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Pyrrolo[2,1-*c*][1,4]benzodiazepine–anthraquinone conjugates. Synthesis, DNA binding and cytotoxicity

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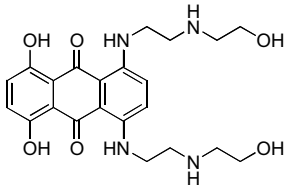
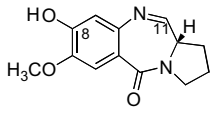
Abstract—New pyrrolobenzodiazepine–anthraquinone hybrids have been designed and synthesized, found to effectively bind to DNA and also exhibit cytotoxicity against many cancer cell lines.

In recent years, combination chemotherapy employing antineoplastic agents with different mechanisms of action is one of the methods that is being adopted to combat cancer. Therefore a single molecule containing two functional groups, each with different type of action could also be beneficial in the treatment of cancer. Intercalating agents such as anthracenediones represent an important class of antineoplastic agents.[1,2] These compounds contain a planar chromophore that inserts between two base pairs in the DNA helix, resulting in miscoding and possible cell death. Mitoxantrone, which is a lead compound in this series is generally used in clinic for treatment of some haematological malignancies, ovarian and breast cancers.[3] Other anthraquinone-type analogues, such as bisantrene,[4] chrysophanol and emodine[5] have exhibited significant in vivo cytotoxic activities.

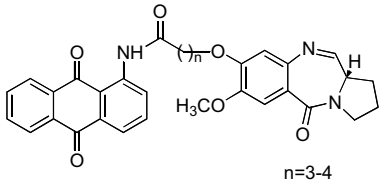
The pyrrolo[2,1-*c*][1,4]benzodazepines (PBDs) are a group of naturally occurring antitumour antibiotics produced by various *Streptomyces* species and examples of which include anthramycin, tomaymycin, sibiromycin and DC-81. The formation of a covalent bond in the minor groove of DNA by nucleophilic attack of 2-amino group of a guanine base to form an aminal linkage to C-11 is responsible for the biological activities of PBDs.[6] X-ray and foot-printing studies on covalent DNA–PBD adducts have demonstrated a high sequence specificity for GC-rich DNA regions in particular, for Pu–G–Pu motifs.[7] In the past few years, several hybrid compounds, in which known antitumour compounds or simple active moieties of known antitumour agents tethered to PBD, have been designed, synthesized and evaluated for their biological activity.[8–11] Recently, we have been involved in the development on new synthetic strategies[12] for the preparation of PBD ring systems and also



mitoxantrone



DC-81



n=3-4

1a-b

Keywords: Anthraquinones; Pyrrolobenzodiazepines; Cytotoxicity; DNA binding.
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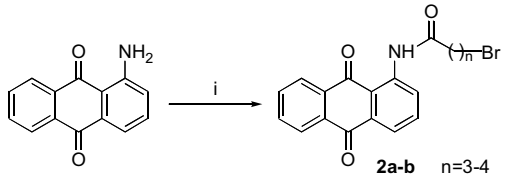
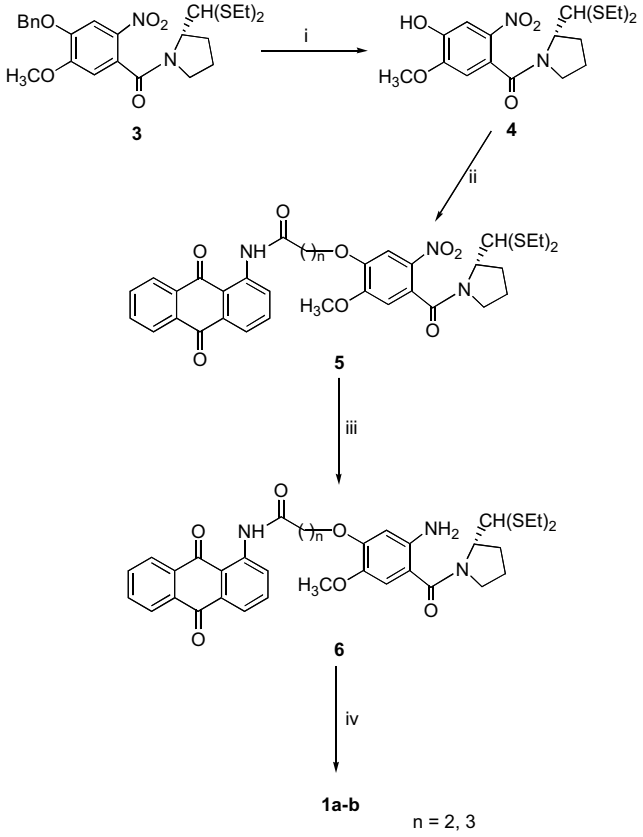
in the design of structurally modified PBDs and their hybrids.[13] In this pursuit some novel PBD hybrids and conjugates have been prepared by linking the PBD moiety to the DNA interactive compounds through suitable alkane spacers. In continuation of these efforts, it has been considered of interest to combine features of intercalating agent like that of anthraquionone family with the DNA binding agent that is, PBD moiety, which led to the synthesis of the compounds **1a** and **1b**. The anthraquinone has been linked through its amino functionality to the C8 position of the A-ring of PBD. It has been envisaged that the combination of DNA intercalating and binding properties in such conjugates could be attractive targets for their DNA binding potential and antitumour activity.

Synthesis of these novel anthraquinone–PBD conjugates has been carried out by employing (9,10-dihydro-9,10-dioxo-1-anthracenyl)-1-bromo-alkanamide **2** as one of the starting materials, which has been obtained by the reaction of an appropriate acid chloride with amino anthraquinone (Scheme 1). The other precursor (2*S*)-N-(4-benzyloxy-5-methoxy-2-nitrobenzoyl)pyrrolidine-2-carboxaldehyde diethyl thioacetal **3** has been prepared by literature method,[14] which on debenzylation gives (2*S*)-N-(4-hydroxy-5-methoxy-2-nitrobenzoyl)pyrrolidine-2-carboxaldehyde diethyl thioacetal **4**. Etherification of **4** by (9,10-dihydro-9,10-dioxo-1-anthracenyl)-1-bromo-alkanamide gives the desired intermediate **5**. This nitrothioacetal has been reduced with $SnCl_2{\cdot}2H_2O$ to give **6**. The deprotection of the thioacetal group afforded the desired compounds **1a**–**b** (Scheme 2).[15]

Compounds **1a**–**b** have been evaluated for their in vitro cytotoxicity in selected human cancer cell lines of colon (HT-29, HCT-15), lung (A-549, HOP-62), cervix (SiHa) origin by using SRB method. Usually, when the concentration of the compound solution is 10^{-6} mol/L, the inhibition of the solution is more than 50%, then compound is considered as an effective agent. According to this standard, it has been observed from Table 1 that both **1a** and **1b** exhibit a strong effect to HT-29, HCT-15, HOP-62 cell lines. However, the in vitro cytotoxicity (IC_{50}) for naturally occurring DC-81[16] is 0.38 and 0.33 μM in L1210 and PC6 cell lines, respectively, and in case of 1,4-disubstituted anthraquinones, for example, mitoxantrone[17] the activity is 0.1 μM in L1210 cells. Therefore, the in vitro cytotoxicity exhibited by these new PBD–anthraquinones is highly significant.



Scheme 1. Reagents and conditions: (i) bromo alkionyl chloride, pyridine, toluene, 60 °C, 82%.



Scheme 2. Reagents and conditions: (i) $EtSH–BF_3OEt_2$, CH_2Cl_2, 12 h, rt, 75%; (ii) compound **2**, K_2CO_3, acetone, 15 h, reflux, 90–92%; (iii) $SnCl_2{\cdot}2H_2O$, MeOH, 4 h, reflux, 76%; (iv) $HgCl_2$, $CaCO_3$, CH_3CN/H_2O, 12 h, rt, 52–56%.

The DNA binding ability for these hybrids has been examined by using thermal denaturation assay with calf thymus (CT) DNA. Interestingly, the compounds **1a** and **1b** elevates the helix melting temperature of CT-DNA by 9.1 and 5.2 °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 these PBD–anthraquinone conjugates have significant DNA binding ability as illustrated in Table 2. In the literature,[17] some substituted amido anthraquinones have shown ΔT_m values in the range of 8.5–10.0 °C. However, mitoxantrone gave a ΔT_m of 15.9 °C with calf thymus DNA at phosphate-drug ratio 10:1. It is observed that the other hybrids such as chrysene[13c] linked PBD have exhibited ΔT_m values in the range of 1.8–2.8 °C. Whereas, the higher T_m values in the case of anthraquinone–PBD hybrids are probably because of the presence of additional carbonyl groups, which could enable to produce additional noncovalent interactions.

In conclusion, these new conjugates synthesized by the combination of both DNA binding pyrrolobenzodiazepines and DNA intercalating anthraquinones have exhibited significant in vitro antitumour activity and DNA binding affinity. The detailed biological and molecular modelling studies are in progress.

Table 1. The percentage growth inhibition data for anthraquinone–PBD hybrids

Compd (mol/L)	Cell lines														
	HT-29			HCT-15			A-549			HOP-62			SiHa		
	10^{-4}	10^{-5}	10^{-6}	10^{-4}	10^{-5}	10^{-6}	10^{-4}	10^{-5}	10^{-6}	10^{-4}	10^{-5}	10^{-6}	10^{-4}	10^{-5}	10^{-6}
1a	93	86	68	NT	66	59	93	27	47	NT	90	74	57	52	41
1b	93	87	73	NT	82	61	93	10	56	NT	97	73	73	49	40

NT: Not tested.

Table 2. Thermal denaturation data for anthraquinone–PBD hybrids with CT-DNA

Compound	Induced ΔT_m °C after incubation at 37 °C	
	0 h	18 h
1a	8.9	9.1
1b	5.1	5.2
DC-81	0.3	0.7

For CT-DNA alone at pH 7.00 ± 0.01, T_m = 69.8 °C ± 0.01 (mean value from 10 separate determinations), all ΔT_m values are ±0.1–0.2 °C. 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].

Acknowledgements

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References and notes

1. Lown, J. W. *Anthracyclines and Anthracenedione-Based Anticancer Agents*; Elsevier Science: Amsterdam, 1988.
2. Reszka, K.; Kolodziejczyk, P.; Hartley, J. A.; Wilson, W. D.; Lown, J. W. In *Bioactive Molecules*; Lown, J. W., Ed.; Elsevier: Amsterdam, 1988; Vol. 6, pp 401–445.
3. (a) Henderson, C.; Allegra, J. C.; Woodcock, T. *J. Clin. Oncol.* **1989**, *7*, 560; (b) Faulds, D.; Balfour, J. A.; Chrisp, P.; Langtry, H. D. *Drugs* **1991**, *41*, 400; (c) Stuart-Harris, R. C.; Bozek, T.; Pavlidis, N. A.; Smith, I. E. *Cancer Chemother. Pharmacol.* **1984**, *12*, 1.
4. Murdock, K. C.; Child, R. G.; Lin, Y. *J. Med. Chem.* **1982**, *25*, 505.
5. (a) Koyama, M.; Takahshi, K. *J. Med. Chem.* **1989**, *32*, 1594; (b) Kupchan, S. M.; Karim, A. *Lloydia* **1976**, *39*, 223.
6. (a) Thurston, D. E. In *Molecular Aspects of Anticancer Drug DNA Interactions*; Neidle, D., Waring, M. J., Eds.; London: Mcmillan, 1993; p 54; (b) Tender, M. D.; Korman, S. *Nature* **1963**, *199*, 501; (c) Hurley, L. H.; Petrusek, R. L. *Nature* **1979**, *282*, 529.
7. Kopka, M. L.; Goodsell, D. S.; Baikalov, I.; Grzeskowiak, K.; Cascio, D.; Dickerson, R. E. *Biochemistry* **1994**, *33*, 13593.
8. (a) Thurston, D. E.; Morris, S. J.; Hartley, J. A. *Chem. Commun.* **1996**, 563; (b) Wilson, S. C.; Howard, P. W.; Forrow, S. M.; Hartley, J. A.; Adams, L. J.; Jenkins, T. C.; Kelland, L. R.; Thurston, D. E. *J. Med. Chem.* **1999**, *42*, 4028.
9. 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.
10. Baraldi, P. G.; Balboni, G.; Cacciari, B.; Guiotto, A.; Manfredini, S.; Romagnoli, R.; Spalluto, G.; Thurston, D. E.; Howard, P. W.; Bianchi, N.; Rutigliano, C.; Mischiati, C.; Gambari, R. *J. Med. Chem.* **1999**, *42*, 5131.
11. Damayanthi, Y.; Reddy, B. S. P.; Lown, J. W. *J. Org. Chem.* **1999**, *64*, 290.
12. (a) Kamal, A.; Laxman, E.; Reddy, P. S. M. M. *Tetrahedron Lett.* **2000**, *41*, 7743; (b) Kamal, A.; Reddy, G. S. K.; Raghavan, S. *Bioorg. Med. Chem. Lett.* **2001**, *13*, 387; (c) Kamal, A.; Reddy, P. S. M. M.; Reddy, D. R. *Tetrahedron Lett.* **2003**, *44*, 2557.
13. (a) 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; (b) Kamal, A.; Reddy, B. S. N.; Reddy, G. S. K.; Ramesh, G. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1933; (c) Kamal, A.; Ramesh, G.; Ramulu, P.; Srinivas, O.; Rehana, T.; Sheelu, G. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3451; (d) Kamal, A.; Ramulu, P.; Srinivas, O.; Ramesh, G. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3517; (e) Kamal, A.; Srinivas, O.; Ramulu, P.; Ramesh, G.; Kumar, P. P. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3577; (f) Kamal, A.; Reddy, P. S. M. M.; Reddy, D. R. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2669.
14. Thurston, D. E.; Murthy, V. S.; Langley, D. R.; Jones, G. B. *Synthesis* **1990**, 81.
15. Spectral data for compound **1a**: ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.05 (m, 2H), 2.20–2.40 (m, 4H), 2.81 (m, 2H), 3.50–3.81 (m, 3H), 3.91 (s, 3H), 4.15–4.26 (m, 2H), 6.76 (s, 1H), 7.42 (s, 1H), 7.55 (d, 1H), 7.80 (m, 3H), 8.0 (d, 1H), 8.2–8.3 (m, 2H), 9.15 (d, 1H), 12.38 (b s, 1H). FAB MS *m/z* = 538 (M+1). Spectral data for compound **1b**: ^{1}H NMR (200 MHz, $CDCl_3$) 1.85–2.40 (m, 8H), 2.60–2.78 (m, 2H), 3.51–3.80 (m, 3H), 3.93 (s, 3H), 4.15–4.20 (m, 2H), 6.78 (s, 1H), 7.42 (s, 1H), 7.60 (d, 1H), 7.65–7.83 (m, 3H), 8.0 (d, 1H), 8.20–8.25 (m, 2H), 9.15 (d, 1H), 12.38 (b s, 1H). FAB MS *m/z* = 552 (M+1).
16. 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.
17. Collier, D. A.; Neidle, S. *J. Med. Chem.* **1988**, *31*, 847.