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Discovery and Characterization of 1*H*-Pyrazol-5-yl-2-phenylacetamides as Novel, Non-Urea-Containing GIRK1/2 Potassium Channel Activators

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Abstract

The G protein-gated inwardly-rectifying potassium channels (GIRK, K_{ir}3) are a family of inward-rectifying potassium channels, and there is significant evidence supporting the roles of GIRKs in a number of physiological processes and as potential targets for numerous indications. Previously reported urea containing molecules as GIRK1/2 preferring activators have had significant

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Author Contributions

C.R.H. oversaw and designed the chemistry. J.M.W., A.K.V., and S.S. performed the synthetic chemistry work. T.M.B., J.S.D., and R.D.M. designed and performed the drug metabolism and pharmacokinetic *in vitro* experiments and *in vivo* studies. K.W. helped with the manuscript and *in vivo* studies. C.D.W. designed and analyzed the *in vitro* pharmacology experiments. K.K.A. performed and analyzed the *in vitro* pharmacology experiments. C.R.H. wrote the manuscript with input from all authors.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acscchemneuro.7b00217. Chemical synthesis of all analogues, *in vitro* pharmacology procedures, *in vitro* PK methods, *in vivo* PK methods (PDF)

Notes

The authors declare the following competing financial interest(s): C.D.W. is the president of WaveFront Biosciences, the maker of the Panoptic kinetic imaging plate reader used to perform the *in vitro* pharmacology experiments. C.D.W. obtains royalties from the sale of Thallol thallium-sensitive fluorescent dye used for the *in vitro* pharmacology experiments. All other authors declare no competing financial interest.

pharmacokinetic (PK) liabilities. Here we report a novel series of 1H-pyrazolo-5-yl-2-phenylacetamides in an effort to improve upon the PK properties. This series of compounds display nanomolar potency as GIRK1/2 activators with improved brain distribution (rodent $K_p > 0.6$).

Graphical Abstract

Keywords

K_{ir3} ; GIRK; activator; thallium flux; pharmacokinetics

The G protein-gated inwardly rectifying potassium channels (GIRK, K_{ir3}) are a family of inward-rectifying potassium channels and are key effectors in G protein-coupled receptor cellular signaling pathways.¹ GIRK channels are tetrameric complexes formed by homo- and heteroassembly among four related subunits (GIRK1–4 or $K_{ir3.1}$ –3.4), which are encoded by the genes *KCNJ3*, *KCNJ6*, *KCNJ9*, and *KCNJ5*, respectively.^{2,3} The GIRK1/2 channel subtype is the most common within the brain, while other subunit combinations have more limited brain distribution; the GIRK1/4 subtype is primarily found in the heart atria, where it plays a key role in regulating heart rate and has been a potential target for the treatment of atrial fibrillation.⁴ There is significant evidence supporting the roles of GIRK channels in a number of physiological processes and as potential targets for numerous indications, such as pain perception, epilepsy, reward/addiction and anxiety.^{1,5–7} Unfortunately, due to a dearth of selective pharmacological tool compounds, the roles of GIRK channels as potential therapeutic targets are not well understood. Herein, we report on a new class of GIRK1/2 channel activators with improved pharmacokinetic properties over the previously reported urea-class of activators.

Previous reports of GIRK1/2 channel activators from our laboratories started from a high-throughput screen of the molecular libraries small molecule repository (MLSMR) compound collection-part of the Molecular Libraries Screening Center Network (MLSCN).⁸ This screen provided the urea-containing compounds, which led to the discovery of **1** (Figure 1).^{9,10} The urea scaffold has been a rich source of a variety of GIRK1/2 channel activators, as well as “molecular switches”, leading to the mode of pharmacology converting from

activator to inhibitor as well as compounds with varying selectivity profiles.^{11,12} Although the urea scaffold has led to the identification of compounds with *in vivo* activity in an antiepileptic model in mice, these compounds suffer from significant pharmacokinetic liabilities, namely, poor brain penetration as well as only modest selectivity against the GIRK1/4 channel subtype.^{9,10} In addition to the urea scaffold, an alternative amide-containing scaffold was reported that resulted from the same MLSCN HTS that yielded the urea scaffold.¹³ A medicinal chemistry effort focused on the amide scaffold led to the discovery of 2; however, this scaffold was significantly less potent as a GIRK1/2 channel activator, as compared to 1, and it was equipotent as an activator against the GIRK1/4 channel.¹³ Mining the previous data led us to postulate that it may be possible to merge these two scaffolds: utilizing the pyrazole “privileged scaffold” from the urea scaffold with the phenylacetamide portion of 2 to develop novel amide compounds that may allow for improved brain penetration as well as improved selectivity for GIRK1/4 (selectivity criteria of >10–30-fold). The results of this effort are presented below.

RESULTS AND DISCUSSION

The synthesis of the pyrazol-5-ylphenylacetamide analogues is outlined in Scheme 1. The appropriately substituted hydrazine, 3, was reacted with 3-aminobut-2-enenitrile, 4, under acidic conditions to generate the substituted aminopyrazoles, 5 in modest yield.¹⁴ The final compounds (7–10) were obtained by PyCIU amide coupling of the acid,¹⁵ 6, with the newly formed (or commercially available) aminopyrazoles, 5.

The initial structure—activity relationship (SAR) library focused on maintaining the substituent on the pyrazole constant (benzyl) and evaluating a variety of acetamides to test whether these were viable replacements of the urea moiety (Table 1). The initial 3,4-difluoro comparator, 7a, was tested and was shown to lose significant activity compared to the urea analogue, 1 ($EC_{50} = 1.97 \mu M$ vs $EC_{50} = 0.073 \mu M$). Replacing the 3,4-difluorophenyl with a 3-chloro-4-fluorophenyl, 7b, produced an ~4-fold increase in potency ($EC_{50} = 0.478 \mu M$), which is still ~5-fold less potent than the urea analogue, but did improve upon the potency of the original amide scaffold (2, $EC_{50} = 1.1 \mu M$). However, 7b provided a slight improvement in the selectivity for GIRK1/2 versus GIRK1/4 channel subtypes (~4-fold selectivity vs ~2-fold selectivity for 1). Unfortunately, other modifications of the phenyl portion did not lead to an improvement over 7b. The unsubstituted phenyl derivative (7c, $EC_{50} = > 10 \mu M$) lost significant activity and we were able to regain some of the activity with other substituents (4-bromo-3-fluoro, 7d, $EC_{50} = 1.35 \mu M$; naphthyl, 7f, $EC_{50} = 1.05 \mu M$; 3,4-dichloro, 7g, $EC_{50} = 1.45 \mu M$), but none of these compounds produced a molecule as active as the urea analogue, 1.

The next round of SAR evaluation was focused on the pyrazole N-substituent (Table 2). For this evaluation, we chose to hold the 3-chloro-4-fluorophenylacetamide constant to obtain a frame of reference for potency comparisons. Replacing the benzyl group with cyclohexyl, 8a, proved to be a productive change as the potency increased ~3-fold over the N-benzylpyrazole analogue (8a, $EC_{50} = 0.165 \mu M$ vs 7b, $EC_{50} = 0.478 \mu M$). The potency was maintained after the insertion of a flexible methylene linker to form the cyclohexylmethyl substituent, 8b ($EC_{50} = 0.135 \mu M$). These changes, although offering an improvement in

GIRK1/2 channel potency, did not dramatically change the selectivity profile of the compounds. Eliminating the cyclohexyl ring and replacing with the sec-butyl group, 8c, led to a loss of activity ($EC_{50} = 0.536 \mu\text{M}$); however, it was similar in potency to the original benzyl analogue. Interestingly, addition of a heteroatom to moieties in this region of the molecule led to significant reduction in activity. Namely, addition of a nitrogen to the benzyl moiety (i.e., 4-pyridylmethyl, 8d, $EC_{50} > 10 \mu\text{M}$) led to an ~20-fold reduction compared to the benzyl counterpart; and addition of an oxygen to the cyclohexyl analogue (i.e., 4-pyran, 8e, $EC_{50} = 3.07 \mu\text{M}$) also led to an ~20-fold reduction in activity. Evaluation of additional alkyl (branched or unbranched, 8g-1) led to the identification of other submicromolar compounds (8j, $EC_{50} = 0.840 \mu\text{M}$; 8k, $EC_{50} = 0.483 \mu\text{M}$; 8l, $EC_{50} = 0.684 \mu\text{M}$), signifying tolerance for flexible groups. However, none of these compounds improved upon the potency or selectivity compared to the cyclohexyl compounds. In addition, cycloalkyl groups were also investigated both directly attached to the pyrazole and those with a methylene spacer group. Contracting the ring system from a 6-membered ring to 3-, 4-, or 5-membered rings was deleterious to the activity, with only the cyclopentyl group showing midnanomolar activity (8p, $EC_{50} = 0.518 \mu\text{M}$). Lastly, direct branched alkyl groups (t-Bu, 8o, inactive; i-Pr, 8q, $EC_{50} = 1.06 \mu\text{M}$) either were inactive or showed significant loss of activity.

To more fully explore the scope of the phenylacetamide substitution tolerance as well as other urea replacements, we synthesized a number of alternative analogues (Table 3). The branching of the methylene group of the phenylacetamide was explored with both quaternary center (cyclopropyl, 9a) and (R)- and (S)-methyl substituents (9b and 9c). Truncation of the phenylacetamide to the benzamide (9d) as well as reverse amide analogues (9e and 9f), both with and without the methylene spacer, and finally squaramide derivatives as complete replacements were all examined. Unfortunately, none of these derivatives were active.

Having established from the previous SAR evaluations that the cyclohexylmethyl and cyclohexyl substituents on the pyrazole were the most productive in terms of activity, we next more deeply investigated the SAR around the acetamide portion of the molecule (Table 4). As seen previously, the 3,4-difluorophenyl derivative, 10a, was an active compound ($EC_{50} = 0.149 \mu\text{M}$) and was a significant improvement over the initial analogue, 7a ($EC_{50} = 1.97 \mu\text{M}$), and offered a slight improvement in selectivity (~5.7-fold selective over the GIRK1/4 subtype). Both the 2-chloro-4-fluoro, 10b ($EC_{50} = 0.343 \mu\text{M}$), and 4-chloro-2-fluoro, 10c ($EC_{50} = 0.382 \mu\text{M}$), derivatives were active, but were less active than the 3,4-substituted variants (8b or 10a). This disparity in activity is also seen in the 2-fluoro-4-trifluoromethoxy (10d, $EC_{50} = 0.600 \mu\text{M}$) versus the 4-fluoro-3-trifluoromethoxy analogue (10g, $EC_{50} = 0.245 \mu\text{M}$). Further carbon substitutions were also tolerated with naphthalene (10f, $EC_{50} = 0.405 \mu\text{M}$) and 2-methylphenyl acetamide (10i, $EC_{50} = 0.293 \mu\text{M}$) having sub-500 nM activity.

Replacing the phenyl group with a variety of heterocyclic analogues was not as productive. The initial analogue, 2-chloropyridine-4-yl acetamide, 10j, retained most of the activity ($EC_{50} = 0.396 \mu\text{M}$); however, many of the other derivatives lost significant activity or were inactive. These include additional pyridine analogues (10k,l) and other 5-membered ring

heteroaryl groups (10m-o) and indole derivatives (10p,q). Further replacement with cycloalkyl groups (indane, 10r; difluorocyclopropyl, 10s) were also not productive analogues as they both lost activity. Lastly, a few analogues with the N- pyrazolcyclohexyl group were also evaluated (10u-z). As was seen previously, the cyclohexyl moiety produced compounds with a similar range of potencies as the cyclohexylmethyl derivatives, and it was not obvious from the potency data that the cyclohexyl derivatives offered any advantages. However, the one analogue where a dramatic increase in potency was observed was the 6-methylpyridin-3-yl acetamide, (10w, $EC_{50} = 0.333 \mu M$ vs 10k, $EC_{50} = >9.4 \mu M$). Although the potency improvement was not obvious, a few cyclohexyl derivatives were more selective against GIRK1/4 (10w, 14-fold selectivity; 10x, 12-fold selectivity; 10z, inactive against GIRK1/4) than their previous counterparts.

Having identified a number of unique 1*H*-pyrazol-5-yl-2- phenylacetamide GIRK1/2 channel activators, we further profiled selected compounds in *in vitro* DMPK assays to assess their human and mouse liver microsomal intrinsic clearance and plasma protein binding (Table 5). All but a few compounds evaluated displayed high intrinsic clearance and were predicted to have high hepatic clearance in vivo ($CL_{hep} > 75\% Q_H$).^{16,17} Both compounds 8e and 8f were predicted to have moderate clearance ($\sim <50\% Q_H$) in both human and mouse. Interestingly, both of these compounds were cycloheteroalkyl substituents (8e, 4-tetrahydropyran; 8f, tetrahydrothiophene-1,1-dioxide). Using equilibrium dialysis, the plasma protein binding of the selected compounds was determined in human and mouse plasma.¹⁸ The results revealed that the compounds had varying degrees of free fraction in human (F_u : 0.003–0.348) and mouse (F_u : 0.010–0.423) plasma; however, a number of compounds were unstable in mouse plasma. Interestingly, both 8e and 8f displayed the highest free fraction in human and mouse, and coupled with the predicted clearance these compounds possess the most attractive *in vitro* DMPK profile; unfortunately, these compounds are not very active as GIRK1/2 channel activators. However, these substituents may inform the next round of compound optimization. Further compounds for evaluation (10f-y) all exhibited high clearance, and many of these compounds were unstable in mouse plasma. Lastly, we evaluated a smaller set of selected compounds (7b, 8a, 8c) in a mouse IP cassette study to assess their ability to cross the blood-brain barrier (BBB).^{19,20} All of the compounds were brain penetrant with K_p values > 0.5 – 0.9 . This represents a significant improvement over the previously reported urea scaffolds where many of those analogues had low brain distribution ($K_p < 0.2$).

■ CONCLUSION

In conclusion, we have identified a novel series of 1*H*-pyrazol-5-yl-2-phenylacetamides as brain-penetrant GIRK1/2 channel activators. The genesis of this series of compounds originated from a merging of two previously disclosed scaffolds that contained a urea moiety and an amide moiety. SAR around the pyrazole identified the cyclohexyl and cyclohexylmethyl groups as the optimal substituents. The acetamide portion allowed for more structural diversity, incorporating substituted phenyl and heteroaryl groups culminating in number of compounds with EC_{50} s between 100 and 200 nM. An improvement in the channel subtype selectivity profile was also seen for selected compounds; however, a universal improvement has not been realized at this point. Lastly, a number of compounds

evaluated displayed moderate-high brain distribution ($K_p = >0.5$), which signifies an improvement over the previous GIRK1/2 channel activators that have been reported. Further *in vivo* evaluation of the lead molecules in models of anxiety is ongoing and will be reported in due course.

■ METHODS

General.

All NMR spectra were recorded on a 400 MHz AMX, AV-400 or Avance III HD 500 MHz Bruker NMR spectrometer. 1H and ^{13}C chemical shifts are reported in δ values in ppm downfield with the deuterated solvent as the internal standard. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, b = broad, m = multiplet), coupling constant (Hz), integration. Low resolution mass spectra were obtained on an Agilent 6120 or 6150 instrument with ESI source. Method A: MS parameters were as follows: fragmentor, 70; capillary voltage, 3000 V; nebulizer pressure, 30 psig; drying gas flow, 13 L/min; drying gas temperature, 350 °C. Samples were introduced via an Agilent 1290 UHPLC apparatus comprising a G4220A binary pump, G4226A ALS, G1316C TCC, and G4212A DAD with ULD flow cell. UV absorption was generally observed at 215 and 254 nm with a 4 nm bandwidth. Column: Waters Acquity BEH C18, 1.0 $\times$ 50 mm, 1.7 μ m. Gradient conditions: 5% to 95% CH₃CN in H₂O (0.1% TFA) over 1.4 min, hold at 95% CH₃CN for 0.1 min, 0.5 mL/min, 55 °C. Method B: MS parameters were as follows: fragmentor, 100; capillary voltage, 3000 V; nebulizer pressure, 60 psig; drying gas flow, 12 L/min; drying gas temperature, 350 °C. Samples were introduced via an Agilent 1260 HPLC apparatus comprising a degasser, G7111A quaternary pump, G4237A ALS, G7116A TCC, G1314 VWD with a ULD flow cell. UV absorption was generally observed at 215 and 254 nm with a 4 nm bandwidth. Column: Poroshell 120, SB-C18, 4.6 $\times$ 75 mm, 2.7 μ m. Gradient conditions: 5% to 80% CH₃CN in H₂O (0.1% TFA) from 0–4 min, 80 to 95% CH₃CN in H₂O (0.1% TFA) from 4–6 min, hold at 95% CH₃CN from 6–8 min, 95% CH₃CN for 0.1 min, 1.5 mL/min, 45 °C. High resolution mass spectra were obtained on a Waters Micromass Q-TOF instrument with ESI source. Solvents for extraction, washing and chromatography were HPLC grade. All reagents were purchased from commercial sources and were used without purification.

Materials and Methods for in Vitro Pharmacology.

Thallium flux assays were performed essentially as previously described.⁹ Briefly, HEK-293 cells expressing either GIRK1 and GIRK2 or GIRK1 and GIRK4 were cultured in α -MEM (Corning, Corning, NY) containing 10% (v/v) fetal bovine serum (Thermo Fisher Scientific, Waltham, MA) plus 1 $\times$ glutagro (Corning, Corning, NY) (referred to hereafter as cell culture medium) at 37 °C in a humidified 5% CO₂ atmosphere. Cells at ~90% confluence were dislodged from the tissue culture vessel using TrypLE Express (Thermo Fisher Scientific, Waltham, MA) and plated at a density of 20 000 cells/well in 20 μ L/well cell culture medium in 384-well, clear-bottom, black-walled, BD PureCoat Amine plates (Corning, Corning, NY) and incubated overnight at 37 °C in a humidified 5% CO₂ atmosphere. On the day of assay, the medium was removed from the plates and replaced with 20 μ L/well of a solution containing assay buffer (Hank's buffered saline solution (Thermo Fisher Scientific,

Waltham, MA) plus 10 mM HEPES (Thermo Fisher Scientific, Waltham, MA)-NaOH, pH 7.2), 1 μ M Thallos (TEFlabs, Austin, TX), 0.5% DMSO, and 0.036% Pluronic F-127 (Sigma-Aldrich, St. Louis, MO). Cell plates containing Thallos solution were incubated 1 h at room temperature. Following incubation the Thallos-containing solution was replaced with 20 μ L/well assay buffer. The Thallos- loaded cell plates were transferred to a Panoptic kinetic imaging plate reader (WaveFront Biosciences, Franklin, TN). Images acquired at 1 Hz, 480/40 nm excitation, and 538/40 nm emission were collected for 10 s, after which time 20 μ L/well of assay buffer containing test compounds at 2-fold over their final concentrations were added. Imaging continued for 4 min, at which time 10 μ L/well of a solution containing 125 mM NaHCO₃, 1.8 mM CaSO₄, 1 mM MgSO₄, 5 mM glucose, 2 mM Ti₂SO₄, and 10 mM HEPES-NaOH pH 7.2 was added and images were collected for an additional 2 min. To quantify test compound effects on GIRK channel activity, the initial slopes of the thallium-evoked changes in fluorescence were fit to a 4-parameter logistic equation using the Excel (Microsoft, Redmond, WA) plugin XLfit (IDBS, Guildford, UK) to obtain potency and efficacy values. Efficacies are relative to a maximally effective concentration of **1**, our most potent and effective activator which shows low selectivity between GIRK1/2 and GIRK1/4 channel subtypes. Ten-point concentration series from 30 μ M to 1.5 nM were generated using an Echo liquid handler (Labcyte, San Jose, CA). Final DMSO concentration, 0.24% (v/v), in the assay was constant across all compound concentrations. Unless otherwise indicated, all buffer salts were obtained from Sigma-Aldrich, St. Louis, MO.

General Procedure for Amide Couplings.

A one dram vial equipped with magnetic stir bar and screw cap vial was charged with the appropriate phenyl acetic acid, **6**, derivative (0.10 mmol), chlorodipyrrolidinocarbenium hexafluorophosphate (PyClU) (33 mg, 0.10 mmol), DMF (0.3 mL), and Hunig's base (0.1 mL, 0.57 mmol) and was stirred for approximately 5 min. The appropriate 5-amino pyrazole, **5**, (0.10 mmol) was added, and the reaction stirred until LCMS analysis indicated significant consumption of the starting materials (10 min to 18 h). The crude reaction mixture was purified via mass-directed acidic reverse phase preparative HPLC to yield the corresponding amide products.

2-(3-Cyano-4-fluorophenyl)-N-(1-(cyclohexylmethyl)-3-methyl-1H-pyrazol-5-yl)acetamide (**10h**).

Following the general procedure above, compound (**10h**) was obtained (25.0 mg, 69%). Analytical LCMS: R_t = 4.135 min; >95% @ 254 nm; MS (ESI⁺) m/z 355.1 [M + H]⁺. ¹H NMR (499 MHz, DMSO-d₆) δ 9.97 (s, 1H), 7.86 (dd, J = 6.3, 2.2 Hz, 1H), 7.74 (ddd, J = 8.0, 5.3, 2.3 Hz, 1H), 7.53 (t, J = 9.1 Hz, 1H), 5.96 (s, 1H), 3.74 (s, 2H), 3.66 (d, J = 7.2 Hz, 2H), 2.08 (s, 3H), 1.68–1.52 (m, 4H), 1.38 (d, J = 12.8 Hz, 2H), 1.07 (q, J = 9.7, 9.0 Hz, 3H), 0.84–0.69 (m, 2H). ¹³C NMR (126 MHz, DMSO-d₆) δ 168.54, 145.98, 137.58, 137.50, 136.60, 134.65, 133.87, 133.85, 117.06, 116.91, 114.41, 100.34, 100.22, 98.95, 53.62, 41.12, 38.30, 30.32, 26.33, 25.61, 14.22. HRMS calcd for C₂₀H₂₃FN₄O: 354.1856; found: 355.1934 [M + H]⁺.

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Figure 1. Structures of the previously reported GIRK1/2 activators and the merged pyrazol-5-ylphenylacetamides

Scheme 1.
Synthesis of the Pyrazol-5-ylphenylacetamide Analogues

Table 1.

SAR Evaluation of the Initial Pyrazol-5-ylphenylacetamides

7a-1			
Cmpd	R	GIRK1/2 ($\mu\text{M} \pm \text{SEM}^a$; % $\pm \text{SEM}^b$)	GIRK1/4 ($\mu\text{M} \pm \text{SEM}^a$; % $\pm \text{SEM}^b$)
1		0.073 $\pm$ 0.007; 96.0 $\pm$ 1.1	0.150 $\pm$ 0.021; 94.0 $\pm$ 1.5
7a		1.97 $\pm$ 0.33; 74.7 $\pm$ 7.8	3.98 $\pm$ 0.19; 46.0 $\pm$ 4.0
7b		0.478 $\pm$ 0.077; 94.5 $\pm$ 5.5	2.0 $\pm$ 0.8; 100 $\pm$ 0
7c		>10; >25	>10; >15
7d		1.35 $\pm$ 0.17; 36.3 $\pm$ 4.9	2.75 $\pm$ 0.74; 17.0 $\pm$ 0.7
7e		inactive	inactive
7f		1.05 $\pm$ 0.14; 32 $\pm$ 1%	1.32 $\pm$ 0.22; 12.0 $\pm$ 0.3%
7g		1.45 $\pm$ 0.35; 62.0 $\pm$ 5.5%	2.01 $\pm$ 0.41; 35.0 $\pm$ 3.0%
7h		>6.9; >56%	>10; >42%
7i		inactive	inactive

^a Potency values were obtained from triplicate determinations; values are average of n = 3.^b Reported efficacy values shown are obtained from triplicate determinations; values are average of n = 3 and are normalized to a standard compound, 1.

Table 2.

Evaluation of the Pyrazole N-Substituent

Cmpd	Structure	GIRK1/2 ($\mu\text{M} \pm \text{SEM}^a$; % $\pm \text{SEM}^b$)	GIRK1/4 ($\mu\text{M} \pm \text{SEM}^a$; % $\pm \text{SEM}^b$)
8a		0.165 ± 0.012 ; $87.3 \pm 0.9\%$	0.720 ± 0.213 ; $50.5 \pm 5.5\%$
8b		0.135 ± 0.024 ; $82.0 \pm 3.5\%$	0.534 ± 0.285 ; $52.3 \pm 7.4\%$
8c		0.536 ± 0.093 ; $99.5 \pm 3.5\%$	3.26 ± 0.44 ; $111 \pm 4\%$
8d		>10 ; $>42\%$	>10 ; $>25\%$
8e		3.07 ± 0.79 ; $86.0 \pm 5.6\%$	>10 ; $>35\%$
8f		5.78 ± 1.52 ; $66.8 \pm 6.8\%$	>10 ; $>33\%$
8g		5.58 ± 0.66 ; $77.0 \pm 11.4\%$	>10 ; $>63\%$
8h		2.63 ± 0.73 ; $97.3 \pm 10.4\%$	6.70 ± 1.68 ; $100.3 \pm 10.0\%$
8i		1.40 ± 0.27 ; $89.7 \pm 9.1\%$	5.93 ± 1.36 ; $80.0 \pm 4.6\%$
8j		0.840 ± 0.180 ; $82.3 \pm 5.8\%$	1.95 ± 0.52 ; $91.7 \pm 8.4\%$
8k		0.483 ± 0.084 ; $57.3 \pm 6.3\%$	1.49 ± 0.31 ; $55.3 \pm 2.5\%$

Cmpd	Structure	GIRK1/2 ($\mu\text{M} \pm \text{SEM}^a$; % $\pm \text{SEM}^b$)	GIRK1/4 ($\mu\text{M} \pm \text{SEM}^a$; % $\pm \text{SEM}^b$)
8l		0.684 ± 0.072 ; $69.5 \pm 5.3\%$	1.74 ± 0.13 ; $30.5 \pm 8.5\%$
8m		>10 ; $>34\%$	>10 ; $>18\%$
8n		1.52 ± 0.41 ; $102.0 \pm 5.5\%$	5.40 ± 1.34 ; $97.7 \pm 6.8\%$
8o		inactive	inactive
8p		0.518 ± 0.091 ; $87.0 \pm 7.3\%$	2.15 ± 0.56 ; $80.3 \pm 8.5\%$
8q		1.06 ± 0.34 ; $101 \pm 2\%$	8.50 ± 1.50 ; $67.5 \pm 0.5\%$

^a Potency values were obtained from triplicate determinations; values are average of $n = 3$.

^b Reported efficacy values shown are obtained from triplicate determinations; values are average of $n = 3$ and are normalized to a standard compound, 1.

Table 3.

SAR of Substituted Phenylacetamides, Reverse Amides, and Urea Replacements

Cmpd	R	GIRK1/2 ($\mu\text{M} \pm \text{SEM}^a$; % $\pm \text{SEM}^b$)	GIRK1/4 ($\mu\text{M} \pm \text{SEM}^a$; % $\pm \text{SEM}^b$)
9a		inactive	inactive
9b		inactive	inactive
9c		inactive	inactive
9d		inactive	inactive
9e		inactive	inactive
9f		inactive	inactive
9g		inactive	inactive

^aPotency values were obtained from triplicate determinations; values are average of $n = 3$.^bReported efficacy values shown are obtained from triplicate determinations; values are average of $n = 3$ and are normalized to a standard compound, 1.

Table 4.

Further Modification of Phenylacetamide and N-Pyrazole Substituents

10a-z				
Cmpd	R	R ₁	GIRK1/2 ($\mu\text{M} \pm \text{SEM}^a$; % $\pm \text{SEM}^b$)	GIRK1/4 ($\mu\text{M} \pm \text{SEM}^a$; % $\pm \text{SEM}^b$)
10a			0.149 $\pm$ 0.009; 71.3 $\pm$ 2.6%	0.850 $\pm$ 0.475; 47.3 $\pm$ 7.5%
10b			0.343 $\pm$ 0.127; 50.0 $\pm$ 4.6%	0.462 $\pm$ 0.019; 21.3 $\pm$ 3.3%
10c			0.382 $\pm$ 0.165; 51.0 $\pm$ 2.3%	1.01 $\pm$ 0.05; 28.7 $\pm$ 4.7%
10d			0.600 $\pm$ 0.201; 53.7 $\pm$ 2.9%	1.22 $\pm$ 0.03; 27.3 $\pm$ 3.7%
10e			0.800 $\pm$ 0.130; 50.0 $\pm$ 5.0%	0.925 $\pm$ 0.220; 18.7 $\pm$ 2.8%
10f			0.405 $\pm$ 0.105; 59.0 $\pm$ 5.6%	0.923 $\pm$ 0.162; 28.0 $\pm$ 5.3%
10g			0.245 $\pm$ 0.063; 81.3 $\pm$ 4.4%	0.751 $\pm$ 0.142; 65.3 $\pm$ 0.9%
10h			0.126 $\pm$ 0.013; 82.3 $\pm$ 3.5%	0.295 $\pm$ 0.071; 75.0 $\pm$ 3.6%
10i			0.293 $\pm$ 0.085; 44.0 $\pm$ 2.1%	0.930 $\pm$ 0.222; 29.7 $\pm$ 4.6%
10j			0.396 $\pm$ 0.029; 79.3 $\pm$ 2.0%	1.28 $\pm$ 0.02; 61.3 $\pm$ 4.7%
10k			>9.4; >40%	inactive
10l			>10; >32%	inactive
10m			<10; >56%	>10; >21%
10n			2.38 $\pm$ 0.77; 61.7 $\pm$ 3.4%	<10; >21%
10o			0.892 $\pm$ 0.107; 59.5 $\pm$ 0.5%	2.59 $\pm$ 0.25; 28.0 $\pm$ 3.3%
10o				

10a-z

Cmpd	R	R ₁	GIRK1/2 ($\mu\text{M} \pm \text{SEM}^a$; % $\pm \text{SEM}^b$)	GIRK1/4 ($\mu\text{M} \pm \text{SEM}^a$; % $\pm \text{SEM}^b$)
10p			inactive	inactive
10q			inactive	inactive
10r			inactive	inactive
10s			1.10 $\pm$ 0.04; 25.5 $\pm$ 0.5%	inactive
10t			2.04 $\pm$ 0.74; 73 $\pm$ 21%	>10; >79%
10u			2.30 $\pm$ 0.02; 91.0 $\pm$ 7.3%	>10; >60%
10v			1.01 $\pm$ 0.30; 83 $\pm$ 12%	>10; >54%
10w			0.333 $\pm$ 0.023; 75 $\pm$ 0%	4.79 $\pm$ 2.78; 23.5 $\pm$ 4.5%
10x			0.150 $\pm$ 0.020; 75.0 $\pm$ 2.5%	1.79 $\pm$ 1.07; 37.7 $\pm$ 1.9%
10y			0.392 $\pm$ 0.072; 76.0 $\pm$ 7.5%	2.06 $\pm$ 0.42; 41.0 $\pm$ 2.5%
10z			0.505 $\pm$ 0.175; 50.0 $\pm$ 9.5%	inactive

^aPotency values were obtained from triplicate determinations; values are average of n = 3.

^bReported efficacy values shown are obtained from triplicate determinations; values are average of n = 3 and are normalized to a standard compound, 1.

Table 5.

In Vitro and in Vivo DMPK Properties of Selected Compounds

cmpd	GIRK1/2 (uM ± SEM)	intrinsic clearance (mL/min/kg)				plasma protein binding (Fu) ^b	
		hCL _{INT}	hCL _{HEP} ^a	mCL _{INT}	mCL _{HEP} ^a	human	mouse
7b	0.478 ± 0.077	ND	ND	690	79.6	ND	0.071
8a	0.165 ± 0.012	246	19.3	622	78.6	0.009	0.136
8b	0.135 ± 0.024	1390	20.7	>5930	88.7	0.003	<i>d</i>
8c	0.536 ± 0.093	156	18.5	1070	83.0	0.016	0.172
8e	3.07 ± 0.79	23.6	11.1	85.7	43.9	0.211	0.423
8f	5.78 ± 1.52	14.3	8.50	18.0	15.0	0.348	0.362
8h	2.63 ± 0.73	47.7	14.6	657	79.2	0.054	0.171
10f	0.405 ± 0.105	2080	20.8	>5930	88.7	0.003	<i>d</i>
10g	0.245 ± 0.063	1900	20.7	>5930	88.7	0.002	0.010
10h	0.126 ± 0.013	648	20.3	2970	87.4	0.020	0.071
10t	2.04 ± 0.74	>2770	20.8	>5930	88.7	0.003	<i>d</i>
10w	0.333 ± 0.023	139	18.2	990	82.5	0.021	0.125
10x	0.150 ± 0.020	1020	20.6	>5930	88.7	0.013	<i>d</i>
10y	0.392 ± 0.072	92.5	17.1	2079	86.3	0.029	<i>d</i>
mouse cassette (IP, 0.25 mg/kg, 0.25 h)							
		plasma (ng/mL)		brain (ng/g)		<i>K_p</i> ^c	
7b		101		63.4		0.61	
8a		87.6		75.5		0.87	
8c		190		104		0.55	

^aPredicted hepatic clearance based on intrinsic clearance in mouse and human liver microsomes using the well-stirred organ CL model (binding terms excluded).

^b*F_u* = fraction unbound.

^c*K_p* = total brain/plasma ratio.

^dUnstable in mouse plasma.