Communications to the Editor

Bis-Quinolinium Cyclophanes: 6,10-Diaza-3(1,3),8(1,4)-dibenzena-1,5(1,4)-diquinolinacyclodecaphane (UCL 1684), the First Nanomolar, Non-Peptidic Blocker of the Apamin-Sensitive Ca^{2+}-Activated K^+ Channel

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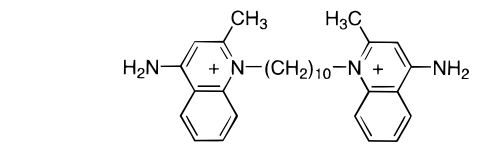
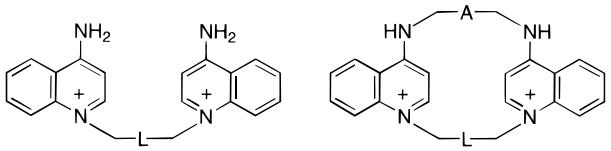
Small conductance Ca^{2+}-activated K^+ (SK_{Ca}) channels comprise a widely distributed but relatively little studied class of K^+ channels.[1] Selective blockade of SK_{Ca} channels may find application in the therapy of myotonic muscular dystrophy,[2] gastrointestinal dysmotilities (Dunn, P. M.; Jenkinson, D. H., unpublished results), disorders of memory,[3] narcolepsy,[4] and alcohol abuse.[5] Furthermore, SK_{Ca} channel blockers will be invaluable tools for further investigations into the role of this K^+ channel subtype in physiological and pathophysiological processes.

Blockers of SK_{Ca} channels include natural peptidic toxins such as apamin,[6] leiurotoxin I,[7] PO5,[8] and Ts K,[9] as well as nonpeptidic compounds such as the neuromuscular blockers tubocurarine, pancuronium, and atracurium.[10] Furthermore, the antiseptic compound, dequalinium (Chart 1), has been shown to be a relatively potent and selective SK_{Ca} channel blocker[11] and has constituted a lead for the development of several series of novel SK_{Ca} channel blockers.[12] We have recently reported the design of novel bis-quinolinium cyclophanes of the general structure **2** (L = group having two aromatic rings, Chart 1) which are submicromolar blockers of the SK_{Ca} channel.[12i] In addition, we have shown that one of them, **2** [UCL 1530, L = methylenebis(benzene-1,4-diyl)], shows selectivity for neuronal over hepatocyte SK_{Ca} channels,[13] which adds to the functional evidence for the existence of SK_{Ca} channel isoforms in different tissues.[14] Such selective compounds should prove to be useful pharmacological tools, especially in view of the recent cloning and expression in *Xenopus* oocytes of SK_{Ca} channel-forming peptides from rat and human brain.[15]

Chart 1



Dequalinium



1 **2**: A = $(CH_2)_8$; **3**: A = aromatic rings

[a] L = aromatic rings, as indicated in Table 1.

Molecular modeling studies suggest that the cyclophanes of type **2** are more rigid than their noncyclic analogues **1** (Chart 1). Thus, the number of distinct conformations within 3 kcal/mol of the calculated global minimum (i.e. the conformations that have a >99% probability of existing at 37 °C) is 2−10 times smaller for cyclophanes **2** compared with the respective noncyclic analogues **1** (Galanakis, D., unpublished results). However, cyclophanes **2** retain substantial conformational mobility, mainly due to the presence of the long aliphatic chain A.

In the present study we have sought to reduce further the conformational freedom of the quinolinium groups of series **2** by replacing part of the aliphatic chain with aromatic rings (A). Initially, we synthesized bis-quinolinium cyclophanes of the general structure **3** (Chart 1 and Table 1) in which each of the linkers A and L contain two aromatic rings. Subsequently, we reduced the size of the linkers by synthesizing cyclophanes in which A and L are benzene rings.

Chemistry. The cyclophanes **3a**−**g** (Table 1) were synthesized according to Scheme 1. The conversion of the novel diquinolines **4a**−**d** to the desired cyclophanes was carried out under high dilution conditions (1−2 mM). The preparations of the necessary dibromides 4,4′-bis(bromomethyl)biphenyl[16] and bis[*p*-(bromomethyl)diphenyl]methane[17] have been described previously. The requisite diamines **5a** and **5b** have been reported[18] but were prepared via the two alternative routes shown in Scheme 2. Higher overall yields were obtained through the diazide route. Amines **5c** and **5d** were commercially available. Final products were purified by high performance liquid chromatography and isolated and analyzed as solid trifluoroacetate hydrates.

Biological Testing. The SK_{Ca} blocking action of the compounds was assessed from their ability to inhibit the after-hyperpolarization (AHP) in cultured rat sympa-

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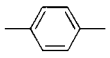
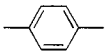
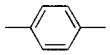
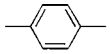
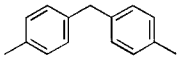
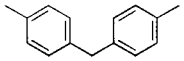
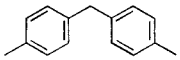
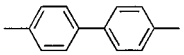
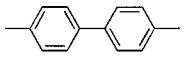
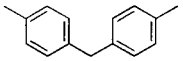
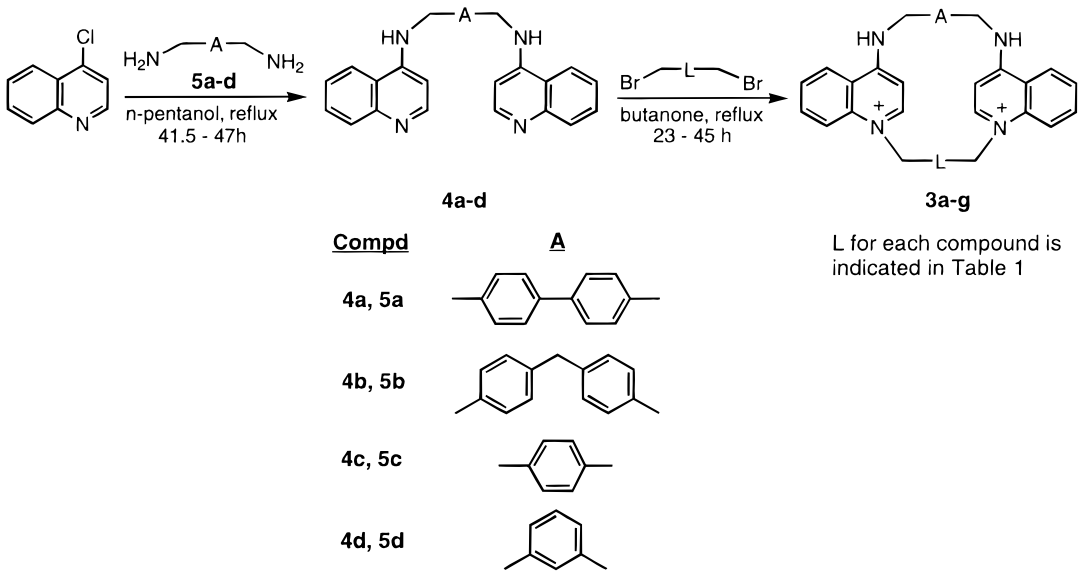
Table 1. Compound Structures and Biological Results for Inhibition of Afterhyperpolarization in Cultured Rat Sympathetic Neurones

Compd	A[a]	L[a]	IC_{50} ± SD (nM)	EMR[b] ± SD	n[c]
Deq.			570 ± 60	1	24
3a			130 ± 10	0.22 ± 0.04	4
3b[d]			3 ± 1	0.01 ± 0.001	4
3c			28 ± 3	0.05 ± 0.01	5
3d			70 ± 40	0.18 ± 0.04	5
3e			260 ± 40	0.31 ± 0.24	5
3f			150 ± 10	0.26 ± 0.12	3
3g			280 ± 10	0.80 ± 0.21	8

[a] See Chart 1 or Scheme 1 for generic structure. [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]UCL 1684.

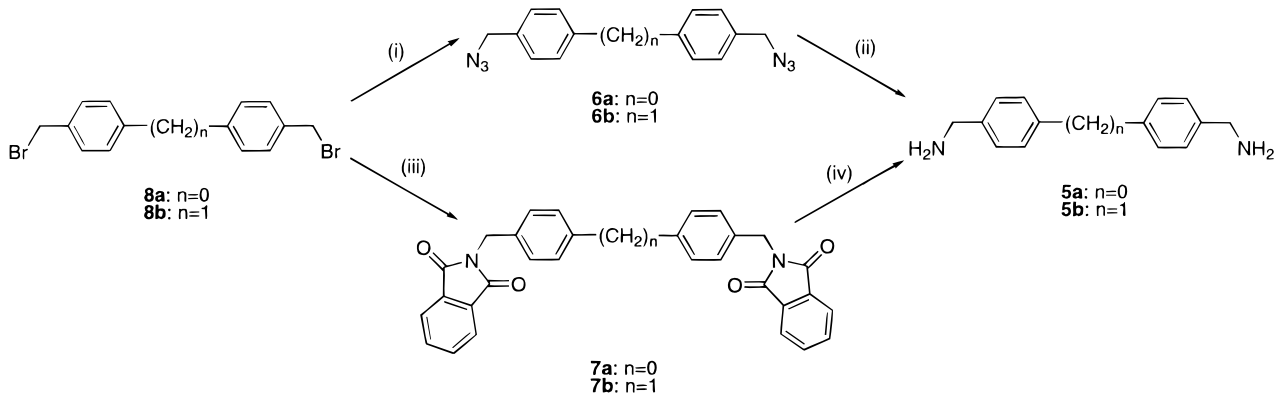
Scheme 1



thetic neurones as described previously.[11b] Briefly, each compound was tested at two to four concentrations on at least three cells. Two to four compounds were examined on a batch of cultured neurones, 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}. However, because there was some variation in the potency of dequalinium during the course of the study, equieffective molar ratios (EMR: relative to dequalinium) were also obtained by simultaneous nonlinear 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.

Results and Discussion. The structures of the cyclophanes and the results of biological testing are presented in Table 1. In those cases where the linkers A and L are large groups containing two or more aromatic rings (**3e**−**g**), the activity of the compound does not seem to be critically dependent upon the nature of the linkers. Hence, cyclophanes **3e**−**g** have similar potencies. However, this is not the case when the linkers A and L are benzene rings (**3a**−**d**). The blocking potency of these smaller cyclophanes is remarkably sensitive to the substitution pattern of the benzene rings. This is exemplified with **3a** and **3b** in which a change in the substitution pattern of A from meta to para causes a 22-fold increase in potency. Compound **3b** (UCL 1684) is the most potent nonpeptidic SK_{Ca} channel blocker reported to date, being 100 times more potent than dequalinium. Furthermore, having an IC_{50}

Scheme 2[a]



[a] (i) NaN_3, DMF, 90 °C, 17 h; **6a**, 97%; **6b**, 95%; (ii) $LiAlH_4$, THF, Reflux, 1 h; **5a**, 93%; **5b**, 87%; (iii) Potassium Phthalimide, DMF, 90 °C, 40 min; **7a**, 87%; **7b**, 88%; (iv) $H_2NNH_2 \cdot H_2O$, DMSO, 120 °C, 1.5 h; **5a**, 98%; **5b**, 35%.

of 3 nM, it constitutes the first synthetic compound to have a potency similar to that of apamin ($IC_{50} = 1$ nM).[6]

The results for the small cyclophanes **3a**−**d** constitute a significant advancement in the structure−activity relationships of the SK_{Ca} channel blockers, when compared with the existing knowledge in the field. So far, large structural changes in the linker(s) joining the two quinolinium groups in several series of SK_{Ca} channel blockers have been shown to have only a small effect on the potency of the molecule. Thus, in dequalinium homologues of structure **1** (Chart 1) in which L was $(CH_2)_n$, the activity of the compound has been reported to be independent of the length of the methylene chain for $5 < n < 12$, while a small decrease in potency has been observed in the 4- and 3-methylene homologues.[12f] Furthermore, in a series of blockers of the general structure **1** which incorporated a large number of semirigid linkers L containing one, two, or three aromatic rings, the change in the biological activity of the molecules was remarkably small, the maximum variation only being approximately 10-fold.[12g] These results are attributable to the residual conformational mobility of the two quinolinium groups and to the extensive delocalization of the positive charge within these groups.[12g] The cyclophanes of series **2** were synthesized in an attempt to reduce further the conformational mobility of the charged heterocycles.[12i] However, the structure−activity trends in series **2** were similar to those observed in the noncyclic analogues **1**, although one compound demonstrated an interesting selectivity profile for blocking the SK_{Ca} channel in different tissues.

Further rigidification in the linker A of series **2** via replacement of most of the methylene chain by groups having two aromatic rings (compounds **3e**−**g**) has not resulted in significant changes in activity. In this context, the small cyclophanes **3a**−**d** represent the first example of closely related analogues in terms of structure, which show substantially different potencies for blocking the SK_{Ca} channel. Preliminary molecular modeling studies indicate that the two quinolinium rings in **3a**−**d** may be "cis" (synperiplanar) or "trans" (antiperiplanar) to each other, or the planes of the two quinolinium rings may form a dihedral angle of approximately 90° ("orthogonal" conformation). Whereas **3a** and **3b** can adopt stable cis and trans conformations of approximately equal energies, **3c** and **3d** adopt orthogonal and trans conformations, also of similar energies. Clearly, these findings do not account for the differences between the potencies of these compounds as channel blockers.

Conclusion. The synthesis and pharmacological testing of novel bis-quinolinium cyclophanes as blockers of the SK_{Ca} channel has been described. Compound **3b** is the most potent nonpeptidic blocker reported to date, showing activity in the low nanomolar range and similar to that of apamin. Furthermore, the results with the present series add significantly to the structure−activity knowledge in the field, since they incorporate the first example of molecules in which the activity depends critically on the nature of the linkers joining the two quinolinium groups.

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