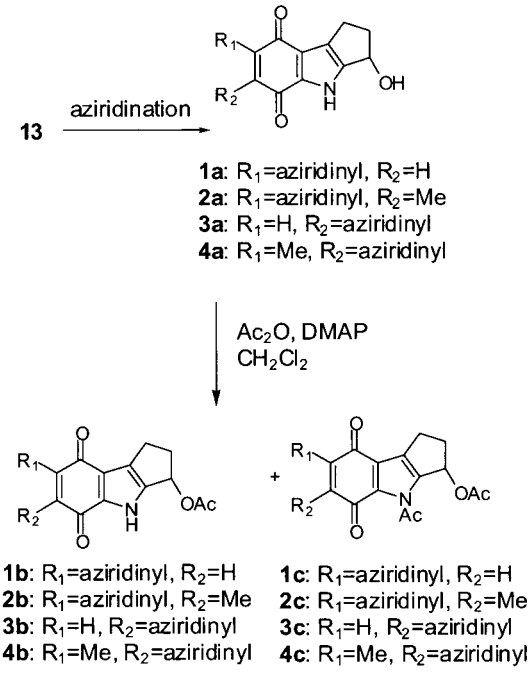
**Synthesis.** The preparation of series **1**−**9** was carried out as outlined in Schemes 1−6. Many of the synthetic procedures utilized for these preparations are straightforward and are briefly outlined below.

Shown in Schemes 1−3 are the synthetic methodologies for the preparation of the cyclopent[*b*]indoles (series **1**−**5**). Preparation of the substituted cyclopent[*b*]indol-3-one system was carried out by the Fischer indole reaction as previously described.[12] Elaboration of the quinone functional group was carried out by nitration, catalytic reduction of the nitro group, and finally Fremy oxidation.[18,19] The 3-hydroxy group (**1a**−**4a** series) was

**Scheme 1**



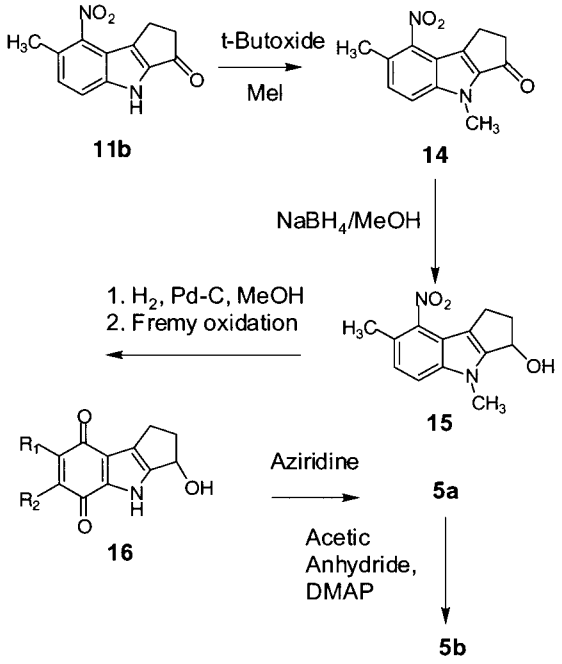

**Scheme 2**



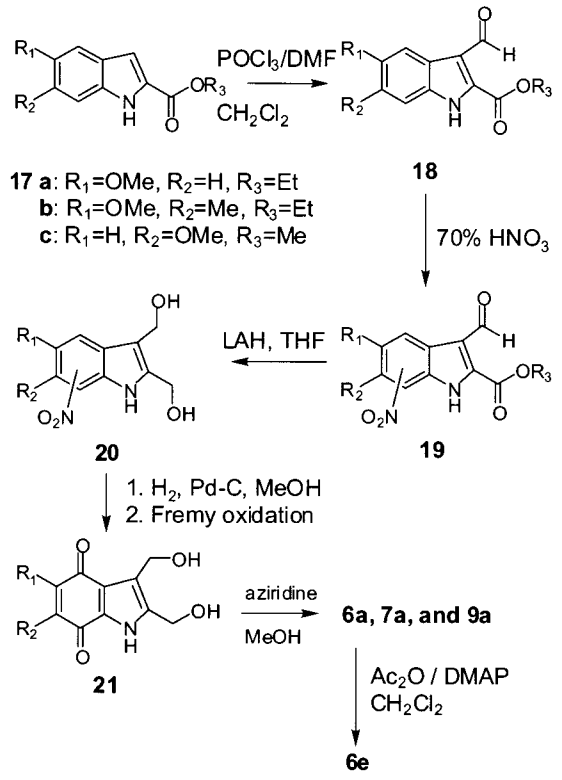

obtained by borohydride reduction of the 3-one group. Acetylation of the 3-hydroxy group afforded a mixture of O- and N-acetylated compounds that constituted the **1b**−**4b** and **1c**−**4c** series. N-Methylation of the cyclopent[*b*]indol-3-one system, followed by quinone and 3-hydroxyl or 3-acetoxy elaboration, provided series **5**.

Shown in Schemes 4−6 are the synthetic methodologies for the preparation of the indoles (series **6**−**9**). Preparation of the 2-(ethylcarboxyl)indole substituted systems were carried out by the Japp−Klingemann/Fischer indole reaction as previously described.[12,20] Vilsmeier formylation and borohydride reduction afforded the 3-hydroxymethyl derivative of these indole systems. The 2-ethylcarboxyl substituent was either retained or reduced with lithium aluminum hydride

**Scheme 3**



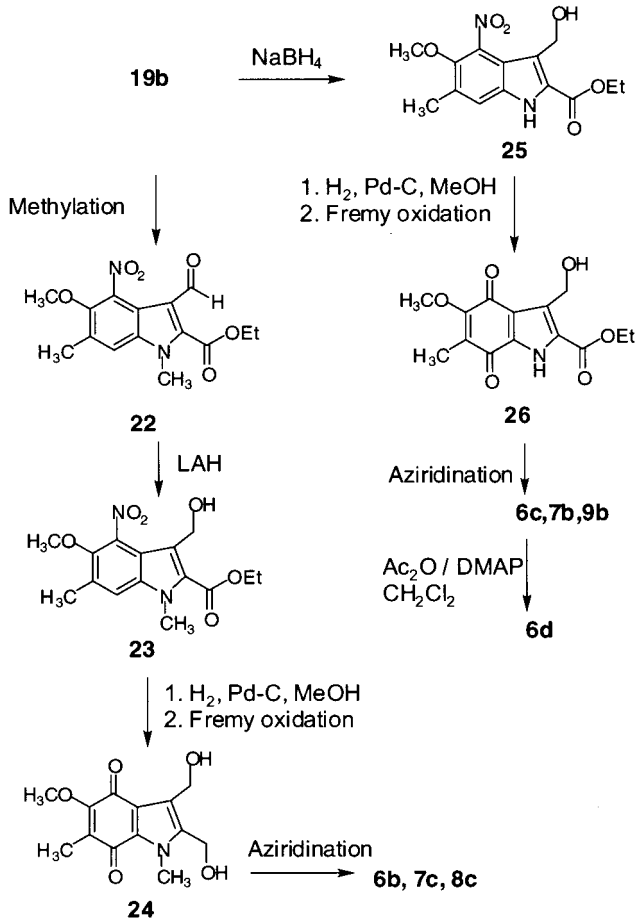

**Scheme 4**



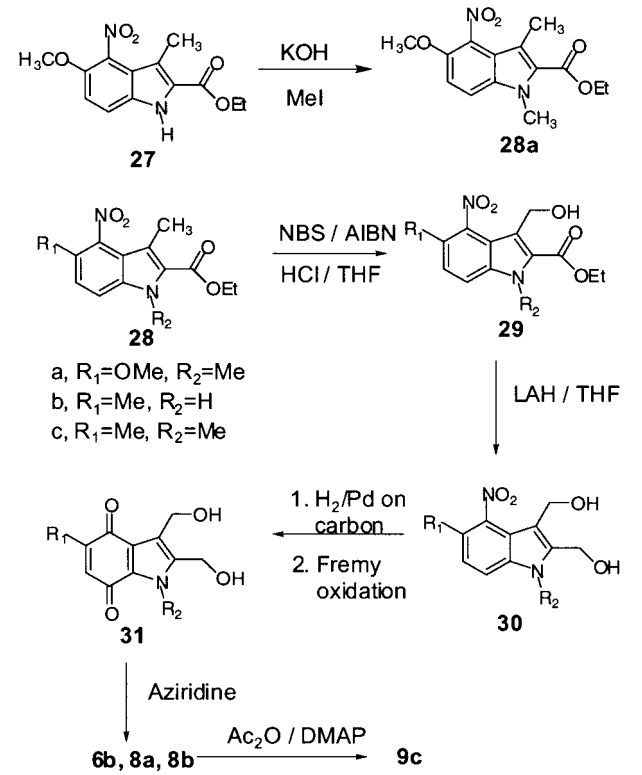

(LAH) to the 2-hydroxymethyl group. The hydroxymethyl groups were either left as is or acetylated to afford the acetate leaving group. Finally, quinone elaboration and aziridination were carried out as previously described.[18,19]

**DT-Diaphorase Substrate Screening of Series 1−9.** The enzyme DT-diaphorase is an NAD(P)H-dependent reducing enzyme that converts quinones and other substrates to the corresponding two-electron reduction products. The hydroquinone reduction product is cytotoxic because its aziridinyl nitrogen is readily

**Scheme 5**




**Scheme 6**




protonated at physiological pH, resulting in alkylation reactions (Scheme 7). The phosphate backbone[21] and the purine N(7)-position[22,23] of DNA are the usual nucleo-

**Scheme 7**

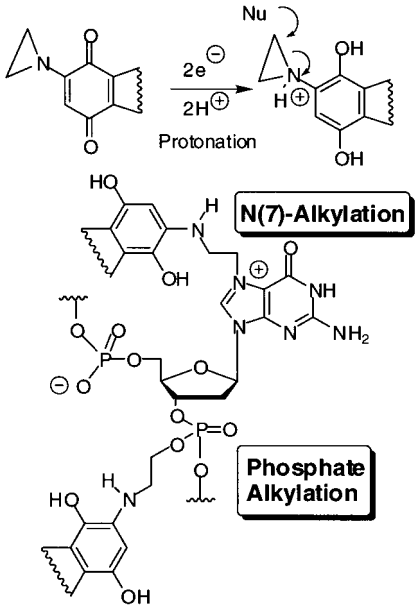
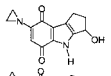
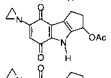
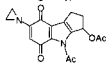
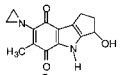
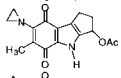
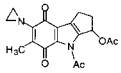



**Table 1.** DT-Diaphorase Kinetic Parameters and DNA Alkylation Percentages for Series **1**−**2**

| COMPOUND | # | $K_M$ (*10$^5$ M) | $V_{max}$ (* 10$^9$ M/s) | $V_{max}/K_M$ (*10$^4$ s$^{-1}$) | DNA alkylation percentage % |
|---|---|---|---|---|---|
|  | 1a | 0.51 | 20.66 | 40.5 | 22.5 |
|  | 1b | 0.073 | 4.75 | 65.1 | 34 |
|  | 1c | 0.10 | 7.05 | 70.5 | 14 |
|  | 2a | 0.149 | 2.19 | 14.7 | 11.2 |
|  | 2b | 0.415 | 2.72 | 6.55 | 21 |
|  | 2c | 0.307 | 4.04 | 13.16 | 1.2 |

phile targets. This enzyme is elevated in some cancers and the reduction process leads to activation of antitumor agents specifically in these cancers.[24−26] The reductive activation process is well-known with substrates such as mitomycin C,[27,28] EO9,[15,29] and the PBIs.[6,30]

Listed in Tables 1−4 are the human DT-diaphorase kinetic parameters for series **1**−**9**, along with the DNA alkylation percentages. These parameters include the apparent dissociation constant $K_M$, the apparent maximal velocity $V_{max}$, and the substrate specificity, $V_{max}/K_M$. The designation apparent refers to the presence of productive and nonproductive constants in $K_M$ and $V_{max}$.[31] These constants cancel in the ratio $V_{max}/K_M$, which is the best parameter to compare substrates. Human DT-diaphorase (from the non-small-cell lung NCI-H460 cell line)[32] was used to obtain these kinetic parameters. This enzyme is the same as that present in other human tissues, cancerous or otherwise.

The human DT-diaphorase enzyme has been cloned and its crystal structure determined (1D4A in the Protein Data Bank).[33] In a study of the DT-diaphorase

**Table 2.** DT-Diaphorase Kinetic Parameters and DNA Alkylation Percentages for Series **3**−**4**

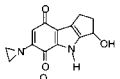
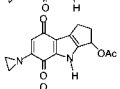
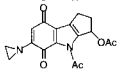
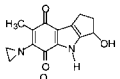
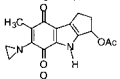
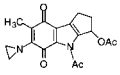
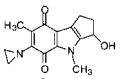
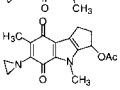
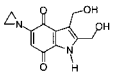
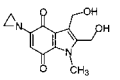
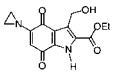
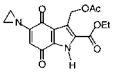
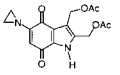
| COMPOUND | # | $K_M$ (*10$^5$ M) | $V_{max}$ (* 10$^9$ M/s) | $V_{max}/K_M$ (*10$^4$ s$^{-1}$) | DNA alkylation percentage % |
|---|---|---|---|---|---|
|  | 3a | 0.70 | 8.93 | 12.8 | 30 |
|  | 3b | 0.046 | 3.69 | 80.22 | 29 |
|  | 3c | 0.11 | 2.58 | 23.45 | 12 |
|  | 4a | 0.777 | 11 | 14.2 | 10 |
|  | 4b | 0.212 | 3.39 | 16 | 44 |
|  | 4c | 0.493 | 2.77 | 5.62 | 9 |

**Table 3.** DT-Diaphorase Kinetic Parameters and DNA Alkylation Percentages for Series **5**−**6**

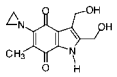
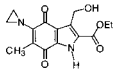
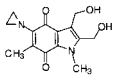
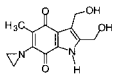
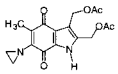
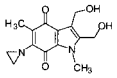
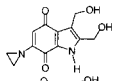
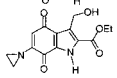
| COMPOUND | # | $K_M$ (*10$^5$ M) | $V_{max}$ (* 10$^9$ M/s) | $V_{max}/K_M$ (*10$^4$ s$^{-1}$) | DNA alkylation percentage % |
|---|---|---|---|---|---|
|  | 5a | 0.179 | 1.53 | 8.55 | 0.3 |
|  | 5b | 0.797 | 2.39 | 3.00 | 1.4 |
|  | 6a | 0.378 | 22.19 | 58.7 | 16.7 |
|  | 6b | 0.05 | 1.51 | 30.2 | 3.8 |
|  | 6c | 0.173 | 74.37 | 430 | 37 |
|  | 6d | 0.459 | 33.13 | 72.18 | 91.2 |
|  | 6e | 0.572 | 9.83 | 17.2 | 43 |

reduction of pyrrolo[1,2-*a*]benzimidazoles (PBIs), the enzyme-bound PBI structure was obtained by superimposition into the human DT-diaphorase active site.[34] Shown in Figure 1 is the active site of DT-diaphorase (1D4A) as well as the same active site containing a PBI aziridinyl quinone. Although the structure shown in Figure 1B was not crystallographically determined, it permitted the rationalization of indole and cyclopent-[*b*]indole substrate specificity as illustrated below.

Inspection of the substrate specificity data in Tables 1−7 reveals the following features:

(1) The presence of a methyl substituent on the quinone ring substantially slows DT-diaphorase reduction. Comparison of series **1** and **2** or of series **6** and **7** shows up to 10-fold changes in specificity when the methyl substituent is present. Steric interactions be-

**Table 4.** DT-Diaphorase Kinetic Parameters and DNA Alkylation Percentages for Series **7**−**9**

| COMPOUND | # | $K_M$ ($*10^5$ M) | $V_{max}$ ($*10^9$ M/s) | $V_{max}/K_M$ ($*10^4$ $s^{-1}$) | DNA alkylation percentage % |
|---|---|---|---|---|---|
|  | **7a** | 3.36 | 27.4 | 8.15 | 2 |
|  | **7b** | 0.436 | 17.58 | 40.3 | 5.6 |
|  | **7c** | 2.05 | 6.68 | 3.26 | 0 |
|  | **8a** | 0.48 | 5.07 | 10.6 | 4 |
|  | **8b** | 0.173 | 2.73 | 15.8 | 37 |
|  | **8c** | 0.333 | 1.39 | 4.17 | 0 |
|  | **9a** | 1.43 | 39.15 | 27.38 | 3.4 |
|  | **9b** | 0.222 | 123 | 554 | 2.2 |

tween residues W105 and F106 (Figure 1) and the methyl substituent are very likely responsible for the lower substrate specificity of methyl-substituted quinones for DT-diaphorase. Indeed, PBI analogues bearing a 7-butyl instead of a 7-methyl were poor substrates for the enzyme.[30]

(2) The position of the aziridinyl substituent on the quinone ring influences substrate specificity to a lesser degree than the methyl substituent; compare **1a** and **3a** or **6a** and **9a**. In these cases, the parallel walls formed by the W105 and F106 residues can accommodate the aziridinyl substituent regardless of its position. These residues likewise accommodate the aziridinyl substituent of EO9-like indole analogues.[17]

(3) The presence of a 3-acetoxy substituent on some of the cyclopent[*b*]indole systems increases substrate specificity markedly over the 3-hydroxy derivatives; compare **1a** and **1b** or **3a** and **3b**. The explanation is that the acetate carbonyl is a hydrogen-bond acceptor for the N−H of His194, Figure 1. Similarily, the 3-acetate and 3-carbamido derivatives of the PBI system can hydrogen-bond with this amino acid.[34] However, if a methyl substituent is present on the quinone ring, the substrate changes position in the active site and the acetate does not hydrogen-bond with His 194; compare **2a** and **2b** or **4a** and **4b**.

(4) The highest specificities for DT-diaphorase observed in this study were for the indoles bearing an ethoxycarbonyl substituent in the 2-position, **6c** and **9b**. The explanation for the high substrate specificities is resonance stabilization of the anion resulting from the Michael transfer of hydride by the $FADH_2$ cofactor. Stabilization of this anionic intermediate by resonance would also stabilize the transition state for hydride transfer, resulting in a high $V_{max}$ value and accordingly

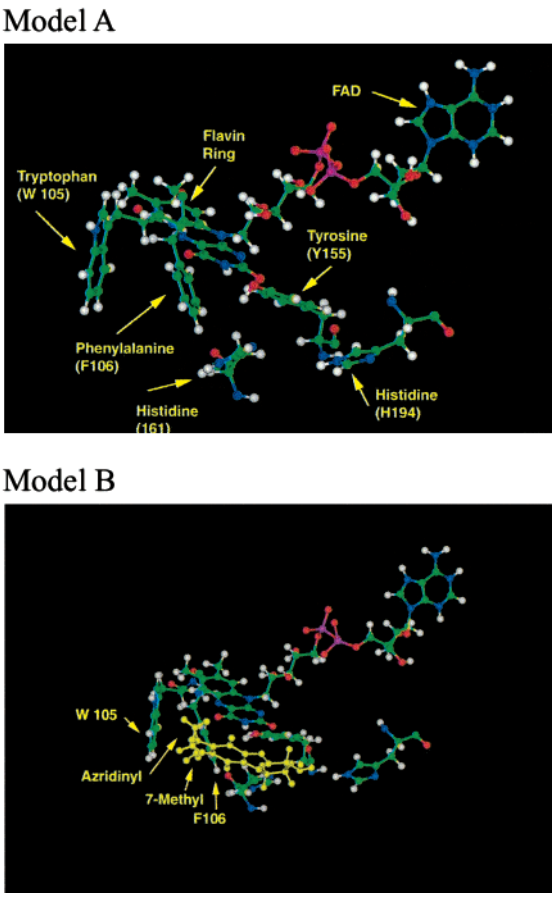



**Figure 1.** Model A: active sites of human DT-diaphorase from NCI-H460 non-small-cell lung cancer obtained from the Protein Data Bank (1D4A). Important amino acid residues are labeled. Model B: S-amino PBI substrate in the DT-diaphorase from NCI-H460 non-small-cell lung cancer.

a high substrate specificity. Both the crystal structure of DT-diaphorase[33] and the reported hydride transfer mechanism to quinones[35] support the Michael-type hydride transfer to the 5-position as shown in Scheme 8. In both **6c** and **9b**, the resulting anion (not to be confused with the radical anion arising from hydrogen atom transfer) can be resonance-delocalized into the ethoxycarbonyl substituent. The 3α-acetoxy substituent of **6d** very likely shifts the substrate in the active site so that hydride transfer occurs to the 6-position rather than the 5-position. In this case, the anion resulting from Michael addition is not stabilized by resonance withdrawal by the ethoxycarbonyl substituent and its substrate specificity is substantially lower than that of **6c**.

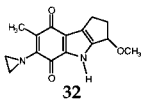
The ideal DT-diaphorase substrate appears to be an N-unsubstituted indoloquinone with an aziridinyl substituent at either the 5- or the 6-position, with a carbonyl substituent at the 2-position and a hydroxymethyl substituent in the 3-position. A following section will present evidence that designing an outstanding DT-diaphorase substrate does not necessarily translate to a good antitumor agent.

**DNA Alkylation Screening of Series 1**−**9.** The extent of DNA reductive alkylation was determined by catalytic reduction of the aziridinyl quinones in the presence of 600 bp calf thymus DNA. Oxidative workup afforded DNA colored blue as a result of the amino-

**Table 5.** Cytostatic ($GI_{50}$ and TGI) and Cytotoxic ($LC_{50}$) Parameters and the Cancer Specificity for Select Members of Series **1**−**9**

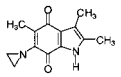
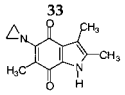
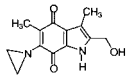
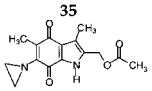
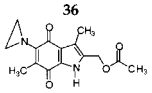
| | log $GI_{50}$, NSC H460 | log TGI, NSC H460 | log $LC_{50}$, NSC H460 | average log $LC_{50}$, 60-cell line | cancer specificity |
|---|---|---|---|---|---|
| **1a** | −8 | −6.99 | −6.53 | −5.66 ± 1.52 | melanoma, CNS, renal |
| **2a** | −8 | −7.67 | −6.07 | −4.74 ± 2.11 | CNS, melanoma |
| | −8 | −7.20 | −5.45 | −5.15 ± 2.14 | CNS, melanoma |
| **3a** | −8.14 | −7.24 | −5.75 | −5.59 ± 1.95 | melanoma, renal |
| **4a** | −8 | −6.97 | −4 | −4.14 ± 1.04 | melanoma, CNS |
| | | | | −4.94 ± 2.85 | melanoma, CNS |
| **4b** | −6.95 | −6.17 | −5.91 | −4.67 ± 1.04 | none |
| **4c** | −7.08 | −6.36 | −5.30 | −4.60 ± 1.12 | melanoma, renal |
| | −6.70 | −5.88 | −4.14 | −4.49 ± 1.31 | melanoma, renal |
| **5a** | −5.9 | −5.12 | −4.30 | −4 | none |
| **6b** | −8 | −8 | −8 | −5.35 ± 2.65 | NSC lung, CNS, melanoma, renal |
| | −8 | −8 | −6.44 | −5.50 ± 2.5 | NSC lung, CNS, melanoma, renal |
| **6c** | −8 | −7 | −6.20 | −5.79 ± 1.98 | CNS, melanoma, renal |
| | −8 | −6.95 | | | CNS, melanoma, renal |
| **6d** | −7.43 | −6.77 | −6.22 | −5.62 ± 1.05 | none |
| **7a** | −8 | −7.49 | −5.93 | −5.54 ± 1.89 | NSC lung, melanoma |
| **7b** | −8 | −7.99 | −7.06 | −5.69 ± 2.31 | NSC lung, melanoma |
| | −8 | −7.96 | −6.11 | −5.75 ± 2.25 | NSC lung, melanoma |
| **8b** | −7.45 | −6.75 | −6.22 | −4.52 ± 1.7 | NSC lung, melanoma |
| | −7.63 | −6.71 | −5.61 | −4.61 ± 2.5 | NSC lung, melanoma |
| **8c** | −7.41 | −6.66 | −6.02 | −4.61 ± 1.64 | melanoma |
| | −7.27 | −6.66 | −6.21 | −4.67 ± 2.48 | melanoma |
| **9a** | −6.86 | −5.94 | −4.93 | −5.07 ± 1.43 | none |
| **9b** | −5.90 | −5.55 | −5.21 | −5.20 ± 1.04 | none |
| | −5.80 | −5.39 | −4.95 | −5.32 ± 1.14 | none |

**Table 6.** In Vivo T/C (Treated/Control) Data from the B16 Melanoma Model[a]

| Compound | 2 mg /Kg/Day | 3 mg /Kg/ Day | 5 mg /Kg/Day |
|---|---|---|---|
| **3b** | NA | NA | Toxic |
| **3c** | Toxic | Toxic | Toxic |
| **4a** | 75% | | 45% |
| **4c** | 37% | 15% | 15% |
|  **32** | NA | NA | NA |

[a] NA, not active; toxic, ≥50% lethality before any control growth deaths.

**Table 7.** In Vivo T/C (Treated/Control) Data from the B16 Melanoma Model[a]

| Compound | 2 mg /Kg/Day | 3 mg /Kg/ Day | 5 mg /Kg/Day |
|---|---|---|---|
| **6a** | Toxic | Toxic | Toxic |
| **6b** | Toxic | Toxic | Toxic |
| **7a** | 36% | Toxic | Toxic |
| **8a** | 54% | Toxic | Toxic |
| **8b** | 74% | Toxic | Toxic |
| **8c** | NA | NA | Toxic |
|  **33** | 22% | Toxic | Toxic |
|  **34** | 10% | 60% | Toxic |
|  **35** | 61% | 37% | 34% |
|  **36** | NA | NA | NA |
|  **37** | 47% | Toxic | Toxic |

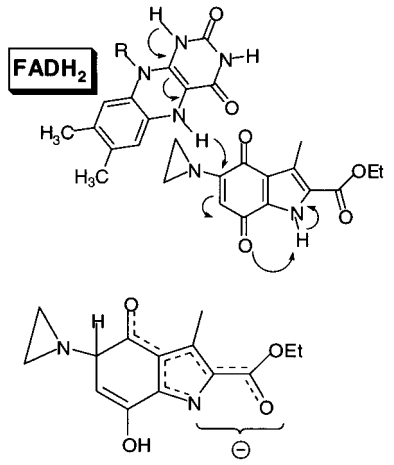
[a] NA, not active; toxic, ≥50% lethality before any control growth deaths.

quinone chromophore. A spectrophotometric determination of absorbance at 550 nm ($\epsilon$ = 800 $M^{-1}$ $cm^{-1}$) permitted the determination of percent alkylation.[21,36] The anaerobic conditions employed for DNA reductive alkylation assays are not meant to mimic the environment of tumor cells. These conditions do permit an accurate assessment of the structural requirements for DNA alkylation by the hydroquinone species. To be sure, agents incapable of akylating DNA under these ideal conditions will not do so in the typical cellular environment. Inspection of the DNA alkylation results in Tables 1−4 revealed the following trends:

(1) The presence of a 6-methyl substituent in the quinone ring of the cyclopent[*b*]indoles substantially reduces the percent DNA alkylation compared to analogues bearing only an aziridinyl substituent (compare **1** with **2**). Noteworthy exceptions are cyclopent[*b*]indoles **3** and **4**, where a 7-methyl does not greatly influence the percent DNA alkylation. Similarly, the PBIs could reductively alkylate DNA with a methyl and even an *n*-butyl in the 7-position.[30] The cyclopent[*b*]indole DNA binding model shown in Figure 2 rationalizes the bulk tolerance at the 7-position and the utility of this system in major-groove recognition.

(2) N-Methylation of the cyclopent[*b*]indole series **5** results in complete loss of DNA alkylation. This observation is consistent with the binding model shown in Figure 2, where the indole NH has a hydrogen-binding

**Scheme 8**




role. N-Acetylation of the cyclopent[*b*]indole system (**1c**−**4c**) only diminishes DNA alkylation somewhat, perhaps because the carbonyl has a hydrogen-bonding role in the DNA major groove.

(3) N-Methylation of the indole series (**7c, 8c**) also results in complete loss of DNA alkylation. It is concluded that the NH of the indole series also plays a role in DNA alkylation.

(4) The presence of an acetate leaving group at the 3α-position of the indole series (**6d, 6e, 8b**) results in high DNA alkylation percentages. A recent publication[37] has provided evidence that elimination of the acetate affords an enimine species capable of trapping DNA nucleophiles (Scheme 9). If an ethoxycarbonyl is present at the 2-position of the indole, the DNA alkylation percent increases with either an acetate or hydroxide leaving group at the 3α-position. The ethoxycarbonyl group lowers the p$K_a$ of the indole NH, facilitating formation of the imine species, even when a weak hydroxide leaving group is present (Scheme 9).

By using the guidelines above, it has been possible to design excellent DNA reductive alkylating agents with a high substrate specificity for DT-diaphorase, such as **6c** and **6d**.

**Cytotoxic and Cytostatic Screening of Series 1**−**9.** Provided in Table 5 are the cytostatic/cytotoxic parameters and cancer specificities for select compounds from series **1**−**9**. The cytostatic parameters include $GI_{50}$ and TGI, which are the concentrations of drug required for 50% growth inhibition and total growth inhibition, respectively. The cytotoxic parameter is the $LC_{50}$, which is the concentration required for 50% cell kill. These in vitro data were obtained under the In Vitro Cell Line Screening Project at the National Cancer Institute.[38,39] By comparing the data in Table 5 with those in Tables 1−4, the following relationships were observed:

(1) The data in Table 5 were used to determine the relationship between H460 DT-diaphorase substrate specificity and cytotoxicity against the H460 cell line. The $-\log LC_{50}$ vs $V_{max}/K_M$ plot shown in Figure 3 includes only DNA alkylating agents exhibiting ≥10% alkylation in our assay. This plot reveals that a saturating relationship exists wherein the $-\log LC_{50}$ value of 6.6 was approached with increasingly large values for substrate specificity ($V_{max}/K_M > 50 \times 10^{-4}$ s$^{-1}$). Compound **6c**, with a $V_{max}/K_M$ value of $430 \times 10^{-4}$ s$^{-1}$ and a $-\log LC_{50}$ value of 6.2, is at saturating cytotoxicity (not shown in Figure 3). The solid curve in Figure 3 was computer-generated from

$$-\log LC_{50} = A(V_{max}/K_m)/[B + (V_{max}/K_m)] \qquad (1)$$

where $-LC_{50}$ is the concentration required for 50% cell kill, $V_{max}/K_M$ is the substrate specificity for DT-diaphorase, and $A$ and $B$ are constants. At high substrate specificities the value of constant $A$ approaches 6.6, the $-\log LC_{50}$ value at saturation.

The presence of a saturating relationship suggests that reductive activation is cytotoxicity-limiting only for substrate specificities lower than $50 \times 10^{-4}$ s$^{-1}$. At substrate specificities somewhat higher than $50 \times 10^{-4}$ s$^{-1}$, reduction is rapid and processes such as DNA alkylation become cytotoxicity-limiting. The DT-diaphorase saturating relationship has also been observed at constant antitumor agent doses, while the concentration of the enzyme was increased in the tumor cell.[40] Increasing the enzyme concentration no doubt results in increasingly rapid reductive activation, eventually leading to constant cytotoxicity.

There have been other DT-diaphorase cytotoxicity structure−activity studies reported in the literature.[16,17] These studies found that cytotoxicity correlated with the velocity of DT-diaphorase reduction but without discernible saturation. Our study was carried out differently in that substrate specificity ($V_{max}/K_M$) rather than velocity was employed to generate the relationship. The use of velocity terms in enzyme kinetic studies is risky because such terms can include nonproductive equilibria constants, whereas such constants cancel out in the $V_{max}/K_M$ expression.[31]

(2) The TGI and $GI_{50}$ parameters are constant even with increasing DT-diaphorase specificity, except for those compounds incapable of reductively alkylating DNA. The cytostatic parameters (TGI and $GT_{50}$) for the NSC H460 cell line shown in Table 5 are all in the range of $-\log = 7-8$. The approximately constant cytostatic activity could be explained by the saturation phenomenon where reductive activation is not the limiting process. The limiting process would be the step following reductive activation, DNA alkylation.

(3) There is another cytotoxic/cytostatic mechanism beside DNA alkylation. Compounds with low DNA alkylation capability, such as **5a** and **9b**, generally exhibit low cytostatic and cytotoxic activity ($-\log = 5-6$). The bar graph in Figure 4 compares the cytostatic/cytotoxic activities of **5a** with the DNA reductive alkylating agent **3a**. Although both compounds possess nearly the same substrate specificity for DT-diaphorase, compound **5a** is inactive against all histological cancer types while **3a** exhibits significant cytostatic/cytotoxic activity. In contrast, some poor DNA alkylating agents, such as quinones **7a** and **9b**, possess significant cytotoxic and cytostatic properties. In addition, a $-\log LC_{50}$ vs $V_{max}/K_M$ plot of only the compounds with low DNA alkylation capability revealed no clear correlation. Clearly DT-diaphorase reductive activation and DNA reductive alkylation is not required for cytotoxic and cytostatic activity. These quinones may be activated by one-electron reduction and afford oxygen radicals as was observed for EO9.[41,42]

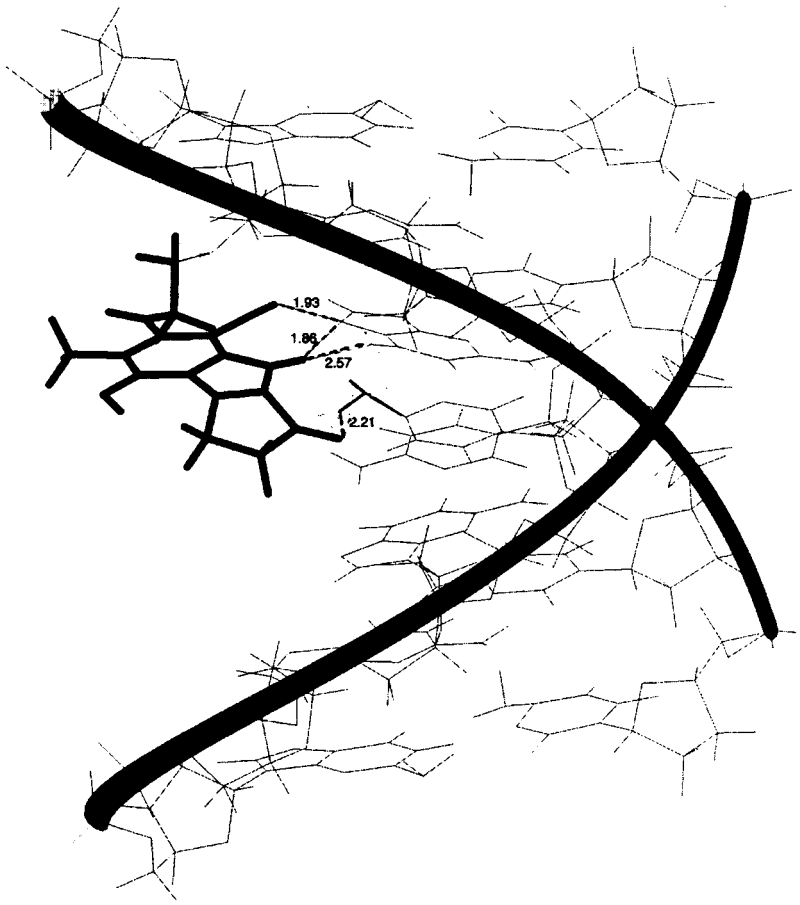
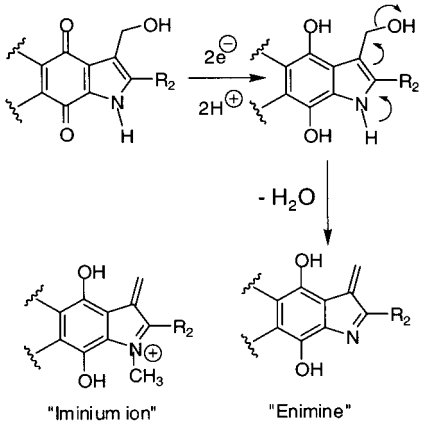



**Figure 2.** Model of an N-unsubstituted cyclopent[*b*]indole hydroquinone in the major groove at a GC base pair with the aziridinyl group reacted with a phosphate. The 3-substituent is a hydrogen-bond acceptor of the cytosine amino NH; length is 2.21 Å. The indole NH is a hydrogen-bond donor to the guanine 6-carbonyl or guanine N(7); lengths are 2.57 and 1.86 Å, respectively. The hydroquinone hydroxyl is a hydrogen-bond donor to the guanine N(7); length is 1.86 Å.

**Scheme 9**




(4) High DT-diaphorase specificity and/or high DNA alkylation percentages do not result in high cancer specificity. Some members of series **1**−**9** possess high DT-diaphorase specificity and/or high DNA alkylation capability. Examples include **6d** (91.2% of DNA bases reductively activated, $72 \times 10^{-4}$ $M^{-1}$ $s^{-1}$ DT-diaphorase specificity), **9b** (2% of DNA bases reductively activated, $554 \times 10^{-4}$ $M^{-1}$ $s^{-1}$ DT-diaphorase specificity), and **6c** (37% of DNA bases reductively activated, $430 \times 10^{-4}$ $M^{-1}$ $s^{-1}$ DT-diaphorase specificity). The best DT-diaphorase substrate, **9b**, and the best DNA alkylating agent, **6d**, possess no specificity for the histological cancer types.

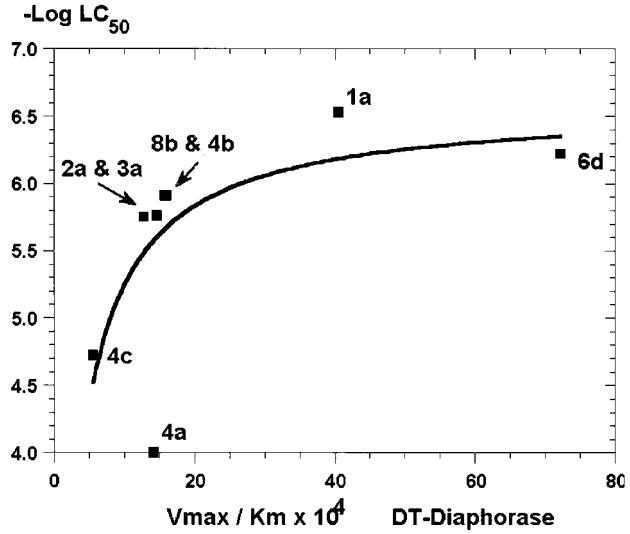



**Figure 3.** Plot of $-\log LC_{50}$ for series **1**−**9** compounds in NCI-H460 non-small-cell lung cancer cell lines versus the specificity for recombinant DT-diaphorase from the same cell line. The solid line was generated from eq 1.

Cancer specificity is measured from the ± value under the average log $LC_{50}$ 60-cell line heading of Table 5. A ± value greater than 2 indicates a greater than 100-fold higher/lower cytotoxicity in some histological cancer types compared to the mean cytotoxicity.

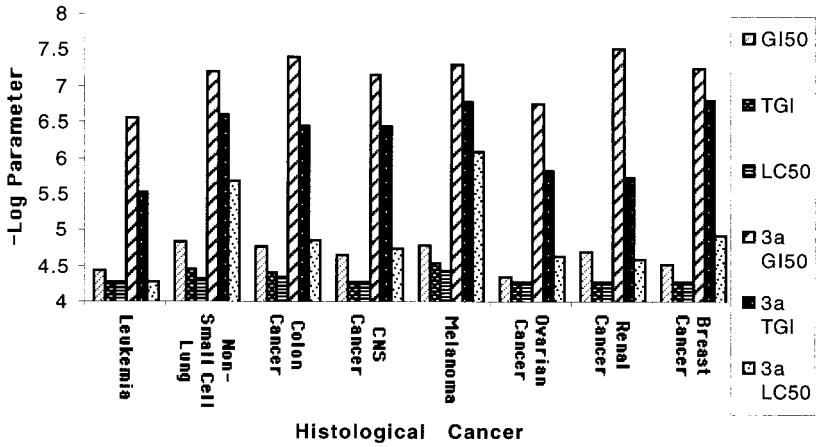



**Figure 4.** Bar graph of −log TGI, $GI_{50}$, and $LC_{50}$ for **5a** and **3a** versus histological cancer type. The −log parameter values are the average of 6−8 cell lines within each histological cancer.

The DT-diaphorase levels in the National Cancer Institute 60-cell line in vitro screen have been determined and correlated with the cytotoxicity of antitumor agents activated by two-electron reduction.[43] Sensitive cancers usually include non-small-cell (NSC) lung and colon cancers [and to a lesser extent central nervous system (CNS), melanoma, and renal cancers] by virtue of their elevated DT-diaphorase levels. An excellent DT-diaphorase substrate such as **9b** will be rapidly reduced regardless of the DT-diaphorase concentration in the cell, resulting in broad cytotoxicity. Generally quinones with DT-diaphorase specificities below $50 \times 10^{-4}$ $M^{-1}$, such as **2a**, **3a**, **7a**, and **7b**, possess high specificities for high DT-diaphorase cancers.

A high percentage of DNA reductive alkylation by **6d** as well as **4b** also results in loss of cancer specificity (± values of ∼1). These substrates are highly efficiently DNA reductive alkylating agents and function even at low DT-diaphorase concentrations, because only a low concentration is needed for DNA alkylation, resulting in the loss of cancer specificity. An exception seems to be **6c**, since it possesses high cancer specificity even though it is an excellent DT-diaphorase substrate and DNA reductive alkylating agent.

**In Vivo Screening Results.** Shown in Tables 6 and 7 are B16 melanoma syngraft assays[44] of some members of series **1**−**9**, as well as related compounds previously reported from this laboratory.[12] Each agent was evaluated at three doses in C57/bl mice: 2, 3, or 5 mg $kg^{-1}$ $day^{-1}$, on days 1, 5, and 9 after subcutaneous tumor implantation of $10^5$ cells in the front flank on day 0. Toxic means that there was early lethality, or ≥50% lethality prior to any deaths in the control group. The treated over control values (T/C) were measured at day 25 of the study. A T/C value <40% is considered active.

The data in Table 6 reveals that cyclopent[*b*]indoles **3b** and **3c** were toxic particularly at the 5 mg $kg^{-1}$ $day^{-1}$ dose. In contrast, cyclopent[*b*]indole **4c** was active at all doses while **4a** possessed modest activity, all without any toxicity. The toxicity of series **3** cyclopent[*b*]indoles may be due to their higher DT-diaphorase substrate specificity and DNA alkylating capability compared to the series **4** analogues, see Table 2. However, the presence of a good leaving group in the 3-position (acetate of **4c**) is required for antitumor activity. Therefore, analogues possessing a poor leaving group at the 3-position, hydroxide of **4a** or methoxide of **32** in Table 6, possess poor in vivo activity.

The in vivo results for the indole-based systems **6**, **7**, and **8** are provided in Table 7 along with the results of simpler indole analogues **33**−**37**.[12] These data clearly show that the presence of hydroxymethyl substituents, particularly in the 3-position, dramatically increases toxicity. In contrast, the simpler methylated indole analogues **33** and **34** exhibit substantial antitumor activity with toxicity only seen at the highest dose. If a hydroxymethyl is present at the 2-position of the indole, **35**, antitumor activity is retained without much toxicity. The ester derivative **36** is inactive at all doses. It is only when the 3-hydroxymethyl group or its ester is present, series **6**, **7**, and **9**, that toxicity overwhelms antitumor activity. The simplest explanation for the above findings is that the "enimine" alkylating agent arising from quinone reduction[37] and then loss of water from 3-hydroxymethyl derivatives, as illustrated in Scheme 9, is largely responsible for toxicity. DNA alkylation studies now underway indicate that this alkylating species reacts with the guanine N(7)- positions randomly. In contrast, the iminium ion that will arise from N-methylated indoles **6b** and **8c** will randomly alkylate oxygen and nitrogen centers on DNA.[45]

## Conclusions

A large number of aziridinyl quinones represented by series **1**−**9** were studied with respect to their DT-diaphorase substrate activity, DNA reductive alkylation, cytostatic/cytotoxic activity, and in vivo activity. As a result, a number of generalizations were made that will be useful in future drug design efforts.

**DT-Diaphorase Substrate Design.** DT-diaphorase substrate specificity of indole- and cyclopent[*b*]indole-based aziridinyl quinones is highly dependent on the substituents present. Methylation of the quinone ring reduces substrate specificity while the position of the aziridinyl ring is less critical. Hydrogen-bond acceptors at the 3-position of the cyclopent[*b*]indole system will also increase substrate specificity, perhaps due to a hydrogen-bonding interaction with the N−H of His194. The presence of an ethoxycarbonyl group at the 2-position of the indole system results in large increases in DT-diaphorase specificity, perhaps due to resonance stablization of the anion arising from hydride transfer.

By varying substituents in a systematic fashion, it was possible to design substrates with a 100-fold range of DT-diaphorase specificity.

**DT-Diaphorase−Cytotoxicity QSAR.** A comparison was made between the substrate specificity for human recombinant DT-diaphorase and the cytotoxicity of DNA alkylating and nonalkylating agents.

The DNA alkylating data were fit to a saturating relationship wherein the cytotoxicity became constant with DT-diaphorase substrate specificities ($V_{max}/K_M$) > $50 \times 10^{-4}$ $s^{-1}$; see eq 1 and Figure 3. The interpretation of this relationship is that reductive activation is no longer rate-limiting at high DT-diaphorase substrate specificities. The evidence presented in this report indicates that high DT-diaphorase substrate specificity, defined as $>50 \times 10^{-4}$ $s^{-1}$, is not a desirable feature in the indole and cyclopent[*b*]indole aziridinyl quinones. High substrate specificity results in a loss of selectivity for histological cancer types with high DT-diaphorase levels as well as toxicity in vivo. The application of these conclusions to other aziridinyl quinones awaits study in our DT-diaphorase and DNA reductive alkylation assays.

The DNA nonalkylating agents did not show a correlation between the cytotoxicity and DT-diaphorase substrate specificities ($V_{max}/K_M$). These quinones are not cytotoxic as a result of two-electron reduction and may be activated by one-electron reduction as was observed for EO9.[41,42]

**DNA Reductive Alkylating Agent Design.** The presence of a 6-methyl in the cyclopent[*b*]indoles substantially reduces DNA reductive alkylation for steric reasons. Likewise, an *N*-methyl in either the indole or the cyclopent[*b*]indole systems hinders DNA reductive alkylation resulting in nearly 0% alkylation in some cases. Perhaps the most important prerequisite for efficient DNA reductive alkylation by the indole system is a leaving group in the 3α-position, either a hydroxide or acetate. When the indole system is N-unsubstituted, leaving group elimination affords the highly reactive enimine functional group. Ongoing studies have revealed that indole systems able to form the enimine upon reductive activation are efficient DNA cross-linkers. The members of series **1**−**9** possess a wide range of DNA reductive alkylation capability, from near 0% to overalkylation at ∼90%. While some DNA alkylation was required for cytostatic and cytotoxic activity, too much alkylation results in loss of cancer selectivity as well as increased in vivo toxicity. Indeed, the most lethal compounds are the indole systems with an acetate or hydroxyl leaving group at the 3α-position. We conclude that poor DNA alkylating agents (according to our assay) show the lowest toxicity with the highest antitumor activity. For these compounds, redox recycling with oxygen radical generation[42] may be responsible for cytotoxicity.

## 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 with a variety of solvents and a fluorescent indicator for visualization. IR spectra were taken as thin films and the strongest absorbances reported. $^1$H NMR spectra were obtained from a 300 MHz spectrometer. All chemical shifts are reported relative to that of TMS.

The synthesis of new compounds is outlined below in the order found in Schemes 1−6.

**Substituted 1,4-dihydrocyclopent[*b*]indol-3(2*H*)-ones (10)** were prepared as previously described for **10b**.[12]

**6-Methoxy-1,4-dihydrocyclopent[*b*]indol-3(2*H*)-one (10a).** Yield 20%; mp 190 °C; TLC (dichloromethane/MeOH 95:5) $R_f$ = 0.75; $^1$H NMR ($CDCl_3$) $\delta$ 9.11 (1H, br s, indole proton), 7.57 (1H, d, $J$ = 9.0 Hz, 8-proton), 6.89 (1H, d, $J$ = 2.4 Hz, 5-proton), 6.83 (1H, dd, $J$ = 2.4 and 9.0 Hz, 7-proton), 3.89 (3H, s, 6-methoxy), 3.09 and 3.00 (4H, m, methylenes of cyclopentyl); IR (KBr pellet) 3450, 3176, 3090, 3026, 2957, 2924, 2843, 1662, 1620, 1535, 1257, 1197, 1136, 1047, 912, and 821 $cm^{-1}$; MS (EI) 201 ($M^+$), 186 ($M^+ - CH_3$), 173, 158, and 130. Anal. Calcd ($C_{12}H_{11}NO_2$): C, H, N.

**7-Methoxy-1,4-dihydrocyclopent[*b*]indol-3(2*H*)-one (10c).** Yield 40%; mp 252 °C; TLC (dichloromethane/MeOH 95:5) $R_f$ = 0.70; $^1$H NMR ($CDCl_3$) $\delta$ 8.42 (1H, br s, indole proton), 7.36 (1H, dd, $J$ = 1.5 and 9.0 Hz, 6-proton), 7.09 (1H, d, $J$ = 1.5 Hz, 8-proton), 7.06 (1H, d, $J$ = 9.0 Hz, 5-proton), 3.87 (3H, s, 7-methoxy), 3.09 and 3.00 (4H, m, methylenes of cyclopentyl); IR (KBr pellet) 3435, 3159, 2997, 2926, 2854, 1589, 1651, 1626, 1537, 1491, 1450, 1404, 1309, 1276, 1217, 1099, and 1045 $cm^{-1}$; MS (EI) 201 ($M^+$), 186 ($M^+ - CH_3$), 173, 158, and 130. Anal. Calcd ($C_{12}H_{11}NO_2$): C, H, N.

**7-Methoxy-6-methyl-1,4-dihydrocyclopent[*b*]indol-3(2*H*)-one (10d).** Yield 23%; mp 222 °C; TLC (dichloromethane/MeOH 95:5) $R_f$ = 0.80; $^1$H NMR ($CDCl_3$) $\delta$ 8.30 (1H, br s, indole proton), 7.19 and 6.99 (2H, 2s, 5,8-protons), 3.89 (3H, s, 7-methoxy), 3.09 and 3.00 (4H, m, methylenes of cyclopentyl), and 2.35 (3H, s, 6-methyl); IR (KBr pellet) 3418, 3176, 2920, 2836, 1666, 1556, 1487, 1446, 1307, 1207, 1095, 1047, and 815 $cm^{-1}$; MS (EI) 215 ($M^+$), 200 ($M^+ - CH_3$), 187, 172, and 115. Anal. Calcd ($C_{13}H_{13}NO_2$): C, H, N.

**Nitro-1,4-dihydrocyclopent[*b*]indol-3(2*H*)-ones (11).** To a solution of 10 mmol of **10** in 200 mL of concentrated $H_2SO_4$, cooled at −20 °C, was added a solution of 10.5 mmol of $KNO_3$ in 20 mL of concentrated $H_2SO_4$. The solution was stirred at that temperature for 40 min and was poured over 500 g of ice, and the solution was extracted five times with 200 mL portions of $CH_2Cl_2$. The extracts were washed with $NaHCO_3$, dried over $Na_2SO_4$, and vacuum-dried. The product was crystallized from $CH_2Cl_2$ and hexane.

**6-Methoxy-5-nitro-1,4-dihydrocyclopent[*b*]indol-3(2*H*)-one (11a).** Yield 32%; mp 228 °C; TLC (dichloromethane/MeOH 95:5) $R_f$ = 0.68; $^1$H NMR ($CDCl_3$) $\delta$ 10.01 (1H, br s, indole proton), 7.94 and 7.00 (2H, 2d, $J$ = 8.7 Hz, 7,8-protons), 4.11 (3H, s, 6-methoxy), 3.12 and 3.04 (4H, m, methylenes of cyclopentyl); IR (KBr pellet) 3460, 3375, 3269 2926, 2850, 1672, 1626, 1550, 1514, 1330, 1246, 1182, 1118, 1006, 962, and 812 $cm^{-1}$; MS (EI) 246 ($M^+$), 231 ($M^+ - CH_3$), 218, 199, 186, 170, 158, and 142. Anal. Calcd ($C_{12}H_{10}N_2O_4$): C, H, N.

**7-Methoxy-8-nitro-1,4-dihydrocyclopent[*b*]indol-3(2*H*)-one (11c).** Yield 65%; mp 293 °C; TLC (dichloromethane/MeOH 95:5) $R_f$ = 0.60; $^1$H NMR ($CDCl_3$) $\delta$ 9.49 (1H, br s, indole proton), 7.69 and 7.23 (2H, 2d, $J$ = 8.7 Hz, 5,6-protons), 4.02 (3H, s, 7-methoxy), 3.30 and 3.00 (4H, m, methylenes of cyclopentyl); IR (KBr pellet) 3437, 3192, 2928, 2854, 2660, 1672, 1628, 1568, 1518, 1329, 1271, 1244, 1182, 1091, 1049, 1001, and 815 $cm^{-1}$; MS (EI) 246 ($M^+$), 229 ($M^+ - OH$), 216, 198, and 170. Anal. Calcd ($C_{12}H_{10}N_2O_4$): C, H, N.

**7-Methoxy-6-methyl-8-nitro-1,4-dihydrocyclopent[*b*]-indol-3(2*H*)-one (11d).** Yield 55%; mp 240 °C; TLC (dichloromethane/MeOH 90:10) $R_f$ = 0.85; $^1$H NMR ($CDCl_3$) $\delta$ 9.45 (1H, br s, indole proton), 7.55 (H, s, 5-protons), 3.95 (3H, s, 7-methoxy), 3.30 and 3.00 (4H, m, methylenes of cyclopentyl), and 2.49 (3H, s, 6-methyl); IR (KBr pellet) 3448, 3203, 3059, 2960, 2926, 2856, 2652, 1672, 1489, 1352, 1276, 1230, 1093, 1022, 972, 864, and 823 $cm^{-1}$; MS (EI) 260($M^+$), 243 ($M^+ - OH$), 230, 213, 185, 156, 143, and 128. Anal. Calcd ($C_{13}H_{12}N_2O_4$): C, H, N.

**3-Hydroxy-(5 or 8)-nitro-1,2,3,4-tetrahydrocyclopent-[*b*]indoles (12).** To a solution of 5 mmol of **11** in 100 mL of MeOH was added 400 mg of $NaBH_4$ and the solution was stirred at room temperature for 20 min. The solution was then mixed with 400 mL of $H_2O$ and extracted four times with 100 mL of $CH_2Cl_2$. The combined extracts were dried over $Na_2SO_4$ and vacuum-dried. The product was crystallized from $CH_2Cl_2$ and hexane.

**3-Hydroxy-6-methoxy-5-nitro-1,2,3,4-tetrahydrocyclopent[*b*]indole (12a).** Yield 94%; mp 192 °C; TLC (dichloromethane/MeOH 95:5) $R_f = 0.45$; $^1$H NMR ($CDCl_3$) $\delta$ 9.80 (1H, br s, indole proton), 7.68 and 6.86 (2H, 2d, $J$ = 8.7 Hz, 7, 8-protons), 5.36 (1H, m, 3-hydroxymethylene proton), 4.05 (3H, s, 6-methoxy), 3.02, 2.75, and 2.38 (4H, m, methylenes of cyclopentyl), and 1.80 (1H, d, $J$ = 7.2 Hz, 3-hydroxymethylene hydroxy); IR (KBr pellet) 3435, 2928, 2852, 1626, 1566, 1508, 1334, 1290, 1184, 1112, 1057, 947, and 804 cm$^{-1}$; MS (EI) 248 ($M^+$), 231 ($M^+ - OH$), 215, 200, 184, 172, 154, and 144. Anal. Calcd ($C_{12}H_{12}N_2O_4$): C, H, N.

**3-Hydroxy-7-methoxy-8-nitro-1,2,3,4-tetrahydrocyclopent[*b*]indole (12c).** Yield 85%; mp 230 °C; TLC (dichloromethane/MeOH 95:5) $R_f = 0.25$; $^1$H NMR ($CDCl_3$) $\delta$ 8.19 (1H, br s, indole proton), 7.45 and 6.93 (2H, 2d, $J$ = 8.7 Hz, 5, 6-protons), 5.32 (1H, m, 3-hydroxymethylene proton), 3.97 (3H, s, 7-methoxy), 3.00, 2.82, and 2.33 (4H, m, methylenes of cyclopentyl); IR (KBr pellet) 3551, 3464, 3416, 3238, 3063, 2972, 2870, 1635, 1572, 1506, 1313, 1276, 1240, 1182, 1082, 954, and 787 cm$^{-1}$; MS (EI) 248 ($M^+$), 231 ($M^+ - OH$), 213, 200, 172, 154, and 143. Anal. Calcd ($C_{12}H_{12}N_2O_4$): C, H, N.

**3-Hydroxy-7-methoxy-6-methyl-8-nitro-1,2,3,4-tetrahydrocyclopent[*b*]indole (12d).** Yield 92%; mp 185 °C; TLC (dichloromethane/MeOH 90:10) $R_f = 0.65$; $^1$H NMR ($CDCl_3$) $\delta$ 8.16 (1H, br s, indole proton), 7.33 (1H, s, 4-protons), 5.30 (1H, m, 3-hydroxymethylene proton), 3.91 (3H, s, 7-methoxy), 3.00, 2.82, and 2.33 (4H, m, methylenes of cyclopentyl), and 2.43 (3H, s, 6-methyl); IR (KBr pellet) 3408, 3175, 3047, 2943, 2856, 1637, 1570, 1516, 1427, 1329, 1288, 1219, 1149, 1076, 1041, 976, and 866 cm$^{-1}$; MS (EI) 262 ($M^+$), 244 ($M^+ - H_2O$), 227, 215, 197, 186, 168, 154, 143, 127, and 115. Anal. Calcd ($C_{13}H_{14}N_2O_4$): C, H, N.

**3-Hydroxy-1,2,3,4-tetrahydrocyclopent[*b*]indole-5,8-diones (13).** A solution of 0.5 mmol of the product above in 10 mL of MeOH and 40 mL of $H_2O$ with 150 mg of 5% Pd on carbon was reduced under 50 psi $H_2$ for 30 min, and the catalyst was filtered off through Celite. The filtrate was mixed with a solution of 400 mg of $KH_2PO_4$ and 800 mg of Fremy salt in 150 mL of $H_2O$, stirred at room temperature for 3 h. The solution was extracted five times with 50 mL portions of $CH_2Cl_2$ to remove the quinone product. The extracts were dried over $Na_2SO_4$ and vacuum-dried. The solid was purified by flash chromatography with 10% acetone in $CH_2Cl_2$ as the eluent. The product was recrystallized from $CH_2Cl_2$ and hexane.

**3-Hydroxy-6-methoxy-1,2,3,4-tetrahydrocyclopent[*b*]-indole-5,8-dione (13a).** Yield 15%; mp 167 °C; TLC (dichloromethane/MeOH 90:10) $R_f$ = 0.70; $^1$H NMR (DMSO-$d_6$) $\delta$ 12.34 (1H, br s, indole proton), 5.72 (1H, s, 5-proton), 5.27 (1H, m, 3-hydroxymethylene proton), 3.74 (3H, s, 6-methoxy), 2.77, 2.46, and 2.15 (4H, m, methylenes of cyclopentyl); IR (KBr pellet) 3493, 3136, 2989, 2939, 2858, 1664, 1628, 1591, 1543, 1402, 1323, 1238, 1120, 1055, 935, and 848 cm$^{-1}$; MS (EI) 233 ($M^+$), 215 ($M^+ - H_2O$), 202, 190, 174, and 158. Anal. Calcd ($C_{12}H_{11}NO_4$): C, H, N.

**3-Hydroxy-7-methoxy-1,2,3,4-tetrahydrocyclopent[*b*]-indole-5,8-dione (13c).** Yield 18%; mp 200 °C; TLC (dichloromethane/MeOH 95:5) $R_f = 0.20$; $^1$H NMR ($CDCl_3$) $\delta$ 9.12 (1H, br s, indole proton), 5.69 (1H, 6-protons), 5.20 (1H, m, 3-hydroxymethylene proton), 3.83 (3H, s, 7-methoxy), 3.01, 2.85, and 2.29 (4H, m, methylenes of cyclopentyl); IR (KBr pellet) 3431, 3205, 3109, 3067, 2937, 2868, 1678, 1645, 1591, 1483, 1458, 1408, 1342, 1292, 1248, 1211, 1180, 1082, 1030, 949, 868, and 842 cm$^{-1}$; MS (EI) 233 ($M^+$), 215 ($M^+ - H_2O$), 200, 190, 187, 176, 172, 162, 158, 144, and 130. Anal. Calcd ($C_{12}H_{11}NO_4$): C, H, N.

**3-Hydroxy-7-methoxy-6-methyl-1,2,3,4-tetrahydrocyclopent[*b*]indole-5,8-dione (13d).** Yield 13%; mp 215 °C; TLC (dichloromethane/MeOH 90:10) $R_f$ = 0.60; $^1$H NMR ($CDCl_3$) $\delta$ 9.10 (1H, br s, indole proton), 5.18 (1H, m, 3-hydroxymethylene proton), 4.02 (3H, s, 7-methoxy), 3.00, 2.78 and 2.32 (4H, m, methylenes of cyclopentyl), and 1.97 (3H, s, 6-methyl); IR (KBr pellet) 3435, 3207, 3013, 2926, 2852, 1637, 1498, 1450, 1408, 1373, 1317, 1290, 1217, 1168, 1105, 1039, 985, 949, and 808 cm$^{-1}$; MS (EMI) 247 ($M^+$), 229 ($M^+ - H_2O$), 214, 204, 201, 190, 186, 176, 172, 158, and 144. Anal. Calcd ($C_{13}H_{13}NO_4$): C, H, N.

**Aziridinyl-3-hydroxy-1,2,3,4-tetrahydrocyclopent[*b*]-indole-5,8-diones (Series a).** To a solution of 0.3 mmol of **13** in 30 mL of MeOH was added 0.5 mL of ethylenimine, and the solution was stirred at room temperature (RT) for 8 h. The solvent was vacuum-removed and the residue was subjected to flash chromatography with 20% acetone in $CH_2Cl_2$ as eluent. The product was crystallized from $CH_2Cl_2$ and hexane.

**7-Aziridinyl-3-hydroxy-1,2,3,4-tetrahydrocyclopent[*b*]-indole-5,8-dione (1a).** Yield 93%; mp 145 °C; TLC (dichloromethane/MeOH 90:10) $R_f$ = 0.55; $^1$H NMR ($CDCl_3$) $\delta$ 9.26 (1H, br s, indole proton), 5.81 (1H, 6-proton), 5.20 (1H, m, 3-hydroxymethylene proton), 2.96, 2.74, and 2.33 (4H, m, methylenes of cyclopentyl), and 2.22 (4H, s, 7-aziridinyl); IR (KBr pellet) 3418, 3283, 2946, 2870, 1626, 1578, 1476, 1364, 1254, 1156, 1078, 946, 846, and 808 cm$^{-1}$; MS (EI) 244 ($M^+$), 226 ($M^+ - H_2O$), 215, 200, 192, 176, and 137. Anal. Calcd ($C_{13}H_{12}N_2O_3$): C, H, N.

**7-Aziridinyl-3-hydroxy-6-methyl-1,2,3,4-tetrahydrocyclopent[*b*]indole-5,8-dione (2a).** Yield 95%; mp 220 °C; TLC (dichloromethane/MeOH 90:10) $R_f = 0.55$; $^1$H NMR ($CDCl_3$) $\delta$ 9.23 (1H, br s, indole proton), 5.18 (1H, m, 3-hydroxymethylene proton), 2.96, 2.72, and 2.36 (4H, m, methylenes of cyclopentyl), 2.32 (4H, s, 7-aziridinyl), and 2.53 (3H, s, 6-methyl); IR (KBr pellet) 3420, 3192, 2928, 2856, 1664, 1629, 1560, 1498, 1458, 1377, 1292, 1230, 1074, 1039, 981, and 856 cm$^{-1}$; MS (EI) 258 ($M^+$), 240 ($M^+ - H_2O$), 229, 214, 204, 190, and 151. Anal. Calcd ($C_{14}H_{14}N_2O_3$): C, 65.10; H, 5.46; N, 10.85. Found: C, 65.16; H, 5.44; N, 10.89.

**6-Aziridinyl-3-hydroxy-1,2,3,4-tetrahydrocyclopent[*b*]-indole-5,8-dione (3a).** Yield 89%; mp 180 °C; TLC (dichloromethane/MeOH 90:10) $R_f$ = 0.50; $^1$H NMR (DMSO-$d_6$) $\delta$ 12.12 (1H, br s, indole proton), 5.73 (1H, s, 7-proton), 5.20 (1H, m, 3-hydroxymethylene proton), 2.70, 2.46, and 2.11(4H, m, methylenes of cyclopentyl), and 2.12 (4H, s, 6-aziridinyl); IR (KBr pellet) 3447, 3198, 3003, 2930, 2864, 1666, 1614, 1577, 1475, 1406, 1294, 1238, 1112, 1041, 949, 852, and 806 cm$^{-1}$; MS (EI) 244 ($M^+$), 226 ($M^+ - H_2O$), 217, 199, 189, and 171. Anal. Calcd ($C_{13}H_{12}N_2O_3$): C, H, N.

**3-Acetoxy-(6 or 7)-aziridinyl-1,2,3,4-tetrahydrocyclopent[*b*]indole-5,8-diones (Series b) and (6 or 7)-Aziridinyl-3-acetoxy-4-acetyl-1,2,3,4-tetrahydrocyclopent[*b*]indole-5,8-diones (Series c).** To a solution of 0.4 mmol of **1**−**3a** in 10 mL of 10% acetone in $CH_2Cl_2$ with 50 mg of DMAP present was added 3 × 60 $\mu$L of acetic anhydride every 3 min. The solution was then directly applied to flash chromatography with 10% acetone in $CH_2Cl_2$ as the eluent. The solution was washed with saturated $NaHCO_3$, dried over $Na_2SO_4$, and vacuum-dried. The product was precipitated from $CH_2Cl_2$ and hexane.

**3-Acetoxy-7-aziridinyl-1,2,3,4-tetrahydrocyclopent[*b*]-indole-5,8-dione (1b).** Yield 42%; mp 165 °C; TLC (dichloromethane/MeOH 90:10) $R_f$ = 0.65; $^1$H NMR ($CDCl_3$) $\delta$ 9.20 (1H, br s, indole proton), 5.83 (1H, 6-proton), 5.66 (1H, m, 3-hydroxymethylene proton), 2.99, 2.76, and 2.21 (4H, m, methylenes of cyclopentyl), 2.21 (4H, s, 7-aziridinyl), and 2.06 (3H, s, 3-acetyl methyl); IR (KBr pellet) 3448, 3225, 3065, 2926, 2872, 1720, 1641, 1585, 1510, 1475, 1367, 1288, 1251, 1163, 1018, 985, and 812 cm$^{-1}$; MS (EI) 286 ($M^+$), 258 ($M^+ - CO$), 243, 226, 211, 199, 170, 142, and 118. Anal. Calcd ($C_{15}H_{14}N_2O_4$): C, H, N.

**3-Acetoxy-7-aziridinyl-6-methyl-1,2,3,4-tetrahydrocyclopent[*b*]indole-5,8-dione (2b).** Yield 45%; mp 165 °C; TLC (dichloromethante:MeOH 90:10) $R_f$ = 0.75; $^1$H NMR ($CDCl_3$)

$\delta$ 9.13 (1H, br s, indole proton), 5.66 (1H, m, 3-hydroxymethylene proton), 2.99, 2.59, and 2.30 (4H, m, methylenes of cyclopentyl), 2.30 (4H, s, 7-aziridinyl), and 2.06 (6H, 2s, 3-acetyl methyl and 6-methyl); IR (KBr pellet) 3454, 3311, 3043, 2926, 2843, 1732, 1663, 1572, 1473, 1382, 1239, 1009, 964, and 800 $cm^{-1}$; MS (EI) 300 ($M^+$), 272 ($M^+ - CO$), 257, 240, 225, 213, 184, 156, and 132. Anal. Calcd ($C_{16}H_{16}N_2O_4$): C, H, N.

**3-Acetoxy-6-aziridinyl-1,2,3,4-tetrahydrocyclopent[*b*]-indole-5,8-dione (3b).** Yield 32%; mp 155 °C; TLC (dichloromethane/acetone 80:20) $R_f = 0.45$; $^1H$ NMR ($CDCl_3$) $\delta$ 9.31 (1H, br s, indole proton), 5.92 (1H, 7-proton), 5.64 (1H, m, 3-hydroxymethylene proton), 3.00, 2.56, and 2.28 (4H, m, methylenes of cyclopentyl), 2.24 (4H, s, 6-aziridinyl), and 2.05 (3H, s, 3-acetyl methyl); IR (KBr pellet) 3437, 3219, 3103, 1995, 1945, 2864, 1724, 1643, 1583, 1481, 1369, 1296, 1236, 1112, 1018, 991, 956, 891, and 850 $cm^{-1}$; MS (EI) 286 ($M^+$), 258 ($M^+ - CO$), 244, 226, 215, 199, and 170. Anal. Calcd ($C_{15}H_{14}N_2O_4$): C, H, N.

**7-Aziridinyl-3-acetoxy-4-acetyl-1,2,3,4-tetrahydrocyclopent[*b*]indole-5,8-dione (1c).** Yield 45%; mp 140 °C; TLC (dichloromethane/acetone 80:20) $R_f = 0.65$; $^1H$ NMR ($CDCl_3$) $\delta$ 6.20 (1H, m, 3-hydroxymethylene proton), 5.90 (1H, s, 6-proton), 2.99, 2.41, and 2.20 (4H, m, methylenes of cyclopentyl), 2.74 (3H, s, 4-acetyl methyl), 2.22 (4H, s, 7-aziridinyl), and 2.06 (3H, s, 3-acetyl methyl); IR (KBr pellet) 3431, 3005, 2928, 2870, 1734, 1672, 1639, 1593, 1491, 1371, 1246, 1186, 1157, 1095, 1020, and 856 $cm^{-1}$; MS (EI) 328 ($M^+$), 300 ($M^+ - CO$), 286, 244, 226, 199, 171, and 132. Anal. Calcd ($C_{17}H_{16}N_2O_5$): C, H, N.

**7-Aziridinyl-3-acetoxy-4-acetyl-6-methyl-1,2,3,4-tetrahydrocyclopent[*b*]indole-5,8-dione (2c).** Yield 48%; mp 156 °C; TLC (dichloromethane/acetone 80:20) $R_f = 0.70$; $^1H$ NMR ($CDCl_3$) $\delta$ 6.26 (1H, m, 3-hydroxymethylene proton), 2.97, 2.43, and 2.22 (4H, m, methylenes of cyclopentyl), 2.72 (3H, s, 4-acetyl), 2.25 (4H, s, 7-aziridinyl), 2.08 and 2.05 (6H, 2s, 6-methyl and 3-acetyl methyl); IR (KBr pellet) 3433, 3011, 2945, 2854, 1734, 1666, 1637, 1593, 1496, 1381, 1350, 1240, 1217, 1157, 1022, 983, and 933 $cm^{-1}$; MS (EI) 342 ($M^+$), 300 ($M^+ -$ acetyl), 283, 258, 240, and 225. Anal. Calcd ($C_{18}H_{18}N_2O_5$): C, H, N.

**6-Aziridinyl-3-acetoxy-4-acetyl-1,2,3,4-tetrahydrocyclopent[*b*]indole-5,8-dione (3c).** Yield 56%; mp 167 °C; TLC (dichloromethane/acetone 80:20) $R_f = 0.65$; $^1H$ NMR ($CDCl_3$) $\delta$ 5.97 (1H, m, 3-hydroxymethylene proton), 5.91 (1H, s, 7-proton), 2.97, 2.43, and 2.21 (4H, m, methylenes of cyclopentyl), 2.77 (3H, s, 4-acetyl methyl), 2.25 (4H, s, 6-aziridinyl), and 2.04 (3H, s, 3-acetyl methyl); IR (KBr pellet) 3448, 3007, 2941, 2862, 1736, 1668, 1637, 1585, 1473, 1361, 1251, 1230, 1112, 1033, 981, and 893 $cm^{-1}$; MS (EI) 328 ($M^+$), 300 ($M^+ - CO$), 286, 244, 226, 199, 171, and 132. Anal. Calcd ($C_{17}H_{16}N_2O_5$): C, 62.19; H, 4.91; N, 8.53. Found: C, 62.16; H, 4.88; N, 8.50.

**4,7-Dimethyl-8-nitro-1,4-dihydrocyclopent[*b*]indol-3(2*H*)-one (14).** To 0.2 mmol of **11b** and 400 mg of KOH in 20 mL of acetone was added 1.0 mL of MeI, followed by stirring at RT for 1 h. The reaction mixture was then neutralized with concentrated HCl and extracted four times with 30 mL portions of $CH_2Cl_2$. The combined extracts were dried ($Na_2SO_4$), concentrated to a residue, and chromographed with $CH_2Cl_2$ as eluent. The product was crystallized from methylene chloride by use of hexane. Yield 92%; mp 207 °C; TLC (dichloromethane) $R_f = 0.45$; $^1H$ NMR ($CDCl_3$) $\delta$ 7.49 and 7.28 (2H, 2d, $J = 8.7$ Hz, 5, 6-protons), 3.96 (3H, s, 4-methyl), 3.14 and 2.97 (4H, m, methylenes of cyclopentyl), and 2.62 (3H, s, 5-methyl); IR (KBr pellet) 3437, 3063, 2939, 2849, 1683, 1521, 14581373, 1338, 1265, 1201, 1047, and 833 $cm^{-1}$; MS (EI) 244 ($M^+$), 227 ($M^+ -$ OH), 197, 184, 169, and 154. Anal. Calcd ($C_{13}H_{12}N_2O_3$): C, H, N.

**4,7-Dimethyl-3-hydroxy-8-nitro-1,2,3,4-tetrahydrocyclopent[*b*]indole (15).** The borohydride reduction of **14** to afford **15** was carried out as described above for the preparation of **12**: Yield 90%; mp 171 °C; TLC (dichloromethane/MeOH 95:5) $R_f = 0.80$; $^1H$ NMR ($CDCl_3$) $\delta$ 7.37 and 7.05 (2H, 2d, $J = 8.4$ Hz, 5, 6-protons), 5.36 (1H, m, 3-hydroxymethylene proton), 3.82 (3H, s, 4-methyl), 3.09, 2.88, and 2.33 (4H, m, methylenes of cyclopentyl), 2.39 (3H, s, 7-methyl), and 1.65 (1H, d, $J = 9.0$ Hz, 3-hydroxymethylene hydroxy); IR (KBr pellet) 3435, 3302, 3202, 2962, 2930, 1637, 1560, 1516, 1460, 1321, 1269, 117, 1045, 952, 908, and 808 $cm^{-1}$; MS (EI) 246 ($M^+$), 229 ($M^+ -$ OH), 212, 199, 182, 167, and 156. Anal. Calcd ($C_{13}H_{14}N_2O_3$): C, H, N.

**4,7-Dimethyl-3-hydroxy-1,2,3,4-tetrahydrocyclopent[*b*]-indole-5,8-dione (16).** The catalytic reduction and Fremy oxidation of **15** to afford **16** was carried out as described for the preparation of **13**: Yield 14%; mp 160 °C; TLC (dichloromethane/MeOH 96:4) $R_f = 0.35$; $^1H$ NMR ($CDCl_3$) $\delta$ 6.35 (1H, q, $J = 1.5$ Hz, 6-proton), 5.22 (1H, m, 3-hydroxymethylene proton), 3.95 (3H, s, 4-methyl), 3.02, 2.73, and 2.34 (4H, m, methylenes of cyclopentyl), 2.04 (3H, d, $J = 1.5$ Hz, 7-methyl), and 1.72 (1H, d, $J = 8.4$ Hz, 3-hydroxymethylene hydroxy); IR (KBr pellet) 3469, 2943, 2872, 1639, 1604, 1475, 1384, 1221, 1159, 1039, 947, and 877 $cm^{-1}$; MS (EI) 231 ($M^+$), 214 ($M^+ -$ OH), 203, 188, 174, and 160. Anal. Calcd ($C_{13}H_{13}NO_3$): C, H, N.

**6-Aziridinyl-4,7-dimethyl-3-hydroxy-1,2,3,4-tetrahydrocyclopent[*b*]indole-5,8-dione (5a)** was prepared by the procedure described for series a: Yield 32%; mp 187 °C; TLC (dichloromethane/MeOH 96:4) $R_f = 0.60$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 5.41 (1H, d, $J = 6.6$ Hz, 3-hydroxyl), 5.04 (1H, m, 3-hydroxymethylene proton), 3.82 (3H, s, 4-methyl), 2.65, 2.50, and 2.10 (4H, m, methylenes of cyclopentyl), and 2.17 (4H, s, 6-aziridine); IR (KBr pellet) 3431, 2995, 2949, 2922, 2858, 1653, 1620, 1479, 1377, 1340, 1153, 1051, and 983 $cm^{-1}$; MS (EI) 272 ($M^+$), 255 ($M^+ -$ OH), 243, 227, and 217. Anal. Calcd ($C_{15}H_{16}N_2O_3$): C, H, N.

**3-Acetyl-6-aziridinyl-4,7-dimethyl-1,2,3,4-tetrahydrocyclopent[*b*]indole-5,8-dione (5b).** Yield 50%; mp 157 °C; TLC (dichloromethane/acetone 96:4) $R_f = 0.62$; $^1H$ NMR ($CDCl_3$) $\delta$ 6.10 (1H, m, 3-hydroxymethylene proton), 3.89 (3H, s, 4-methyl), 2.96, 2.79, and 2.40 (4H, m, methylenes of cyclopentyl), 2.30 (4H, s, 6-aziridinyl), 2.09 and 2.08 (6H, 2s, 7-methyl and 3-acetyl methyl); IR (KBr pellet) 3447, 3020, 2922, 2854, 1734, 1653, 1626, 1583, 1375, 1336, 1232, 1151, 1030, and 856 $cm^{-1}$; MS (EI) 314 ($M^+$), 299 ($M^+ - CH_3$), 272, 255, 239, 227, and 215. Anal. Calcd ($C_{17}H_{18}N_2O_4$): C, H, N.

**2-(Ethoxycarbonyl)indoles (17).** The synthesis of **17a** and **17c** was reported in the literature,[46] and **17b** was synthesized following the procedure described for **16a**.

**2-(Ethoxycarbonyl)-5-methoxy-6-methylindole (17b).** Yield 63%; mp 165 °C; TLC ($CHCl_3$) $R_f = 0.55$; $^1H$ NMR ($CDCl_3$) $\delta$ 8.65 (1H, br s, indole proton), 7.26, 7.11, and 6.99 (3H, 3s, 3, 4, 7-proton), 4.38 (2H, q, $J = 7.2$ Hz, methylene of 2-ethoxyl), 3.87 (3H, s, 5-methoxyl), 2.33 (3H, s, 6-methyl), and 1.39 (3H, t, $J = 7.2$ Hz, methyl of 2-ethoxyl); IR (KBr pellet) 3445, 3325, 2982, 2941, 2839, 1682, 1520, 1246, 1213, 1168, 1136, 1026, 864, and 833 $cm^{-1}$; MS (EI) 233 ($M^+$), 218, 204, 187, 172, and 144. Anal. Calcd ($C_{13}H_{15}NO_3$): C, H, N.

**2-(Ethoxycarbonyl)-3-formylindoles (18).** A solution of 6 mL of distilled DMF with 2 mL of distilled $POCl_3$ in 40 mL of dried $CH_2Cl_2$ was stirred at 0 °C for 30 min. To this solution was slowly added 50 mmol of **18** in 30 mL of dried $CH_2Cl_2$, and the reaction mixture was stirred at 0 °C for 30 min. To the completed reaction was added $NaHCO_3$ saturated solution until neutral. The product was removed from the neutralized reaction by extracting five times with 100 mL portions of methylene chloride. The product was crystallized from dichloromethane and hexane.

**2-(Ethoxycarbonyl)-3-formyl-5-methoxyindole (18a).** Yield 87%; mp 231 °C; TLC ($CHCl_3$) $R_f = 0.30$; $^1H$ NMR ($CDCl_3$) $\delta$ 10.74 (1H, s, 3-formyl), 9.20 (1H, br s, indole proton), 7.90 (1H, d, $J = 2.7$ Hz, 4-proton), 7.35 (1H, d, $J = 8.7$ Hz, 7-proton), 7.07 (1H, dd, $J = 2.7$ and 8.7 Hz, 6-proton), 4.52 (2H, q, $J = 7.2$ Hz, methylene of 2-ethoxy), 3.91 (3H, s, 5-methoxy), and 1.45 (3H, t, $J = 7.2$ Hz, methyl of 2-ethoxy); IR (KBr pellet) 3448, 3157, 2985, 2833, 1709, 1637, 1581, 1531, 1467, 1433, 1398, 1371, 1244, 1201, 1122, 1028, 983, and 804

$cm^{-1}$; MS (EI) 247 ($M^+$), 218 ($M^+ - HCO$), 200, 186, 173, 158, 144, 130, and 119. Anal. Calcd ($C_{13}H_{13}NO_4$): C, H, N.

**2-(Ethoxycarbonyl)-3-formyl-5-methoxy-6-methylindole (18b).** Yield 90%; mp 223 °C; TLC ($CHCl_3$) $R_f = 0.45$; $^1H$ NMR ($CDCl_3$) $\delta$ 10.72 (1H, s, 3-formyl), 9.20 (1H, br s, indole proton), 7.81 and 7.21 (2H, 2s, 4,7-protons), 4.51 (2H, q, $J$ = 7.2 Hz, methylene of 2-ethoxy), 3.93 (3H, s, 5-methoxy), 2.34 (3H, s, 6-methyl), and 1.45 (3H, t, $J$ = 7.2 Hz, methyl of 2-ethoxy); IR (KBr pellet) 3435, 3105, 3053, 2993, 2939, 2835, 1718, 1639, 1572, 1531, 1469, 1433, 1390, 1371, 1263, 1205, 1147, 1093, 1060, 962, 866, and 812 $cm^{-1}$; MS (EI) 261 ($M^+$) 232 ($M^+ - HCO$), 214, 200, 187, 172, 158, 144, and 133. Anal. Calcd ($C_{14}H_{15}NO_4$): C, H, N.

**3-Formyl-2-(methoxycarbonyl)-6-methoxyindole (18c).** Yield 90%; mp 185 °C; TLC ($CHCl_3$/acetone 95:5) $R_f = 0.65$; $^1H$ NMR ($CDCl_3$) $\delta$ 10.69 (1H, s, 3-formyl), 9.10 (1H, br s, indole proton), 8.32 (1H, d, $J$ = 8.7 Hz, 4-proton), 7.01 (1H, dd, $J$ = 2.7 and 8.7 Hz, 5-proton), 6.85 (1H, d, $J$ = 2.7 Hz, 7-proton), 4.04 and 3.87 (6H, 2s, 6-methoxy and ester methoxy); IR (KBr pellet) 3311, 3173, 3130, 2953, 2926, 2856, 1712, 1641, 1579, 1444, 1250, 1213, 1161, 1097, 1026, and 827 $cm^{-1}$; MS (EI) 233 ($M^+$) 218, 201, 186, 173, 158, and 144. Anal. Calcd ($C_{12}H_{11}NO_4$): C, H, N.

**2-(Ethoxycarbonyl)-3-formyl-(4 or 7)-nitroindoles (19).** A solution of 2 mmol of **18** was stirred in 25 mL of 70% $HNO_3$ for 25 min, followed by addition of 100 g of ice. The resulting mixture was extracted three times with 100 mL portions of methylene chloride. The combined extracts were washed with saturated $NaHCO_3$ and then dried over $Na_2SO_4$. The product was crystallized from $CH_2Cl_2$ and hexane.

**2-(Ethoxycarbonyl)-3-formyl-5-methoxy-4-nitroindole (19a).** Yield 73%; mp 220 °C; TLC ($CHCl_3$) $R_f = 0.15$; $^1H$ NMR ($CDCl_3$) $\delta$ 10.6 (1H, s, 3-formyl), 9.42 (1H, br s, indole proton), 7.58 and 7.24 (2H, 2d, $J$ = 8.7 Hz, 6,7-protons), 4.54 (2H, q, $J$ = 7.2 Hz, methylene of 2-ethoxy), 3.97 (3H, s, 5-methoxy), and 1.47 (3H, t, $J$ = 7.2 Hz, methyl of 2-ethoxy). IR (KBr pellet) 3437, 3269, 3080, 2999, 2928, 1689, 1637, 1547, 1523, 1477, 1444, 1390, 1269, 1219, 1184, 1126, 1085, 1012, 869, and 833 $cm^{-1}$; MS (EI) 292 ($M^+$) 274 ($M^+ - H_2O$), 263, 246, 229, 216, 198, 188, 170, 160, 144, and 130. Anal. Calcd ($C_{13}H_{12}N_2O_6$): C, H, N.

**2-(Ethoxycarbonyl)-3-formyl-5-methoxy-6-methyl-4-nitroindole (19b).** Yield 50%; mp 198 °C; TLC ($CHCl_3$) $R_f$ = 0.40; $^1H$ NMR ($CDCl_3$) $\delta$ 10.61 (1H, s, 3-formyl), 9.40 (1H, br s, indole proton), 7.41 (1H, s, 7-proton), 4.53 (2H, q, $J$ = 7.2 Hz, methylene of 2-ethoxy), 3.91 (3H, s, 5-methoxy), 2.48 (3H, s, 6-methyl), and 1.47 (3H, t, $J$ = 7.2 Hz, methyl of 2-ethoxy); IR (KBr pellet) 3367, 3105, 2985, 2941, 1726, 1658, 1539, 1464, 1429, 1394, 1253, 1197, 1178, 1016, 977, 864, and 808 $cm^{-1}$; MS (EI) 306 ($M^+$) 288 ($M^+ - H_2O$), 277, 261, 243, 230, 202, 196, 174, 156, 144, and 130. Anal. Calcd ($C_{14}H_{14}N_2O_6$): C, H, N.

**3-Formyl-2-(methylcarboxyl)-6-methoxy-7-nitroindole (19c).** Yield 92%; mp 197 °C; TLC (acetone/dichloromethane 20:80) $R_f = 0.80$; $^1H$ NMR ($CDCl_3$) $\delta$ 10.74 (1H, s, 3-formyl), 10.61 (1H, br s, indole proton), 8.71 and 7.15 (2H, 2d, $J$ = 8.7 Hz, 4,5-protons), 4.12 and 4.08 (6H, 2s, 6-methoxyl and 2-ester methyl); IR (KBr pellet) 3396, 2960, 2883, 1716, 1662, 1545, 1442, 1411, 1342, 1253, 1192, 1089, 962, and 810 $cm^{-1}$; MS (EI) 278 ($M^+$) 263, 260, 245, 218, 202, 187, and 169. Anal. Calcd ($C_{12}H_{10}N_2O_6$): C, H, N.

**2,3-Di(hydroxymethyl)nitroindoles (20).** To a solution of 100 mg of lithium aluminum hydride (LAH) in 15 mL of dry tetrahydrofuran (THF) cooled to −15 °C was added a solution of 0.5 mmol of **19** in 2 mL of dry THF. The solution was stirred at the same temperature for another 5 min and then 5 mL of ethyl acetate was added, followed by the addition of 2 mL of water. The solid was filtered off and the solution was vacuum-dried, followed by flash chromatography with ethyl acetate as the eluent. The product was recrystallized from ethyl acetate and hexane.

**2,3-Di(hydroxymethyl)-5-methoxy-4-nitroindole (20a).** Yield 40%; mp 127 °C; TLC (ethyl acetate) $R_f = 0.30$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 11.4 (1H, br s, indole proton), 7.51 and 7.02 (2H, 2d, $J$ = 9 Hz, 6,7-protons), 5.30 and 4.35 (2H, 2t, $J$ = 5.1 Hz, hydroxyls of 2,3-hydroxymethyls), 4.63 and 4.38 (4H, 2d, $J$ = 5.1 Hz, methylenes of 2,3-hydroxymethyls), and 3.92 (3H, s, 6-methoxyl); IR (KBr pellet) 3348, 2960, 2752, 1633, 1579, 1514, 1464, 1435, 1357, 1321, 1261, 1199, 1103, 1039, 979, and 829 $cm^{-1}$; MS (EI) 252 ($M^+$) 234 ($M^+ - H_2O$), 219, 205, 191, 177, 163, 159, and 121. Anal. Calcd ($C_{11}H_{12}N_2O_5$): C, H, N.

**2,3-Di(hydroxymethyl)-5-methoxy-6-methyl-4-nitroindole (20b).** Yield 44%; mp 158 °C; TLC (ethyl acetate/methanol 95:5) $R_f = 0.50$; $^1H$ NMR ($CDCl_3$) $\delta$ 8.52 (1H, br s, indole proton), 7.30 (H, 1s, 7-protons), 4.90 and 4.65 (4H, 2d, $J$ = 5.7 Hz, methylenes of 2,3-hydroxymethyls), 4.02 (3H, s, 5-methoxyl), 2.48 (3H, s, 6-methyl), 2.23 and 2.04 (2H, 2t, $J$ = 5.7 Hz, hydroxyls of 2,3-hydroxymethyls); IR (KBr pellet) 3447, 3385, 2924, 2852, 1637, 1560, 1475, 1429, 1356, 1327, 1278, 1228, 1176, 1101, 1001, 916, 866, and 815 $cm^{-1}$; MS (EI) 266 ($M^+$) 248 ($M^+ - H_2O$), 231, 219, 215, 201, 187, 162, 158, 143, and 130. Anal. Calcd ($C_{12}H_{14}N_2O_5$): C, H, N.

**2,3-Di(hydroxymethyl)-6-methoxy-7-nitroindole (20c).** Yield 35%; mp 172 °C; TLC (dichloromethane/methanol 95:5) $R_f = 0.18$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 11.05 (1H, br s, indole proton), 7.91 and 7.01 (2H, 2d, $J$ = 9 Hz, 4,5-protons), 5.07 and 4.76 (2H, 2t, $J$ = 6.0 Hz, hydroxyls of 2,3-hydroxymethyls), 4.63 and 4.62 (4H, 2d, $J$ = 6.0 Hz, methylenes of 2,3-hydroxymethyls), and 3.94 (3H, s, 6-methoxyl); IR (KBr pellet) 3443, 3221, 3013, 2924, 2881, 2852, 1629, 1570, 1510, 1421, 1345, 1300, 1253, 1199, 1091, 1012, 958, 846, and 894 $cm^{-1}$; MS (EI) 252 ($M^+$), 235 ($M^+ - OH$), 221, 205, and 158. Anal. Calcd ($C_{11}H_{12}N_2O_5$): C, H, N.

**2,3-Di(hydroxymethyl)indole-4,7-diones (21).** A solution of 0.2 mmol of **20** in 15 mL of methanol and 60 mL of water in the presence of 80 mg of 5% Pd on carbon was hydrogenated at 50 psi $H_2$ for 25 min. The catalyst was filtered off through Celite, and the filtrate was combined with a solution consisting of 50 mg of $KH_2PO_4$ and 100 mg of Fremy salt in 50 mL of $H_2O$. The solution was stirred at room temperature for 6 h and then extracted six times with 50 mL portions of ethyl acetate. The combined extracts were dried over $Na_2SO_4$ and vacuum-dried to a residue that was purified by flash chromatography with ethyl acetate as the eluent. The product was recrystallized from ethyl acetate and hexane.

**2,3-Di(hydroxymethyl)-5-methoxyindole-4,7-dione (21a).** Yield 15%; mp 208 °C; TLC (ethyl acetate) $R_f = 0.20$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 12.8 (1H, br s, indole proton), 5.76 (1H, s, 6-proton), 4.99 and 4.63 (2H, 2t, $J$ = 5.4 Hz, hydroxyls of 2,3-hydroxymethyls), 4.58 and 4.46 (4H, 2d, $J$ = 5.4 Hz, methylenes of 2,3-hydroxymethyls), and 3.95 (3H, s, 5-methoxy); IR (KBr pellet) 3425, 2926, 2856, 1647, 1599, 1533, 1465, 1398, 1340, 1249, 1167, 1122, 1037, and 880 $cm^{-1}$; MS (EI) 237 ($M^+$), 219 ($M^+ - H_2O$), 204, 190, 176, 162, 148, 134, and 106. Anal. Calcd ($C_{11}H_{11}NO_5$): C, H, N.

**2,3-Di(hydroxymethyl)-5-methoxy-6-methylindole-4,7-dione (21b).** Yield 26%; mp 178 °C; TLC (ethyl acetate/methanol 95:5) $R_f = 0.48$; $^1H$ NMR ($CDCl_3$) $\delta$ 9.43 (1H, br s, indole proton), 4.75 and 4.67 (4H, 2d, $J$ = 6.0 Hz, methylenes of 2,3-hydroxymethyls), 4.02 (3H, s, 5-methoxy), 3.82 and 2.20 (2H, 2t, $J$ = 6.0 Hz, hydroxyls of 2,3-hydroxymethyls), and 1.98 (3H, s, 6-methyl); IR (KBr pellet) 3437, 3227, 3122, 2961, 2852, 1693, 1635, 1604, 1492, 1444, 1375, 1303, 1240, 1188, 1103, 1051, 1024, and 929 $cm^{-1}$. MS (EI) 251 ($M^+$), 233 ($M^+ - H_2O$), 218, 104, 190, 176, 162, 146, and 134. Anal. Calcd ($C_{12}H_{13}NO_5$): C, H, N.

**2,3-Di(hydroxymethyl)-6-methoxyindole-4,7-dione (21c).** Yield 10%; mp 217 °C; TLC (dichloromethane/methanol 90:10) $R_f = 0.30$; $^1H$ NMR (DMSO-$d_6$) $\delta$ 12.6 (1H, br s, indole proton), 5.75 (1H, s, 5-proton), 5.07 and 4.73 (2H, 2t, $J$ = 6.0 Hz, hydroxyls of 2,3-hydroxymethyls), 4.60 and 4.47 (4H, 2d, $J$ = 6.0 Hz, methylenes of 2,3-hydroxymethyls), and 3.75 (3H, s, 6-methoxy). IR (KBr pellet) 3433, 3173, 3117, 2951, 2876, 1670, 1633, 1597, 1248, 1112, 997, and 852 $cm^{-1}$; MS (EI) 237 ($M^+$), 219 ($M^+ - H_2O$), 204, 190, 176, 162, 148, and 134. Anal. Calcd ($C_{11}H_{11}NO_5$): C, H, N.

**Aziridinyl-2,3-di(hydroxymethyl)indole-4,7-diones (6, 7, 9a).** To 0.2 mmol of **21** in 20 mL of methanol was added 0.6

mL of ethylenimine, and the resulting mixture was stirred at room temperature for 10 h. The solvent was removed and the residue was purified by flash chromatography with 20% acetone in ethyl acetate as the eluent. The product was recrystallized from ethyl acetate and hexane.

**5-Aziridinyl-2,3-di(hydroxymethyl)indole-4,7-dione (6a).** Yield 74%; mp 167 °C; TLC (ethyl acetate/methanol 95:5) $R_f$ = 0.35; $^1$H NMR (DMSO-$d_6$) $\delta$ 12.3 (1H, br s, indole proton), 5.78 (1H, s, 6-proton), 4.99 and 4.65 (2H, 2t, $J$ = 5.7 Hz, hydroxyls of 2,3-hydroxymethyls), 4.60 and 4.44 (4H, 2d, $J$ = 5.7 Hz, methylenes of 2,3-hydroxymethyls), and 2.14 (4H, s, 5-aziridinyl); IR (KBr pellet) 3445, 3209, 3047, 2926, 2856, 1631, 1579, 1498, 1465, 1361, 1271, 1159, 1095, 1003, 846, and 804 cm$^{-1}$; MS (EI) 248 ($M^+$), 230 ($M^+ - H_2O$), 215, 201, 189, and 174. Anal. Calcd ($C_{12}H_{12}N_2O_4$): C, H, N.

**5-Aziridinyl-2,3-di(hydroxymethyl)-6-methylindole-4,7-dione (7a).** Yield 75%; mp 165 °C; TLC (ethyl acetate/methanol 95:5) $R_f$ = 0.38; $^1$H NMR (DMSO-$d_6$) $\delta$ 12.3 (1H, br s, indole proton), 4.94 and 4.62 (2H, 2t, $J$ = 5.1 Hz, hydroxyls of 2,3-hydroxymethyls), 4.58 and 4.40 (4H, 2d, $J$ = 5.1 Hz, methylenes of 2,3-hydroxymethyls), 2.23 (4H, s, 5-aziridinyl), and 1.92 (3H, s, 6-methyl); IR (KBr pellet) 3404, 3209, 2949, 2879, 1626, 1587, 1562, 1500, 1458, 1377, 1350, 1248, 1188, 1149, 1103, 1020, 995, and 819 cm$^{-1}$; MS (EI) 262 ($M^+$), 247 ($M^+ - CH_3$), 244 ($M^+ - H_2O$), 229, 215, and 203. Anal. Calcd ($C_{13}H_{14}N_2O_4$): C, H, N.

**6-Aziridinyl-2,3-di(hydroxymethyl)indole-4,7-dione (9a).** Yield 52%; mp 175 °C; TLC (ethyl acetate/methanol 95:5) $R_f$ = 0.38; $^1$H NMR (DMSO-$d_6$) $\delta$ 12.4 (1H, br s, indole proton), 5.76 (1H, s, 5-proton), 5.04 and 4.72 (2H, 2t, $J$ = 5.7 Hz, hydroxyls of 2,3-hydroxymethyls), 4.57 and 4.46 (4H, 2d, $J$ = 5.7 Hz, methylenes of 2,3-hydroxymethyls), and 2.14 (4H, s, 6-aziridinyl); IR (KBr pellet) 3394, 3232, 3038, 2928, 2874, 1660, 1610, 1500, 1392, 1275, 1132, 1020, and 879 cm$^{-1}$; MS (EI) 248 ($M^+$), 230, 202, 189, 174, 160, and 146. Anal. Calcd ($C_{12}H_{12}N_2O_4$): C, H, N.

**5-Aziridinyl-2,3-di(acetoxymethyl)indole-4,7-dione (6e).** To a solution of 0.2 mmol of **6a** in 5 mL of $CH_2Cl_2$ and 1 mL of acetone containing 50 mg of DMAP was added 100 $\mu$L of acetic anhydride. The solution was stirred at room temperature for 5 min and directly flash-chromatographed with ethyl acetate as the eluent. The eluted solution containing the product was washed with $NaHCO_3$, dried over $Na_2SO_4$, and vacuum-dried. The product was recrystallized from ethyl acetate and hexane. Yield 82%; mp 167 °C; TLC (dichloromethane/methanol 95:5) $R_f$ = 0.6; $^1$H NMR ($CDCl_3$) $\delta$ 8.8 (1H, br s, indole proton), 5.72 (1H, s, 6-proton), 4.63 and 4.46 (4H, 2s, methylenes of 2,3-hydroxymethyls), 2.24 (4H, s, 5-aziridinyl), 2.10 and 2.08 (6H, 2s, 2,3-acetyl methyls); IR (KBr pellet) 3447, 3420, 3194, 3047, 2966, 1739, 1674, 1628, 1570, 1506, 1475, 1377, 1246, 1165, 1145, 1112, 1033, 977, 949, 850, and 804 cm$^{-1}$; MS (EI) 332 ($M^+$), 290 ($M^+$ − acetyl), 279, 261, 243, 226, 217, and 188. Anal. Calcd ($C_{16}H_{16}N_2O_6$): C, H, N.

**2-(Ethoxycarbonyl)-3-formyl-1,6-dimethyl-5-methoxy-4-nitroindole (22).** To 0.2 mmol of **19b** in 20 mL of acetone with 500 mg of KOH was added 1.0 mL of MeI; the mixture was stirred at room temperature for 1 h, neutralized with HCl, and extracted four times with 30 mL portions of $CH_2Cl_2$. The combined extracts was dried with $Na_2SO_4$ and dried, followed by chromatography with $CH_2Cl_2$ as the eluent. The product was crystallized from $CH_2Cl_2$ and hexane. Yield 55%; mp 148 °C; TLC ($CHCl_3$) $R_f$ = 0.55; $^1$H NMR ($CDCl_3$) $\delta$ 10.42 (1H, s, 3-formyl), 7.40 (H, s, 7-proton), 4.52 (2H, q, $J$ = 7.2 Hz, methylene of ethoxy), 4.06 and 3.91 (6H, 2s, 1-methyl and 5-methoxyl), 2.51 (3H, s, 6-methyl), and 1.46 (3H, t, $J$ = 7.2 Hz, methyl of ethoxy); IR (KBr pellet) 3435, 2999, 2930, 1712, 1666, 1545, 1475, 1404, 1238, 1161, 1031, 979, 908, and 779 cm$^{-1}$; MS (EI) 320 ($M^+$), 307, 287, 275, 244, and 216. Anal. Calcd ($C_{15}H_{16}N_2O_6$): C, H, N.

**2,3-Di(hydroxymethyl)-1,6-dimethyl-5-methoxy-4-nitroindole (23).** Synthesis of this compound followed the same procedure as the synthesis of **21**. Yield 55%; mp 197 °C; TLC (EtOAc/MeOH 95:5) $R_f$ = 0.65; $^1$H NMR (DMSO-$d_6$) $\delta$ 7.58 (1H, 1s, 7-proton), 5.13 and 4.44 (2H, 2t, $J$ = 5.4 Hz, hydroxyls of 2,3-hydroxymethyls), 4.61 and 4.42 (4H, 2d, $J$ = 5.4 Hz, methylenes of 2,3-hydroxymethyls), 3.77 and 3.75 (6H, 2s, 1-methyl and 5-methoxy), and 2.40 (3H, s, 6-methyl). IR (KBr pellet) 3447, 2945, 2891, 1637, 1525, 1477, 1377, 1329, 1174, 1124, 993, 854, and 765 cm$^{-1}$; MS (EI) 280 ($M^+$), 262, 245, 229, 215, 186, and 173. Anal. Calcd ($C_{13}H_{16}N_2O_5$): C, H, N.

**2,3-Di(hydroxymethyl)-1,6-dimethyl-5-methoxyindole-4,7-dione (24).** Yield 16%; mp 160 °C; TLC (ethyl acetate/methanol 95:5) $R_f$ = 0.56; $^1$H NMR (DMSO-$d_6$) $\delta$ 5.13 and 4.67 (2H, 2t, $J$ = 5.4 Hz, hydroxyls of 2,3-hydroxymethyls), 4.59 and 4.54 (4H, 2d, $J$ = 5.4 Hz, methylenes of 2,3-hydroxymethyls), 3.90 and 3.87 (6H, 2s, 1-methyl and 5-methoxy), and 1.85 (3H, s, 6-methyl); IR (KBr pellet) 3335, 3194, 2943, 2852, 1682, 1643, 1506, 1464, 1313, 1122, 1008, 989, 900, and 740 cm$^{-1}$; MS (EI) 265 ($M^+$), 247 ($M^+ - H_2O$), 232, 218, 204, 190, 176, 148, 132, and 120. Anal. Calcd ($C_{13}H_{15}NO_5$): C, H, N.

**5-Aziridinyl-2,3-di(hydroxymethyl)-1,6-dimethylindole-4,7-dione (7c).** Yield 50%; mp 165 °C; TLC (ethyl acetate/methanol 95:5) $R_f$ = 0.48; $^1$H NMR (DMSO-$d_6$) $\delta$ 5.08 and 4.59 (2H, 2t, $J$ = 5.4 Hz, hydroxyls of 2,3-hydroxymethyls), 4.58 and 4.52 (4H, 2d, $J$ = 5.4 Hz, methylenes of 2,3-hydroxymethyls), 3.89 (3H, s, 1-methyl), 2.23 (4H, s, 5-aziridinyl), and 1.93 (3H, s, 6-methyl); IR (KBr pellet) 3435, 3335, 3057, 2958, 2852, 1666, 1631, 1508, 1336, 1151, 1043, 989, and 875 cm$^{-1}$; MS (EI) 276 ($M^+$), 258, 243, 229, 214, 202, and 188. Anal. Calcd ($C_{14}H_{16}N_2O_4$): C, H, N.

**2-(Ethoxycarbonyl)-3-(hydroxymethyl)nitroindoles (25).** To a solution of 2 mmol of **19** in 100 mL of MeOH was added 1 g of $NaBH_4$, and the solution was stirred at room temperature for 20 min, followed by the addition of 200 mL of water. The solution was then extracted five times with 100 mL portions of chloroform. The extract was dried over $Na_2SO_4$ and vacuum-dried to a residue. The product was crystallized from $CH_2Cl_2$ and hexane.

**2-(Ethoxycarbonyl)-3-(hydroxymethyl)-5-methoxy-4-nitroindole (25a).** Yield 90%; mp 165 °C; TLC (chloroform/acetone 80:20) $R_f$ = 0.40; $^1$H NMR ($CDCl_3$) $\delta$ 10.45 (1H, br s, indole proton), 7.54 and 7.28 (2H, 2d, $J$ = 8.7 Hz, 6,7-protons), 5.12 (2H, d, $J$ = 6.6 Hz, 3-methylene), 5.00 (1H, t, $J$ = 6.6 Hz, 3-hydroxyl), 4.54 (2H, q, $J$ = 7.2 Hz, methylene of ethoxy), 4.01 (3H, s, 5-methoxy), and 1.48 (3H, t, $J$ = 7.2 Hz, methyl of ethoxy); IR (KBr pellet) 3562, 3452, 3313, 2993, 2949, 2849, 1682, 1533, 1475, 1384, 1255, 1180, 1120, 1012, and 827 cm$^{-1}$; MS (EI) 294 ($M^+$), 276, 248, 231, 212, and 186. Anal. Calcd ($C_{13}H_{14}N_2O_6$): C, H, N.

**2-(Ethoxycarbonyl)-3-(hydroxymethyl)-5-methoxy-6-methyl-4-nitroindole (25b).** Yield 90%; mp 147 °C; TLC (chloroform/acetone 80:20) $R_f$ = 0.50; $^1$H NMR ($CDCl_3$) $\delta$ 8.91 (1H, br s, indole proton), 7.35 (1H, s, 7-proton), 4.97 (2H, d, $J$ = 6.3 Hz, 3-methylene), 4.45 (2H, q, $J$ = 7.2 Hz, methylene of ethoxy), 3.90 (3H, s, 5-methoxy), 2.49 (1H, t, $J$ = 6.3 Hz, 3-hydroxyl), 2.48 (3H, s, 6-methyl), and 1.44 (3H, t, $J$ = 7.2 Hz, methyl of ethoxy); IR (KBr pellet) 3431, 3238, 3001, 2957, 2889, 1718, 1701, 1527, 1465, 1261, 1188, 1155, 1006, and 779 cm$^{-1}$; MS (EI) 308 ($M^+$), 291 ($M^+$ − OH), 279, 261, 245, 226, 215, 200, 187, and 174. Anal. Calcd ($C_{14}H_{16}N_2O_6$): C, H, N.

**3-(Hydroxymethyl)-6-methoxy-2-(methoxycarbonyl)-7-nitroindole (25c).** Yield 93%; mp 150 °C; TLC (chloroform/methanol 90:10) $R_f$ = 0.35; $^1$H NMR ($CDCl_3$) $\delta$ 10.25 (1H, br s, indole proton), 8.03 and 7.01 (2H, 2d, $J$ = 9.3 Hz, 4,5-protons), 5.07 (2H, d, $J$ = 6.9 Hz, 3-methylene), 4.90 (1H, t, $J$ = 6.9 Hz, 3-hydroxyl), 4.10 and 4.02 (6H, 2s, 6-methoxy and ester methyl); IR (KBr pellet) 3433, 2920, 2850, 1736, 1629, 1560, 1521, 1464, 1253, 1180, 1091, 970, and 804 cm$^{-1}$; MS (EI) 280 ($M^+$), 263 ($M^+$ − OH), 247, 231, 219, 200, 172, and 157. Anal. Calcd ($C_{12}H_{12}N_2O_6$): C, H, N.

**2-(Ethoxycarbonyl)-3-(hydroxymethyl)indole-4,7-diones (26).** A solution of 2 mmol of **25** in 80 mL of methanol with 300 mg of 5% Pd on carbon was reduced under 50 psi $H_2$ for 25 min, and the catalyst was filtered off through Celite. The solvent was vacuum-dried and the residue was dissolved in 5 mL of acetone, which was then mixed with a solution of 800 mg of $KH_2PO_4$ and 1.6 g of Fremy salt in 200 mL of $H_2O$.

The solution was stirred at room temperature for 5h and extracted five times with 200 mL of methylene chloride. The extract was dried and purified by flash chromatography with 10% acetone in methylene chloride as the eluent. The product was precipitated from $CH_2Cl_2$ and hexane.

**2-(Ethoxycarbonyl)-3-(hydroxymethyl)-5-methoxyindole-4,7-dione (26a).** Yield 64%; mp 213 °C; TLC (chloroform/methanol 90:10) $R_f = 0.30$; $^1$H NMR ($CDCl_3$) $\delta$ 9.77 (1H, br s, indole proton), 5.85 (1H, s, 6-proton), 5.07 (2H, d, $J = 7.2$ Hz, 3-methylene), 4.41 (2H, q, $J = 7.2$ Hz, methylene of ethoxy), 4.22 (1H, t, $J = 7.2$ Hz, 3-hydroxyl), 3.89 (3H, s, 5-methoxy), and 1.41 (3H, t, $J = 7.2$ Hz, methyl of ethoxy); IR (KBr pellet) 3433, 3122, 3072, 2989, 2924, 2785, 1712, 1649, 1597, 1560, 1487, 1467, 1340, 1280, 1161, 1031, and 844 cm$^{-1}$; MS (EI) 279 ($M^+$), 261, 250, 233, 218, 205, 190, and 177. Anal. Calcd ($C_{13}H_{13}NO_6$): C, H, N.

**2-(Ethylcarboxyl)-3-(hydroxymethyl)-5-methoxy-6-methylindole-4,7-dione (26b).** Yield 41%; mp 183 °C; TLC ($CHCl_3$/MeOH 90:10) $R_f = 0.45$; $^1$H NMR ($CDCl_3$) $\delta$ 9.75 (1H, br s, indole proton), 5.05 (2H, d, $J = 7.2$ Hz, 3-methylene), 4.41 (2H, q, $J = 7.2$ Hz, methylene of ethoxy), 4.34 (1H, t, $J = 7.2$ Hz, 3-hydroxyl), 4.08 (3H, s, 5-methoxy), 2.01 (3H, s, 6-methyl), and 1.41 (3H, t, $J = 7.2$ Hz, methyl of ethoxy); IR (KBr pellet) 3435, 3211, 3063, 2991, 2920, 2852, 1710, 1647, 1606, 1496, 1294, 1232, 1112, 1074, 1033, and 866 cm$^{-1}$; MS (EI) 293 ($M^+$), 275, 264, 247, 232, 219, 204, and 186. Anal. Calcd ($C_{14}H_{15}NO_6$): C, H, N.

**3-(Hydroxymethyl)-6-methoxy-2-(methoxycarbonyl)-indole-4,7-dione (26c).** Yield 42%; mp 150 °C; TLC (chloroform/methanol 90:10) $R_f = 0.35$; $^1$H NMR ($CDCl_3$) $\delta$ 9.70 (1H, br s, indole proton), 5.91 (1H, s, 5-proton), 5.04 (2H, d, $J = 7.8$ Hz, 3-methylene), 4.59 (1H, t, $J = 7.8$ Hz, 3-hydroxyl), 3.96 and 3.88 (6H, 2s, 6-methoxy and ester methyl); IR (KBr pellet) 3431, 3238, 3001, 2957, 2889, 1718, 1701, 1527, 1465, 1398, 1261, 1188, 1155, 1006, and 779 cm$^{-1}$; MS (EI) 265 ($M^+$), 250 ($M^+ - Me$), 233, 218, 205, 190, 162, 148, and 120. Anal. Calcd ($C_{12}H_{11}NO_6$): C, H, N.

**Aziridinyl-2-(ethoxycarbonyl)-3-(hydroxymethyl)indole-4,7-diones.** To a solution of 1 mmol of product above in 50 mL of methanol was added 1 mL of aziridine, and the reaction was stirred at room temperature for 20 h. The solvent was evaporated and the residue was purified by flash chromatography on silica gel with 10% acetone in $CH_2Cl_2$ as the eluent. The product was recrystallized from $CH_2Cl_2$ and hexane.

**5-Aziridinyl-2-(ethylcarboxyl)-3-(hydroxymethyl)indole-4,7-dione (6c).** Yield 86%; mp 170 °C; TLC (chloroform/methanol 90:10) $R_f = 0.32$; $^1$H NMR ($CDCl_3$) $\delta$ 9.78 (1H, br s, indole proton), 5.96 (1H, s, 6-proton), 5.07 (2H, d, $J = 7.2$ Hz, 3-methylene), 4.41 (2H, q, $J = 7.2$ Hz, methylene of ethoxy), 4.34 (1H, t, $J = 7.2$ Hz, 3-hydroxyl), 2.30 (4H, s, 5-aziridinyl), and 1.41 (3H, t, $J = 7.2$ Hz, methyl of ethoxy); IR (KBr pellet) 3450, 3259, 3024, 2899, 2862, 1703, 1656, 1581, 1550, 1491, 1363, 1278, 1240, 1149, 1028, and 842 cm$^{-1}$; MS (EI) 290 ($M^+$), 279, 260, 244, 233, 226, 215, 199, 188, and 171. Anal. Calcd ($C_{14}H_{14}N_2O_5$): C, H, N.

**5-Aziridinyl-2-(ethylcarboxyl)-3-(hydroxymethyl)-6-methylindole-4,7-dione (7b).** Yield 76%; mp 197 °C; TLC (chloroform/methanol 90:10) $R_f = 0.40$; $^1$H NMR ($CDCl_3$) $\delta$ 9.61 (1H, br s, indole proton), 5.04 (2H, d, $J = 7.2$ Hz, 3-methylene), 4.45 (2H, q, $J = 7.2$ Hz, methylene of ethoxy), 4.38 (1H, t, $J = 7.2$ Hz, 3-hydroxyl), 2.39 (4H, s, 5-aziridinyl), 2.10 (3H, s, 6-methyl), and 1.41 (3H, t, $J = 7.2$ Hz, methyl of ethoxy); IR (KBr pellet) 3448, 3252, 3088, 2997, 2922, 2854, 1709, 1651, 1587, 1550, 1492, 1377, 1209, 1261, 1176, 1147, 1035, 997, 962, and 871 cm$^{-1}$; MS (EI) 304 ($M^+$), 286 ($M^+ - H_2O$), 275, 258, 240, 229, 212, and 202. Anal. Calcd ($C_{15}H_{16}N_2O_5$): C, H, N.

**6-Aziridinyl-3-(hydroxymethyl)-2-(methylcarboxyl)indole-4,7-dione (9b).** Yield 52%; mp 258 °C; TLC (chloroform/methanol 90:10) $R_f = 0.45$; $^1$H NMR ($CDCl_3$) $\delta$ 9.78 (1H, br s, indole proton), 6.02 (1H, s, 5-proton), 5.02 (2H, d, $J = 6.9$ Hz, 3-methylene), 4.59 (1H, t, $J = 6.9$ Hz, 3-hydroxyl), 3.93 (3H, s, ester methyl), and 2.28 (4H, s, 6-aziridinyl); IR (KBr pellet) 3420, 3257, 3074, 2997, 2958, 2922, 2852, 1705, 1674, 1626, 1587, 1545, 1267, 1157, 1078, 1030, 952, 868, and 804 cm$^{-1}$; MS (EI) 276($M^+$), 244 ($M^+ - CO$), 229, 216, 188, 160, and 132. Anal. Calcd ($C_{13}H_{12}N_2O_5$): C, H, N.

**3-(Acetoxymethyl)-5-aziridinyl-2-(ethoxycarbonyl)indole-4,7-dione (6d).** Synthesis of this compound followed the procedure described for the acetylation of **6e**. Yield 73%; mp 185 °C; TLC (chloroform/acetone 90:10) $R_f = 0.45$; $^1$H NMR ($CDCl_3$) $\delta$ 9.76 (1H, br s, indole proton), 5.96 (1H, s, 6-proton), 5.54 (2H, s, 3-methylene), 4.42 (2H, q, $J = 7.2$ Hz, methylene of ethoxy), 2.29 (4H, s, 5-aziridinyl), 2.07 (3H, s, 3-acetoxymethyl), and 1.39 (3H, t, $J = 7.2$ Hz, methyl of ethoxy); IR (KBr pellet) 3445, 3234, 2995, 2920, 2852, 1726, 1701, 1676, 1583, 1556, 1500, 1363, 1280, 1244, 1153, 1024, and 819 cm$^{-1}$; MS (EI) 332 ($M^+$), 318, 290, 279, 260, 244, and 217. Anal. Calcd ($C_{16}H_{16}N_2O_6$): C, H, N.

**2-(Ethoxycarbonyl)-5-methoxy-3-methyl-4-nitroindole (27a).** To 1.17 g (5 mmol) of 2-(ethylcarboxyl)-5-methoxy-3-methylindole[46] in 180 mL of $CH_2Cl_2$, cooled by a dry ice bath at −20 °C, was added 0.5 mL of 70% $HNO_3$, and the solution was stirred for 10 min. The solution was then neutralized with $NaHCO_3$ aqueous solution and extracted four times with 50 mL portions of methylene chloride. The extracts were dried over $Na_2SO_4$ and vacuum-dried to a residue that was purified by flash chromotography on silica gel with $CH_2Cl_2$ as the eluent. Yield 880 mg (69%); mp 175−177 °C; TLC ($CHCl_3$) $R_f = 0.5$; $^1$H NMR($CDCl_3$) $\delta$ 8.81(1H, br s, indole proton), 7.45 and 7.12 (2H, d, $J = 9$ Hz, 6,7-protons), 4.44 (2H, q, $J = 7.5$ Hz, methylene of ethoxy), 3.94 (3H, s, 5-methoxy), 2.46 (3H, s, 3-methyl), and 1.43 (3H, t, $J = 7.5$, methyl of ethoxy); IR (KBr pellet) 3423, 3340, 2933, 2847, 1680, 1628, 1541, 1475, 1384, 1251, 1182, 1120, 1049, 1016, and 796 cm$^{-1}$; MS (EI) 278 ($M^+$), 261 ($M^+ - OH$), 249, 232, 215, 203, 187, 174, and 156. Anal. Calcd ($C_{13}H_{14}N_2O_5$): C, H, N.

**2-(Ethoxycarbonyl)-1,3-dimethyl-4-nitroindoles (28).** To 2 mmol of **27** in 20 mL of acetone containing 400 mg of KOH was added 1.0 mL of MeI, and the mixture was stirred at room temperature for 1 h. The reaction mixture was then neutralized with HCl and extracted four times with 30 mL portions of $CH_2Cl_2$. The combined extracts were dried over $Na_2SO_4$ and evaporated to a residue that was purified by chromatography on silica gel with $CH_2Cl_2$ as the eluent. The purified product was recrystallized from $CH_2Cl_2$ and hexane.

**2-(Ethoxycarbonyl)-5-methoxy-1,3-dimethyl-4-nitroindole (28a).** Yield ∼100%; mp 120−122 °C; TLC ($CHCl_3$) $R_f = 0.45$; $^1$H NMR ($CDCl_3$) $\delta$ 7.42 and 7.13 (2H, d, $J = 9.3$ Hz, 6,7-protons), 4.42 (2H, q, $J = 7.2$ Hz, 2-methylene of ethyl), 3.98 and 3.94 (6H, 2s, 1-methyl and 5-methoxy), 2.40 (3H, s, 3-methyl), and 1.43 (3H, t, $J = 7.2$, 2-methyl of ethyl); IR (KBr pellet) 3441, 3003, 2947, 2912, 1709, 1637, 1527, 1465, 1375, 1236, 1165, 1112, 1045, 912, and 794 cm$^{-1}$; MS (EI) 292 ($M^+$), 275 ($M^+ - OH$), 263, 244, 216, 188, 163, and 149. Anal. Calcd ($C_{14}H_{16}N_2O_5$): C, H, N.

**2-(Ethoxycarbonyl)-1,3,5-trimethyl-4-nitroindole (28b).** Yield 90%; mp 65−68 °C; TLC ($CHCl_3$) $R_f = 0.50$; $^1$H NMR ($CDCl_3$) $\delta$ 7.38 and 7.18 (2H, d, $J = 9$ Hz, 6,7-protons), 4.46 (2H, q, $J = 7.5$ Hz, 2-methylene of ethoxy), 3.99 (3H, s, 1-methyl), 2.42 and 2.39 (6H, 2s, 3,5-methyls), and 1.43 (3H, t, $J = 7.5$, 2-methyl of ethoxy); IR (KBr pellet) 3338, 2978, 2928, 2854, 1699, 1521, 1373, 1290, 1251, 1155, 1085, 1014, and 802 cm$^{-1}$; MS (EI) 276 ($M^+$), 259 ($M^+ - OH$), 247, 229, 213, 202, 174, 157, 142, 131, and 115. Anal. Calcd ($C_{14}H_{16}N_2O_4$): C, H, N.

**2-(Ethoxycarbonyl)-3-(hydroxymethyl)-4-nitroindoles (29).** To 1.50 mmol of **28** in 15 mL of $CCl_4$ at reflux were added 258 mg of *N*-bromosuccinimide (NBS) and 26 mg of 2,2′-azo-bis-isobutyrylnitrile (AIBN). The reaction was refluxed for 1.5 h with additional AIBN added every 30 min (13 mg × 3). Hexane was added to the cooled reaction mixture in order to precipitate the crude product as a light yellow solid. A solution of the crude product above in 25 mL of THF, 20 mL of $H_2O$, and 4 mL of HCl was stirred at room temperature for 1.5 h. This mixture was neutralized with saturated $NaHCO_3$ solution and then extracted three times with 50 mL portions of $CH_2$-

$Cl_2$. The extracts were dried over $Na_2SO_4$ and concentrated to a residue. The product was recrystallized from $CH_2Cl_2$ and hexane.

**2-(Ethoxycarbonyl)-3-(hydroxymethyl)-5-methoxy-1-methyl-4-nitroindole (29a).** Yield 34%; mp 120−122 °C; TLC ($CHCl_3$/MeOH 9:1) $R_f = 0.55$; $^1$H NMR ($CDCl_3$) $\delta$ 7.48 and 7.17 (2H, d, $J$ = 8.7 Hz, 6,7-protons), 4.87 (2H, d, $J$ = 6.6 Hz, 3-hydroxymethyl), 4.47 (2H, q, $J$ = 7.2 Hz, 2-methylene of ethyl), 4.01 and 3.96 (6H, 2s, 1-methyl and 5-methoxyl), 2.31 (1H, t, $J$ = 6.6 Hz, 3-hydroxy), and 1.46 (3H, t, $J$ = 7.2, 2-methyl of ethyl); IR (KBr pellet) 3497, 3412, 3232, 2947, 1685, 1621, 1609, 1534, 1287, 1174, 1000, and 823 cm$^{-1}$; MS (EI) 308 ($M^+$), 291 ($M^+ - OH$), 261, 244, 235, 216, 201, 177, 163, 149, and 105. Anal. Calcd ($C_{14}H_{16}N_2O_6$): C, H, N.

**2-(Ethoxycarbonyl)-3-(hydroxymethyl)-5-methyl-4-nitroindole (29b).** Yield 80%; mp 134−136 °C; TLC (chloroform/methanol 9:1) $R_f$ = 0.45; $^1$H NMR ($CDCl_3$) $\delta$ 9.08 (1H, br s, indole proton), 7.46 and 7.23 (2H, d, $J$ = 9 Hz, 6,7-protons), 5.01 (2H, d, $J$ = 6 Hz, 3-hydroxymethyl), 4.46 (2H, q, $J$ = 7.5 Hz, 2-methylene of ethoxy), 2.48 (3H, s, 5-methyl), 2.35 (1H, t, $J$ = 6 Hz, 3-hydroxy), and 1.47 (3H, t, $J$ = 7.5, methyl of ethoxy); IR (KBr pellet) 3447, 3194, 2980, 2928, 1718, 1678, 1527, 1465, 1365, 1265, 1180, 1116, 989, and 808 cm$^{-1}$; MS (EI) 278 ($M^+$), 261 ($M^+ - OH$), 245, 231, 215, 186, 171, and 149. Anal. Calcd ($C_{13}H_{14}N_2O_5$): C, H, N.

**2-(Ethoxycarbonyl)-3-(hydroxymethyl)-1,5-dimethyl-4-nitroindole (29c).** Yield 31%; mp 113−115 °C; TLC (chloroform/methanol 9:1) $R_f$ = 0.50; $^1$H NMR ($CDCl_3$) $\delta$ 7.43 and 7.23 (2H, d, $J$ = 9 Hz, 6,7-protons), 4.88 (2H, d, $J$ = 6 Hz, 3-hydroxymethyl), 4.48 (2H, q, $J$ = 7.5 Hz, methylene of ethoxy), 4.01 and 2.48 (6H, 2s, 1,5-methyl), 2.35 (1H, t, $J$ = 6 Hz, 3-hydroxy), and 1.46 (3H, t, $J$ = 7.5 Hz, methyl of ethoxy); IR (KBr pellet) 3489, 3425, 3244, 2928, 1685, 1637, 1618, 1525, 1263, 1168, 1014, and 810 cm$^{-1}$; MS (EI) 292 ($M^+$), 275 ($M^+ - OH$), 245, 228, 219, 200, 185, and 172. Anal. Calcd ($C_{14}H_{16}N_2O_5$): C, H, N.

**2,3-Di(hydroxymethyl)-4-nitroindoles (30).** To a solution of 0.3 mmol of **29** in 4 mL of dry THF cooled to −10 °C was added 60 mg of LAH, and the solution was stirred for 5 min. The unreacted LAH was quenched by gradually adding 5 mL of ethyl acetate followed by 2 mL of $H_2O$. The ethyl acetate layer was separated and the aqueous layer was washed five times with 20 mL portions of ethyl acetate. The combined extracts were dried over $Na_2SO_4$ and concentrated to a residue. Purification was carried out by flash chromatography on silica gel with ethyl acetate as the eluent.

**2,3-Di(hydroxymethyl)-5-methoxy-1-methyl-4-nitroindole (30a).** Yield 66%; mp 163−165 °C; TLC (chloroform/methanol 9:1) $R_f$ = 0.35; $^1$H NMR ($CDCl_3$) $\delta$ 7.42 and 7.02 (2H, 2d, $J$ = 8.7 Hz, 6,7-protons), 4.85 and 4.69 (4H, 2d, $J$ = 6 Hz, methylenes of 2,3-hydroxymethyls), 3.95 and 3.84 (6H, 2s, 1-methyl and 5-methoxy), 2.33 and 2.19 (2H, 2t, $J$ = 6 Hz, hydroxys of 2,3-hydroxymethyls); IR (KBr pellet) 3425, 2924, 2847, 1628,1521, 1475, 1421, 1365, 1300, 1263, 1105, 1062, 1001, 974, and 891 cm$^{-1}$; MS (EI) 266 ($M^+$), 249 ($M^+ - OH$), 237, 208, 194, 189,165, and 121. Anal. Calcd ($C_{12}H_{14}N_2O_5$): C, H, N.

**2,3-Di(hydroxymethyl)-5-methyl-4-nitroindole (30b).** Yield 60%; mp 132−134 °C; TLC (chloroform/methanol 9:1) $R_f$ = 0.3; $^1$H NMR (DMSO-$d_6$) $\delta$ 11.5 (1H, br s, indole proton), 7.45 and 7.03 (2H, d, $J$ = 9 Hz, 6,7-protons), 5.31 and 4.38 (2H, 2t, $J$ = 6 Hz, hydroxys of 2,3-hydroxymethyls), 4.64 and 4.40 (4H, 2d, $J$ = 6 Hz, methylenes of 2,3-hydroxymethyls), and 2.34 (3H, s, 5-methyl); IR (KBr pellet) 3394, 2995, 2926, 1637, 1510, 1475, 1363, 1321, 1151, 1014, and 974 cm$^{-1}$; MS (EI) 236 ($M^+$), 219 ($M^+ - OH$), 207, 177, 163, 149, and 105. Anal. Calcd ($C_{11}H_{12}N_2O_4$): C, H, N.

**2,3-Di(hydroxymethyl)-1,5-dimethyl-4-nitroindole (30c).** Yield 59%; mp 119−121 °C; TLC (chloroform/methanol 9:1) $R_f$ = 0.34; $^1$H NMR (DMSO-$d_6$) $\delta$ 7.63 and 7.16 (2H, d, $J$ = 9 Hz, 6,7-protons), 5.18 and 4.40 (2H, 2t, $J$ = 6 Hz, hydroxys of 2,3-hydroxymethyls), 4.63 and 4.45 (4H, 2d, $J$ = 6 Hz, methylenes of 2,3-hydroxymethyls), 3.78 and 2.34 (6H, 2s, 1,5-methyls); IR (KBr pellet) 3425, 2972, 2925, 1647, 1518, 1465, 1373, 1342, 1300, 1182, 1069, 1055, 989, and 802 cm$^{-1}$; MS (EI) 250 ($M^+$), 233 ($M^+ - OH$), 221, 191, 177, 163,149, and 105. Anal. Calcd ($C_{12}H_{14}N_2O_4$): C, H, N.

**2,3-Di(hydroxymethyl)indole-4,7-diones (31).** A solution of 0.5 mmol of product above with 295 mg of 5% Pd−C in 40 mL of methanol was reduced under 50 psi $H_2$ for 30 min. The catalyst was Celite-filtered off, and the filtrate was vacuum-dried. The solid residue was dissolved in 4 mL of acetone and mixed with a solution of 250 mg of $KH_2PO_4$ and 500 mg of Fremy salt in 25 mL of water. The solution was stirred at room temperature for 4 h and extracted five times with 50 mL portions of ethyl acetate. The extracts were dried over $Na_2SO_4$ and vacuum-dried and then purified by flash column chromatography on silica gel with ethyl acetate as the eluent.

**2,3-Di(hydroxymethyl)-5-methoxy-1-methylindole-4,7-dione (31a).** Yield 25%; mp 191−193 °C; TLC (chloroform/methanol 9:1) $R_f$ = 0.45; $^1$H NMR ($CDCl_3$) $\delta$ 6.23 (1H, s, 6-proton), 4.70 and 4.65 (4H, 2d, $J$ = 6 Hz, 2,3-methylenes of hydroxymethyls), 4.11 and 1.98 (2H, 2t, $J$ = 6 Hz, hydroxys of 2,3-hydroxymethyls), 4.04 and 3.84 (6H, 2s, 1-methyl and 5-methoxy); IR (KBr pellet) 3465, 3332, 2945, 2931, 1649, 1537, 1419, 1374, 1233, 950, and 811 cm$^{-1}$; MS (EI) 251 ($M^+$), 233 ($M^+ - H_2O$), 218, 204, 190, 176, 162, and 120. Anal. Calcd ($C_{12}H_{13}NO_5$): C, H, N.

**2,3-Di(hydroxymethyl)-5-methylindole-4,7-dione (31b).** Yield 52%; mp 181−183 °C; TLC (chloroform/methanol 9:1) $R_f$ = 0.24; $^1$H NMR (DMSO-$d_6$) $\delta$ 12.4 (1H, br s, 1-indole proton), 6.44 (1H, q, $J$ = 1.5 Hz, 6-proton), 5.03 and 4.66 (2H, 2t, $J$ = 6 Hz, hydroxys of 2,3-hydroxymethyls), 4.59 and 4.46 (4H, 2d, $J$ = 6 Hz, methylenes of 2,3-hydroxymethyls), and 1.94 (3H, s, 5-methyl); IR (KBr pellet) 3423, 3101, 2953, 2930, 1707, 1523, 1465, 1384, 1253, 1159, 1109, 991, and 806 cm$^{-1}$; MS (EI) 221 ($M^+$), 203 ($M^+ - H_2O$), 190, 174, 147, 118, and 107. Anal. Calcd ($C_{11}H_{11}NO_4$): C, H, N.

**2,3-Di(hydroxymethyl)-1,5-dimethylindole-4,7-dione (31c).** Yield 32%; mp 136−138 °C; TLC (chloroform/methanol 9:1) $R_f$ = 0.50; $^1$H NMR ($CDCl_3$) $\delta$ 6.40 (1H, q, $J$ = 1.5 Hz, 6-proton), 4.72 and 4.67 (4H, 2d, $J$ = 6 Hz, methylenes of 2,3-hydroxymethyls), 4.04 and 1.94 (2H, 2t, $J$ = 6 Hz, hydroxys of 2,3-hydroxymethyls), 4.04 (3H, s, 1-methyl), and 2.34 (3H, s, 5-methyl); IR (KBr pellet) 3439, 3377, 2955, 2928, 1643, 1608, 1508, 1465, 1377, 1242, 1020, 993, and 802 cm$^{-1}$; MS (EI) 235 ($M^+$), 217($M^+ - H_2O$), 202, 188, 174, 161, 146, and 133. Anal. Calcd ($C_{12}H_{13}NO_4$): C, H, N.

**5-Aziridinyl-2,3-di(hydroxymethyl)-1-methylindole-4,7-dione (6b).** Yield 87%; mp 145−147 °C; TLC (chloroform/methanol 9:1) $R_f$ = 0.40; $^1$H NMR ($CDCl_3$) $\delta$ 5.82 (1H, s, 6-hydrogen), 4.38 and 2.22 (2H, 2t, $J$ = 5.4 Hz, hydroxys of 2,3-hydroxymethyls), 4.71 and 4.66 (4H, 2d, $J$ = 5.4 Hz, methylenes of 2,3-hydroxymethyls), 4.01 (3H, s, 1-methyl), and 2.22 (4H, s, 5-aziridinyl); IR (KBr pellet) 3539, 3362, 2954, 2919, 1683, 1623, 1514, 1472, 1376, 1250, 1031, and 810 cm$^{-1}$; MS (EI) 262 ($M^+$), 244 ($M^+ - H_2O$), 229, 215, 201, 169, 159, and 146. Anal. Calcd ($C_{13}H_{14}N_2O_4$): C, H, N.

**6-Aziridinyl-2,3-di(hydroxymethyl)-5-methylindole-4,7-dione (8a).** Yield 33%; mp 193−194 °C; TLC (chloroform/methanol 9:1) $R_f$ = 0.20; $^1$H NMR (DMSO-$d_6$) $\delta$ 12.3 (1H, br s, 1-indole proton), 5.03 and 4.70 (2H, 2t, $J$ = 5.4 Hz, hydroxys of 2,3-hydroxymethyls), 4.57 and 4.44 (4H, 2d, $J$ = 5.4 Hz, methylenes of 2,3-hydroxymethyls), 2.26 (4H, s, 6-aziridinyl), and 1.91 (3H, s, 5-methyl); IR (KBr pellet) 3465, 3097, 2943, 2928, 1617, 1543, 1465, 1375, 1234, 1108, 994, and 812 cm$^{-1}$; MS (EI) 262 ($M^+$), 244 ($M^+ - H_2O$), 229, 215, 201, 188, 169, 159, 146, 134, and 108. Anal. Calcd ($C_{13}H_{14}N_2O_4$): C, H, N.

**6-Aziridinyl-2,3-di(acetoxymethyl)-5-methylindole-4,7-dione (8b).** Yield 88%; mp 135−137 °C; TLC (chloroform/acetone 9:1) $R_f$ = 0.60; $^1$H NMR ($CDCl_3$) $\delta$ 9.4 (1H, br s, 1-indole proton), 5.31 and 5.19 (4H, 2s, methylenes), 2.28 (4H, s, 6-aziridinyl), 2.10, 2.08, and 2.05 (9H, 3s, methyls); IR (KBr pellet) 3390, 3088, 2956, 2917, 1635, 1498, 1364, 1211, 1134, 940, and 825 cm$^{-1}$; MS (EI) 346 ($M^+$), 303 ($M^+$ − acetate), 287, 244, 226, and 215. Anal. Calcd ($C_{17}H_{18}N_2O_6$): C, H, N.

**6-Aziridinyl-2,3-di(hydroxymethyl)-1,5-dimethylindole-4,7-dione (8c).** Yield 69%; mp 140−141 °C; TLC (chloroform/

methanol 9:1) $R_f = 0.46$; $^1$H NMR ($CDCl_3$) $\delta$ 4.66 and 4.63 (4H, 2d, $J = 5.4$ Hz, methylenes), 4.49 and 2.15 (2H, 2t, $J = 5.4$ Hz, hydroxyls), 3.99 (3H, s, 1-methyl), 2.32 (4H, s, 6-aziridinyl), and 1.91 (3H, s, 5-methyl); IR (KBr pellet) 3512, 3384, 2959, 2926, 1678, 1622, 1514, 1467, 1381, 1235, 1017, 992, and 810 $cm^{-1}$; MS (EI) 276 ($M^+$), 258 ($M^+ - H_2O$), 243, 230, 215, 202, 188, 174, 160, 145, 132, 122, and 108. Anal. Calcd ($C_{14}H_{16}N_2O_4$): C, H, N.

**Kinetics Studies with Recombinant DT-Diaphorase.** Kinetics studies were carried out in 0.05 M Tris buffer, pH 7.4, under anaerobic conditions, employing Thunberg cuvettes. A 4.0 mM quinone stock solution was prepared in dimethyl sulfoxide (DMSO). To the top port of the cuvette was added the quinone stock solution, and to the bottom port were added the recombinant DT-diaphorase and NADH stock solutions in Tris buffer. Both portions were purged with argon for 20 min and equilibrated at 30 °C for 10 min. The ports were then mixed and the reaction was followed at 336 nm for 10 min to obtain initial rates. The final concentrations of the mixture were 0.3 mM NADH, $1.3 \times 10^{-6}$ to $6.7 \times 10^{-5}$ M quinone substrate, and $1.4 \times 10^{-9}$ M recombinant DT-diaphorase. To calculate $V_{max}$, the value of $\Delta\epsilon$ must be obtained. $\Delta\epsilon$ was calculated from the initial and final absorbances for complete quinone reduction; all values are between 8000 and 9000 $M^{-1}$ $cm^{-1}$. All data were fitted to a Lineweaver−Burk plot from which $V_{max}$ and $K_M$ were obtained.

**Modeling into the DT-Diaphorase Active Site.** Crystallographic coordinates for human DT-diaphorase (1D4A) were obtained from the Protein Data Bank. The coordinates were used as downloaded from the Protein Data Bank and are unrefined. For modeling purposes, INSIGHT II from Molecular Simulations, Inc. (San Diego, CA), was used as previously described.[34,47]

**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 Tris buffer, pH 7.4, and 2 mg of Pd on carbon was added a 5:1 base pair equivalent amount of the indoloquinone dissolved in 0.5 mL of dimethyl sulfoxide. 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 $\mu$M syringe filter. The filtrate was adjusted to 0.3 M acetate with a 3 M stock solution of acetate, pH 5.1, and then diluted with 2 volumes of ethanol. The mixture was chilled at −20 °C for 12 h and the DNA pellet was collected by centrifugation 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′-chloroethyl aminoquinones for extinction coefficient determination were prepared by treatment of the indoloquinone with HCl.[48]

**In Vivo Evaluation.** The B-16 melanoma in C57/bl mice syngraft model was employed to determine in vivo activity.[44] Each agent was evaluated at three doses: 2, 3, or 5 mg $kg^{-1}$ $day^{-1}$, on days 1, 5, and 9 after subcutaneous tumor implantation of $10^5$ cells in the front flank on day 0. Toxic means that there was early lethality, or ≥50% lethality prior to any deaths in the control group. The treated over control values (T/C) were measured at day 25 of the study. A T/C value <40% is considered active. NA means that the compound was not active. The control was obtained with drug-free animals.

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