# Identification of Hydroxybenzoic Acids as Selective Lactate Receptor (GPR81) Agonists with Antilipolytic Effects

Curt A. Dvorak,* Changlu Liu, Jonathan Shelton, Chester Kuei, Steven W. Sutton, Timothy W. Lovenberg, and Nicholas I. Carruthers

Janssen Research & Development, LLC, San Diego, California 92121, United States

 Supporting Information

ABSTRACT: Following the characterization of the lactate receptor (GPR81), a focused screening effort afforded 3-hydroxybenzoic acid **1** as a weak agonist of both GPR81 and GPR109a (niacin receptor). An examination of structurally similar arylhydroxy acids led to the identification of 3-chloro-5-hydroxybenzoic acid **2**, a selective GPR81 agonist that exhibited favorable in vivo effects on lipolysis in a mouse model of obesity.
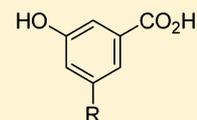
KEYWORDS: lactate receptor, GPR81, HCA1, nicotinic acid receptor, GPR109a, agonist, lipolysis, flushing, FFA

GPR81 (HCA1) is a member of a subfamily of recently deorphanized G-protein-coupled receptors (GPCRs) consisting of GPR81, GPR109a (HM74A), and GPR109b (HM74).[1] All three receptors are predominantly expressed on adipocytes and are activated by the endogenous ligands 2-hydroxypropanoate (lactate), 3-hydroxybutyrate, and 3-hydroxyoctanoate, respectively. These GPCRs may be considered to be a family of hydroxy-carboxylic acid receptors and in turn share homology with the orphan receptor GPR31, the 5-oxo-6,8,11,14-eicosatetraenoic acid (5-oxo-ETE) receptor OXER1, the succinate receptor (GPR91), and the α-ketoglutarate receptor (GPR99). This family of receptors all exhibits a highly conserved arginine residue in transmembrane helix 3 that presumably interacts with the corresponding endogenous ligands.

Stimulation of GPR81, GPR109a, or GPR109b in adipose tissue leads to a decrease in the lipolysis of triglycerides by hormone-sensitive lipase, which results in reduced transport of fatty acids to the liver and a decrease in hepatic triglycerides.[2,3] The beneficial effects of niacin (**3**) result from its interaction with GPR109a, leading to a host of beneficial outcomes including the lowering of triglycerides (35−45%), increasing HDL-C (30−40%), and lowering LDL-C. However, patient compliance is generally limited due to niacin's target related side effects, which include pruritus and flushing, that are attributed to a GPR109a-dependent production of PDG_2 and PDE_2 by immune cells.[4,5] The development of a small molecule agonist of GPR81, expressed predominantly on adipocytes, with selectivity over other GPCRs, may provide a novel treatment for dyslipidemia without the undesirable side effects associated with the stimulation of skin immune cells.[6] Consequently, there have been reports of 3H-imidazo[4,5-b]pyridin-5-ol derivatives as GPR81 agonists but with unknown selectivity.[7] To this end, we embarked on the search for small molecule GPR81 agonists that were selective over GPR109a.

We chose to evaluate several hundred low molecular weight organic acids for GPR81 functional activity given the conserved arginine residue in transmembrane helix 3, which provides an anchor point for the carboxylic acid moiety of the endogenous ligand.[8] As recently reported, this led to the identification of 3-hydroxybenzoic acid (**1**; Chart 1), which exhibited moderate

Chart 1

1
hGPR81 EC50 μM = 184
hGPR109a EC50 μM = 215

4
hGPR81 EC50 μM = 377
hGPR109a EC50 μM > 1000

3
hGPR81 inactive at 10 mM
hGPR109a EC50 μM = 0.78

activation of both GPR81 and GPR109a.[9] The presence of a 3-hydroxyl is essential for GPR81 activity, and thus, removal eliminates activity (e.g., benzoic acid), and relocation to the 2- or 4-position on the aromatic ring eliminates activity at both of these receptors. However, in contrast to a report describing phenolic acids capable of activating GPR109a, we were pleased to discover that the addition of a second hydroxyl in the orientation as found in 3,5-dihydroxybenzoic acid (**4**) was indeed selective for GPR81, albeit slightly less potent than **1**.[10] The other dihydroxybenzoic acid isomers were determined to be inactive at GPR81 (vida supra).

These observations prompted an examination of the SAR around **4**, which is the subject of this current report. The objectives were to improve affinity at GPR81 while maintaining selectivity over GPR109a and ultimately determine the effects on adipocyte lipolysis in vivo with a compound that provided suitable exposure after oral administration. Two approaches were pursued; the first was to scan a range of substituted 3-hydroxybenzoic acids to determine the effects of replacing one of the hydroxyl groups, and the second was to evaluate common carboxylic acid isosteres. These structures are summarized in Table 1 and Figure 1, respectively. The

**Table 1. Potency of 3-Hydroxy-5-Substituted Benzoic Acids in the hGPR81 Assay**
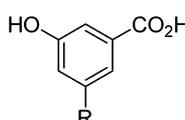
compd	R	EC_{50} (μM)[a]
5	=N−	>1000
6	=N(O)−	>1000
4	OH	377 ± 88
7	OCH$_3$	>1000
8	CH$_3$	42 ± 16
9	tBu	>1000
10	Ph	>1000
11	CF$_3$	>1000
12	CN	>1000
13	Br	129 ± 26
2	Cl	16 ± 9
14	F	38 ± 1
15−18		>1000

[a]GTPγS binding assays were performed as described previously.[12] Results are reported as an average of at least two determinations with standard deviations.
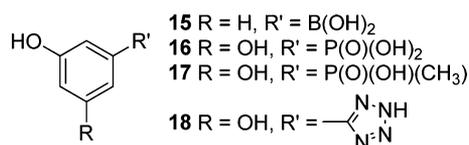
Figure 1. Carboxylic acid isomeres.

compounds evaluated were commercially available with the exception of those whose syntheses are depicted in Scheme 1. The phosphonic acid **16** and phosphinic acid **17** were both prepared from a common bromide precursor **19**. Palladium-catalyzed cross-coupling of either diethyl phosphite or ethyl methylphosphinate with 1-bromo-3,5-dimethoxybenzene (**19**) provided the corresponding intermediates **20** and **21** in good yield. The methyl ethers were then cleaved with BBr$_3$ followed by hydrolysis of the ethoxy group(s), thus providing **16** and **17**, respectively. The tetrazole analogue **18** was prepared by the condensation of 3,5-dihydroxybenzonitrile and Bu$_3$SnN$_3$ in 1,4-dioxane followed by aqueous hydrochloric acid. The N-oxide of 5-hydroxynicotinic acid (**6**) was obtained by treatment of 5-hydroxynicotinic acid (**5**) with 30% H$_2$O$_2$ in glacial acetic acid.
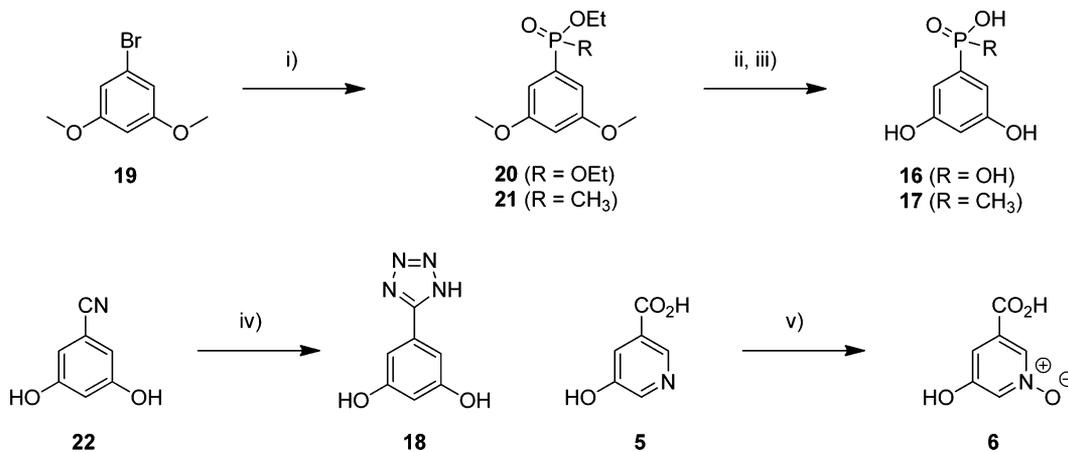
An examination of the SAR around **4** with holding the 3-hydroxybenzoic acid core constant is shown in Table 1. The introduction of a hydroxyl onto the nicotinic acid structure to afford **5** significantly reduces GPR109a activity (EC_{50} = 368 μM) without imparting any GPR81 activity. The corresponding N-oxide (**6**), which places the oxygen atoms in relatively the same orientation as **4**, was found to have no activity at either receptor. Capping one of the hydroxyl groups with a methyl group thereby giving the methyl ether **7** was not tolerated and led to a loss of activity. However, the replacement of the hydroxyl with a simple methyl group, as in **8**, exhibited moderate GPR81 activity at ~42 μM. Simple hydrophobic groups of varying sizes were then explored. The larger tert-butyl and aryl set in compounds **9** and **10** resulted in a loss of potency at GPR81. The more lipophilic trifluoromethyl, **11**, and the more polar nitrile **12** were also not tolerated. Gratifyingly, the halogenated series of compounds, **13**, **2**, and **14** all exhibited a significant increase of GPR81 activity over that of 3,5-dihydroxybenzoic acid (**4**). The chloro derivative, 3-chloro-5-hydroxybenzoic acid (**2**), was determined to have the best potency with an EC_{50} ~ 16 μM. All of the compounds in Table 1 were also counter screened against GPR109a and found to be inactive at the concentrations tested (EC_{50} > 1 mM). An exploration of carboxylic acid replacements was less fruitful with compounds **15−18**, found to be inactive at both GPR81 and GPR109a. The lack of potency seen for **18** was somewhat surprising given the occurrence of tetrazoles as a carboxylic acid replacement for GPR109a agonists.[11] Compound **2** was then selected for further profiling and examined in more detail.

The activity of 3-chloro-5-hydroxybenzoic acid (**2**) at GPR81 was well conserved across all relevant preclinical species tested except the dog−human (EC_{50} = 16 μM), monkey (EC_{50} = 17 μM), dog (EC_{50} = 67 μM), rat (EC_{50} = 7 μM), mouse (EC_{50} = 22 μM), and hamster (EC_{50} = 27 μM). The pharmacokinetic data for compound **2** were obtained to establish the exposure upon oral dosing in mice (Table 2). The 30 mg/kg po dose provided a C_{max} of 67 μM at approximately 3-fold over the in

Scheme 1. Syntheses of Compounds 6, 16−18, 20 and 21[a]

[a](i) Pd(PPh$_3$)$_4$, Et$_3$N, toluene Δ; HP(O)OEt$_2$ (R = OEt) or HP(O)OEt(CH$_3$) (R = CH$_3$). (ii) BBr$_3$, −78 °C, CH$_2$Cl$_2$. (iii) 6 M HCl, 100 °C. (iv) Bu$_3$SnN$_3$, 1,4-dioxane, 100 °C 24 h; 1 M HCl, 16 h. (v) 30% H$_2$O$_2$, AcOH.

Table 2. Pharmacokinetic Parameters of 3-Chloro-5-hydroxybenzoic Acid (2) in Mice

dose (mg/kg)	t_{max} (h)	AUC_{inf} (h ng/mL)	C_{max} (ng/mL)	C_{max} (μM)	$t_{1/2}$ (h)
30	0.5	9356	11689.50	67.2	1.47
100	0.5	51312	55252.80	341.9	1.10

vitro EC_{50} with an AUC_{inf} of 54 h μM and a short $t_{1/2}$ of 1.5 h, and the 100 mg/kg po dose attained a C_{max} of 342 μM at greater than 15-fold over the observed in vitro EC_{50}. Compound 2 was then tested acutely by measuring nonesterified free fatty acid cholesterol (NEFAc) as a marker for lipolysis in vivo at 30, 100, and 300 mg/kg po in overnight fasted C57Bl6/J mice fed high fat chow for 10 weeks (DIO mice). Shown in Figure 2, it was determined that compound 2
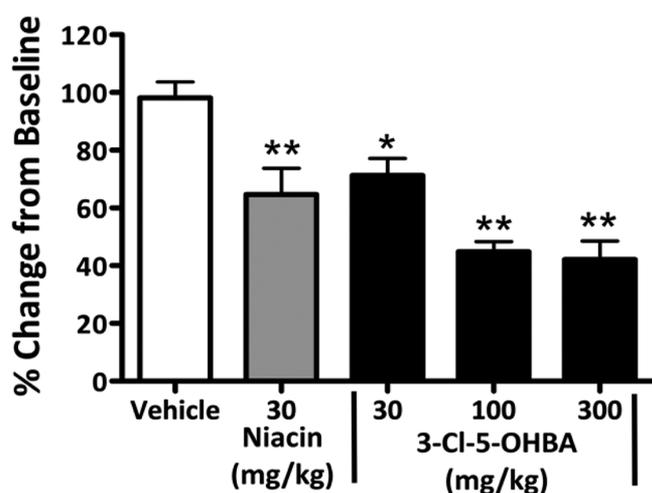

Figure 2. Nonesterified free fatty acid cholesterol (NEFAc) following treatment with niacin at 30 or compound 2 at 30, 100, and 300 mg/kg po in overnight fasted C57Bl6/J mice fed high fat chow for 10 weeks (DIO mice). *$P < 0.05$ vs vehicle and **$P < 0.01$ vs vehicle.

gave significant reductions in NEFAc at all doses tested. This resulted in compound 2 having a minimum efficacious dose equivalent to that of niacin for the reduction of observed NEFAc. The potential differentiating factor would be that compound 2 should not cause the flushing that is associated with niacin due to a lack of GPR109a cross-reactivity.

Comparing the pharmacokinetic data for compound 2 and the observed pharmacodynamics at the two lower doses of the lipolysis study demonstrate a good correlation between potency, exposure, and in vivo efficacy. The reduction in free fatty acids plateaus at the higher doses examined.

In conclusion, a series of phenolic benzoic acids were determined to have activity at GPR81 with selectivity over GPR109a. An examination of the SAR around the 3-hydroxybenzoic acid afforded the discovery of 3-chloro-5-hydroxybenzoic acid as a potent and selective GPR81 agonist. The compound was examined in vivo for effects on lipolysis, and it was found that 3-chloro-5-hydroxybenzoic acid gave significant reductions in free fatty acids at all doses tested, resulting in a minimum efficacious dose of 30 mg/kg. Further investigations of GPR81 agonists are ongoing and will be the subject of future publications.

■ ASSOCIATED CONTENT

ⓢ Supporting Information

Experimental procedures for assay protocols, in vivo studies, and the synthesis and characterization of compounds 6, 16−18, 20 and 21. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: 858-784-3284. E-mail: cdvorak@its.jnj.com.

Notes

The authors declare no competing financial interest.

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