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Original article

Synthesis and antitumor evaluation of novel Benzo[*d*]pyrrolo[2,1-*b*]thiazole derivatives

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ABSTRACT

A series of novel 2,3-bis(hydroxymethyl)benzo[*d*]pyrrolo[2,1-*b*]thiazoles and their bis(alkylcarbamate) derivatives were synthesized starting from benzothiazole via reaction with dimethyl acetylenedicarboxylate (DMAD)/*tetra*-fluoro boric acid, catalytic hydrogenation, and alkylcarbamoylation. The antiproliferative activity of these agents against human leukemia and various solid tumor cell growth in vitro was studied. The structure–activity relationship studies revealed that the bis(alkylcarbamates) derivatives are generally more cytotoxic than the corresponding bis(hydroxymethyl) congeners in inhibiting human lymphoblastic leukemia CCRF-CEM and various human solid tumor cell growth in culture. These agents have no cross-resistance to taxol or vinblastine. Studies on the therapeutic effect against human breast carcinoma MX-1 xenograft showed that complete tumor remission (CR) were achieved by treating with C1-4′-F- or C1-4′-Cl-Ph-bis(*i*-propylcarbamates) derivatives (**19b** and **19c**, respectively) and more than 99% tumor suppression by the corresponding bis(ethylcarbamates) **18b** and **18c** at the maximal tolerated dose. Alkaline agarose gel shifting assay revealed that the newly synthesized compounds are able to induce DNA interstrand cross-linking. The present studies generated a series of new potent DNA interstrand cross-linking agents, which have potential for further antitumor drug development.

1. Introduction

The naturally occurring antibiotic antineoplastic Mitomycin C (MMC **1**, Fig. 1), isolated from *Streptomyces caespitosis*, is a bioreductive DNA bisalkylating agent [1–3]. This natural product can induce DNA cross-linking via bioreactivation by reductase under anaerobic conditions [4]. Thereby, this agent is effective for treating hypoxia tumors. Another antitumor natural product, retrorsine (**2**), which also contains pyrrolizidine pharmacophore, is able to induce DNA cross-linking by a similar mechanism of action as that of MMC [5]. However, the interaction of the pyrrolizidine alkaloids with DNA double strands can proceed without the catalyst of the reductase. Several synthetic compounds containing bis(hydroxymethyl)pyrrolidine or pyrrolizidine pharmacophore can serve as DNA bifunctional alkylating agents. These agents are thioimidazoles (i.e., carmethizole, **3**) [6], 3,4-bis(methylcarbamate) pyrroles (**4**) [7], 2,3-dihydroxy-6,7-bis(hydroxymethyl)pyrrolizines (IPP, **5**) [8], and their thio analogues 6,7-bis(hydroxymethyl)pyrrolo[1,2-*c*]thiazole (**6**) [9], and dihydropyrrolo[2,1-*b*]thiazole (**7**) [10]. Among these derivatives, carmethizole hydrochloride (**3**) was showed to have potent inhibitory activity against murine P388 leukemias. It is also reported that compound **5** has significant

Abbreviations: DMAD, dimethyl acetylenedicarboxylate; CR, complete tumor remission; MMC, Mitomycin C; ATO, arsenic trioxide; CCRF-CEM, human lymphoblastic leukemia; SAR, structure–activity relationship; MX-1, human breast carcinoma; SK-OV-3, ovarian adenocarcinoma; HCT-116, human colon carcinoma; H1299, human lung cancer; PC-3, human prostate adenocarcinoma; OECM-1, oral cancer; U87, glioma; H460, human large-cell lung carcinoma; (Q2D × 2), every other days; two times, (Q2D × 3); every other days, three times; (Q2D × 4), every other days; four times, (Q2D × 5); every other days, five times; DMF, *N*,*N*-dimethylformamide; THF, tetrahydrofuran; DMSO, dimethyl sulfoxide; PI, propidium iodide; PBS, phosphate buffered saline.

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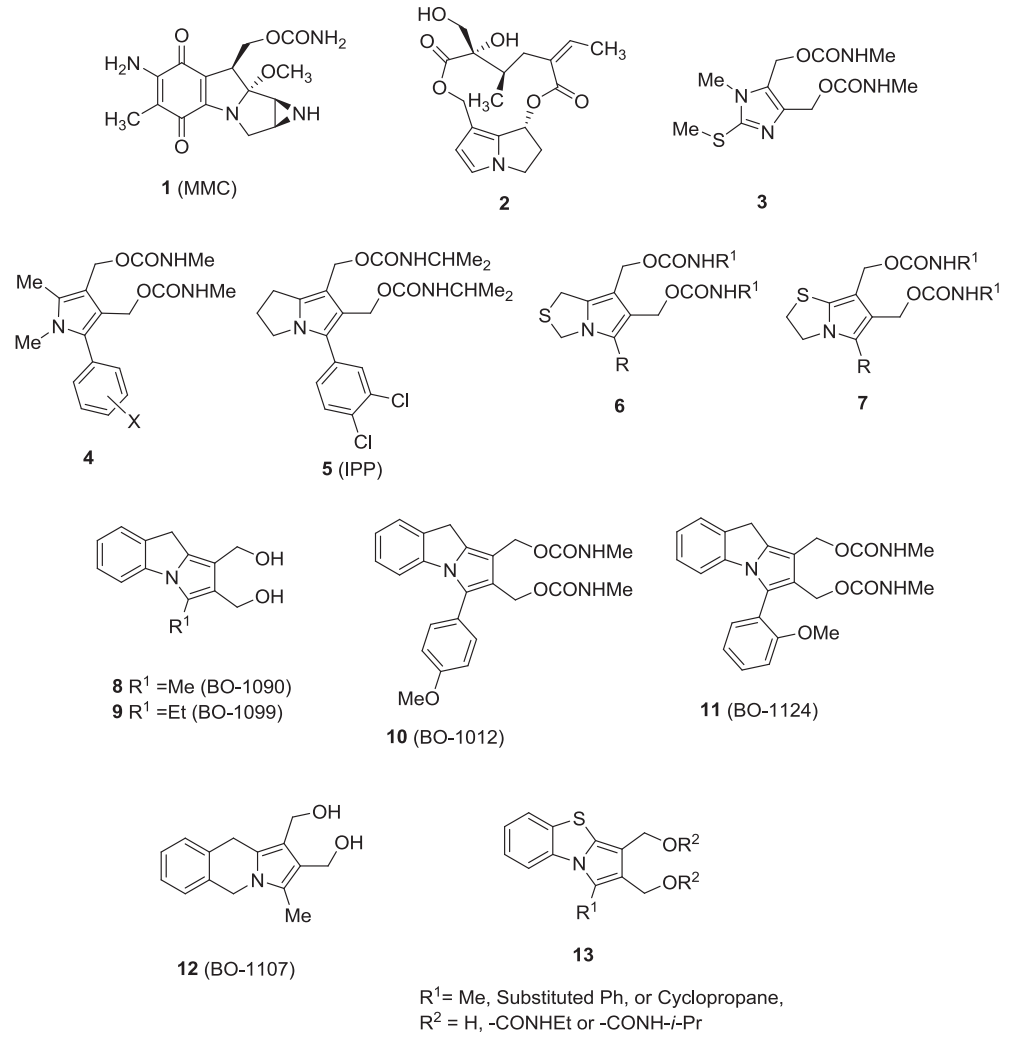


Fig. 1. Chemical structure of some DNA bifunctional alkylating agents.

antitumor activity against a broad range of experimental marine neoplasias and human tumor xenograft in nude athymic mice [8]. Lalezari *et. al.* reported that dihydropyrrolo[2,1-*b*]thiazole (**7**) was as cytotoxic as IPP [10].

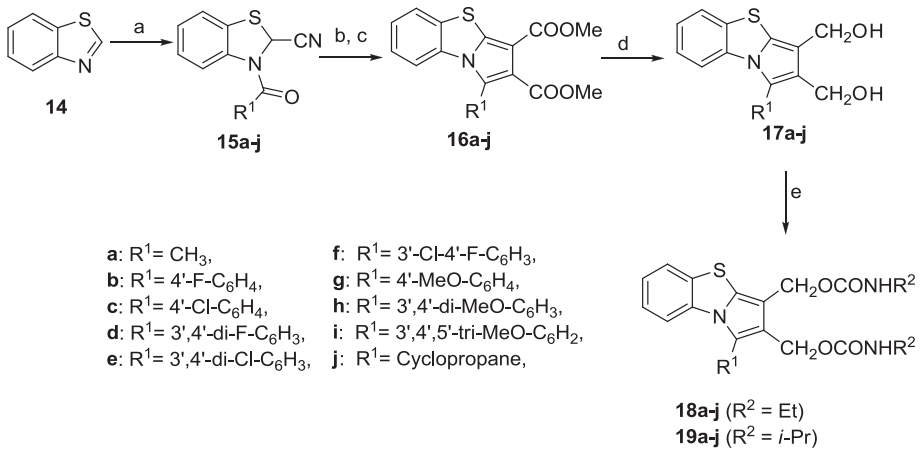
Recently, we have synthesized a series of bis(hydroxymethyl)-8*H*-3*a*-azacyclopenta[*a*]indene-1-yl and their bis(methylcarbamate) derivatives, which can considered as a "benzologue" derivatives of pyrrolizines (**5**) [11]. We have demonstrated that these agents possess significant cytotoxicity in inhibiting human lymphoblastic leukemia and a variety of human tumors in vitro and have potent therapeutic efficacy in xenograft model. For example, the bis(hydroxymethyl) derivatives [BO-1090 (**8**) and BO-1099 (**9**)] and bis(-methylcarbamates) derivatives, BO-1012 (**10**) and BO-1124 (**11**) are able to induce complete tumor remission (CR) in nude mice bearing human breast carcinoma MX-1 xenograft. Moreover, the bis(hydroxymethyl) derivatives, BO-1099 (**9**) significantly suppressed (>95%) human prostate adenocarcinoma PC-3 xenograft. Remarkably, we found that the combination treatment of BO-1012 (**10**) with arsenic trioxide (ATO, DNA repair inhibitor) achieved more than 82% and 92% tumor suppression in nude mice bearing human large-cell lung carcinoma H460 and cisplatin-resistant human bladder carcinoma NTUB1/P xenografts, respectively [12]. More recently, we have also synthesized a series of new bis(hydroxymethyl)pyrrolo[1,2-*b*] isoquinolines and their bis(alkylcarbamate) derivatives for antitumor evaluation [13]. Of these derivatives, BO-1107 (**12**) was shown to have potent antitumor activity against human breast carcinoma MX-1 and ovarian adenocarcinoma SK-OV-3 xenografts.

Study on the mechanism of action and the chemical properties of thioimidazoles (e.g. **3**, Fig. 1) or dihydropyrrolo[2,1-*b*]thiazole (**7**) suggested that the sulfur atom participates in the expulsion of the hydroxyl or carbamate moiety leading to the nucleophilic attack by DNA [14]. To continue our research and development of new bifunctional DNA-cross linking agents for antitumor application, we utilized the known benzo[*d*]pyrrolo[2,1-*b*]thiazole diesters [15,16] to prepare 2,3-bis(hydroxymethyl)-4*H*-benzo[*d*]pyrrolo[2,1-*b*]thiazoles and their bis(alkylcarbamate) derivatives (**13**, Fig. 1) for antitumor studies. These derivatives can be considered as the "benzologue" of dihydropyrrolo [2,1-*b*]thiazole (**7**). One can expect that the sulfur atom of compound **13** may also involve in the DNA cross-linking as shown in thioimidazoles (e.g. **3**, Fig. 1) or dihydropyrrolo[2,1-*b*]thiazole (**7**). Herein, we report the anti-proliferative activity against various human tumor cell growth in vitro, therapeutic efficacy in xenograft model, as well as the mechanism of actions of the new benzo[*d*]pyrrolo[2,1-*b*]thiazole analogues.

2. Results and discussion

2.1. Chemistry

The synthetic route for the preparation of (benzo[*d*]pyrrolo[2,1-*b*] thiazole- 2,3-diyl)dimethanol (**17a**–**j**) and their bis(alkylcarbamates)



Scheme 1. Reaction Condition: (a) Acid Chloride/$AlCl_3$, trimethylsilyl cynide/CH_2Cl_2, rt; (b) tetrafluoroboric acid in ether; (c) DMAD/DMF, ambient temperature; (d) $LiAlH_4$, CH_2Cl_2/ether 0 °C; (e) R_2NCO/TEA.

(**18a**–**j** and **19a**–**j**) is shown in Scheme 1. Compound **15a**–**j** was synthesized from the commercially available benzothiazole **14** according to the literature procedure [17]. By following the method developed by Berrabah et al., [15], compounds **15a**–**j** were treated with *tetra*-fluoro boric acid in ether, and directly further reacted with dimethyl acetylenedicarboxylate (DMAD) to yield the diester **16a**–**j** in low yields. It should be noted that many attempts have been made to synthesize compound **16** having a phenyl substituent at C1 (wherein R^1 = Ph) under various conditions failed; a gummy polymer with a complex mixture was obtained instead. The diester functions of **16a**–**j** were reduced to the corresponding bis-alcohol derivatives **17a**–**j** by reacting with $LiAlH_4$ in a mixture of ether/CH_2Cl_2 in an ice bath. Treatment of **17a**–**j** with ethyl or *iso*-propyl isocyanate in the presence of triethylamine afforded the desired bis(ethylcarbamate) or

Table 1
The cytotoxicity of newly synthesized 2,3-bis(hydroxymethyl)benzo[*d*]pyrrolo[2,1-*b*]thiazole (**17a**–**j**) and their bis(alkylcarbamate) derivatives (**18a**–**j** and **19a**–**j**) against human lymphoblastic leukemia (CCRF-CEM), its drug-resistant sublines (CCRF-CEM/Taxol and CCRF-CEM/VBL).

Compd.	R^1	R^2	Cell growth inhibition (IC_{50} μM)[a]		
			CCRF-CEM	CCRF-CEM/Taxol[b]	CCRF-CEM/VBL[b]
17a	Me	H	0.97 ± 0.03	1.88 ± 0.01 [1.93 ×][c]	1.86 ± 0.01 [1.91 ×]
17b	4′-F-C_6H_4	H	1.06 ± 0.04	2.56 ± 0.01 [2.41 ×]	3.72 ± 0.18 [3.50 ×]
17c	4′-Cl-C_6H_4	H	2.61 ± 0.03	5.22 ± 0.03 [2.00 ×]	4.09 ± 0.03 [1.56 ×]
17d	3′,4′-di-F-C_6H_3	H	0.19 ± 0.02	0.19 ± 0.01 [1.00 ×]	0.28 ± 0.01 [1.47 ×]
17e	3′,4′-di-Cl-C_6H_3	H	1.04 ± 0.02	ND[d]	ND
17f	3′-Cl–4′–F-C_6H_3	H	3.09 ± 0.04	ND	ND
17g	4′-MeO-C_6H_4	H	1.13 ± 0.09	2.17 ± 0.02 [1.9 ×]	2.63 ± 0.03 [2.30 ×]
17h	3′,4′- di-MeO-C_6H_3	H	1.82 ± 0.03	ND	ND
17i	3′,4′,5′-tri-MeO-C_6H_2	H	4.03 ± 0.02	ND	ND
17j	Cyclopropane	H	0.97 ± 0.07	2.31 ± 0.01 [2.37 ×]	2.44 ± 0.01 [2.51 ×]
18a	Me	Et	0.33 ± 0.01	0.45 ± 0.01 [1.30 ×]	0.43 ± 0.01 [1.30 ×]
18b	4′-F-C_6H_4	Et	0.07 ± 0.0002	0.38 ± 0.01 [5.42 ×]	0.45 ± 0.03 [6.42 ×]
18c	4′-Cl-C_6H_4	Et	0.21 ± 0.01	0.32 ± 0.01 [1.52 ×]	0.39 ± 0.01 [1.85 ×]
18d	3′,4′-di-F-C_6H_3	Et	0.21 ± 0.01	0.34 ± 0.01 [1.61 ×]	0.31 ± 0.01 [1.47 ×]
18e	3′,4′-di-Cl-C_6H_3	Et	0.38 ± 0.001	ND	ND
18f	3′-Cl–4′–F-C_6H_3	Et	0.38 ± 0.01	ND	ND
18g	4′-MeO-C_6H_4	Et	0.19 ± 0.002	0.37 ± 0.01 [1.90 ×]	0.42 ± 0.01 [2.20 ×]
18h	3′,4′- di-MeO-C_6H_3	Et	0.49 ± 0.003	ND	ND
18i	3′,4′,5′-tri-MeO-C_6H_2	Et	0.32 ± 0.001	ND	ND
18j	Cyclopropane	Et	0.17 ± 0.03	0.68 ± 0.01 [4.01 ×]	0.54 ± 0.01 [3.18 ×]
19a	Me	*i*-Pr	0.13 ± 0.01	0.22 ± 0.01 [1,69 ×]	0.25 ± 0.01 [1.92 ×]
19b	4′-F-C_6H_4	*i*-Pr	0.05 ± 0.001	0.16 ± 0.01 [3.20 ×]	0.19 ± 0.01 [3,80 ×]
19c	4′-Cl-C_6H_4	*i*-Pr	0.08 ± 0.0003	0.39 ± 0.01 [4.87 ×]	0.42 ± 0.01 [5.25 ×]
19d	3′,4′-di-F-C_6H_3	*i*-Pr	0.33 ± 0.001	0.19 ± 0.01 [0.57 ×]	0.30 ± 0.01 [0.90 ×]
19e	3′,4′-di-Cl-C_6H_3	*i*-Pr	2.29 ± 0.04	ND	ND
19f	3′-Cl–4′–F-C_6H_3	*i*-Pr	0.50 ± 0.02	ND	ND
19g	4′-MeO-C_6H_4	*i*-Pr	0.14 ± 0.002	0.170.01 [1.17 ×]	0.25 ± 0.01 [1.47 ×]
19h	3′,4′- di-MeO-C_6H_3	*i*-Pr	0.11 ± 0.02	ND	ND
19i	3′,4′,5′-tri-MeO-C_6H_2	*i*-Pr	1.05 ± 0.03	ND	ND
19j	Cyclopropane	*i*-Pr	0.10 ± 0.01	0.73 ± 0.01 [7.30 ×]	0.90 ± 0.01 [9.00 ×]
Vinblastine			0.0007 ± 0.0001	0.08 ± 0.01 [106 ×]	0.50 ± 0.12 [680 ×]
Taxol			0.0012 ± 0.0001	0.43 ± 0.04 [358 ×]	1.27 ± 0.05 [980 ×]

[a] Data represent the mean ± SD of each compound from three to six independent experiments.
[b] CCRF-CEM/VBL and CCRF-CEM/Taxol are sub-cell lines of CCRF-CEM cells with 680-fold resistance to vinblastine and 358-fold resistance to taxol, respectively, relative to the IC_{50} value of the parent cell line.
[c] Numbers in bracket are measures of cross-resistance as determined by comparison with the corresponding IC_{50} value of the parent cell line.
[d] Not determined.

Table 2
The cytotoxicity of benzo[*d*]pyrrolo[2,1-*b*]thiazole derivatives against human solid tumors (breast carcinoma MX-1, colon carcinoma HCT-116, lung carcinoma H1299, prostate carcinoma PC-3, oral carcinoma OECM1 and glioma U87) cell growth in vitro.

Compd	IC_{50} (μM)[a]					
	MX-1	HCT-116	H1299	PC3	OECM-1	U87
17a	7.10 ± 0.037	4.44 ± 0.08	ND[b]	ND	ND	ND
17b	2.77 ± 0.005	9.59 ± 0.19	9.27 ± 1.69	13.78 ± 2.15	6.03 ± 1.20	15.46 ± 1.31
17c	10.40 ± 0.003	9.26 ± 0.049	12.43 ± 0.44	20.41 ± 3.43	14.60 ± 1.13	20.66 ± 2.26
17d	ND	ND	21.35 ± 3.74	15.22 ± 1.86	8.48 ± 0.67	17.36 ± 1.86
17g	ND	ND	9.94 ± 1.87	8.81 ± 1.20	5.00 ± 0.78	18.10 ± 3.71
17j	ND	ND	13.32 ± 2.23	10.74 ± 2.29	7.12 ± 0.95	28.70 ± 4.32
18b	0.48 ± 0.005	0.52 ± 0.025	13.55 ± 2.03	23.70 ± 3.05	11.47 ± 2.63	29.24 ± 2.63
18c	0.91 ± 0.01	0.70 ± 0.008	4.33 ± 0.45	13.85 ± 3.78	11.61 ± 2.84	26.01 ± 2.27
18d	1.49 ± 0.04	1.10 ± 0.015	10.33 ± 1.11	19.27 ± 3.44	14.60 ± 3.39	38.48 ± 7.21
18g	ND	ND	8.85 ± 1.53	17.90 ± 3.59	9.39 ± 1.90	29.47 ± 4.86
18j	ND	ND	20.64 ± 1.89	24.19 ± 2.96	26.28 ± 4.44	74.50 ± 8.70
19a	0.13 ± 0.001	0.97 ± 0.018	9.70 ± 1.10	ND	ND	ND
19b	0.62 ± 0.014	0.35 ± 0.010	33.03 ± 3.20	24.19 ± 4.17	11.89 ± 2.90	53.50 ± 9.97
19c	1.19 ± 0.004	1.70 ± 0.006	8.75 ± 0.88	13.71 ± 1.28	7.54 ± 0.95	29.04 ± 4.49
19d	1.00 ± 0.002	0.41 ± 0.005	30.75 ± 6.92	29.56 ± 1.83	15.10 ± 1.34	74.85 ± 15.69
19g	ND	ND	8.19 ± 1.74	17.49 ± 0.80	7.23 ± 1.57	60.88 ± 13.43
19j	ND	ND	20.44 ± 1.65	13.38 ± 0.84	9.67 ± 1.06	45.49 ± 6.19
Cisplatin	ND	ND	16.53 ± 0.90	4.71 ± 0.66	2.44 ± 0.53	54.57 ± 3.33

[a] Data represent the mean ± SD of each compound from three to six independent experiments.
[b] Not determined.

bis(*iso*-propylcarbamate) derivatives (**18a**–**j** and **19a**–**j**, respectively) in good yield.

2.2. Biological activity

2.2.1. In vitro cytotoxicity

In our anticancer agent screening program, we use human lymphoblastic leukemia (CCRF-CEM) and its drug-resistant sublines resistant to Taxol (CCRF-CEM/Taxol) and Vinblastine (CCRF-CEM/VBL) cell lines for evaluating the primary anti-proliferative activity and structure–activity relationship (SAR) studies of the tested compound. Those compounds warranted in vitro cytotoxicity against these cancer cell lines are further selected for evaluating their anti-proliferative activity against other human solid tumor cell growth in culture. Table 1 shows proliferative activities of the newly synthesized bis(hydroxylmethyl)benzo[*d*]pyrrolo[2,1-*b*]thiazole derivatives (**17a**–**j**), bis(ethylcarbamates) (**18a**–**j**), and bis(*iso*-propylcarbamates) derivatives (**19a**–**j**) against CCRF-CEM and CCRF-CEM/Taxol and CCRF-CEM/VBL cell growth in vitro. Generally, the bis(alkylcarbamate) derivatives (**18a**–**j** or **19a**–**j**) are more potent than the corresponding bis(hydroxymethyl) derivatives (**17a**–**j**). In the series of bis(hydroxymethyl) derivatives (**17a**–**j**), compound having 3′,4′-di-F-Ph (**17d**) at C1 is the most potent derivative with IC_{50} value 0.19 μM against CCRF/CEM cell growth in culture. While, C1-Me (**17a**), 4′-F-Ph (**17b**), 3′,4′-di-Cl-Ph (**17e**), 4′-OMe-Ph (**17g**), cyclopropyl (**17j**) substituted derivatives are equally potent, but somewhat more cytotoxic than other phenyl substituted derivatives. In the C1-halo substituted phenyl derivatives, 4′-F-Ph (**17b**) is 2-fold more potent than 4′-Cl-Ph (**17c**), but the order of the potency for di-halo substituted derivatives is 3′,4′-di-F-Ph (**17d**) > 3′,4′-di-Cl-Ph (**17e**) > 3′-Cl,4′-F-Ph (**17f**). While, in the series of compounds having an electron-donating MeO function on the C1-Ph derivatives, the cytotoxicity decreases when the number of the MeO function increases [e. g. 4′-MeO-Ph (**17g**) > 3′,4′-$(MeO)_2$-Ph (**17h**) > 3′,4′,5′-$(MeO)_3$-Ph (**17i**)].

In comparison with the cytotoxicity of bis(ethylcarbamates) (**18a**–**j**) and bis(*iso*-propylcarbamates) (**19a**–**j**), one can see that the latter congeners are more or equal cytotoxic than the former derivatives. The SAR study of bis(alkylcarbamates) derivatives reveals that the order of their potency is almost as same as that of the bis(hydroxymethyl) derivatives. Of these derivatives, compounds **18b**, **19b**, and **19c**, were shown to have most significant cytotoxicity with IC_{50} values of 0.07, 0.05 and 0.08 μM, respectively, against CCRF-CEM cell growth in culture.

Our previous research on the SAR studies of 1,2-bis(hydroxylmethyl)cyclopenta[*a*]indenes and their counterparts

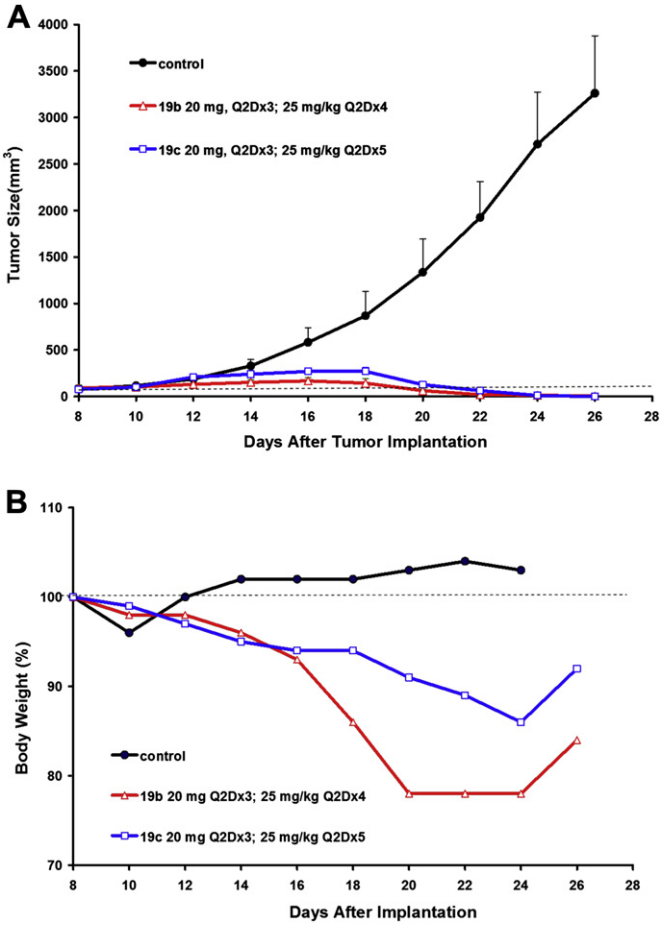


Fig. 2. Therapeutic effects of **19b** and **19c** in nude mice bearing MX-1 human mammary xenograft (*i.v.* inj., $n = 3$, $p < 0.01$). A: average tumor size changes. B: average body weight changes.

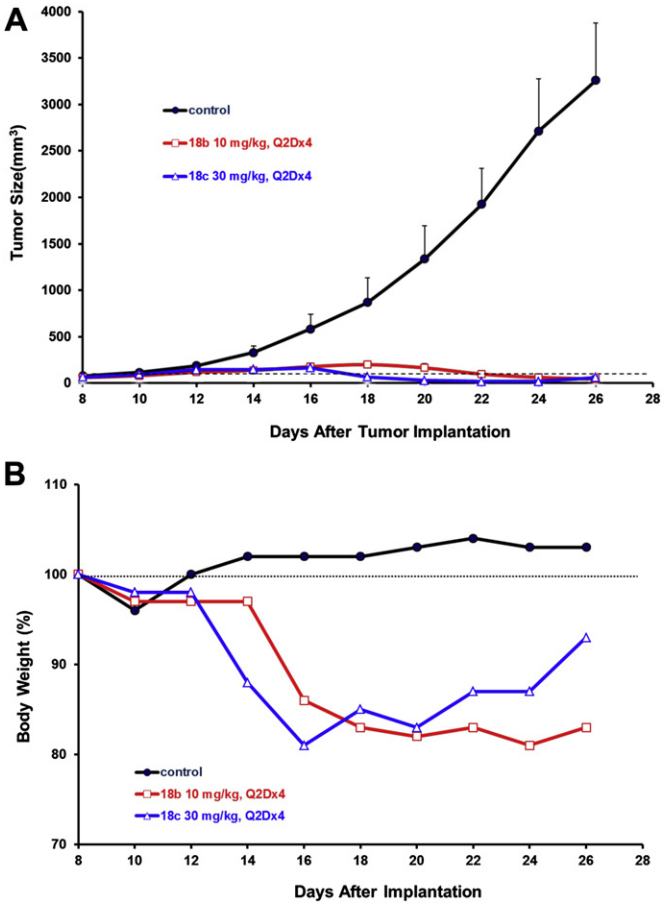


Fig. 3. Therapeutic effects of **18b** and **18c** in nude mice bearing MX-1 human mammary xenograft (*i.v.* inj., $n = 3$, $p < 0.01$). A: average tumor size changes. B: average body weight changes.

1,2-bis(methylcarbamate) derivatives (**8**–**11**, Fig. 1) demonstrated that the size and the electron property of the substituents at the C1 position affected the cytotoxicity of these agents [11]. More recently, our studies on the SAR of pyrrolo[1,2-*b*]isoquinolines reveal that their cytotoxicity is mainly affected by the size of the substituents at the C1 position rather than the electron properties, although the C1-methoxyphenyl derivatives are somewhat more cytotoxic than the corresponding halophenyl compounds [13]. In the present studies, however, we found that the halophenyl derivatives are generally have better cytotoxicity than the MeO substituted phenyl counterparts. It indicates that the electron properties of the substituent(s) on the phenyl ring have little influence over their potency. The size of the substituent on C1 affects the cytotoxicity of the bis(hydroxymethyl) derivatives, but this does not happen in the bis(alkylcarbamate) derivatives.

Our previous report demonstrated that cyclopenta[*a*]indenes (e.g. **8**–**11**) or bis(hydroxylmethyl)pyrrolo[1,2-*b*]isoquinolines (e.g. **12**) did not have multi-drug resistance to antitumor agents such as Taxol and Vinblastine [11,13]. To realize whether the newly synthesized pyrrolo[1,2-*b*]isoquinoline derivatives are cross-resistant to these two natural products, we evaluated their cytotoxicity against CCRF-CEM/Taxol and CCRF-CEM/VBL, which are 330-fold resistant to Taxol, and 680-fold resistant to Vinblastine, respectively. As shown in Table 1, the newly synthesized benzo[*d*]-pyrrolo[2,1-*b*]thiazole derivatives 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.

On the basis of the in vitro anti-proliferative activity (IC_{50} values) against CCRF/CEM cell lines, the selected compounds were further examined for their anti-proliferative activity against a variety of human solid tumor cell growth in vitro as shown in Table 2. The results showed that bis(alkylcarbamates) derivatives are generally more active than bis(hydroxymethyl) derivatives against human breast cancer MX-1 and colon cancer HCT-116. It also revealed that the tested compounds are generally more cytotoxic against MX-1 and HCT-116 than other tested tumor cell lines. Over all, we found that most of compounds are more potent than the positive control cisplatin in inhibiting human lung cancer H1299 and glioma U87 cell lines. It also showed that several derivatives have significant anti-proliferative activity against human lung H1299, prostate PC-3, oral cancer OECM-1, and glioma U87 cell line growth in vitro. However, it is unable to conclude a SAR among the tested compounds and tumor cell lines examined.

2.2.2. *In vivo antitumor activity*

To further investigate the antitumor activity of benzo[*d*]pyrrolo[2,1-*b*]thiazole derivatives, we selected compounds **18b**, **18c**, **19b** and **19c** for evaluating their therapeutic efficacy in human tumor xenograft models, since these agents exhibit a broad spectrum of antitumor activity in vitro. The results showed that complete tumor remission was observed in all three tested mice subcutaneously implanted with MX-1 xenograft when treated with **19c** at the submaximal tolerated dose of 20 mg/kg, every other days, three times (Q2D × 3) and then 25 mg/kg [Q2D × 5, intravenous injection (iv. inj.)] on D24, D26, and D26, respectively, (Fig. 2A). When the same tumor bearing mice were treated with **19b** (20 mg/kg, Q2D × 3 and then 25 mg/kg, Q2D × 4, iv. inj.), we found that complete tumor remission was seen in two out of three mice on

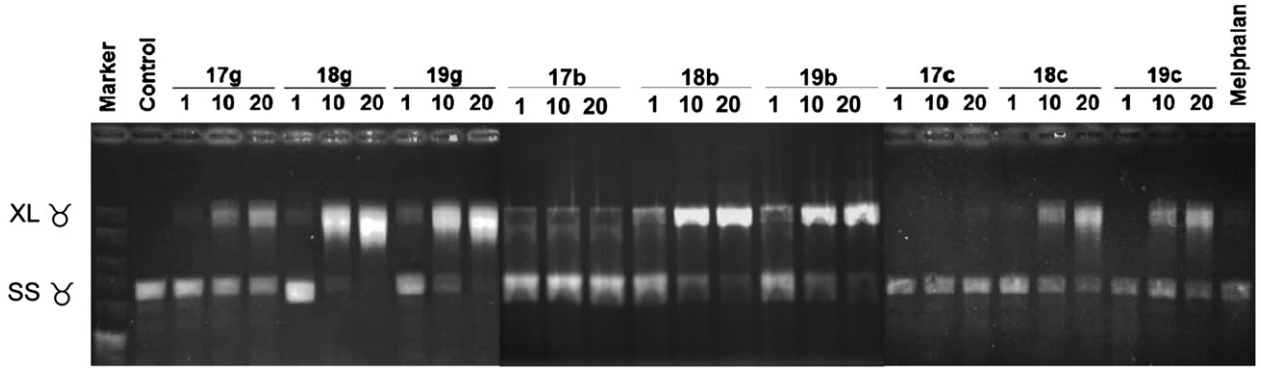


Fig. 4. Representative DNA cross-linking gel shift assay for bis(hydroxymethyl) derivatives (**17b**, **17c** and **17g**) and their corresponding bis(alkylcarbamate) derivatives (**18b**, **18c**, **18g**, **19b**, **19c** and **19g**) at the concentrations of 1, 10, and 20 μM. Melphalan (1 μM) was used as a positive control. XL stands for cross-linking DNA, and SS refers to single strand DNA.

D26 and 28 (Fig. 2A). In another experiment, it revealed that more than 99% tumor suppression was achieved when the tumor bearing mice were treated with **18b** (10 mg/kg, Q2D × 4) or **18c** (30 mg/kg, Q2D × 4) via iv. inj. (Fig. 3). As shown in Fig. 2B and Fig. 3B, one can see that the tested mice had about 20% average body weight loss during the drug treatment, with an exception of **19c**, which caused about 10% body weight loss. However, the body weight recovered after ceasing of treatment, indicating that the test compounds have a little toxicity to the host.

2.2.3. DNA cross-linking study

Our previous reports demonstrated that bis(hydroxymethyl)-8*H*-3*a*-azacyclopenta[*a*]indene-1-yl or bis(hydroxymethyl)pyrrolo[1,2-*b*]isoquinolines and their bis(methylcarbamate) derivatives are able to induce DNA interstrand cross-linking. To realize whether the newly synthesized compounds are also capable of cross-linking with DNA, linearized pBR322 DNA was treated with bis(hydroxymethyl) derivatives (**17b**, **17c** and **17g**) and their corresponding bis(alkylcarbamate) derivatives (**18b**, **18c**, **18g**, **19b**, **19c** and **19g**) at various concentrations (1, 10, and 20 μM) using alkaline agarose gel shifting assay. Melphalan (1 μM) was used as the positive control. As revealed in Fig. 4, it revealed that bis(hydroxymethyl) derivatives **17b, 17c** and **17g** are much less efficient DNA-cross-linking agent than bis(alkylcarbamate) compounds **18b**, **18c**, **18g**, 1**9b**, **19c** and **19g**, probably due to the latter derivatives exhibit better leaving groups (e.g., alkylcarbamoyl function) than the former (e.g., hydroxymethyl group). It should be noted to show that **17b** and **17c** are weak cross-linking agents, but they are as cytotoxic as **18g** in inhibiting various solid tumors in culture (see Table 2). These observations suggests, in addition to DNA cross-linking, other mechanism of action may be involved. Other possible mechanism of action of bis(hydroxymethyl) derivatives (such as **17c**) will be elucidated latter.

2.2.4. Cell cycle inhibition

Previously, we demonstrated that 3*a*-aza-cyclopenta[*a*]indenes are able to induce cell cycle perturbation in H1299 cells [11]. We found that the S phase cell accumulation was observed when the tested cells were treated with the bis(methylcarbamates) derivatives at 24 h and followed by the increase of the G2/M phase at 48 h and 72 h. Additionally, increased sub-G1 population were noticed at 72 h. To investigate whether the newly synthesized benzo[*d*]pyrrolo[2,1-*b*]thiazole derivatives are able to interfere with cell cycle progression, H1299 cells were treated with compound **17c**, **18c**, and **19c** at 0.5-, 1- and 2-fold of IC_{50} dose for 24 h and 48 h respectively. Then the cell cycle distributions were analyzed by flow cytometry. From the cell cycle data (Fig. 5), we observed that **17c** at the highest dose used (25 μM) reduced the G1 population at 24 h, but resulted in increased accumulation of the G1 phase at 48 h. These results revealed that high dose of **17c** slightly delay the cell cycle progression at G2/M first and then resulted in G1 arrest. While **18c** treated cells notably accumulate in sub-G1 and G2/M phase but reduced G1 population was observed after 24 h and 48 h by a dose dependent manner. Similarly, it also revealed that, the cell progression was arrested at G2/M phase after treating with the **19c** after 24 h at doses of 4.5 and 9 μM. After 48 h, the G2/M population was decreased whereas the sub-G1 population increased, indicating that the progress of disturbed cell cycle resulted in cell death. However, **19c** at 18 μM apparently inhibited the cell cycle progression after 24 h treatment, while the cell cycle progression was resumed to result in increased G2/M population as well as sub-G1 population. These results implicated that cell cycle disturbance by **18c** and **19c** play crucial role in triggering cell death. Consistent to our results shown in Fig. 4, the weak induction of DNA interstrand cross-linking as well as cell cycle progression of **17c** as compared to **18c** and **19c** suggests that **17c** may have different mode of action from compounds **18c** and **19c**.

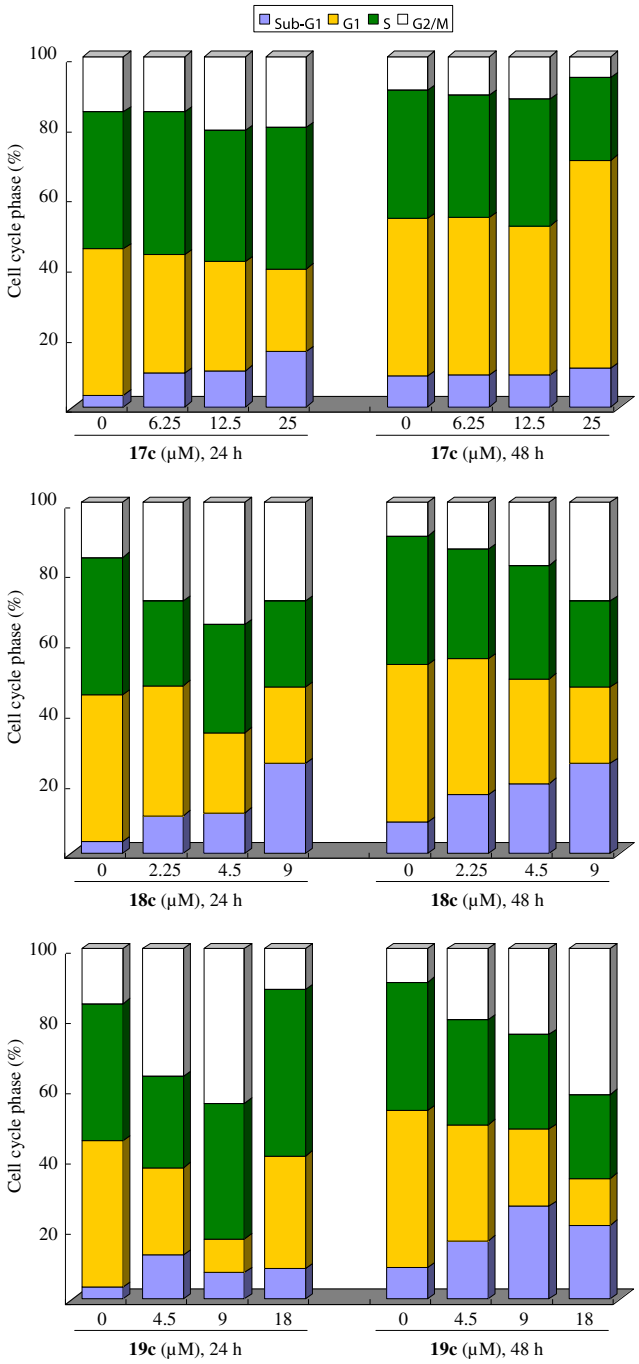


Fig. 5. Effect of compounds on cell cycle progression in human non-small cell lung adenocarcinoma H1299 cells. The cells were cultured without compound (control), with **17c** (6.25 μM, 12.5 μM, 25 μM), **18c** (2.25 μM, 4.5 μM, 9 μM), and **19c** (4.5 μM, 9.0 μM, 18 μM) for 24 h and 48 h. Then cells were subjected for cell cycle analysis with a flow cytometer.

3. Conclusions

In the present studies we have synthesized a series of substituted 2,3-bis(hydroxymethyl)-4*H*-benzo[*d*]pyrrolo[2,1-*b*]thiazoles (**17a**–**j**) and their bis(alkylcarbamate) derivatives (**18a**–**j** and **19a**–**j**) for antitumor studies. A variety of substituent(s) were introduced to the C1 position of the pyrrole ring for studying their

SAR. The preliminary antitumor studies revealed that these agents exhibited significant antitumor activity in inhibiting various human tumor cell growth in vitro with no or a little cross-resistance to either Taxol or Vinblastine. Among the newly synthesized derivatives, compounds **18b**, **19b**, and **19c** were found to have potent therapeutic efficacy against MX-1 in xenograft model, which was used as a primary drug screening model for selecting lead compounds for further evaluation of anticancer activity against other solid tumor xenografts in our Drug Development Program. Based on the results of preliminary therapeutic efficacy studies, compound **19c** will be selected as a lead compound for studying its anticancer activity alone or in combination with DNA repair inhibitors in inhibiting human solid tumors, which are resistant to chemotherapeutic agents, in xenograft model and the results will be reported in a due course.

4. Experimental protocols

4.1. Material and methods

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 was performed on silica gel G60 F254 (Merck) with short-wavelength UV light for visualization. Elemental analysis was done on a Heraeus CHN–O Rapid instrument. High-performance liquid chromatography was performed on Elite instrument: column, Mightysil RP-18 (250 × 4.6 mm). Compounds were detected by UV at 260 nm. The mobile phase was MeCN/THF (50:50 v/v) with 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 Bruker AVANCE 600 DRX and 400 MHz, Bruker Top-Spin spectrometers in the solvents indicated. The Proton chemical shifts were reported in parts per million (δ ppm) relative to $(CH_3)_4Si$ and coupling constants (J) in Hertz (Hz) and s, d, t, m, br s, refer to singlet, doublet, triplet, multiplet, broad respectively.

4.2. Chemistry: general methods

4.2.1. 3-Acetyl-2,3-dihydrobenzo[d]thiazole-2-carbonitrile (**15a**)

Acetyl chloride (4.0 g, 51.0 mmol) was added drop wise to a stirring solution of benzothiazole (6.8 g, 50.0 mmol) in dichloromethane (40 mL) under argon atmosphere. A catalytic amount of $AlCl_3$ and trimethylsilylcyanide (5.1 g, 52.0 mmol) were then added into the reaction mixture. After being stirred for 17 h at room temperature, the reaction mixture was evaporated to dryness in vacuo and the residue was crystallized from ether to give **15a**. Yield: 8.2 g (80%); mp: 92–93 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 2.39 (s, 3H, Me), 7.05 (s, 1H, ArH), 7.16–7.26 (m, 2H, 2 × ArH), 7.49–7.51 (m, 1H, ArH), 7.65 ppm (br s, 1H, C2–H); ^{13}C NMR ([D$_6$]DMSO): δ = 22.6, 52.6, 117.4, 118.4, 123.5, 125.8, 126.3, 131.2, 134.5, 168.8 ppm; Anal. calcd for $C_{10}H_8N_2OS$: C 58.80, H 3.95, N 13.72, S 15.70, found: C 58.85, H 3.95, N 13.78, S 15.76.

By following the same synthetic procedure as that for **15a**, the following compounds were synthesized:

4.2.2. 3-(4-Fluorobenzoyl)-2,3-dihydrobenzo[d]thiazole-2-carbonitrile (**15b**)

Compound **15b** was prepared from **14** (5.4 g, 40.0 mmol), 4-fluorobenzoyl chloride (6.5 g, 41.0 mmol), and trimethylsilylcyanide (4.1 g, 42.0 mmol). Yield: 9.3 g (82%); mp: 150–151 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 6.71 (br s, 1H, C2–H), 6.85 (s, 1H, ArH), 7.04–7.07 (m, 1H, ArH), 7.12–7.16 (m, 1H, ArH), 7.38–7.40 (m, 2H, 2 × ArH), 7.52–7.54 (m, 1H, ArH), 7.64–7.67 ppm (m, 2H, 2 × ArH); ^{13}C NMR ([D$_6$]DMSO): δ = 54.5, 116.0, 116.2, 117.5, 118.7, 123.9, 125.9, 128.1, 129.7, 130.8, 130.9, 136.3, 163.1, 165.0, 166.9 ppm; Anal. calcd for $C_{15}H_9FN_2OS$: C 63.37, H 3.19, N 9.85, S 11.28, found: C 63.02, H 3.27, N 9.81, S 11.31.

4.2.3. 3-(4-Chlorobenzoyl)-2,3-dihydrobenzo[d]thiazole-2-carbonitrile (**15c**)

Compound **15c** was prepared from **14** (5.4 g, 40.0 mmol), 4-chlorobenzoyl chloride (7.2 g, 41.0 mmol), and trimethylsilylcyanide (4.1 g, 42.0 mmol); Yield: 10.1 g (84%); mp: 115–117 °C (lit.: 115–118 °C [18]); ^{1}H NMR ([D$_6$]DMSO): δ = 6.75 (br s, 1H, C2-H), 6.85 (s, 1H, ArH), 7.05–7.09 (m, 1H, ArH), 7.13–7.17 (m, 1H, ArH), 7.52–7.54 (m, 1H, ArH), 7.58–7.61 ppm (m, 4H, 4 × ArH); ^{13}C NMR ([D$_6$]DMSO): δ = 54.4, 117.5, 118.8, 124.0, 125.9, 126.0, 128.2, 129.1, 130.0, 132.0, 136.2, 136.8, 166.9 ppm.

4.2.4. 3-(3,4-Difluorobenzoyl)-2,3-dihydrobenzo[d]thiazole-2-carbonitrile (**15d**)

Compound **15d** was prepared from **14** (6.8 g, 50.0 mmol), 3,4-difluorobenzoyl chloride (9.0 g, 51.0 mmol), and trimethylsilylcyanide (5.1 g, 52.0 mmol); Yield: 11.9 g (79%); mp: 149–150 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 6.77 (br s, 1H, C2–H), 6.84 (s, 1H, ArH), 7.06–7.10 (m, 1H, ArH), 7.13–7.17 (m, 1H, ArH), 7.45 (m, 1H, ArH), 7.52–7.54 (m, 1H, ArH), 7.58–7.65 (m, 1H, ArH), 7.73–7.76 ppm (m, 1H, ArH); ^{13}C NMR ([D$_6$]DMSO): δ = 54.3, 117.4, 117.9, 118.1, 118.3, 118.4, 118.8, 123.9, 125.6, 125.9, 126.0, 128.2, 130.7, 136.0, 148.3, 148.4, 150.3, 150.4, 150.5, 150.6, 152.5, 152.6, 165.7 ppm; Anal. calcd for $C_{15}H_8F_2N_2OS$: C 59.60, H 2.67, N 9.27, S 10.61, found: C 59.24, H 2.90, N 9.17, S 10.53.

4.2.5. 3-(3,4-Dichlorobenzoyl)-2,3-dihydrobenzo[d]thiazole-2-carbonitrile (**15e**)

Compound **15e** was prepared from **14** (6.8 g, 50.0 mmol), 3,4-dichlorobenzoyl chloride (10.4 g, 51.0 mmol), and trimethylsilylcyanide (5.1 g, 52.0 mmol); Yield: 13.5 g (81%); mp: 166–167 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 6.84 (br s, 2H, C2–H and ArH), 7.08–7.11 (m, 1H, ArH), 7.14–7.18 (m, 1H, ArH), 7.52–7.54 (m, 2H, 2 × ArH), 7.80–7.82 (m, 1H, ArH), 7.88–7.90 ppm (m, 1H, ArH); ^{13}C NMR ([D$_6$]DMSO): δ = 54.3, 117.4., 118.9, 123.9, 125.9, 126.2, 128.1, 128.2, 130.2, 131.2, 131.8, 133.7, 134.7, 135.9, 165.5 ppm; Anal. calcd for $C_{15}H_8Cl_2N_2OS$: C 53.75, H 2.41, N 8.36, S 9.57, found: C 53.55, H 2.50, N 8.31, S 9.18.

4.2.6. 3-(3-Chloro-4-fluorobenzoyl)-2,3-dihydrobenzo[d]thiazole-2-carbonitrile (**15f**)

Compound **15f** was prepared from **14** (6.8 g, 50.0 mmol), 3-chloro-4-fluorobenzoyl chloride (9.9 g, 51.0 mmol), and trimethylsilylcyanide (5.1 g, 52.0 mmol); Yield: 13.2 g (83%); mp: 187–188 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 6.81(br s, 1H, C2–H), 6.84 (s, 1H, ArH), 7.06–7.10 (m, 1H, ArH), 7.13–7.17 (m, 1H, ArH), 7.52–7.56 (m, 1H, ArH), 7.58–7.60 (m, 2H, 2 × ArH), 7.86–7.88 ppm (m, 1H, ArH); ^{13}C NMR ([D$_6$]DMSO): δ = 54.8, 117.8, 117.9, 118.1, 119.3, 120.8, 120.9, 124.4, 126.4, 126.5, 128.7, 129.6, 129.7, 131.3, 131.5, 136.5, 158.6, 160.6, 166.1 ppm; Anal. calcd for $C_{15}H_8ClFN_2OS$: C 56.52, H 2.53, N 8.79, S 10.06, found: C 56.41, H 2.92, N 8.78, S 9.69.

4.2.7. 3-(4-Methoxybenzoyl)-2,3-dihydrobenzo[d]thiazole-2-carbonitrile (**15g**)

Compound **15g** was prepared from **14** (5.4 g, 40.0 mmol), 4-methoxybenzoyl chloride (7.0 g, 41.0 mmol), and trimethylsilylcyanide (4.1 g, 42.0 mmol); Yield: 8.2 g (70%); mp: 122–123 °C (lit.: 122–123.5 °C [18]); ^{1}H NMR ([D$_6$]DMSO): δ = 3.82 (s, 3H, MeO), 6.72 (br s, 1H, C2–H), 6.84 (s, 1H, ArH), 7.01–7.14 (m, 4H, 4 × ArH), 7.52–7.55 ppm (m, 3H, 3 × ArH); ^{13}C NMR ([D$_6$]DMSO): δ = 54.8, 55.5, 114.2, 117.7, 118.6, 123.9, 124.9, 125.6, 125.8, 128.0, 130.3, 136.7, 162.3, 167.6 ppm .

*4.2.8. 3-(3,4-Dimethoxybenzoyl)-2,3-dihydrobenzo[d]thiazole-2-carbonitrile (**15h**)*

Compound **15h** was prepared from **14** (6.8 g, 50.0 mmol), 3,4-dimethoxybenzoyl chloride (10.3 g, 51.0 mmol), and trimethylsilylcyanide (5.1 g, 52.0 mmol); Yield: 10.4 g (64%); mp: 219–220 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 3.76 (s, 3H, MeO), 3.84 (s, 3H, MeO), 6.82 (br s, 2H, C2–H and ArH), 7.07–7.11 (m, 2H, 2 × ArH), 7.12–7.17 (m, 3H, 3 × ArH), 7.51–7.53 ppm (m, 1H, ArH); ^{13}C NMR ([D$_6$]DMSO): δ = 54.9, 55.5, 55.7, 111.3, 117.8, 118.9, 121.6, 123.9, 124.9, 125.7, 125.8, 128.0, 136.7, 148.6, 151.9, 167.7 ppm; Anal. calcd for $C_{17}H_{14}N_2O_3S$: C 62.56, H 4.32, N 8.58, S 9.82, found: C 62.31, H 4.25, N 8.39, S 9.68.

*4.2.9. 3-(3,4,5-Trimethoxybenzoyl)-2,3-dihydrobenzo[d]thiazole-2-carbonitrile (**15i**)*

Compound **15i** was prepared from **14** (6.8 g, 50.0 mmol), 3,4,5-trimethoxybenzoyl chloride (11.5 g, 51.0 mmol), and trimethylsilylcyanide (5.1 g, 52.0 mmol); Yield: 15.8 g (88%); mp: 189–190 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 3.75 (s, 3H, MeO), 3.78 (s, 6H, 2 × MeO),6.81 (s, 1H, ArH), 6.91 (s, 2H, 2 × ArH), 7.02 (br s, 1H, C2–H), 7.13–7.20 (m, 2H, 2 × ArH), 7.51–7.54 ppm (m, 1H, ArH); ^{13}C NMR ([D$_6$]DMSO): δ = 54.8, 56.1, 60.2, 105.4, 117.9, 119.4, 123.7, 125.9, 126.1, 128.2, 128.5, 136.4, 140.3, 153.1, 167.7 ppm; Anal. calcd for $C_{18}H_{16}N_2O_4S$: C 60.66, H 4.53, N 7.86, S 9.00, found: C 60.36, H 4.45, N 7.76, S 9.11.

*4.2.10. 3-(Cyclopropanecarbonyl)-2,3-dihydrobenzo[d]thiazole-2-carbonitrile (**15j**)*

Compound **15j** was prepared from **14** (6.8 g, 50.0 mmol), cyclopropylcarbonyl chloride (5.2 g, 51.0 mmol), and trimethylsilylcyanide (5.1 g, 52.0 mmol); Yield: 6.3 g (55%); mp: 113–114 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 0.97 (m, 2H, CH$_2$), 1.15 (m, 2H, CH$_2$), 2.13 (m, 1H, CH), 7.17–7.27 (m, 3H, 3 × ArH), 7.52–7.54 (m, 1H, ArH), 7.74 ppm (br s, 1H, C2–H); ^{13}C NMR ([D$_6$]DMSO): δ = 8.8, 9.8, 13.4, 52.8, 117.5, 118.4, 123.7, 125.7, 126.4, 128.2, 136.3, 171.4 ppm; Anal. calcd for $C_{12}H_{10}N_2OS$: C 62.59, H 4.38, N 12.16, S 13.92, found: C 62.56, H 4.29, N 12.26, S 13.93.

*4.2.11. Dimethyl 1-methylbenzo[d]pyrrolo[2,1-b]thiazole-2,3-dicarboxylate (**16a**)*

To a solution of **15a** (5.0 g, 24.5 mmol) in dichloromethane (50 mL) was added drop wise 7 mL of tetrafluoroboric acid (HBF$_4$). The solution was stirred for 3 h at room temperature. The brown precipitates appeared were collected by filtration and the filter cake was washed with ether to give hydrofluoroborate salt. The solid salt was added to a solution of dimethyl acetylenedicarboxylate (10.4 g, 73.0 mmol) in DMF (30 mL) and then warmed at 35 °C for 13 h. The reaction mixture was concentrated in vacuo and the residue was crystallized from methanol to give **16a**. Yield: 1.7 g (23%); mp: 143–144 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 2.85 (s, 3H, Me), 3.83 (s, 3H, COOMe), 3.85 (s, 3H, COOMe), 7.50 (t, J = 7.6 Hz, 1H, ArH), 7.58 (t, J = 7.6 Hz, 1H, ArH), 8.07 (d, J = 8.0 Hz, 1H, ArH), 8.12 ppm (d, J = 8.0 Hz, 1H, ArH); ^{13}C NMR ([D$_6$]DMSO): δ = 12.2, 51.4, 51.7, 102.9, 114.7, 117.6, 124.5, 125.2, 126.4, 128.9, 131.2, 134.2, 136.1, 162.4, 164.6 ppm; Anal. calcd for $C_{15}H_{13}NO_4S$: C 59.39, H 4.32, N 4.62, S 10.57, found: C 59.02, H 4.23, N 4.87, S 10.42.

By following the same synthetic procedure as that for **16a**, the following compounds were synthesized:

*4.2.12. Dimethyl 1-(4-fluorophenyl)benzo[d]pyrrolo[2,1-b]thiazole-2,3-dicarboxylate (**16b**)*

Compound **16b** was prepared from **15b** (8.1 g, 28.0 mmol), HBF$_4$ (8 mL), and DMAD (12.2 g, 84.0 mmol); Yield: 2.5 g (23%); mp: 186–187 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 3.63 (s, 3H, COOMe), 3.83 (s, 3H, COOMe), 6.78–6.80 (m, 1H, ArH), 7.28–7.30 (m, 1H, ArH), 7.30–7.44 (m, 3H, 3 × ArH), 7.62–7.66 (m, 2H, 2 × ArH), 8.03–8.05 ppm (m, 1H, ArH); ^{13}C NMR ([D$_6$]DMSO): δ = 51.6, 51.9, 103.4, 113.8, 115.7, 115.9, 120.1, 124.9, 125.4, 126.3, 128.3, 131.4, 133.0, 133.1, 133.7, 136.9, 161.9, 162.4, 163.8, 164.0 ppm; Anal. calcd for $C_{20}H_{14}FNO_4S$: C 62.65, H 3.68, N 3.65, S 8.36, found: C 62.68, H 3.46, N 3.62, S 8.76.

*4.2.13. Dimethyl 1-(4-chlorophenyl)benzo[d]pyrrolo[2,1-b]thiazole-2,3-dicarboxylate (**16c**)*

Compound **16c** was prepared from **15c** (9.1 g, 30.0 mmol), HBF$_4$ (9 mL), and DMAD (12.7 g, 90.0 mmol); Yield: 2.5 g (21%); mp: 172–173 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 3.64 (s, 3H, COOMe), 3.83 (s, 3H, COOMe), 6.85–6.87 (m, 1H, ArH), 7.30–7.42 (m, 2H, ArH), 7.60–7.66 (m, 4H, 4 × ArH), 8.04–8.06 ppm (m, 1H, ArH); ^{13}C NMR ([D$_6$]DMSO): δ = 51.7, 52.0, 103.5, 133.9, 120.3, 125.0, 125.4, 126.3, 127.9, 128.0, 128.9, 131.4, 132.4, 133.7, 134.6, 137.1, 162.4, 164.0 ppm; Anal. calcd for $C_{20}H_{14}ClNO_4S \cdot 0.5H_2O$: C 58.75, H 3.70, N 3.43, S 7.84, found: C 58.76, H 3.45, N 3.59, S 7.73.

*4.2.14. Dimethyl 1-(3,4-difluorophenyl)benzo[d]pyrrolo[2,1-b]thiazole-2,3-dicarboxylate (**16d**)*

Compound **16d** was prepared from **15d** (10.2 g, 33.0 mmol), HBF$_4$ (10 mL), and DMAD (14.1 g, 99.0 mmol); Yield: 3.8 g (28%); mp: 183–184 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 3.65 (s, 3H, COOMe), 3.83 (s, 3H, COOMe), 6.86–6.88 (m, 1H, ArH), 7.31–7.35 (m, 1H, ArH), 7.40–7.44 (m, 2H, 2 × ArH), 7.61–7.68 (m, 1H, ArH), 7.73–7.78 (m, 1H, ArH), 8.04–8.06 ppm (m, 1H, ArH); ^{13}C NMR ([D$_6$]DMSO): δ = 51.8, 52.4, 113.7, 118.6, 122.3, 122.5, 125.6, 125.9, 126.7, 127.0, 134.6, 135.6, 148.2, 149.6, 152.2, 156.4, 163.7, 165.0 ppm; Anal. calcd for $C_{20}H_{13}F_2NO_4S$: C 59.85, H 3.26, N 3.49, S 7.99, found: C 59.56, H 3.53, N 3.32, S 8.12.

*4.2.15. Dimethyl 1-(3,4-dichlorophenyl)benzo[d]pyrrolo[2,1-b]thiazole-2,3-dicarboxylate (**16e**)*

Compound **16e** was prepared from **15e** (12.1 g, 35.9 mmol), HBF$_4$ (11 mL), and DMAD (15.2 g, 107.0 mmol); Yield: 5 g (32%); mp: 198–199 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 3.64 (s, 3H, COOMe), 3.81 (s, 3H, COOMe), 6.88–6.90 (m, 1H, ArH), 7.31–7.42 (m, 2H, 2 × ArH), 7.57–7.60 (m, 1H, ArH), 7.81–7.83 (m, 1H, ArH), 7.91 (s, 1H, ArH), 8.03–8.05 ppm (m, 1H, ArH); ^{13}C NMR ([D$_6$]DMSO): δ = 51.7, 52.0, 103.7, 114.0, 120.4, 124.8, 125.4, 126.4, 126.8, 129.6, 130.9, 131.4, 132.5, 132.7, 133.6, 137.5, 162.3, 163.8 ppm; Anal. calcd for $C_{20}H_{13}Cl_2NO_4S$: C 55.31, H 3.02, N 3.23, S 7.38, found: C 55.36, H 3.02, N 3.13, S 7.35.

*4.2.16. Dimethyl 1-(3-chloro-4-fluorophenyl)benzo[d]pyrrolo[2,1-b]thiazole-2,3-dicarboxylate (**16f**)*

Compound **16f** was prepared from **15f** (10.0 g, 31.4 mmol), HBF$_4$ (9 mL), and DMAD (13.3 g, 94.0 mmol); Yield: 2.2 g (17%); mp: 196–197 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 3.65 (s, 3H, COOMe), 3.83 (s, 3H, COOMe), 6.83–6.85 (m, 1H, ArH), 7.32–7.43 (m, 2H, 2 × ArH), 7.62–7.64 (m, 2H, 2 × ArH), 7.89–7.90 (m, 1H, ArH), 8.05–8.07 ppm (m, 1H, ArH); ^{13}C NMR ([D$_6$]DMSO): δ = 51.6, 51.9, 103.6, 114.0, 117.2, 117.4, 119.8, 119.9, 120.3, 124.9, 125.5, 126.4, 126.7, 128.8, 127.1, 131.3, 131.7, 131.8, 133.0, 133.7, 137.2, 157.2, 158.8, 162.4, 163.8 ppm; Anal. calcd for $C_{20}H_{13}ClFNO_4S$: C 57.49, H 3.14, N 3.35, S 7.67, found: C 57.79, H 3.16, N 3.55, S 7.41.

*4.2.17. Dimethyl 1-(4-methoxyphenyl)benzo[d]pyrrolo[2,1-b]thiazole-2,3-dicarboxylate (**16g**)*

Compound **16g** was prepared from **15g** (5.3 g, 17.9 mmol), HBF$_4$ (5 mL), and DMAD (7.6 g, 54.0 mmol); Yield: 1.7 g (24%); mp: 167–168 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 3.63 (s, 3H, COOMe), 3.82 (s, 3H, COOMe), 3.86 (s, 3H, Me), 6.84–6.86 (m, 1H, ArH), 7.12 (d, J = 8.4 Hz, 2H, 2 × ArH), 7.28–7.32 (m, 1H, ArH), 7.37–7.40 (m, 1H, ArH), 7.48 (d, J = 8.4 Hz, 2H, 2 × ArH), 8.03–8.05 ppm (m, 1H, ArH);

^{13}C NMR ([D$_6$]DMSO): δ = 52.0, 52.3, 55.7, 103.7, 114.3, 144.6, 120.4, 121.3, 125.3, 125.7, 126.6, 129.6, 131.8, 132.5, 134.3, 136.8, 160.6, 163.0, 164.7 ppm; Anal. calcd for $C_{21}H_{17}NO_5S$: C 63.79, H 4.33, N 3.54, S 8.11, found: C 63.81, H 4.33, N 3.55, S 8.10.

4.2.18. Dimethyl 1-(3,4-dimethoxyphenyl)benzo[d]pyrrolo[2,1-b] thiazole-2,3-dicarboxylate (**16h**)

Compound **16h** was prepared from **15h** (5.8 g, 17.7 mmol), HBF_4 (5 mL), and DMAD (7.5 g, 53.0 mmol); Yield: 2.3 g (30%); mp: 226–227 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 3.65 (s, 3H, COOMe), 3.73 (s, 3H, Me), 3.82 (s, 3H, COOMe), 3.86 (s, 3H, Me), 6.93–6.95 (m, 1H, ArH), 7.07–7.16 (m, 3H, 3 × ArH), 7.30–7.34 (m, 1H, ArH), 7.37–7.41 (m, 1H, ArH), 8.03–8.05 ppm (m, 1H, ArH); ^{13}C NMR ([D$_6$]DMSO): δ = 51.6, 51.9, 55.5, 55.6, 103.2, 111.5, 113.9, 114.1, 119.9, 120.8, 123.2, 124.8, 125.3, 126.2, 129.2, 131.3, 133.9, 136.3, 148.4, 149.7, 162.5, 164.3 ppm; Anal. calcd for $C_{22}H_{19}NO_6S$: C 62.11, H 4.50, N 3.29, S 7.54, found: C 62.12, H 4.44, N 3.38, S 7.48.

4.2.19. Dimethyl 1-(3,4,5-trimethoxyphenyl)benzo[d]pyrrolo[2,1-b] thiazole-2,3-dicarboxylate (**16i**)

Compound **16i** was prepared from **15i** (10.2 g, 28.0 mmol), HBF_4 (8 mL), and DMAD (11.9 g, 84.0 mmol); Yield: 2.8 g (22%); mp: 244–245 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 3.69 (s, 3H, COOMe), 3.76 (s, 6H, 2 × Me), 3.79 (s, 3H, Me), 3.83 (s, 3H, COOMe), 6.89 (s, 2H, 2 × ArH), 7.03–7.05 (m, 1H, ArH), 7.35–7.43 (m, 2H, 2 × ArH), 8.03–8.05 ppm (m, 1H, ArH); ^{13}C NMR ([D$_6$]DMSO): δ = 51.6, 51.9, 53.8, 56.1, 60.2, 103.3, 108.5, 114.3, 119.9, 123.9, 124.7, 125.3, 126.2, 129.0, 131.3, 133.8, 136.5, 138.4, 152.8, 162.4, 164.3 ppm; Anal. calcd for $C_{23}H_{21}NO_7S$: C 60.65, H 4.65, N 3.08, S 7.04; found: C 60.38, H 4.67, N 3.15, S 7.13.

4.2.20. Dimethyl 1-cyclopropylbenzo[d]pyrrolo[2,1-b]thiazole-2,3-dicarboxylate (**16j**)

Compound **16j** was prepared from **15j** (5.6 g, 24.3 mmol), HBF_4 (7 mL), and DMAD (10.3 g, 73.0 mmol); Yield: 1.8 g (22%); mp: 157–158 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 0.71 (m, 2H, CH_2), 1.13 (m, 2H, CH_2), 2.26 (m, 1H, CH), 3.77 (s, 3H, COOMe), 3.82 (s, 3H, COOMe), 7.45–7.49 (m, 1H, ArH), 7.55–7.59 (m, 1H, ArH), 8.03–8.05 (m, 1H, ArH), 8.36–8.38 ppm (m, 1H, ArH); ^{13}C NMR ([D$_6$]DMSO): δ = 7.1, 51.4, 52.1, 103.0, 115.2, 119.8, 124.4, 125.0, 126.4, 130.2, 131.2, 134.2, 135.2, 162.3, 165.2 ppm; Anal. calcd for $C_{17}H_{15}NO_4S$: C 61.99, H 4.59, N 4.25, S 9.74, found: C 61.78, H 4.48, N 4.29, S 9.66.

4.2.21. [1-Methylbenzo[d]pyrrolo[2,1-b]thiazole-2,3-diyl] dimethanol (**17a**)

A solution of **16a** (2.0 g, 6.6 mmol) in anhydrous dichloromethane (30 mL) was added drop wise to a stirred mixture of lithium aluminum hydride (0.6 g, 16.0 mmol) in anhydrous ether (20 mL) at −5 °C to 0 °C. The reaction mixture was allowed to stir at this temperature for 20 min. The excess hydride was decomposed by adding water (1 mL) followed by NH_4OH (1 mL) and water (1 mL) at −5 °C to 0 °C. The mixture was filtered through a pad of Celite, washed with several times with dichloromethane. The combined filtrate and washings were washed successively with brine solution and water. The organic layer was dried (Na_2SO_4) and concentrated to dryness in vacuo. The residue was crystallizes from ether to give **17a**. Yield: 1.3 g (82%); mp: 138–139 °C; ^{1}H NMR ([D$_6$] DMSO): δ = 2.63 (s, 3H, Me), 4.39 (d, J = 5.2 2H, Hz, CH_2), 4.56 (d, J = 5.2 Hz, 2H, CH_2), 4.60 (t, J = 5.6 Hz, 1H, exchangeable, OH), 4.97 (t, J = 5.6 Hz, 1H, exchangeable, OH), 7.21–7.25 (m, 1H, ArH), 7.34–7.38 (m, 1H, ArH), 7.76–7.78 (m, 1H, ArH), 7.83–7.85 ppm (m, 1H, ArH); ^{13}C NMR (125 MHz [D$_6$]DMSO): δ = 11.9, 54.4, 56.1, 113.0, 113.6, 121.4, 122.3, 123.5, 124.4, 124.8, 125.9, 131.6, 135.6 ppm; Anal. calcd for $C_{13}H_{13}NO_2S$: C 63.13, H 5.30, N 5.66, S 12.97, found: C 62.94, H 5.24, N 5.83, S 12.90.

By following the same synthetic procedure as that for **17a**, the following compounds were synthesized:

4.2.22. [1-(4-Fluorophenyl)benzo[d]pyrrolo[2,1-b]thiazole-2,3-diyl] dimethanol (**17b**)

Compound **17b** was prepared from **16b** (2.4 g, 6.6 mmol) and $LiAlH_4$ (0.6 g, 16.0 mmol); Yield: 1.5 g (76%); mp: 155–156 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 4.22 (d, J = 5.1 Hz, 2H, CH_2), 4.66 (d, J = 5.1 Hz, 2H, CH_2), 4.73 (t, J = 5.1 Hz, 1H, exchangeable, OH), 5.13 (t, J = 5.1 Hz, 1H, exchangeable, OH), 6.83–6.85 (m, 1H, ArH), 7.15–7.19 (m, 2H, 2 × ArH), 7.37–7.41 (m, 2H, 2 × ArH), 7.53–7.57 (m, 2H, 2 × ArH), 7.79–7.81 ppm (m, 1H, ArH); ^{13}C NMR ([D$_6$]DMSO): δ = 54.7, 56.1, 112.7, 114.7, 115.9, 123.9, 123.5, 124.4, 124.7, 125.6, 126.8, 127.8, 131.8, 133.2, 133.3, 134.8 ppm; Anal. calcd for $C_{18}H_{14}FNO_2S$: C 66.04, H 4.31, N 4.28, S 9.79, found: C 66.25, H 4.22, N 4.14, S 9.76.

4.2.23. [1-(4-Chlorophenyl)benzo[d]pyrrolo[2,1-b]thiazole-2,3-diyl]dimethanol (**17c**)

Compound **17c** was prepared from **16c** (2.1 g, 5.0 mmol) and $LiAlH_4$ (0.45 g, 12.5 mmol); Yield: 1.5 g (88%); mp: 151–152 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 4.22 (d, J = 4.4 Hz, 2H, CH_2), 4.66 (d, J = 4.4 Hz, 2H, CH_2), 4.76 (t, J = 5.2 Hz, 1H, exchangeable, OH), 5.14 (t, J = 5.2 Hz, 1H, exchangeable, OH), 6.91–6.93 (m, 1H, ArH), 7.16–7.22 (m, 2H, 2 × ArH), 7.53 (d, J = 8.4 Hz, 2H, 2 × ArH), 7.61 (d, J = 8.4 Hz, 2H, 2 × ArH), 7.79–7.82 ppm (m, 1H, ArH); ^{13}C NMR ([D$_6$]DMSO): δ = 54.6, 56.1, 112.9, 114.9, 124.0, 124.2, 124.8, 125.5, 125.7, 127.0, 129.1, 130.3, 131.8, 132.7, 133.5, 134.8 ppm; Anal. calcd for $C_{18}H_{14}ClNO_2S$: C 62.88, H 4.10, N 4.07, S 9.33, found: C 62.73, H 4.09, N 4.06, S 9.19.

4.2.24. [1-(3,4-Difluorophenyl)benzo[d]pyrrolo[2,1-b]thiazole-2,3-diyl]dimethanol (**17d**)

Compound **17d** was prepared from **16d** (1.6 g, 4.0 mmol) and $LiAlH_4$ (0.37 g, 10.0 mmol); Yield: 1.1 g (83%); mp: 154–155 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 4.22 (d, 2H, J = 4.6 Hz, CH_2), 4.66 (d, J = 4.6 Hz, 2H, CH_2), 4.76 (t, J = 5.0 Hz, 1H, exchangeable, OH), 5.14 (t, J = 5.0 Hz, 1H, exchangeable, OH), 6.91–6.93 (m, 1H, ArH), 7.18–7.21 (m, 2H, 2 × ArH), 7.37 (m, 1H, ArH), 7.58–7.65 (m, 2H, 2 × ArH), 7.81–7.83 ppm (m, 1H, ArH); ^{13}C NMR ([D$_6$]DMSO): δ = 54.6, 56.0, 112.9, 114.8, 118.1, 120.0, 123.3, 124.0, 124.7, 125.8, 127.2, 128.1, 128.2, 128.8, 131.8, 134.7, 148.7, 150.7 ppm; Anal. calcd for $C_{18}H_{13}F_2NO_2S$: C 62.60, H 3.79, N 4.06, S 9.28, found: C 62.46, H 3.84, N 3.90, S 9.16.

4.2.25. [1-(3,4-Dichlorophenyl)benzo[d]pyrrolo[2,1-b]thiazole-2,3-diyl]dimethanol (**17e**)

Compound **17e** was prepared from **16e** (4.0 g, 9.2 mmol) and $LiAlH_4$ (0.85 g, 23.0 mmol); Yield: 2.8 g (84%); mp: 159–160 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 4.23 (s, 2H, CH_2), 4.66 (s, 2H, CH_2), 4.83 (br s, 1H, exchangeable, OH), 5.16 (br s, 1H, exchangeable, OH), 6.95–6.99 (m, 1H, ArH), 7.19–7.24 (m, 2H, 2 × ArH), 7.49–7.52 (m, 1H, ArH), 7.78–7.84 ppm (m, 3H, 3 × ArH); ^{13}C NMR ([D$_6$]DMSO): δ = 54.5, 56.0, 113.0, 115.1, 122.9, 124.1, 124.8, 125.8, 126.2, 127.6, 131.0, 131.1, 131.3, 131.7, 131.8, 132.0, 132.3, 134.7 ppm; Anal. calcd for $C_{18}H_{13}Cl_2NO_2S$: C 57.15, H 3.46, N 3.70, S 8.48, found: C 57.20, H 3.48, N 3.66, S 8.33.

4.2.26. [1-(3-chloro-4-fluorophenyl)benzo[d]pyrrolo[2,1-b] thiazole-2,3-diyl]dimethanol (**17f**)

Compound **17f** was prepared from **16f** (2.0 g, 4.7 mmol) and $LiAlH_4$ (0.45 g, 12.0 mmol); Yield: 1.3 g (75%); mp: 178–179 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 4.22 (d, J = 4.4 Hz, 2H, CH_2), 4.65 (d, J = 4.4 Hz, 2H, CH_2), 4.78 (t, J = 4.8 Hz, 1H, exchangeable, OH), 5.14 (t, J = 4.8 Hz, 1H, exchangeable, OH), 6.89–6.91 (m, 1H, ArH), 7.17–7.23 (m, 2H, 2 × ArH), 7.51–7.53 (m, 1H, ArH), 7.57–7.62 (m, 1H, ArH), 7.75–7.77 (m, 1H, ArH), 7.80–7.82 ppm (m, 1H, ArH); ^{13}C

NMR ([D$_6$]DMSO): δ = 54.6, 56.0, 112.8, 114.8, 117.6, 120.0, 123.1, 124.0, 124.8, 125.6, 125.8, 127.3, 129.2, 131.8, 132.8, 134.7 ppm; Anal. calcd for $C_{18}H_{13}ClFNO_2S$: C 59.75, H 3.62, N 3.87, S 8.86, found: C 59.72, H 3.61, N 3.86, S 8.81.

*4.2.27. [1-(4-Methoxyphenyl)benzo[d]pyrrolo[2,1-b]thiazole-2,3-diyl]dimethanol (**17g**)*

Compound **17g** was prepared from **16g** (1.7 g, 4.3 mmol) and $LiAlH_4$ (0.4 g, 10.0 mmol); Yield: 1.3 g (91%); mp: 156–157 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 3.85 (s, 3H, MeO), 4.22 (d, J = 4.8 Hz, 2H, CH_2), 4.66 (m, 3H, CH_2 and exchangeable OH), 5.09 (t, J = 4.8 Hz, 1H, exchangeable, OH), 6.85–6.87 (m, 1H, ArH), 7.08–7.19 (m, 4H, 4 × ArH), 7.39–7.42 (m, 2H, 2 × ArH), 7.76–7.79 ppm (m, 1H, ArH); ^{13}C NMR ([D$_6$]DMSO): δ = 54.8, 55.7, 56.2, 112.8, 114.4, 114.5, 123.5, 123.8, 124.3, 124.6, 125.5, 125.6, 126.4, 131.8, 132.4, 135.0, 159.7 ppm; Anal. calcd for $C_{19}H_{17}NO_3S$: C 67.24, H 5.05, N 4.13, S 9.45, found: C 67.54, H 5.00, N 4.00, S 9.30.

*4.2.28. [1-(3,4-Dimethoxyphenyl)benzo[d]pyrrolo[2,1-b]thiazole-2,3-diyl]dimethanol (**17h**)*

Compound **17h** was prepared from **16h** (2.2 g, 5.0 mmol) and $LiAlH_4$ (0.5 g, 13.0 mmol); Yield: 1.7 g (91%); mp: 215–216 °C ^{1}H NMR ([D$_6$]DMSO): δ = 3.74 (s, 3H, OMe), 3.85 (s, 3H, MeO), 4.25 (s, 2H, CH_2), 4.66 (br s, 3H, CH_2 and exchangeable OH), 5.09 (br s, 1H, exchangeable, OH), 6.93–6.95 (m, 1H, ArH), 7.01–7.06 (m, 2H, 2 × ArH), 7.10–7.18 (m, 3H, 3 × ArH), 7.76–7.78 ppm (m, 1H, ArH); ^{13}C NMR ([D$_6$]DMSO): δ = 54.9, 55.9, 56.0, 56.2, 112.0, 113.0, 114.5, 114.7, 123.5, 123.6, 123.8, 124.3, 124.6, 125.5, 125.7, 126.4, 131.8, 135.0, 148.9, 149.4 ppm; Anal. calcd for $C_{20}H_{19}NO_4S\cdot 0.5H_2O$: C 63.47, H 5.33, N 3.70, S 8.47, found: C 63.37, H 5.13, N 3.67, S 8.28.

*4.2.29. [1-(3,4,5-Trimethoxyphenyl)benzo[d]pyrrolo[2,1-b]thiazole-2,3-diyl]dimethanol (**17i**)*

Compound **17i** was prepared from **16i** (2.4 g, 5.2 mmol) and $LiAlH_4$ (0.5 g, 13.0 mmol); Yield: 1.5 g (74%); mp: 160–161 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 3.76 (s, 9H, 3 × MeO), 4.26 (d, J = 4.4 Hz, 2H, CH_2), 4.66 (d, J = 4.4 Hz, 2H, CH_2), 4.71 (t, J = 4.8 Hz, 1H, exchangeable, OH), 5.09 (t, J = 4.8 Hz, 1H, exchangeable, OH), 6.80 (s, 2H, 2 × ArH), 7.03–7.05 (m, 1H, ArH), 7.17–7.22 (m, 2H, 2 × ArH), 7.77–7.79 ppm (m, 1H, ArH); ^{13}C NMR ([D$_6$]DMSO): δ = 54.9, 56.1, 56.5, 60.7, 108.5, 113.2, 114.6, 123.9, 124.6, 124.7, 125.6, 125.8, 126.5, 126.6, 131.8, 134.9, 137.9, 153.2 ppm; Anal. calcd for $C_{21}H_{21}NO_5S$: C 63.14, H 5.30, N 3.51, S 8.03, found: C 63.17, H 5.35, N 3.49, S 8.14.

*4.2.30. [1-Cyclopropylbenzo[d]pyrrolo[2,1-b]thiazole-2,3-diyl]dimethanol (**17j**)*

Compound **17j** was prepared from **16j** (1.8 g, 5.4 mmol) and $LiAlH_4$ (0.5 g, 13.0 mmol); Yield: 1.0 g (67%); mp: 153–154 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 0.71 (m, 2H, CH_2), 1.12 (m, 2H, CH_2), 2.04 (m, 1H, CH), 4.47 (d, J = 4.8 Hz, 2H, CH_2), 4.55 (m, 3H, CH_2 and exchangeable OH), 4.96 (t, J = 4.8 Hz, 1H, exchangeable, OH), 7.23 (t, J = 7.6 Hz, 1H, ArH), 7.38 (t, J = 7.6 Hz, 1H, ArH), 7.77 (d, J = 8 Hz, 1H, ArH), 8.20 ppm (d, J = 8 Hz, 1H, ArH); ^{13}C NMR ([D$_6$]DMSO): δ = 6.8, 7.6, 54.7, 56.0, 113.6, 114.0, 122.7, 123.5, 124.2, 125.8, 126.1, 126.6131.6, 135.5 ppm; Anal. calcd for $C_{15}H_{15}NO_2S$: C 65.91, H 5.53, N 5.12, S 11.73, found: C 65.63, H 5.42, N 5.10, S 11.54.

*4.2.31. General procedure for preparation of bis(alkylcarbamate) derivatives (**18** and **19**)*

A solution of bis(hydroxymethyl) derivatives (**17a**–**j**, 1.0 equivalent) in anhydrous THF was treated with excess triethylamine (6.0 equivalent) followed by excess alkylisocyanate (4.0 equivalent). The reaction mixture was stirred at ambient temperature under an argon atmosphere. After completion of the reaction (for 4–20 h), the reaction mixture was evaporated to dryness in vacuo. The desired product was obtained by crystallization.

*4.2.32. [1-Methylbenzo[d]pyrrolo[2,1-b]thiazole-2,3-diyl]bis(methylene)bis(ethylcarbamate) (**18a**)*

Compound **18a** was prepared from **17a** (0.24 g, 1.0 mmol), Et_3N (0.5 mL), and ethylisocyanate (0.28 g, 4.0 mmol); Yield: 0.3 g (85%); mp: 158–159 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 0.98 (t, J = 7.0 Hz, 6H, 2 × Me), 2.67 (s, 3H, Me), 2.98 (q, J = 7.0 Hz, 4H, CH_2), 5.01 (s, 2H, CH_2), 5.03 (s, 2H, CH_2), 7.02–7.05 (br s, 2H, exchangeable, NH), 7.29 (t, J = 8.0 Hz, 1H, ArH), 7.41 (t, J = 8.0 Hz, 1H, ArH), 7.84 (d, J = 8.0 Hz, 1H, ArH), 7.91 ppm (d, J = 8.0 Hz, 1H, ArH); ^{13}C NMR ([D$_6$]DMSO): δ = 11.4, 14.9, 34.9, 35.0, 56.2, 57.0, 108.3, 113.2, 120.4, 123.1, 123.7, 124.0, 125.0, 125.7, 130.6, 134.8, 155.9, 156.1 ppm; Anal. calcd for $C_{19}H_{23}N_3O_4S$: C 58.59, H 5.95, N 10.79, S 8.23, found: C 58.26, H 5.62, N 10.46, S 8.60.

*4.2.33. [1-(4-Fluorophenyl)benzo[d]pyrrolo[2,1-b]thiazole-2,3-diyl]bis(methylene)bis(ethylcarbamate) (**18b**)*

Compound **18b** was prepared from **17b** (0.33 g, 1.0 mmol), Et_3N (0.5 mL), and ethylisocyanate (0.28 g, 4.0 mmol); Yield: 0.36 g (79%); mp: 172–173 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 0.98 (t, J = 7.0 Hz, 6H, 2 × Me), 2.98 (q, J = .0 Hz, 4H, CH_2), 4.81 (s, 2H, CH_2), 5.12 (s, 2H, CH_2), 6.79–6.81(m, 1H, ArH), 7.05–7.11 (br s, 2H, exchangeable, NH), 7.19–7.26 (m, 2H, 2 × ArH), 7.38–7.43 (m, 2H, 2 × ArH), 7.56–7.59 (m, 2H, 2 × ArH), 7.86–7.88 ppm (m, 1H, ArH); ^{13}C NMR ([D$_6$]DMSO): δ = 14.9, 15.0, 34.9, 35.0, 56.7, 57.0, 109.2, 112.7, 115.6, 115.8, 122.4, 124.0, 124.4, 125.6, 126.4, 127.3, 130.8, 132.8, 132.9, 134.1, 155.7, 156.1, 161.4, 163.3 ppm; Anal. calcd for $C_{24}H_{24}FN_3O_4S$: C 61.39, H 5.15, N 8.95, S 6.83, found: C 61.36, H 5.26, N 8.94,S 7.14.

*4.2.34. [1-(4-Chlorophenyl)benzo[d]pyrrolo[2,1-b]thiazole-2,3-diyl]bis(methylene)bis(ethylcarbamate) (**18c**)*

Compound **18c** was prepared from **17c** (0.25 g, 0.7 mmol), Et_3N (0.4 mL), and ethylisocyanate (0.23 g, 3.0 mmol); Yield: 0.28 g (78%); mp: 190–191 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 1.00 (t, J = 7.2 Hz, 6H, 2 × Me), 2.99 (q, J = 7.2 Hz, 4H, CH_2), 4.83 (s, 2H, CH_2), 5.13 (s, 2H, CH_2), 6.87–6.89 (m, 1H, ArH), 7.07–7.14 (br s, 2H, exchangeable, NH), 7.21–7.29 (m, 2H, 2 × ArH), 7.55 (d, J = 8.4 Hz, 2H, 2 × ArH), 7.63 (d, J = 8.4 Hz, 2H, 2 × ArH), 7.88–7.90 ppm (m, 1H, ArH); ^{13}C NMR ([D$_6$]DMSO): δ = 14.9, 15.0, 34.9, 35.0, 56.6, 57.0, 109.4, 112.8, 122.6, 124.1, 124.4, 125.3, 125.5, 127.7, 128.7, 128.9, 130.7, 132.3, 133.6, 134.0, 155.7, 156.0 ppm; Anal. calcd for $C_{24}H_{24}ClN_3O_4S$: C 59.31, H 4.98, N 8.65, S 6.60, found: C 59.44, H 4.95, N 8.52, S 6.72.

*4.2.35. [1-(3,4-Difluorophenyl)benzo[d]pyrrolo[2,1-b]thiazole-2,3-diyl]bis(methylene)bis(ethylcarbamate) (**18d**)*

Compound **18d** was prepared from **17d** (0.2 g, 0.6 mmol), Et_3N (0.3 mL), and ethylisocyanate (0.17 g, 2.4 mmol). Yield, 0.24 g (85%); mp: 167–168 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 1.00 (t, J = 7.2 Hz, 6H, 2 × Me), 2.98 (q, J = 7.2 Hz, 4H, CH_2), 4.84 (s, 2H, CH_2), 5.12 (s, 2H, CH_2), 6.87–6.88 (m, 1H, ArH), 7.06–7.13 (br s, 2H, exchangeable, NH), 7.20–7.28 (m, 2H, 2 × ArH), 7.37–7.38 (m, 1H, ArH), 7.59–7.68 (m, 2H, 2 × ArH), 7.87–7.89 ppm (m, 1H, ArH); ^{13}C NMR ([D$_6$] DMSO): δ = 14.9, 15.0, 34.9, 35.0, 56.5, 57.0, 109.3, 112.9, 117.8, 119.8, 122.8, 124.1, 124.4, 125.6, 127.5, 127.8, 130.7, 134.0, 148.3, 148.9, 150.2, 150.8, 155.7, 156.0 ppm; Anal. calcd for $C_{24}H_{23}F_2N_3O_4S$: C 59.13, H 4.76, N 8.62, S 6.58, found: C 59.36, H 4.65, N 8.32, S 6.80.

*4.2.36. [1-(3,4-Dichlorophenyl)benzo[d]pyrrolo[2,1-b]thiazole-2,3-diyl]bis(methylene)bis(ethylcarbamate) (**18e**)*

Compound **18e** was prepared from **17e** (0.3 g, 0.8 mmol), Et_3N (0.4 mL), and ethylisocyanate (0.23 g, 3.0 mmol); Yield: 0.37 g (90%); mp: 178–179 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 1.00 (t, J = 7.0 Hz, 6H, 2 × Me), 2.99 (q, J = 7.0 Hz, 4H, CH_2), 4.85 (s, 2H, CH_2), 5.13 (s,

2H, CH_2), 6.93–6.95 (m, 1H, ArH), 7.08–7.14 (br s, 2H, exchangeable, NH), 7.23–7.30 (m, 2H, 2 × ArH), 7.52–7.54 (m, 1H, ArH), 7.81–7.83 (m, 2H, 2 × ArH), 7.88–7.90 ppm (m, 1H, ArH); ^{13}C NMR ([D_6]DMSO): δ = 14.9, 15.0, 35.0, 35.1, 56.5, 56.9, 109.6, 112.9, 123.2, 123.9, 124.2, 124.5, 125.6, 128.4, 130.7, 130.8, 131.4, 131.6, 132.1, 134.0, 155.7, 156.0 ppm; Anal. calcd for $C_{24}H_{23}Cl_2N_3O_4S$: C 55.39, H 4.45, N 8.07, S 6.16, found: C 55.27, H 4.45, N 8.09, S 6.00.

4.2.37. [1-(3-chloro-4-fluorophenyl)benzo[d]pyrrolo[2,1-b] thiazole-2,3-diyl]bis(methylene)bis(ethylcarbamate) (**18f**)

Compound **18f** was prepared from **17f** (0.36 g, 1.0 mmol), Et_3N (0.5 mL), and ethylisocyanate (0.28 g, 4.0 mmol); Yield: 0.41 g (82%); mp: 158–159 °C; ^{1}H NMR ([D_6]DMSO): δ = 1.00 (t, J = 7.2 Hz, 6H, 2 × Me), 2.99 (q, J = 7.2 Hz, 4H, CH_2), 4.84 (s, 2H, CH_2), 5.13 (s, 2H, CH_2), 6.86–6.88 (m, 1H, ArH), 7.01–7.12 (br s, 2H, exchangeable, NH), 7.21–7.29 (m, 2H, 2 × ArH), 7.54–7.56 (m, 1H, ArH), 7.59–7.63 (m, 1H, ArH), 7.77–7.79 (m, 1H, ArH), 7.88–7.90 ppm (m, 1H, ArH); ^{13}C NMR: ([D_6]DMSO): δ = 14.9, 15.0, 35.0, 35.1, 56.5, 57.0, 109.4, 112.8, 117.1, 119.8, 122.9, 124.1, 124.4, 125.6, 127.8, 130.7, 131.5, 131.6, 132.6, 134.0, 155.7, 156.0, 156.5, 158.5 ppm; Anal. calcd for $C_{24}H_{23}ClFN_3O_4S$: C 57.20, H 4.60, N 8.34, S 6.36, found: C 57.26, H 4.47, N 8.04, S 6.50.

4.2.38. [1-(4-Methoxyphenyl)benzo[d]pyrrolo[2,1-b]thiazole-2,3-diyl]bis(methylene)bis(ethylcarbamate) (**18g**)

Compound **18g** was prepared from **17g** (0.2 g, 0.6 mmol), Et_3N (0.3 mL), and ethylisocyanate (0.17 g, 2.4 mmol); Yield: 0.24 g (85%); mp: 146–147 °C; ^{1}H NMR ([D_6]DMSO): δ = 1.00 (t, J = 7.2 Hz, 6H, 2 × Me), 2.99 (q, J = 7.2 Hz, 4H, CH_2), 3.87 (s, 3H, OMe), 4.80 (s, 2H, CH_2), 5.11 (s, 2H, CH_2), 6.83–6.85 (m, 1H, ArH), 7.04–7.10 (br s, 2H, exchangeable, NH), 7.11–7.13 (m, 2H, 2 × ArH), 7.18–7.26 (m, 2H, 2 × ArH), 7.41–7.43 (m, 2H, 2 × ArH), 7.84–7.86 ppm (m, 1H, ArH); ^{13}C NMR ([D_6]DMSO): δ = 14.9, 15.0, 34.9, 35.0, 56.1, 56.9, 57.1, 109.0, 112.7, 114.1, 121.9, 122.0, 123.9, 124.3, 125.4, 126.7, 130.7, 132.0, 134.2, 155.8, 156.1, 159.6 ppm; Anal. calcd for $C_{25}H_{27}N_3O_5S$: C 62.35, H 5.65, N 8.73, S 6.66, found: C 62.19, H 5.55, N 8.75, S 7.03.

4.2.39. [1-(3,4-Dimethoxyphenyl)benzo[d]pyrrolo[2,1-b]thiazole-2,3-diyl]bis(methylene)bis(ethylcarbamate) (**18h**)

Compound **18h** was prepared from **17h** (0.2 g, 0.5 mmol), Et_3N (0.3 mL), and ethylisocyanate (0.14 g, 2.0 mmol); Yield: 0.25 g (90%); mp: 145–146 °C; ^{1}H NMR ([D_6]DMSO): δ = 1.00 (t, J = 7.0 Hz, 6H, 2 × Me), 2.99 (q, J = 7.0 Hz, 4H, CH_2), 3.74 (s, 3H, MeO), 3.87 (s, 3H, MeO), 4.85 (s, 2H, CH_2), 5.12 (s, 2H, CH_2), 6.93–6.95 (m, 1H, ArH), 7.03–7.08 (br s, 2H, exchangeable, NH), 7.09–7.11 (m, 2H, 2 × ArH), 7.13–7.15 (m, 1H, ArH), 7.21–7.26 (m, 2H, 2 × ArH), 7.84–7.86 ppm (m, 1H, ArH); ^{13}C NMR ([D_6]DMSO): δ = 15.0, 34.9, 35.0, 55.4, 57.0, 57.1, 109.1, 111.6, 113.0, 114.0, 121.8, 122.0, 123.1, 124.0, 124.3, 125.4, 126.8, 127.0, 130.8, 134.3, 148.5, 149.2, 155.9, 156.1 ppm; Anal. calcd for $C_{26}H_{29}N_3O_6S$: C 61.04, H 5.71, N 8.21, S 6.27, found: C 60.81, H 5.71, N 8.11, S 6.19.

4.2.40. [1-(3,4,5-Trimethoxyphenyl)benzo[d]pyrrolo[2,1-b]thiazole-2,3-diyl]bis(methylene)bis(ethylcarbamate) (**18i**)

Compound **18i** was prepared from **17i** (0.39 g, 1.0 mmol), Et_3N (0.5 mL), and ethylisocyanate (0.28 g, 4.0 mmol); Yield: 0.48 g (89%); mp: 196–197 °C; ^{1}H NMR ([D_6]DMSO): δ = 0.99 (t, J = 7.2 Hz, 6H, 2 × Me), 2.99 (q, J = 7.2 Hz, 4H, CH_2), 3.76 (s, 6H, 2 × OMe), 3.78 (s, 3H, MeO), 4.85 (s, 2H, CH_2), 5.12 (s, 2H, CH_2), 6.80 (s, 2H, 2 × ArH), 7.01–7.03 (m, 1H, ArH), 7.07–7.12 (br s, 2H, exchangeable, NH), 7.24–7.27 (m, 2H, 2 × ArH), 7.85–7.87 ppm (m, 1H, ArH); ^{13}C NMR ([D_6]DMSO): δ = 14.9, 15.0, 34.9, 35.0, 55.9, 57.0, 60.1, 107.9, 109.3, 113.2, 121.9, 124.1, 124.3, 125.1, 125.4, 127.0, 127.2, 130.8, 134.2, 137.7, 152.8, 155.8, 156.1 ppm; Anal. calcd for $C_{27}H_{31}N_3O_7S$: C 59.87, H 5.77, N 7.76, S 5.92, found: C 59.49, H 5.72, N 7.44, S 5.78.

4.2.41. [1-Cyclopropylbenzo[d]pyrrolo[2,1-b]thiazole-2,3-diyl] bis(methylene)bis(ethylcarbamate) (**18j**)

Compound **18j** was prepared from **17j** (0.2 g, 0.66 mmol), Et_3N (0.5 mL), and ethylisocyanate (0.18 g, 2.6 mmol); Yield: 0.23 g (75%); mp: 187–188 °C; ^{1}H NMR ([D_6]DMSO): δ = 0.76 (m, 2H, CH_2), 0.99 (t, J = 7.2 Hz, 6H, 2 × Me), 1.16 (m, 2H, CH_2), 2.10 (m, 1H, CH), 3.00 (q, J = 7.2 Hz, 4H, CH_2), 5.00 (s, 2H, CH_2), 5.07 (s, 2H, CH_2), 7.03–7.08 (br s, 2H, exchangeable, NH), 7.30 (t, J = 8.4 Hz, 1H, ArH), 7.43 (t, J = 8.4 Hz, 1H, ArH), 7.84 (d, J = 8 Hz, 1H, ArH), 8.27 ppm (d, J = 8 Hz, 1H, ArH); ^{13}C NMR ([D_6]DMSO): δ = 6.2, 7.1, 15.0, 34.9, 35.0, 56.8, 57.0, 108.2, 114.1, 121.5, 123.7, 123.9, 125.5, 125.6, 127.9, 130.6, 134.8, 155.9, 156.1 ppm; Anal. calcd for $C_{21}H_{25}N_3O_4S\cdot 0.5H_2O$: C 59.92, H 6.13, N 9.98, S 7.62, found: C 59.60, H 5.87, N 9.89, S 7.53.

4.2.42. [1-Methylbenzo[d]pyrrolo[2,1-b]thiazole-2,3-diyl] bis(methylene)bis(iso-propylcarbamate) (**19a**)

Compound **19a** was prepared from **17a** (0.24 g, 1.0 mmol), Et_3N (0.5 mL), and isopropylisocyanate (0.34 g, 4.0 mmol); Yield: 0.33 g (79%); mp: 172–173 °C; ^{1}H NMR ([D_6]DMSO): δ = 1.03 (d, J = 6.4 Hz, 12H, 4 × Me), 2.67 (s, 3H, Me), 3.59 (m, 2H, CH), 5.02 (s, 2H, CH_2), 5.04 (s, 2H, CH_2), 6.97–6.99 (br s, 2H, exchangeable, NH), 7.29 (t, J = 8.0 Hz, 1H, ArH), 7.41 (t, J = 8.0 Hz, 1H, ArH), 7.84 (d, J = 8.0 Hz, 1H, ArH), 7.91 ppm (d, J = 8.0 Hz, 1H, ArH); ^{13}C NMR ([D_6]DMSO): δ = 11.4, 22.5, 42.2, 42.3, 56.1, 56.9, 66.9, 108.3, 113.1, 120.5, 123.1, 123.7, 124.0, 125.1, 125.7, 130.6, 134.8, 155.3, 155.4 ppm; Anal. calcd for $C_{21}H_{27}N_3O_4S$: C 60.41, H 6.52, N 10.06, S 7.68, found: C 60.08, H 6.24, N 9.76, S 7.97.

4.2.43. [1-(4-Fluorophenyl)benzo[d]pyrrolo[2,1-b]thiazole-2,3-diyl] bis(methylene)bis(iso-propylcarbamate) (**19b**)

Compound **19b** was prepared from **17b** (0.33 g, 1.0 mmol), Et_3N (0.5 mL), and isopropylisocyanate (0.34 g, 4.0 mmol); Yield: 0.47 g (95%); mp: 174–175 °C; ^{1}H NMR ([D_6]DMSO): δ = 1.04 (d, J = 6.6 Hz, 12H, 4 × Me), 3.59 (m, 2H, CH), 4.81 (s, 2H, CH_2), 5.12 (s, 2H, CH_2), 6.79–6.81 (m, 1H, ArH), 6.99–7.05 (br s, 2H, exchangeable, NH), 7.18–7.26 (m, 2H, 2 × ArH), 7.38–7.42 (m, 2H, 2 × ArH), 7.55–7.58 (m, 2H, 2 × ArH), 7.86–7.88 ppm (m, 1H, ArH); ^{13}C NMR ([D_6] DMSO): δ = 22.5, 42.2, 42.3, 56.5, 56.9, 109.2, 112.7, 115.6, 115.8, 122.4, 124.1, 124.4, 125.5, 125.6, 126.4, 127.4, 130.8, 132.9, 134.1, 155.1, 155.4, 161.4, 163.3 ppm; Anal. calcd for $C_{26}H_{28}FN_3O_4S$: C 62.76, H 5.67, N 8.44, S 6.44, found: C 62.46, H 5.61, N 8.30, S 6.48.

4.2.44. [1-(4-Chlorophenyl)benzo[d]pyrrolo[2,1-b]thiazole-2,3-diyl]bis(methylene)bis(iso-propylcarbamate) (**19c**)

Compound **19c** was prepared from **17c** (0.17 g, 0.5 mmol), Et_3N (0.3 mL), and isopropylisocyanate (0.17 g, 2.0 mmol); Yield: 0.2 g (78%); mp: 171–172 °C; ^{1}H NMR ([D_6]DMSO): δ = 1.04 (d, J = 6.4 Hz, 12H, 4 × Me), 3.59 (m, 2H, CH), 4.82 (s, 2H, CH_2), 5.12 (s, 2H, CH_2), 6.87–6.89 (m, 1H, ArH), 6.97–7.05 (br s, 2H, exchangeable, NH), 7.20–7.28 (m, 2H, 2 × ArH), 7.54 (d, J = 8.4 Hz, 2H, 2 × ArH), 7.63 (d, J = 8.4 Hz, 2H, 2 × ArH), 7.87–7.89 ppm (m, 1H, ArH); ^{13}C NMR ([D_6] DMSO): δ = 22.5, 42.2, 42.3, 56.4, 56.8, 109.4, 112.8, 122.6, 124.1, 124.4, 125.3, 125.5, 127.8, 128.7, 128.9, 130.8, 132.3, 133.6, 134.0, 155.0, 155.4 ppm; Anal. calcd for $C_{26}H_{28}ClN_3O_4S\cdot 0.5H_2O$: C 59.70, H 5.59, N 8.03, S 6.13, found: C 59.38, H 5.32, N 7.87, S 6.50.

4.2.45. [1-(3,4-Difluorophenyl)benzo[d]pyrrolo[2,1-b]thiazole-2,3-diyl]bis(methylene)bis(iso-propylcarbamate) (**19d**)

Compound **19d** was prepared from **17d** (0.17 g, 0.5 mmol), Et_3N (0.3 mL), and isopropylisocyanate (0.17 g, 2.0 mmol); Yield: 0.2 g (79%); mp: 173–174 °C; ^{1}H NMR ([D_6]DMSO): δ = 1.03 (d, J = 6.6 Hz, 12H, 4 × Me), 3.58 (m, 2H, CH), 4.83 (s, 2H, CH_2), 5.12 (s, 2H, CH_2), 6.87–6.89 (m, 1H, ArH), 6.98–7.04(br s, 2H, exchangeable, NH), 7.20–7.28 (m, 2H, 2 × ArH), 7.38 (m, 1H, ArH), 7.59–7.67 (m, 2H, 2 × ArH), 7.87–7.89 ppm (m, 1H, ArH); ^{13}C NMR ([D_6]DMSO):

δ = 22.5, 42.2, 42.3, 56.3, 56.8, 109.4, 112.9, 117.8, 117.9, 119.8, 119.9, 122.9, 124.1, 124.4, 125.6, 127.5, 127.9, 130.8, 134.0, 148.3, 150.3, 155.0, 155.4 ppm; Anal. calcd for $C_{26}H_{27}F_2N_3O_4S$: C 60.57, H 5.28, N 8.15, S 6.22, found: C 60.22, H 5.02, N 7.94, S 6.36.

4.2.46. *[1-(3,4-Dichlorophenyl)benzo[d]pyrrolo[2,1-b]thiazole-2,3-diyl]bis(methylene)bis(iso-propylcarbamate) (**19e**)*

Compound **19e** was prepared from **17e** (0.25 g, 0.66 mmol), Et_3N (0.4 mL), and isopropylisocyanate (0.22 g, 2.6 mmol); Yield: 0.32 g (88%); mp: 181–182 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 1.04 (d, J = 6.6 Hz, 12H, 4 × Me), 3.59 (m, 2H, CH), 4.85 (s, 2H, CH_2), 5.13 (s, 2H, CH_2), 6.94–6.96 (m, 1H, ArH), 7.00–7.06 (br s, 2H, exchangeable, NH), 7.23–7.30 (m, 2H, 2 × ArH), 7.52–7.54 (m, 1H, ArH), 7.81–7.83(m, 2H, 2 × ArH), 7.88–7.91 ppm (m, 1H, ArH); ^{13}C NMR ([D$_6$]DMSO): δ = 22.5, 42.2, 42.3, 56.3, 56.7, 109.6, 112.9, 123.2, 123.9, 124.2, 124.4, 125.6, 128.4, 130.7, 130.8, 131.4, 131.6, 132.0, 134.0, 155.0, 155.4 ppm; Anal. calcd for $C_{26}H_{27}Cl_2N_3O_4S$: C 56.94, H 4.96, N 7.66, S 5.85, found: C 56.66, H 4.93, N 7.54, S 5.68.

4.2.47. *[1-(3-chloro-4-fluorophenyl)benzo[d]pyrrolo[2,1-b]thiazole-2,3-diyl]bis(methylene)bis(iso-propylcarbamate) (**19f**)*

Compound **19f** was prepared from **17f** (0.18 g, 0.5 mmol), Et_3N (0.4 mL), and isopropylisocyanate (0.17 g, 2.0 mmol); Yield: 0.2 g (75%); mp: 178–179 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 1.04 (d, J = 6.6 Hz, 12H, 4 × Me), 3.60 (m, 2H, CH), 4.84 (s, 2H, CH_2), 5.13 (s, 2H, CH_2), 6.86–6.88 (m, 1H, ArH), 6.99–7.06 (br s, 2H, exchangeable, NH), 7.21–7.29 (m, 2H, 2 × ArH), 7.53–7.55 (m, 1H, ArH), 7.62–7.64 (m, 1H, ArH), 7.77–7.79 (m, 1H, ArH), 7.88–7.90 ppm (m, 1H, ArH); ^{13}C NMR ([D$_6$]DMSO): δ = 22.5, 23.2, 42.2, 42.3, 56.3, 56.8, 109.4, 112.8, 117.3, 119.9, 123.0, 124.1, 124.4, 125.6, 127.9, 130.8, 131.4, 131.5132.6, 134.0, 155.0, 155.4, 156.5, 158.5 ppm; Anal. calcd for $C_{26}H_{27}ClFN_3O_4S$: C 58.70, H 5.12, N 7.90, S 6.03, found: C 58.80, H 5.20, N 7.88, S 5.73.

4.2.48. *[1-(4-Methoxyphenyl)benzo[d]pyrrolo[2,1-b]thiazole-2,3-diyl]bis(methylene)bis(iso-propylcarbamate) (**19g**)*

Compound **19g** was prepared from **17g** (0.25 g, 0.7 mmol), Et_3N (0.4 mL), and isopropylisocyanate (0.24 g, 2.8 mmol); Yield: 0.32 g (86%); mp: 141–142 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 1.04 (d, J = 6.6 Hz, 12H, 4 × Me), 3.59 (m, 2H, CH), 3.87 (s, 3H, MeO), 4.81 (s, 2H, CH_2), 5.11 (s, 2H, CH_2), 6.83–6.85 (m, 1H, ArH), 6.97–7.04 (br s, 2H, exchangeable, NH), 7.12 (d, J = 8.4 Hz, 2H, 2 × ArH), 7.17–7.25 (m, 2H, 2 × ArH), 7.42 (d, J = 8.4 Hz, 2H, 2 × ArH), 7.84–7.86 ppm (m, 1H, ArH); ^{13}C NMR ([D$_6$]DMSO): δ = 22.5, 42.2, 42.3, 55.2, 56.7, 57.0, 109.1, 112.7, 114.1, 122.0, 123.9, 124.3, 125.4, 126.7, 130.8, 132.0, 134.3, 155.1, 155.4, 159.6 ppm; Anal. calcd for $C_{27}H_{31}N_3O_5S$: C 63.63, H 6.13, N 8.25, S 6.29, found: C 63.38, H 6.06, N 8.05, S 6.61.

4.2.49. *[1-(3,4-Dimethoxyphenyl)benzo[d]pyrrolo[2,1-b]thiazole-2,3-diyl]bis(methylene)bis(iso-propylcarbamate) (**19h**)*

Compound **19h** was prepared from **17h** (0.18 g, 0.5 mmol), Et_3N (0.4 mL), and isopropylisocyanate (0.17 g, 2.0 mmol); Yield: 0.23 g (89%); mp: 145–146 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 1.04 (d, J = .6 Hz, 12H, 4 × Me), 3.59 (m, 2H, CH), 3.74 (s, 3H, MeO), 3.86 (s, 3H, MeO), 4.85 (s, 2H, CH_2), 5.12 (s, 2H, CH_2), 6.94–6.96 (m, 1H, ArH), 6.96–7.01 (br s, 2H, exchangeable, NH), 7.02–7.06 (m, 2H, 2 × ArH), 7.14–7.17 (m, 1H, ArH), 7.19–7.29 (m, 2H, 2 × ArH), 7.85–7.87 ppm (m, 1H, ArH); ^{13}C NMR ([D$_6$]DMSO): δ = 22.5, 23.2, 42.2, 42.3, 55.4, 56.9, 109.1, 111.6, 113.0, 114.0, 121.8, 122.1, 123.1, 124.0, 124.3, 125.4, 126.8, 127.0, 130.8, 134.3, 148.5, 149.2, 155.2, 155.4 ppm; Anal. calcd for $C_{28}H_{33}N_3O_6S$: C 62.32, H 6.16, N 7.79, S 5.94, found: C 62.11, H 5.90, N 7.45, S 6.15.

4.2.50. *[1-(3,4,5-Trimethoxyphenyl)benzo[d]pyrrolo[2,1-b]thiazole-2,3-diyl]bis(methylene)bis(iso-propylcarbamate) (**19i**)*

Compound **19i** was prepared from **17i** (0.2 g, 0.5 mmol), Et_3N (0.4 mL), and isopropylisocyanate (0.17 g, 2.0 mmol); Yield: 0.25 g (88%); mp: 195–196 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 1.04 (d, J = 6.4 Hz, 12H, 4 × Me), 3.59 (m, 2H, CH), 3.77 (s, 6H, 2 × MeO), 3.79 (s, 3H, MeO), 4.85 (s, 2H, CH_2), 5.12 (s, 2H, CH_2), 6.80 (s, 2H, 2 × ArH), 7.00–7.02 (m, 1H, ArH), 7.03–7.08 (br s, 2H, exchangeable, NH), 7.24–7.27 (m, 2H, 2 × ArH), 7.86–7.88 ppm (m, 1H, ArH); ^{13}C NMR ([D$_6$]DMSO): δ = 22.5, 23.2, 42.2, 42.3, 55.9, 56.8, 56.9, 60.1, 107.8, 107.9, 109.4, 113.2, 121.9, 124.1, 124.3, 125.1, 125.4, 127.0, 127.3, 130.8, 134.2, 137.7, 152.8, 155.2, 155.4 ppm; Anal. calcd for $C_{29}H_{35}N_3O_7S$: C 61.14, H 6.19, N 7.38, S 5.63, found: C 60.77, H 6.06, N 7.00, S 5.78.

4.2.51. *[1-Cyclopropylbenzo[d]pyrrolo[2,1-b]thiazole-2,3-diyl]bis(methylene)bis(iso-propylcarbamate) (**19j**)*

Compound **19j** was prepared from **17j** (0.13 g, 0.5 mmol), Et_3N (0.4 mL), and isopropylisocyanate (0.17 g, 2.0 mmol); Yield: 0.17 g (77%); mp: 215–216 °C; ^{1}H NMR ([D$_6$]DMSO): δ = 0.77 (m, 2H, CH_2), 1.04 (d, J = 6.4 Hz, 12H, 4 × Me), 1.16 (m, 2H, CH_2), 2.10 (m, 1H, CH), 3.60 (m, 2H, CH), 5.01 (s, 2H, CH_2), 5.07 (s, 2H, CH_2), 6.95 (br s, 2H, exchangeable, NH), 7.31 (t, J = 7.6 Hz, 1H, ArH), 7.44 (t, J = 7.6 Hz, 1H, ArH), 7.85 (d, J = 7.6 Hz, 1H, ArH), 8.27 ppm (d, J = 7.6 Hz, 1H, ArH); ^{13}C NMR ([D$_6$]DMSO): δ = 6.2, 7.1, 22.5, 42.2, 56.6, 56.8, 108.2, 114.1, 121.6, 123.7, 123.9, 125.5, 125.6, 127.9, 130.6, 134.8, 155.3, 155.4 ppm; Anal. calcd for $C_{23}H_{29}N_3O_4S$: C 62.28, H 6.59, N 9.47, S 7.23, found: C 62.02, H 6.68, N 9.22, S 7.40.

4.3. Biological assays

4.3.1. Cytotoxicity assays

The cytotoxic effects of the newly synthesized compounds were determined in T-cell acute lymphocytic leukemia (CCRF-CEM) and their resistant sub-cell lines (CCRF-CEM/Taxol and CCRF-CEM/VBL) by the XTT assay [19] and human solid tumor cells (i.e. breast carcinoma MX-1 and colon carcinoma HCT-116) by the SRB assay [20] in a 72 h incubation using a microplate spectrophotometer as previously described [21]. After the addition of phenazine methosulfate-XTT solution, incubated at 37 °C for 6 h and absorbance at 450 and 630 nm was detected on a microplate reader (EL 340). The cytotoxicity of the newly synthesized compounds against non-small cell lung carcinoma H1299, prostate cancer PC-3, oral cancer OECM-1 and glioma U87 were determined by the Alamar blue assay [22] in a 72 h incubation using a microplate spectrophotometer as previously described. 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 using the CompuSyn software by Chou and Martin [23] based on the median-effect principle and plot [24,25]. Ranges given for the values of IC_{50} were mean ± SE (n = 4).

4.3.2. In vivo studies

Athymic nude mice bearing the nu/nu gene were obtained from NCI, Frederick, MD and used for all human tumor xenografts. Male nude mice 6 weeks or older weighing 20–24 g or more were used. Compounds were administered via the tail vein for i.v. injection as described previously [21]. Tumor volume was assessed by measuring length × width × height (or width) by using a caliper. Vehicle used was 50 μL DMSO and 40 μL Tween 80 in 160 μL saline. The maximal tolerate dose of the tested compound was determined and applied for the in vivo therapeutic efficacy assay. For tumor-bearing nude mice during the course of the experiment, the body weight refers to total weight minus the weight of the tumor. All animal studies were conducted in accordance with the guidelines for the National Institute of Health Guide for the Care and Use of Animals and the protocol approved by the Institutional Animal Care and Use Committee.

4.3.3. Alkaline agarose gel shift assay

Formation of DNA cross-linking was analyzed by alkaline agarose gel electrophoresis as previously described [13]. In brief, purified pEGFP-N1 plasmid DNA (1500 ng) was mixed with various concentrations (1–20 μM) of **17b**, **18b**, **19b**, **17c**, **18c**, **19c**, **17g**, **18g**, and **19g** 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 (1000 ng) was mixed with 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, the DNA was then visualized under UV light.

4.3.4. Cell cycle analysis

The effects of compounds **17c**, **18c**, and **19c** on cell cycle progression were analyzed with a flow cytometer as described previously [26]. Briefly, Human non-small cell lung carcinoma H1299 cells were treated with these compounds at 0.5-, 1- and 2-fold dose of IC_{50} values for 24 h and 48 h before harvested and fixed with ice-cold 70% ethanol. Then the cells were 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, and then subjected to flow cytometric analysis (FACScan flow cytometer; Becton Dickinson, San Jose, CA). The cell cycle phase distribution was analyzed using ModFit LT 3.0 software (Verity Software House, Topsham, ME) based on DNA histograms.

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