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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-1*H*-indol-5-yl)-ethyl acrylamides

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Abstract—Bioisosteric replacement studies led to the identification of *N*-(1-benzo[1,3]dioxol-5-yl-ethyl)-3-(2-chloro-phenyl)-acrylamide ((*S*)-**3**) as a highly potent KCNQ2 opener, and 3-(2,6-difluoro-phenyl)-*N*-[1-(2,3-dihydro-benzofuran-5-yl)-ethyl]-acrylamide ((*S*)-**4**), and *N*-[1-(2,3-dihydro-1*H*-indol-5-yl)-ethyl]-3-(2-fluoro-phenyl)-acrylamide ((*S*)-**5**) as highly efficacious KCNQ2 openers. In contrast, their respective *R* enantiomers showed significantly less or no appreciable KCNQ2 opener activity even at the highest concentration tested (10 μM). Because of its high potency and moderate efficacy as well as its convenient synthesis, (±)-**3** was selected as a reference compound for analyzing efficacies of KCNQ openers in electrophysiology studies. Compounds (*S*)-**4** and (*S*)-**5** demonstrated significant activity in reducing neuronal hyperexcitability in rat hippocampal slices. The synthesis and the KCNQ2 opener activity of these acrylamides are described.

1. Introduction

KCNQ2 and KCNQ3 potassium channel heteromultimers are the principal molecular substrates of the M-channels.[1,2] The M-channels have been identified with a high degree of CNS localization, including regions linked to epilepsy, migraine, and neuropathic pain, such as the cortex, hippocampus trigeminal nucleus caudalis, and dorsal horn. The M-channels are also expressed in the peripheral nervous system, including regions linked to migraine and neuropathic pain, such as trigeminal ganglion and dorsal root ganglia. Modulators of the M-channel have been under clinical investigation: blockers (e.g., linopirdine) for cognition enhancement[3] and openers (e.g., retigabine, Fig. 1) for epilepsy.[4] The M-channels play a critical role in controlling the excitability of neurons and nociceptors.[5] Thus, retigabine attenuated nociceptive behaviors in rat models of persistent and neuropathic pain.[6] In addition, retigabine

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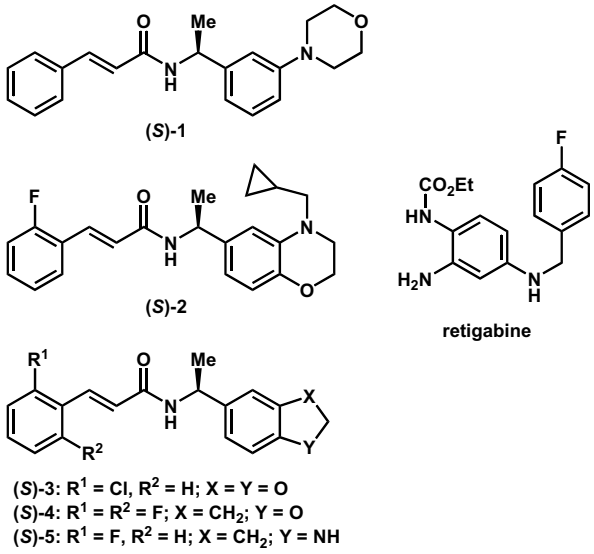


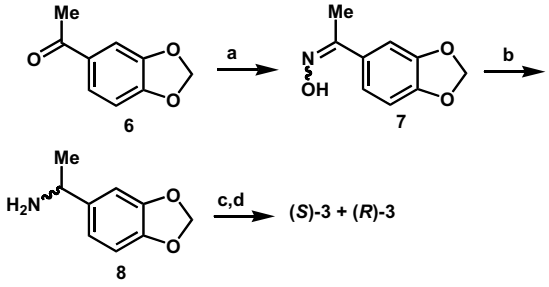
Figure 1.

reduced the behavioral manifestation of nociceptive activity in a model of inflammatory pain.[5] This has been attributed to the M-current enhancement as the pain reduction mediated by retigabine was blocked by the concurrent application of the M-current blocker XE991.[5] Recently, we reported that (*S*)-*N*-[1-(3-morpholin-4-yl-phenyl)-ethyl]-3-phenyl-acrylamide ((*S*)-**1**), a novel KCNQ2 opener, demonstrated significant oral activity in the cortical spreading depression model of migraine.[7] Thus, enhancement of KCNQ/M-channel activity may represent a novel approach for the treatment of epilepsy, migraine, neuropathic pain, and perhaps other disorders of neuronal excitability.[8–10]

As part of our continuous efforts toward the identification of KCNQ2 openers with improved potency and efficacy over retigabine and acrylamide (*S*)-**1**, we recently identified (*S*)-*N*-[1-(4-cyclopropylmethyl-3,4-dihydro-2*H*-benzo[1,4]oxazin-6-yl)-ethyl]-3-(2-fluoro-phenyl)-acrylamide ((*S*)-**2**) as a potent and efficacious KCNQ2 opener.[11] The discovery of acrylamide (*S*)-**2** prompted us to further investigate this class of acrylamides. Thus, we explored the bioisosteric replacement of the 3,4-dihydro-2*H*-benzo[14] oxazin moiety of (*S*)-**2**, and these efforts culminated in the identification of *N*-(1-benzo[1,3]dioxol-5-yl-ethyl)-3-(2-chloro-phenyl)-acrylamide ((*S*)-**3**) as a highly potent KCNQ2 opener, and 3-(2,6-difluoro-phenyl)-*N*-[1-(2,3-dihydro-benzofuran-5-yl)-ethyl]-acrylamide ((*S*)-**4**), and *N*-[1-(2,3-dihydro-1*H*-indol-5-yl)-ethyl]-3-(2-fluoro-phenyl)-acrylamide ((*S*)-**5**) as highly efficacious KCNQ2 openers. This report describes the effects of these acrylamides and their respective enantiomers on KCNQ mediated currents and the activity on neuronal hyperexcitability in rat hippocampal slices.

2. Chemistry

Scheme 1 describes the synthesis of (*S*)-**3** and (*R*)-**3**. Commercially available 1-benzo[1,3]dioxol-5-yl-ethanone (**6**) was converted to oxime **7**, which was reduced to amine (±)-**8**. This amine was coupled with 3-(2-chloro-phenyl)-acrylic acid in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDAC·HCl), 4-dimethylaminopyridine (DMAP), and triethylamine, and the resulting (±)-**3** was separated by chiral HPLC to furnish two enantiomers: (*S*)-**3**[12] and (*R*)-**3**. The absolute configuration of (*R*)-**3** was determined by X-ray diffraction analysis (Fig. 2).



Scheme 1. Reagents and conditions: (a) $NH_2OH{\cdot}HCl$, Et_3N, EtOH, 25 h; (b) H_2, Ra-Ni, 30% $NH_3{\cdot}H_2O$ in MeOH, 100% over two steps; (c) 3-(2-chloro-phenyl)-acrylic acid, EDAC·HCl, DMAP, Et_3N, CH_2Cl_2, 91%; (d) Chiral HPLC (AD column).

The synthesis of acrylamide **4**[13] is analogous to that of **3** (Scheme 2). The absolute configuration of (*R*)-**4** was determined by X-ray diffraction analysis of the urea derivative (**12**) of its degradation product (*R*)-**11** shown in Scheme 3 (Fig. 3).

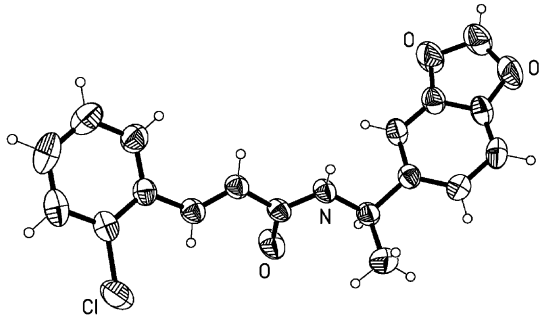
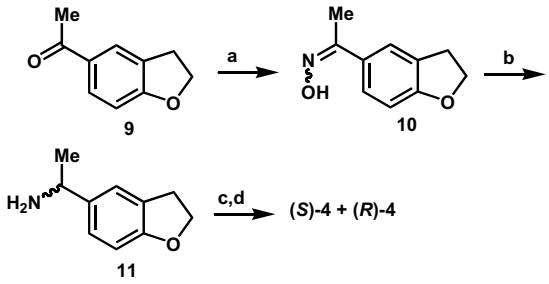
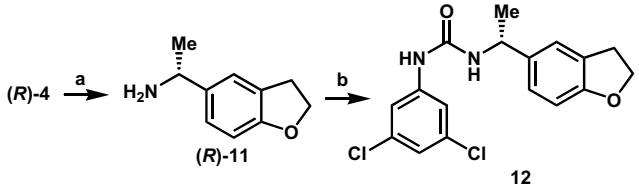


Figure 2. Thermal ellipsoid plot (35% ellipsoids) of crystalline (*R*)-**3**. Crystallographic atom numbering.



Scheme 2. Reagents and conditions: (a) $NH_2OH{\cdot}HCl$, Et_3N, EtOH, 25 h; (b) H_2, Ra-Ni, 30% $NH_3{\cdot}H_2O$ in MeOH, 100% over two steps; (c) 3-(2,6-difluoro-phenyl)-acrylic acid, EDAC·HCl, DMAP, Et_3N, CH_2Cl_2, 91%; (d) Chiral HPLC (AD column).



Scheme 3. Reagents and conditions: (a) NH_2NH_2, EtOH, 80 °C, 75%; (b) 3,5-dichlorophenyl isocyanate, *i*-Pr_2NEt, CH_2Cl_2, 85%.

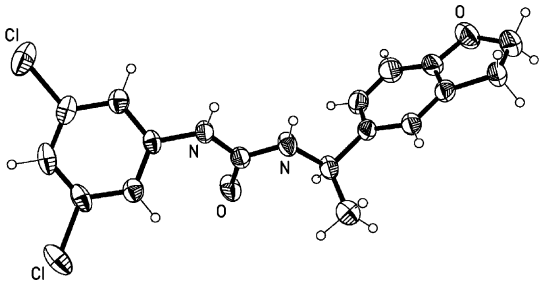
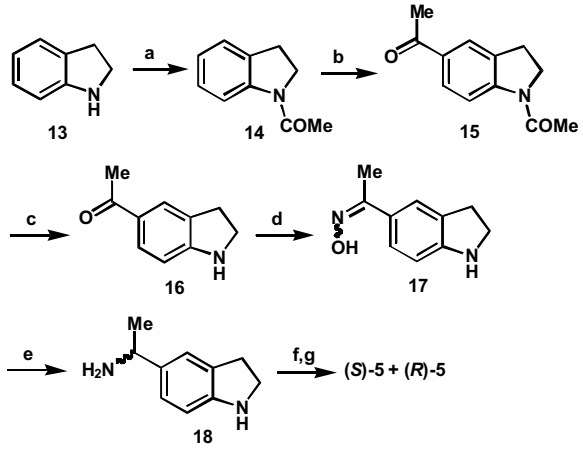
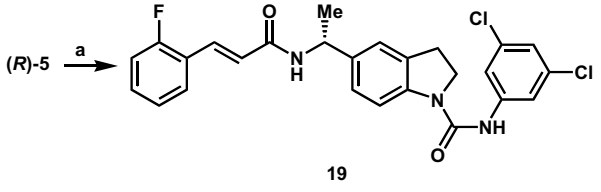


Figure 3. Thermal ellipsoid plot (35% ellipsoids) of crystalline **12**. Crystallographic atom numbering.

Scheme 4 depicts the synthesis of acrylamide **5**. Commercially available 2,3-dihydro-1*H*-indole (**13**) was converted to its acetamide **14**, which underwent a regioselective Friedel–Crafts reaction to furnish methyl ketone **15**. Deprotection of the acetamide moiety was achieved upon exposure to concentrated hydrochloric acid, and the resulting methyl ketone **16** was converted to (*S*)-**5**[14] and (*R*)-**5** in the same fashion as that described for (*S*)-**3** and (*R*)-**3** (Scheme 1). The absolute configuration of (*R*)-**5** was determined by X-ray diffraction analysis of its urea derivative **19** shown in Scheme 5 (Fig. 4).



Scheme 4. Reagents and conditions: (a) CH_3COCl, Et_3N, DMAP, CH_2Cl_2, 99%; (b) CH_3COCl, $AlCl_3$, CS_2, 40%; (c) concd HCl, 100%; (d) $NH_2OH{\cdot}HCl$, Et_3N, EtOH, 25 h; (e) H_2, Ra-Ni, 30% $NH_3{\cdot}H_2O$ in MeOH, 100% over two steps; (f) 3-(2-fluoro-phenyl)-acrylic acid, EDAC·HCl, DMAP, Et_3N, CH_2Cl_2, 91%; (g) Chiral HPLC (AD column).



Scheme 5. Reagents and conditions: (a) 3,5-dichlorophenyl isocyanate, *i*-Pr_2NEt, CH_2Cl_2, 85%.

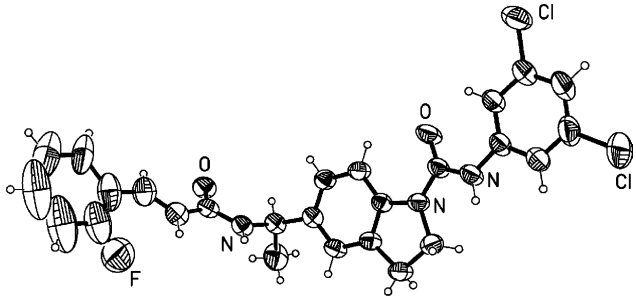


Figure 4. Thermal ellipsoid plot (35% ellipsoids) of crystalline **19**. Crystallographic atom numbering.

3. Results and discussion

The effects of test compounds on outward potassium currents were determined using the whole-cell patch-clamp recordings from HEK 293 cells stably expressing cloned mouse (m) KCNQ2 channels as described previously.[7] Currents were evoked by stepping from −80 mV holding potential to −40 mV. The evaluation was conducted in the presence of the test compound and compound-free control conditions. Compound effects were expressed as the percent of compound-free control current using current responses measured at −40 mV. The data at −40 mV were used for preliminary structure activity relationship studies as this membrane potential is most likely related to physiological conditions. The EC_{50}'s shown in Table 1 were calculated from the concentration–response relationship of the test compounds. The efficacies of test compounds were normalized to the efficacy of (±)-**3** to yield an E/E_{ref} ratio (Table 1). The ratio was calculated by dividing the maximum current amplitude produced by the test compound by the maximum current amplitude produced by the reference compound. Compounds which provide a higher E/E_{ref} ratio are more efficacious KCNQ2 channel openers.

Table 1. Whole-cell patch-clamping data (−40 mV)

Compd	EC_{50} (μM)	E/E_{ref} [a]
(*S*)-**3**	0.0200 ± 0.0001	1.1 ± 0.1
(±)-**3**	0.072 ± 0.002	1.0 ± 0.1
(*S*)-**4**	0.33 ± 0.01	2.4 ± 0.1
(*S*)-**5**	0.43 ± 0.02	2.4 ± 0.2
(*R*)-**5**	NA	0.41 ± 0.04
(*S*)-**1**	3.2 ± 0.6	1.4 ± 0.1
(*S*)-**2**	0.06 ± 0.01	1.83 ± 0.02
Retigabine	1.30 ± 0.04	1.60 ± 0.05

NA: not available.

[a] The efficacy of the test compound relative to (±)-**3**.

As shown in Table 1, the methylene dioxybenzene analog (*S*)-**3** is most potent (EC_{50} 20 ± 1 nM) but least efficacious among the three (*S*) enantiomers described in this report. Both the dihydrobenzofuran analog (*S*)-**4** and the dihydroindole derivative (*S*)-**5** showed comparable moderate potency in opening KCNQ2 channels, but they have improved efficacy over (*S*)-**3**, retigabine, and even (*S*)-**2** by approximately 140%, 50%, and 31%, respectively. In contrast with their (*S*) counterparts, both (*R*)-**3** and (*R*)-**4** exhibited no appreciable KCNQ2 opener activity when tested at 10 μM. The effects of (*R*)-**5** on the enhancement of mKCNQ2 currents at 3–5 μM were weak to moderate, whereas (*S*)-**5** showed robust activity in this concentration range (198 ± 60% vs 1665 ± 464% of control current at 3 μM; 140 ± 26% vs 1701 ± 484% of control current at 10 μM). The high (*S*) enantiospecificity for augmenting KCNQ2 currents observed in **3**, **4**, and **5** is consistent with that of **1** and **2** as reported previously.[11,16] Thus, the (*S*) absolute configuration at the (1-phenyl)ethyl moiety may play an important role in opening KCNQ2 channels for this series of acrylamides.

It is important to note that (±)-**3** is still a highly potent KCNQ2 opener (EC_{50} 72 ± 2 nM) even though it is approximately 3-fold less potent than its active enantiomer (*S*)-**3**. Interestingly, (±)-**3** exhibited virtually the same efficacy as (*S*)-**3**. Because of its high potency and moderate efficacy in activating KCNQ2 channels, and its convenient synthesis, we chose (±)-**3** as a reference compound for analyzing efficacies of KCNQ openers in electrophysiology studies as described earlier. The benefit of using an E/E_{ref} value is that it allows ranking of compound efficacies independent of the amount of basal KCNQ2 current expression.

Two pairs of enantiomers of **4** and **5** were examined for their ability to reduce spontaneous neuronal discharges in rat hippocampal slices. In this assay, the induction of spontaneous neuronal bursting was achieved by bathing slices to an artificial cerebrospinal fluid (CSF) buffered with HEPES (10 mM) and containing zero calcium ($CaCl_2$) and low magnesium ($MgSO_4$ 1.3 mM).[15] The resulting multiple-unit extracellular electrical activity was recorded by advancing an electrode 50–150 μm into the CA1 region of the hippocampal slice. This electrical activity was then amplified using a differential amplifier, and the number of events per minute was collected and analyzed. The analyzed data was expressed in hertz and reported as percent inhibition of compound-free control.

In order to establish a concentration–response curve for (*S*)-**4** (Fig. 5A) and (*S*)-**5** (Fig. 5B), slices were exposed to a range of concentrations (100 nM–2.5 μM). A minimum of three slices were used for each compound concentration. The EC_{50}'s calculated for (*S*)-**4** and (*S*)-**5** were 0.94 and 0.46 μM, respectively. It should be noted that (*R*)-**4** had only a negligible effect (15.2 ± 4.4% inhibition, $n = 11$) on neuronal discharge when tested at the concentration producing maximal inhibition by (*S*)-**4** (5.0 μM) (Fig. 5), which is consistent with the inability of this concentration of (*R*)-**4** to activate KCNQ2 channels expressed in HEK 293 cells (data not shown). However, when (*R*)-**5** was similarly tested at 5 M, it produced a moderate inhibition (43.3 ± 2.5%, $n = 11$) compared to the maximal effect (91.8 ± 2.2% inhibition, $n = 11$) generated by its opposite enantiomer (*S*)-**5** at 5 μM. Again, this is consistent with the moderate activity of (*R*)-**5** to activate KCNQ2 channels expressed in HEK 293 cells at this concentration (data not shown).

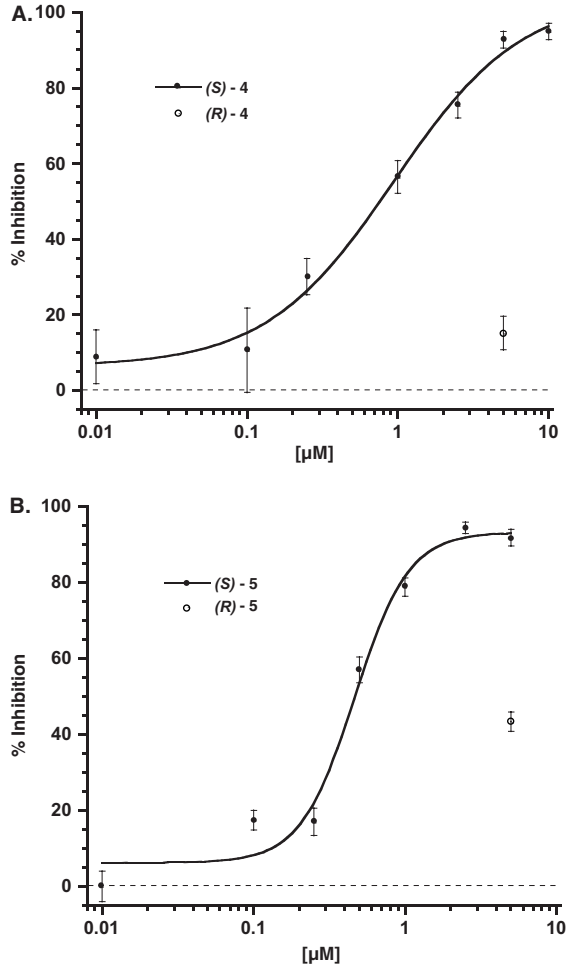


Figure 5. Group concentration–response relationships generated for (*S*)-**4** (A) and (*S*)-**5** (B) in hippocampal slices ($n = 3$–18 slices).

We have hypothesized that a KCNQ opener would be effective in inhibiting the induced neuronal hyperexcitability in rat hippocampal slices 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, the two active enantiomers (*S*)-**4** and (*S*)-**5**, like (*S*)-**2**,[11] demonstrated significant activity in reducing neuronal hyperexcitability in rat hippocampal slices, while significant less activity was seen with their respective (*R*) enantiomers. These results are in agreement with their activity in augmenting KCNQ2 channels expressed in HEK 293 cells. It should be noted that the EC_{50}'s of (*S*)-**4** (0.46 μM), (*S*)-**5** (0.94 μM) and (*S*)-**2** (0.70 μM) in the slice studies are within the same range despite the fact that (*S*)-**2** is six- and seven-fold more potent than (*S*)-**4**

and (*S*)-**5**, respectively, in the whole-cell patch-clamping studies at −40 mV. This is not unexpected as the media for current slice studies is slightly different from that reported for (*S*)-**2**,[11] and in addition, the activity in hippocampal slices also depends on the physical properties of the test compounds, such as solubility and permeability. More studies are needed to further understand the correlation between the whole-cell patch-clamp data and the slice data.

4. Conclusion

Compound (*S*)-**3** was identified as a highly potent KCNQ2 opener, and (*S*)-**4** and (*S*)-**5** as highly efficacious KCNQ2 openers. These compounds, along with (*S*)-**2**, which is a highly potent and efficacious KCNQ2 opener, are expected to be valuable tools in further studying the pharmacology of KCNQ2 and possibly other KCNQ channels. The readily available and highly potent (±)-**3** has been shown to be a useful reference compound for ranking compound efficacies. The (*S*) enantiospecific KCNQ2 interactions observed with acrylamide **3**, **4**, and **5** suggests that the (*S*) absolute configuration at the benzylamine moiety is part of the pharmacophore in the acrylamide series. The significant inhibitory activity of (*S*)-**4** and (*S*)-**5** in hyperexcited hippocampal slices suggests that KCNQ2 openers may represent a novel class of agents for the treatment of CNS disorders of neuronal excitability such as epilepsy, migraine, and neuropathic pain.

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12. Data for (*S*)-**3**: ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.51 (3H, d, $J = 10.0$ Hz), 5.16 (1H, quintet, $J = 10.0$ Hz), 5.92 (2H, s), 5.96 (1H, d, $J = 5.0$ Hz), 6.38 (1H, d, $J = 15.6$ Hz), 6.75 (1H, d, $J = 10.0$ Hz), 6.82 (2H, m), 7.24 (2H, m), 7.37 (1H, d, $J = 5.0$ Hz), 7.51 (1H, d, $J = 5.0$ Hz), and 7.97 (1H, d, $J = 15.6$ Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 21.84, 49.00, 101.15, 107.02, 108.42, 119.60, 123.67, 126.99, 127.64, 130.25, 130.48, 133.29, 134.86, 137.21, 137.25, 146.93, 148.00, and 164.54; HRMS m/z calcd for $C_{18}H_{17}ClNO_3$ $(M+H)^+$ 330.0897, found 330.0894; $[\alpha]_D^{20}$ −51.6 (*c* 2.00, CH_2Cl_2).
13. Data for (*S*)-**4**: ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.52 (3H, d, $J = 7.0$ Hz), 3.17 (2H, t, $J = 8.6$ Hz), 4.54 (2H, t, $J = 8.6$ Hz), 5.19 (1H, quintet, $J = 8.8$ Hz), 5.89 (1H, d, $J = 6.7$ Hz), 6.68 (1H, d, $J = 16.2$ Hz), 6.72 (1H, m), 6.89 (2H, m), 7.09 (1H, d, $J = 8.2$ Hz), 7.23 (2H, m), 7.71 (1H, d, $J = 16.2$ Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 21.76, 29.81, 48.91, 71.43, 109.33, 111.82 (d, $J = 26.3$ Hz), 111.83 (d, $J = 16.3$ Hz), 112.90 (t, $J = 15.0$ Hz), 123.41, 126.03, 126.81 (t, $J = 8.8$ Hz), 127.49 (d, $J = 7.5$ Hz), 130.41 (t, $J = 11.3$ Hz), 135.32, 159.58, 161.75 (dd, $J = 7.5$, 252.5 Hz), and 164.70; HRMS m/z calcd for $C_{19}H_{17}FN_2O_2Na$ $(M+Na)^+$ 352.1125, found 352.1142; $[\alpha]_D^{20}$ −41.5 (*c* 1.59, CH_2Cl_2).
14. Data for (*S*)-**5**: ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.52 (3H, d, $J = 6.7$ Hz), 3.01 (2H, t, $J = 8.2$ Hz), 3.55 (2H, t, $J = 8.2$ Hz), 5.16 (1H, t, $J = 7.1$ Hz), 5.80 (1H, d, $J = 7.0$ Hz), 6.48 (1H, d, $J = 15.9$ Hz), 6.62 (1H, d, $J = 7.9$ Hz), 7.03 (4H, m), 7.28 (1H, m), 7.45 (1H, m), and 6.67 (1H, d, $J = 15.9$ Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 21.60, 29.86, 47.54, 48.97, 109.69, 116.20 (d, $J = 21.3$ Hz), 123.05 (d, $J = 11.3$ Hz), 123.17, 124.08 (d, $J = 7.5$ Hz), 124.43 (d, $J = 3.8$ Hz), 125.53, 129.81 (d, $J = 2.5$ Hz), 130.20, 133.84, 134.10, 150.60, 161.45 (d, $J = 251.3$ Hz), and 164.74; HRMS m/z calcd for $C_{19}H_{20}FN_2O$ $(M+H)^+$ 311.1560, found 311.1555; $[\alpha]_D^{20}$ −40.3 (*c* 1.09, CH_2Cl_2).
15. This media is slightly different from the one we used in the evaluation of (*S*)-**2** (zero magnesium ($MgSO_4$) and low calcium ($CaCl_2$ 1.5 mM)) (see Ref. 11).
16. Wu, Y.-J.; He, H.; Sun, L.-S.; L'Heureux, A.; Chen, J.; Dextraze, P.; Starrett, J. E., Jr.; Boissard, C. G.; Gribkoff, V. K.; Natale, J.; Dworetzky, S. I. *J. Med. Chem.* **2004**, *47*, 2887–2896.