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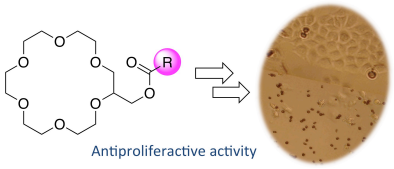

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Synthesis and biological evaluation of crown ether acyl derivatives

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ABSTRACT

A set of crown ethyl acyl derivatives based on 18-crown-6 moiety was synthesized and evaluated for biological activity. *In vitro* antiproliferative profiling demonstrated significant activities against HBL-100, HeLa, SW1573 and WiDr human cell lines. The most active compound exhibited GI_{50} values in the range of 3.7-5.6 μM. Antimicrobial evaluation showed that three polyaromatic compounds were active against *Staphylococcus aureus* (MIC_{90} values from 8.3 μM to 50 μM), whereas a (decyloxy)benzene substitution exhibited moderate activity against *Candida albicans* (MIC_{90} values 36 μM). According to SAR evaluation, the size of the crown ether and the acyl side chain had a significant effect on the bioactivity. Aromatic moieties close to the acyl group led to improved bioactivity as exemplified by some of the tested compounds. These results provide further evidence on the potential of crown ethyl structure as a scaffold for developing new biological probes and lead candidates for drug development.

Crown ethers, first described by Pedersen et al.[1] are macrocyclic polyethers well known for their noncovalent ion binding properties. Their common names include the number and type of atoms in the polyether ring. The ring size of these molecules controls their binding selectivity for a range of metal ions, thus 18-crown-6 has high affinity for potassium while 15-crown-5 selects sodium cations. To explain this, a high complex stability has been associated with greater penetration of the metal cation into the polyether cavity.

Crown ether macrocycles are known in all ring sizes from 9 to at least 60, leading to great variety in structures (exceeding 10000 examples).[2] Therefore, crown ethers have been extensively studied from different aspects, including sensor applications, biological model systems (for example, abiotic ion channels)[3] and biological functionality, especially for anticancer and antimicrobial effects.[2,4,5] Crown ether derivatives function similarly to natural ionophores (such as gramicidin) and have thus been used to study several biological processes, in particular due to their channel forming and ion transport capabilities through lipid membranes.[5] Despite, large macrocylic compounds do not comply with the druglike, "rule-of-five" properties,[6] the potential of macrocycles for drug discovery has attracted considerable attention in the recent years.[7]

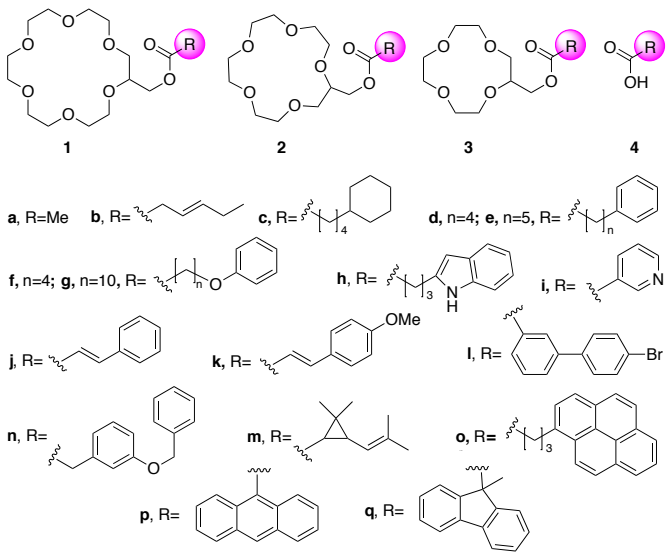

Figure 1. Structure of the crown ether acyl derivatives.

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Table 1. Antiproliferative activity (GI_{50}) against human solid tumour and non-tumour cells[a]

Compound	HBL-100 (breast)	HeLa (cervix)	SW1573 (lung)	WiDr (colon)	BJ-hTert (fibroblast)	*c*LogP[b]
1a	>100	>100	>100	>100		-0.96
1b	>100	>100	>100	>100		0.86
1c	72.0 (± 13.0)	71.0 (± 9.3)	51.0 (± 6.0)	33.0 (± 0.8)		3.27
2c	>100	>100	>100	>100		3.46
3c	>100	>100	>100	>100		3.66
1d	69.0 (± 23.0)	52.0 (± 3.4)	>100	>100		2.04
1e	64.0 (± 24.0)	79.0 (± 25.0)	78.0 (± 5.4)	>100		2.56
1f	>100	>100	>100	>100		1.46
1g	20.0 (± 6.2)	41.0 (± 28.0)	24.0 (± 2.5)	66.0 (± 12.0)	24 (± 4.0)	4.63
1h	>100	>100	>100	>100		1.50
1i	>100	>100	>100	>100		-0.59
1j	57.0 (± 2.1)	48.0 (± 7.6)	48.0 (± 5.9)	>100		1.20
1k	50.0 (± 2.6)	56.0 (± 4.5)	54.0 (± 3.2)	56.0 (± 4.5)	>100	1.12
1l	6.4 (± 0.4)	7.8 (± 6.5)	4.4 (± 0.7)	6.2 (± 3.8)	8.2 (± 0.1)	3.51
1m	40.0 (± 3.4)	63.0 (± 7.3)	54.0 (± 3.2)	56.0 (± 4.5)	>100	2.40
1n	29.0 (± 2.6)	35.0 (± 2.2)	36.0 (± 2.9)	24.0 (± 6.5)	22 (± 9.6)	2.34
1o	21.0 (± 1.0)	17.0 (± 0.4)	18.0 (± 4.4)	19.0 (± 1.4)	19 (± 1.5)	3.94
1p	5.0 (± 0.9)	3.7 (± 0.7)	3.8 (± 0.1)	5.6 (± 1.3)	5.4 (± 2.4)	3.10
1q	11.0 (± 0.6)	12.0 (± 1.3)	8.8 (± 1.0)	12.0 (± 1.4)	18 (± 3.8)	2.51
CDDP	1.9 (± 0.2)	2.0 (± 0.3)	3.0 (± 0.4)	26.0 (± 5.3)		
VP-16	1.4 (± 0.1)	3.3 (± 1.6)	15.0 (± 1.5)	23.0 (± 3.1)		

[a] Values are given in μM and are means of two to three experiments; standard deviation is given in parentheses.

[b] *c*LogP values were calculated using the Schrodinger small-molecule drug discover suite conditions.

Herein we report the synthesis of a series of crown ether acyl derivatives and the evaluation of their antimicrobial and antiproliferative properties. The antimicrobial activity was tested against Gram (+) and Gram (-) bacteria as well as against the model yeast *Candida albicans*. Previous reports describe dialkyldiaza-18-crown-6 ethers as inhibitors of bacterial growth.[8,9] In addition, the antiproliferative profile was evaluated *in vitro* against a panel of human solid tumour cell lines. Thus we first synthetized compounds **1c**, **2c** and **3c** where the same cyclohexyl side-chain was attached to 18-6, 15-5 and 12-4 crown ether moieties. Based on their antiproliferative activity (Table 1) we selected 18-crown-6 derivatives for further development, changing the length of the side chain and the size of the attached cyclic moiety.

Table 2. Antimicrobial primary screening of compounds. Ciprofloxacin and amphotericin B were used as positive controls. Data is presented as average inhibition% (±standard deviation, $n = 3$) at 50 μM concentration. Inhibition results >90% are in bold

Compound	*C. albicans* ATCC 90028	*E. faecalis* ATCC 29212	*S. aureus* ATCC 25923	*E. coli* ATCC 25922
1a	nt	nt	nt	nt
1b	9.4 (± 5.5)	4.7 (± 5.9)	6.9 (± 0.6)	4.3 (± 3.6)
1c	0	0	61.0 (± 8.6)	0
2c	0	0	0	3.1 (± 2.7)
3c	0	0	0	4.9 (± 2.2)
1d	5.5 (± 17.0)	0.3 (± 1.6)	0	5.0 (± 2.8)
1e	11.0 (± 23.0)	0	0	5.8 (± 4.2)
1f	1.6 (± 16.0)	3.5 (± 4.6)	0	8.1 (± 3.6)
1g	**101.0** (± 0.1)	2.1 (± 4.2)	29.0 (± 20.0)	0
1h	nt	nt	nt	nt
1i	24.0 (± 9.6)	6.8 (± 4.8)	0	6.2 (± 1.7)
1j	36.7 (± 13.0)	3.3 (± 2.7)	3.4 (± 18.0)	5.9 (± 1.0)
1k	0	0	0	7.3 (± 7.4)
1l	1.0 (± 26.0)	0	**98.0** (± 0.1)	6.5 (± 4.3)
1m	0	0	0	0
1n	0	0	0	3.6 (± 2.5)
1o	**101.0** (± 0.1)	0	**99.0** (± 0.3)	0
1p	4.3 (± 18.0)	0	**98.0** (± 0.7)	7.8 (± 5.4)
1q	0	0	0	0.1 (± 1.1)

The general synthetic approach used included two alternatives: first the "Steglich esterification" using the acid of the selected side chain and a crown ether alcohol, utilizing dicyclohexylcarbodiimide as coupling agent and 4-dimethylaminopyridine as catalytic base.[10] The second approach consisted in the usage of the corresponding acyl chloride as described in the supporting information. Using this simple strategy we obtained the corresponding derivatives **1a-q** with yields ranging 60-95% (Fig. 1).

The *in vitro* antiproliferative activity of the crown ether acyl derivatives was determined in HBL-100, HeLa, SW1573 and WiDr human solid tumor cells. Table 1 shows the results (expressed as GI_{50}) using the SRB assay.[11] The standard anticancer drugs cisplatin (CDDP) and etoposide (VP-16) were used as positive controls. The most active compound of the series was **1p** and exhibited GI_{50} values against all cells in the range 3.7-5.6 μM. When compared to CDDP and VP-16, compound **1p** showed an improved biological activity in the resistant cell line WiDr. In addition, the non-tumor cell line BJ-hTert (telomerase-immortalized human foreskin fibroblasts) was used to study the effect on a subset of the most potent crown ether acyl derivatives of the series. The results show that compounds 1k and 1m are inactive (GI50 > 100 μM), thus indicating some selectivity, whilst the other derivatives showed no discrimination between tumor and non-tumor cell lines. Further experiments will be necessary to explain this outcome.

Furthermore, the antimicrobial activity of the compounds was evaluated against a set of strains typically used in clinical antimicrobial testing by using the broth microdilution method according EUCAST and CLSI guidelines.[12,13] All samples were initially tested at 50 μM. As can be seen from the primary screening results in Table 2, compounds **1g** and **1o** were highly active against *C. albicans* ATCC 90028 and compounds **1l**, **1o** and **1p** displayed significant activity against *Staphylococcus aureus* ATCC 25923, fully inhibiting the growth of this Gram-positive bacterium. Confirmatory dose-response experiments were carried out for these compounds.

Compounds **1l**, **1o** and **1p** showed MIC_{90} values of 25 μM, 8.3 μM and 50 μM, respectively, against *S. aureus* (Table 3). Against *C. albicans* **1g** and **1o** exhibited MIC_{90} values of 36 and 42 μM, respectively. All the compounds were inactive against the Gram-negative *E. coli*, possibly due to its outer membrane structure. Antimicrobial properties of crown ether derivatives have previously been studied by Leevy et al.[4] who demonstrated that activity against *Escherichia coli* is altered by the spacer length. Furthermore, Gram-positive *Bacillus subtilis* has been demonstrated to be in general more susceptible to different crown ether derivatives than *E. coli*.[4,14] Interestingly, the activity of compound **1p**, the most active compound in the antiproliferative experiments, was about 4-fold lower against *S. aureus* based on comparison between the GI_{50} and MIC_{50} values (Table 1, Table 3). On the other hand, compound **1o** with MIC_{50} of 7.2 μM against *S. aureus* was only moderately active against the cancer cells (GI_{50} values in the range of 17-21 μM, Table 1).

Table 3. Minimum inhibitory concentrations (MIC_{90}, MIC_{50}) for the most active compounds against *S. aureus* ATCC 25923 and *C. albicans* ATCC 90028. Ciprofloxacin (MIC_{90} against *S. aureus* 0.5 μg/mL) and amphotericin B (MIC_{90} against *C. albicans* 0.5 μg/mL) were used as positive controls

	C. albicans		*S. aureus*	
Compound	MIC_{90}[a]	MIC_{50}	MIC_{90}	MIC_{50}
1g	16.7	n.d.[b]	n.d.	n.d.
1l	n.d.	n.d.	11.3	6.8
1o	21.7	20.1	4.3	3.7
1p	n.d.	n.d.	27.8	11.1

[a] Values are given in μg/mL.

[b] Not determined.

The structure-activity relationship (SAR) study clearly demonstrated that the size of the crown ether and the acyl side chain influence the bioactivity. Initially, it was established that the preferred crown ether was [18]-crown-6. The selection was based on the GI_{50} values obtained for derivatives **1c**, **2c** and **3c** in the antiproliferative profiling (Table 1). Then, further efforts were devoted to study the influence of the acyl side chain on the antiproliferative activity. A clear trend could not be inferred from the biological data. For instance, a larger aliphatic linker improves the antiproliferative activity of **1g** when compared to **1f**. The side-chain length affects significantly the hydrophobicity of the compound, and thus calculated logP values for **1f** and **1g** are markedly different (1.46 and 4.63, respectively, Table 1). According to Supek et al.,[14] logP of the molecule is the most important molecular descriptor in determining the biological activity of 18-crown-6 ethers, and not generally affected by features such as the side chain length or molecular symmetry. For our compounds, the calculated logP values vary from -0.96 to 4.63 (Table 1). The most active compounds have logP values >3, but some of the moderately active or inactive compounds have similar values, and thus the logP does not seem to be clearly correlated with biological activity according to our data. Based on our results, improved activity was obtained when the aromatic structure was close to the acyl group, as exemplified with compounds **1l**, **1p** and **1q**. Further experiments are necessary to discern if the observed differences correlate to diverse mechanisms of action.

In conclusion, in this study we described an efficient strategy for synthesizing a series of crown ether acyl derivatives. Of the crown ether moieties 18-6, 15-5 and 12-4, 18-crown-6 ether core structure showed initially the best potential based on antiproliferation assays on human cancer cell lines and was thus chosen for synthesising a series of acyl derivatives. Compounds were evaluated for antiproliferative activity against HBL-100, HeLa, SW1573 and WiDr human solid tumor cell lines, and the most active compound **1p** displayed GI_{50} values in the range of 3.7-5.6 μM. Antimicrobial evaluation yielded compounds active against *S. aureus* and *C. albicans*; most active was compound **1o** against *S. aureus* with MIC_{90} value of 8.3 μM. These results will be helpful in understanding the biological effects of crown ether derivatives, and may thus aid the development of novel anticancer and antimicrobial agents based on crown ether moieties.

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Supplementary Material

Supplementary material associated with this article including compound preparation and characterization as well as experimental details on biological assays, can be found in the online version.

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