Analogues of Acifran: Agonists of the High and Low Affinity Niacin Receptors, GPR109a and GPR109b

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Abstract: Recently identified GPCRs, GPR109a and GPR109b, the high and low affinity receptors for niacin, may represent good targets for the development of HDL elevating drugs for the treatment of atherosclerosis. Acifran, an agonist of both receptors, has been tested in human subjects, yet until recently very few analogs had been reported. We describe a series of acifran analogs prepared using newly developed synthetic pathways and evaluated as agonists for GPR109a and GPR109b, resulting in identification of compounds with improved activity at these receptors.

Niacin (nicotinic acid) is a water-soluble vitamin that, at high doses in humans, favorably affects essentially all serum lipid and lipoprotein parameters.[1] Niacin's ability to increase high-density lipoprotein (HDL) to a greater extent than other currently used drugs is of particular interest, and it has been used for many years in the treatment of atherosclerotic disease.[2] Recent mechanistic investigations have shown that niacin may exert its beneficial effects on lipids through activation of a G-protein coupled receptor (GPCR) located on adipocytes.[3] The resultant decrease in intracellular cAMP leads to inhibition of lipolysis via negative modulation of hormone-sensitive lipase activity, thereby decreasing plasma free fatty acid levels and, ultimately, it has been postulated, raising HDL. Two G_i-coupled orphan GPCRs that share 95% identity and which are both expressed in human adipocytes have been identified as possible molecular targets for niacin.[4] GPR109a (HM74a) is the human orthologue of the previously described rodent receptor (PUMA-G),[5] whereas GPR109b (HM74) appears to have arisen from a very late gene duplication. It differs from GPR109a and PUMA-G mainly in the C-terminal tail portion and has no rodent equivalent.[6] Niacin has been shown to activate GPR109a, with an EC_{50} of 250 nM in a GTPγS assay and displaces ^{3}H-niacin from GPR109a, expressing CHO cell membranes with an IC_{50} of 81 nM.[4] It is a much weaker ligand for GPR109b, with an EC_{50} in the millimolar range. Unfortunately, the use of niacin as a therapeutic is limited by a number of associated side-effects, including an uncomfortable cutaneous flushing response and free fatty acid rebound. The development of novel agonists of the niacin receptor that retain the beneficial effect on atherosclerosis but with fewer side effects would clearly be of value.

With the discovery of these putative molecular targets for niacin, we and others quickly identified the known lipid modulating agents, acifran and acipimox (Figure 1) as functional agonists of GPR109a (Table 1). In our hands, these compounds are able to decrease forskolin-induced elevation of cAMP levels in cells stably transfected with the receptor. Acifran is less active at this receptor than niacin but more potent than the clinically used agent acipimox.[4] We found the agonist potency of acifran in a whole cell cAMP assay was around 1 μM for GPR109a and it showed only a 3−4-fold selectivity for this receptor over GPR109b (Table 1), whereas niacin and acipimox showed no effect up to 30 μM in the GPR109b assay. Recent data has shown that β-hydroxybutyrate can function as ligand for GPR109a, with an EC_{50} in the mM range. Despite the low affinity, under various stressful conditions in vivo, the plasma levels of β-hydroxybutyrate can significantly exceed this concentration.[7] Our data indicate, therefore, that acifran should be considered to be a full agonist of GPR109a, as it has both comparable agonist efficacy to this purported natural ligand and it is able to fully reverse the cAMP elevating effect of forskolin. There is no known natural ligand for GPR109b, but again acifran was able to fully reverse the cAMP elevating effect of forskalin in the GPR109b assay. While acifran was clinically evaluated and was shown to lower free fatty acids in humans, in the absence of a molecular target, only four analogs were prepared and reported.[8] A recent paper described further investigations around the acifran series, but significant improvements in potency were not achieved. Only the 4-fluorophenyl derivative of acifran was equipotent with the parent molecule, and derivatives with substitution on the furan ring or four-membered ring analogues were all inactive or considerably less potent than acifran. Chiral versions of acifran were also prepared via a Sharpless asymmetric dihydroxylation, and all of the activity of racemic acifran could be assigned to the (*S*)-enantiomer, as the (*R*)-enantiomer was completely inactive.[9]

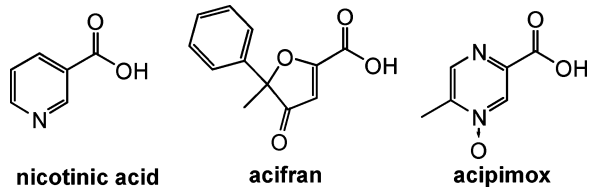

Figure 1. Structures of known antilipolytic compounds shown to be agonists of GPR109a.

The above observations, coupled with the availability of a high-throughput functional screening assay, prompted us to investigate the use of acifran as a starting point for the discovery of new receptor agonists and ultimately new agents to elevate HDL.

We quickly found the scope of the reported procedure for the preparation of acifran (Scheme 1) to be somewhat limited and, hence, inadequate for fully exploring the SAR. These limitations were identified as the instability of the α-*tert*-hydroxy ketone intermediate **3** under the acid hydrolysis conditions employed for the conversion of the alkyne **2** to **3** and the lack of reproducibility of the conversion of ketone **3** to acifran analog **5** via the six-membered intermediate **4**. In particular, when the substituent R_1 was heterocyclic in nature, no formation of the desired analogues **5** was detected. The search for new approaches to acifran analogs led us to investigate a route making use of dithiane chemistry so that α-*tert*-hydroxy ketone intermediate **3** could be produced under neutral conditions (Scheme 1). The dithiane anion derived from **6** was added to various ketones, resulting in *tert*-alcohols **7** in good yields. The resulting

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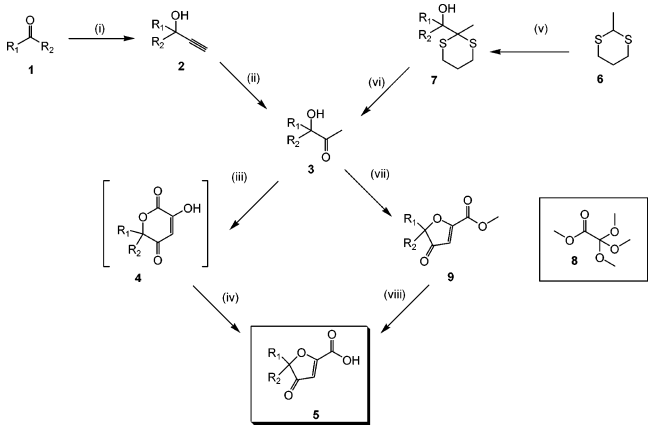
Table 1. cAMP Whole Cell Assay Results for Racemic Acifran Analogues **5a**−**5z**

cmpd	R_1	R_2	GPR109a EC_{50},[a] μM (*n*)	GPR109b EC_{50},[a] μM (*n*)
5a (acifran)	Ph	Me	1.3 (36)	4.2 (6)
5b	Ph	Et	24 (3)	4.8 (3)
5c	vinyl	Me	>50 (2)	n.d.
5d	Et	Me	>50 (2)	n.d.
5e	1-spiro	indane	19 (2)	NA
5f	spirocyc	lopentane	>100 (2)	n.d.
5g	1-cyclopentenyl	Me	2.5 (6)	5.1 (3)
5h	1-cyclohexenyl	Me	2.0 (6)	n.d.
5i	3-pyridine	Me	33 (3)	NA
5j	4-pyridine	Me	NA	NA
5k	2-furan	Me	46 (3)	NA
5l	2,5-diCl-Ph	Me	41 (3)	NA
5m	2,4-diF-Ph	Me	12 (6)	24 (3)
5n	3,4-diF-Ph	Me	7.2 (6)	19 (3)
5o	2,6-diF-Ph	Me	6.1 (15)	36 (3)
5p	3,5-diF-Ph	Me	2.6 (15)	33 (6)
5q	2-F-Ph	Me	5.7 (6)	n.d.
5r	4-F-Ph	Me	5.4 (9)	9.9 (6)
5s	3-F-Ph	Me	1.2 (6)	n.d.
5t	3-Cl-Ph	Me	1.6 (6)	22 (3)
5u	3-Br-Ph	Me	0.57 (33)	4.9 (6)
5v	3-I-Ph	Me	0.43 (6)	5.1 (3)
5w	3-Me-Ph	Me	5.4 (6)	NA
5x	3-Et-Ph	Me	1.1 (6)	6.2 (3)
5y	3-CF_3-Ph	Me	4.2 (15)	12.6 (3)
5z	3-OMe-Ph	Me	23 (6)	NA
niacin			0.12 (>100)	NA
acipimox			5.3 (27)	NA

[a] Values are means of multiple determinations. The number of experiments is listed in parentheses. The standard deviations where calculable were <30% of the mean. N.A. = less than 50% activity observed at any concentration up to 100 μM. n.d = not determined.

Scheme 1. Synthetic Routes for the Preparation of Acifran and Its Phenyl Analogs[a]



[a] Reagents and conditions: (i) sodium acetylide; (ii) HgO, H_2SO_4; (iii) NaH, ethyl oxalate, THF; (iv) (a) NaOH; (b) HCl; (v) (a) *n*-BuLi, −78 −10 °C; (b) R_1COR_2, −78 °C−rt; (vi) $Hg(ClO_4)_2$, MeOH, rt; (vii) NaH, THF, **8**, 65 °C; (viii) LiOH or HCl.

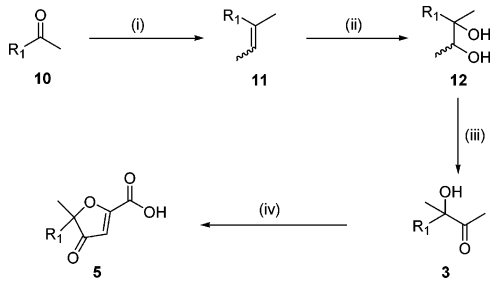
compounds **7** were smoothly deprotected with 2 equiv of $Hg(ClO_4)_2$ in methanol at room temperature. In most cases, the α-*tert*-hydroxy ketones **3** could be used directly in the next reaction after simple extractive workup and without the need for column chromatography. In a second modification to the previously reported method, α-hydroxy-ketones **3** were condensed with the ortho ester **8**[10] instead of ethyl oxalate, allowing the direct formation of the desired five-membered ring. This route generated a range of acifran analogs **9** as methyl esters that were readily hydrolyzed to the free acids **5** under basic or acidic conditions. We were thus able to avoid the generation of the six-membered ring product **4**, which required ring opening, followed by recyclization to provide the desired five-membered ring, a process that we found to be highly substituent dependent.

When this synthetic procedure was used, compounds **5a**−**z** were prepared and evaluated for their agonist activity at the GPR109a and GPR109b receptors using a cAMP whole cell assay.[11] The resulting data are summarized in Table 1. We first examined the effect of changing the R_2 group from methyl (**5a**, acifran) to ethyl (**5b**),[9] which, while significantly reducing activity for GPR109a, appeared to be tolerated by GPR109b, generating a compound with modest activity but some selectivity for GPR109b. Combining the two substituents in a spirocycle (**5e**, **5f**) was not tolerated, and we found that such changes significantly decreased activity at both receptors relative to acifran. Changing the R_1 group from phenyl (acifran) to small aliphatic chains (**5c**, **5d**) also significantly decreased the agonist activity. However, replacement of the phenyl group with cycloalkene substituents (**5g**, **5h**) resulted in compounds that retained good activity at GPR109a. The analogues incorporating nitrogen or oxygen containing heterocycles (**5i**−**k**) in place of the phenyl group also lost GPR109a activity relative to the parent compound, suggesting that the binding pocket for the R_1 side chain in GPR109a is somewhat hydrophobic. With this in mind, we next prepared a number of substituted phenyl analogs (**5l**−**z**), essentially all of which retained some agonist activity at the GPR109a receptor. In general, *ortho*- and *para*-substitutions decreased the activity moderately, whereas *meta*-substitution was well tolerated and even suggested a modest enhancement of activity. The *meta*-fluorophenyl analog (**5s**) and the *meta*-chlorophenyl analog (**5t**) showed activity comparable to acifran (the former result confirming the previous observation that this compound was essentially equipotent with acifran itself[9]), while the *meta*-bromophenyl analog (**5u**) and *meta*-iodophenyl analog (**5v**) were 2−3-fold more potent. Replacement of the *meta*-halogen with other groups (**5w**−**z**) decreased the activity with the exception of the *meta*-ethyl analog (**5x**). In general, we did not observe any significant change in the selectivity of this series for GPR109a over GPR109b and for all compounds in which only the phenyl group had been substituted (**5l**−**z**), the ratio of activities remained in the range of 3−10. The observation that a *meta*-ethyl-substituted phenyl analogue was more active than its methyl counterpart (**5w**) and the apparent ability to accommodate very large halogen atoms, led us to explore larger substituents in the *meta*-position.

Using the aforementioned bromophenyl analog (**5u**) as a starting material, Suzuki coupling reactions[12] under microwave conditions ($RB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, dioxane, 140 °C, 3 min microwave heating) provided access to various alkenyl-, aryl-, and heteroaryl-substituted phenyl analogs. However, the cAMP assay for these compounds showed that only vinyl (EC_{50} = 4.81 μM) and 1-hexenyl (EC_{50} = 7.05 μM) substitution was tolerated but that all of the longer alkenyl as well as all 10 aryl- and heteroaryl-substituted analogs prepared were inactive (see Supporting Information).

With this apparent size restriction and preference for lipophilic groups identified, we next investigated the utility of a substituted thiophene in this position, a well characterized phenyl isostere in which the ring is somewhat smaller while retaining similar hydrophobicity. However, the synthetic route that we employed for the preparation of compounds **5b**−**z** did not allow ready access to α-halogen-substituted thiophene analogs. The problem was traced to incompatibility of α-halo-thiophenes with the use of the dithiane anion that we used in our previous route to the key hydroxyl ketone **3**. We thus identified a third route to **3**

Scheme 2. Synthetic Pathway to Thiophene Analogs of Acifran[a]



[a] Reagents and conditions: (i) triphenylethyl phosphonium bromide, KO*t*-Bu, THF, 0 °C−rt; (ii) NMO, OsO_4, acetone/H_2O; (iii) Swern oxidation; (iv) (a) NaH, THF, **8**, 65 °C; (b) HCl.

Table 2. cAMP Whole Cell Assay Results for Racemic Thiophene-Substituted Acifran Analogues **5aa**−**5aj**

cmpd	R_1	GPR109a EC_{50},[a] μ**M** (*n*)	GPR109b EC_{50},[a] μ**M** (*n*)
5a (acifran)	Ph	1.3 (36)	4.2 (6)
5aa	2-thienyl	5.0 (21)	n.d.
5ab	5-Cl-2-thienyl	2.1 (15)	3.7 (6)
5ac	5-Me-2-thienyl	0.48 (9)	0.27 (6)
5ad	4-Br-2-thienyl	0.74 (21)	4.1 (12)
5ae	4-Me-2-thienyl	2.8 (6)	1.8 (3)
5af	4-Br-5-Me-2-thienyl	7.0 (6)	10.4 (3)
5ag	3-thienyl	2.4 (6)	4.2 (3)
5ah	5-Cl-3-thienyl	0.20 (9)	1.7 (3)
5ai	5-Br-3-thienyl	0.11 (9)	1.5 (3)
5aj	5-Me-3-thienyl	0.37 (6)	0.18 (3)

[a] Values are means of multiple determinations. The number of experiments is listed in parentheses. The standard deviations where calculable were <30% of the mean. n.d = not determined.

using a dihydroxylation approach (Scheme 2). Wittig reaction of ketones **10** with triphenylethyl phosphonium bromide afforded olefinic mixtures **11**, which were transformed to the diols **12** through an osmium tetroxide catalyzed dihydroxylation. The oxidation of the diols **12** to α-*tert*-hydroxy ketones **3** was first attempted with pyridinium chlorochromate (PCC). However, the diol underwent cleavage to revert to the starting ketone **10** without formation of the desired hydroxy-ketone. Oxidation with Dess−Martin periodinane gave a mixture of the starting ketone and the desired product, whereas a Swern oxidation method was able to transform the diol cleanly to the desired α-*tert*-hydroxy ketone without cleavage. α-*tert*-Hydroxy ketones **3** were transformed to acifran analogs by condensation with ortho ester **8**, followed by acidic hydrolysis of the resultant esters as described in the previous scheme.

When either this synthetic procedure or that shown in Scheme 1 was used, compounds **5aa**−**aj** were prepared and evaluated for their agonist activity at the GPR109a and GPR109b receptors using our cAMP whole cell assays, and the resultant data are shown in Table 2. Replacement of the phenyl ring of acifran with substituted thiophene groups was well tolerated. Whereas the unsubstituted 2- and 3-thiophene analogs (compound **5aa** and **5ag**) were marginally less potent than acifran, monosubstitution on the thiophene ring appeared to improve activity. In particular, 5-substituted-3-thienyl analogs showed quite potent agonist activity. The 5-chloro-3-thienyl analog (**5ah**) and the 5-bromo-3-thienyl analog (**5ai**) were several times more potent than acifran itself at GPR109a and showed around 10-fold selectivity for this receptor over GPR109b. The 5-methyl-3-thienyl analogue (**5aj**) was in the same potency range for GPR109a, but showed improved activity at GPR109b relative to other compounds in the series. All of the compounds prepared

Table 3. cAMP Whole Cell Assay Results for Selected Chiral Acifran Analogues

cmpd[a]	% ee[b]	GPR109a EC_{50},[c] μ**M** (*n*)	GPR109b EC_{50},[c] μ**M** (*n*)
(+)-**5a**	>99	0.52 (6)	3.0 (3)
(−)-**5a**	>99.9	NA	NA
(+)-**5u**	>98	0.29 (6)	14 (3)
(−)-**5u**	>98	>25 (3)	n.d.
(+)-**5aj**	>98	0.24 (3)	0.18 (3)
(−)-**5aj**	>92	>20 (6)	5.0 (3)
(+)-**5ah**	>98	0.066 (6)	0.69 (3)
(−)-**5ah**	>98	4.68 (6)	16 (3)
(+)-**5ai**	>98	0.048 (6)	0.37 (3)

[a] Chiral **5a** and **5aj** were prepared by chiral HPLC column (CHIRALCEL OD) separation. Chiral **5a** was also prepared by classical resolution as described previously.[7] The enantiomers of halogenated analogues, **5u**, **5ah**, and **5ai**, were not separable using chiral HPLC. These enantiomers were prepared by formation of pairs of diasteromeric amides with chiral α-methyl benzylamine; separation of the pairs by column chromatography followed by acidic hydrolysis to return the enantiomers. [b] All compounds were >99.5% chemical purity by LCMS. The enatiomeric purity of chiral **5a** and **5aj** was determined by chiral HPLC. The enantiomeric purity of chiral **5u**, **5ah**, and **5ai** was determined by ^{1}H NMR of the corresponding separated diastereomeric amides before hydrolysis to the acids. [c] Values are means of multiple determinations. The number of experiments is listed in parentheses. The standard deviations where calculable were <30% of the mean. N.A. = less than 50% activity observed at any concentration up to 100 μM. n.d = not determined.

for which the potency was sufficient to generate complete concentration−response curves appeared to be full agonists in the GPR109a assay, in that their efficacy was comparable to β-hydroxybutyrate, the only known naturally occurring ligand for the receptor. Similarly all the compounds were able to fully reverse the effect of forskolin in either receptor assay.

Thus far we had prepared all of our acifran analogues in racemic form. We next prepared a number of examples in chiral form by one of three methods. First we used the classical resolution approach to prepare (+)- and (−)-acifran, as has been previously reported.[8] This method was not generally applicable in a timely manner, however, so other examples were prepared either by formation of a diasteromeric amide with chiral α-methyl benzylamine, the resulting diastereomers separated by column chromatography and subjected to acidic hydrolysis to return the separated enantiomers, or by chiral HPLC on a semipreparative column. Interestingly, in preliminary studies, we were able to prepare such analogues in moderate enantiomeric excess (>50% ee) by application of a Sharpless chiral dihydroxylation protocol for the transformation of **11** to **12** (Scheme 2). The ee obtained, however, was limited by the modest *E*/*Z* ratio obtained in the alkene formation step, and this approach was not further optimized.

The EC_{50} values of the separated acifran enantiomers for the GPR109a and 109b receptors (Table 3) showed that, as with acifran itself,[8] the (+)-enantiomers were essentially twice as active as the racemic mixtures, whereas the activity of the (−)-enantiomers was more variable and highly dependent on purity. With increasing enantiomeric excess for the (−)-enantiomers, the activity at the GPR109a tended to zero, and although receptor agonist activity could not be ruled out, all of the observed GPR109a activity could be accounted for by the contaminating presence of small amounts of the (+)-enantiomer. This outcome is consistent with previous observations described above when chiral acifran was prepared via a Sharpless asymmetric dihydroxylation.[9] In that case, all of the activity of racemic acifran could be attributed to the (*S*)-enantiomer, and hence, from this precedent, we would assign the (+)-enantiomers

prepared in this study to the *S*-configuration. However, the absolute configuration was not confirmed experimentally.

The activity for GPR109b tracked closely with that for GPR109a, and no improvement in selectivity was obtained following resolution. This is perhaps not surprising given the exceptionally high homology that these two receptors share, although selective agonists of each receptor have been described.[3,13] It is likely though, that the binding interactions for acifran analogs are essentially identical with either receptor.

In summary, we have developed two new synthetic pathways suitable for the preparation of a wide range of acifran analogs and synthesized a number of compounds to explore the SAR. *meta*-Halo-phenyl analogs appeared more potent than acifran in our agonist functional assay at GPR109a, the high affinity niacin receptor. Our initial SAR studies on the synthetic analogs, demonstrated that a small hydrophobic ring is preferred for good activity at the position of phenyl ring of acifran. This led us to investigate thiophene analogs, resulting in the discovery of a number of compounds with superior activity to acifran itself. Resolution of a small number of analogues showed that the (+)-enantiomers were consistently more active than their antipodes, providing examples such as (+)-**5ah** and (+)-**5ai** with activity around 20-fold more potent than that of (racemic) acifran and 3-fold better than niacin at GPR109a in our hands and that show as much as 10-fold selectivity for that receptor over GPR109b.

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Supporting Information Available: Description of the general synthetic methods used. This material is available via Internet at http://pubs.acs.org.

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