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Anthranilic acid replacements in a niacin receptor agonist

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ABSTRACT

Niacin is an effective drug for raising HDL cholesterol. However, niacin must be taken in large doses and significant side effects are often observed, including facial flushing, loss of glucose tolerance, and liver toxicity. An anthranilic acid was identified as an agonist of the niacin receptor. In order to improve efficacy and provide structural diversity, replacements for the anthranilic acid were investigated and several compounds with improved properties were identified.

Low HDL cholesterol levels are a risk factor for atherosclerotic coronary disease, a leading cause of death in the developed world. However, current strategies for raising HDL are limited.[1,2] Exercise and weight loss can afford modest HDL increases (3–9%). In addition, several pharmaceuticals also provide modest HDL increases. For example, statins show a 5–15% HDL increase and fibrates show a 10–25% increase.[3] Niacin, a drug in use since the 1950s, shows the most dramatic HDL elevation (20–35%), but its use has been limited by side effects. Patients on niacin therapy experience intense facial reddening called flushing, which causes low patient compliance with treatment. Additionally, glucose tolerance is reduced in patients taking niacin, limiting its use in diabetic and prediabetic patients. Liver toxicity can be a problem as well.[4]

Early efforts to improve upon niacin met with little success because its mechanism of action was not known. Recent renewed interest in HDL raising drugs spurred efforts to identify niacin's cellular target, and a G_i coupled GPCR, GPR109A, also referred to as HM74A or 19CD, was identified in fat cells. In vitro, this receptor showed a high affinity for niacin.[5] Animal studies involving GPR109A were also undertaken. While it is not possible to show HDL effects in mice because of their naturally high HDL levels, a decrease in free fatty acids can be used as a surrogate marker for HDL changes. With niacin treatment, a decrease in free fatty acid is seen in humans, which is then followed by HDL changes. Upon niacin treatment, GPR109A knockout mice did not show a decrease in free fatty acids that is seen in wild type mice, further supporting GPR109A as the likely target of niacin. Unfortunately, niacin receptor knockout mice also failed to show a niacin induced flush, indicating that flushing was in fact mediated through the niacin receptor.

We felt that cloning of the receptor would allow for the identification of a novel GPR109A agonist. Furthermore, it was felt that pharmacokinetics would be the key to reduced flushing and improved HDL levels. Niacin itself is rapidly metabolized resulting in a very short half life in man. Thus, a high dose of niacin (2–4 g/day) must be taken by patients to achieve the maximum HDL elevation (35%). A niacin receptor agonist with lower clearance and a longer half life may show less severe flushing at an efficacious dose. We believed that a longer half life may also increase the HDL levels achieved. The short half life of niacin led us to question whether full 24 h coverage of the niacin receptor was being achieved using the current treatment regime. Although the receptor may not be fully activated, HDL increases of 25–35% are seen. Perhaps, even greater changes in HDL could be obtained by compounds with longer occupancy of the receptor.

Cloning of the niacin receptor allowed for high throughput screening, and a structurally distinct molecule that activated

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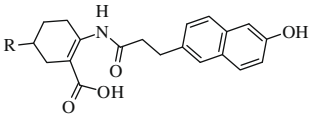
GPR109A was identified. Further optimization led to the identification of compound **6** (Table 1).[6] We sought to modify the cyclohexenyl ring of **6** with the goal of improving the potency and pharmacokinetic properties. Alkyl and aryl substitution in the C4-, 5-, and 6-position were explored. This Letter discusses our SAR efforts at the C4-position.

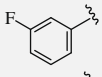
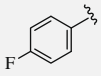
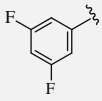
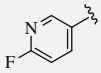
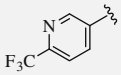
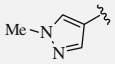
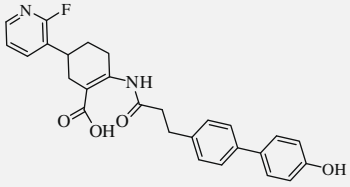
As shown in Scheme 1, compounds containing a C4 substituted cyclohexenyl ring were synthesized from the corresponding ketone **1**. Deprotonation with LiHMDS followed by quenching of the anion with Mander's reagent provided ketoester **2**. Treatment of **2** with sodium hydride at −78 °C followed by Comins reagent gave vinyl triflate **3** as a single regioisomer.[7] Palladium catalyzed coupling of vinyl triflate **3** with amide **4** gave **5a**. Hydrolysis of the ester provided the desired acid **5b**.

All compounds were evaluated in a binding assay and a functional assay.[8] For the binding assay, membrane preparations were made from CHO-KI cells stably expressing the niacin receptor. Displacement of radiolabeled niacin from the membrane preparation was measured in the absence and presence of human serum. For the functional assay, the same membrane preparations were incubated with compound and then [^{32}S]GTPγS. Retention of [^{32}S]GTPγS on the membrane preparation was measured to determine activation of the G proteins coupled to the niacin receptor, generating EC_{50} values. In this assay, the full response to niacin was defined as 100% and was used as a reference for other compounds.

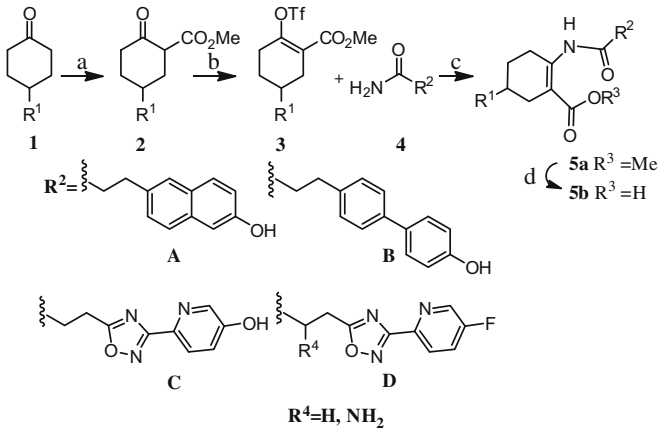
We first synthesized a series of C-4 alkyl substituted cyclohexenyl compounds. A methyl group in the C4-position, resulted in a

Table 1
Binding and functional activity of compounds for niacin receptor



Compound	R =	h-^{3}H-Niacin IC_{50} (nM)	hGTPγS EC_{50} (nM)	Serum shift fold change
	NIACIN	140	1000	1
6	H	8	76	
7	Me	43	430	
8	Pr	8	10	>400
9	Bu	6	30	
10	Ph	34	92	
11	OH	420	nd	
12	CN	7% at 25 μM	5300	
13		11	31	>400
14		7.4	28	>400
15		8.6	130	
16		3	11	>400
17		52	410	100
18		16	64	90
19		18	94	
20		14	480	200
21		120	2650	>400
22		36	137	

Compounds containing chiral centers are racemic unless otherwise indicated. Binding to niacin receptor determined by displacement of [^{3}H]niacin. Functional assays were run using binding of hGTPγS to membrane preparations. Data are an average of two independent titrations having calculated standard errors below 15%. The assay-to-assay variation was generally ±2-fold.



Scheme 1. Reagents and conditions: (a) LiHMDS $CNCO_2Me$, THF, −78 °C to rt, 90%; (b) NaH, Comins reagent, THF, −78 °C to rt, 90%; (c) Pd_2dba_3, Xanthphos, Cs_2CO_3, dioxane, 90 °C; (d) LiOH, MeOH/THF, 5–70%.

fivefold decrease in binding and functional activity (compound **7**, Table 1), but larger alkyl chains, including propyl and butyl (compounds **8** and **9**), showed similar binding activity compared to **6**, along with a 2–7-fold improvement in the functional assay. Polar substituents such as OH and CN (**11** and **12**) resulted in a 50-fold decrease in binding activity.

Installation of a phenyl group (compound **10**) in the C4-position of the cyclohexene resulted in a fourfold decrease in binding activity but similar functional activity compared to compound **6** (Table 1). We then explored substitution on the phenyl ring to determine if further enhancements in potency could be achieved. The 2- and 3-fluoro phenyl substituted analogs (**13** and**14**) had a threefold improvement in the binding and functional assay compared to **10**. The 4-fluoro phenyl substituted analog **15**, on the other hand, showed no improvement compared to **10**. The 2,5-difluorophenyl compound **16** was synthesized next and had a 9–10-fold improvement in binding activity and functional activity compared to compound **10**.

While compounds **8**, **13**, **14**, and **16** had excellent activity in both the binding and functional assays in the absence of serum, in the presence of serum the activity decreased by 400-fold. As this was a concern, we sought to replace the phenyl ring with more polar groups with the goal of reducing the serum shift. Thus, a series of fluorinated 3-pyridines were synthesized (compounds **17**–**19**). The 2-fluoro 3-pyridine compound, **17**, had comparable binding activity to compound **10**, but a fourfold decrease in functional activity with a 100-fold serum shift. The 4-fluoro 3-pyridine compounds **18** had activity comparable to compound **10**, but had only a 90-fold serum shift. The methyl pyrazole analogs **20** and **21** were also synthesized. Compound **20** had good binding activity (comparable to compound **10**), but poor functional activity and large serum shift (200-fold), while the regioisomer, **21**, had poor activity and >400-fold serum shift.

Having identified substituted cyclohexenyl compounds with good activity and an improved serum shift, we investigated the pharmacokinetic properties of this series. Along with the naphthol sidechain, we had also identified a biphenol side chain, **B**, as a potent group in the anthranilic acid series. When this side chain was coupled with the 2-fluoropyridine cyclohexenyl group to provide **22**, a threefold improvement in functional activity over compound **17** was obtained. Therefore, compound **22** was selected to be evaluated for its pharmacokinetics in mice (Table 2). It had a modest half life (1.8 h) and low clearance (5.4 mL/min/Kg) with poor oral bioavailability (12%). This profile was comparable to the reference anthranilic acid analog **23**, indicating that the substituted cyclohexenyl ring had a relatively neutral effect on pharmacokinetics.

Next, we combined the substituted cyclohexenyl group with an oxadiazole hydroxypyridine side chain **C** (Scheme 1) that had previously demonstrated reduced serum shift and good in vitro activity in the anthranilic acid series.[8] This allowed us to explore whether the combination of the substituted cyclohexenyl head group and the oxadiazole hydroxypyridine side chain would have additive effects on potency and/or better pharmacokinetics. However, the substitutions that had improved potency in combination with side chain **A** had no effect on compounds with side chain **C** (Table 3, compounds **25**–**27**). The propyl compound **25**, 3-fluorophenyl compound **26**, and 4-fluoro-3-pyridine compound **27** had binding and functional activity comparable to the unsubstituted cyclohexenyl compound **24** and had no improvement in serum shift. Interestingly, disubstituted cyclohexenyl compound **28** had greater activity than the unsubstituted cyclohexenyl compound **24**, achieving an EC_{50} of 6 nM in the functional assay.

We next sought to determine the effect of the substituted cyclohexenyl groups in combination with side chain **C** on pharmacokinetics (Table 2). Therefore, we decided to investigate the pharmacokinetics of compound **29**, the mono fluoro analog of compound **28**. Compound **29** had comparable clearance to the unsub-

Table 2
Pharmacokinetics of niacin receptor agonists

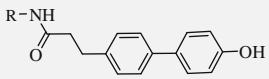
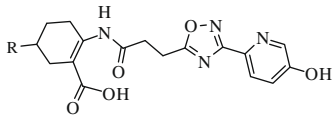
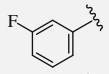
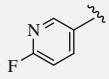
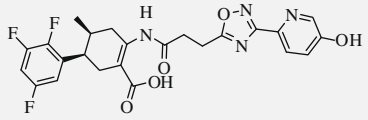
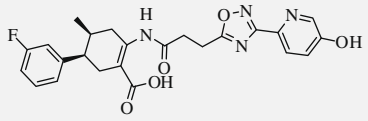
	Compound	Clp (mL/min/kg)	V_{dss} (L/kg)	$t_{1/2}$ (h)	AUC (μM h kg/mg)	C_{max} (μM)	*F* (%)
Mouse							
	**22**	5.4	0.42	1.8	6.8	1.2	12
	**23**	8.7	0.78	2.4	4.0	4.0	35
	24	2.0	0.21	1.3	3.0	3.9	13
	29	5.6	0.76	3.9	0.16	0.3	2
	31	0.5	1.4	14	82	16	87
Rat	**35**	2.3	2.4	4.8	13	3.8	44

Table 3
Binding and functional activity of compounds for niacin receptor



Compound	R =	h-^{3}H-Niacin IC_{50} (nM)	hGTPγS EC_{50} (nM)
	NIACIN	140	1000
24	H	4	21
25	Pr	4	17
26		7	27
27		9	98
28		12	6
29		8	42

See legend of Table 1.

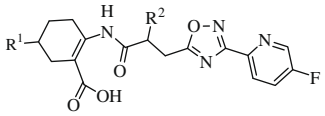
stituted compound **24** and a longer half life (3.9 h), but also had much lower oral AUC and bioavailability (%F = 2).

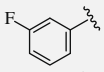
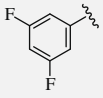
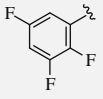
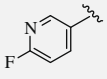
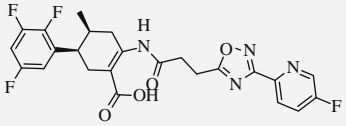
Although the results with the oxadiazole hydroxypyridine side chain **C** were disappointing, they did indicate that a substituted cyclohexenyl could provide significant improvements in the functional assay. Therefore, for the next round of compounds, we sought a side chain with excellent pharmacokinetics that was in need of improvement in the binding and functional activity (Table 4). Side chain **D**, with a fluoropyridine in place of the hydroxypyridine moiety, conferred excellent pharmacokinetics, but poor potency and large serum shifts (>400-fold). Compound **31** with an unsubstituted cyclohexenyl group and an amine in the linker region was evaluated for its pharmacokinetics in mice and had low clearance (0.5 mL/min/Kg), a long half life (14 h), and good bioavailability (87%). As hoped, the combination of side-chain **D** with several substituted cyclohexenyl groups provided dramatic improvements in potency. The 3-fluorophenyl compounds **32** and **33** showed twofold improvements in functional activity and the 3,5-difluorophenyl compound **34** and 2,3,5-trifluorocompounds **36** and **37** provided 20- and 10-fold improvements in the functional assay compared to compounds **30** and **31**. In addition, compounds **34** and **35** showed improved serum shift. On the other hand, a heterocyclic substituted head group exemplified by fluoropyridine **38**, as well as the disubstituted cyclohexenyl group in **39**, were poorly tolerated in combination with side chain **D**.

Compound **35** was selected for pharmacokinetic studies in a rat (Table 2). Gratifyingly, compound **35** had low clearance (2.3 mL/min/Kg), good oral AUC (13.2 μM h), good bioavailability (44%), and a long half life (4.8 h). In compound **35**, we have identified a potent GPR109A agonist with good pharmacokinetics.

In summary, the effects of substituted cyclohexenyl groups on potency and pharmacokinetics were investigated. The substituted cyclohexenyl groups had a neutral or slightly detrimental effect on pharmacokinetics, but showed significant potency improvements. This allowed for the use of a side chain with excellent pharmacokinetics that was hindered by a lack of potency. The combination of side chain **D** with a difluoro substituted phenyl cyclohexenyl head group provided compound **35**, which had the desired potency and pharmacokinetic properties.

Table 4
Binding and functional activity of compounds for niacin receptor



Compound	R^1 =	R^2 =	h-^{3}H-Niacin IC_{50} (nM)	hGTPγS EC_{50} (nM)	Serum shift fold change
NIACIN			140	1000	
30	H	H	35	490	>400
31		NH_2	32	460	>400
32		H	57	230	
33		NH_2	85	250	
34		H	19	22	30
35		NH_2	28	100	30
36		H	9	45	
37		NH_2	15	46	
38		H	280	7400	
39		H	6	75	

See legend of Table 1.

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