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THE SAR OF UK-78,282: A NOVEL BLOCKER OF HUMAN T CELL Kv1.3 POTASSIUM CHANNELS

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Abstract. UK-78,282 was identified in a human T cell ^{86}Rb efflux high-throughput screen of our compound libraries. This compound was found to be a potent and selective blocker of human T cell voltage-gated K^+ channels and to inhibit T cell activation. The SAR around UK-78,282 and a general pharmacophore hypothesis are presented in this communication. © 1997 Elsevier Science Ltd.

Introduction. Cyclosporin A (CsA) and FK-506 have demonstrated potent immunosuppressive activity in man and, in combination with azathioprine and steroids, these drugs have provided successful maintenance therapy and rejection episode management following organ transplantation.[1] However, the low therapeutic indices and significant side effects of both CsA and FK-506 have severely limited the use of these drugs to treat chronic immunologic disorders such as rheumatoid arthritis, psoriasis, and type I diabetes.[2] Thus, there is an intensive effort within the pharmaceutical industry to identify effective immunosuppressive agents with reduced toxicity.

Discovery of the voltage-gated K^+ channel, Kv1.3, which is abundantly expressed on human T cells and is involved in control of membrane potential, IL-2 production, and T cell proliferation, provides a novel pharmacological target for immunosuppressive therapy.[3] The protein constituent of the *Centruroides margaritatus* scorpion venom, margatoxin (MgTX), is an exceptionally potent and selective blocker of the Kv1.3 K^+ channel. MgTX inhibits Ca^{2+}-dependent T cell activation and proliferation via membrane depolarization caused by Kv1.3 blockade.[4]

We initiated a high-throughput screen for identification of small-molecule Kv1.3 channel blockers via the inhibition of ^{86}Rb efflux from T cells. The protocol involved loading human T cells with ^{86}Rb overnight, washing cells, adding test compounds, and then depolarizing cells by increasing the concentration of extracellular K^+.[5] Various aryl-substituted quinuclidines, piperidines, and acyclic amines were identified as potential lead structures, with inhibitory potencies in the 1-10 micromolar range, and showed structural features in common with some known potassium channel blockers such as tetraethylammonium (**1**), glyburide (**2**), clofilium (**3**), sotalol (**4**), and dofetilide (**5**). Ultimately, UK-78,282 (4-[diphenylmethoxymethyl]-1-[3-(4-methoxyphenyl)-propyl]- piperidine) was chosen as the lead compound because of its in vitro potency (IC_{50} = 0.4 μM in Rb efflux and 0.2 μM in Kv1.3 patch-clamp studies) and relatively good selectivity.[6] A synthetic chemistry program was initiated around this piperidine structure with the goal of improving the in vitro potency below 100 nanomolar.

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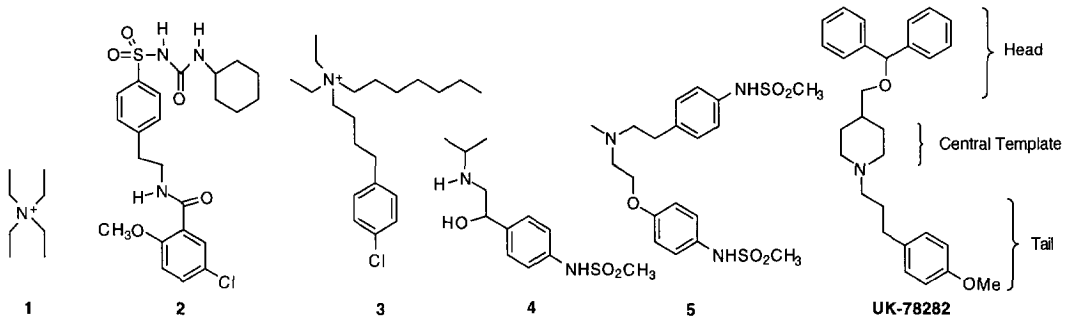


1 2 3 4 5 UK-78282

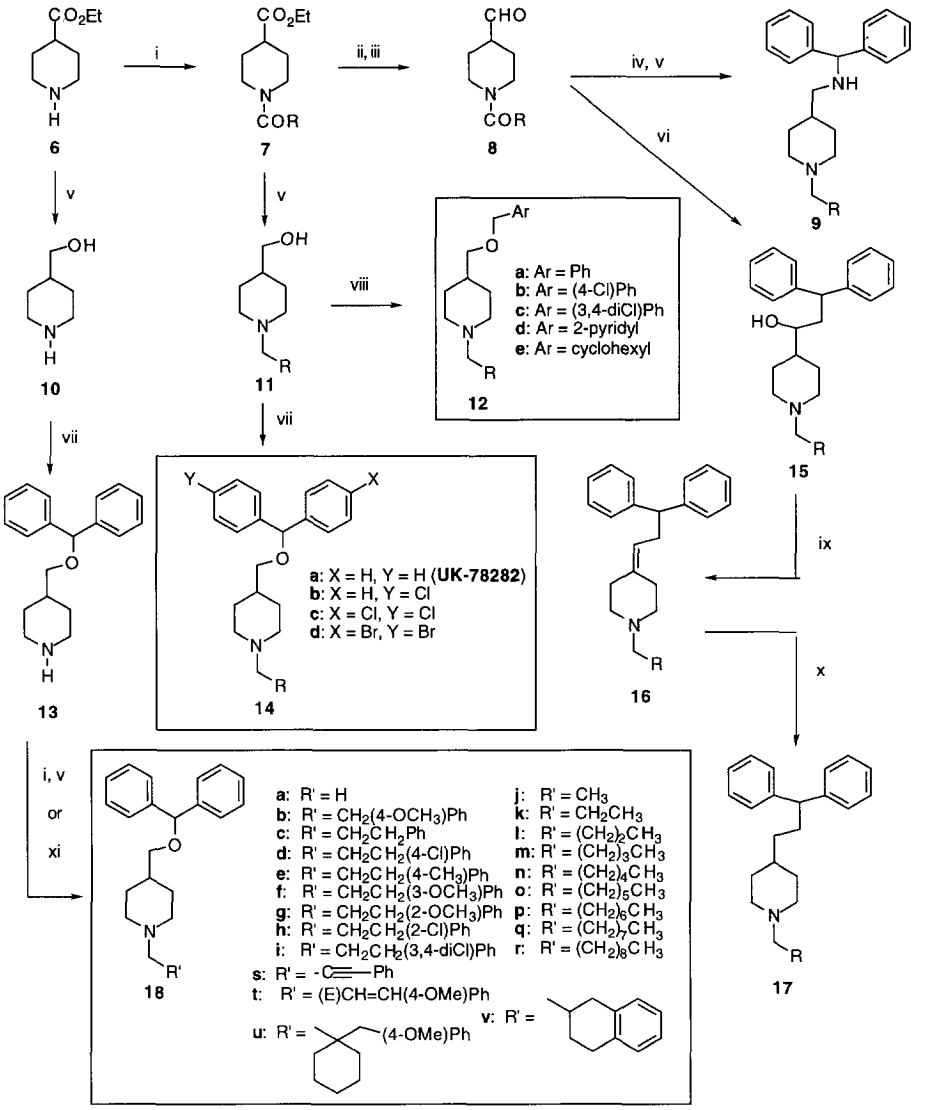
Results. UK-78,282 was divided into three regions for SAR investigation and interpretation: (1) head group, (2) central template, and (3) tail group (see above).

The synthesis of these compounds was generally straightforward with reasonable yields (50-70%) and is detailed in Scheme 1 (**R** = $CH_2CH_2(4\text{-}OCH_3)Ph$).[7] Ethyl isonipecotate (**6**) served as a versatile starting material for the preparation of almost all the head and tail group analogs. The major synthetic obstacle encountered in these manipulations was a reliable and safe method for the production of benzhydrol ethers from primary alcohols (**10** to **13** or **11** to **14**). Although the use of crystalline diphenyldiazomethane was successful, the preparation of this reagent resulted in the generation of large amounts of mercuric waste.[8] The application of a literature procedure[9] designed to trap the diphenylmethyl carbocation generated from benzhydrol (Ph_2CHOH) under acidic conditions (H_2SO_4) worked in low yields (<25%). Ultimately, a variation of this latter procedure (benzhydrol with catalytic p-toluenesulfonic acid in benzene at reflux) with the pre-formed hydrochloride salt of **11** resulted in good yields of the desired ethers (**14a** 75%; **14b** 72%; **14c** 77%; **14d** 64%).

In the head group of piperidine UK-78,282, removal of the benzhydryl ether (**11**) resulted in a dramatic drop in potency (see Table 1), whereas the mono-benzyl ether (**12a**) was intermediate in its potency. Substitution of the phenyl ring of the mono-benzyl ether was then briefly explored. The 4-chloro derivative (**12b**) restored potency and prompted the preparation of other Topliss tree[10] analogs including the 3,4-dichlorophenyl ether (**12c**), which showed improved potency. When this substitution was applied to the original benzhydryl series, however, the corresponding dihalogenated diphenyl ethers were not as active (**14c-d**).

Other head group variations included the replacement of the ether oxygen with nitrogen (**9**), which generated reduced potency, and the carbon analog (**17**), which was slightly more potent. Cyclohexyl and pyridyl replacements were also weak blockers (**12d**, **e**) and a compound that restricts the geometry of the carbon tether was also prepared (**16**) with no change in potency.

In the tail group of UK-78,282, replacement of the p-methoxy phenpropyl unit with a methyl (**18a**) resulted in a loss of potency (Table 2). Similarly, contraction of this three carbon tether (**18b**) or removal of the para methoxy substituent (**18c**) compromised channel blockade. The preparation of Topliss aryl substituents (**18d-i**) revealed that the 4-methyl analog was a slightly better channel blocker than UK-78,282.



Scheme 1: R = CH_2CH_2(4-OCH_3)Ph. (i) RCOCl, TEA, THF; (ii) $LiBH_4$, THF; (iii) Swern; (iv) RNH_2, $HB(OAc)_3$, AcOH; (v) $LiAlH_4$, $AlCl_3$ (3:1), THF, Δ; (vi) $RMgBr$, Et_2O; (vii) $Ph_2C{=}N_2$, benzene, Δ; **OR** Ph_2CHOH, p-TsOH (cat.), benzene, Δ; (viii) KH, $ArCH_2X$; (ix) Burgess Reagent; (x) H_2, 5%Pd/C, EtOH; (xi) RCH_2X, K_2CO_3, DMF, Δ.

Compound	IC_{50} ± SEM (n) (Rb Efflux, μM)
11	>20 (2)
12a	2.1 ± 0.5 (4)
12b	0.5 ± 0.1 (2)
12c	0.2 ± 0.0 (3)
12d	5.9 ± 2.8 (3)
12e	3.0 ± 0.5 (2)
14a	0.4 ± 0.2 (35)
14b	0.2 ± 0.0 (2)
14c	1.1 ± 0.1 (2)
14d	3.2 ± 0.3 (2)
9	2.8 ± 1.6 (4)
15	1.5 ± 0.4 (4)
16	0.4 ± 0.2 (4)
17	0.2 ± 0.1 (3)

Table 1
IC_{50} Values for Blockade of Human T Cell ^{86}Rb Efflux [5]

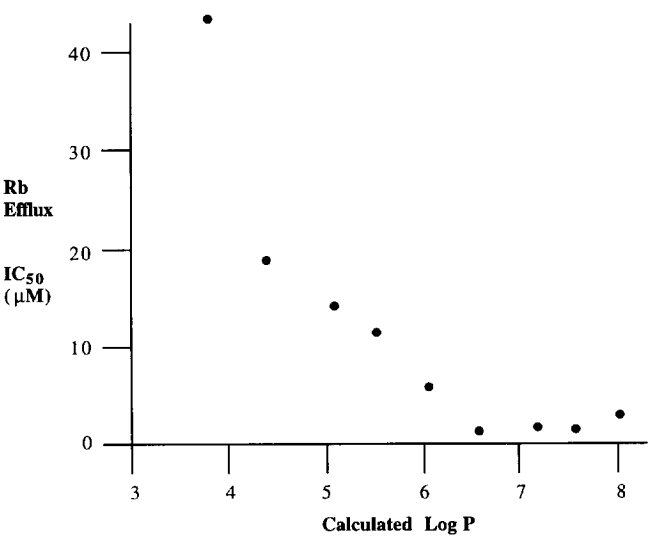
Although this SAR was not very pronounced, there were indications that the degree of lipophilicity in the tail group influenced channel blockade. A series of compounds in which the tail group of UK-78,282 was replaced with simple alkyl chains (**18j-r**) shows a relationship between channel-blocking activity (inhibition of Rb efflux) and lipophilicity (cLog P)[11], as shown in Figure 1.

As in the head group series of analogs, conformational rigidity in the tail group was also explored. However, several spatially demanding variations of the UK-78,282 tail (**18s-v**) were found to be only moderately active.

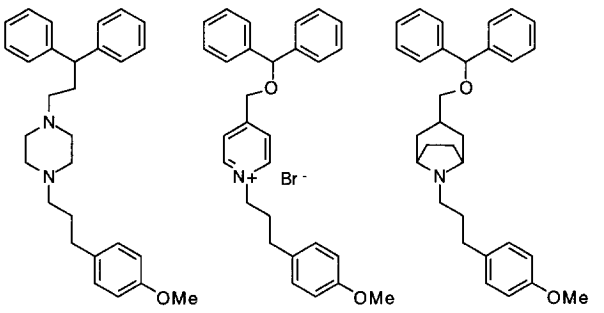
Table 2 IC_{50} Values for Blockade of Human T Cell ^{86}Rb Efflux[5]

Compound	IC_{50} ± SEM (n) (Rb Efflux, μM)
18a	>20 (1)
18b	2.9 (1)
18c	0.7 ± 0.5 (3)
18d	0.5 ± 0.1 (3)
18e	0.2 ± 0.0 (2)
18f	1.5 ± 0.4 (4)
18g	2.4 ± 0.6 (4)
18h	0.8 ± 0.4 (2)
18i	2.2 ± 0.2 (2)
18j	>20 (2)
18k	17.8 (1)
18l	14.0 ± 2.1 (2)
18m	10.8 ± 0.5 (2)
18n	5.5 ± 0.7 (2)
18o	0.5 ± 0.1 (2)
18p	0.7 ± 0.2 (3)
18q	0.7 (1)
18r	2.6 ± 0.7 (2)
18s	>20 (3)
18t	4.3 ± 0.9 (3)
18u	3.4 ± 3.0 (2)
18v	5.6 ± 1.9 (2)

Figure 1



Finally, the SAR of the piperidine template of UK-78,282 was investigated (see below). Piperidine replacement with a piperazine (**19**) or pyridine ring (**20**) generated a loss of activity, whereas a more conformationally rigid tropane replacement (**21**) maintained activity.



19 IC_{50} = 2.7 μM ± 0.6 μM (2)

20 IC_{50} > 20 μM (2)

21 IC_{50} = 0.4 μM ± 0.2 μM (4)

Discussion. From the SAR generated around UK-78,282, the essential pharmacophore that emerged consists of a benzhydryl (or mono-benzyl ether) head group and a lipophilic tail connected to a basic nitrogen embedded within a flexible framework. From the results in Table 1, the importance of the benzhydryl head group seems apparent (**14a** vs. **12a** or **11**). Evidence has been reported for a specific molecular interaction between a benzhydryl moiety and a histidine residue in a G-protein-coupled receptor,[12] and we hypothesized that a similar interaction may be occurring between histidine 404 in the pore region of the Kv1.3 channel and the benzhydryl group in the UK-78,282 series of channel blockers.[6] The importance of a $\sigma+$ $\pi+$ substitution was implied from the data on mono-benzyl ethers in Table 1 (**12a-e**). However, when this substitution was applied to the benzhydryl group (**14b-d**), it provided compounds of either equal or greatly diminished activity, indicating that perhaps these two series of inhibitors are binding at different sites or that overall lipophilicity (π) is also an essential parameter. Finally, the lack of a significant binding interaction for the ether oxygen in UK-78,282 (**14a**) was illustrated by the carbon analog **17**.

In the tail group of UK-78,282, lipophilicity was determined to be the most important physical property (Table 2). Not only did a Hansch analysis indicate a $\pi+$ dependence, but the effect of cLog P on activity (Figure 1) illustrates that hydrophobicity is necessary for K^+ channel blockade. The necessity of a basic nitrogen in order to achieve significant activity was implicit from the results of our initial high-throughput screen, and the inactivity of pyridine salt **20** supported this conclusion. Due to the conformational flexibility of these channel blockers, computational methods were not successful in defining an optimal spatial disposition of the three binding elements in the essential pharmacophore. Moreover, no significant insights were gleaned from the preparation of several conformationally biased compounds (**16**, **18s-v**, and others not shown). Recent studies have clearly demonstrated that UK-78,282 is a relatively selective use-dependent blocker that also binds to the closed state of Kv1.3.[6]

In summary, UK-78,282 was identified as a moderately potent and selective blocker of the Kv1.3 voltage-gated human T cell potassium channel. The SAR around this piperidine revealed three crucial elements for activity: a benzhydryl head group, a lipophilic tail, and a central basic nitrogen.

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