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The Synthesis and In Vitro Cytotoxic Studies of Novel Bis-naphthalimidopropyl Polyamine Derivatives

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Abstract—Bis-naphthalimidopropyl putrescine (BNIPPut), spermidine (BNIPSpd), spermine (BNIPSpm) and oxa-putrescine (BNIPOPut) were synthesised and their growth-inhibitory properties characterised. All these compounds except for BNIPOPut, showed high in vitro cytotoxic activity (with mean GI_{50} values between 0.5 and 8.45 μM) and selectivity against cancer cells derived from nine different human tumours. The increased content of nitrogen atoms in the linker chain of BNIPSpd and BNIPSpm significantly improved their aqueous dissolution properties with a marginal decrease in their cytotoxic activity. © 2000 Elsevier Science Ltd. All rights reserved.

In the 1970s, naphthalimides were synthesised as potential antitumour agents,[1] which have resulted in the development of two compounds, namely mitonafide and amonafide.[2,3] In the early 1990s bis-naphthalimido compounds which contain two naphthalimido rings, covalently attached at the end of a linker chain containing two nitrogen atoms were synthesised and their anticancer properties evaluated.[4] Among the numerous compounds synthesised in this series, DMP 8408[5] and LU 795539[6] (Fig. 1) have shown promise for cancer therapy.

With regard to their mode of action, it was suggested that bis-naphthalimido compounds bind to DNA double helix via the major groove.[7] Although most of the bis-naphthalimides have shown higher cytotoxic activity than the monomeric compounds, they are however very insoluble in aqueous solutions,[8] making their testing and potential development into chemotherapeutics difficult. There have been a number of modifications undertaken on the napthalimido rings of these compounds in an attempt to improve their solubility and activity.[8] Bis-naphthalimido compounds which have been synthesised and tested so far have only one or two nitrogen atoms in the linker chain.[8] We report in this paper the synthesis of bis-naphthalimidopropyl polyamine derivatives, which contain 2, 3 and 4 nitrogen atoms in the linker chain. Our approach to increase the aqueous solubility of bis-naphthalimido compounds was to introduce more heteroatoms in the linker chain. The cytotoxic properties of the newly synthesised bis-naphthalimidopropyl putrescine (BNIPPut), spermidine BNIPSpd), spermine (BNIPSpm) and oxa-putrescine (BNIPOPut) against different panels of human cancer cell lines are also presented.

Bis-naphthalimido compounds with linker chain containing 2 nitrogens were previously synthesised by simply reacting the corresponding alkyltetraamine with 1,8-naphthalic anhydride.[8] In order to introduce more heteroatoms in the linker chain, *N*-alkylation reaction was chosen according to a modified method which we recently reported.[9] The common intermediate for the synthesis of BNIPPut, BNIPSpd, BNIPSpm and BNIPOPut is toluenesulfonyloxypropylnaphthalimide **2**. This was prepared by first reacting 1,8-naphthalic anhydride with aminopropanol to give *N*-(3-hydroxypropyl)naphthalimide which upon reaction with tosyl chloride gave **2** in 60% yield (Scheme 1). It is important to note that during the tosylation reaction we found that the best condition to obtain a maximum yield for **2** is to use four times excess of tosyl chloride in a small volume of solvent.[10] Under other conditions (e.g., with equimolar or 2 molar quantities of tosyl chloride), a mixture of **2** and **3**, *N*-(3-chloropropyl)naphthalimide is always formed thus reducing the overall yield of the reaction and also, renders purification very difficult.

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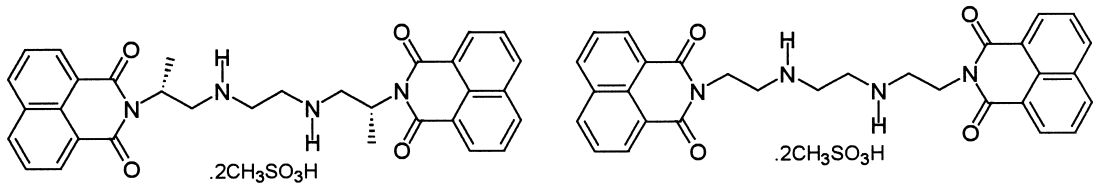
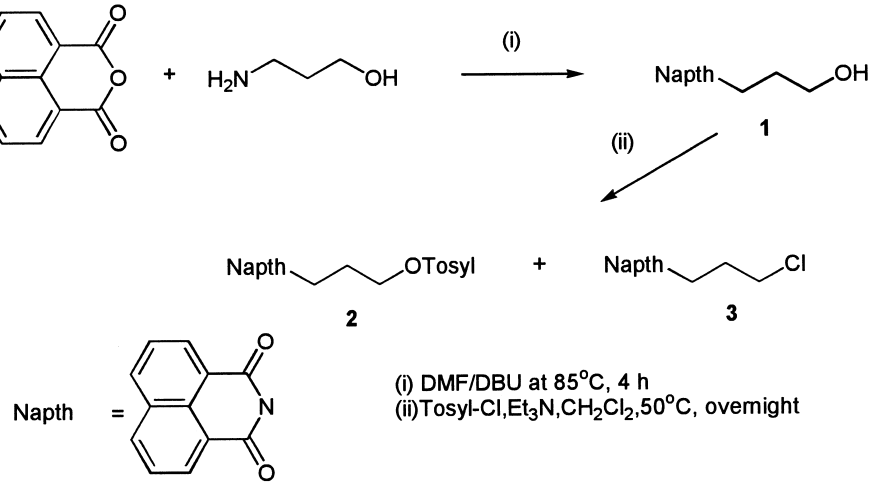
To obtain bis-naphthalimides BNIPPut, BNIPSpd, BNIPSpm and BNIPOPut (Scheme 2), polyamines **4**–**7** were first protected with 2,4,5-trimethylsulphonyl chloride (Mts-Cl) in pyridine followed by their *N*-alkylation with compound **2** to yield the fully protected bis-naphthalimido compounds which, on subsequent deprotection with hydrobromic acid/glacial acetic acid in dichloromethane afforded BNIPPut, BNIPSpd, BNIPSpm and BNIPOPut as their hydrobromide salts.

The growth-inhibitory effects of BNIPPut, BNIPSpd, BNIPSpm and BNIPOPut on cancer cells derived from different human tumours were carried out at the National Cancer Institute (NCI) in the USA. Data from the in vitro testing by the standard procedures of the NCI Drug Discovery and Developmental Therapeutic Programme[11] are shown in Table 1 and Table 2. Each compound was routinely tested at five 10-fold dilutions with a highest concentration of 100 μM. Cell growth and

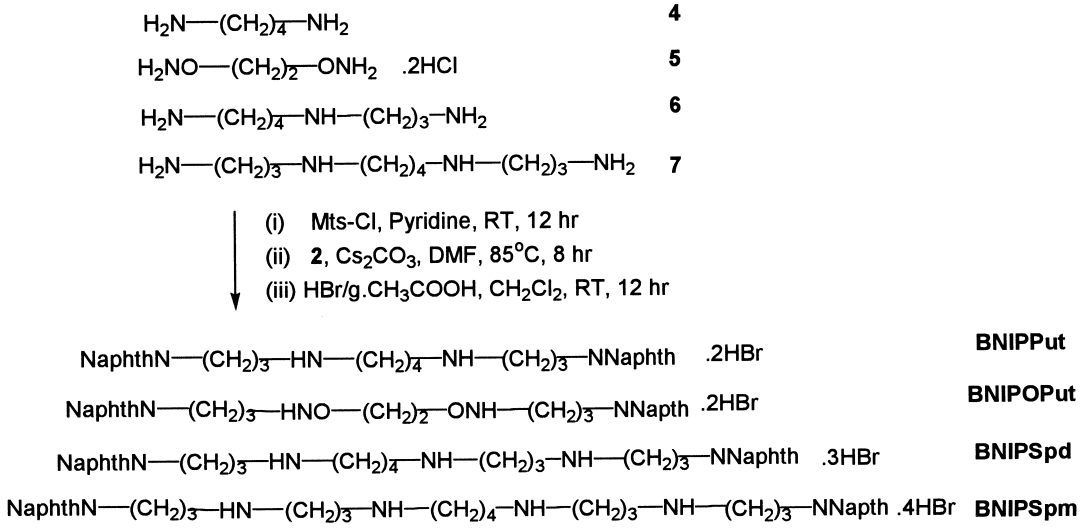


DMP 8408 **LU 795553**

Figure 1. Chemical structure of bis-naphthalimides DMP 8408 and LU 795553.



Scheme 1.



Scheme 2.

Table 1. Growth-inhibitory activity of bis-naphthalimidopropyl polyamines against different panels of human cancer cell lines

Panel of cell lines[a]	Compound/cytotoxicity (GI_{50} (μM))[b,c,d]			
	BNIPPut	BNIPSpd	BNIPSpm	BNIPOPut[e]
Leukaemia (6)	0.08	0.25	1.43	25.72
Non small cell lung cancer (9)	0.53	0.58	3.51	72.65
Colon cancer (7)	0.59	2.35	4.23	73.19
CNS cancer (6)	0.29	1.16	4.07	65.88
Melanoma (8)	0.44	1.17	4.04	77.99
Ovarian cancer (6)	0.34	0.79	6.12	81.03
Renal cancer (7)	0.57	2.49	7.43	65.39
Prostate cancer (2)	0.13	0.46	3.46	77.55
Breast cancer (7)	2.26	3.49	4.49	77.57
Mean value[e]	0.58	1.42	4.31	68.55

[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.

Table 2. Cytotoxic activity of BNIPPut, BNIPSpd and BNIPSpm against different human cancer cell lines

Panel of cell lines	Cell line	Compound/cytotoxicity (GI_{50} (μM))[a,b]		
		BNIPPut	BNIPSpd	BNIPSpm
Leukemia	HL-60 TB	0.222	0.156	0.033
	K-562	0.012	1.170	3.600
Non small	EKVX	1.370	1.050	4.500
cell lung Cancer	NCI-H522	0.013	0.030	0.825
Colon cancer	HCT-15	2.660	10.900	12.800
	SW-620	0.037	0.449	1.160
CNS cancer	SF-268	0.029	0.111	0.722
	SF-295	0.488	4.780	5.350
Ovarian Cancer	IGROV1	0.082	0.267	0.856
	SK-OV-3	0.722	1.310	10.800
Renal cancer	786-0	0.024	0.185	0.555
	UO-31	1.910	10.600	10.800
Breast cancer	NCI/ADR-RES	13.10	19.500	15.100
	HS 578T	0.065	0.044	0.630

[a]Data obtained from NCI's in vitro tumour cells screen.
[b]Data are an average of at least two testings.

viability were estimated by the sulforhodamine B method after 48 h drug exposure.[11] The growth-inhibitory properties of the bis-naphthalimidopropyl polyamines were expressed as a GI_{50} value defined as the drug concentration that causes 50% growth inhibition.

The cell lines used in the in vitro screen were the following: 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 our preliminary experiments BNIPPut and BNIPOPut were found to be insoluble in either water or 10% dimethylsulfoxide (soluble in dimethylsulfoxide on warming). However, BNIPSpd and BNIPSpm showed good solubility especially in 10% dimethylsulfoxide at 100-fold higher concentration than the highest test concentration in the cytotoxic study. Thus, it was concluded that the increase of the solubility was assisted by an increase of the number of nitrogens in the molecule. The results from the cytotoxic studies (Table 1) show that BNIPPut having 2 nitrogen atoms is the most active compound according to the mean GI_{50} values against different panels of cancer cell lines. However, the newly synthesised BNIPSpd and BNIPSpm with 3 and 4 nitrogen in the linker chain atoms respectively retain high cytotoxic activity (Table 1). Thus, the better solubility of BNIPSpd and especially of BNIPSpm in aqueous media was achieved without any significant drop in their anticancer activity. Apart from the bis-naphthalimido groups, the polyamine linker appears to be important for the cytotoxic properties of these compounds since the introduction of oxygen atoms in the linker of the most active BNIPPut led to a dramatic increase in the GI_{50} values of the corresponding compound BNIPOPut (Table 1). BNIPPut, BNIPSpd and BNIPSpm were demonstrated to possess a high selectivity in their cytotoxic effects against different panels of cell lines (Table 1). The data in Table 2 show high selectivity in the cytotoxic action of bis-naphthalimidopropyl polyamines against different cell lines in each panel (Table 2). For example BNIPPut, BNIPSpd and BNIPSpm were found to be 202-, 443- and 24-fold respectively more active against HS 578T than NCI/ADR-RES cell lines in the panel of Breast Cancer. Nevertheless, with a few exemptions BNIPPut was the most active compound against each cell line, followed by BNIPSpd and BNIPSpm.

After repeat of the primary screen, BNIPPut, BNIPSpd and BNIPSpm were recently selected by the NCI Biological Evaluation Committee for further in vivo testing. The mechanism of action of the bis-naphthalimidopropyl polyamines is currently under investigation in our laboratory. These compounds possess rapid cytotoxic activity and strongly bind to DNA which may contribute to their cytotoxic action (unpublished data).

The increased solubility and promising cytotoxic effects of the newly synthesised BNIPSpd and BNIPSpm are good basis for their further development as anticancer drugs.

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10. Synthesis of toluenesulfonyloxypropylnaphthalimide **2**.To a suspension of 1,8-naphthalic anhydride (9.98 g, 0.05 mol) in dry DMF (100 mL) were added 3-amino-propanol (3.75 g, 0.05 mol) and DBU (7.45 mL). The reaction mixture was stirred for 4 h at 85 °C. The solvent was removed under vacuum. The residue was poured into cold water to give *N*-(3-hydroxypropyl)naphthalimide **1** (10.0 g) as a white solid which was used without further purification. Compound **1** (5.0 g, 0.019 mol) was dissolved in anhydrous CH_2Cl_2 (50 mL) followed by the addition of 4-methylbenzenesulfonylchloride (14.22 g, 0.074 mol) and triethylamine (15 mL). The mixture was left overnight at 50 °C. After washings with a saturated solution of $NaHCO_3$ and water, the solvent was removed under vacuum to give a crude, which was recrystallised from ethanol to the pure product **2** as a white solid, mp 200 °C, 60% yield.
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