Inhibitors of Topoisomerase II Based on the Benzodiimidazole and Dipyrroloimidazobenzimidazole Ring Systems: Controlling DT-Diaphorase Reductive Inactivation with Steric Bulk

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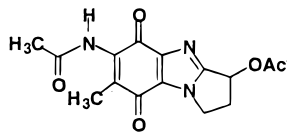
Described herein are the synthesis, cytotoxic properties, and topoisomerase II inhibition assays of benzodiimidazole and dipyrroloimidazobenzimidazole structural variants of the pyrrolo[1,2-*a*]benzimidazole or APBI ring system. These ring variants were designed to inhibit topoisomerase II, much as the APBIs are able to do. Since only the quinone form of the APBIs can intercalate DNA, two-electron reduction to the hydroquinone by DT-diaphorase is known to deactivate these compounds. Indeed, the APBIs possess a high inverse correlation with the cellular concentration of DT-diaphorase. Therefore one feature of the ABPI structural variants is the excessive bulk about the quinone ring, which was predicted to diminish DT-diaphorase substrate activity. Another feature is the presence of one or two alkylating centers, which would permit alkylation of DNA and/or topoisomerase II. Inhibition assays for topoisomerase II-mediated relaxation of supercoiled DNA indicate that the benzodiimidazole and dipyrroloimidazobenzimidazole quinone ring systems are catalytic inhibitors of topoisomerase II. Both quinone systems exhibit cytotoxicity perhaps due to the lack of inactivation by DT-diaphorase as well as topoisomerase II inhibition. One quinone displayed the novel feature of cytotoxicity selectively against melanoma cell lines. In conclusion, the benzodiimidazole and dipyrroloimidazobenzimidazole quinone ring systems will be subjected to future analogue development and structure−activity studies.

Introduction

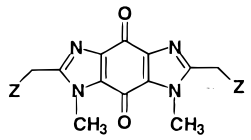
The design of antitumor agents utilizing the two-electron reducing enzyme DT-diaphorase is envisioned either by reductive activation or by the absence of reductive inactivation. The reductive activation of antitumor agents by DT-diaphorase specifically in cancer cell lines is well-known in the literature.[1,2] The reductive activation process usually involves reduction of a quinone species to the hydroquinone by DT-diaphorase followed by leaving group elimination to afford an alkylating quinone methide species.[3,4] DT-diaphorase is also known to play a role in the detoxification of toxins,[1] and therefore cancers low in DT-diaphorase could be sensitive to some agents based on the absence of reductive inactivation

The 6-acetamidopyrrolo[1,2-*a*]benzimidazole-based antitumor agents, the APBIs in Chart 1, have been investigated in this laboratory for some time.[5−8] The APBIs are inactivated by two-electron reduction and therefore show a strong inverse correlation with DT-diaphorase levels (the highest inverse correlation of 20 000 compounds in the National Cancer Institute's archives).[6] The unchanged (oxidized) APBI, rather than the reduced form, appears to act as an inhibitor of the first step of topoisomerase II-mediated relaxation of supercoiled DNA.[5,7] These findings prompted a study of structural variants of the pyrrolo[1,2-*a*]benzimidazole ring system: the benzodiimidazole and dipyrroloimidazobenzimidazole quinone systems shown in Chart 1, along with their complete ring names.

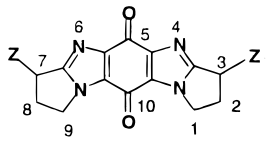
Chart 1



APBI-A



BENZO[1, 2 - *d*]DIIMIDAZOLE



PYRROLO[1, 2 -*a*]PYRROLO[1' , 2' : 1 , 2] IMIDAZO[4, 5 - *f*] BENZIMIDAZOLE

The rationale for studying these ring variants is that the increased steric bulk about the quinone ring, compared to the APBIs, would decrease the DT-diaphorase substrate activity. Since the 7-butyl APBI derivatives were slowly reduced by DT-diaphorase,[7] the bulkier benzodiimidazoles and the dipyrroloimidazobenzimidazoles should be even more resistant to reduction resulting in enhanced cytotoxicity. Previous studies of the APBIs revealed the importance of a substituent at the 3-position, which is usually an acetoxy derivative,[6]

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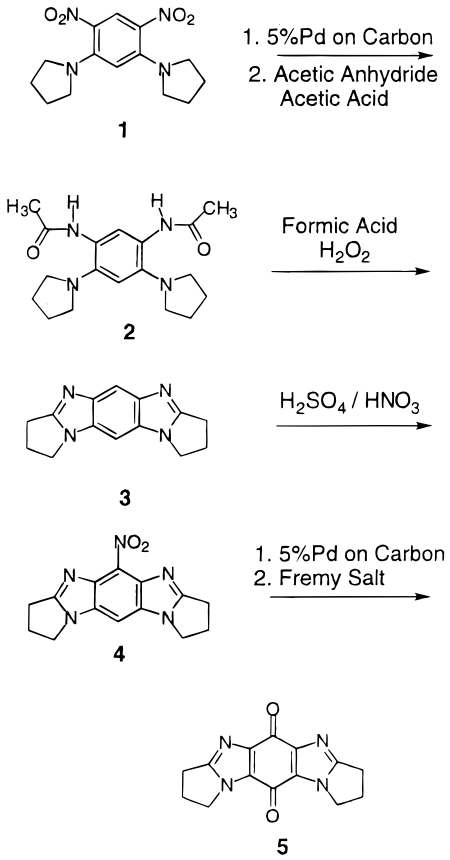
Scheme 1



as well as the importance of the 3-center configuration on DT-diaphorase reductase activity.[7] Therefore, both the *cis*- and *trans*-diacetoxy derivatives of the dipyrroloimidazobenzimidazole system were investigated. The benzodiimidazoles had to be substituted with chloride, rather than acetoxy groups, to observe cytotoxicity. The present report provides a description of the synthesis, cytotoxicity, DT-diaphorase substrate activity, and topoisomerase II activity of the ring variants shown in Chart 1.

Results and Discussion

Synthesis. The preparation of the unsubstituted dipyrroloimidazobenzimidazole quinone **5** was carried out as outlined in Scheme 1. Treatment of 1,5-dibromo-2,4-dinitrobenzene with pyrrolidine afforded **1**, which was converted to **2** by reduction and then acetylation. Cyclization of **2** to **3** was carried out as previously described for the synthesis of the ABPIs.[9,10] Quinone elaboration involved nitration, nitro group reduction to the amine, and finally Fremy oxidation of the amine.[9−11]

Preparation of the benzodiimidazoles **11**−**13** was carried out starting with **6** as outlined in Scheme 2. Reduction and tetrachloroacetylation of **6** afforded **8**, which was ring-closed to the benzodiimidazole system **9** by a Phillips type reaction.[12] Quinone elaboration by the usual method[9−11] afforded a mixture of **11**−**13**, all of which were important to the present study.

The preparation of the dipyrroloimidazobenzimidazole derivatives **19a**,**b** and **20** was carried out as outlined in Schemes 3 and 4. Annelation of both of the pyrroloimidazo rings was carried out stepwise, **14** → **15** and **16** → **17**, employing the "*tert*-amino effect".[10,13,14] The second annelation gives rise to cis/trans isomers, which are not readily separated until converted to the quinone products. Quinone elaboration[9−11] afforded three products: the cis and trans isomers of **19** and the deacetylated product **20.** The latter product very likely arose from reductive removal of one of the acetates during nitro group reduction. Identification of the cis and trans forms of **19** was based on the independent synthesis outlined in Scheme 4. Nucleophilic substitution of 1,5-dibromo-2,4-dinitrobenzene with (*R*)- 4-amino-2-hydroxybutanoic acid[15] afforded **22**, which was converted to *trans*-**17** by an " internal Phillips reaction".[10] Conversion of authentic *trans*-**17** to the quinone species resulted in a product spectrally identical to **19b**.

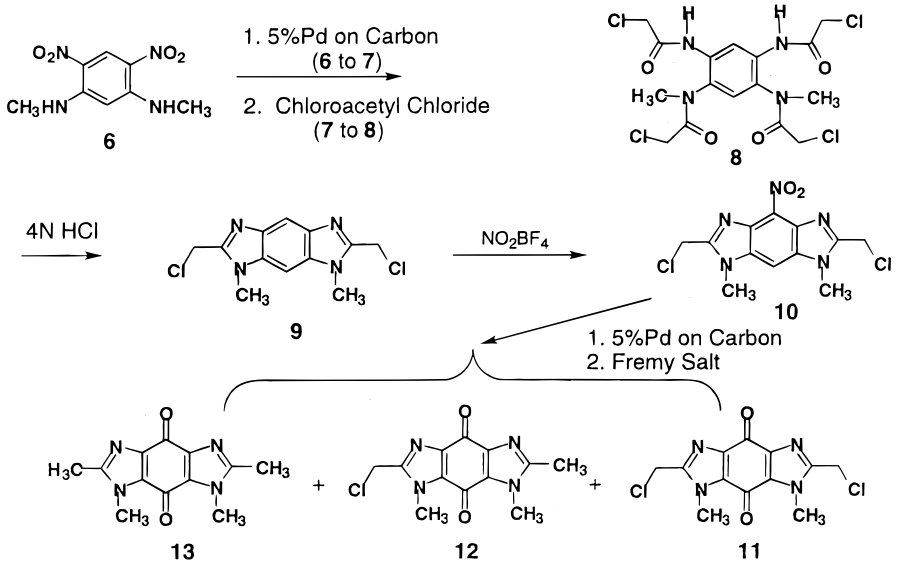
Differential Cytotoxicity. This section presents a discussion of the relative cytotoxicities of **5**, **19a,b**, and **11**−**13** against seven histological types of cancer: leukemia, non-small-cell lung, colon, CNS, melanoma, ovarian, and renal. The measure of cyotoxicity employed is the LC_{50} value, which is the concentration of drug lethal to 50% of cancer cells. The LC_{50} data were obtained by screening the compound against up to 60 human cancer cell lines in the National Cancer Institute's in vitro screen.[16,17] The LC_{50} data in Figures 1 and 2 are the average values for each type of cancer expressed as the $-\log(LC_{50})$ on the *y*-axis. The *x*-axis shows the histological cancer type, and the *z*-axis shows the compound. Lower LC_{50} values represent higher potency, and therefore higher $-\log(LC_{50})$ values represent higher potency.

Inspection of Figure 1 reveals that unsubstituted benzodiimidazole quinone **5** possesses a high specificity toward melanoma cell lines. In contrast the agent APBI-A, which shows in vivo activity against melanoma,[6] displays high specificity toward both melanoma and renal cancers. The combination of the structural features of **5** and APBI-A, represented by **19a**,**b**, results in either complete loss of cytotoxicity (**19b**) or retained cytotoxicity but with complete loss of specificity (**19a** in Figure 1). Compound **19b** was tested as the racemate, and therefore neither enantiomer (*RR* or *SS*) is active. In conclusion, the meso form (**19a**) appears to be the most suitable stereoisomer for further analogue development.

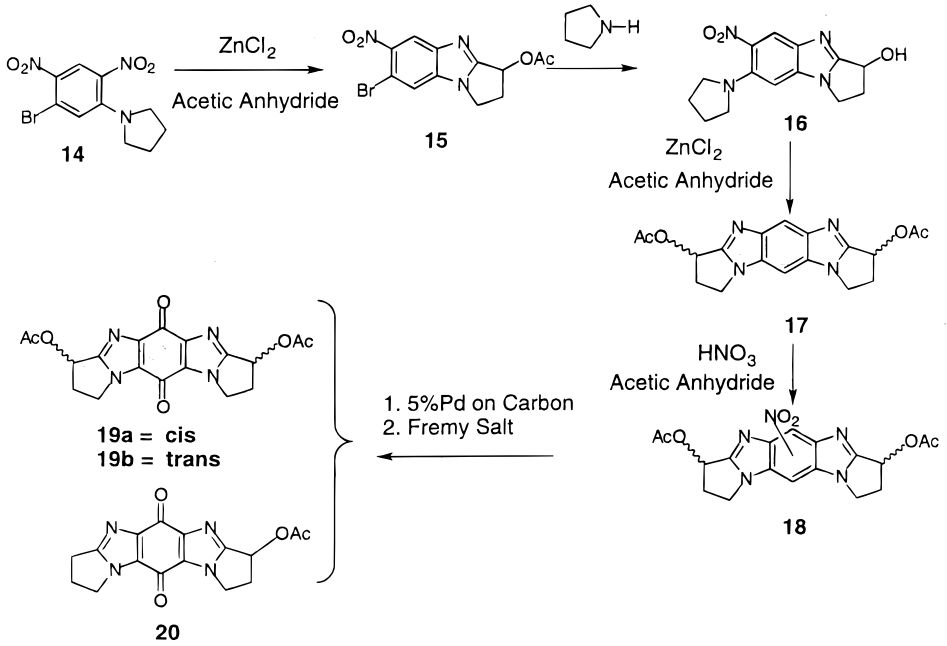
The data in Figure 2 reveal that the unsubstituted benzodiimidazole quinone **(13)** possesses no cytotoxicity while analogues bearing leaving groups (**11** and **12**) possess cytotoxicity, but with little or no specificity. In order for the benzodiimidazole quinones to show appreciable cytotoxicity, substitution with an excellent leaving group (chloride as opposed to acetate) appears to be required. The nearly equivalent cytotoxicity of **11** and **12** suggests that alkylation, but not necessarily cross-linking, is occurring. In contrast to the benzodiimidazoles, the dipyrroloimidazobenzimidazoles and the APBIs are still cytotoxic when substituted with acetate or even when unsubstituted.

DT-Diaphorase Substrate Activity. The compounds evaluated for rat liver DT-diaphorase substrate activity include **5**, **11**−**13**, **19a,b**, and **20.** The substrate specificity was compared with that of the known antitumor agent APBI-A, Chart 1. We had proposed that increasing the steric bulk about the quinone ring of APBI-A would slow DT-diaphorase-mediated reduction.

Scheme 2



Scheme 3



However, the dipyrroloimidazobenzimidazole quinones **5** and **20** were substantially better substrates for the enzyme than ABPI-A, Figure 3. Both **5** and **20** had similar V_{max}/K_m values (6.24 and 7.1 $\times$ 10^{-4} s^{-1}) compared to that of 2.6 $\times$ 10^{-4} s^{-1} for APBI-A. These results suggest that the presence of one or more fused pyrrolo rings is important for the DT-diaphorase substrate activity of these systems. Indeed, none of the benzodiimidazole quinones **11**−**13** were reduced by rat liver DT-diaphorase. The presence of the fused pyrrolo ring in mitomycin C may be in part responsible for its efficient reductve activation in tumor cells. When the dipyrroloimidazobenzimidazole ring possesses two acetate substituents, **19a,b**, DT-diaphorase substrate activity was lost perhaps for steric reasons.

Some of the cytotoxicity results presented in Figures 1 and 2 show a relationship between the absence of DT-diaphorase substrate activity and the lack of specific cancer cell cytotoxicity. Thus **19a**, **11**, and **12** exhibit cytotoxicity against all cancer panels, while the less bulky APBIs (represented by APBI-A) possess cytotoxicity toward melanoma and renal cancers. The lack of cancer cell specificity we observe with **19a**, **11**, and **12** may originate with the absence of DT-diaphorase inactivation in all the cancer cell lines. In contrast compound **20** was inactive against all cancer cell lines, perhaps due to the efficient reductive inactivation by DT-diaphorase.

The quinones **5** and **19b** do not fit into the relationship between DT-diaphorase substrate activity and specific cytotoxicity discussed above. Quinone **5** is an excellent substrate for DT-diaphorase and also has a high specificity toward melanoma cell lines. Quinone **19b**, on the other hand, is not reduced (inactivated) by DT-diaphorase, is an inhibitor of topoisomerase II, and is noncytotoxic. Clearly other factors beside DT-diaphorase and topoisomerase II must be involved in the biological activity of these quinones.

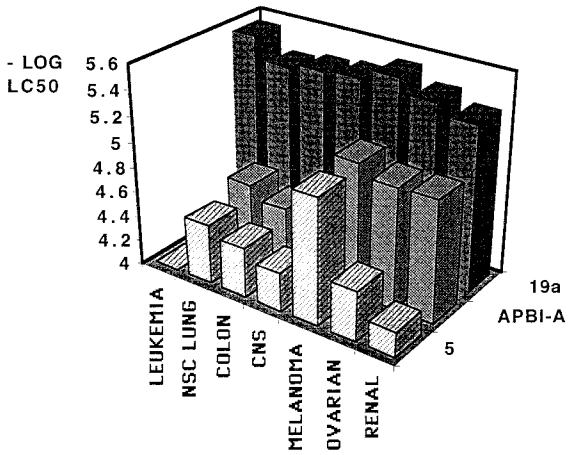
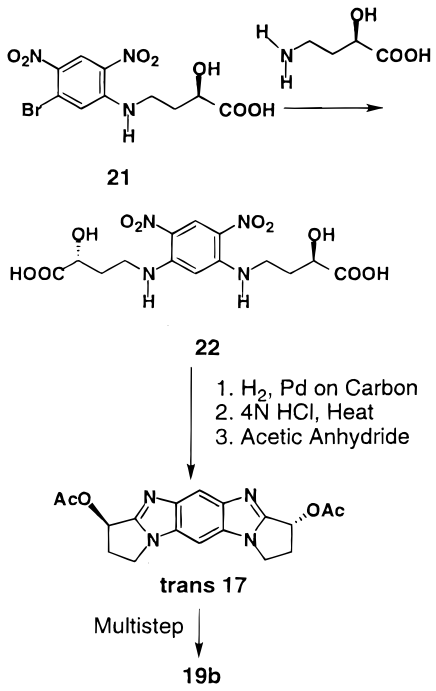


Figure 1. Plots of $-\log(LC_{50})$ versus cancer type for **5**, APBI-A, and **19a**. Each cancer type represents the average of six to eight different cancer cell lines. Compound **5** has high specificity for melanoma, while APBI-A has specificity for melanoma, ovarian, and renal cancers. In contrast, **19a** is completely nonspecific.

Scheme 4



To gain insight into other possible cytotoxicity mechanisms, we employed the National Cancer Institute's COMPARE program.[16] This program along with the DISCOVERY program[18] were developed to compare the patterns of cytotoxicity in 60-cell line cancer screens with those of other compounds. Antitumor agents with identical mechanisms of action possess identical or nearly identical cytotoxicity patterns (correlation coefficient > 0.8). For example anthracycline analogues (doxorubicin, rubidazone, daunamycin) have a high correlation (>0.9) with each other as do the DNA alkylating agents (chlorambucil, thiotepa, triethylenemelamine). Quinone **5** does not correlate well with any known compound in the National Cancer Institute's archives suggesting a unique mechanism of cytotoxicity. In contrast, compound **19a** is active against all cell lines and there is no pattern to utilize in correlations. A

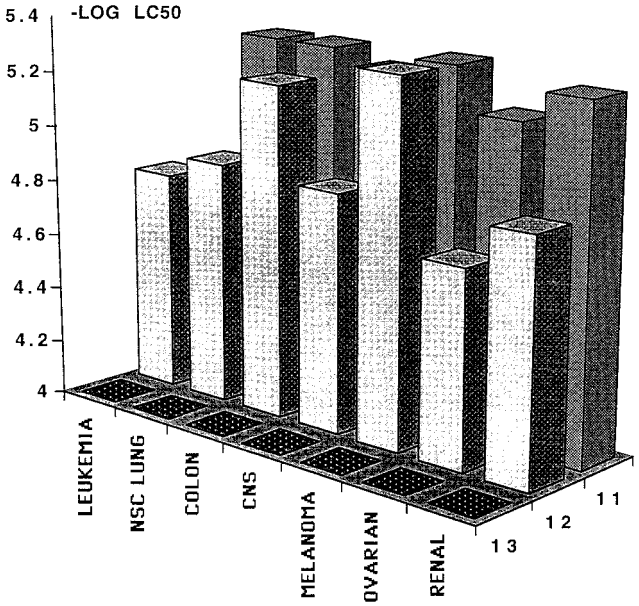


Figure 2. Plots of $-\log(LC_{50})$ versus cancer type for **11**−**13**. Each cancer type represents the average of six to eight different cancer cell lines. Compound **13** is considered to be devoid of activity; i.e., $-\log(LC_{50})$ is 3−4. Both **11** and **12** are cytotoxic but with little or no selectivity for cancer type.

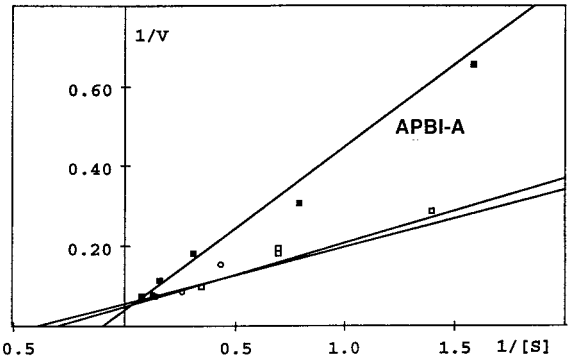


Figure 3. Reciprocal plots of velocity (nM/s) versus concentration (10^{-5} M) for substrate activity of APBI-A, **5**, and **20** with purified rat liver DT-diaphorase. Both **5** and **20** fall on the same plot due to similar V_{max}/K_m values. The relative specificity or k_{cat}/K_m of these substrates was calculated from the V_{max}/K_m value and the concentration of enzyme.

COMPARE correlation of 0.9 was observed between **19a** and tamoxifen, which is also active against all cell lines.

Topoisomerase II Inhibition. Previous studies with the APBIs indicated that they are catalytic inhibitors of topoisomerase II rather than poisons.[7,19] Topoisomerase II poisons are usually clinically active and act at the religation step by stabilizing the enzyme−DNA complex formed upon double-strand cleavage. Examples of agents which stabilize the cleavable complex include *m*-AMSA, etoposide, doxorubicin,[20−22] naturally occurring naphthoquinones,[23−25] and many other structurally diverse compounds.[26,27] However, catalytic inhibitors of topoisomerase II are being reported more frequently in the literature and may also be clinically useful.[28−31] Of particular note are the pyrroloiminoquinones,[32,33] which are mechanistic and structurally related to the APBIs and imino-APBIs. Both the pyrroloiminoquinones and the ABPIs are known to inhibit the topoisomerase II-mediated relaxation of supercoiled DNA by intercalation.[5] The data shown in Figures 4 and

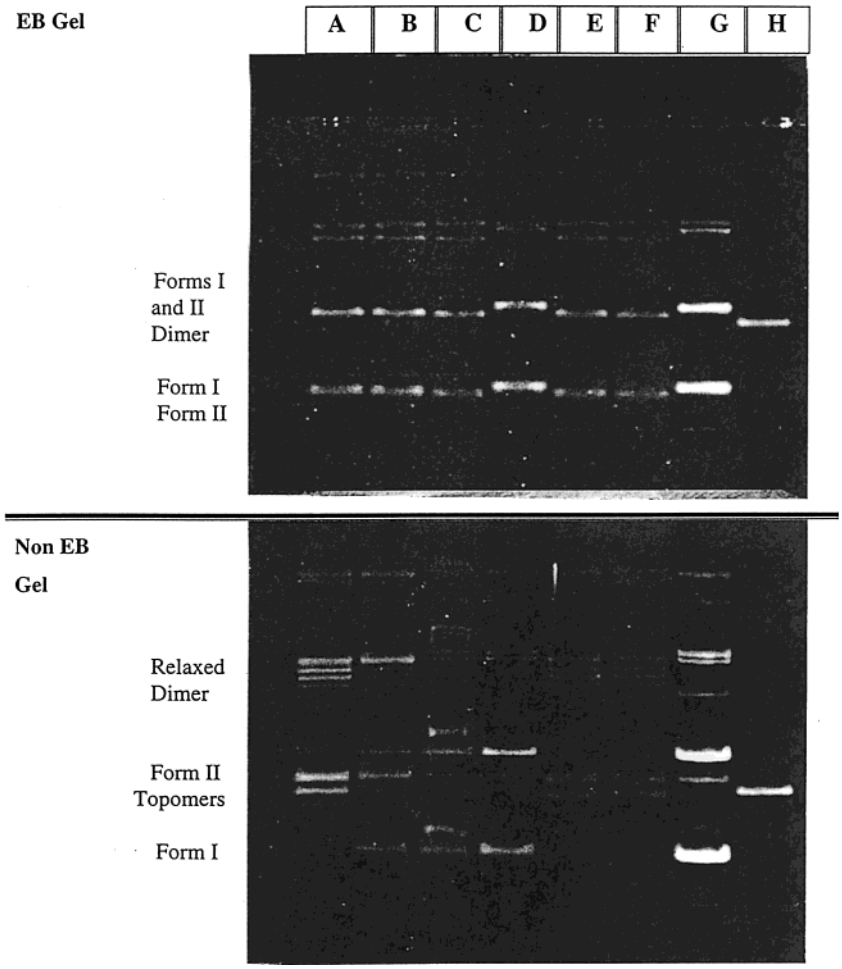


Figure 4. Agarose gels of topoisomerase II-catalyzed relaxations of pRYG supercoiled DNA (form I along with supercoiled dimer and trimer) run in the presence or absence of ethidium bromide. In both Figures 4 and 5, lanes E−H are controls: lane G is unrelaxed pRYG supercoiled DNA, lane H is the linear form of pRYG supercoiled DNA, lanes E and F are relaxation reactions carried out with and without DMSO. Lanes A and B are relaxation reactions carried out in the presence of 0.19 and 0.39 mM **19a** and lanes C and D likewise carried out in the presence of 0.13 and 0.26 mM **19b**. The higher concentrations of both compounds show inhibition with **19b** causing complete inhibition.

5 indicate that the benzodiimidazole and dipyrroloimidazobenzimidazole quinone systems likewise inhibit topoisomerase II.

Relaxation assays were carried out with recombinant human topoisomerase II, and completed reactions were assayed on agarose gels run with or without ethidium bromide (EB). The EB-containing gels will readily detect the presence of linear DNA, but these gels bearly resolve form I and form II DNAs (form II traveled slightly faster than form I). If linear DNA is present, then the agent acts as a topoisomerase II poison; i.e., the agent stabilizes the cleavable complex. In contrast, non-EB-containing gels will readily resolve the form II topomers arising from the supercoiled or form I DNA, and catalytic inhibition could then be documented by noting the decrease in relaxation with increaasing inhibitor concentration.

Shown in Figure 4 are the assays for the relaxation of pRYG supercoiled DNA by p170 human topoisomerase II in the presence of **19a** (lanes A and B) and **19b** (lanes C and D). The other lanes are either controls or references (see legend of Figure 4). Inspection of the non-EB gel of Figure 4 reveals the absence of linear DNA indicating the absence of topoisomerase II poisoning. Inspection of the non-EB gel shown in Figure 4 reveals significant amounts of form I DNA in lanes B−D indicating that both **19a**,**b** can inhibit topoisomerase II-mediated relaxation. Consistent with the results obtained with the non-EB gel, 0.38 mM **19b** completely inhibited relaxation, lane D.

The non-EB-containing gel in Figure 5 shows that both **5** and **11** can inhibit topoisomerase II by virtue of the presence of significant amounts of form I DNA in lanes A−D, with both concentrations of **11** (0.25 and 0.5 mM) causing complete inhibition.

Conclusions

The goal at the outset of this research was to develop more effective cytotoxic analogues of the pyrrolo[1,2-*a*]-benzimidazole (APBI) system previously developed in this laboratory. The strategy employed was to add fused pyrrolo and fused imidazo rings to the APBI system in the hope of increasing the bulk about the quinone ring so as to prevent DT-diaphorase reductive inactivation. The resulting dipyrroloimidazobenzimidazole quinones **5** and **20** were found to be even better substrates for DT-diaphorase than the APBIs. These results suggest that the presence of one or more fused pyrrolo rings is important for the DT-diaphorase substrate activity of these systems. Indeed, none of the benzodiimidazole

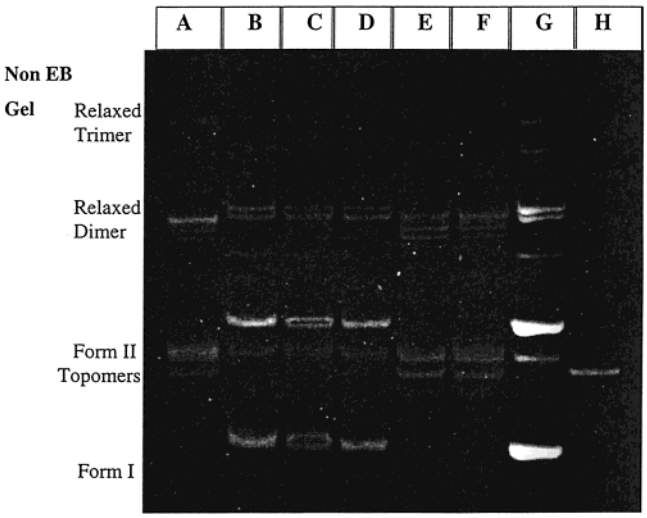


Figure 5. Agarose gels of topoisomerase II-catalyzed relaxations of pRYG supercoiled DNA (form I along with supercoiled dimer and trimer) run in the absence of ethidium bromide. The control lanes E−H are the same as described in the Figure 4 legend. Lanes A and B are relaxation reactions carried out in the presence of 0.1 and 0.2 mM **5** and lanes C and D likewise carried out in the presence of 0.25 and 0.5 mM **11**. Compound **11** shows complete inhibition at both concentrations.

quinones **11**−**13** were reduced by rat liver DT-diaphorase. When **5** was substituted with acetates to afford **19a,b**, neither quinone was reduced by DT-diaphorase presumably for steric reasons.

The lack of cancer cell specificity we observe with **19a**, **11**, and **12** may originate with the absence of DT-diaphorase inactivation in all the cancer cell lines. Thus the absence of DT-diaphorase inactivation in all cells results in cytotoxicity in all panels. In contrast compound **20** was inactive against all cancer cell lines, perhaps due to the efficient reductive inactivation by DT-diaphorase.

The quinones **5** and **19b** do not fit into the relationship between DT-diaphorase substrate activity and specific cytotoxicity discussed above. Quinone **5** is an excellent substrate for DT-diaphorase and also has a high specificity toward melanoma cell lines. Quinone **19b,** on the other hand, is not reduced by DT-diaphorase and is noncytotoxic. There are no others compounds in the National Cancer Institute's archives with the LC_{50} mean graph profile of **5** suggesting the presence of a novel cytotoxicity profile.

The benzodiimidazole and dipyrroloimidazobenzimidazole quinones **19a,b**, **5,** and **11** were found to inhibit topoisomerase II suggesting a possible cytotoxicity mechanism. The absence of linear DNA formation in the topoisomerase II assays indicates the absence of cleavable complex stabilization as seen in the clinically used topoisomerase II poisons.

This study has succeded in identifying novel lead compounds for further development. Quinone **5** is a highly selective cytotoxic agent whose mechanism of action must be investigated. The rational design of selective anticancer compounds would be possible based on these mechanistic studies. On the other hand, quinones **19a** and **11** possess broad-spectrum cytotoxicity and could also be effective cancer chemotherapeutic agents. These systems will also be subjected to mechanistic and structure−activity studies.

Experimental Section

All solutions and buffers for DT-diaphorase and topoisomerase II assays used doubly distilled water. Human recombinant p170 topoisomerase II and pRYG plasmid were purchased from TopoGEN Inc. All analytically pure compounds were dried under high vacuum in a drying pistol over refluxing toluene. Elemental analyses were run at Atlantic Microlab, Inc., Norcross, GA. All TLCs were performed on silica gel plates using a variety of solvents and a fluorescent indicator for visualization. IR spectra were taken as thin films and the strongest absorbances reported. ^{1}H NMR spectra were obtained from a 300-MHz spectrometer. All chemical shifts are reported relative to TMS.

DT-Diaphorase Reduction Kinetics Studies. Rat liver DT-diaphorase was isolated as previously described.[7,34] Kinetic studies were carried out in 0.05 M pH 7.4 Tris·HCl buffer, under anaerobic conditions, employing Thunberg cuvettes. A 2 mM stock solution of the appropriate quinone was prepared in dimethyl sulfoxide (DMSO). To the top port was added the quinone stock and to the bottom port was added DT-diaphorase and NADH in the Tris buffer. The top and bottom ports were purged with argon for 20 min and equilibrated to 30 °C. The ports were then mixed and the reaction was followed at 296 nm for 25 min to obtain initial rates. The concentrations upon after mixing were 0.3 mM NADH, $1-20 \times 10^{-5}$ M quinone, and 14.5 nM (based on flavin) enzyme active sites. The value of $\Delta\epsilon$ was calculated from the initial and final absorbance values for complete quinone reduction; usual value for ϵ is 6000−8000 M^{-1} cm^{-1}. The value of $\Delta\epsilon$ was used to calculate V_{max} in M s^{-1}. The results were fitted to a Lineweaver−Burke plot from which k_{cat}/K_m values were calculated based on 14.5 nM active sites.

Cytotoxicity Studies. Cytotoxicity studies were carried out at the National Cancer Institute.[16,35] The submitted sample (∼5 mg) was subjected to the 60-cell line in vitro assay, which resulted in mean graph data including LC_{50} (lethal concentration, 50%), GI_{50} (growth inhibition concentration, 50%), and TGI (total growth inhibition concentration). Average LC_{50} values off each cancer type are plotted in Figures 1 and 2.

Topoisomerase II Assays. The topoisomerase relaxation reactions were carried out with 0.25 μg of pRYG supercoiled DNA (Form I), 4 units of topoisomerase II in 20 μL of 50 mM Tris·Cl, pH 8, containing 120 mM KCl, 10 mM $MgCl_2$, 0.5 mM dithiothreitol, and 0.5 mM ATP. Varying amounts of the quinone-based inhibitors were added to some reactions. The reactions were run for 45 min at 37 °C and then mixed with 2 μL of a stop solution consisting of 10% SDS. After the reaction was stopped, 1 μL of a solution of 1 mg of proteinase K in 1 mL of water was added and the resulting mixture incubated at 37 °C for 15 min. The reaction mixture was extracted once with a 20-μL solution of chloroform:isoamyl alcohol (24:1) and then combined with 2 μL of 10X loading solution (0.25% bromophenol blue and 50% aqueous glycerol). The relaxation reactions were assayed in 1X TAE buffer (50X is 242 g of Tris base, 57.1 mL of acetic acid, and 100 mL of 0.5 M EDTA in 1 L of dd water) using a 1% agarose gel. The gel and running buffer either did or did not have 0.5 μg/mL ethidium bromide. The gel was loaded with 20 μL of reaction mixture/gel loading solution and run at 2 V/cm for 6−8 h. The gels without ethidium were soaked in a solution of 0.5 μg/mL ethidium bromide in the TAE buffer followed by washing for 30 min in the same buffer without ethidium. Gels with ethidium bromide were washed for 30 min in the TAE buffer.

1,5-Diacetamido-2,4-dipyrrolidinobenzene (2) was prepared by the two-step procedure described as follows. To 1.0 g (3.26 mmol) of 1,3-dibromo-4,6-dinitrobenzene in a round-bottom flask was added 1.5 mL of pyrrolidine dropwise. After addition of the pyrrolidine the solution was heated at 90 °C until starting material had disappeared by TLC, about a 12-h reaction time. The completed reaction was poured over 100 g of ice resulting in precipitation of the product. The product was filtered off and dried under vacuum. An analytical sample was prepared by flash chromatography using silica gel with chloroform as the eluent. The product **1** was recrystallized from

chloroform/hexane: 892 mg (89%) yield; TLC ($CHCl_3$) R_f = 0.37; mp >190 °C dec; IR (KBr pellet) 2874, 1599, 1554, 1489, 1354, 1323, 1309, 1259 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.55 and 5.90 (2H, 2s, aromatic protons), 3.28 (8H, t, J = 6.6 Hz, pyrrolo protons), 2.00 (8H, quintet, J = 3.3 Hz, pyrrolo protons); MS (EI mode) m/z 306 (M^+), 289 ($M^+ - OH$).

A solution consisting of 4.0 g (13.7 mmol) of **1** and 800 mg of 5% Pd on carbon in 350 mL of acetic acid was shaken under 50 psi of H_2 for 12 h and then filtered through Celite into a flask containing 100 mL of acetic anhydride. An additional 50 mL of acetic anhydride was then added to the reaction mixture followed by stirring at room temperature for 1.5 h. The reaction was then concentrated in vacuo to an oil to which 350 mL of diethyl ether was added. The product crystallized from solution as an off-white solid pure enough for the next step. An analytical sample was prepared by recrystallization from chloroform/hexane: 4.0 g (88%) yield; mp 205 °C; TLC (chloroform/methanol [90:10]) R_f = 0.43; IR (KBr pellet) 3539, 3485, 1645, 1562, 1516, 1427, 1371, 1298, 972, 815 cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6) δ 6.94 and 6.15 (2H, 2s, aromatic protons), 3.16 (4H, t, J = 6.4 Hz, pyrrolidino protons), 2.5 (4H, t, J = 6.4 Hz, pyrrolodino protons); MS (EI mode) m/z 330 (M^+), 287 (M^+ − ketene). Anal. ($C_{18}H_{26}N_4O_2 \cdot 0.5H_2O$) C, H, N.

3*H*,7*H*-1,2,8,9-Tetrahydropyrrolo[1,2-*a*]pyrrolo[1′,2′:1,2]imidazo[4,5-*f*]benzimidazole (3). To a solution consisting of 6 mL of 96% formic acid and 3 mL of 30% hydrogen peroxide was added 1.5 g (4.5 mmol) of **2**, and the reaction stirred at 70 °C for 2 h. The reaction was then cooled and diluted with 25 mL of water and the pH adjusted to ∼7 with concentrated ammonium hydroxide. The resulting precipitate as filtered off and the remaining solution extracted five times with 25-mL portions of chloroform. The chloroform extracts were dried (Na_2SO_4), filtered, and concentrated to afford the crude product. An analytical sample was obtained by recrystallization from chloroform/hexane: 165 mg (10%) yield; mp 240 °C dec; T1C (chloroform/methanol [80:20]) R_f = 0.40; IR (KBr pellet) 1545, 1491, 1433, 1354, 1302, 1228, 1118, 943, 848, 669 cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6) δ 7.62 and 7.40 (2H, 2s, aromatic protons) 4.10 (4H, t, J = 6.87 Hz, C(1) and C(9) methylenes), 2.95 (4H, t, J = 7.0 Hz, C(3) and C(7) methylenes); MS (EI mode) m/z 238 (M^+). Anal. ($C_{14}H_{14}N_4 \cdot 0.2H_2O$) C, H, N.

5-Nitro-3*H*,7*H*-1,2,8,9-tetrahydropyrrolo[1,2-*a*]pyrrolo-[1′,2′:1,2]imidazo[4,5-*f*]benzimidazole (4). To a solution consisting of fuming nitric acid and concentrated sulfuric acid (50:50), cooled in an ice/salt bath to 0 °C, was added 316 mg (1.33 mmol) of **3** and the reaction was stirred for 15 min. The reaction was then poured over crushed ice and pH adjusted to 6.0−6.5 with concentrated ammonium hydroxide. The resulting solution was then extracted five times with 25-mL portions of chloroform. The chloroform extracts were combined, dried (Na_2SO_4), filtered, and concentrated. Pure product was obtained by recrystallization from chloroform/hexane. An analytical sample was prepared by preparative TLC using chloroform/methanol (9:1) as the eluent: 343 mg (90%) yield; mp > 260 °C; TLC (chloroform/methanol [90:10]) R_f = 0.23; IR (KBr pellet) 3510, 3448, 3171, 1498, 1460, 1340, 1236, 1157, 1033, 675 cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6) δ 7.93 (1H, s, aromatic proton), 4.20 (4H, t, J = 7.08 Hz, C(1) and C(9) methylenes), 3.04 (4H, t, J = 7 Hz, C(3) and C(7) methylenes) 2.68 (4H, quintet, J = 7.68 Hz, C(2) and C(8) methylenes); MS (EI mode) m/z 238 (M^+). Anal. ($C_{14}H_{13}N_5O_2$) C, H, N.

3*H*,7*H*-1,2,8,9-Tetrahydropyrrolo[1,2-*a*]pyrrolo[1′,2′:1,2]imidazo[4,5-*f*]benzimidazole-5,10-dione (5). A solution consisting of 250 mg (0.88 mmol) of **4**, 62 mg of 5% palladium on activated carbon, and 80 mL of methanol was shaken at 50 psi H_2 for 4 h. The reaction was then filtered through Celite and the methanol removed in vacuo to afford the amine as a solid residue. To the amine was added a solution consisting of 1 g of monobasic potassium phosphate in 25 mL of water. To this solution was added a second solution consisting of 1.8 g of Fremy's salt, 1.5 g of monobasic potassium phosphate, and 75 mL of water. The reaction was stirred at room temperature for 2 h and then extracted thrice with 25-mL portions of chloroform. The chloroform extracts were dried (Na_2SO_4), filtered, and concentrated in vacuo to afford crude product. The product was further purified by column chromatography using silica gel and chloroform/methanol (99:1) as eluent. The purified product was recrystallized from chloroform/hexane: 95 mg (40%) yield; mp >260 °C; TLC (chloroform/methanol [80:20]) R_f = 0.47; IR (KBr pellet) 1676, 1656, 1521, 1485, 1438, 1408, 1307, 740 cm^{-1}; ^1H NMR ($CDC1_3$) δ 4.25 (4H, t, J = 7.13 Hz, C(1) and C(9) methylene), 2.96 (4H, t, J = 6.1 Hz, C(3) and C(7) methylene), 2.72 (4H, quintet, J = 7.77 Hz, C(2) and C(8) methylene); ^{13}C NMR ($CDC1_3$) δ 175, 168, 160, 147, 129, 45, 26, 22; MS (EI mode) m/z 268 (M^+). Anal. ($C_{14}H_{12}N_4O_2$) C, H, N.

1,5-Bis(methylamino)-2,5-dinitrobenzene (6). To 2.48 g (7.6 mmol) of 1,5-dibromo-2,4-dinitrobenzene was added 25 mL of dimethylformamide followed by 15 mL of 17% methylamine in dimethylformamide. The reaction was stirred at room temperature for 15 min and then 30 mL of ice water was added to precipitate the product. The yellow product was filtered off, washed with ice water, and dried under vacuum: 1.62 g (95%) yield; mp 260 °C dec; TLC (CH_2Cl_2) R_f = 0.62; IR (KBr pellet) 3385, 1629, 1593, 1541, 1425, 1410, 1342, 1294, 1195, 1010, 800 cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6) δ 8.97 and 5.69 (2H, 2s, C(5) and C(2) protons), 8.5 (2H, s, amine protons), 3.0 (6H, s, *N*-methyl's); MS (EI mode) m/z 226. Anal. ($C_8H_{10}N_4O_4$) C, H, N.

1,5-Diamino-2,4-bis(methylamino)benzene·HC1 (7). A suspension consisting of 1.05 g (4.66 mmol) of **6** and 200 mg of 5% Pd on carbon in 150 mL of methanol was shaken under 50 pi H_2 for 2 h. The reaction mixture was filtered through Celite into 5 mL of concentrated hydrochloric acid and the methanol was removed in vacuo to afford the HCl salt as a greenish solid: 1.36 g (93%) yield; mp 130−145 °C dec; IR (KBr pellet) 3346, 3219, 3022, 2858, 1531, 1460, 1288, 1130, 887, 468 cm^{-1}; MS (EI mode) m/z 166 (M^+), 155 (M^+ − methyl), 124 (M^+ − methyl, NH_2).

1,5-Bis(chloroacetamido)-2,4-bis(*N*-methylchloroacetamido)benzene (8). To a solution consisting of 100 mL of dry dimethylformamide and 2.5 mL of pyridine was added 815 mg (2.6 mmol) of **7** followed by the addition of 1.0 mL (13 mmol) of chloroacetyl chloride. The reaction was stirred for 2 h and then the dimethylformamide was removed in vacuo to afford a solid residue. To the residue was added 75 mL of water and the solution was neutralized with aqueous sodium bicarbonate. The resulting precipitate was filtered off and dried in vacuo. The supernatant was extracted thrice with 50-mL portions of chloroform. The chloroform extracts were dried (Na_2SO_4), filtered, and evaporated to a solid residue. The precipitate and extracted solids were combined and recrystallized from chloroform/hexane: 848 mg (69%) yield; mp 193−200 °C dec; TLC (chloroform/methanol [90:10]) R_f = 0.42; IR (KBr pellet) 1670, 1597, 1534, 1534, 1507, 1428, 1385, 1344, 1255, 1252, 1125, cm^{-1}. Anal. (C_{16} $H_{18}Cl_4N_4O_4$) H; N: found 11.31; found 11.86. This intermediate has some triacetylated impurity; all acetylated species were converted to pure **9**.

2,6-Bis(chloromethyl)-1,7-dimethylbenzo[1,2-*d*:4,5-*d*′]-diimidizole (9). To a 50-mL solution of 4 N HCl, held at 80 °C, was added 821 mg (1.74 mmol) of **8** and the reaction stirred for 2 h at 80 °C. The completed reaction was cooled and neutralized with aqueous sodium bicarbonate. The resulting precipitate was filtered off and dried under high vacuum: 438 mg (88%) yield; mp 214−215 °C; TLC (1-butanol/acetic acid/water [5:3:2]) R_f = 0.55; IR (KBr pellet) 1528, 1470, 1424, 1370, 1257, 936, 909, 801 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.12 and 7.17 (2H, 2s, C(4) and C(8) protons), 4.88 (4H, s, C(2) and C(6), chloromethyls), 3.93 (6H, s, N(1) and N(7) methyls); MS (EI mode) m/z 282 (M^+), 284 (M + 2). Anal. ($C_{12}H_{12}Cl_2N_4$) C, H, N.

2,6-Bis(chloromethyl)-1,7-dimethyl-4-nitrobenzo[1,2-*d*:4,5-*d*′]diimidizole (10). To 20 mL of dry acetonitrile was added 50 mg (0.17 mmol) of **9** followed by 46 mg of (85%) nitronium tetrafloroborate. The reaction was stirred for 1 h; then the acetonitrile was removed in vacuo. The dry residue was then dissolved in 25 mL of water and the pH adjusted to

7.5 with aqueous sodium bicarbonate. It was then extracted thrice with 20-mL portions of chloroform. The chloroform extracts were dried (Na_2SO_4), filtered, and concentrated. The resulting yellow solid was recrystallized from chloroform/hexane: 36 mg (63%); mp 256 °C dec; TLC (chloroform/methanol [90:10]) $R_f = 0.21$; IR (KBr pellet) 1525, 1504, 1471, 1433, 1404, 1365, 1257, 1188, 1132, 1003 cm^{-1}; ^1H NMR ($CDC1_3$) δ 7.52 (1H, s, aromatic proton), 4.97 (4H, s, C(2)- and C(6)-chloromethyls), 4.02 (6H, s, N(1)- and N(7)-methyls); MS (EI mode) *m/z* 327 (M^+), 329 (M + 2), 292 ($M^+ - C1$), 246 ($M^+ - C1 - NO_2$). Anal. ($C_{12}H_{11}C1_2N_5O_2$) C, H, N.

2,6-Bis(chloromethyl)-1,7-dimethylbenzo[1,2-*d*:4,5-*d*′]-diimidazole-4,8-dione (11), 2-chloromethyl-1,6,7-trimethylbenzo[1,2-*d*:4,5-*d*′]diimidizole-4,8-dione (12), and 1,2,6,7-tetramethylbenzo[1,2-*d*:4,5-*d*′]diimidizole-4,8-dione (13) were synthesized by the following two-step procedure. A suspension consisting of 207 mg (0.57 mmol) of **10** was dissolved in 25 mL of chloroform and placed in a separatory funnel. To the separatory funnel was added a sodium dithionite solution, consisting of 800 mg sodium dithionite and 5 mL of pH 7.0 phosphate buffer, and the funnel was vigorously shaken. After addition of a second portion of sodium dithionite followed by shaking, the chloroform layer was removed and the aqueous dithionite layer was extracted thrice with 50-mL portions of chloroform. The chloroform extracts were combined, dried (Na_2SO_4), filtered, and concentrated to afford crude amine product which was crystallized from chloroform/methanol/hexane: 70 mg (40.4%) yield.

To a suspension of 117 mg (0.39 mmol) of the crude amine obtained above in 10 mL of water/dimethylformamide [9:1] was added 20 mL of a solution consisting of 450 mg (1.56 mmol) of Fremy's salt and 35 mg of monobasic potassium phosphate. The reaction was stirred for 8 h and then extracted thrice with 25-mL portions of chloroform. The chloroform extracts were dried (Na_2SO_4), filtered, and concentrated to a solid residue. The quinone products were purified by flash chromatography using silica gel and chloroform/methanol [97:3] as the eluent.

11: 23 mg (17.5%) yield; mp 232 °C dec; TLC (chloroform/methanol [90:10]) $R_f = 0.51$; IR (KBr pellet) 3043, 1668, 1500, 1404, 1344, 1085, 1006, 839, 754, 692, 636 cm^{-1}; ^1H NMR ($CDC1_3$) δ 4.74 (4H, s, C(2) and C(6) methylenes), 4.09 (6H, s, N(1) and N(7) methyls); MS (EI mode) *m/z* 312 (M^+), 314 (M + 2), 277 ($M^+ - Cl$). Anal. ($C_{12}H_{10}Cl_2N_4O_2 \cdot 0.25H_2O$) C, H; N: calcd, 17.64; found, 17.18. The low nitrogen percent found is due to slow hydrolysis to the alcohol.

12: 2.16 mg (2.3%) yield; mp 198 °C dec; TLC (chloroform/methanol [90:10]) $R_f = 0.45$; IR (KBr pellet) 3447, 3016, 2316, 1978, 1500, 1321, 1082, 1004, 748, 640 cm^{-1}; ^1H NMR δ ($CDC1_3$) δ 4.73 (2H, s, C(2)-methylene), 4.08 and 3.93 (6H, 2s, N(1) and N(7) methyls), 2.51(3H, s, C(6)-methyl); MS (EI mode) *m/z* 312 (M^+), 314 ($M^+ + 2$), 277 ($M^+ - Cl$). Anal. ($C_{12}H_{11}ClN_4O_2$) C, H, N.

13: 3.03 mg (3.16%) yield; mp > 300 °C dec; TLC (chloroform/methanol [90:10]) $R_f = 0.34$; IR (KBr pellet) 2924, 1670, 1643, 1498, 1369, 1315, 1080, 991, 742, 628 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.91 (6H, s, N(1)- and N(7)-methyls), 2.48 (6H, s, C(2)- and C(6)-methyls); MS (EI mode) *m/z* 244 (M^+), 229 ($M^+ -$ methyl), 216 ($M^+ -$ CO). Anal. ($C_{12}H_{12}N_4O_2$).

1-Bromo-2,4-dinitro-5-pyrrolidinobenzene (14). To 4.0 g (0.012mol) of 1,5-dibromo-2,4-dinitrobenzene, suspended in 50 mLof ethanol and cooled in an ice bath, was added 2.2 mL of pyrrolidine and the reaction was stirred for 1 h with continued cooling. The resulting yellow precipitate was filtered off, rinsed with ethanol, and vacuum-dried: 3.32 g (92%) yield; TLC ($CHCl_3$/hexane [80:20]) $R_f = 0.54$; mp 165−171 °C; IR (KBr pellet) 3113, 2876, 1579, 1554, 1510, 1369, 1321, 1273, 1155, 991 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.60 (1 H, s, aromatic proton), 7.18 (1 H, s, aromatic proton), 3.32 (4H, m, pyrrolidine methylenes), 2.06 (4H, m, pyrrolidine methylenes); MS (EI mode) *m/z* 315 and 317 (M^+, ^{79}Br and ^{81}Br). Anal. ($C_{10}H_{10}BrN_3O_4$) C, H, N.

3-Acetoxy-7-bromo-6-nitro-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole (15). A mixture consisting of 906 mg (3 mmol) of **14**, 10 mL of acetic anhydride, and 408 mg of 98% $ZnCl_2$ was heated at 120 °C for 20 h. (Note: Yield of products decreases if anhydrous $ZnCl_2$ is employed.) The reaction mixture was allowed to cool and then poured into 25 mL of H_2O followed by extraction thrice with 25-mL portions of chloroform. The chloroform extracts were dried (Na_2SO_4), filtered, and concentrated to a solid residue. Pure products were obtained by flash chromatography using silica gel with chloroform/hexane [99:1] as the eluent followed by recrystallization from ethyl acetate/hexane: 571.7 mg (56%) yield; mp > 115 °C dec; TLC (chloroform/methanol [90:10]) $R_f = 0.60$; IR (KBr pellet) 1743, 1624, 1575, 1527, 1460, 1433, 1371, 1348, 1232, 1080 cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6) δ 8.40 and 8.22 (2H, 2s, C(5)- and C(8)-aromatic protons), 6.18 (1H, dd, $J = 7.6$ Hz, $J = 4$ Hz, C(3)-proton), 4.34 (2H, m, C(1) methylene), 3.13 and 2.59 (2H, 2m, C(2) methylene), 2.09 (3H, s, acetate methyl); MS (EI mode) *m/z* 339 and 341 (M^+ ^{79}Br and ^{81}Br), 298 and 300 ($M^+ -$ acetyl), 234 and 236 ($M^+ -$ acetyl − nitro). Anal. ($C_{12}H_{10}BrN_3O_4 \cdot 0.4H_2O$) C, H, N.

3-Hydroxy-6-nitro-7-pyrrolidino-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole (16). To 1.0 g (2.9 mmol) of **15** was added 30 mL of pyrrolidine followed by heating at reflux for 12 h. The reaction mixture was concentrated in vacuo and the residue diluted with 90 mL of chloroform. The resulting solution was washed thrice with 30-mL portions of 20% aqueous acetic acid and twice with 30-mL portions of water. The chloroform solution was then dried (Na_2SO_4), filtered, and concentrated to a solid residue. Recrystallization from chloroform/hexane afforded pure product: 730 mg (87%) yield; mp 205−208 °C; TLC (chloroform/methanol [90:10]) $R_f = 0.42$; IR (KBr pellet) 3161, 1645, 1579, 1514, 1450, 1413, 1363, 1319, 1288, 825 cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6) δ 8.00 and 7.02 (2H, 2s, aromatic protons), 6.05 (1H, d, $J = 5.7$ Hz, hydroxyl), 5.15 (1H, m, C(3)-proton), 4.29 and 4.12 (2H, 2m, C(1)-diastereomeric methylene), 3.12 (4H, t, $J = 6.6$ Hz, 2- and 5-pyrrolidine protons), 2.88 and 2.39 (2H, 2m, C(2) diastereomeric methylene), 1.92 (4H, q, $J = 3.3$ Hz, 3- and 4-pyrrolidine protons); MS (EI mode) *m/z* 288 (M^+), 271 ($M^+ -$ OH), 241 ($M^+ - HNO_2$). Anal. ($C_{14}H_{16}N_4O_3 \cdot 0.1H_2O$) C, H, N.

***cis*- and *trans*-3,7-Diacetoxy-3*H*,7*H*-1,2,8,9-tetrahydropyrrolo[1,2-*a*]pyrrolo[1′,2′:1,2]imidazo[4,5-*f*]benzimidazole (17).** To 576 mg (2 mmol) of **16** in 20 mL of acetic anhydride was added 272 mg of $ZnCl_2$ and the reaction was heated at reflux for 5 h. The reaction was concentrated in vacuo to an oil followed by dilution with 100 mL of chloroform. The chloroform solution was extracted twice with water and dried over sodium sulfate. Chromatographic purification was carried out employing a silica gel column utilizing chloroform/methanol [9:1] as the eluent. The cis/trans mixture eluted off just after a fast moving orange band corresponding to acetylated **16**. The fractions corresponding to the cis/trans mixture were combined and concentrated to a solid residue: 165 mg (23%) yield; mp >300 °C dec; TLC (chloroform/methanol [90:10]) $R_f = 0.36$; IR (KBr pellet) 1743, 1639, 1548, 1437, 1373, 1300, 1232, 1006, 1232, 1080 cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6) δ 7.92 and 7.70 (2H, 2s, aromatic protons), 6.17 (2H, dd, $J = 7.2$ Hz, $J = 3.9$ Hz), 4.23 (4H, m, C(1) and C(9) diastereomeric methylenes), 3.15 and 2.58 (4H, 2m, C(2) and C(8) diastereomeric methylenes), 2.09 (6H, s, acetate methyl); MS (EI mode) *m/z* 354 (M^+) 311 ($M^+ -$ acetyl), 295 ($M^+ -$ acetate), 251 ($M^+ -$ acetic acid & acetyl). Anal. ($C_{18}H_{18}N_4O_4$) C, H, N.

***cis*- and *trans*-3,7-Diacetoxy-5(and 10)-nitro-3*H*,7*H*-1,2,8,9-tetrahydropyrrolo[1,2-*a*]pyrrolo[1′,2′:1,2]imidazo[4,5-*f*]benzimidazole (18)**. To a solution consisting of 81 mg (0.25 mmol) of **17** in 25 mL of acetonitrile was added 8 equiv of (85%) nitronium tetrafloroborate and the resulting reaction mixture was stirred for 16 h at room temperature. The solvent was removed in vacuo and the residue was dissolved in 20 mL of H_2O. The pH was adjusted to 6.0−6.5 with aqueous sodium bicarbonate and the solution was then extracted thrice with 15-mL portions of chloroform. The chloroform extracts were dried over sodium sulfate, filtered, and concentrated to a solid residue. The product was purified using silica gel preparative TLC plates employing chloroform/

methanol [90:10] as the developing solvent: 27 mg (30%) combined yield of two products; TLC (chloroform/methanol [90:10]) $R_f = 0.57$ and 0.26; ^{1}H NMR of high R_f product (DMSO-d_6) δ 8.37 (1H, s, aromatic), 6.20 (2H, dd, $J = 2.7$ Hz, $J = 5.8$ Hz, C(3) and C(7) methines), 4.55 (4H, m, C(1)- and C(9)-methylenes), 3.07 and 2.55 (4H, 2m, C(2) and C(8) methylenes), 2.11 (6H, s, acetate methyls); ^{1}H NMR of low R_f product (DMSO-d_6) δ 8.19 (1H, s, aromatic), 6.21 (2H, m, C(3) and C(7) methine), 4.39 and 4.28 (4H, 2m, C(1) and C(9) methylene), 3.21 and 2.65 (4H, 2m, C(2) and C(8) methylenes), 2.11 (6H, s, acetate methyls).

***cis*- and *trans*-3,7-Diacetoxy-3*H*,7*H*-1,2,8,9-tetrahydropyrrolo[1,2-*a*]pyrrolo[1′,2′:1,2]imidazo[4,5-*f*]benzimidazole-5,10-dione (19a,b)** was synthesized from crude **18** by the following two-step procedure. A solution consisting of 138 mg (0.34 mmol) of **18**, 75 mL of methanol, and 32 mg of 5% palladium on activated carbon was shaken under 50 psi for 4 h. The reaction mixture was then filtered through a Celite cake to remove the catalyst and concentrated to an off-white solid, which was dissolved in 25 mL of H_2O containing 200 mg of monobasic potassium phosphate. To this solution was added a second solution consisting of 50 mL of H_2O, 1 g of monobasic potassium phosphate, and 726 mg of Fremy's salt. The resulting mixture was stirred at room temperature. until it turned from purple to orange. The solution was then extracted thrice with 75-mL portions of chloroform. The extracts were dried over sodium sulfate and concentrated to a residue, which was purified by flash chromatography on silica gel employing chloroform/methanol (95:5) as the eluent. The major yellow band afforded 86 mg of *cis*/*trans*-**19** and the minor slower moving yellow band afforded **20**. Separation of the cis and trans forms of **19** was accomplished by using a 34-cm silica gel column employing chloroform/methanol (98:2) as the eluent. The separated products were recrystallized from ethyl acetate.

***trans*-19:** 23.7 mg (18%) yield; TLC (chloroform/methanol [90:10]) $R_f = 0.52$; mp >260 °C; IR (KBr pellet) 1743, 1672, 1520, 1498, 1437, 1375, 1226, 1089, 1043 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.09 (2H, dd, $J = 3.3$ Hz, $J = 7.78$ Hz, C(3) and C(7) protons), 4.36 (4H, m, C4 and C(9) protons), 3.17 and 2.66 (4H, 2m, C(2) and C(8) protons), 2.12 (6H, s, acetate methyl); MS (EI mode) *m*/*z* 384 (M^+), 341 (M^+ − acetyl), 325, 281. Anal. ($C_{18}H_{16}N_4O_6$) C, H, N.

***cis*-19:** 27 mg (21%) yield; TLC (chloroform/methanol [90:10]) $R_f = 0.46$; mp 239−240 °C; IR (KBr pellet) 1739, 1670, 1521, 1498, 1437, 1373, 1228, 1080, 1047 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.08 (2H, dd, $J = 2.7$ Hz, $J = 7.8$ Hz, C(3) and C(7) methine), 4.36 (4H, m, C(1) and C(9) methylenes), 3.20 and 2.65 (4H, 2m, C(2) and C(8) methylenes), 2.116 (6H, s, acetate methyls), MS (EI mode) *m*/*z* 384 (M^+), 341 (M^+ − acetyl), 325, 281. Anal. ($C_{18}H_{16}N_4O_6$) C, H, N.

20: 11 mg (10%) yield; TLC (chloroform/methanol [90:10]) $R_f = 0.31$; mp >240 °C; IR (KBr pellet) 1736, 1685, 1670, 1521, 1498, 1224, 1041 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.09 (1H, dd, $J = 3.3$ Hz, $J = 8.5$ Hz, C(3) methine), 4.34 (4H, m, C(1) and C(9) methylenes), 3.18−2.64 (6H, 3m, C(2), C(7) and C(8) methylenes), 2.11 (3H, s, acetate methyl); MS (EI mode) *m*/*z* 326 (M^+), 383 (M^+ − acetyl). Anal. ($C_{16}H_{14}N_4O_4$) C, H, N.

(*R*)-1-Bromo-5-(3-carboxy-3-hydroxypropylamino)-2,4-dinitrobenzene (21). To a solution consisting of 375 mg (3.15 mmol) of (*R*)-β-hydroxy-4-aminobutyric acid and 250 mg of sodium bicarbonate in 75 mL of H_2O was added a solution of 500 mg (1.5 mmol) of 1,5-dibromo-2,4-dinitrobenzene dissolved in 50 mL of acetone. The reaction mixture was heated at reflux for 22 h and then cooled to room temperature. The pH of the reaction mixture was adjusted to 8 with sodium bicarbonate and then extracted twice with 20-mL portions of chloroform to remove unreacted 1,5-dibromo-2,4-dinitrobenzene. The aqueous layer was acidified with 4 N hydrochloric acid and then extracted five times with 50-mL portions of chloroform. The chloroform extracts were dried over sodium sulfate, filtered, and concentrated to a solid residue, which was recrystallized from chloroform/hexane: 347 mg (63%) yield; TLC (chloroform/methanol [80:20]) $R_f = 0.63$; mp 140−160 °C; IR (KBr pellet) 3497, 3317, 1737, 1618, 1568, 1539, 1417, 1319, 1267, 1244, 1184, 1116 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 12.56 (1H, bs, acid proton), 8.94 (1H, t, $J = 5.4$ Hz, amine proton), 8.82 (1H, s, aromatic proton), 7.49 (1H, s, aromatic proton), 5.61 (1H, bs, hydroxy proton), 4.10 (1H, dd, $J = 3.3$ Hz, $J = 7.8$ Hz, methine), 3.61 (2H, m, methylene), 2.00 & 1.88 (2H, 2m, methylene); MS (EI mode) *m*/*z* 363 and 365 (M^+, ^{79}Br & ^{81}Br), 274 (M^+ − $CH_2CHOHCOOH$). Anal. ($C_{10}H_{10}BrN_3O_7$) H, N; C: calcd, 32.98; found, 33.74. This somewhat impure intermediate was converted to the pure intermedeiate **22**.

(*R*,*R*)-1,5-Bis(3-carboxy-3-hydroxypropylamino)-2,4-dinitrobenzene (22). To a solution consisting of 250 mg (0.68) mmol) of **21** and 75 mL of H_2O were added 200 mg of sodium bicarbonate and 165 mg (1.38 mmol) of (*R*)-4-amino-β-hydroxybutyric acid. The reaction mixture was refluxed for 20 h and then allowed to cool to room temperature. The solution was then concentrated and placed on a 100-g phenyl Baker Bond reverse-phase column and the product was eluted with H_2O. The product fractions were combined and then acidified with concentrated hydrochloric acid resulting in crystallization of the product: 220 mg (80.6%) yield; TLC (butanol/acetic acid/water [5:2:3]) $R_f = 0.43$; mp 149−157 °C; IR (KBr pellet) 3333, 1761, 1720, 1626, 1577, 1404, 1329, 1302, 1219, 1107 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.97 and 5.94 (2H, 2s, aromatic protons), 8.63 (2H, t, $J = 4.8$ Hz, amine protons), 4.12 (2H, dd, $J = 8.1$ Hz, $J = 4.0$ Hz, methines), 3.49, 2.06, &1.93 (8H, 3m, methylenes). Anal. ($C_{14}H_{18}N_4O_{10}\cdot 0.25H_2O$), C, H, N.

(*R*,*R*)-3,7-Diacetoxy-3*H*,7*H*-1,2,8,9-tetrahydropyrrolo-[1,2-*a*]pyrrolo[1′,2′:1,2]imidazo[4,5-*f*]benzimidazole (*trans*-17) was synthesized by the following sequence. A suspension consisting of 200 mg (0.49) mmol) of **22** in 75 mL of 4 N HCl and 40 mg of 5% Pd on activated carbon was shaken under 50 psi H_2 for 4 h. The solution was then filtered through Celite into a round-bottom flask and heated at 90 °C for 15 h. The reaction mixture was then allowed to cool and the solvent was removed under vacuum. The resulting solid was redissolved in a minimum volume of water and the pH adjusted to 5.5−6.0 with aqueous sodium hydroxide. The crystallized product was filtered and dried under vacuum: 110 mg (82%) yield; mp >260 °C; TLC (butanol/acetic acid/water [5:2:3]) $R_f = 0.14$; IR (KBr pellet) 3138, 2860, 1543, 1498, 1438, 1327, 1302, 1120, 1105, 956 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.76 and 7.50 (2H, 2s, aromatic protons), 5.84 (2H, d, $J = 6$ Hz, C(3) and C(7) hydroxy), 5.10 (2H, m, C(3) and C(7) methines), 4.22−4.06 (4H, m, C(1) and C(9) methylenes), 2.95 and 2.42 (4H, 2m, C(2) and C(8) methylenes); MS (EI mode) *m*/*z* 270 (M^+) 253 (M^+ − OH).

To a solution consisting of 48 mg (0.17 mmol) of the above product in 15 mL of dry methylene chloride was added 100 μL of acetyl chloride followed by 400 μL of dry pyridine. The reaction mixture was stirred for 4 h at room temperature and then diluted with 10 mL of chloroform. The resulting mixture was extracted five times with 25-mL portions of water and the chloroform layer was dried over sodium sulfate, filtered, and concentrated to a residue which was recrystallized from ethyl acetate/hexane: 38 mg (62%) yield; ^{1}H NMR (DMSO-d_6) δ 7.89 and 7.63 (2H, 2d, $J = 1$ Hz, aromatic), 6.13 (2H, dd, $J = 3.3$ Hz, $J = 7.8$ Hz, C(3) and C(7) protons), 4.24 (4H, m, C(1) and C(9) methylene), 3.12 and 2.54 (4H, 2m, C(2) and C(8) methylenes), 2.06 (6H, s, acetate methyls); MS (EI mode) *m*/*z* 354 (M^+).

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