Synthesis and Biological Evaluation of Imidazoquinoxalinones, Imidazole Analogues of Pyrroloiminoquinone Marine Natural Products

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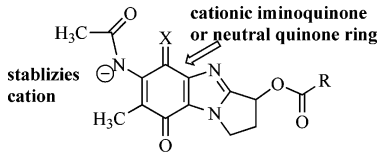
This report describes the synthesis and biological activity of imidazoquinoxalines, benzimidazole-based analogues of indole-based pyrroloiminoquinone marine natural products. Our analogues consist of series **1**, which possesses the ethylene tether and extended amidine feature found in the pyrroloiminoquinone natural products, and series **2**, which also has the ethylene tether but with an electrostatically stabilized iminoquinone rather than a resonance stabilized iminoquinone (i.e., extended amidine). The biological properties of series **1** analogues, bearing electron-rich side chain rings (indole and phenol), display cytostatic and cytotoxic properties similar to that of the pyrroloiminoquinone natural products. In contrast, COMPARE analysis suggests that analogues bearing benzyl and phenethyl side chains possess a different cytotoxicity mechanism. Hollow fiber assays of analogs of **1** indicate promising antitumor activity and acceptable levels of toxicity. One analogue of **2** is active only against breast cancer cell lines, but the cellular target is as yet unknown.

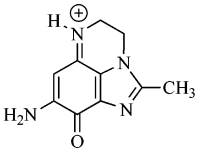
Introduction

The interest in biologically active pyrroloiminoquinone-based alkaloid pigments from marine sponges, chiefly the *Latrunculia* and *Zyzzya* species, began in the early 1990s[1−3] and has continued to the present day.[4−9] Various cellular targets may be responsible for the biological properties of these alkaloids, including enzymes involved in DNA relaxation, topoisomerase I and II, and signal transduction receptors. For example, both catalytic inhibition and poisoning (stabilization of the cleavable complex) of topoisomerase II was observed for makaluvamine derivatives.[10] The makaluvamine structure, shown in Chart 1, possesses the pyrroloiminoquinone substructure with the vinylogous amidine functional group. Vinylogous amidine resonance is responsible for the red to blue pigmentation as well as the iminoquinone hydrolytic stability.

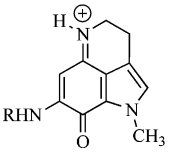
At about the time the pyrroloiminoquinones were discovered, our laboratory designed hydrolytically stable iminoquinones based on the 6-acetamidopyrrolo[1,2-*a*]benzimidazoles (APBI[a]) ring system, Chart 1.[11−14] Iminoquinone stability arises from electrostatic stabilization by the neighboring acetamido anion[11] rather than by the vinylogous amidine resonance. It is noteworthy that both the APBIs and the pyrroloiminoquinone natural products inhibit topoisomerase II. Perhaps the similar ring sizes (indole versus benzimidazole) as well as the presence of the iminoquinone cation result in similar mechanisms of action. The features of the pyrroloiminoquinone natural products (ethylene tether and extended amidine) and the APBIs (benzimidazole and zwitterionic iminoquinone) were combined to afford the imidazoquinoxalinones **1** and **2** shown in Chart 2. It was anticipated that the imidazoquinoxalines could possess new

Chart 1





Imidazoquinoxalinone



Makaluvamines (pyrroloiminoquinones)

biological and chemical properties: the presence of the purine-like benzimidazole ring may affect signaling enzymes, the ethylene tether may prevent the irreversible iminoquinone hydrolysis observed with the imino-APBIs,[11] and the zwitterionic iminoquinones may be more bio-available than extended amidines.

Recently, we reported the preparation of imidazoquinoxalines **1** and **2** without the amino substituents seen in the pyrroloiminoquinone natural products.[15] These minimally substituted imidazoquinoxalines displayed little activity in the NCI 60-cell-line cancer panel.[16] The present study describes the synthesis of substituted analogues of **1** and **2** and the evaluation of their biological properties. Two of the anticipated biological and chemical properties were realized. Derivatives of **1** and **2** show greater hydrolytic stability compared to the APBIs. Although derivatives of **2** show little biological activity, select derivatives

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[a] Abbreviations: BAD, Bcl-2/Bcl-X_L-associated death promotor; Bcl, B cell lymphoma/leukemia; APBI, 6-acetamidopyrrolo[1,2-*a*]benzimidazoles; HBr, hydrobromic acid; HCl, hydrochloric acid; DMSO, dimethyl sulfoxide; GI_{50}, growth inhibition 50%; TGI, total growth inhibition; LC_{50}, lethal concentration 50%; akt/PKB, protein kinase B; RSK, ribosomal S6 kinase; IP, intraperitonial; SC, subcutaneaous; MALDI, matrix assisted laser desorption/ionization; TLC, thin layer chromatography.

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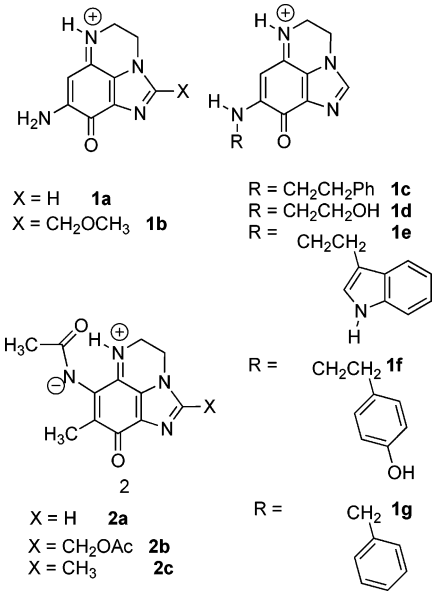
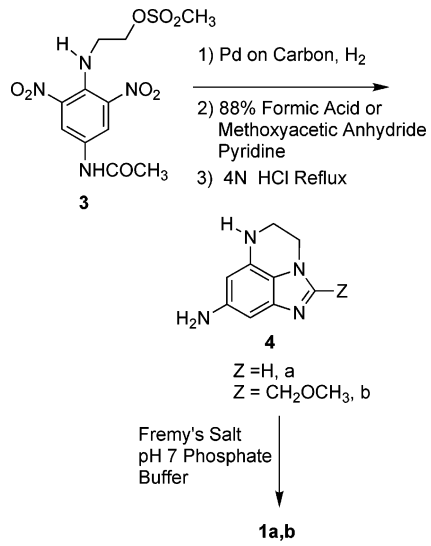
Chart 2



Scheme 1



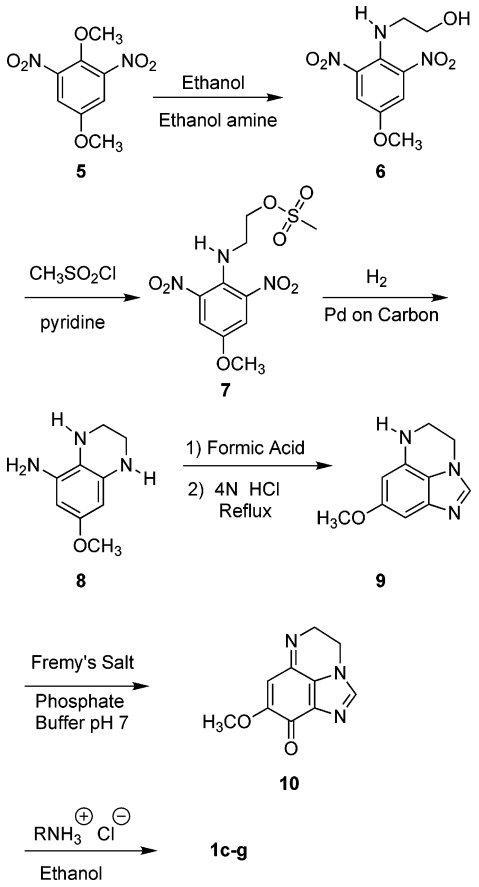
of **1** show inhibition of topoisomerase II-mediated relaxation of supercoiled DNA and may promote apoptosis.

Results and Discussion

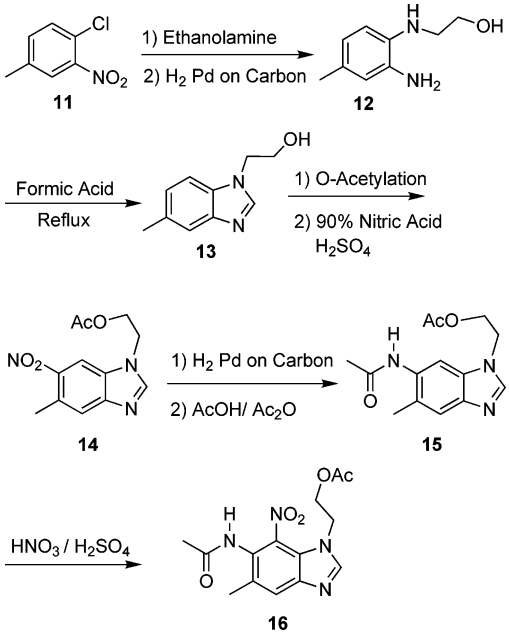
Synthesis. The preparation of compounds **1a**,**b** started with the functionalized benzene compound **3** that was previously reported, Scheme 1.[15] Catalytic reduction of the nitro groups of **3** was followed by peracylation of the resulting amines with either formic acid or methoxyacetic anhydride. Treatment of the acylated product with 4 N HCl resulted in imidazo ring closure and deacylation to afford intermediate **4**. Oxidation of **4** with Fremy's salt in pH 7 phosphate buffer[11] afforded the extended amidine products **1a**,**b**.[15] The purpose of preparing **1a**,**b** was to determine the biological activity of imidazoquinoxalines devoid of substituents at the 8-amino center.

The preparation of substituted 8-amino derivatives **1c**−**g** was carried out by methanol displacement from the methoxy derivative **10** using diverse alkylamine hydrochlorides, Scheme 2. Similarly, White et al. had prepared diverse makaluvamine derivatives from methoxy pyrroloquinolines.[17] The preparation of **10** was carried out starting with the known compound **5**[18] in seven steps, Scheme 2. These synthetic steps are analogous to

Scheme 2



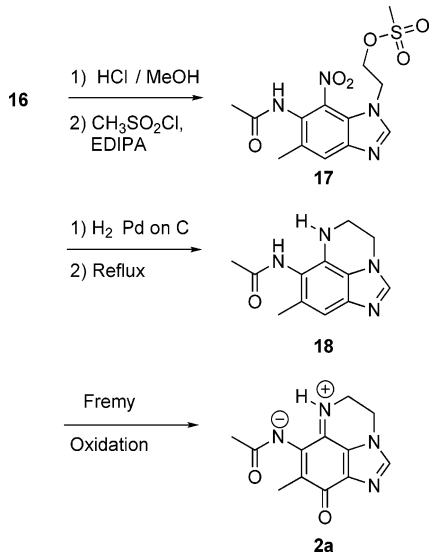
Scheme 3



those previously reported for the preparation of simple imidazoquinoxalines.[15]

The preparation of **2a** began by nucleophilic aromatic substitution of 4-chloro-3-nitrotoluene (**11**) with ethanolamine, Scheme 3. Benzimidazole ring formation was carried out via a Phillips cyclization reaction[19] of the reduction product **12**, using formic acid in refluxing 4 N HCl, to give **13** in 82% yield. The alcohol group of **13** was protected by acetylation with acetic anhydride followed by nitration to obtain the key benzimidazole

Scheme 4



intermediate **14**, with an overall yield of 16%. Catalytic reduction of **14** followed by amide formation with acetic anhydride gave **15**, which was selectively nitrated to afford the 7-nitro isomer **16**.

Deprotection of **16** via acid-catalyzed *trans*-esterification gave the alcohol as an HCl salt that was immediately used in the next step, Scheme 4. Methanesulfonation of the alcohol intermediate using diisopropyl ethylamine and methane sulfonyl chloride gave the sulfonate ester **17**. Catalytic reduction of **17** followed by stirring in refluxing methanol afforded the ring-closed quinoxaline intermediate that was Fremy oxidized[20] to yield **2a**.

Peracetylation of **12** with methoxyacetyl chloride in dry dichloromethane, containing diisopropyl ethylamine, afforded **19** in 98% yield, Scheme 5. Nitration of **19** with fuming nitric acid gave **20** in 55% yield, which was treated with 48% HBr. The HBr treatment causes deacetylation, imidazo ring closure, and *O*-demethylation, resulting in formation of benzimidazole **21** in 74% yield. Catalytic reduction of **21** with 5% palladium on carbon in methanol afforded the aniline intermediate that was peracetylated with acetic anhydride in acetic acid to give **22** in 70% yield from **21**.

Selective nitration of **22** with a solution of 90% HNO_3 and H_2SO_4 gave the 7-nitro isomer **23** as the sole product. Alcohol deacetylation via the acid-catalyzed *trans*-esterification of **23** with concentrated HCl in methanol followed by sulfonation with methanesulfonyl chloride resulted in an unexpected product containing the 2-chloromethyl substituent (**24**) instead of the expected 2-sulfonoxymethyl substituent. The 2-sulfonoxymethyl group likely formed, but rapidly reacted with chloride due to the benzylic character of this center. The reduction and cyclization of this intermediate proved futile due to hydrogenolysis of the chloro during catalytic reduction to afford the 2-methyl substituent. To circumvent this problem, an acetate group was substituted via an S_N2 reaction with sodium acetate in dimethyl sulfoxide (DMSO) to yield **25** as pale yellow crystals in 75% yield. This substituent maintained the function of a leaving group but was more robust than the chloro group and was not susceptible to hydrogenolysis. Compound **2b** was obtained by the catalytic reduction of **25** followed by refluxing in methanol to give the ring-closed quinoxaline intermediate, which was Fremy oxidized[20] in phosphate buffer (pH = 7), with an overall yield from **25** of 66% as yellow crystals.

Scheme 5

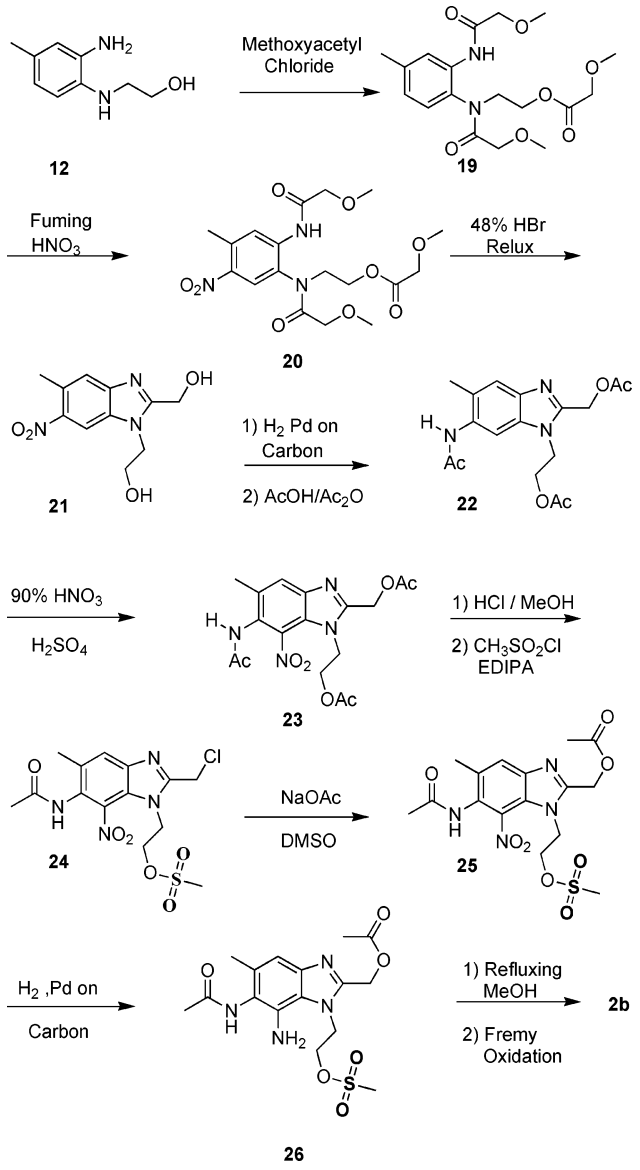


Table 1. In Vitro Assay Results of Imidazoquinoxalinones **1**

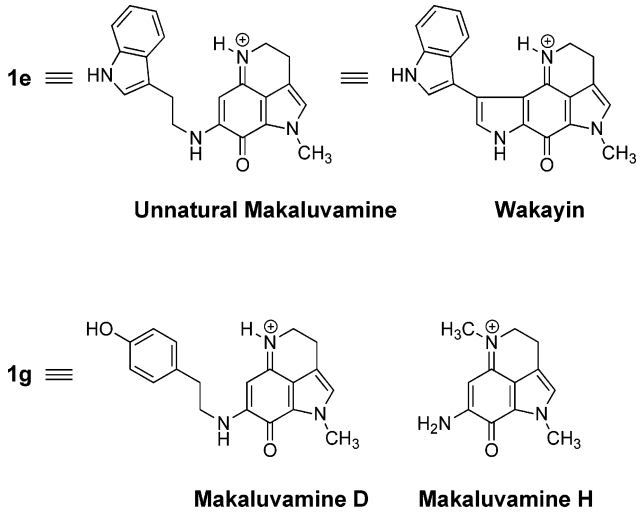
cmpd	mid-range Log GI_{50} (range)	mid-range Log TGI and range	mid-range Log LC_{50} and range
1a	−5.34 (1.86)	−4.91 (1.52)	−4.45 (1.25)
1c	**−6.78 (2.62)**	−5.97 (4.0)	−5.26 (4.0)
1d	−5.98 (3.38)	−5.42 (3.78)	−4.89 (3.61)
	−5.96 (3.28)	−5.37 (4.0)	−4.7 (3.42)
1e	**−6.72 (2.37)**	**−6.13 (2.57)**	**−5.35 (3.32)**
	−6.63 (2.34)	**−6.06 (3.64)**	**−5.17 (3.3)**
1f	**−5.60 (3.76)**	**−4.85 (3.47)**	**−4.39 (3.18)**
1g	−6.21 (3.02)	−5.55 (3.65)	−4.83 (3.02)
	−6.29 (3.2)	−5.69 (3.52)	−5.18 (4.0)

Cell Line Assays and COMPARE Analysis. The mean cytostatic and cytotoxic parameters of **1** and **2**, measured in a 60 human cancer cell line panel, are provided in Tables 1 and 2, respectively. The cytostatic parameters include GI_{50} and TGI, which are the concentrations of drug required for 50% growth inhibition and total growth inhibition, respectively. The cytotoxic parameter is the LC_{50}, which is the concentration required for 50% cell kill. These data were obtained under the In Vitro Cell Line Screening Project at the National Cancer Institute.[16,21,22] Also provided in Tables 1 and 2 are range values, which represent the log of the maximum concentration difference

Table 2. In Vitro Assay Results of Imidazoquinoxalinones **2**

cmpd	mid-range GI_{50} (range)	mid-range TGI and range	mid-range LC_{50} and range
2a	−4.33 (0.8)	inactive	inactive
2b	inactive	inactive	inactive
2c	**−4.28 (4.00)**	−4.09(1.48)	inactive
X = CH_3			

Chart 3



between the least-sensitive and the most-sensitive cell line. This range parameter has been used to gain insights into the selectivity of antitumor agents, because it provides a measure of histological specificity.[23]

The criteria used for judging the activity of compounds include: log mean values <-4 with log range values of >2, and with high selectivity for 10 or more cell lines and selectivity for one or more histological cancers. Compounds exhibiting the highest activity according to these criteria are highlighted in bold in Tables 1 and 2. Analogues of **1** bearing a two-carbon tether and an electron-rich aromatic ring (**1e** and **1f**) are the most active, with histological selectivity exhibited toward melanoma and colon cancer. Both of these compounds resemble the natural products wakayin and makaluvamine, respectively. Compound **1e** resembles a wakayin-like synthetic makaluvamine reported in the literature,[24] Chart 3. Compounds **1c** and **1g** are also potent compounds, but exhibit distinctly different histological specificity than **1e** or **1f**, Figure 1. The *N*-unsubstituted version **1a** has little histologic specificity, while intermediate **10** in Scheme 2 is devoid of cytotoxic and cytostatic activity. Thus, the presence of an aromatic substituent at the 8-position is required for cytostatic/cytotoxic activity by **1**. The presence of a 2-methoxymethyl group in **1b** resulted in cytotoxic activity in two cell lines even though only an 8-amino is present, Figure 2. The importance of the 2-substituent on the cytostatic/cytotoxic activity by **1** will require additional studies.

The only analogue of **2** with activity is **2c** (X = CH_3, Chart 2), which exhibited cytostatic activity exclusively against breast cancer cell lines.[15] Observed log GI_{50} values in breast cancer cell lines include: BT-549, <-8; MDA-MB-231/ATCC, −4.89; MDA-MB-435, −4.72; and MDA-N, −4.73. These cell lines are also prominent in the TGI panel. The data in Table 2 shows that increasing the size of X beyond CH_3, **2c**, causes complete loss of activity, while **2a** (X = H) possesses only modest activity. These findings do not reflect the structure−activity relationship of the APBIs, which showed a tolerance of bulky leaving groups at the 3-position of the pyrrolobenzimidazole ring.[12]

COMPARE analysis[16,25] provided insights into what might be the molecular target of active analogues exhibiting high histologic selectivity (compounds in bold font in Tables 1 and 2). The mean graphs of compounds with log range values of >2 and cell line selectivity would have distinctive patterns amenable to a meaningful COMPARE analysis. In contrast, mean graphs with low log range values are essentially flat and will correlate with other flat mean graphs, providing little meaningful target information. The COMPARE analysis correlates mean graph data with known molecular target levels in cell lines and thereby generates hypotheses concerning the agent's mechanism of action. A molecular target is a protein or enzyme that has been measured in the National Cancer Institute's panel of 60 human tumor cell lines. The levels of over 1000 biologically relevant molecular targets have been determined in these tumor cell lines from measurements of mRNA and enzyme activity levels.[26]

COMPARE correlations indicated that the cytostatic and cytotoxic parameters of compounds **1** and **2** represent three mechanistically distinct classes. The GI_{50}, TGI, and LC_{50} parameters of compounds **1e** and **1f** correlate (0.5−0.6 correlation coefficient) with the same molecular target: the ABC (ATP binding cassette) family of membrane transporters.[27] These transporters are important in the transport of drugs into or out of cells and could likely play a role in the transport of **1e** and **1f** into cancer cells. COMPARE correlations using **1e** as a seed against the natural products database revealed a good correlation with makaluvamine H (Chart 3), giving correlation coefficients of 0.748 (GI_{50}) and 0.670 (TGI), <0.69 in LC_{50}. The topoisomerase II poisoning exhibited by **1e** is consistent with a cytotoxic mechanism similar to that of the makaluvamines, see the next section.

In contrast, COMPARE analysis of **1c** revealed a dependence on levels of the proteins BAD and cytochrome C without any correlations with makaluvamine-type compounds. The molecular target correlations suggest that **1c** may be promoting apoptosis by a BAD phosphorylation by either akt/PKB or p90RSK. Compound **1g** may promote apoptosis, with its cytostatic properties (GI_{50}) dependent on the levels ErbB proteins.[28−30] At this point, we can only assert that compounds **1c**,**g** have different mean graphs than compounds **1e**,**f**, suggesting different mechanisms of cytotoxicity. The possible role of **1c**,**g** in promoting apoptosis will be addressed in another study.

Human Topoisomerase II Cleavage Assays. Makaluvamines are reported to be topoisomerase II poisons with potencies similar to that of the clinically used drug etoposide. To determine if **1e** shares this feature, relaxation assays were carried out with recombinant human topoisomerase II (p170) in the presence of pRYG supercoiled DNA using etoposide as a positive control. We assayed relaxation reactions on agarose gels in the presence of ethidium bromide, Figure 3. The ethidium bromide-containing gels readily detect the presence of linear DNA, but will 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, that is, the agent stabilizes the cleavable complex. Inspection of the agarose gel shown in Figure 3 confirms that **1e**, along with the etoposide control, are topoisomerase II poisons. However, comparison of the mean graph of **1e** with that of etoposide revealed no similarities. Etoposide displays potent cytostatic/cytotoxic activity ($<10^{-6}$ M) against all histological cancer types.

Hollow Fiber Assays. To provide insights into drug toxicity and antitumor efficacy, the National Cancer Institute determined both acute toxicity of **1g** and its activity in the hollow fiber

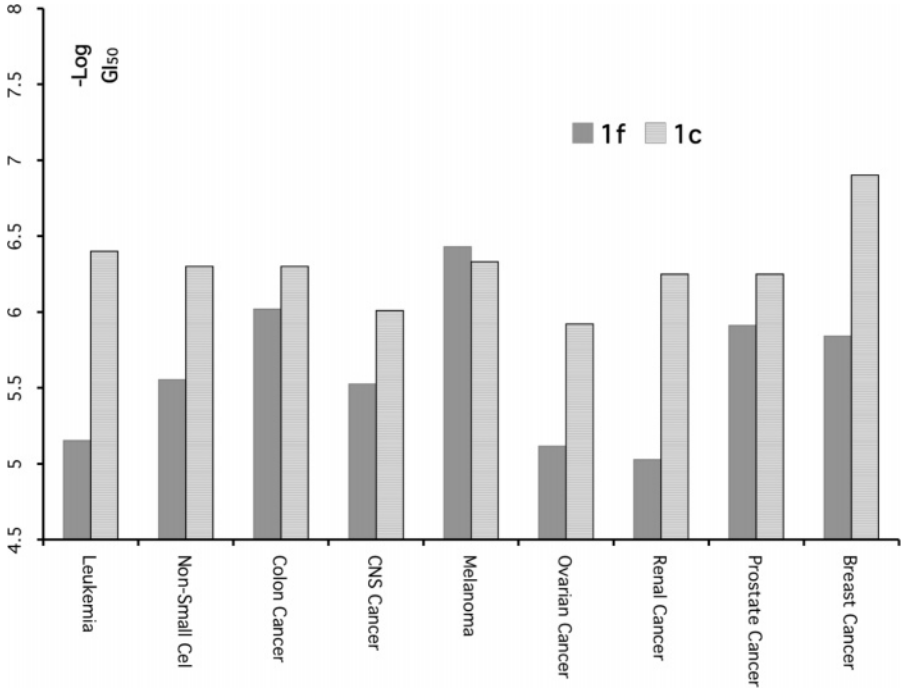


Figure 1. Bar graphs of the average $-\log GI_{50}$ values for **1f** and **1c** against histological cancer types. The different bar graph patterns are due to the cytostatic mechanisms for **1f** (resembles pyrroloiminoquinone natural products) and **1c** (a promoter of apoptosis).

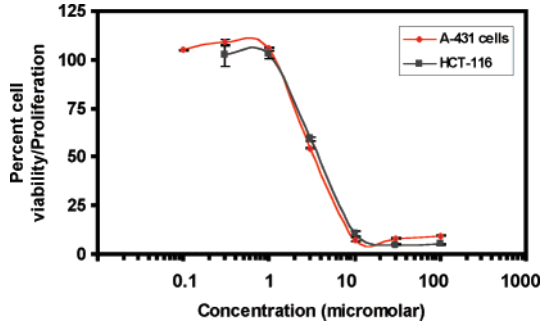


Figure 2. Plots of percent cell viability/proliferation versus concentration of **1b** against human colon tumor HCT-116 cells and human epidermoid carcinoma A-431 cells. The IC_{50} for both tumor cell lines is 3 μM.

assay. The maximum tolerated dose (MTD) for **1g** is 12.5 mg/kg (see Experimental Section for details). The hollow fiber assays[31,32] are carried out as previously described. The candidate drug is injected intraperitoneal (IP) at 3.1 mg/kg/dose, according to the schedule QD × 4, for 3 days. The score is broken down into an IP and a subcutaneaous (SC) score that is based on the percent of cancer left in the fiber (<50% = 2 and >50% = 0) The highest total score (IP + SC) is 96. The typical antitumor agent only has a hollow fiber score of ~5, while the highest score being 64 for the natural product cyclin-dependent kinase inhibitor flavopiridol.[33] A good SC score (≥8) indicates that the drug is able to get to the tumor site (subcutaneous) from a distant site (intraperitoneal) of injection. Compound **1g** had an IP score of 4/48 and an SC score of 6/48. These results indicate that compound **1g** has minimal antitumor activity in this assay. This find has prompted the synthesis of diverse analogues of series **1** to optimize activity.

Conclusions. The synthesis of imidazoquinoxalines, benzimidazole-based analogues of the indole-based pyrroloiminoquinone marine natural products, was successfully carried out employing multistep syntheses. Series **1** possesses the ethylene tether and extended amidine features found in pyrroloiminoquinone natural products. The incorporation of various amino side chains into **1** was readily carried out from the reaction of intermediate **10** and diverse amines, Scheme 2. Currently, more diverse libraries of series **1** are being prepared using this methodology to optimize antitumor activity. In contrast, the structural diversification of series **2** was made early in the synthesis (Schemes 3−5), resulting in only a few analogues of **2**. Nevertheless, we were able to evaluate the biological properties of these novel compounds.

Series **1** structurally resembles pyrroloiminoquinone natural products, specifically the makaluvamines. COMPARE analysis verified that **1e**,**g** possess similar mean graph profiles to those of this class of natural products. Visual comparison of the published mean graph of makaluvamine H[34] with that of **1g** supports the COMPARE results. However, the mean graph data indicate that **1e** and **1g** are less potent than makaluvanine H. The major reason is likely the presence of the electron-deficient benzimidazole ring (compared to the indole ring of the natural products), resulting in a protonated amidine pK_a of 8.2. Thus, a substantial fraction of **1e**,**g** will be neutral at physiological pH, while cationic makaluvamines are likely the active form given the activity of makaluvamine H. The presence of neutral imidazoquinoxalines at physiological pH may be responsible for the change in molecular target observed with some analogues of series **1**. Our COMPARE results suggest that Compound **1c** has a different mechanism of action than **1e**,**g** based on a different spectrum of cytostatic/cytotoxic activity. The pharmacological basis for this difference will be the subject of additional study.

Experimental Section

Elemental analyses (CHN) were obtained for intermediates and final products. Melting points and decomposition points were determined with a Mel-Temp apparatus All TLCs were run with VWR silica gel, 60 F_{254} plates, employing various solvents and a fluorescent indicator for visualization. IR spectra were taken as KBr pellets using a Nicolet MX-1 FTIR spectrophotometer; the strongest IR absorbances are reported. ^{1}H NMR spectra were obtained with

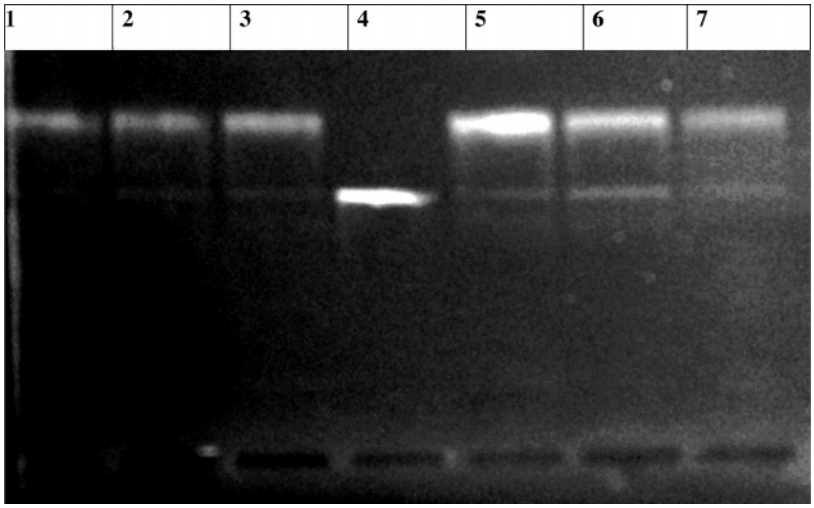


Figure 3. Agarose gel of topoisomerase IIa catalyzed relaxations of pRYG supercoiled DNA (form I) run in the absence of ethidium bromide. The control lanes include relaxation reaction with pRYG supercoiled DNA in lane 1, pRYG supercoiled DNA only in lane 2, relaxation reactions carried out with DMSO in lane 3, linear DNA standard in lane 4, relaxation reactions carried out in the presence of etoposide in lane 5, and relaxation reactions carried out in the presence of 0.05 and 0.10 mM of **1e** in lanes 6 and 7.

a 300 or 500 MHz spectrometer. All NMR chemical shifts (δ) are reported relative to tetramethylsilane (TMS). Uncorrected melting points were determined with an electrothermal Mel-Temp apparatus. MS measurements were carried out with a high-resolution electron impact mass spectrometer at the University of Nebraska, Center for Mass Spectrometry or a Voyager DE STR MALDI-TOF mass spectrometer.

Compounds in series **1** and **2** were screened in the National Cancer Institute's 60-cell-line screen.[16,35] Mean graph results were then analyzed with COMPARE online (http://itbwork.nci.nih.gov/PublicServer/jsp/Form_Upload.jsp).[16,25]

8-Amino-5,6-dihydro-4*H*-imidazo[1,5,4-*de*]quinoxaline (4a). A solution consisting of 200 mg (0.64 mmol) of **3** in 50 mL of methanol was hydrogenated under 50 psi of H_2 in the presence of 100 mg of 5% Pd on carbon at room temperature for 24 h. The catalyst was filtered off using celite filter aid, and the filtrate was concentrated in vacuo to afford a dark oil. To this oil was added 3 mL of 88% formic acid, and the resulting mixture was refluxed for 45 min. The solution was then concentrated in vacuo to an oil, and 3 mL of 48% HBr was added followed by heating at reflux for 40 min. The reaction was then concentrated in vacuo to a solid, which was recrystallized from ethanol/ethyl acetate as the trihydrobromide salt (150 mg, overall 56% yield); mp > 270 °C dec; TLC (*n*-butanol, acetic acid, H_2O [5:2:3]) $R_f = 0.38$; FTIR 3633, 3011, 2864, 2598, 2362, 1666, 1521, 1384, 1315, 1226, 1086, 815, 583 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.37 (1s, 1H, imidazole), 6.84 and 6.51 (2d, $J = 1$ Hz, 2H, aromatic), 4.49 and 3.58 (2t, $J = 5$ Hz, 4H, ethylene); MALDI *m*/*z* 175 (M + 1).

8-Amino-5,6-dihydro-4*H*-2-(methoxymethyl)-imidazo[1,5,4-*de*]quinoxaline (4b). Compound **4b** was prepared by the following three-step procedure. Reductive cyclization of **3** was carried out as described under the preparation of **4a**. To a solution of 100 mg of the reductive cyclization product, dissolved in 5 mL of dry pyridine, was added 200 mg of methoxyacetic anhydride. The reaction mixture was stirred at room temperature for 3 h and then evaporated in vacuo to an oil that was purified by column chromatography using silica gel and acetone/ethyl acetate (1:1) as eluent. Recrystallization of the product, 7-acetamido-1,4,5-tri(methoxyacetamido)-1,2,3,4-tetrahydroquinoxaline, was carried out using chloroform/hexane (40 mg, 45% yield); mp 165−170 °C; TLC (acetone/ethyl acetate [1:1]) $R_f = 0.18$; FTIR 3284, 3005, 2926, 2870, 1672, 1604, 1529, 1467, 1425, 1267, 1118, 750, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.9 (bs, 1H, amide NH), 8.0 and 7.6 (2s, 2H, aromatic), 4.40, 4.35 and 4.03 (3 very broad s, 6H, methylenes), 3.53, 3.47 and 3.37 (3 bs, 9H, methoxy), 2.18 (s, 3H, acetyl). Multiple amide resonance structures cause the ethylene group resonances to become very broad.

A solution consisting of 25 mg of the above product dissolved in a minimal amount of 48% HBr was refluxed for 5 min, then cooled, and concentrated to an oily liquid under vacuum. Adding a small volume of ethanol and then ethyl acetate resulted in crystallization of the trihydrobromide salt of **4b** (15 mg, 56% yield). This compound is somewhat unstable and was used immediately in the Fremy oxidation step.

8-Amino-2-methyl-4,5-dihydroimidazo[1,5,4-*de*]quinoxalin-9-one (1a). To a solution of 60 mg (0.14 mmol) of the trihydrobromide of **2** in 5 mL of 0.2 M pH 7.0 phosphate buffer was added 150 mg of Fremy's salt. The reaction mixture immediately turned dark purple. The reaction mixture was placed on a 100 mL Baker Phenyl reverse phase column and eluted with distilled H_2O to remove salts. The product eluted as a purple band with 0.5 M HCl. Concentration of the fractions containing product and then precipitation of the residue from ethanol/ethyl acetate afforded the dihydrochloride salt of **1a** (15 mg, 41% yield); mp > 233 °C dec; TLC (*n*-butanol, acetic acid, H_2O [5:2:3]) $R_f = 0.23$; FTIR 3420, 3203, 3080, 2976, 1705, 1635, 1541, 1329 cm−1; ^1H NMR (DMSO-d_6) δ 11.49, 9.81, and 8.94 (3s, 3H, NH protons), 8.21 (s, 1H, imidizole), 5.86 (s, 1H, 7-H), 4.4 and 3.9 (2t, $J = 7$ Hz, 4H, ethylene bridge); MALDI *m*/*z* calcd for $C_9H_9N_4O$ (M + 1), 189.07; found, 189.067. Anal. ($C_9H_8N_4O$·2HCl) C, H, N.

8-Amino-2-(methoxymethyl)-4,5-dihydroimidazo[1,5,4-*de*]quinoxalin-9-one (1b). To a mixture of 50 mg (0.11 mmol) of **4b** in 5.0 mL of 0.2 M pH 7.4 phosphate buffer was added 120 mg of Fremy's salt, and the resulting mixture was stirred for 5 min. The reaction mixture was then placed on a 100 mL Baker Phenyl reverse phase column and eluted with distilled H_2O to remove salts and excess Fremy's salt. The product was then eluted as a purple band with 1.0 N HCl. Concentration of the product fractions and then precipitation of the residue from ethanol/ethyl acetate to afford the dihydrochloride salt (23 mg, 68% yield); mp > 220 °C dec; TLC (*n*-butanol, acetic acid, H_2O [5:2:3]) $R_f = 0.27$; FTIR 3510, 3421, 3227, 3053, 3003, 2951, 2843, 1707, 1629, 1537, 1349, 1348, 1253, 1107, 1049, 952, 839, 738 cm^{-1}; ^1H NMR (DMSO-d_6) δ 5.58 (s, 1H, 7-H), 4.58 (s, 2H, methylene), 4.37 and 3.98 (2t, $J = 7$ Hz, 4H, ethylene bridge), 3.30 (s, 3H, methoxy); MALDI *m*/*z* calcd for $C_{11}H_{13}N_4O_2$ (M + 1), 233.104; found, 233.098. Anal. ($C_{11}H_{12}N_4O_2$·2HCl) C, H, N.

4-(2-Hydroxyethylamino)-3,5-dinitroanisole (6). A mixture of 800 mg (3.5 mmol) of **5** and 2 mL of ethanolamine in 20 mL of ethanol was heated until dissolution of solids and then stirred without heating for 24 h. The product slowly crystallized from solution as red needles upon chilling in a freezer (550 mg, 61% yield); mp 101−102 °C; TLC (ethyl acetate) $R_f = 0.62$; FTIR 3506, 3387, 1526, 1444, 1355, 1252, 1225, 1047, 894, 763, 697 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.1 (broad s, 1H, amino), 7.781 (s, 1H), 3.85 (s, 3H), 3.84 (q, J= 4.8 Hz, 2H), 3.12 (q, J = 4.8 Hz, 2H), 1.60 (t, J = 4.8 Hz, 1H, hydroxyl). Anal. ($C_9H_{11}N_3O_6$) C, H, N.

4-(2-Methanesulfonoxyethylamino)-3,5-dinitroanisole (7). To a solution consisting of **6** (3 mmol) in 5 mL of pyridine was added 0.5 mL of methanesulfonyl chloride, and the resulting mixture stirred for 1 h. The reaction mixture was combined with 100 mL of methylene chloride and extracted 2× (100 mL) with H_2O followed by 1× (100 mL) with 1 N HCl. Compound **7** remained in the organic layer after 1 N HCl extraction. The organic layer was dried with Na_2SO_4, concentrated to a small volume, and diluted with hexane, resulting in **7** as orange crystals. The product was filtered off and dried without further purification (94% yield); mp 109−110 °C; TLC (ethyl acetate) $R_f = 0.64$; IR 3302, 1666, 1535, 1350, 1283, 1062, 979, 526 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.78 (s, 2H, aromatic), 4.36 and 3.37 (2t, 4H, J = 4.8 Hz ethylene bridge), 3.85 (s, 3H, methoxy), 3.05 (s, 3H, methylsulfonyl). Anal. ($C_{10}H_{13}N_3O_8S$) C, H, N.

8-Methoxy-4,5-dihydro-imidazo[1,5,4-*de*]quinoxalin-9-one (10). A solution of 100 mg (0.30 mmol) of **7** in 50 mL of methanol was hydrogenated under 50 psi of H_2 in the presence of 80 mg of 5% Pd on carbon for 24 h. The catalyst was filtered off using celite, and the filtrate was concentrated in vacuo to afford **8** as a dark oil. To this oil was added 3 mL of 88% formic acid, and the mixture was refluxed for 1 h. After completion of reflux, the solution was then concentrated in vacuo, 4 mL of 37% HCl was added, and the mixture was refluxed for 2 h. The reaction was then concentrated in vacuo to afford **9** as a dark oil. This oil was then dissolved in 10 mL of 0.2 M pH 7.0 phosphate buffer, to which was added 250 mg of Fremy's salt. After a 5 min reaction time, the aqueous solution was extracted 3× (15 mL) with chloroform. The organic layer was dried with Na_2SO_4 and concentrated in vacuo, resulting in an oil that was precipitated using ethyl acetate/hexanes to afford **10** (6.5 mg, 11% yield after three synthetic steps); mp > 120 °C dec; TLC (10% MeOH in $CHCl_3$) $R_f = 0.43$; FTIR 3369, 3115, 3043, 2939, 2850, 1649, 1539, 1246, 1087, 1028, 839, 661 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.69 (1s, 1H, imidazole), 6.09 (1s, 1H, 7-H), 4.33 and 4.21 (2t, J = 6 Hz, 4H, ethylene), 3.86 (1s, 3H, methoxy); MALDI *m/z* calcd for $C_{10}H_{10}N_3O_2$ (M + 1), 204.08; found, 204.075.

General Procedure for the Synthesis of 8-Substituted 4,5-Dihydroimidazo[1,5,4-*de*]quinoxalin-9-ones (1c−g). To a solution of 10 mg (0.005 mmol) of **10** in 15 mL of methanol was added 1.0 equiv of the amine hydrochloride. The reaction was allowed to stir at room temperature for 24 h. The reaction mixture was then placed on a 100 mL Baker Phenyl reverse phase column and eluded with distilled H_2O to remove salts and unreacted amine. The product was eluted as a purple band with 25% methanol in 0.5 M HCl. Concentration of the fractions containing product and then precipitation of the residue from ethanol/ethyl acetate afforded the dihydrochloride salt of **1c−g**. Physical properties are provided below.

8-(2-Phenethylamino)-4,5-dihydroimidazo[1,5,4-*de*]quinoxalin-9-one (1c). Yield, 6.0 mg (33%); mp > 218 °C dec; TLC (*n*-butanol, acetic acid, H_2O [5:2:3]) $R_f = 0.32$; FTIR 3421, 3024, 2924, 2852, 1699, 1633, 1535, 1350, 1259, 1099, 752, 704 cm^{-1}; ^1H NMR (CD_3OD) δ 8.13 (1s, 1H, 2-H), 7.28 (mult, 5H, aromatic), 5.58 (s, 1H, 7-H), 4.51 and 4.07 (2t, J = 6 Hz, 4H, ethylene bridge), 3.70 and 3.04 (2t, J = 7 Hz, 4H, ethylene side chain); MALDI *m/z* calcd for $C_{17}H_{17}N_4O$ (M + 1), 293.140; found, 293.137. Anal. ($C_{17}H_{16}N_4O$·2HCl) C, H, N.

8-(2′-Hydroxyethylamino)-4,5-dihydroimidazo[1,5,4-*de*]quinoxalin-9-one (1d). Yield, 5.8 mg (34%); mp > 198 °C dec; TLC (*n*-butanol, acetic acid, H_2O [5:2:3]) $R_f = 0.12$; IR 3431, 3213, 3047, 2937, 1699, 1639, 1562, 1541, 1450, 1356, 1126, 1066, 949, 814, 540, 451 cm^{-1}; ^1H NMR (CD_3OD) δ 8.13 (1s, 1H, 2-H), 5.74 (s, 1H, 7-H), 5.52 and 4.09 (t, J = 7 Hz, 4H, ethylene bridgehead), 3.84 and 3.60 (2t, J = 5 Hz, 4H, ethylene side chain); MALDI *m/z* calcd for $C_{11}H_{13}N_4O_2$ (M + 1), 233.104; found, 233.098. Anal. ($C_{11}H_{13}N_4O_2$·2HCl·0.1H_2O) C, H, N.

8-[2-(1*H*-Indol-3′-yl)-ethylamino]-4,5-dihydroimidazo[1,5,4-*de*]quinoxalin-9-one (1e). Yield, 4.7 mg (22%); mp > 205 °C dec; TLC (*n*-butanol, acetic acid, H_2O [5:2:3]) $R_f = 0.48$; IR 3450, 3080, 3024, 2931, 2831, 1631, 1535, 1350, 1236, 1182, 1099, 752, 702, 626 cm^{-1}; ^1H NMR (CD_3OD) δ 7.99 (s, 1H, 2-H), 7.47 (d, J = 7 Hz, 1H, 7-indole), 7.35 (d, J = 8 Hz, 1H, 4-indole), 7.04 (s, 1H, 2-indole) 6.97 (t, J = 9 Hz, 1H, 5-indole), 6.89 (t, J = 8 Hz 6-indole), 5.36 (s, 1H, 7-H), 4.36 and 3.91 (t, J = 7 Hz, 2H, ethylene bridgehead), 3.68 and 3.11 (2t, J = 7 Hz, 4H, ethylene chain); MALDI *m/z* calcd for $C_{19}H_{18}N_5O_2$ (M + 1), 332.151; found, 332.148. Anal. ($C_{19}H_{17}N_5O$·3HCl·0.4H_2O) C, H, N.

8-[2-(4′-Hydroxyphenyl)-ethylamino]-4,5-dihydroimidazo-[1,5,4-*de*]quinoxalin-9-one (1f). Yield, 4.3 mg (23%); mp > 196 °C dec; TLC (*n*-butanol, acetic acid, H_2O [5:2:3]) $R_f = 0.32$; IR 3441, 3225, 3117, 2935, 1639, 1562, 1516, 1352, 1234, 1105, 835, 648 cm^{-1}; ^1H NMR (CD_3OD) δ 8.0 (1s, 1H, 2-H), 6.98 and 6.63 (2d, 4H, J = 8 Hz, aromatic), 5.86 (s, 1H, 7-H), 4.38 and 3.97 (2t, J = 7 Hz, 4H, ethylene bridge), 3.55 and 2.84 (2t, J = 7 Hz, 4H, ethylene chain); MALDI *m/z* calcd for $C_{17}H_{17}N_4O_2$ (M + 1), 309.135; found, 309.142. Anal. ($C_{19}H_{17}N_5O$·2HCl) C, H, N.

8-Benzylamino-4,5-dihydroimidazo[1,5,4-*de*]quinoxalin-9-one (1g). Yield, 5.8 mg (34%); mp > 240 °C dec; TLC (*n*-butanol, acetic acid, H_2O [5:2:3]) $R_f = 0.32$; IR 3136, 3045, 1637, 1537, 1404, 1234, 1084, 700 cm^{-1}; ^1H NMR (CD_3OD) δ 8.12 (1s, 1H, 2-H), 7.44 (mult, 5H, aromatic), 5.61 (s, 1H, 7-H), 4.49 (s, 2H, methylene), 4.49 and 4.06 (2t, J = 6 Hz, 4H, ethylene bridgehead); MALDI *m/z* calcd for $C_{16}H_{15}N_4O$ (M + 1), 279.125; found, 279.126. Anal. ($C_{16}H_{15}N_4O$·2HCl) C, H, N.

2-(2-Hydroxyethylamino)-5-methylaniline (12). Compound **12** was prepared according to the following two-step process. A solution of 4 mL (5.2 g, 0.03 mol) of 4-chloro-3-nitrotoluene (**11**) in 4 mL (4.08 g, 0.07 mol) of ethanolamine was refluxed for 2 h. The reaction was then cooled to room temperature and diluted with dichloromethane and H_2O. The organic phase was dried with sodium sulfate and concentrated, giving a red oil. Triturating the oil with hexane yielded 4-(2-hydroxyethylamino)-3-nitrotoluene, a red solid. The solid was filtered, washed with hexanes, and dried in the air. The crude product was recrystallized with DCM to give red prisms (4.44 g, 75% yield); mp 80−81 °C; TLC (chloroform/methanol [90:10]) $R_f = 0.44$; IR (KBr pellet) 3445, 3346, 2957, 2926, 2872, 1637, 1568, 1523, 1433, 1406, 1356, 1311, 1219, 1178, 1151, 1039, 922, 812 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.06 (s, 1H, N-4-amino), 7.98 (s, 1H, C-2 aromatic proton), 7.29 (d, J = 8.7 Hz, 1H, C-5 aromatic proton), 6.83 (dd, J = 8.7 Hz, 1H, C-6 aromatic proton), 3.95 and 3.53 (t, J = 4.8 Hz, 4H, methylenes), 2.27 (3H, s, C-1 methyl); MS (EI mode) *m/z* 196 (M^+).

A solution of 8.1 g (142 mmol) of 4-(2-hydroxyethylamino)-3-nitrotoluene in 100 mL of methanol was purged with nitrogen. To this solution was added 2 g of 5% activated Pd on carbon. This mixture was hydrogenated under 50 psi of H_2 at room temperature for 3 h. The catalyst was then filtered off, and the solids were washed with DMF. The filtrates were combined and concentrated in vacuo, resulting in a purple solid. This solid was recrystallized with DCM to afford light purple crystals (6.3 g, 93% yield); mp 137−138 °C; TLC (ethyl acetate) $R_{xc4} = 0.55$; IR (KBr pellet) 749, 868, 1053, 1155, 1269, 1462, 1521, 1620, 2939, 3198, 3356 cm^{-1}; ^1H NMR (DMSO-d_6) δ 6.36 (1H, s), 6.29 (2H, d), 4.62 (t, J = 5.7 Hz, 1H), 4.353 (br s, 2H), 4.11 (t, J = 6 Hz, 1H), 3.57 (q, J = 6 Hz, 2H,), 3.02 (q, J = 6 Hz, 2H), 2.06 (3H, s, methyl); MS (EI mode) *m/z* 166 (M^+). Anal. ($C_9H_{14}N_2O$) C, H, N.

1-(2-Hydroxyethyl)-5-methylbenzimidazole (13). To a solution consisting of 276 mg (6 mmol) of 96% formic acid and 20 mL of 4 N HCl was added 500 mg (3 mmol) of **12**. The reaction mixture was refluxed for 1 h, cooled to room temperature, and placed in an ice bath. The cooled reaction was neutralized with ammonium

hydroxide (pH = 8). The resulting crystals were filtered and washed with cold H_2O and air-dried to afford **13** (435 mg, 82% yield); mp 135−136 °C; TLC (20% methanol in chloroform) $R_{xc4} = 0.4$; IR (KBr pellet) 790, 869, 1076, 1178, 1253, 1329, 1386, 1510, 1780, 2868, 3090, 3171, cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.71 (s, 1H), 7.18 (d, $J = 8$ Hz, 1H), 7.11 (s, 1H), 7.03 (d, $J = 8$ Hz, 1H), 4.26 (t, $J = 5$ Hz, 2H), 4.04 (t, $J = 5$ Hz, 2H), 2.35 (s, 3H, methyl). Anal. ($C_{10}H_{12}N_2O$) C, H, N.

1-(2-Acetoxyethyl)-5-methyl-6-nitrobenzimidazole (14). To a solution consisting of 25 mL of acetic anhydride and 25 mL of acetic acid was added 4 g (0.02 mol) of **13**, and the resulting mixture was refluxed for 1 h. The acetic acid was removed in vacuo leaving an oily liquid (*O*-acetylated **13**), which was pure by TLC and used without further characterization.

A 4:1 solution of 90% HNO_3 and H_2SO_4 (20 mL) was cooled to −5 °C with an ice-salt bath. To the cooled nitric acid solution was added *O*-acetylated **13** in 10 mL of acetic acid dropwise over 1 h, while keeping the temperature between −5 and 0 °C. After the addition was complete, the reaction was warmed to 10 °C and stirred for 1 h. The reaction was then warmed to room temperature, followed by pouring over ice and a pH adjustment with sodium bicarbonate (pH ∼ 5). This solution was extracted with dichloromethane, and the extracts were dried with Na_2SO_4 and concentrated, giving an oily solid consisting of both nitro isomers. A small portion of the desired isomer was isolated by column chromatography with silica gel (20% acetone in ethyl acetate). The pure crystals were then used to crystallize selectively the desired isomer from ethanol to afford pale yellow crystals (1.5 g, 25% yield); mp 129−130 °C; TLC (20% methanol in chloroform) $R_{xc4} = 0.71$; FTIR (KBr pellet) 889, 1051, 1232, 1325, 1423, 1516, 1745, 2982, 3109, cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.24 (s, 1H), 8.08 (s, 1H), 7.71 (s, 1H), 4.47 (m, 4H), 2.73 (s, 3H, methyl), 2.04 (s, 3H, methyl). Anal. ($C_{12}H_{13}N_3O_4$) C, H, N.

6-Acetamido-1-(2-acetoxyethyl)-5-methylbenzimidazole (15). A solution consisting of 100 mg (0.44 mmol) of **14** in 25 mL of methanol was purged with nitrogen gas. To this solution was added 50 mg of 5% Pd on carbon. The mixture was hydrogenated under 50 psi of H_2 at room temperature for 3 h. The catalyst was filtered off and washed with methanol. The methanol solution was concentrated, giving a clear residue that was dissolved in a solution consisting of 2 mL of acetic acid and 2 mL of acetic anhydride, and stirred for 24 h under a drying tube. The solution was concentrated, giving a clear residue that was solidified with acetone to afford **15** as white crystals (50 mg, 41% yield). When the reaction was scaled up to 800 mg and the catalyst was washed with DMF, the yield increased to 58%; mp 191−193 °C; TLC (20% methanol in chloroform) $R_{xc4} = 0.5$; IR (KBr pellet) 869, 1045, 1240, 1371, 1494, 1537, 1649, 1741, 3281, cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.14 (s, 1H), 7.85 (s, 1H), 7.59 (s, 1H), 7.10 (br s, 1H), 4.39 (s, 4H), 2.39 (s, 3H, methyl), 2.27 (s, 3H, methyl), 2.03 (s, 3H, methyl). Anal. ($C_{14}H_{17}N_3O_3 \cdot 0.7AcOH$) C, H, N.

6-Acetamido-1-(2-acetoxyethyl)-5-methyl-7-nitrobenzimidazole (16). To a 4:1 solution containing 90% HNO_3 and H_2SO_4 (12 mL/3 mL) was added 550 mg (2 mmol) of **15** in small portions, keeping the temperature between 0 and 5 °C. After the addition, the reaction was stirred for 20 min at 0 °C, quenched with ice, and the pH was adjusted to 8 with $NaHCO_3$. The neutral solution was then extracted with dichloromethane, dried with Na_2SO_4, and concentrated, giving a yellow solid. The solid was recrystallized with ethyl acetate and hexanes, yielding pale yellow crystals (400 mg, 63% yield); mp 186−187 °C; TLC (20% methanol in chloroform) $R_{xc4} = 0.6$; IR (KBr pellet) 1045, 1238, 1375, 1529, 1660, 1737, 3074, 3286, cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.91, (s, 1H), 7.87 (s, 1H), 7.58 (br s, 1H), 4.44 (t, $J = 4$ Hz, 2H, ethylene), 4.25 (t, $J = 4$ Hz, 2H, ethylene) 2.41 (s, 3H methyl), 2.26 (s, 3H methyl), 1.96 (3H, s, methyl). Anal. ($C_{14}H_{16}N_4O_5$) C, H, N.

6-Acetamido-1-(2-methanesulfonoxyethyl)-5-methyl-7-nitrobenzimidazole (17). A solution of methanol (50 mL) containing 350 mg (1.1 mmol) of **16** and 6 mL of concentrated HCl was stirred at room temperature for 12 h. The reaction solution was evaporated giving a yellow HCl salt of the *O*-deacetylated product. Under nitrogen gas, the HCl salt (343 mg, 1.1 mmol) was suspended in 50 mL of dry dichloromethane. To this suspension was added of 574 μL of diisopropyl ethylamine and the mixture was stirred for 5 min followed by dropwise addition of 255 μL (3.3 mmol) of methane sulfonyl chloride. The reaction was stirred for 2 h, concentrated, and chromatographed on silica gel (5% methanol in dichloromethane) to afford yellow crystals (250 mg, 64% yield); mp 164−166 °C; TLC (20% methanol in chloroform) $R_{xc4} = 0.48$; IR (KBr pellet) 526, 800, 912, 1172, 1354, 1531, 1666, 3022, 3304, cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.99 (s, 1H), 7.90 (s, 1H), 7.51 (br s, 1H), 4.52 (t, $J = 4.5$ Hz, 2H, ethylene), 4.44 (t, $J = 4.5$ Hz, 2H, ethylene), 2.92 (s, 3H methyl), 2.41 (s, 3H, methyl), 2.23 (s, 3H, methyl). Anal. ($C_{13}H_{16}N_4O_6S$) C, H, N.

7-Acetamido-8-methyl-4,5-dihydroimidazo[1,5,4-*de*]quinoxalin-9-one (2a). A solution containing 100 mg of **17** in 25 mL of methanol was purged with nitrogen. To this was added 200 mg of 5% Pd on carbon. This mixture was hydrogenated under 50 psi of H_2 at room temperature for 30 min. The catalyst was filtered off, and the clear methanol solution was refluxed for 2 h. The methanol was evaporated giving 60 mg of ring-closed product **18**, which was too unstable for complete characterization. The ring-closed product was dissolved in 50 mL of phosphate buffer containing 300 mg of Fremy's salt. This mixture was stirred for 10 min followed by extraction with dichloromethane. The organic extracts where dried with Na_2SO_4 and concentrated to a yellow solid (20 mg, 29% yield); mp > 185 °C dec; TLC (decomposed on TLC plate to form new product, thus, no R_f value was calculated); ^1H NMR ($CDCl_3$) δ, 7.65 (s, 1H), 7.45 (br s, 1H), 4.34 (t, $J = 7.2$ Hz, 2H), 4.23 (t, $J = 7.2$ Hz, 2H), 2.24 (s, 3H, methyl), 2.05 (s, 3H, methyl); MS (EI mode) *m*/*z* 244 (M^+). Anal. ($C_{12}H_{12}N_4O_2 \cdot 0.5H_2O$) C, H (the nitrogen % deviated significantly from the calculated value). Structure was verified using high-resolution MALDI calcd for $C_{12}H_{12}N_4O_2$ (M + 2), 246.111; found, 246.113.

3-Methoxyacetamido-4-[*N*-methoxyacetyl-2-(2-methoxyacetoxy)-ethylamino] Toluene (19). To an ice cold suspension of 1 g (6 mmol) of **12** in 50 mL of dry dichloromethane was added 3.2 mL (183 mmol) of diisopropylethylamine with stirring. To this mixture was added 1.7 mL (18 mmol) of methoxyacetyl chloride dropwise. The reaction was allowed to warm to room temperature while monitoring the progress of the reaction by TLC. At this time, an additional 0.5 mL of methoxyacetyl chloride and 0.5 mL of diisopropylethylamine were then added dropwise. When the reaction was complete, 25 mL of H_2O was added to quench the reaction. The organic phase was separated and washed once with 25 mL of H_2O, followed by a washing with 25 mL of saturated $NaHCO_3$, and then with 25 mL of saturated brine solution. The organic phase was then dried with Na_2SO_4 and concentrated, yielding a brown oil that solidified upon triturating with hexanes, giving a cream-colored solid (98% yield); mp 92−93 °C; TLC (1:1 acetone and ethyl acetate) $R_{xc4} = 0.41$; IR (KBr pellet) 1026, 1120, 1192, 1286, 1419, 1541, 1662, 1743, 2822, 2931, 3267 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.52 (s, 1H, amide), 8.16 (s, 1H), 7.09 (d, $J = 8$ Hz, 1H), 6.98 (d, $J = 8$ Hz, 1H), 4.36 (t, $J = 5.4$ Hz, 2H), 4.01−3.59 (m, 8H) 3.45, 3.38, and 3.28 (3s, 9H, methoxy), 2.39 (s, 3H, methyl); MS (HRE) *m*/*z* (M^+) calcd for $C_{18}H_{26}N_2O_7$, 382.17; found, 382.173. Anal. ($C_{18}H_{26}N_2O_7$) C, H, N.

3-Methoxyacetamido-4-[*N*-methoxyacetyl-2-(2-methoxyacetoxy)ethylamino]-6-nitrotoluene (20). To 5 mL of 90% nitric acid, cooled to 0 °C with an ice bath, was added 1 g (2.6 mmol) of **19** portionwise. During the addition, the temperature was kept below 10 °C, followed by stirring at 0 °C for 4 h. The completed reaction was quenched with ice, and the product was extracted with dichloromethane. The organic phase was dried with Na_2SO_4 and concentrated to a yellow oil. The oil was crystallized with ethanol to afford **20** as pale yellow crystals (614 mg, 55% yield); mp 126−128 °C; TLC (1:1 acetone/ethyl acetate) $R_{xc4} = 0.43$; IR (KBr pellet) 686, 754, 844, 954, 1028, 1122, 1188, 1344, 1508, 1670, 1747, 2822, 2933, 3059 cm^{-1}; ^1H NMR ($CDCl_3$) δ 876 (s, 1H, amide), 8.52 (s, 1H), 8.01 (s, 1H), 4.4 (m, $J = 2.1$ Hz, 2H), 4.04−3.63 (m, 8H) 3.47 (s, 3H, methoxy), 3.37(s, 3H, methoxy), 3.27 (s, 3H,

methoxy), 2.69 (s, 3H, methyl); MS (HRE) *m/z* (M^+) calcd for $C_{18}H_{25}N_3O_9$, 427.16; found, 427.1527. Anal. ($C_{18}H_{25}N_3O_9$) C, H, N.

1-(2-Hydroxyethyl)-2-(hydroxymethyl)-5-methyl-6-nitrobenzimidazole (21). A solution of 3.04 g (7 mmol) of **20** in 50 mL of 48% HBr was refluxed at 130 °C for 22 min. The reaction was then cooled in an ice bath immediately, and the pH was adjusted to 8 with $NaHCO_3$. The precipitated solids were filtered, washed with H_2O, and partially dried by vacuum filtration. The damp crystals were recrystallized with ethanol yielding cream-colored needles (1.32 g, 74% yield); mp 211−213 °C; TLC (1:1 acetone/ethyl acetate) $R_{xc4} = 0.44$; FTIR (KBr pellet) 879, 1074, 1332, 1431, 1516, 2908, 3171, 3344 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.36 (s, 1H), 7.62 (s, 1H), 5.7 (t, $J = 6$ Hz, 1H), 5.01 (t, $J = 5$ Hz, 1H), 4.76 (d, $J = 5$ Hz, 2H), 4.43 (t, $J = 5$ Hz, 2H), 3.74 (q, $J = 5.7$ Hz, 2H), 2.59 (t, 3H, CH_3); MS (HRE) *m/z* (M^+) calcd for $C_{11}H_{13}N_3O_4$, 251.09; found, 251.089. Anal. ($C_{11}H_{13}N_3O_4 \cdot 0.75H_2O$) C, H, N.

6-Acetamido-1-(2-acetoxyethyl)-2-(acetoxymethyl)-5-methylbenzimidazole (22). A solution of 2.18 g (8.6 mmol) of **21** in 110 mL of methanol was degassed with nitrogen. To this was added 492 mg of 5% Pd on carbon, and the mixture was hydrogenated under 50 psi of H_2 at room temperature for 3 h. The catalyst was filtered off and washed with methanol. The methanol filtrate was concentrated in vacuo leaving a white solid. The solid was dissolved in a solution of 55 mL of acetic anhydride and 55 mL of acetic acid and stirred at room temperature for 18 h. The completed reaction was concentrated leaving a light purple solid that was taken up in 25 mL of saturated $NaHCO_3$ solution and 50 mL of dichloromethane. The organic layer was dried with Na_2SO_4 and concentrated to a white solid that was recrystallized with chloroform and hexane to afford **22** as white crystals (2.11 g, 70% yield); mp 161−162 °C; TLC (10% methanol in chloroform) $R_{xc4} = 0.47$; IR (KBr pellet) 879, 1074, 1332, 1431, 1516, 2908, 3171, 3344 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.19 (s, 1H), 7.56 (s, 1H), 7.12 (s, 1H), 5.36 (s, 2H), 4.48 (t, $J = 6$ Hz, 2H), 4.39 (t, $J = 6$ Hz, 2H), 2.38 (s, 3H, methyl), 2.25 (s, 3H, methyl) 2.15 (s, 3H, methyl), 2.02 (s, 3H, methyl); MS (HRE) *m/z* (M^+) calcd for $C_{17}H_{21}N_3O_5$, 347.15; found, 347.1479. Anal. ($C_{17}H_{21}N_3O_5$) C, H, N.

6-Acetamido-1-(2-acetoxyethyl)-2-(acetoxymethyl)-5-methyl-7-nitrobenzimidazole (23). A solution of 12 mL of 90% nitric acid and 3 mL of concentrated sulfuric acid was cooled to 0 °C on an ice bath. To this mixture was added 525 mg (2 mmol) of **22** in small portions while keeping the temperature below 5 °C. After the addition was complete, the reaction was stirred at 0 °C for 75 min, followed by pouring over ice. The resulting mixture was neutralized with sodium bicarbonate, and the product was extracted with dichloromethane. The organic phase was dried with sodium sulfate and concentrated to a yellow oil that crystallized with ethyl acetate and hexane to afford light yellow crystals (462 mg, 78% yield); mp 158−159 °C; TLC (10% methanol in chloroform) $R_{xc4} = 0.45$; IR (KBr pellet) 1049, 1240,1357, 1435, 1527, 1660, 1753, 2982, 3292 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.78 (s, 1H), 7.58 (s, 1H, amide), 5.38 (s, 2H, methylene), 4.46 (t, $J = 5$ Hz, 2H), 4.21 (t, $J = 5$ Hz, 2H), 2.37 (s, 3H, methyl), 2.21 (s, 3H, methyl), 2.16 (s, 3H, methyl), 1.94 (s, 3H, methyl); MS (HRE) *m/z* (M^+) calcd for $C_{17}H_{20}N_4O_7$, 392.13; found, 392.1328. Anal. ($C_{17}H_{20}N_4O_7$) C, H, N.

6-Acetamido-2-(chloromethyl)-1-(2-methansulfonoxyethyl)-5-methyl-7-nitrobenzimidazole (24). To a solution of 500 mg (1.3 mmol) of **23** in 55 mL of methanol was added 7 mL of concentrated HCl. The reaction was stirred at room temperature for 48 h. The methanol was removed in vacuo leaving a yellow residue that was crystallized from methanol and ethyl acetate to afford 408 mg of yellow crystals.

This product was suspended in dry dichloromethane under a nitrogen atmosphere. To this suspension was added 630 μL (36 mmol) of diisopropyl ethyl amine dropwise, and the solution was stirred at room temperature for 5 min, followed by the dropwise addition of 557 μL (7.2 mmol) of methane sulfonyl chloride. The reaction was stirred for 12 h at room temperature and then concentrated in vacuo to afford a yellow oil that was chromatographed on silica gel using ethyl acetate as the eluent. The fractions containing product were concentrated, giving a yellow residue that was crystallized with methanol to afford **24** as yellow crystals (190 mg, 40% yield); mp > 180 °C dec; TLC (10% methanol in chloroform) $R_{xc4} = 0.2$; FTIR (KBr pellet) 925, 974, 1020, 1167, 1348, 1523, 1668, 3323, 3418, 3464 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.84 (s, 1H, amide), 7.90 (s, 1H), 5.13 (s, 2H, methylene), 4.55 (t, $J = 4$ Hz, 2H), 4.396 (t, $J = 4$ Hz, 2H), 3.01 (s, 3H, methyl), 2.31 (s, 3H, methyl), 2.04 (s, 3H, methyl); MS (EI mode) *m/z* 404 (M^+). Anal. ($C_{14}H_{17}ClN_4O_6S$) C, H, N.

6-Acetamido-2-(acetoxymethyl)-1-(2-methansulfonoxyethyl)-5-methyl-7-nitrobenzimidazole (25). To a solution of 122 mg (0.3 mmol) of **24** in 3 mL of DMSO was added 61 mg (0.732 mmol) of sodium acetate dissolved in a minimum amount of methanol. The reaction was stirred at room temperature for 5 h. After 5 h, H_2O was added to quench the reaction (exothermic), resulting in the formation of pale yellow crystals. The crystals were filtered off and dried to afford **25** (96 mg, 75% yield); mp > 215 °C dec; TLC (10% methanol in chloroform) $R_{xc4} = 0.29$; IR (KBr pellet) 810, 922, 1041, 1165, 1249, 1357, 1521, 1666, 1736, 3016, 3323 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.85 (s, 1H), 7.36 (s, 1H), 5.40 (s, 2H, methylene), 4.58 (t, $J = 5$ Hz, 2H), 4.41 (t, $J = 5$ Hz, 2H), 2.90 (s, 3H, methyl), 2.41 (s, 3H, methyl), 2.22 (s, 3H, methyl), 2.17 (s, 3H, methyl); MS (HRE) *m/z* 428.0995 (M^+); Anal. ($C_{16}H_{20}N_4O_8S$) C, H, N.

7-Acetamido-2-acetoxymethyl-5, 6-dihydro-8-methyl-4*H*-imidazo[1,5,4-*de*]quinoxaline (26). To a suspension of 96 mg (0.22 mmol) of **25** in 25 mL of methanol was added 256 mg of 5% Pd on carbon. The mixture was hydrogenated under 50 psi of H_2 for 30 min. The catalyst was filtered off and washed with methanol. The methanol filtrate was heated at 60 °C for 1 h and concentrated, giving a white powder that was recrystallized with methanol and ethyl acetate to afford white crystals (52 mg, 78% yield); mp > 225 °C dec; TLC (20% methanol in chloroform) $R_{xc4} = 0.4$; IR (KBr pellet) 1033, 1219, 1500, 1643, 1755, 3009, 3329 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.16 (s, 1H), 6.85 (s, 1H), 4.49 (s, 2H, methylene) 4.45 (t, $J = 4.5$ Hz, 2H), 3.57 (t, $J = 4.5$ Hz, 2H), 2.28 (s, 3H, methyl), 2.21 (s, 3H, methyl), 2.05 (s, 3H, methyl); MS (HRE) *m/z* (M^+) calcd for $C_{15}H_{18}N_4O_3$, 302.1379; found, 302.138.

7-Acetamido-2-acetoxymethyl-8-methyl-4,5-dihydroimidazo-[1,5,4-*de*]quinoxalin-9-one (2b). To a solution consisting of 10 mL of phosphate buffer (pH = 7) and 300 mg of Fremy's salt was added 40 mg (0.132 mmol) of **26** at room temperature. The reaction was stirred for 10 min, followed by extraction with dichloromethane. The organic layer was dried with sodium sulfate and concentrated, giving a yellow solid. The solid was recrystallized with chloroform and hexane to afford yellow crystals (22 mg, 56% yield); mp > 205 °C dec; TLC (20% methanol in chloroform) $R_{xc4} = 0.33$; IR (KBr pellet) 1035, 1238, 1373, 1535, 1662, 1743, 2987, 3161, cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.54 (s, 1H), 5.21 (s, 2H, methylene), 4.32 (m, methylene, 2H), 4.21 (m, methylene, 2H), 2.32 (s, 3H, methyl), 2.11 (s, 3H, methyl), 2.04 (s, 3H, methyl); MS (HRE) *m/z* (M^+) calcd for $C_{15}H_{16}N_4O_4$, 316.1172; found, 316.1165. Anal. ($C_{15}H_{16}N_4O_4 \cdot 0.75H_2O$) C, H, N.

Cell Culture and MTT Assay. Cancer cell line HCT-116 (human colon tumor) was grown at 37 °C in 5% CO_2 in McCoy's 5a medium containing 10% fetal bovine serum, penicillin (50 IU/mL), streptomycin (50 μg/mL), and 2 mM L-glutamine. Human epidermoid carcinoma A-431 cells were maintained at 37 °C in 5% CO_2 in Dulbecco's Modified Eagle's Medium containing 10% fetal bovine serum, penicillin (50 IU/mL), streptomycin (50 μg/mL), and 4 mM L-glutamine. Cells were plated at a density of 10 000 cells/well in a 96-well plate. A total of 24 hrs after plating, they were exposed to medium containing compound at a concentration of 0.3 to 100 μM (HCT-116) and 0.1 to 100 μM (A-431) for 48 h at 37 °C. After a 48 h exposure of cells to compounds, 100 μL of fresh culture medium containing MTT at a final concentration

of 0.3 mg/mL was added to each well and incubated for 3 h at 37 °C. The formazan crystals were solubilized in 100 μL of DMSO. The absorbance of each well was measured by a microplate reader (Multi-skan lab systems) at 570 nm. The percentage cytotoxicity was calculated by comparison of the A_{570} reading from treated versus control cells. Makaluvamine C was used as positive control at a concentration of 10 μM.

Topoisomerase II Inhibition Assays. The topoisomerase II relaxation reactions were carried out with 0.25 μg of pRYG supercoiled DNA (form I) and 4 units of topoisomerase II in a total volume of 20 μL 50 mM Tris buffer (pH 8.0) containing 120 mM of KCl, 10 mM of $MgCl_2$, 0.5 mM of dithiothreitol, and 0.5 mM of ATP in microcentrifuge tubes. Varying amounts of drugs were added to each reaction. The reactions were run at 37 °C for 45 min. The reactions were then stopped by the addition of 2 μL of 10% SDS to each tube, and 1 μL of proteinase K (1 mg/mL) was also added. The resulting mixtures were incubated for 15 min at 37 °C. Each reaction mixture was extracted once with 20 μL of chloroform/isoamyl alcohol (24:1) and then combined with 2 μL of 10× loading dye (0.25% bromophenol blue and 50% aqueous glycerol). The resulting mixtures were then loaded onto a 1% agarose gel in 1× TAE buffer and run at 2 V/cm for 5−7 h. The gels either contained or did not contain 0.5 μg/mL of ethidium bromide. After running, gels without ethidium bromide were soaked in 1× TAE buffer containing 0.5 μg/mL of ethidium bromide for 30 min, followed by destaining in 1× TAE buffer for 20 min.

In Vitro Screening Procedures. Compounds from series **1** and **2** were tested against all cell lines included in the 60-cell-line panel, as previously described.[22]

Acute Toxicity Assays. The drug was prepared at a concentration consistent with administration of 400 mg/kg intraperitoneally in a volume of 1 mL. Each mouse was treated with a single dose of 400, 200, and 100 mg/kg (1 mL, 0.5 mL, and 0.25 mL/mouse). The body weight and strain of mouse used should be consistent with that to be used in the subsequent hollow fiber assay. The mice are held for a period of 14 days, observing for morbidity (body weight loss) and mortality. If the mice do not survive for 14 days, administer a 50% lower dose to a single mouse and continue this process (50 mg/kg/25 mg/kg/12.5 mg/kg/etc.) until a tolerated dosage is determined. Use this maximum tolerated dosage as the basis for selecting doses for antitumor drug evaluations.

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Supporting Information Available: Elemental analyses of reported compounds are included. This material is available free of charge via the Internet at http://pubs.acs.org.

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