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Bioorganic & Medicinal Chemistry Letters
ELSEVIER
Bioorganic & Medicinal Chemistry Letters 14 (2004) 471–474

Synthesis and antitumour activity of pyrene-linked pyrrolo [2,1-*c*][1,4]benzodiazepine hybrids

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Received 7 August 2003; accepted 23 October 2003

Abstract—Pyrene-linked pyrrolobenzodiazepine hybrids have been synthesized that exhibit potential anticancer activity in a number of human tumour cell lines. These hybrids also exhibit much enhanced DNA-binding ability in comparison to the parent pyrrolobenzodiazepine ring system (DC-81).

The pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs) are naturally occurring antitumour antibiotics isolated from *Streptomyces* species.[1] These compounds are known to exhibit antitumour activity based on their covalent binding ability to the N2 of the guanine residues in the minor groove of DNA. These properties have been investigated in detail for such compounds that form covalent adducts in the minor groove of DNA and show sequence specificity for GC-rich DNA regions, in particular for Pu-G-pu triplets.[2] In recent years, a number of PBD based symmetrical and unsymmetrical DNA cross-linking agents have been designed and synthesized that exhibit significant DNA binding and anticancer activity.[3] In our studies, we have investigated the non-cross linking PBD dimers that exhibit remarkable DNA binding in the absence of cross linking.[4] In this context many PBD conjugates have been synthesized and investigated for their anticancer activity.[5] A number of polyaromatic hydrocarbons (PAH) and their derivatives in view of their planar ring system are known to intercalate with DNA resulting in the anticancer activity.[6] Some of the polyaromatic hydrocarbons (PAH) based on pyrene and chrysene derivatives like (1-pyrenylmethyl)amino alcohol derivatives **1**, crisnatol **2** (770U82) have undergone different stages of clinical trials.[7] Recently, we have synthesized some hybrid molecules **3** wherein chrysene is linked to PBD at C8 position through an alkylamide spacer.[8] It has been envisaged that such hybrids of PBD that are linked to polyaromatic hydrocarbons (PAH) may produce enhanced anticancer activity. Our interest in the structural modifications and development of new synthetic strategies on the PBD ring system[9] has lead to the design and synthesis of pyrene-linked PBD hybrids as potential DNA binding anticancer agents.[10]

Synthesis of these pyrene-linked PBD hybrids has been carried out by employing aminopyrene **6** as one of the starting materials and this has been obtained by the reduction of the nitropyrene **5** by $SnCl_2{\cdot}2H_2O$ in EtOAc (Scheme 1). Whereas, (2*S*)-*N*-(4-benzyloxy-5-methoxy-2-nitro-benzoyl)pyrrolidine-2-carboxaldehyde diethylthioacetal **7** has been prepared by literature method,[11] which upon debenzylation gives **8**. Etherification of (2*S*)-*N*-(4-hydroxy-5-methoxy-2-nitrobenzoyl)pyrrolidine-2-carboxaldehyde diethylthioacetal **8** by methyl bromoalkanoates provides **9a–b**. Basic hydrolysis of these esters **9a–b** give the desired precursor acids **10a–b**. The key intermediates **11a–b** have been prepared by amidation of **6** with the acid precursors **10a–b**. Further, these upon reduction and followed by deprotection of aminothioacetal precursors **11a–b** afford the target pyrene-linked PBD hybrids **4a–b** (Scheme 2).[12]

Amongst these hybrids **4a** has shown promising anticancer activity in 60-cell line panel. The LC_{50} values of compound **4a** against leukemia (SR), non-small cell lung cancer (NCI-H226), colon (HCC-2298), CNS (SF-539 and SNB-19), melanoma (M14 and A498) and prostate (DU-145) have been illustrated in Table 1. The in vitro cytotoxicity (IC_{50}) for the naturally occurring

Keywords: Pyrrolobenzodiazepines; Pyrene amine; Cytotoxity; DNA-binding affinity.
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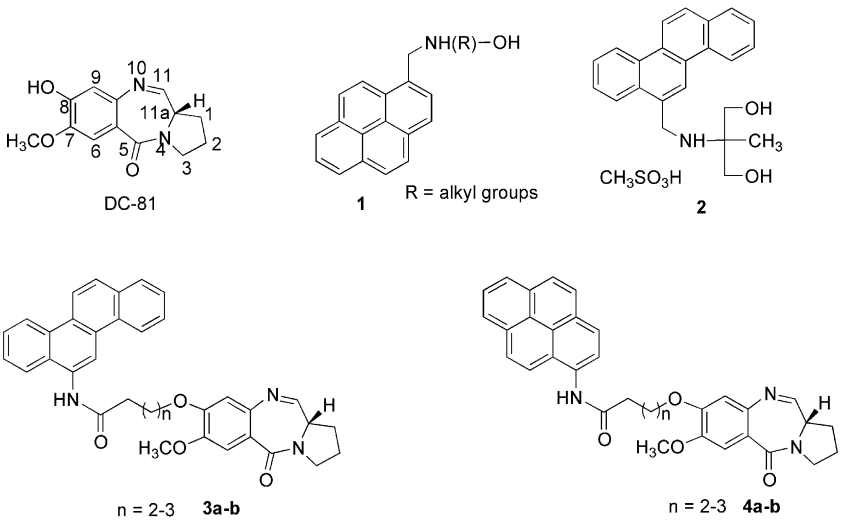
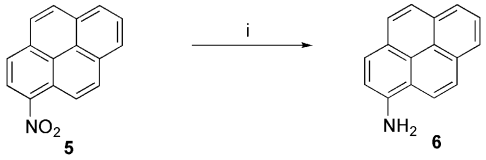
doi:10.1016/j.bmcl.2003.10.050





Scheme 1. (i) $SnCl_2{\cdot}2H_2O$, EtOAc, 3 h, 76%.

Table 1. In vitro cytotoxicity of compound **4a** in selected human cancer cell lines

Cancer panel/cell line	LC_{50} (μM)
Leukemia	
SR	0.05
Non-small cell lung	
NCI-H226	<0.01
Colon	
HCC-2298	0.01
CNS	
SF-539	0.18
SNB-19	0.42
Melanoma	
M14	0.05
A498	0.04
Prostate	
DU-145	0.24

DC-81[13] is 0.38 and 0.33 μM in L1210 and PC6 cell lines, respectively. It is interestingly to observe that compound **4a** has promising anticancer activity in number of cancer cell lines.

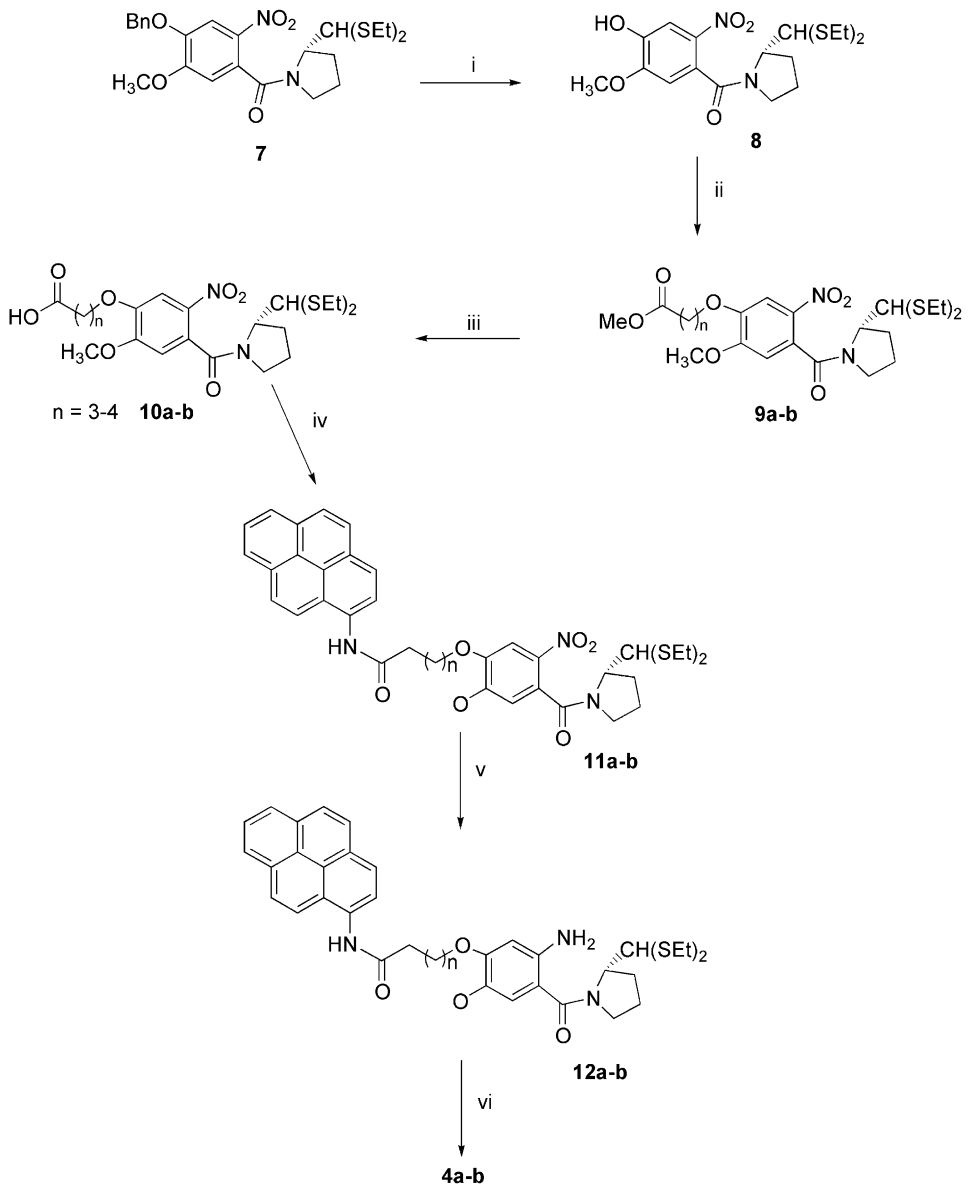
The DNA binding ability for these pyrene-linked PBD hybrids has been examined by thermal denaturation studies using calf thymus (CT) DNA. Interestingly, in this assay one of the pyrene-linked PBD hybrid (**4a**) elevates the helix melting temperature of CT-DNA by a 6.5 °C after incubation for 18 h at 37 °C. On the other hand, the naturally occurring DC-81 exhibits a ΔT_m of 0.7 °C. This demonstrates that hybrid **4a** has significant DNA binding affinity as illustrated in Table 2. This increase of melting temperature could be attributed to the additional effect of intercalation with DNA by the pyrene subunit apart from the covalent binding that may take place by the PBD ring system.

In conclusion, one of the new pyrene-linked PBD hybrid (**4a**) not only exhibits promising cytotoxic activity in a number of cancer cell lines but also shows good DNA-binding ability. The detailed biological and molecular modeling studies of chrysene and pyrene linked PBD hybrids are in progress.

Table 2. Thermal denaturation data for pyrene-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
4a	1:5	6.1	6.5
4b	1:5	3.6	3.8
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).



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 **6**, 12 h, rt, 55–56%; (v) $SnCl_2{\cdot}2H_2O$, EtOAc, reflux, 3 h, 70–72%; (vi) $HgCl_2$–$CaCO_3$, CH_3CN–H_2O, 12 h, rt, 49–54%.

Acknowledgements

We thank the National Cancer Institute, Maryland, USA for the in vitro anticancer assay in human cancer cell lines. We are also grateful to CSIR, New Delhi for the award of research fellowships to G.R., O.S. and P.R.

References and notes

1. Kamal, A.; Rao, M. V.; Laxman, N.; Ramesh, G.; Reddy, G. S. K. *Current Medicinal Chemistry — Anti-Cancer Agents* **2002**, *2*, 215.
2. Kopka, M. L.; Goodsell, D. S.; Baikalov, I.; Grzeskowiak, K.; Cascio, D.; Dickerson, R. E. *Biochemistry* **1994**, *33*, 13593.
3. (a) Gregson, S. J.; Howard, P. W.; Hartley, J. A.; Brooks, N. A.; Adams, L. J.; Jenkins, T. C.; Kelland, L. R.; Thurston, D. E. *J. Med. Chem.* **2001**, *44*, 737. (b) Zhou, Q.; Duan, W.; Simmons, D.; Shayo, Y.; Raymond, M. A.; Dorr, R. T.; Hurley, L. H. *J. Am. Chem. Soc.* **2001**, *123*, 4865. (c) Tercel, M.; Stribbling, S. M.; Sheppard, H.; Siim, B. G.; Wu, K.; Pullen, S. M.; Botting, K. J.; Wilson, W. R.; Denny, W. A. *J. Med. Chem.* **2003**, *46*, 2132.
4. Kamal, A.; Ramesh, G.; Laxman, N.; Ramulu, P.; Srinivas, O.; Neelima, K.; Kondapi, A. K.; Srinu, V. B.; Nagarajaram, H. M. *J. Med. Chem.* **2002**, *45*, 4679.
5. (a) Reddy, B. S. P.; Damayanthi, Y.; Reddy, B. S. N.; Lown, W. *J. Anti-Cancer Drug Design* **2000**, *15*, 225. (b) Kamal, A.; Laxman, N.; Ramesh, G.; Srinivas, O.; Ramulu, P. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1917. (c) Kamal, A.; Reddy, B. S. N.; Reddy, G. S. K.; Ramesh, G. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1933.
6. (a) Venitt, S.; Crofton-Sleigh, C.; Agbandje, M.; Jenkins, T. C.; Neidle, S. *J. Med. Chem.* **1998**, *41*, 3748. (b) Perry, P. J.; Gowan, S. M.; Reszka, A. P.; Polucci, P.; Jenkins,

T. C.; Kelland, L. R.; Neidle, S. *J. Med. Chem.* **1998**, *41*, 3253.
7. (a) Banik, B. K.; Becker, F. F. *Bioorg. Med. Chem.* **2001**, *9*, 593. (b) Becker, F. F.; Banik, B. K. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2877. (c) Bair, K. W.; Andrews, C. W.; Tuttle, R. L.; Knick, V. C.; Cory, M.; McKee, D. D. *J. Med. Chem.* **1991**, *34*, 1983. (d) Bair, K. W.; Andrews, C. W.; Tuttle, R. L.; Knick, V. C.; Cory, M.; McKee, D. D. *J. Med. Chem.* **1990**, *33*, 2385.
8. Kamal, A.; Ramesh, G.; Ramulu, P.; Srinivas, O.; Rehana, T.; Sheelu, G. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3451.
9. (a) Kamal, A.; Ramulu, P.; Srinivas, O.; Ramesh, G. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3517. (b) Kamal, A.; Srinivas, O.; Ramulu, P.; Ramesh, G.; Kumar, P. P. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3577. (c) Kamal, A.; Ramulu, P.; Srinivas, O.; Ramesh, G. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3955. (d) Kamal, A.; Reddy, P. S. M. M.; Reddy, D. R. *Tetrahedron Lett.* **2003**, *44*, 2857.
10. Kamal, A.; Ramesh, G.; Ramulu, P.; Srinivas, O. Indian Patent Appl. No. 456/DEL/03, US and PCT Patent Applied (March 2003).
11. Thurston, D. E.; Murty, V. S.; Langley, D. R.; Jones, G. B. *Synthesis* **1990**, 81.
12. Spectral data for compound **4a**: ^{1}H NMR ($CDCl_3$) δ, 1.40–2.40 (m, 8H), 2.60–2.90 (m, 2H), 3.40–4.05 (m, 4H), 4.10–4.40 (m, 2H), 6.85 (s, 1H), 7.40 (s, 1H), 7.65 (d, 1H, $J = 4.5$ Hz), 7.75–8.20 (m, 8H), 8.20–8.40 (m, 1H), 9.0–9.10 (m, 1H), MS (FAB) 532 $[M+H]^+$.
13. Bose, D. S.; Thompson, A. S.; Smellie, M.; Berardini, M. D.; Hartley, J. A.; Jenkins, T. C.; Neidle, S.; Thurston, D. E. *J. Chem. Soc., Chem. Commun.* **1992**, 1518.