Design and Synthesis of Two Cytotoxic Analogs of the Novel Pyrrolo[1′,2′:1,2][1,4]diazepin [7,6-*b*]indol-5(6*H*)-one Nucleus

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The design and synthesis of the two cytotoxic derivatives **15** and **16** of the novel pyrrolo[1′,2′:1,2][1,4]diazepin[7,6-*b*]indol-5(6*H*)-one nucleus is described. Readily available methyl 2-indolecarboxylates **5** and **6** are nitrosated with $NaNO_2$ in AcOH to give the analogs **7** and **8**, which are then oxidized with $KMnO_4$ in aq. NaOH to provide the 3-NO_2 acids **9** and **10**. These, in turn, are subjected to amidation with (2*S*)-pyrrolidine-2-carboxaldehyde diethyl thioacetal in the presence of EDCI and HOBt and then to a 7-*exo*-trig cyclization reaction to give the target molecules **15** and **16**. The new analogs were evaluated in the human leukemic K_{562} cell line and were shown to have micromolar potency.

The natural product anthramycin **1** and its naturally occurring (e.g. DC-81, **2**) and synthetic pyrrolo[2,1-*c*][1,4]benzodiazepine (PBD) congeners (e.g. **3** and **4**)[1,2] have in recent years generated significant interest as the basis for novel types of antitumour agents (Figure 1).[3] The PBDs interact within the minor groove of DNA by forming a covalent bond between their electrophilic C11-position and the exocyclic C2-NH_2 moiety of a guanine residue.[4] Most of the known PBDs, including anthramycin, have a tricyclic skeleton which consists of an aromatic A-ring, a 1,4-diazepin-5-one B-ring bearing a N10-C11 imine functionality (or the equivalent) and a pyrrolidine C-ring. Through extensive Structure-Activity Relationship (SAR) studies, each of these rings is known to play a distinct role in the interaction with DNA. For example, the (*S*) configuration of the C11a position at the B-C junction provides the molecules with the appropriate 3-dimensional shape to fit into the DNA minor groove. Similarly, the aromatic A-ring substituents can enhance binding of the PBD through the formation of hydrogen bonds to DNA bases.[4,5]

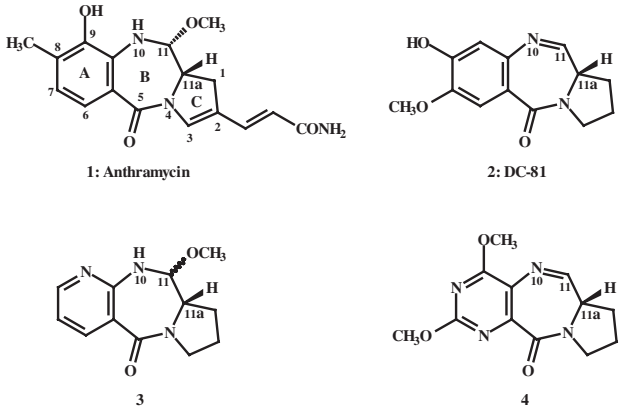
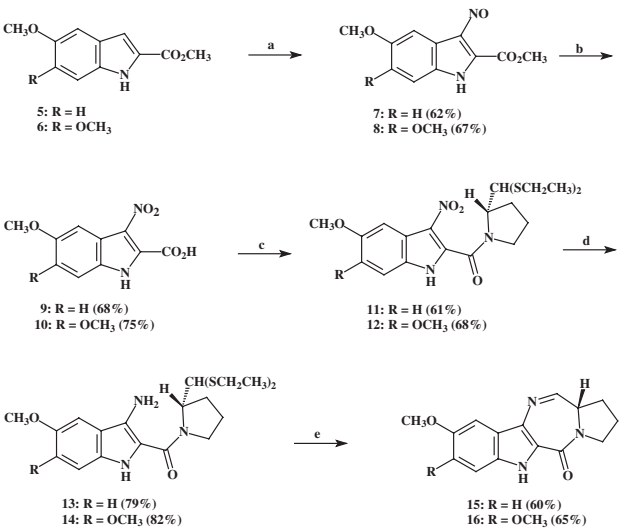


Figure 1.

It is known that the presence of a hydroxyl group at the C-9 position of the A-ring can lead to cardiotoxicity.[6] In an attempt to circumvent this, various analogs have been synthesized which bear nitrogen-containing aromatic heterocycles (e.g. **3**) in place of the usual aromatic A-ring. We report here the design and synthesis of the first indolic PBD analogs **15** and **16** (Scheme 1). The indole nucleus was chosen because it is known to increase minor groove binding affinity without significantly affecting sequence selectivity.[7] Furthermore, the methoxyl groups in the indole moiety were considered to be potential sources of hydrogen bonds. In the structures of the new tetracyclic analogs **15** and **16** the B and C-rings of the parent PBDs have been retained.

The strategy for the synthesis of the target molecules **15** and **16** is shown in Scheme 1. Methyl 5-methoxy-2-indolecarboxylate (**5**) was prepared from commercially available 5-methoxyindole-2-carboxylic acid by reaction with thionyl chloride



Scheme 1. Reagents and conditions: (a) $NaNO_2$, AcOH, 2 h; (b) $KMnO_4$, aq. NaOH (2M), 30 min; (c) (2*S*)-pyrrolidine-2-carboxaldehyde diethyl thioacetal, EDCI, HOBt, Et_3N, CH_2Cl_2-DMF (2:1), 18 h; (d) 10% Pd-C, H_2 (50 atm), MeOH, 3.5 h; (e) $HgCl_2$, $CaCO_3$, CH_3CN-H_2O (4:1), 8 h.

in methanol. Its bis-methoxy counterpart **6** was made from 3,4-dimethoxybenzaldehyde by the two-step method reported by Bunker et al.[8] As direct nitration of the carboxylates **5** and **6** did not lead to the corresponding 3-NO_2 indoles, **5** and **6** had to be first nitrosated with $NaNO_2$ in AcOH to give the analogs **7** and **8** followed by oxidation with $KMnO_4$ in aq. NaOH to provide the desired 3-NO_2 acids **9** and **10**. Implementation of classical methods for the formation of the amides **11** and **12**, such as initial reaction of **9** and **10** with $SOCl_2$ or $(COCl)_2$ followed by coupling to (2*S*)-pyrrolidine-2-carboxaldehyde diethyl thioacetal[9] or direct amidation of **9** and **10** with the thioacetal in the presence of 1,1′-carbonyldiimidazole (CDI), were not successful. Their synthesis was eventually effected by a modified literature procedure using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole hydrate (HOBt) in a mixture of CH_2Cl_2-DMF.[10] The C-3 NO_2 groups of **11** and **12** were catalytically hydrogenated to the corresponding amines **13** and **14**, which then underwent a 7-*exo*-trig cyclization reaction to the target molecules **15** and **16** using the method described by Thurston and co-workers.[9] The overall yield from methyl 5-methoxy-2-indolecarboxylate (**5**) to the new derivative **15** was 12%, while that of its congener **16**[11] from the ester **6** was 18%.

The new analogs **15** and **16** were evaluated in the human leukemic K_{562} cell line and were shown to have micromolar potency (Table 1). The three values for each compound appearing in Table 1 represent evaluations on consecutive weeks using the same stock solution. The decreasing activity suggests that the new analogs are not as stable when stored in solution as parent PBD molecules such as **1**–**4**. A DNA footprinting experiment involving incubation of **15** and **16** with plasmid DNA at concentrations of up to 100 μM for 5 h and using anthramycin **1** as a control indicated that **15** and **16** showed no evidence of selective interaction with DNA. Given that the molecules are significantly less cytotoxic than the equiv. PBDs with benzenoid A-rings (e.g. the IC_{50} value for DC-81 **2** in the K_{562} cell line after 1 h exposure is 3.0 μM), this suggests that they may be exerting their effect either through nonselective interaction with DNA or through an as yet unidentified non-DNA-interactive mechanism. The difference in activity between analogues **15** and **16** suggest that a full SAR study should be feasible and the synthesis of further analogues is underway in order to study stability and biological activity in this series.

Table 1. Cytotoxicity of compounds **15** and **16** in the K_{562} cell line[a]

IC_{50} (μM)[b]	
15	**16**
18[c]	30[c]
47	38
50	58

[a]K_{562} is a human leukemia cell line in which IC_{50} values were measured using a microculture tetrazolium assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide, MTT) following a 1 h exposure to drug at 37 °C.
[b]Dose of drug required to inhibit cell growth by 50% compared to drug-free control.
[c]The three values for each compound represent evaluations on consecutive weeks using the same stock solutions stored at −20 °C in DMSO.

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11 Selected data for **16**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.03–2.14 (m, 2H, 2 × H1), 2.32–2.45 (m, 2H, 2 × H2), 3.66–3.84 (m, 3H, 2 × H3 + H12a), 3.90 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 6.86 (s, 1H, H7(10)), 7.22 (s, 1H, H10(7)), 7.42 (d, 1H, $J = 3.9$ Hz, H12), 10.40 (bs, 1H, NH); ^{13}C NMR (50 MHz, $CDCl_3$): δ 24.4, 30.1, 46.1, 55.7, 56.0, 94.3, 99.8, 117.5, 121.6, 129.9, 130.6, 146.2, 150.0, 155.4, 160.1; $[a]_D^{20}$ + 440.6° (*c* 0.014, $CHCl_3$). Anal. Calcd. for $C_{16}H_{17}N_3O_3$: C, 64.20; H, 5.72; N, 14.04%. Found: C, 64.11; H, 5.75; N, 13.96%.

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