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Novel Oxa-Spermine Homologues: Synthesis and Cytotoxic Properties

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Abstract—New oxa-spermine homologues **5**–**9** were synthesised and their anticancer properties were evaluated against a broad spectrum of cancer cells. All compounds, except **9** showed average GI_{50} values in the range of 1.89–7.56 μM. SAR studies showed that the cytotoxic activity of these novel oxa-spermines depended on the length of the alkyl chain, the position of the oxa-amino functionality and also, on the type of sulphonamido group in the molecule. Although the mechanism of action of these compound remains to be elucidated, it would appear that direct drug–DNA interactions are not involved in the mode of action of these drugs.

Introduction

Natural polyamines such as spermidine (1,8-diamino-4-azaoctane), spermine (1,12-diamino-4,9-diazadodecane) and putrescine (1,4-diaminobutane), play an important role in cell growth and differentiation. The metabolism of these polyamines is a promising target for cancer chemotherapy and chemoprevention.[1–3] During the last 20 years, several types of polyamine analogues which possess growth inhibitory activity and also are able to affect the natural polyamine metabolism have been synthesized and studied.[3–8] We have for a number of years been synthesizing novel type of spermidine and spermine homologues and derivatives with aminooxy functionality (i.e., oxa-polyamines) within the polyamine chain.[9–11] We previously reported that oxa-polyamine derivatives affect polyamine metabolism in 3T3 Swiss cells.[12,13] Furthermore, in a recent communication, we described the synthesis of a series of novel oxa-spermidine homologues and derivatives which showed good anticancer activity.[14] We have since extended our studies in the design and synthesis of oxa-spermine analogues and homologues. In this paper we report the synthesis and the in vitro cytotoxic properties of novel oxa-spermine homologues (**5**–**9**) (Fig. 1) against a number of different panels of cancer cell lines.

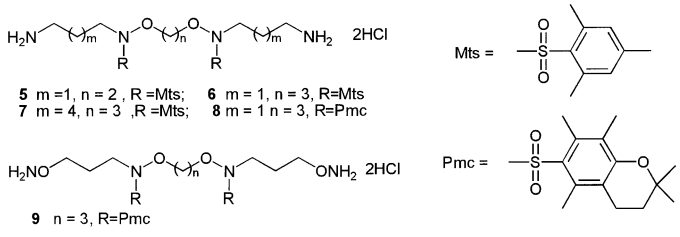

Figure 1. Oxa-spermine homologues.

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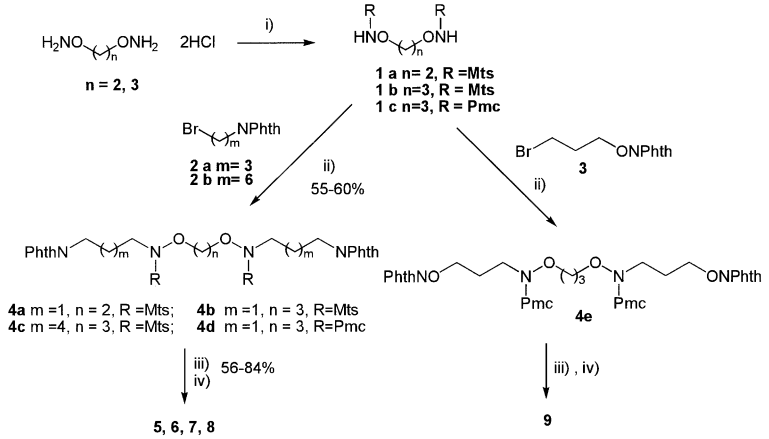
Results and Discussion

Compounds **5**–**9** were synthesized according to the methods described in our previous report[10] and the strategy adopted is depicted in Scheme 1. 2-Bis(aminooxy)ethane and 1,3-bis(aminooxy)propane dihydrochlorides were prepared from the reaction between the corresponding dibromoalkanes and *N*-hydroxyphthalimide followed by acid hydrolysis.[15] Their subsequent sulphonation with either mesitylenesulphonyl chloride (Mts-Cl) or 2,2,5,7,8-pentamethyl-3,4-dihydro-2H-chromen-6-sulphonylchloride (Pmc-Cl) in pyridine gave the bis(sulphonylaminooxy) alkanes **1a**–**c** in 60–75% yields. *N*-Alkylation of **1a**–**c** with the appropriate ω-bromoalkylphthalimide (**2a,2b**) gave the fully protected oxa-spermine homologues **4a**–**e** with yields varying between 55 and 60%. The removal of the phthaloyl groups from **4a**–**d** was performed in hydrazine/ethanol. The resulting diamines were converted directly to their dihydrochloride salts to give compounds **5**–**8**. For the synthesis of oxa-polyamine with terminal aminooxy groups such as in **9**, bromopropyloxyphthalimide **3** was first prepared from the reaction between 1,3-dibromopropane and *N*-hydroxyphthalimide according to procedure previously reported.[16] Compound **3** underwent alkylation with **1b** to give a product which upon hydrazinolysis and treatment with acid afforded **9**.

The growth inhibition properties of the new oxa-spermine homologues **5, 6, 7**, **8** and **9** against a wide range of cancer cells, representing human leukaemia, lung, colon, CNS, melanoma, ovarian, renal, prostate and breast tumors were investigated at the National Cancer Institute (NCI) in the USA. The following cell lines were used in the in vitro screen: leukaemia [CCRF-CEM, HL-60 (TB), K-562, MOLT-4, RPMI-8226, SR]; non-small cell lung cancer (A549/ATCC, EKVX, HOP-62, HOP-92, NCI-H226, NCI-H23, NCI-H322M, NCI-H460, NCI-H522); colon cancer (COLO 205, HCC-2998, HCT-116, HCT-15, HT-29, KM-12, SW-620); CNS cancer (SF-268, SF-295, SF-539, SNB-19, SNB-75, U251); melanoma (LOXIMVI, MALME-3M, M14, SK-MEL-2, SK-MEL-28, SK-MEL-5, UACC-257, UACC-62); ovarian cancer (IGROV1, OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, SK-OV-3); renal cancer (786-0, A498, ACHN, CAKI-1, RXF 393, TK-10, UO-31); prostate cancer (PC-3, DU-145); breast cancer (MCF-7, NCI/ADR-RES, MDA-MB-231/ATCC, HS 578T, MDA-MB-435, MDA-N, BT-549). Each compound was routinely tested at five 10-fold dilutions with a highest concentration of 100 μM. The anticancer potency of oxa-spermine homologues is summarized in Table 1 and is expresssed as their GI_{50} (drug concentrations required to produce 50% growth inhibition), TGI (drug concentrations required to produce total growth inhibition) and LC_{50} values (drug concentrations that are lethal to the survival of 50% cells). The selectivity in the anticancer activity (as GI_{50}, TGI and LC_{50} values) of all the compounds against different cell lines in each panel is presented in Table 2.

The new oxa-spermine homologues showed good anticancer activity against all the cell lines used. The *anticancer potency* of these compounds generally depended on the length of the alkyl chain and on the type of the sulphonamido functionality (i.e., Mts or Pmc groups). When the length of the alkyl chain between the two oxygen atoms was increased from two (**5**) to three carbon atoms (**6**), the growth inhibitory activity, expressed as mean GI_{50} values, showed a general increase (Table 1). A significant decrease in the TGI and LC_{50} values of compound **6** was also observed. When the sulphonamido group, Pmc is introduced instead of the Mts group into the chain as shown in compound **8**, this contributed to a further increase in the growth inhibitory activity (Table 1). Furthermore the extension in the length of the terminal alkyl chain (**7**) also exhibited a modest increase in anticancer activities (Table 1). However, the introduction of two oxygen atoms next to the terminal amino groups (**9**) resulted in a marked decrease in the anticancer potency and was considered to be inactive by the NCI criteria ($GI_{50} > 100$ μM) (data not shown). It is interesting to mention that an increase in drug concentration from 10 to 100 μM was accompanied by a decrease in the cytotoxicity of compound **8** against most of the cell lines tested. Similar paradoxical effect has recently been reported for other type of compounds tested by the NCI[17] and appears not to be related to any technical errors.



Scheme 1. Reagents and conditions: (i) R-Cl, pyridine, rt, 24 h; (ii) K_2CO_3, DMF, 85 °C, 8 h; (iii) $N_2H_4{\cdot}H_2O$, EtOH, reflux overnight; (iv) satd HCl/Et_2O, Et_2O/MeOH.

Table 1. Cytotoxicity of oxa-spermine homologues **5**–**8** against different panels of human cancer cell lines

Panel of cell lines[a,b]	Compound/Anticancer activity (μM)[c,d,e]											
	5			**6**			**7**			**8**		
	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
L (6)	2.73	84.3	>100	2.46	4.29	76.9	2.97	>100	>100	1.85	3.52	ND[f]
NSCLC (9)	5.19	40.7	>100	2.36	5.39	14.9	1.69	2.99	6.08	1.80	3.39	ND
CC (7)	7.73	49.5	86.7	1.94	9.87	34.5	1.89	23.1	38.4	1.72	3.32	ND
CNSC (6)	5.8	43.2	>100	2.93	6.98	15.3	1.79	3.22	6.07	1.88	3.18	ND
M (8)	5.32	55.5	85.6	2.20	5.21	12.4	1.73	3.57	6.2	1.93	3.78	ND
OC (6)	4.52	21.7	22.9	2.39	4.28	22.7	1.67	3.48	6.79	1.7	3.21	ND
RC (7)	16.5	65.7	>100	9.13	18.9	49.2	2.00	19.0	25.7	2.21	19.5	37.2
PC (2)	13.5	ND	>100	1.77	3.40	6.09	1.61	2.77	5.44	1.81	3.42	ND
BC (7)	6.8	56.4	>100	4.27	9.79	22.8	3.51	7.22	13.8	2.12	5.97	68.6
Mean Value[g]	7.56			3.27			2.09			1.89		

[a]L, leukemia; NSCLC, non-small cell lung cancer; CC, colon cancer; CNSC, CNS cancer; M, melanoma; OC, ovarian cancer; RC, renal cancer; PC, prostate cancer; BC, breast cancer.
[b]The number of cell lines routinely tested is shown in parentheses.
[c]Data obtained from NCI's in vitro tumour cells screen.
[d]Data are mean values of the corresponding panel.
[e]The value of each cell line (not shown) is an average of at least two determinations.
[f]Not determined.
[g]Mean GI_{50} values over all cell lines tested.

Table 2. Cytotoxicity of oxa-spermine homologues against different human cancer cell lines

Cell lines[a]	Compound/Anticancer activity (μM)[b,c]											
	5			**6**			**7**			**8**		
	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
L												
K-562	3.32	>100	>100	2.01	5.05	>100	4.74	>100	>100	1.93	ND[d]	ND
MOLT-4	3.78	>100	>100	1.35	3.09	ND	3.00	>100	>100	1.68	ND	ND
NSCLC												
NCI-H226	9.74	62.5	>100	1.46	3.16	6.82	1.81	4.10	ND	1.75	3.46	ND
NCI-H522	2.39	4.91	>100	1.16	2.42	5.08	1.76	3.27	6.07	1.89	3.42	ND
CC												
HCT-15	22.5	59.3	>100	21.0	41.4	81.6	1.82	3.60	7.12	1.77	3.31	ND
KM-12	2.31	5.28	>100	1.88	3.56	6.72	2.12	4.35	8.91	1.74	2.40	ND
CNSC												
SF-268	2.08	5.93	>100	1.21	2.49	5.12	1.83	3.42	6.37	1.61	3.18	ND
SF-295	22.7	83.6	>100	10.9	26.0	62.4	1.83	3.41	6.33	1.98	ND	ND
M												
LOX IMVI	2.97	>100	>100	2.03	4.04	ND	1.74	3.19	5.83	1.62	3.03	ND
UACC-257	2.92	>100	>100	1.73	3.23	6.04	1.75	3.27	6.10	2.02	4.21	ND
OC												
IGROV-1	1.75	5.67	>100	2.05	4.00	7.80	1.73	3.22	5.99	1.71	ND	ND
OVCAR-8	18.9	79.2	>100	2.70	4.22	8.98	1.78	3.34	6.22	1.81	3.45	ND
RC												
TK-10	3.74	35.4	>100	3.32	14.5	40.4	1.65	3.07	5.73	1.71	3.29	ND
UO-31	27.7	76	>100	16.9	30.6	55.3	2.16	4.83	12.3	1.95	3.97	ND
PC												
PC-3	1.46	3.83	>100	1.88	3.41	6.19	1.76	3.15	5.64	1.7	ND	ND
DU-145	25.6	>100	>100	1.68	3.39	5.99	1.46	2.38	5.23	1.91	3.42	ND
BC												
NCI/ADR-RES	24.6	51.3	>100	21.7	44.0	89.3	14.7	27.9	52.8	3.59	15.8	ND
HS 578T	2.67	30.3	>100	1.08	2.53	5.92	2.13	4.32	8.72	1.88	3.70	ND

[a]L, leukemia; NSCLC, non-small cell lung cancer; CC, colon cancer; CNSC, CNS cancer; M, melanoma; OC, ovarian cancer; RC, renal cancer; PC, prostate cancer; BC, breast cancer.
[b]Data obtained from NCI's in vitro tumour cells screen.
[c]Data are an average of at least two determinations.
[d]Not determined.

Table 3. Drug-induced alterations in calf thymus DNA melting behavior (ΔT_m) at different Drug: DNA molar ratio

Molar ratio (drug/DNA[a])	Induced ΔT_m (°C)[b]	
	Compound **6**	Spermine
1:20	0	28.3
1:10	0	17.0
1:5	2.4	9.2

[a][DNA] = 100 μM.
[b]$\Delta T_m = T_m$ (DNA + drug) − T_m (DNA).

Oxa-spermine homologues **5**–**8** were also shown to possess some degree of selectivity in their action against different cell lines in each panel (Table 2). Although an increase in the anticancer activity of oxa-spermine homologues was observed with the elongation of either the terminal or internal alkyl chain (Table 1), this was accompanied by a decrease in their selectivity (Table 2). For example, in the panel of renal tumors the GI_{50} values for compound **5** were 3.74 and 27.7 μM, for compound **6**, its GI_{50} were 3.32 and 16.9 μM and for compound **7** its GI_{50} were 1.65 and 2.16 μM against TK-10 and UO-31 cell lines, respectively. It is interesting to note that Pmc containing compound such as **8** exhibited low anticancer selectivity. It is worth mentioning that, with regard to their further development as anticancer agents, oxa-spermine homologue **6** appears to be the most interesting one since it demonstrated high anticancer activity and at the same time, retained a relatively high selectivity. When the cytotoxic properties of the recently described oxa-spermidine homologues and derivatives[14] and the oxa-spermine homologues presented here were compared, the latter group of compounds showed a higher cytotoxicity profile.

It is well known that spermidine and spermine interact with DNA and this is at least partially responsible for their multifunctional roles in biology.[1] Furthermore in vitro studies had shown that spermidine and spermine cause strong stabilization of *calf thymus* DNA duplex. We recently synthesized a series of bis-naphthalimidopropyl spermidine and spermine derivatives possessing anticancer properties[18] and these compounds were shown to strongly stabilize the thermal helix→coil transition of *calf thymus* DNA duplex, when compared with spermidine and spermine.[19] The cytotoxic activity of the bis-naphthalimidopropyl polyamines was due, at least in part, to their effects on DNA.

Considering the possibility DNA–drug interactions to be part of the mechanism of action of the oxa-spermine homologues, we investigated the influence of oxa-spermine homologue **6** on the DNA double helix stability using thermal denaturation experiments. In addition, natural polyamine spermine was used as a control. The results from these experiments are shown in Table 3. In contrast with spermine, which exhibits strong stabilization of DNA duplex against thermal denaturation with all the concentrations used, only a weak stabilizing effect of compound **6** was observed at 1:5 (drug/DNA) molar ratio.

The data from the in vitro screening of oxa-spermines (**5**–**8**) were processed by the NCI COMPARE analysis which allowed a prediction of the biochemical mechanism of novel drugs.[20] The analysis showed no correlation with the mode of action of any of the compounds in the NCI Standard Agent Database (Pearson correlation coefficients <0.6), suggesting the involvement of yet unknown biochemical targets in the cytotoxicity of our compounds.

The negative NCI COMPARE analysis of our compounds, showing no correlation with drugs targeting DNA was in accordance with the results from the thermal denaturation experiments with *calf thymus* DNA, since only small effects of compound **6** were observed at high drug concentration.

Conclusion

We have designed and synthesized novel oxa-spermine homologues (**5**–**9**). These compounds were tested by the NCI in an in vitro preclinical screening program against 60 human tumor cell lines. Oxa-spermine homologues (**5**–**8**) showed good anticancer activity against all the cell lines used with GI_{50} values in the low micro-molar range (1.89–7.56 μM). The cytotoxic activity of these compounds generally depended on the length of the alkyl chain and on the type of the sulphonamido functionality (i.e., Mts or Pmc groups). On the basis of thermal denaturation studies and the negative NCI COMPARE analysis, it appears that direct interactions with DNA are not involved in the mechanism of action of these compounds. The most promising compound **6** was selected for further in vivo testing by the NCI Biological Evaluation Committee. The precise mechanism of action of the oxa-spermine derivatives is currently under investigation in our laboratory. These compounds appear to be good inducers of apoptosis in MCF-7 human breast cancer cells treated with compound **6** (unpublished). The optimization of these compounds from the SAR point of view and the elucidation of the mechanism of their action might lead to the development of a novel type of anticancer drugs.

Experimental

All reagents were purchased from Aldrich, Fluka and Lancaster and were used without purification. 2,2,5,7,8-Pentamethyl-3,4-dihydro-2H-chroman-6-sulphonylchloride was synthesized by previously reported method[21] and can also be purchased from Novabiochem. Melting points (mp) were determined on Gallenkamp melting point apparatus in open capillaries and are uncorrected. TLC was performed on Kieselgel plates (Merck) 60 F_{254} in chloroform/methanol (97:3 or 99:1). IR spectra were recorded on a Nicolet 5ZDX FT-IR spectrophotometer or a Perkin–Elmer 781 IR spectrophotometer. FAB-mass spectra were obtained on a VG Analytical AutoSpec (25Kv) spectrometer, EC/CI spectra were performed on a Micromass Quatro II (low resolution) or a VG Analytical ZAB-E instrument (accurate mass).

^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-EX90 FT NMR spectrometer.

1,2-Bis(aminooxy)ethane and 1,3-bis(aminooxy)propane dihydrochlorides. There were synthesized according to our method previously reported.[15]

Bis[(sulphonyl)aminooxy]alkanes 1a and 1b. General procedure. Bis(aminooxy)alkane dihydrochloride (0.0302 M) was dissolved in pyridine (120 cm^3). Aryl sulphonylchloride (0.0757 mol) was added to the solution and the mixture was left stirring overnight at room temperature. All the solvent was removed under vacuo to give a precipitate. This was dissolved in chloroform (200 cm^3) and transferred in a separating funnel (500 cm^3). The organic phase was washed with (i) dilute hydrochloric acid (1 M) (ii) water (iii) saturated solution of sodium bicarbonate (iv) water. The chloroform layer was dried with sodium sulphate and filtered. Removal of the solvent gave a solid which was recrystallised from toluene.

1,2-Bis[*N*,*N*′-(2-mesitylsulphonyl)aminooxy]ethane 1a. This was obtained in 91% yield. Mp 116–117 °C (dec, toluene) ^{1}H NMR ($CDCl_3$): δ 7.32 (s, 2H, NH), 7.04 (s, 4H), 7.32 (s, 2H, NH), 4.00 (s, 4H), 2.65 (s, 12H), 2.30 (s, 6H).). ^{13}C NMR ($CDCl_3$): δ 143.6, 140.8, 132.1, 130.8 (arom C), 73.8 (CH_2O), 23.0, 21.0 (CH_3). IR cm^{-1} (Nujol): 3250, 3060, 2820, 1600, 1350, 1160. LRMS (FAB): calcd for $C_{20}H_{28}N_2O_6S_2$ 456.14, found: 457 $[MH]^+$.

1,3-Bis[*N*,*N*′-(2-mesitylsulphonyl)aminooxy]propane 1b. This was obtained in 79% yield. Mp 157–158 °C (EtOH) ^{1}H NMR ($CDCl_3$): δ 7.49 (s, 2H, NH), 7.24 (s, 4H), 4.01 (s, 4H), 2.84 (s, 12H), 2.55 (s, 6H), 1.99 (m, 2H).). ^{13}C NMR ($CDCl_3$): δ 143.6, 140.7, 132.1, 130.8 (aromatic C), 73.5, 26.8, 23.0, 21.0. IR cm^{-1} (Nujol): = 3200, 1330,1170. LRMS (FAB): calcd for $C_{21}H_{30}N_2O_6S_2$ 470.15, found: 471 $[MH]^+$.

1,3-Bis[*N*,*N*′-(2,2,5,7,8-pentamethyl-3,4-dihydro-2H-chroman-6-sulphonylchloride)amino-oxy]propane 1c. This was obtained in 79% yield. ^{1}H NMR ($CDCl_3$): δ 7.35 (s, 2H, NH), 3.92 (t, 4H), 2.61 (s, 12H), 2.22 (s, 6H), 1.63–2.00 (m, 6H), 1.42 (s, 12H), ^{13}C NMR ($CDCl_3$): δ 155.5, 138.1, 125.7, 124.8, 118.6 (arom C), 74.2, 73.5, 32.5, 26.8, 21.3, 18.5, 17.5, 12.1. IR cm^{-1} (Nujol): 3200, 1550,1330,1155. LRMS (FAB): calcd for $C_{31}H_{46}N_2O_8S_2$ 638.82, found: 639 $[MH]^+$.

6-Bromohexylphthalimide 2b and 3-bromopropyloxyphthalimide 3. There were synthesised according to the method reported previously.[15]

α,ω - Di[phthalimidoalkyl] - *N*,*N*′ - di(arylsulphonyl) - α,ωbis-bis-(aminooxy)alkanes 4a–c. General *N*-alkylation reaction. A mixture of arylsulphoamide (3.13 mmol), ω-Bromoalkyl(oxy)phthalimide (6.4 mmol), anhydrous K_2CO_3 were dissolved in anhydrous DMF (8 mL) was allowed to stir at rt for 24 h. The solvent was removed under vacuo and the residue dissolved in chloroform (50 mL). The resulting solution was washed with 2M HCl, saturated bicarbonate solution, water, dried over Na_2SO_4 and filtered through silica gel. The solvent was removed under vacuo followed by the addition of Et_2O. The mixture was allowed to stir for 20 min at room temperature, cooled to give white crystals. The latter was filtered off and washed with Et_2O.

1,12 - Diphthalimido - 4,9 - di(2 - meitylenesulphonyl) - 4,9 - diaza-5,6-dioxadodecane 4a. This was obtained in 60% as a white solid; mp 183–184 °C (dec) (EtOH). ^{1}H NMR ($CDCl_3$): δ 7.70–7.93 (m, 8H), 7.01 (s, 4H), 3.83 (t, 4H), 3.55 (s, 4H), 3.35 (t, 4H), 2.66 (s, 12H), 2.37 (s, 6H), 1.79–2.46 (m, 4H). ^{13}C NMR ($CDCl_3$): δ 168.0 (C=O), 143.7, 141.9, 133.9, 132.0, 129.6, 132.1 (arom C), 73.5, 47.5, 35.7, 25.6, 23.1, 21.0. IR cm^{-1} (Nujol): 3100, 1710 (C=O), 1600, 1325, 1165 (SO_2). HRMS (FAB): calcd for $C_{42}H_{46}N_4O_{10}S_2$ 830.27, $[MH]^+$ 831.2734, found: 831.2747 $[MH]^+$.

1,13-Diphthalimido-4,10-di(2-meitylenesulphonyl)-4,10-diaza-5,9-dioxatridecane 4b. This was obtained in 82% as a white solid; ^{1}H NMR ($CDCl_3$): δ 7.57–7.81 (m, 8H, arom CH, Phth), 6.92 (s, 4H, arom H of Mts group), 3.79 (t, 4H, 2×CH_2O), 3.14–3.71 (m, 8H, 4×CH_2N), 2.60 (s, 12H, 4×CH_3), 2.26 (s, 6H, 2×CH_3), 2.02 (m, 4H, 2×CH_2), 1.47 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$): δ 168.0 (C=O), 143.9, 141.9, 133.9, 132.0, 129.6, 123.2 (arom C), 73.0 (CH_2–O), 47.5, 35.8, 27.2, 26.0, 23.1, 21.0 (CH_3). IR cm^{-1} (Nujol): 3100, 1710 (C=O), 1600, 1325, 1165 (SO_2). HRMS (FAB): calcd for $C_{43}H_{48}N_4O_{10}S_2$ 844.28, $[MH]^+$ 845.2890, found: 845.2929 $[MH]^+$.

1,19-Diphthalimido-7,13-di(2-meitylenesulphonyl)-7,13-diaza-8,12-dioxatnanodecane 4c. This was obtained in 83% as a white solid; ^{1}H NMR ($CDCl_3$): δ 7.59–7.84 (m, 8H, arom CH, Phth), 6.87 (s, 4H, arom H of Mts group), 3.56 (t, 4H, 2×CH_2O), 2.90–3.28 (m, 8H, 4×CH_2N), 2.51 (s, 12H, 4×CH_3), 2.33 (s, 6H, 2×CH_3), 1.00–1.72 (m, 20H, 10×CH_2). ^{13}C NMR ($CDCl_3$): δ 168.2 (C=O), 142.1, 140.0, 133.8, 133.4, 132.1, 131.8, 123.0 (arom C), 45.3, 45.1, 37.8, 28.3, 27.0, 26.2, 26.1, 24.7, 22.7, 20.8 (CH_2, CH_3). IR cm^{-1} (Nujol): 3100, 1710 (C=O), 1600, 1325, 1165 (SO_2). LRMS (FAB): calcd for $C_{49}H_{60}N_4O_{10}S_2$ 928.38, found: 929 $[MH]^+$.

1,13-Diphthalimido-4,10-di(2,2,5,7,8-pentamethyl-3,4-dihydro-2H-chromen-6-sulphonyl)-4,10-diaza-5,9-dioxatridecane 4d. This was obtained in 95% as a white solid. ^{1}H NMR ($CDCl_3$): δ 7.75–7.92 (m, 8H), 3.82 (t, 4H), 3.61 (t, 4H), 3.36 (t, 4H), 2.73 (t, 4H), 2.61 (s, 12H), 2.19 (s, 6H), 1.84–2.08 (m, 10H), 1.4 (s, 12H). ^{13}C NMR ($CDCl_3$): δ 167.9 (C=O), 155.7, 139.2, 139.1, 133.8, 132.0, 124.8, 124.7, 123.1, 118.7. (arom C), 74.3, 72.9, 47.5, 35.8, 32.5, 27.3, 26.6, 25.9, 21.3, 18.5, 17.5, 17.5, 12.1.(CH_2 and CH_3 groups). IR cm^{-1} (Nujol): 3000, 1710 (C=O), 1600, 1325, 1155 (SO_2). LRMS (FAB): calcd for $C_{53}H_{64}N_4O_{12}S_2$ 1013.22, found: 1013 $[M]^+$.

1,12-Diphthalimidooxy-4,9-di(2,2,5,7,8-pentamethy3,4-dihydro-2H-chromen-6-sulphonyl)-4,9-diaza-5,6-dioxadodecane 4e. This was obtained in 90% as a white solid;

^{1}H NMR ($CDCl_3$): δ 7.85 (m, 8H, arom CH, Phth), 4.38 (t, 4H, 2×CH_2O), 3.51–3.58 (m, 8H, 4×CH_2N), 2.74 (t, 8H), 2.62 (s, 12H), 2.39 (m, 4H), 2.20 (s, 6H), 1.84–2.00 (m, 6H), 1.40 (s, 12H). ^{13}C NMR ($CDCl_3$): δ 163.0 (C=O), 155.7, 139.2, 139.1, 134.4, 128.9, 124.9, 124.9, 124.8, 123.4, 118.7 (arom C), 75.9, 74.3, 72.6, 46.7, 32.6, 26.0, 21.4, 18.6, 17.5, 12.2 (CH_2 and CH_3). IR cm^{-1} (Nujol): 3100, 1730 (C=O), 1600, 1340, 1155 (SO_2). LRMS (FAB): calcd for $C_{53}H_{64}N_4O_{14}S_2$ 1045.22, found: 1045 $[M]^+$.

Hydrazinolysis (removal of the phthaloyl group). Compounds **4a–e** (1.94 mmol) were dissolved in ethanol (30 mL) and $N_2H_4{\cdot}H_2O$ (4.0 mmol) was added dropwise. The mixture was left refluxing overnight whereby a white precipitate was formed. The white solid was filtered off and washed with ethanol. The filtrate was reduced to dryness and the residue resuspended in chloroform. The solution with a white turbidity was filtered again and the filtrate was reduced to dryness to afford the free diamine compound as a yellow oil in quantitative yield. To form the corresponding dihydrochloride salt the diamine was dissolved in ether/methanol (5:1, 10 mL) followed by the addition of a saturated solution of HCl/ether (3 mL) with cooling. After filtration and drying, the dihydrochloride salt was obtained as a white solid.

1,12-Diamino-4,9-bis(2-meitylenesulphonyl)-4,9-diaza-5,8-dioxadodecane dihydrochloride 5. This was obtained as a white solid in 56%; ^{1}H NMR (DMSO-d_6): δ 8.32 (broad s, 6H, 2×NH_3^+), 7.17 (s, 4H), 4.26 (s, 4H), 2.78–3.35 (m, 8H), 2.57 (s, 12H), 2.36 (s, 6H), 1.52–2.16 (m, 4H). ^{13}C NMR (DMSO-d_6): δ 143.9, 141.1, 131.9, 129.1 (arom C), 73.0, 46.8, 37.3, 24.2, 22.5, 20.5, (CH_2 and CH_3). IR cm^{-1} (Nujol): = 3400 (N–H), 1950 (NH_3^+), 1600, 1340,1165 (SO_2). HRMS (FAB): calcd for $C_{26}H_{44}N_4O_6S_2Cl_2$ 643.25, ($[M-2HCl]^+$ 571.2624), found: 571.2615 $[M-2HCl]^+$.

1,13-Diamino-4,10-di(2-meitylenesulphonyl)-4,10-diaza-5,9-dioxatridecane dihydrochloride 6. was obtained in 60% yield as a white solid. ^{1}H NMR (DMSO-d_6): δ 8.40 (broad s, 6H, 2×NH_3^+), 7.20 (s, 4H), 3.14–3.52 (m, 8H, 4×CH_2N), 2.78–3.10 (m, 4H), 2.64 (s, 12H), 2.38 (s, 6H), 2.00 (m, 4H), 1.40 (m, 2H). ^{13}C NMR (DMSO-d_6): δ 144.2, 141.3, 132.2, 129.5, 72.9, 47.2, 26.9, 24.6, 22.8, 20.8. HRMS (FAB): calcd for $C_{27}H_{44}N_4O_6S_2Cl_2$ 658.27 ($[M-2HCl]^+$ 585.2781), found: 585.2785 $[M-2HCl]^+$.

1,19-Diphthalimido-7,13-di(2-meitylenesulphonyl)-7,13-diaza-8,12-dioxananodecane dihydrochloride 7. This was obtained in 60% yield as a white solid; ^{1}H NMR (DMSO-d_6): δ 8.28 (broad s, 6H, 2×NH_3^+), 7.17 (s, 4H), 2.97–3.40 (m, 8H, 4×CH_2N), 2.68–2.95 (t, 4H), 2.62 (s, 12H), 2.35 (s, 6H), 1.10–1.86 (m, 18H). ^{13}C NMR (DMSO-d_6): δ 143.7, 140.8, 131.8, 129.4, 72.3, 48.9, 26.7, 26.5, 25.9, 25.7, 25.3, 22.4, 20.4. HRMS (FAB): calcd for $C_{33}H_{58}N_4O_6S_2Cl_2$ 741.37 ($[M-2HCl]^+$ 669.3720), found: 669.3738 $[M-2HCl]^+$.

4,10-Bis(2,2,5,7,8-pentamethy-3,4-dihydro-2H-chromen-6-sulphonyl)-4,10-diaza-5,9-dioxatridecane-1,14,diamine dihydrochloride 8. This was obtained as a white solid in 60% yield; ^{1}H NMR (DMSO-d_6): δ 8.42 (broad s, 6H, 2×NH_3^+), 2.68–3.25 (m, 16H), 2.49 (s, 12H), 2.03 (s, 6H), 1.73–1.95 (m, 10H), 1.33 (s,12H). ^{13}C NMR (DNSO-d_6): δ 155.4, 139.0, 138.7, 124.8, 124.4, 119.3 (arom C), 74.6, 72.8, 42.6, 32.1, 32.0, 26.6, 25.5, 21.1, 18.6, 17.5, 12.2 (CH_2 and CH_3). IR cm^{-1} (Nujol): 3400 (N–H), 2700, 2555, 1325,1155 (SO_2). LRMS (FAB): calcd for $C_{37}H_{62}N_4O_8S_2Cl_2$ 825.92, found: 753 $[M-2HCl]^+$.

4,9-Bis(2,2,5,7,8-pentamethy-3,4-dihydro-2H-chromen-6-sulphonyl)-5,9-dioxa-4,10-diazotetradecane 9. This was obtained in 72% yield as a white solid; ^{1}H NMR ($CDCl_3$): δ 6.22 (broad s, 4H), 4.14 (t, 4H), 3.24 (m, 12H), 2.49 (s, 12H), 2.12 (s, 6H), 1.87 (m, 10H), 1.34 (s, 12H). ^{13}C NMR ($CDCl_3$): δ 155.1, 138.6, 124.4, 124.0,118.9 (arom C), 74.3, 72.4, 45.9, 34.0, 31.7, 26.2, 25.0, 20.7, 18.2, 17.2, 11.9 (CH_2 and CH_3). IR cm^{-11} (Nujol): 3400, 2800, 1325, 1155 (SO_2). LRMS (FAB): calcd for $C_{37}H_{62}N_4O_{10}S_2Cl_2$ 825.92, found: 753 $[M-2HCl]^+$.

Anticancer studies

The in vitro anticancer screening tests were carried out at the National Cancer Institute. Details of the assay procedures have been reported previously.[22] A 48 h drug exposure protocol was followed and a sulforhodamine B assay was used to estimate cell viability and cell growth.

Thermal denaturation studies

Optical thermal denaturation experiments were performed in stoppered quartz cuvettes using a Perkin-Elmer Lambda 2 UV–vis spectrophotometer fitted with a Peltier circulating heating/cooling temperature programmer and water supply, according to the published procedure.[23] *Calf thymus* DNA (Sigma) working solutions (100 μM) were prepared by dilution of a DNA stock solution in SHE buffer. The DNA–drug solutions were prepared by the addition of the drug (5% DMSO/water) to give the desired final concentration. The binding affinity (i.e., T_m) was measured by determining the change in midpoint of the thermal denaturation curves of DNA in 0.01 M SHE buffer (pH 7.0). Drug-induced alterations in DNA melting behaviour are given by ΔT_m. T_m for the drug-free *Calf thymus* DNA was determined to be 67 °C.

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