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# 3-Nitro-4-amino benzoic acids and 6-amino nicotinic acids are highly selective agonists of GPR109b

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**Abstract**—A series of 3-nitro-4-substituted-aminobenzoic acids were prepared and found to act as potent and highly selective agonists of the orphan human GPCR GPR109b, a low affinity receptor for niacin. No activity was observed at the closely homologous high affinity niacin receptor, GPR109a. A second series, comprising 6-amino-substituted nicotinic acids was, also prepared and several analogues showed comparable activity to the nitroaryl series.


The discovery that the human G-protein coupled receptors GPR109a (HM74A) and GPR109b (HM74) are high and low affinity receptors, respectively, for niacin (**1**) (Fig. 1) has garnered significant recent attention.[1,2] Niacin has been used for many years for the treatment of lipid disorders including dyslipidemia, for the prevention of atherosclerosis, and is of particular interest because of its ability to raise levels of high density lipoproteins (HDL).[3] Evidence suggests that the anti-lipolytic activity of niacin (**1**) is mediated by GPR109a and not via GPR109b,[4] indeed, the function of GPR109b currently remains unknown. However, given the identical receptor coupling, the high homology and the significant overlap in the expression profile between GPR109a and GPR109b, it is likely that activation of GPR109b could also inhibit lipolysis.

A challenge in the development of GPR109b as a molecular target remains the lack of a rodent ortholog. 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 ortholog, PUMA-G,[5] whereas GPR109b does not, and a search of publicly available genomes only identified the presence of a GPR109b ortholog in chimpanzee. Lower species, including even Rhesus monkey, showed no evidence of the receptor. However, the identification of ligands selective for GPR109b could provide useful tools for further exploring the pharmacology of this receptor. A selective activator of GPR109b may display an anti-lipolytic response, and hence provide an alternative strategy for the therapeutic control of lipid levels. Furthermore, it is 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 PUMA-G,[7] and via receptor expressed in epidermal Langerhans cells.[8] Thus minor expressional changes between GPR109a and GPR109b may eliminate the flushing response and avoid this unpleasant side effect of GPR109a activation.

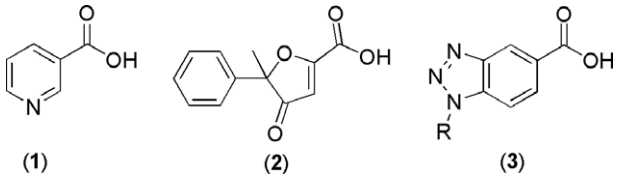


**Figure 1.** Ligands for GPR109a and GPR109b.

*Keywords*: Niacin; GPR109a; GPR109b; HM74; HM74A; PUMA-G; HDL; Lipolysis.
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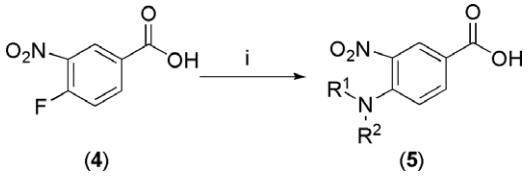
Until recently, the only other reported ligand for the GPR109b receptor, in addition to the very weak agonist niacin, was acifran (**2**)[9] ($EC_{50}$ = 4.2 μM),[1,10] an agent previously shown to elevate HDL in rodents and humans.[11] However, acifran lacks selectivity over GPR109a ($EC_{50}$ = 1.3 μM).[1,10] Further studies on somewhat larger series of acifran analogs have shown a similar lack of selectivity between the two receptors, despite improvements in potency.[12,13] We recently reported the discovery of a series of 1-alkyl-benzotriazole-5-carboxylic acids (**3**) as the first potent and selective agonists of GPR109b.[14] We herein describe the identification and initial SAR around two related series of selective agonist ligands for the GPR109b receptor which may be useful tools with which to further explore the function of this receptor.

The 1-alkyl-benzotriazole-5-carboxylic acids (**3**) described in our previous work[14] were synthesized in three steps from 4-fluoro-3-nitrobenzoic acid (**4**) via substitution of the aryl fluoride with a series of primary amines to give the associated 4-substituted amino-3-nitrobenzoic acids (**5**) (Scheme 1). Catalytic reduction to the 3,4-diamine, and formation of the benzotriazole via condensation with an azide source afforded the desired benzotriazoles. However, during the course of this investigation we discovered that the intermediate 4-amino-3-nitrobenzoic acids also displayed significant activity at GPR109b while retaining selectivity against GPR109a. As a result of this discovery, a wider range of 4-substituted amino-3-nitrobenzoic acids were synthesized and tested in a GPR109b cAMP whole cell assay. Seventeen compounds were found to display agonist responses with $pEC_{50}$ greater than 6. Of these, four compounds displayed agonist responses with $pEC_{50}$ of 7 or greater. 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. As may be seen from Table 1, the best activity was observed for small linear alkyl groups, such as *n*-ethyl (**5b**) and *n*-butyl (**5e**), with the greatest potency observed for the *n*-propyl derivative (**5c**). Substitution α to the amine was tolerated, albeit with modest reductions in potencies, and with the greatest potency observed for the 3-pentyl (**5i**) analog. Cyclic substituents (**5j–l**) were tolerated, but generally resulted in reduced potency compared to the equivalent non-cyclized analogs. Substitution β to the amine was also tolerated. *Bis*-N-substituted analogs were tolerated with modest reductions in potency relative to the mono-N-substituted analogs, with the exception of the pyrrolidine analog (**5x**)




**Scheme 1.** Reaction and conditions: (i) $R^1R^2NH$, $H_2O$, $NaHCO_3$, 150 °C, 20 min μW.

**Table 1.** GPR109b agonist activity of selected 4-substituted amino-3-nitro benzoic acids (**5**)[a]

| Compound | $R^1$ | $R^2$ | GPR109b $pEC_{50}$ (*n*) |
|---|---|---|---|
| **5a** | Me | H | 5.72 ± 0.11 (4) |
| **5b** | Et | H | 6.64 ± 0.22 (4) |
| **5c** | *n*-Pr | H | 7.53 ± 0.13 (4) |
| **5d** | Allyl | H | 7.00 ± 0.31 (3) |
| **5e** | *n*-Bu | H | 6.80 ± 0.19 (4) |
| **5f** | *n*-Pentyl | H | 6.55 ± 0.13 (4) |
| **5g** | *i*-Pr | H | 6.59 ± 0.19 (4) |
| **5h** | 2-Butyl | H | 6.82 ± 0.07 (4) |
| **5i** | 3-Pentyl | H | 7.24 ± 0.12 (4) |
| **5j** | *c*-Pr | H | 6.51 ± 0.09 (4) |
| **5k** | *c*-Bu | H | 6.70 ± 0.15 (4) |
| **5l** | *c*-Pentyl | H | 6.51 ± 0.10 (4) |
| **5n** | –CH(Me)CH(Me)$_2$ | H | 6.44 ± 0.14 (4) |
| **5o** | Ph | H | 5.61 ± 0.10 (4) |
| **5p** | Bz | H | 5.51 ± 0.23 (4) |
| **5q** | –$CH_2CH_2OMe$ | H | 6.23 ± 0.18 (4) |
| **5r** | $CHMeCH_2OMe$ | H | 5.85 ± 0.15 (4) |
| **5s** | –$CH_2CH$-[$O(CH_2)_3$]– | H | 6.51 ± 0.14 (4) |
| **5t** | Me | Me | 5.69 ± 0.23 (4) |
| **5u** | Me | *n*-Pr | 7.28 ± 0.18 (3) |
| **5v** | Et | Et | 6.20 ± 0.09 (4) |
| **5w** | *n*-Pr | *n*-Pr | 6.92 ± 0.07 (4) |
| **5x** | –[$(CH_2)_4$]– | | <5 (4) |

Errors are ± log SD. Compounds that 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.

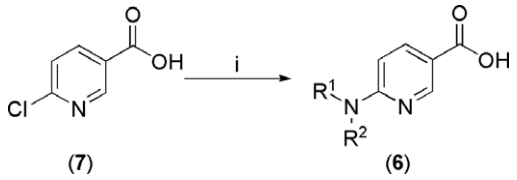
[a] Activities were measured from 30 pM to 100 μM and are provided as the negative log of the molar value of $EC_{50}$.

which was essentially inactive. None of the compounds prepared showed any activity at GPR109a at concentrations up to 30 μM.

It was clear from this SAR that in addition to a modest improvement in potency, the 4-substituted amino-3-nitrobenzoic acid series allowed a greater range of substitution than the 1-alkyl-benzotriazole-5-carboxylic acid series investigated earlier.[14] The nitro benzene moiety takes up a smaller footprint than the benzotriazole scaffold, and we attributed this wider range of active compounds to an increase in available space within the binding site. We further hypothesized that a pyridyl ring could make a suitable electronic isosteric replacement of the nitro aryl moiety and avoid putative metabolic liabilities in addition to providing a smaller footprint. Thus, we decided to investigate a series of 6-amino substituted nicotinic acids (**6**) as possible alternative ligands for the GPR109b receptor.

The 6-amino substituted nicotinic acids were readily obtained in a single step from 6-chloro-nicotinic acid (**7**) (Scheme 2). Substitution of the chloro-nicotinic acid by the relevant primary or secondary amine was carried out in 2-propanol under microwave irradiation, albeit in very low yield. The biological activity of each member of the series was measured using the cAMP whole cell assay. Thirteen compounds were found to display agonist responses with $pEC_{50}$ greater than 6, and of these, four displayed agonist responses with a $pEC_{50}$ greater than 7. Again, in each case the compounds were able to fully

reverse the cAMP elevating effect of forskolin, suggesting that they are likely to be full agonists of the receptor. As may be seen from Table 2, the SAR is very similar between the two series showing that the pyridyl substitution was indeed a good mimic of the nitro-phenyl moiety. Again, the greatest potency was observed for small linear mono-substitutions, with *n*-propyl (**6c**) being the most potent analog prepared. α-Branched cyclic substituents (**6j–l**) and substitution β to the amine were tolerated, indeed the cyclobutyl analog (**6k**) was amongst the most potent compounds identified. However, in this series, formation of tertiary amines led to a significant reduction in potency. The phenyl (**6o**) and benzyl (**6p**) analogs, despite being somewhat less active than (**6c**), may allow sites for further functionalization. Selectivity over GPR109a remained high, none of the compounds prepared showed any activity at GPR109a at concentrations up to 30 μM with the exception of **6j** ($EC_{50}$ = 20 μM) and **6m** ($EC_{50}$ = 35 μM) which showed weak activities.




**Scheme 2.** Reaction and conditions: (i) $R^1R^2NH$, *i*-PrOH, 180 °C, 40 min μW.

**Table 2.** GPR109b agonist activity of selected 6-substituted amino-nicotinic acids (**6**)[a]

| Compound | $R_1$ | $R_2$ | GPR109b $pEC_{50}$ (*n*) |
|---|---|---|---|
| **6a** | Me | H | 5.13 ± 0.22 (4) |
| **6b** | Et | H | 6.40 ± 0.004 (3) |
| **6c** | *n*-Pr | H | 7.29 ± 0.22 (4) |
| **6d** | Allyl | H | 7.15 ± 0.09 (6) |
| **6e** | *n*-Bu | H | 7.00 ± 0.17 (4) |
| **6f** | *n*-Pentyl | H | 6.82 ± 0.23 (4) |
| **6g** | *i*-Pr | H | 6.22 ± 0.27 (4) |
| **6h** | 2-Butyl | H | 6.42 ± 0.18 (3) |
| **6i** | 3-Pentyl | H | 6.57 ± 0.14 (3) |
| **6j** | *c*-Pr | H | 6.49 ± 0.23 (4) |
| **6k** | *c*-Bu | H | 7.14 ± 0.24 (4) |
| **6l** | *c*-Pentyl | H | 6.41 ± 0.12 (4) |
| **6m** | *i*-Bu | H | 5.97 ± 0.18 (4) |
| **6n** | –CH(Me)CH(Me)$_2$ | H | 5.88 ± 0.18 (4) |
| **6o** | Ph | H | 5.49 ± 0.21 (4) |
| **6p** | Bz | H | 5.80 ± 0.22 (4) |
| **6q** | –$CH_2CH_2OMe$ | H | 6.03 ± 0.15 (4) |
| **6r** | $CHMeCH_2OMe$ | H | 5.49 ± 0.14 (3) |
| **6s** | –$CH_2CH$-[$O(CH_2)_3$]– | H | 6.22 ± 0.15 (4) |
| **6t** | Me | Me | <5 (4) |
| **6u** | Me | *n*-Pr | 5.79 ± 0.17 (4) |
| **6v** | Et | Et | <5 (4) |
| **6w** | *n*-Pr | *n*-Pr | 5.20 ± 0.11 (4) |
| **6x** | –[$(CH_2)_4$]– | | NA |

Errors are ± log SD. Compounds that 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.

[a] Activities were measured from 30 pM to 100 μM and are provided as the negative log of the molar value of $EC_{50}$.

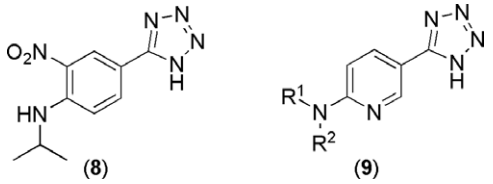



**Figure 2.** Tetrazole analogs prepared.

We further probed the nature of the ligand binding site by synthesizing and testing tetrazole analogs from each series. *N*-Isopropyl-2-nitro-4-(2*H*-tetrazole-5-yl)benzenamine (**8**) was synthesized in one step from 4-isopropylamino)-3-nitrobenzonitrile via condensation with sodium azide. 5-(2*H*-Tetrazol-5-yl)pyridine-2-amines (**9**) (*n*-propyl (**9c**); *i*-propyl (**9g**); benzyl (**9p**); methyl, *n*-propyl (**9u**)) were prepared in two steps via substitution of 2-chloro-4-cyanopyridine with the relevant amine, and subsequent cyclization with sodium azide. None of the tetrazoles displayed significant activity, further highlighting the steric constraint around the acid portion of the binding site (Fig. 2).

In summary, a series of N-functionalized 3-nitro-4-amino benzoic acids were prepared that displayed good in vitro agonist activity at GPR109b in a whole cell cAMP assay. The observed activity was highest amongst small linear and α-branched alkyl chains. Pyridine was shown to act as a suitable replacement for the aryl nitro functionality by preparing a series of N-functionalized 6-amino nicotinic acids that showed similar in vitro activities at GPR109b. All active substances displayed what was assumed to be a full agonist effect in the absence of a reference endogenous ligand. Significant selectivity of >200-fold over the closely related receptor GPR109a was observed, as none of the compounds prepared displayed any significant activity against this receptor at the highest concentration tested. We have previously demonstrated that GPR109b selective agonists can inhibit isoproterenol induced lipolysis in human cadaver subcutaneous adipose tissue,[14] indicating that activation of this receptor may be capable of inhibiting lipolysis. The further development of selective GPR109b agonists, such as those described here, is essential to further explore the therapeutic utility of this receptor.

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.09.058.

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