

3-(1*H*-Tetrazol-5-yl)-1,4,5,6-tetrahydro-cyclopentapyrazole (MK-0354): A Partial Agonist of the Nicotinic Acid Receptor, G-Protein Coupled Receptor 109a, with Antilipolytic but No Vasodilatory Activity in Mice

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The discovery and profiling of 3-(1*H*-tetrazol-5-yl)-1,4,5,6-tetrahydro-cyclopentapyrazole (**5a**, MK-0354), a partial agonist of GPR109a, is described. Compound **5a** retained the plasma free fatty acid lowering effects in mice associated with GPR109a agonism, but did not induce vasodilation at the maximum feasible dose. Moreover, preadministration of **5a** blocked the flushing effect induced by nicotinic acid but not that induced by PGD₂. This profile made **5a** a suitable candidate for further study for the treatment of dyslipidemia.

Introduction

Nicotinic acid (sometimes called niacin) is a water-soluble vitamin that at high doses in humans favorably modulates essentially all serum lipid and lipoprotein parameters. As a result, nicotinic acid has been used for the treatment of cardiovascular disease for many years.¹ Nicotinic acid can lower very low-density lipoprotein cholesterol (VLDL-c),^a low-density lipoprotein cholesterol (LDL-c), and lipoprotein(a) (Lp(a)), but the recent upsurge in interest in this area has focused on nicotinic acid's ability to increase high density lipoprotein-cholesterol (HDL-c) to a greater extent than other currently marketed drugs, as HDL-c levels are inversely correlated with the risk of coronary heart disease.² Indeed, in the Coronary Drug Project, nicotinic acid was shown to reduce the number of cardiac events over a six-year dosing period and to reduce all cause mortality by 11% after 15 years.^{3,4} Subsequently, combinations of nicotinic acid with the LDL-lowering statin class of drugs have been shown to slow the progression of atherosclerosis, decrease the number of cardiac events, and provide a therapeutic benefit beyond that of statins alone.^{5,6}

The use of nicotinic acid as a therapeutic, however, is limited by a number of associated side-effects, most notably a highly uncomfortable cutaneous flushing response that generally manifests itself on the upper body and face which can limit patient compliance.⁷ Hence the development of novel agents with nicotinic acid-like effects on plasma lipid parameters and atherosclerosis, but that do not induce flushing, has been considered to be a high value goal for some time.

Mechanistic investigations showed that nicotinic acid binds to a G-protein coupled receptor (GPCR) expressed in rat spleen and adipocytes,⁸ a finding that sparked a resurgence in the field. Two G_i-coupled orphan GPCRs that share 95% identity and that are both expressed in human adipocytes were subsequently cloned and identified as putative molecular targets for nicotinic acid.⁹ GPR109a (also called HM74a) is the human orthologue of the previously described mouse receptor (PUMA-G, called mGPR109a hereafter),¹⁰ whereas GPR109b (also called HM74) differs from hGPR109a and mGPR109a mainly in the intracellular C-terminal tail portion of the receptor and is not expressed in rodents.⁹ Nicotinic acid was shown to activate hGPR109a in a guanine nucleotide exchange assay and displace ³H-nicotinic acid from hGPR109a expressing CHO cell membranes with activity in the tens of nanomolar range, but is a much weaker ligand for GPR109b.⁹ Further evidence in mGPR109a knockout mice has demonstrated that the free fatty acid (FFA) and triglyceride lowering effects of nicotinic acid are ablated in the absence of this receptor.¹⁰ These data, coupled with the highly restricted species expression of GPR109b, has brought hGPR109a to the forefront as the more interesting potential drug target of the two. The question still remains however, as to whether either receptor is the molecular target responsible for the lipid remodeling and antiatherogenic properties of nicotinic acid in humans. The demonstration that other known compounds previously shown to raise HDL in humans, acipimox¹¹ and acifran,¹² are also agonists for hGPR109a¹³ is supportive of this idea, but conclusive evidence is still lacking. A hypothesis has been described whereby the initial activation of GPR109a decreases intracellular cAMP levels in adipocytes, leading to reduced protein kinase A (PKA) activity. This in turn results in a decrease in hormone sensitive lipase activity, thereby reducing intracellular triglyceride (TG) hydrolysis and FFA secretion. It has been further postulated that this decrease in FFA levels directly results in decreased production of TG and VLDL in the liver. The use of knockout mice indicates that these antilipolytic effects of nicotinic acid are receptor dependent.¹⁴ What is much less clear is whether the acute effects on plasma FFA and TG can eventually lead to increases in HDL levels. It has been hypothesized, though, that the reduction in the number

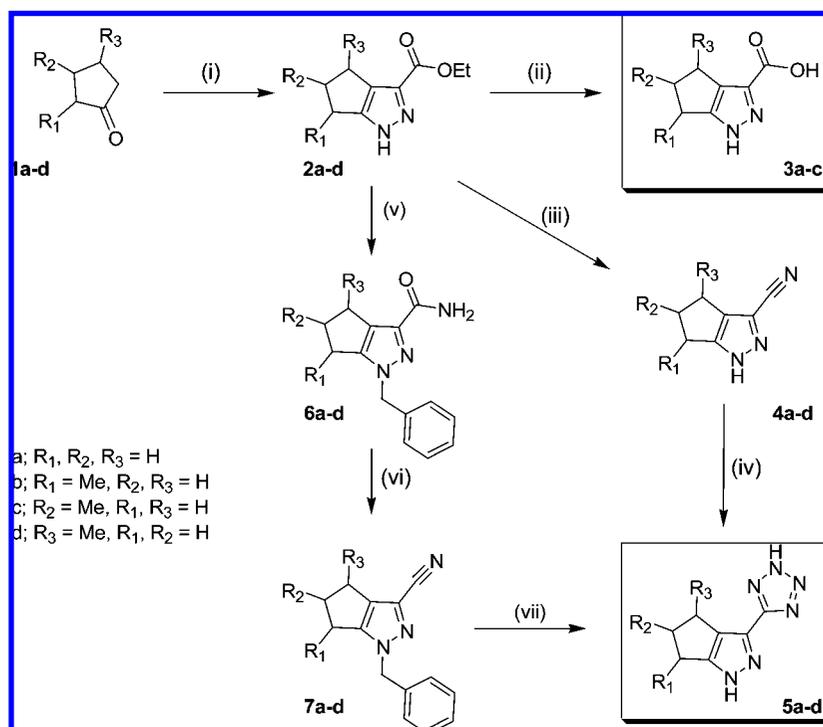
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^a Abbreviations: GPR109a, G-protein coupled receptor 109a (also known as HM74a); GPR109b, G-protein coupled receptor 109b (also known as HM74); PUMA-G, protein upregulated in macrophages by interferon gamma (the mouse orthologue of GPR109a); VLDL-c, very low-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; HDL-c, high density lipoprotein-cholesterol; Lp(a), lipoprotein(a); CHO cells, Chinese hamster ovary cells; TG, triglycerides; FFA, Free fatty acids; cAMP, 3',5'-cyclic adenosine monophosphate; MAPK, Mitogen activated protein kinase; ip, intraperitoneal; PGD₂, prostaglandin D₂.

Scheme 1^a

^a Reagents and conditions: (i) (a) NaOEt, EtOH, (EtOCO)₂, 75 °C; (b) H₂NNH₂ (aq.), 75 °C. (ii) Aqueous LiOH, THF/MeOH, 50–80 °C, 3 h. (iii) (a) NH₃, MeOH, reflux; (b) NaCl, POCl₃, MeCN, r.t. 3 h. (iv) NaN₃, DMF, 200 °C, microwave. (v) (a) NH₃, MeOH, reflux or NH₄OH, 1,4-dioxane, r.t.; (b) BnBr, base, 1,4-dioxane, r.t. (vi) SOCl₂, DMF, r.t. (vii) (a) NaN₃, ZnBr₂, DMF 120 °C or 200 °C, microwave; (b) DMSO, air, KO^tBu, r.t. or HCO₂H/MeOH, Pd (black), r.t.

of VLDL particles may limit the cholesterol ester transfer protein (CETP)-mediated exchange of cholesterol from HDL to VLDL, and TG from VLDL to HDL, thereby leading to a net increase in HDL levels.¹⁴ Clearly the identification of new agonists of the receptor with plasma FFA lowering activity *in vivo* would be of great interest to further explore this hypothesis. In addition, the identification of compounds that lack the characteristic flushing effect of nicotinic acid may lead to an improvement in patient compliance.

At the outset of our program, it was unclear as to the mechanism of the flushing effect and it appeared possible that the two known pharmacological effects of niacin could be separated with a molecule that selectively activated GPR109a but did not interact with whichever protein was responsible for the flushing. However, it has since been shown that both reduction of plasma FFA and vasodilation by nicotinic acid in mice requires the presence of mGPR109a.¹⁶ From these data, it would appear unlikely that a separation of these two key pharmacological effects can be achieved. Despite this supposition, we herein report the discovery of a new partial agonist of GPR109a that retains the plasma FFA lowering effects in rodents associated with receptor activation but that does not induce any vasodilation in mice at the maximum feasible dose and that acts as a competitive antagonist of nicotinic acid-induced vasodilation in the same model.

Our lead identification strategy, which involved an extensive SAR investigation starting from known small molecule compounds that had been shown to activate the receptor, has been described previously.¹⁷ This *in vitro* exploration of 5- and 6-membered heterocyclic acids led us to focus on a series of pyrazole acid derivatives. Further screening and elaboration provided a series of 5,5-fused pyrazole acids as suitable lead compounds. We have also demonstrated for a series of 4,5- and 5-substituted pyrazoles, replacement of the acid functionality

with a tetrazole resulted in a loss in potency of 1–2 orders of magnitude for GPR109a, although, on the whole, receptor selectivity was maintained.¹⁸ Despite this unpromising precedent, we sought to apply this isosteric replacement to the 5,5-fused pyrazole analogues, and so a series of acids and tetrazoles was prepared as outlined in Scheme 1.

Starting from the appropriate cyclic ketone (**1a–d**), acylation with diethyl oxalate followed by cyclization of the resultant diketo ester with hydrazine provided the bicyclic pyrazole esters **2a–d**. Base catalyzed hydrolysis of the ester function provided the acids **3a–c**. Our original route to the tetrazole series consisted of direct amidolysis of **2a–d** to provide the primary amides that were dehydrated by treatment with POCl₃ to provide the nitriles **4a–d** (R = H). Dipolar cycloaddition of **4a–d** with sodium azide under microwave heating provided the tetrazole analogues **5a–d**. All of the compounds containing alkyl groups on the cyclopentyl ring were prepared only as racemates. As a result of the very low yields observed in the latter two steps of the tetrazole synthesis, we also carried out a similar reaction sequence via the benzyl protected pyrazole amides **6a–d**. Dehydration to provide the nitriles **7a–d**, followed by dipolar cycloaddition with sodium azide and benzyl group deprotection, again provided the tetrazole analogues **5a–d**. The addition of these two steps significantly improved the overall yield, but the synthetic sequence was somewhat more cumbersome. For the preparation of **5a** on a larger scale, an alternative route was developed whereby ketone **1a** was acylated with the sodium salt of 1H-tetrazole-5-carboxylic acid ethyl ester and the resultant tetrazole diketone treated with hydrazine to form the pyrazole tetrazole directly.¹⁹

A comparison of the *in vitro* agonist activity data of the compounds synthesized is shown in Table 1. The 5,5-bicyclic acid (**3a**)¹⁷ showed good potency and full efficacy at both the cloned hGPR109a and mGPR109a receptors, whereas simple

Table 1. Agonist activity of bicyclic pyrazoles in the whole cell cAMP assay at the homologous hGPR109a and mGPR109a receptors

compound	R ₁	R ₂	R ₃	hGPR109a EC ₅₀ , μM (n) ^a	efficacy (% nicotinic acid response)	mGPR109a EC ₅₀ , μM (n) ^a	efficacy (% nicotinic acid response)
3a	H	H	H	0.86 ± 0.05 (2)	106 ± 13	0.5 ± 0.14 (2)	97 ± 4
3b	Me	H	H	8.3 ± 0.2 (3)	93 ± 14	12.2 ± 0.36 (2)	94 ± 5
3c	H	Me	H	n.e. ^d	n.e.	n.e.	n.e.
5a	H	H	H	1.65 ± 0.22 (23) ^b	59 ± 15	1.08 ± 0.33 (16) ^c	71 ± 15
5b	Me	H	H	n.e.	n.e.	n.e.	n.e.
5c	H	Me	H	n.e.	n.e.	n.e.	n.e.
5d	H	H	Me	n.e.	n.e.	n.e.	n.e.

^a EC₅₀ from multiple determinations. Errors shown are ± SEM. ^b 95% confidence interval = 1.3–2.0 μM. ^c 95% confidence interval = 0.7–1.6 μM. ^d n.e. = no effect at maximum concentration tested (100 μM).

methyl substitution around the cyclopentyl ring (**3b–c**) gave a significant reduction in potency or ablation of activity. In contrast to our previous experience, when the carboxylic acid moiety was replaced by a tetrazole, the unsubstituted analogue **5a** retained comparable potency to the parent. None of the methyl substituted bicyclic pyrazole tetrazole analogues, however, showed significant receptor activity in the cAMP assay. Furthermore, **5a** demonstrated clear and statistically significant partial agonism in the cAMP assays for both the mouse and human receptors with efficacy approximately 60–70% of that of either nicotinic acid or β-hydroxy butyrate, a putative physiologically relevant ligand for hGPR109a,²⁰ in the same assay platform. In addition, the compound showed no activation of GPR109b in the cAMP assay at any concentration up to 100 μM. Following these interesting observations, we then prepared a number of other 5,5-fused pyrazoles analogous to those that showed receptor activity in our earlier studies. For example, insertion of either an oxygen or sulfur heteroatom into the 5-membered ring fused to the pyrazole, while maintaining modest activity when the acid functionality was a carboxylate,¹⁷ showed barely measurable activity in the GPR109a cyclase assay when this group was replaced by a tetrazole. Hence, compound **5a** appeared to be somewhat unique among the members of the pyrazole tetrazole series in having reasonable receptor activity. As it was the first compound that we had discovered that showed clear partial agonist character in our in vitro cAMP assays for both hGPR109a and mGPR109a, we selected this compound for more extensive profiling and closer comparison with **3a**.

Further characterization of **5a** in a hGPR109a GTPγS assay (EC₅₀ = 2.3 ± 0.4 μM, n = 4; efficacy 72% of nicotinic acid response) confirmed the partial agonist character observed in the cAMP assay, whereas **3a** was again a full agonist in this assay (EC₅₀ = 10.4 ± 3.4 μM; n = 4; efficacy 99% of nicotinic acid response). Importantly for future in vivo studies, this partial agonist activity was maintained on the rodent receptors (**5a**; mGPR109a GTPγS assay, EC₅₀ = 0.4 ± 0.06 μM; n = 3; efficacy 68% of nicotinic acid response; rat GPR109a GTPγS assay, EC₅₀ = 2.3 ± 0.6 μM, n = 3; efficacy 38% of nicotinic acid response). In binding studies, **5a** was a competitive inhibitor of ³H-nicotinic acid binding to hGPR109a (**5a**; K_i = 505 ± 40 nM, n = 6; nicotinic acid; K_i = 50 ± 4 nM, n = 6). In a further demonstration of competition between **5a** and nicotinic acid, **5a** was shown to antagonize the effect of nicotinic acid in the hGPR109a cAMP assay, with a maximum antagonist efficacy consistent with its partial agonist character (see Supporting Information). Despite being a partial agonist of the cAMP pathway, **5a** was able to fully inhibit isoproterenol stimulated lipolysis in human adipocytes (**5a**; IC₅₀ = 3.1 ± 0.1 μM, n = 5) as, less surprisingly, was **3a**, and so both of these compounds were progressed into further studies. In line with the hypotheses outlined above, we focused our in vivo screening first on testing

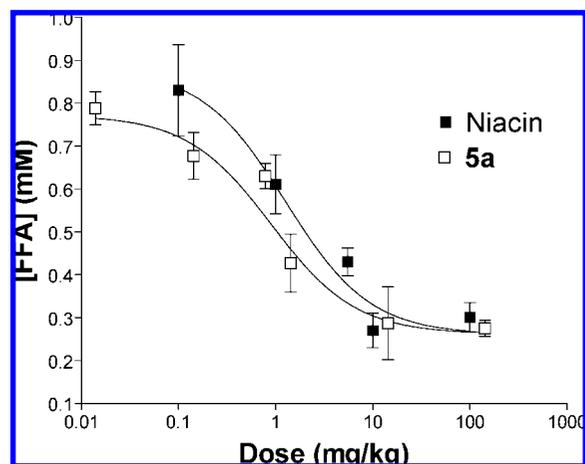


Figure 1. Effect of acute **5a** and nicotinic acid on plasma FFA levels in fasted C57/BL6 mice. Compounds were administered ip in saline 20 min prior to sample collection.

for acute effects on plasma FFA as a potential marker for longer term lipid profile modification and second on characterizing the flushing side effect in a quantitative manner.

The time course of the nicotinic acid-induced plasma FFA reduction was determined in mouse, and dose–response experiments with **5a** were performed at a single time point (20 min), the time of maximum efficacy of nicotinic acid in this model (Figure 1). Compound **5a** was similar in efficacy and marginally more potent than nicotinic acid in this acute test (ED₅₀ for **5a** = 3.1 ± 0.9 mg/kg; ED₅₀ for nicotinic acid = 9.7 ± 3.6 mg/kg), confirming what we had observed in vitro, that a partial agonist was capable of fully suppressing lipolysis in these systems. It has been previously shown that the antilipolytic effect of nicotinic acid requires the presence of the receptor as the compound has no effect on FFA in mGPR109a knockout mice. Compound **5a** was also without effect on FFA in mGPR109a knockout mice (see Supporting Information). The measured pharmacokinetic parameters in mice (Table 2) were consistent with the observed in vivo pharmacodynamic effect and plasma FFA measurements were made and further confirmed the pharmacokinetic–pharmacodynamic relationship.

Partial agonists of GPR109a have been previously described along with a prediction that such compounds may have tissue specific effects, but no in vivo data was included.²¹ More recently, it has been reported that other agonists of the receptor, both full and partial, including 5-isopropyl pyrazole-3-carboxylate, did not induce flushing in mice.²² In this paper, it was concluded that some GPR109a agonists may differentially activate parallel downstream receptor signaling pathways and that differences in flushing in vivo were not necessarily a function of efficacy at the receptor. Compounds that inhibit the

Table 2. Pharmacokinetic Parameters in Mouse for **5a**

parameter ^a	
Cl _p (mL/min/kg)	52
V _d (L/kg)	13
T _{1/2} (h)	10
C _{max} (μM)	16
T _{max} (h)	0.083
F _{oral} (%)	93

^a Cl_p, plasma clearance (blood clearance for mice); V_d, volume of distribution; T_{1/2}, terminal half-life; C_{max}, observed maximal plasma concentration following oral dosing; T_{max}, time to reach the C_{max}; F_{oral}, oral bioavailability. IV doses were formulated in PBS and injected at 5.0 mg/kg to male C57BL/6 mice. Peroral doses were formulated in PBS and given by oral gavage at 10 mg/kg.

cAMP pathway were able to inhibit lipolysis. Compounds that stimulated MAPK-induced phosphorylation of ERK1 and ERK2 were able to induce flushing, whereas those compounds that did not signal through MAPK but were still able to inhibit adenylate cyclase did not induce flushing. Consistent with these observations is that the production of PGD₂ requires the generation of arachidonic acid and its subsequent metabolism to prostaglandins, and this process is known to be regulated by activation of the MAPK pathway.²³ In our assays, **3a** but not **5a** was able to activate MAPK signaling in cells, overexpressing either mGPR109a or hGPR109a. In addition, **5a** was also a competitive antagonist of nicotinic acid-induced MAPK signaling in this model, showing that it can occupy the receptor but still fail to initiate signaling through MAPK.²⁴ Also consistent with these observations is that **5a** did not induce receptor internalization, a process known to be β-arrestin and MAPK-dependent and was able to block nicotinic acid-induced receptor internalization (data not shown). Hence it would be predicted that **3a** but not **5a** would be able to induce flushing in vivo in mice. It is likely that this ability of **5a** to distinguish between receptor activation pathways is due, at least in part, to its partial agonist character.

The vasodilation effect (a component of, and surrogate for, flushing) may be quantified in anesthetized mice by use of a laser-Doppler instrument to measure blood flow changes in the exposed ear.¹⁵ Nicotinic acid induces a dose-dependent increase in blood flow after ip injection in this model (Figure 2a), such that a dose of 100 mg/kg ip results in a 100% increase over baseline blood flow compared to vehicle treatment after 5 min. Compound **3a** also showed a similar dose-dependent effect in mice in the same dose range (data not shown). In contrast, as predicted from the in vitro data, there was no effect of increasing ip doses of **5a**, up to the maximal feasible dose (based on solubility of **5a** in the administration vehicle) of 400 mg/kg (Figure 2). Subsequent analysis of plasma levels of **5a** verified that concentrations of at least 30-fold higher than those that produced a maximal effect in the plasma FFA-lowering model in mouse were achieved (510 ± 230 μM following a dose of 100 mg/kg ip; plasma levels not measured at 400 mg/kg). In further experiments to characterize the effect of **5a** in this model, the compound was preadministered at 100 mg/kg ip to mice that were challenged 5 min later with a dose of nicotinic acid (30 mg/kg, ip) that normally produces robust vasodilation. A complete inhibition of the expected nicotinic acid-induced flushing effect was observed (Figure 3), consistent with the competitive antagonist effect of **5a** observed on the nicotinic acid-induced activation of MAPK signaling in vitro. As would also be expected, **5a** failed to antagonize flushing induced by PGD₂, which acts downstream of GPR109a.¹⁵

In summary, we have described the discovery and profiling of 3-(1*H*-tetrazol-5-yl)-1,4,5,6-tetrahydro-cyclopentapyrazole

(**5a**, MK-0354). This compound was found to be a partial agonist of GPR109a in all species tested and was shown to compete with nicotinic acid binding and activity in both a conventional radioligand binding assays and in vitro functional systems measuring cAMP accumulation. In vivo, **5a** possessed plasma FFA lowering effects in mice comparable to those of nicotinic acid. However, in contrast to nicotinic acid and the closely related pyrazole acid analogue **3a**, **5a** did not induce vasodilation in mice even at very high doses and was able to block the vasodilation induced by nicotinic acid in the same model. In addition, **5a** showed no interaction with any other target tested in a panel of over 120 other proteins, including the hERG channel, was not an inhibitor of any of the major CYP isoforms and had no effect in dog cardiovascular or mouse CNS safety pharmacology models (data not shown). From these data, **5a** was identified as a compound of sufficient interest to progress into further pharmacological studies in animals and eventually into human trials, to test the hypothesis that lowering plasma FFA by activation of GPR109a would result in similar HDL-c elevating lowering effect observed with nicotinic acid. Data from these advanced preclinical and clinical studies will be described elsewhere in due course.

Experimental Section

Proton and carbon nuclear magnetic resonance (¹H and ¹³C NMR) spectra were recorded on a Varian Mercury VX-400 equipped with a four-nucleus auto switchable probe and z-gradient or a Bruker Avance-400 equipped with a Quad Nucleus Probe (QNP) or a Broad Band Inverse (BBI) and z-gradient. 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. Microwave irradiations were carried out using the Emyrs synthesizer (Personal Chemistry). Thin-layer chromatography (TLC) was performed on silica gel 60 F₂₅₄ (Merck), and column chromatography was carried out on prepacked silica gel columns using KP-Sil supplied by Biotage. Evaporation was performed in vacuo on a Buchi rotary evaporator. Celite 545 was used for stated filtrations. Strong cation exchange (SCX) columns were purchased from Phenomenex (Strata SCX 55 μm, 70 Å). All other reagents were purchased from Aldrich.

Analytical HPLC/MS was conducted on an AB/MDS Sciex API 150EX mass spectrometer with an electrospray source, using a Shimadzu Inc. LC-10AD VP HPLC-pump, Shimadzu Inc. SCL-10A VP HPLC system controller, Shimadzu Inc. SPD-10A VP UV detector, monitoring at 214 nm, Leap Scientific CTC HTS, PAL autosampler, Analyst 1.2 software and an (a) Alltech Prevail C18 column (5 μ, 50 mm × 4.6 mm), using a gradient of 5% v/v CH₃CN (containing 1% v/v TFA) in H₂O (containing 1% v/v TFA) (t = 0.0 min) gradient to 100% v/v CH₃CN in H₂O (t = 20.0 min), 3.5 mL/min, or (b) Waters YMC ODS-A C18 column (5 μ, 50 mm × 4.6 mm), using a gradient of 5% v/v CH₃CN (containing 1% v/v TFA) in H₂O (containing 1% v/v TFA) (t = 0.0 min) gradient to 95% v/v CH₃CN in H₂O (t = 4.0 min), 3.5 mL/min.

Preparative HPLC was conducted on a Varian Prostar reverse phase HPLC using a Phenomenex Luna C18 column (10 μ, 250 mm × 50 mm), 5% (v/v) CH₃CN (containing 0.1% v/v TFA) in H₂O (containing 0.1% v/v TFA) gradient to 95% CH₃CN, 60 mL/min, λ = 220 nm, or using a Phenomenex Luna C18 column (10 μ, 250 mm × 21.20 mm), 5% (v/v) CH₃CN (containing 0.1% v/v TFA) in H₂O (containing 0.1% v/v TFA) gradient to 95% CH₃CN, 20 mL/min, λ = 220 nm. Compound **3a** was purchased from Fluorochem but for larger amounts was also prepared using the synthesis described below.

General Procedure for the Synthesis of 1*H*-Pyrazole-3-carboxylic acid ethyl esters (2a–d). The appropriate ketone (**1a–d**) was dissolved in ethanol (5 mL/mmol), and diethyl oxalate (1.2 eq.) and

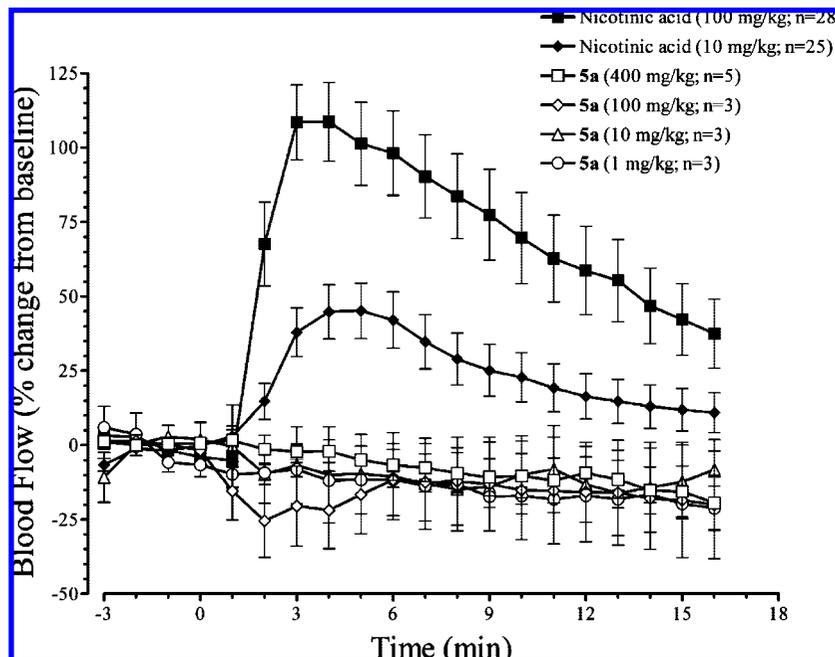


Figure 2. Quantification of the flushing response of nicotinic acid and **5a** as measured by laser-Doppler recordings of blood flow in the ear of male C57/BL6 mice.

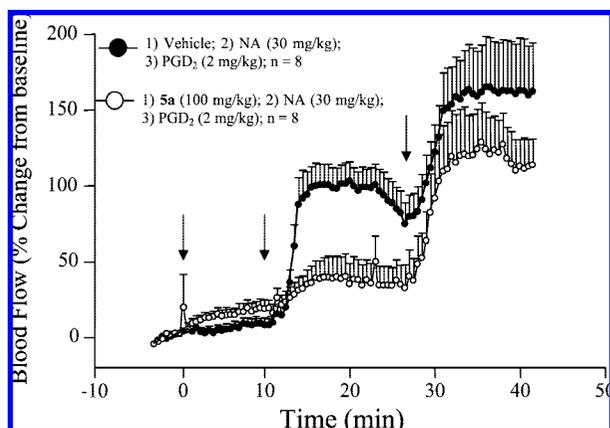


Figure 3. **5a** attenuates the vasodilation response induced by nicotinic acid but not that induced by PGD₂ as measured by laser-Doppler recordings of blood flow in the ear of male C57/BL6 mice following sequential treatment with compound (or vehicle), nicotinic acid, and PGD₂ at the time points shown. The first treatment in the sequence (see legend) was injected at $t = 0$, the second treatment at $t = 10$ min, and the third treatment at $t = 28$ min. NA = nicotinic acid.

sodium ethoxide (1.1 eq.) were added at r.t. The mixture was heated at 75 °C for 30 min and cooled to 4 °C in an ice bath. An aqueous solution of hydrazine (2 equiv, 2 mL/mmol) was added, and the resulting mixture was heated at 75 °C for 1 h. Solvent was removed under reduced pressure, and the crude residue was partitioned between DCM and water. The organic portion was separated and solvent was removed under reduced pressure, and the residue was either purified by column chromatography (*n*-hexane, to 50% EtOAc/*n*-hexane, silica) or hydrolyzed directly to the corresponding 1*H*-pyrazole-3-carboxylic acid (**3**) without further purification or analysis.

1,4,5,6-Tetrahydro-cyclopentapyrazole-3-carboxylic Acid Ethyl Ester (2a). (16.16 g, 90.0 mmol, 76%). m/z (ES⁺): 181 [M + H]⁺. ¹H NMR (CD₃OD): δ 4.34 (q, 2H, $J = 7.1$, OCH₂CH₃), 2.78 (t, 2H, $J = 7.0$), 2.72 (br s, 2H), 2.49 (br s, 2H), 1.36 (t, 3H, $J = 7.1$, OCH₂CH₃).

6-Methyl-1,4,5,6-tetrahydro-cyclopentapyrazole-3-carboxylic Acid Ethyl Ester (2b). (0.603 g, 3.11 mmol, 62%). m/z (ES⁺): 195 [M + H]⁺, 149 [M-OEt]⁺. ¹H NMR (CDCl₃): δ 10.7 (br s, 1H, NH),

4.35 (q, 2H, $J = 7.1$, OCH₂CH₃), 3.16 (sextet, 1H, $J = 7.10$, C(6)-H), 2.87–2.77 (m, 1H), 2.77–2.62 (m, 2H), 1.36 (t, 3H, $J = 7.1$, OCH₂CH₃), 1.29 (d, 3H, $J = 6.9$, CH₃). HPLC/MS: (column b) 94%, $t_r = 1.75$ min.

4- and 5-Methyl-1,4,5,6-tetrahydro-cyclopentapyrazole-3-carboxylic Acid Ethyl Ester (2c and 2d). (0.570 g, 2.94 mmol, 59%, ca. 1.3:1 mixture of 5-methyl (**2d**) and 4-methyl (**2c**) regioisomers). m/z (ES⁺): 195 [M + H]⁺, 149 [M-OEt]⁺. Major regioisomer 5-methyl-1,4,5,6-tetrahydro-cyclopentapyrazole-3-carboxylic acid ethyl ester (**2d**) ¹H NMR (CDCl₃): δ 10.9 (br s, 1H, NH), 4.42–4.30 (m, 2H, OCH₂CH₃), 3.02–2.90 (m, 2H), 2.43–2.32 (m, 2H), 2.08–1.98 (m, 1H), 1.40–1.34 (m, 3H, OCH₂CH₃), 1.21 (d, 3H, $J = 6.3$, CH₃); Minor regioisomer 4-methyl-1,4,5,6-tetrahydro-cyclopentapyrazole-3-carboxylic acid ethyl ester (**2c**) m/z (ES⁺): 195 [M + H]⁺, 149 [M-OEt]⁺. ¹H NMR (CDCl₃): δ 10.9 (br s, 1H, NH), 4.42–4.30 (m, 2H, OCH₂CH₃), 3.24 (sextet, 1H, $J = 6.7$, C(4)-H), 3.02–2.90 (m, 1H), 2.88–2.60 (m, 3H), 1.40–1.34 (m, 3H, OCH₂CH₃), 1.29 (d, 3H, $J = 6.9$, CH₃).

General Procedure for the Synthesis of 1*H*-Pyrazole-3-carboxylic acids (3a–c). The appropriate pyrazole-3-carboxylate ethyl ester (**2a–c**) was dissolved in a solution of 1:5:1 MeOH:THF:1 M aq LiOH (70 mL) or aq sodium hydroxide and heated to 50–80 °C for 3 h or until hydrolysis was complete. Solvent was removed under reduced pressure, and the resulting solid suspended in water (50 mL). The mixture was acidified to pH 1 by the addition of 1 M HCl and then extracted with ethyl acetate (100 mL). The organic portion was separated and solvent removed under reduced pressure and the residue purified by preparative reverse-phase HPLC to give the pyrazole-3-carboxylic acid (**3a–c**) as an off-white solid.

1,4,5,6-Tetrahydrocyclopenta[*c*]pyrazole-3-carboxylic Acid (3a). (5.10 g, 71%) HPLC/MS: (column a) 99%, $t_r = 2.18$ min, m/z (ES⁺): 153 [M + H]⁺, 135 [M-OH]⁺. ¹H NMR (DMSO-*d*₆): δ 2.70–2.60 (m, 4H, C(4)-H and C(6)-H), 2.42–2.32 (m, 2H, C(5)-H).

6-Methyl-1,4,5,6-tetrahydrocyclopenta[*c*]pyrazole-3-carboxylic Acid (3b). HPLC/MS: (column a) 99%, $t_r = 3.05$ min, m/z (ES⁺): 167 [M + H]⁺, 149 [M-OH]⁺. ¹H NMR (DMSO-*d*₆): δ 2.98 (sextet, 1H, $J = 6.2$, C(6)-H), 2.70–2.47 (m, 3H, C(4)-H and C(5)-H), 1.90–1.80 (m, 1H, C(5)-H), 1.11 (d, 3H, $J = 6.9$, CH₃).

5-Methyl-1,4,5,6-tetrahydrocyclopenta[*c*]pyrazole-3-carboxylic acid (3c). HPLC/MS: (column a) 99%, $t_r = 2.53$ min, m/z (ES⁺): 167 [M + H]⁺, 149 [M-OH]⁺. ¹H NMR (DMSO-*d*₆): δ 2.90–2.45 (m, 3H), 2.22–2.12 (m, 2H), 1.07 (t, 3H, $J = 6.3$, CH₃).

Preparation of 1-Benzyl-1,4,5,6-tetrahydro-cyclopentapyrazole-3-carboxylic acid amide (6a). 1,4,5,6-Tetrahydro-cyclopentapyrazole-3-carboxylic acid ethyl ester (**2a**) (0.808 g, 4.48 mmol) was suspended in methanolic ammonia (ca. 7 M, 12 mL) and the mixture heated under reflux overnight. The solution was cooled and the resulting precipitate (1,4,5,6-tetrahydro-cyclopentapyrazole-3-carboxylic acid amide) collected by vacuum filtration as a white crystalline solid (0.438 g, 2.90 mmol, 65%). m/z (ES⁺): 152 [M + H]⁺. ¹H NMR (CD₃OD): δ 2.79 (t, 2H, $J = 6.9$), 2.73 (t, 2H, $J = 7.3$), 2.55 (br s, 2H).

To a stirred solution of 1,4,5,6-tetrahydro-cyclopentapyrazole-3-carboxylic acid amide (3.77 g, 25.0 mmol) in DMF (50 mL) at 25 °C was added K₂CO₃ (12.1 g, 87.4 mmol) followed by benzyl bromide (11.7 g, 62.4 mmol). The reaction was heated to 55 °C and stirred for 16 h. After cooling to ambient temperature, the mixture was diluted with EtOAc (100 mL) and filtered. The filtrate was washed with H₂O (2 × 100 mL), the organic portion dried over MgSO₄, filtered, and the solvent removed under reduced pressure. Purification by column chromatography (50–95% EtOAc/hexanes, silica) provided the title compound (1.14 g, 4.73 mmol, 19% yield) as a white solid. m/z (ES⁺): 242 [M + H]⁺. ¹H NMR (CDCl₃): δ 7.37–7.30 (m, 3H), 7.19 (m, 2H), 6.67 (br s, 1H), 5.34 (br s, 1H), 5.19 (s, 2H), 2.82 (m, 2H), 2.51 (m, 4H).

Preparation of 1-Benzyl-6-methyl-1,4,5,6-tetrahydro-cyclopentapyrazole-3-carboxylic Acid Amide (6b). 6-Methyl-1,4,5,6-tetrahydro-cyclopentapyrazole-3-carboxylic acid ethyl ester (**2b**) (0.603 g, 3.11 mmol) was dissolved in 1,4-dioxane (3.5 mL) and ammonium hydroxide (25 mL) added. The resulting solution was stirred overnight at room temperature. Solvent was removed under reduced pressure and the residue dissolved in 1,4-dioxane (30 mL) and 5 M aqueous sodium hydroxide (0.72 mL, 3.64 mmol) added, followed by benzyl bromide (0.56 g, 3.30 mmol). The resulting solution was stirred at 25 °C for 20 h. An additional 5 M aqueous sodium hydroxide solution (0.30 mL, 1.5 mmol) and benzyl bromide (0.25 g, 1.50 mmol) was added, and the solution was stirred at 25 °C for an additional 20 h. Solvent was removed under reduced pressure and the residue partitioned between ethyl acetate and water. The organic portion was separated, solvent removed under reduced pressure, and the resulting residue purified by column chromatography (30–60% EtOAc/hexanes, silica) to provide the title compound (0.470 g, 1.84 mmol, 61% yield) as a colorless oil. HPLC/MS: (column b) $t_r = 2.35$ min, m/z (ES⁺): 256 [M + H]⁺, 239 [M-NH₂]⁺. ¹H NMR (CDCl₃): δ 7.35–7.25 (m, 3H), 7.13 (d, 2H, $J = 7.2$), 6.79 (br s, 1H, CONHH), 6.26 (br s, 1H, CONHH), 5.21 (q, 2H, $J = 15.7$, CH₂Ph), 3.00–2.90 (m, 1H), 2.90–2.78 (m, 1H), 2.78–2.65 (m, 2H), 2.10–2.00 (m, 1H), 1.10 (d, 3H, $J = 6.9$, CH₃). ¹³C NMR (CDCl₃): δ 165.0 (CONH₂), 155.4, 138.9, 136.3, 128.7 (C(2')), 128.5, 127.9 (C(4')), 126.9 (C(3')), 54.4 (CH₂Ph), 40.6 (C(5)), 32.0 (C(6)), 22.7 (C(4)), 19.3 (CH₃).

Preparation of 1-Benzyl-5-methyl-1,4,5,6-tetrahydro-cyclopentapyrazole-3-carboxylic Acid Amide (6c) and 1-Benzyl-4-methyl-1,4,5,6-tetrahydro-cyclopentapyrazole-3-carboxylic Acid Amide (6d). A mixture of 5- and 4-methyl-1,4,5,6-tetrahydro-cyclopentapyrazole-3-carboxylic acid ethyl ester (**2c** and **2d**) (0.570 g, 2.94 mmol) was dissolved in 1,4-dioxane (3.5 mL) and ammonium hydroxide (25 mL) was added. The resulting solution was stirred for 2 days at room temperature. Solvent was then removed under reduced pressure and the residue dissolved in 1,4-dioxane (30 mL) and 5 M aqueous sodium hydroxide (0.72 mL, 3.64 mmol) added, followed by benzyl bromide (0.56 g, 3.30 mmol). The resulting solution was stirred at room temperature for 20 h. Additional 5 M aqueous sodium hydroxide solution (0.30 mL, 1.5 mmol) and benzyl bromide (0.25 g, 1.50 mmol) was added, and the solution was stirred at room temperature for an additional 20 h. Solvent was removed under reduced pressure and the residue partitioned between ethyl acetate and water. The organic portion was separated, solvent removed under reduced pressure, and the resulting residue purified

by column chromatography (30–75% EtOAc/hexanes, silica) to give 1-benzyl-4-methyl-1,4,5,6-tetrahydro-cyclopentapyrazole-3-carboxylic acid amide **6d** (0.162 g, 0.64 mmol, 21% yield) as a colorless oil. HPLC/MS: (column b) $t_r = 2.39$ min, m/z (ES⁺): 256 [M + H]⁺, 239 [M-NH₂]⁺. ¹H NMR (CDCl₃): δ 7.37–7.26 (m, 3H), 7.17 (d, 2H, $J = 7.3$), 6.73 (br s, 1H, CONHH), 5.71 (br s, 1H, CONHH), 5.16 (s, 2H, CH₂Ph), 2.51 (sextet, 1H, $J = 6.6$, C(4)-H), 2.75–2.62 (m, 1H), 2.58–2.48 (m, 1H), 2.46–2.35 (m, 1H), 2.10–2.00 (m, 1H), 1.30 (d, 3H, $J = 6.9$, CH₃). ¹³C NMR (CDCl₃): δ 164.7 (CONH₂), 151.2, 139.0, 135.9, 134.1, 128.9 (C(2')), 128.2 (C(4')), 127.7 (C(3')), 55.0 (CH₂Ph), 39.9 (C(5)), 32.4 (C(4)), 23.3 (C(6)), 21.1 (CH₃).

Also obtained was 1-benzyl-5-methyl-1,4,5,6-tetrahydro-cyclopentapyrazole-3-carboxylic acid amide **6c** (0.213 g, 0.84 mmol, 28% yield) as a white solid. ¹H NMR (CDCl₃): δ 7.37–7.28 (m, 3H), 7.19–7.14 (m, 2H), 6.68 (br s, 1H, CONHH), 5.72 (br s, 1H, CONHH), 5.17 (s, 2H, CH₂Ph), 3.08–2.97 (m, 2H), 2.65 (dd, 1H, $J_1 = 15.3$, $J_2 = 7.5$), 2.41 (dd, 1H, $J_1 = 18.1$, $J_2 = 8.7$), 2.09 (dd, 1H, $J_1 = 15.51$, $J_2 = 6.2$), 1.150 (d, 3H, $J = 6.5$, CH₃). ¹³C NMR (CDCl₃): δ 164.9 (CONH₂), 150.9, 139.2, 136.0, 129.0 (C(2')), 128.23 (C(4')), 128.18, 127.7 (C(3')), 55.1 (CH₂Ph), 41.2 (C(5)), 32.7, 32.5, 21.8 (CH₃).

Preparation of 3-(2H-Tetrazol-5-yl)-1,4,5,6-tetrahydro-cyclopentapyrazole (5a). 1,4,5,6-Tetrahydro-cyclopentapyrazole-3-carboxylic acid amide (0.210 g, 1.39 mmol) was added to anhydrous acetonitrile (12 mL), heated to 80 °C, and sodium chloride (2.0 g, 34 mmol) was added. After 15 min, phosphorus oxychloride (0.128 g, 0.83 mmol) was added and the solution was heated at 80 °C overnight, cooled, filtered, and the collected solid washed with acetonitrile. Solvent was removed from the combined solutions under reduced pressure, and the resulting solid purified by preparative HPLC to provide 1,4,5,6-tetrahydro-cyclopentapyrazole-3-carbonitrile as a deep-purple-colored solid (0.031 g, 0.23 mmol, 17%). m/z (ES⁺): 134 [M + H]⁺. ¹H NMR (CD₃OD): δ 2.79 (t, 2H, $J = 7.3$), 2.73 (t, 2H, $J = 7.1$), 2.65–2.55 (m, 2H).

1,4,5,6-Tetrahydro-cyclopentapyrazole-3-carbonitrile (0.022 g, 0.165 mmol) and sodium azide (0.086 g, 1.30 mmol) were dissolved in DMF (3 mL) and heated under microwave irradiation at 175 °C for 20 min. The mixture was cooled to room temperature, filtered, and the filtered solid washed with ethyl acetate. The combined solutions were added to saturated aqueous sodium bicarbonate (20 mL) and washed with ethyl acetate (20 mL). The aqueous layer was acidified to pH 1 with the addition of 1 M aqueous hydrochloric acid and extracted with ethyl acetate (2 × 20 mL). The ethyl acetate extracts were combined and solvent removed under reduced pressure and the resulting solid purified by preparative HPLC to give 3-(2H-tetrazol-5-yl)-1,4,5,6-tetrahydro-cyclopentapyrazole (**5a**) as a white solid (0.012 g, 0.068 mmol, 41%).

Alternative Preparation of 3-(2H-tetrazol-5-yl)-1,4,5,6-tetrahydro-cyclopentapyrazole (5a). Thionyl chloride (760 mg, 6.39 mmol) was added dropwise to a solution of 1-benzyl-1,4,5,6-tetrahydro-cyclopentapyrazole-3-carboxylic acid amide (**6a**, 1.03 g, 4.27 mmol) in DMF (17 mL) at room temperature. The reaction mixture was stirred for 17 h at which time NaHCO₃ (saturated aq, 2 mL) was added to quench excess thionyl chloride. The mixture was diluted with EtOAc (100 mL) and washed sequentially with NaHCO₃ (saturated aq, 75 mL) and H₂O (2 × 100 mL). The organic portion was separated and dried over MgSO₄. The mixture was filtered and solvent removed under reduced pressure to provide 1-benzyl-1,4,5,6-tetrahydro-cyclopentapyrazole-3-carbonitrile (**7a**, 760 mg, 3.41 mmol, 80% yield) as a yellow oil, which was not purified further. ¹H NMR (CDCl₃): δ 7.40–7.34 (m, 3H), 7.23 (m, 2H), 5.22 (s, 2H), 2.70 (m, 2H), 2.52 (m, 4H).

To a solution of **7a** (700 mg, 3.14 mmol) in DMF (6.8 mL) in a heavy walled reaction vessel was added sequentially ZnBr₂ (1.30 g, 4.98 mmol) and NaN₃ (775 mg, 11.9 mmol). The vessel was sealed and heated at 120 °C for 18 h. The resultant mixture was cooled to room temperature and HCl (3 M aq, 2 mL) was added whereupon stirring was continued for 5 min. The mixture was diluted with EtOAc (50 mL) and washed with HCl (1M, aq, 50 mL). The organic portion was dried over MgSO₄, the mixture

filtered, and solvent removed under reduced pressure. Purification by silica gel chromatography (50:50:0.2, hexanes:EtOAc:AcOH) gave 1-benzyl-3-(2*H*-tetrazol-5-yl)-1,4,5,6-tetrahydro-cyclopentapyrazole (450 mg, 1.69 mmol, 54% yield) as a white solid.

Air was bubbled through a stirring solution of 1-benzyl-3-(2*H*-tetrazol-5-yl)-1,4,5,6-tetrahydro-cyclopentapyrazole (450 mg, 1.69 mmol) and KO*t*-Bu (11.8 mL of a 1 M solution in THF) in DMSO (10 mL) for 1.5 h. The reaction mixture was acidified by the addition of 3 M aq HCl (5 mL). The reaction mixture was then adjusted to pH 3 by cautious addition of solid K₂CO₃. The mixture was filtered and solvent removed under reduced pressure. The residue was purified by reverse-phase HPLC to provide 3-(2*H*-tetrazol-5-yl)-1,4,5,6-tetrahydro-cyclopentapyrazole (207 mg, 1.18 mmol, 70% yield) (**5a**) as a white solid. HPLC/MS: (column a) 99%, *t_r* = 2.28 min, *m/z* (ES+): 177 [M + H]⁺, 149 [M-N₂ + H]⁺. ¹H NMR (CD₃OD): δ 2.88 (t, 2H, *J* = 7.0), 2.82 (t, 2H, *J* = 7.3), 2.64 (quintet, 2H, *J* = 7.1).

Preparation of 6-Methyl-3-(2*H*-tetrazol-5-yl)-1,4,5,6-tetrahydro-cyclopentapyrazole (5b). Thionyl chloride (5.5 mL, 0.5 M in DMF, 2.75 mmol) was added to a solution of 1-benzyl-6-methyl-1,4,5,6-tetrahydro-cyclopentapyrazole-3-carboxylic acid amide (**6b**, 0.467 g, 1.83 mmol) in DMF (17 mL) and the resulting mixture stirred at room temperature for 3 h. NaHCO₃ was then added to quench excess thionyl chloride and the solution stirred for an additional 30 min. The mixture was extracted with EtOAc (2 × 30 mL) and the combined extracts washed with brine, dried over MgSO₄, filtered, and the solvent removed under reduced pressure to give 1-benzyl-6-methyl-1,4,5,6-tetrahydro-cyclopentapyrazole-3-carbonitrile (**7b**), which was directly without further purification.

Zinc bromide (0.506 g, 2.25 mmol) and sodium azide (0.238 g, 3.66 mmol) were added to a solution of **7b** in DMF (3 mL). The resulting solution was heated under microwave irradiation to 200 °C for 10 min. Additional sodium azide (0.100 g, 1.54 mmol) was added, and the solution was heated to 200 °C for a further 10 min. Solvent was removed under reduced pressure, and the resulting solid was suspended in aqueous 3 M hydrochloric acid and extracted with ethyl acetate (2 × 20 mL). The organic solution was dried over magnesium sulfate and solvent removed under reduced pressure to give 1-benzyl-6-methyl-3-(2*H*-tetrazol-5-yl)-1,4,5,6-tetrahydro-cyclopentapyrazole, which was used directly without further purification. HPLC/MS: (column b) 99%; *t_r* = 2.43 min; *m/z* (ES+): 281 [M + H]⁺, 253 [M-N₂ + H]⁺.

1-Benzyl-6-methyl-3-(2*H*-tetrazol-5-yl)-1,4,5,6-tetrahydro-cyclopentapyrazole was dissolved in formic acid (10% in methanol, 2.5 mL) and palladium black (350 mg) added. The resulting solution was stirred at room temperature for 2 days, filtered, and the residue applied to a 10 g SCX cartridge, which was then eluted with methanol to remove no acidic species. Elution with ammonia (2 M in ethanol) provided 6-methyl-3-(2*H*-tetrazol-5-yl)-1,4,5,6-tetrahydro-cyclopentapyrazole ammonium salt (**5b**) as a white solid (0.111 g, 0.53 mmol). HPLC/MS: (column a) 97%; *t_r* = 3.03 min; *m/z* (ES+): 191 [M + H]⁺, 163 [M-N₂ + H]⁺. ¹H NMR (CD₃OD): δ 3.15 (sextet, 1H, *J* = 7.1, C(6)-H), 2.95–2.85 (m, 2H), 2.82–2.68 (m, 2H), 2.12–2.00 (m, 1H), 1.29 (d, 3H, *J* = 6.9, CH₃).

Preparation of 5-Methyl-3-(2*H*-tetrazol-5-yl)-1,4,5,6-tetrahydro-cyclopentapyrazole (5c). **5c** was prepared using the same method described above for **5b** and the title compound isolated as a white solid (0.053 g, 0.25 mmol). HPLC/MS: (column a) 98%; *t_r* = 3.08 min; *m/z* (ES+): 191 [M + H]⁺, 163 [M-N₂ + H]⁺. ¹H NMR (CD₃OD): δ 3.65–3.55 (m, 1H), 3.12–2.85 (m, 2H), 2.41 (ddd, 1H, *J*₁ = 43.5, *J*₂ = 14.4, *J*₃ = 5.2), 1.28–1.13 (m, 3H, CH₃).

Preparation of 4-Methyl-3-(2*H*-tetrazol-5-yl)-1,4,5,6-tetrahydro-cyclopentapyrazole (5d). **5d** was prepared using the same method described above for **5b** and the title compound isolated as a white solid (0.032 g, 0.15 mmol). HPLC/MS: (column a) 99%; *t_r* = 3.30 min; *m/z* (ES+): 191 [M + H]⁺, 163 [M-N₂ + H]⁺. ¹H NMR (CD₃OD): δ 3.40 (sextet, 1H, *J* = 6.51, C(4)-H), 2.85–2.60 (m, 3H), 1.26 (d, 3H, *J* = 6.8, CH₃).

In Vitro Assays. [³H] Nicotinic Acid Binding. Radioligand binding assays were carried out on membranes derived from stably transfected Chinese hamster ovary (CHO) cells. The derivation of

the cell lines and the radioligand binding protocol have been described previously.²⁰

[³²S] GTPγS Binding Assay. [³²S] GTPγS binding assays were performed as previously described.²⁰

Measurement of Adenylyl Cyclase Inhibition. A 96-well adenylyl cyclase activation flashplate assay kit (Perkin-Elmer) protocol was developed and applied as described previously.²²

Human Subcutaneous Fat Lipolysis Assay. Cultured human subcutaneous adipocytes were received from Zen Bio., Inc. plated in 96-well plates two weeks prior to performing the lipolysis assay. Upon arrival, all media was removed and pooled (ZenBio Adipocyte Maintenance Medium). Then 150 μL of this media was realiquoted to each well. Cells were kept in a sterile, humidified incubator at 37 °C. On the day of the lipolysis assay, cells were washed twice with 150 μL of Zen Bio Wash buffer. After the second wash and removal of wash buffer, 75 μL of test compounds were added to each well in triplicate. Compounds were prepared in Zen Bio Assay buffer plus 1 μM theophylline. Cells were incubated for 5 h at 37 °C. Glycerol was determined using a free glycerol reagent from Sigma (reagent A). Adipocyte media (50 μL) was removed and transferred to a flat-bottom 96-well plate. Reagent A (50 μL) was then added to each well. After 15 min, absorbance was read at OD₅₄₀ on a Spectramax 340PC microplate reader (Molecular Devices). The amount of glycerol released was calculated based on regression analysis of known glycerol concentrations using a Glycerol Standard (Sigma).

In Vivo Assays. Animals. Animal studies were performed according to the Guide for the Care and Use of Laboratory Animals published by the National Academy of Sciences (1996) and approved by the Arena Pharmaceuticals and Merck Research Laboratories Animal Care and Use Committees. All mice used were C57Bl/6 males, at least 8 weeks old (The Jackson Laboratories West, Sacramento, CA). Animals were housed under standard laboratory conditions with a 12 h dark, 12 h light cycle with constant temperature and humidity. Mice were fed a standard rodent diet, and had ad libitum access to food and water.

In Vivo Mouse Lipolysis. Prior to study, mice were fasted for 16 h. Compound or vehicle (0.5% methylcellulose) was administered by oral gavage (po), and animals were euthanized 20 min postdose by CO₂ asphyxiation. Blood was collected via the inferior vena cava, anticoagulated in EDTA, and plasma separated by centrifugation on a tabletop microcentrifuge. Plasma was used for the measurement of FFA, using an enzymatic method (NEFA C Free Fatty-Acid Assay; WAKO Chemicals USA, Richmond, VA) and for the measurement of compound levels, by API-4000 LC-MS/MS after acetonitrile precipitation.

In Vivo Mouse Vasodilation. Mouse vasodilation was measured by laser Doppler flowmetry as previously described.²² Briefly, male C57Bl/6 mice (8–10 weeks old; ~25 g) were anesthetized with Nembutal via ip injection (80 mg/10 mL/kg). After 10 min, the mouse was placed under an LDPI laser Doppler (PeriScan PIM II; Perimed, Stockholm) and a needle and syringe containing vehicle (PBS; 40% hydroxypropyl-β-cyclodextrin (HPBCD) or 0.5% methylcellulose) or drug was placed in the intraperitoneal space and a slight back pressure was applied to prevent premature delivery of compound. The mouse's right ear was turned inside-out to expose the ventral side using forceps. The laser Doppler was focused in the center of the ventral right ear and adjusted as follows: repeated data collection; 15 × 15 image format, auto interval start, 20 s delay, medium resolution, very fast scan speed, and 8–9 V intensity (~4.5 cm from ear). After a three minute baseline reading, vehicle or compound was administered into the ip space (5 mL/kg through the preinserted syringe) and readings continued for approximately 15 min. Vasodilation was expressed as “% change of perfusion over baseline values”. At the end of the studies, mice were euthanized and a blood sample was collected by cardiac puncture and anticoagulated in EDTA. Plasma was obtained by centrifugation and used for determination of compound concentration by LC-MS/MS.

Supporting Information Available: HPLC-MS spectra, dose-response curves for **5a**, **5a** antagonizes nicotinic acid, PK-PD relationship for **5a** and nicotinic acid in c57/bl6 mice, effect of **5a** and nicotinic acid in PUMA-G knockout mice. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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