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Identification of (*R*)-*N*-(4-(4-methoxyphenyl)thiazol-2-yl)-1-tosylpiperidine-2-carboxamide, ML277, as a novel, potent and selective K_v7.1 (KCNQ1) potassium channel activator

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Abstract

A high-throughput screen utilizing a depolarization-triggered thallium influx through KCNQ1 channels was developed and used to screen the MLSMR collection of over 300,000 compounds. An iterative medicinal chemistry approach was initiated and from this effort, ML277 was identified as a potent activator of KCNQ1 channels (EC₅₀ = 260 nM). ML277 was shown to be highly selective against other KCNQ channels (>100-fold selectivity versus KCNQ2 and KCNQ4) as well as against the distantly related hERG potassium channel.

Keywords

KCNQ1 activator; MLPCN probe; Potassium channels; Voltage-gated ion channels; ML277

Long QT syndrome (LQTS) is a disorder of the electrical activity of the heart in which the heart muscles take longer than normal to recover after each heart beat and is named from the abnormal pattern on an electrocardiogram (EKG).¹ In response to exercise or stress, LQTS can lead to dangerous arrhythmias causing uncontrollable heartbeats which can be fatal. This syndrome can either be caused by genetic mutations, or from pharmacological intervention.

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To date, six genes have been identified that are responsible for LQTS and are subclassified as LQT1-LQT6 depending on the responsible gene. The two most common forms are LQTS1 – caused by mutations in the KCNQ1 gene (nearly half of the cases), and LQTS2 – caused by human Ether-a-go-go-related gene (hERG) (responsible for ~35% of the cases).^{1a} KCNQ1 (K_v7.1; K_vLQT1) is a voltage-gated potassium channel gene that plays a key physiological role in the repolarization of the cardiac tissue following an action potential.^{1c,2} In cardiac muscle cells, KCNQ1 forms a channel complex with the KCNE1 β -subunit (minK) to produce the delayed rectifier current I_{Ks} , and is responsible, in part, for terminating the action potential.^{1c,3} Thus, activators of KCNQ1 channels could be useful treatments of LQTS.

Unfortunately, there are relatively few selective KCNQ1 channel activators that have been reported in the literature (Figure 1).² The reported small molecule activators range from marketed drugs and drug-like compounds such as the NSAID, mefenamic acid, **1**,⁴ and L364,373, **2**,⁵ to nondrug-like molecules such as phenylboronic acid, **3**,⁶ and zinc pyrithione (ZnPy), **4**.⁷ These compounds have reported activities in the micromolar range against KCNQ1 and are either not selective versus other members of the K_v7 family^{4, 6–7}, or have no selectivity data reported.⁵ Based on this, we were interested in finding potent and selective activators of KCNQ1 and herein we report our efforts toward this endeavor.

As members of the Molecular Libraries Production Center Network (MLPCN), a screen of the >300,000 NIH Molecular Library Small Molecule Repository (MLSMR) compound collection using a depolarization-triggered influx assay (Pubchem Assay Identifier, AID: 2648) to identify activators of the KCNQ1 channel was performed.⁸ From the initial library screen, 1,082 compounds were deemed “hits” and after a round of triage and selectivity screens (parental CHO cells and KCNQ2 channels), 26 compounds were identified as KCNQ1 activators. One particular series was considered a starting “hit” series due to high potency against KCNQ1 (EC₅₀ = 660 nM), chemical tractability and favorable selectivity profile as deemed by PubChem assay statistics; compound **5** (PubChem Compound Identifier, CID: 4880560) has been evaluated in 597 assays and shows activity in only four confirmatory assays (Figure 2).

As **5** represents a racemic mixture, and initial testing was performed from the DMSO stock solutions, we started our medicinal chemistry campaign with a resynthesis of the original hit, as well as both the *R*- and *S*-enantiomers (from the commercially available starting materials). The resynthesized material reconfirmed with slightly lower potency than the original HTS hit (EC₅₀ = 1.7 μ M) (Table 1). Interestingly, when the purified enantiomers were evaluated, only the *R*-isomer showed any activity (*R*-isomer, EC₅₀ = 1.5 μ M; *S*-isomer, EC₅₀ > 30 μ M) and was equipotent with the racemic material. These results were very promising and we initiated an iterative medicinal chemistry effort aimed at evaluating each of the three distinct areas of the molecule – the upper amide portion, the piperidine, and the lower sulfonamide (see Figure 1).

The first area of SAR evaluation was the sulfonamide portion of the molecule. The synthesis of these molecules followed known synthetic protocols and is shown in Scheme 1. Starting from the commercially available (*R*)-*N*-Boc-2-piperidine carboxylic acid which was coupled with 4-(4-methoxyphenyl)thiazol-2-amine, **7**, using HATU and DIEA to yield **8**. The Boc group was removed (TFA, DCM) and a library of sulfonamides (or amides) was completed via sulfonyl chloride coupling under basic conditions.

The SAR evaluation started with replacement of the initial hit compound phenylsulfonamide portion with substituted aryl and heteroaryl groups (Table 2). Addition of an *ortho*-methyl group provided an equipotent compound (**10a**, 1.38 μ M), while a *meta*-substituted tolyl

group provided a modest improvement in potency (**10b**, 890 nM). Moving the methyl group to the 4-position resulted in a significant improvement in potency (**10c**, 260 nM). The *para* position tolerated an increase in bulk to a certain degree with the propyl group being well tolerated (**10d**, 700 nM); however, as the size increased the potency decreased (*iso*-propyl, **10e**, 2.59 μ M; *tert*-butyl, **10f**, >30 μ M). Replacement of the methyl group with the corresponding trifluoromethyl group led to a diminution in potency for all regioisomers (**10g–10i**, 1–5 μ M). Other substitution groups were tolerated in terms of potency (cyano, methoxy, fluoro and chloro; 1 μ M), with the 4-chloro compound (**10t**; 420 nM) being the most potent analog. Adding additional substituents, such as the addition of a methyl spacer group (benzyl) led to a complete loss of activity (**10u–10z**). Interestingly, replacement of the aryl group with 1-isoquinoline led to the discovery of a weak KCNQ1 inhibitor (**10aa**; IC₅₀ = 8 μ M). The 3-pyridyl replacement was equipotent with the phenyl (**10bb**; 1.36 μ M); however, other heteroaryl groups were not active, nor were the amide replacements for the sulfonamide group (**10ee–10gg**).

Utilizing the optimized 4-methylphenylsulfonamide group, along with the initial 4-(4-methoxyphenyl)thiazol-2-amine group, the internal piperidine ring system was evaluated (Table 3). The syntheses of compounds in Table 3 (**11a–l**) were completed as outlined in Scheme 1. Structural changes around this portion of the molecule were not well tolerated, as shown with the one carbon expansion of the ring (**11a**; >30 μ M); or the contraction of the ring system (**11b**; 2.86 μ M; **11c**; >30 μ M). Although the pyrrolidine ring system was active, **11b** was ~10-fold less potent than the piperidine analog (**10c**). Addition of a heteroatom to the ring was somewhat tolerated with the morpholine (**11d**; 4.38 μ M) and thiomorpholine (**11e**; 600 nM) producing moderately active compounds. However, the piperazine analog was inactive (**11f**). Lastly, moving the nitrogen and amide linker to a 1,3-arrangement was detrimental to activity as was removal of the ring system and replacement with straight (or branched) chain linkers (**11g–l**).

The final SAR library evaluated the upper amide portion of the molecule and the synthesis of these molecules is outlined in Scheme 2. For compounds **14a–h**, the synthesis started with a sulfonamide formation with the commercially available (*R*)-piperidine-2-carboxylic acid, **12**, (RSO₂Cl, TEA, DCM) followed by aniline coupling (R¹NH₂, HATU, DIEA, DMF) to yield the desired compounds **14a–h**. In order to access compounds **14i–o**, a further Suzuki coupling step was employed. To this end, **13** was coupled with an appropriately halogen substituted heteroaryl amine, **15** (HATU, DIEA, DMF) as outlined previously. Finally, **16** was subjected to Suzuki cross-coupling conditions (R²B(OH)₂, K₃PO₄, Pd(OAc)₂, THF:H₂O, 80 °C) leading to the isolation of the desired compounds.

The SAR evaluation started with minor changes to the right-hand phenyl group with replacements for the 4-methoxy group (Table 4). Unfortunately, alkyl, halogen and nitro replacements of the 4-methoxy group led to a total loss of activity against KCNQ1 (**14a–f**). In addition, truncation of the molecule into the thiazole (**14g**) or cyclization into the 2-benzo[d]thiazole (**14h**) was also not tolerated. Cyclization of the 4-methoxy group into the benzofuran moiety, **14i**, was tolerated; however there was 5-fold loss of potency (1.32 μ M). Even substituting the 4-methoxy with a 4-trifluoromethoxy group was not tolerated (**14j**). Finally, modification of the thiazole from the 2,4-substitution pattern to 2,5-substitution (**14k** and **14m**), or addition of an nitrogen (thiadiazole moiety) were not tolerated (**14l**, **14n–o**) highlighting the very narrow SAR window in this portion of the molecule, suggesting the 4-(4-methoxyphenyl)-2-methylthiazole moiety is an integral part of the binding on this molecule to KCNQ1.

To further profile our lead compound, **10c**, we performed a number of ancillary pharmacological assays to assess the overall selectivity of **10c**. Selectivity of **10c** for

blocking or activating KCNQ channel family members was assessed utilizing automated electrophysiology methods (AID: 493009) (Table 5). **10c** was shown to be >100-fold selective versus KCNQ2 and KCNQ4 (no significant change in amplitude, <25% activation up to 30 μ M). In addition, **10c** was evaluated against the hERG potassium channel and was inactive against this channel up to 30 μ M. Lastly, **10c** was evaluated against Ricerca Biosciences¹⁰ Lead Profiling assay which evaluates up to 68 GPCRs, ion channels and transports for % inhibition at 10 μ M. **10c** was found to bind to only 6 of the 68 assays conducted.¹¹ However, these assays are single-point values and functional assay selectivity will need to be evaluated. This point is highlighted by the fact that **10c** was shown to have 80% inhibition of the hERG binding assay; however, when evaluated in a functional assay **10c** showed no inhibition up to 30 μ M. Based on the potent activation of KCNQ1 and the clean selectivity profile, compound **10c** has been declared an MLPCN probe and redesignated as ML277.^{12,13}

Finally, we evaluated ML277 in our Tier 1 *in vitro* pharmacokinetic assays (Table 6) in order to gauge the metabolic stability and predicted clearance in rat (r) and human (h). Utilizing rapid equilibrium dialysis, we determined the protein binding of ML277 in human and rat plasma. ML277 showed high plasma protein binding in both human and rat ($F_u < 0.01$). ML277 was also assessed for its intrinsic clearance in hepatic microsomes in order to predict the *in vivo* clearance (CL_{HEP}) in rat and human. ML277 was unstable towards oxidative metabolism in both species and was therefore predicted to display high clearance in both human and rat microsomes (18.0 and 64.7 mL/min/kg, respectively). In order to better understand the metabolic instability of ML277 *in vitro*, we identified the primary pathways of biotransformation using mass spectrometric techniques. The results of this analysis revealed the principal pathways of metabolism were NADPH-dependent oxidation of the tolyl moiety and oxidative *O*-demethylation of the methoxy group. In an effort to block these sites of oxidation, and subsequently attenuate the metabolic instability, we replaced both the tolyl moiety and 4-methoxy groups with 4-trifluoromethoxy which resulted in a compound with significantly improved *in vitro* clearance (CL_{HEP} , 7.9 and 20.6 mL/min/kg, respectively for human and rat). While this compound proved inactive against KCNQ1, its improved DMPK properties provided valuable metabolism SAR in order to improve the PK properties of ML277.

In conclusion, we report the discovery of a novel and potent KCNQ1 activator (ML277) after a medicinal chemistry effort stemming hits obtained from a high-throughput screen of the MLSMR compound collection. The SAR analysis revealed that the (*R*)-isomer was the active enantiomer and further SAR studies showed the most active southern portion was the tolyl moiety. Further studies revealed that the 4-(4-methoxyphenyl)thiazole right-hand portion was critical for activity as all other replacements were inactive. Lastly, we have shown that ML277 is selective against KCNQ2 and KCNQ4. ML277 is an MLPCN probe and as such is freely available upon request.

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8. **Automated electrophysiology assay:** KCNQ1 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. CHO cells stably expressing KCNQ1 channels were freshly dislodged from flasks and dispensed into a 384-well population patch clamp (PPC) plate. The cell plating density was 6,000 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 KCNQ1 was then measured with the recording protocol as followings. Leak currents were linearly subtracted by extrapolating from the current elicited by a 100-ms step to -90 mV from a holding potential of -80 mV. During the voltage pulse protocol, cells were held at -80 mV, followed by a 2,000 ms depolarization from -80 mV to +40 mV, and then back to -80mV. The currents were measured at the end of the depolarization pulse to +40 mV before and after the application of compounds for 3 min. Only cells with a current amplitude more than 0.6–0.8 (depending on cell lot) nA at +40 mV and a seal resistance >30 MΩ were included in the data analysis. Compound effects were assessed by the percentage changes in the KCNQ1 steady state currents, which were calculated by dividing the difference between pre- and post-compound KCNQ1 currents by the respective pre-compound currents in the same well. No corrections for liquid junction potentials (estimated as -20 mV by comparing the KCNQ1 reversal potential with the calculated Nernst potential for potassium) were applied. The current signal was sampled at 0.625 kHz. A similar protocol was used to evaluate compound effects on KCNQ2 and KCNQ4 channels. For KCNQ4, the cells were depolarized to +40 mV from a holding potential of -70 mV. Currents were measured at the step current at +40 mV. Compound effects on hERG channels were evaluated using an IonWorks™ automated electrophysiology assay employing CHO cells stably expressing hERG channels, which is similar to the assay used for KCNQ1 channels with the exceptions noted below. Compound effects were examined using a pulse protocol consisting of a 100 ms step to -30 mV, a conditioning prepulse (2 s duration, 45 mV) followed by a test pulse (2 s duration, -30 mV) from a holding potential at -70 mV. hERG current amplitudes are calculated from the initial peak currents measured during the test pulses to -30 mV minus currents measured during the initial steps to -30 mV. Leak currents are estimated using a 100 ms step to -80 mV from the holding potential (-70 mV) and a linear correction is applied to the data.
9. All final compounds were purified by high-throughput HPLC and characterized by LCMS and/or ¹H NMR and found to be in agreement with their structures (>95% purity).
10. www.ricerca.com.
11. Ricerca Lead Profiling Results (% inhibition at 10 µM, species): Adenosine, A₃ (73%, human); Calcium Channel L-type (52%, rat); Cannabinoid, CB₁ (97%, human); Potassium channel, hERG (80%, human); Serotonin (5-hydroxytryptamine), 5-HT_{2B} (66%, human); Transporter, dopamine (DAT) (54%, human).
12. (*R*)-*N*-(4-(4-methoxyphenyl)thiazol-2-yl)-1-tosylpiperidine-2-carboxamide (ML277, CID 53347902). To a solution of 4-(4-methoxyphenyl)thiazol-2-amine, **2**, in DMF (0.65 mmol, 0.4 M) was added in order *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium

hexafluorophosphate (HATU; 0.72 mmol), *N,N*-diisopropylethylamine (DIEA; 2 mmol), and (*R*)-1-(tert-butoxycarbonyl)piperidine-2-carboxylic acid, **1**, (0.65 mmol). The mixture was stirred overnight at room temperature. The reaction mixture was dissolved in 5x volume H₂O and extracted with dichloromethane (DCM), then concentrated in vacuo. The product was purified by column chromatography (ethyl acetate and hexanes, ramp to 50% ethyl acetate). The Boc-protected intermediate was subsequently dissolved in minimal DCM. To this was added equivolume TFA and the deprotection proceeded at room temperature for 2 hours, at which time the reaction mixture was concentrated *in vacuo*. The final compound was afforded by dissolving the deprotected intermediate (0.57 mmol) in DCM (0.1 M). To this solution was added triethylamine (1.14 mmol) and *p*-toluenesulfonyl chloride (TsCl; 0.63 mmol). After stirring overnight at room temperature, the reaction mixture was washed with saturated sodium bicarbonate and brine and passed through a phase separator. The mixture was concentrated in vacuo and purified by HPLC. ¹H NMR: (400 MHz, CDCl₃) δ (ppm): 9.98 (br. s., 1 H), 7.81 (m, 4 H), 7.39 (d, *J* = 8.1 Hz, 2 H), 7.05 (s, 1 H), 6.98 (dt, *J* = 8.9, 2.6 Hz, 2 H), 4.75 (d, *J* = 4.8 Hz, 1 H), 4.08 (dd, *J* = 14.7, 2.2 Hz, 1 H), 3.88 (s, 3 H), 3.14 (td, *J* = 14, 2.4 Hz, 1 H), 2.48 (s, 3 H), 2.33 (d, *J* = 14 Hz, 1 H), 1.63-1.12 (m, 5 H); ¹³C NMR: (100 MHz, CDCl₃) δ (ppm): 168.06, 159.55, 156.86, 149.71, 144.18, 136.77, 130.11, 127.33, 127.19, 126.93, 114.05, 105.90, 56.21, 55.28, 43.94, 23.54, 23.07, 21.55, 19.75. LCMS: R_T = 0.845 min., *m/z* = 472.1 [M + H]⁺ (>99% @ 215 and 254 nm).

13. **10c**, VU0458298 (ML277) has been declared a probe via the Molecular Libraries Probe Production Centers Network (MLPCN) and is available through the network, see: <http://mli.nih.gov/mli/>.

Figure 1.
Literature reported activators of KNCQ1.

5**CID: 4880560****KCNQ1 EC₅₀ = 660 nM**

Figure 2.
Structure of the initial HTS hit.

Scheme 1.

(a) HATU, DIEA, DMF, 4-(4-methoxyphenyl)thiazol-2-amine (**7**) (42–77%), (b) TFA, DCM, (c) RSO₂Cl (or ROCl), TEA, DCM.⁹

Scheme 2.

(a) *p*-TsCl or *p*-chlorophenylSO₂Cl, TEA, DCM (R = CH₃, 57–72%; R = Cl, 37 – 43%); (b) R¹NH₂, HATU, DIEA, DMF; (c) HATU, DIEA, DMF (R = CH₃, 12–79%; R = Cl, 19–75%); (d) R²B(OH)₂, K₃PO₄, Pd(OAc)₂, THF:H₂O, 80 °C.⁹

Table 1

Resynthesis and synthesized enantiomer activity.

Cmpd	Structure	EC ₅₀ (μM) ^a	%Max ^b
5		1.69 ± 0.35	285.0 ± 30.3
(R)-5		1.47 ± 0.58	358.9 ± 34.5
(S)-5		>30	

^aEC₅₀'s were generated from 8-point concentration response curves in duplicate with 3-fold dilutions starting from the maximal concentration (30 μM).

^bThe % Max values list the percent increase in KCNQ1 currents recorded at +40 mV at saturating compound concentrations in dose-response experiments for each compound. These maximal measured increases can vary for each compound and along with the EC₅₀ would contribute to compound efficacy in tissue or animal experiments.

Table 2SAR evaluation of the sulfonamide analogs, **10a–gg**.

Cmpd	R	EC ₅₀ (μM) ^a	%Max ^b
(R)-5		1.47 ± 0.58	358.9
10a		1.38 ± 0.24	145.6
10b		0.89 ± 0.05	252
10c		0.26 ± 0.03	265.9
10d		0.70 ± 0.12	172
10e		2.59 ± 0.56	109

Cmpd	R	EC ₅₀ (μM) ^a	%Max ^b
10f		>30	
10g		4.74 ± 0.62	131.0
10h		1.00 ± 0.19	125.1
10i		1.71 ± 1.00	60.4
10j		1.78 ± 0.30	32.2
10k		0.81 ± 0.06	256.6

Cmpd	R	EC ₅₀ (μM) ^a	%Max ^b
10l		0.78 ± 0.05	178
10m		>30	20.9
10n		0.88 ± 0.14	132.5
10o		1.63 ± 0.10	88
10p		1.16 ± 0.03	231.8
10q		1.03 ± 0.03	231.9
10r		0.73 ± 0.09	52

Cmpd	R	EC ₅₀ (μM) ^a	%Max ^b
10s		1.41 ± 0.05	178.6
10t		0.42 ± 0.06	161.0
10u		>30	
10v		>30	
10w		>30	20.2
10x		>30	31.1
10y		>30

Cmpd	R	EC ₅₀ (μM) ^a	%Max ^b
10z		>30	
10aa		7.97 ± 0.87	(inhibitor)
10bb		1.36 ± 0.14	87.3
10cc		>30	
10dd		>30	
10ee		6.13 ± 3.98	47.8
10ff		8.16 ± 12.4	68.0
10gg		>30	

^aEC₅₀'s were generated from 8-point concentration response curves in duplicate with 3-fold dilutions starting from the maximal concentration (30 μM). 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 calculated EC₅₀ value. EC₅₀ values are expressed as EC₅₀ ± SD, using estimated standard deviations provided by the fitting software (Origin 6.0).

^bThe % Max values list the percent increase in KCNQ1 currents recorded at +40 mV at saturating compound concentrations in dose-response experiments for each compound. These maximal measured increases can vary for each compound and along with the EC₅₀ would contribute to compound efficacy in tissue or animal experiments.

Table 3

SAR of internal ring system (**11a–l**).

Cmpd		EC ₅₀ (μM) ^a	%Max ^b
11a		>30	
11b		2.86 ± 0.17	64
11c		>30	
11d		4.38 ± 0.87	54
11e		0.60 ± 0.14	88
11f		>30	
11g		>30	
11h		>30	
11i		>30

Cmpd		EC ₅₀ (μM) ^a	%Max ^b
11j		>30	
11k		>30	
11l		12.9 ± 1.4	121

^aEC₅₀'s were generated from 8-point concentration response curves in duplicate with 3-fold dilutions starting from the maximal concentration (30 μM). 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 calculated EC₅₀ value. EC₅₀ values are expressed as EC₅₀ ± SD, using estimated standard deviations provided by the fitting software (Origin 6.0).

^bThe % Max values list the percent increase in KCNQ1 currents recorded at +40 mV at saturating compound concentrations in dose-response experiments for each compound. These maximal measured increases can vary for each compound and along with the EC₅₀ would contribute to compound efficacy in tissue or animal experiments.

Table 4SAR evaluation of the amide analogs, **14a–x**.

Cmpd	R ¹	R	EC ₅₀ (μM) ^a	%Max ^b
10c		Me	0.26 ± 0.03	265.9
14a		Me	>30	
14b		Me	>30	
14c		Me	>30	
14d		Me	>30	
14e		Me	>30	
14f		Me	>30	
14g		Me	>30	
14h		Me	>30	
14i		Me	1.32 ± 0.42	30
14j		Me	>30	
14k		Me	27.4 ± 61.3	Inhibitor

Cmpd	R ¹	R	EC ₅₀ (μM) ^a	%Max ^b
14l		Me	>30	
14m		Me	>30	
14n		Me	>30	
14o		Cl	>30	

^aEC₅₀'s were generated from 8-point concentration response curves in duplicate with 3-fold dilutions starting from the maximal concentration (30 μM). 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 calculated EC₅₀ value. EC₅₀ values are expressed as EC₅₀ ± SD, using estimated standard deviations provided by the fitting software (Origin 6.0).

^bThe % Max values list the percent increase in KCNQ1 currents recorded at +40 mV at saturating compound concentrations in dose-response experiments for each compound. These maximal measured increases can vary for each compound and along with the EC₅₀ would contribute to compound efficacy in tissue or animal experiments.

Table 5

Effects of **10c** on activation of KCNQ2, KCNQ4 and inhibition of hERG channels. EC₅₀ values are plotted for KCNQ1, KCNQ2 and KCNQ4 channels. IC₅₀ value is given for hERG.

Cmpd	EC ₅₀ /IC ₅₀ (μM)			
	KCNQ1	KCNQ2	KCNQ4	hERG
10c	0.26	>30	>30	>30

Table 6In vitro pharmacokinetic properties of **10c** (ML277).

10c, ML277	
MW	407.5
cLogP	5.04
hPPB (% fu)	0.6
rPPB (% fu)	0.7
hCL _{HEP} (mL/min/kg)	18.0
rCL _{HEP} (mL/min/kg)	64.7