Discovery of Novel Tricyclic Full Agonists for the G-Protein-Coupled Niacin Receptor 109A with Minimized Flushing in Rats

Hong C. Shen,*[,†] Fa-Xiang Ding,[†] Qiaolin Deng,[†] Larissa C. Wilsie,[‡] Mihajlo L. Krsmanovic,[‡] Andrew K. Taggart,[‡] Ester Carballo-Jane,[§] Ning Ren,[‡] Tian-Quan Cai,[‡] Tsuei-Ju Wu,[‡] Kenneth K. Wu,[‡] Kang Cheng,[‡] Qing Chen,[#] Michael S. Wolff,[#] Xinchun Tong,[#] Tom G. Holt,[#] M. Gerard Waters,[‡] Milton L. Hammond,[†] James R. Tata,[†] and Steven L. Colletti[†]

Departments of Medicinal Chemistry, Cardiovascular Diseases, Pharmacology, and Drug Metabolism, Merck Research Laboratories, Merck & Co., Inc., Rahway, New Jersey 07065-0900

Received February 5, 2009

Tricyclic analogues were rationally designed as the high affinity niacin receptor G-protein-coupled receptor 109A (GPR109A) agonists by overlapping three lead structures. Various tricyclic anthranilide and cycloalkene carboxylic acid full agonists were discovered with excellent in vitro activity. Compound **2g** displayed a good therapeutic index regarding free fatty acids (FFA) reduction and vasodilation effects in rats, with very weak cytochrome P450 2C8 (CYP2C8) and cytochrome P450 2C9 (CYP2C9) inhibition, and a good mouse pharmacokinetics (PK) profile.

Introduction

Niacin (nicotinic acid), a B group vitamin, has been used in humans to treat hyperlipidaemia since 1955. Niacin favorably alters lipid profiles by lowering total plasma cholesterol, triglycerides (TG[a]), very-low-density lipoprotein cholesterol (VLDL-C), and low-density lipoprotein cholesterol (LDL-C) and by raising high-density lipoprotein cholesterol (HDL-C).[1] Specifically, niacin increases HDL-C by approximately 25% at a daily dose of 1−2 g.[2] In addition, niacin reduces 30% of serum lipoprotein a (Lp(a)), which is also an independent risk factor for cardiovascular disease. Furthermore, niacin has been shown to slow the progression of atherosclerosis[3] and reduce coronary disease morbidity and mortality when taken alone or in combination with statins.[4] Despite these attributes, niacin treatment often causes intense cutaneous flushing that limits patient compliance. Thus, an extended release form of niacin has been introduced to attenuate the drug peak plasma levels, thereby reducing the severity of flushing.[5] More recently, Merck has developed a combination of an extended release form of niacin and a prostaglandin D_2 (PGD_2) receptor DP1 antagonist to reduce the niacin-induced flushing while maintaining the favorable lipid profile change.[6]

Prior to the identification of the niacin receptors, efforts seeking compounds with the desirable pharmacological properties of niacin were limited to simple heterocyclic carboxylic acids or tetrazoles, such as 5-(3-pyridyl)tetrazole,[7] "acipimox",[8] pyrazole carboxylic acid,[9] isoxazole carboxylic acid,[10] and acifran.[11]

In 2001, the niacin receptor in mouse was discovered and named "protein up-regulated in macrophages by interferon γ", or PUMA-G.[12] Subsequently, GPR109A and GPR109B, two closely related human homologues of PUMA-G, were found to bind niacin with high and low affinity, respectively.[13] Both GPR109A and PUMA-G are G_i-coupled receptors are expressed mainly in adipose, spleen, and myeloid lineage leukocytes.

Despite its long history of clinical use, niacin's precise mechanism of action toward HDL-C elevation and regression of atherosclerosis has not been well understood. It has been shown that the binding of niacin to GPR109A leads to the decrease of cAMP levels, which results in the downstream adipose lipolysis suppression. As a result, the release of free fatty acids (FFA) is decreased. It is postulated that depriving the liver of FFA substrates for TG synthesis may alter the lipid profile.[14] Importantly, the FFA reduction is GPR109A-dependent, since the niacin-induced lipolysis suppression was abrogated in PUMA-G knockout mice.[13b] On the other hand, the vasodilation effect of niacin is also dependent on GPR109A.[15] Therefore, it appears that a separation of these two pharmacological effects would be impossible. However, we[16] and Arena[17] both reported niacin receptor agonists that successfully achieved a superior therapeutic index of FFA reduction over vasodilation in the mouse model. Although the mechanistic rational for such observation is unclear, it is conceivable that these compounds might possess different tissue distribution and/or binding kinetics from niacin, which could contribute to the separation of FFA reduction and vasodilation effects.[18]

Since the discovery of the niacin receptors, extensive efforts have been devoted to develop niacin receptor agonists[19] by GlaxoSmithKline,[20] Arena,[21] Merck,[22] Roche,[23] Schering-Plough,[24] Incyte,[25] and the Ijzerman group.[26] To develop niacin receptor agonists with superior therapeutic profiles, reduced adverse effects, and distinctive structures, we explored novel tricyclic full agonists possessing remarkable affinity and selectivity for GPR109A. In addition, as rats and dogs are the most common species used for studies of niacin-induced inhibition of lipolysis,[27] a rat model was adopted to evaluate FFA reduction

* To whom correspondence should be addressed. Address: Merck Research Laboratories, Mail Code RY121-252, P.O. Box 2000, 126 East Lincoln Avenue, Rahway, New Jersey 07065-0900. Phone: (732) 594-1755. Fax: (732) 594-9473. E-mail: hong_shen@merck.com.

† Department of Medicinal Chemistry.

‡ Department of Cardiovascular Diseases.

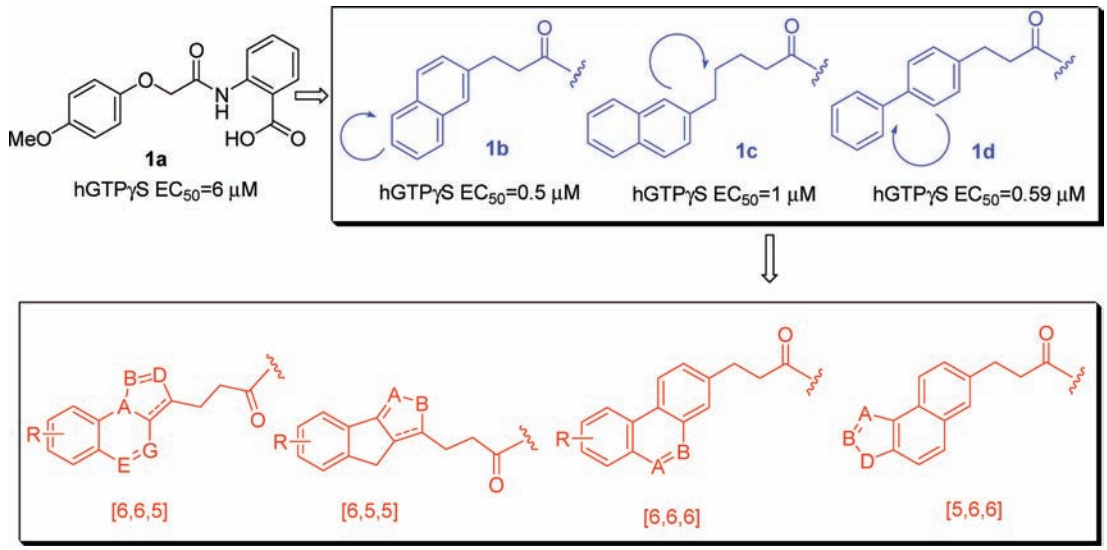
§ Department of Pharmacology.

Department of Drug Metabolism.

[a] Abbreviations: GPR109A, G-protein-coupled receptor 109A; FFA, free fatty acids; CYP2C8, cytochrome P450 2C8; CYP2C9, cytochrome P450 2C9; PK, pharmacokinetics; TG, triglycerides; VLDL-C, very-low-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; Lp(a), lipoprotein a; PGD_2, prostaglandin D_2; PUMA-G, protein up-regulated in macrophages by interferon γ; GPR109B, G-protein-coupled receptor 109B; SAR, structure−activity relationship; AUC, area under the curve; Cl_p, plasma clearance; DDQ, 2,3-dichloro-5,6-dicyano-*p*-benzoquinone; TI, therapeutic index.

10.1021/jm900151e CCC: $40.75 © 2009 American Chemical Society
Published on Web 03/23/2009

Scheme 1



and vasodilation. Herein we report the structure−activity relationship (SAR) of novel tricyclic agonists for GPR109A and pharmacological properties of several potent analogues.

Design

On the basis of the high-throughput screening hit **1a** and preliminary SAR study, three anthranilic acid derivatives (**1b**−**d**) were identified as leads. It was envisioned that the overlay of these structures might offer novel structures that could provide optimized interactions with the receptor (Scheme 1). Thus, four different scaffolds including tricyclic [6,6,5], [6,5,5], [6,6,6], and [5,6,6] systems were designed. Furthermore, the replacement of anthranilide fragment with cyclohexene or cyclopentene carboxylic acids that were applied in the biaryl series[16b] was also adopted.

In Vitro SAR

The in vitro activity of our compounds was measured by their competitive binding to GPR109A against [^{3}H]niacin and the guanine nucleotide exchange ([^{35}S]GTPγS) functional assay. Meanwhile, compound-induced inhibition of lipolysis was ascertained by the human adipocyte lipolysis assay.[28] This assay quantifies the reduction of the glycerol release by adipocytes resulting from the binding of agonists with the receptor.

Tables 1 and 2 summarize the SAR of tricyclic anthranilides, and Table 3 summarizes the SAR of the cycloalkene carboxylic acid class. These compounds were highly selective for GPR109A over GPR109B, as supported by the observation that a subset of compounds were completely inactive against GPR109B.

Analogues bearing different inner heterocycles, such as pyrazole, isoxazole, imidazole, or triazole, had good to excellent affinity for GPR109A. In general, a hydrogen bond donor, such as a hydroxyl group at the terminal position of the tricyclic scaffold, significantly increased the affinity. For example, analogue **2k** was 3- and 6-fold more active than **2l** in the binding and functional assay, respectively. The employment of a pyrazole to mimic the phenol hydroxyl group proved to be effective in that the activity of analogue **2p** (EC_{50} = 0.27 μM in hGTPγS assay) matched the corresponding naphthol compound **2q** (EC_{50} = 0.34 μM in hGTPγS assay). The advantage of pyrazole **2p** over naphthol **2q** is to avoid potential covalent protein binding that results from the oxidation of the naphthol moiety.[29]

The size of the middle ring of the tricyclic skeleton appeared to be crucial for activity. For instance, analogue **2e** containing the [6,6,5] ring system was almost 100-fold more active than **2m**, the [6,5,5] tricyclic counterpart. The use of a sulfur atom in **2k**, as a bioisostere for an ethylene group, gave a 30-fold higher activity than **2m**, where only one methylene group was used to bridge the phenol and isoxazole moiety. The presence of a nitrogen atom in the bridge of the tricyclic structure seemed to be detrimental, as shown by analogues **2i**, **2j**, and **2o**.

With the realization that plasma protein binding could lead to discrepancies between the activity of compounds in the absence of serum and the IC_{50} values of the human lipolysis assay in the presence of bovine serum (1%), as well as their in vivo efficacy, 4% of human serum was applied in an additional [^{3}H]niacin binding assay to estimate the serum shift. In general, high serum shift was observed for the tricyclic class (250- to 5000-fold). It was, however, unexpected that minor structural changes could lead to a significant difference in the magnitude of serum shift. For example, greater than 20-fold reduction in serum shift was observed for compound **2b** with respect to analogues **2a** and **2c**. Also, cyclopentene carboxylic acid derivatives typically had 8- to 50-fold smaller serum shift than the corresponding cyclohexene carboxylic acid analogues (**3g** vs **3a**; **3h** vs **3f**). Despite the large serum shift, analogues **2d**, **2h**, and **3e** were superior to niacin in the human adipocyte lipolysis inhibition assay, in which 1% of bovine serum was present.

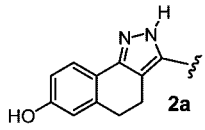
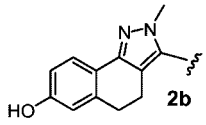
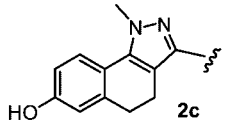
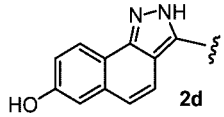
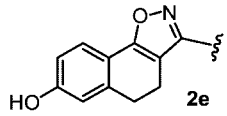
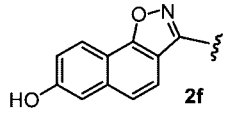
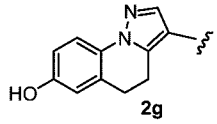
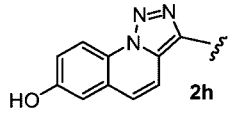
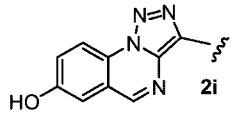
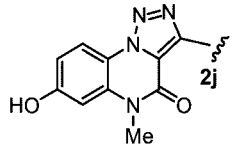
Chemistry

The syntheses of analogues **2a**−**f** commenced with tetralone **4**.[30] In general, the α-acylation of **4** was followed by a cyclization with hydrazines or hydroxamine to furnish the tricyclic scaffold. The subsequent amide formation and functional group manipulations afforded the desired analogues (Schemes 2 and 3). In both cases described in Schemes 2 and 3, the cyclization step was concomitant with esterification, and therefore, a subsequent hydrolysis was required to afford the corresponding acid.

As shown in Scheme 4, the synthesis of analogue **2g** involved a Cu-catalyzed intramolecular N-arylation as the key step to construct the tricyclic core.[31]

The preparation of **2h** featured a diazo transfer and the ensuing cyclization to form the triazole system (Scheme 5).

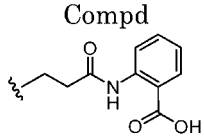
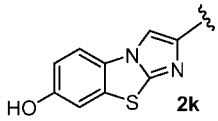
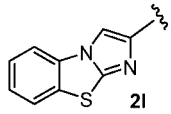
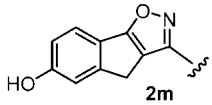
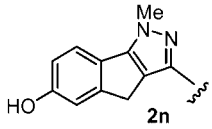
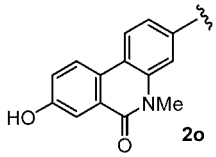
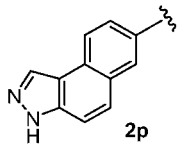
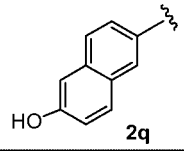
Table 1. In Vitro SAR of [6,6,5] Tricyclic Anthranilide Analogues[a]

Compd	GPR109A ^{3}H Niacin IC_{50} (μM)	GPR109A GTPγ-S EC_{50} (μM)	Serum shift[b] (fold)	h-adipocyte IC_{50} (μM)
Niacin	0.14	1.0	0	0.07
 **2a**	0.017	0.074	>5000	0.28
 **2b**	0.035	0.17	250	-[c]
 **2c**	0.13	0.84	>5000	-
 **2d**	0.005	0.034	>5000	0.06
 **2e**	0.004	0.05	1000	-
 **2f**	0.003	0.051	5000	0.15
 **2g**	0.013	0.064	360	0.11
 **2h**	0.008	0.040	1000	0.02
 **2i**	inactive	inactive	-	-
 **2j**	0.36	>5	-	-

[a] Values are based on one or two experiments, each in triplicate, and within 20% deviation upon repeat. [b] Ratio of compound binding IC_{50} in the presence and absence of 4% human serum. [c] Hyphens indicate that compounds were not tested.

To incorporate an additional nitrogen atom in the linker of the tricyclic ring, analogue **2i** was designed. The synthesis of **2i** was accomplished via two key steps. The first step was a nucleophilic aromatic substitution using the diazonium salt generated in situ as the electrophile and using sodium azide as the nucleophile (**24** and **25**). The second step incorporated

Table 2. In Vitro SAR of [6,5,5], [6,6,6], [5,6,6] Tricyclic and Naphthol Anthranilide Analogues[a]

Compd	GPR109A ^{3}H Niacin IC_{50} (μM)	GPR109A GTPγ-S EC_{50} (μM)	Serum shift [b] (fold)	h-adipocyte IC_{50} (μM)
				
 **2k**	0.011	0.12	1000	0.20
 **2l**	0.031	0.69	3000	-[c]
 **2m**	0.38	>10	>5000	-
 **2n**	2.1	7.3	>5000	-
 **2o**	0.34	2.6	>5000	-
 **2p**	0.018	0.27	>5000	-
 **2q**	0.021	0.34	3500	-

[a] Values are based on one or two experiments, each in triplicate, and within 20% deviation upon repeat. [b] Ratio of compound binding IC_{50} in the presence and absence of 4% human serum. [c] Hyphens indicate that compounds were not tested.

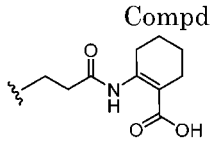
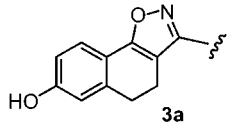
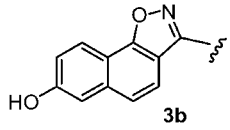
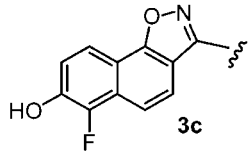
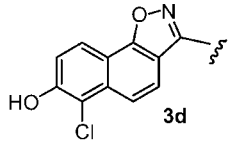
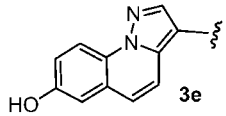
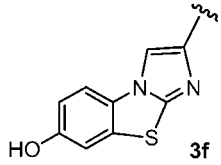
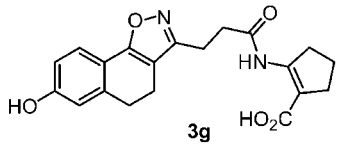
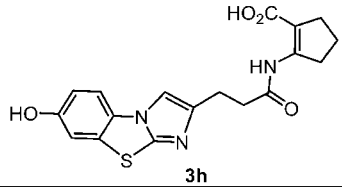
an elegant cyclization sequence involving the addition of malononitrile to the azide followed by the subsequent addition of the triazo group to the nitrile. The resulting enamine then condensed with the aldehyde to generate the tricyclic core (Scheme 6). It is noted that the hydrogenation of the double bond of intermediate **30** utilized *p*-$TsNHNH_2$ as the reducing reagent. Standard Pd/C hydrogenation gave a complex mixture presumably due to the reduction of the imine functionality.

The preparation of **2j** started with known intermediate **32** (Scheme 7),[32] which was formed in a manner analogous to the preparation of **2i**. The final demethylation step chemoselectively removed the methyl group of the ester and ether and left the lactam methyl group intact.

To generate analogue **2o** (Scheme 8), the key transformation involved a C−H activation followed by an intramolecular C−C bond formation.[33] A mixture of three products was obtained, including the dehalogenated product and two tricyclic regioisomers. Because of the difficult separation, the mixture (**42**) was carried on to a later step in which the pure intermediate **43** was obtained. Finally, demethylation of compound **43** afforded **2o**.

The synthesis of pyrazole analogue **2p** is shown in Scheme 9. The Heck reaction[34] of aryl bromide **45** and acrylamide **46** afforded bicyclic anthranilide **47**. The pyrazole ring was then constructed via a sequence of a regioselective bromination to afford **49** and via a Suzuki coupling to install the methyl group of intermediate **50**. An interesting *N*-nitroso formation followed by the base-mediated ring closure afforded compound **51**, which was subsequently transformed to analogue **2p**.

Table 3. In Vitro SAR of Tricyclic Cycloalkene Carboxylic Acid Analogues[a]

Compd	hGPR109A ^{3}H Niacin IC_{50} (μM)	hGPR109A GTPγ-S EC_{50} (μM)	Serum shift[b] (fold)	h-adipocyte IC_{50} (μM)
				
 **3a**	0.004	0.019	10000	-[c]
 **3b**	0.003	0.0065	30000	-
 **3c**	0.006	0.024	3000	-
 **3d**	0.001	0.043	20000	-
 **3e**	0.001	0.048	>4000	0.06[d]
 **3f**	0.005	0.076	4000	2.6
 **3g**	0.013	0.11	200	-
 **3h**	0.023	0.62	500	-

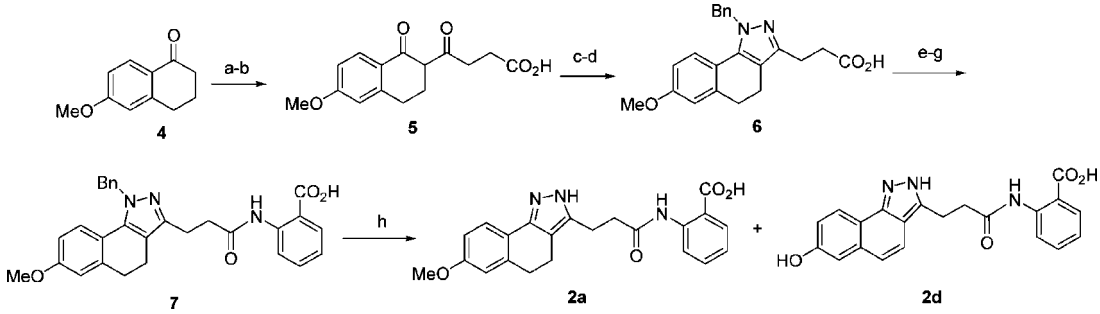
[a] On average, repeat determinations differed by ±20%. [b] Ratio of compound binding IC_{50} in the presence and absence of 4% human serum. [c] Hyphens indicate that compounds were not tested. [d] In this experiment, the niacin control has IC_{50} of 0.44 μM. The IC_{50} of **3e** was adjusted accordingly.

A representative cycloalkene carboxylic acid derivative is depicted in Scheme 10. The α-carbonylation of tetralone **4** using diethyl oxalate gave considerably higher yield than the corresponding reaction with 4-chloro-4-oxobutyrate (Scheme 2), in which the O-acylation was a severe side reaction. The fully conjugated tricyclic moiety was assembled via condensation of dicarbonyl compound **53** with hydroxylamine, followed by a DDQ dehydrogenation. A chain extension sequence allowed for the formation of acid intermediate **56**. An ensuing sequence of phenol protection, amide formation, and phenol deprotection afforded the desired analogue **3b**.

CYP Inhibition

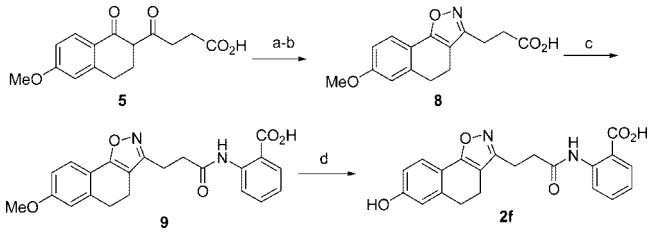
In a previous report,[16a] several structurally related analogues of **1a**−**d** induced strong CYP2C8 and 2C9 inhibition. Therefore, we screened the tricyclic compounds against these two CYP enzymes (Table 4). It was found that the inner ring of the

Scheme 2[a]



[a] Reagents and conditions: (a) LDA, $ClCO(CH_2)_2CO_2Me$, THF, −78 °C to room temp; (b) LiOH, THF/MeOH/H_2O, room temp, 100%, 27% over two steps; (c) $BnNHNH_2$ HCl salt, Et_3N, EtOH, 80 °C, 97%; (d) LiOH, THF/MeOH/H_2O, room temp, 100%; (e) MsCl, Et_3N, then methylanthranilide, 0 to 40 °C; (f) LiOH, THF/MeOH/H_2O, room temp; (g) 10% Pd/C, H_2, HCl, MeOH, room temp, 7% over three steps; (h) BBr_3, CH_2Cl_2, 0 °C to room temp, 40% for **2a**, 31% for **2d**.

Scheme 3[a]



[a] Reagents and conditions: (a) hydroxylamine hydrochloride, Et_3N, EtOH, 80 °C, 81%; (b) LiOH, THF/MeOH/H_2O, room temp, 28% over two steps; (c) $SOCl_2$, then anthranilic acid, 75 °C, 88%; (d) BBr_3, CH_2Cl_2, 0 °C to room temp, 67%.

tricyclic scaffold played a key role in this regard, and the presence of an inner pyrazole resulted in a pronounced drop of CYP inhibition (**2a**, **2c**, and **2g**). In addition, the full conjugation of the tricyclic system seemed to correlate to more potent CYP inhibition (**2d** vs **2a**; **2f** vs **2e**). With cycloalkene carboxylic acid replacing the anthranilide moiety, the CYP2C8 and 2C9 inhibition was considerably reduced (**3a** and **3g** vs **2e**; **3f** and **3h** vs **2k**; **3b** vs **2f**). Furthermore, within the subset of cyclohexene carboxylic acid analogues, the introduction of a fluoride or chloride increased the CYP 2C9 liability (**3c** and **3d** vs **3a**).

Pharmacology

The PK profiles of a subset of compounds in mice are shown in Table 5. Analogs **2a**, **2e**, **2g**, and **2h** generally have low clearance, low volume of distribution, low to moderate bioavailability, and moderate oral exposure. In contrast, the PK profiles of **2o**, **2p**, and **3f** were significantly improved as shown by their better half-life, excellent oral exposure, and good C_{max} despite modest bioavailability.

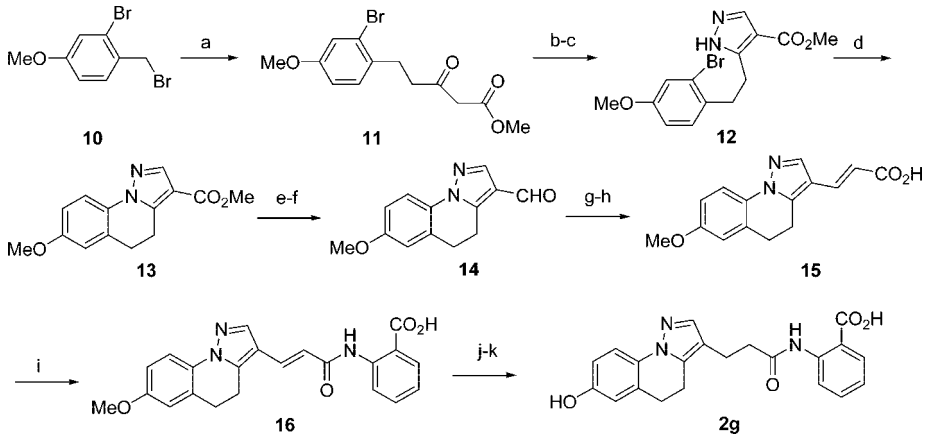
As FFA reduction is GPR109A-dependent,[13] FFA were measured as the primary biomarker. In this experiment, plasma samples were used to measure both FFA and drug levels after oral dosing of fasted rats. Niacin (EC_{50} = 0.67 μM in rat GTPγS assay) displayed dose-dependent FFA reduction and subsequent rebound at doses ranging from 3 to 100 mg/kg (Figure 1). The correlation of drug level and FFA reduction established the IC_{50} (∼5 μM) for niacin to reduce FFA (Figure 2). At 100 mg/kg (po), compound **2g** (EC_{50} = 2.7 μM in rat GTPγS assay) displayed similar maximum FFA reduction with respect to niacin (Figure 3). Furthermore, the FFA suppression lasted longer than niacin at 100 mg/kg dose in the time course of the study. The estimated EC_{50} for **2g** was ∼60 μM based on the correlation of FFA reduction and plasma drug level (Figure 4). The high plasma drug level required for efficacy was presumably due to the in vivo serum shift of compounds.

To ascertain the level of vasodilation induced by compounds in rats, the blood perfusion change of the rat ear was measured by applying laser Doppler flowmetry.[27] All animals were anesthetized and dosed subcutaneously. In sharp contrast to the niacin-elicited intense flushing at ≥3 mg/kg doses (Figure 5), compound **2g** induced only moderate flushing at 100 mg/kg (Figure 6). It was assumed that the extent of vasodilation correlated with the compound level at which the maximum FFA reduction was achieved. To measure the compound level 5−35 min after dosing in the vasodilation study, a parallel PK study was conducted with a second cohort of rats. In this study, rats were treated under the identical conditions as used in the flushing experiment, and blood was collected after the subcutaneous dosing of compound at 30 and 100 mg/kg. The peak plasma concentration of **2g** at which very moderate flushing occurred was ∼500 μM corresponding to the 100 mg/kg dose (Figure 7). In a similar manner, the peak drug level corresponding to niacin's vasodilation threshold dose (1 mg/kg) was approximately 7.6 μM. With the therapeutic index (TI) being defined as the ratio of the plasma level at the threshold dose for vasodilation (∼7.6 μM for niacin and ∼500 μM for **2g**) over the IC_{50} for FFA suppression (∼5 and ∼60 μM for niacin and compound **2g**, respectively), compound **2g** had a TI of at least 8 (∼500 μM/∼60 μM) whereas niacin had a much smaller TI of 1.5 (∼7.6 μM/∼5 μM) based on the same criteria.[27] Assuming that the rat model is predictive of the pharmacology for these compounds in humans, this observation indicated the possibility of developing niacin receptor agonists with a better therapeutic window regarding FFA reduction and vasodilation.

Conclusion

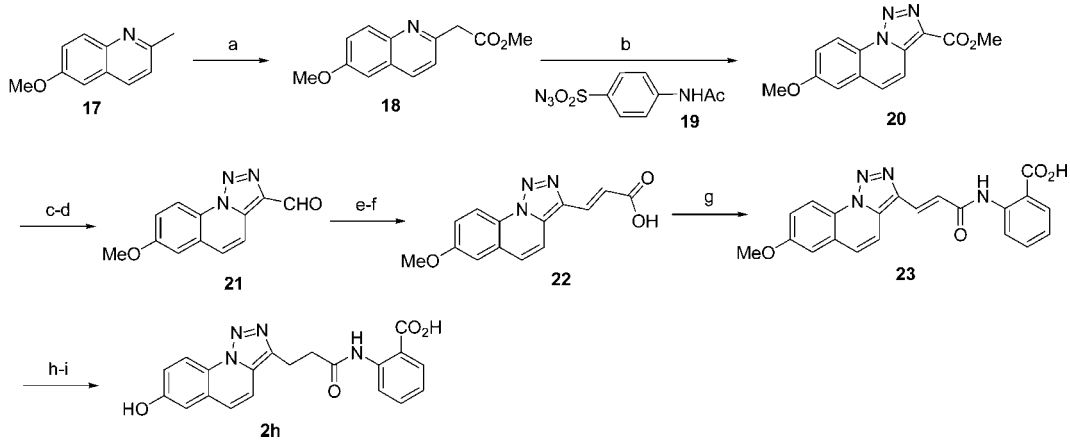
In summary, we have discovered novel, potent, and selective tricyclic agonists for the high affinity niacin receptor GPR109A. Three analogues (**2d**, **2h**, and **3e**) exhibited more efficacious lipolysis inhibition in human adipocytes than niacin. The introduction of the cycloalkene carboxylic acid moiety to replace the anthranilide led to a reduction of CYP inhibition in several cases. With very weak CYP2C8 and 2C9 inhibition and a moderate PK, analogue **2g** demonstrated good FFA suppression (100 mg/kg, po) with minor vasodilation (100 mg/kg, subcutaneous) in rats. The significantly larger TI of **2g** with respect to niacin suggested the potential of developing niacin receptor agonists with niacin's desired therapeutic effects but minimal vasodilation. In addition, the success of utilizing tricyclic

Scheme 4[a]



[a] Reagents and conditions: (a) LDA, methyl acetoacetate, THF, −78 to 0 °C, 79%; (b) $HC(OEt)_3$, Ac_2O, 80 °C; (c) NH_2NH_2, MeOH, 65 °C, 71% over two steps; (d) CuI, K_2CO_3, *N*,*N*′-dimethylethylenediamine, 110 °C, 41%; (e) Dibal-H, CH_2Cl_2, 0 °C, 67%; (f) diacetoxyiodobenzene, TEMPO, CH_2Cl_2, room temp, 99%; (g) $MeO_2CCH_2P(O)(OMe)_2$, *n*-BuLi, 0 °C, 95%; (h) LiOH, THF/MeOH/H_2O, room temp, 99%; (i) $SOCl_2$, then anthranilic acid, 100 °C, 45%; (j) 10% Pd/C, 42 psi of H_2, MeOH, room temp, 82%; (k) BBr_3, CH_2Cl_2, 0 °C to room temp, 70%.

Scheme 5[a]



[a] Reagents and conditions: (a) LDA, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine, THF, then $ClCO_2Me$, −78 °C to room temp, 41%; (b) NaH, THF, **19**, 78 °C to room temp, 20%; (c) DIBAL-H, CH_2Cl_2, 0 °C to room temp; (d) diacetoxyiodobenzene, TEMPO, CH_2Cl_2, room temp; (e) $MeO_2CCH_2P(O)(OMe)_2$, *n*-BuLi, THF, 0 °C to room temp; (f) LiOH, THF/MeOH/H_2O, room temp, 41% over four steps; (g) $SOCl_2$, 50 °C, then anthranilic acid, toluene, 120 °C; (h) 10% Pd/C, 45 psi of H_2, MeOH, room temp; (i) BBr_3, CH_2Cl_2, 0 °C to room temp, 12% over four steps.

scaffolds to rigidify biaryl conformation may serve as a general strategy in medicinal chemistry.

Experimental Section

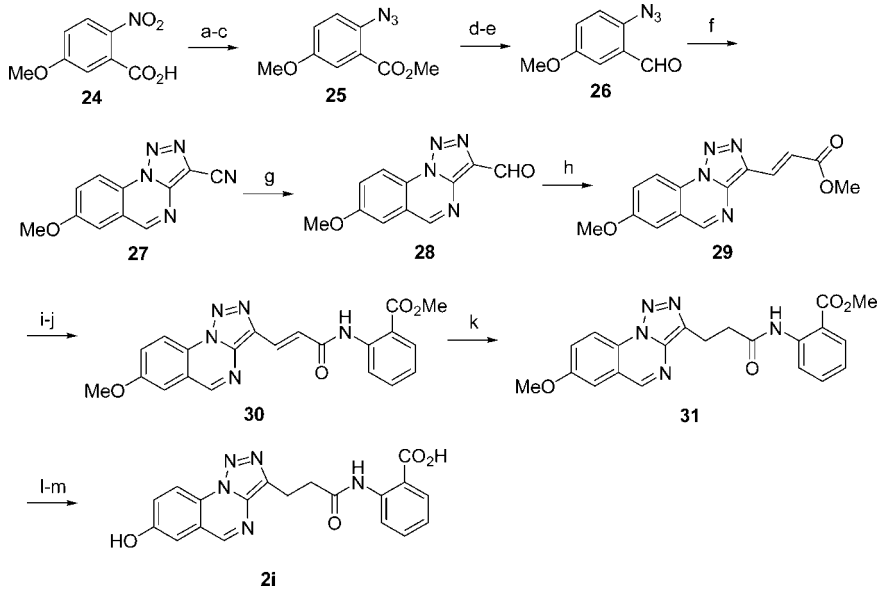
Nuclear magnetic resonance (NMR) spectra were recorded on a 500 MHz NMR spectrometer. Chemical shifts are given in parts per million (ppm) with the residual solvent signal used as reference. Coupling constants are reported in Hz. NMR abbreviations are used as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, dt = doublet of triplets, br = broad.

LC/MS data were recorded by the LC/MS system with an electrospray source, UV detector monitoring at 254 nm, a C18 column (5 μm, 50 mm × 4.6 mm) using a gradient of 5% v/v CH_3CN (containing 0.05% v/v TFA) in H_2O (containing 0.05% v/v TFA) (t = 0.0 min) to 100% v/v CH_3CN in H_2O (t = 20.0 min), and flow rate of 3.5 mL/min.

Flash chromatography was performed using Si 25 S/M cartridges. Analytical thin layer chromatography (TLC) was performed with 0.25 mm silica gel 60-F commercial silica gel plates. Visualization was accomplished with UV light or potassium permanganate stain, followed by heating. Preparative HPLC was conducted on a reverse phase HPLC using a C18 column (10 μm, 250 mm × 20 mm), 5% (v/v) CH_3CN (containing 0.1% v/v TFA) in H_2O (containing 0.1% v/v TFA) gradient to 100% CH_3CN, 20−50 mL/min, λ = 254 nm. All compounds have >90% purity by both NMR and RP-HPLC. In most cases, compounds have >95% purity by NMR and RP-HPLC. For retention time and NMR spectra of all final analogues, refer to the Supporting Information.

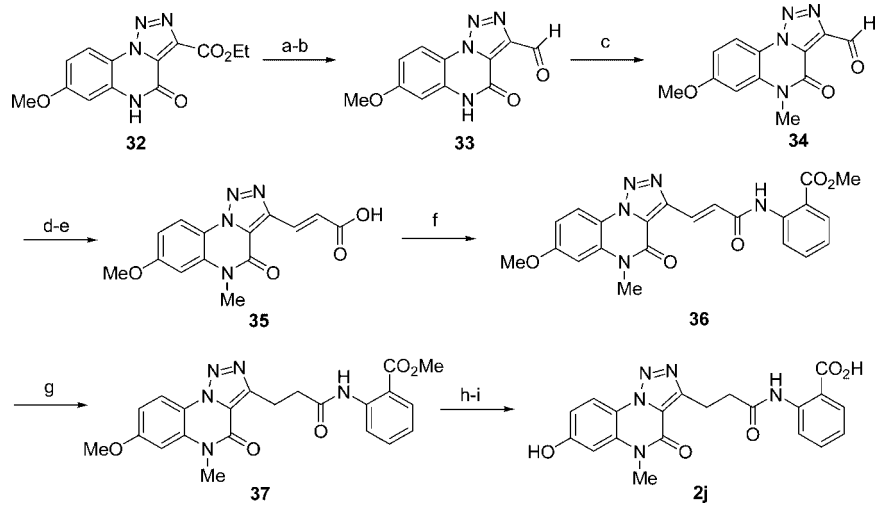
Procedure for the Synthesis of 2a. To a solution of diisopropylamine (5.3 g, 52 mmol) in 200 mL of THF was added *n*-butyllithium (22.4 mL, 56 mmol, 2.5 M in hexane) at −78 °C. The resulting solution was stirred at −78 °C for 30 min and then at room temperature for an additional 30 min. The solution was cooled to −78 °C, and to this solution was dropwise added a solution of tetralone **4** (7.03 g, 39.9 mmol) in 80 mL of THF. After 1 h at −78 °C, to the above solution was added 4-chloro-4-oxobutyrate (8.43 g, 6.84 mL, 56 mmol) in one portion. The resulting solution was warmed to room temperature over 2 h. The solvent was then evaporated, and the residue was diluted with 200 mL of THF/MeOH/water (v/v/v, 3:1:1). To this mixture was added 100 mL of lithium hydroxide (1 M in water), and the resulting solution was stirred overnight. After removal of some solvent in vacuo, the remaining aqueous layer was extracted with ethyl acetate (100 mL × 3). The aqueous phase was acidified with HCl until pH 3 was attained. The mixture was extracted with ethyl acetate (100 mL × 2). The combined organic fractions were dried with sodium sulfate and concentrated in vacuo to give the product **5** as

Scheme 6[a]



[a] Reagents and conditions: (a) $TMSCHN_2$, MeOH, room temp; (b) Pd/C, 40 psi of H_2, room temp; (c) $NaNO_2$, HCl, then NaN_3, 87% over three steps; (d) DIBAL-H, CH_2Cl_2, room temp; (e) PCC, CH_2Cl_2, room temp, 71% over two steps; (f) malononitrile, piperidine, room temp, 87%; (g) DIBAL-H, DME, CH_2Cl_2, −78 °C, 13% (53% based on recovered starting material); (h) $MeCO_2CH_2P(O)(OMe)_2$, *n*-BuLi, THF, 0 °C to room temp; (i) LiOH, THF/MeOH/H_2O, room temp; (j) $SOCl_2$, 50 °C, then anthranilic acid, toluene, 120 °C; (k) *p*-$TsNHNH_2$, MeOH, room temp, 12% over four steps; (l) LiOH, THF/MeOH/H_2O, room temp; (m) BBr_3, CH_2Cl_2, 0 °C to room temp, 75% over two steps.

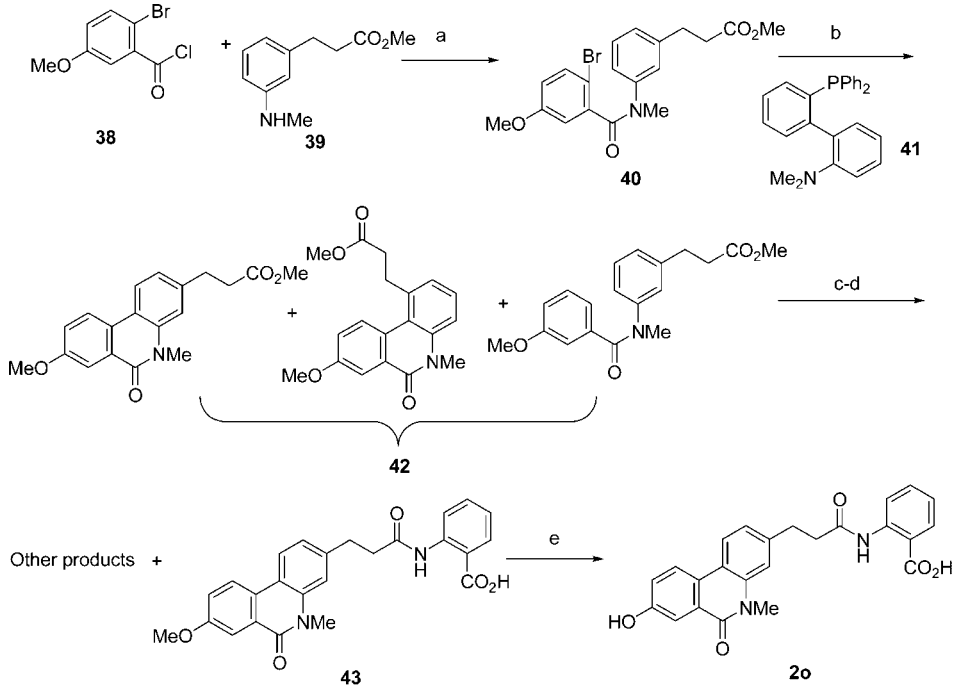
Scheme 7[a]



[a] Reagents and conditions: (a) $LiBH_4$, THF, 65 °C; (b) Dess−Martin reagent, CH_2Cl_2, room temp, 84% over two steps; (c) MeI, K_2CO_3, DMF, 80 °C, 15%; (d) $MeO_2CCH_2P(O)(OMe)_2$, *n*-BuLi, THF, 0 °C to room temp; (e) LiOH, THF/MeOH/H_2O, room temp, 81% over two steps; (f) $(COCl)_2$, cat. DMF, CH_2Cl_2, then methyl anthranilate, triethylamine, 29%; (k) *p*-$TsNHNH_2$, MeOH, reflux, 50%; (h) LiOH, THF/MeOH/H_2O, room temp, 90%; (i) BBr_3, CH_2Cl_2, 0 °C to room temp, 14%.

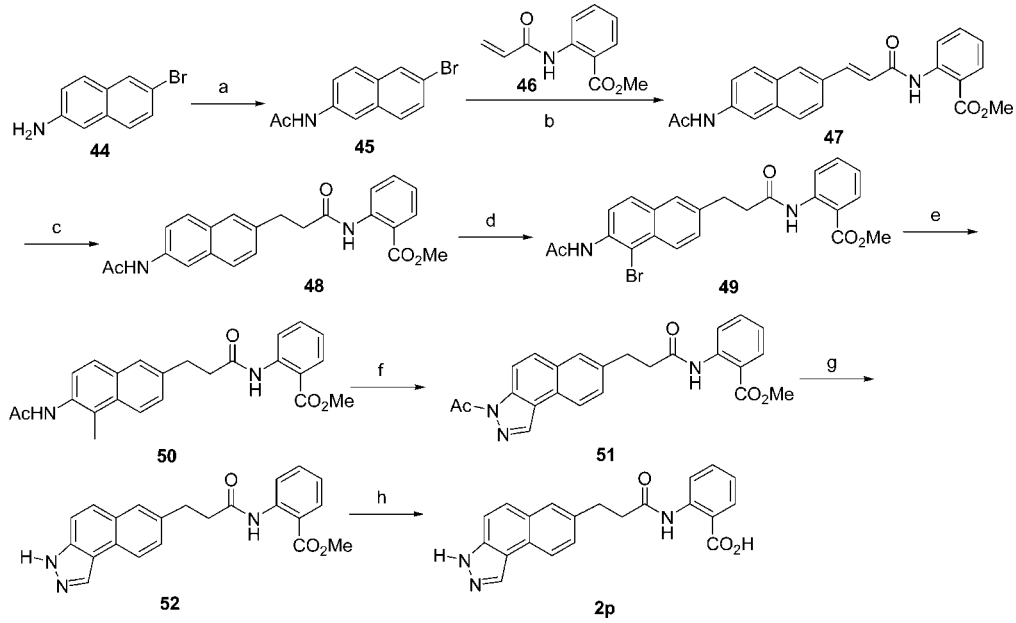
a gray solid (3.0 g, 10.9 mmol, 27% over two steps). To **5** (0.434 g, 1.57 mmol) was added benzylhydrazine dihydrochloride salt (338 mg, 1.73 mmol), triethylamine (0.35 g, 3.46 mmol), and ethanol (100 mL). The mixture was heated at reflux for 2 h. After removal of the solvent, the residue was purified by RP-HPLC to give **6** (0.55 g, 1.52 mmol, 97%) as an oil. To the solution of **6** (0.34 g, 0.94 mmol) in methylene chloride (30 mL) was added trifluoromethylsulfonyl chloride (215 mg, 1.88 mmol) and triethylamine (0.53 mL, 3.75 mmol) at 0 °C. After 30 min, the resulting solution was warmed to room temperature for 20 min and then cooled to 0 °C again. Methyl anthranilate (354 mg, 2.35 mmol) was added to the above solution at 0 °C, and the resulting solution was heated at 40 °C overnight. After removal of the solvent, the residue was purified by RP-HPLC to give the amide intermediate. To this intermediate (70 mg) was added a mixture solvent of THF/MeOH/water (10 mL, v/v/v, 3/1/1) and lithium hydroxide (1 mL, 1 N), and the resulting mixture was stirred at room temperature overnight. After removal of the organic solvents, the aqueous phase was acidified to pH 4 by HCl (1 N). The precipitate was extracted with ethyl acetate. The organic phase was washed with brine, dried over sodium sulfate, and concentrated in vacuo to give the desired intermediate. To this intermediate (65 mg) was added methanol (50 mL), one drop of concentrated hydrochloride, and palladium on carbon (10%, 30 mg). The resulting mixture was subjected to hydrogenation for 8 h before it was filtered and concentrated in vacuo. The resulting residue was purified by RP-HPLC to give **7** (25 mg, 0.064 mmol, 7% over three steps). To the suspension of **7** (25 mg, 0.064 mmol) in methylene chloride (30 mL) was added boron tribromide (0.64 mL, 1 N) at 0 °C. The resulting mixture was stirred at room temperature overnight before it was quenched by water at 0 °C. The aqueous portion was extracted with ethyl acetate. The combined organic phase was concentrated in vacuo.

Scheme 8[a]



[a] Reagents and conditions: (a) Et_3N, CH_2Cl_2, room temp, 100%; (b) $Pd(OAc)_2$ (0.13 equiv), **41** (0.21 equiv), K_2CO_3, 125 °C, 77%; (c) LiOH, THF/MeOH/H_2O, room temp; (d) MsCl, triethylamine, anthranilic acid, 0 °C to room temp, 3% over two steps; (e) BBr_3, CH_2Cl_2, 75%.

Scheme 9[a]

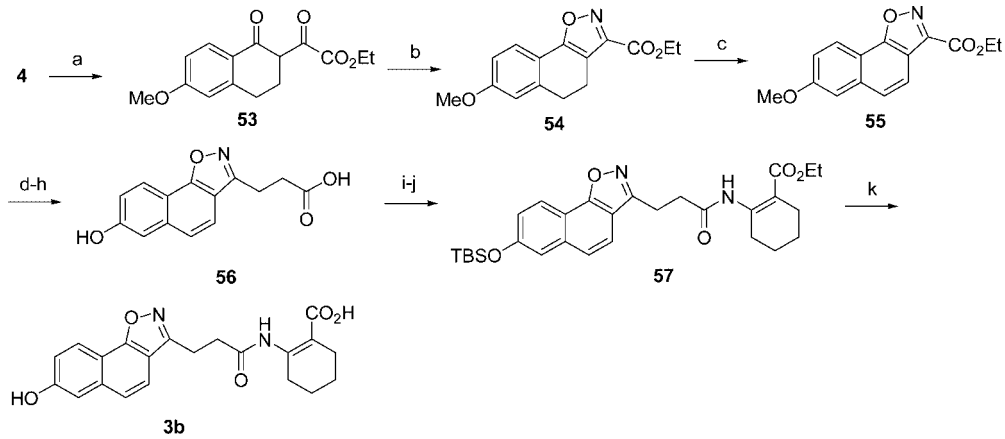


[a] Reagents and conditions: (a) acetic anhydride, Et_3N, cat. DMAP, CH_2Cl_2, room temp, 90%; (b) $Pd(OAc)_2$, P(*O*-Tol)$_3$, Et_3N, Bu_4NCl, 4 Å molecular sieves, DMF, 109 °C, 77%; (c) $Pd(OH)_2$/C, H_2, CH_2Cl_2/MeOH, room temp, 33%; (d) Br_2, $CHCl_3$, 0 °C, 89%; (e) $Pd(PPh_3)_4$, $MeB(OH)_2$, aq K_2CO_3, dioxane, 100−110 °C, 38%; (f) amyl nitrite, KOAc, HOAc, $CHCl_3$, 18-crown-6, 64%; (g) NaOEt, MeOH; (h) LiOH, THF/MeOH/H_2O, room temp, 68% over two steps.

The residue was purified by RP-HPLC to give **2a** (10 mg, 0.026 mmol, 41%) and **2d** (7.2 mg, 0.019 mmol, 30%). **2a**: ^{1}H NMR (CD_3OD, 500 MHz) δ 8.54 (1H, d), 8.05 (1H, d), 7.54 (1H, t), 7.47 (1H, d), 7.13 (1H, t), 6.76 (1H, s), 6.72 (1H, dd), 3.14 (2H, t), 2.89 (4H, m), 2.76 (2H, t); LCMS *m*/*z* 378 ($M^+ + 1$). HRMS calcd for $C_{21}H_{19}O_4N_3$ [$M + 1^+$]: 378.1454. Found: 378.1392. **2d**: ^{1}H NMR (CD_3OD, 500 MHz) δ 8.54 (1H, d), 8.14 (1H, d), 8.03 (1H, dd), 7.64 (1H, d), 7.53 (1H, m), 7.31 (1H, d), 7.23 (1H, d), 7.13 (1H, dd), 7.11 (1H, t), 3.43 (2H, t), 2.97 (2H, t); LCMS *m*/*z* 376 ($M^+ + 1$). HRMS calcd for $C_{21}H_{17}O_4N_3$ [$M + 1^+$]: 376.1297. Found: 376.1283.

Procedure for the Synthesis of 2e. To a solution of **5** (81 mg, 0.29 mmol) in ethanol (20 mL) was added hydroxylamine hydrochloride salt (41 mg, 0.59 mmol). The mixture was heated at reflux overnight. After removal of the solvent, to the residue was added lithium hydroxide (1 N) in THF and methanol. The mixture was stirred at room temperature for 5 h and concentrated. The residue was then purified by RP-HPLC to give a mixture of two isoxazole

Scheme 10[a]



[a] Reagents and conditions: (a) LDA, $(EtOCO)_2$, −78 °C to room temp, 71%; (b) hydroxylamine hydrochloride, Et_3N, ethanol, 80 °C, 69%; (c) DDQ, dioxane, 100 °C, 84%; (d) DIBAL-H, −78 °C, 89%; (e) $MeO_2CCH_2P(O)(OMe)_2$, *n*-BuLi, THF, 0 °C to room temp; (f) LiOH, THF/MeOH/H_2O, room temp; (g) BBr_3, CH_2Cl_2, 0 °C to room temp; (h) 10% Pd/C, H_2, MeOH, room temp, 53% over five steps; (i) TBSCl, Et_3N, CH_2Cl_2; (j) oxalyl chloride, cat. DMF, CH_2Cl_2, then ethyl-2-amino-1-cyclohexene carboxylate; (k) LiOH, THF/MeOH/H_2O, room temp, 14% over three steps.

Table 4. CYP2C8 and CYP2C9 Inhibition[a]

compd	CYP2C8/2C9, IC_{50}[b] (μM)	compd	CYP2C8/2C9, IC_{50}[b] (μM)
2a	23/26	**2p**	-/<0.14
2c	-/17	**3a**	44/11
2d	5.8/9.2	**3b**	-/12
2e	5.9/2.2	**3c**	-/2
2f	-/0.99	**3d**	-/1.5
2g	11/18	**3e**	-/19
2h	-/1.5	**3f**	-/12
2l	-/4.4	**3g**	47/47
2m	20/8.5	**3h**	-/12
2o	-/4.7		

[a] The CYP2C8 inhibition assay in human liver microsomes used paclitaxel and montelukast as the substrate and positive control, respectively. The CYP2C9 inhibition assay in human liver microsomes used diclofenac and sulfaphenazole as the substrate and positive control, respectively. [b] Hyphens indicated that compounds were not tested.

Table 5. Mouse PK Profiles[a]

compd	*F* (%)	Cl_p ((mL/min)/kg)	Vd_{ss} (L/kg)	C_{max} (μM)	$T_{1/2}$ (h)	$AUCN_{po}$ (μM·h·kg/mg)
2a	1.4	2.4	0.44	0.073	2.1	0.26
2e	8.2	9.7	1.1	0.62	1.8	0.38
2g	17	12	1.8	0.35	2.9	0.66
2h	6	7	0.95	0.17	1.7	0.4
2o	10	1.4	0.26	1.26	2.3	3.1
2p	24	0.6	0.19	3.9	3.5	20
3f	25	0.9	0.32	3.8	4.4	15.6
3g	7.5	4.1	0.20	1.83	1.9	1.7

[a] Values are an average of three male C57BL/6 mice. The iv doses were formulated at 1 mg/kg and the oral doses were formulated at 2 mg/kg in DMSO/Tween/water and given by oral gavage (5:10:85). *F*, oral bioavailability; Cl_p, plasma clearance; Vd, volume of distribution; C_{max}, observed maximal plasma concentration following oral dosing; $T_{1/2}$, terminal half-life; $AUCN_{po}$, normalized area under the curve for oral dosing.

annulated regioisomers. To the major isomer **8** (22 mg, 0.08 mmol) was added 1 mL of thionyl chloride. The resulting clear solution was heated at 75 °C for 90 min, and thionyl chloride was removed in vacuo. To the residue was added toluene (10 mL) and then anthranilic acid (22 mg, 0.16 mmol). The mixture was heated at 75 °C for 2 h. The resulting yellow slurry was concentrated and purified by RP-HPLC to give **9** (28 mg, 0.07 mmol, 88%) as a brown solid. To this intermediate (28 mg, 0.07 mmol) in 15 mL of dichloromethane was added boron tribromide (0.57 mL, 0.57 mmol, 1 M in dichloromethane) at 0 °C. The mixture was warmed to room temperature and stirred overnight. The mixture was then quenched with water at 0 °C and warmed to room temperature. The mixture was concentrated and purified by RP-HPLC to give the desired

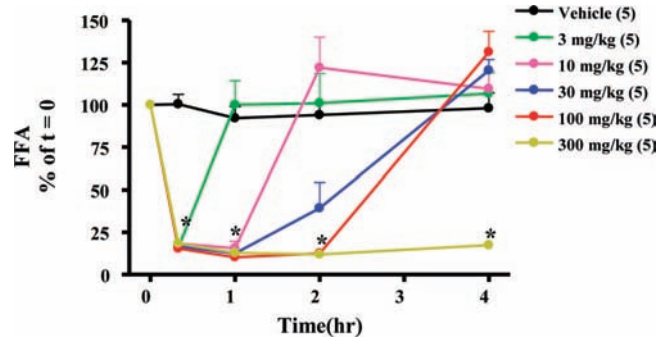


Figure 1. Effect of niacin on FFA reduction in Sprague−Dawley rats (fasted, oral dosing, 5% HPBCD).

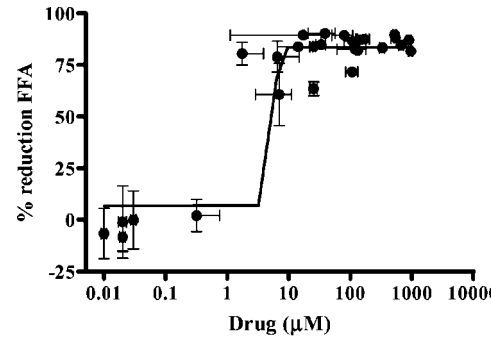


Figure 2. Correlation of FFA reduction with drug level of niacin ($IC_{50} \approx 5$ μM).

compound **2e** (18 mg, 0.047 mmol, 67%) as a white solid. ^1H NMR (CD_3OD, 500 MHz) δ 8.56 (1H, d), 8.07 (1H, dd), 7.55 (1H, m), 7.42 (1H, d), 7.14 (1H, t), 6.75 (1H, s), 6.71 (1H, dd), 3.07 (2H, t), 2.94 (2H, t), 2.87 (2H, t), 2.72 (2H, t); LCMS *m*/*z* 379 ($M^+ + 1$). HRMS calcd for $C_{21}H_{18}O_5N_2$ [$M + 1^+$]: 379.1294. Found: 379.1291.

Procedure for the Synthesis of 2g. To a solution of diisopropylamine (5.1 g, 7.1 mL, 50 mmol) in 80 mL of THF was added *n*-butyllithium (22 mL, 55 mmol, 2.5 M in hexane) at 0 °C. After 30 min, the resulting solution was cooled to −78 °C. To this solution at −78 °C was added a solution of methyl acetoacetate (2.3 g, 2.2 mL, 20 mmol) in 20 mL of THF dropwise. After 30 min, to this solution was added *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (2.3 g, 3.0 mL, 20 mmol). The resulting red solution was warmed to 0 °C and stirred for 0.5 h. To this solution was slowly added a solution of **10** (5.6 g, 20 mmol) in 20 mL of THF. The

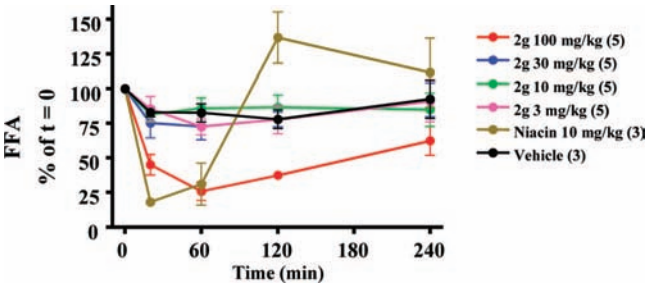


Figure 3. Effect of **2g** on FFA reduction in Sprague−Dawley rats (fasted, oral dosing, 5% HPBCD).

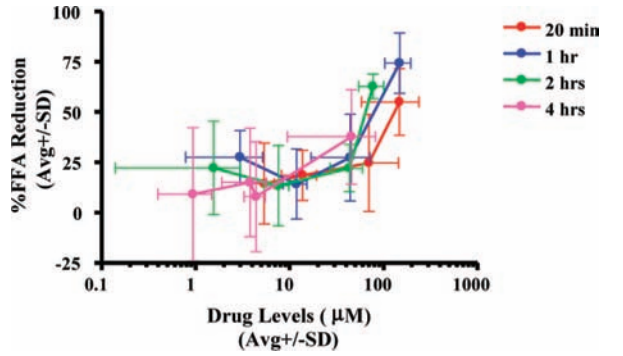


Figure 4. Correlation of FFA reduction with drug level of **2g** ($IC_{50} \approx 60\ \mu M$).

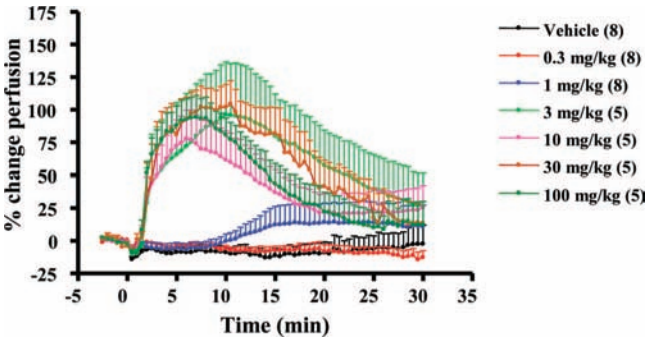


Figure 5. Effect of niacin on Sprague−Dawley rat vasodilation (5% HPBCD, subcutaneous dosing).

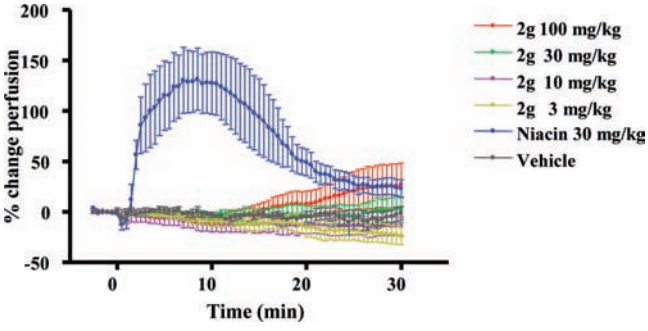


Figure 6. Effect of **2g** on Sprague−Dawley rat vasodilation (5% HPBCD, subcutaneous dosing).

resulting solution was slowly warmed to room temperature and stirred for 2 h. The solution was then quenched with 1 N HCl (15 mL). The mixture was then extracted with ethyl acetate (100 mL). The organic layer was dried with sodium sulfate and concentrated. The residue was purified by silica gel chromatography (2−20% ethyl acetate−hexanes) to provide **11** as a light-yellow oil (5.0 g, 16 mmol, 79%). A mixture of this intermediate ketoester (5.0 g, 16 mmol), acetic anhydride (3.25 g, 3 mL, 32 mmol), and triethyl

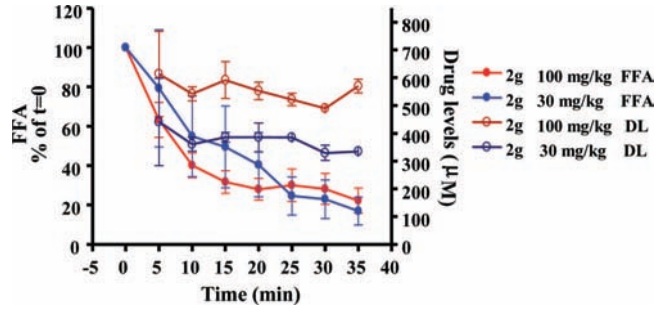


Figure 7. Correlation of FFA reduction and the drug level of **2g** (5% HPBCD, subcutaneous dosing, DL = drug level).

orthoformate (2.3 g, 2.75 mL, 16 mmol) was heated at 135 °C for 1.5 h. The crude was quickly purified by silica gel chromatography using 5−20% ethyl acetate−hexanes to give a dark oil, which was then added to a mixture of hydrazine monohydrate (20 mL, 64−65%) and ethanol (300 mL). The mixture was heated overnight, concentrated, and purified by silica gel chromatography (5−50% ethyl acetate−hexanes) to provide compound **12** and its ethyl ester (upon ester exchange) as an off-white solid (3.8 g, 11.2 mmol, 71% over two steps). A mixture of **12** and its ethyl ester (30 mg, 0.088 mmol), copper(I) iodide (1 mg, 0.0044 mmol), *N*,*N*′-dimethylethylenediamine (1.6 mg), potassium carbonate (26 mg, 0.18 mmol), and toluene (2 mL) was heated at 110 °C under nitrogen overnight. The mixture was purified by RP-HPLC to give a white solid **13** (16 mg, 0.062 mmol, 70%). Following a similar sequence as described for the preparation of **2h** from **20** provided the desired compound **2g** (10 mg) as a white solid. ^{1}H NMR (acetone-d_6, 500 MHz) δ 11.2 (1H, d), 8.75 (1H, d), 8.08 (1H, dd), 7.61 (2H, m), 7.45 (1H, s), 7.13 (1H, t), 6.76 (2H, m), 2.88 (2H, t), 2.70 (2H, t); LCMS *m*/*z* 378 ($M^+ + 1$). HRMS calcd for $C_{21}H_{19}O_4N_3$ [$M + 1^+$]: 378.1454. Found: 378.1392.

Procedure for the Synthesis of 2h. To a solution of diisopropylamine (2.34 g, 3.3 mL, 23 mmol) in 50 mL of THF was added *n*-butyllithium (16 mL, 25.3 mmol, 1.6 M in hexane) at −78 °C. After 10 min, the resulting solution was warmed to 0 °C and stirred for 30 min. To this solution at −78 °C was added a solution of **17** (2 g, 11.5 mmol) in 25 mL of THF dropwise. After 5 min, to this solution was added *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (2.67 g, 3.5 mL, 23 mmol). The resulting red solution was stirred at −78 °C for 1 h. To this solution was then slowly added methyl chloroformate (2.17 g, 1.77 mL, 23 mol). The resulting solution was slowly warmed to room temperature. The solution was then quenched with water (250 mL). The mixture was then extracted with ethyl acetate (100 mL). The organic layer was dried and concentrated. The residue was purified by silica gel chromatography (2−25% ethyl acetate−hexanes) to give a mixture of products, which was further purified by RP-HPLC to give **18** (1.1 g, 4.76 mmol, 41%, washed with sodium carbonate) as a brown solid. To **18** (1.1 g, 4.76 mmol) and sodium hydride (230 mg, 5.71 mmol, 60% in petroleum oil) was added 80 mL of THF at −78 °C. The mixture was slowly warmed to room temperature. After 30 min, to this mixture was added 4-acetamidobenzenesulfonyl azide **19** (1.37 g, 5.71 mmol) in one portion. The slurry was stirred at room temperature for 3 h. To this mixture was added water, and the resulting mixture was extracted with dichloromethane (100 mL × 5). The combined organic layer was dried and concentrated. The residue was taken up with methanol and filtered. The solid was washed with methanol and became light-yellow. The filtrate was concentrated and purified by RP-HPLC to give **20** (300 mg, 1.17 mmol, 20%), which was combined with the collected light-yellow solid. To a solution of **20** (300 mg, 1.17 mmol) in 20 mL of dichloromethane was added DIBAL-H (3.5 mL, 3.5 mmol, 1 M in toluene) at 0 °C. The mixture was warmed to room temperature and stirred for 4 h. The mixture was then quenched with water and saturated Rochelle's salt (100 mL). The aqueous layer was then extracted with 30% isopropyl alcohol in chloroform. The combined fractions were dried with sodium sulfate and concentrated in vacuo

to give the desired alcohol as a light-yellow solid that contained some inorganic salt. To a solution of this alcohol in 30 mL of dichloromethane were added diacetoxyiodobenzene (450 mg, 1.4 mmol) and 15 mg of TEMPO. The resulting slurry turned clear. After 16 h at room temperature, the mixture was washed with sodium sulfite solution and extracted twice with 30% isopropanol in chloroform (100 mL). The combined organic fractions were dried with sodium sulfate and concentrated in vacuo to give **21** as a yellow solid. To a solution of trimethylphosphonoacetate (499 mg, 0.44 mL, 2.74 mmol) in 30 mL of THF was added *n*-butyllithium (1.21 mL, 2.5 M in hexane, 3.0 mmol) at 0 °C. After 15 min, the mixture was warmed to room temperature and transferred to a solution of the aldehyde (310 mg, 1.37 mmol) in 10 mL of THF. The resulting slurry was stirred at room temperature for 2 h, and to this mixture was added 50 mL of water. The mixture was then extracted with 100 mL of ethyl acetate and 100 mL of 30% isopropanol in chloroform. The combined organic fractions were dried with sodium sulfate and concentrated to give the enoate as a yellow solid. To this enoate were added 50 mL of THF/methanol/water (3:1:1, 50 mL) and 1 N lithium hydroxide solution (15 mL). After 4 h, the clear yellow solution was washed with ethyl acetate (100 mL). The aqueous layer was acidified with concentrated HCl until precipitate appeared. This mixture was extracted four times with 30% isopropanol in chloroform (50 mL). The combined organic layers were dried with sodium sulfate and concentrated in vacuo to give compound **22** (130 mg, 0.48 mmol, 41% over four steps) as a yellow solid. To compound **22** (130 mg, 0.48 mmol) was added 3 mL of thionyl chloride. The resulting clear solution was heated at 50 °C for 30 min, and thionyl chloride was removed in vacuo. To the residue was added toluene (20 mL) and then anthranilic acid (137 mg, 1.0 mmol). The mixture was heated at 120 °C for 3 h. The resulting yellow slurry was washed with acetone and methanol and filtered to give **23** as a yellow solid. To a slurry of this intermediate (155 mg, 0.40 mmol) in 20 mL of methanol was added 50 mg of Pd/C (10%). The mixture was held under 45 psi of hydrogen gas overnight. The slurry was filtered, and the solid was washed with acetone (50 mL) and 30% isopropanol in chloroform (500 mL). The filtrate was concentrated to give a yellow solid. To this methyl ether (40 mg, 0.10 mmol) in 5 mL of dichloromethane was added boron tribromide (3 mL, 3 mmol, 1 M in dichloromethane) at 0 °C. The mixture was warmed to room temperature and stirred for 12 h. The mixture was then quenched with water at −78 °C and warmed to room temperature. The mixture was concentrated and purified by RP-HPLC to give **2h** (21 mg, 0.056 mmol, 12% over four steps) as a white solid. ^{1}H NMR (CD_3OD, 500 MHz) δ 8.53 (2H, t), 8.03 (1H, dd), 7.66 (1H, d), 7.54 (1H, m), 7.50 (1H, d), 7.30 (1H, dd), 7.26 (1H, d), 7.13 (1H, t), 3.44 (2H, t), 2.99 (2H, t); LCMS *m*/*z* 377 ($M^+ + 1$). HRMS calcd for $C_{20}H_{16}O_4N_4$ [$M + 1^+$]: 377.1250. Found: 377.1252.

Procedure for the Synthesis of 2i. To 2-nitro-5-methoxybenzoic acid **24** (4 g, 20.3 mmol) in 35 mL of methanol was added trimethylsilyldiazomethane (35 mL, 70 mmol, 2 M in dichloromethane) at room temperature dropwise. The mixture was stirred at room temperature for 10 h. To the mixture was added several drops of acetic acid. The resulting solution was concentrated in vacuo to give the methyl ester of **24** as a brown solid. To this intermediate was added 150 mg of Pd/C (10%). The mixture was stirred under 40 psi of hydrogen gas for 5 h. The mixture was filtered and washed with dichloromethane. The filtrate was concentrated in vacuo to give the aniline intermediate as a dark-red oil. To this aniline intermediate were added 30 mL of ethanol and 5 mL of concentrated HCl. To this mixture at 0 °C was dropwise added a solution of sodium nitrite (5.6 g, 81.2 mmol) in 15 mL of water to form the diazonium salt. After 1 h at 0 °C, to the resulting dark-red solution was slowly added sodium azide (8.6 g, 132 mmol) in 15 mL of water. After 1 h at 0 °C, the slurry was filtered and washed with saturated sodium carbonate solution and water to give azide **25** (3.6 g, 18 mmol, 87% over three steps) as a red solid. The same DIBAL-H reduction procedure as described in the synthesis of **2g** gave the corresponding benzyl alcohol as a dark-red oil. To this oil in 100 mL of dichloromethane was added PCC (8 g) at 0 °C. The mixture was stirred at room temperature for 4 h and purified by silica gel chromatography (2−20% ethyl acetate−hexanes) to give the aryl azide aldehyde intermediate **26** (1.1 g, 6.3 mmol, 71% over two steps) as a light-yellow solid. To a solution of **26** (1.1 g, 6.3 mmol), malononitrile (423 mg, 0.40 mL, 6.4 mmol), and 15 mL of dichloromethane was added a solution of piperidine (145 mg, 0.17 mL, 1.7 mmol) in 5 mL of dichloromethane. After 2 h at room temperature, the mixture was filtered and the solid was washed with dichloromethane to give the tricycle **27** (1.23 g, 5.5 mmol, 87%) as a brown solid. To compound **27** (0.64 g, 2.8 mmol) in 10 mL of DME and 20 mL of dichloromethane was added Dibal-H (7 mL, 7 mmol, 1 M in hexane) at −78 °C. The mixture was stirred at −78 °C for 3 days. The mixture was then quenched with water and saturated Rochelle's salt (200 mL) at −78 °C. The aqueous layer was then extracted with 30% isopropyl alcohol in chloroform. The combined fractions were dried with sodium sulfate and concentrated in vacuo. The residue was purified by RP-HPLC to give aldehyde **28** (85 mg, 0.37 mmol, 13%, 53% based on recovered starting material) as a light-yellow solid. A similar homologation sequence described in the preparation of **2h** gave the intermediate enamide **30**. To a slurry of this enamide **30** in 150 mL of methanol was added *p*-toluenesulfonylhydrazide (400 mg). The mixture was heated at reflux overnight. After removal of the solvent, the residue was purified by RP-HPLC to give **31** (18 mg, 0.044 mmol, 12% over four steps) as a pale-yellow solid. Following similar hydrolysis and demethylation procedures as described for the preparation of **2e**, the desired compound **2i** (14 mg, 0.033 mmol, 75% over two steps) was obtained as a white solid. ^{1}H NMR (CD_3OD, 500 MHz) δ 8.50 (1H, d), 8.47 (1H, d), 8.00 (1H, d), 7.84 (1H, s), 7.52 (1H, t), 7.34 (1H, dd), 7.29 (1H, d), 7.11 (1H, t), 3.50 (2H, t), 3.06 (2H, t); LCMS *m*/*z* 378 ($M^+ + 1$).

Procedure for the Synthesis of 2j. Compound **32** was obtained following the literature procedures.[33] To **32** (1.42 g, 4.9 mmol) in 80 mL of THF was added $LiBH_4$ (129 mg, 5.9 mmol). The mixture was heated at reflux for 1 h. To this mixture was then added 80 mL of THF and $LiBH_4$ (81 mg, 3.7 mmol). The mixture was heated at reflux until the reaction mixture became clear. It was then quenched with 1 N HCl until gas evolution ceased. The solvent was removed, and the solid was washed with water, ether, and azeotropically dried using toluene to give the corresponding alcohol (1.16 g, 4.7 mmol, 96%) as a solid. To a mixture of this alcohol (180 mg, 0.74 mmol) in 30 mL of DCM was added Dess−Martin reagent (0.31 g, 0.74 mmol). The mixture was stirred at room temperature overnight and concentrated. The solid was washed with DCM and methanol to give **33** (158 mg, 0.65 mmol, 88%) as a solid. To **33** (158 mg, 0.65 mmol) were added MeI (184 mg, 1.3 mmol), potassium carbonate (446 mg, 3.2 mmol), and DMF (10 mL). The mixture was heated at 80 °C for 1.5 h, diluted in ethyl acetate, and washed with brine. The organic layer was dried over sodium sulfate, concentrated, and purified by silica gel chromatography, eluting with 40−74% ethyl acetate−hexanes to give **34** (25 mg, 0.097 mmol, 15%). A similar chain extension, amide formation, reduction of the double bond, hydrolysis of the methyl ester, and demethylation using boron tribromide for the preparation of **2i** gave **2j**. ^{1}H NMR (CD_3OD, 500 MHz) δ 8.55 (1H, d), 8.26 (1H, d), 8.03 (1H, d), 7.53 (1H, t), 7.12 (1H, t), 6.98 (1H, s), 6.89 (1H, d), 3.62 (3H, s), 3.54 (2H, t), 2.99 (2H, t); LCMS *m*/*z* 408 ($M^+ + 1$).

Procedure for the Synthesis of 2o. At room temperature, to a solution of **39** (0.49 g, 1.94 mmol) in dichloromethane (20 mL) was slowly added **38** (0.375 g, 1.94 mmol) followed by the addition of triethylamine (0.5 mL). The mixture was stirred for additional 2 h before it was quenched with 1 N HCl (15 mL). To this mixture was then added dichloromethane (50 mL). The organic layer was washed with water, dried with sodium sulfate, concentrated in vacuo, and purified by silica gel chromatography (2−25% ethyl acetate−hexanes) to give **40** (0.79 g, 1.95 mmol, 100%) as a colorless oil. To **40** (112 mg, 0.28 mmol), potassium carbonate (114 mg), and 2 mL of dimethylacetamide were added palladium acetate (8 mg, 0.036 mmol) and ligand **41** (22 mg, 0.058 mmol)

under nitrogen. The mixture was heated at 125 °C for 3 days and purified by silica gel chromatography (2−15% ethyl acetate−hexanes) to give a mixture of three products (70 mg in total, 0.22 mmol, 77%), which were dissolved in 5 mL of THF/MeOH/water (3:1:1). To this solution was added LiOH (2 mL, 1 N). The mixture was stirred at room temperature overnight, concentrated, and acidified using HCl. To the resulting white slurry was added water (20 mL). The mixture was extracted with 30% isopropanol in chloroform (2 × 50 mL). The organic layer was dried with sodium sulfate, concentrated, and purified by RP-HPLC to give a mixture of acids (25 mg in total, 0.080 mmol, 37%). To this mixture (25 mg, 0.080 mmol) in 2 mL of dichloromethane was added MsCl (8 mg) and then triethylamine (11 mg) at 0 °C. The mixture was warmed to room temperature and stirred for 30 min. To this almost clear yellow solution was added anthranilic acid (10 mg). The resulting mixture was stirred overnight, concentrated, and purified by RP-HPLC to give **43** (3 mg, 0.007 mmol, 3% over two steps) as a white solid. Repeating the above procedures afforded 7 mg of **43** in total. To a mixture of **43** (7 mg, 0.016 mmol) in 3 mL of dichloromethane was added boron tribromide (0.16 mL, 1 M in dichloromethane) at 0 °C. The mixture was warmed to room temperature and stirred overnight. It was then quenched with a few drops of water and purified by RP-HPLC to give **2o** (5 mg, 0.012 mmol, 75%) as a white solid. ^{1}H NMR (acetone-d_6, 500 MHz) δ 11.35 (1H, s), 8.79 (1H, d), 8.46 (1H, d), 8.12 (1H, d), 8.01 (1H, d), 7.63 (1H, t), 7.46 (3H, m), 7.32 (1H, d), 7.17 (1H, t), 3.78 (3H, s), 3.72 (2H, t), 2.97 (2H, t); LCMS *m*/*z* 417 ($M^+ + 1$).

Procedure for the Synthesis of 2p. To a solution of aminobromonaphthalene **44** (1.81 g, 8.2 mmol) in 80 mL of dichloromethane at 0 °C were added acetic anhydride (1.15 mL, 12.2 mmol), triethylamine (2.86 mL, 20 mmol), and a small amount of DMAP. The solution was warmed to room temperature and stirred for 3 h. The solvent was removed, and the residue was dissolved in ethyl acetate, washed with water, 1 N HCl, water, 1 N NaOH, saturated sodium bicarbonate solution, and brine, successively. The organic layer was then dried with sodium sulfate and concentrated in vacuo to give acetamide **45** (1.95 g, 7.4 mmol, 90%) as a pink solid. A mixture of **45** (1.95 g, 7.4 mmol), **46** (1.51 g, 7.4 mmol), palladium acetate (249 mg, 1.1 mmol), tri-*o*-tolylphosphine (494 mg, 1.6 mmol), triethylamine (3.1 mL, 22 mmol), tetrabutylammonium chloride (2.1 g, 7.4 mmol), and 4 Å molecular sieves was added to DMF (20 mL) under argon. The mixture was heated at 109 °C in a sealed tube overnight. The mixture was then filtered, concentrated, and partitioned between water and dichloromethane. The organic layer was washed with water, dried with sodium sulfate, and concentrated. The residue was treated with methanol to give **47** (1.47 g, 3.79 mmol, 51%) as a yellow solid, which was collected by filtration. To **47** (1.47 g, 3.79 mmol) was added 300 mg of Pd/C (10%) and 100 mL of MeOH/DCM. To the mixture was then added palladium hydroxide (100 mg), and the slurry was agitated under 45 psi of hydrogen in a Parr shaker overnight. The slurry was filtered, concentrated, and purified by silica gel chromatography (5% methanol/DCM) to give **48** (0.49 g, 1.26 mmol, 33%) as a sticky oil. To a solution of **48** (522 mg, 1.34 mmol) in 60 mL of chloroform at 0 °C was added dropwise a solution of bromine (83 μL, 1.6 mmol). The mixture was stirred at 0 °C for 5 min and quenched with 1% sodium sulfite. The aqueous phase was extracted with chloroform three times. The combined organic layers were washed with saturated aqueous sodium bicarbonate and dried over sodium sulfate to give **49** (0.56 g, 1.19 mmol, 89%). A mixture of this bromide intermediate **49** (0.56 g, 1.19 mmol), methyl boronic acid (93 mg, 1.55 mmol), potassium carbonate (494 mg, 3.58 mmol), palladium tetrakistriphenylphosphine (138 mg, 0.l2 mmol), 2 mL of water, and 20 mL of dioxane was degassed with argon and heated at 100 °C overnight. After concentration, the residue was purified by silica gel chromatography to give **50** (0.18 g, 0.45 mmol, 38%) as a white solid. To a solution of this methylated intermediate **50** (89 mg, 0.22 mmol) in 5 mL of chloroform were added potassium acetate (44 mg, 0.44 mmol), acetic acid (26 mg, 0.44 mmol), acetic anhydride (45 mg, 0.44 mmol), 18-crown-6 (10 mg), and amylnitrite (74 uL, 0.63 mmol). The mixture was heated at 70 °C overnight. The reaction mixture was then purified by silica gel chromatography to give **51** (58 mg, 0.14 mmol, 64%) as a white solid. To a suspension of this tricyclic acetamide intermediate **51** (58 mg, 0.14 mmol) in 40 mL of methanol was added sodium ethoxide (226 uL, 21% in methanol). After 5 min, to the mixture was added 10 mL of aqueous 1 N lithium hydroxide solution, and the mixture was stirred for 30 min. The solvent was evaporated, and the aqueous residue was acidified and extracted with 30% isopropanol in chloroform. After removal of the solvent, the residue was purified by RP-HPLC to give the desired product **2p** (34 mg, 0.094 mmol, 68% over two steps) as a white solid. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 11.2 (1H, s), 8.55 (1H, s), 8.47 (1H, d), 8.24 (1H, d), 7.95 (1H, d), 7.84 (1H, s), 7.67 (1H, d), 7.59 (3H, m), 7.12 (1H, t), 3.12 (2H, t), 2.83 (2H, t); LCMS *m*/*z* 360 ($M^+ + 1$).

Procedure for the Synthesis of 3b. To a solution of diisopropylamine (2.5 g, 14.2 mmol) in 80 mL of THF was added *n*-butyllithium (12.4 mL, 19.9 mmol, 1.6 M in hexane) at −78 °C. The resulting solution was stirred at −78 °C for 1 h and then at room temperature for an additional 30 min. The solution was recooled to −78 °C, and to this solution was added dropwise a solution of 6-methoxytetralone **4** (2.5 g, 14.2 mmol) in 20 mL of THF. After 1 h at −78 °C, to the above solution was added diethyl oxalate (1.91 g, 2.70 mL, 19.88 mmol) in one portion, and the mixture was aged for 1 h. The resulting solution was warmed to room temperature for 2 h. The solution was diluted with 200 mL of ethyl acetate and washed with 1 N HCl, water, saturated sodium bicarbonate, dried over sodium sulfate, and concentrated in vacuo. The residue was purified by chromatography (SiO_2) using 15% ethyl acetate−hexanes to afford **53** (2.79 g, 10.1 mmol, 71%) as an oil. To the solution of **53** (2.79 g, 10.1 mmol) in ethanol (100 mL) was added hydroxylamine hydrochloride (0.84 g, 12.1 mmol), and the resulting mixture was heated at reflux for 2 h. After removal of the solvent, the residue was dissolved in ethyl acetate (200 mL) and washed with water, saturated sodium bicarbonate, and concentrated to give **54** (1.9 g, 6.96 mol, 69%). To the solution of **54** (0.90 g, 3.3 mmol) in dioxane (100 mL) was added DDQ (2.3 g, 9.9 mmol), and the resulting mixture was heated at reflux for 6 h. After removal of the solvent, the residue was dissolved in ethyl acetate and washed with sodium hydroxide (1 N, 100 mL), saturated sodium bicarbonate (100 mL), brine (200 mL), dried over sodium sulfate, and concentrated in vacuo to afford **55** (0.75 g, 2.8 mmol, 84%) as an off-white solid. To the solution of **55** (0.75 g, 2.77 mmol) in dichloromethane (80 mL) was added Dibal-H (6.6 mL, 1 N, 6.6 mmol) at −78 °C. The resulting solution was stirred at −78 °C for 1.5 h before it was quenched by methanol at −78 °C. To the above solution was added saturated potassium tartrate (300 mL) at room temperature, and the mixture was stirred for 2 h. The water phase was extracted with methylene chloride (200 mL), and the combined organic phase was washed with brine (3 × 200 mL), dried over sodium sulfate, and concentrated in vacuo. The residue was purified on silica gel chromatography using 10% ethyl acetate−hexanes to afford the aldehyde (0.57 g, 2.5 mmol, 91%) as a white solid. To a solution of trimethylphosphonoacetate (300 mg, 1.65 mmol) in 20 mL of THF was added *n*-butyllithium (1.1 mL, 1.6 M in hexane, 1.8 mmol) at 0 °C. After 15 min, the mixture was warmed to room temperature and transferred to a solution of aldehyde (0.288 g, 1.27 mmol) in 10 mL of THF. The resulting slurry was stirred at room temperature for 2 h, and to this mixture was added 50 mL of water. The mixture was then extracted with 100 mL of ethyl acetate and 100 mL of 30% isopropanol in chloroform. The combined organic portions were dried with sodium sulfate and concentrated to give the corresponding α,β-unsaturated ester (0.32 g, 1.20 mmol, 95%) as a white solid. To the solution of the ester (0.34 g, 1.20 mmol) in a mixture solvents of THF/MeOH/water (100 mL, v/v/v, 3/1/1) was added lithium hydroxide (1 N, 10 mL). The resulting solution was stirred at room temperature for 1 h. After removal of the organic solvent, the alkaline mixture was acidified to pH 4 with hydrochloride (1 N). The precipitate was extracted with 30% isopropanol in chloroform. The organic phase was concentrated in vacuo to afford the corresponding acid (290

mg, 1.08 mmol, 90%). To the suspension of the acid (290 mg, 1.08 mmol) in methylene chloride (100 mL) was added boron tribromide (11 mL, 11 mmol) at 0 °C. The resulting mixture was stirred at room temperature overnight. The reaction was then quenched by water at 0 °C. The mixture was extracted with 30% isopropanol/chloroform, and the organic phase was washed with water and concentrated in vacuo. The residue was purified by RP-HPLC to give the corresponding phenol (0.25 g, 0.98 mmol, 91%) as a white solid. The solution of phenol (0.25 g, 0.98 mmol) in methanol (50 mL) in the presence of palladium on carbon (10%, 46 mg) was subjected to hydrogenation at room temperature for 1 h. After filtration through Celite under nitrogen, the filtrate was concentrated to give **56** (190 mg, 0.74 mmol, 75%). To the solution of **56** (190 mg, 0.74 mmol) in methylene chloride (10 mL) was added dimethyl-*tert*-butylsilyl chloride (0.33 g, 2.2 mmol), triethylamine (0.22 g, 2.2 mmol), and a small amount of DMAP at room temperature. After being stirred for 3 h, the mixture was diluted in methylene chloride (100 mL), washed with water, dried over sodium sulfate, and concentrated in vacuo to give the TBS-silyl ether as a crude product. To this crude product in methylene chloride (10 mL) was added DMF (5 μL) and oxalyl chloride (0.64 mL, 1.28 mmol, 2M) at 0 °C. The mixture was stirred at room temperature for 2 h. The excess of oxalyl chloride and solvent was removed in vacuo. The residue was treated with ethyl 2-amino-1-cyclohexene carboxylate (216 mg, 1.28 mmol), and the mixture was aged overnight. The mixture was concentrated and purified by RP-HPLC to give **57**. To **57** was added a mixture solvent of THF/MeOH/water (10 mL, v/v/v, 3/1/1) and lithium hydroxide (1 N, 1 mL) and stirred at room temperature overnight. After removal of the organic solvents, the aqueous phase was acidified to pH 4, and the precipitate was extracted with 30% isopropanol in chloroform. The combined organic phase was washed with water and concentrated in vacuo. The residue was purified by RP-HPLC to afford **3b** (39 mg, 0.10 mmol, 14% over three steps) as a white solid. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 11.7 (s, 1H), 10.2 (s, 1H), 8.19 (s, 1H), 7.67 (d, 2H), 7.32 (d, 2H), 3.42 (m, 2H), 2.84 (m, 2H), 2.51 (bs, 2H), 2.22 (bs, 2H), 1.53 (bs, 4H); LCMS *m*/*z* 381 ($M^+ + 1$). HRMS calcd for $C_{21}H_{20}O_5N_2$ [$M + 1^+$]: 381.1451. Found: 381.1443.

Biological Assays. [^{3}H]Niacin Binding Assay. Radioligand binding assays were conducted on membranes derived from stably transfected Chinese hamster ovary (CHO) cells. The derivation of the cell lines and the applied radioligand binding protocol are described in the Supporting Information of the previous paper.[16b]

[^{35}S]GTPγS Binding Assay. The [^{35}S]GTPγS binding assay protocol was described in the Supporting Information of the previous paper.[16b]

Human Subcutaneous Adipocyte Lipolysis Assay.[28] Primary human subcutaneous preadipocytes were purchased from Cambrex (now Lonza) and differentiated into adipocytes in 96-well tissue culture plates according to the manufacturer's directions and proprietary media. Sixteen hours prior to assay, the differentiation medium was completely removed from the wells and replaced with 150 μL per well of PGM (preadipocyte growth medium, a proprietary medium supplied by the manufacturer). The plates were incubated overnight at 37 °C, 5% CO_2, 90% humidity. The 96-well plates containing the differentiated adipocytes were washed three times with PBS (no Ca^{2+} or Mg^{2+}) in 100, 100, and 50 μL volumes in a nonsterile environment. Treatment began with the addition of 50 μL of Krebs buffer to all wells followed by 50 μL of test compound or buffer (controls). The plates were incubated for 5 min at room temperature, and then 50 μL/well of the nonspecific phosphodiesterase inhibitor, IBMX, was added at a working concentration of 1 mM or buffer was added to the controls. Thus, a 150 μL volume was achieved for all assay wells with a final DMSO concentration of 1.04%. The purpose of the IBMX is to elevate intracellular cAMP, which can subsequently be suppressed via niacin receptor GPR109A signaling. The compound was applied to the cells first, and shortly thereafter the stimulant IBMX was added. The whole mixture incubated for 5 h, and lipolysis data could be read out by measuring glycerol. After 5 h of incubation at 37 °C, 5% CO_2, 90% humidity, a 100 μL volume was removed from each well and transferred to a clear, flat-bottomed 96-well plate. A standard curve was run in column 12 of the plate by diluting the Sigma glycerol standard in Krebs buffer to make 0, 1, 2.5, 5, 10, and 20 mg/dL in individual 100 μL volumes. Glycerol content in the wells was assayed by adding 100 μL of free glycerol reagent (Sigma) followed by a 10 min incubation at room temperature, approximately 23 °C. Absorbance was determined at 540 nm in a Molecular Devices ThermoMax microplate reader, and free glycerol content was quantitated on the basis of a standard curve. The amount of glycerol released was calculated on the basis of regression analysis of known glycerol concentrations using a glycerol standard (Sigma).

Measurement of Rat Vasodilation.[27] Compound **2g** was titrated in the rat ear vasodilation model at 100, 30, 10, and 3 mg/kg. The compound was formulated in 5% HPBCD. For vasodilation studies, rats were not fasted. All animals were anesthetized with sodium pentobarbital (50 mg/kg) 30 min before placing the animals under the laser Doppler. Dosing was done subcutaneously after measuring baseline perfusion for 2.5 min. After dosing, perfusion measurements continued for 30 min, and blood for drug levels was collected at 35 min postdose.

Laser Doppler was performed with a PeriScan PIMII laser Doppler perfusion imager system, from Perimed (repeated mode, image size of 25 × 25, autointerval of 30 s, medium resolution, very high scan speed, 40 images recorded, intensity of 8−9 V. Perfusion color scale: user defined, 0−3 V).

The laser beam was positioned in the center of the ear or in an area with suitable basal perfusion. Baseline measurements were taken for 3 min (first seven frames). At this point, the compounds or vehicle was injected subcutaneously, and the recording continued for the remaining 33 frames.

Calculations were performed using the analysis tools in the LDPIwin 2.3 software, defining a Region of Interest (ROI) and exporting the data to Excel. Graphs were prepared using the Prism software.

In Vivo Rat Lipolysis. Rat lipolysis studies were conducted as previously described.[27] For all studies, **2g** was formulated in 5% hydroxypropyl β-cyclodextrin (HPBCD) and pH was adjusted to 7−8. Male CD rats from Charles River were used in all of the studies. For the FFA reduction study, rats were fasted for 18 h on wire. Dosing was done po (100, 30, 10, and 3 mg/kg), and blood samples (EDTA) were collected at 0, 20 min, 1 h, 2 h, and 4 h postdosing. FFA were measured with an enzymatic method (WAKO Chemicals GmbH 994-75409 NEFA C free fatty acid assay). Plasma samples were also used to measure compound levels.

MiniPK Studies. In order to calculate the C_{max} at which flushing does not occur, a miniPK study was conducted. In this study, rats were treated in the exact same conditions as the animals used in the flushing studies, and blood was collected at 5 min intervals after subcutaneous dosing of compound. In this case, the doses tested were 100 and 30 mg/kg.

Supporting Information Available: Experimental procedures for compound preparation, characterization data, biological assay protocols, and LC/MS and ^{1}H NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Carlson, L. A. Nicotinic acid: the broad-spectrum lipid drug. A 50th anniversary review. *J. Intern. Med.* **2005**, *258*, 94–114. (b) Knopp, R. H. Drug treatment of lipid disorders. *N. Engl. J. Med.* **1999**, *341*, 498–511.

(2) Knopp, R. H.; Davidson, M.; Goldberg, A. C.; Kafonek, S. D.; Kashyap, M.; Sprecher, D.; Superko, H. R.; Jenkins, S.; Marcovina, S. Equivalent efficacy of a time-release form of niacin (Niaspan) given once-a-night vs plain niacin in the management of hyperlipidemia. *Metabolism* **1998**, *47*, 1097–1104.

(3) (a) Mack, W. J.; Selzer, R. H.; Hodis, H. N.; Erickson, J. K.; Liu, C. R.; Liu, C. H.; Crawford, D. W.; Blankenhorn, D. H. One-year reduction and longitutudinal analysis of carotid intima-media thickness associated with colestipol/niacin therapy. *Stroke* **1993**, *24*, 1779–1783. (b) Nikkila, E. A.; Viikinkoski, P.; Valle, M.; Frick, M. H. Prevention of progression of coronary atherosclerosis by treatment of hyperlipi-

daemia: a seven year prospective angiographic study. *Br. Med. J.* **1984**, *289*, 220–223.

(4) (a) Canner, P. L.; Berge, K. G.; Wenger, N. K.; Stamler, J.; Friedman, L.; Prineas, R. J.; Friedewald, W. Fifteen year mortality in coronary drug project patients: long-term benefit with niacin. *J. Am. Coll. Cardiol.* **1986**, *8*, 1245–1255. (b) Pritzker, L. B. Do lipid lowering drugs reduce the risk of coronary heart disease? *Crit. Rev. Clin. Lab. Sci.* **1998**, *35*, 603–621. (c) Taylor, A. J.; Sullenberger, L. E.; Lee, H. J.; Lee, J. K.; Grace, K. A. Arterial biology for the investigation of the treatment effects of reducing cholesterol (ARBITER) 2. *Circulation* **2004**, *110*, 3512–3517.

(5) McGovern, M. Niaspan: creating a new concept for raising HDL-cholesterol. *Eur. Heart J. Suppl.* **2005**, *7*, F41−F47.

(6) Cheng, K.; Wu, T. J.; Wu, K. K.; Sturino, C.; Metters, K.; Gottesdiener, K.; Wright, S. D.; Wang, Z.; O'Neill, G.; Lai, E.; Waters, M. G. Antagonism of the prostaglandin D2 receptor 1 suppresses nicotinic acid-induced vasodilation in mice and humans. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 6682–6687.

(7) Pereira, J. N.; Holland, G. F.; Hochstein, F. A.; Gilgore, S.; Defelice, S.; Pinson, R. The pharmacology of 5-(3-pyridyl)tetrazole, a hypocholesteremic lipolysis inhibitor. *J. Pharmacol. Exp. Ther.* **1968**, *162*, 148–154.

(8) Stuyt, P. M. J.; Kleinjans, H. A. J.; Stalenhoef, A. F. H. Tolerability and effects of high doses acipimox as additional lipid-lowering therapy in familial hypercholesterolemia. *Neth. J. Med.* **1998**, *53*, 228–233, and references therein.

(9) Gundersen, K.; Demissianos, H. V. The Effects of 5-Methylpyrazole-3-Carboxylic Acid (U-19,425) and Nicotinic Acid (NA) on Free Fatty Acids (FFA), Triglycerides (TG) and Cholesterol in Man. In *Drugs Affecting Lipid Metabolism*; Holmes, W. L., Carlson, L. A., Paoletti, R., Eds.; Plenum Press: New York, 1969; pp 213−226 and references therein.

(10) Eastwood, G. R.; Kellett, D. N. Some effects of 3-chloroisoxazole-5-carboxylic acid on lipid and carbohydrate mechanism. *Biochem. Pharmacol.* **1969**, *18*, 569–577, and references therein.

(11) (a) Larosa, J. C.; Miller, V. T.; Edwards, K. D. G.; DeBovis, M. R.; Stoy, D. B. Acifran: a double-blind, randomized, placebo-controlled efficacy study in type IIa hyperlipoproteinemic patients. *Artery* **1987**, *14*, 338–350, and references therein. (b) Jung, J.-K.; Johnson, B. R.; Duong, T.; Decaire, M.; Uy, J.; Gharbaoui, T.; Boatman, P. D.; Sage, C. R.; Chen, R.; Richman, J. G.; Connolly, D. T.; Semple, G. Analogues of acifran: agonists of the high and low affinity niacin receptors, GPR109a and GPR109b. *J. Med. Chem.* **2007**, *50*, 1445–1448.

(12) Schaub, A.; Futterer, A.; Pfeffer, K. PUMA-G, an IFN-γ-inducible gene in macrophages is a novel member of the seven transmembrane spanning receptor superfamily. *Eur. J. Immunol.* **2001**, *31*, 3714–3725.

(13) (a) Wise, A.; Foord, S. M.; Fraser, N. J.; Barnes, A. A.; Elshourbagy, N.; Eilert, M.; Ignar, D. M.; Murdock, P. R.; Steplewski, K.; Green, A.; Brown, A. J.; Dowell, S. J.; Szekeres, P. G.; Hassall, D. G.; Marshall, F. H.; Wilson, S.; Pike, N. B. Molecular identification of high and low affinity receptors for nicotinic acid. *J. Biol. Chem.* **2003**, *278*, 9869–9874. (b) Tunaru, S.; Kero, J.; Schaub, A.; Wufka, C.; Blaukat, A.; Pfeffer, K.; Offermanns, S. PUMA-G and HM74 are receptors for nicotinic acid and mediate its anti-lipolytic effect. *Nat. Med.* **2003**, *9*, 352–355. (c) Offermanns, S. The nicotinic acid receptor GPR109A (HM74A or PUMA-G) as a new therapeutic target. *Trends Pharmacol. Sci.* **2006**, *27*, 384–390.

(14) (a) Karpe, F.; Frayn, K. N. The nicotinic acid receptor, a new mechanism for an old drug. *Lancet* **2004**, *363*, 1892–1894. (b) Pike, N. B.; Wise, A. Identification of a nicotinic acid receptor: Is this the molecular target for the oldest lipid-lowering drug? *Curr. Opin. Invest. Drugs* **2004**, *5*, 271–275.

(15) Benyó, Z.; Gille, A.; Kero, J.; Csiky, M.; Suchánková, M. C.; Nüsing, R.; Moers, A.; Pfeffer, K.; Offermanns, S. GPR109A (PUMA-G/HM74A) mediates nicotinic acid-induced flushing. *J. Clin. Invest.* **2005**, *115*, 3634–3640.

(16) (a) Shen, H. C.; Szymonifka, M.; Deng, Q.; Carballo-Jane, E.; Cheng, K.; Wu, K.; Wu, T.-J.; Wang, J.; Tong, X.; Ren, N.; Taggart, A.; Cai, T.; Waters, G.; Hammond, M.; Tata, J. R.; Colletti, S. L. Discovery of orally bioavailable and novel urea agonists for the high affinity niacin receptor GPR109A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6723–6728. (b) Shen, H. C.; Ding, F.-X.; Taggart, A.; Cheng, K.; Carballo-Jane, E.; Ren, N.; Chen, Q.; Wang, J.; Wolff, M.; Waters, G.; Hammond, M.; Tata, J. R.; Colletti, S. L. Discovery of biaryl anthranilides as full agonists for the high affinity niacin receptor. *J. Med. Chem.* **2007**, *50*, 6303–6306. (c) Raghavan, S.; Tria, G. S.; Shen, H. C.; Ding, F.-X.; Taggart, A. K. P.; Ren, N.; Wilsie, L. C.; Krsmanovic, M. L.; Holt, T. G.; Wolff, M. S.; Waters, M. G.; Hammond, M. L.; Tata, J. R.; Colletti, S. L. Tetrahydroanthranilic acid as a surrogate for anthranilic acid: application to the discovery of potent niacin receptor agonists. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3163–3167.

(17) Semple, G.; Skinner, P. J.; Gharbaoui, T.; Shin, Y.-J.; Jung, J.-K.; Cherrier, M. C.; Webb, P. J.; Tamura, S. Y.; Boatman, P. D.; Sage, C. R.; Schrader, T. O.; Chen, R.; Colletti, S. L.; Tata, J. R.; Water, M. G.; Cheng, K.; Taggart, A. K.; Cai, T.-Q.; Carballo-Jane, E.; Behan, D. T.; Connolly, D. T.; Richman, J. G. 3-(1*H*-Tetrazol-5-yl)-1,4,5,6-tetrahydro-cyclocyclopentapyrazole (MK-0354): a partial agonist of the nicotinic acid receptor, G-protein coupled receptor 109a, with antilipolytic but no vasodilatory activity in mice. *J. Med. Chem.* **2008**, *51*, 5101–5108.

(18) (a) Benyó, Z.; Gille, A.; Bennett, C. L.; Clausen, B. E.; Offermanns, S. Nicotinic acid-induced flushing is mediated by activation of epidermal langerhans cells. *Mol. Pharmacol.* **2006**, *70*, 1844–1849. (b) Morrow, J. D.; Awad, J. A.; Oates, J. A.; Roberts, L. J., II. Identification of skin as a major site of prostaglandin D2 release following oral administration of niacin in humans. *J. Invest. Dermatol.* **1992**, *98*, 812–815.

(19) (a) Semple, G.; Boatman, P. D.; Richman, J. G. Recent progress in the discovery of niacin receptor agonists. *Curr. Opin. Drug Discovery Dev.* **2007**, *10*, 452–459. (b) Boatman, P. D.; Richman, J. G.; Semple, G. Nicotinic acid receptor agonists. *J. Med. Chem.* **2008**, *51*, 7653–7662.

(20) (a) Campbell, M.; Hatley, R. J.; Heer, J. P.; Mason, A. M.; Pinto, I. L.; Rahman, S. S.; Smith, I. E. D. Chemical Compounds. Patent WO 2005016867, 2005; GlaxoSmithKline. (b) Campbell, M.; Hatley, R. J.; Heer, J. P.; Mason, A. M.; Nicholson, N. H.; Pinto, I. L.; Rahman, S. S.; Smith, I. E. D. 2-Substituted Benzoic Acid Derivatives as GPR109A Receptor Agonists. Patent WO 2005016870, 2005; GlaxoSmithKline. (c) Pinto, I. L.; Rahman, S. S.; Nicholson, N. H. Novel Compounds. Patent WO 2005077950, 2005; GlaxoSmithKline. (d) Campbell, M.; Mason, A. M.; Pinto, I. L.; Pollard, D. R.; Smith, I. E. D. Anthranilic Acid Derivatives and Their Use in Treatment of Diseases of Lipid Metabolism, in Particular Dyslipidaemia. Patent WO 2006085108, 2006; GlaxoSmithKline. (e) Mason, A. M.; Pinto, I. L.; Rahman, S. S. Anthranilic Acid Derivatives Active at the GPR109A Teceptor. Patent WO 2006085111, 2006s; GlaxoSmithKline. (f) Hatley, R. J.; Mason, A. M.; Pinto, I. L.; Smith, I. E. D. Anthranilic Acid Derivatives as GPR109A Receptor Agonists. Patent WO 2006085112, 2006; GlaxoSmithKline. (g) Pinto, I. L.; Simpson, J. K. Chemical Compounds. Patent WO 2006085113, 2006; GlaxoSmithKline. (h) Hatley, R. J. D.; Pinto, I. L. Patent WO 2006/045564 A1, 2006. (i) Hatley, R. J. D.; Pinto, I. L. Xanthine Derivatives with HM74A Receptor Activity. Patent WO 2006045565, 2006; GlaxoSmithKline. (j) Heer, J. P.; Smith, I. E. D. Xanthine Derivatives as Selective HM74A Agonists Patent WO 2007017265, 2007; GlaxoSmithKline.

(21) (a) Boatman, D. P.; Schrader, T. O.; Semple, G.; Skinner, P. J.; Jung, J.-K. Fused Pyrazole Derivatives and Methods of Treatment of Metabolic-Related Disorders Thereof. Patent WO 2006069242, 2006; Arena. (b) Gharbaoui, T.; Skinner, P. J.; Shin, Y.-J.; Averbuj, C.; Jung, J.-K.; Johnson, B. R.; Duong, T.; Decaire, M.; Uy, J.; Cherrier, M. C.; Webb, P. J.; Tamura, S. Y.; Zou, N.; Rodriguez, N.; Boatman, P. D.; Sage, C. R.; Lindstrom, A.; Xu, J.; Schrader, T. O.; Chen, R.; Richman, J. G.; Connolly, D. T.; Colletti, S. L.; Tata, J. R.; Semple, G. Agonist lead identification for the high affinity niacin receptor GPR109a. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4914–4919. (c) Skinner, P. J.; Cherrier, M. C.; Webb, P. J.; Shin, Y.-J.; Gharbaoui, T.; Lindstrom, A.; Hong, V.; Tamura, S. Y.; Dang, H. T.; Chen, R.; Richman, J. G.; Connolly, D. T.; Semple, G. Fluorinated pyrazole acids are agonists of the high affinity niacin receptor GPR109a. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5620−5623.

(22) (a) Semple, G.; Schrader, T.; Skinner, P. J.; Colletti, S. L.; Gharbaoui, T.; Imbriglio, J. E.; Jung, J.-K.; Liang, R.; Raghavan, S.; Schmidt, D.; Tata, J. R. A Preparation of Tetrazole Derivatives, Useful as Modulators of RUP25 Receptor. Patent WO 2005044816, 2005; Arena and Merck. (b) Colletti., S. L.; Beresis, R. T.; Chen, W.; Tata, J. R.; Shen, H. C.; Marley, D. M.; Deng, Q.; Frie, J. L.; Ding, F. Niacin Receptor Agonists, Compositions Containing Such Compounds and Methods of Treatment. Patent WO 2006052555, 2006; Merck. (c) Colletti, S. L.; Tata, J. R.; Shen, H. C.; Ding, F.-X.; Frie, J. L.; Imbriglio, J. E.; Chen, W. Niacin Receptor Agonists, Compositions Containing Such Compounds and Methods of Treatment. Patent WO 2006057922, 2006; Merck. (d) Raghavan, S.; Colletti, S. L.; Ding, F.-X.; Shen, H.; Tata, J. R.; Lins, A. R.; Smenton, A. L.; Chen, W.; Schmidt, D. Y.; Tria, G. S. Niacin Receptor Agonists, Compositions Containing Such Compounds and Methods of Treatment. Patent WO 2006002557, 2006; Merck. (e) Colletti, S. L.; Shen, H.; Tata, J. R.; Szymonifka, M. J. Niacin Receptor Agonists, Compositions Containing Such Compounds and Methods of Treatment. Patent WO 2007027532, 2007; Merck. (f) Colletti, S. L.; Imbriglio, J. E.; Beresis, R. T.; Frie, J. L. Niacin Receptor Agonists, Compositions Containing Such Compounds and Methods of Treatment. Patent WO 2007035478, 2007; Merck.

(23) (a) Dehmlow, H.; Grether, U.; Kratochwil, N. A.; Narquizian, R.; Panousis, C.; Peters, J.-U. Novel Anthranilic Acid Derivatives. Patent US 20060281810, 2006; Hoffmann-La Roche. (b) Dehmlow, H.; Grether, U.; Kratochwil, N. A.; Narquizian, R.; Panousis, C. Novel Thiophene Derivatives Which Are HM74A Agonists. Patent US 20070072873, 2007; Hoffmann-La Roche.

(24) Palani, A.; Su, J.; Xiao, D.; Huang, X.; Rao, A. U.; Chen, X.; Tang, H.; Qin, J.; Huang, Y.; Aslanian, R. G.; Mckittrick, B. Heterocycles as Nicotinic Acid Receptor Agonists for the Treatment of Dyslipidemia. Patent WO 2006124490, 2006; Schering.

(25) Cao, G.; Xue, C.-B.; Anand, R.; Huang, T.; Kong, L.; Glenn, J.; Feng, H. Disubstituted Thienyl Compounds and Their Use as Pharmaceuticals. Patent WO2007015744, 2007; Incyte.

(26) For niacin receptor partial agonists, see the following: Herk, T.; Brussee, J.; Nieuwendijk, A. M. C. H.; Klein, P. A. M.; Ijzerman, A. P.; Stannek, C.; Brumeister, A.; Lorenzen, A. Pyrazole derivatives as partial agonists for the nicotinic acid receptor. *J. Med. Chem.* **2003**, *46*, 3945–3951.

(27) (a) Richman, J. G.; Kanemitsu-Parks, M.; Gaidarov, I.; Cameron, J. S.; Griffin, P.; Zheng, H.; Guerra, N. C.; Cham, L.; Behan, D. P.; Boatman, P. D.; Chen, R.; Skinner, P. J.; Ornelas, P.; Semple, G.; Connolly, D. T. Nicotinic acid receptor agonists differentially activate downstream effectors. *J. Biol. Chem.* **2007**, *282*, 18028–18036. (b) Carballo-Jane, E.; Gerckens, L. S.; Luell, S.; Parlapiano, A. S.; Wolff, M.; Colletti, S. L.; Tata, J. R.; Taggart, A. K.; Waters, M. G.; Richman, J. G.; McCann, M. E.; Forrest, M. J. Comparison of rat and dog models of vasodilation and lipolysis for the calculation of a therapeutic index for GPR109A agonists. *J. Pharmacol. Toxicol. Methods* **2007**, *56*, 308–316.

(28) Taggart, A. K.; Kero, J.; Gan, X.; Cai, T. Q.; Cheng, K.; Ippolito, M.; Ren, N.; Kaplan, R.; Wu, K.; Wu, T. J.; Jin, L.; Liaw, C.; Chen, R.; Richman, J.; Connolly, D. T.; Offermanns, S.; Wright, S. D.; Waters, M. G. (D)-beta-Hydroxybutyrate inhibits adipocyte lipolysis via the nicotinic acid receptor PUMA-G. *J. Biol. Chem.* **2005**, *280*, 26649–26652.

(29) Doherty, M. D.; Cohen, G. M. Metabolic activation of 1-naphthol by rat liver microsomes to naphthoquinone and covalent binding species. *Biochem. Pharmacol.* **1984**, *33*, 3201–3208.

(30) Pandit, U. K.; van der Vlugt, F. A.; van Dalen, A. C. A facile approach to ring C modified steroids. *Tetrahedron Lett.* **1969**, *10*, 3693–3696.

(31) Antilla, J. C.; Baskin, J. M.; Barder, T. E.; Bachwald, S. L. Copper-diamine-catalyzed N-arylation of pyrroles, pyrazoles, indazoles, imidazoles, and triazoles. *J. Org. Chem.* **2004**, *69*, 5578–5587.

(32) Bertelli, L.; Biagi, G.; Giorgi, I.; Manera, C.; Livi, O.; Scartoni, V.; Betti, L.; Giannaccini, G.; Trincavelli, L.; Barili, P. L. 1,2,3-Triazolo[1,5-*a*]quinoxalines: synthesis and binding to benzodiazepine and adenosine receptors. *Eur. J. Med. Chem.* **1998**, *33*, 113–122.

(33) Campeau, L.-C.; Parisien, M.; Leblanc, M.; Fagnou, K. Biaryl synthesis via direct arylation: establishment of an efficient catalyst for intramolecular processes. *J. Am. Chem. Soc.* **2004**, *126*, 9186–9187.

(34) Heck, R. F. Palladium-catalyzed vinylation of organic halides. *Org. React.* **1982**, *27*, 345–390.

JM900151E