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Synthesis of C8-linked pyrrolo[2,1-*c*][1,4]benzodiazepine–benzimidazole conjugates with remarkable DNA-binding affinity

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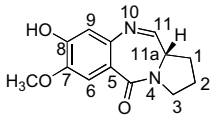
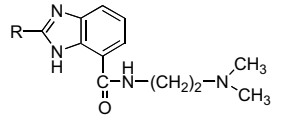
Abstract—Two types of benzimidazoles have been synthesized and linked to DC-81 at C8-position through different alkyl chain spacers. These PBD conjugates have exhibited remarkable DNA-binding affinity, and a representative compound shows promising in vitro anticancer activity.

The pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs) are a family of low molecular weight DNA-interactive antitumour antibiotics originally isolated from *Streptomyces* species,[1] well known examples include anthramycin, sibiromycin and DC-81 (**1**). These compounds are known to exert their cytotoxic potency effect by covalently binding to the exocyclic C2–NH_2 of guanine residues within the minor groove of DNA. Naturally occurring and synthetic derivatives possessing PBD ring system are generally of interest as potential anticancer and gene targeting agents due to their sequence specificity while binding to duplex DNA with a preference for 5′-Pu–G–Pu motifs.[2] In the literature, large number of PBD conjugates have been synthesized and evaluated for their biological activity, particularly for their antitumour potential.[3] Recently, Thurston and co-workers have synthesized C8-cyclic amine PBD conjugates and evaluated for their antitumour activity and also demonstrated for their DNA-binding affinity.[4] We have designed and synthesized noncross-linking mixed imine–amine PBD dimers that have significant DNA-binding ability and potent antitumour activity,[5] and also synthesized a number of PBD conjugates that have shown potent anticancer activity and significant DNA-binding ability.[6]

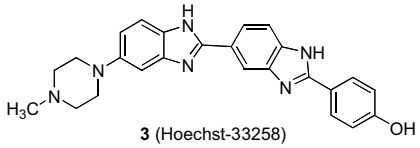
The benzimidazoles are potent antitumour, antifungal and antiparasitic agents, whose mode of action is thought to result from their inhibition of microtubule formations.[7] Substituted benzimidazoles have proven as drug leads, which have exhibited pharmacological interest.[8] In addition, 2-substituted benzimidazoles cover a broad range of biological activities, including antitumour (**2**). A series of 2-phenylbenzimidazole-4-carboxamides have shown in vitro and in vivo antitumour activity and DNA-binding affinity.[9]



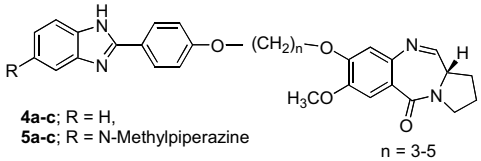
1 (DC-81)



2
R = Ph, 2-Furyl, 2, 3-thienyl, 2-Pyrrolyl



3 (Hoechst-33258)



4a-c; R = H,
5a-c; R = N-Methylpiperazine

n = 3-5

Further, some 2,5-disubstituted benzimidazoles are known to act as topoisomerase I poisons and also shown cytotoxic activity against human lymphoblastoma, and

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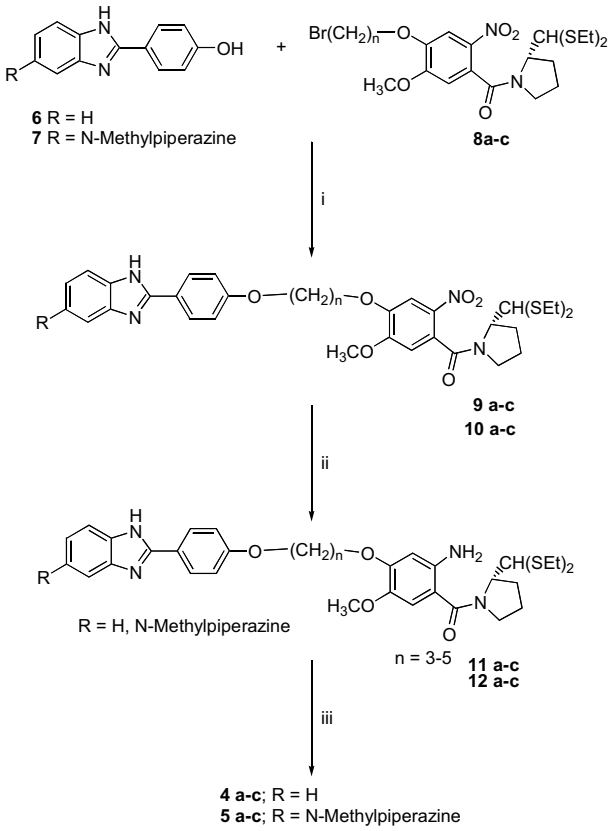
RPMI 8402 cells.[10] Several groups have explored the possibility of utilizing the pharmacophore like benzimidazoles motif derived from the well established bis-benzimidazole dye Hoechst-33258.[11,12] This compound not only shows in vitro antitumour activity but also act as an inhibitor of DNA topoisomerase I. It has been revealed from the foot printing and related structural methods that Hoechst-33258 recognizes 3–4 consecutive A/T base pairs by hydrogen bonding to base edges in 1:1 complexes.[13]

This is in continuation to our efforts towards the structural modifications of PBD ring system and also the development of new synthetic strategies[14] for this ring system. We herein report the synthesis of substituted benzimidazole analogues that have been linked through different alkyl chain spacers at C8-position of DC-81 (**1**). These PBD conjugates have shown remarkable DNA-binding affinity and promising in vitro anticancer activity for a typical example.[15]

Synthesis of benzimidazole–PBD conjugates has been carried out by employing the PBD precursors (**8a**–**c**) and 4-(benzimidazol-2-yl)phenol (**6**) or 4-[6-(4-methyl-1-piperazinyl)-benzimidazol-2-yl]phenol (**7**). The precursor **6** has been prepared by the condensation of *o*-phenylenediamine and 4-hydroxybenzaldehyde. While the precursor **7** has been prepared by replacing the chlorine of 5-chloro-2-nitroaniline with *N*-methylpiperazine followed by reduction of nitro group and is further condensed with 4-hydroxybenzaldehyde as shown in Scheme 1.

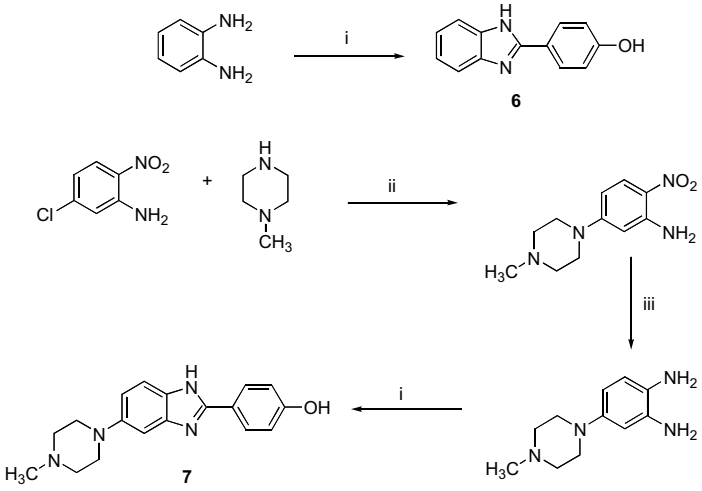
Whereas, the preparation of other PBD precursors **8a**–**c** has been carried out by employing the commercially available vanillin by earlier reported methods[16] that involves oxidation of vanillin followed by esterification, monoalkylation with dibromoalkanes, nitration followed by ester hydrolysis and coupling of 2(*S*)-pyrrolidine carboxaldehyde diethylthioacetal afforded **8a**–**c**. The key intermediates **9a**–**c** and **10a**–**c** have been prepared by linking compounds of **6** and **7** to compounds



Scheme 2. Reagents and conditions: (i) K_2CO_3, DMF, rt, 48 h, 72–76%; (ii) $SnCl_2{\cdot}2H_2O$, MeOH, reflux, 6–8 h, 80–85%; (iii) $HgCl_2$, $CaCO_3$, CH_2CN–H_2O (4:1), rt, 12 h, 55–61%.

8a–**c**, and these nitro compounds upon reduction by $SnCl_2{\cdot}2H_2O$ in methanol gives the aminothioacetal precursors **11a**–**c** and **12a**–**c** which upon deprotection of thioacetal by using $HgCl_2/CaCO_3$ affords the desired PBD-conjugates **4a**–**c** and **5a**–**c** (Scheme 2).[17]

The DNA-binding activity for these substituted benzimidazoles and C8-linked PBD conjugates has been examined by thermal denaturation studies using calf thymus



Scheme 1. Reagents and conditions: (i) 4-hydroxy benzaldehyde, $Na_2S_2O_5$, EtOH/H_2O, reflux, 80 °C; (ii) K_2CO_3, DMF, 90 °C; (iii) H_2, 10% Pd/C, EtOH, rt.

Table 1. Thermal denaturation data for PBD-benzimidazole conjugates with calf thymus (CT) DNA

PBD conjugates	[PBD]/[DNA] molar ratio[b]	ΔT_m (°C)[a] after incubation at 37 °C	
		0 h	18 h
7	1:5	2.9	2.9
4a	1:5	11.0	11.4
4b	1:5	3.8	4.0
4c	1:5	10.3	11.0
5a	1:5	16.6	22.5
5b	1:5	15.5	15.5
5c	1:5	22.4	22.6
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 20 separate determinations), all ΔT_m values are ±0.05–0.1 °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).

(CT) DNA. Melting studies show that these compounds stabilize the 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. In this assay, compound **6** has not shown any significant ΔT_m while ΔT_m of compound **7** is 2.9 °C. Interestingly, in this study PBD conjugates have shown significantly high melting temperature values (4.0–22.6 °C). The ΔT_m of compound **5a** is 16.6 °C at 0 h while the melting temperature increases to 22.5 °C upon incubation for 18 h at 37 °C. However, compound **5c** elevates the helix melting temperature of CT-DNA by 22.4 °C at 0 h and there is not much difference in the melting temperatures even after incubation for 18 h (22.6 °C). Data for compound **7**, **4a**–**c**, **5a**–**c** and DC-81 are included in Table 1. It is interestingly to observe that both the PBD-conjugates **4** and **5** the ΔT_m values are higher when the length of alkyl chain spacers is 3 or 5. Further, it is to be noted that in case of **5c** the ΔT_m value is remarkably significant even at 0 h of incubation at 37 °C. The typical DNA thermal denaturation profiles for compounds **5a**–**c** after 18 h of incubation period have been shown in Figure 1. Another aspect which has been seen from the DNA melting data is the difference in the ΔT_m values after incubation for 18 h is not noticeable except in case of **5a**.

As a representative member compound **5a** has been evaluated in the standard 60 cell line cancer screen of NCI. This compound shows significant cytotoxic potency in a wide spectrum of cell lines with LC50 values ranging from 0.01 to 50 μM (Table 2), especially this compound is more potent in case of melanoma. Moreover, this compound **5a** exhibits less than 10 nM potency as seen from the GI50 values in almost all the 60 cancer cell lines. The in vitro cytotoxicity (IC_{50}) for the

Table 2. In vitro anticancer activity of **5a** in selected human cancer cell lines

Cancer panel/cell line	LC_{50} (μM)
Nonsmall cell lung	
NCI-H522	0.01
EKVX	25.2
HOP-62	17.4
NCI-H226	23.5
Colon	
KM12	45.2
CNS	
SF-539	0.01
SF-68	27.8
SNB-19	47.5
Melanoma	
SK-MEL-2	0.01
SK-MEL-5	0.01
UACC-62	0.01
MALME-3M	34.4
M14	0.01
Ovarian	
IGROVI	45.3
OVCAR-3	34.6
OVCAR-5	33.9
OVCAR-8	21.0
Renal	
A 498	0.01
RXF 393	0.01
786-0	31.2
SN12-C	18.7
TK-10	39.7
Prostate	
PC-3	16.4
DV-145	33.7
Breast	
MDA-MB-435	0.01
MDA-MB-231/ATCC	13.5
BT-549	12.6
T47D	42.1

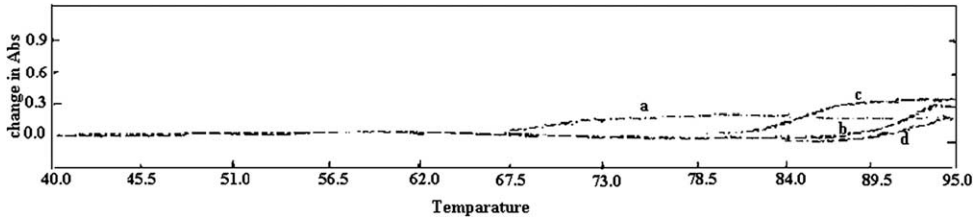


Figure 1. Thermal melting curves for CT-DNA and its ligand complexes: (a) CT-DNA with no ligand, (b) CT-DNA + **5a**, (c) CT-DNA + **5b** and (d) CT-DNA + **5c**.

naturally occurring DC-81[18] is 0.38 and 0.33 μM in L1210 and PC6 cell lines, respectively.

In conclusion, C8-linked PBD–benzimidazole conjugates have been synthesized that exhibit remarkable DNA-binding ability. Moreover, **5a** exhibits potential anticancer activity in a number of cancer cell lines. This investigation further reveals the significance of combining a noncovalent DNA-binding component (substituted benzimidazoles) to the covalent binding PBD moiety. The detailed mechanistic and molecular modeling studies for these PBD conjugates are in progress.

Acknowledgements

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17. Selected data for compound **4c**: ^{1}H NMR ($CDCl_3$) δ 1.60–2.19 (m, 8H), 2.15–2.20 (m, 2H), 3.55–3.90 (m, 6H), 3.99–4.20 (m, 4H), 6.74–6.90 (m, 3H), 7.19–7.30 (m, 3H), 7.59–7.70 (m, 3H), 7.90–8.0 (d, 2H, J = 8.6 Hz); MS (FAB) 525 $[M+1]^{+\cdot}$. Compound **5c**: ^{1}H NMR ($CDCl_3$) δ 1.60–2.10 (m, 10H), 2.30 (s, 3H), 2.55–2.62 (m, 4H), 3.08–3.19 (m, 4H), 3.60–3.79 (m, 3H), 3.82 (s, 3H), 3.92–4.05 (m, 4H), 6.64 (s, 1H), 6.79–6.90 (m, 3H), 6.96 (s, 1H), 7.35–7.88 (d, 1H, J = 4.4 Hz), 7.92–7.96 (d, 2H, J = 9.0 Hz); MS (FAB) 623 $[M+1]^{+\cdot}$.
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