Voltage-gated potassium channels as therapeutic targets

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Abstract | The human genome encodes 40 voltage-gated K^+ channels (K_V), which are involved in diverse physiological processes ranging from repolarization of neuronal and cardiac action potentials, to regulating Ca^{2+} signalling and cell volume, to driving cellular proliferation and migration. K_V channels offer tremendous opportunities for the development of new drugs to treat cancer, autoimmune diseases and metabolic, neurological and cardiovascular disorders. This Review discusses pharmacological strategies for targeting K_V channels with venom peptides, antibodies and small molecules, and highlights recent progress in the preclinical and clinical development of drugs targeting the K_V1 subfamily, the K_V7 subfamily (also known as KCNQ), K_V10.1 (also known as EAG1 and KCNH1) and K_V11.1 (also known as HERG and KCNH2) channels.

Inwardly rectifying
Describes K^+ or Ca^{2+} channels that are closed at depolarized membrane potentials and open with steep voltage dependence on hyperpolarization. They are called inward rectifiers because current more readily flows through them into than out of the cell.

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After protein kinases and G protein-coupled receptors, voltage-gated-like ion channels (VGICs) constitute the third largest group of signalling molecules encoded by the human genome[1]. With 78 members, K^+ channels make up about half of this extended gene superfamily and can be divided into four structural types based on their mode of activation and the number of their transmembrane segments: inwardly rectifying 2-transmembrane K^+ channels (K_{ir}), 2-pore 4-transmembrane K^+ channels (K_{2P}), Ca^{2+}-activated 6-transmembrane or 7-transmembrane K^+ channels (K_{Ca}), and voltage-gated 6-transmembrane K^+ channels (K_V).

This Review focuses on the largest gene family in the K^+ channel group, the K_V channels, which in humans are encoded by 40 genes and are divided into 12 subfamilies. Similar to the K_V channel that was first cloned — the *Drosophila Shaker* channel[2] — all mammalian K_V channels consist of four α-subunits, each containing six transmembrane α-helical segments, S1–S6, and a membrane-re-entering P-loop, which are arranged circumferentially around a central pore as homotetramers or heterotetramers. This ion conduction pore is lined by four S5–P–S6 sequences. The four S1–S4 segments, each containing four positively charged arginine residues in the S4 helix, act as voltage sensor domains and 'gate' the pore by 'pulling' on the S4–S5 linker[3,4]. Excellent reviews have been written on the current understanding of electro-mechanical coupling mechanisms during the gating process[5–7].

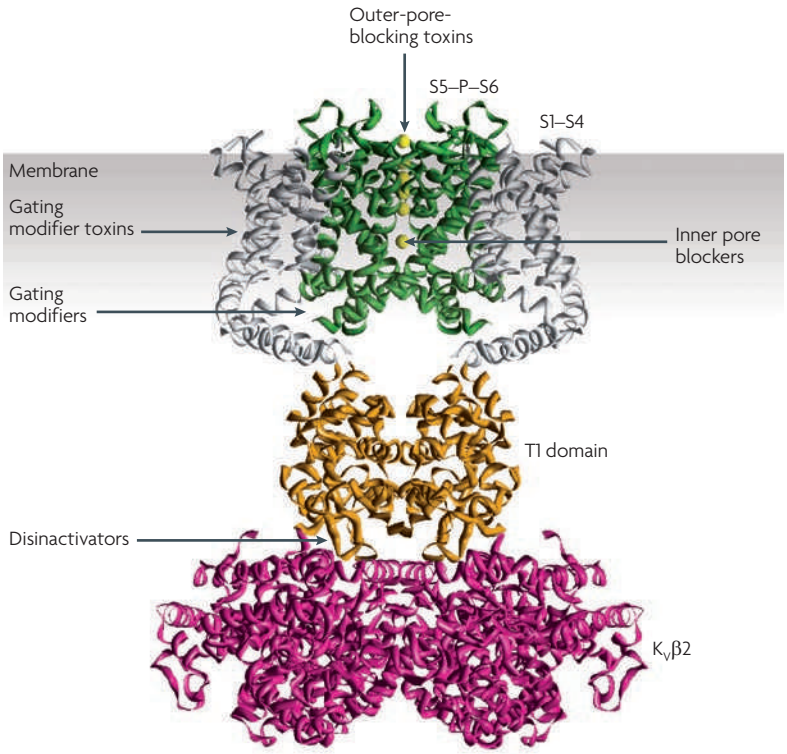
All 40 K_V channels encoded by the human genome have been cloned and their biophysical properties characterized in minute detail. However, it often remains a challenge to determine precisely which channel underlies a K^+ current in a native tissue. This is because, within subfamilies, such as the K_V1 or K_V7 families, the α-subunits can heteromultimerize relatively freely, resulting in a wide range of possible channel tetramers with different biophysical and pharmacological properties[8]. The properties of K_V channel α-subunit complexes can be further modified by association with intracellular β-subunits. For example, K_V1 family channels interact through their amino-terminal tetramerization domain with K_Vβ1–3 proteins, which form a second symmetrical tetramer on the intracellular surface of the channel (see the figure in BOX 1) and modify the gating of the α-subunits. K_V channel-interacting proteins 1–4 enhance the surface expression and alter the function of K_V4 channel α-subunits[8]. In addition to this 'mixing and matching' of α- and β-subunits, K_V channel properties can be further modified by phosphorylation, dephosphorylation, ubiquitylation, sumoylation and palmitoylation. In terms of drug discovery, this molecular diversity creates a challenge. However, it also provides an opportunity to achieve selectivity by designing modulators that discriminate between homotetramers and heteromultimers or that bind to tissue-specific β-subunits[9].

Because of the concentration gradient for K^+ that exists across the cell surface membrane, the opening of K_V channels results in an efflux of positive charge, which can serve to repolarize or even hyperpolarize the membrane. In excitable cells such as neurons or cardiac myocytes, K_V channels are therefore often expressed together with voltage-gated Na^+ and/or voltage-gated Ca^{2+} (Ca_V)

Box 1 | **Interactions of venom peptides and small molecules with K_V channels**

The figure illustrates the structure of the voltage-gated K^+ channel 1.2 (K_V1.2; also known as KCNA2) (REF. 3) with the S5–P–S6 region coloured green, the voltage sensor domain (S1–S4) coloured light grey, the tetramerization (T1) domain coloured orange and the intracellular $K_V\beta$2 subunit coloured magenta. For clarity, only two of the four subunits are shown.

Peptide toxins (see REF. 234 for a systematic nomenclature) typically contain 18–60 amino-acid residues and are cross-linked by 2–4 disulphide bridges, forming compact molecules that are remarkably resistant to denaturation. Peptide toxins can affect K_V channels by two mechanisms: 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 into the channel pore, occluding it like a cork in a bottle[235–237]. By contrast, spider toxins, such as hanatoxin, interact with the voltage sensor domain of K_V channels and increase the stability of the closed state[238,239]. The resulting rightward shift in activation voltage and acceleration of deactivation means that the channel is more difficult to open (that is, it requires more membrane depolarization) and closes faster. These 'gating-modifier' toxins typically contain a cluster of hydrophobic residues on one face of the molecule and seem to partition into the membrane when they bind to the voltage sensor[240,241]. In contrast to peptide toxins, which affect K_V channels from the extracellular side, most small molecules bind to the inner pore, the gating hinges or the interface between the α- and β-subunit.



channels and are responsible for repolarization after action potential firing. Pharmacological activation of K^+ channels in excitable cells consequently reduces excitability, whereas channel inhibition has the opposite effect and increases excitability (FIG. 1). In both excitable and non-excitable cells, K_V channels also play an important part in Ca^{2+} signalling, volume regulation, secretion, proliferation and migration. In proliferating cells, such as lymphocytes or cancer cells, K_V channels provide the counterbalancing K^+ efflux for the Ca^{2+} influx through store-operated inward-rectifier Ca^{2+} channels — such as the Ca^{2+} release-activated Ca^{2+} channel (CRAC)[10,11] or transient receptor potential (TRP) channels — which is necessary for cellular activation. In this case, K_V channel blockers inhibit proliferation and suppress cellular activation[10,12]. It is well established that both migration and metastases require Ca^{2+} influx through CRAC[13] or TRP subfamily V member 2 (REF. 14). In this context, K^+ channels have been traditionally viewed as modulators of the driving force for Ca^{2+} influx. However, although no K_V channels have been found to have intrinsic catalytic functions (in the sense of the protein kinase activity of TRP subfamily M (TRPM) channels), they often form part of large supramolecular complexes. The behaviour of these complexes can be influenced by the channel in the absence of ion flow. Therefore, non-canonical (non-conductive) properties of K_V channels are increasingly found to be of importance[15–18]. K_V channels can also play a key part in preventing depolarization following activation of electrogenic transporters, including Na^+-coupled glucose and amino-acid transporters in cells such as proximal tubule endothelial cells, which have to sustain large fluxes of cations or anions[19].

Overall, K_V channels therefore constitute potential drug targets for the treatment of diverse disease processes ranging from cancer to autoimmune diseases to metabolic, neurological and cardiovascular disorders. However, K_V channels — in particular K_V11.1 (also known as HERG and KCNH2) with its promiscuous blocker-binding pocket and its relevance for cardiac repolarization — also present a challenge to drug discovery, owing to drug-induced arrhythmias. The therapeutic potential of K_V channel modulation is further underscored by the phenotypes of transgenic mice and is associated with various human 'channelopathies' that are caused by mutations in K_V channel genes (discussed below and in TABLE 1). This article considers pharmacological strategies for targeting K_V channels with venom peptides, antibodies and small molecules, and reviews recent progress in the preclinical and clinical development of drugs that target the K_V1 subfamily, the K_V7 subfamily (also known as KCNQ), K_V10.1 (also known as EAG1 and KCNH1) and K_V11.1 channels. Channels for which there is currently no pharmacological data will not be discussed in detail but are listed in TABLE 1 together with their potential therapeutic importance.

Pharmacological channel modulation strategies

Agents that modulate K_V channels can be broadly divided into three chemical categories: metal ions, organic small molecules (molecular weight 200–500 Da) and venom peptides (molecular weight 3–6 kDa)[9]. These substances affect K_V channel function by blocking the ion-conducting pore from the external or internal side, or modifying channel gating through binding to the voltage sensor domain or auxiliary subunits (BOX 1). Similar to other proteins expressed on the cell surface, K_V channels can be targeted with antibodies (molecular weight 150 kDa), which can inhibit channel function, lead to channel internalization or deplete channel-expressing cells by complement- or cell-mediated cytotoxicity. Antibodies and toxins can also be engineered to serve as carriers for the delivery of active compounds to channel-expressing cells, or can be conjugated to cytotoxic drugs, isotopes

Venom peptide
A peptide toxin from the venoms of scorpions, sea anemones, cone snails, snakes, spiders or tarantulas. Many venom peptides target voltage- or ligand-gated ion channels.

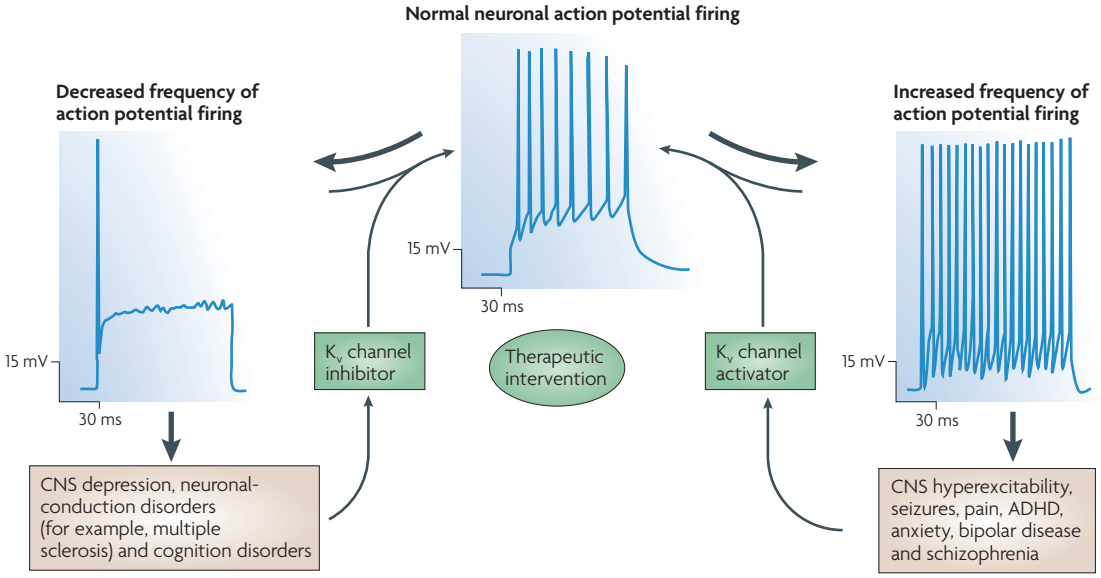


Figure 1 | **Theoretical effects of K_V channel inhibitors and activators on pathologically altered neuronal activity.** Transmission of information in the nervous system is encoded in the frequency of electrical action potential firing in nerve fibres. Pathological changes in action potential firing frequency can lead to various neurological and psychological disorders. As voltage-gated K^+ channels (K_V) play important parts in defining the action potential waveform, modulators of these channels are expected to have therapeutic utility in such disorders. For example, under conditions in which action potential firing is decreased (specifically, in depression and cognitive dysfunction) K_V channel blockers should restore normal firing. By contrast, K_V channel activators should be useful to reduce pathological hyperexcitability (specifically, in epilepsy and pain) by reducing action potential firing. ADHD, attention deficit–hyperactivity disorder; CNS, central nervous system.

or other molecules. In terms of channel inhibition, one study has reported the development of monoclonal antibodies (specific for K_V10.1), although polyclonal antibodies have been obtained in several cases using extracellular parts of the pore loop as antigen[20].

Peptide toxins typically bind either to the outer vestibule or the voltage sensor of K_V channels. By contrast, small molecules — as exemplified by the hydrophobic cations tetrabutylammonium (compound 1) (FIG. 2), *d*-tubocurarine (compound 2) and verapamil (compound 3) — block K_V channels by physically occluding the inner pore and inserting their ammonium group into the ion permeation pathway (BOX 1). The inner pore of K_V channels can also be targeted by nucleophilic molecules such as the K_V1 channel blocker correolide (compound 11), which fits neatly into the hydrophobic surface of the S6 helix with its lipophilic domain and chelates a permeating K^+ with its polar acetyl groups[21]. Typical blockers of K_V11.1 enter the channel from the intracellular side and seem to reside in a pocket in the inner mouth, where they interact mostly with two aromatic residues[22]. The wide range of drugs that this pocket can accommodate might be due to the lack of a cluster of proline residues, which induces a kink in the intracellular channel pore opening of K_V channels, in contrast to other K^+ channel families[23]. This produces a broader opening in K_V11.1 that allows entry of a wide range of molecules of varying sizes and shapes[24]. In addition to the inner pore, small molecules can also bind to the 'gating hinges'. This occurs in the case of the K_V7 channel activator retigabine, which has been found by mutagenesis to bind to a putative hydrophobic pocket that is formed following channel opening between the cytoplasmic parts of S5 and S6 (REF. 25).

Another interesting mechanism of action for channels with β-subunits are the so-called disinactivators that disrupt the interaction between α- and β-subunits and thereby modify channel behaviour[26,27]. However, rational design of K_V channel modulators is extremely difficult because no crystal structures have yet been solved for medically important K_V channels, such as K_V1.5 (also known as KCNA5), K_V7.2 (also known as KCNQ2) or K_V11.1. Only two crystal structures in the K_V channel field have been resolved — the bacterial K_VAP and the mammalian K_V1.2 (also known as KCNA2) channels (both in the open state) — and no structure of a channel with a drug molecule bound has been resolved. K_V channel modulators are therefore typically identified through high-throughput screening (BOX 2) or serendipity and then optimized through classical medicinal chemistry. Lead identification is usually achieved by ion flux assays (mostly using isotopes and/or atomic absorption spectroscopy) or fluorescent dye assays[28]. More recently, it has been achieved through automated electrophysiology, which can offer quality levels comparable to that of manual patch clamp assays and has a reasonable throughput. Detailed studies on functional drug–target interactions can be achieved through patch clamping, which allows the behaviour of a single ion channel to be studied on the microsecond timescale.

Table 1 | **Properties and therapeutic opportunities for K_V channels***

Channel (alternative names)[‡§]	Expression	Channelopathy	Phenotype of transgenic mice	Therapeutic importance
$K_V1.1$ (KCNA1)	CNS (medulla, pons, cerebellum, midbrain, hippocampus and auditory nuclei), node of Ranvier and kidney	Missense mutations cause episodic ataxia[33] and primary hypomagnesaemia[43]	$K_V1.1^{-/-}$: epilepsy with spontaneous seizures[31]; hyperalgesia; and fail to follow high-frequency amplitude-modulated sound	$K_V1.1$ disinactivators reduce PTZ-induced seizures[27]; suggested for epilepsy and neuropathic pain; $K_V1.1$ blockers in clinical trials for MS[40,41] and spinal cord injury[38]
$K_V1.2$ (KCNA2)	CNS (pons, medulla, cerebellum, hippocampus, thalamus, cerebral cortex and spinal cord)	Not reported	$K_V1.2^{-/-}$: die on post-natal day 17 from generalized seizures[32]; reduced NREM sleep	$K_V1.2$ activators or disinactivators might be useful for seizure disorders
$K_V1.3$ (KCNA3)	T and B cells, macrophages, microglia, osteoclasts, platelets, CNS (prominent in the olfactory bulb) and testis	A variant in the promoter is associated with impaired glucose tolerance and lower insulin sensitivity	$K_V1.3^{-/-}$: increased sense of smell ('supersmellers')[258], increased insulin sensitivity, lower body weight[70,71]; and no immune phenotype[259]	$K_V1.3$ blockers preferentially inhibit $CCR7^-$ effector memory T cells[54], treat rat models of MS, rheumatoid arthritis, type 1 diabetes[55], contact dermatitis[68] and periodontal bone resorption
$K_V1.4$ (KCNA4)	CNS (olfactory bulb, corpus striatum and hippocampus), heart, skeletal and smooth muscle and pancreatic islets	Not reported	$K_V1.4^{-/-}$: occasionally spontaneous seizures; no changes in cardiac I_{to}	Not determined
$K_V1.5$ (KCNA5)	Cardiac myocytes (I_{Kur}), CNS (hippocampus, cortex and pituitary), microglia, Schwann cells, macrophages and vascular smooth muscle	No human mutations reported; $K_V1.5$ expression reduced in chronic atrial fibrillation	$K_V1.5^{-/-}$: no LPS-induced nitric oxide release in microglia SWAP mice (mouse $K_V1.5$ replaced with rat $K_V1.1$): resistant to drug-induced QT prolongation	$K_V1.5$ blockers are in development as antiarrhythmics for atrial fibrillation[85]
$K_V1.6$ (KCNA6)	Spinal cord, CNS, oligodendrocyte progenitor cells, astrocytes and pulmonary artery smooth muscle	Not reported	$K_V1.6^{-/-}$ mice are commercially available; phenotype not characterized	Not determined
$K_V1.7$ (KCNA7)	Heart, skeletal muscle, liver, lung, placenta and CNS	Not reported	$K_V1.7^{-/-}$ mice are commercially available; phenotype not characterized	Might be a target for atrial fibrillation similar to $K_V1.5$
$K_V1.8$ (KCNA10)	Kidney, CNS, heart and skeletal muscle	Not reported	Not reported	Not determined
$K_V2.1$ (KCNB1)	CNS (cerebral cortex, hippocampus, cerebellum), pancreatic β-cells, insulinomas and gastric cancer cells	Not reported	$K_V2.1^{-/-}$: reduced fasting blood glucose levels and increased serum insulin levels[106]	$K_V2.1$ blockers suggested as hypoglycaemic agents for type 2 diabetes
$K_V2.2$ (KCNB2)	CNS (olfactory bulb, cortex, hippocampus and cerebellum) and pancreatic δ-cells	Not reported	Not reported	Not determined
$K_V3.1$ (KCNC1)	CNS (cerebellum, substantia nigra, cortical and hippocampal interneurons, inferior colliculi, cochlear and vestibular nuclei), skeletal muscle and mouse $CD8^+$ T cells	Not reported	$K_V3.1^{-/-}$: reduced body weight, impaired motor skills and sleep loss; $K_V3.1$–$K_V3.3$ double knockout: severe myoclonus and hypersensitivity to ethanol	Not determined
$K_V3.2$ (KCNC2)	CNS (fast spiking GABAergic interneurons), pancreatic islets, Renshaw cells (spinal interneurons), pancreatic β-cells	Not reported	$K_V3.2^{-/-}$: alterations in cortical electroencephalographic patterns and increased seizure susceptibility	Not determined
$K_V3.3$ (KCNC3)	CNS (brainstem, cerebellum, forebrain, Purkinje cells, motorneurons and auditory brainstem)	Missense mutations cause spinocerebellar ataxia 13	$K_V3.1$–$K_V3.3$ double knockout: severe myoclonus and hypersensitivity to ethanol; $K_V3.3^{-/-}$: no overt effects on phenotype	Not determined
$K_V3.4$ (KCNC4)	CNS (brainstem and hippocampal granule cells) and skeletal muscle	Missense mutation in the β-subunit KCNE3 (also known as MIRP2) causes periodic paralysis[111]	Not reported	$K_V3.4$ blockers suggested for Alzheimer's disease[112,113]

Table 1 (cont.) | **Properties and therapeutic opportunities for K_V channels***

Channel (alternative names)[‡§]	Expression	Channelopathy	Phenotype of transgenic mice	Therapeutic importance
K_V4.1 (KCND1)	CNS, heart, liver, kidney, thyroid gland and pancreas	Not reported	Not reported	Not determined
K_V4.2 (KCND2)	CNS (cerebellum, hippocampus, thalamus, forebrain and dorsal horn neurons) and rodent heart	Truncation mutations cause temporal lobe epilepsy	$K_V4.2^{-/-}$: enhanced sensitivity to tactile and thermal stimuli; $I_{to,f}$ eliminated (in mice, $I_{to,f}$ is mediated by a heteromultimer of K_V4.2 and K_V4.3)	K_V4.2 activators might be useful for inflammatory pain[119]
K_V4.3 (KCND3)	CNS (cortex and cerebellum), atrial and ventricular myocytes (I_{to}) and smooth muscle	Not reported	Not reported	K_V4.3 blockers might be useful as antiarrhythmics (in humans $I_{to,f}$ is mediated by a K_V4.3 homotetramer[117])
K_V7.1 (KCNQ1)	Heart, ear, skeletal muscle, liver, epithelia in kidney, lung and gastrointestinal tract	Loss-of-function mutations: type 1 LQTS[123] or Jervell and Lange–Nielsen syndrome[124]; gain-of-function mutations: familial atrial fibrillation[126], short QT syndrome[125] or type 2 diabetes[146]	$K_V7.1^{-/-}$ mice are deaf and have abnormal cardiac ECG T wave and P wave morphologies and prolongation of the QT interval	K_V7.1 inhibitors are in development for treating atrial arrhythmias[134] K_V7.1 openers suggested for treatment of LQTS
K_V7.2 (KCNQ2)	CNS (hippocampus, cortex, thalamus, cerebellum, brain stem and nodes of Ranvier) and sympathetic and dorsal root ganglia	Loss-of-function mutations lead to BFNC[149]	$K_V7.2^{-/-}$ mice die within a few hours after birth; $K_V7.2^{+/-}$ mice show hypersensitivity to PTZ-induced seizures	$K_V7.2/K_V7.3$ inhibitors historically developed for treatment of learning and memory disorders[154,155] $K_V7.2/K_V7.3$ activators are in development for the treatment of epilepsy[160,162,166] and pain[168]; suggested for treatment of migraine, ADHD, bipolar disease, schizophrenia[182] and bladder contractility disorders
K_V7.3 (KCNQ3)	CNS (hippocampus, cortex, thalamus, cerebellum, brain stem), nodes of Ranvier and sympathetic and dorsal root ganglia	Loss-of-function mutations lead to BFNC	Mouse models of human BFNC involving K_V7.3 (and K_V7.2) mutations exhibit seizures	As above
K_V7.4 (KCNQ4)	Outer hair cells and neurons of the auditory system and vascular smooth muscle	Loss-of-function mutations cause deafness autosomal dominant 2a (REFS 152,153)	$K_V7.4^{-/-}$ mice have a degenerative loss of outer hair cells and accompanying loss of hearing	K_V7.4 activators may be useful in the treatment of hearing disorders
K_V7.5 (KCNQ5)	CNS (hippocampus, cortex and thalamus), skeletal muscle and vascular smooth muscle	Not reported	Phenotype not reported	Not determined
K_V10.1 (KCNH1 and EAG1)	CNS	Aberrantly expressed in cancer[12,188]	Slight tendency to seizures	K_V10.1 inhibitors for cancer[204]
K_V10.2 (KCNH5 and EAG2)	CNS, muscle, heart, placenta, lung, liver, kidney and pancreas	Not reported	Not reported	Not determined
K_V11.1 (KCNH2, ERG1 and HERG)	Heart, CNS, endocrine cells and lymphocytes	K_V11.1 mutations cause type 2 LQTS[24,206]	Paroxistic bradycardia; N629D is lethal owing to cardiac malformation	K_V11.1 blockers suggested for arrhythmia (liability for drug-induced LQTS) and cancer treatment

*For a complete reference list containing gene and protein accession numbers, chromosomal location, splice variants, expression, physiological role, mutations and pharmacology, see the IUPHAR database of voltage-gated K^+ channels (K_V) at http://www.iuphar-db.org/PRODLC/FamilyMenuForward?familyId=16. ‡K_V5, K_V6, K_V8 and K_V9 channels are not functional alone; they coassemble with K_V2 subunits and modify their function. §K_V11.2 is expressed in the CNS and on endocrine cells; K_V11.3 and K_V12.1– K_V12.3 are expresssed in the CNS; no data are reported on these channels regarding channelopathies, transgenic mice and therapeutic importance. ADHD, attention deficit–hyperactivity disorder; BFNC, benign familial neonatal convulsions; CCR7, CC-chemokine receptor 7; CNS, central nervous system; GABA, γ-aminobutyric acid; I_{Kur}, ultra rapid delayed rectifier K^+ current; I_{to}, transient outward K^+ current; $I_{to,f}$, fast transient outward K^+ current; LPS, lipopolysaccharide; LQTS, long QT syndrome; MS, multiple sclerosis; NREM, non-rapid eye movement; PTZ, pentylenetetrazole.

K_V1 family channels

Channels belonging to the K_V1 or mammalian *Shaker*-family are widely expressed throughout the nervous system. Of the eight known pore-forming subunits of this family (K_V1.1 (also known as KCNA1)–K_V1.8 (also known as KCNA10)), most have been shown to form heteromultimers in the central nervous system (CNS). The exact composition of neuronal K_V1 channels remains to be fully elucidated. However, in general, most forms of neuronal K_V1 channels are thought to contain at least one K_V1.1 and/or K_V1.2 subunit[29], and these two channels are therefore regarded as targets for various CNS disorders. K_V1 family channels are also found in peripheral tissues such as the heart, the vasculature and the immune system. In these tissues, K_V1.5 and K_V1.3 (also known as KCNA3) are under investigation as targets for atrial fibrillation and immunosuppression, respectively. The therapeutic relevance of K_V1.4 (also known as KCNA4), K_V1.6 (also known as KCNA6), K_V1.7 (also known as KCNA7) and K_V1.8 is currently not clear.

K_V1.1 and K_V1.2. The importance of K_V1.1 and K_V1.2 in controlling neuronal excitability has been shown by the ability of K_V1 channel-inhibiting venom toxins such as dendrotoxin to produce seizures in rodents[30]. Furthermore, K_V1.1-knockout mice exhibit spontaneous seizures and CNS structural changes[31]. Similarly, knockout of K_V1.2 in mice is associated with increased susceptibility to seizures[32]. In humans, several loss-of-function mutations in K_V1.1 have been linked to partial seizures, episodic ataxia and myokymia disorders[33]. Moreover, loss-of-function mutations in leucine-rich glioma-inactivated protein 1 (LGI1), which is co-expressed with K_V1.1, have been associated with temporal lobe epilepsy[34]. Normal LGI1 protein inhibits the $K_V\beta$1 subunit-mediated inactivation of K_V1.1–K_V1.4 heteromultimeric channels, which increases the K^+ current and reduces neuronal excitability. By contrast, mutated LGI1 lacks the ability to abrogate $K_V\beta$ subunit-mediated inactivation[34].

Several small-molecule agents have been identified that are functionally equivalent to LGI1 and reverse or prevent $K_V\beta$1 subunit-mediated inactivation of K_V1.1. Various techniques including a yeast two hybrid-based screen have been used to identify disinactivators of protein–protein interactions between β-subunits and pore-forming α-subunits[26,27]. Several structural classes of compounds (see FIG. 2 for examples) have been reported to interact directly with the $K_V\beta$1 N-terminus or its receptor site on K_V1.1, preventing inactivation of the channel. In addition to increasing current flow, these K_V1.1 disinactivators effectively reduce pentylenetetrazole (PTZ)-induced and maximal electric-shock-induced seizures in mice[27]. Accordingly, compounds that act by this mechanism have the potential to reduce neuronal hyperexcitability in epilepsy and pain disorders. However, the current development status of this therapeutic strategy is unknown. Using a different screening strategy termed Leptics technology[35], investigators have recently identified both activators and inhibitors of K_V1.1 function that modulate β-subunit protein–protein interactions with K_V1 pore-forming α-subunits[36].

Whereas activation of K_V1.1 and K_V1.2 channels is expected to reduce neuroexcitability (FIG. 2), there are physiological and pathophysiological situations in which electrical signalling in the nervous system is reduced and needs to be amplified. Damage to nerves caused by trauma (specifically, spinal cord injury) or disease (specifically, multiple sclerosis) is often associated with a decreased ability to generate and propagate action potentials[37,38]. Neuronal damage is typically manifested as a loss of myelin, resulting in the uncovering of juxtaparanodal K_V1.1 and K_V1.2 channels and their redistribution along damaged axons[37,39]. The presence of newly exposed K_V channels slows and sometimes prevents conduction of electrical signals along the axon. Studies have shown that inhibition of these axonal K_V1.1 and K_V1.2 channels by the non-selective K^+ channel inhibitor 4-aminopyridine (4-AP) (compound 4) improves impulse conduction in damaged nerve fibres. This resulted in speculation that 4-AP might provide a treatment opportunity for spinal cord injury[37]. Indeed, Phase II clinical trial data for a slow-release formulation of 4-AP, fampridine SR, to treat spinal cord injury were encouraging. However, in two subsequent larger Phase III clinical studies in patients with spinal cord injury, the drug failed to produce any statistically significant reduction in spasticity[38]. However, in a separate set of Phase III clinical studies, fampridine SR was found to improve walking ability in patients with multiple sclerosis[40,41]. While these findings represent important progress in treating the symptoms of multiple sclerosis, the impact of fampridine SR on disease progression remains to be determined.

Although K_V1.1 is typically considered to be a neuronal channel, it has recently been linked to human autosomal dominant hypomagnesaemia[42]. A loss-of-function mutation in K_V1.1 reduces TRPM6-mediated Mg^{2+} reabsorption in the kidney — a function that depends on K_V1.1 setting a negative membrane potential[43]. Because of its fundamental role in many cellular functions, abnormalities in Mg^{2+} levels can result in widespread organ dysfunction, which can precipitate potentially fatal complications (for example, ventricular arrhythmia, coronary artery vasospasm and seizures). Pharmacological enhancement of available K_V1.1 channel activity might provide a therapeutic opportunity for treating hypomagnesaemia.

K_V1.3. K_V1.3 was discovered in human T cells in 1984 (REFS 10,44,45). It was proposed as a target for immunosuppression because non-selective K^+ channel blockers such as 4-AP (compound 4) inhibit T cell proliferation and interleukin-2 secretion[44]. These findings were subsequently confirmed with the more K_V1.3-selective scorpion toxin margatoxin[46], which was also found to suppress delayed-type hypersensitivity in miniature pigs, providing the first evidence that K_V1.3 blockade can inhibit immune responses *in vivo*[47]. K_V1.3 blockers exert their immunosuppressive effect by depolarizing the T cell membrane[46] and thus reducing the driving force for Ca^{2+} entry through the CRAC channel[10], which consists of the endoplasmic reticulum Ca^{2+}-sensor stromal interaction molecule 1 and the pore-forming protein

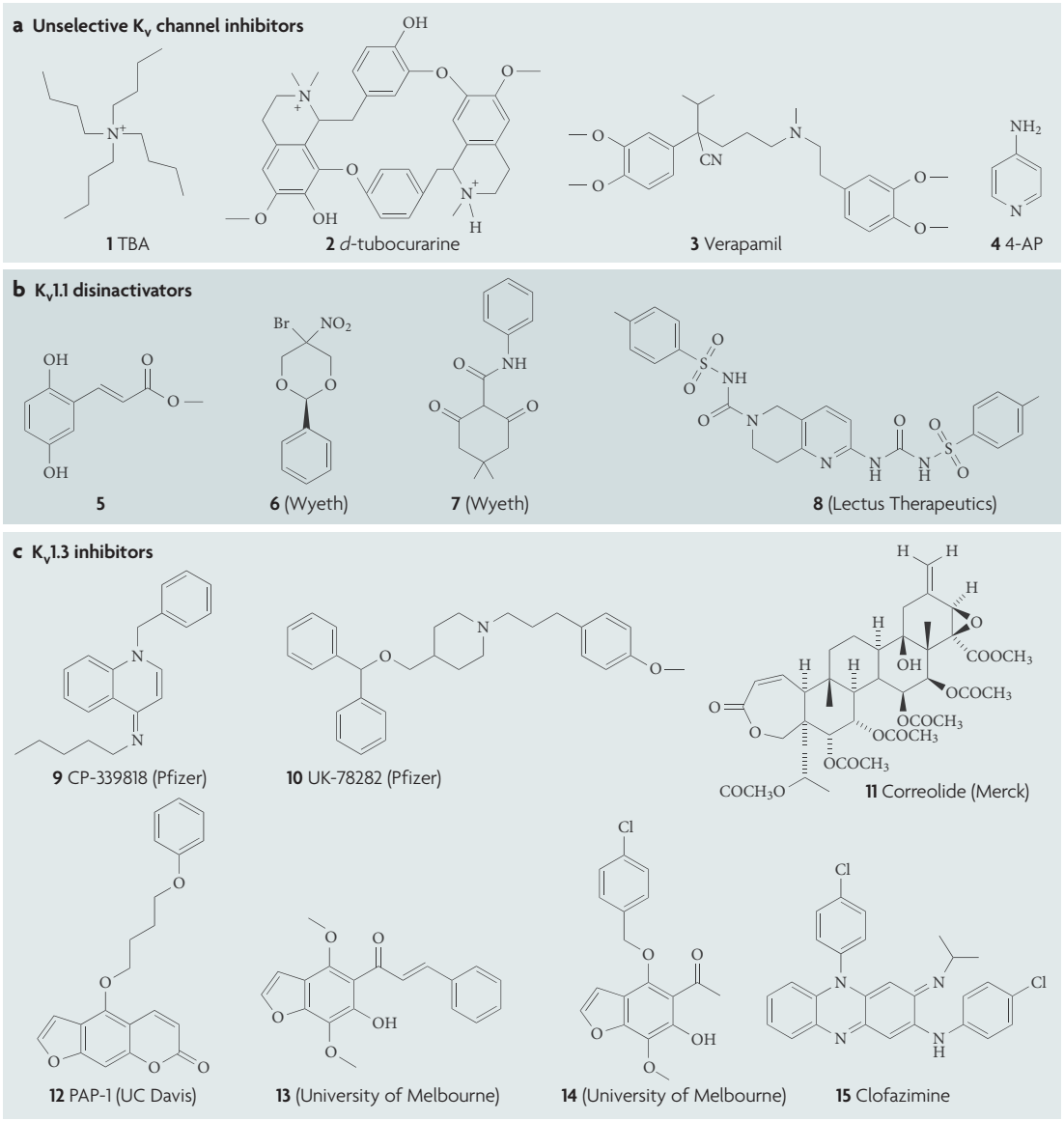


Figure 2 | **Structures of unselective K_v channel blockers and K_v1 family channel modulators.** Originators of the compounds are provided in brackets. **a** | Unselective inhibitors of voltage-gated K^+ channels (K_v) channels. Phase III trials of 4-aminopyridine (4-AP) for multiple sclerosis were recently completed[40,41]. **b** | K_v1.1 (also known as KCNA1) disinactivators prevent seizures in mice and have been suggested for the treatment of epilepsy and pain. Compound 5, compounds 6 and 7, and compound 8 are from REFS 26,27,36, respectively. **c** | K_v1.3 (also known as KCNA3) inhibitors provide effective treatment in rat and pig models of autoimmune disease and are therefore regarded as promising new immunosuppressants. Compounds 12, 13, 14 and 15 are from REFS 65,66,67,69, respectively. TBA, tetrabutyl ammonium.

ORAI1 (REFS 11,48–50). As T cells are small and have no substantial intracellular Ca^{2+} stores, this Ca^{2+} influx through the inward rectifier CRAC is necessary for the translocation of nuclear factor of activated T cells to the nucleus and the ultimately resulting cytokine secretion and T cell proliferation. To be fully activated, the T cell must therefore retain a negative membrane potential by a counterbalancing K^+ efflux through K_v1.3 and/or the other T cell K^+ channel, K_{Ca}3.1.

Small-molecule K_v1.3-targeted discovery programmes that were initiated in the mid-1990s failed to identify compounds that were sufficiently selective for *in vivo* use[51]. The Pfizer compounds CP-339818 (compound 9) (FIG. 2) and UK-78282 (compound 10) lacked selectivity over Na^+ channels or K_v1.4, and the molecular complexity of Merck's nortriterpene correolide (compound 11) was too great for successful analogue development. Interest in K_v1.3 as a target for immunosuppression subsequently waned, partly because differences in T cell K^+ channel expression between mice and humans made it impossible to use the well-established mouse models of autoimmune diseases to evaluate K_v1.3 blockers. Interestingly, mice express additional K_v channels, such as K_v1.1, K_v1.6 and K_v3.1 (also known as KCNC1), in their T cells[47,52,53] and do not rely on K_v1.3 to set their resting membrane potential.

Box 2 | **Ion channel screening technologies**

Drug discovery efforts to target voltage-gated K^+ channels (K_V) present substantial challenges. One reason for this is that the traditional technologies used to measure ion channel function are not always translatable to the high-throughput world of drug discovery. Electrophysiology techniques, such as cellular voltage clamp and in particular the patch clamp variant of this technique, have been the 'gold standard' for measuring ion channel function for nearly three decades[242]. It is a high-fidelity but low-throughput platform that requires skilled operators. This technology is useful for investigating the biophysical properties and modulation of ion channels in general and K_V channels in particular. However, it can only be used to examine a few compounds per day and is impractical in modern drug discovery, for which hundreds of thousands, and sometimes millions, of compounds need to be tested for activity.

To facilitate drug discovery programmes that target ion channels, a number of technologies have been developed. As with many drug target classes, radioligand-binding studies have proved successful in the identification of modulators of K_V channels. Radioiodinated venom toxins such as margatoxin[243] or tritiated natural products such as correolide[244] have been used to identify modulators of $K_V1.3$ (also known as KCNA3) channels; radiolabelled dofetilide is regularly used to investigate potential modulators of $K_V11.1$ (also known as HERG and KCNH2)[245].

Radioligand binding assays can have high throughput, but ligands identified by this technique do not always have functional activity. Examining K_V channel function more directly in ion flux assays can overcome this issue. Historically, radiolabelled[86] rubidium (Rb^+) ions have been used as a surrogate for K^+ in high-throughput ion flux assays for various K^+ channel targets[246]. Radioactive Rb^+ can also be replaced by unlabelled Rb^+ and then detected by atomic absorption spectroscopy[247]. More recently, thallium, to which K^+ channels are permeant, has been used successfully in high-throughput screening assays, in which it interacts with a preloaded intracellular fluorescent dye after passing through open K^+ channels[248]. Membrane potential-sensitive fluorescent dyes have also been used to examine compound interactions with K_V channels[249].

Perhaps the most important advancement in ion channel drug discovery in recent years has been the development of higher throughput electrophysiological platforms. These range from the medium-throughput systems — such as the high-fidelity PatchXpress (Molecular Devices)[250], Qpatch (Sophion)[251,252] or PatchLiner (Nanion)[253], which can test up to 100 compounds per day — to higher-throughput platforms such as IonWorks HT and Quattro (Molecular Devices)[254,255] and more recently Qpatch HTX (Sophion) that can test thousands of compounds per day. Although not truly high throughput, when used in conjunction with other screening technologies, these new electrophysiology platforms have allowed for a higher fidelity and more direct approach to K_V channel drug discovery than was previously possible. More detailed discussions of screening for ion channel modulators can be found in several recent reviews[28,256,257].

However, there has recently been a revival of interest in $K_V1.3$ as a drug target following the discovery that $K_V1.3$ blockers selectively inhibit the Ca^{2+} signalling, proliferation and *in vivo* migration of CC-chemokine receptor 7 $(CCR7)^-$ effector memory T cells (T_{EM})[54–56]. Drugs that target $K_V1.3$ might therefore constitute immunomodulators rather than general immunosuppressants[57]. T_{EM} are a memory T cell subset that is negative for CCR7 and has been implicated in the pathogenesis of T cell-mediated autoimmune diseases such as multiple sclerosis, type 1 diabetes, rheumatoid arthritis and psoriasis[55,58–61]. In keeping with this observation, myelin antigen-reactive T cells in the blood from patients with multiple sclerosis, islet antigen-reactive T cells from children with new-onset type 1 diabetes, as well as synovial fluid T cells from patients with rheumatoid arthritis and brain-infiltrating T cells in postmortem brain sections from patients with multiple sclerosis, have all been shown to be $K_V1.3^{high}$ $CCR7^-$ T_{EM} cells[54,55,61]. Similar to humans, rats, pigs and primates can upregulate $K_V1.3$ in their T_{EM} cells, making it possible to evaluate the immunosuppressive effects of $K_V1.3$ blockers in these species.

The possibility that $K_V1.3$ could serve as a target for T_{EM}-specific immunosuppression has led to the recent development of both peptidic and small-molecule $K_V1.3$ blockers. Sea anemone peptide K^+ channel toxin ShK effectively treats adoptive-transfer experimental autoimmune encephalomyelitis (EAE) in rats[62]. Subsequently, it was shown that ShK-L5 (REF. 63), a ShK derivative with improved selectivity over $K_V1.1$, is therapeutically beneficial in pristane-induced arthritis and chronic relapsing EAE in rats[55,56]. A close structural analogue of ShK-L5 is currently in preclinical development for multiple sclerosis, and attempts are being made to prolong the short half-life of venom peptides such as ShK or the scorpion peptide OSK1 by conjugating them to Fc antibody fragments[64].

Starting from two natural products, the psoralen 5-methoxypsoralen from the rue plant and the benzofuran khellinone from the toothpickweed, several classes of $K_V1.3$ inhibitors with affinities in the nanomolar to low micromolar range have been developed[65–67]. The most potent of these compounds, the psoralen PAP-1 (compound 12), inhibits $K_V1.3$ with a half-maximal inhibitory concentration (IC_{50}) of 2 nM. It has been shown to effectively treat rat allergic contact dermatitis[68], which is a simple animal model of psoriasis, and to prevent spontaneous autoimmune diabetes in diabetes-prone Biobreeding Worcester rats[55]. The khellinone-type $K_V1.3$ blockers (exemplified by the chalcone (compound 13) and the 4-substituted khellinone (compound 14)) are currently being optimized for development for multiple sclerosis. Studies on clofazimine (compound 15) recently provided further evidence that $K_V1.3$ could be a target for immunosuppression in humans. Clofazimine is a drug that is marketed as Lamprene by Novartis and has been clinically used since the 1960s for leprosy, pustular psoriasis, skin graft-versus-host disease and discoid lupus erythematosis. It inhibits $K_V1.3$ with an IC_{50} of 400 nM and prevents the rejection of transplanted human foreskin in immunodeficient mice reconstituted with human T cells[69]. Clofazimine could therefore be used as a template for the design of $K_V1.3$ blockers of a different chemotype or it could directly

Effector memory T cells (T_{EM}). Terminally differentiated memory T cells that home to inflamed tissue and secrete large amounts of inflammatory cytokines. T_{EM} cells are involved in the pathogenesis of T cell-mediated autoimmune diseases and in the clearance of chronic viral infections.

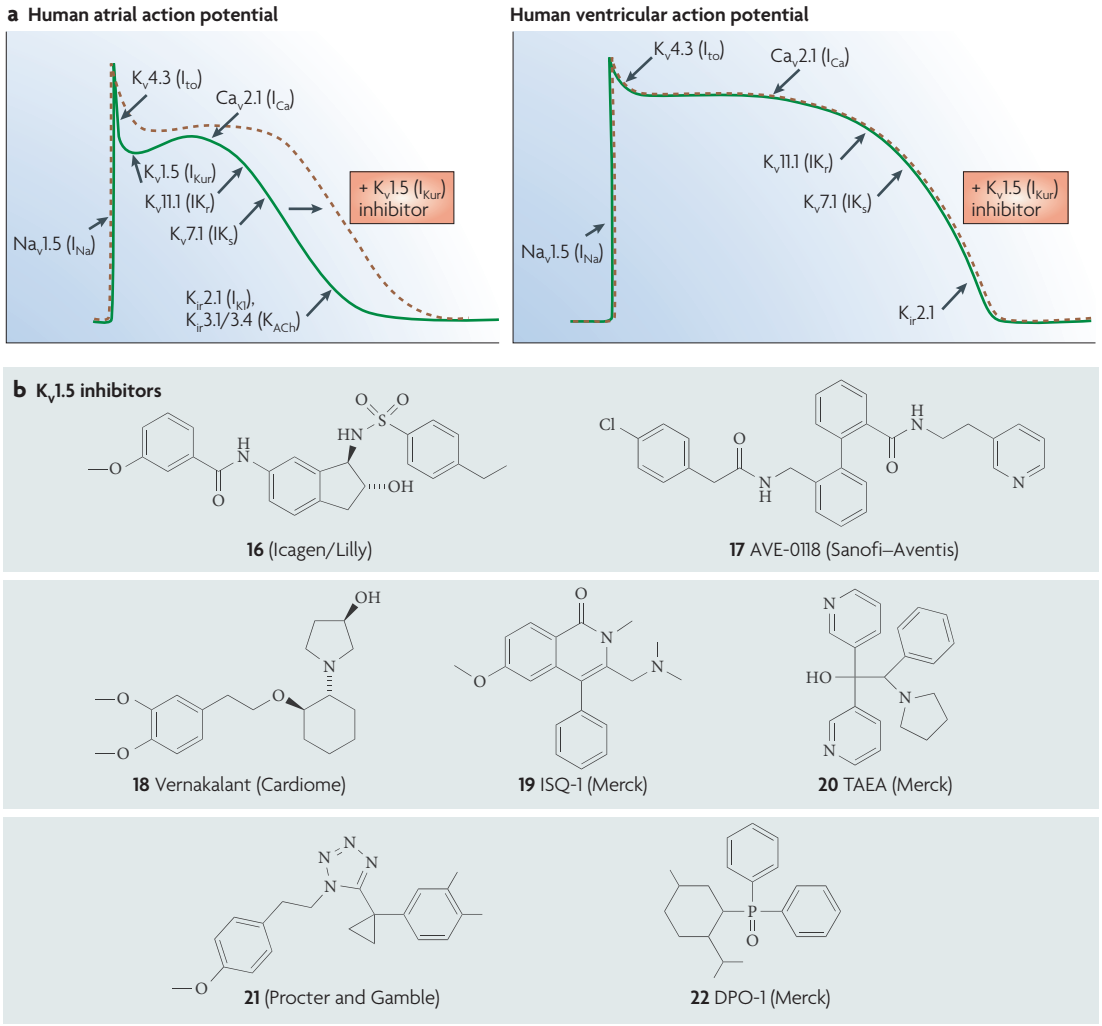


Figure 3 | **K_V1.5 inhibitors as atrium-selective antiarrhythmic agents. a** | Schematics of a human atrial and ventricular action potential and the underlying ionic conductances that define the waveform. The current for which the channel is responsible is shown in brackets. Voltage-gated K^+ channel 1.5 (K_V1.5; also known as KCNA5) is only expressed in atrial myocytes, and K_V1.5 blockers therefore selectively prolong the action potential duration in the atrium (indicated by the dashed line). **b** | Structures of K_V1.5 inhibitors. Originators of the compounds are provided in brackets. Several K_V1.5 blockers have been or are in clinical trials for the treatment of atrial fibrillation. Compound 16 is from REF. 86. Compound 17 is from REFS 89,90. Compound 18 is from REFS 96, 97. Compounds 19 and 20 are from REF. 93. Compounds 21 and 22 are from REFS 95, 92, respectively. Ca_v, voltage-gated Ca^{2+} channel; I_{Ca}, Ca^{2+} current; I_{K1}, inward rectifier K^+ current 1; I_{KACh}, acetylcholine-dependent K^+ current; I_{Kr}, rapid delayed rectifier K^+ current; I_{Ks}, slow delayed rectifier K^+ current; I_{Kur}, ultra-rapid delayed rectifier K^+ current; I_{Na}, Na^+ current; I_{to}, transient outward K^+ current; Na_v, voltage-gated Na^+ channel.

enter clinical trials after careful consideration of its benefit versus its known risks, such as gastrointestinal intolerance and skin discolorations. Results obtained with clofazimine should be interpreted with caution as the compound has multiple activities on other targets and pathways, such as stimulation of phospholipases, increasing phagocytosis by macrophages or interactions with DNA.

Based on experiments with $K_V1.3^{-/-}$ mice, these channels have also been suggested as a target for the treatment of type 2 diabetes and obesity[70]. $K_V1.3^{-/-}$ mice gained less weight on a high-fat diet than control mice and had increased insulin sensitivity owing to increased glucose uptake into adipose tissue and skeletal muscle. In these tissues in normal mice, blockade of K_V1.3 with margatoxin facilitates the translocation of glucose transporter type 4 to the plasma membrane and so improves insulin sensitivity[71]. Intriguingly, knockout of K_V1.3 can also reduce adiposity and increase lifespan in a genetic model of obesity. K_V1.3 and melanocortin receptor 4 (MC4R) double-knockout mice had a lower bodyweight and an increased lifespan and reproductive success compared with $MC4R^{-/-}$ mice[72]. However, although it is certain that mouse adipocytes express K_V1.3 protein, electrophysiological studies on neonatal brown fat cells[73,74] and white adipocytes from rats and adult humans[75,76] show K_V currents with different pharmacological and biophysical characteristics to that carried by K_V1.3 channel homotetramers. It is therefore unclear whether K_V1.3 could be a target for the improvement of insulin sensitivity and weight reduction in type 2 diabetes in humans.

K_V1.5. Although K_V1.5 is expressed in various tissues in humans[77–79], its functional expression in atrial but not ventricular muscle in the heart[77] has made this channel the focus of considerable interest in the pharmaceutical industry. Studies in the early 1990s showed that K_V1.5 was the primary molecular component of the channel underlying the ultra rapid delayed rectifier current (I_{Kur})[80,81]. This human atrium-specific K^+ current plays an important part in the early phases of atrial action potential repolarization[82] (FIG. 3a). This function, and its regiospecific localization, suggested K_V1.5 as an attractive target for the development of safer pharmacological interventions for atrial arrhythmias, particularly atrial fibrillation. The absence of functional K_V1.5 expression in the human ventricle reduces the risk of serious ventricular arrhythmias that can be induced by treatments targeting channels with broader expression in the heart[83,84]. Given the ubiquitous expression of other K_V1 channels, efforts have been made to identify and develop K_V1.5-selective agents. Development has been complicated by the fact that the importance of I_{Kur}, or the contribution of K_V1.5 to I_{Kur}-like currents, in atrial repolarization in the hearts of mice, rats, rabbits and dogs may be different from humans, making it difficult to evaluate antiarrhythmic efficacy in these species[84,85].

Despite these challenges, a number of pharmaceutical companies have attempted to develop K_V1.5 inhibitors for atrial fibrillation (FIG. 3b). More than 50 patent applications for K_V1.5 inhibitors have been submitted (see REF. 85 for a comprehensive review). One of the earliest attempts identified a number of potent K_V1.5 inhibitors, including arylsulphonamidoindanes[86] (compound 16) and subsequently tetrahydronapthalenes. However, these compounds were abandoned because of poor pharmacokinetic profiles. Other compounds from these discovery programmes entered human clinical trials but did not progress beyond Phase I. Other companies[83,87–99] developing K_V1.5 inhibitors (compounds 17–22) have demonstrated varying degrees of atrium-specific modulation of action potential repolarization. However, the majority of these compounds have not progressed beyond animal efficacy testing, owing to pharmacodynamic or pharmacokinetic issues. However, the bisaryls AVE-0118 (compound 17) and AVE-1231 from Sanofi–Aventis[89,90,100,101], although at best weakly selective for K_V1.5, progressed into human testing, with AVE-0188 reaching Phase IIa trials before development ceased. Vernakalant (compound 18) is currently in the final stages of development after a completed Phase III study gained conditional approval from the US Food and Drug Administration (FDA) for intravenous conversion of atrial fibrillation to sinus rhythm. This compound has previously been shown to reduce atrial fibrillation in various animal models[97,98]. Although it has been suggested that the primary target of vernakalant is K_V1.5, its mechanism of action probably involves blockade of several ion channels, including those responsible for the transient outward K^+ current (I_{to}) and the fast Na^+ current[97] (FIG. 3a). A selective K_V1.5 inhibitor, XEN-D0101 (REF. 85), was effective in two preclinical canine models of atrial fibrillation[102,103] and is currently undergoing Phase I evaluation as an intravenous treatment to terminate atrial fibrillation.

Delayed rectifier
A slowly activating and very slowly inactivating ion channel through which K^+ preferentially passes out of, rather than into, the cell.

Transient outward K^+ current
A rapidly activating and inactivating K^+ current.

Antiarrhythmic
An agent that decreases the incidence of arrhythmias. Class I agents interfere with the cardiac Na^+ current. Class II agents are anti-sympathetic nervous system agents (mostly beta blockers). Class III agents affect K^+ channels. Class IV agents affect voltage-gated Ca^{2+} channels and the atrioventricular node. Class V agents work by other or unknown mechanisms.

K_V2.1 channels

K_V2.1 encodes a classical delayed rectifier channel that is involved in neuronal repolarization. Its function can be diversified through heteromultimerization with the 'silent' K_V5, K_V6, K_V8 and K_V9 subunits (TABLE 1), which modify inactivation, trafficking, drug sensitivity and expression of K_V2.1 (REFS 104,105). K_V2.1 has recently been implicated in exocytic processes both in neurons and in pancreatic β-cells. In β-cells, inhibition of K_V2.1 enhances insulin secretion, suggesting a potential therapeutic strategy for type 2 diabetes[106,107]. This effect apparently occurs, at least in part, through non-conducting functions — namely, a physical interaction with syntaxin (a component of the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex) that facilitates vesicle fusion[108,109].

K_V3.4 channels

Of the *Shaw*-related family of mammalian K_V channels, so far only K_V3.4 has been proposed as a drug target. K_V3.4 co-assembles with K^+ voltage-gated channel subfamily E member 3 (KCNE3; also known as MIRP2) to give rise to I_{to} in skeletal muscle and neurons[110]. In muscle, alterations in the function of the complex owing to mutations in the accessory subunit KCNE3 are associated with periodic paralysis[111]. Additionally, in nervous tissue, K_V3.4 has been related to neuronal death induced by β-amyloid peptides in Alzheimer's disease[112,113]. K^+ depletion through hyperactivity of K_V channels contributes to apoptotic neuronal death[114], and blockade of K^+ channels has neuroprotective effects[115]. The expression of K_V3.4 is increased in the early stages of Alzheimer's disease and increases further as the disease advances[112]. In addition to these higher expression levels, the current carried by K_V3.4 is enhanced by β-amyloid peptide. The K_V3.4-blocking anemone toxin BDS simultaneously abolishes the increase in current and neuronal death[113]. Hence, blockade of K_V3.4 in the context of Alzheimer's disease could reduce neuronal loss and thereby cognitive impairment.

K_V4.2 and K_V4.3 channels

The *Shal*-type K_V4.2 and K_V4.3 channels are expressed at high levels in the brain and the heart, where they contribute to I_{to} (FIG. 3a). One remarkable feature of K_V4 channels is the complexity of their association with various ancillary subunits or scaffolding proteins and their extensive post-translational modification[116]. In terms of drug discovery, atrial and ventricular K_V4.3 channels could constitute targets for antiarrhythmic therapy. Indeed, inhibition of I_{to}, which in humans is mediated by a K_V4.3 homotetramer[117], seems to be one of the mechanisms of action of the class III antiarrhythmic agent tedisamil. However, in addition to I_{to}, tedisamil also inhibits the rapid delayed rectifier K^+ current (I_{Kr}), the slow delayed rectifier K^+ current (I_{Ks}), I_{Kur} and the ATP-dependent K^+ current ($I_{K\text{-}ATP}$)[118]. The FDA recently rejected an application for the use of tedisamil for the treatment of atrial arrhthymias. The future development of this compound remains unclear.

In addition to the potential utility of inhibitors, the important role of K_V4.2 in pain plasticity in dorsal

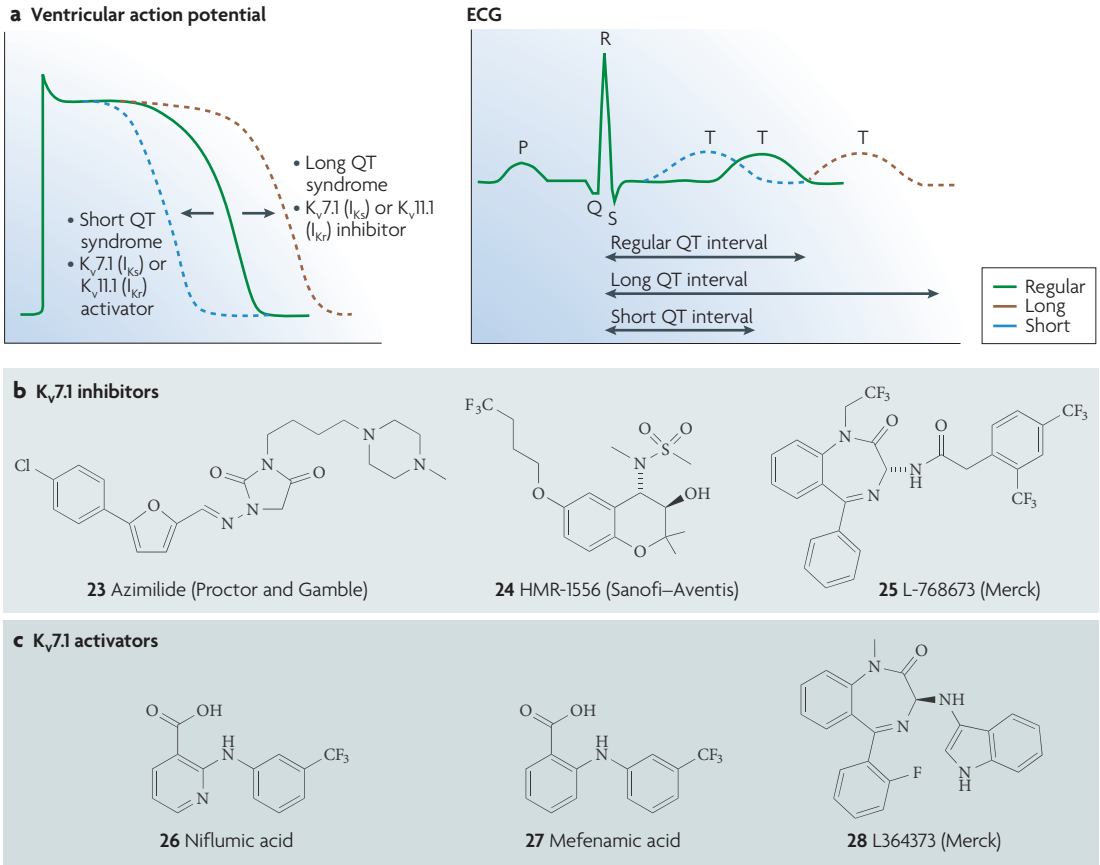


Figure 4 | **$K_V7.1$ and $K_V11.1$ are crucial for determining the length of the cardiac action potential. a** | Illustration of a ventricular action potential and electrocardiogram (ECG) showing the effects of long and short QT syndrome as well as pharmacological modulators of voltage-gated K^+ channel 7.1 ($K_V7.1$; also known as KCNQ1) or $K_V11.1$ (also known as HERG and KCNH2) on action potential duration and length of QT interval. The current for which the channel is responsible is shown in brackets. Inhibition of $K_V7.1$ and $K_V11.1$ prolongs ventricular action potential duration. This is similar to acquired or hereditary long QT syndrome. Activators of $K_V7.1$ or $K_V11.1$ reduce the duration of cardiac action potential, which is manifested as a shorter QT interval. **b** | $K_V7.1$ inhibitors. Azimilide has been shown to reduce atrial fibrillation in clinical trials[128,129], and HMR-1556 (REF. 134) and L-768673 (REF. 138) are effective in dog models of this condition. Originators of the compound are provided in brackets. **c** | $K_V7.1$ activators. Compounds 26 and 27 are from REF. 139. Compound 28 is from REF. 141. I_{Kr}, rapid delayed-rectifier K^+ current; I_{Ks}, slow delayed-rectifier K^+ current.

horn neurons in the spinal cord[119] suggests that $K_V4.2$ activators might be useful for the treatment of inflammatory pain.

K_V7 family channels

The K_V7 family comprises five members: $K_V7.1$ (also known as KCNQ1), $K_V7.2$, $K_V7.3$ (also known as KCNQ3), $K_V7.4$ (also known as KCNQ4) and $K_V7.5$ (also known as KCNQ5). Whereas $K_V7.1$ is predominantly found in peripheral tissues, $K_V7.2$–$K_V7.5$ seem to be most widely expressed in the nervous system[120,121].

$K_V7.1$. $K_V7.1$ is present in cardiac muscle, in which it is co-expressed with the auxiliary subunits KCNE1, KCNE2 and KCNE3 to form the functional channel responsible for I_{Ks}[120,122]. This current has an important role in controlling repolarization, and thus the duration, of the cardiac action potential (FIG. 4a). In humans, numerous loss-of-function mutations in $K_V7.1$ or KCNE subunits (resulting in reduced current flow and prolongation of cardiac action potentials) have been identified in potentially life-threatening cardiac abnormalities — such as long QT syndrome (LQTS), in which the QT interval is prolonged[120,123]. Several of these loss-of-function mutations in $K_V7.1$ are associated with Jervell and Lange–Nielsen syndrome[124], a condition with auditory abnormalities in addition to cardiac rhythm defects. Gain-of-function mutations in $K_V7.1$ increase current flow through the channel and lead to shortening of the cardiac action potential. They are associated with cardiac rhythm disorders such as short QT syndrome[125] and atrial fibrillation[126].

For more than a decade, the $K_V7.2$/KCNE-associated cardiac K^+ current has remained a target of interest for the development of antiarrhythmic drugs. Some marketed antiarrhythmic agents (for example, amiodarone) may produce their clinical effects in part through modulation of $K_V7.1$ or KCNE activity[127]. Azimilide (compound 23) (FIG. 4b) is a mixed inhibitor of $K_V7.1$ (which underlies I_{Ks}) and $K_V11.1$ (which underlies I_{Kr}) that has shown efficacy in various animal models of arrhythmia[128,129].

QT interval
On an electrocardiogram, the QT interval represents the time between the electrical activation and the repolarization of the ventricles. It is measured from the onset of the Q wave to the end of the T wave.

However, when assessed in clinical trials, only limited efficacy in the conversion of atrial fibrillation to sinus rhythm was observed[130–132]. The current development status of azimilide is unknown. More selective inhibitors of $K_V7.1$, such as the chromanol HMR-1556 (compound 24)[133,134] and L-768673 (compound 25), have also been reported to prolong cardiac action potentials and reduce the incidence of arrhythmias in animal models. HMR-1556, which has greater than 1000-fold selectivity for I_{Ks} over I_{Kr}, restores sinus rhythm and prevents heart failure in pigs with persistent atrial fibrillation[135,136]. In a canine model of vagal atrial fibrillation, HMR-1556 prolonged the atrial effective refractory period. However, it had only a modest effect on the duration of induced atrial fibrillation[137]. The acyl benzodiazepine L-768673 has been reported to increase ventricular refractoriness in conscious dogs[138]. Despite the promising activities of these selective $K_V7.1$ inhibitors in animal models, neither seems to have been developed sufficiently to be assessed for clinical efficacy in humans.

In addition to inhibitors, several pharmacological activators of $K_V7.1$ (with or without KCNE1) channels have been reported. Niflumic acid (compound 26) and the structurally related mefenamic acid (compound 27) increase current flow through $K_V7.1$–KCNE1 by inducing hyperpolarizing shifts in the voltage dependence of activation[139]. The benzodiazepine L-364373 (compound 28) potently activates homomeric $K_V7.1$ channels but is considerably weaker when $K_V7.1$ is co-expressed with the auxiliary subunit KCNE1 (as occurs in the heart)[140,141]. The therapeutic utility of $K_V7.1$ activators remains to be explored.

Although most well characterized in the heart, $K_V7.1$ is found in the inner ear and epithelial tissues of the kidney, lung and gastrointestinal tract[120]. In contrast to the heart, $K_V7.1$ channels in epithelial cells seem to be co-expressed primarily with KCNE3 to form a conductance that exhibits little time dependence with regard to activation and only weak sensitivity to membrane potential[142]. Gating of the channel is modulated through various second-messenger pathways, including cyclic AMP pathways[143,144]. Epithelial $K_V7.1$ channels have an important role in maintaining the driving force for proximal tubular and intestinal Na^+ absorption, gastric acid secretion, and cAMP-induced jejunal Cl^- secretion[120,145]. Recent studies have also revealed an association of $K_V7.1$ with susceptibility to type 2 diabetes[146]. $K_V7.1$ activity seems to counteract the stimulation of cellular K^+ uptake into the liver by insulin and thereby influences K^+-dependent insulin signalling[147]. The therapeutic utility of targeting $K_V7.1$ for diabetes or epithelial fluid transport disorders has yet to be explored.

$K_V7.2$–$K_V7.5$. Over the past decade, there has been considerable interest within the pharmaceutical industry to develop modulators of the neuronal K^+ conductance referred to as the M-current. It is so called because of its sensitivity to inhibitory modulation by various G protein-coupled receptor ligands, most notably muscarinic acetylcholine receptor agonists[148]. This current was first identified in the late 1970s and was subsequently shown to modulate synaptic plasticity and neuronal excitability in many areas of the brain[121,148]. The molecular nature of the M-current only became evident following the characterization of loss-of-function mutations in a rare hereditary human epilepsy called benign familial neonatal convulsions[149]. At around the time of these studies, it was shown that $K_V7.2$ and $K_V7.3$, in heteromultimeric combination, were the molecular components of at least one form of the channel underlying the neuronal M-current[150]. Subsequent studies have indicated that heteromultimeric combinations of $K_V7.3$ and $K_V7.5$ may also underlie M-currents in some areas of the brain[151]. The contribution of $K_V7.4$ to the M-current is less clear. However, $K_V7.4$ is evidently important in auditory physiology, given its expression in hair cells of the cochlea. Furthermore, loss-of-function mutations or single-nucleotide polymorphisms in $K_V7.4$ are associated with autosomal dominant deafness 2A and age-related hearing impairment[152,153].

Given the importance of $K_V7.2$–$K_V7.5$ in a wide range of neuronal processes, it is not surprising that considerable effort has been directed towards developing therapeutic agents that target these channels. More than 20 patents for new modulators of $K_V7.2$–$K_V7.5$ have been issued, and over 100 US patent applications are currently at various stages of approval. Early studies with M-current inhibitors, such as linopirdine (compound 29) (FIG. 5), demonstrated improvements in learning and memory performance in animals[154]. However, clinical trials did not provide conclusive results for the treatment of cognitive disorders[155]. Although second-generation inhibitors, such as XE-991 (compound 30) and DMP-543 (compound 31) were developed[156], no further clinical efficacy studies investigating improvement of cognitive function have been reported.

In contrast to the abandoned inhibitors, there remains widespread interest in the pharmaceutical industry to develop M-current activators. The first agent that was proven to enhance M-current activity was retigabine (compound 32). Activation of recombinant $K_V7.2/K_V7.3$ by retigabine was confirmed independently by a number of investigators, who showed that current enhancement by retigabine resulted from a profound hyperpolarizing shift in the voltage dependence of channel activation[157–159]. When examined *in vivo*, retigabine exhibited anticonvulsant activity in a broad range of seizure models including PTZ-induced seizures, maximal electric shock, audiogenic seizures in DBA/2J mice as well as seizures produced by amygdala kindling[160]. Based on these findings, retigabine has been the subject of numerous clinical studies to assess its anticonvulsant activity in humans. Phase II[161,162] and Phase III efficacy trials[163,164] have been successfully completed. Retigabine is currently awaiting FDA approval as a new first-in-class epilepsy therapy.

A number of other $K_V7.2$–$K_V7.5$ activators have been identified including the benzanilide $K_V7.2/K_V7.3$ opener ICA-27243 (compound 34), which exhibits >30-fold selectivity for $K_V7.2/K_V7.3$ over $K_V7.3/K_V7.5$ heteromultimeric channels, or $K_V7.1$, $K_V7.4$ and $K_V7.5$ homomultimeric channels[165]. Like retigabine, ICA-27243

M-current
A slowly activating and deactivating K^+ current that exhibits substantial conductance in the voltage range of action potential generation and plays an important part in determining neuronal excitability. It is called M-current because of its inhibition by muscarinic agonists.

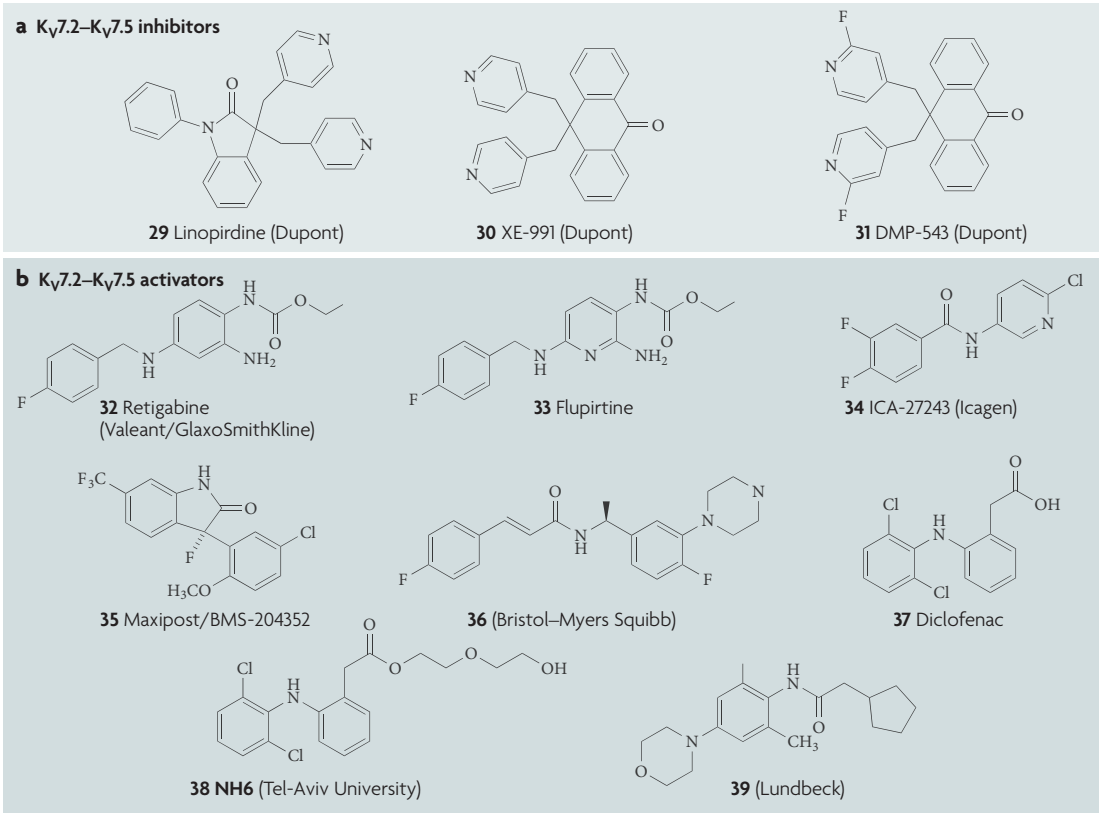


Figure 5 | **Structures of $K_V7.2–K_V7.5$ channel modulators. a** | Voltage-gated K^+ channel 7.2 ($K_V7.2$)–$K_V7.5$ inhibitors. K_V7 channel inhibitors had been proposed to improve learning and memory but failed in clinical trials. Compound 29 is from REF. 154. Compounds 30 and 31 are from REF. 156. **b** | $K_V7.2–K_V7.5$ activators are effective anticonvulsants in rodent models and clinical trials, and have been proposed for the treatment of neuropathic pain, anxiety disorders, mania, migraine, attention deficit–hyperactivity disorder and schizophrenia, based on rodent studies. Originators of the compound are provided in brackets. Compound 32 is from REFS 157–159. Compound 33 is from REFS 173,174. Compound 34 is from REFS 165,166. Compounds 35 and 36 are from REFS 170,178. Compound 37 is from REF. 180. Compound 38 is from REF. 181. Compound 39 is from REFS 185,186.

shows efficacy in various animal seizure models[166], providing evidence that selective activation of $K_V7.2/K_V7.3$ is sufficient to achieve anticonvulsant activity. Despite the promising *in vivo* activity of ICA-27243 (and a more advanced related compound, ICA-69673) in animal models, this class of agents has not been developed beyond Phase I clinical trials. However, a new, structurally distinct $K_V7.2/K_V7.3$ activator chemotype, exemplified by ICA-105665, is in development and is currently undergoing Phase II clinical trials[167].

The clear role of K_V7 channels in controlling neuronal excitability, combined with their expression in sensory and central neurons that are involved in nociceptive signalling[168,169], has further prompted the exploration of $K_V7.2–K_V7.5$ activators for the treatment of pain[170,171]. Both retigabine and its structural analogue flupirtine (compound 33) produce analgesic activity in rat models of neuropathic pain[172–174]. Flupirtine has been in clinical use as an analgesic in Europe since 1984 and is currently in Phase II clinical trials in the United States for the treatment of fibromyalgia. However, a recently completed Phase IIa clinical trial of retigabine in patients with post-herpetic neuralgia failed to demonstrate significant antinociceptive activity. The $K_V7.2/K_V7.3$-selective activator ICA-27243 has shown significant oral antinociceptive activity in animal models of inflammatory, chronic and neuropathic pain[175,176]. Furthermore, numerous $K_V7.2–K_V7.5$ activator chemotypes (compounds 35 and 36) are reportedly effective in diabetic neuropathy and other rodent neuropathic pain models following intravenous administration[170,177,178]. A patent application has also been filed for the use of $K_V7.2–K_V7.5$ activators for the treatment of migraine pain[179]. Interestingly, diclofenac (compound 37), an 'old' non-steroidal anti-inflammatory drug that is used clinically to treat inflammation and pain associated with arthritis, activates $K_V7.2$ channels, as do a number of related compounds (such as meclofenamic acid)[180]. Structural analogues of diclofenac such as NH6 (compound 38), which retain $K_V7.2$ channel-opening activity but lack cyclooxygenase-inhibiting activity, have recently been synthesized[181] and may allow assessment of the contribution of K_V7 channel opening to the analgesic activity of this class of agents.

Both selective and non-selective $K_V7.2/K_V7.3$ activators also exhibit efficacy in animal models of neuropsychiatric disorders such as anxiety, attention deficit–hyperactivity

disorder, mania, bipolar disease and schizophrenia[182]. Retigabine and ICA-27243, but not the $K_V7.4$–$K_V7.5$-preferring activator BMS-204352, are effective in an amphetamine- and chlordiazepoxide-induced hyperactivity model of mania[183]. Similarly, retigabine has been shown to inhibit avoidance responses in a conditioned avoidance response model of antipsychotic activity. This effect was blocked by the K_V7 inhibitor XE-991 (REF. 184). Furthermore, retigabine was able to inhibit hyper-locomotor responses in phencyclidine-sensitized animals, which is often used as a model of schizophrenia[184]. Similar effects are also produced by compound 39 (REFS 185,186).

Most of the interest in developing $K_V7.2$–$K_V7.5$ activators as therapeutic agents has focused on neurological or psychological disorders. However, the presence of these channels in the bladder and other urological tissues, together with the finding that $K_V7.2$–$K_V7.5$ activators can modulate bladder contraction and micturition responses in animal models, suggests that these agents might also be useful in the treatment of incontinence and related disorders[187].

$K_V10.1$

$K_V10.1$ gives rise to a slowly activating, non-inactivating K^+ current in heterologous systems. $K_V10.1$ mRNA[12,188,189] and protein[190] are abundant in the brain, but in peripheral tissues protein expression is restricted to particular cell populations[188]. Paradoxically, the only characterized physiological role of $K_V10.1$ is in skeletal muscle development, in which it is expressed during a limited time window when myoblasts exit the cell cycle and fuse[191]. Deletion of exon 1 of the gene encoding $K_V10.1$ in mice results only in a mild increase in sensitivity to seizures (H. Menke, Dissertation, Univ. Göttingen, 1998). Most of the interest in $K_V10.1$ arises from its expression in up to 70% of tumour cell lines and human cancers. These include colon carcinoma[192,193] (in which amplification of the gene has been detected by fluorescence *in situ* hybridization in 3.5% of cases and correlates with poor prognosis), gastric[194] and mammary tumours[188] and sarcomas[195] (in some of which channel expression also correlates with a poor outcome). Efforts to determine the mechanism underlying this expression pattern have been largely unsuccessful, although it has been reported that $K_V10.1$ expression is initiated after immortalization by papillomavirus oncogenes[196]. $K_V10.1$ expression might offer an advantage to tumours through increased vascularization and resistance to hypoxia[18]. However, this does not explain the observation that the proliferation of cell lines, derived from the tumour types mentioned above, is reduced by inhibiting the expression or function of $K_V10.1$ (REF. 197). Additionally, $K_V10.1$ expression seems to also affect cytoskeletal organization, which might influence proliferation and other properties of tumour cells, such as migration and metastasis[198].

Two potent blockers of $K_V10.1$, astemizole (compound 40) (FIG. 6) and imipramine (compound 41) have been shown to decrease tumour cell proliferation *in vitro* and, in the case of astemizole, also *in vivo*[195,199–201]. In mouse models, oral doses of astemizole that are well below the toxic range reduced the progression of established subcutaneous tumours (melanoma and pancreatic and mammary carcinomas) and the frequency of metastasis in lung carcinoma models with a potency comparable to the maximal tolerable dose of the established chemotherapeutic agent cyclophosphamide[18]. Tests in humans will of course need to be conducted to clarify the predictive value of these observations. Additionally, both imipramine and astemizole block $K_V11.1$ and therefore pose cardiac risks (for example, see REF. 202); it was for this reason that the antihistamine astemizole was withdrawn from the market in 2000. However, as discussed below for $K_V11.1$, the risk to benefit ratio for these drugs might need to be reconsidered for repositioning.

The intracellular channel pore openings of $K_V10.1$ and $K_V11.1$ are similar, although not identical[203]. Nevertheless, all known $K_V10.1$ blockers are also effective blockers of $K_V11.1$ and therefore share their cardiac safety problems. This has prompted the search for biological modulators that can differentiate between the two channel classes. A specific peptide toxin that can achieve this has yet to be reported. However, a monoclonal antibody (mAb56) has been developed that specifically blocks $K_V10.1$ without affecting $K_V11.1$ or the close relative $K_V10.2$ (REF. 204). The antibody showed efficacy *in vitro* against several tumour cell lines, and *in vivo* in certain tumour models, but the doses required were high and the reduction of tumour growth was modest. The experiments were performed in immunodeficient mice, so that the antibody could in principle act exclusively as a channel blocker. Interestingly, the role of $K_V10.1$ in tumour biology is not entirely mediated by K^+ permeation, as a non-conducting mutant preserves part of the pro-neoplastic properties of the wild-type channel[18].

$K_V11.1$

$K_V11.1$ plays a crucial part in cardiac repolarization (FIG. 4a), especially in the later phases of the action potential owing to its unique kinetics. Upon depolarization — that is, in the ascending phase of the action potential — $K_V11.1$ opens rapidly, but K^+ flux is quickly terminated by channel inactivation. Following repolarization, release of inactivation is fast and is followed by slow deactivation. In this way, the channel is active during the depolarization of the action potential and during part of the diastolic phase of the cardiac cycle. In the later phase, the membrane potential is set at values at which the driving force for K^+ flux is low, but K^+ conductance buffers incoming depolarizations[202,205]. Therefore, $K_V11.1$ has a pivotal role in setting the duration of the effective refractory period of the cardiac action potential.

$K_V11.1$ mutations cause LQTS type 2 because deficient $K_V11.1$ function reduces repolarization and increases the possibility of torsades de pointes, ventricular fibrillation and sudden death[24,206]. The considerable interest of the pharmaceutical industry in $K_V11.1$ is due to the involvement of this channel in drug-induced or acquired LQTS (aLQT). $K_V11.1$ blockers such as dofetilide (compound

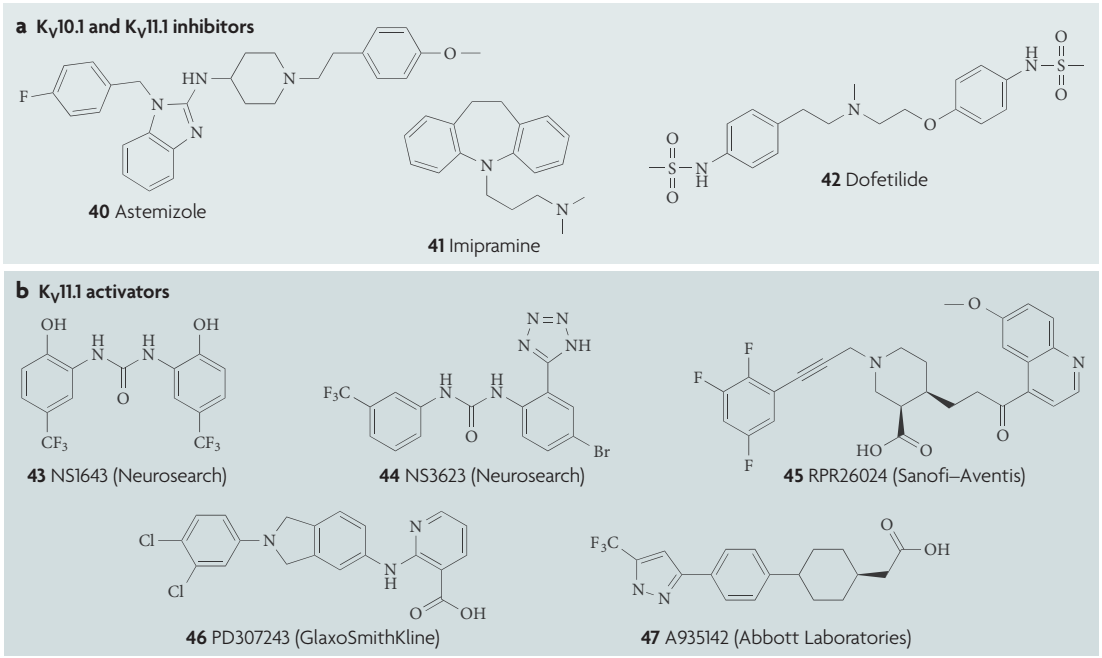


Figure 6 | **Modulators of K_V10.1 and K_V11.1. a** | Voltage-gated K^+ channel 10.1 (K_V10.1; also known as EAG1 and KCNH1) and K_V11.1 (also known as HERG and KCNH2) inhibitors have been proposed for the treatment of cancer[9,179]. K_V11.1 inhibitors prolong the QT interval and can be both antiarrythmic and proarrythmic (for example, recall from the market of the antihistamine astemizole by the US Food and Drug Administration because of long QT induction). Compound 40 is from REFS 199–202. Compound 41 is from REF. 199. **b** | K_V11.1 activators have been proposed as potential antiarrythmics[205]. Originators of the compounds are provided in brackets. Compounds 43–47 are from REFS 208–212, respectively.

42) have been used for many years as class III antiarrythmics[207]. Drugs in this class are efficacious in preventing and reverting atrial fibrillation and flutter, but their intrinsic arrhythmogenic activity largely restricts their use (often to intensive-care settings). As discussed above, K_V11.1 can be blocked by many structurally diverse compounds, and regulatory agencies request that all new drug candidates are tested for this possibility.

The large number of compounds identified as channel modulators has made it possible to identify several K_V11.1 activators in recent years (FIG. 6). Of these, six are small molecules (NS1643 (compound 43)[208], NS3623 (compound 44)[209], RPR260243 (compound 45)[210], PD307243 (compound 46)[211] and A935142 (compound 47)[212]), and one is a natural toxin (mallotoxin[213]). Owing to its complex kinetics, the activity of K_V11.1 can be increased by altering its activation, inactivation or deactivation. All of these properties are modified by the various K_V11.1 activators: NS1643 and NS3623 reduce inactivation, RPR260243 delays deactivation and PD307243 and A935142 alter all three of these properties.

These activators have two potential therapeutic applications. First, they could be used to rescue aLQT. Second, K_V11.1 activators could become a novel class of antiarrhythmics, as they have been reported to reduce electrical heterogeneity in the myocardium and thereby the possibility of re-entry[205]. Concerns about the feasibility of such an antiarrhythmic approach have been raised by a recently described cardiac condition, termed short QT syndrome[214], in which a faster repolarization results in a shorter QT interval. However, experimental models suggest that shortening of the QT interval poses a low risk of arrhythmia[205].

Of the 300 LQTS-inducing mutations in K_V11.1 that have been identified, a large proportion results in defective channel trafficking[215]. Interestingly, K_V11.1 blockers also increase the cell surface expression of the channel. However, there is no direct relationship between channel blocking efficiency and trafficking, as compounds that do not block the channels, such as thapsigargin or fexofenadine, also increase surface expression[216]. Compounds such as these could directly improve membrane targeting of the channel by acting as molecular chaperones. It is therefore conceivable to use modifiers of K_V11.1 trafficking to ameliorate LQTS that originates from surface expression defects of K_V11.1 (REF. 217).

Besides its relevance in cardiac physiology, overexpression of a primate-specific, brain isoform of K_V11.1 (encoded by *KCNH2-3.1*) — which lacks an N-terminal domain crucial for slow deactivation and therefore induces high-frequency, non-adaptive firing patterns in cultured cortical neurons — has recently been linked to an increased risk of schizophrenia[218]. It was suggested that isoform-specific K_V11.1 inhibitors might be useful for the treatment of schizophrenia. K_V11.1 has also been extensively characterized in tumours[219]. As discussed for K_V10.1, the expression of K_V11.1 seems to be required for tumour cell proliferation. Indeed, K_V11.1 blockers impair the proliferation of tumour

cells. K_V11.1 also interacts with integrins to regulate survival and migration, and is implicated in the regulation of apoptosis[220–226].

Thus, available data suggest K_V11.1 as a target for cancer therapy, but the concomitant inhibition of I_{Kr} would initially seem a considerable hurdle for such an approach. Several considerations should be made in this regard. The risk to benefit profile of an anticancer drug is radically different from that of compounds for the treatment of benign conditions. Additionally, there are at least three alternative transcripts of K_V11.1 (REFS 227,228), which are differentially expressed in the heart and in tumour cells. This opens the possibility to selectively inhibit the channel in tumours while preserving heart function, in a similar way as discussed above for schizophrenia.

Finally, it has recently been shown that an anticancer compound (the cyclin-dependent kinase inhibitor roscovitine) that is in Phase II clinical trials[229] is an efficient blocker of K_V11.1 but does not induce arrhythmia, probably owing to its low affinity for the closed and inactivated states of the channel. However, K_V11.1 inhibition could not only directly contribute to the prevention of tumour progression but might also treat some collateral effects of neoplasia. For example, K_V11.1 expression is required for muscle wasting related to inactivity and neoplasia[230], presumably through its role in the activation of ubiquitin-dependent protein degradation.

Outlook and challenges

Since the first cloning of a K_V channel more than 20 years ago, remarkable progress has been made in our understanding of the diverse physiological and pathophysiological roles of this class of channels. However, owing to the difficulties of targeting ion channels in general, medicinal chemistry efforts in this area have considerably lagged behind drug development in the fields of G protein-coupled receptors and protein kinases. K_V channel drug discovery faces the same general problems as all other target fields — particularly that transgenic approaches can be misleading for target evaluation. Although heterozygous $K_V7.2^{+/-}$ mice and K_V7.2-transgenic mice in which channel expression was drastically reduced[231,232] have been used to validate K_V7.2 as a target for anticonvulsive therapy, many other transgenic approaches have been disappointing. There are many possible reasons for this, ranging from developmental compensation to different physiological roles of particular K_V channels in different species. Striking examples of such species-specific differences are the lack of importance of K_V1.3 in mouse T cell function (see the section on K_V1.3) or the different roles of K_V1.4, K_V1.5, K_V4.2 and K_V4.3 in the cardiac action potential of different species[233].

Another hurdle to K_V channel drug discovery is the fact that traditional methods developed for high-throughput screening of ion channels, such as binding assays or voltage-sensitive fluorescent probes, measure ion channel activity indirectly and can therefore miss compounds that interact with a particular conformational (gating) state of the channel. Furthermore, these assays can be susceptible to potentially misleading actions of compounds with poor physiochemical properties (such as low solubility, 'sticky' hydrophobic compounds), which can result in incorrect 'hit' identification or can cause active compounds to be missed (see BOX 2 for an overview of screening technologies). However, with the recent advent of high- or at least medium-throughput electrophysiology, which measures K_V channel function directly and is able to identify state-dependent modulators, this situation is currently changing. As a result, pharmaceutical companies and academic screening centres are becoming increasingly successful at identifying potent and selective K_V channel modulators.

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. Currently, a co-crystal of the bacterial KcsA channel with tetrabutyl ammonium is the only visualized example of a ligand bound in the inner pore of a K^+ channel. However, results of mutational, electrophysiological and ligand-binding experiments are increasingly interpreted in structural terms using homology modelling and ligand docking.

Despite this impressive progress, true channel structure-based drug design is currently not possible for K_V channel modulators. It is hoped that co-crystal structures will eventually be resolved for medically important channels, such as K_V1.5, K_V7.2 and K_V11.1, with drug molecules bound. It remains a challenge to decide which of the available structures to use for homology modelling, as the inner-pore geometry varies substantially between the KcsA, K_VAP and K_V1.2 structures[9]. Other crucial issues are the possible coexistence of several drug-binding modes and an incomplete understanding of the influence of protein dynamics on high-affinity drug binding. Like all ion channels, K_V channels are 'moving targets' that undergo large conformational changes, switching between open, closed and inactivated states on a millisecond timescale. These changes in gating state are often accompanied by substantial changes in the conformation of drug binding sites, resulting in a phenomenon referred to as state-dependent inhibition. It is not yet possible to model the 'trapping' of the channel in one of its many possible conformations by a drug.

Based on the current status of the K_V channel field, it is to be expected that drugs modulating the channels discussed in this article will reach the clinic within the next few years. Non-selective K_V channel modulators such as fampridine may have already found a niche in the potential treatment of multiple sclerosis. The $K_V7.2/K_V7.3$ activator retigabine has completed Phase III clinical trials for the treatment of epilepsy and is currently the most advanced novel K_V channel modulator. Next-generation modulators of $K_V7.2/K_V7.3$ channels are only a few years behind retigabine in their development. However, despite more than 20 years of work, a K_V channel modulator specifically designed for a particular target has yet to reach the market. Other K_V channels, such as K_V2.1 or K_V3.4, may offer attractive therapeutic opportunities in the future, but need

to be explored further before they can be regarded as valid drug targets. It will also be interesting to see whether there will be any repositioning of existing marketed drugs in the K_V channel field. For example, could an 'old' drug such as clofazimine find new life as a $K_V1.3$ inhibitor-based immunosuppressant? Therefore, although the development of drugs that target K_V channels is at an early stage and presents many challenges, there are considerable opportunities for future success.

Note added in proof

Another interesting mode of interaction has recently been identified between $K_V3.1$ and the polycyclic ciguatera toxin gambierol, which is produced by the dinoflagellate *Gambierdiscus toxicus*. This lipophilic ether toxin inserts into the space between S5 and S6 outside of the permeation pathway on the side of the helices facing the lipid and prevents the channel from opening by stabilizing the closed state[260].

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Competing interests statement
The authors declare competing financial interests: see web version for details.

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