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Discovery of pyrazolyl propionyl cyclohexenamide derivatives as full agonists for the high affinity niacin receptor GPR109A

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ABSTRACT

A series of pyrazolyl propionyl cyclohexenamides were discovered as full agonists for the high affinity niacin receptor GPR109A. The structure–activity relationship (SAR) studies were aimed to improve activity on GPR109A, reduce Cytochrome P450 2C8 (CYP2C8) and Cytochrome P450 2C9 (CYP2C9) inhibition, reduce serum shift and improve pharmacokinetic (PK) profiles.

Niacin (nicotinic acid), as a group B vitamin (>15–20 mg/day), plays its physiological role as the precursor for NAD^+ and $NADP^+$.[1] As an anti-dyslipidemic drug dosed 500–2000 mg/day, niacin demonstrates its pharmacological utility by modifying lipid profiles.[2] Particularly, niacin elevates high density lipoprotein cholesterol (HDL-C), and reduces total plasma cholesterol, triglyceride (TG), very low-density lipoprotein cholesterol (VLDL-C), low-density lipoprotein cholesterol (LDL-C) and lipoprotein a (Lp(a)).[3] In clinical studies including the Coronary Drug Project (CDP),[4] Stockholm Ischaemic Heart Disease Study,[5] HATS Trial,[6] and ARBITER 2 trial,[2d] niacin reduced the morbidity and mortality of patients with coronary heart disease, and slowed the progression of atherosclerosis by mono-therapy or combination therapy with simvastatin. The most common adverse effect of niacin treatment is severe skin vasodilation.

The beneficial role of niacin and the discovery of GPR109A, the high affinity niacin receptor,[7] have stirred the interests of both academia[8] and pharmaceutical industry[9] in pursuing niacin receptor agonists as potential therapeutic agents for cardiovascular disease yet devoid of the skin vasodilatory effects of niacin. Previously, we disclosed biaryl and tricyclic anthranilides, ureas, and cycloalkenes as full agonists for GPR109A.[10] Herein we report the discovery of pyrazole analogs for the high affinity niacin receptor GPR109A. To enhance activity against GPR109A, improve PK profiles and reduce CYP liability, we identified pyrazole, hydroxypyridine, cyclohexene and an aryl substituent of cyclohexene as fragments for optimized GPR109A agonists.

First, we studied a series of anthranilides (Fig. 1) with their binding and [^{35}S]GTPγS assay data shown in Table 1. Having simi-

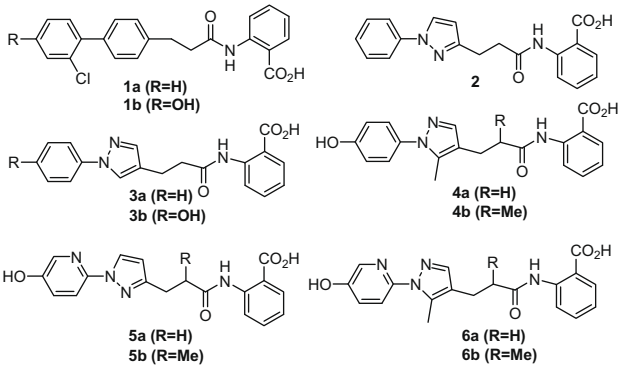

Figure 1. Structures of pyrazolyl anthranilides.

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Table 1
In vitro SAR against GPR109A for anthranilides **1–6**

Compds	R	^{3}H-niacin binding IC_{50}[a] (μM)	Serum shift[b]	hGTPγS EC_{50}[a] (μM)
Niacin		0.14	1	1.0
1a	H	0.019		0.12
1b	OH	0.004	>5000	0.045
2		0.31		9.5
3a	H	0.26	40	3.9
3b	OH	0.032	70	0.34
4a	H	0.024		0.093
4b	Me	0.077	60	1.1
5a	H	0.058	170	1.3
5b	Me	0.025	200	0.94
6a	H	0.015		0.75
6b	Me	0.091		3.0

[a] Value are means of at least three experiments, average standard deviation is about 20%.
[b] ^{3}H-Niacin binding with 4% human serum.

lar maximum level of response as niacin in the GTPγS assay, all analogs described in this Letter are characterized as full agonists. Compound **1b** containing a terminal hydroxyl group was 3–5-fold more active against GPR109A than **1a**. Despite its good activity, compound **1b** had a poor PK profile with only 5% bioavailability (Table 5) and more than 5000-fold serum shift in the competitive binding assay with 4% human serum. Furthermore, **1b** was a potent inhibitor for CYP2C8 and 2C9 (Table 4), and potentially had bioactivated covalent binding due to the phenolic moiety. To overcome these issues, our first approach was to replace the inner phenyl group with a pyrazole, as shown by analogs **2–6** in Figure 1. Although less active than the biphenyl analogs **1a** and **1b**, analogs bearing the inner pyrazole group had significantly reduced serum shift in the binding assay (compounds **3–6**, Table 1). Similar to analog **1b**, by incorporating a terminal hydroxyl group in the pyrazole class, **3b** was approximately 10-fold more active than **3a**. Concerning the potential covalent binding of the electron-rich phenol group, we replaced 4-hydroxyphenyl with 4-hydroxy-2-pyridyl, as shown in compound **5** and **6**, to reduce the electron density of the aromatic ring. This structural change gave no significant change of activity in the binding assay, despite some loss of activity in the GTPγS assay (**6a** vs **4a**, **6b** vs **4b**, Table 1). The methyl substitution at the five position of the pyrazole in **4a** slightly increased the binding affinity with respect to **3b**. The introduction of α-methyl group to **4a** and **6a** resulted in less active compounds, **4b** and **6b**. Conversely, the α-methyl group was beneficial to activity in the case of **5b**, a more active compound than **5a**.

After adopting the 4-hydroxypyridyl-pyrazole as the optimized biaryl group, we replaced the anthranilide moiety with the cyclohexenamide shown in Figure 2.[11] In comparison with **6a**, the replacement of a 2-carboxyphenyl with a 2-carboxy 1-hexenyl group in **8a** improved the binding affinity (IC_{50} = 7 nM for **8a** vs IC_{50} = 15 nM for **6a**) and functional activity (EC_{50} = 0.28 μM for **8a** vs EC_{50} = 0.75 μM for **6a**) (Table 2). Despite the higher affinity of 1,3-disubstituted pyrazole **10a** with respect to 1,4-disubstituted pyrazole **9a** in the competitive binding assay (IC_{50} = 5 nM for **10a** vs IC_{50} = 26 nM for **9a**), **9a** and **10a** had similar functional activity. The α-methyl group of the amide led to slightly reduced activities, as shown by the activity of **8a** versus **8b**, **9a** versus **9b**, and **10a** versus **10b**. Further saturation of the cyclohexene ring such as cis racemic 1,2-substituted cyclohexane isomers **11** resulted in a drastic loss of activity.

To improve the activity, we modified the 4-position of the cyclohexene moiety of **10a** with various alkyl and aryl substituents. With excellent binding activity (IC_{50}s <20 nM), the aryl-substituted analogs exhibited excellent functional activity.[12] In particular, **12d** and **12e** demonstrated remarkable binding affinity (6 nM and 5 nM, respectively) and functional activity (67 nM and 62 nM, respectively).

We then measured the compound-induced inhibition of lipolysis in human adipocytes (Table 3). Compounds **4a**, **4b** and **7** suppressed the lipolysis similar to niacin, indicating the efficacy of these compounds in whole cells.

The biphenyl series generally posed issues on CYP2C8 and 2C9 inhibition. One such example was **1b**, a potent inhibitor of both CYP2C8 and 2C9 enzymes with submicromolar IC_{50}s (Table 4). The presence of pyrazole as the inner ring, the substitution pattern of pyrazole, and cyclohexene replacement of anthranilide (Fig. 2) all played a profound role in reducing CYP inhibition. With respect to **1b**, the 1,3-disubstituted pyrazole analog **2** reduced inhibition of both CYP2C8 and 2C9 with IC_{50}s in a low micromolar range. Furthermore, compounds **3a**, **4a**, and **4b** containing a 1,4-disubstituted pyrazole, had further reduced inhibition against both CYP enzymes (IC_{50} = 15–34 μM). Finally, the cyclohexene analogs **7** and **9a** completely removed CYP2C8 and 2C9 inhibition. The incorporation of

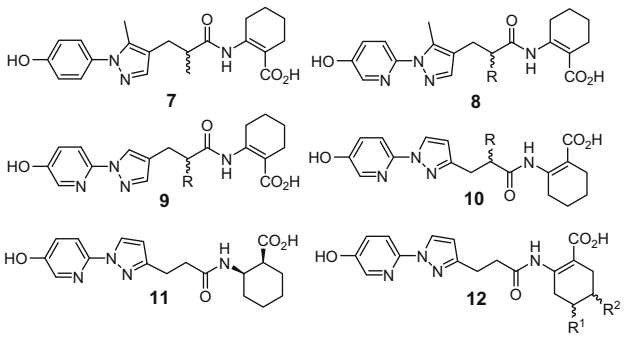


Figure 2. Structures of pyrazolyl propionyl cyclohexenamides.

Table 2
In vitro SAR against GPR109A for cylohexenamides **7–10**

Compds	R	^{3}H-niacin binding IC_{50}[a] (μM)	Serum shift[b]	hGTPγS EC_{50}[a] (μM)
7		0.026	50	0.35
8a	H	0.007		0.28
8b	CH_3	0.046		0.77
9a	H	0.026	240	0.20
9b	CH_3	0.022		0.54
10a	H	0.005	1000	0.23
10b	CH_3	0.010	900	0.37
11		1.31		17.9
12a	R^1 = CH_3 (*R*); R^2 = H	0.014	290	0.17
12b	R^1 = $CH_2CH_2CH_3$; R^2 = H	0.009	110	0.13
12c	R^1 = 3-F-phenyl; R^2 = H	0.012	40	0.23
12d	R^1 = 2-F-3-F-phenyl; R^2 = H	0.006	30	0.067
12e	R^1 = H; R^2 = 3-F-5-F-phenyl	0.005	100	0.062
12f	R^1 = 3-F-4-F-5-F-phenyl; R^2 = H	0.015	30	0.095

[a] Value are means of at least three experiments, average standard deviation is about 20%.
[b] ^{3}H-Niacin binding with 4% human serum.

Table 3
In vitro inhibition of lipolysis in human adipocytes[a]

Compd	Niacin	**4a**	**4b**	**7**
IC_{50} (μM)	0.08	0.04	0.06	0.07

[a] Free glycerol was measured to calculate IC_{50}s for the lipolysis inhibition assay.

Table 4
CYP2C8 and 2C9 inhibition for compounds[a]

Compd	1b	2	3a	4a	4b	7	9a	12e
CYP2C8 IC_{50} (μM)	<0.4	2.7	—	19	34	>100	—	7.7
CYP2C9 IC_{50} (μM)	<0.4	2.5	15	15	22	>100	>50	23

[a] The CYP2C8 inhibition assay in human liver microsomes used taxol and montelukast as the substrate and positive control, respectively. The CYP2C9 inhibition assay in human liver microsomes used diclofenac and sulfaphenazole as the substrate and positive control, respectively.

Table 5
Mouse or rat PK[a]

Compd	*F*%	Cl (mL/min/kg)	Vd_{ss} (L/kg)	C_{max} (μM)	$T_{1/2}$ (h)	AUC_{po} (μM h kg/mg)
1b[b]	4.6	18	0.35	0.93	2.0	0.11
3b[b]	21	17	3.56	0.48	3.5	0.58
4a[b]	2.5	26	0.88	0.03	1.1	0.1
7[b]	52	3.5	0.68	4.78	4.8	6.5
9a[b]	46	1.2	0.40	11.53	3.3	19
12d[c]	4.5	28	0.36	0.03	0.74	0.06
12e[c]	39	8.6	0.53	1.58	5.5	1.6

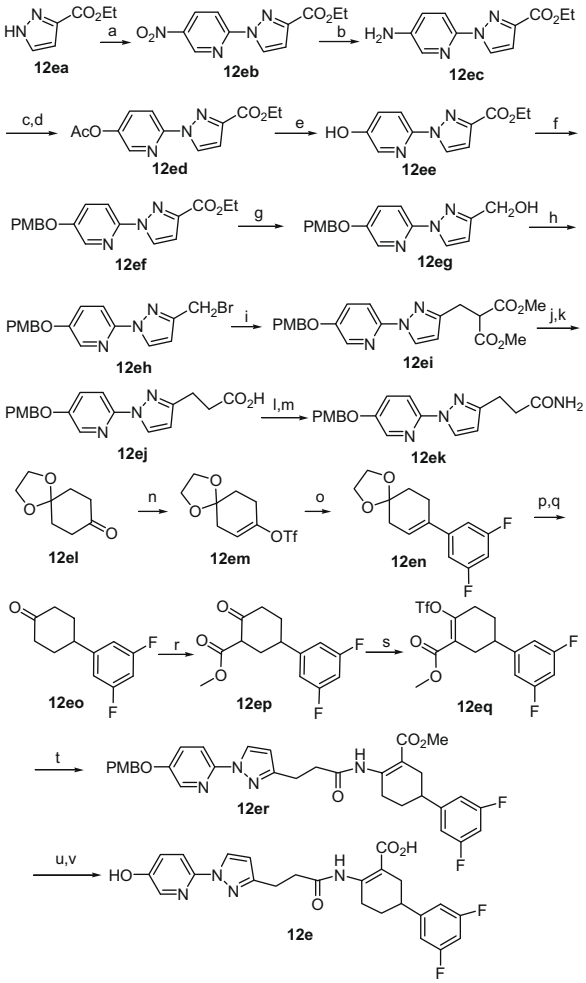
[a] Formulations: 0.2 mg/mL ethanol/PEG/water (10:40:50). IV dose: 1 mg/kg ($n = 3$). PO dose: 2 mg/kg ($n = 3$). Blood concentration was determined by LC/MS/MS following protein precipitation with acetonitrile.
[b] Mouse PK.
[c] Rat PK.

an aryl group in **12e** increased the CYP inhibition, but was still significantly better than the lead compound **1b**.

Besides reducing CYP2C8 and 2C9 liability (Table 4), in general, the replacement of the inner phenyl group with a pyrazole, and the anthranilide moiety with a cyclohexenamide provided significantly improved mouse or rat PK parameters (**7** and **9a** vs **1b**, **3b**, and **4a** in Table 5). In addition, the fluoroaryl substitution position of the cyclohexenyl group appeared to have a substantial impact on rat PKs of analogs, as exemplified by **12d** and **12e**. With respect to **12d**, compound **12e** had an eightfold higher bioavailability and half life, a threefold lower clearance, and a 26-fold higher oral exposure. Comparing **3b** and **4a**, it appeared that the extra methyl group present in **4a** led to the significant reduction of C_{max}, half life, normalized AUC and bioavailability.

The synthesis of analog **12e**, as a representative example, was achieved over 22 steps in total as shown in Scheme 1. The preparation of the key intermediate **12ek** was commenced with commercially available pyrazole **12ea**. The nucleophilic aromatic substitution of 2-bromo-5-nitropyridine with **12ea** provided **12eb**. The reduction of the nitro group in **12eb** was followed by a three-step sequence to convert the resulting amino group to a hydroxyl group via an intermediate diazonium tetrafluoroborate salt. The hydroxyl group of **12ee** was subsequently protected as a PMB ether. The two-carbon chain extension led to carboxylic acid **12ej**, which was then converted to amide **12ek** through an active succinimidyl ester intermediate in excellent yield. Intermediate **12eq** was synthesized in six steps starting from **12el**. The Pd-catalyzed amidation of **12eq** with **12ek** formed **12er**. Finally, the deprotection of the PMB group followed by hydrolysis afforded the desired product **12e**. The overall synthesis has a convergent nature.

In conclusion, we have identified a new class of pyrazolyl propionyl cyclohexenamides as highly active full agonists for the niacin receptor GPR109A. The sequential employment of pyrazole, hydroxypyridine, cyclohexene, and an aryl substitution of cyclohexene eventually led to the discovery of **12e** with excellent activity against GPR109A, very weak CYP2C8 and 2C9 inhibition, and a good rat PK profile.



Scheme 1. Reagents and conditions: (a) 2-bromo-5-nitropyridine, NaH, DMF, 0 °C to rt, 0.5 h, 88%; (b) Zn, AcOH, 60 °C, 0.5 h, 99%; (c) $NaNO_2$, 40% HBF_4, 0 °C, 2 h; (d) Ac_2O, 65 °C, 16 h, 52% in two steps; (e) cat. H_2SO_4, EtOH, reflux, 16 h, 97%; (f) NaH, PMB-Cl, cat. NaI, DMF, 90 °C, 1 h, 95%; (g) $LiBH_4$, THF, reflux, 24 h, 100%; (h) Ph_3P, NBS, pyridine, CH_2Cl_2, 0 °C, 1.5 h, 77%; (i) dimethyl malonate, NaH, DMF, 0 °C to rt, 1 h, 98%; (j) LiOH, THF/MeOH/H_2O (3:1:1), rt, 1 h, 92%; (k) DMF, 130 °C, 20 min, 94%; (l) *N*-hydroxy succinimide, EDCI, CH_2Cl_2, 3 h, 99%; (m) 28% NH_3/H_2O; dioxane, 2 h, 97%; (n) LiHMDS, 2-(*N*,*N*-bistrifluoro-methylsulfonylamino)-5-chloropyridine, THF, −78 °C to rt, 2 h, 72%; (o) 3,5-difluorophenylboronic acid, $Pd(PPh_3)_2Cl_2$, Na_2CO_3, THF, rt, 2 h, 86%; (p) H_2, 10% Pd/C, MeOH, rt, 16 h; (q) 3 N HCl, THF, rt, 5 h, 80% in two steps; (r) LiHMDS, methyl cyanoformate, THF, −78 °C to rt, 3 h, 62%; (s) NaH, 2-(*N*,*N*-bis-trifluoromethylsulfonylamino)-5-chloropyridine, THF, 0 °C to rt, 2 h, 68%; (t) $Pd_2(dba)_3$, **12ek**, xantphos, Cs_2CO_3, dioxane, 80 °C, 16 h; 49%; (u) TFA, triisopropylsilane, CH_2Cl_2; 0 °C to rt, 20 min; (v) LiOH, THF/MeOH/H_2O(3:1:1), 0 °C to rt, 15 h, 40% in two steps.

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