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SYNTHESIS AND QSAR OF DEQUALINIUM ANALOGUES AS K^+ CHANNEL BLOCKERS. INVESTIGATIONS ON THE ROLE OF THE 4-AMINO GROUP.

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Abstract: Dequalinium (**1**) is a potent and selective non-peptidic blocker of the SK_{Ca} channel. The contribution of the 4-amino group to activity was investigated by replacing it by other groups R^4. The size or lipophilicity of R^4 was found to be unimportant and a good correlation was obtained between σ_R for R^4 and the blocking potency of the analogues, suggesting that the role of the NH_2 group is electronic.

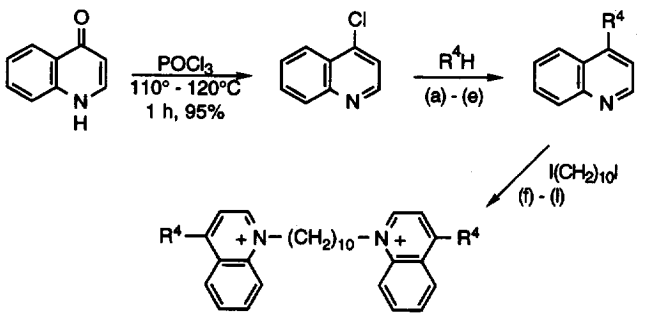
Introduction. In recent years there has been an increasing interest in compounds that modulate K^+ channels as potential therapeutic agents.[1,2] With at least 20 subtypes described to date,[3] most of them need pharmacological exploration. One of the least well studied subtypes is that of small conductance Ca^{2+} - activated K^+ (SK_{Ca}) channels.[4,5] SK_{Ca} channels have been implicated in myotonic muscular dystrophy.[6,7] Dequalinium (**1**), a bis-quinolinium compound that has been used for many years as an antiseptic,[8] has recently been shown to be a potent and selective non - peptidic blocker of the SK_{Ca} channel.[9,10] This compound therefore constitutes a useful lead for the development of more potent blockers to assist investigations of the physiological and pathophysiological role of the SK_{Ca} channels. We have therefore initiated studies towards identifying the pharmacophore of dequalinium for SK_{Ca} channel blockade.[11,12]

Synthesis. Two structural features of dequalinium that merit examination are the 2-methyl and 4-amino groups. Hence analogues have been synthesised in which these have been removed or replaced by other groups to probe their potential contribution to blocking activity. The compounds for this study (**Table 1**) were synthesised via **Scheme 1.** Substituent R^4 was introduced via a nucleophilic displacement of the chlorine atom of 4-chloroquinoline and the resulting intermediates were reacted with 1,10-diiododecane either in MEK or in 4-methylpentan-2-ol to yield the final products **2** - **8**.

Biological testing. The SK_{Ca} blocking action of the compounds was assessed from their ability to inhibit the after - hyperpolarisation (AHP) in cultured rat sympathetic neurones as described previously.[10] Each compound was tested at 2 to 4 concentrations on at least three cells and dequalinium was also tested on the same cells 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 obtained by simultaneous non linear least squares fitting of the data with the Hill equation. 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.[13]

Discussion of results. It is clear that the contribution of the 2-methyl group of dequalinium is very small since its removal does not result in much loss of potency (compound **2**). On the other hand, the 4-amino group seems to be important as the desamino analogue[12] **8** is an order of magnitude less potent than **2**. The NH_2 group is an excellent H-bond donor particularly since it participates in the delocalisation of the ring charge and, hence, carries a fractional positive charge which makes the hydrogens more acidic. However, it clearly does not act as an H-bond donor since its replacement by the NMe_2 group (compound **3**) does not result in loss of potency. On the other hand, it is unlikely that it acts as a H-bond acceptor, as its lone pair of electrons is involved in the delocalisation of the ring charge and is not readily available for H-bonding.

Scheme 1[a]



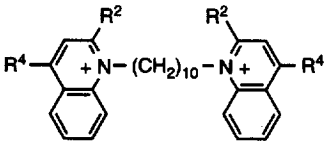
[a]Methods: (a) $R^4 = NH_2$, dry NH_3 (gas)/PhOH, 180°C, 4 h, 99%; (b) $R^4 = NMe_2$, $Me_2NH{\cdot}HCl$, 170°C, 30 min, 76%; (c) $R^4 = NHPh$, $PhNH_2$/glacial AcOH, reflux, 3 h, 86%; (d) $R^4 = OPh$, PhOH/KOH, 125° - 130°C, 1 h, 100%; (e) $R^4 = NHCOCH_3$, 4-aminoquinoline made under (a) was acetylated using Ac_2O/pyridine, 80°C, 24 h, 100%; (f) **2**, 4-methylpentan-2-ol, reflux, 2.5 h, 59%; (g) **3**, 4-methylpentan-2-ol, reflux, 22 h, 17%; (h) **4**, methyl ethyl ketone (MEK), reflux, 96 h, 82%; (i) **5**, 4-methylpentan-2-ol, reflux, 30 h, 71%; (j) **6**, MEK, reflux, 96 h, 16%; (k) **7**, MEK, reflux, 96 h, 25%; (l) **8**, see ref. 12.

The importance of the delocalisation of the charge was examined by replacing the NH_2 group by other groups having different electronic properties (**Table 1**). It should be noted that all the substituents used have an overall electron releasing effect or are neutral. This was because quinolines with electron withdrawing substituents at position 4 are very weak nucleophiles and we were unable to alkylate the ring nitrogen in the final quaternization step (**Scheme 1**). The 2-methyl was also removed to facilitate the synthesis. From **Table 1** it is evident that there is no correlation between the size or lipophilicity (as expressed by the sum of the hydrophobic fragmental constants[14] Σf) of R^4 with EMR. However, it is also clear that the more electron releasing is R^4 the more potent is the compound. This trend can be quantified using the Hammett constant for "para" substitution[15] σ_P and the correlation equation that results is **1**:

$$pEMR = -1.16\ (\pm 0.38)\ \sigma_P - 1.05\ (\pm 0.17) \qquad \mathbf{1}$$
$$n = 7,\ r = -0.80,\ s = 0.317$$

where n is the number of compounds, r is the correlation coefficient and s is the standard deviation.

Table 1. Structure, biological results and parameter values for the compounds.



Compd	R^4	R^2	Σf^1	$\sigma_P{}^2$	$\sigma_I{}^3$	$\sigma_R{}^4$	pEMR[5]	EMR[6]±SD	$IC_{50} \pm SD$[7]	n[8]
1	NH_2	CH_3	—	—	—	—	0	1	0.74 ± 0.05	18
2	NH_2	H	-0.842	-0.66	0.17	-0.80	-0.11	1.3 ± 0.5	1.4 ± 0.3	4
3	NMe_2	H	0.473	-0.83	0.17	-0.88	-0.15	1.4 ± 0.6	1.4 ± 0.3	3
4	NHPh	H	0.893	-0.40	0.30	-0.86	-0.53	3.4 ± 1.8	2.4 ± 0.5	8
5	$NHCOCH_3$	H	-0.589	0.00	0.28	-0.35	-0.74	5.5 ± 1.0	4.5 ± 0.3	3
6	OPh	H	1.401	-0.03	0.40	-0.48	-0.81	6.5 ± 1.9	2.7 ± 1.6	5
7	CH_3	H	0.701	-0.17	-0.01	-0.16	-1.41	26 ± 14	15 ± 3.8	3
8	H	H	0.182	0.00	0.00	0.00	-1.18	15 ± 7.9	21 ± 5.0	4

[1] Σf: sum of hydrophobic fragmental constants for substituent R^4 (ref. 14). [2] σ_P: Hammett constant for "para" substitution (ref. 15). [3] σ_I: Electronic parameter for inductive effects (ref. 16). [4] σ_R: Electronic parameter for resonance effects (ref. 16). [5] pEMR: -log(EMR). [6] EMR: equi - effective molar ratio: the ratio of the concentration of the test compound and of dequalinium that cause 50% inhibition of the AHP, as determined in the same experiment. [7] In μM. [8] Number of cells tested.

Equation 1 is only a moderate correlation at first glance. It should be noted, however, that σ_P is a descriptor of the *overall* electronic effect of R^4. When this is separated into its inductive and resonance components as represented by σ_I[16] and σ_R[16] respectively interesting correlations result:

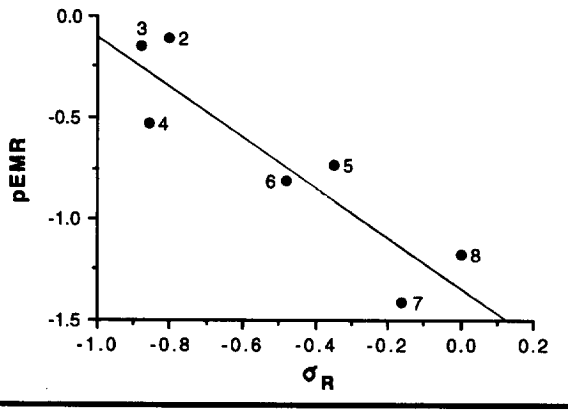
$$\text{pEMR} = 1.43\ (\pm 1.27)\ \sigma_I - 0.97\ (\pm 0.30) \qquad 2$$
$$n = 7,\ r = 0.45,\ s = 0.477$$

$$\text{pEMR} = -1.25\ (\pm 0.26)\ \sigma_R - 1.33\ (\pm 0.16) \qquad 3$$
$$n = 7,\ r = -0.90,\ s = 0.228$$

The blocking potency of the compounds does not correlate with the inductive effect of R^4 but correlates well with its resonance effect. This is consistent with conventional chemical concepts. R^4 is "para" to the positively charged ring nitrogen and is in direct conjugation with it. Therefore, it is likely that the inductive effect of R^4 only operates to a small extent from such a distance, while its resonance effect, being less dependent on distance, would become the dominant factor. The greater resonance effect of R^4 would obviously cause better delocalisation of the positive charge. A plot of pEMR vs σ_R is shown in **Figure 1**.

In conclusion, the 2-methyl group of dequalinium is not important for SK_{Ca} channel blocking activity whereas the 4-amino group makes a substantial contribution. The latter acts neither as a H-bond donor nor as a H-bond acceptor but its replacement by other groups and QSAR on the resultant compounds suggests that its role is electronic via delocalisation of the positive charge of the quinolinium ring.

Figure 1. Plot of pEMR vs σ_R for the compounds of **Table 1** showing the least squares fitted regression line



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