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Perspective

Recent Developments in the Biology and Medicinal Chemistry of Potassium Channel Modulators: Update from a Decade of Progress

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Introduction

Investigation of potassium channel biology continues to be an ever-expanding field of research. The ability of K^+ channels to regulate membrane potential accords them a central role in varied cellular processes that govern excitability, action potential characteristics, stimulus−secretion coupling, cell volume regulation, and epithelial electrolyte transport. Medicinal chemistry attention to K^+ channels as drug targets has grown with the realization that a variety of K^+ channel inhibitors and openers offer significant therapeutic opportunities in cardiac, smooth muscle, neuronal, immune, and secretory systems. Building on the efforts of earlier studies,[1] the state of the art includes newly discovered channels and ongoing characterization of their pharmacology, physiology, and structure. Progressive improvements in molecular biology have enabled regular cloning of potassium channels of interest, and defined families of these channels have facilitated a comprehensive understanding of their function. Advances in electrophysiology have made key contributions to the characterization of these proteins, yet pharmacology in native tissues remains a crucial determinant of physiological function. Most importantly, many families of increasingly selective small molecules have emerged as tools for target validation and clinical proof of principle. As expected, an enormous number of publications characterize the exponential growth of this area, and many reviews have appeared summarizing the therapeutic potential of these channels.[2−4] The scope of this Perspective is to update the key advances in potassium channel biology which lend themselves to a deeper understanding and more expedient identification of potassium channels of therapeutic relevance, and to review developments in medicinal chemistry from three of the families of potassium channels where such molecules would have considerable clinical potential.

K^+ Channel Biology

Since the first postulation of a selective K^+ permeability in excitable cells by Julius Bernstein in 1902,[5] much has been learned about the nature, diversity, architecture, and function of K^+ channels. Potassium channels are membrane proteins that selectively conduct K^+ ions across the cell membrane along its electrochemical gradient at a rate of 10^6−10^8 ions/s.[6] In the resting state, the concentration of K^+ ions outside the cell membrane is some 25-fold lower than the concentration in the intracellular fluid, and consequently, an outward current due to the efflux of positively charged ions is generated by the opening of K^+ channels. This efflux of K^+ is a mechanism that permits recovery (repolarization) and/or lowering (hyperpolarization) of the resting potential of the cells. Thus, opening of K^+ channels offers a mechanism to counteract, dampen, or restrict depolarizing activity triggered by influx of cations (Na^+ and Ca^{2+}) or efflux of anions (Cl^-). Activators of K^+ channels tend to dampen or stabilize cellular excitability or lower the effectiveness of excitatory inputs whereas blockers of K^+ channels have the op-

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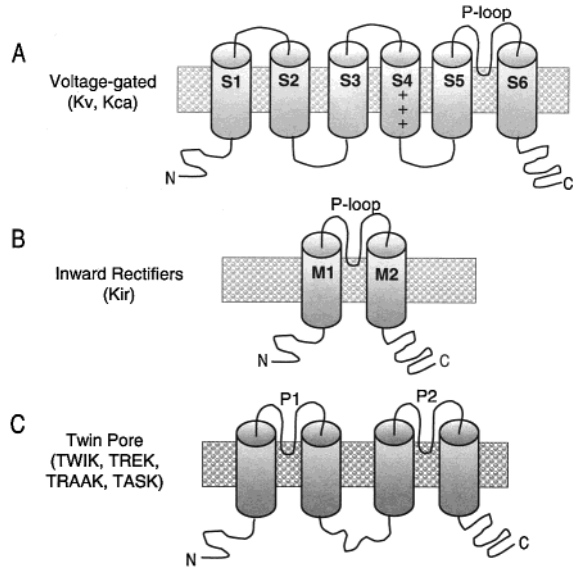


Figure 1. Structural classification of K^+ channels: (A) six-transmembrane−one-pore family includes voltage-gated K^+ channels including Kv1−4 (*Shaker*, *Shal*, *Shab*, *Shaw*), KCNQ, *erg*, and Ca^{2+} activated types; (B) two-transmembrane−one-pore family includes inward rectifiers; (C) four-transmembrane−two-pore channels including TASK, TREK, TRAAK, and TASK.

posite effect. In addition to controlling cellular excitability, K^+ channels are also critical to fluid and electrolyte transport and cell proliferation.

General Properties and Classification. A unique set of functional determinants characterizes potassium channels and forms the basis for this superfamily of membrane proteins as drug targets. These include (i) a water-filled permeation pathway (pore) that allows K^+ ions to flow across the cell membrane, (ii) a selectivity filter that specifies K^+ as permeant ion species, and (iii) a gating mechanism that serves to switch between open and closed channel conformations.[6] The ion conducting (or pore containing) subunit is generally referred to as the principal or α-subunit. In some cases, the auxiliary subunits (for example, *β*-subunits) coassemble with the α-subunit to regulate expression, biophysical, and pharmacological properties. With many K^+ channels, the α-subunit serves as the principal binding site for potassium channel openers and blockers although there are clear exceptions where ligand-binding sites reside within an auxiliary subunit.

K^+ channels are classified on the basis of primary amino acid sequence of the pore-containing unit (α-subunit) into three major families depicted in Figure 1: (i) voltage-gated potassium channels (Kv) containing six transmembrane regions with a single pore, (ii) inward rectifiers (Kir) containing only two transmembrane regions and a single pore, and (iii) two-pore tandem K^+ channels containing four transmembranes with two pores. A tripeptide sequence Gly-Tyr(Phe)-Gly is common to the pore of all K^+ channels and constitutes the signature motif for determining K^+ ion selectivity. The general architecture of K^+ channel families is shown in Figure 1.

Since the first isolation of the gene encoded by the *Shaker* (Sh) locus in *Drosophila melanogaster*,[7] over 50 genes encoding voltage-gated K^+ channels, inward rectifier, and two-pore channels have been isolated and extensively studied in terms of biophysical and molecular pharmacological properties during the past decade. Many excellent reviews have dealt with the biophysical, structure−function, and modulation of K^+ channels.[3,4,8] A simplified classification is provided in Table 1. For current details of this continuously growing list of channels and standardized gene nomenclature, the reader is encouraged to consult additional publications[4,9] or Internet resources.[10]

Table 1. Classes of Potassium Channels

	voltage-gated	inward rectifiers	two pore
Shaker	Kv1 (1.1−1.7, 1.10)		
	Kv2 (2.1, 2.2)	Kir1 (1.1−1.3)	TWIK (TWIK1−2)
	Kv3 (3.1−3.4)	Kir2 (2.1−2.4)	TREK
	Kv4 (4.1−4.3)	Kir3 (3.1−3.4)	TASK
	Kv5 (5.1)	Kir4 (4.1)	TRAAK
	Kv6 (6.1, 6.2)	Kir6 (6.1−6.2)	
	Kv8 (8.1)	Kir7 (7.1)	
	Kv9 (9.1−9.3)		
ether-a-go-go	EAG		
	hERG		
LQT-related	KCNQ1 (KvLQT1)		
	KCNQ 2−5		
Ca^{2+}-activated	Slo (α)		
	SK (SK1−3)		
	IK (IK1)		
auxiliary subunits	Kv (*β*1-*β*3)	SUR1, SUR2	
	K_{Ca} (*β*1−*β*4)		
	Mink		
	miRP1, miRP2		

Voltage-Gated K^+ Channels. The electrical properties of excitable cells are determined in large part by the voltage-gated K^+ channels (Kv's) they possess. The Kv channels control the falling phase of the action potential in excitable cells such as cardiac myocytes and nerve cells, whereas in nonexcitable cells, they may contribute to volume regulation, hormone secretion, or activation by mitogens. About 20 mammalian Kv genes have been cloned and assigned to subfamilies (e.g., Kv1, Kv2, Kv3, etc.) on the basis of sequence similarities. Kv channels function as oligomeric proteins composed of four α-subunits each containing six transmembrane segments (S1 through S6). The transmembrane segment S4 contains positively charged residues and serves as the voltage sensor. The tripeptide sequence motif Gly-Tyr(Phe)-Gly is located within transmembranes S5 and S6; four of these segments derived from four individual α-subunits form a functional K^+-conducting pore.

Inward Rectifiers. The inward rectifiers (Kirs) belong to a distinct family of channels with four subunits each containing two transmembrane domains and a segment with pore elements in between. These channels conduct K^+ currents prominently in the inward direction. This inward rectification is due to blockade of outward currents by intracellular Mg^{2+} ions and by the naturally occurring polyamines, spermine, and spermidine.[11] Although these channels are organized as homotetramers, complex octameric arrangements have also been described in combination with auxiliary subunits.[12]

Twin-Pore K^+ Channels. The twin-pore K^+ channels including TWIK, TREK, TRAAK, and TASK are weak inward rectifiers with four putative transmembrane domains and two pore domains.[13] The Gly-Tyr-(Phe)-Gly residues of the K^+-signature motif are pre-

served in the first pore-loop of these channels, but they are replaced by Gly-Phe-Gly or Gly-Leu-Gly in the second pore loop; two such subunits are thought to form a functional channel retaining the tetrameric arrangement. Members of this family lack the voltage sensing S4 transmembrane domain characteristics of voltage-gated K^+ channels; thus these channels carry a voltage-independent outward K^+ leak current at resting potentials.

Auxiliary Subunits. The biophysical and pharmacological properties of the α-subunit of K^+ channels can be modified by the presence of auxiliary subunits.[14] These include the various β-subunits that assemble with Kv and K_{Ca} channels, minK and minK-related peptides that coassemble respectively with hERG and KvLQT1 subunits, and the sulfonylurea receptors that integrate with certain Kirs. These subunits play diverse roles ranging from modulation of current kinetics, cell surface expression and/or trafficking of the ion channel complex, to serving as binding sites for both endogenous and exogenous ligands. Association of the sulfonylurea receptor with Kir6.1 or Kir6.2 is necessary for ligand-dependent activation or inhibition of the K_{ATP} channel. Chaperone proteins such as KChAP have been identified that regulate the function and expression of some Kv channels.[15] Certain α-subunits do not form functional channels by themselves but associate with α-subunits of other subfamily members to regulate expression, biophysical, and pharmacologic properties.[16,17] Given the diversity of K^+ channel subunits and the potential to vary the constituents to form α−α or α−β heteromeric channel complexes that influence biophysical and pharmacological properties, it is difficult to know with precision the exact composition of channel complexes in vivo. Although invaluable for drug development, this information is seldom available to guide medicinal chemistry efforts.

K^+ Channel Structure. The structural determinants of K^+ channels noted above have initially been elucidated by a combination of mutagenesis and biophysical approaches. More recently, the three-dimensional structure of a conduction pore from the crystal structure analysis of a bacterial K^+ channel has become available.[18] The KcsA channel, cloned from *Streptomyces lividans* is a non-voltage-dependent channel that contains two transmembrane domains with an intervening pore loop. X-ray analysis at 3.2 Å resolution revealed four identical subunits arranged to form an inverted cone, cradling the selectivity filter of the pore in its outer end. The selectivity filter is 12 Å long and lined with the carbonyl groups from the Gly-Tyr-Gly signature sequence whereas the remainder of the pore is wider and lined with hydrophobic amino acid residues. Functional analysis has suggested that the dimensions of the pore in mammalian Kv channels may be different from those of the non-voltage-gated bacterial KcsA structure.[19] More recently, the structure of a β-subunit from a Kv channel has been published.[20] This subunit also forms a 4-fold symmetric structure; each subunit is an oxidoreductase enzyme complete with a nicotinamide cofactor in its active site. X-ray crystallography at 2.1 Å resolution revealed a cytoplasmic assembly that includes the tetramerization domain of the α-subunit with the oxidoreductase β-subunit as a 4-fold symmetric complex.[20,21] A detailed understanding of the structure of other regions of K^+ channels, particularly those of the ligand binding domains, will enable utilization of a structure-based approach to refine and/or design selective compounds targeting K^+ channels. Indeed, a structure-based design strategy has recently been applied to prepare selective blockers of IK_{Ca} channels (vide infra).[22]

K^+ Channels as Therapeutic Targets

The intent of the next several sections is not to review all known K^+ channels but rather to highlight many of the therapeutically relevant members and to cover recent advances in the synthesis and biological activities of organic modulators. Although members of the voltage-gated K^+ channel family were the first to be cloned, medicinal chemistry efforts were largely focused on the ATP-sensitive K^+ channels. These efforts were pioneered by the discovery of nicorandil and cromakalim which were demonstrated to relax vascular muscle with associated membrane hyperpolarization and/or K^+ channel activation.

ATP-Sensitive K^+ Channels (K_{ATP}): Background. ATP-sensitive potassium (K_{ATP}) channels, inhibited by intracellular ATP, belonging to the family of weak inward rectifiers are unique in that they couple cellular energy metabolism to membrane electrical activity.[23] In addition to ATP, membrane phospholipids, particularly phosphatidylinositol 4,5-bisphosphate, can modulate the physiological nucleotide sensitivity of K_{ATP} channels by antagonizing ATP inhibition of these channels.[24] Although the molecular properties of these channels were only elucidated during the past few years, these channels have been the most widely explored of K^+ channels in terms of their therapeutic potential.[2,25]

First-Generation Openers and Indications. The first K_{ATP} openers were investigated for the treatment of hypertension, with diazoxide **1** and pinacidil **2** having advanced to the stage of commercial pharmaceutical products. Nicorandil, a K_{ATP} opener and guanylate cyclase activator, is currently available for use in angina pectoris. Although (−)-cromakalim **3** completed phase III trials in hypertension, K_{ATP} openers, in general, have failed to demonstrate any clear advantages over established antihypertensives such as the calcium channel blockers or ACE inhibitors. Therefore, more recent research in this area has focused on examining the utility of K_{ATP} openers in the treatment of disorders such as myocardial ischemia, angina, asthma, bladder overactivity, alopecia, dysmenorrhea, and Raynaud's syndrome.[26,27] Due to the presence of K_{ATP} channels in the vasculature, a tremendous challenge to the medicinal chemist has been the design of target organ selective agents with reduced hemodynamic liabilities.

A number of structural classes have historically been known to possess K_{ATP} channel opening properties, and these are summarized in Figure 2 along with a prototype from each series. Recently the tertiary carbinols such as ZD-6169 **4** and novel variations of dihydropyridines such as ZM-244085 **5** have emerged as newer chemotypes. In the benzopyran class, in particular, a seemingly limitless number of variations or subclasses have appeared, many of which could reasonably be

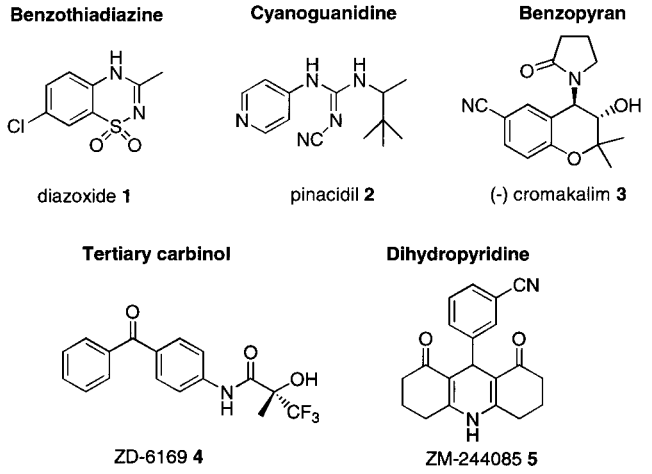
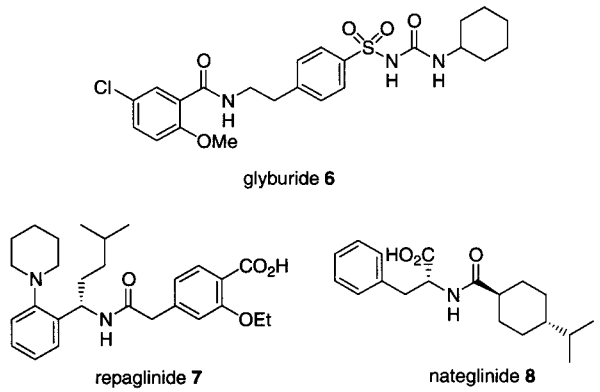


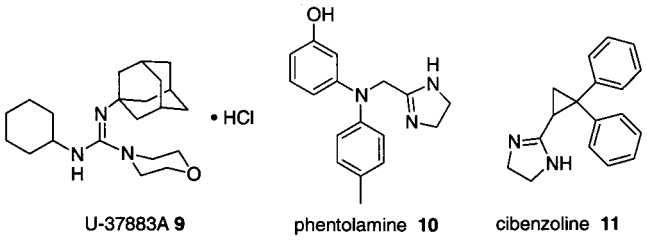
Figure 2. First-Generation K_{ATP} Channel Modulators.

considered as unique structural series themselves. However, since most of these families are well represented in the literature, the reader is referred to earlier reviews[28,29] for a more detailed description of the structure−activity relationships of these classes.

First-Generation Blockers and Indications. Compared to developments in K_{ATP} channel openers, medicinal chemistry research in the area of K_{ATP} blockers has seen less activity. Glyburide **6** has been in use since 1984 for the treatment of type II diabetes, and the pharmacology of this agent has been thoroughly described in the literature.[30] While the sulfonylureas continue to be useful in the pharmacotherapy of type II diabetes, several new agents have entered the clinical arena or are in later stages of investigation including those that stimulate insulin secretion, improve insulin action, or reduce carbohydrate absorption.[31] These agents include nonsulfonylurea derivatives that are also insulin secretagogues. For example, repaglinide **7**, a benzoic acid derivative of the meglitinide family approved for the treatment of diabetes, is reportedly a more potent insulinotropic agent than glyburide, and unlike sulfonylureas it appears to require the presence of glucose to close K_{ATP} channels and induce calcium influx. Nateglinide **8**, a phenylalanine derivative with insulinotropic action, is in clinical development for the treatment of type II diabetes. More recently, two oral antidiabetic drugs, glyburide **6** and metformin (Glucophage R), together in a single formulation have been approved as a unique approach to manage type II diabetes as this combination addresses the underlying defects of insulin deficiency and insulin resistance.[32]



The vascular selective K_{ATP} blocker U-37883A **9** was shown preclinically to possess diuretic and natriuretic activity.[33] The adverse cardiovascular side effects seen with U-37883A in dogs and rats,[34] although not believed to be due to its K_{ATP} blocking properties, precluded its further development. Other types of K_{ATP} blockers known are weak and nonselective; these include phentolamine (imidazolines), tetraethylammonium (quaternary ammonium salt), 5-hydroxydecanoate, guanethidine, and bretylium. In contrast to the sulfonylurea analogues that interact at SUR1 and SUR2 proteins (vide infra), phentolamine **10** and cibenzoline **11** have been suggested to directly block the channel pore-forming subunit of the K_{ATP} channel.[35] Whether these two mechanisms for channel blockade offer differential advantages in the development of therapeutic agents is unclear.



Molecular and Pharmacological Differentiation of K_{ATP} Channels. Recently, progress has been made in the cloning and expression of K_{ATP} channel subunits, elucidation of their subunit composition, and structure−function analysis of diverse K_{ATP} channel combinations.[12,36] K_{ATP} channels have been shown to be heterooctameric complexes composed of four inward rectifying K^+ channels belonging to the Kir 6.*x* subfamily and four regulatory proteins, the sulfonylurea receptor (SUR). The Kir subunit consists of two transmembrane segments, M1 and M2, with an extracellular pore loop and cytoplasmic N- and C-termini. This subunit is responsible for ion permeation, and it is the primary site of ATP inhibition of K_{ATP} channel activity. The SUR subunit confers sensitivity to most KCOs and sulfonylureas. In addition, the SUR subunit binds ATP at the first nucleotide-binding fold (NBF-1), and cooperative interaction between the two NBFs of SUR1 appears to be important for K_{ATP} channel regulation.[37] The SUR subunit belongs to the ATP-binding cassette (ABC) superfamily and has two members, SUR1 and SUR2, that are encoded by two genes containing 39 and 38 exons, respectively. In addition, the SUR2 gene has been shown to undergo alternative splicing at exon 38, generating two splice variants SUR2A and SUR2B. Expression of the SURs with Kir6.1 or Kir6.2 subunits has revealed K_{ATP} channels with distinct biophysical and pharmacological properties (Table 2). The pancreatic β-cell K_{ATP} channel is composed of Kir6.2 and SUR1, assembled in a heterooctameric stoichiometry. SUR1-Kir6.2 is also the major subunit combination of K_{ATP} channels not only in the pancreas but also in neurons. In contrast, the cardiac K^+ channel is thought to be composed of SUR2A-Kir6.2, whereas SUR2B in conjunction with Kir6.2 or Kir6.1 is thought to constitute diverse smooth muscle type K_{ATP} channels.[12,38]

Table 2. Diversity of K_{ATP} Channels

K_{ATP}-type	tissue expression	pharmacology
SUR1-Kir6.2	pancreas, brain	activated by diazoxide; high affinity glyburide inhibition
SUR2A-Kir6.2	cardiac myocytes, skeletal muscle	activated by cromakalim, pinacidil, but not diazoxide; low affinity glyburide inhibition
SUR2B-Kir6.1, SUR2B-Kir6.2	smooth muscle (various)	activated by cromakalim, pinacidil, and diazoxide; low affinity glyburide inhibition
MitoK_{ATP}	heart, liver	activated by diazoxide, inhibited by 5-hydroxydecanoate

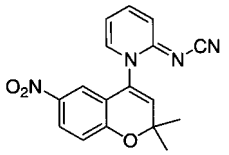
Additional splicing of the SUR genes may offer yet another level of diversity.

Studies using recombinant subunits have shown that the Kir subunit can also influence the pharmacology of the putative vascular-selective K_{ATP} channel inhibitor U-37783A. Currents generated by expression of Kir6.1-SUR1 or Kir6.2-SUR1 were effectively inhibited by 1 μM glyburide. On the other hand, only currents from Kir6.1-SUR1 were inhibited by U-37883 ($IC_{50} = 32\ \mu M$). These data suggest that the pharmacology of K_{ATP} channels may be determined not only by the sulfonylurea receptor but also by the nature of the pore-forming subunit.[39]

The molecular constituents of K_{ATP} channels in tissues such as the brain and heart appear to be more complex than those precisely defined by already known SUR-Kir combinations. For example, the nature of K_{ATP} channels in the mitochondria whose pharmacological profile and subcellular distribution are distinct from sarcolemmal type K_{ATP} channels remains uncertain.[40] By assessing flavoprotein fluorescence, an index of mitochondrial redox state, and sarcolemmal K_{ATP} currents, it was shown that diazoxide targets mitochondrial but not sarcolemmal K_{ATP} channels, implying that mitochondrial K_{ATP} channels are mediators of ischemic preconditioning.[41,42] With such emerging diversity of K_{ATP} channels, it appears possible to target certain isoforms involved in various functions ranging from transmitter release to ischemic protection for the identification of tissue-selective compounds.

Ligand Binding Domains. The profiles of KCOs were originally elucidated by glyburide-sensitive in vitro functional assays. Initial attempts to define specific binding site of [^{3}H]cromakalim were not successful[43] which limited efforts to correlate functional data with receptor binding. Subsequently, binding sites for cyanoguandine ([^{3}H]P 1075)[44] and nitroethylene ([^{3}H]Bay X 9228)[45] were described in intact cells and tissues; however, efforts to characterize binding sites in membrane preparations were unsuccessful. More recently, it was demonstrated that KCO binding is critically dependent on the presence of nucleotides (MgATP) as revealed by the Mg dependence of [^{3}H] P 1075 binding to native membrane preparations.[46,47] Similar nucleotide requirements were noted for the heterologously expressed SUR2A and SUR2B subunits.[48] Atwal et al.[47] noted a good correlation between [^{3}H] P 1075 binding affinitites of a set of KATP channel openers to their cardioprotective potencies ($r^2 = 0.88$), although it remains to be established that the binding sites in cardiac membranes are relevant to the functional cardioprotective properties of these compounds. With the relatively low binding affinity of [^{3}H] P 1075 ($K_D \sim 10-20$ nM), clearly, there is a need for higher affinity KCOs to facilitate high throughput binding assays to define the structure−activity relationship of diverse KCO chemotypes in various tissues.

Residues that underlie KCO binding have recently been elucidated by exploiting pharmacologic differences between SUR isoforms. As noted above, the diversity of SURs confers the basis of tissue-specific pharmacology within K_{ATP} channels. For example, the SUR2 isoforms confer high sensitivity to KCOs such as cromakalim and low sensitivities to sulfonylureas and, conversely, SUR1 imparts high sensitivity to sulfonylureas. By exchanging segments from SUR1 and SUR2A, it was shown initially that the C-terminal set of transmembrane segments were important for stimulation of K_{ATP} channels by SR 47063 **12**, a cromakalim analogue.[49] Subsequently two smaller segments were identified: a cytosolic loop between helices 13 and 14 (Tyr^{1059}-Leu^{1087}) and a stretch encompassing transmembrane helices 16 and 17 (Arg^{1218}-Asn^{1320}) in rat SUR2 as critical for ligand binding and current stimulation.[50] Consistent with this notion, patch clamp studies showed that the SUR2 segment containing the transmembrane domains (TM) 12−17 confers sensitivity to the benzopyran cromakalim **3** and the cyanoguanidine, pinacidil **2**. By an elegant chimeric approach, Moreau et al.[51] showed that a minimal set of two residues within the transmembrane helix 17, Thr^{1253} and to a lesser extent Leu^{1249}, are determinants of opener sensitivity at SUR2A for structurally distinct KCOs including benzopyran analogues such as SR 47063 **12** and cyanoguandines. On the other hand, these residues did not appear critical to the activity of benzothiadiazine, diazoxide **1**. In fact, a SUR1 segment that includes TMs 6−11 and the nucleotide-binding fold NBF-1 appears to govern sensitivity to diazoxide, although the precise regions remain to be fully elucidated.[52]



SR 47063 **12**

The interaction domains of sulfonyl ureas such as glyburide **6** are positioned near cytoplasmic loops of TM12−17 of SUR1 based on the identification of segments required for high affinity inhibition and [^{3}H]-glyburide binding.[50,53] In fact, high affinity sulfonylurea binding was imparted on SUR2B by substituting the region separating the two KCO binding segments with the corresponding domain from SUR1. These studies show that the sulfonylurea binding pocket lies in close proximity to the KCO binding sites within the second set (12−17 segments) of transmembrane domains,[50,54]

in support of pharmacological data indicating that these sites may be closely coupled in an allosteric fashion. Although a model integrating functional topology of SUR and Kir has been proposed,[54] the mechanisms underlying K_{ATP} channel activation or inhibition remain to be elucidated. A detailed analysis of channel inhibition and binding processes has shown that occupation of one of the four SUR receptor sites per channel complex is sufficient to induce K_{ATP} channel closure.[54] Similarly, KCO-induced channel activation appears to be mediated by interaction with a single binding site per tetrameric SUR complex.[55]

Unlike sulfonylurea analogues, the interactions of imidazolines such as phentolamine **10** appear to reside on the inward rectifier Kir6.2 as revealed by studies using a truncated Kir6.2 mutant that expresses in the absence of a sulfonylurea receptor.[56] Understanding the molecular basis and mechanisms underlying opener and blocker sensitivities across various SUR-Kir combinations as well as SUR-independent ligand actions will be of key importance in the design of tissue-specific compounds.

K_{ATP} Channel Openers: Recent Developments. Although very few entirely novel pharmacophores have appeared, many of the newer variations have nonetheless provided fascinating compounds with unique pharmacological profiles. The focus of research efforts has continued to be directed toward identifying compounds that show selectivity for the target organ in vivo, for whole tissues in vitro, or more recently for one of the cloned SUR-Kir combinations. We have focused on novel structural types and those agents that have demonstrated in vitro or in vivo advantages such as improved channel selectivity.

Myocardial Ischemia. A significant body of literature has accumulated demonstrating the importance of cardiac K_{ATP} channel opening in myocardial preconditioning, a paradoxical form of cardioprotection wherein brief ischemic insults can reduce damage to the heart caused by subsequent prolonged ischemia.[29,57] Although this role was first attributed to the classical sarcolemmal K_{ATP} channels, recent evidence implicates the mitochondrial K_{ATP} channels, whose pharmacological and histochemical characteristics and molecular identity differ importantly from surface K_{ATP} channels.[58] By using flavoprotein fluorescence to assess the redox potential of the mitochondrial matrix, it was shown that the mito-K_{ATP} channels are activated equally by diazoxide and pinacidil and that 5-hydoxydecanoate is an effective blocker (Figure 3).[42] These observations contrast with the pharmacology of surface K_{ATP} channels, which are strongly activated by pinacidil and more weakly by diazoxide and are insensitive to 5-hydroxydecanoate.[58−60]

Early studies with cromakalim **3**[61] showed it to possess cardioprotective effects in vitro and in vivo; however, its utility was limited by its potent systemic vasodilator effects at comparable doses.[62] The first cardioselective K_{ATP} opener to be described was BMS-180448 **13**.[63,64] This compound was identified by its relative in vitro potency in a rat whole heart assay versus that observed in the rat aorta. When compared to cromakalim (see Table 3), BMS-180448 **13** was

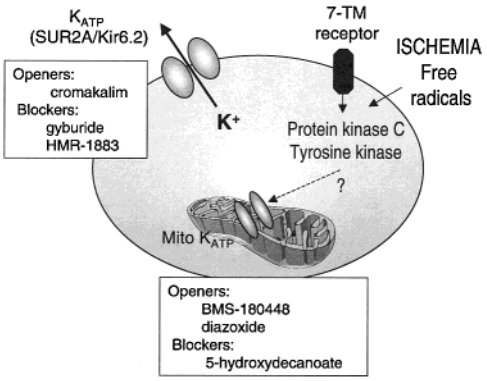
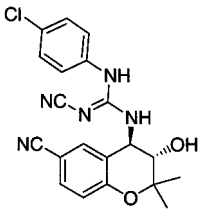


Figure 3. Schematic representation of plasmalemmal and myocardial K_{ATP} channels and the interactions of K_{ATP} channel modulators. K_{ATP} channels in the plasma membrane composed of SUR2A-Kir6.2 are activated by cromakalim and inhibited by glyburide and HMR-1883. Mitochondrial K_{ATP} channels are activated by diazoxide and inhibited by 5-hydroxydecanoate. Ischemia leading to the release of adenosine, neurohormones, and free radicals collectively activates protein kinases which modulate mitochondrial K_{ATP} channels.

Table 3. Cardioprotective and Vasorelaxant Potencies of K_{ATP} Openers[69]

compd	antiischemic EC_{25} (μM)[a]	vasorelaxant IC_{50} (μM)[b]	ratio
cromakalim	8.9	0.032	278
BMS-180448	2.5	1.8	1.4
BMS-191095	1.4	>30	<0.05
15	0.040	9.4	<0.004

[a] EC_{25} is determined by measurement of the increase in time to contractions in globally ischemic perfused rat hearts. [b] IC_{50} values are for the relaxation of rat aorta.

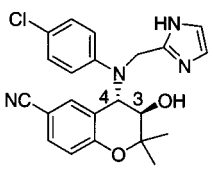


BMS-180448 **13**

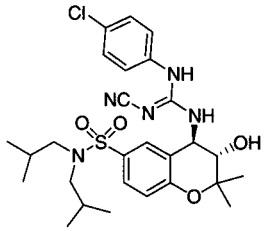
approximately 200-fold more selective for antiischemic effects. Although BMS-180448 did not demonstrate absolute in vitro cardioselectivity, the comparison with cromakalim nonetheless provided an index of selectivity that was predictive of greater in vivo selectivity. In the dog, cardioprotective effects were seen with BMS-180448 at a dose of 1−2 mg/kg administered iv (2.5−5 μmol/kg) whereas a dose of 21.5 μmol/kg was required to reduce blood pressure by 20%.[64] BMS-180448 is reportedly devoid of hemodynamic effects at the efficacious dose. Subsequent mechanistic studies revealed that BMS-180448 evokes cardioprotective effects at concentrations that do not affect action potential shortening via activation of the plasma membrane bound (sarcolemmal) cardiac channel.[65] The cardioprotective action of BMS-180448, however, was abolished by 5-hydroxydecanoate (5-HD), a K_{ATP} blocker selective for the mito-K_{ATP} channel.[66] BMS-180448 has been under clinical evaluation through phase II for myocardial ischemia although the recent development status of this compound is unclear. It remains to be validated whether the selectivity seen in the preclinical animal models are

predictive of the clinical separation of cardioprotective versus hypotensive effects.

Research efforts at Bristol-Myers Squibb have continued toward identifying K_{ATP} openers with enhanced cardioselectivity. The recently described BMS-191095 **14**[67] displayed more than a 20-fold improvement in selectivity for cardioprotective over vasorelaxant effects in vitro compared with BMS-180448 (Table 3). BMS-191095 has reportedly entered phase I clinical trials for the treatment of myocardial ischemia.[68] It is interesting to note that the absolute stereochemistries at the 3 and 4 positions of the benzopyran for BMS-191095 are opposite that of BMS-180448 and cromakalim. More recent SAR studies on the aromatic ring substitution of the benzopyran nucleus of BMS-180448 were aimed at determining the effects of replacement of the cyano with a variety of substituents with primary emphasis on the sulfonamide group. The derivative **15** displayed enhanced cardioprotective potency (EC_{25} = 0.04 μM) with greater than a 100-fold improvement in cardiac selectivity relative to BMS-180448 (Table 3).[69] The range of selectivity seen within this group of compounds including cromakalim illustrates the versatility of the benzopyran nucleus as a platform for designing tissue-selective agents.



BMS-191095 **14**



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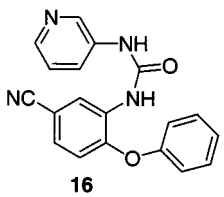
Continued research seeking to identify novel scaffolds for more cardioselective agents led to the synthesis of the biaryl ether **16**.[70] The design strategy involved ring opening of the pyran of BMS-180448 and attachment of an aryl ring to mimic the lipophilic gem dimethyl group that was found to be critical for K_{ATP} activator potency in the earlier series. This novel structural type showed similar potencies to BMS-180448 in the rat whole heart (EC_{25} = 2.0 μM) and rat aorta (IC_{50} = 1.0 μM) assays. From the available in vitro data, **16** does not appear to possess superior cardioselectivity relative to earlier members of the benzopyran series; however, it is clearly the first of a new chemotype which is presumably unoptimized.



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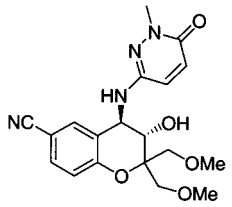
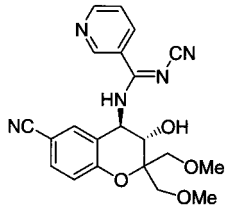
Angina. On the basis of the initial observations that nicorandil showed efficacy in many types of angina pectoris, K_{ATP} openers from other structural classes have been investigated to some extent. The mechanism of action of such agents is likely to involve opening of K_{ATP} channels in the resistance vessels of the coronary vasculature. A member of the benzopyran class, JTV-506 **17**, has been under clinical investigation for the treatment of angina.[71] In anesthetized dogs, JTV-506 produced an approximately 100% increase in coronary blood flow (CBF) with only a 5% reduction in mean arterial pressure (MAP) at doses of 3−5 μg/kg admin-



JTV-506 **17**



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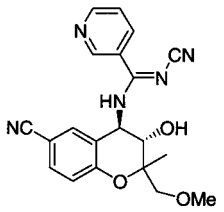

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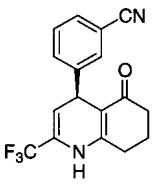
istered iv.[72] (−)-Cromakalim, at equiefficacious doses, produced a somewhat greater decrease in MAP (8−10%). In this study, JTV-506 was nonselective versus effects on heart rate (HR) as the latter was increased from 10 to 50% over the efficacious dose range. A similar separation of CBF and MAP was observed on oral dosing in dogs, once again with a slight improvement in selectivity compared to (−)-cromakalim.[72] In vitro studies showed JTV-506 to be marginally more potent at the porcine coronary artery (IC_{50} = 0.11 μM) compared to the rat aorta (IC_{50} = 0.3 μM) and 3-fold more selective relative to cromakalim.[73] In this case, the relative in vitro selectivity for these two tissue types agrees well with that observed in vivo in the dog. The gem methoxymethyl group of JTV-506 appears to exert a beneficial influence on selectivity as replacement with a gem dimethyl group eliminates any in vivo selectivity.[73] The structurally related compounds **18** and **19** also possessing the methoxymethyl substitution at the C-2 position of the benzopyran likewise were shown to have in vivo coronary artery selectivity of a similar or greater magnitude as JTV-506 although both of these compounds were 20−30-fold less potent.

Bladder Overactivity. K_{ATP} openers such as cromakalim **3** and ZD-6169 **4** have been shown in vitro to relax bladder smooth muscle from humans, pigs, rats, or guinea pigs.[74,75] In addition, in vivo effects to inhibit bladder overactivity in animal models have been demonstrated with a number of K_{ATP} openers.[75−80] Whether the effects observed in preclinical animal models will translate into clinical efficacy is currently not known. To date, clinical data showing efficacy for K_{ATP} openers is limited. The only published clinical study,[81] which examined the effects of cromakalim, was not placebo controlled, and there was no indication of the dose administered. Although 35% of those in the trial reported improvements in objective and subjective symptoms, the absence of a control group and the small size of the trial make it difficult to attribute the effects to the drug. While it remains an attractive target for this

therapy, the utility of a K_{ATP} opener for the treatment of bladder overactivity remains to be proven.

Identifying bladder-selective K_{ATP} openers with minimal hemodynamic effects has continued to be a significant challenge. In general, the available in vitro assays for assessing bladder/vascular selectivity have not been predictive of results observed in whole animal models. For example, in vivo bladder-selective actions have been claimed in a number of studies;[78,82,83] however, the same compounds showed no selectivity for isolated tissues in vitro. This point is illustrated by considering the case of ZM-244085 **5**, a K_{ATP} opener with reported in vivo bladder selectivity.[78] Over the dose range from 0.1 to 3.0 mg/kg po, ZM-244085 inhibited bladder activity in conscious rats without effects on mean arterial pressure (MAP), giving it at least 30-fold in vivo selectivity. However, in vitro, ZM-244085 showed greater potency for the guinea pig portal vein ($IC_{50} = 0.75\ \mu M$) than for guinea pig bladder strips ($IC_{50} = 4.2\ \mu M$). In the same study, cromakalim was nonselective, showing both bladder effects and significant drops in MAP at 1 mg/kg po. In vitro, cromakalim was approximately 4-fold less selective than ZM-244085 for the bladder. Given the magnitude of the difference in apparent in vivo selectivity between ZM-244085 and cromakalim, one would have expected a much greater separation in vitro if these assays were truly predictive. Despite the apparently promising preclinical profile of ZM-244085, there is no reported clinical development of this compound.

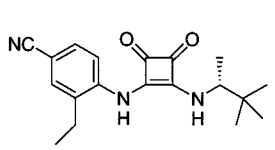
ZD-6169 **4**, a novel tertiary carbinol K_{ATP} opener, has been of considerable interest following the initial report of its bladder-selective properties in dogs and male rats.[76] Although a related study using smaller female rats did not report significant effects of ZD-6169 on bladder function relative to vehicle,[84] other studies using different in vivo models described bladder effects at nonhypotensive doses. Researchers were able to show that ZD-6169 inhibited PGE_2-induced bladder overactivity either dosing orally (3 mg/kg) or intraarterially (1 mg/kg).[84] In another rat model of bladder overactivity, 3 mg/kg po of ZD-6169 was able to reduce spontaneous bladder contractions by 55%.[75] This oral dose of ZD-6169 in rats has been variously reported to either have no effect[76] or minimal effect[75] on MAP. Consequently, the 3 mg/kg oral dose of ZD-6169 has been demonstrated to significantly inhibit bladder overactivity in two independent animal models, but this is also the apparent threshold dose for hypotensive effects. The degree of selectivity for ZD-6169, although less than originally reported, is clearly an improvement relative to older, vascular-selective K_{ATP} openers such as cromakalim.[75,77] Whether the effects seen in these animal models are clinically significant is unknown. ZD-6169 advanced as far as phase II clinical trials; however, the development of this compound has been reportedly terminated in favor of ZD-0947 **20**.[85]



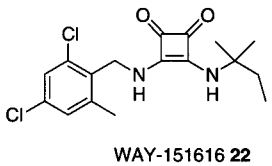
ZD-0947 **20**

Although K_{ATP} openers affect bladder function primarily via smooth muscle relaxation, recent investigations into elucidating the mechanism of the bladder-selective actions of ZD-6169 suggest the involvement of an inhibitory effect on bladder C-fiber afferents. When infused intravesically, ZD-6169 was able to block acetic acid-induced bladder overactivity in a manner similar to that of the C-fiber afferent neurotoxin capsaicin.[79] In another study, pretreatment of capsaicin abolished the effects of ZD-6169.[80] Since it was administered directly into the bladder, the relevance to effects seen on oral dosing is not clear in the absence of objective evidence that ZD-6169 is renally eliminated.

With regard to new developments in the design of bladder-selective K_{ATP} openers, researchers at Wyeth-Ayerst have described the development and SAR studies of a series of arylsquarates exemplified by WAY-133537 **21**[82] and WAY-151616 **22**.[86] This series originated by the novel replacement of the cyanoguanidine moiety of pinacidil **2** with the bioisosteric diaminosquarate. WAY-



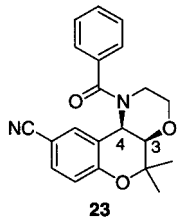
WAY-133537 **21**



WAY-151616 **22**

133537 and WAY-151616 were effective in vitro at relaxing rat bladder strips with respective IC_{50} values of 0.09 and 0.10 μM. Both compounds are reported to possess in vivo bladder selectivity in conscious rats after oral dosing. Comparing the ED_{50} for inhibition of unstable bladder contractions and the ED_{20} for MAP lowering, WAY-133537 was 18-fold selective and WAY-151616 was 166-fold selective. WAY-133537 showed less selectivity toward changes in heart rate (HR) that are likely compensatory in nature secondary to vasodilation. (HR data is not available for WAY-151616.) In the same model, ZD-6169 **4** was 3-fold selective. The origin of the reported selectivity of WAY-133537 and WAY-151616 is unclear. Similar to the results reported for ZM-244085 **5**, WAY-133537 did not display in vitro tissue selectivity for bladder over the rat aorta. Although the selectivity comparison against a 20% reduction in MAP is useful for comparison purposes, the clinical significance of this value is questionable given that this is well beyond a tolerable blood pressure drop for an otherwise healthy individual.

A compound with reportedly modest in vitro tissue selectivity for the rat bladder smooth muscle ($IC_{50} = 8.15\ \mu M$) over the rat portal vein ($IC_{50} = 34.5\ \mu M$) is shown below.[87] Compound **23** is derived from the benzo-



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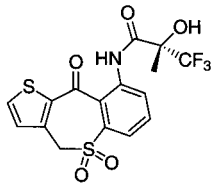
pyran class, but differs from most other K_{ATP} openers of this type in that the 3 and 4 position substituents

are oriented cis rather than trans and are connected via an ethylene bridge. Relative to (−)-cromakalim, this represents a selectivity improvement of 27-fold in these two assays. Once again, the basis of this selectivity is unclear, and no in vivo data are available to determine if this level of in vitro selectivity translates into greater in vivo selectivity.

Two novel variants **24** and **25** from the tertiary carbinol class with reported bladder selectivity in vivo[88] differ from ZD-6169 and related analogues in two major respects: (1) the tertiary carbinol is no longer located in the para position relative to the second aromatic ring, and (2) the second aromatic ring is connected to the first via a seven-membered heterocyclic ring. In vitro, both



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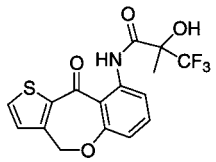
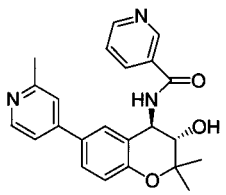


25

compounds possess similar potency to ZD-6169 to relax 15 mM KCl-stimulated guinea pig bladder strips (compound **25** IC_{50} = 2.1 μM and compound **24** IC_{50} = 2.5 μM). In conscious rat cystometry at an oral dose of 3 mg/kg, compounds **24** and **25** increased the interval between bladder contractions by 65% and 54%, respectively, at the 4 h time point with minimal or no effect on blood pressure or heart rate. The bladder effect appears to be time-dependent as the interval between bladder contractions increases over the first 4 h then stabilizes at hours 4 and 5. This time-dependent effect on bladder capacity is similar to that seen with vehicle from other studies.[84] Compound **25** is also reported to increase the intercontraction interval at 1 mg/kg iv in an anesthetized rat model of bladder hyperreflexia with no effects on blood pressure. No tissue selectivity data are given for either of these agents or related analogues.

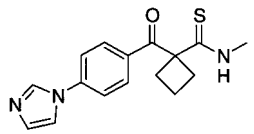
Airway Hyperreactivity. K_{ATP} openers have been investigated clinically for the treatment of asthma; however, cerebral vasodilatation leading to headache has been observed as a limiting side effect, even with an inhaled formulation.[89] A more recent K_{ATP} opener, SDZ-217-744 **26**, is reported to have improved selectivity in guinea pigs for inhibition of airway hyperreactivity



SDZ-217-744 **26**

relative to (−)-cromakalim or bimakalim.[90] The ED_{50} value for inhibition of immune complex-induced airway hyperreactivity is 0.08 μg/kg whereas the ED_{20} for reduction of MAP is over 100 μg/kg upon intratracheal administration. Chronic administration of SDZ-217-744 also reversed the salbutamol-induced airway hyperreactivity. SDZ-217-744 differs structurally from cromakalim in that the 6-cyano has been replaced by a 2-methyl-4-pyridyl group and the substituent at C4 is a 3-pyridinecarboxamide. Whether the improvement in the preclinical profile of SDZ-217-744 translates to the clinical environment remains to be seen.

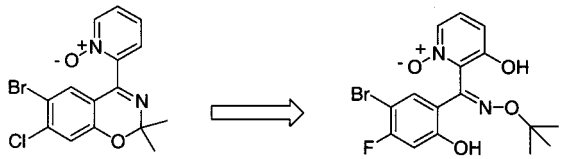
Novel Chemotypes: Thioamides. A novel structural variation on the thioamide class is MCC-134 **27**. This compound has the interesting profile of fully (100%) activating SUR2B-Kir6.2 (K_d = 5.2 μM) while only partially (22%) activating SUR2A-Kir6.2 (K_d = 8.5 μM).



MCC-134 **27**

In contrast, the compound was partially efficacious (40%) at inhibiting SUR1/Kir6.2 combination.[91] MCC-134 relaxed rat aorta strips with an IC_{50} of 12 μM.[92] Inhibition of this effect was reportedly not competitively antagonized by glyburide, and it was suggested that this compound has other unspecified pharmacological actions that directly inhibit the contractile machinery of the cell. In rabbit portal vein smooth muscle cells, MCC-134 activated currents in a glyburide-sensitive manner with an EC_{50} value of 5.3 μM, comparable to that of (−)-cromakalim (6.4 μM), but was weaker in efficacy (50% relative to (−)-cromakalim), slower in onset, and longer lasting (due to less marked desensitization). At higher concentrations (50−100 μM), MCC-134 suppressed voltage-dependent Ca^{2+} and norepinephrine-induced cation currents and concomitant elevations in intracellular Ca^{2+} concentration.[93] As an antihypertensive agent, MCC-134 has been shown to have a slower onset of action with less tachycardia and longer duration in comparison with (−)-cromakalim.[94] It remains to be determined whether the interesting preclinical pharmacology of MCC-134 translates into a clinical advantage in treating hypertension or other disorders.

Pyridyloximes. An interesting approach to generating a novel structural class from the 1,3-benzoxazine TCV-295 **28**[95] involves opening the 1,3-benzoxazine ring to reveal the unusual (*tert*-butoxyimino)benzylpyridine system. Compound **29** was shown to possess potent K_{ATP} activity with vasorelaxant effects on the rat aorta (EC_{50} = 0.28 μM) in vitro and the dog coronary artery in vivo.



TCV-295 **28** **29**

This compound demonstrated a stable, long lasting (>7 h) hypotensive effect with minimal tachycardia and a slower onset of action relative to cromakalim when administered orally to spontaneously hypertensive rats (SHRs). In this series, the (*Z*)-*tert*-butyloxime shown was active whereas the corresponding (*E*)-isomer was not, and the pyridine *N*-oxide was not required for activity as a number of the pyridine analogues were equipotent in vitro. Substitution on the phenyl with one or two halogens and another electron-withdrawing group provided optimal potency. Yet analogues with a single electron-withdrawing group were also active

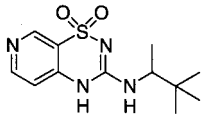
Table 4. In Vitro K_{ATP} Opening Activity of Pyridothiadiazines[97]

compd	rat pancreatic β-cells % insulin release[a]	rat aorta rings relaxation[b]
diazoxide	28.8	19.3
BPDZ-44	7.1	154
BPDZ-79	98.9	7.5
BPDZ-83	41.5	2.3

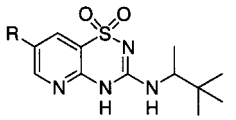
[a] Percentage of residual insulin release at 50 μM test compound in the presence of glucose. [b] ED_{50} values (μM).

although less potent. It is unclear what if any advantages this newer structural class of K_{ATP} opener might possess over earlier generation compounds such as TCV-295 either as antihypertensive agents or for some other application such as angina. Nonetheless, this does provide a novel scaffold from which it might be possible to further explore the potential for SUR/Kir subtype-selective compounds.

Pyridothiadiazines. The pyridothiadiazine BPDZ-44 **30** was described[96] as a novel K_{ATP} opener with improved selectivity and potency relative to diazoxide for pancreatic β-cells over vascular smooth muscle tissues such as the rat aorta. More recent SAR investigations in this series have uncovered structurally similar analogues with the opposite tissue selectivity.



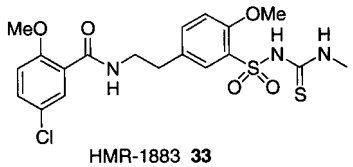
BPDZ-44 **30**



R=H; BPDZ-79 **31**
R=Cl; BPDZ-83 **32**

As shown in Table 4, both BPDZ-79 **31** and BPDZ-83 **32** were found to be more selective for relaxing the rat aorta over inhibiting insulin release in pancreatic β-cells relative to BPDZ-44 or diazoxide.[97] BPDZ-79 differs structurally from BPDZ-44 only in the position of the pyridyl nitrogen whereas BPDZ-83 incorporates the added change of a chloro substituent on the aromatic ring. All of these compounds were considerably less potent than pinacidil to relax the rat aorta. Although a clear therapeutic benefit for these newer compounds has yet to emerge, these results do illustrate how subtle structural changes can affect tissue selectivity.

K_{ATP} Channel Blockers: Recent Advances. Ventricular Arrhythmias. Considerable attention recently has been directed toward the potential of selective K_{ATP} blockers in the treatment of ventricular arrhythmias.[98] HMR-1883 **33** (clamikalant) is a novel sulfonylurea K_{ATP} blocker, currently being investigated in phase II clinical trials as the sodium salt (HMR-1098) for the treatment of ventricular arrhythmias and sudden cardiac death. The properties of HMR-1098 have recently been reviewed.[99]



HMR-1883 **33**

HMR-1883 differs structurally from glyburide in several respects: (i) exchange of the sulfonylurea for a sulfonylthiourea, (ii) the smaller size of the lipophilic substituent attached to the nitrogen of the sulfonylthiourea, (iii) the position of attachment of the sulfonylthiourea to the aromatic ring relative to the left-hand portion, and (iv) the addition of a methoxy substituent adjacent to the sulfonylthiourea. Although less potent than glyburide, HMR-1883 shows selectivity for cardiac K_{ATP}s (guinea pig cardiomyocytes or recombinant SUR2A-Kir6.2) over the pancreatic K_{ATP} channels (rat insulinoma or recombinant SUR1-Kir6.2) shown in Table 5.

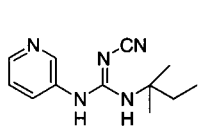
Table 5. Inhibition of K_{ATP} Subtypes in Vitro[a,209]

compd	cardiomyocytes[b]	SUR2A-Kir6.2[c]	RINm5F[d]	SUR1-Kir6.2[e]
HMR-1883	0.8	4.3	7.5	8.2
glyburide	0.008	0.22	0.0016	0.0043

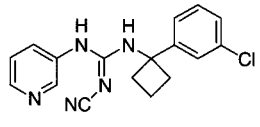
[a] IC_{50} values shown (μM). [b] Inhibition of HOE-234 activation in guinea pig cardiomyocytes. [c] Inhibition of HOE-234 activation in human SUR2A-Kir6.2 expressed in *Xenopus* oocytes. [d] Inhibition of diazoxide activation in the rat insulinoma cell line RINm5F. [e] Inhibition of diazoxide activation in human SUR1/Kir6.2 expressed in CHO cells.

Glyburide, on the other hand, shows greater potency at the pancreatic cell types. HMR-1883 inhibited rimakalim-induced shortening of action potential duration in papillary muscle and cardiac myocytes at concentrations 10−50-fold lower than those required for inhibition K_{ATP} channels in pancreatic β-cells.[100] This in vitro selectivity was mirrored in vivo where HMR-1883 prevented ventricular fibrillation in dogs at a dose of 3.0 mg/kg iv without effects on plasma insulin or blood glucose.[101] HMR-1883 is further distinguished from glyburide in that it does not show effects on the mito-K_{ATP} nor does it block the cardioprotective effects of diazoxide **1** or ischemic preconditioning.[60,102] Action potential recordings in anesthetized pigs under conditions of coronary ischemia demonstrated that HMR-1883 effectively blocked action potential shortening and improved upstroke velocity.[103] HMR-1883 therefore shows a favorable profile as a potential therapeutic for the treatment of ventricular arrythmias without pancreatic side effects or the liabilities of nonselective blockers under ischemic conditions.

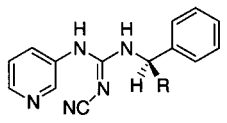
Novel Chemotypes: PNU-99963. The sulfonylurea K_{ATP} channel blockers are believed to bind to a region of the SUR subunit that is adjacent to, but distinct from, that of K_{ATP} openers such as P 1075 **34** or cromakalim.[52,104] Indeed, the absence of obvious structural overlap between openers and blockers supports this. It is intriguing, therefore, that the potent K_{ATP} blocker PNU-99963 **35** is a member of the cyanoguanidine



P 1075 **34**



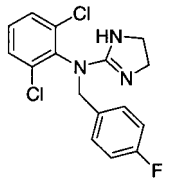
PNU-99963 **35**



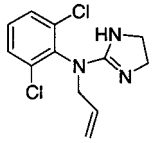
R=Me: PNU-94126 **36**
R=Et: PNU-96293 **37**

class,[105] more known for openers such as P 1075 and pinacidil. PNU-99963 shows greater potency as a K_{ATP} blocker than glyburide with an IC_{50} of 18 nM vs 72 nM for glyburide. Close structural analogues of PNU-99963 lacking the chloro substitution and having gem-dimethyl substitution in place of the cyclobutane were also found to possess K_{ATP} blocking properties albeit with lower potency. An interesting stereochemical observation was also made in these SAR studies. Both PNU-94126 (R = Me) **36** and PNU-96293 (R = Et) **37**, possessing the (*R*) absolute stereochemistry, were found to block K_{ATP} channels; however, the respective antipodes were inactive. This stereochemical preference for K_{ATP} blockade with the (*R*) enantiomers mirrors the findings with cyanoguanidine openers such as pinacidil **2**.[106] The site of interaction of PNU-99963 is presently unknown, but this compound is an interesting new tool to probe interactions between openers and blockers at the SUR/Kir combinations.

IMID-4F. Compound **38**, a K_{ATP} blocker related structurally to alinidine **39** and phentolamine **10**, has recently been described.[107] This newer agent shows a 40-fold greater potency relative to alinidine ($pK_B = 5.5$) as an antagonist in the rat thoracic aorta with a pK_B equal to 7.10. This potency value is similar to that of



IMID-4F **38**



alinidine **39**

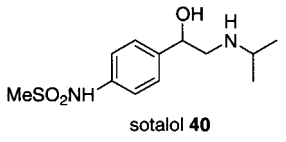
glyburide in vascular preparations.[108] Unlike glyburide, IMID-4F is a noncompetitive antagonist of (−)-cromakalim-induced tissue relaxation. IMID-4F also did not inhibit [^{3}H]P 1075 binding, indicating a distinct site of interaction with the SUR/Kir proteins. It has been suggested, therefore, that IMID-4F may interact directly with the channel pore similar to the behavior of phentolamine in pancreatic K_{ATP} channels.[109] The greatly enhanced potency of this new imidazoline K_{ATP} blocker makes it a more useful tool with which to probe the therapeutic utility of this distinct class of K_{ATP} blocker. However, information regarding the ancillary pharmacological properties of IMID-4F would aid in assessing the full utility of this agent.

Voltage-Gated K^+ Channels

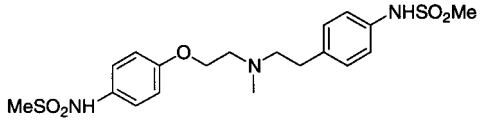
Although Kv channels were the first to be cloned and served as prototypes to elucidate structure−function relationship of K^+ channels, many of the organic modulators including tetraethylammonium and 4-aminopyridine are weak and nonselective. Peptide toxins that bind with high specificity to block the channel pore or alter gating features have served as important tools for the analysis of the structure and function of these K^+ channels.[110] More recently, the identification of the molecular components of many cardiac and neuronal voltage-gated K^+ channels has renewed medicinal chemistry efforts to identify selective openers and blockers of various channel types.

Cardiac Delayed Rectifier K^+ Channels. Major efforts of antiarrhythmic drug discovery have been directed toward compounds that prolong the cardiac action potential and refractoriness.[111] In principle, agents that reduce outward repolarizing potassium K^+ currents in the myocardium can achieve this result. Drugs that fall into this mode of action are commonly referred to as class III antiarrhythmic agents. The cardiac myocytes express many types of delayed rectifier currents that contribute to various phases of repolarization. These include rapidly activating delayed rectifier currents designated ultrarapid (I_{Kur}), rapid (I_{Kr}), and slow (I_{Ks}) components, whose corresponding molecular counterparts have now been identified and reviewed.[112] The goal of developing class III antiarrhythmics effective against ventricular arrhythmias should now, in principle, be accelerated, targeting the molecular components of these cardiac delayed rectifiers such as I_{Ks} (the combination of KvLQT1+minK), I_{Kr} (the combination of hERG+minK-related peptide or miRP), and I_{Kur} (where Kv1.5 is the principal component).

The currently available class III antiarrhythmic drugs reduce the rate of recurrence of atrial fibrillation, the most commonly sustained cardiac arrhythmia in clinical practice. However, these agents are of limited use due to a variety of adverse effects including the risk of ventricular proarrhythmia.[113] Available drugs in this class including sotalol **40** have been shown to possess

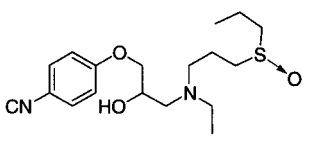


sotalol **40**

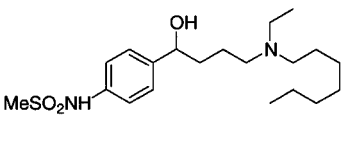


dofetilide **41**

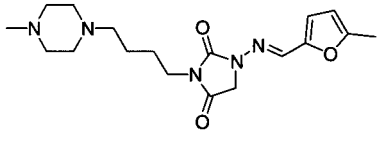
interesting class III properties, but these are not selective. More recently developed antiarrhythmics include compounds that more specifically block an ionic current (e.g., dofetilide **41** and almokalant **42** that block I_{Kr}) or block multiple ionic currents in order to prolong atrial and ventricular action potentials with minimal undesirable pharmacologic effects. The latter include compounds such as ibutilide **43** and azimilide **44**, which is in clinical development for the treatment of atrial fibrillation and blocks both I_{Ks} and I_{Kr}, unlike dofetilide. Compound **44** appears to be devoid of frequency-dependent effects on repolarization and is reported to have a low incidence of in vivo proarrhythmic effects such as torsades de pointes.[114]



almokalant **42**



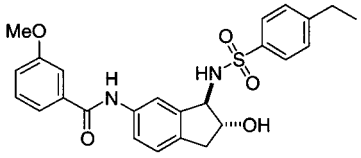
ibutilide **43**



azimilide **44**

***I*Kur/Kv1.5.** The Kv1.5 subunit is the major component of the cardiac ultrarapid delayed rectifier in human atria as revealed by localization and antisense oligonucleotides. Because of its rapidity of activation and slow inactivation, I_{Kur} is believed to contribute significantly to repolarization in human atrium. Consequently, a specific blocker of I_{Kur} would theoretically overcome the shortcomings and disadvantages of currently used agents for the treatment of atrial flutter and/or atrial fibrillation.

***I*Kur (Kv1.5) Blockers.** Many thiazolidinone and tetrahydrothiazinone analogues that block Kv1.5 channels have been claimed for treatment of cardiac arrhythmia.[115,116] Compound **45** inhibited Kv1.5 currents in CHO cells with an IC_{50} value of 0.1 μM.[116] In human atrial myocytes, 1 μM of compound **45** inhibited I_{Kur} currents and prolonged action potential duration by >50%. However, compound **45** was nonselective as it



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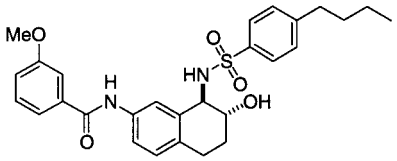

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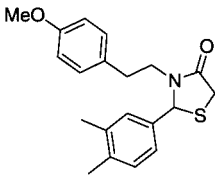


47

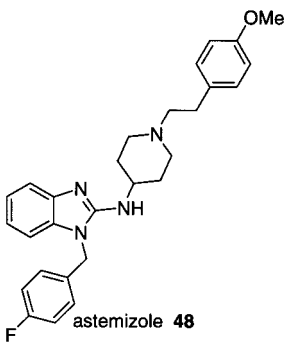
effectively inhibited Kv1.3 current, $^{86}Rb^+$ efflux, and PHA-evoked lymphocyte proliferation, suggesting that this compound may also have immunosuppressant effects. In CHO cells transfected with human Kv1.5, compound **46** potently inhibited currents ($IC_{50} \sim 0.05$ μM) but was somewhat less potent in inhibiting cation flux ($IC_{50} \sim 2.9$ μM). On the other hand, compound **47** inhibited Kv1.5 currents (0.2 μM) and efflux (0.9 μM) with comparable potencies. The selectivity of these two molecules versus other Kv channels, particularly Kv1.3, is unclear.

***I*Kr (hERG+miRP).** Since compounds that block I_{Kr} can prolong cardiac repolarization and increase action potential duration and refractoriness both in atria and ventricle without affecting conduction per se, theoretically they represent potentially useful agents for the treatment of arrhythmias such as atrial fibrillation. However, blockage of I_{Kr} typically causes maximal action potential or QT prolongation at slow heart rates (known as reverse rate dependence) rather than the desired effect of greatest efficacy during rapid rates or tachycardia. Consequently, these agents may have a liability in that they may have an enhanced risk of proarrhythmia at normal heart rates and may lead to an unpredictable development of excessive QT prolongation leading to polymorphic ventricular tachycardia or torsades de pointes which can, in susceptible individuals, cause syncope and sudden death.

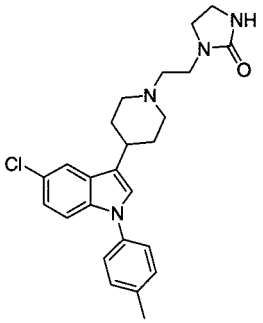
I_{Kr} currents are derived from "*ether-a-go-go* related" subunits. Recently, a small membrane subunit, minK-related peptide 1 (MiRP1), was found to coassemble with the human gene hERG (*human ether-a-go-go related gene*) to generate currents with gating and sensitivity to antiarrythmics similar to native cardiac I_{Kr}.[117] Mutations in the hERG gene including missense, deletion, and splice donor mutations result in chromosome 7-linked congenital long QT syndrome LQT 2. These mutations result in a loss of channel function via multiple mechanisms including abnormal protein tracking, generation of nonfunctional channels, and altered channel gating. Evidence that some hERG blockers can correct the defective protein trafficking of the Asn470Asp hERG mutant and normalize currents sets the stage for a new therapeutic paradigm to use small molecule blockers to rectify disease-induced ion channel functional defects.[118]

***I*Kr (hERG+miRP) Blockers.** Methanesulfonanilides such as dofetilide **41** are class III antiarrhythmics that selectively block the rapid component of the delayed rectifier outward potassium current. Although residues in the pore region hERG interact with dofetilide, an intact C-type inactivation was crucial for high affinity binding.[119] Dofetilide was recently approved for conversion and maintenance of sinus rhythm in patients with atrial fibrillation and atrial flutter.

The cardiotoxicity of many H_1 receptor antagonists such as astemizole **48**, antipsychotics such as sertindole **49**, certain tricyclic antidepressants, antiemetics, and antibiotics have been linked to their potent inhibition of I_{Kr} channels; an effect that leads to the rare occurrence of torsades de pointes in susceptible individuals. For example, the antipsychotic agent sertindole **49**



astemizole **48**



sertindole **49**

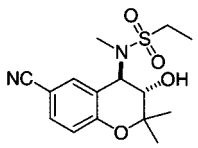
blocked hERG currents with an IC_{50} value of 14 nM when tail currents at −40 mV were measured after a 2 s depolarization to +20 mV. When currents were measured at the end of prolonged (20 s) depolarizing pulses, the IC_{50} of sertindole was ~3 nM.[120] On the other hand, sertindole was approximately 1000-fold less active at blocking Kv1.5, displaying an IC_{50} value of 2 μM. The sertindole core may provide a useful starting point for the development of very high affinity ligands for the hERG channel.

Another important aspect of blockade of the hERG channel lies in its use as an indicator of QT prolongation. Due to the possibility of proarrhythmia associated with lengthened QT intervals, recommendations have appeared for rigorous assessment of this risk prior to human dosing.[121] Although opinions vary,[122] this evalu-

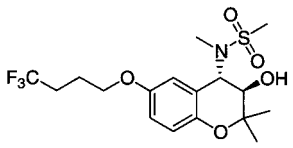
ation was typically done via measurement of action potentials in canine cardiac Purkinje fibers. The linkage between hERG inhibition and QT prolongation has become another component of preclinical safety evaluation,[123] and compound evaluation for blockade of this channel is also used as a preliminary assessment of proarrythmic liability. While not as definitive as measurement of action potentials in Purkinje fibers or QT intervals in canine ECGs, this assay does enable early assessment of cardiac safety for lead compounds. Consequently, hERG evaluation could be routinely used as a general tool for preclinical evaluation mindful of the potential regulatory consequences of this observation and the possibility that hERG activity does not necessarily predict QT prolongation.

***I_{Ks}* (LvLQT1+mink).** The slowly activating component of the delayed rectifier (I_{Ks}) potentially overcomes some of the limitations of I_{Kr} blockers associated with ventricular arrhythmias. However, because of its slow activation kinetics, the role of I_{Ks} in atrial repolarization may be limited due to the relatively short action potential duration of the atrium. Accordingly, it is thought that selective blockers of I_{Ks} may lead to a greater drug effect at faster rates and thus improved efficacy, but with a reduction in the toxicity that occurs at normal heart rates.[124] Selective inhibitors of I_{Ks} and its molecular counterpart, KvLQT1+minK, are of considerable interest as targets of the development of novel class III antiarrhythmic agents.

***I_{Ks}* (LvLQT1+minK) Blockers.** Chromanol 293B **50** is a prototypical inhibitor of I_{Ks} that is currently employed as a pharmacological tool. This compound is a racemate obtained by structural modifications of the benzopyran K_{ATP} channel opener HOE-234. The (−)-



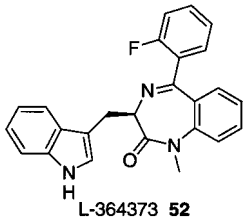
chromanol 293B **50**



HMR-1556 **51**

(3*R*,4*S*) enantiomer of chromanol 293B is more potent at inhibiting the I_{Ks} complex (IC_{50} = 1.4 μM) versus other cardiac ion channels including hERG, Kv1.5, and Kv4.3.[125] It was also noted in this study that the original stereochemical designation for the active enantiomer was incorrectly assigned. HMR-1556 **51**, like chromanol 293B, inhibits I_{Ks} currents in *Xenopus* oocytes with an IC_{50} value of 120 nM and shows selectivity versus hERG channels (10% inhibition at 10 μM). HMR-1556 also possesses the (3*R*,4*S*) absolute stereochemistry, opposite that of the active benzopyran core of cromakalim.[126] Numerous patents have also appeared recently claiming a variety of structural modifications of these benzopyrans as I_{Ks} blockers.[127]

Although the exact structure is not published, L-735821 is a benzodiazepine that blocks I_{Ks} and prolongs action potential duration of guinea pig ventricular myocytes.[128] This compound not only inhibits KvLQT1 currents (EC_{50} = 0.08 μM), but it also blocks KCNQ2 currents (EC_{50} = 1.5 μM).[129] In contrast, L-364373 **52** is structurally related to L-735821, yet it



L-364373 **52**

is an activator of I_{Ks} and shortens action potential duration. The concentration response curve for activation in guinea pig myocytes was biphasic with activation of currents at low concentrations (100 nM to 1 μM) and diminished response at concentrations greater than 3 μM. The increase in current was only observed for the (*R*) enantiomer. L-364373 also activated the cloned I_{Ks} channels depending on the ratio of KvLQT1 and minK subunits expressed. L-364373 activated cloned KvLQT1 channels, but it did not affect channels formed by coassembly of KvLQT1 and minK subunits, suggesting that the association of minK with KvLQT1 prevents the binding of L-364373 or precludes channel activation.[130]

Kv1.3. Background. K^+ channels play an essential role in the stimulation and maintenance of cellular proliferation of T cells, B cells, macrophages, and brown adipocytes. In T lymphocytes, mitogens cause a shift in K^+ conductance with activated T cells showing a substantial increase in K^+ conductance versus quiescent cells. In human peripheral T lymphocytes, the Kv1.3 channel plays a critical role in mediating the K^+ current (I_{Kn}) and Ca^{2+} influx which enables proliferation.[131] In vitro studies have shown that blocking Kv1.3 inhibits stimulated T cell proliferation, thereby making this channel an attractive target for immunosuppressant agents.[132] Additional studies demonstrated the Kv1.3-selective peptide margatoxin (MgTx) had immunosuppressant activity in vivo in a minipig model of delayed type hypersensitivity (DTH),[133] lending added support for this channel as a target for immunomodulation. High throughput screening and continued structural studies have provided a much more comprehensive understanding of Kv1.3, and the discovery of the first small molecule blockers of this channel has been reviewed.[134] Additional information regarding the structure of Kv1.3, the mode of binding of existing blockers, and novel chemotypes with improved selectivity continue to argue in favor of this target as an exciting area of potassium channel research.

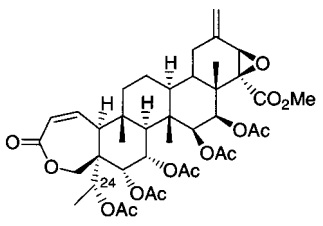
A comparison of the binding conformations of various toxins with Kv1.3 has provided some structural information describing the outer portion of this ion channel and, specifically, the binding interaction of these small peptides with residues in the vestibule of Kv1.3.[135] These studies suggest that a diad in the toxin comprised of lysine and a hydrophobic residue constitute a minimal functionality for Kv1.*x* binding with Kv1.3 selectivity arising from additional interactions of Arg or Asp residues in toxins with His^{404} in the vestibule of the channel. The functional protein is believed to be a homotetramer, and the ability of toxins to capitalize on the resulting symmetry of this assembly in their binding conformations is reported in the same study.[136] Of the Kv-associated toxins, Shk, a 35 amino acid toxin from a sea anemone, is a highly potent blocker with a well-defined conformation. This peptide shares the diad

noted above for other toxins (Lys^{22} and Tyr^{23} in Shk), and when Lys^{22} is replaced with diaminopropionic acid (Dap), the resulting Shk-Dap^{22} demonstrates excellent Kv1.3 selectivity.[137] A model of tetrameric Kv1.3 was generated based on the published structure of KcsA,[18] and docking models of Shk and Shk-Dap^{22} indicated three functionally important residues with closer proximity in the binding conformation of Shk-Dap,[22] with additional changes in the side chains involved in Kv binding. The association with His^{404} in Kv1.3 is maintained with both peptides, and in cellular assays of immunosuppression, the Dap^{22} variant is equipotent to native Shk and MgTx.[137]

Data using toxins as 'molecular calipers' provides added structural information about the tetrameric Kv1.3 channels, which assist in the design of novel ligands. Expression of a mutant form of Kv1.3 in CV-1 cells has enabled purification, and lipid reconstitution provides functional Kv1.3 identical to native channels upon comparison of biophysical characterization using electrophysiology and toxin binding.[138] Immunoblotting and quantification of this construct supports a homotetrameric complex comprised of 64 kDa subunits, and impressively, the tetrameric complexes were visualized using staining and electron microscopy revealing a 65 × 65 Å *X*-*Y* dimension and an overall mass of 270 kDa. A central portion of this visualized tetramer suggests a site for the pore whose dimensions are consistent with those predicted from earlier studies using scorpion toxins.[131,139] Studies pursuant to higher resolution protein structures are presumably underway which may enable definition of an environment around His^{404} and facilitate more specific ligand design.

Kv1.3 Blockers. The biophysical properties of Kv1.3 have been well studied,[131,139] and earlier reviews have detailed efforts directed toward identifying small molecule blockers of this ion channel.[134,140] Key aspects of these compounds have since been disclosed which provide SAR data and insight into the nature of binding for most of the known Kv1.3 ligands.

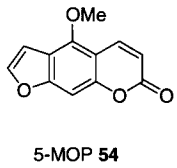
Of the small molecules which block Kv1.3, correolide **53** is among the best characterized. This triterpene natural product is isolated from the extracts of *Spachea correae*,[141] and it has been shown to be a highly potent and selective blocker of the Kv1.3 ion channel (IC_{50} = 86 nM in transfected CHO cells).[142] Data from these binding studies and evaluation of selectivity versus ancillary ion channels and receptors has enabled the use of this compound as an in vitro and in vivo probe of Kv1.3 function in immunosuppression.[143]



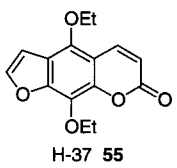
correolide **53**

Early structural studies indicated that the potentially reactive functional groups in correolide such as the epoxide and the exocyclic olefin do not participate in covalent modification of Kv1.3,[142] and assessment of correolide binding using chimeric Kv channels has provided evidence that this triterpene binds to specific residues of the S5 and S6 transmembrane regions of this channel.[144] Accessibility of correolide to its binding site is believed to be influenced by conformational changes associated with inactivation of the channel at its C terminus[144]—a trait shared among the majority of the known Kv1.3 blockers. In in vitro models of immunosuppression, correolide inhibits CD-23-induced increases in intracellular calcium, and it also inhibits Ca^{2+}-dependent thymocyte proliferation. In vivo, the parent natural product induced hyperactivity in swine models of delayed-type hypersensitivity; consequently two analogues with modifications at C-24 were characterized for in vivo studies. These compounds had comparable in vitro potencies to the parent natural product with enhanced pharmacokinetics, and in vivo, these derivatives inhibited the DTH response to tuberculin toxin in Yucatan minipigs with efficacies comparable to those observed for MgTx.[143] To date, the published SAR of correolide is limited, yet several patents have appeared claiming analogues as potent immunosuppressant agents.[140,145] Presumably another derivative of this triterpene is in advanced stages of characterization.

The observation that the 5-methoxypsoralen (5-MOP) **54** was found to be a selective Kv blocker[146] has prompted efforts to separate the potassium channel modulation effects from the photoreactivity associated with these structures.[147] While initial efforts resulted in analogues with Kv blocking activity and reduced photomutagenic potential,[148] more recent studies revealed that H-37 **55** potently and selectively inhibits potassium channels in T cells.[149] In stimulated T cells, H-37 inhibited proliferation and IFN-γ production with no photoinduced DNA intercalation commonly seen with psoralen analogues. Immunosuppressant effects were also observed using H-37 in vivo where induction of IFN-γ secretion was reduced in Lewis rats challenged with experimental immune encephalomyelitus (EAE). While alkoxypsoralens have the desired in vitro and in vivo profiles, the published analogues are nonselective for the Kv1.*x* family.[149] Future efforts would presumably discriminate between these subtypes as this SAR develops.



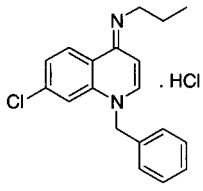
5-MOP **54**



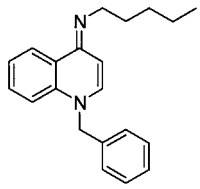
H-37 **55**

Other Kv1.3 blockers have emerged as potential immunosuppressants.[134] The 4-iminodihydroquinolines such as WIN-17317-3 **56** were reported as Kv1.3 antagonists which originally demonstrated selectivity versus the other Kv1.*x* channels and in vitro efficacy in models of immunosuppression using stimulated T cells (EC_{50} = 1 μM).[150] A close analogue of WIN-17317-3, CP-339818 **57**, has also been shown to block Kv1.3 with the ability to inhibit stimulated T cell activation at comparable concentrations.[151] Studies using Kv mutants again revealed that C-type inactivation of Kv1.3 had an effect on the binding of these iminoquinolines. In the case of CP-339818, binding is optimal with residues exposed in the vestibule of the channel which become progres-

sively less accessible upon conformationally induced inactivation.[151] However, as a class, these iminoquinolines demonstrate additional affinity for other ion channels. WIN-17317-3 is reported to have a high affinity for voltage-gated Na^+ channels[152] found in the brain, and CP-339818 has an equal affinity for the Kv1.4 channel expressed in heart and brain.[151] To date, structure activity studies are limited. However, ongoing efforts could plausibly incorporate the structural data from mutant studies[151] into the design of more selective iminoquinoline analogues.

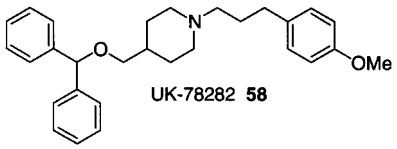


WIN-17317-3 **56**



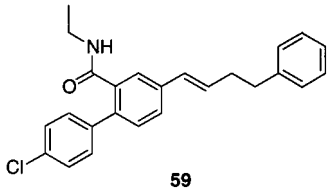
CP-339818 **57**

UK-78282 **58** appears to be the prototype for the benzhydryl piperidine Kv1.3 blockers originally described as leads from a high throughput $^{86}Rb^+$ efflux screen.[153] Although UK-78282 has a comparable affinity for Kv1.4, it is selective versus the remainder of the Kv1.*x* channels. Once again, binding of these compounds to Kv1.3 is influenced by C-type inactivation. Mutation of His^{404} in the vestibule of the channel provides mutants with varied degrees of C-type inactivation, and in these mutants, UK-78282 potently blocks channels which can rapidly enter the C-type inactive conformation with much less of an effect on slowly inactivating variants.[153] UK-78282 inhibits stimulated T cell proliferation with potencies comparable to the other Kv1.3 blockers, and some SAR has been disclosed showing that UK-78282 is preferred among the analogues described.[154]



UK-78282 **58**

De novo ligand design has begun using structural information from Kv1.3 mutants as well as key components of existing Kv1.3 blockers. A model employing key residues in the Kv1.3 active site (His^{404}, Asp^{402}, Tyr^{400}) coupled with combinatorial synthesis of candidate Kv channel blockers provided biphenyl analogues such as **59**, which have comparable activity ($IC_{50} = 1-10\ \mu M$) to analogues of UK-78282.[155] Although only Kv1.3 binding data is provided, this is the first reported use of structural information from voltage-gated channels to be used with combinatorial chemistry pursuant to the design of completely novel K^+ channel modulators.

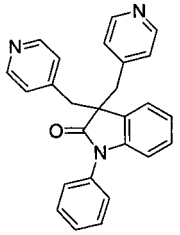


59

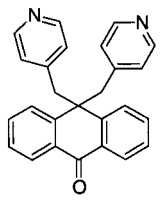
To date, most of the known small molecule Kv1.3 blockers have some association with the key residues in the vestibule of the channel such as His^{404}, and the binding conformation of these ligands is typically affected by C-type inactivation. Future structural studies identifying residues associated with known Kv1.3 ligands in the vestibule of the channel and continued mapping of this pharmacophore will likely lead to improved blockers for immunomodulation.

KCNQ-Derived Channels. The M-type current is a voltage-gated K^+ current that plays a critical role in regulating neuronal excitability in the nervous system.[17,156] It is now known that combinations of the KCNQ3 subunit with KCNQ2, KCNQ4, or KCNQ5 counterparts gives rise to diverse heteromeric channels that underlie these currents. Mutations in KCNQ2 or KCNQ3 genes result in a loss of ion channel function and an increase in cellular excitability leading to benign familial neonatal convulsion, a generalized form of epilepsy. On the other hand, mutations in KCNQ4 cause autonomic dominant progressive hearing loss. Because of their important physiological functions, KCNQ channels have clear potential as drug targets.

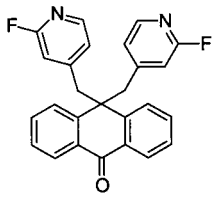
KCNQ Blockers. The potential for targeting the KCNQ3-containing channels is underscored by the fact that compounds developed as cognition enhancers such as linopirdine **60** and XE-991 **61** are blockers of these currents.[157] It has been suggested that blockade of the M channel underlies, at least in part, the enhancement of transmitter release by these drugs. Linopirdine, a



linopirdine **60**



XE-991 **61**

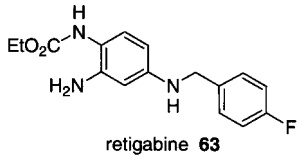


DMP-543 **62**

putative cognition-enhancing drug, increases acetylcholine release in rat brain tissue and improves performance in animal models of learning and memory.[158] Although clinical data with linopirdine were inconclusive, DuPont has developed analogues such as XE-991 with superior pharmacological and pharmacodynamic properties as orally active acetylcholine-releasing agents with potential in Alzheimer's disease. Heteromers derived from KCNQ1/minK that underlie cardiac I_{Ks} current are 14−18-fold less sensitive to XE-991 blockade compared to either KCNQ1 alone or neuronal KCNQ2/3 combination, demonstrating selectivity for this class of compounds for neurotransmitter release over cardiac function.[159] Replacement of the 4-pyridyl pendant group in linopirdine with 2-fluoro-4-pyridyl moiety resulted in DMP-543 **62**, a compound 10−20 times more potent in releasing ACh from hippocampus and with improved half-life (4-fold) and brain:plasma (6-fold) distribution compared to linopridine.[160] With the more recent availability of cloned KNCQ subunits, more potent compounds that selectively inhibit M-currents useful for treating cognitive deficits in neurodegenerative diseases are likely to be forthcoming.

KCNQ Activators. Genetic evidence linking mutations in KCNQ family members to benign familial

neonatal convulsions has prompted a reexamination of this type of K^+ channel opener as potential antiepileptic agents. The antiepileptic agent retigabine **63** has been shown to activate KCNQ2/3 expressed in CHO cells in a partially linopirdine-sensitive manner, suggesting that M-channel activation may be a new mode of action for anticonvulsant drugs.[161]



retigabine **63**

Calcium-Activated K^+ Channels

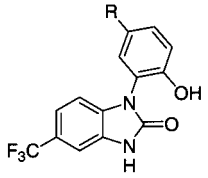
Unlike voltage-gated K^+ channels, the calcium-activated K^+ channels are regulated not only by membrane depolarization but also by changes in intracellular Ca^{2+} levels. These channels have been initially described on the basis of biophysical (conductance) and differential toxin sensitivities. For example, on the basis of single-channel conductance (measured in picosiemens (pS)), K_{Ca} channels are classified as large (100−250 pS), intermediate (25−100 pS), and small (2−25 pS) conductance channels. More recently, this classification has been complemented by the emergence of three distinct genes encoding these subfamilies, i.e., large conductance BK_{Ca} (α-slo together with $\beta1-\beta4$ subunits), intermediate conductance IK_{Ca} (IK), and small conductance channels SK_{Ca} (SK1, SK2, and SK3).[162−164] The recent emergence of the molecular diversity of calcium-activated potassium channel subunits has renewed considerable enthusiasm in the development of modulators for these classes of ion channels.

BK_{Ca} Channels: Background. The high conductance or maxi-K (BK_{Ca}) channels are activated by an increase in intracellular calcium concentration and membrane depolarization. These channels are sensitive to blockade by iberiotoxin. The cloning of multiple splice variants of the pore-forming α-subunit (*mSlo*, *hSlo* following the nomenclature of the initially cloned *Drosophila slowpoke* (*dSlo*) calcium-activated K^+ channel) and multiple β-subunits has recently generated considerable diversity within the BK_{Ca} family. This, together with the widespread distribution of BK_{Ca} channels throughout the CNS and in peripheral tissues offers rich opportunities for discovering novel therapeutic agents as well as significant challenges in the form of tissue and organ specificity. Therapeutic applications for channel openers have focused on stroke, epilepsy, and bladder overactivity although there is evidence for utility in the treatment of asthma, hypertension, gastric hypermotility, and psychoses.[165] In general, the state of the art in the area of designing selective BK_{Ca} openers, although more extensively developed than those of IK_{Ca} or SK_{Ca} channels, is far less mature compared to that of openers of the K_{ATP} channels.

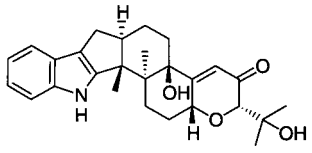
BK_{Ca} Openers. A number of different structural classes of BK openers have appeared in the literature and are listed in Table 6. Benzimidazolone analogues such as NS-4 **64** and NS-1619 **65** stimulate BK_{Ca} activity leading to membrane hyperpolarization. NS-1619 activates BK_{Ca} currents at 10−30 μM in vascular and nonvascular smooth muscle, although over similar concentration ranges, the compound also inhibits delayed rectifiers and calcium currents. NS-1608 is a diphenylurea analogue that enhances BK_{Ca} activity by shifting current activation to more negative potentials at micromolar concentrations and does not require the presence of the β-subunit. Other known BK_{Ca} openers include glycosylated triterpene activators (dehydrosoyasaponin-I) and several indole diterpene blockers such as paxilline **66**.[166] In general, most of the early BK_{Ca} channel openers are relatively weak agents or they are known to possess ancillary pharmacology (see Table 6)[165,167] which limits their utility as therapeutic agents or as probes to study the in vivo therapeutic relevance of BK_{Ca} channels.

Table 6. Structural Classes of BK_{Ca} Openers

structural class	prototype	ancillary pharmacology
benzimidazolones	NS-1619	K_{ATP}, L-type Ca^{2+} channel
benzimidazolones	NS-4	K_{ATP}, Kv, Cl^- channel, and Ca^{2+} channel
benzopyrans	cromakalim	K_{ATP}
dihydropyridines	nitrendipine	L-type Ca^{2+} channel
biarylamines	niflumic acid	Cl^- channel, NSAID
biarylamines	MCI-154	
biarylureas	CGS-7181	
biarylureas	NS-1608	
3-aryloxindoles	BMS-204352	
terpenoid	dehydrosoyasaponin-I	
terpenoid	Maxi-K diol	Ca^{2+} channel
flavoid	phloretin	Na^+ channel



R = Cl: NS-4 **64**
R = CF_3: NS-1619 **65**

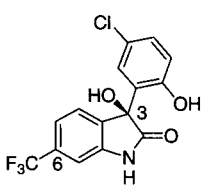


paxilline **66**

In general, activation of BK_{Ca} currents has been assessed by determining the ability of a single concentration of the test compound to increase cloned mammalian (*mSlo* or *hSlo*) currents in *Xenopus* oocytes or cell lines, which limits strict comparison of compound potencies. Furthermore, selectivity of available openers across various ion channel types, and more importantly evaluation versus the cloned BK_{Ca} channels containing diverse β subunit combinations, remains to be investigated.

Stroke. Bristol-Myers Squibb has reported novel aryloxindole BK channel openers.[168] Structurally these

bear a close resemblance to the benzimidazolones with the primary exception being the oxindole replacement for the benzimidazolone. The (−) enantiomer of compound **67** has been shown to increase BK_{Ca} currents in



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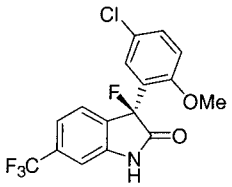
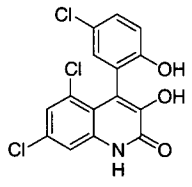


BMS-204352 **68**

the *mSlo* channel expressed in *Xenopus* oocytes by 41% over control at 20 μM. In the same study, the reference oxindole derivative, NS-4 **64**, activated BK_{Ca} currents 32% at 20 μM. Some stereodifferentiation was seen in this series as the (+) enantiomer activated currents only 24% at 20 μM. The related 3-fluoro analogue BMS-204352 **68** is reported to be neuroprotective and reduce infarct size in two preclinical rat stroke models.[169] Efficacy was observed over a dose range of 10 ng/kg to 3 mg/kg. Interestingly, at doses greater than 1 mg/kg, BMS-204352 displayed an inverted-U dose−response relationship. It reduced electrically evoked hippocampal field potentials when administered iv at 30 ng/kg. BMS-204352 is also reported to have no effects on heart rate and mean arterial pressure (MAP) in conscious dogs up to 3 mg/kg iv. In vitro, in hippocampal slices it was effective at reducing glutamate release (IC_{50} = 352 nM). The 3-fluoro substitution of BMS-204352 was introduced specifically to improve metabolic stability. Currently, BMS-204352 is undergoing clinical trials for stroke.[168] When tested as the racemate, BMS-204352 activated BK_{Ca} currents similar to compound **67** in cloned *Slo* channels.[168] A number of other analogues lacking the 3-fluoro or 3-hydroxy groups showed even greater potentiation of BK_{Ca} currents in vitro, indicative that these substitutions are not absolute requirements. Replacement of the 6-CF_3 group with 6-iodo, 6-phenyl, or a fused phenyl was well tolerated.[168]

The 3-hydroxy and 3-amino-4-aryl-quinolin-2(1*H*)one structures have recently been disclosed as novel chemotypes with potent BK opening activity in vitro and in vivo.[170] Within this series, the trend continues wherein the 5-halo-2-hydroxy substitution pattern is present on one of the aromatic rings.

The vast majority of analogues for which data was disclosed possess the 5-chloro-2-hydroxyphenyl or 5-chloro-2-methoxyphenyl groups. Compound **69** activated currents by >50% at 20 μM in *mSlo* or *hSlo* channels. Compound **70** similarly activated BK currents >50% at 20 μM. In vivo, this latter compound reduced infarct volume by 14% when dosed (0.001 mg/kg, iv) in a focal stroke model in rats.



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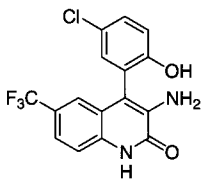
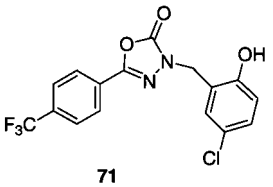


70

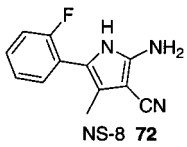
The 1,3,4-oxadiazolone compound **71** depicted below exemplifies another novel structural series from Bristol-Myers Squibb.[171] Once again, the 5-halo-2-hydroxyphenyl moiety is present, in this case linked to the second aromatic ring via the oxadiazolone ring. This compound



71

is reported to potentiate BK_{Ca} currents by 26% at 1 μM. In vivo, **71** reduced infarct volume by 18% in a focal stroke model in Wistar rats at a dose of 10 μg/kg, administered iv. Similarly, in an equivalent model in spontaneously hypertensive rats, infarct size was reduced by 14% at a dose of 10 mg/kg (ip). A number of structural analogues of this compound have also been claimed to activate BK_{Ca} currents in vitro. All of those analogues with reported data again possess the 5-halo-2-hydroxyphenyl group with variations in the spacer heterocycle or additional aromatic ring substituents.

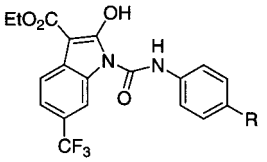
Bladder Overactivity. The structurally novel arylpyrrole NS-8 **72** has recently been disclosed as a BK opener selective for the bladder smooth muscle.[172] This compound bears little obvious structural similarity to other known BK openers. NS-8 activates BK currents



NS-8 **72**

in guinea pig bladder cells and relaxes guinea pig bladder strips contracted with 20 mM KCl with an IC_{50} = 0.54 μM. It has also been shown to suppress the excitability of dorsal root ganglion neurons. In vivo NS-8 was efficacious in two different models of bladder overactivity. In rats, the compound increased bladder capacity 60−80% in a dose-dependent manner at 3 and 10 mg/kg id. NS-8 also inhibited bladder contractions triggered by filling in rats when given either intravenously over the dose range of 0.03 to 1 mg/kg iv or intravesically at concentrations from 30 μg/mL to 300 μg/mL. Intracerebroventricular administration failed to show an effect. It is speculated that NS-8 may exert its bladder inhibitory effects via the afferent signaling pathway or at the bladder smooth muscle per se. The data reported tends to rule out the involvement of a purely central mechanism or effects on the efferent pathway to the bladder. NS-8 is reportedly in phase I clinical trials for pollakiuria.[173] Interestingly, NS-8 has also been claimed to possess activity versus cyclooxygenase-2 (Cox-2).[174] Prostanoids, generated from cyclooxygenase isozymes, play a role in the physiological function of the lower urinary tract, and inhibition of Cox-2 prevents or reverses the urodynamic changes associated with experimentally induced bladder inflammation.[175] Whether this ancillary activity plays a role in mediating the effects of NS-8 is currently not known.

Several indole-3-carboxylic acid esters have been disclosed as BK openers.[176] CGS-7184 **73** and CGS-7181 **74** are representative examples from this series. These compounds bear structural resemblance to other BK_{Ca} openers by the presence of the urea moiety and the juxtaposition of two substituted aromatic rings. The urea NH may be viewed as an isostere of the 2-hydroxy substituent of earlier BK_{Ca} openers.

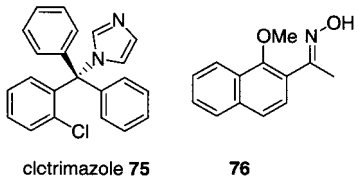


R=Cl: CGS-7184 **73**
R=Me: CGS-7181 **74**

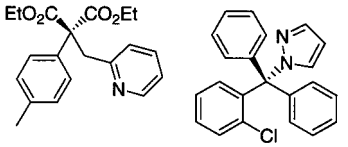
CGS-7184 and its analogues are reported to potently open BK_{Ca} channels in both coronary artery and urinary bladder smooth muscle cells. Although a substantial delay (6−8 min) between drug application and maximal activation of BK_{Ca} current was noted, current enhancement in both coronary and bladder cells with CGS-7181 and CGS-7184 was comparable. These compounds appear to be more potent than the benzimidazole analogues as the effective concentration for current enhancement is some 20−50-fold lower than NS-1619. Channel opening activity was noted at the whole cell level when CGS-7184 was applied to the extracellular side or at the single-channel level whereas it increased open state probability when applied to the intracellular side of the membrane. In this regard, these indole carboxylate compounds are similar to NS-4 and NS-1619, but distinct from maxi-K diol, which is reported to act only from the cytosolic side.[166,167] In vivo effects of CGS-7184 or its analogues have not been reported.

IK_{Ca} Channels: Background. Studies in T lymphocytes identified a high affinity binding site for the scorpion toxin charybdotoxin (ChTx), which was originally associated with a voltage-dependent potassium channel (Kv1.3).[177] Upon further study, ChTx also demonstrated a high affinity for an intermediate conductance calcium-dependent channel in T cells identified as human IK.[178] Early studies in red blood cells using synthetic ChTx, [^{125}I]ChTx, iberiotoxin, and their recombinant variants effectively blocked IK in erythrocytes and thymocytes.[177,179] Both IK and Kv1.3 are involved in thymocyte regulation and proliferation. Of these two channels, blockade of Kv1.3 has been more thoroughly studied as a potential immunosuppressant treatment;[134] however, in stimulated T cells, IK is upregulated to a greater extent (15× for IK vs 1.3× for Kv1.3).[180] In addition, selective blockade of IK using clotrimazole resulted in a greater inhibition of T cell proliferation compared with selective Kv1.3 blockade using agitoxin-2.[180] IK is also reported to have a dominant effect on Ca^{2+} influx in thymocytes needed for sustained proliferation.[181] These data suggest a prevailing role for IK in the modulation of T cell proliferation. Homology models of IK and Kv1.3 have been generated based on the published structure of the KcsA channel,[18] and refined docking models using ChTx and its variants revealed differences in the turret region of the channel: Kv1.3 possesses a unique trio of residues not present in IK. ChTx variations at residue 32 capitalized on this difference resulting in analogues that were 60-fold more selective for IK versus Kv1.3.[22] These selective toxins allow for discrimination of IK-mediated processes in T cells, and these studies provide support for using the structure of KcsA as an early guide for the design of selective K channel modulators.

IK_{Ca} Blockers. Antagonists of the intermediate conductance potassium channel (IK) have potential therapeutic uses as modulators of thymocytes and erythrocytes. Nonselective IK blockers such as clotrimazole **75** have demonstrated antiproliferative effects in lymphocytes in vitro and in vivo. These compounds



clotrimazole **75** **76**

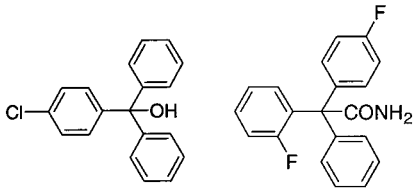


77 TRAM-34 **78**

reduced T cell proliferation and IFN-γ secretion in T lymphocytes stimulated with a variety of antigens (concanavalin A, PHA, tetanus toxin),[181] and recently, structures have emerged with more selective pharmacology. Oxime and malonate derivatives such as **76** and **77**[182] as well as newer analogues of nitrendipine[183] are claimed to selectively inhibit IK function. A new triarylmethane, TRAM-34 **78**, is reported as a potent and highly selective IK blocker with in vitro activity in immunosuppressant models using stimulated thymocytes.[184] In contrast to clotrimazole, TRAM-34 does not inhibit CYP3A liver enzymes, and a preliminary in vivo assessment suggests an acceptable safety index for this analogue. Although SAR data is limited, subtle changes in the triarylmethane core result in demonstrable improvement. For example, alteration of the heterocycle from imidazole in clotrimazole to pyrazole in TRAM-34 is key to an improved metabolic profile for these candidate immunosuppressants.

Blockers of the IK channel are better characterized as modulators of erythrocytes for the treatment of sickle cell disease. Dehydration of red blood cells potentiates the polymerization of the hemoglobin S in patients with homozygous point mutations in the β-globin gene resulting in erythrocyte sickling. IK channel blockers (originally described as erythrocyte Gardos channels) are believed to inhibit red cell dehydration, and consequently such blockers have been proposed as a therapy for sickle cell anemia.[185] Clotrimazole is also an inhibitor of the IK channel in red blood cells, and consequently this compound was identified as a potential treatment of sickle cell disease.[186] More recently, novel IK blockers have appeared which are claimed for the same use. Triphenylmethane derivatives such as **79** from academic[187] and ICA-15451 **80** from industrial sources[188]

have emerged, and data indicate that agents such as ICA-15451 have desirable potency and ion channel selectivity (IC_{50} = 9 nM for the Gardos channel vs 2000 nM for the cardiac I_{Ks} channel). Clinical evaluation of the first of the candidates from this series is believed to be underway.



79 ICA-15451 **80**

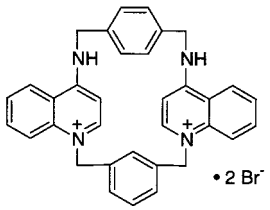
It has been suggested that blockers of IK may be beneficial in other disorders of cell proliferation. Clotrimazole has been shown to inhibit the rate of cellular proliferation of cancer cell lines in vitro and this IK blocker also reduced the proliferation of metastases in SCID mice inoculated with human melanoma cells.[189] The IK channel also serves as an effector for mitogenic Ras/MAPK signaling in fibroblasts and other cell types including prostate cancer cells.[190] Consequently, a selective IK blocker may have a role in controlling proliferative disorders in other tissues.

IK_{Ca} Openers. Agonists of IK channels may also be therapeutically beneficial in hypertension, cystic fibrosis, and peripheral vascular disease. Although not highly specific, 1-ethyl-2-benzimidazolinone (1-EBIO) and clinically used benzoxazoles are described as pharmacological activators of the IK channel.[191] Recent patents have claimed isatin derivatives as IK openers; however, no specific data is provided.[192]

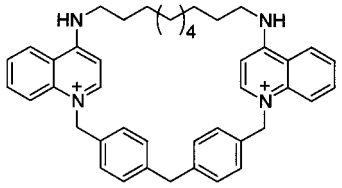
SK_{Ca} Channels: Background. The small conductance Ca^{2+}-activated potassium (SK_{Ca})[163] is found in a variety of different cell types including sympathetic neurons, intestinal smooth muscle, bladder smooth muscle, hepatocytes, and brown adipocytes.[193] Like BK_{Ca} channels, SK_{Ca} channels are activated by changes in intracellular calcium concentrations and possess a unitary conductance of 2−25 pS. In excitable cells, the SK_{Ca} channels are responsible for the slow afterhyperpolarization that often follows action potentials. Three mammalian genes, SK1, SK2, and SK3, encoding SK_{Ca} channels have been cloned which demonstrate a high degree of structural homology and Ca^{2+} sensitivity.[194] Only SK2 and SK3 are apamin-sensitive. Calmodulin, a ubiquitous mediator of calcium-dependent processes, is constitutively associated with the SK_{Ca} α-subunits, and it is the Ca^{2+} binding to calmodulin that induces SK_{Ca} channel gating. The domains in both calmodulin and SK_{Ca} subunits responsible for constitutive interactions as well as for Ca^{2+}-induced conformational changes have recently been elucidated.[195]

The existence of both apamin-sensitive and -insensitive SK channels suggests that development of subtype-selective agents should be feasible. From a molecular perspective, both SK1 and SK2 modulation is thought to be important in diseases involving loss of synaptic plasticity, as for example, age-related loss of memory and learning as in Alzheimer's disease. SK channels, particularly SK2 and SK3, also play a role in the innervation and control of motility of the gastrointestinal and genitourinary tracts where hyperpolarization and relaxation responses are mediated by spontaneous transient outward currents composed of BK_{Ca} and SK_{Ca} channels.[196] The SK3 gene was targeted by homologous recombination by insertion of a gene switch that permitted experimental regulation of SK3 expression while retaining normal SK3 promoter function.[197] SK3 overexpression induced abnormal respiratory responses to hypoxic challenge and compromised parturition. These results implicate SK3 channels as potential therapeutic targets for disorders such as sleep apnea and for regulating uterine contractions during labor. Modulators of SK_{Ca} channel subtypes have been suggested to potentially have utility in the treatment of disorders such as myotonic muscular dystrophy, gastrointestinal dismotilities, memory disorders, epilepsy narcolepsy, and alcohol intoxication.[198]

SK_{Ca} Blockers. Three general classes of SK_{Ca} blockers are known: (i) natural peptide toxins apamin and leiurotoxin I (scyllatoxin); (ii) bis-quinolinium blockers such as UCL-1684 **81** and UCL-1530 **82**, and (iii) neuromuscular blockers such as tubocurarine. UCL-1530,[199] UCL-1684,[200] and the peptide toxins are active in the low nanomolar range whereas the others show less potency (typically ∼1 μM). UCL-1684, in particular, is the most potent nonpeptidic SK_{Ca} blocker with an IC_{50} value of 3 nM.



UCL-1684 **81**

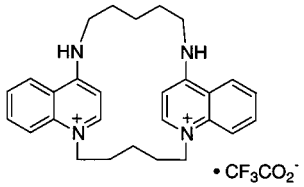


UCL-1530 **82**

The blockers described above have proven useful primarily as tools in elucidating the physiological relevance of SK_{Ca} channels and probing their functional diversity in various tissues.[162] For example, UCL-1530 has been reported to discriminate between neuronal SK_{Ca} channels and those in either hepatocytes or jejunum[201] whereas dequalinium shows roughly equal potency in the three tissues. Thus far there have been no reported in vivo studies with these agents; therefore, the relevance of the promising in vitro selectivity of UCL-1530 remains unclear.

Extensive SAR investigations have been described for the bis-quinolinium blockers, exploring the importance of the two linker portions, and the heterocycle.[198] The presence of a charged moiety is important for activity, either a quaternized nitrogen or one sufficiently basic as to be protonated under physiological conditions. Indeed, the charged groups have been suggested to mimic the two arginines of apamin.[202] Very recently, another variation of these structures has appeared with comparable potency. UCL-1848 **83**, bearing two pentylene linkers between the quinolinium rings, has an IC_{50} of 1−2 nM in assays of SK_{Ca} channel blockade.[203]

Additional patents have also appeared describing structurally related SK_{Ca} blockers.[204]



UCL-1848 **83**

Other K^+ Channel Types

The above discussion has covered most, if not all, K^+ channels where currently available molecular, genetic, and biophysical evidence presents certain members as attractive therapeutic targets amenable to exploitation by the medicinal chemist. Although it may be argued that many K^+ channels critical to cellular excitability have already been cloned and evaluated, it is quite possible, with heightened genomic efforts, i.e., expressed sequence tag (EST) analysis, genome sequencing, and microarray techniques, to reveal novel members or, more likely, auxiliary subunits and proteins that are critical to the in vivo regulation of the primary subunit. For example, as many as 50 genes in the *Caenorhabditis elegans* genome may encode K^+ channels belonging to the novel structural class of two-pore channels.[205] These two-pore channels are thought to function as background channels involved in the modulation of resting membrane potential in various cell types. Although less well studied at the present time, some neuroprotective agents such as riluzole and volatile general anesthetics such as chloroform and isoflurane have been shown to target members of these channels.[206]

Conclusions and Perspective

Over the past decade, potassium channels have emerged as attractive targets for medicinal chemistry efforts. The fundamental role of these channels in physiological processes including neuronal signaling, vascular and nonvascular muscle contractility, cardiac and auditory function, hormone secretion, immune function, and cell proliferation has been underscored by recent discoveries linking K^+ channel mutations to various diseases. Molecular cloning and expression of diverse K^+ channels now offers a platform for the medicinal chemist to examine compounds targeting defined subtypes. This has also eliminated the need for strictly using native cells/tissues that contain multiple and, in many cases, poorly defined receptor combinations expressed in relatively low amounts inadequate for a robust in vitro screen. These advances are catalyzing a transformation in the discovery of novel potassium channel modulating compounds, with high throughput screening promising to greatly accelerate the process of identifying newer pharmacophores with potentially unique pharmacology.

However, many challenges remain to be resolved. Much less progress has been made in the biochemical characterization of K^+ channel proteins and in our understanding of mechanisms that control transcription, functional expression, and regulation of these proteins both in normal and diseased patients. Further progress will require, among other things, an enhanced understanding of the expression patterns of potassium channel subunits, knowledge of specific composition of heteromultimeric subunits, and the regulation of channel proteins in disease states. As noted previously, given the diversity of K^+ channel subunits and the potential to vary the constituents to form heteromeric channel complexes that exhibit different pharmacological properties, it is imperative to know the precise composition of channel complexes in vivo. This information would help guide medicinal chemistry efforts by targeting the desired subtype combination(s) to optimize molecular selectivity. Reviews of clinical data from early K^+ channel modulators will also aid in directing these efforts, with particular attention being paid to the unanticipated mechanism-based side effects observed in patients treated with a breadth of chemotypes.[26]

The opportunities and issues outlined in the preceding paragraphs can be illustrated by considering the challenges encountered in the discovery of organ-selective K_{ATP} openers. The task of identifying molecules selective against hypotensive effects has been hampered by the absence of predictive high throughput in vitro assays, necessitating the use of labor-intensive in vivo assays for a definitive assessment. Although some gauge of hypotensive liability can be obtained from testing compounds in vitro in conduction vessels such as the aorta or the portal vein, the control of blood pressure in vivo is likely to be governed by effects on resistance vessels whose channel composition may be different. Methodologies to analyze subunits in defined vascular cell types have recently become available,[207] the utilization of which will enable targeting the appropriate subtype combinations. In addition, assessment of efficacy versus side effects using in vitro comparisons between isolated tissue experiments rarely provides a reliable measure of absolute selectivity due to variable stimulation methods and the measurement of unrelated endpoints. This can lead to a poor correlation with effects seen at the in vivo level, especially when it is not clear what degree of efficacy an in vitro model relates to a given in vivo effect. These obstacles have become particularly evident in the design of cardioselective and bladder-selective agents. The more recent cloning and functional analysis of various subunit combinations should prove extremely valuable in ameliorating this situation as correlation between effects on the cloned subunit combinations and in vivo effects are established.

Enhanced utilization of structure-based drug design could emerge from future studies on the architecture of mammalian K^+ channels. Tissue delivery of genes, perhaps in concert with small molecules, may be envisaged as an avenue enabling specific modulation of ion channel function and improved drug selectivity. In fact, gene transfer of potassium channels has recently been shown to be a novel and effective strategy to suppress certain conditions as, for example, arrhythmias caused by unstable repolarization.[208]

Judging by the volume of publications over the last 10 years, medicinal chemistry research in the K_{ATP} field continues to outstrip efforts directed toward other potassium channel classes. This situation is likely to continue for the short term as the newer methods of

compound screening against cloned subtypes are fully exploited. However, given the maturity of the field and the clinical experience to date, this trend will not persist indefinitely without future clinical successes or the discovery of molecules that can more selectively modulate channel subtype activity. It is hoped that research activity will depart from this long-examined family, and an increased interest in emerging areas of calcium-activated potassium channels, KCNQ channels, and Kv1.3 channels will emerge. Given the significant market potential for the therapeutic applications being explored, these targets offer some promise. Yet even in these newer areas, the challenges associated with identifying tissue- or organ-specific agents will remain a significant hurdle. Clinical experience with these newer pharmacological agents is currently lacking; however, prototype compounds such as BMS-204352 have advanced to the point where crucial efficacy trials are underway. The clinical fate of such compounds will set the direction for the next stage of K^+ channel research. With enhanced understanding of the molecular components of the various delayed rectifiers that mediate cardiac repolarization, the development of class III antiarrhythmics agents effective against ventricular arrhythmias has reemerged as an active area of investigation. The past decade has seen limited medicinal chemistry research in this area, perhaps due to the difficulties associated with working in the complex environment of native cell or tissue-based assays and the availability of compounds lacking the required potency and selectivity. The recent cloning and expression studies coupled with knowledge of genetically linked mutations has enabled a more precise understanding of how these K^+ channels act in concert to regulate cardiac excitability. The availability of various cloned subunit combinations should facilitate identification of compounds that selectively interact with defined channel subunits, a fact supported by the increase in the number of patent applications for I_{Ks} and Kv1.5 blockers in the past few years.

Remarkable progress has been made in elucidating the biological and biophysical aspects of various K^+ channels. As further definitions of the role and regulation of these ion channel proteins continue, it could be envisaged that the stage is set for another decade of enthusiasm and progress where medicinal chemists can fully exploit the pharmacological potential of many existing and novel K^+ channels.

Biographies

Michael J. Coghlan obtained a B.S. degree in chemistry from Loyola University of Chicago followed by M.S. and Ph.D. degrees in organic chemistry from Northwestern University under the direction of Prof. James A. Marshall. Afterward he did postdoctoral studies in organic chemistry at The Ohio State University with Prof. Leo Paquette. He joined Abbott Laboratories in 1992 where he is currently a Project Leader in Neurological and Urological Diseases Research, Pharmaceutical Products Division.

William A. Carroll obtained a B.S. degree in chemistry from the University of Illinois at Urbana-Champaign and joined Abbott Laboratories in 1985 where he worked for two years prior to pursuing graduate studies. He subsequently completed a Ph.D. degree in organic chemistry at Indiana University under the direction of Prof. Paul A. Grieco. He rejoined Abbott Laboratories in 1992 and is currently a Group Leader in Neurological and Urological Diseases Research, Pharmaceutical Products Division.

Murali Gopalakrishnan obtained his Bachelor of Pharmacy degree from Banaras Hindu University, India, and Ph.D. degree in biochemical pharmacology at the State University of New York at Buffalo with Prof. David J. Triggle. Following postdoctoral research at Baylor College of Medicine, Houston, TX, with Prof. Arthur M. Brown, he joined Abbott Laboratories in 1993 where he is currently a Group Leader in Neurological and Urological Diseases Research, Pharmaceutical Products Division.

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