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Design, synthesis and in vitro cytotoxic studies of novel bis-pyrrolo[2,1][1,4] benzodiazepine-pyrrole and imidazole polyamide conjugates

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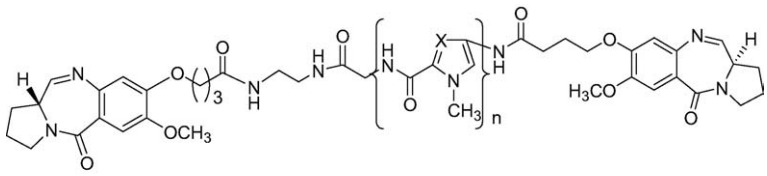
Abstract

The design, synthesis and biological evaluation of novel pyrrolo [2,1][1,4] benzodiazepine (PBD) dimers **38**–**43** linked with pyrrole and imidazole polyamides from either side by a flexible methylene chain of variable length are described, which involved mercuric chloride mediated cyclization of the corresponding amino diethyl thioacetals. The compounds were prepared with varying numbers of pyrrole and imidazole containing polyamides to determine the structural requirements for optimal in vitro antitumor activity. These compounds were tested against a panel of 60 human cancer cells by the National Cancer Institute, and demonstrated that, of the compounds bis-PBD-pyrrole polyamides (**38**–**40**) and bis-PBD-imidazole polyamides (**41**–**43**) certain of the bis-PBD-pyrrole and imidazole polyamide conjugates are active for individual cancer cell lines (Table 1). However, this study found that bis-PBD-pyrrole and imidazole polyamide conjugates **38**–**43** in general are potent against many human cancer cell lines.



38 n = 1, X = CH **41** n = 1, X = N
39 n = 2, X = CH **42** n = 2, X = N
40 n = 3, X = CH **43** n = 3, X = N

Keywords: Cytotoxicity; Polyamide; Pyrrolo [2,1-c] [1,4] benzodiazepines; DNA minor groove binders; PBD-polyamide conjugates

1. Introduction

DNA has for many years been a traditional target for chemotherapeutic intervention [1] in human cancers, especially for those where high proliferation rates of some tumor cell types have resulted in sensitivity to drugs, which block replication and transcription of their DNA [2]. Substantial progress has been made in understanding the fundamental principles responsible for the sequence-selective recognition of DNA by small organic molecules [3] including a range of naturally occurring antitumor antibiotics. Three fundamental issues that arise in the examination of DNA binding agents are: the origin of binding affinity, binding selectivity and reaction selectivity including DNA alkylation or cleavage. Each factor can independently assert levels of control on the sequence-selective recognition of DNA and the relative role and origin of these effects remain a primary objective of many investigations. A powerful complement to such tools in the examination of naturally derived DNA binding agents is the

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preparation and subsequent examination of key partial structure modifications or variations in the natural product and their corresponding unnatural enantiomers.

In addition, DNA sequence specificity or selectivity has recently become recognized as an important component of many cytotoxic agents [4,5] e.g. CC-1065 and duocarmycins [6], saramycin [7], distamycin [8–10], netropsin [8–10], pyrrolo [1,4] benzodiazepinone [11], bleomycin [12,13], several of which are of clinical interest in the treatment of human malignancies. In this context PBDs (pyrrolo [2,1-*c*][1,4] benzodiazepines), represent a group of exceptionally potent naturally occurring antitumor antibiotics, derived from *Streptomyces* species [14]. Their interactions with DNA are unique since they bind within the minor groove of DNA forming a covalent aminal bond between the C11 position of the central B-ring and the N2 amino group of a guanine base [14,15]. They differ in the number, type and position of substituent in both their aromatic A-ring and pyrrolidine C-rings, and in the degree of saturation of the C-rings which can be either fully saturated or unsaturated at either the C2–C3 (endocyclic) or C2 (exocyclic) positions. There is either an imine or carbinolamine methyl ether moiety at the N10–C11 position [16–19]. This latter is an electrophilic center responsible for alkylating DNA.

In addition studies on netropsin, distamycin and related compounds have led to the concept of polyamides as information reading agents [20]. A predominantly 4–5 AT base pair sequence is recognized by netropsin and distamycin in the minor groove of DNA. In our group attempts have been made to link PBD with pyrrole [21,22] and imidazole [23,24] and glycosylated pyrrole and imidazole polyamide [25], the well-established DNA minor groove binders. It was found that some PBD-glycosylated polyamides [25] conjugates exhibit good cytotoxicity against different human cancer cells. Studies have also shown that some synthetic compounds, which contain two PBD moieties linked from two possible positions by a flexible methylene chain of variable length, are significantly more potent than PBD naturally occurring compounds both in vitro and in vivo [26,27]. Recently a large number of structurally modified PBDs compounds have also been prepared and evaluated for their biological activity, particularly their antitumor potential [28,29]. The first PBD dimer comprising two unsubstituted PBD units joined through their C7–C7′ positions was reported by Farmer et al. [30,31] in 1988. Dimers with this linkage had only weak DNA cross-linking activity and no cytotoxicity data were reported. The first dimer with an C8–C8′ linkage was reported [32–34] in 1992. Dimers of this type have significant DNA interstrand cross-linking activity, which forms a symmetric interstrand cross link with duplex DNA involving a four base pairs bonding site but spanning six base pairs overall [35] and pronounced in vitro cytotoxicity and in vivo antitumor activity. More recently, we have also reported C2–C2′ linked dimers and their cytotoxicity [22,36]. A number of these dimeric compounds have been selected for preclinical studies but unfortunately most of them did not proceed beyond that stage. To date only a few PBD dimers have been prepared to examine interstrand cross-linking of DNA, which are linked from two possible positions by a flexible methylene chain of variable length. To our knowledge no attempt has been made to synthesize bis-pyrrolo [2,1-c][1,4] benzodiazepines (PBD)-pyrrole and imidazole polyamide conjugates **38**–**43** (Bis-PBDs dimers with pyrrole and imidazole polyamide conjugates).

In view of the commonly observed activity of these PBDs dimers and PBDs polyamides conjugates we attempted to conjugate two PBDs units with pyrrole and imidazole bearing polyamide from either side by a flexible methylene chain of variable length. In order to investigate the structure–activity relationship systematically as well as their cytotoxicity against human cancer cells, we herein describe the first synthesis and report of the cytotoxic activity of novel bis-pyrrolo [2,1-c][1,4] benzodiazepines (PBD)-pyrrole and imidazole polyamide conjugates **38**–**43** (Bis-PBDs dimers with pyrrole and imidazole polyamide conjugates), which contain two PBDs moieties linked from two different positions with pyrrole and imidazole bearing polyamides by a flexible methylene chain of variable length.

2. Results and discussion

2.1. Synthesis

In our previous work the (2*S*)-*N*-[5-methoxy-4-[3-(carboxy) propyloxy]-2-nitrobenzoyl] pyrrolidine-2-carboxaldehyde diethyl thioacetal (**1**) was synthesized [22] by using convenient routes in good yield. Condensation of the (2*S*)-*N*-[5-methoxy-4-[3-(carboxy) propyloxy]-2-nitrobenzoyl] pyrrolidine-2-carboxaldehyde diethyl thioacetal (**1**) with [2-[*N*-(benzyloxycarbonyl) amino]ethyl]amine in the presence of EDCI and HOBt in dry DMF at room temperature gave the [2-(4-{4-[2-(bis-ethylsulfanyl-methyl)-pyrrolidine-1-carbonyl]-2-methoxy-5-nitro-phenoxy}-butyrylamino)-ethyl]-carbamic acid benzyl ester (**2**) in 70% yield. Removal of the CBZ group from this compound **2** with EtSH and $BF_3{\cdot}OEt_2$ gave the free amino *N*-(2-amino-ethyl)-4-{4-[2-(bis-ethylsulfanyl-methyl)-pyrrolidine-1-carbonyl]-2-methoxy-5-nitro-phenoxy}-butyramide (**3**) in 70% yield (Scheme 1). Owing to the sensitivity of this amine to oxidation, it was used for the next reaction immediately.

The (2*S*)-*N*-[5-methoxy-4-[3-(carboxy) propyloxy]-2-nitrobenzoyl] pyrrolidine-2-carboxaldehyde diethyl thioacetal (**1**) was then coupled with the amine moiety of 1-methyl-4-nitro-1*H*-pyrrole-2-carboxylic acid methyl ester (**4**), using EDCI and HOBt as the coupling agents, in dry DMF at room temperature for about 12 h to afford the corresponding coupled compound **5** in 80% yield which, upon hydrolysis with 0.5 N NaOH at room temperature then acidification, produced the corresponding acid **6** in 70% yield. The polyamide acid **6** was treated with the *N*-(2-amino-ethyl)-4-{4-[2-(bis-ethylsulfanyl-methyl)-pyrrolidine-1-carbonyl]-2-methoxy-5-