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The Synthesis and In Vitro Cytotoxic Studies of Novel Oxa-Spermidine Derivatives and Homologues

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Abstract—A series of oxa-spermidine derivatives and homologues were prepared and their anticancer properties were evaluated. All these compounds showed an average GI_{50} value in the range of 3.9–28.9 μM. SAR studies showed that the presence of a sulphonamido functionality and the length of the alkyl chain are important factors for an enhanced activity.

For the last two decades polyamine analogues and derivatives have been synthesised as biological tools to elucidate the physiological role of the natural polyamines (putrescine, spermidine and spermine) and also, as therapeutic agents.[1,2] More recently polyamine analogues bearing a primary amino-oxy moiety have generated great interest among the scientific community and their use as probes to understand the functions of polyamines in biological systems is well documented.[3–5] Others have designed and synthesised aminoxy compounds as potential anticancer agents.[6,7] For the last 10 years we have been designing and synthesising compounds with a secondary amino-oxy functionality within the polyamine chain.[8,9] Recently, we have shown the effects of an oxa-spermidine derivative on the polyamine metabolism in 3T3 Swiss cells.[10,11] We would like to report in this paper the in vitro cytotoxic properties of oxa-spermidine derivatives and homologues (**1a–d**, **2a–d**, **3**) (Fig. 1) against a number of different panels of cancer cell lines.

Compounds **1a–d**, **2a–d**, **3** were prepared in an overall yield of 60–85% starting from compound **4** according to our previously reported method[8,9] as shown in Scheme 1.

The key intermediate, *N*-(aminooxypropyl)phthalimide **4** was prepared from a rearrangement reaction involving 3-bromopropylamine and *N*-hydroxyphthalimide in the presence of DBU. The aminooxy group was activated by sulphonation using either mesitylsulphonyl chloride (Mts-Cl) or 2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-chromen-6-sulphonyl chloride (Pmc-Cl) to yield the corresponding *N*-(phthalimido)propyloxysulphonamides **5**. Subsequent *N*-alkylation with the appropriate bromoalkylphthalimide yielded the fully protected oxapolyamine derivatives/homologues **6a–d**, **7a–d**, **8**. Selective removal of the phthalimido groups from **6a–d**, **7a–d**, **8** were achieved by refluxing with hydrazine hydrate in ethanol. Treatment of the resulting diamines with saturated HCl/Et_2O gave the final products **1a–d**, **2a–d**, **3** as the corresponding dihydrochloride salt. The parent oxa-spermidine homologue **9** was obtained by the deprotection of compound **1a** or **2a** with hydrobromic acid/glacial acetic acid in dichloromethane as shown in Scheme 2.

The ability of the new oxa-spermidine derivatives/homologues **1a–d**, **2a–d**, **3** to inhibit the in vitro growth of cancer cells derived from human leukaemia, lung, colon, CNS, melanoma, ovarian, renal, prostate and breast tumours was evaluated at the National Cancer Institute (NCI) in the USA. The results from the in vitro-testing of the compounds **1b–d**, **2a–d**, **3** by the standard procedures of the NCI Drug Discovery and Developmental Therapeutic Programme[12] are summarised in Table 1. The cells were assayed by the sulforhodamine B method after a 48-h period of drug exposure.[12] The growth inhibitory properties the oxa-spermidine derivatives/homologues were expressed as a

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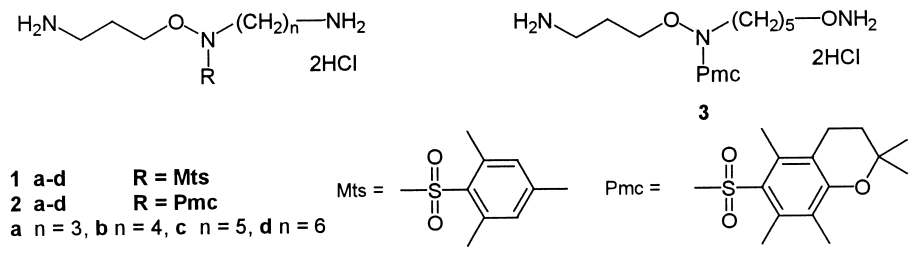
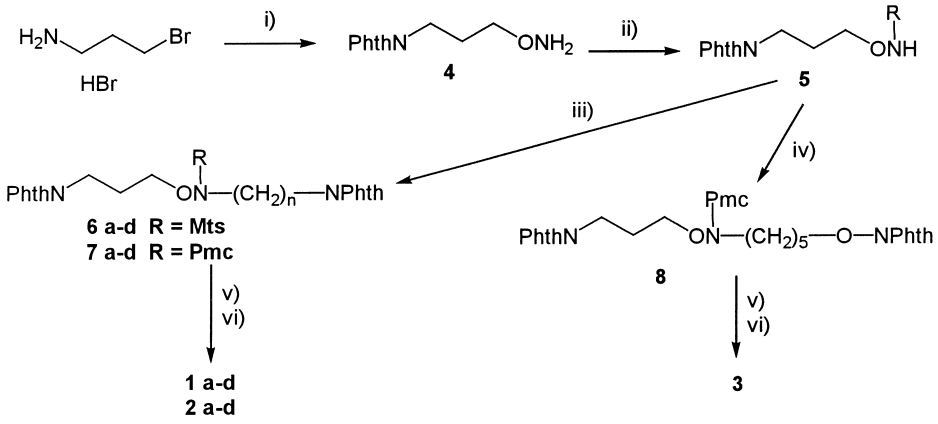
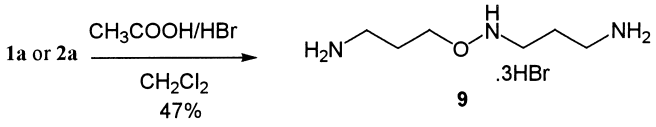


Figure 1. Structures of oxa-spermidine derivatives/homologues.



Scheme 1. Reagents and conditions: (i) *N*-hydroxyphthalimide, DBU, DMF, 85 °C, 8 h; (ii) Mts-Cl pr Pmc-Cl, pyridine, rt, 8 h; (iii) $Br(CH_2)_nNPhth$, K_2CO_3, DMF, 85 °C, 8 h; (iv) $Br(CH_2)_5ONPhth$, K_2CO_3, DMF, 85 °C, 8 h; (v) $H_2NNH_2.H_2O$, EtOH, reflux overnight; (vi) Sat. HCl/Et_2O, Et_2O:MeOH.



Scheme 2.

mean GI_{50} value for each panel of human tumour cell lines. The GI_{50} value is defined as the concentration that causes 50% growth inhibition.

Each compound was routinely evaluated at five 10-fold dilutions with a highest concentration of 100 μM. The cell lines used in the in vitro screen were as follows: 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).

In the present study, we report the discovery and the anticancer properties of novel oxa-spermidine derivatives/homologues against a broad spectrum of human tumour cell lines. Most compounds tested (**1b**–**d**, **2a**–**d**, **3**), showed good anticancer activity against all the cell lines. However, some degree of selectivity in the cytotoxicity was observed, depending on both, the panel (Table 1) and the cell line used, especially in the case of compounds **2c** and **2d**. For example in the panel of ovarian cancer cells, compound **7** showed a GI_{50} value of 3.27 and 52.5 μM against IGROV1 and OVCAR-5 cell lines respectively. Furthermore, in the panel of colon cancer cells, compound **2d** exhibited a GI_{50} value of 1.98 and 34.5 μM with HCC-2998 and SW-620 cell lines respectively. The cytotoxic activity of our compounds depends on the presence of a sulphonamido functionality i.e., either a Mts or a Pmc group and also, on the length of the alkyl chain located at the nitrogen of the aminooxy group. It is noteworthy to mention that the parent oxa-spermidine analogue **9** (also tested by NCI) was found to be inactive (GI_{50} >100 μM) against all cell lines. Compound **1a** ($n=3$), bearing a Mts group did not show any cytotoxic activity (GI_{50} >100 μM against the majority of cell lines tested, data not shown). However when the alkyl chain was increased from a propyl ($n=3$) to a butyl ($n=4$) group (**1b**), a dramatic increase in the cytotoxicity was observed ($GI_{50}=16.4$ μM). Further chain elongation in this series of compounds i.e., **1c**, **1d** ($n=5,6$) did not significantly affect the level of cytotoxic activity (Table 1). When a different

Table 1. Growth inhibition of cultured cells derived from a variety of human tumours by oxa-spermidine derivatives/homologues

Panel of cell lines[1a]	Compound/Cytotoxicity [GI_{50} (μM)][b,c,d]							
	1b	**1c**	**1d**	**2a**	**2b**	**2c**	**2d**	**3**
Leukaemia (6)	11.8	15.3	19.5	13.0	26.8	3.9	4.1	6.3
Non small cell lung cancer (9)	18.5	16.9	22.4	14.2	15.3	11.5	6.3	13.5
Colon cancer (7)	13.3	16.2	16.7	13.8	20.3	9.8	7.2	12.8
Cns cancer (6)	18.1	16.9	16.5	12.9	18.0	9.6	5.6	14.9
Melanoma (8)	18.1	18.2	15.7	14.8	19.1	10.9	10.7	13.7
Ovarian cancer (6)	17.2	17.9	18.0	12.6	13.5	12.7	4.7	12.4
Renal cancer (7)	18.2	16.1	17.5	14.7	20.9	16.5	11.3	14.3
Prostate cancer (2)	14.5	15.7	15.4	14.0	14.5	16.1	16.3	10.6
Breast cancer (7)	17.8	20.2	23.0	15.8	28.4	10.0	10.2	13.8
Mean value[e]	16.4	15.2	18.3	14.0	19.6	11.2	8.5	12.5

[a]The number of cell lines routinely tested is shown in parentheses.
[b]Data obtained from NCI's in vitro tumour cells screen.
[c]Data are mean values of the corresponding panel.
[d]The value of each cell line (not shown) is an average of at least two testings.
[e]Mean values over all cell lines tested.

sulphonamido group (i.e., Pmc) was used instead of the Mts group in analogous oxa-polyamine homologues (**2a**–**d**), the inhibitory activity of these novel compounds showed a general increase. In contrast to the Mts derivatives (**1a**–**d**), chain elongation in Pmc substituted oxa-spermidine homologues led to a modest increase in cytotoxicity. The introduction of a primary aminooxy group (ONH_2, **3**) at the end of the alkyl chain of the most active compound **2d** showed no significant change in the cytotoxicity (Table 1).

The NCI COMPARE analysis, allowing a theoretical prediction of the biochemical mechanism of action of novel drugs on the basis of the data from their in vitro screening[13] was conducted on compounds **2c** and **2d**. COMPARE analysis, however, 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 new non-classical biochemical targets in the anticancer activity of our compounds. After a repeat of the primary screen, compounds **2c** and **2d** were recently selected by the NCI Biological Evaluation Committee for further in vivo testing.

The mechanism of action of the oxa-spermidine derivatives/homologues is currently under investigation in our laboratory. These compounds possess very rapid cytotoxic activity and appear to be good inducers of apoptosis which was recently demonstrated in MCF-7 human breast cancer cells treated with compound **2d** (unpublished observations).

The newly synthesised oxa-spermidine analogues/homologues (**1b**–**d**, **2a**–**d**, **3**) show good potential for their further development as anticancer drugs.

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