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Tetrahydro anthranilic acid as a surrogate for anthranilic acid: Application to the discovery of potent niacin receptor agonists

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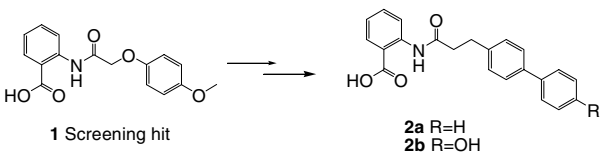
Abstract—The design, synthesis, and biological activity of a series of cycloalkene acid-based niacin receptor agonists are described. This led to the discovery that tetrahydro anthranilic acid is an excellent surrogate for anthranilic acid. Several compounds were identified that were potent against the niacin receptor, had enhanced cytochrome P450 selectivity against subtypes CYP2C8 and CYP2C9, and improved oral exposure in mice.

Coronary heart disease (CHD) is the leading cause of death in industrialized nations.[1] An elevated level of LDL-cholesterol and a reduced level of HDL-cholesterol are two of the major risk factors for CHD. While statins have helped significantly to reduce LDL levels in patients at risk for CHD, they have only a modest effect on raising HDL. Therefore, new therapies for cholesterol management that address HDL elevation are highly desirable.

Nicotinic acid (Niacin) is currently the most efficacious drug of choice on the market for raising HDL levels (15–35%).[2] Among its other benefits are significant lowering of free fatty acids, triglycerides, VLDL/LDL levels, and lipoprotein (a).[3] Furthermore, niacin, when combined with a LDL lowering agent such as lovastatin, has resulted in regression of atherosclerosis.[4] Despite its many advantages, niacin has some limitations. It has a very short half-life in man. Therefore, to achieve the maximum elevation of HDL (35%), patients on niacin therapy are required to take large doses of drug (2–4 g/day). Additionally, about 90% of the patients on niacin therapy experience acute cutaneous flushing in the face and neck. This unpleasant side effect causes a significant reduction in patient compliance. Therefore, a drug that is as efficacious as niacin at raising HDL, that has better pharmacokinetic properties, and does not cause flushing would be an advance in cholesterol therapy. The recent discovery of a G-protein coupled receptor GPR109A (also referred to as HM74A or PUMA-G in rodent) which showed high affinity for niacin[5] has spurred wider interest in identifying other potent niacin receptor agonists.[6–12]

Screening the Merck sample collection against GPR109A identified compound **1**, an anthranilic acid derivative with modest activity in the functional assay (hGTPγS = 6 μM). Optimization of the side chain led to the discovery of biphenyl anthranilic acid derivatives **2a** and **2b** that were full agonists against the niacin receptor (Fig. 1).[13]



1 Screening hit

2a R=H
2b R=OH

Figure 1. Early anthranilic acid leads.

Keywords: Niacin receptor agonists; Tetrahydro anthranilic acid; Surrogate for anthranilic acid.
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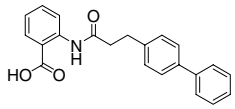
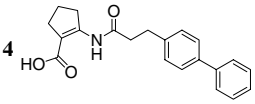
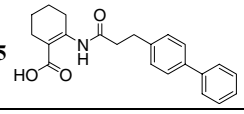
Table 1. Binding and functional activity for niacin receptor agonists[a]

Compound	h-^{3}H-Niacin IC_{50} (μM)	hGTPγS EC_{50} (μM)
Niacin	0.14	1.0
2a 	0.094	0.59
4 	0.13	0.84
5 	0.021	0.38

[a] Data are an average of two experiments with a calculated standard error below 20%.

Compound **2a** (Table 1) had activity similar to niacin in the binding assay but was 2-fold more potent in the functional assay. More significantly, **2a** had a better pharmacokinetic profile in mice (low clearance and longer $t_{1/2}$) compared to niacin with oral bioavailability of 13% (Table 2). Anthranilic acid is a good pharmacophore that is frequently used in drug discovery programs.[14] In the niacin receptor program however, we discovered that several anthranilic acid analogs had undesirable cytochrome P450 activity against subtypes CYP2C8 and CYP2C9. In addition, these analogs were also highly protein bound, causing significant serum shift in binding and functional assays. Because of these shortcomings, we began a search for anthranilic acid surrogates. In this preliminary letter, we report that tetrahydro anthranilic acid can be an ideal replacement for anthranilic acid. The resulting analogs that incorporated the tetrahydro anthranilic acid scaffold led to compounds with improved in vitro activity and superior PK profiles (higher oral AUC). Furthermore, these compounds also exhibited good selectivity against cytochrome P450 subtypes CYP2C8 and CYP2C9.

Our approach discussed herein was to replace the anthranilic acid moiety while keeping the bi-aryl side chain in **2a** and **2b** constant. Towards this end, the effect of replacing anthranilic acid with saturated 5- and 6-membered rings was examined (Fig. 2, **3a**, **3b**). Surprisingly, this led to completely inactive compounds (both *cis* and *trans* isomers).

We hypothesized that the amide NH in **2a** makes an intramolecular hydrogen bond with the carbonyl group of the acid and helps preorganize the molecule in a favorable bioactive conformation for receptor binding (Fig. 2). With the ring system saturated (as in **3a/3b**) it would be difficult to preserve this interaction and thus explain the lack of activity. Furthermore, deletion of this amide NH via methylation (NMe) resulted in complete loss of activity. We reasoned that this intramolecular H bond, which was critical for receptor affinity, could be preserved only by keeping the relevant atoms (the amide NH and the carboxyl group) in the same plane. Perhaps this arrangement could be achieved by incorporating a double bond in the 5- or 6-membered ring to generate a cyclopentene or cyclohexene ring system. In order to test this hypothesis, we undertook the synthesis of the compounds **4** and **5** (Fig. 2).

The compounds described herein were prepared using two different synthetic routes (Schemes 1 and 2).[15] Treatment of the commercially available methyl 2-oxo-

Table 2. Pharmacokinetic parameters of niacin receptor agonists dosed in mice (n = 3 mice, 1 mpk iv, 2 mpk po; niacin, 10 mpk iv, 20 mpk po)

Compound	Cl (mL/min/kg)	Vd_{ss} (L/kg)	$t_{1/2}$ (h)	C_{max} (μM)	AUC_N (μM/h/mg)	F%
Niacin	167	1.0	0.02	71	1.0	~100
2a	4.8	0.2	1.9	4.1	1.4	13
4	1.2	0.4	2.8	15	27.7	64

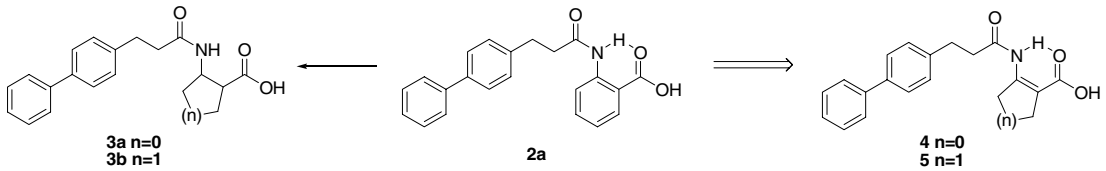
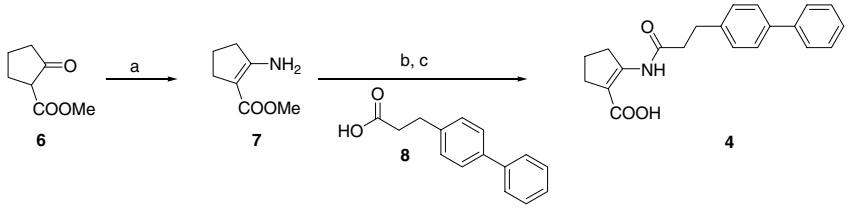
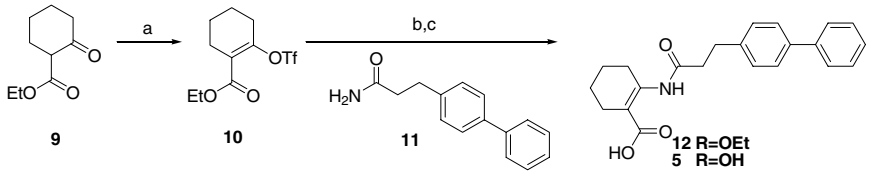


Figure 2. Intramolecular H bond possible with anthranilic acid and cycloalkene.



Scheme 1. Reagents and conditions: (a) NH_4OAC, MeOH, 99%; (b) **8**, MsCl, DMAP, DCM, 45%; (c) 0.5 N NaOH, H_2O–THF.



Scheme 2. Reagents and conditions: (a) NaH, Comins reagent, THF, 90%; (b) **11**, Xantphos, $Pd_2(dba)_3$, Cs_2CO_3, 1,4-dioxane, 55 °C, 70%; (c) 1 N NaOH, THF–MeOH–H_2O.

cylopentane carboxylate **6** with ammonium acetate in methanol gave the desired iminoester **7** in quantitative yield. This was coupled to the biphenyl acid **8** using MsCl, DMAP in DCM. The ester was saponified under standard conditions using aqueous NaOH–THF–H_2O to give the desired product **4**.

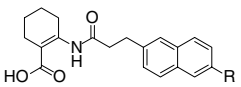
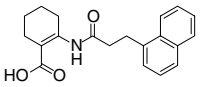
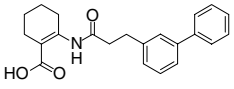
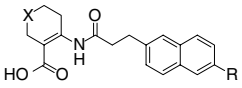
Alternatively, compounds were synthesized via a palladium mediated cross-coupling reaction (Scheme 2).[16] Commercially available ethyl-2-oxocyclohexane carboxylate was transformed to the vinyl triflate **10** using sodium hydride and Comins reagent.[17] Reaction of the triflate **10** with the amide **11** in the presence of $Pd_2(dba)_3$, Xantphos and Cs_2CO_3 in 1,4-dioxane gave the desired product **12** in 70% yield. Saponification of the ethyl ester using NaOH–THF–MeOH afforded **5**.

All synthesized compounds (**2a**, **4**, and **5**) were tested in a niacin receptor binding and functional assay. In the binding assay, membranes were prepared from the niacin receptor expressed in CHO cells. Displacement of radiolabeled niacin from the membrane was measured affording the IC_{50} values. In addition, the same membrane preparations were incubated with compound followed by [^{32}S]GTPγS for determining functional activity. Retention of [^{32}S]GTPγS on the membrane was measured, 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. The data for compounds **4** and **5** are summarized in Table 1.

Replacing anthranilic acid with the cyclopentene moiety (**4**) resulted in the binding and functional activity being maintained (compared to **2a**). More notably, we observed a remarkable improvement in the pharmacokinetic properties (Table 2). Compound **4** when dosed in mice, showed a 4-fold decrease in clearance, an increase in half-life, and about 20-fold increase in AUC (po) with an oral bioavailability of 64% compared to **2a** (13%). Interestingly, the larger cyclohexene moiety (**5**) resulted in further enhancements in activity in both binding and functional assays. Encouraged by these results, we decided to explore the SAR of this series of compounds further.

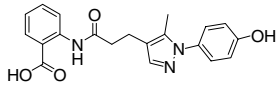
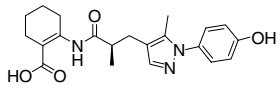
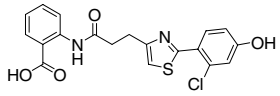
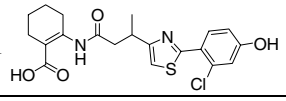
While synthesizing these compounds, we observed that the cyclopentene containing analog **4** appeared chemically unstable and was prone to amide bond cleavage under strongly acidic and basic conditions. As this was a serious concern, we undertook a study to determine the stability of this compound. Thus **4** was subjected to pH = 2.5 aqueous buffer solution at 40 °C. After 6 h, an analysis of the solution by LC-MS revealed that less than 5% of **4** remained. By contrast, the cyclohexene (or tetrahydro anthranilic acid) analogs fared much better and were stable at a pH of 2.5. Therefore, we decided to focus our attention on **5** wherein we modified the side chain with the goal of increasing the in vitro potency. The data for the synthesized compounds (**13**–**17**) are summarized below (Table 3).

Table 3. Binding and functional activity for tetrahydro anthranilic acid analogs[a]

Compound			h-^{3}H-Niacin IC_{50} (μM)	hGTPγS EC_{50} (μM)
		Niacin	0.14	1.0
13a	**R = H**		0.038	0.69
13b	**R = OH**		0.083	
14			0.1	1.21
15			0.14	1.16
16	**X = NH**		—	14% at 100 μM
	R = H			
17	**X = O**		0.9	6.3
	R = OH			

[a] Data are an average of two experiments with a calculated standard error below 20%.

Table 4. Modification of the side chain[a]

Compound	h-^{3}H-Niacin IC_{50} (μM)	hGTPγS EC_{50} (μM)	CYP2C8 IC_{50} (μM)	CYP2C9 IC_{50} (μM)	pH = 2.5 stability (40 °C, 6 h)
18 	0.024	0.11	28	22	
19 	0.026	0.35	>100	>100	100%
20 	0.001	0.006	2.8	1.2	
21 	0.002	0.018	—	5.1	97%

[a] Data are an average of two experiments with a calculated standard error below 20%.

We examined the effect of placing a β-naphthyl group on the side chain (**13a**). Compared to **5**, this resulted in the binding activity being maintained, with a 2-fold decrease in functional activity. Placing a hydroxyl group at the C-6 position (**13b**) resulted in a 5-fold increase in binding and functional activity. On the other hand, a α-naphthyl in the side chain resulted in a less potent compound (**14**) with an activity comparable to niacin. Similarly, meta bi-phenyl analog **15** was about 5-fold less active than its para bi-phenyl counterpart **5**.

We also examined the effect of placing a heteroatom such as nitrogen or oxygen in the tetrahydro anthranilic acid ring. Our initial thought was that this could help improve the serum shift for these compounds. Thus, compounds **16** and **17** were prepared. Among them, the oxygen analog **17** had modest activity. Nevertheless, compound **17** was less active than **13b** indicating that for the niacin receptor, a heteroatom in the ring is not optimal.

Next, we combined the tetrahydro anthranilic acid moiety (in **5**) with side chains that had previously demonstrated good in vitro activity with anthranilic acid.[13] (Table 4). The binding and functional activity for tetrahydro anthranilic acid analogs **19** and **21** remained about the same when compared to the anthranilic acid counterparts **18** and **20**. More importantly, a 4- to 6-fold improvement in CYP2C8 and CYP2C9 selectivity was observed (compare **18** vs **19** and **20** vs **21**). Compounds **19** and **21** were also stable in pH = 2.5 aqueous buffer at 40 °C.

Table 5. Pharmacokinetic parameters of niacin receptor agonists dosed in mice (n = 3 mice, 1 mpk iv, 2 mpk po)

Compound	Cl (mL/min/kg)	Vd_{ss} (L/kg)	$t_{1/2}$ (h)	C_{max} (μM)	AUC_N (μM/h/mg)	F%
18	26	0.88	1.1	0.03	0.04	2
19	3.52	0.68	4.8	4.78	6.4	52
20	7.3	0.37	1.6	2.14	2.1	36
21	0.3	0.16	6.1	11.3	71.6	54

Compounds **18**–**21** were also evaluated in mouse for their pharmacokinetic properties (Table 5). As anticipated, replacing anthranilic acid with tetrahydro anthranilic (**19** and **21**) resulted in a robust increase in oral AUCN and half-life with the concomitant decrease in clearance and considerable improvement in oral bioavailability.

In summary, we have designed cyclopentene and cyclohexene moieties as replacements for anthranilic acid. This led to the discovery that tetrahydro anthranilic acid is an excellent surrogate for anthranilic acid. We applied this design to the niacin receptor agonist program, and identified several compounds that were highly potent against the receptor, had improved cytochrome P450 selectivity against subtypes CYP2C8 and CYP2C9, and improved oral exposure. We anticipate that this tetrahydro anthranilic acid scaffold will have broader scope with application to other anthranilic acid-based drug discovery programs.

Acknowledgments

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