Discovery, Synthesis, and Structure−Activity Relationship of a Series of N-Aryl-bicyclo[2.2.1]heptane-2-carboxamides: Characterization of ML213 as a Novel KCNQ2 and KCNQ4 Potassium Channel Opener

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Ⓢ Supporting Information

ABSTRACT: Herein we report the discovery, synthesis, and evaluation of a series of N-aryl-bicyclo[2.2.1]heptane-2-carboxamides as selective KCNQ2 (K_v7.2) and KCNQ4 (K_v7.4) channel openers. The best compound, **1** (ML213), has an EC_{50} of 230 nM (KCNQ2) and 510 nM (KCNQ4) and is selective for KCNQ2 and KCNQ4 channels versus a large battery of related potassium channels, as well as affording modest brain levels. This represents the first report of unique selectivity profiles for KCNQ2 and KCNQ4 over the other channels (KCNQ1/3/5) and as such should prove to be a valuable tool compound for understanding these channels in regulating neuronal activity.

KEYWORDS: KCNQ2, KCNQ4, activator, Kv7, ion channels, ML218, MLPCN probe
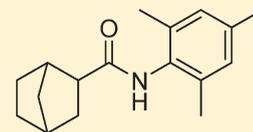
ML213, 1
SID: 103073346
CID: 3111211
KCNQ2 EC_{50} = 230 nM
KCNQ4 EC_{50} = 510 nM
>80-fold vs. other K+ Channels

The KCNQ (K_v7) family of channels includes six transmembrane domain voltage gated K^+ channels consisting of five members (K_v7.1−K_v7.5).[1] In order to form functional channels, four K_v7 subunits are required, leading to increased complexity in composition and functional diversity of this family of channels.[2] It is known that all five channel subunits can form homomeric channels in vitro; however, the formation of heteromeric channels is predominantly seen for KCNQ2 and KCNQ3 subunits. In contrast with other K_v channel families, KCNQ (or K_v7) channels commonly display activation at voltages close to neuronal resting membrane potentials and are regulated by G-protein coupled receptor (GPCR) signaling, notably by muscarinic receptors.[3] In addition, these channels are activated at subthreshold membrane potential. Thus, KCNQ channels are critical for setting up the excitation threshold of action potentials. Among the channels, K_v7.2−K_v7.5 are predominantly expressed (in rodents) in the peripheral and central nervous system, including hippocampal cells, cortical cells, and dorsal root ganglion. The K_v7.1 (or KCNQ1) is expressed mainly in cardiac tissue, peripheral epithelial cells, and smooth muscle cells.[2]

A growing body of evidence now exists supporting the premise that neuronal KCNQ2/3 channels represent interesting targets for the treatment of diseases involving altered neuronal excitability, such as epilepsy and chronic pain.[2,4−6] This evidence includes genetic studies where mutations in either KCNQ2 or KCNQ3 cause benign familial neonatal convulsions (BFNC), a

rare form of epilepsy,[7−9] the distribution of these channels in the central nervous system (CNS), and pharmacological experiments.[2,4] Activation of these channels hyperpolarizes neuronal membranes, resulting in an increase of firing threshold and hence lessening of action potential firing. Two structurally related compounds that act as KCNQ2/3 activators are in advanced clinical trials or are marketed drugs. Ezogabine (Retigabine in Europe) is currently in phase III clinical trials for the treatment of partial epilepsies,[10−13] and flupirtine is a marketed analgesic for pain.[14] Supported by this data, a number of groups have initiated efforts to develop novel KCNQ activators.[15−20] Although there has been extensive research into the discovery and development of selective K_v7 (KCNQ) small molecule activators, there still remains the question of selectivity. Most of the reported structures are either nonselective KCNQ activators or have limited selectivity for KCNQ2/Q3. Few have some degree of isoform preference of neuronal KCNQ channels.[21]

As members of the Molecular Libraries Production Center Network (MLPCN), we screened a library of approximately 300 000 compounds from the NIH Molecular Libraries Small Molecule Repository (MLSMR) compound collection for KCNQ2 activation.[22,23] A number of structural classes were identified from this
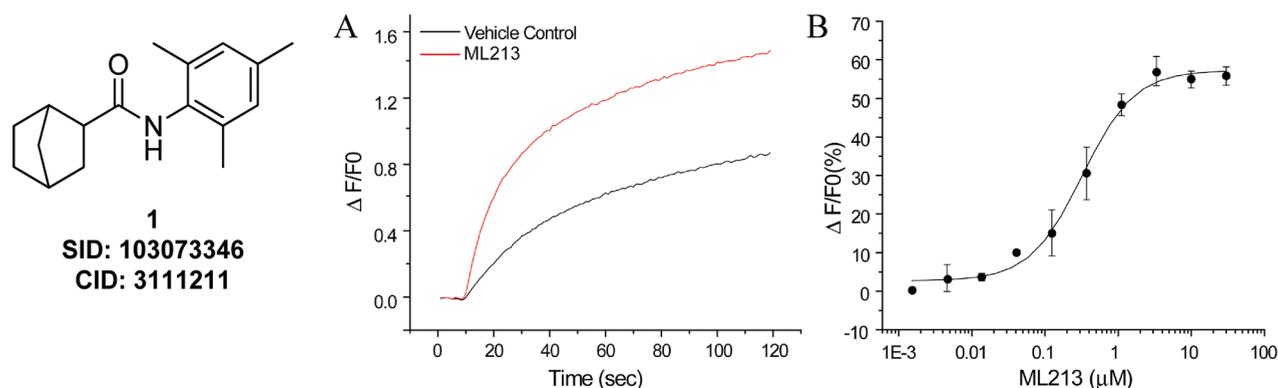
Figure 1. Initial KCNQ2 activator from the HTS, ML213 (**1**). (A) ML213 produced a concentration-dependent increase in Tl+ influx; (B) ML213 affords an EC_{50} value of 359 nM and a maximal increase of 56% in 10-point CRC.

screen, but the most promising compound was, **1** (SID: 103073346, CID: 3111211), a [2.2.1]bicycloamide with an EC_{50} = 359 nM (Figure 1). Based on the distribution of KCNQ2 in the CNS, and previous work showing efficacy of KCNQ2 activators in a number of CNS related in vivo animal models, compound **1** represents an attractive lead molecule for a CNS indication. The calculated properties of **1** are in line with most known molecules with CNS exposure (MW < 300 (257), cLogP = 2−4 (4.1), tPSA <75 (29.1)).

The structure−activity relationship (SAR) analysis began by keeping the bicyclo[2.2.1]heptylamide constant and evaluating a variety of anilines (Table 1). The evaluation started by deletion of one of the methyl groups, leaving the 2,4-dimethyl derivative (**2**, 220 nM) which was equipotent with **1** (330 nM, after reconfirmation). Moving the methyl groups around the ring revealed interesting SAR: the 2,5-dimethyl compound (**5**, 400 nM) was also equipotent, but the 2,6-dimethyl, **3**, and 2,3-dimethyl, **6**, derivatives were significantly less potent (3240 nM and 1000 nM, respectively). Moving the dimethyl groups to the meta positions (3,5-dimethyl, **7**) led to a much less active compound (47.4% maximal change at 30 μM). Deletion of the two ortho methyl groups led to a 2-fold reduction in potency (**4**, 750 nM). The simple phenyl, **11**, derivative also showed a potentiation of the maximal change (136% at 30 μM; EC_{50} not determined). Extending the substitution in the 4-position to either an ethyl group (**8**, 920 nM), an isopropyl group (**9**, 720 nM), or a 2-naphthyl group (**10**; 600 nM) was tolerated, and all were equipotent to the tolyl compound, **4**. Interestingly, replacing the 4-methyl with a 4-trifluoromethyl group led to an inactive compound, **12**. Finally, addition of halogens (2,4-dichloro, **13**, 1520 nM; 2,4-difluoro, **14**, 10 050 nM; 4-fluoro, **15**, EC_{50} not determined) was not tolerated. Replacement of the phenyl with a pyridyl, **17**, was also not tolerated (inactive), although a substituted pyridyl, **16**, was active (1810 nM).

The next round of SAR kept the mesityl (2,4,6-trimethyl) aniline constant, and a variety of alkyl, cycloalkyl, and aryl amides were evaluated (Table 2). The SAR around the amide group was not very productive. Replacement of the bicyclo[2.2.1]heptane group with a simple cyclohexyl group, **18** (i.e., removal of the bridge head group), led to a 5-fold loss in activity (1570 nM). Simple alkyl or branched alkyl groups were not tolerated (**19**, 103% maximal change, EC_{50} not determined; **20**, 79% maximal change, EC_{50} not determined; **21**, 38% maximal change, EC_{50} not determined), nor was the bulkier adamantyl group (**22**, inactive). The only group that gave comparable activity to ML213 was the

cyclopentylethyl group (**24**, 490 nM). Replacement with aryl groups or substituted aryl groups led to inactive compounds (**26**−**32**). Unlike other reported amide activators of KCNQ2, ML213 is the first non-biaryl amide reported in the literature.

To further profile ML213, currents through KCNQ2 channels were recorded using an IonWorks automated electrophysiology instrument in Population Patch Clamp mode (Figure 2); the results show ML213 displays a concentration-dependent enhancement of KCNQ2 currents. Membrane currents provide a linear measure of channel activity and provide a direct determination of compound effects on channel function. To evaluate potency for ML213 at different test potentials, 8-point concentration response curves for ML213 were generated at a variety of voltage steps. The EC_{50} values were comparable at voltages greater than −10 mV (Figure 2A), although the relative increases in current were decreased at higher levels of depolarization due to higher levels of channel open probability in control. At −10 mV step potential, ML213 exhibited a concentration-dependent enhancement of KCNQ2 currents with an EC_{50} value of 230 nM and a maximal increase of 445% (Figure 2B). The close agreement between EC_{50} values in the electrophysiological and fluorescent assays supports use of both assay methods in selectivity studies. To evaluate the mechanism for KCNQ2 potentiation by ML213 and determine an estimate of potency that was not dependent on test potentials, voltage activation curves were determined with and without ML213. In the presence of 5 μM ML213, the $V_{1/2}$ (voltage required for half-maximal activation) of KCNQ2 was left-shifted by 37.4 ± 3.0 mV compared with control values in the same cells (Figure 2C). In addition, ML213 caused a concentration-dependent shift in the $V_{1/2}$ for KCNQ2 activation with an EC_{50} 340 ± 70 nM and a maximal shift of 37.4 mV (Figure 2D).

Compound **1** (ML213) was further evaluated for its selectivity in fluorescent and electrophysiological assays against six related channels from the K_v7 (KCNQ) family and against two distantly related potassium channels (hERG and Kir2.1) (Table 3). For comparison, ML213 displayed EC_{50} values for KCNQ2 activation of 359 nM in a fluorescent Tl^+ influx assay and 230 nM in an IonWorks (IW) electrophysiological assay. As can be seen in Table 3, ML213 displays excellent selectivity against KCNQ1, KCNQ1/KCNE1, KCNQ3, and KCNQ5 (>80-fold selective). This compound does show equivalent potency in the IonWorks against KCNQ2/Q3 (370 nM) and is active against KCNQ4 (Tl^+, 2400 nM; IW, 510 nM), with different selectivity depending on the assay. In addition, ML213 was tested on Ricerca's

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dx.doi.org/10.1021/cn200065b |*ACS Chem. Neurosci.* 2011, 2, 572−577

Table 1. SAR Evaluation of the Aniline Portion of 1
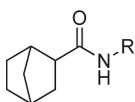
Entry	R	KCNQ2 EC_{50} (nM)[a] (% at 30μM)[b]
1		330 ± 110[c]
2		220 ± 70
3		3240 ± 1000
4		750 ± 290
5		400 ± 130
6		1000 ± 470
7		(47.4)
8		920 ± 250
9		720 ± 190
10		600 ± 190
11		(135.9)
12		Inactive
13		1520 ± 200
14		10050 ± 2490
15		(61.0)
16		1810 ± 620
17		Inactive

[a] EC_{50}'s were generated from 8-point concentration response curves with 3-fold dilutions starting from the maximal concentration (30 μM) with quadruplicates in automated electrophysiological assay. Activity definition: The compound will be defined as inactive if the compound exhibits less than 30% activation at 30 μM. Otherwise, the compound will be defined as an activator with the EC_{50} value. [b] In cases in which a saturating response was not obtained at the highest tested concentration, (% at 30 μM) is listed. [c] EC_{50} values were obtained from five sets of experiments with quadruplicate measurements for each experiment. EC_{50} values are expressed as $EC_{50} \pm$ SD, using estimated standard deviations provided by software Origin 6.0.

(formerly MDS Pharma's) Lead Profiling Screen (binding assay panel of 68 GPCRs, ion channels and transporters screened at 10 μM) and was found to have no significant activities (no inhibition of radio ligand binding >50% at 10 μM; see the Supporting Information). Lastly, ML213 has been tested in 254 assays performed with the MLPCN network and was active in only

Table 2. SAR Evaluation of the Left-Hand Amide Portion of 1
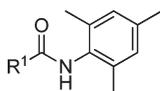
Entry	R[1]	KCNQ2 EC_{50} (nM)[a] (% at 30μM)[b]
18		1570 ± 300
19		(103.1)
20		(79.0)
21		(38.0)
22		Inactive
23		Inactive
24		490 ± 230
25		1600 ± 720
26		Inactive
27		Inactive
28		(31.8)
29		(57.3)
30		(32.3)

[a] EC_{50}'s were generated from 8-point concentration response curves with 3-fold dilutions starting from the maximal concentration (30 μM) with quadruplicates in automated electrophysiological assay. Activity definition: The compound will be defined as inactive if the compound exhibits less than 30% activation at 30 μM. Otherwise, the compound will be defined as an activator with EC_{50} value. [b] In cases in which a saturating response was not obtained at the highest tested concentration, (% at 30 μM) is listed. EC_{50} values are expressed as $EC_{50} \pm$ SD, using estimated standard deviations provided by software Origin 6.0.

one assay (AID 2314) that was not dependent on KCNQ2. Combined, these profiling exercises demonstrate the broad selectivity of ML213 and highlight the value of ML213 as a highly selective probe to prosecute KCNQ2/4 function.

In order to evaluate the potential of ML213 to serve as an in vivo probe of KCNQ2/4 function, ML213 and some closely related analogues were profiled in our tier 1 in vitro DMPK battery (Table 4). In CYP$_{450}$ assays, ML213 (1) was clean (>30 μM) against all of the CYP enzymes (CYP3A4, 2D6, 2C9, 1A2) evaluated. Other analogues of ML213 also displayed a favorable profile against CYP inhibition with the exception of 10, which showed inhibition of CYP1A2 (0.83 μM) and CYP2C9 (7.68 μM) and 6, which inhibited CYP1A2 (7.75 μM). All other compounds tested were >10 μM against all CYPs. The metabolic stability of ML213 and analogues was evaluated in assays which can predict rat and human hepatic clearance from in vitro microsomal clearance values (Table 4). This allows for a rank ordering of compounds that would be predicted to have poor stability in in vivo testing protocols (after oral dosing). Unfortunately, all of the compounds evaluated showed high (near hepatic blood flow) clearance in human and rat liver microsomes. However, ML213 did display an acceptable
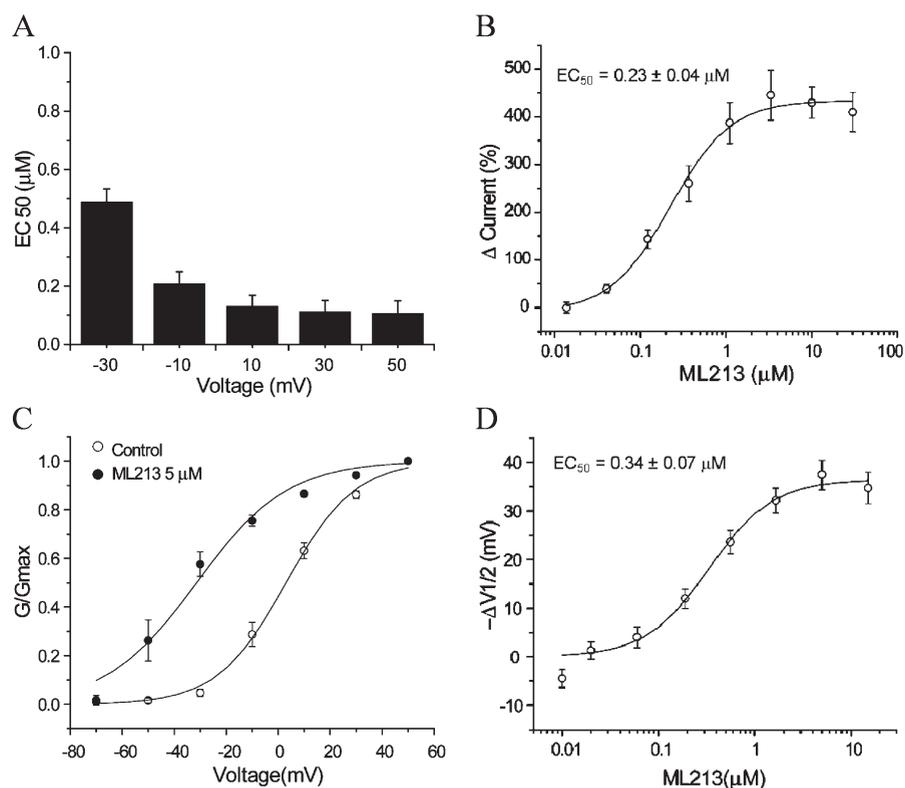
Figure 2. ML213 effects on voltage-dependent activation of KCNQ2 channels using automated electrophysiology. (A) EC_{50} values of ML213 were determined at different voltage steps. (B) Concentration-dependent activation of KCNQ2 channels measured at -10 mV. (C) KCNQ2 conductance−voltage curves were determined in the absence and presence of 5 μM ML213. (D) $\Delta V_{1/2}$ values were calculated from the differences of $V_{1/2}$ between control and ML213 treatment in the same cells and plotted against ML213 concentrations.

Table 3. Selectivity of ML213 on KCNQ Family Members and Other Potassium Channels Using Tl+ Flux Assay and IonWorks (IW) Automated Electrophysiological Assay

Channel	Tl$^+$ EC_{50} (nM)	Fold selectivity	IW EC_{50} (nM)	Fold selectivity
KCNQ1	>30	>80	>30	>80
KCNQ1/E1	>30	>80	>30	>80
KCNQ2	360		330	
KCNQ2/3	nd^a		370	1
KCNQ3	>30	>80		
KCNQ4	2400	6.7	510	1.4
KCNQ5	>30	>80		
Kir2.1	>10	>27		
hERG			>10	>28

^{a} nd: Not determined.

free fraction profile in plasma protein binding studies (6.5% and 1.2% free unbound for human and rat, respectively). Other analogues also showed free fraction profile > 1−3% as well.

The metabolic stability data suggests that ML213 would likely not be appropriate for oral dosing; however, several other dosing options are available for compounds that undergo significant first-pass metabolism (e.g., subcutaneous (SC), intravenous (IV), or intraperitoneal (IP) dosing). Thus, due to the therapeutic importance and distribution of KCNQ2 and KCNQ4 in the CNS, we further profiled ML213 in a snapshot in vivo PK study[23] to assess the plasma and brain levels after a single time

point (1 h, 10 mg/kg) following IP administration. ML213 displayed plasma levels at 1 h greater than 1 μM (1.26 μM) with brain levels of 0.476 μM, resulting in a moderate brain$_{AUC}$/plasma$_{AUC}$ of 0.37, an acceptable value for a first generation probe.

In summary, we have discovered a potent, selective, and brain penetrant KCNQ2 and KCNQ4 activator, ML213, from an MLPCN high-throughput screening campaign. Although there are several reported KCNQ2/3 activators in the primary literature, ML213 (CID 3111211) has several unique features, especially being the first KCNQ2 and KCNQ4 selective probe compound, and, as such, ML213 will be an important tool for understanding the roles of these channels in regulating neuronal excitability. The probe may be used for investigations of the roles of KCNQ2 and KCNQ4 channels in antiepileptogenesis in mechanistic cellular studies and tissue slice experiments. Further in vivo evaluation of this probe will be reported in due course. ML213 is an MLPCN probe and as such is freely available upon request.

■ METHODS

General. The syntheses of selected compounds are described below. The general chemistry, experimental information, and syntheses of all other compounds are supplied in the Supporting Information. Purity of all final compounds was determined by HPLC analysis as >95%.

***N*-Mesitylbicyclo[2.2.1]heptane-2-carboxamide (ML213, 1).** To a solution of 2-norbornanecarbonylchloride (1.0 eq, 250 mg, 1.6 mmol) in DMF (1 mL) was added 2,4,6-trimethylaniline (213 mg, 1.6 mmol) and triethylamine (440 μL, 3.2 mmol). After stirring for 24 h,

Table 4. Intrinsic Clearance and Protein Binding of ML213 and Analogues

| | Intrinsic clearance | | Protein binding (% fu) | | CYP inhibition (μM) | | | |
| | Human | Rat | | | | | | |
Compd	CL_{INT}	CL_{INT}	Human	Rat	1A2	2C9	3A4	2D6
1	64.8	645.1	6.5	1.2	>30	>30	>30	>30
2	36.5	496.9	6.4	2.0	10.51	>30	>30	>30
4	41.9	277.6	4.7	3.9	10.37	>30	>30	>30
6	55.9	691.9	6.2	4.4	7.75	>30	>30	22.22
9	42.4	1659.0	0.6	0.7	12.23	27.04	>25	>30
10	68.4	406.2	0.5	0.2	0.83	7.68	>30	>30

the reaction mixture was filtered and the supernatant purified by mass directed chromatography to provide ML213 (124.3 mg, 0.5 mmol, 30%) as a white crystalline solid. LCMS: R_T = 0.766 min, m/z = 258.4 $[M + H]^+$, >98% at 215 and 254 nm. ^{1}H NMR (400 MHz, CDCl$_3$) δ 6.89 (s, 2H), 6.61 (bs, 1H), 2.90−2.84 (m, 1H), 2.58 (d, J = 5.2 Hz, 1H), 2.37−2.31 (m, 1H), 2.26 (s, 3H), 2.19 (s, 3H), 2.18 (s, 3H), 1.79−1.73 (m, 1H), 1.67−1.21 (m, 8H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 172.3, 136.6, 135.1, 131.5, 128.8, 47.4, 41.3, 36.9, 34.3, 31.5, 29.3, 24.4, 20.8, 18.5, 18.3. HRMS calcd for C$_{17}$H$_{24}$NO $[M + H]^+$, 258.1858; found, 258.1860.

Thallium-Based Fluorescence Assay. Activity of potassium channels was monitored by the influx of a surrogate ion for potassium, thallium (Tl+). Thallium influx was detected through the use of a thallium-sensitive fluorescent dye, FluxOR (Invitrogen, Carlsbad, CA). Cells were plated using a Multidrop (Thermo scientific, Hudson, New Hampshire) at 6500 cells/well into BD Biocoat poly-D-lysine coated 384-well plates and incubated overnight (16−20 h) at 37 °C and 5% CO$_2$. The thallium-based fluorescence assay protocol was adapted from the manufacturer's recommended protocol. Briefly, medium was removed; cells were loaded with 1× FluxOR dye solution, 25 μL/well, for 90 min at room temperature in the dark; the 1× FluxOR solution was replaced by assay buffer (Hank's balanced salt solution containing 5.8 mM potassium; catalog # 14065, Invitrogen), 20 μL/well; test compounds at final concentration 10 μM from a 5 mM stock supplied by the NIH Molecular Libraries Small Molecule Repository (BioFocus DPI, San Francisco, CA) or controls in assay buffer were then added to cells, 4 μL/well; 20 min later, cell plates were loaded to a Hamamatsu FDSS 6000 kinetic imaging plate reader (Hamamatsu Photonics, Hamamatsu city, Japan); after establishing fluorescence baseline by 1 Hz scanning for 10 s, the KCNQ2 channels were activated by addition of 6 μL/well stimulus buffer containing 12.5 mM K$_2$SO4 and 12.5 mM Tl$_2$SO4 to the assay buffer giving a final potassium concentration of 15.8 mM and final Tl+ concentration of 5 mM; fluorescence measurement was continued at 1 Hz for another 110 s. To evaluate the robustness of the HTS thallium-based fluorescence assays, ZTZ240 at 10 μM was applied as positive activator control, while assay buffer was employed as negative control. Both the positive and negative controls are prepared with 0.2% (v/v) DMSO, corresponding to the test concentration 10 μM. The fluorescence ratio, $F(max - min)/F_0$ ($\Delta F/F_0$), was calculated for each well using the entire 120 s detection window and then normalized to the positive and negative control wells. Hit selection was based on the B scores of test compounds calculated from the fluorescence ratios. If the B score of the test compound was more than 3 times the standard deviation (SD) of the B scores of ratios of the library compounds, and the B score of initial fluorescence intensity is within 2 times the standard deviation of the B scores of the library compounds, then the compound is designated as an activator of KCNQ2 channels. Otherwise, it is designated as inactive.

Automated Electrophysiology Assay. KCNQ2 activity was examined in an electrophysiological assay using the population patch clamp mode on the IonWorks Quattro (MDC, Sunnyvale, CA), an automated patch clamp instrument. The CHO cells stably expressing KCNQ2 channels were freshly dislodged from flasks and dispensed into a 384-well population patch clamp (PPC) plate. The cell plating density was 7000 cells/well suspended in the extracellular solution, composed of (in mM) 137 NaCl, 4 KCl, 1 MgCl2, 1.8 CaCl2, 10 HEPES, and 10 glucose, pH 7.4 adjusted with NaOH.

After dispensing, seal resistance of cells was measured for each well and cells were perforated by incubation with 50 μg/mL amphotericin B (Sigma, St. Louis, MO), which was dissolved in the internal solution composed of (in mM) 40 KCl, 100 K-Gluconate, 1 MgCl2, 5 HEPES, 2 CaCl2, pH 7.2 adjusted with KOH. Activity of KCNQ2 was then measured with the recording protocol as follows. Leak currents were linear subtracted extrapolating the current elicited by a 100 ms step to −100 mV from a holding potential of −90 mV. During the voltage pulse protocol, cells were held at −90 mV, followed by by a 2000 ms depolarizing step from −90 to −10 mV, and then back to −90 mV for 2000 ms. The currents were measured at the end of the depolarization pulse before and after the application of compounds for 3 min. Only cells with a current amplitude more than 100 pA at −10 mV and a seal resistance > 30 MΩ were included in the data analysis.

Compound effects were assessed by the percentage changes in the KCNQ2 steady state currents, which were calculated by dividing the difference between pre- and postcompound KCNQ2 currents by the respective precompound currents in the same well. When constructing conductance−voltage curves, conductance values were calculated by dividing the steady state outward currents measured during the voltage steps by the driving force (step voltage minus the calculated potassium reversal potential).

The KCNQ2 protocol was also used for KCNQ2/KCNQ3 recording. But for KCNQ1 and KCNQ4, the cells were depolarized to +40 mV from the holding potential −70 mV. Currents were measured at the step current at +40 mV. And for KCNQ1/KCNE1, cells were stimulated by a 3000 ms depolarizing step from −70 to +40 mV, followed by hyperpolarization to −20 mV for 500 ms. Currents were measured at the steady state of +40 mV voltage step.

No corrections for liquid junction potentials (estimated as −20 mV by comparing the KCNQ2 reversal potential with the calculated Nernst potential for potassium) were applied. The current signal was sampled at 0.625 kHz.

■ ASSOCIATED CONTENT

ⓢ Supporting Information. Experimental procedures and spectroscopic data for selected compounds, detailed pharmacology and DMPK methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

Professors Lindsley and Hopkins directed and designed the chemistry. Professor Daniels oversaw the pharmacokinetic studies and analysis. Dr. Townsend helped design compounds and performed synthetic chemistry. Drs. Yu, Wu and Zou andMr. Long performed experiments. Drs. Lindsley, Hopkins, Li, McManus, Yu and Wu participated in writing or editing the manuscript.

■ REFERENCES

(1) Hille, B. (2001) *Ion Channels of Excitable Membranes*, 3rd ed., Sinauer Associates, Sunderland, MA.

(2) Munro, G., and Dalby-Brown, W. (2007) K_v7 (KCNQ) Channel Modulators and Neuropathic Pain. *J. Med. Chem. 50*, 2576–2582.

(3) Brown, D. A., and Adams, P. R. (1980) Muscarinic suppression of a novel voltage-sensitive K^+ current in a vertebrate mouse. *Nature 283*, 673–676.

(4) Wickenden, A. D., Roeloffs, R., McNaughton-Smith, G., and Rigdon, G. C. (2004) KCNQ potassium channels: drug targets for the treatment of epilepsy and pain. *Expert Opin. Ther. Pat. 14*, 1–13.

(5) Gribkoff, V. K. (2008) The therapeutic potential of neuronal K_v7 (KCNQ) channel modulators: an update. *Expert Opin. Ther. Pat. 12*, 565–581.

(6) Xiong, Q., Gao, Z., Wang, W., and Li, M. (2008) Activation of K_v7 (KCNQ) voltage-gated potassium channels by synthetic compounds. *Trends Pharmacol. Sci. 29*, 99–107.

(7) Singh, N. A., Singh, N. A., Westenskow, P., Charlier, C., Pappas, C., Leslie, J., Dillon, J., BFNC Physician Consortium, Anderson, V. E., Sanguinetti, M. C., Leppert, M. F. (2003) KCNQ2 and KCNQ3 potassium channel genes in benign familial neonatal convulsions: expansion of the functional and mutation spectrum. *Brain 126*, 2726−2737.

(8) Charlier, C., Singh, N. A., Ryan, S. G., Lewis, T. B., Reus, B. E., Leach, R. J., and Leppert, M. (1998) A pore mutation in a novel KQT-like potassium channel gene in an idiopathic epilepsy family. *Nat. Genet. 18*, 53–55.

(9) Singh, N. A., Charlier, C., Stauffer, D., DuPont, B. R., Leach, R. J., Melis, R., Ronen, G. M., Bjerre, I., Quattlebaum, T., Murphy, J. V., McHarg, M. L., Gagnon, D., Rosales, T. O., Peiffer, A., Anderson, V. E., and Leppert, M. (1998) A novel potassium channel gene, *KCNQ2*, is mutated in an inherited epilepsy of newborns. *Nat. Genet. 18*, 25–29.

(10) Rostock, A., Tober, C., Rundfeldt, C., Bartsch, R., Engel, J., Polymeropoulos, E. E., Kutscher, B., Löscher, W., Hönack, D., White, H. S., and Wolf, H. H. (1996) D-23129: a new anticonvulsant with a broad spectrum of activity in animal models of epileptic seizures. *Epilepsy Res. 23*, 211–223.

(11) Rundfeldt, C. (1997) The new anticonvulsant retigabine (D-23129) acts as an opener of K^+ channels in neuronal cells. *Eur. J. Pharmacol. 336*, 243–249.

(12) Porter, R. J., Patriot, A., Sachedo, R., Nohria, V., and Alves, M. (2007) Randomized, multicenter, dose-ranging trial of retigabine for partial-onset seizures. *Neurology 68*, 1197–1204.

(13) www.clinicaltrials.gov.

(14) Ilyin, V. I., Carlin, K. P., Hodges, D. D., Robledo, S., and Woodward, R. M. (2002) Flupertine. A positive modulator of heteromeric KCNQ2/Q3 channels. *Soc. Neurosci. Abstr. 758*, 10.

(15) Wu, Y.-J., Sun, L.-Q., He, H., Chen, J., Starrett, J. E., Jr., Dextraze, P., Daris, J. P., Boissard, C. G., Pieschl, R. L., Gribkoff, V. K., Natale, J., Knox, R. J., Harden, D. G., Thompson, M. W., Fitzpatrick, W., Weaver, D., Wu, D., Gao, Q., and Dworetzky, S. I. (2004) Synthesis and KCNQ2 opener activity of *N*-(1-benzo[1,3]dioxol-5-yl-ethyl, *N*-[1-(2,3-dihydro-benzofuran-5-yl)-ethyl, and *N*-[1-(2,3-dihydro-1H-indol-5-yl)-ethyl acrylamides. *Bioorg. Med. Chem. Lett. 14*, 4533.

(16) Gribkoff, V. K., Starrett, J. E., Jr., Dworetzky, S. I., Hewawasam, P., Boissard, C. G., Cook, D. A., Frantz, S. W., Heman, K., Hibbard, J. R., Huston, K., Johnson, G., Krishnan, B. S., Kinney, G. G., Lombardo, L. A., Meanwell, N. A., Molinoff, P. B., Myers, R. A., Moon, S. L., Ortiz, A., Pajor, L., Pieschl, R. L., Post-Munson, D. J., Signor, L. J., Srinivas, N., Taber, M. T., Thalody, G., Trojnacki, J. T., Wiener, H., Yeleswaram, K., and Yeola, S. W. (2001) Targeting acute ischemic stroke with a calcium-sensitive opener of maxi-K potassium channels. *Nat. Med. 7*, 471–477.

(17) Roeloffs, R., Wickenden, A. D., Crean, C., Werness, S., McNaughton-Smith, G., Stables, J., McNamara, J. O., Ghodadra, N., and Rigdon, G. C. (2008) In vivo profile of ICA-27243 [*N*-(6-chloro-pyridin-3-yl)-3,4-difluoro-benzamide], a potent and selective KCNQ2/Q3 (Kv7.2/Kv7.3) activator in rodent anticonvulsant models. *J. Pharmacol. Exp. Ther. 326*, 818–828.

(18) Amato, G., Roeloffs, R., Rigdon, G. C., Antonio, B., Mersch, T., McNaughton-Smith, G., Wickenden, A. D., Fritch, P. C., and Suto, M. J. (2011) *N*-Pyridyl and pyrimidine benzamides as KCNQ2/Q3 potassium channel openers for the treatment of epilepsy. *ACS Med. Chem. Lett. 2*, 481–484.

(19) Fritch, P. C., McNaughton-Smith, G., Amato, G. S., Burns, J. F., Eargle, C. W., Roeloffs, R., Harrison, W., Jones, L., and Wickenden, A. D. (2010) Novel KCNQ2/Q3 agonists as potential therapeutics for epilepsy and neuropathic pain. *J. Med. Chem. 53*, 887–896.

(20) Qi, J., Zhang, F., Mi, Y., Fu, Y., Xu, W., Zhang, D., Wu, Y., Du, X., Jia, Q., Wang, K., and Zhang, H. (2011) Design, synthesis and biological activity of pyrazolo[1,5-*a*]pyrimidin-7(4*H*)-ones as novel Kv7/KCNQ potassium channel activators. *Eur. J. Med. Chem. 46*, 934–943.

(21) Gao, Z., Zhang, T., Wu, M., Xiong, Q., Sun, H., Zhang, Y., Zu, L., Wang, W., and Li, M. (2010) Isoform-specific prolongation of Kv7 (KCNQ) potassium channel opening mediated by new molecular determinants for drug-channel interactions. *J. Biol. Chem. 285*, 28322–28332.

(22) For information on the Molecular Libraries Probe Production Centers Network (MLPCN), see http://mli.nih.gov/mli/.

(23) For assay details, see the Supporting Information.