Chemistry of the Pyrrolo[1,2-*a*]benzimidazole Antitumor Agents: Influence of the 7-Substituent on the Ability To Alkylate DNA and Inhibit Topoisomerase II

Ru Zhou and Edward B. Skibo*

Department of Chemistry and Biochemistry, Box 871604, Arizona State University, Tempe, Arizona 85287-1604

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This study addresses the influence the 7-substituent on the cytotoxicity of pyrrolo[1,2-*a*]-benzimidazole quinones possessing a 6-aziridinyl group (PBIs) and a 6-acetamido group (APBIs). Reduction of a PBI to the aziridinyl hydroquinone results in both nucleophile trapping (alkylation) and 1,5-sigmatropic shift reactions. The latter process is essentially an internal redox reaction wherein the hydroquinone causes reductive opening of the aziridinyl ring. The 7-substituent controls the fate of the aziridinyl ring by means of steric and electronic effects. An electron-rich 7-substituent favors the 1,5-sigmatropic shift reaction. If the 7-substituent distorts the 6-aziridinyl group from the conformation required for the 1,5-sigmatropic shift, then nucleophile trapping occurs. The 7-methyl substituent results in significant nucleophilic trapping, and the 7-unsubstituted and 7-methoxy substituents favor the 1,5-sigmatropic reaction. Thus, the 7-methyl PBIs show the most cytotoxicity of the analogues studied. The APBIs are cytotoxic only as quinones, and reduction to the hydroquinone results in loss of activity. Consistent with this observation, the change from 7-methyl to the more electron-rich 7-methoxy results in a substantial loss of APBI cytotoxicity as well as decreased topoisomerase II inhibition. The mechanism of inhibition is thought to involve the intercalation of only electron deficient APBIs into DNA.

The pyrrolo[1,2-*a*]benzimidazoles represent a new class of antitumor agent exhibiting cytotoxic activity against a variety of cancer cell lines.[1−6] Since the first reported synthesis of the pyrrolobenzimidazoles in 1990,[1] two patents have been granted[7,8] and several analogues are currently undergoing *in vivo* trials at the National Cancer Institute. Typically, the pyrrolo[1,2-*a*]benzimidazoles possess a 7-methyl substituent and either a 6-acetamido group (APBIs) or a 6-aziridinyl group (PBIs), inset of Chart 1. The PBI analogues alkylate DNA upon two-electron reduction as a result of nucleophile-mediated opening of the protonated aziridine ring.[3] In contrast, the APBIs are not reductive alkylating agents and exhibit cytotoxicity in the oxidized form.[6] In order to evaluate further the chemical and cytotoxic properties of the pyrrolo[1,2-*a*]benzimidazoles, analogues bearing 7-methoxy, 7-unsubstituted, and 7-aziridinyl substituents were prepared and studied, **1**−**4** in Chart 1. This report describes these synthetic efforts along with the results of hydrolysis studies and of cytotoxicity screening.

The results of these studies indicate that the 7-substituent greatly influences the cytotoxicity of the pyrrolo[1,2-*a*]benzimidazoles. The PBIs afford aziridinyl hydroquinones upon reduction which undergo two competing reactions: a 1,5-sigmatropic shift of hydrogen (reaction A) and nucleophile-mediated ring opening (reaction B), Chart 2. Only the latter reaction results in DNA alkylation and cytotoxicity. The 7-substituent controls the balance between the two competing reactions with the 7-methyl substituent favoring the alkylation reaction. Presumably both the electronic and steric natures of the 7-substituent control the fate of the aziridinyl ring. The 7-substituent controls the electronic character of the APBI and thereby the ability to inhibit topoisomerase II. As the APBI becomes more electron rich, or is reduced to the hydroquinone, the inhibition of this enzyme decreases. Thus the 7-methoxy APBI **3** is much less cytotoxic than the relatively electron deficient 7-methyl derivative.

Chart 1

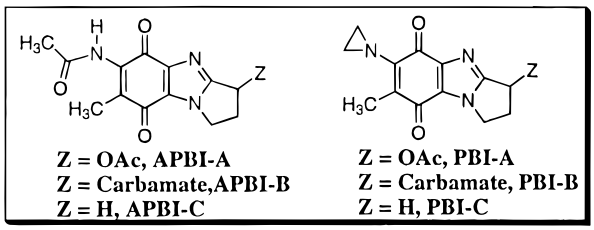
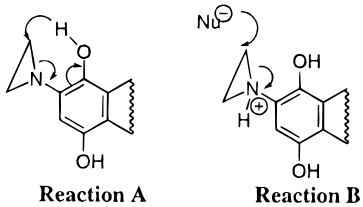


Chart 2



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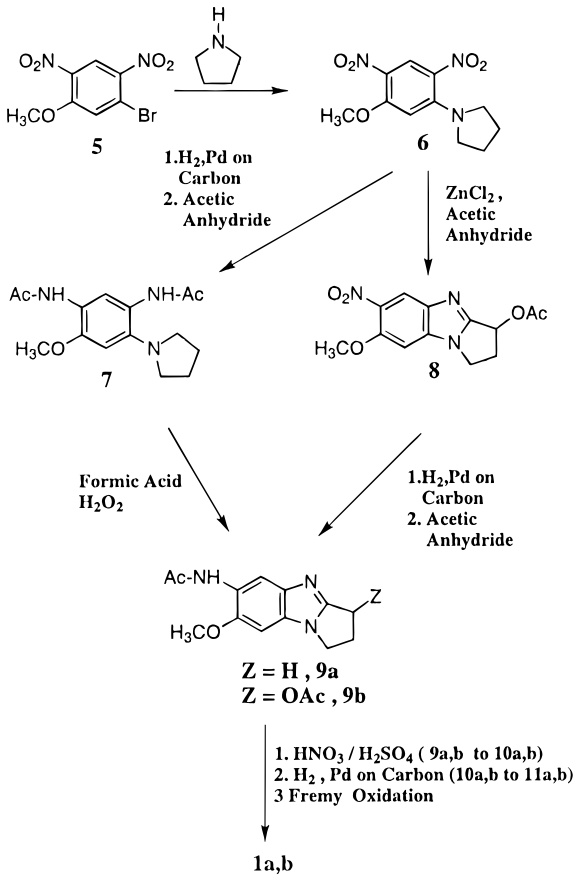
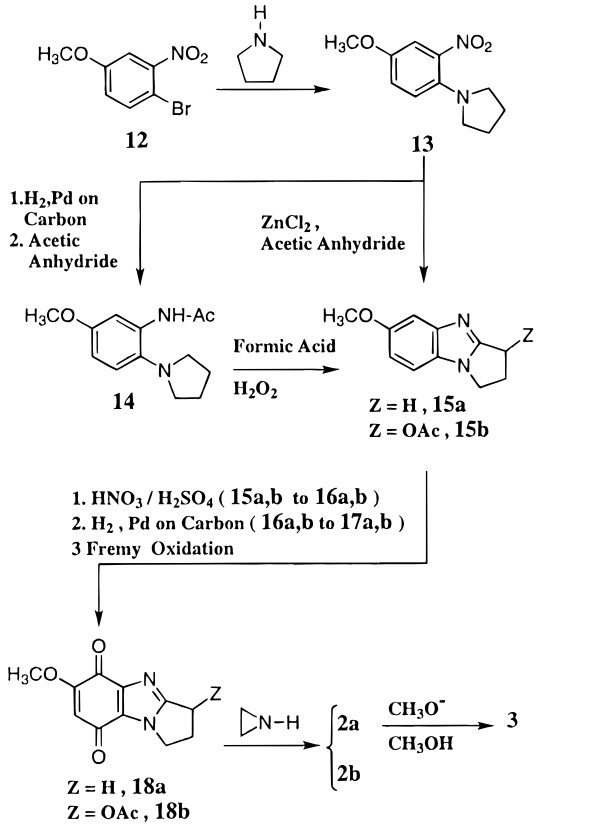
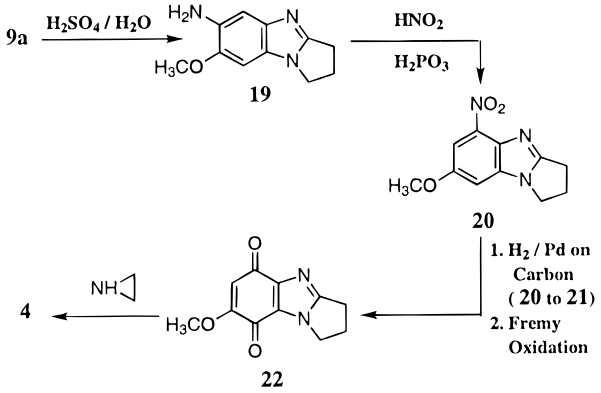
Scheme 1



Scheme 2



Scheme 3



The findings reported herein are relevant to the design of more active pyrrolo[1,2-*a*]benzimidazoles as well as to the chemistry of aziridinyl quinone antitumor agents and to the design of topoisomerase II inhibitors.

Results and Discussion

Synthesis of the Pyrrolo[1,2-*a*]benzimidazole Ring System. The presence of a substituent ortho to an *N*,*N*-dialkylaniline gives rise to the "*tert*-amino effect".[9] This effect either involves nucleophilic attack by the tertiary nitrogen or oxidation of the tertiary nitrogen to an iminium ion, which is then subjected to nucleophilic attack. Cyclizations of this type have been employed in the preparation of various benzimidazole derivatives by Meth-Cohn and co-workers.[9−11]

The ring closure reactions **7** → **9a** and **6** → **8** shown in Scheme 1 both likely occur via an iminium ion intermediate.[11] Oxidation of **7** by performic acid affords the iminium ion which is then subjected to nucleophilic attack by the adjacent acetamido nitrogen, eventually resulting in formation of the pyrrolo[1,2-*a*]benzimidazole **9a**.[9] On the basis of previous studies, the ring closure of **6** to **8** is proposed to utilize a Lewis acid-catalyzed internal redox reaction to afford the *o*-nitroso iminium ion, which undergoes ring closure.[11−13]

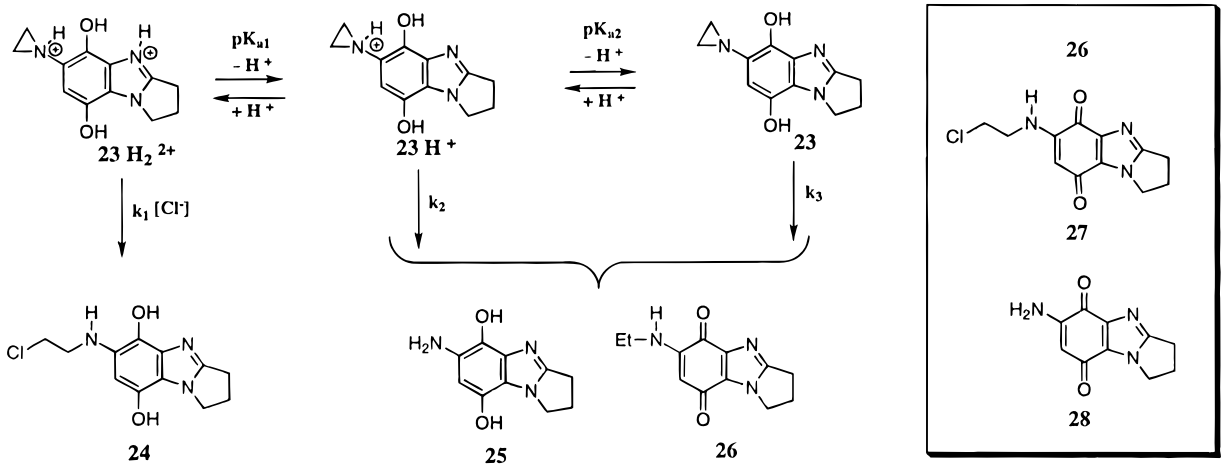
Completion of the synthesis of APBIs **1a,b**, starting from the pyrrolo[1,2-*a*]benzimidazoles **8** and **9a**, is outlined in Scheme 1. Quinone elaboration involved nitration and then reduction to the amino derivative and finally Fremy oxidation of the amine.[1]

Preparation of the PBIs **2a,b** and **3** is outlined in Scheme 2. The preparation of the pyrrolo[1,2-*a*]benzimidazoles **15a,b** was carried out as described above followed by quinone elaboration. Displacement of the methoxy group from quinones **18a,b** with aziridine afforded the target PBIs **2a,b**. Reductive addition of methoxide to **2a** in the presence of air afforded the 7-methoxy derivative **3**.

The preparation of **4**, starting with **9a**, was carried out as outlined in Scheme 3. The synthetic steps involved the acid hydrolysis of the 6-acetamido group of **9a** resulting in **19**, which was then converted to **20** in a one pot-reaction. The conversion of **20** to **4** involved reduction, Fremy oxidation, and substitution with aziridine as previously reported.[1]

Hydrolytic Chemistry of Reduced PBIs. The goal of these studies was to correlate the fate of reduced PBIs in aqueous buffer with their cytotoxicity and DNA alkylation capability. Another goal was to obtain a detailed mechanistic study of the aziridinyl hydroquinone fate. The results of this study may be pertinent to the fate of reduced aziridinyl quinones currently used in chemotherapy.

Scheme 4



The hydrolysis of reduced **2a** (**23**) was studied in anaerobic buffer (μ = 1.0, KCl) over the pH range of 2−10 at 30.0 ± 0.2 °C. Preparative anaerobic hydrolysis of **23** followed by aerobic workup revealed the presence of quinones **26**−**28** shown in Scheme 4 (see the Experimental Section). The formation of these products was pH dependent: **26** and **28** were isolated in 37% and 18% yields, respectively, at pH 5.5 and 7.4, and only **27** was isolated in 56% yield at pH 2.0. The quinone products are deep blue in color (λ_{max} = 550 nm) due to the presence of the aminoquinone chromophore. When anaerobic reactions held at pH 5.5 or 7.4 were followed by repetitive scanning in the UV−vis region, the buildup of blue product was noted (attributed to the formation of **26**). Upon completion of the anaerobic process, the reaction was opened to the air resulting in the formation of additional blue product (attributed to the oxidation of **25** to **28**). When an anaerobic reaction held at pH 2 was performed, there was no buildup of blue product indicating the formation of the hydroquinone product **24**, which oxidized to **27** upon workup.

In order to assess the influence of pH on the fate of **23**, the pH−rate profile for hydrolysis shown in plot A of Figure 1 was obtained over the pH range of 2−10. Absorbance vs time(s) measurements for hydrolysis were made at 310 nm. All absorbance vs time(s) plots were first-order in character, and the resulting k_{obsd} (s^{-1}) values were independent of buffer concentration but dependent on pH. Found in plot A of Figure 1 are log k_{obsd} vs pH data for the hydrolysis of **23**. Careful inspection of these data reveal the presence of three regions: (i) a plateau below pH 4, (ii) a plateau between pH 5 and 6 which presents as an inflection, and (iii) a plateau above pH 8. The presence of three plateaus is an indication that the three forms of **23** shown in Scheme 4 are involved in the hydrolysis reactions. The rate law for the hydrolysis of an equilibrating mixture of **23**, **23**H$^+$, and **23**H$_2^{2+}$ is shown in eq 1:

$$k_{obsd} = \frac{k_1 a_H^2 + k_2 K_{a1} a_H + k_3 K_{a1} K_{a2}}{a_H^2 + K_{a1} a_H + K_{a1} K_{a2}} \qquad (1)$$

where the rate and equilibrium constants are those shown in Scheme 4. Computer fitting of the data in plot A of Figure 1 to eq 1 provided the following solution: $k_1 = 6.37 \times 10^{-2}$ s^{-1}, $k_2 = 1.08 \times 10^{-3}$ s^{-1}, $k_3 = 1.36 \times 10^{-5}$ s^{-1}, pK_{a1} = 3.85, and pK_{a2} = 6.20. This solution was used to generate the solid line in plot A of Figure 1.

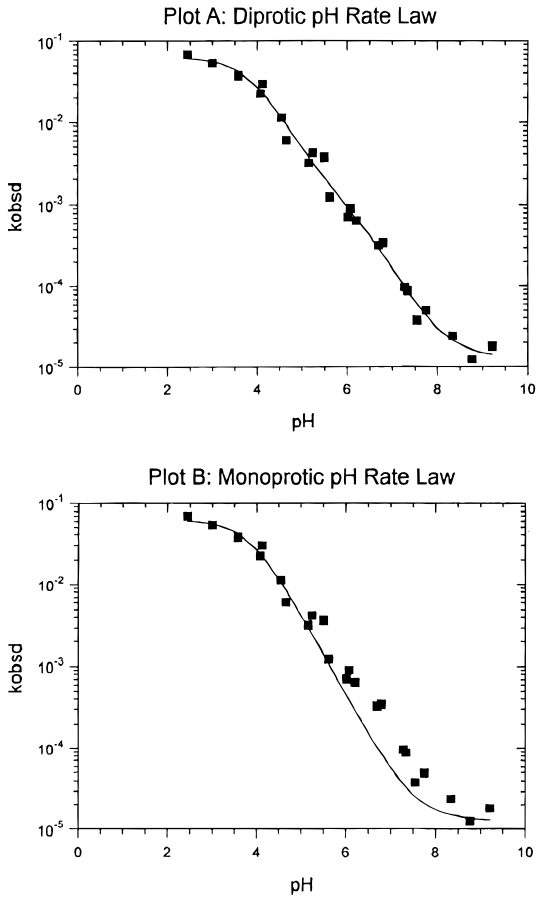
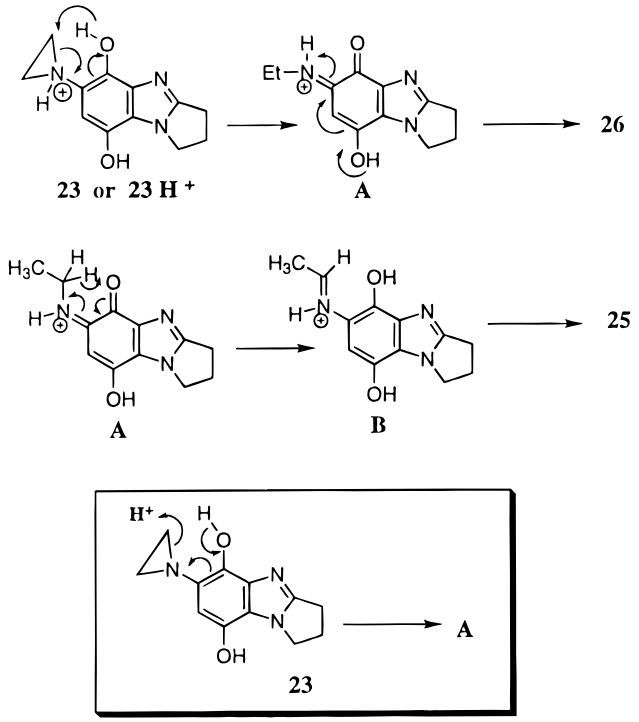


Figure 1. Plot of log k_{obsd} vs pH for the hydrolysis of **23** and its protonated forms in anaerobic aqueous buffer at μ = 1.0 (KCl) and 30.0 ± 0.2 °C.

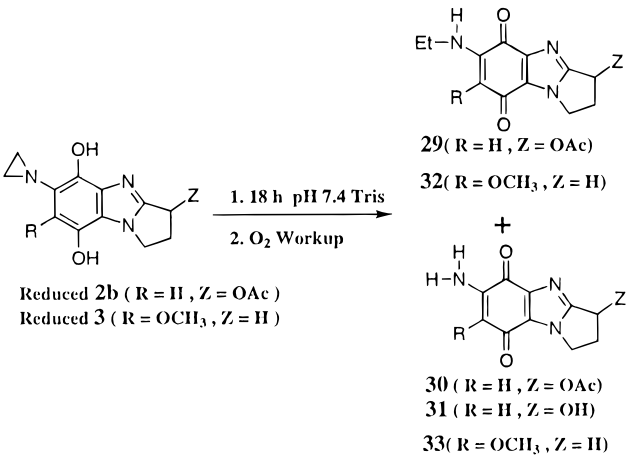
The plateau between pH 5 and 6 is difficult to see on casual inspection of the data shown. Rather than the diprotic rate law in eq 1, a monoprotic pH−rate law could conceivably fit the data. Fitting the data to $k_{obsd} = (k_1 a_H + k_2 K_{a1} a_H)/(a_H + K_{a1})$ provided the curve shown in plot B of Figure 1. The curve in this plot misses the high-pH data due to the inflection actually present between pH 5 and 6.

The pK_a assignments for the diprotic rate law in eq 1 have been made as illustrated in Scheme 4. Compound

Scheme 5



Scheme 6



23 has two basic nitrogens: the aziridinyl nitrogen and the N(4)-position. At low pH values (<3.0) both nitrogens are protonated to afford **23**H_2^{2+}. The more basic of the two nitrogens is difficult to assess, however. A protonated benzimidazole hydroquinone nitrogen (protonated N(4)-position of **23**) generally has pK_a values in the range of 4[14]−6.[1] Typically, N-protonated benzimidazoles have a pK_a value of 5.5.[15] The aziridine nitrogen is quite basic, and the pK_a values of protonated forms are usually >8.[16] The presence of an aromatic substituent on the aziridinyl nitrogen should decrease the pK_a to ∼6, based on the pK_a values of other protonated amino hydroquinones.[17] Since the aziridinyl nitrogen may be somewhat more basic than the N(4)-position of **23**, the structure of **23**H^+ shows the proton on this nitrogen. The presence of aziridinyl alkylation reactions by reduced PBIs near neutrality,[6] in fact, suggests the presence of a protonated aziridinyl nitrogen, which would be required for the nucleophile-mediated opening of the aziridine ring to occur.[18]

The pH−rate profile and product studies indicate that both **23** and **23**H^+ involve the formation of intermediate **A** by a 1,5-sigmatropic shift of hydrogen as illustrated in Scheme 5. A prototropic shift converts **A** to the quinone **26**, and oxidative dealkylation of **A** via intermediate **B** affords **25**. The conversion of **23** or **23**H^+ to **A** is essentially an internal redox reaction wherein the hydroquinone brings about the reductive opening of the aziridine ring. Protonation of the N(4)-position will result in an electron deficient hydroquinone incapable of reducing the aziridine ring. Therefore, **23**H_2^{2+} only traps chloride nucleophile to afford **24**.

The mechanism for the formation of intermediate **A** will now be discussed. There are two possible mechanisms: the concerted, sigmatropic process already discussed and the prototropic process shown in the inset of Scheme 5.[13] The observation that **23** decomposes in the neutral form at $k_3 = 1.36 \times 10^{-5}$ s^{-1} favors a concerted mechanism which does not require external protons. Consistent with a concerted process, neutral **23** decomposes to **26** in dry anaerobic dimethyl sulfoxide at approximately k_3 (s^{-1}).[13] The decomposition of **23**H^+ could involve the prototropic process shown in the inset of Scheme 5 where the transition state consists of an external proton and **23**. If this is the case, eq 1 must be rewritten as eq 2, where k_2 is a second-order rate constant:

$$k_{obsd} = \frac{k_1 a_H^2 + k_2 K_{a1} K_{a2} a_H + k_3 K_{a1} K_{a2}}{a_H^2 + K_{a1} a_H + K_{a1} K_{a2}} \qquad (2)$$

This equation is kinetically equivalent to eq 1 and still fits the data shown in plot A of Figure 1. The value of k_2 obtained from the computer fit is 1733 M^{-1} s^{-1}.

Alternatively, **23**H^+ may spontaneously decompose at $k_2 = 1.08 \times 10^{-3}$ s^{-1} by a sigmatropic process. The 79-fold rate enhancement in the sigmatropic process (k_2 (s^{-1}) compared to k_1 (s^{-1}) in eq 1 for the decomposition **23**H^+ and **23**, respectively) would be the result of the transformation of a localized cation (**23**H^+) to a more stabilized delocalized cation (**A**). An analogy to this rate enhancement is the oxy Cope rearrangement, where a large change in oxygen anion stability accelerates the reaction.[19]

Although the two possible decomposition mechanisms for **23**H^+ cannot be rigorously distinguished, we favor the sigmatropic shift mechanism. One reason is the presence of this mechanism in the decomposition of **23** which suggests that **23**H^+ could likewise react in this fashion. Furthermore, if an external proton were involved (prototropic mechanism) in the reaction, general acids should participate in the prototropic process. However, the rate law for the decomposition of **23**, eq 1, does not contain a buffer catalysis term. Finally, the sigmatropic shift mechanism explains the differences in alkylation reactivity observed between the various 7-substituted PBIs (see the next section).

In order to determine the influence of the 3- and 7-substituents on the course of reduced PBI decomposition, the preparative hydrolysis of reduced **2b** and reduced **3** was carried out in anaerobic Tris buffer, Scheme 6. Reduced **2b** differs from reduced **2a** (**23**) only with respect to the presence of a 3-acetoxy substituent. Preparative hydrolysis of reduced **2b** afforded **29** and **30** in 31% and 40% yields, respectively. An additional

Table 1. Mean log GI_{50}, log TGI, and log LC_{50} Values Obtained from 60 Cancer Cell Line Mean Graphs for Substituted PBIs

PBI	log GI_{50}	log TGI	log LC_{50}
PBI-C (Chart 1)	−7.74	−6.98	−5.69
PBI-A (Chart 1)	−7.92	−7.50	−6.61
2a	−6.44	−5.87	−5.26
2b	−6.57	−5.97	−5.28
3	−6.15	−5.60	−4.87
4	>−4	>−4	>−4

product, **31** (8%), resulted from the ester hydrolysis of **30**. Reduced **3** differs from **23** only with respect to the presence of a 7-methoxy substituent. The products resulting from the hydrolysis of reduced **3** are structurally similar to those obtained from **23**: **32** (22%), **33** (11%), and unreacted **3** (15%). The results cited above indicate that reduced **2b** and reduced **3** form hydrolysis products structurally similar to those formed from reduced **2a** (**23**).

Comparative Hydrolytic Chemistry and Cytotoxicity of 7-Substituted PBIs. The results cited in the previous section indicate that the 7-unsubstituted and the 7-methoxy PBIs share identical hydrolytic chemistry upon reduction. It is noteworthy that neither system traps nucleophiles at neutrality. For example, **23** did not trap water nor did it alkylate 5′-dAMP or calf thymus DNA in anaerobic pH 7.4 Tris buffer (see the Experimental Section). Since the electron-releasing effect of methyl lies between that of the -H and -OCH_3 substituents, the 7-methyl PBIs should behave like the 7-unsubstituted and 7-methoxy PBIs upon reduction. Verification that the 7-methyl PBI is more electron rich than the 7-unsubstituted derivative was obtained by measuring the E_m (NHE) for two-electron reduction of both PBI-C (Chart 1) and **2a** in pH 7.4 buffer ($\mu = 1.0$, $NaClO_4$). PBI-C possesses an E_m value of −50 mV, whereas **2a** possesses an E_m value of −20 mV at this pH. Electron release by the methyl is expected to decrease the E_m value of the quinone.[20]

In contrast to the 7-unsubstituted and the 7-methoxy PBIs, the reduced 7-methyl PBIs are alkylating agents and trap water as well as the phosphates of 5′-dAMP and calf thymus DNA.[3,4,6] The alkylation of DNA followed by aerobic workup affords a blue product due to the presence of the aminoquinone chromophore. The DNA alkylation reaction is responsible for the cytotoxicity/antitumor activity of the 7-methyl PBIs.[3] Consistent with their apparent lack of alkylation activity, the 7-unsubstituted and 7-methoxy PBIs possess significantly less cytotoxicity than the 7-methyl analogues.

Shown in Table 1 are the cytotoxicity parameters for various 7-substituted PBI analogues. The GI_{50} and TGI are the concentrations of PBI required to inhibit cancer cell growth by 50% and 100%, respectively. The LC_{50} is the concentration of PBI required to cause 50% cancer cell lethality. The GI_{50}, TGI, and LC_{50} values in Table 1 are mean values obtained from 60 cancer cell line assays carried out at the National Cancer Institute.[21] Lower cytotoxicity parameters represent greater potency as a cytotoxic agent. The data in Table 1 show that the 7-methyl analogues (PBI-C and PBI-A) are more potent than either the 7-unsubstituted PBIs (by 1 order of magnitude) or the 7-methoxy PBI (by 2 orders of magnitude). The placement of the aziridinyl group in the 7-position is known to result in a substantial decrease in PBI cytotoxicity.[4] Indeed, the 7-aziridinyl derivative **4** was found to be inactive in all cancer cell lines.

Table 2. Mean log GI_{50}, log TGI, and log LC_{50} Values from 60 Cancer Cell Line Mean Graphs for Substituted APBIs

APBI	log GI_{50}	log TGI	log LC_{50}
APBI-A	−5.80	−5.32	−4.73
APBI-C	−5.84	−5.43	−4.97
1b	−4.73	−4.41	−4.13

An explanation for the anomalous behavior of the 7-methyl PBIs invokes possible steric effects by the 7-substituent on the rotational conformation of the aziridinyl group as well as the 1,5-sigmatropic shift of hydrogen discussed in the previous section. The fate of a reduced PBI pertains to nucleophile trapping in competition with the 1,5-sigmatropic shift. Indeed, hydrolytic reactions of reduced 7-methyl PBIs provide both types of products.[5,6] The 1,5-sigmatropic shift should be sensitive to the conformation of the aziridinyl group, which must be positioned correctly for the pericyclic reaction involving the hydroquinone ring. If the 7-substituent interferes with this conformation, then the competing nucleophile-trapping reaction will be favored. Therefore, the steric influence of the 7-methyl substituent may be responsible for the nucleophile-trapping reactions and hence the cytotoxicity/antitumor activity of the PBIs. The methoxy substituent is approximately the same size as the methyl substituent, and therefore **3** should trap nucleophiles upon reduction. However, reduced **3** only undergoes the 1,5-sigmatropic shift perhaps because the electron-releasing methoxy substituent does not favor the trapping of a nucleophile.

Work recently submitted for publication from this laboratory featured the 7-*n*-butyl PBIs. Our steric interpretation presented above correctly predicted that this PBI derivative would reductively alkylate DNA. However, the bulky 7-*n*-butyl substituent slowed reductive activation by DT-diaphorase and substantially decreased the cytotoxicity of 7-*n*-butyl PBIs.

Comparative Cytotoxicity of the 7-Substituted APBIs. The APBIs are cytotoxic as the quinones, and cellular reduction to the hydroquinone by DT-diaphorase results in complete loss of activity.[6] It has been observed that the quinone derivatives intercalate DNA and the electron-rich hydroquinones do not.[22] If the electron-rich character of the APBI is important in DNA intercalation and cytotoxicity, then more electron-rich APBIs (i.e., 7-methoxy derivatives) should be less cytotoxic than the 7-methyl derivatives. The cytotoxic parameters of the 7-methoxy APBIs (**1a,b**) are, in fact, 1 order of magnitude greater (less active) than those of the 7-methyl analogues, Table 2.

A recent review presented evidence that the APBI intercalation of DNA prevents topoisomerase II relaxation of supercoiled DNA by inhibiting the double-strand cleavage step leading to the enzyme−DNA complex.[22] Intercalating agents are known to inhibit topoisomerase I and II resulting in antitumor activity.[23] Indeed, the activity of the topoisomerases is important in cell division, and many types of cancer possess elevated levels of these enzymes.[24] Examples of topoisomerase II inhibitors that possess structural similarities to the APBIs (presence of the benzimidazole ring or the presence of a quinonoid system) are known in

the literature.[25−27] Unlike the APBIs, these inhibitors stabilize the enzyme−DNA complex formed upon double-strand cleavage by topoisomerase II and thereby inhibit the religation to relaxed DNA. The result is the formation of linear DNA upon treatment of the complex with SDS/proteinase K. There is another class of compounds[28] that behave like the APBIs and inhibit the double-strand cleavage step leading to the DNA−enzyme complex. The antitumor activity of these topoisomerase inhibitors[22,28] suggests that compounds of this type could be useful in cancer chemotherapy.

In contrast to other APBIs, which inhibit topoisomerase II-mediated relaxation of SV-40 DNA at concentrations ≥0.3 mM,[22] **1a** caused little inhibition at concentrations ≥0.6 mM. The presence of the relatively electron-rich PBI-A resulted in no inhibition of topoisomerase II-mediated relaxation of the DNA.

These results support the proposal that electron deficient APBIs are required for intercalation into DNA and topoisomerase II inhibition. The electron-rich analogues **1a** and PBI-A show little or no inhibition of topoisomerase II, while the more electron deficient APBIs inhibit the enzyme.

Conclusions

Conclusions are drawn with respect to the influence of the 7-substituent on the cytotoxicity of pyrrolo[1,2-*a*]benzimidazole antitumor agents. These conclusions will be of value in the design of new PBI and APBI analogues as antitumor agents.

The ring-opening reactions of aziridines usually involve N-protonation followed by nucleophilic attack.[18] Aziridinyl quinone antitumor agents are known to alkylate DNA by this process upon reduction to the hydroquinone.[29] In the present report, we show that PBI aziridinyl hydroquinones can also undergo a 1,5-sigmatropic transfer of hydrogen. This process is essentially an internal redox reaction wherein the electron-rich hydroquinone brings about reductive ring opening of the aziridinyl group. This process appears to be influenced by the adjacent 7-substituent, which may control the favored rotational conformations of the 6-aziridinyl group as well as control the electronic character of the hydroquinone ring. In order for the 1,5-sigmatropic reaction to occur, the aziridinyl group must achieve a particular conformation with respect to the hydroquinone ring. If the 7-substituent interferes with this conformation, then the competing nucleophile-trapping process can occur. An electron-rich hydroquinone ring, as in reduced **3**, would favor the 1,5-sigmatropic shift by increasing the reducing capability of this ring. Ongoing work in this laboratory with aziridinyl benzoquinones related to known antitumor agents[29] showed that hydrolysis products, which could arise by the 1,5-sigmatropic shift reaction, are formed upon reduction. Future publications from this laboratory will show that hydrolysis products of this type are actually common.

From the results of the hydrolytic study, it is apparent that the 7-substituent of the PBI hydroquinone controls the fate of the aziridinyl group: nucleophile trapping (alkylation) vs the 1,5-sigmatropic shift. Therefore, the influence of the 7-substituent is an important factor in PBI cytotoxicity, which relies on DNA alkylation. The 7-methyl PBI analogue undergoes aziridinyl alkylation reactions upon hydroquinone reduction, whereas the 7-unsubstituted and the 7-methoxy analogues predominately undergo the 1,5-sigmatropic reaction. Correspondingly, the 7-methyl-substituted PBIs are the most cytotoxic of these analogues. We conclude that new cytotoxic PBI analogues must have the 7-methyl substituent.

The influence of the 7-substituent on the cytotoxicity of the APBI agents is also discussed. The change from the 7-methyl to the 7-methoxy is accompanied by a 10-fold decrease in cytotoxicity as well as a decrease in topoisomerase II inhibition. The conclusion is that increasing the electron-rich character of APBIs will not lead to effective antitumor agents. Currently, electron deficient APBIs, which cannot be reduced by DT-diaphorase, are being developed.

Experimental Section

All analytically pure compounds were dried under high vacuum in a drying pistol heated with refluxing methanol. Some compounds contained water or chloroform of crystallization that was determined from the elemental analyses found. The presence of chloroform was verified by an ^{1}H NMR spectrum in dimethyl sulfoxide-d_6. Elemental analyses were run at Atlantic Microlab, Inc., Norcross, Ga. Uncorrected melting points 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.

Electrochemistry. The determination of the E_m value was carried out with BAS 27 voltammograph. Measurements were carried out in μ = 1.0 ($NaClO_4$) aqueous buffer at 25−26 °C under an atmosphere of argon with a graphite mull working electrode and with a BAS Ag/AgCl gel electrode as reference. The electrode was calibrated against the E_o value of the benzoquinone/hydroquinone couple (699 mV, NHE). The midpount potential E_m was determined from the average of the anodic ($E_{p,a}$) and cathodic ($E_{p,c}$) potentials.

Kinetic Studies. The kinetic studies were carried out in buffers prepared with doubly distilled water and adjusted to μ = 1.0 with KCl. The following buffer systems were employed to hold pH: acetic acid/acetate (pK_a = 4.61), phosphate monobasic/phosphate dibasic (pK_a = 6.56), and boric acid/borate (pK_a = 9.2). These pK_a values were obtained at 30.0 ± 0.2 °C in μ = 1.0 (KCl) aqueous solutions. Measurements of pH were made with a combination electode. The hydrolytic studies of the hydroquinones were carried out in anaerobic aqueous buffers employing Thunberg cuvettes. A dimethyl sulfoxide stock of the hydroquinone to be studied was prepared fresh, and 100 μL of this stock was added to 2.90 mL of buffer. The hydroquinone stock solutions were prepared by dissolving 2.5 mg of quinone in 2 mL of dimethyl sulfoxide containing 5 mg of Pd on carbon. This mixture was purged with argon and then purged with H_2 gas until the solution became colorless. The reduced solution was placed in a glovebox and filtered through a Millex-SR filter. The absorbance vs time data were collected on a UV−vis spectrophotometer in thermostated cells held at 30.0 ± 0.2 °C. These data were computer-fit to a single first-order rate law.

Preparation of New Compounds and Their Physical Properties. 5-Bromo-2,4-dinitroanisole (5). To 50 mL of fuming nitric acid frozen at −78 °C was added 14.8 g (79.5 mmol) of 3-bromoanisole over 2 min, and the mixture was allowed to warm to 10 °C over 25 min. The reaction mixture was then poured onto ice and the resulting precipitate collected and dried. Recrystallization from ethanol afforded **5**: 13.6 g (62%) yield; mp 83 °C; TLC (chloroform/hexane, 1:1) R_f = 0.45; IR (KBr pellet) 3106, 1601, 1582, 1534, 1481, 1435, 1339, 1275, 1186, 1105, 1015, 949, 903, 826 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.63

and 7.27 (2H, 2s, aromatic protons), 4.10 (3H, s, methoxy); MS (EI mode) m/z 277 (M^+). Anal. ($C_7H_5BrN_2O_5$) C, H, Br, N.

2,4-Dinitro-5-(*N*-pyrrolidino)anisole (6). To a solution of 9 g (32.6 mmol) of **5** in 200 mL of ethanol was added 13.5 mL of pyrrolidine over 1 min. The mixture was allowed to sit at room temperature for 1 h, and then the yellow precipitate was filtered off and dried. Recrystallization from ethanol afforded **6**: 4.95 g (57%) yield; mp 151.5−152.5 °C; TLC (chloroform) $R_f = 0.26$; IR (KBr pellet) 3447, 3090, 2980, 2943, 2868, 1611, 1564, 1507, 1452, 1339, 1316, 1273, 1078, 1001, 920, 872, 804 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.69 and 6.23 (2H, 2s, aromatic protons), 4.00 (3H, s, methoxy), 3.34 (4H, m, methylenes adjacent to pyrrolidine nitrogen), 2.06 (4H, m, other pyrrolidine methylenes); MS (EI mode) m/z 267 (M^+). Anal. ($C_{11}H_{13}N_3O_5$) C, H, N.

2,4-Diacetamido-5-(*N*-pyrrolidino)anisole (7). A solution of 5 g (18.7 mmol) of **6** in 25 mL of acetic anhydride and 5 mL of acetic acid was shaken under 50 psi of H_2 in the presence of 200 mg of 5% Pd on carbon for 10 h. The completed reaction was filtered through Celite and the filtrate diluted with 500 mL of diethyl ether. The resulting white precipitate was filtered off and dried under vacuum. Recrystallization from chloroform/hexane gave **7**: 4.2 g (78%) yield; mp 203 °C; TLC (chloroform/methanol, 95:5) $R_f = 0.28$; IR (KBr pellet) 3277, 2969, 2868, 1641, 1545, 1507, 1445, 1414, 1370, 1261, 1213, 1146, 1032, 1110, 604 cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6) δ 9.06 and 8.91 (2H, 2s, amide protons), 7.53 and 6.39 (2H, 2s, aromatic protons), 3.79 (3H, s, methoxy), 3.33 (4H, m, methylenes adjacent to pyrrolidine nitrogen), 2.00 and 1.96 (6H, 2s, acetyl methyls), 1.83 (4H, m, other pyrrolidine methylenes); MS (EI mode) m/z 291 (M^+), 248 (M^+ − acetyl), 233 (M^+ − acetamido). Anal. ($C_{15}H_{21}N_3O_3 \cdot 0.2H_2O$) C, H, N.

3-Acetoxy-7-methoxy-6-nitro-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole (8). A mixture consisting 5.34 g (20 mmol) of **6**, 5.44 g (40 mmol) of anhydrous $ZnCl_2$, and 20 mL of acetic anhydride was refluxed at 90−100 °C for 4 h. The reaction mixture was cooled, diluted with 100 mL of water, and then extracted with 3 × 100 mL portions of chloroform. The dried extracts (sodium sulfate) were concentrated to a brown residue, which was flash chromatographed on a silica gel column using ethyl acetate/methanol (95:5) as the eluant. Evaporation of the eluant afforded the pure product, which was recrystallized from ethyl acetate/hexane: 940 mg (16.2% yield); mp 164 °C; TLC (ethyl acetate/methanol, 95:5) $R_f = 0.38$; IR (KBr pellet) 3596, 3416, 3092, 2945, 1740, 1640, 1588, 526, 1435, 1356, 1317, 1246, 1704, 1208, 986, 837, 760 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.27 and 6.92 (2H, 2s, aromatic protons), 6.16 (1H, dd, $J = 7.6$, 3.4 Hz, C(3) proton), 4.29 and 4.14 (2H, 2m, C(1) diastereomeric methylene), 3.98 (3H, s, methoxy), 3.21 and 2.70 (2H, 2m, C(2) diastereomeric methylene), 2.12 (3H, s, acetate methyl); MS (EI mode) m/z 291 (M^+), 249 (M^+ − ketene), 234 (M^+ − ketene and methyl). Anal. ($C_{13}H_{13}N_3O_5$) C, H, N.

6-Acetamido-7-methoxy-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole (9a). A mixture of 2.5 g (8.59 mmol) of **7**, 14 mL of 96% formic acid, and 7 mL of 30% hydrogen peroxide was stirred at 50 °C for 30 min. The reaction mixture was then diluted with water and neutralized to pH 7.00 with concentrated ammonium hydroxide. After the neutralized solution was chilled, the precipitated crystals were filtered off and dried. Recrystallization from chloroform/hexane afforded **9a**: 1.1 g (52.2%) yield; mp 217 °C; TLC (chloroform/methanol, 9:1) $R_f = 0.54$; IR (KBr pellet) 3348, 1674, 1593, 1530, 1479, 1460, 1441, 1254, 1204, 1105, 953, 880, 804 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.68 and 6.77 (2H, 2s, aromatic protons), 7.75 (1H, bs, amide protons), 4.06 (2H, t, $J = 7.6$ Hz, C(1) methylene), 3.93 (3H, s, methoxy), 3.03 (2H, t, $J = 7.6$ Hz, C(3) methylene), 2.70 (2H, quint, $J = 7.5$ Hz, C(2) methylene), 2.22 (3H, s, acetamido methyl); MS (EI mode) m/z 245(M^+), 203 (M^+ − ketene), 188 (M^+ − acetamido). Anal. ($C_{13}H_{15}N_3O_2$) C, H, N.

6-Acetamido-3-acetoxy-7-methoxy-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole (9b). A solution of 180 mg (0.62 mmol) of **8** in 50 mL of methanol was shaken under 50 psi of H_2 in the presence of 80 mg of 5% Pd on carbon for 5 h. The completed reaction was filtrated through Celite into a flask containing 0.35 mL of acetic acid, and the filtrate was evaporated *in vacuo* to remove the methanol. The residue was combined with 1.0 mL of acetic anhydride and then stirred for 30 min at room temperature. The completed reaction was diluted with 40 mL of diethyl ether and chilled in a refrigerator overnight resulting in crystallization of **9b**. Recrystallization from ethyl acetate/hexane afforded pure **9b**: 154 mg (82%) yield; TLC (chloroform/methanol, 9:1) $R_f = 0.33$; mp 216−218 °C; IR (KBr pellet) 3314, 2936, 1744, 1670, 1591, 1539, 1478, 1414, 1368, 1256, 1227, 1207, 1105, 1017, 886, 732, 624 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.78 and 6.79 (2H, 2s, aromatic protons), 7.75 (1H, s, amine proton), 6.13 (1H, dd, $J = 7.5$, 3.1 Hz, C(3) proton), 4.21and 4.07 (2H, 2m, C(1) diastereomeric methylene), 3.92 (3H, s, methoxy), 3.15 and 2.63 (2H, 2m, C(2) diastereomeric methylene), 2.20 (3H, s, acetate methyl), 2.10 (3H, s, acetamido methyl); mass sprectrum (EI mode) m/z 303 (M^+), 260 (M^+ − acetyl), 244 (M^+ − acetate), 202 (M^+ − acetate and ketene), 187 (M^+ − acetate, ketene, and methyl). Anal. ($C_{15}H_{17}N_3O_4$) C, H, N.

6-Acetamido-7-methoxy-8-nitro-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole (10a). To a mixture of 3.5 mL of fuming nitric acid and 0.5 mL of concentrated sulfuric acid, chilled at −20 °C, was added 376 mg (2.0 mmol) of **9a**. The reaction mixture was stirred for 10 min and then poured into 60 mL of cold water. The mixture was neutralized carefully to pH 7.00 using saturated sodium bicarbonate solution and extracted with 3 × 100 mL portions of chloroform followed by drying (sodium sulfate) of the extracts. Evaporation of the solvent afforded a yellow solid which was a mixture of the 8-nitro isomer with a trace of the 5-nitro isomer. Chromatography on silica gel employing chloroform/methanol (9:1) as the eluant afforded the 8-nitro isomer **10a**: 336 mg (58%) yield; TLC (chloroform/methanol, 9:1) $R_f = 0.19$; mp 254−256 °C; IR (KBr pellet) 2986, 1663, 1530, 1470, 1414, 1366, 1262, 1049, 748 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.36 (1H, bs, amide protons), 6.97 (1H, s, aromatic proton), 4.11 (2H, t, $J = 7.1$ Hz, C(1) methylene), 3.91 (3H, s, methoxy), 3.08 (2H, t, $J = 7.7$ Hz, C(3) methylene), 2.75 (2H, quint, $J = 7$ Hz, C(2) methylene), 2.15 (3H, s, acetamido methyl); MS (EI mode) m/z 290 (M^+), 248 (M^+ − ketene), 244 (M^+ − nitro), 233 (M^+ − acetamido). Anal. ($C_{13}H_{14}N_4O_4 \cdot 0.85CHCl_3$) C, H, N.

6-Acetamido-3-acetoxy-7-methoxy-8-nitro-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole (10b). To a mixture of 1 mL of fuming nitric acid and 0.15 mL of sulfuric acid, chilled in an ice−water bath (−20 °C), was added 195 mg (0.65 mmol) of **9b**. The reaction mixture was stirred in the ice−water bath for 10 min and then poured over a mixture 20 mL of chloroform and 20 g of ice. The pH of this mixture was adjusted to 7.0 by adding saturated sodium bicarbonate. The chloroform layer was separated, and the aqueous layer was then extracted with 3 × 30 mL portions of chloroform. The combined chloroform extracts were dried (sodium sulfate) and evaporated to a residue. Chromatography on silica gel employing chloroform/methanol (9:1) as the eluant afforded **10b**. Recrystallization was carried out from chloroform: 132 mg (58%) yield; TLC (ethyl acetate/methanol, 95:5) $R_f = 0.50$; mp 242−244 °C; IR (KBr pellet) 3341, 3362, 2928, 2361, 1750, 1701, 1535, 1478, 1371, 1223, 1111, 1055, 966, 828 cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6) δ 9.67 (1H, s, amine proton), 7.61 (1H, s, aromatic proton), 6.11 (1H, dd, $J = 7.7$, 3.5 Hz, C(3) proton), 4.25 (2H, m, C(1) diastereomeric methylene), 3.90 (1H, s, methoxy), 3.14 and 2.63 (2H, m, C(2) diastereomeric methylene), 2.08 (1H, s, acetate methyl), 1.99 (1H, s, acetamido methyl); MS (EI mode) m/z 348 (M^+), 306 (M^+ − ketene), 302 (M^+ − nitro), 289 (M^+ − acetate). Anal. ($C_{15}H_{16}N_4O_6 \cdot 0.90CHCl_3$) C, H, N.

6-Acetamido-8-amino-7-methoxy-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole (11a). A solution of 200 mg (0.69 mmol) of **10a** in 20 mL of methanol was shaken under 50 psi of H_2 in the presence of 60 mg of 5% Pd on carbon for 16 h. The catalyst was removed by filtration through Celite, and the solvent was removed *in vacuo*, 109 mg (51.8%) yield. Further purification of the unstable amine was not attempted, and it was carried directly to the next step.

6-Acetamido-3-acetoxy-8-amino-7-methoxy-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole (11b). A suspension of 205 mg (0.59 mmol) of **10b** and 50 mL of methanol was shaken under 50 psi of H_2 in the presence of 100 mg of 5% Pd on

carbon for 12 h. The catalyst was removed by filtration through Celite, and the solvent was removed *in vacuo*, 170 mg (90%) yield. Further purification of the unstable amine was not attempted, and it was carried directly to the next step.

6-Acetamido-7-methoxy-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]-benzimidazole-5,8-dione (1a). To a suspension of 109 mg (0.42 mmol) of **11a** in 10 mL of water containing 200 mg of potassium phosphate monobasic was added a solution of 1.0 g of Fremy's salt in 30 mL of water containing 500 mg of potassium phosphate monobasic (pH = 3.0). The reaction mixture was stirred at room temperature for 1.5 h and then extracted with 3 × 30 mL portions of chloroform. The extracts were dried (sodium sulfate) and concentrated to afford a brick-red solid. Purification was carried out by chromatography on silica gel employing chloroform/methanol (9:1) as the eluant. Recrystallization from chloroform/hexane afforded 34 mg (18%) yield: mp 182−184 °C; TLC (chloroform/methanol, 9:1) R_f = 0.43; IR (KBr pellet) 3403, 3156, 2986, 1663, 1530, 1470, 1418, 1366, 1262, 1227, 1184, 1111, 1049, 748 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.14 (1H, s, amide proton), 4.23 (2H, t, J = 7.2 Hz, C(1) methylene), 4.09 (3H, s, methoxy), 2.95 (2H, t, J = 7.5, C(3) methylene), 2.71 (2H, quint, J = 7 Hz, C(2) methylene), 2.19 (3H, s, acetamido methyl); MS (EI mode) m/z 275 (M^+), 233 (M^+ − ketene), 218 (M^+ − ketene and methyl). Anal. ($C_{13}H_{13}N_3O_4$) C, H, N.

6-Acetamido-3-acetoxy-7-methoxy-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole-5,8-dione (1b). To a suspension of **11b**, 170 mg (0.53 mmol), in 10 mL of water containing 200 mg of potassium phosphate monobasic was added a solution of 1.0 g of Fremy's salt in 30 mL of water containing 500 mg of potassium phosphate monobasic (pH = 3.0). The reaction mixture was stirred for 5 h and then extracted with 3 × 50 mL portions of chloroform. The extracts were dried (sodium sulfate) and concentrated, and the residue was chromatographed on silica gel using chloroform/methanol (9:1) as the eluant. Recrystallization from chloroform/ hexane afforded pure **1b**: 42 mg (21.4%) yield; TLC (chloroform/methanol, 9:1) R_f = 0.48; mp 166−168 °C; IR (KBr pellet) 3459, 3225, 3005, 2961, 1736, 1687, 1655, 1595, 1530, 1370, 1327, 1236, 1047, 748, 725, 594 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.17 (1H, s, amide proton), 6.06 (1H, dd, J = 7.7, 3.0 Hz, C(3) proton), 4.31 (2H, m, C(1) diastereomeric methylene), 4.10 (3H, s, methoxy), 3.15 and 2.63 (2H, 2m, C(2) diastereomeric methylene), 2.19 (3H, s, acetate methyl), 2.08 (3H, s, acetamido methyl); MS (EI mode) m/z 333 (M^+), 291 (M^+ − ketene), 276 (M^+ − ketene and methyl). Anal. ($C_{15}H_{15}N_3O_6$) C, H, N.

3-Nitro-4-(*N*-pyrrolidino)anisole (13). A solution consisting of 4.0 g (17.3 mmol) of 4-bromo-3-nitroanisole (**12**) and 6 mL of pyrrolidine (69.2 mol) in 10 mL of ethanol was heated at reflux for 30 h. The solvent was then removed under vacuum, and the residue was poured over 200 g of cracked ice. The resulting mixture was extracted with 3 × 50 mL portions of chloroform. The dried extracts (sodium sulfate) were concentrated to an oily residue, which was placed on a silica gel flash column. The product was eluted with ethyl acetate/methanol (95:5). Evaporation of the eluant afforded a red oil which slowly solidified in the refrigerator: 3.67 g (96%) yield; TLC (ethyl acetate/methanol, 95:5) R_f = 0.65; mp 53−54 °C; IR (KBr pellet) 2988, 2947, 2859, 1561, 1518, 1489, 1462, 1364, 1333, 1269, 1221, 1159, 1069, 1044, 918, 872, 808, 743, 631 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.31 (1H, d, J_{meta} = 3.1 Hz, aromatic C(2) proton), 7.14 and 6.83 (2H, abx, J_{ortho} = 9.2 Hz, J_{meta} = 3.1 Hz, J_{para} = 0 Hz, C(5) and C(6) protons), 3.79 (3H, s methoxy), 3.18 (4H, m, pyrrolidine methylene adjacent to nitrogen), 1.98 (4H, m, other pyrrolidine methylenes); MS (EI mode) m/z 222 (M^+), 205 (M^+ − OH), 175 (M^+ − OH and NO). Anal. ($C_{11}H_{14}N_2O_3 \cdot 0.2H_2O$) C, H, N.

3-Acetamido-4-(*N*-pyrrolidino)anisole (14). A mixture consisting of 3.2 g (14.4 mmol) of **13**, 750 mg of 5% Pd on carbon, 25 mL of acetic anhydride, and 3.2 mL of acetic acid was shaken under 50 psi of H_2 for 2 h. The completed reaction was filtered through Celite. Concentration of the filtrate *in vacuo* afforded a brownish oil. Chromatography of this oil on silica gel employing ethyl acetate/methanol (95:5) as the eluant afforded the product as a yellow oil: 3.3 g (98%) yield; TLC (ethyl acetate/methanol, 95:5) R_f = 0.62; IR (neat) 3318, 2963, 2876, 2834, 1669, 1613, 1370, 1040, 945, 862, 806, 754 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.550 (1H, br s, amide proton), 8.06 (1H, d, J_{meta} = 2.7, Hz, aromatic C(2) proton), 7.51 and 6.50 (2H, abx, J_{ortho} = 8.8 Hz, J_{meta} = 2.3 Hz, J_{para} = 0 Hz), 3.80 (3H, s, methoxy), 2.95 (4H, m, pyrrolidine methylene adjacent to nitrogen), 2.19 (3H, s, acetamido methyl), 1.952 (4H, m, other pyrrolidine methylenes); MS (EI mode) m/z 234 (M^+), 219 (M^+ − methyl), 191 (M^+ − acetyl), 176 (M^+ − acetamido). Anal. ($C_{13}H_{18}N_2O_2 \cdot 0.2H_2O$) C, H, N.

6-Methoxy-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole (15a). A mixture consisting of 1.855 g (8.1 mmol) of **14**, 9.5 mL of 98% formic acid, and 4.5 mL of 30% hydrogen peroxide was stirred at 50 °C for 60 min. The reaction mixture was then poured onto 200 mL of ice water and neutralized with concentrated ammonia hydroxide. Extraction of the neutralized solution with 3 × 100 mL portions of chloroform, drying of the extracts (sodium sulfate), and then concentration of the extracts afforded a dark solid residue. Chromatography of this residue on silica gel, employing chloroform/methanol as the eluant (9:1), afforded **15a** as a brown solid: 1.23 g (81%) yield; TLC (chloroform/methanol, 95:5) R_f = 0.30; mp 128−129 °C; IR (KBr pellet) 3424, 3046, 2959, 2899, 2839, 1586, 1528, 1489, 1447, 1300, 1242, 1198, 1150, 1103, 1034, 941, 891, 806, 719 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.20 (1H, d, J_{meta} = 2.3 Hz, C(8) aromatic proton), 7.18 and 6.86 (2H, abx, J_{ortho} = 8.6 Hz, J_{meta} = 2.3 Hz, C-5 and C-6 aromatic protons, respectively), 4.08 (2H, t, J = 7.0 Hz C(1) methylene), 3.86 (3H, s, methoxy), 3.05 (2H, t, J = 7.6 Hz, C(3) methylene), 2.69 (2H, quint, J = 7.5 Hz C(2) methylene); MS (EI mode) m/z 188 (M^+), 173 (M^+ − methyl), 157 (M^+ − methoxy). Anal. ($C_{11}H_{12}N_2O \cdot 0.2H_2O$) C, H, N.

3-Acetoxy-6-methoxy-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]-benzimidazole (15b). A solution consisting of 3.49 g (16 mmol) of **14** and 4.35 g (32 mmol) of anhydrous $ZnCl_2$ in 16 mL of acetic anhydride was refluxed at 90−100 °C for 5 h. The reaction mixture was cooled to room temperature, diluted with 100 mL of water, and then extracted with 3 × 100 mL portions of chloroform. The dried extracts (sodium sulfate) were concentrated to a brown residue, which was flash chromatographed on a silica gel column using ethyl acetate/methanol (95:5) as the eluant. Evaporation of the eluants afforded the pure product, which was recrystallized from ethyl acetate/hexane: 520 mg (13% yield); mp 155−156 °C; TLC (ethyl acetate) R_f = 0.19; IR (KBr pellet) 2961, 1746, 1626, 1582, 1530, 1495, 1423, 1370, 1246, 1146, 1080, 1028, 947, 814 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 7.45 (1H, d, J_{ortho} = 8.8 Hz, C(8) aromatic proton), 7.72 and 6.62 (2H, abx, J_{ortho} = 8.8 Hz, J_{meta} = 2.3 Hz, J_{para} = 0, C(8) and C(7) protons, respectively), 6.10 (1H, dd, J = 7.6, 3.2 Hz, C(3) proton), 4.16 (2H, m, C(1) diastereomeric methylene), 3.78 (3H, s, methoxy), 3.13 (3H, s, acetate methyl), 3.11 and 2.59 (2H, 2m, C(2) diastereomeric methylene); MS (EI mode) m/z 246 (M^+), 203 (M^+ − acetyl), 187 (M^+ − acetate). Anal. ($C_{13}H_{14}N_2O_3$) C, H, N.

6-Methoxy-5-nitro-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole (16a). To 4.2 mL of fuming nitric acid, chilled in a 2-propanol−dry ice bath, was added 450 mg (2.4 mmol) of **15a**. The reaction mixture was then warmed by placing it in a salt−ice water bath for 5 min. The reaction mixture was poured over 100 g of cracked ice and resulting solution adjusted to pH 7 with saturated sodium bicarbonate solution. The neutralized solution was extracted three times with 100 mL portions of chloroform, and the dried extracts (sodium sulfate) were concentrated to a yellow solid. Purification of this solid was carried out by flash chromatography on silica gel using ethyl acetate/methanol (95:5) as the eluant followed by recrystallization from chloroform/hexane: 212 mg (38%) yield; mp 182 °C; TLC (ethyl acetate/methanol, 95:5) R_f = 0.17; IR (KBr pellet) 3432, 2953, 2915, 2367, 2346, 1584, 1524, 1449, 1408, 1373, 1333, 1298, 1269, 1209, 1088, 955, 874, 799 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.57 and 6.76 (2H, abq, J_{ortho} = 8.9 Hz, C(8) and C(7) aromatic proton, respectively), 4.15 (2H, t, J = 7.1 Hz, C(1) methylene), 3.97 (3H, s, methoxy), 3.13 (2H, t, J = 7.6 Hz, C(3) methylene), 2.76 (2H, quint, J = 7.5 Hz, C(2) methylene); MS (EI mode) m/z 233 (M^+), 218 (M^+ − methyl),

203 ($M^+ - NO$), 186 ($M^+ - NO$ and OH). Anal. ($C_{11}H_{11}N_3O_3 \cdot 0.1H_2O$) C, H, N.

3-Acetoxy-6-methoxy-5-nitro-2,3-dihydro-1*H*-pyrrolo-[1,2-*a*]benzimidazole (16b). To 0.7 mL of fuming nitric acid and 0.7 mL of acetic anhydride cooled in an ice bath was added 98 mg (0.40 mmol) of **15b**. The reaction mixture was stired for about 5 min and then poured over 50 mg of cracked ice. The resulting mixture was adjusted to pH 7 with saturated sodium bicarbonate solution and then extracted three times with 50 mL portions of chloroform. Drying the combined chloroform extracts (sodium sulfate) followed by concentration afforded a yellow solid, which was flash chromatographed on silica gel using ethyl acetate as the eluant. The purified product was recrystallized from chloroform/hexane: 72 mg (68%) yield; TLC (ethyl acetate) $R_f = 0.28$; mp 144−146 °C; IR (KBr pellet) 2947, 1746, 1626, 1530, 1450, 1443, 1370, 1246, 1080, 1028, 843 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.68 and 6.87 (2H, abq, $J = 8.9$ Hz, C(8) and C(7) aromatic protons, respectively), 6.16 (2H, dd, $J = 7.5$, 3.2 Hz, C(3) proton), 4.32 and 4.18 (2H, 2m, C(1) diastereomeric methylene), 3.98 (3H, s, methoxy), 3.25 and 2.68 (2H, 2m, C(2) diastereomeric methylene), 2.13 (3H, s, acetate methyl); MS (EI mode) m/z 291 (M^+), 248 ($M^+ -$ acetyl), 232 ($M^+ -$ acetate). Anal. ($C_{13}H_{13}N_3O_5$) C, H, N.

5-Amino-6-methoxy-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]-benzimidazole (17a). A suspension of 50 mg (0.21 mmol) of **16a** in 20 mL of methanol containing 70 mg of 5% Pd on carbon was shaken under 50 psi of H_2 for 3 h. The reaction mixture was filtered through Celite and the filter cake washed with methanol. Evaporation of the solvent afforded crude **17a**. The resulting solid was unstable and used in the Fremy oxidation without further purification.

3-Acetoxy-5-amino-6-methoxy-2,3-dihydro-1*H*-pyrrolo-[1,2-*a*]benzimidazole (17b). A suspension of 136 mg (0.47 mmol) of **16b** in 20 mL of methanol containing 100 mg of 5% Pd on carbon was shaken under 50 psi of H_2 for 2 h. The reaction mixture was filtered through Celite and the filter cake washed with methanol. Evaporation of solvent afforded crude **17b** which was used without further purification.

6-Methoxy-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole-5,8-dione (18a). To a suspension of the crude **17a** in 10 mL of water, containing 80 mg of monobasic potassium phosphate, was added a solution of 400 mg of Fremy's salt in 30 mL of water containing 200 mg of monobasic potassium phosphate. The reaction mixture was stirred at room temperature for 2 h and then extracted three times with 50 mL portions of chloroform. The dried extracts (sodium sulfate) were concentrated and then purified by chromatography on silica gel employing chloroform/methanol (95:5) as the eluant. Recrystallization from chloroform/hexane afforded **18a** in 29 mg (63.3%) yield: TLC (chloroform/methanol, 95:5) $R_f = 0.40$; mp 221 °C; IR (KBr pellet) 3490, 3054, 2980, 2942, 1692, 1647, 1589, 1476, 1298, 1244, 1134, 1084, 1040, 868, 723 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.70 (1H, s, C(7) aromatic proton), 4.25 (2H, t, $J = 7.2$ Hz, C(1) methylene), 3.86 (3H, s, methoxy), 2.97 (2H, t, $J = 7.6$ Hz, C(3) methylene), 2.72 (2H, quint, $J = 7.5$ Hz, C(2) methylene); MS (EI mode) m/z 218 (M^+), 203 ($M^+ -$ methyl). Anal. ($C_{11}H_{10}N_2O_3 \cdot 0.1H_2O$) C, H, N.

3-Acetoxy-6-methoxy-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]-benzimidazole-5,8-dione (18b). To a suspension of the crude **17b** in 10 mL of water, containing 200 mg of monobasic potassium phosphate, was added a solution of 1.5 g of Fremy's salt in 50 mL of water containing 500 mg of monobasic potassium phosphate. The reaction mixture was stirred at room temperature for 2 h and extracted with 3 × 50 mL portions of chloroform. The dried extracts (sodium sulfate) were concentrated to a residue, which was then chromatographed over silica gel employing chloroform/methanol (95:5) as eluant. Recrystallization from chloroform/hexane afforded 52 mg (39%) yield: TLC (chloroform/methanol, 95:5) $R_f = 0.56$; mp 189−191 °C dec; IR (KBr pellet) 2994, 1748, 1638, 1582, 1530, 1371, 1275, 1213, 799 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.09 (1H, dd, $J = 7.7$, 3.2 Hz, C(3) proton), 5.78 (1H, s, C(7) aromatic proton), 4.35 (2H, m, C(1) diastereomeric methylene), 3.88 (3H, s, methoxy), 3.16 and 2.66 (2H, 2m, C(2) diastereomeric methylene), 2.11 (3H, s, acetate methyl); MS (EI mode) m/z 276 (M^+), 233 ($M^+ -$ acetyl), 217 ($M^+ -$ acetate). Anal. ($C_{13}H_{12}N_2O_5$) C, H, N.

6-(*N*-Aziridinyl)-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benz-imidazole-5,8-dione (2a). To a solution of 25 mg (0.11 mmol) of **18a** in 4 mL of dry methanol was added 0.5 mL of ethylenimine, and the resulting mixture was stirred at room temperature for 2 h. The solvent was removed *in vacuo* and the red residue purified by flash chromatography on silica gel employing chloroform/methanol (95:5) as the eluant. The purified product was recrystallized from chloroform/hexane: 22 mg (83.5%) yield; TLC (chloroform/methanol, 95:5) $R_f = 0.31$; mp 206 °C; IR (KBr pellet) 3435, 2998, 2961, 2928, 2375, 1676, 1640, 1574, 1476, 1402, 1362, 1304, 1265, 1130, 1067, 993, 868 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.83 (1H, s, C(7) aromatic proton), 4.22 (2H, t, $J = 7.2$ Hz, C(1) methylene), 2.96 (2H, t, $J = 7.7$ Hz, C(3) methylene), 2.70 (2H, quint, $J = 7.5$ Hz, C(2) methylene), 2.26 (4H, s, aziridine protons); MS (EI mode) m/z 229 (M^+). Anal. ($C_{12}H_{11}N_3O_2$) C, H, N.

3-Acetoxy-6-(*N*-aziridinyl)-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole-5,8-dione (2b). To a solution of 42 mg (0.15 mmol) of **18b** in 5 mL of dry methanol was added 0.2 mL of ethylenimine. The reaction mixture was then stirred at room temperature for 2 h. The solvent was removed *in vacuo* and the red residue flash chromatographed on silica gel employing chloroform/methanol (95:5) as the eluant. The purified product was recrystallized from chloroform/hexane: 33 mg (78%) yield; TLC (chloroform/methanol, 95:5) $R_f = 0.23$; mp 164−166 °C; IR (KBr pellet) 2992, 1748, 1678, 1572, 1510, 1364, 1257, 1235, 1064, 1024, 997, 893 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.08 (1H, dd, $J = 7.5$, 3.0 Hz, C(3) proton), 5.90 (1H, s, C(7) aromatic proton), 4.32 (2H, m, C(1) diastereomeric methylene), 3.15 and 2.64 (2H, 2m, C(2) diastereomeric methylene), 2.28 (4H, s, aziridinyl protons), 2.10 (3H, s, acetate methyl); MS (EI mode) m/z 287 (M^+), 244 ($M^+ -$ acetyl), 230 ($M^+ -$ acetate). Anal. ($C_{14}H_{13}N_3O_4 \cdot 0.2H_2O$) C, H, N.

6-(*N*-Aziridinyl)-7-methoxy-2,3-dihydro-1*H*-pyrrolo-[1,2-*a*]benzimidazole-5,8-dione (3). To a mixture of sodium methoxide prepared by reacting 40 mg of sodium metal in 5 mL of methanol was added 12.3 mg (0.054 mmol) of **2a**. The mixture was stirred at room temperature for 10 min and then diluted with 10 mL of water. The resulting mixture was extracted three times with 20 mL portions of chloroform. The dried extracts (sodium sulfate) were concentrated and chromatographed on silica gel employing chloroform/methanol as the eluant. Recrystallization from chloroform/hexane afforded 8 mg (57% yield) of **3**: mp 156−158 °C dec; TLC (chloroform/methanol, 95:5) $R_f = 0.44$; IR (KBr pellet) 1701, 1645, 1580, 1514, 1343, 1306, 1148, 1067, 1018 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.22 (2H, t, $J = 7.2$ Hz, C(1) methylene), 3.93 (3H, s, methoxy), 2.94 (2H, t, $J = 7.7$ Hz, C(3) methylene), 2.70 (2H, quint, $J = 7.5$ Hz, C(2) methylene), 2.33 (4H, s, aziridinyl protons); MS (EI mode) m/z 259 (M^+), 244 ($M^+ -$ methyl). Anal. ($C_{13}H_{13}N_3O_3 \cdot 1.5CHCl_3$) C, H, N.

6-Amino-7-methoxy-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]-benzimidazole (19). A solution of 326 mg (1.9 mmol) of **9a** in 8 mL of concentrated sulfuric acid and 8 mL of water was warmed at 80 °C for 8 h. The mixture was poured onto ice and adjusted to pH 8.0 with concentrated ammonium hydroxide solution. Extraction of the cooled solution with 3 × 50 mL of chloroform followed by drying (sodium sulfate) of the extracts and evaporation of the solvent afforded **19** as a crude solid: 370 mg (96%) yield; TLC (chlorform/methanol, 9:1) $R_f = 0.51$; mp 205−207 °C; ^1H NMR ($CDCl_3$) δ 7.06 and 6.73 (2H, 2s, aromatic protons), 4.04 (2H, t, $J = 7.0$ Hz, C(1) methylene), 3.90 (3H, s, methoxy), 3.01 (2H, t, $J = 7.6$ Hz, C(3) methylene), 2.69 (2H, quint, $J = 7.2$ Hz, C(2) methylene); MS (EI mode) m/z 203 (M^+), 172 ($M^+ -$ methoxy).

7-Methoxy-5-nitro-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benz-imidazole (20). A mixture of 370 mg (1.83 mmol) of **19** in 12 mL of concentrated sulfuric acid and 5 mL of acetic acid was cooled to −10 °C, and then 0.88 g of sodium nitrite in 4 mL of water was added while maintaining the reaction temperature at less than 10 °C. After the addition was complete, the reaction mixture was stirred at 0 °C for 45 min. The resulting mixture was added to 12 mL of 50% hypophosphous acid while maintaining the temperature at less than 5 °C. The reaction

mixture was then stirred at room temperature for 90 min, after which it was diluted with 25 mL of water and neutralized to pH 7.00 with concentrated ammonia hydroxide solution. The neutralized mixture was then extracted three times with 50 mL portions of chloroform, and the extracts were dried (sodium sulfate) and concentrated. Chromatography on silica gel employing chloroform/methanol (9:1) as the eluant afforded pure **20**, which was recrystallized from chloroform/hexane: 67 mg (15.7%) yield; mp 157−158 °C; TLC (chloroform/methanol, 95:5) $R_f = 0.49$; IR (KBr pellet) 2986, 1626, 1576, 1508, 1443, 1393, 1285, 1252, 1192, 1088, 995, 824 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.69 and 7.08 (2H, 2d, $J = 2.4$ Hz, aromatic proton), 4.13 (2H, t, $J = 7.1$ Hz, C(1) methylene), 3.89 (3H, s, methoxy), 3.14 (2H, t, $J = 7.7$ Hz, C(3) methylene), 2.76 (2H, quint, $J = 7.4$ Hz, C(2) methylene); MS (EI mode) m/z 233 (M^+), 188 ($M^+ - NO_2$). Anal. ($C_{11}H_{11}N_3O_3$) C, H, N.

5-Amino-7-methoxy-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole (21). A solution of 63 mg (0.27 mmol) of **20** in 20 mL of methanol was shaken under 50 psi of H_2 in the presence of 50 mg of 5% Pd on carbon for 4 h. The catalyst was removed by filtration through Celite, and the solvent was removed under vacuum. Evaporation of the solvent afforded crude **21** which was used without further purification.

7-Methoxy-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole-5,8-dione (22). To a suspension of crude **21** in 10 mL of water containing 200 mg of monobasic potassium phosphate was added a solution of 0.5 g of Fremy's salt in 30 mL of water containing 500 mg of potassium phosphate monobasic. The reaction mixture was stirred at room temperature for 2 h and then extracted with 3 × 30 mL portions of chloroform. Drying of the extracts (sodium sulfate) and concentration afforded a yellow solid, which was purified by chromatography on silica gel employing chloroform/methanol (9:1) as the eluant. The purified product was recrystallized from chloroform/hexane: 18 mg (31%) yield; mp 214−216 °C; TLC (chloroform/methanol, 95:5) $R_f = 0.51$; IR (KBr pellet) 3102, 1672, 1645, 1589, 1512, 1335, 1252, 1184, 1163, 1090, 1030, 872 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.78 (1H, s, aromatic proton), 4.28 (2H, t, $J = 7.2$ Hz, C(1) methylene), 3.85 (3H, s, methoxy), 2.99 (2H, t, $J = 7.6$ Hz, C(3) methylene), 2.74 (2H, quint, $J = 7.3$ Hz, C(2) methylene); MS (EI mode) m/z 218 (M^+). Anal. ($C_{11}H_{10}N_2O_3$) C, H, N.

7-(*N*-Aziridinyl)-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole-5,8-dione (4). To a solution of 10 mg (0.046 mmol) of **22** in 2 mL of methanol, chilled at 0 °C, was added 0.2 mL of ethylenimine. After stirring at 0 °C for 2 h, the solvent was removed and the orange residue chromatographed on silica gel employing ethyl acetate/methanol (9:1) as the eluant. Final purification was carried out by recrystallization from ethyl acetate/hexane: 3.5 mg (33%) yield; mp 142 °C; TLC (chloroform/methanol, 95:5) $R_f = 0.28$; IR (KBr pellet) 2926, 2233, 1666, 1643, 2579, 1512, 1359, 1163, 985, 732 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.87 (1H, s, aromatic proton), 4.23 (2H, t, $J = 7.2$ Hz, C(1) methylene), 2.94 (2H, t, $J = 7.6$ Hz, C(3) methylene), 2.69 (2H, quint, $J = 7.3$ Hz, C(2) methylene), 2.18 (4H, s, aziridinyl proton); MS (EI mode) m/z 229 (M^+), 187 (M^+ − aziridine).

Hydrolysis of Reduced 2a (23). To 10 mL of the buffer were added 11 mg (0.04 mmol) of **3** in 2 mL of dimethyl sulfoxide and 10 mg of 5% Pd on carbon. The mixture was purged with argon for 10 min, and then hydrogen gas was passed through the deaerated mixture for 15 min, resulting in the formation of a colorless solution. Excess hydrogen was removed from this solution by purging with argon for ∼5 min. Incubation of the reaction mixture for 18 h at 30 °C resulted in the formation of a purple solution, which was opened to the air and filtered through Celite. The filtrate was extracted with 3 × 20 mL portions of chloroform. The extracts were washed two times with water, dried (sodium sulfate), and then concentrated to a residue. Silica gel chromatography employing chloroform/methanol (95:5) as the eluant successfully separated hydrolysis products.

When 0.05 M, pH 7.4 and 5.5, Tris buffers were employed, **26** and **28** were obtained in 37% and 18% yields, respectively. When 1.0 M, pH 2.0, formate buffer ($\mu = 1.0$, KCl) was employed, the only product was **27** (56% isolated yield). Physical properties of **26**−**28** are provided below.

26: ^1H NMR ($CDCl_3$) δ 6.00 (1H, bs, amino proton), 5.20 (1H, s, aromatic proton), 4.24 (2H, t, $J = 7.2$ Hz, C(1) methylene), 3.19 (2H, m, methylene of ethyl), 2.93 (2H, t, $J = 7.5$ Hz, C(3) methylene), 2.70 (2H, quint, $J = 7.3$ Hz, C(2) methylene), 1.32 (3H, t, $J = 7.2$ Hz, methyl of ethyl); MS (EI mode) m/z 289 (M^+), 246 (M^+ − acetyl), 230 (M^+ − acetate).

27: ^1H NMR ($CDCl_3$) δ 6.28 (1H, bs, amine), 5.25 (1H, s, aromatic), 4.25 (2H, t, $J = 7.2$ Hz, C (1) methylene), 3.72 (2H, t, $J = 5.7$ Hz, methylene next to chloro), 3.53 (2H, q, $J = 6.0$ Hz, methylene next to amino), 2.95 (2H, t, $J = 7.5$ Hz, C(3)-methylene), 2.70 (2H, quint, $J = 7.2$ Hz, C(2)methylene); MS (EI mode) m/z 265 (M^+), 230 (M^+ − Cl), 216(M^+ − chloromethyl).

28: ^1H NMR ($CDCl_3$) δ 5.50 (1H, s, aromatic proton), 5.21 (2H, bs, amino proton), 4.23 (2H, t, $J = 7.8$ Hz, C(1) methylene), 2.94 (2H, t, $J = 7.8$ Hz, C(3) methylene), 2.70 (2H, quint, $J = 6.9$ Hz, C(2) methylene); MS (EI mode) m/z 261 (M^+), 218 (M^+ − acetyl).

Hydrolysis of Reduced 2b. The reduction of **2b** in 0.05 M, pH 7.4, Tris buffer and workup of the hydrolysis products were carried out as described for **2a**. Silica gel chromatography of the chloroform extracts employing chloroform/methanol (95:5) as the eluant separated products **29** and **30**. The yields, which were obtained upon evaporation of collected fractions, were 31% and 40%, respectively. The aqueous layer which had been extracted with chloroform was added to a 20 g Bakerbond phenyl (40 μm) reverse-phase column prepared with water. The column was eluted with water resulting in removal of a purple product (**33**), 8% yield upon concentration of the product fraction. Physical properties of **29**−**31** are provided below.

29: ^1H NMR ($CDCl_3$) δ 6.08 (1H, dd, $J = 7.5$, 3.0 Hz, C(3) proton), 6.05 (1H, bs, amine proton), 5.28 (1H, s, aromatic proton), 4.35 (2H, m, C(1) diastereomeric methylene), 3.21 (2H, m, methylene of ethyl), 3.19 and 2.63 (2H, 2m, C(2) diastereomeric methylene), 2.11 (3H, s, acetate methyl), 1.33 (3H, t, $J = 7.2$ Hz, methyl of ethyl); MS (EI mode) m/z 298 (M^+), 246 (M^+ − acetyl), 230 (M^+ − acetate).

30: ^1H NMR ($CDCl_3$) δ 6.08 (1H, dd, $J = 7.5$, 3.0 Hz, C(3) proton), 5.57 (1H, s, aromatic proton), 5.29 (2H, bs, amino proton), 4.34 (2H, m, C(1) diastereomeric methylene), 3.15 and 2.64 (2H, 2m, C(2) diastereomeric methylene), 2.11 (3H, s, acetate methyl); MS (EI mode) m/z 261 (M^+), 218 (M^+ − acetyl), 202 (M^+ − acetate).

31: ^1H NMR (dimethyl sulfoxide-d_6) δ 7.28 (2H, bs, amino proton), 5.85 (1H, m, C(3) proton), 5.34 (1H, s, aromatic proton), 4.96 (1H, m, hydroxyl proton), 4.19 and 4.07 (2H, 2m, C(1) diastereomeric methylene), 2.88 and 2.35 (2H, 2m, C(2) diastereomeric methylene); MS (EI mode) m/z 219 (M^+), 202 (M^+ − OH).

Hydrolysis of Reduced 3. The reduction of **3** in 0.05 M, pH 7.4, Tris buffer and workup of the hydrolysis products were carried out as described for **2a**. The concentrated chloroform extracts were subjected to preparative thin layer chromatographic separation employing chloroform/methanol (95:5) as the eluant. The isolated compounds included **32** (22%), **33** (11%), and unreacted **3** (15%). Physical properties of **32** and **33** are provided below.

32: ^1H NMR ($CDCl_3$) δ 5.60 (1H, bs, amine proton), 4.23 (2H, t, $J = 7.2$ Hz, C(1) methylene), 3.78 (3H, s, methoxy), 3.63 (2H, quint, $J = 6.6$ Hz, methylene of ethyl), 2.91 (2H, t, $J = 7.2$ Hz, C(2) methylene), 2.69 (2H, quint, $J = 7.2$ Hz, C(3) methylene), 1.27 (3H, t, $J = 7.2$ Hz, methyl of ethyl); MS (EI mode) m/z 261 (M^+), 246 (M^+ − methyl).

33: ^1H NMR ($CDCl_3$) δ 5.03 (2H, bs, amino proton), 4.22 (2H, t, $J = 7.2$ Hz, C(1) methylene), 3.89 (3H, s, methoxy), 2.92 (2H, t, $J = 7.5$ Hz, C(3) methylene), 2.69 (2H, quint, $J = 7.2$ Hz, C(2) methylene); MS (EI mode) m/z 233 (M^+), 218 (M^+ − methyl).

Calf Thymus DNA Treated with Reduced 2a. The reaction mixture consisted of the following components: 7.18 mg of sonicated calf thymus DNA dissolved in 5 mL of 0.05 M, pH 7.4, Tris buffer, 4.87 mg (0.0213 mmol) of **2a** dissolved in 2 mL of dimethyl sulfoxide, and 10 mg of 5% Pd on carbon. The combination of these components, deaeration, and catalytic reduction were carried out as described under Hydrolysis

of Reduced **2a**. The anaerobic incubation of the reaction was carried out at 30 °C for 24 h. The completed reaction was opened to the air and filtered throught Celite to remove the catalyst. The filtrate was extracted three times with 50 mL portions of chloroform to remove hydrolysis products. These products were separated and yields obtained as described under Hydrolysis of Reduced **2a**. The aqueous layer was adjusted to 0.3 M sodium acetate with 3.0 M, pH 5.1, sodium acetate stock and then diluted with 3 vol of ethanol. This mixture was chilled at −20 °C for 24 h and then centrifuged at 5000*g* for 15 min. The DNA pellet was washed by suspending in ethanol and centrifuging. Weight of the vacuum-dried DNA pellet was 5.87 mg, and the yields of hydrolysis products were **26**, 35%, and **28**, 10%.

Topoisomerase II Assays. The topoisomerase relaxation reactions were carried out with 0.50 μg of SV-40 supercoiled (form I) DNA and 20 units of *Drosophila melanogaster* topoisomerase II (United States Biochemical) in 20 λ of 100 mM Tris·HCl (pH 7.9) containing 500 mM NaCl, 500 mM KCl, 50 mM $MgCl_2$, 1 mM EDTA, 150 μg/mL BSA, and 10 mM ATP. The reactions were run for 30 min at 37 °C and then combined with 1 λ of a solution of proteinase K and SDS (3.75 and 75 mg, respectively, in 1.5 mL of H_2O). After incubation at 37 °C for 45 min further, the reaction mixtures were combined with 4 λ of 6× gel loading solution (0.25% bromophenol blue, 0.25% xylene cyanol FF, 30% glycerol in water). A 1.3% agarose gel run for 18 h in 0.045 M Tris borate buffer with 1 mM EDTA was employed to assay the relaxation reactions. The DNA bands were visualized with ethidium bromide.

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