Synthesis and Structure−Activity Relationships of Cetiedil Analogues as Blockers of the Ca^{2+}-Activated K^+ Permeability of Erythrocytes†

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Cetiedil, [2-cyclohexyl-2-(3-thienyl)ethanoic acid 2-(hexahydro-1*H*-azepin-1-yl)ethyl ester], which blocks the intermediate calcium-activated potassium ion permeability (IK_{Ca}) in red blood cells, was used as a lead for investigating structure−activity relationships with the aim of determining the pharmacophore and of synthesizing agents of greater potency. A series of compounds having structures related to cetiedil was made and tested on rabbit erythrocytes. Channel blocking activity within the series was found to correlate well with octanol−water partition coefficients but not with the specific chemical structure of the acid moiety. However, whereas log *P* for the compounds spans a range of values over 4 orders of magnitude, potency only increases by 2 orders. This suggests that hydrophobic interactions with an active site on the channel are probably not the main determinants of activity. It seems more likely that increased lipophilicity enhances access to the channel, probably from within the cell membrane. In keeping with this interpretation, cetiedil methoiodide was found to be inactive. Triphenylethanoic was found to be a more effective acid grouping than 2-cyclohexyl-2-(3-thienyl)ethanoic, and its 2-(hexahydro-1*H*-azepin-l-yl)ethyl ester (**11**) was approximately 3 times more potent than cetiedil. The 9-benzylfluoren-9-yl carboxylic acid ester (**21**) was found to be approximately 9 times more active than cetiedil, and replacing $-CO_2-$ in **21** by an ethynyl (−C≡C−) linkage (compound **26**, UCL 1608) increased potency by some 15-fold over that of cetiedil.

Introduction

K^+ channels constitute a remarkably diverse family of membrane-spanning proteins that have a wide range of functions in electrically excitable and inexcitable cells. One important class opens in response to an increase in the concentration of calcium within the cytosol. This was first demonstrated in red blood cells with which it was found that treatments, such as metabolic inhibition, which caused cytosolic Ca^{2+} to rise also resulted in a large increase in K^+ permeability (the Gárdos effect[1]). The change in permeability was subsequently shown to be mediated by the opening of Ca^{2+}-activated K^+ channels (K_{Ca}) in the membrane of the red cell. Later work, based initially on pharmacological and electrophysiological evidence (for reviews see refs 2−4) and more recently based on structural evidence from cloning studies,[5] has established that there are several kinds of Ca^{2+}-activated K^+ channels. Those in the red cell belong to the intermediate conductance (IK_{Ca}) subtype, so named because its single channel conductance (10−30 pS) lies between that of the small conductance (SK_{Ca}) and high conductance (BK_{Ca}) subtypes (reviewed in refs 2 and 3). The gene (KCNN4) coding for the IK_{Ca} channel has now been cloned[6−9] and its expression in a variety of cells results in the formation of an intermediate conductance Ca^{2+}-activated K^+ channel that has been variously termed SK4, KCa4, IK1, and $IK_{Ca}1$. The same channel occurs in T lymphocytes, where it is concerned in the control of proliferation, raising the possibility that selective IK_{Ca} channel blockers could provide a novel means of immunosupression.[10]

Several compounds have been shown to block the IK_{Ca}-mediated Ca^{2+}-activated K^+ permeability in red blood cells. These include quinine and quinidine,[11,12] some carbocyanine dyes,[13] clotrimazole[14,15] (Chart 1), and some related compounds such as TRAM-34[10b,c,15b] (Chart 1), charybdotoxin,[16] dequalinium and some of its derivatives[17] (Chart 1), nifedipine,[18] nitrendipine,[19] and cetiedil [20−23] (Chart 1), which is the starting point of the present work. Cetiedil [(±)-2-cyclohexyl-2-(3-thienyl)ethanoic acid 2-(hexahydro-1*H*-azepin-1-yl)ethyl ester, **1**; Chart 1, Table 1] is one of a series of compounds with spasmolytic, local anaesthetic, and analgesic actions[24] and is of particular interest because it has been suggested,[20] though by no means proven, that the ability of cetiedil to block the Ca^{2+}-activated K^+ permeability in red blood cells may contribute to its reported efficacy in the treatment of sickle-cell disease (e.g., see ref 25). It is argued that cetiedil's ability to reduce the loss of K^+ from the cells may diminish the reduction in cell volume that can trigger the sickling of erythrocytes (see refs 26 and 27 for reviews). Cetiedil is also of interest because it blocks other K^+ channels that are involved in cell volume regulation in hepatocytes[28] and lymphocytes.[29,30] As part of a broader investigation of the medicinal chemistry of nonpeptidic K^+ channel blocking

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Chart 1

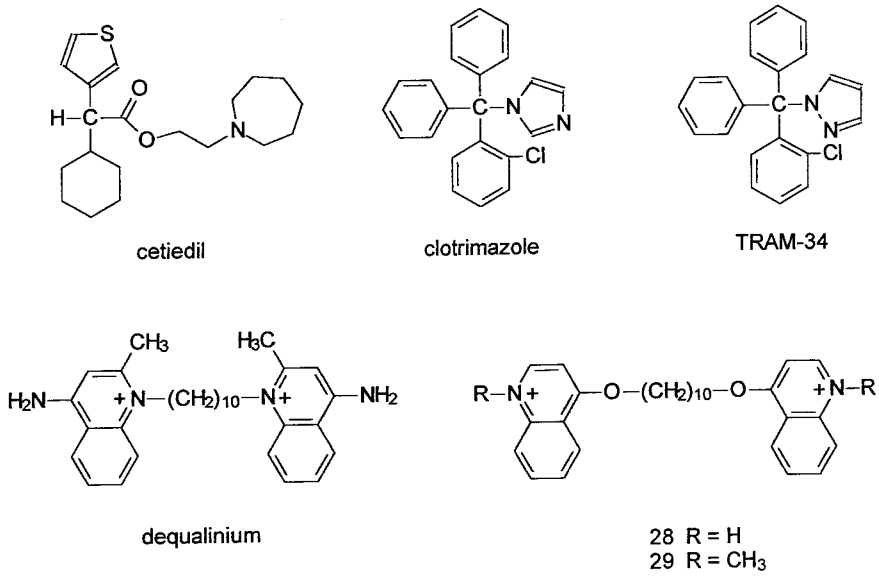
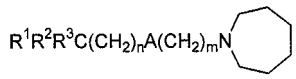


Table 1. Cetiedil Analogues: Molecular Formulas, Melting Points, and Solvent for Crystallation



	R_1	R_2	R_3	*n*	A	*m*	formula[a]	mp, °C	crystn solvent
1	C_6H_{11}	3-thienyl	H	0	COO	2	$C_{20}H_{31}NO_2S\cdot 2CF_3CO_2H$ *b*	oil[c]	HPLC
2	C_6H_{11}	H	H	0	COO	2	$C_{16}H_{29}NO_2\cdot 1.1HCl\cdot 0.5H_2O$	158−159	MeCN:EtOH (10:1)
3	H	3-thienyl	H	0	COO	2	$C_{14}H_{21}NO_2S\cdot HCl$	141.5−142.5	EtOAc:EtOH
4	C_6H_{11}	3-thienyl	OH	0	COO	2	$C_{20}H_{31}NO_3\ S\cdot HCl$ *d*	176−177[e]	EtOAc:EtOH (10:1)
5	C_6H_{11}	3-furyl	OH	0	COO	2	$C_{20}H_{21}NO_4\cdot C_2H_2O_4$ *f*	142−144	MeOH:Et_2O (1:3)
6	C_6H_{11}	C_6H_{11}	H	0	COO	2	$C_{22}H_{39}NO_2\cdot 1.1HCl\cdot 0.2H_2O$	222−224	EtOAc:EtOH (5:1)
7	C_6H_{11}	Ph	H	0	COO	2	$C_{22}H_{33}NO_2\cdot HCl$	170−172[g]	EtOAc:EtOH (10:1)
8	Ph	Ph	H	0	COO	2	$C_{22}H27NO_2\cdot HCl$	156−157[h]	EtOAc:EtOH (5:1)
9	Ph	Ph	H	1	COO	2	$C_{23}H_{29}NO_2\cdot 0.7C_2H_2O_4$	183−185	EtOAc:MeOH (5:1)
10	Ph	3-thienyl	H	0	COO	2	$C_{20}H_{25}NO_2S\cdot C_2H_2O_4$	179−180[i]	washed EtOH
11	Ph	Ph	Ph	0	COO	2	$C_{28}H_{31}NO_2\cdot HCl\cdot 0.5H_2O$ *j*	220−221	EtOAc:EtOH (10:1)
12	Ph	Ph	Ph	1	COO	2	$C_{29}H_{33}NO_2\cdot C_2H_2O_4\cdot 0.1H_2O$ *k*	204−205	ppd from EtOH
13	Ph	Ph	Ph	0	COO	3	$C_{29}H_{33}NO_2\cdot HCl\cdot 0.25H_2O$	214−215	EtOH:Et_2O (6:1)
14	C_6H_{11}	3-thieny l	H	0	CONH	2	$C_{20}H_{32}N_2OS\cdot CF_3CO_2H\cdot 1.5H_2O$ *l*	oil	HPLC
15	Ph	Ph	Ph	2	O	2	$C_{31}H_{37}NO_5\cdot 1.25C_2H_2O_4$	180−183	EtOH

[a] All compounds had C, H, N analyses within ±0.4% for the formula shown. All hydrochloride salts also had Cl analysis within ±0.4%, in accord with the formula indicated. [b] Cetiedil trifluoroacetate. [c] Citrate mp 115°, hydrochloride mp 152°. [d] UCL 1269. [e] Hydrochloride[24] mp 156°. [f] $C_2H_2O_4$ = oxalic acid. [g] Hydrochloride[43] mp 178°. [h] Hydrochloride[44] mp 142−143°. [i] Hydrochloride[45] mp 124°. [j] UCL 1274. [k] Hygroscopic. [l] 95% purity according to HPLC analysis; N analysis 0.6% low (calcd, 5.7; found, 5.1).

agents,[31−34] we have initiated a study of the structure−activity relationships and mechanism of action[23,35] of cetiedil and its congeners as inhibitors of the Ca^{2+}-activated K^+ permeability in mammalian erythrocytes. Our aims are to characterize the pharmacophore for cetiedil and to develop more potent and selective blockers of the intermediate conductance Ca^{2+}-activated K^+ channel.

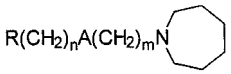
Chemistry

The final products **1**−**27** are listed together with melting points and crystallization solvents in Tables l and 2.

The esters **2**−**12** and **17**−**25** were synthesized by heating the sodium salt of the acid (generated from sodium in 2-PrOH) in 2-PrOH under reflux or in DMF (generated with NaH) with 1-(chloroalkyl)hexahydro-1*H*-azepine, as exemplified for compound **5** (Scheme 1). Esters **13** and **16** were made by treating the acid chloride with the appropriate 1-(hydroxyalkyl)hexahydro-1*H*-azepine (Scheme 2.) The requisite acids for esters **1**, **4**, and **5**, and amide **14** were prepared as outlined in Scheme 3 using the published procedures. For compound **4**, the requisite carboxylic acid, 2-cyclohexyl-2-hydroxy-2-(3-thienyl)ethanoic acid, was synthesized as previously described;[23] for **5**, the synthesis is given in the Experimental Section. For **10**, 2-phenyl-2-(3-thienyl)ethanoic acid was prepared according to the method of ref 36. For **14**, 2-cyclohexyl-2-(3-thienyl)-ethanoic acid was prepared as published.[23] Other acids were obtained commercially. The respective acids for esters **21**−**25** were made from 9-carboxyfluorene by alkylation of the disodium salt in liquid ammonia[37] using the appropriate *p*-substituted benzyl halide (Scheme 4).

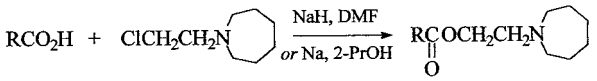
The amide **14** was prepared by coupling the acid with 1-(2-aminoethyl)hexahydro-1*H*-azepine using *n*-butyl chloroformate (Scheme 5). The ether **15** was obtained by a Williamson synthesis from triphenylpropanol and 1-(2-chloroethyl)hexahydro-1*H*-azepine using sodium

Table 2. Cetiedil Analogues: Molecular Formulas, Melting Points, and Solvent for Crystallization



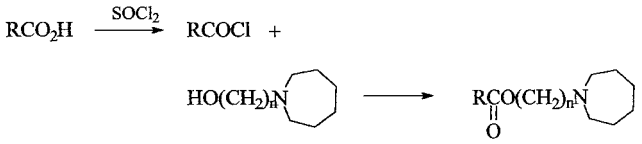
	R	*n*	A	*m*	formula[a]	mp, °C	crystn solvent
16	1-adamantyl	0	COO	2	$C_{19}H_{31}NO_2Cl$	203−205	EtOAc:EtOH (5:1)
17	1-naphthyl	0	COO	2	$C_{19}H_{23}NO_2Cl{\cdot}0.25H_2O$	199−200	EtOH
18	2-naphthyl	0	COO	2	$C_{19}H_{23}NO_2Cl{\cdot}0.25H_2O$	196−198	EtOH
19	9-anthryl	0	COO	2	$C_{23}H_{25}NO_2{\cdot}Cl{\cdot}0.5H_2O$	181−182	EtOH
20	9-fluorenyl	0	COO	2	$C_{22}H_{25}NO_2{\cdot}C_2H_2O4{\cdot}0.75H_2O$	169−170	EtOH
21	9-benzylfluoren-9-yl	0	COO	2	$C_{29}H_{31}NO_2{\cdot}C_2H_2O_4{\cdot}0.25H_2O$	160−170	EtOH
22	9-(*p*-methoxybenzyl)fluoren-9-yl	0	COO	2	$C_{30}H_{33}NO_3{\cdot}C_2H_2O_4$	130−132	EtOH
23	9-(*p*-fluorobenzyl)fluoren-9-yl	0	COO	2	$C_{29}H_{30}FNO_2{\cdot}C_2H_2O_4$	161−162	EtOH
24	9-(*p*-chlorobenzyl)fluoren-9-yl	0	COO	2	$C_{29}H_{30}ClNO_2{\cdot}C_2H_2O$	140−141	2-PrOH
25	9-(*p*-nitrobenzyl)fluoren-9-yl	0	COO	2	$C_{29}H_{30}N_2O_4{\cdot}C_2H_2O_4{\cdot}0.5H_2O$	170−172	MeOH
26	9-benzylfluoren-9-yl	1	CtC	1	$C_{30}H_{31}N{\cdot}0.8C_2H_2O_4{\cdot}0.25C_3H_7OH$	153−155	2-PrOH
27	cetiedil methoiodide				$C_{21}H_{34}NO_2SI$	144−145	EtOAc:EtOH (1:1)

Scheme 1



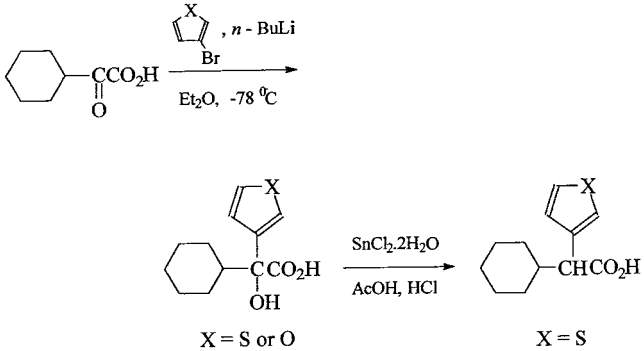
2 - 12, 17 - 25

Scheme 2



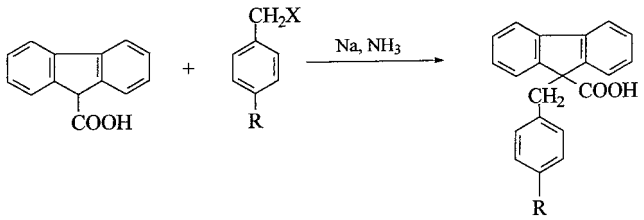
13: n = 3, **16:** n = 2

Scheme 3



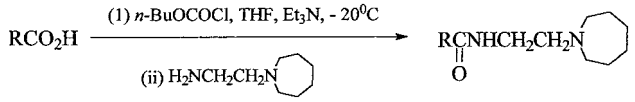
X = S or O X = S

Scheme 4



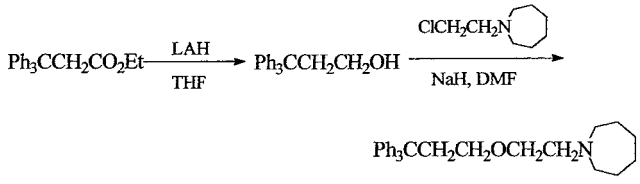
hydride in DMF (Scheme 6). The acetylene derivative **26** was synthesized by alkylation of 9-benzylfluorene anion (generated from NaH in DMSO) with 1-chloro-4-(hexahydro-1*H*-azepin-1-yl)but-2-yne (Scheme 7), which was made by the action of thionyl chloride on the corresponding carbinol. The latter was synthesized via a Mannich reaction between propargyl alcohol, formaldehyde, and hexahydroazepine catalyzed with cuprous chloride.[38] Cetiedil methoiodide (**27**) was prepared from cetiedil base using methyl iodide in ether.

Scheme 5



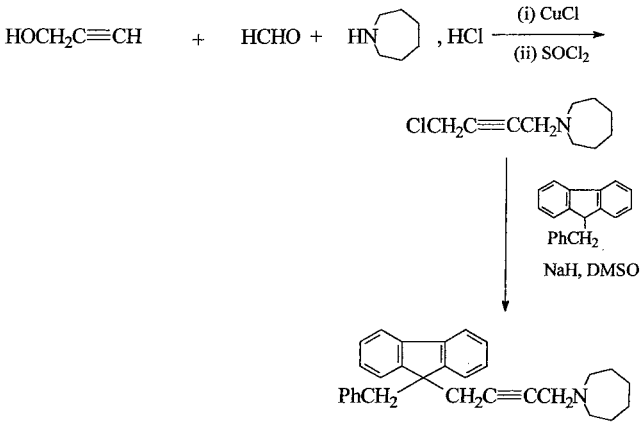
14

Scheme 6



15

Scheme 7



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Biological Testing

The potency of the compounds was assessed from their ability to inhibit the net loss of cell K^+ that follows the application of the Ca^{2+} ionophore A23187 to rabbit erythrocytes in suspension. The ionophore increases the cytosolic concentration of Ca^{2+}, with the consequences that the Ca^{2+}-activated K^+ channels in the membrane open and K^+ leaves the cells. The amount of K^+ lost was measured using a K^+-sensitive electrode placed in the suspension. This provides a convenient, if indirect, measure of changes in the number of open K^+ channels. The method has recently been used to study the action of a range of IK_{Ca} blocking agents, including cetiedil, charybdotoxin, and clotrimazole.[17,35c] As before, the

experiments have been done with a low K^+ (0.1 mM) solution because cetiedil then becomes approximately 3-fold more active.[35c] This has the additional advantage that small changes in the concentration of K^+ in the cell suspension are more readily measured using the K^+-sensitive electrode technique. The relation between the concentration of each test compound and its inhibitory action was analyzed by using the Hill equation to obtain an estimate (± the approximate standard deviation) of the concentration (IC_{50}) that causes 50% inhibition of the K^+ loss initiated by A23187. In preliminary analyses, the Hill coefficient (n_H) was allowed to vary and was consistently observed to be in the range 2−3.5, as in earlier work.[35c] A common value of n_H (usually ∼2.5) was assumed when fitting sets of assays done at the same time, and the maximum inhibition was taken to be 100%, in keeping with the observations. Fitting was done using a least-squares minimization curve fitting program (CVFIT, written by Prof. D. Colquhoun, University College London, and available at www.ucl.ac.uk/pharmacol/dc.html).

Similar results were obtained (ref 35d; unpublished observations by Babaoglu, Benton, and Haylett) when the response measured was the A23187-initiated increase in the influx of ^{86}Rb in erythrocytes rather than the net loss of K^+ as assessed using a K^+-sensitive electrode. This provides a more direct indication of IK_{Ca} activation. In the same experiments, the inhibitory action of cetiedil analogues on ^{86}Rb influx was unaffected by simultaneous blockade of Na^+K^+ ATPase and the Na^+K^+ $2Cl^-$ cotransporter.

Results and Discussion

To determine the pharmacophore of cetiedil (**1**) we have to ask how critical are the various chemical features encompassed by cetiedil's chemical structure. We noted the presence of a cyclohexane ring, a 3-thienyl group, an ester functionality, and a two-carbon chain connecting to a cyclic tertiary amine in a seven-membered aliphatic ring. The molecule is also chiral.

In our initial approach to this problem we synthesized five analogues in which the hexahydroazepinylethyl ester was maintained as a constant feature. Removing the thienyl ring (**2**) or the cyclohexyl residue (**3**) reduced potency by approximately 6−12-fold (Table 3). Replacing 3-thienyl by phenyl (**7**) did not change activity, thus establishing that the point of substitution of thiophene or even the presence of the S atom were apparently not of importance. The carbinol (**4**), an intermediate in the synthesis of cetiedil that now has three substituting groups on the acidic functionality, was about 6-fold less active. On the other hand, the ester with three phenyl substituting groups (**11**) showed a 3-fold potency increase.

Is there a pattern in these results? It seemed that the introduction of OH, a polar group that reduced activity, in contrast to the introduction of a phenyl group that increased activity, was particularly informative, and this led us to seek for a possible correlation with lipophilicity. Estimates for the octanol−water partition coefficients by the use of fragmental constants (*f*) of Rekker[39] and by the CLOG P program[40] are listed in Table 3.

The correlation equations (1 and 2) for the activities of cetiedil plus the five analogues, (i.e. **1**−**4**, **7**, **11**) according to the estimate of log *P* by using *f* values or CLOG P, respectively, are given below:

Table 3. Cetiedil Analogues: IK_{Ca} Channel Blocking Activities and Octanol−Water Partition Estimates

compd	IC_{50} ± SD,[a] μM	log *P*[b]	CLOG P[c]
1	25 ± 1	6.59	5.98
2	290 ± 30	5.18	4.93
3	142 ± 36	3.45	3.55
4	155 ± 10	4.94	5.22
5	200 ± 10	4.41	4.75
6	14 ± 1	8.33	6.86
7	18 ± 3	6.88	5.93
8	164 ± 42	5.44	5.25
9	43 ± 4	5.96	5.67
10	47 ± 4	5.15	4.90
11	8.2 ± 0.3	7.14	6.69
12	9.3 ± 1.2	7.65	7.11
13	9.2 ± 0.8	7.65	6.40
14	75 ± 3	5.15	5.11
15	5.5 ± 0.3	7.83	7.35
16	178 ± 12	5.60	4.38
17	222 ± 26	5.16	5.18
18	285 ± 16	5.16	5.18
19	28 ± 1	6.45	6.36
20	42 ± 9	5.47	5.24
21	4.0 ± 0.2	7.68	7.18
22	2.8 ± 0.3	7.75	7.09
23	4.1 ± 0.8	7.92	7.32
24	2.9 ± 0.3	8.41	7.89
25	4.6 ± 0.6	7.44	6.92
26	1.5 ± 0.1	9.10	7.90
27	≫200[d]		

[a] Concentration of the test compound that causes 50% inhibition of K^+ loss from rabbit blood cells treated with the Ca^{2+} ionophore A23187. [b] log *P* calculated for the free base using the revised hydrophobic fragmental values (*f*) of Mannhold and Rekker et al.,[39] where $CH_2 = 0.519$, $CH = 0.315$, $C = 0.110$, $C_6H_5 = 1.903$, thienyl (C_4H_3S) = 1.613, furyl (C_4H_3O) = 1.086, naphthyl = 3.191, anthryl = 4.478, fluorenyl = 4.149, adamantyl = 4.170, aliphatic $-CO_2 = -1.200$, $-N(CH_2)_6 = 1.442$, aliphatic $-CONH = -2.435$, aliphatic $-O- = -1.545$, aliphatic-OH = −1.448, aryl OMe = 0.274, aryl F = 0.444, aryl Cl = 0.933, aryl $NO_2 = -0.039$. A correction of $+2C_M = +0.438$ was applied to each of the hydrocarbon residues C_6H_{11} and $N(CH_2)_6$. No proximity effect corrections were applied. [c] ref 40. [d] Insufficiently active to allow accurate measurement

$$-\log IC_{50} = 0.26 \log P + 2.61$$
$$r = 0.86,\ n = 6,\ F = 11.0 \quad (1)$$
$$-\log IC_{50} = 0.38\ \text{CLOG P} + 2.10$$
$$r = 0.79,\ n = 6,\ F = 6.7 \quad (2)$$

These correlations suggest that activity is determined predominantly by the lipophilicity of the compound: there appears to be little (if any) structural requirement for channel interaction encoded in the carboxylic acid part of the molecule. In keeping with this conclusion, the synthesis and testing of the two chiral enantiomers of cetiedil showed them to be similarly active as erythrocyte IK_{Ca} channel blockers.[23]

Cetiedil is estimated to be a very lipophilic molecule (log *P* for the free base = 6.59 by *f* values, or 5.98 by CLOG P) and there is little prospect of obtaining more potent and clinically useful agents by merely increasing lipophilicity. Even so, it seemed possible that interesting and potentially valuable pharmacological tools might be obtained by this approach. Also, some other structural features of the molecules that might affect potency were thought worth investigating. Thus, 3-furyl was introduced in place of 3-thienyl (**4**), giving compound **5**, which

was slightly less active than **4**, indicating that there was no advantage in increasing the hydrogen-bond acceptor property of the heterocyclic ring. Incorporating two cyclohexyl groups (**6**) (log $P = 8.33$ by f values) increased the potency about 2-fold relative to cetiedil, in keeping with the higher lipophilicity. Homologous esters were also investigated: the ester (**9**) of diphenylpropionic acid was 3−4 times more potent than the lower homologue **8,** but this accords with its higher lipophilicity. The homologous esters **12** and **13** were not significantly more potent than **11**. Thus the distances between the aryl groups and the amine do not appear to be critical. Changing the polarity of the ester also does not seem to be of importance for activity; the more polar amide (**14**) isostere of cetiedil (**1**) was approximately 3 times less active, but it is also less lipophilic. The less polar ether (**15**) isostere of **12** was slightly more active. The influence of bulk was explored with the adamantyl ester **16**; it was less active than cetiedil and less lipophilic.

Some fused-ring structures were also examined to explore the possibility that a larger aromatic area might be advantageous for activity. Thus, 1-naphthyl (**17**) and 2-naphthyl (**18**) were less active than the thienyl (**3**) carboxylic ester, implying that extension of the ring area, which might have improved binding by stacking interactions, was not effective. Fusing a third ring, however, as in 9-anthryl (**19**) and 9-fluorenyl (**20)** carboxylic esters, did increase potency; they can be considered as ring-fused analogues of the diphenyl analogue **8** and are some 5 times more potent than **8**. For **19**, the increased potency accords with the increase in lipophilicity (extra CH_2 group), but **20** is of interest since it has similar lipophilicity to **8**, suggesting that there may be something special about fluorenyl. This ring system was therefore explored further. The 9-benzyl analogue (**21**) was approximately 10 times more potent than **20**; however, when this fluorenyl compound is compared with the triphenyl analogue **11**, the improvement is seen to be smaller. Nevertheless, fluorene has an important advantage for synthesis, and the benzyl group was used to probe for possible electronic contributions to binding interaction with the channel. Substituents (MeO, F, Cl, NO_2) were therefore introduced into the para position of the benzyl group of the 9-benzyl-9-fluorenyl ester. There was only a small range of potencies ($IC_{50} = 2.8-4.6$ μM), offering little indication of an electronic contribution to binding. Since there was a strong possibility that the ester grouping might be subject to hydrolytic cleavage in vivo, the ester group of **21** was also replaced by the electron rich, but hydrolytically more stable, ethynyl group to give the aminohydrocarbon **26** (UCL 1608), which was actually the most active of this series of compounds ($IC_{50} = 1.5$ μM), being approximately 15 times more potent than cetiedil. The activities of all of these compounds, however, appear to correlate reasonably well with lipophilicity so that no specific structural features stand out. Considering all 26 compounds (**1**−**26**) gives correlation eqs 3 and 4 (relating to log P values and CLOG P, respectively):

$$-\log IC_{50} = 0.455 \log P + 1.638 \quad (3)$$

$$r = 0.91,\ n = 26,\ F = 121$$

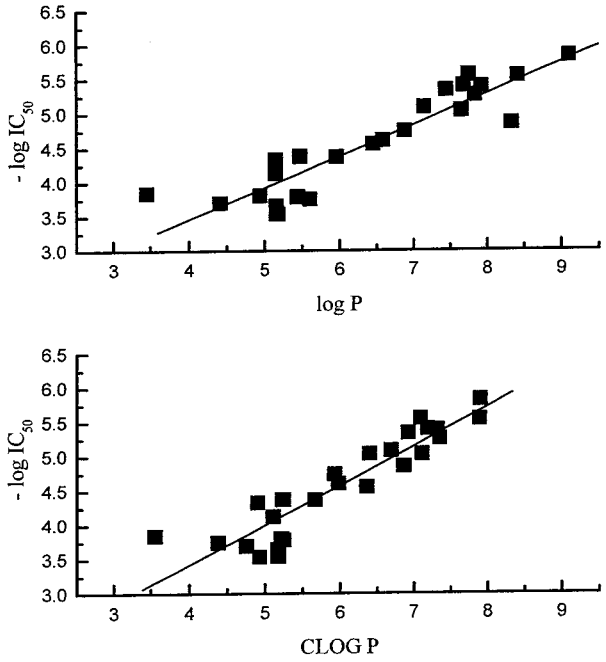

Figure 1. The relationship between the log IC_{50} (M) values (Table 3) for compounds **1**−**26** and the calculated values of log P (upper) and CLOG P (lower). The lines have been drawn according to eqs 3 and 4, respectively.

$$-\log IC_{50} = 0.574 \text{ CLOG P} + 1.128 \quad (4)$$

$$r = 0.92,\ n = 26,\ F = 124$$

The correlation between log P (determined from f values) and CLOG P for these structures is reasonably good as indicated by eq 5. It is interesting to note, however, that the coefficient for log P is not 1; it appears that for this group of compounds CLOG P is generally less than the value of log P estimated by summing fragmental constants.

$$\text{CLOG P} = 0.76(\pm 0.05) \log P + 1.09(\pm 0.30) \quad (5)$$

$$r = 0.960,\ n = 26$$

Inspection of the plot of activity versus lipophilicity in Figure 1 shows that, whereas log P increases over 4 orders of magnitude, potency only increases by 2 orders. This is energetically very unfavorable. Such a poor activity yield from the increased lipophilicity suggests that hydrophobic interactions with the active site on the channel are probably not the main determinants of potency. Rather, it seems likely that lipophilicity is important for access to the active site, presumably because the compounds have to enter the cell membrane. This is in keeping with earlier work on the mechanism of action of cetiedil and its congeners.[35] A study[35c] of how the block varied with the time for which the cells had been preincubated with the agent showed that cetiedil reached its maximum action after 20−30 min as compared with 60−120 min for the less lipophilic hydroxycetiedil (**4**, UCL 1269). Consistent with this, the more lipophilic triphenylethanoic ester (**11**, UCL 1274) acted rapidly, producing a maximal effect within 3 min.[35c] This and other evidence[35b,c] suggested that cetiedil, in contrast to clotrimazole, acts on the channel (probably in its open state[35c]) from within the membrane rather than at its outer or inner surfaces.

In accord with this, the most likely explanation of the lack of activity of the methoiodide derivative (**27**) of cetiedil is the inability of this permanently charged compound to enter the membrane and hence to reach the site of action. However, cetiedil methoiodide differs from cetiedil in another important way, namely, the absence of a dissociable proton. This raises the possibility that the N^+-H group in cetiedil could either act as a hydrogen-bond donor or become deprotonated to give the conjugate base, which might be imagined to be the active form, or essential for access to the site of action. Recent work on a related type of IK_{Ca} blocker suggests that the latter mechanisms are unlikely. It was found[17] that certain bisquinolino and bisquinolinium derivatives of the SK_{Ca} blocker dequalinium (Chart 1) are potent inhibitors of the IK_{Ca}-mediated loss of K^+ from rabbit red cells, studied by the same methods as used in the present work. One of the most active of the series is the dioxy analogue[33d] 1,10-bis(quinolin-4-yloxy)decane (**28**) (Chart 1), which had an IC_{50} of 1.22 ± 0.04 μM. The calculated log *P* for this compound is 7.93 and eq 3 predicts a corresponding IC_{50} of 5.2 μM. Since there are two basic nitrogen centers in this symmetrical molecule, which on statistical grounds would make a log difference of 0.3 if it is assumed that either center can interact with the channel, the result is in fair agreement. Furthermore, the characteristics of the blocking action of these bisquinolino compounds closely resemble those of cetiedil but not clotrimazole (Chart 1). Thus, although **28** has a very different chemical structure from cetiedil, it appears to act in the same way. The *N*,*N*-dimethyl quaternary ammonium salt (**29**) of this compound was also active,[17] but much less so (IC_{50} of 78 ± 15 μM); however, it still has approximately one-third of the potency of cetiedil, and since it does not possess an NH^+ group, this finding shows that the molecule does not have to be a hydrogen-bond donor or become deprotonated in order to be able to block the channel. By implication, neither does cetiedil. The potency difference between compounds **28** and **29** (a factor of approximately 65) accords with the idea that a molecule that is permanently doubly charged would be much less able to penetrate the cell membrane. In contrast, the cetiedil cation can dissociate a proton to give the uncharged and highly lipophilic, and therefore membrane permeable, conjugate base. The base becomes reprotonated when it leaves the lipophilic region to act in its positively charged form on the channel.

The findings of the present work together with these mechanistic considerations suggest that cetiedil and its congeners act in the charged form but reach their site of action from within the membrane. Viewed in this light, cetiedil methoiodide is ineffective because, when tested at practicable concentrations, not enough of it can reach the membrane region from which the compounds gain access to the channel. Nevertheless, the effect of the positive charge can be offset if the rest of the molecule is sufficiently lipophilic. More generally, our work also shows that designing compounds that act specifically at the cetiedil site associated with the IK_{Ca} channel is a challenging task because of the conflicting requirements of lipophilicity (with its concomitant reduction in aqueous solubility) and the presence of a positive charge. Although much progress has been made in developing IK_{Ca} channel blockers that act in the same way as clotrimazole but with greater potency and selectivity,[10b,c] the potential value of having several different pharmacological classes of IK_{Ca} blocker available for immunosupression, as well as for the possible treatment of sickle cell disease, makes this a goal worth pursuing.

Experimental Section

Melting points were determined in open glass capillary tubes with an Electrothermal electrically heated copper block apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 983 spectrometer. ^{1}H NMR spectra were recorded at 200 MHZ on a Varian VXR 400 spectrometer in solutions of $CDCl_3$ using TMS as an internal reference. Multiplicities are reported as (s) singlet, (d) doublet, (t) triplet, (q) quartet, and (m) multiplet. Assignments of hydroxyl and ammonium protons were checked by deuterium exchange. Mass spectra were recorded on a VG 7070H double focusing mass spectometer interfaced with a Finnagen INCOS data system. Thin-layer chromatography was carried out on glass plates (Merck Kiesegel 60 F_{254}) and column chromatography used Merck 7734 silica gel (63−20 mm). Preparative and analytical HPLC was performed using a Gilson binary grading system with an LB diode array detector, set at either 215, 240, or 254 nm. Elemental analyses were determined in house by A. A. T. Stones and were within ±0.4% unless otherwise indicated.

General Procedure for the Preparation of Compounds 2−12 (Scheme 1). The appropriately substituted carboxylic acid (9 mmol) was added to dry DMF (50 mL) containing a stirred suspension of NaH (0.4 g, 10 mmol as a 60% dispersion in mineral oil). 1-(2-Chloroethyl)-hexahydro-1*H*-azepine hydrochloride (2.0 g, 10 mmol from Aldrich) in dry DMF (50 mL) containing NaH (0.4 g, 10 mmol) as a 60% dispersion in mineral oil was added. The mixture was stirred at room temperature overnight (approximately 20 °C) and then filtered and concentrated under reduced pressure. Water (30 mL) was added to the resulting oil and the mixture was extracted (3×) with ethyl acetate. The combined extracts were dried (Na_2SO_4) and concentrated, and the resulting oil was dissolved in methanol, treated with oxalic acid, and diluted with 5 volumes of ethyl acetate to yield the product as a hydrogen oxalate, which was washed well with ether and then recrystallized (solvent indicated in Table 1). Alternatively, the oily residue was dissolved in chloroform and treated with anhydrous HCl gas; the chloroform was removed under reduced pressure and the resulting oil was crystallized from EtOAc:EtOH (Table 1) to afford the product as hydrochloride. Yields 40−50%.

2-(Hexahydro-1*H*-azepin-1-yl)ethyl cyclohexylethanoate hydrochloride hemihydrate (2): ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.6 (t, J = 3.2 Hz, 2H, OCH_2), 3.6−3.0 (m, 4H, NCH_2), 2.2 (d, J = 7.6 Hz, 2H, CH_2CO), 2.15−0.8 (m, 19H, CCHC).

2-(Hexahydro-1*H*-azepin-1-yl)ethyl 3-thienylethanoate hydrochloride (3): ^{1}H NMR (200 MHz, $CDC1_3$) δ 12.5 (s, 1H, NH), 7.25−7.0 (m, 2H, thienyl), 7.1 (s, 1H, thienyl) 4.5 (t, J = 5.8 Hz, 2H, OCH_2), 3.75 (s, 2H,CH_2CO), 3.25−2.8 (m, 4H, NCH_2).

2-(Hexahydro-1*H*-azepin-1-yl)ethyl cyclohexylhydroxy-3-thienylethanoate hydrochloride (4): ^{1}H NMR (400 MHz, CD_3OD) δ 7.41 (s, 1H, thienyl-2H), 7.31 (m, 1H, thienyl-4H), 7.20 (dd, 1H, thienyl-4H) 4.44 (t, J = 2.5 Hz, 2H, OCH_2), 3.47−3.31 (m, 6H, NCH_2), 2.1−1.0 (m, 19H, CCH_2C); IR (Nujol mull) ν 3226 (OH), 1736 (C═O), 732 (thienyl) cm^{-1}.

2-(Hexahydro-1*H*-azepin-1-yl)ethyl Cyclohexyl-3-furylhydroxyethanoate Hydrogen Oxalate (5). 2-Cyclohexyl-2-(3-furyl)ethanoic acid was synthesized according to the procedure described[23] for 2-cyclohexyl-2-(3-thienyl)ethanoic acid. Thus 3-lithiofuran was prepared from 3-bromofuran (4.85 g, 33 mmol) in anhydrous Et_2O (80 mL) at −78 °C in a nitrogen atmosphere and *n*-butyllithium in hexane (6.6 mL, 60 mmol. Then, 2-cyclohexyl-2-ketoethanoic acid[23] (3.68 g, 24 mmol) in

dry Et_2O (40 mL) at −78 °C was added and the mixture was stirred for 5 h and then warmed to −10 °C and hydrolyzed with ice. The aqueous layer was separated off, acidified with 2 N HCl, and extracted with Et_2O. The combined extracts were dried and evaporated and the residue was crystallized from petroleum spirits to give 2-cyclohexyl-2-(3-furyl)ethanoic acid, mp 131−133 °C; yield 1.8 g (33%). The corresponding aminoethyl ester had ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.5 (s, 1H, furyl-5H), 7.4 (s, 1H, furyl-2H), 6.4 (s, 1H, furyl-4H), 4.55−3.20 (m, 8H, OCH_2, NCH_2), 2.0−1.1 (m, 19H, CCH_2C); IR (KBr) ν 3439 (OH), 1734 (C=O), 1639 (furyl) cm^{-1}.

2-(Hexahydro-1*H*-azepin-1-yl)ethyl dicyclohexylethanoate hydrochloride (6): ^{1}H NMR (200 MHz, $CDC1_3$) δ 4.6 (t, 2H, OCH_2), 3.6−3.1 (m, 6H, NCH_2), 2.2−2.1 (m, 1H, CHCO), 2.0−0.8 (m, 30H, CCH_2C).

2-(Hexahydro-1*H*-azepin-1-yl)ethyl cyclohexylphenylethanoate hydrochloride (7): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.3 (m, 5H, Ph), 4.6 (m, 2H, OCH_2), 3.3 (m, 2H, NCH_2) 3.25 (d, J = 10.6 Hz, 1H, COCH), 3.15 (m, 2H, NCH_2), 2.7−2.5 (m, 2H, NCH_2), 2.05−0.7 (m, 19H, CCH_2C).

2-(Hexahydro-1*H*-azepin-1-yl)ethyl diphenylethanoate hydrochloride (8): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.5−7.3 (m, 10H, Ph), 5.2 (s, 1H, PhCHCO), 4.7 (t, 2H, OCH_2), 3.3−2.6 (m, 6H, NCH_2), 2.0−1.5 (m, 8H, CCH_2C).

2-(Hexahydro-1*H*-azepin-1-yl)ethyl 3,3-diphenylpropionate hydrogen oxalate (9): ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.3−7.1 (m, 10H, Ph), 4.4 (t, J = 7.9 Hz, 1H, PhCH), 4.2 (t, J = 5.0 Hz, 2H, OCH_2), 3.2 (t, J = 5.2 Hz, 2H, CH_2N), 3.15 (d, J = 8.1 Hz, 2H, CH_2CO), 3.1 (m, 4H, NCH_2), 1.7−1.0 (m, 8H, CCH_2C).

2-(Hexahydro-1*H*-azepin-1-yl)ethyl phenyl-3-thienylethanoate hydrogen oxalate (10): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.5−7.0 (m, 8H, Ph, thienyl), 5.3 (s, 1H, PhCHCO), 4.4 (m, 2H, OCH_2), 3.3−3.03 (m, 6H, NCH_2), 1.65−1.5 (m, 8H, CCH_2); IR (Nujol mull) ν 3445 (N^+H), 1733 (C=O), 712 (thienyl) cm^{-1}.

2-(Hexahydro-1*H*-azepin-1-yl) 2,2,2-triphenylethanoate hydrochloride hemihydrate (11): ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.4−7.2 (m, l5H, Ph), 4.8 (bs, 1H, NH), 4.3 (t, 2H, OCH_2), 2.7 (t, 2H, CH_2N), 2.5 (m, 4H, NCH_2), 1.6−1.4 (m, 8H, CCH_2).

2-(Hexahydro-1*H*-azepin-1-yl)ethyl 3,3-diphenylpropionate hydrogen oxalate (12): ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.3−7.2 (m, l5H, Ph), 4.0 (t, 2H, OCH_2), 3.8 (s, 2H, CH_2CO), 3.0 (m, 6H, NCH_2), 1.7−1.5 (m, 8H, CCH_2C); IR (Nujol mull) ν 3440 (N^+H), 1744 (C=O) cm^{-1}.

3-(Hexahydro-1*H*-azepin-1-yl)propyl 2,2,2-Triphenylethanoate Hydrochloride (13) (Scheme 2). 3-Chloropropanol (1.5 g, 16 mmol) was heated with hexahydroazepine (1.6 g, 16 mmol) in DMF (50 mL) with anhydrous K_2CO_3 (3 g) under reflux for 72 h, filtered, cooled, treated with decolorizing charcoal, and evaporated under reduced pressure to afford 1-(3-hydroxypropyl)hexahydro-1*H*-azepine, which was purified by column chromatography using a EtOAc:MeOH (1:4) mixture.

Triphenylethanoic acid (1.2 g, 4.2 mmol) in dry THF (40 mL) was treated with freshly distilled thionyl chloride (10 mL) and then heated under reflux for 6 h. The mixture was evaporated under reduced pressure and the residue was crystallized from cyclohexane to afford triphenylethanoyl chloride, mp 129−131 °C (yield 0.41 g, 32%). The latter (13 mmol), in dry Et_2O (20 mL), was added with stirring to 1-(3-hydroxypropyl)hexahydro-1*H*-azepine (0.2 g, 13 mmol) in dry Et_2O (20 mL), and the mixture was heated under reflux for 2 h and then cooled and filtered to afford the hydrochloride **13** (0.46 g), after being recrystallized from EtOH:Et_2O (6:1) mixture: mp 214−215 °C (dec); yield 0.14 g (25%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.3−7.2 (m, 15H, Ph), 4.3 (t, 2H, OCH_2), 2.6−2.1 (m, 6H, NCH_2), 2.0−1.5 (m, 10H, CCH_2C). Anal. ($C_{29}H_{33}NO_2$·HCl·0.25H_2O) C, H, N, Cl.

***N*-[2-(Hexahydro-1H-azepin-1-yl)ethyl]-2-cyclohexyl-2-(3-thienyl)ethanoamide Trifluoroacetate Sesquihydrate (14) (Scheme 5).** 2-Cyclohexyl-2-(3-thienyl)ethanoic acid[23] (1.5 g, 6.2 mmol) in freshly distilled THF (30 mL) at −20 °C with Et_3N (0.63 g, 6.2 mmol) was treated with *n*-butyl chloroformate (0.9 mL, 7.1 mmol) with stirring for 30 min. Then 1-(2-aminoethyl)hexahydro-1*H*-azepine (0.89 g, 6.2 mmol, prepared from 1-(2-chloroethyl)-hexahydro-1*H*-azepine and 0.880 ammonia in EtOH) was added and the mixture was stirred at −20 °C for 5 h and then allowed to warm to 0 °C and basified. The THF layer was separated, dried, and concentrated to yield the product as an oil which was chromatographed to 95% purity by preparative HPLC and isolated as an oily trifluoroacetate (yield 0.49 g, 23%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.3 (s, 1H, NH), 7.2−7.05 (m, 3H, thienyl), 3.5−3.39 (m, 4H, NCH_2), 3.3 (s, 1H, NH), 3.21 (d, J = 10.4 Hz, 1H, thienyl CHCO), 3.2−2.67 (m, 4H, NCH_2), 1.9−0.7 (m, 19H, CCH_2C); IR ($CDCl_3$) ν 3607 (NH), 3293 (N^+H), 1665 (C=O), 734 (thienyl) cm^{-1}. Anal. ($C_{20}H_{32}N_2OS$·CF_3CO_2H·1.5H_2O) C, H; N: calcd, 5.7; found, 5.1.

1-[2-(6,6,6-Triphenyl-3-oxa-1-hexanyl)]hexahydro-1*H*-azepine Hydrogen Oxalate (15) (Scheme 6). Ethyl 3,3,3-triphenylpropionate (1 g, 3.16 mmol) in dry THF (10 mL) was added during 20 min to a stirred suspension of $LiA1H_4$ (0.48 g, 12 mmol) in dry THF (20 mL) at 0 °C. The mixture was stirred at 20 °C for 5 h and then excess $LiA1H_4$ was decomposed by the cautious addition of aqueous KOH (5 mL of 20% w/v). The mixture was filtered and the organic layer was dried ($MgSO_4$) and evaporated. The resulting oil was crystallized from hexane to afford 0.6 g (66% yield) of 3,3,3-triphenylpropan-1-ol, mp 104−106 °C (lit.[41] mp 106−108 °C).

1-(2-Choroethyl)-hexahydro-1*H*-azepine hydrochloride (0.27 g, 1.39 mmol) in dry DMF (15 mL) was added dropwise to a stirred suspension of NaH (0.066 g, 2.77 mmol) in dry DMF (15 mL) at 20 °C. After 10 min, a solution of 3,3,3-triphenylpropan-1-ol (0.40 g, 1.39 mmol) in dry DMF (10 mL) was added slowly and the stirred mixture was heated at 115 °C for 20 h in a nitrogen atmosphere. The mixture was then filtered, the solvent was removed under reduced pressure, and the resulting oil was purified by column chromatography using ethanol to give the amine product. The latter was treated with oxalic acid in ethanol and then with ether to form the insoluble hydrogen oxalate; this was collected, washed with ether, and recrystallized twice from ethanol to afford the pure product (0.6 g, 66% yield): mp 180−183 °C; ^{1}H NMR (200 MHz, CD_3OD) δ 7.21−7.12 (m, 15H, Ph), 3.32 (t, 2H, $PhCCH_2$), 3.13 (dd, 2H, OCH_2), 2.91 (dd, 2H, OCH_2), 2.63 (m, 6H, NCH_2), 1.57 (s, 8H, CCH_2C). Anal. ($C_{28}H_{36}NO$·1.25$C_2H_2O_4$) C, H, N.

2-(Hexahydro-1*H*-azepin-1-yl)ethyl 1-Adamantylethanoate Hydrochloride (16) (Scheme 2). 1-(2-Hydroxyethyl)-hexahydro-1*H*-azepine (1.44 g, 10 mmol) in dry $CHCl_3$ (20 mL) was added dropwise over l h to 1-adamantylcarboxyl chloride (2 g, 10 mmol, from Aldrich) in dry $CHCl_3$ (100 mL) at 0 °C with stirring. The mixture was evaporated under reduced pressure and the resulting oil was taken into EtOAc: EtOH (5:1) and allowed to crystallize in a refrigerator. The product was collected and recrystallized to yield **16** (0.88 g, 26% yield): mp 203−205 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.5 (s, 1H, NH), 4.6 (t, J = 5.1 Hz, 2H, OCH_2), 3.55−3.0 (m, 6H, NCH_2), 2.3−1.5 (m, 23H, CH). Anal. ($C_{19}H_{31}NO_2$·HCl) C, H, N, Cl.

General Procedure for Preparation of Compounds 17−20. 1-(2-Chloroethyl)-hexahydro-1*H*-azepine hydrochloride (2.3 g, 11 mmol) was added to a solution of Na (0.26 g, 11 mmol) in dry 2-propanol (30 mL) and the mixture was stirred for 10 min. The carboxylic acid (5.8 mmol) was added and the mixture was heated under reflux for 24 h. The mixture was then cooled, filtered, and evaporated under reduced pressure; the resulting residue was dissolved in 2 N NaOH (20 mL) and extracted with $CHCl_3$ (4 × 20 mL). The combined extracts were concentrated and the resulting oil was purified by column chromatography eluting with $CHCl_3$:MeOH (100:1). The resulting purified oil in EtOH was treated with anhydrous HCl, and the resulting hydrochloride salt was collected and recrystallized from EtOH. The fluorenyl derivative (**20**) was too hygroscopic and was therefore converted into the hydrogen oxalate and recrystallized from EtOH.

2-(Hexahydro-1*H*-azepin-l-yl)ethyl 1-naphthoate hydrochloride (17): ^{1}H NMR (400 MHz, CD_3OD) δ 8.92−7.55 (m, 7H, naphthyl), 4.77 (t, 2H, OCH_2), 3.68−3.48 (m, 6H, CH_2N), 1.94−1.74 (m, 8H, CCH_2C).

2-(Hexahydro-1*H*-azepin-1-yl)ethyl 2-naphthoate hydrochloride (18): ^{1}H NMR (400 MHz, CD_3OD) δ 8.71 (s, 1H, naphthyl-1H), 8.05−7.77 (m, 6H, naphthyl 4.74 (t, 2H, OCH_2), 3.69−3.05 (m, 6H, NCH_2), 1.96−1.75 (br s, 8H, CCH_2C).

2-(Hexahydro-1*H*-azepin-1-yl)ethyl 9-anthracenecarboxylate hydrochloride hemihydrate (19): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.72 (s, 1H, anthracenyl-10H), 8.15 (m, 5H, Ar−H), 7.58 (m, 5H, Ar−H), 4.64 (t, J = 6.0 Hz, 2H, OCH_2), 2.87 (br t, 2H, NCH_2) 2.72 (br t, J = 5.7 Hz, 4H, NCH_2), 1.64−1.56 (m, 8H, CCH_2C).

2-(Hexahydro-1*H*-azepin-1-yl)ethyl 9-fluorenylcarboxylate hydrogen oxalate (20): ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.89−7.36 (m, 8H, Ar−H), 5.13 (s, 1H, fluorenyl-9H), 4.38 (t, J = 6 Hz, 2H, OCH_2), 3.10 (br s, 6H, NCH_2), 1.68−1.54 (m, 8H, CCH_2C).

General Procedure for Preparation of Compounds 21−25 (Scheme 4). 9-Carboxyfluorene (3.7 g, 17 mmol) was added to a stirred suspension of Na metal (0.85 g, 37 mmol) in liquid ammonia[37] (100 mL) and then anhydrous Et_2O (65 mL) was added. After 1 h the appropriate *p*-substituted benzyl chloride (18 mmol) was added (for **23**, *p*-fluorobenzyl bromide was used) and stirring was continued for 4 h. The liquid ammonia was removed, water (35 mL) was added, and the aqueous phase was washed with Et_2O (2 × 35 mL) and acidified with 2 N HCl, and the resulting precipitate was collected dried and crystallized from EtOAc:petroleum ether mixture to give the 9-(*p*-substituted benzyl)-9-carboxyfluorene in 21−62% yield. The following melting points were obtained [substituent (% yield), mp (°C), analysis]: H (61%), 195−199 (lit.[42] mp 204−205); OMe (21%), 200−202, $C_{22}H_{18}O_3$; F (62%), l95−196, $C_{21}H_{15}FO_2$; Cl (37%), 190−191, $C_{21}H_{15}ClO_2$; NO_2 (51%), 201−203, $C_{21}H_{18}NO_4$.

1-(2-Chloroethyl)hexahydro-1*H*-azepine hydrochloride (2.34 g, 11.6 mmol) was added to a solution of Na metal (0.27 g, 11.6 mmol) in anhydrous 2-propanol (35 mL) and the mixture was stirred at 20 °C for 30 min. The 9-(*p*-substituted benzyl)-9-carboxyfluorene (5.8 mmol) was added and the mixture was heated with stirring under reflux for 36 h. The mixture was then cooled, filtered, and evaporated under reduced pressure; the resulting residue was dissolved in 2 N NaOH (20 mL) and extracted with $CHCl_3$ (4 × 20 mL). The combined extracts were concentrated and the residual oil was purified by column chromatography eluting with $CHCl_3$:MeOH (100:1). The resulting purified oil in Et_2O (10 mL) was treated with anhydrous oxalic acid (1 mmol) in MeOH (0.5 mL) to give a precipitate that was crystallized from the solvent indicated in Table 2 to afford the corresponding product, 2-(hexahydro-1*H*-azepin-1-yl)ethyl 9-(*p*-substituted benzyl) fluorene-9-carboxylate hydrogen oxalate (yield 30−45%).

21: ^{1}H NMR (400 MHz, CD_3OD) δ 7.63−6.68 (m, 13H, Ar−H), 4.34 (t, 2H, OCH_2), 3.46 (s, 2H, $PhCH_2$), 3.30 (m, 4H, NCH_2), 2.21 (m, 2H, NCH_2), 1.52 (m, 8H, CCH_2C).

22: ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.71−7.34 (m, 8H, Ar−H), 6.52 (d, J = 8.8 Hz, 2H, C_6H_4), 6.43 (d, J = 8.8 Hz, 2H, C_6H_4), 4.26 (t, J = 4.0 Hz, 2H, OCH_2), 3.61 (s, 2H, $PhCH_2$) 3.53 (s, 3H, OCH_3), 3.10 (t, J = 4.0 Hz, 2H, NCH_2), 1.49 (m, 8H, CCH_2C).

23: ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.68−7.35 (m, 8H, Ar−H) 6.67 (t, 2H, C_6H_4), 6.57 (dd, 2H, C_6H_4), 4.27 (t, 2H, OCH_2), 3.69 (s, 2H, $PhCH_2$), 3.10 (t, 2H, NCH_2) 2.77 (m, 4H, NCH_2), 1.49 (m, 8H, CCH_2C).

24: ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.69−7.35 (m, 8H Ar−H), 6.90 (d, J = 8.4 Hz, 2H, C_6H_4), 6.55 (d, J = 8.4 Hz, 2H, C_6H_4), 4.27 (t, J = 4.5 Hz, 2H, OCH_2), 3.70 (s, 2H, $PhCH_2$), 3.10 (t, J = 4.5 Hz, 2H, NCH_2) 2.77 (m, 4H, NCH_2), 1.49 (m, 8H, CCH_2C).

25: ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.72−7.37 (m, 10H, Ar−H + C_6H_4), 6.82 (d, J = 8.8 Hz, 2H, C_6H_4), 4.28 (t, J = 3.9 Hz, 2H, OCH_2), 3.88 (s, 2H, $PhCH_2$), 3.10 (t, J = 4.3 Hz, 2H, NCH_2) 2.77 (m, 4H, NCH_2), 1.49 (m, 8H, CCH_2C).

1-[(9-Benzyl)fluoren-9-yl]-4-(hexahydro-1*H*-azepin-1-yl)but-2-yne Hydrogen Oxalate (26). (Scheme 7). Aqueous propargyl alcohol (8.1 g of 39% solution, 143 mmol), formaldehyde (28 mL of 37% aqueous solution), hexahydroazepine hydrochloride (21.8 g, 161 mmol), and CuCl were heated together at 100 °C for 4 h. The mixture was then cooled, acidified (2 N HCl), and washed with $CHCl_3$ (3 × 20 mL). The aqueous layer was basified (K_2CO_3 and 33% NH_4OH) and extracted with $CHCl_3$ (10 × 20 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated to an oil, which was purified by column chromatography eluting with $CHCl_3$:MeOH (4:1) to afford 4-(hexahydro-1*H*-azepin-1-yl)but-2-yn-1-ol (18 g, 75% yield).

To the above aminobutynol (5.15 g, 31 mmol) in CH_2Cl_2 (12 mL), cooled to 0 °C, was added dropwise thionyl chloride (4.0 g, 30 mmol) in CH_2Cl_2 (3 mL), and the mixture was left at 20 °C for 6 h. The mixture was then basified by cautious addition of NH_4OH and extracted with $CHCl_3$ (4 × 20 mL). The combined extracts were dried ($MgSO_4$) and evaporated to an oil, which was purified by column chromatography eluting with Et_2O to give 1-chloro-4-(hexahydro-1*H*-azepin-1-yl)but-2-yne (3.61 g, 63% yield).

9-Benzylfluorene (1.5 g, 5.7 mmol) was cautiously added in portions to NaH (0.3 g, 7.5 mmol, of 60% dispersion in mineral oil) in dry DMSO (4 mL) under a nitrogen atmosphere. The mixture was stirred for 15 min at 20 °C and then the above chlorobutyne (1.07 g, 5.7 mmol) in dry DMSO (1.5 mL) was added, and the mixture was stirred for 1 h at 20 °C. Water (10 mL) was then added and the mixture was extracted with Et_2O (5 × 10 mL). The combined ether extracts were washed (5 × 10 mL), dried ($MgSO_4$), and concentrated to afford an oil, which was purified by column chromatography (SiO_2 and Et_2O: petroleum ether, 3:2) to give the product (**26**, base, 1.0 g, 43% yield). A portion of the latter (0.2 g) in Et_2O (7 mL) was added to a solution of anhydrous oxalic acid (0.053 g) in MeOH (0.4 mL), and the resulting precipitate was collected by filtration and crystallized from 2-PrOH to give the product **26** hydrogen oxalate: mp 153−155 °C (yield 0.17 g, 71% recovery); recrystallization did not alter the melting point; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.69−6.54 (m, 13H, Ar−H), 3.52 (s, 2H, $PhCH_2$), 3.36 (s, 2H, $C{\equiv}CCH_2$) 3.08 (s, 2H, NCH_2), 2.44 (m, 4H, NCH_2), 1.49 (m, 8H, CCH_2C); IR (Nujol mull) ν 2248 (w, C≡C) cm^{-1}. Anal. ($C_{30}H_{31}N{\cdot}0.8C_2H_2O_4{\cdot}0.25C_3H_7OH$) C, H, N.

2-(Hexahydro-1*H*-azepin-1-yl)ethyl 2-Cyclohexyl-2-(3-thienyl)ethanoate Methoiodide (Cetiedil Methoiodide, 27). Cetiedil base (0.5 g, 1.43 mmol) in dry Et_2O (20 mL) at −20 °C was treated with methyl iodide (0.22 g, 1.55 mmol) and then left for 12 h at 0 °C.

Methanol (20 mL) was added and the mixture was heated at 50 °C for 4 h and then evaporated under reduced pressure. The resulting solid was crystallized from EtOAc:EtOH (1:1) to give the product (0.38 g, 54% yield): mp 144−145 °C; IR (Nujol mull) ν 1732 (C═O), 728 (thienyl) cm^{-1}. Anal. ($C_{21}H_{34}NO_2S{\cdot}I$) C, H, N, I.

Pharmacology. Blood (2−5 mL) was drawn from the ear vein of adult New Zealand white rabbits and mixed with heparin (20 units/mL whole blood). After centrifugation (3 min at 1600*g*) the supernatant and buffy coat were aspirated and discarded, leaving a pellet of packed cells. This was then resuspended in 5 volumes of a Ca^{2+}-free solution containing (in mM) NaC1, 145; KCl, 0.1; $MgSO_4$, 1; EDTA, 1; TRIS, 10; inosine, 10; pH adjusted to 7.4 by adding 1 M NaOH. The suspension was centrifuged as before, and the pellet resuspended twice. After the final centrifugation, the cells were stored as a pellet in this solution at 4 °C, for up to 4 days.

The assays were done using a solution containing (mM) NaCl, 145; KCl, 0.1; $MgSO_4$ 1; $CaCl_2$ 1; inosine 10; TRIS 10 (pH adjusted to 7.4 by the addition of 1 M NaOH). Because earlier experiments[35c] had shown that the action of cetiedil develops slowly (half-time 5 min), the cells were incubated with the test compounds (with the exception of **21**−**26**) for 40−60 min before the tests proper. This period was extended to 120 min in measurements with compound **4** (UCL 1269). The test compounds were added as a small volume (typically ≤ 4 μL)

of a stock solution in DMSO. "Control" cells were incubated under the same conditions (20 μL of packed cells added to 2 mL of the low-K^+ solution at 37 °C; hematocrit ~1%). The cell suspension was then transferred to a water-jacketed bath that contained the K^+-sensitive and reference electrodes and was maintained at 37 °C. When the signal from the K^+ electrode had become steady, A23187 (2 μM) was added, and the resulting loss of K^+ from the cells was recorded for 3 min. Finally, digitonin (100 μM) was introduced in order to lyse the cells and allow their total K^+ content to be estimated (see ref 35c for further details). The amount of K^+ lost in response to the 3 min application of A23187 could then be calculated as a fraction of this total. The value observed in the presence of the test compound was expressed as a percentage of the K^+ loss from the "control" cells. Each measurement at a given concentration was repeated at least three times and concentration-inhibition curves were constructed from the means of the values obtained (see refs 17 and 35c,d).

The preincubation period was omitted in experiments with highly lipophilic compounds (**21**−**26**), which were instead added as a small volume (again typically = 4 μL) of a stock solution in DMSO to the cell suspension (20 μL of packed cells plus 2 mL of the standard low K^+ physiological solution) in the recording bath 3 min prior to the introduction of A23187.

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References

(1) Gárdos, G. The function of calcium in the potassium permeability of human erythocytes. *Biochim. Biophys. Acta* **1958**, *30*, 653−654.

(2) Cook, N. S.; Quast, U. Potassium channel pharmacology. In *Potassium Channels*; Cook, N. S., Ed.; Ellis Horwood Ltd.: Chichester, 1990; pp 70−95.

(3) Haylett, D. G.; Jenkinson, D. H. Calcium-activated potassium channels. In *Potassium Channels*; Cook, N. S., Ed.; Ellis Horwood Ltd.: Chichester, 1990; pp 70−95.

(4) Castle, N. A. Recent advances in the biology of small conductance calcium-activated potassium channels. *Perspectives Drug Discovery Des.* **1999**, *15/16*, 131−154.

(5) Vergara, C.; LaTorre, R.; Marrion, N. V.; Adelman, J. P. Calcium-activated potassium channels. *Curr. Opin. Neurobiol.* **1998**, *8*, 321−329.

(6) Joiner, W. J.; Wang, L. Y.; Tang, M. D.; Kaczmarek, L. K. hSK4, a member of a novel subfamily of calcium-activated potassium channels. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 11013−11018.

(7) Ishii, T. M.; Silvia, C.; Hirschberg, B.; Bond, C. T.; Adelman, J. P.; Maylie, J. A human intermediate conductance calcium-activated potassium channel. *Proc. Nat. Acad. Sci. U.S.A.* **1997**, *94*, 11651−11656.

(8) Logsdon, N. J.; Kange, J.; Togo, J. A.; Christian, E. P.; Aiyar, J. A novel gene, hKCa4, encodes the calcium-activated potassium channel in human T lymphocytes. *J. Biol. Chem.* **1997**, *272*, 32734−32726.

(9) Jensen, B. S.; Strøbæk, D.; Christophersen, P.; Jorgensen, T. D.; Hansen, C.; Silahtaroglu, A.; Olesen, S. P.; Ahring, P. K. Characterization of the cloned human intermediate-conductance Ca^{2+} − activated K^+ channel. *Am. J. Physiol.* **1998**, *275*, C848−C856.

(10) (a) Jensen, B. S.; Odum, N.; Jorgensen, N. K.; Christophersen, P.; Olesen, S. P. Inhibition of T cell proliferation by selective block of Ca^{2+} -activated K^+ channels. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 10917−10921. (b) Wulff, H.; Miller, M. J.; Hansel, W.; Grissmer, S.; Cahalan, M. D.; Chandy, K. G. Design of a potent and selective inhibitor of the intermediate-conductance Ca^{2+}-activated K^+ channel, $IK_{Ca}1$: a potential immunosuppressant. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 8151−8156. (c) Ghanshani, S.; Wulff, H.; Miller, M. J.; Rohm, H.; Neben, A.; Gutman, G. A.; Cahalan, M. D.; Chandy, K. G. Up-regulation of the $IK_{Ca}1$ potassium channel during T-cell activation−molecular mechanism and functional consequences. *J. Biol. Chem.* **2000**, *275*, 37137−37149.

(11) Armando-Hardy, M.; Ellory, J. C.; Ferreira, H. G.; Fleminger, S.; Lew, V. L. Inhibition of the calcium-induced increase in the potassium permeability of human red blood cells by quinine. *J. Physiol.* **1975**, *250*, 32P.

(12) Reichstein, E.; Rothstein, A. Effects of quinine on Ca^{2+}-induced K^+ efflux from human red cells. *J. Membr. Biol.* **1981**, *59*, 57−63.

(13) Simons, T. J. B. Actions of a carbocyanine dye on calcium-dependent potassium transport in human red cell ghosts. *J. Physiol.* **1979**, *288*, 481−507.

(14) Alvarez, J.; Montero, M.; Garcia-Sancho, J. High-affinity inhibition of Ca^{2+}-dependent K^+ channels by cytochrome- P-450 inhibitors. *J. Biol. Chem.* **1992**, *267*, 11789−11793.

(15) (a) Brugnara, C.; De Franceschi, L.; Alper, S. L. Inhibition of Ca^{2+}-dependent K^+ transport and cell dehydration in sickle erythrocytes by clotrimazole and other imidazole derivatives. *J. Clin. Invest.* **1993**, *92*, 520−526. (b) Rittenhouse, A. R.; Vandorpe, D. H.; Brugnara, C.; Alper, S. L. The antifungal imidazole clotrimazole and its major in vivo metabolite are potent blockers of the calcium-activated potassium channel in murine erythroleukemia cells. *J. Membr. Biol.* **1997**, *157*, 177−191.

(16) (a) Wolff, D.; Cecchi, X.; Spalvins, A.; Canessa, M. Charybdotoxin blocks with high affinity the Ca-activated K^+ channel of Hb-A and Hb-S red-cells − individual differences in the number of channels. *J. Membr. Biol.* **1988**, *106*, 243−25. (b) Brugnara, C.; De Franceschi, L.; Alper, S. L. Ca^{2+}-activated K^+ transport in erythrocytes—Comparison of binding and transport inhibition by scorpion toxins. *J. Biol. Chem.* **1993**, *268*, 8760−8768.

(17) Malik-Hall, M.; Ganellin, C. R.; Galanakis, D.; Jenkinson, D. H. Compounds that block both intermediate-conductance (IK_{Ca}) and small-conductance (SK_{Ca}) calcium-activated potassium channels. *Br. J. Pharmacol.* **2000**, *129*, 1431−1438.

(18) Kaji, D. M. Nifedipine inhibits calcium-activated K-transport in human erythrocytes. *Am. J. Physiol.* **1990**, *259*, C332−C339.

(19) Ellory, J. C.; Kirk, K.; Culliford, S. J.; Nash, G. B.; Stuart, J. Nitrendipine is a potent inhibitor of the Ca^{2+}-activated K^+ channel of human erythrocytes. *FEBS Lett.* **1992**, *296*, 219−221.

(20) Berkowitz, L. R.; Orringer, E. P. Effects of cetiedil on monovalent cation permeability in the erythrocyte: an explanation for the efficacy of cetiedil in the treatment of sickle cell anemia. *Blood Cells* **1982**, *8*, 283−288.

(21) Berkowitz, L. R.; Orringer, E. P. An analysis of the mechanism by which cetiedil inhibits the Gardos phenomenon. *Am. J. Hemat.* **1984**, *17*, 217−223.

(22) Christophersen, P.; Vestergaard-Bogind, B. Cetiedil, a common inhibitor of the Ca^{2+}-activated K^+ channels and Ca^{2+} pump in human erythrocytes. *Acta Physiol. Scand. Suppl.* **1985**, *542*, 167.

(23) Roxburgh, C. J.; Ganellin, C. R.; Shiner, M. A. R.; Benton, D. C. H.; Dunn, P. M.; Ayalew, Y.; Jenkinson, D. H. The synthesis and some pharmacological actions of the enantiomers of the K^+-channel blocker cetiedil. *J. Pharm. Pharmacol.* **1996**, *48*, 851−857.

(24) Robba, M.; Le Guen, Y. Dérivés thiophenique β-substitués II. Synthése et étude pharmacologique d'esters amines des acides cyclopentyl et cyclohexyl(thiényl-3) glycoliques. *Chim. Thérap.* **1966**, *1*, 238−245. β-Substituted Thiophene Derivatives. II. Synthesis and pharmacological study of aminoesters of cyclopentyl and cyclohexyl 3-thienyl glycolic acids.

(25) Benjamin, L. J.; Berkowitz, L. R.; Orringer, E.; Mankad, V. N.; Prasad, A. S.; Lewkow, L. M.; Chillar, R. K.; Peterson, C. M. A collaborative, double-blind randomized study of cetiedil citrate in sickle cell crisis. *Blood* **1986**, *67*, 1442−1447.

(26) Joiner, C. H. Cation-transport and volume regulation in sickle red-blood-cells. *Am. J. Physiol.* **1993**, *264*, C251−C270.

(27) Steinberg, M. H. Management of sickle cell disease. *New Eng. J. Med.* **1999**, *340*, 1021−1030.

(28) Sandford, C. A.; Sweiry, J. H.; Jenkinson, D. H. Properties of a cell volume-sensitive potassium conductance in isolated guinea-pig and rat hepatocytes. *J. Physiol.* **1992**, *447*, 133−148.

(29) Sarkadi, B.; Cheung, R.; Mack, E.; Grinstein, S.; Gelfand, E. W.; Rothstein, A. Cation and anion transport pathways in volume regulatory response of human lymphocytes to hyposmotic media. *Am. J. Physiol.* **1985**, *248*, C480−C487.

(30) Decoursey, T. E.; Chandy, K. G.; Gupta, S.; Cahalan, M. D. Two types of potassium channels in murine T-lymphocytes. *J. Gen. Physiol.* **1987**, *89*, 379−404.

(31) Demonchaux, P.; Ganellin, C. R.; Dunn, P. M.; Jenkinson, D. H. Search for the pharmacophore of the K^+ channel blocker, apamin. *Eur. J. Med. Chem.* **1991**, *26*, 915−920.

(32) Dunn, P. M.; Benton, D. C. H.; Campos Rosa, J.; Ganellin, C. R.; Jenkinson, D. H. Discrimination between subtypes of apamin-sensitive Ca^{2+}-activated K^+ channels by gallamine and a novel bis-quaternary quinolinium cyclophane, UCL 1530. *Br. J. Pharmacol.* **1996**, *117*, 35−42.

(33) (a) Galanakis, D.; Davis, C. A.; Del Rey Herrero, B.; Ganellin, C. R.; Dunn, P. M.; Jenkinson, D. H. Synthesis and structure−activity relationships of dequalinium analogues as K^+ channel blockers. Investigations on the role of the charged heterocycle. *J. Med. Chem.* **1995**, *38*, 595−606. (b) Galanakis, D.; Davis, C. A.; Del Rey Herrero, B.; Ganellin, C. R.; Dunn, P. M.; Jenkinson, D. H. Synthesis and QSAR of dequalinium analogues as K^+ channel blockers. Investigations on the role of the 4-amino group.

Bio. Med. Chem. Lett. **1995**, *5*, 559−562. (c) Galanakis, D.; Ganellin, C. R.; Dunn, P. M.; Jenkinson, D. H. On the concept of a bivalent pharmacophore for SK_{Ca} channel blockers: Synthesis, pharmacological testing, and radioligand binding studies on mono-, bis-, and tris-quinolinium compounds. *Arch. Pharm.-Pharm. Med. Chem.* **1996**, *329*, 534−528. (d) Galanakis, D.; Davis, C. A.; Ganellin, C. R.; Dunn, P. M. Synthesis and quantitative structure−activity relationship of a novel series of small-conductance Ca^{2+}-activated K^+ channel blockers related to dequalinium. *J. Med. Chem.* **1996**, *39*, 359−370.

(34) Campos Rosa, J.; Beckwith-Hall, B. M.; Galanakis, D.; Ganellin, R. R.; Dunn, P. M.; Jenkinson, D. H. Bis-quinolinium cyclophanes: A novel class of potent blockers of the apamin-sensitive Ca^{2+}-activated K^+ channel. *Bio. Med. Chem. Lett.* **1997**, *7*, 7−10.

(35) (a) Benton, D. C. H.; Athmani, S.; Roxburgh, C. J.; Shiner, M. A. R.; Haylett, D. G.; Ganellin, C. R.; Jenkinson, D. H. Effects of cetiedil and its analogues on the Ca^{2+}-activated K^+ permeability of rabbit erythrocytes and on levcromakalim-stimulated $^{86}Rb^+$ efflux from rat aorta. *Br. J. Pharmacol.* **1994**, *112*, 466P. (b) Dunn, P. M. The action of blocking agents applied to the inner face of Ca^{2+}-activated K^+ channels from human erythrocytes. *J. Membr. Biol.* **1998**, *165*, 133−143. (c) Benton, D. C. H.; Roxburgh, C. J.; Ganellin, C. R.; Shiner, M. A. R.; Jenkinson, D. H. Differences in the actions of some blockers of the calcium-activated potassium permeability in mammalian red cells. *Br. J. Pharmacol.* **1999**, *126*, 169−178. (d) Babaoglu, M. O.; Miscony, Z.; Ganellin, C. R.; Benton, D. C. H.; Haylett, D. G.; Jenkinson, D. H. Effects of some cetiedil congeners on the calcium-activated potassium permeability of human erythrocytes. *FASEB J.* **2000**, *14*, A1360, 290P.

(36) MacDowell, D. W. H.; Jeffries A. T. The chemistry of indenothiophenes. III. The metalation of 3-benzylthiophene and an alternative synthesis of 4*H*-indeno[1,2-*b*] thiophene-4-carboxylic Acid. *J. Org. Chem.* **1971**, *36*, 1053−1056.

(37) Murphy, W. S.; Hauser, C. R. Alkylations of fluorene and of 9-fluorenylcarboxylic acid by means of alkali amides in liquid ammonia. Alkylations by *n*-butyllithium. *J. Org. Chem.* **1966**, *31*, 85−88.

(38) Karaev, S. F.; Janez Quintana, G. R.; Movsumzade, M. M. Synthesis and properties of 4-perhydroazepino-2-butyn-1-ol and its derivatives. *J. Org. Chem. USSR* (Engl. Transl.) **1983**, 2014−2017 (*Zh. Org. Khim.* **1983**, *19*, 2307−2310). *Chem. Abstr.* **1984**, *100*, 85580v.

(39) Mannhold, R.; Rekker, R. F.; Dross, K.; Bijloo, G.; deVries, G. The lipophilic behavior of organic compounds: 1. An updating of the hydrophobic fragmental constant approach. *Quant. Struct.-Act. Relat.* **1998**, *17*, 517−536.

(40) CLOG P for Windows, Version 2.0.0.b, BioByte Corp., Claremont, CA.

(41) McPhee, W. D.; Lindsrom, E. G. The reaction of triphenylmethylsodium with esters of α,*β*-unsaturated acids. *J. Am. Chem. Soc.* **1943**, *65*, 2177−2180.

(42) Bavin, P. M. G. Aliphatic chemistry of fluorene. Part V. Spiroketones. *Can. J. Chem.* **1960**, *38*, 1099−1103.

(43) Robba, M. F.; Duval, D. J. C. Salts of an ester of (3-thienyl)-cyclohexyl-acetic acid, and their preparation and use. *Brit. Pat.* 1, 392, 671 **1973**; *Ger. Offen.* 2,313,338. *Chem. Abstr.* **1974**, *80*, 3401*u*.

(44) Blicke, F. F. (Polymethylenimino)ethyl esters of diphenylacetic and benzilic acids. U. S. Pat 2,735,847. *Chem. Abstr.* **1956**, *50*, 15602*i*.

(45) Robba, M.; Le Guen, Y. Derivés thiophéniques *β*-substitués III. Esters aminés des acides α-phenyl-α-cyclopentyl et α-cyclohexyl-α-(thienyl-3) acétiques. *β*-substituted thiophene derivatives III. Aminoesters of α-phenyl-α-cyclopentyl and α-cyclohexyl-α-(3-thienyl) acetic acids. *Chim. Thér.* **1967**, *2*, 120−124.

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