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1,2,4-Benzothiadiazine linked pyrrolo[2,1-*c*][1,4]benzodiazepine conjugates: Synthesis, DNA-binding affinity and cytotoxicity

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Abstract—Benzothiadiazine–pyrrolobenzodiazepine conjugates linked through different alkane spacers have been prepared. These new classes of hybrid molecules exhibit cytotoxicity against many cancer cell lines. Their DNA thermal denaturation studies have been carried out and one of the compounds (**4b**) elevates the DNA helix melting temperature of the CT-DNA by 6.7 °C after incubation for 36 h.

In recent years, combination chemotherapy with different mechanisms of action is one of the methods that is being adopted to treat cancer. Therefore, a single molecule containing more than one pharmacophore, each with different mode of action, could be beneficial for the treatment of cancer. Sulfonylurea derivatives constitute an important class of therapeutic agents in medicinal chemistry.[1] Compound LY186641 (**1**) was reported to possess a broad spectrum of activity in several solid tumour models,[2–4] and reached the clinical trials based on its impressive preclinical activity and apparent lack of toxicity to proliferating normal tissues.[5,6] The mode of action of these compounds differs from the traditional anticancer drugs which typically inhibit DNA, RNA, or protein synthesis. Further, it was found to accumulate in the cell mitochondria which may be target site for antitumour activity of these compounds.[7,8] 1,2,4-Benzothiadiazine 1,1-dioxide ring system and 2,10-dihydro-10-hydroxy-3*H*-imidazo[1,2-*b*][1,2,4]benzothiadiazine 6,6-dioxide (**2**) contain a built-in sulfonylhydroxyguanidine moiety. Compound **2** exhibits its activity against several tumour cell lines, which is considered to combine the imino group of guanidine along with the hydroxylamino group of hydroxyurea. The potent antiviral and anticancer activities of this compound are exhibited by inhibition of ribonucleotide reductase.[9,10]

The pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs) are a group of naturally occurring antitumour antibiotics, members of which include anthramycin, tomamycin, neothramycins A and B, sibiromycin, mazethramycin, chicamycin, prothracarin, DC-81 (**3**) and dextochrysin.[11] The formation of a covalent bond in the minor groove of DNA by nucleophilic attack of 2-amino group of guanine base to form an amino linkage to C-11 is responsible for the biological activities of PBDs.[12] In the past few years, several hybrid compounds, in which known antitumour agents tethered to PBD moiety, have been designed, synthesized and evaluated for their biological activity.[13–15] Recently, we have been involved in the development of new synthetic strategies[16] for the preparation of PBD ring system and also in the design of structurally modified PBDs and their hybrids for the development of more potent anticancer agents.[17]

A number of piperazine derivatives have been synthesized for their chemotherapeutic use in the area of medicinal chemistry.[18] Michejda and co-workers[19] reported symmetrical bifunctional agents as a promising antitumour class of compounds with remarkable selectivity against colon cancers that possess a piperazine moiety in its linker spacer. Recently, *trans*-diamine dichloroplatinum(II) complexes with piperazine ligands have exhibited significant cytotoxicity.[20] In these, platinum–piperazine complexes are taken up by the cancer

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cells and bind to DNA much faster than the cisplatin. Moreover, some of the PBD conjugates with piperazinyl alkane spacers have also shown promising anticancer activity.[21]

The 1,2,4-benzothiadiazine has been linked through 3-piperazinyl alkane spacer to the C8-position of the A ring of PBD ring system to explore their potential as new class of anticancer agents. Such conjugates with the combination of DNA binding and DNA, RNA or protein synthesis inhibitors could be attractive targets for their DNA-binding potential as well as antitumour activity (Fig. 1).

Synthesis of these benzothiadiazine–pyrrolobenzodiazepine conjugates has been carried out by employing 3-chloro-1,2,4-benzothiadiazines (**8a**–**b**), which have been obtained by previously reported methods[22] and the precursors **5a**–**b** have also been prepared by the methods reported in our earlier studies.[17] These precursors have been treated with *N*-Boc piperazine to give **6a**–**b**, which on Boc deprotection yield intermediates **7a**–**b**. 10-Substituted 3-chloro-1,2,4-benzothiadiazines (**8a**–**b**) are then coupled with **7a**–**b** in the presence of triethylamine to afford **9a**–**b**, which upon reduction and cyclisation give the target molecules **4a**–**d** [23] (Scheme 1).

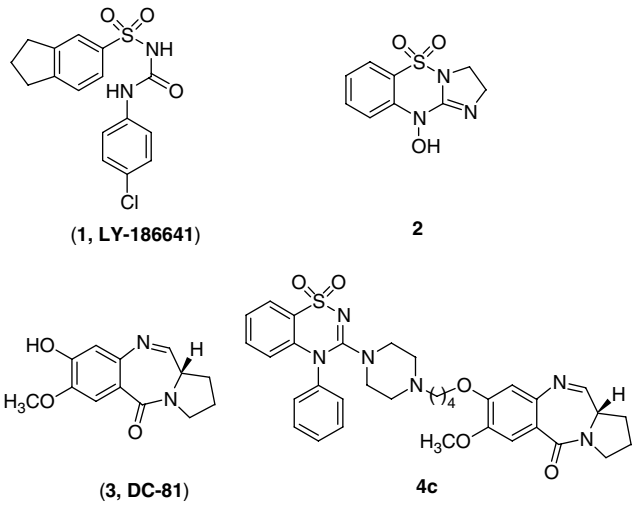
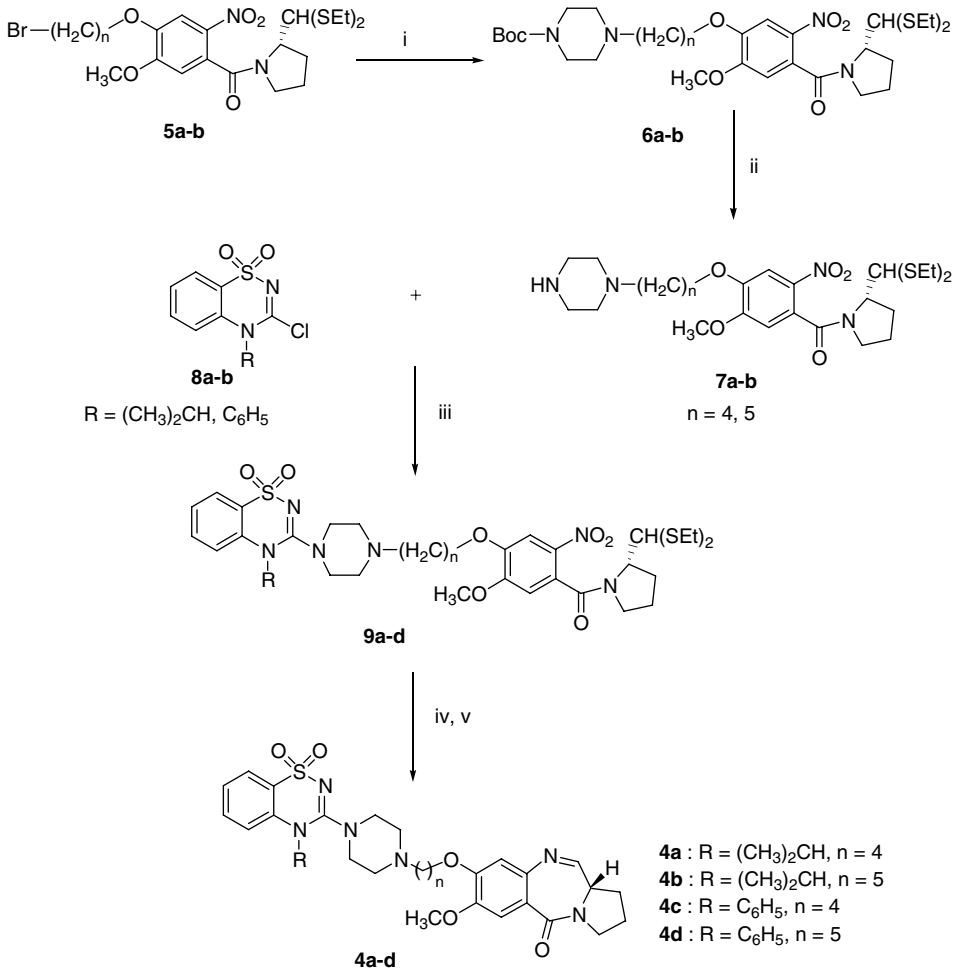


Fig. 1. Chemical structures of sulfonylurea derivative (**1**, LY-186641), imidazo benzothiadiazine derivative (**2**), DC-81 (**3**) and benzothiadiazine–PBD conjugate (**4c**).



Scheme 1. Reagents and conditions: (i) *N*-Boc-piperazine, K_2CO_3, acetone, reflux, 24 h, 85–90%; (ii) TFA, CH_2Cl_2, 0 °C, 8 h, 75–80%; (iii) THF, Et_3N, rt, 6–8 h, 75–80%; (iv) $SnCl_2{\cdot}2H_2O$, MeOH, 4 h, reflux; (v) $HgCl_2$, $CaCO_3$, CH_3CN/H_2O, 12 h, rt, 55–60%.

Table 1. IC_{50} values[a] (in μM) for compounds **4a–d** in selected human cancer cell lines

Compound	A549[b]	Hop62[b]	Zr-75-1[c]	DWD[d]	KB[d]	Gurav[d]	PC3[e]
4a	—[f]	343.00	2.24	—[f]	26.10	—[f]	—[f]
4b	30.30	27.90	0.12	27.7	2.43	26.50	27.80
4c	—[f]	369.00	0.15	—[f]	29.40	—[f]	357.00
4d	30.20	—[f]	—[f]	—[f]	2.30	356.00	2.35
ADR	1.92	—[f]	2.32	24.90	0.17	0.16	2.26

[a] Mean of three determinations.
[b] Lung cancer.
[c] Breast cancer.
[d] Oral cancer.
[e] Prostate cancer.
[f] IC_{50} value not attained at the concentrations used in the assay; ADR, adriamycin.

Compounds **4a–d** have been evaluated for their in vitro cytotoxicity in selected human cancer cell lines of lung, breast, oral and prostate by using Sulforhodamine (SRB) method.[24,25] The compounds exhibiting $IC_{50} \leqslant 10^{-5}$ M are considered to be active on the respective cell lines. Table 1 reveals that compounds **4a–c** have exhibited strong effect against Zr-75-1 cell line (IC_{50} 0.12–2.24 μM) in comparison to adriamycin (IC_{50} 2.32 μM). The in vitro cytotoxicity (IC_{50}) for compound **4b** is 2.43 μM in KB cell lines and in case of **4d**, the activity is 2.30 and 2.35 μM in KB and PC3 cell lines, respectively, whereas, this value for adriamycin is 0.17 and 2.26 μM for the same cell lines. Therefore, the in vitro cytotoxicity exhibited by these new PBD–benzothiadiazine conjugates is highly significant.

The DNA-binding ability for these benzothiadiazine–PBD conjugates has been determined by thermal denaturation studies using calf thymus (CT)-DNA. These studies were carried out at PBD/DNA molar ratio 1:5. Interestingly, all the compounds (**4a–d**) elevate the helix melting temperature of CT-DNA in the range of 3.9–6.1 °C at 0 h and also examined after 18 and 36 h incubation at 37 °C. Compound **4b** showed highest ΔT_m of 6.1 °C at 0 h and increased up to 6.7 °C after 36 h incubation, whereas the naturally occurring DC-81 (**1**) exhibits a ΔT_m of 0.7 °C after incubation under similar conditions (Table 2). This result indicates the effect on DNA-binding affinity introducing the benzothiadiazine moiety to PBD through piperazine with different alkane piperazine spacers at C8-position of the DC-81 possesses good DNA-binding ability.

Table 2. Thermal denaturation data for benzothiadiazine–PBD conjugates with calf thymus (CT) DNA

Compound	[PBD]:[DNA] molar ratio[b]	ΔT_m (°C)[a] after incubation at 37 °C		
		0 h	18 h	36 h
4a	1:5	3.9	4.2	4.3
4b	1:5	6.1	6.4	6.7
4c	1:5	4.0	4.1	4.1
4d	1:5	4.2	4.4	4.6
DC-81	1:5	0.3	0.7	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 is 100 μM and ligand concentration is 20 μM in aqueous sodium phosphate buffer [10 mM sodium phosphate + 1 mM EDTA, pH 7.00 ± 0.01].

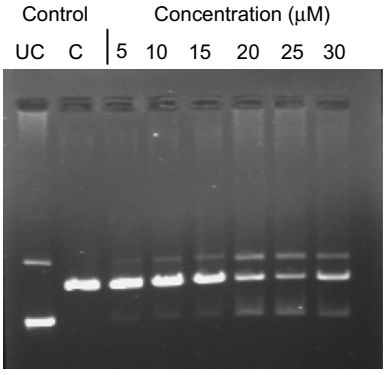


Fig. 2. RED_{100}-restriction endonuclease digestion assay for a C8-linked PBD with CT-DNA inhibitory activity of **4c** on the cleavage of plasmid pBR322 by restriction endonuclease BamH1 (20 U in 2 μL) for 1 h at 37 °C. The cut (C) and uncut (UC) products were separated by agarose gel electrophoresis and visualized by ethidium bromide staining under UV illumination. Lane 1: control pBR322; lane 2: complete digest of pBR322 by BamH1; lanes 3–8: increasing concentration of **4c**.

The restriction endonuclease inhibition assay carried out on these molecules also confirms the relative binding affinity of these PBD conjugates. The experimental protocol is described in the previous study.[26,27] The result of a representative compound **4c** has been shown in Figure 2, suggesting the inhibition of BamH1 by this PBD hybrid.

In conclusion, these new synthesized benzothiadiazine–PBD conjugates have exhibited significant in vitro antitumour activity against breast, oral and prostate human cancer cell lines and remarkable DNA-binding ability. Further, the detailed biological and molecular modelling studies are in progress.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.08.018.

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23. Spectral data for compounds **4b**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.90 (d, J = 2.34 Hz, 1H), 7.68 (d, J = 3.90 Hz, 1H), 7.57 (d, J = 2.34 Hz, 1H), 7.52 (t, J = 2.34 Hz, 2H), 7.21 (s, 1H), 7.16 (s, 1H), 7.68 (s, 1H), 4.06 (d, J = 7.03 Hz, 1H), 3.94 (s, 3H), 3.45–3.90 (m, 10H), 2.55–2.65 (m, 4H), 2.42 (t, J = 7.03 Hz, 1H), 2.20–2.38 (m, 6H), 1.90 (t, J = 7.03 Hz, 1H), 1.40–1.70 (m, 6H), 0.88 (t, J = 6.25 Hz, 2H). FAB-MS m/z 623 $(M+1)^+$; IR (KBr) (ν_{max}/cm^{-1}): 2928, 2866, 1602, 1532, 1451, 1304, 1255, 1146 (SO_2), 877. Compound **4c**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.95 (d, J = 7.62 Hz, 1H), 7.65 (d, J = 5.08 Hz, 1H), 7.28–7.58 (m, 10H), 6.77 (s, 1H), 4.02 (d, J = 5.92 Hz, 1H), 3.90 (s, 3H), 3.40–3.85 (m, 6H), 2.20–2.40 (m, 3H), 1.90–2.18 (m, 6H), 1.80 (q, J = 7.62 Hz, 2H), 1.55 (t, J = 7.62 Hz, 2H), 0.86 (t, J = 6.77 Hz, 2H). FAB-MS m/z 643 $(M+1)^+$; IR (KBr) (ν_{max}/cm^{-1}): 2928, 1597, 1547, 1428, 1301, 1263, 1164 (SO_2), 759. The detail spectral data of other compounds and experimental procedures are available in Supplementary information.
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