1-Alkyl-benzotriazole-5-carboxylic Acids Are Highly Selective Agonists of the Human Orphan G-Protein-Coupled Receptor GPR109b

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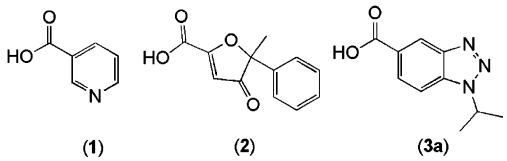
Abstract: 1-Substituted benzotriazole carboxylic acids have been identified as the first reported examples of selective small-molecule agonists of the human orphan G-protein-coupled receptor GPR109b (HM74), a low-affinity receptor for the HDL-raising drug niacin. No activity was observed at the highly homologous high-affinity niacin receptor GPR109a (HM74A). The high degree of selectivity was attributed to a difference in the amino acid sequence adjacent to a key arginine−ligand interaction allowing somewhat larger ligands to be tolerated by GPR109b.

Niacin (**1**, Figure 1) has long been used for the treatment of lipid disorders and for the prevention of atherosclerosis, 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 hormone sensitive lipase activity, thereby decreasing plasma free fatty acid levels and ultimately raising HDL. Two closely related human orphan G-protein-coupled receptors, both of which are expressed in human adipocytes and are negatively coupled to cyclic AMP (termed GPR109b, or HM74 and GPR109a, or HM74A; 95% identity), have recently been identified as possible molecular targets for niacin.[3,4]

GPR109a is the human orthologue of the previously described rodent receptor (PUMA-G),[5] whereas GPR109b appears to have arisen from a very late gene duplication. It differs from GPR109a and PUMA-G mainly in the C-terminal tail region and has no rodent equivalent. 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.[3] It is a much weaker ligand for GPR109b with an EC_{50} in the millimolar range. Recent evidence suggests that the antilipolytic activity of niacin is mediated by GPR109a and not GPR109b,[6] but it is plausible that a selective activator of GPR109b may elicit an effect similar to that of niacin on lipolysis in adipose cells expressing the receptor. It remains unconfirmed as to whether niacin elicits its characteristic and uncomfortable cutaneous flushing side effect in humans, which occurs in >90% of patients, through interaction with either of these receptors. Given the high concentrations of niacin required to activate GPR109b, although it would appear unlikely that this receptor is involved, near-millimolar plasma levels can be achieved in patients after treatment with niacin. However, our unpublished observations of a vasodilation effect in vivo in rodents in response to niacin imply that GPR109b is not necessary for the vasodilation or flushing response because the receptor is not present in these species. Hence, it is possible that a selective agonist of GPR109b may be able to provide the beneficial HDL elevating effect of niacin while having a reduced liability with regard to flushing.



(**1**) (**2**) (**3a**)

Figure 1. Ligands for GPR109a and GPR109b.

Another agent shown to elevate HDL in rodents and humans, acifran (**2**)[7] is the only other reported agonist of the GPR109b receptor (EC_{50} = 20μM), but it lacks selectivity for that receptor over GPR109a (EC_{50} = 2.1μM),[3] and again, flushing was reported as a side effect in clinical studies.[8] We herein describe the identification and early SAR investigation around the first series of selective agonist ligands for GPR109b, which should prove to be useful tools with which to explore the function and therapeutic utility of this receptor.

1-Isopropylbenzotriazole-5-carboxylic acid (**3a**) was identified as an agonist of GPR109b by high-throughput screening of our compound collection by measuring the ability of compounds to decrease cAMP levels in cells stably transfected with GPR109b and activated with forskolin. Our initial hit had an EC_{50} of 400 nM at the GPR109b receptor in this assay, with no detectable activity up to 1 mM at GPR109a or in nontransfected cells using an identical assay platform.

To explore the SAR around this promising but isolated hit, 1-substituted benzotriazole-5-carboxylic acids were synthesized in three steps from 4-fluoro-3-nitrobenzoic acid (**4**) as shown in Scheme 1.

Substitution of the aryl fluoride occurred readily in refluxing ethanol, THF, or water with a primary amine to give a series of 4-amino-3-nitrobenzoic acids (**5**) in near-quantitative yield. The reaction was also found to occur rapidly under microwave irradiation at 150 °C. For the purposes of library synthesis, the reaction was optimal in water because the desired amine (**5**) could be readily isolated by filtration to provide a bright-yellow solid. The application of microwave irradiation allowed the minimization of solvent volumes, which aided precipitation of the product upon cooling.

Reduction of the nitrophenyl moiety occurred readily under an atmospheric pressure of hydrogen with activated palladium on carbon to give the bisamine (**6**), which could be isolated but was best reacted immediately with sodium nitrite in acetic acid to form the desired benzotriazole (**3**) in good yield. This conversion was also readily achieved using polymer-bound nitrite resin as the nitrite source. The use of this polymer-supported reagent allowed isolation of the desired benzotriazole (**3**) in good yield and purity after removal of the acetic acid. To the best of our knowledge, this constitutes the first use of this resin for such a ring-forming application.[9]

The direct alkylation of benzotriazole-5-carboxylic acid with alkyl halides was also investigated (Scheme 2). As expected,

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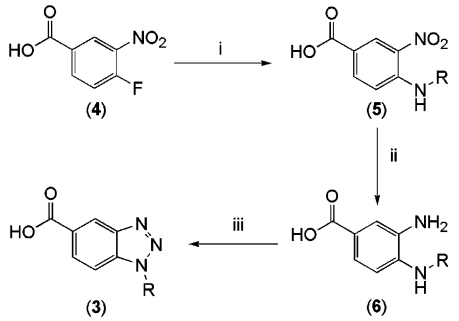
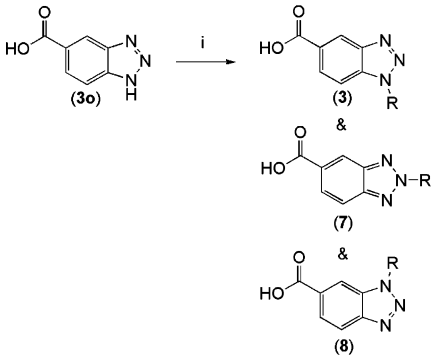
Scheme 1[a]



[a] Reaction conditions: (i) RNH_2, H_2O, $NaHCO_3$, 150 °C, 20 min, μW; (ii) H_2, Pd/C, EtOAc, 3 h, room temp; (iii) PS-NO_2, HOAc, 18 h, room temp.

Scheme 2[a]



[a] Reaction conditions: RBr, K_2CO_3, MeCN, 60 °C, DMA.

direct alkylation gave a mixture of 1- and 2-substituted benzotriazole-5-carboxylic acids (**3** and **7**, respectively) and 1-substituted benzotriazole-6-carboxylic acids (**8**), which in most cases could only be separated by lengthy HPLC purification, and were inseparable in a number of cases. Although this direct alkylation provided a shorter synthetic pathway, the difficulties in purification and the facile nature of the regiospecific route led us to utilize Scheme 1 to synthesize a series of 35 1-substituted benzotriazole-5-carboxylic acids **3** from **4** and 16 1-substituted benzotriazole-6-carboxylic acids **8** from the isomeric 3-fluoro-4-nitrobenzoic acid.

The biological activity of each member of the series was measured using a cAMP whole-cell assay. Ten compounds were found to display agonist responses with pEC_{50} between 6 and 7 for GPR109b (Table 1). 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 in Table 1, branching α to N(1) in the 1-substituted benzotriazole-5-carboxylic acid series leads to similar or improved activity compared to analogues substituted with a linear alkyl chain. Surprisingly, cycloalkyl-substituted derivatives such as cyclopropyl- (**3r**), cyclobutyl- (**3s**), and cyclohexylbenzotriazole-5-carboxylic acid (**3u**) had significantly lower activity, the exception being the cyclopentyl derivative (**3t**), which retained a pEC_{50} of around 6. Incorporation of ether or thioether linkers γ to N(1) was well tolerated and in some examples in the ether series provided a modest improvement in potency when compared to the corresponding alkyl analogue. 1-Benzylbenzotriazole-5-carboxylic acid (**3v**) also had measurable activity and may provide alternatives for investigating further functionalization; however, our initial investigations of such derivatives (**3x**−**z**) resulted in no significant potency enhancement.

Table 1. GPR109b Agonist Activity of Selected Compounds[a]

compd	R	GPR109b pEC_{50} (*n*)
3a	^{i}Pr	6.40 ± 0.36 (10)
3b	$CHMeCH_2OMe$	6.70 ± 0.27 (9)
3c	$-CH_2CH_2OEt$	6.59 ± 0.29 (8)
3d	^{t}Bu	6.59 ± 0.40 (4)
3e	1-pentyl	6.54 ± 0.11 (6)
3f	$-CH_2CH_2SEt$	6.52 ± 0.34 (5)
3g	$-CH_2CH_2OMe$	6.50 ± 0.22 (11)
3h	2-butyl	6.48 ± 0.27 (8)
3i	^{n}Bu	6.16 ± 0.18 (7)
3j	Et	6.05 ± 0.21 (8)
3k	Me	5.70 ± 0.15 (7)
3l	3-pentyl	5.52 ± 0.35 (5)
3m	^{n}Pr	5.13 ± 0.24 (6)
3n	H	NA (2)
3o	allyl	NA (2)
3p	CH_2-c-Pr	NA (2)
3q	Ph	NA (2)
3r	c-Pr	5.63 ± 0.15 (5)
3s	c-Bu	5.51 (2)
3t	cyclopentyl	5.96 ± 0.24 (6)
3u	cyclohexyl	5.23 ± 0.23 (3)
3v	Benzyl	5.04 ± 0.36 (6)
3w	$CHPh_2$	<5 (2)
3x	*p*-methoxybenzyl	NA (2)
3y	*m*-methoxybenzyl	5.30 ± 0.12 (5)
3z	3,5-difluorobenzyl	<5 (2)

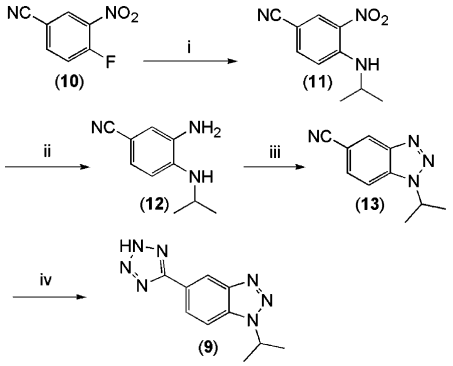
[a] Activities were measured from 30 pM to 100 μM and are provided as the negative log of the molar value of IC_{50}. Errors are ± log SD. Compounds that showed no response are designated NA (not active). Compounds displaying only a weak response at high concentration are designated <5. Accurate pEC_{50} values for these compounds were not determined.

Perhaps the most remarkable observation in light of the exceptionally high homology between the two receptors, particularly in the transmembrane regions where an agonist ligand may be expected to interact, was the excellent selectivity that these compounds showed for GPR109b vs GPR109a. No activity could be detected for any of the examples prepared at concentrations up to 100 μM at GPR109a.

A number of GPCR ligands have been shown to interact with their receptors by binding to residues in the third transmembrane helical region (TM3). This knowledge, the selectivity data, and the observation that there are so few differences within the transmembrane domains of GPR109b and GPR109a led us to hypothesize that the acid portion of the agonist molecules may interact with Arg-111 on TM3. Adjacent to this arginine, there is a single amino acid difference in the sequences between the two receptors. While the difference (Leu to Phe substitution) is very subtle, we believe that it may allow for slightly larger (i.e., 6,5-fused bicyclic) ligands to be tolerated by GPR109b. Conversely, smaller ligands such as niacin would be preferred by GPR109a and would bind less tightly in the GPR109b binding site. The absence of other sequence differences adjacent to basic amino acid residues in the transmembrane regions makes this one of the few plausible explanations for the observed specificity, and the suggestion that Arg-111 is involved in the critical binding interaction is well supported by recent mutagenesis and modeling data; however, niacin was the only tool available for this study, and so further experiments with selective GPR109b ligands would be required to further substantiate our hypothesis.[10] Interestingly, none of the GPR109b-selective compounds showed any activation of the homologous mouse receptor PUMA-G. This is consistent with the proposed binding model, since PUMA-G is identical to GPR109a and not GPR109b around the proposed binding site and again prefers smaller ligands such as niacin.

The specificity of the interactions with the receptor could be further illustrated by the observation that none of the 1-substi-

Scheme 3[a]



[a] Reaction conditions: (i) $^{i}PrNH_2$, H_2O, $NaHCO_3$, 150 °C, 20 min, μW; (ii) H_2, Pd/C, EtOAc, 3 h, room temp; (iii) PS-NO_2, AcOH, 18 h, room temp; (iv) H_2O, NaN_3, 180 °C, 20 min, μW.

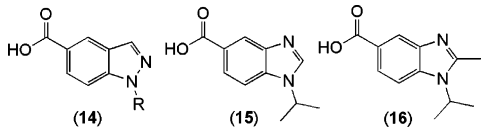


Figure 2. Analogues of benzotriazole series prepared.

tuted benzotriazole-6-carboxylic acids (**8**) had any significant activity toward GPR109b, showing that side chain groups are only tolerated in a single direction relative to the key acid−receptor interaction.

In an effort to further determine the effect of the size of the interacting ligand on activity and to explore the limits of the binding pocket, we prepared the bioisosteric tetrazole analogue **9** (Scheme 3). Employing a similar route to that used for preparation of the acid series, 1-isopropyl-benzotriazole-5-tetrazole (**9**) was synthesized starting from 4-fluoro-3-nitrobenzonitrile (**10**). The resulting 1-isopropylbenzotriazole-5-nitrile (**13**) was readily converted to the tetrazole (**9**) by treatment with sodium azide in water under microwave irradiation conditions. This compound, which would be expected to have a similar pK_a to **3a** but in which the acidic moiety is somewhat larger, had no activity in our GPR109b assay, suggesting that the bicyclic acid scaffold was close to the size limit for the receptor and that further enlargement could not be tolerated in the acid region of the molecule.

We briefly investigated a number of other bicyclic heterocyclic species closely related to **3** in search of more potent agonist molecules, and as part of this investigation, a small series of 1-functionalized indazole-5-carboxylic acids (**14**, Figure 2) were prepared via direct alkylation of ethyl 1*H*-indazole-5-carboxylate and hydrolysis of the resulting alkylated ester. All of these examples were also found to be inactive, suggesting that the nitrogen in the 3-position of the 1-benzotriazole series may be making an important, presumably hydrogen-bonding, interaction with the receptor. In support of this notion, the 1-isopropylbenzimidazole-5-carboxylic acid analogue (**15**), prepared by condensation of intermediate **6** with trimethylorthoformate, was found to have modest activity at GPR109b (pEC_{50} = 5.47 ± 0.19, n = 5). However, the related 1-isopropyl-2-methylbenzimidizole-5-carboxylic acid (**16**), in turn formed by a similar condensation of **6** with trimethylorthoacetate, was inactive, further illustrating the strict size constraints in the receptor agonist binding site.

In summary, a series of 1-functionalized benzotriazole-5-carboxylic acids were prepared that displayed good in vitro agonist activity at GPR109b in our whole-cell cAMP assay. The observed activity was highest among substrates with small

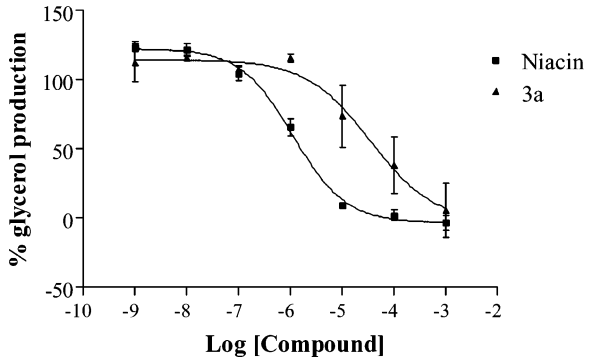


Figure 3. Inhibition of isoproterenol-stimulated lipolysis in human subcutaneous adipocytes.

branched chains and alkyloxy derivatives. All active substrates displayed what, in the absence of a reference endogenous ligand, we assume to be a full agonist effect. All compounds with activity for GPR109b were highly selective against the closely related receptor GPR109a.

As discussed above, these analogues had no activity for the mouse PUMA-G receptor, and with no rodent GPR109b orthologue, we were unable to probe the function of this receptor in vivo. Hence, we opted to further study the functional utility of the series at the GPR109b receptor using adipocytes freshly isolated from human cadaver subcutaneous adipose tissue. Stimulation of such isolated cells with isoproterenol (which elevates cAMP levels by interaction with the β-adrenergic receptor) stimulates lipolysis, measured by monitoring glycerol production, an effect that is dose-dependently inhibited by increasing concentrations of niacin or **3a** (Figure 3). The intrinsic potency of **3a** in this assay is somewhat lower than that of niacin, which may reflect the lower expression level of the GPR109b receptor (relative to GPR109a) in these cells, but comparable efficacy was observed in reversing the effect of isoproterenol. Importantly, neither niacin nor **3a** showed any affinity for the β-adrenergic receptor. Hence, we have demonstrated that selective activation of GPR109b or GPR109a receptors has the ability to inhibit isoproterenol stimulated lipolysis in human adipocytes. These data are strongly suggestive that agonists of GPR109b may have potential as antilipolytic agents and may thus find utility in the treatment of dyslipidemia and the prevention of atherosclerosis. GPR109b may thus provide an alternative target to the high-affinity niacin receptor, GPR109a, for such indications. The GPR109b receptor will be of further interest and importance if it can be demonstrated that the activation of this receptor avoids the characteristic flushing side effect of niacin. We believe that the compounds described in this communication will be useful in future studies to further elucidate the therapeutic utility of GPR109b and its agonists.

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Supporting Information Available: Receptor alignment chart, synthetic methods, spectroscopic data, and assay conditions. This material is available free of charge via the Internet at http://pubs.acs.org.

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