# A Comparison of the Cytotoxic and Physical Properties of Aziridinyl Quinone Derivatives Based on the Pyrrolo[1,2-*a*]benzimidazole and Pyrrolo[1,2-*a*]indole Ring Systems

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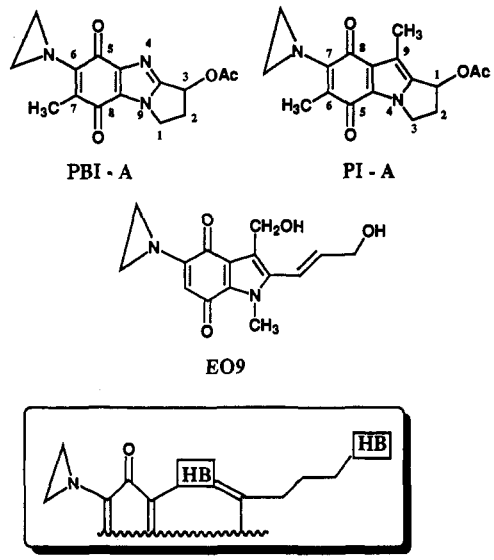
The cytotoxicity and physical properties of the pyrrolo[1,2-*a*]benzimidazole (PBI) and pyrrolo-[1,2-*a*]indole (PI) aziridinyl quinones were compared in order to assess the influence of the benzimidazole ring on antitumor activity and DNA reductive alkylation. Our studies show that the PI system possesses none of the cytotoxicity of the PBI systems. Unlike the PBIs, the PI system does not reductively alkylate DNA. Apparently, the benzimidazole ring favors reductive alkylation due to its electron deficient character compared to indole. In addition, the benzimidazole ring may provide the hydrogen bonding interactions required for the interaction with DNA. Our findings resulted in the elucidation of a PBI pharmacophore. Inspection of the literature revealed another drug sharing the PBI pharmacophore, 5-(1-aziridinyl)-3-(hydroxymethyl)-2-(3-hydroxy-1-propenyl)-1-methyl-1*H*-indole-4,7-dione (EO9), which remarkably has cytotoxic properties similar to those of the PBIs

## Introduction

The pyrrolo[1,2-*a*]benzimidazoles (PBIs) represent a new class of antitumor agent recently discovered in this laboratory.[1-5] One of the most potent analogues, PBI-A, is shown in Chart 1. Although the PBI system resembles the mitomycin system (pyrrolo[1,2-*a*]indole), it possesses a unique spectrum of cytotoxicity and mechanism of action. Thus, PBIs show cytotoxicity toward solid tumors but not toward leukemia.[6] The mechanism of PBI cytotoxicity involves DNA cleavage presumably as a result of binding to the major groove followed by phosphate backbone alkylation.[6,7] The benzimidazole ring of the PBI system is thought to bind to the major groove as a result of Hoogsteen-type hydrogen bonds. In order to assess the role of the benzimidazole ring in PBI antitumor activity, we compared the cytotoxic and physical properties of PBI-A with the similarly functionalized pyrrolo[1,2-*a*]-indole system PI-A.

The results of this comparative study, which led to the elucidation of the pharmacophore shown in the inset of Chart 1, are presented herein. Since PI-A is completely noncytotoxic, we concluded that a heteroatom is required in the ring of the pharmacophore. This heteroatom may influence cytotoxicity by providing a hydrogen-bonding donor or acceptor and by exerting an electronic effect. In contrast, the 9-methyl of PI-A prevents any hydrogen-bonding donor or acceptor interactions. Ongoing studies have provided evidence that a side-chain hydrogen-bonding moiety also contributes to cytotoxicity. It is noteworthy that a recently discovered antitumor agent, 5-(1-aziridinyl)-3-(hydroxymethyl)-2-(3-hydroxy-1-propenyl)-1-methyl-1*H*-indole-4,7-dione (EO9),[8] possesses a structure which resembles the pharmacophore in the inset of Chart 1 and a spectrum of cytotoxicity similar to that of PBI-A. In additon, both EO9 and PBI-A have the unique property of not suppressing bone marrow.

**Chart 1**




## Synthesis

The preparation of PI-A was carried out as outlined in Scheme 1. The synthetic steps leading to PI-A are based on previous reports from this laboratory[1] and elsewhere.[9-11]

In order to introduce a methyl group into the 9-position of the final product, the potassium salt **1**[9] was treated with methyl iodide. The presence of this methyl group in the final product was required in order to prevent hydrogen bonding interactions and thereby assess the role of these interactions in cytotoxicity. A 9-unsubstituted analogue was deemed unsuitable for this purpose since the 9-position is susceptible to electrophilic substitution. The methylated product was then reductively cyclized to afford **2**. Annelation of the pyrrolo ring was carried out by treatment of **2** with methyl acrylate in the presence of potassium *tert*-butoxide. The annelation product **3** was decarboxylated to **4**, which was then nitrated to afford **5**. Reduction of **5** and then Fremy oxidation of the reduction reaction mixture afforded three products: **6**, **7**, and **8**. The minor products (**7** and **8**) were formed as a result of methanolysis

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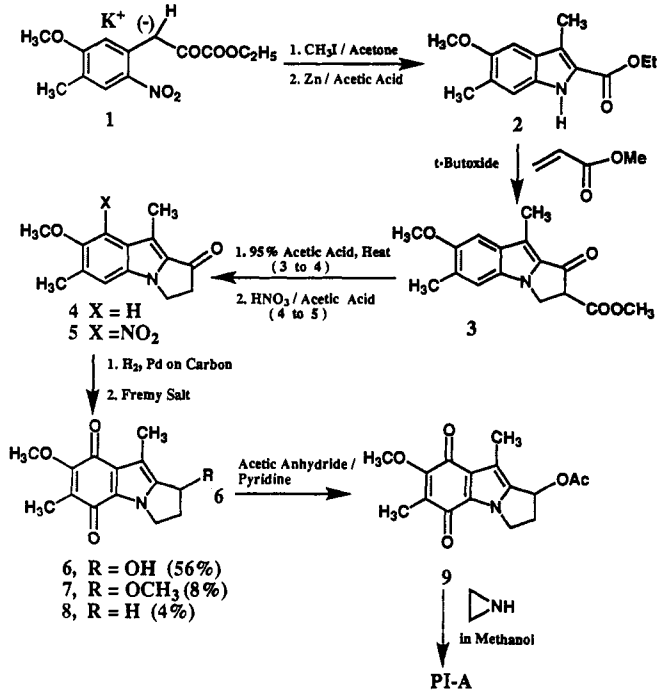
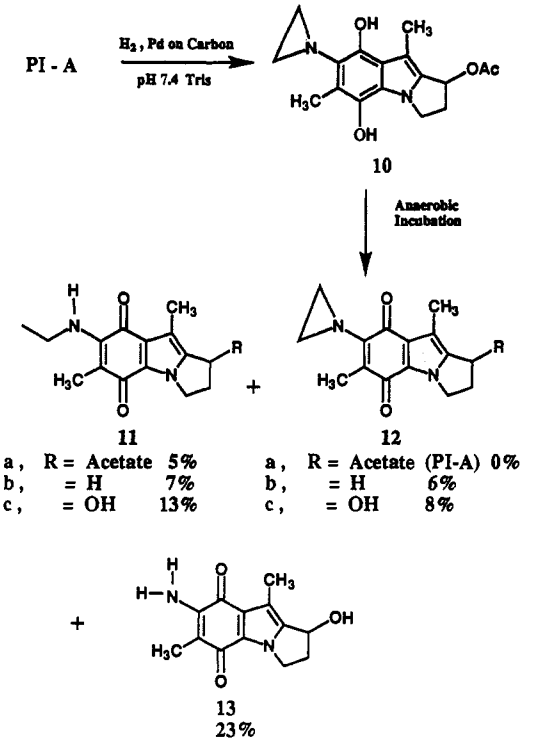
Scheme 1




Scheme 2




of the 3-hydroxyl group and overreduction (3-hydroxy to methylene), respectively, during the catalytic reduction step. Acetylation of **6** to afford **9** was followed by aziridination in methanol to afford PI-A.

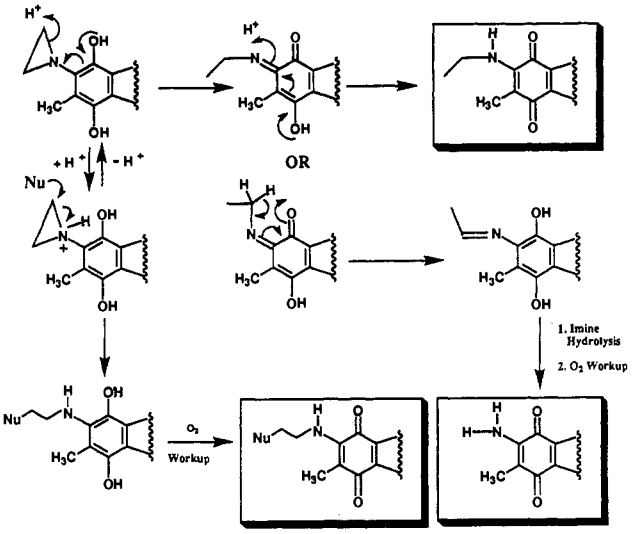
## Fate upon Reductive Activation

The hydrolytic chemistry of reduced PI-A (**10**) and reduced PBI-A (**14**) were compared in anaerobic 0.05 M Tris pH 7.4 buffer. The goal was to determine the influence of the indole and benzimidazole rings on the reactivity of the aziridinyl and acetate groups.

The products obtained upon reducing PI-A to **10**, followed by anaerobic incubation and then aerobic workup, are shown in Scheme 2. If the reduction of PI-A was immediately followed by aeration, only unreacted PI-A was obtained. Thus, none of the products shown in Scheme 2 were the result of the catalytic reduction step. The hydrolysis of **10** involved both the aziridinyl and acetate groups and provided a total of six hydrolysis products. Proton trapping of the aziridinyl hydroquinone afforded compounds **11** and **13**. The mechanisms accounting for the formation of the ethylamino and amino group of **11** and **13** are shown in Scheme 3. The relatively electron rich (low reduction potential) character of the indole ring very likely accounts for the exclusive proton trapping by the aziridinyl ring.[12] There was no apparent trapping of water or hydroxide nucleophiles by the aziridinyl group to afford the (hydroxyethyl)amino product (Nu = OH) shown in Scheme 3. In addition, the aziridinyl group of **10** did not react with 5′-dAMP and did not significantly alkylate calf thymus DNA, Figure 1.

The absorbance vs wavelength plots shown in Figure 1 were obtained for ~600 bp calf thymus DNA (A) and the same DNA alkylated by reduced PI-A (B) and by reduced PBI-A (C). The aziridinyl alkylation products are blue ($\lambda_{max}$ = 550 nm) due to the presence of the amino quinone chromophore formed upon aerobic workup of the DNA alkylation reaction.[6] The presence of this chromophore is apparent from the absorbance vs wavelength plot for DNA treated with reduced PBI-A. In contrast, the same

Scheme 3




plot for DNA treated with reduced PI-A shows very little absorbance at 550 nm.

Recent $^{31}$P-NMR and isolations studies have confirmed that the site of PBI reductive alkylation on DNA is at the phosphate oxygen. The results of these studies will be published in due course.

Quinone methide formation, followed by proton and nucleophile trapping reactions, account for the presence of R = H and R = OH, respectively, in the products shown in Scheme 2. The quinone methide mechanism is illustrated in Scheme 4. The quinone methide shown in Scheme 4 resembles the mitomycin quinone methide with respect to structure as well as to nucleophile- and proton-trapping capability.[13]

The hydrolysis of reduced PBI-A (**14**) in anaerobic 0.05 M pH 7.4 Tris buffer involved only the aziridinyl ring; no quinone methide products resulting from the elimination of acetate were detected. The isolated aziridinyl proton-

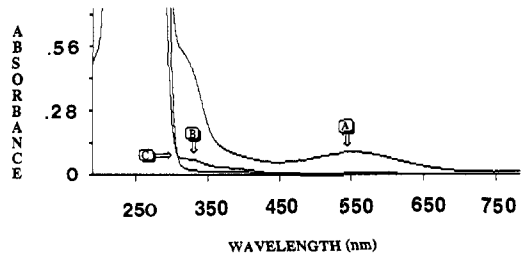
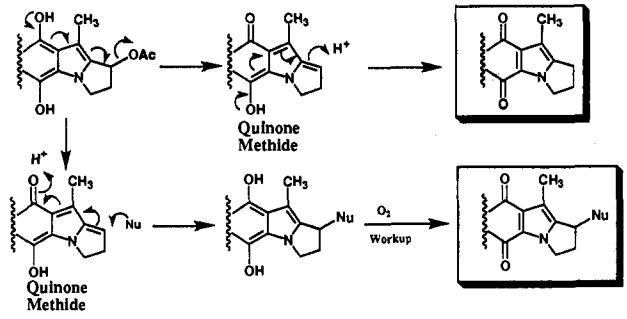
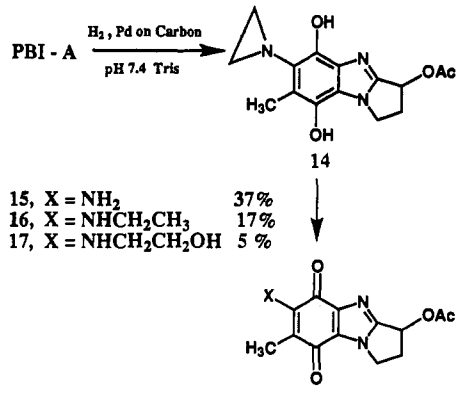



**Figure 1.** UV–visible spectra of reductively alkylated sonicated calf thymus DNA employing PBI-A (A), PI-A (B), and a drug-free control (C).

**Scheme 4**




**Scheme 5**




trapping products were **15** and **16** and the isolated aziridinyl nucleophile-trapping product was **17**, Scheme 5. See Scheme 3 for the mechanism involved in proton/nucleophile trapping by the aziridinyl ring. The difference in reactivity observed between reduced PBI-A and PI-A is very likely due to the electronic difference between the indole and benzimidazole rings. The benzimidazole ring is more electron deficient than the indole ring due to the additional nitrogen, and therefore reduced PBI-A is less likely to eliminate the acetate anion. Previous work in this laboratory showed that the rate of leaving group elimination leading to a quinone methide decreases with increasing electron deficient character (see the linear free energy plot in ref 14).

## Cytotoxicity Studies

PBI-A possesses a mean log $LC_{50}$ value of −6.61 against a panel of 60 cancer cell lines ($LC_{50}$ is the concentration needed for 50% cell kill).[6] Values of log $LC_{50}$ range from <−8.0 for melanoma cell lines to >−4.0 for leukemia cell lines.[6] In contrast, the pyrrolo[1,2-*a*]indole PI-A is completely devoid of activity (mean log $LC_{50}$ > −5). Obviously, the 4-nitrogen of the PBI system is crucial for cytotoxicity. The hydrolysis studies described in this report indicate that the 4-nitrogen promotes aziridinyl alkylation reactions as a result of electron withdrawal. Also this nitrogen can act as a hydrogen-bonded acceptor (or hydrogen-bond donor when protonated) upon reduction of the PBI quinone. The hydrogen-bonding interactions are thought to be important in PBI binding to the major groove of DNA. The 9-methyl group of PI-A would of course preclude any such interaction in the major groove.

**Table 1.** COMPARE Correlations of $GI_{50}$, TGI, and $LC_{50}$ Response Parameters for E09 and PBI-A

| drug | maximal concentration[a] (M) | correlation with E09 $GI_{50}$ | TGI | $LC_{50}$ |
|---|---|---|---|---|
| PBI-A | $1 \times 10^{-4}$ | 0.325 | 0.415 | 0.619 |
| PBI-A | $1 \times 10^{-5}$ | 0.704 | 0.729 | 0.372 |
| PBI-A | $1 \times 10^{-6}$ | 0.677 | 0.605 | 0.558 |

[a] The highest concentration of drug employed in the cytotoxicity assay. For PBI-A three maximal concentrations were employed, whereas the E09 assay (from NCI files) was carried out at only one concentration ($10^{-4}$ M).

The cytotoxicity of the 6-acetamido PBIs (APBI's) suggests that the 4-nitrogen does more than just promote aziridinyl alkylation. The APBI's do not possess the 6-aziridinyl group and therefore do not under go reductive alkylation. Nevertheless they possess significant cytotoxicity, although not as much as the PBIs. For example, the 6-acetamido derivative of PBI-A possesses a mean log $LC_{50}$ value of −5.67 against a panel of 60 cancer cell lines. Indeed, both PBI and APBI analogues are currently undergoing *in vivo* trials at the National Cancer Institute. We believe that the APBI's bind to the DNA major groove upon quinone reduction in the same fashion as proposed for the reduced PBI's.

The recently discovered indole-based antitumor agent E09[8] bears a strong structural resemblance to PBI-A. The 3-position of E09 (analogous to the 4-position of PBI-A) is substituted with a hydroxymethyl group, which could provide the hydrogen-bonding and electron-withdrawing interactions required for cytotoxicity according to our pharmacophore. In addition, E09 possesses a side-chain hydrogen-bonding moiety (hydroxyl), which is also part of our pharmacophore. In fact, E09 and PBI-A possess similar mean log $LC_{50}$ values (−6.5 and −6.6, respectively) as well as similar mean graphs. Both drugs are inactive against leukemias *in vivo* and *in vitro*, and both are active against solid tumors. For example, both E09 and PBI-A show high activity against nearly all cell lines in the melanoma panel.

In order to quantitate the similarities between PBI-A and E09, we used the National Cancer Institute COMPARE results to calculate correlation coefficients for the $GI_{50}$, TGI, and $LC_{50}$ drug-response parameters.[15,16] These respective parameters represent drug concentration at which the percentage growth is 50% (growth inhibition), 0% (total growth inhibition), and −50% (50% lethality). The correlation coefficients summarized in Table 1 indicate that there are significant similarities between E09 and PBI-A (correlation coefficients >0.6). Previous COMPARE studies indicated moderate to good correlations (0.6–0.7) between PBI-A and antitumor agents known to undergo reductive alkylation and cleave DNA, features which are shared by E09.

The most interesting property shared by PBI-A and E09 is the absence of substantial bone marrow suppression. Shown in Figure 2 are the results of bone marrow suppression studies on C57B1/6 female mice. From the bar graphs in this figure, it is apparent that there was no

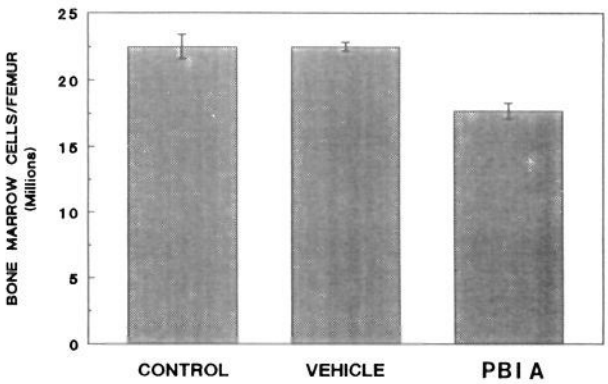



**Figure 2.** Bar graphs of bone marrow counts of untreated C57BI/6 mice (control), the same mice treated with dimethyl sulfoxide (vehicle), and the same mice treated ip daily with 2 mg/kg PBI-A for 4 days. Graphs are the averaged results of two studies consisting of five mice each.

dramatic decreases in bone marrow counts of drug-treated mice from the control and vehicle counts. In the same study, blood platelets were virtually unaffected by PBI-A, but leukocytes showed a substantial decrease (51% of vehicle values).

## Conclusions

The change from the PBI (pyrrolo[1,2-*a*]benzimidazole) to the PI (pyrrolo[1,2-*a*]indole) system is accompanied by a dramatic change in reductive alkylation capability and cytotoxicity. These changes are very likely due to alterations in the electronic character (PI's are more electron rich than PBI's), the hydrogen-bonding capabilities (the 4-nitrogen of the PBI system can undergo H-bonding interactions whereas the PI system bears a C-methyl at this position), and the steric effect of 9-methyl of the PI. These findings contributed to the elucidation of the pharmacophore shown in the inset of Chart 1. It is noteworthy that an antitumor agent E09 possesses some of the features of this pharmacophore and accordingly possesses some of the cytotoxic properties of PBI-A. In addition, both E09 and PBI-A do not result in bone marrow suppression.

## Experimental Section

All analytically pure compounds were dried under high vacuum in a drying pistol heated with refluxing methanol. Some compounds still contained water of crystallization that was determined from the elemental analyses. Elemental analyses were run at Atlantic Microlab, Inc., in Norcross, GA. Uncorrected melting and decomposition points were determined with a Mel-Temp apparatus. All TLC was run with silica gel plates with a fluorescent indicator employing a variety of solvents. IR spectra were taken as KBr pellets or thin films; the strongest IR absorbances are reported. $^1$H NMR spectra were obtained on a 300-MHz spectrometer, and chemical shifts are reported relative to TMS.

**Preparation of New Compounds and Their Physical Properties. Ethyl 5-Methoxy-3,6-dimethylindole-2-carboxylate (2).** To a stirred mixture of 18.0 g (56.4 mmol) of **1** and 300 mL of dry acetone was slowly added 17.0 g (120 mmol) of iodomethane at room temperature. After completion of addition, stirring was continued for 4 h, at which time the initial purple color of the reaction had faded and the potassium iodide salt precipitated from solution. The reaction mixture was filtered and the solid was washed two times with 50-mL portions of acetone. The filtrate and washings were combined and evaporated to a red oil which was dissolved in 200 mL of glacial acetic acid and treated with 24 g of zinc dust under efficient mechanical stirring at 40–45 °C. After being stirred for 3 h, the reaction mixture was filtered, and the solid residue was washed 2 times with 25-mL portions of acetic acid. The filtrate and washings were diluted with 800 mL of ice-cold water and extracted two times with 200-mL portions of ethyl acetate. The extracts were washed with water and dried ($Na_2SO_4$). Concentration of the extracts afforded a brown solid product, which was subjected to silica gel flash chromatography employing chloroform as eluant. Recrystallization from methanol afforded **2** as white crystals: 3.6-g (26%) yield; mp 162–164 °C; TLC (chloroform) $R_f$ = 0.75; IR (KBr pellet) 3327, 2990, 1674, 1535, 1472, 1285, 1253, 1204, 1018 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 8.42 (1H, bs, -NH proton), 7.09 and 6.91 (2H, 2 s, aromatic protons), 4.37 (2H, q, $J$ = 7.2 Hz, methylene protons), 3.87 (3H, s methoxy), 2.55 and 2.30 (6H, 2 s, 3,6-dimethyl), 1.40 (3H, t, $J$ = 7.2 Hz, methyl); mass spectrum (EI), $m/z$ 247 ($M^+$), 201 ($M^+ - CH_3CH_2OH$), 186, 172, 158. Anal. ($C_{14}H_{17}NO_3$) C, H, N.

**Methyl 2,3-Dihydro-7-methoxy-6,9-dimethyl-1-oxo-1*H*-pyrrolo[1,2-*a*]indole-2-carboxylate (3).** To a mechanically stirred suspension of 1.57 g (14 mmol) of potassium *tert*-butoxide in 30 mL of dry benzene were added a solution of 3.5 g (14 mmol) of **2** in 100 mL of dry benzene and then 2.41 g (28 mmol) of methyl acrylate. The reaction mixture was refluxed for 4 h and then stirred at room temperature for 48 h. The reaction was treated with 400 mL of water and acidified with concentrated hydrochloric acid to pH 4. The acidified mixture was extracted two times with 200-mL portions of methylene dichloride, and then the extracts were washed with water and dried ($Na_2SO_4$). Concentration of the extracts afforded **3** as a white solid which was recrystallized from methanol: 1.90-g (48%) yield; mp 168–170 °C; TLC (chloroform) $R_f$ = 0.50; IR (KBr pellet) 2951, 2922, 1726, 1701, 1568, 1456, 1336, 1250, 1168, 1035 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 7.12 and 6.90 (2H, 2 s, aromatic protons), 4.67 (1H, dd, $J$= 4.3 Hz, $J$ = 10.8 Hz, 3-diastereomeric proton), 4.44 (1H, dd, $J$ = 8.2 Hz, $J$ = 10.8 Hz, 2-methine proton), 4.16 (1H, dd, $J$ = 4.2 Hz, $J$ = 8.2 Hz, 3-diastereomeric proton), 3.87 and 3.81 (6H, 2 s, methoxy and ester methyl), 2.50 and 2.33 (6H, 2 s, 6,9-dimethyl); mass spectrum (EI), $m/z$ 287 ($M^+$), 272 ($M^+ - CH_3$), 255 ($M^+ - CH_3OH$), 228 ($M^+ - OCOCH_3$), 213, 186, 158. Anal. ($C_{16}H_{17}NO_4$) C, H, N.

**2,3-Dihydro-7-methoxy-6,9-dimethyl-1*H*-pyrrolo[1,2-*a*]indol-1-one (4).** A solution of 2.40 g (8.4 mmol) of **3** in 80 mL of 95% acetic acid was heated at reflux for 18 h. The reaction mixture was diluted with 300 of mL water, resulting in precipitation of a white solid product. The product was filtered, washed two times with 50-mL portions of water, and vacuum dried. Recrystallization from methanol afforded **4** as a white crystalline solid: 1.77-g (93%) yield; mp 196–198 °C; TLC (chloroform/ethyl acetate, 90:10) $R_f$ = 0.40; IR (KBr pellet) 2970, 2918, 1699, 1630, 1562, 1485, 1402, 1321, 1230, 1168, 1037 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 7.11 and 6.92 (2H, 2 s, aromatic protons), 4.27 (2H, t, $J$ = 6.2 Hz, 3-methylene protons), 3.88 (3H, s, methoxy), 3.13 (2H, t, $J$ = 6.6 Hz, 2-methylene protons), 2.51 and 2.33 (6H, 2 s, 6,9-dimethyl); mass spectrum (EI), $m/z$ 229 ($M^+$), 214 ($M^+ - CH_3$), 201, 186, 172, 158. Anal. ($C_{14}H_{15}NO_2$) C, H, N.

**2,3-Dihydro-7-methoxy-6,9-dimethyl-8-nitro-1*H*-pyrrolo-[1,2-*a*]indol-1-one (5).** To a mixture of 1.0 g (4.36 mmol) of **4** and 30 mL of glacial acetic acid was added 1.5 mL of 69% nitric acid dropwise with stirring while maintaining a temperature of 10–15 °C. After being stirred for 10 min at this temperature range, the reaction mixture was combined with 200 mL of ice–water and then extracted two times with 50-mL portions of dichloromethane. The combined extracts were washed with water and dried ($Na_2SO_4$). Concentration of the dried extracts afforded a red gum, which was purified by flash chromatography on silica gel using chloroform as the eluant. The product was eluted from the column as a fast-moving yellow band. Recrystallization of the isolated product from ethyl acetate afforded **5** as a yellow crystalline solid: 600-mg (50%) yield; mp 188–190 °C; TLC (chloroform/ethyl acetate, 90:10) $R_f$ = 0.75; IR (KBr pellet) 2945, 2918, 1701, 1568, 1525, 1392, 1317, 1275, 1176, 1124, 1026 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 7.29 (1H, s, aromatic protons), 4.33 (2H, t, $J$ = 6.5 Hz, methylene protons), 3.87 (3H, s, methoxy), 3.18 (2H, t, $J$ = 6.5 Hz, methylene protons), 2.45 and 2.37 (6H, 2 s, 6,9-dimethyl); mass spectrum (EI), $m/z$ 274 ($M^+$), 257, 241, 227, 198, 158. Anal. ($C_{14}H_{14}N_2O_4$) C, H, N.

**2,3-Dihydro-1-hydroxy-7-methoxy-6,9-dimethyl-1*H*-pyrrolo[1,2-*a*]indole-5,8-dione (6).** A solution of 210 mg (0.77 mmol) of **5** in 100 mL of methanol containing 210 mg of 5% Pd on charcoal was shaken under 50 psi of $H_2$ for 12 h. The reaction mixture was filtered through Celite, and the filtercake was washed two times with 25-mL portions of methanol. The combined filtrates were concentrated to afford the crude amine product which was used in the next step without purification.

To a solution of 2.0 g of Fremy salt and 2.2 g of potassium dihydrogen phosphate in 100 mL of water was added a solution of the crude amine in 100 mL of acetone. The reaction was stirred for 12 h and then diluted with 300 mL of water. The resulting solution was extracted two times with 100 mL of dichloromethane. The extracts were washed with water, dried ($Na_2SO_4$), and concentrated to afford an orange solid. Flash chromatography of this solid on silica gel using chloroform/ethyl acetate (90:10) as the eluant afforded **6** along with small amounts of **7** and **8**.

Recrystallization of **6** from chloroform/hexane (80:20) afforded red crystals: 112-mg (56%) yield; mp 199–200 °C; TLC (chloroform/ethyl acetate, 90:10) $R_f$ = 0.20; IR (KBr pellet) 3423, 2947, 2852, 1661, 1618, 1487, 1321, 1113 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 5.22–5.18 (1H, m, methine proton), 4.35–4.16 (2H, m, methylene protons), 3.98 (3H, s, methoxy), 2.90–2.75 (1H, m, 3-diastereomeric methylene), 2.51–2.40 (1H, m, 2-diastereomeric methylene proton), 2.33 and 1.92 (6H, 2 s, 6,9-dimethyl), 1.81 (1H, d, $J$ = 5.6 Hz, OH proton); mass spectrum (EI), $m/z$ 261 ($M^+$), 246 ($M^+ - CH_3$), 218, 190, 162. Anal. ($C_{14}H_{15}NO_4$) C, H, N.

Recrystallization of **2,3-dihydro-1,7-dimethoxy-6,9-dimethyl-1*H*-pyrrolo[1,2-*a*]indole-5,8-dione (7)** from hexane afforded orange crystals: 17-mg (8%) yield; mp 110–111 °C; TLC (chloroform/ethyl acetate, 90:10) $R_f$ = 0.60; IR (KBr pellet) 2988, 2955, 2895, 1659, 1634, 1599, 1483, 1319, 1111 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 4.69 (1H, dd, $J$ = 1.6 Hz, $J$ = 6.1 Hz, methine proton), 4.26–4.22 (2H, m, 3-diastereomeric methylene), 3.98 and 3.33 (6H, 2 s, 1,7-dimethoxys), 2.67–2.50 (2H, m, 2-diastereomeric methylene), 2.35 and 1.92 (6H, 2 s, 6,9-dimethyl); mass spectrum (EI), $m/z$ 275 ($M^+$), 260 ($M^+ - CH_3$), 244 ($M^+ - CH_3$), 216, 201, 186, 172. Anal. ($C_{15}H_{17}NO_4$) C, H, N.

Recrystallization of **2,3-dihydro-7-methoxy-6,9-dimethyl-1*H*-pyrrolo[1,2-*a*]indole-5,8-dione (8)** from hexane afforded red crystals: 7.5-mg (4%) yield; mp 170–171 °C; TLC (chloroform/ethyl acetate, 90:10) $R_f$ = 0.80; IR (KBr pellet) 2944, 2847, 1655, 1632, 1595, 1495, 1319, 1273, 1111 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 4.17 (2H, t, $J$ = 7.1 Hz, 3-methylene protons), 3.95 (3H, s, methoxy), 2.74 (2H, t, $J$ = 7.2 Hz, 1-methylene protons), 2.51 (2H, quintet, $J$ = 7.1 Hz, 2-methylene protons), 2.21 and 1.91 (6H, 2 s, 6,9-dimethyl), Anal. ($C_{14}H_{15}NO_3 \cdot 0.10H_2O$) C, H, N.

**2,3-Dihydro-1-acetoxy-6,9-dimethyl-7-methoxy-1*H*-pyrrolo-[1,2-*a*]indole-5,8-dione (9).** To a solution of 100 mg (0.38 mmol) of **6** in 15 mL of dry pyridine was added 10 mL of freshly distilled acetic anhydride, and the mixture was stirred at room temperature for 10 h. The reaction mixture was diluted with 100 mL of water and extracted two times with 50-mL portions of ethyl acetate. The extracts were washed with water, dried ($Na_2SO_4$), and concentrated to afford a red gum. Flash chromatography of the gum over silica gel using chloroform as the eluant provided **7** as orange-yellow crystals: 98-mg (85%) yield; mp 125–126 °C; TLC (chloroform) $R_f$ = 0.60; IR (KBr pellet) 2942, 2842, 1740, 1640, 1489, 1371, 1234, 1111 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 6.06 (1H, dd, $J$ = 1.8 Hz, $J$ = 6.8 Hz, methine proton), 4.35–4.02 (2H, m, 3-diastereomeric methylene), 3.99 (3H, s, methoxy), 2.95–2.82 (1H, m, 2-diastereomeric methylene proton), 2.56–2.49 (1H, m, 2-diastereomeric methylene proton), 2.29 (3H, s, acetate), 2.05 and 1.93 (6H, 2 s, 6,9-dimethyls); mass spectrum (EI), $m/z$ 303 ($M^+$), 244 ($M^+ - OCOCH_3$), 228, 201, 172, 144. Anal. ($C_{16}H_{17}NO_5 \cdot 0.5\ H_2O$) C, H, N.

**7-(1-Aziridinyl)-2,3-dihydro-1-acetoxy-6,9-dimethyl-1*H*-pyrrolo[1,2-*a*]indole-5,8-dione (PI-A).** To a solution of 80 mg (0.26 mmol) of **9** in 20 mL of methanol, chilled at 0 °C, was added 0.8 mL of ethylenimine. After being stirred at 0 °C for 30 min, the reaction mixture was stirred at room temperature for 1 h. The solvent was removed in vacuo to obtain a red solid. Flash chromatography of this solid on silica gel using chloroform/ethyl acetate (90:10) as the eluant and the recrystallization of the purified solid from hexane gave PI-A as orange crystals: 60-mg (72%) yield; mp 157–159 °C; TLC (chloroform/ethyl acetate, 90:10) $R_f$ = 0.30; IR (KBr pellet) 2995, 2958, 2916, 1739, 1657, 1622, 1495, 1373, 1338, 1240, 1202, 1145 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 6.05 (1H, dd, $J$ = 1.7 Hz, $J$ = 6.7 Hz, methine proton), 4.30–4.22 (2H, m, 3-diastereomeric methylene), 2.91–2.80 (1H, m, 2-diastereomeric methylene proton), 2.55–2.48 (1H, m, 2-diastereomeric methylene protons), 2.28 (7H, bs, aziridine and ester methyl protons), 2.04 and 2.02 (6H, 2 s, 6,9-dimethyl); mass spectrum (EI), $m/z$ 314 ($M^+$), 287 ($M^+ - HCN$), 271 ($M^+ - O{=}C{=}NH$), 255 ($M^+ - OCOCH_3$), 239, 227, 186. Anal. ($C_{17}H_{18}N_2O_4 \cdot 0.25\ H_2O$) C, H, N.

**Hydrolysis of 7-(1-aziridinyl)-2,3-dihydro-1-acetoxy-5,8-dihydroxy-6,9-dimethyl-1*H*-pyrrolo[1,2-*a*]indole (10).** A solution of 20 mg (0.064 mmol) of PI-A in 5 mL of dimethyl sulfoxide was added to a suspension of 20 mg of 5% Pd on carbon in 250 mL of 0.05 M pH 7.4 Tris buffer. The reaction mixture was degassed with argon for 1 h and then purged with hydrogen until the red solution turned colorless (~5 min). The reaction mixture was purged with argon for 30 min and kept at 30 °C for 8 h while maintaining anaerobic conditions. The reaction solution turned purple during the incubation time due to the formation of quinone hydrolysis products. The reaction was opened to air, stirred for 15 min, and filtered through Celite. The filtercake was washed two times with 25-mL portions of chloroform. The filtrate was extracted two times with 75-mL portions of chloroform. The combined chloroform extracts were washed with water, dried ($Na_2SO_4$), and concentrated to a purple solid mass. Preparative thin-layer chromatographic separation on silica gel using chloroform/ethyl acetate (90:10) as eluant afforded six products: **11a–c, 12b,c,** and **13**. The properties of these products are provided below.

**7-(*N*-Ethylamino)-2,3-dihydro-1-acetoxy-6,9-dimethyl-1*H*-pyrrolo[1,2-*a*]indole-5,8-dione (11a)** was recrystallized from diethyl ether resulting in purple crystals: 0.95-mg (5%) yield; mp 138–140 °C; TLC (chloroform/ethyl acetate, 90:10) $R_f$ = 0.50; IR (KBr pellet) 3318, 2928, 1740, 1657, 1593, 1493, 1310, 1227, 1134, 1040 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 6.05 (1H, dd, $J$ = 1.7 Hz, $J$ = 6.5 Hz, methine proton), 5.80 (1H, bs, NH), 4.39–4.23 (2H, m, 3-diastereomeric methylene proton), 3.56 (2H, q, $J$ = 7.2 Hz, methylene protons of ethylamino), 2.89–2.82 (1H, m, 2-diastereomeric methylene proton), 2.57–2.52 (1H, m, 2-diastereomeric methylene proton), 2.28 (3H, s, ester methyl), 2.09 and 2.07 (6H, 2 s, 6,9-dimethyl), 1.25 (3H, t, $J$ = 7.2 Hz, methyl of ethylamino); mass spectrum (EI), $m/z$ 316 ($M^+$), 301 ($M^+ - CH_3$), 287 ($M^+ - CH_2CH_3$), 257 ($M^+ - OCOCH_3$), 241, 227, 186,146. Anal. ($C_{17}H_{20}N_2O_4 \cdot 0.25\ H_2O$) C, H, N.

**7-(*N*-Ethylamino)-2,3-dihydro-6,9-dimethyl-1*H*-pyrrolo-[1,2-*a*]indole-5,8-dione (11b)** was recrystallized from hexane, resulting in purple crystals: 1.2 mg (7%) yield; mp 148–150 °C; TLC (chloroform/ethyl acetate, 90:10) $R_f$ = 0.70; IR (KBr pellet) 3310, 2970, 2918, 1657, 1593, 1524, 1505, 1371, 1317, 1227, 1126 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 5.68 (1H, bs, NH), 4.17 (2H, t, $J$ = 7.0 Hz, 3-methylene protons), 3.50 (2H, quintet, $J$ = 7.2 Hz, 2-methylene protons), 2.69 (2H, t, $J$ = 7.3 Hz, 1-methylene protons), 2.48 (2H, quintet, $J$ = 7.0 Hz, methylene protons of ethylamino), 2.18 and 2.04 (6H, 2 s, 6,9-dimethyl), 1.21 (3H, t, $J$ = 7.2 Hz, methyl of ethylamino); mass spectrum (EI), $m/z$ 258 ($M^+$), 243 ($M^+ - CH_3$), 229 ($M^+ - CH_2CH_3$), 215 ($M^+ - NCH_2CH_3$), 174, 148, 121. Anal. ($C_{15}H_{18}N_2O_2 \cdot 0.25H_2O$) C, H, N.

**7-(*N*-Ethylamino)-2,3-dihydro-1-hydroxy-6,9-dimethyl-1*H*-pyrrolo[1,2-*a*]indole-5,8-dione (11c)** was recrystallized from chloroform/hexane, resulting in purple crystals: 2.2-mg (13%) yield; mp 186–188 °C; TLC (chloroform/ethyl acetate, 90:10) $R_f$ = 0.30; IR (KBr pellet) 3437, 3319, 2922, 2872, 1657, 1593, 1491, 1308, 1229, 1130, 1061 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 5.73 (1H, bs, NH), 5.19 (1H, doublet of triplet, $J$ = 2.5 Hz, $J$ = 6.0 Hz, methine proton), 4.37–4.22 (2H, m, 3-diastereomeric methylene proton), 3.56 (2H, quintet, $J$ = 7.1 Hz, methylene protons of ethylamino), 2.86–2.74 (1H, m, 2-diastereomeric methylene proton), 2.51–2.43 (1H, m, 2-diastereomeric methylene proton), 2.33 and 2.08 (6H, 2 s, 6,9-dimethyl), 1.63 (1H, d, $J$ = 5.6 Hz, OH proton), 1.26 (3H, t, $J$ = 7.2 Hz, methyl protons of ethylamino); mass spectrum (EI), $m/z$ 274 ($M^+$), 259 ($M^+ - CH_3$), 245 ($M^+ - CH_2CH_3$), 231 ($M^+ - NCH_2CH_3$), 215, 202, 190, 174, 163. Anal. ($C_{15}H_{18}N_2O_3 \cdot 0.25H_2O$) C, H, N.

**7-(1-Aziridinyl)-2,3-dihydro-6,9-dimethyl-1*H*-pyrrolo[1,2-*a*]indole-5,8-dione (12b)** was recrystallized from hexane resulting

in purple crystals: 1.0-mg (6%) yield; mp 167–168 °C; TLC (chloroform/ethyl acetate, 90:10) $R_f$ = 0.40; IR (KBr pellet) 2916, 1655, 1620, 1497, 1331, 1229, 1142, 1080 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 4.19 (2H, t, $J$ = 7.1 Hz, 3-methylene protons), 2.75 (2H, t, $J$ = 7.1 Hz,1-methylene proton), 2.53 (2H, quintet, $J$ = 7.1 Hz, 2-methylene protons), 2.28 (4H, s, aziridinyl protons), 2.24 and 2.04 (6H, 2 s, methyl); mass spectrum (EI), $m/z$ 256 ($M^+$), 241 ($M^+ - CH_3$), 227, 186, 170, 149, 128, 100. Anal. ($C_{15}H_{16}N_2O_2$) C, H, N.

**7-(1-Aziridinyl)-2,3-dihydro-1-hydroxy-6,9-dimethyl-1*H*-pyrrolo[1,2-*a*]indole-5,8-dione (12c)** was recrystallized from chloroform/hexane, resulting in orange crystals: 1.5-mg (8%) yield; mp 172–174 °C; TLC (chloroform/ethyl acetate, 90:10) $R_f$ = 0.20; IR (KBr pellet) 3443, 2987, 2922, 1662, 1630, 1493, 1339, 1231, 1138, 1065 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 5.25 (1H, doublet of triplet, $J$ = 2.3 Hz, $J$ = 6.2 Hz, methine proton), 4.38–4.17 (2H, m, 3-diastereomeric methylene protons), 2.52–2.43 (1H, m, 2-diastereomeric methylene protons), 2.33 and 2.04 (6H, 2 s, 6,9-dimethyl), 2.29 (4H, s, aziridinyl protons), 2.07 (1H, d, $J$ = 6.0 Hz, OH); mass spectrum (EI), $m/z$ 272 ($M^+$), 255 ($M^+ - OH$), 227, 216, 199, 149, 111. Anal. ($C_{15}H_{16}N_2O_3$) C, H, N.

**7-Amino-2,3-dihydro-1-hydroxy-6,9-dimethyl-1*H*-pyrrolo-[1,2-*a*]indole-5,8-dione (13)** was recrystallized from chloroform, resulting in deep red crystals: 3.6-mg (23%) yield; mp 196–198 °C; TLC (chloroform/ethyl acetate, 90:10) $R_f$ = 0.10; IR (KBr pellet) 3457, 3422, 3354, 2924, 1667, 1607, 1489, 1393, 1352, 1107, 1072 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 5.17 (1H, doublet of triplet, $J$ = 1.9 Hz, $J$ = 6.1 Hz, methine proton), 4.85 (2H, bs, $NH_2$), 4.35–4.23 (2H, m, 3-diastereomeric methylene protons), 2.82–2.72 (1H, m, 2-diastereomeric methylene proton), 2.49–2.41 (1H, m, 2-diastereomeric methylene proton), 2.32 and 1.80 (6H, 2 s, 6,9-dimethyl), 1.74 (1H, d, $J$ = 5.5 Hz, OH); mass spectrum (EI), $m/z$ 246 ($M^+$), 229 ($M^+ - OH$), 202, 161, 134, 106, 91. Anal. ($C_{13}H_{14}N_2O_3 \cdot 0.25H_2O$) C, H, N.

**Hydrolysis of 6-(1-Aziridinyl)-2,3-dihydro-3-acetoxy-5,8-dihydroxy-7-methyl-1*H*-pyrrolo[1,2-*a*]benzimidazole (14).** A solution of 20 mg (0.066 mmol) of PBI-A in 3 mL of dimethyl sulfoxide was added to a suspension of 20 mg of 5% Pd on carbon in 250 mL of 0.05 M pH 7.4 Tris buffer. The reaction mixture was degassed with argon for 1 h and then purged with hydrogen for 5 min, during which time the orange reaction mixture turned colorless. The reaction mixture was degassed with argon for 30 min to remove the hydrogen and then kept at 30 °C for 8 h. During the incubation, the reaction solution went from colorless to purple due to quinone fromation. The reaction vessel was opened to air, stirred for 15 min, and then filtered through Celite. The filtercake was washed two times with 25-mL portions of chloroform, and the filtrate was extracted two times with 75-mL portions of chloroform. The combined chloroform extracts were washed with water, dried ($Na_2SO_4$), and concentrated to a purple solid mass. Preparative thin-layer chromatographic separation of the purple solid over silica gel using chloroform/methanol (95:5) as eluant afforded three products: **15**, **16**, and **17**. The properties of these products are provided below.

**6-Amino-2,3-dihydro-3-acetoxy-7-methyl-1*H*-pyrrolo[1,2-*a*]benzimidazole-5,8-dione (15)** was recrystallized from chloroform, resulting in red crystals: 6.13-mg (34%) yield; mp 218–220 °C; TLC (chloroform/methanol, 95:5) $R_f$ = 0.50; IR (KBr pellet) 3457, 3370, 2928, 1744, 1626, 1601, 1512, 1235, 1082 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 6.06 (1H, dd, $J$ = 3.0 Hz, $J$ = 7.6 Hz, methine proton), 5.07 (2H, bs, $NH_2$), 4.40–4.22 (2H, m, 1-diastereomeric methylene protons), 3.20–3.08 (1H, m, 2-diastereomeric methylene proton), 2.67–2.58 (1H, m, 2-diastereomeric methylene proton), 2.11 (3H, s, ester methyl), 1.88 (3H, s, 7-methyl); mass spectrum (EI), $m/z$ 275 ($M^+$), 232 ($M^+ - COCH_3$), 215, 204, 178, 158, 133. Anal. ($C_{13}H_{13}N_3O_4 \cdot 0.3H_2O$) C, H, N.

**6-(*N*-Ethylamino)-2,3-dihydro-3-acetoxy-7-methyl-1*H*-pyrrolo[1,2-*a*]benzimidazole-5,8-dione (16)** was recrystallized from hexane, resulting in purple crystals: 3.40-mg (17%) yield; mp 123–125 °C; TLC (chloroform/methanol, 95:5) $R_f$ = 0.70; IR (KBr pellet) 3297, 2972, 2934, 1750, 1692, 1616, 1591, 1512, 1311, 1244, 1111, 1047 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 6.07 (1H, dd, $J$ = 3.0 Hz, $J$ = 7.6 Hz, methine proton), 5.89 (1H, bs, NH), 4.40–4.25 (2H, m, 1-diastereomeric methylene protons), 3.63 (2H, quintet, $J$ = 7.17 Hz, methylene protons of ethylamino), 3.19–3.07 (1H, m, 2-diastereomeric methylene proton), 2.67–2.59 (1H, m, 2-diastereomeric methylene proton), 2.12 and 2.11 (6H, 2 s, methyl), 1.29 (3H, t, $J$ = 7.23 Hz, methyl of ethylamino); mass spectrum (EI), $m/z$ 303 ($M^+$), 260 ($M^+ - COCH_3$), 243, 233, 214, 189, 133. Anal. ($C_{15}H_{17}N_3O_4 \cdot 0.6H_2O$) C, H; N: calcd 13.37, found 12.39.

**6-[*N*-(2′-Hydroxyethyl)amino]-2,3-dihydro-3-acetoxy-7-methyl-1*H*-pyrrolo[1,2-*a*]benzimidazole-5,8-dione (17)** was recrystallized from hexane, resulting in dark brown crystals: 1.1-mg (5%) yield; mp 102–105 °C dec; TLC (chloroform/methanol, 95:5) $R_f$ = 0.25; IR (KBr pellet) 3433, 3331, 2945, 1744, 1686, 1586, 1524, 1317, 1231, 1117, 1061 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 6.19 (1H, bs, NH), 6.05 (1H, dd, $J$ = 3.0 Hz, $J$ = 7.6 Hz, methine proton), 4.40–4.22 (2H, m, 1-diastereomeric methylene protons), 3.86 (2H, t, $J$ = 4.6 Hz, methylene protons), 3.74 (2H, q, $J$ = 4.4 Hz, methylene protons), 3.20–3.10 (1H, m, 2-diastereomeric methylene proton), 2.70–2.58 (1H, m, 2-diastereomeric methylene proton), 2.11 (3H, s, ester methyl), 2.10 (3H, s, 7-methyl); mass spectrum (EI), $m/z$ 319 ($M^+$), 288 ($M^+ - OCH_3$) 259 ($M^+ - CH_3$-OOH), 228, 201, 173, 133. Anal. ($C_{15}H_{17}N_3O_5$) C, H, N.

**Preparation of Calf Thymus DNA Adducts.** A solution of reduced PBI-A or reduced PI-A was prepared as described below. The quinone (0.016 mmol) was dissolved in 1 mL of dimethyl sulfoxide, and the resulting solution was added to 20 mL of 0.05 M pH 7.4 Tris buffer along with 3 mg of 5% Pd on carbon. This mixture was deaerated with argon for 20 min and then purged with $H_2$ gas to reduce the quinone (for about 5 min). The excess $H_2$ gas was removed by purging with argon for 15 min. The hydroquinone solution was then placed in an $N_2$ glove box employed above. The catalytic reduction apparatus consisted of a 50-mL flask equipped with an inlet tube and an outlet tube. The inlet tube reached into the reaction solution for purging with $H_2$ or argon gases, and the outlet tube was above the reaction solution.

The reduced quinone solution was filtered through a Millex-SR 0.5-$\mu$m syringe filter and combined with sonicated calf thymus DNA in the $N_2$ glovebox. The resulting mixture was incubated at 25 °C for 24 h and then removed from the box. After adjusting the reaction mixture to 0.3 M NaOAc with a 3.0 M pH 5.1 NaOAc solution, 3 volumes of absolute ethanol was added and the resulting mixture chilled at −20 °C for 24 h. During this time the DNA precipitated from the solution (as blue threads when PBI-A was the quinone). The DNA was pelleted by centrifuging at 5000$g$ for 15 min. The pellet was then twice resuspended in ethanol and centrifuged to remove any drug hydrolysis products.

The vacuum dried pellet was weighed (9.5-mg yield for PBI-A-treated DNA and 8.80-mg yield for PI-A-treated DNA) and dissolved in 0.05 M pH 7.4 Tris at 2 mg/mL for UV–visible spectral studies.

**Cytotoxicity Studies.** These studies were carried out at the National Cancer Institute (NCI) employing published procedures.[15] The COMPARE studies of PBI-A (NSC 624149) and E09 (NSC 382459) were also carried out at the NCI.

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