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Design and Synthesis of Novel Chrysene-Linked Pyrrolo[2,1-*c*][1,4]-benzodiazepine Hybrids as Potential DNA-Binding Agents

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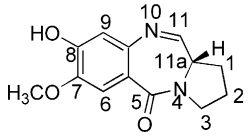
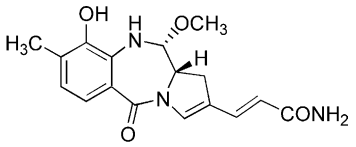
Abstract—Chrysene-linked pyrrolobenzodiazepine hybrids have been prepared that posses cytotoxicity in some cancer cell lines. They also exhibit promising DNA-binding affinity and this is supported by molecular modeling studies.

The pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs) are a family of DNA interactive antitumour antibiotics derived from *Streptomyces* species.[1] The PBD class of compounds that include the naturally occurring anthramycin and DC-81, owes its DNA-interactive ability and resultant biological effects to an N10–C11 carbinol amine/imine moiety in the central B-ring, which is capable of covalently binding to the N2 of the guanine residues in the minor groove of DNA. X-ray and DNA foot printing studies on covalent adducts have demonstrated a high sequence specificity for G-C rich DNA regions, in particular for Pu-G-pu triplets.[2]



DC-81



Anthramycin

A large number of PBD conjugates have been prepared and evaluated for their biological activity, particularly their antitumour potential.[3] Thurston and co-workers have synthesized novel C2-aryl pyrrolobenzodiazepines (PBDs) as potential antitumour agents.[4] Recently, we have designed and synthesized non-cross-linking mixed imine–amide PBD dimers that have significant DNA-binding ability and potent antitumour activity.[5] Many compounds that have planar ring systems possess chemotherapeutic activity by intercalating with DNA.[6]

Recently, chrysene derivatives have been synthesized and evaluated for their antitumour activity and DNA intercalating potential. Among them, some of the of 2-[(arylmethyl)amino]-1,3-propanediol derivatives such as 770U82 (crisnatol), 773U82 and 502U83 are currently in different phases of clinical trials as potential antitumour agents.[7] Therefore, based on these findings, it has been considered of interest to link the polycyclic aromatic rings like chrysene at C8 position of PBD ring system through an alkylamide spacer. The design of such hybrids of PBD linked to highly lipophilic aromatic amines could result in primarily, selective interactions with cancer cells as a major affect in cell killing. We have been engaged in the last few years in the structural modifications[8] and the development of new synthetic strategies[9] for the PBD-based ring systems. In continuation of these efforts, we herein report the design, synthesis, DNA-binding affinity and anticancer potential of chrysene linked PBD hybrids.

Synthesis of the novel PBD hybrids has been carried out employing the amino chrysene **2** have been obtained by the reduction of the nitro chrysene **1** by $SnCl_2.2H_2O$ in EtOAc (Scheme 1). The (2*S*)-*N*-(4-benzyloxy-5-methoxy-2-nitrobenzoyl)pyrrolidine-2-carboxaldehyde diethylthioacetal **3** has been prepared by literature method,[10] which upon debenzylation gives **4**. Etherification of (2*S*)-*N*-(4-hydroxy-5-methoxy-2-nitrobenzoyl)-pyrrolidine-2-carboxaldehyde diethylthioacetal **4** by methyl bromoalkanoates provides **5a–b**. Basic hydrolysis of these esters **5a–b** gives the desired precursor acids **6a–b**. Amidation of these with chrysene amine **2** in presence of isobutyl chloroformate triethyl amine affords the corresponding nitrothioacetal intermediates

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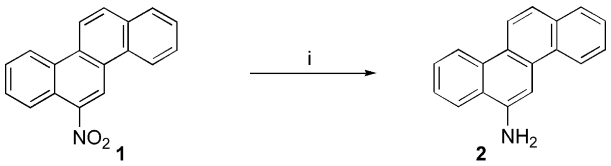


Scheme 1. (i) $SnCl_2{\cdot}2H_2O$, EtOAc, 3 h, 80%.

7a–b in moderate yields. Further, these upon reduction by $SnCl_2.2H_2O$ in EtOAc and followed by deprotection of aminothioacetal precursors **8a–b** using $HgCl_2/CaCO_3$ affords the chrysene liked PBD hybrids **9a–b** (Scheme 2).[11]

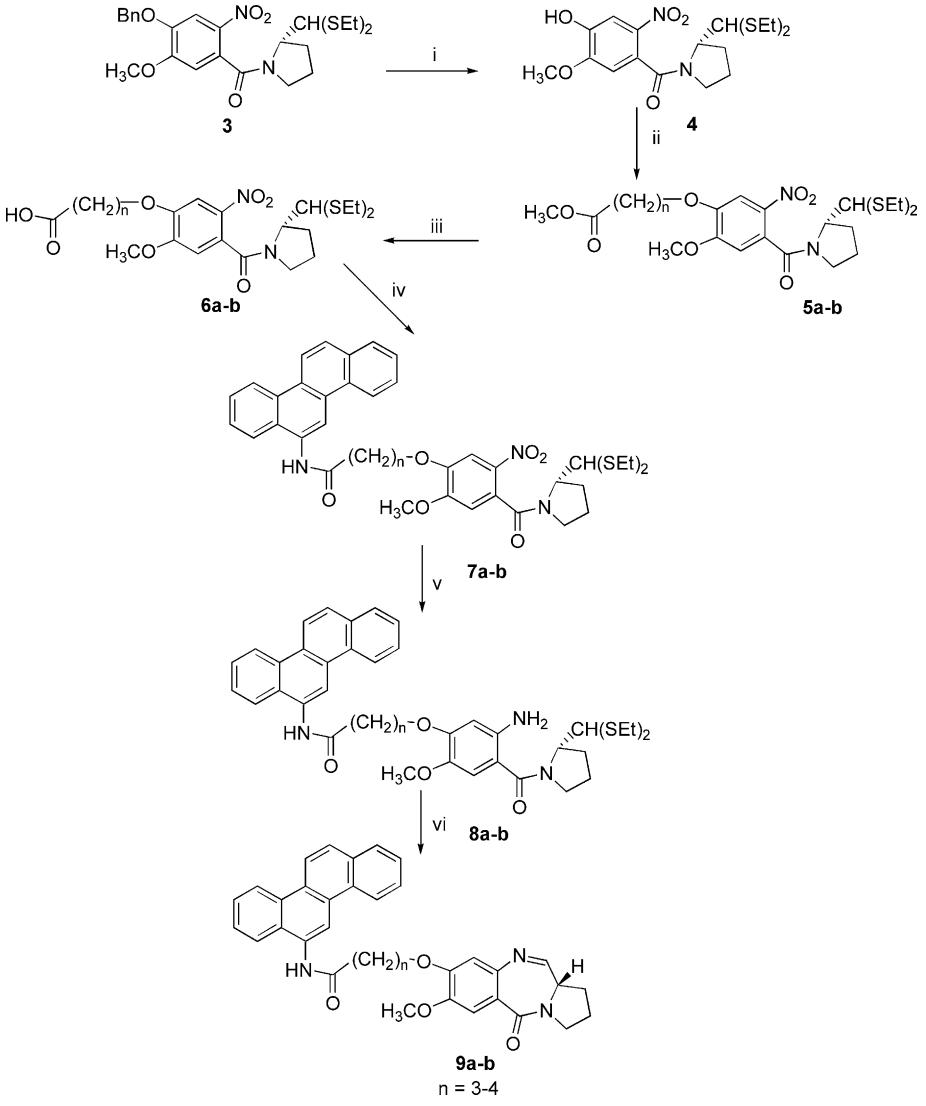
Compounds **9a–b** have been evaluated for the primary anticancer activity in the standard three-cell line panel consisting of the MCF7 (breast), NCI-H460 (lung) and SF-268 (CNS). The results are described in Table 1. Amongst these hybrids **9b** have promising anticancer activity and further, the PBD hybrid **9b** has shown to possess <10 micro molar potency (at the LC_{50} level) against one non-small cell lung cancer (NCI-H226, 4.5 μM), one melanoma cancer (UACC-62, 4.07 μM) and one renal cancer (A498, 6.29 μM) in the 60-cell line panel. The in vitro cytotoxicity (IC_{50}) for the naturally occurring DC-81[12] is 0.38 and 0.33 μM in L1210 and PC6 cell lines, respectively.

Table 1. In vitro one dose primary anticancer assay[a] chrysene linked PBD hybrids[a]

PBD hybrids	Growth percentages		
	(Lung) NCI-H460	(Breast) MCF7	(CNS) SF-268
9a	105	56	121
9b	0	1	12

[a]One dose of **9a–b** at 10^{-4} molar concentration.

The DNA binding activity for these novel chrysene linked PBD hybrids has been examined by thermal denaturation studies using calf thymus (CT) DNA.[5] Melting studies show that these compounds stabilize the



Scheme 2. (i) EtSH–BF_3OEt_2, CH_2Cl_2, 12 h, rt, 75%; (ii) methyl bromoalkanoate, K_2CO_3, DMF, 24 h, rt, 89–91%; (iii) 1 N LiOH, THF–MeOH–H_2O, 12 h, rt, 74–78%; (iv) isobutyl chloroformate, Et_3N, compound **2**, 12 h, rt, 59–62%; (v) $SnCl_2{\cdot}2H_2O$, EtOAc, reflux, 3 h, 72–72%; (vi) $HgCl_2$–$CaCO_3$, CH_3CN–H_2O, 12 h, rt, 50–52%.

Table 2. Thermal denaturation data for chrysene linked PBD hybrids with CT-DNA

PBD hybrids	[PBD]/[DNA] molar ratio[b]	ΔT_m (°C)[a] after incubation at 37 °C for	
		0 h	18 h
9a	1:5	2.6	2.8
9b	1:5	1.2	1.8
DC-81	1:5	0.3	0.7

[a]For CT-DNA alone at pH 7.00 ± 0.01, T_m = 69.2 °C ± 0.01 (mean value from 10 separate determinations), all ΔT_m values are ± 0.1–0.2 °C.
[b]For a 1:5 molar ratio of [PBD]/[DNA], where CT-DNA concentration = 100 μM and ligand concentration = 20 μM in aqueous sodium phosphate buffer [10 mM sodium phosphate + 1 mM EDTA, pH 7.00 ± 0.01].

Table 3. The values of energy of interactions calculated for the DNA-PBD hybrid complexes

Complex	Energy of interaction (E_{int}) in kcal mol^{-1}
DNA–9a	−65.6
DNA–9b	−62.4

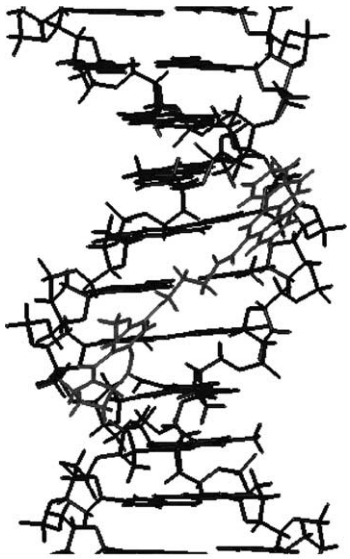


Figure 1. Projection diagram showing the **DNA-9a** complex, side-on view.

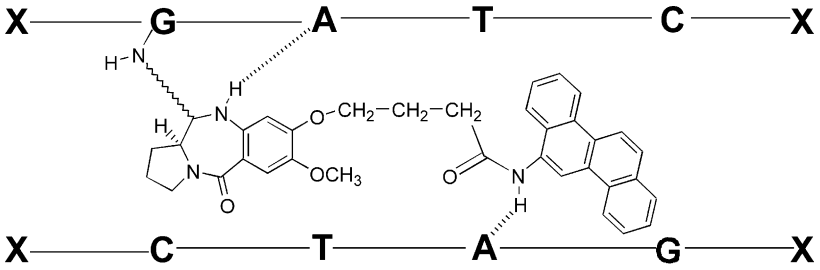


Figure 2. Schematic representation of the preferred DNA binding sites of chrysene linked PBD hybrid **9a**.

thermal helix-coil or melting stabilization (ΔT_m) for the CT-DNA duplex at pH 7.0, incubated at 37 °C, where PBD/DNA molar ratio is 1:5. Interestingly, in this assay, one of the chrysene-linked PBD hybrid (**9a**) elevates the helix melting temperature of CT-DNA by a 2.8 °C after incubation for 18 h at 37 °C. On the other hand, the naturally occurring DC-81 exhibits a ΔT_m of 0.7 °C. This demonstrates the hybrid **9a** has moderate DNA-binding affinity, as illustrated in Table 2.

The difference of DNA binding affinity with respect to the linker length in this class of hybrids has been investigated by molecular modeling studies. Energetically favorable models of the DNA–PBD hybrid complexes for **9a–b** molecules were built using the systematic procedure as described in our previous report,[5] involving molecular modeling and docking followed by detailed molecular dynamic stimulations. The DNA sequence that has been used for this study is 5′-CGCAGATCTGCG. The energy of interaction (E_{int}) between the DNA and the PBD-hybrid molecule in a complex was calculated as a measure of stability of that complex. The values of E_{int} obtained for the DNA–PBD complexes are shown in Table 3. The lowest value of E_{int} corresponds to the DNA-**9a** complex indicating that the molecule **9a** forms energetically the most stable complex with DNA (Figs 1 and 2). Further, it has been observed that in these compounds as the carbon chain number reduces from three to two the stability of their DNA complexes decreases (calculated E_{int} values of chrysene-linked PBD hybrids for the two carbon chain from molecular modeling are 57.4 kcal mol^{-1}). From the above data it appears that there is no clear relationship between anticancer activity and DNA-binding affinity for these compounds while membrane interaction as the primary effect for anticancer activity cannot be ruled out. This aspect of membrane interaction has been discussed in detail by Banik et al.[7] on the mechanism of antitumour activity of polyaromatic hydrocarbons.

In conclusion, new chrysene-linked PBD hybrids have been synthesized that exhibit cytotoxic activity in some cancer cell lines. Some of these hybrids have promising DNA-binding ability that has been supported by the molecular modeling studies. The detailed mechanistic studies of these PBD hybrids is in progress.

Acknowledgements

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11. Spectral data for compound **9a** ^{1}H NMR ($CDCl_3$) δ 1.40–2.50 (m, 8H), 2.85–3.00 (m, 2H), 3.45–3.85 (m, 4H), 4.10–4.32 (m, 2H), 6.85 (s, 1H), 7.40–7.70 (m, 5H), 7.85–8.05 (m, 3H), 8.40–8.80 (m, 4H), 9.0–9.10 (m, 1H); MS (FAB) 558 $[M+H]^{+\cdot}$. Compound **9b** ^{1}H NMR ($CDCl_3$) δ 1.42–2.53 (m, 10H), 2.81–3.05 (m, 2H), 3.40–3.85 (m, 4H), 4.15–4.30 (m, 2H), 6.84 (s, 1H), 7.40–7.72 (m, 5H), 7.85–8.10 (m, 3H), 8.40–8.80 (m, 4H), 9.05–9.12 (m, 1H); MS (FAB) 572 $[M+H]^+$.
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