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Use of Kv1.3 Blockers for Inflammatory Skin Conditions

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Abstract

Recent results using animal models of inflammatory skin conditions have shown that blockers of the voltage-gated potassium channel, Kv1.3 hold great promise for clinical utility. Kv1.3 blockers act as immunosuppressants by modulating the various subsets of inflammatory T and B cells involved in autoimmune disorders. While peptidic inhibitors based on naturally occurring venoms demonstrate potent and selective Kv1.3 blockade, these require parenteral administration and may face potential immunogenicity problems. Small molecule blockers show considerable diversity, however selectivity over other Kv1- family channels has been difficult to achieve. More recent advances have added to the evidence that Kv1.3 channels are a suitable therapeutic target and that the development of novel and selective agents will herald new drugs for inflammatory skin disorders.

Keywords

Psoriasis; Kv1.3 channel; inflammation; drug design; autoimmune disorder

Introduction

Autoimmune disorders can be treated using immunosuppressive agents that also address aspects of the chronic inflammation associated with these illnesses. Quite often these drugs are non-specific in their mode of action resulting in various side effects. In extreme cases, the compounds compromise the immune system making it difficult to fight infections. For example, natalizumab (Tysabri), which is used to treat multiple sclerosis (MS) and Crohn's disease by preventing T cells from leaving the vasculature and entering tissue, has been associated with patients developing progressive multifocal leukoencephalopathy (PML), a rare and typically fatal viral infection [1,2]. These disproportionate effects of some immunosuppressants indicate that there is a clear need for agents that are more specific for treating autoimmune disorders. Numerous companies are involved in developing immunomodulatory agents with the potential market expected to reach $50 billion in the coming years [3].

Psoriasis is one of the most common autoimmune-mediated skin disorders, affecting about 2.0- 2.5% of the world's population [4–6]. The most common form of psoriasis is plaque psoriasis which is characterized by red, elevated patches of skin often covered with silvery scales. Other forms of psoriasis include flexural, pustular, guttate, nail and erythrodermic

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psoriasis as well as psoriatic arthritis [7]. In some cases plaque psoriasis and other variants can coexist or overlap in the same individual. Since most suffers remain affected for the remainder of their lives psoriasis is associated with a significant reduction in the quality of life and an enormous economic burden on the health care system for the “biologics” that are currently increasingly used for treatment [8,9].

The majority of individuals with psoriasis are mildly affected by the disease and are most frequently treated with topical agents including emollients, corticosteroids, retinoids and vitamin D_3 analogues [10]. Less frequently used topical compounds include coal tar and dithranol (anthralin), which have low cosmetic appeal and concerns regarding their safety. Of the standard treatments, these have been associated with a range of side effects such as skin irritation and variable efficacy [11]. When topical treatments prove ineffective, phototherapy either in the form of ultraviolet B (UVB) or ultraviolet A following pretreatment with an orally administered photosensitizer such as methoxsalen (i.e. PUVA therapy) can be used [12]. Unfortunately, the risk of non-melanoma skin cancer is marginally increased in these patients [13]. Patients with severe psoriasis that do not respond well to either topical treatment or phototherapy may be treated systemically with retinoids or immunosuppressants [10]. Methotrexate is able to inhibit both DNA synthesis and neutrophil chemotaxis [14] and is the most commonly prescribed systemic immunosuppressive drug used for psoriasis. Long term use of methotrexate can lead to hepatotoxicity and myelosuppression requiring patients to undergo regular monitoring [15]. Due to general advances in the field of immunology many monoclonal antibodies targeting specific immune cells surface receptors or cytokines have been introduced for the treatment of rheumatoid arthritis and psoriasis in the last 5 years. This new class of agents is referred to as “biologics”[16] and includes the TNF-α neutralizing agents etanercept, infliximab and adalimumab which have been approved by the FDA for treatment of psoriasis [17]. While these agents have greatly improved the management of psoriasis and other autoimmune diseases, their long-term therapeutic efficacy in psoriasis is only 60% and more trials are needed to firmly establish them as being beneficial relative to their risk of side effects such as increased rates of serious infections and cancer. In addition, their high cost makes them inaccessible to most individuals. As a consequence, there is a need for improved treatments that are low in cost with acceptable side effect profiles. One rational approach is to seek new targets that affect the underlying immune mechanisms of psoriasis in a more specific manner. This review will focus on one such target, the voltage-gated potassium channel Kv1.3, which plays an important role in the activation and proliferation of so-called effector memory T cells [18,19]. Small molecule inhibitors of Kv1.3 will be outlined together with the more recent patent literature. Finally, the role of serendipity will be explored in the discovery of Kv1.3 blockers and we will introduce our current work in this field.

Pathogenesis of psoriasis

While the precise mechanisms for psoriasis pathogenesis is still unknown, it is clearly associated with an overactive immune system triggered by the activation of T lymphocytes resulting in keratinocyte hyperproliferation [20,21].

T cell mediated immune responses in psoriasis

Psoriasis has a complex pathology with genetic, immunologic, and environmental factors all contributing to its pathogenesis [22,23]. However, activated T cells seem to play a crucial role, which is strongly substantiated by the following observations: (i) immunotherapy targeted against $CD4^+$ T cells clears active plaques of psoriasis [24] and (ii) in SCID mice, transplanted nonlesional psoriatic skin converts to a psoriatic plaque subsequent to intradermal administration of activated T cells [25]. T cell activation during psoriasis is most likely the result of an unknown antigen triggering the immune response through antigen

presenting cells (APC, primarily dendritic cells) [16]. This interaction between the APC and T cells activates an immune response resulting in T cells migrating to the dermis and epidermis. Once there, they are reactivated and secrete cytokines, which induce other cells to produce cytokines (e.g. TNF-α, IL-8 and GM-CSF) altering the pathology of the keratinocytes. This cascade of events leads to the excessive proliferation seen in psoriatic plaques [16]. Additionally, in a normal immune response, antigens are eliminated by T cell-stimulated pathways in the skin leading to the termination of the immune response. In psoriasis, this process persists chronically due to an irregularity in the feedback cycle [16,21]. A functional analysis conducted on isolated and cloned T cells obtained from psoriatic skin lesions showed that they were capable of evoking epidermal keratinocyte proliferation by secreting a variety of mediators including IFN-γ, a cytokine implicated in inflammation and T cell proliferation [26].

Central memory and effector memory T cells

Two subsets of human memory T cells; central memory (T_{CM}) and effector memory (T_{EM}) cells have been described based on the expression of the cell surface protein C-C chemokine receptor type 7 (CCR7) [27]. Naïve and memory T cell numbers are roughly maintained in constant numbers to initiate effective immune responses to new antigens whilst keeping high levels of memory cells to deal with previously encountered pathogens [28,29]. CCR7 is used by naïve T cells to gain access into lymph nodes, where they then encounter antigen and are activated to become naïve-effectors that proliferate and secrete inflammatory cytokines. Naïve-effectors then leave the lymph node for the site of antigenic challenge where they are able to carry out their protective role [30]. A number of naïve cells differentiate into long-lived T_{CM} cells, which retain a memory of the specific antigen. These T_{CM} cells also use CCR7 to enter the lymph node where they transform into T_{CM}-effectors after antigen encounter and then migrate to inflamed tissues [30,31]. A repeated antigenic stimulation arising from a chronic infection or autoimmune disease can induce T cells to differentiate into T_{EM} cells which do not need to migrate to lymph nodes for antigen induced activation [31]. T_{EM} cells can become activated in peripheral tissues or typical memory T cell compartments such as the lamina propria or subcutaneous fat pads and then become T_{EM}-effectors that quickly migrate to inflamed sites and secrete large volumes of cytokines to perform immediate effector functions [30].

Kv1.3 Potassium Channels

Role of potassium channels in T cells

Human T cells express two types of K^+ channels, the voltage-gated Kv1.3 and the calciumactivated KCa3.1 channel [32–34]. Both Kv1.3 and KCa3.1 play a role in regulating membrane potential and calcium signaling during the activation of T cells [34]. Upon engagement of the T cell receptor there occurs an immediate release of calcium through IP_3 receptors from the ER. The resulting depletion of the ER is “sensed” by the single-EF hand protein STIM1 and then initiates a process to assemble calcium-release activated calcium (CRAC) channels in the membrane of the cell [35–37]. Further calcium enters the cell through the CRAC channel ultimately leading to T cell proliferation though activation of calcineurin and the transcription factor NFAT [37]. However, this calcium influx, which is crucial to the process, is only possible if the T cells are able to maintain a negative membrane potential through a counterbalancing potassium efflux via Kv1.3 and/or KCa3.1 [34,38,39]. As has been known for some time, blockade of these potassium channels results in inhibition of T cell activation/proliferation and cytokine secretion [34,39,40].

Kv1.3 and KCa3.1 channels in naïve, T_{CM} and T_{EM} cells

Blockade of Kv1.3 or KCa3.1 channels would appear to be a suitable target for immunomodulatory agents, however we must be careful to avoid a general diminution of immune function. Fortunately, the expression of Kv1.3 and KCa3.1 channels differs across the various immune cell types [31,41]. Both Kv1.3 (250 channels/cell) and KCa3.1 (5–35 channels/cell) channel expression in the resting state of the cells is similar across the naïve, T_{CM} and T_{EM} cell types (Table 1) [18,30]. When activated, naïve and T_{CM} cells up-regulate KCa3.1 to ~500 channels per cell with little or no change in Kv1.3 expression [31,34]. However, in activated T_{EM} cells (both CD4+ and CD8+ subsets), Kv1.3 channels are increased to ~1500–2000 channels per cell with little or no change in KCa3.1 expression [18,30,31]. The dominant channel in T_{EM} cells is therefore the Kv1.3 channel which provides an attractive opportunity for intervention by therapeutic agents.

Potential use of Kv1.3 blockers in psoriasis therapy

Studies on the involvement of the different T cell subsets typically find that T_{EM} cells are present in the target tissue of autoimmune diseases and seem to play an important role in their pathogenesis [42]. For example, in post mortem samples from multiple sclerosis lesions T_{EM} cells are found in abundance [31]. Activated T cells that express CD2 and the IL-2 receptor CD25, and are mainly T_{EM} cells of the CD45RO+ phenotype are also found in psoriasis lesions [43]. Biopsies from psoriasis patients showed that during the early phase of the psoriatic process, CD8+ T cells along with $CD45RO^+$, $CD2^+$ and $CD25^+$ phenotypes are found in the skin [44]. The later phase of psoriasis involves $CD94^-$ and CD161-expressing cells [44]. The presence of the cells is also associated with the release of cytokines such as IFN-γ, IL-2 and TNF-α [45,46]. Given the cell types involved in psoriasis and the differential distribution of Kv1.3 channels in the active and inactive states of T cells, there is good reason to believe that Kv1.3 blockers could specifically suppress the disease-causing T_{EM} cells without significantly affecting naïve and T_{CM} cells. This rationale also forms the basis for treatment with the biologic Alefacept (anti-CD2) which reduces the levels of T_{EM} cells while leaving T_{CM} cells and naïve cells mostly unaffected [47]. The biologics used for psoriasis themselves can be classified into two groups; those that neutralize TNF-α (etanercept, infliximab, adalimumab) and agents that modulate pathogenically activated T cells (alefacept, efalizumab). A more extensive discussion on the biologics and psoriasis can be found in Sobell *et al.* [48,49]. Initial success with the use of biologic agents (particularly alefacept and efalizumab) in psoriasis strengthens the link between T cells and the disorder, and has provided further impetus to explore the Kv1.3 channel as a drug target.

Evidence showing the benefits of blocking the Kv1.3 channel for the treatment of autoimmune disorders has been accumulating for over two decades. The initial work in this area emerged from the use of various peptide toxins particularly following the report of an MS patient who experienced a two month remission from the disorder after being stung by a scorpion [50]. The scorpion toxin in this case probably was charybdotoxin (ChTX) [51] which blocks Kv1.3 channels (IC_{50} 3 nM) and depolarizes human T cells [52–54]. These peptide inhibitors are sourced mainly from venoms derived from sea anemones, scorpions, snakes and marine snails [30]. Particular focus has been placed on ShK (IC_{50} 11 pM) from the sea anemone *Stichodactyla helianthus* but a lack of selectivity for Kv1.3 led to the synthesis of numerous derivatives [55]. Of these analogues, ShK-186 has proven effective in experimental autoimmune encephalomyelitis [56], delayed type hypersensitivity (DTH) [56] and rheumatoid arthritis [57]. In addition, initial safety tests appear promising [57] and protective immune responses to acute infections like influenza and chlamydia were not compromised [56]. While selectivity has been improved for these peptides as well as their safety profile, the peptide-based inhibitors require parenteral administration and may face potential immunogenicity problems [57]. A detailed overview of peptide inhibitors can be

found in Rangaraju *et al.* [42]. However, despite the undoubted efficacy and selectivity of the peptide Kv1.3 blockers it would be desirable to have orally or topically available small molecule Kv1.3 blockers, which would avoid many of the hurdles facing peptide development.

Small molecule Kv1.3 blockers

The search for drug-like small molecule blockers of Kv1.3 channels is currently being undertaken by a number of groups in academia and industry. The first known inhibitors were standard potassium channel blockers such as 4-aminopyridine [40], tetraethylammonium and the calcium channel blockers verapamil, nifedipine and diltiazem [30]. In addition to these compounds many other small molecule inhibitors have been developed specifically targeting Kv1.3 channels. An occurring problem is the lack of selectivity of these compounds within the Kv1-family of potassium channels; in particular over Kv1.4 and Kv1.5 which are associated with cardiac function [32]. The following discussion provides a brief overview of each of the structural classes of small molecule Kv1.3 blockers highlighting the diversity of chemotypes and providing functional data where available. The reader is also directed to previous reviews on inhibitors of Kv1.3 channels in the following references [18,19,30,58]. This current review will primarily focus on series of compounds where SAR information is available. Other individual compounds will be mentioned where their structure is thought to contribute to the discussion.

Dihydroquinolines

To circumvent the potential drawbacks of peptidic Kv1.3 blockers, several groups have undertaken screening campaigns to find potent small molecule Kv1.3 channel blockers. The first nanomolar compound, WIN-17317 (**1**) (Figure 1), was found during a high-throughput ^{125}I-ChTXdisplacement screen at Sterling-Winthrop in 1995. While showing only modest affinity (~200 nM) this molecule was functionally active and inhibited T cell proliferation and IL-2 production [59]. Three other analogues were developed, CP-339818 (**2**), CP-393223 (**3**) and CP-394322 (**4**) (Figure 1) showing improved blockade of Kv1.3 channels (120 nM, 70 nM and 90 nM, respectively) [60]. Substitution at the N1 position of the dihydroquinoline ring influenced activity markedly with compound **5** (CP-393224) showing a 10–200 fold reduction in activity across a series of assays [60]. The reduction in activity was attributed to the reduced lipophilicity of the N1 substituent. While CP-339818 (**2**) was initially promising, this compound showed poor selectivity for Kv1.3 as it also blocked Kv1.4 at comparable concentrations [60]. The initial hit, WIN-17317-3 (**1**) was also later shown to block neuronal sodium channels with an IC_{50} of 9 nM, [61] suggesting that all Kv1.3 blockers belonging to this chemotype might have this problem.

Correolide

Correolide (**6**) (Figure 1), a natural product from the Costa Rican tree *Spachea correa* was discovered by scientists at Merck using a high-throughput ^{86}Rb-flux assay and found to block Kv1.3 with an IC_{50} of 90 nM [62]. Unfortunately, correolide was not specific for Kv1.3 as it bound with equal potency to all Kv1-family channels [63]. Other simplified analogues were synthesized including the C18-correolide analogue 43 (**7**) (Figure 1) which inhibited Kv1.3 with an IC_{50} of 37 nM in ^{86}Rb-flux assays [64]. To date, the Kv1.3 specificity of this compound has not been reported as the synthesis of these analogues is hampered by the limited supply of the natural parent compound [64].

Benzamides

Further ^{86}Rb-flux screening by Merck identified PAC (**8**) (Figure 1), a cyclohexyl-substituted benzamide, which blocked Kv1.3 with an IC_{50} of 270 nM. A more potent

derivative *trans-N*-propylcarbamoyloxy- PAC (**9**) (Figure 1), was subsequently synthesized showing improved activity (IC_{50} 50 nM) [65]. Once again only modest selectivity (2–6 fold) over other Kv1 channels was exhibited by these compounds. From an SAR perspective, removal of the phenyl ring, methylation of the amide nitrogen or replacement of the amide carbonyl with a sulfonyl group significantly reduced activity. Modifications to the 2-position of the 2-methoxy phenyl ring were tolerated while substituents in the 3 or 4 position reduced potency. The *trans* C1 carbamate compounds also showed better selectivity for Kv1.3 over the other Kv1 channels, although at present this appears to be insufficient for clinical utility [65].

Piperidines

UK-78,282 (**10**) (Figure 2), a piperidine blocker of Kv1.3 was identified by Pfizer following a large scale screen using a high-throughput human T cell ^{86}Rb efflux assay. This compound blocks Kv1.3 with an IC_{50} of 200 nM showing 100-fold selectivity for Kv1.3 over most other Kv1-family channels, the only exception being Kv1.4. Albeit a somewhat large molecule (MW 429.6), this compound was chosen as a lead to develop more potent and selective Kv1.3 blockers. The SAR exploration focused on three regions; the head group, central template and tail. The benzhydryl head group was shown to be required for good activity while replacement with a simpler benzyl group (CP-190,325, **11**) (Figure 2) reduced activity about 10 fold [66]. The ether oxygen in the head group could be replaced with a carbon atom showing a very marginal increase in activity. Replacement of the central template with piperazine or pyridine lost potency, however a tropane ring maintained activity (400 nM). Deviation away from a para-methoxy phenpropyl group in the tail section of the molecule also reduced potency. While there was a very weak relationship between activity and lipophilicity, the logP of UK-78,282 is estimated to be over 7.0 highlighting the extreme hydrophobicity of these molecules [67]. Interestingly, competition experiments suggest that UK-78,282 can bind to residues at the inner surface of the channel, overlapping with the site of action of verapamil.

Phenyl Stilbenes

In 1999 Chamberlin and Lew, researchers at the University of California, Irvine, used a *de novo* ligand based design method to design a new class of micromolar Kv1.3 blockers [68]. A model of the outer vestibule of the Kv1.3 channel was generated and compounds were designed to interact with residues known to be important for toxin binding (Gly380, Asp383 and His404) [69]. Fragments of compounds were joined through suitable spacer groups to form single molecules, which led to the selection of a phenyl stilbene scaffold (**12**) (Figure 2) for further elucidation. Parallel combinatorial synthesis was undertaken to produce a library of 400 compounds where a number of these compounds showed low micromolar activity in a ^{125}I-ChTX displacement assay. Compound **13** (Figure 2) was the most active compound (IC_{50} 2.9 μM) [68] although it has an estimated logP of over 8.0.

Psoralens

Starting from the natural product 5-methoxypsoralen (5-MOP, **14**) (Figure 2) a number of highly potent Kv1.3 blockers were developed by groups at the University of California, Davis and the University of Kiel. The template 5-MOP was initially extracted from the common rue, a plant that had been reported to have beneficial effects in multiple sclerosis, and was found to have modest activity on the neuronal Kv1.2 and the T cell Kv1.3 channel [70]. The development of 5-MOP analogues followed where the methyl group in the 5 position was replaced with a series of substituents of various structure and length. The simplest derivative, Psora-1 (**15**) (Figure 2), which contains an additional phenyl ring on a single methylene linker, already showed nanomolar activity (IC_{50} ~ 350 nM). The optimal

length for the chain linking the psoralen and aromatic rings was found to be four carbons (Psora-4, **16**, 3 nM) [71]. Selectivity of Psora-4 against Kv1.5 was poor however, and this led to the design of PAP-1 (**17**) (Figure 2) which has comparable Kv1.3 potency (2 nM) but showed greater selectivity (23-fold over Kv1.5) [72]. PAP-1 also demonstrates 33–125 fold selectivity over related Kv1-family channels and 1000-fold selectivity for more distantly related K^+ channels like KCa3.1, Kv2.1, Kv3.1 and Kv11.1 (HERG).

PAP-1 has also been considered as a potential human therapeutic agent and has undergone preliminary screens showing that it did not exhibit any cytotoxic or phototoxic effects [72]. This study also demonstrated that PAP-1 was able to suppress the activation of $CCR7^-$ human T_{EM} cells (IC_{50} 10 nM) and prevented DTH in Lewis rats dosed either orally or by i.p. injection (at 3 mg/kg). Both effects were ascribed to the Kv1.3 blocking action of PAP-1. The action of PAP-1 in allergic contact dermatitis was further investigated in a rat model using the skin sensitizer oxazolone [73]. Immunohistochemistry showed that PAP-1 was able to prevent $CD8^+$ T cells from infiltrating the skin and reduced the production of the inflammatory cytokines, IFN-γ, IL-2 and IL-17. Topical application also proved effective which is encouraging for a drug that may have clinical utility in dermatology settings [73]. This study further showed that PAP-1 did not act as a skin sensitizer or skin irritant, which is a prerequisite for a topical drug. Taken together this data shows that PAP-1 has great potential for development as a topical drug for the treatment of psoriasis.

Khellinones

In 2004, researchers at the Walter and Eliza Hall Institute (WEHI) of Medical Research in Australia developed two novel classes of Kv1.3 blockers from khellinone (1-(6-hydroxy-4,7- dimethoxy-3a,7a-dihydrobenzofuran-5-yl)ethanone) (**18**) (Figure 3), itself a weak Kv1.3 blocker (IC_{50} = 45 μM) [74]. These two classes were khellinone dimers and khellinone chalcones. When khellinone was dimerised at the 6-hydroxy position with a ρ-xylene linker to yield **19** (Figure 3), the potency was greatly increased to an IC_{50} of 280 nM. Using aliphatic linkers the authors demonstrated that optimal activity was a balance between chain length and the flexibility of the linker. Introduction of an ether into the chain reduced activity suggesting hydrophobicity also influenced biological activity. **19** itself has 10-fold selectivity for Kv1.3 over the related channels, Kv1.1, Kv1.2 and 3-fold selectivity over Kv1.5 [74]. The khellinone chalcones were formed by the reaction of aryl aldehydes with the 5-acetyl group of khellinone. Interestingly, the khellinone chalcone **20** (Figure 3) was found to block Kv1.3 with an IC_{50} of 400 nM. This compound has 3-fold selectivity over Kv1.1 and has no observable effect on Kv1.2 channels as well as 20-fold selectivity over Kv1.5 [74].

Further work by this group on the khellinone scaffold yielded derivatives selectively alkylated on either the 4- or 7-position phenolic groups [75]. Once again submicromolar activity was found with modest selectivity over other potassium channels. Compounds **21** (IC_{50} 480 nM) and **22** (IC_{50} 400 nM) represent the most potent of the derivatives modifying the 4 and 7-positions, respectively (Figure 3). The SAR demonstrated that a range of substituents were tolerated on the benzyl group such as halogen atoms. The correlation between the activities of 19 pairs of derivatives (i.e. comparing the same substituent either in the 4 or 7 position) gave an R value of 0.74 possibly suggesting a common binding mode between these series of compounds.

Recent patents from the WEHI illustrate continued interest in khellinone and related scaffolds [76–78]. In the first of these patents further substitution on positions 5, 6 and 7 of the khellinone ring led to highly potent compounds [76]. **23** was able to block the Kv1.3 channel with an IC_{50} of 110 nM. Optimisation within this series led to **24** where replacement

of the methoxy substituent with a methyl group and the morpholine ring with tetrazol-5-amine gave a 5-fold increase in activity (EC_{50} = 23 nM) (Figure 3) [76].

In a separate patent filing, synthesis of a series of chromenone derivatives were described using the natural product khellin (**25**) (Figure 3) as the scaffold [77]. Khellin is found in the plant *Ammi visnaga* and is structurally related to khellinone (**18**). Once again a phenoxypropyl side chain was used together with a morpholine group which showed reasonable potency (**26**, IC_{50} = 190 nM). In accord with the khellinone series a methyl substituent was more potent than a methoxy group and branching on the phenoxypropyl chain improved activity (**27**, IC_{50} = 14 nM) (Figure 3) [77]. The phenoxypropyl side chain is of course reminiscent of the series of compounds based on 5-MOP (e.g. Psora-4, **15**). Indeed, it would be interesting to speculate what activity and/or selectivity could be obtained if the same side chain as PAP-1 were adopted within this series of compounds.

One further patent has shown the furan ring of khellinone can be opened with little affect on potency. For example, compound **28** (Figure 3) employed a related branched phenoxypropyl chain to give a potency that was quoted as less than 50 nM. Importantly for this compound it showed 45- fold selectivity over Kv1.1 and 37-fold selectivity against Kv1.5 [78]. Taking together all three patents [76–78] and the previous studies [74,75], it seems that the khellin scaffold can be trimmed back to a single aromatic ring and exploited to derive potent and reasonably selective Kv1.3 blockers. Recent disclosures from the company associated with the patents (Bionomics Ltd.) show that they are pursuing Kv1.3 blockers for a wide range of autoimmune disorders [79]. The compounds appear to have undergone preclinical testing and show efficacy in animal models of both MS and DTH [79].

Thienopyridines

In 2007, a novel class of Kv1.3 blockers was reported by scientists at Xention Ltd. [80] based on thienopyridines, a known class of ADP receptor/P2Y12 inhibitors [81]. Compound **29** showed modest Kv1.3 blockade (46% at 1 μM) while substitution of the pyridine with a thiophene ring (**30**) increased activity (91% at 1 μM) (Figure 4). Modifications to the phenyl ring were also tolerated and substitution of the thienyl group of **30** with a phenylbutyl chain (**31**) (Figure 4) maintained activity (83% at 1 μM) [80]. Unfortunately, this set of compounds show no selectivity over Kv1.5. Interestingly, the features of two aromatic systems separated by a chain of 4–6 atoms is found in many compounds as well as this thienopyridine set of molecules.

Tetraphenylporphyrins

In 2003, researchers at the University of California, Berkley reported the synthesis of a new class of ligands for Kv1.3 using a tetraphenylporphyrin core [82]. The overall design was based on the four-fold symmetry of the homotetrameric structure of the potassium channel. Three of the compounds potently displaced radiolabeled peptide toxins (**32**, 20 nM; **33**, 26 nM; **34**, 13 nM) (Figure 4), but inhibited Kv1.3 currents in patch-clamp experiments only at low micromolar concentrations. Although the tetraphenylporphyrins had originally been designed with the idea that their positively charged side chains could interact with four conserved aspartate residues in the outer vestibule in a four-fold symmetry, [82] later solid-phase NMR studies with a KcsA-Kv1.3 chimeric channel demonstrated that one of the four arms of the tetraphenylporphyrin actually penetrated into the selectivity filter [83] and that the compounds were standing upright in the pore like a cross instead of covering it as initially intended [82]. The highly charged nature of these molecules together with their large size will no doubt preclude them from being therapeutic agents, however they represent useful and interesting research tools and could potentially be used for the synthesis of metalloporphyrins for imaging and crystallographic studies.

Dihydrophenanthridines

In 2009, Pegaro *et al.* reported the discovery of two structurally new classes of Kv1.3 and KCa3.1 inhibitors based on a virtual high throughput screening approach [84]. A homology model of the Kv1.3 channel was created from the crystal structure of the bacterial KcsA channel and used for a virtual screen of around 3.3 million compounds. The search focused on the inner vestibule of the ion channel as this is thought to be the binding site for other Kv1.3 blockers such as verapamil and PAP-1 [71]. From a list of the best 500 docked structures, 37 were selected for patch-clamp testing based on structural diversity and docking scores. Two compounds including the dihydrophenanthridine derivative (**35**) and dibenzocycloheptanedione derivative (**36**) were identified giving IC_{50} values of 5 μM and 30 μM respectively (Figure 4). Variations on the 5–6 dihydrophenanthridine derivative at positions N5 and C6 were performed to yield a variety of compounds showing similar or slightly improved activity with **37** being the most potent (IC_{50} 0.71 μM). Compound **37** was also selective over KCa3.1 showing only 12% inhibition at 20 μM in patchclamp assays. The introduction of an additional methylene group in the main scaffold and derivatisation of the sulfonyl substituents led to other active compounds such as **38** (IC_{50} 5.8 μM) (Figure 4). Compound **38** also showed anti-inflammatory potential in a mouse DTH model, though this may be due to its affect on the IK1 channel (IC_{50} = 2.1 μM) as Kv1.3 is not upregulated in mouse T_{EM} cells. It has been proposed that these compounds be used as leads for further optimization [84].

Serendipity and new uses for old drugs

Most of the starting points for medicinal chemistry in the Kv1.3 field have come from testing known potassium channel blockers, extracting medicinal plants, screening of actual or virtual libraries or other rational approaches for the design of ligands. In other cases, findings have come from clinical observations. By way of example, in 1976 a 28-year old male with an inflammatory skin condition was treated with clofazimine (**39**) following the failure of standard therapies. In this case, the leprosy drug clofazimine was able to successfully treat the condition with only minor skin inflammation being observed one year after the treatment was stopped [85]. Testing in 2008 by Ren *et al.* identified clofazimine as a novel inhibitor of the TCR signaling pathway that worked via the blockade of Kv1.3 channels (IC_{50} 300 nM) and showed that it prevents the rejection of transplanted human foreskin in immunodeficient mice reconstituted with human T cells [86]. The potential link between Kv1.3 blockade and the treatment of an inflammatory skin condition with clofazimine is thus of great interest. It has even been proposed that clofazimine could be used as a new treatment for autoimmune diseases such as psoriasis [86]. When considering this data it is tantalizing to think that this provides consolidating evidence for the utility of Kv1.3 blockers in psoriasis.

In our laboratories we have followed up a similar success story on the use of diphenoxylate for inflammatory skin disorders. In the 1970's, physician Earl Lanier prescribed a 67-year old woman Lomotil (containing diphenoxylate, **40**) to treat her acute diarrhoea. Astonishingly, not only did the drug prove effective in treating her diarrhoea but the patient also experienced a four-month remission of her psoriasis [87]. A further eight patients were treated in open studies with a topical preparation of diphenoxylate. An improvement was observed in all cases after an average of four weeks with many of these patients having no reoccurrence of their lesions while others kept their psoriasis under satisfactory control [87,88]. At the beginning of the 1980's, Lanier subsequently conducted a larger clinical trial on 35 individuals with an array of inflammatory skin conditions using topical and oral preparations of diphenoxylate, yielding further successful results [89]. Again, many of these patients had no reoccurrence of their symptoms after a short time period while administering the diphenoxylate preparations [89]. However, since Lanier's work, these promising

observations have not been investigated further. Recent work in our laboratory exploring diphenoxylate (**40**) has found that this compound blocks Kv1.3 with an IC_{50} of 5 μM in patch-clamp experiments (unpublished results). The possibility exists that the anti-inflammatory action of diphenoxylate observed by Lanier was mediated by the blockade of Kv1.3 channels. Indeed, this is the present subject of our research representing a medicinal chemistry campaign to optimize the activity of diphenoxylate analogues for Kv1.3 activity and selectivity.

Molecular similarity

Our interest in diphenoxylate was sparked by the structure of verapamil (**41**) (Figure 4), itself a low micromolar Kv1.3 blocker (IC_{50} 6 μM) [90]. From a 2D perspective both molecules share a number of structural similarities such as the two aromatic regions at each end of the molecule, a cyano group and a basic nitrogen atom. In isolation this observation is tenuous, however we noted related groups in UK-78,282 (**10**) and often observed a separation of two aromatic regions by a chain of around 4–6 atoms in other potent Kv1.3 blockers like PAP-1 (**17**). Using the Phase software within the Maestro package (Schrodinger Inc. Portland, USA) we were able to generate a simplistic pharmacophore based on compounds **9**, **10**, **17**, **23**, **27**, **28**, **31**, **40** and **41**. This consists of two aromatic regions separated by 8.8 Å and a hydrogen bond acceptor feature (Figure 5A). While the model can accommodate a range of Kv1.3 chemotypes it only provides three features and is perhaps not specific enough for drug discovery purposes. As a general filter however, it may be useful to reduce the number of compounds selected for screening. Another feature of many Kv1.3 blockers is their high lipophilicity. Table 2 gives the calculated logP and $logD_{7.4}$ values of the compounds used to derive the pharmacophore. The average value of $logD_{7.4}$ across these compounds is 4.95 illustrating the high lipophilicity of these molecules. While the compounds listed in Table-2 might not be ideal for oral formulation because of their relatively high logP values, which typically result in large variations in oral availability and so-called “food-effects”, their lipophilicity makes them well suited for topical application. The optimal logP values for transdermal delivery have of course been shown to be in the range of 1–3 [91,92]. However, for the treatment of psoriasis the aim is not to deliver the compounds into the circulation through the skin (which would require a high permeability and mobility), but to deliver them to the thickened and inflamed epidermis and retain them there. As shown by Cross *et al.* the epidermis and dermis are actually much more lipophilic environments than originally believed and compounds with logP values of 4 and higher very effectively partition into the epidermis and dermis while leaving a considerable reservoir in the stratum corneum [93]. Other properties that are advantageous for dermal drug delivery include; molecular weight <500, high potency, melting point well below 200°C, non-irritant and non-immunogenic [92]. Many of the above mentioned Kv1.3 blockers fulfill many of these criteria and should therefore be suitable for topical use. PAP-1 (**17**) in fact has already been shown to effectively treat allergic contact dermatitis in rats following topical application [73] at 2% in the lipophilic crème base Eucerin®. (B) Diagram showing the structure of all four subunits of the Kv1.2 channel with the S1–S4 region colored green and the S5-P-S6 region in blue. The potassium ions are shown as yellow spheres while the T1 domain and β-subunit are colored magenta and gold, respectively. The cell membrane is shown in its anticipated position relative to the protein.

Alongside our computational work, Saini and co-workers [94] have applied QSAR analyses to a series of khellinone derivatives described by Baell et al. [74]. The equation that was generated for a set of 22 compounds showed that lipophilicity was a major factor in understanding their biological activity [94]. In addition, it was found that molar refractivity and polarizability played a lesser role and notably molecules that were too bulky were of

lower potency. While this work was able to provide a reasonable equation, the ability to employ this QSAR analysis for other series of compounds remains untested.

Design and future directions

Much of the discussion so far has focused on several series of small molecules with associated SAR but without any information on how they bind to the Kv1.3 channel. To better understand the target macromolecule it is necessary to look at the structure of potassium channels in general. However, with respect to crystal structures one needs to realize that the ion channel field significantly falls behind the kinase field and that we currently only have the structures of a few prokaryotic channels like KcsA [95] and one mammalian voltage-gated K^+ channel, the rat Kv1.2 channel [96,97]. Given the high sequence homology between Kv1-family members we should be able to use the 3-D coordinates of Kv1.2, which is assumed to have been crystallized in the open state, as an approximation to gain insights into the structure of Kv1.3. As shown in Figure 5B, Kv1.2 can be broadly "divided" into three parts: transmembrane (TM) domain, tetramerization domain (T1) and β-subunit. The TM domain consists of 24 membrane-spanning helices, 6 from each of the 4 identical α-subunits of the channel. Each 6TM α-subunit consists of a voltage-sensor domain comprising TM segments S1 to S4 and a pore domain composed of segments S5, S6 and a membrane-reentering P-loop. Below the selectivity filter (which in Figure 5B is shown filled with permeating K+ ions) is a hydrophobic cavity (inner vestibule) approximately 9 Å across, which is filled with water [96]. Below the membrane spanning part of the channel is the T1 region, which holds the channel tetramer together and which forms the docking platform for the β subunit for channels that have a β subunit. The β subunit is related to oxido-reductases and the crystal structure of Kv1.2 contains bound molecules of NADP+. This subunit may have a catalytic function and potentially could regulate the activity of the potassium channel [96].

In general, drugs modulating K_V channels can interact with these large proteins at multiple sites. Peptide toxins from the venoms of snakes, scorpions, sea anemones and cone snails bind to the outer vestibule of Kv channels and in most cases insert a lysine side chain into the channel pore to occlude it [98–100]. Spider toxins like hanatoxin, which typically contain a cluster of hydrophobic amino acids, partition into the membrane and interact with the voltage-sensor [101,102]. Small molecules like the hydrophobic cations tetrabutylammonium (which was co-crystallized with KcsA [95]) or verapamil (**41**) occlude the inner pore and have been proposed to project into "niches" between the N-terminal parts of S6 and the P-loop in the case of larger molecules like verapamil [19]. The inner pore of Kv channels can also be targeted by lipophilic molecules like correolide (**6**), which has been shown through a combination of mutagenesis [103] and molecular modeling to "snuggle" into the hydrophobic surface of the S6 helix with its lipophilic part and to chelate a permeating potassium ion with its polar acetyl groups [104]. Small molecules can further bind to the "gating-hinges" as in the case of the Kv7 channel activator retigabine, which has been found by mutagenesis to bind to a putative hydrophobic pocket formed upon channel opening between the cytoplasmic parts of S5 and S6 [105]. It is further possible for small molecules to bind at the interface between the α- and the β-subunit and disrupt their interaction [106,107]. Another interesting mode of interaction was recently identified for the polycyclic cigutera toxin gambeirol, which is produced by the dinoflagellate *Gambierdiscus toxicus.* This highly lipophilic molecule (estimated logP 5.41) inserts into the space between S5 and S6 outside of the permeation pathway on the side of the helices facing the lipid and prevents the channel from opening by stabilizing the closed state [108].

Of course it would be ideal to have co-crystals of all the different Kv1.3 blockers with the Kv1.3 channel to ultimately determine where they bind. However, this is currently not

feasible because of the great technical difficulties associated with crystallizing membrane proteins of the size of Kv channels and we believe the existing mutagenesis data is sufficient to demonstrate that at least correolide and verapamil bind in the inner vestibule. Because of the structural similarity of many of the compounds described above to verapamil it is reasonable to assume that compounds like PAP-1 (**17**) and UK-78,282 (**10**) also bind in the inner vestibule, but their exact orientation is currently impossible to predict without very detailed mapping studies. But it is of course also possible that some of the compounds discussed above bind to other sites, which could include:

- The side portals described by Long and co-workers [96] which lie above the T1 domain. These regions have a number of negatively charged amino acids that can attract compounds with positive charges.
- The outer pore
- The “inner” gating hinges, which form the site of action of retigabine.
- The voltage-sensor domain
- Interfaces between the TM domains into which lipophilic compounds could insert in a similar manner to gambeirol
- The β-subunit would theoretically be a possibility but is unlikely for any of the abovediscussed Kv1.3 blockers since most of the screening assays were performed on expression systems that only contained the α-subunit of the channel.

It may be that the binding sites do not include any of the suggestions above and will need to be identified experimentally for each compound class, ideally through a crystal structure, which then would allow true structure-based drug design. In the interim, classic medicinal chemistry efforts will be needed to optimize activity and selectivity as well as the physicochemical properties of suitable leads.

Conclusions

From the work conducted so far on Kv1.3 channels it is patently clear that this is a good target to pursue for autoimmune disorders such as psoriasis, MS and type-1 diabetes. Animal models have demonstrated *in vivo* efficacy and if we include unsubstantiated human clinical data, then the case is compelling to further explore Kv1.3 inhibitors as drugs. If we take as an example rat models of MS, chronic administration of ShK-186 was able to significantly reduce a set of key indicators for disease progression [109]. For a fuller discussion on the utility of Kv1.3 blockers in models of inflammation the following reviews are recommended [19,30,42,58]. For all researchers working in this field, a key transition is to successfully progress from animal models to properly conducted human clinical trials.

This review has highlighted a selected set of compounds where SAR data was available, representing a wide range of chemotypes. For many of the potent ligands, a common pharmacophore was found which included two aromatic regions. Use of the pharmacophore may help future design efforts and be of utility in identifying the binding site of these ligands on the K1.3 channel. The lipophilicity of these molecules was also discussed with regard to potential topical use. The range of physicochemical properties that are desirable for topical drugs was underscored which may form the basis of future strategies for Kv1.3 molecular design. Finally, to be able to contribute significantly to this research, structure-based drug design is needed, however this would require gargantuan efforts and is it likely that standard medicinal chemistry practices will dominate in order to optimize a molecule that will ultimately find its way to market.

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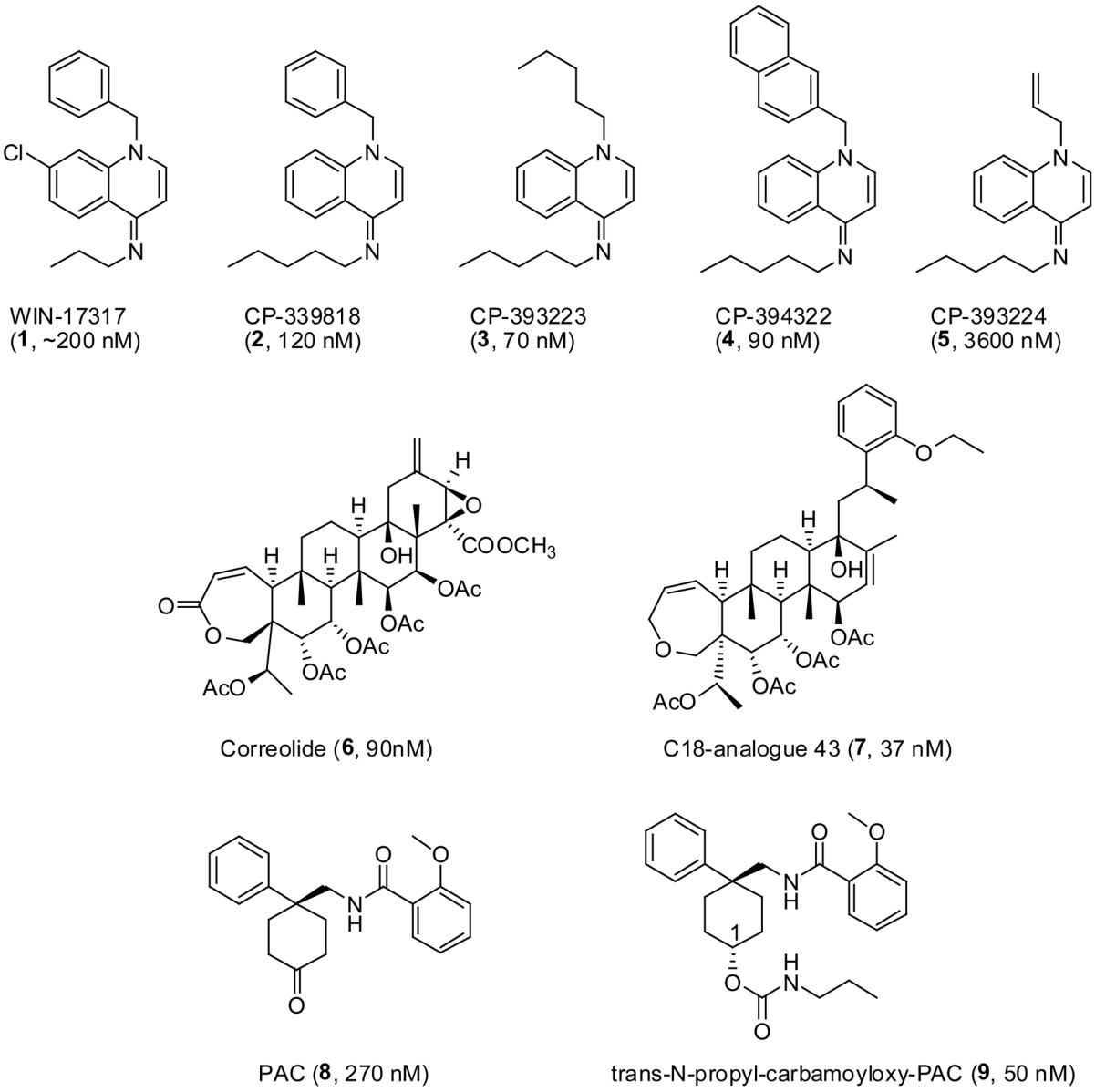

Figure 1.
Structures of the dihydroquinoline, correolide and benzamide analogues.

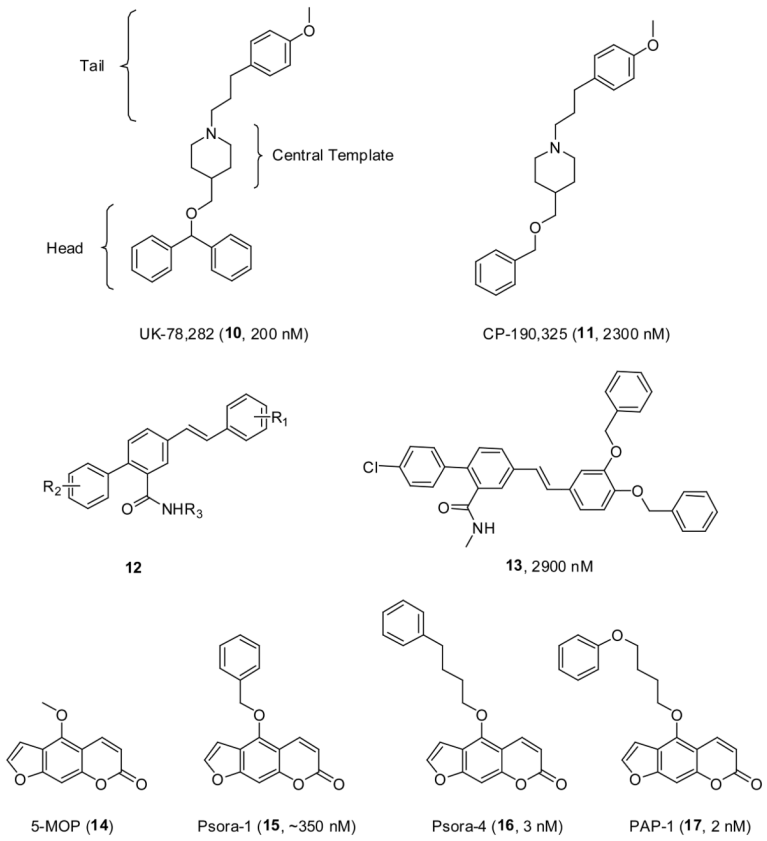

Figure 2.
Structures of the piperidine, phenyl stilbene and psoralen series of compounds.

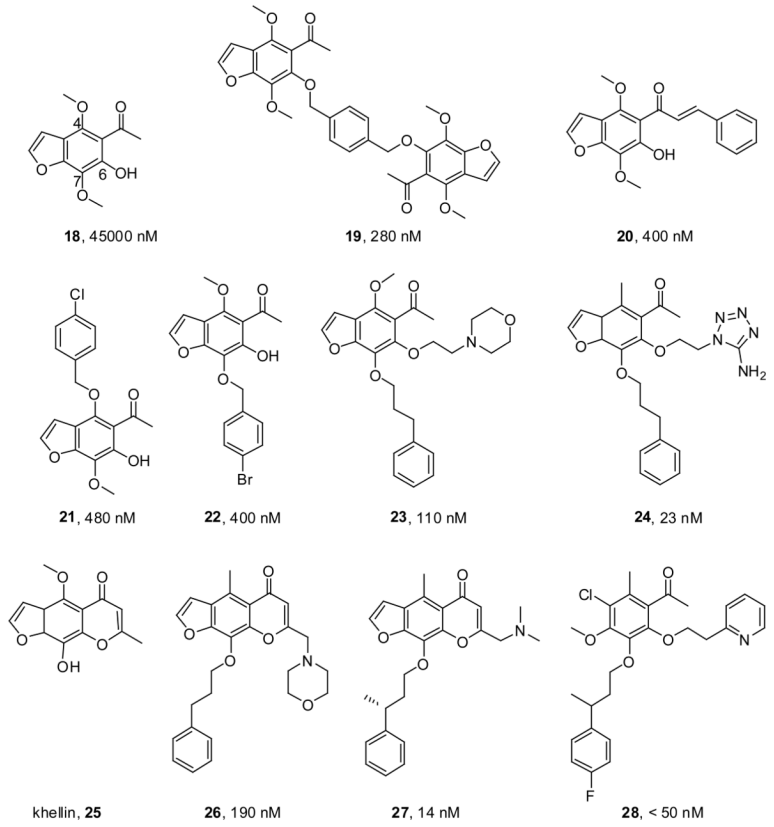

Figure 3.
Structures of compounds based on khellin and khellinone.

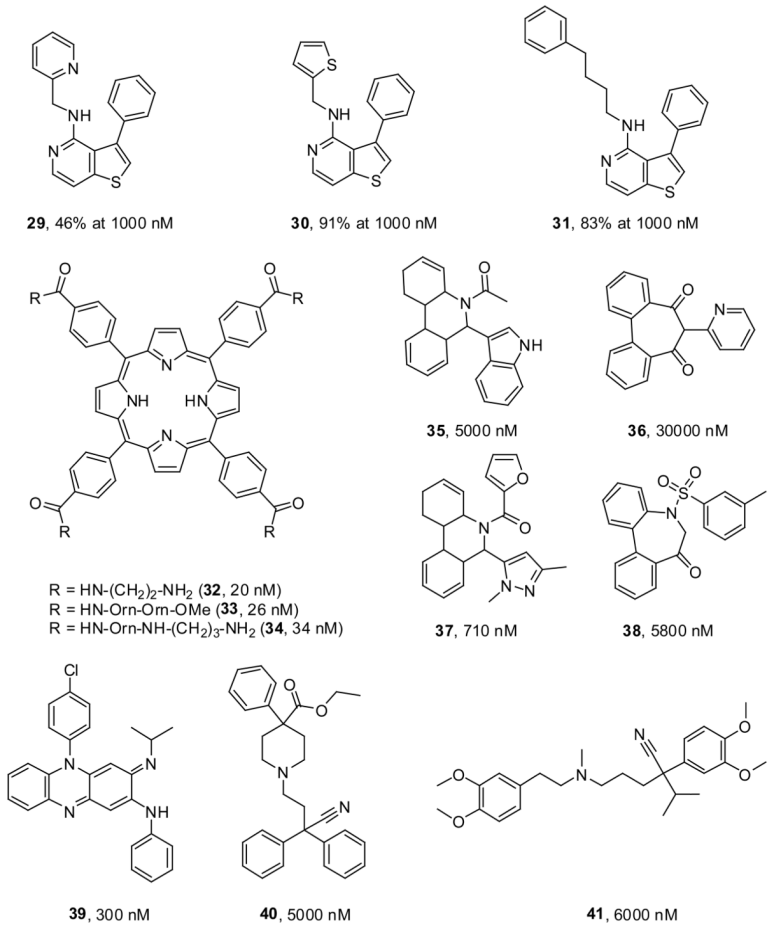

Figure 4.

Structures of the thienopyridine, tetrahydroporphyrin, dihydrophenanthridine derivatives together with established drugs that inhibit Kv1.3 channels.

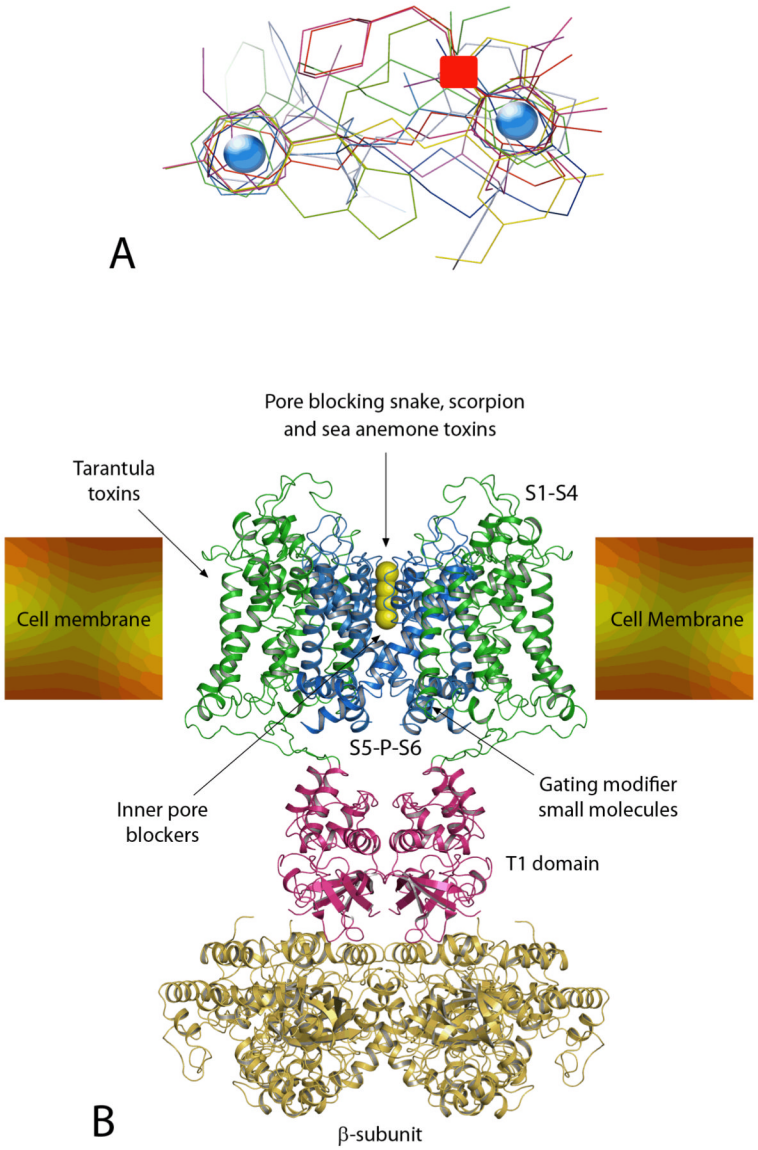

Figure 5.

(A) Pharmacophore model developed from compounds **9**, **10**, **17**, **23**, **27**, **28**, **31**, **40** and **41** featuring two aromatic groups (indicated with blue circles) and a hydrogen bond acceptor feature (red square).

Table 1

Distribution of potassium channels over T cell subtypes [34]. The values are given as the average number of channels/cell.

State	Channel	Naïve ($CCR7^+$ $CD45RA^+$)	T_{CM} ($CCR7^+$ $CD45RA^-$)	T_{EM} ($CD4^+CCR7^-$ $CD45RA^-$ and $CD8^+CCR7^-$ $CD45RA^-$; $CD8^+CCR7^-$ $CD45RA^+$)
Inactive	Kv1.3	250	250	250
	KCa3.1	5	5	35
Active	Kv1.3	300	300	1500
	KCa3.1	500	500	50

Table 2

Physicochemical properties of the compounds used to derive the pharmacophore model.

Compound number	ClogP	$ClogD_{7.4}$	MW
9	4.55	4.55	424.5
10	7.24	5.27	429.6
17	5.05	5.05	350.4
23	4.23	4.19	453.5
27	5.14	2.98	405.5
28	6.75	6.75	486.0
31	8.04	7.99	358.5
40	5.88	5.45	452.6
41	3.90	2.33	454.6