Khellinone Derivatives as Blockers of the Voltage-Gated Potassium Channel Kv1.3: Synthesis and Immunosuppressive Activity

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The voltage-gated potassium channel Kv1.3 constitutes a promising new target for the treatment of T-cell-mediated autoimmune diseases such as multiple sclerosis. In this study, we report the discovery of two new classes of Kv1.3 blockers based on the naturally occurring compound khellinone, 5-acetyl-4,7-dimethoxy-6-hydroxybenzofuran: (1) khellinone dimers linked via the alkylation of the 6-hydroxy groups and (2) chalcone derivatives of khellinone formed by Claisen−Schmidt condensation of the 5-acetyl group with aryl aldehydes. In particular, the chalcone 3-(4,7-dimethoxy-6-hydroxybenzofuran-5-yl)-1-phenyl-3-oxopropene (**16**) and several of its derivatives inhibited Kv1.3 with K_d values of 300−800 nM and a Hill coefficient of 2, displayed moderate selectivity over other Kv1-family K^+ channels, suppressed T-lymphocyte proliferation at submicromolar concentrations, and showed no signs of acute toxicity in mice. Because of their relatively low molecular weight and lipophilicity and their high affinity to Kv1.3, aryl-substituted khellinone derivatives represent attractive lead compounds for the development of more potent and selective Kv1.3 blocking immunosuppressants.

Introduction

The voltage-gated potassium channel Kv1.3 is critically involved in the activation of human T cells and has therefore long been pursued as a novel target for immunosuppressive therapy. The recent report[1] that terminally differentiated effector memory T cells express much higher levels of Kv1.3 and lower levels of the calcium-activated potassium channel IKCa1 ($K_{Ca}3.1$) than other T cells has considerably increased the attractiveness of targeting Kv1.3. Autoreactive effector memory T cells play a crucial role in the pathogenesis of T-cell-mediated autoimmune diseases. For example, in multiple sclerosis, T cells directed against components of the myelin sheath of the central nervous system were found to be predominantly effector memory T cells and to exhibit the $Kv1.3^{high}$ phenotype.[1] The same study showed that this special K^+ channel phenotype rendered the proliferation of effector memory T cells highly sensitive to inhibition by Kv1.3 blockers. Naïve and central memory T cells were only affected at 10-fold higher concentrations of Kv1.3 blockers and could escape Kv1.3 inhibition during subsequent stimulation through up-regulation of the calcium-activated potassium channel IKCa1. It thus seems plausible to preferentially target the disease-inducing effector memory T cell population with a selective Kv1.3 blocker without much effect on the normal immune response. In proof of this concept, the Kv1.3-blocking polypeptides kaliotoxin[2] and *Stichodactyla helianthus* toxin (ShK)[3] have been shown to prevent and treat experimental autoimmune encephalomyelitis in rats, an animal model for multiple sclerosis. Examination of Kv1.3-deficient mice recently revealed another, previously unrecognized role for Kv1.3.[4] Studies conducted with these mice demonstrated that Kv1.3 is involved in body weight regulation and that blockade of Kv1.3 increased basal metabolic rate suggesting that Kv1.3 might also be a new pharmacological target for the treatment of obesity.

Despite their exquisite potency and demonstrated efficacy polypeptides have the disadvantage of requiring parenteral administration and extensive formulation to compensate for their short circulating half-lives. Several groups in academia and in the pharmaceutical industry have therefore tried to develop potent and selective small molecule Kv1.3 blockers and have identified eight chemically distinct classes of compounds.[5,6] The best characterized of these are the nortriterpene correolide,[7,8] originally isolated from the bark of *Spachea correa*, and the recently described cyclohexyl-substituted benzamides,[9,10] both developed by scientists at Merck. However, despite extensive screening efforts, none of these compound classes have so far rendered an analogue with appropriate characteristics for clinical development, suggesting the necessity to explore other templates for the development of small molecule Kv1.3 blocker.

Khellinone (**1a**) and visnaginone (**1b**) in Figure 1 are naturally occurring benzofuran derivatives isolated from *Ammi visnaga*. While khellinone itself only weakly blocks Kv1.3 with a K_d of 45 μM, we show here that it serves as a versatile starting material for at least two

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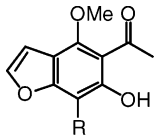
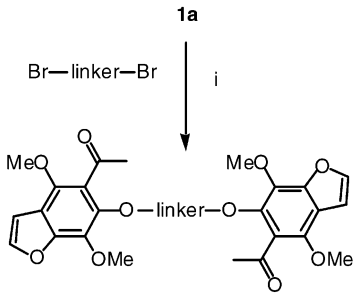


1a R=OMe

1b R=H

Figure 1. Structures of khellinone **1a** and visnaginone **1b**.

Scheme 1. Synthesis of Khellinone (**1a**) Dimers **2**−**9**[a]



Linker	Product
p-xylyl	**2**
m-xylyl	**3**
o-xylyl	**4**
-$(CH_2)_4$-	**5**
-$(CH_2)_5$-	**6**
-$(CH_2)_6$-	**7**
-$(CH_2)_7$-	**8**
-$(CH_2)_2O(CH_2)_2$-	**9**

[a] Reagents: (i) Cs_2CO_3, DMF.

different classes of new Kv1.3 blockers that block Kv1.3 currents and inhibit anti-CD3 activated T-cell proliferation at submicromolar concentrations.

Chemistry

Dimers **2**−**9** of khellinone (**1a**) were readily made by alkylation of the phenolic oxygen atom with various bis-bromo hydrocarbons using cesium carbonate in DMF (General Procedure A; Scheme 1). To improve the solubility characteristics of **2**, a tetra(ethyleneglycol) derivative was prepared through NBS-based bis-bromination of the aryl methyl groups of methyl 2,5-dimethylbenzoate **11** to give **12**, which was used to bis-alkylate khellinone, rendering **13** (Scheme 2). Transesterification with tetra(ethyleneglycol) monomethyl ether using titanium isopropoxide as catalyst according to the method of Ferezou et al.[11] rendered **15**, while direct ester hydrolysis with lithium hydroxide gave carboxylic acid analogue **14**. The alkylated compounds **21**, an *O*-benzyl analogue of khellinone, and **20**, a khellinone trimer (Scheme 3), were made according to the general alkylation procedure A.

Claisen−Schmidt condensation of the 5-acetyl group of khellinone with benzaldehyde yielded the chalcone derivative **16** (Scheme 3). The dimer **22**, the cinnamoyl derivative **23**, the thiophenes **24** and **25** and the chalcones **26**−**28** were prepared analogously by reacting khellinone with the respective aldehydes (Scheme 3). Chalcone **16** was selectively reduced using ionic hydrogenation[12] with triethylsilane/TFA to give hydrocinnamophenone **17**. Hydrogenation with 10% Pd/C as catalyst yielded the tetrahydro derivative **18**. Chalcone **16** was also dimerized by the standard alkylation procedure to give **19**.

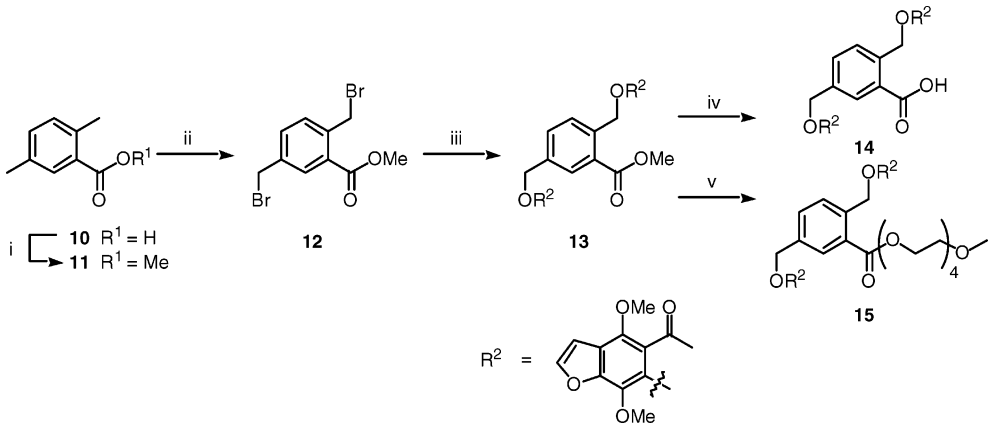
Unexpectedly, reaction of **1a** with succinic dihydrazide resulted in the dihydrazone **29**. The identity of **29** was confirmed by the reaction of khellinone with hydrazine dihydrate. A Beilstein search indicated the transformation to be without precedent.

Dimer **30** was made as a more soluble analogue of **2** by reducing the carbonyl groups of **2** using tetrabutylammonium borohydride in DCM as shown in Scheme 4. The known visnaginone chalcone **31**[13] was prepared from visnaginone **1b**, as shown in Scheme 5, while the visnaginone dimer **32** resulted from alkylation of visnaginone with dibromo-*p*-xylene using cesium carbonate in DMF.

Results and Discussion

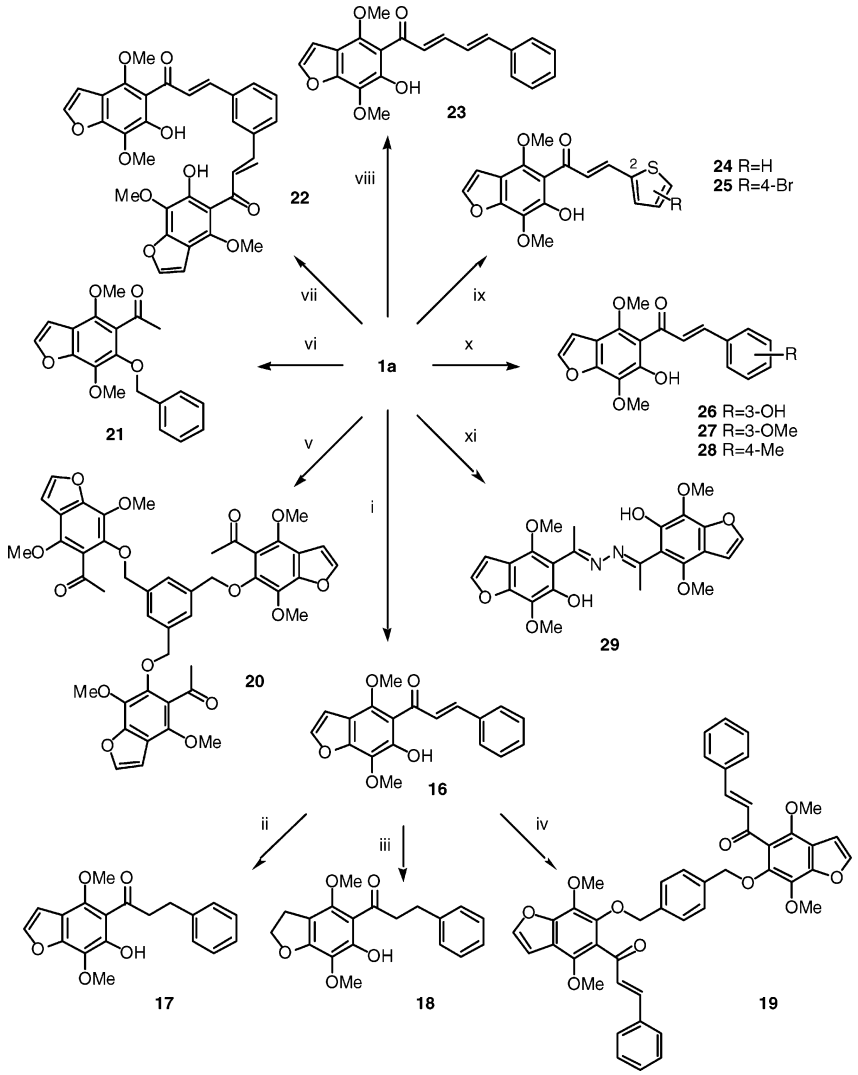
SAR of Khellinone Dimers. As shown in Table 1, when khellinone (**1a**) was dimerized through a *p*-xylene linker, the potency of blocking Kv1.3 greatly increased from a K_d of 45 μM for khellinone to 0.3 μM for **2**. It is tempting to speculate that this is due to the utilization of a second binding site on the tetrameric Kv1.3 channel. The progressive drop in activity in going from **2** to the meta- and ortho-substituted xylene dimers **3** and **4**, respectively, is in line with this hypothesis. The change from an aromatic linker to an aliphatic linker was not favorable as the *n*-butyl-linked dimer **5** exhibited a K_d of 3.5 μM. To help elucidate the possible binding geometry of **2**, a crystal structure was obtained from crystals grown in acetic acid (Figure 2). The resulting structure revealed that the poor activity of dimer **5** could simply be due to the fact that the alkyl linker is too short. This was supported by the observation of a higher potency for the *n*-pentane- and *n*-hexane-linked dimers **6** and **7** with respective K_ds of 0.82 μM and 0.68 μM. Replacement of the central methylene unit in **6** with an oxygen atom was not favorable as **9** had a K_d of 4 μM. Together, these data suggested that hydrophobicity in the linker was a prerequisite for tighter binding. The heptane-linked dimer **8** (K_d 3.0 μM), however, was clearly too long and flexible. To investigate the role of the linker itself in binding to Kv1.3, the *O*-benzyl derivative **21** was synthesized and found to block Kv1.3 with a K_d of 10 μM, indicating that the presence of a bulky and hydrophobic substituent alone considerably increases the affinity of khellinone to the channel but is not sufficient to reach the level of activity of the dimers themselves. In an attempt to locate a possible third khellinone binding site, the trimer **20** was tested, but was found to be inactive. Since **2** was very poorly soluble in aqueous medium ($ClogS_w = -6.55$) and this was anticipated to limit its usefulness as a lead compound, we synthesized the more hydrophilic poly(ethylene)glycol conjugate **15** ($ClogS_w = -4.32$). However, **15** did not exhibit any activity on Kv1.3. The methyl ester precursor **13** and the carboxylic acid **14** displayed some activity against Kv1.3 with respective K_d values of 3 μM and 5 μM but were still significantly less active than the unsubstituted dimer **2**. This demonstrates the detrimental effect of polar substituents and confirms what has been previously reported for the structurally related alkoxypsoralens[14] and furoquinoli-

Scheme 2. Synthesis of Pegylated Khellinone Dimer **15**[a]



[a] Reagents: (i) H_2SO_4, MeOH; (ii) NBS, CCl_4; (iii) Cs_2CO_3, **1a**, DMF; (iv) LiOH, H_2O; (v) $Ti(OCHMe_2)_4$, $HO(CH_2CH_2O)_4Me$.

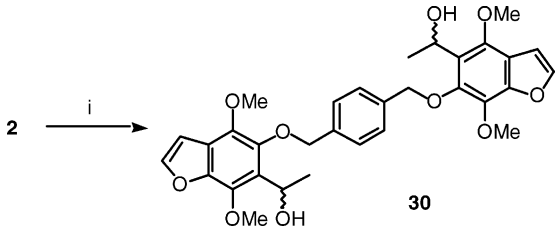
Scheme 3. Various Functionalizations of Khellinone[a]



[a] Reagents: (i) PhC(=O)H, NaOH, H_2O; (ii) Et_3SiH/TFA, DCM; (iii) H_2, 10%Pd/C, EtOAc; (iv) Cs_2CO_3, p-$BrCH_2PhCH_2Br$, DMF; (v) Cs_2CO_3, α,α′,α″-tribromomesitylene, DMF, (vi) K_2CO_3, $PhCH_2Br$, acetone; (vii) *m*-HC(=O)PhC(=O)H, NaOH, H_2O; (viii) PhCH=CHC(=O)H, NaOH, H_2O; (ix) (substituted)thiophene-2-carboxaldehyde, NaOH, H_2O; (x) (substituted)benzaldehyde, NaOH, H_2O; (xi) $(NH_2NH_2C(=O)CH_2)_2$, MeOH.

nones[15] as Kv1.3 blockers. Removal of the 7-methoxy group as per visnaginone dimer **32** resulted in a significant drop in activity. Selective manipulation of the 4- and 7-methoxy groups to higher alkoxy groups did not lead to any dimers with improved activity (data not shown). Likewise, functional manipulation of the ketone group to a variety of oximes, hydrazones, alkenes and chalcones, ketone reduction, and dimers linked through the acetyl group by formation of symmetrical hydrazones or chalcones did not improve activity. Some

Scheme 4. Synthesis of Bis-alcohol **30**[a]



[a] Reagents: (i) Bu_4NBH_4, DCM.

of these dimers are exemplified by compounds **19**, **22**, **29**, and **30** in Scheme 3 and Scheme 4 and in Table 1.

In summary, hydrophobic linkers are required for optimum activity of the dimeric khellinones linked via alkylation of the 6-hydroxy groups. The optimum linker length is around 7.2 Å as defined by the intra-atomic distance between the two oxygen atoms at the 6-position. This value is 7.17 Å in the crystal structure of **2** and 7.27 Å in a molecular model of **6** (generated using Sybyl, Tripos Associates) assuming a staggered pentane conformation. The respective intra-atomic aryl 6-C atom distances are also similar at 9.85 Å and 9.82 Å, respectively. Weaker activity in the longer, heptane-linked dimer **8** may be due to its excessive flexibility. The higher activity of **2** suggests that π-electron density in the linkers may be helpful but this could merely be due to greater conformational constraint of the xylene linker. The 7-methoxy group was required for potent activity, as was the carbonyl functionality in the 5-acetyl group.

SAR of Khellinone Chalcones at Kv1.3. In the course of preparing khellinone dimers functionalized at the 5-acetyl group, all monomeric precursors were also tested for blockade of Kv1.3. Serendipitously, chalcone **16** was found to inhibit Kv1.3 with a K_d of 0.4 μM. Selective reduction of the alkene group gave **17**, which was still quite potent (K_d 0.6 μM). Further reduction of the furan ring rendered **18**, which was a little less potent (K_d 1.5 μM). Extension of the alkene chain in the cinnamoyl derivative **23** was not useful and rendered the resulting compound completely inactive. The visnaginone-derived chalcone **31** was not a potent blocker of Kv1.3, indicating that the 7-OMe group in **16** is important for binding to Kv1.3. We subsequently embarked on a more detailed SAR investigation with the synthesis of several chalcones bearing various substituents such as Cl, Br, F, CH_3, CN, COOH, OH, OCH_3, NO_2, $N(C_2H_5)$ in ortho, meta, and para positions of the styryl phenyl ring. The most potent of these are listed in Table 1 (see Supporting Information for examples of less potent analogues of this series). *m*-Hydroxy and *m*-methoxy substitution rendered **26** and **27** with K_ds of 0.7 μM and 0.8 μM, respectively. Introduction of a *p*-methyl group resulted in **28** with a K_d of 0.7 μM. A number of heterocyclic chalcones were also investigated, where the styryl phenyl ring was replaced by variously substituted quinoline, pyrrole, pyridine, indole, or thiophene rings (see Supporting Information for examples). Of these only the thiophenes **24** and **25** blocked Kv1.3 with respective K_d values of 1 and 0.8 μM.

In summary, the optimal functionalization of the acetophenone group in khellinone was as a chalcone, formed from Claisen−Schmidt condensation with aryl aldehydes. The preferred aryl ring was phenyl or thiophene-2-yl. The side-chain phenyl ring tolerated meta-substitution with OH or OMe, or para-substitution with Me, while the thiophene-2-yl ring tolerated a 4-Br substituent. The 7-OMe of the benzofuran ring was also required for optimal binding.

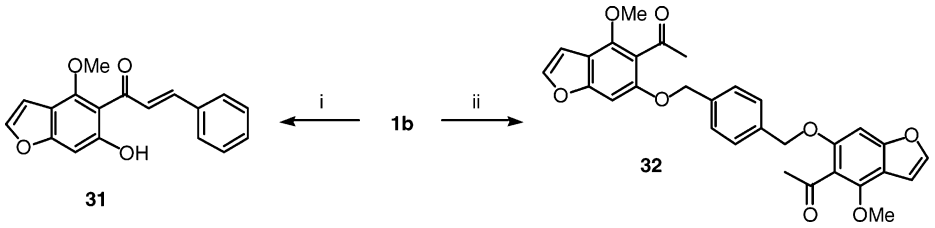
Nature of Block. Following application of both the khellinone dimers and the khellinone chalcones to cells stably expressing *Kv1.3* in whole-cell patch-clamp experiments, blockade of Kv1.3 current increased during consecutive depolarizing pulses and reached a steady-state after 3−8 min, a phenomenon termed "use-dependent inhibition" (Figure 3a). However, the two compound classes differed markedly in their kinetics of block and their Hill coefficients. While the dimers exhibited Hill coefficients close to 1 consistent with a 1:1 stoichiometry of interaction between blocker and channel protein and did not affect the inactivation kinetics of Kv1.3 (Figure 3b, left), the chalcones exhibited Hill coefficients of 2, suggesting that two molecules of blocker interact with one Kv1.3 channel tetramer, and considerably accelerated the inactivation of Kv1.3 (Figure 3b, right). The chalcones thus resemble the cyclohexyl-substituted benzamides in their Kv1.3-blocking behavior. This class of compounds[9] also speeds up inactivation and blocks Kv1.3 with a Hill coefficient of 2. A recent competition binding study conducted with a radiolabeled benzamide suggests the presence of two receptor sites for the benzamides on the Kv1.3 protein that display positive allosteric cooperativity.[16] Whether something similar is the case for the chalcones remains to be determined.

Although we have not mapped it in this study, we suspect that the binding site for both the dimers and the chalcones is located in a hydrophobic pocket on the intracellular site of the Kv1.3 protein, most likely in the water-filled cavity below the selectivity filter of the channel. Both the binding sites for correolide[17] and verapamil[18] have been previously identified to lie in this region.

Selectivity. Of the dimers, **2** and **7** were tested for selectivity over other Kv1-family channels. As shown in Table 2, **2** was more than 10-fold selective for Kv1.3 over Kv1.1 and Kv1.2, whereas **7** was actually a more potent inhibitor of Kv1.1 and Kv1.2 with K_ds of 0.32 μM and 0.25 μM, respectively. The latter pharmacological profile was cause for concern, as blockade of Kv1.1 and Kv1.2 is suspected to be the reason for correolide's gastrointestinal side-effects. When administered to minipigs, correolide caused hyperactivity[8] and also elicited twitches in guinea-pig ileum by stimulating the enteric nervous system and enhancing neurotransmitter release.[19] For this reason, these alkane-linked dimers were not pursued further in the context of this project. Compound **2**, on the other hand, was more widely tested and maintained selectivity for Kv1.3, being several times less active on Kv1.5 and inactive on the calcium-activated K^+ channels IKCa1.

Of the khellinone chalcones **16**, **26**, and **27** were tested for selectivity as shown in Table 2. While only 2.5- or 3-fold less active on Kv1.1, they exhibited 20-fold selectivity over the cardiac potassium channels Kv1.5 and Kv1.7 and had no effect on Kv1.2 and IKCa1. This selectivity profile renders the khellinone chalcones the

Scheme 5. Synthesis of Visnaginone Derivatives **31** and **32**[a]



[a] Reagents: (i) PhC(=O)H, NaOH, H_2O; (ii) Cs_2CO_3, *p*-$BrCH_2PhCH_2Br$, DMF.

Table 1. K_d Values of Khellinone Analogues for Block of Kv1.3 Current

compd	K_d (μM)	compd	K_d (μM)
ShK[43]	11 pM[a]	**17**	0.6[b]
4-AP[39]	195[a]	**18**	1.5[b]
correolide[44]	0.1[a]	**19**	no effect[d]
1a	50[a]	**20**	no effect[c]
1b	100[a]	**21**	10[a]
2	0.28[a]	**22**	no effect[c]
3	0.56[a]	**23**	no effect[d]
4	7.1[a]	**24**	1[b]
5	3.5[a]	**25**	0.8[b]
6	0.82[a]	**26**	0.7[b]
7	0.68[a]	**27**	0.8[b]
8	3.0[a]	**28**	0.7[b]
9	4.0[a]	**29**	70[a]
13	3.1[a]	**30**	2.0[a]
14	5.0[a]	**31**	17[b]
15	no effect[c]	**32**	1.0[a]
16	0.4[b]		

Each compound was tested 2−3 times at 3−5 concentrations. Values for the dissociation constant K_d and the Hill coefficient n_H were determined by fitting the Hill equation to the reduction of area under the K^+ current curve. Standard deviations were between 2−10% in each case. [a] $n_H = 1$; [b] $n_H = 2$; [c] Tested at 10 μM; [d] Tested at 1 μM. The K_ds of the most potent peptidic Kv1.3 blocker, the sea anemone toxin ShK (*Stichodactyla helianthus* toxin),[43] of the unselective small molecule K^+ channel blocker 4-AP (4-aminopyridine)[39] and of Merck's small molecule Kv1.3 blocker correolide[44] are given for comparison.

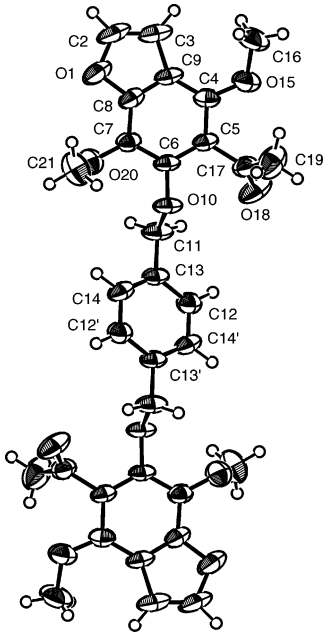


Figure 2. ORTEP illustration of dimer **2**. Primed atoms (′) are related to the unprimed atoms by a center of symmetry.

first known small molecule Kv1.3 blockers that are selective over Kv1.2 and Kv1.5.[6,20]

Inhibition of Proliferation. On the basis of the promising pharmacological profile of the khellinone chalcones, compounds **16**, **26** and **27** were tested for toxicity against Jurkat E6-1 and MEL cells. Also included were thiophenes **24** and **25**. The poor aqueous solubility of dimer **2** made it too difficult to work with, and this compound was not investigated further in this project. While the thiophenes **24** and **25** proved toxic at 10 μM, compounds **16**, **26**, and **27** were found to be nontoxic at concentrations of 0.1, 1, 2.5, and 10 μM and were therefore selected for T-cell proliferation assays. As shown in Table 3, these compounds and the dimer **7** inhibited the anti-CD3 antibody-stimulated proliferation of peripheral blood T cells stimulated with EC_{50} values between 0.6 and 1 μM.

Acute Toxicity in Vivo. To determine if the compounds exhibited any unexpected acute toxic effects in vivo, mice ($n = 5$) were injected intravenously with a single dose of **7**, **16**, **26**, or **27** at 5 mg/kg as a bolus. The animals appeared normal immediately after the injection and did not exhibit any signs of acute toxicity for 7 days afterward. The body weights in treated animals (day 1: 18.0 g; day 7: 27.0 g) were similar to control mice injected with the vehicle (day 1: 18.0 g; day 7: 28.0 g). Collectively, the data from this limited toxicity study suggests that the khellinone chalcones **16**, **26**, and **27** are not acutely toxic and constitute promising lead compounds for the development of more potent small molecule Kv1.3 blockers as immunosuppressants.

Conclusion

Khellinone (**1a**) is a weak inhibitor of the voltage-gated potassium channel, Kv1.3, with a K_d of 45 μM. However, being small (mw 234) and containing both a phenolic group that can be alkylated and an acetyl group that readily undergoes Claisen−Schmidt condensation, it serves as a versatile template for the design of new small molecule Kv1.3 blockers. In this sense, it complies with developing notions that smaller, more polar molecules make the most druggable templates, because they give the medicinal chemist more room to move during the optimization process.[21] Dimerization of **1a** through a *p*-xylyl linker to give **2** increased affinity for Kv1.3 150-fold. While **2** was selective for Kv1.3 over a range of other Kv1-family channels, poor aqueous solubility limited its further use. Dimers **6** and **7** were found to block Kv1.1 and Kv1.2 more potently than Kv1.3 and might thus be of interest for enhancing impulse propagation in demyelinated neurons[22] in multiple sclerosis and diabetic neuropathy, where destruction of the myelin sheath uncovers normally silent potassium channels in the Node of Ranvier[23] that contribute to the conduction failure observed following demyelination.[24] Using the dimer **7** as a template for

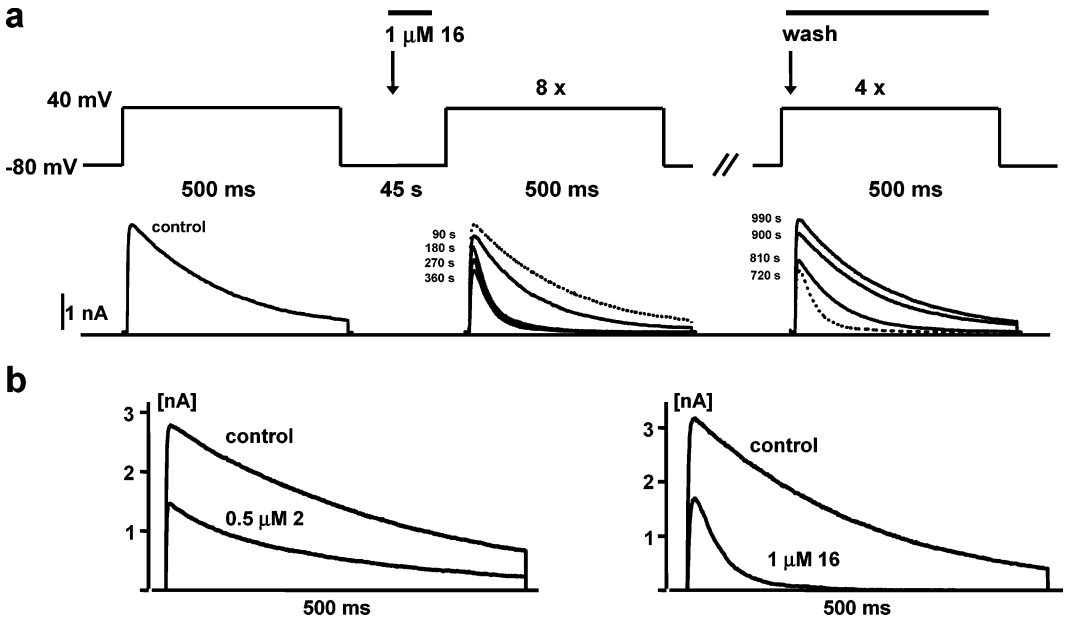


Figure 3. (a) Typical experiment showing onset of block and washout of 1 μM of compound **16**. Currents were elicited by depolarizing steps from −80 to 40 mV for 500 ms applied every 45 s (see pulse protocol above the current traces). With this pulse protocol no "run-down" of the Kv1.3 current was observed over periods of 20−60 min. After establishing a stable control current (left) 1 μM compound **16** was perfused over the cell. Equilibrium block was reached after 360 s (middle). Every second pulse is shown; control current dashed for comparison. After 720 s compound **16** was washed out (right) through continuous perfusion of Ringer solution (~30 mL). Blocked current dashed for comparison. (b) Effect of 0.5 μM compound **2** and 1 μM compound **16** on Kv1.3 currents. Equilibrium block is shown in both cases.

Table 2. Selectivity (K_d, μM) for Kv1.3 over Other K^+ Channels[a]

	Kv1.1	Kv1.2	Kv1.3	Kv1.5	Kv1.7	IKCa1
ShK[43]	16 pM	10 nM	11 pM	>100 nM	12 nM	28 nM
4-AP[39]	290	590	195	270	nd	29
2	3.1	.2	0.28	1.1	nd	>100
7	0.3	0.25	0.68	nd	nd	nd
16	1.2	>50	0.4	5.1	10	>100
26	1.7	>50	0.7	12	50	>100
27	2.0	>50	0.8	20	>50	>100

[a] K_d values (mean of at least three experiments with standard deviations between 2−10% in each case) for blockade of other Kv1-family channels and the calcium-activated K^+ channel IKCa1. nd: not determined. The selectivities of ShK (*Stichodactyla helianthus* toxin) and 4-AP (4-aminopyridine) are given for comparison.

Table 3. Inhibition of T-Cell Proliferation

compd	EC_{50} (μM)
ShK[44]	0.04
correolide[44]	0.3
7	0.8
16	0.6
26	1
27	1

the development of Kv1.1/Kv1.2 inhibitors as symptomatic therapeutics for the therapy of demyelinating diseases appears valid in the light that **7** did not display any signs of acute toxicity when administered to mice.

Derivatization of khellinone, through its acetyl group as a chalcone, resulted in the more Kv1.3-selective compounds **16**, **26**, and **27,** which inhibited Kv1.3 with K_d values of 0.4−0.8 μM and half-maximally suppressed anti-CD3 antibody stimulated T-cell proliferation at submicromolar levels. Perhaps because of their ease of synthesis, chalcones have been widely used as templates for various SAR studies and have been reported to display a wide range of biological activities such as antifungal,[25] antiprotozoal,[26] and antiproliferative[27] effects. Fluorinated chalcones in particular have been shown to exhibit antiinflammatory properties through inhibition the 5-lipoxygenase [28] and reduction of nitric oxide production.[29] However, the pharmacophores proposed in these studies differ considerably from the pharmacophore described here as necessary for Kv1.3 inhibition. On the basis of the similar potencies with which **16**, **26**, and **27** block Kv1.3 and suppress T-cell proliferation, we therefore suggest that their antiproliferative effects are due to Kv1.3 inhibition. It has also been proposed that chalcones in general should be viewed with caution,[30] because of their propensity as α,β-unsaturated ketones to act as a Michael Acceptor and thus possibly undergo covalent reactions with proteins.[31,32] However, the potency of the reduced derivative **17** together with the fact that the effect of the chalcones in electrophysiological experiments is easily reversible by washing with Ringer solution demonstrates that this is not the case here.

In conclusion, we have generated two new classes of small molecule Kv1.3 blockers in this study: khellinone dimers and khellinone chalcones. The chalcones in particular constitute promising templates to further improve potency and selectivity for Kv1.3. They are the first small molecule Kv1.3 blockers that are selective over the cardiac potassium channel Kv1.5 and the neuronal potassium channel Kv1.2, and their small size easily allows for further chemical modification in order to develop a truly Kv1.3 selective small molecule as an immunosuppressant.

Experimental Section

Melting points were determined in open capillary tubes using an electrothermal melting point apparatus (Model 9200) and are uncorrected. Mass spectra (MS) were recorded on a Kratos MS80 RFA mass spectrometer at the University of Canterbury. Elemental analyses were performed for carbon,

nitrogen, hydrogen, sulfur, and bromine at the University of Otago microanalytical laboratory. Proton nuclear magnetic resonance (^{1}H NMR) and carbon nuclear magnetic resonance (^{13}C NMR) were performed on a Bruker Avance DRX 300 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 $CHCl_3$ at δ_H 7.24 ppm and $CDCl_3$ 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. IR spectra were obtained with a FTIR spectrophotometer, using a diffuse reflectance accessory with a KBr background. Spectra were run either in chloroform solution, neat, or as a Nujol mull. Analytical thin-layer chromatography 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.[33] using ACS analytical grade solvents. All moisture sensitive experiments were performed in oven-dried glassware under an atmosphere of nitrogen. Tetrahydrofuran was distilled from sodium benzophenone ketal immediately prior to use. Anhydrous dimethylformamide was purchased from Aldrich. Petroleum ether describes a mixture of hexanes in the bp range 30−50 °C.

General Procedure A. To a suspension of the benzofuran (0.5 mmol) and cesium carbonate (0.5 mmol) in DMF under nitrogen was added the dibromide (0.25 mmol), and the reaction mixture was stirred at 55 °C for 16 h. The reaction mixture was diluted with ethyl acetate (10 mL), washed with 10% aqueous citric acid (3 × 10 mL) and brine (10 mL), dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by flash chromatography.

General Procedure B. A mixture of the benzofuran (1.0 mmol) and the aldehyde (1.2 mmol) in 1 M NaOH in methanol/water (2:1) was stirred at room temperature 16 h, and 10% aqueous citric acid was added. If a solid precipitated, the solid was collected by filtration and recrystallized. If an oil developed, the oil was extracted with ethyl acetate (2 × 10 mL), and the pooled organics were washed with brine (10 mL), dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was recrystallized to give the pure chalcone.

1,4-Bis(5-acetyl-4,7-dimethoxybenzofuran-6-yloxymethyl)benzene (2). Khellinone **1a** (236 mg, 1.0 mmol) and α,α′-dibromo-*p*-xylene (132 mg, 0.5 mmol) were treated as described in General Procedure A. The crude product was recrystallized from methanol to give **2** (170 mg, 59%) as a colorless solid: Mp 192 °C (MeOH); ^{1}H NMR ($CDCl_3$) δ: 2.44 (s, 6H), 3.99 (s, 6H), 4.08 (s, 6H), 5.09 (s, 4H), 6.88 (d, J = 2.3 Hz, 2H), 7.43 (s, 4H), 7.59 (d, J = 2.3 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ: 32.4, 60.8, 60.8, 76.1, 104.7, 116.3, 124.3, 128.2, 134.2, 136.7, 143.7, 144.0, 148.3, 201.6; IR (KBr, chloroform) 1701; MS (thermospray, LC/MS) m/z 575 (M + H$^+$); Anal. ($C_{32}H_{30}O_{10}$): C, H.

1,3-Bis(5-acetyl-4,7-dimethoxybenzofuran-6-yloxymethyl)benzene (3). Khellinone **1a** (236 mg, 1.0 mmol) and α,α′-dibromo-*m*-xylene (132 mg, 0.5 mmol) were treated as described under General Procedure A. The crude product was recrystallized from methanol to give **3** (162 mg, 56%) as a colorless solid: Mp 112 °C (MeOH); ^{1}H NMR ($CDCl_3$) δ: 2.45 (s, 6H), 3.99 (s, 6H), 4.10 (s, 6H), 5.10 (s, 4H), 6.88 (d, J = 2.3 Hz, 2H), 7.40 (m, 3H), 7.50 (s, 1H), 7.59 (d, J = 2.3 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ: 32.7, 61.2, 61.2, 76.6, 105.0, 116.7, 124.7, 128.2, 128.3, 128.6, 134.6, 137.3, 144.1, 144.3, 144.6, 148.6, 201.9; IR (KBr) 1696; MS (thermospray, LC/MS) m/z 575 (M + H$^+$); Anal. ($C_{32}H_{30}O_{10}$): C, H.

1,2-Bis(5-acetyl-4,7-dimethoxybenzofuran-6-yloxymethyl)benzene (4). Khellinone **1a** (236 mg, 1.0 mmol) and α,α′-dibromo-*o*-xylene (132 mg, 0.5 mmol) were treated as described under General Procedure A. The crude product was recrystallized from ethanol to give **4** (167 mg, 58%) as a colorless solid: Mp 140 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ: 2.39 (s, 6H), 3.98 (s, 6H), 4.03 (s, 6H), 5.27 (s, 4H), 6.87 (d, J = 2.3 Hz, 2H), 7.34 (m, 2H), 7.50 (s, 2H), 7.58 (d, J = 2.3 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ: 32.6, 61.1, 61.2, 73.8, 105.0, 116.7, 124.7, 128.3, 129.3, 134.7, 135.6, 144.0, 144.2, 144.6, 148.6, 201.7; IR (KBr) 1689; MS (thermospray, LC/MS) m/z 575 (M + H$^+$); Anal. ($C_{32}H_{30}O_{10}$): C, H.

1,4-Bis(5-acetyl-4,7-dimethoxybenzofuran-6-yloxy)butane (5). Khellinone **1a** (236 mg, 1.0 mmol) and 1,4-dibromobutane (59 μL, 0.5 mmol) were treated as described under General Procedure A. The crude product was purified by flash chromatography eluting with ethyl acetate/cyclohexane (3:7) to afford **5** (221 mg, 84%) as white needles: Mp 119−120 °C; ^{1}H NMR ($CDCl_3$) δ: 1.87 (m, 4H), 2.50 (s, 6H), 3.95 (s, 6H), 4.06 (s, 6H), 4.09 (m, 4H), 6.83 (d, J = 2.1 Hz, 2H), 7.54 (d, J = 2.1 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ: 26.6, 32.7, 61.0, 61.1, 74.7, 104.9, 116.3, 124.5, 134.4, 143.9, 144.4, 144.6, 148.6, 201.8; IR (KBr, chloroform) 1702; MS (ES$^+$) m/z 527 (M + H$^+$); HRMS (ES$^+$) calcd $C_{28}H_{30}O_{10}$ (M + H$^+$) 526.1917, found 526.1912.

1,5-Bis(5-acetyl-4,7-dimethoxybenzofuran-6-yloxy)pentane (6). Khellinone **1a** (236 mg, 1.0 mmol) and 1,5-dibromopentane (68 μL, 0.5 mmol) were treated as described in General Procedure A. The crude product was purified by flash chromatography eluting with ethyl acetate/cyclohexane (1:9 to 2:8) to give **6** (177 mg, 65%) as a gray oil: ^{1}H NMR ($CDCl_3$) δ: 1.57 (m, 2H), 1.77 (tt, J = 7.0, 7.0 Hz, 4H), 2.51 (s, 6H), 3.95 (s, 6H), 4.08 (m, 10H), 6.84 (d, J = 2.1 Hz, 2H), 7.54 (d, J = 2.1 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ: 22.2, 29.7, 32.7, 61.0, 61.1, 74.9, 104.9, 116.3, 124.5, 134.4, 143.9, 144.4, 144.7, 148.6, 201.8; IR (KBr, chloroform) 1705; MS (ES$^+$) m/z 541 (M + H$^+$); HRMS (ES$^+$) calcd $C_{29}H_{32}O_{10}$ (M + H$^+$) 541.2074, found 541.2070.

1,6-Bis(5-acetyl-4,7-dimethoxybenzofuran-6-yloxy)hexane (7). Khellinone **1a** (236 mg, 1.0 mmol) and 1,6-dibromohexane (77 μL, 0.5 mmol) were treated as described under General Procedure A. The crude product was purified by flash chromatography eluting with ethyl acetate/cyclohexane (1:9 to 2:8) to give **7** (215 mg, 77%) as a colorless solid: Mp 89−90 °C (EtOAc/cyclohexane); ^{1}H NMR ($CDCl_3$) δ: 1.48 (m, 4H), 1.75 (m, 4H), 2.52 (s, 6H), 3.97 (s, 6H), 4.06 (m, 10H), 6.85 (d, J = 2.4 Hz, 2H), 7.55 (d, J = 2.4 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ: 25.6, 26.8, 32.7, 61.0, 61.2, 75.1, 104.6, 116.3, 124.5, 134.4, 143.9, 144.4, 144.8, 148.6, 201.8; IR (KBr, chloroform) 1702; MS (ES$^+$) m/z 555 (M + H$^+$); Anal. ($C_{30}H_{34}O_{10}$): C, H.

1,7-Bis(5-acetyl-4,7-dimethoxybenzofuran-6-yloxy)heptane (8). Khellinone **1a** (236 mg, 1.0 mmol) and 1,7-dibromoheptane (85 μL, 0.5 mmol) were treated as described in General Procedure A. The crude product was purified by flash chromatography eluting with ethyl acetate/cyclohexane (1:9) to give **8** (243 mg, 86%) as a yellow oil: ^{1}H NMR ($CDCl_3$) δ: 1.39 (m, 6H), 1.69 (m, 4H), 2.49 (s, 6H), 3.94 (s, 6H), 4.03 (m, 10H), 6.82 (d, J = 2.3 Hz, 2H), 7.52 (d, J = 2.3 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ: 25.7, 29.0, 30.0, 32.7, 60.9, 61.1, 75.1, 104.8, 116.2, 124.5, 134.3, 143.8, 144.4, 144.8, 148.6, 201.8; IR (film) 1705; MS (ES$^+$) m/z 569 (M + H$^+$); Anal. ($C_{31}H_{36}O_{10}$): C, H.

Bis(5-acetyl-4,7-dimethoxybenzofuran-6-yloxyethyl)-ether (9). To a suspension of **1a** (236 mg, 1.0 mmol) and anhydrous potassium carbonate (138 mg, 1.0 mmol) in DMF (2 mL) under nitrogen was added di(2-bromoethyl) ether (63 μL, 0.5 mmol), and the reaction mixture was stirred at room temperature for 2 d. It was diluted with ethyl acetate (10 mL), washed with 10% aqueous citric acid (3 × 10 mL) and then brine (10 mL), dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by flash chromatography eluting with ethyl acetate/cyclohexane (1:9 to 3:7) to afford the product **9** (99 mg, 37%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ: 2.53 (s, 6H), 3.77 (t, J = 4.8 Hz, 4H), 3.96 (s, 6H), 4.06 (s, 6H), 4.24 (t, J = 4.8 Hz, 6H), 6.85 (d, J = 2.1 Hz, 2H), 7.55 (d, J = 2.1 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ: 32.7, 61.0, 61.2, 70.0, 73.7, 104.9, 116.5, 124.5, 134.3, 144.0, 144.5, 144.8, 148.6, 201.7; IR (film) 1689; MS (ES$^+$) m/z 543 (M + H$^+$); HRMS (ES$^+$) calcd $C_{28}H_{30}O_{11}$ (M + H$^+$) 543.1866, found 543.1873.

Methyl 2,5-Dimethylbenzoate (11). A mixture of 2,5-dimethylbenzoic acid (**10**) (1.50 g, 10 mmol), sulfuric acid (0.11 mL, 2.0 mmol), and methanol (4 mL) was heated at reflux for 4 h. The mixture was poured into water (40 mL) and extracted with ethyl acetate (2 × 30 mL). The pooled organics were washed with saturated sodium bicarbonate (2 × 20 mL) and

brine (20 mL), dried over $MgSO_4$, and concentrated in vacuo to give **11** (1.14 g, 70%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ: 2.34 (s, 3H), 2.56 (s, 3H), 3.88 (s, 3H), 7.12 (d, J = 7.8 Hz, 1H), 7.24 (d, J = 7.8 Hz, 1H), 7.73 (s, 1H); ^{1}H NMR lit.[34] ($CDCl_3$) δ: 2.34 (s, 3H), 2.54 (s, 3H), 3.88 (s, 3H), 7.12 (d, J = 7.8 Hz, 1H), 7.21 (dd, J = 1.7, 7.8 Hz, 1H), 7.70 (d, J = 1.7 Hz, 1H,).

Methyl 2,5-Di(bromomethyl)benzoate (12). To a solution of **11** (328 mg, 2.0 mmol) in carbon tetrachloride (10 mL) was added *N*-bromosuccinimide (890 mg, 5.0 mmol) and 2,2′-azobisisobutyronitrile (16 mg, 0.1 mmol), and the reaction mixture was heated at reflux for 4 h. The resulting suspension was filtered, and the residue was washed with chloroform (3 × 5 mL). The pooled organics were concentrated in vacuo to give a mixture of product and succinimide as determined by ^{1}H NMR. The mixture was dissolved in dichloromethane (20 mL) and washed with water (2 × 10 mL). The dichloromethane phase was dried over $MgSO_4$ and concentrated in vacuo, and the resulting residue was purified by flash chromatography eluting with 2% ether in petroleum ether to furnish the product (0.50 g, 78%) as a white solid: Mp 79.5−80 °C (MeOH); lit.[35] 81−83 °C (MeOH); ^{1}H NMR ($CDCl_3$) δ: 3.98 (s, 3H), 4.49 (s, 2H), 4.94 (s, 2H), 7.46 (d, J = 7.8 Hz, 1H), 7.53 (dd, J = 1.8, 7.8 Hz, 1H), 8.00 (d, J = 1.8 Hz, 1H).

Methyl 2,5-Bis(5-acetyl-4,7-dimethoxybenzofuran-6-yloxymethyl)benzoate (13). Khellinone **1a** (147 mg, 0.62 mmol) and **12** (100 μL, 0.31 mmol) were treated as described in General Procedure A. Purification by flash chromatography eluting with ethyl acetate/cyclohexane (1:4 to 2:3) afforded **13** (149 mg, 76%) as a straw-colored solid: Mp 87−89 °C; ^{1}H NMR ($CDCl_3$) δ: 2.48 (s, 3H), 2.48 (s, 3H), 3.87 (s, 3H), 3.98 (s, 3H), 3.99 (s, 3H), 4.03 (s, 3H), 4.09 (s, 3H), 5.11 (s, 2H), 5.55 (s, 2H), 6.87 (d, J = 2.7 Hz, 1H), 6.88 (d, J = 2.7 Hz, 1H), 7.57 (d, J = 2.7 Hz, 1H), 7.59 (d, J = 2.7 Hz, 1H), 7.67 (dd, J = 1.5, 8.1 Hz, 1H), 7.85 (d, J = 8.1 Hz, 1H), 8.07 (d, J = 1.5 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ: 32.7, 32.7, 51.9, 61.1, 61.1, 61.2, 61.2, 74.1, 76.0, 104.9, 105.0, 116.6, 116.7, 124.3, 124.5, 127.5, 127.7, 130.2, 132.2, 134.3, 134.5, 136.2, 139.7, 144.0, 144.1, 144.4, 144.5, 144.6, 144.6, 148.5, 148.7, 166.9, 201.5, 201.7; IR (film) 1718, 1701; MS (ES^+) m/z 633 (M + H^+); HRMS (ES^+) calcd $C_{34}H_{32}O_{12}$ (M + H^+) 633.1972, found 633.1981.

2,5-Bis(5-acetyl-4,7-dimethoxybenzofuran-6-yloxymethyl)benzoic Acid (14). To a solution of **13** (63 mg, 0.10 mmol) in THF (3 mL) was added 0.25 M lithium hydroxide in methanol/water (2:1, 3 mL), and the reaction mixture was stirred at 80 °C for 16 h. The mixture was then concentrated to one-third volume, diluted with water (10 mL), and extracted with ether (15 mL). The aqueous phase was cooled, acidified to pH 4 with 1 M HCl, and extracted with ethyl acetate (2 × 20 mL). The pooled organics were washed with brine (10 mL), dried over $MgSO_4$, and evaporation to afford a residue that was purified by flash chromatography, eluting with methanol/dichloromethane (1:25) to give **14** (26 mg, 42%) as a colorless oil: ^{1}H NMR (d_6-acetone) δ: 2.43 (s, 3H), 2.44 (s, 3H), 4.01 (s, 3H), 4.01 (s, 3H), 4.03 (s, 3H), 4.15 (s, 3H), 5.23 (s, 2H), 5.60 (s, 2H), 7.13 (d, J = 2.4 Hz, 1H), 7.13 (d, J = 2.4 Hz, 1H), 7.75 (dd, J = 1.8, 7.8 Hz, 1H), 7.86 (d, J = 2.4 Hz, 1H), 7.86 (d, J = 2.4 Hz, 1H), 7.91 (d, J = 7.8 Hz, 1H), 8.18 (d, J = 1.8 Hz, 1H); ^{13}C NMR (d_6-acetone) δ: 31.9, 31.9, 60.4, 60.5, 60.5, 60.6, 74.1, 75.6, 105.1, 105.1, 116.6, 116.7, 124.6, 124.6, 127.0, 127.4, 130.3, 131.9, 135.4, 135.6, 136.6, 139.9, 144.4, 144.5, 145.2, 149.4, 167.2, 204.1, 204.2; IR (film) 1612, 1700; MS (ES^+) m/z 619 (M + H^+); Anal. ($C_{33}H_{30}O_{12}$): C, H.

2,5-Bis(5-acetyl-4,7-dimethoxybenzofuran-6-yloxymethyl)benzoic Acid, Methoxytetrakis(ethylene glycol) Ester (15). To a suspension of **13** (80 mg, 0.13 mmol) in tetrakis(ethylene glycol) monomethyl ether (720 μL, 3.80 mmol) was added titanium(IV) isopropoxide (36 μL, 0.12 mmol), and the reaction mixture was stirred under nitrogen at 100 °C for 14 h. The reaction was quenched with 1 M HCl (1 mL) and resulting mixture partitioned over 1 M HCl/ethyl acetate (1:1, 120 mL). The organic phase was washed with saturated sodium bicarbonate (60 mL), dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by flash chromatography, eluting with ethyl acetate/petroleum ether (3:2) to afford the starting material **13** (30 mg). Further elution with ethyl acetate/petroleum ether (7:3) furnished **15** (32 mg, 50% by returned starting material) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ: 2.49 (s, 3H), 2.49 (s, 3H), 3.35 (s, 3H), 3.51 (m, 2H), 3.58−3.68 (m, 12H), 3.81 (t, J = 4.8 Hz, 2H), 3.99 (s, 3H), 4.00 (s, 3H), 4.03 (s, 3H), 4.11 (s, 3H), 4.44 (t, J = 4.8 Hz, 2H), 5.12 (s, 2H), 5.56 (s, 2H), 6.88 (d, J = 2.1 Hz, 1H), 6.90 (d, J = 2.1 Hz, 1H), 7.59 (d, J = 2.1 Hz, 1H), 7.61 (d, J = 2.1 Hz, 1H), 7.67 (d, J = 8.1 Hz, 1H), 7.86 (d, J = 8.1 Hz, 1H), 8.09 (s, 1H); ^{13}C NMR ($CDCl_3$) δ: 32.7, 32.7, 58.9, 61.1, 61.1, 61.3, 61.3, 63.5, 69.0, 70.4, 70.5, 71.8, 74.2, 76.0, 104.9, 105.0, 116.6, 116.7, 124.6, 124.6, 127.4, 127.5, 130.3, 132.3, 134.4, 134.6, 136.2, 139.9, 144.0, 144.1, 144.4, 144.5, 144.6, 148.5, 148.7, 166.4, 201.5, 201.7; IR (film) 1703, 1713; MS (ES^+) m/z 826 (M + NH_4^+); HRMS (ES^+) calcd $C_{42}H_{49}O_{16}$ (M + H^+) 809.303, found 809.302.

3-(4,7-Dimethoxy-6-hydroxybenzofuran-5-yl)-1-phenyl-3-oxopropene (16). Khellinone **1a** (236 mg, 1.0 mmol) and benzaldehyde (152 μL, 1.5 mmol) were treated as described in General Procedure B. The crude product was recrystallized from methanol to give **16** (252 mg, 78%) as red needles: Mp 125−126 °C (MeOH) lit.[36] 129 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ: 4.03 (s, 3H), 4.07 (s, 3H), 6.86 (d, J = 2.3 Hz, 1H), 7.34−7.42 (m, 3H), 7.50 (d, J = 2.3 Hz, 1H), 7.63 (m, 2H), 7.85 (m, 2H); ^{13}C NMR ($CDCl_3$) δ: 61.0, 61.9, 105.2, 111.8, 112.7, 127.0, 128.5, 129.0, 129.5, 130.4, 135.1, 143.4, 144.1, 150.7, 151.9, 153.2, 194.7.

3-(4,7-Dimethoxy-6-hydroxybenzofuran-5-yl)-1-phenyl-3-oxopropane (17). A solution of **16** (49 mg, 0.15 mmol) in dichloromethane (1 mL) was treated with triethylsilane (74 μL. 0.45 mmol) and trifluoroacetic acid (77 μL, 1.0 mmol) and stirred under nitrogen for 3 h. The reaction mixture was diluted with cyclohexane, and on concentrating, a solid precipitated. The solid was filtered to afford **17** (46 mg, 93%) as yellow needles: Mp 112−113 °C (cyclohexane); ^{1}H NMR ($CDCl_3$) δ: 3.04 (t, J = 7.5 Hz, 2H), 3.41 (t, J = 7.5 Hz, 2H), 4.03 (s, 3H), 4.09 (s, 3H), 6.87 (d, J = 2.3 Hz, 1H), 7.17−7.29 (m, 5H), 7.48 (d, J = 2.3 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ: 30.6, 46.0, 60.6, 61.0, 105.7, 110.5, 110.8, 126.0, 128.4, 128.4, 128.9, 141.3, 143.8, 151.3, 152.1, 153.3, 206.6; IR (film) 1620, 3152; MS (ES^+) m/z 327 (M + H^+); Anal. ($C_{19}H_{18}O_5$): C, H.

3-(2,3-Dihydro-4,7-dimethoxy-6-hydroxybenzofuran-5-yl)-1-phenyl-3-oxopropane (18). A suspension of **16** (162 mg, 0.50 mmol) and 10% Pd on carbon (60 mg) in ethyl acetate (3 mL) was treated with hydrogen at one atmosphere for 16 h. The reaction mixture was filtered through Celite and washed with ethyl acetate, and the filtrate was concentrated in vacuo. The resulting solid was recrystallized from methanol to give **18** (103 mg, 63%) as pale yellow needles: Mp 113−114 °C (methanol); ^{1}H NMR ($CDCl_3$) δ: 3.00 (t, J = 7.5 Hz, 2H), 3.29 (m, 4H), 3.84 (s, 3H), 3.87 (s, 3H), 4.65 (t, J = 8.5 Hz, 2H), 7.16−7.31 (m, 5H); ^{13}C NMR ($CDCl_3$) δ: 28.1, 30.7, 45.0, 59.4, 60.6, 72.9, 108.0, 108.4, 125.9, 128.4, 128.4, 141.5, 154.0, 158.6, 159.2, 204.8; IR (film) 1621; MS (ES^+) m/z 329 (M + H^+); Anal. ($C_{19}H_{20}O_5$): C, H.

1,4-Bis[4,7-dimethoxy-5-(1-phenyl-3-oxopropen-3-yl)-benzofuran-6-yloxymethyl]benzene (19). Compound **16** (34 mg, 0.10 mmol) and α,α′-dibromo-*p*-xylene (13 mg, 0.05 mmol) were treated as described in General Procedure A. The crude product was recrystallized from methanol to give **19** (25 mg, 67%) as a colorless solid: Mp 161−162 °C (methanol); ^{1}H NMR ($CDCl_3$) δ: 3.93 (s, 6H), 4.05 (s, 6H), 4.97 (s, 4H), 6.89 (d, J = 2.2 Hz, 2H), 6.92 (d, J = 15.9 Hz, 2H), 7.24 (d, J = 15.9 Hz, 2H), 7.30−7.35 (m, 6H), 7.39−7.44 (m, 4H), 7.60 (d, J = 2.2 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ: 61.1, 61.2, 105.0, 116.8, 122.4, 128.3, 128.4, 128.8, 128.9, 130.4, 134.6, 136.8, 144.6, 144.9, 145.1, 145.6, 148.8, 194.3; IR (film) 1648; MS (ES^+) m/z 751 (M + H^+); Anal. ($C_{46}H_{38}O_{10}$): C, H.

1,3,5-Tris(5-acetyl-4,7-dimethoxybenzofuran-6-yloxymethyl)benzene (20). Khellinone **1a** (71 mg, 0.3 mmol) and α,α′,α″-tribromomesitylene (36 mg, 0.1 mmol) were treated as described in General Procedure A. The crude product was purified by flash chromatography eluting with ethyl acetate/

dichloromethane (1:19) to afford **20** (60 mg, 73%) as a colorless solid: Mp 122−126 °C; ^{1}H NMR ($CDCl_3$) δ: 2.48 (s, 6H), 3.99 (s, 6H), 4.11 (s, 6H), 5.13 (s, 4H), 6.88 (d, J = 2.1 Hz, 2H), 7.51 (s, 2H), 7.59 (d, J = 2.1 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ: 32.6, 61.1, 61.2, 76.5, 105.0, 116.7, 124.7, 127.7, 134.6, 137.6, 144.0, 144.3, 144.6, 148.6, 201.7; IR (film) 1699; MS (ES^+) m/z 824 ($M + H^+$); HRMS (ES^+) calcd $C_{45}H_{43}O_{15}$ ($M + H^+$) 823.260, found 823.262.

5-Acetyl-6-benzyloxy-4,7-dimethoxybenzofuran (21). To a solution of **1a** (5.0 g, .21 mmol) in acetone (200 mL) were added potassium carbonate (10 g) and benzyl bromide (3.6 g, 21 mmol). The reaction mixture was heated at reflux with vigorous stirring. After 8 h the solid was filtered and the filtrate was evaporated to dryness. The resulting residue was recrystallized from ethanol to give **21** (5.9 g, 85%) as a colorless solid: Mp 88 °C; lit.[37] 86 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ: 2.44 (s, 3H), 3.98 (s, 3H), 4.09 (s, 3H), 5.08 (s, 2H), 6.88 (d, J = 2.3 Hz, 1H), 7.45−7.34 (m, 5H), 7.59 (d, J = 2.3 Hz, 1H).

1,3-Bis[3-(4,7-dimethoxy-6-hydroxybenzofuran-5-yl)-3-oxopropen-1-yl]benzene (22). Khellinone **1a** (94 mg, 0.4 mmol) and isophthalaldehyde (27 mg, 0.2 mmol) were treated as described in General Procedure B. The crude product was recrystallized from THF/ethanol to give **22** (80 mg, 70%) as a brown solid: Mp 166−170 °C (THF/ethanol); ^{1}H NMR ($CDCl_3$) δ: 4.07 (s, 6H), 4.09 (s, 6H), 6.89 (d, J = 2.1 Hz, 2H), 7.49 (t, J = 7.8 Hz, 1H), 7.53 (d, J = 2.1 Hz, 2H), 7.69 (ddd, J = 1.5, 1.5, 7.8 Hz, 2H), 7.84 (d, J = 15.9 Hz, 2H), 7.85 (t, J = 1.5 Hz, 1H), 7.93 (d, J = 15.9 Hz, 2H), 12.62 (s, 2H); ^{13}C NMR ($CDCl_3$) δ: 60.9, 61.9, 105.1, 111.8, 112.7, 127.9, 128.5, 129.8, 135.9, 142.2, 144.2, 150.7, 152.0, 153.1, 196.5; IR (KBr) 1630, 3134; MS (ES^+) m/z 571 ($M + H^+$); HRMS (ES^+) calcd $C_{32}H_{27}O_{10}$ ($M + H^+$) 571.1604, found 571.1599.

1-(4,7-Dimethoxy-6-hydroxybenzofuran-5-yl)-5-phenylpenta-2,4-dien-1-one (23). Khellinone **1a** (236 mg, 1.0 mmol) and cinnamaldehyde (126 μL, 1.0 mmol) were treated as described in General Procedure B. The crude product was recrystallized from dichloromethane/ethanol to give **23** (94 mg, 27%) as an orange solid: Mp 123−125 °C (DCM/ethanol); ^{1}H NMR ($CDCl_3$) δ: 4.02 (s, 3H), 4.06 (s, 3H), 6.85 (d, J = 2.2 Hz, 1H), 7.01 (d, J = 5.4 Hz, 2H), 7.30−7.42 (m, 7H), 7.67 (ddd, J = 5.4, 5.4, 14.8 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ: 60.6, 61.5, 104.9, 105.3, 111.4, 112.2, 126.9, 127.0, 128.5, 128.9, 129.1, 130.0, 135.8, 141.6, 143.7, 143.8, 150.3, 151.5, 152.9, 164.1; IR (film) 1625, 3120; MS (ES^+) m/z 373 ($M + Na^+$); HRMS (ES^+) calcd $C_{21}H_{19}O_5$ ($M + H^+$) 351.123, found 351.122.

3-(4,7-Dimethoxy-6-hydroxybenzofuran-5-yl)-1-(thiophen-2-yl)-3-oxopropene (24). Khellinone **1a** (94 mg, 0.4 mmol) and thiophene-2-carboxaldehyde (37 μL, 0.4 mmol) were treated as described in General Procedure B. The crude product was recrystallized from methanol to give **24** (80 mg, 61%) as an orange solid: Mp 129 °C (MeOH) lit.,[38] 125 °C; ^{1}H NMR ($CDCl_3$) δ: 4.05 (s, 3H); 4.06 (s, 3H), 6.87 (d, J = 2.3 Hz, 1H), 7.08 (dd, J = 3.7, 5.0 Hz, 1H), 7.35 (d, J = 3.7 Hz, 1H), 7.40 (d, J = 5.0 Hz, 1H), 7.50 (d, J = 2.3 Hz, 1H), 7.69 (d, J = 15.3 Hz, 1H), 7.98 (d, J = 15.3 Hz, 1H).

1-(4-Bromothiophen-2-yl)-3-(4,7-dimethoxy-6-hydroxybenzofuran-5-yl)-3-oxopropene (25). Khellinone **1a** (236 mg, 1.0 mmol) and 4-bromo-2-thiophenecarboxaldehyde (118 μL, 1.0 mmol) were treated as described in General Procedure B. The crude product was recrystallized from dichloromethane/ethanol to give **25** (160 mg, 39%) as orange needles: Mp 139 °C (DCM/ethanol); ^{1}H NMR ($CDCl_3$) δ: 4.04 (s, 3H), 4.05 (s, 3H), 6.86 (d, J = 2.3 Hz, 1H), 7.03 (d, J = 3.9 Hz, 1H), 7.07 (d, J = 3.9 Hz, 1H), 7.49 (d, J = 2.3 Hz, 1H), 7.57 (d, J = 15.3 Hz, 1H), 7.84 (d, J = 15.3 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ: 61.0, 61.7, 105.3, 111.5, 112.5 116.3, 126.2, 129.5, 131.4, 132.2, 135.1, 142.4, 144.1, 150.7, 152.0, 153.3, 193.6; IR (film) 1616; MS (ES^+) m/z 409, 411 ($M + H^+$); Anal. ($C_{17}H_{13}BrO_5S$): C, H, Br, S.

3-(4,7-Dimethoxy-6-hydroxybenzofuran-5-yl)-1-(3-hydroxyphenyl)-3-oxopropene (26). Khellinone **1a** (709 mg, 3.0 mmol) and 3-hydroxybenzaldehyde (440 mg, 3.6 mmol) were treated as described in General Procedure B. The crude product was recrystallized from methanol/water to give **26** (609 mg, 60%) as red needles: Mp 157−159 °C (methanol/water); ^{1}H NMR (d_6-acetone) δ: 3.94 (s, 3H), 4.13 (s, 3H), 6.94 (d, J = 7.8 Hz, 1H), 7.15 (d, J = 2.1 Hz, 1H), 7.23 (m, 2H), 7.30 (dd, J = 7.8, 7.8 Hz, 1H), 7.69 (m, 2H), 7.78 (d, J = 2.1 Hz, 1H), 8.55 (br s, 1H); ^{13}C NMR (d_6-acetone) δ: 60.9, 61.8, 106.5, 112.9, 113.0, 115.4, 118.6, 121.1, 128.3, 129.9, 131.0, 140.4, 144.1, 145.3, 151.3, 152.7, 153.0, 158.7, 195.4; IR (film) 1626, 3429; MS (ES^+) m/z 341 ($M + H^+$); Anal. ($C_{19}H_{16}O_6$): C, H.

3-(4,7-Dimethoxy-6-hydroxybenzofuran-5-yl)-1-(3-methoxyphenyl)-3-oxopropene (27). Khellinone **1a** (236 mg, 1.0 mmol) and 3-methoxybenzaldehyde (122 μL, 1.0 mmol) were treated as described in General Procedure B. The crude product was recrystallized from dichloromethane/ethanol to give **27** (213 mg, 60%) as deep red needles: Mp 99 °C (DCM/ethanol); ^{1}H NMR ($CDCl_3$) δ: 3.84 (s, 3H), 4.02 (s, 3H), 4.07 (s, 3H), 6.86 (d, J = 2.1 Hz, 1H), 6.95 (d, J = 7.8 Hz, 1H), 7.14 (s, 1H), 7.23 (d, J = 7.8 Hz, 1H), 7.32 (dd, J = 7.8, 7.8 Hz, 1H), 7.50 (d, J = 2.1 Hz, 1H), 7.81 (m, 2H); ^{13}C NMR ($CDCl_3$) δ: 55.3, 61.0, 61.9, 105.2, 111.9, 112.7, 113.6, 116.1, 121.1, 127.3, 129.5, 129.9, 136.5, 143.3, 144.1, 150.7, 151.9, 153.2, 159.9, 194.7; IR (film) 1623, 3123; MS (ES^+) m/z 355 ($M + H^+$); Anal. ($C_{20}H_{18}O_6$): C, H.

3-(4,7-Dimethoxy-6-hydroxybenzofuran-5-yl)-1-(4-methylphenyl)-3-oxopropene (28). Khellinone **1a** (236 mg, 1.0 mmol) and 4-tolualdehyde (118 μL, 1.0 mmol) were treated as described in General Procedure B. The crude product was recrystallized from dichloromethane/methanol to give **28** (90 mg, 27%) as an orange powder: Mp 148 °C (DCM/methanol); ^{1}H NMR ($CDCl_3$) δ: 2.38 (s, 3H), 4.01 (s, 3H), 4.06 (s, 3H), 6.85 (d, J = 2.1 Hz, 1H), 7.21 (d, J = 7.8 Hz, 2H), 7.49 (d, J = 2.1 Hz, 1H), 7.53 (d, J = 7.8 Hz, 2H), 7.83 (m, 2H); ^{13}C NMR ($CDCl_3$) δ: 21.5, 61.0, 62.0, 105.2, 111.9, 112.8, 125.9, 128.5, 129.5, 129.7, 132.4, 141.1, 143.7, 144.1, 150.7, 151.8, 153.2, 194.7; IR (film) 1625, 3126; MS (ES^+) m/z 339 ($M + H^+$); HRMS (ES^+) calcd $C_{20}H_{18}O_5$ ($M + H^+$) 339.123, found 339.124; Anal. ($C_{20}H_{18}O_5$): C: calcd 71.0 found 69.4, H.

***N,N′*-Bis[1-(4,7-dimethoxy-6-hydroxybenzofuran-5-yl)-ethylidene-1-yl]hydrazine (29). Method 1:** A suspension of succinic acid dihydrazide (146 mg, 1.0 mmol) and khellinone **1a** (472 mg, 2.0 mmol) in methanol (2 mL) was heated at reflux for 36 h. The reaction mixture was concentrated in vacuo, and the resulting residue was filtered, suspended in dichloromethane (25 mL), and washed with dichloromethane (3 × 25 mL). The filtrate was purified by flash chromatography eluting with ethyl acetate/dichloromethane (1:9) to afford **29** (167 mg, 36%) as a yellow powder. An analytical sample was obtained by recrystallization from dichloromethane/ethanol: Mp 167−168 °C (DCM/ethanol); ^{1}H NMR ($CDCl_3$) δ: 2.68 (s, 6H), 4.02 (s, 6H), 4.11 (s, 6H), 6.86 (d, J = 2.1 Hz, 2H), 7.50 (d, J = 2.1 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ: 19.6, 60.9, 61.1, 105.0, 110.9, 112.6, 129.3, 143.4, 149.2, 149.2, 149.8, 168.3; IR (KBr) 1616, 3157; MS (ES^+) m/z 469 ($M + H^+$); Anal. ($C_{24}H_{24}N_2O_8$): C, H, N. **Method 2:** A solution of hydrazide hydrate (25 μL, 0.5 mmol) and khellinone **1a** (236 mg, 1.0 mmol) in methanol was heated at reflux for 16 h. The reaction mixture was concentrated in vacuo and was purified by flash chromatography, eluting with ethyl acetate/dichloromethane (1:9) to afford **29** (60 mg, 26%) as a yellow powder.

1,4-Bis(4,7-dimethoxy-5-[(1*RS*)-hydroxyethyl)]benzofuran-6-yloxy)xylene (30). A mixture of **2** (115 mg, 0.2 mmol) and tetrabutylammonium borohydride (307 mg, 1.2 mmol) in dichloromethane (1 mL) was stirred at room temperature for 48 h, and the reaction mixture was purified by flash chromatography, eluting with dichloromethane to afford **30** (64 mg, 55%) as a colorless solid: Mp 182−187 °C; ^{1}H NMR ($CDCl_3$) δ: 1.52 (d, J = 6.6 Hz, 6H), 3.83 (d, J = 9.0 Hz, 2H), 4.04 (s, 6H), 4.09 (s, 6H), 5.19 (AB, J = 10.8 Hz, 4H), 5.32 (dq, J = 6.9, 9.0 Hz, 2H), 7.05 (d, J = 2.1 Hz, 2H), 7.62 (s, 4H), 7.80 (d, J = 2.1 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ: 25.1, 60.8, 61.1, 65.1, 75.8, 104.8, 116.6, 124.6, 128.4, 134.5, 137.2, 144.1, 145.3, 145.6, 147.4; IR (KBr) 3550; MS (ES^+) m/z 601 ($M + Na^+$); Anal. ($C_{32}H_{34}O_{10}$): C, H.

3-(6-Hydroxy-4-methoxybenzofuran-5-yl)-1-phenyl-3-oxopropene (31). Compound **31** was prepared according to

the General Procedure B from visnaginone **1b** (103 mg, 0.50 mmol) and benzaldehyde (56 μL, 0.55 mmol) and was recrystallized from ethanol (37 mg, 25%) as orange plates: Mp 100−101 °C (EtOH) lit.[36] 106 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ: 4.15 (s, 3H), 6.81 (s, 1H), 6.88 (d, J = 2.4 Hz, 1H), 7.40−7.44 (m, 3H), 7.47 (d, J = 2.4 Hz, 1H), 7.64 (m, 2H), 7.85 (m, 2H); ^{13}C NMR ($CDCl_3$) δ: 61.1, 94.9, 105.1, 110.7, 111.2, 127.3, 128.3, 128.9, 130.3, 135.2, 142.8, 143.6, 155.9, 160.5, 162.1, 194.4.

1,4-Bis(5-acetyl-4-methoxybenzofuran-6-yloxymethyl)-benzene (32). Compound **32** was prepared from visnaginone **1b** (150 mg, 0.73 mmol) and α,α′-dibromo-*p*-xylene (96 mg, 0.36 mmol) according to General Procedure A. Purification by flash chromatography eluting with ethyl acetate/cyclohexane (1:9 to 2:8) afforded the product (20 mg, 11%) as a white solid: Mp 196−197 °C; ^{1}H NMR ($CDCl_3$) δ: 2.51 (s, 6H), 4.05 (s, 6H), 5.10 (s, 4H), 6.80 (s, 2H), 6.86 (d, J = 2.3 Hz, 2H), 7.40 (s, 4H), 7.49 (d, J = 2.3 Hz, 2H); IR (film) 1609; MS (thermospray, LC/MS) m/z 515 ($M + H^+$); Anal. ($C_{30}H_{26}O_8$): C, H

Electrophysiology. The effectiveness of the generated compounds in blocking Kv1.3 was assayed on L929 cells stably expressing mKv1.3[39] or on activated human T cells. 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, Kv1.7, and IKCa1 currents were recorded from cells either stably or transciently expressing these currents as previously described.[39−41] K_d values were determined by fitting the reduction of area under the current curve to the Hill equation.

Calculation of Aqueous Solubility. Calculated aqueous solubility utilized the General Solubility Equation:[42] $\log S_w = 0.5 - \log K_{ow} - 0.01(MP - 25)$, where K_{ow} is the octanol−water partition coefficient of the solute and MP is its melting point in °C (for oils, MP is set to 25). In our case, we used ClogP values for the lipophilicity term, and hence have renamed log S_w to ClogS_w. For compounds **2**, **6**, **7**, **8**, **15**, **16**, **26,** and **27** this gave respective ClogS_w values of −6.55, −4.38, −5.55, −5.44, −4.32, −4.81, −4.38, and −4.47.

Cytoxoticity as Flow Cytometric Measurement of Cell Viability. Jurkat E6-1 and MEL were seeded at 5×10^5 cells/mL in 12-well plates. Compounds **16** and **24**−**27** at 100 nM, 1 μM, 2.5 μM, and 10 μM) were added in a final DMSO concentration of 0.01%. After 48 h of incubation, cells were harvested by sucking them off the plates. Cells were centrifuged, resuspended in 0.5 mL of PBS containing 1 μg/mL propidium iodide (PI), and red fluorescence measured on a FACScan flow cytometer (Becton Dickinson) after 20 min (10^4 cells of every sample being analyzed). The percentage of dead cells was determined by their PI uptake. Incubation with 20% DMSO served as a control for setting the gates of the flow cytometer for dead cells.

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 (60 min) and then stimulated with 50 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 measured in a scintillation counter. All experiments were done in triplicate. Results are reported as normalized for maximum [^{3}H]thymidine incorporation for controls.

Acute in Vivo Toxicity Determination. Five CF-1BR mice (17−19 g) were injected intravenously with a single 1.0 mL dose of 5 mg/kg **7**, **16**, **26**, or **27** in mammalian Ringer solution with 1% ethanol and 2.5% bovine serum albumin. Five control mice were injected with an equal volume of the vehicle. Mice were observed for adverse effects immediately after dosing, at 4 h after injection and daily for 7 days.

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Supporting Information Available: Structures and Kv1.3 blocking data for less active compounds. Crystal data, atomic coordinates, anisotropic displacement parameters, and geometrical parameters of **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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