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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 17 (2007) 5620–5623

Fluorinated pyrazole acids are agonists of the high affinity niacin receptor GPR109a

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Received 1 June 2007; revised 23 July 2007; accepted 24 July 2007
Available online 23 August 2007

Abstract—A series of 5-alkyl pyrazole-3-carboxylic acids were prepared and found to act as potent and selective agonists of the human GPCR, GPR109a, the high affinity nicotinic acid receptor. No activity was observed at the highly homologous low affinity niacin receptor, GPR109b. A further series of 4-fluoro-5-alkyl pyrazole-3-carboxylic acids were shown to display similar potency. One example from the series was shown to have improved properties in vivo compared to niacin.

Niacin (**1**) (Fig. 1) has long been used for the treatment of lipid disorders and for the prevention of cardiovascular disease, the leading cause of death in the U.S., as a result of its ability to raise high-density lipoprotein (HDL) levels.[1] Recent mechanistic investigations have shown that niacin may exert its beneficial action through activation of a G-protein-coupled receptor (GPCR) located on adipocytes.[2] The consequent decrease in intracellular cAMP is believed to result in inhibition of lipolysis by negative modulation of lipase activity and perilipin phosphorylation, thereby decreasing plasma free fatty acid (FFA) levels which has been postulated to result in increased HDL.[3] Two closely related human orphan G_i-protein coupled receptors, both of which are expressed in human adipocytes (termed GPR109b, or HM74 and GPR109a, or HM74A; 95% identity), have recently been identified as possible molecular targets for niacin.[4,5]

GPR109a is the human ortholog of the previously described rodent receptor (PUMA-G),[6] whereas GPR109b appears to have arisen from evolutionary late gene duplication. It differs from GPR109a and PUMA-G mainly in the C-terminal region and a search of available genomic databases reveals it is found only in chimpanzees and humans. Niacin has been shown to activate GPR109a with an EC_{50} of 250 nM in a GTPγS assay and displaces ^{3}H-niacin from GPR109a expressing Chinese hamster ovary (CHO) cell membranes with an IC_{50} of 81 nM.[4] It is a much weaker ligand for GPR109b with an EC_{50} in the millimolar range. The lack of a GPR109b ortholog in rodents suggests that GPR109a is sufficient for the antilipolytic activity of niacin in vivo.[7] Recent evidence shows that the cutaneous flushing side effect in mice requires the presence of PUMA-G,[8] and thus suggests that the human flushing response, observed in the majority of patients, most likely occurs via GPR109a.

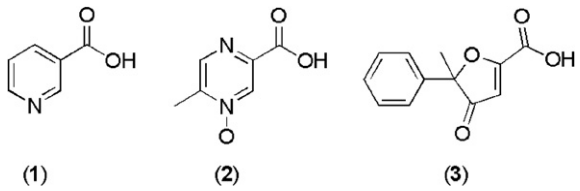

Figure 1. Known ligands for GPR109a.

Keywords: Niacin; GPR109a; GPR109b; GPCRs; Lipolysis; Free fatty acids; Selectfluor; Cutaneous flushing; Pyrazole carboxylic acids; HM74; HM74a; PUMA-G.
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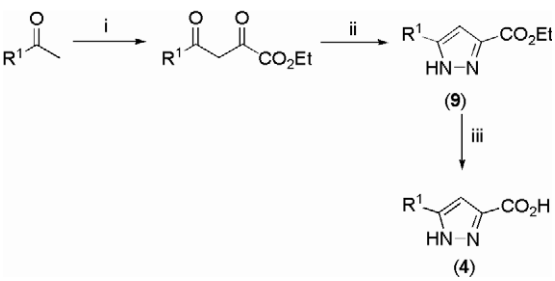
doi:10.1016/j.bmcl.2007.07.101

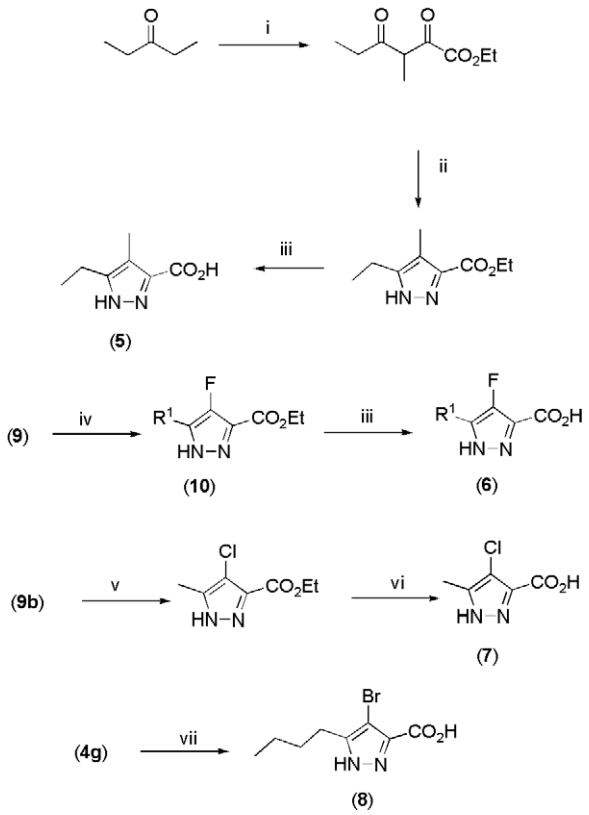
In addition to Niacin (**1**) two other agents that interact with GPR109a have been shown to elevate HDL in rodents and humans. Acipimox (**2**, EC_{50} = 2.0 μM) was launched in 1985 by Pharmacia (now Pfizer) for the treatment of hyperlipidemia and cutaneous flushing is reported as a side effect with this compound.[9] Acifran[10] (**3**) also raises HDL in humans; however, it lacks selectivity for GPR109a (EC_{50} = 2.1 μM) over GPR109b (EC_{50} = 20 μM)[4] and also induces cutaneous flushing.[11] Additional agents have been shown to mediate lipid levels in rats and to bind or activate GPR109a in vitro. 5-*n*-Propyl- (**4d**), 5-*i*-propyl- (**4e**) and 5-*n*-butyl-pyrazole-3-carboxylic acid (**4g**) have been shown to induce hypolipidemia in rats.[12] An extension of the alkyl pyrazole series also recently incorporated functionalized 5-benzyl-pyrazole-3-carboxylic acids which were proposed as partial agonists of GPR109a.[13] Recent investigations have also extended the scope of the acifran series, however, selectivity for GPR109b remains an issue.[14,15] As a part of our ongoing studies to develop selective agonists of GPR109a, we investigated a series of 4-functionalized-5-alkyl-pyrazole-3-carboxylic acids (**4**–**8**).

5-Alkyl-pyrazole-3-carboxylic acids (**4**) were readily synthesized via Claisen condensation of diethyl oxalate with alkyl methyl ketones to give the corresponding α,γ-diketo esters (Scheme 1). Cyclization with hydrazine hydrochloride afforded the pyrazole-3-carboxylic acid ethyl esters (**9**), which upon hydrolysis provided the desired 5-alkyl-pyrazole-3-carboxylic acids (**4**). 5-Ethyl-4-methylpyrazole-3-carboxylic acid (**5**) was prepared in a similar manner from 3-pentanone (Scheme 2). Synthesis of 5-alkyl-4-fluoro-pyrazole-3-carboxylic acids (**6**) was achieved via Selectfluor™ fluorination of the pyrazole-3-carboxylic acid ethyl esters (**9**) in acetonitrile to give 5-alkyl-4-fluoro-pyrazole-3-carboxylic acid ethyl esters (**10**) and subsequent hydrolysis. Chlorination of 4-methylpyrazole-3-carboxylic acid ethyl ester (**9b**) with *N*-chlorosuccinimide in CCl_4 smoothly gave 3-chloro-4-methylpyrazole-3-carboxylic acid ethyl ester which was hydrolyzed to give 3-chloro-4-methylpyrazole-3-carboxylic acid (**7**). 4-Butylpyrazole-3-carboxylic acid (**4g**) was also readily brominated with bromine in acetic acid to provide 3-bromo-4-butylpyrazole-3-carboxylic acid (**7**).

The biological activity at GPR109a of each of the 4-functionalized-5-alkyl-pyrazole-3-carboxylic acids (**4**–**8**)



Scheme 1. Reagents and conditions: (i) EtO_2CCO_2Et, KO*t*-Bu, EtOH, 25 °C, 3 h; (ii) N_2H_4·HCl, EtOH, H_2O, 80°; (iii) LiOH (aq), MeOH, THF, 25 °C, 3 h.

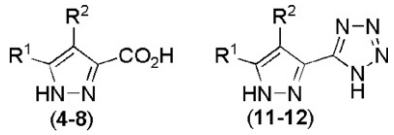


Scheme 2. Reagents and conditions: (i) EtO_2CCO_2Et, KO*t*-Bu, EtOH, 25 °C, 3 h; (ii) N_2H_4·HCl, EtOH, H_2O, 80°; (iii) LiOH (aq), MeOH, THF, 25 °C, 3 h; (iv) Selectfluor™, MeCN, 65 °C, 18 h; (v) NCS, CCl_4; (vi) NaOH (aq); (vii) Br_2, AcOH.

was measured using a cAMP whole cell Dynamic2 Homogenous Time-Resolved Fluorescence (HTRF) assay (Table 1). Twelve compounds were found to exhibit an EC_{50} below 1 μM, and of these seven exhibited EC_{50} values of 100 nM or lower. All of the compounds prepared, which had measurable activity and complete dose–response curves, displayed efficacy values of 95–100% relative to niacin (**1**) suggesting that they are all full agonists. Niacin (**1**) has previously been shown to be equally efficacious with β-hydroxybutyrate, which has been proposed as a physiologically relevant ligand for the receptor.[17] Optimum activity was found for small alkyl substituents at C(5)-R^1, namely methyl, ethyl, cyclopropyl, and butyl. Fluorination at C(4)-R^2 was well tolerated, resulting in compounds that were equipotent with the non-functionalized analogs. Bromination, chlorination, and insertion of a methyl group at C(4)-R^2 however, resulted in significant loss of activity in the HTRF assay. None of the compounds displayed any activity on GPR109b at concentrations up to 50 μM with the exception of **4e**, **4g**, and **4i**, which displayed activities two to three orders of magnitude less potent than at GPR109a.

The effect of replacing the carboxylic acid moiety with the commonly used tetrazole isostere was also investigated. Formation of 5-functionalized-1H-pyrazol-3-yl-1H-tetrazoles (**11a**, **b**, **e**) was achieved in three steps from the related ethyl carboxylic ester (**9**) (Scheme 3). Ami-

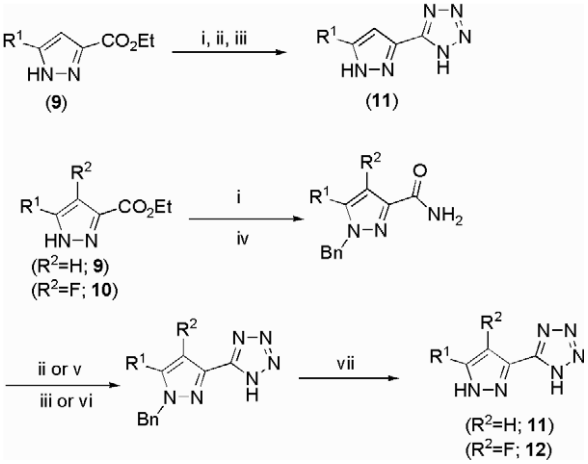
Table 1. GPR109a and GPR109b agonist activity of selected 4,5-disubstituted pyrazole-3-carboxylic acids and 4,5-disubstituted pyrazole-3-tetrazoles[a]



Compound	C(5)-R^1	C(4)-R^2	GPR109a pEC_{50} (*n*)	GPR109b pEC_{50} (*n*)
1	—	—	8.06 ± 0.19 (17)	4.12 ± 0.35 (8)
4a[b]	H	H	6.11 ± 0.10 (2)	NA (2)
4b[c,d]	Me	H	7.61 ± 0.26 (5)	NA (2)
4c[d]	Et	H	7.10 ± 0.17 (5)	NA (2)
4d[b,d]	Pr	H	6.74 ± 0.15 (5)	NA (2)
4e[b,c,d]	*i*-Pr	H	6.96 ± 0.19 (5)	4.38 ± 0.33 (2)
4f[d]	*c*-Pr	H	7.00 ± 0.18 (5)	NA (2)
4g[b,c,d]	Bu	H	7.07 ± 0.18 (5)	4.93 ± 0.19 (4)
4h[d]	*c*-Bu	H	5.65 ± 0.14 (4)	NA (2)
4i[c,d]	Pentyl	H	6.96 ± 0.22 (5)	4.73 ± 0.08 (4)
5	Et	Me	5.53 ± 0.19 (3)	NA (2)
6B	Me	F	7.38 ± 0.27 (5)	NA (2)
6C	Et	F	7.24 ± 0.28 (5)	NA (2)
6F	*c*-Pr	F	6.57 ± 0.30 (5)	NA (2)
6G	Bu	F	7.10 ± 0.25 (5)	NA (2)
7	Me	Cl	5.60 ± 0.22 (5)	NA (2)
8	Bu	Br	5.36 ± 0.17 (3)	NA (2)
11a	H	H	4.77 ± 0.07 (2)	NT
11b	Me	H	5.82 ± 0.11 (2)	NT
11d	Pr	H	5.11 ± 0.31 (5)	6.14 ± 0.18 (5)
11e	*i*-Pr	H	4.39 ± 0.56 (2)	NT
11f	*c*-Pr	H	4.74 ± 0.49 (5)	5.49 ± 0.08 (5)
11g	Bu	H	4.73 ± 0.27 (6)	5.12 ± 0.21 (5)
12b	Me	F	5.82 ± 0.07 (7)	NA (3)
12c	Et	F	6.12 ± 0.19 (7)	4.91 ± 0.18 (2)
12f	*c*-Pr	F	5.23 ± 0.78 (4)	NA (2)
12g	Bu	F	5.46 ± 0.18 (5)	NA (3)

[a] Activities were measured from 30 pM to 100 μM and are provided as the negative log of the molar value of EC_{50}. Errors are ± log SD. Compounds that showed no response are designated NA (not active). Compounds that were untested are designated NT. Efficacies were observed as 95–100% relative to niacin where accurately measurable.
[b] Previously described by van Herk et al.[13]
[c] Previously described by Seki et al.[12]
[d] Previously described by Gharbaoui et al.[16]

nolysis provided the primary amide which was reduced to the nitrile using phosphorus oxychloride allowing formation of the desired tetrazole by cyclization with sodium azide. Yields for the reduction step however, were low, hence preparation of further tetrazoles (**11d**, **11f**, **11g**, **12b**, **12c**, **12f**, **12g**) was achieved in five steps via a benzyl protected pyrazole. Benzyl protection of the primary amide, reduction and cyclization with azidotrimethylsilane or sodium azide gave the intermediate benzyl protected tetrazole. Debenzylation in aerated DMSO gave the desired pyrazole-tetrazoles (**17** and **18**). However, only moderate activity on GPR109a was observed; all of the examples, with the exception of **12c**, having EC_{50} values of greater than 1 μM. In addition, all the tetrazoles were significantly less potent than the analogous carboxylic acids. As the pK_a values of the tetrazole and carboxylic acids are similar, this is highly suggestive that the acid binding region of the



Scheme 3. Reagents and conditions: (i) NH_3, MeOH, 50 °C, 18 h; (ii) $POCl_3$, MeCN, NaCl, 80 °C, 18 h; (iii) NaN_3, ZnBr, DMF, 175 °C, 20 mins; (iv) BnBr, K_2CO_3, DMF, 55 °C, 18 h; (v) $SOCl_2$, DMF, rt, 18 h; (vi) N_3SiMe_3, DMF, 175 °C, 20 mins, μW; (vii) DMSO, THF, O_2, 2 h.

receptor is sterically constrained and thus highly selective in this region. Weak activity at GPR109b was observed for **12c** and **11g**, and moderate activity for **11d** (EC_{50} = 860 nM) and **11f** (EC_{50} = 3.6 μM) with an accompanying reversal of selectivity over GPR109a. Increasing activity at GPR109b with larger substituents is consistent with a predicted increase in the size of the binding region of GPR109b over GPR109a.[18] The remaining tetrazoles (**11** and **12**) displayed no activity at GPR109b.

The reduction of free fatty acid (FFA) levels induced by oral administration of 4-fluoro-5-methyl-pyrazole-3-carboxylic acid (**6b**) was measured in fasted male Sprague–Dawley rats (Fig. 2). Significant reductions in plasma FFA levels were observed upon administration of both 1 and 10 mg/kg **6b** that were essentially equivalent in magnitude to the response elicited by a 10 mg/kg dose of niacin, but the effects were significantly longer lasting. Only a very modest reduction of FFA levels was observed following oral administration of 0.1 mg/kg of **6b** which was not statistically significant. Cutaneous vasodilation was also measured in male C57 Bl/6 mice as a surrogate for the flushing side effect (Fig. 3). **6b**, administered at 100 mg/kg, elicited a cutaneous vasodilation response that appeared notably less than that

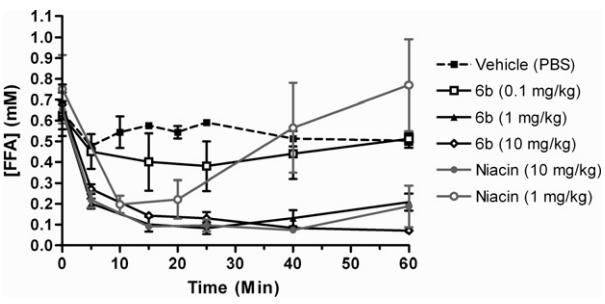


Figure 2. Free fatty acid reduction in male Sprague–Dawley rats with niacin (**1**) and (**6b**).

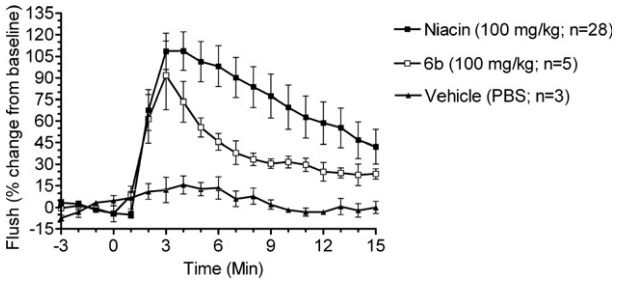


Figure 3. Cutaneous flushing response in male C57 BL/6 mice with niacin (**1**) and (**6b**).

elicited by an equivalent dose of niacin (**1**) ($p = 0.07$ at 6 min).

In summary, a series of 5-alkyl-pyrazole-3-carboxylic acids were prepared and assessed for their activity at the human GPCR GPR109a. Functionalization at the 4-position with either chlorine, bromine, or methyl substituents led to significant loss in potency relative to the unsubstituted analogs. Compounds functionalized with 4-fluoro substituents retained activity, however. The most potent compound from this series, 4-fluoro-5-methyl-pyrazole-3-carboxylic acid (**6b**, $EC_{50} = 42$ nM), was shown to effectively decrease plasma free fatty acid levels in male Sprague–Dawley rats, an effect that was much longer lasting than acute treatment with niacin at 10 mg/kg. Although some cutaneous flushing was also observed, flushing was notably lower than for a high (100 mg/kg) dose of niacin, suggesting that there may be a window of separation between the flushing and antilipolytic effects of compounds of this type in rodents.

Supplementary data

Synthetic methods, spectroscopic data and assay conditions. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.07.101.

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