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Synthesis of C-8 Methanesulphonate Substituted Pyrrolobenzodiazepines as Potential Antitumour Agents

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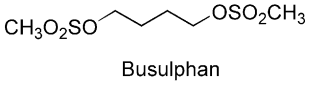
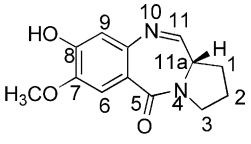
Abstract—The facile synthesis of C-8 methanesulphonate substituted pyrrolobenzodiazepines is described. These have been prepared by linking the methanesulphonate at C-8 position with alkanol spacer and their in vitro cytotoxicity have been described.

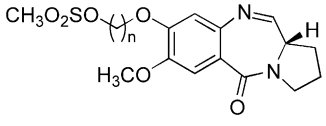
Pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs) are of considerable current interest due to their ability to recognize and subsequently form covalent bonds to specific base sequences of double stranded DNA. Such agents have potential therapeutic targets, in the therapy of genetic based diseases (e.g., cancer) and validation of DNA sequences.[1–3] The pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs) are a family of DNA interactive antitumour antibiotics derived from *Streptomyces* species that include anthramycin, tomaymycin, sibiromycin and DC-81.[4]



Busulphan



DC-81



n = 2-3 **1a-b**

The cytotoxicity and antitumour activity of these compounds are attributed to their property by forming a covalent bond between the C11 position of DNA and the N2 amino group of a guanine base, and they usually span approximately three base pairs with a preference of Pu-G-Pu.[5]

A large number of PBD conjugates have been prepared and evaluated for their biological activity, particularly their antitumour potential.[6] Thurston and co-workers have synthesized novel PBD dimers as cross-linking agents.[7] Recently, we have designed and synthesized non-cross-linking mixed imine-amide PBD dimers that have significant DNA-binding ability and potent antitumour activity.[8] Alkanediol dimethanesulphonates of general formula $CH_3SO_2O(CH_2)_nOSO_2CH_3$ constitute a very interesting class of bifunctional chemotherapeutic agents. Busulphan is one of these homologous series, which is in the treatment of chronic myeloid leukemia for last 30 years.[9] Recently, two long-chain busulphan analogues have been prepared to improve the drug effectiveness.[10]

Furthermore, Indisulam and related sulphonamides have shown interesting anticancer avtivity.[11] Therefore, it has been considered of interest to design and synthesize C8 linked alkanol methylsulphonate (alkylating part of busulphan) PBD hybrids as cross-linking bifunctional chemotherapeutic agents. We have been engaged in the last few years in the structural modifications[12] and the development of new synthetic strategies[13] for the PBD based ring systems. In continuation of these efforts, we herein report the synthesis and anticancer activity of PBD ring system linked to methanesulphonate with different alkanol spacers at C8 position.

The compounds (**1a–b**) were prepared from the (2*S*)-*N*-(4-benzyloxy-5-methoxy-2-nitrobenzoyl)pyrrolidine-2-carboxaldehyde diethylthioacetal **2**. This compound **2** has been prepared by literature method,[14] which upon

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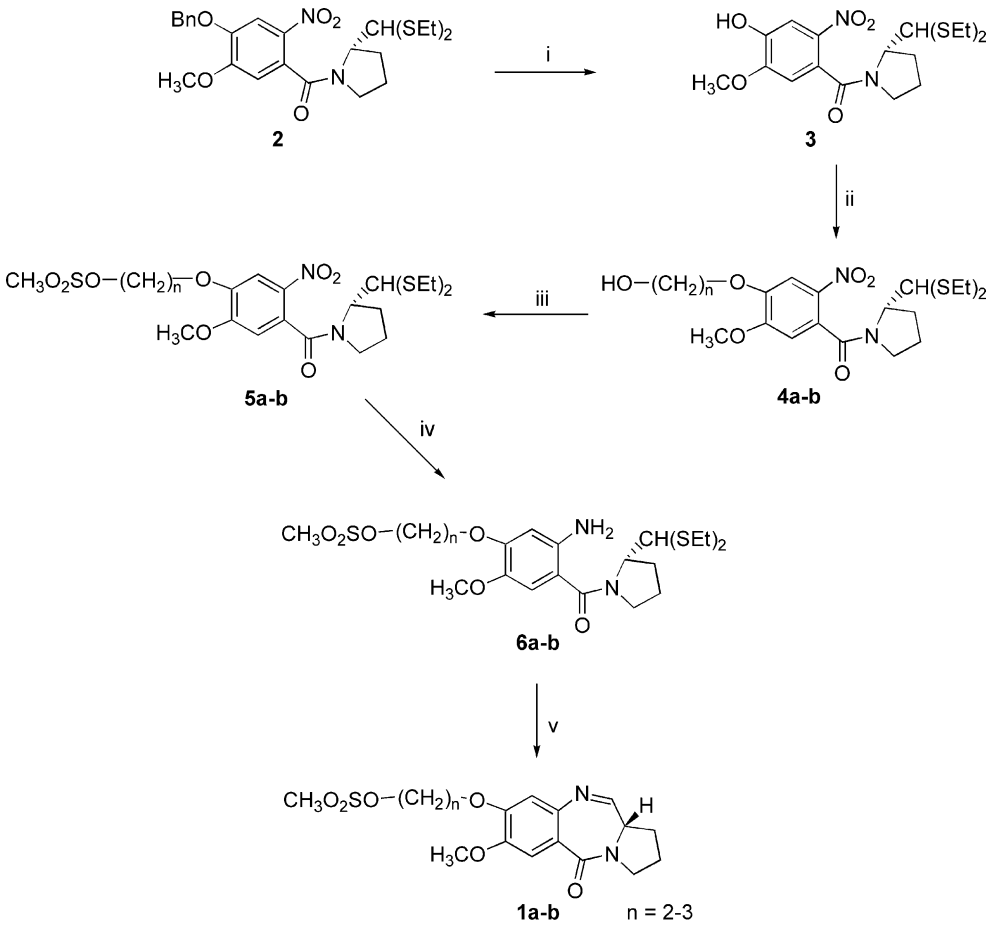

Scheme 1. (i) EtSH–BF_3OEt_2, CH_2Cl_2, 12 h, rt, 75%; (ii) bromoalkanol, K_2CO_3, DMF, 24 h, rt, 94–96%; (iii) CH_3SO_2Cl, Et_3N, CH_2Cl_2, 8 h, 0 °C, 90–91%; (iv) $SnCl_2{\cdot}2H_2O$, MeOH, reflux, 4 h, 85–87%; (vi) $HgCl_2$–$CaCO_3$, CH_3CN–H_2O, 12 h, rt, 68–71%.

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

Cancer panel/cell line	GI_{50} (μM)
Leukemia	
CCRF-CEM	14.5
SR	11.1
RPMI-8226	12.2
Non-small cell lung	
NCI-H226	15.4
NCI-H23	12.2
NCI-H522	10.9
Colon	
HCT-116	18.5
HCT-15	16.5
SW-620	14.6
CNS	
SF-539	18.6
U251	17.1
Melanoma	
LOX EMVI	15.7
MALME-3M	13.6
M14	18.7
SK-MEL-5	16.9
UACC-257	18.2
UACC-62	19.4
Ovarian	
OVCAR-3	16.3
Renal	
786-0	17.0
A498	17.1
ACHN	16.0
CAKI-1	17.7
TK-10	18.1
UO-31	15.2
Breast	
NCI/ADR-RES	13.5
MDA-MB-231/ATCC	16.5

debenzylation gives **3**. Etherification of (2*S*)-*N*-(4-hydroxy-5-methoxy-2-nitrobenzoyl)pyrrolidine-2-carboxaldehyde diethylthioacetal **3** by bromoalkanols provides **4a–b**. Mesylation of these alcohols **4a–b** gives the desired precursors **5a–b**. Further, these upon reduction followed by deprotection of thioacetal group affords the target compounds **1a–b**[15] (Scheme 1).

Compounds **1a–b** have been evaluated for cytotoxicity activities against nine panels containing 60 human cell lines. A 48 h continuous drug exposure protocol was used and a sulfurhodamine B protein assay was used to estimate cell viability or growth.

Compounds **1a–b** possess cytotoxic potency against many cell lines. Compound **1a** (Table 1) exhibits a wide spectrum of activity against 26 cell lines in eight cell panels, with GI_{50} value of <20 μM. The average GI_{50} value of compound **1a** against leukemia cancer CCRF-CEM, RFMI-8226 and SR are 14.5, 12.2 and 11.1 μM, respectively. In non-small cell lung cancer panel, the growth of NCI-H226, NCI-H23 and NCI-H522 cell lines are affected by compound **1a** with an GI_{50} value as 15.4, 12.2 and 10.9 μM, respectively. The GI_{50} value of colon cancer HCT-116, HCT-15 and SW-620 cell lines is 18.5, 16.5 and 14.6 μM respectively. In CNS cancer SF-539 and U251 cell lines are affected with GI_{50} values are 18.6 and 17.1 μM. Most of the cell lines in melanoma cell panel were affected by **1a** at 13.6–19.4 μM concentrations. In the ovarian cancer panel the growth of the OVCAR-3 cell line was affected by compound **1a** with GI_{50} value as 16.3 μM. The six cell lines in the renal cancer were affected by compound **1a** with GI_{50} value at 15.2 to 18.1 μM concentrations. Compound **1a** exhibits cytotoxic potency in breast cancer panel in which NCI/ADR-RES and MDA-MB-231/ATCC cell lines were affected with GI_{50} values of 13.5 and 16.5 μM, respectively.

Compound **1b** exhibits cytotoxic potency against leukemia cancer cell lines CCRF–CEM and SR with the GI_{50} value of 52.8 and 62.9 μM, respectively, and its also exhibits cytotoxicity against SNB-75 (CNS cancer) and OVCAR-4 (ovarian cancer) cell lines with the GI_{50} value of 94.7 and 70.3 μM, respectively.

In conclusion, new methanesulphonate–PBD hybrids have been synthesized that exhibit cytotoxic activity in some cancer cell lines. The detailed mechanistic studies of these PBD hybrids are in progress.

Acknowledgements

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15. Selected data for compound **1a** ^{1}H NMR ($CDCl_3$) δ 1.80–2.40 (m, 4H), 3.00 (s, 3H), 3.40–3.85 (m, 2H), 3.95 (s, 3H), 4.05–4.40 (m, 3H), 4.40–4.55 (m, 2H), 6.82 (s, 1H), 7.5 (s, 1H), 7.65 (d, 1H, $J=4.4$ Hz); MS (EI) m/z 368.