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Novel Antitumor Indolizino[6,7-*b*]indoles with Multiple Modes of Action: DNA Cross-Linking and Topoisomerase I and II Inhibition

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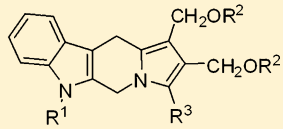
Supporting Information

ABSTRACT: A series of bis(hydroxymethyl)indolizino[6,7-*b*]indoles and their bis(alkylcarbamates) were synthesized for antitumor studies. These agents were designed as hybrid molecules of β-carboline (topoisomerase inhibition moiety) and bis(hydroxymethyl)pyrrole (DNA cross-linking moiety). The preliminary antitumor studies indicated that these agents exhibited significant cytotoxicity against a variety of human tumor cells in vitro. Treatment of human breast carcinoma MX-1 xenograft-bearing nude mice with compounds **18b** and **28c** achieved more than 99% tumor remission. We also observed that **18a** displayed potent therapeutic efficacy against human lung adenocarcinoma A549 and colon cancer HT-29 xenografts. These results revealed that compound **18a** was more potent than irinotecan against HT-29 cells and was as potent as irinotecan against A549 cells in xenograft models. Furthermore, we demonstrated that these derivatives possess multiple modes of action, such as induction of DNA cross-linking, inhibition of topoisomerase I and II, and cell-cycle arrest at the S-phase.



R^1 = Me, Et, or CH_2Ph
R^2 = H, CONHEt, or CONH-*i*-Pr
R^3 = Alkyl or substituted phenyl

INTRODUCTION

A hybrid molecule comprising two pharmacophoric groups is often used for the design of new drugs.[1] The hybrid may have increased potencies and/or modified selectivity profiles as receptor ligands compared to the corresponding single drug. Accordingly, several hybrid compounds in which the known antitumor compounds have been tethered to distamycin and netropsin frames have been designed, synthesized, and subjected to biological studies.[2−4] We have also designed and synthesized various DNA-directed alkylating agents, which were prepared by linking various DNA-affinic molecules, such as 9-anilinoacridines, acridines, or quinolines, with phenyl *N*-mustard, a DNA alkylator.[5,6] Our studies demonstrated that these conjugates were generally more cytotoxic than the individual intercalator or alkylating agent alone. Azatoxin (**1**, Figure 1), a hybrid of etoposide, which is a topoisomerase II (topo II) inhibitor,[7] and ellipticine, a DNA intercalator, topo II inhibitor, and DNA binding agent,[8] was reported to have a dual mechanism of action, including the inhibition of topo II and tubulin polymerization.[9,10] As a result, we designed and synthesized novel hybrid indolizino[6,7-*b*]indole derivatives, which contain biologically active β-carboline and bis(hydroxymethyl)pyrrole pharmacophores, as shown in Figure 2.

β-Carboline alkaloids were originally identified in nature. Naturally occurring β-carboline alkaloids and synthetic derivatives of β-carboline preserve a unique pharmacophore and exhibit a wide spectrum of biological activities,[11−17] such as antimalarial, antithrombotic, sedative, anti-HIV, and anticancer activities. Of these derivatives, naturally occurring β-carboline alkaloids, such as callophycin A (**2**)[18] and fascaplysin (**3**),[19] displayed antiproliferative effects in vitro against a variety of human cancer cell lines. Many synthetic β-carboline derivatives,[20,21] including **1**,[9] were also observed to have moderate to good cytotoxicity against tumor cell growth in vitro. These reports indicated that β-carboline derivatives exhibit antitumor activity through DNA intercalation[21] and the inhibition of topoisomerase I (topo I) and II,[20−22] cyclin-dependent kinase (CDK),[23] and IkK kinase complex (IkK).[24]

Another pharmacophoric group in the target compounds is bis(hydroxymethyl)pyrrole, which was observed to induce DNA cross-linking. Previous reports revealed that bis(hydroxymethyl)pyrroles (**4** and **5**)[25] or pyrrolizines (**6** and **7**)[26] displayed significant antitumor activity. The bis(hydroxymethyl) functionality located at the adjuvant position of these agents can form two electrophilic centers, leading to DNA double-strand cross-linking. The proposed mechanism of action for DNA interstrand cross-linking induced by these derivatives occurs most likely via an S_N1 electrophilic

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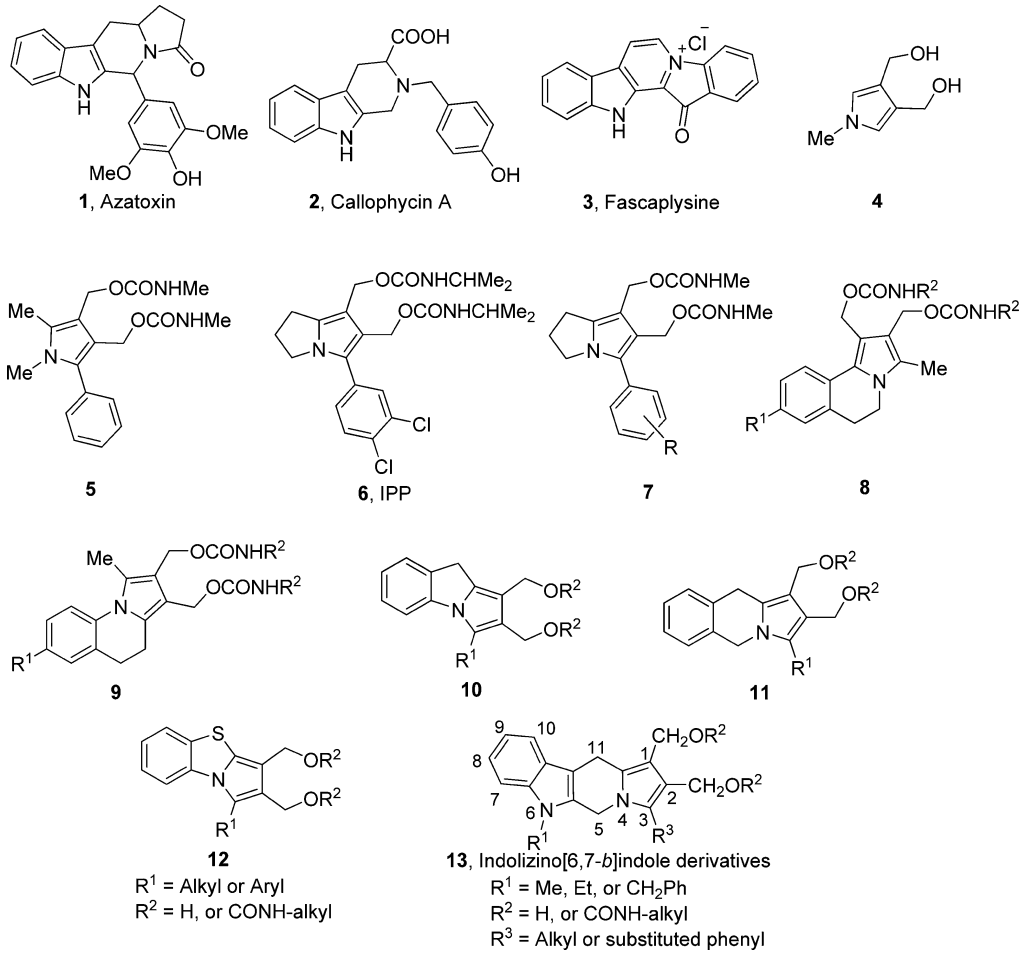

Figure 1. Structures of several anticancer β-carboline derivatives (**1**–**3**), bis(hydroxymethyl)pyrrole and their carbamate derivatives (**4**–**12**), and the newly synthesized novel tetracyclic indolizino[6,7-*b*]indole derivatives (**13**).

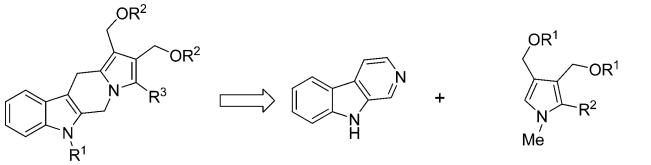

Figure 2. Novel indolizino[6,7-*b*]indole derivatives comprising β-carboline and bis(hydroxymethyl)pyrroles moieties.

reaction.[27] The potential electrophilic reactivity of these agents, in which the carbamate moieties act as leaving groups in an alkyl–oxygen cleavage mechanism, would be modulated by the degree of electronic perturbation in the participating pyrrole.[27] Of these analogues, compound **6** was found to have significant antitumor activity against a broad range of human tumor xenografts.[28] After further structural modification, pyrrolo[2,1-*a*]isoquinolines (**8**) and pyrrolo[1,2-*a*]quinolines (**9**) were synthesized and found to have a broad spectrum of activity against a wide range of tumors.[29]

We have previously designed and synthesized a series of bis(hydroxymethyl)-8*H*-3*a*-azacyclopenta[*a*]indene-1-yl and their bis(methylcarbamate) derivatives (**10**).[30] These analogues exhibited significant cytotoxicity against human lymphoblastic leukemia CCRF-CEM and a variety of human tumors in vitro and showed potent therapeutic efficacy in xenograft models. We also found that these derivatives possessed significant synergistic antitumor effects when coadministered with DNA repair inhibitors, such as arsenic trioxide (ATO), in nude mice transplanted with cisplatin-resistant lung and bladder cancer cells.[31] Recently, we further synthesized a set of bis-(hydroxymethyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline analogues (**11**)[32] and bis(hydroxymethyl)benzo[*d*]pyrrolo[2,1-*b*]thiazoles (**12**),[33] which can be considered "bioisoteres" of 3*a*-azacyclopenta[*a*]indene (**10**). We demonstrated that these analogues also exhibited a broad spectrum of antitumor activity against the growth of various human solid tumor cells both in vitro and in xenograft models.

To continue the investigation of bis(hydroxymethyl)pyrrole analogues as potential antitumor agents, we synthesized novel tetracyclic indolizino[6,7-*b*]indole derivatives (**13**), which were designed as a hybrid molecule of β-carboline, a DNA intercalating agent and topoisomerase inhibitor, and bis-(hydroxymethyl)pyrroles, a DNA double-strand cross-linking agent. We anticipate that the hybrid may possess an interesting mechanism of action and may display potent antitumor activity. Here, we report the chemical modification of β-carboline derivatives for a better understanding of the structure–activity relationships (SAR), antitumor activity, and mechanism of action of these newly synthesized compounds.

RESULTS AND DISCUSSION

Chemistry. The synthesis of 1,2-bis(hydroxymethyl)-indolizino[6,7-*b*]indole derivatives (**18a**–**c**) and their bis-

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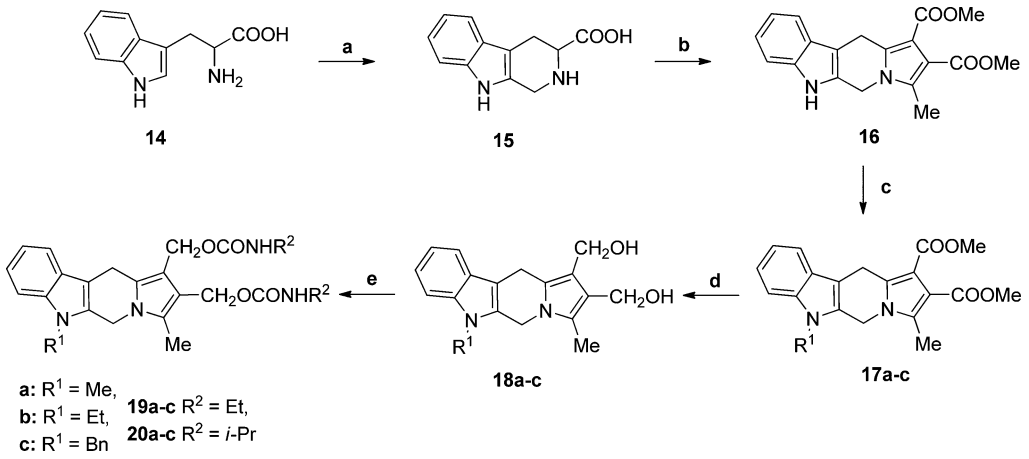
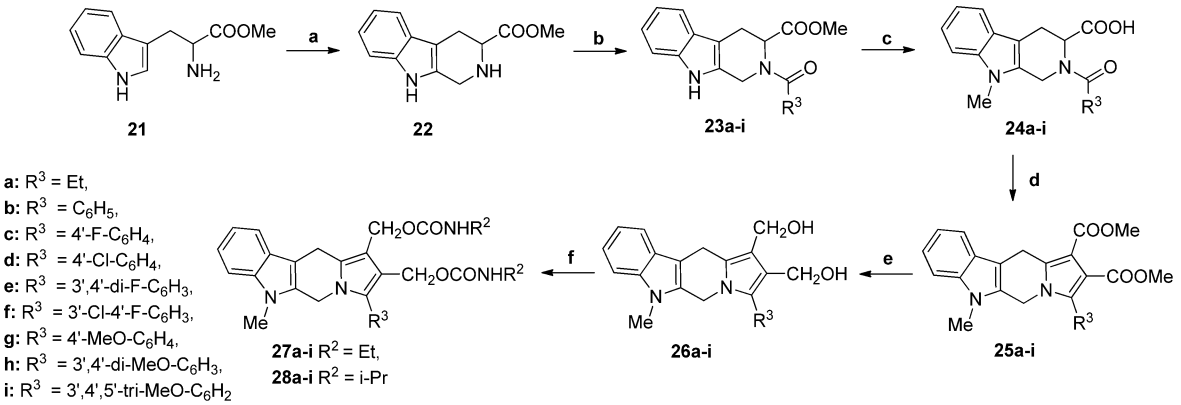
Scheme 1. 6-Substituted Indolizino[6,7-*b*]indole Derivatives[a]



[a]Reagents and conditions: (a) formaldehyde, sulfuric acid, rt; (b) DMAD, Ac_2O, 70 °C; (c) NaH, DMF, R^1-I or R^1-Br; (d) LAH, ether, DCM, 0 °C; (e) R^2NCO, TEA.

Scheme 2. 3-Substituted Indolizino[6,7-*b*]indole Derivatives[a]



[a]Reagents and conditions: (a) formaldehyde, MeOH; (b) R^3COCl, TEA; (c) NaH, MeI, THF; (d) DMAD, Ac_2O, 60−75 °C; (e) LAH, ether, DCM, 0 °C; (f) R^2NCO, TEA.

(alkylcarbamate) derivatives (**19a−c** and **20a−c**) is depicted in Scheme 1. Compound **15** was prepared by reacting commercially available L-tryptophan (**14**) with 37% formaldehyde in the presence of sulfuric acid according to the procedure described in the literature.[34] The reaction of **15** with dimethyl acetylenedicarboxylate (DMAD) in acetic anhydride (Ac_2O) yielded the diester **16**,[35] which was N-alkylated by treatment with sodium hydride (NaH) and either an alkyl or benzyl halide, such as iodomethane (MeI), iodoethane (EtI), or benzyl bromide, in dimethylformamide (DMF) at ambient temperature to yield **17a−c**. The ester functions of **17a−c** were reduced to the corresponding bis(hydroxymethyl) derivatives (**18a−c**) by reaction with lithium aluminum hydride (LAH) in a mixture of ether/dichloromethane (DCM) in an ice bath. Treatment of **18a−c** with alkyl isocyanates, such as ethyl isocyanate or isopropyl isocyanate, in the presence of triethylamine (TEA) resulted in a good yield of the desired bis(alkylcarbamate) derivatives **19a−c** and **20a−c**.

Because the solubility of compound **15** is very poor and is not suitable for N-acylation with other alkyl acid chlorides or benzoyl chlorides, an alternative method for synthesizing the target compounds was developed. We used L-tryptophan methyl ester hydrochloride (**21**) as the starting material (Scheme 2). Compound **22** was synthesized from **21** by Pictet−Spengler cyclization according to a previously described method.[36] Acylation of **22** with various acid chlorides in the presence of TEA produced the corresponding *N*-acyl derivatives **23a−i**.[37] Similarly, compounds **23a−i** were treated with NaH and MeI in tetrahydrofuran (THF) at ambient temperature to yield the carboxylic acids **24a−i**, which were further converted into the diesters **25a−i** by treatment with DMAD in Ac_2O at 60−75 °C. The diesters **25a−i** were reduced to the bis(hydroxymethyl) derivatives **26a−i** by reaction with LAH in a mixture of ether/DCM in an ice bath. Similarly, treatment of **26a−i** with alkyl isocyanates resulted in the desired bis(alkylcarbamate) derivatives **27a−i** and **28a−i**.

In Vitro Cytotoxicity. In our anticancer-agent-screening program, we used the CCRF-CEM cell line for evaluating the primary antiproliferative activity of the tested compounds for SAR studies. Compounds that exhibited significant in vitro cytotoxicity against this cancer cell line were selected for further evaluation of their antiproliferative activity against the growth of a variety of human solid tumor cells in culture. The antiproliferative activities of the newly synthesized bis(hydroxymethyl)indolizino[6,7-*b*]indole (**18a−c**, **26a−i**), bis(ethylcarbamate) (**19a−c**, **27a−i**), and bis(isopropylcarbamate) derivatives (**20a−c**, **28a−i**) against

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Table 1. Cytotoxicity of Newly Synthesized 2,3-Bis(hydroxymethyl)-5*H*-indolizino[6,7-*b*]indoles and Their Bis(alkylcarbamate) Derivatives against CCRF-CEM Cells and Solid Tumor Cell Growth in Vitro

	IC_{50} (μM)[a]									
compd	CCRF/CEM	PC3	OECM1	MX-1	HCT-116	CL141T	A549	H460	HT-29	HEL299
18a	0.04 ± 0.0003	0.61 ± 0.10	0.96 ± 0.38	0.22 ± 0.019	1.00 ± 0.15	0.51 ± 0.16	1.14 ± 0.30	0.38 ± 0.09	3.35 ± 1.27	2.79 ± 1.08
18b	0.10 ± 0.002	2.66 ± 0.47	1.17 ± 0.89	ND	1.71 ± 0.59	0.79 ± 0.33	2.97 ± 1.00	0.73 ± 0.35	6.15 ± 2.41	2.24 ± 1.38
18c	0.29 ± 0.003	4.52 ± 1.17	4.17 ± 0.61	ND	ND	2.76 ± 0.86	ND	ND	ND	ND
19a	0.04 ± 0.0005	4.11 ± 1.50	1.90 ± 0.74	0.46 ± 0.001	2.33 ± 0.39	2.84 ± 0.94	4.83 ± 1.17	ND	11.29 ± 1.71	15.48 ± 7.96
19b	0.14 ± 0.001	4.61 ± 0.66	4.90 ± 1.79	ND	3.22 ± 0.34	1.40 ± 0.30	ND	5.14 ± 1.71	ND	ND
19c	0.14 ± 0.004	5.05 ± 0.55	5.42 ± 1.41	ND	3.84 ± 0.62	ND	ND	8.31 ± 3.15	ND	ND
20a	0.03 ± 0.0007	3.03 ± 1.29	1.45 ± 0.79	0.25 ± 0.002	1.26 ± 0.12	2.43 ± 0.71	3.81 ± 0.042	ND	10.78 ± 1.93	ND
20b	0.14 ± 0.001	3.28 ± 0.75	2.27 ± 0.79	ND	2.20 ± 0.29	1.88 ± 0.59	2.64 ± 0.72	ND	6.36 ± 0.81	ND
20c	0.10 ± 0.002	4.18 ± 0.06	4.20 ± 1.61	ND	3.92 ± 0.32	4.66 ± 1.15	ND	7.26 ± 3.90	ND	ND
26a	0.20 ± 0.002	3.32 ± 0.69	2.11 ± 1.04	ND	ND	1.32 ± 0.47	3.15 ± 1.20	ND	8.56 ± 2.01	4.24 ± 2.22
26b	1.17 ± 0.024	ND	ND	ND	ND	ND	ND	ND	ND	ND
26c	1.46 ± 0.012	ND	ND	ND	ND	ND	ND	ND	ND	ND
26d	4.58 ± 0.378	12.90 ± 1.67	17.16 ± 1.85	11.28 ± 0.42	17.46 ± 2.01	23.75 ± 3.28	ND	ND	ND	ND
26e	7.94 ± 0.017	ND	ND	ND	ND	ND	ND	ND	ND	ND
26f	14.37 ± 0.001	ND	ND	ND	ND	ND	ND	ND	ND	ND
26g	1.30 ± 0.041	ND	ND	ND	ND	ND	ND	ND	ND	ND
26h	0.96 ± 0.018	37.85 ± 11.94	37.22 ± 4.04	ND	ND	33.11 ± 7.83	ND	ND	ND	ND
26i	4.45 ± 0.144	ND	ND	ND	ND	ND	ND	ND	ND	ND
27a	0.16 ± 0.002	5.00 ± 0.41	3.90 ± 0.45	ND	1.80 ± 0.31	2.85 ± 0.88	ND	ND	ND	ND
27b	0.23 ± 0.008	9.13 ± 2.78	5.19 ± 0.24	ND	ND	10.50 ± 0.75	ND	ND	ND	ND
27c	0.39 ± 0.005	14.61 ± 8.69	8.60 ± 0.75	ND	ND	12.43 ± 1.75	ND	ND	ND	ND
27d	0.35 ± 0.013	4.95 ± 0.60	9.01 ± 2.07	1.83 ± 0.082	1.17 ± 0.009	7.88 ± 0.91	ND	ND	ND	ND
27e	0.13 ± 0.011	6.76 ± 0.34	14.37 ± 2.89	ND	ND	9.61 ± 2.29	ND	18.95 ± 5.05	ND	ND
27f	1.80 ± 0.027	ND	ND	ND	ND	ND	ND	ND	ND	ND
27g	0.10 ± 0.003	8.30 ± 2.24	6.15 ± 1.12	ND	7.10 ± 0.59	8.13 ± 2.98	ND	ND	ND	ND
27h	0.30 ± 0.006	8.03 ± 2.87	5.29 ± 1.42	ND	ND	11.24 ± 1.84	ND	ND	ND	ND
27i	0.62 ± 0.011	ND	ND	ND	ND	ND	ND	ND	ND	ND
28a	0.16 ± 0.0009	2.10 ± 0.79	2.02 ± 0.63	ND	2.22 ± 0.63	2.66 ± 0.24	ND	ND	ND	ND
28b	0.13 ± 0.001	7.84 ± 0.52	4.87 ± 0.71	ND	ND	9.60 ± 1.66	ND	4.72 ± 4.01	ND	ND
28c	0.11 ± 0.004	6.71 ± 1.65	6.56 ± 1.15	2.19 ± 0.03	6.09 ± 0.59	7.46 ± 1.10	ND	ND	ND	25.91 ± 9.89
28d	0.44 ± 0.003	13.09 ± 1.31	12.67 ± 1.47	12.39 ± 0.050	9.28 ± 0.52	13.96 ± 3.12	ND	ND	ND	ND
28e	0.44 ± 0.009	34.21 ± 15.98	15.19 ± 5.24	ND	ND	18.28 ± 5.91	ND	ND	ND	ND
28f	1.91 ± 0.002	ND	ND	ND	ND	ND	ND	ND	ND	ND
28g	0.12 ± 0.006	6.84 ± 1.87	4.17 ± 0.93	ND	5.93 ± 0.75	4.00 ± 0.54	ND	9.52 ± 3.37	ND	ND
28h	0.30 ± 0.008	ND	ND	ND	ND	ND	ND	ND	ND	ND
28i	0.95 ± 0.004	ND	ND	ND	ND	ND	ND	ND	ND	ND
cisplatin	ND	6.32 ± 1.00	3.77 ± 1.07	ND	9.93 ± 1.98	2.45 ± 0.52	28.76 ± 4.58	8.88 ± 1.84	33.31 ± 3.01	1.80 ± 0.41

[a]The data represent the mean ± STDEV of each compound from three to six independent experiments. ND: not determined.

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Table 2. Cytotoxicity of Selected 2,3-Bis(hydroxymethyl)-5*H*-indolizino[6,7-*b*]indoles and Their Bis(alkylcarbamate) Derivatives against KB, KBvin10, MES-SA, and MES-SA/dx5 As Measured by Cell Growth in Vitro[a]

	compd						
	18a[b]	**18b**[b]	**19a**[b]	**26a**[b]	**28c**[b]	vincristine[c]	doxorubicin[b]
KB	2.51 ± 1.14	1.47 ± 0.40	2.75 ± 0.46	1.97 ± 0.39	14.22 ± 6.57	2.11 ± 0.45	ND
KBvin10	1.31 ± 0.20 [0.52×][d]	0.90 ± 0.51 [0.62×][d]	2.60 ± 0.50 [0.94×][d]	1.56 ± 0.51 [0.79×][d]	15.60 ± 2.61 [1.1×][d]	303.2 ± 34.12 [143.7×][d]	ND
MES-SA	0.24 ± 0.04	0.40 ± 0.13	0.78 ± 0.14	0.77 ± 0.08	ND	ND	0.038 ± 0.0028
MES-SA/dx5	0.12 ± 0.02 [0.52×][d]	0.24 ± 0.04 [0.60×][d]	0.28 ± 0.06 [0.36×][d]	0.47 ± 0.12 [0.61×][d]	ND	ND	2.30 ± 0.98 [60.52×][d]

[a]The data represent the mean ± STDEV of each compound from three to six independent experiments. ND: not determined. [b]IC_{50} values in μM. [c]IC_{50} values in nM. [d]Resistance factor.

CCRF-CEM cells are shown in Table 1. We synthesized compounds with a methyl, ethyl, and benzyl substituent at the hetero nitrogen atom (N^6) at the indole ring because we observed that the *N*-Me derivatives, such as compounds **18a**, **19a**, and **20a**, are generally more cytotoxic than the corresponding *N*-Et (**18b**, **19b**, and **20b**) and *N*-Bn derivatives (**18c**, **19c**, and **20c**) Therefore, we focused primarily on the *N*-Me derivatives for our antitumor studies. In the series of 6-methyl-1,2-bis(hydroxymethyl) derivatives, **18a** and **26a−i**, the order of potency in this series is **18a** (C3-Me) > **26a** (C3-Et) > **26b−i** (C3-Ph), indicating that the size of the substituent may affect the antiproliferative activity. The C3-Me derivative (**18a**) is the most cytotoxic, with an IC_{50} of 0.04 μM against the CCRF-CEM cell line in vitro. Among the C3-Ph substituted derivatives **26b−i**, the C3-4′-F-Ph (**26c**) is more potent than the chloro-substituted derivatives (**26b−f**), and the C3-4′-OMe and C3-3′,4′-di-OMe derivatives **26g** and **26h**, respectively, are more cytotoxic than the C3-3′,4′,5′-tri-OMe derivative (**26i**). However, the OMe substituted derivatives are generally more cytotoxic than the halogen-substituted compounds.

For the bis(alkylcarbamate) derivatives, we found that the bis(ethylcarbamate) derivatives, **27a−i**, are more potent than the corresponding bis(isopropylcarbamate) compounds, **28a−i**, for the inhibition of cancer cell growth. Unlike the 1,2-bis(hydroxymethyl) derivatives, the substituent(s) on the phenyl ring of the bis(alkylcarbamate) derivatives do not have a large influence on potency. However, compounds with a 3′-Cl,4′-F-Ph substituent at C3, such as **26f**, **27f**, and **28f**, are less cytotoxic than compounds with other substituent(s). For the compounds with a OMe group on the C3-substituted phenyl derivatives, the cytotoxicity is decreased when the number of OMe group is increased (i.e., 4′-MeO-Ph > 3′,4′-di-MeO-Ph > 3′,4′,5′-tri-MeO-Ph). In general, the bis-(alkylcarbamate) derivatives **27b−i** and **28b−i** are more potent than the corresponding bis(hydroxymethyl) congeners, **26b−i**, with the exception of the C3-Me- (**18a**, **19a**, and **20a**) and C3-Et-substituted derivatives (**18b**, **19b**, and **20b**).

To further investigate the antiproliferative activity of the newly synthesized derivatives, the cytotoxicity of the selected compounds was evaluated with regard to the inhibition of cell growth in vitro of other human solid tumors, such as human prostate cancer (PC3), human oral epidermoid carcinoma (OECM1), human breast cancer (MX-1), human colon cancer (HCT-116), human colon adenocarcinoma (HT-29), and human lung adenocarcinoma (CL141T, A549, and H460). As summarized in Table 1, all of the compounds tested displayed a broad spectrum of antitumor activity against the solid tumor cell lines tested in culture. In general, the bis(alkylcarbamate) derivatives are more cytotoxic than the corresponding bis-(hydroxymethyl) derivatives against all the tumor cells tested. The most cytotoxic compound of this series is compound **18a**, which has significant cytotoxicity against the tested tumor cells in vitro. We also examined the cytotoxic effects of several newly synthesized compounds on human embryonic lung fibroblast HEL299 cells. As shown in Table 1, compounds **19a** and **28c** were much less toxic to the HEL299 cell line than to the other tested tumor cell lines, whereas compounds **18a**, **18b**, and **26a** were slightly less or similarly cytotoxic to the HEL299 cells. Notably, the HEL299 cell line is relatively more susceptible to cisplatin than the other tested tumor cells.

Overcoming the multidrug resistance (MDR) of resistant tumor cells is a major concern for new drug discovery and development. To determine whether tumor cells are resistant to the newly synthesized derivatives in a manner similar to currently used anticancer drugs, we selected five compounds (**18a**, **18b**, **19a**, **26a**, and **28c**) and evaluated their in vitro cytotoxicity against human drug-sensitive cancer cell lines and their resistant subcell lines, including a human oral cancer cell line KB, the subcell line resistant to vincristine KBvin10,[38] a human uterine sarcoma cell line MES-SA, and the subcell line resistant to doxorubicin MES-SA/dx5. As shown in Table 2, almost all the tested compounds were relatively more potent against the MDR cell lines than the parental lines. This finding indicates that these cell lines, which are resistant to vincristine or doxorubicin, are not cross-resistant to the tested compounds.

In Vivo Antitumor Activity. On the basis of the in vitro antiproliferative effects of the newly synthesized derivatives, we selected compounds **18a**, **18b**, and **28c** and evaluated their therapeutic efficacy against a variety of solid tumors in xenograft models. In all experiments, the tumors were subcutaneously implanted in nude mice, and the maximum tolerated dose (MTD) of the tested compound was administered via intravenous injection (iv inj). The MX-1 xenograft-bearing mice were treated with compound **18b** at 25 mg/kg once per day for 3 consecutive days (QD×3) or compound **28c** at 35 mg/kg once per day for 4 consecutive days (QD×4). In all cases, greater than 99% tumor suppression and no relapses were observed on day 20 (D20) (Figure 3A). The average body weight of all the drug-treated mice recovered after the cessation of treatment (Figure 3B). In another study, we treated HT-29 xenograft-bearing nude mice with either compound **18a** or irinotecan, which is used as a standard chemotherapeutic for advanced colorectal cancer. As shown in Figures 4, compound **18a** significantly suppressed tumor growth at the same treatment regime (25 mg/kg, QD×4, iv inj). Treatment with irinotecan also significantly suppressed the growth of HT-29 cells in nude mice; however, the tumor began growing on D22 after the tumor cells were implanted. These

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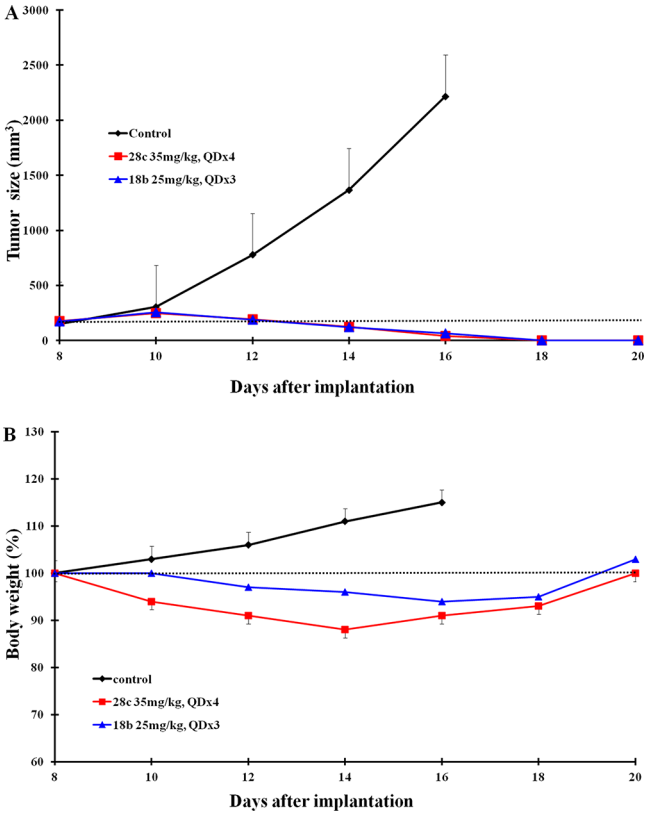

Figure 3. Therapeutic effect of **28c** and **18b** in MX-1 xenograft-bearing nude mice (iv inj, $n = 4$): (A) average tumor size changes; (B) average body weight changes.

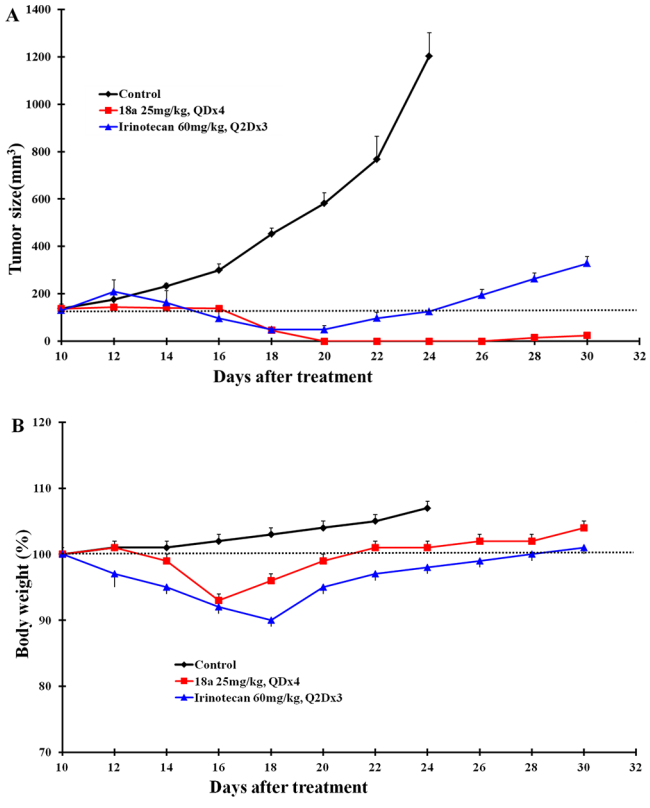

Figure 4. Therapeutic effects of **18a** and irinotecan in HT-29 xenograft-bearing nude mice: (A) average tumor size changes; (B) average body weight changes.

results indicated that compound **18a** is more potent than irinotecan against HT-29 cells. We further evaluated the antitumor activity of compound **18a** against A549 cells in a xenograft model and compared the activity of compound **18a** to that of irinotecan (Figure 5). Under the same treatment conditions (25 mg/kg, QD×4 via iv inj), compound **18a** was as potent as irinotecan (60 mg/kg, every 2 days for three times (Q2D×3), iv inj). Both compounds suppressed tumor growth by approximately 50%. For the changes in body weight (Figures 4B and 5B) in the drug-treated mice, we found that compound **18a** was less toxic than irinotecan. Our current studies demonstrate that compound **18a** is most likely more potent than irinotecan against the human colon and lung tumor xenograft models and is also less toxic to the host based on the average body weight loss.

Cell-Cycle Inhibition. Guan et al. synthesized a series of β-carboline derivatives, which could induce DNA damage and G2/M arrest in human epithelial carcinoma (HeLa) cells.[21] Additionally, a distinct S phase arrest was observed, which may be caused by the inhibition of topo I and II at the beginning of the DNA synthesis. Recently, we demonstrated that treatment of human lung adenocarcinoma (H1299) cells with bis-(hydroxymethyl) derivatives of benzo[*d*]pyrrolo[2,1-*b*]thiazole at higher concentrations for 24 h induced the accumulation of the G1 population. This treatment also resulted in accumulation at the G1 and G2/M phases and the appearance of a sub-G1 population at 48 h.[33] In the present studies, we investigated the effects of compounds **18a**, **18b**, and **28c** on cell-cycle progression in CL141T cells. We treated CL141T cells with compounds **18a**, **18b**, and **28c** at 0.5-, 1-, and 2-fold their IC_{50} values for 24, 48, and 72 h and analyzed the cell-cycle distributions by flow cytometry. The IC_{50} values of **18a**, **18b**, and **28c** were 0.5, 0.8, and 7.5 μM, respectively. As shown in Figure 6, the distribution of S phase of the CL141T cells was increased by treatment with the bis(hydroxymethyl) derivatives, **18a** and **18b**, at 0.5-fold IC_{50} for 24 h. Increasing the concentrations to 1- and 2-fold IC_{50}, we observed an accumulation of the G1 and S phases. However, treatment of CL141T cells with bis(isopropylcarbamate) **28c** caused an S phase delay after 24 h at various concentrations. The previous S-phase delayed cells were progressed to the G2/M phase after 48 and 72 h of treatment, when the cells were treated with 0.5- and 1-fold IC_{50}. However, these cells did not progress to the G2/M phase when the cells were treated with 2-fold concentration of IC_{50}. In general, all three compounds, **18a**, **18b**, and **28c**, induced S-phase delay initially, followed by G2 arrest. Compounds **18a** and **18b**, but not **28c**, treated cells at 1- and 2-fold IC_{50} resulted in the delay of G1 to S progression, suggesting that **18a** and **18b** produced more severe DNA damage than **28c**. In addition to cell cycle disruption, we observed an increase of a sub-G1 population in CL141T cells treated with various doses of **18a**, **18b**, and **28c** for 72 h, suggesting that these compounds can trigger apoptotic cell death.

Induction of Apoptosis. Our cell-cycle progression studies suggested that newly synthesized compounds could cause apoptosis of cancer cells. Therefore, we determined the induction of apoptosis by compounds **18a** and **18b** with an annexin V binding assay[39] in H460 cells. As shown in Figure 7A, after a 24 h treatment with compounds **18a** and **18b**, apoptotic cells increased from 4.10% to 15.09% and 12.92% and was significantly increased to 61.35% and 43.52%,

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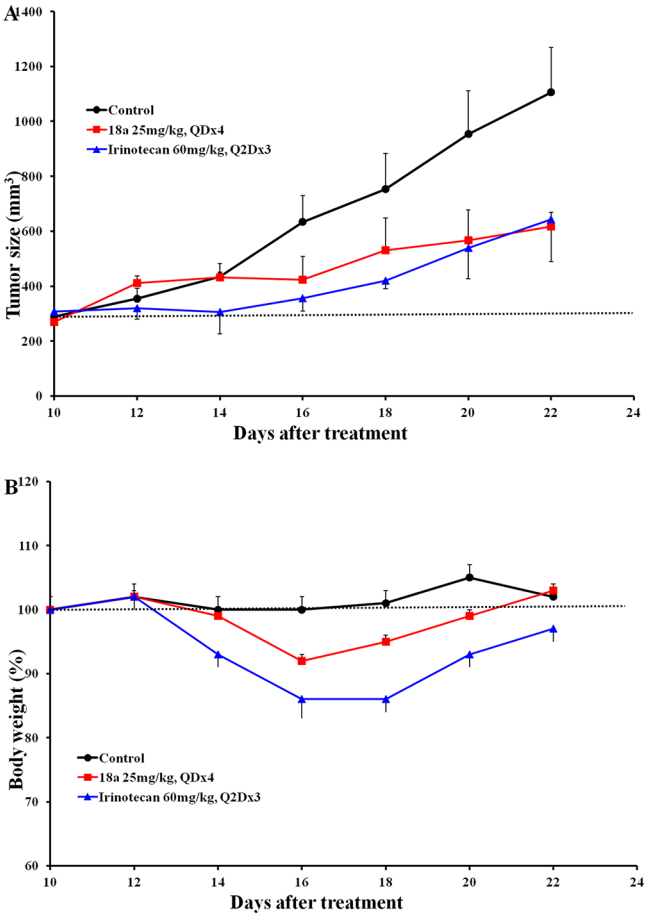

Figure 5. Therapeutic effects of **18a** and irinotecan in A549 xenograft-bearing nude mice: (A) average tumor size changes; (B) average body weight changes.

respectively, after 72 h. These results confirmed that the damage induced by the tested compounds can trigger apoptotic cell death. We also investigated the expression of caspase-3, caspase-7, and poly ADP-ribose polymerase (PARP), indicator proteins of apoptosis,[40,41] in H460 cells treated with compounds **18a** and **18b** using Western blot analysis. As shown in Figure 7B, we found that cleaved caspase-3 (c-caspase-3), cleaved caspase-7 (c-caspase-7), and cleaved PARP (c-PARP) were significantly increased after treatment with compounds **18a** and **18b** at the IC_{50} dose after 24 and 72 h. This finding indicates that these compounds cause cancer cell apoptosis via reduction of the membrane integrity and increase of the levels of c-caspase-3, c-caspase-7, and c-PARP.

DNA Cross-Linking Study. Our previous studies revealed that bis(hydroxymethyl)pyrrole analogues[30,32,33] are capable of forming DNA interstrand cross-links. To determine whether the newly synthesized compounds are also able to produce DNA cross-links, we used an alkaline agarose gel shift assay in which linearized pEGFP-N1 plasmid DNA was treated with bis(hydroxymethyl) derivatives (**18a** and **18b**) and bis(alkylcarbamate) derivatives (**19b**, **20b**, and **28c**) at various concentrations (1, 5, and 10 μM). The results show that compounds **18a**, **18b**, **19b**, and **20b** formed DNA interstrand cross-links efficiently, with the exception of compound **28c**, which produced less DNA cross-linking (Figure 8). This finding suggests that DNA cross-linking correlates with in vitro cytotoxicity and is the main mechanism of inhibiting cancer cell growth.

Inhibition of Topoisomerase I. As noted previously, the novel tetracyclic indolizino[6,7-*b*]indole derivative is designed as a hybrid molecule of β-carboline and a bis(hydroxymethyl)-pyrrole. We demonstrated that the newly synthesized derivatives with a bis(hydroxymethyl)pyrrole moiety can induce DNA cross-linking. Because these agents bear a β-carboline moiety, we also investigated whether the synthesized derivatives act as inhibitors of topo I. A supercoiled plasmid DNA relaxation assay was used to determine whether topo I activity was inhibited by the newly synthesized compounds. Irinotecan was used as a positive control. As shown in Figure 9, irinotecan, **18a**, and **19a** interfered with the ability of topo I to relax DNA supercoils. These results demonstrate that the newly synthesized compounds exhibit inhibitory effects against topo I. However, **18a** and **19a** are less potent than irinotecan in inhibiting topo I.

Inhibition of Topoisomerase II. Previous studies have shown that β-carboline analogues also exhibit inhibitory effects against topo II.[7,8] To determine whether the newly synthesized compounds also inhibit topo II, an ATP-dependent topo II mediated relaxation assay was performed. Etoposide was used as a positive control. The results for the representative compounds (**18a** and **19a**) are shown in Figure 10, where etoposide, **18a**, and **19a** inhibit DNA topo II activation in a concentration-dependent manner (25−200 μM). These results demonstrate that the newly synthesized derivatives exhibit inhibitory activity against topo II but are less potent than etoposide.

Stability of Compound 18a in Rat Plasma. A bioanalytical system was used to evaluate the plasma stability of compound **18a** in rat plasma. The analytical validations were performed according to the U.S. Food and Drug Administration (U.S. FDA) guidelines.[42] The stability tests of compound **18a** in rat plasma samples were performed for short-term stability at concentrations of 1 and 10 μg/mL incubated for 1, 2, 3, and 4 h at room temperature. The degradation of compound **18a** was detected by high-performance liquid chromatography (HPLC). HPLC indicated that compound **18a** is stable in rat plasma with a long half-life ($t_{1/2}$ = 12.4 ± 1.25 h, n = 3). These results demonstrate that the newly prepared bis(hydroxymethyl)indolizino[6,7-*b*]indole derivatives are chemically and metabolically stable.

CONCLUSIONS

In this study, we synthesized a series of new, substituted bis(hydroxymethyl)indolizino[6,7-*b*]indole derivatives and their bis(alkylcarbamate) derivatives for antitumor studies. These agents were designed as hybrid molecules of β-carboline, a topo I and II inhibitory moiety, and bis(hydroxymethyl)-pyrrole, a DNA cross-linking moiety. The preliminary antitumor studies revealed that the newly synthesized compounds exhibited a broad spectrum of significant antitumor activity by inhibiting the growth of various human tumor cell lines with no cross-resistance to vinblastine and doxorubicin. Among the newly synthesized derivatives, compounds **18a**, **18b**, and **28c** were selected for further evaluation of their therapeutic efficacy against human solid tumors in xenograft models. We observed that the synthesized compounds displayed multiple modes of action. These compounds are capable of inducing DNA cross-linking and exhibit inhibitory effects against topo I and II. To the best of our knowledge, this

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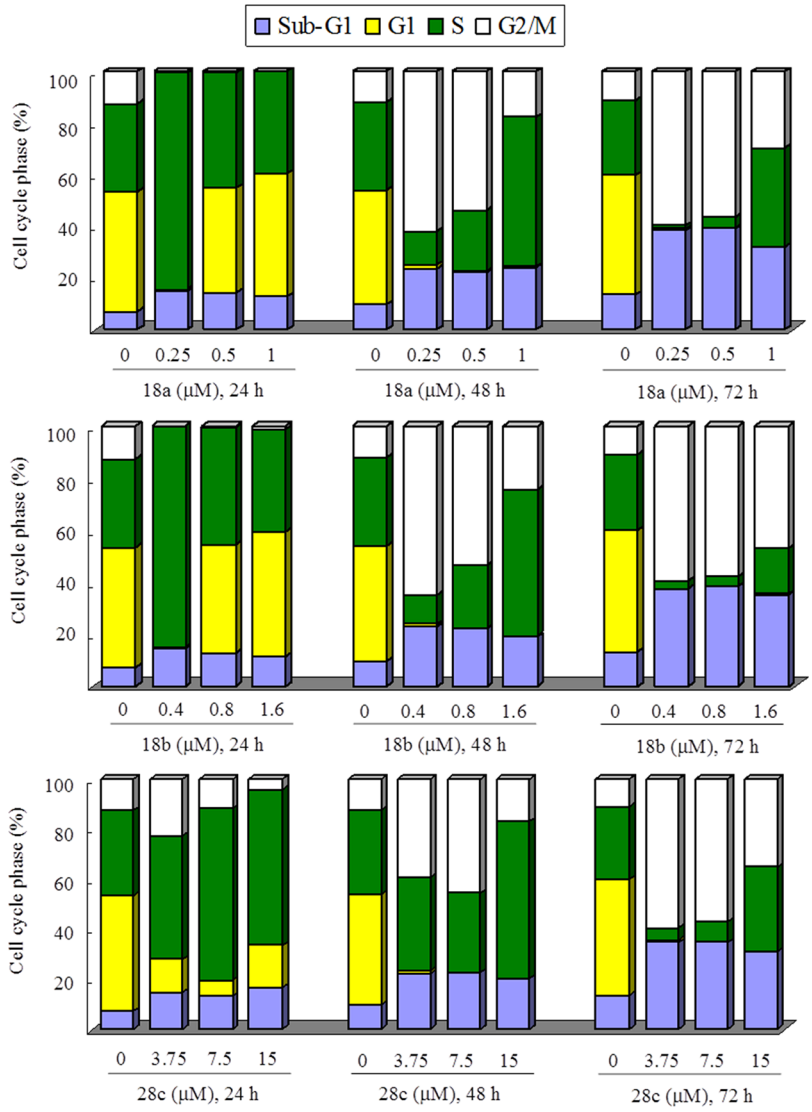

Figure 6. Effects of various compounds on cell-cycle progression in CL141T cells. The cells were treated with **18a** (0.25, 0.5, 1 μM), **18b** (0.4, 0.8, 1.6 μM), and **28c** (3.25, 7.5, 15 μM) for 24, 48, and 72 h. The cells were then harvested and analyzed for cell-cycle progression by flow cytometry.

is the first time bis(hydroxymethyl)indolizino[6,7-*b*]indole derivatives have been synthesized as DNA cross-linkers and topo I and II inhibitors. Flow cytometry studies of cell-cycle distribution indicated that the bis(hydroxymethyl) derivatives may have different modes of action from the bis-(alkylcarbamate) derivatives. Further studies on the levels of the apoptotic proteins, caspase-3, caspase-7, and PARP suggest that the studied compounds are able to cleave these proteins and able to induce apoptosis. Of these agents, compound **18a** was found to have superior antitumor activity to irinotecan in A549 and HT-29 xenograft-bearing nude mice and with less toxicity. This derivative may potentially be selected as a candidate for preclinical studies.

EXPERIMENTAL SECTION

General Chemistry. All commercial chemicals and solvents were reagent grade and were used without further purification unless otherwise specified. Melting points were determined in open capillaries on a Fargo melting point apparatus and are uncorrected. Thin-layer chromatography (TLC) was performed on silica gel G60 F254 (Merck, Merck KGaA, Darmstadt, Germany), and short-wavelength ultraviolet (UV) light was used for visualization. Elemental analysis was performed on a Heraeus CHN-O Rapid instrument. HPLC was performed on an Elite instrument with a Mightysil RP-18 (250 mm × 4.6 mm) column. Compounds were detected by UV at 260 nm. The mobile phase was acetonitrile/THF (80:20 v/v) with a flow rate of 1 mL/min. The purity of all tested compounds was ≥95% based on analytical HPLC. ^{1}H NMR spectra and ^{13}C NMR spectra were recorded on a Bruker AVANCE 600 DRX and/or 400 MHz Bruker Top-Spin spectrometer in the solvents indicated. The proton chemical shifts were reported in parts per million (δ ppm) relative to $(CH_3)_4Si$ (TMS), and coupling constants (J) were reported in hertz (Hz). Abbreviations used are the following: s, singlet; d, doublet; t, triplet; m, multiplet; br s, broad peak.

1,2,3,4-Tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylic Acid (15). Formalin [37% (80 mL)] was added to a mixture of L-tryptophan (**14**, 50 g, 245 mmol) and 0.1 N H_2SO_4 (150 mL). The resulting mixture was stirred at room temperature for 4 h. A white solid separated out and was filtered, washed with water, and dried. The resulting powder weighed 14.41 g (78%); mp 275−276 °C (lit.[34] mp 280−282 °C). ^{1}H NMR (DMSO-d_6) δ 2.84 (1H, m, CH_2), 3.16 (1H, m, CH_2), 3.66 (1H, m, CH_2), 4.23 (1H, m, CH_2), 4.38 (1H, m, CH), 6.98−7.02 (1H, m, ArH), 7.13−7.16 (1H, m, ArH), 7.32−7.34 (1H, m, ArH), 7.45 −7.48 (1H, m, ArH), 11.03 (1H, brs, exchangeable, NH).

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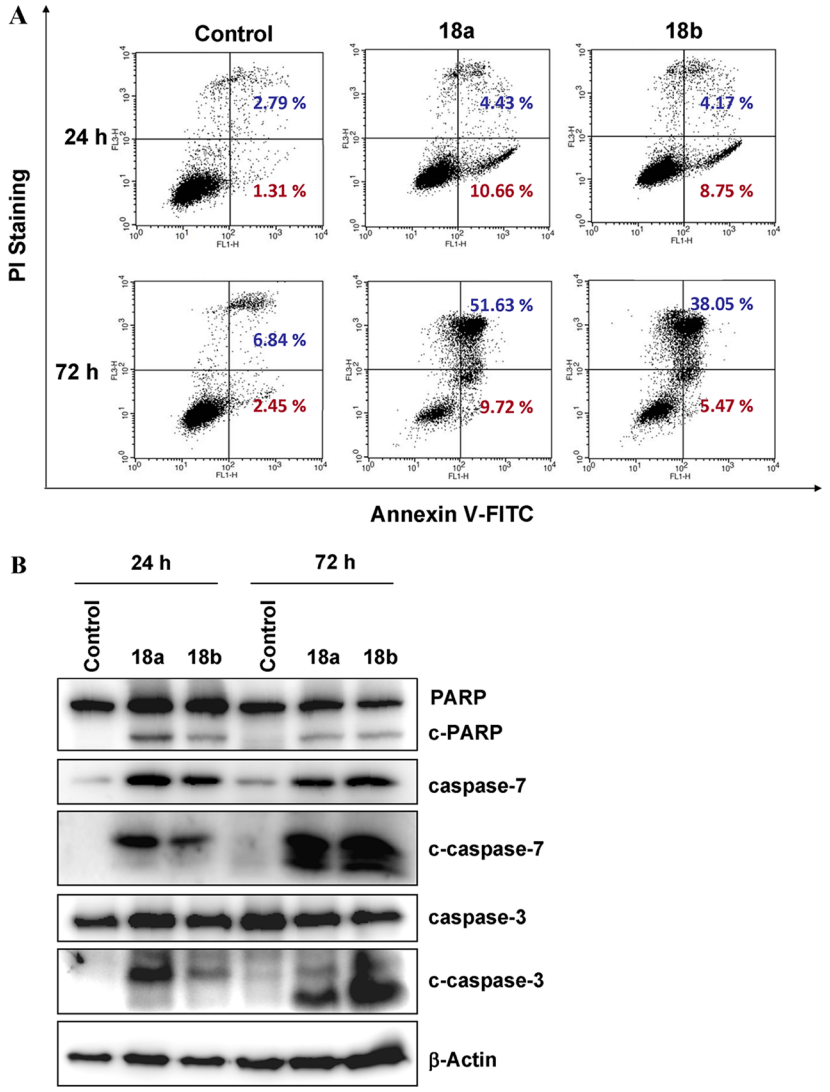

Figure 7. (A) Effects of compounds **18a** and **18b** on the induction of apoptosis in human non-small-cell lung adenocarcinoma H460 cells. The cells were harvested and analyzed for apoptosis by flow cytometric analysis of phosphatidylserine externalization (annexin V binding) and cell membrane integrity (PI staining) after treatment with **18a** (0.4 μM) and **18b** (0.8 μM) for 24 and 72 h. (B) Effect of compounds **18a** and **18b** on the levels of the apoptotic proteins, caspase-3, caspase-7, and PARP and their cleaved forms using Western blot analysis.

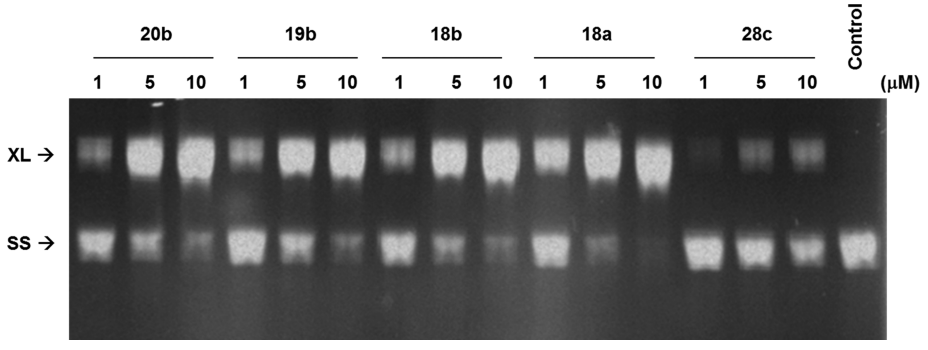

Figure 8. Representative DNA cross-linking gel shift assay for bis(hydroxymethyl) derivatives (**18a** and **18b**) and bis(alkylcarbamate) derivatives (**19b**, **20b**, and **28c**) at 1, 5, and 10 μM: XL, cross-linking DNA; SS, single-strand DNA.

Dimethyl 3-Methyl-6,11-dihydro-5*H*-indolizino[6,7-*b*]indole-1,2-dicarboxylate (16). DMAD (8.4 g, 59.5 mmol) was added to a solution of **15** (10 g, 39.6 mmol) in Ac_2O (70 mL), and the reaction mixture was heated at 70 °C for 2 h with stirring. The reaction mixture was evaporated to dryness in vacuo, and the residue was recrystallized from MeOH to give **16**, 11.5 g (86%); mp 252−253 °C (lit.[35] mp 255−260 °C). ^{1}H NMR (DMSO-d_6) δ 2.43 (3H, s, Me), 3.73 (3H, s, COOMe), 3.75 (3H, s, COOMe), 4.16 (2H, s, CH_2), 5.21 (2H, s, CH_2), 7.02−7.06 (1H, m, ArH), 7.11−7.14 (1H, m, ArH), 7.39 (1H, d, J = 8.0 Hz, ArH), 7.52 (1H, d, J = 7.8 Hz, ArH), 11.12 (1H, brs, exchangeable, NH).

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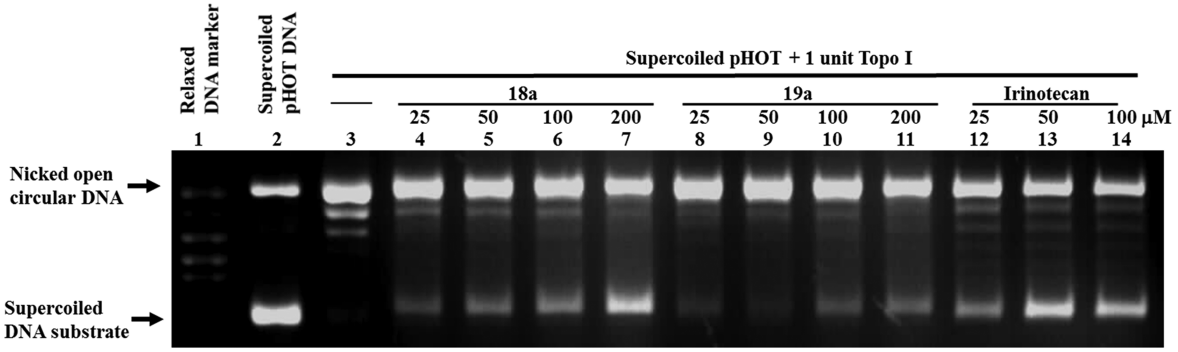

Figure 9. Inhibition of topo I activity by **18a** and **19a**: lane 1, marker for relaxed pHOT1 DNA; lane 2, supercoiled pHOT DNA, no enzyme; lane 3, DNA plus 1 unit of topo I; lanes 4−7, DNA plus 1 unit of topo I in the presence of 25, 50, 100, and 200 μM **18a**; lanes 8−11, DNA plus 1 unit of topo I in the presence of 25, 50, 100, and 200 μM **19a**; lanes 12−14, DNA plus 1 unit of topo I in the presence of 25, 50, and 100 μM irinotecan. All reaction samples were separated by electrophoresis on a 1% agarose gel, stained with ethidium bromide, and photographed under UV light as described in the topo I mediated DNA relaxation assay.

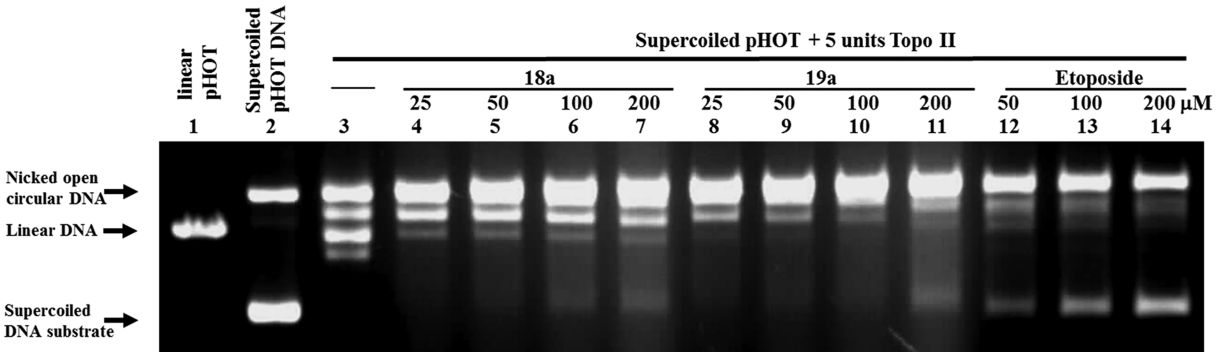

Figure 10. Inhibition of topo II catalytic activity by **18a** and **19a**: lane 1, marker for linear pHOT1 DNA; lane 2, supercoiled pHOT DNA, no enzyme; lane 3, DNA plus 5 units of topo II; lanes 4−7, DNA plus 5 units of topo II in the presence of 10, 50, 100, and 200 μM **18a**; lanes 8−11, DNA plus 5 units of topo II in the presence of 10, 50, 100, and 200 μM **19a**; lanes 12−14, DNA plus 5 units of topo II plus 50, 100, and 200 μM etoposide. All reaction samples were electrophoresed in 1% agarose gels as described in the topo I mediated DNA relaxation assay.

Dimethyl 3,6-Dimethyl-6,11-dihydro-5*H*-indolizino[6,7-*b*]-indole-1,2-dicarboxylate (17a). Compound **16** (6 g, 17.7 mmol) was dissolved in DMF (150 mL) and cooled to 0−5 °C. NaH (0.63 g, 26.5 mmol) was then added. After the mixture was stirred for 15 min, MeI (2.5 g, 17.7 mmol) was added, and the reaction mixture was stirred for 1 h at 0−5 °C and then at room temperature for 9 h. The excess hydride was decomposed with MeOH, and the reaction mixture was evaporated to dryness in vacuo. The residue was crystallized from MeOH to give **17a**, 5.8 g (94%); mp 240−241 °C. ^{1}H NMR (DMSO-d_6) δ 2.45 (3H, s, Me), 3.73 (6H, s, COOMe and Me), 3.74 (3H, s, COOMe), 4.16 (2H, s, CH_2), 5.27 (2H, s, CH_2), 7.05−7.09 (1H, m, ArH), 7.17−7.21 (1H, m, ArH), 7.47−7.49 (1H, m, ArH), 7.52−7.54 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 10.3, 20.8, 29.4, 51.0, 51.3, 102.3, 109.2, 109.4, 112.8, 117.9, 118.9, 121.4, 125.2, 128.4, 132.4, 132.5, 137.4, 164.5, 165.5. Anal. Calcd for $C_{20}H_{20}N_2O_4$: C, 68.17; H, 5.72; N, 7.95. Found: C, 67.79; H, 5.73; N, 7.92.

Following the same synthetic procedure as for **17a**, the following compounds were prepared.

Dimethyl 6-Ethyl-3-methyl-6,11-dihydro-5*H*-indolizino[6,7-*b*]indole-1,2-dicarboxylate (17b). Compound **17b** was prepared from **16** (5.1 g, 15 mmol), NaH (0.54 g, 22.5 mmol), and EtI (2.3 g, 15 mmol). Yield 5.1 g (92%); mp 202−203 °C. ^{1}H NMR (DMSO-d_6) δ 1.29 (3H, t, J = 6.9 Hz, Me), 2.46 (3H, s, Me), 3.73 (3H, s, COOMe), 3.74 (3H, s, COOMe), 4.17 (2H, s, CH_2), 4.20 (2H, q, J = 6.9 Hz, CH_2), 5.29 (2H, s, CH_2), 7.05−7.09 (1H, m, ArH), 7.16−7.20 (1H, m, ArH), 7.49−7.51 (1H, m, ArH), 7.53−7.55 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 10.4, 15.3, 20.8, 37.6, 51.0, 51.2, 102.6, 109.2, 109.5, 112.9, 118.1, 119.0, 121.5, 125.5, 127.7, 132.4, 132.6, 136.4, 164.5, 165.5. Anal. Calcd for $C_{21}H_{22}N_2O_4$: C, 68.84; H, 6.05; N, 7.65. Found: C, 68.46; H, 6.06; N, 7.59.

Dimethyl 6-Benzyl-3-methyl-6,11-dihydro-5*H*-indolizino-[6,7-*b*]indole-1,2-dicarboxylate (17c). Compound **17c** was prepared from **16** (5.1 g, 15 mmol), NaH (0.54 g, 22.5 mmol), and benzyl bromide (2.65 g, 15 mmol). Yield 6.0 g (90%); mp 195−196 °C. ^{1}H NMR (DMSO-d_6) δ 2.39 (3H, s, Me), 3.72 (3H, s, COOMe), 3.74 (3H, s, COOMe), 4.21 (2H, s, CH_2), 5.23 (2H, s, CH_2), 5.46 (2H, s, NCH_2), 7.06−7.14 (4H, m, 4 × ArH), 7.21−7.23 (1H, m, ArH), 7.24−7.29 (2H, m, 2 × ArH), 7.40−7.42 (1H, m, ArH), 7.56−7.58 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 10.3, 20.9, 46.2, 51.0, 51.2, 103.3, 109.3, 110.1, 112.9, 118.2, 119.4, 121.8, 125.6, 126.6, 127.3, 128.3, 128.7, 132.3, 132.6, 137.1, 137.9, 164.5, 165.4. Anal. Calcd for $C_{26}H_{24}N_2O_4$: C, 72.88; H, 5.65; N, 6.54. Found: C, 72.65; H, 5.64; N, 6.46.

[3,6-Dimethyl-6,11-dihydro-5*H*-indolizino[6,7-*b*]indole-1,2-diyl]dimethanol (18a). A solution of **17a** (3.5 g, 10.0 mmol) in anhydrous DCM (35 mL) was added dropwise to a stirred suspension of LAH (0.9 g, 25.0 mmol) in anhydrous diethyl ether (20 mL) at 0 to −5 °C. The reaction mixture was further stirred for 15 min after the addition was completed. The excess hydride was destroyed by the sequential addition of water (1 mL), 15% aqueous (aq) NaOH (1 mL), and water (1 mL) at 0 °C. The mixture was filtered through a pad of Celite and washed with DCM. The combined filtrate and washings were evaporated to dryness in vacuo. The residue was crystallized from ether to give **18a**. Yield 2.3 g. ^{1}H NMR (DMSO-d_6) δ 2.29 (3H, s, Me), 3.73 (3H, s, NMe), 3.99 (2H, s, CH_2), 4.38 (4H, br s, CH_2 and exchangeable, OH), 4.44 (2H, s, CH_2), 5.13 (2H, s, CH_2), 7.05 (1H, t, J = 7.4 Hz, ArH), 7.16 (1H, t, J = 7.4 Hz, ArH), 7.46 (1H, d, J = 7.8 Hz, ArH), 7.53 (1H, d, J = 7.8 Hz, ArH). ^{13}C NMR (DMSO-d_6) δ 9.4, 18.6, 29.3, 54.1, 54.2, 103.5, 109.2, 116.8, 117.9, 118.7, 119.2, 121.1, 121.2, 123.6, 125.6, 130.0, 137.3. Anal. Calcd for

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$C_{18}H_{20}N_2O_2 \cdot 0.5H_2O$: C, 70.80; H, 6.93; N, 9.17. Found: C, 70.85; H, 6.65; N, 9.02.

Following the same synthetic procedure as for **18a**, the following compounds were prepared.

[6-Ethyl-3-methyl-6,11-dihydro-5*H*-indolizino[6,7-*b*]indole-1,2-diyl]dimethanol (18b). Compound **18b** was prepared from **17b** (3.6 g, 10 mmol) and LAH (0.92 g, 25 mmol). Yield 2.5 g. ^{1}H NMR (DMSO-d_6) δ 1.29 (3H, t, J = 7.1 Hz, Me), 2.30 (3H, s, Me), 4.00 (2H, s, CH_2), 4.21 (2H, q, J = 7.1 Hz, CH_2), 4.38 (3H, br s, CH_2 and exchangeable, OH), 4.44 (3H, br s, CH_2 and exchangeable, OH), 5.13 (2H, s, CH_2), 7.04−7.07 (1H, m, ArH), 7.14−7.18 (1H, m, ArH), 7.47−7.49 (1H, m, ArH), 7.53−7.55 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 9.4, 15.3, 18.6, 37.5, 54.2, 54.3, 103.8, 109.3, 116.8, 118.0, 118.7, 119.2, 121.1, 121.2, 123.7, 125.9, 129.1, 136.3. Anal. Calcd for $C_{19}H_{22}N_2O_2$: C, 73.52; H, 7.14; N, 9.03. Found: C, 73.68; H, 6.93; N, 8.66.

[6-Benzyl-3-methyl-6,11-dihydro-5*H*-indolizino[6,7-*b*]-indole-1,2-diyl]dimethanol (18c). Compound **18c** was prepared from **17c** (5.3 g, 12 mmol) and LAH (1.10 g, 30 mmol). Yield 3.6 g. ^{1}H NMR (DMSO-d_6) δ 2.21 (3H, s, Me), 4.04 (2H, s, CH_2), 4.36 (3H, br s, CH_2 and exchangeable, OH), 4.44 (3H, br s, CH_2 and exchangeable, OH), 5.04 (2H, s, CH_2), 5.47 (2H, s, NCH_2), 7.04−7.07 (3H, m, 3 × ArH), 7.08−7.10 (1H, m, ArH), 7.20−7.22 (1H, m, ArH), 7.26−7.30 (2H, m, 2 × ArH), 7.41−7.43 (1H, m, ArH), 7.57−7.59 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 9.4, 18.7, 46.1, 54.1, 54.2, 104.6, 109.9, 116.9, 118.2, 119.1, 119.4, 121.5, 121.9, 123.6, 125.9, 126.3, 127.2, 128.7, 129.7, 137.1, 138.1. Anal. Calcd for $C_{24}H_{24}N_2O_2$: C, 77.39; H, 6.49; N, 7.52. Found: C, 77.19; H, 6.28; N, 7.16.

General Procedure for the Preparation of Bis-(alkylcarbamate) Derivatives (19a−c and 20a−c). Alkyl isocyanate (5 equiv) was added to a solution of bis(hydroxymethyl) derivative (**17**, 1.0 equiv) and TEA (2−3 equiv) in anhydrous DMF. The reaction mixture was stirred at ambient temperature (for 8−12 h) under argon. After completion of the reaction, the reaction mixture was evaporated to dryness in vacuo. The residue was triturated with ether, and the separated solid was collected by filtration. The desired product was obtained by either recrystallization or column chromatography.

[3,6-Dimethyl-6,11-dihydro-5*H*-indolizino[6,7-*b*]indole-1,2-diyl]bis(methylene) Bis(ethylcarbamate) (19a). Compound **19a** was prepared from **18a** (0.29 g, 1 mmol), TEA (0.3 mL), and ethyl isocyanate (0.28 g, 4 mmol). Yield 0.3 g. ^{1}H NMR (DMSO-d_6) δ 0.98 (6H, t, J = 6.9 Hz, 2 × Me), 2.32 (3H, s, Me), 2.98 (4H, q, J = 6.9 Hz, CH_2), 3.73 (3H, s, NMe), 4.04 (2H, s, CH_2), 4.94 (2H, s, CH_2), 4.98 (2H, s, CH_2), 5.17 (2H, s, CH_2), 6.89 (2H, br s, exchangeable, NH), 7.04−7.08 (1H, m, ArH), 7.15−7.19 (1H, m, ArH), 7.46−7.48 (1H, m, ArH), 7.52−7.54 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 9.4, 15.1, 18.6, 29.4, 34.9, 56.8, 56.9, 103.2, 109.3, 112.4, 114.6, 117.9, 118.8, 121.2, 124.5, 125.5, 126.2, 129.7, 137.4, 156.3. Anal. Calcd for $C_{24}H_{30}N_4O_4$: C, 65.73; H, 6.90; N, 12.78. Found: C, 65.53; H, 6.75; N, 12.44.

[6-Ethyl-3-methyl-6,11-dihydro-5*H*-indolizino[6,7-*b*]indole-1,2-diyl]bis(methylene) Bis(ethylcarbamate) (19b). Compound **19b** was prepared from **18b** (0.62 g, 2 mmol), TEA (0.6 mL), and ethyl isocyanate (0.56 g, 8 mmol). Yield 0.52 g. ^{1}H NMR (DMSO-d_6) δ 0.98 (6H, t, J = 7.1 Hz, 2 × Me), 1.29 (3H, t, J = 7 Hz, Me), 2.33 (3H, s, Me), 2.97 (4H, q, J = 7.1 Hz, CH_2), 4.04 (2H, s, CH_2), 4.21 (2H, q, J = 7 Hz, CH_2), 4.94 (2H, s, CH_2), 4.99 (2H, s, CH_2), 5.17 (2H, s, CH_2), 6.89 (2H, br s, exchangeable, NH), 7.04−7.08 (1H, m, ArH), 7.15−7.18 (1H, m, ArH), 7.47−7.49 (1H, m, ArH), 7.52−7.54 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 9.4, 15.1, 15.2, 18.6, 34.9, 37.5, 56.8, 56.9, 103.4, 109.4, 112.4, 114.6, 118.0, 118.8, 121.2, 124.4, 125.8, 128.8, 136.3, 156.3. Anal. Calcd for $C_{25}H_{32}N_4O_4 \cdot 0.5H_2O$: C, 65.06; H, 7.21; N, 12.14. Found: C, 64.71; H, 7.09; N, 12.03.

[6-Benzyl-3-methyl-6,11-dihydro-5*H*-indolizino[6,7-*b*]-indole-1,2-diyl]bis(methylene) Bis(ethylcarbamate) (19c). Compound **19c** was prepared from **18c** (0.37 g, 1 mmol), TEA (0.3 mL), and ethyl isocyanate (0.28 g, 4 mmol). Yield 0.3 g. ^{1}H NMR (DMSO-d_6) δ 0.98 (6H, t, J = 7.1 Hz, 2 × Me), 2.24 (3H, s, Me), 2.97 (4H, q, J = 7.1 Hz, CH_2), 4.09 (2H, s, CH_2), 4.92 (2H, s, CH_2), 4.99 (2H, s, CH_2), 5.09 (2H, s, CH_2), 5.47 (2H, s, NCH_2), 6.89 (2H, br s, exchangeable, NH), 7.06−7.09 (3H, m, 3 × ArH), 7.11−7.14 (1H, m, ArH), 7.20−7.22 (1H, m, ArH), 7.27−7.30 (2H, m, 2 × ArH), 7.40−7.42 (1H, m, ArH), 7.57−7.59 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 9.3, 15.1, 18.7, 34.9, 46.1, 56.8, 56.9, 104.2, 109.9, 112.5, 114.7, 118.1, 119.1, 121.6, 124.4, 125.9, 126.4, 127.2, 128.6, 129.3, 137.1, 138.0, 156.3, 156.4. Anal. Calcd for $C_{30}H_{34}N_4O_4$: C, 70.02; H, 6.66; N, 10.89. Found: C, 69.76; H, 6.64; N, 10.52.

[3,6-Dimethyl-6,11-dihydro-5*H*-indolizino[6,7-*b*]indole-1,2-diyl]bis(methylene) Bis(isopropylcarbamate) (20a). Compound **20a** was prepared from **18a** (0.29 g, 1 mmol), TEA (0.3 mL), and *i*-Pr isocyanate (0.34 g, 4 mmol). Yield 0.32 g. ^{1}H NMR (DMSO-d_6) δ 1.02 (12H, d, J = 6.4 Hz, 4 × Me), 2.31 (3H, s, Me), 3.56 (2H, m, CH), 3.73 (3H, s, NMe), 4.03 (2H, s, CH_2), 4.94 (2H, s, CH_2), 4.98 (2H, s, CH_2), 5.16 (2H, s, CH_2), 6.82 (2H, br s, exchangeable, NH), 7.04−7.07 (1H, m, ArH), 7.15−7.18 (1H, m, ArH), 7.45−7.47 (1H, m, ArH), 7.52−7.54 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 9.4, 18.6, 22.6, 23.3, 29.3, 42.1, 56.6, 56.8, 103.2, 109.3, 112.4, 114.6, 117.9, 118.8, 121.2, 124.4, 125.5, 126.1, 129.7, 137.4, 155.6. Anal. Calcd for $C_{26}H_{34}N_4O_4$: C, 66.93; H, 7.35; N, 12.01. Found: C, 66.79; H, 7.15; N, 11.73.

[6-Ethyl-3-methyl-6,11-dihydro-5*H*-indolizino[6,7-*b*]indole-1,2-diyl]bis(methylene) Bis(isopropylcarbamate) (20b). Compound **20b** was prepared from **18b** (0.62 g, 2 mmol), TEA (0.6 mL), and *i*-Pr isocyanate (0.68 g, 8 mmol). Yield 0.61 g. ^{1}H NMR (DMSO-d_6) δ 1.01 (12H, d, J = 6.4 Hz, 4 × Me), 1.29 (3H, t, J = 7 Hz, Me), 2.33 (3H, s, Me), 3.57 (2H, m, CH), 4.04 (2H, s, CH_2), 4.21 (2H, q, J = 7 Hz, CH_2), 4.94 (2H, s, CH_2), 4.99 (2H, s, CH_2), 5.17 (2H, s, CH_2), 6.82 (2H, br s, exchangeable, NH), 7.04−7.08 (1H, m, ArH), 7.15−7.18 (1H, m, ArH), 7.45−7.49 (1H, m, ArH), 7.52−7.54 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 9.4, 15.2, 18.6, 22.6, 37.6, 42.1, 56.6, 56.8, 103.4, 109.3, 112.4, 114.7, 118.0, 118.8, 121.2, 124.4, 125.8, 126.2, 128.8, 136.3, 155.6, 155.7. Anal. Calcd for $C_{27}H_{36}N_4O_4$: C, 67.48; H, 7.55; N, 11.66. Found: C, 67.26; H, 7.59; N, 11.54.

[6-Benzyl-3-methyl-6,11-dihydro-5*H*-indolizino[6,7-*b*]-indole-1,2-diyl]bis(methylene) Bis(isopropylcarbamate) (20c). Compound **20c** was prepared from **18c** (0.18 g, 0.5 mmol), TEA (0.2 mL), and *i*-Pr isocyanate (0.17 g, 2 mmol). Yield 0.18 g. ^{1}H NMR (DMSO-d_6) δ 1.01 (12H, d, J = 6.4 Hz, 4 × Me), 2.25 (3H, s, Me), 3.57 (2H, m, CH), 4.09 (2H, s, CH_2), 4.92 (2H, s, CH_2), 4.99 (2H, s, CH_2), 5.10 (2H, s, CH_2), 5.47 (2H, s, NCH_2), 6.81 (2H, br s, exchangeable, NH), 7.06−7.09 (3H, m, 3 × ArH), 7.11−7.14 (1H, m, ArH), 7.20−7.24 (1H, m, ArH), 7.27−7.30 (2H, m, 2 × ArH), 7.41−7.43 (1H, m, ArH), 7.56−7.58 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 9.3, 18.7, 22.6, 42.1, 46.1, 56.6, 56.8, 104.2, 109.9, 112.6, 114.8, 118.1, 119.2, 121.6, 124.3, 125.9, 126.1, 127.2, 128.6, 129.3, 137.1, 138.0, 155.6. Anal. Calcd for $C_{32}H_{38}N_4O_4$: C, 70.82; H, 7.06; N, 10.32. Found: C, 70.55; H, 6.93; N, 10.03.

Methyl 1,2,3,4-Tetrahydro-9*H*-pyrido[3,4-*b*]indole-3-carboxylate (22). Formalin [37% (12.5 mL)] was added to L-tryptophan methyl ester hydrochloride (**21**, 25.4 g, 100 mmol) in aqueous MeOH (170 mL; ratio 10:1). The resulting mixture was stirred at room temperature for 5 h. The reaction mixture was evaporated to dryness in vacuo. The residue was treated with sodium bicarbonate. A white solid separated out and was filtered, washed with water, and dried. The resulting powder weighed 14.4 g (63%); mp 161−162 °C (lit.[35] mp 164−165 °C). ^{1}H NMR (DMSO-d_6) δ 2.78 (1H, m, CH_2), 2.98 (1H, m, CH_2), 3.66 (3H, s, COOMe), 3.85 (1H, m, CH), 4.02 (2H, q, J = 15.8 Hz, NCH_2), 6.92−6.96 (1H, m, ArH), 7.00−7.04 (1H, m, ArH), 7.27−7.29 (1H, m, ArH), 7.37−7.39 (1H, m, ArH), 10.78 (1H, br s, exchangeable NH).

Methyl 2-Propionyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]-indole-3-carboxylate (23a). A mixture of **22** (6.9 g, 30 mmol), propionyl chloride (2.7 g, 30 mmol), and TEA (4.8 mL) in anhydrous THF (150 mL) was refluxed for 9 h. The reaction mixture was concentrated under vacuo and then diluted with water. The solid separated out and was filtered and washed with water and hexane to give **23a**. Yield 5.1 g (60%); mp 73−74 °C. ^{1}H NMR (DMSO-d_6) δ 1.05 (3H, m, Me), 2.50 (2H, m, CH_2), 3.02 (1H, m, CH_2), 3.34 (1H, m, CH_2), 3.55 and 3.57 (3H, s, COOMe), 4.14 and 5.06 (1H, d, J = 17

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Hz, NCH_2), 4.60 and 4.90 (1H, d, J = 15.8 Hz, NCH_2), 5.29 and 5.72 (1H, d, J = 5.2 Hz, CH), 6.94−6.98 (1H, m, ArH), 7.03−7.07 (1H, m, ArH), 7.27−7.31 (1H, m, ArH), 7.41−7.43 (1H, m, ArH), 10.89 and 10.90 (1H, br s, exchangeable NH). ^{13}C NMR (DMSO-d_6) δ 9.0, 22.6, 23.3, 38.6, 52.2, 67.0, 104.2, 111.0, 117.6, 118.6, 118.7, 120.9, 126.2, 129.8, 136.2, 171.4, 173.7. Anal. Calcd for $C_{16}H_{18}N_2O_3 \cdot 0.55H_2O$: C, 64.87; H, 6.50; N, 9.46. Found: C, 65.03; H, 6.75; N, 9.06.

Following the same synthetic procedure as for **23a**, the following compounds were prepared.

Methyl 2-(Benzoyl)-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]-indole-3-carboxylate (23b). Compound **23b** was prepared from **22** (6.9 g, 30 mmol), benzoyl chloride (4.2 g, 30 mmol), and TEA (4.8 mL). Yield 7.5 g (75%); mp 193−194 °C (lit.[43] 218−219 °C); ^{1}H NMR (DMSO-d_6) δ 3.09 (1H, m, CH_2), 3.33 (1H, m, CH_2), 3.54 and 3.65 (3H, s, COOMe), 4.39 and 5.18 (1H, d, J = 17.1 Hz, NCH_2), 4.52 and 4.64 (1H, d, J = 16.6 Hz, NCH_2), 4.86 and 5.81 (1H, m, CH), 6.95−6.99 (1H, m, ArH), 7.04−7.06 (1H, m, ArH), 7.24−7.31 (1H, m, ArH), 7.43−7.45 (2H, m, 2 × ArH), 7.46−7.51 (4H, m, 4 × ArH), 10.66 and 10.95 (1H, br s, exchangeable NH). ^{13}C NMR (DMSO-d_6) δ 22.4, 43.8, 52.5, 64.9, 104.5, 111.1, 117.7, 118.7, 121.2, 126.2, 126.5, 128.7, 129.4, 130.0, 135.7, 136.1, 170.8, 171.1. Anal. Calcd for $C_{20}H_{18}N_2O_3$: C, 71.84; H, 5.43; N, 8.38. Found: C, 71.80; H, 5.42; N, 8.46.

Methyl 2-(4-Fluorobenzoyl)-1,2,3,4-tetrahydro-9*H*-pyrido-[3,4-*b*]indole-3-carboxylate (23c). Compound **23c** was prepared from **22** (6.9 g, 30 mmol), 4-fluorobenzoyl chloride (4.7 g, 30 mmol), and TEA (4.8 mL). Yield 9.2 g (88%); mp 191−192 °C. ^{1}H NMR (DMSO-d_6) δ 3.10 (1H, m, CH_2), 3.33 (1H, m, CH_2), 3.55 and 3.65 (3H, s, COOMe), 4.38 and 5.16 (1H, d, J = 17.1 Hz, NCH_2), 4.54 and 4.66 (1H, d, J = 16.2 Hz, NCH_2), 4.89 and 5.80 (1H, m, CH), 6.96−7.00 (1H, m, ArH), 7.05−7.07 (1H, m, ArH), 7.26−7.33 (3H, m, 3 × ArH), 7.43−7.45 (1H, m, ArH), 7.53−7.57 (2H, m, 2 × ArH), 10.68 and 10.95 (1H, br s, exchangeable NH). ^{13}C NMR (DMSO-d_6) δ 22.4, 43.9, 52.5, 57.0, 104.6, 111.1, 115.8, 117.7, 118.7, 121.2, 126.3, 129.3, 132.1, 136.2, 161.9, 170.3, 171.0. Anal. Calcd for $C_{20}H_{17}FN_2O_3$: C, 68.17; H, 4.86; N, 7.95. Found: C, 68.43; H, 4.85; N, 7.77.

Methyl 2-(4-Chlorobenzoyl)-1,2,3,4-tetrahydro-9*H*-pyrido-[3,4-*b*]indole-3-carboxylate (23d). Compound **23d** was prepared from **22** (6.9 g, 30 mmol), 4-chlorobenzoylchloride (5.2 g, 30 mmol), and TEA (4.8 mL). Yield 9 g (82%); mp 243−244 °C (lit.[35,37] 245−246 °C). ^{1}H NMR (DMSO-d_6) δ 3.10 (1H, m, CH_2), 3.35 (1H, m, CH_2), 3.55 and 3.65 (3H, s, COOMe), 4.41 and 5.18 (1H, d, J = 17.4 Hz, NCH_2), 4.51 and 4.64 (1H, d, J = 16.4 Hz, NCH_2), 4.88 and 5.82 (1H, d, J = 5.4 Hz, CH), 6.96−6.99 (1H, m, ArH), 7.03−7.08 (1H, m, ArH), 7.25−7.32 (1H, m, ArH), 7.42−7.60 (5H, m, 5 × ArH), 10.65 and 10.95 (1H, br s, exchangeable NH). ^{13}C NMR (DMSO-d_6) δ 22.5, 43.8, 52.5, 67.0, 104.6, 111.1, 117.7, 118.7, 121.1, 126.1, 128.6, 128.8, 129.2, 134.5, 136.1, 169.4, 170.8.

Methyl 2-(3,4-Difluorobenzoyl)-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole-3-carboxylate (23e). Compound **23e** was prepared from **22** (4.6 g, 20 mmol), 3,4-difluorobenzoylchloride (3.5 g, 20 mmol), and TEA (3.2 mL). Yield 5.8 g (78%); mp 81−82 °C. ^{1}H NMR (DMSO-d_6) δ 3.10 (1H, m, CH_2), 3.35 (1H, m, CH_2), 3.55 and 3.65 (3H, s, COOMe), 4.36 and 5.14 (1H, d, J = 16.6 Hz, NCH_2), 4.55 and 4.67 (1H, d, J = 15.5 Hz, NCH_2), 4.92 and 5.78 (1H, d, J = 5.2 Hz, CH), 6.96−6.99 (1H, m, ArH), 7.04−7.06 (1H, m, ArH), 7.27−7.33 (2H, m, 2 × ArH), 7.43−7.45 (1H, m, ArH), 7.52−7.57 (2H, m, 2 × ArH), 10.67 and 10.94 (1H, br s, exchangeable NH). ^{13}C NMR (DMSO-d_6) δ 22.5, 43.8, 52.6, 56.9, 104.6, 111.1, 116.5, 117.7, 118.2, 118.7, 121.1, 124.1, 126.2, 129.2, 132.9, 136.2, 149.4, 169.4, 170.7. Anal. Calcd for $C_{20}H_{16}F_2N_2O_3$: C, 64.86; H, 4.35; N, 7.56. Found: C, 64.63; H, 4.23; N, 7.18.

Methyl 2-(3-Chloro-4-fluorobenzoyl)-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole-3-carboxylate (23f). Compound **23f** was prepared from **22** (4.6 g, 20 mmol), 3-chloro-4-fluorobenzoyl chloride (3.8 g, 20 mmol), and TEA (3.2 mL). Yield 6.1 g (79%); mp 97−98 °C. ^{1}H NMR (DMSO-d_6) δ 3.09 (1H, m, CH_2), 3.32 (1H, m, CH_2), 3.56 and 3.65 (3H, s, COOMe), 4.37 and 5.15 (1H, d, J = 17.4 Hz, NCH_2), 4.55 and 4.67 (1H, d, J = 16.2 Hz, NCH_2), 4.93 and 5.79 (1H, d, J = 4.9 Hz, CH), 6.96−6.99 (1H, m, ArH), 7.05−7.08 (1H, m, ArH), 7.25−7.31 (1H, m, ArH), 7.48−7.55 (3H, m, 3 × ArH), 7.68−7.72 (1H, m, ArH), 10.67 and 10.95 (1H, br s, exchangeable NH). ^{13}C NMR (DMSO-d_6) δ 22.6, 43.8, 52.6, 56.9, 104.6, 111.1, 117.5, 118.7, 120.2, 121.2, 126.3, 127.7, 129.4, 130.4, 131.5, 133.4, 136.2, 158.3, 164.1, 168.9, 170.8. Anal. Calcd for $C_{20}H_{16}ClFN_2O_3$: C, 62.10; H, 4.17; N, 7.24. Found: C, 61.89; H, 4.03; N, 6.97.

Methyl 2-(4-Methoxybenzoyl)-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole-3-carboxylate (23g). Compound **23g** was prepared from **22** (6.9 g, 30 mmol), 4-methoxybenzoyl chloride (5.1 g, 30 mmol), and TEA (4.8 mL). Yield 7.3 g (67%); mp 159−160 °C. ^{1}H NMR (DMSO-d_6) δ 3.11 (1H, m, CH_2), 3.36 (1H, m, CH_2), 3.54 and 3.64 (3H, s, COOMe), 3.82 (3H, s, OMe), 4.37 and 5.11 (1H, d, J = 17.6 Hz, NCH_2), 4.66 (1H, m, NCH_2), 4.97 and 5.76 (1H, m, CH), 6.96−6.99 (1H, m, ArH), 7.03−7.06 (3H, m, 3 × ArH), 7.25−7.28 (1H, m, ArH), 7.43−7.46 (3H, m, 3 × ArH), 10.67 and 10.94 (1H, br s, exchangeable NH). ^{13}C NMR (DMSO-d_6) δ 22.6, 44.1, 52.6, 55.2, 57.1, 104.6, 111.1, 114.1, 117.7, 118.7, 121.1, 126.2, 127.6, 128.7, 129.6, 130.0, 136.2, 160.5, 170.9, 171.4. Anal. Calcd for $C_{21}H_{20}N_2O_4$: C, 69.22; H, 5.53; N, 7.69. Found: C, 69.07; H, 5.58; N, 7.57.

Methyl 2-(3,4-Dimethoxybenzoyl)-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole-3-carboxylate (23h). Compound **23h** was prepared from **22** (6.9 g, 30 mmol), 3,4-dimethoxybenzoyl chloride (6 g, 30 mmol), and TEA (4.8 mL). Yield 7.2 g (61%); mp 195−196 °C. ^{1}H NMR (DMSO-d_6) δ 3.12 (1H, m, CH_2), 3.34 (1H, m, CH_2), 3.55 and 3.64 (3H, s, COOMe), 3.78 (3H, s, OMe), 3.81 (3H, s, OMe), 4.37 and 5.13 (1H, d, J = 17.8 Hz, NCH_2), 4.67 (1H, m, NCH_2), 5.02 and 5.74 (1H, m, CH), 6.96−6.99 (1H, m, ArH), 7.02−7.08 (4H, m, 4 × ArH), 7.26−7.31 (1H, m, ArH), 7.44−7.46 (1H, m, ArH), 10.66 and 10.94 (1H, br s, exchangeable NH). ^{13}C NMR (DMSO-d_6) δ 22.7, 44.2, 51.3, 52.6, 57.1, 104.6, 110.3, 111.3, 117.7, 118.7, 121.1, 123.2, 126.3, 127.7, 129.7, 136.2, 149.4, 170.1, 171.4. Anal. Calcd for $C_{22}H_{22}N_2O_5$: C, 66.99; H, 5.62; N, 7.10. Found: C, 66.63; H, 5.62; N, 6.89.

Methyl 2-(3,4,5-Trimethoxybenzoyl)-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole-3-carboxylate (23i). Compound **23i** was prepared from **22** (4.6 g, 20 mmol), 3,4,5-trimethoxybenzoyl chloride (4.6 g, 20 mmol), and TEA (3.2 mL). Yield 4.9 g (58%); mp 207−208 °C. ^{1}H NMR (DMSO-d_6) δ 3.11 (1H, m, CH_2), 3.34 (1H, m, CH_2), 3.57 and 3.65 (3H, s, COOMe), 3.71 (3H, s, OMe), 3.80 (6H, s, 2 × OMe), 4.36 and 5.16 (1H, d, J = 16.9 Hz, NCH_2), 4.64 (1H, m, NCH_2), 4.99 and 5.76 (1H, m, CH), 6.72 (2H, s, 2 × ArH), 6.96−6.99 (1H, m, ArH), 7.04−7.07 (1H, m, ArH), 7.31−7.33 (1H, m, ArH), 7.42−7.44 (1H, m, ArH), 10.66 and 10.94 (1H, br s, exchangeable NH). ^{13}C NMR (DMSO-d_6) δ 22.6, 43.9, 52.5, 56.1, 60.1, 104.1, 111.1, 117.7, 118.1, 121.1, 126.3, 129.5, 130.9, 136.2, 138.6, 153.1, 170.5, 171.1. Anal. Calcd for $C_{23}H_{24}N_2O_6$: C, 65.08; H, 5.70; N, 6.60. Found: C, 64.79; H, 5.75; N, 6.45.

2-Propionyl-9-methyl-1,2,3,4-tetrahydropyrido[3,4-*b*]-indole-3-carboxylic Acid (24a). Compound **23a** (4.2 g, 15 mmol) was dissolved in THF (150 mL), and NaH (0.72 g, 30 mmol) was added to the reaction mixture at 0−5 °C. After the mixture was stirred for 15 min, MeI (2.1 g, 15 mmol) was added and the reaction mixture was stirred for 1 h at 0−5 °C and then at room temperature for 8 h. The excess of hydride was decomposed by the treatment of MeOH. The reaction mixture was concentrated in vacuo and diluted with water. The pH was adjusted to 6 with acetic acid to give 2.8 g (67%); mp 246−247 °C. ^{1}H NMR (acetic acid-d_4) δ 1.20 (3H, m, Me), 2.67 (2H, m, CH_2), 3.09 (1H, m, CH_2), 3.52 (1H, m, CH_2), 3.62 and 3.64 (3H, s, NMe), 4.45 and 5.28 (1H, d, J = 17.5 Hz, NCH_2), 4.83 and 4.92 (1H, d, J = 15.2 Hz, NCH_2), 5.23 and 6.02 (1H, d, J = 5.2 Hz, CH), 7.02−7.07 (1H, m, ArH), 7.12−7.17 (1H, m, ArH), 7.28−7.30 (1H, m, ArH), 7.44−7.46 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 9.1, 22.6, 26.2, 29.1, 37.9, 67.0, 104.3, 109.2, 117.7, 118.7, 120.9, 125.8, 131.1, 136.9, 172.4, 173.8. Anal. Calcd for $C_{16}H_{18}N_2O_3$: C, 67.12; H, 6.34; N, 9.78. Found: C, 66.97; H, 6.72; N, 9.43.

Following the same synthetic procedure as for **24a**, the following compounds were prepared.

2-(Benzoyl)-9-methyl-1,2,3,4-tetrahydropyrido[3,4-*b*]indole-3-carboxylic Acid (24b). Compound **24b** was prepared from **23b** (6.7 g, 20 mmol), NaH (0.96 g, 40 mmol), and MeI (2.8 g, 20 mmol).

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Yield 4.8 g (73%); mp 164−165 °C (lit.[44] 175−176 °C). ^{1}H NMR (acetic acid-d_4) δ 3.18 (1H, m, CH_2), 3.52 (1H, m, CH_2), 3.43 and 3.68 (3H, s, NMe), 4.64 and 5.40 (1H, m, NCH_2), 4.71 and 4.91 (1H, m, NCH_2), 5.03 and 6.14 (1H, m, CH), 7.03−7.06 (1H, m, ArH), 7.14−7.17 (1H, m, ArH), 7.30−7.33 (1H, m, ArH), 7.50−7.56 (4H, m, 4 × ArH), 7.57−7.59 (2H, m, 2 × ArH). ^{13}C NMR (DMSO-d_6) δ 22.5, 29.1, 38.3, 56.8, 104.4, 109.3, 117.8, 118.7, 120.9, 125.8, 126.4, 128.8, 129.8, 130.9, 136.1, 136.9, 171.5, 171.9. Anal. Calcd for $C_{20}H_{18}N_2O_3$: C, 71.84; H, 5.43; N, 8.38. Found: C, 71.68; H, 5.37; N, 8.36.

2-(4-Fluorobenzoyl)-9-methyl-1,2,3,4-tetrahydropyrido[3,4-*b*]indole-3-carboxylic Acid (24c). Compound **24c** was prepared from **23c** (3.5 g, 10 mmol), NaH (0.46g, 20 mmol), and MeI (1.4 g, 10 mmol). Yield 2.6 g (74%); mp 266−267 °C. ^{1}H NMR (acetic acid-d_4) δ 3.18 (1H, m, CH_2), 3.56 (1H, m, CH_2), 3.46 and 3.68 (3H, s, NMe), 4.64 and 5.40 (1H, d, J = 17.1 Hz, NCH_2), 4.70 and 4.93 (1H, d, J = 15.7 Hz, NCH_2), 5.04 and 6.11 (1H, d, J = 4.7 Hz, CH), 7.03−7.07 (1H, m, ArH), 7.14−7.18 (1H, m, ArH), 7.21−7.25 (2H, m, 2 × ArH), 7.31−7.33 (1H, m, ArH), 7.45−7.49 (1H, m, ArH), 7.60−7.66 (2H, m, 2 × ArH). ^{13}C NMR (DMSO-d_6) δ 23.2, 43.9, 50.9, 57.1, 104.8, 111.1, 115.8, 117.6, 118.6, 121.1, 126.3, 129.2, 132.5, 136.2, 161.2, 170.6, 172.0. Anal. Calcd for $C_{20}H_{17}FN_2O_3$: C, 68.17; H, 4.86; N, 7.95. Found: C, 68.21; H, 5.21; N, 7.88.

2-(4-Chlorobenzoyl)-9-methyl-1,2,3,4-tetrahydropyrido[3,4-*b*]indole-3-carboxylic Acid (24d). Compound **24d** was prepared from **23d** (7.3 g, 20.0 mmol), NaH (0.96 g, 40.0 mmol), and MeI (2.8 g, 20.0 mmol). Yield 5.4 g (74%); mp 262−263 °C. ^{1}H NMR (acetic acid-d_4) δ 3.18 (1H, m, CH_2), 3.56 (1H, m, CH_2), 3.45 and 3.68 (3H, s, NMe), 4.64 and 5.40 (1H, d, J = 15.8 Hz, NCH_2), 4.70 and 4.93 (1H, d, J = 17.1 Hz, NCH_2), 5.02 and 6.12 (1H, d, J = 5.4 Hz, CH), 7.04−7.07 (1H, m, ArH), 7.15−7.18 (1H, m, ArH), 7.31−7.33 (1H, m, ArH), 7.45−7.47 (1H, m, ArH), 7.50−7.55 (3H, m, 3 × ArH), 7.59−7.61 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 23.1, 29.2, 50.8, 56.8, 104.4, 109.3, 117.8, 118.8, 121.0, 125.8, 128.7, 130.7, 134.6, 136.9, 170.6, 171.7. Anal. Calcd for $C_{20}H_{17}ClN_2O_3$: C, 65.13; H, 4.65; N, 7.60. Found: C, 64.77; H, 4.79; N, 7.68.

2-(3,4-Difluorobenzoyl)-9-methyl-1,2,3,4-tetrahydropyrido-[3,4-*b*]indole-3-carboxylic Acid (24e). Compound **24e** was prepared from **23e** (5.5 g, 15 mmol), NaH (0.72 g, 30 mmol), and MeI (2.1 g, 15 mmol). Yield 4.2 g (76%); mp 231−232 °C. ^{1}H NMR (acetic acid-d_4) δ 3.17 (1H, m, CH_2), 3.55 (1H, m, CH_2), 3.46 and 3.68 (3H, s, NMe), 4.64 and 5.37 (1H, m, NCH_2), 4.69 and 4.92 (1H, m, NCH_2), 5.02 and 6.08 (1H, m, CH), 7.03−7.08 (1H, m, ArH), 7.13−7.17 (1H, m, ArH), 7.31−7.38 (3H, m, 3 × ArH), 7.47−7.51 (2H, m, 2 × ArH). ^{13}C NMR (DMSO-d_6) δ 23.0, 29.2, 50.9, 56.9, 104.6, 109.3, 111.1, 116.3, 117.7, 118.3, 121.0, 123.9, 130.6, 136.2, 136.9, 170.6, 171.8. Anal. Calcd for $C_{20}H_{16}F_2N_2O_3$: C, 64.86; H, 4.35; N, 7.56. Found: C, 64.63; H, 4.47; N, 7.57.

2-(3-Chloro-4-fluorobenzoyl)-9-methyl-1,2,3,4-tetrahydropyrido[3,4-*b*]indole-3-carboxylic Acid (24f). Compound **24f** was prepared from **23f** (6 g, 15 mmol), NaH (0.72 g, 30 mmol), and MeI (2.1 g, 15 mmol). Yield 5.1 g (85%); mp 183−184 °C. ^{1}H NMR (acetic acid-d_4) δ 3.18 (1H, m, CH_2), 3.57 (1H, m, CH_2), 3.47 and 3.69 (3H, s, NMe), 4.65 and 5.37 (1H, d, J = 17.2 Hz, NCH_2), 4.71 and 4.94 (1H, d, J = 15.8 Hz, NCH_2), 5.03 and 6.09 (1H, d, J = 4 Hz, CH), 7.04−7.08 (1H, m, ArH), 7.15−7.19 (1H, m, ArH), 7.32−7.37 (2H, m, 2 × ArH), 7.45−7.51 (2H, m, 2 × ArH), 7.68−7.70 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 23.1, 29.1, 50.6, 57.4, 104.3, 109.2, 117.3, 118.7, 120.1, 129.0, 130.7, 136.9, 170.8, 171.5. Anal. Calcd for $C_{20}H_{16}ClFN_2O_3$: C, 62.10; H, 4.17; N, 7.24. Found: C, 61.88; H, 4.37; N, 7.31.

2-(4-Methoxybenzoyl)-9-methyl-1,2,3,4-tetrahydropyrido-[3,4-*b*]indole-3-carboxylic Acid (24g). Compound **24g** was prepared from **23g** (3.6 g, 10 mmol), NaH (0.48 g, 20 mmol), and MeI (1.4 g, 10 mmol). Yield 2.3 g (64%); mp 222−223 °C. ^{1}H NMR (acetic acid-d_4) δ 3.19 (1H, m, CH_2), 3.56 (1H, m, CH_2), 3.47 and 3.68 (3H, s, NMe), 3.85 (3H, s, OMe), 4.66 and 5.37 (1H, d, J = 17.2 Hz, NCH_2), 4.81 and 4.97 (1H, d, J = 15.2 Hz, NCH_2), 5.16 and 6.10 (1H, d, J = 5 Hz, CH), 7.01−7.05 (3H, m, 3 × ArH), 7.14−7.17 (1H, m, ArH), 7.31−7.33 (1H, m, ArH), 7.46−7.52 (3H, m, 3 × ArH). ^{13}C NMR (DMSO-d_6) δ 23.2, 29.2, 50.8, 55.3, 56.9, 104.4, 109.3, 114.1, 117.8, 118.8, 120.9, 125.8, 128.0, 128.5, 129.1, 131.0, 136.9, 160.4, 170.3, 171.9. Anal. Calcd for $C_{21}H_{20}N_2O_4$: C, 69.22; H, 5.53; N, 7.69. Found: C, 69.11; H, 5.77; N, 7.96.

2-(3,4-Dimethoxybenzoyl)-9-methyl-1,2,3,4-tetrahydropyrido[3,4-*b*]indole-3-carboxylic Acid (24h). Compound **24h** was prepared from **23h** (5.9 g, 15 mmol), NaH (0.72 g, 30 mmol), and MeI (2.1 g, 15 mmol). Yield 4.7 g (79%); mp 197−198 °C. ^{1}H NMR (acetic acid-d_4) δ 3.19 (1H, m, CH_2), 3.56 (1H, m, CH_2), 3.47 and 3.68 (3H, s, NMe), 3.86 (3H, s, OMe), 3.88 (3H, s, OMe), 4.66 and 5.38 (1H, d, J = 17.4 Hz, NCH_2), 4.86 and 4.98 (1H, d, J = 15.6 Hz, NCH_2), 5.22 and 6.09 (1H, d, J = 5.2 Hz, CH), 6.99−7.02 (1H, m, ArH), 7.04−7.07 (1H, m, ArH), 7.14−7.18 (2H, m, 2 × ArH), 7.21−7.25 (1H, m, ArH), 7.31−7.33 (1H, m, ArH), 7.46−7.48 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 23.4, 29.2, 50.8, 55.5, 57.4, 104.8, 109.2, 110.4, 111.5, 117.7, 118.6, 119.4, 120.8, 131.3, 136.9, 148.5, 170.9, 172.3. Anal. Calcd for $C_{22}H_{22}N_2O_5$: C, 66.99; H, 5.62; N, 7.10. Found: C, 66.73; H, 5.85; N, 6.81.

2-(3,4,5-Trimethoxybenzoyl)-9-methyl-1,2,3,4-tetrahydropyrido[3,4-*b*]indole-3-carboxylic Acid (24i). Compound **24i** was prepared from **23i** (4.2 g, 10 mmol), NaH (0.48 g, 20 mmol), and MeI (1.4 g, 10 mmol). Yield 3.2 g (76%); mp 280−282 °C. ^{1}H NMR (acetic acid-d_4) δ 3.21 (1H, m, CH_2), 3.56 (1H, m, CH_2), 3.47 and 3.69 (3H, s, NMe), 3.84 (3H, s, OMe), 3.87 (6H, s, 2 × OMe), 4.66 and 5.39 (1H, d, J = 16.9 Hz, NCH_2), 4.83 and 4.95 (1H, d, J = 16.1 Hz, NCH_2), 5.18 and 6.08 (1H, d, J = 5.2 Hz, CH), 6.86 (2H, s, 2 × ArH), 7.04−7.07 (1H, m, ArH), 7.15−7.18 (1H, m, ArH), 7.32−7.34 (1H, m, ArH), 7.45−7.47 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 24.0, 29.2, 56.1, 59.0, 60.2, 104.3, 109.1, 117.8, 118.5, 120.6, 126.5, 131.8, 132.9, 136.9, 138.1, 152.9, 170.8, 172.5. Anal. Calcd for $C_{23}H_{24}N_2O_6$: C, 65.08; H, 5.70; N, 6.60. Found: C, 64.89; H, 5.31; N, 6.87.

Dimethyl 3-(Ethyl)-6-methyl-6,11-dihydro-5*H*-indolizino-[6,7-*b*]indole-1,2-dicarboxylate (25a). DMAD (1.7 g, 12 mmol) was added to a solution of **24a** (2.2 g, 8 mmol) in Ac_2O (30 mL), and the reaction mixture was heated at 70 °C for 2 h with stirring. The reaction mixture was evaporated to dryness in vacuo, and the residue was recrystallized from MeOH to give 1.8 g (64%); mp 231−232 °C. ^{1}H NMR (DMSO-d_6) δ 1.19 (3H, t, J = 7.4 Hz, Me), 2.87 (2H, q, J = 7.4 Hz, CH_2), 3.73 (3H, s, COOMe), 3.74 (3H, s, COOMe), 3.75 (3H, s, NMe), 4.17 (2H, s, CH_2), 5.34 (2H, s, CH_2), 7.06−7.09 (1H, m, ArH), 7.17−7.21 (1H, m, ArH), 7.47−7.49 (1H, m, ArH), 7.53−7.55 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 14.3, 17.7, 20.9, 29.5, 51.1, 51.3, 102.3, 109.4, 112.6, 118.0, 119.0, 121.5, 125.2, 128.7, 132.2, 137.5, 137.8, 164.6, 165.5. Anal. Calcd for $C_{21}H_{22}N_2O_4$: C, 68.84; H, 6.05; N, 7.65. Found: C, 68.73; H, 5.96; N, 7.64.

Following the same synthetic procedure as for **25a**, the following compounds were prepared:

Dimethyl 3-(Phenyl)-6-methyl-6,11-dihydro-5*H*-indolizino-[6,7-*b*]indole-1,2-dicarboxylate (25b). Compound **25b** was prepared from **24b** (3.3 g, 10 mmol) and DMAD (2.1 g, 15 mmol) in AC_2O (40 mL). Yield 3.0 g, (74%); mp 249−250 °C. ^{1}H NMR (DMSO-d_6) δ 3.58 (6H, s, 2 × COOMe), 3.77 (3H, s, NMe), 4.30 (2H, s, CH_2), 5.17 (2H, s, CH_2), 7.06−7.9 (1H, m, ArH), 7.16−7.19 (1H, m, ArH), 7.44−7.46 (1H, m, ArH), 7.47−7.53 (5H, m, 5 × ArH), 7.55−7.57 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 21.3, 29.4, 41.9, 51.2, 51.7, 102.2, 109.2, 116.4, 118.0, 119.1, 121.5, 125.1, 128.5, 129.7, 130.3, 133.0, 133.9, 137.4, 164.1, 165.8. Anal. Calcd for $C_{25}H_{22}N_2O_4$: C, 72.45; H, 5.35; N, 6.76. Found: C, 72.34; H, 5.33; N, 6.72.

Dimethyl 3-(4-Fluorophenyl)-6-methyl-6,11-dihydro-5*H*-indolizino[6,7-*b*]indole-1,2-dicarboxylate (25c). Compound **25c** was prepared from **24c** (1.7 g, 4.8 mmol) and DMAD (1.0 g, 7.2 mmol) in AC_2O (15 mL). Yield 1.45 g (73%); mp 253−254 °C. ^{1}H NMR (DMSO-d_6) δ 3.59 (6H, s, 2 × COOMe), 3.78 (3H, s, NMe), 4.31 (2H, s, CH_2), 5.16 (2H, s, CH_2), 7.07−7.10 (1H, m, ArH), 7.17−7.21 (1H, m, ArH), 7.33−7.37 (2H, m, 2 × ArH), 7.45−7.47 (1H, m, ArH), 7.56−7.59 (3H, m, 3 × ArH). ^{13}C NMR (DMSO-d_6) δ 21.3, 29.4, 41.8, 51.2, 51.6, 102.2, 109.3, 115.4, 116.3, 117.9, 119.0, 121.5, 125.1, 126.0, 128.7, 132.2, 133.8, 137.5, 164.0, 165.5. Anal. Calcd for

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$C_{25}H_{21}FN_2O_4$: C, 69.44; H, 4.89; N, 6.48. Found: C, 69.21; H, 4.93; N, 6.47.

Dimethyl 3-(4-Chlorophenyl)-6-methyl-6,11-dihydro-5*H*-indolizino[6,7-*b*]indole-1,2-dicarboxylate (25d). Compound **25d** was prepared from **24d** (4 g, 10.8 mmol) and DMAD (2.3 g, 16.3 mmol) in AC_2O (30 mL). Yield 3.5 g, (72%); mp 264−265 °C. ^{1}H NMR (DMSO-d_6) δ 3.60 (6H, s, 2 × COOMe), 3.78 (3H, s, NMe), 4.30 (2H, s, CH_2), 5.18 (2H, s, CH_2), 7.06−7.10 (1H, m, ArH), 7.17−7.20 (1H, m, ArH), 7.45−7.47 (1H, m, ArH), 7.54−7.59 (5H, m, 5 × ArH). ^{13}C NMR (DMSO-d_6) δ 21.3, 29.5, 41.9, 51.3, 51.7, 102.1, 109.3, 116.5, 118.0, 119.1, 121.6, 125.1, 128.6, 131.9, 132.3, 133.7, 134.1, 137.5, 164.0, 165.5. Anal. Calcd for $C_{25}H_{21}ClN_2O_4$: C, 66.89; H, 4.72; N, 6.24. Found: C, 66.82; H, 4.63; N, 6.24.

Dimethyl 3-(3,4-Difluorophenyl)-6-methyl-6,11-dihydro-5*H*-indolizino[6,7-*b*]indole-1,2-dicarboxylate (25e). Compound **25e** was prepared from **24e** (3.7 g, 10 mmol) and DMAD (2.1 g, 15 mmol) in AC_2O (50 mL). Yield 3.2 g (71%); mp 253−254 °C. ^{1}H NMR (DMSO-d_6) δ 3.61 (6H, s, 2 × COOMe), 3.78 (3H, s, NMe), 4.29 (2H, s, CH_2), 5.19 (2H, s, CH_2), 7.06−7.08 (1H, m, ArH), 7.17−7.20 (1H, m, ArH), 7.36−7.38 (1H, m, ArH), 7.45−7.47 (1H, m, ArH), 7.55−7.59 (2H, m, 2 × ArH), 7.63−7.64 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 21.2, 29.4, 41.8, 51.2, 51.6, 102.1, 109.4, 116.5, 118.0, 119.1, 121.5, 125.1, 128.7, 131.2, 133.9, 137.5, 164.0, 165.2. Anal. Calcd for $C_{25}H_{20}F_2N_2O_4$: C, 66.66; H, 4.48; N, 6.22. Found: C, 66.63; H, 4.47; N, 6.22.

Dimethyl 3-(3-Chloro-4-fluorophenyl)-6-methyl-6,11-dihydro-5*H*-indolizino[6,7-*b*]indole-1,2-dicarboxylate (25f). Compound **25f** was prepared from **24f** (4.6 g, 12 mmol) and DMAD (2.5 g, 18 mmol) in AC_2O (90 mL). Yield 4.1 g (73%); mp 250−251 °C. ^{1}H NMR (DMSO-d_6) δ 3.60 (6H, s, 2 × COOMe), 3.78 (3H, s, NMe), 4.28 (2H, s, CH_2), 5.17 (2H, s, CH_2), 7.05−7.09 (1H, m, ArH), 7.16−7.19 (1H, m, ArH), 7.44−7.46 (1H, m, ArH), 7.54−7.58 (3H, m, 3 × ArH), 7.75−7.78 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 21.2, 29.5, 41.8, 51.2, 51.6, 102.1, 109.5, 116.5, 118.0, 119.1, 121.6, 125.1, 128.7, 131.3, 132.7, 133.9, 137.5, 164.0, 165.2. Anal. Calcd for $C_{25}H_{20}ClFN_2O_4$: C, 64.31; H, 4.32; N, 6.00. Found: C, 63.92; H, 4.09; N, 5.92.

Dimethyl 3-(4-Methoxyphenyl)-6-methyl-6,11-dihydro-5*H*-indolizino[6,7-*b*]indole-1,2-dicarboxylate (25g). Compound **25g** was prepared from **24g** (2 g, 5.4 mmol) and DMAD (1.2 g, 8.2 mmol) in AC_2O (15 mL). Yield 1.5 g (61%); mp 211−212 °C. ^{1}H NMR (DMSO-d_6) δ 3.59 (6H, s, 2 × COOMe), 3.77 (3H, s, NMe), 3.83 (3H, s, OMe), 4.29 (2H, s, CH_2), 5.14 (2H, s, CH_2), 7.04−7.09 (3H, m, 3 × ArH), 7.16−7.20 (1H, m, ArH), 7.43−7.47 (3H, m, 3 × ArH), 7.56−7.58 (1H, m, ArH). 13C NMR (DMSO-d_6) δ 21.4, 29.4, 41.8, 51.2, 51.6, 55.2, 102.3, 109.0, 113.9, 116.0, 118.0, 119.1, 121.5, 125.1, 128.8, 131.8, 132.9, 133.6, 133.9, 137.5, 159.5, 164.1, 165.8. Anal. Calcd for $C_{26}H_{24}N_2O_5$: C, 70.26; H, 5.44; N, 6.30. Found: C, 69.94; H, 5.43; N, 6.21.

Dimethyl 3-(3,4-Dimethoxyphenyl)-6-methyl-6,11-dihydro-5*H*-indolizino[6,7-*b*]indole-1,2-dicarboxylate (25h). Compound **25h** was prepared from **24h** (4.3 g, 11 mmol) and DMAD (2.3 g, 16.5 mmol) in AC_2O (60 mL). Yield 3.7 g (72%); mp 241−242 °C. ^{1}H NMR (DMSO-d_6) δ 3.60 (3H, s, COOMe), 3.61 (3H, s, COOMe), 3.78 (3H, s, NMe), 3.79 (3H, s, OMe), 3.83 (3H, s, OMe), 4.30 (2H, s, CH_2), 5.19 (2H, s, CH_2), 7.05−7.10 (4H, m, 4 × ArH), 7.17−7.20 (1H, m, ArH), 7.45−7.48 (1H, m, ArH), 7.56−7.58 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 21.3, 29.4, 41.9, 51.1, 51.6, 55.5, 102.3, 109.4, 111.5, 113.9, 116.1, 118.0, 119.0, 121.5, 125.1, 128.9, 133.3, 137.5, 148.3, 164.1, 165.9. Anal. Calcd for $C_{27}H_{26}N_2O_6$: C, 68.34; H, 5.52; N, 5.90. Found: C, 68.60; H, 5.90; N, 5.61.

Dimethyl 3-(3,4,5-Trimethoxyphenyl)-6-methyl-6,11-dihydro-5*H*-indolizino[6,7-*b*]indole-1,2-dicarboxylate (25i). Compound **25i** was prepared from **24i** (3 g, 7 mmol) and DMAD (1.5 g, 10.5 mmol) in AC_2O (40 mL). Yield 3.0 g (84%); mp 253−254 °C. ^{1}H NMR (DMSO-d_6) δ 3.62 (3H, s, COOMe), 3.63 (3H, s, COOMe), 3.73 (3H, s, OMe), 3.77 (3H, s, NMe), 3.80 (6H, s, 2 × OMe), 4.30 (2H, s, CH_2), 5.26 (2H, s, CH_2), 6.82 (2H, s, 2 × ArH), 7.05−7.09 (1H, m, ArH), 7.15−7.19 (1H, m, ArH), 7.45−7.47 (1H, m, ArH), 7.55−7.57 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 21.4, 29.5, 51.1, 51.7, 56.1, 60.0, 102.2, 107.8, 116.4, 118.0, 121.5, 125.1, 128.9, 132.7, 133.0, 133.7, 152.7, 164.0, 165.9. Anal. Calcd for $C_{28}H_{28}N_2O_7$: C, 66.66; H, 5.59; N, 5.55. Found: C, 66.31; H, 5.63; N, 5.45.

[3-Ethyl-6-methyl-6,11-dihydro-5*H*-indolizino[6,7-*b*]indole-1,2-diyl]dimethanol (26a). A solution of **25a** (1.46 g, 4 mmol) in anhydrous DCM (30 mL) was added dropwise to a stirred suspension of LAH (0.37 g, 10 mmol) in anhydrous diethyl ether (10 mL) at 0 to −5 °C. The reaction mixture was further stirred for 30 min after the addition was completed. The excess hydride was decomposed by the sequential addition of water (1 mL), 15% aqueous NaOH (1 mL), and water (1 mL) at 0 °C. The mixture was filtered through a pad of Celite, and the solid residue was washed with DCM. The filtrate was washed with water and brine solution. The organic layer was dried over anhydrous Na_2SO_4 and concentrated to dryness in vacuo. The oily residue was crystallized from ether to give 0.81 g. ^{1}H NMR (DMSO-d_6) δ 1.17 (3H, t, J = 7.4 Hz, Me), 2.74 (2H, q, J = 7.4 Hz, CH_2), 3.75 (3H, s, NMe), 4.00 (2H, s, CH_2), 4.34 (1H, br s, exchangeable, OH), 4.36 (2H, s, CH_2), 4.42 (3H, br s, CH_2 and exchangeable, OH), 5.19 (2H, s, CH_2), 7.04−7.07 (1H, m, ArH), 7.15−7.19 (1H, m, ArH), 7.45−7.47 (1H, m, ArH), 7.53−7.55 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 15.7, 16.8, 18.6, 19.4, 54.1, 54.2, 103.5, 109.2, 116.9, 117.9, 118.7, 118.9, 121.1, 121.9, 125.6, 129.8, 130.0, 137.4. Anal. Calcd for $C_{19}H_{22}N_2O_2$: C, 73.52; H, 7.14; N, 9.03. Found: C, 73.16; H, 7.18; N, 8.92.

Following the same synthetic procedure as for **26a**, the following compounds were prepared.

[3-Phenyl-6-methyl-6,11-dihydro-5*H*-indolizino[6,7-*b*]-indole-1,2-diyl]dimethanol (26b). Compound **26b** was prepared from **25b** (2.1 g, 5.0 mmol) and LAH (0.46 g, 12.5 mmol). Yield 1.48 g. ^{1}H NMR (DMSO-d_6) δ 3.60 (3H, s, NMe), 4.10 (2H, s, CH_2), 4.30 (2H, m, CH_2), 4.57 (4H, br s, CH_2 and 2 × OH), 5.15 (2H, s, CH_2), 7.04−7.08 (1H, m, ArH), 7.14−7.18 (1H, m, ArH), 7.38−7.42 (2H, m, 2 × ArH), 7.45−7.48 (2H, m, 2 × ArH), 7.51−7.58 (3H, m, 3 × ArH). ^{13}C NMR (DMSO-d_6) δ 18.9, 29.3, 41.2, 54.2, 54.4, 103.3, 109.3, 117.9, 118.3, 118.8, 121.2, 121.7, 124.6, 125.5, 127.1, 128.3, 129.5, 130.2, 130.3, 131.8, 137.4. Anal. Calcd for $C_{23}H_{22}N_2O_2$: C, 77.07; H, 6.19; N, 7.82. Found: C, 76.83; H, 5.94; N, 7.50.

[3-(4-Fluorophenyl)-6-methyl-6,11-dihydro-5*H*-indolizino-[6,7-*b*]indole-1,2-diyl]dimethanol (26c). Compound **26c** was prepared from **25c** (1.3 g, 3 mmol) and LAH (0.27 g, 7.5 mmol). Yield 0.86 g. ^{1}H NMR (DMSO-d_6) δ 3.61 (3H, s, NMe), 4.10 (2H, s, CH_2), 4.27 (2H, s, CH_2), 4.56 (4H, br s, CH_2 and exchangeable, 2 × OH), 5.13 (2H, s, CH_2), 7.04−7.08 (1H, m, ArH), 7.14−7.18 (1H, m, ArH), 7.31−7.35 (2H, m, 2 × ArH), 7.44−7.46 (1H, m, ArH), 7.56−7.60 (3H, m, 3 × ArH). ^{13}C NMR (DMSO-d_6) δ 18.9, 29.3, 41.1, 54.2, 54.3, 103.3, 109.3, 115.1, 115.3, 117.9, 118.2, 118.8, 121.2, 121.8, 124.6, 125.5, 128.1, 128.5, 130.1, 132.3, 132.4, 137.4. Anal. Calcd for $C_{23}H_{21}FN_2O_2$: C, 73.39; H, 5.62; N, 7.44. Found: C, 73.16; H, 5.33; N, 7.09.

[3-(4-Chlorophenyl)-6-methyl-6,11-dihydro-5*H*-indolizino-[6,7-*b*]indole-1,2-diyl]dimethanol (26d). Compound **26d** was prepared from **25d** (3.2 g, 7 mmol) and LAH (0.6g, 17.8 mmol). Yield 2.2 g. ^{1}H NMR (DMSO-d_6) δ 3.62 (3H, s, NMe), 4.10 (2H, s, CH_2), 4.29 (2H, m, CH_2), 4.55 (2H, s, CH_2), 4.59 (2H, br s, exchangeable, 2 × OH), 5.16 (2H, s, CH_2), 7.04−7.08 (1H, m, ArH), 7.14−7.18 (1H, m, ArH), 7.43−7.45 (1H, m, ArH), 7.53−7.59 (5H, m, 5 × ArH). ^{13}C NMR (DMSO-d_6) δ 18.9, 29.3, 41.2, 54.1, 54.2, 103.3, 109.3, 117.9, 118.4, 118.8, 121.2, 122.1, 125.1, 125.4, 128.3, 128.4, 130.1, 130.6, 131.9, 137.4. Anal. Calcd for $C_{23}H_{21}ClN_2O_2{\cdot}0.5H_2O$: C, 68.74; H, 5.52; N, 6.97. Found: C, 68.72; H, 5.16; N, 6.94.

[3-(3,4-Difluorophenyl)-6-methyl-6,11-dihydro-5*H*-indolizino[6,7-*b*]indole-1,2-diyl]dimethanol (26e). Compound **26e** was prepared from **25e** (2.7 g, 6 mmol) and LAH (0.55 g, 15 mmol). Yield 1.8 g. ^{1}H NMR (DMSO-d_6) δ 3.63 (3H, s, NMe), 4.10 (2H, s, CH_2), 4.28 (2H, m, CH_2), 4.55 (2H, s, CH_2), 4.62 (2H, br s, exchangeable, 2 × OH), 5.19 (2H, s, CH_2), 7.05−7.08 (1H, m, ArH), 7.14−7.16 (1H, m, ArH), 7.39−7.41 (1H, m, ArH), 7.44−7.46 (1H,

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m, ArH), 7.54−7.59 (2H, m, 2 × ArH), 7.64−7.68 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 18.9, 29.3, 411, 54.1, 54.2, 103.2, 109.3, 117.3, 117.4, 117.9, 118.4, 118.8, 119.0, 119.1, 121.2, 122.3, 125.2, 125.5, 127.3, 127.5, 129.3, 130.1, 137.4. Anal. Calcd for $C_{23}H_{20}F_2N_2O_2$: C, 70.04; H, 5.11; N, 7.10. Found: C, 69.86; H, 4.95; N, 7.24.

[3-(3-Chloro-4-fluorophenyl)-6-methyl-6,11-dihydro-5*H*-indolizino[6,7-*b*]indole-1,2-diyl]dimethanol (26f). Compound **26f** was prepared from **25f** (3.7 g, 8 mmol) and LAH (0.74 g, 20 mmol). Yield 2.6 g. ^{1}H NMR (DMSO-d_6) δ 3.61 (3H, s, NMe), 4.08 (2H, s, CH_2), 4.26 (2H, m, CH_2), 4.54 (2H, s, CH_2), 4.62 (2H, br s, exchangeable, 2 × OH), 5.17 (2H, s, CH_2), 7.03−7.07 (1H, m, ArH), 7.13−7.17 (1H, m, ArH), 7.42−7.44 (1H, m, ArH), 7.52−7.56 (3H, m, 3 × ArH), 7.76−7.78 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 18.9, 29.3, 41.1, 54.1, 54.2, 103.2, 109.3, 116.7, 116.8, 117.9, 118.3, 118.8, 119.3, 119.5, 121.2, 122.3, 125.2, 125.4, 127.2, 129.6, 129.7, 130.1, 130.9, 131.0, 137.4. Anal. Calcd for $C_{23}H_{20}ClFN_2O_2$: C, 67.23; H, 4.91; N, 6.82. Found: C, 66.86; H, 5.00; N, 6.57.

[3-(4-Methoxyphenyl)-6-methyl-6,11-dihydro-5*H*-indolizino-[6,7-*b*]indole-1,2-diyl]dimethanol (26g). Compound **26g** was prepared from **25g** (1.3 g, 3 mmol) and LAH (0.27 g, 7.5 mmol). Yield 0.9 g. ^{1}H NMR (DMSO-d_6) δ 3.61 (3H, s, NMe), 3.83 (3H, s, OMe), 4.09 (2H, s, CH_2), 4.27 (2H, s, CH_2), 4.55 (4H, br s, CH_2 and exchangeable, 2 × OH), 5.10 (2H, s, CH_2), 7.04−7.08 (3H, m, 3 × ArH), 7.14−7.18 (1H, m, ArH), 7.44−7.47 (3H, m, 3 × ArH), 7.56−7.58 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 15.1, 18.9, 29.3, 41.1, 54.5, 55.1, 103.4, 109.3, 113.8, 117.8, 117.9, 118.8, 121.1, 121.2, 123.9, 124.0, 125.5, 129.3, 130.2, 131.6, 137.4, 158.5. Anal. Calcd for $C_{24}H_{24}N_2O_3$: C, 74.21; H, 6.23; N, 7.21. Found: C, 73.94; H, 6.11; N, 7.48.

[3-(3,4-Dimethoxyphenyl)-6-methyl-6,11-dihydro-5*H*-indolizino[6,7-*b*]indole-1,2-diyl]dimethanol (26h). Compound **26h** was prepared from **25h** (3.3 g, 7 mmol) and LAH (0.64 g, 17.5 mmol). Yield 2.3 g. ^{1}H NMR (DMSO-d_6) δ 3.62 (3H, s, NMe), 3.80 (3H, s, OMe), 3.82 (3H, s, OMe), 4.09 (2H, s, CH_2), 4.29 (2H, s, CH_2), 4.52 (1H, br s, exchangeable, OH) 4.55 (3H, br s, CH_2 and exchangeable, OH), 5.14 (2H, s, CH_2), 7.04−7.08 (3H, m, 3 × ArH), 7.12 (1H, s, ArH), 7.14−7.18 (1H, m, ArH), 7.44−7.46 (1H, m, ArH), 7.56−7.58 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 18.9, 29.3, 41.1, 54.3, 54.5, 55.5, 55.6, 103.4, 109.3, 111.7, 114.3, 117.9, 118.0, 118.7, 121.1, 121.2, 122.6, 124.0, 124.2, 125.5, 129.6, 130.3, 137.4, 148.1, 148.4. Anal. Calcd for $C_{25}H_{26}N_2O_4$: C, 71.75; H, 6.26; N, 6.69. Found: C, 71.66; H, 6.29; N, 6.53.

[3-(3,4,5-Trimethoxyphenyl)-6-methyl-6,11-dihydro-5*H*-indolizino[6,7-*b*]indole-1,2-diyl]dimethanol (26i). Compound **26i** was prepared from **25i** (2.5 g, 5 mmol) and LAH (0.46 g, 12.5 mmol). Yield 1.7 g. ^{1}H NMR (DMSO-d_6) δ 3.64 (3H, s, NMe), 3.73 (3H, s, OMe), 3.82 (6H, s, 2 × OMe), 4.10 (2H, s, CH_2), 4.31 (2H, s, CH_2), 4.55 (4H, br s, CH_2 and exchangeable, 2 × OH), 5.20 (2H, s, CH_2), 6.83 (2H, s, 2 × ArH), 7.04−7.08 (1H, m, ArH), 7.14−7.18 (1H, m, ArH), 7.44−7.46 (1H, m, ArH), 7.56−7.58 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 18.9, 29.4, 41.3, 54.2, 54.5, 56.1, 60.0, 103.3, 107.9, 109.3, 117.9, 118.2, 118.8, 121.2, 121.5, 124.4, 125.5, 127.2, 129.8, 130.3, 136.7, 137.4, 152.7. Anal. Calcd for $C_{26}H_{28}N_2O_5$: C, 69.63; H, 6.29; N, 6.25. Found: C, 69.25; H, 6.30; N, 6.08.

General Procedure for Preparing Bis(alkylcarbamate) Derivatives (27a−i and 28a−i). Alkyl isocyanate (5 equiv) was added to a solution of bis(hydroxymethyl) derivatives (**26a−i**, 1.0 equiv) and TEA (2−3 equiv) in anhydrous DMF or THF. The reaction mixture was stirred at ambient temperature (for 8−12 h) under argon. After completion of the reaction, the reaction mixture was evaporated to dryness in vacuo. The residue was triturated with ether, and the separated solid was collected by filtration. The desired product was obtained by either recrystallization or column chromatography.

[3-Ethyl-6-methyl-6,11-dihydro-5*H*-indolizino[6,7-*b*]indole-1,2-diyl]bis(methylene) Bis(ethylcarbamate) (27a). Compound **27a** was prepared from **26a** (0.15 g, 0.5 mmol), TEA (0.2 mL), and ethyl isocyanate (0.14 g, 2 mmol). Yield 0.18 g. ^{1}H NMR (DMSO-d_6) δ 0.99 (6H, t, J = 7.1 Hz, 2 × Me), 1.17 (3H, t, J = 7.4 Hz, Me), 2.77 (2H, q, J = 7.4 Hz, CH_2), 2.99 (4H, q, J = 7.1 Hz, CH_2), 3.75 (3H, s, NMe), 4.04 (2H, s, CH_2), 4.95 (2H, s, CH_2), 4.99 (2H, s, CH_2), 5.23 (2H, s, CH_2), 6.92 (2H, br s, exchangeable, NH), 7.05−7.08 (1H, m, ArH), 7.16−7.20 (1H, m, ArH), 7.46−7.48 (1H, m, ArH), 7.53−7.55 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 15.1, 15.4, 16.8, 18.6, 29.4, 34.9, 56.7, 57.0, 103.1, 109.3, 112.4, 114.3, 117.9, 118.8, 121.2, 122.6, 124.4, 125.5, 129.7, 132.1, 137.4, 156.3, 156.4. Anal. Calcd for $C_{25}H_{32}N_4O_4$: C, 66.35; H, 7.13; N, 12.38. Found: C, 66.19; H, 7.23; N, 12.28.

[3-Phenyl-6-methyl-6,11-dihydro-5*H*-indolizino[6,7-*b*]-indole-1,2-diyl]bis(methylene) Bis(ethylcarbamate) (27b). Compound **27b** was prepared from **26b** (0.36 g, 1 mmol), TEA (0.3 mL), and ethyl isocyanate (0.28 g, 4 mmol). Yield 0.32 g. ^{1}H NMR (DMSO-d_6) δ 1.00 (6H, t, J = 6.8 Hz, 2 × Me), 2.99 (4H, q, J = 6.8 Hz, CH_2), 3.60 (3H, s, NMe), 4.15 (2H, s, CH_2), 4.81 (2H, s, CH_2), 5.08 (2H, s, CH_2), 5.16 (2H, s, CH_2), 6.98 (2H, br s, exchangeable, NH), 7.05−7.09 (1H, m, ArH), 7.15−7.19 (1H, m, ArH), 7.45−7.47 (2H, m, 2 × ArH), 7.51−7.55 (4H, m, 4 × ArH), 7.56−7.58 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 15.1, 18.6, 29.3, 35.0, 41.3, 56.9, 57.3, 103.0, 109.3, 113.6, 116.7, 117.9, 118.8, 121.2, 125.3, 126.8, 127.7, 128.5, 129.8, 130.4, 130.9, 131.5, 137.4, 156.1, 156.4. Anal. Calcd for $C_{29}H_{32}N_4O_4$: C, 69.58; H, 6.44; N, 11.19. Found: C, 69.26; H, 6.25; N, 10.83.

[3-(4-Fluorophenyl)-6-methyl-6,11-dihydro-5*H*-indolizino-[6,7-*b*]indole-1,2-diyl]bis(methylene) Bis(ethylcarbamate) (27c). Compound **27c** was prepared from **26c** (0.2 g, 0.5 mmol), TEA (0.2 mL), and ethyl isocyanate (0.14 g, 2 mmol). Yield 0.2 g. ^{1}H NMR (DMSO-d_6) δ 0.99 (6H, t, J = 7.1 Hz, 2 × Me), 2.99 (4H, q, J = 7.1 Hz, CH_2), 3.60 (3H, s, NMe), 4.14 (2H, s, CH_2), 4.79 (2H, s, CH_2), 5.07 (2H, s, CH_2), 5.12 (2H, s, CH_2), 6.97 (2H, br s, exchangeable, NH), 7.05−7.08 (1H, m, ArH), 7.15−7.19 (1H, m, ArH), 7.32−7.36 (2H, m, 2 × ArH), 7.44−7.46 (1H, m, ArH), 7.54−7.57 (3H, m, 3 × ArH). ^{13}C NMR (DMSO-d_6) δ 15.1, 18.9, 29.3, 35.0, 56.9, 57.2, 102.9, 109.3, 113.5, 115.3, 115.5, 117.9, 118.8, 121.3, 125.4, 126.8, 127.4, 129.8, 132.5, 132.6, 137.4, 156.1, 156.4, 160.9. Anal. Calcd for $C_{29}H_{31}FN_4O_4$: C, 67.17; H, 6.03; N, 10.80. Found: C, 67.48; H, 5.96; N, 10.63.

[3-(4-Chlorophenyl)-6-methyl-6,11-dihydro-5*H*-indolizino-[6,7-*b*]indole-1,2-diyl]bis(methylene) Bis(ethylcarbamate) (27d). Compound **27d** was prepared from **26d** (0.4 g, 1 mmol), TEA (0.4 mL), and ethyl isocyanate (0.28 g, 4 mmol). Yield 0.31 g. ^{1}H NMR (DMSO-d_6) δ 0.98 (6H, t, J = 6.4 Hz, 2 × Me), 2.98 (4H, q, J = 6.4 Hz, CH_2), 3.60 (3H, s, NMe), 4.13 (2H, s, CH_2), 4.80 (2H, s, CH_2), 5.07 (2H, s, CH_2), 5.15 (2H, s, CH_2), 6.97 (2H, br s, exchangeable, NH), 7.04−7.08 (1H, m, ArH), 7.14−7.18 (1H, m, ArH), 7.43−7.45 (1H, m, ArH), 7.54−7.60 (5H, m, 5 × ArH). ^{13}C NMR (DMSO-d_6) δ 15.1, 18.9, 29.3, 35.0, 41.3, 56.8, 57.1, 102.9, 109.3, 113.7, 117.1, 117.9, 118.8, 121.3, 125.3, 127.1, 128.5, 129.8, 130.1, 132.2, 132.7, 137.4, 156.1, 156.4. Anal. Calcd for $C_{29}H_{31}ClN_4O_4 \cdot 0.5H_2O$: C, 64.02; H, 5.93; N, 10.30. Found: C, 63.97; H, 6.01; N, 10.29.

[3-(3,4-Difluorophenyl)-6-methyl-6,11-dihydro-5*H*-indolizino[6,7-*b*]indole-1,2-diyl]bis(methylene) Bis-(ethylcarbamate) (27e). Compound **27e** was prepared from **26e** (0.2 g, 0.5 mmol), TEA (0.2 mL), and ethyl isocyanate (0.14 g, 2 mmol). Yield 0.15 g. ^{1}H NMR (DMSO-d_6) δ 0.99 (6H, t, J = 7 Hz, 2 × Me), 2.98 (4H, q, J = 7 Hz, CH_2), 3.62 (3H, s, NMe), 4.14 (2H, s, CH_2), 4.82 (2H, s, CH_2), 5.07 (2H, s, CH_2), 5.17 (2H, s, CH_2), 6.97 (2H, br s, exchangeable, NH), 7.05−7.09 (1H, m, ArH), 7.15−7.19 (1H, m, ArH), 7.35−7.38 (1H, m, ArH), 7.44−7.46 (1H, m, ArH), 7.53−7.57 (2H, m, 2 × ArH), 7.63−7.65 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 15.1, 15.7, 18.9, 29.4, 34.0, 35.0, 41.2, 56.8, 57.0, 102.8, 109.3, 113.7, 117.4, 117.6, 118.0, 118.9, 119.4, 119.5, 121.3, 125.3, 127.1, 127.6, 128.5, 129.2, 129.8, 137.4, 156.0, 156.3. Anal. Calcd for $C_{29}H_{30}F_2N_4O_4$: C, 64.91; H, 5.64; N, 10.44. Found: C, 65.57; H, 6.01; N, 10.07.

[3-(3-Chloro-4-fluorophenyl)-6-methyl-6,11-dihydro-5*H*-indolizino[6,7-*b*]indole-1,2-diyl]bis(methylene) Bis-(ethylcarbamate) (27f). Compound **27f** was prepared from **26f** (0.4 g, 1 mmol), TEA (0.4 mL), and ethyl isocyanate (0.28 g, 4 mmol). Yield 0.32 g. ^{1}H NMR (DMSO-d_6) δ 0.97 (6H, t, J = 7.1 Hz, 2 × Me), 2.97 (4H, q, J = 7.1 Hz, CH_2), 3.61 (3H, s, NMe), 4.13 (2H, s, CH_2), 4.80 (2H, s, CH_2), 5.07 (2H, s, CH_2), 5.16 (2H, s, CH_2), 6.98

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(2H, br s, exchangeable, NH), 7.04−7.08 (1H, m, ArH), 7.14−7.18 (1H, m, ArH), 7.43−7.45 (1H, m, ArH), 7.53−7.57 (3H, m, 3 × ArH), 7.72−7.74 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 15.1, 15.7, 18.9, 29.4, 34.0, 35.0, 41.2, 56.7, 57.0, 102.8, 109.3, 113.7, 116.8, 116.9, 117.4, 117.9, 118.8, 119.5, 121.3, 125.3, 127.2, 128.8, 129.0, 129.7, 131.2, 132.3, 137.4, 155.9, 156.3, 157.9. Anal. Calcd for $C_{29}H_{30}ClFN_4O_4$: C, 62.98; H, 5.47; N, 10.13. Found: C, 62.64; H, 5.60; N, 10.34.

[3-(4-Methoxyphenyl)-6-methyl-6,11-dihydro-5*H*-indolizino-[6,7-*b*]indole-1,2-diyl]bis(methylene) Bis(ethylcarbamate) (27g). Compound **27g** was prepared from **26g** (0.2 g, 0.5 mmol), TEA (0.2 mL), and ethyl isocyanate (0.14 g, 2 mmol). Yield 0.2 g. ^{1}H NMR (DMSO-d_6) δ 1.00 (6H, t, J = 6.9 Hz, 2 × Me), 2.99 (4H, q, J = 6.9 Hz, CH_2), 3.61 (3H, s, NMe), 3.84 (3H, s, OMe), 4.14 (2H, s, CH_2), 4.79 (2H, s, CH_2), 5.07 (2H, s, CH_2), 5.11 (2H, s, CH_2), 6.97 (2H, br s, exchangeable, NH), 7.06−7.09 (3H, m, 3 × ArH), 7.15−7.19 (1H, m, ArH), 7.43−7.47 (3H, m, 3 × ArH), 7.56−7.58 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 15.1, 18.8, 29.3, 35.0, 41.3, 55.1, 56.9, 57.0, 103.0, 109.3, 113.9, 118.0, 118.8, 121.2, 123.1, 125.4, 131.7, 137.4, 158.9. Anal. Calcd for $C_{30}H_{34}N_4O_5$: C, 67.91; H, 6.46; N, 10.56. Found: C, 67.60; H, 6.35; N, 10.25.

[3-(3,4-Dimethoxyphenyl)-6-methyl-6,11-dihydro-5*H*-indolizino[6,7-*b*]indole-1,2-diyl]bis(methylene) Bis-(ethylcarbamate) (27h). Compound **27h** was prepared from **26h** (0.21 g, 0.5 mmol), TEA (0.2 mL), and ethyl isocyanate (0.14 g, 2 mmol). Yield 0.15 g. ^{1}H NMR (DMSO-d_6) δ 0.98 (6H, t, J = 7 Hz, 2 × Me), 2.98 (4H, q, J = 7 Hz, CH_2), 3.61 (3H, s, NMe), 3.78 (3H, s, OMe), 3.82 (3H, s, OMe), 4.13 (2H, s, CH_2), 4.79 (2H, s, CH_2), 5.06 (2H, s, CH_2), 5.15 (2H, s, CH_2), 6.97 (2H, br s, exchangeable, NH), 7.04−7.10 (4H, m, 4 × ArH), 7.14−7.18 (1H, m, ArH), 7.44−7.46 (1H, m, ArH), 7.55−7.57 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 15.1, 18.8, 25.1, 29.4, 34.9, 35.0, 41.3, 55.5, 57.5, 67.0, 103.0, 109.3, 111.7, 113.4, 114.0, 116.2, 118.0, 118.8, 121.2, 122.7, 123.3, 125.4, 130.0, 137.4, 148.5, 156.2, 156.4. Anal. Calcd for $C_{31}H_{36}N_4O_6$: C, 66.41; H, 6.47; N, 9.99. Found: C, 66.02; H, 6.18; N, 9.63.

[3-(3,4,5-Trimethoxyphenyl)-6-methyl-6,11-dihydro-5*H*-indolizino[6,7-*b*]indole-1,2-diyl]bis(methylene) Bis-(ethylcarbamate) (27i). Compound **27i** was prepared from **26i** (0.45 g, 1 mmol), TEA (0.4 mL), and ethyl isocyanate (0.28 g, 4 mmol). Yield 0.35 g. ^{1}H NMR (DMSO-d_6) δ 0.98 (6H, t, J = 7.1 Hz, 2 × Me), 2.98 (4H, q, J = 7.1 Hz, CH_2), 3.64 (3H, s, NMe), 3.74 (3H, s, OMe), 3.81 (6H, s, 2 × OMe), 4.14 (2H, s, CH_2), 4.81 (2H, s, CH_2), 5.07 (2H, s, CH_2), 5.22 (2H, s, CH_2), 6.79 (2H, m, 2 × ArH), 6.96 (2H, br s, exchangeable, NH), 7.05−7.08 (1H, m, ArH), 7.15−7.18 (1H, m, ArH), 7.45−7.47 (1H, m, ArH), 7.56−7.58 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 15.1, 18.9, 29.4, 34.9, 35.0, 41.4, 55.9, 56.8, 57.5, 60.0, 102.9, 107.7, 109.3, 113.7, 116.4, 117.9, 118.8, 121.3, 125.4, 126.3, 126.6, 130.0, 131.7, 137.0, 137.4, 152.8, 156.2, 156.3. Anal. Calcd for $C_{32}H_{38}N_4O_7$: C, 65.07; H, 6.48; N, 9.49. Found: C, 64.69; H, 6.52; N, 9.26.

[3-Ethyl-6-methyl-6,11-dihydro-5*H*-indolizino[6,7-*b*]indole-1,2-diyl]bis(methylene) Bis(isopropylcarbamate) (28a). Compound **28a** was prepared from **26a** (0.31 g, 1 mmol), TEA (0.3 mL), and *i*-Pr isocyanate (0.36 g, 4 mmol). Yield 0.32 g. ^{1}H NMR (DMSO-d_6) δ 1.00 (12H, d, J = 6.4 Hz, 4 × Me), 1.17 (3H, t, J = 7.4 Hz, Me), 2.77 (2H, q, J = 7.4 Hz, CH_2), 3.59 (2H, m, CH), 3.75 (3H, s, NMe), 4.03 (2H, s, CH_2), 4.94 (2H, s, CH_2), 4.99 (2H, s, CH_2), 5.22 (2H, s, CH_2), 6.83 (2H, br s, exchangeable, NH), 7.04−7.08 (1H, m, ArH), 7.15−7.19 (1H, m, ArH), 7.46−7.48 (1H, m, ArH), 7.52−7.54 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 15.4, 16.8, 18.6, 22.6, 23.3, 29.4, 42.2, 45.6, 56.5, 56.8, 103.1, 109.3, 112.5, 117.9, 118.8, 121.2, 124.4, 125.5, 129.7, 132.1, 137.4, 155.6, 155.7. Anal. Calcd for $C_{27}H_{36}N_4O_4$: C, 67.48; H, 7.55; N, 11.66. Found: C, 67.27; H, 7.77; N, 12.01.

[3-Phenyl-6-methyl-6,11-dihydro-5*H*-indolizino[6,7-*b*]-indole-1,2-diyl]bis(methylene) Bis(isopropylcarbamate) (28b). Compound **28b** was prepared from **26b** (0.36 g, 1 mmol), TEA (0.3 mL), and *i*-Pr isocyanate (0.34 g, 4 mmol). Yield 0.34 g. ^{1}H NMR (DMSO-d_6) δ 1.01 (12H, d, J = 7 Hz, 4 × Me), 3.58 (2H, m, CH), 3.60 (3H, s, NMe), 4.14 (2H, s, CH_2), 4.80 (2H, s, CH_2), 5.07 (2H, s, CH_2), 5.15 (2H, s, CH_2), 6.89 (2H, br s, exchangeable, NH), 7.04−7.08 (1H, m, ArH), 7.14−7.18 (1H, m, ArH), 7.44−7.46 (2H, m, 2 × ArH), 7.48−7.52 (4H, m, 4 × ArH), 7.55−7.57 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 18.9, 22.6, 23.3, 29.3, 40.6, 41.3, 42.2, 56.8, 57.2, 102.9, 109.3, 113.6, 116.7, 117.9, 118.8, 121.2, 125.3, 126.7, 127.7, 128.5, 129.8, 130.4, 130.9, 137.4, 155.4, 155.7. Anal. Calcd for $C_{31}H_{36}N_4O_4$: C, 70.43; H, 6.86; N, 10.60. Found: C, 70.25; H, 7.15; N, 10.75.

[3-(4-Fluorophenyl)-6-methyl-6,11-dihydro-5*H*-indolizino-[6,7-*b*]indole-1,2-diyl]bis(methylene) Bis(isopropylcarbamate) (28c). Compound **28c** was prepared from **26c** (0.2 g, 0.5 mmol), TEA (0.2 mL), and *i*-Pr isocyanate (0.18 g, 2 mmol). Yield 0.19 g. ^{1}H NMR (DMSO-d_6) δ 1.02 (12H, d, J = 6.4 Hz, 4 × Me), 3.55 (2H, m, CH), 3.60 (3H, s, NMe), 4.13 (2H, s, CH_2), 4.79 (2H, s, CH_2), 5.07 (2H, s, CH_2), 5.13 (2H, s, CH_2), 6.88 (2H, br s, exchangeable, NH), 7.05−7.08 (1H, m, ArH), 7.15−7.18 (1H, m, ArH), 7.31−7.35 (2H, m, 2 × ArH), 7.44−7.46 (1H, m, ArH), 7.54−7.57 (3H, m, 3 × ArH). ^{13}C NMR (DMSO-d_6) δ 18.9, 22.6, 29.3, 41.2, 42.2, 56.7, 57.0, 102.9, 109.2, 113.5, 115.3, 115.5, 116.9, 117.9, 118.8, 121.2, 125.3, 126.7, 127.4, 129.8, 130.3, 132.5, 132.6, 137.4, 155.4, 155.7, 160.9, 162.8. Anal. Calcd for $C_{31}H_{35}FN_4O_4$: C, 68.11; H, 6.45; N, 10.25. Found: C, 68.41; H, 6.33; N, 9.96.

[3-(4-Chlorophenyl)-6-methyl-6,11-dihydro-5*H*-indolizino-[6,7-*b*]indole-1,2-diyl]bis(methylene) Bis(isopropylcarbamate) (28d). Compound **28d** was prepared from **26d** (0.4 g, 1 mmol), TEA (0.4 mL), and *i*-Pr isocyanate (0.34 g, 4 mmol). Yield 0.34 g. ^{1}H NMR (DMSO-d_6) δ 1.02 (12H, d, J = 6.4 Hz, 4 × Me), 3.57 (2H, m, CH), 3.61 (3H, s, NMe), 4.14 (2H, s, CH_2), 4.81 (2H, s, CH_2), 5.08 (2H, s, CH_2), 5.16 (2H, s, CH_2), 6.88 (2H, br s, exchangeable, NH), 7.05−7.09 (1H, m, ArH), 7.15−7.19 (1H, m, ArH), 7.44−7.46 (1H, m, ArH), 7.54−7.58 (5H, m, 5 × ArH). ^{13}C NMR (DMSO-d_6) δ 18.9, 22.6, 23.3, 29.3, 41.3, 42.2, 56.7, 56.9, 102.9, 109.3, 113.7, 117.2, 117.9, 118.8, 121.3, 123.1, 125.3, 127.1, 128.5, 129.7, 129.8, 130.0, 132.1, 132.6, 137.4, 155.4, 155.7. Anal. Calcd for $C_{31}H_{35}ClN_4O_4 \cdot 0.5H_2O$: C, 65.08; H, 6.34; N, 9.79. Found: C, 64.94; H, 6.21; N, 9.73.

[3-(3,4-Difluorophenyl)-6-methyl-6,11-dihydro-5*H*-indolizino[6,7-*b*]indole-1,2-diyl]bis(methylene) Bis-(isopropylcarbamate) (28e). Compound **28e** was prepared from **26e** (0.2 g, 0.5 mmol), TEA (0.2 mL), and *i*-Pr isocyanate (0.18 g, 2 mmol). Yield 0.22 g. ^{1}H NMR (DMSO-d_6) δ 1.03 (12H, d, J = 6.4 Hz, 4 × Me), 3.57 (2H, m, CH), 3.63 (3H, s, NMe), 4.14 (2H, s, CH_2), 4.82 (2H, s, CH_2), 5.08 (2H, s, CH_2), 5.18 (2H, s, CH_2), 6.92 (2H, br s, exchangeable, NH), 7.06−7.10 (1H, m, ArH), 7.16−7.20 (1H, m, ArH), 7.36−7.39 (1H, m, ArH), 7.45−7.47 (1H, m, ArH), 7.55−7.62 (3H, m, 3 × ArH). ^{13}C NMR (DMSO-d_6) δ 18.9, 22.6, 23.3, 29.4, 41.2, 42.2, 56.6, 56.8, 102.8, 109.2, 113.7, 117.4, 117.6, 117.9, 118.8, 119.4, 119.5, 121.3, 125.3, 127.1, 127.5, 129.2, 129.7, 137.4, 148.2, 155.3, 155.7. Anal. Calcd for $C_{31}H_{34}F_2N_4O_4$: C, 65.94; H, 6.07; N, 9.92. Found: C, 65.67; H, 6.07; N, 9.71.

[3-(3-Chloro-4-fluorophenyl)-6-methyl-6,11-dihydro-5*H*-indolizino[6,7-*b*]indole-1,2-diyl]bis(methylene) Bis-(isopropylcarbamate) (28f). Compound **28f** was prepared from **26f** (0.2 g, 0.5 mmol), TEA (0.2 mL), and *i*-Pr isocyanate (0.17 g, 2 mmol). Yield 0.2 g. ^{1}H NMR (DMSO-d_6) δ 1.02 (12H, d, J = 6.5 Hz, 4 × Me), 3.58 (2H, m, CH), 3.62 (3H, s, NMe), 4.14 (2H, s, CH_2), 4.80 (2H, s, CH_2), 5.07 (2H, s, CH_2), 5.17 (2H, s, CH_2), 6.91 (2H, br s, exchangeable, NH), 7.05−7.09 (1H, m, ArH), 7.15−7.19 (1H, m, ArH), 7.44−7.46 (1H, m, ArH), 7.54−7.58 (3H, m, 3 × ArH), 7.71−7.73 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 18.9, 22.6, 25.1, 29.4, 41.2, 42.2, 56.6, 56.8, 66.99, 102.8, 109.3, 113.7, 116.8, 117.0, 117.5, 117.9, 118.8, 119.5, 119.6, 121.3, 125.3, 127.2, 128.8, 128.9, 129.7, 131.2, 132.3, 137.4, 155.3, 155.6, 155.9, 157.9. Anal. Calcd for $C_{31}H_{34}ClFN_4O_4$: C, 64.08; H, 5.90; N, 9.64. Found: C, 63.89; H, 5.76; N, 9.39.

[3-(4-Methoxyphenyl)-6-methyl-6,11-dihydro-5*H*-indolizino-[6,7-*b*]indole-1,2-diyl]bis(methylene) Bis(isopropylcarbamate) (28g). Compound **28g** was prepared from **26g** (0.2 g, 0.5 mmol), TEA (0.2 mL), and *i*-Pr isocyanate (0.17 g, 2 mmol). Yield 0.21 g. ^{1}H NMR (DMSO-d_6) δ 1.03 (12H, d, J = 6.3 Hz, 4 × Me), 3.57 (2H, m, CH), 3.60 (3H, s, NMe), 3.83 (3H, s, OMe), 4.13 (2H, s, CH_2), 4.78 (2H, s, CH_2), 5.07 (2H, s, CH_2), 5.10 (2H, s, CH_2), 6.88 (2H, br s, exchangeable, NH), 7.05−7.08 (3H, m, 3 × ArH), 7.15−7.18 (1H, m, ArH), 7.42−7.46 (3H, m, 3 × ArH), 7.55−7.57 (1H, m, ArH). ^{13}C

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NMR (DMSO-d_6) δ 18.9, 22.6, 23.3, 29.3, 41.2, 42.2, 56.8, 57.2, 103.1, 109.2, 113.3, 113.9, 116.3, 117.9, 118.8, 121.3, 123.1, 125.4, 126.2, 129.9, 131.3, 137.4, 155.5, 155.7, 158.9. Anal. Calcd for $C_{33}H_{38}N_4O_5$: C, 66.80; H, 6.86; N, 10.03. Found: C, 66.57; H, 6.55; N, 9.81.

[3-(3,4-Dimethoxyphenyl)-6-methyl-6,11-dihydro-5*H*-indolizino[6,7-*b*]indole-1,2-diyl]bis(methylene) Bis-(isopropylcarbamate) (28h). Compound **28h** was prepared from **26h** (0.21 g, 0.5 mmol), TEA (0.2 mL), and *i*-Pr isocyanate (0.17 g, 2 mmol). Yield 0.18 g. ^{1}H NMR (DMSO-d_6) δ 1.02 (12H, d, J = 6.3 Hz, 4 × Me), 3.58 (2H, m, CH), 3.61 (3H, s, NMe), 3.78 (3H, s, OMe), 3.82 (3H, s, OMe), 4.13 (2H, s, CH_2), 4.78 (2H, s, CH_2), 5.06 (2H, s, CH_2), 5.15 (2H, s, CH_2), 6.89 (2H, br s, exchangeable, NH), 7.04–7.10 (4H, m, 4 × ArH), 7.14–7.18 (1H, m, ArH), 7.44–7.46 (1H, m, ArH), 7.55–7.57 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 18.9, 22.0, 22.6, 23.3, 29.3, 41.3, 42.2, 55.5, 56.8, 57.4, 103.0, 109.3, 111.7, 113.4, 114.1, 116.2, 117.9, 118.8, 121.2, 122.7, 123.3, 125.4, 126.2, 129.9, 131.6, 137.4, 148.5, 155.5, 155.7. Anal. Calcd for $C_{33}H_{40}N_4O_6$: C, 67.33; H, 6.85; N, 9.52. Found: C, 67.04; H, 7.14; N, 9.16.

[3-(3,4,5-Trimethoxyphenyl)-6-methyl-6,11-dihydro-5*H*-indolizino[6,7-*b*]indole-1,2-diyl]bis(methylene) Bis-(isopropylcarbamate) (28i). Compound **28i** was prepared from **26i** (0.22 g, 0.5 mmol), TEA (0.2 mL), and *i*-Pr isocyanate (0.17 g, 2 mmol). Yield 0.21 g. ^{1}H NMR (DMSO-d_6) δ 1.02 (12H, d, J = 6.3 Hz, 4 × Me), 3.59 (2H, m, CH), 3.64 (3H, s, NMe), 3.74 (3H, s, OMe), 3.82 (6H, s, 2 × OMe), 4.15 (2H, s, CH_2), 4.81 (2H, s, CH_2), 5.07 (2H, s, CH_2), 5.23 (2H, s, CH_2), 6.79 (2H, m, 2 × ArH), 6.92 (2H, br s, exchangeable, NH), 7.05–7.09 (1H, m, ArH), 7.15–7.19 (1H, m, ArH), 7.45–7.47 (1H, m, ArH), 7.56–7.58 (1H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 18.9, 22.6, 23.3, 29.4, 41.4, 42.1, 55.9, 57.4, 60.0, 102.9, 107.7, 109.3, 116.3, 117.9, 118.8, 121.2, 125.4, 126.3, 126.6, 129.9, 131.7, 137.0, 137.4, 152.8, 155.5, 155.7. Anal. Calcd for $C_{34}H_{42}N_4O_7$: C, 66.00; H, 6.84; N, 9.06. Found: C, 65.70; H, 6.79; N, 8.86.

Biological Assays. *Cytotoxicity Assays.* The cytotoxic effects of the newly synthesized compounds were determined in the T-cell acute lymphocytic leukemia cell line CCRF-CEM by the XTT assay[45] and in human solid tumor cell lines such as MX-1 cell line by the SRB assay.[46] The cells were incubated for 72 h, and the assays were read using a microplate spectrophotometer as previously described.[47] After the addition of phenazine methosulfate-XTT solution, the cells were incubated at 37 °C for 6 h. The absorbance at 450 and 630 nm was detected on a microplate reader (EL 340). The cytotoxicity of the newly synthesized compounds against CL141T (the parental cell, CL141, was isolated from a lung cancer patient and was kindly provided by Dr. Pan-Chyr Yang, Academia Sinica, Taiwan, and the CL141T cell line was isolated from a xenograft tumor grown in a SCID mouse), the lung adenocarcinoma cell lines A549 and H460, the prostate cancer cell line PC3, the oral cancer cell line OECM1, the oral carcinoma cell line KB, the vincristine-resistant oral carcinoma cell line KBvin10, and the colorectal cancer cell lines HCT-116 and HT-29 was determined by Alamar blue assay[48] in a 72 h incubation using a microplate spectrophotometer as previously described. After addition of the Alamar blue solution, the cells were incubated at 37 °C for 10–12 h. PrestoBlue (Invitrogen, U.S.) assay was used to evaluate the cytotoxicity of the newly synthesized compounds against the MES-SA and MES-SA/dx5 cell lines. The MES-SA and MES-SA/dx5 cells were kindly provided by Dr. Hong-Lin Chan, College of Life Science, National Tsing-Hua University. In detail, cells were incubated for 72 h using a microplate spectrophotometer. After addition of the PrestoBlue reagent, the cells were incubated at 37 °C for 3 h. The absorbance at 570 and 600 nm was detected on a microplate reader. The IC_{50} values were determined from the dose–effect relationship with six or seven concentrations of each drug using the CompuSyn software by Chou and Martin,[49] which is based on the median-effect principle and plot.[50,51] The ranges given for the IC_{50} values were the mean ± STDEV (n = 3).

In Vivo Studies. Athymic nude mice bearing the nu/nu gene were obtained from National Cancer Institute (NCI), Frederick, MD, and used for all human tumor xenografts. Male nude mice 6 weeks old or older weighing 20–24 g or more were used. The compounds were administered via tail vein iv inj as described previously.[47] Tumor volume was assessed by measuring the length × width × height (or width) using a caliper. The vehicle used was 50 μL of DMSO and 40 μL of Tween 80 in 160 μL of saline. The MTD of the tested compound was determined and used for the in vivo therapeutic efficacy assay. For tumor-bearing nude mice during the course of the experiment, the body weight refers to the total weight minus the weight of the tumor. All animal studies were conducted in accordance with the guidelines for the National Institutes of Health Guide for the Care and Use of Animals, and the protocol was approved by the Institutional Animal Care and Use Committee.

Alkaline Agarose Gel Shift Assay. Formation of DNA cross-links was analyzed by alkaline agarose gel electrophoresis as previously described.[33] Briefly, purified pEGFP-N1 plasmid DNA (1500 ng) was mixed with various concentrations (1, 5, and 10 μM) of **18a**, **18b**, **19b**, **20b**, and **28c** in 40 μL of binding buffer (3 mM sodium chloride/1 mM sodium phosphate, pH 7.4, and 1 mM EDTA). The reaction mixture was incubated at 37 °C for 2 h. At the end of the reaction, the plasmid DNA was linearized by digestion with *Bam*HI followed by precipitation with EtOH. The DNA pellets were dissolved and denatured in alkaline buffer (0.5 N NaOH and 10 mM EDTA). An aliquot of 20 μL of DNA solution (1500 ng) was mixed with 4 μL of 6× alkaline loading dye and then electrophoretically resolved on a 0.8% alkaline agarose gel with NaOH–EDTA buffer at 4 °C. The electrophoresis was performed at 18 V for 22 h. After the gels were stained with ethidium bromide solution, the DNA was visualized under UV light.

Cell-Cycle Analysis. The effects of compounds **18a**, **18b**, and **28c** on cell-cycle progression were analyzed with a flow cytometer as described previously.[52] Briefly, CL141T cells were treated with the compounds at 0.5-, 1- and 2-fold IC_{50} for 24, 48, and 72 h before the cells were harvested and fixed with ice-cold 70% EtOH. The cells were then stained with 4 μg/mL propidium iodide (PI) in phosphate buffered saline (PBS) containing 1% Triton X-100 and 0.1 mg/mL RNase A. The cells were then subjected to flow cytometric analysis (FACScan flow cytometer; Becton Dickinson, San Jose, CA). The cell-cycle phase distribution was analyzed using the ModFit LT 3.0 software (Verity Software House, Topsham, ME) and was based on the DNA histograms.

Annexin V-FITC Apoptosis Detection. An annexin V-FITC apoptosis detection kit was purchased from eBioscience Inc. (San Diego, CA) and was used to study apoptosis induced by the synthesized compounds. This study was performed according to the instructions provided by the manufacturer. H460 cells were treated with compounds **18a** and **18b** at the IC_{50} dose for 24 and 72 h prior to harvesting. The cells were resuspended in annexin V-FITC containing binding buffer. After incubation at room temperature for 10 min, the cells were washed and resuspended with PI and subjected to flow cytometric analysis (FACScan flow cytometer; Becton Dickinson, San Jose, CA).

Western Blot Analysis. For electrophoresis, the proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). The proteins were then transferred to a nitrocellulose membrane, which was blocked with 5% skimmed milk in phosphate buffered saline Tween-20 (PBST). A specific primary antibody was added to bind the target proteins for either 1 h at room temperature or overnight at 4 °C. A horseradish peroxidase (HRP) conjugated secondary antibody was added to the membrane after the primary antibody was washed off. All signals were detected after the HRP was activated by enhanced chemiluminescence. The antibodies anti-caspase-3, anti-cascase-7, and anti-PARP (no. 9662, no. 9492, and no. 9542, Cell Signaling, Danvers, MA) and anti-β-actin (GTX100313, GeneTex, San Antonio, TX) were used for analyzing the protein levels of caspase-3, cascase-7, and PARP, as well as their cleaved forms by different exposure periods and β-actin.

Topoisomerase I Mediated DNA Relaxation Assay. The topo I kit from TopoGEN Inc. (Port Orange, FL, U.S.) was used to determine whether the synthesized compounds inhibited the topo I mediated relaxation of supercoiled DNA. The assay was performed according to the manufacturer's instructions. The reaction mixture (20 μL) contained 200 ng of plasmid pHOT DNA and drug in an incubation

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buffer (10 mM Tris-HCl, pH 7.9, and 1 mM EDTA). The mixture was incubated at 37 °C and was initiated by the addition of 1 unit of topo I. After reaction for 30−45 min, the reaction was stopped by the addition of 2 μL of 10% SDS. The samples were then loaded onto a 1% agarose gel and electrophoresed at 50 V for 90 min. The gels were stained with ethidium bromide, destained with water, and photographed.

Topoisomerase II Mediated DNA Relaxation Assay. The topo II kit from TopoGEN Inc. (Port Orange, FL, U.S.) was used to evaluate the inhibitory activity of the synthetic compounds against the topo II mediated relaxation of supercoiled DNA. The assay was performed according to the manufacturer's instructions. The reaction mixture (20 μL) contained 125 ng of plasmid pHOT DNA and drug in an assay incubation buffer (0.5 M Tris-HCl, pH 8.0, 1.5 M NaCl, 100 mM $MgCl_2$, 5 mM dithiothreitol, 300 μg/mL BSA, and 20 mM ATP). The mixture was warmed to 37 °C. The reaction was initiated by the addition of 6 units of topo II. The mixture was incubated for 30−45 min, and the reaction was stopped by the addition of 2 μL of 10% SDS. The samples were then loaded onto a 1% agarose gel and electrophoretically separated at 50 V for 90 min. The gels were stained with ethidium bromide, destained with water, and photographed.

Stability Assay of Compound 18a in Rat Plasma. *HPLC Conditions.* A reversed-phase C18 column (4.6 mm × 150 mm, 5 μm, Extend-C18, Agilent, Palo Alto, CA, U.S.) was used as a stationary phase for the separation of compound **18a**. The mobile phase comprised acetonitrile/10 mM monosodium phosphate (pH 4.5) (32:68, v/v) with at flow rate of 1.1 mL/min. The identification and quantification of compound **18a** were performed on an HPLC system equipped with a chromatographic pump (LC-20AT, Shimadzu, Kyoto, Japan), diode array detector (SPD-M20A, Shimadzu), autosampler (SIL-20AT, Shimadzu), and degasser (DG-2410). The UV wavelength of detection was set at 228 nm, and the injection volume of all samples was 20 μL.

Method Validation. The stock solution of compound **18a** in MeOH (0.5 mg/mL) was diluted with 50% acetonitrile to yield serial working standard solutions (1.25, 2.5, 5, 25, 50, and 250 μg/mL). Propyl paraben (10 μg/mL) was dissolved in acetonitrile as the internal standard. The sample was extracted by acetonitrile protein precipitation as described in the sample preparation section. The calibration curves were constructed as follows: compound **18a** peak area/IS peak area for the *y*-axis and compound **18a** concentration for the *x*-axis.

Short-Term Stability. The stability analysis followed the U.S. FDA guidelines. Short-term temperature stability for the spiked plasma samples was assessed by incubation for 1, 2, 3, and 4 h at room temperature. The samples were then vortex-mixed with 140 μL of internal standard solution containing 10 μg/mL propyl paraben dissolved in acetonitrile for protein precipitation. The stability was represented as the mean relative error (%) of at least three replicates per concentration of the freshly prepared samples and the samples prepared for stability analysis as the peak area ratio of compound **18a** and propyl paraben (internal standard). The sample stability was defined as the stability ($[(C_{obs} - C_{pre})/C_{pre}] \times 100\%$) within 15% deviation of the freshly prepared samples and the samples under a short-term cycle.

Sample Preparation. The plasma was prepared by centrifugation of the blood samples at 3000*g* for 10 min at 4 °C. The plasma samples were stored at −20 °C until analysis. For each sample, 14 μL of compound **18a** was spiked into 56 μL of plasma and was vortex-mixed with 140 μL of IS solution containing 10 μg/mL propyl paraben dissolved in acetonitrile for protein precipitation. The sample was then centrifuged at 16000*g* for 10 min at 4 °C. The supernatant was filtered through a 0.22 μm filter, and 50 μL of filtrate was transferred to autosampler vials. A fixed sample loop volume (20 μL) was injected into the HPLC−photodiode array system.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Physical data of the newly synthesized bis(hydroxymethyl)-indolizino[6,7-*b*]indole derivatives (**18a−c**, **26a−i**), bis-(ethylcarbamates) (**19a−c**, **27a−i**), and bis-(isopropylcarbamate) derivatives (**20a−c**, **28a−i**). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

MX-1, human breast carcinoma; A549, human lung adenocarcinoma; CL141T, human lung adenocarcinoma; HT-29, human colon cancer; HCT-116, human colon cancer; topo I, topoisomerase I; topo II, topoisomerase II; IkK, IkK kinase complex; ATO, arsenic trioxide; DMAD, dimethyl acetylenedicarboxylate; NaH, sodium hydride; MeI, iodomethane; EtI, iodoethane; TEA, triethylamine; Ac_2O, acetic anhydride; CCRF-CEM, human lymphoblastic leukemia; PC3, human prostate cancer; OECM1, human oral epidermoid carcinoma; HEL299, human embryonic lung fibroblast; KB, human oral cancer; KBvin10, human oral cancer resistant to vincristine; MES-SA, human uterine sarcoma cell line; MES-SA/dx5, human uterine sarcoma cell line resistant to doxorubicin; iv inj, intravenous injection; QD×3, once per day for 3 days; QD×4, once per day for 4 days; Q2D×3, every 2 days for three times; HeLa, human epithelial carcinoma; H1299, human lung adenocarcinoma; PARP, poly ADP-ribose polymerase; c-caspase-3, cleaved caspase-3; c-caspase-7, cleaved caspase-7; c-PARP, cleaved poly ADP-ribose polymerase; U.S. FDA, U.S. Food and Drug Administration; PI, propidium iodide; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; PBST, phosphate buffered saline Tween-20; HRP, horseradish peroxidase; Topo, topoisomerases

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