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Discovery of pyrazolopyrimidines as the first class of allosteric agonists for the high affinity nicotinic acid receptor GPR109A

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ABSTRACT

Pyrazolopyrimidines were discovered as the first class of allosteric agonists for the high affinity nicotinic acid receptor GPR109A. In addition to its intrinsic activity, compound **9n** significantly enhances nicotinic acid binding to the receptor, thereby potentiating the functional efficacy of nicotinic acid.

Nicotinic acid is a group B vitamin that in high doses reduces morbidity and mortality from cardiovascular disease.[1,2] These benefits presumably arise via alterations in the serum lipid profile including elevated high density lipoprotein cholesterol (HDL-C), and reduced total plasma cholesterol, very low-density lipoprotein cholesterol (VLDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride (TG), and lipoprotein a (Lp(a)).[3] However, the adverse effect of cutaneous vasodilation (flushing) often limits patient compliance to therapy.

GPR109A (also called PUMA-G in mice; protein-upregulated in macrophages by interferon γ) is a G_i-protein coupled receptor for nicotinic acid that is highly expressed in adipocytes and myeloid-lineage immune cells.[4,5] Binding of agonists to GPR109A induces reduction of intracellular cAMP levels which, in adipocytes, is thought to impair triglyceride lipolysis and thus reduce levels of circulating free fatty acid (FFA).[6] Decreased plasma FFA is thought to be responsible for the other observed changes in serum lipids (e.g., reduction in LDL-C and increase of HDL-C).[5] GPR109A expressed in epidermal Langerhans' cells has also been shown to mediate cutaneous flushing.[6]

G-protein-coupled receptor (GPCR) ligands can be classified as either orthosteric or allosteric modulators.[7] Orthosteric and endogenous ligands bind to the same site, which is topologically distinct from the allosteric site. In general, the binding domains of different orthosteric ligands may not exactly superimpose, but do overlap to an extent that two orthosteric ligands cannot bind simultaneously. Therefore, orthosteric ligands are competitive in equilibrium binding assays, and the binding of one orthosteric ligand does not change the dissociation kinetics of another. In contrast, with little or no overlap of their binding sites, an allosteric modulator and an orthosteric ligand can bind to the same receptor simultaneously. As a result, an allosteric modulator may modulate the affinity of an orthosteric ligand, either positively or negatively.[8]

Various orthosteric GPR109A agonists have been reported by GlaxoSmithKline,[9] Arena,[10] Roche,[11] Schering-Plough,[12] Incyte,[13] A.P. Ijzerman's group at Leiden University,[14] and ourselves,[15] respectively. In contrast, to the best of our knowledge, allosteric modulators of the nicotinic acid receptor have not been documented. We speculated that a positive allosteric modulator might improve the potency of β-hydroxybutyrate, the proposed endogenous ligand for GPR109A,[16] thereby mimicking, and possibly improving on, nicotinic acid's therapeutic effects. It would also be intriguing to examine whether allosteric modulation of the receptor would induce the adverse effects of nicotinic acid treatment, such as cutaneous flushing. Herein, we report our discovery of pyrazolopyrimidines as the first class of allosteric agonists for the nicotinic acid receptor GPR109A. In addition, we present the in vitro characteristics of a representative analog **9n**.

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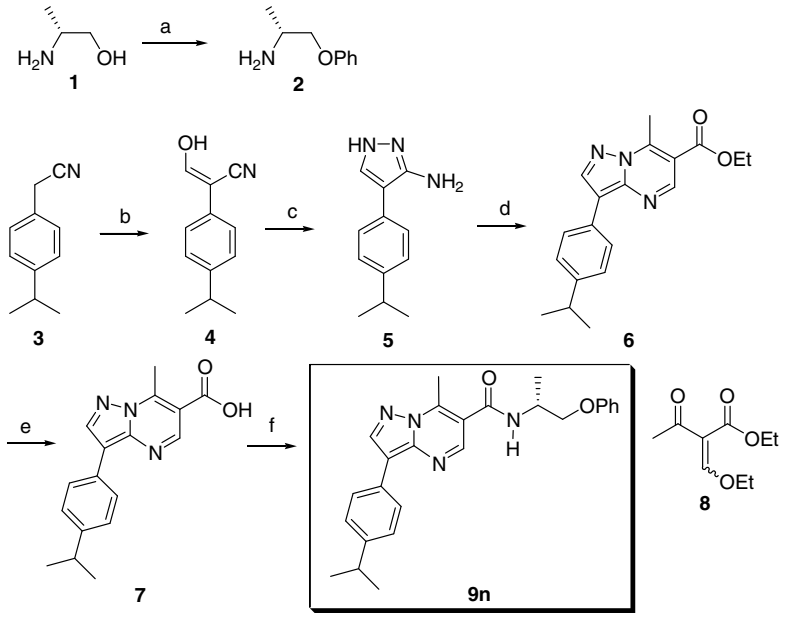


Scheme 1. Reagents and conditions: (a) PhI, CuI (0.05 equiv), Cs_2CO_3, pentanitrile, 125 °C, 16 h, 50%; (b) NaH, ethyl formate, *tert*-amyl alcohol, toluene, 75 °C, 12 h, 70%; (c) hydrazine, acetic acid, EtOH, reflux, 16 h, 98%; (d) **8**, ethanol, reflux, 1 h, 85%; (e) LiOH, THF/MeOH/water (3:1:1), rt, 2 h, 99%; (f) **2**, HOBT, EDCI, CH_2Cl_2, rt, 12 h, 77%.

The synthetic route to pyrazolopyrimidine **9n** as a representative example is summarized in Scheme 1. Starting with 4-isopropylphenylacetonitrile **3**, an α-formylation followed by condensation with hydrazine afforded aminopyrazole **5**. The ensuing reaction of **5** with α-ethoxyvinylidene ethyl acetoacetate **8** generated pyrazolopyrimidine **6**.[17] The subsequent hydrolysis of **6** led to acid **7**, which was then coupled with 1-phenoxy-2-propylamine to give **9n**. The requisite 1-phenoxy-2-propylamine **2** was derived from a CuI-catalyzed chemoselective *O*-arylation.[18] This sequence was adopted to prepare most pyrazolopyrimidine analogs in this letter.

The activity of compounds against GPR109A was measured both by ^{3}H-nicotinic acid competition binding assays and agonist-induced exchange of GDP for ^{35}S-GTPγS on cell membranes prepared from CHO cells overexpressing human GPR109A.[16] The maximum response to nicotinic acid in the hGTPγS assay was defined as 100%, based on which we measured the maximum response level of our compounds. The SAR of the pyrazolopyrimidine class regarding equilibrium binding in the ^{3}H-nicotinic acid and efficacy in the hGTPγS assays is summarized in Table 1. The pyrazolopyrimidine core of these analogs appeared to be crucial for activity against GPR109A since modification of the core rendered compounds inactive (data not shown). Extensive SAR studies of the pyrazolopyrimidine series established the importance of the 1,2-aminoalkoxy functionality. For example, **9b** was active, whereas its carbon homolog **9c** was not. The substitution pattern of the phenoxy group in **9b** clearly influenced activity. The introduction of *para*- or *meta*-methoxy group improved the EC_{50} values in the hGTPγS assay, but with lower maximum response (41% and 55%, respectively). Similar observations were also made with chloro-, fluoro-, and methyl-substituted analogs (**9h–l**). The substitution of methyl group of the 1-methoxy-2-propylamino moiety with phenyl or benzyl groups (**9p–q**) improved the EC_{50} values in the hGTPγS assay by twofold and gave a similar maximum response level as **9a**.

By combining the structural features of the α-methyl group (with respect to the amide nitrogen atom) in **9a** and the phenoxy group in **9b**, we were able to obtain compound **9m** with improved activity as a racemate in the hGTPγS assay (EC_{50} = 0.7 μM (74%)). Further elaboration using enantiomerically pure 1-phenoxy-2-propylamine gave two enantiomers with considerably different affinities for GPR109A. The *R*-enantiomer **9n** was 19-fold more active in the binding assay, and 17-fold more active in the hGTPγS assay than its *S* enantiomer **9o** (Table 1). As shown in the hGTPγS assay, **9n** gave approximately 75% of nicotinic acid's maximum response (Fig. 1).

In addition to their functional activation of GPR109A, compounds of the pyrazolopyrimidine class also enhanced the binding of nicotinic acid to the receptor. For instance, **9n** increased the binding of ^{3}H-nicotinic acid to the receptor (black curve) in a concentration-dependent manner, whereas unlabeled nicotinic acid displaced ^{3}H-nicotinic acid from the receptor (red curve) (Fig. 2). We interpret enhanced ^{3}H-nicotinic acid binding as indicative of positive allosteric modulation of the receptor, which taken together with the agonist properties of **9n** designates this compound as an allosteric agonist of GPR109A. The mechanism by which **9n** enhances ^{3}H-nicotinic acid binding will be described elsewhere.

Nicotinic acid has been shown to inhibit forskolin-stimulated cAMP accumulation in CHO-hGPR109A cells.[19] A prediction of enhanced nicotinic acid binding to GPR109A in the presence of **9n** would be that the dose–response curve for nicotinic acid in this assay might be left-shifted. Like nicotinic acid, **9n** alone also induced the reduction of cAMP levels but with less magnitude (the bottom curve in Fig. 3). Furthermore, **9n** potentiated the nicotinic acid-induced cAMP reduction in a dose-dependent fashion. As shown in Figure 3, the effect of nicotinic acid on the cAMP accumulation was significantly left-shifted with increasing concentration of **9n** displaying a sensitization of nicotinic acid activity.

In summary, we have discovered a pyrazolopyrimidine class of allosteric agonists of the nicotinic acid receptor GPR109A. To our knowledge, this is the first report of allosteric agonists of this receptor. The observation of GPR109A allosteric agonism

Table 1
In vitro SAR for pyrazolopyrimidine analogs

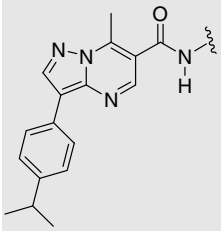
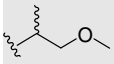
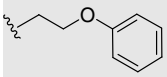
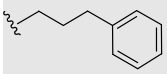
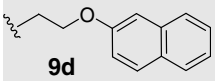
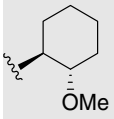
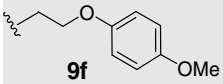
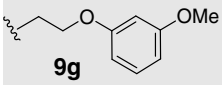
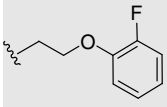
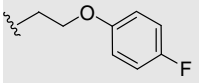
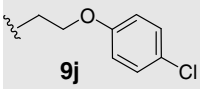
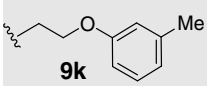
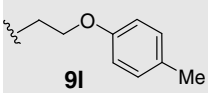
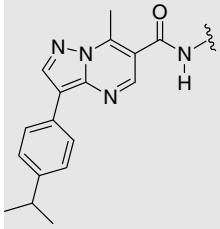
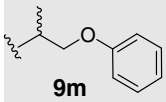
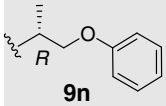
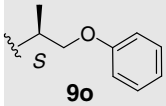
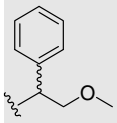
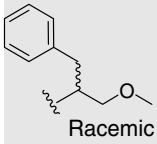
Compound	^{3}H-nicotinic acid binding IC_{50}, μM[a]	hGTPγS EC_{50}, μM[a]	(%) at 100 μM
			
Nicotinic acid	0.15	1.0	100
 **9a** Racemate	3.4	3.2	43
 **9b**	1.0	6.0	76
 **9c**	na	na	0
 **9d**	—	2.3	21
 **9e**	na	na	0
 **9f**	—	1.3	41
 **9g**	—	0.93	55
 **9h**	—	1.8	40
 **9i**	—	0.46	24
 **9j**	—	2.3	32
 **9k**	—	1.3	35
 **9l**	—	2.1	22

Table 1 (*continued*)

Compound	^{3}H-nicotinic acid binding IC_{50}, μM[a]	hGTPγS EC_{50}, μM[a]	(%) at 100 μM
			
 **9m**	1.3	0.7	74
 **9n**	0.17	0.12	75
 **9o**	3.3	3	42
 **9p** Racemic	—	1.9	37
 **9q** Racemic	—	1.4	31

[a] Values were means of at least three experiments, average standard deviation is about 20% (na, not active).

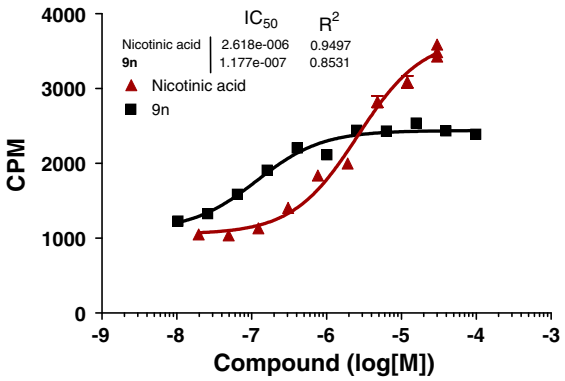


Figure 1. GTPγS curve of **9n** ($n = 25$).

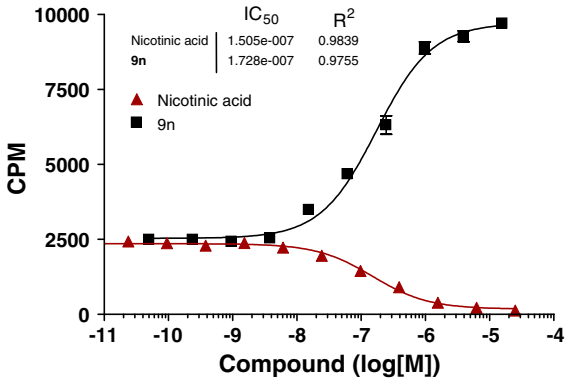


Figure 2. Binding curve of **9n** and nicotinic acid ($n = 8$).

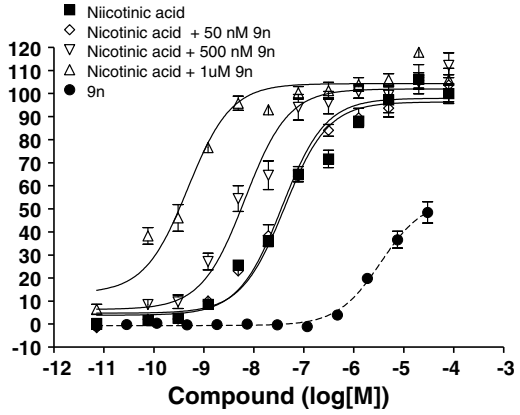


Figure 3. Effects of **9n** on cAMP accumulation in CHO-hGPR109A cells.

as reflected by these analogs may offer interesting implications, worthy of further pharmacological investigation.

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