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BIS-QUINOLINIUM CYCLOPHANES: A NOVEL CLASS OF POTENT BLOCKERS OF THE APAMIN-SENSITIVE Ca^{2+}-ACTIVATED K^+ CHANNEL

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Abstract. Based on the structure-activity analysis of two series of blockers of the small conductance Ca^{2+}-activated K^+ (SK_{Ca}) channel, a novel class of bis-quinolinium cyclophane blockers has been designed and synthesised. These compounds exhibit submicromolar activity; UCL 1530 (**4**) is a useful agent since it has been shown (elsewhere) to be selective for the neuronal SK_{Ca} channel (IC_{50} = 80 nM) relative to hepatocyte channels.

Introduction and design considerations. Small conductance Ca^{2+}-activated K^+ (SK_{Ca}) channels[1,2] form a physiologically important subtype which may play a role in myotonic muscular dystrophy.[3-5] This channel is blocked selectively by apamin with nanomolar potency.[6-8] Recently, it has been shown that dequalinium (Figure 1) is also a selective blocker of the SK_{Ca} channel.[9,10] Analogues of the general structure **I** have been synthesised and quantitative structure-activity analysis has suggested a possible relationship between blocking potency and the energy of the lowest unoccupied molecular orbital of the compound.[11,12] Furthermore, the role of the aliphatic chain of dequalinium has been investigated by varying the number of methylene groups in L (Figure 1) with the finding that the length of the chain is not critical for activity.[13] In addition to this, rigidification of L has been explored by replacing the alkyl chain with semi-rigid aromatic systems (series **II**, Figure 1; analogues **1a-6a** of Table 1 belong to this series).[14] Most of the compounds of series **II** were slightly more potent than dequalinium but the activity was found not to be critically dependent upon the nature of the linker L. This was attributed in part to the extensive delocalisation of the positive charge within the quinolinium group[15] and to the conformational mobility of the quinolinium rings in series **II** despite the presence of a rigid linker.[14] Therefore, it became of interest to seek a reduction in the mobility of the quinolinum groups to see whether this might lead to further increases in activity. It has also been shown that compounds of the general structure **III** in which the quinolinium groups are linked via an exocyclic heteroatom X are effective blockers of the SK_{Ca} channel.[16] One of the most potent analogues in series **III** had n=10, X=NH, R^1=CH_2Ph and R^2=R^7=H. Thus, it seemed plausible that the combination of the structural features of series **II** and **III**, that is, linking the exocyclic N atoms of compounds of series **II** via a methylene chain, to give series **IV** (Figure 1), could be tolerated and perhaps provide an increase in potency via constraining the quinolinium groups. A ten-methylene chain was selected as the linker, since this would permit a direct comparison of the analogues of series **IV** with those of series **III**. Moreover, molecular modelling studies suggested that a ten carbon linker would provide sufficient separation between the exocyclic N atoms and, therefore, should not present any synthetic difficulties. To test this working

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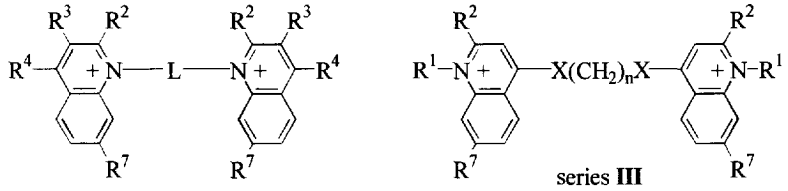
hypothesis, the novel cyclophanes **1-6** (Table 1) were synthesised and tested for inhibition of the afterhyperpolarisation (AHP) of rat sympathetic neurones.

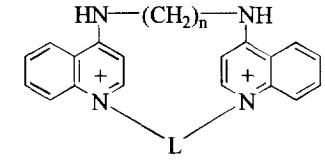


Dequalinium: R^2=CH_3, R^4=NH_2
R^3=R^7=H, L=$(CH_2)_{10}$

series **I**: L=$(CH_2)_{10}$

series **II**: L=CH_2ArCH_2

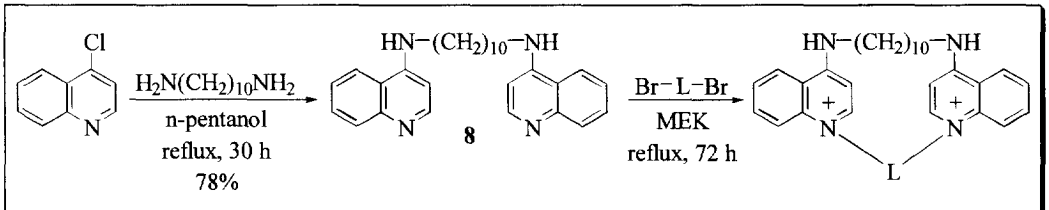
series **III**



IV

Figure 1

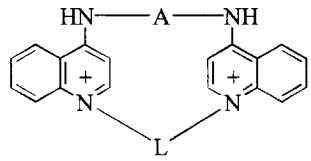
Synthesis. Compounds **1-6** were prepared via Scheme 1. Reaction of 4-chloroquinoline[17] with 1,10-diaminodecane in pentanol afforded the intermediate diquinoline **8**, which was dialkylated with the appropriate dibromomethyl compound in methyl ethyl ketone (MEK) under dilution conditions (0.019 M) to give the desired cyclophanes **1-6**. Attempts to obtain analytically pure samples of **1-6** for biological testing using conventional purification methods failed and, therefore, reverse phase (RP) preparative HPLC was performed. A Kromasil C18 5 μm column and solvent mixtures of H_2O + 0.1% trifluoroacetic acid (TFA) : MeOH + 0.1% TFA were used. All compounds were isolated and analysed as the trifluoroacetates.

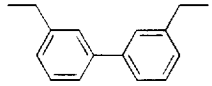
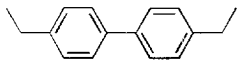
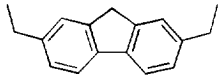
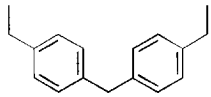
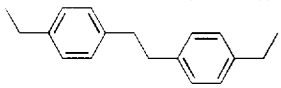
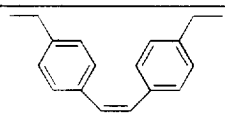


Scheme 1

Biological testing. The SK_{Ca} blocking action of the compounds was assessed from their ability to inhibit the AHP in cultured rat sympathetic neurones as described previously.[10] Briefly, each compound was tested at 2 to 4 concentrations on at least three cells. Between 1 and 3 compounds were examined at a time, and in each such series of experiments, dequalinium was also included as a reference compound. The Hill equation was fitted to the data to obtain estimates of the IC_{50} (Table 1). However, because there was some variation in the potency of dequalinium during the course of the study, equi-effective molar ratios (EMR: relative to dequalinium) were also obtained by simultaneous non-linear least squares fitting of the data with the Hill equation. These are also listed in Table 1 and it is these values which have been used for the comparison between compounds, bearing in mind that the smaller the value of EMR the more potent is the compound.

Table 1. Structures and biological results for the cyclophanes **1-6** (series **IV**) and their acyclic analogues **1a-6a** (series **II**) and **7a** (series **III**).



Compd	A	L	mp (°C)[a]	IC_{50} ± SD (μM)	EMR[b] ± SD	n[c]
1	$-(CH_2)_{10}-$		112-114	0.11 ± 0.05	0.28 ± 0.14	3
1a	H, H			0.29 ± 0.07[d]	0.55 ± 0.3[d]	6
2	$-(CH_2)_{10}-$		280-282	0.17 ± 0.05	0.28 ± 0.17	7
2a	H, H			0.38 ± 0.04[d]	0.40 ± 0.1[d]	3
3	$-(CH_2)_{10}-$		214-216	0.09 ± 0.05	0.12 ± 0.07	7
3a	H, H			0.31 ± 0.04[d]	0.33 ± 0.1[d]	4
4[e]	$-(CH_2)_{10}-$		181-186	0.08 ± 0.02	0.16 ± 0.1	9
4a	H, H			0.45 ± 0.15[d]	0.39 ± 0.12[d]	4
5	$-(CH_2)_{10}-$		202-204	0.18 ± 0.03	0.58 ± 0.34	3
5a	H, H			0.41 ± 0.12[d]	0.71 ± 0.19[d]	4
6	$-(CH_2)_{10}-$		184-186	0.15 ± 0.02	0.28 ± 0.13	3
6a	H, H			0.19 ± 0.03[d]	0.24 ± 0.08[d]	6
7a	$-(CH_2)_{10}-$	$PhCH_2$, $PhCH_2$		0.7 ± 0.1	1.0 ± 0.4[f]	7

[a] Melting points refer to trifluoroacetate salts. [b] Equieffective molar ratio: the ratio of the concentrations of the test compound and dequalinium that cause 50% inhibition of the AHP, as determined in the same experiment. [c] Number of neurones tested. [d] Data from ref. 14. [e] UCL 1530. [f] Data from ref. 16.

Discussion of results. All the cyclophanes **1-6** exhibit submicromolar activity, with **3** being almost an order of magnitude more potent than dequalinium and the most effective non-peptidic inhibitor of the AHP on rat sympathetic neurones identified so far. The structure-activity relationships previously observed[14] in the acyclic analogues **1a-6a** (series **II**, Figure 1) are generally preserved in the cyclophane series. Thus, shift of the substitution position in the biphenyl **1** from meta- to para- (**2**), rigidification by fusion into a fluorene (**3**), or increasing intramolecular flexibility by insertion of a methylene group between the phenyl rings (**4**) has little significant effect on potency. Further increase in flexibility by insertion of two methylene groups (**5**) or rigidification with a cis-ethene bridge (**6**) also has only a modest influence on potency. Overall, the transition from the acyclic compounds **1a-6a** to the cyclophanes **1-6** results either in retention, or in a small increase, of potency.

The cyclophanes **1-6** may also be compared with the dibenzyl analogue[16] **7a**, which belongs to series **III** (Figure 1). In this case it is clear that linking the phenyl rings, directly or via a bridge, leads to a significant increase in potency.

In addition, it has been shown that compound **4** (UCL 1530) and dequalinium differ in their rank order of potencies in blocking the SK_{Ca} channel in rat sympathetic neurones and in guinea-pig hepatocytes,[18,19] with **4** being more selective for the neuronal SK_{Ca} channel. This constitutes additional evidence for the existence of SK_{Ca} channel subtypes in different tissues.[20] Compound **4** (UCL 1530) is the first example of a dequalinium-based blocker to show such tissue selectivity. Earlier studies with series **I** (Figure 1) did not demonstrate differences in the blocking activity of the compounds in the above two tissues.[21] Thus, bis-quinolinium cyclophanes may be useful tools for the pharmacological characterisation of putative SK_{Ca} subtypes.

In conclusion, a novel class of bis-quinolinium cyclophane SK_{Ca} channel blockers has been designed. These compounds have IC_{50} values in the submicromolar range and may constitute leads for the development of more potent and selective SK_{Ca} channel blockers.

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