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Novel bifunctional alkylating agents, 5,10-dihydropyrrolo[1,2-*b*]isoquinoline derivatives, synthesis and biological activity

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ABSTRACT

A series of linear pyrrolo[1,2-*b*]isoquinoline derivatives was synthesized for antitumor evaluation. The preliminary antitumor studies reveal that both bis(hydroxymethyl) and their bis(alkylcarbamate) derivatives show significant antitumor activity in inhibiting various human tumor cell growth in vitro. 1,2-Bis(hydroxymethyl)-3-methyl-5,10-dihydropyrrolo[1,2-*b*]isoquinoline (**20a**) was selected for antitumor studies in animal models. The results show that this agent can induce complete tumor remission or significant suppression in nude mice bearing human breast (MX-1) xenograft and ovarian (SK-OV-3) xenografts, respectively. Alkaline agarose gel shifting assay showed that **20a** is able to cross-link with DNA. Studies on the cell cycle inhibition revealed that this agent induces cell arrest at G2/M phase. The results warrant further antitumor investigation against other human tumor growth in animal models.

1. Introduction

Among DNA alkylating agents, the naturally occurring antitumor alkylating agent, mitomycin C (MMC, **1**, Fig. 1) is a clinically useful chemotherapeutic agent for treating various cancer patients.[1,2] MMC and its synthetic analogue indoloquinone EO9 (**2**),[3] which bears two reactive nucleophilic centers in the molecule, were reported to be capable of cross-linking with DNA. The quinone moiety of these agents plays an important role in their antitumor activity. It requires bioreductive activation to switch on the nucleophilic centers on the pyrrole ring to allow interaction with DNA, which forms biadducts.[4]

Another class of bifunctional DNA alkylators, such as thioimidazoles (i.e., carmethizole, **3**, Fig. 1),[5] bis(hydroxymethyl)pyrrole derivatives (i.e., **4**[6], **5**[7] and **6**[8]), 2,3-dihydroxy-6,7-bis(alkylcarbamates)pyrrolizines [e.g., **7** (IPP) and **8**][9] were developed originally from the pyrrolizine alkaloids. Of the bis(alkylcarbamates)pyrrolizine analogues, compound **7** was found to have significant antitumor activity against a broad range of experimental human tumor xenografts.[10] It was demonstrated that the electronic properties of the substituent(s) on the phenyl ring as well as the lipophilicity and planarity of the molecule may affect the antitumor activity and toxicity of compounds belonging to this class.[10] Later, Anderson et al. synthesized bis(carbamoylmethyl) derivatives of pyrrolo[2,1-*a*]isoquinolines (**9**) and pyrrolo[1,2-*a*]quinolines (**10**), which bear angular tricyclic structures to limit the deviation from co-planarity of the phenyl and pyrrolo rings.[11] The results showed that these agents exhibited a broad spectrum of antitumor activity against a wide range of tumors.

Recently, we have synthesized a series of bis(hydroxymethyl)azacyclopenta[*a*]indenes and their bis(methylcarbamate) derivatives.[12] These agents can be considered as 'benzologues' of bis(hydroxymethyl)pyrrolizines and were able to cross-link to DNA double strands. These analogues exhibited potent cytotoxicity and antitumor activity against human lymphoblastic leukemia and various solid tumors.[12] Remarkably, complete tumor remission (CR) in nude mice bearing human breast carcinoma MX-1 xenograft by bis(hydroxymethyl) derivatives (**11** and **12**, Fig. 1) and bis(methylcarbamate) derivatives (**13** and **14**) and significant suppression against prostate adenocarcinoma PC3 xenograft by **12** were achieved.

One of the drawbacks of using DNA alkylating agents is that these drugs may lose antitumor activity because tumor cells possess DNA repair mechanisms to fix DNA damage. More recently, we found that the combination treatment of **13** and arsenic trioxide (ATO, DNA repair inhibitor) significantly suppressed human large cell lung carcinoma H460 xenograft (>82%) and cisplatin-resistant NTUB1/P human bladder carcinoma xenografts (>92%) in nude mice.[13] These exciting results prompted us to continue designing and

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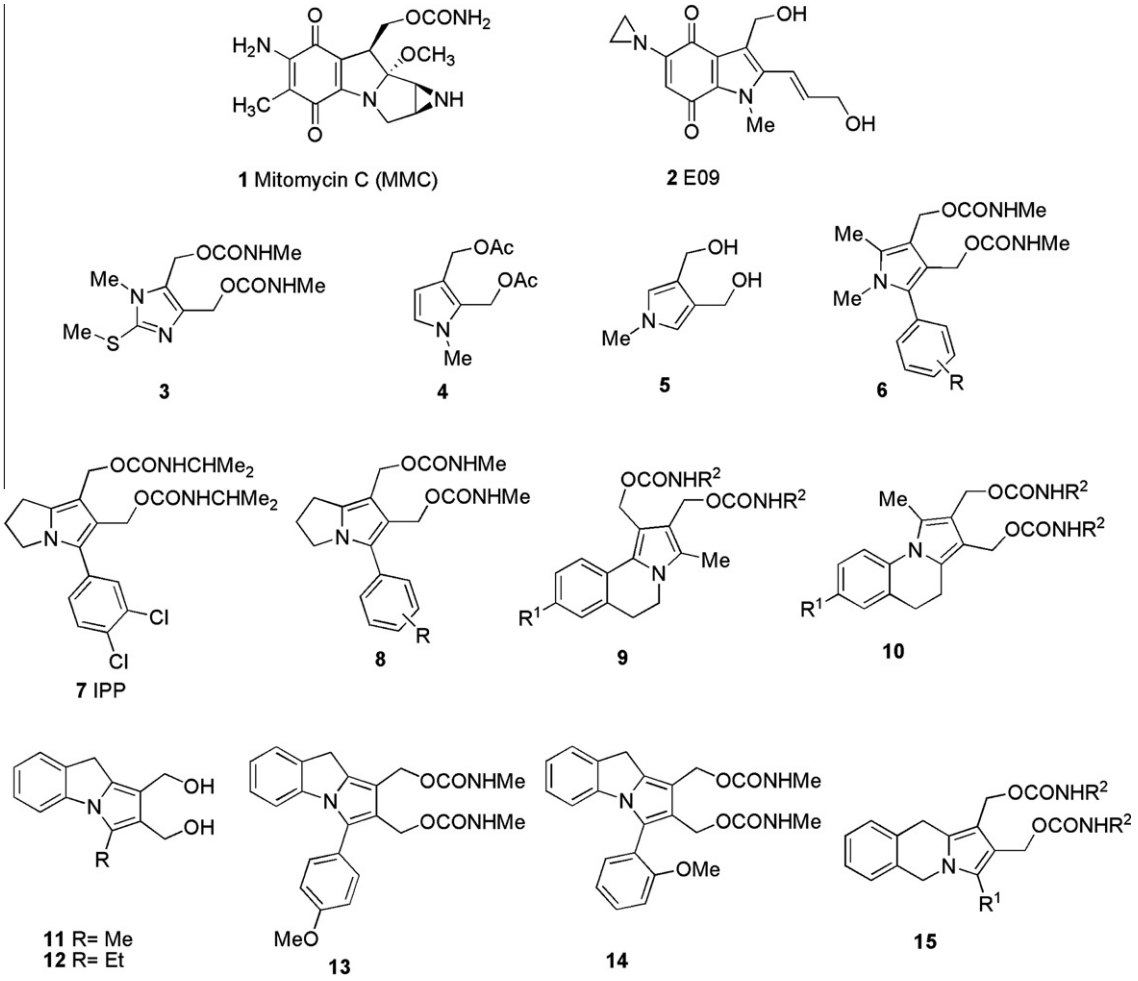


Figure 1. Chemical structure of some DNA bifunctional alkylating agents.

synthesizing new bis(hydroxymethyl)pyrrolizine analogues for antitumor studies.

As mentioned previously, pyrrolo[2,1-*a*]isoquinolines (**9**) and pyrrolo[1,2-*a*]quinolines (**10**) bear an angular tricyclic ring system. To investigate whether derivatives with a linear tricyclic ring system also possess potent antitumor activity and/or have superior potency than the corresponding bis(hydroxymethyl)azacyclopenta[*a*]indenes, we have synthesized a series of linear 5,10-dihydropyrrolo[1,2-*b*]isoquinolines and their bis(alkylcarbamates) (**15**), all which were subjected to antitumor evaluation. The results show that the newly synthesized analogues exhibit significant antitumor activity and are able to induce DNA interstrand cross-linking. Herein, we report the antitumor activity of these agents against various human tumor cell growths both in vitro and in vivo and mechanism of action studies.

2. Results and discussion

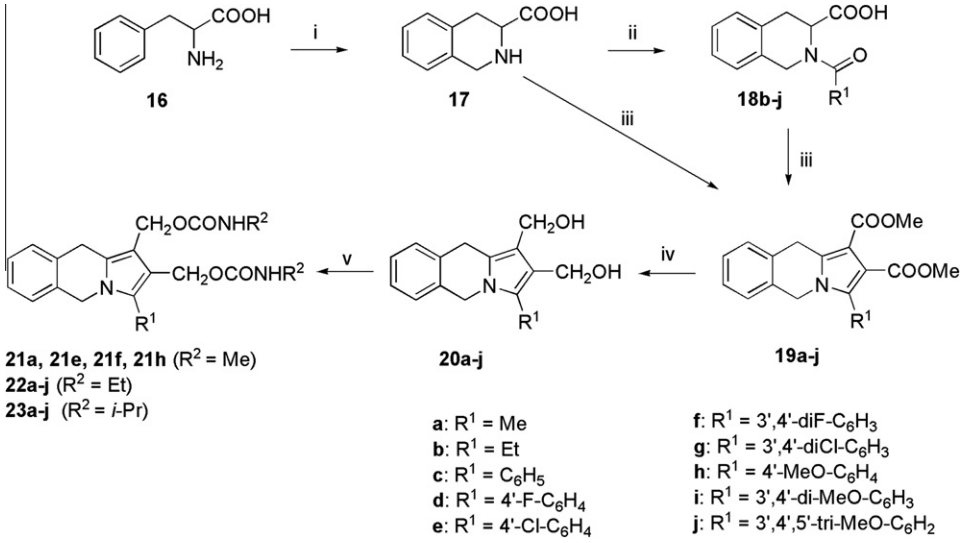
2.1. Chemistry

The synthetic route for bis(hydroxymethyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinolin-1-yl derivatives (**20**) and their bis(alkylcarbamate) derivatives (**21**–**23**) is shown in Scheme 1. The known 3-carboxy-1,2,3,4-tetrahydroisoquinoline (**17**) was synthesized by treating the commercially available D,L-phenylalanine (**16**) with formaldehyde and concd HCl by following the procedure developed by Dean.[14] Compound **17** was N-acylated by treating with various acid chlorides (R^1COCl) in presence of 2 N NaOH to give *N*-acyl-3-carboxy-1,2,3,4-tetrahydroisoquinolines (**18b–j**) by following the literature procedure.[15,16] Compounds **18b–j** were then reacted with dimethyl acetylenedicarboxylate (DMAD) in acetic anhydride at 60–70 °C yielded 5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-dicarboxylic acid dimethyl esters (**19b–j**). The methyl ester derivative **19a** (R^1 = Me) was prepared in good yields directly from **17** by reacting with acetic anhydride and DMAD.[17] The ester function of **19a–j** was reduced to bis(hydroxymethyl) derivatives **20a–j** by treating with $LiAlH_4$ in a mixture of ether/CH_2Cl_2 at 0–5 °C. Compounds **20a–j** were then further treated with methyl-, ethyl- or *iso*-propylisocyanate in presence of triethylamine (TEA) to furnish the desired bis(alkylcarbamate)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1-yl derivatives (**21**–**23**) in good to high yields.

2.2. Biological results

2.2.1. In vitro cytotoxicity

Table 1 shows the antiproliferative activities of the newly synthesized bis(hydroxylmethyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline derivatives (**20**) and their bis(alkylcarbamate) derivatives (**21**–**23**) against human lymphoblastic leukemia (CCRF-CEM) and its drug-resistant sublines resistant to Taxol (CCRF-CEM/Taxol) and Vinblastine (CCRF-CEM/VBL) cell growth in vitro. It demonstrated that the newly synthesized conjugates possess significant cytotoxicity with IC_{50} in submicro molar range. In the series of



Scheme 1. Reagents and conditions: (i) 37% formalin/concd HCl, reflux; (ii) acid chloride, 2 N NaOH/acetone, room temperature; (iii) DMAD/Ac_2O, 60–70 °C; (iv) $LiAlH_4$, CH_2Cl_2/Et_2O, 0–5 °C; (v) R^2NCO/Et_3N, room temperature.

bis(hydroxymethyl) derivatives, it showed that 3-alkyl (Me or Et) substituted derivatives are more cytotoxic than the 3-phenyl substituted compounds. The order of their potency, for example, is **20a** (3-Me) > **20b** (3-Et) > **20c** (3-Ph), indicating that the compounds having a smaller size of substituent at C3 have greater cytotoxicity. A similar observation is found in 3-phenyl derivatives: the cytotoxicity decreases when the number of the methoxy functions increases (**20h** vs **20i** vs **20j**). Furthermore, the cytotoxicity decreases when the number of the halo function increases in the halo substituted 3-phenyl derivatives (**20d** > **20f** and **20e** > **20g**). In this study, one can observe that the C3-methoxyphenyl derivatives are somewhat more cytotoxic than the corresponding halophenyl compounds. This suggests that the electron properties of the substituent(s) on the phenyl ring have very little influence over their potency.

In the series of bis(alkylcarbamates) derivatives, the alkylcarbamate moiety may serve as a better leaving group than that of the OH group. Thus, the bis(alkylcarbamates) derivatives may easily generate nucleophilic cations on both methylene functions that allow the cations to become more favorable targets of DNA. In the series of 3-phenyl substituted derivatives, the bis(alkylcarbamates) are generally more potent than their corresponding bis(hydroxymethyl) derivatives, except for compound bis(ethylcarbamate) **22i**, which is as potent as the corresponding bis(hydroxymethyl) **20i** and more cytotoxic than bis(*iso*-propylcarbamates) **23i**. However, in the series of C3-alkyl derivatives, the bis(hydroxymethyl) derivatives (**20a** and **20b**) are more potent than their corresponding bis(alkylcarbamates) (**21a**, **22a**, and **23a**; **22b** and **23b**, respectively).

Our previous research on the SAR studies of 1,2-bis(hydroxylmethyl)cyclopenta[*a*]indenes and their counterparts 1,2-bis(methylcarbamate) derivatives (**11–14**, Fig. 1) demonstrated that the size and the electron property of the substituents at the C3 position affected the cytotoxicity of these agents.[12] However, we found that the cytotoxicity of pyrrolo[1,2-*b*]isoquinolines is mainly affected by the size of the substituents at the C3 position rather than the electron property in the current studies. In comparison with the potency of both these series, the bis(hydroxymethyl) derivatives of pyrrolo[1,2-*b*]isoquinoline are more cytotoxic than the corresponding cyclopenta[*a*]indenes. In contrast, the bis(alkylcarbamate) derivatives of cyclopenta[*a*]indenes are more potent than the corresponding pyrrolo[1,2-*b*]isoquinolines.

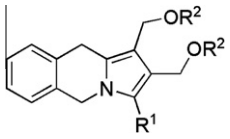
Our previous report demonstrated that cyclopenta[*a*]indenes have no multi-drug resistance toward antitumor agents such as Taxol and Vinblastine. To realize whether the newly synthesized pyrrolo[1,2-*b*]isoquinoline derivatives also have no cross-resistance to these two agents, we evaluated their cytotoxicity against CCRF-CEM/Taxol and CCRF-CEM/VBL, which are subcell lines of CCRF-CEM cells that are 330-fold resistant to Taxol, and 680-fold resistant to Vinblastine, respectively. As shown in Table 1, the newly synthesized pyrrolo[1,2-*b*]isoquinolines have no cross-resistance to either Taxol or Vinblastine. This suggests that all derivatives are neither a good substrate of membrane multi-drug resistance transporters (i.e., *p*-glycoprotein) nor mutated tubulin.

The antiproliferative activity of the selected pyrrolo[1,2-*b*]isoquinoline derivatives in inhibiting human solid tumors such as breast carcinoma MX-1, colon carcinoma HCT-116, non-small cell lung carcinoma H1299, prostate PC3, oral carcinoma OECM1 and glioma U87 cell growth in vitro (Table 2) were also investigated. Of these compounds, C3-Me derivative (**20a**) was found to have potent cytotoxicity in inhibiting MX-1 cell growth in vitro with IC_{50} values of 0.66 μM. Compounds **20a**, **21e**, and **21f** exhibited potent inhibitory activity against human colon carcinoma HCT-116 with IC_{50} values of 0.29, 0.04 and 0.36 μM, respectively. The tested compounds have good to moderate effects against H1299, PC3, OECM1 and U87 cell growth in vitro.

2.2.2. In vivo antitumor activity

To investigate the antitumor activity of pyrrolo[1,2-*b*]isoquinoline derivatives in animal models, we selected compound **20a** for evaluating its therapeutic efficacy in animal models since this agent has the most potent cytotoxicity among all of the compounds tested and exhibits a broad spectrum of antitumor activity in inhibiting both CCRF/CEM and other solid tumors in vitro. Nude mice implanted with human breast carcinoma MX-1 xenograft were given 30 mg/kg, every two days for two times (Q2D × 2), intravenous injection (iv injection) on day 8 and 10 (Fig. 2). Remarkably, it shows that complete tumor remission against human breast carcinoma MX-1 in nude mice was achieved (Fig. 2A). This concentration was established from tolerability studies. Under this dosage, one can see that nude mice's body weights recovered after cessation of the treatment, indicating the low toxicity of the compound to the host (Fig. 2B). In another experiment, we found that **20a** was able to effectively suppress

Table 1
The cytotoxicity of newly synthesized bis(hydroxymethyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinolin-1-yl derivatives (**20**) and their bis(alkylcarbamate) derivatives (**21**–**23**) against human lymphoblastic leukemia (CCRF-CEM) and its drug-resistant sublines (CCRF-CEM/Taxol and CCRF-CEM/VBL)[a]



Compd	R^1	R^2	Cell growth inhibition (IC_{50} μM)		
			CCRF-C EM	CCRF-CEM/Taxol[b]	CCRF-CEM/VBL[b]
20a	Me	H	0.08 ± 0.02	0.10 ± 0.001 [1.22×][c]	0.09 ± 0.01 [1.1×]
20b	Et	H	0.18 ± 0.01	0.21 ± 0.003 [1.15×]	0.19 ± 0.02 [1.03×]
20c	C_6H_5	H	0.51 ± 0.01	0.50 ± 0.04 [0.99×]	0.29 ± 0.02 [0.56×]
20d	4′-F-C_6H_4	H	0.51 ± 0.01	0.88 ± 0.03 [1.72×]	0.49 ± 0.002 [0.95×]
20e	4′-Cl-C_6H_4	H	0.60 ± 0.002	0.77 ± 0.17 [1.28×]	0.72 ± 0.18 [1.2×]
20f	3′,4′-Di-F–C_6H_3	H	1.14 ± 0.22	1.26 ± 0.26 [1.11×]	0.95 ± 0.21 [0.84×]
20g	3′,4′-Di-Cl–C_6H_3	H	8.44 ± 0.06	4.82 ± 0.01 [0.57×]	6.42 ± 0.05 [0.76×]
20h	4′-MeO–C_6H_4	H	0.23 ± 0.03	0.25 ± 0.001 [1.02×]	0.11 ± 0.001 [0.48×]
20i	3′,4′-Di-MeO–C_6H_3	H	1.10 ± 0.04	0.76 ± 0.02 [0.69×]	0.62 ± 0.01 [0.56×]
20j	3′,4′,5′-Tri-MeO–C_6H_2	H	1.97 ± 0.46	2.88 ± 0.05 [1.45×]	2.78 ± 0.09 [1.40×]
21a	Me	CONHMe	0.13 ± 0.01	0.11 ± 0.002 [0.86×]	0.08 ± 0.001 [0.59×]
21e	4′-Cl–C_6H_4	CONHMe	0.19 ± 0.01	0.22 ± 0.004 [1.18×]	0.16 ± 0.05 [0.87×]
21f	3′,4′-Di-F–C_6H_3	CONHMe	0.49 ± 0.09	0.45 ± 0.20 [0.91×]	0.47 ± 0.05 [0.97×]
21h	4′-MeO–C_6H_4	CONHMe	0.18 ± 0.03	0.13 ± 0.03 [0.72×]	0.09 ± 0.02 [0.49×]
22a	Me	CONHEt	0.24 ± 0.002	0.11 ± 0.001 [0.48×]	0.12 ± 0.003 [0.51×]
22b	Et	CONHEt	0.44 ± 0.01	0.21 ± 0.01 [0.48×]	0.24 ± 0.01 [0.53×]
22c	C_6H_5	CONHEt	0.26 ± 0.01	0.26 ± 0.03 [1.01×]	0.23 ± 0.001 [0.90×]
22d	4′-F–C_6H_4	CONHEt	0.31 ± 0.01	0.43 ± 0.05 [1.39×]	0.57 ± 0.01 [1.85×]
22e	4′-Cl–C_6H_4	CONHEt	0.59 ± 0.01	0.35 ± 0.01 [0.59×]	0.57 ± 0.06 [0.97×]
22f	3′,4′-Di-F–C_6H_3	CONHEt	2.08 ± 0.02	1.25 ± 0.02 [0.60×]	0.67 ± 0.003 [0.32×]
22g	3′,4′-Di-Cl–C_6H_3	CONHEt	2.37 ± 0.02	0.90 ± 0.002 [0.38×]	1.19 ± 0.04 [0.50×]
22h	4′-MeO–C_6H_4	CONHEt	0.37 ± 0.02	0.22 ± 0.003 [0.58×]	0.35 ± 0.01 [0.93×]
22i	3′,4′-Di-MeO–C_6H_3	CONHEt	1.07 ± 0.001	0.81 ± 0.01 [0.75×]	0.65 ± 0.02 [0.60×]
22j	3′,4′,5′-Tri-MeO–C_6H_2	CONHEt	1.41 ± 0.02	2.14 ± 0.08 [1.52×]	3.13 ± 0.03 [2.23×]
23a	Me	CONH-*i*-Pr	0.27 ± 0.004	0.23 ± 0.002 [0.84×]	0.14 ± 0.003 [0.50×]
23b	Et	CONH-*i*-Pr	0.57 ± 0.01	0.32 ± 0.01 [0.56×]	0.18 ± 0.0002 [1.42×]
23c	C_6H_5	CONH-*i*-Pr	0.45 ± 0.01	0.23 ± 0.02 [0.50×]	0.25 ± 0.01 [0.55×]
23d	4′-F–C_6H_4	CONH-*i*-Pr	0.76 ± 0.03	0.23 ± 0.01 [0.30×]	0.23 ± 0.001 [0.31×]
23e	4′-Cl–C_6H_4	CONH-*i*-Pr	0.54 ± 0.003	0.63 ± 0.01 [1.16×]	0.57 ± 0.04 [1.06×]
23f	3′,4′-Di-F–C_6H_3	CONH-*i*-Pr	1.33 ± 0.01	0.78 ± 0.003 [0.58×]	0.61 ± 0.05 [0.46×]
23g	3′,4′-Di-Cl–C_6H_3	CONH-*i*-Pr	3.55 ± 0.02	2.47 ± 0.07 [0.69×]	1.35 ± 0.04 [0.38×]
23h	4′-MeO–C_6H_4	CONH-*i*-Pr	0.34 ± 0.002	0.19 ± 0.002 [0.56×]	0.16 ± 0.001 [0.47×]
23i	3′,4′-Di-MeO–C_6H_3	CONH-*i*-Pr	1.29 ± 0.10	0.60 ± 0.02 [0.46×]	0.77 ± 0.05 [0.59×]
23j	3′,4′,5′-Tri-MeO–C_6H_2	CONH-*i*-Pr	0.89 ± 0.01	1.20 ± 0.01 [1.35×]	1.42 ± 0.05 [1.59×]
Taxol			0.003 ± 0.0003	0.43 ± 0.05 [330×]	1.27 ± 0.05 [980×]
Vinblastine			0.0007 ± 0.001	0.08 ± 0.01 [106.2×]	0.50 ± 0.12 [679.5×]

[a] Cell growth inhibition was measured by the XTT assay[20] for leukemic cells after 72-h incubation using a microplate spectrophotometer as described previously.[22] Similar in vitro results were obtained by using the Cell Counting Kit-8 for the CCK-8 assays as described by technical manual of Dojindo Molecular Technologies, Inc. (Gaithersburg, MD; Website: www.dojindo.com). IC_{50} values were determined from dose-effect relationship at six or seven concentrations of each drug by using the CompuSyn software by Chou and Martin[24] based on the median-effect principle and plot using the serial deletion analysis.[25,26] Ranges given for Taxol and vinblastine were mean ± SE (n = 4).

[b] CCRF-CEM/Taxol and CCRF-CEM/VBL are subcell lines of CCRF-CEM cells that are 330-fold resistant to Taxol, and 680-fold resistant to vinblastine, respectively, when comparing with the IC_{50} of the parent cell line.

[c] Numbers in the brackets are fold of cross-resistant determined by comparison with the corresponding IC_{50} of the parent cell line.

human ovarian tumor SK-OV-3 implanted in nude mice on day 22 at the dose of 20 mg/kg, every day for four times (QD × 4), iv injection (Fig. 3A and B). The results of MX-1 and SK-OV-3 xenografts studies show the potential utility of compound **20a** in inhibiting the growth of both tumors.

2.2.3. DNA cross-linking study

To realize whether the newly synthesized compounds are capable of cross-linking with DNA double strands, pEGFP-N1 plasmid DNA was treated with bis(hydroxymethyl) derivatives (**20a** and **20h**) and their corresponding bis(alkylcarbamate) derivatives (**22a** and **23h**, respectively) at various concentrations as indicated (1, 10, and 20 μM) using alkaline agarose gel shifting assay (Fig. 4).[18] Melphalan (1, 5, and 10 μM) was used as the positive control. As revealed in Figure 4, one can see that all of the tested compounds were able to induce DNA interstrand cross-linking, suggesting that DNA cross-linking may be the main mechanism of action for these agents.

2.2.4. Cell cycle inhibition

It is well known that DNA interacting agents can alter the cell cycle progression by arresting the cell cycle at the G2/M phase.[19] Previously, we have demonstrated that 3a-aza-cyclopenta[*a*]indene derivatives were able to induce G2/M arrest.[12] We therefore studied the inhibitory effect of **20a** on cell cycle distribution (Table 3). The human non-small lung carcinoma H1299 cells were treated with **20a** at the concentrations of 1.25, 2.5, and 5 μM for 24 h. The cells were harvested, stained with propidium iodide (PI) and analyzed with a flow cytometer. It clearly shows that **20a** remarkably accumulated the cells at G2/M phase. Furthermore, increased sub-G1 populations were noticed in cells treated with **20a** at each concentration.

Table 2
The cytotoxicity of newly synthesized bis(hydroxymethyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinolin-1-yl derivatives (**20**) and their bis(alkylcarbamate) derivatives (**21**–**23**) against human solid tumors (breast carcinoma MX-1, colon carcinoma HCT-116, lung carcinoma H1299, prostate carcinoma PC3, oral carcinoma OECM1 and glioma U87) cell growth in vitro

Compd	Cell growth inhibition (IC_{50} μM)					
	MX-1[a]	HCT-116[a]	H1299[b]	PC3[b]	OECM1[b]	U87[b]
20a	0.66 ± 0.03	0.29 ± 0.01	2.48 ± 1.22	6.07 ± 1.70	6.10 ± 1.11	27.91 ± 3.90
20d	4.01 ± 0.05	2.46 ± 0.08	8.76 ± 1.15	5.93 ± 1.52	6.99 ± 0.12	23.25 ± 0.26
20e	3.28 ± 0.01	3.06 ± 0.46	24.73 ± 8.16	9.85 ± 0.79	12.05 ± 0.67	26.68 ± 5.79
20f	6.99 ± 0.35	3.21 ± 0.21	ND[c]	ND	ND	ND
20h	1.99 ± 0.07	1.13 ± 0.07	13.94 ± 0.83	10.93 ± 1.21	6.12 ± 0.21	10.57 ± 0.23
21a	1.68 ± 0.01	1.17 ± 0.02	ND	ND	ND	ND
21e	1.18 ± 0.01	0.04 ± 0.002	ND	ND	ND	ND
21f	2.73 ± 0.05	0.36 ± 0.001	ND	ND	ND	ND
21h	1.33 ± 0.02	1.07 ± 0.05	ND	ND	ND	ND
22a	2.27 ± 0.01	0.90 ± 0.03	19.42 ± 0.42	15.45 ± 1.84	11.34 ± 2.07	13.89 ± 4.90
22d	1.51 ± 0.03	0.79 ± 0.05	3.94 ± 1.13	9.59 ± 1.31	12.52 ± 1.70	28.36 ± 4.86
22e	1.69 ± 0.06	0.62 ± 0.10	37.25 ± 0.89	10.98 ± 1.14	25.32 ± 0.18	33.59 ± 3.91
22h	1.27 ± 0.05	0.75 ± 0.004	27.61 ± 1.65	18.54 ± 0.82	10.48 ± 0.13	17.06 ± 1.78
23a	1.28 ± 0.01	1.38 ± 0.02	9.77 ± 3.75	16.87 ± 7.42	11.80 ± 2.58	26.61 ± 4.21
23d	1.17 ± 0.19	0.63 ± 0.001	8.13 ± 1.34	12.29 ± 1.34	11.05 ± 2.61	36.87 ± 5.98
23e	1.70 ± 0.09	0.69 ± 0.01	25.15 ± 4.05	9.97 ± 1.32	26.10 ± 7.86	22.72 ± 2.03
23h	0.98 ± 0.03	0.97 ± 0.04	13.16 ± 1.94	6.79 ± 1.01	8.83 ± 0.74	21.04 ± 0.67
Taxol	0.035 ± 0.00514	0.0013 ± 0.0005	ND	ND	ND	ND
Vinblastine	0.0029 ± 0.0002	0.0018 ± 0.0004	ND	ND	ND	ND
Cisplatin	ND	ND	4.95 ± 0.60	26.65 ± 4.19	ND	ND

[a] Cell growth inhibition was measured by the SRB assay[21] for solid tumor cells after 72-h incubation using a microplate spectrophotometer as described previously.[22]
[b] Cell growth inhibition was determined by the Alamar blue assay[23] in a 72 h incubation using a microplate spectrophotometer as described previously.
[c] Not determined.

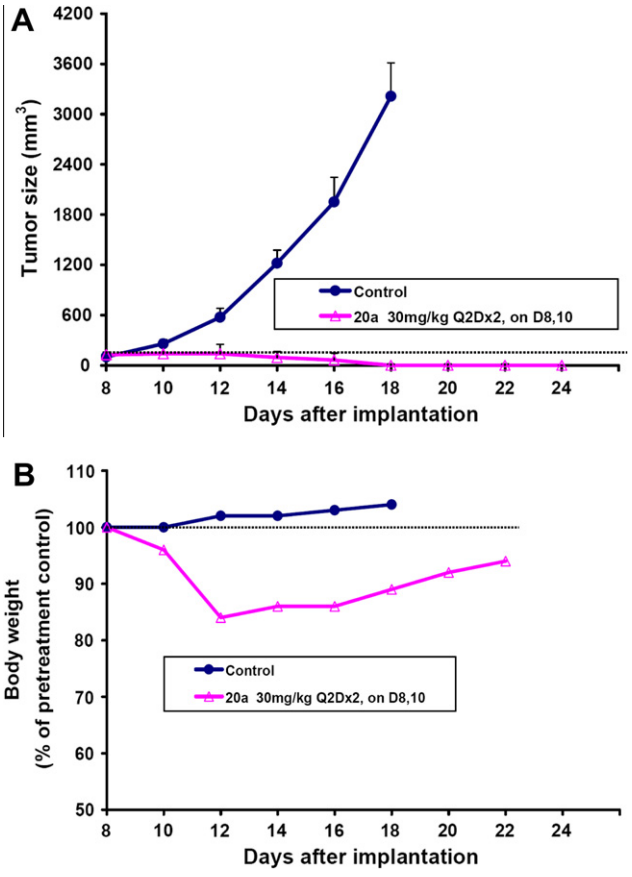


Figure 2. Therapeutic effects of **20a** in nude mice bearing MX-1 human mammary xenograft (iv injection, $n = 4$). (A) Average tumor size changes. (B) Average body weight changes.

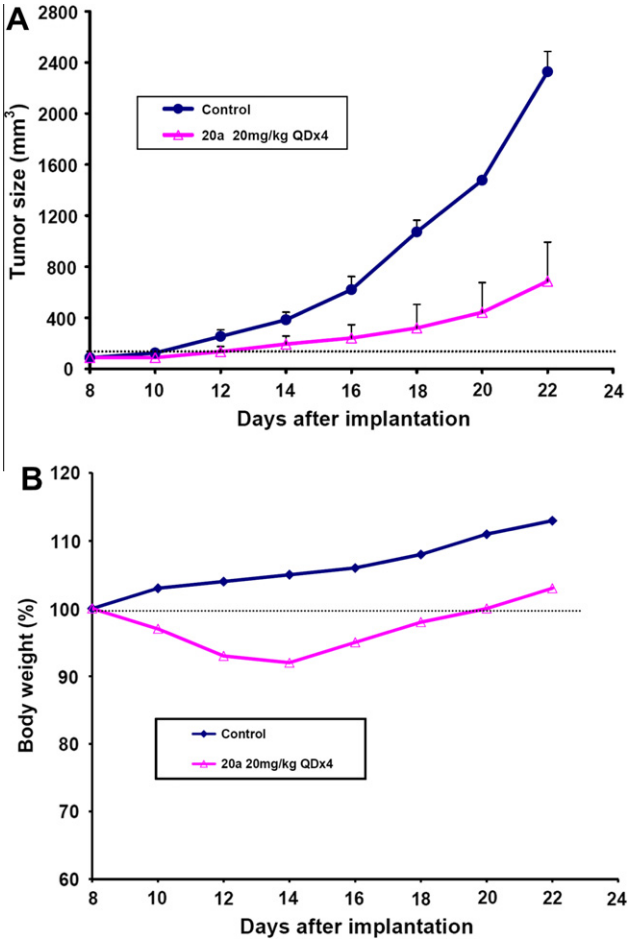


Figure 3. Therapeutic effects of **20a** in nude mice bearing ovarian adenocarcinoma SK-OV-3 xenograft (iv injection, $n = 4$). (A) Average tumor size changes. (B) Average body weight changes.

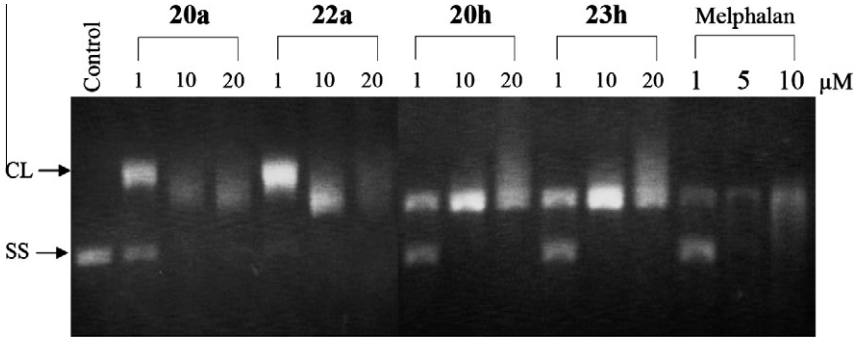
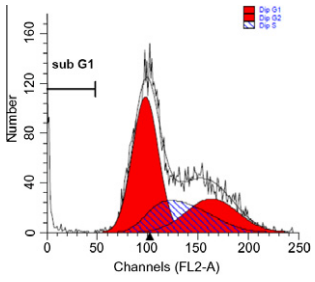
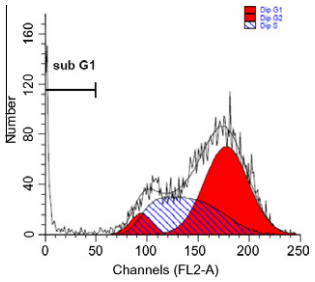
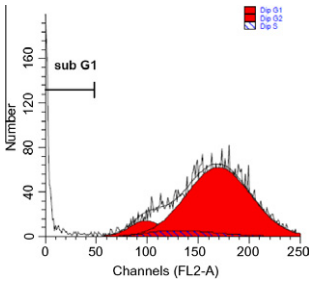
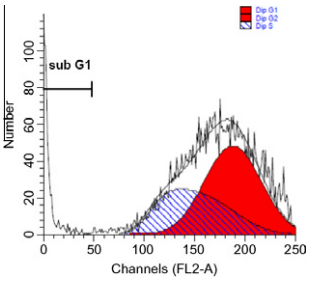


Figure 4. Representative DNA cross-linking gel shift assay for bis(hydroxymethyl) derivatives (**20a** and **20h**) and their corresponding bis(alkylcarbamate) derivatives (**22a** and **23h**, respectively) at various concentrations as indicated. Control lane shows single-stranded DNA (SS), while CL shown in all tested lanes is DNA double-stranded cross-linking. Melphalan (1, 5, and 10 μM) was used as a positive control.

Table 3
Effects of compound **20a** on cell cycle progress in human non-small cell lung adenocarcinoma H1299

Concentration (μM)	0	1.25	2.5	5
				
Sub G1	14.1 ± 0.7	21.6 ± 4.2	26.6 ± 0.7	26.5 ± 3.0
G1	42.9 ± 0.8	5.6 ± 0.6	7.7 ± 0.7	0.5 ± 0.3
S	22.5 ± 0.3	32.7 ± 5.9	18.3 ± 8.4	24.7 ± 4.6
G2/M	20.5 ± 0.9	40.1 ± 6.5	47.3 ± 2.1	48.4 ± 2.2

3. Conclusion

In the present studies, we have synthesized a series of linear pyrrolo[1,2-*b*] isoquinoline derivatives for antitumor evaluation. Both bis(hydroxymethyl) and their bis(alkylcarbamate) derivatives show potent antitumor activity in inhibiting various human tumor xenografts in vitro. Among these analogues, we discovered compound **20a**, which was selected for antitumor studies in animal models, exhibits potent therapeutic efficacy against human breast MX-1 xenograft in nude mice, as complete tumor remission was observed. This agent is also able to significantly suppress human ovarian tumors implanted in nude mice. The results reported herein warrant further investigation to optimize the schedule and dosage to get greater suppression of other human tumor growth in animal models. Additional, the evaluation of the antitumor activity of **20a** in combination with DNA repair inhibitor (e.g., ATO) is currently undergoing in our laboratory.

4. Experimental section

4.1. General methods and materials

All commercial chemicals and solvents were reagent grade and were used without further purification unless otherwise specified. Melting points were determined on a Fargo melting point apparatus and are uncorrected. Thin-layer chromatography was performed on Silica Gel G60 F_{254} (Merck) with short-wavelength UV light for visualization. All reported yields are isolated yields after chromatography or crystallization. Elemental analyses were done on a Heraeus CHN-O Rapid instrument. ^{1}H NMR spectra were recorded on a 600 MHz, Brucker AVANCE 600 DRX and 400 MHz, Brucker Top-Spin spectrometers in the indicated solvent. The chemical shifts were reported in ppm (δ) relative to TMS and coupling constants (J) in Hertz (Hz) and s, d, t, m, br s, refer to singlet, doublet, triplet, multiplet, broad, respectively. High performance liquid chromatography analysis for checking purity of synthesized compounds were recorded on a Hitachi D-2000 Elite instrument: column, Mightysil RP-18 GP 250–4.6 (5 μm); mobile phase, MeCN/THF (50:50 v/v); flow rate, 1 mL/min; injected sample 10 μL, column temp, 27 °C; wavelength, 254 nm. The purity of all tested compounds was ⩾95% based on analytical HPLC.

4.2. 1,2,3,4-Tetrahydroisoquinoline-3-carboxylic acid hydrochloride (17)

A mixture of D,L-phenylalanine (**16**, 50 g, 3.03 mol), concd HCl (325 mL) and 37% formalin (110 mL) was heated to a gentle reflux with vigorous stirring. After 30 min, another portion of formalin (50 mL) and concd HCl (110 mL) was added. The reaction mixture was further stirred and heated for 4 h and cooled to room temperature. The white solid separated out was filtered and washed with methanol (30 mL) to give **17** 64.1 g, yield 98%; mp >280 °C (lit.[14] mp 286–290 °C). ^{1}H NMR (DMSO-d_6) δ 3.15 (1H, m, CH), 3.32 (1H, m, CH), 4.30 (2H, m, CH_2), 4.43 (1H, m, CH), 7.20–7.35 (4H, m, 4 × ArH), 9.89 (1H, br s, exchangeable, NH); 10.06 (1H, br s, exchangeable, COOH).

4.3. 2-Propionyl-1,2,3,4-tetrahydroisoquiniline-3-carboxylic acid (18b)

To a suspension of 1,2,3,4-tetrahydroisoquinoline-3-carboxylic-acid hydrochloride (**17**, 10 g, 46.8 mmol) in acetone (60 mL) was added 2 N NaOH (40 mL) solution at room temperature. The clear solution obtained then added dropwise into a solution of propionyl chloride (5.2 g, 57 mmol) in acetone (20 mL) at room temperature, simultaneously 2 N NaOH was added dropwise and pH maintained above 10. The reaction mixture was stirred at room temperature for 2 h, the solvent was evaporated under reduce pressure. The solution was acidified to pH 5–6 with 3 N HCl. The white solid separated, filtered it and dried to give **18b** 9 g, yield 87%; mp 173–174 °C. ^{1}H NMR (DMSO-d_6) δ 1.03 (3H, m, Me), 2.45 (2H, m, CH_2), 3.13 (2H, m, CH_2), 4.52 (2H, m, CH_2), 5.09 (1H, m, CH), 7.16–7.20 (4H, m, 4 × ArH), 12.63 (1H, br s, exchangeable, COOH). Anal. Calcd for ($C_{13}H_{15}NO_3$): C, 66.94; H, 6.48; N, 6.00. Found: C, 66.64; H, 6.48; N, 5.89.

By following the same synthetic procedure as that for **18b**, the following compounds were synthesized.

4.3.1. 2-Benzoyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (18c)

Compound **18c** was prepared from **17** (8.0 g, 37.4 mmol) and benzoyl chloride (6.43 g, 45.6 mmol). Yield, 9.9 g (77%); mp 168–169 °C (lit.[15] mp 168–169 °C). ^{1}H NMR (DMSO-d_6) δ 3.18 (2H, m, CH_2), 4.52 (2H, m, CH_2), 5.08 (1H, m, CH), 7.14–7.22 (4H, m, 4 × ArH), 7.41–7.49 (5H, m, 5 × ArH), 12.85 (1H, br s, exchangeable, COOH).

4.3.2. 2-(4-Fluorobenzoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (18d)

Compound **18d** was prepared from **17** (10.0 g, 46.8 mmol) and 4-fluorobenzoyl chloride (9.91 g, 57.0 mmol). Yield, 13.5 g (97%); mp 179–180 °C; ^{1}H NMR (DMSO-d_6) δ 3.20 (2H, m, CH_2), 4.52 (2H, m, CH_2), 5.06 (1H, m, CH), 7.19–7.33 (6H, m, 6 × ArH), 7.48–7.52 (2H, m, 2 × ArH), 12.76 (1H, br s, exchangeable, COOH). Anal. Calcd for ($C_{17}H_{14}FNO_3$): C, 68.22; H, 4.71; N, 4.68. Found: C, 68.08; H, 4.57; N, 4.42.

4.3.3. 2-(4-Chlorobenzoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (18e)

Compound **18e** was prepared from **17** (10.0 g, 46.8 mmol) and 4-chloroenzoyl chloride (10.1 g, 57.0 mmol). Yield, 13.8 g (93%), mp 77–79 °C; ^{1}H NMR (DMSO-d_6) δ 3.29 (2H, m, CH_2), 4.49 (2H, m, CH_2), 5.02 (1H, m, CH), 7.01–7.17 (4H, m, 4 × ArH), 7.40–7.58 (4H, m, 4 × ArH), 12.81 (1H, br s, exchangeable, COOH). Anal. Calcd for ($C_{17}H_{14}ClNO_3$): C, 64.67; H, 4.47; N, 4.44. Found: C, 64.52; H, 4.56; N, 4.33.

4.3.4. 2-(3,4-Difluorobenzoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (18f)

Compound **18f** was prepared from **17** (10.0 g, 46.8 mmol) and 3,4-difluorobenzoyl chloride (10.2 g, 57.0 mmol). Yield, 14.2 g (95%); mp 89–91 °C; ^{1}H NMR (DMSO-d_6) δ 3.18 (2H, m, CH_2), 4.48 (2H, m, CH_2), 5.02 (1H, m, CH), 7.09–7.15 (4H, m, 4 × ArH), 7.33 (1H, s, ArH), 7.46–7.54 (2H, m, 2 × ArH), 12.84 (1H, br s, exchangeable, COOH). Anal. Calcd for ($C_{17}H_{13}F_2NO_3$): C, 64.35; H, 4.13; N, 4.41. Found: C, 64.21; H, 4.26; N, 4.56.

4.3.5. 2-(3,4-Dichlorobenzoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (18g)

Compound **18g** was prepared from **17** (10.0 g, 46.8 mmol) and 3,4-dichlorobenzoyl chloride (12.1 g, 57.0 mmol). Yield, 14.5 g (89%); mp 101–102 °C; ^{1}H NMR (DMSO-d_6) δ 3.27 (2H, m, CH_2), 4.45 (2H, m, CH_2), 4.99 (1H, m, CH), 7.08–7.13 (4H, m, 4 × ArH), 7.44–7.46 (1H, m, ArH), 7.66–7.70 (2H, m, 2 × ArH), 12.67 (1H, br s, exchangeable, COOH). Anal. Calcd for ($C_{17}H_{17}Cl_2NO_3$): C, 58.31; H, 3.74; N, 4.00. Found: C, 58.19; H, 4.04; N, 3.87.

4.3.6. 2-(4-Methoxybenzoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (18h)

Compound **18h** was prepared from **17** (10.0 g, 46.8 mmol) and 4-methoxybenzoyl chloride (9.8 g, 57.0 mmol). Yield, 12.6 g (86%); mp 178– 180 °C; ^{1}H NMR (DMSO-d_6) δ 3.28 (2H, m, CH_2), 3.85 (3H, s, MeO), 4.60 (2H, m, CH_2), 5.02 (1H, m, CH), 6.90–6.94 (3H, m, 3 × ArH), 7.18–7.22 (3H, m, 3 × ArH), 7.42–7.48 (2H, m, 2 × ArH), 12.66 (1H, br s, exchangeable, COOH). Anal. Calcd for ($C_{18}H_{17}NO_4$): C, 69.44; H, 5.50; N, 4.50. Found: C, 69.12; H, 5.62; N, 4.27.

4.3.7. 2-(3,4-Dimethoxybenzoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (18i)

Compound **18i** was prepared from **17** (10.0 g, 46.8 mmol) and 3,4-dimethoxybenzoyl chloride (12.5 g, 57 mmol). Yield, 11.9 g (61%); mp 235–236 °C; ^{1}H NMR (DMSO-d_6) δ 3.19 (2H, m, CH_2), 3.77 (6H, s, 2 × MeO), 4.56 (2H, m, CH_2), 5.04 (1H, m, CH), 6.99–7.04 (4H, m, 4 × ArH), 7.17–7.23 (3H, m, 3 × ArH), 12.88 (1H, br s, exchangeable, COOH). Anal. Calcd for ($C_{19}H_{19}NO_5$): C, 66.85; H, 5.61; N, 4.10. Found: C, 66.65; H, 5.66; N, 3.86.

4.3.8. 2-(3,4,5-Trimethoxybenzoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (18j)

Compound **18j** was prepared from **17** (7.0 g, 32.7 mmol) and 3,4,5-trimethoxybenzoyl chloride (9.3 g, 39.9 mmol). Yield, 12.0 g (98%); mp 186–189 °C; ^{1}H NMR (DMSO-d_6) δ 3.19 (2H, m, CH_2), 3.70 (3H, s, MeO), 3.80 (6H, s, 2 × MeO), 4.59 (2H, m, CH_2), 5.02 (1H, m, CH), 6.70 (2H, s, 2 × ArH), 7.13–7.28 (4H, m, 4 × ArH), 12.72 (1H, br s, exchangeable, COOH). Anal. Calcd for ($C_{20}H_{21}NO_6$): C, 64.68; H, 5.70; N, 3.77. Found: C, 64.44; H, 5.50; N, 3.44.

4.4. 3-Methyl-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-dicarboxylic acid dimethyl ester (19a)

Dimethyl acetylenedicarboxylate (6.39 g, 45.0 mmol) was added into a mixture of 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (**17**, 10 g, 46.8 mmol) in acetic anhydride (70 mL) and the reaction mixture was heated at 70 °C with stirring for 1.5 h. The reaction mixture was evaporated to dryness in vacuo. The residue was recrystallized from MeOH to give **19a**, 11.0 g (74%); mp 152–154 °C (lit.[17] mp 140–142 °C). ^{1}H NMR (DMSO-d_6) δ 2.40 (3H, s, Me), 3.70 (3H, s, COOMe), 3.72 (3H, s, COOMe), 4.18 (2H, s, CH_2), 5.06 (2H, s, CH_2), 7.21–7.32 (2H, m, 2 × ArH), 7.35–7.39 (2H, m, 2 × ArH).

4.4.1. 3-Ethyl-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-dicarboxylic acid dimethyl ester (19b)

A mixture of dimethyl acetylenedicarboxylate (DMAD) (6.39 g, 45.0 mmol) and **18b** (7.0 g, 30.0 mmol) in acetic anhydride (50 mL) was heated at 65 °C with stirring for 1.5 h. The reaction mixture was evaporated to dryness in vacuo and the residue was recrystallized from MeOH to give **19b**, 8.77 g (93%); mp 88–89 °C. ^{1}H NMR (DMSO-d_6) δ 1.15 (3H, t, J = 7.6 Hz, Me), 2.85 (2H, q, J = 7.6 Hz, CH_2), 3.71 (6H, s, 2 × COOMe), 4.19 (2H, s, CH_2), 5.12 (2H, s, CH_2), 7.29–7.33 (2H, m, 2 × ArH), 7.38–7.43 (2H, m, 2 × ArH). Anal. Calcd for ($C_{18}H_{19}NO_4$): C, 68.99; H, 6.11; N, 4.47. Found: C, 68.96; H, 6.05; N, 4.38.

By following the same synthetic procedure as that for **19b**, the following compounds were synthesized.

4.4.2. 3-Phenyl-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-dicarboxylic acid dimethyl ester (19c)

Compound **19c** was prepared from DMAD (6.0 g, 41.0 mmol) and **18c** (8.0 g, 28.4 mmol). Yield, 7.0 g (68%); mp 137–138 °C. ^{1}H NMR (DMSO-d_6) δ 3.58 (3H, s, COOMe), 3.76 (3H, s, COOMe), 4.33 (2H, s, CH_2), 4.98 (2H, s, CH_2), 7.21–7.32 (3H, m, 3 × ArH), 7.41–7.53 (6H, m, 6 × ArH). Anal. Calcd for ($C_{22}H_{19}NO_4$): C, 73.12; H, 5.30; N, 3.88. Found: C, 72.91; H, 5.30; N, 3.53.

4.4.3. 3-(4-Fluorophenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-dicarboxylic acid dimethyl ester (19d)

Compound **19d** was prepared from DMAD (10.7 g, 65.0 mmol) and **18d** (15.0 g, 50.1 mmol). Yield, 11.1 g (58%); mp 149–150 °C. ^{1}H NMR (DMSO-d_6) δ 3.58 (3H, s, COOMe), 3.76 (3H, s, COOMe), 4.32 (2H, s, CH_2), 4.96 (2H, s, CH_2), 7.23–7.30 (1H, m, ArH), 7.32–7.36 (4H, m, 4 × ArH), 7.40–7.42 (1H, m, ArH), 7.49–7.52 (2H, m, 2 × ArH). Anal. Calcld for ($C_{22}H_{18}FNO_4$): C, 69.65; H, 4.78; N, 3.69. Found: C, 69.54; H, 4.82; N, 3.78.

4.4.4. 3-(4-Chlorophenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-dicarboxylic acid dimethyl ester (19e)

Compound **19e** was prepared from DMAD (6.8 g, 47.0 mmol) and **18e** (10.0 g, 31.6 mmol). Yield, 8.0 g (64%); mp 164–166 °C. ^{1}H NMR (DMSO-d_6) δ 3.60 (3H, s, COOMe), 3.76 (3H, s, COOMe), 4.32 (2H, s, CH_2), 4.98 (2H, s, CH_2), 7.20–7.26 (1H, m, ArH), 7.27–7.33 (2H, m, 2 × ArH), 7.39–7.43 (1H, m, ArH), 7.48 (2H, d, J = 8.5 Hz, 2 × ArH), 7.57 (2H, d, J = 8.5 Hz, 2 × ArH). Anal. Calcd for ($C_{22}H_{18}ClNO_4$): C, 66.75; H, 4.58; N, 3.54. Found: C, 66.52; H, 4.85; N, 3.66.

4.4.5. 3-(3, 4-Difluorophenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-dicarboxylic acid dimethyl ester (19f)

Compound **19f** was prepared from DMAD (6.71 g, 47.0 mmol) and **18f** (10.0 g, 31.5 mmol). Yield, 8.4 g (67%); mp 142–144 °C. ^{1}H NMR (DMSO-d_6) δ 3.61 (3H, s, COOMe), 3.76 (3H, s, COOMe), 4.31 (2H, s, CH_2), 5.00 (2H, s, CH_2), 7.22–7.42 (5H, m, 5 × ArH), 7.54–7.61 (2H, m, 2 × ArH). Anal. Calcd for ($C_{22}H_{17}F_2NO_4$): C, 66.50; H, 4.31; N, 3.52. Found: C, 66.15; H, 4.44; N, 3.36.

4.4.6. 3-(3,4-Dichlorophenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-dicarboxylic acid dimethyl ester (19g)

Compound **19e** was prepared from DMAD (8.5 g, 60.0 mmol) and **18g** (14.0 g, 40.0 mmol). Yield, 11.0 g (63%); mp 169–170 °C. ^{1}H NMR (DMSO-d_6) δ 3.61 (3H, s, COOMe), 3.77 (3H, s, COOMe), 4.31 (2H, s, CH_2), 5.02 (2H, s, CH_2), 7.22–7.33 (3H, m, 3 × ArH), 7.40–7.47 (2H, m, 2 × ArH), 7.76–7.78 (2H, m, 2 × ArH). Anal. Calcd for ($C_{22}H_{17}Cl_2NO_4$): C, 61.41; H, 3.98; N, 3.26. Found: C, 61.33; H, 3.95; N, 2.89.

4.4.7. 3-(4-Methoxyphenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-dicarboxylic acid dimethyl ester (19h)

Compound **19h** was prepared from DMAD (7.5 g, 53.0 mmol) and **18h** (10.0 g, 35.1 mmol). Yield, 8.4 g (66%); mp 155–158 °C. ^{1}H NMR (DMSO-d_6) δ 3.58 (3H, s, COOMe), 3.75 (3H, s, COOMe), 3.82 (3H, s, MeO), 4.31 (2H, s, CH_2), 4.95 (2H, s, CH_2), 7.05–7.07 (2H, m, 2 × ArH), 7.21–7.24 (1H, m, ArH), 7.28–7.31 (2H, m, 2 × ArH), 7.37–7.42 (3H, m, 3 × ArH). Anal. Calcd for ($C_{23}H_{21}NO_5$): C, 70.58; H, 5.41; N, 3.58. Found: C, 70.25; H, 5.44; N, 3.49.

4.4.8. 3-(3,4-Dimethoxyphenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-dicarboxylic acid dimethyl ester (19i)

Compound **19i** was prepared from DMAD (5.6 g, 39.0 mmol) and **18i** (9.0 g, 26.4 mmol). Yield, 9.0 g (81%); mp 202–203 °C. ^{1}H NMR (DMSO-d_6) δ 3.60 (3H, s, COOMe), 3.66 (3H, s, MeO), 3.78 (3H, s, COOMe), 3.82 (3H, s, MeO), 4.32 (2H, s, CH_2), 5.00 (2H, s, CH_2), 6.99 (1H, dd, J = 2.0 and 8.0 Hz, ArH), 7.02 (1H, d, J = 2.0 Hz, ArH), 7.07 (1H, d, J = 8.0 Hz, ArH), 7.22–7.25 (1H, m, ArH), 7.25–7.32 (2H, m, 2 × ArH), 7.40–7.42 (1H, m, ArH). Anal. Calcd for ($C_{24}H_{23}NO_6$): C, 68.40; H, 5.50; N, 3.32. Found: C, 68.02; H, 5.53; N, 2.94.

4.4.9. 3-(3,4,5-Trimethoxyphenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-dicarboxylic acid dimethyl ester (19j)

Compound **19i** was prepared from DMAD (5.7 g, 40.0 mmol) and **18j** (10.0 g, 26.9 mmol). Yield, 6.2 g (51%); mp 165–166 °C. ^{1}H NMR (DMSO-d_6) δ 3.63 (3H, s, COOMe), 3.74 (6H, s, COOMe and MeO), 3.80 (6H, s, 2 × MeO), 4.32 (2H, s, CH_2), 5.00 (2H, s, CH_2), 6.75 (2H, s, 2 × ArH), 7.22–7.26 (1H, m, ArH), 7.28–7.32 (1H, m, ArH), 7.35–7.37 (1H, m, ArH), 7.40–7.42 (1H, m, ArH). Anal. Calcd for ($C_{25}H_{25}NO_7$): C, 66.51; H, 5.58; N, 3.10. Found: C, 66.25; H, 5.59; N, 3.01.

4.5. [3-Methyl-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-diyl]dimethanol (20a)

A solution of **19a** (8.0 g, 26.7 mmol) in anhydrous dichloromethane (20 mL) was added dropwise in to a stirred suspension of $LiAlH_4$ (2.5 g, 66.0 mmol) in anhydrous diethyl ether (50 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 (2.5 mL), 15% aqueous NaOH (2.5 mL), and water (2.5 mL) at 0 °C. The mixture was filtered through a pad of Celite, the solid residue was washed with dichloromethane. The combined filtrate and washings were evaporated to dryness in vacuo. The residue was recrystallized from ether to give **20a**, 4.2 g (64.0%); mp 99–101 °C. ^{1}H NMR (DMSO-d_6) δ 2.22 (3H, s, Me), 3.96 (2H, s, CH_2), 4.33 (2H, m, CH_2), 4.38 (4H, m, CH_2 and exchangeable, 2 × OH), 4.93 (2H, s, CH_2), 7.24–7.28 (2H, m, 2 × ArH), 7.34–7.36 (2H, m, 2 × ArH). Anal. Calcd for ($C_{15}H_{17}NO_2$): C, 74.05; H, 7.04; N, 5.76. Found: C, 74.24; H, 6.97; N, 5.71.

By following the same synthetic procedure as that for **20a**, the following compounds were synthesized.

4.5.1. [3-Ethyl-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-diyl]dimethanol (20b)

Compound **20b** was prepared from **19b** (5.0 g, 15.9 mmol) and $LiAlH_4$ (1.5 g, 39.0 mmol). Yield, 3.15 g (76%) as syrup; ^{1}H NMR (DMSO-d_6) δ 1.10 (3H, t, J = 7.6 Hz, Me), 2.66 (2H, q, J = 7.6 Hz, CH_2), 3.95 (2H, s, CH_2), 4.33 (3H, m, CH_2 and exchangeable, OH), 4.39 (3H, m, CH_2 and exchangeable, OH), 4.96 (2H, s, CH_2), 7.23–7.28 (2H, m, 2 × ArH), 7.33–7.39 (2H, m, 2 × ArH).

4.5.2. [3-Phenyl-5,10-dihydropyrrolo[1,2-b]isoquinoline-1,2-diyl]dimethanol (20c)

Compound **20c** was prepared from **19c** (7.0 g, 19.4 mmol) and $LiAlH_4$ (1.8 g, 48.0 mmol). Yield, 4.7 g (80%); mp 150–152 °C. ^{1}H NMR (DMSO-d_6) δ 4.06 (2H, s, CH_2), 4.28 (2H, d, J = 4.8 Hz, CH_2), 4.50 (2H, d, J = 4.8 Hz, CH_2), 4.54 (2H, br s, exchangeable OH), 4.98 (2H, s, CH_2), 7.18 (1H, t, J = 7.2 Hz, ArH), 7.26 (2H, t, J = 7.2 Hz, 2 × ArH), 7.36–7.39 (2H, m, 2 × ArH), 7.45–7.51 (4H, m, 4 × ArH). Anal. Calcd for ($C_{20}H_{19}NO_2$): C, 78.66; H, 6.27; N, 4.59. Found: C, 78.48; H, 6.42; N, 4.41.

4.5.3. [3-(4-Fluorophenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-diyl]dimethanol (20d)

Compound **20d** was prepared from **19d** (10.0 g, 26.4 mmol) and $LiAlH_4$ (2.5 g, 65.0 mmol). Yield, 6.36 g (74%); mp 135–136 °C. ^{1}H NMR (DMSO-d_6) δ 4.05 (2H, s, CH_2), 4.26 (2H, d, J = 4.8 Hz, CH_2), 4.49 (2H, d, J = 4.8 Hz, CH_2), 4.53 (2H, t, J = 4.8 Hz, exchangeable, OH), 4.96 (2H, s, CH_2), 7.17–7.21 (1H, m, ArH), 7.26–7.34 (4H, m, 4 × ArH), 7.38 (1H, m, ArH), 7.48–7.52 (2H, m, 2 × ArH). Anal.

Calcd for ($C_{20}H_{18}FNO_2$): C, 74.29; H, 5.61; N, 4.33. Found: C, 74.38; H, 5.68; N, 3.98.

4.5.4. [3-(4-Chlorophenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-diyl]dimethanol (20e)

Compound **20e** was prepared from **19e** (7.0 g, 17.7 mmol) and $LiAlH_4$ (1.63 g, 44 mmol). Yield, 3.1 g (52%); mp 170–172 °C. ^1H NMR (DMSO-d_6) δ 4.06 (2H, s, CH_2), 4.28 (2H, d, J = 4.8 Hz, CH_2), 4.50 (2H, d, J = 4.8 Hz, CH_2), 4.55 (2H, m, exchangeable, OH), 4.99 (2H, s, CH_2), 7.18–7.20 (1H, m, ArH), 7.21–7.27 (2H, m, 2 × ArH), 7.29 (1H, d, J = 7.6 Hz, ArH), 7.38 (2H, d, J = 7.2 Hz, 2 × ArH), 7.48 (2H, d, J = 7.2 Hz, 2 × ArH). Anal. Calcd for ($C_{20}H_{18}ClNO_2$): C, 70.69; H, 5.34; N, 4.12. Found: C, 70.42; H, 5.37; N, 4.05.

4.5.5. [3-(3,4-Difluorophenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-diyl]dimethanol (20f)

Compound **20f** was prepared from **19f** (7.5 g, 18.0 mmol) and $LiAlH_4$ (1.73 g, 47 mmol). Yield, 5.3 g (83%); mp 145–146 °C. ^1H NMR (DMSO-d_6) δ 4.05 (2H, s, CH_2), 4.27 (2H, d, J = 5.2 Hz, CH_2z), 4.48 (2H, d, J = 5.2 Hz, CH_2), 4.53 (1H, t, J = 5.2 Hz, exchangeable, OH), 4.60 (1H, t, J = 5.2 Hz, exchangeable, OH), 5.01 (2H, s, CH_2), 7.18–7.33 (4H, m, 4 × ArH), 7.37–7.39 (1H, m, ArH), 7.52–7.54 (2H, m, 2 × ArH). Anal. Calcd for ($C_{20}H_{17}F_2NO_2$): C, 70.37; H, 5.02; N, 4.10. Found: C, 70.08; H, 5.01; N, 3.76.

4.5.6. [3-(3,4-Dichlorophenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-diyl]dimethanol (20g)

Compound **20g** was prepared from **19g** (9.0 g, 20.9 mmol) and $LiAlH_4$ (1.9 g, 52.0 mmol). Yield, 5.7 g (72%); mp 150–151 °C. ^1H NMR (DMSO-d_6) δ 4.05 (2H, s, CH_2), 4.26 (2H, d, J = 4.8 Hz, CH_2), 4.48 (2H, d, J = 4.8 Hz, CH_2), 4.53 (1H, t, J = 4.8 Hz, exchangeable OH), 4.63 (1H, t, J = 4.8 Hz, exchangeable, OH), 5.03 (2H, s, CH_2), 7.18–7.21 (1H, m, ArH), 7.23–7.27 (2H, m, 2 × ArH), 7.28–7.30 (1H, m, ArH), 7.38–7.40 (1H, m, ArH), 7.46–7.48 (2H, m, 2 × ArH). Anal. Calcd for ($C_{20}H_{17}Cl_2NO_2$): C, 64.18; H, 4.58; N, 3.74. Found: C, 63.89; H, 4.40; N, 3.87.

4.5.7. [3-(4-Methoxyphenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-diyl]dimethanol (20h)

Compound **20h** was prepared from **19h** (7.5 g, 19.0 mmol) and $LiAlH_4$ (1.77 g, 47 mmol). Yield, 4.3 g (67%); mp 174–176 °C. ^1H NMR (DMSO-d_6) δ 3.82 (3H, s, MeO), 4.04 (2H, s, CH_2), 4.26 (2H, s, CH_2), 4.48 (4H, m, CH_2 and exchangeable, OH), 4.93 (2H, s, CH_2), 7.04–7.06 (2H, m, 2 × ArH), 7.17–7.21 (1H, m, ArH), 7.24–7.27 (2H, m, 2 × ArH), 7.36–7.39 (3H, m, 3 × ArH). Anal. Calcd for ($C_{21}H_{21}NO_3$): C, 75.20; H, 6.31; N, 4.18. Found: C, 75.36; H, 6.30; N, 4.13.

4.5.8. [3-(3,4-Dimethoxyphenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-diyl]dimethanol (20i)

Compound **20i** was prepared from **19i** (8.0 g, 18.9 mmol) and $LiAlH_4$ (1.75 g, 47 mmol). Yield, 5.34 g (77%); mp 145–146 °C. ^1H NMR (DMSO-d_6) δ 3.79 (6H, s, 2 × MeO), 4.04 (2H, s, CH_2), 4.29 (2H, s, CH_2), 4.52 (4H, br s, CH_2 and exchangeable, OH), 4.97 (2H, s, CH_2), 6.96–6.98 (1H, m, ArH), 7.05–7.07 (2H, m, 2 × ArH), 7.19–7.28 (3H, m, 3 × ArH), 7.37–7.39 (1H, m, ArH). Anal. Calcd for ($C_{22}H_{23}NO_4$): C, 72.31; H, 6.34; N, 3.83. Found: C, 72.25; H, 6.34; N, 3.95.

4.5.9. [3-(3,4,5-Trimethoxyphenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-diyl]dimethanol (20j)

Compound **20j** was prepared from **19j** (6.0 g, 13.3 mmol) and $LiAlH_4$ (1.2 g, 33.0 mmol). Yield, 3.1 g (58%); mp 146–147 °C. ^1H NMR (DMSO-d_6) δ 3.74 (3H, s, MeO), 3.82 (6H, s, 2 × MeO), 4.05 (2H, s, CH_2), 4.31 (2H, s, CH_2), 4.50 (2H, s, CH_2), 4.53 (2H, s, exchangeable, OH), 5.04 (2H, s, CH_2), 6.73 (2H, s, 2 × ArH), 7.21–7.27 (2H, m, 2 × ArH), 7.33–7.38 (2H, m, 2 × ArH). Anal. Calcd for ($C_{23}H_{25}NO_5$): C, 69.86; H, 6.37; N, 3.54. Found: C, 69.71; H, 6.37; N, 3.45.

4.6. General procedure for preparing bis(alkylcarbamate) derivatives (21–23)

To a solution of bis(hydroxymethyl) derivatives (**20a**–**20j**, 1.0 equiv) and triethylamine (2–3 equiv) in anhydrous dichloromethane was added alkylisocyanate (5 equiv). The reaction mixture was stirred at ambient temperature (for 3–20 h) under an argon atmosphere. After the completion of the reaction, the reaction mixture was evaporated to dryness in vacuo. The residue was triturated with ether, and solid separated was collected by filtration. The desired product was either obtained by recrystallization or liquid chromatography.

4.6.1. [3-Methyl-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-diyl]bis(methylene) bis(methylcarbamate) (21a)

Compound **21a** was synthesized from **20a** (0.80 g, 3.2 mmol), Et_3N (1 mL) and methylisocyanate (1.14 g, 20 mmol). Yield, 0.53 g (44%); mp 174–176 °C. ^1H NMR (DMSO-d_6) δ 2.23 (3H, s, Me), 2.53 (6H, m, 2 × Me), 3.99 (2H, s, CH_2), 4.88 (2H, s, CH_2), 4.92 (2H, s, CH_2), 4.95 (2H, s, CH_2), 6.74 (2H, br s, exchangeable, NH), 7.21–7.32 (2H, m, 2 × ArH), 7.34–7.38 (2H, m, 2 × ArH). Anal. Calcd for ($C_{19}H_{23}N_3O_4$): C, 63.85; H, 6.49; N, 11.76. Found: C, 63.54; H, 6.57; N, 11.64.

4.6.2. [3-(4-Chlorophenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-diyl]bis(methylene) bis(methylcarbamate) (21e)

Compound **21e** was synthesized from **20e** (1.0 g, 3.0 mmol), Et_3N (1 mL), and methylisocyanate (0.85 g, 15 mmol). Yield, 1.1 g (82%); mp 194–196 °C. ^1H NMR (DMSO-d_6) δ 2.54 (6H, m, 2 × Me), 4.09 (2H, s, CH_2), 4.80 (2H, s, CH_2), 4.97 (2H, s, CH_2), 5.03 (2H, s, CH_2), 6.81 (2H, br s, exchangeable, NH), 7.17–7.23 (1H, m, ArH), 7.24–7.30 (2H, m, 2 × ArH), 7.36–7.40 (1H, m, ArH), 7.45–7.47 (2H, m, 2 × ArH), 7.55–7.57 (2H, m, 2 × ArH). Anal. Cacld for ($C_{24}H_{24}ClN_3O_4$): C, 63.50; H, 5.33; N, 9.26. Found: C, 63.33; H, 5.35; N, 9.20.

4.6.3. [3-(3,4-Difluorophenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-diyl]bis(methylene) bis(methylcarbamate) (21f)

Compound **21f** was synthesized from **20f** (1.0 g, 2.9 mmol), Et_3N (1 mL), and methylisocyanate (0.85 g, 15 mmol). Yield, 1.0 g (77%); mp 190–192 °C; ^1H NMR (DMSO-d_6) δ 2.54 (6H, m, 2 × Me), 4.09 (2H, s, CH_2), 4.81 (2H, s, CH_2), 4.99 (2H, s, CH_2), 5.03 (2H, s, CH_2), 6.83 (2H, br s, exchangeable, NH), 7.18–7.23 (1H, m, ArH), 7.25–7.31 (3H, m, 3 × ArH), 7.36–7.40 (1H, m, ArH), 7.52–7.61 (2H, m, 2 × ArH). Anal. Calcd for ($C_{24}H_{23}F_2N_3O_4$): C, 63.29; H, 5.09; N, 9.23. Found: C, 63.00; H, 5.12; N, 9.16.

4.6.4. [3-(4-Methoxyphenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-diyl]bis(methylene) bis(methylcarbamate) (21h)

Compound **21h** was synthesized from **20h** (1.0 g, 2.9 mmol), Et_3N (1 mL), methylisocyanate (1.6 g, 29 mmol). Yield, 1.2 g (87%); mp 182–184 °C. ^1H NMR (DMSO-d_6) δ 2.53 (6H, m, 2 × Me), 3.85 (3H, s, MeO), 4.08 (2H, s, CH_2), 4.78 (2H, s, CH_2), 4.93 (2H, s, CH_2), 5.01 (2H, s, CH_2), 6.81 (2H, br s, exchangeable, NH), 7.06–7.08 (2H, m, 2 × ArH), 7.17–7.23 (1H, m, ArH), 7.24–7.30 (2H, m, 2 × ArH), 7.32–7.40 (3H, m, 3 × ArH). Anal. Calcd for ($C_{25}H_{27}N_3O_5$): C, 66.80; H, 6.05; N, 9.35. Found: C, 66.72; H, 6.15; N, 9.24.

4.7. [3-Methyl-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-diyl]bis(methylene)bis (ethylcarbamate) (22a)

Compound **22a** was synthesized from **20a** (1.0 g, 4.1 mmol), Et_3N (0.5 mL), and ethylisocyanate (1.1 g, 16 mmol). Yield, 0.83 g (53%); mp 208–209 °C; ^1H NMR (DMSO-d_6) δ 0.97 (6H, t, J = 8 Hz, 2 × Me), 2.25 (3H, s, Me), 2.95 (4H, q, J = 8 Hz, CH_2), 4.00 (2H, s, CH_2), 4.89 (2H, s, CH_2), 4.93 (2H, s, CH_2), 4.96 (2H, s, CH_2), 6.86 (2H, br s, exchangeable, NH), 7.23–7.29 (2H, m, 2 × ArH), 7.34–7.35 (2H, m, 2 × ArH). Anal. Calcd for ($C_{21}H_{27}N_3O_4$): C, 65.44; H, 7.06; N, 10.90. Found: C, 65.29; H, 7.14; N, 10.89.

4.7.1. [3-Ethyl-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-diyl]bis(methylene)bis (ethylcarbamate) (22b)

Compound **22b** was synthesized from **20b** (0.5 g, 1.9 mmol), Et_3N (0.5 mL), ethylisocyanate (0.55 g, 7.7 mmol). Yield, 0.39 g (50%); mp 127–129 °C. ^1H NMR (DMSO-d_6) δ 0.95 (6H, t, J = 7.2 Hz, 2 × Me), 1.10 (3H, t, J = 7.2 Hz, Me), 2.69 (2H, q, J = 7.2 Hz, CH_2), 2.97 (4H, m, CH_2), 3.99 (2H, s, CH_2), 4.89 (2H, s, CH_2), 4.93 (2H, s, CH_2), 4.99 (2H, s, CH_2), 6.84 (2H, br s, exchangeable, NH), 7.23–7.29 (2H, m, 2 × ArH), 7.34–7.39 (2H, m, 2 × ArH). Anal. Calcd for ($C_{22}H_{29}N_3O_4$): C, 66.14; H, 7.32; N, 10.52. Found: C, 66.35; H, 7.53; N, 10.74.

4.7.2. [3-Phenyl-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-diyl]bis(methylene)bis (ethylcarbamate) (22c)

Compound **22c** was synthesized from **20c** (1.0 g, 3.2 mmol), Et_3N (1.0 mL), and ethylisocyanate (1.2 g, 16.0 mmol). Yield, 1.17 g (81%); mp 170–171 °C; ^1H NMR (DMSO-d_6) δ 0.97 (6H, t, J = 7.2 Hz, 2 × Me), 2.98 (4H, q, J = 7.2 Hz, CH_2), 4.10 (2H, s, CH_2), 4.80 (2H, s, CH_2), 4.97 (2H, s, CH_2), 5.03 (2H, s, CH_2), 6.94 (2H, br s, exchangeable, NH), 7.18–7.20 (1H, m, ArH), 7.26–7.29 (2H, m, 2 × ArH), 7.36–7.40 (4H, m, 4 × ArH), 7.42–7.44 (2H, m, 2 × ArH). Anal. Calcld for ($C_{26}H_{29}N_3O_4$): C, 69.78; H, 6.53; N, 9.39. Found: C, 69.64; H, 6.54; N, 9.37.

4.7.3. [3-(4-Fluorophenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-diyl]bis(methylene) bis(ethylcarbamate) (22d)

Compound **22d** was synthesized from **20d** (1.0 g, 3.0 mmol), Et_3N (1.2 mL), and (1.1 g, 15.0 mmol). Yield, 1.08 g (76%); mp 183–184 °C. ^1H NMR (DMSO-d_6) δ 0.98 (6H, t, J = 6.8 Hz, 2 × Me), 2.97 (4H, q, J = 6.8 Hz, CH_2), 4.09 (2H, s, CH_2), 4.79 (2H, s, CH_2), 4.95 (2H, s, CH_2), 5.02 (2H, s, CH_2), 6.93 (2H, br s, exchangeable, NH), 7.20–7.22 (1H, m, ArH), 7.27–7.41 (5H, m, 5 × ArH), 7.46–7.48 (2H, m, 2 × ArH). Anal. Calcd for ($C_{26}H_{28}FN_3O_4$): C, 67.08; H, 6.06; N, 9.03. Found: C, 67.24; H, 6.02; N, 8.88.

4.7.4. [3-(4-Chlorophenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-diyl]bis(methylene) bis(ethylcarbamate) (22e)

Compound **22e** was synthesized from **20e** (1.0 g, 2.9 mmol), Et_3N (1.0 mL), and ethylisocyanate (0.84 g, 18.0 mmol). Yield, 0.92 g (64%); mp 191–192 °C. ^1H NMR (DMSO-d_6) δ 0.98 (6H, t, J = 7.2 Hz, 2 × Me), 2.97 (4H, q, J = 7.2 Hz, CH_2), 4.09 (2H, s, CH_2), 4.80 (2H, s, CH_2), 4.97 (2H, s, CH_2), 5.02 (2H, s, CH_2), 6.92 (2H, br s, exchangeable, NH), 7.18–7.22 (1H, m, ArH), 7.25–7.27 (2H, m, 2 × ArH), 7.28–7.30 (1H, m, ArH), 7.37–7.39 (2H, m, 2 × ArH), 7.44–7.46 (2H, m, 2 × ArH). Anal. Calcd for ($C_{26}H_{28}ClN_3O_4$): C, 64.79; H, 5.86; N, 8.72. Found: C, 64.54; H, 5.84; N, 8.82.

4.7.5. [3-(3,4-Difluorophenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-diyl]bis(methylene)bis (ethyl carbamate) (22f)

Compound **22f** was synthesized from **20f** (1.0 g, 2.9 mmol), Et_3N (1 mL), and ethylisocyanate (0.8 g, 11.7 mmol). Yield, 1.0 g (70%); mp 189–190 °C. ^1H NMR (DMSO-d_6) δ 0.98 (6H, t, J = 6.8 Hz, 2 × Me), 2.98 (4H, q, J = 6.8 Hz, CH_2), 4.09 (2H, s, CH_2), 4.81 (2H, s, CH_2), 5.00 (2H, s, CH_2), 5.02 (2H, s, CH_2), 6.95 (2H, br s, exchangeable, NH), 7.19–7.21 (1H, m, ArH), 7.25–7.39 (4H, m, 4 × ArH), 7.53–7.60 (2H, m, 2 × ArH). Anal. Calcd for ($C_{26}H_{27}F_2N_3O_4$): C, 64.59; H, 5.63; N, 8.69. Found: C, 64.50; H, 5.60; N, 8.69.

4.7.6. [3-(3,4-Dichlorophenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-diyl)bis(methylene]bis (ethylcarbamate) (22g)

Compound **22g** was synthesized from **20g** (1.0 g, 2.6 mmol), Et_3N (1.0 mL), and ethylisocyanate (0.85 g, 10.7 mmol). Yield, 1.15 g (83%); mp 208–209 °C. ^1H NMR (DMSO-d_6) δ 0.98 (6H, t, J = 6.8 Hz, 2 × Me), 2.97 (4H, q, J = 6.8 Hz, CH_2), 4.09 (2H, s, CH_2), 4.82 (2H, s, CH_2), 5.02 (4H, s, 2 × CH_2), 6.95 (2H, br s, exchangeable, NH), 7.19–7.22 (1H, m, ArH), 7.26–7.31 (2H, m, 2 × ArH), 7.39–7.45 (2H, m, 2 × ArH), 7.70–7.72 (2H, m, 2 × ArH). Anal. Calcd for ($C_{26}H_{27}Cl_2N_3O_4$): C, 60.47; H, 5.27; N, 8.14. Found: C, 60.34; H, 5.13; N, 8.10.

4.7.7. [3-(4-Methoxyphenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-diyl]bis(methylene) bis(ethylcarbamate) (22h)

Compound **22h** was synthesized from **20h** (1.0 g, 2.9 mmol), Et_3N (1.0 mL), and ethylisocyanate (0.84 g, 11.8 mmol). Yield, 0.92 g (65%); mp 165–166 °C. ^1H NMR (DMSO-d_6) δ 0.99 (6H, t, J = 6.8 Hz, 2 × Me), 2.98 (4H, q, J = 6.8 Hz, CH_2), 3.83 (3H, s, MeO), 4.09 (2H, s, CH_2), 4.78 (2H, s, CH_2), 4.94 (2H, s, CH_2), 5.02 (2H, s, CH_2), 6.92 (2H, br s, exchangeable, NH), 7.05–7.08 (2H, m, 2 × ArH), 7.19–7.22 (1H, m, ArH), 7.27–7.29 (2H, m, 2 × ArH), 7.35–7.39 (3H, m, 3 × ArH). Anal. Calcd for ($C_{27}H_{31}N_3O_5$): C, 67.91; H, 6.54; N, 8.80. Found: C, 67.73; H, 6.86; N, 8.57.

4.7.8. [3-(3,4-Dimethoxyphenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-diyl]bis(methylene) bis(ethylcarbamate) (22i)

Compound **22i** was synthesized from **20i** (1.0 g, 2.7 mmol), Et_3N (1 mL), and ethylisocyanate (0.77 g, 10.9 mmol). Yield, 0.96 g (69%); mp 124–125 °C. ^1H NMR (DMSO-d_6) δ 0.98 (6H, t, J = 6.8 Hz, 2 × Me), 2.98 (4H, q, J = 6.8 Hz, CH_2), 3.77 (3H, s, MeO), 3.82 (3H, s, MeO), 4.08 (2H, s, CH_2), 4.79 (2H, s, CH_2), 4.98 (2H, s, CH_2), 5.02 (2H, s, CH_2), 6.93 (2H, br s, exchangeable NH), 6.96–6.98 (2H, m, 2 × ArH), 7.07–7.09 (1H, m, ArH), 7.18–7.22 (1H, m, ArH), 7.25–7.28 (2H, m, 2 × ArH), 7.30–7.37 (1H, m, ArH). Anal. Calcd for ($C_{28}H_{33}N_3O_6$): C, 66.26; H, 6.55; N, 8.28. Found: C, 66.34; H, 6.45; N, 8.11.

4.7.9. [3-(3,4,5-Trimethoxyphenyl)-5,10-dihydropyrrolo[1,2-*b*] isoquinoline-1,2-diyl]bis (methylene)bis(ethylcarbamate) (22j)

Compound **22j** was synthesized from **20j** (0.79 g, 2.0 mmol), Et_3N (0.8 mL), and ethylisocyanate (0.57 g, 8.0 mmol). Yield, 0.94 g (99%); mp 170–171 °C. ^1H NMR (DMSO-d_6) δ 0.97 (6H, t, J = 6.8 Hz, 2 × Me), 2.98 (4H, q, J = 6.8 Hz, CH_2), 3.73 (3H, s, MeO), 3.80 (6H, s, 2 × MeO), 4.09 (2H, s, CH_2), 4.81 (2H, s, CH_2), 5.02 (2H, s, CH_2), 5.05 (2H, s, CH_2), 6.70 (2H, s, 2 × ArH), 6.95 (2H, br s, exchangeable, NH), 7.19–7.21 (1H, m, ArH), 7.23–7.26 (1H, m, ArH), 7.27–7.29 (1H, m, ArH), 7.33–7.35 (1H, m, ArH). Anal. Calcd for ($C_{29}H_{35}N_3O_7$): C, 64.79; H, 6.56; N, 7.82. Found: C, 64.68; H, 6.42; N, 7.97.

4.8. [3-Methyl-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-diyl]bis(methylene)bis(*iso*-propylcarbamate) (23a)

Compound **23a** was synthesized from **20a** (0.5 g, 2.0 mmol), Et_3N (0.4 mL), and isopropylisocyanate (0.69 g, 8.0 mmol). Yield,

0.55 g (65%); mp 215–218 °C. ^{1}H NMR (DMSO-d_6) δ 0.99 (12H, d, J = 6.4 Hz, 4 × Me), 2.25 (3H, s, Me), 3.54 (2H, m, CH), 3.99 (2H, s, CH_2), 4.89 (2H, s, CH_2), 4.93 (2H, s, CH_2), 4.96 (2H, s, CH_2), 6.78 (2H, br s, exchangeable, NH), 7.25–7.29 (2H, m, 2 × ArH), 7.34–7.35 (2H, m, 2 × ArH). Anal. Calcd for ($C_{23}H_{31}N_3O_4$): C, 66.81; H, 7.56; N, 10.16. Found: C, 66.69; H, 7.66; N, 10.10.

4.8.1. [3-Ethyl-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-diyl]bis(methylene)bis(*iso*-propylcarbamate) (23b)

Compound **23b** was synthesized from **20b** (0.26 g, 1.0 mmol), Et_3N (0.4 mL), and isopropylisocyanate (0.34 g, 4.0 mmol). Yield, 0.25 g (58%); mp 155–158 °C. ^{1}H NMR (DMSO-d_6) δ 1.00 (12H, d, J = 6.4 Hz, 4 × Me), 1.10 (3H, t, J = 7.6 Hz, Me), 2.69 (2H, q, J = 7.6 Hz, CH_2), 3.55 (2H, m, CH), 3.99 (2H, s, CH_2), 4.89 (2H, s, CH_2), 4.93 (2H, s, CH_2), 4.99 (2H, s, CH_2), 6.79 (2H, br s, exchangeable, NH), 7.25–7.29 (2H, m, 2 × ArH), 7.33–7.39 (2H, m, 2 × ArH). Anal. Calcd for ($C_{24}H_{33}N_3O_4$): C, 67.42; H, 7.78; N, 9.83. Found: C, 67.11; H, 7.55; N, 9.71.

4.8.2. [3-Phenyl-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-diyl]bis(methylene)bis(*iso*-propylcarbamate) (23c)

Compound **23c** was synthesized from **20c** (0.5 g, 1.6 mmol), Et_3N (0.5 mL), and isopropylisocyanate (0.54 g, 6.4 mmol). Yield, 0.52 g (74%); mp 175–176 °C. ^{1}H NMR (DMSO-d_6) δ 0.99 (12H, d, J = 6.8 Hz, 4 × Me), 3.62 (2H, m, CH), 4.10 (2H, s, CH_2), 4.80 (2H, s, CH_2), 4.98 (2H, s, CH_2), 5.03 (2H, s, CH_2), 6.86 (2H, br s, exchangeable, NH), 7.18–7.22 (1H, m, ArH), 7.26–7.28 (2H, m, 2 × ArH), 7.38–7.42 (4H, m, 4 × ArH), 7.49–7.51 (2H, m, 2 × ArH). Anal. Calcd for ($C_{28}H_{33}N_3O_4$): C, 70.71; H, 6.99; N, 8.84. Found: C, 70.59; H, 6.92; N, 8.67.

4.8.3. [3-(4-Fluorophenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-diyl]bis(methylene)bis(*iso*-propylcarbamate) (23d)

Compound **23d** was synthesized from **20d** (0.5 g, 1.5 mmol), Et_3N (0.5 mL), and isopropylisocyanate (0.51 g, 6.0 mmol). Yield, 0.62 g (81%); mp 190–191 °C. ^{1}H NMR (DMSO-d_6) δ 0.99 (12H, d, J = 6.4 Hz, 4 × Me), 3.60 (2H, m, CH), 4.09 (2H, s, CH_2), 4.79 (2H, s, CH_2), 4.95 (2H, s, CH_2), 5.02 (2H, s, CH_2), 6.86 (2H, br s, exchangeable, NH), 7.18–7.22 (1H, m, ArH), 7.26–7.29 (5H, m, 5 × ArH), 7.31–7.33 (2H, m, 2 × ArH). Anal. Calcd for ($C_{28}H_{32}FN_3O_4$): C, 68.14; H, 6.53; N, 8.51. Found: C, 68.31; H, 6.40; N, 8.60.

4.8.4. [3-(4-Chlorophenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-diyl]bis(methylene)bis(*iso*-propylcarbamate) (23e)

Compound **23e** was synthesized from **20e** (0.45 g, 1.3 mmol), Et_3N (0.5 mL), and isopropylisocyanate (0.42 g, 5.0 mmol). Yield, 0.43 g (64%); mp 191–192 °C. ^{1}H NMR (DMSO-d_6) δ 1.01 (12H, d, J = 6.4 Hz, 4 × Me), 3.57 (2H, m, CH), 4.09 (2H, s, CH_2), 4.80 (2H, s, CH_2), 4.97 (2H, s, CH_2), 5.02 (2H, s, CH_2), 6.86 (2H, br s, exchangeable, NH), 7.18–7.22 (1H, m, ArH), 7.25–7.30 (2H, m, 2 × ArH), 7.37–7.39 (1H, m, ArH), 7.46 (2H, d, J = 8.0 Hz, 2 × ArH), 7.55 (2H, d, J = 8.0 Hz, 2 × ArH). Anal. Calcd for ($C_{28}H_{32}ClN_3O_4$): C, 65.94; H, 6.32; N, 8.24. Found: C, 65.76; H, 6.17; N, 8.35.

4.8.5. [3-(3,4-Difluorophenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-diyl]bis(methylene)bis(*iso*-propylcarbamate) (23f)

Compound **23f** was synthesized from **20f** (0.5 g, 1.4 mmol), Et_3N (0.5 mL), and isopropylisocyanate (0.49 g, 5.8 mmol). Yield, 0.62 g (83%); mp 176–178 °C. ^{1}H NMR (DMSO-d_6) δ 1.02 (12H, d, J = 6.4 Hz, 4 × Me), 3.58 (2H, m, CH), 4.09 (2H, s, CH_2), 4.81 (2H, s, CH_2), 5.00 (2H, s, CH_2), 5.02 (2H, s, CH_2), 6.87 (2H, br s, exchangeable, NH), 7.19–7.21 (1H, m, ArH), 7.26–7.30 (3H, m, 3 × ArH), 7.37–7.39 (1H, m, ArH), 7.51–7.59 (2H, m, 2 × ArH). Anal. Calcld for ($C_{28}H_{31}F_2N_3O_4$): C, 65.74; H, 6.11; N, 8.21. Found: C, 65.51; H, 6.05; N, 8.38.

4.8.6. [3-(3,4-Dichlorophenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-diyl]bis(methylene)bis(*iso*-propylcarbamate) (23g)

Compound **23g** was synthesized from **20g** (0.5 g, 1.3 mmol), Et_3N (0.5 mL), and isopropylisocyanate (0.45 g, 5.3 mmol). Yield, 0.6 g (82%); mp 192–193 °C. ^{1}H NMR (DMSO-d_6) δ 1.01 (12H, d, J = 6.4 Hz, 4 × Me), 3.57 (2H, m, CH), 4.09 (2H, s, CH_2), 4.81 (2H, s, CH_2), 5.02 (4H, s, 2 × CH_2), 6.87 (2H, br s, exchangeable, NH), 7.19–7.21 (1H, m, ArH), 7.25–7.27 (2H, m, 2 × ArH), 7.30–7.37 (1H, m, ArH), 7.43–7.45 (1H, m, ArH), 7.69 (1H, s, ArH) 7.73–7.75 (1H, m, ArH). Anal. Calcd for ($C_{28}H_{31}N_3O_4Cl_2$): C, 61.77; H, 5.74; N, 7.72. Found: C, 61.84; H, 5.67; N, 7.56.

4.8.7. [3-(4-Methoxyphenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-diyl]bis(methylene)bis(*iso*-propylcarbamate) (23h)

Compound **23h** was synthesized from **20h** (0.5 g, 1.4 mmol), Et_3N (0.5 mL), and isopropylisocyanate (0.5 g, 5.9 mmol). Yield, 0.49 g (65%); mp 199–200 °C. ^{1}H NMR (DMSO-d_6) δ 1.00 (12H, d, J = 6.0 Hz, 4 × Me), 3.57 (2H, m, CH), 3.82 (3H, s, MeO), 4.07 (2H, s, CH_2), 4.77 (2H, s, CH_2), 4.92 (2H, s, CH_2), 5.01 (2H, s, CH_2), 6.85 (2H, br s, exchangeable, NH), 7.04–7.06 (2H, m, 2 × ArH), 7.17–7.21 (1H, m, ArH), 7.25–7.28 (2H, m, 2 × ArH), 7.34–7.36 (3H, m, 3 × ArH). Anal. Calcd for ($C_{29}H_{35}N_3O_5$): C, 68.89; H, 6.98; N, 8.31. Found: C, 68.93; H, 6.96; N, 8.14.

4.8.8. [3-(3,4-Dimethoxyphenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-diyl]bis(methylene) bis(*iso*-propylcarbamate) (23i)

Compound **23i** was synthesized from **20i** (0.37 g, 1.0 mmol), Et_3N (0.4 mL), and isopropylisocyanate (0.34 g, 4 mmol). Yield, 0.30 g (56%); mp 188–189 °C. ^{1}H NMR (DMSO-d_6) δ 1.01 (12H, d, J = 6.0 Hz, 4 × Me), 3.57 (2H, m, CH), 3.77 (3H, s, MeO), 3.82 (3H, s, MeO), 4.08 (2H, s, CH_2), 4.78 (2H, s, CH_2), 4.98 (2H, s, CH_2), 5.01 (2H, s, CH_2), 6.84 (2H, br s, exchangeable, NH), 6.95–6.97 (2H, m, 2 × ArH), 7.06–7.08 (1H, m, ArH), 7.18–7.21 (1H, m, ArH), 7.25–7.29 (2H, m, 2 × ArH), 7.30–7.37 (1H, m, ArH). Anal. Calcd for ($C_{30}H_{37}N_3O_6$): C, 67.27; H, 6.96; N, 7.84. Found: C, 67.27; H, 7.06; N, 7.62.

4.8.9. [3-(3,4,5-Trimethoxyphenyl)-5,10-dihydropyrrolo[1,2-*b*]isoquinoline-1,2-diyl]bis (methylene)bis(*iso*-propylcarbamate) (23j)

Compound **23j** was synthesized from **20j** (0.39 g, 1.0 mmol), Et_3N (0.4 mL), and isopropylisocyanate (0.34 g, 4 mmol). Yield, 0.32 g (57%); mp 189–190 °C. ^{1}H NMR (DMSO-d_6) δ 1.01 (12H, d, J = 6.4 Hz, 4 × Me), 3.57 (2H, m, CH), 3.73 (3H, s, MeO), 3.80 (6H, s, 2 × MeO), 4.08 (2H, s, CH_2), 4.79 (2H, s, CH_2), 5.01 (2H, s, CH_2), 5.05 (2H, s, CH_2), 6.69 (2H, s, 2 × ArH), 6.87 (2H, br s, exchangeable, NH), 7.19–7.29 (2H, m, 2 × ArH), 7.33–7.39 (2H, m, 2 × ArH). Anal. Calcld for ($C_{31}H_{39}N_3O_7$): C, 65.82; H, 6.95; N, 7.43. Found: C, 65.68; H, 6.74; N, 7.11.

4.9. Biological experiments

4.9.1. Cytotoxicity assays

The effects of the newly synthesized compounds on cell growth were determined in T-cell acute lymphocytic leukemia CCRF-CEM) and their resistant subcell lines (CCRF-CEM/Taxol and CCRF-CEM/VBL) by the XTT assay[20] and human solid tumor cells (i.e., breast carcinoma MX-1 and colon carcinoma HCT-116) the SRB assay[21] in a 72 h incubation using a microplate spectrophotometer as described previously.[22] After the addition of phenazine methosulfate-XTT

solution at 37 °C for 6 h, absorbance at 450 and 630 nm was detected on a microplate reader (EL 340; Bio-Tek Instruments Inc., Winooski, VT). The cytotoxicity of the newly synthesized compounds against non-small cell lung cancer H1299, human prostate cancer PC3, oral carcinoma OECM1 and human glioma U87 were determined by the Alamar blue assay[23] in a 72 h incubation using a microplate spectrophotometer as described previously. After the addition of Alamar blue solution, it was incubated at 37 °C for 6 h. Absorbance at 570 and 600 nm was detected on a microplate reader. IC_{50} values were determined from dose-effect relationship at six or seven concentrations of each drug by using the CompuSyn software by Chou and Martin[24] based on the median-effect principle and plot.[25,26] Ranges given for Taxol and vinblastine were mean ± SE (n = 4).

4.9.2. In vivo studies

Athymic nude mice bearing the nu/nu gene were used for human breast tumor MX-1 and human ovarian adenocarcinoma SK-OV-3 xenograft. Outbred Swiss-background mice were obtained from the National Cancer Institute (Frederick, MD). Male mice 8 weeks old or older weighing about 22 g were used for the experiments. Drug was administrated via the tail vein by iv injection.[22] Tumor volumes were assessed by measuring length × width × height (or width) by using caliper. Vehicle used was DMSO (50 μL) and Tween 80 (40 μL) in saline (160 μL). The maximal tolerable dose of the tested compound was determined and applied for the in vivo antitumor activity assay. All animal studies were conducted in accordance with the guidelines of the National Institutes of Health Guide for the Care and Use of Animals and the protocol approved by the Memorial Sloan-Kettering Cancer Center's Institutional Animal Care and Use Committee.

4.9.3. Alkaline agarose gel shift assay

Formation of DNA cross-linking was analyzed by alkaline agarose gel electrophoresis. In brief, purified pEGFP-N1 plasmid DNA (1.5 μg) was mixed with various concentrations (1–20 μM) of **20a**, **22a**, **20h** and **23h** in 40 μL 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 reaction, the plasmid DNA was linearized by digestion with *Bam*HI and followed by precipitation with ethanol. The DNA pellets were dissolved and denatured in alkaline buffer (0.5 N NaOH–10 mM EDTA). An aliquot of 20 μL of DNA solution (1 μg) was mixed with a 4 μL of 6 X alkaline loading dye and then electrophoretically resolved on a 0.8% alkaline agarose gel with NaOH–EDTA buffer at 4 °C. The electrophoresis was carried out at 18 V for 22 h. After staining the gels with an ethidium bromide solution, and the DNA was then visualized under UV light.

4.9.4. Flow cytometric analysis

The effects of **20a** on cell cycle distribution were analyzed with a flow cytometer as previously described.[12] Briefly, human non-small cell lung carcinoma H1299 cells were treated with **20a** at 1.25, 2.5, and 5 μM for 24 h. The attached cells were then trypsinized, washed with phosphate buffer saline (PBS), and fixed with ice-cold 70% ethanol for 30 min. The cells were stained with 4 μg/ml propidium iodide (PI) in PBS containing 1% Triton X-100 and 0.1 mg/ml RNase A. The stained cells were then analyzed using the FACS SCAN flow cytometer (Becton Dickinson, San Joes, CA, USA). The percentage of the cells in each cell cycle phase was determined using the ModFit LT 2.0 software based on the DNA histograms.

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