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Synthesis of novel C2-aryl pyrrolobenzodiazepines (PBDs) as potential antitumour agents

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Three novel C2-aryl substituted pyrrolobenzodiazepines (PBDs) have been synthesised and evaluated in a number of cell lines revealing selective cytotoxicity at the sub-nanomolar level towards melanoma and ovarian cancer cell lines.

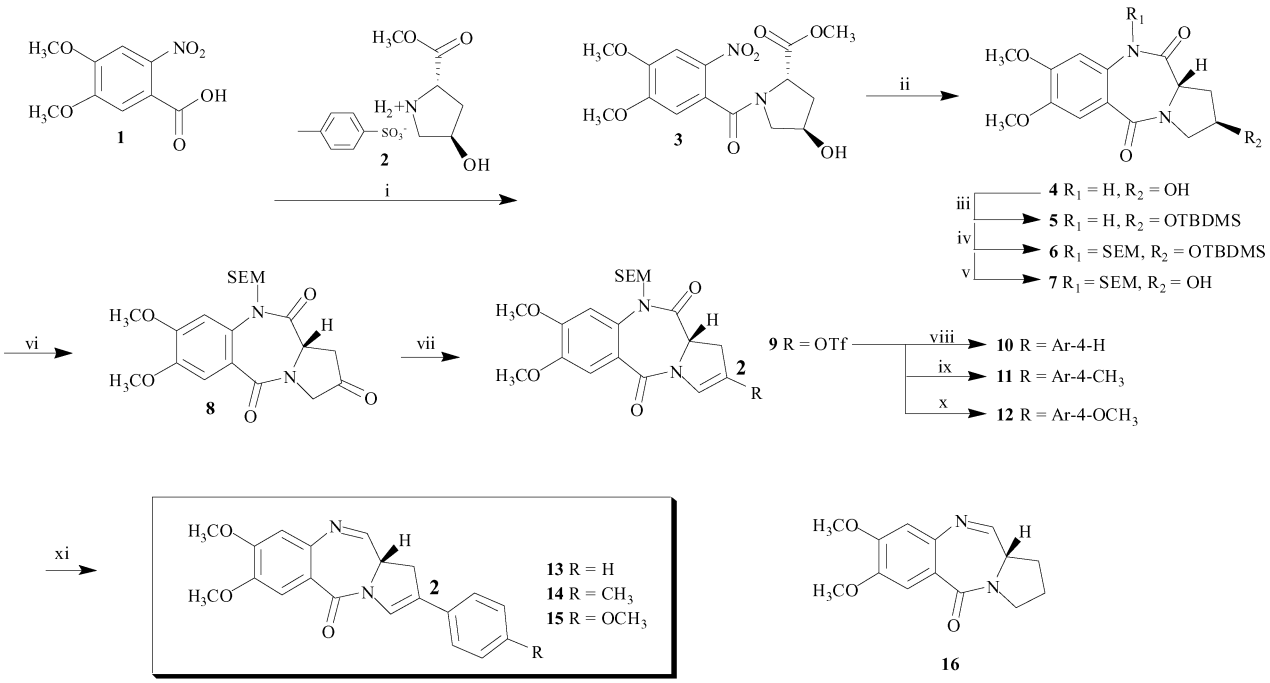
Anthramycin, the first example of a series of PBD antitumour antibiotics, was isolated from *Streptomyces refuineus* in 1965 by Leimgruber *et al*.[1] Many other PBD natural products have since been found in *Streptomyces* species, and a large number of synthetic analogues have been investigated and reported.[2] The PBDs exert their biological activity by binding covalently to the N2-position of guanine within the minor groove of DNA in a sequence-selective manner, preferring to interact with purine-guanine-purine sequences.

Using a new synthetic approach, we report here the synthesis of a series of C2-aryl C2/C3-unsaturated PBDs that represent a structural sub-class not observed in nature. Compared to PBD monomers with other substitution patterns, these C2-aryl analogues have remarkably selective cytotoxicity at the sub-nanomolar level towards melanoma and ovarian cell lines.

The synthetic route starts from commercially available 6-nitroveratric acid (**1**) and the C-ring building block **2** to provide the target molecules **13**–**15** in nine steps (Scheme 1). Although the initial synthetic steps are based on the classical approach of Leimgruber *et al*.,[3] a novel use of an N10-hemiaminal protecting group is employed. Furthermore, towards the latter stages of the synthesis, the Suzuki reaction is used for the first time on a PBD skeleton to introduce the C2-aryl substituents.

The pre-formed C-ring[4] was coupled to **1** to provide the A–C ring backbone **3**. For the next step involving reduction of the nitro group, several methods were investigated including the sodium dithionite method originally employed by Leimgruber *et al*.[3] Catalytic hydrogenation was selected as the preferred method. On a scale of greater than 1 g, it was found that, on formation of the amine spontaneous cyclisation occurred to give **4**, thus eliminating the need for the two-day acid-treatment step employed by Leimgruber. This spontaneous cyclisation was not encountered using other reduction methods. Following cyclisation, the C2 alcohol was protected as a TBDMS ether.

The PBD dilactam **5** was next treated with SEM-Cl under strongly basic conditions to provide the SEM version of the N10 hemiaminal shown by Mori *et al*.[5] to promote C[11]-lactam reduction. Having successfully derivatized the N10 position, the C2-TBDMS ether was selectively cleaved with TBAF at room temperature without affecting the orthogonal N10-SEM protecting group. Swern oxidation furnished the C2 ketone **8**,



Scheme 1 *Reagents and conditions*: i, $(COCl)_2$, DMF, TEA, anhydrous DCM, RT, 57%; ii, 10% Pd/C, EtOH, H_2, 75%; iii, TBDMS-Cl, imidazole, anhydrous DMF, RT, 95%; iv, SEM–Cl, NaH, anhydrous DMF, 0 °C, 77%; v, TBAF, THF, RT, 70%; vi, $(COCl)_2$, anhydrous DMSO, anhydrous DCM, TEA, N_2, −55 °C, 49%; vii, anhydrous pyridine, anhydrous DCM, anhydrous triflic anhydride, RT, 60%; viii, benzeneboronic acid, benzene, EtOH, water, N_2, Na_2CO_3, $Pd(PPh_3)_4$, RT, 78%; ix, methylbenzeneboronic acid, benzene, EtOH, water, N_2, Na_2CO_3, $Pd(PPh_3)_4$, RT, 66%; x, methoxybenzeneboronic acid, benzene, EtOH, water, N_2, Na_2CO_3, $Pd(PPh_3)_4$, RT, 90%; xi, $NaBH_4$, anhydrous EtOH, anhydrous THF, wet silica gel, N_2, RT, **13**:‡ 74%, **14**:§ 38%, **15**:¶ 72%; *Note*: The C2-unsubstituted PBD **16** shown for comparative purposes was not synthesised using this route.

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Table 1 Cytotoxicity of compounds **13**–**15**

	GI_{50}[ad]/nM				TGI[bd]/nM				LC_{50}[cd]/nM			
Cell line	**16**[e]	**15**	**14**	**13**	**16**[e]	**15**	**14**	**13**	**16**[e]	**15**	**14**	**13**
MALME3M (Melanoma)	2275	0.73	2.4	11.09	4850	1.67	4.71	21.63	53715	6.69	61.02	84.92
SKMEL28 (Melanoma)	3175	3.32	5.95	9.9	9720	6.74	12.37	43.17	23305	17.25	71.36	82.89
SKOV3 (Ovarian)	3490	0.03	0.04	0.05	NA	6.22	4.67	25.87	> 100000	> 10	> 10	> 10
MCF7 (Breast)	2490	2.89	4.47	17.24	> 100000	9.27	99.51	1001.63	> 100000	> 10	> 10	> 10
HCT116 (Colon)	2730	3.83	2.31	3.83	> 100000	8.01	7.59	9.55	> 100000	9.17	9.04	82.54
H460 (Lung)	2925	4.09	3.51	9.14	> 100000	8.2	8.03	1707.32	> 100000	9.68	9.97	5984.73

[a] Dose required to inhibit cell growth by 50% compared to PBD-free controls after incubation for 48 h at 37 °C; [b] Dose required for complete inhibition of cell growth compared to PBD-free controls after incubation for 48 h at 37 °C; [c] Dose required to kill 50% of cells compared to PBD-free controls after incubation for 48 h at 37 °C; [d] The MTT assay was used to measure cytotoxicity, and PBDs were dissolved in DMSO/culture medium prior to addition to the culture medium; [e] Data for compound **16** were obtained from the NCI's 60 cell line panel.

which was converted to the C2–C3 enol triflate **9** using trifluoromethanesulfonic anhydride in the presence of pyridine.[6]

Three trial Suzuki reactions were performed on the enoltriflate **9**. The reactions with methoxybenzeneboronic (x) methylbenzeneboronic (ix) and benzeneboronic acids (viii) in benzene proceeded at room temperature.[7] This observation coupled with the diverse array of commercially available arylboronic acids suggested the possibility of generating libraries of analogues through parallel combinatorial synthetic methodologies.

Reduction of the dilactam in the presence of the N10-SEM group was achieved using sodium borohydride in anhydrous EtOH/THF. The resulting unstable N10-SEM protected carbinolamines underwent spontaneous cleavage in the presence of wet silica gel to afford the required C2-aryl PBDs **13**, **14** and **15** in their imine forms without the need for treatment with additional reagents such as TBAF.

Compound **15** was evaluated in the standard NCI 60-cell line screen and was shown to have nanomolar potency (at the LC_{50} level) against six melanoma (MALME-3M, M14, SK-MEL-2, SK-MEL-5, UACC-257, UACC-62), two non-small cell lung (NCI-H460, NCI-H522), one CNS (SF-539) and two colon cancer lines (COLO 205, HCC-2998).

Compounds **13**, **14** and **15** were subsequently tested in a more focused 6-cell line panel and the results are shown in Table 1. Data for the C-ring unsubstituted analogue **16** have been included for comparative purposes. All analogues exhibit nanomolar potency at the GI_{50} level against the two melanoma lines, and picomolar potency against SKOV3, an intrinsically cisplatin-resistant ovarian cancer cell line.

Efforts are now underway to carry out a Structure Activity Relationship (SAR) study through the synthesis of further C2-aryl analogues in order to maximise the cytotoxicity in key tumour cell lines and to elucidate the precise mechanism of action. Compound **15** has been selected for *in vivo* studies and these results will be reported elsewhere.

Notes and references

† Cytotoxicity studies were carried out at the Cancer Research Laboratories, University of Nottingham, University Park, Nottingham, UK NG7 2RD.
‡ ^{1}H (250 MHz, $CDCl_3$) NMR: δ 7.91 (d, 1H, *J* 5 Hz, H-11), 7.53 (s, 1H, H-9), 7.52 (s, 1H, H-3), 7.43–7.11 (m, 5H, Ar-H), 6.84 (s, 1H, H-6), 4.44 (ddd, 1H, *J* 2.5, 5, 10 Hz, H-11a), 3.98 & 3.95 (2s, 6H, 7,8-MeO), 3.60 (ddd, 1H, *J* 2.5, 12.5, 17.5 Hz, H-1), 3.42 (ddd, 1H, *J* 2.5, 5, 17.5 Hz, H-1).
§ ^{1}H (250 MHz, $CDCl_3$) NMR: δ 7.90 (d, 1H, *J* 3.9 Hz, H-11), 7.53 (s, 1H, H-9), 7.47 (br s, 1H, H-3), 7.31 (d, 2H, *J* 8.15 Hz, Ar-Tolyl), 7.18 (d, 2H, *J* 8.04 Hz, Ar-Tolyl), 6.84 (s, 1H, H-6), 4.42 (ddd, 1H, *J* 4.1, 5.2, 11.4 Hz, H-11a), 3.98 & 3.95 (2s, 6H, 7,8-MeO), 3.59 (ddd, 1H, *J* 1.9, 11.5, 16.3 Hz, H-1), 3.39 (ddd, 1H, *J* 1.9, 5.3, 16.6 Hz, H-1), 2.36 (s, 3H, Ar-Me).
¶ ^{1}H (250 MHz, $CDCl_3$) NMR: δ 7.91 (d, 1H, *J* 2.5 Hz, H-11),7.53 (s, 1H, H-3), 7.43–7.30 (m, 3H, H-6 & Ar-Phenyl), 6.94-6.84 (m, 3H, H-9 & Ar-Phenyl), 4.46–4.38 (ddd, 1H, *J* 2.5, 5, 12.5 Hz, H-11a), 3.98 & 3.95 (2s, 6H, 7,8-MeO), 3.81 (s, 3H, Ar–MeO), 3.60 (ddd, 1H, *J* 2.5, 12.5, 17.5 Hz, H-1) 3.41 (ddd, 1H, *J* 2.5, 5.0, 17.5 Hz).

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