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Synthesis and Antitumor Activity of a New Class of Pyrazolo[4,3-e]pyrrolo[1,2-a][1,4]diazepinone Analogs of Pyrrolo[1,4]benzodiazepines (PBDs)

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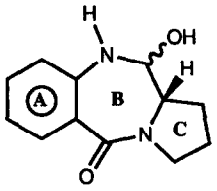
Abstract: A new class of Pyrrolo[1,4]benzodiazepines (PBDs) analogs featuring a pyrazolo[4,3-e]pyrrolo[1,2-a][1,4]diazepinone ring system has been designed and synthesized. In these compounds the A-benzene ring, characteristic of PBDs, has been replaced by a dimethylpyrazole ring, a modification suggested by modelling studies performed on the PBD base structure. Biological evaluation revealed appreciable antitumor activity for compounds **14** and **15** (8.84-22.4 μM) which encourages further investigation of the N^6 or N^7 alkyl pyrazole analogs.

Pyrrolo[1,4]benzodiazepines (PBDs) are potent antitumor antibiotics derived from various Streptomyces species showing interesting properties from both synthetic and biological standpoints[1]. Natural products belonging to this family include Anthramycin, Tomaymycin, Neothramycins A and B, Sibiromycin and Chicamycin. The antitumor activity of these natural products is believed to involve the formation of a labile covalent aminal linkage between the carbinolamine carbon (C11) of the antibiotics and the 2-amino group of guanine residues within the minor groove of DNA[2]. Reaction with guanines in specific sequences results in DNA sequence specificity. The resulting DNA-antibiotic adduct inhibits DNA replication.



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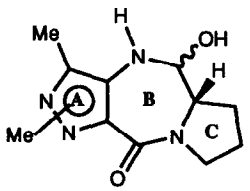

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In the case of Anthramycin the precise structure of the drug-DNA adduct has been elucidated[3], whereas for Tomaymycin NMR and fluorescence studies on the drug-DNA adduct[4] have been made. Recent studies[5] performed with dimeric PBD analogues have demonstrated that these new agents exhibit irreversible DNA cross-linking properties, and increased sequence specificity. The major limits to the clinical use of Anthramycin and other members of P[1,4]B family is their dose-limiting cardiotoxicity and tissue necrosis[6]. The P[1,4]B cardiotoxicity mechanism has demonstrated to be very similar to that of anthracyclines and it appears to be related to the formation of ortho quinone imine by oxidation[7].

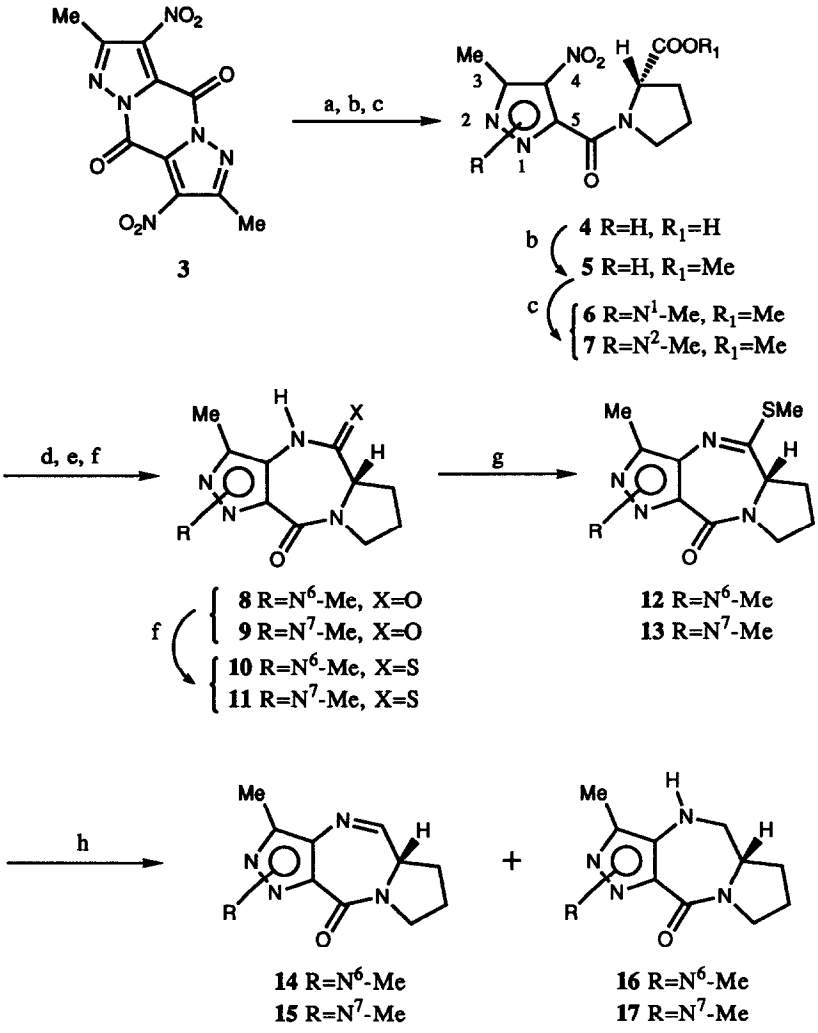
A rational approach to the development of clinically useful drugs in this series has been proposed, and some medicinal chemistry groups[8] have begun to investigate synthetic methods for the preparation of rationally designed analogues to delineate more complete structure-activity relationships. In a project aimed to design new analogues of P[1,4]B lacking both cardiotoxicity and tissue necrosis, we have substituted the A benzene ring of the PBD skeleton (**1**) with a 1,3-dimethyl- or 1,5-dimethyl-pyrazole nucleus, a modification which, according to the CPK model proposed by Thurston and Hurley[7], maintains all the structural requirements necessary for antitumor activity.

The unknown pyrazolo[4,3-e]pyrrolo[1,2-a][1,4]diazepinone ring system **2** could be obtained through standard synthetic routes established for PBD's involving direct coupling of pyrazolic diketopiperazine **3** with L-proline followed by reduction and cyclization to the dilactam and elaboration to the corresponding target compounds. Reaction of the readily available diketopiperazine **3** with L-proline in a 1:1 mixture of DMF-water in the presence of 1,1,3,3-tetramethylguanidine (TMG) furnished the acid **4** in good yield, which was in turn converted into the ester **5**. Methylation of **5** with methyl iodide in the presence of sodium methoxide in methanol solution gave a 1:1 mixture of the corresponding 1,3-dimethyl and 1,5-dimethyl derivatives **6** and **7**.

After chromatographic separation, the pure nitro derivatives **6** and **7** were both reduced with aqueous $TiCl_3$[10] in methanol solution and cyclized in situ by sodium methoxide to the corresponding dilactams **8** and **9** in good overall yield. In order to introduce the imine functionality in the 1,4-diazepine ring of **8** and **9**, we took advantage of the general and mild method developed by Kaneko et al.,[11] namely the aluminum-amalgam reduction of imino thioethers. Thiation of secondary amide function of **8** and **9** was achieved selectively with Lawesson's reagent to give the thioamides **10** and **11** which were in turn methylated to the corresponding imino thioethers **12** and **13** in fairly good yield. Treatment of **12** and **13** with Al-Hg in aqueous THF at 0-5°C for 24h gave a mixture of products which after purification led to the target compounds **14** (35%) and **15** (42%) along with minor amounts of the over reduction products **16** (15%) and **17** (12%). Compounds **14** and **15** were in the imine form, as was unambiguously established by ^1H and ^{13}C NMR spectroscopy[12].

Antitumor activity of compounds **12-17** was evaluated *in vitro* on a L 1210 tumor cell line[13]. Compounds **14** (IC_{50} = 22.4 μM) and **15** (IC_{50} = 8.84 μM) were 100-1000 fold less potent than the natural product Tomaymycin (IC_{50} = 0.01 μM) and comparable to those of L-PAM (IC_{50} = 11.6 μM) used as a reference compound. Compounds **12,13** and **16,17**, lacking the imine function characteristic of PBDs, were devoid of any antitumor activity. These results clearly indicate that the substitution of the A-ring of PBDs by a 1,3-dimethyl or a 1,5-dimethyl pyrazole ring still produces active compounds, whereas modification of the imine functionality results in loss of activity. This benzene/pyrazole substitution looks promising for the preparation of N^6 and N^7 alkyl derivatives to modulate the DNA binding in this new class of anthramycin analogs.

Scheme



a: L-proline, TMG; **b:** MeOH, H^+; **c:** MeI, MeONa; **d:** $TiCl_3$; **e:** MeONa; **f:** Lawesson's reagent; **g:** MeI, K_2CO_3, THF; **h:** Al/Hg, THF/H_2O, 0°C.

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12. (10aS)-1,2,3,5,10a-hexahydro-6,8-dimethyl-pyrazolo[4,3-e]pyrrolo[1,2-a][1,4]diazepin-5-one
14: yellow oil; ^{1}H NMR ($CDCl_3$) δ : 1.94-2.02 (m,2H); 2.26 (s,3H); 2.28-2.35 (m,2H);3.51-3.58 (m,2H) 3.6-3.7 (m,1H); 4.07 (s,3H);7.34 (d,J=3.8 Hz,1H); ^{13}C NMR: 10.6, 23.9, 29.9 , 39.6, 45.9, 54.9, 127.8, 131.1,144.1, 157.8, 158.9.
(10aS)-1,2,3,5,10a-hexahydro-7,8-dimethyl-pyrazolo[4,3-e]pyrrolo[1,2-a][1,4]diazepin-5-one
15: mp 139-140°C (EtOAc); ^{1}H NMR (CDCl3) δ: 1.92-1.97 (m,2H); 2.25 (s,3H); 2.30 (m,2H); 3.6-3.8 (m,2H); 3.8-3.85 (m,1H); 3.85 (s,3H); 7.37 (d,J=3.8Hz,1H). 2D NOE (NOESY) experiments showed cross-peak between the N^7-methyl (δ = 3.85) and C^8-methyl (δ = 2.25) groups. ^{13}C NMR: 8.9, 24.1, 30.3, 37.3, 46.2, 55.3, 129.6, 136.1, 137.9, 159.9, 160,9.
13. Dr.G.Pezzoni and Dr.M.Bergomi, personal communication.