Synthesis of novel non-cross-linking pyrrolobenzodiazepines with remarkable DNA binding affinity and potent antitumour activity

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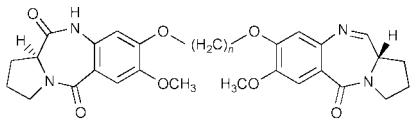
Mixed imine–amide pyrrolobenzodiazepine dimers have been prepared which exhibit potent antitumour activity and have significant DNA binding affinity; one of them, 1c, has been shown to cause a remarkable rise in the melting temperature of calf thymus DNA.

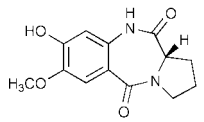
There has been increasing interest in discovering and developing small molecules that are capable of interacting with nucleic acids in a sequence-selective manner.[1–3] Such compounds have potential in the therapy of genetic-based diseases (some cancers),[4] diagnostics, and validation of DNA sequences. The pyrrolo[2,1-*c*][1,4]benzodiazepines (PBD's) are a well known class of antitumour antibiotics with sequence-selective DNA binding ability that are derived from various *Streptomyces* species. Their interaction with DNA has been extensively studied and is considered unique since they bind within the minor groove of DNA, forming a covalent aminal bond between the C11 position of PBD B-ring and the N2-amino group of a guanine base.[5] Further, the requirement of (*S*)-stereochemistry at C11a for these compounds enables a snug fit in the minor groove of DNA.[6] It has also been shown that PBD's inhibit both endonuclease activity and *in vitro* transcription in a sequence-selective manner.[7]

The naturally occurring PBD's (*e.g.* anthramycin and tomaymycin) span approximately 3 base pairs with a preference for purine–guanine–purine sequences. Thurston and co-workers[8] have synthesized C8 diyldioxy ether-linked PBD dimers (DSB-120), that span approximately six base pairs of DNA and in which the sequence selectivity is also increased (*e.g.* purine–GATC–pyrimidine for DSB-120). Moreover, the cytotoxic potency and large change in calf thymus (CT) DNA melting temperature has been attributed to its ability to irreversibly cross-link DNA *via* guanine residues on opposite strands because of the presence of two active sites (*i.e.* two imine functionalities). Recently, another new cross-linking PBD dimer (SJG-136) having C2/C2′-*exo* unsaturation has been prepared by the same group and exhibits extraordinary DNA binding affinity.[9] It has been established that the imine functionality or its equivalent methanolamine form is a primary requirement for the covalent binding, whereas the non-covalent interactions of PBD's with DNA bases help in rationalising the sequence selectivity and drug orientations.[10] Therefore, it has been considered of interest to design and synthesize C8 linked PBD-dimers, wherein one ring of PBD has the imino function while the other has an amide group. It has been envisaged that such a mixed dimer could offer more insight into not only the covalent binding but also the role played by non-covalent interactions with DNA bases.

In recent years a number of hybrid molecules containing the PBD ring system have been synthesized to improve upon the DNA binding ability and sequence selectivity.[11] We have been interested in the structural modifications of the PBD ring system and the development of new synthetic strategies.[12–16] In continuation of these efforts, we report a new synthesis of novel mixed dimers of PBD containing the imino function in one of the PBD rings and an amido group in the other, linked at the C8 position by a suitable alkane spacer. Interestingly, the larger sized spacer (n = 5) increases the melting temperature of CT DNA by a significant 17 °C after 18 h incubation at a (PBD) : (DNA) ratio of 1 : 5 for **1c**.



1a n = 3
1b n = 4
1c n = 5



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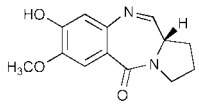
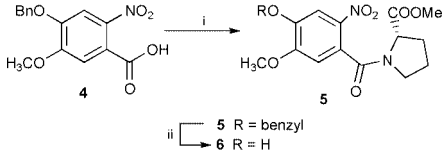


3 (DC-81)

Synthesis of the imine–amide mixed dimers of PBD has been carried out employing the commercially available vanillin. Oxidation of vanillin followed by benzylation and nitration by literature methods[17] provides the starting material **4**. L-Proline methyl ester has been coupled to **4** followed by debenzylation with BF_3·OEt_2–EtSH to give the nitro ester **6** (Scheme 1). Another precursor has been prepared from vanillic acid methyl ester **7** by its etherification with dibromoalkanes to afford **8**. The mono alkylation of **7** has been achieved by using 3 molar equivalents of the dibromo alkanes. Nitration of **8** followed by ester hydrolysis and coupling (2*S*)-pyrrolidinecarbaldehyde diethyl thioacetal gives **11**. The key intermediate has been prepared by linking **6** and **11**. Reduction of both nitro groups provides **13** which has a dilactam moiety at one end and the amino functionality on the other. Deprotection of the thioacetal group by using the method of Thurston and coworkers[8] affords the target molecules **1a**–**c** (Scheme 2).

Interestingly, the data presented in Table 1 show that as the size of the linker spacer increases from n = 3 to 5 the DNA stabilization is also enhanced. In this assay for a 1 : 5 molar ratio of (PBD) : (DNA), one of these mixed imine–amide PBD dimers (**1c**) elevates the helix melting temperature of CT DNA by a remarkable to 17.0 °C after incubation for 18 h at 37 °C. Under similar conditions, the dimer having two imino functionalities *i.e.* DSB-120 provides a ΔT_m of 15.4 °C. On the other hand, the naturally occurring DC-81 having only one imino group



5 R = benzyl
6 R = H

Scheme 1 *Reagents and conditions*: i, $SOCl_2$ L-proline methyl ester hydrochloride, Et_3N, H_2O, 0 °C, 30 min, 85%; ii, BF_3·OEt_2–EtSH, CH_2Cl_2, rt, 8 h, 88%.

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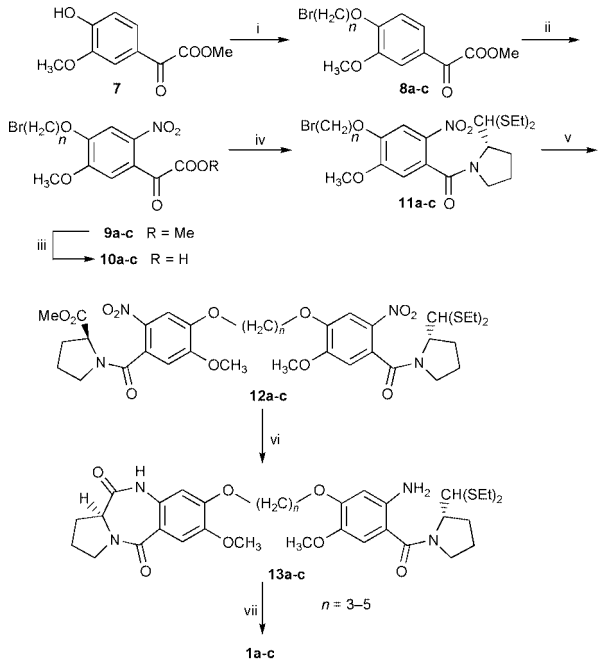

Scheme 2 *Reagents and conditions*: i, $Br(CH_2)_nBr$, K_2CO_3, CH_3COCH_3, reflux, 48 h, 82–86%; ii, $SnCl_4$–HNO_3, CH_2Cl_2, −25 °C, 5 min, 88–91%; iii, 1 M LiOH, THF, MeOH, H_2O (3 : 1 : 1), rt, 12 h, 89–93%; iv, $SOCl_2$ then DMF, THF, H_2O, 2(*S*)-pyrrolidinecarbaldehyde diethyl thioacetal, Et_3N, 3 h, 89–92%; v, **6**, K_2CO_3, CH_3COCH_3, reflux, 48 h, 85–90%; vi, $SnCl_2{\cdot}2H_2O$, MeOH, reflux, 40 min, 80–85%; vii, $HgCl_2$, $CaCO_3$, CH_3CN–H_2O (4 : 1), 3–8 h, 55–61%.

Table 1 Thermal denaturation with calf thymus DNA,[a] at a [PBD] : [DNA] molar ratio of 1 : 5[b] and *in vitro* one dose primary anticancer assay[c] in the NCI-H460, MCF 7 and SF-268 for **1a–c**

Induced ΔT_m/°C[ab] after incubation at 37 °C for			Growth percentages		
Compound	0 h	18 h	(Lung) NCI-H460	(Breast) MCF7	(CNS) SF-268
1a	6.5	7.0	4	10	11
1b	5.0	8.5	−16	−41	−81
1c	14.0	17.0	−39	7	−21
DC-81(**3**)	0.3	0.7	—	—	—
DSB-120	10.2	15.4	—	—	—

[a] For CT-DNA at pH 7.00 ± 0.01, ΔT_m = 66.5 °C ± 0.01 (mean value from 60 separate determinations), all ΔT_m values ± 0.1–0.2 °C. [b] For a 1 : 5 molar ratio of [ligand] : [DNA], where CT-DNA concentration = 100 μM in aqueous buffer [10 mM sodium phosphate + 1 M EDTA, pH 7.00 ± 0.01]. [c] One dose of **1a–c** at 10^{-4} molar concentration.

exhibits a ΔT_m of 0.7 °C. This demonstrates that compound **1c** containing a single imino functionality has a very significant DNA binding affinity. To the best of our knowledge, this is the first synthetic non-cross-linking molecule to exhibit a remarkable DNA binding effect similar to the naturally occurring sibiromycin (ΔT_m = 16.3 °C at 18 h).[11] These data indicates that non-covalent interactions play an important role for the enhancement of DNA binding affinity. The preliminary anticancer assays carried out on three human cell lines; lung (NCI-H460), breast (MCF7) and CNS (SF-268), exhibit significant anticancer activity for these compounds as illustrated in Table 1.

In summary, the synthesis of **1a–c**† reported here describes the importance of non-covalent interactions for increasing the DNA binding affinity and potent antitumour activity of the non-cross-linking mixed imine–amide PBD dimers. These findings may allow researchers to design newer analogues with improved therapeutic potential, particularly for antitumour activity. The sequence selectivity, endonuclease activity and detailed anticancer activity of these non-cross-linking PBD compounds will be reported elsewhere.

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Notes and references

† *Selected data for compound* **1a**: δ_H(200 MHz, DMSO-d_6 + $CDCl_3$) 1.89–2.5 (m, 10H), 3.4–4.05 (m, 13H), 4.1–4.4 (m, 3H), 6.6 (s, 1H), 6.82 (s, 1H), 7.4 (s, 1H), 7.5 (s, 1H), 7.65 (d, 1H), 9.9 (s, 1H, NH exchangeable); ν_{max} (KBr)/cm^{-1} 3450–3460 (br), 2993, 2345, 1686, 1654, 1606, 1518, 1491, 1437, 1384, 1265, 1228, 1119, 1021, 839, 784; $[\alpha]_D^{30}$ +202.6 (*c* 0.5, $CHCl_3$); *m/z* (FAB): 549 $(M + H)^+$ (calc. for $C_{29}H_{31}N_4O_7$).

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