(*S*)-*N*-[1-(3-Morpholin-4-ylphenyl)ethyl]-3-phenylacrylamide: An Orally Bioavailable KCNQ2 Opener with Significant Activity in a Cortical Spreading Depression Model of Migraine

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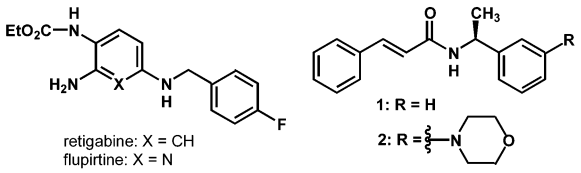
Abstract: (*S*)-*N*-[1-(3-Morpholin-4-ylphenyl)ethyl]-3-phenylacrylamide (**2**) was synthesized as an orally bioavailable KCNQ2 potassium channel opener. In a rat model of migraine, **2** demonstrated significant oral activity in reducing the total number of cortical spreading depressions induced by potassium chloride.

Introduction. Potassium (K^+) channels are considered to be the most diverse class of ion channels with greater than 60 genes identified. One or more types of K^+ channels are usually present on excitable cell membranes, where they have critical roles in cell function. This has been demonstrated in neurons where they are responsible, in part, for determining cell excitability by contributing to membrane repolarization following depolarization, resting membrane potential, and regulation of neurotransmitter release. The biophysical and pharmacological characteristics of different K^+ channels vary widely, contributing to the complex physiology of excitable cells. Within the past several years, several members of the KCNQ gene family have been identified with some high degree of CNS specificity. These channels are the molecular substrate of the cholinergically inhibited "M channel",[1] which regulates, in part, the subthreshold electrical activity of many different neurons. The M channel has been reported to consist of heterotetramers of KCNQ2 and KCNQ3 proteins and possibly KCNQ3 and KCNQ5. Modulators of the M channel have been under clinical investigation,

Chart 1



i.e., blockers for cognition enhancement[2] and openers for epilepsy.[3] Retigabine (Chart 1) has been shown to activate human KCNQ2, KCNQ2/3, and KCNQ5 mediated currents. The activation was induced by a shift of the opening threshold of both the homomeric and heteromeric potassium channels to more negative potentials.[4−6] Flupirtine (Chart 1), a close analogue of retigabine, also shifts the activation curves toward negative voltages, but it is less effective than retigabine.[7] Both compounds demonstrated significant analgesic activity on tactile allodynia in Chung lesioned rats, indicating the therapeutic potential of KCNQ openers for the treatment of neuropathic pain.[7]

High-throughput screening of the Bristol-Myers Squibb compound collection using thallium(I) influx assay[8] identified (*S*)-3-phenyl-*N*-(1-phenylethyl)acrylamide (**1**) as a lead, and subsequent lead optimization led to **2** (Chart 1) as an orally bioavailable KCNQ2 opener. Acrylamide **2** was evaluated in a model of cortical spreading depression (CSD). CSD is defined as a wave of brief neuronal excitation followed by a long-lasting inhibition that originates from a cerebral focal point and spreads out at a rate of 2−3 mm/min.[9,10] Visual aura and other prodromal symptoms in patients with classic migraine appear to proceed in a temporal fashion similar to experimentally induced spreading depression in animals.[11,12] Therefore, it has been suggested that CSD may underlie prodromal events that precede classic migraine, particularly visual aura.[13] Recently, spreading depression has been visualized in migraine patients using functional magnetic resonance imaging.[14] Experimentally, CSD has been shown to produce a transient, localized hyperemia and plasma protein extravasation, mimicking an inflammatory process.[15] This in turn is believed to sensitize trigeminal primary afferent neurons, leading to enhanced pain perception.[15,16] Antimigraine drugs are believed to interfere with these processes.[13,14] This lends support to the hypothesis that CSD may mediate the visual aura, and possibly other prodomal symptoms,[9] that precede classic migraine attacks and the ensuing pain.[15] Thus, compounds that interrupt cortical spreading depression may be useful for the treatment of some types of migraine headache. This report describes the synthesis of acrylamide **2**, its effects on KCNQ2-mediated currents, and its effect on cortical spreading depression in rats.

Chemistry. The synthesis of **2** is shown in Scheme 1. Coupling of 3-phenylacrylic acid (**3**) with (*S*)-1-(3-methoxyphenyl)ethylamine provided amide **4**. The methoxy group of **4** was cleaved with boron tribromide, and the resulting phenol was converted to triflate **5**. Palladium-catalyzed coupling of triflate **5** with morpholine furnished acrylamide **2**.

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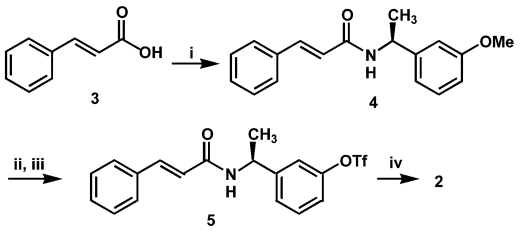
Scheme 1[a]



[a] Reagents: (i) (*S*)-1-(3-methoxyphenyl)ethylamine, EDAC·HCl, DMAP, Et_3N, CH_2Cl_2, 70%; (ii) BBr_3, CH_2Cl_2, −78 °C, 95%; (iii) Tf_2O, pyridine, CH_2Cl_2, −78 °C, 80%; (iv) morpholine, $Pd_2(dba)_3$ (5 mol %), 2-(di-*tert*-butylphosphino)biphenyl (10 mol %), K_3PO_4, DME, 80 °C, 67%.

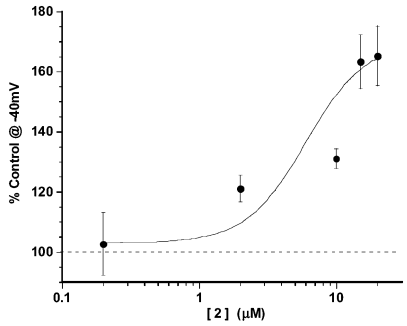


Figure 1. Effects of **2** on mKCNQ2 currents at a single membrane voltage (−40 mV) in oocytes (n = 4−7).

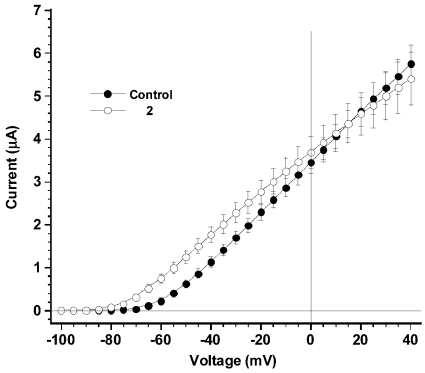


Figure 2. Effects of **2** (20 μM) on the mKCNQ2 current−voltage relationship in oocytes (n=7).

Results and Discussion. The effects of **2** on outward potassium current were determined by using two electrode voltage clamp recordings from *Xenopus laevis* oocytes expressing cloned mKCNQ2 channels. The evaluation in Figure 1 was conducted at a single membrane voltage (−40 mV), and the effects of this compound on mKCNQ2 current were expressed as the percent of compound free control current. Figure 1 demonstrates the concentration−response relationship obtained from the application of compound **2** to oocytes expressing the mKCNQ2 construct. The estimated EC_{50} for the compound is 6.0 μM.

Figure 2 describes the current−voltage ($I-V$) relationship (−100 to +40 mV) for compound free control mKCNQ2 currents (●) and for the effects of **2** (20 μM; ○) on the same oocytes. Application of **2** had a marked effect on the mKCNQ2 $I-V$ relationship. First, application of **2** generated a hyperpolarizing shift in the threshold for current activation from approximately −70 to −85 mV. Second, compound **2** produced a significant increase in outward current from approximately −70 to +10 mV; at higher voltages the compound resulted in a decrease in the apparent amplitude of the currents. Observations similar to this were reported in oocytes expressing hKCNQ2 currents following application of retigabine.[5]

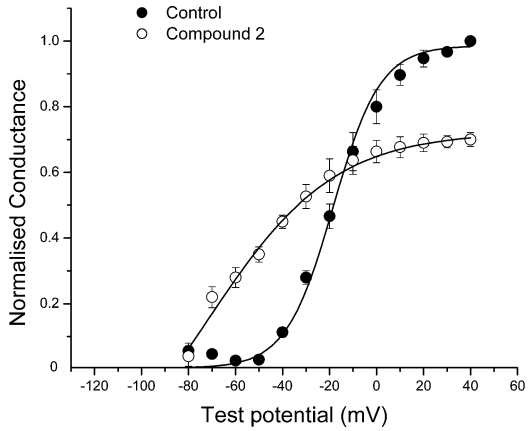


Figure 3. Effect of **2** (10 μM) on mouse KCNQ2 conductance. Note the "crossover" in the $G-V$ relationship produced by compound **2**, where there is a hyperpolarizing shift in the voltage dependence of activation until approximately −20 mV but a pronounced inhibition of KCNQ2 conductance at potentials above −15 mV. Data shown are the mean ± SEM for n = 5 cells.

The experiments described above were carried out in *Xenopus* oocytes expressing mKCNQ2. However, oocytes have been shown to express endogenous KCNQ1 channels,[17] and it is unknown if they likewise express other endogenous family members. Thus, we examined the effects of **2** in an alternative expression system to rule out any oocyte-specific actions of **2**. Figure 3 shows the changes in outward conductance of mKCNQ2 stably expressed in HEK 293 cells produced by local superfusion of **2** (10 μM). Compound **2** appears to linearize the voltage dependence of activation of KCNQ2 in the membrane potential range (−110 to about −30 mV), suggesting that **2** may remove the voltage dependence of activation of KCNQ2 channels. The voltage for half-activation ($V_{0.5}$) was −18.3 mV in control conditions and −52.1 mV in the presence of 10 μM compound **2**, a 33.8 mV shift in the hyperpolarizing direction, thus greatly increasing the open probability (P_o) of KCNQ2 channels at membrane potentials where the channel open probability is normally close to zero. Under in vivo physiological conditions, one might expect **2** to produce profound hyperpolarization of neurons expressing KCNQ channels, and indeed, we have shown **2** to hyperpolarize HEK 293 cells expressing mKCNQ2 (data not shown). Similar to the oocyte results, compound **2** also inhibited mKCNQ2 conductance at more positive test potentials, in this case, at potentials more positive than approximately −15 mV. It will be interesting to determine if such a "crossover" in the conductance−voltage relationship occurs in KCNQ2/heteromultimer combinations such as KCNQ2/Q3 and KCNQ3/Q5, which are thought to constitute some native M channels. In general, the mechanism and physiological significance of facilitated conductance at negative potentials and inhibited conductance at more positive potentials are unknown but would depend on the "crossover" voltage of a compound. Electrophysiological measurements from neurons would

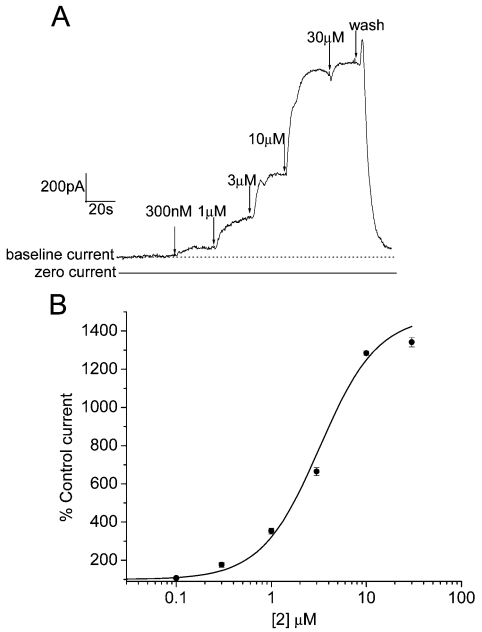


Figure 4. Dose-dependent effects of **2** on mKCNQ2 currents expressed in an HEK 293 stable cell line: (A) concentration-dependent augmentation of KCNQ2 current in a single cell voltage-clamped at −40 mV; (B) logistic fit to the mean ± SEM concentration−response data obtained from three cells.

help to reveal the physiological effects of this voltage-dependent alteration of KCNQ2 channels.

Figure 4 shows the concentration-dependent facilitation of KCNQ2 current from single cells voltage-clamped at −40 mV. The threshold concentration of compound **2** for augmenting KCNQ2 current was approximately 300 nM. The onset of compound **2** action was fast (1−2 s) and reached a plateau in approximately 15 s. As shown in Figure 4A, the effect of compound **2** was readily reversible after application of compound free control saline (wash). Measurements from three such experiments were used to construct the concentration−response curve shown in Figure 4B. The EC_{50} for this compound in this system is about 3.3 ± 0.6 μM and again is in general agreement with the effects in the oocyte expression system.

The effects of **2** on membrane potential were also investigated using a fluorescent membrane potential assay employing voltage-sensitive dyes in SH-SY5Y human neuroblastoma cells[18] expressing native KCNQ channels. The relative fluorescence intensity change was used as an indication of the change in membrane potential.[19] As shown in Figure 5, application of **2** led to a concentration-dependent hyperpolarization of the cells. A mean EC_{50} of 0.69 ± 0.08 μM was calculated. This greater potency, compared to electrophysiology measurements, likely reflects differences in the cell types and assays used in the experimentation and possibly other KCNQ channels found in these cells.

Compound **2** was characterized in several pharmacokinetic studies (Table 1). Oral bioavailability was excellent in both rat and dog, 93% and 100%, respectively. The favorable oral bioavailability is presumably due to its good absorption, consistent with the excellent Caco-2 permeability (Pc 260 nm/s) and moderate clearance. The oral absorption was rapid in the dog, with peak concentration occurring within 0.5 h, whereas the compound was slowly absorbed in rat. Acrylamide **2** was also shown to have moderate brain penetration based on the samples taken at the approximate t_{max} in both species (Table 2).

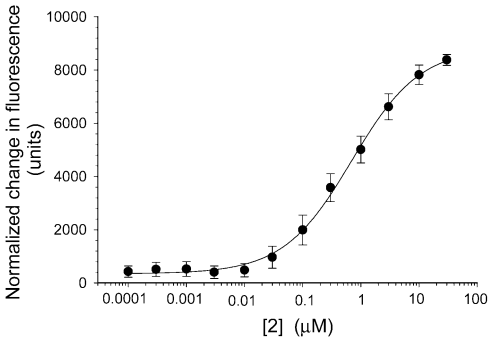


Figure 5. Effects of **2** on resting membrane potential in SH-SY5Y human neuroblastoma cells (n = 4).

Table 1. Pharmacokinetic Parameters of **2**

PK parameters	rat[a]	dog[b]
iv		
dose, mg/kg	5	1
$t_{1/2}$, h	2.1 ± 0.5	3 ± 0.3
Cl, mL/(min·kg)	41 ± 4	12.7 ± 0.2
V_{dss}, L/kg	2.5 ± 0.6	3 ± 0.8
po (solution)		
dose, mg/kg	11	3
t_{max}, h	5 ± 1	0.5 ± 0.25
F, %	93	100

[a] Compound dosed in Sprague−Dawley rats as a solution in 90% PEG-400/10% Tween 80 (n = 3), iv and in 90% PEG-400/10% Tween 80 (n = 3), po. [b] Compound dosed in Beagle dogs (n = 3) as a solution in 50% PEG-400/50% H_2O, iv and po.

Table 2. Brain, Plasma, and Brain/Plasma Data of **2**

	rat[a]	dog[b]
brain concn, ng/g	631 ± 136	538
plasma concn, ng/mL	804 ± 279	640
B/P ratio	0.78	0.84

[a] Compound dosed in Sprague−Dawley rats as a solution in 100% PEG-400 at 20 mg/kg, po (n = 3), and samples were taken at 6 h. [b] Compound dosed in a Beagle dog as a solution in 90% PEG-400/10% Tween 80 at 3 mg/kg, po (n = 1), and samples were taken at 30 min.

Compound **2** was examined for its ability to reduce the total number of CSDs following intragastric (IG) administration. CSD was produced by a 10 min application of crystalline potassium chloride to a small region in the rat parietal cortex. The total number of depolarizations in the distal lead of each animal was used to determine the mean number of depolarizations per animal. These were pooled within each treatment group and analyzed. Since the data were distributed in a non-Gaussian fashion, intergroup comparison of depolarizations using the nonparametric Kruskal−Wallis test was performed. As shown in Figure 6, **2** produced a dose-related reduction in the total number of depolarizations. Both 10 and 30 mg/kg doses were effective in significantly reducing the total number of depolarization events. Comparison of the proximal depolarizations between treatment groups also yielded an identical pattern [vehicle (PEG 400), 36 ± 1; **2**, 3 mg/kg, 36 ± 4; **2**, 10 mg/kg, 25 ± 4 ($p < 0.05$); **2**, 30 mg/kg, 23 ± 2 ($p < 0.001$)].

The ratio of proximal to distal depolarizations (P/D ratio) was 1.07 ± 0.03 in the vehicle-treated control

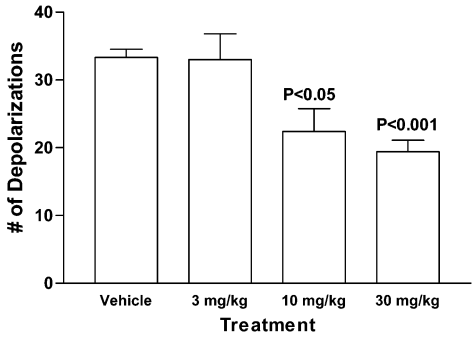


Figure 6. Effect of **2** (IG) on KCl-induced cortical depolarizations. Mean depolarizations in the distal lead from individual rats organized as columns based on treatment. Horizontal bar represents the group mean ± SEM.

group. This ratio showed a dose-related increase across treatment groups (3 mg/kg, 1.10 ± 0.03; 10 mg/kg, 1.15 ± 0.06; 30 mg/kg, 1.22 ± 0.06). The increase in *P*/*D* ratio for the 30 mg/kg treated group was significant in comparison with the vehicle-treated group ($p < 0.05$, Kruskal−Wallis test with Dunn's posttest).

We have hypothesized that a KCNQ opener would be efficacious in a cortical model of hyperexcitability by virtue of increasing K^+ efflux from the cells, stabilizing the cell membrane, and thus making it harder for depolarizing stimuli to spread. Consistent with this hypothesis, **2** reduced the incidence of depolarizations in a dose-related fashion. The highest dose tested showed a 42% reduction in the incidence of depolarizations. This pattern of dose-related reduction was also observed with the depolarizing events recorded by the proximal lead. Moreover, the ratio of the proximal to distal depolarizations was significantly higher in the 30 mg/kg treated dose. Thus, in addition to reducing the incidence of cortical depolarizations per se, **2** also appears to significantly reduce their spread.

By use of a fluorescent dye in a FLIPR-based screen,[8] preliminary evidence showed **2** to activate other members of the KCNQ family. Although **2** activated these channels, further electrophysiological characterization is required. In this report we focused on the effects of **2** on KCNQ2. Given that **2** is most likely nonselective, the activity on homomeric KCNQ2 currents still may be sufficient for in vivo CSD efficacy, since many neuronal populations contain only the KCNQ2 channel as determined by in situ hybridization (data not shown). Finally, we can estimate the effective brain concentration as close to or less than the in vitro EC_{50}. At a 10 mg/kg IG dose of **2**, the brain concentration is approximately 1 μM, which effectively reduced the total number of CSDs. The exact effective brain concentration remains to be established.

Conclusion. **2** was identified as a KCNQ2 opener with excellent oral bioavailability in dogs and rats. In vivo testing of **2** in the CSD model suggests that KCNQ2 openers may have potential for the treatment of CNS disorders characterized by hyperexcitability, such as migraine headache with aura.

Supporting Information Available: Experimental details for the synthesis of **2** and the biological assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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