Angular Methoxy-Substituted Furo- and Pyranoquinolinones as Blockers of the Voltage-Gated Potassium Channel Kv1.3

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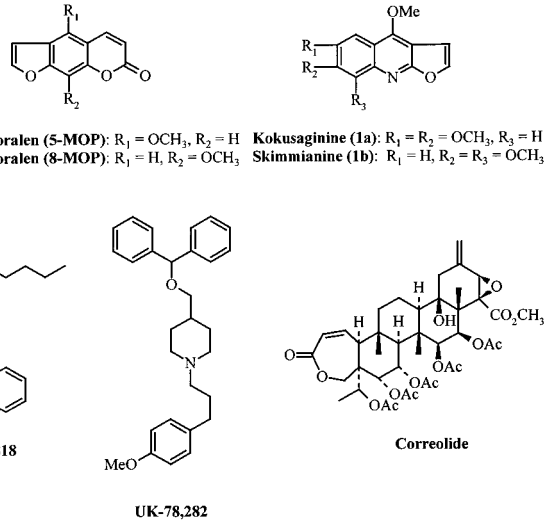
The voltage-gated potassium channel Kv1.3 constitutes an attractive target for immunosuppression because of its role in T-lymphocyte activation and its functionally restricted expression to lymphocytes. Blockade of Kv1.3 channels by margatoxin has previously been shown to prevent T-cell activation and attenuate immune responses in vivo. In the present study, several furo- and pyranoquinoline derivatives were synthesized and screened for their blocking activities of Kv1.3 channels, stably expressed in mice-fibroblasts L929. In addition the activities of the compounds on Kv currents of the neuroblastoma cell line N1E-115 were determined. The most potent compounds, the angular furoquinolinone 8-methoxy-2-(1′-methylethyl)-5-methyl-4,5-dihydrofuro[3,2-*c*]quinolin-4-one (**8c**) and the angular pyranoquinolinone 9-methoxy-2,2,6-trimethyl-2,6-dihydro-5*H*-pyrano[3,2-*c*]quinolin-5-one (**9a**), inhibited Kv1.3 channels with half-blocking concentrations of 5 and 10 μM, respectively, and displayed 8-fold (**8c**) and 2-fold (**9a**) selectivity over Kv currents of N1E-115 cells. Thus, compounds **8c** and **9a** might function as a template for the development of novel immunosuppressants.

Introduction

In voltage clamp experiments on amphibian nodes of Ranvier psoralens, benzofurans, coumarins, acridones, and furoquinolines occurring in *Ruta graveolens* were found to block delayed rectifier K^+ currents (Chart 1).[1] Initially our group focused on alkoxypsoralens as promising compounds which in addition were tested for their affinities to a number of homomeric K^+ channels.[2] The results of these testings revealed that in addition to moderate affinities to the *Shaker*-type K^+ channels Kv1.1 and Kv1.2, which are proposed to form the paranodal K^+ channels in mammals,[3] alkoxypsoralens have high affinities for Kv1.3. The *Shaker*-related K^+ channel Kv1.3 is involved in the control of membrane potential, production of lymphokines, and proliferation of human T-lymphocytes.[4,5] Inhibition of Kv1.3 channels causes depolarization which reduces the electrochemical driving force for the increase in intracellular Ca^{2+} concentration required for lymphocyte activation: stimulation of gene transcription, production of lymphokines (e.g. interkeukin 2, IL-2), DNA synthesis, and finally cell division.[4−6] The Kv1.3 channel has a limited distribution in the periphery, being found mainly in B-cells, macrophages, platelets, osteoclasts, and fibroblasts, besides T-lymphocytes. In addition, the K^+ channel in human T-cells is a homomultimer of Kv1.3 subunits in contrast to the heterotetrameric form of channels in brain, where Kv1.3 is combined with at least Kv1.1 and Kv1.2. Thus, a selective Kv1.3 blocker may have ion channel selectivity against other tissues.[6]

Using peptidyl inhibitors, such as margatoxin (MgTX), several groups have demonstrated that blockade of Kv1.3 channels suppresses activation and proliferation of human T-cells.[7] MgTX has been shown to suppress delayed-type hypersensitivity and allogenic−antibody responses in miniswine,[8] providing in vivo evidence that Kv1.3 is a novel pharmacological target for immunosuppressive therapy.[6,9] Potent, specific Kv1.3 inhibitors have the potential to be novel immunosuppressive agents with utility in transplantation, autoimmune disease, and inflammation therapy.[6] Efforts to identify a small, nonpeptidyl inhibitor of Kv1.3 channels have resulted in the discovery of submicromolar Kv1.3 blockers such as the dihydroquinoline CP-339,818,[10] the piperidine UK-78,282,[11] and correolide, a triterpene isolated from the root and bark of a Costa Rican tree, *Spachea correae* (Chart 1).[12−14] However, both CP-339,-818 (half-blocking concentration, IC_{50}, for Kv1.3 channels: 0.23 μM)[10] and UK-78,282 (IC_{50}(Kv1.3) = 0.28 μM)[11] block Kv1.3 and Kv1.4, a *Shaker*-related K^+

Chart 1. Chemical Structures of Potassium Channel Blocking Psoralens and Furoquinolines from *R. graveolens* and of Kv1.3 Blockers



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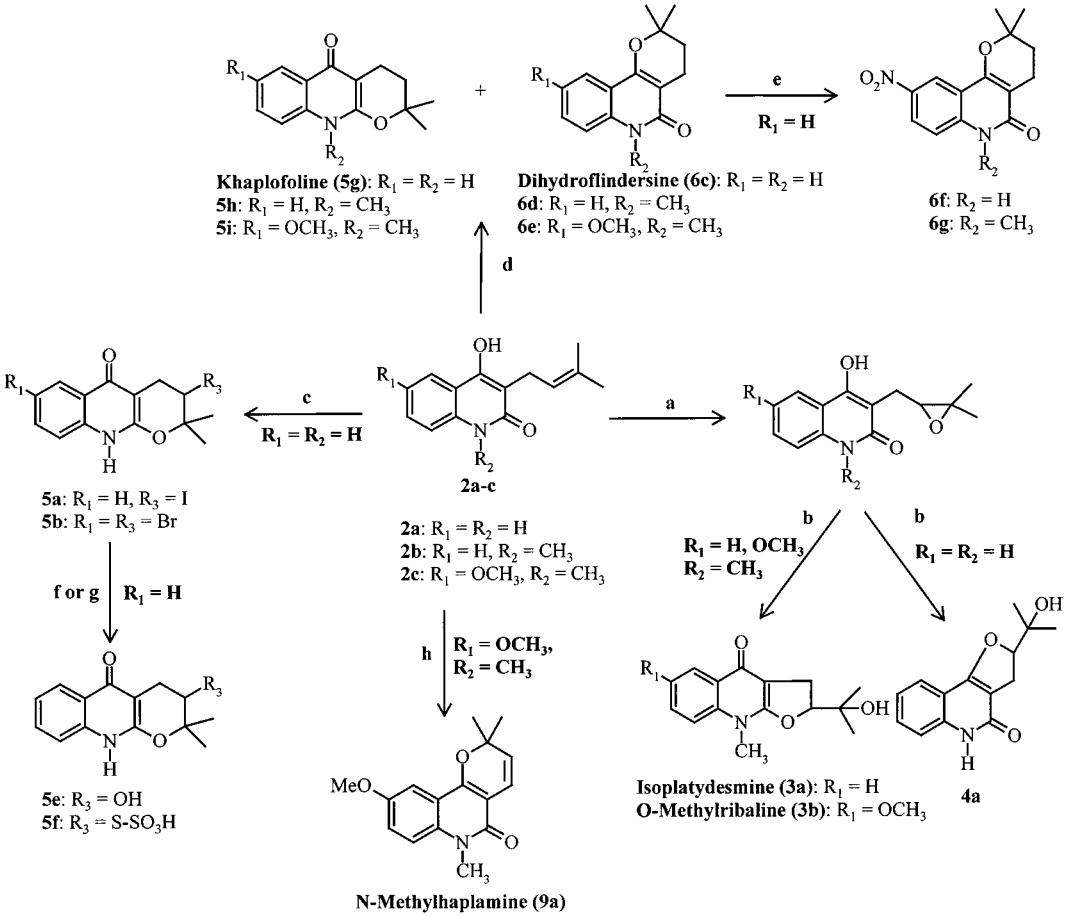
Scheme 1[a]



[a] Reagents: (a) *m*-chloroperbenzoic acid, $CHCl_3$; (b) 3 M HCl or 3 M NaOH; (c) I_2 or Br_2, AgOAc, AcOH; (d) concd HCl; (e) concd HNO_3/concd H_2SO_4, 7 min (**6f**) or 25 min (**6g**); (f) 0.05 M K_2CO_3 (**5e**); (g) $Na_2S_2O_3$, 0.5 M NaOH (**5f**); (h) DDQ.

channel expressed in the heart and brain, with similar potency,[10,11] thus lacking selectivity. The most promising compound seems to be correolide, which has a 4−14-fold selectivity for Kv1.3 over other *Shaker*-related Kv channels.[14]

By investigating the furoquinoline alkaloid kokusaginine (**1a**) for its affinity to homomeric *Shaker*-type K^+ channels, our group identified the compound (Chart 1) as a blocker of Kv1.3 in the low-micromolar range.[15] This finding raised the possibility of discovering more Kv1.3 blockers among the alkaloids from *R. graveolens*.

In the present study, we screened linear and angular furo- and pyranoquinoline alkaloids on mice-fibroblasts L929, stably transfected with *m*Kv1.3. In addition all compounds were investigated for their effects on Kv and Na^+ channels of the neuroblastoma cell-line N1E-115 to determine potential selectivity for Kv1.3.

Chemistry

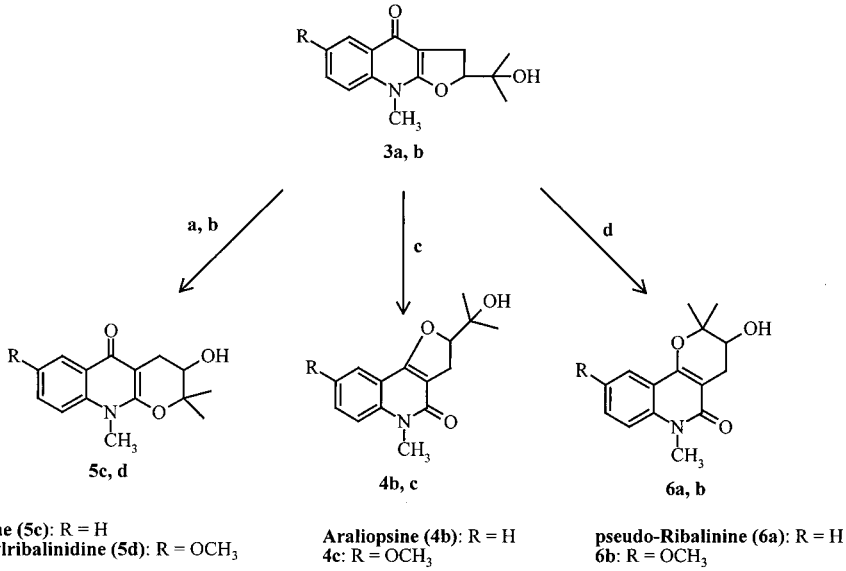
Central intermediates for the synthesis of both dihydrofuro- and dihydropyranoquinolinones are substituted 4-hydroxy-3-(3-methylbut-2-enyl)quinolin-2-ones **2a**−**c**.[16−19] Oxidative cyclization of **2b**,**c** with *m*-chloroperbenzoic acid and ring closure of the intermediate epoxide with 3 M hydrochloric acid gave the linear dihydrofuro-[2,3-*b*]quinolinones isoplatydesmine (**3a**) and *O*-methylribaline (**3b**) (Scheme 1).[17,18] In contrast to earlier findings[19] basic workup of these epoxides also resulted in the linear products. The epoxide of **2a**, however, exclusively afforded the angular dihydrofuro[3,2-*c*]-quinolinone **4a** under both acidic and basic conditions (Scheme 1).

Oxidative cyclization of **2a** with iodine and silver acetate gave the linear dihydropyrano[2,3-*b*]quinolinone **5a** according to the method of Subramanian et al. (Scheme 1).[20] Utilizing bromine instead of iodine, the main product is the linear 3,7-dibromodihydropyrano-quinolinone **5b** (Scheme 1).

The formation of pyranoquinolinones under these conditions in contrast to the formation of furoquinolinones in the epoxide route can be accounted for mechanistically. In the reaction pathway with halogen, the halogen is first added to the double bond of the prenylquinolinone, which then spontaneously undergoes intramolecular cyclization by an S_N1 reaction involving a tertiary carbonium ion. Thus, the cyclization proceeds via the electronically favored 6-*endo* process. In contrast, S_N2 cyclization of the epoxides and ring closure by the stereochemically favored 5-*exo* process is the most plausible explanation for the formation of furoquinolinones in the peracid route. This explanation agrees with Baldwin's suggestions that in the opening of three-membered rings to form cyclic structures *exo* modes are generally preferred on stereochemical grounds.[21]

The reaction pathway with iodine and silver acetate failed in the case of the *N*-methyl-substituted adducts **2b**,**c**. Thus, the isomeric linear dihydropyranoquinolinones **5c,d** to isoplatydesmine (**3a**) and *O*-methylriba-

Scheme 2. Rearrangement Reactions of Linear Dihydrofuroquinolinones[a]



[a] Reagents: (a) pyridine, $(Ac)_2O$; (b) 0.3 M NaOH, MeOH; (c) $NaOCH_3$, MeOH; (d) 3 M NaOH, MeOH.

line (**3b**) were synthesized by rearrangement reaction of the dihydrofuroquinolinones in pyridine and acetic anhydride according to the method of Rapoport and Holden (Scheme 2).[22]

The corresponding angular dihydrofuro- and dihydropyranoquinolinones were also obtained via rearrangement reactions of linear dihydrofuroquinolinones. Utilizing the methods of James and Grundon, angular dihydrofuroquinolinones are formed with sodium methoxide in methanol for 20 h at room temperature, whereas angular dihydropyranoquinolinones are obtained with sodium hydroxide in methanol when heated for 18 h under reflux (Scheme 2).[23]

James and Grundon intensely studied the base-catalyzed rearrangement reactions by means of the dihydrofuroquinolinone balfourodine and its isomers.[23] They found that starting with either the linear dihydrofuroquinolinone or the linear dihydropyranoquinolinone, the angular dihydrofuroquinolinone is formed at room temperature under kinetic control and that the thermodynamically more stable angular dihydropyranoquinolinone results from an equilibrium-controlled reaction at elevated temperatures. Furthermore, under thermodynamic control the angular dihydrofuroquinolinone isomerizes to the angular dihydropyranoquinolinone, the latter being unaffected by treatment with alkali.

Our findings for isoplatydesmine (**3a**) and *O*-methylribaline (**3b**) and their isomers are in accordance with the results of James and Grundon. Table 1 shows the calculated heats of formation of isoplatydesmine (**3a**) and *O*-methylribaline (**3b**) and their isomers after geometry optimization with AM1.

Thus, the linear dihydrofuroquinolinones are thermodynamically the least stable isomers, whereas the angular dihydropyranoquinolinones are the most stable isomers, with the linear dihydropyranoquinolinones and the angular dihydrofuroquinolinones having intermediate stabilities (Scheme 1).

The linear dihydropyranoquinolinones **5e,f** were synthesized as illustrated in Scheme 1. The synthesis of **5e** is a modification of the method of Subramanian et al.[20]

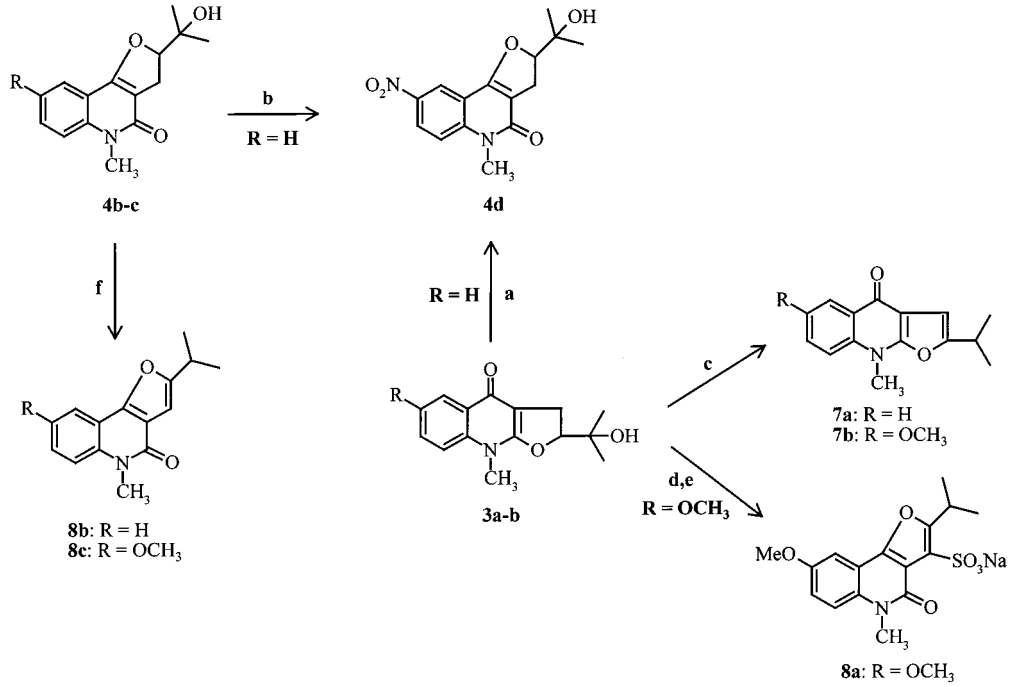
Table 1. Heats of Formation (kcal/mol) Calculated by AM1, HyperChem, Version 5.0

linear dihydrofuro-quinolinones	linear dihydropyrano-quinolinones	angular dihydrofuro-quinolinones	angular dihydropyrano-quinolinones
−70.8 (**3a**)	−74.9 (**5c**)	−76.6 (**4b**)	−86.5 (**6a**)
−108.2 (**3b**)	−112.2 (**5d**)	−113.5 (**4c**)	−123.4 (**6b**)
	−42.8 (**5g**)		−50.2 (**6c**)
	−34.7 (**5h**)		−42.8 (**6d**)
	−72.0 (**5i**)		−79.7 (**6e**)
	−39.2 (isomer to **6f**)		−46.8 (**6f**)
	−31.3 (isomer to **6g**)		−39.5 (**6g**)
−67.1 (isomer to **4d**)		−73.3 (**4d**)	

Dihydropyranoquinolinones without substitution in position 3 are obtained via acid-catalyzed ring closure of 3-prenylquinolinones. Refluxing **2a**−**c** in concentrated hydrochloric acid resulted in mixtures of linear (**5g**−**i**) and angular (**6c**−**e**) dihydropyranoquinolinones, the latter being the main products (Scheme 1).[24,25] Calculated heats of formation for the compounds support higher thermodynamic stability of the angular over the linear dihydropyranoquinolinones (Table 1).

The dihydropyranoquinolinones **6f**,**g** were obtained via nitration of **6c**,**d** (Scheme 1). Analytical samples of the linear isomers were synthesized by nitration of khaplofoline (**5g**) and **5h** to establish structural identity of **6f**,**g**. The angular dihydrofuroquinolinone **4d** was prepared by nitration of either isoplatydesmine (**3a**) by a reaction time of 15 h or araliopsine (**4b**) for 30 min (Scheme 3). Nitration of isoplatydesmine for 30 min gave the linear 5-nitroisoplatydesmine in small amount. Acid-catalyzed rearrangements of linear dihydrofuroquinolinones are reported previously, but in combination with dehydration.[26] This is the first study to present acid-catalyzed isomerization of a linear dihydrofuroquinolinone (isoplatydesmine, **3a**) to an angular dihydrofuroquinolinone (8-nitroaraliopsine, **4d**) without dehydration.

Scheme 3. Nitration and Acid-Catalyzed Dehydration and Isomerization of Dihydrofuroquinolinones[a]



[a] Reagents: (a) concd HNO_3/concd H_2SO_4, 15 h; (b) concd HNO_3/concd H_2SO_4, 30 min; (c) concd H_2SO_4, 1 min; (d) concd H_2SO_4, 24 h; (e) 3 M NaOH; (f) concd H_2SO_4, 3 min (**8b**) or 15 min (**8c**).

Gaston and Grundon obtained a mixture of dehydrated linear and angular furoquinolinones when keeping linear dihydrofuroquinolinones in sulfuric acid with the proportion of the two isomers depending on the reaction time.[26] After treatment of linear dihydrofuroquinolinones with sulfuric acid for a few minutes, linear furoquinolinones are the main products, whereas angular furoquinolinones are predominant after 1 or 2 h. Our findings concerning the dehydration of isoplatydesmine (**3a**) or *O*-methylribaline (**3b**) with sulfuric acid for a few minutes confirm the formation of linear furoquinolinones (**7a**,**b**) under these conditions (Scheme 3).

When treating **3a**,**b** with sulfuric acid for 2 h or longer the isolation of the angular furoquinolinones was complicated by the partial sulfonation in position 3 of the angular furoquinolinones. Treatment of *O*-methylribaline (**3b**) with sulfuric acid for 24 h gave the sodium salt of the angular furoquinoline-3-sulfonic acid **8a** (Scheme 3). Thus, direct dehydration of angular dihydrofuroquinolinones **4b**,**c** was our favored method for synthesizing the angular, dehydrated furoquinolinones **8b**,**c** (Scheme 3). Again the calculated heats of formation for the furoquinolinones **7a,b** and **8b,c** support the higher thermodynamic stability of the angular isomers: **7a**: −2.1 kcal/mol and **8b**: −8.6 kcal/mol; **7b**: −39.4 kcal/mol and **8c**: −45.6 kcal/mol.

N-Methylhaplamine (**9a**) was prepared via dehydrocyclization of **2c** with DDQ (2,3-dichloro-5,6-dicyanobenzoquinone) according to the analogous synthesis of haplamine (Scheme 1).[27]

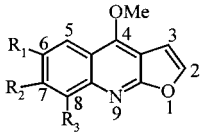
Pharmacology

Blockade of Kv Channels of the Neuroblastoma Cell Line N1E-115. To rapidly assess the effects of the quinolines on Kv channels, they were screened on the neuroblastoma cell-line N1E-115 in the whole-cell configuration of the patch-clamp technique. The K^+ current of N1E-115 cells is generated by at least two types of channels. The first has gating kinetics best classified as a delayed rectifier; the second type of channel activates more slowly and − in contrast to the delayed rectifier which partially inactivates during a 100-ms pulse − does not inactivate during this time.[28] The delayed rectifier of N1E-115 cells is generated by the *Shaw*-type K^+ channel Kv3.1.[29]

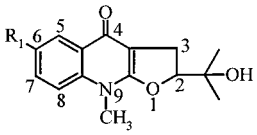
The blockade of peak K^+ currents (B_K) was determined by normalizing the peak steady-state K^+ currents in the test solution at pulse amplitudes of 40 mV to the corresponding K^+ currents in normal bathing medium. The accompanying blockade of peak Na^+ currents (B_{Na}), measured at pulse amplitudes of 0 mV, was defined in the same way. The results are shown in Tables 2 and 3. Unless otherwise stated the compounds were tested at 50 μM.

Blockade of Kv1.3 Channels. To investigate the effects of the quinolines on Kv1.3 channels, most compounds were tested for their ability to block K^+ currents of mice fibroblasts L929, stably transfected with *m*Kv1.3 using the whole-cell configuration of the patch-clamp technique. Blockade of the steady-state peak K^+ currents (B_K) was determined in the same way as described above for the N1E-115 cells. The results are shown in Tables 2 and 3. Again the compounds were tested at 50 μM unless otherwise stated. For the most potent compounds we determined half-blocking concentrations for the blockade of Kv1.3 currents as well as for the blockade of Kv channels of N1E-115 by testing different concentrations and fitting a modified Hill equation to the data points. The results are given in Table 4.

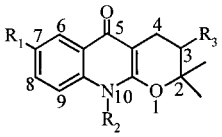
Table 2. Compound Structures of Linear Furoquinolines and Linear Furo- and Pyranoquinolinones and Block of Peak K^+ Currents of N1E-115 and Kv1.3[a]



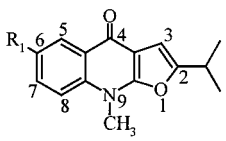
1a-c



3a-c



5b-j



7a-b

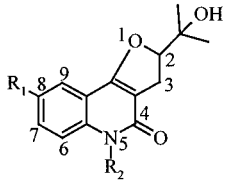
compd	R_1	R_2	R_3	B_K (%) N1E-115	B_{Na} (%) N1E-115	B_K (%) Kv1.3
1a	OCH_3	OCH_3	H	22	0	51
1b	H	OCH_3	OCH_3	20	6	13
1c	H	H	H	31	0	23
3a	H			0	0	1
3b	OCH_3			0 (25 μM)	0 (25 μM)	1 (25 μM)
3c	OH			7 (100 μM)	13 (100 μM)	nd
5b	Br	H	Br	32	1	35
5c	H	CH_3	OH	0	0	nd
5d	OCH_3	CH_3	OH	6	3	nd
5e	H	H	OH	0 (100 μM)	0 (100 μM)	nd
5f	H	H	S_2O_3H	0 (100 μM)	6 (100 μM)	1 (25 μM)
5g	H	H	H	6	−18	7
5h	H	CH_3	H	nd	nd	4 (25 μM)
5i	OCH_3	CH_3	H	7	0	10
5j	OH	CH_3	OH	0 (100 μM)	3 (100 μM)	nd
7a	H			15	0	23
7b	OCH_3			33	1	44

[a] B_K N1E-115: blockade of peak steady-state K^+ currents of neuroblastoma cells N1E-115 normalized to the corresponding K^+ currents in normal bathing medium (pulse amplitude 40 mV, pulse duration 100 ms); B_{Na} N1E-115: accompanying blockade of peak Na^+ currents (pulse amplitude 0 mV); B_K Kv1.3: blockade of peak steady-state K^+ currents of Kv1.3 channels (pulse amplitude 40 mV, pulse duration 200 ms). Compounds were tested at 50 μM unless otherwise stated; blockades are given as mean of at least three experiments; nd, not determined.

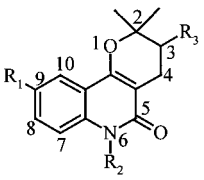
Discussion

The most striking result of the patch-clamp experiments is the higher potency of angular isomers in blocking Kv channels as compared to linear isomers. Angular dihydrofuroquinolinones (**4a**−**d**) which were found to be ineffective (Table 3) are the only exception. Linear dihydrofuroquinolinones (**3a**−**c**) and linear dihydropyranoquinolinones (**5b**−**j**) affected neither the Kv channels of N1E-115 nor the Kv1.3 currents (Table 2). The only exception was **5b**, which showed a weak blocking effect. The blocking activity of **5a** was not determined due to its limited solubility. Angular dihydropyranoquinolinones were in general more potent than their linear isomers (Table 3): angular dihydropyranoquinolinones bearing hydroxy groups (**6a,b**) displayed weak blocking activities, whereas angular dihydropyranoquinolinones lacking the hydroxy group (**6c**−**e**) were found to block both Kv channels of N1E-115 and Kv1.3 currents and N-alkylation increased the blocking activity of the angular dihydropyranoquinolinones on Kv currents of both cell lines (Table 3). The substituent in position 9 of the angular dihydropyranoquinolinones was important for their selectivity: Compounds **6c**,**d** without substitution in position 9 were found to be more potent blockers of Kv channels of N1E-115, whereas a methoxy group (**6e**) increased the blocking activity of Kv1.3 currents. Nitro substituents (**6f,g**) greatly diminished the potency (Table 3).

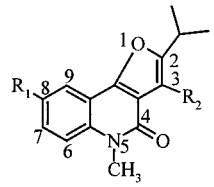
Table 3. Compound Structures of Angular Furo- and Pyranoquinolinones and Block of Peak K^+ Currents of N1E-115 and Kv1.3[a]



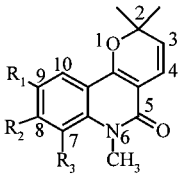
4a-d



6a-g



8a-c



9a-b

compd	R_1	R_2	R_3	B_K (%) N1E-115	B_{Na} (%) N1E-115	B_K (%) Kv1.3
4a	H	H		7	0	nd
4b	H	CH_3		4 (25 μM)	0 (25 μM)	3 (25 μM)
4c	OCH_3	CH_3		0 (25 μM)	0 (25 μM)	0 (25 μM)
4d	NO_2	CH_3		4	0	nd
6a	H	CH_3	OH	15	4	4 (25 μM)
6b	OCH_3	CH_3	OH	21	2	4
6c	H	H	H	37	3	15
6d	H	CH_3	H	65	7	48
6e	OCH_3	CH_3	H	39 (25 μM)	3 (25 μM)	57 (25 μM)
				52	4	83
6f	NO_2	H	H	23	8	nd
6g	NO_2	CH_3	H	14 (25 μM)	0 (25 μM)	7 (25 μM)
8a	OCH_3	SO_3Na		0 (25 μM)	0 (25 μM)	nd
8b	H	H		25 (25 μM)	0 (25 μM)	44 (25 μM)
				42	11	70
8c	OCH_3	H		32 (25 μM)	5 (25 μM)	98 (25 μM)
				57	11	98
9a	OCH_3	H	H	44 (25 μM)	1 (25 μM)	88 (25 μM)
				64	12	93
9b	H	OCH_3	OCH_3	55	12	41

[a] B_K N1E-115: blockade of peak steady-state K^+ currents of neuroblastoma cells N1E-115 normalized to the corresponding K^+ currents in normal bathing medium (pulse amplitude 40 mV, pulse duration 100 ms); B_{Na} N1E-115: accompanying blockade of peak Na^+ currents (pulse amplitude 0 mV); B_K Kv1.3: blockade of peak steady-state K^+ currents of Kv1.3 channels (pulse amplitude 40 mV, pulse duration 200 ms). Compounds were tested at 50 μM unless otherwise stated; blockades are given as mean of at least three experiments; nd, not determined.

Table 4. Half-Blocking Concentrations of Selected Furo- and Pyranoquinolines for Blockade of Kv1.3 Channels and N1E-115 Kv Currents[a]

	$IC_{50} \pm$ SEM (μM)		
compd	Kv1.3	N1E-115	$S \pm$ SEM
8c	5 ± 1	38 ± 3	7.6 ± 2.2
6e	18 ± 1	42 ± 3	2.3 ± 0.3
9a	10 ±1	31 ± 4	3.1 ± 0.7
1a	49 ± 7	200 ± 30	4.1 ± 1.2

[a] IC_{50} values are the mean of at least three experiments. *S*: selectivity of Kv1.3 current blockade over N1E-115 Kv channel blockade given by IC_{50}(N1E-115)/IC_{50}(Kv1.3).

The second remarkable result is the higher potency of furoquinoline derivatives with an aromatic furan ring compared to dihydrofuroquinolinones. The linear furoquinolines kokusaginine (**1a**), skimmianine (**1b**), and dictamnine (**1c**) as well as the linear dehydrated furoquinolinones (**7a,b**) were in general more potent than dihydrofuroquinoline derivatives (Table 2). Of these linear furoquinolines kokusaginine (**1a**) seems to be the most promising compound, blocking Kv1.3 currents in the low-micromolar range and with higher potency than the N1E-115 K^+ current. Again the angular isomers (**8b,c**) displayed higher affinities with the exception of **8a**, the sodium salt of a sulfonic acid, being ineffective, thus corroborating that hydrophilic substituents diminish potency (Table 3). Finally, the pyranoquinolinone **9a** showed a slightly higher blocking activity than the corresponding hydrogenated derivative (**6e**). The dehydrated pyranoquinolinone veprisine (**9b**) bearing two methoxy groups in positions 7 and 8 was less effective than **9a**. Moreover, in contrast to veprisine (**9b**) compound **9a** as well as **8b**,**c** displayed higher blocking activities of Kv1.3 currents compared to Kv channels of N1E-115 (Table 3). Na^+ currents were largely uneffected by all compounds tested (Tables 2 and 3). Thus, the effective quinolines are selective blockers of Kv channels. The compounds **6e**, **9a**, and especially **8c** were found to be the most potent and moreover selective blockers of Kv1.3 channels, showing a much higher potency than the furoquinoline kokusaginine. For these effective compounds we determined the half-blocking concentrations for N1E-115 and Kv1.3. The IC_{50} values are listed in Table 4.

In summary, the following pharmacophoric model for the Kv channel blocking activity of the quinolines can be deduced. First, an angular structured furo- or pyranoquinolinone seemed to be required for optimal interaction with a hydrophobic pocket of limited size at the receptor site of Kv1.3 channels as well as Kv channels of N1E-115 cells. Moreover, it is striking that the most potent compounds (**8c** and **9a**) on Kv currents have a double bond in the furan or pyran ring in common, thus pointing toward $\pi-\pi$ interaction with the receptor site. Hydrophilic substituents such as hydroxy or sulfonic groups in this region diminish potency almost completely. For a favored binding to the receptor site of Kv1.3 the methoxy group in position 8 of the angular furoquinolinones **8c** or in position 9 of the angular pyranoquinolinones **6e** and **9a** is of crucial importance. Compound **8c** reveals 8-fold selectivity (Table 4) for Kv1.3 over Kv channels of N1E-115 cells, whereas **8b** lacking a substituent in the quinoline ring is much less selective. Compound **6e** reveals 2-fold selectivity (Table 4) for Kv1.3, and **6d** lacking the methoxy group is even more potent for Kv currents of N1E-115 cells just like the 7,8-dimethoxy-substituted angular pyranoquinolinone veprisine (**9b**). Thus, the substitution pattern in the quinoline ring is critical for Kv1.3 selectivity.

Conclusion

The synthesis, pharmacological testing, and resulting structure−activity relationship (SAR) of a series of furo- and pyranoquinolines as blockers of voltage-gated K^+ channels have been described. With **8c**, **6e**, and **9a** we succeeded in synthesizing compounds with Kv1.3 channel blocking activity greater than that of kokusaginine. Moreover they were shown to be selective for Kv1.3 compared to the Kv channel blockade of N1E-115 cells. The selectivity must be further investigated, using other closely related homomeric Kv channels. Although the substances might still lack the high selectivity and potency required for a therapeutic drug, they might serve as templates for the design of Kv1.3-selective K^+ blockers.

Experimental Section

Melting points were determined with a Reichert Thermovar melting point apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker ARX 300 spectrometer, and chemical shifts (ppm) are reported relative to TMS. Signals are designated as follows: br (broad), s (singlet), d (doublet), dd (doublet of doublets), ddd (doublet of doublets of doublets), t (triplet), sept (septet), m (multiplet), ex (exchangeable with D_2O). To establish structural identity of the new compounds the key problem is to distinguish between linear and angular isomers as well as between furo and pyrano isomers. NMR data of known isomeric alkaloids are essential to confirm structures by comparison. These data as well as ^{13}C NMR data of new compounds recorded on a 300-MHz spectrometer are available as Supporting Information. Elemental analyses were performed by a Hewlett-Packard CHN-autoanalyzer and were within ±0.4% of the theoretical values. Elemental analyses of **5f**, **8a**,**c** were carried out by Ilse Beetz, Mikroanalytisches Laboratorium, Industriestr. 10, 96317 Kronach, and are within ±0.4% of the theoretical values. Yields were not optimized. Compounds **2a**,[16] **2b**,[18] **2c**,[19] **3a**,[18] **3b**,[19] **4c**,[19] **5a**,[20] **5c**,[18] **5d**,[18] **5g**,[25] **6c**,[25] **6d**,[30] **7a,b**[26] were synthesized as described previously. Ribaline (**3c**)[31] and ribalinidine (**5j**)[31] were a friendly gift of Dr. L. Jurd, Western Regional Research Center, Berkeley, CA. Veprisine (**9b**),[32] dictamnine (**1c**),[33] and skimmianine (**1b**)[34] were a generous gift of Prof. Dr. O. Schimmer, Friedrich-Alexander-University, Erlangen-Nürnberg. Kokusaginine (**1a**) was isolated from the herb of *R. graveolens* as described previously.[35]

2-(1′-Hydroxy-1′-methylethyl)-2,3,4,5-tetrahydrofuro-[3,2-*c*]quinolin-4-one (4a). A solution of **2a** (460 mg, 2 mmol) and *m*-chloroperbenzoic acid (55% purity, 700 mg, 4 mmol) in $CHCl_3$ (20 mL) was left 3 days at room temperature. To remove the *m*-chlorobenzoic acid the $CHCl_3$ phase was extracted once with 20 mL 2 M Na_2CO_3, washed with water and dried. After evaporation of the $CHCl_3$, the residue was suspended in 10 mL Me_2CO, collected by vacuum filtration and washed with 10 mL Me_2CO (210 mg, 40%): mp 239 dec; ^1H NMR (300 MHz, DMSO-d_6) δ 1.17 and 1.19 (2 × s, 6H, $C(CH_3)_2$), 2.99 (br dd, 2H, $-CH_2-$), 4.72 (br s, 1H, ex, OH), 4.83 (br dd, 1H, 2-H), 7.17 (ddd, 1H, $^3J = 7.5$ Hz and $^4J = 1.0$ Hz, 8-H), 7.33 (br dd, 1H, $^3J = 8.1$ Hz, 6-H), 7.50 (ddd, 1H, $^3J = 7.8$ Hz and $^4J = 1.2$ Hz, 7-H), 7.61 (dd, 1H, $^3J = 8.0$ and $^4J = 1.2$ Hz, 9-H), 11.33 (s, br, 1H, ex, NH). Anal. ($C_{14}H_{15}NO_3 \cdot H_2O$) C, H, N.

2-(1′-Hydroxy-1′-methylethyl)-5-methyl-8-nitro-2,3,4,5-tetrahydrofuro[3,2-*c*]quinolin-4-one (4d). H_2SO_4 (1.2 mL of 18 M) was added dropwise to 65% HNO_3 (1 mL) with ice cooling. To the resulting solution, solid **3a** (520 mg, 2 mmol) was added slowly in portions with intense stirring. The mixture was stirred 15 h at room temperature, then poured into cold water (40 mL) and neutralized with 3 M NaOH. The precipitate was collected by vacuum filtration, washed with water and recrystallized from water (243 mg, 40%): mp 189 °C dec; ^1H NMR (300 MHz, DMSO-d_6) δ 1.16 and 1.24 (2 × s, 6H, $C(CH_3)_2$), 3.07 (d, 2H, $^3J = 9.2$ Hz, $-CH_2-$), 3.64 (s, 3H, $-N-CH_3$), 4.79 (br s, 1H, ex, OH), 4.92 (t, 1H, $^3J = 9.2$ Hz, 2-H), 7.74 (d, 1H, $^3J = 9.2$ Hz, 6-H), 8.36−8.44 (m, 2H, 7-H and 9-H). Anal. ($C_{15}H_{16}N_2O_5$) C, H, N.

3,7-Dibromo-2,2-dimethyl-2,3,4,10-tetrahydro-5*H*-pyrano[2,3-*b*]quinolin-5-one (5b). 2a (920 mg, 4 mmol) was dissolved in glacial AcOH (60 mL) and AgOAc (670 mg, 4 mmol) was added. The suspension was stirred at room temperature and 3.2 g (40 mmol) Br_2 were added. Stirring was

continued for 12 h. The precipitated AgBr was filtered and washed with $CHCl_3$. The combined filtrate and the washings were diluted with water and extracted with $CHCl_3$. The extract was successively washed with dilute solutions of $NaHCO_3$, $Na_2S_2O_3$ and finally with water and dried. The $CHCl_3$ was evaporated and the residue was repeatedly recrystallized from aqueous EtOH (230 mg, 15%): mp 241 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.50 and 1.53 (2 × s, 6H, C(CH_3)$_2$), 2.94 (dd, 1H, ^2J = 17.6 Hz and ^3J = 5.1 Hz, 4-H), 3.15 (dd, 1H, ^2J = 17.6 Hz and ^3J = 5.0 Hz, 4-H), 4.75 (br dd, 1H, 3-H), 7.38 (d, 1H, ^3J = 8.8 Hz, 9-H), 7.73 (dd, 1H, ^3J = 8.8 Hz and ^4J = 2.3 Hz, 8-H), 8.09 (d, 1H, ^4J = 2.3 Hz, 6-H), 12.11 (br s, 1H, ex, NH). Anal. ($C_{14}H_{13}NO_2Br_2$) C, H, N.

3-(2′-Hydroxy-2′,2′-dioxo-2′λ^6-disulfanyl)-2,2-dimethyl-2,3,4,10-tetrahydro-2*H*-pyrano[2,3-*b*]quinolin-5-one (5f). **5a** (355 mg, 1 mmol) and $Na_2S_2O_3$ (158 mg, 1 mmol) were heated in aqueous NaOH (0.5%, 15 mL) at 70 °C for 3 h. After cooling to room temperature the mixture was filtrated and the filtrate was acidified with concentrated HCl. The precipitate formed was collected by vacuum filtration and recrystallized from aqueous EtOH (273 mg, 80%): mp 261 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.53 and 1.67 (2 × s, 6H, C(CH_3)$_2$), 3.31 (dd, 1H, ^2J = 13.7 Hz and ^3J = 7.1 Hz, 4-H), 3.40 (dd, 1H, ^2J = 13.7 Hz and ^3J = 6.4 Hz, 4-H), 3.92 (br dd, 1H, 3-H), 7.59 (ddd, 1H, ^3J = 7.7 Hz and ^4J = 1.1 Hz, 7-H), 7.69 (br dd, 1H, 9-H), 7.85 (ddd, 1H, ^3J = 7.7 Hz and ^4J = 1.3 Hz, 8-H), 8.25 (dd, 1H, ^3J = 8.3 Hz and ^4J = 1.3 Hz, 6-H). Anal. ($C_{14}H_{15}NO_5S_2$) C, H, N, O, S.

7-Methoxy-2,2,10-trimethyl-2,3,4,10-tetrahydro-5*H*-pyrano[2,3-*b*]quinolin-5-one (5i). **2c** (550 mg, 2 mmol) were refluxed with concentrated HCl (20 mL) for 7 h. After cooling to room temperature the solution was diluted with water (100 mL) and neutralized with 2 M Na_2CO_3. The precipitate formed (**5i** plus **6e**) was collected by vacuum filtration and recrystallized from EtOH/water, 3:1. From this solution **5i** crystallized first and was collected by vaccuum filtration and again recrystallized from aqueous EtOH (137 mg, 25%): mp 199 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.48 (s, 6H, C(CH_3)$_2$), 1.95 (t, 2H, ^3J = 6.4 Hz, 3-CH_2), 2.77 (t, 2H, ^3J = 6.5 Hz, 4-CH_2), 3.87 (s, 3H, −N−CH_3), 3.90 (s, 3H, −O−CH_3), 7.53 (dd, 1H, ^3J = 9.3 Hz and ^4J = 2.4 Hz, 8-H), 7.71 (d, 1H, ^4J = 2.4 Hz, 6-H), 7.93 (d, 1H, ^3J = 9.3 Hz, 9-H). Anal. ($C_{16}H_{19}NO_3$) C, H, N.

3-Hydroxy-9-methoxy-2,2,6-trimethyl-2,3,4,6-tetrahydro-5*H*-pyrano[3,2-*c*]quinolin-5-one (6b). **3b** (280 mg, 1 mmol) was refluxed with MeOH (6 mL) and 3 M NaOH (30 mL) for 18 h. After cooling to room temperature the precipitate formed was collected by vacuum filtration and washed with Et_2O (261 mg, 85%): mp 179 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 and 1.47 (2 × s, 6H, C(CH_3)$_2$), 2.09 (d, 1H, ^3J = 6.3 Hz, ex, OH), 2.72 (dd, 1H, ^2J = 17.9 Hz and ^3J = 5.3 Hz, 4-H), 2.93 (dd, 1H, ^2J = 18.0 Hz and ^3J = 5.0 Hz, 4-H), 3.66 (s, 3H, −N−CH_3), 3.89 (s, 3H, −O−CH_3), 3.93 (m, 1H, 3-H), 7.15 (dd, 1H, ^3J = 9.2 Hz and ^4J = 2.9 Hz, 8-H), 7.24 (d, 1H, ^3J = 9.2 Hz, 7-H), 7.41 (d, 1H, ^4J = 2.9 Hz, 10-H). Anal. ($C_{16}H_{17}NO_3 \cdot 2H_2O$) C, H, N.

9-Methoxy-2,2,6-trimethyl-2,3,4,6-tetrahydro-5*H*-pyrano[3,2-*c*]quinolin-5-one (6e). Compound **6e** was prepared from **2c** according to the method given for **5i**. After separation of **5i** the filtrate was evaporated until **6e** precipitated. It was collected by vacuum filtration and recrystallized from aqueous EtOH (380 mg, 70%): mp 112 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.37 (s, 6H, C(CH_3)$_2$), 1.81 (t, 2H, ^3J = 6.6 Hz, 3-CH_2), 2.47 (t, 2H, ^3J = 6.6 Hz, 4-CH_2), 3.57 (s, 3H, −N−CH_3), 3.81 (s, 3H, −O−CH_3), 7.22 (dd, 1H, ^3J = 9.1 Hz and ^4J = 2.9 Hz, 8-H), 7.28 (d, 1H, ^4J = 2.9 Hz, 10-H), 7.44 (d, 1H, ^3J = 9.1 Hz, 7-H). Anal. ($C_{16}H_{19}NO_3$) C, H, N.

2,2-Dimethyl-9-nitro-2,3,4,6-tetrahydro-5*H*-pyrano[3,2-*c*]quinolin-5-one (6f). H_2SO_4 (1.2 mL of 18 M) was added dropwise to 65% HNO_3 (1 mL) with ice-cooling. To the resulting solution, solid **6c** (460 mg, 2 mmol) was added slowly in portions with intense stirring. The mixture was stirred 7 min at room temperature, then poured into cold water (40 mL). The precipitate was collected by vacuum filtration and washed with water (165 mg, 30%): mp >300 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.41 (s, 6H, C(CH_3)$_2$), 1.85 (t, 2H, ^3J = 6.5 Hz, 3-CH_2), 2.47 (t, 2H, ^3J = 6.6 Hz, 4-CH_2), 7.41 (d, 1H, ^3J = 9.1 Hz, 7-H), 8.31 (dd, 1H, ^3J = 9.1 Hz and ^4J = 2.6 Hz, 8-H), 8.49 (d, 1H, ^4J = 2.6 Hz, 10-H), 11.99 (s, 1H, ex, NH). Anal. ($C_{14}H_{14}N_2O_4$) C, H, N.

2,2,6-Trimethyl-9-nitro-2,3,4,6-tetrahydro-5*H*-pyrano[3,2-*c*]quinolin-5-one (6g). H_2SO_4 (1.2 mL of 18 M) was added dropwise to 65% HNO_3 (1 mL) with ice cooling. To the resulting solution, solid **6d** (490 mg, 2 mmol) was added slowly in portions with intense stirring. The mixture was stirred 25 min at room temperature, then poured into cold water (40 mL). The precipitate was collected by vacuum filtration, washed with water and recrystallized from water (115 mg, 20%): mp 213 °C dec; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.42 (s, 6H, C(CH_3)$_2$), 1.86 (t, 2H, ^3J = 6.6 Hz, 3-CH_2), 2.50 (t, 2H, ^3J = 6.7 Hz, 4-CH_2), 3.64 (s, 3H, −N−CH_3), 7.73 (d, 1H, ^3J = 9.3 Hz, 7-H), 8.37 (dd, 1H, ^3J = 9.3 Hz and ^4J = 2.7 Hz, 8-H), 8.56 (d, 1H, ^4J = 2.7 Hz, 10-H). Anal. ($C_{15}H_{16}N_2O_4$) C, H, N.

8-Methoxy-2-(1′-methylethyl)-5-methyl-4-oxo-4,5-dihydrofuro[3,2-*c*]quinoline 3-Sulfonate Sodium (8a). A solution of **3b** (290 mg, 1 mmol) in sulfuric acid (5 mL of 18 M) was stirred 24 h at room temperature, then poured into cold water (40 mL) and neutralized with 3 M NaOH. The precipitate was collected by vacuum filtration, washed with water and recrystallized from aqueous EtOH (300 mg, 80%): mp >300 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.30 and 1.32 (2 × s, 6H, C(CH_3)$_2$), 3.64 (s, 3H, −N−CH_3), 3.89 (s, 3H, −OCH_3), 4.15 (br sept, 1H, ^3J = 6.8 Hz, −C*H*(CH_3)$_2$), 7.24 (dd, 1H, ^3J = 9.3 Hz and ^4J = 2.4 Hz, 7-H), 7.38 (d, 1H, ^4J = 2.6 Hz, 9-H), 7.57 (d, 1H, ^3J = 9.4 Hz, 6-H). Anal. ($C_{16}H_{16}NO_6SNa$) C, H, N, O, S, Na.

Cell Culture. The N1E-115 cell line was maintained at 36.5 °C and 5% CO_2 in Dulbecco's modified Eagle medium supplemented with 10% fetal calf serum and 50 μg/mL kanamycin. The L929 cells stably transfected with *m*Kv1.3 were maintained at 36.5 °C and 5% CO_2 in Dulbecco's modified Eagle medium supplemented with 10% fetal calf serum and 300 μg/mL Geneticin (G418). The transfection has been described previously.[36]

Electrophysiology. Membrane currents were recorded in the whole-cell mode of the patch-clamp technique[37] using an EPC-9 patch-clamp amplifier (HEKA, Germany) interfaced to a PC running acquisition and analysis software (Pulse and PulseFit). Recordings were carried out at room temperature in a control bathing solution with 5% DMSO. For N1E-115 cells the bathing medium contained 125 mM NaCl, 5.5 mM KCl, 1.5 mM $CaCl_2$, 2.0 mM $MgCl_2$, 25 mM glucose and 5 mM HEPES (pH 7.4); the patch pipets contained 120 mM KF, 10 mM KCl, 10 mM NaCl, 10 mM HEPES, 10 mM EGTA (pH 7.2). For L929 cells stably transfected with *m*Kv1.3 the bathing medium contained 160 mM NaCl, 4.5 mM KCl, 2.0 mM $CaCl_2$, 1.0 mM $MgCl_2$ and 5 mM HEPES (pH 7.4); the patch pipets contained 120 mM KF, 20 mM KCl, 10 mM HEPES, 10 mM EGTA (pH 7.2). All membrane currents were recorded with 50% series-resistance compensation, leak currents were subtracted using a P/10 protocol, and capacitive currents were compensated using the internal clamp circuitry. The holding potential in all experiments was −80 mV. K^+ currents were elicited by a 100-ms (N1E-115) or 200-ms (Kv1.3) pulse with an amplitude of 40 mV and Na^+ currents by a 100-ms pulse with an amplitude of 0 mV. Test compounds were dissolved in DMSO and diluted with the bathing solutions (5% DMSO) to the required concentrations. The test solutions were applied using a peristaltic pump (Reglo-Digital, Ismatec, Germany).

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Supporting Information Available: Synthetic procedures for the preparation of compounds **4b**, **5e**,**h**, **6a**, **8b**,**c**, and **9a** and NMR data of all known compounds as well as ^{13}C NMR data of new compounds which are essential for distinguishing between linear and angular as well as furo and pyrano isomers. This material is available free of charge via the Internet at http://pubs.acs.org.

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