Notes

Synthesis and Pharmacological Evaluation of Isoindolo[1,2-*b*]quinazolinone and Isoindolo[2,1-*a*]benzimidazole Derivatives Related to the Antitumor Agent Batracylin

Sanath K. Meegalla,† Gregory J. Stevens,‡ Charlene A. McQueen,‡ Allan Y. Chen,§ Chiang Yu,§ Leroy F. Liu,§ Louis R. Barrows,⊥ and Edmond J. LaVoie*,†

Department of Pharmaceutical Chemistry, Rutgers, The State University of New Jersey, Piscataway, New Jersey 08855, Department of Pharmacology and Toxicology, The University of Arizona, College of Pharmacy, Tucson, Arizona 85721, Department of Pharmacology, The University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, Piscataway, New Jersey 08855, and Department of Pharmacology and Toxicology, The University of Utah, Salt Lake City, Utah 84112

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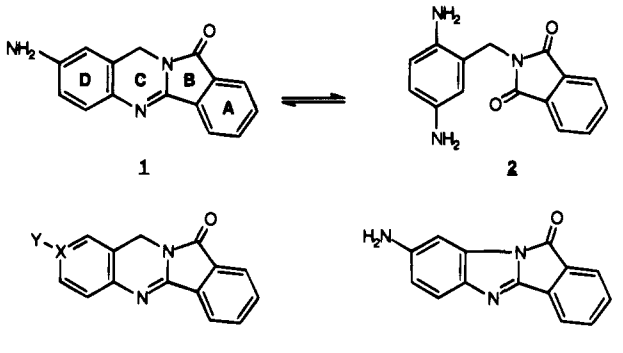
The synthesis and pharmacological activity of isoindolo[1,2-*b*]quinazolin-12(10*H*)-ones and isoindolo[2,1-*a*]benzimidazoles related to batracylin are described. The acute toxicity of batracyclin has been associated with the formation of its *N*-acetyl metabolite which is a potent inducer of unscheduled DNA synthesis in rat hepatocytes. The desamino derivative and the 8-aza analog of batracylin retained the ability to inhibit topoisomerase II but did not induce unscheduled DNA synthesis. While less active than batracylin, these analogs were cytotoxic to CCRF CEM leukemia cells. The isoindolo[2,1-*a*]benzimidazole derivatives were inactive as topoisomerase II inhibitors and, in general, failed to exhibit comparable antitumor activity or to induce unscheduled DNA synthesis.

Introduction

Batracylin (**1**), 8-aminoisoindolo[1,2-*b*]quinazolin-12-(10*H*)-one (Chart 1), exhibits antineoplastic activity *in vivo* against murine leukemia P-388 sublines resistant to adriamycin, cisplatin, and methotrexate.[1,2] These results are particularly noteworthy as the adriamycin-resistant P388 leukemia subline cell line is known to possess multidrug resistance. Batracylin is also active as an antitumor agent in mice bearing early-stage and advanced colon carcinoma 38, a relatively refractory solid tumor model.[1] Oral administration of batracylin is effective against other murine solid tumors including pancreatic ductal adenocarcinoma, colon adenocarcinoma #51, and hepatoma 129.[3] Batracylin is inactive against B16 melanoma, CD8F1 mammary carcinoma, L1210 leukemia, Lewis lung carcinoma, and human MX-1 mammary xenograft.[1] For those susceptible tumor cell lines implanted in mice, high doses of batracylin are required, with a total po administration of 400–800 mg/kg required to observe an increase in life span. Batracylin is known to hydrolyze reversibly to produce a ring-opened product **2** under acidic conditions.[4] While this ring-opened product was not detected in the plasma of mice administered batracylin, one cannot preclude the possibility that it could contribute to the antineoplastic activity observed with batracylin, particularly in those instances in which batracylin was administered by gavage.

There are major differences observed in the toxicity of batracylin in mice and rats. One-tenth of the oral

Chart 1



LD_{10} dose in mice (1855 mg/kg) was fatal to all treated rats.[5] Renal, gastrointestinal, and testicular toxicity were observed in rats administered 2.2 mg/kg per day for 9 days. Consistent with the observation that rats are more rapid acetylators than mice,[6] it has been suggested that N-acetylation of batracylin may be linked to its toxicity.[7] Recent studies have demonstrated that batracylin induced unscheduled DNA synthesis (UDS) in primary rat hepatocytes. In this assay, *N*-acetylbatracylin is as potent an inducer of UDS as batracylin.[8] These data suggest that *N*-acetylbatracylin could be associated with some of the adverse effects of batracylin.

The major limitations associated with the chemotherapeutic potential of batracylin are the high dose levels required for antineoplastic activity and its systemic toxicity, especially in rats. In the present study 8-desaminobatracylin (**3**) and 8-azabatracylin (**4**) were synthesized in an effort to develop analogs with de-

† Rutgers, The State University of New Jersey.
‡ The University of Arizona.
§ The University of Medicine and Dentistry of New Jersey.
⊥ The University of Utah.
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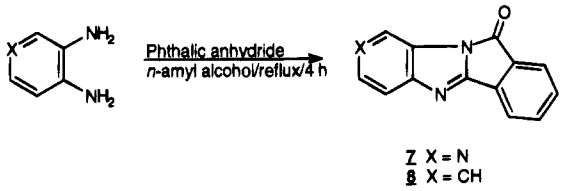
Scheme 1



creased toxicity. To examine the influence of contracting the "C" ring of batracylin from a 6-membered ring to a 5-membered ring (Chart 1), the benzimidazole analog of batracylin (**5**) and several of its derivatives were also prepared. The relative cytotoxic activities of these analogs were evaluated. Comparative studies were also performed to assess their potential to induce DNA damage in rat hepatocytes, as well as to inhibit mammalian topoisomerase II. We also explored several methods for the synthesis and isolation of the ring-opened form of batracylin, **2**. The propensity of **2** to cyclodehydrate to form batracylin precluded our ability to directly assess its potential to contribute to the pharmacological activity observed with batracylin. Several ring-opened derivatives associated with the benzimidazole analogs of batracylin were synthesized, isolated in pure form, and evaluated for biological activity.

Chemistry

Batracylin **1** and 8-desaminobatracylin **3** were prepared by reaction of the appropriate 2-aminobenzylamine with phthalic anhydride under conditions similar to those previously described.[11-14] While batracylin was previously prepared using 2,5-diaminobenzylamine, formation of 5-nitro-2-aminobenzylamine from the commercially available 5-nitro-2-aminobenzonitrile by reduction with BH_3·THF provided a convenient route to **6** which upon catalytic hydrogenation gave batracylin in good yield. 8-Azabatracylin, isoindolo[2,1-*a*]pyrimidino[5,4-*c*]pyridin-10(12*H*)-one, **4**, was similarly prepared by reacting 4-amino-3-(aminomethyl)pyridine with phthalic anhydride. Consistent with the greater nucleophilicity of the 3-aminomethyl moiety,[15] a single cyclized product was formed.

The reaction of *o*-phenylenediamine or 3,4-diaminopyridine with phthalic anhydride, as shown in Scheme 1, gave isoindolo[2,1-*a*]imidazo[5,4-*c*]pyridin-10-one, **7**, and isoindolo[2,1-*a*]benzimidazole, **8**, respectively. Attempts to prepare **5** by reaction of 3-bromo-4-nitroacetanilide with potassium phthalimide resulted in the formation of 8-(*N*-acetylamino)isoindolo[2,1-*a*]benzimidazole which proved resistant to either chemical (hydrazine/EtOH at 90 °C)[16] or enzymatic (hog kidney acylase) hydrolysis.[17] Efforts to prepare **5** by reacting phthalic anhydride directly with 4-nitro-*o*-phenylenediamine or reacting potassium phthalimide with 2-bromo-4-nitroaniline were also unsuccessful.

The benzimidazole analog of batracylin was prepared as outlined in Scheme 2 by reacting the (trimethylsilyl)-ethylsulfonamide[18] of 3-bromo-4-nitroaniline, **9**, with potassium phthalimide. Reduction of the *N*-arylphthalimide **10** with Fe in acetic acid resulted in the formation of an isoindolo[2,1-*a*]benzimidazole, which upon treatment with CsF in DMF gave **5**.

N-Arylphthalimides (**11a-13a**) were prepared from several appropriately substituted aniline derivative as outlined in Scheme **3**. Deprotection of the *o*-*N*-acetamides **11a** and **12a** provided compounds **11b** and **12b**. Cyclization of these *N*-(2-aminophenyl)phthalimides resulted in the formation of the isoindolo[2,1-*a*]benzimidazole derivatives **11c-12c**. Reduction of the *N*-(*o*-nitrophenyl)phthalimide **13a** with Fe/AcOH provided 6-methyl-11-oxoisoindolo[2,1-*a*]benzimidazole, **13b**. Treatment of *N*-(aminophenyl)phthalimide **11b** with HBr/AcOH followed by cyclization resulted in the formation of compound **14**.

Table 1. Biological Evaluation of Batracylin Derivatives

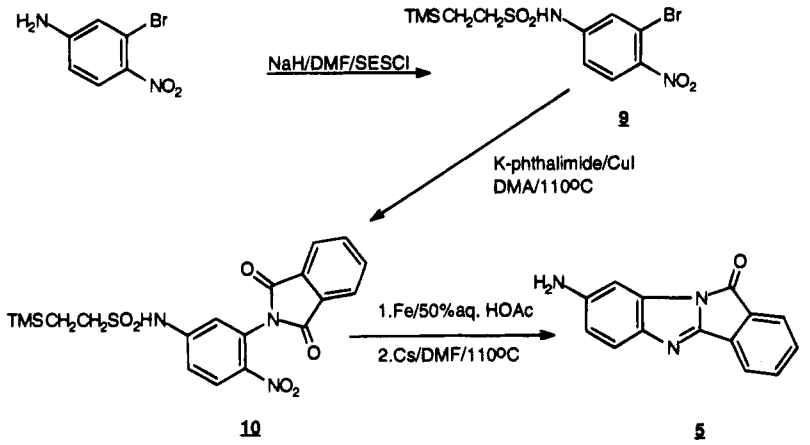
compd	cytotoxic activity IC_{50} (μM)[a]	Topo II-mediated DNA cleavage[b]	DNA repair (UDS) net grain counts[c]	DNA repair (UDS) concn (M)[d]
1	14	1.0	93.1 ± 20.8	5×10^{-7}
3	60	1.0	−4.5 ± 3.5	1×10^{-5}
4	102	0.1	−5.2 ± 2.5	5×10^{-5}
5	>200	0	−4.4 ± 2.6	5×10^{-4}
6	>150	0	10.5 ± 13.9	5×10^{-4}
7	>200	0	−10.5 ± 5.9	5×10^{-4}
8	68	0	−7.4 ± 4.0	5×10^{-4}
11b	>200	0	−8.9 ± 1.7	1×10^{-4}
11c	>200	0	−3.6 ± 2.5	1×10^{-4}
12b	71	0	−8.7 ± 4.4	1×10^{-4}
12c	>200	0	−3.7 ± 2.1	5×10^{-4}
13a	>200	0	−4.1 ± 2.8	1×10^{-4}
13b	>200	0	−3.5 ± 1.4	5×10^{-4}
14	>200	0	nd	nd
VP-16	0.002	1.0×10^3	−7.5 ± 4.5	1×10^{-4}

[a] Inhibition of CCRF CEM T-cell leukemia. [b] Relative activity to induce topoisomerase-mediated DNA cleavage is based on the relative potency of these compounds to cause 90% of topoisomerase-mediated cleavage of liner 8.4-kb YEPG DNA. The potency of batracylin to stimulate topoisomerase-mediated DNA cleavage in this assay is taken as 1.0. [c] Net grain counts refer to the nuclear count minus the highest cytoplasmic count determined for each cell. A positive response requires a net grain count >0 and a positive dose response. [d] The concentration provided is either the highest concentration at which toxicity was not observed or the concentration which provided the highest number of net grain counts.

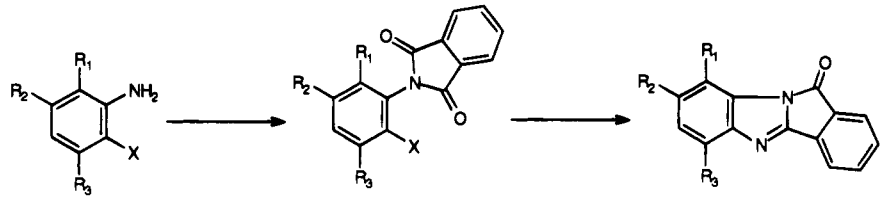
Biological Results and Discussion

Batracylin did exhibit modest cytotoxicity when assayed in CCRF CEM T-cell leukemia cells. This is in contrast to the absence of any noteworthy cytotoxicity observed with several other cell lines including SK-MEL-5 (malignant human melanoma), PC-3 (human prostate adenocarcinoma), HCT 116 (human colon tumor), and A 549 (human lung carcinoma). Evaluation of cytotoxicity in CCRF CEM cells provides a basis for comparing the relative cytotoxic activity of 8-desaminobatracylin, **3**, 8-azabatracylin, **4**, and the benzimidazole analogs **5**, **8**, **11c**, **12c**, **13b**, and **14**. The IC_{50} values of these compounds in this cell line are provided in Table 1. While **3** was more cytotoxic than **4**, both of these analogs were less active than batracylin. In the case of **3**, similar potency to batracylin as an inhibitor of topoisomerase II-mediated DNA cleavage was observed. While **4** did exhibit some weak activity as a topoisomerase II inhibitor, it was less active than batracylin. 8-Nitroisoindolo[1,2-*b*]quinazolin-12(10*H*)-one, **6**, was devoid of any significant cytotoxicity and had no effect on topoisomerase II-mediated DNA cleavage. Several isoindolo[2,1-*a*]benzimidazoles were synthesized which resemble batracylin derivatives in which the "C" ring is contracted from a 6- to a 5-membered ring. Most all of the isoindolo[2,1-*a*]benzimidazoles (**5**, **8**, **11c**, **12c**, **13b**, **14**) as well as isoindolo[2,1-*a*]imidazo[5,4-*c*]pyridin-10-one, **7**, did not exhibit comparable cytotoxicity to that

Scheme 2



Scheme 3



11a X = $NHCOCH_3$ R_1 = H R_2 = OCH_3 R_3 = H
11b X = NH_2 R_1 = H R_2 = OCH_3 R_3 = H
12a X = $NHCOCH_3$ R_1 = CH_3 R_2 = H R_3 = H
12b X = NH_2 R_1 = CH_3 R_2 = H R_3 = H
13a X = NO_2 R_1 = H R_2 = H R_3 = CH_3

11c R_1 = H R_2 = OCH_3 R_3 = H
12c R_1 = CH_3 R_2 = H R_3 = H
13b R_1 = H R_2 = H R_3 = CH_3
14 R_1 = H R_2 = OH R_3 = H

observed with batracylin. It was also evident that several of the ring-opened intermediates such as compounds **11b** and **13a**, which are precursors for these tetracyclic derivatives, were less cytotoxic than batracylin or desaminobatracylin. While **8** and **12b** did exhibit weak cytotoxic activity,' neither of these compounds influenced topoisomerase II-mediated cleavage of DNA.

The results of assays performed to assess the ability of these compounds to induce unscheduled DNA synthesis (UDS) in rat hepatocytes are also summarized in Table 1. This assay provides a measure of the potential of a compound to produce DNA damage and consequently induce DNA repair. Hepatocytes generally consist of a nondividing cell population with less than 1% of the cells in primary culture in S-phase. Therefore, topoisomerase II inhibitors are not necessarily expected to induce DNA repair in hepatocytes. This is consistent with the lack of DNA repair observed with several potent topoisomerase inhibitors including adriamycin,[19] *m*-AMSA (unpublished results, G. Stevens and C. A. McQueen), and etoposide (VP-16). The vehicle control (1.0% DMSO) produced a net grain count of -5.88 ± 3.68. Batracylin induced DNA repair ranging from 44.5 to 137 net grain counts at concentrations ranging from 5×10^{-8} to 1.0×10^{-6} M. In addition to batracylin, the only derivatives which exhibited any effect on topoisomerase II-mediated DNA cleavage were **3** and **4**. Neither **3** nor **4** induced UDS. These results indicate that analogs of batracylin can be developed which retain their potential to inhibit topoisomerase II but have diminished potential to modify DNA in nonproliferating cells. None of the ring-opened derivatives (**11b**, **12b**, **13a**) or the isoindolo[2,1-*a*]benzimidazoles (**5**, **8**, **11c**, **12c**, **13b**, **14**) as well as isoindolo[2,1-*a*]imidazo-[5,4-*c*]pyridin-10-one, **7**, affected UDS.

Conclusions

Batracylin is an experimental antitumor agent which is active as a topoisomerase II inhibitor and induces unscheduled DNA synthesis in nonproliferating cells. None of the analogs of batracylin in which the "C" ring was contracted from a 6- to a 5-membered ring was active as a topoisomerase inhibitor or induced UDS in rat hepatocytes. It has been suggested that a metabolite of batracylin, *N*-acetylbatracylin, may be associated with its acute toxicity and potential to initiate a genotoxic response in nonproliferating cells. This study indicates that both 8-desaminobatracylin and 8-azabatracylin retain activity as inhibitors of topoisomerase II. In contrast to batracylin, neither of these compounds induce UDS. The potential of batracylin to inhibit topoisomerase II, therefore, is not likely to be linked to its ability to induce DNA repair in nonproliferating cells. While both 8-desaminobatracylin, **3**, and 8-azabatracylin, **4**, are less cytotoxic than batracylin, these analogs may also exhibit less acute toxicity *in vivo*.

Experimental Section

Chemistry. Melting points were determined with a Thomas-Hoover unimelt capillary melting point apparatus. Infrared spectral data (IR) were obtained on a Perkin-Elmer 1600 Fourier transform spectrophotometer and are reported in cm^{-1}. Proton (^1H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance were recorded on a Varian Gemini-200 Fourier Transform spectrometer. NMR spectra (200 MHz ^1H and 50 MHz ^{13}C) were recorded in DMSO (unless otherwise noted) with chemical shifts reported in δ units downfield from tetramethylsilane (TMS). Coupling constants are reported in hertz. Mass spectra were obtained from Midwest Center for

Mass Spectrometry within the Department of Chemistry at the University of Nebraska—Lincoln. Combustion analyses were performed by Atlantic Microlabs, Inc., Norcross, GA, and were within ±0.4%. DMF was stirred with CaH_2 for 12 h and distilled prior to use. Dioxane was purified prior to use by drying over Na followed by distillation. The *n*-amyl alcohol used in this study was dried over 4 Å molecular sieves and distilled.

8-Aminoisoindolo[1,2-*b*]quinazolin-12(10*H*)-one (1). Hydrogenation of 8-nitroisoindolo[1,2-*b*]quinazolin-12(10*H*)-one, **7**, was accomplished at 50 psi for 25 min using 200 mg (0.7 mmol) of this nitro compound and 10% Pd–C (35.8 mg) in 10 mL of glacial HOAc. The reaction mixture was filtered and concentrated *in vacuo* to half the volume, and the yellow solid which precipitated was collected by suction filtration. The product was dried *in vacuo* to obtain 1 (160.5 mg, 90%): mp 288 °C (lit.[13] mp 287–8 °C); HRMS calcd for $C_{15}H_{11}N_3O$ 249.0902, found 249.0899. Anal. ($C_{15}H_{11}N_3O$) C, H, N.

Isoindolo[1,2-*b*]quinazolin-12(10*H*)-one (3). 2-Aminobenzylamine (122 mg, 1.0 mmol) and phthalic anhydride (140 mg, 1.0 mmol) in DMF (10 mL) were heated at reflux under N_2 for 3 h. DMF was then distilled off and the oil obtained chromatographed on silica (EtOAc:hexane, 1:9). A yellow solid was isolated and recrystallized twice (CH_2Cl_2/hexane then THF/hexane) to obtain (100 mg, 43%) of **4**: mp 180–182 °C (lit.[12] mp 182–183 °C); IR 1726 cm^{-1}; ^1H NMR δ 4.9 (2H, s, CH_2), 7.2 (3H, m, H8 + H7 + H6), 7.5 (1H, d, J = 8, H9), 7.6 (2H, m, H3 + H4), 7.8 (1H, m, H2), 8.04 (1H, d, J = 7.3, H1), ^{13}C NMR δ 41.1, 121.7, 122.6, 123.6, 123.9, 127.3, 128.1, 128.9, 129.2, 132.5, 133.1, 134.9, 140.7, 149.3, 167.3; HRMS calcd for $C_{15}H_{10}N_2O$: 234.0782, found: 234.0788. Anal. ($C_{15}H_{10}N_2O$) C, H.

Isoindolo[2,1-*a*]pyrimidino[4,3-*d*]pyridin-10(12*H*)-one (4). 4-Amino-3-(aminomethyl)pyridine (123 mg, 1 mmol) and phthalic anhydride (148 mg, 1 mmol) in DMF (30 mL) were heated at reflux using a Dean–Stark trap. After 5 h the reaction mixture was concentrated to half the volume, and ether (30 mL) was added. The reaction mixture was then kept at 4 °C for 5 h. The dark purple solid which formed was collected by suction filtration and dried over P_2O_5 under vacuum to give 138 mg (59%) of **5**: mp 254–256 °C; IR (Nujol) 1725 cm^{-1}; ^1H NMR (DMSO) δ 4.9 (2H, s, CH_2), 7.36 (1H, d, J = 5, H6), 8.0 (4H, m, aromatic H), 8.53 (2H, m, H7 + H9); ^{13}C NMR δ 41.1 117.7, 121, 121.8, 123.4, 130.5, 133.6, 133.9, 134, 146.9, 148.9, 150.6, 153, 163.3; HRMS calcd for $C_{14}H_9N_3O$ 235.0745, found 235.0734.

8-Amino-11-oxoisoindolo[2,1-*a*]benzimidazole (5). The *N*-phenylphthalimide (**10**) (250 mg, 0.6 mmol) was heated together with Fe powder (267 mg, 4.7 mmol) and 50% aqueous HOAc (10 mL) at 100 °C for 4 h. The reaction mixture was allowed to cool to room temperature, and water (10 mL) was added. The reaction mixture was repeatedly extracted six times with EtOAc:*t*-BuOH (9:1). The organic layer was dried and concentrated to give a brown solid which was chromatographed on silica (MeOH:EtOAc 5% and then 10%) to give a colorless solid. This compound (243 mg, 0.6 mmol) and CsF (297 mg, 1.9 mmol) in DMF (5 mL) was heated under N_2 at 120 °C for 12 h. DMF was distilled off, and the resulting yellow solid was chromatographed on silica (MeOH:EtOAc, 4:5) to give a greenish yellow solid which was recrystallized (THF: hexane) to give 105 mg (80%) of **6**: mp 175–176 °C dec; IR 3336, 3214, 1636; ^1H NMR δ 6.5 (1H, dd, J_1 = 2, J_2 = 8.6, H7), 6.68 (1H, d, J = 1.9, H9), 7.3 (1H, d, J = 8.5, H6), 7.4 (2H, m, H2 + H3), 7.77 (1H, dd, J_1 = 1.5, J_2 = 7.2, H4), 8.16 (1H, d, J = 6.3, H1); ^{13}C NMR δ 96.6, 112.1, 116.9, 127.9, 128.7, 128.8, 130.8, 133.3, 138.1, 138.4, 144.9, 150, 171; HRMS calcd for $C_{14}H_9N_3O$ 235.0745, found 235.0739. Anal. ($C_{14}H_9N_3O$) C, H.

8-Nitroisoindolo[1,2-*b*]quinazolin-12(10*H*)-one (6). 5-Nitro-2-aminobenzylamine (1.6 g, 9.5 mmol) and phthalic anhydride (1.4 g, 10 mmol) in *n*-amyl alcohol (50 mL) were refluxed overnight under N_2. The resulting yellow solution was concentrated to 10 mL and kept at 4 °C for 5 h. The golden yellow crystals that formed were collected by suction filtration and washed with *n*-amyl alcohol (15 mL) and dried *in vacuo* to give 1.5 g of **7** (76.9%): mp 282–285 °C; IR 1727; ^1H NMR 5.07 (2H, s, CH_2), 7.61 (1H, J = 9.2, H4), 7.8 and 8.07 (3H and 1H, respectively, m, H6 + H4 + H3 + H2 and H1) 8.18 (1H, dd, J_1 = 2.6, J_2 = 8.7, H7) 8.3 (1H, d, J = 2.6, H9); ^{13}C NMR ($CDCl_3$) 68.8, 150 7, 151.1, 151.8, 155.7, 156.7, 158.3, 159.8, 161.4, 161.7, 162, 162.3, 174.2; HRMS calcd for $C_{15}H_9N_3O$ 279.0643, found 279.0642. Anal. ($C_{15}H_9N_3O$) C, H.

Isoindolo[2,1-*a*]imidazo[5,4-*c*]pyridin-10-one (7). 3,4-Diaminopyridine (500 mg, 4.5 mmol) and phthalic anhydride (700 mg, 4.7 mmol) in dioxane was refluxed for 6 h under N_2. The reaction mixture was concentrated, and the resulting precipitate was collected by suction filtration. The crude material was then suspended in 1,2,3-trimethylbenzene and refluxed under N_2 for 3 h using a Dean–Stark trap to remove water. After 3 h solvent was distilled and resulting oil was chromatographed on silica (EtOH:MeOH, 4:1) to give 378 mg (38%) of **7**: mp > 200 °C; IR 1625 cm^{-1}; ^1H NMR 7.6 (3H, m, aromatic H), 7.9 (2H, m, aromatic H), 8.33 (1H, d, J = 6, aromatic H) 8.9 (1H, s, H-9); ^{13}C NMR δ 109.6, 129.7, 129.9, 130.1, 130.3, 130.7, 131.1, 134.17, 138.7, 140.4, 142.9, 155, 168; HRMS calcd for $C_{13}H_7N_3O$ 221.0589, found 221.0578. Anal. ($C_{13}H_7N_3O$) C, H.

11-Oxoisoindolo[2,1-*a*]benzimidazole (8). 1,2-Phenylenediamine (250 mg, 2.3 mmol) and phthalic anhydride (350 mg, 2.5 mmol) in *n*-amyl alcohol (10 mL) were heated at reflux for 2 h. The reaction mixture was allowed to cool to room temperature, and the colorless precipitate formed was collected by suction filtration. This solid was washed several times with ether (50 mL) and vacuum dried for 2 days to obtain 197 mg (39%) of **8**: mp >290 °C; IR (Nujol) 1645 cm^{-1}; ^1H NMR δ 7.2 (2H, m), 7.6 (4H, m), 7.8 (3H, m); ^{13}C NMR δ 115.3, 122.2, 122.4, 129.8, 130, 130.3, 131.1, 133.5, 139.1, 151.5, 168.9; HRMS (EI) calcd for $C_{14}H_8N_2O$ 220.0635, found 220.0647. Anal. Calcd for $C_{14}H_8N_2O$: C, 76.36; H, 8.18. Found: C, 76.09; H, 7.82.

3-Bromo-4-nitro-*N*-[[2-(trimethylsilyl)ethyl]sulfonyl]-aniline (9). 3-Bromo-4-nitroaniline (217 mg, 1 mmol) in DMF (0.5 mL) was slowly added to NaH (24 mg, 1 mmol) in DMF (0.5 mL) under N_2. The resulting brownish yellow solution was stirred for 1 h. SESCl (290 mg, 1.4 mmol) in DMF (1 mL) was then added slowly. The reaction mixture was stirred for 2 h and poured into ice water (20 mL). The aqueous layer was extracted with EtOAc (20 × 3 mL), and organic layer was dried (Na_2SO_4) and concentrated. The yellow oil obtained was chromatographed on silica (10% EtOAc:hexane) to obtain the title compound which was recrystallized (CH_2Cl_2:hexane) to give 232 mg (61%) of **9** as a yellow solid; mp 145–146 °C; IR 3237 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.04 (9H, s, TMS), 1.03 and 3.16 (2H each, m, CH_2s), 7.30 (1H, dd, J_1 = 4, J_2 = 7, aromatic H), 7.5 (1H, d, J = 4, aromatic H), 7.7 (NH, brs), 7.97 (1H, d, J = 7, aromatic H); ^{13}C NMR ($CDCl_3$) δ −1.5, 10.8, 50.10, 117.2, 117.3, 124.1, 128.2, 142.5, 145.2; HRMS (EI) calcd for $C_{11}H_{17}BrN_2OSiS$ 379.9861, found 379.9899. Anal. ($C_{11}H_{17}BrN_2OSiS$) C, H.

***N*-[2-Nitro-5-[[(trimethylsilyl)sulfonyl]amino]phenyl]-phthalimide (10).** 3-Bromo-4-nitro-*N*-[2-(trimethylsilyl)ethyl]sulfonylaniline (381 mg, 1 mmol), CuI (380 mg, 2 mmol), and potassium phthalimide (185 mg, 1 mmol) in DMA (10 mL) were heated at reflux for 4 h while passing a thin stream of N_2 over the reaction mixture. The DMA was distilled off from the reaction mixture and the resulting solid suspended in EtOAc. This suspension was chromatographed on silica (50% EtOAc:hexane) to give 192 mg (43%) of **10** as a yellow solid: mp 145–149 °C: IR 1731; ^1H NMR δ 0.002 (9H, s, TMS), 1.03 and 3.19 (2H each, m, CH_2s), 7.3 (2H, m), 7.8 and 7.9 (4H, m), 8.15 (2H, m); ^{13}C NMR δ −1.6, 10.6, 49.9, 118.2, 119.7, 124.7, 127.7, 128.3, 132.1, 135.4, 140.8, 143.9, 166.8; HRMS calcd for $C_{19}H_{21}N_3O_6SiS$ 447.0920, found 447.0892.

***N*-(2-Amino-5-methoxyphenyl)phthalimide (11b).** 4-Methoxy-2-nitroacetanilide (2.5 g, 11.9 mmol) in EtOH (300 mL) was hydrogenated at 50 psi over Pd–C (300 mg) for 2.5 h. The reaction mixture was filtered through Celite (20 g) and concentrated to obtain 2-amino-4-methoxyacetanilide (1.7 g, 80%): mp 148–179 °C (lit.[27] mp 147–148 °C). 2-Amino-4-methoxyacetanilide (1.8 g, 10 mmol) and phthalic anhydride (1.4 g, 10 mmol) in DMF (20 mL) was heated at reflux for 4 h. The reaction mixture was allowed to cool to room temperature

and extracted with EtOAc (3 × 50 mL). The EtOAc extracts were combined, dried (Na_2SO_4), and concentrated *in vacuo* to obtain a colorless solid which was chromatographed on silica (EtOAc:hexane, 3:7) to obtain 930 mg (30%) of **11a**: mp 227–229 °C. Anal. ($C_{17}H_{14}O_4N_2$) C, H. **11a** (310 mg, 1 mmol) in 2 N HCl (10 mL) was refluxed under N_2 for 4 h. The reaction mixture was allowed to cool to room temperature and kept at 5 °C for 12 h. The colorless crystals formed were collected by suction filtration and dried over P_2O_5 to give 77 mg (29%) of **11b**: mp 239–243 °C.

8-Methoxy-11-oxoisoindolo[2,1-*a*]benzimidazole (11c). Aminophthalimide **11b** (268 mg, 1 mmol) in 1,2,3-trimethylbenzene was heated at reflux for 6 h using a Dean–Stark trap to remove water. The reaction mixture was allowed to cool to room temperature and kept at 5 °C for 12 h. The yellow solid formed was collected by suction filtration, washed with ether (3 × 50 mL), and dried over P_2O_5 to give 195 mg (78%) of **12c**: mp 255–257 °C; IR (Nujol) 1633 cm^{-1}; ^1H NMR (DMSO) δ 3.82 (3H, s, OMe), 6.87 (1H, dd, $J_1 = 2.1$, $J_2 = 8.7$, H-7), 7.1 (1H, d, $J = 2.1$, H9), 7.48 (1H, d, $J = 8.8$, H6), 7.7 (3H, m, aromatic H), 7.8 (d, 1H, $J = 7.3$, H1); ^{13}C NMR (DMSO) δ 55.8, 97.4, 112, 116.4, 129.6, 129.9, 130.1, 131, 133.4, 134.2, 139, 150, 156, 169; HRMS (EI) calcd for $C_{15}H_{10}N_2O_2$ 250.0742, found 250.0745. Anal. ($C_{15}H_{10}N_2O_2$) C, H.

***N*-(2-Amino-6-methylphenyl)phthalimide (12b).** 2-Amino-3-methylacetanilide (492 mg, 3 mmol) and phthalic anhydride (420 mg, 3 mmol) in *n*-amyl alcohol (10 mL) were heated at reflux for 1.5 h. The reaction mixture was allowed to cool to room temperature and diluted with ether (20 mL). The resulting mixture was kept at 4 °C for 8 h. The white solid formed was collected by suction filtration. The crude product (**12a**) was suspended in 2 N HCl (10 mL) and heated at 100 °C for 4 h under N_2. The reaction mixture was allowed to cool to room temperature and kept at 4 °C for 12 h. The colorless crystals formed were collected by suction filtration and dried over P_2O_5 under vacuum to give *N*-(2-amino-6-methylphenyl)phthalimide (136 mg, 18%): mp >250 °C.

9-Methyl-11-oxoisoindolo[2,1-*a*]benzimidazole (12c). Aminophthalimide **12b** (252 mg, 1 mmol) in 1,2,3-trimethylbenzene (25 mL) was heated at reflux using a Dean–Stark trap to remove water. After 4 h the reaction mixture was allowed to cool to room temperature, diluted with ether (20 mL), and kept at 4 °C for 12 h. The colorless solid formed was collected by suction filtration, washed with ether (3 × 10 mL), and dried over P_2O_5 under vacuum to give 142 mg (61%) of **12c**: mp >290 °C; IR (Nujol) 1627 cm^{-1}; ^1H NMR (DMSO) δ 7.06 (1H, d, $J = 7.3$, H8), 7.16 (1H, t, $J = 7.3$, H7), 7.43 (1H, d, $J = 7.3$, H6), 7.7 (2H, m, H2 + H3), 7.85 and 7.93 (1H each, d, $J = 7.1$, H1 + H4); ^{13}C NMR δ 17.01, 112.6, 122.5, 122.8, 124.9, 129.8, 130, 130.2, 130.7, 131.3, 133.2, 138.1, 138.2, 151, 168.6; HRMS calcd for $C_{15}H_{10}N_2O$ 234.0793, found 234.0784. Anal. ($C_{15}H_{10}N_2O$) C, H.

***N*-(2-Nitro-3-methylphenyl)phthalimide (13a).** 2-Nitro-3-methylaniline (300 mg, 2.0 mmol) and phthalic anhydride (280 mg, 2 mmol) in *n*-amyl alcohol (10 mL) were heated at reflux for 12 h. Amyl alcohol was distilled off, and the resulting solid was chromatographed on silica (EtOAc:hexane, 1:5). The solid obtained was recrystallized twice (CH_2Cl_2:hexane) to obtain 83.6 mg (15%) of **13a**: mp 198–200 °C.

6-Methyl-11-oxoisoindolo[2,1-*a*]benzimidazole (13b). *N*-(2-Nitro-3-methylphenyl)phthalimide (**13a**) (169 mg, 0.6 mmol), Fe powder (267 mg, 4.7 mmol), and 50% aqueous HOAc were heated at 100 °C for 4 h. The mixture was allowed to cool to room temperature, and water (10 mL) was added. The resulting solution was extracted six times with EtOAc:*t*-BuOH (9:1). The organic layer was dried and concentrated to give a brown solid which was chromatographed on silica (2% MeOH in EtOAc) to give 57.4 mg of **13b** (41%) as a colorless solid: mp >290 °C; IR (Nujol) 1627 cm^{-1}; ^1H NMR (DMSO) δ 2.55 (3H, s, CH_3), 7.02 (1H, d, $J = 7.3$, H4), 7.1 (1H, t, $J = 7.4$, H8), 7.6 (2H, m, aromatic H), 7.88 (2H, m, H1 + H4); ^{13}C NMR (DMSO) δ 17, 112.8, 122.3, 122.5, 124.9, 129.7, 130, 130.3, 130.4, 130.8, 134.3, 138.5, 138.7, 151.3, 169.2; HRMS (EI) calcd for $C_{15}H_{10}N_2O$ 234.0793, found 234.0789. Anal. ($C_{15}H_{10}N_2O$) C, H.

8-Hydroxy-11-oxoisoindolo[2,1-*a*]benzimidazole (14). Aminophthalimide **11b** (268 mg, 1 mmol) was heated at reflux for 12 h in HBr (5 mL) and HOAc (5 mL). The solvents were distilled off, and the resulting solid was dried *in vacuo*. The crude product was suspended in 1,2,3-trimethylbenzene and refluxed for 12 h using a Dean–Stark trap to remove water. The mixture was allowed to cool to room temperature and kept at 5 °C for 6 h. The precipitate was collected by suction filtration to give 63.8 mg (29%) of **14**: mp >290 °C; IR 3443, 1631 cm^{-1}; ^1H NMR (DMSO) δ 6.94 (1H, d, $J = 8.6$, H7), 710 (1H, brs, H9), 7.55 (1H, d, $J = 8.7$, H6), 7.8 (3H, m, aromatic H), 8.04 (1H, d, $J = 6.8$, H1); ^{13}C NMR (DMSO) δ 99.4, 115.4, 117.9, 126, 126.4, 132.8, 133, 133.1, 134.1, 134.2, 134.4, 150.6, 158.6, 167.9; HRMS calculated for $C_{14}H_8N_2O_2$ 236.0585, found 236.0588. Anal. ($C_{14}H_8N_2O_2$) C, H.

Biological Methods. Topoisomerase II-Mediated DNA Cleavage Assay. DNA topoisomerase II was purified from calf thymus gland by previously detailed procedures.[20] Plasmid YEPG was also purified by the alkali lysis methods followed by phenol deproteination and by CsCl/ethidium isopycnic centrifugation.[21] The end-labeling of the plasmid was accomplished as previously reported.[22] The cleavage assays were performed as reported previously.[23]

Unscheduled DNA Synthesis in Rat Primary Hepatocytes. Hepatocytes were isolated from F-344 rats (220–300 g) as previously described.[24] Approximately 5 × 10^5 cells were seeded into 6-well dishes containing plastic thermonox coverslips. After 2 h, cultures were washed, refed with serum free Williams medium E, and simultaneously exposed to 10 μCi/mL tritiated thymidine (50–90 Ci/mmol), and test compounds were dissolved in DMSO. After 18–20 h, the coverslips were washed, and incorporation of labeled thymidine was determined by autoradiography.[25,26] Twenty cells were counted per slide, and each concentration was evaluated in triplicate. Data are expressed as the mean ± SD of two experiments.

Evaluation of Cytotoxicity in T-cell Leukemia, CCRF CEM Cells. The MTT-microtiter plate tetrazolium cytotoxicity assay (MTA) was used in this study to evaluate relative cytotoxicity.[27–29] CCRF CEM cells (30 000 cells/well) were seeded in 200 μL of growth medium in Corning 96-well microtiter plates and allowed to attach. Each dose of drug was evaluated in four replicate plates. Each plate contained eight replicate control wells which were treated with DMSO only.

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