A New Class of Blockers of the Voltage-Gated Potassium Channel Kv1.3 via Modification of the 4- or 7-Position of Khellinone

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The voltage-gated potassium channel Kv1.3 constitutes an attractive target for the selective suppression of effector memory T cells in autoimmune diseases. We have previously reported the natural product khellinone, **1a**, as a versatile lead molecule and identified two new classes of Kv1.3 blockers: (i) chalcone derivatives of khellinone, and (ii) khellinone dimers linked through the 6-position. Here we describe the multiple parallel synthesis of a new class of khellinone derivatives selectively alkylated at either the 4- or 7-position via the phenolic OH and show that several chloro, bromo, methoxy, and nitro substituted benzyl derivatives inhibit Kv1.3 with submicromolar potencies. Representative examples of the most potent compounds from each subclass, **11m** (5-acetyl-4-(4′-chloro)benzyloxy-6-hydroxy-7-methoxybenzofuran) and **14m** (5-acetyl-7-(4′-bromo)benzyloxy-6-hydroxy-4-methoxybenzofuran), block Kv1.3 with EC_{50} values of 480 and 400 nM, respectively. Both compounds exhibit moderate selectivity over other Kv1-family channels and HERG, are not cytotoxic, and suppress human T cell proliferation at low micromolar concentrations.

Introduction

Human T cells express two types of potassium channels, the voltage-gated potassium channel Kv1.3 and the calcium-activated potassium channel KCa3.1 (also known as IKCa1, SK4), both of which are critically involved in membrane potential regulation and calcium signaling during T cell activation.[1−4] Engagement of the T-cell receptor by an antigen-presenting cell during an immune response initiates a calcium flux into the T cell that raises the cytosolic calcium concentration into the micromolar range and that ultimately results in cytokine secretion and T-cell proliferation.[3,5] This crucial calcium influx is only possible if the T cell can keep its membrane potential negative through a counterbalancing potassium efflux through Kv1.3 and/or KCa3.1.[1−4] Blockade of these two potassium channels depolarizes the T cell membrane and thus inhibits calcium signaling and consequently prevents T-cell activation.[1,4−6]

While it was initially supposed that lymphocyte potassium channel blockade would lead to a general immunosuppression similar to the calcineurin inhibitor cyclosporin, it has recently become apparent that Kv1.3 and KCa3.1 are differentially expressed in different T and B cell subsets and that Kv1.3 blockers preferentially affect terminally differentiated effector memory T (T_{EM}) cells.[7,8] Kv1.3 blockade has therefore been proposed as a new therapeutic approach for the treatment of autoimmune diseases such as multiple sclerosis (MS), type-1 diabetes, rheumatoid arthritis, psoriasis, and chronic-graft-versus-host diseases where T_{EM} cells are involved in the pathogenesis.[4,9] The best evidence that a Kv1.3-based approach will be effective currently exists for the case of MS: the Kv1.3-blocking peptides kaliotoxin, ShK, and its more selective derivative ShK(L5) prevent and treat experimental autoimmune encephalomyelitis in rats, an animal model of MS.[10−12] Furthermore, recent studies with autoreactive T cells from MS patients showed that these cells expressed much higher levels of Kv1.3 than T cells from controls, and that their proliferation was inhibited by selective Kv1.3 blockade with ShK toxin under conditions that did not affect normal peripheral blood T cells. A subsequent study on postmortem brain sections from MS patients identified $Kv1.3^{high}$ T cells in active MS plaques,[13] demonstrating that $Kv1.3^{high}$ T_{EM} cells are indeed actively involved in the disease process of MS.

While peptide toxins, most notably the toxin ShK, derived from the anemone *Stichodactyla helianthus*, and its various analogues, were valuable tools for elucidating the structure and function of Kv1.3,[14] they have the disadvantage of requiring parenteral administration when used as drugs. A number of groups in academia and industry[4,15] have therefore actively searched for small molecule Kv1.3 blockers since Kv1.3 was cloned in 1993[16] and since cell lines stably expressing the channel have become available. Peptidomimetics of the active residues of ShK exhibited moderate levels of Kv1.3 blockade (EC_{50} 75−95 μM in whole-cell patch-clamp) and are noteworthy as examples of the few successful de novo designed mimetics.[17,18] A more potent small molecule Kv1.3 blocker is the natural product correolide (Figure 1A), which inhibited Rb^+ efflux through the Kv1.3 channel with an EC_{50} of 86 nM[19] and blocked the channel with an EC_{50} of 110 nM in patch-clamp experiments.[20] In proof of correolide's immunosuppressive potential, scientists at Merck showed that it inhibited T-cell proliferation and attenuated delayed-type hypersensitivity in miniswine.[21] However, development of correolide as a therapeutic was hampered by its lack of selectivity over other Kv1-family channels[22] and its molecular complexity that precluded a cost-effective total synthesis.[15,23] Other more recent examples of small molecule Kv1.3 blockers include the cyclohexyl-substituted benzamides[23] (Figure 1A), which were identified in a high-throughput screen and which exhibited EC_{50} values as low as 50 nM, and the 5-phenylalkoxypsoralen Psora-4 (EC_{50} 3 nM, Figure 1A), which was developed out of the natural product 5-methoxypsoralen and which currently constitutes the most potent small molecule Kv1.3 blocker.[24]

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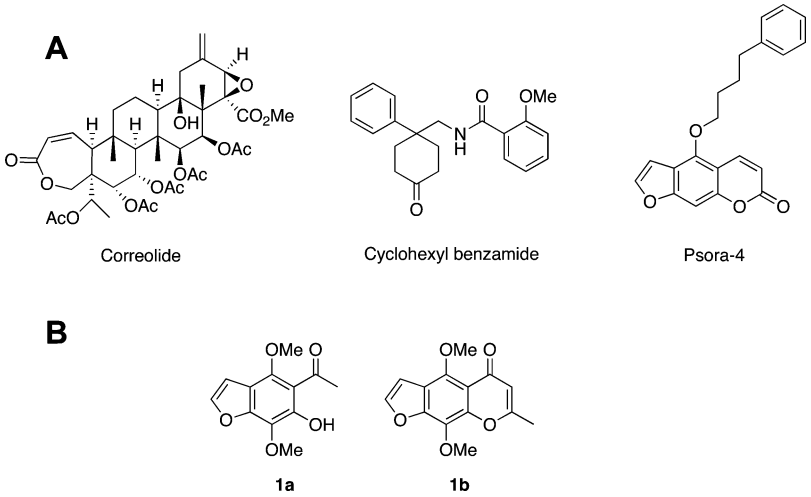


Figure 1. Figure 1. (A) Structures of correolide, a representative cyclohexyl benzamide and the 5-phenylalokoxypsoralen Psora-4. (B) Structures of khellinone **1a** and khellin **1b**. The 7-position in khellinone corresponds to the 9-position in khellin as indicated in Table 1.

In a previous report, we identified the natural product khellinone **1a** (Figure 1B) as a versatile lead molecule for the development of selective Kv1.3 blockers.[25] While khellinone itself inhibited Kv1.3 with an EC_{50} of 45 μM, its facile conversion to chalcones or to 6-linked dimers afforded respective jumps in activity to 0.3 and 0.7 μM. The khellinone chalcones in particular were seen as an interesting class because they are the only small molecule Kv1.3 blockers to show selectivity over both the Kv1.2 and Kv1.5 channels. Khellinone offers more room for development beyond the existing series. Here we describe further efforts to derivatize this versatile starting material, this time with a view to developing structure−activity relationships around the 4- and 7-positions.

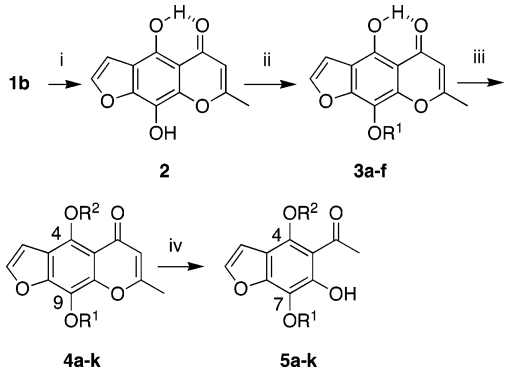
Chemistry

We report here a versatile approach to the preparation of 4- and/or 7-alkoxy analogues of khellinone. Limited derivatization at these positions in khellinone and the related khellin has been previously reported in the literature. An early study from 1952 described the formation of 4-alkoxy khellins by the selective demethylation of khellin with acid and subsequent alkylation.[26] A restricted number of compounds with C1−C4 alkoxy substituents in the 4-position in combination with any alkoxy substitution at the 7-position were afforded in a six-step synthesis of khellinone derivatives from pyrogallol.[27] Other workers bis-demethylated khellin to dinorkhellin either in a single step using magnesium iodide in ether,[28] or in two steps through the khellin quinone,[29,30] followed by alkylation to give 4- or 9-alkoxy-substituted khellins.[3] The methodologies to obtain dinorkhellin were either low yielding or unnecessarily time-consuming. We improved the synthesis of dinorkhellin and developed a route to selectively alkylate this diphenol to develop a general and reliable approach for the introduction of diversity at the 4- and 7-positions of khellinone. Once the route was in hand we tailored the methodology for multiple parallel synthesis (MPS) application in order to synthesize a small library of asymmetrically substituted khellinone derivatives.

As a first step khellin (**1b**) was treated with boron tribromide to afford the diphenol (see Table 1). Selective alkylation of **2** with 1 equiv of alkylating agent in the presence of potassium carbonate gave the 9-alkyl-4-hydroxykhellin analogues **3a**−**f**. Selectivity at the 9-position was obtained because the pK_a of the free phenol is lower than the pK_a of the intramolecularly hydrogen-bonded 4-hydroxyl group.[29] Cesium carbonate in dimethylformamide provided sufficiently strong conditions for alkylation at the 4-position. Finally, the khellin analogues **4a**−**k** were subjected to alkaline hydrolysis to afford the khellinone analogues **5a**−**k**. The order of alkylation was verified using NOE NMR experiments on **5f**, showing through-space proximity between the 4-ethoxy group and the acetyl group (see Supporting Information).

The synthesis of the 4-alkoxy and 7-alkoxy khellinones was adapted to MPS by performing the reactions on a 24-block MPS reactor and simplifying the workup of the alkylation reactions to a protocol of acidification, filtration, and desiccation. By this

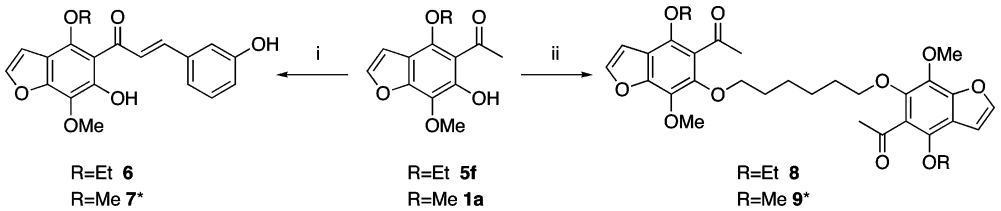
Table 1. Synthesis of Monoalkylated Khellinone Analogues and Their Blockade of Kv1.3[a]



R^1 (7)	R^2 (4)	compound[c]	EC_{50} (μM)
Me	Me	**1a**	45
Et	Me	**5a**	10
Pr	Me	**5b**	8
Pn	Me	**5c**	3
Bn	Me	**5d**	1.2
2-Npm*	Me	**5e**	5
Me	Et	**5f**	7
Me	Pr	**5g**	5
Me	Pn	**5h**	4
Me	Bn	**5i**	2.5
Me	1-Npm[b]	**5j**	8
Me	2-Npm[b]	**5k**	5

[a] Reagents: (i) BBr_3, DCM, −78 °C; (ii) Me_2SO_4 or RX, K_2CO_3, DMF; (iii) RX, Cs_2CO_3, DMF; (iv) NaOH, H_2O, EtOH. [b] Npm = naphthylmethyl. [c] Each compound was tested 2−3 times at three concentrations. Values for the EC_{50} were determined by fitting the Hill equation to the reduction of area under the K^+ current curve. Standard deviations were between 5 and 15%.

Scheme 1. Synthesis of the Chalcone **6** and the Dimer **8** of the 4-Ethyl Analogue **5f**[a]



[a] Reagents: (i) 3-hydroxybenzaldehyde, NaOH, H_2O; (ii) 1,6-dibromohexane, K_2CO_3, DMF. *The synthesis of **7** and **9** is described elsewhere.[25]

method we generated a library of compounds and investigated the structure activity relationships around the phenyl ring on the 4- or 7-positions (Table 1) by whole-cell patch-clamp.

The khellinone analogue **5f** was subjected to further modifications. From it chalcone **6** was readily prepared in 67% yield by Claisen−Schmidt condensation and the dimer **8** in 90% yield by alkylation of the phenolic OH with a bis-haloalkane (Scheme 1). The six-carbon alkyl linker was chosen for the dimer because it was shown that this length was optimal for activity against Kv1.3 in the previously reported khellinone dimer series.[25,31]

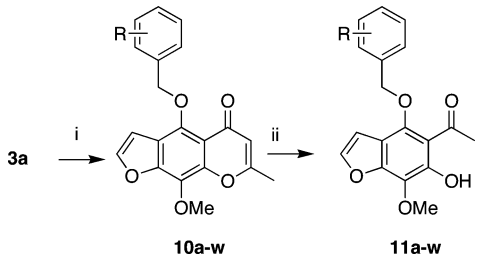
Results and Discussion

SAR of 4- and 7-Substituted Khellinones on Kv1.3. We screened all newly synthesized compounds by manual whole-cell patch-clamp on L929 cells stably expressing Kv1.3. We chose this technique instead of a faster Rb^+-flux or binding assay[32] despite its low throughput and its extreme labor-intensity, because whole-cell patch-clamp is considered the "gold-standard" for testing ion channel modulators[33] and allows to study channel inhibition under physiological conditions. Rb^+ flux assays normally work with high concentrations of extracellular potassium. This prevents the inactivation of Kv1.3[34] and leads to an underestimation of the potency of compounds that exhibit a use- and/or state-dependent block. Binding assays have the disadvantage that they only detect whether a compound binds to the Kv1.3 protein and do not provide information on channel function.

Table 1 lists the effects that varying the 7-substituent has on Kv1.3 blocking activity (**5a**−**e**). The potency improved markedly with any alkyl group larger than methyl, giving rise to around a 10−20-fold increase depending on the length of the alkyl chain. The benzyl ether **5d** and the pentyl ether **5c** were the most effective blockers of Kv1.3 in this series with EC_{50} values of 1.2 μM and 3 μM, respectively. Interestingly, the activity trend observed in the analogues where the 4-substituent has been changed (**5f**−**k**) mirrored the trend for the 7-substituted compounds (**5a**−**e**).

Since the benzyl-substituted compounds **5d** and **5i** proved to be the most potent compounds of the 4- and 7-substituted khellinone series, and because many benzyl halides are commercially available, we selected **5d** and **5i** as templates to test the effect that varying the physicochemical properties of substituents has on channel blocking activity. As shown in Table 2, a variety of ortho and meta substituents did not produce any significant changes in potency relative to the unsubstituted benzyl ether **5i** with the exception of the 3-methoxy- and the 3-trifluoromethoxy-substituted **11k** and **11j**, which were approximately 4-fold more potent. A clear outlier here is the 3-carboxy **11h**, which is not measurably active. Since the carboxy group is negatively charged at physiological pH, inactivity could be either due to poor membrane permeability resulting in little or no compound reaching the binding site, or intolerance of such a polar group in the binding site, or a combination of these two effects.

Table 2. Synthesis and Channel Blocking Activity Kv1.3 of Substituted 4-benzyl Ethers[a]



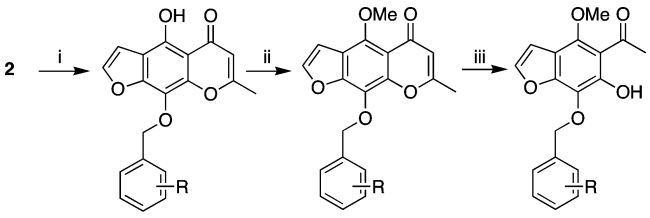
compd[b]	R	EC_{50} (μM)	compd[b]	R	EC_{50} (μM)
11a	2-F	1.7	**11m**	4-Cl	0.48
11b	2-Cl	1.2	**11n**	4-Br	0.50
11c	2-Me	2.0	**11o**	4-CF_3	0.51
11d	2-Ph	2.0	**11p**	4-^{i}Pr	0.70
11e	3-F	1.2	**11q**	4-^{t}Bu	1.5
11f	3-Cl	1.4	**11r**	4-Ph	0.90
11g	3-Me	1.0	**11s**	4-OBn	8.8
11h	3-COOH	>10	**11t**	4-COPh	0.50
11i	3-NO_2	1.4	**11u**	4-COOH	no effect[c]
11j	3-OCF_3	0.55	**11v**	4-OCF_3	1.8
11k	3-OMe	0.68	**11w**	4-OMe	1.5
11*l*	4-F	1.0			

[a] Reagents: (i) RX, Cs_2CO_3, DMF; (ii) NaOH, H_2O, EtOH. Compounds **11a** to **11w** were prepared as described in General Procedure E (Supporting Information). [b] Each compound was tested two times at 4−5 concentrations. Values for the EC_{50} were determined by fitting the Hill equation to the reduction of area under the K^+ current curve. Standard deviations were between 5 and 15%. The Hill coefficient was 1.2 for all compounds. [c] Tested at 10 μM.

The para position could also accommodate a variety of different substituents and yielded several compounds that were 2−5 times more potent than the unsubstituted benzyl ether **5i**, the most potent of which included **11m** with a 4-chloro substituent, **11n** with a 4-bromo substituent, and **11o** with a 4-trifluoromethyl. Each of these compounds blocked Kv1.3 with EC_{50} values of around 0.5 μM. Bulky groups in **11q** (4-^{t}Bu), **11r** (4-Ph), and **11t** (4-COPh) could all be tolerated but the longest and most bulky group of 4-OBn in **11s** gave rise to a noticeable drop in activity (EC_{50} 8.8 μM), suggesting that there is a limit to the size of the para substituent that can be accommodated. The inactivity of the *p*-carboxy compound **11u** could arise for the same reasons as those for the inactive *m*-carboxy compound **11h** discussed above.

Table 3 lists the effects that varying the substitution on the 7-benzyl ether has on Kv1.3-blocking activity. A number of different substituents could be tolerated at the ortho, meta, and para positions and the para position could also accommodate bulky groups in **14q** and **14s** but less so the bulky 4-OBn group of **14r**. The most active compounds in this series bore the same substitution as the most active analogues from the 7-benzyl ether series, including the *p*-chloro derivative **14l,** the *para*-bromo derivative **14m**, and the *p*-trifluoromethyl **14n**, each with an EC_{50} close to 0.4 μM.

Table 3. Synthesis and Channel Blocking Activity Kv1.3 of Substituted 7-Benzyl Ethers[a]



12a-u **13a-u** **14a-u**

compd[b]	R	EC_{50} (μM)	compd[b]	R	EC_{50} (μM)
14a	2-F	2.5	**14l**	4-Cl	0.42
14b	2-Cl	0.7	**14m**	4-Br	0.40
14c	2-Me	1.9	**14n**	4-CF_3	0.42
14d	2-Ph	1.1	**14o**	4-^{i}Pr	1.0
14e	3-F	0.75	**14p**	4-^{t}Bu	5.8
14f	3-Cl	0.60	**14q**	4-Ph	1.2
14g	3-Me	1.2	**14r**	4-OBn	6.3
14h	3-COOH	no effect[c]	**14s**	4-COPh	0.50
14i	3-NO_2	0.60	**14t**	4-COOH	no effect[c]
14j	3-OCF_3	0.45	**14u**	4-OCF_3	0.60
14k	4-F	0.70			

[a] Reagents: (i) RX, K_2CO_3, DMF; (ii) MeI, Cs_2CO_3, DMF; (iii) NaOH, H_2O, EtOH. Compounds **14a** to **14u** were prepared as described in General Procedure F (Supporting Information). [b] Each compound was tested two times at 4−5 concentrations. Values for the EC_{50} were determined by fitting the Hill equation to the reduction of area under the K^+ current curve. Standard deviations were between 5 and 15%. The Hill coefficient was 1.8 for all compounds. [c] Tested at 10 μM.

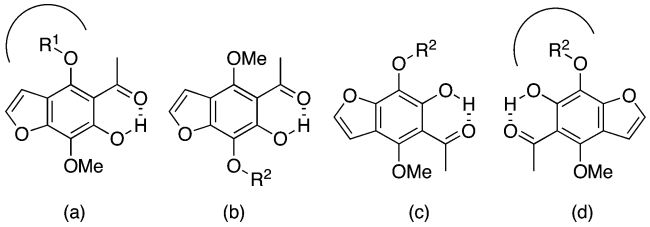
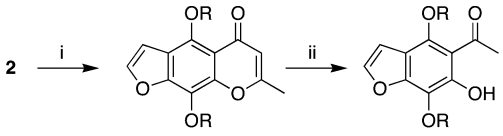


Figure 2. Pseudo-symmetry in the khellinone analogues.

Generally, we found that the SAR of the 7-benzyl ethers paralleled the SAR for the 4-substituents, not just for the ortho-, meta- and para-substituted benzyl compounds, but also for the alkyl and naphthylmethyl compounds. To explain this phenomenon, we considered two possibilities relating to the binding mode of these compounds. First, there may be a unique binding pocket that accommodates the benzyl ether or alkyl ether group (Figure 2 (a)), and the pseudo-symmetry of the 5-acetyl-6-hydroxybenzofuran scaffold allows the 4-substituted compounds to occupy this pocket similarly to 7-substituted compounds in Figure 2d after flipping (Figure c) and inverting (Figure 2b) the scaffold. The pseudo-symmetry of the compounds arises because the furan and hydrogen-bonded salicyl groups can be considered as bioisosteric. If the compounds cannot flip/invert in the channel to exploit their pseudo-symmetry, an alternate explanation has the scaffold locked in a single orientation, with pseudosymmetry in the tetrameric Kv1.3 channel protein offering two similar binding sites in the channel pore corresponding to both the 4- and 7-positions. Compounds **16a** and **16b** were prepared to test these theories. The potency of blockade of these analogues (Table 1, **16a**, EC_{50} = 6 μM and **16b**, EC_{50} = 1.2 μM) is comparable to their unsymmetrically alkylated counterparts (**5a**, EC_{50} = 10 μM, **5f**, EC_{50} = 7 μM and **5d**, EC_{50} = 1.2 μM, **5i** EC_{50} = 2.5 μM), suggesting that the latter hypothesis is probably false because an additional substituent, able to access an appropriate binding site, would be expected to increase potency. However the lack of change in potency does not negate the flip/invert theory.

Table 4. Dialkylated Khellinone Analogues[a]



15a R = Et **16a** R = Et

15b R = Bn **16b** R = Bn

compound	EC_{50} (μM)
16a	6.0[a]
16b	1.2[b]

[a] Hill coefficient of 2. [b] Hill coefficient of 1.

Table 5. Kv1.3 Blockade by Chalcones and Dimers

compound	EC_{50} (μM)
6	2.5
7[a]	0.7
8	0.8
9[a]	0.7

[a] Compounds **7** and **9** were reported previously.[25]

Our previous report showed that chalcone derivatives of khellinone gave marked increases in activity relative to khellinone.[25] The 3′-hydroxychalcone **7** (Scheme 1), for example, blocked Kv1.3 with an EC_{50} of 0.7 μM, corresponding to a 60−70-fold increase over khellinone (EC_{50} of 45 μM). The chalcone **6** was therefore synthesized from **5f**, which was already more than 6-fold more potent than khellinone with an EC_{50} of 7 μM, and tested against Kv1.3 to determine whether the sharp gains in activity conferred by chalcones of khellinone would map onto the alkoxykhellinone analogues. However, with an EC_{50} of 2.5 μM the chalcone **6** only showed a 3-fold increase in activity over its parent **5f** (Table 5). Similarly, we have previously reported that khellinone dimers such as **9** are more than 50-fold more potent than khellinone itself in blocking Kv1.3. However, dimerization of the more potent khellinone analogue **5f** resulted in dimer **8** (Table 5) that exhibited a more modest 10-fold increase in potency relative to parent molecule **5f**. These results together suggest that these separated activity-enhancing modifications to khellinone, that is, increasing the size of the 4-alkyl ether combined with chalcone formation or dimerization, are not necessarily additive when combined in one molecule.

In summary, khellinone analogues with any ether larger than methyl in the 4- and 7- positions showed markedly increased activity over khellinone. From a library of monosubstituted benzyl ethers at the 4- or 7-positions, optimal potency of between 0.4 and 0.5 μM was observed for a number of examples. One compound from each subclass, namely the *p*-chlorobenzyl ether in the 4-position (**11m**) and *p*-bromobenzyl ether in the 7-position (**14m**), was selected for further testing on their mechanism of block, selectivity, and antiproliferative function.

Nature of Block. While both the 4- and the 7-series compounds blocked Kv1.3 with comparable potencies and SAR, they markedly differed in their mechanism of block. Figure 3 shows exemplary experiments performed with **11m** and **14m**. We first applied a 500-ms depolarizing pulse to +40 mV to elicit a control current from cells stably transfected with Kv1.3 and then clamped the membrane back to −80 mV. After 2 min, a time interval which is sufficient to allow all Kv1.3 channels to recover from their characteristic C-type inactivation,[35,36] we perfused blocking concentrations of the sea anemone toxin ShK,

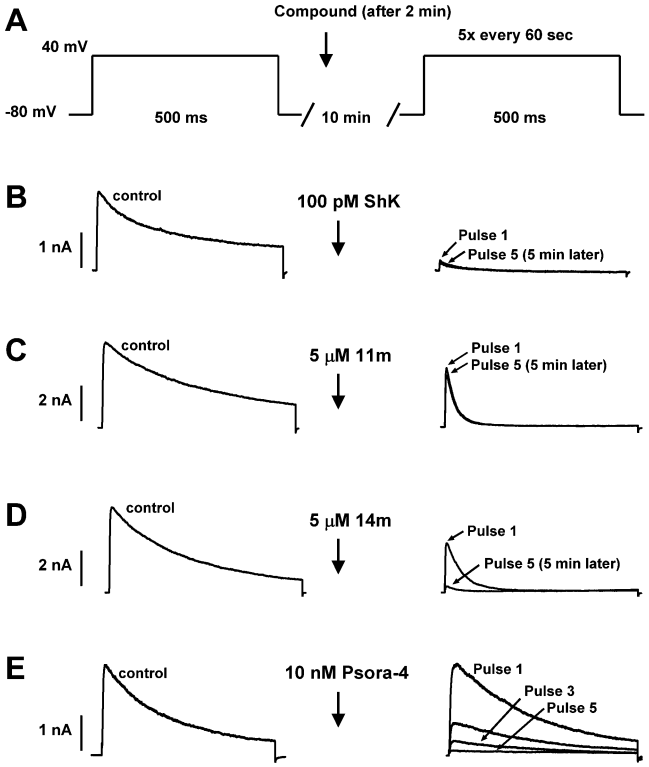


Figure 3. (A) Pulse protocol for the whole-cell patch-clamp experiments on Kv1.3 shown in B, C, D, and E. After a control current had been elicited the membrane was held at −80 mV for 2 min before blockers were perfused and for another 8 min before the next depolarizing pulse. Afterward pulses were applied every 60 s until no further increase in effect was observed. (B) Effect of 100 pM ShK. (C) Effect of 5 μM **11m**. (D) Effect of 5 μM **14m**. (E) Effect of 10 nM Psora-4.

11m, **14m**, or the alkoxypsoralen Psora-4 onto the closed channel ensemble and then gave the compounds another 8 min to diffuse to their respective binding sites (see pulse protocol in Figure 3A). When we then opened the channels with another depolarizing pulse, we observed very different kinetics of block for the four blockers. ShK immediately showed full block and had no effect on the inactivation kinetics because it can bind to the channel in both the closed and the open state (Figure 3B). Compound **11m** in contrast closely resembled the open channel blocker verapamil[37,38] in its kinetics of block and in its accelerating effect on Kv1.3 inactivation (Figure 3C). Like the peptide ShK, **11m** exhibited its full blocking potency on the first pulse and its effect did not increase with subsequent depolarizing pulses. Similar results were obtained with other 4-series compounds such as **11b**, **11f**, **11o**, and **11r** (data not shown). Surprisingly, the 7-series compounds as exemplified by **14m** (Figure 3D) differed from 4-series compounds and displayed blocking kinetics that are "a mixture" between the open channel block of 4-series and the blockade of the C-type inactivated state by Psora-4[24] (Figure 3E). Similar to **11m**, **14m** greatly accelerated the inactivation of Kv1.3 but in contrast to **11m**, its blocking effect increased considerably with subsequent depolarizing pulses until it reached a steady-state block after five pulses. Other 7-series compounds such as **14b**, **14f**, and **14s** displayed the same blocking kinetics (data not shown).

For the 4-series compounds, the observed Hill coefficient of 1.2 (Table 2) indicates a 1:1 interaction between blocker and channel molecule and is consistent with a single binding-site in the open state of the channel. In contrast, the 7-series compounds exhibit a Hill coefficient of 1.8 (Table 3), suggesting that two blocker molecules bind to one channel molecule in a cooperative manner. This observation is in accordance with two binding sites for the 7-series on Kv1.3, one in the open state and an additional site in the C-type inactivated state. These two different stoichiometries of interaction are not necessarily inconsistent with our flip/invert model. We hypothesize that the first binding site in the open state of the channel binds both the 4- and the 7-series compounds but that the second site in the C-type inactivated state recognizes khellinones only in the inverted orientation as presented by 7-benzyl ether khellinones and that therefore only the 7-series compounds can occupy this second site. However, without mapping their binding sites on the Kv1.3 channel protein we are currently not able to more definitively interpret these different mechanisms of block by compounds from the 4- and 7-series and it is certainly possible to explain our results differently.

Table 6. Selectivity of **7**, **11m**, **14m**, and **16b** over Other K^+ Channels[a]

channel	**7**[b] EC_{50} (μM)	**11m** EC_{50} (μM)	**14m** EC_{50} (μM)	**16b** EC_{50} (μM)
Kv1.1	1.7	1.7	1.2	7.0
Kv1.2	>50	2.5	1.4	10
Kv1.3	0.7	0.5	0.4	1.2
Kv1.4	nd	4.8	4.3	>20
Kv1.5	12	0.7	0.9	5.0
HERG	nd	5.0	9.0	nd

[a] EC_{50} values for blockade of other K^+ channels. Compounds were tested 2−3 times at 3−5 different concentrations (standard deviations 2−15%). nd: not determined. [b] Compound **7** was reported previously.[25]

Selectivity. As representative examples of the most active compounds from each subclass **11m** and **14m** were tested for selectivity over other Kv1-family channels and over the cardiac K^+ channel HERG (Kv11.1). As shown in Table 6, both **11m** and **14m** exhibited a very similar selectivity profile: 3-fold selectivity over Kv1.1, 5-fold over Kv1.2, 10-fold over Kv1.4, 2-fold over Kv1.5, and 10 to 20-fold over HERG. Interestingly, this selectivity profile is very different from the previously described chalcone derivatives of khellinone, such as **7** (Scheme 1),[25] which exhibited close to 20-fold selectivity over the cardiac potassium channels Kv1.5 and had no effect on the neuronal potassium channel Kv1.2 (Table 6). The symmetrically alkylated **16b** exhibited a similar selectivity profile to that of **11m** and **14m** with poor selectivity over Kv1.2 and Kv1.5 but was slightly more selective over Kv1.1 (6-fold) and Kv1.4 (>20-fold). In their lack of selectivity over Kv1.5 the 4- and 7-substituted khellinones **11m** and **14m** and the symmetric **16b** thus more closely resemble the alkoxypsoralen Psora-4[24] and the nortriterpene correolide[22] than the structurally related khellinone chalcones.[25] A reason for these differences in selectivity could be that binding of the phenyl ring in the 5-position-derivatized khellinone chalcones orients the furosalicyl scaffold differently in the channel pores relative to the 4- or 7-substituted analogues **11m** and **14m**.

Inhibition of Proliferation. To determine if **11m** and **14m** indeed exhibit immunosuppressive activity as expected from their submicromolar inhibition of Kv1.3, we tested their effect on the anti-CD3 antibody stimulated proliferation of human peripheral blood T cells. As shown in Figure 4, both compounds suppressed T cell proliferation with an EC_{50} of 2.5 μM. To ensure that this suppression of proliferation was not caused by unspecific cytotoxicity, we tested both compounds against Jurkat E6-1 and MEL cells and found that at concentrations ranging from 250 nM to 20 μM neither compound significantly reduced cell viability as measured by trypan blue exclusion after 48 h.

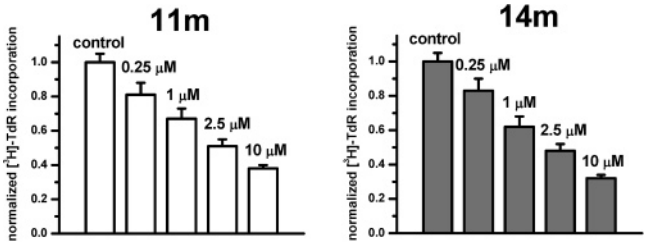


Figure 4. Inhibition of T-cell proliferation. Effect of increasing concentrations of **11m** and **14m** on the anti-CD3 antibody stimulated [^{3}H]-thymidine incorporation of human T cells. Control: 41 500 ± 1500 counts.

Conclusion

We reported here a new class of khellinone-based blockers of the voltage-gated potassium channel Kv1.3. These were obtained by replacing either the 4-methoxy or the 7-methoxy groups in khellinone with a substituted benzyloxy group via selective synthetic manipulation starting from khellin. The SAR around the benzyl ring at the 4-position of khellinone paralleled the SAR around the benzyl ring at the 7-position of khellinone. However, taken together this SAR was relatively shallow, demonstrating that the binding site for this class of compounds on Kv1.3 must be relatively large and can accommodate compounds of varying size. One of the more potent 4-benzyloxy derivatives, **11m**, contained a *p*-chloro substituent in the benzyl ring and had an EC_{50} of 0.48 μM, while an representative potent compound from the 7-benzyloxy series, **14m**, with a *p*-bromo substituent in the benzyl ring and had an EC_{50} of 0.40 μM. Intriguingly, the two compound series were found to possess quite different mechanisms of blockade. Compounds from the 4-series accelerated inactivation, displayed full blocking potency on the first depolarizing pulse and a Hill coefficient close to 2. In contrast, 7-series compounds**,** while also accelerating inactivation, showed a use-dependent blockade and a Hill coefficient close to unity. Both **11m** and **14m,** which were tested as representatives of the 4- and 7-series, inhibited the proliferation of human T cells with an EC_{50} of 2.5 μM and were not appreciably cytotoxic at concentrations of up to 20 μM. Both compounds were slightly to moderately selective for Kv1.3 over a panel of other ion channels tested, with the exception of Kv1.5 where selectivity was marginal. Both compounds were 10−20-fold selective for Kv1.3 over the cardiac potassium channel HERG.

Experimental Section

Melting points were determined in open capillary tubes using an Electrothermal melting point apparatus (Model 9200) and are uncorrected. Multiple parallel synthesis was performed on a Mettler Toledo Autochem MiniBlock XT with a 6 × 4 reaction grid. Mass spectra (MS) were recorded on a Finnigan LQC Advantage MAX mass spectrometer. High-resolution mass spectra (HRMS) were recorded on a Kratos MS80 RFA mass spectrometer at the University of Canterbury, New Zealand. Elemental analyses were performed for carbon, hydrogen, and chlorine at the University of Otago, New Zealand. Proton nuclear magnetic resonance (^{1}H NMR) were performed on a Bruker Avance DRX 300 (300 MHz) with the solvents indicated, and carbon nuclear magnetic resonance (^{13}C NMR) were performed on a Bruker Avance DRX 300 (75 MHz) with the solvents indicated. Chemical shifts were reported in parts per million (ppm) on the δ scale and were referenced to the appropriate solvent peaks: $CDCl_3$ referenced to C*H*Cl_3 at δ_H 7.24 ppm and *C*DCl_3 at δ_C 77.0 ppm; acetone-d_6 referenced to $(CD_3)(CHCD_2)CO$ at δ_H 2.17 ppm and $(CD_3)_2CO$ at δ_C 29.2 ppm. Analytical thin-layer chromatography (TLC) was performed on Merck silica gel 60F_{254} aluminum-backed plates. Flash chromatography was performed on Merck silica 60 following the guidelines given by Still et al.[39] using ACS analytical grade solvents. All moisture sensitive experiments were performed in oven-dried glassware under an atmosphere of nitrogen. Anhydrous dimethylformide was purchased from Aldrich. Petroleum ether describes a mixture of hexanes in the bp range 30−50 °C.

General Procedure A. A suspension of **3f** (1.0 equiv, 0.2 M), cesium carbonate (2.0 equiv), and alkyl or benzyl halide (generally 1.2 equiv) in anhydrous dimethylformamide was stirred under nitrogen at room temperature until completion as determined by TLC (typically 16 h). The reaction mixture was then diluted with ethyl acetate and washed twice with 2 M HCl and brine, dried over $MgSO_4$, and concentrated in vacuo. The crude product was purified by flash chromatography.

General Procedure B. A suspension of **2** (1.0 equiv, 0.25 M), potassium carbonate (2.0 equiv) and alkyl or benzyl halide (1.1 equiv) in anhydrous dimethylformamide was stirred under nitrogen at 60 °C until completion as determined by TLC (typically 2−4 h). The reaction mixture was then diluted with ethyl acetate and washed twice with 2 M HCl and brine, dried over $MgSO_4$, and concentrated in vacuo. The crude product was purified by flash chromatography.

General Procedure C. A suspension of the phenol (1.0 equiv, 0.15 M), cesium carbonate (5.0 equiv) and iodomethane (5.0 equiv) in anhydrous dimethylformamide was stirred under nitrogen at room temperature for 16 h. The reaction mixture was partitioned over 2 M HCl and ethyl acetate and the organic phase was extracted twice with 2 M HCl then washed with brine. After drying over $MgSO_4$ and evaporation, the residue was either purified by flash chromatography or used directly in the following step.

General Procedure D. To a solution of the khellin analogue (0.01−0.1 M) in ethanol (2 parts) at reflux was added slowly 3 M NaOH (1 part), and the resulting solution was stirred at 70 °C for 3 h. The reaction mixture was concentrated in vacuo to one-third of the original volume and then acidified to pH 2 by Universal indicator with 2 M HCl. If precipitation occurred, the solid was collected by filtration washing with water. The solid was then dried in the presence of P_2O_5. If precipitation did not occur, the acidified reaction mixture was extracted twice with ethyl acetate and the pooled organic layers were washed with brine, dried over $MgSO_4$, and concentrated in vacuo.

4,9-Dihydroxy-7-methyl-furo[3,2-*g*]chromen-5-one (2). To a suspension of **1** (10.4 g, 40 mmol) in dichloromethane (100 mL) at −78 °C boron tribromide (1 M in dichloromethane, 80 mL, 80 mmol) was added over 75 min. The reaction mixture was allowed to warm to room temperature and then stirred for 16 h. On cooling the reaction mixture to 0 °C, the reaction was quenched with water by dropwise addition at first, but at an increasing rate until 50 mL had been added. The reaction mixture was concentrated in vacuo to remove the dichloromethane, and the resulting suspension was filtered, washing with water. The orange solid was heated as a suspension in propan-2-ol (200 mL) at 70 °C for 20 min, during which time the solid became pale yellow. The suspension was filtered and the solid was dried to afford **2** (8.4 g, 90%) as a pale yellow solid: Mp 283−284 °C (^{i}PrOH) lit.[40] 285−287 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ: 2.42 (s, 3H), 6.19 (s, 1H), 7.05 (d, J = 2.1 Hz, 1H), 8.02 (d, J = 2.1 Hz, 1H), 9.84 (br s, 1H), 12.9 (s, 1H); ^{13}C NMR (DMSO-d_6) δ: 20.2, 104.4, 105.1, 107.0, 112.9, 123.3, 141.8, 145.7, 146.3, 148.2, 168.5, 183.9; MS (ES+) *m*/*z* 233.2 ($M + H^+$).

4-Hydroxy-9-methoxy-7-methyl-furo[3,2-*g*]chromen-5-one (3f). To a solution of **2** (2.32 g, 10 mmol) in acetonitrile (45 mL) were added K_2CO_3 (2.76 g, 20 mmol) and dimethyl sulfate (1.00 mL, 10.5 mmol), and the reaction mixture was stirred at 80 °C for 90 min. The reaction was quenched by addition of 10% aqueous citric acid solution (30 mL) followed by 15 min stirring at room temperature. The reaction mixture was then concentrated to half volume, and the resulting suspension was partitioned over chloroform (100 mL) and 10% citric acid solution (100 mL). The phases were separated, and the aqueous phase was extracted with chloroform (2 × 100 mL) to give **3f** (2.33 g, 95%) as a yellow solid: Mp 201−202 °C lit.[41] 204 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ: 2.46 (s, 3H), 4.04 (s, 3H), 6.30 (s, 1H), 7.13 (d, J = 2.1 Hz, 1H), 8.09 (d, J = 2.1 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ: 20.7, 61.8, 104.7, 105.6, 107.8, 113.8, 125.9, 114.9, 145.4, 149.9, 151.0, 167.4, 184.2; MS (ES^+) m/z 247.2 ($M + H^+$).

4-Ethoxy-9-methoxy-7-methyl-furo[3,2-*g*]chromen-5-one (4f). Compound **3f** (110 mg, 0.45 mmol) and ethyl iodide (0.35 mL, 4.5 mmol) were treated as described under General Procedure A to afford **4f** (110 mg, 90%) as a colorless solid: Mp 104−106 °C lit.[26] 109−110 °C (EtOH/H_2O); ^{1}H NMR ($CDCl_3$) δ: 1.43 (t, J = 10.5 Hz, 3H), 2.35 (s, 3H), 4.16 (m, 5H), 6.03 (s, 1H), 6.93 (d, J = 3.2 Hz), 7.58 (d, J = 3.2 Hz); ^{13}C NMR ($CDCl_3$) δ: 15.6, 20.0, 61.3, 71.2, 105.2, 110.4, 120.5, 129.9, 145.4, 146.0, 147.0, 148.5, 164.0, 178.1, 196.2; MS (ES^+) m/z 275.1 ($M + H^+$).

5-Acetyl-4-ethoxy-6-hydroxy-7-methoxybenzofuran (5f). Compound **4f** (49 mg, 0.20 mmol) was treated as described under General Procedure D to afford **5f** (32 mg, 75%) as a colorless solid: Mp 94−95 °C; lit.[42] 93−95 °C (EtOH/H_2O); ^{1}H NMR ($CDCl_3$) δ: 1.52 (t, J = 6.9 Hz, 3H), 2.72 (s, 3H), 4.05 (s, 3H), 4.40 (q, J = 6.9 Hz, 2H), 6.84 (d, J = 2.4 Hz, 1H), 7.49 (d, J = 2.4 Hz, 1H), 13.08 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 15.2, 33.0, 60.6, 69.2, 110.5, 110.7, 123.1, 128.4, 143.3, 150.4, 151.9, 153.1, 205.0; MS (ES^+) m/z 251.0 ($M + H^+$).

9-Benzyloxy-4-Hydroxy-7-methyl-furo[3,2-*g*]chromen-5-one (3d). Compound **2** (348 mg, 1.5 mmol) and benzyl bromide (214 μL, 1.8 mmol) were treated as described under General Procedure B to afford **3d** (370 mg, 77%) as a colorless solid: Mp 110−112 °C lit[43] 114 °C; ^{1}H NMR ($CDCl_3$) δ: 2.33 (s, 3H), 5.25 (s, 2H), 6.00 (s, 1H), 6.97 (d, J = 2.2 Hz, 1H), 7.32 (m, 3H), 7.45 (m, 2H), 7.59 (d, J = 2.2 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ: 20.5, 76.1, 104.7, 105.5, 107.6, 113.7, 124.3, 128.3, 128.4, 136.8, 144.9, 146.0, 150.2, 151.5, 167.3, 184.1; MS (ES^+) m/z 323.1 ($M + H^+$).

9-Benzyloxy-4-methoxy-7-methyl-furo[3,2-*g*]chromen-5-one (4d). Compound **3d** (300 mg, 0.93 mmol) was treated as described under General Procedure C to afford **4d** (290 mg, 93%) as a colorless solid: Mp 98−100 °C; ^{1}H NMR ($CDCl_3$) δ: 2.30 (s, 3H), 4.04 (s, 3H), 5.32 (s, 2H), 6.02 (s, 1H), 6.99 (d, J = 2.2 Hz, 1H), 7.31 (m, 3H), 7.47 (m, 2H), 7.60 (d, J = 2.2 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ: 19.6, 61.9, 75.5, 104.9, 110.2, 113.2, 118.7, 127.8, 128.0, 136.5, 145.2, 147.4, 147.6, 149.3, 163.6, 177.9; MS (ES^+) m/z 337.1 ($M + H^+$); Anal. ($C_{20}H_{16}O_5$): C, H.

5-Acetyl-7-benzyloxy-6-hydroxy-4-methoxybenzofuran (5d). Compound **4d** (200 mg, 0.59 mmol) was treated as described under General Procedure D, and the crude product was purified by flash chromatography eluting with ethyl acetate/petroleum ether (1:9) to afford **5d** (90 mg, 48%) as a yellow solid: Mp 78−80 °C; ^{1}H NMR ($CDCl_3$) δ: 2.68 (s, 3H), 4.09 (s, 3H), 5.23 (s, 2H), 6.84 (d, J = 2.3 Hz, 1H), 7.32 (m, 3H), 7.60 (d, J = 2.3 Hz, 1H), 7.54 (m, 2H); ^{13}C NMR ($CDCl_3$) δ: 32.9, 60.0, 74.5, 105.4, 110.1, 110.2, 127.1, 127.6, 127.9, 137.2, 143.4, 151.5, 152.5, 153.6, 204.9; MS (ES^+) m/z 312.9 ($M + H^+$); Anal. ($C_{18}H_{16}O_5$): C, H.

3-(4-ethoxy-6-hydroxy-7-methoxybenzofuran-5-yl)-1-(3-hydroxyphenyl)-3-oxopropene (6). Compound **5f** (15 mg, 0.060 mmol) and 3-hydroxybenzaldehyde (9.0 mg, 0.075 mmol) were suspended in 2 M NaOH (0.5 mL) and stirred at 80 °C for 3 h. The reaction mixture was diluted with 10% aqueous citric acid (10 mL) and then extracted with ethyl acetate (2 × 10 mL). The pooled organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo. The resulting residue was purified by flash chromatography eluting with ethyl acetate/hexane (1:4) to afford **6** (14 mg, 67%) as a deep red solid: Mp 153−155 °C; ^{1}H NMR ($CDCl_3$) δ: 1.39 (t, J = 6.9 Hz, 3H), 4.10 (s, 3H), 4.22 (q, J = 6.9 Hz, 2H), 6.83 (m, 1H), 6.90 (d, J = 7.8 Hz, 1H), 7.11 (s, 1H), 7.26 (m, 2H), 7.51 (m, 1H), 7.76 (d, J = 15.6 Hz, 1H), 7.93 (d, J = 15.6 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ: 15.3, 60.6, 71.0, 105.0, 112.1, 113.3, 114.5, 117.3, 121.0, 127.3, 129.2, 129.8, 136.4, 142.4, 143.8, 149.5, 151.4, 152.7, 155.7; MS (ES^+) m/z 355.2 ($M + H^+$); HRMS (EI) calcd $C_{20}H_{18}O_6$ (M^+) 354.1103, found 354.1106.

1,6-Bis(5-acetyl-4-ethoxy-7-methoxybenzofuran-6-yloxy)hexane (8). A suspension of **5f** (20 mg, 0.080 mmol), 1,6-dibromohexane (10 mg, 0.042 mmol), and potassium carbonate (11 mg, 0.080 mmol) in dimethylformamide (0.5 mL) was stirred at 70 °C for 6 h, and the reaction mixture was diluted with 10% aqueous citric acid (20 mL) and extracted with ethyl acetate (3 × 10 mL). The pooled organic layers were dried over $MgSO_4$ and concentrated in vacuo to give a residue that was purified by flash chromatography eluting with ethyl acetate/hexane (gradient from 1:9 to 1:4) to afford **7** (21 mg, 90%) as a colorless solid: Mp 134−135 °C; ^{1}H NMR ($CDCl_3$) δ: 1.49 (m, 4H), 1.76 (m, 4H), 2.53 (s, 3H), 4.06 (m, 10H), 4.20 (q, J = 7.2 Hz, 4H), 6.81 (d, J = 2.1 Hz, 2H), 7.56 (d, J = 2.1 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ: 15.6, 25.7, 29.7, 30.1, 32.8, 61.1, 69.9, 75.2, 105.1, 117.1, 125.3, 134.2, 143.1, 144.5, 144.9, 148.6; MS (ES^+) m/z 583.3 ($M + H^+$); Anal. ($C_{32}H_{38}O_{10}$): C, H.

5-Acetyl-4-(4′-chloro)benzyloxy-6-hydroxy-7-methoxybenzofuran (11m). Compound **3f** (27 mg, 0.11 mmol) was treated as described under General Procedure E (Supporting Information) to afford **11m** (20 mg, 44% over two steps) as a yellow solid: Mp 106−108 °C; ^{1}H NMR ($CDCl_3$) δ: 2.61 (s, 3H), 4.06 (s, 3H), 5.27 (s, 2H), 6.75 (d, J = 2.1 Hz, 1H), 7.38 (m, 4H), 7.49 (d, J = 2.1 Hz, 1H), 12.94 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 33.3, 61.0, 75.2, 105.4, 111.4, 111.8, 129.0, 129.1, 129.4, 134.5, 134.6, 144.1, 150.0, 152.0, 153.3, 205.1; MS (ES^-) m/z 345.3 ($M - H^+$); Anal. ($C_{19}H_{17}O_5Cl$): C, H, Cl.

5-Acetyl-7-(4′-bromo)benzyloxy-6-hydroxy-4-methoxybenzofuran (14m). Compound **2** (59 mg, 0.26 mmol) was treated as described under General Procedure F (Supporting Information) to afford **14m** (31 mg, 31% over three steps) as a yellow solid: Mp 106−107 °C; ^{1}H NMR ($CDCl_3$) δ: 2.70 (s, 3H), 4.12 (s, 3H), 5.18 (s, 2H), 6.86 (d, J = 2.4 Hz, 1H), 7.37−7.46 (m, 5H); ^{13}C NMR ($CDCl_3$) δ: 33.2, 60.4, 74.0, 105.7, 110.6, 110.6, 121.9, 127.2, 129.9, 131.4, 136.5, 143.8, 152.0, 152.7, 153.9, 205.2; MS (ES^+) m/z 390.8, 392.8 ($M + H^+$); HRMS (EI) calcd $C_{18}H_{15}O_5Cl$ (M^+) 346.0608, found 346.0614.

4,9-Diethoxy-7-methyl-furo[3,2-g]chromen-5-one (15a). A suspension of **2** (174 mg, 0.75 mmol), cesium carbonate (731 mg, 2.25 mmol), and ethyl iodide (182 μL, 2.25 mmol) was stirred at room temperature for 6 h, then diluted with ethyl acetate (30 mL) and washed with 2 M HCl (3 × 15 mL) and brine (15 mL). The resulting solution was dried over $MgSO_4$ and concentrated in vacuo to afford **15a** (205 mg, 95%) as a brown solid: Mp 90 °C; lit.[28] 94 °C (petroleum ether); ^{1}H NMR ($CDCl_3$) δ: 1.46 (m, 6H), 2.37 (s, 3H), 4.20 (q, J = 7.0 Hz, 2H), 4.41 (q, J = 7.1 Hz, 2H), 6.05 (s, 1H), 6.95 (d, J = 2.2 Hz, 1H), 7.59 (d, J = 2.2 Hz, 1H).

4,9-Dibenzyloxy-7-methyl-furo[3,2-*g*]chromen-5-one (15b). A suspension of **2** (174 mg, 0.75 mmol), cesium carbonate (731 mg, 2.25 mmol), and benzyl bromide (267 μL, 2.25 mmol) was stirred at 60 °C for 3 h, then diluted with ethyl acetate (30 mL) and washed with 2 M HCl (3 × 15 mL) and brine (15 mL). This solution was dried over $MgSO_4$ and concentrated in vacuo to give a residue that was purified by flash chromatography, eluting with ethyl acetate/petroleum ether (3:7), to afford **15b** (263 mg, 85%) as a colorless solid: Mp 128−130 °C; ^{1}H NMR ($CDCl_3$) δ: 2.34 (s, 3H), 5.17 (s, 2H), 5.36 (s, 2H), 6.05 (s, 1H), 6.71 (d, J = 1.9 Hz, 1H), 7.33−7.35 (m, 6H), 7.45−7.76 (m, 5H); ^{13}C NMR ($CDCl_3$) δ: 20.0, 75.8, 77.4, 105.3, 110.5, 114.2, 120.8, 128.0, 128.2, 128.3, 128.7, 128.9, 136.8, 137.3, 145.5, 146.2, 147.8, 149.2, 164.0, 178.2; MS (ES^+) m/z 413.2 ($M + H^+$); Anal. ($C_{26}H_{20}O_5$): C, H.

5-Acetyl-4,7-diethoxy-6-hydroxybenzofuran (16a). Compound **15a** (144 mg, 0.50 mmol) was treated as described in General Procedure D, and the crude product was purified by flash chro-

matography, eluting with ethyl acetate/petroleum ether (1:4), to furnish **16a** (73 mg, 55%) as a yellow solid: Mp 72−74 °C; ^{1}H NMR ($CDCl_3$) δ: 1.36 (t, J = 7.0 Hz, 2H), 1.46 (t, J = 7.0 Hz, 2H), 2.70 (s, 3H), 4.22 (q, J = 7.0 Hz, 2H), 4.36 (q, J = 7.0 Hz, 2H), 6.79 (d, J = 2.3 Hz, 1H), 7.42 (d, J = 2.3 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ: 15.4, 15.5, 33.3, 68.8, 69.4, 105.8, 110.7, 110.8, 143.5, 150.7, 152.8, 153.8, 205.2; MS (ES^+) m/z 265.1 ($M + H^+$); Anal. ($C_{14}H_{16}O_5$): C, H.

5-Acetyl-4,7-benzyloxy-6-hydroxybenzofuran (16b). Compound **15b** (124 mg, 0.30 mmol) was treated as described in General Procedure D, and the crude product was purified by flash chromatography, eluting with ethyl acetate/petroleum ether (1:9), to furnish **16b** (45 mg, 41%) as a yellow solid: Mp 74−76 °C; ^{1}H NMR ($CDCl_3$) δ: 2.60 (s, 3H), 5.26 (s, 2H), 5.30 (s, 2H), 6.75 (d, J = 2.3 Hz, 1H), 7.25−7.78 (m, 11H); ^{13}C NMR ($CDCl_3$) δ: 33.4, 74.9, 105.6, 111.4, 111.6, 118.1, 127.8, 128.0, 128.3, 128.6, 128.8, 136.0, 137.4, 144.0, 150.6, 152.7, 153.8, 205.3; MS (ES^+) m/z 389.3 ($M + H^+$); Anal. ($C_{24}H_{20}O_5$): C, H.

Electrophysiology. The effectiveness of the generated compounds in blocking Kv1.3 was assayed on L929 cells stably expressing *mKv1.3*.[44] All experiments were conducted in the whole-cell configuration of the patch-clamp technique with a holding potential of −80 mV. Currents were recorded in normal Ringer solution (160 mM NaCl, 4.5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, pH 7.4, 290−310 mOsm) with an internal pipet solution containing 134 mM KF, 2 mM $MgCl_2$, 10 mM HEPES, 10 mM EGTA (pH 7.2, 290−310 mOsm). If currents exceeded 2 nA 60−80% series resistance compensation was used. Depolarizing pulses to 40 mV were applied every 45 s for 500 ms. Kv.1.1, Kv1.2, Kv1.5, Kv1.4, and HERG currents were recorded from cells stably expressing these currents as previously described.[44−46] EC_{50} values were determined by fitting the reduction of area under the current curve to the Hill equation.

Cytotoxicity Assay. Jurkat E61 and MEL cells were seeded at 5×10^5 cells/mL in twelve-well plates. Compounds were added at concentrations of 2.5, 10, and 20 μM in a final DMSO concentration of 0.1%, which was found not to affect cell viability. After 48 h the cells in each well were well mixed and resuspended and the number of trypan blue positive cells in three aliquots from each well determined under a light microscope. The test was repeated twice.

T-Cell Proliferation. Peripheral blood mononuclear cells were isolated from the blood of healthy volunteers with the help of a density gradient. Cells were washed and seeded at 2×10^5 cells per well in medium (RPMI 1640 supplemented 10% fetal calf serum, 2 mM glutamine, 1 mM sodium pyruvate, 1% nonessential amino acids, 100 units/mL penicillin, 100 μg/mL streptomycin, and 50 μM β-mercaptoethanol) in flat-bottom 96-well plates (final volume 200 μL). Cells preincubated with compound (30 min) and then stimulated with 5 ng/mL anti-CD3 antibody (Biomeda) for 48 h. [^{3}H]-Thymidine (1 μCi per well) was added for the last 6 h. Cells were harvested onto glass fiber filters, and radioactivity was measured in a scintillation counter. All experiments were done in triplicate. Results are reported as normalized for maximum [^{3}H]-thymidine incorporation for controls.

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Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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