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Synthesis and In Vitro Cytotoxicity of Novel Long Chain Busulphan Analogues

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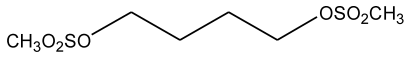
Abstract—Two novel long chain alkanediol dimethanesulphonates, analogues of busulphan, were synthesized. Their in vitro cytotoxicity was evaluated against six solid tumor cell lines (A2780, H322, LL, WiDr, C26-10 and UMSCC-22B). 2-Tetradecylbutane-1,4-diol dimethanesulphonate was proved to be the most active compound exhibiting IC_{50} values between 20.82 and 26.36 μM.

Many chemotherapeutic agents used in the treatment of cancer are bifunctional and are able to crosslink biological macromolecules.[1] Their most important cellular target is believed to be DNA, and in the case of alkylating agents the formation of interstrand crosslinks may be the most relevant cytotoxic lesion. Crosslinking starts by an initial covalent reaction of drug with an electrophilic site on the DNA to form a monoadduct, followed by a second reaction with the other DNA strand.

Alkanediol dimethanesulphonates of general formula $CH_3SO_2O(CH_2)_nOSO_2CH_3$ constitute a very interesting class of bifunctional chemotherapeutic agents. The best known agent of this homologous series is busulphan ($n=4$) (**1**), which has been one of the drugs of choice in the treatment of chronic myeloid leukemia for the last 30 years.[2,3] It was suggested that busulphan produced crosslinking in DNA through a GG bridge, but no distinction could be made as to whether this was derived from inter- or intrastrand crosslinks.[4,5] It has also been reported that busulphan induced DNA–protein crosslinks in human cell lines and mouse leukemia cell lines after prolonged drug treatment.[6]

Bifunctional alkylating agents, like busulphan analogues, could be of great importance as new anticancer agents. Hepsulfam (**2**), an anticancer agent with structural similarity to busulphan,[7] was found to have not only excellent antileukemic activity against P388 and L1210 mouse leukemia in vivo, but also good activity against some solid tumors.[6]



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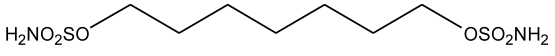

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Recently we have been involved in the synthesis and study of novel lipophilic cytotoxic agents.[8,9] In this paper the synthesis and in vitro cytotoxic activity of two new long chain busulphan analogues is described. The presence of the long chain confers lipophilicity to these analogues that may facilitate transport across the cellular membrane and increase intacellular drug accumulation thus improving drug effectiveness. Since structurally similar alkylating agents can differ in their anticancer activity, we designed two analogues with different relative positions of the two alkylating centers.

Chemistry

Commercially available hexadecane-1,2-diol (**3**) was chosen as starting material. Long chain diol **3** was converted into the corresponding dimethanesulphonate **4**

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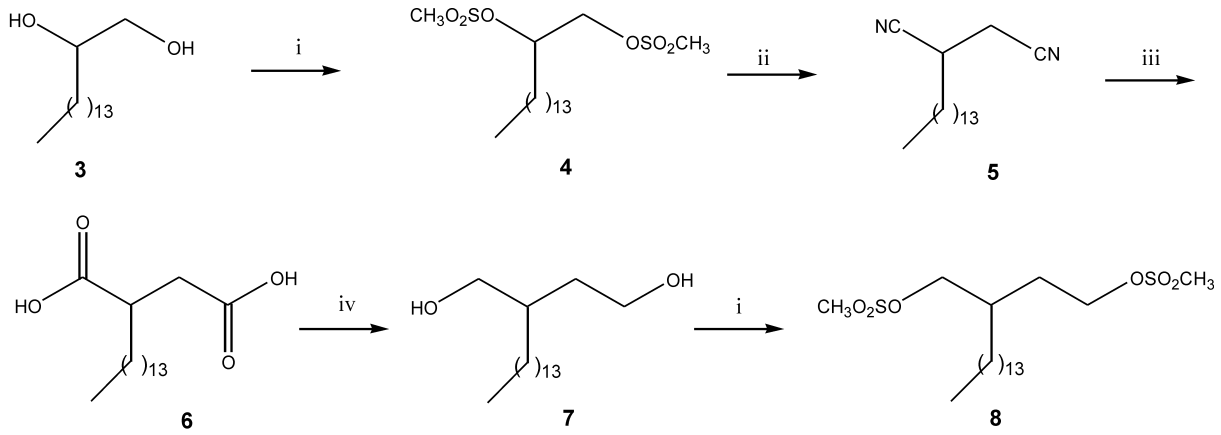

Scheme 1. (i) MsCl, Et_3N; (ii) NaCN, DMF; (iii) H_2SO_4; (iv) $LiAlH_4$.

by treatment with methanesulphonyl chloride in the presence of triethylamine. Methanesulphonyloxy groups were replaced by cyano groups by treatment with sodium cyanide. The dinitrile **5** was hydrolyzed under acidic conditions and the diacid **6** was reduced by $LiAlH_4$. 2-Tetradecylbutane-1,4-diol (**7**) was converted into the target busulphan anologue **8**. Busulphan was also prepared by treatment of 1,4-butanediol with methanesulphonyl chloride (Scheme 1).

All intermediates and final products gave satisfactory analytical and spectroscopic data in full accord with their assigned structures.[10]

In vitro cytotoxicity assay

Cell culture: cells were grown in monolayers in RPMI 1640 supplemented with 5% heat inactivated fetal calf serum and 2 mM L-glutamine in a 37 °C, 5% CO_2, 95% humidified air incubator. Exponentially growing cells were trypsinized and resuspended in antibiotic containing medium (50 μg gentamicin/mL); single cell suspensions displaying $\geq$97% viability by trypan blue dye exclusion were subsequently counted. After counting, dilutions were made to give the appropriate cell densities for inoculation onto the microtiter plates. Cells were inoculated in a volume of 100 μL per well at densities of 5000 and 20,000 cells per well, based on their doubling times.[11,12]

Chemosensitivity testing: the assay was performed in 96-well plates using the National Cancer Institute protocol.[13] Briefly, pure compounds were initially dissolved in DMSO at 400 times the desired final maximum test concentrations. Each agent was tested at five different dilutions. Immediately after preparation of these intermediate dilutions, 100 μL aliquots of each dilution were added to the appropriate 96-well plate wells. Drug incubation times were 72 h, after which cells were precipitated with 25 μL ice-cold 50% (w/v) trichloroacetic acid and fixed for 60 min. Then the sulforhodamine B (SRB) assay was performed.[14,15] The optical density (OD) of each well was measured at 492 nm using a 96-well plate reader (Titertek Multiscan MCC/340; Flow Laboratories).

Results and Discussion

The long chain dimethanesulphonates **4** and **8** as well as busulphan were tested for their cytotoxicity against six cell lines of various origin. The in vitro experiments were performed with the following cell lines: A2780, a human ovarian cancer cell line; H322, a human non-small cell lung cancer (NSCLC) cell line (subtype BAC, NCI); LL, a murine NSCLC cell line; WiDr, a human colon carcinoma cell line; C26-10, a murine colon carcinoma cell line; and UMSCC-22B, a human head and neck squamous cell carcinoma cell line.[16] The cytotoxicity of the compounds is expressed as IC_{50}, which is the drug concentration causing a 50% growth inhibition. The IC_{50} values exhibited by the compounds are summarized in Table 1.

Busulphan was proved inactive against all these solid tumors. 2-Tetradecylbutane-1,4-diol dimethanesulphonate (**8**) gave the best results against all the cell lines exhibiting IC_{50} between 20.82 μM and 26.36μM. Hexadecane-1,2-diol dimethanesulphonate (**4**) exhibited weaker activity (IC_{50} between 25.59 and 59.72 μM). In the case of LL cell line the activity of compound **8** was slightly higher than that of compound **4**, and in WiDr and C26-10 comparable. However, in the case of A2780, H322 and 22B cell lines an approximate 2-fold increase of the activity was observed.

It has been recognized that the relative position of the two alkylating centers within bifunctional alkylating agents is of importance with regard to their anticancer

Table 1. In vitro cytotoxicity of busulphan and analogues **4** and **8**

Cell line	IC_{50} (μM)[a]		
	Busulphan	**4**	**8**
A270	>100	45.45 (±13.37)	25.89 (±1.51)
H322	>100	59.72 (±6.72)	25.12 (±3.12)
LL	>100	28.43 (±5.88)	20.82 (±0.64)
WiDr	>100	25.59 (±5.59)	24.85 (±1.97)
C26-10	92.50 (±9.56)	30.97 (±4.92)	26.26 (±4.22)
22B	>100	46.47 (±5.85)	26.36 (±3.87)

[a]Values are means of three experiments, standard deviation is given in parentheses.

activity.[17] The ability of bifunctional dimethanesulphonates to span only selected target nucleophilic distances, coupled with the availability and reactivity of these sites, is crucial to the type and quantity of DNA adducts that may be produced.[18,19] The results obtained in this study show that 1,4-distance between the alkylating centers leads to better results in comparison with 1,2-distance. Furthermore, both lipophilic derivatives **4** and **8** are more active than busulphan, indicating that the presence of the long chain facilitates the molecular interactions with DNA increasing the cytotoxic activity.

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10. For example: compound **4**: mp 52–54 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm: 0.85 (t, 3H, $J=7$ Hz, CH_3), 1.25 (m, 24H, $12\times CH_2$), 1.70 (m, 2H, CH_2CHOSO_2), 3.05 (s, 6H, $2\times CH_3OSO_2$), 4.30 (m, 2H, CH_2OSO_2), 4.85 (m, 1H, CH). Analysis for $C_{18}H_{38}O_6S_2$ (414.62): calcd C 52.14, H 9.24%. Found C 52.28, H 9.37%. Compound **8**: mp 53–55 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm: 0.86 (t, 3H, CH_3), 1.24 (m, 26H, $13\times CH_2$), 1.88 (m, 3H, CH, CH_2), 3.05 (s, 6H, $2\times CH_3OSO_2$), 4.20 (m, 4H, $2\times CH_2$). Analysis for $C_{18}H_{38}O_6S_2$ (442.68): calcd C 54.26, H 9.56%. Found C 54.39, H 9.62%.
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