Published in final edited form as:

Bioorg Med Chem Lett. 2013 September 15; 23(18): 5195–5198. doi:10.1016/j.bmcl.2013.07.002.

Discovery and SAR of a novel series of GIRK1/2 and GIRK1/4 activators

Susan J. Ramos-Hunter^{d,e}, Darren W. Engers^{a,b,c}, Kristian Kaufmann^b, Yu Du^b, Craig W. Lindsley^{a,b,c,d,e}, C. David Weaver^{a,e}, and Gary A. Sulikowski^{d,e,*}

^aDepartment of Pharmacology, Vanderbilt University Medical Center, Nashville, TN 37232, USA

^bVanderbilt Center for Neuroscience Drug Discovery, Vanderbilt University Medical Center, Nashville, TN 37232, USA

^cVanderbilt Specialized Chemistry Center for Probe Development (MLPCN), Nashville, TN 37232, USA

^dDepartment of Chemistry, Vanderbilt University, Nashville, TN 37232, USA

^eVanderbilt Institute of Chemical Biology, Vanderbilt University, Vanderbilt University Medical Center, Nashville, TN 37232, USA

Abstract

This Letter describes a novel series of GIRK activators identified through an HTS campaign. The HTS lead was a potent and efficacious dual GIRK1/2 and GIRK1/4 activator. Further chemical optimization through both iterative parallel synthesis and fragment library efforts identified dual GIRK1/2 and GIRK1/4 activators as well as the first examples of selective GIRK1/4 activators. Importantly, these compounds were inactive on GIRK2 and other non-GIRK1 containing GIRK channels, and SAR proved shallow.

Keywords

GIRK; K_{ir}3.x; Activators; Thallium flux

The G protein regulated Inwardly Rectifying Potassium (K⁺) (GIRK) channels are a family of inward-rectifying potassium channels also known as the K_{ir}3 family, whose physiological role is to modulate the excitability of the various cell types in which they are expressed.¹⁻⁴ Four GIRK channel subunits are expressed, as either homo- or heterodimers, in mammals: GIRK1 (K_{ir}3.1), GIRK2 (K_{ir}3.2), GIRK3 (K_{ir}3.3) and GIRK4 (K_{ir}3.4). GIRK1–GIRK3 are the predominant subunits in the CNS, with GIRK4 expressed at low levels.¹⁻⁷ Multiple lines of evidence support important roles for GIRK in a variety of physiological processes including the control of heart rate and electrical excitability in a variety of neuronal populations, leading to postulates that GIRK channel modulation as potential target for a variety of therapeutic indications including pain, epilepsy, and reward/addiction.¹⁻⁷

However, a complete lack of selective and effective GIRK activators has prevented further target validation for the many indications where GIRK activation is speculated to be of potential benefit.¹⁻⁷ Indeed, the only compounds that are known to activate GIRK are alcohols (e.g., ethanol) at concentrations of hundreds of millimolar and, recently, the compound naringin has been identified as a GIRK activator; however, the reported EC₅₀ is in excess of 100 μ M.⁷⁻⁹

Based on this limitation in the field, we elected to develop a suite of small molecule GIRK probes. Towards this goal, we identified GIRK activator leads by mining HTS hits obtained from an MLSCN¹⁰ mGlu₈ screening project, where mGlu₈ activity was measured via a thallium flux-based readout of GIRK1/GIRK2.¹¹ Once hits for mGlu₈ (mGlu₈/Gq₁₉ calcium flux assay) were triaged, multiple hits remained that were putative GIRK activators with potencies ranging from ~1 μ M to 10 μ M. We recently reported on the one series (Fig. 1) from this effort, represented by HTS hit **1** (VU0032230), that was subsequently optimized to afford the first selective (~10-fold vs GIRK1/4, inactive on GIRK2/3 and inactive on other K⁺ channels) GIRK1/2 activator **2** (VU0456810, also known as ML297).¹² Importantly, **2** was centrally penetrant and established anti-epileptic properties in mice via GIRK1/2 activation. The HTS also identified another attractive, and structurally distinct, dual GIRK1/2 and GIRK1/4 activator **3** (VU0259369), and in this letter, we detail the synthetic strategy and SAR for this lead.

The optimization strategy for **3** is shown in Figure 2. Here, we elected to pursue divergent approaches in parallel, that consisted of iterative parallel library synthesis and a fragment library approach, to enable both the optimization as well as an attempt to identify a minimum pharmacophore.^{13,14} In short order, over 100 analogs were synthesized (via standard acylation chemistry) and assayed in the GIRK1/2 and GIRK1/4 thallium flux assays, and SAR proved to be shallow for this series, with >50% of the analogs displaying no GIRK activity. The iterative parallel synthesis effort, surveying modifications to the parent **3**, afforded few actives (Fig. 3). Of the actives, bromine effectively replaced the chlorine atom, as in **3a**, in contrast deletion of the methyl group, providing **3b**, maintained potency and efficacy at GIRK1/4, but diminished potency and lost efficacy at GIRK1/2. Moving the halogen of **3** from the 2- to the 4-position, as in **3c** and **3d**, also led to a diminution in potency at both GIRK1/2 and GIRK1/4. Figure 4 highlights the GIRK1/2 and GIRK1/4 concentration response curves for **3** and **3a**. All attempts to replace or modify the *N,N*-dimethyl sulfonamide group, incorporation of heterocycles or modification of the linker moiety led to a complete loss of GIRK activity. Fortunately, the fragment libraries proved more productive.

For the fragment libraries (Fig. 2), we held the 5-(*N,N*-dimethyl-sulfonamide)-4-methylphenyl moiety **4** constant, and prepared libraries of amide **5** and urea congeners **6** (Scheme 1). In parallel, we held the 2-(2-chlorophenyl)acetamide portion constant, and treated the acid chloride **7** with a variety of anilines to provide analogs **8**. Yields for these libraries ranged from 22% to 95% based on the coupling partners employed.¹⁵

The amide congeners **5** proved to be uniformly inactive, with the exception of a few C4- to C8-aliphatic amide congeners; however, the GIRK1/2 EC₅₀s were in the 4–10 μ M range

with modest efficacies (23–91%). In contrast, the urea congeners **6** possessed robust SAR, low micromolar potencies, high efficacy (71–120%) and engendered a slight preference for GIRK1/2 (Table 1). This effort provided dual GIRK1/2 and GIRK1/4 activators such as **6b**, **6f** and **6j**, with a range of full to partial efficacies. This is an important finding, as it is not yet clear if a full or partial GIRK activator will be more ideal in terms of in vivo efficacy, safety and tolerability, and having a range of chemical tools will enable these issues to be addressed. Analogs such as **6h**, with a 4-CF₃ moiety, proved to be selective for GIRK1/2 (EC₅₀ = 1.5 μM) over GIRK1/4 (EC₅₀ >10 μM), but with low efficacy (26%). Electron rich congeners, such as **6i**, were uniformly inactive at both GIRK1/2 and GIRK1/4.

In analogs **8**, where the eastern 2-(2-chlorophenyl)acetamide portion was held constant and diverse anilines were surveyed, interesting SAR emerged (Table 2). SAR was very shallow, and unlike analogs **6**, all analogs **8** were devoid of activity at GIRK1/2, affording GIRK1/4 preferring activators. A 4-Cl,3-CF₃phenyl amide derivative **8e**, was a very potent GIRK1/4 activator (EC₅₀ = 0.24 - μM), but displayed weak, partial activation (17%) of the channel, and was inactive on GIRK1/2. Other electron withdrawing substituents in the 3-position, such as Cl (**8g**) or CF₃ (**8h**), were selective, partial GIRK1/4 activators; interestingly, electron donating substituents in the 3-position, such as **8i**, were inactive at both GIRK1/2 and GIRK1/4. None of the analogs **6** or **8** were active at non-GIRK1 containing GIRK channels, further enabling them as valuable tool compounds to better understand selective GIRK channel activation (see Fig. 5).

In summary, we have described the synthesis and one of the first accounts of SAR for a novel series of rarely described GIRK activators, indicating that GIRK activators can be identified from HTS campaigns and optimized. SAR proved shallow, and an iterative parallel synthesis approach provided little improvement in terms of potency or efficacy at GIRK1/2 or GIRK1/4. Instead a fragment library approach afforded a diverse array of GIRK activators that included dual GIRK1/2 and GIRK1/4 activators, GIRK1/2 preferring activators and GIRK1/4 selective activators possessing a wide range of efficacy (from weak partial to full activation), and devoid of activity at non-GIRK1-containing GIRK channels. Additional characterization and refinements are in progress and will be reported in due course.

Acknowledgments

Vanderbilt is a member of the MLPCN and houses the Vanderbilt Specialized Chemistry Center for Accelerated Probe Development. This work was supported by the NIH/MLPCN grant U54 MH084659 (C.W.L.), the Vanderbilt Department of Pharmacology and William K. Warren, Jr. who funded the William K. Warren, Jr. Chair in Medicine (to C.W.L.). Funding for the NMR instrumentation was provided in part by a grant from NIH (S10 RR019022).

References and notes

1. Kubo Y, Reuveny E, Slesinger PA, Jan YN, Jan LY. *Nature*. 1993; 364:802. [PubMed: 8355805]
2. Lesage F, Duprat F, Fink M, Guillemare E, Coppola T, Lazdunski M, Hugnot JP. *FEBS Lett*. 1994; 353:37. [PubMed: 7926018]
3. Kobayashi T, Ikeda K, Ichikawa T, Abe S, Togashi S, Kumanishi T. *Biochem Biophys Res Commun*. 1995; 208:1166. [PubMed: 7702616]
4. Karschin C, Dissmann E, Stuhmer W, Karschin A. *J Neurosci*. 1996; 16:3559. [PubMed: 8642402]

5. Luscher C, Slesinger P. Nat Rev Neurosci. 2010; 11:301. [PubMed: 20389305]
6. Krapivinsky G, Gordon EA, Wickman K, Velimirovi B, Krapivinsky L, Clapham DE. Nature. 1995; 374:135. [PubMed: 7877685]
7. Kobayashi T, Ikeda K, Kojima H, Niki H, Yano R, Yoshioka T, Kumanishi T. Nat Neurosci. 1999; 2:1091. [PubMed: 10570486]
8. Yow TT, Pera E, Absalom N, Heblinski M, Johnston GA, Hanrahan JR, Chebib M. Br J Pharmacol. 2011; 163:1017. [PubMed: 21391982]
9. Aryal P, Dvir H, Choe S, Slesinger PA. Nat Neurosci. 2009; 12:988. [PubMed: 19561601]
10. The MLSCN evolved into the MLPCN in 2008. For more information on the MLPCN and further details on the HTS effort, see: www.mli.nih.gov/ml/mlpcn
11. *Thallium flux assay protocol*: Compounds were dissolved in DMSO, transferred to 384-well polypropylene plates, and serially diluted in DMSO using and Agilent Bravo (11-points, threefold dilutions). Serially diluted plates were dispensed to daughter plates using a Labcyte Echo555 and diluted to twofold over their target assay concentration with 20 mM HEPES-buffered (pH 7.4) Hanks Balanced Salt Solution (HBSS), hereafter referred to as Assay Buffer, using a Thermo Fisher Combi. Twenty-thousand HEK-293 cells/well stably transfected with the ion channel subunits of interest (e.g., GIRK1/GIRK2, GIRK1/GIRK4) were plated into 384-well, black-walled, clear-bottom, aminocoated coated plates in 20 μ L/well alpha-minimal essential medium (MEM) supplemented with 10% (v/v) fetal bovine serum and incubated overnight in a 5% CO₂ incubator at 37 °C. Cell culture medium was removed from cell plates and replaced with 20 μ L/well Assay Buffer. Twenty microliters/well of 0.5 μ M of the thallium-sensitive dye, Thallous-AM (TEFlabs, Austin TX) in Assay Buffer was added to cell plates. Cell plates were incubated for ~60 min at room temperature and then dye-loading solution was removed from cell plates and replaced with 20 μ L/well Assay Buffer. Dye loaded and washed cell plates were transferred to a Hamamatsu FDSS 6000 and a double-addition protocol was initiated. After 10s, 20 μ L/well of 20 μ M test compound in 0.2% DMSO and Assay Buffer was added. After 4 min 10 μ L/well of a 5 $\times$ sodium bicarbonate-based thallium stimulus buffer (20 mM HEPES pH 7.4, 135 mM NaHCO₃, 2 mM CaSO₄, 1 mM MgSO₄, 5 mM glucose, 12 mM Ti₂SO₄) was added and 2 more minutes of data collection followed. Fluorescence data were collected at 1 Hz. *Data analysis*: Waveform signals (fluorescence intensity vs time normalized by dividing each fluorescence value (*F*) by the initial fluorescence value for each trace (*F*₀)) were reduced to single values by subtracting the average normalized waveform from vehicle control wells from each wave on the plate followed by obtaining the slope of the change in fluorescence immediately after the addition of the thallium stimulus. Slope values were normalized as a percentage of the slope obtained by the addition of a maximally effective concentration of the GIRK activator, ML297 (e.g., an efficacy value of 100% means that the compound's maximum apparent efficacy equals that of ML297). Curve fits for normalized slope values were obtained using a fourparameter logistic equation in the Excel add-in, XLfit.
12. Kauffman, K.; Days, E.; Romaine, I.; Du, Y.; Sliwoski, G.; Morrison, R.; Denton, J.; Niswender, CM.; Daniels, JS.; Sulikowski, GA.; Xie, S.; Lindsley, CW.; Weaver, CD. ACS Chem Neurosci. 2013. in press. <http://dx.doi.org/10.1021/cn400062a>
13. Kennedy JP, Williams L, Bridges TM, Daniels RN, Weaver D, Lindsley CW. J Comb Chem. 2008; 10:345. [PubMed: 18220367]
14. Lindsley CW, Wisnoski DD, Leister WH, O'Brien JA, Lemiare W, Williams DL Jr, Burno M, Sur C, Kinney GG, Pettibone DJ, Tiller PR, Smith S, Duggan ME, Hartman GD, Conn PJ, Huff JR. J Med Chem. 2004; 47:5825. [PubMed: 15537338]
15. *General urea library*: To a solution of 5-amino-*N,N*,2-trimethylbenzenesulfonamide (**4**) (15 mg, 0.07 mmol, 1.0 equiv) in DMF (1.0 mL) was added an isocyanate (0.07 mmol, 1.0 equiv) at rt. After 12 h, the reaction was purified by reverse phase HPLC to afford the desired urea. *General Amide Library*: To a solution of 5-amino-*N,N*,2-trimethylbenzenesulfonamide (**4**) (15 mg, 0.07 mmol, 1.0 equiv) in DMF and TEA (4:1, 1.0 mL) was added the acid chloride (0.084 mmol, 1.1 equiv) at rt. Once the reaction was complete by LCMS, the reaction was filtered and purified by reverse phase HPLC to afford the desired amide. *General urea library*: To a solution of 5-amino-*N,N*,2-trimethylbenzenesulfonamide (**4**) (15 mg, 0.07 mmol, 1.0 equiv) in DMF and TEA (4:1, 1.0 mL) was added the acid chloride (0.084 mmol, 1.1 equiv) at rt. Once the reaction was complete by LCMS, the reaction was filtered and purified by reverse phase HPLC to afford the desired amide.

1, VU0032230
GIRK1/2 EC_{50} = 1.4 μ M, 97%
GIRK1/4 EC_{50} = 0.69 μ M, 93%
inactive on GIRK2/3

2, VU0456810 (ML297)
GIRK1/2 EC_{50} = 0.16 μ M, 194%
GIRK1/4 EC_{50} = 1.8 μ M, 93%
inactive on GIRK2/3

3, VU0259369
GIRK1/2 EC_{50} = 1.1 μ M, 100%
GIRK1/4 EC_{50} = 1.1 μ M, 104%
inactive on GIRK2/3

Figure 1.
Structures and GIRK activities of HTS leads **1** and **3**, and the optimized GIRK1/2 activator **2**.

Figure 2.
Chemical optimization plan for 3, consisting of iterative parallel synthesis and fragment libraries.

3a, VU0474527GIRK1/2 EC₅₀ = 1.4 μM, 108%GIRK1/4 EC₅₀ = 1.6 μM, 114%

inactive on GIRK2/3

3b, VU0474530GIRK1/2 EC₅₀ = 3.3 μM, 55%GIRK1/4 EC₅₀ = 1.6 μM, 123%

inactive on GIRK2/3

3c, VU0474523GIRK1/2 EC₅₀ = 2.3 μM, 71%GIRK1/4 EC₅₀ = 2.2 μM, 64%

inactive on GIRK2/3

3d, VU0474532GIRK1/2 EC₅₀ = 3.6 μM, 107%GIRK1/4 EC₅₀ = 5.2 μM, 108%

inactive on GIRK2/3

Figure 3.Representative active GIRK activators from iterative parallel synthesis effort around **3**.

Figure 4. GIRK activator concentration response curves (CRC) measuring thallium flux. (A) GIRK1/2 ($EC_{50} = 1.1 \mu\text{M}$, 110%) and GIRK1/4 ($EC_{50} = 1.1 \mu\text{M}$, 104%) CRCs for the HTS hit **3**; (B) GIRK1/2 ($EC_{50} = 1.4 \mu\text{M}$, 108%) and GIRK1/4 ($EC_{50} = 1.6 \mu\text{M}$, 114%) CRC for related analog **3a**.¹¹

Figure 5. GIRK activator concentration response curves (CRC) measuring thallium flux. (A) GIRK1/4 ($EC_{50} = 0.24 \mu\text{M}$, 17%) CRC for **8e**; (B) GIRK1/4 ($EC_{50} = 1.8 \mu\text{M}$, 22%) CRCs for **8g**.¹¹

Scheme 1.

Reagents and conditions: (a) RCOCl, TEA, DMF, rt, 16 h, 45–95%; (b) RNCO, DMF, rt, 22–88%; (c) ArNH₂, TEA, DMF, rt, 37–92%.

Table 1

Structures and GIRK activities of analogs **6**

Compd	Ar	GIRK1/2		GIRK1/4	
		EC ₅₀ ^a (μM)	Efficacy ^a (%)	EC ₅₀ ^a (μM)	Efficacy ^a (%)
6a	Ph	1.4	94	3.9	100
6b	4-ClPh	3.3	92	2.8	72
6c	3-Cl,4-MePh	2.9	91	6.2	115
6d	4-OMePh	1.7	101	5.6	104
6e	2-FPh	3.9	111	7.8	72
6f	3-FPh	1.1	89	1.8	55
6g	2-ClPh	2.7	94	6.8	106
6h	4-CF ₃ Ph	1.5	26	>10	ND
6i	2,5-diOMePh	>10	ND	>10	ND
6j	3-CNPh	3.9	83	3.3	29

^a Potency values were obtained from triplicate determinations and the reported efficacy values shown are standardized to the efficacy of **2**, arbitrarily designated to 100%.
 ND = not determined.

Table 2

Structures and GIRK activities of analogs **8**

Compd	Ar	GIRK1/2		GIRK1/4	
		EC ₅₀ ^a (μM)	Efficacy ^a (%)	EC ₅₀ ^a (μM)	Efficacy ^a (%)
8a	4-F,3-SO ₂ MePh	>10	ND	>10	ND
8b	3- <i>t</i> -BuPh	5.9	44	2.7	76
8c	3-OCHF ₂ Ph	>10	ND	7.8	119
8d	3-Cl,5-CF ₃ Ph	>10	ND	>10	ND
8e	4-Cl,3-CF ₃ Ph	>10	ND	0.24	17
8f	4-ClPh	>10	ND	>10	ND
8g	3-ClPh	>10	ND	1.8	22
8h	4-CF ₃ Ph	>10	ND	2.1	20
8i	3-MePh	>10	ND	>10	ND

^a Potency values were obtained from triplicate determinations and the reported efficacy values shown are standardized to the efficacy of **2**, arbitrarily designated to 100%.
ND = not determined.