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Bis-quinolinium cyclophanes: toward a pharmacophore model for the blockade of apamin-sensitive SK_{Ca} channels in sympathetic neurons

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Abstract—The synthesis, pharmacological evaluation, and molecular modeling studies of unsymmetrical bis-alkylene bis-quinolinium cyclophanes and xylylene–alkylene bis-quinolinium cyclophanes is described. Two important structural features of the pharmacophore for SK_{Ca} channel blockade have been identified. These are (i) an optimum distance of ca. 5.8 Å between the centroids of the pyridinium rings of the two quinolinium groups and (ii) a preference for conformations having the quinolinium groups in a synperiplanar orientation.

Small conductance Ca^{2+}-activated K^+ (SK_{Ca}) channels comprise an important subclass of K^+ channels.[1,2] Functional, pharmacological, and structural data have suggested the existence of subtypes of the SK_{Ca} channel.[3–6] In accordance with these observations, three SK_{Ca} subunits have been identified by DNA cloning, namely SK1, SK2, and SK3.[7,8] Though apamin, a peptidic toxin from bee venom, potently and selectively blocks SK_{Ca} channels and has been invaluable in their study,[9–11] there is considerable interest in the discovery of nonpeptidic blockers of the SK_{Ca} channel. Such compounds, in addition to being useful pharmacological tools, may have important therapeutic applications, as the SK_{Ca} channel is implicated in a number of pathophysiological conditions.[12–21]

Dequalinium (Chart 1) has been shown to be a relatively potent and selective SK_{Ca} channel blocker.[22,23] Restriction of the conformational flexibility of the quinolinium groups through their incorporation into a cyclophane

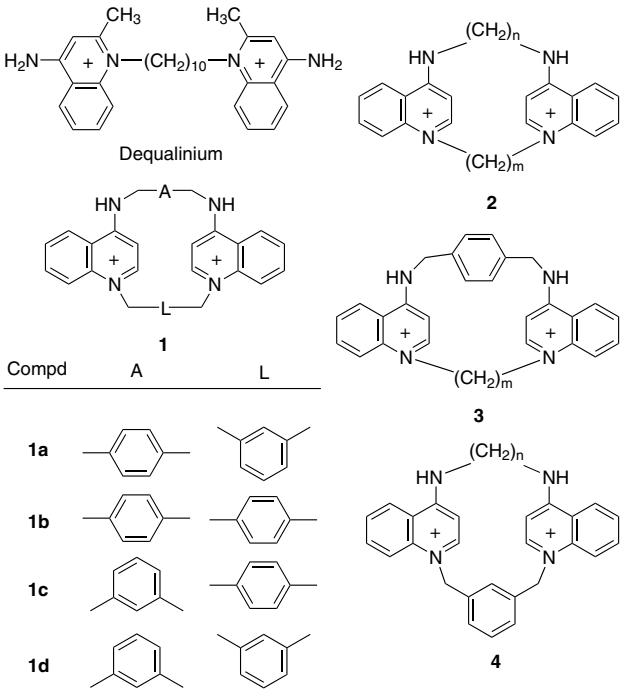

Chart 1. General structures for the cyclophanes considered in this study.

Keywords: Quinolinium; Cyclophane; Pharmacophore; SK3 channel; Apamin; Conformation; Synperiplanar; Centroid.
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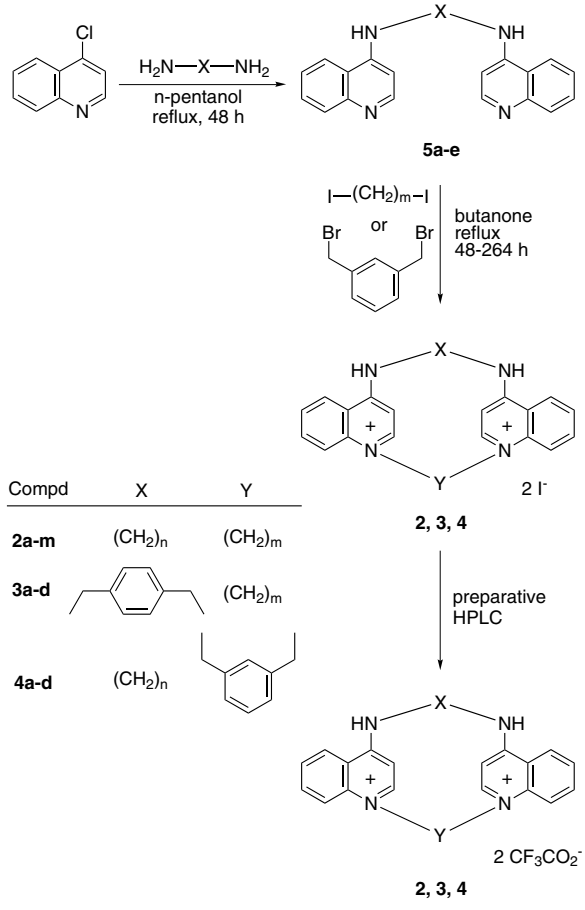
structure has yielded potent bis-xylyl cyclophane blockers **1**[24,25] (Chart 1). Replacement of the bis-xylyl linkers by bis-alkylene groups has afforded symmetric cyclophanes **2** ($n = m$), which are nanomolar blockers of the SK_{Ca} channel found in rat superior cervical ganglion neurons.[26] This channel is most probably a homomer composed of SK3 subunits.[27]

In the present study, we have achieved a 'fine tuning' of the separation of the quinolinium groups via the synthesis of unsymmetrical bis-alkylene cyclophanes **2** ($n \neq m$). Furthermore, using **1a** (Chart 1), the most potent of the bis-xylyl blockers as a lead, we have synthesized mixed xylyl-alkylene cyclophanes **3** and **4** and have varied the length of the alkylene chain.

The cyclophanes of the general structures **2**, **3**, and **4** were synthesized according to Scheme 1. The diquinolines **5a**–**e** were obtained via treatment of 4-chloroquinoline with the necessary α,ω-diaminoalkane or *p*-xylylenediamine in pentanol. The conversion of the diquinolines **5a**–**e** to the desired cyclophanes **2**, **3**, and **4** was carried out under concentrations ranging from 3.7 to 19 mM. In all cases the synthesis of the cyclophanes involved reaction of the respective diquinoline **5** with either an α,ω-diiodoalkane or 1,3-bis-(bromomethyl)benzene in butanone for a prolonged period of time. The final compounds were purified by preparative HPLC[25] (purity >99.2%) and analyzed as ditrifluoroacetate salts.



Scheme 1.

The SK_{Ca} channel blocking action of the compounds was assessed from their ability to inhibit the afterhyperpolarization (AHP) in cultured rat sympathetic neurons as described previously.[23,27] Briefly, individual cells were impaled with an intracellular microelectrode, which was used both to elicit and to record action potentials. During a successful run, the impaled cell was exposed to several concentrations of one or more of the compounds under test and also of dequalinium, which was used as a reference agent. All the compounds were made up as 1–10 mM stock solutions in dimethyl sulfoxide (DMSO) and were thereafter diluted first in water and finally in the physiological bathing fluid. This had the composition (mM): NaCl, 118; KCl, 4.8; $CaCl_2$, 2.5; $MgSO_4$, 1.19; $NaHCO_3$, 25; KH_2PO_4, 1.18, and glucose 11. It was warmed to 30–31 °C and equilibrated with 95% O_2, 5% CO_2. The final concentration of DMSO was always <0.1%. The new compounds were examined in batches of up to 4 and each was tested at 3–4 concentrations on at least 3 cells. Dequalinium was also applied at 3–4 different concentrations. The Hill equation was fitted to the results to obtain an estimate of the IC_{50}. However, because there was some variation in the apparent potency of dequalinium during the course of the study, equieffective molar concentration ratios (EMR relative to dequalinium) were also determined by simultaneous nonlinear least squares fitting of the data obtained with each compound, taken together with the values observed with dequalinium in that set of assays. As before, the Hill equation was used to fit the data: a common Hill coefficient was assumed. The EMR values are also listed in Table 1 and it is these values that have been used for the comparison between compounds.

All cyclophanes presented in Table 1 blocked the putative SK3 channel at submicromolar concentrations, some of them acting in the low nanomolar range. The activity of the bis-alkylene cyclophanes **2a**–**m** increased dramatically to a peak as the length of the linkers was reduced from $n = m = 10$ to $n = m = 5$ (**2f**) and then dropped with further shortening of the chains to $n = m = 3$. The novel unsymmetrical bis-alkylene cyclophanes follow the activity pattern observed in this homologous series. Furthermore, replacement of only the *m*-xylyl linker L of **1a** by alkylene groups to give **3a**–**d** resulted in an overall reduction in the blocking potency. Varying the alkylene linker in **3a**–**d** from 3 to 6 methylene groups had only a 3-fold effect on potency. Substitution of only the *p*-xylyl linker A of **1a** for alkylene groups to provide **4a**–**d** resulted in retention or a slight loss of activity, the maximum variation within the subseries **4a**–**d** being again only 3-fold. Interestingly, the blocking potency peaks for $n = 4$ (**4c**) in this subseries. On the whole, the replacement of the *p*-xylyl group A by aliphatic linkers is better tolerated than the replacement of the *m*-xylyl group L.

We have previously suggested that the groups joining the two quinolinium rings in the cyclophane molecule do not interact with the channel directly but, rather, pro-

Table 1. Structures and pharmacological results for the cyclophanes

Compd	n[a]	m[a]	$IC_{50} \pm SD$ (nM)	EMR[b] ± SD	Centroids[c]
Deq.	—	—	900 ± 100	1	
1a[d]	—	—	3 ± 1	0.010 ± 0.001	6.48
1b[d]	—	—	28 ± 3	0.050 ± 0.010	7.13
1c[d]	—	—	70 ± 40	0.180 ± 0.040	6.7
1d[d]	—	—	130 ± 10	0.220 ± 0.040	6.15
2a[e]	10	10	260 ± 5	0.440 ± 0.170	11.39
2b[e]	8	8	190 ± 20	0.430 ± 0.170	8.81
2c[e]	6	6	60 ± 6	0.100 ± 0.030	6.75
2d	5	6	16 ± 4	0.025 ± 0.015	6.2
2e	6	5	12 ± 2	0.017 ± 0.009	6.36
2f[e]	5	5	2 ± 0.5	0.003 ± 0.001	5.78
2g	4	5	9 ± 4	0.010 ± 0.005	5.61
2h	5	4	15 ± 3	0.020 ± 0.010	5.12
2i	3	5	30 ± 6	0.072 ± 0.016	5.09
2j[e]	4	4	40 ± 5	0.090 ± 0.030	4.99
2k	3	4	130 ± 60	0.270 ± 0.070	4.29
2l[e]	3	3	630 ± 20	0.350 ± 0.100	3.84
2m[e]	3	3	380 ± 10	0.770 ± 0.250	3.74
3a	—	6	65 ± 9	0.079 ± 0.028	6.92
3b	—	5	60 ± 3	0.068 ± 0.019	6.28
3c	—	4	150 ± 20	0.170 ± 0.070	5.91
3d	—	3	150 ± 60	0.220 ± 0.150	4.95
4a	6	—	35 ± 7	0.045 ± 0.016	6.59
4b	5	—	31 ± 1	0.033 ± 0.012	5.68
4c	4	—	10 ± 1	0.015 ± 0.005	5.33
4d	3	—	25 ± 3	0.031 ± 0.012	5.11

[a] See Chart 1 for generic structures.
[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] Distance (in Å) between the centroids of the two pyridinium rings.
[d] Data from Ref. 25.
[e] Data from Ref. 26.

vide a scaffold for the appropriate spatial arrangement of the quinolinium moieties for effective interaction with the channel.[25,26] The impact of the variation of the linkers on the distance of the two quinolinium groups in cyclophanes **1**–**4** was examined using molecular modeling techniques. Thus, the global minimum energy conformer for each molecule was identified through an extensive conformational search[33] and the distance between the centroids of the two pyridinium rings of the quinolinium groups was recorded and is presented in Table 1.

A plot of the centroid distances versus $-\log(\mathrm{EMR} * 10^{-6})$ is presented in Figure 1. The blocking potency of the bis-alkylene cyclophanes **2** increases steeply to a maximum as the distance of the quinolinium groups increases from 3.7 to 5.8 Å and then drops as the distance increases further to 11.4 Å. Thus, is appears that the optimum quinolinium distance for maximum potency is ca. 5.8 Å (as in **2f**) in this series of cyclophanes. This is not the case, however, for the bis-xylyl cyclophanes **1**. Although **1a** and **1b** fit this model well, an unexpected drop in potency is observed in the cases of **1c** and **1d**. The quinolinium distance (6.15 Å) in the latter compound approaches closely the optimum distance of 5.8 Å but its potency is approximately 2 orders of magnitude lower than predicted from the model. In fact, **1d** is the least potent cyclophane within series **1**. Furthermore, in series **3**, cyclophanes **3a**, **3b**,

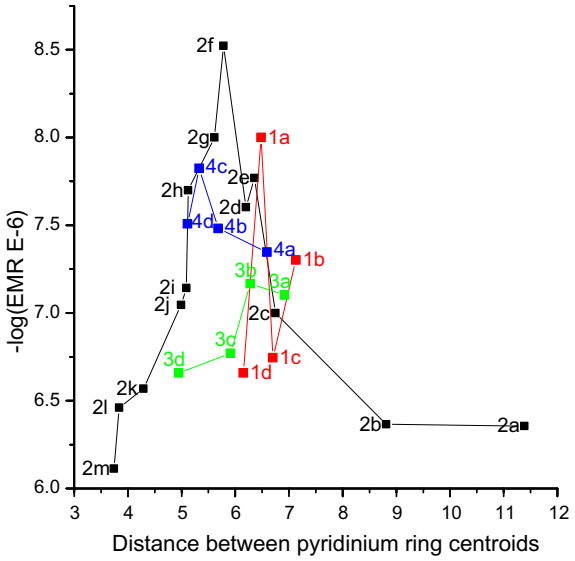


Figure 1. Plot of $-\log(\mathrm{EMR} * 10^{-6})$ versus the distance between the pyridinium ring centroids in Å, for the cyclophanes of Table 1. The EMR values of Table 1 have been multiplied by 10^{-6} to normalize the scale. The four cyclophane series are color-coded.

and **3d** fit the model reasonably well (Fig. 1), but the potency does not reach a maximum in the case of **3c** (centroid distance = 5.9 Å) as would be predicted. A similar anomalous case is observed in series **4** with

Table 2. Conformers and energies for the cyclophanes

Compd[a]	Conformation[b]	E (6-31G*)[c]	ΔE[d]
2f_(8)	S	−814,173.51	0.00
2f_(23)	S	−814,172.89	0.62
2f_(305)	S	−814,171.48	2.03
2f_(14)	S	−814,171.04	2.47
2f_(82)	A	−814,170.90	2.61
4b_(21)	S	−884,732.02	0.00
4b_(217)	A	−884,731.17	0.85
4b_(103)	A	−884,731.06	0.96
4b_(193)	S	−884,730.55	1.47
4b_(1)	S	−884,730.55	1.47
4b_(197)	S	−884,730.26	1.76
4b_(49)	A	−884,729.98	2.04
4b_(3)	S	−884,729.78	2.24
4b_(117)	A	−884,729.44	2.58
4b_(5)	S	−884,729.43	2.59
4b_(53)	A	−884,729.22	2.80
3c_(9)	A	−860,232.33	0.00
3c_(15)	S	−860,230.25	2.08
3c_(35)	S	−860,230.16	2.17
3c_(3)	A	−860,229.95	2.38
3c_(39)	S	−860,229.72	2.61

[a] The conformer number is shown in parentheses.
[b] S, synperiplanar; A, antiperiplanar.
[c] Energy of the conformer (in kcal/mol) calculated at the 6-31G* level.
[d] $E_{\text{conformer}} - E_{\text{global minimum conformer}}$. Only conformers with $\Delta E < 3$ kcal/mol are presented.

molecule **4b** (centroid distance = 5.7 Å), despite the fact that **4a**, **4c**, and **4d** fit the model extremely well.

The existence of the above outliers (**4b**, **3c**, **1d**, and **1c**) strongly suggests that the distance between the quinolinium groups is not the sole parameter that determines the blocking potency of the molecule. We have previously demonstrated that, for the cyclophanes of series **1**, the quinolinium groups in the molecule may adopt synperiplanar or antiperiplanar conformations.[25] The most potent cyclophane **1a** shows an energetic preference for the synperiplanar conformation, while the least potent cyclophanes **1c** and **1d** show increasing preference for the antiperiplanar conformation.[25] An extensive conformational analysis was performed for cyclophanes **2f** (which has the same maximal potency) in comparison with the anomalously active compounds **3c** and **4b**[33] and results are presented in Table 2. Cyclophane **2f** prefers almost exclusively synperiplanar conformations, **4b** shows preference for the synperiplanar conformation but adopts also antiperiplanar conformations of relatively low energies, while **3c** exists primarily in antiperiplanar conformations. These results, along with the previously published results for series **1**,[25] suggest that the conformation of the molecule is an important structural feature that, along with the quinolinium distance, determine the potency of the blockers.

In conclusion, the molecular modeling analysis of the cyclophanes of series **1**–**4** has identified two important features of the pharmacophore of the bis-quinolinium cyclophanes as blockers of the putative SK3 channel. These are the distance between the centroids of the pyridinium rings of the quinolinium groups (the optimum being ca. 5.8 Å) and the conformation of the molecule, higher activity being associated with a preference for synperiplanar arrangement of the quinolinium groups.

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References and notes

1. Haylett, D. G.; Jenkinson, D. H. In *Potassium Channels*; Cook, N. S., Ed.; Ellis Horwood Limited: Chichester, 1990; pp 70–95.
2. Castle, N. A. *Perspect. Drug Discov. Des.* **1999**, *15/16*, 131.
3. Wadsworth, J. D.; Doorty, K. B.; Strong, P. N. *J. Biol. Chem.* **1994**, *269*, 18053.
4. Wadsworth, J. D.; Torelli, S.; Doorty, K. B.; Strong, P. N. *Arch. Biochem. Biophys.* **1997**, *346*, 151.
5. Wadsworth, J. D.; Doorty, K. B.; Ganellin, C. R.; Strong, P. N. *Biochemistry* **1996**, *35*, 7917.
6. Dunn, P. M.; Benton, D. C. H.; Campos Rosa, J.; Ganellin, C. R.; Jenkinson, D. H. *Br. J. Pharmacol.* **1996**, *117*, 35.
7. Kohler, M.; Hirschberg, B.; Bond, C. T.; Kinzie, J. M.; Marrion, N. V.; Maylie, J.; Adelman, J. P. *Science* **1996**, *273*, 1709.
8. Chandy, K. G.; Fantino, E.; Wittekindt, O.; Kalman, K.; Tong, L. L.; Ho, T. H.; Gutman, G. A.; Crocq, M. A.; Ganguli, R.; Nimgaonkar, V.; Morris-Rosendahl, D. J.; Gargus, J. J. *Mol. Psychiatry* **1998**, *3*, 32.
9. Banks, B. E. C.; Brown, C.; Burgess, G. M.; Burnstock, G.; Claret, M.; Cocks, T. M.; Jenkinson, D. H. *Nature* **1979**, *282*, 415.
10. Burgess, G. M.; Claret, M.; Jenkinson, D. H. *J. Physiol. (Lond.)* **1981**, *317*, 67.
11. Lazdunski, M. *Cell Calcium* **1983**, *4*, 421.
12. Gater, P. R.; Haylett, D. G.; Jenkinson, D. H. *Br. J. Pharmacol.* **1985**, *86*, 861.
13. Murphy, M. E.; Brayden, J. E. *J. Physiol. (Lond.)* **1995**, *489*, 723.
14. Renaud, J. F.; Desnuelle, C.; Schmid-Antomarchi, H.; Hugues, M.; Serratrice, G.; Lazdunski, M. *Nature* **1986**, *319*, 678.
15. Behrens, M. I.; Vergara, C. *Am. J. Physiol.* **1992**, *263*, C794.
16. Behrens, M. I.; Jalil, P.; Serani, A.; Vergara, F.; Alvarez, O. *Muscle Nerve* **1994**, *17*, 1264.
17. Bevan, M. D.; Wilson, C. J. *J. Neurosci.* **1999**, *19*, 7617.
18. Messier, C.; Mourre, C.; Bontempi, B.; Sif, J.; Lazdunski, M.; Destrade, C. *Brain Res.* **1991**, *551*, 322.
19. Belcadi-Abbassi, W.; Destrade, C. *Neuroreport* **1995**, *6*, 1293.
20. Benington, J. H.; Woudenberg, M. C.; Heller, H. C. *Brain Res.* **1995**, *692*, 86.
21. Yamamoto, H. A.; Harris, R. A. *Eur. J. Pharmacol.* **1983**, *88*, 357.
22. Castle, N. A.; Haylett, D. G.; Morgan, J. M.; Jenkinson, D. H. *Eur. J. Pharmacol.* **1993**, *236*, 201.
23. Dunn, P. M. *Eur. J. Pharmacol.* **1994**, *252*, 189.
24. Campos Rosa, J.; Galanakis, D.; Ganellin, C. R.; Dunn, P. M.; Jenkinson, D. H. *J. Med. Chem.* **1998**, *41*, 2.

25. Campos Rosa, J.; Galanakis, D.; Piergentili, A.; Bhandari, K.; Ganellin, C. R.; Dunn, P. M.; Jenkinson, D. H. *J. Med. Chem.* **2000**, *43*, 420.
26. Chen, J. Q.; Galanakis, D.; Ganellin, C. R.; Dunn, P. M.; Jenkinson, D. H. *J. Med. Chem.* **2000**, *43*, 3478.
27. Hosseini, R.; Benton, D. C. H.; Dunn, P. M.; Jenkinson, D. H.; Moss, G. W. J. *J. Physiol. (Lond.)* **2001**, *535*, 323.
28. Vinter, J. G.; Davis, A.; Saunders, M. R. *J. Comput. Aided Mol. Des.* **1987**, *1*, 31.
29. Morley, S. D.; Abraham, R. J.; Haworth, I. S.; Jackson, D. E.; Saunders, M. R.; Vinter, J. G. *J. Comput. Aided Mol. Des.* **1991**, *5*, 475.
30. Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902.
31. Granovsky, A. A. http://classic.chem.msu.su/gran/gamess/index.html.
32. Schmidt, M. W.; Baldridge, K. K.; Boatz, J. A.; Elbert, S. T.; Gordon, M. S.; Jensen, J. J.; Koseki, S.; Matsunaga, N.; Nguyen, K. A.; Su, S.; Windus, T. L.; Dupuis, M.; Montgomery, J. A. *J. Comput. Chem.* **1993**, *14*, 1347.
33. The conformational search for each molecule was performed using the conformational search module of the XED/COSMIC[28,29] molecular modeling software running on a Silicon Graphics O^2 workstation, using a dielectric constant of 8 to attenuate the influence of the electrostatics. All unique conformers were fully minimized using the AM1[30] Hamiltonian as implemented in the PC GAMESS version[31] of the GAMESS (US) quantum mechanics package.[32] Duplicate conformers were discarded and all unique conformers were subjected to 6-31G* single point calculations using PC GAMESS to evaluate the energy of the conformer at the ab initio level.