Synthesis, Molecular Modeling, and K$^+$ Channel-Blocking Activity of Dequalinium Analogues Having Semirigid Linkers[⊥]

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Dequalinium [1,1′-(decane-1,10-diyl)bis(2-methyl-4-aminoquinolinium)] is an effective blocker of the small conductance Ca^{2+}-activated K$^+$ channel. It has been shown that the number of methylene groups in the alkyl chain linking the two quinolinium rings of this type of molecule is not critical for activity. To further investigate the role of the linker, analogues of dequalinium have been synthesized, in which the alkyl chain has been replaced by CH$_2$XCH$_2$ where X is a rigid or semirigid group containing aromatic rings. The compounds have been tested for blockade of the slow after-hyperpolarization on rat sympathetic neurons. The most potent compounds have X = phenanthryl, fluorenyl, *cis*-stilbene, and C$_6$H$_4$(CH$_2$)$_n$C$_6$H$_4$, where *n* = 0−4. The conformational preferences of the compounds were investigated using the XED/COSMIC molecular modeling system. Although there is some dependence of the potency of the analogue on the conformational properties of the linker (X), overall, X groups having substantial structural differences are tolerated. It seems that X provides a support for the two quinolinium groups and does not interact with the channel directly. The intramolecular separation between the quinolinium rings, which is provided by rigid groups X, is not critical for activity; this may be attributed to the residual conformational mobility of the heterocycles and to the extensive delocalization of the positive charge. These two factors may permit favorable contacts between the quinolinium groups and the channel over a range of intramolecular separations.

Introduction

Compounds that modulate K$^+$ channels have attracted considerable attention in recent years.[1,2] K$^+$ channels form the most diverse family of ion channels,[3] and potent and selective modulators are available only for a few subtypes. The availability of modulators for the less well-studied subtypes will enable the better pharmacological and physiological characterization of these K$^+$ channels and may lead to novel therapeutic approaches for disease states.[1,2] Small conductance Ca^{2+}-activated K$^+$ (SK$_{Ca}$) channels are activated by an increase in the intracellular concentration of Ca^{2+} and have a unitary conductance of 6−14 pS.[4,5] They are found in many tissues, but in general their physiological role is not fully understood, although it has been identified in some cases.[6−11] It has been suggested that these channels have a physiological role in the central nervous system, since an endogenous ligand with SK$_{Ca}$ channel-blocking activity has been isolated from pig brain.[12] Such an endogenous modulator has also been characterized in pheochromocytoma cells.[13] Furthermore, SK$_{Ca}$ channels have been suggested to play a role in myotonic muscular dystrophy.[14,15] There is evidence that SK$_{Ca}$ blockade in this disease can lead to a reduction in basal muscle electrical activity and suppression of myotonic discharges.[16]

The most potent SK$_{Ca}$ channel blockers known are the natural peptidic toxins apamin,[17−19] leiurotoxin I,[20] and PO5.[21] Due to the limited availability of the three peptides, research has been initiated toward the discovery of small, synthetic SK$_{Ca}$ channel blockers. A starting point was the discovery that simple bis-charged molecules such as tubocurarine are active.[22−24] Dequalinium (**1a**, Chart 1) was then identified as the most potent and selective available nonpeptidic bis-quaternary blocker of the SK$_{Ca}$ channel.[25,26] The stereoelectronic requirements for optimal SK$_{Ca}$ channel blockade have since been probed in various series of dequalinium analogues.[27−31] Thus, it has been found that the quinolinium groups can be replaced by other charged heterocycles to provide effective blockers.[28] The substituents of the quinolinium group in molecules of the general structure **1b** and **2a** have been suggested to modulate the blocking activity of the compound by altering the energy of the LUMO.[30,31] Moreover, it has been established that, surprisingly, the number of methylene groups in the alkylene chain of dequalinium is not critical for activity.[32] Thus, the potency of the compound is not significantly altered when reducing the number of C atoms in the chain from 12 to 5, though a small decrease is observed on further descending to 4 and 3 methylene groups. The remarkably low sensitivity of potency to the length of the linker has led to the suggestion that the molecule may adopt a folded conformation at the binding site.[32] In the present study, the flexible alkyl chain of dequalinium has been replaced with rigid or semirigid groups to further probe the role of the linker. The choice of the type of linking group in the present analogues was based upon the previously established structure−activity relationships of series **1b** and **2a** (Chart 1). Thus, optimal activity had been obtained with *N*-benzyl substituents as in **1c** and **2b**. Hence, it seemed reasonable to replace the

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Chart 1

1a

2a

1b

2b

1c

3

Scheme 1

4 - 19

alkylene chain of dequalinium with benzylic groups to give compounds of the general structure **3** (Chart 1). In these analogues, X can be a mono-, bi-, or tricyclic aromatic moiety. The use of aromatic groups as more rigid substitutes of the alkane chain of dequalinium has the additional advantage of maintaining a high lipophilic character in the linker. The 2-Me group of dequalinium was omitted in this series, since it is known to contribute little to potency while causing problems in the synthesis by hindering the ring N atom and making it difficult for the latter to undergo alkylation.[30]

Chemistry

Compounds **4**−**19** (Table 1) were prepared via reaction of 4-aminoquinoline[33] with the appropriate dibromide as detailed in Scheme 1. The synthesis of all dibromides has been reported in the literature (see the Experimental Section for each individual one). However, different routes from the published ones were used for the preparation of **6c**, **10c**, **11c**, **17c** (Scheme 3), and **12f** (Scheme 4). Thus, **6c**, **10c**, **11c**, and **17c** were conveniently synthesized via $LiAlH_4$ reduction of the corresponding methyl esters of the diacids to give the respective diols, which were brominated using PBr_3. The methyl ester **6a** was prepared via photocyclization of the *trans*-(*E*)-stilbene diester **11a** (Scheme 2). The synthesis of **12f** (Scheme 4) involved bromination of **12c** to the *meso* dibromide **12d**, double dehydrobromination to the diphenylacetylene **12e**, and free radical bromination of this to give the desired compound. The purification of the final compounds was achieved through recrystallization except in the case of **5** and **7**, where reverse phase preparative HPLC had to be used.

An interesting isomerization was observed in the case of the *cis*-(*Z*)-stilbene **10**. A solution of this compound
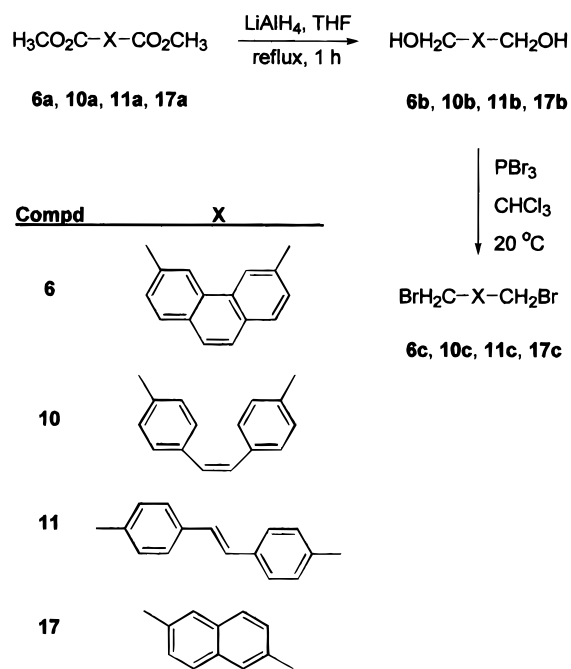
Scheme 2

11a

hv, I_2
PhH
5 days
48%

6a
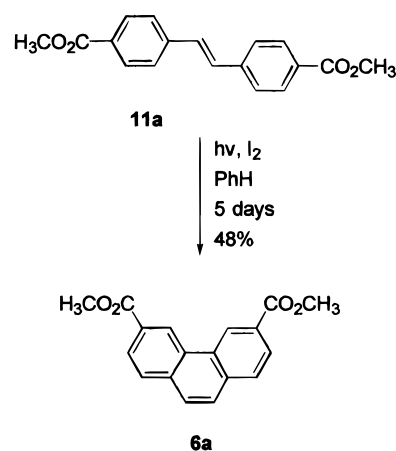
in DMSO-d_6 equilibrated on standing for 25 days to a mixture of the cis- (**10**) to trans- (**11**) isomers in the ratio of 1:3, respectively (data not shown). To avoid any complications arising from this slow isomerization, fresh solutions of the compounds were used in the biological testing.

Biological Testing

The SK_{Ca}-blocking action of the compounds was assessed from their ability to inhibit the after-hyperpolarization (AHP) in cultured rat sympathetic neurons[26] (see the Experimental Section). Briefly, each compound was tested at two to four concentrations on at least three cells. Between one and four 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}. 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. It should be noted that the compounds were applied in a continuously

Scheme 3

extension of the linker is similarly reflected in the 6-fold potency difference between the *cis-* and *trans*-stilbenes **10** and **11**. Finally, reducing the separation between the quinolinium groups by attaching them to *p-* or *m*-xylene (**18**, **19**) markedly reduces potency. Thus, although considerable variation of X is tolerated, there appears to be an optimum between the limits represented by X = benzene-1,4-diyl (**18**) and 1,1':4',1''-terphenyl-4,4''-diyl (**16**).

We previously reported that for dequalinium homologues there is little dependence of the SK_{Ca} channel-blocking potency on the length of the aliphatic chain (above $(CH_2)_4$) connecting the two quinolinium groups.[32] This observation, however, does not of itself provide a convincing answer to the question of whether the alkyl chain binds to the channel. Any contribution to binding from the hydrocarbon linker would be expected to be hydrophobic in nature. The putative binding of the flexible chain to the channel would also be associated with an entropy loss resulting from the restriction of the rotatable bonds of the chain. Thus, if the energy gain from the hydrophobic binding were approximately equal to the entropy loss due to conformational restriction, then little change in the free energy of binding would be expected on varying the chain length. Such a compensation between favorable and unfavorable components of the free energy of binding has been observed in carbonic anhydrase inhibitors bearing chains of variable length.[34,35] The chains in these molecules consisted of glycine units which appeared to make only hydrophobic contacts with the enzyme, and there was little dependence of the dissociation constants of the inhibitors on the number of glycine units.

If the linker X of the present series of analogues were binding to the channel, then it would be expected that restriction of rotatable bonds within X, without significant drop in the lipophilicity or change in the conformational preferences of the group, would result in less entropy loss upon binding and, hence, higher potency. However, on comparing various pairs of compounds, e.g., **4**−**6**, **5**−**7**, and **6**−**10**, it is clear that the freezing of degrees of freedom of X has no effect on potency. This constitutes strong evidence that the linking chain of dequalinium analogues does not bind directly to the channel, and therefore, any change in the potency of the compound as a result of alteration in X should be attributed to modulation of other molecular properties such as the conformation.

flowing solution to isolated cells, so that differences in depletion as a consequence of variation in lipophilicity are unlikely to have been a complicating factor.

Results and Discussion

Structures and biological results for the compounds are given in Table 1. It can be seen that the alkyl chain of dequalinium can be replaced effectively with various aromatic groups, with a modest increase in activity in about one-half of the examples. The variation in the structure of the linker is considerable, yet the maximum difference in potency observed in the series is only approximately 1 order of magnitude (cf. compounds **10** and **18**), and this has important implications for the role of the linker. Certain features do stand out, however.

The meta-disubstituted biphenyl **4** is similar in potency to rigid analogues of **4** such as the phenanthrene **6** and the *cis*-stilbene **10**. Separation of the two benzene rings of the para-disubstituted biphenyl **5** by a poly(methylene) chain, $(CH_2)_n$, where $n = 1-4$ (**8**, **9**, **13**, **15**), has very little influence on potency, but rigidification to maintain the chain in an extended form (**11**, **12**, **16**) substantially reduces potency. The effect of

Scheme 4

Table 1. Structures, Biological Results, and Distances between the Benzylic C Atoms for the Compounds

$$H_2N \cdots \overset{+}{N}-CH_2-X-CH_2-\overset{+}{N} \cdots -NH_2$$

Compd	X	$D_1{}^a$ (Å)	$D_4{}^b$ (Å)	$IC_{50} \pm SD$ (μM)	$EMR^c \pm SD$	r^d
Deq.		11.34	11.38	0.65 ± 0.04	1	89
4[e]		7.61	7.11	0.29 ± 0.07	0.55 ± 0.3	6
5		10.14	10.17	0.38 ± 0.04	0.40 ± 0.10	3
6		7.46	7.31	0.20 ± 0.007	0.33 ± 0.14	4
7		9.89	9.89	0.31 ± 0.04	0.33 ± 0.10	5
8		9.95	9.85	0.45 ± 0.15	0.39 ± 0.12	4
9		12.19	6.15	0.41 ± 0.12	0.71 ± 0.19	4
10		8.38	8.05	0.19 ± 0.03	0.24 ± 0.08	6
11		12.37	12.44	0.94 ± 0.05	1.46 ± 0.66	5
12		12.68	12.70	1.00 ± 0.20	1.41 ± 0.69	5
13		12.27	11.91	0.20 ± 0.015	0.31 ± 0.13	4
14		11.80	11.88	0.36 ± 0.02	0.59 ± 0.34	4
15		14.52	14.60	0.37 ± 0.09	0.44 ± 0.18	3
16		14.39	14.43	1.9 ± 0.36	2.3 ± 1.0	5
17		8.07	8.08	0.49 ± 0.09	0.83 ± 0.22	5
18		5.87	5.87	2.8 ± 0.25	3.7 ± 1.4	4
19		5.07	5.05	0.8 ± 0.07	1.32 ± 0.33	5

[a] The interatomic distance between the two benzylic C atoms connected to the quinolinium ring N atom, in the lowest energy conformation, using a dielectric constant of 1.0. [b] As D_1 but using a dielectric constant of 4.0. [c] 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. [d] Number of neurons tested. [e] Compound **4** can also formally be represented in an anti-conformation, but this is of higher energy (≅2 kcal/mol); the distances D_1 and D_4 are 8.54 and 8.53 Å, respectively; in either case, the benzene rings are not coplanar but form a dihedral angle of 50.5−53.6°.

To gain insight into the conformational behavior of compounds **4**−**19**, molecular modeling studies were performed using the XEDMININ routine within the XED/COSMIC package.[36,37] Partial atomic charges were obtained from a semiempirical AM1[38] calculation. Initially, a conformational search was performed for
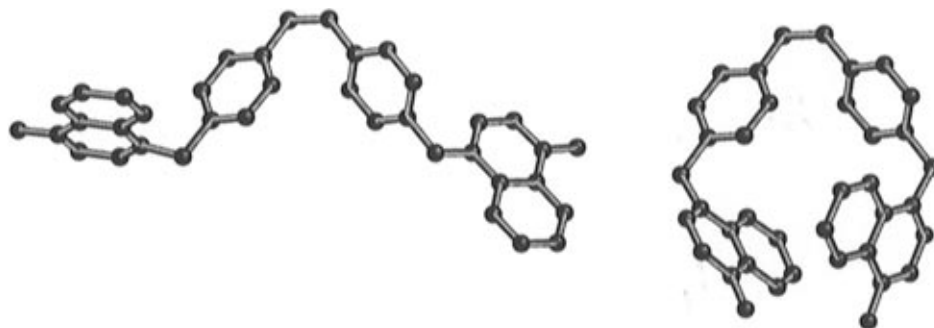
Figure 1. Two of the low-energy conformations of the *cis*-stilbene derivative **10**. Hydrogen atoms are omitted for clarity. The conformers were obtained as detailed in the Experimental Section, using a dielectric constant of 4.0.

each of the compounds of Table 1 using a dielectric constant of 1.0. Since the molecules possess two positively charged quinolinium groups, their conformational preferences may depend upon the dielectric constant used, and under vacuum conditions, the repulsions between the quinolinium groups may be overestimated. Since nothing is known with certainty about the biological milieu in which these compounds act, the choice of a biologically relevant dielectric constant is problematic. Nevertheless, the conformational search was repeated for each of the compounds using the higher dielectric constant of 4.0, to investigate whether the behavior of the molecule would be altered. The value of 4 is commonly used in modeling studies to simulate a protein environment.[39]

The results of the modeling studies demonstrate that the position in space of the quinolinium group, with respect to the aromatic ring to which it is attached through the benzylic C atom, is not well defined. This is due to the presence of two single bonds between each of the quinolinium ring N atoms and the aryl ring which give rise to several low-energy conformations. The quinolinium group, being always connected to a benzylic C atom, adopts similar conformations for all the analogues and so the analysis has to focus on differences in the conformational behavior of the linker X in order to explain changes in blocking activity.

In compounds with relatively flexible linkers, such as **9**, **13**, and **15**, the conformation of X is dependent upon the dielectric constant used. As the dielectric is increased, the repulsions between the charged quinolinum groups are attenuated and the molecule tends to adopt partially folded conformations more easily. This is exemplified by **9**. The lowest energy vacuum conformation of X in this molecule has the ethylene bridge in a trans-arrangement, giving a distance between the two benzylic C atoms of 12.19 Å (Table 1). However, increasing the dielectric to 4.0 causes the ethylene group to adopt a gauche conformation which reduces the distance by one-half. This conformation is stabilized through π-stacking between the two phenyl rings as well as between the two quinolinium groups. The lowest energy conformations of **13** and **15** have the propylene and butylene chains, respectively, in the trans-arrangement, both when using a dielectric of 1.0 or 4.0. Nevertheless, low-energy partially folded conformers do exist in the latter case, and it would be expected that, in an aqueous environment, folding would be promoted even more by hydrophobic effects. Thus, the ability of **9**, **13**, and **15** to fold may enable them to simulate the conformation of **10**, and this may explain their potency.

These results are in accord with the previously suggested "folded" pharmacophore for the dequalinium analogues.[32] Further support for this argument is provided by the drop in activity observed when the ability of X to fold is removed (cf. compounds **10**−**11**, **10**−**12**, and **15**−**16**). In rigid analogues in which the benzylic C atoms occupy well-defined positions in space (**5**−**8**, **10**) distances between these atoms ranging from ≈7 to ≈10 Å have little effect on potency. This tolerance may be explained by the relative mobility of the quinolinium rings. Figure 1 shows two representative low-energy conformations of the *cis*-stilbene **10**, one with the quinolinium groups in an "extended" arrangement and the other with these groups in a "folded" conformation. Although the distance between the benzylic C atoms in both conformers is essentially the same, the distance between the exocyclic N atoms varies from 19 Å in the extended conformer to 4.5 Å in the folded one.

It has been established that the quinoline rings have to be charged for potent blockade of the SK_{Ca} channel in dequalinium analogues.[31] In addition, it has been shown that the charge is extensively delocalized in the quinolinium group resulting in a large, ring-shaped positive electrostatic field around this group.[28] Hence, the mobility of the quinolinium rings and the large positive field around them may permit favorable interactions with the channel in compounds with significantly different benzylic C−C distances. This assumes binding to an anionic site, but a similar argument can be developed for binding to an aromatic site.[28]

Conclusion

The alkyl chain of dequalinium can be replaced effectively with rigid or semirigid linkers containing aryl rings to give analogues with slightly increased potency in many cases. The linker does not seem to interact directly with the channel, but rather, its role seems to be that of a "scaffold" for the two quinolinium rings. Although the conformational properties of the linker are of some importance, the dependence of the blocking activity of the analogue on the distance between the "anchoring" benzylic C atoms is not critical. This may be due to a combination of two factors: the conformational mobility of the quinolinium groups and the extensive delocalization of the positive charge, which gives rise to a large positive field around these groups.

Experimental Section

(a) Chemistry. Melting points (mp) were obtained either on an Electrothermal melting point apparatus or on a Kofler apparatus equipped with a microscope RCH and are uncor-

rected. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian XL-200 (200 MHz) or VXR-400 (400 MHz) spectrometer. The ^{1}H NMR spectra of all compounds were in accord with the structures. Mass spectra were run on a ZAB SE or VG 7070H spectrometer, using FAB in a matrix of *m*-nitrobenzyl alcohol or thioglycerol (5, 7–10). All compounds showed mass numbers corresponding to [M − H]$^+$ except 6 [M]$^+$. Analytical high-performance liquid chromatography (HPLC) was performed on a Shimadzu HPLC apparatus with a UV detector and a Kromasil C18 5 μm column. Solvent mixtures of A = water + 0.1% TFA and B = MeOH + 0.1% TFA were used at a flow rate of 1 mL/min. The ratio A:B was 1:1 except for compounds 6 (45:55), 15 (40:60), and 17 (55:45), and in every case the absorption was >97% of the total, using a detector set at λ 215 nm (4, 5, 13, 16), 254 nm (6, 10–12, 15, 17–19), 300 nm (7–9), or 315 nm (14). For preparative HPLC a Gilson apparatus was used with a UV detector and a Kromasil C18 5 μm column. The solvent mixtures were as above, and the flow rate was 20 mL/min. All compounds were dried at 60 °C and 0.1 mmHg for 15 h, but many held on tenaciously to solvent molecules, especially water which appears to be a solvate. 3,3′-Bis(bromomethyl)biphenyl,[40] 4,4′-bis(bromomethyl)biphenyl,[41,42] 2,7-bis(bromomethyl)fluorene,[43] bis[*p*-(bromomethyl)diphenyl]methane,[44] bis-*p*-(bromomethyl)-bibenzyl,[44] 1,3-bis[4-(bromomethyl)phenyl]propane,[44] 2,6-bis-[4-(bromomethyl)phenyl]pyridine,[45] 4-bis[4-(bromomethyl)-phenyl]butane,[44] and 4,4″-bis(bromomethyl)-1,1′:4′,1″-terphenyl[46] were synthesized according to literature procedures, and α,α′-dibromo-*p*-xylene and α,α′-dibromo-*m*-xylene were obtained from Aldrich.

General Experimental Procedure for the Preparation of Compounds 1,1′-[Biphenyl-3,3′-diylbis(methylene)]-bis(4-aminoquinolinium) Dibromide Hydrate (4), 1,1′-[Biphenyl-4,4′-diylbis(methylene)]bis(4-aminoquinolinium) Ditrifluoroacetate (5), 1,1′-(Phenanthrene-3,6--diylbis(methylene)]bis(4-aminoquinolinium) Dibromide Hydrate Ethanoate (6), 1,1′-[Fluorene-2,7-diylbis(methylene)]bis(4-aminoquinolinium) Ditrifluoroacetate (7), 1,1′-[Methylenebis(benzene-1,4-diylmethylene)]bis(4-aminoquinolinium) Dibromide Hydrate (8), 1,1′-[Ethylenebis-(benzene-1,4-diylmethylene)]bis(4-aminoquinolinium) Dibromide Hydrate (9), (Z)-1,1′-[Stilbene-4,4′-diylbis(methylene)]bis(4-aminoquinolinium) Dibromide Sesquihydrate (10), (E)-1,1′-[Stilbene-4,4′-diylbis(methylene)]bis-(4-aminoquinolinium) Dibromide Dihydrate (11), 1,1′-[Ethyne-1,2-diylbis(benzene-1,4-diylmethylene)]bis(4-aminoquinolinium) Dibromide Sesquihydrate (12), 1,1′-[Propane-1,3-diylbis(benzene-1,4-diylmethylene)]bis(4-aminoquinolinium) Dibromide Hemihydrate Ethanoate (13), 1,1′-[Pyridine-2,6-diylbis(benzene-1,4-diylmethylene)]bis(4-aminoquinolinium) Dibromide Hydrate (14), 1,1′-[Butane-1,4-diylbis(benzene-1,4-diylmethylene)]bis-(4-aminoquinolinium) Dibromide Hydrate (15), 1,1′-[1,1′:4′,1″-Terphenyl-4,4″-diylbis(methylene)]bis(4-aminoquinolinium) Dibromide Trihydrate (16), 1,1′-[Naphthalene-2,6-diyl(bis(methylene)]bis(4-aminoquinolinium) Dibromide Hydrate (17), 1,1′-[Benzene-1,4-diylbis(methylene)]bis(4-aminoquinolinium) Dibromide Dihydrate (18), and 1,1′-[Benzene-1,3-diylbis(methylene)]bis(4-aminoquinolinium) Dibromide Hemihydrate (19). 4-Aminoquinoline[33] (1.58 mmol), the corresponding bis(bromomethyl) compound BrCH$_2$XCH$_2$Br (0.79 mmol), and MEK (25 mL) were heated under reflux for 72 h. After filtration and washing thoroughly with MEK and CHCl$_3$, the solid product was purified by crystallization from EtOH or EtOH/MeOH. In the case of 5 and 7, the compounds were purified by reverse phase preparative HPLC and obtained as poly(trifluoroacetate)s. Yields, melting points, and analytical data are given in Table 2.

Dimethyl 3,6-Phenanthrenedicarboxylate (6a). A solution of dimethyl 4,4′-stilbene-(E)-dicarboxylate (11a; 1.95 g, 6.58 mmol) in benzene (700 mL) containing I$_2$ (0.362 mmol) was irradiated for 5 days using a medium-pressure mercury lamp (450 W, Hanovia) in a quartz well, while O$_2$ was slowly bubbled through the reaction mixture. After removal of the solvent *in vacuo*, the residue was taken up in CHCl$_3$. Addition

Table 2. Yields, Melting Points, and Analytical Data for Compounds 4−19[a]

compd	yield (%)	mp (°C)	mol formula[b]
4	64	289−291	C$_{32}$H$_{28}$N$_4$$^{2+}$·2Br$^-$·H$_2$O
5	38	235−237	C$_{32}$H$_{28}$N$_4$$^{2+}$·2CF$_3CO_2$$^-$·3CF$_3CO_2$H
6	52	266−268	C$_{34}$H$_{28}$N$_4$$^{2+}$·2Br$^-$·H$_2$O·C$_2H_5$OH
7	31	227−228 dec	C$_{33}$H$_{30}$N$_4$$^{2+}$·2CF$_3CO_2$$^-$·2.3CF$_3CO_2$H
8	69	248−250	C$_{33}$H$_{30}$N$_4$$^{2+}$·2Br$^-$·H$_2$O
9	77	207−209	C$_{34}$H$_{32}$N$_4$$^{2+}$·2Br$^-$·H$_2$O
10	61	218−220	C$_{34}$H$_{30}$N$_4$$^{2+}$·2Br$^-$·1.5H$_2$O
11	84	264−266	C$_{34}$H$_{30}$N$_4$$^{2+}$·2Br$^-$·2H$_2$O
12	77	299−301	C$_{34}$H$_{28}$N$_4$$^{2+}$·2Br$^-$·1.5H$_2$O
13	46	209−212	C$_{35}$H$_{34}$N$_4$$^{2+}$·2Br$^-$·C$_2H_5$OH·0.5H$_2$O
14	63	293−295 dec	C$_{37}$H$_{31}$N$_5$$^{2+}$·2Br$^-$·H$_2$O
15	67	228−230	C$_{36}$H$_{36}$N$_4$$^{2+}$·2Br$^-$·H$_2$O
16	80	243−245	C$_{38}$H$_{32}$N$_4$$^{2+}$·2Br$^-$·3H$_2$O
17	62	240−242	C$_{30}$H$_{26}$N$_4$$^{2+}$·2Br$^-$·H$_2$O[c]
18	82	319−321	C$_{26}$H$_{24}$N$_4$$^{2+}$·2Br$^-$·2H$_2$O
19	43	321−322	C$_{26}$H$_{24}$N$_4$$^{2+}$·2Br$^-$·0.5H$_2$O

[a] Compounds were recrystallized from EtOH except for 4 and 14 (MeOH/EtOH) and 5 and 7 which were purified by reverse phase preparative HPLC as detailed in the Experimental Section and obtained as poly(trifluoroacetates). [b] All compounds had CHN analyses within ±0.4% of the values calculated for the indicated molecular formulae. [c] N: calcd, 9.03; found, 8.48.

of MeOH precipitated a white solid (0.96 g) which, on recrystallization (CH$_2$Cl$_2$/MeOH mixture), gave 0.93 g (48%) of white prisms: mp 208−210 °C. Anal. (C$_{18}$H$_{14}$O$_4$) C, H.

General Procedure for the Synthesis of Compounds 6b, 10b, 11b, and 17b. A solution (suspension in the case of 11a) of the corresponding methyl diester in dry THF was added dropwise with stirring to a solution of LiAlH$_4$ (2.8 mol excess) in dry THF held at room temperature under a N$_2$ atmosphere. When the addition was complete, the mixture was boiled under reflux for 1 h and then decomposed with saturated aqueous Na$_2$SO$_4$ (a white precipitate formed). The products were obtained after filtration, drying of the organic layer, and concentration.

3,6-Bis(hydroxymethyl)phenanthrene (6b): mp 157−158 °C (CHCl$_3$) (lit.[47] mp 159−160 °C).

General Procedure for the Synthesis of Compounds 6c, 10c, 11c, and 17c. To a suspension of 6b, 10b, 11b, or 17b (2.06−2.87 mmol) in CHCl$_3$ (20 mL) was added PBr$_3$ (2.96 mmol) dropwise with stirring. After the end of the addition, the suspension was stirred for 15 h. The CHCl$_3$ layer was washed with water (20 mL), dried (Na$_2$SO$_4$), and concentrated to give the products.

3,6-Bis(bromomethyl)phenanthrene (6c): mp 173−175 °C (CH$_2$Cl$_2$/hexane) (lit.[47] mp 173−175 °C).

(Z)-4,4′-Bis(hydroxymethyl)stilbene (10b): mp 110−113 °C; lit.[48] mp 110−113 °C).

(Z)-4,4′-Bis(bromomethyl)stilbene (10c): mp 71−73 °C (lit.[48] mp 69−69.5 °C).

(E)-4,4′-Bis(hydroxymethyl)stilbene (11b): mp 266−267 °C (lit.[49] mp 268−270 °C).

(E)-4,4′-Bis(bromomethyl)stilbene (11c): mp 191−193 °C (lit.[50] mp 192−193 °C).

(E)-4,4′-Dimethylstilbene (12c): mp 179−180 °C (CCl$_4$) (lit.[51] mp 175−177 °C).

Meso-1,2-dibromo-1,2-bis(4-methylphenyl)ethane (12d). A suspension of 12c (2 g, 9.6 mmol) and pyridinium hydrobromide perbromide[52] (4 g, 12.5 mmol) in glacial AcOH (40 mL) was stirred at room temperature. After 1.5 h the reagent was consumed and the precipitate of colorless, crystalline product was collected, washed with MeOH, and dried (3.6 g, 88.2%): mp 208−210 °C. Anal. (C$_{16}$H$_{16}$Br$_2$) C, H.

1,2-Bis(4-methylphenyl)ethyne (12e): mp 138−140 °C (EtOH) (lit.[50] mp 136 °C).

1,2-Bis[4-(bromomethyl)phenyl]ethyne (12f): mp 190−192 °C (CH$_2$Cl$_2$/hexane) (lit.[49] mp 187−190 °C).

2,6-Bis(hydroxymethyl)naphthalene (17b): mp 172−174 °C (lit.[53] mp 170−170.5 °C).

2,6-Bis(bromomethyl)naphthalene (17c): mp 178−179 °C (lit.[54] mp 182−184 °C).

(b) Molecular Modeling. The structures of all compounds were first built and minimized (with no charges on them) using the XED module in the XED/COSMIC[36,37] system running on a Silicon Graphics Indigo/2 workstation. The minimized structures were saved and the ".dat" files were converted to Sybyl 6.03[55] ".mol" structure files. The MOPAC package was then used interfaced with Sybyl to perform a point semiempirical MO calculation on the molecules using the AM1 Hamiltonian[38] and Mulliken population analysis. The Mulliken charges were loaded and the molecules saved as ".mol" files. These were converted back to ".dat" COSMIC files which were read into XED and minimized. The minimized structures were then subjected to conformational searching using the XEDMININ routine. Typically, the rotational increment of single bonds was between 30° and 90° depending on the number of such bonds in the structure, and 200 randomizations of the molecule were performed. The dielectric constant was set to either 1.0 or 4.0, and structures within 10 kcal/mol (upper search limit) of the global minimum were stored. The structures from this initial search were subsequently fully minimized using the COSMIC force field. Visualization and manipulation of the resultant conformers were achieved using the XEDA routine.

(c) Pharmacology. Stock solutions (2 mM) of all compounds were prepared in MeOH. Superior cervical ganglia from 17-day-old rats were treated with collagenase and trypsin and then dissociated using a fire-polished pipet. The resultant cell suspension was plated onto laminin-coated plastic dishes and maintained in tissue culture for 3–10 days. Culture dishes were placed on the stage of an inverted microscope and perfused with physiological salt solution of the following composition (in 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; they were then warmed to 30 °C and equilibrated with 95% O_2:5% CO_2. Intracellular recordings were made with conventional microelectrodes filled with 1 M KCl (resistance 80–120 MΩ), connected to the headstage of a Neurolog NL 102 amplifier. Action potentials were evoked by injection of 30 ms depolarizing current pulses every 5 s. Data acquisition and analysis were performed on a microcomputer using the pClamp suite of programs (Axon Instruments). Inhibition of the AHP was measured by digitally subtracting records obtained in the presence of blocker from controls and expressing the peak difference as a percentage of the control AHP at the same time point.

Supporting Information Available: ¹H NMR and mass spectral data for all compounds (6 pages). Ordering information is given on any current masthead page.

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