Pyrrolo[1,2-*a*]benzimidazole-Based Quinones and Iminoquinones. The Role of the 3-Substituent on Cytotoxicity

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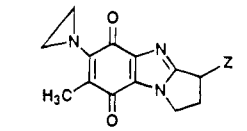
The influence of the 3-substituent on the cytotoxicity of the 6-aziridinylpyrrolo[1,2-*a*]-benzimidazole quinones (PBIs), the 6-acetamidopyrrolo[1,2-*a*]benzimidazole quinones (APBIs), and the 6-acetamidopyrrolo[1,2-*a*]benzimidazole iminoquinones (imino-APBIs) was investigated by comparing LC_{50} mean graphs consisting of 60 cancer lines. Increasing lipophilicity of the 3-substituent of PBIs and APBIs increased the cytotoxicity specifically in melanoma cell lines. The 3-substituent does not influence DNA cleavage by reduced PBIs, except for the 3-carbamate derivative which shows enhanced cleavage. This property of the 3-carbamate is rationalized in terms of the PBI major groove binding model. The imino-APBIs show enhanced cytotoxicity in melanoma and renal cancer cell lines; the correlation coefficient for log LC_{50} vs log lipophilicity is 0.8 to 0.9. COMPARE correlations revealed that the PBIs are activated by DT-diaphorase but that the APBIs and imino-APBIs are inactivated by this enzyme. Thus, the latter two agents are cytotoxic only as quinones. It was noted that APBIs possess a similar cytotoxic profile to three anthracycline analogues. This observation suggests mechanistic similarities between both types of cytotoxic agents. Major conclusions of this study pertain to the design of agents displaying cytotoxicity specifically against melanoma and renal cancers and to the use of 60-cell line mean graphs and COMPARE in cancer drug QSAR.

Introduction

The pyrrolo[1,2-*a*]benzimidazoles (PBIs) shown in Chart 1 represent a new class of antitumor agent exhibiting cytotoxic activity against a variety of cancer cell lines.[1-7] Also shown in Chart 1 are the structurally related 6-acetamido quinone (APBI) and 6-acetamido iminoquinone (imino-APBI) derivatives. The APBI derivatives tend to possess lower cytotoxic potency against cancer cells than do the PBIs. However, the APBI and imino-APBI derivatives possess high specificity toward melanoma cell lines. The question posed at the start of this study dealt with the role of the 3-substituent in both cytotoxicity and DNA alkylation. In this report evidence is presented that the presence of an ester group at the 3-position is required for optimal cytotoxicity in the PBIs, APBIs, and imino-APBIs. The role of this substituent is to provide lipophilicity and, in the case of carbamate, a hydrogen-bonding functionality.

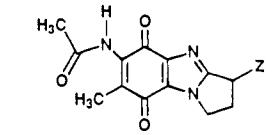
The results of the studies described herein and in a previous article[8] have permitted the elucidation of the pharmacophore shown in Chart 2. As discussed previously,[8] at least one antitumor agent discovered outside this laboratory (EO9[9]) possesses the essential features outlined in Chart 2. Starting from the left in Chart 2, these features are briefly discussed below. First, the 6-aziridinyl substituent produces greater cytotoxicity than does the 6-acetamido substituent, probably as a result of aziridinyl DNA alkylation at the phosphate backbone.[5,6] Second, both the quinone and iminoquinone analogues are cytotoxic. In the PBIs, bioreduction of the quinone will afford a hydroquinone, which will activate the aziridine center as an alkylating agent and be involved in hydrogen-bonding interactions with DNA. The APBIs and imino-APBIs appear to be cytotoxic without bioreduction, however. Third, the hydrogen-bonding feature (HB) is an absolute requirement for antitumor activity.[8] Finally, the 3-ester substituent provides the required lipophilicity, which appears to be particularly important for cytotoxicity in the iminoquinone (imino-APBI) analogues.

Chart 1

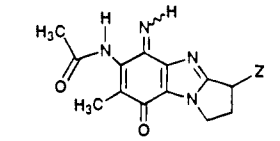


PBI-*

(Pyrrolo[1, 2-*a*]benzimidazole)



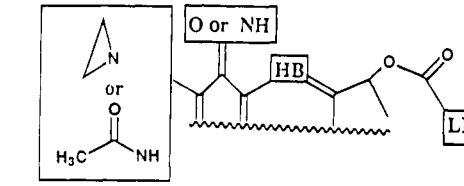
APBI-*



imino-APBI-*

Z	*
OAc	A
Carbamate	B
H	C
OH	D
Chloroacetate	E
Propionate	F
Benzoate	G
Valerate	H
Methoxyacetate	I

Chart 2



Synthetic Chemistry. The preparation of the PBI, APBI, and imino-APBI analogues is discussed in con-

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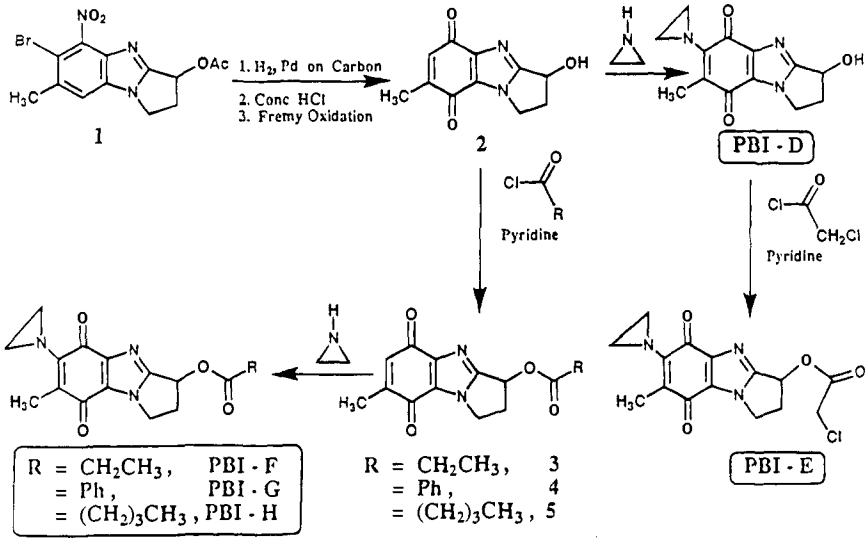
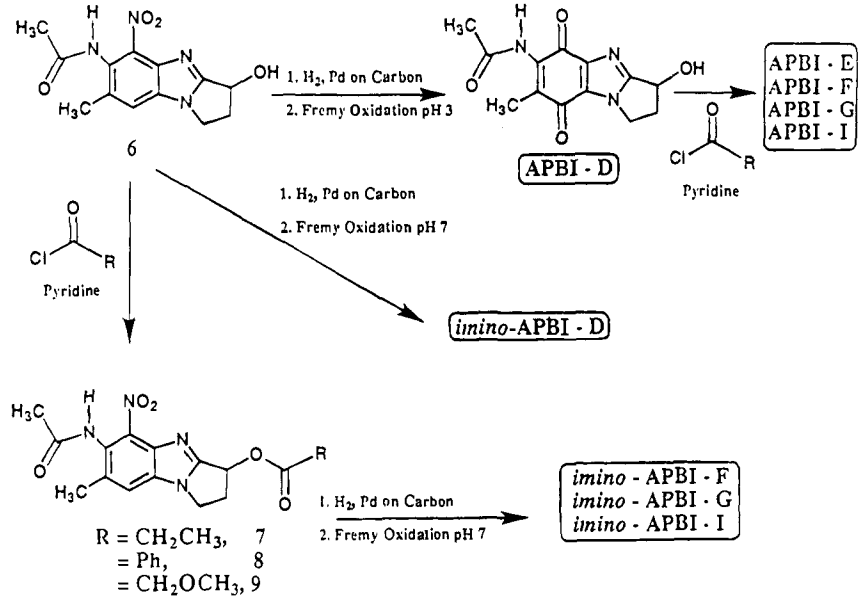
Scheme 1



Scheme 2



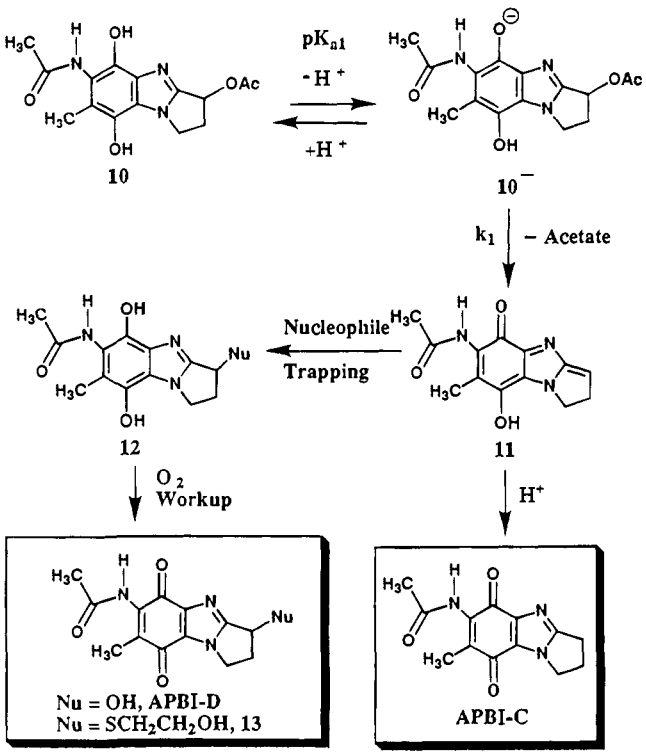
junction with Schemes 1 and 2. The steps leading to the PBI analogues, starting with **1**,[2] are outlined in Scheme 1. Conversion of **1** to **2** was followed either by acetylation to afford **3**–**5** or by aziridination to afford PBI-D. Acylation of PBI-D with chloroacetyl chloride in the presence of pyridine afforded PBI-E. The chloroacetyl group of PBI-E is reactive toward nucleophiles, and therefore aziridination had to be carried out before acylation. For the more stable acyl derivatives **3**–**5**, aziridination was carried out as the last step resulting in PBI-F,G,H.

The APBI derivatives were prepared from **6** as outlined in Scheme 2. Reduction of **6** and Fremy oxidation at pH 3 gave APBI-D, which was acylated to afford the other APBI quinone derivatives shown in Scheme 2. To obtain the iminoquinones imino-APBI-I,G,F, compound **6** was acylated to afford **7**–**9**, which were reduced and then subjected to Fremy oxidation at pH 7. Reduction of **6** and then Fremy oxidation afforded imino-APBI-D.

Hydrolytic Studies. In order to assess the fate of the leaving group upon reductive activation under physiological conditions, the hydrolytic chemistry of reduced APBI and imino-APBI derivatives was studied in anaerobic aqueous buffer. The results of these studies indicate that the elimination of the acetate leaving group from reduced APBI-A is not an important process at pH 7.4. Loss of the leaving group does become important in the case of reduced APBI-E due to the good leaving ability of chloroacetate. Reduction of the aminoquinone to aminophenol did not result in elimination of the 3-substituent. The results of the above studies indicate that the 3-substituent does not generally act as a leaving group upon reductive activation. The 3-substituent of the PBIs is likewise not activated as a leaving group upon reductive activation.[8] Outlined below are details of the hydrolytic studies of reduced APBI and imino-APBI derivatives.

The previously reported quinone APBI-A was catalytically reduced to the hydroquinone (designated as **10**), which was isolated as the hydrochloride salt. Product studies were carried out by incubating **10** in anaerobic buffers held at pH 7.5 and 8.0 for 60–72 h and then working up the reactions under aerobic conditions. The major product obtained from these product studies was APBI-A in 76% (pH 7.5) and 48% (pH 8.0) yields. At

Scheme 3



pH 9.0, a 72 h incubation of **10** resulted in extensive decomposition: only 2% of APBI-A was isolated along with 5% of APBI-D and 12% of APBI-C upon aerobic workup.

The formation of APBI-C,D at pH 9.0 is an indication that the quinone methide species **11** is involved in the hydrolysis of **10** in strong base. As shown in Scheme 3, **11** can either trap a proton to afford APBI-C or trap water to afford **12** (Nu = OH), which is then converted to APBI-D upon aerobic workup. An alternative route to APBI-D is a $B_{AC}2$ ester hydrolysis to the alcohol followed by oxidative workup. Evidence supporting a quinone methide mechanism was obtained from the preparative hydrolysis of **10** in anaerobic pH 9.26 buffer containing mercaptoethanol. The nucleophile-trapping product **13** was obtained from this reaction in 5% yield upon aerobic workup. This product could arise from the addition of the sulfur nucleophile to the quinone methide. Previous studies in the laboratory showed that a benzimidazole-based quinone methide can readily trap a sulfur nucleophile.[10] Further evidence of quinone methide formation was obtained from pH rate studies on **10**. The rate of reaction was followed spectrophotometrically at 290 nm under strict anaerobic conditions at 30 °C. These data (not shown) show that **10** eliminates acetate as illustrated in Scheme 3. The rate law for the elimination is $k_{obsd} = k_1K_{a1}/(a_H + K_{a1})$ where k_{obsd} is the observed first-order rate constant, k_1 is the first-order rate constant for the elimination of acetate, K_{a1} is the hydroquinone acid dissociation constant, and a_H is the proton activity determined with a pH meter. Fitting the pH–rate data to this law provided pK_{a1} = 8.95 and $k_1 = 3.90 \times 10^{-4}$. If the reaction of **10** was simply a $B_{AC}2$ hydrolysis process, the k_{obsd} values would increase with pH without a slope change to zero.

Increasing the leaving ability of the 3-substituent of an APBI hydroquinone increases the amount of 3-hydroxy derivative formed during hydrolysis. For example, reduction of APBI-E in pH 7.4 buffer afforded a 70% yield of the 3-hydroxy derivative (APBI-D). This species can form by means of a $B_{AC}2$ ester hydrolysis reaction or by a process involving a quinone methide species. The former mechanism is more likely since the quinone methide proton-trapping product (APBI-C) is not observed in the hydrolysis reactions. This finding is relevant to the cytotoxicity studies, which indicate that APBI-E,I possess a lower cytotoxicity than expected, which may be the result of hydrolysis of the lipophilic ester (see cytotoxicity studies).

Table 1. Medium log LC_{50} values obtained from 60 Cancer Cell Line Screens of PBIs and APBIs[a]

	LC_{50}		
substituent	PBI	APBI	imino-APBI
A	−6.60	−4.70	−4.29
B	−5.75	–	–
C	−5.87	−4.94	–
D	−5.85	> −4	−4.21
E	−5.73	−4.3	–
F	−5.80	−4.90	−4.91
G	−6.30	−4.79	−4.75
H	−6.30	−4.79	−4.75
I	–	−4.32	−4.35

[a] (LC_{50} is the concentration in moles per liter required for 50% lethality. The medium log LC_{50} values are the average of two or more screens.

All of the imino-APBIs are resistant to hydrolysis of the 3-substituent upon reduction. The high redox potential of these analogues[1] would preclude leaving group elimination to afford a quinone methide. Previous studies in this laboratory provided evidence of a linear free energy relationship between the rate constant for leaving group elimination and the redox potential.[11] The relationship shows that increasing the redox potential of the quinone (i.e., more electron deficient) decreases the rate constant for loss of an anion leaving group from the hydroquinone. The hydrolytic stability of the imino-APBIs resulted in excellent correlations between cytotoxicity and the lipophilicity of the 3-substituent.

Cytotoxicity Studies of PBIs and APBIs. From the mean LC_{50} data[12] shown in Table 1, it appears that changing the leaving ability and lipophilicity of the 3-substituent does not substantially change the cytotoxicity of either the PBIs or the APBIs. The COMPARE correlations[13] shown in Table 2 indicate that there are good (but not excellent) correlations between PBI-A and other PBIs as well as between APBI-A and other APBIs. Typically, good COMPARE correlations indicate the presence of common features of the cytotoxic agents under study (see COMPARE studies in ref 7). For the PBIs, these common features are likely the reductive activation requirements and the presence of DNA alkylation and cleavage. However, structurally related drugs usually have excellent correlation coefficients (>0.8).[13] Thus it is concluded that although the various 3-substituted PBIs and APBIs have similar medium cytotoxicities in the 60-cell line screen, there must be differences in the cytotoxicity against individual cell lines that account for the absence of excellent COMPARE correlations.

The differences in cytotoxicity between lipophilic and relatively hydrophilic 3-substituted PBIs are readily apparent by comparing the mean graph LC_{50} data for the 3-hydroxy (PBI-D) and 3-valerate (PBI-I) derivatives. The cytotoxicity of PBI-I against all cell lines in

Table 2. COMPARE Correlations of LC_{50} (moles per liter) Response Parameters from a 60-Cancer Cell Line Screen for PBI-A, APBI-A, and imino-APBI-A with Their Respective Analogues

analogue	PBI-A	analogue	APBI-A	analogue	imino-APBI-A
PBI-A	1.0	APBI-A	1.0	imino-APBI-A	1
PBI-B	0.84	APBI-C	0.70	imino-APBI-D	0.09
PBI-C	0.71	APBI-I	0.71	imino-APBI-F	0.26
PBI-F	0.72			imino-APBI-G	0.58
				imino-APBI-I	0.52

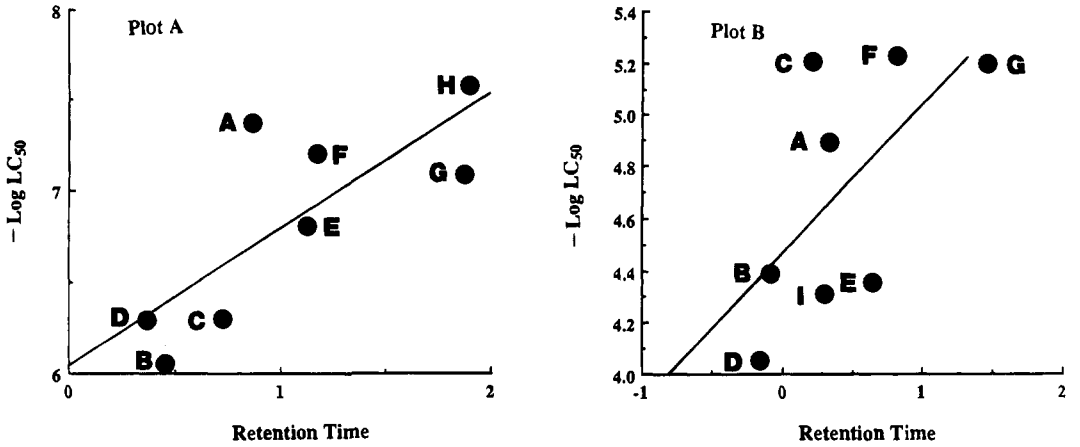


Figure 1. Plots of the average -log LC_{50} value of cell lines in the melanoma panel vs the log retention time of the cytotoxic agent determined on a C_{18} reverse phase column. Plot A shows the data obtained for the PBIs and plot B shows the data obtained for the APBIs.

the melanoma panel is consistently higher than that of PBI-D. In contrast, other panels (leukemia, lung, colon, CNS, ovarian) either do not respond or show a random response to the 3-substituent change (higher or lower LC_{50}). The cytotoxicity of the APBIs also shows a dependence on the lipophilicity of the 3-substituent. Thus, the 3-hydroxy derivative (APBI-D) is inactive whereas the 3-benzoate derivative (APBI-G) is cytotoxic against all melanoma cell lines.

Figure 1 illustrates the relationship between the average cytotoxicity against the melanoma panel and the lipophilicity of the PBIs (plot A) and APBIs (plot B). The lipophilicity was determined from the retention time of the PBIs and APBIs on a reverse phase column.[14] The correlation coefficient for the PBI in plot A is good, 0.614, while that for the APBIs in plot B is poor, 0.374. The scatter observed in both plots may arise from ester hydrolysis of some of the 3-substituents (e.g., the chloroacetate and methoxyacetate substituents, see Hydrolytic Studies). For example, the hydrolytically labile analogues APBI-I,E possesses lower cytotoxicity than expected (-log LC_{50} values are below the line of plot B), perhaps due to hydrolysis to the inactive 3-hydroxy analogue APBI-D.

In conclusion, the absence of excellent COMPARE correlations for the PBIs and APBIs is due to the greater sensitivity of melanoma cell lines to the lipophilicity of the 3-substituent compared to other types of cancer cell lines. Hydrolysis of the 3-substituent likely is an important cause of the data scatter seen in Figure 1. However, the data in plot A of Figure 1 clearly shows the positive influence of lipophilicity on cytotoxicity.

Cytotoxicity Studies of Imino-APBIs. The medium log LC_{50} data for the imino-APBIs shown in Table 1 indicate that increasing the lipophilicity of the 3-substituent results in increasing cytotoxicity against cell lines, especially melanoma and renal cancer. For example, the relatively lipophilic benzoate derivative (imino-APBI-G) has a lower medium log LC_{50} than the methoxyacetate derivative (imino-APBI-I). Comparison of mean graph data for both compounds reveals that not all of the cancer types are affected by the substitution change. However, quantitation of the similar cytotoxicity pattern was achieved using COMPARE correlations, which are shown in Table 2. The poor COMPARE correlations between the imino-APBIs is due to the differential effects of lipophilicity on cytotoxicity against cell lines. The correlation of cytotoxicity with the lipophilicity of the imino-APBIs was determined for both the melanoma and renal cancer cell lines, Figure 2. The lipophilicity of the imino-APBIs was determined from the retention time on a reverse phase column.[14] The correlation coefficients for melanoma in plot A (0.8) and for renal cancer in plot B (0.9) are both excellent. The presence of excellent correlations with lipophilicity in this plot is due to the hydrolytic stability of the lipophilic 3-substituent of the imino-APBIs (see Hydrolytic Studies).

Mechanism of Cytotoxicity. The mechanism of PBI cytotoxicity has been the subject of a series of publications from this laboratory.[6,7,15] The most recent publication provides evidence that reduced PBIs interact at the DNA major groove and alkylate the phosphate backbone. Cleavage of DNA results from nucleophile-assisted breakdown of the resulting phosphotriester. Shown in Scheme 4 is the proposed cleavage mechanism for reduced PBI-B at an A–T base pair. The requirement for reductive activation of the PBI quinone was recently documented by correlating the DT-diaphorase levels and cytotoxicity in 60 cancer cell lines.[16] The COMPARE correlation coefficient for PBI-F with DT-diaphorase levels is 0.414. Mitomycin C, which is known to be activated by DT-diaphorase,[17,18] has a correlation coefficient of 0.422. The streptonigrin-based antitumor agents, which are also activated by quinone

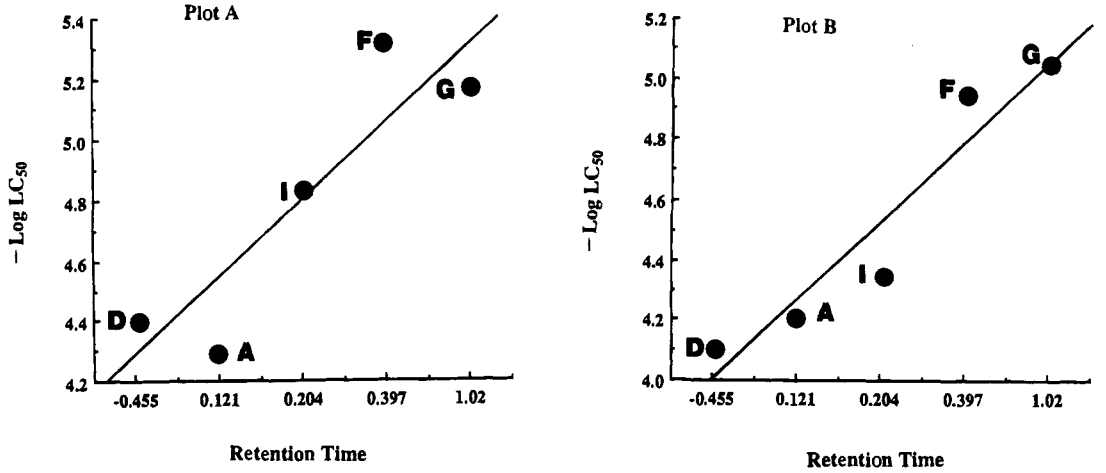
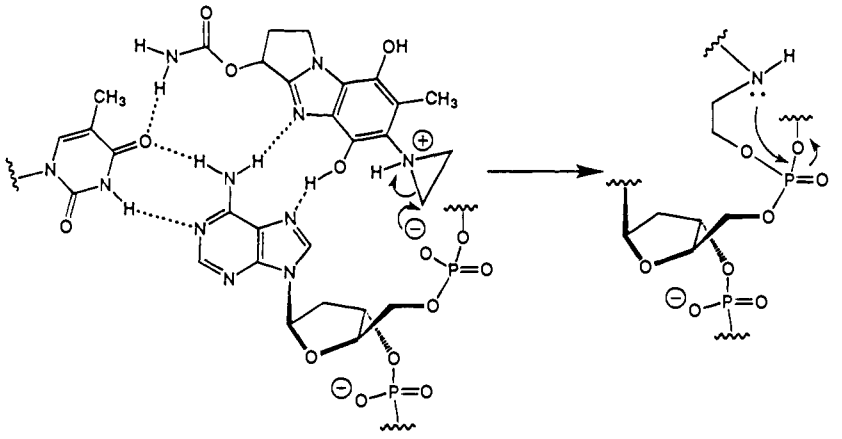


Figure 2. Plots of the average -log LC_{50} values of cell lines in the melanoma (plot A) and the renal cancer (plot B) panels, obtained with the imino-APBIs, vs the log retention time of the cytotoxic agent determined on a C_{18} reverse phase column.

Scheme 4



reduction,[18] have the highest correlations with DT-diaphorase (0.56–0.497).

In order to determine the influence of the 3-substituents on the DNA cleavage reaction, 600 bp calf thymus DNA was treated with reduced PBIs under anaerobic conditions and the amount of "blue DNA" was quantitated by absorbance measurements at 550 nm. The PBI reductive cleavage reaction affords pieces of DNA bearing the blue aminoquinone chromophore resulting from nucleophile trapping by the aziridinyl hydroquinone followed by air oxidation.[7,8,14]

Shown in Figure 3 are the results of cleavage studies of PBI-B (plot 1), PBI-C (plot 2), PBI-D (plot 3), and PBI-A,G (plots 4 and 5). From the nearly identical absorbance values at 550 nm for plots 2–5, it is apparent that the size of the 3-substituent does not influence the DNA cleavage reaction. This observation is consistent with the major groove binding model wherein the 3-substituent would lie along the major groove and not interfere with binding or alkylation/cleavage.

A 3-substituent could interact with the major groove if H-bonding functionalities are present and appropriately placed. This seems to be the case with reduced PBI-B which can form three hydrogen bonds in the major groove (Scheme 4). The relatively large amount of PBI-B reductive alkylation and cleavage of DNA (plot 1) may be due to stronger major groove interactions. The DNA cleavage capability of PBI-B does not result in noteworthy cytotoxic properties; however, the presence of the hydrophilic carbamate group very likely reduced

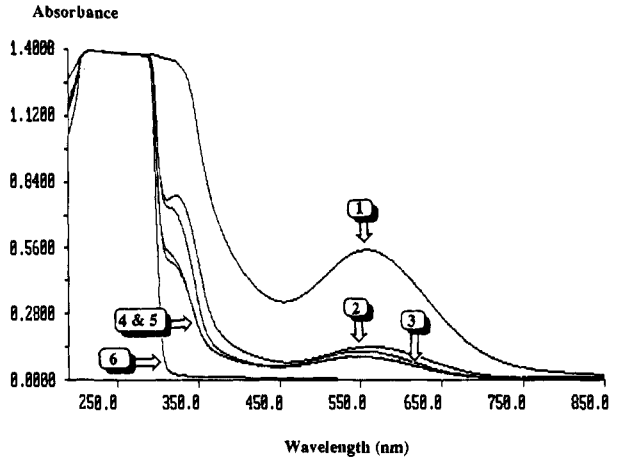


Figure 3. Absorbance vs wavelength plots of 600 bp calf thymus DNA (2 mg in 2 mL of pH 7.4 Tris buffer) which had been reductively alkylated by PBIs: plot 1, PBI-B; plot 2, PBI-C; plot 3, PBI-D; plots 4 and 5, PBI-A,G; plot 6, native DNA.

lipophilicity resulting in low cytotoxicity (point B of plot A, Figure 1).

The cytotoxicity mechanism of the APBIs and imino-APBIs is still under investigation, and only preliminary results can be presented here. Neither the APBIs nor the imino-APBIs require reductive activation. In fact, reduction leads to a loss of cytotoxicity. Thus, the APBIs and imino-APBIs show a high correlation between cytotoxicity and the absence of DT-diaphorase in the cell line. Coefficients obtained from COMPARE by

correlating low DT-diaphorase levels with cytotoxicity against 60 cancer cell lines are as follows: APBI-I, 0.669; APBI-D, 0.602; APBI-E, 0.588; imino-APBI-I, 0.465.[16] The correlation coefficient for APBI-I is the highest obtained among the over 20 000 compounds surveyed at the NCI. The absence of a reductive activation step leading to cytotoxicity of the APBIs and imino-APBIs is consistent with the hydrolysis studies, which indicate that there is no formation of an alkylating species upon reduction at physiological pH.

Further insights into the mechanism of action were obtained from COMPARE wherein the LC_{50} mean graphs of selected compounds (APBI-G and imino-APBI-G) were compared with those of antitumor compounds in the NCI archives. Compounds with similar or identical mechanisms of action show high COMPARE correlation coefficients. APBI-G shows good correlations with the anthracyclines aclacinomycin A (0.67), rubidazone (0.64), and deoxydoxorubicin (0.63). In contrast, imino-APBI-G did not show good correlations (coefficient >0.6) with any known antitumor agent—the best correlation obtained was with the steroid tamoxifen (0.59). This finding suggests that the APBIs may intercalate DNA and act as topoisomerase inhibitors, properties which are characteristic of anthracyclines.

Conclusions

The lipophilicity of the 3-substituent specifically increases the cytotoxicity of PBIs and APBIs against melanoma cell lines. Other types of cell lines are not greatly affected by the lipophilicity change. Plots of log LC_{50} against melanoma cell lines vs log of the lipophilicity (determined from retention times) show scatter due to hydrolysis of the 3-substituent. We have concluded that melanoma specific agents could be designed by increasing the lipophilicity of the 3-substituent, particularly if analogues are developed which are resistant to hydrolysis.

The imino-APBIs show increasing cytotoxicity against both melanoma and renal cell lines as the lipophilicity of the 3-substituent increases. Plots of log LC_{50} vs log of the lipophilicity possess excellent correlation coefficients due to the hydrolytic stability of the 3-substituent. We have concluded that new cytotoxic imino-APBIs could be designed by simply increasing the lipophilicity of the 3-substituent.

The DNA cleavage by PBIs is unaffected by the size of the 3-substituent, presumably because this substituent lies along the major groove. The presence of the 3-carbamate substituent greatly increases DNA cleavage. This enhanced cleavage may be the result of an additional hydrogen-bonding interaction in the major groove involving the 3-carbamate. The low lipophilicity of this substituent results in low cytotoxicity, however. We have concluded that PBIs showing enhanced DNA cleavage could be designed by preparing analogues with lipophilic 3-carbamate substituents.

Conclusions have been made pertaining to bioactivation and mechanism of action of the title compounds. We have concluded from COMPARE data provided to us[16] that PBIs require activation by DT-diaphorase. However, DT-diaphorase reduction of the APBIs and imino-APBIs substantially decreases their cytotoxicity. The mechanism of action of the APBIs and imino-APBIs was investigated using COMPARE. The APBIs appear to resemble anthracycline analogues and may inhibit topoisomerases. The cytotoxicity of imino-APBIs do not correlate well with that of any known antitumor agent, however.

A final conclusion deals with the use of 60-cell line mean graphs and COMPARE in cancer QSAR studies. The use of the NCI 60-cell line screen, rather than a single cell line, presents a broad view of cytotoxicity. If PBI cytotoxicity had been carried out with a leukemia cell line, the inevitable conclusion would have been that these agents are inactive. COMPARE permits comparison of cytotoxicity results for a series of analogues wherein systematic structural changes were made. In the present report, less than excellent correlation coefficients provided evidence that structural changes influenced cytotoxicity in a cell line specific fashion.

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., 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. ^1H NMR spectra were obtained on a 300 MHz spectrometer, and chemical shifts are reported relative to TMS.

3-Hydroxy-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole-5,8-dione (2) was prepared in two steps as described below: A suspension consisting of 1.36 g (4.3 mmol) of **1**, 200 mg of 5% Pd on carbon, and 300 mL of methanol was shaken under 50 psi H_2 for 18 h. The completed reaction was then filtered through Celite into a flask containing 5 mL of concentrated HCl. The filtrate was evaporated *in vacuo* to afford the amine hydrochloride salt. This salt was dissolved in 150 mL of H_2O containing 3.0 g of monobasic potassium phosphate. Another solution consisting of 7.9 g of Fremy salt dissolved in 250 mL of water containing 1.5 g of monobasic potassium phosphate was then added to the amine hydrochloride salt solution and the resulting mixture stirred at room temperature for 7.5 h. The reaction mixture was then extracted five times with 100 mL portions of chloroform. The extracts were dried (Na_2SO_4), filtered, and concentrated to a residue. The pure product was obtained by flash chromatography of the residue on silica gel using chloroform/methanol (98:2) as the eluant. Recrystallization was carried out from chloroform/hexane: 583 mg (73%) yield; mp 155 °C dec; TLC (acetone) $R_f = 0.43$; IR (KBr pellet) 3242, 1656, 1518, 1477, 1302, 1267, 1155, 1111, 968, 812 cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6 with a drop of D_2O) δ 6.52 (1H, q, $J = 1.5$ Hz, C(6)-proton coupled with C(7)-methyl), 5.26 (1H, dd, $J = 3$ and 7.3 Hz, C(3)-proton), 4.34 (2H, m, C(1)-diastereomeric methylene), 3.02 and 2.73 (2H, 2m, C(2)-diastereomeric methylene), 2.10 (3H, d, $J = 1.5$ Hz, C(7)-methyl split with C(6)-proton); MS (EI mode) *m/z* 218 (M^+), 201 (M^+ − hydroxy). Anal. ($C_{11}H_{10}N_2O_3 \cdot 0.9H_2O$) C,N; H: calcd, 5.07; found, 4.15.

3-Hydroxy-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole-5,8-dione 3-Propionate (3). To a solution of 207 mg (0.95 mmol) of **2** in 20 mL of methylene chloride were added 90 μL (1.15 mmol) of pyridine and then 130 μL (1.1 mmol) of propionic anhydride. The resulting mixture was refluxed for 28 h, and then the solvent was removed *in vacuo* to afford a solid residue. The residue was purified by flash chromatography on silica gel using chloroform as the eluant. The purified product was then recrystallized from chloroform/hexane: 151 mg (58%) yield; TLC (chloroform/methanol, 90:10) $R_f = 0.64$; mp 116–117 °C; IR (KBr pellet) 1739, 1656, 1523, 1437, 1259, 1169, 1085, 977, 900, 733 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.55 (1H, q, $J = 2$ Hz, C(6)-proton coupled to C(7)-methyl), 6.10 (1H, dd, $J = 3$ and 7.6 Hz, C(3)-proton), 4.35

(2H, m, C(1)-diastereomeric methylene), 3.18 and 2.65 (2H, 2m, C(2)-diastereomeric methylene), 2.38 (2H, quartet of doublets, $J = 3$ and 7.4 Hz, methylene of propionate), 2.11 (3H, d, $J = 2$ Hz, C(7)-methyl coupled to C(6)-proton), 1.15 (3H, t, $J = 7.5$ Hz, methyl of propionate); MS (EI mode) m/z 274 (M^+), 217 ($M^+ - CH_3CH_2C{=}O$), 201 (M^+ − propionic acid). Anal. ($C_{14}H_{14}N_2O_4$) C,H,N.

3-Hydroxy-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole-5,8-dione 3-Benzoate (4). To a solution of 47 mg (0.215 mmol) of **2** in 15 mL of methylene chloride were added 130 μL (1.76 mmol) of pyridine and then 100 μL (0.88 mmol) of benzoyl chloride. The reaction mixture was refluxed for 44 h and then allowed to cool to room temperature. The completed reaction mixture was washed twice with 25 mL portions of water, once with a 25 mL portion of pH 5.5 acetate buffer, and once with a 30 mL portion of water. The methylene chloride layer was dried (Na_2SO_4), filtered, and concentrated *in vacuo* to a solid residue, which was recrystallized from acetone/hexane: 59 mg (82%) yield; TLC (chloroform/methanol, 90:10) $R_f = 0.63$; mp 163−164 °C dec; IR (KBr pellet) 3065, 2958, 2922, 1724, 1666, 1601, 1450, 1265, 1107, 1068 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.02 (2H, d, $J = 7.5$ Hz, C(2)- and C(6)-phenyl protons), 7.5 (1H, t, $J = 7.3$ Hz, C(4)-phenyl protons), 7.43 (2H, t, $J = 7.7$ Hz, C(3)- and C(5)-phenyl protons), 6.57 (1H, bs, C(6)-proton), 6.32 (1H, dd, $J = 3.0$ and 7.5 Hz, C(3)-proton), 4.42 (2H, m, C(1)-diastereomeric methylene), 3.30 and 2.82 (2H, 2m, C(2)-diastereomeric methylene), 2.12 (3H, d, $J = 1.5$ Hz, C(7)-methyl split by C(6)-proton); MS (EI mode) m/z 322 (M^+), 217 ($M^+ - PhC{=}O$). Anal. ($C_{18}H_{14}N_2O_4 \cdot 0.1H_2O$) C,H,N.

3-Hydroxy-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole-5,8-dione 3-Valerate (5). To a solution of 70 mg (0.32 mmol) of **2** in 20 mL of methylene chloride containing 100 μL (1.28 mmol) of pyridine was added 76 μL (0.64 mmol) of valeryl chloride while maintaining a temperature of 10 °C. The reaction mixture was stirred for 5 min at 10 °C and then for 5 h at room temperature. The completed reaction mixture was washed twice with 20 mL portions of saturated aqueous sodium bicarbonate, twice with 20 mL portions of pH 5.5 acetate buffer, and finally twice with 20 mL portions of water. The methylene chloride layer was then dried (Na_2SO_4), filtered, and concentrated *in vacuo* to a solid residue. Recrystallization was carried out from chloroform/hexane: 75.3 mg (78%) yield; TLC (chloroform/methanol, 95:5) $R_f = 0.68$; mp 106−107 °C; IR (KBr pellet) 2960, 1739, 1658, 1674, 1514, 1251, 1161, 1111, 977, 729 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.55 (1H, q, $J = 1.5$ Hz, C(6)-proton split by C(7)-methyl), 6.09 (1H, dd, $J = 3$ and 7.6 Hz, C(3)-proton), 4.35 (2H, m, C(1)-diastereomeric methylene), 3.17 and 2.64 (2H, 2m, C(2)-diastereomeric methylene), 2.35 (2H, triplet of doublets, $J = 2$ and 7.5 Hz, C(2)-methylene of valerate), 2.11 (3H, d, $J = 1.5$ Hz, C(7)-methyl), 1.62 (2H, quint, $J = 7.5$ Hz, C(3)-methylene of valerate), 1.35 (2H, sextet, $J = 7.3$ Hz, C(4)-methylene of valerate), 0.909 (3H, t, $J = 7.5$ Hz, C(5)-methyl of valerate); MS (EI mode) m/z 302 (M^+), 201 (M^+ − valerate). Anal. ($C_{16}H_{18}N_2O_4$) C,H,N.

6-(1-Aziridinyl)-3-hydroxy-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole-5,8-dione (PBI-D). To a solution of 208 mg (0.79 mmol) of **2** in 8 mL of dry methanol, chilled to 4 °C by means of an ice bath, was added 2 mL of ethylenimine. The reaction mixture was stirred at 4 °C for 30 min and then for 12 h at room temperature. The product crystallized from solution during the 12 h period and was filtered off and dried under vacuum. Purification was accomplished by chromatography on silica gel with chloroform/methanol (98:2) as the eluant. Recrystallization was carried out from a hot mixture of ethyl acetate/methanol: 85.5 mg (42%) yield; TLC (chloroform/methanol, 82:20) $R_f = 0.59$; mp 140 °C dec; IR (KBr pellet) 3194, 1676, 1639, 1521, 1315, 1205, 1099, 985, 825, 738 cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6) δ 5.92 (1H, d, $J = 5.7$ Hz, C(3)-hydroxy), 4.98 (1H, m, C(3)-proton), 4.14 (2H, m, C(1)-diastereomeric methylene) 2.88 and 2.35 (2H, 2m, C(2)-diastereomeric methylene), 2.30 (4H, s, aziridine protons), 1.94 (3H, s, C(7)-methyl); MS (EI mode) m/z 259 (M^+), 232 ($M^+ - CH_2{=}CH$). Anal. ($C_{13}H_{13}N_3O_3 \cdot 0.4H_2O$) C,H,N.

6-(1-Aziridinyl)-3-hydroxy-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole-5,8-dione 3-Chloroacetate (PBI-E). To a suspension of 106 mg (0.41 mmol) of PBI-D in 10 mL of methylene chloride were added 308 μL (4.1 mmol) of dry pyridine and then 39 μL (0.49 mmol) of chloroacetyl chloride. The reaction mixture was stirred for 15 min and then washed twice with 20 mL portions of water. The dried (Na_2SO_4) organic layer was filtered and concentrated to a residue, which was purified by flash chromatography on silica gel employing chloroform/methanol (99:1) as the eluant. The purified product was then recrystallized from chloroform/hexane: 49 mg (36%) yield; mp 164−167 °C dec; TLC (chloroform/methanol, 90:10) $R_f = 0.53$; IR (KBr pellet) 3003, 1755, 1674, 1635, 1570, 1560, 1356, 1317, 1070, 870 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.18 (1H, dd, $J = 3$ and 7.5 Hz, C(3)-proton), 4.35 (2H, m, C(1)-diastereomeric methylene), 4.09 (2H, d, $J = 3$ Hz, chloromethyl) 3.28 and 2.73 (2H, 2m, C(2)-diastereomeric methylene), 2.37 (4H, aziridine protons), 2.08 (3H, s, C(7)-methyl); MS (EI mode) m/z 335 (M^+, ^{35}Cl), 337 ($M^+ + 2$, ^{37}Cl), 240, 214. Anal. ($C_{16}H_{14}ClN_3O_4$) C,H,N.

6-(1-Aziridinyl)-3-hydroxy-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole-5,8-dione 3-Benzoate (PBI-G). To a solution of 58.2 mg (0.15 mmol) of **4** in 4 mL of dry methanol, chilled by means of an ice bath to 0°C, was added 0.5 mL of ethylenimine. The reaction mixture was stirred at 0 °C for 0.5 h and then removed from the ice bath and stirred for 1 h. Removal of the reaction solvent *in vacuo* afforded a red solid, which was purified by flash chromatography on silica gel employing chloroform as the eluant. Recrystallization was carried out from chloroform/hexane: 31.8 mg (58%) yield; TLC (chloroform/methanol, 90:10) $R_f = 0.71$; mp 158−160 °C; IR (KBr pellet) 3001, 2362, 2335, 1724, 1680, 1637, 1518, 1267, 1107, 711 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.02 (2H, dd, $J = 2$ and 8.4 Hz, C(2)- and C(6)-phenyl protons), 7.56 (1H, triplet of doublets, $J = 1.2$ and 7 Hz, C(4)-phenyl proton), 7.43 (2H, triplet of doublets, $J = 1.7$ and 6.7 Hz, C(3)- and C(5)-phenyl protons), 6.28 (1H, dd, $J = 2.9$ and 7.5 Hz, C(3)-protons), 4.37 (2H, m, C(1)-diastereomeric methylene), 3.25 and 2.79 (2H, 2m, C(2)-diastereomeric methylene), 2.38 (4H, s, aziridine protons), 2.01 (3H, s, C(7)-methyl); MS (EI mode) m/z 363 (M^+), 258 ($M^+ - PhC{=}O$). Anal. ($C_{19}H_{17}N_3O_4 \cdot 0.5H_2O$) C,H,N.

6-(1-Aziridinyl)-3-hydroxy-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole-5,8-dione 3-Propionate (PBI-F). To a solution of 60 mg (0.22 mmol) of **3** in 2.5 mL of dry methylene chloride was added 0.5 mL of ethylenimine, and the resulting mixture was stirred at room temperature for 1 h. The reaction mixture was placed on a silica gel column prepared with chloroform. The product was eluded with chloroform and then recrystallized from chloroform/hexane: 17 mg (22%) yield; TLC (chloroform/methanol, 90:10) $R_f = 0.65$; mp 166−169 °C; IR (KBr pellet) 1741, 1674, 1637, 1574, 1521, 1438, 1313, 1140, 995, 736 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.07 (1H, dd, $J = 3$ and 7.5 Hz, C(3)-proton), 4.30 (2H, m, C(1)-diastereomeric methylene), 3.15 and 2.62 (2H, 2m, C(2)-diastereomeric methylene), 2.36 (4H, s, aziridinyl protons), 2.35 (2H, quartet of doublets, $J = 2.7$ and 7.5 Hz, methylene of propionate), 2.08 (3H, s, C(7)-methyl), 1.15 (3H, t, $J = 7.6$ Hz, methyl of propionate); MS (EI mode) m/z 315 (M^+), 258 ($M^+ - CH_3CH_2C{=}O$), 240 ($M^+ - H$ − propionic acid), 216 (M^+ − aziridine − $H_3CCH{=}C{=}O$). Anal. ($C_{16}H_{17}N_3O_4 \cdot 0.1H_2O$) C,H,N.

6-(1-Aziridinyl)-3-hydroxy-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole-5,8-dione 3-Valerate (PBI-H). To a solution of 50 mg (0.165 mmol) of **5** in 4 mL of dry methanol, chilled to 0 °C by means of an ice bath, was added 0.5 mL of ethylenimine. The reaction mixture was stirred at 0 °C for 30 min and then removed from the ice bath and stirred for 1 h. The reaction solvent was removed *in vacuo* and the residue subjected to flash chromatography on silica gel with chloroform as the eluant. The purified product was recrystallized from chloroform/hexane: 14.7 mg (26%) yield; TLC (chloroform/methanol, 98:2) $R_f = 0.67$; mp 123−126 °C dec; IR (KBr pellet) 2960, 1741, 1678, 1635, 1518, 1313, 1163, 1141, 1035, 993 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.05 (1H, dd, $J = 3$ and 7.6 Hz, C(3)-proton), 4.31 (2H, m, C(1)-diastereomeric methylene), 3.15 and 2.62 (2H, 2m, C(2)-diastereomeric methylene),

2.36 (4H, s, aziridinyl protons), 2.34 (2H, triplet of doublets, J = 2.2 and 7.6 Hz, C(2)-methylene of valerate), 2.08 (3H, s, C(7)-methyl), 1.61 (2H, quint, J = 7.6 Hz, C(3)-methylene of valerate), 1.33 (2H, sextet, J = 7.6 Hz, C(4)-methylene of valerate), 0.907 (3H, t, J = 7.3 Hz, C(5)-methyl of valerate); MS (EI mode) m/z 343 (M^+), 258 ($M^+ - H_3C(CH_2)_3C{=}O$). Anal. ($C_{18}H_{21}N_3O_4$) C,H,N.

6-Acetamido-3-hydroxy-7-methyl-5-nitro-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole (6). A solution of 205 mg (0.62 mmol) of 6-acetamido-3-hydroxy-7-methyl-5-nitro-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole 3-acetate in 10 mL of methanol was combined with 88 mg of potassium carbonate. After 20 min, the reaction mixture was concentrated to dryness and then chromatographed on a silica gel column using chloroform/methanol (98:2) as the eluant. The isolated product was recrystallized by dissolution in a minimum volume of chloroform/methanol (4:1) and then addition of a few drops of hexane: 141 mg (78%) yield; TLC (chloroform/methanol, 80:20) R_f = 0.33; mp 259 °C dec; IR (KBr pellet) 3336, 3211, 3181, 3173, 3160, 3152, 1667, 1531, 1404, 1370 cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6) δ 9.67 (1H, bs, acetamido NH), 7.67 (1H, s, aromatic), 5.97 (1H, d, J = 5 Hz, C(3)-hydroxy), 5.09 (1H, m C(3)-proton), 4.14 (2H, m C(1)-diastereomeric methylene), 2.90 and 2.36 (2H, 2m, C(2)-diastereomeric methylene), 2.27 (3H, s, C(7)-methyl), 1.98 (3H, s, acetamido methyl); MS (EI mode) m/z 290 (M^+), 272 ($M^+ - H_2O$). Anal. ($C_{13}H_{14}N_4O_4{\cdot}0.7H_2O$) C,H,N.

6-Acetamido-3-hydroxy-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole-5,8-dione (APBI-D). A suspension consisting of 756 mg (2.6 mmol) of **6**, 150 mg of 5% Pd on carbon, and 130 mL of methanol was shaken under 50 psi H_2 for 18 h. The reaction mixture was then filtered through Celite and the filtrate concentrated to afford the amine as a white solid. The amine was dissolved in 200 mL of water containing 2.0 g of monobasic potassium phosphate. Another solution consisting of 4.65 g of Fremy's salt dissolved in 150 mL of water containing 3 g of monobasic potassium phosphate was added to the amine solution and the resulting mixture stirred for 2.5 h at room temperature. The reaction mixture was then extracted 10 times with 100 mL portions of chloroform. The extracts were dried (Na_2SO_4), filtered, and concentrated to a yellow solid, which was recrystallized from chloroform/hexane: 149 mg (21%) yield; TLC (chloroform/methanol, 90:10) R_f = 0.34; mp 232–238 °C dec; IR (KBr pellet) 3275, 1678, 1645, 1508, 1315, 1248, 1099, 1010, 740, 597 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.62 (1H, s, amide proton), 5.92 (1H, d, J = 5.6 Hz, C(3)-hydroxy), 5.02 (1H, m, C(3)-proton), 4.24 (2H, m, C(1)-diastereomeric methylene), 2.95 and 2.42 (2H, 2m, C(2)-diastereomeric methylene), 2.07 (3H, s, C(7)-methyl), 1.82 (3H, s, acetamido methyl); MS (EI mode) m/z 275(M^+), 233 ($M^+ - CH_2{=}C{=}O$), 259 ($M^+ - O$). Anal. ($C_{13}H_{13}N_3O_4{\cdot}0.4H_2O$) C,H,N.

General Procedure for Preparing APBIs. To a mixture of 32 mg (0.11 mmol) of APBI-D in 3 mL of methylene chloride were added 10 μL (0.108 mmol) of pyridine and 0.10 mmol of the appropriate acid chloride. The reaction mixture was stirred at room temperature for 1 h and then evaporated *in vacuo* to a solid. Purification of the solid was carried out utilizing preparative silica gel TLC with chloroform/methanol (96:4) as the solvent. The product was removed from the TLC plate by extraction with chloroform/methanol (95:5) and recrystallized from chloroform/hexane. Physical properties of the APBIs are provided below.

6-Acetamido-3-hydroxy-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole-5,8-dione 3-chloroacetate (APBI-E): 55% yield; TLC (chloroform/methanol, 90:10) R_f = 0.55; mp 200–202 °C dec; IR (KBr pellet) 1757, 1658, 1520, 1367, 1315, 1215, 1778, 1082, 1035, 738 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.70 (1H, s, acetamido N-H), 6.17 (1H, dd, J = 2.9 and 7.6 Hz, C(3)-proton), 4.37 (2H, m, C(1)-diastereomeric methylene), 4.1 (2H, d, J = 2.2 Hz, chloromethyl), 3.24 and 2.72 (2H, 2m, C(2)-diastereomeric methylene), 2.25 (3H, s, C(7)-methyl), 1.98 (3H, s, C(6)-acetamido methyl); MS (EI mode) m/z 351 (M^+), 309 ($M^+ - H_2C{=}C{=}O$), 259, 217, 251 (M^+ – chloroacetic acid – $H_2C{=}C{=}O$). Anal. ($C_{15}H_{14}ClN_3O_5{\cdot}0.2H_2O$) C,H,N.

6-Acetamido-3-hydroxy-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole-5,8-dione 3-propionate (APBI-F): 55% yield; TLC (chloroform/methanol, 90:10) R_f = 0.47; mp 173–175 °C; IR (KBr pellet) 1741, 1676, 1510, 1371, 1311, 1246, 1186, 1105, 1033, 597 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.71 (1H, s, acetamido NH), 6.10 (1H, dd, J = 2.5 and 7.7 Hz, C(3)-proton), 4.35 (2H, m, C(1)-diastereomeric methylene), 3.18 and 2.65 (2H, 2m, C(2)-diastereomeric methylene), 2.36 (2H, quartet of doublets, J = 2.5 and 7.5 Hz, methylene of propionate), 2.25 (3H, s, C(7)-methyl), 1.98 (3H, s, acetamido methyl), 1.15 (3H, t, J = 7.3 Hz, methyl of propionate); MS (EI mode) m/z 331 (M^+), 289 ($M^+ - CH_2{=}C{=}O$), 257 (M^+ – propanoic acid). Anal. ($C_{16}H_{17}N_3O_5$) C,H,N.

6-Acetamido-3-hydroxy-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole-5,8-dione 3-benzoate (APBI-G): 25% yield; TLC (chloroform/methanol, 90:10) R_f = 0.53; mp 206–208 °C; IR (KBr pellet) 1720, 1658, 1520, 1489, 1315, 1261, 1105, 1070, 1026, 717 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.02 (1H, dd, J = 1.4 and 8.3 Hz, C(3)- and C(6)-phenyl), 7.10 (1H, s, acetamido NH) 7.59 (1H, triplet of doublets, J = 1.4 and 7.4 Hz, C(4)-phenyl protons), 7.41 (2H, t, J = 6.9 Hz, C(3)- and C(5)-phenyl protons), 6.31 (1H, dd, J = 1.8 and 8.7 Hz, C(3)-proton), 4.42 (2H, m, C(1)-diastereomeric methylene), 3.28 and 2.82 (2H, 2m, C(2)-diastereomeric methylene), 2.25 (3H, s, C(7)-methyl), 2.00 (3H, s, C(6)-acetamido methyl); MS (EI mode) m/z 379 (M^+), 337 ($M^+ - H_2C{=}C{=}O$), 257 (M^+ – benzoic acid). Anal. ($C_{20}H_{17}N_3O_5{\cdot}0.3H_2O$) C,H,N.

6-Acetamido-3-hydroxy-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole-5,8-dione 3-methoxyacetate (APBI-I): 73% yield; TLC (chloroform/methanol, 90:10) R_f = 0.40; mp 152–156 °C; IR (KBr pellet) 2361, 2333, 1753, 1653, 1521, 1435, 1383, 1195, 1024, 1037 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.70 (1H, s, acetamido NH), 6.19 (1H, dd, J = 2.9 and 7.6 Hz, C(3)-proton), 4.36 (2H, m, C(1)-diastereomeric methylene), 4.08 (2H, s, methylene of methoxymethyl), 3.45 (3H, s, methyl of methoxymethyl), 3.23 and 2.73 (2H, 2m, C(2)-diastereomeric methylene), 2.25 (3H, s, C(7)-methyl), 1.98 (3H, s, acetamido methyl); MS (EI mode) m/z 347 (M^+), 305 ($M^+ - H_2C{=}C{=}O$), 257 (M^+ – methoxyacetic acid). Anal. ($C_{16}H_{17}N_3O_6$) C,H,N.

***syn/anti*-6-Acetamido-5-imino-3-hydroxy-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazol-8-one (imino-APBI-D)** was prepared in two steps as described below. A suspension consisting of 300 mg (1.05 mmol) of **6**, 60 mg of 5% Pd on carbon, and 50 mL of methanol was shaken under 50 psi H_2 for 2.5 h. The completed reaction mixture was then filtered through Celite and the filtrate concentrated *in vacuo* to a solid, which was the reduced (5-amino) form of **6**. Recrystallization was carried out from chloroform/hexane: 262 mg (97%) yield; mp 190–220 °C dec; TLC (chloroform/methanol, 80:20) R_f = 0.39; IR (KBr pellet) 3358, 3230, 1651, 1527, 1456, 1298, 1084, 1014, 806, 750 cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6) δ 8.92 (1H, s, amide proton), 6.55 (1H, s, C(8)-proton) 5.76 (1H, m, C(3)-hydroxy), 5.04 (1H, m, C(3)-proton), 4.82 (2H, s, amine), 4.00 (2H, m, C(1)-diastereomeric methylene), 2.87 and 2.38 (2H, 2m, C(2)-diastereomeric methylene), 2.15 (3H, s, C(7)-methyl), 2.04 (3H, s, acetamido methyl).

To a solution of 150 mg (0.57 mmol) of reduced **6** in 20 mL of 0.2 M pH 7.0 phosphate buffer (μ = 1.0, KCl) was added 718 mg of Fremy salt. The mixture was stirred for 1 h followed by addition of 10 mL of water. The precipitated iminoquinone was filtered off, washed three times with 10 mL portions of water, and then vacuum-dried: 77 mg (49%) yield; TLC (acetone/methanol, 80:20) R_f = 0.40; mp 250–320 °C dec; IR (KBr pellet) 3311, 3138, 2814, 1626, 1589, 1500, 1427, 1396, 1155, 972, 742 cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6) δ 9.27 (1H, s, imino proton) 6.67 (1H, s, C(6)-acetamido), 5.85 (1H, q, J = 5.9 Hz, C(3)-hydroxy), 4.97 (1H, m, C(3)-proton), 4.15 (2H, m, C(1)-diastereomeric methylene), 2.87 and 2.35 (2H, 2m, C(2)-diastereomeric methylene), 1.73 (3H, s, C(7)-methyl), 1.59 (3H, s, C(6)-acetamido methyl); MS (EI mode) m/z 274 (M^+), 232 ($M^+ - H_2C{=}C{=}O$). Anal. ($C_{13}H_{14}N_4O_3{\cdot}1.3H_2O$) C,H,N.

6-Acetamido-3-hydroxy-7-methyl-5-nitro-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole 3-Propionate (7). To a suspension of 200 mg (0.69 mmol) of **6** in 10 mL of dry

methylene chloride were added 11 μL (1.38 mmol) of pyridine and 10 μL (0.76 mmol) of propionic anhydride. The reaction mixture was refluxed for 48 h, and then the same portions of pyridine and propionic anhydride were added again followed by another 48 h reflux. The reaction mixture was diluted with 30 mL of methylene chloride and washed twice with 25 mL portions of water, twice with 25 mL portions of 10% aqueous acetic acid, and finally twice with 25 mL portions of water. Evaporation of the methylene chloride afforded the solid product, which was recrystallized from hot chloroform: 221 mg (93%) yield; mp 198–200 °C dec; TLC (acetone/methanol, 90:10) R_f = 0.45; IR (KBr pellet) 2978, 1745, 1680, 1525, 1464, 1380, 1273, 1167, 1084, 1057 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.90 (1H, s, amide), 7.48 (1H, s, C(8)-proton), 6.19 (1H, dd, J = 7.5 and 3.2 Hz, C(3)-proton), 4.24 (2H, m, C(1)-diastereomeric methylene), 3.25 and 2.46 (2H, 2m, C(2)-diastereomeric methylene), 2.43 (3H, s, C(7)-methyl), 2.38 (2H, quartet of doublets, J = 3.2 and 7.8 Hz, methylene of propyl), 2.22 (3H, s, amide methyl), 1.16 (3H, t, J = 7.5 Hz, methyl of propyl); MS (EI mode) m/z 346 (M^+), 328 ($M^+ - H_2O$), 273 (M^+ – propionate), 230 (M^+ – propionic acid – CH_2=C=O), 212 (M^+ – propionic acid – CH_2=C=O – H_2O), 184 (M^+ – propionic acid – CH_2=C=O – NO_2). Anal. ($C_{16}H_{18}N_4O_5$) C,H,N.

6-Acetamido-3-hydroxy-7-methyl-5-nitro-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole 3-Benzoate (8). To a suspension consisting of 400 mg (1.38 mmol) of **6** and 50 mL of dry methylene chloride were added 580 μL (4.14 mmol) of triethylamine and 240 μL (2.07 mmol) of benzoyl chloride. The reaction mixture was refluxed for 10 h and the precipitated product filtered off, washed with water, and vacuum-dried: 514 mg (94%) yield; mp 270 °C dec; TLC (chloroform/methanol, 90:10) R_f = 0.51; IR (KBr pellet) 2970, 1722, 1680, 1525, 1464, 1357, 1271, 1111, 1070, 719 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 9.78 (1H, s, amide), 7.99 (2H, d, J = 8.1 Hz, C(2)- and C(6)-phenyl proton), 7.82 (2H, t, J = 8.3 Hz, C(3)- and C(5)-phenyl proton), 6.44 (1H, dd, J = 4.3 and 6.8 Hz, C(3)-proton), 4.32 (2H, m, C(1)-diastereomeric methylene), 3.25 and 2.78 (2H, 2m, C(2)-diastereomeric methylene), 2.35 (3H, s, C(7)-methyl), 2.03 (3H, s, amide methyl); MS (EI mode) m/z 394 (M^+), 376 ($M^+ - H_2O$), 364(M^+ – NO), 348 ($M^+ - NO_2$), 247 (M^+ – PhC=O – H_2C=C=O). Anal. ($C_{20}H_{18}N_4O_5 \cdot 0.7H_2O$) C,N; H: calcd, 4.80; found, 4.47.

6-Acetamido-3-hydroxy-7-methyl-5-nitro-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole 3-Methoxyacetate (9). To a suspension consisting of 400 mg (1.38 mmol) of **6** and 50 mL of dry methylene chloride were added 311 μL (4.14 mmol) of pyridine and then 100 μL (1.52 mmol) of methoxyacetyl chloride. The reaction mixture was stirred at room temperature for 4 h and the product filtered off, washed with water, and vacuum-dried: 303 mg (61%) yield; mp 210 °C dec; TLC (chloroform/methanol, 90:10) R_f = 0.36; IR (KBr pellet) 2960, 1766, 1682, 1525, 1464, 1361, 1257, 1178, 1057 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 9.78 (1H, s, amide proton), 7.79 (1H, s, C(8)-proton), 6.24 (1H, dd, J = 3.5 and 7.7 Hz, C(3) proton), 4.31 (2H, m, C(1)-diastereomeric methylene), 4.13 (2H, s, methylene of methoxymethyl), 3.16 and 2.65 (2H, 2m, C(2)-diastereomeric methylene) 2.49 (3H, s, methyl of methoxymethyl), 2.33 (3H, s, C(7)-methyl), 2.02 (3H, s, amide methyl); MS (EI mode) m/z 362 (M^+), 344 ($M^+ - H_2O$), 316 ($M^+ - NO_2$). Anal. ($C_{16}H_{18}N_4O_6$) C,H,N.

***syn/anti*-6-Acetamido-5-imino-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazol-8-one 3-Propionate (imino-APBI-F).** A suspension consisting of 180 mg (0.52 mmol) of **7**, 36 mg of 5% Pd in carbon, and 75 mL of methanol was shaken under 50 psi H_2 for 3.5 h. The completed reaction mixture was filtered through Celite and the methanol removed *in vacuo*. The solid residue was suspended in 25 mL of 0.2 M pH 7.0 phosphate buffer (μ = 1.0, KCl). To this suspension were added a solution of 750 mg of Fremy salt in 40 mL of the same buffer and then 10 mL of H_2O. The reaction mixture was stirred at room temperature for 1 h. The precipitated product was filtered off, washed with water, and vacuum-dried: 78 mg (46%) yield; TLC (chloroform/methanol, 90:10) R_f = 0.42; mp 244–300 °C dec; IR (KBr pellet) 3129, 2362, 1736, 1712, 1626, 1601, 1496, 1421, 1317, 1178 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 11.02 (1H, s, imine proton), 8.34 (1H, s, amide proton), 6.08 (1H, dd, J = 3 and 7.5 Hz, C(3)-proton), 4.35 (2H, m, C(1)-diastereomeric methylene), 3.14 and 2.65 (2H, 2m, C(2)-diastereomeric methylene), 2.41 (2H, quartet of doublets, J = 2.8 and 7.6 Hz, methylene of propyl), 2.25 (3H, s, C(7)-methyl), 2.00 (3H, s, acetamido methyl), 1.17 (3H, t, J = 7.5 Hz, methyl of propyl); MS (EI mode) m/z 330 (M^+), 288 ($M^+ - H_2C$=C=O), 241(M^+ – propionic acid – methyl), 213 ($M^+ - H_2C$=C=O – CH_3CH_2C=O – H_2O). Anal. ($C_{16}H_{18}N_4O_4 \cdot H_2O$) C,H,N.

***syn/anti*-6-Acetamido-5-imino-3-hydroxy-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazol-8-one 3-benzoate (imino-APBI-G)** was prepared in the two steps described as follows. A suspension consisting of 200 mg (0.51 mmol) of **8**, 40 mg of 5% Pd on carbon, and 75 mL of methanol was shaken under 50 psi H_2 for 2 h. The reaction mixture was filtered through Celite and concentrated *in vacuo* to a solid, which was recrystallized from chloroform/hexane: 176 mg (94%) yield; TLC (chloroform/methano, 80:20) R_f = 0.56; mp 203–209 °C dec; IR (KBr pellet) 3360, 1718, 1655, 1527, 1492, 1302, 1267, 1109, 1070, 709 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 8.92 (1H, s, amide proton), 7.98 (2H, d, J = 7.3 Hz, C(2)- and C(6)-phenyl), 7.68 (1H, t, J = 7.6 Hz, C(4)-phenyl), 7.52 (2H, t, J = 7.8 Hz, C(3)- and C(5)-phenyl), 6.62 (1H, s, C(8)-proton), 6.38 (1H, dd, J = 2.9 and 7.5 Hz, C(3)-proton), 4.96 (2H, m, amino), 4.17 (2H, m, C(1)-diastereomeric methylene), 3.21 and 2.72 (2H, 2m, C(2)-diastereomeric methylene), 2.17 (3H, s, C(7)-methyl), 2.04 (3H, s, methyl of acetamido); MS (EI mode) m/z 364 (M^+), 347 (M^+ – amino).

To a solution of 151 mg (0.42 mmol) of the amine in 12.5 mL of dimethyl formamide were added 25 mL of 0.2 M pH 7.0 phosphate buffer (μ = 1, KCl) and 700 mg of Fremy salt. This mixture was stirred at room temperature for 1 h and then extracted three times with 25 mL portions of chloroform. The chloroform extracts were dried (Na_2SO_4) and concentrated to a red solid, which was recrystallized from acetone/hexane: 68 mg (43%) yield; TLC (chloroform/methanol, 90:10) R_f = 0.60; mp 160 °C dec; IR (KBr pellet) 2990, 1722, 1653, 1602, 1491, 1491, 1313, 1267, 1109, 1026, 713 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 11.71, 9.38, and 6.68 (1H, 3s, syn/anti protons), 9.67 and 6.73 (1H, 2s, syn/anti amide protons), 7.98 (2H, dd, J = 1.2 and 9 Hz, C(2)- and C(6)-phenyl), 7.69 (1H, q, J = 7 and 2 Hz, C(4)-phenyl), 7.54 (2H, t, J = 7.5 Hz, C(3)- and C(5)-phenyl), 6.35 and 6.28 (1H, 2m, C(3)-proton), 4.33 (2H, m, C(1)-diastereomeric methylene), 3.23 and 2.71 (2H, 2m, C(2)-diastereomeric methylene), 2.09 and 1.71 (3H, 2s, C(7) syn/anti methyl), 1.84 (3H, s, methyl of acetamido), 1.58 (3H, d, syn/anti amide methyl). Anal. ($C_{20}H_{18}N_4O_4 \cdot 2H_2O$) C,H; N: calcd, 14.76; found, 14.21.

***syn/anti*-6-Acetamido-5-imino-3-hydroxy-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazol-8-one 3-methoxyacetate (imino-APBI-I)** was prepared in the two steps described as follows. A suspension consisting of 250 mg (0.69 mmol) of **9**, 58 mg of 5% Pd on carbon, and 75 mL of methanol was shaken under 50 psi H_2 for 2 h. The reaction mixture was filtered through Celite and concentrated to afford the amine form of **9**. Recrystallization was carried out from acetone/hexane: 198 mg (85%) yield; TLC (chloroform/methanol, 80:20) R_f = 0.51; mp 180 °C dec; IR (KBr pellet) 3352, 1753, 1726, 1664, 1620, 1529, 1491, 1307, 1263, 1126, cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 8.91 (1H, s, amide proton), 6.18 (1H, dd, J = 2.7 and 7.5 Hz, C(3)-proton), 4.95 (2H, s, amine), 4.13 (2H, m, C(1)-diastereomeric methylene), 4.10 (2H, s, methylene of methoxymethyl), 3.14 and 2.59 (2H, 2m, C(2)-diastereomeric methylene), 2.54 (3H, s, methyl of methoxymethyl), 2.16 (3H, s, C(7)-methyl), 2.04 (3H, s, amide methyl); MS (EI mode) m/z 332 (M^+), 290 ($M^+ - H_2C$=C=O).

To a solution of 150 mg (0.45 mmol) of the amine obtained above in 20 mL of 0.2 M pH 7.0 phosphate buffer (μ = 1.0, KCl) was added 700 mg of Fremy salt. The reaction mixture was stirred for 1 h and then extracted three times with 20 mL portions of chloroform. The chloroform extracts were dried (Na_2SO_4) and concentrated to a red solid, which was recrystallized from acetone/hexane: 35 mg (22%) yield; TLC (chloroform/methanol, 90:10) R_f = 0.42; mp 98–104 °C dec; IR (KBr pellet) 2991, 1757, 1656, 1610, 1489, 1265, 1248, 1186, 1124, 1030 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 11.68 and 9.37 (1H,

2s, syn/anti imine proton), 9.67 and 6.68 (1H, 2s, syn/anti amide proton), 6.12 (1H, m, C(3)-proton), 4.25 (2H, m, C(1)-diastereomeric methylene), 3.09 and 2.56 (2H, 2m, C(2)-diastereomeric methylene), 4.10 (2H, d, $J = 3$ Hz, methylene of methoxymethyl), 3.46 (3H, s, methyl of methoxymethyl), 1.75 and 1.59 (6H, 2s, 7-methyl and methyl of acetamido, no assignments made); MS (EI mode) m/z 346 (M^+) 304 ($M^+ - CH_2{=}C{=}O$). Anal. ($C_{16}H_{18}N_4O_5$) C,H,N.

Hydrolysis Studies of Reduced APBI-A. The hydroquinone form of APBI-A (**10**) was prepared by H_2 reduction of the quinone in methanol for 1 h employing 5% Pd on carbon as the catalyst. The reduced solution was filtered through Celite and the filtrate acidified with concentrated HCl. Evaporation of the filtrate afforded **10** as the hydrochloride salt. Recrystallization from methanol/ethyl acetate afforded pure product as a colorless crystalline solid, which slowly air-oxidized back to the quinone.

To 100 mL of anaerobic buffer was added 20 mg of **10**·HCl followed by incubation at 30 °C for 60–72 h. Buffers employed were 0.2 M pH 7.5 and 8.0 phosphate and 0.2 M pH 9.0 borate, all of which were $\mu = 1.0$, KCl. The completed reaction was opened to the air and the hydroquinone species allowed to oxidize to quinones over a 1 h period. Extraction of the quinones was carried out by extraction with chloroform. The dried extracts (Na_2SO_4) were concentrated and separated by preparative TLC employing chloroform as the developing solvent. Products obtained from the hydrolysis of **10** followed by the oxidative workup afforded APBI-A,D,C. Identification of these products was based on spectral comparisons with authentic materials.

Hydroquinone **10** was hydrolyzed in the presence of 2-mercaptoethanol resulting in isolation of the nucleophile-trapped product **13** upon aerobic workup. To 15 mL of 0.2 M pH 9.26 borate buffer ($\mu = 1.0$, KCl) containing 70 μL of mercaptoethanol was added 40 mg of **10**·HCl under strict anaerobic conditions. The reaction mixture was stored at 30 °C for 34 h and then exposed to the air for 1 h. Extraction of the reaction mixture with 3 × 50 mL portions of chloroform was followed by drying of the extracts (Na_2SO_4), concentration of the extracts, and preparative TLC of the residue employing $CHCl_3$/MeOH (98:2) as the developing solvent. The polar product **13** was isolated in 1.77 mg (5.3%) yield and identified from the ^{1}H NMR spectrum: ^{1}H NMR ($CDCl_3$) δ 7.70 (1H, s, amide proton) 5.27 (1H, dd, $J = 7.2$ and 3.3 Hz C(3) proton), 4.40 and 4.24 (2H, 2m, C(1)-diastereomeric methylene), 3.92 (1H, t, $J = 5.8$ Hz, methylene of side chain), 3.01 and 2.70 (2H, 2m, C(2)-diastereomeric methylene), 2.89 (2H, t, $J = 5.8$ Hz, methylene of side chain), 2.24 (3H, s, methyl), 1.97 (3H, s, acetamido methyl); MS (EI mode) m/z 275 ($M^+ - SCH_2CH_2$), 233 ($M^+ - H_2C{=}C{=}O - SCH_2CH_2$).

Hydrolysis Studies of Reduced APBI-E. The presence of the chloroacetate group in APBI-E precluded hydroquinone preparation by catalytic reduction due to hydrogenolysis of the chloro group. Reduction was carried out in anaerobic 0.2 M pH 7.4 buffer by titration with a dithionite solution in the same buffer until the yellow quinone color disappeared. Incubation at 30 °C for 60 h was followed by the workup as described under APBI-A hydrolysis. The major hydrolysis product was APBI-D, 70% yield.

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