Synthesis and Preliminary Biological Evaluations of CC-1065 Analogues: Effects of Different Linkers and Terminal Amides on Biological Activity

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Received October 12, 1999

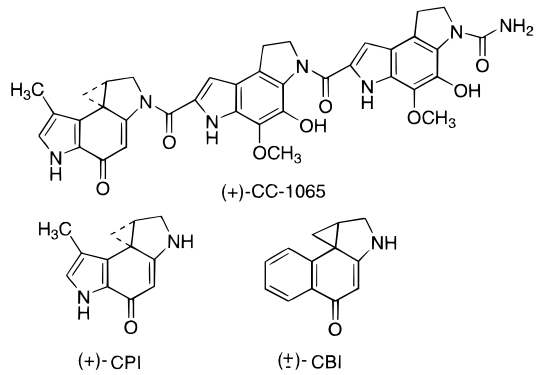
CC-1065 analogues possessing a biologically active CBI functional group and amide-substituted indole and benzofuran were synthesized. The IC_{50} values of compounds **26**, bearing two indoles, and **25**, bearing only one indole, are 0.4 and 3 nM, respectively, against U937 leukemia cells in vitro. The IC_{50} values of compounds **28**, bearing a butyramino group, and **27**, bearing an acetamino group, are 0.008 and 0.4 nM, respectively, against U937 leukemia cells in vitro. Compound **29**, bearing a double-bond linker, is about 4-fold more potent than **25**, bearing no double-bond linker. Compound **26** is highly potent against all cell lines tested in the NCI in vitro screening with IC_{50} values in the 0.1−5 nM range for most cell lines. Compounds **26** and **30** are highly active against L1210 leukemia in mice. Compound **26** is also active against B16BL6 melanoma in mice. Most importantly, **26** and **30** are not myelosuppressive at therapeutically effective doses. The mechanism of tumor cell death is through induction of apoptosis, and is accompanied by DNA fragmentation.

Introduction

CC-1065 (Chart 1) was first isolated from *Streptomyces zelensis* by scientists at the Upjohn Company[1] and was found to have potent antitumor and antimicrobial activity both in vitro and in experimental animals.[1−4] The duocarmycins discovered later were found to possess chemical structures and biological activities similar to those of CC-1065.[5−9] CC-1065 binds to double-stranded B-DNA within the minor groove with the sequence preference of 5′-d(A/GNTTA)-3′ and 5′-d(AAAAA)-3′ and alkylates the N3 position of the 3′-adenine with its left-hand CPI segment.[10−12] CC-1065 also inhibits gene transcription by interfering with binding of the TATA box binding protein to its target DNA.[13] Despite its high potency and broad spectrum of antitumor activity, CC-1065 cannot be used in humans because it causes delayed death in experimental animals.[14]

To pursue compounds retaining the potent antitumor activity but devoid of the toxic side effects of the parent compound, many CC-1065 analogues have been synthesized and tested.[15−41] Among them are the CC-1065 analogues adozelesin (U-73975), bizelesin (U-77779), and carzelesin (U-080244), as well as the duocarmycin analogue KW-2189. These compounds are currently in clinical trials.[42] CC-1065 analogues, in which the right-hand indole was replaced by C4-substituted pyrrole linked to the CPI unit by different linkers, were synthesized by Wang et al.[24,25,28,31,36] A trans double-bond linking the CPI and the pyrrole substantially increased the cytotoxicity of the agent. A C4-terminal amide group also greatly increased cytotoxicity. Furthermore, a trans double-bond and a C4-amide group act synergistically to increase the potency in vitro.[25,31] Herein, we report the synthesis and preliminary biological evaluations of new CC-1065 analogues in which a biologically active CBI group was linked to substituted indole and benzofuran by different linkers and terminal amides (Chart 2).

Chart 1. Structures of CC-1065, CBI, and CPI



Results

Chemical Synthesis. Synthesis of acids **11** and **12** is illustrated in Scheme 1. Ethyl 5-nitroindole-2-carboxylate, **1**, and methyl 5-nitrobenzofuran-2-carboxylate, **2**, were reductively transformed to their corresponding amines **3** and **4**, respectively, by hydrogenolysis over Pd/C. Amines **3** and **4** were converted to their corresponding acetamino esters **5** and **6** by treatment with acetic anhydride in ethyl acetate with very high yields. Esters **5** and **6** were then hydrolyzed, respectively, using dilute sodium hydroxide solution (3 N), followed by neutralization using dilute hydrochloric acid (20%) to afford their corresponding acids **7** and **8**. Acids **7** and **8** were coupled to amine **3** in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDCI) to afford esters **9** and **10**, respectively. Esters **9** and **10** were then converted to their corresponding acids **11** and **12** by treatment with sodium hydroxide solution, followed by neutralization using dilute hydrochloric acid.

Acid **17** was synthesized employing a procedure similar to that for the synthesis of acid **12** (Scheme 2).

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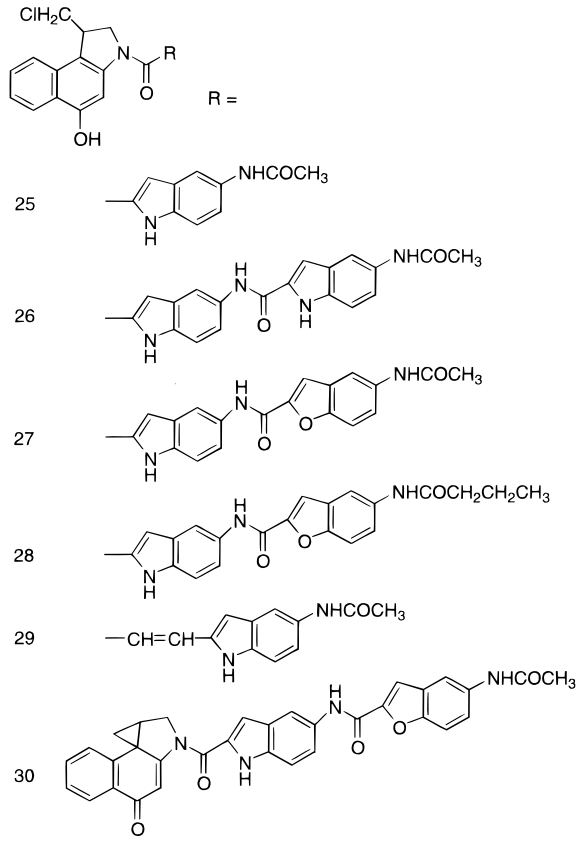
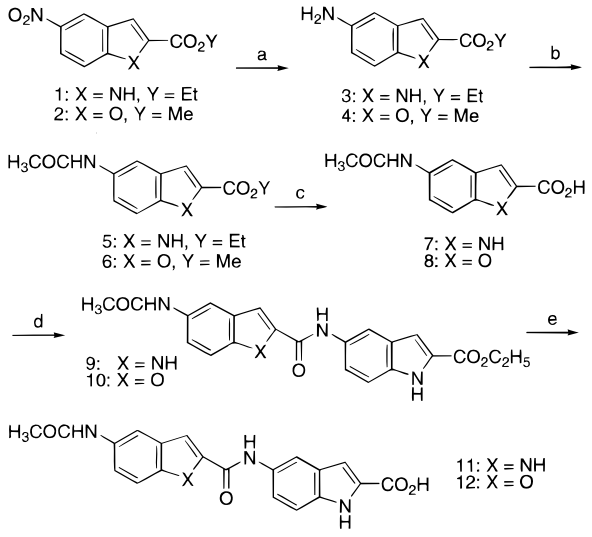
10.1021/jm990514c CCC: $19.00
Published on Web 03/17/2000

Chart 2. Structures of New CC-1065 Analogues



Scheme 1. Synthesis of Compounds **11**−**12**

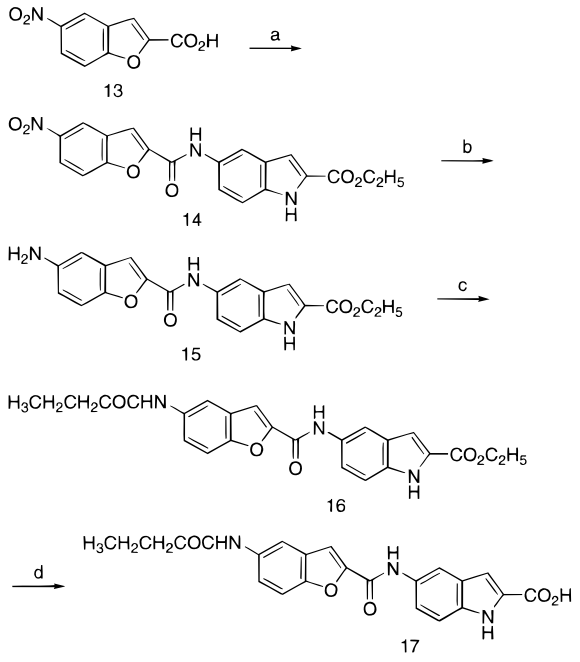


(a) H_2, Pd/C; (b) Ac_2O; (c) NaOH then HCl; (d) **3**, EDCI; (e) NaOH then HCl.

5-Nitrobenzofuran-2-carboxylic acid, **13**, was treated with amine **3** in the presence of EDCI to afford **14**. The latter was hydrogenated to give amine **15**, which was then reacted with butyric anhydride to afford butyramino ester **16**. The latter was hydrolyzed using sodium hydroxide solution to give **17**, after neutralization using dilute hydrochloric acid solution.

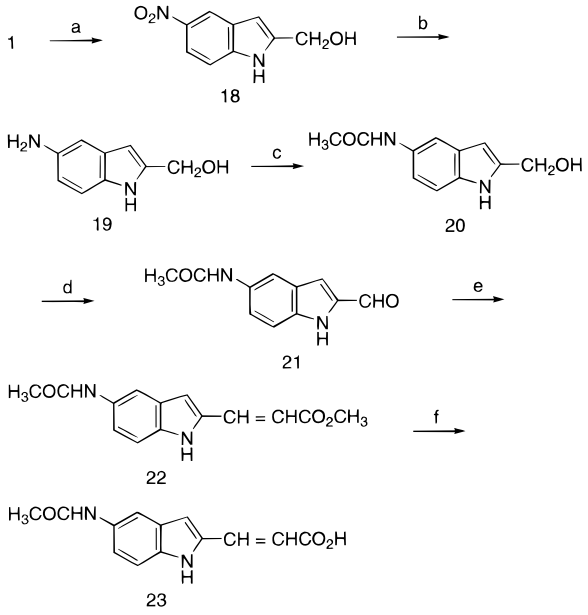
A strategy previously developed for the synthesis of 2-pyrroleacrylic acid was adapted to synthesize 2-indoleacrylic acid, **23** (Scheme 3).[28,31] Treatment of ethyl 5-nitroindole-2-carboxylate, **1**, with lithium aluminum

Scheme 2. Synthesis of Compound **17**



(a) **3**, EDCI; (b) H_2, Pd/C; (c) $(C_3H_7CO)_2O$; (d) NaOH then HCl.

Scheme 3. Synthesis of Compound **23**

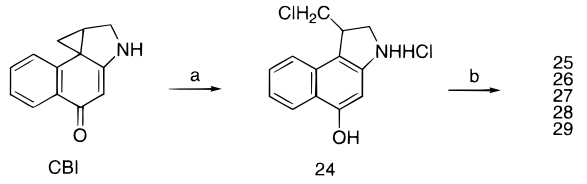


(a) $LiAlH_4$; (b) H_2, Pd/C; (c) AcCl then NaOH; (d) MnO_2; (e) $Ph_3P{=}CHCO_2CH_3$; (f) NaOH then HCl.

hydride in the presence of sulfuric acid at 0 °C for 30 min cleanly reduced the ester into alcohol **18** with high yield (90%). The latter was hydrogenated over Pd/C to afford amine **19**. Treatment of **19** with acetyl chloride followed by basic hydrolysis transformed the 5-amino to a 5-acetamino group. The 2-hydroxymethyl group of **20** was oxidized using manganese dioxide in ethanol to its corresponding aldehyde **21**. The aldehyde was then refluxed with methyl (triphenylphosphoranylidene)-acetate in toluene for 3 days to afford ester **22**. Basic hydrolysis of the latter afforded 5-acetamino-2-indoleacrylic acid, **23**.

The CBI-bearing compounds **25**−**29** were synthesized as shown in Scheme 4. Treatment of acids **7**, **11**, **12**,

Scheme 4. Synthesis of Compounds **25**−**29**



(a) anhydrous HCl in EtOAc; (b) RCO_2H, EDCI, RCO_2H = **7**, **11**, **12**, **17** and **23**.

Table 1. Cytotoxicity against U937 Leukemia Cells

compd	IC_{50} (nM)[a]	compd	IC_{50} (nM)[a]
25	3.0	**29**	0.9
26	0.4	**30**	0.2
27	0.4	Adr	100
28	0.008		

[a] IC_{50} values are defined as the minimal drug concentration necessary to inhibit incorporation of [^{3}H]thymidine by 50% and are the averages of three experiments.

17, and **23**, respectively, with amine **24**[22] in the presence of EDCI afforded targets **25**−**29**. Compound **27** was treated with triethylamine, acetonitrile, and water to give **30** (85% yield).

Biological Studies. 1. Cytotoxicity. The new agents were tested in vitro against U937 leukemia cells, and the results are summarized in Table 1. Compound **26**, bearing two indoles with an IC_{50} value of 0.4 nM, is 7-fold more potent than **25**, bearing only one indole with an IC_{50} value of 3 nM. Compound **28**, bearing a butyramino group with an IC_{50} value of 0.008 nM, is 50-fold more potent than **27**, bearing an acetamino group with an IC_{50} value of 0.4 nM. Compound **26**, bearing two indoles, is as potent as **27**, bearing one indole and one benzofuran. Compound **29**, bearing a double-bond linker, is about 4-fold more potent than **25**, bearing no double-bond linker. Compound **30**, with an IC_{50} value of 0.2 nM, is 2-fold more potent than its corresponding uncyclized precursor **27**.

Cytotoxicity of **26** was also tested by the National Cancer Institute (NCI), and the results are shown in Table 2. As expected, **26** is highly potent against all cell lines tested. For all cell lines IC_{50} values were less than 10 nM for a 48-h drug exposure, with most in the 0.1−5 nM range.

2. DNA Fragmentation and Apoptosis. In general, cell death occurs through either necrosis or apoptosis. It is now recognized that most anticancer drugs, including the CC-1065 and duocarmycin classes of agents, induce apoptosis.[44−46] To examine the mechanism of cytotoxic action of these new compounds, **27** was chosen for further studies using U937 cells. At a 10 nM concentration, **27** caused DNA fragmentation in about 12%, 20%, 40%, and 80% of U937 cells for incubation times of 2, 3, 4, and 6 h, respectively (Figure 1). Cells exhibited the morphological changes typical of apoptosis. The kinetics of appearance of morphologically apoptotic cells correlated well with DNA fragmentation. More than 80% of cells were in apoptotic state after 6 h of incubation. Cell death was not obvious until 5 h of incubation, and all cells were dead after 20 h of incubation. These results agree with those found for duocarmycin analogues.[46]

3. Antitumor Screening in Mice. The antitumor activity of **26** and **30** was tested against L1210 leukemia cells (inoculated with 10^5 cells/mouse) in mice, and the results are shown in Table 3. At a dose of 70 μg/kg, **26** and **30** produced an increase in life span (ILS) of 107% and 93%, respectively. Cyclophosphamide (CP) produced an ILS of 173% at a dose of 125 mg/kg and 100% cures at a dose of 188 mg/kg.

The antitumor activity of **26**, **28**, and **30** was also tested in mice inoculated with a low burden of L1210 leukemia cells (100 cells/mouse), and the results are shown in Table 4. At a dose of 30 μg/kg, **26**, **28**, and **30** produced 40%, 20%, and 60% of long-term survivors (60 days), respectively. In contrast, at a dose of 30 mg/kg, cyclophosphamide produced 50% of long-term survivors, and taxol did not produce any long-term survivors.

Compound **26** was also tested in mice bearing B16 melanoma, and the results are shown in Table 5. In this experiment, **26** is not as effective as adriamycin (Adr).

Table 2. Cytotoxicity of **26** against NCI Tumor Panels[a]

panel/cell line	IC_{50} (nM)	panel/cell line	IC_{50} (nM)
leukemia:		melanoma:	
CCRF-CEM	3.14	LOX IMVT	0.577
HL-60 (TB)	1.40	M14	1.48
K-562	3.76	SK-MEL-2	2.11
MOLT-4	0.375	SK-MEL-28	2.21
RPMI-8226	8.98	SK-MEL-5	1.11
SR	0.562	UACC-257	2.46
non-small-cell lung cancer:		UACC-62	0.419
A549/ATCC	1.49	ovarian cancer:	
EKVX	2.39	IGROV1	1.01
HOP-62	1.25	OVCAR-3	3.51
HOP-92	2.11	OVCAR-8	1.60
NCI-H23	1.36	SK-OV-3	4.33
NCI-H322M	2.13	renal cancer:	
NCI-H460	1.48	786-0	1.66
NCI-H522	0.238	A498	3.95
colon cancer:		ACHN	1.28
COLO 205	2.99	CAKI-1	4.27
HCC-2998	4.03	SN12C	3.01
HCT-116	0.495	TK-10	3.13
HCT-15	>10	UO-31	4.05
HT29	1.34	prostate cancer:	
KM12	1.78	PC-3	2.03
SW-620	2.36	DU-145	0.982
CNS cancer:		breast cancer:	
SF-268	0.212	MCF7	0.476
SF-295	2.64	NCI/ADR-RES	8.56
SF-539	1.24	MDA-MB-231/ATCC	5.97
SNB-19	2.59	HS 578T	1.63
SNB-75	0.513	MDA-MB-435	1.86
U251	0.719	MDA-N	2.22
		BT-549	3.29
		T-47D	1.68

[a] The assay was performed by NCI using the SRB method (48-h incubation).[43]

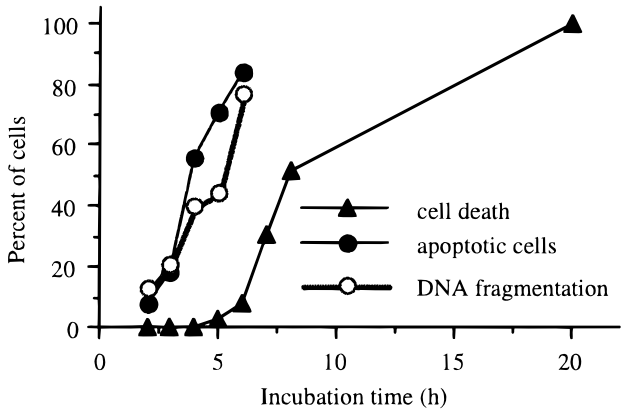


Figure 1. DNA fragmentation, apoptosis, and cell death.

Table 3. Antitumor Activity in Mice Bearing L1210 Leukemia[a]

compd	dose (/kg)	% weight change[b]	% ILS	30-day survivors
26	25 μg	+23	67	0
	42 μg	+16	107	0
	70 μg	+9	107	1
30	25 μg	+23	53	0
	42 μg	+18	80	1
	70 μg	−15	93	0
CP	125 mg	−5	173	0
	188 mg	−16		6
	250 mg	−22		3

[a] BDF_1 male mice (4−6 week old, 6/group) were used. Each mouse was inoculated with 10^5 cells (0.1 mL) ip on day 0. Drugs were administered on days 1, 5, and 9 ip. Antitumor activity was determined by comparing the median survival time of the treated groups (T) with that of a control group (C) and are expressed as a percentage of ILS. The median number of days the vehicle-treated mice died was 7.5. [b] Group body weight change between days 0 and the day when the animal weight was the lowest (days 9 for CP and 12 for **26** and **30**).

Table 4. Antitumor Activity in Mice Bearing L1210 Leukemia[a]

compd	dose (/kg)	60-day survivors (%)
26	30 μg	40
28	30 μg	20
30	30 μg	60
CP	30 mg	50
taxol	30 mg	0

[a] One hundred L1210 leukemia cells were injected ip to female CDF_1 mice (10 mice/group) on day 0. Drugs were administered on days 1, 5, and 9 ip. The median number of days the untreated group of mice (given the vehicle only) died was 23.

Table 5. Antitumor Activity in Mice Bearing B16BL6 Melanoma[a]

compd	dose (/kg)	weight change (g/mouse)[b]	% ILS	90-day survivors
26	25 μg	1.1 ± 0.6	50	0
	50 μg	0.3 ± 0.6	59	0
	100 μg	0.9 ± 0.9	91[c]	1
Adr	5 mg	0.5 ± 0.6	44[d]	0
	10 mg	1.0 ± 1.3	159	2

[a] BDF_1 female mice (4−6 week old, 8/group) were used. Each mouse was inoculated with 10^6 cells (0.1 mL) ip on day 0. Drugs were administered on days 1, 5, and 9 ip. Antitumor activity was determined by comparing the median survival time of the treated groups (T) with that of a control group (C) and are expressed as a percentage of ILS. The median number of days the untreated mice (given the vehicle only) died was 16. [b] Mean body weight change between days 1 and 10. [c] One mouse died on day 12. [d] One mouse died on day 14.

However, **26** may be useful in the treatment of adriamycin-resistant cells because **26** is not cross-resistant with adriamycin in B16 melanoma cells (data not shown).

4. Hematological Measurements. At a dose of 50 μg/kg, 27 days after the drugs were given to non-tumor-bearing CDF_1 mice, **26** and **30** had no effect on white blood cell (WBC) and platelet counts compared with controls (Table 6). However, the conventional alkylating agents cyclophosphamide and busulfan significantly suppressed both WBC and platelets. As expected, taxol and 5-FU had little effect on WBC and platelets. All of the compounds tested had little effect on red blood cell counts (RBC) and the mean corpuscular hemoglobin concentration (MCHC).

Table 6. Hematological Effects in Mice[a]

drug	dose (/kg)	WBC (10^3/μL)	platelet (10^6/μL)	RBC (10^6/μL)	MCHC (g/dL)
saline		12.20	1.13	9.56	33.70
30% DMSO/ 0.5% glucose		12.85	1.16	8.50	34.45
26	50 μg	13.10	1.10	8.72	36.70
30	50 μg	13.95	1.01	7.81	35.50
CP	30 mg	11.22	0.77	7.67	37.25
taxol	30 mg	13.40	1.04	8.33	36.50
5-FU	30 mg	12.40	1.24	8.82	35.40
busulfan	30 mg	7.00	0.71	8.45	35.65

[a] Female CDF_1 mice (10 mice/group) were used. Drugs were administered on day 1 ip. On day 27, blood samples were taken and hematological measurements were performed. Data are presented as the median value calculated from 10 mice.

Discussion

Consistent with previous observations, the major factors determining the potency of the CC-1065 class of compounds include hydrophobic interaction and van der Waals contacts between the drug and DNA. The fact that compounds **26**, with two indoles, and **27**, with one indole and one benzofuran, are more potent than **25**, with only one indole, further supports this hypothesis. That compound **28**, with a more hydrophobic butyramino group, is approximately 50-fold more potent than **27**, with a less hydrophobic acetamino group, is also in agreement with this hypothesis. Whereas in the case of the CPI-pyrrole agents a trans double-bond linker between the CPI and the right-hand pyrrole drastically enhanced their cytotoxicity (IC_{50}: 1.0×10^{-6} nM against KB cells in vitro),[28,31] by contrast, **29** (IC_{50}: 0.9 nM), also bearing a trans double-bond linker, is only slightly more potent than **25** (IC_{50}: 3 nM), bearing no such linker. The reason for this dramatic effect of a trans double-bond linker on CPI and CBI compounds is not well-understood.

Similar to other CC-1065 and duocarmycin classes of agents,[46] the new agents induced DNA fragmentation and apoptosis. The link between DNA alkylation, DNA fragmentation, and apoptosis caused by them is not clear. We showed previously that apoptosis induced by **27** was inhibited by a serine protease inhibitor DK120.[47] DK120 inhibits a novel serine protease termed AP24 (apoptotic protease of 24 kDa).[48] AP24 apparently functions downstream of a family of cysteine proteinases called caspases in apoptosis induced by various chemotherapeutic agents.[48−49] Activation of AP24 then transmits signals to the nucleus to induce DNA fragmentation, and thus inhibition of this protease prevents apoptotic cell death. The CC-1065 class of compounds binds to DNA and alkylates N3 of adenine. However, it is not clear whether **27** acts directly or indirectly via DNA alkylation to activate AP24, which then triggers apoptosis.

As seen from Tables 3 and 4, compounds **26** and **30** showed significant activity in mice bearing L1210 leukemia cells. Compound **26** was also active in B16 melanoma-bearing mice. Most importantly, in comparison to cyclophosphamide and busulfan, they had little suppressive effects on WBC and platelet counts at a dose of 50 μg/kg. However, CC-1065, at a dose of 25 μg/kg, severely suppressed WBC.[14] These results suggest that a terminal acetamino group may preserve antitumor activity and reduce myelosuppression, the main side effects associated with this class of compounds. The four CC-1065 and duocarmycin analogues, adozelesin, bizelesin, carzelesin, and KW-2189, currently under clinical

development, have remarkable antitumor activity in mice. However, suppression of human bone marrow undermines their clinical effectiveness. Further experiments to confirm these preliminary results are in progress, and we will report the results in due course.

Experimental Section

Chemistry. ^{1}H NMR spectra were recorded at ambient temperature on an NT-360 spectrometer. Elemental analysis was performed by Atlantic Microlab, Inc. at Norcross, GA. High-resolution mass spectra (FABHRMS) were recorded on a modified MS50 mass spectrometer equipped with a VG 11-250J data system. Analytical thin-layer chromatography was performed on silica-coated plastic plates (silica gel 60 F-254, Merck) and visualized under UV light. Preparative separations were performed by flash chromatography on silica gel (Merck, 70−230 mesh).

Ethyl 5-Acetaminoindole-2-carboxylate (5). To ethyl 5-nitroindole-2-carboxylate, **1** (500 mg, 2.14 mmol), in ethyl acetate (100 mL) was added 5% Pd/C (100 mg) and the reaction mixture was hydrogenated for 1 h at a pressure of 60 lb/in.^{2} at room temperature. The reaction mixture was filtered, and the solvent was removed in vacuo to afford **3** as a yellow solid. Without further purification, **3** was dissolved in ethyl acetate (10 mL) and treated with acetic anhydride (2 mL). The reaction mixture was stirred at room temperature for 1 h. Methanol (2 mL) was added, and the reaction mixture was stirred for an additional 30 min. Solvent was removed and a gray powder was obtained (540 mg, 100% yield). An analytical sample was recrystallized in ethyl acetate, mp 202−203 °C. ^{1}H NMR (DMSO-d_6, ppm): 11.74 (s, 1H, NH), 9.78 (s, 1H, NH), 7.99 (s, 1H, Ar−H), 7.38−7.30 (m, 2H, Ar−H), 7.09−7.08 (m, 1H, Ar−H), 4.36−4.30 (q, 2H, J = 7.0, 13.7 Hz, CH_2CH$_3$), 2.03 (s, 3H, CH$_3$CO), 1.36−1.31 (t, 3H, J = 7.0 Hz, CH$_2$CH_3). Anal. ($C_{13}H_{14}N_2O_3$) C, H, N.

Methyl 5-Acetaminobenzofuran-2-carboxylate (6). Methyl 5-acetaminobenzofuran-2-carboxylate, **6**, was made from methyl 5-nitrobenzofuran-2-carboxylate, **2**, using a procedure similar to that used for synthesis of **5**. The yield was 100%. An analytical sample was recrystallized in ethyl acetate, mp 158−159 °C. ^{1}H NMR (DMSO-d_6, ppm): 10.03 (s, 1H, NH), 8.19−8.18 (d, 1H, J = 1.6 Hz, Ar−H), 7.76−7.75 (d, 1H, J = 1.1 Hz, Ar−H), 7.65−7.63 (d, 1H, J = 9.0 Hz, Ar−H), 7.55−7.52 (dd, 2H, J = 2.3, 8.9 Hz, Ar−H), 3.89 (s, 1H, OCH$_3$), 2.07 (s, 3H, CH$_3$CO). Anal. ($C_{12}H_{11}NO_4$) C, H, N.

5-Acetaminoindole-2-carboxylic Acid (7). A sodium hydroxide solution (3 N, 2 mL) was added to a solution of **5** (250 mg, 1.02 mmol) in methanol (7 mL) and the reaction mixture was stirred overnight at room temperature. Methanol was removed, and water (5 mL) was added. The solution was acidified to pH 2 using 20% hydrochloric acid. The precipitate was filtered and washed with water. Compound **7** was obtained as a gray powder (158 mg, 71% yield), mp 260 °C dec. ^{1}H NMR (DMSO-d_6, ppm): 11.62 (s, 1H, NH), 9.77 (s, 1H, NH), 7.98−7.97 (d, 1H, J = 1.4 Hz, Ar−H), 7.36−7.28 (m, 2H, Ar−H), 7.02 (d, 1H, J = 1.5 Hz, Ar−H), 2.03 (s, 3H, CH$_3$CO). Anal. ($C_{11}H_{10}N_2O_3$·0.6H$_2$O) C, H, N.

5-Acetaminobenzofuran-2-carboxylic Acid (8). A sodium hydroxide solution (3 N, 2 mL) was added to a solution of **6** (302 mg, 1.3 mmol) in methanol (20 mL) and the reaction mixture was stirred for 48 h at room temperature. Solvent was evaporated, and water (20 mL) was added. The solution was acidified to pH 2 using 20% hydrochloric acid. The precipitate was filtered and washed with water. Compound **8** was obtained as a gray powder (231 mg, 81% yield), mp >300 °C. ^{1}H NMR (DMSO-d_6, ppm): 13.30 (s, 1H, COOH), 10.01 (s, 1H, NH), 8.16−8.15 (d, 1H, J = 1.9 Hz, Ar−H), 7.65−7.60 (m, 2H, Ar−H), 7.53−7.50 (dd, 2H, J = 1.6, 8.4 Hz, Ar−H), 2.07 (s, 3H, CH$_3$CO). Anal. ($C_{11}H_9NO_4$·0.3H$_2$O) C, H, N.

5-[(5-Acetamino-1*H*-indol-2-ylcarbonyl)amino]-1*H*-indole-2-carboxylic Acid (11). EDCI (268 mg) was added to a solution of **3** (94 mg, 0.46 mmol) and **7** (101 mg, 0.46 mmol) in DMF (3 mL), and the reaction mixture was stirred overnight at room temperature. Ethyl acetate (40 mL) was added, and the mixture was washed with saturated sodium carbonate solution (10 mL) followed by water (20 mL × 2). The solution was dried using sodium sulfate, and solvent was removed in vacuo. A gray powder **9** was obtained (129 mg, 69% yield). ^{1}H NMR (DMSO-d_6, ppm): 11.82, (s, 1H, NH), 11.57 (s, 1H, NH), 10.09 (s, 1H, NH), 9.77 (s, 1H, NH), 8.13−7.15 (m, 8H, Ar−H), 4.38−4.32 (q, 2H, J = 7.0, 13.7 Hz, CH_2CH$_3$), 2.04 (s, 3H, CH$_3$CO), 1.37−1.33 (t, 3H, J = 7.0 Hz, CH$_2$CH_3). MS (ion spray, m/z): 404. Without further purification, a sodium hydroxide solution (3 mL) was added to a solution of **9** (103 mg, 0.25 mmol) in DMF (3 mL) and methanol (15 mL). The reaction mixture was stirred overnight at room temperature. Methanol was removed, and water (5 mL) was added. The solution was acidified to pH 2 using 20% hydrochloric acid. The precipitate was filtered and washed with water. Compound **11** was produced as a gray powder (45 mg, 48% yield), mp >300 °C. ^{1}H NMR (DMSO-d_6, ppm): 12.80 (br, 1H, COOH), 11.69, (s, 1H, NH), 11.57 (s, 1H, NH), 10.06 (s, 1H, NH), 9.77 (s, 1H, NH), 8.12−7.09 (m, 8H, Ar−H), 2.05 (s, 3H, CH$_3$CO). Anal. ($C_{20}H_{16}N_4O_4$·1.2H$_2$O) C, H, N.

5-[(5-Acetamino-1*H*-benzofuran-2-ylcarbonyl)amino]-1*H*-indole-2-carboxylic Acid (12). EDCI (523 mg) was added to a solution of **3** (186 mg, 0.91 mmol) and **8** (200 mg, 0.91 mmol) in DMF (3 mL) and THF (3 mL). The reaction mixture was stirred overnight at room temperature. Ethyl acetate (40 mL) was added, and the mixture was washed with saturated sodium carbonate solution (10 mL) followed by water (20 mL × 2), diluted hydrochloric acid (10 mL), and water (20 mL). The solution was dried using sodium sulfate, and solvent was removed in vacuo. Ether (20 mL) was added, and a gray powder **10** was obtained (270 mg, 73% yield): ^{1}H NMR (DMSO-d_6, ppm): 11.84, (s, 1H, NH), 10.38 (s, 1H, NH), 10.03 (s, 1H, NH), 8.15−7.15 (m, 8H, Ar−H), 4.38−4.32 (q, 2H, J = 6.9, 13.7 Hz, CH_2CH$_3$), 2.08 (s, 3H, CH$_3$CO), 1.37−1.33 (t, 3H, J = 6.8 Hz, CH$_2$CH_3). MS (ion spray, M + 2H): 406. Without further purification, a 3 N sodium hydroxide solution (3 mL) was added to a solution of **10** (140 mg, 0.35 mmol) in DMF (2 mL), acetone (10 mL), methanol (15 mL) and water (5 mL). The reaction mixture was stirred overnight at room temperature. Acetone and methanol were removed, and water (20 mL) was added. The solution was acidified to pH 2 using 20% hydrochloric acid, and the precipitate was filtered and washed with water. Compound **12** was obtained as a gray powder (54 mg, 41% yield), mp >300 °C. ^{1}H NMR (DMSO-d_6, ppm): 12.80 (br, 1H, COOH), 11.72, (s, 1H, NH), 10.36 (s, 1H, NH), 10.03 (s, 1H, NH), 8.15−7.10 (m, 8H, Ar−H), 2.08 (s, 3H, CH$_3$CO). Anal. ($C_{20}H_{14}N_3O_5$·1.1H$_2$O) C, H, N.

Ethyl 5-[(5-Nitro-1*H*-benzofuran-2-ylcarbonyl)amino]-1*H*-indole-2-carboxylate (14). EDCI (612 mg) was added to a solution of **3** (218 mg, 1.07 mmol) and **13** (221 mg, 1.07 mmol) in DMF (8 mL) and THF (10 mL). The reaction mixture was stirred overnight at room temperature. Solvent was removed, and ethyl acetate (100 mL) was added. The reaction mixture was washed with water (100 mL × 3). The organic solution was dried using sodium sulfate, and solvent was removed in vacuo. Ether was added. The product was filtered and washed with ether to afford a yellow solid (220 mg, 52% yield). An analytical sample was recrystallized in ethyl acetate. ^{1}H NMR (DMSO-d_6, ppm): 11.87, (s, 1H, NH), 10.62 (s, 1H, NH), 8.84−7.17 (m, 8H, Ar−H), 4.38−4.32 (q, 2H, J = 7.2, 14.0 Hz, CH_2-CH$_3$), 1.37−1.33 (t, 3H, J = 7.2, 14.0 Hz, CH$_2$CH_3). Anal. ($C_{20}H_{15}N_3O_6$) C, H, N.

5-[(5-Butyramino-1*H*-benzofuran-2-ylcarbonyl)amino]-1*H*-indole-2-carboxylic Acid (17). Compound **14** (200 mg, 0.51 mmol) was first dissolved in DMF (5 mL) and ethyl acetate (20 mL) was then added. Pd/C (10%, 20 mg) was added to the solution, and the reaction mixture was hydrogenated at 1 atm for 1 h. Amine **15** was filtered over Celite, and the filter cake was washed with methanol. Solvent was removed in vacuo. Without further purification, the product was dissolved in DMF (2 mL). Butyric anhydride (1 mL) and pyridine (1 mL) were added subsequently. The reaction mixture was stirred at room temperature for 1 h. Ethyl acetate (50 mL)

was added, and the solution was washed subsequently with 20 mL of water, sodium hydrogen carbonate solution and brine. The solution was dried using sodium sulfate, and solvent was removed in vacuo. To the flask was added ether, and the solid was filtered and washed with ether to produce 150 mg of white powder **16** (68% yield from **14**). Without further purification, ester **16** (100 mg, 0.23 mmol) was dissolved in DMF (3 mL), and a 3 N sodium hydroxide solution (2 mL) was then added. The reaction mixture was stirred overnight at room temperature. Solvent was removed, and water was added to give a suspension, which was then filtered. Hydrochloric acid (10%) was added to the filtrate and the precipitate was filtered. The resulting solid was washed with water and dried to afford an off-white solid (12 mg, 13%). ^{1}H NMR (DMSO-d_6, ppm): 11.72 (br, 1H, NH), 10.37, (s, 1H, NH), 10.01 (s, 1H, NH), 8.18−7.10 (m, 8H, Ar−H), 2.34−2.30 (t, 2H, J = 7.0, 14.4 Hz, CH_2CH$_2$-CH$_3$), 1.68−1.62 (m, 2H, CH$_2$CH_2CH$_3$), 0.96−0.92 (t, 3H, J = 7.4, 14.7 Hz, CH$_2$CH$_2$CH_3). Anal. ($C_{22}H_{18}N_3O_5$) C, H, N.

2-Hydroxymethyl-5-nitroindole (18). Concentrated sulfuric acid (1.27 mL) was added dropwise to lithium aluminum hydride (1.89 g) in THF (100 mL) at 0 °C under nitrogen. The reaction mixture was stirred for 20 min at 0 °C after the addition was complete. A solution of **1** (2 g, 8.5 mmol) in THF (80 mL) was then added slowly. The reaction mixture was stirred for an additional 30 min at 0 °C. Ice (10 g) was added carefully, and the mixture was filtered. The filter cake was washed with ethyl acetate (200 mL). The mixture was then washed with water (50 mL). The organic phase was dried using sodium sulfate and filtered. Solvent was removed in vacuo to produce a gray solid (1.48 g, 90% yield). An analytical sample was recrystallized in ethyl acetate, mp 156−157 °C. ^{1}H NMR (DMSO-d_6, ppm): 11.77 (brs, 1H, NH), 8.49−8.48 (d, 1H, J = 3.5 Hz, Ar−H), 7.97−7.93 (dd, 1H, J = 2.4, 9.0 Hz, Ar−H), 7.49−7.46 (d, 1H, J = 9.2 Hz, Ar−H), 6.57 (s, 1H, Ar−H), 5.42−5.39 (t, 1H, J = 5.6 Hz, OH), 4.66−4.64 (d, 1H, J = 5.5 Hz, CH$_2$OH). Anal. ($C_9H_8N_2O_3$) C, H, N.

5-Amino-2-hydroxymethylindole (19). To a solution of **18** (862 mg, 4.49 mmol) in methanol (50 mL) was added 5% Pd/C (50 mg). The reaction mixture was hydrogenated for 1 h at a pressure of 50 lb/in.^{2}. The reaction mixture was filtered through Celite, which was washed with methanol. Solvent was removed in vacuo, and 707 mg (97% yield) of gray powder was obtained, mp 159−160 °C. ^{1}H NMR (DMSO-d_6, ppm): 10.44 (s, 1H, NH), 7.01−6.99 (d, 1H, J = 8.5 Hz, Ar−H), 6.60 (d, 1H, J = 2.0 Hz, Ar−H), 6.43−6.40 (dd, 1H, J = 2.4, 8.5 Hz, Ar−H), 5.97 (brs, 1H, Ar−H), 5.07 (brs, 1H, OH), 4.50 (brs, 2H, CH$_2$OH), 4.30 (brs, 2H, NH$_2$). Anal. ($C_9H_{10}N_2O$) C, H, N.

5-Acetamino-2-hydroxymethylindole (20). To a solution of **19** (200 mg, 1.25 mmol), (dimethylamino)pyridine (20 mg) and triethylamine (0.94 mL) in THF (10 mL) cooled to 0 °C under nitrogen, was added a solution of acetyl chloride (0.30 mL, 4.16 mmol) in THF (5 mL) dropwise. The reaction mixture was allowed to warm to room temperature and stirred for 3 h. THF was removed in vacuo, and ethyl acetate (40 mL) was added. The solution was washed with water (20 mL × 2). The organic phase was dried using sodium sulfate, and solvent was removed in vacuo. The residue was dissolved in methanol (5 mL), and 3 N sodium hydroxide solution (1 mL) was added. The reaction was allowed to proceed overnight. Methanol was removed, and water (10 mL) was added. The product was extracted with ethyl acetate (30 mL × 3), and the solvent was removed in vacuo. The solution was dried using sodium sulfate. The product was crystallized in ethyl acetate to afford 128 mg (50% yield) of gray powder, mp 161 °C. ^{1}H NMR (DMSO-d_6, ppm): 10.84 (s, 1H, NH), 9.62 (s, 1 H, NH), 7.75 (d, 1H, J = 2.1 Hz, Ar−H), 7.22−7.10 (m, 2H, Ar−H), 6.20 (s, 1H, Ar−H), 6.43−6.40 (dd, 1H, J = 2.4, 8.5 Hz, Ar−H), 5.18−5.15 (t, 1H, J = 5.5 Hz, OH), 4.57−4.56 (d, 2H, J = 5.4 Hz, CH$_2$OH), 2.01 (s, 3H, CH$_3$). Anal. ($C_{11}H_{12}N_2O_2$) C, H, N.

5-Acetamino-2-indolecarboxaldehyde (21). To a solution of **20** (100 mg, 0.5 mmol) in ethanol (10 mL) was added manganese dioxide (250 mg), and the reaction mixture was stirred for 3 h at room temperature. The reaction mixture was filtered, and the solid was washed with ethanol. Solvent was removed in vacuo to produce a gray solid (97 mg, 100% yield). An analytical sample was recrystallized in ethyl acetate, mp 200 °C dec. ^{1}H NMR (DMSO-d_6, ppm): 11.80 (s, 1H, NH), 9.84 (s, 1H, NH), 9.81 (s, 1H, CHO), 8.09 (s, 1H, Ar−H), 7.38−7.32 (m, 3H, Ar−H), 2.04 (s, 3H, CH$_3$). Anal. ($C_{11}H_{10}N_2O_2$·0.4H$_2$O) C, H, N.

5-Acetamino-2-indoleacrylic Acid (23). Compound **21** (140 mg, 0.7 mmol) was added to a solution of methyl (triphenylphosphoranylidene)acetate (257 mg, 0.77 mmol) in toluene (30 mL), and the reaction mixture was heated to reflux for 3 days. Solvent was removed after the mixture was cooled to room temperature. Ethyl acetate (10 mL) was added to the residue, and the resulting precipitate was filtered, MS (ion spray, M + H) 259. Without further purification, the product was dissolved in DMF (3 mL) and methanol (5 mL). Sodium hydroxide solution (3 N, 2 mL) was added, and the reaction mixture was stirred overnight. Methanol was removed, and water (10 mL) was added. The solution was acidified to pH 2 using 20% hydrochloric acid. The resulting precipitate was filtered and washed with water. The product (44 mg, 26% yield) was obtained as a yellow solid, mp 238−239 °C. ^{1}H NMR (DMSO-d_6, ppm): 12.25 (brs, 1H, COOH), 11.42 (s, 1H, NH), 9.74 (s, 1H, NH), 7.88 (s, 1H, Ar−H), 7.55−7.50 (d, 1H, J = 16.0 Hz, CH═CH), 7.28 (s, 2H, Ar−H), 6.80 (d, 1H, J = 2.0 Hz, Ar−H), 6.44−6.40 (d, 1H, J = 15.8 Hz, CH═CH), 2.03 (s, 3H, CH$_3$). Anal. ($C_{13}H_{12}N_2O_3$·0.3H$_2$O) C, H, N.

Compounds **25**−**29** were synthesized using a procedure similar to that described below for the synthesis of **25**.

3-[(5-Acetamino-1*H*-indol-2-yl)carbonyl]-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benz[*e*]indole (25). Anhydrous hydrochloride in ethyl acetate (3 N, 4 mL) was added to CBI (20 mg, 0.1 mmol), which was synthesized according a reported procedure.[22] The reaction mixture was stirred for 30 min at room temperature in the dark. Solvent was removed to produce **24**. The latter was dissolved in DMF (1 mL). Compound **7** (23 mg, 0.11 mmol) was added, followed by the addition of EDCI (58 mg). The reaction mixture was stirred overnight at room temperature. The product was purified by thin-layer chromatography eluting with ethyl acetate. A gray powder **25** was obtained (9.5 mg, 22% yield). ^{1}H NMR (DMSO-d_6, ppm): 11.57 (s, 1H, NH), 10.52 (s, 1H, OH), 9.89 (s, 1H, NH), 8.25−7.23 (m, 9H, Ar−H), 4.90−4.85 (dd, 1H, J = 9.6, 11.0 Hz, NHH), 4.75−4.71 (dd, 1H, J = 1.8, 10.6 Hz, NHH), 4.35−4.28 (m, 1H, ClCH$_2$CHCH$_2$), 4.14−4.10 (dd, 1H, J = 3.4, 11.1 Hz, CHHCl), 3.97−3.92 (dd, 1H, J = 8.0, 11.1 Hz, CHHCl), 2.12 (s, 3H, CH$_3$). FABHRMS: calcd for ($C_{24}H_{21}ClN_3O_3$) 434.1271, found 434.1256.

3-[[5-[(5-Acetamino-1*H*-indol-2-ylcarbonyl)amino]-1*H*-indol-2-yl]carbonyl]-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benz[*e*]indole (26). Gray powder (80% yield). ^{1}H NMR (DMSO-d_6, ppm): 11.63 (s, 1H, NH), 11.61 (s, 1H, NH), 10.51 (s, 1H, OH), 10.12 (s, 1H, NH), 9.84 (s, 1H, NH), 8.38−7.29 (m, 13H, Ar−H), 4.92−4.86 (dd, 1H, J = 9.6, 11.0 Hz, NHH), 4.77−4.74 (dd, 1H, J = 1.8, 10.6 Hz, NHH), 4.36−4.28 (m, 1H, ClCH$_2$CHCH$_2$), 4.15−4.11 (dd, 1H, J = 3.0, 10.8 Hz, CHHCl), 3.98−3.93 (dd, 1H, J = 7.9, 11.0 Hz, CHHCl), 2.04 (s, 3H, CH$_3$). FABHRMS: calcd for ($C_{33}H_{27}ClN_5O_4$) 592.1752, found 592.1733.

3-[[5-[(5-Acetamino-1*H*-benzofuran-2-ylcarbonyl)amino]-1*H*-indol-2-yl]carbonyl]-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benz[*e*]indole (27). Gray powder (53% yield). ^{1}H NMR (DMSO-d_6, ppm): 11.66 (s, 1H, NH), 10.51 (s, 1H, OH), 10.46 (s, 1H, NH), 10.10 (s, 1H, NH), 8.42−7.30 (m, 13H, Ar−H), 4.92−4.88 (t, 1H, J = 10.2 Hz, NHH), 4.77−4.74 (dd, 1H, J = 1.8, 10.9 Hz, NHH), 4.36−4.28 (m, 1H, ClCH$_2$CHCH$_2$), 4.15−4.11 (dd, 1H, J = 2.7, 10.7 Hz, CHHCl), 3.98−3.93 (dd, 1H, J = 7.7, 11.0 Hz, CHHCl). FABHRMS: calcd for ($C_{33}H_{25}$-ClN_4O_5) 592.1508, found 592.1506.

3-[[5-[(5-Butyramino-1*H*-benzofuran-2-ylcarbonyl)amino]-1*H*-indol-2-yl]carbonyl]-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benz[*e*]indole (28). Gray powder (42% yield). ^{1}H NMR (DMSO-d_6, ppm): 11.75 (s, 1H, NH), 10.45 (s, 1H, OH), 10.43 (s, 1H, NH), 10.00 (s, 1H, NH), 8.21−7.23 (m, 13H, Ar−H), 4.85−4.80 (t, 1H, J = 10.2 Hz, NHH), 4.60−4.56 (m,

1H, NH*H*), 4.26−4.20 (m, 1H, $ClCH_2CHCH_2$), 4.06−4.00 (m, 1H, C*H*HCl), 3.92−3.88 (m, 1 H, CH*H*Cl). FABHRMS: calcd for ($C_{35}H_{30}ClN_4O_5$) 621.1905, found 621.1898.

3-[(5-Acetamino-1*H*-indol-2-yl)acrylyl]-1-(chloromethyl)-5-hydroxy-1,2-dihydro-3*H*-benz[*e*]indole (29). Gray powder (13% yield). ^{1}H NMR (DMSO-d_6, ppm): 11.69 (s, 1H, NH), 10.52 (s, 1H, OH), 9.82 (s, 1H, NH), 8.28−6.90 (m, 11H, Ar−H), 4.55−4.54 (d, 1H, J = 4.7 Hz, N*H*H), 4.27−4.23 (m, 2H, NHH, $ClCH_2CHCH_2$), 4.12−4.08 (dd, 1H, J = 3.1, 11.0 Hz, C*H*HCl), 3.91−3.85 (dd, 1H, J = 8.8, 11.1 Hz, CH*H*Cl), 2.12 (s, 3H, CH_3). FABHRMS: calcd for ($C_{26}H_{23}ClN_3O_3$) 460.1428, found 460.1417.

2-[[5-[(5-Acetamino-1*H*-benzofuran-2-ylcarbonyl)amino]-1*H*-indol-2-yl]carbonyl]-1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one (30). To a solution of **27** (10 mg) in DMF (0.5 mL) were added triethylamine (0.5 mL), water (0.5 mL) and acetonitrile (0.5 mL) sequentially. The reaction mixture was stirred at room temperature for 30 min. Solvent was removed in vacuo and the product was washed with water. Ether was added. The solid was filtered and washed with ether to afford **30** (85% yield). ^{1}H NMR (DMSO-d_6, ppm): 11.77 (s, 1 H, NH), 11.45 (s, 1 H, NH),10.28 (s, 1 H, NH), 8.21−6.96 (m, 13 H, Ar−H), 4.66−4.61 (dd, 1 H, J = 4.6, 10.0 Hz, N*H*H), 4.52−4.49 (d, 1 H, J = 10.3 Hz, NH*H*), 3.05−2.95 (m, 1 H), 2.08 (s, 3 H), 1.77−1.73 (dd, 1H, J = 4.4, 7.9 Hz), 1.72−1.70 (t, 1H, J = 4.8 Hz). FABHRMS: calcd for ($C_{33}H_{24}N_4O_5$) 556.1741, found 556.1741.

Cell Lines. The human monocytic leukemia, U937, was obtained from ATCC and cultured in RPMI-1640 plus 10% FCS in the absence of antibiotics. The cells were routinely tested for mycoplasma contamination and always found to be negative.

Drugs. For in vitro studies, drugs were dissolved in DMF to provide a stock solution of l mg/mL and were stored at −20 °C. For each experiment, drug solutions were freshly prepared from the stock solution by addition of sterile H_2O to afford concentrations suitable for the experiment.

Cytotoxicity. Cytotoxic effects of the drugs were measured by inhibition of DNA synthesis. U937 cells in RPMI-1640 plus 10% FCS medium were seeded at 5×10^4 cells/well in a 96-well plate. Drugs (10 μL) at increasing concentrations were added to each well and the total volume was adjusted to 0.1 mL/well using the same medium. The plate was incubated for 24 h at 37 °C followed by addition of 10 μL of [^{3}H]thymidine (20 μCi/mL). The plate was incubated for another 24 h. The cells were harvested and radioactivity was counted using the Packard Matrix 96 beta counter. The results are expressed as the percentage growth inhibition (IC_{50}) calculated as follows: % growth inhibition (IC_{50}) = [(total cpm − experimental cpm)/total cpm] × 100.

DNA Fragmentation. U937 cells (5×10^6 cells) in RPMI 1640 plus 10% FCS were labeled with 20 μCi of [^{3}H]thymidine for 20 h at 37 °C. Cells were washed three times and resuspended at 1×10^6 cells/mL in the same medium containing 2.5% FCS. The cells (25-μL aliquot containing 2.5×10^3 cells/well) were then placed into a 96-well plate. Wells for total counts received an additional 25 μL of medium, whereas experimental wells received 10 μL of drug solution and 15 μL of medium. The plates were incubated for the indicated length of time at 37 °C. After incubation, 100 μL of 10 mM Tris, 10 mM of EDTA and 0.3% Triton X-100 was added to each well. Intact DNA was collected on glass fiber filter paper using a Packard harvester and samples were counted in a scintillation counter. The percentage DNA release was calculated as follows: % DNA release = [(total cpm − experimental cpm)/total cpm] × 100.

Apoptosis Assay. U937 cells were cultured under the same conditions as for the DNA fragmentation assay, except that the cells were not labeled with [^{3}H]thymidine. The plates were incubated at 37 °C, and at different time points aliquots were removed and examined microscopically in the presence of trypan blue. Morphologically apoptotic cells are defined as those exhibiting at least two or more prominent membrane protuberances. This change occurs prior to cell death, which is defined as the inability to exclude trypan blue. In most experiments, the cultures were coded and counted blindly. At least 100 cells were counted for each sample and data are reported as the percentage of apoptotic cells.

Antitumor Screening in Mice. L1210 leukemia cells (10^5-cells/mouse, 0.1 mL) were injected ip to male BDF_1 mice (6 mice/group) on day 0. Drugs dissolved in DMF and diluted with a vehicle containing 30% DMSO in 0.5% glucose at different doses were administered on days 1, 5, and 9 ip. Antitumor activity was determined by comparing the median survival time of the treated groups (T) with that of a control group (C) and was expressed as a percentage of ILS [increase of life span, where % ILS = (T/C − 1) × 100]. These calculations considered dying animals only. Long-term (30 days) survivors were noted separately. Cyclophosphamide was used as a positive control. The median number of days the untreated group of mice (given the vehicle only) died was 7.5.

For the experiment with low number of tumor inoculation, 100 L1210 leukemia cells were injected ip to female CDF_1 mice (10 mice/group) on day 0. Drugs dissolved in DMSO and diluted with a vehicle containing 30% DMSO in 0.5% glucose at different doses were administered on days 1, 5, and 9 ip. Long-term (60 days) survivors were expressed as a percentage of the total number of mice in that group. Cyclophosphamide and taxol were used as positive controls. The median number of days the untreated group of mice (given the vehicle only) died was 23.

B16BL6 melanoma cells were grown in Dulbecco's modified Eagle's complete minimal essential medium supplemented with 10% heat-inactivated FBS, sodium pyruvate, nonessential amino acids, 2-fold vitamin soluton, L-glutamine, 100 μg/mL penicillin and 100 μg/mL streptomycin. Cultures incubated as monolayers and harvested with EDTA/trypsin. Cells were washed three times with medium and were adjusted to 10^7 cells/mL. BDF_1 female mice (4−6 week old, 8/group) were used. Each mouse was inoculated with 10^6 cells (0.1 mL) ip on day 0. Drugs were dissolved in dimethylacetamide (DMA) and diluted with a vehicle containing DMA, ceremorphor and water (1:5:44, v/v). Mice were injected with 0.1 mL of drug preparation ip on days 1, 5, and 9. Antitumor activity was determined by comparing the median survival time of the treated groups (T) with that of a control group (C) and was expressed as a percentage of ILS [increase of life span, where % ILS = (T/C − 1) × 100]. These calculations considered dying animals only. Adriamycin was used as a positive control. The median number of days the untreated mice (given the vehicle only) died was 16.

Hematological Measurements. Female CDF_1 mice (10 mice/group) were used. Drugs were dissolved in DMSO,diluted with a vehicle containing 30% DMSO in 0.5% glucose, and administered on day 1 ip. On day 27, blood samples were taken and hematological measurements were performed.

Acknowledgment. We thank Dr. Gary G. Meadows, Department of Pharmaceutical Sciences, Washington State University, Pullman, WA, for determining the antitumor activity of compound **26** in mice bearing the B16BL6 melanoma and Dr. Dieter Hermann, Boehringer-Mannheim, Germany, for determining the antitumor activity of compounds **26**, **28**, and **30** in mice inoculated with 100 L1210 leukemia cells and for measuring the hematological effects. We also thank Jolande Murray for help with the manuscript. This work was supported in part by a grant from the National Institutes of Health (CA79357-01 to Y.W.).

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JM990514C