Structure-Activity Studies of Antitumor Agents Based on Pyrrolo[1,2-a]benzimidazoles: New Reductive Alkylating DNA Cleaving Agents

Imadul Islam and Edward B. Skibo*[1]

Department of Chemistry, Arizona State University, Tempe, Arizona 85287-1604

Robert T. Dorr and David S. Alberts

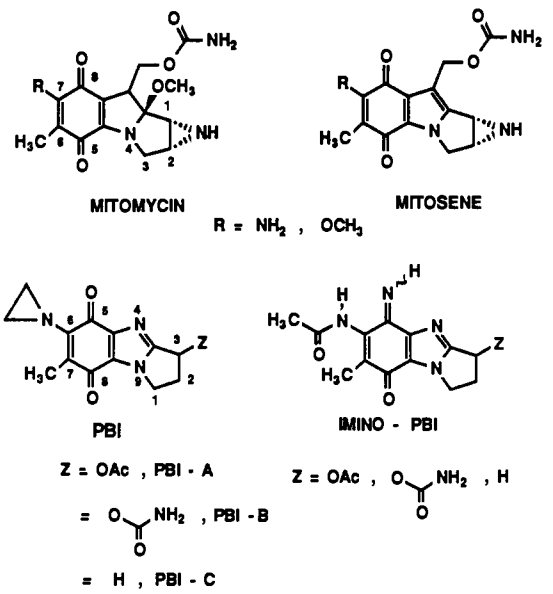
Arizona Cancer Center, School of Medicine, University of Arizona, Tucson, Arizona 85724. *Received March 12, 1991*

Described herein are structure-activity studies of new antitumor agents based on the pyrrolo[1,2-a]benzimidazole (PBI) ring system. These compounds were designed as new DNA cross-linkers mimicking the mitomycin antitumor agents. Actually, the PBI derivatives were found to have anthracycline-like features: (i) shared cross resistance with doxorubicin in a human myeloma line, (ii) cardiotoxicity, and (iii) excellent DNA strand cleaving capability. The DNA strand cleavage is thought to result from reductive alkylation of DNA followed by the generation of reactive oxygen radicals. The best antitumor agent studied is 6-*N*-aziridinyl-3-hydroxy-7-methyl-2,3-dihydro-1*H*-pyrrolo-[1,2-a]benzimidazole-5,8-dione 3-acetate (PBI-A), which possesses nanomolar IC_{50} values against various human ovarian and colon cancer cell lines.

The pyrrolo[1,2-a]benzimidazoles (PBI)[2] are new classes of antitumor agents showing activity against ovarian and colon cancers. In fact, some of the PBI derivatives are much more active than mitomycin C against ovarian cancer cell lines. Presented herein are structure-activity studies of these agents as well as a toxicological assessment of the most active PBI analogue.

The pyrrolo[1,2-a]benzimidazoles were designed as reductive cross-linkers of DNA. Inspection of the structures below reveals their similarities to mitomycins and mitosenes. Placement of a leaving group at the 3-position of the pyrrolo[1,2-a]benzimidazole system should permit the formation of an alkylating quinone methide species upon quinone reduction and leaving-group elimination.[3] Previous studies in one of our laboratories (E.B.S.) showed that benzimidazole-based quinone methides can act as alkylating agents.[4] The 6-aziridinyl group of the pyrrolo[1,2-a]benzimidazoles is present to provide the second alkylating center and thereby to produce a DNA cross-link. Remers and co-workers[5] have found that placement of a 7-aziridinyl group on mitomycins and mitosenes increases their antitumor activity, perhaps due to the presence of a third alkylating center. The imino-PBI derivatives represent hydrolytically stable (at physiological pH) iminoquinones possessing a much higher reduction potential than the corresponding quinones. With these compounds we were able to study the influence of reduction potential on antitumor activity.

Chart I



Shown in Chart I are the three most active pyrrolo-[1,2-a]benzimidazole antitumor agents, PBI-A, PBI-B, and PBI-C. Structure-activity studies revealed that the 6-aziridinyl group is largely responsible for the antitumor activity. The leaving group in the 3-position influences antitumor activity to a lesser degree. The most active of all the analogues studied, PBI-A, was screened in fresh human tumor cloning assays against ovarian cancers and found to be quite active. The level of activity in these assays suggest that PBI-A could have clinical utility in treating ovarian cancer.[6]

Chemistry

The synthesis of PBI-A and PBI-B, as well as a number of related derivatives, has been reported in a previous publication.[2] Our structure-activity studies required the preparation of new analogues possessing various 3- and 6-substituents as well as high reduction potential iminoquinone analogues. The goals were to determine the role

(1) National Institutes of Health research career development award recipient (CA01349), 1988-1993.

(2) Islam, I.; Skibo, E. B. Synthesis and Physical Studies of Azamitosene and Iminoazamitosene Reductive Alkylating Agents. Iminoquinone Hydrolytic Stability, Syn/Anti Isomerization, and Electrochemistry. *J. Org. Chem.* **1990**, *55*, 3195-3205.

(3) Moore, H. W.; Czerniak, R. Naturally Occurring Quinones as Potential Bioreductive Alkylating Agents. *Med. Res. Rev.* **1981**, *1*, 249-280.

(4) Skibo, E. B. Studies of Extended Quinone Methides. The Hydrolysis Mechanism of 1-Methyl-2-(bromomethyl)-4,7-dihydroxybenzimidazole. *J. Org. Chem.* **1986**, *51*, 522-527.

(5) (a) Iyengar, B. S.; Lin, H.-J.; Cheng, L.; Remers, W. A.; Bradner, W. T. Development of New Mitomycin C and Porfiromycin Analogues. *J. Med. Chem.* **1981**, *24*, 975-981. (b) Hodges, J. C.; Remers, W. A.; Bradner, W. T. Synthesis and Antineoplastic Activity of Mitosene Analogues of the Mitomycins. *J. Med. Chem.* **1981**, *24*, 1184-1191. (c) Also see: Orlemans, E. O. M.; Verboom, W.; Scheltinga, M. W.; Reinhoudt, D. N.; Lelieveld, P.; Fiebig, H. H.; Winterhalter, B. R.; Double, J. A.; Bibby, M. C. Synthesis, Mechanism of Action, and Biological Evaluation of Mitosenes. *J. Med. Chem.* **1989**, *32*, 1612-1620.

(6) (a) Von Hoff, D. D. He's Not Going to Talk About In Vitro Predictive Assays Again, Is He? *J. Natl. Cancer Inst.* **1990**, *82*, 96-101. (b) Von Hoff, D. D.; Sandbach, J. F.; Clark, G. M.; Turner, J. N.; Forseth, B. F.; Piccart, M. J.; Colombo, N.; Muggia, F. M. Selection of Cancer Chemotherapy for a Patient by an In Vitro Assay Versus a Clinician. *J. Natl. Cancer Inst.* **1990**, *82*, 110-116.

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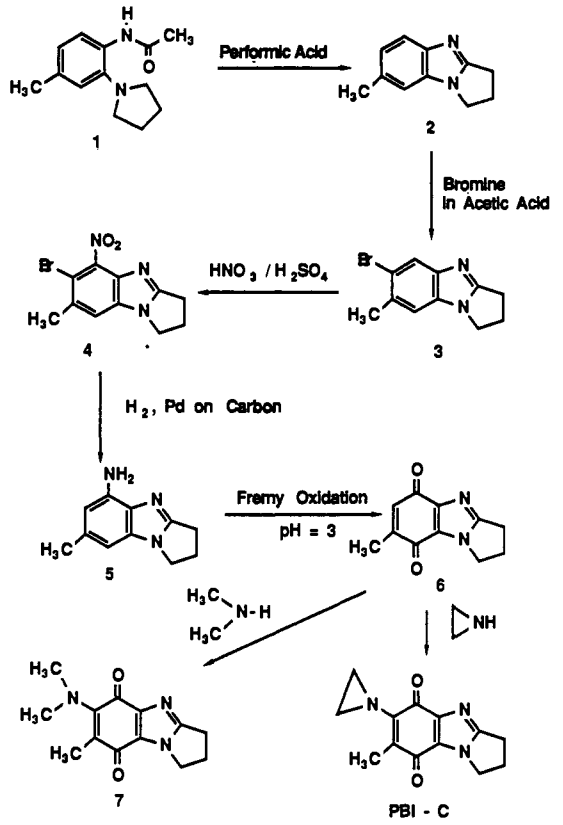
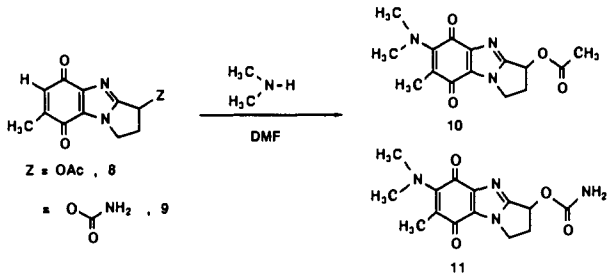
Scheme I



Scheme II



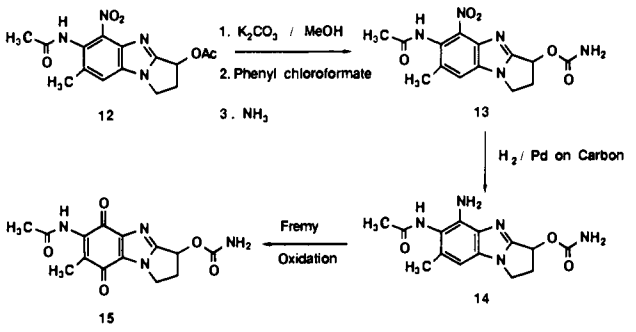
of these substituents and the imino functionality on antitumor activity.

Shown in Scheme I are steps leading to quinone analogues without a substituent in the 3-position, 7 and PBI-C. Oxidative cyclization of 1 by performic acid afforded 2.[7] The bromination of 2, to provide 6-bromo derivative 3, was carried out so as to direct nitration to the 5-position in the next step. The resulting 6-bromo, 5-nitro derivative 4 was subjected to catalytic reduction in order to both remove the 6-bromo substituent and reduce the nitro group. Fremy oxidation of the reduction product 5 afforded quinone 6, which was converted to 7 and PBI-C by oxidative addition of dimethylamine and ethylenimine, respectively.

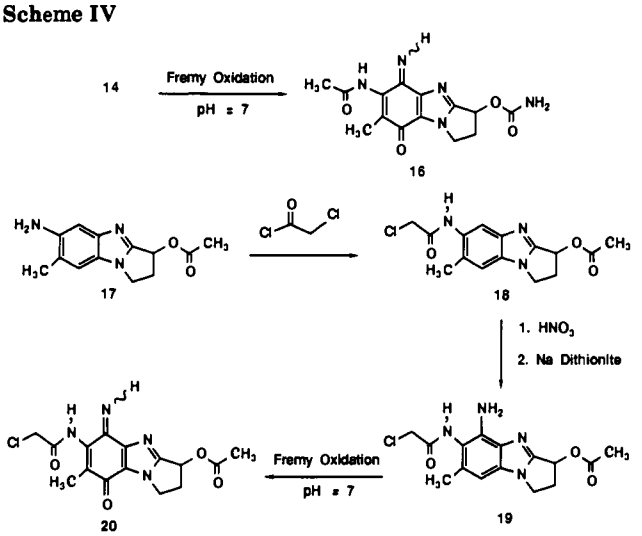
Other quinone derivatives necessary for our studies were prepared as shown in Schemes II and III. Derivatives possessing either an acetate or a carbamate leaving group in the 3-position, and a dimethylamine group in the 6-position, were prepared by oxidative addition of dimethylamine to the reported compounds 8 and 9[2] as shown erase-mediated removal of the 3-acetate group. The an-

(7) (a) Nair, M. D.; Adams, R. Benzimidazole Syntheses by Oxidative Cyclization with Peroxytrifluoroacetic Acid. *J. Am. Chem. Soc.* **1961**, *83*, 3518. (b) Meth-Cohn, O.; Suschitzky, H. Syntheses of Heterocyclic Compounds. Part IV. Oxidative Cyclization of Aromatic Amines and Their *N*-Acyl Derivatives. *J. Chem. Soc.* **1963**, 4666.

Scheme III



Scheme IV



in Scheme II. A derivative possessing a carbamate leaving group in the 3-position and an acetamido group in the 6-position (15) was prepared as shown in Scheme III.

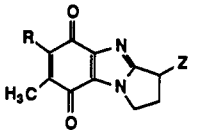
The new iminoquinone derivatives (16 and 20) were prepared as described in Scheme IV. The synthesis of iminoquinones 21 and 22 was reported in a previous publication.[2] Access to the iminoquinones was possible by carrying out Fremy oxidations of amines at neutral pH (14 → 16 and 19 → 20). An acetamido group is always present at the 6-position of iminoquinones so as to stabilize the imine by intramolecular hydrogen bonding.[2]

In Vitro Antitumor Screening

The series of pyrrolo[1,2-*a*]benzimidazole derivatives in Table I was screened for activity against WiDr colon cancer. Since the pyrrolo[1,2-*a*]benzimidazoles were designed to mimic mitomycin C, comparisons were made with this clinically used drug. The results of studies suggest that the pyrrolo[1,2-*a*]benzimidazoles have anthracycline-like qualities and are not cross-linkers.

The IC_{50} data (inhibitory concentration in 50% of treated cells) shown in Table II indicate that PBI-A and PBI-C are as active as mitomycin C (MMC) against this cell line. These data also show that the 6-aziridinyl group is essential for antitumor activity. Replacement of the 6-aziridinyl group of PBI-A and PBI-B with dimethylamino (10 and 11), with acetamido (16 and 15), and with an H substituent (8 and 9) all result in substantial losses of inhibitory activity. The group at the 3-position also influences the antitumor activity; an acetate at this position always results in better inhibitory activity than a carbamate. For example, PBI-A has a lower IC_{50} value than PBI-B, and 8 has a lower IC_{50} value than 9. However, the derivative without a substituent at the 3-position (PBI-C) is as active as PBI-A. It is very likely that the absence of a bulky substituent at the 3-position results in low IC_{50} values and that PBI-A is active only due to est-

Table I. Pyrrolo[1,2-*a*]benzimidazole and Mitomycin C Inhibition of WiDr Colon Cancer



compound	R	Z	IC_{50} (WiDr), nM
PBI-A			10
PBI-B			100
PBI-C		H	12–15
mitomycin C			10
7		H	13000
8	H		300
9	H		10000
10			10000
11			13000
15			16000
16			6000
17		H	500

Table II. IC_{50} (nM) for PBI-A, PBI-B, and Mitomycin C (MMC) Inhibition of Myeloma, Ovarian Cancer, and Colon Cancer Cell Lines

cancer	PBI-A	PBI-B	MMC
2780/DOX (ovarian)	16	200	200
2780/S (ovarian)	0.6	100	100
2008/S (ovarian)	7	NA	NA[a]
2008/R (ovarian)	7	NA	NA
SW480 (colon)	9	19	7
7226/S (myeloma)	100	140	200

[a] NA: not achievable.

titumor activity in the absence of the 3-position leaving group indicates that a cross-linking cytotoxicity mechanism is not in operation.

Shown in Table II are IC_{50} values for PBI-A, PBI-B, and MMC inhibitory activity against ovarian, colon, and myeloma cancer cell lines. These data show that PBI-A is much more active than MMC against ovarian cancer and that both of these agents have about the same activity against myeloma and colon cancer. The greater activity of 3-acetate substitution, compared to 3-carbamate substitution, is apparent in Table II: PBI-A is more active than PBI-B against all ovarian and colon cancers. Another noteworthy observation is the shared cross resistance of PBI-A with doxorubicin. The IC_{50} value for PBI-A against doxorubicin-sensitive ovarian cancer (2780/S) is 0.6 nM whereas the IC_{50} value is 16 nM against the doxorubicin-resistant strain (2780/DOX).

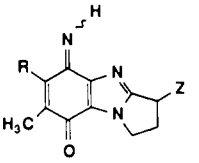
The fresh human tumor cloning assay has been shown to be of value in predicting in vivo activity.[6] The activity of PBI-A against ovarian cancers in vitro (Table II)

Table III. Fresh Human Ovarian Tumor Cloning Assays for Cancers from 10 Patients[a]

entry	IC_{50}, μg/mL	entry	IC_{50}, μg/mL
1	0.00442	6	0.0165
2	0.0985	7	0.00437
3	0.004	8	0.821
4	unachievable	9	0.0856
5	0.0074	10	1.53

[a] IC_{50} values were obtained after 1-h exposure to PBI-A.

Table IV. Iminoquinone Inhibition of WiDr Colon Cancer



compound	R	Z	IC_{50} (WiDr), nM
16			16000
20			280
21		H	10000
22			150

prompted the screening of this drug against fresh human ovarian cancers. The results of this assay indicate that the median IC_{50} is 0.05 μg/mL with an achievable range from 0.004 to 1.53 μg/mL. Only one ovarian cancer displayed resistance to PBI-A (Table III).

Shown in Table IV are IC_{50} values for iminoquinone activity against WiDr colon cancer. The presence of an acetate in the 3-position of these analogues (**20** and **22**) is essential for antitumor activity. The 6-chloroacetamido group of **20** could provide an additional alkylating center, but **20** showed somewhat less antitumor activity than 6-acetamido analogue **22**.

A noteworthy observation is the greater antitumor activity of **22** (IC_{50} = 150 nM) compared to its quinone analogue **16** (IC_{50} = 6000 nM). The opposite change occurs on going from iminoquinone **21** (IC_{50} = 10000 nM) to its quinone analogue **17** (IC_{50} = 500 nM). The change from iminoquinone to quinone is accompanied by a large decrease in reduction potential ($E_{7.4}$ = 445 mV for **21** and $E_{7.4}$ = 28 mV for **17**, vs NHE). One consequence of the lower reduction potential of the quinone derivative is the capability of generating oxygen radicals: enzymatic reduction of the quinone to the hydroquinone would be followed by facile oxygen reoxidation to afford the quinone and a potentially cytotoxic oxygen radical species. In contrast, reduction of the iminoquinone analogue affords an aminophenol which is relatively unreactive toward oxygen.[2] Perhaps the antitumor activity of **17** is due to oxygen radical generation and the lack of activity of **21** is due to its inability to produce oxygen radicals. Iminoquinones bearing an acetate group, **20** and **22**, may eliminate acetic acid upon reduction to afford an alkylating quinone methide species, resulting in cellular alkylation. In contrast, alkylation involving the 3-position of the quinones is not an important factor in antitumor activity (loc cit, this section) and thus **16** is nearly inactive.

Toxicity Assessment

The toxicity of PBI-A was evaluated in female CD2F1 mice and beating neonatal rat myocytes.

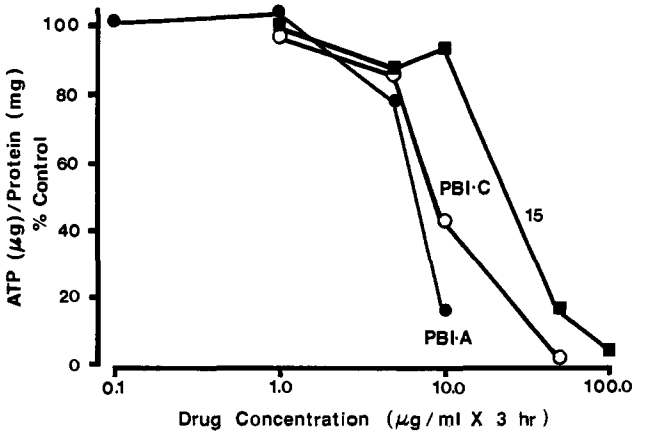


Figure 1. Plots of ATP (μg)/protein (mg) as percent of control vs drug concentration for PBI-A (●), PBI-C (○), and 15 (■) in cardiac myocytes from Sprague–Dawley rats.

The in vivo results are based on a 14-day toxicity test with the above mice. These results indicate that doses of 100, 30, and 10 mg/kg of PBI-A were 100% lethal, while doses of 1 and 3 mg/kg were nonlethally toxic. Evaluation of PBI-A against ip-implanted P-388 leukemia was also carried out. There was no observed reduction in tumor load and there were no survivors by day 14. PBI-A has a nonlethal toxicity dose the same as that of mitomycin C (1–3 mg/kg), but unlike mitomycin C, shows no activity against P388 leukemia.

The data in Figure 1 indicate that the pyrrolo[1,2-*a*]benzimidazoles produce cardiotoxic effects in vitro. Mitomycin C possesses an IC_{50} value >200 μg/mL against neonatal rat heart myocytes while PBI-A and PBI-C possess IC_{50} values of 6 and 8 μg/mL, respectively. Replacement of the aziridinyl group of PBI-B with the acetamido group (**15**) results in substantial loss of antitumor activity (see Table I) with retention of the cardiotoxicity, IC_{50} = 24 μg/mL. These results indicate that the aziridinyl group, which is necessary for antitumor activity, is not responsible for cardiotoxicity. The generation of radical species during cycling between quinone and hydroquinone forms very likely causes cardiotoxicity in the pyrrolo[1,2-*a*]benzimidazoles.[8] The high reduction potential iminoquinone derivatives are thus candidates for low cardiotoxicity drugs since cycling between the iminoquinone and aminophenol forms would be slow.[2] Currently, we are incorporating the aziridinyl group into the iminoquinones so as to retain antitumor activity and design out cardiotoxicity.

Mechanism of Cytotoxicity

Pyrrolo[1,2-*a*]benzimidazole antitumor activity appears to be due to reductive alkylation of DNA followed by strand cleavage. Both alkaline elution assays and the nicking of supercoiled DNA provided evidence of strand cleavage.

Found in Figure 2 are results of alkaline elution assays to treated and untreated myleoma 8226 cells. Cross-linking of DNA was detected by late retention times with MMC, and DNA strand cleavage was detected by rapid elution of DNA with the pyrrolo[1,2-*a*]benzimidazole. Treatment with the cross-linking drug mitomycin C produced the anticipated late retention of DNA (Plot B) compared to

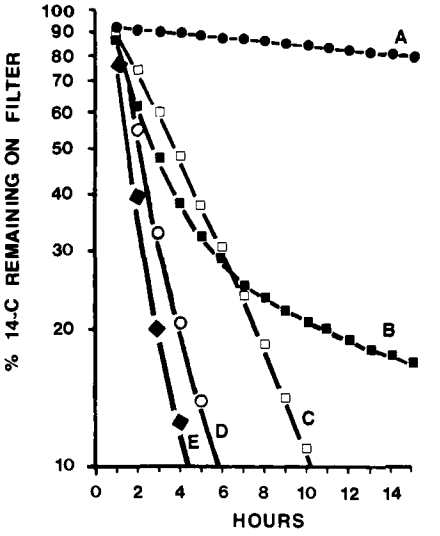
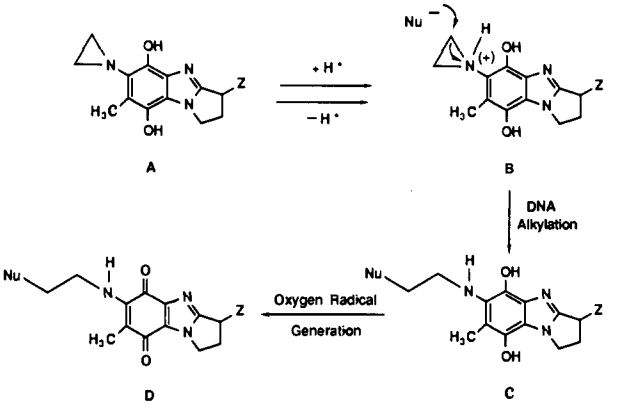


Figure 2. Plots of percent ^{14}C remaining on a polycarbonate filter vs time for lysed 8226 human myeloma cells treated as follows: A, control; B, mitomycin C treatment (5 μg/mL); C, PBI-A treatment (10 μg/mL); D, 650 cGy of radiation; E, PBI-A (10 μg/mL) and 650 cGy of radiation.

Table V. Dose–Response Relationship for DNA Single-Strand Cleavage with PBI-A in Human 8226 Multiple Myeloma Cells

concentration, μg/mL × 1 h	rd-equiv DNA single-strand cleavage
0.1	59 ± 12
1.0	321 ± 22
10.0	469 ± 59

Scheme V



irradiated (650 cGy) control cells (Plot D). PBI-A treatment produced a significant degree of strand cleavage (Plot C), which was somewhat less than that of 650 cGy of DNA damage (Plot D). Cells receiving X-ray treatment after PBI-A treatment produced additive DNA strand cleavage (Plot E). The data in Table V show that a dose–response relationship exists for PBI-A mediated DNA cleavage.

The alkaline elution assay results are consistent with the results of ethidium cross-linking assays (see Experimental Section) and the data in Table I, both of which indicate that cross-linking by PBI-A does not occur.

Plasmid nicking studies suggest that PBI-A cleaves DNA by the process shown in Scheme V. Relaxation of supercoiled DNA accompanying nicking is only observed after the DNA is incubated with reduced PBI-A, structure A in Scheme V, under anaerobic conditions, followed by incubation in the presence of oxygen. Reductive alkylation by PBI-A may pertain to equilibrium protonation of the basic aziridinyl nitrogen of A at physiological pH followed by nucleophilic attack and ring opening to afford C.[9] In

(8) (a) Doroshow, J. H. Effect of Anthracycline Antibiotics on Oxygen Radical Formation in Rat Heart. *Cancer Res.* **1983**, *43*, 460–472. (b) Begleiter, A. Cytocidal Action of the Quinone Group and Its Relationship to Antitumor Activity. *Cancer Res.* **1983**, *43*, 481–484.

this respect, the pyrrolo[1,2-a]benzimidazoles bear a resemblance to aziridinylquinone antitumor agents. Unlike the aziridinylquinones, the pyrrolo[1,2-a]benzimidazoles are not cross-linking agents. The introduction of oxygen converts C to D with concomitant radical generation. Incubation of DNA with unreduced PBI-A does not lead to nicking, perhaps because the aziridinyl nitrogen of PBI-A is a vinylogous amide and the protonation step leading to alkylation cannot occur at physiological pH.

Conclusion

The pyrrolo[1,2-a]benzimidazoles represent a new class of antitumor agents whose mechanism of cytotoxicity appears to involve reductive alkylation of DNA followed by strand cleavage. Alkaline elution assays and a plasmid nicking assay both show that PBI-A cleaves DNA. Pyrrolo[1,2-a]benzimidazole structure–activity studies show that the 6-aziridinyl group is required for antitumor activity while the leaving group in the 3-position is not. This finding is consistent with a monoalkylation, rather than a cross-linking mechanism of cytotoxicity. The iminoquinone derivatives require the leaving group in the 3-position to show antitumor activity. Details of the iminoquinone cytotoxicity mechanism are not yet available, however.

Although the pyrrolo[1,2-a]benzimidazoles were designed on the basis of mitomycin chemistry and cross-linking behavior, they now appear to be more similar to the anthracyclines. PBI-A, for example, shows shared cross resistance with doxorubicin against doxorubicin resistant (2780/DOX) ovarian cancer (see Table II). Furthermore, doxorubicin, like PBI-A, is cardiotoxic[10] and produces DNA strand cleavage[11] probably by a free-radical mechanism.[12,13] Currently, the sequence specificity and mode of DNA interaction (intercalation, minor-groove binding, etc.) of the pyrrolo[1,2-a]benzimidazoles are under study.

Experimental Section

All analytically pure compounds were dried under high vacuum at room temperature or in a drying pistol heated with refluxing methanol. Uncorrected melting and decomposition points were determined with a Mel-Temp apparatus. All TLC was run with Merck silica gel 60 (F_{254}) plates, 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 Bruker AM-400 spectrometer and chemical shifts are reported relative to TMS. Nitrogen elemental analyses of quinones and iminoquinone analogues (PBI-C, 11, 15, 16, and 20) consistently deviated from theoretical percentages even upon repurification and reanalysis. This problem was observed previously[2] and may be due to incomplete combustion. The poor results of the elemental analysis of compound 20 may be due to an active halide and basic nitrogens in the same molecule. ^1H NMR, mass spectra, and TLC indicate these compounds are pure, however. Elemental analyses were not obtained for all intermediates of routine chemical transformations.

Microculture Tetrazolium Assay. Cleavage of the MTT tetrazolium dye was employed as an indicator of cancer cell viability.[14] This assay measures mitochondrial dehydrogenase activity, which reduces the MTT dye to a colored formazan compound (λ_{max} 570). Cells were plated at 50000/well in 96-well, flat-bottomed plastic trays (Falcon). Drug stock solutions were prepared in dimethyl sulfoxide and then diluted in buffer for addition to the wells. Inhibitory concentrations in 50% of the treated cells (IC_{50} values) were calculated from concentration–survival curves generated for each compound over a range of concentrations producing 0–100% survival.

Fresh human tumor chemosensitivity assays[15] were carried out at the Arizona Cancer Center with ovarian cancers surgically removed from patients, minced, and plated on soft agar as a single cell suspension of cells.

Short-Term Toxicity and in Vivo Activity against P388 Leukemia. These studies were carried out at Southern Research Institute. Normal, non-tumor-bearing CD2F1 mice were treated intraperitoneally (ip) with doses of **PBI-A** of 100, 30, 10, 3, and 1 mg/kg on a schedule of every fourth day for three doses. The mice (three per dose) were observed for a maximum of 14 days after the last treatment. Doses used in the P388 study were based on the results of this toxicity study. The CD2F1 mice were implanted with P388/0 leukemia (10^6 cells/mouse) such that there were six mice for each of the five doses of drug and 30 controls.

In vitro cardiotoxicity studies were carried out as previously described[16] by utilizing hearts from 1-2-day-old neonatal Sprague–Dawley rats as the source of cardiac myocytes. Briefly, the hearts are minced and subjected to serial trypsin exposures to yield a mixture of myocytes and fibroblasts. The latter are reduced by their rapid attachment to plastic allowing a myocyte-rich supernatant to be plated into 6-well plastic culture wells (Falcon Plasticware). Three days after plating, myocytes synchronously beat at ca. 60–180 contractions/min. The cells are then exposed to the anticancer agent for 1 h, washed with fresh medium, and returned to a 37 °C incubator for 3 days. Afterward the cells are lysed and protein is measured by the Bio-Rad method (Sigma Diagnostics, Sigma Chemical Co., St. Louis, MO) and ATP is measured by bioluminescence (LKB Wallac Model 1251 Luminometer) using purified firefly luciferase (Turner Instruments and Reagents, Mountain View, CA). The ratio of ATP/protein normalizes cell viability (ATP content) to the number of myocytes present in each well (protein content).

Alkaline DNA elution studies[17] were performed with 8226 human myeloma cells exposed to drugs for 1 h, then washed, radiated (some groups), lysed with proteinase K (E. Merck and

(9) Protonation of the aziridinyl group is required for the covalent crosslinking of DNA by bifunctional aziridinyl quinones: Akhtar, M. H.; Begleiter, A.; Johnson, D.; Lown, J. W.; McLaughlin, L.; Sim, S.-K. Studies Related to Antitumor Antibiotics. Part IV. Correlation of Covalent Cross-Linking of DNA by Bifunctional Aziridinoquinones with Their Antineoplastic Activity. *Can. J. Chem.* **1975**, *53*, 2891–2905.

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Co., Darmstadt, Germany), and eluted over 15 h at pH 12.1 through a polycarbonate filter (Nucleopore Inc., Richmond, CA; 2.0-μm pore size).

Reaction of PBI-A with Plasmid DNA. The DNA cleavage activity was determined with 11 kb pHPV6 DNA obtained from Microprobe Inc., Bothell, WA. The reaction mixture, which produced nicked DNA, consisted of 0.13 nm DNA, 4.0 μM PBI-A, and 7.0 μM sodium dithionite in 100 μL of 0.02 M, pH 7.2, phosphate buffer. The reaction was carried out under strict anaerobic conditions by degassing the DNA/PBI-A mixture with argon and then adding the degassed dithionite stock to reduce PBI-A. Incubation of the reaction mixture at 37 °C under an argon atmosphere was then carried out for 12 h. Control reactions were as follows: (1) DNA only with no degassing but with the incubation step, (2) DNA and dithionite with the degassing and incubation steps, and (3) DNA and PBI-A only with no degassing but with the incubation step.

At the end of incubation, air was introduced into anaerobic reactions and the presence of nicking determined by agarose gel electrophoresis. The electrophoresis was carried out in 0.04 M Tris/acetate pH 8.0 buffer containing 1 mM EDTA. Agarose gel (standard low M_r, Bio-Rad Laboratories) was prepared in the same buffer at 0.8% with 0.25 μg/mL of ethidium bromide. The DNA bonds were visualized by transillumination with 300-nm UV light. Nicked DNA (travels slower than supercoiled DNA) was not present at the end of incubation in any of the runs. After aerobic incubation at 37 °C of these reactions for another 12 h, however, the run with DNA/PBI-A/dithionite consisted of only nicked DNA and the other runs contained unchanged DNA.

Ethidium bromide cross-linking assays were carried out according to published procedures.[18] In these assays λ-DNA (New England Biolabs) was incubated with an excess of reduced PBI-A in 0.02 M, pH 7.4 phosphate buffer at 30 °C under an argon atmosphere for times ranging from 1 h to 1 day. Ethidium–DNA fluorescence after heating assay solutions to 96 °C for 2 min was not observed for an PBI-A reaction. Control-reactions with the cross-linker mitomycin C produced the expected after-heat fluorescence.

The preparations of new compounds are described below.

4-Acetamido-5-(1-pyrrolidino)toluene (1). A mixture consisting of 6.2 g (30.09 mmol) of 3-(1-pyrrolidino)-4-nitrotoluene,[2] 500 mg of 5% Pd/C, and 250 mL of methanol was shaken under 50 psi for 4 h. The completed reaction was filtered through Celite into a flask containing 10 mL of acetic acid. The filtrate was then evaporated in vacuo to an acetic acid/amine mixture, to which was added 10 mL of acetic anhydride. The mixture was stirred at room temperature for 30 min and then diluted with 300 mL of diethyl ether. Pure product crystallized from the mixture after sonication and then chilling overnight (5.46 g, 83% yield). Recrystallization was carried out from chloroform/hexane: mp 92–94 °C; TLC (chloroform) R_f = 0.75; IR (KBr pellet) 1655, 1644, 1605, 1534, 1509, 1489, 1418, 1373, 1355, 1329 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 9.03 (1 H, s, amide proton), 7.05 and 6.57 (2 H, ABX, J_{ortho} = 7.8 Hz, J_{meta} = 0 Hz, J_{para} = 0 Hz, C(5) and C(6) protons), 6.62 (1 H, s, C(2) proton), 3.14 (4 H, m, pyrrolidine methylenes adjacent to N), 2.21 (3 H, s, methyl), 1.98 (3 H, s, acetamido methyl), 1.84 (4 H, m, other pyrrolidine methylenes); mass spectrum (EI mode), m/z 218 (P^+ - acetyl). Anal. ($C_{13}H_{18}N_2O$) C, H, N.

7-Methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole (2). A mixture consisting of 1.0 g (4.58 mmol) of 1, 3 mL of 96% formic acid, and 1.5 mL of 30% H_2O_2 was stirred at 70 °C for 40 min. The yellow reaction mixture was then cooled to room temperature, diluted with water, and neutralized to pH 7.00 with concentrated ammonium hydroxide. Extraction of the neutralized solution with 3 × 100 mL portions of chloroform, drying of the extracts (sodium sulfate), and concentration of the extracts afforded a yellowish-brown crude solid. Chromatography on silica gel employing 80:20 chloroform/hexane as the eluant afforded a white solid (550 mg, 69% yield). An analytical sample was prepared by crystallization from diethyl ether/hexane: mp 118–120 °C; TLC (chloroform) R_f = 0.66; IR (KBr pellet) 2980, 1524, 1486, 1463, 1452, 1418, 1293, 1281, 1218, 803 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 7.36, and 6.72 (2 H, ABX, J_{ortho} = 8.2 Hz, J_{meta} = 0 Hz, J_{para} = 0 Hz, C(5) and C(6) aromatic protons, respectively), 7.20 (1 H, s, C(8) proton), 4.04 (2 H, t, $J \approx 7$ Hz, C(1) methylene), 2.90 (2 H, t, $J \approx 7$ Hz, C(3) methylene), 2.61 (2 H, quintet, $J \approx 7$ Hz, C(2) methylene), 2.40 (3 H, s, 7-methyl); mass spectrum (EI mode), m/z 172 (P^+). Anal. ($C_{11}H_{12}N_2$) C, H, N.

6-Bromo-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole (3). To a solution of 1 g (5.81 mmol) of 2 in 30 mL of glacial acetic acid, heated at 100 °C, was added 300 μL of bromine in 3 mL glacial acetic acid. After the addition, the reaction mixture was heated at 100–110 °C for 4 h. The cooled reaction mixture was diluted with 100 mL of water and neutralized with aqueous sodium bicarbonate. The product crystallized from solution as a light yellow solid. Yield upon drying the collected solid was 1.37 g (88%). Recrystallization from chloroform/hexane afforded analytically pure 3: mp 167–170 °C; TLC (chloroform/methanol 90:10) R_f = 0.56; IR (KBr pellet) 2949, 2929, 1581, 1521, 1482, 1461, 1448, 1418, 1291, 872 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 7.71, and 7.43 (2 H, 2 × s, aromatic protons), 4.04 (2 H, t, $J \approx 7$ Hz, C(1) methylene), 2.91 (2 H, t, $J \approx 7$ Hz, C(3) methylene), 2.59 (2 H, quintet, $J \approx 7$ Hz, C(2) methylene), 2.39 (3H, s, 7-methyl); mass spectrum (EI mode), m/z 250 and 252 (P^+, ^{79}Br and P^+, ^{81}Br), 171 (P^+ - Br). Anal. ($C_{11}H_{11}BrN_2 \cdot 0.25H_2O$) C, H, N.

6-Bromo-7-methyl-5-nitro-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole (4). A solution of 245 mg (0.97 mmol) of 3 in a 5-mL mixture of fuming nitric acid and concentrated sulfuric acid (4:1) was stirred in an ice bath for 10 min. The completed reaction was poured over cracked ice and the pH of resulting solution adjusted to 6.5 with aqueous sodium bicarbonate. Extraction of this solution with 3 × 50 mL portions of chloroform, drying of the extracts (sodium sulfate), and then concentration afforded a yellow solid (165 mg, 57%). Recrystallization from chloroform/hexane afforded analytically pure material: mp 201–203 °C; TLC (chloroform/methanol 95:5) R_f = 0.61; IR (KBr pellet) 1537, 1517, 1449, 1413, 1383, 1374, 1342, 1297, 884, 860 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 7.77 (1 H, s, aromatic proton), 4.15 (2 H, t, $J \approx 7$ Hz, C(1) methylene), 2.99 (2 H, t, $J \approx 7$ Hz, C(3) methylene), 2.67 (2 H, quintet, $J \approx 7$ Hz, C(2) methylene); 2.52 (3 H, s, 7-methyl); mass spectrum (EI mode), m/z 295 and 297 (P^+, ^{79}Br and P^+, ^{81}Br), 249 and 251 ($P^+ - NO_2$). Anal. ($C_{11}H_{10}BrN_3O_2$) C, H, N.

5-Amino-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole (5). A suspension of 550 mg (1.85 mmol) of 4 in 100 mL of methanol containing 90 mg of 5% palladium on carbon was shaken under 50 psi H_2 for 8 h. The reaction mixture was filtered through Celite and the filter cake washed with methanol. Acidification of filtrate with a few drops of 1 N HCl and evaporation in vacuo afforded the dihydrochloride salt of 5. Recrystallization was carried out from ethyl acetate/methanol: 350 mg, 72% yield; mp 320 °C dec; TLC (chloroform/methanol 90:10) R_f = 0.39; IR (KBr pellet) 3369, 3316, 3206, 2918, 2886, 2871, 1652, 1569, 1495, 1386 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 6.74 and 6.54 (2 H, 2 × s, C(6) and C(8) aromatic protons), 4.25 (2 H, t, $J \approx 7$ Hz, C(1) methylene), 3.30 (2 H, t, $J \approx 7$ Hz, C(3) methylene), 2.74 (2 H, quintet, $J \approx 7$ Hz, C(2) methylene), 2.33 (3 H, s, 7-methyl); mass spectrum (EI mode), m/z 187 (P^+ for 5). Anal. ($C_{11}H_{13}BrN_2 \cdot 2HCl \cdot 0.25 H_2O$) C, H, N.

7-Methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole-5,8-dione (6). To a suspension of 218 mg (0.83 mmol) of 5 in 10 mL of water, containing 200 mg of monobasic potassium phosphate, was added a solution of 1.34 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 and then chromatographed over silica gel, employing chloroform as the eluant, to afford yellow 6 (105 mg, 61% yield). Recrystallization from chloroform/hexane afforded analytically pure 6: mp 162–164 °C; TLC (methanol chloroform 10:90) R_f = 0.62; IR (KBr pellet) 1675, 1659, 1647, 1515, 1154 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 6.54 (1 H, quartet, J = 1.6 Hz, C(6) proton coupled to 7-methyl), 4.16 (2 H, t, $J \approx 7$ Hz, C(1) methylene), 2.86 (2 H, t, $J \approx 7$ Hz, C(3) methylene), 2.62 (2 H, quintet, $J \approx 7$ Hz, C(2) methylene),

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2.00 (3 H, d, J = 1.6 Hz, 7-methyl coupled to C(6) proton); mass spectrum (EI mode), m/z 202 (P^+), 174 (P^+ - CO), 146 (P^+ - 2 CO). Anal. ($C_{11}H_{10}N_2O_2$) C, H, N.

6-(Dimethylamino)-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole-5,8-dione (7). To a solution of 50 mg (0.24 mmol) of **6** in 3 mL of dry DMF, chilled to 0 °C, was added an excess of methanolic dimethylamine. After the addition, the reaction mixture was stirred at room temperature for about 30 min and then the solvent was evaporated in vacuo. The residue was chromatographed on silica gel employing chloroform as the eluant. Blue **7** was obtained in 71% yield (42 mg). An analytical sample was prepared by dissolving the residue in a small amount of chloroform, adding hexane, and then chilling the mixture overnight in a refrigerator: mp 158 °C dec; TLC (chloroform/methanol 90:10) R_f = 0.56; IR (KBr pellet) 1675, 1659, 1623, 1524, 1314, 1067 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.16 (2 H, t, $J \approx 7$ Hz, C(1) methylene), 3.04 (6 H, s, dimethylamino), 2.87 (2 H, t, $J \approx 7$ Hz, C(3) methylene), 2.64 (2 H, quintet, $J \approx 7$ Hz, C(2) methylene), 1.93 (3 H, s, 7-methyl); mass spectrum (EI mode), m/z 245 (P^+), 230 (P^+ - CH_3), 217 (P^+ - CO). Anal. ($C_{13}H_{15}N_3O_2 \cdot 0.25H_2O$) C, H, N.

6-(1-Aziridinyl)-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]-benzimidazole-5,8-dione (PBI-C). To a solution of 35 mg (0.17 mmol) of **6** in 4 mL of dry methanol, chilled at 0 °C, was added 0.5 mL of ethylenimine. The reaction was stirred at 0 °C for 30 min and then at room temperature for 1 h. The solvent was removed in vacuo and the red residue flash chromatographed on silica gel using chloroform as the eluant. The purified product was recrystallized from chloroform/hexane: 20.5 mg, 48% yield; mp 192–194 °C dec; TLC (acetone) R_f = 0.73; IR (KBr pellet) 1674, 1632, 1575, 1518, 1377, 1338, 1315, 1137, 988 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.17 (2 H, t, $J \approx 7$ Hz, C(1) methylene), 2.89 (2 H, t, $J \approx 7$ Hz, C(3) methylene), 2.65 (2 H, quintet, $J \approx 7$ Hz, C(2) methylene); 2.31 (4 H, s, aziridine protons), 2.02 (3 H, s, 7-methyl); mass spectrum (EI mode), m/z 243 (P^+), 228 (P^+ - methyl). Anal. ($C_{13}H_{13}N_3O_2$) C, H; N: calcd, 17.27; found, 16.76.

6-(Dimethylamino)-3-hydroxy-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole-5,8-dione 3-Acetate (10). To a solution of 36 mg (0.13 mmol) of **8**[2] in 2 mL of dry DMF, chilled at 0 °C, was added 500 μL of methanolic dimethylamine. The reaction mixture was stirred at 0 °C for 15 min and the solvent then evaporated in vacuo. The resulting blue solid was flash chromatographed on silica gel using chloroform as eluant. The purified product was recrystallized from chloroform/hexane: 28 mg, 71% yield; mp 74 °C; TLC (chloroform/methanol 90:10) R_f = 0.67; IR (KBr pellet) 1676, 1615, 1597, 1587, 1522, 1480, 1469, 1448, 1442, 1315 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.98 (1 H, dd, J = 7.6 Hz, J = 3.0 Hz, C(3) proton), 4.23 (2 H, m, C(1) diastereomeric methylene), 3.09 and 2.64 (2 H, 2 × m, C(2) diastereomeric methylene), 3.03 (6 H, s, dimethylamino), 2.02 (3 H, s, 7-methyl), 1.91 (3 H, s, acetate methyl); mass spectrum (EI mode), m/z 303 (P^+), 243 (P^+ - acetic acid).

6-(Dimethylamino)-3-hydroxy-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole-5,8-dione 3-Carbamate (11). To a solution of 52 mg (0.19 mmol) of **9**[2] in 2 mL of dry DMF, chilled at 0 °C, was added 500 μL of dimethylamine solution in methanol (0.22 g/mL). The reaction mixture was stirred at 0 °C for 10 min and then at room temperature for 30 min. The solvent was evaporated in vacuo and the residue flash chromatographed on silica gel employing a mixture (98:2) of chloroform/methanol as the eluant: 55 mg, 95% yield; mp 123–125 °C; TLC (acetone) R_f = 0.57; IR (KBr pellet) 3463, 3401, 1725, 1680, 1622, 1618, 1525, 1383, 1317, 1089 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.98 (1 H, dd, J = 7 Hz, J = 3.2 Hz, C(3) proton), 4.76 (2 H, br s, amide proton), 4.31 (2 H, m, C(1) diastereomeric methylene), 3.11 (6 H, s, dimethylamino), 3.12 and 2.7 (2 H, 2 × m, C(2) diastereomeric methylene), 2.01 (3 H, s, 7-methyl); mass spectrum (EI mode), m/z 304 (P^+), 261 (P^+ - O=C=NH), 243 (P^+ - carbamic acid). Anal. ($C_{14}H_{16}N_4O_4 \cdot 0.25H_2O$) C, H; N: calcd, 18.14; found, 17.02.

6-Acetamido-3-hydroxy-7-methyl-5-nitro-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole 3-Carbamate (13). To a suspension of 205 mg (0.61 mmol) of **12** in 10 mL of dry methanol was added 88 mg of K_2CO_3. The reaction was stirred at room temperature for 20 min and then concentrated in vacuo. The black residue was flash chromatographed on silica gel using a chloroform/methanol (98:2) mixture as the eluant. The yellow product was recrystallized by dissolving it in a minimum amount of chloroform/methanol (4:1), adding hexane, and then chilling; 141 mg (78%) yield: mp 259 °C dec; TLC (chloroform:methanol 20:80) R_f = 0.35; IR (KBr pellet) 3237, 3181, 3065, 1741, 1700, 1667, 1632, 1574, 1532, 1404 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 9.67 (1 H, s, amide proton), 7.66 (1 H, s, C(8) proton), 5.97 (1 H, d, J = 5.3 Hz, hydroxyl proton), 5.09 (1 H, m, C(3) proton), 4.22 and 4.05 (2 H, 2 × m, C(1) diastereomeric methylene), 2.90 and 2.37 (2 H, 2 × m, C(2) diastereomeric methylene) 2.27 (3 H, s, 7-methyl proton), 1.97 (3, H, s, acetamido methyl); mass spectrum (EI mode), m/z 290 (P^+), 272 (P^+ - H_2O), 248 (P^+ - ketene), 244 (P^+ - NO_2).

To a solution of 200 mg (0.68 mmol) of the alcohol obtained above in 25 mL of dry pyridine, chilled at 0 °C, was added 800 μL of phenyl chloroformate. The reaction was stirred at 0 °C for 30 min and then at room temperature for 2 h. The pyridine was evaporated in vacuo and the yellowish-red residue dissolved in 50 mL of chloroform. The chloroform solution was washed two times with dilute aqueous acetic acid (5%) and then two times with water. The extract was dried over Na_2SO_4 and then concentrated to a residue, which was dissolved in a chloroform/methanol (9:1) solution. Hexane was added to this solution until it became cloudy; chilling the solution in a refrigerator afforded pure phenyl carbonate: 194 mg, 69% yield; mp 245 °C dec; TLC (chloroform/methanol 80:20) R_f = 0.44; IR (KBr pellet) 1764, 1683, 1525, 1496, 1487, 1359, 1353, 1246, 1216, 1092 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 9.79 (1 H, s, amide proton), 7.82 (1 H, s, C(8) proton), 7.47 and 7.32 (5 H, 2 × m, phenyl), 6.23 (1 H, dd, J = 7.7 Hz, J = 3.2 Hz, C(3) proton), 4.39 and 4.27 (2 H, 2 × m, C(1) diastereomeric methylene), 3.25 and 2.87 (2 H, 2 × m, C(2) diastereomeric methylene), 2.36 (3 H, s, 7-methyl), 2.04 (3 H, s, acetamido methyl); mass spectrum (EI mode), m/z 410 (P^+), 392 (P^+ - H_2O), 364 (P^+ - $PhOCO_2$).

To a solution of 199 mg (0.48 mmol) of the phenyl carbonate obtained above in 150 mL of dichloromethane and methanol (9:1) was added 100 mL of liquid ammonia. The solution was kept at −76 °C for 30 min and then allowed to come to room temperature over a 3-h period. The solvent was evaporated and the solid residue recrystallized from a mixture of chloroform/methanol (90:10) and hexane: mp 266 °C dec; TLC (chloroform/methanol 80:20) R_f = 0.3; IR (KBr pellet) 3320, 3245, 1744, 1721, 1659, 1528, 1372, 1359, 1310, 1299, 1258, 1087 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 9.74 (1 H, s, amide proton), 6.01 (1 H, dd, J = 7.9 Hz, J = 3.9 Hz, C(3) proton), 4.24 (2 H, m, C(1) diastereomeric methylene), 3.11 and 2.53 (2 H, 2 × m, C(2) diastereomeric methylene), 2.37 (3 H, s, 7-methyl), 2.02 (3 H, s, acetamido methyl); mass spectrum (EI mode), m/z 333 (P^+), 290 (P^+ - O=C=NH), 272 (P^+ - carbamic acid). Anal. ($C_{14}H_{15}N_5O_5$) C, H, N.

5-Amino-6-acetamido-3-hydroxy-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole 3-Carbamate (14). A suspension of 130 mg (0.39 mmol) of **13** in 50 mL of methanol was shaken for 4 h under 50 psi in the presence of 50 mg of 5% Pd on carbon. The catalyst was removed by filtration through Celite and the filterate concentrated to dryness. The glassy residue was dissolved in 10 mL of a 90:10 chloroform/methanol mixture, to which hexane was added until cloudiness appeared. Chilling this mixture resulted in crystallization of white **14**: 107 mg, 90% yield; mp 229 °C dec; TLC (chloroform/methanol 80:20) R_f = 0.27; IR (KBr pellet) 3361, 3319, 3277, 1752, 1729, 1657, 1614, 1376, 1315, 1303 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 8.89 (1 H, s, amide proton), 6.68 (2 H, br s, amide protons), 6.58 (1 H, s, C(8) proton, 5.95 (1 H, dd, J = 7.4 Hz, J = 3.2 Hz, C(3) proton), 4.92 (2 H, br s, amino), 4.07 (2 H, m, C(1) diastereomeric methylene), 3.05 and 2.49 (2 H, 2 × m, C(2) diastereomeric methylene), 2.15 (3 H, s, 7-methyl), 2.04 (3 H, s, acetamido methyl); mass spectrum (EI mode), m/z 303 (P^+), 260 (P^+ - HN=C=O), 242 (P^+ - carbamic acid). Anal. ($C_{14}H_{17}N_5O_3 \cdot 0.25H_2O$) C, H, N.

6-Acetamido-3-hydroxy-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole-5,8-dione 3-Carbamate (15). To a suspension of 50 mg (0.16 mmol) of **14** in 5 mL of water, containing 25 mg of monobasic potassium phosphate, was added a solution of 320 mg of Fremy's salt in 20 mL of water containing 200 mg of monobasic potassium phosphate. The reaction mixture was stirred at room temperature for 2 h and then extracted with 6 × 20 mL portions of chloroform. The combined extracts were dried over Na_2SO_4 and concentrated to a residue, which was

recrystallized with chloroform/hexane: 28 mg, 58% yield; mp 205 °C dec; TLC (methanol/chloroform 20:80) R_f = 0.46; IR (KBr pellet) 3467, 3410, 3377, 1789, 1753, 1694, 1675, 1611, 1520 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 9.68 (1 H, s, amide proton), 6.79 (2 H, br s, amide proton), 5.92 (1 H, dd, J = 7.6 Hz, J = 3.2 Hz, C(3) proton), 4.27 (2 H, m, C(1) diastereomeric methylene), 3.08 and 2.52 (2 H, 2 × m, C(2) diastereomeric methylene), 2.09 (3 H, s, 7-methyl), 1.83 (3 H, s, acetamido methyl); mass spectrum (EI mode), m/z 318 (P^+), 276 (P^+ - ketene), 257 (P^+ - carbamic acid). Anal. ($C_{14}H_{14}N_4O_5 \cdot 0.5H_2O$) C, H; N: calcd, 17.12; found, 16.55.

6-Acetamido-3-hydroxy-5-imino-7-methyl-2,3,5,8-tetrahydro-1*H*-pyrrolo[1,2-*a*]benzimidazol-8-one 3-Carbamate (16). To a suspension of 50 mg (0.16 mmol) 14 in 5 mL of 0.2 M pH 7.0 phosphate buffer (μ = 1.0 KCl) was added the following: 130 mg of Fremy's salt solution in 10 mL of this buffer and then 5 mL of water. This mixture was stirred at room temperature for 40 min and the resulting red solution was extracted with 6 × 20 mL portions of chloroform. The combined extracts were dried over Na_2SO_4 and concentrated to a residue, which was recrystallized from chloroform/hexane: 19 mg, 37% yield; mp 259 °C dec; TLC (chloroform/methanol 80:20) R_f = 0.35; IR (KBr pellet) 3419, 3365, 1722, 1652, 1624, 1603, 1496, 1385, 1323, 1086 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 9.33 (1 H, s, amide proton), 6.63 (3 H, m, amide and imine protons), 5.86 (1 H, m, C(3) proton), 4.39 (2 H, m, C(1) diastereomeric methylene), 3.19 and 2.48 (2 H, 2 × m, C(2) diastereomeric methylene), 1.75 and 1.60 (6 H, 2 × s, 7-methyl and acetamido methyl); mass spectrum (EI mode), m/z 317 (P^+), 275 (P^+ - ketene), 274 (P^+ - O=C=NH), 256 (P^+ - carbamic acid). Anal. ($C_{14}H_{15}N_5O_4 \cdot 0.25H_2O$) C, H; N: calcd, 21.76; found, 19.16.

6-Amino-3-hydroxy-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole 3-Acetate Dihydrochloride (17·2HCl). A suspension of 400 mg (1.45 mmol) of the nitro derivative[2] and 100 mg of 5% Pd on charcoal in 100 mL of methanol was shaken under 50 psi of H_2 for 4 h. The reaction mixture was filtered through Celite under an atmosphere of nitrogen, and the filter cake was then washed with methanol. Acidification of filtrate with few drops of 1 N HCl followed by evaporation in vacuo afforded the dihydrochloride salt of 17. Recrystallization was carried out from methanol/ethyl acetate: 338 mg, 84% yield; mp 179 °C dec; TLC (chloroform/methanol 80:20), R_f = 0.64; IR (KBr pellet) 3430, 3423, 3417, 1728, 1643, 1475, 1467, 1303, 1242 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 7.12 and 6.85 (2 H, 2 × s, aromatic protons), 6.01 (1 H, dd, J = 7.52 Hz, J = 3.03 Hz, C(3) proton), 4.06 (2 H, m, C(1) diastereomeric methylene), 3.05 and 2.48 (2 × m, C(2) diastereomeric methylene), 2.16 and 2.04 (6 H, 2 × s, C(7) methyl and acetate methyl); mass spectrum (EI mode), m/z 245 (P^+), 202 (P^+ - acetyl).

6-(Chloroacetamido)-3-hydroxy-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole 3-Acetate (18). To a suspension of 350 mg (1.10) mmol of 17·2HCl in 30 mL of acetone was added 1.5 mL of chloroacetyl chloride. The reaction mixture was stirred at room temperature for 1 h and the solvent was evaporated in vacuo. The residue was dissolved in 75 mL of chloroform and washed with water three times. Drying the extract (sodium sulfate) and concentration in vacuo afforded the product as a yellow solid. Recrystallization was carried out from chloroform/diethyl ether: 220 mg, 62% yield; mp 207 °C dec; TLC (chloroform/methanol 90:10) R_f = 0.58; IR (KBr pellet) 3442, 3384, 1744, 1696, 1681, 1528, 1226, 1174, 1141, 1083 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.29 (1 H, br s, amide proton), 8.14 and 7.23 (2 H, 2 × s, aromatic protons), 6.17 (1 H, dd, J = 7.53 Hz, J = 3.36 Hz, C(3) proton), 4.24 (2 H, s, methylene of chloroacetamido, 4.25 and 4.14 (2 H, 2 × m, C(1) diastereomeric methylene), 3.19 and 2.66 (2 H, 2 × m, C(2) diastereomeric methylene), 2.42 (3 H, s, 7-methyl), 2.14 (3 H, s, acetate methyl); mass spectrum (EI mode), m/z 321 (P^+), 278 (P^+ - acetyl).

5-Amino-6-(chloroacetamido)-3-hydroxy-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole 3-acetate (19) was prepared in two steps from 18 as described below.

To a mixture of 4 mL of fuming nitric acid and 0.5 mL of concentrated sulfuric acid, chilled in a dry ice–acetone bath, was added 200 mg (0.62 mmol) of 18 portionwise. The reaction mixture was removed from the ice bath and stirred at room temperature for 15 min while coming to room temperature and then poured into a mixture of 150 g of ice and 50 mL of chloroform. Saturated sodium bicarbonate was added to the above mixture with vigorous stirring until the pH was neutral. The chloroform layer was separated and the aqueous layer was extracted twice with 50-mL portions of chloroform. The extracts were dried (sodium sulfate) and then concentrated to give the yellow 5-nitro derivative, which was recrystallized from chloroform/hexane: 140 mg, 63% yield; mp 211 °C dec; TLC (chloroform/methanol 9:1) R_f = 0.42; IR (KBr pellet) 3463, 3420, 1746, 1695, 1523, 1496, 1358, 1222 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.78 (1 H, br s, amide proton), 7.47 (1 H, s, aromatic proton), 6.15 (1 H, dd, J = 7.5 Hz, J = 3.15 Hz, C(3) proton), 4.28 and 4.17 (2 H, 2 × m, C(1) diasteromeric methylene), 4.18 (2 H, s, methylene of choloacetamido), 3.22 and 2.66 (2 H, 2 × m, C(2) diastereomeric methylene), 2.40 (3 H, s, 7-methyl), 2.10 (3 H, s, acetate methyl); mass spectrum (EI mode), m/z 366 (P^+), 348 (P^+ - H_2O), 320 (P^+ - NO_2).

To a suspension of 50 mg (0.13 mmol) of the product obtained above in 1 mL of methanol was added a 100-mg portion of sodium dithionite in 1.5 mL of pH 7.00 phosphate buffer three times at 5-min intervals. The reaction mixture was then extracted three times with 25-mL portions of chloroform. The dried extracts (sodium sulfate) were concentrated to give 19 as a white solid: 15 mg, 34% yield; mp 276 °C dec; TLC (chloroform/methanol 85:15) R_f = 0.67; IR (KBr pellet) 3335, 1736, 1672, 1622, 1528, 1492, 1372, 1305, 1236, 1038 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.91 (1 H, br s, amide proton), 6.68 (1 H, s, aromatic proton), 6.12 (1 H, dd, J = 7.1 Hz, J = 2.52 Hz, C(3) proton), 4.30 (2 H, s, methylene of chloroacetamido) 4.23 and 4.08 (2 H, 2 × m, C(1) diasteromeric methylene), 3.17 and 2.63 (2 H, 2 × m, C(2) diasteromeric methylene), 2.35 (3 H, s, 7-methyl) 2.13 (3 H, s, acetate methyl); mass spectrum (EI mode), m/z 366 (P^+).

6-(Chloroacetamido)-3-hydroxy-5-imino-7-methyl-2,3,5,8-tetrahydro-1*H*-pyrrolo[1,2-*a*]benzimidazol-8-one 3-Acetate (20). To a suspension of 33 mg (0.09 mmol) of 19 in 2 mL of 0.2 M, pH 7.0 phoshate buffer (μ = 1.0 KCl) was added a suspension of 150 mg of Fremy's salt in 10 mL of the same phosphate buffer. This mixture was stirred at room temperature for 1 h, and the resulting red solution was then extracted with 3 × 25 mL portions of chloroform. The combined extracts were dried (Na_2SO_4) and concentrated to a solid residue, which was recrystallized from chloroform/hexane: 15 mg, 43% yield; mp 216 °C dec; TLC (chloroform/methanol 90:10) R_f = 0.28; IR (KBr pellet) 3447, 2926, 1744, 1677, 1671, 1659, 1619, 1611, 1531, 1383, 1231 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 9.30 (1 H, br s, amide proton) 7.81 (1 H, s, imine proton) 6.00 (1 H, m, C(3) proton) 4.27 (2 H, s, methylene of chloroacetamido) 4.23 and 3.68 (2 H, 2 × m, C(1) diastereomeric methylene), 3.07 and 2.51 (2 H, 2 × m, C(2) diasteromeric methylene), 2.05 and 1.75 (6 H, 2 × s, 7-methyl and acetate methyl); mass spectra (EI mode), m/z 350 (P^+). Anal. ($C_{15}H_{15}ClN_4O_4 \cdot 1.5H_2O$) C, H; N: calcd, 14.85; found, 13.16.

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Registry No. 1, 135513-32-5; 2, 59007-79-3; 3, 135513-33-6; 4, 135513-34-7; 5, 135513-35-8; 6, 135513-36-9; 7, 135513-37-0; 8, 123567-10-2; 9, 123567-23-7; 10, 135513-38-1; 11, 135513-39-2; 12, 123567-26-0; 13, 135513-40-5; 14, 135513-41-6; 15, 135513-42-7; 16, 135535-67-0; 17, 135513-43-8; 17·2HCl, 135513-53-0; 18, 135513-44-9; 19, 135513-45-0; 20, 135513-46-1; 21, 135513-47-2; 22, 135513-48-3; PBI-A, 123567-24-8; BPI-B, 123567-25-9; BPI-C, 135513-52-9; Fremy's salt, 14293-70-0; 3-(1-pyrrolidino)-4-nitrotoluene, 123567-04-4; dimethylamine, 124-40-3; ethylenimine, 151-56-4; 6-acetamido-3-hydroxy-7-methyl-5-nitro-2,3-dihydro-1*H*-pyrrolo[1,2,*a*]benzimidazol-3-ol, 135513-49-4; phenyl chloroformate, 1885-14-9; chloroacetyl chloride, 79-04-9; 6-(chloroacetamido)-3-hydroxy-7-methyl-5-nitro-2,3-dihydro-1*H*-pyrrolo-[1,2-*a*]benzimidazole-3-acetate, 135513-51-8; sodium dithionite, 7631-94-9; 6-acetamido-3-hydroxy-7-methyl-5-nitro-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazol-3-ol phenylcarbonate, 135513-50-7.