Bioorganic & Medicinal Chemistry Letters 19 (2009) 4207–4209

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

5-N,N-Disubstituted 5-aminopyrazole-3-carboxylic acids are highly potent agonists of GPR109b

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ARTICLE INFO

Article history:
Received 8 April 2009
Revised 23 May 2009
Accepted 27 May 2009
Available online 30 May 2009

Keywords:
Niacin
GPR109a
GPR109b
HM74
HM74a
HDL
LDL
GPCR
Dyslipidemia
Atherosclerosis
Flushing
Pyrazole
Reductive amination

ABSTRACT

A series of 5-N,N-disubstituted-5-aminopyrazole-3-carboxylic acids were prepared and found to act as highly potent and selective agonists of the G-Protein Coupled Receptor (GPCR) GPR109b, a low affinity receptor for niacin and some aromatic D-amino acids. Little activity was observed at the highly homologous higher affinity niacin receptor, GPR109a.

The high and low affinity human G-protein coupled receptors for niacin (**1**), namely GPR109a (HM74A) and GPR109b (HM74) respectively, have been the subject of significant recent attention,[1] given the ability of niacin (**1**) to raise levels of high density lipoproteins (HDL) and thus treat lipid disorders including dyslipidemia and atherosclerosis.[2] Evidence suggests that the antilipolytic activity of niacin is mediated by GPR109a,[3] but GPR109b has been shown to inhibit isoproterenol-induced lipolysis in primary human adipocytes.[4] Despite this evidence, the physiological function of GPR109b currently remains unknown. Recently, a number of aromatic D-amino acids have been shown to activate the receptor in the μM range which may help to uncover the true function of the receptor.[5] However, given the identical receptor coupling, the high homology and the significant overlap in the expression profile between GPR109a and GPR109b, it is very likely that GPR109b may also be involved in modulation of lipolysis and hence could represent an interesting target for the treatment of dyslipidemia. Furthermore, it remains possible that selective GPR109b activators may avoid the characteristic and uncomfortable cutaneous flushing response elicited by niacin in humans.[6] Niacin-induced flushing has been shown to be mediated by GPR109a and by PUMA-G[7] expressed in epidermal Langerhans cells.[8] Thus ligands selective for GPR109b over GPR109a may potentially avoid the flushing response associated with GPR109a activation.

A challenge in the development of GPR109b as a molecular target remains the lack of a satisfactory rodent orthologue. Given the high (95% identity) homology between the two receptors, GPR109b appears to have arisen from a very late gene duplication of GPR109a. GPR109a has a rodent orthologue, PUMA-G,[9] whereas GPR109b does not, and a search of available genomes identified the presence of a GPR109b orthologue only in chimpanzee. Lower species, even lower primates such as Rhesus monkey, show no evidence of the receptor. However, development of ligands selective for GPR109b could provide useful tools for further exploring the pharmacology of this receptor.

We have recently investigated a number of novel ligands for the GPR109b receptor. Before our studies commenced, the only

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doi:10.1016/j.bmcl.2009.05.108

reported ligand for the GPR109b receptor, in addition to the very weak agonist niacin, was acifran (**2**)[10] (EC_{50} = 4.2 μM).[11] Acifran has been shown to elevate HDL in rodents and humans,[12] however acifran lacks selectivity over GPR109a (EC_{50} = 1.3 μM).[11] Additional acifran analogs have shown a similar lack of selectivity between the two receptors, despite improvements in potency.[11,13] We initially reported the discovery of a series of 1-alkyl-benzotriazole-5-carboxylic acids (**3**) as the first potent and selective agonists of GPR109b.[4] Subsequently we also reported a series of 6-aminonicotinic acids (**4**) as isosteres of the 4-substituted amino-3-nitrobenzoic acids (**5**) that were active intermediates on the route to **3**.[14] These two series of compounds showed improved potency compared to the benzotriazole series. We hypothesized that 5-aminopyrazole-3-carboxylic acid may provide a reasonable replacement for the 6-aminonicotinic acid moiety and thus decided to investigate this motif as potential ligands for GPR109b (Fig. 1).

Mono- or symmetrically di-substituted 5-aminopyrazole-3-carboxylic acids (**6a–r**) were synthesized in 4 steps from 5-nitropyrazole -3-carboxylic acid (**7**) (Scheme 1). Protection of 5-nitropyrazole-3-carboxylic acid (**7**) as the ethyl ester (**8**) and subsequent reduction under a hydrogen atmosphere with palladium on carbon gave the intermediate 5-aminopyrazole-3-carboxylic acid ethyl ester (**9**). Reductive amination with an appropriate aldehyde provided the mono- or symmetrically di-substituted 5-aminopyrazole-3-carboxylic acid ethyl ester (**10**), which was subsequently hydrolyzed under acidic or basic conditions to give the desired mono- or symmetrically di-substituted 5-aminopyrazole-3-carboxylic acid (**6**).

Agonist dose responses for Gi-coupled GPR109a and GPR109b were generated using a cAMP Homogenous Time-Resolved Fluorescence (HTRF) assay in CHO stable cell lines. Positive controls were defined from the amount of cAMP generated by 5 μM forskolin stimulated cells with a GPR109a or GPR109b agonist, namely niacin (**1**) (pEC_{50} = 7.57) or 6-(allylamino)nicotinic acid (pEC_{50} = 7.38) respectively. Negative controls were defined as cAMP generated by 5 μM forskolin stimulated cells.

It was immediately obvious that although some mono-functionalized amines (**6a, 6b**) displayed weak agonist responses (pEC_{50} = 5.35, 5.17), a significant increase in potency was observed for some of the symmetric disubstituted amines (**6d–r**). Specifically the dibenzyl (**6j**, pEC_{50} = 6.07) and bis-2′-thiophenylmethyl (**6n**, pEC_{50} = 6.59) analogs displayed agonist responses with pEC_{50} greater than 6, and the di-3′thiophenylmethyl (**6o**, pEC_{50} = 8.50) analog displayed the greatest agonist response we had hitherto observed. In each case the compounds were able to fully reverse the cAMP elevating effect of forskolin in stably transfected CHO-K1 cells, suggesting that they are likely to be full agonists of the receptor. In addition, significant selectivity over GPR109a were

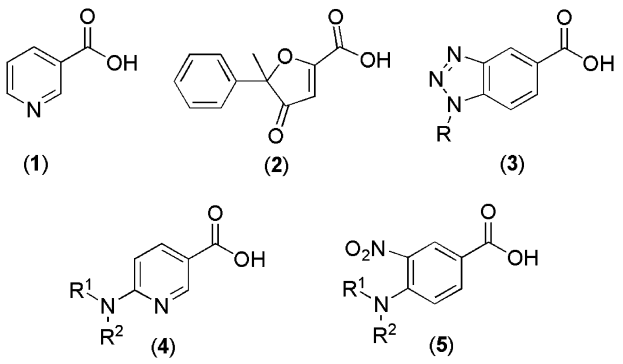
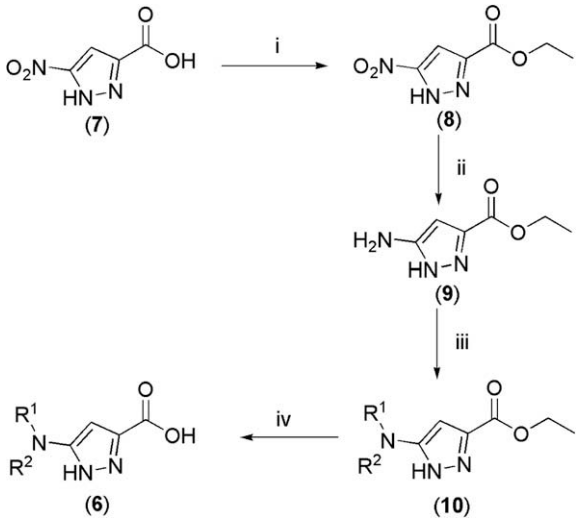


Figure 1. Ligands for GPR109a and GPR109b.



Scheme 1. Reaction conditions: (i) AcCl, EtOH, 80 °C, 18 h; (ii) Pd/C (10%), EtOH, H_2 (1 atm), 25 °C, 18 h; (iii) RCHO, $NaBH(OAc)_3$, DCE, 60 °C, 18 h (mono addition) or 170 °C 20 min μW (di addition); (iv) 1:5:1 MeOH/THF/1 M (aq) LiOH or HCl.

observed, a lack of potency against GPR109a prevented accurate measurement of selectivity for **6k** and **6n**, however **6o** displayed an agonist response against GPR109a around 3000 times less than that for GPR109b (Table 1).

Interestingly, the incorporation of additional functionality onto the thiophenyl or benzyl moieties led to complete, or near complete, loss of potency. In addition, replacement of the 3′-thiophenyl moieties with the smaller 3′-furanyl moieties also led to near complete loss of potency.

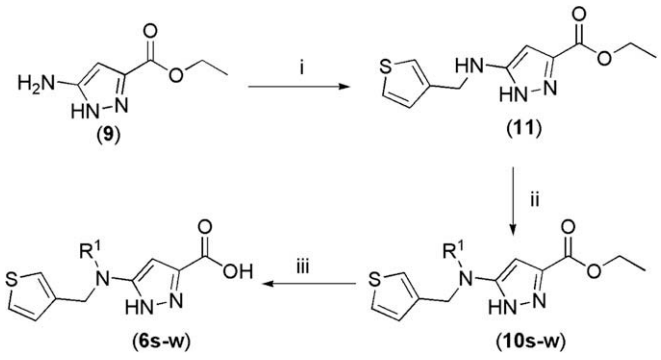
In an attempt to investigate if the 3′thiophenylmethyl moiety was optimal for both amine substitution positions we prepared a small series of non-symmetrically disubstituted amines (**6s–w**) in three steps from ethyl 5-amino-1*H*-pyrazole-3-carboxylate (**9**) (Scheme 2). Reductive amination with a single equivalent of thiophene-3-carbaldehyde gave ethyl 5-(thiophen-3-ylmethylamino)-1*H*-pyrazole-3-carboxylate (**11**) which was then subjected a second orthogonal reductive amination to provide the non-symmetrically di-substituted amine ester (**10s–w**). Subsequent hydrolysis then gave the desired non-symmetrically di-substituted amines (**6s–w**). However, the only examples which displayed agonist responses with pEC_{50} greater than 6 were the benzyl (**6s**, pEC_{50} = 7.84) and 2′thiophenylmethyl (**6u**, pEC_{50} = 6.85) analogs with neither showing improved potencies over the symmetrically di-3′thiophenylmethyl analog (**6o**).

In summary, a series of N,N-difunctionalized 5-aminopyrazole-3-carboxylic acids were prepared that displayed excellent in vitro agonist activity at GPR109b in a whole cell cAMP assay. The observed activity was highest for the dibenzyl amine, and for analogs utilizing thiophene as an isosteric replacement for the phenyl moiety. Optimal potency was observed for the di-3′thiophenylmethyl amine. Replacement of one of these 3′-thiophenyl moieties with benzyl, 2′thiophene or 2′- or 3′-furan led to a decrease in potency, All active substances displayed a full agonist effect as compared to niacin (**1**) or 6-(allylamino)nicotinic acid. None of the compounds prepared displayed any significant activity against the closely related receptor GPR109a, with the exception of the monobenzyl analog (**6c**) which was inactive at GPR109b and which may prove to be a very interesting starting point for the further elaboration of new GPR109a agonists. We believe that the further development of selective GPR109b agonists, such as those described herein, is essential to further explore the therapeutic utility of this receptor.

Table 1
GPR109b agonist activity of selected N-substituted 5-aminopyrazole-3-carboxylic acids (**6**)[a]

Compd	R^1	R^2	GPR109b pEC_{50} (n)	GPR109a pEC_{50} (n)
6a	2-Butyl	H	5.35 ± 0.39 (4)	NA (4)
6b	2-Pentyl	H	5.17 ± 0.24 (3)	NA (4)
6c	Benzyl	H	NA (4)	6.92 ± 0.23 (4)
6d	*n*-Propyl	*n*-Propyl	5.08 ± 0.23 (3)	<5 (4)
6e	*n*-Hexyl	*n*-Hexyl	<5 (4)	<5 (4)
6f	CH_2 *c*-propyl	CH_2 *c*-propyl	5.40 ± 0.29 (4)	NA (4)
6g	CH_2 *c*-hexyl	CH_2 *c*-hexyl	NA (4)	<5 (4)
6h	$CH_2CH_2CH(CH_3)_2$	$CH_2CH_2CH(CH_3)_2$	<5 (4)	<5 (4)
6i	CH_2CH_2Ph	CH_2CH_2Ph	5.25 ± 0.36 (3)	<5 (4)
6j	Benzyl	Benzyl	6.07 ± 0.12 (4)	<5 (4)
6k	2′-Fluorobenzyl	2′-Fluorobenzyl	5.06 ± 0.30 (4)	NA (4)
6l	3′-Fluorobenzyl	3′-Fluorobenzyl	<5 (4)	5.03 ± 0.79 (4)
6m	4′-Fluorobenzyl	4′-Fluorobenzyl	<5 (4)	NA (4)
6n	CH_2-2′-thiophenyl	CH_2-2′-thiophenyl	6.59 ± 0.07 (4)	<5 (4)
6o	CH_2-3′-thiophenyl	CH_2-3′-thiophenyl	8.50 ± 0.18 (4)	5.04 ± 0.72 (4)
6p	CH_2-3′-furanyl	CH_2-3′-furanyl	5.05 ± 0.08 (3)	NA (4)
6q	CH_2-2′-(5′-CH_3)thiophenyl	CH_2-2′-(5′-CH_3)thiophenyl	NA (4)	<5 (4)
6r	CH_2-2′-(5′-Br)thiophenyl	CH_2-2′-(5′-Br)thiophenyl	NA (4)	<5 (4)
6s	Benzyl	CH_2-3′-thiophenyl	7.84 ± 0.13 (4)	<5 (4)
6t	4′-Fluorobenzyl	CH_2-3′-thiophenyl	NA (4)	<5 (4)
6u	CH_2-2′-thiophenyl	CH_2-3′-thiophenyl	6.85 ± 0.07 (4)	<5 (4)
6v	CH_2-2′-furanyl	CH_2-3′-thiophenyl	<5 (4)	<5 (4)
6w	CH_2-3′-furanyl	CH_2-3′-thiophenyl	NA(4)	<5 (4)

[a] Activities were measured at concentrations from 30 pM to 100 μM. Errors are ± log SD. Compounds which showed no response are designated NA (not active). Compounds displaying only a weak response at high concentration are designated <5. Accurate pEC_{50} values for these compounds were not determined.



Scheme 2. Reaction conditions: (i) Thiophene-3-carbaldehyde, $NaBH(OAc)_3$, DCE, 60 °C, 18 h; (ii) RCHO, $NaBH(OAc)_3$, DCE, 65 °C, 18 h, (iii) 1:5:1 MeOH/THF/1 M (aq) LiOH.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.05.108.

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