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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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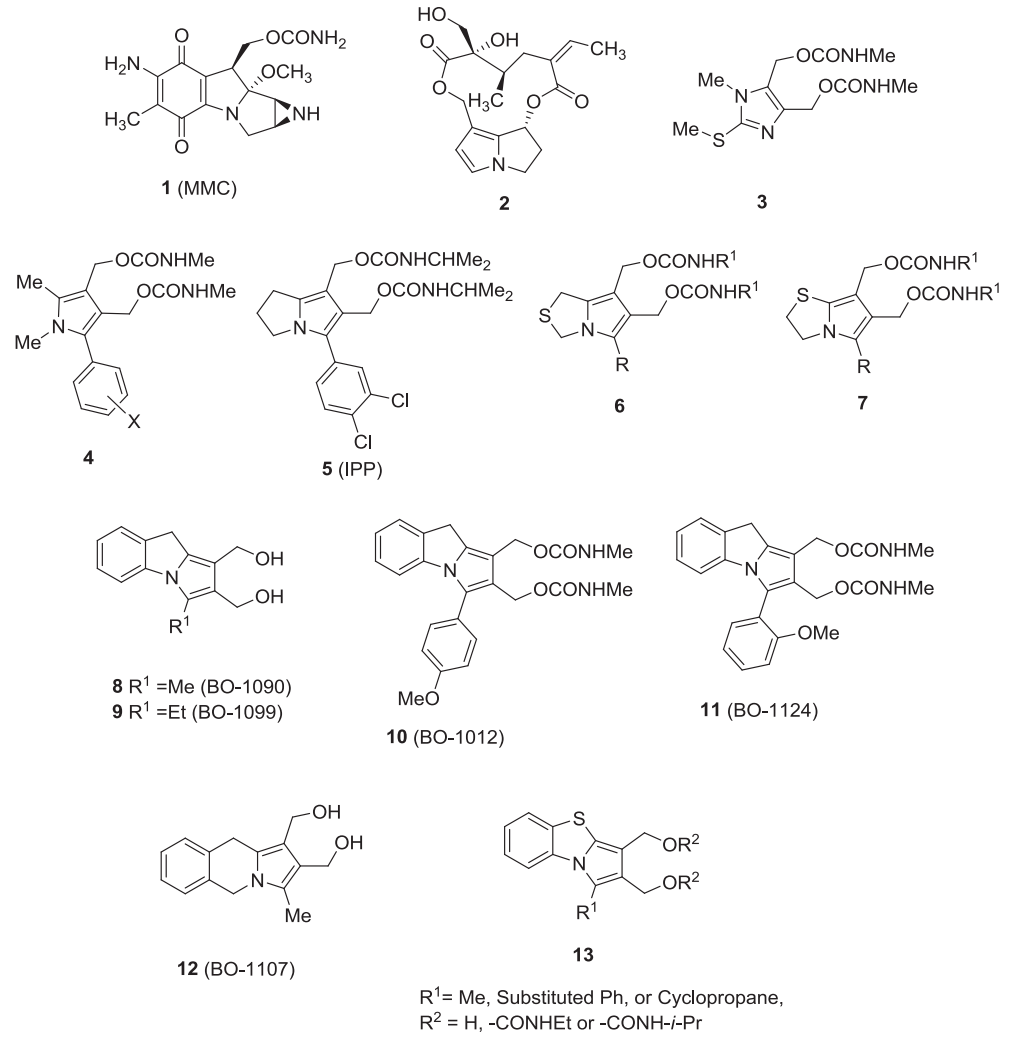

Fig. 1. Chemical structure of some DNA bifunctional alkylating agents.

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)