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Discovery of orally bioavailable and novel urea agonists of the high affinity niacin receptor GPR109A

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Abstract—A urea class of high affinity niacin receptor agonists was discovered. Compound **1a** displayed good PK, better in vivo efficacy in reducing FFA in mouse than niacin, and no vasodilation in a mouse model. Compound **1q** demonstrated equal affinity to GPR109A as niacin.

Niacin (nicotinic acid) is a drug that reduces coronary heart disease either administered alone or in combination with statins.[1,2] Well known for its effects in elevating high density lipoprotein cholesterol (HDL-C), niacin also plays a beneficial role in other lipid profiles by reducing total plasma cholesterol, triglycerides (TG), very low density lipoprotein cholesterol (VLDL-C), low density lipoprotein cholesterol (LDL-C), and lipoprotein a (Lp(a)).[3a] The most common adverse effect of niacin is severe cutaneous flushing which limits patient compliance.

Recently PUMA-G (protein-upregulated in macrophages by interferon-γ), the G-protein coupled niacin receptor in mouse, was discovered.[4] Subsequently HM74A and HM74, also referred as GPR109A and GPR109B, two related human homologs of PUMA-G, were found to bind niacin with high and low affinity, respectively.[5] GPR109A is expressed mainly in adipose tissue, spleen, and myeloid lineage leukocytes. Despite its long history in man, the precise mechanism of niacin's therapeutic effect remains unclear.[6] It has been well accepted that niacin binds GPR109A in adipose tissue. This leads to a decrease of cAMP levels through the inhibition of the adenylyl cyclase. Reduced levels of cAMP result in the suppression of protein kinase A (PKA), leading to less phosphorylation of hormone sensitive lipase (HSL). As phosphorylation of HSL is required for its function, the HSL-mediated lipolysis of triglycerides from adipose tissue is suppressed leading to reduction in free fatty acids (FFA). Suppression of lipolysis commonly serves as the basis for an in vivo PD assay and can signify receptor engagement.[7] The connection between FFA and HDL-C, and the pathway to regression of atherosclerosis is speculative.[3b]

Given the well-established beneficial effects of niacin in man, we envisioned developing niacin receptor agonists to treat dyslipidemias, atherosclerosis, and metabolic syndrome while minimizing the adverse flushing effects that are associated with niacin therapy.

Several classes of selective agonists for GPR109A have been identified. We,[8] GlaxoSmithKline (GSK),[9] and Roche[10] have disclosed anthranilic acid derivatives in addition to xanthine derivatives (GSK),[11] pyrazolotetrazoles (Merck, Arena),[12] and thiophenes (Roche[13] and Incyte[14]). Partial agonists of the niacin receptor were also reported by the University of Heidelberg group.[15]

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Targeting GPR109A, our design of niacin receptor agonists featured a urea moiety that links acylated anthranilic acid and a heterocycle directly attached to a terminal aromatic ring (Fig. 1). The structure activity relationship (SAR) studies herein were focused on the terminal ring (Table 1) and piperazine modification (Table 2). The affinities were determined by competitive binding of compounds with the receptor against [^{3}H]-niacin. Functional activities of compounds and response levels relative to niacin were measured by [^{35}S]GTPγS assay. In this assay, the full response of niacin was defined as 100%, which was used as a reference for other agonists.

We first probed the terminal aromatic ring of the pharmacophore in Figure 1 (Table 1). The quinoxaline ring of **1a** played an important role for its activity. The absence, or the different location of one or two nitrogen atoms of **1a**, led to a significant loss of activity (**1b–d**). Other bicyclic or monocyclic systems, including quinazoline, benzoisothiazole, benzoxazole, isoquinoline, quinoline, pyrimidine, pyridine, and naphthylene analogs, gave significantly less activities than **1a**.

Identifying the quinoxaline as an optimized terminal ring, we then studied the SAR of the quinoxaline substitution. The position of a hydroxyl group greatly influenced the affinity as revealed by analogs **1l**, **1n**, and **1q**. Specifically, the hydroxyl group in **1q** consistently offered a 4-fold increase of activity with respect to **1a**. To understand the plausible interactions between the agonist and the receptor, we employed a homology model of hGPR109A which was constructed based on the X-ray crystal structure of bovine rhodopsin (PDB entry: 1L9H). In our docking model of **1q** with its putative binding pocket in hGPR109A, the hydroxyl group was placed in a polar pocket consisting of Asn171, Ser179, and His259. A hydrogen bond was thus conceivable between the hydroxyl group and the side chain of residue Asn171 (Fig. 2b). Placing the hydroxyl group at other positions of the quinoxaline ring did not offer such putative hydrogen bonding interactions, which might explain the poorer binding affinities of **1l** and **1n**. A weak hydrogen bond donor such as an amino group present in **1o** did not improve the activity over the parent compound **1a**. Capping the hydrogen bond donor, as with hydrogen bond acceptor **1p**, greatly reduced activity.

Table 2 summarizes the efforts to modify the piperazine ring. First, the introduction of a methyl group (**1r**, **1s**), or additional conformational constraints (**1t**, **1u**) to the piperazine ring, did not improve activity. The replacement of piperazine with homopiperazine had no substantial effect (**1v**), and the replacement with piperidine

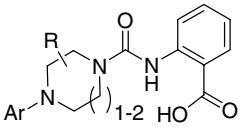


Figure 1. Pharmacophore of urea anthranilides.

Table 1. In vitro SAR in hGPR109A transfected cells[a]

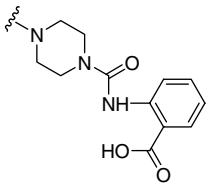
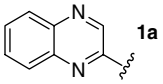
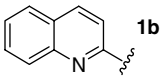
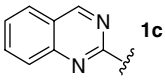
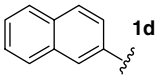
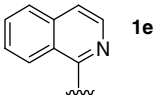
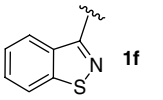
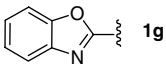
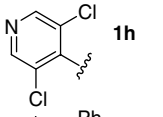
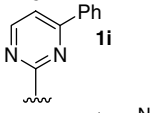
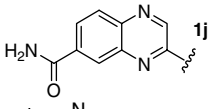
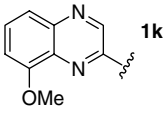
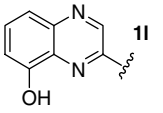
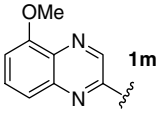
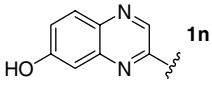
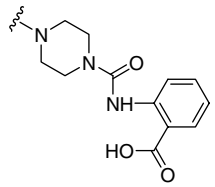
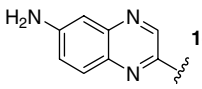
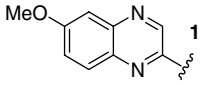
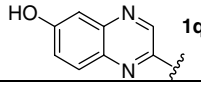
Compound	^{3}H-niacin binding IC_{50}, μM[a]	hGTPγS EC_{50}, μM[a]	(%) at 100 μM
			
Niacin	0.14	1.0	100
 **1a**	0.55	1.6	94
 **1b**	2.5	5.9	77
 **1c**	—	7.8	87
 **1d**	76	13	54
 **1e**	2.2	9.0	85
 **1f**	1.2	5.0	36
 **1g**	5.7	22	79
 **1h**	20	—	—
 **1i**	2.1	13	84
 **1j**	—	7.9	49
 **1k**	—	11	33
 **1l**	0.87	11	77
 **1m**	0.29	3.3	85
 **1n**	0.79	5.8	49

Table 1 (*continued*)

Compound	^{3}H-niacin binding IC_{50}, μM[a]	hGTPγS EC_{50}, μM[a]	(%) at 100 μM
			
 **1o**	6.7	17	74
 **1p**	—	—	37
 **1q**	0.14	0.47	83

[a] Values are based on one or two experiments, each in triplicate, and within 20% deviation upon repetation. Missing data were due to inconclusive reading as a result of the curve shape.

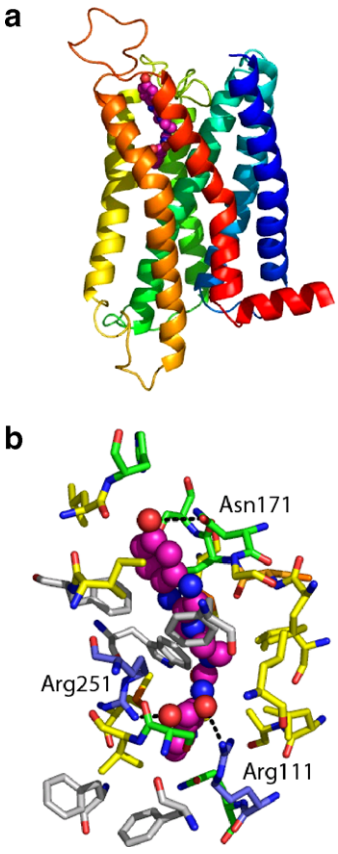
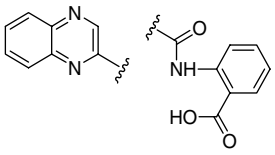
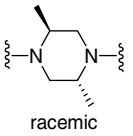
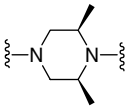
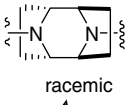
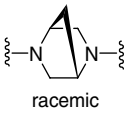
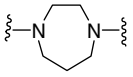
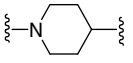
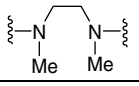


Figure 2. Modeling view of **1q** (purple spheres) in the putative binding pocket of GPR109A. (a) A full view of seven transmembrane helix shown in ribbon representation in rainbow colors. (b) A closer look of compound **1q** inside the binding pocket. Residues located within 4 Å of compound **1q** are shown in stick form with the carbons in yellow for hydrophobic residues, in gray for aromatic residues, in green for polar residues, in blue for Arg, and in orange for Cys. The dashed lines illustrate atom pairs within hydrogen bond distance. The pictures were prepared by PyMOL (Delano Scientific LLC, Sounth San Franscisco, CA).

Table 2. In vitro SAR in hGPR109A transfected cells[a]

Compound	^{3}H-niacin binding IC_{50}, μM[a]	hGTPγS EC_{50}, μM[a]	(%) at 100 μM
			
 **1r** racemic	0.65	4.4	77
 **1s**	1.1	9.1	72
 **1t** racemic	—	—	54
 **1u** racemic	—	—	0
 **1v**	0.8	4.6	70
 **1w**	1.6	6.3	55
 **1x**	—	—	35

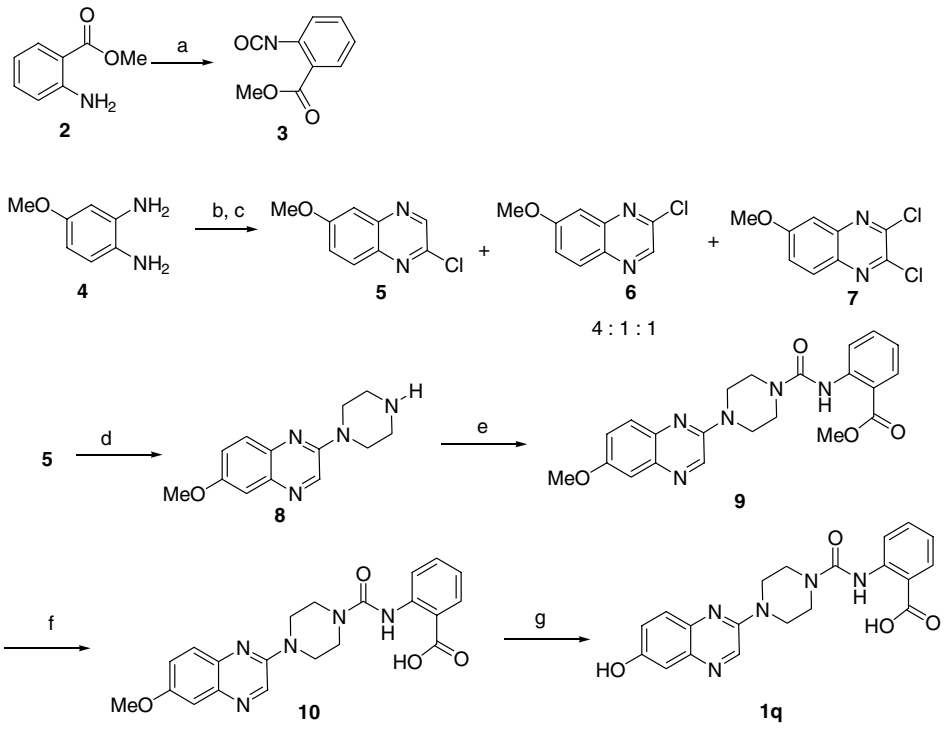
[a] Values are based on one or two experiments, each in triplicate, and within 20% deviation upon repetition. Missing data were due to inconclusive reading as a result of the curve shape.

(**1w**) or ethylenediamine (**1x**) led to a pronounced decrease of activity.

A representative synthesis of urea analogs is illustrated in Scheme 1. The condensation of ethyl glyoxylate and diaminobenzene **4** followed by chlorination of the hydroxyl quinoxaline intermediate afforded chloroquinoxaline **5**, which could be separated from **6** and **7** by flash chromatography.[16] The subsequent C–N bond formation between piperazine and quinoxaline **5** was a microwave-assisted aromatic nucleophilic substitution reaction. It should be noted that the reaction using microwave was significantly faster than conventional heating (30 min, 150–170 °C vs. reflux for 16 h).[17] The next key step in the syntheses of most analogs was the urea formation between a secondary amine and isocyanate **3**. Finally, hydrolysis and demethylation gave the desired analog **1q**.

The pharmacokinetic (PK) profile of compound **1a** in mouse is shown in Table 3. Good bioavailability, half-life and oral exposure were observed.

We were interested in studying the anti-lipolytic and flushing effects of **1a** as directly compared to niacin. Gratifyingly, **1a** exhibited excellent in vivo pharmacody-



Scheme 1. Reagents and conditions: (a) *p*-nitrophenylchloroformate, diisopropylethyl amine, CH_2Cl_2, THF, 0 °C to rt, overnight; (b) $HCOCO_2Et$, EtOH, H_2O, reflux 2 h; (c) $POCl_3$, reflux, 1 h; (d) piperazine, butanol, microwave, 150–170 °C, 30 min; (e) **3**, THF, CH_2Cl_2, rt, 3 h; (f) LiOH (1 N), THF/MeOH/H_2O (3:1:1), rt, 1 h; (g) dodecanethiol, NaH, DMF, 120 °C, 6 h.[18]

Table 3. Mouse PK for **1a**[a]

Compound	F%	Cl (mL/min/kg)	Vd_{ss} (L/kg)	C_{max} (μM)	$T_{1/2}$ (h)	$AUCN_{po}$ (μM h kg/mg)
1a	65	25	5.1	0.48	4.4	1.2

[a] Formulation: 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.

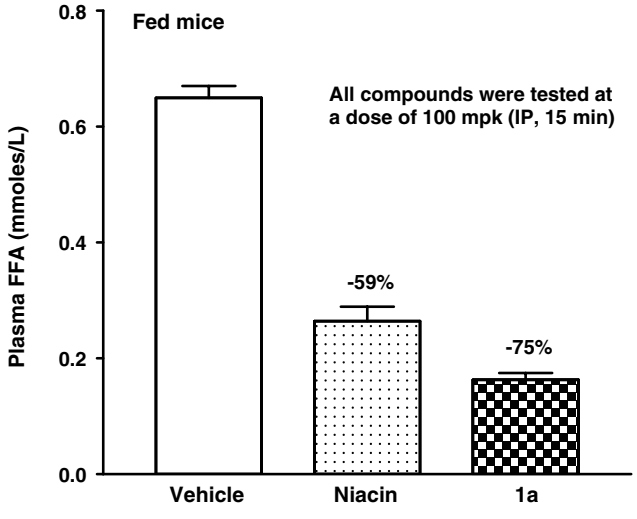


Figure 3. Effect of **1a** and niacin on FFA suppression in mice (mpk = mg/kg).

namics (PD) on free fatty acid (FFA) reduction in mice (Fig. 3). In this model, fed male C57BL/6 mice were used (n = 8). The mice were treated with vehicle (5% β-cyclodextrin) and niacin as well as **1a** at 100 mpk via intraperitoneal dosing (ip) for 15 min. We were pleased to find that **1a** (GTPγS in PUMA-G, EC_{50} = 11 μM), at a dose of 100 mpk, decreased plasma FFA by 75%, significantly more than the maximal suppression (59%) typically elicited by niacin in this model (GTPγS in PUMA-G, EC_{50} = 0.27 μM for niacin).

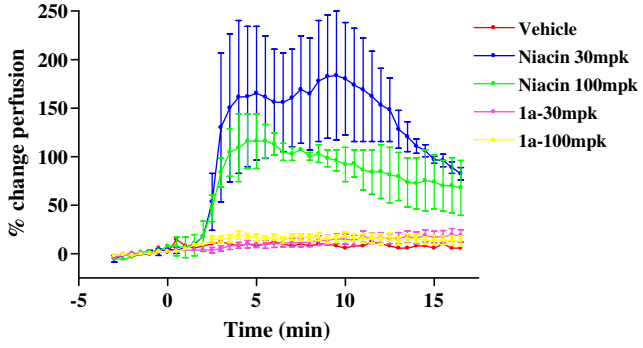


Figure 4. Effect of **1a** and niacin on mouse vasodilation (mpk = mg/kg).

Vasodilation (flushing) has been a major side effect of niacin therapy. To test whether our compounds could lead to vasodilation, we established a mouse model

where we measured the extent of flushing by the change in blood perfusion of the mouse ear via laser Doppler. In this study, **1a** did not induce any flushing at either 30 mg/kg or 100 mg/kg (ip), in contrast to the intense flushing caused by the niacin control at these doses (Fig. 4). As a balanced compound regarding its activity and PK, **1a** was selected for study in the mouse vasodilation model. It is unclear to us whether the lack of vasodilation effect of **1a** is a class effect until more compounds have been tested.

In conclusion, we have identified a novel urea class of anthranilic acid derivatives which bound and activated the niacin receptor GPR109A. The SAR established the importance of the piperazine and quinoxaline moieties. Compound **1q** bearing a terminal hydroxyl group matched the potency of niacin indicating the added favorable interaction of the hydroxyl group with the receptor. Compound **1a** had a good PK profile and better efficacy in reducing FFA devoid of any flushing at 100 mg/kg in the mouse model. These data suggested the possibility of developing niacin-like compounds with reduced or no flushing.

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18. The corresponding experimental procedures to Scheme 1 are shown below: To a suspension of diaminomethoxybenzene bis-HCl salt (2.1 g, 10 mmol) in 25 mL of water and 10 mL of ethanol was added sodium bicarbonate (1.7 g, 20 mmol). To the resulting mixture was added ethyl glyoxylate (2.04 g, 2 mL, 11 mmol) and the mixture was then under reflux for 2 h. The mixture was cooled and filtered. The collected solid was dissolved in DMSO and purified by RP-HPLC to afford a mixture of alcohols (1.76 g). To this mixture of alcohols was added 40 mL of $POCl_3$.The resulting mixture was under reflux for 1 h. The mixture was concentrated by distilling off the solvent. The residue was poured into ice and basified with a saturated sodium carbonate solution. The mixture was then extracted with ethyl acetate. The organic layer was washed with brine, dried with sodium sulfate, and concentrated in vacuo. The residue was purified by flash chromatography eluting with 5% of ethyl acetate in hexanes to obtain 5 (220 mg, 1.13 mmol, 11% over 2 steps). A mixture of 5 (220 mg, 1.13 mmol), piperazine (440 mg, 5.1 mmol), and 4 mL of butanol was heated at 150 °C in microwave for 15 min, then at 170 °C in microwave for additional 15 min. The mixture was purified by RP-HPLC to give 8 (300 mg, 0.84 mmol, 74%) as a yellow oil. A solution of 8 (300 mg, 0.84 mmol) in 4 mL of dichloromethane was treated with a stock solution of isocyanate **3** (0.2 M, 2 mL, 0.40 mmol). After 1 h, the solution was concentrated and purified by RP-HPLC to afford **9** (119 mg, 0.28 mmol, 34%) as a yellow oil. To **9** (42 mg, 0.1 mmol) in 4 mL of THF/water/methanol (3:1:1) was added 1 mL of LiOH (1 N) at rt. The mixture was stirred at rt for 1 h, and concentrated. To this

mixture was added HCl (1 N) slowly until pH 3. The mixture was extracted with 30% isopropanol in chloroform. The organic layer was dried with sodium sulfate and concentrated to give **10** (38 mg, 0.093 mmol, 93%) as a yellow solid. ^{1}H NMR (acetone-d_6, 500 MHz) δ 8.80 (1H, m), 8.42 (1H, d), 7.96 (1H, d), 7.58 (2H, m), 7.29 (2H, d), 7.03 (1H, t), 3.86 (3H, s), 3.81 (4H, m), 3.65 (4H, m); LCMS *m*/*z*: 408 (M+1). The isocyanate stock solution was prepared by mixing methyl anthranilate **2**(907 mg, 6 mmol), diisopropylethyl a mine (3.1 g, 4.2 mL, 24 mmol), and *p*-nitrophenylchloroformate (1.21 g, 6 mmol) in 30 mL of dichloromethane at 0 °C, and the subsequent stirring at room temperature overnight. To sodium hydride (10 mg, 0.25 mmol, 60%) in 3 mL of DMF at 0 °C was added dodecanethiol (25 mg, 0.25 mmol). The mixture was warmed to room temperature and stirred for 15 min. To this mixture was added a solution of 10 (10 mg, 0.025 mmol) in 3 mL of DMF. The mixture was heated at 120 °C for 6 h. The mixture was filtered and purified by RP-HPLC to afford **1q**(2 mg, 0.005 mmol, 20%) as a brown oil. 1H NMR (acetone-d_6 500 MHz)δ 8.80 (1H, m), 8.66 (1H, d), 8.00 (1H, s), 7.59 (2H, m), 7.28 (2H, d), 7.05 (1H, t), 3.90 (m, 4H), 3.78 (m, 4H); LCMS *m*/*z*: 394 (M+1).