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The discovery of high affinity agonists of GPR109a with reduced serum shift and improved ADME properties

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ABSTRACT

Amino-anthranilic acid derivatives have been identified as a new class of low serum shifted, high affinity full agonists of the human orphan G-protein-coupled receptor GPR109a with improved ADME properties.

Nicotinic acid (niacin) has been a leading treatment for dyslipidemia and for the prevention of atherosclerosis for over 40 years.[1] Long term clinical studies have demonstrated niacin's ability to reduce the incidence of mortality from coronary heart disease.[2] In spite of niacin's clinical significance, patients treated with niacin show low compliance of use due to an intense flushing side effect.[3] In response to the maladies of niacin therapy, a number of drug discovery programs have focused on the development of a 'flush-free' niacin-like therapy. Despite considerable effort in the field, the absence of a niacin-related target and/or mechanism of action, has limited such investigations.

Recently, a high affinity G-protein coupled receptor, GPR109a, has been identified for niacin.[4] Mechanistic studies have suggested that niacin may exert its therapeutic effect through the activation of GPR109a located on adipocytes.[5] Experiments have shown that niacin activation of GPR109a results in a reduction of intracellular cAMP. It is believed that this down regulation of cAMP in adipocytes, triggers a cascade of events that results in the lipid altering affects associated with niacin treatment (i.e., raise in HDL, lower triglycerides and LDL).

In control experiments, GPR109a receptor knockout mice, when treated with niacin therapy, failed to show the characteristic reduction in plasma FFA that is observed upon niacin treatment in wild type mice. Furthermore, niacin receptor knockout mice, when treated with niacin therapy, failed to show a niacin-induced flush. As a result, it is concluded that the niacin-associated flush and anti-lipolytic effects are both mediated through the niacin receptor.[5a]

Following the identification of the high affinity niacin receptor (GPR109a), our group initiated a drug discovery program focused on the development of a 'flush-free' agonist. A high-throughput screen of our compound collection,[6] followed by a hit to lead effort, identified anthranilic acid derivative **1** as a promising lead structure.[7] Despite an excellent in vitro profile, the modest ADME properties and the high serum-potency-shift (>10,000-fold) of compound **1** highlighted the need for further structure based improvements.

Our initial medchem effort focused on strategies to alleviate the large serum shift effect observed for the class. Previous work in the group revealed a tight binding environment around the anthranilic acid moiety and the terminal phenol group.[7] With this in mind, our strategy involved the localization of an amino moiety on the carbon chain connecting the head-anthranilic acid group and the biphenyl tail region. As illustrated in Table 1, the incorporation of an amino group into the architecture of **1** had a significant effect on the serum shift. Analysis of **2** in our in vitro binding assay, in the presence of 4% human serum, revealed a 20-fold reduction in serum-potency-shift, when compared to the des-amino derivative **1**. Based on this result, we were motivated to look at other members of this lead class. In this regard, introduction of the amino group into the high affinity thiazole derivative **3** and the

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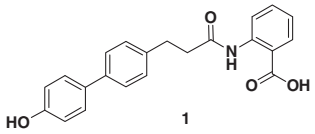
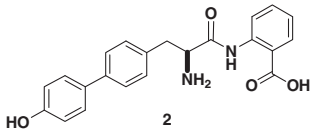
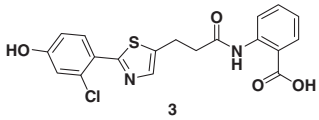
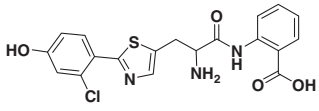
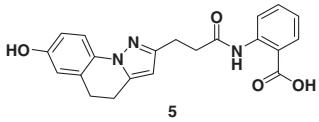
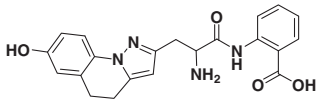
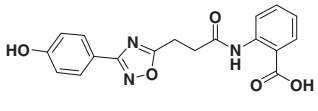
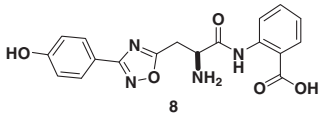
Table 1
The effect of amine substitution on serum shift[a]

Compound	Serum shift	h-^{3}H-niacin[b] IC_{50} (μM)	hGTPγS[c] EC_{50} (μM)
 1	>10,000-fold hSS	0.016	0.06

Species[d]	Clp (mL/min/kg)	$t_{½}$[e] (h)	AUCN po (μM.h.kg/mg)	F (%)[e]
Mouse	8.7	2.4	2.0	35

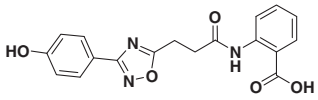
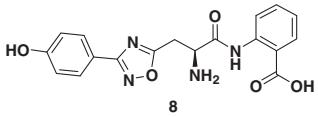
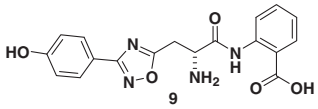
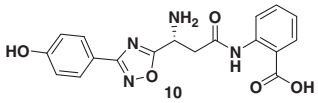
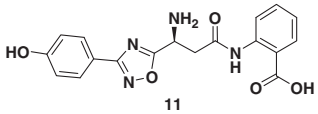
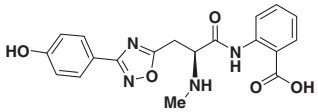
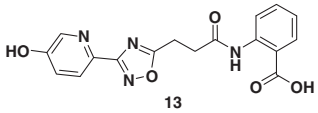
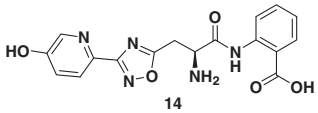
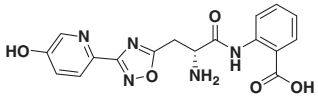
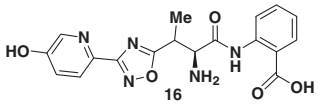
Compound	Serum shift	h-^{3}H-niacin[b] IC_{50} (μM)	hGTPγS[c] EC_{50} (μM)
 2	500-fold hSS	0.15	3.2
 3	300-fold hSS	0.001	0.006
 4	30-fold hSS	0.10	0.93
 5	500-fold hSS	0.013	0.065
 6	70-fold hSS	0.120	1.0
 7	40-fold hSS	0.010	0.120
 8	7-fold hSS	0.042	0.290

[a] Values are an average of two or greater individual assays; serum shift refers to the ratio of the binding affinity as determined by [^{3}H]-Nicotinic acid competition in the presence and absence of 4% human serum.
[b] [^{3}H]-Nicotinic acid binding competition assay.
[c] Membrane binding of compounds versus [γ-35S] GTP in active recombinant CHO-K1cells stably expressing the niacin receptor.
[d] C57BL/6-mice.
[e] Dose: 1 mg/kg iv; 2 mg/kg po; $t_{½}$ = plasma half-life (0–8 h).

tricyclic-pyrazole derivative **5** showed a similar 10-fold reduction in serum shift. However, despite the amino group's favorable effect on serum shift, the reduced affinity for serum proteins was accompanied by a 10-fold loss in potency on the receptor.

While this trend was consistent amongst most members of the class, oxadiazole **7** was less affected by the change. In this case, amino-oxadiazole **8** showed a characteristic 5-fold reduction in serum shift, relative to the parent, and comparable in vitro activity.

Table 2
High affinity, low serum shifted, full agonists of GPR109a[a]

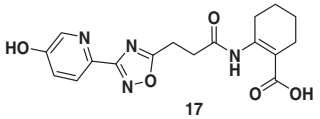
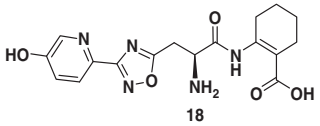
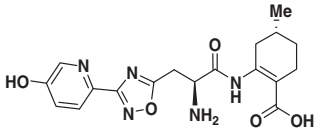
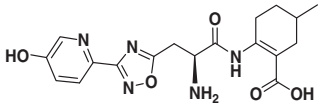
Compound	Serum shift	h-^{3}H-niacin[b] IC_{50} (μM)	hGTPγS[c] EC_{50} (μM)
 7	40-fold hSS	0.010	0.120
 8	7-fold hSS	0.042	0.290
 9	9-fold hSS	0.041	0.89
 10	8-fold hSS	0.108	2.96
 11	6-fold hSS	0.158	3.42
 12	5-fold hSS	0.115	1.5
 13	30-fold hSS	0.006	0.027
 14	5-fold hSS	0.001	<0.052
 15	20-fold hSS	0.004	0.036
 16	6-fold hSS	0.10	0.5

[a] Values are an average of two or greater individual assays; serum shift refers to the ratio of the binding affinity as determined by [^{3}H]-Nicotinic acid competition in the presence and absence of 4% human serum.
[b] [^{3}H]-Nicotinic acid binding competition assay.
[c] Membrane binding of compounds versus [γ-^{35}S] GTP in active recombinant CHO-K1cells stably expressing the niacin receptor.

SAR around amino-derivative **8** revealed a number of interesting trends related to the serum shift and in vitro activity (Table 2). A comparison of derivative **8**, and its epimer **9**, showed that the stereochemistry had little effect on the serum shift and the intrinsic affinity for the receptor.

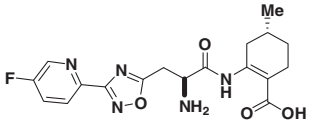
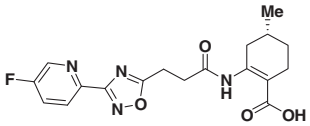
In contrast, re-positioning of the amino-group adjacent to the oxadiazole moiety, as in derivative **10**, resulted in a significant loss of activity in both our binding (h-^{3}H-niacin) and functional assay (h-GTPγS). Further substitution of the amine, as in methyl-amine **12**, demonstrated a modest reduction in serum shift along with a concomitant loss of in vitro potency. The conversion of the phenol moiety to a hydroxy-pyridine group improved the in vitro profile of the amino-series. In this case, amino-hydroxypyridine **14**

Table 3
THA based agonists of GPR109a[a]

Compound	Serum shift	h-^{3}H-niacin[b] IC_{50} (μM)	hGTPγS[c] EC_{50} (μM)
 **17**	40-fold hSS	0.003	0.024
 **18**	6-fold hSS	0.005	0.039
 **19**	8-fold hSS	0.011	0.085
 **20 (mixture of diastereomers)**	40-fold hSS	0.020	0.721

[a] Values are an average of two or greater individual assays; serum shift refers to the ratio of the binding affinity as determined by [^{3}H]-Nicotinic acid competition in the presence and absence of 4% human serum.
[b] [^{3}H]-Nicotinic acid binding competition assay.
[c] Membrane binding of compounds versus [γ-^{35}S] GTP in active recombinant CHO-K1cells stably expressing the niacin receptor.

Table 5
Amino-fluoropyridine agonists of GPR109a[a]

Compound	Serum shift	h-^{3}H-niacin[b] IC_{50} (μM)	hGTPγS[c] EC_{50} (μM)
 **21**	100-fold hSS	0.012	0.214
 **22**	1000-fold hSS	0.021	0.410

Cmpd	species[d]	Clp (mL/min/kg)	$t_{½}$[g] (h)	AUCN po (μM.h.kg/mg)	F (%)[f]
21	Mouse	1.7	5.6	26.2	100
	Rat	0.7	5.5	49.3	81
22	Mouse	3.3	4.7	15.2	54

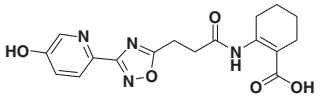
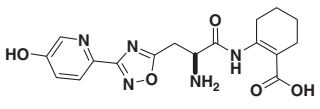
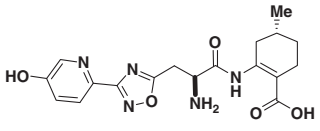
[e] Rat Sprague–Dawley.
[a] Values are an average of two or greater individual assays; serum shift refers to the ratio of the binding affinity as determined by [^{3}H]-Nicotinic acid competition in the presence and absence of 4% human serum.
[b] [^{3}H]-Nicotinic acid binding competition assay.
[c] Membrane binding of compounds versus [γ-^{35}S] GTP in active recombinant CHO-K1cells stably expressing the niacin receptor.
[d] C57BL/6-mice.
[f] Dose: 1 mg/kg iv; 2 mg/kg po.
[g] $t_{½}$ = plasma half-life (0–8 h).

demonstrated excellent affinity and functional activity with a 6-fold reduction in serum shift, when compared to the des-amino analog **13**. Additional stereochemical modifications, as in stereo-isomer **15**, showed improved in vitro potency, relative to phenol **9**, but suffered from an increased affinity for serum proteins. The introduction of a methyl group, as a means of gearing the conformation of the molecule, as in derivative **16**, resulted in a significant loss in affinity for the receptor.

Previous work in the group, identified the tetrahydroanthranilic acid (THA) moiety as an advantageous surrogate to the anthranilic acid group.[8] Replacement of the anthranilic acid piece with the THA moiety in the amino series, as in compound **18**, (Table 3). Methyl substitution on both the cyclohexene moiety, as in **19** and **20**, was also well tolerated.

With a promising in vitro profile and a reduced serum shift, we proceeded to investigate the ADME properties of this amino-THA series. As illustrated in Table 4, the amino-THA class had favorable clearance and half-lives, similar to the des-amino derivative **17**. However, the generally low bioavailability prohibited further development of this series and motivated additional structural modifications. In this regard, we had data to suggest that the terminal hydroxy-pyridine moiety was negatively effecting the oral exposure of the compounds. As a result, we focused our

Table 4
Mouse and rat pharmacokinetic studies of GPR109a agonists[a,b]

Compound	Species	Clp (mL/min/kg)	$t_{½}$[d] (h)	AUCN po (μM.h.kg/mg)	F (%)[c]
 **17**	Mouse	2.0	1.4	2.99	13
	Rat	1.0	2.2	3.64	8.1
 **18**	Mouse	2.5	4.5	1.6	9
	Rat	0.7	2.3	1.8	2
 **19**	Mouse	9.22	2.64	0.35	7.5

[a] C57BL/6-mice.
[b] Rat Sprague–Dawley.
[c] Dose: 1 mg/kg iv; 2 mg/kg po.
[d] $t_{½}$ = plasma half-life (0–8 h).

attention on finding a suitable replacement of the hydroxy-pyridine moiety and ultimately discovered fluoropyridine **21**. As illustrated in Table 5, this simple functional group exchange significantly improved the ADME properties for the series. The clearance and half-lives, in both mouse and rat species, for **21** were quite favorable, however, it was the bioavailability that showed the most notable improvement. In mice, the bioavailability showed a 10-fold improvement when compared to hydroxypyridine **18**. The rat pharmacokinetic parameters showed a similar trend, with a bioavailability of 81%, good oral exposure, clearance less than 1 mL/min/kg, and a half-life of 5 h. Finally, the comparison of amino-fluoropyridine **21** to the des-amino derivative **22** illustrates clearly the positive effect the amino group has on the serum shift, GPR109a activity, and the pharmacokinetic profile.

In summary, we have disclosed a new class of amino-anthranilic acid agonists of GPR109a[9] that are potent agonists, have reduced serum shift and excellent ADME properties.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.11.116.

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9. For details on the synthesis of the disclosed agonists refer to WO2007075749A2.