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Discovery of 'molecular switches' within a GIRK activator scaffold that afford selective GIRK inhibitors

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ABSTRACT

This letter describes a multi-dimensional SAR campaign based on a potent, efficacious and selective GIRK1/2 activator (~10-fold versus GIRK1/4 and inactive on nonGIRK 1-containing GIRKs, GIRK 2 or GIRK2/3). Further chemical optimization through an iterative parallel synthesis effort identified multiple 'molecular switches' that modulated the mode of pharmacology from activator to inhibitor, as well as engendering varying selectivity profiles for GIRK1/2 and GIRK1/4. Importantly, these compounds were all inactive on nonGIRK1 containing GIRK channels. However, SAR was challenging as subtle structural modifications had large effects on both mode of pharmacology and GIRK1/2 and GIRK1/4 channel selectivity.

The $K_{ir}3.1$–3.4 family of inward-rectifying potassium channels, also referred to as G protein regulated Inwardly Rectifying Potassium (K^+) or GIRK1-4 channels, modulate cell excitability, and have been implicated in a number of crucial physiological processes.[1–7] Due a lack of potent and selective small molecule GIRK activators (the only known activators are alcohols (e.g., ethanol) and naringin with EC_{50}s >100 μM) and inhibitors (only the weak and unselective SCH23390 IC_{50}s ~4–8 μM), target validation for GIRK activation and inhibition in multiple therapeutic areas has been hindered.[1–9] With this in mind, we identified GIRK activator **1** (VU0032230) from an MLSCN[10] HTS campaign that employed a thallium-flux based readout of GIRK1/GIRK2.[11] Subsequent chemical optimization, via parallel synthesis (Fig. 1), evaluated alternate ureas and linkage moieties, that ultimately afforded the first selective (~10-fold versus GIRK1/4, inactive on GIRK2, GIRK2/3 and on the K^+ channels tested) GIRK1/2 activator **2** (VU0456810, also known as ML297).[11] ML297 was shown to possess a favorable DMPK profile, was centrally penetrant and established proof of concept for GIRK1/2 activation in preclinical epilepsy models.[11] In this Letter, we describe further multi-dimensional SAR exploration of **2** that resulted in

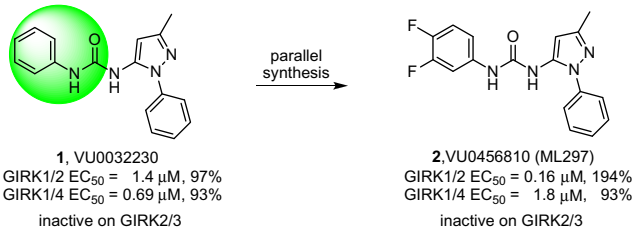

Figure 1. Structures and GIRK activities of HTS GIRK activator lead **1** and the optimized GIRK1/2 and GIRK1/4 activator **2** (ML297).

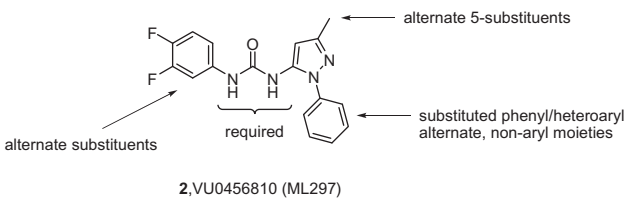

Figure 2. Chemical optimization plan for **2**, employing parallel synthesis.

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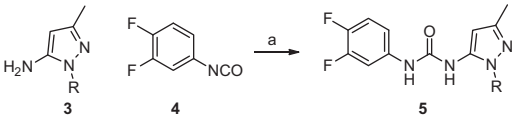


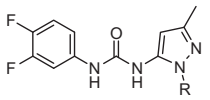
Scheme 1. Synthesis of analogs **5**. Reagents and conditions. (a) CH_2Cl_2, rt, 16 h, 73–98%.

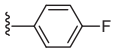
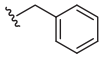
the identification of additional GIRK activators, and 'molecular switches'[12–14] that afforded the selective GIRK inhibitors.

Figure 2 highlights the chemical optimization plan for **2** to survey multiple dimensions of the molecule. In the previous work that afforded **2**, SAR was limited to alternate ureas and alternate linkages for the urea moiety; moreover, the urea linkage was found to be required, and not even *N*-Me congeners of either NH possessed any GIRK activity. Interestingly, all of the analogs assayed were either GIRK activators or inactive.

For our first library, we held the 3-methyl pyrazole and the 3,4-difluorophenyl moieties of **2** constant, and surveyed a variety of alternatives for the *N*-Ph group. In this instance, all of the *N*-functionalized-5-amino-3-methylpyrrazoles **3** were commercially available, and simply reacted with 3,4-difluorophenyl isocyanate **4** to deliver analogs **5** (Scheme 1). This library afforded a number of GIRK activators, including the benzyl congener **5d**, the most potent dual GIRK1/2 (EC_{50} = 0.07 μM, 87%) and GIRK1/4 (EC_{50} = 0.11 μM, 99%) activator identified to date. All attempts to incorporate a basic amine, represented by pyridyl analogs **5c** and **5e**, resulted in a substantial loss of GIRK activity. While comparable

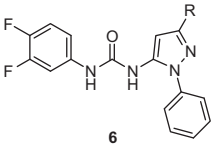
Table 1
Structures and GIRK activities of analogs **5**



Compd	R	GIRK1/2 EC_{50} (μM)[a]	Efficacy[b] (%)	GIRK1/4 EC_{50} (μM)[a]	Efficacy[b] (%)
5a		0.46	96	1.3	103
5b		0.19	78	0.54	57
5c		>10	78	>30	ND
5d		0.07	87	0.11	99
5e		>10	88	>30	ND

ND = not determined.
[a] Potency values were obtained from triplicate determinations.
[b] Reported efficacy values shown are standardized to the efficacy of **2**, arbitrarily designated to 100%.

Table 2
Structures and GIRK activities of analogs **6**



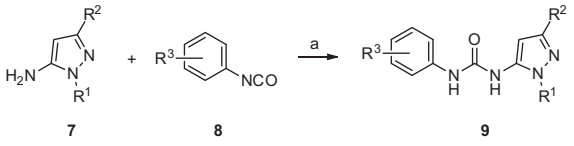
Compd	R	GIRK1/2		CAT	GIRK1/4		CAT
		EC_{50} (μM)[a]	Effic.[b] (%)	ACT	EC_{50} (μM)[a]	Effic.[b] (%)	ACT
		IC_{50} (μM)[a]	IC_{50} (μM)[a]	INH	IC_{50} (μM)[a]	Effic.[b] (%)	INH
6a		>30	ND	ACT	30	ND	ACT
		30	ND	INH	30	ND	INH
6b		0.41	32	ACT	30	ND	ACT
		NA	NA	INH	NA	NA	INH
6c		NA	NA	ACT	NA	NA	ACT
		2.0	136	INH	1.4	150	INH
6d		30	ND	ACT	30	ND	ACT
		30	ND	INH	30	ND	INH
6e		NA	NA	ACT	NA	NA	ACT
		5.1	85	INH	4.1	88	INH
6f		30	ND	ACT	30	ND	ACT
		30	ND	INH	30	ND	INH
6 g		NA	NA	ACT	NA	NA	ACT
		0.65	95	INH	0.56	67	INH

ND = not determined; ACT = activator; INH = inhibitor; NA = not applicable.
[a] Potency values were obtained from triplicate determinations.
[b] Reported efficacy values are standardized to the efficacy of **2**, arbitrarily designated to 100% for activators, and standardized to SCH3320, arbitrarily designated to full inhibition (100%), for inhibitors.

to the parent **2**, both the 4-fluorophenyl congener **5b** and the chemically distinct trifluoroethyl analog **5a**, were substitutions worthy of further analogs. Additional analogs of **5d**, surveying alternate ureas, led to a family of dual GIRK1/2 and GIRK1/4 activators. Importantly, all analogs **5** were inactive on nonGIRK1 containing channels (Table 1).

In parallel, a library of analogs **6** was constructed that held the 3,4-difluorophenyl and the *N*-phenyl moieties constant, and evaluated alternative substituents in the 3-position of the pyrazole, following a slight perturbation of Scheme 1. This effort generated unique, textured SAR and, for the first time for GIRK, the identification of 'molecular switches'[12–14] that modulated the mode of pharmacology from activator to inhibitor (Table 2). As with the activators, where we normalize efficacy to **2**, we employ the only known GIRK inhibitor SCH23390 (a D_1 antagonist with off-target GIRK activity),[15] and normalize percent inhibition with SCH23390 affording 100% inhibition.

Here, replacement of the 3-methyl group with either a phenyl (**6a**), CF_3 (**6f**) or cyclobutyl moiety (**6d**), led to a complete loss of GIRK activity. A cyclopropyl group (**6b**) proved to be a selective, GIRK1/2 partial activator (EC_{50} = 0.41 μM, 32%); in contrast, an isopropyl derivative (**6c**) was a dual GIRK1/2 (IC_{50} = 2.5 μM, 136%) and GIRK1/4 (IC_{50} = 4.1 μM, 88%) inhibitor, and represented the first example of a GIRK 'molecular switch'.[12–14] While this phenomenon is common amongst Family A and C allosteric GPCR ligands[12,13] and, more recently, KCNQ2 ion channel ligands[14] and Ca channel modulators,[16] it has not yet been reported for GIRK ligands. Ring expansion from the inactive cyclobutyl (**6d**) to a cyclopentyl analog (**6e**) restores GIRK activity, but as a weak dual GIRK1/2 and GIRK1/4 inhibitor. Quite unexpectedly, a cyclopropyl methyl congener (**6g**) was a potent, and fully efficacious GIRK1/2 (IC_{50} = 0.65 μM, 95%) and GIRK1/4 (IC_{50} = 0.56 μM, 67%) inhibitor, whereas **6b**, the cyclopropyl analog was a selective GIRK1/2 activator. Importantly, all analogs **6** were inactive on nonGIRK1 containing GIRK channels. Based on the SAR data generated from these two libraries, it was time to explore additional isocyanates within **6** and to merge productive modifications noted in analogs **5** and **6**,



Scheme 2. Synthesis of analogs **9**. Reagents and conditions. (a) CH_2Cl_2, rt, 16 h, 64–95%.

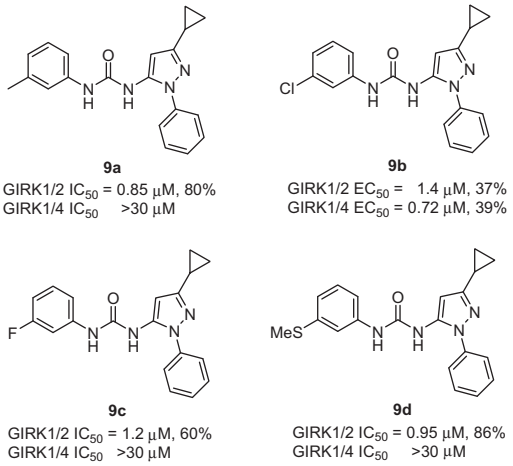


Figure 3. Selected analogs **9** where nature of phenyl substituent converts a 3-cyclopropyl GIRK1/2 activator scaffold into GIRK1/2 selective inhibitors.

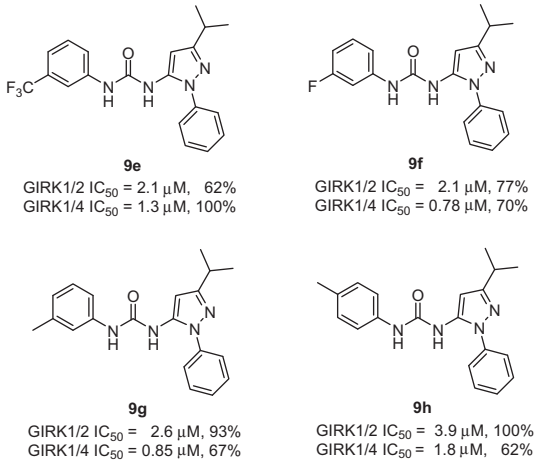


Figure 4. Selected analogs **9** where the 3-isopropyl moiety engenders exclusive GIRK inhibition, often slightly favoring GIRK1/4.

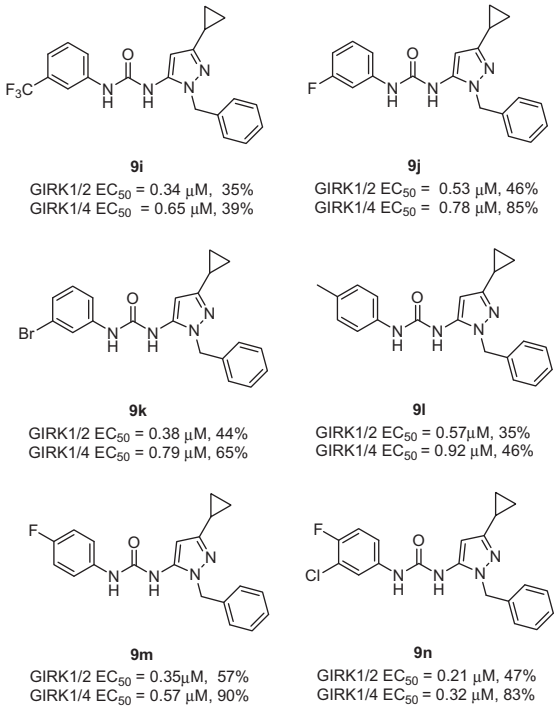


Figure 5. Selected analogs **9** where a 3-cyclopropyl, *N*-benzyl GIRK1/2 activator scaffold uniformly affords potent, dual GIRK1/2 and GIRK1/4 activators with variable efficacies.

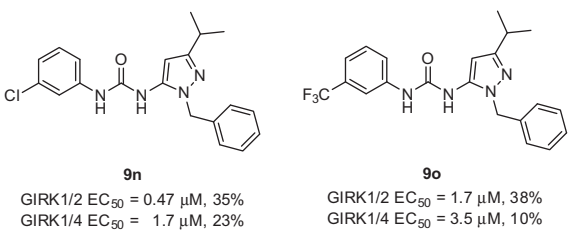


Figure 6. Selected analogs **9** with 3-isopropyl, *N*-benzyl motif affords low efficacy GIRK1/2 and dual GIRK1/2 and GIRK1/4 activators.

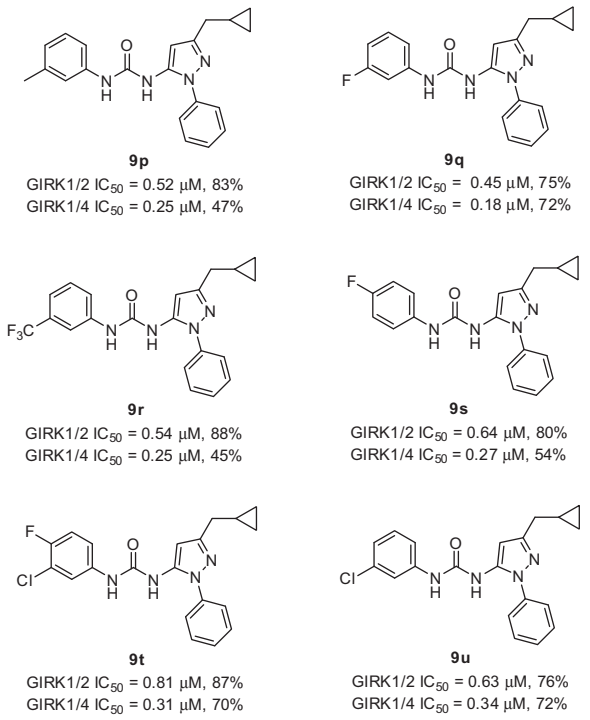


Figure 7. Selected analogs **9** with 3-cyclopropyl, *N*-phenyl motif afford highly potent dual GIRK1/2 and GIRK1/4 inhibitors.

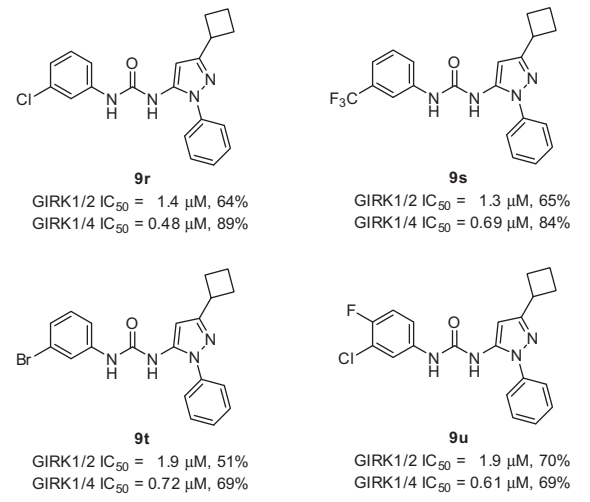


Figure 8. Selected analogs **9** with 3-cyclobutyl, *N*-phenyl motif afford GIRK1/4 preferring inhibitors.

and assess if these would either be additive or if additional 'molecular switches'[12–14] be discovered (Scheme 2).

First, we elected to employ the *N*-phenyl and *N*-benzyl moieties in analogs **5**, with the cyclopropyl, isopropyl and cyclopropyl methyl groups found in active analogs **6**, and explored a diverse array of isocyanates to deliver 120 analogs **9** from a matrix library ($2 \times 3 \times 20$). These new analogs displayed a wide range of GIRK1/2 and GIRK1/4 selectivity profiles, as well as promiscuous modulation in the mode of pharmacology from activator to inhibitor. Whereas the cyclopropyl group in **6b** afforded a selective GIRK1/2 partial activator, other substitutions on the phenyl ring of the urea afforded both weak activators (EC_{50}s 0.9–5 μM, 13–65%) and potent, GIRK1/2 selective inhibitors. Figure 3 highlights some representative 3-cyclopropyl, *N*-phenyl analogs **9a**–**d**, where the nature of the 3-substitutent modulates the mode of pharmacology.

In sharp contrast, additional analogs of the isopropyl derivative **6c** were exclusively either GIRK inhibitors, or inactive, and many displayed a slight preference for GIRK1/4 over GIRK1/2 (Fig. 4). Interestingly, the optimal phenyl substituents in the expanded **6b** library (analogs **9a**–**d**), were not optimal for the **6c** scaffold (**9e**–**h**), yet substituents in the 3-position were uniformly more active.

A library combining the 3-cyclopropyl moiety with the *N*-benzyl group, functionalities that both imparted GIRK activation, provided highly potent and efficacious dual GIRK1/2 and GIRK1/4 activators (Fig. 5). The SAR within this series was shallow, with virtually all substituents on the aryl ring affording submicromolar, dual GIRK1/2 and GIRK1/4 activators.

The corresponding 3-isopropyl, *N*-benzyl congener library afforded fewer active compounds, and with the exception of a few low efficacy (<10%) GIRK1/2 preferring activators, the majority of these analogs were either GIRK1/2 selective or dual GIRK1/2 and GIRK1/4 inhibitors (Fig. 6).

The cyclopropylmethyl derivative **6g** was a dual GIRK1/2 and GIRK1/4 inhibitor, and replacement of the *N*-phenyl with an *N*-benzyl group provided only weak to inactive analogs. However, further evaluation of alternate ureas within the *N*-phenyl **6g** series provided a number of sub-micromolar dual GIRK1/2 and GIRK1/4 inhibitors (Fig. 7). Here, SAR lacked texture, with virtually any substituents on the urea phenyl ring providing GIRK inhibitors of comparable potency.

Finally, these library efforts highlighted the impact of slight structural variations leading to dramatic changes in GIRK channel selectivity, mode of GIRK pharmacology, and in some cases, an order of magnitude gain or loss of GIRK activity. Therefore, we prepared an expanded library around the 3-cylobutyl analog **6d** to determine if GIRK activity could be achieved with alternate functionality. While >90% of these analogs were inactive, several emerged that displayed activity as GIRK inhibitors, with a slight preference for GIRK1/4 (Fig. 8). As seen earlier, substituents in the 3-position are preferred, and groups in the 4-position generally abolish GIRK1/4 inhibition.

In general, these analogs possess high *c*log*P*s (>4), but experimental log*P* values range from 3.2 to 3.9. The majority of GIRK ligands reported herein display moderate protein binding in rat (1–3% free) and human (1–4% free), moderate intrinsic clearance (CL_{INT} <40 mL/min/kg) and very clean CYP profiles (>20 μM versus 3A4, 2D6, 2C9 and 1A2). Studies probing in vivo PK and CNS exposure are in progress.

In summary, we detailed a multi-dimensional SAR campaign based on a potent, efficacious and selective GIRK1/2 activator (~10-fold versus GIRK1/4 and inactive on GIRK2/3) ML297. Further chemical optimization through an iterative parallel synthesis effort identified multiple 'molecular switches' that modulated the mode of pharmacology from activator to inhibitor, as well as engendering varying selectivity profiles for GIRK1/2 and GIRK1/4. Importantly, these compounds were all inactive on nonGIRK1 containing GIRK channels. However, SAR was challenging as subtle structural modifications had large effects on both mode of pharmacology and GIRK1/2 and GIRK1/4 channel selectivity. Despite the optimization challenges, this effort afforded potent and selective GIRK inhibitors, activators with improved potency/efficacy, and a valuable set of tool compounds to further dissect the roles of GIRK channels in various pathological states. Detailed molecular pharmacology studies are underway (e.g., developing mutants that exchange various domains between GIRK1/2 with GIRK 2/3) to understand the mode/site of binding of these novel GIRK ligands and attempt to

elucidate the origins of the 'molecular switches'. Further efforts and refinements are in progress and will be reported in due course.

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