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K Channel Modulators for the Treatment of Neurological Disorders and Autoimmune Diseases

Heike Wulff, and Boris S. Zhorov

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K^+ Channel Modulators for the Treatment of Neurological Disorders and Autoimmune Diseases

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1. Introduction

Potassium channels are tetrameric membrane proteins that selectively conduct K^+ across cellular membranes. With 78 family members, K^+ channels make up about half of the extended superfamily of 143 voltage-gated ion channels in the human genome, the third largest family of signaling molecules, following G-protein coupled receptors and protein kinases.[1] K^+ channels have probably evolved from an ancestral gene encoding a simple 2-transmembrane segment (TM) protein, like the bacterial KcsA channel. Subsequent gene duplication or addition of a 4 TM voltage sensor domain or intracellular domains for ligand binding have produced a large family of extraordinarily versatile signaling molecules: 15 inwardly rectifying 2-TM K^+ channels (K_{ir}),[2] 15 two-pore 4-TM K^+ channels (K_{2P}),[3] 8 calcium-activated 6- or 7-TM K^+ channels (K_{Ca}),[4] and 40 voltage-gated K^+ channels (K_V).[5] Each of these 78 K^+ channels has a unique expression pattern allowing cells in a complex multicellular organism to "fine tune" their membrane potential and their excitability according to their respective physiological functions. Specific modulation of individual K^+ channel types therefore offers an enormous potential for the development of physiological tool compounds and new drugs. To name just a few examples, K^+ channel modulators are already clinically used as drugs for the treatment of type-2 diabetes and cardiac arrhythmia and are widely pursued in academia and the pharmaceutical industry as novel targets for epilepsy, memory disorders, chronic pain, cardiac and brain ischemia, hypertension, bladder over-reactivity, immunosuppression, and cancer. In this Review, we will first give an overview of K^+ channel pharmacology in general and then discuss the medicinal chemistry of the K^+ channels which constitute targets for the treatment of neurological disorders ($K_V7.2$–7.5, $K_{Ca}1.1$, $K_{Ca}2.1$–2.3) and autoimmune diseases ($K_V1.3$, $K_{Ca}3.1$) in more detail.

1.1. K^+ Channels as Drug Targets

The extracellular K^+ concentration of 4 mM is about 40-times lower than the intracellular K^+ concentration of 160 mM. The opening of K^+ channels consequently generates an efflux of positive charge, which hyperpolarizes or repolarizes the cellular membrane. In excitable cells, such as neurons or cardiac myocytes, K^+ channels are generally expressed together with voltage-gated Na^+ (Na_V) or Ca^{2+} (Ca_V) channels and are responsible for the repolarization after action potential firing. Pharmacological activation of K^+ channels in excitable cells therefore reduces excitability whereas channel inhibition has the opposite effect and increases excitability. For example the $K_V7.2/7.3$ activator retigabine acts as an anticonvulsant, while the unselective K^+ blocker 4-aminopyridine (4-AP) induces seizures.[6] In both excitable and nonexcitable cells K^+ channels further play an important role in Ca^{2+} signaling, volume regulation, secretion, proliferation, and migration. In proliferating cells, such as lymphocytes or dedifferentiated smooth muscle cells, K^+ channels are often found together with store-operated inward-rectifier Ca^{2+}channels like CRAC (calcium-release activated Ca^{2+} channel)[7,8] or transient receptor potential

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channels like TRPC1[9] and provide the counterbalancing K^+ efflux for the Ca^{2+} influx, which is necessary for cellular activation. In this case, K^+ channel inhibitors like the $K_{Ca}3.1$ blocker TRAM-34 inhibit proliferation.[10,11]

However, despite the fact that all K^+ channels in the human genome have been cloned and their biophysical properties characterized in great detail,[1] it still often is a challenge to precisely determine which of the 78 K^+ channels underlies a native K^+ conductance in a specific cell type. In contrast to voltage-gated Na^+ and Ca^{2+} channels, which contain four homologous domains in a single large polypeptide chain, K^+ channels consist of four individual α-subunits arranged circumferentially around a central pore as a homo- or heterotetramer. Each subunit includes six transmembrane α-helical segments S1−S6 and a membrane-re-entering P-loop (P) consisting of an extracellular S5−P linker (a turret), a pore helix (P-helix), an ascending limb containing the signature sequence TVGYG, and the extracellular linker P−S6.[12] Four voltage-sensing domains S1−S4 are linked to the pore-forming domain composed of four S5−P−S6 sequences.

Within a K^+ channel subfamily (see Tables 1−4), like the K_V1-family or the K_V7-family (KCNQ), the α-subunits can heterotetramerize relatively freely leading to a wide variety of different channel tetramers with different biophysical and pharmacological properties.[5] The properties of K^+ channel α-subunit complexes can further be modified by association with intracellular or membrane-spanning β-subunits. For example, K_V1-family channels interact through their N-terminal T1 domain with $K_V\beta1$–3 proteins, which form a second symmetric tetramer on the intracellular surface of the channel and modify the gating of the α-subunits. K_V4 channels interact with the so-called "K^+ channel interacting proteins" KChIp1–4, which enhance surface expression and alter the functional properties of the K_V4 α-subunits.[5] $K_{Ca}1.1$ (BK) channels associate in a tissue specific manner with four different 2-TM domain β-subunits β1–4 (KCNMB1–4),[13] which influence Ca^{2+}-sensitivity, inactivation, and in the case of β4, render the channel complexes resistant to the $K_{Ca}1.1$ blockers charybdotoxin and iberiotoxin.[14] The inward rectifiers $K_{ir}6.1$ and $K_{ir}6.2$ associate with two members of the ATP binding cassette proteins, the sulfonylurea receptors SUR1 or SUR2A/B to form ATP-sensitive K^+ channels in the pancreas or cardiovascular system.[15,16] In addition to "mixing" with α-subunits from the same channel subfamily and combining with different β-subunits, the α-subunits of many K^+ channel gene families can be alternately spliced to generate additional K^+ channel diversity. (The exceptions are the K_V1-family channels, which contain intronless coding regions.)[5] K^+ channel properties can be further altered through post-translational modifications like phosphorylation,[17,18] ubiquitinylation,[19] and palmitoylation.[20] In terms of drug discovery, this molecular diversity not only constitutes a considerable challenge but also offers opportunities for achieving tissue specificity by targeting tissue-specific β-subunits or for designing modulators that selectively target homotetramers over hetermultimers and vice versa.

However, rational design of selective K^+ channel modulators is difficult because there are currently no X-ray structures for medically important channels like $K_V11.1$ (hERG), $K_V7.1$–7.5, or $K_{Ca}1.1$. Investigators are therefore building homology models of these channels with inner-pore blockers based on the available X-ray structures of KcsA, MthK, K_VAP, and $K_V1.2$ and data from mutational studies.[21–27] Because the use of absolute numbers of residues, which are different in different channels, is inconvenient in a Review we are using a universal labeling scheme.[28] This scheme is shown in Figure 3A for $K_V1.2$, where residues facing either the inner pore or niches between the extracellular halves of S6s and P-helices are marked by asterisks. For well-known residues, we also show absolute numbers in brackets. The above-mentioned models visualize contacts between ligands and ligand-sensing residues, which were identified in mutational studies, and therefore are useful for designing new mutational experiments. However, experimental data on

Table 1. K_V Channels

channel	inhibitors	activators
K_V1.1 (KCNA1)	DTX, DTX-κ, ShK, HgTX, KTX, 4-AP, TEA	unknown
K_V1.2 (KCNA2)	ChTx, DTX, MTX, NTX, HgTX, 4-AP, TEA	unknown
K_V1.3 (KCNA3)	ChTX, MgTX, ShK, OSK1, HgTX, KTX, NTX, correolide, PAP-1	unknown
K_V1.4 (KCNA4)	UK78282, 4-AP	unknown
K_V1.5 (KCNA5)	AVEO118, S9947, clofilium, 4-AP	unknown
K_V1.6 (KCNA6)	HgTX, ShK, DTX, 4-AP	unknown
K_V1.7 (KCNA7)	flecainide, 4-AP	unknown
K_V1.8 (KCNA10)	Ba^{2+}, TEA, ketoconazole	cGMP
K_V2.1 (KCNB1)	hanatoxin, TEA	linoleic acid
K_V2.2 (KCNB2)	quinine, TEA	unknown
K_V3.1 (KCNC1)	4-AP, TEA	unknown
K_V3.2 (KCNC2)	ShK, verapamil, 4-AP, TEA	unknown
K_V3.3 (KCNC3)	4-AP, TEA	unknown
K_V3.4 (KCNC4)	BDS-1, TEA	unknown
K_V4.1 (KCND1)	4-AP, TEA	unknown
K_V4.2 (KCND2)	heteropodatoxins, PATX1, PATX2	unknown
K_V4.3 (KCND3)	PATX1, PATX2, nicotine	unknown
K_V5.1 (KCNF1)	K_V5 and K_V6 channels are not functional alone they coassemble with K_V2 subunits and act as modifiers or silencers	
K_V6.1 (KCNG1)		
K_V6.2 (KCNG2)		
K_V6.3 (KCNG3)		
K_V6.4 (KCNG4)		
K_V7.1 (KCNQ1, KVLQT)	L735821, chromanol 293B, mefloquine	L364373, mefenamic acid
K_V7.2 (KCNQ2)	XE991, linopirdine	retigabine, BMS204352, S-1
K_V7.3 (KCNQ3)	linopirdine, XE991	retigabine, BMS204352, diclofenac
K_V7.4 (KCNQ4)	linopirdine, XE991, bepridil	retigabine, BMS204352, S-1
K_V7.5 (KCNQ5)	linopirdine, XE991	retigabine, BMS204352, S-1
K_V8.1 (KCNV1)	K_V8 and K_V9 channels are not functional alone, coassemble with K_V2 subunits and modify their properties	
K_V8.2 (KCNV2)		
K_V9.1 (KCNS1)		
K_V9.2 (KCNS2)		
K_V9.3 (KCNS3)		
K_V10.1 (KCNH1, eag-1)	quinidine	unknown
K_V10.2 (KCNH5, eag-2)	quinidine	unknown
K_V11.1 (KCNH2, erg-1, HERG)	astemizole, BeKM-1, ergtoxin, E4031, sertindole, dofetilide, terfenadine	mallotoxin, RPR260243
K_V11.2 (KCNH6, erg-2)	sipatrigine	unknown
K_V11.3 (KCNH7, erg-3)	sertindole, pimozide	unknown
K_V12.1 (KCNH8, elk-1)	Ba^{2+}	unknown
K_V12.2 (KCNH3, elk-2)	none	unknown
K_V12.3 (KCNH14, elk-3)	Ba^{2+}	unknown

Table 2. K_{Ca} Channels

channel	inhibitors	activators
K_{Ca}1.1 (KCNMA1, BK, slo)	IbTX, ChTX, paxilline, slotoxine, TEA	NS1609, NS1619, BMS204352, DHS-1, estradiol
K_{Ca}2.1 (KCNN1, SK1)	UCL1684, apamin, tamapin, leiurotoxin, NS8593	NS309, DC-EBIO, riluzole, EBIO
K_{Ca}2.2 (KCNN2, SK2)	tamapin, apamin, leiurotoxin, UCL1684, Lei-Dab[7], NS8593	NS309, DC-EBIO, riluzole, EBIO, CyPPA
K_{Ca}2.3 (KCNN3, SK3)	leiurotoxin, apamin, UCL1684, NS8593	NS309, DC-EBIO, riluzole, EBIO, CyPPA
K_{Ca}3.1 (KCNN4, IK1, SK4)	MTX, ChTX, TRAM-34, ICA-17043	NS309, DC-EBIO, riluzole, EBIO
K_{Ca}4.1 (KCNT1, slack)	TEA, quinidine, bepridil	bithionol
K_{Ca}4.2 (KCNT2, slick)	TEA, quinidine	unknown
K_{Ca}5.1 (KCNU1, slo3)	TEA	unknown

Table 3. K_{ir} Channels

channel	inhibitors	activators
$K_{ir}1.1$ (KCNJ1, ROMK1)	tertiapin, Ba^{2+}, Cs^+	none
$K_{ir}2.1$ (KCNJ2, IRK1)	spermine, spermidine, Ba^{2+}, Cs^+	unknown
$K_{ir}2.2$ (KCNJ12, IRK2)	spermine, spermidine, Ba^{2+}, Cs^+	unknown
$K_{ir}2.3$ (KCNJ4, IRK3)	spermine, spermidine, Ba^{2+}, Cs^+	arachidonic acid, tenidap
$K_{ir}2.4$ (KCNJ14, IRK4)	Ba^{2+}, Cs^+	unknown
$K_{ir}3.1$ (KCNJ3, GIRK1)	Ba^{2+}, Cs^+	unknown
$K_{ir}3.2$ (KCNJ6, GIRK2)	tertiapin, halothane, Ba^{2+}, Cs^+	unknown
$K_{ir}3.3$ (KCNJ9, GIRK3)	none	unknown
$K_{ir}3.4$ (KCNJ5, GIRK4)	Ba^{2+}, Cs^+, 4-AP, TEA	unknown
$K_{ir}4.1$ (KCNJ10, BIRK1)	Ba^{2+}, Cs^+	unknown
$K_{ir}4.2$ (KCNJ15, $K_{ir}1.3$)	Ba^{2+}, Cs^+	unknown
$K_{ir}5.1$ (KCNJ16)	Ba^{2+}, Cs^+	unknown
$K_{ir}6.1$ (KCNJ8)	glibenclamide and other sulfonylureas (sites on associated SUR subunits and channel)	diazoxide, pinacidil, nicorandil (for associated SUR subunits)
$K_{ir}6.2$ (KCNJ11)	glibenclamide and other sulfonylureas phentolamine ($K_{ir}6.2$)	diazoxide, pinacidil, cromokalim, nicorandil
$K_{ir}7.1$ (KCNJ13, $K_{ir}1.4$)	Ba^{2+}, Cs^+	unknown

Table 4. K_{2P} Channels

channel	inhibitors	activators
$K_{2P}1.1$ (KCNK1, TWIK-1)	unknown	unknown
$K_{2P}2.1$ (KCNK2, TREK-1)	Ba^{2+}, quinidine	arachidonic acid, volatile anesthetics riluzole
$K_{2P}3.1$ (KCNK3, TASK-1)	anandamide, Ba^{2+}	halothane, isofluorane
$K_{2P}4.1$ (KCNK4, TRAAK)	Gd^+	arachidonic acid, lysopholipids, riluzole
$K_{2P}5.1$ (KCNK5, TASK-2)	clofilium, lidocaine, quinidine	halothane
$K_{2P}6.1$ (KCNK6, TWIK-2)	Ba^{2+}, quinidine, volatile anesthetics	arachidonic acid
$K_{2P}7.1$ (KCNK7)	does not form functional channels	
$K_{2P}9.1$ (KCNK9, TASK-3)	ruthenium red	unknown
$K_{2P}10.1$(KCNK10, TREK-2)	quinidine	arachidonic acid, halothane, isoflurane, riluzole
$K_{2P}12.1$(KCNK12, THIK-2)	does not form functional channels	
$K_{2P}13.1$(KCNK13, THIK-1)	Ba^{2+}, halothane	arachidonic acid
$K_{2P}15.1$ (KCNK15, TASK-5)	does not form functional channels	
$K_{2P}16.1$ (KCNK16, TALK-1)	Ba^{2+}, quinidine, chloroform	isoflurane, nitric oxide
$K_{2P}17.1$ (KCNK17, TASK-4)	Ba^{2+}, chloroform	nitric oxide
$K_{2P}18.1$ (KCNK18)	quinine, quinidine, free fatty acids	volatile anesthetics

specific contacts between the functional groups of the ligands and the functional groups of the ligand-sensing residues is usually unavailable. Multiple drug-binding modes may coexist and their population is highly sensitive to ligand–receptor energy, and hence to the inner-pore geometry. The latter varies substantially in the available X-ray structures of open channels. For example, mutations of the $K_V11.1$-channel pore-facing $Phe^{i22(656)}$ significantly decreased the affinity of the antiarrhytmic drug MK-499, cisapridine, and several other ligands.[29] In matching positions of K_VAP[30] and $K_V1.2$,[31] the distances between C^{β} atoms in diagonally opposed α-subunits are 17.4, and 13.3 Å, respectively, and therefore vary by as much as 4 Å (the distances were measured in the biological-unit structures, PDB indexes 1ORQ and 2A79, respectively). Usually it is unclear which X-ray structure would be a better template for a homology model. Another problem is that C-type inactivation usually,[32–35] but not universally,[32,36,37] enhances ligand binding and that the structural changes that ligand-binding sites undergo upon C-type inactivation are unknown.[37] In these circumstances, the predicted free energy of the ligand–receptor complex cannot be the decisive criterion to choose the most promising binding mode for rational drug design. Additional (and very important) information that may help to choose the correct binding mode may be obtained from considering structure–activity relations in a series of ligands. For example, if a particular ligand-binding mode explains the structure–activity relationships, it may be favored over other binding modes that may be better in terms of ligand–receptor energy, but does not explain the structure–activity relations.

Another challenging aspect for the development of K^+channel modulators is the fact that K^+ channels, like all ion channels, are "moving targets" that undergo large conformational changes switching between open and closed states on a millisecond time scale. These changes in "gating state" are often accompanied by dramatic changes in the conformation of drug binding sites resulting in a phenomenon referred to as "state-dependent inhibition". For ion channels in excitable cells firing action potentials like neurons, state-dependent inhibition translates into what is called "use-dependent inhibition" because channel blocking increases as channels are "used" during rapid cycling from closed into open states and back and the number of blocked channels increases with time. To determine the true effect of an ion channel modulator on a channel, it is therefore advisable to use functional assays rather than binding assays.

1.2. K^+ Channel Pharmacology

Chemicals modulating K^+ channel function fall into three general categories: metal ions, organic small molecules, and venom-derived peptides. These substances can affect K^+ channels by blocking the ion-conducting pore from the external or internal side or by modulating channel gating through binding to the voltage-sensor domain or auxiliary subunits. Tables 1−4 contain a list of the most

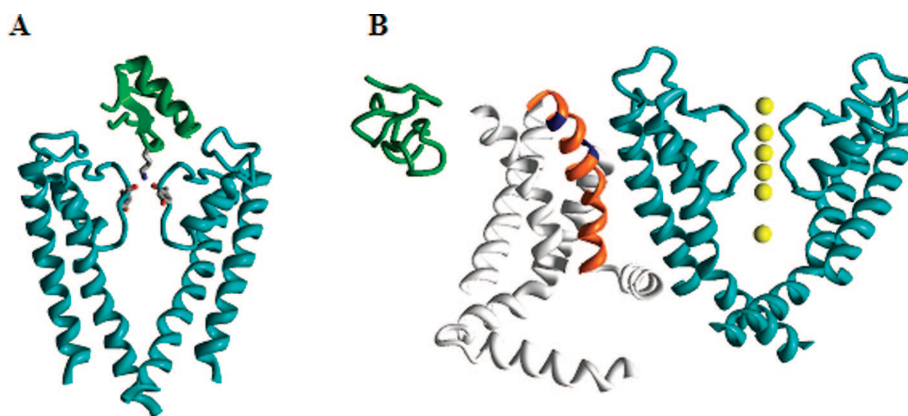


Figure 1. Action of peptide toxins on potassium channels. (A) Complex of KcsA with charybdotoxin (PDB index 2A9H). (B) NMR structure of hanatoxin1 (PDB index 1D1H) on the same scale as the X-ray structure of $K_V1.2$ (PDB index 2A79).

commonly used K_V, K_{Ca}, K_{ir}, and K_{2P} channel inhibitors and activators. In this Review, we will not discuss the actions of metal ions such as Cs^+, Ba^{2+}, Cd^{2+}, Pb^{2+}, Co^{2+}, and Ni^{2+} because most of these cations block K^+ channels only in the millimolar range and are not very specific. Instead, we will first give a brief overview of venom-derived peptide toxins and small-molecule K^+ channel modulators and then focus on the medicinal chemistry of K^+ channel modulators, which hold promise for the treatment of diseases of the nervous or immune system.

1.2.1. Peptides

Venomous animals such as snakes, spiders, scorpions, sea anemones, cone snails, and bees produce a large variety of peptide toxins, which target ion channels. The first peptide toxins that were found to inhibit K^+ channels in the late 1970s and early 1980s were the bee venom apamin, which blocks $K_{Ca}2$ (SK) channels,[38,39] and the scorpion toxins noxiustoxin (NTX)[40] and charybdotoxin (ChTX),[41] which were isolated from the venoms of the scorpions *Centruroides noxius* and *Leiurus quinquestriatus.*

While NTX only blocks the K_V1-family channels $K_V1.2$ and $K_V1.3$, ChTX turned out to be relatively promiscuous: it inhibits both the K_{Ca} channels $K_{Ca}1.1$ and $K_{Ca}3.1$, as well as $K_V1.2$, $K_V1.3$, and $K_V1.6$.[42] Apamin, ChTX, and the K_V1-family channel blocking α-dendrotoxins[43] from the venoms of the black mamba *Dendroaspis polylepis* and the green mamba *Dendroaspis angusticeps* quickly became popular neuroscience tools. They have been widely used to elucidate the physiological function of K^+ channels even before the *Drosophila Shaker* channel in 1987[44] and the mammalian K^+ channels, starting with $K_V1.1$, $K_V1.2$, and $K_V1.3$ in 1990,[45] were cloned. However, K^+ channel blocking peptide toxins are not just pharmacological tools. They also proved to be tremendously useful for gaining structural information about the K^+ channel proteins themselves and continue to be widely used to study K^+ channel gating and subunit composition. Starting in the mid-1990s, the groups of Chris Miller, Roderick MacKinnon, and George Chandy deduced the dimensions of the outer vestibule of *Shaker* and $K_V1.3$ using ChTX and the related scorpion toxins agiotoxin-2 and kaliotoxin, whose structures had been determined by NMR,[46–48] as molecular calipers in mutant cycle analysis experiments.[47,49] All three groups estimated that the outer vestibule of the pore is 9–14 Å wide at the top and then tapers down to a width of 4–5 Å at a depth of 5–7 Å.[47,49–52] These predicted dimensions proved to be remarkably accurate when compared with the crystal structure of the bacterial K^+ channel KcsA published three years later in 1998.[12]

During the last 20 years many laboratories have identified about 200 K^+ channel-targeting peptide toxins not only in snake and scorpion venoms but also in sea anemones, marine cone snails, and tarantulas (see ref 53 for a systematic nomenclature). The toxins contain between 18 and 60 amino acid residues and are cross-linked by 2−4 disulfide bridges, forming compact molecules, which are remarkably resistant to denaturation. On the basis of the arrangement and number of β-strands and α-helices in their structures, the toxins can be categorized into eight different folds: βββ, hairpinlike and crosslike αα, 3_{10}αα (helical-capping motif), 3_{10}ββ, and the three α/β scaffolds αββ, βαββ, and 3_{10}ββα.[54] Taken together, these peptide toxins and their synthetic derivatives constitute a large pharmacological armamentarium to target K_V and K_{Ca} channels with high potency and specificity. Interestingly, so far only one K_{ir} blocking peptide, the bee venom toxin tertiapin,[55] and no toxins targeting K_{2P} channels[56] have been identified.

Venom-derived peptide toxins produce effects on K^+ channels by two different mechanisms (Figure 1). The toxins from scorpions, sea anemones, snakes, and cone snails bind to the outer vestibule of K^+ channels and in most cases insert a lysine side chain (position 27 in charybdotoxin) into the channel pore to occlude it.[54,57–59] This mechanism has often been compared with a cork being inserted into a bottle. The toxin molecule forms several secondary contacts with channel residues in the outer vestibule to further stabilize the binding. Figure 1A illustrates this binding mode by showing the complex between ChTX and KcsA determined by NMR.[60] Only two of the four KcsA subunits are shown to allow a view into the pore revealing that the Lys residue of the toxin binds to the backbone carbonyls groups of four tyrosines from the signature-sequence GYG motifs in the ion channel pore. Spider toxins like hanatoxin (HaTX), which was isolated from the venom of the Chilean rose tarantula *Grammostola spatulata*,[61] in contrast, interact with the voltage sensor domain of K_V channels and increase the stability of the closed state.[62,63] The resulting rightward shift in activation voltage and acceleration of deactivation means that the channel is "harder" to open (i.e., membrane requires more depolarization) and closes faster. However, in contrast to channels inhibited by pore-blocking toxins, channels inhibited by so-called "gating modifier" toxins can still be opened by strong depolarizations. Gating-modifier toxins

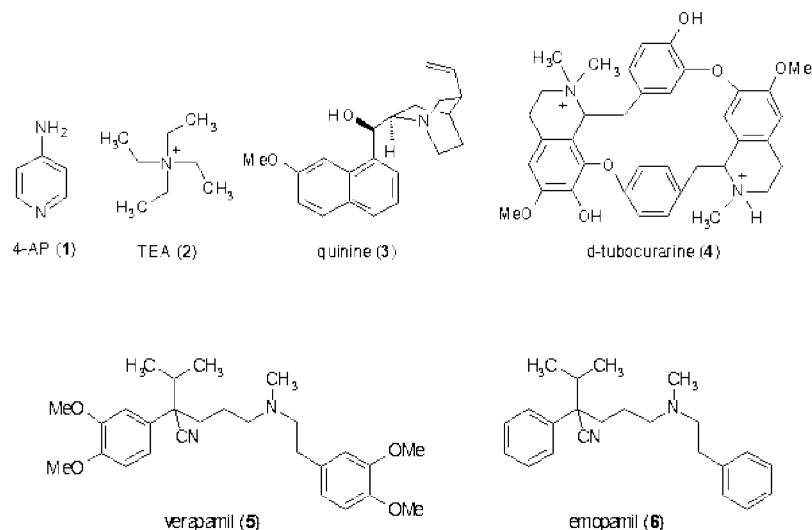


Figure 2. Structures of unselective K^+channel blockers.

differ from pore blocking toxins in two more aspects: the stoichiometry of the toxin-channel interaction and the location of the binding site. While pore blocking toxins bind with a 1:1 stoichiometry to the receptor site involving multiple amino acid residues in the outer vestibule of the channel, experiments with saturating concentrations of gating-modifier toxins suggest that 3−4 toxin molecules bind to the four voltage-sensor domains of a single channel molecule.[62,64] The combined evidence from studies on the kinetics of inhibition, mutagenesis, and membrane partition experiments further suggests that the amphiphilic gating-modifier toxins, which contain a cluster of hydrophobic residues on one face of the molecule, partition into the membrane when they bind to the voltage sensor.[64,65] This nonspecific membrane partitioning makes it difficult to design specific gating modifier molecules for use as drugs as pointed out by Maria Garcia in a commentary on the work by Lee and MacKinnon characterizing the binding of the tarantula toxin VSTX1 to the bacterial K_VAP channel.[65,66] Figure 1B shows the NMR structures of hanatoxin1[67] on the same scale as the X-ray structure of $K_V1.2$.[31] For clarity, only one of the four voltage-sensing domains (gray with orange S4) and only two of the four pore-domain subunits (cyan) are shown. Two positions in S4, whose mutations affect the toxin binding in $K_V2.1$,[63] are marked in blue. However, it should be remembered that this figure is only a cartoon illustrating the binding mode because the structure of the toxin-channel complex has not been experimentally determined.

1.2.2. Small Molecules

In the early days of studying ion channels, the two agents that were used to pharmacologically identify K^+ channels were 4-aminopyridine (4-AP, **1**) and tetraethylammonium (TEA, **2**), which inhibit many K^+ channels in the high-micromolar or millimolar range, but have little or no effect on Na^+ and Ca^{2+} channels. For some K^+ channels, like $K_V3.1$, which have no other known peptide or small molecule inhibitors, 4-AP (**1**) and TEA (**2**) still remain the only available blockers (see Tables 1−4). Other broadly active K^+ channel blockers include quinine (**3**), D-tubocurarine (**4**), and verapamil (**5**) (Figure 2). These drugs are organic cations and block open K^+ channels by binding in the inner pore. However, before their binding sites are discussed, it is necessary to mention binding sites for inorganic cations in K^+ channels. Seven K^+ binding sites are seen in the crystal of the KcsA−FAB complex in high-K^+concentration.[68] Sometimes these sites are designated S1−S7, but since S1–S6 are generally used for transmembrane helical segments in P-loop channels, we use designations T1−T7 (Figure 3B). Occupancy of sites T1−T4 in the selectivity filter by ions and water molecules alternates during the permeation process[69] and may depend on the channel state and physiological conditions. A metal ion in the center of the cavity (site T5) is stabilized by electrostatic interactions with P-helixes.[70] Another site at the cytoplasmic entrance of the selectivity filter is seen in the complex of KcsA with Cs^+.[71] We designate this site T4′. Ions at sites T4′ and T5 do not form multiple coordinating bonds with the channel and may oscillate in broader regions than the octa-coordinated ions in sites T1–T4. Therefore, T4′ and T5 designate regions rather than points.

Tetraalkylammonium compounds like TEA (**2**) have long been known to block K^+ channels from the cytoplasmic side.[72,73] The X-ray structures of cocrystals of KcsA and tetrabutylammonium (TBA) show the TBA molecule trapped between sites T4′ and T5 below the selectivity filter of the closed channel (Figure 4A).[68,74–76] Reconstruction of the experimental electron-density maps from molecular-dynamics data indicates that the exact position of TBA along the pore axis depends on how the selectivity filter is occupied by permeating ions.[75] Understandably, electrostatic repulsion between an ion in site T4, which is most proximal to the inner pore, shifts TBA in the cytoplasmic direction.[75] In KcsA, the shift of the trapped TBA in the cytoplasmic direction is limited by the closed activation gate (Figure 4A), but in the open channel, the effect of the selectivity-filter occupancy on the binding mode of a cationic ligand may be more pronounced as predicted by a model of a local anesthetic in an open Na^+ channel.[77] In the absence of X-ray structures of open K^+ channels with an open-channel blocker bound, structures of such complexes can only be suggested from studies, which combine experimental and molecular-modeling approaches. Docking of the dicationic D-tubocurarine (**4**) in the $K_V1.2$-based homology model of $K_V1.3$ with K^+ ions in sites T1 and T3 (Figure 4B)[25] predicted that an ammonium nitrogen occupies approximately the same position between sites T4′ and T5 as the nitrogen atom of TBA in $K_{cs}A$.[68,74–76] The second ammonium group and ether

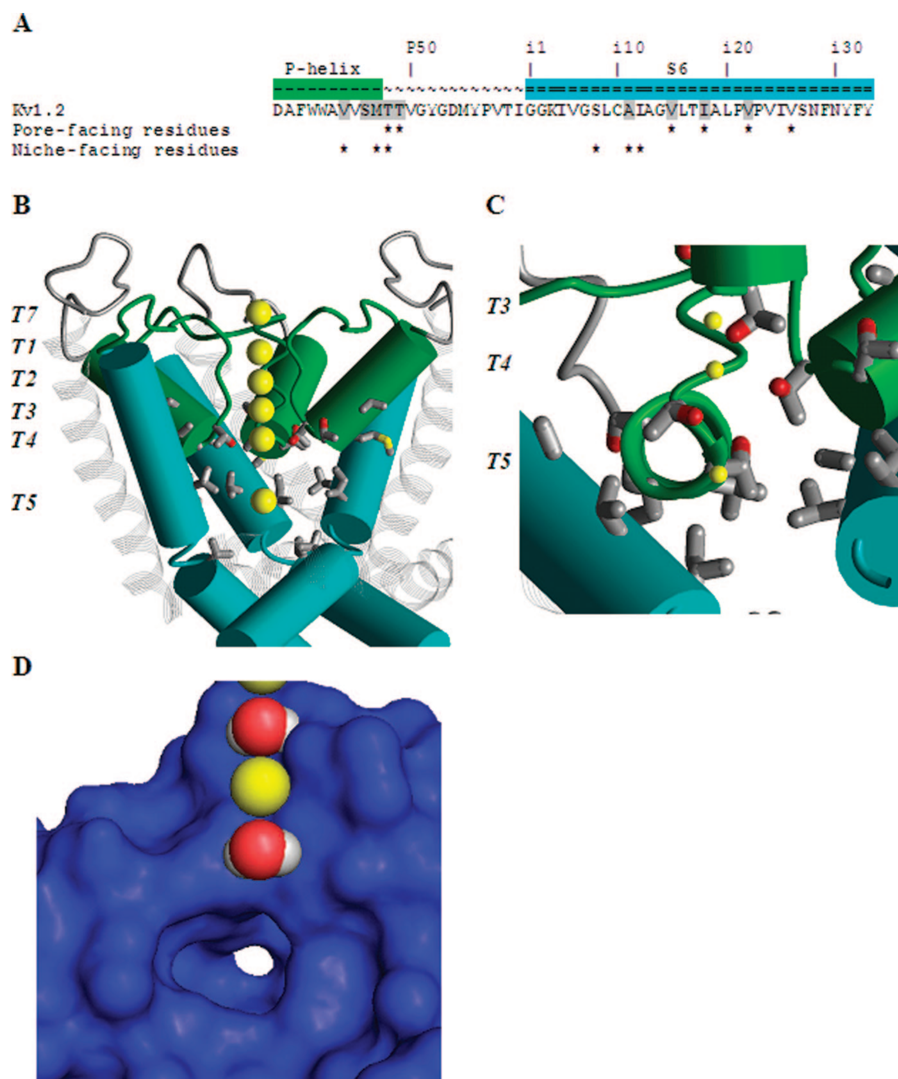


Figure 3. Ligand-binding regions in the inner pore and niches of K^+channels. (A) P−S6 sequence of $K_V1.2$ with residue labels.[28] Positions where ligand-sensing residues have been identified in various K_V channels are highlighted. Residues whose $C^{\alpha}-C^{\beta}$ bonds face the inner pore or the niches are marked by asterisks. (B) Side view of the $K_V1.2$ structure[31] with P-helices (green), S6s (cyan), and S5s (gray strands). One subunit is removed for clarity. Yellow spheres show K^+ ions in the center of the inner pore (K^+_{T5}), at the extracellular side of the channel (K^+_{T7}), and in the outer pore (K^+_{T1}, K^+_{T2}, K^+_{T3}, and K^+_{T4}). Sidechains of residues highlighted in panel A are shown. In the inner pore, the ligand-sensing positions are exemplified by T^{p48} and T^{p49} at the level of K^+_{T4}, V^{i15} at the level of K^+_{T5}, and I^{i18} and V^{i22} at the helix kink. (C) View from inside the pore along a P-helix shown by the rod. Side chains of V^{p44}, S^{p46}, M^{p47}, T^{p48}, and T^{p49} in the helix represent ligand-sensing position in various K_V channels.[28] K^+_{T3}, K^+_{T4}, and $K^+_{T5}$are shown by small yellow spheres. (D) View at the fraction of the protein surface from inside the pore. K^+_{T2} and K^+_{T4} are replaced with water molecules. K^+_{T5} is removed to show a niche with a white opening at the far end. In K^+ channels, the niches contain ligand-sensing residues.

oxygens of D-tubocurarine (**4**) are not involved in strong electrostatic or H-bonding interaction,[25] which can explain the rather low affinity of the drug to $K_V1.3$.

N-terminal parts of S6s and P-loops line four "niches" in the inner pore (Figure 3C) that can harbor terminal moieties of some ligands, for example, four methyl groups of TBA. Verapamil (**5**), a cardiovascular drug targeting L-type Ca^{2+} channels, also blocks the inner pore of the open $K_V1.3$ and cysteine substitution of $Ala^{i11(413)}$, which lines the niche, dramatically reduced verapamil potency.[33] Intriguingly, verapamil (**5**) blocks the mutant with 1:1 stoichiometry, but its derivative emopamil (**6**), which lacks all four methoxy groups, blocks the mutant with 2:1 stoichiometry.[33] To explain this observation, Dreker and Grissmer proposed that the unsubstituted phenyl rings of emopamil (**6**) penetrate the niches deeper than the dimethoxy-substituted phenyl rings of verapamil (**5**) and that the inner pore therefore can accommodate two molecules of emopamil (**6**) but only one molecule of verapamil (**5**). Despite the fact that the protonation state of the tertiary amino groups of the two emopamil (**6**) molecules and their position in the inner pore remain unknown, this study suggests that residues lining the niches interact with drugs. In some K^+ channels, the niches have polar residues, which may be targets for subtype-specific ligands. For example, $K_V1.2$ channels are insensitive to the antiarrhythmic drug propafenone, but replacement of the VVS^{p46} motif with the TIT motif from the propafenone-sensitive channel $K_V2.1$ confers propafenone sensitivity to $K_V1.2$.[78]

Hydrophobic cations, such as TBA, D-tubocurarine (**4**), and verapamil (**5**), block K^+ channels by physically occluding the inner pore and inserting their ammonium group into the ion permeation pathway. Another mechanism is described for 4-AP (**1**), which blocks the *Shaker* channel by promoting

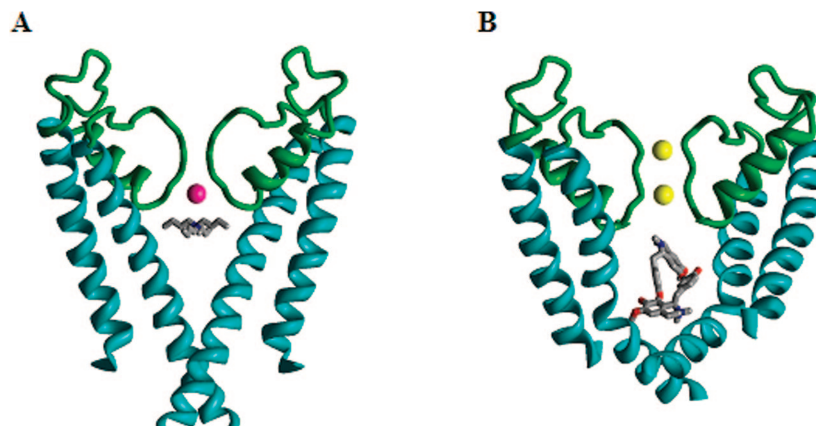


Figure 4. Cationic ligands in the inner pore of K^+channels. (A) X-ray structure (PDB index 2BOB) of tetrabutylammonium trapped in the closed conformation of KcsA. The thallium ion in position 4 of the selectivity filter is magenta. (B) $K_V1.2$-based model of the open $K_V1.3$ with D-tubocurarine. K^+ ions in positions 1 and 3 are yellow. In both complexes, the ammonium group of the ligand is at the focus of the macrodipoles of the P-loop helices (green).

activation-gate closure.[79–81] Interestingly, 4-AP (**1**) demonstrates some features characteristic for hydrophobic cations: it enters the open *Shaker*, competes with TEA (**2**) for binding in the open channel, and remains trapped in the closed channels.[80] However, the small size of 4-AP (**2**) and lack of groups ionizable at physiological pH does not allow the drug to physically occlude the permeation pathway.

In addition to the above-described relatively low-affinity and unselective small molecule K^+ channel blockers, medicinal chemistry efforts in both the pharmaceutical industry and in academia have identified a number of small molecule K^+ channel modulators, which are potent and selective enough to be developed into drugs. Some of these compounds, such as the K_V7 channel activator flupirtine, are already in clinical use, and several other compounds are currently in various stages of clinical trials. However, it is not our intent to review all ongoing K^+ channel drug discovery efforts in all therapeutic areas in this article. In the following two sections, we will focus on K^+ channel modulators for the treatment of neurological disorders and autoimmune diseases and use these compounds as examples of the evolving medicinal chemistry of K^+ channels.

2. K^+ Channel Modulators for the Treatment of Neurological Diseases

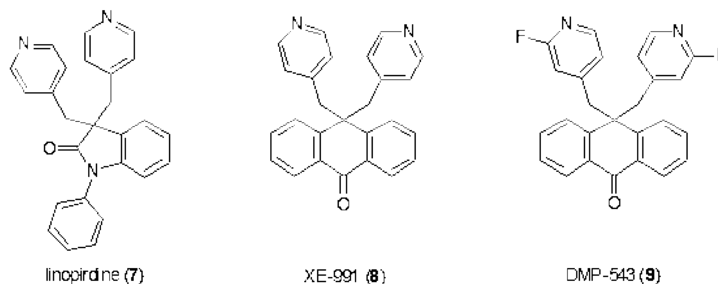
K^+ channels have equilibrium potentials close to –80 mV. They are therefore ideally suited to set the resting membrane potential, a task they perform in most cells. In neurons and other excitable cells, K^+ channels are also crucial for determining the shape, the duration, and the frequency of action potential firing. To adjust these functions to the specific requirements of a particular neuron, the different K^+ channels often show subfamily specific patterns of cellular and subcellular localization. For example, K_V1 channels are predominantly found on axons and nerve terminals, K_V2 channels in the soma and in dendrites, K_V3 channels in dendritic or axonal domains, and K_V4 channel in somatodendritic membranes.[82]

The neuronal K^+ channels that we are focusing on in this review, the voltage-gated $K_V7.2$–7.5 channels and the calcium-activated $K_{Ca}1.1$ and $K_{Ca}2.1$–2.3 channels, also serve very specific physiological functions in certain types of neurons. $K_V7.2$–7.5 channels underly the so-called M-current in hippocampal and cortical neurons, which plays a critical role in determining the subthreshold excitability and responsiveness to synaptic inputs. The Ca^{2+}-activated $K_{Ca}1.1$ and $K_{Ca}2$ channels are involved in the neuronal afterhyperpolarization (AHP), which prevents the immediate initiation of a second action potential, and K_{Ca} channels thus act as modulators of action potential frequency. Although not necessarily found together in all neuronal subtypes, three components of the AHP have been described: a fast component lasting 50 ms (fAHP) carried by $K_{Ca}1.1$, a medium component decaying in 200 ms (mAHP) partly carried by $K_{Ca}2$ channels, and a slow component of unknown molecular identity, that decays in roughly 1–2 s (sAHP). As discussed in the following, activation or inhibition of neuronal K_V7 and K_{Ca} channels offers exciting therapeutic possibilities for intervening in epilepsy, ataxia, dementia, stroke, and neurophatic pain.

2.1. $K_V7.2$–7.5 (KCNQ) Channels

K_V7 (KCNQ) channels are classical K_V channels with six transmembrane segments, including a voltage-sensor segment S4 and a long intracellular C-terminus, which contains binding sites for regulatory molecules such as calmodulin and phosphatidylinositol-4,5-biphosphate (PIP_2).[83] The family consists of five members, $K_V7.1$–7.5, which share between 30% and 65% amino acid identity.[5] While $K_V7.1$ (KCNQ1, K_VLQT) channels are found in the heart, peripheral epithelia, and smooth muscle, $K_V7.2$–7.5 (KCNQ2–5) channels are expressed in hippocampal and cortical neurons and in regions of the nervous system involved in neuropathic pain such as the dorsal and ventral horn of the spinal cord and dorsal root and trigeminal ganglion neurons. In these neurons, $K_V7.2$–7.5 channels underlie the so-called M-current, a noninactivating current, which exhibits significant conductance in the voltage range of action potential generation. Consequently, the M-current tends to allow the firing of single action potentials but opposes sustained membrane depolarization and repetitive firing. The M-current has therefore often been likened to a "brake" on neuronal firing. The current was first described by Brown and Adams in 1980 in frog sympathetic neurons and named M-current (I_M) because of its inhibition by muscarinic agonists.[84] Today it is relatively well established that several common neurotransmitters such as acetylcholine, substance P, or bradykinin close M channels by stimulating G-protein-coupled receptors such as M1 muscarinic acetylcholine receptors or bradykinin B_2R receptors.[83] Although downstream signaling from these receptors is complex, the

Kv7.2-7.5 blockers:



Kv7.2-7.5 activators:

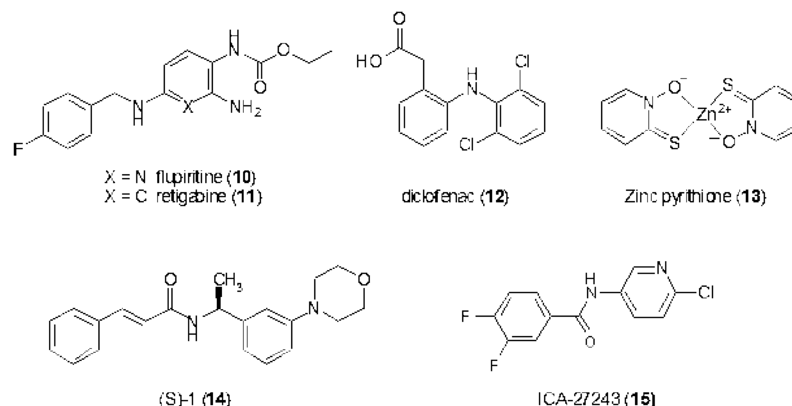


Figure 5. K_V7 channel modulators.

primary mechanism for receptor-induced M-channel closure seems to be the fall in membrane PIP_2levels following the receptor mediated activation of phospholipase-Cβ, which hydrolyzes PIP_2 in the inner leaflet of the plasma membrane. When PIP_2 levels fall below the levels, which are required for K_V7 channel activity,[85,86] the channels close, resulting in membrane depolarization and leading to increased neuronal excitability.[83]

As demonstrated by the group of David McKinnon, the classical M channel in sympathetic neurons consists of $K_V7.2/K_V7.3$ heteromultimers.[87] However, $K_V7.4$ and $K_V7.5$ can also form functional M channels and have been proposed to be involved in certain neuronal pathways like the auditory system ($K_V7.4$) or the cerebral cortex ($K_V7.5$).[83] In keeping with the physiological importance of the M-current, homozygous $K_V7.2^{-/-}$ mice die within a few hours after birth, while heterozygous $K_V7.2^{+/-}$ mice with decreased expression of $K_V7.2$ showed hypersensitivity to pentylenetetrazole-induced seizures.[88] In contrast, mice in which $K_V7.2$ expression was drastically reduced but not completely abolished through a dominant-negative suppression approach exhibited spontaneous seizures, behavioral hyperactivity, and morphological changes in the hippocampus.[89] Reduction of $K_V7.2$ expression after the completion of hippocampal development in adult mice produced deficits in hippocampus-dependent spatial memory suggesting a critical role for M channels in cognitive performance.[89] In humans missense or deletion mutations in $K_V7.2$ or $K_V7.3$ cause benign familial neonatal convulsions (BFNC), an autosomal dominant epilepsy of infancy.[90–92] Although the seizures in BFNC typically resolve by 3 months of age, BFNC is associated with an increased incidence of various forms of epilepsy later in life suggesting a strong link between $K_V7.2$ and $K_V7.3$ mutations and epilepsy.[6] Loss of function mutations in $K_V7.4$ in contrast result in hearing loss presumably because of an important function of $K_V7.4$ in sensory outer hair cells.[93]

2.1.1. K_V7 Channel Blockers

In the late 1980s, DuPont initiated clinical trials with the phenylindolinone DuP996, which is now generally called linopirdine (**7**, Figure 5), for Alzheimer's disease.[94] Linopirdine was regarded as a promising drug because it enhanced the release of acetylcholine in cholinergic nerve terminals in the brain only when its release was triggered and improved learning and memory in rodents and primates. Although clinical trials with linopirdine remained largely inconclusive[95] or showed that it did not improve memory performance in elderly subjects,[96] linopirdine became a valuable pharmacological tool. In 1995 Aiken et al. reported that linopirdine blocks the M-current in rat CA1 pyramidal neurons and suggested that the I_M blockade might be responsible for the enhancement of neurotransmitter release by linopirdine because the two effects had similar IC_{50}/EC_{50} values.[97] This observation was later confirmed when $K_V7.2$ and $K_V7.3$ were cloned in 1998 and linopirdine was found to inhibit $K_V7.2$ channels or heteromultimers consisting of $K_V7.2$ and $K_V7.3$ with the same IC_{50} of 4 μM[87] as the M-current in sympathetic neurons.[98] Two more potent anthracenone analogs of linopirdine are XE991 (**8**) and DPM-543 (**9**), which enhance [^{3}H]-acetylcholine release from rat brain slices about 5–10 fold more potently than linopirdine[99] and in the case of XE991 (**8**) inhibit $K_V7.2/K_V7.3$ heteromultimers with an IC_{50} of 600 nM.[87] However, after the failure of linopirdine the pharmaceutical industry ceased to show interest in K_V7 channel inhibitors and no further compounds were developed. The impairment in hippocampus-dependent spatial memory observed in the above-mentioned mice, where $K_V7.2$ was conditionally suppressed,[89] also raises doubts about whether

K_V7 channel inhibition indeed constitutes a valid target for cognition enhancement. Interestingly, so far no venom-derived peptides have been identified that block K_V7 channels.

2.1.2. K_V7 Channel Activators

In contrast to K_V7 channel blockers, K_V7 activators are currently generating a lot of interest as potential drugs for the treatment of epilepsy and neuropathic pain after several clinically used drugs were retrospectively shown to activate K_V7 channels.[6,100,101] In the early 1990s, Asta Medica submitted a collection of compounds including flupirtine (**10**), a non-opioid centrally acting analgesic, which had been marketed in Europe since 1984 for the treatment for postoperative and cancer pain,[102] to the NIH Anticonvulsant Screening Program, which randomly tests compounds submitted by academic laboratories or pharmaceutical companies in mouse models of epilepsy (http://www.ninds.nih.gov/funding/research/asp/). Since flupirtine was found to have potent anticonvulsant effects, chemists at Asta Medica in collaboration with scientists from the NIH Antiepileptic Drug Development Program optimized the molecule for anticonvulsant activity by removing the basic nitrogen atom in the pyridine ring and reported desazaflupirtine (D-23129) in 1996, now called retigabine (**11**), as an orally active broad spectrum anticonvulsant.[103] Retigabine subsequently entered clinical trials at Valeant Pharmaceuticals Inc. It recently was reported to reduce seizure frequency in a dose-dependent manner in Phase-2 clinical trials[104] and is currently undergoing two large Phase-3 clinical trials,[105] which are expected to be completed in summer 2008. After retigabine (**11**) was initially believed to enhance GABAergic transmission, it was independently shown by three groups in 2000 to activate $K_V7.2/K_V7.3$ channels with an EC_{50} of about 1 μM by shifting the voltage dependence of activation by 20−30 mV to more negative potentials and by markedly slowing channel deactivation kinetics.[106–108] Subsequent studies demonstrated that retigabine (**11**) also activates $K_V7.2$ and $K_V7.3$ homotetramers as well as $K_V7.4$ and $K_V7.3/K_V7.5$ channels with roughly the same potency but only affects the cardiac $K_V7.1$ channel at 100-fold higher concentrations.[109] Through site-directed mutagenesis Wuttke et al. located retigabine's binding site in a putative hydrophobic pocket formed upon channel opening between the cytoplasmic parts of S5 and S6 involving Trp^{236} in S5 and $Gly^{i14(301)}$ in S6, which is proposed to be the "gating-hinge".[110] Two other "old drugs", which were recently reported by a group from Tel Aviv University to activate $K_V7.2/K_V7.3$ channels are the cyclooxygenase (COX) inhibitors meclofenac (EC_{50} 25 μM) and diclofenac (**12**, EC_{50} 2.6 μM).[111] Both compounds enhanced the native M-current in cultured cortical neurons and diclofenac (**12**) effectively suppressed electroshock-induced seizures in mice with an ED_{50} of 43 mg/kg.[111] These findings suggest that activation of neuronal K_V7 channels could contribute to the analgesic effects of diclofenac.

Reflecting the great interest of the pharmaceutical industry K_V7 activators belonging to many different chemotypes have been reported in the last 5 years. These include: benzanilides, benzisoxazoles, indazoles, oxindoles including the $K_{Ca}1.1$ activators BMS-204352 (**24**), quinolinones, 2,4-disubstituted pyrimidine-5-carboxamides, phenylacrylamides, 5-carboxamide-thiazoles, benzothiazoles, quinazolinones and salicylic acid derivatives. The interested reader is referred to several recent review articles and book chapters on neuronal K_V7 activators.[100,101,112] Out of these compounds, we here only show the morpholino-substituted phenylacrylamide (S)-1 (**14**, EC_{50} 3 μM for $K_V7.2$), which is effective in a rat model of migraine,[113] the recently reported zinc pyrithione (**13**, EC_{50} 1.5 μM for $K_V7.2$), which was identified at the High Throughput Biology Center at Johns Hopkins University,[114] and the benzamide ICA-27243 (**15**), which was recently described by Wickenden et al. at Icagen.[115] ICA-27243 (**15**) activates $K_V7.2$/K7.3 channels with an EC_{50} of 200–400 nM and suppresses electroshock-induced seizures in rodents following oral administration.[115] Another K_V7 channel activator of undisclosed structure from Icagen (ICA-105665) has recently entered Phase-1 clinical trials for the treatment of epilepsy.

Taken together, the neuronal $K_V7.2$–7.5 channels constitute promising new targets for the treatment of epilepsy, pain of various etiologies, and possibly migraine and anxiety.[116] It is currently not clear whether highly subtype specific activators will exhibit any advantages over the currently available more broadly active compounds, but it is feasible that subtype selective activators might cause less dizziness and somnolence than retigabine.

2.2. $K_{Ca}1.1$ (BK, Maxi-K) Channels

$K_{Ca}1.1$ channels are activated by intracellular Ca^{2+}, depolarization, or a combination of both Ca^{2+} and voltage and are characterized by an unusually high single channel conductance of 250 pS, which is responsible for the different names the channel has received: BK for "big", large-conductance, or Maxi-K. The channel was first cloned in 1991 from a *Drosophila* mutant[117] (named slowpoke because of its lethargic phenotype) and two years later by the group of Lawrence Salkoff from mouse brain.[118] In contrast to other K_V and K_{Ca} channels, $K_{Ca}1.1$ has seven transmembrane segments (S0−S6) instead of six, an extremely long intracellular C-terminus containing four additional hydrophobic segments (S7−S10), and a so-called "calcium-bowl", which together with the RCK (regulators of conductance of K^+) domain in S7 and S8 is responsible for the complex calcium-dependent gating of the channel (see Salkoff et al.[13] for a recent review). Another salient feature that distinguishes $K_{Ca}1.1$ from other K^+ channels are two rings of negative charges in the inner and outer vestibules, which have been proposed to increase K^+ concentration in the vicinity of the selectivity filter and could explain $K_{Ca}1.1$'s high single channel conductance.[119,120] $K_{Ca}1.1$ channels in different tissues can vary significantly in their sensitivity to Ca^{2+}, inactivation properties, and even pharmacology because of alternative splicing of the α-subunit and coassembly with four different auxiliary β-subunits.[13] While $\beta1$ is primarily expressed in smooth muscle, hair cells, and some neurons, $\beta2$ is found in ovary and endocrine tissue, $\beta3$ in testis, and $\beta4$ is the most abundant β-subunit in the brain.[121]

$K_{Ca}1.1$ channels are widely expressed throughout the body, however their physiological role is currently best understood in neurons, smooth muscle, secretory endocrine cells and sensory receptors. In the brain $K_{Ca}1.1$ is found in the soma, dendrites, and presynaptic terminals of neurons[122,123] and is thought to underlie the fast afterhyperpolarization current and to regulate synaptic transmission by limiting the Ca^{2+} influx through Ca_V channels. In peripheral tissue, $K_{Ca}1.1$ is involved in regulating the tone of vascular, uterine, gastrointestinal, airway, and bladder smooth muscle. However, contrary to initial reports by Ahluwalia et al.,[124] $K_{Ca}1.1$ is not essential

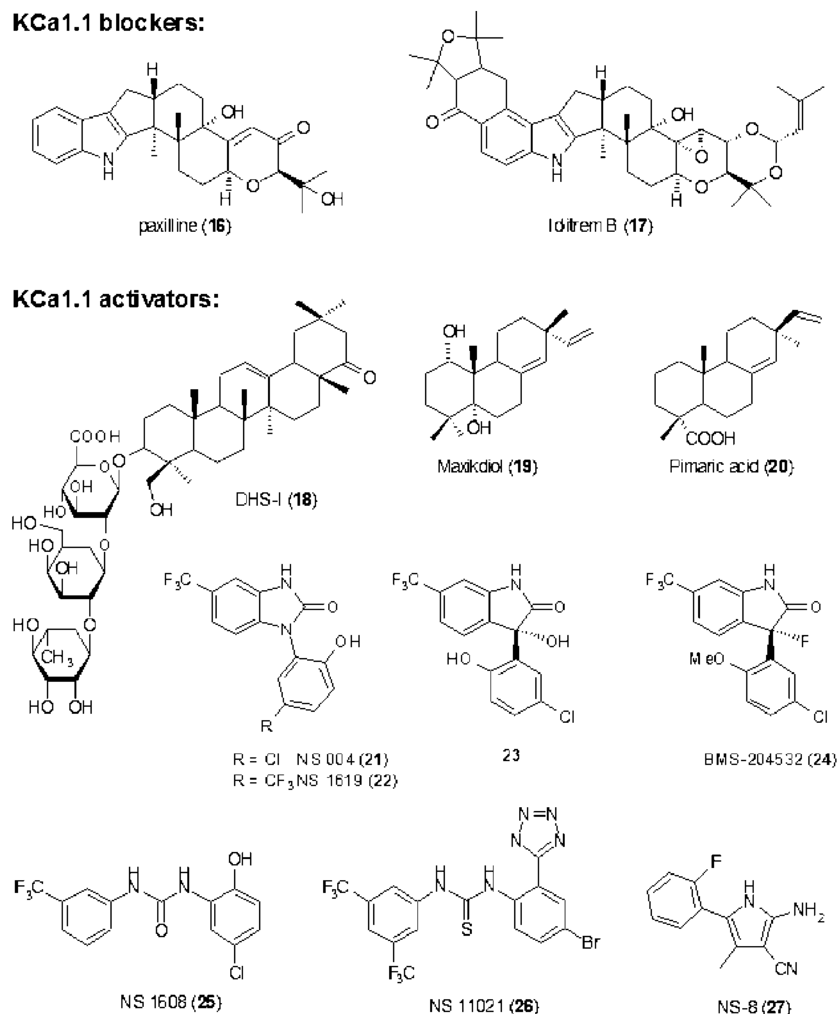


Figure 6. $K_{Ca}1.1$ modulators.

for innate immunity. Two subsequently published studies demonstrate that neither human nor mouse neutrophils express $K_{Ca}1.1$ and that pharmacological inhibition or genetic deletion of $K_{Ca}1.1$ has no effect on neutrophile function.[125–127] $K_{Ca}1.1$ knockout mice are viable and exhibit a number of surprisingly mild phenotypes including ataxia, high-frequency hearing loss, hypertension, bladder over-reactivity, and erectile dysfunction.[128–132] In humans a mutation resulting in increased $K_{Ca}1.1$ channel function has been found to be responsible for a rare form of generalized epilepsy.[133] This seemingly counterintuitive phenotype is most likely caused by faster repolarization resulting from increased $K_{Ca}1.1$ function, which reduces the refractory period following action potentials. However, despite this human gain-of-function mutation, which sounds a note of caution against indiscriminately activating $K_{Ca}1.1$, $K_{Ca}1.1$ activators have often been proposed to be ideally suited to potentiate existing negative feed-back mechanisms in both neurons and vascular smooth muscle.

2.2.1. $K_{Ca}1.1$ Channel Blockers

$K_{Ca}1.1$ is most commonly pharmacologically characterized by its sensitivity to the scorpion toxins ChTX and iberiotoxin. While ChTX also blocks $K_V1.2$, $K_V1.3$, $K_V1.6$, and $K_{Ca}3.1$,[42] iberiotoxin (IbTX, $IC_{50} \approx 10$ nM) is selective for $K_{Ca}1.1$.[134] Other more recently identified and less commonly used peptide blockers of $K_{Ca}1.1$ are slotoxin (IC_{50} 1.5 nM)[135] and BmP09 from the Chinese scorpion *Buthus martensi Karsch* (IC_{50} 27 nM).[136] An interesting feature of peptide inhibition of $K_{Ca}1.1$ is that the β-subunit participates in the formation of the high-affinity toxin binding site either through a direct interaction between four residues in its large extracellular loop and the toxin bound in the channel pore or through an allosteric effect on the α-subunit.[137,138] The presence of different β-subunits can thus drastically change the sensitivity of $K_{Ca}1.1$ to ChTX and IbTX. While $\beta1$ enhances toxin binding, complexes of $\beta4$ with $K_{Ca}1.1$ are resistant to both toxins.[14]

$K_{Ca}1.1$ blockers have been suggested for the possible treatment of depression and memory impairment. However, no major efforts have been seemingly done yet to identify nonpeptide $K_{Ca}1.1$ blockers. The exception is some work performed by the Membrane Biochemistry and Biophysics group at Merck in the early 1990, where Maria Garcia and co-workers investigated whether the tremorgenic indole alkaloids, which are produced by fungi of the *Penicillium, Aspergillus*, and *Claviceps* genera and which are known to cause staggers syndromes in animals that feed on contaminated grain, might block $K_{Ca}1.1$.[139] The group found that several indole diterpenes including the now commonly used paxilline (**16**, IC_{50} 2–50 nM, depending on the intracellular Ca^{2+} concentration; Figure 6),[140,141] indeed potently inhibit $K_{Ca}1.1$, but concluded that their tremorgenicity might be unrelated to $K_{Ca}1.1$ block.[139] Paxilline binds to $K_{Ca}1.1$ at an

intracellular site that is involved in channel gating and seems to be coupled to the calcium binding sites because of the antagonism between paxilline and Ca^{2+}.[140] However, the exact location of this site has not been identified. A more recently reported indole diterpene, which potently inhibits $K_{Ca}1.1$, is the mycotoxin lolitrem B (**17**, IC_{50} 3.7 nM; Figure 6) from the endophyte *Neotyphodium lolii*, which commonly infects rye grass seeds.[142]

2.2.2. $K_{Ca}1.1$ Channel Activators

In contrast to the relatively small number of $K_{Ca}1.1$ inhibitors, a large number of both natural and synthetic $K_{Ca}1.1$ activators have been reported. Two $K_{Ca}1.1$ activators, BMS-204352 (**24**) and NS-8 (**27**), even advanced into clinical trials for stroke and overactive bladder. In 1993, the same group at Merck that had identified the $K_{Ca}1.1$ blockers IbTX and paxilline reported the isolation of three $K_{Ca}1.1$ activators from *Desmodium adscendens*, a medicinal herb used in Ghana as a treatment for asthma.[143] NMR and mass spectroscopic analysis revealed the three active components: the known triterpenoid glycosides dehydrosoyasaponin I (DHS-I, **18**), and its derivatives soyasaponin I, and soyasaponin III. DHS-I was reported to be a noncompetitive inhibitor of ^{125}I-ChTX binding to smooth muscle membranes containing BK channels, suggesting a binding site located outside of the pore but allosterically coupled to it. Subsequent studies performed by McManus et al. on oocytes injected with either $K_{Ca}1.1$ alone or $K_{Ca}1.1$ together with $\beta1$ revealed that DHS-I stimulates $K_{Ca}1.1$ activity only in the presence of the β-subunit.[144] However, DHS-I (**18**) is difficult to study in physiological preparations despite its low nanomolar potency because it is poorly membrane-permeable and needs to be applied intracellularly in electrophysiological experiments to reach its intracellular binding site.[143] A diterpenoid that activates $K_{Ca}1.1$ less potently than DHS-I is the 1,5-dihydroxyisoprimane maxikdiol (**19**), which was identified in 1994 at Merck in the fermentation broth of an unidentified coelomycite.[145] A SAR study of diterpenoids structurally related to maxikdiol more recently showed that relatively small structural changes have a significant effect on the activity of terpenoid $K_{Ca}1.1$ activators.[146] While maxikdiol (**19**) is only active if applied intracellularly and requires the presence of the $\beta1$ subunit, the pine resin acid pimaric acid (**20**) activates $K_{Ca}1.1$ channels consisting of only α-subunits ($EC_{50} \approx 3$ μM) and is freely membrane-permeable. Interestingly, sandaracopimaric acid, the diasteromer of pimaric acid was equipotent at increasing $K_{Ca}1.1$ activity, while abietic acid, which lacks the extracyclic double-bond, was ineffective.[146] Another natural product that activates $K_{Ca}1.1$ is the benzopyran mallotoxin ($EC_{50} = 0.5$ μM) also known as rottlerin.[147] However, mallotoxin is not specific for $K_{Ca}1.1$ and also activates $K_V11.1$ (hERG) channels.[148] An endogenous compound that activates $K_{Ca}1.1$ channels by binding to an extracellular site on the $\beta1$-subunit is the female hormone 17β-estradiol (EC_{50} 2.4 μM).[149] This $K_{Ca}1.1$ activation has been suggested to be at least partially responsible for the direct vasorelexant effect of estrogens.

The first synthetic $K_{Ca}1.1$ activators (Figure 6) were the benzimidazolones NS 004 (**21**) and NS 1619 (**22**), which were identified by the Danish company NeuroSearch A/S in 1992 and which have become widely used pharmacological tool compounds. In 1994 Olesen et al. reported that NS 004 (**21**) and NS 1619 (**22**) increased $K_{Ca}1.1$ currents in cerebellar granule and aortic smooth muscle cells at concentrations between 3 and 30 μM by shifting their current–voltage relationship toward more negative membrane potentials.[150,151] In a subsequent study, the same group showed that opening of the benzimidazolone ring into the diphenylurea NS 1608 (**25**) resulted in a 10-fold more potent $K_{Ca}1.1$ activator ($EC_{50} = 2.1$ μM).[141] NS 1608 (**25**) caused $K_{Ca}1.1$ channels expressed in HEK-293 cells to open at less depolarized potentials (maximal shift of the *I*/*V* curve by 70 mV) and to deactivate more slowly. The effect of NS 1619 (**22**) and NS 1608 (**25**) is independent of the presence of internal Ca^{2+}demonstrating that the compound does not act by increasing the Ca^{2+} sensitivity of the channel. In contrast to DHS-I (**18**), NS 1619 (**22**) directly activates the α-subunit and does not require the presence of a β-subunit. This difference in the action of NS 1619 (**22**) and DHS-1 (**18**) has subsequently often been used to determine whether a β-subunit is present in native tissue. For example, Papassotiriou et al. concluded that BK channels in endothelium are composed of α-subunits without associated β-subunits because the current could be activated by NS 1619 but not by DHS-I,[152] while Tanaka et al. argued that the BK channels in human coronary smooth muscle cells consist of both α- and β-subunits due to their sensitivity to DHS-I.[153] A recently described more potent and selective diphenylurea $K_{Ca}1.1$ activator from NeuroSearch is the tetrazole substitute thiourea NS 11021 (**26**).[154] NS 11021 activates $K_{Ca}1.1$ with an EC_{50} of 400 nM and in contrast to NS 1609 exerts no effect on L- or T-type Ca^{2+} channels at 30 μM. However, NS 11021 (**26**) is not perfectly selective and activates $K_V7.4$ and $\alpha7$ nicotinic acetylcholine receptors at concentrations of 10–30 μM.[154] It remains to be seen if NS 11021 will replace the earlier NeuroSearch $K_{Ca}1.1$ activators as the most frequently used tool compound.

Scientists at Bristol-Myers Squibb demonstrated that one of the nitrogen atoms of the benzimidazolones could be replaced by a carbon and generated a number of aryloxindole $K_{Ca}1.1$ activators exemplified by **23**.[155] However, **23** and its des-hydroxy analog were not further pursued as neuroprotective drug candidates in favor of the more metabolically stable BMS-204352 (**24**, Flindokalner or MaxiPost), which entered clinical trials for stroke. BMS-204352 (**24**) activates $K_{Ca}1.1$ channels expressed in HEK cells with an EC_{50} of 350 nM in a strongly Ca^{2+}-dependent manner.[156] With a calculated logP of 5.1 BMS-204352 enters the brain quickly and reaches roughly 10-fold higher concentrations in the brain than in plasma. In permanent middle cerebral artery occlusion, an animal model of stroke, BMS-204352 reduced infarct areas measured at 24 h in both normotensive and hypertensive rats by 20–30% when administered 2 h after the beginning of brain ischemia at doses between 1 μg/kg and 1 mg/kg.[156] However, BMS 204352 (**24**) displayed an interesting inverted-U-shaped dose–response relationship and at doses of 3 mg/kg no longer reduced infarct sizes. It is currently not clear if this effect might be related to the activation of $K_V7.2$–7.5 channels seen at higher concentrations of BMS-204352.[157,158] Because of the promising results in rodent models of stroke, BMS-204352 (**24**) entered clinical trials and was found to be well tolerated and safe in both Phase-1 and Phase-2 studies. However, BMS-204352 failed to show efficacy compared to placebo in a Phase-3 study involving about 2000 patients and was therefore discontinued.[159] The idea behind using $K_{Ca}1.1$ activators as neuroprotectants was to hyperpolarize neurons and protect them from "excitotoxic" cell death. Following an ischemic stroke,

neurons surrounding the core of the infarct tend to die through a combination of low oxygen tension, excessive excitatory amino acid release, and elevation in intracellular Ca^{2+} (Ca^{2+}-overload). $K_{Ca}1.1$ activators are thought to be able to block Ca^{2+} entry through Ca_V channels and NMDA receptors by hyperpolarizing neurons and to thus prevent neurotransmitter release and Ca^{2+}-overload.[156] The reasons that BMS-204352 (**24**) failed to show benefit in humans might be numerous, but the major reason was probably the timing of the administration after the infarct. In rats, BMS-204352 was always administered 2 h after the occlusion. However, for logistic reasons, this was not possible in stroke patients, and the drug was given at various times within the first 48 h after stroke onset. Other CNS indications for which $K_{Ca}1.1$ activators have been proposed include epilepsy and pain. However, very little has been published in this area, and it is currently not clear how useful $K_{Ca}1.1$ activators will be for these indications and whether they exhibit any advantages over the K_V7 channel activators.

In addition to CNS indications, $K_{Ca}1.1$ activators could also be useful for a number of peripheral indications such as urinary incontinence, erectile dysfunction, asthma, and hypertension because of their ability to relax smooth muscle. One $K_{Ca}1.1$ activator, the arylpyrrole NS-8 (**27**) identified by Nippon Shinyaku, even advanced into Phase-2 clinical trials for overactive bladder,[160] but its development was stopped in January 2007 because it lacked sufficient efficacy at the expected therapeutic dosage.

2.3. $K_{Ca}2$ (SK) Channels

Small-conductance Ca^{2+}-activated K^+channels were first described in 1982[161] and later found to be encoded by three closely related genes, when they were cloned by Kohler et al. in 1996.[162]Similar to the K_V channels, $K_{Ca}2.1$ (SK1), $K_{Ca}2.2$ (SK2), and $K_{Ca}2.3$ (SK3) channels have six transmembrane segments (S1−S6) and intracellular N- and C-termini.[4] However, $K_{Ca}2$ channels only contain two positively charged amino acids in the S4 segment and are therefore insensitive to changes in membrane voltage. Instead, $K_{Ca}2$ channels are highly sensitive to increases in intracellular Ca^{2+}. The latter activates the channels with EC_{50} values of 300–700 nM[163] by binding to calmodulin, which is constitutively associated with the intracellular C-terminus of the channel in a 1:1 stoichiometry.[164] In expression systems, both homomeric and heteromeric $K_{Ca}2$ channels are formed,[165,166] but the existence of heteromeric $K_{Ca}2$ channels has so far not been confirmed in native tissues. Similar to $K_{Ca}1.1$ channels, $K_{Ca}2$ channels can be alternatively spliced to generate channels with modified calmodulin binding as in the case of $K_{Ca}2.1$,[167] with an extended N-terminus dubbed SK2-L as in the case of $K_{Ca}2.2$,[168] or without the N-terminus and first transmembrane segment as in the case of $K_{Ca}2.3$.[169,170] Alternative splicing of $K_{Ca}2.3$ resulting in the insertion of fifteen additional amino acids in the outer pore region can also produce $K_{Ca}2.3$ channels, which are insensitive to the peptide toxins apamin and leiurotoxin-I.[171]

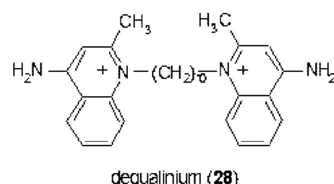
Despite their small conductance of only a few pS, $K_{Ca}2$ channels are powerful modulators of electrical excitability and exert profound physiological effects both within and outside the nervous system. In the mature rat brain, $K_{Ca}2.1$, $K_{Ca}2.2$, and $K_{Ca}2.3$ have a partially overlapping but clearly distinct distribution patterns. While $K_{Ca}2.1$ and $K_{Ca}2.2$ are coexpressed in the neocortex, hippocampus, and thalamus,[163]$K_{Ca}2.3$ is primarily expressed in subcortical regions and in many monoaminergic neurons including the dopaminergic neurons of the substantia nigra.[172,173] In all these neurons $K_{Ca}2$ channels underlie the apamin-sensitive medium afterhyperpolarization (mAHP).[174,175] However, depending on the type of neuron and the other ion channels it expresses, the function of $K_{Ca}2$ channels varies from contributing to the instantaneous firing rate, over setting the tonic firing frequency, regulating burst firing, and potentially catecholamine release.[163] Mice lacking $K_{Ca}2.1$, $K_{Ca}2.2$, or $K_{Ca}2.3$ are viable and do not exhibit any overt neurological phenotypes.[175,176] $K_{Ca}2.2^{-/-}$ mice lack the apamin-sensitive mAHP current in CA1 hippocampal neurons,[175] while mice conditionally overexpressing $K_{Ca}2.2$ exhibit reduced synaptic plasticity and show impaired hippocampal learning and memory.[176] Conditional $K_{Ca}2.3$ overexpression, in contrast, resulted in a greater bladder capacity, abnormal breathing patterns in response to hypoxia, and compromised parturition with protracted labor suggesting that $K_{Ca}2.3$ channels play a role in neurons regulating breathing patterns and uterine and bladder smooth muscle tone.[177,178] Furthermore, $K_{Ca}2.3$ polymorphisms such as polyglutamine repeats and a truncation mutation,[179] which can act as a dominant-negative suppressor,[180] have been associated with schizophrenia[181,182] and cerebellar ataxia.[183]

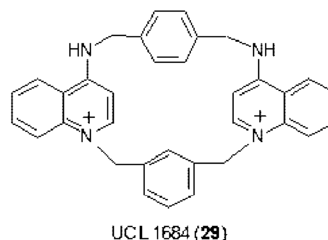
Outside of the nervous system, $K_{Ca}2$ channels are involved in blood pressure regulation[184] and metabolism. The role of $K_{Ca}2$ channels in blood pressure regulation seems to involve both catecholamine release from chromaffin cells in the adrenal gland,[185,186] as well as participation of $K_{Ca}2.3$ channels in the endothelium-derived hyperpolarization factor (EDHF) response.[187] For more details on other peripheral indications the interested reader is referred to a recent extensive review on the physiology and pharmacology of $K_{Ca}2$ channels.[188]

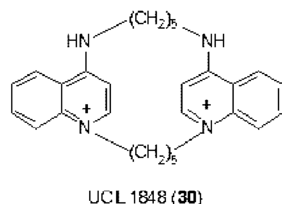
2.3.1. $K_{Ca}2$ Channel Blockers

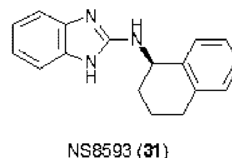
Since the early 1980s, the 18-amino-acid bee venom toxin apamin has been the main pharmacological tool to distinguish $K_{Ca}2$ channels from the apamin-insensitive K_{Ca} channels $K_{Ca}1.1$ and $K_{Ca}3.1$.[39] Following the cloning of the three $K_{Ca}2$ channels, apamin was found to be most potent on $K_{Ca}2.2$ channels (IC_{50} = 30–200 pM) and to block $K_{Ca}2.1$ (IC_{50} = 1–12 nM) and $K_{Ca}2.3$ (IC_{50} = 1–20 nM) channels with 10–50-fold lower affinity.[162,163] Other pore blocking peptide toxins, which inhibit $K_{Ca}2$ channels, are the 31-amino acid scorpion toxins leiurotoxin-I (scyllatoxin)[189,190] and tamapin.[191] Both toxins have roughly the same potency as apamin and show comparable (leiurotoxin-I) or somewhat greater (tamapin) preference for $K_{Ca}2.2$. A common structural feature of apamin and leiurotoxin-I is the presence of a RXCQ motif,[192,193] which is changed to RXCE in tamapin. This motif seems to be crucial for high-affinity blockade of $K_{Ca}2$ channels because other scorpion toxins, which lack this motif, show little or no effect on $K_{Ca}2.2$ and $K_{Ca}2.3$ currents.[194] By replacing Met^7 in the RMCQ sequence of leiurotoxin-I with the unnatural amino acid diaminobutanoic acid (Dab), Shakkottai et al. generated Lei−Dab^7, which blocks $K_{Ca}2.2$ with 650-fold higher potency than $K_{Ca}2.3$ and $K_{Ca}2.1$.[194] However, currently there are no natural toxins or analogs that selectively inhibit $K_{Ca}2.1$ or $K_{Ca}2.3$.

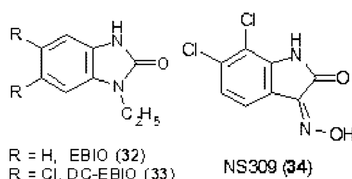
Apamin has been an invaluable tool for determining both the physiological role and the therapeutic potential of $K_{Ca}2$ channels. Intrathecal or systemic administration of apamin has been shown to enhance learning and memory responses

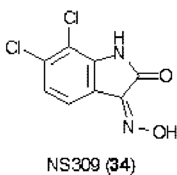


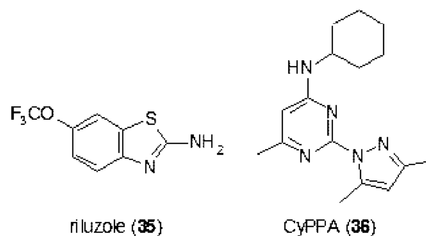












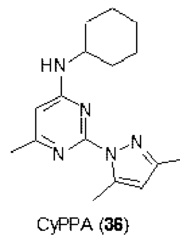


Figure 7. $K_{Ca}2$ channel modulators.

in both mice and rats.[195–198] Taken together with the observation that mice overexpressing $K_{Ca}2.2$ exhibit impaired hippocampal learning and memory,[176] these findings suggest that selective $K_{Ca}2.2$ blockers might be able to function as "memory enhancers" and improve cognitive performance in dementia. However, higher doses of systemically administered apamin induce seizures cautioning that the therapeutic window for this application might be narrow. Because apamin reduces immobility time as effectively as amitriptyline in the mouse forced swimming tests,[199] $K_{Ca}2$ blockers have also been suggested for the treatment of depression and Parkinson's disease.[173] Selective $K_{Ca}2.3$ blockers might be particularly interesting in this respect because $K_{Ca}2.3$ underlies the mAHP in dopaminergic neurons of the substantia nigra.[172,173]

$K_{Ca}2$ channels are also potently blocked by a number of compounds containing two permanently charged or protonatable nitrogens, which are incorporated into aromatic rings connected through relatively variable linkers and separated by a distance of a little less than 6 Å.[200,201] Work on these compounds started in the early 1980s when it was noticed that D-tubocurarine (**4**) and the antiseptic dequalinium (**28**, Figure 7) inhibited small-conductance K_{Ca} channels in the micromolar range.[202–204] Using dequalinium (**28**) as a template, Ganellin, Jenkinson, and Galanakis at the Departments of Pharmacology and Chemistry at the University College of London (UCL) carefully explored the SAR around dequalinium in a large series of papers[205–211] and eventually designed the bis-quinolinium cyclophane UCL 1684 (**29**), which blocks $K_{Ca}2$ channels as potently and as selectively as apamin. Like apamin, UCL 1684 inhibits $K_{Ca}2.2$ (IC_{50} = 200 pM) 3- and 50-fold more potently than $K_{Ca}2.1$ and $K_{Ca}2.3$, respectively.[212,213] The subsequently published UCL 1848 (**30**), in which the aromatic xylyl linkers of UCL 1684 (**29**) were replaced by aliphatic pentylene groups,[214,215] is even more potent and blocks $K_{Ca}2.2$ channel with an IC_{50} of 110 pM.[166] Because of their relatively large molecular weight and permanent charge, these bis-quinolinium cyclophanes are not viable drug candidates. However they have become popular tool compounds because they wash out better than apamin from physiological preparations.

A recently reported $K_{Ca}2$ channel blocker of a completely different chemical structure is the benzimidazole derivative NS8593 (**31**).[216] In contrast to apamin and the UCL compounds, which can all displace apamin and are therefore presumably also pore blockers, NS8593 (**31**) is an inhibitory gating modulator that decreases the Ca^{2+} sensitivity of $K_{Ca}2$ channels by shifting their Ca^{2+} activation curve roughly 10-fold to the right. At an intracellular Ca^{2+} concentration of 500 nM NS8593 inhibits $K_{Ca}2.1$, $K_{Ca}2.2$, and $K_{Ca}2.3$ with IC_{50} values of 420, 600, and 730 nM, respectively.[216] It is currently not known what the in vivo effects of NS8593 (**31**) are and whether it exhibits less toxicity than the pore blocker apamin. Since NS8593 reduces the apparent Ca^{2+} sensitivity of $K_{Ca}2$ channels, it could potentially be more active in relatively slow-firing neurons with low Ca^{2+} concentrations and less effective in fast-firing neurons with presumably higher Ca^{2+} levels.[216] $K_{Ca}2$ channel blockade has further been proposed to contribute to the therapeutic effects and possibly the side effects of tricyclic and phenothiazine antidepressants like amitryptiline, imipramine, and trifluoperazine, which block $K_{Ca}2.2$ channels at concentrations of 8−60 μM.[217,218] However, it is currently not clear if this really is the case.

Taken together, selective $K_{Ca}2.2$ blockers could potentially be useful as cognition enhancers in dementias such as Alzheimer's disease, while $K_{Ca}2.3$ blockers might be helpful for the treatment of depression and Parkinson's disease. However, in the absence of any druglike and brain-penetrable small-molecule $K_{Ca}2$ channel blockers, these exciting possibilities remain untested. Another challenge for medicinal chemists will be the design of subtype-specific $K_{Ca}2$ blockers

and only the future will show if compounds suitable for clinical development will emerge out of the ongoing efforts.

2.3.2. $K_{Ca}2$ Channel Activators

$K_{Ca}2$ channels are activated by several relatively simple heterocyclic molecules like ethylbenzimidazolone (EBIO, **32**, EC_{50} 87–450 μM) and its more potent derivative dichloro-EBIO (**33**, EC_{50} 12–27 μM; Figure 7).[219,220] In contrast to the $K_{Ca}1.1$ activating benzimidazolones NS 004 (**21**) and NS 1619 (**22**), which seem to activate $K_{Ca}1.1$ channels in a Ca^{2+} independent fashion, $K_{Ca}2$ channel activators increase $K_{Ca}2$ channel function by increasing Ca^{2+}sensitivity and therefore absolutely require the presence of at least 30–50 nM of Ca^{2+} to exert their effects.[220] The structurally related oxime NS309 (**34**) is significantly more potent and activates all three $K_{Ca}2$ channels at submicromolar concentrations.[221,222] In contrast to EBIO (**32**), NS309 (**34**) selectively activates the mAHP in hippocampal pyramidal neurons and has no effect on the slow afterhyperpolarization,[222] which is carried by a currently not identified ion channel. Unfortunately, NS309 inhibits the cardiac K^+ channel $K_V11.1$ (hERG) with an IC_{50} of 1 μM,[221] which precludes its possible clinical use. Other compounds that show some structural similarity to EBIO (**32**) are the centrally acting muscle relaxants chlorzoxazone and zoxazolamine, which both activate $K_{Ca}2.2$ at high micromolar concentrations,[219,223] and the more potent neuroprotectant riluzole (**35**), which activates $K_{Ca}2.1$, $K_{Ca}2.2$, and $K_{Ca}2.3$ with EC_{50} values of 2–10 μM.[188,224] Similar to EBIO and NS309, the benzothiazole riluzole (**35**) shifts the Ca^{2+} sensitivity of $K_{Ca}2$ channels causing them to open at lower intracellular Ca^{2+} concentrations.[225] A recently reported $K_{Ca}2$ channel activator of a chemically different structure is the aminopyrimidine CyPPA (**36**) from Neurosearch, which activates $K_{Ca}2.3$ and $K_{Ca}2.2$ currents with EC_{50} values of 6 and 14 μM but has no effect on $K_{Ca}2.1$ or $K_{Ca}3.1$ at concentrations up to 100 μM.[226] Although the potency of this compound is not high, its design demonstrates that it is in principle possible to obtain subtype-specific $K_{Ca}2$ channel activators.

In contrast to $K_{Ca}2$ blockers, which in general increase neuronal excitability, $K_{Ca}2$ channel activators reduce excitability and have therefore been proposed for the treatment of CNS disorders that are characterized by hyperexcitability. On the basis of the observation that overexpression of the $K_{Ca}2.3$ isoform SK3–1B, which suppressed $K_{Ca}2$ channels in deep cerebellar neurons of transgenic mice and thus increased hyperexcitability of these neurons, lead to severe ataxia with in-coordination, tremor, and altered gait,[227] $K_{Ca}2$ activators have been suggested for the symptomatic treatment of cerebellar ataxia.[227,228] Because there are currently no approved treatments for cerebellar ataxia, a Phase-2 clinical trial with the unselective $K_{Ca}2$ channel activator riluzole (**35**) is currently ongoing to test this exciting hypothesis (ClinicalTrials.gov identifier NCT00202397). $K_{Ca}2$ channel activators could potentially also be used as antiepileptic drugs. EBIO (**32**) inhibits epileptiform activity in an in vitro hippocampal slice model of epilepsy[229] and reduces seizures induced by either electroshock or pentylenetetrazole when injected subcutaneously at concentrations of 10–80 mg/kg into mice.[230] However, at 80 mg/kg EBIO induced profound sedation causing the authors of this study to conclude that $K_{Ca}2$ channel activation probably has a smaller therapeutic window than most currently used antiepileptic drugs. Unfortunately, the fact that EBIO also increases the slow APH and not only the $K_{Ca}2$ channel mediated medium APH,[220,222] makes it hard to interpret both the in vitro and in vivo results with EBIO. In future it will be interesting to see whether more potent and selective $K_{Ca}2$ activators than EBIO are effective in epilepsy models. It further remains to be seen whether $K_{Ca}2$ activators impair learning and memory as suggested by the phenotype of mice overexpressing $K_{Ca}2.2$.[176] On the basis of findings made in mice overexpressing $K_{Ca}2.3$, selective $K_{Ca}2.3$ activation further constitutes a potential new therapeutic target for the treatment of hypertension and urinary incontinence.[177,184] Taken together, $K_{Ca}2$ channel activation offers many therapeutic possibilities that await the discovery of more potent and ideally $K_{Ca}2$ channel subtype specific activators to be fully explored.

2.4. Other K^+ Channel Modulators with CNS Activity

Neuronal K^+ channels also seem to be the targets of several drugs, which have been clinically used for a long time. The two-pore K^+ channels $K_{2P}2.1$ (TREK-1) and several other K_{2P} channels are activated by the volatile anesthetics halothane and isoflurane, which seem to bind to a region in the C-terminus of these channels.[231] Because $K_{2P}2.1^{-/-}$ mice are resistant to anesthesia by volatile anesthetics, it seems likely that $K_{2P}2.1$ channel activation indeed contributes considerably to the physiological effect of volatile anesthetics.[232] $K_{2P}2.1^{-/-}$ mice further show increased sensitivity to ischemia and epilepsy induction in comparison to $K_{2P}2.1^{+/+}$ mice suggesting that $K_{2P}2.1$ activators might be useful as anticonvulsants and neuroprotective agents. Interestingly, the neuroprotectant riluzole (**35**) activates $K_{2P}2.1$ and $K_{2P}4.1$ channels at concentrations of 10–100 μM,[233] and it has been suggested that this effect contributes to riluzole's ability to reduce infarct areas in mice subjected to focal cerebral ischemia.[234] See Mathie et al. for a recent comprehensive review about the therapeutic potential of K_{2P} channels.[56]

Two other K^+ channel blockers, which have long been used for the treatment of multiple sclerosis and Lambert-Eaton syndrome are 4-AP (**1**) and its derivative 3,4-diaminopyridine.[42] The use of 4-AP was originally based on the observation that 4-AP could improve conductivity in experimentally demyelinated nerve fibers.[235] However, more recent work by Smith et al. demonstrated that at clinically used doses 4-AP fails to restore conduction in demyelinated rat axons but potentiates synaptic transmission by increasing transmitter release at synapses.[236] The potential targets for 4-AP include all 4-AP sensitive K^+ channels in the K_V1, K_V3, and K_V4 families, and it is currently not clear which of these channels is the primary target. It is further possible that inhibition of $K_V1.3$ in T cells contributes to the clinically observed effect.[42,237] Unfortunately, 4-AP has a very narrow therapeutic window, and its usefulness as a drug for multiple sclerosis is limited because of its propensity to induce seizures at higher doses.[42]

3. K^+ Channel Blockers for the Treatment of Autoimmune Diseases and Inflammation

K^+ channels do not only play an important role in the central nervous and cardiovascular system, they are also critically involved in regulating calcium signaling, proliferation, secretion, and migration in nonexcitable cells. In the immune system, K^+ channels are expressed in cells of both the innate and the adaptive immune system. The evolutionary older innate immune system involves a number of mecha-

nisms and cell types that defend us from infections by recognizing a variety of microbe-derived molecules such as LPS (lipopolysaccaride), peptidoglycans, bacterial lipoproteins, unmethylated CpG DNA, or double-stranded RNA through so-called pattern recognition receptors. Cells belonging to the innate immue system include natural killer cells, mast cells, eosinophils, basophils, and the phagocytic cells monocytes/macrophages, neutrophils, and dendritic cells. These cells can generate and release toxic molecules, such as reactive oxygen species, engulf or kill microbes, and in the case of dendritic cells and macrophages, present antigen to T cells. They are therefore also called antigen-presenting cells (APCs). Most innate immune cells seem to express K^+ channels, although their function in the different cells types is currently not well understood and their expression insufficiently characterized. Human natural killer cells were reported to express $K_V1.3$-like K_V channels more than 20 years ago[238,239] but have not been studied after the cloning of the various K^+ channels, and it is currently not clear which other K^+ channels they express. Human mast cells express $K_{Ca}3.1$ and probably also K_{ir} channels.[240,241] Human eosinophils require proton channels and do not seem to exhibit any significant K^+ conductance.[125] Human basophils have not been studied. Monocytes/macrophages are particularly confusing and depending on the species, the source of cells, the culture conditions, and their activation and differentiation status have been reported to express $K_V1.3$, $K_V1.5$, $K_{Ca}1.1$, $K_{Ca}3.1$, and $K_{ir}2.1$.[242–254] The K_V current in macrophages is most likely a heteromultimer consisting of $K_V1.3$ and $K_V1.5$.[255,256] Microglia, which are brain-resident macrophages, resembles macrophages in many ways and have been shown to express $K_{ir}2.1$, $K_V1.3$, $K_V1.5$, and the K_{Ca} channels $K_{Ca}2.3$ and $K_{Ca}3.1$.[257–262] Neutrophils require proton channels for their oxidative burst[125] and have recently been described to also express $K_{Ca}2.3$.[263] The expression of K^+ channels in dendtritic cells has not been studied.

In contrast to the innate immune system, K^+ channel expression has been studied much more systematically in the adaptive immune system, which provides vertebrates with the ability to remember specific antigens (immunological memory). Following repeated encounters with the same antigen, both T and B cells can differentiate into more reactive and long-lived memory cells. In the B cell lineage, memory cells produce high-affinity antibodies, while memory T cells produce special cytokines and are more effective at providing help to B cells and macropages ($CD4^+$ = helper T cells) or at killing target cells ($CD8^+$ = cytotoxic T cells.) In parallel with this differentiation from naïve into memory cells, expression of the voltage-gated $K_V1.3$ and the Ca^{2+}-activated $K_{Ca}3.1$ channel changes in both human T and B cells during activation and differentiation. In both lineages naïve and early memory cells (IgD^+ B cells and $CCR7^+$ T cells) express about 200–300 $K_V1.3$ channels per cell and very few $K_{Ca}3.1$ channels in the resting state. Following activation, naïve and early memory cells upregulate $K_{Ca}3.1$ to 500 channels per cell with no change in $K_V1.3$ expression.[264–266] "Late" memory cells ($CCR7^-$ effector memory T cells and IgD^-CD27^+memory B cells), in contrast, increase $K_V1.3$ to 1500 or 2000 channels per cell with no increase in $K_{Ca}3.1$ expression.[265,266] Because of this differential expression pattern, $K_{Ca}3.1$ is the functionally dominating K^+ channel in naïve and early memory T and B cells, while $K_V1.3$ is the dominating K^+ channel in effector memory T cells and class-switched memory B cells.[7,266]

The exact reason for why T and B cells change K^+ channel expression during differentiation is currently not known, but it has been hypothesized that different $K_V1.3$ to $K_{Ca}3.1$ ratios allow cells to generate a differently shaped calcium signal, which in turn allows cells to express different genes.[7] Figure 8 summarizes our current understanding of the roles of $K_V1.3$ and $K_{Ca}3.1$ during the activation of naïve human T cells. Antigen presented on the surface of an antigen-presenting cell activates the T cell receptor/CD3 complex. Through a number of tyrosine kinases, which are not shown in the cartoon, this leads to the downstream activation of phospholipase-C, which catalyzes the hydrolysis of membrane phosphatidylinosito-4,5-biphosphate into the two second messengers: inositol-1,4,5-triphosphate (IP_3) and diacylglycerol (DAG). IP_3 binds to and opens IP_3 receptors on the membrane of the endoplasmic reticulum (ER), resulting in an initial small rise in cytoplasmic Ca^{2+}. However, T cells are small and do not have sufficiently large Ca^{2+} stores in the ER to sustain T cell activation. T cells therefore require additional Ca^{2+} influx from the extracellular space through a voltage-independent Ca^{2+}channels called CRAC for Ca^{2+}-release activated Ca^{2+} channel.[267,268] The CRAC channel, which is encoded by the ORAI1 or CRACM1 gene,[8,269] is activated by the stromal interacting molecule 1 (STIM1), which "senses" the decrease in the Ca^{2+} content of the ER through a Ca^{2+}-binding EF-hand motif.[270,271] However, the exact mechanism by which STIM1 activates the CRAC channel is currently not completely understood. Calcium influx through CRAC and the resulting increase in cytosolic Ca^{2+} lead to the translocation of NFAT (nuclear factor of activated T cells) and other transcription factors to the nucleus. The initiation of new transcription then ultimately results in cytokine production and T cell proliferation.[268,272,273]

How are $K_V1.3$ and $K_{Ca}3.1$ involved in this process? CRAC is an inward-rectifier Ca^{2+}channel that can only bring Ca^{2+} into cells at negative membrane potentials and closes at more positive potentials. The opening of $K_V1.3$ and $K_{Ca}3.1$ and the resulting K^+ efflux generates a negative membrane potential of approximately –60 to –70 mV and thus facilitates and maintains Ca^{2+}entry for the entire duration of T cell activation.[7,268,273] In other words, $K_V1.3$ and $K_{Ca}3.1$ provide the counterbalancing K^+ efflux for the Ca^{2+} influx during T cell activation and therefore constitute pharmacological targets for inhibiting T cell activation.[7,274] However, on the basis of the differential expression of $K_{Ca}3.1$ and $K_V1.3$ in naïve versus memory T and B cells, $K_{Ca}3.1$ blockers are probably more suitable for treating acute immune responses, while $K_V1.3$ blockers constitute promising new immunosuppressants for autoimmune diseases, such as multiple sclerosis, rheumatoid arthritis, and psoriasis, where effector memory T cells are involved in the pathogenesis.[237,275]

3.1. $K_V1.3$ Channels

$K_V1.3$ is a classical *Shaker*-type K^+ channel with six transmembrane segments including a voltage sensor in S4. The channel was cloned in 1990[276,277] and shown to encode the K_V channel that had been discovered in human T cells in 1984.[278,279] Apart from T cells, $K_V1.3$ channels are expressed in B cells,[266] macrophages,[254,280] microglia,[258,261] fat cells,[281] oligodendrocytes,[282] and the olfactory bulb.[283,284] In the brain, $K_V1.3$ is generally only found as part of a heteromultimer with other K_V1-family subunits.[285]

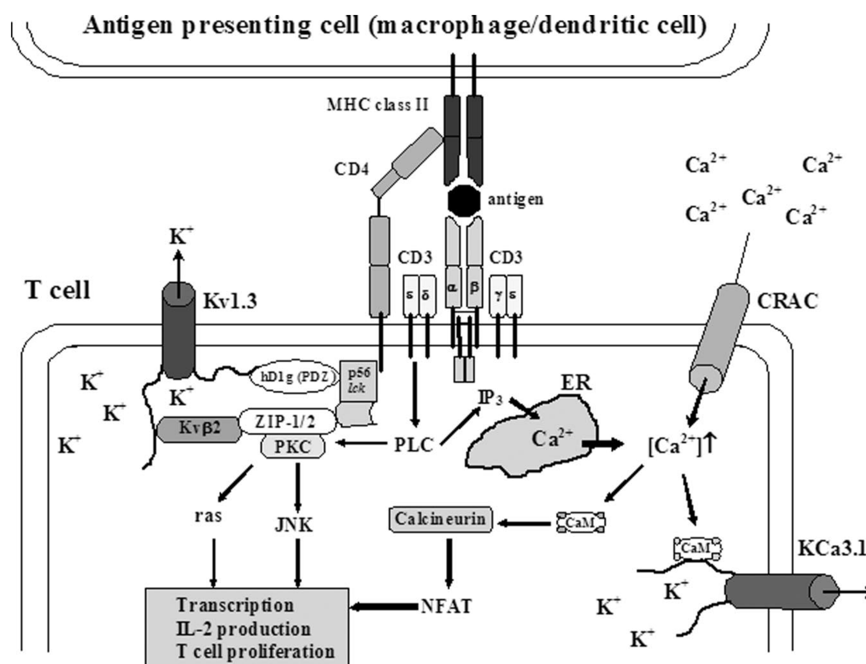


Figure 8. Cartoon showing involvement of $K_V1.3$, $K_{Ca}3.1$, and CRAC channels in the activation of a $CD4^+$ T cell by an antigen-presenting cell (APC). Engagement of the T-cell receptor/CD3 complex through at an antigenic peptide presented in the context of major histocompatibility complex (MHC) class II activates protein kinase C (PKC) and generates IP_3, which liberates Ca^{2+} from intracellular stores. The rise in $[Ca^{2+}]_i$ activates the phosphatase calcineurin, which then dephosphorylates the transcription factor nuclear factor of activated T cells (NFAT) enabling it to accumulate in the nucleus and bind to the promoter of the interleukin-2 (IL-2) gene. Parallel activation of the c-Jun N-terminal kinase (JNK) and ras by PKC results in the activation of other transcription factors and initiates transcription of various genes and finally T cell proliferation. CaM: calmodulin. hD1g: human homologue of the Drosophila disk-large tumor suppressor protein.

On the basis of the observation that unspecific K^+ channel blockers like 4-AP (**1**) and TEA (**2**) inhibited T-cell proliferation,[278] George Chandy at the University of California, Irvine proposed $K_V1.3$ as a new potential target for immunosuppression in 1984. Investigators at Merck corroborated this hypothesis in 1997 by demonstrating that continuous infusion of the $K_V1.3$ blocking peptide margatoxin prevented delayed type hypersensitivity in mini-pigs.[286] Initially, $K_V1.3$ blockers were viewed as general immunosuppressants and often proposed for the prevention of transplant rejection because it was assumed that they inhibited calcium influx in all T cell subsets equally well. They therefore seemed to simply constitute potential alternatives to the general immunosuppressants cyclosporine and FK506.[287] However, the above-described more recent discovery that only $CCR7^-$ effector memory T cells rely on $K_V1.3$, while $CCR7^+$ naïve T and central memory T cells rely on $K_{Ca}3.1$, has changed this concept. $K_V1.3$ blockers are now regarded as immunomodulators that can selectively inhibit human effector memory T cells and spare naïve and central memory T cells.[7,275] One fact that complicates $K_V1.3$ drug development is a species difference in lymphocyte K^+ channel expression between mice and other animals. While $K_V1.3$ is up-regulated in activated human, rat, pig, and primate effector memory T cells,[265,288,289] mouse effector memory T cells do not up-regulate $K_V1.3$ expression.[290] In addition to $K_V1.3$ and $K_{Ca}3.1$, mouse T cells express $K_V1.1$, $K_V1.2$, and $K_V1.6$ in $CD4^+$ T cells[291] and $K_V3.1$ in $CD8^+$ T cells.[290,292] As expected from this species difference, $K_V1.3^{-/-}$ mice exhibit no defects in the immune system.[293] The other phenotype observed in these mice, a decrease in body weight and improved insulin sensitivity,[294,295] might also be a species difference. While mouse adipocytes certainly express $K_V1.3$ protein, electrophysiological studies performed with neonatal brown fat cells[281,296] and cultured white adipocytes from rats and adult humans[297,298] show K_V currents with properties that do not fit the pharmacological and biophysical characteristics of a current carried by $K_V1.3$ homotetramers (e.g., no use-dependence or ChTX sensitivity).

Similar to all other K_V channels, $K_V1.3$ activates in response to membrane depolarization. However, $K_V1.3$ shows two unique biophysical properties: C-type inactivation and a pronounced use-dependence during repetitive depolarizing pulses. With an inactivation time constant of 250 ms $K_V1.3$ inactivates slower than classical A-type currents but faster than classical delayed-rectifiers, which inactivate with time constants of more than 1 s. This type of inactivation, called C-type inactivation, is distinct from the N-terminal ball-and-chain mechanism of *Shaker* and is caused through a rearrangement of the external vestibule of the channel.[299] The structural changes that occur during C-type inactivation are probably profound because $K_V1.3$ requires a considerable time, 30–60 s depending on the length of the depolarizing pulses, to be ready to open again. Consequently, if a channel ensemble is subjected to fast depolarizing pulses, for example, a 200 ms pulse every second, fewer and fewer channels are ready to open again with every pulse, a phenomenon that is called use-dependence. C-type inactivation is slowed down considerably by increasing the extracellular K^+ concentration, presumably because K^+ interacts with residues in the external vestibule that are involved in C-type inactivation.[300] When testing $K_V1.3$ blockers, it is important to take these two properties of the channel into consideration. When using a manual or automated patch-clamp, the pulse protocol has to be chosen appropriately to obtain a stable control current. It is further important to be aware of the fact that all assays, which use high extracellular K^+ to depolarize the membrane and thus activate $K_V1.3$ channels, slow down C-type inactivation and can make $K_V1.3$

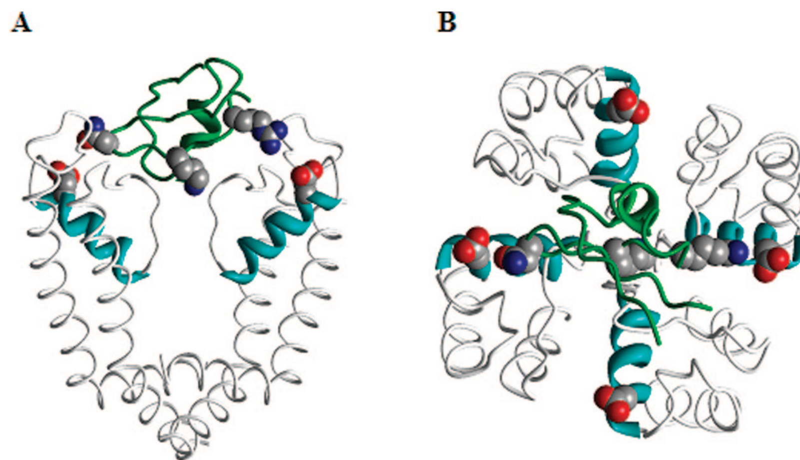


Figure 9. Schematic presentation of the $K_V1.3$-KTX complex reconstructed from the model of Lange et al.[315] (A) Side view with two channel subunits removed for clarity. (B) Top view with cytoplasmic half of the channel removed for clarity.

blockers that bind to the C-type inactivated state appear less potent than they would be under physiological conditions.

3.1.1. Peptidic $K_V1.3$ Blockers

$K_V1.3$ seems to be quite a popular target for venom-derived peptide toxins and is blocked by a large number of scorpion, snake, sea anemone, and cone snail toxins at picomolar to nanomolar concentrations. The scorpion toxins are particularly numerous and include charybdotoxin (IC_{50} = 3 nM),[301,302] kaliotoxin (IC_{50} = 650 pM),[303] margatoxin (IC_{50} = 110 pM),[304,305] agitoxin-2 (IC_{50} = 200 pM),[48] noxiustoxin (IC_{50} = 1 nM),[306] *Heterometrus spinnifer* toxin 1 (IC_{50} = 12 pM).[307] hongotoxin (IC_{50} = 86 pM),[308] *Pandinus imperator* toxin 1 and 2 (IC_{50} = 11 nM and 50 pM, respectively),[309] anuroctoxin (IC_{50} = 730 pM),[310] and *Orthochirus scrobiculosus toxin* 1 (OSK1, IC_{50} = 14 pM).[311] The most potent and selective toxin inhibitor of $K_V1.3$ is currently the OSK1 derivative OSK1-Lys[16]Asp[20], which blocks $K_V1.3$ with an IC_{50} value of 3 pM and shows >300-fold selectivity over closely related channels.[311]

As mentioned in section 1.2.1, the group of George Chandy used kaliotoxin (KTX) as a molecular probe to deduce the dimensions of the outer vestibule and the depths of the pore of $K_V1.3$.[47,49] Using an early molecular model of *Shaker* by Durrell and Guy[312] and the results from their mutant cycle analysis, this group then generated a molecular model of the S5−P−S6 region of the $K_V1.3$ channel.[47] In collaboration with Raymond Norton's group in Melbourne, KTX was manually docked in this model by guiding Lys[27] into the pore such that it would lie near the selectivity filter, and the toxin was then rotated about the central pore axis until Arg[24] in the toxin aligned with $Asp^{p38(386)}$ in $K_V1.3$ in a salt bridge suggested by the mutagenesis.[47] Using high-resolution solid-state NMR spectroscopy, Lange et al. demonstrated that high-affinity binding of KTX to a chimeric KcsA-$K_V1.3$ channel involves structural rearrangements in both molecules.[313] In particular, significant chemical-shift changes upon complex formation were observed for the channel residues Asp^{p38} and $GYGD^{p54}$, which are directly involved in the KTX binding. Figure 9 shows a schematic presentation of the $K_V1.3$-KTX complex reconstructed from the Lange et al. model.[313] The X-ray structure of $K_V1.2$[31] is superimposed on the solid-state NMR structure of KTX[314] (PDB index 1SWX). The KTX backbone is green with space-filled residues R24, K27, and N30. The pore domain of $K_V1.3$ is shown as a white backbone, cyan P-helices, and space-filled $Asp^{p38(386)}$. It should be noted that side-chain conformations taken from nonbound structures of KTX and $K_V1.3$ and shown in Figure 9 are likely to differ from those in the KTX-$K_V1.3$ complex. Furthermore, because the reconstruction was not energy-minimized, details of the mutual disposition of KTX and $K_V1.3$ may differ from those obtained by Lange et al.[315] with the help of NMR-derived constraints and molecular dynamics. Despite these limitations, the reconstruction shows that KTX blocks the ion permeation by inserting Lys[27] in the outer pore, while R24 and N30 stabilize the complex by interacting with two of the four Asp^{p38} residues in the channel P-helices.

In the mid-1990s, $K_V1.3$ blocking peptides were also discovered in the two Caribbean sea anemones *Stichodactyla heliantus* and *Bunadosoma granulifera,* which contain ShK and BgK, respectively.[316–319] Of these two peptides the 35-residue ShK with reported IC_{50} values ranging from 0.9 to 11 pM[320–322] is the more potent and more widely studied molecule. However, in addition to potently blocking $K_V1.3$, ShK also displays picomolar affinity for $K_V1.1$ (IC_{50} = 25 pM) and $K_V1.6$ (200 pM) and blocks $K_V3.2$[323,324] and $K_{Ca}3.1$ (IC_{50} = 28 nM)[320] in the nanomolar range. The NMR solution structure of ShK[325] shows a $3_{10}\alpha\alpha$ fold[54] and a salt bridge between Asp[5] and Lys,[30] which is conserved in all sea anemone K^+ channel toxins.[318,325] By performing extensive SAR around ShK in combination with mutant cycle analysis, George Chandy and co-workers refined their previous $K_V1.3$ vestibule model[47] using restrained molecular dynamics simulations and the KcsA structure as a template.[320] Interestingly, docking models of ShK and its more $K_V1.3$-selective derivative ShK-Dap,[22] which contains diaminopropionic acid (DAP) in place of the critical Lys[22], show that the two peptides bind with a different orientation.[326] While ShK occupies the pore with its positively charged Lys[22] similar to the above-described scorpion toxin KTX, the equivalent Dap[22] in ShK-Dap[22] interacts with $His^{p56(404)}$ and $Asp^{p38(386)}$. On the basis of these models, attempts were made by Baell, Harvey, and Norton at the Biomedical Research Institute in Australia to design type-III ShK peptidomimetics. However, their most potent compounds, which mimic ShK's Lys[22]−Tyr[23] diad plus Arg[11] or Arg[24], block $K_V1.3$ only with IC_{50} values of 75 or 95 μM.[327,328]

ShK and its recently described more $K_V1.3$-selective analog ShK(L5), in which L-phosphotyrosine is attached to the N-terminus via an aminoethyloxyethyloxy-acetyl linker,[324] served

as important tools to further validate $K_V1.3$ as a target for the treatment of effector memory T cell-mediated autoimmune disease. Both compounds suppress delayed type hypersensitivity and effectively prevent or treat adoptive-transfer experimental autoimmune encephalomyelitis in Lewis rats,[288,324] an animal model of multiple sclerosis induced by the transfer of $CD4^+$ myelin specific memory T cells. ShK-L5 has further been shown to significantly reduce the number of affected joints and to improve radiological and histopathological findings in pristane induced arthritis in Dark Agoti rats.[237] On the basis of these experiments, an analogue of ShK-L5 is currently in preclinical development for the treatment of multiple sclerosis and possibly other autoimmune diseases. The short plasma half-life of the peptides does not seem to constitute a major obstacle because ShK-L5 was extremely effective in rats with once daily subcutaneous administration.[237] This could be because the peptide partitions into a deep compartment that prevents its rapid elimination through filtration in the kidney.[324] Alternatively, it may be possible that once daily suppression of activated effector memory T cells is enough to inhibit an ongoing immune reaction. Interestingly, the recently published D-diastereomer of ShK,[329] which is resistant to proteolysis, has the same short half-life of 20−30 min, demonstrating that plasma clearance of peptide toxins is predominantly renal. With a MW of roughly 4000 Da these peptides are freely filtered (size exclusion of the filter ~ 60 kDa). D-allo-ShK (IC_{50} = 36 nM) retains biological activity but is more than 1000-fold less effective than naitive ShK (IC_{50} = 11 pM). Models of D-allo-ShK in the $K_V1.3$ pore suggest that it makes different contacts with the vestibule, some of which are less favorable than for native ShK.[329]

The high affinity of peptide toxins to $K_V1.3$ also makes these toxins attractive as fluorophore-tagged tools for channel visualization in living cells. This is especially useful for flow cytometry because there currently is no good monoclonal antibody that recognizes an extracellular epitope of $K_V1.3$ and that could be used to identify $K_V1.3^{high}$ effector memory T cells. By attaching fluorescein-6-carboxyl (F6CA) through a hydrophilic aminoethyloxyethyloxy-acetyl linker to the N-terminal Arg-1 of ShK, Beeton et al. generated such a probe and demonstrated that it specifically stained $K_V1.3$ expressing cells and was useful in flow cytometry.[330] Using a similar approach, Pragle et al. visualized K_V1-family channels in unpermeabilized rat brain sections with hongotoxin conjugated to an Alexa dye via Cys^{19}on the "backside" of the toxin.[331]

3.1.2. Small Molecule $K_V1.3$ Blockers

In a manner similar to that of the other K_V1-family channels, $K_V1.3$ is blocked by 4-AP (**1**) and TEA (**2**) at micromolar to millimolar concentrations and by a number of promiscuous small molecule ion channel inhibitors like quinine (**3**), D-tubocuraine (**4**), verapamil (**5**), diltiazem, and nifedipine.[7,332] The more potent and selective $K_V1.3$ blockers, which were described during the last 10 years, fall into two general groups. The first group includes typical combinatorial library compounds like the dihydroquinolone CP-339818, the piperidine UK-78282, and phenyl-stilbene A, which are relatively simple structures that are rich in nitrogen and halogen atoms. The second group consists of natural products or natural-product-derivatives, such as the terpenoids correolide and candelalide B, the psoralens, and the khellinones, which are rich in oxygen atoms and have a more complex stereochemistry. Because we and others have previously extensively reviewed $K_V1.3$ blockers,[7,42,274,275,332,333] we concentrate here on the more recently described compounds, their mode of action, and their potential to be developed into drugs (Figure 10).

Using a high-throughput ^{86}Rb-flux assay, scientists at Merck identified the *nor*-triterpene correolide (**37**),which contains an unusual α,β-unsaturated-7-membered lactone ring, in extracts from the bark and roots of the Costa Rican tree *Spachea correa*.[334] Correolide blocks $K_V1.3$ with an IC_{50} of 90 nM[264,335] and two correolide derivatives, which cause less hyperactivity than correolide in vivo, have been shown to suppress delayed type hypersensitivity in minipigs.[336] However, because of their lack of selectivity over $K_V1.2$, $K_V1.5$, $K_V1.6$, and especially $K_V1.1$,[337] correolide (**37**) and its derivatives increase the peristaltic activity of the gastrointestinal tract by increasing acetylcholine and tachykinin release.[338,339] Because correolide (**37**) contains a total of 15 chiral carbon atoms, chemists at Merck have attempted to simplify it by synthesizing a number of derivatives in which the E-ring was removed.[340] The most potent of these compounds, the C18-analog 43 (**38**), inhibited $K_V1.3$ with an EC_{50} of 37 nM in ^{86}Rb-flux assays and suppressed T cell proliferation 15-times more potently than correolide.[340] However, the $K_V1.3$-specificity of this compound has not been reported, and it still requires correolide (**37**) as a starting material. The Merck group also isolated three novel diterpenoid pyrones called candelalides A−C from the fermentation broth of *Sesquicillium candelabrum* and reported that the most potent of these compounds, candelalide B (**39**), blocks ^{86}Rb efflux through $K_V1.3$ channels with an IC_{50} of 1.2 μM.[341]

Through site-directed mutagenesis Hanner et al. revealed several correolide-sensing residues in segments S5 and S6 of $K_V1.3$, including the pore-facing residues Val^{i15}, Ala^{i19}, and $Val^{i22}$and concluded that correolide (**37**) binds in the inner pore between the selectivity filter and the activation gate. As described in section 1.2.2, residues in positions i15, i19, and i22 are also involved in the binding of permanently charged hydrophobic cations such tetraalkylammonium to KcsA[68,74−76] and *Shaker*.[73,342] The center of the drug-binding region coincides with site T5 for permeating metal ions, which in the absence of drugs are stabilized there by electrostatic interactions with P-helices.[70] How can a nucleophilic molecule like correolide (**37**) lacking a positive charge have the same binding site as cationic ligands in the region of the channel, which is close to the selectivity filter and hence evolved to provide stabilization for permeating metal ions? Furthermore, correolide (**37**) has an elongated shape and should bind inside the pore with a vertex approaching the selectivity filter and the long axis collinear to the pore axis.[343] In this orientation, multiple oxygen atoms at the sides of correolide would lack H-bonding partners because the pore-facing positions in the inner helices have only hydrophobic residues. To address this problem, Bruhova and Zhorov used Monte Carlo-minimization to search for the correolide binding site from 20 000 starting positions/orientations of the drug in models of $K_V1.3$ based on the X-ray structure of $K_V1.2$.[343] When a drug blocks the ion permeation pathway, ions obviously do not permeate but can remain in the channel and occupy some of the sites T1−T5 in an unknown pattern. Cationic blockers targeting the inner pore would compete with metal ions and displace them from sites T4′ and T5, where metal ions make few or no direct

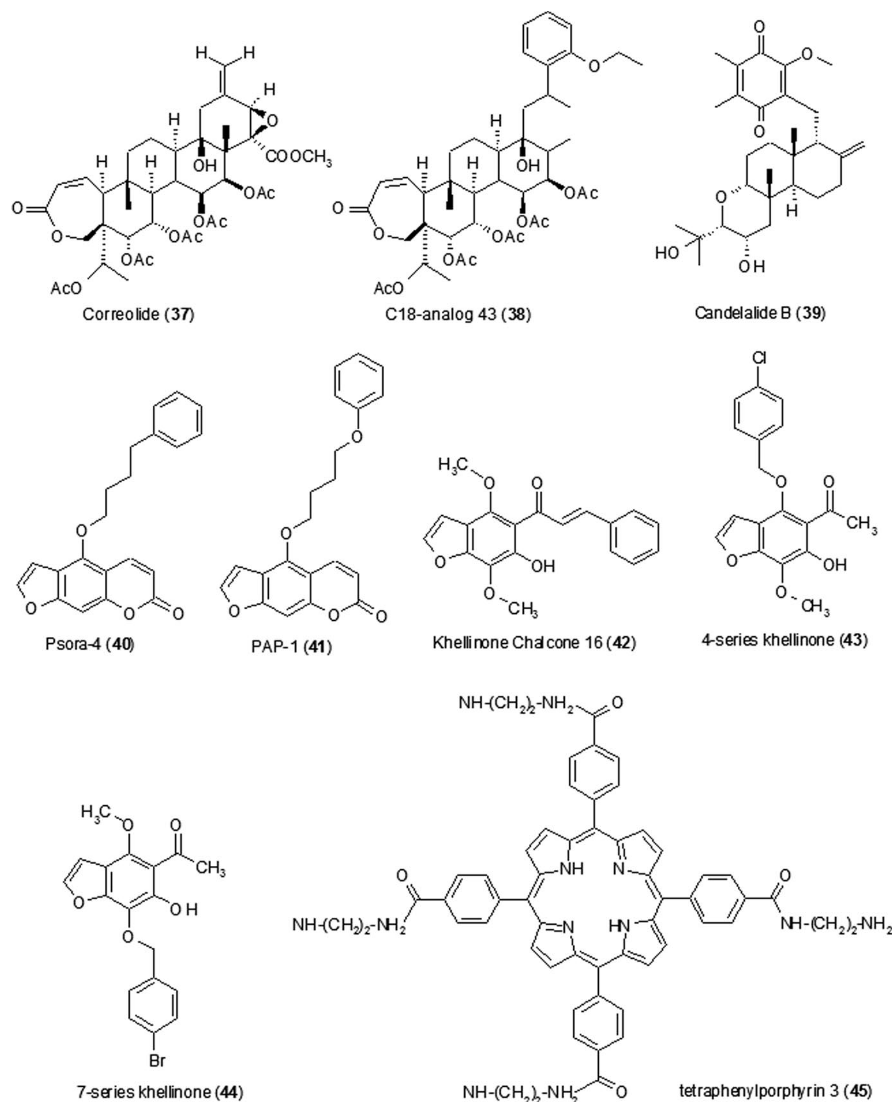


Figure 10. $K_V1.3$ blockers.

contacts with the protein atoms, but nucleophilic drugs like correolide (**37**) could bind a metal ion in the pore. To explore this possibility, two models of $K_V1.3$ were generated with K^+ binding sites T2/T4 or T1/T3/T5 loaded with K^+ ions. During energy optimization of the T2/T4 model, correolide coordinated the K^+ion in site T4. In the T1/T3/T5 model, the third K^+ ion was initially placed in site T5. During energy optimization, this K^+ ion was readily chelated by three acetoxy groups of correolide (Figure 11A and B), shifted upward into site T4′ and occurred approximately in the same position as the ammonium groups of TBA and D-tubocurarine (Figure 4). In both models, correolide (**37**) further directly interacted with the pore-facing residues, whose mutation decreased correolide binding in experiments by Hanner et al.[34] A model in which correolide coordinates the K^+ ion at site T4′ may also explain why correolide derivatives without the epoxy group and the seven-membered ring retain the channel-blocking activity.[340]

The direct experimental validation of this ternary-complex model would require a high resolution X-ray structure. However, there is a simple analogy. When a proton binds to a nucleophilic amino group of a ligand, the proton−ligand complex is considered as a protonated ligand, which can bind to a nucleophilic group of the protein. Similarly, when a ligand binds K^+, the complex may be considered as a K^+-bound ligand that can bind to a nucleophilic site of the ion channel. Many X-ray structures in which a metal ion is coordinated between a ligand and a protein can be found in the Protein Databank. In most cases, the ternary complexes involve a transition metal with well-defined coordination geometry and strong coordinating bonds. In contrast, ternary complexes involving an alkali or alkaline earth metal ions are weak. However, the high local concentration of permeating ions in ion channels should promote the formation of ternary complexes. Such complexes have long been predicted to explain paradoxes in SAR[344–346] and the ability of some ion-channel drugs to bind metal ions has been experimentally demonstrated.[347–349] A number of ion channel models with pore-bound ligands chelating metal ions further support the possible involvement of metal ions in ligand–receptor complexes.[22,343,350–353] For Na^+ channels, a recent model explained the intriguing observation that two molecules of the nucleophilic local anesthetic benzocaine bind to a site, where a single molecule of a cationic local anesthetic ligand, such as lidocaine binds.[354] According to a classical concept, batrachotoxin, veratridine, and other Na^+ channel agonists activate Na^+ channels allosterically.[355] On the basis of the ternary-complex idea, a new mechanism of action of these important pharmacological tools was proposed, according to which a Na^+ channel agonist molecule binds in the central

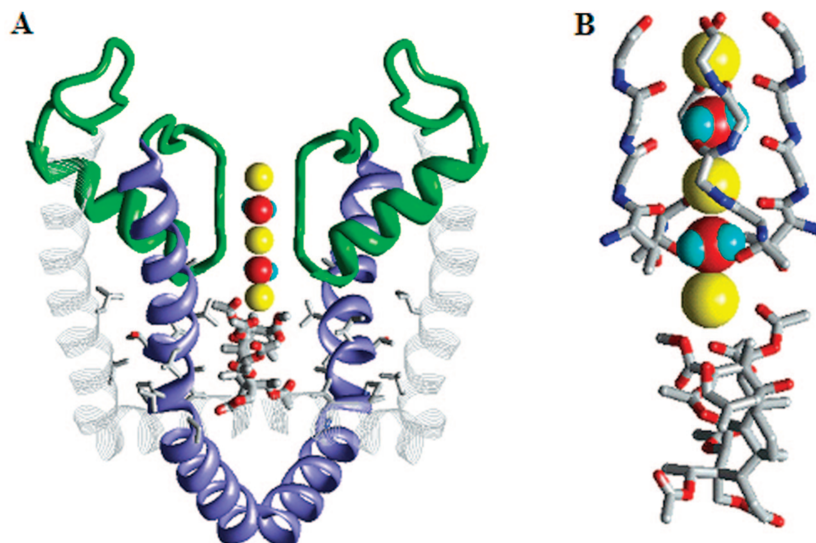


Figure 11. $K_V1.2$-based model of the open $K_V1.3$ with correolide (**37**).[343] (A) Side view of the model with only two of the four S5−P−S6 chains shown for clarity. The selectivity-filter area is loaded with two K^+ ions (positions 1 and 3) and two water molecules (positions 2 and 4). The third K^+ ion was initially placed in position 5. During energy optimization, it was chelated by three acetoxy groups of correolide, shifted upward and occurred approximately in the same position as the ammonium groups of TBA and D-tubocurarine (Figure 4). Sticks show side chains of correolide-sensing residues[34] in the inner helixes (blue) and outer helices (gray strands). (B) Close-up view of the complex, in which the signature-sequence TVGYG^{p53} backbones and Thr^{p49} side chains are shown as sticks. Ions K^+_{T1} and K^+_{T3} are octa-coordinated by the backbone carbonyls. $K^+_{T4'}$ is coordinated by three acetoxy groups of correolide and a water molecule in site T4. In addition, four Thr^{p49} side chain oxygens are within 4 Å from $K^+_{T4'}$ and P-helices' macrodipoles can additionally stabilize this ion.

pore but leaves a path for ion permeation between its nucleophilic face and a nucleophilic residue in the inner pore.[353] Subsequent mutational, electrophysiological, and ligand-binding experiments confirmed important predictions of the new model of action of sodium agonists.[356–358]

Two other classes of oxygen-rich natural products that potently block $K_V1.3$ channels are psoralens and khelliones bearing lipophilic phenylalkyl or phenoxyalkyl side-chains.[275,332] Following up on anecdotal reports that tea prepared from leaves of *Ruta graveolens,* the common rue, had beneficial effects in multiple sclerosis, the groups of Wolfram Hänsel and Eilhard Koppenhöfer at the University of Kiel in Germany extracted the low-affinity $K_V1.3$ blocker 5-methoxypsoralen from *Ruta* and demonstrated that it reduced visual field defects in single-case studies in multiple sclerosis patients.[359] However, 5-methoxypsoralen, which is a drug used for the treatment of psoriasis, was not ideal for the treatment of multiple sclerosis because of its phototoxicity. Our own group therefore performed detailed SAR investigations around 5-methoxypsoralen with the aim of eliminating the phototoxicity and increasing potency and selectivity for $K_V1.3$.[360] After identifying Psora-4 (**40**), the first nanomolar $K_V1.3$ blocker (IC_{50} = 3 nM) in 2003,[361] we designed PAP-1 (**41**) through a classical medicinal chemistry approach in 2005.[35] Although PAP-1 (IC_{50} = 2 nM) is only slightly more potent than Psora-4, it is less lipophilic and more selective than Psora-4 and therefore more suitable for in vivo use. PAP-1 is 25–125-fold selective over $K_V1.1$, $K_V1.2$, $K_V1.4$, $K_V1.5$, $K_V1.6$, and $K_V1.7$ and more than 1000-fold selective over more distantly related K^+ channels like $K_V2.1$, $K_V3.1$, $K_V4.2$, $K_V11.1$ (hERG), and the K_{Ca} channels.[35] PAP-1 further displays no in vitro and in vivo toxicity, is orally available, and has a half-life of 3 h in rats[35,237,362] and 6 h in rhesus macaques.[289] As a first proof of efficacy, Schmitz et al. demonstrated that PAP-1 (**41**) suppresses delayed-type hypersensitivity, a reaction mediated by skin homing CD4$^+$ effector memory T cells, when administered at 3 mg/kg intraperitoneally.[35] Azam at al.[362] next tested whether PAP-1 (**41**) could also suppress allergic contact dermatitis (ACD), an inflammatory skin reaction that is mediated by CD8$^+$ effector memory T cells and resembles psoriasis in some aspects. In keeping with the selective effect of $K_V1.3$ blocker on effector memory T cells, PAP-1 (**41**) did not prevent antigen presentation during the sensitization phase of ACD but potently suppressed oxazolone-induced inflammation by inhibiting the infiltration of CD8$^+$ T cells and reducing the production of the inflammatory cytokines INF-γ, IL-2, and IL-17, when administered intraperitoneally or orally during the elicitation phase.[362] PAP-1 was equally effective when applied topically in a cream demonstrating that it penetrates skin well and could potentially be developed into a topical for the treatment of psoriasis.[362] Oral PAP-1 treatment at 50 mg/kg further significantly reduces diabetes incidence and delays diabetes onset in a rat model of type-1 diabetes.[237]

Starting with the 4,7-dimethoxy-substituted benzofuran khellinone isolated from *Ammi visnaga*, which can be regarded as a lactone ring-opened version of the psoralen ring system, Baell et al.[363,364] synthesized three other classes of $K_V1.3$ blockers through multiple parallel synthesis: 3-substituted khellinone chalcones (**42**), khellinone dimers, and khellinone derivatives alkylated at either the 4- or 7-position (**43** and **44**). All three compound classes inhibit $K_V1.3$ with IC_{50} values of 100–400 nM and display moderate 3–10-fold selectivities over other K_V1-family channels, are not cytotoxic, and suppress human T cell proliferation at low micromolar concentrations.[363,364] A more potent khellinone derivative of undisclosed structure is currently in preclinical development at the Australian company Bionomics. Interestingly, the different khellinone derivatives and the psoralens exhibit different mechanisms of blocking despite their structural similarity. While the psoralens Psora-4 (**40**) and PAP-1 (**41**) block $K_V1.3$ by binding to the C-type inactivated state and therefore only reach their full blocking potency after repeated channel openings,[35] the 3-substituted khellinone chalcones like chalcone-16 (**42**) and the 4-substituted khellinones (**43**) seem to be open channel blockers and reach

full blocking potency on the first depolarizing pulse.[363,364] Intriguingly, the 7-series khellinones (**44**) display blocking kinetics that are a mixture between open-channel and use-dependent blocking.[364] The compound classes further differ in the stoichiometry of their interaction with $K_V1.3$. Psora-4, PAP-1, the khellinone chalcones and the 7-substituted khelliones exhibit a Hill coefficient of 2, suggesting that 2 blocker molecules bind to one channel molecule. Although cooperativity has not yet been rigorously proven for all these compounds, it is highly likely on the basis of detailed binding studies performed by Schmalhofer et al. with disubstituted cyclohexyl type $K_V1.3$ blockers, which demonstrate the presence of two receptor sites on the $K_V1.3$ channel protein that display positive allosteric cooperativity.[365] In contrast, the khellinone dimers and the 4-substituted khellinones have Hill coefficients close to unity indicating a 1:1 interaction.

Dirk Trauner's group at the University of California, Berkeley, took a peptidomimetic approach for designing $K_V1.3$ blockers. Using the tetraphenylporphyrin system (**45**) as a scaffold, Gradl et al.[366] put four positively charged groups at the optimal distance to form salt bridges with four Asp^{p38} residues in the outer vestibule of $K_V1.3$. However, no mutational or computational studies have yet been performed to confirm the binding mode of the tetraphenylporphyrins. The tetraphenylporphyrins displace radiolabeled peptides from $K_V1.3$ with K_d values of 20–150 nM and inhibit $K_V1.3$ currents in patch-clamp experiments at low micromolar concentrations. The tetraphenylporphyrins may not be ideal drug candidates because of their permanent charge and their relatively high molecular weight. However, these crosslike compounds, which have been suggested to simultaneously form four salt bridges with four Asp^{p38} residues, may serve as the basis for the attachments of fluorophores as alternatives to fluorophore-tagged peptides or antibodies. The tetraphenylporphyrins could also be used for the synthesis of metalloporphyrins for imaging and crystallographic studies.[366]

3.2. $K_{Ca}3.1$ Channels

A calcium-dependent K$^+$ efflux, which is now known to be carried by $K_{Ca}3.1$, was first described in 1958 by the Hungarian scientist Gardos in human erythrocytes.[367] $K_{Ca}3.1$ is therefore also often referred to as the "Gardos channel". Other names for the channel include SK4, KCNN4, and "intermediate-conductance" K_{Ca} because its single-channel conductance is larger than the conductance of the $K_{Ca}2$ channels but smaller than the conductance of $K_{Ca}1.1$ channels. $K_{Ca}3.1$ was cloned nearly simultaneously by three groups in 1997[368–370] and found to show about 42–44% sequence identity to the $K_{Ca}2$ channels (see section 2.3). The channel is voltage-independent and, like the $K_{Ca}2$ channels, binds calmodulin in its C-terminus, which renders the channel sensitive to submicromolar Ca^{2+} concentrations.[371] $K_{Ca}3.1$ is primarily expressed in placenta, lung, salivary gland, distal colon, and lymphoid organs, but it is mostly absent from cardiac and neuronal tissue.[264,369,370,372] In naïve T and B cells,[264,266] fibroblasts,[373] dedifferentiated vascular smooth muscle cells,[11,374] and vascular endothelial cells,[375] $K_{Ca}3.1$ is an important regulator of proliferation that exerts its effect by hyperpolarizing the cell membrane and thus facilitating Ca^{2+}-entry, a prerequisite for cell proliferation. In all these cell types, stimulation with either growth factors (in the case of fibroblast or vascular smooth muscle cells) or antigen or mitogen (in the case of T and B cells) leads to transcriptional up-regulation of $K_{Ca}3.1$ expression[11,264,376] and $K_{Ca}3.1$ blockade suppresses proliferation. In tissues that are involved in salt and fluid transport like the colon, the lung, and salivary glands, $K_{Ca}3.1$ activity is found on the basolateral side of the respective epithelium, where it recycles K$^+$ together with $K_V7.1$ and thus helps to facilitate chloride secretion.[377,378] In vascular endothelia, $K_{Ca}3.1$ mediates the so-called endothelium-derived hyperpolarization (EDHF) response together with $K_{Ca}2.3$.[379,380] $K_{Ca}3.1$ channels have further been shown to be involved in the migration of macrophages,[253] microglia,[262] vascular smooth muscle cells,[381,382] and mast cells.[241] In microglia, $K_{Ca}3.1$ also seems to play a role in the oxidative burst, nitric oxide production, and microglia-mediated neuronal killing.[261,383] Two independently generated $K_{Ca}3.1^{-/-}$ mice[384,385] were both viable, of normal appearance, produced normal litter sizes, did not show any gross abnormalities in any of their major organs, and exhibited rather mild phenotypes: impaired volume regulation in erythrocytes and lymphocytes[384] and a reduced EDHF response together with a mild ~7 mmHg increase in blood pressure.[385] So far no human disease involving $K_{Ca}3.1$ mutations have been described. Readers interested in more details on the expression and physiological function of $K_{Ca}3.1$ are referred to a recent review.[188]

3.2.1. Peptidic $K_{Ca}3.1$ blockers

The most potent peptidic blocker of $K_{Ca}3.1$ is maurotoxin (MTX, $IC_{50} = 1$ nM), a 34-residue scorpion toxin with an $\alpha\beta\beta$ fold.[386,387] However, MTX has even higher affinity ($IC_{50} = 100$ pM) for $K_V1.2$.[386,388] Another scorpion toxin that inhibits $K_{Ca}3.1$ is ChTX ($IC_{50} = 5$ nM), which has been traditionally used to distinguish "charybdotoxin-sensitive" from "apamin-sensitive" K_{Ca} channels despite the fact that ChTX also inhibits $K_V1.3$ and $K_{Ca}1.1$ (see section 2.2.1). Mutational studies by Rauer et al.,[321,389] which used ChTX and the sea anemone toxins ShK and BgK as molecular calipers, demonstrated that the outer vestibules of $K_{Ca}3.1$ and $K_V1.3$ have very similar dimensions, which partly explains this cross-reactivity. On the basis of this information, the authors designed the ChTX analog ChTX-Glu^{32}, whose negatively charged Glu^{32} is repelled by negatively charged residues in the outer vestibule of $K_V1.3$, resulting in 30-fold selectivity for $K_{Ca}3.1$ over $K_V1.3$.[389] However, this modification did not increase selectivity over $K_{Ca}1.1$.

3.2.2. Small Molecule $K_{Ca}3.1$ Blockers

Because we recently reviewed the pharmacology of $K_{Ca}3.1$ in detail,[188] we here only briefly describe the most commonly used $K_{Ca}3.1$ blockers and comment on their therapeutic applications. Compounds that inhibit $K_{Ca}3.1$ in the micromolar range include the chinchona alkaloid quinine ($IC_{50} = 100\ \mu M$), the vasodilator cetiedil ($IC_{50} = 25\ \mu M$), and the L-type Ca^{2+} channel blockers nifidipine ($IC_{50} = 4\ \mu M$) and nitrendipine ($IC_{50} = 1\ \mu M$).[188] The first nanomolar $K_{Ca}3.1$ blocker that was identified is the azole antimycotic clotrimazole (**46**, Figure 12), which inhibits both the Gardos channel in human erythrocytes and the cloned $K_{Ca}3.1$ channel with IC_{50} values of 70−250 nM.[188,390,391] Using clotrimazole (**46**) as a tool compound, Carlo Brugnara and co-workers at Harvard University demonstrated that pharmacological $K_{Ca}3.1$ blockade has beneficial effects in a transgenic mouse model of sickle-cell anemia.[392] The group further provided evidence that clotrimazole could reduce erythrocyte dehydration in a small number of patients with sickle cell anemia.[393]

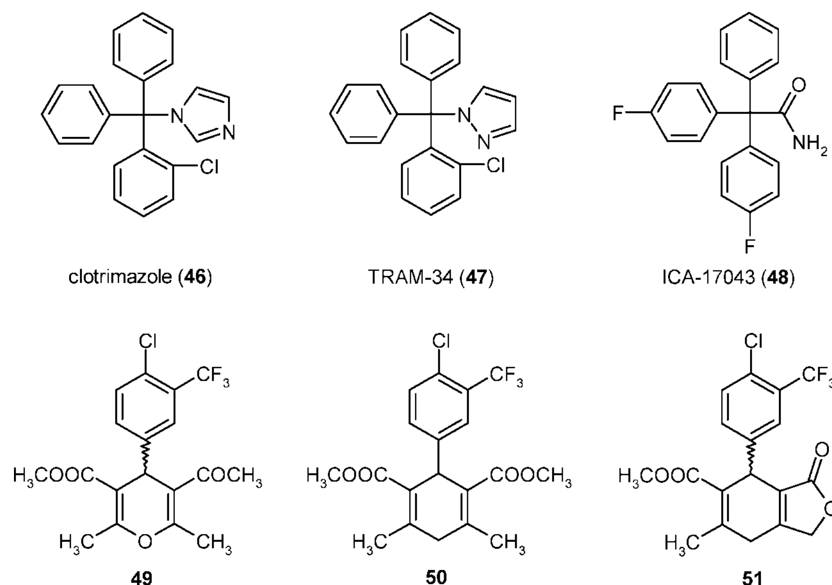


Figure 12. KCa3.1 blockers.

However, clotrimazole (**46**) itself was not an ideal drug for long-term use because of its acute inhibition and chronic induction of human cytochrome P450-dependent enzymes leading to liver damage. Three groups, including us therefore used clotrimazole (**46**) as a template for the design of triarylmethane based $K_{Ca}3.1$ blockers, which are free of cytochrome P450 inhibition.[188] By replacement of clotrimazole's azole ring, which is responsible for the compounds strong P450 inhibition, with a pyrazole ring, our own group identified TRAM-34 (**47**), a compound that inhibits $K_{Ca}3.1$ with an IC_{50} of 20 nM and displays 200–1000-fold selectivity over K_V and other K_{Ca} channels.[10] Through site-directed mutagenesis we later demonstrated that TRAM-34 (**47**) and clotrimazole (**46**) block $K_{Ca}3.1$ by interacting with $Thr^{p49(250)}$ in the pore loop and $Val^{i15(275)}$ in S6.[394] Scientists at Icagen developed the fluorinated triphenyl acetamide ICA-17043 (**48**), which inhibits $K_{Ca}3.1$ with an IC_{50} of 11 nM.[395] ICA-17043 had been found to be both effective and safe in a phase-2 trial for sickle-cell anemia,[396] but recently failed in Phase-3 clinical trials apparently because of a lack of efficacy. A chemically different class of $K_{Ca}3.1$ blockers are the 4-phenyl-4*H*-pyrans[397] and the related cyclohexadiens.[398] Using the dihydropyridine nifedipine as a template, Urbahns et al. at Bayer replaced the NH in the dihydropyridine ring system, which is required for Ca_V channel blockade, first with an O atom in **49** (IC_{50} = 8 nM)[397] and then with CH_2 in the cyclohexadiene **50** (IC_{50} = 1.5 nM).[398] However, the cyclohexadienes showed a tendency toward 3,6/3,5 double bond isomerization, and the authors therefore also synthesized a series of corresponding cyclohexadiene lactones exemplified by **51** (IC_{50} = 8 nM), in which the second ring prevents isomerization.[398] Compound **51** was subsequently shown to exhibit good selectivity over ion channels and to reduce infarct volumes, brain edema, and intracranial pressure following traumatic brain injury in rats.[399] The authors of this study demonstrated that $K_{Ca}3.1$ mRNA increases in the brain following injury, but they did not determine whether this increase was on microglia, brain infiltrating macrophages and T cells, or possibly on cells of the blood brain barrier endothelium. However, taken together with reports by the group of Lyanne Schlichter showing that TRAM-34 (**47**) reduces microglia-mediated neuronal killing both in vitro and in an optic nerve transection model in vivo,[383] this study suggests the possibility of using $K_{Ca}3.1$ blockers for the treatment of traumatic brain injury and possibly ischemic stroke and other neurodegenerative disorders with an inflammatory component.

On the basis of the expression of $K_{Ca}3.1$ in T cells, B cells, macrophages, and mast cells, $K_{Ca}3.1$ blockers have also been suggested as novel immunosuppressants.[7,188] Our own group demonstrated that TRAM-34 (**47**) suppresses the proliferation of human $CCR7^+$ T cells and IgD^+ B cells in vitro,[10,266] while scientists at Schering-Plough reported that TRAM-34 effectively prevents experimental autoimmune encephalomyelitis (EAE) induced by immunization with MOG peptide in mice.[400] While $K_{Ca}3.1$ blockers might not be optimal for the treatment of diseases such as multiple sclerosis where the brain-infiltrating T cells are of a $K_V1.3^{high}$ effector memory phenotype,[401,402] they could potentially be useful for the treatment of rheumatoid arthritis, transplant rejection, asthma, and primary biliary cirrhosis (PBC). A small clinical trial, conducted by Wotjtulewski at al. in 1980 reported that clotrimazole (**46**) was superior to ketoprofen in improving rheumatoid arthritis.[403] In T cell proliferation assays, TRAM-34 (**47**) further synergizes with cyclosporine suggesting that it could be used in combination therapy in transplant rejection to reduce cyclosporine toxicity.[10] $K_{Ca}3.1$ blockade with TRAM-34 (**47**) also inhibits human lung mast cell migration[241] and antimitochondrial antibody secretion by B cells from PBC patients[404] suggesting that $K_{Ca}3.1$ blockade might be useful for the therapy of asthma and PBC. $K_{Ca}3.1$ blockade also constitutes a potential new therapeutic approach to cardiovascular diseases such as restenosis and possibly atherosclerosis based on $K_{Ca}3.1$'s role in driving vascular smooth muscle cell and fibroblast proliferation.[11,376,382,405] In proof of this concept, TRAM-34 (**47**) and clotrimazole (**46**) significantly reduce intimal hyperplasia following balloon catheter injury in rats,[11] demonstrating that $K_{Ca}3.1$ blockers can prevent restenosis, a common complication of angioplasty. Finally, $K_{Ca}3.1$ blockers might also be useful to inhibit tumor angiogenesis because of their proven ability to inhibit angiogenesis in the mouse matrigel plug assay.[375]

From a drug-design standpoint, both the triarylmethane- and the phenyl-pyran/cyclohexadiene-type $K_{Ca}3.1$ blockers are perfect examples of the power of what Camille Wermuth has

termed the "SOSA" approach, the selective optimization of the side activity of an "old" drug for new target.[406] In both cases careful consideration of the previously known SAR for cytochrome P450 inhibition or $Ca_v1.2$ blockade made it possible to design potent and selective $K_{Ca}3.1$ blockers.

3.2.3. $K_{Ca}3.1$ Activators

Presumably because of their similar mode of activation through a C-terminally bound calmodulin, $K_{Ca}3.1$ channels are activated by the same compounds that activate $K_{Ca}2$ channels (see section 2.2.2). The only reported exception so far is the aminopyrimidine CyPPA (**36**), which activates $K_{Ca}2.3$ and $K_{Ca}2.2$ currents but not $K_{Ca}3.1$ and $K_{Ca}1.1$.[226] All other $K_{Ca}2$ activators, namely, EBIO (**32**), DC-EBIO (**33**), NS309 (**34**), and riluzole (**35**) activate $K_{Ca}3.1$ about 5-fold more potently than $K_{Ca}2$ channels.[188] The most potent $K_{Ca}3.1$ activator is currently NS309 (**34**) with an EC_{50} of 27 nM.[188,221] Therapeutically $K_{Ca}3.1$ activators might be useful for the treatment of cystic fibrosis because they could potentially increase the diminished chloride secretion onto the lung surface by activating the basolateral $K_{Ca}3.1$ channel on the lung epithelium.[407] Because of the expression of $K_{Ca}3.1$ in vascular endothelium and the role of $K_{Ca}3.1$ in the EDHF response, $K_{Ca}3.1$ activators might also be able to lower blood pressure. However, as discussed above (sections 2.3 and 3.2) the EDHF response is carried by both $K_{Ca}2.3$ and $K_{Ca}3.1$ channels, and it would be necessary to have subtype specific activators before attempting to make any predictions about which of the two channels is physiologically more important. It is currently not clear what the effects, if any, of $K_{Ca}3.1$ activation will be on the immune system since none of the existing activators have been tested on immune cell function in vitro or in vivo.

4. Conclusion and Perspectives

During recent years remarkable progress has been made in our understanding of the physiological and pathophysiological role of K^+ channels. All 78 K^+ channels in the human genome have been cloned, and a large and steadily increasing body of literature is available describing their tissue distribution, subcellular localization, involvement in signaling, and role in disease processes. However, because of the difficulties of targeting ion channels in general, chemistry efforts in this area have considerably lagged behind the large-scale medicinal chemistry programs targeting G-protein coupled receptors and protein kinases, and there are currently no clinically used drugs that have been rationally developed to target a particular K^+ channel. With the recent advent of high- or, at least, medium-throughput electrophysiology, this situation is currently changing rapidly and academic screening centers and pharmaceutical companies are increasingly screening for K^+ channel modulators as physiological tool compounds and as potential drug candidates. K^+ modulators are particularly attractive for the treatment of neurological disorders, autoimmune diseases, and inflammation. It is therefore to be expected that drugs modulating the channels discussed here ($K_V7.2$–7.5, $K_{Ca}1.1$, and $K_{Ca}2.1$–2.3 for neurological disorders and $K_V1.3$ and $K_{Ca}3.1$ for autoimmune diseases and inflammation) will be developed within the next 5–10 years. The success of these efforts is often assumed to critically depend on the ability of medicinal chemists to identify subtype selective modulators. However, the example of retigabine shows that a relatively low potency and not subtype-selective compound can be developed as a drug, and only the future will show if more potent and selective $K_V7.2$–7.5 channel activators will offer any advantages.

The discovery of K^+ channel modulating drugs is also increasingly assisted by structural information. The X-ray structures of K^+ channels in the open and closed states have revolutionized our knowledge about how drugs target K^+ channels during the last 10 years. Although cocrystals of KcsA with TBA currently are the only visualized example of a drug bound in the inner pore of a K^+ channel, results of numerous mutational, electrophysiological, and ligand-binding experiments are increasingly interpreted in structural terms using homology modeling and ligand docking. However, it should be noted that the inner-pore dimensions between the X-ray structures of MthK, K_VAP, and $K_V1.2$, which were all crystallized in the open state, vary widely. It is therefore advisable to interpret homology models of medicinally important K^+ channels like $K_V11.1$ (hERG), which are based on these templates, with caution. This restricts reliability of predicted ligand–receptor complexes, which are based only on energy criteria. Another complication is the huge diversity in the chemical structure of drugs modulating the same channel protein. In particular, the paradoxical observations that both cationic and nucleophilic ligands target the same region in the inner pore, which evolved to enable fast flow of K^+ ions via the low-dielectric energy barrier in the membrane environment, suggest that metal ions may be invisible but important receptor components for nucleophilic ligands. Future high-resolution structures of medically important ion channels in the closed, open, and slow-inactivated states should make it possible to perform more precise energy calculations and more reliable structure-based drug design. Meanwhile, homology modeling and ligand docking is likely to become an increasingly popular approach to rationalize huge and often controversial data on structure–activity relations of available ligands. In some cases, this approach may provide important information for structure-assisted design of new potent and specific drugs.

5. Abbreviations

AHP	afterhyperpolarization
4-AP	4-aminopyridine
BFNC	benign familial neonatal convulsions
BgK	*Bunadosoma granulifera* K^+ channel toxin
Ca_V	volgate-gated Ca^{2+} channel
ChTX	charybdotoxin
CNS	central nervous system
COX	cyclooxygenase
CRAC	calcium release activated Ca^{2+} channel
DAP	diaminopropionic acid
DC-EBIO	dichloroethylenbenzimidazolone
DHS	dehydrosoyasaponin
EAE	experimental autoimmune encephamolyelitis
EBIO	ethylenbenzimidazolone
EDHF	endothelium-derived hyperpolarization factor
F6CA	fluorescein-6-carboxyl
GABA	γ-aminobutyric acid
HaTX	hanatoxin
hERG	human ether-à-go-go related gene ($K_V11.1$)
IbTX	iberiotoxin
I_M	M current (neuronal K^+ current inhibited by muscarinic agonists)
K_{2P}	two-pore K^+ channel
KTX	kaliotoxin
K_{Ca}	calcium-activated K^+ channel

K_{ir}	inward-rectifier K^+ channel
K_V	voltage-gated K^+ channel
mAHP	medium afterhyperpolarization
MgTX	margatoxin
MTX	maurotoxin
PBC	primary biliary cirrhosis
PIP_2	phosphatidylinositol-4,5-biphosphate
Na_V	voltage-gated Na+ channel
NMDA	*N*-methyl-D-aspartic acid
RCK	regulator of conductance of K^+
SAR	Structure-activity relationship
ShK	*Stichodactyla helianthus* K^+ channel toxin
SK	small-conductance K_{Ca}
SOSA	selective optimization of a side activity
SUR	sulfonylurea receptor
TBA	tetrabutylammonium
TEA	tetraethylammonium
TM	transmembrane segment
VSTX1	voltage sensor toxin 1 from *Grammostola spatulata*

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7. References

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