Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 3041–3053
Bioorganic & Medicinal Chemistry

Synthesis of a novel C2-aryl pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-dione library: Effect of C2-aryl substitution on cytotoxicity and non-covalent DNA binding

Dyeison Antonow,[a] Terence C. Jenkins,[b,†] Philip W. Howard[a] and David E. Thurston[a,*]

[a]*Cancer Research UK Gene Targeted Drug Design Research Group, The School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX, UK*

[b]*Morvus Technology Limited, Building 115, Porton Down Science Park, Salisbury, Wiltshire SP4 0JQ, UK*

Received 7 December 2006; revised 22 January 2007; accepted 31 January 2007
Available online 2 February 2007

Abstract—A 23-member C2-aryl pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-dione (PBD dilactam) library has been synthesized using Suzuki coupling, and the effect of base upon racemisation at the C11a-position during the cross-coupling reaction studied. Three library members (**21**, **30** and **33**) were sufficiently cytotoxic in the NCI's preliminary screen to warrant further evaluation, and one (**30**, R = *p*-Br) was found to be cytotoxic at the sub-micromolar level in the A498 renal cancer cell line. DNA thermal denaturation studies suggested that this activity may be associated with non-covalent DNA interaction, and also demonstrated that introduction of C2–C3 unsaturation and addition of C2-aryl functionalities to the PBD dilactam skeleton significantly enhanced helix stabilisation compared to the unsubstituted PBD dilactam (**6**).

1. Introduction

The pyrrolobenzodiazepines (PBDs) are a family of naturally occurring antitumour agents that usually interact covalently with DNA in a sequence-selective manner.[1] PBD monomers such as anthramycin (**1**, Fig. 1), tomaymycin (**2**) and DC-81 (**3**) are known to monoalkylate DNA by covalently binding to the N2 of a guanine base in the DNA minor groove through their electrophilic N10–C11 imine (or equivalent carbinolamine) moiety, a process which can block transcription and lead to cytotoxicity.[2] The development of viable synthetic pathways[3] has allowed many analogues of PBD monomers to be explored, including the joining of two PBD units together through their C8-positions to create a new family of PBD dimers.[4,5] These analogues can covalently cross-link DNA, and one example of a dimer, SJG-136 (**4**),[6] has recently entered clinical trials.[7,8]

PBDs containing an N10–C11 lactam moiety instead of an electrophilic N10–C11 imine or carbinolamine, and thus unable to interact covalently with DNA, are also known. For example, Kaneko and co-workers first reported[9] that the PBD dilactam **5a** has significant in vivo antitumour activity in a P388 lymphocytic leukaemia mouse model but did not propose a mechanism of action. Jones and co-workers followed up this observation by demonstrating, through DNA thermal denaturation studies, that dilactam **5a** and a number of related analogues such as **5b** can still bind to DNA but through a non-covalent mechanism which was suggested to account for the biological activity.[10] Based on this, models were proposed in which dilactams of this type, which are isohelical in shape with the DNA minor groove, were docked into duplex DNA and held in place by hydrogen bonding interactions (e.g., Fig. 2).

There has been significant recent research activity in the PBD area.[11–16] For the N10–C11 imine-containing PBD monomer and dimer families it has been demonstrated that inclusion of C2/C3-*endo* unsaturation in association with C2-aryl substitution can significantly enhance DNA-binding affinity and cytotoxicity.[17–20] This prompted us to prepare an analogous library of pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-diones (PBD

Keywords: PBD; Dilactams; Suzuki coupling; Racemisation; Antitumour agents.
* Corresponding author. Tel.: +44 (0) 207 753 5931; fax: +44 (0) 207 753 5964; e-mail addresses: terry.jenkins@morvus.com; philip.howard@pharmacy.ac.uk; david.thurston@pharmacy.ac.uk
† Tel.: +44 (0) 1980 556622; fax: +44 (0) 1980 556630.

0968-0896/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2007.01.054

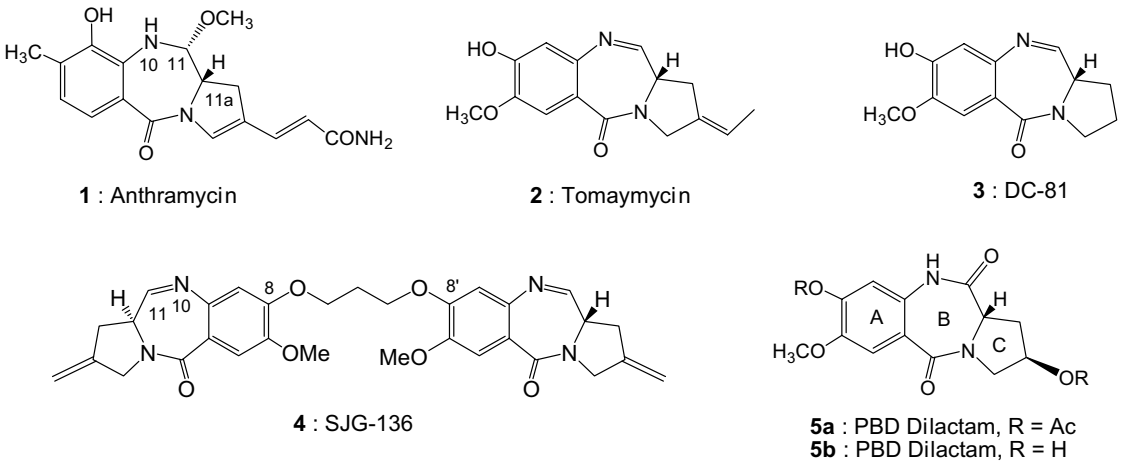


Figure 1. The naturally occurring electrophilic pyrrolobenzodiazepine (PBD) monomers anthramycin (**1**), tomaymycin (**2**) and DC-81 (**3**) that monoalkylate DNA in the minor groove, the synthetic PBD dimer SJG-136 (**4**) that forms covalent interstrand cross-links and the PBD dilactams (**5a** and **5b**) that lack an electrophilic N10–C11 imine moiety but that still bind to DNA through weaker non-covalent interactions.

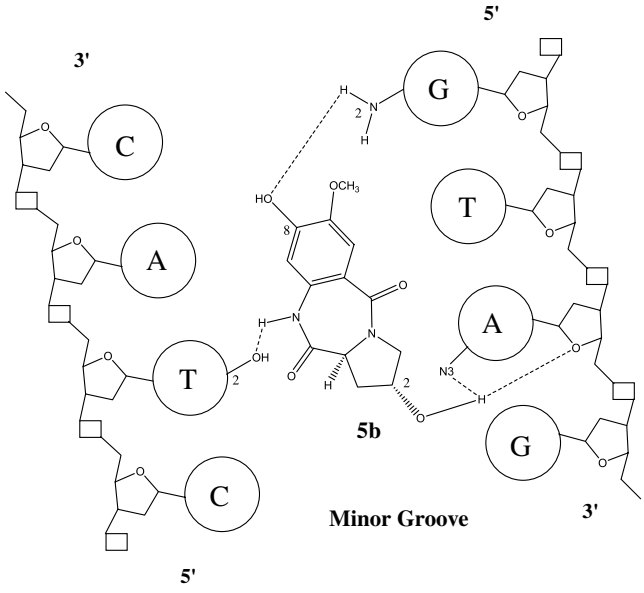


Figure 2. Diagram based on molecular modelling studies[10] rationalising the mechanism of non-covalent DNA interaction of the PBD dilactams (e.g., **5b** depicted in this model). The molecules are thought to interact through a combination of hydrogen bonds (dashed lines) and van der Waals interactions depending upon the base pair sequence and the substitution pattern of the PBD A- and C-rings.

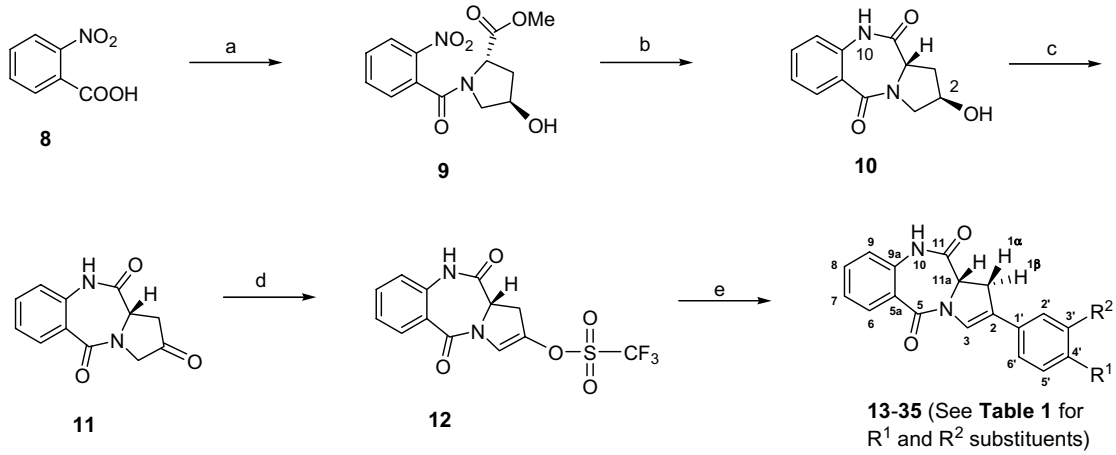
dilactams) to establish whether similar C2–C3 modifications might influence non-covalent DNA-binding affinity and cytotoxicity in comparison to the previously known unsubstituted PBD dilactam **6** (see Table 6). From a library of 23 novel dilactams, three (**21**, **30** and **33**) were found to be sufficiently cytotoxic in the NCI preliminary screen to warrant progression to the full 60-cell line panel. The most cytotoxic dilactam (**30**) had a mean GI_{50} of 7.41 μM across the whole panel, but was particularly active in the A498 renal cell line with a GI_{50} in the sub-micromolar range (0.51 μM). Seven members of the library (including **21**, **30** and **33**) were also examined for DNA-binding affinity through thermal denaturation studies and were found to raise the melting temperature of DNA by 1.0–2.3 °C, supporting earlier observations[10] of the ability of substituted PBD dilactams to stabilise DNA. As the unsubstituted parent dilactam **6** increases ΔT_m by <0.5 °C, these results clearly demonstrate that addition of C2-aryl substituents with C2–C3 unsaturation can enhance non-covalent DNA binding affinity and potentially introduce cytotoxicity.

2. Results and discussion

2.1. Synthesis

The synthetic pathway (Scheme 1) started from commercially available 2-nitrobenzoic acid **8** which was treated with oxalyl chloride and then coupled to 2(*S*),4(*R*)-4-hydroxyproline methyl ester in the presence of base to give the amide **9** in excellent yield. B-ring formation was expected to take place spontaneously after reduction of the nitro group, as reported previously for 7,8-dimethoxy-substituted PBD systems.[20] However, ring closure did not occur in this case, possibly due to the absence of electron-donating substituents in the aromatic ring, and so several other methods to effect ring closure were investigated. Acid treatment in H_2O/THF[21] was found to be the best method for generating the ring closed intermediate **10**.

Suzuki coupling has been previously explored as a means to attach aryl substituents to the C2-position of electrophilic N10–C11 imine-containing PBDs.[20] The usual approach uses a C2–C3 enol triflate intermediate of type **12** as starting material for cross-coupling with boronic acids. The enol-triflate is prepared from the corresponding C2-ketone, itself prepared by oxidising the C2-hydroxyl group. In the previously published procedure,[20] triflation was carried out only after N10 protection, which required prior C2-hydroxyl protection and then deprotection before oxidation. However, for this dilactam library, the C2-hydroxyl group of **10** could be subjected directly to Jones' oxidation and the resulting



Scheme 1. Reagents and conditions: (a) $(COCl)_2$, DMF, CH_2Cl_2, rt, overnight; then methyl-2(*S*),4(*R*)-4-hydroxy-2-prolinate (4-toluenesulfonate salt), TEA, THF, −40 °C, 5 h, 98%; (b) 10% Pd/C, EtOH, H_2 (45 psi), 2 h; then Conc HCl/H_2O/THF (0.03:10:1 v/v), rt, 20 h, 82%; (c) Jones' reagent (freshly prepared), acetone, rt, 2 h, 76%; (d) Trifluoromethanesulfonic anhydride, pyridine, $CHCl_3$, rt, 2 h, 75%; (e) Various boronic acids or pinacol esters of boronic acids, TEA, $Pd(PPh_3)_4$, toluene/ethanol/H_2O (1:1:0.3 v/v), reflux, 30 min (see Table 1 for yields).

C2-ketone (**11**) treated with pyridine and triflic anhydride to provide the enol triflate **12** in 75% yield. This triflation yield was significantly higher than for our previously reported method,[22] demonstrating that a large excess of reagents is critical for the success of this step.

The Suzuki coupling was performed using **12** and a variety of commercially available boronic acids or pinacol esters of boronic acids in the presence of $Pd(PPh_3)_4$ as catalyst. Initially, reactions were performed in refluxing toluene/ethanol/H_2O using Na_2CO_3 as base in a parallel reaction station. During these reactions, compounds **13–35** (Table 1) crystallised out from the solution in good yields, allowing straight-forward work up by filtration. However, optical rotation measurements and chiral chromatography analyses[23] of the final products indicated that most of them had racemised at their C11a positions during cross-coupling. One possible explanation for this is the proximity of the 11a-proton to the amidic C11 carbonyl which may increase its acidity, thus facilitating deprotonation under basic conditions.

Table 1. Structures and yields of members of the PBD dilactam library (**13–35**) obtained via Suzuki coupling of enol triflate **12** with the various boronic acids or pinacol esters of boronic acids (Scheme 1)

Dilactams	R^1	R^2	Yields (%)
13	H	H	58
14	CH_3	H	55
15	OCH_3	H	68
16	$N(CH_3)_2$	H	67
17	Cl	H	66
18	F	H	44
19	CN	H	64
20	COOH	H	42
21	Cl	Cl	55
22	H	NO_2	72
23	H	CN	57
24	H	Cl	48
25	H	OCH_3	31
26	H	$NHCOCH_3$	76
27	NH_2	H	39
28	H	NH_2	60
29	OH	H	43
30	Br	H	45
31	CHO	H	62
32	$NHCOCH_3$	H	71
33	1-Naphthyl		41
34	2-Naphthyl		61
35	*trans*-2-(Phenyl)vinyl		32

In order to study the effect of different bases on this racemisation process, several experiments were performed using 4-chlorophenylboronic acid as a model reactant to give **17**. From the optical rotation values (Tables 2 and 3) and the chiral HPLC data (Fig. 3), it is evident that racemisation is minimised by using bases milder than Na_2CO_3, and with TEA providing the best yields. Based on these data, Suzuki coupling products **13–35** were re-synthesized for cytotoxicity screening and DNA-binding studies using triethylamine (TEA) as base (as shown in Scheme 1).

2.2. Biological activity

Compounds **13–35** were evaluated in the preliminary National Cancer Institute (NCI) cytotoxicity screen in

Table 2. Effect of different bases on the yield and optical purity of batches of **17** produced by Suzuki coupling of enol triflate **12** with 4-chlorophenylboronic acid

Base	Yield (%)	$[\alpha]_D^T$	cHPLC[a] (%)
Na_2CO_3	52	+177	~50
NaOAc	34	+786	~100
CsF	58	+794	~100
TEA	66	+703	~100
Imidazole	No reaction	—	—

[a] Relative area under the curve (AUC, %) for the required 11a(*S*)-isomer of **17** based on Chiral HPLC (cHPLC) analysis (see Fig. 3). See Section 4 for concentration and temperature (*T*) details.

Table 3. Effect of base on the optical rotation of a selection of Suzuki products

Dilactams	$[\alpha]_D^{T\ a,b}$	$[\alpha]_D^{T\ b,c}$
13	0	+760
14	+47	+772
15	+11	+706
17	0	+703
18	+14	+745
19	+76	+752
20	+286	+571
21	+51	+659
24	+8.1	+707
25	+11	+694
34	+145	+609

[a] Conditions: Na_2CO_3 (1.1 equiv), toluene/EtOH/water (5:5:1.5 v/v), boronic acid, reflux, 40 min.
[b] Optical rotations measured in DMF (see Section 4 for concentration and temperature (*T*) details).
[c] Conditions: TEA (3.0 equiv.), toluene/EtOH/water (5:5:1.5 v/v), boronic acid, reflux, 40 min.

MCF7 (Breast), NCI-H460 (Lung) and SF-268 (CNS) cell lines. Compounds **21**, **30** and **33** had sufficient cytotoxicity (see Table 4) to be progressed by the NCI for evaluation in their full 60 cell line panel (Table 5). Although their mean GI_{50} values were relatively high (with only **30** being <10 μM), dilactams **30** and **33** were particularly active in the A498 renal cell line with **30** having a sub-micromolar GI_{50} value (0.51 μM) only slightly higher than that for anthramycin (0.195 μM) in the same cell line.

2.3. Thermal denaturation studies

In order to investigate DNA interaction as a potential mechanism for the observed cytotoxicity, thermal denaturation studies were carried out on a selection of dilactams (including **21**, **30** and **33**), to establish whether they could stabilise the DNA helix (Table 6). ΔT_m shift measurements were taken before and after incubation at

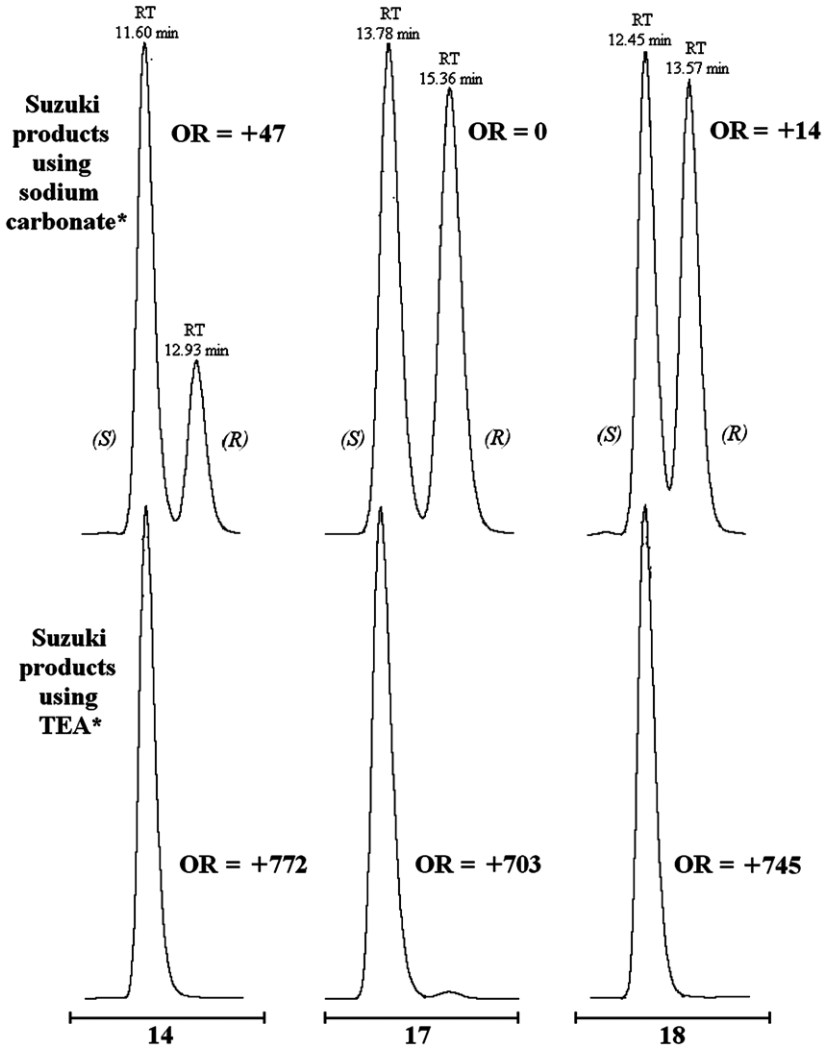


Figure 3. Chiral chromatograms obtained for **14**, **17** and **18** after Suzuki coupling with the relevant boronic acids using either 1.1 equiv of Na_2CO_3 (top traces) or 3.0 equiv of TEA (bottom traces) in toluene/EtOH/water (5:5:1.5 v/v), refluxing for 40 min. *Conditions: Waters 2695 separator module, Chiralcel™ OD column (25 cm × 4.6 mm) coupled to a Waters 2996 photodiode array detector, mobile phase: hexane/*i*-PrOH (1:1 v/v) at a flow rate of 0.6 mL/min. OR = optical rotation in DMF (see Section 4 for concentration and temperature details). RT = retention time.

Table 4. Results of the NCI 3-cell line primary cytotoxicity screen for dilactams **21**, **30** and **33**

Dilactams	Growth percentage[a] (%)		
	MCF7 (breast)	NCI-H460 (lung)	SF-268 (CNS)
21	51	5	86
30	61	20	109
33	42	29	73

Values are reported as percentage growth of treated cells compared to untreated control cells. In this preliminary screen, only compounds that reduce the growth of any one of the cell lines to 32% or less of treatment-free controls are progressed to evaluation in the full 60-cell line panel.

[a] Conditions: 48 h exposure to 50 μM at 37 °C, Alamar blue determination (for further details see: www.dtp.nci.nih.gov/branches/btb/ivclsp.html). All other library members were inactive in this screen.

Table 5. Cytotoxicity of dilactams **21**, **30**, **33** and, for comparative purposes, anthramycin (**1**) in A498 (renal cancer) cells and across the full NCI 60-cell line panel (as mean GI_{50} values)

Cell line	GI_{50}[a] (μM)			
	21	**30**	**33**	**1**
A498 (renal cancer)	>50	0.51	3.16	0.195
Mean (full panel)	46.8	7.41	14.8	0.029

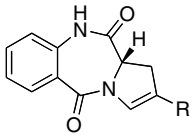
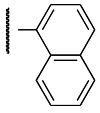
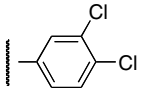
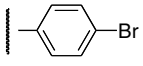
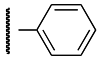
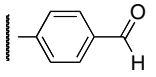
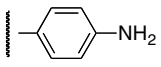
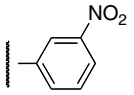
[a] Dose required to inhibit growth by 50% compared to treatment-free control; Conditions: 48 h incubation at 37 °C, sulforhodamine B (SRB) staining agent (for further details see: www.dtp.nci.nih.gov/branches/btb/ivclsp.html).

37 °C with calf thymus (CT) DNA duplex to gauge whether there was a kinetic component to the binding process, although time-dependence would not normally be expected for a reversible DNA binding interaction. The results show that, for the dilactams examined, the ΔT_m shifts are significantly higher than for the unsubstituted dilactam **6** or the C2-chloro/C2–C3-unsaturated analogue **7**, neither of which contains C2-aryl substituents.

One explanation for these observations is that the rigid planar C2-aromatic rings lie along the DNA minor groove and generate hydrophobic interactions with DNA bases thus enhancing binding affinity. In no case was a time-dependent effect upon binding behaviour observed, confirming that the binding mode is entirely non-covalent and hence reversible. Furthermore, the melting curves showed a differential effect upon the high-(G/C) rather than the low-temperature (A/T) domains (data not shown), suggesting a weak GC > AT preference for binding sites on DNA.

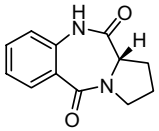
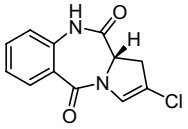
Interestingly, of the library members selected for the thermal denaturation study, **21**, **30** and **33**, which were the only ones to show any significant cytotoxicity, also produced the highest ΔT_m values. Furthermore, all selected library members had ΔT_m values significantly

Table 6. ΔT_m values for the stabilisation of native double-stranded calf thymus (CT) DNA by a selection of the C2-functionalised PBD dilactams **13**, **21**, **22**, **27**, **30**, **31** and **33.** Data for the previously reported PBD dilactams **6** and **7**, and the N10–C11 imine-containing natural products anthramycin (**1**), tomaymycin (**2**) and DC-81 (**3**) are included for comparative purposes

Dilactams	 **R =**	Induced ΔT_m[a] (°C) after incubation at 37 °C for			
		0 h	4 h	18 h	72 h
33		2.3	2.3	2.3	2.4
21		2.1	2.1	2.2	2.2
30		1.9	2.0	2.0	2.1
13		1.6	1.5	1.5	1.6
31		1.5	1.5	1.4	1.5
27		1.2	1.1	1.1	1.1
22		1.0	0.9	1.0	1.0

(*continued on next page*)

Table 6 (*continued*)

Comparative Data		Induced ΔT_m[a] (°C) after incubation at 37 °C for			
		0 h	4 h	18 h	72 h
6[10]		<0.5	—	—	—
7[10]		<0.5	—	—	—
3[25]	DC-81	0.3	0.5	0.7	—
2[25]	Tomaymycin	1.0	2.4	2.6	—
1[25]	Anthramycin	9.4	11.2	13.0	—

[a] For CT-DNA alone, T_m = 67.82 ± 0.07 °C (mean from >110 experiments). All ΔT_m values are ± 0.1–0.2 °C. For a fixed 1:5 molar ratio of [ligand]/[DNA], DNA concentration = 100 μM (DNAp) or 50 μM (bp) and ligand concentration = 20 μM, in aqueous sodium phosphate buffer [10 mM sodium phosphate + 1 mM EDTA, pH 7.00 ± 0.01]. Working solutions were incubated at 37.0 ± 0.1 °C for the time shown[10]. Dilactams **5a** (Fig. 1, R = Ac)[10] and **5b** (Fig. 1, R = H)[10] had ΔT_m values of 2.9 and 3.3 °C, respectively, at 0 h and 37 °C.

higher than that for the N10–C11 imine-containing natural product DC-81 (**3**) which bonds to DNA covalently. This suggests that substitution with a C2-aryl functionality in association with C2–C3 unsaturation may be more important for optimising DNA binding affinity than the presence of either an N10–C11 imine moiety or A-ring substituents.

Furthermore, the dilactam **33** gave a ΔT_m value (2.3 °C) similar to that for the N10–C11 imine-containing tomaymycin (**2**) after 4–72 h incubation with DNA, suggesting that C2-aryl substitution may be as important for DNA interaction as an electrophilic N10–C11 imine moiety, A-ring substituents or C2-*exo* unsaturation.

3. Conclusions

The preparation of intermediate **12** using excess reagents at the triflation step has allowed the successful synthesis of a 23-member library of C2-aryl substituted PBD dilactams (**13–35**). Suzuki coupling was shown to be an efficient method for installing aryl substituents at the C2 position, although the use of base to avoid racemisation at the C11a position was found to be critical, with TEA providing the best (i.e., non-racemising and highest yielding) results. This methodology should be generally applicable to the triflation of N10-unprotected PBD dilactams, thus significantly shortening the number of steps required compared to previously published synthetic routes for N10–C11 imine-containing PBDs.[20,21]

Given the lack of an electrophilic N10–C11 imine moiety (or the equivalent) and A-ring substituents for all library members, dilactams **21**, **30** and **33** showed a surprising degree of cytotoxicity in NCI-H460 (lung) cells in the preliminary NCI screen, with **30** having a similar sub-micromolar potency to anthramycin (**2**) in the A498 renal cancer cell line.

The thermal denaturation data for the dilactams studied showed that they cause a significant degree of DNA helix stabilisation through a non-covalent interaction which may be responsible for the cytotoxicity of dilactams **21**, **30** and **33**. Furthermore, comparison of the ΔT_m values with those for the control molecules **6** (completely unsubstituted), **7** (C2–C3 unsaturation, and C2-chloro) and **3** (N10–C11 imine, A-ring substituents, no C-ring substituents or unsaturation) highlights the importance of a C2-aryl functionality for optimising DNA binding affinity. It is also worth noting that the cytotoxic dilactams **21**, **30** and **33** gave the largest ΔT_m shifts compared to the limited number of other library members examined. The observation that the ΔT_m values for the previously reported dilactams **5a** and **5b** are slightly higher than those for **21**, **30** and **33** suggests that incorporation of A-ring substituents in the latter might enhance ΔT_m by providing further hydrogen bonding opportunities with functional groups in the DNA minor groove (see Fig. 2), and this will be explored in future studies.

In summary, these results support the previous proposal of Jones and co-workers[10] that the activity of dilactam **5a** in a P388 model[9] may be due to its non-covalent interaction with DNA. They also demonstrate that, for PBD dilactams, inclusion of a C2-aryl functionality along with C2–C3 unsaturation can significantly enhance non-covalent DNA interaction even in the absence of A-ring substituents, and can potentially introduce cytotoxic properties. Dilactam **30** is presently being evaluated in an in vivo model of A498 renal cancer, and the results will be reported elsewhere.

4. Experimental

4.1. General synthetic methods

^{1}H and ^{13}C NMR spectra were acquired at 400 and 100 MHz, respectively, using a Brüker Avance NMR spectrometer at 300 K. Chemical shifts are reported relative to Me_4Si (TMS, $\delta = 0.0$ ppm) and are designated as s (singlet), d (doublet), t (triplet), dt (double triplet), dd (doublet of doublets), ddd (double doublet of doublets) or m (multiplet). Coupling constants (J values) are reported in Hertz. A pro-PBD numbering system is used for carbon and proton assignments for intermediate **9** (i.e., based on the final tricyclic ring system). In addition, assignments marked with primes (e.g., H1′ and C2′) indicate positions on C2-aryl substituents. Mass spectra were obtained using a Finnigan Navigator Single Quadrupole Mass Spectrometer coupled to a Waters Alliance HPLC and autosampler (Model 2690). The conditions were: mobile phase = 50:50 v/v (H_2O + 0.1% TFA):(acetonitrile + 0.1% TFA), run time = 3 min, method = isocratic, mode = electrospray positive ($ES^{\cdot+}$), cone = 25, offset = 1, skimmer = 1.5, RF lens = 0.1, source heater = 150 °C, gas flow = 400 L/h. IR spectra were recorded on a Perkin-Elmer Spectrum 1000 FT IR Spectrometer. Melting points were measured on a digital Electrothermal™ melting point apparatus. Thin-layer chromatography (TLC) was performed on silica gel aluminium plates (Merck 60 F_{254}), and flash chromatography was carried out using Merck 60 (230–400 mesh ASTM) silica gel. Optical rotations and chiral HPLC analyses were carried out according to the published methodologies of Tercel and co-workers.[23] Optical rotations were measured using an ADP 220 polarimeter (Bellingham Stanley Ltd), and concentrations (c) are reported in g/100 mL. Chiral HPLC was performed using a Waters 2695 separator module and a Chiralcel™ OD column (25 cm × 4.6 mm) coupled to a Waters 2996 photodiode array detector. A mobile phase of hexane/*i*-PrOH (1:1 v/v) was used at a flow rate of 0.6 mL/min.

4.1.1. 4-Hydroxy-1-(2-nitrobenzoyl)pyrrolidine-2-carboxylic acid methyl ester (9). Oxalyl chloride (11.81 g or 8.1 mL, 93.1 mmol; 1.3 equiv) and DMF (0.2 mL) were added to a suspension of 2-nitrobenzoic acid (11.96 g, 71.6 mmol; 1 equiv) in dry CH_2Cl_2 (500 mL). The mixture was allowed to stir at room temperature for 20 h. CH_2Cl_2 and excess oxalyl chloride were removed under vacuum until a viscous yellow oil was obtained. The residue was dissolved in dry THF (500 mL) and quickly transferred to a dropping funnel and then added dropwise over a period of 5 h to a solution of 2*S*,4*R*-4-hydroxy-2-proline methyl ester (25.0 g, 78.8 mmol; 1.1 equiv) and Et_3N (15.93 g or 21.9 mL, 157.5 mmol; 2.2 equiv) in dry THF (1 L) at −40 °C. The reaction mixture was then allowed to warm to room temperature (1 h). The mixture was concentrated under vacuum, and the residue was redissolved in EtOAc (500 mL) followed by washing with saturated $NaHCO_3$ (2 × 150 mL). The aqueous layer was extracted several times with EtOAc (until no UV activity was observed by TLC). The organic extracts were combined and concentrated under vacuum, then washed with water (2 × 100 mL). This second aqueous layer was back extracted with EtOAc, and organic phases were combined and concentrated under vacuum to afford the product **9** as a yellow-green oil (20.6 g, 98%). IR (film, ν_{max}/cm^{-1}): 3427 (OH), 3070, 2954, 1745 (C=O), 1633 (C=O), 1532, 1488, 1435, 1349 (NO_2), 1205, 1084, 854; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.09–2.12 (m, 1H, 1α), 2.34–2.37 (m, 1H, 1β), 3.11 (dd, 1H, J = 1.7, 11.1 Hz, H3α), 3.48 (dd, 1H, J = 4.3, 11.0 Hz, H3β), 4.37 (br m, 1H, H2), 4.73 (t, 1H, J = 8.1 Hz, H11a), 7.48 (d, 1H, J = 7.6 Hz, H6), 7.56 (t, 1H, J = 8.4 Hz, H8), 7.70 (t, 1H,J = 7.5 Hz, H7), 8.1 (d, 1H, J = 8.4 Hz, H9); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 172.3 (C11), 166.7 (C5), 145.1 (C9a), 134.6 (C7), 132.7 (C5a), 130.2 (C8), 128.6 (C6), 124.6 (C9), 70.0 (C2), 57.4 (C11a), 56.6 (C3), 52.5 (OCH_3), 38.0 (C1); MS (ESI) *m*/*z* (relative intensity): 295 ($[M+H]^{\cdot+}$, 100%), 263 (20), 235 (42).

4.1.2. 2*R*-Hydroxy-1,2,3,10,11,11a*S*-hexahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-dione (10). Palladium on charcoal catalyst (0.35 g, 10% w/w) was added as a slurry in EtOAc (10 mL) (CAUTION: pyrophoric) to a solution of **9** (3.45 g, 11.7 mmol; 1 equiv) in ethanol (50 mL). The reaction mixture was agitated under an atmosphere of hydrogen (45 psi) in a Parr apparatus for 2 h. The mixture was filtered through celite and the solvent removed under reduced pressure. The resulting oil was diluted with THF (4 mL) and water (40 mL) containing 0.12 mL of concentrated HCl. The solution was allowed to stir for 20 h at room temperature. The product crystallised out of solution as a colourless solid which was collected by vacuum filtration, and was recrystallised in EtOAc/MeOH to furnish **10** as a colourless solid (2.23 g, 82%). Mp 229–230 °C; $[\alpha]_D^{24}$ +450° (c 0.25, MeOH); IR (KBr pellet, ν_{max}/cm^{-1}): 3509, 3460, 3207, 2883, 1685 (C=O), 1609 (C=O), 1573, 1481, 1449, 1355, 1250, 1216, 1059, 1030, 965; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.96–2.02 (m, 1H, H1α), 2.74 (dt, 1H, J = 13.5, 5.5 Hz, H2β), 3.53 (dd, 1H, J = 12.4, 4.9 Hz, H3β), 3.68 (dd, 1H, J = 12.4, 2.9 Hz, H3α), 4.19 (dd, 1H, J = 8.09, 5.8 Hz, H11a), 4.40 (m, 1H, H2), 7.03 (d, 1H, J = 8.1 Hz, H9), 7.18 (t, 1H, J = 7.6 Hz, H7), 7.44 (t, 1H, J = 8.3 Hz, H8), 7.77 (d, 1H, J = 7.3 Hz, H6); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 172.1 (C11), 168.3 (C5), 137.7 (C9a), 133.9 (C8), 131.6 (C6), 127.4 (C5a), 125.8 (C7), 122.6 (C9), 69.5 (C2), 57.0 (C11a), 55.2 (C3), 35.4 (C2); MS (ESI) *m*/*z* (relative intensity): 233 ($[M+H]^{\cdot+}$, 100%); Elem. Anal. Calcd for $C_{12}H_{12}N_2O_3$: C, 62.06; H, 5.21; N, 12.06%. Found: C, 62.05; H, 5.22; N, 12.00%.

4.1.3. 1,2,3,10,11,11a*S*-Hexahydro-5*H*-pyrrolo[2,1-*c*]-[1,4]benzodiazepine-2,5,11-trione (11). A freshly prepared solution of Jones' reagent (11.42 g, CrO_3, 9.3 mL H_2SO_4 and 33.2 mL H_2O) was added dropwise to a solution of **10** (13.26 g, 57.1 mmol; 1 equiv) in acetone (1.0 L) in an ultra-sound bath. The reaction mixture was sonicated at room temperature for over 4 h whilst monitoring by TLC ($CHCl_3$/MeOH 10:1). The reaction mixture was quenched with MeOH to consume the excess oxidant and allowed to stand before decanting the precipitated chromium salts. The remaining solution was concentrated under vacuum and the residue

redissolved in EtOAc and washed with water. The aqueous layer was back-extracted with EtOAc (until no UV activity was observed by TLC). The organic layers were combined, dried over $MgSO_4$, concentrated under vacuum and the resulting solid collected by vacuum filtration and washed with EtOAc/ether 7:3 to afford the ketone **11** as a colourless solid (9.93 g, 76%). Mp 231–233 °C; $[\alpha]_D^{23.1}$ +548° (*c* 0.124, MeOH); IR (KBr pellet, ν_{max}/cm^{-1}): 3224 (N–H), 2917, 1772 (C=O), 1698 (C=O), 1616 (C=O), 1579, 1482, 1455, 1254, 1165, 1059, 760; ^1H NMR ($CDCl_3$, 400 MHz): δ 2.84 (dd, 1H, J = 19.4, 10.2, H1α), 3.62 (dd, 1H, J = 19.5, 3.5 Hz, H1β), 3.95 (d, 1H, J = 20.2 Hz, H3β), 4.30 (d, 1H, J = 20.2 Hz, H1α), 4.61 (dd, 1H, J = 10.2, 3.5 Hz, H11a), 7.08 (d, 1H, J = 8.1 Hz, H9), 7.34 (t, 1H, J = 7.8 Hz, H7), 7.56 (t, 1H, J = 8.0 Hz, H8), 8.01 (d, 1H, J = 7.9 Hz, H6), 8.6 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 206.5 (C2), 169.5 (C11), 166.1 (C5), 134.9 (C9a), 133.2 (C8), 131.4 (C6), 125.90 (C7), 125.88 (C5a), 121.3 (C9), 54.0 (C11a), 52.8 (C3), 36.6 (C1); MS (ESI) *m*/*z* (relative intensity): 347 ($[M+H]^{\cdot+}$, 100%), 115 (50), 100 (70).

4.1.4. Trifluoromethanesulfonic acid 5,11-dioxo-1,10,11,11a *S*-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-2-yl ester (12). Triflic anhydride (18.87 g or 11.2 mL; 66.9 mmol; 7 equiv) was rapidly added to a cold (ice bath) vigorously stirred solution of **11** (2.20 g, 9.56 mmol; 1 equiv) and pyridine (5.29 g or 5.4 mL; 66.9 mmol; 7 equiv) in $CHCl_3$ (250 mL). The reaction mixture was allowed to warm to room temperature and stirred for 2 h. The reaction mixture was washed consecutively with water, saturated $CuSO_4$ solution and saturated $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$, concentrated under vacuum and the residue purified by flash chromatography ($CHCl_3$) to give **12** as a brown solid. (2.60 g, 75%). Mp 170–172 °C; $[\alpha]_D^{23}$ +379° (*c* 0.186, $CHCl_3$); IR (KBr pellet, ν_{max}/cm^{-1}): 3224 (N–H), 3065, 2982, 2946, 1698 (C=O), 1617 (C=O), 1483, 1444, 1329 (S=O assym.) 1205, 1135 (S=O sym.), 922, 755; ^1H NMR ($CDCl_3$, 400 MHz): δ 3.21 (ddd, 1H, J = 16.5, 11.4, 2.4 Hz, H1α), 3.96 (ddd, 1H, J = 16.5, 4.1, 1.8 Hz, H1β), 4.63 (dd, 1H, J = 11.4, 4.1 Hz, H11a), 7.06 (d, 1H, J = 8.0 Hz, H9), 7.21 (t, 1H, J = 1.9 Hz, H3) 7.35 (t, 1H, J = 8.3 Hz, H7), 7.56 (dt, 1 H, J = 7.9, 1.6 Hz, H8), 8.01 (dd, 1H, J = 7.9, 1.4 Hz, H6), 8.26 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 167.7 (C11), 162.9 (C5), 137.9 (C2), 134.5 (C9a), 133.5 (C8), 131.8 (C6), 125.9 (C7), 125.0 (C5a), 121.4 (C9), 118.5 (q, J = 319 Hz from ^{13}C $^{19}F_3$ coupling, CF_3), 119.4 (C3), 55.7 (C11a), 30.3 (C1); MS (ESI) *m*/*z* (relative intensity): 363 ($[M+H]^{\cdot+}$, 100%). Elem. Anal. Calcd for $C_{12}H_{10}N_2O_3$: C, 43.10; H, 2.50; N, 7.73%. Found: C, 43.13; H, 2.35; N, 7.65%.

4.2. General procedure for Suzuki coupling

Palladium catalyst $Pd(PPh_3)_4$ (13 mg, 0.011 mmol, 0.02 equiv) was added to a solution of **12** (200 mg, 0.55 mmol, 1 equiv) and aryl boronic acid (or pinacol ester of boronic acid) (0.71 mmol, 1.3 equiv) in toluene (5 mL), EtOH (5 mL) and water (1.5 mL) in the presence of Et_3N (167 mg or 0.3 mL, 1.65 mmol, 3 equiv). The reaction vessels were flushed with nitrogen in a Carousel Reaction Station and the reaction mixtures were heated at reflux for 30 min with magnetic stirring when TLC (EtOAc/hexane 8.5:1.5 v/v) showed complete consumption of starting material. Water (10 mL) was then added to the reaction mixtures with shaking. The products crystallised out of solution and were collected by vacuum filtration, washed with water, ethanol, and finally $CHCl_3$ (HPLC grade). Some filtrates contained significant amounts of dissolved product, which were purified by flash chromatography and combined with the precipitated material to afford products **13**–**35**.

4.2.1. 2-Phenyl-1,10,11,11a*S*-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-dione (13). Colourless solid, 93 mg (58%); Mp 323–324 °C; $[\alpha]_D^{27.1}$ +760° (*c* 0.121, DMF); IR (disc, ν_{max}/cm^{-1}) 3112 (NH), 2928, 1699 (C=O), 1598 (C=O), 1482, 1446, 1385, 1303, 1222, 1174, 864, 753, 702; ^1H NMR (d_6-DMSO, 400 MHz): δ 3.17 (ddd, 1H, J = 16.2, 10.9, 2.1 Hz, H1α), 3.71 (ddd, 1H, J = 16.3, 3.6, 1.6 Hz, H1β), 4.81 (dd, 1H, J = 10.9, 3.5 Hz, H11a), 7.20 (d, 1H, J = 8.1 Hz, H9) 7.27 (t, 1H, J = 7.3 Hz, H4′), 7.30 (t, 1H, J = 8.1 Hz, H7), 7.37 (t, 2 H, J = 7.8 Hz, H3′ and H5′), 7.56–7.59 (m, 3 H, H8, H2′ and H6′), 7.60 (t, 1H, J = 1.5 Hz, H3), 7.87 (dd, 1H, J = 7.9, 1.5 Hz, H6), 10.73 (s, 1H, NH); ^{13}C NMR (d_6-DMSO, 100 MHz): δ 168.9 (C11), 161.5 (C5), 136.1 (C9a), 133.4 (C1′), 132.5 (C8), 130.6 (C6), 128.6 (C3′ and C5′), 127.3 (C4′), 125.6 (C5a), 125.0 (C2′ and C6′), 124.7 (C2), 124.2 (C7), 122.6 (C3), 121.7 (C9), 56.1 (C11a), 30.5 (C1); MS (ESI) *m*/*z* (relative intensity): 290.9 ($[M+H]^{\cdot+}$, 100%); Elem. Anal. Calcd for $C_{18}H_{14}N_2O_2$: C, 74.47; H, 4.86; N, 9.65%. Found: C, 74.49; H, 4.62; N, 9.57%.

4.2.2. 2-(4-Tolyl)-1,10,11,11a*S*-tetrahydro-5*H*-pyrrolo-[2,1-*c*][1,4]benzodiazepine-5,11-dione (14). Brown solid, 92 mg (55%); Mp 302–303 °C; $[\alpha]_D^{26.4}$ +772° (*c* 0.103, DMF); IR (disc, ν_{max}/cm^{-1}) 3214 (NH), 2919, 1681 (C=O), 1643 (C=O), 1483, 1447, 1419, 1307, 1272, 1215, 1174, 894, 869, 837, 804, 755, 698; ^1H NMR (d_6-DMSO, 400 MHz): δ 2.31 (s, 3 H, 4′-CH_3), 3.14 (ddd, 1H, J = 16.2, 10.9, 2.1 Hz, H1α), 3.67 (ddd, 1H, J = 16.3, 3.5, 1.5 Hz, H1β), 4.79 (dd, 1H, J = 10.8, 3.5 Hz, H11a), 7.18 (d, 2 H, J = 8.0 Hz, H3′ and H5′), 7.20 (d, 1H, J = 7.4 Hz, H9), 7.30 (dt, 1H, J = 8.0, 0.9 Hz, H7), 7.45 (d, 2 H, J = 8.1 Hz, H2′ and H6′), 7.54 (s, 1H, H3), 7.58 (dt, 1H, J = 8.2, 1.6 Hz, H8), 7.86 (dd, 1H, J = 7.9, 1.5 Hz, H6), 10.75 (s, 1H, NH); ^{13}C NMR (d_6-DMSO, 100 MHz): δ 169.0 (C11), 161.4 (C5), 136.7 (C9a), 136.0 (C4′), 132.5 (C8), 130.6 (C6), 130.5 (C1′), 129.2 (C3′ and C5′), 125.7 (C5a), 125.0 (C2′ and C6′), 124.8 (C2), 124.2 (C7), 121.8 (C3), 121.6 (C9), 56.1 (C11a), 30.5 (C1), 20.8 (4′-CH_3); MS (ESI) *m*/*z* (relative intensity): 305 ($[M+H]^{\cdot+}$, 100%); Elem. Anal. Calcd for $C_{19}H_{16}N_2O_2$: C, 74.98; H, 5.30; N, 9.20%. Found: C, 74.79; H, 5.17; N, 9.17%.

4.2.3. 2-(4-Methoxyphenyl)-1,10,11,11a*S*-tetrahydro-5*H*-pyrrolo[2,1-c][1,4]benzodiazepine-5,11-dione (15). Colourless solid, 85 mg (68%, from 140 mg of **12**); $[\alpha]_D^{26.8}$ +706° (*c* 0.107, DMF); Mp 324–326 °C; IR (disc, ν_{max}/cm^{-1}) 3219 (NH), 2928, 2836, 1694 (C=O),

1608 (C=O), 1514, 1444, 1255, 1216, 1181, 1033, 819, 753; ^{1}H NMR (d_6-DMSO, 400 MHz): δ 3.13 (ddd, 1H, J = 16.1, 10.8, 1.7 Hz, H1α), 3.67 (dd, 1H, J = 16.3, 1.9 Hz, H1β), 4.77 (dd, 1H, J = 10.7, 3.4 Hz, H11a), 6.93 (d, 2 H, J = 8.7 Hz, H3′ and H5′) 7.19 (d, 1H, J = 8.0 Hz, H9), 7.29 (t, 1H, J = 7.4 Hz, H7), 7.46 (s, 1H, H3), 7.48 (t, 2 H, J = 8.7 Hz, H2′ and H6′), 7.57 (dt, 1H, J = 8.1, 1.3 Hz, H8) 7.85 (dd, 1H, J = 7.8 Hz, 1.0 Hz, H6), 10.70 (br s, 1H, NH); ^{13}C NMR (d_6-DMSO, 100 MHz): δ 169.0 (C11), 161.2 (C5), 158.6 (C4′), 136.0 (C9a), 132.4 (C8), 130.6 (C6), 126.5 (C2′ and C6′), 125.9 (C1′), 125.7 (C2), 124.7 (C5a), 124.2 (C7), 121.6 (C9), 120.8 (C3), 114.1 (C3′ and C5′), 56.0 (C11a), 55.1 (4′-OCH_3), 30.7 (C1); MS (ESI) m/z (relative intensity): 321 ($[M+H]^{\cdot+}$, 100%).

4.2.4. 2-(4-Dimethylaminophenyl)-1,10,11,11a*S*-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-dione (16). Yellow solid, 310 mg (67%, from 500 mg of **12**); Mp 315–316 °C; $[\alpha]_D^{26.8}$ +563° (c 0.214, DMF); IR (disc, ν_{max}/cm^{-1}) 3112 (NH), 2921, 1697 (C=O), 1617 (C=O), 1528, 1482, 1444, 1362, 1260, 1201, 1175, 1062, 946, 868, 817, 752, 696; ^{1}H NMR (d_6-DMSO, 400 MHz, 323 K): δ 2.94 (s, 6 H, 4′-N(CH_3)$_2$), 3.11 (ddd, 1H, J = 16.2, 10.9, 2.2 Hz, H1α), 3.68 (ddd, 1H, J = 16.3, 3.6, 1.6 Hz, H1β), 4.73 (dd, 1H, J = 10.8, 3.6 Hz, H11a), 6.73 (d, 2H, J = 8.9 Hz, H3′ and H5′), 7.20 (d, 1H, J = 8.1 Hz, H9), 7.28 (t, 1H, J = 8.1 Hz, H7), 7.30 (s, 1H, H3), 7.36 (d, 2H, J = 8.8 Hz, H2′ and H6′), 7.55 (dt, 1H, J = 8.2, 1.6 Hz, H8), 7.86 (dd, 1H, J = 7.9, 1.5 Hz, H6) 10.57 (s, 1H, NH); ^{13}C NMR (d_6-DMSO, 100 MHz, 323 K): δ 169.1 (C11), 160.9 (C5), 149.7 (C4′), 135.9 (C9a), 132.3 (C8), 130.5 (C6), 126.1 (C2′ and C6′), 125.9 (C5a), 125.4 (C1′), 124.2 (C7), 121.6 (C9), 121.0 (C2), 118.9 (C3), 112.2 (C3′ and C5′), 55.9 (C11a), 40.1 (4′-N(CH_3)$_2$), 30.7 (C1); MS (ESI) m/z (relative intensity): 334 ($[M+H]^{\cdot+}$, 100%).

4.2.5. 2-(4-Chlorophenyl)-1,10,11,11a*S*-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-dione (17). Colourless solid, 118 mg (66%); Mp 330–332 °C; $[\alpha]_D^{24.5}$ +703° (c 0.190, DMF); IR (disc, ν_{max}/cm^{-1}) 3213 (NH), 2982, 1682 (C=O), 1651 (C=O), 1483, 1445, 1418, 1273, 1215, 1089, 830, 777, 678; ^{1}H NMR (d_6-DMSO, 400 MHz): δ 3.15 (dd, 1H, J = 15.1, 11.9 Hz, H1α), 3.68 (d, 1H, J = 14.9 Hz, H1β), 4.82 (dd, 1H, J = 10.6, 2.8 Hz, H11a), 7.20 (d, 1H, J = 8.0 Hz, H9) 7.30 (t, 1H, J = 7.5 Hz, H7), 7.41 (d, 2H, J = 8.3 Hz, H3′ and H5′), 7.57-7.61 (m, 3 H, H8, H2′ and H5′), 7.67 (s, 1H, H3), 7.86 (d, 1H, J = 7.6 Hz, H6), 10.74 (s, 1H, NH); ^{13}C NMR (d_6-DMSO, 100 MHz): δ 168.9 (C11), 161.6 (C5), 136.1 (C9a), 132.6 (C8), 132.4 (C4′), 131.5 (C5a), 130.7 (C6), 128.5 (C3′ and C5′), 126.8 (C2′ and C6′), 125.6 (C2), 124.3 (C7), 123.6 (C3), 121.7 (C9), 56.2 (C11a), 30.5 (C1); MS (ESI) m/z (relative intensity): 324.9 ($[M+H]^{\cdot+}$, 100%).

4.2.6. 2-(4-Fluorophenyl)-1,10,11,11a*S*-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-dione (18). Colourless solid, 244 mg (44%); Mp 318–320 °C (dec); $[\alpha]_D^{27.1}$ +745° (c 0.098, DMF); IR (disc, ν_{max}/cm^{-1}) 3110, 2976, 1696 (C=O), 1602 (C=O), 1504, 1483, 1446, 1418, 1223, 1104, 864, 753, 698; ^{1}H NMR (d_6-DMSO, 400 MHz): δ 3.15 (ddd, 1H, J = 16.1, 10.9, 1.7 Hz, H1α), 3.68 (dd, 1H, J = 16.3, 2.0 Hz, H1β), 4.80 (dd, 1H, J = 10.8, 3.4 Hz, H11a), 7.17–7.21 (m, 3 H, H9, H3′ and H5′), 7.29 (t, 1H, J = 7.4 Hz, H7), 7.56–7.63 (m, 4 H, H8, H2′ and H6′), 7.85 (dd, 1H, J = 7.8, 1,1 Hz, H6), 10.72 (s, 1H, NH); ^{13}C NMR (d_6-DMSO, 100 MHz): δ 168.9 (C11), 161.5 (C5), 160.4 (d, J = 123 Hz, C4′ coupling to 4′-^{19}F), 136.1 (C9a), 132.5 (C8), 130.6 (C6), 129.4 (C1′), 127.1 (d, J = 8 Hz, C2′ and C6′ long range coupling to 4′-^{19}F), 125.6 (C2), 124.3 (C7), 123.8 (C5a), 122.6 (C3), 121.7 (C9), 115.4 (d, J = 22 Hz, C3′ and C5′ coupling to 4′-^{19}F), 56.2 (C11a), 30.7 (C1); MS (ESI) m/z (relative intensity): 308.9 ($[M+H]^{\cdot+}$, 100%).

4.2.7. 4-(5,11-Dioxo-1,10,11,11a*S*-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-2-yl)benzonitrile (19). Colourless solid, 111 mg (64%); Mp 356–358 °C; $[\alpha]_D^{27.3}$ +752° (c 0.111, DMF); IR (disc, ν_{max}/cm^{-1}) 3290 (NH), 3095, 2221, 1695 (C=O), 1651 (C=O), 1602, 1481, 1440, 1414, 1384, 1275, 1212, 1172, 842, 752; ^{1}H NMR (d_6-DMSO, 500 MHz): δ 3.18 (ddd, 1H, J = 16.1, 11.0, 2.0 Hz, H1α), 3.71 (ddd, 1H, J = 16.3, 3.5, 1.4 Hz, H1β), 4.88 (dd, 1H, J = 10.9, 3.5 Hz, H11a), 7.21 (d, 1H, J = 7.85 Hz, H9) 7.31 (dt, 1H, J = 8.0, 0.8 Hz, H7), 7.60 (dt, 1H, J = 8.2, 1.5 Hz, H8), 7.76 (d, 2H, J = 8.5 Hz, C3′ and C5′) 7.80 (d, 2 H, J = 8.6 Hz, C2′ and C6′), 7.87 (dd, 1H, J = 8.0, 1.4 Hz, H6), 7.88 (s, 1H, H3), 10.77 (s, 1H, NH); ^{13}C NMR (d_6-DMSO, 125 MHz): δ 168.7 (C11), 161.9 (C5), 138.4 (C9a), 136.1 (C1′), 132.8 (C8), 132.4 (C3′ and C5′), 130.7 (C6), 126.3 (C3), 125.7 (C2, and C6′), 125.4 (C5a), 124.3 (C7), 123.1 (C2), 121.7 (C9), 119.0 (CN), 108.9 (C4′), 56.4 (C11a), 30.2 (C1); MS (ESI) m/z (relative intensity): 316.1 ($[M+H]^{\cdot+}$, 100%).

4.2.8. 4-(5,11-Dioxo-1,10,11,11a*S*-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-2-yl)-benzoic acid (20). Yellow solid, 100 mg (42%). Mp 371 °C (dec); $[\alpha]_D^{23.4}$ +571° (c 0.099, DMF); IR (disc, ν_{max}/cm^{-1}) 3300–3000 (COOH), 3091, 2975, 1694 (C=O), 1654 (C=O), 1604 (C=O), 1581, 1536, 1481, 1448, 1388, 1271, 1218, 1174, 834, 795, 755, 685; ^{1}H NMR (d_6-DMSO, 500 MHZ): δ 3.21 (dd, 1H, J = 15.2, 11.4 Hz, H1α), 3.75 (d, 1H, J = 14.5 Hz, H1β), 4.84 (dd, 1H, J = 10.6, 3.1 Hz, H11a), 7.25 (d, 1H, J = 8.0 Hz, H9) 7.33 (t, 1H, J = 7.5 Hz, H7), 7.49 (d, 2 H, J = 8.0 Hz, C2′ and C6′), 7.61–7.63 (m, 2H, H3 and H8) 7.89 (d, 3 H, J = 7.9 Hz, H6), 10.82 (s, 1H, NH); ^{13}C NMR (d_6-DMSO, 125 MHz): δ 169.2 (C11), 168.9 (COOH), 161.5 (C5), 139.2 (C1′), 136.1 (C9a), 133.6 (C4′), 132.5 (C8), 130.6 (C6), 129.3 (C3′ and C5′), 125.7 (C5a), 124.9 (C2), 124.2 (C7), 123.9 (C3′ and C5′), 122.5 (C3), 121.7 (C9), 56.1 (C11a), 30.6 (C1); MS (ESI) m/z (relative intensity): 335.1 ($[M+H]^{\cdot+}$, 100%).

4.2.9. 2-(3,4-Dichlorophenyl)-1,10,11,11a*S*-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-dione (21). Colourless solid, 139.5 mg (55%); Mp 364–367 °C; $[\alpha]_D^{23.9}$ +659° (c 0.088, DMF); IR (disc, ν_{max}/cm^{-1}) 3230 (NH), 2930, 1703 (C=O), 1631 (C=O), 1578, 1481, 1439, 1302, 1279, 1207, 1136, 1027, 877, 814, 774, 750;

^{1}H NMR (d_6-DMSO, 500 MHz): δ 3.16 (ddd, 1H, J = 16.1, 11.0, 2.0 Hz, H1α), 3.67 (ddd, 1H, J = 16.2, 3.4, 1.3 Hz, H1β), 4.84 (dd, 1H, J = 10.9, 3.5 Hz, H11a), 7.20 (d, 1H, J = 8.0 Hz, H9) 7.30 (t, 1H, J = 7.3 Hz, H7), 7.58 (dt, 1H, J = 8.6, 1.9 Hz, H8), 7.59-7.61 (m, 2H, H5′ and H6′) 7.81 (s, 1H, H3), 7.86 (dd, 1H, J = 6.3, 1.4 Hz, H6), 7.87 (s, 1H, H2′), 10.76 (s, 1H, NH); ^{13}C NMR (d_6-DMSO, 125 MHz): δ 168.8 (C11), 161. 8 (C5), 136.1 (C9a), 134.4 (C1′), 132.7 (C8), 131.5 (C3′), 130.7 (C5′), 130.6 (C2′), 129.1 (C4′), 126.7 (C6), 125.4 (C5a), 125.2 (C6′), 125.1 (C3), 124.3 (C7), 122.4 (C2), 121.7 (C9), 56.3 (C11a), 30.4 (C1); MS (ESI) m/z (relative intensity): 359 ([M+H]$^{\cdot+}$, 100%).

4.2.10. 2-(3-Nitrophenyl)-1,10,11,11a*S*-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-dione (22). Yellowish solid, 133 mg (72%); Mp 324–325 °C (dec); $[\alpha]_D^{25.2}$ +703° (c 0.078, DMF); IR (disc, ν_{max}/cm^{-1}) 3214 (NH), 2973, 1701 (C=O), 1623 (C=O), 1526 (NO_2 asym.), 1481, 1424, 1345 (NO_2 sym.), 1211, 874, 735, 679; ^{1}H NMR (d_6-DMSO, 400 MHz): δ 3.24 (dd, 1H, J = 15.8, 11.2 Hz, H1α), 3.74 (d, 1H, J = 16.2 Hz, H1β), 4.87 (dd, 1H, J = 10.8, 3.3 Hz, H11a), 7.20 (d, 1H, J = 8.0 Hz, H9), 7.30 (t, 1H, J = 7.5 Hz, H7), 7.59 (t, 1H, J = 7.3 Hz, H8), 7.64 (t, 1H, J = 8.0 Hz, H5′) 7.87 (d, 1H, J = 7.8 Hz, H6), 7.90 (s, 1H, H3), 8.07 (t, 2 H, J = 7.9 Hz, H4′ and H6′), 8.28 (s, 1H, H2′), 10.76 (s, 1H, NH); ^{13}C NMR (d_6-DMSO, 100 MHz): δ 168.8 (C11), 161.9 (C5), 148.3 (C3′), 136.1 (C9a), 135.4 (C1′), 132.7 (C8), 131.3 (C6′), 130.7 (C6), 130.0 (C5′), 125.6 (C3), 125.4 (C2), 124.3 (C7), 122.6 (C5a), 121.7 (C9), 121.5 (C4′), 119.2 (C2′), 56.3 (C11a), 30.5 (C1); MS (ESI) m/z (relative intensity): 336 ([M+H]$^{\cdot+}$, 100%).

4.2.11. 3-(5,11-Dioxo-1,10,11,11a*S*-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-2-yl)benzonitrile (23). Colourless solid, 249 mg (57%, from 500 mg of **12**); $[\alpha]_D^{26.5}$ +536° (c 0.112, DMF); Mp 339–340 °C; IR (disc, ν_{max}/cm^{-1}) 3199 (NH), 2978, 2229 (CN), 1693 (C=O), 1656 (C=O), 1483, 1446, 1413, 1302, 1212, 884, 840, 792, 750, 681; ^{1}H NMR (d_6-DMSO, 500 MHZ): δ 3.24 (ddd, 1H, J = 16.0, 10.9, 1.5 Hz, H1α), 3.78 (dd, 1H, J = 16.3, 2.1 Hz, H1β), 4.92 (dd, 1H, J = 10.8, 3.4 Hz, H11a), 7.20 (d, 1H, J = 8.1 Hz, H9) 7.30 (t, 1H, J = 7.6 Hz, H7), 7.55 (t, 1H, J = 7.8 Hz, H5′), 7.59 (t, 1H, J = 7.2 Hz, H8), 7.69 (d, 1H, J = 7.6 Hz, H4′), 7.84 (s, 1H, H3), 7.86 (d, 1H, J = 7.7 Hz, H6), 7.91 (d, 1H, J = 8.0 Hz, H6′), 8.09 (s, 1H, H2′) 10.75 (s, 1H, NH); ^{13}C NMR (d_6-DMSO, 125 MHz): δ 168.8 (C11), 161.8 (C5), 136.1 (C9a), 134.9 (C1′), 132.7 (C8), 130.7 (C4′), 130.4 (C5′), 129.7 (C6′), 129.5 (C6), 128.5 (C2′), 125.5 (C5a), 125.1 (C3), 124.3 (C7), 122.8 (C2), 121.7 (C9), 118.7 (CN), 111.8 (C3′), 56.3 (C11a), 30.3 (C1); MS (ESI) m/z (relative intensity): 316 ([M+H]$^{\cdot+}$, 100%).

4.2.12. 2-(3-Chlorophenyl)-1,10,11,11a*S*-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-dione (24). Colourless solid, 110 mg (48%); Mp 332–333 °C; $[\alpha]_D^{23.5}$ +707° (c 0.099, DMF); IR (disc, ν_{max}/cm^{-1}) 3168 (NH), 2976, 1701 (C=O), 1619 (C=O), 1481, 1446, 1383, 1227, 898, 869, 757, 684; ^{1}H NMR (d_6-DMSO, 400 MHz): δ 3.16 (ddd, 1H, J = 16.0, 11.0, 1.5 Hz, H1α), 3.68 (dd, 1H, J = 16.1, 1.8 Hz, H1β), 4.83 (dd, 1H, J = 10.8, 3.4 Hz, H11a), 7.20 (d, 1H, J = 8.0 Hz, H9) 7.28–7.32 (m, 2 H, H7 and H4′), 7.39 (t, 1H, J = 7.9 Hz, H5′), 7.53 (d, 1H, J = 7.7 Hz, H6′), 7.59 (dt, 1H, J = 7.0, 1.2 Hz, H8), 7.65 (s, 1H, H2′), 7.74 (s, 1H, H3), 7.86 (d, 1H, J = 6.9 Hz, H6), 10.74 (s, 1H, NH); ^{13}C NMR (d_6-DMSO, 100 MHz): δ 168.8 (C11), 161.7 (C5), 136.1 (C9a), 135.8 (C1′), 133.5 (C3′), 132.6 (C8), 130.7 (C6), 130.4 (C5′), 126.9 (C4′), 125.5 (C5a), 124.7 (C2′), 124.4 (C7), 124.3 (C3), 123.7 (C6′), 123.3 (C2), 121.7 (C9), 56.2 (C11a), 30.4 (C1); MS (ESI) m/z (relative intensity): 324.9 ([M+H]$^{\cdot+}$, 100%).

4.2.13. 2-(3-Methoxyphenyl)-1,10,11,11a*S*-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-dione (25). Near-colourless solid, 71.5 mg (31%); Mp 288–289 °C; $[\alpha]_D^{24.2}$ +694° (c 0.072, DMF); IR (disc, ν_{max}/cm^{-1}) 3204 (NH), 2935, 1684 (C=O), 1642 (C=O), 1453, 1419, 1270, 1220, 1166, 1050, 866, 778, 755, 688; ^{1}H NMR (d_6-DMSO, 400 MHz): δ 3.16 (ddd, 1H, J = 16.1, 10.9, 1,8 Hz, H1α), 3.68 (dd, 1H, J = 16.2, 2.0 Hz, H1β), 3.80 (s, 3 H, 3′-OCH_3), 4.81 (dd, 1H, J = 10.8, 3.4 Hz, H11a), 6.84 (d, 1H, J = 9.2 Hz, C4′), 7.10–7.12 (m, 2 H, H2′ and H6′), 7.20 (d, 1H, J = 8.0 Hz, H9), 7.26–7.32 (m, 2 H, H7 and H5′), 7.59 (dt, 1H, J = 8.6, 1.4 Hz, H8), 7.67 (s, 1H, H3), 7.86 (dd, 1H, J = 7.8, 1.3 Hz, H6), 10.72 (s, 1H, NH); ^{13}C NMR (d_6-DMSO, 100 MHz): δ 173.9 (C11), 169.0 (C5), 159.4 (C3′), 136.1 (C9a), 134.8 (C1′), 132.6 (C8), 130.7 (C6), 129.6 (C5′), 124.7 (C5a), 124.3 (C7), 123.2 (C3), 121.7 (C9), 117.6 (C6′), 113.4 (C4′), 110.0 (C2′), 56.1 (C11a), 55.1 (3′-OCH_3), 30.6 (C1); MS (ESI) m/z (relative intensity): 321 ([M+H]$^{\cdot+}$, 100%); Elem. Anal. Calcd for $C_{19}H_{16}N_2O_3$: C, 71.24; H, 5.03; N, 8.74%. Found: C, 71.02; H, 5.12; N, 8.80%.

4.2.14. *N*-[3-(5,11-Dioxo-1,10,11,11a*S*-tetrahydro-1*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-2-yl)-phenyl]acetamide (26). Colourless solid, 308 mg (76%, from 425 mg of **12** using Na_2CO_3 as base); Mp 308–311 °C; $[\alpha]_D^{25}$ +16° (c 0.157, DMF); IR (disc, ν_{max}/cm^{-1}) 3213 (NH), 2930, 1683 (C=O), 1644 (C=O), 1612 (C=O), 1558, 1484, 1451, 1410, 1215, 1177, 880, 802, 754, 695; ^{1}H NMR (d_6-DMSO, 400 MHz): δ 2.06 (s, 3H, 3′-OCH_3), 3.12 (ddd, 1H, J = 16.1, 10.9, 2.0 Hz, H1α), 3.68 (ddd, 1H, J = 16.2, 3.5, 1.5 Hz, H1β), 4.81 (dd, 1H, J = 10.8, 3.5 Hz, H11a), 7.15–7.32 (m, 4 H, H7, H9, H5′ and H6′), 7.46–7.49 (m, 2 H, H3 and H4′), 7.59 (dt, 1H, J = 8.2, 1.6 Hz, H8), 7.72 (s, 1H, H2′), 7.86 (dd, 1H, J = 7.9, 1.5 Hz, H6), 9.96 (s, 1H, 3′-NH), 10.76 (s, 1H, NH); ^{13}C NMR (d_6-DMSO, 100 MHz): δ 168.9 (C11), 168.4 (3′-NHC=O), 161.5 (C5), 139.6 (C3′), 136.1 (C9a), 133.7 (C1′), 132.6 (C8), 130.6 (C6), 128.9 (C5′), 125.6 (C5a), 125.3 (C7), 124.7 (C2), 122.5 (C3), 121.7 (C9), 119.9 (C6′), 117.9 (C4′), 115.4 (C2′), 56.1 (C11a), 30.5 (C1), 24.0 (3′-OCH_3); MS (ESI) m/z (relative intensity): 348 ([M+H]$^{\cdot+}$, 100%).

4.2.15. 2-(4-Aminophenyl)-1,10,11,11a*S*-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-dione (27). Yellow-

green solid, 82 mg (39%, from 250 mg of **12**); Mp 258–259 °C; $[\alpha]_D^{27}$ +845° (*c* 0.058, DMF); IR (disc, ν_{max}/cm^{-1}) 3389 (NH), 3225 (NH), 2914, 1693 (C=O), 1620 (C=O), 1518, 1454, 1398, 1305, 1269, 1171, 866, 818, 785, 759; ^1H NMR (d_6-DMSO, 400 MHz): δ 3.07 (ddd, 1H, J = 16.2, 10.8, 2.1 Hz, H1α), 3.62 (ddd, 1H, J = 16.3, 3.5, 1.5 Hz, H1β), 4.71 (dd, 1H, J = 10.8, 3.5 Hz, H11a), 5.28 (s, 2H, NH_2), 6.56 (d, 2 H, J = 8.6 Hz, H3′ and H5′), 7.18 (d, 1H, J = 8.0 Hz, H9), 7.22 (d, 2 H, J = 8.5 Hz, H2′ and H6′), 7.25 (s, 1H, H3), 7.28 (t, 1H, J = 8.1 Hz, H7), 7.55 (dt, 1H, J = 8.2, 1.6 Hz, H8), 7.84 (dd, 1H, J = 7.9, 1.5 Hz, H6), 10.67 (s, 1H, NH); ^{13}C NMR (d_6-DMSO, 100 MHz): δ 169.1 (C11), 160.8 (C5), 148.4 (C4′), 135.9 (C9a), 132.2 (C8), 130.5 (C6), 126.2 (C2′ and C6′), 125.9 (C5a), 125.8 (C1′), 124.2 (C7), 121.6 (C9), 120.7 (C2), 118.2 (C3), 113.8 (C3′ and C5′), 55.9 (C11a), 30.7 (C1); MS (ESI) *m*/*z* (relative intensity): 306.1 ($[M+H]^{\cdot+}$, 100%).

4.2.16. 2-(3-Aminophenyl)-1,10,11,11a*S*-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-dione (28). Red-brown solid, 130 mg (60%); Mp 284–286 °C; $[\alpha]_D^{24}$ +758° (*c* 0.095, DMF); IR (disc, ν_{max}/cm^{-1}) 3436, 3358 (NH_2), 3213 (NH), 2921, 1681 (C=O), 1610 (C=O), 1447, 1416, 1312, 1277, 1238, 1217, 1170, 1110, 997, 963, 868, 849, 759, 704, 685; ^1H NMR (d_6-DMSO, 400 MHz): δ 3.09 (ddd, 1H, J = 16.2, 10.9, 2.1 Hz, H1α), 3.66 (ddd, 1H, J = 16.3, 3.6, 1.6 Hz, H1β), 4.77 (dd, 1H, J = 10.8, 3.5 Hz, H11a), 5.06 (s, 2H, NH_2), 6.49 (d, 1H, J = 7.2 Hz, H4′), 6.71–6.74 (m, 2 H, H2′ and H6′), 7.01 (t, 1H, J = 7.6 Hz, H5′), 7.19 (d, 1H, J = 7.7 Hz, H9), 7.29 (dt, 1H, J = 8.1, 0.9 Hz, H7), 7.36 (t, 1H, J = 1.6 Hz, H3), 7.58 (dt, 1H, J = 8.1, 1.6 Hz, H8), 7.86 (dd, 1H, J = 7.9, 1.5 Hz, H6), 10.70 (s, 1H, NH); ^{13}C NMR (d_6-DMSO, 100 MHz): δ 169.0 (C11), 161.4 (C5), 148.7 (C3′), 136.0 (C9a), 133.7 (C1′), 132.5 (C7), 130.6 (C6), 129.1 (C5′), 125.7 (C5a), 125.6 (C2), 124.2 (C7), 121.6 (C9), 121.5 (C3), 113.3 (C4′), 113.0 (C6′), 110.4 (C2′), 56.0 (C11a), 30.6 (C1); MS (ESI) *m*/*z* (relative intensity): 306 ($[M+H]^{\cdot+}$, 100%).

4.2.17. 2-(4-Hydroxyphenyl)-1,10,11,11a*S*-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-dione (29). Yellow-green solid, 94 mg (43%); Mp 317–318 °C; $[\alpha]_D^{23.9}$ +728° (*c* 0.101, DMF); IR (disc, ν_{max}/cm^{-1}) 3424 broad (OH), 3209 (NH), 2904, 1694 (C=O), 1614 (C=O), 1514, 1452, 1270, 1227, 1174, 1105, 1004, 866, 823, 758, 700; ^1H NMR (d_6-DMSO, 400 MHz): δ 3.10 (ddd, 1H, J = 16.2, 10.9, 2.1 Hz, H1α), 3.65 (ddd, 1H, J = 16.3, 3.5, 1.6 Hz, H1β), 4.75 (dd, 1H, J = 10.8, 3.5 Hz, H11a), 6.77 (d, 2 H, J = 8.7 Hz, H3′ and H5′), 7.19 (d, 1H, J = 8.1 Hz, H9), 7.29 (dt, 1H, J = 8.1, 1.0 Hz, H7), 7.37 (s, 1H, H3), 7.38 (d, 2 H, J = 8.6 Hz, H2′ and H6′), 7.56 (dt, 1H, J = 8.1, 1.6 Hz, H8), 7.85 (dd, 1H, J = 7.9, 1.5 Hz, H6), 9.56 (s, 1H, OH), 10.70 (s, 1H, NH); ^{13}C NMR (d_6-DMSO, 100 MHz): δ 169.0 (C11), 161.1 (C5), 157.0 (C4′), 136.0 (C9a), 132.3 (C8), 130.6 (C6), 126.5 (C2′ and C6′), 125.8 (C1′), 125.1 (C5a), 124.3 (C2), 124.2 (C7), 121.6 (C9), 119.9 (C2), 115.5 (C3′ and C5′), 56.0 (C11a), 30.7 (C1); MS (ESI) *m*/*z* (relative intensity): 307 ($[M+H]^{\cdot+}$, 100%).

4.2.18. 2-(4-Bromophenyl)-1,10,11,11a*S*-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-dione (30). Yellow-white solid, 119 mg (45%); Mp 294–298 °C; $[\alpha]_D^{23.9}$ +561° (*c* 0.098, DMF); IR (disc, ν_{max}/cm^{-1}) 3108 (NH), 2924, 1698 (C=O), 1624 (C=O), 1482, 1443, 1279, 1213, 1175, 1074, 1005, 956, 871, 814, 756, 694; ^1H NMR (d_6-DMSO, 400 MHz): δ 3.14 (ddd, 1H, J = 16.3, 11.0, 2.2 Hz, H1α), 3.68 (ddd, 1H, J = 16.3, 3.6, 1.6 Hz, H1β), 4.81 (dd, 1H, J = 10.9, 3.6 Hz, H11a), 7.19 (d, 1H, J = 8.1 Hz, H9), 7.29 (dt, 1H, J = 8.2, 1.0 Hz, H7), 7.54–7.60 (m, 4 H, H2′,H3′,H5′ and H6′), 7.58 (dt, 1H, J = 8.1, 1.6 Hz), 7.67 (s, 1H, H3), 7.85 (dd, 1H, J = 7.9, 1.5 Hz, H6), 10.73 (s, 1H, NH); ^{13}C NMR (d_6-DMSO, 100 MHz): δ 168.9 (C11), 161.6 (C5), 136.1 (C9a), 132.8 (C1′), 132.6 (C8), 131.4 (C3′ and C5′), 130.7 (C6), 127.1 (C2′ and C6′), 125.5 (C5a), 124.3 (C7), 123.62 (C3), 123.60 (C4′), 121.7 (C9), 120.1 (C2), 56.2 (C11a), 30.4 (C1); MS (ESI) *m*/*z* (relative intensity): 370.7 ($[M+H]^{\cdot+}$, 95%), 372.1 ($[M]^{\cdot+}$, 100%).

4.2.19. 4-(5,11-Dioxo-1,10,11,11a*S*-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-2-yl)benzaldehyde (31). Yellow-white solid, 140 mg (62%); Mp 293–294 °C; $[\alpha]_D^{23.3}$ +769° (*c* 0.093, DMF); IR (disc, ν_{max}/cm^{-1}) 3445, 3273 (NH), 2849 and 2753, 1711 (C=O), 1690 (C=O), 1602 (C=O), 1566, 1482, 1453, 1421, 1396, 1312, 1275, 1215, 1170, 830, 813, 755, 728, 668; ^1H NMR (d_6-DMSO, 400 MHz): δ 3.21 (ddd, 1H, J = 16.2, 11.0, 2.1 Hz, H1α), 3.76 (ddd, 1H, J = 16.2, 3.6, 1.5 Hz, H1β), 4.88 (dd, 1H, J = 10.9, 3.5 Hz, H11a), 7.21 (d, 1H, J = 7.5 Hz, H9), 7.31 (dt, 1H, J = 8.2, 1.0 Hz, H7), 7.60 (dt, 1H, J = 7.4, 1.6 Hz, H8), 7.79 (d, 2 H, J = 8.3 Hz, H2′ and H6′), 7.86 (t, 1H, J = 1.7 Hz, H3), 7.88 (dd, 1H, J = 7.9, 1.6 Hz, H6), 7.89 (d, 2 H, J = 8.4 Hz, H3′ and H5′), 9.98 (s, 1H, CHO), 10.76 (s, 1H, NH); ^{13}C NMR (d_6-DMSO, 100 MHz): δ 192.3 (CHO), 168.8 (C11), 161.9 (C5), 139.6 (C1′), 136.1 (C9a), 134.5 (C4′), 132.8 (C8), 130.7 (C6), 129.8 (C3 and C5′), 125.9 (C3), 125.5 (C2′ and C6′), 125.4 (C5a), 124.3 (C7), 123.7 (C2), 121.7 (C9), 56.4 (C11a), 30.4 (C1); MS (ESI) *m*/*z* (relative intensity): 319 ($[M+H]^{\cdot+}$, 100%).

4.2.20. *N*-[4-(5,11-Dioxo-1,10,11,11a*S*-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-2-yl)-phenyl]acetamide (32). Yellow-green solid, 176 mg (71%); Mp 331–333 °C; $[\alpha]_D^{23.2}$ +631° (*c* 0.088, DMF); IR (disc, ν_{max}/cm^{-1}) 3244 (NH), 2992, 1690 (C=O), 1659 (C=O), 1596 (C=O), 1535, 1483, 1458, 1414, 1375, 1327, 1281, 1219, 1172, 1143, 962, 814, 785, 755; ^1H NMR (d_6-DMSO, 400 MHz): δ 2.06 (s, 3 H, 4′-OCH_3), 3.12 (ddd, 1H, J = 16.2, 10.9, 2.1 Hz, H1α), 3.68 (ddd, 1H, J = 16.3, 3.5, 1.5 Hz, H1β), 4.78 (dd, 1H, J = 10.8, 3.5 Hz, H11a), 7.19 (d, 1H, J = 8.1 Hz, H9), 7.29 (dt, 1H, J = 8.1, 1.0 Hz, H7), 7.49 (d, 2H, J = 8.6 Hz, H2′ and H6′), 7.50 (s, 1H, H3), 7.58 (dt, 1H, J = 8.8, 1.6 Hz, H8), 7.59 (d, 2 H, J = 8.5 Hz, H3′ and H5′), 7.86 (dd, 1H, J = 7.9, 1.5 Hz, H6), 9.99 (s, 1H, 4-NH), 10.71 (s, 1H, NH); ^{13}C NMR (d_6-DMSO, 100 MHz): δ 169.0 (C11), 168.2 (4′-NHC=O), 161.3 (C5), 138.5 (C4′), 136.0 (C9a), 132.4 (C8), 130.6 (C6), 128.1 (C1′), 125.7 (C5a), 125.6 (C2′ and C6′), 124.7 (C2), 124.2 (C7), 121.6 (C9), 121.4 (C3), 118.9 (C3′ and C5′), 56.1

(C11a), 30.6 (C1), 24.0 (4′-OCH_3); MS (ESI) *m*/*z* (relative intensity): 348 ($[M+H]^{\cdot+}$, 100%).

4.2.21. 1-Naphthalen-1-yl-1,10,11,11a*S*-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-dione (33). Colourless solid, 77.2 mg (41%); Mp 262–264 °C; $[\alpha]_D^{27.2}$ +609° (*c* 0.096, DMF); IR (disc, ν_{max}/cm^{-1}) 3127 (NH), 1682 (C=O), 1613 (C=O), 1462, 1396, 1304, 1265, 1225, 1169, 1113, 1043, 1000, 852, 776, 702; ^1H NMR (d_6-DMSO, 400 MHz): δ 3.38 (ddd, 1H, J = 16.3, 10.8, 2.1 Hz, H1α), 3.86 (ddd, 1H, J = 16.4, 3.4, 1.5 Hz, H1β), 4.86 (dd, 1H, J = 10.7, 3.4 Hz, H11a), 7.24 (d, 1H, J = 8.0 Hz, H9), 7.28 (s, 1H, H3), 7.32 (dt, 1H, J = 8.0, 0.9 Hz, H7), 7.52–7.64 (m, 5 H, H8, H2′, H3′, H6′ and H7′), 7.88–7.92 (m, 2H, H6 and H4′), 7.99 (d, 1H, J = 7.7 Hz, H5′), 8.25 (d, 1H, J = 8.3 Hz, H8′), 10.81 (s, 1H, NH); ^{13}C NMR (d_6-DMSO, 100 MHz): δ 169.0 (C11), 161.7 (C5), 136.2 (C9a), 133.6 (C1′), 132.6 (C8), 131.6 (C4a'), 130.7 (C6), 130.5 (C8a'), 128.7 (C5′), 128.0 (C4′), 126.6 (C7′), 126.0 (C2′), 125.7 (C5a), 125.5 (C3′), 125.3 (C3), 124.8 (C8′), 124.3 (C7), 123.7 (C2), 121.7 (C9), 55.7 (C11a), 34.3 (C1); MS (ESI) *m*/*z* (relative intensity): 341 ($[M + H]^{\cdot+}$, 100%).

4.2.22. 2-Naphthalen-2-yl-1,10,11,11a*S*-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-dione (34). Grey-white solid, 148 mg (61%); Mp 319–322 °C; $[\alpha]_D^{23.4}$ +761° (*c* 0.115, DMF); IR (disc, ν_{max}/cm^{-1}) 3308 (NH), 3104, 3051, 2921, 1698 (C=O), 1636 (C=O), 1479, 1440, 1389, 1298, 1258, 1213, 1171, 1001, 870, 822, 785, 759, 725, 627; ^1H NMR (d_6-DMSO, 400 MHz, 323 K): δ 3.30 (ddd, 1H, J = 16.0, 10.9, 1.8 Hz, H1α), 3.89 (dd, 1H, J = 16.2, 2.2 Hz, H1β), 4.87 (dd, 1H, J = 10.8, 3.6 Hz, H11a), 7.23 (d, 1H, J = 8.1 Hz, H9), 7.31 (t, 1H, J = 7.5 Hz, H7), 7.47–7.54 (m, 2 H, H6′ and H7′), 7.59 (dt, 1H, J = 8.3, 1.2 Hz, H8), 7.72 (s, 1H, H3), 7.84–7.95 (m, 6 H, H6, H3′, H4′, H5′, H8′ and H1′), 10.63 (s, 1H, NH); ^{13}C NMR (d_6-DMSO, 100 MHz, 323 K): poor solubility impeded the shim adjustment, thus leading to a high level of noise on the spectrum; MS (ESI) *m*/*z* (relative intensity): 341 ($[M+H]^{\cdot+}$, 100%).

4.2.23. trans-2-(Phenyl)vinyl-1,10,11,11a*S*-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-dione (35). Yellow-white solid, 90 mg (32%); Mp 309–311 °C; $[\alpha]_D^{23.8}$ +770° (*c* 0.085, DMF); IR (disc, ν_{max}/cm^{-1}) 3230 (NH), 3170, 3061, 3022, 2938, 1693 (C=O), 1630 and 1601 (C=O, Fermi resonance), 1577, 1485, 1441, 1417, 1346, 1266, 1216, 1170, 1145, 1051, 955, 812, 778, 753, 692, 672; ^1H NMR (d_6-DMSO, 400 MHz): δ 2.99 (dd, 1 H, J = 15.6, 11.6 Hz, H1α), 3.62 (dd, 1H, J = 16.1, 3.3 Hz, H1β), 4.80 (dd, 1H, J = 10.8, 3.5 Hz, H11a), 6.57 (d, 1H, J = 16.2 Hz, *trans*-1-H), 7.19-7.26 (m, 4 H, H3, H9, *trans*-2-H, H4′), 7.29 (dt, 1H, J = 8.0, 0.8 Hz, H7), 7.36 (t, 2 H, J = 7.5 Hz, H3′ and H5′), 7.51 (d, 2 H, J = 7.5 Hz, H2′ and H6′), 7.58 (dt, 1H, J = 8.2, 1.6 Hz, H8), 7.85 (dd, 1H, J = 7.9, 1.4 Hz, H6), 10.71 (s, 1H, NH); ^{13}C NMR (d_6-DMSO, 100 MHz): δ 168.8 (C11), 161.3 (C5), 137.1 (C9a), 136.0 (*trans*-2-C), 132.6 (C8), 130.6 (C6), 128.9 (C1′), 128.7 (C3′ and C5′), 127.4 (C4′), 126.3 (*trans*-1-C), 126.2 (C2′ and C6′), 125.6 (C5a), 125.5 (C2), 124.3 (C7), 122.3 (C3), 121.7 (C9), 56.4 (C11a), 29.2 (C1); MS (ESI) *m*/*z* (relative intensity): 317 ($[M+H]^{\cdot+}$, 100%).

4.3. Thermal denaturation assay

Stock solutions of all dilactams were freshly prepared in HPLC-grade DMSO, and working solutions were produced by appropriate dilution in aqueous buffer, as required. Test compounds were subjected to DNA thermal melting (denaturation) studies using calf thymus DNA (CT-DNA, type-I, highly polymerized sodium salt; 42% G + C [Sigma]) at a fixed 100 μM (in DNAp, equivalent to 50 μM in base pairs [bp]) concentration, quantitated using an extinction coefficient of 6600 (M phosphate)$^{-1}$ cm^{-1} at 260 nm.[10,24] Solutions were prepared in pH 7.00 ± 0.01 aqueous buffer containing 10 mM NaH_2PO_4/Na_2HPO_4 and 1 mM Na_2EDTA (all AnalaR™ grade). Working assay mixtures containing CT-DNA and the test compound (0–20 μM, as required) were incubated at 37.0 ± 0.1 °C for 0–72 h using a Grant GD120 water bath. Samples were monitored at 260 nm using a Cary 4000 UV–visible spectrophotometer fitted with a Peltier heating accessory. A precision probe calibrated to ±0.01 °C in the −10 to +120 °C range was used for temperature measurements. Heating was applied at a rate of 1 °C min^{-1} in the 50–98 °C range, with optical data sampling at 0.10 °C intervals. A separate experiment was carried out using buffer alone, and this baseline was subtracted from each DNA melting curve before data treatment. Optical data were imported into the Origin 5 program (MicroCal Inc., Northampton, MA) for analysis. DNA helix → coil transition temperatures (T_m) were determined at the midpoint of the normalized melting profiles. Results for each compound are shown as means ± standard deviation from at least three determinations. Ligand-induced alterations in DNA melting behaviour (ΔT_m) are given by:

$$\Delta T_m = T_m(\text{DNA} + \text{ligand}) - T_m(\text{DNA})$$

where the T_m value determined for native CT-DNA is 67.82 ± 0.07 °C (averaged from ~110 runs). Working solutions with the candidate dilactams contained ⩽0.3% v/v DMSO, and T_m results were corrected for the effects of DMSO co-solvent using a previously described[10,24] linear correction term determined for calibration mixtures. For kinetic experiments, working solutions of DNA–dilactam mixtures at the fixed 5:1 molar ratio were incubated at 37 °C and evaluated after fixed time intervals of 0 (i.e., no incubation), 4, 18 and 72 h.

Acknowledgments

Cancer Research UK (C180/A1060 to DET) and the Engineering and Physical Sciences Research Council (GR/R47646/01) are thanked for providing financial support for the research and NMR/MS instrumentation, respectively. The National Cancer Institute (USA) is acknowledged for providing the cytotoxicity data.

References and notes

1. Thurston, D. E. In *Molecular Aspects of Anticancer Drug-DNA Interactions*; Neidle, S., Waring, M. J., Eds.; The Macmillan Press Ltd: London, UK, 1993; Vol. 1, p 54.
2. Puvvada, M. S.; Forrow, S. A.; Hartley, J. A.; Stephenson, P.; Gibson, I.; Jenkins, T. C.; Thurston, D. E. *Biochemistry* **1997**, *36*, 2478.
3. Thurston, D. E.; Bose, D. S. *Chem. Rev.* **1994**, *94*, 433.
4. Bose, D. S.; Thompson, A. S.; Ching, J. S.; Hartley, J. A.; Berardini, M. D.; Jenkins, T. C.; Neidle, S.; Hurley, L. H.; Thurston, D. E. *J. Am. Chem. Soc.* **1992**, *114*, 4939.
5. Thurston, D. E.; Bose, D. S.; Thompson, A. S.; Howard, P. W.; Leoni, A.; Croker, S. J.; Jenkins, T. C.; Neidle, S.; Hartley, J. A.; Hurley, L. H. *J. Org. Chem.* **1996**, *61*, 8141.
6. Gregson, S. J.; Howard, P. W.; Jenkins, T. C.; Kelland, L. R.; Thurston, D. E. *Chem. Commun.* **1999**, 797.
7. Alley, M. C.; Hollingshead, M. G.; Pacula-Cox, C. M.; Waud, W. R.; Hartley, J. A.; Howard, P. W.; Gregson, S. J.; Thurston, D. E.; Sausville, E. A. *Cancer Res.* **2004**, *64*, 6700.
8. Hartley, J. A.; Spanswick, V. J.; Brooks, N.; Clingen, P. H.; McHugh, P. J.; Hochhauser, D.; Pedley, R. B.; Kelland, L. R.; Alley, M. C.; Schultz, R.; Hollingshead, M. G.; Schweikart, K. M.; Tomaszewski, J. E.; Sausville, E. A.; Gregson, S. J.; Howard, P. W.; Thurston, D. E. *Cancer Res.* **2004**, *64*, 6693.
9. Kaneko, T.; Wong, H.; Doyle, T. W.; Rose, W. C.; Bradner, W. T. *J. Med. Chem.* **1985**, *28*, 388.
10. Jones, G. B.; Davey, C. L.; Jenkins, T. C.; Kamal, A.; Kneale, G. G.; Neidle, S.; Webster, G. D.; Thurston, D. E. *Anti-Cancer Drug Des.* **1990**, *5*, 249.
11. Carlier, P. R.; Zhao, H.; MacQuarrie-Hunter, S. L.; DeGuzman, J. C.; Hsu, D. C. *J. Am. Chem. Soc.* **2006**, *128*, 15215.
12. Kamal, A.; Babu, A. H.; Ramana, A. V.; Ramana, K. V.; Bharathi, E. V.; Kumar, M. S. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2621.
13. Kamal, A.; Reddy, D. R.; Reddy, P. S. M. M.; Rajendar *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1160.
14. Kitamura, T.; Sato, Y.; Mori, M. *Tetrahedron* **2004**, *60*, 9649.
15. Wang, J. J.; Shen, Y. K.; Hu, W. P.; Hsieh, M. C.; Lin, F. L.; Hsu, M. K.; Hsu, M. H. *J. Med. Chem.* **2006**, *49*, 1442.
16. Kamal, A.; Reddy, K. L.; Devaiah, V.; Shankaraiah, N.; Reddy, G. S. K.; Raghavan, S. *J. Comb. Chem.* **2007**, *9*, 29.
17. Gregson, S. J.; Howard, P. W.; Barcella, S.; Nakamya, A.; Jenkins, T. C.; Kelland, L. R.; Thurston, D. E. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1849.
18. Gregson, S. J.; Howard, P. W.; Corcoran, K. E.; Barcella, S.; Yasin, M. M.; Hurst, A. A.; Jenkins, T. C.; Kelland, L. R.; Thurston, D. E. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1845.
19. Gregson, S. J.; Howard, P. W.; Corcoran, K. E.; Jenkins, T. C.; Kelland, L. R.; Thurston, D. E. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2859.
20. Cooper, N.; Hagan, D. R.; Tiberghien, A.; Ademefun, T.; Matthews, C. S.; Howard, P. W.; Thurston, D. E. *Chem. Commun.* **2002**, 1764.
21. Pena, M. R.; Stille, J. K. *J. Am. Chem. Soc.* **1989**, *111*, 5417.
22. Chen, Z.; Gregson, S. J.; Howard, P. W.; Thurston, D. E. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1547.
23. 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.
24. McConnaughie, A. W.; Jenkins, T. C. *J. Med. Chem.* **1995**, *38*, 3488.
25. Thurston, D. E.; Bose, D. S.; Howard, P. W.; Jenkins, T. C.; Leoni, A.; Baraldi, P. G.; Guiotto, A.; Cacciari, B.; Kelland, L. R.; Foloppe, M. P.; Rault, S. *J. Med. Chem.* **1999**, *42*, 1951.