

Synthesis and Agonistic Activity at the GPR35 of 5,6-Dihydroxyindole-2-carboxylic Acid Analogues

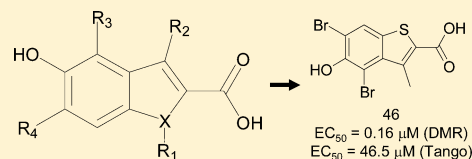
Huayun Deng and Ye Fang*

Biochemical Technologies, Science and Technology Division, Corning Inc., Corning, New York 14831, United States

S Supporting Information

ABSTRACT: 5,6-Dihydroxyindole-2-carboxylic acid (DHICA), an intermediate of melanin synthesis and an eumelanin building block, was recently discovered to be a GPR35 agonist with moderate potency. Here, we report the synthesis and pharmacological characterization of a series of DHICA analogues against GPR35 using both label-free dynamic mass redistribution and Tango β -arrestin translocation assays. This led to identification of novel GPR35 agonists with improved potency and/or having biased agonism.

KEYWORDS: GPR35, 5,6-dihydroxyindole-2-carboxylic acid, biased agonism, melanin synthesis



GPR35, an orphan G protein-coupled receptor (GPCR), is implicated in inflammation, pain, cardiovascular diseases, and metabolic disorders.¹ Recent pharmacological screens have identified several clinically used drugs to be GPR35 agonists; these drugs include the antiasthma drugs cromolyn and dicumarol,² the loop diuretics bumetanide and furosemide,³ the catechol-O-methyl transferase inhibitor drug entacapone used for the treatment of Parkinson's disease,⁴ and the antinociception niflumic acid.^{5,6} Certain abundant natural phytochemicals including myricetin, morin, and ellagic acid,⁶ and gallic acid⁷ also have been found to be GPR35 agonists with moderate potency. Coupled with up-regulation of GPR35 in several types of inflamed cells,^{1,2,8} these findings provide rationales for proposing activation of GPR35 as a therapeutic mechanism with opportunity for development in certain diseases, including inflammatory disorders. Therefore, we have been interested in discovering GPR35 agonists as potential therapeutics.

The natural agonists for GPR35 remain controversial. The possible natural agonists include kynurenic acid,⁸ 2-acyl lysophosphatidic acid,⁹ and more recently, 5,6-dihydroxyindole-2-carboxylic acid (DHICA, 3).¹⁰ DHICA is a building block of eumelanin, the black to brown pigment produced by melanin and found in human skin, hair, and eyes. To expand pharmacological tools for GPR35 as well as to understand the structural basis of DHICA to activate GPR35, we synthesized a series of DHICA analogues and characterized them using both label-free dynamic mass redistribution (DMR) and Tango β -arrestin translocation assays. The DMR assay is based on whole cell phenotypic responses,^{11,12} while Tango is an end point measurement of gene reporter activity linked to the GPR35 activation-mediated β -arrestin translocation.^{4,6}

DHICA is an indolic intermediate of melanin synthesis. Recently, we found that DHICA is a GPR35 agonist.¹⁰ It triggers a robust DMR in HT-29, a native colon cancer cell line endogenously expressing GPR35,⁴ with an EC_{50} of 23.6 μ M; and it exhibits comparable potency (EC_{50} , 23.2 μ M) to cause a

β -arrestin translocation signal in an engineered U2OS cell line stably expressing a C-terminal modified GPR35 (U2OS-GPR35-*bla*).¹⁰ DHICA presents several positions for structure activity relationship (SAR) modifications (Figure 1). We and

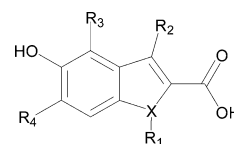


Figure 1. Structures of DHICA analogues. DHICA: X = N, $R_1 = R_2 = R_3 = H$, $R_4 = OH$.

others have shown that the carboxylic acid group of several different classes of GPR35 agonists identified to date is critical to activate GPR35.^{5,13} Thus, we decided to focus on analogues bearing modifications at other positions of DHICA.

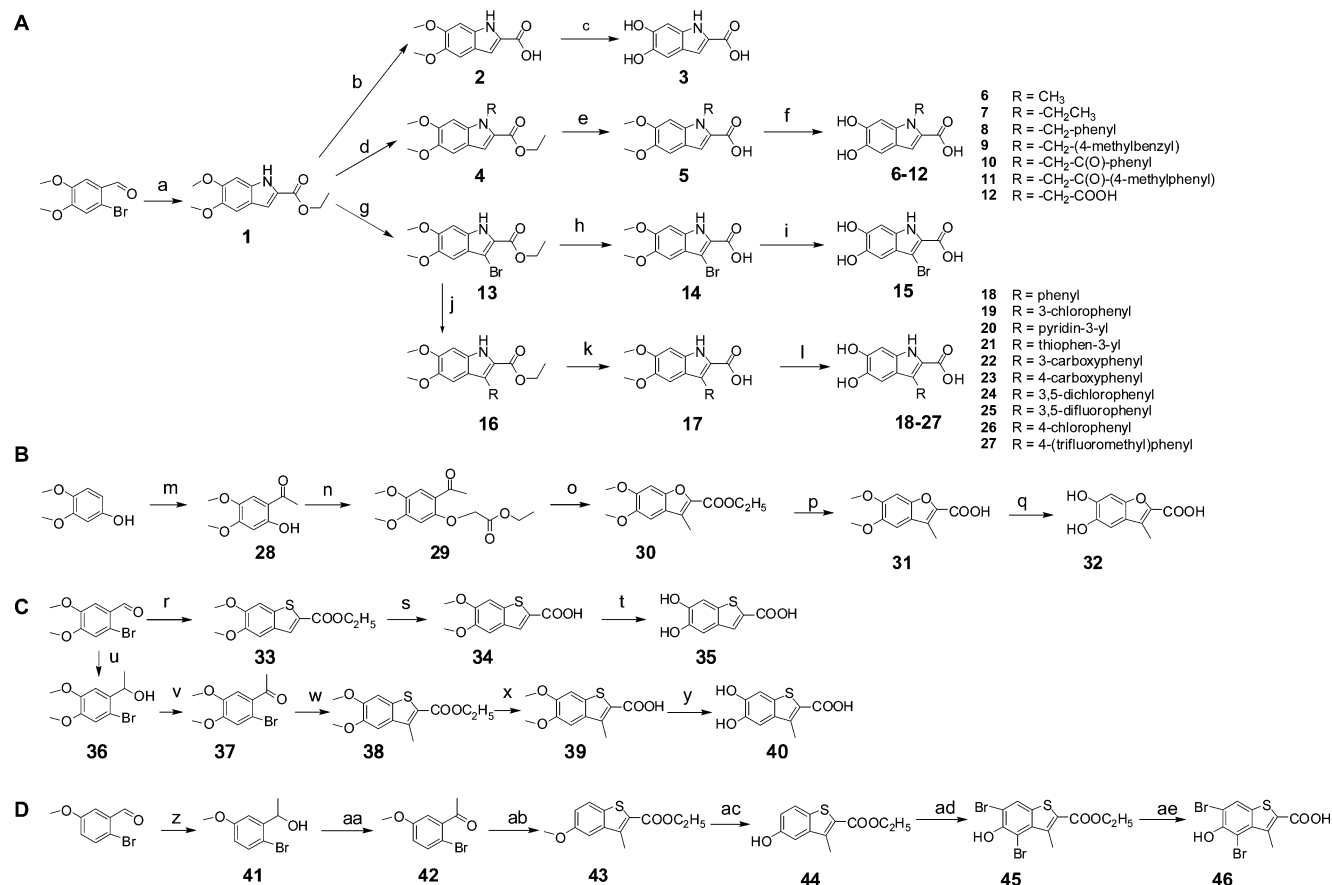
The synthesis of all DHICA analogues is outlined in Scheme 1. We first synthesized N-alkylated derivatives of DHICA from 2-bromo-4,5-dimethoxybenzaldehyde (6–12) (Scheme 1A). The route commences with copper-catalyzed domino four-component coupling and cyclization of 2-bromo-4,5-dimethoxybenzaldehyde with ethyl 2-isocyanoacetate using CuI to produce the ethyl ester 1, N-alkylation to produce the intermediate 4, hydrolysis to produce the carboxylic acid intermediate 5, and then demethylation to give 1-alkylated analogues 6–12 in good yield. Next, we synthesized 3-substituted DHICA analogues 15 and 18–27, whose syntheses were also outlined in Scheme 1A. Intermediate 1 was initially reacted with N-bromosuccinimide (NBS) to produce intermediate 13, then hydrolyzed to produce 14, and demethylated to produce 3-bromo-DHICA 15. For targets 18–27, the route begins with Suzuki cross-coupling of the intermediate 13 with

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Scheme 1. Synthesis of DHICA Analogues



Reagents and conditions: (A) (a) CuI, Cs₂CO₃, ethyl 2-isocyanoacetate in DMSO, 80 °C, 60%; (b) NaOH, 1:1 EtOH/H₂O, rt, 75%; (c) BBr₃, CH₂Cl₂, dropwise/78 °C, rt, 43%; (d) CH₃I, CH₃CH₂I, benzyl bromide, 4-methylbenzyl bromide, or methyl bromoacetate for **6**, **7**, **8**, **9**, or **12**, respectively, NaH/THF, rt; or 2-bromo-1-phenylethanone, or 2-bromo-1-p-tolyethanone for **10** or **11**, respectively; DMF, 95 °C, 65–85%; (e) LiOH/EtOH, 50 °C, 86–93%; (f) BBr₃, CH₂Cl₂, dropwise/−15 °C, rt, 65–91%; (g) NBS, DMF, 0 °C, 76%; (h) LiOH, 1:1 EtOH/H₂O, rt, 79%; (i) BBr₃, CH₂Cl₂, rt, 19%; (j) phenylboronic acid, 3-chlorophenylboronic acid, pyridin-3-ylboronic acid, thiophen-3-ylboronic acid, 3-(methoxycarbonyl)phenylboronic acid, 4-(methoxycarbonyl)phenylboronic acid, 3,5-dichlorophenylboronic acid, 3,5-difluorophenylboronic acid, 4-chlorophenylboronic acid, or 4-(trifluoromethyl)phenylboronic acid for **18** to **27**, respectively, Na₂CO₃, [1,1'-bis(diphenylphosphino)ferrocene]-dichloropalladium(II)/CH₂Cl₂ (1:1), dioxane/water, 100 °C, 63–81%; (k) LiOH, EtOH/H₂O, 78–92%, rt to 50 °C; (l) BBr₃, CH₂Cl₂, rt, 4–31%. (B) (m) BF₃Et₂O, Ac₂O, 90 °C, 17%; (n) methyl 2-bromoacetate, K₂CO₃, 100 °C, 81%; (o) Na/MeOH, 60 °C, 88%; (p) NaOH, EtOH/H₂O, rt, 82%; (q) BBr₃, CH₂Cl₂, rt, 33%. (C) (r) ethyl 2-mercaptoacetate, K₂CO₃, DMF, 18-crown-6 (catalytic amount), 80 °C, 73%; (s) NaOH, EtOH/H₂O, rt, 56%; (t) BBr₃, CH₂Cl₂, rt, 33%. (D) (z) methylmagnesium bromide, −78 °C, N₂, 88%; (aa) pyridinium chlorochromate, CH₂Cl₂, rt, 95%; (ab) ethyl 2-mercaptoacetate, catalytic amount of 18-crown-6, K₂CO₃, DMF, 80 °C, 63%; (ac) BBr₃, CH₂Cl₂, rt, 21%; (ad) Br₂, HOAc, rt, 30%; (ae) LiOH, EtOH/H₂O, rt, 29%.

boronic acid to produce 3-substituted ethyl ester **16**, then hydrolysis to produce **17**, and demethylation to produce 3-substituted DHICA analogues **18–27**. All compounds with purity greater than 95% were obtained from the contract research organization BioDuro Co. (Beijing, China).

We employed both DMR and Tango β -arrestin translocation assays to characterize the pharmacological activity of DHICA analogues at the GPR35. First, we recorded the dose responses of all DHICA analogues in native HT-29 cells using DMR agonist assays. Results showed that out of the 18 analogues only compound **12** was inactive at a dose up to 1 mM. All other analogues led to a clear dose-dependent DMR whose characteristics were similar to those of the known GPR35 agonists, including zaprinast, kynurenic acid,⁴ and DHICA.¹⁰ Their dose responses were best fitted with a monophasic sigmoidal nonlinear regression, leading to a single EC₅₀ for each compound (Table 1). SAR analysis showed that, compared to

DHICA, its N-alkylated derivatives generally caused a decrease in potency, while, except for **20**, **22**, and **23**, most of its 3-substituted analogues increased its potency. The most potent analogue was **24** (3-(3,5-dichlorophenyl)-DHICA), whose EC₅₀ was $1.06 \pm 0.12 \mu\text{M}$ (two independent measurements, each in duplicate, $n = 4$). Compared to DHICA, 3-bromo-DHICA (**15**) exhibited a 6-fold increase in potency; similarly, 3-(3-chlorophenyl)-DHICA (**19**), 3-(3,5-difluorophenyl)-DHICA (**25**), and 3-(4-chlorophenyl)-DHICA (**26**) also displayed increased potency. However, the efficacy, based on the maximal DMR signal amplitude, was found to be compound-dependent (Table 1). Except for **6**, **7**, and **21**, all other DHICA analogues led to DMR signals that were smaller than those for DHICA.

Second, we measured the ability of DHICA analogues to cause β -arrestin translocation using the Tango assay in the engineered U2OS-GPR35-*bla* cell line. This cell line stably

Table 1. Compounds, and Their EC₅₀ and Efficacy (That Is, the Maximal Responses) as Measured Using DMR and Tango Assays^a

compd	EC ₅₀ (μM)		efficacy	
	DMR	Tango	DMR (pm)	Tango (% ZAP)
Zaprinast	0.14 ± 0.02	5.3 ± 0.7	245	100
3 (DHICA)	23.6 ± 2.5	23.2 ± 2.1	256	75
6	64.2 ± 5.5	45.4 ± 3.2	275	85
7	77.1 ± 6.5	45.7 ± 3.7	232	52
8	43.5 ± 3.2	inactive	184	
9	17.3 ± 1.5	>500	170	>25
10	43.7 ± 3.7	inactive	132	
11	39.7 ± 3.1	inactive	150	
12	inactive	inactive		
15	4.10 ± 0.31	166 ± 12	188	48
18	20.2 ± 1.9	inactive	138	
19	2.48 ± 0.20	34.4 ± 3.4	162	19
20	84.0 ± 7.5	inactive	150	
21	10.3 ± 0.7	61.2 ± 5.1	261	18
22	24.9 ± 1.9	75.7 ± 6.4	210	17
23	22.6 ± 1.8	10.8 ± 1.9	172	40
24	1.06 ± 0.12	24.2 ± 1.9	102	25
25	4.36 ± 0.31	109 ± 7	175	48
26	2.54 ± 0.37	387 ± 24	137	89
27	12.9 ± 1.1	inactive	194	
32	16.2 ± 1.3	133 ± 11	214	27
35	7.35 ± 0.51	520 ± 41	216	86
40	6.17 ± 0.44	90.6 ± 7.4	218	109
46	0.16 ± 0.02	46.5 ± 3.2	197	94

^aThe data represents mean ± SD from two independent measurements (*n* = 4). ZAP, zaprinast.

expresses two fusion proteins: human GPR35 linked to a Gal4-VP16 transcription factor via a TEV protease site, and β -arrestin/TEV protease fusion protein. The cell line also stably expresses the β -lactamase reporter gene under the control of a UAS response element. The activation of GPR35 by agonists leads to the recruitment of β -arrestin-TEV protease fusion protein to the activated GPR35, leading to cleavage of Gal4-VP16 transcription factor from the receptor by the protease. The transcription factor then translocates to the nucleus and activates the expression of β -lactamase. Such a β -arrestin translocation is often specific to the GPR35 activation, given that the test compound is neither fluorescent nor toxic to cells.⁶ All Tango assay results were reported after being normalized to the maximal response of zaprinast within the same plate. Results showed that only a subset of analogues including 8, 10, 11, 12, 18, 20, and 27 were inactive, while the rest led to clear

dose-dependent responses. Compared to DMR assay results, the potency observed was mostly right-shifted, except for DHICA, 6, 7, and 23. Among all indole analogues tested, the most potent agonist was 3-(3-carboxyphenyl)-DHICA (23), with an EC₅₀ of 10.8 μM. Furthermore, compared to DHICA, only *N*-methyl-DHICA (6) and 3-(4-chlorophenyl)-DHICA (26) led to an increase in efficacy (85% and 89% of the zaprinast maximal response, respectively). Together with DMR results, these results suggest that except for 12 all other DHICA analogues are GPR35 agonists, but with distinct potency and efficacy.

Previously, we had discovered that certain thieno[3,2-*b*]thiophene-2-carboxylic acid derivatives are GPR35 agonists; particularly, YE210 (6-bromo-3-methylthieno[3,2-*b*]thiophene-2-carboxylic acid) displayed a potency better than that of zaprinast, and an efficacy comparable to that of zaprinast.¹³ Together with the observations that *N*-alkylation and 3-substitution of DHICA altered the potency and efficacy of DHICA, we designed and characterized several benzofuran and benzo[*b*]thiophene analogues of DHICA. 5,6-Dihydroxy-3-methylbenzofuran-2-carboxylic acid (3-methyl-DHFCA, 32) was synthesized according to Scheme 1B. The synthesis began with acetylation of 3,4-dimethoxyphenol with acetic anhydride and BF₃·Et₂O to produce 28, the alkylation with methyl 2-bromoacetate in the presence of K₂CO₃ to produce 29, the ring closure with NaOCH₃ to produce 30, and the sequential hydrolysis and demethylation to produce the product 32. 5,6-Dihydroxybenzo[*b*]thiophene-2-carboxylic acid (DHTCA, 35) was synthesized according to Scheme 1C. The synthesis begun with a one-step ring closure of 2-bromo-4,5-dimethoxybenzaldehyde with ethyl 2-mercaptoacetate in the presence of K₂CO₃ to produce 33, then the sequential hydrolysis and demethylation to produce the product 35. 5,6-Dihydroxy-3-methylbenzo[*b*]thiophene-2-carboxylic acid (3-methyl-DHTCA, 40) and its analogue 46 were synthesized following a similar procedure (Scheme 1C and D).

DMR assays showed that, for compounds 32, 35, and 40, their DMR characteristics were similar to those of the GPR35 full agonists including zaprinast and YE210 (Figure 2a). However, the DMR characteristics of 46 were almost identical to those of DHICA (Figure 2b). All four analogues displayed higher potency than DHICA, and compound 46 gave rise to the highest potency with an EC₅₀ of 0.16 μM, which is comparable to that of zaprinast.⁴ Interestingly, compared to DHICA, all gave rise to slightly lower efficacy (Figure 2c, Table 1).

The Tango assay revealed a different pattern. First, all four compounds resulted in lower potency than DHICA (Figure 2d). Second, except for 32, which exhibited lower efficacy than DHICA, all the other three compounds gave rise to an efficacy

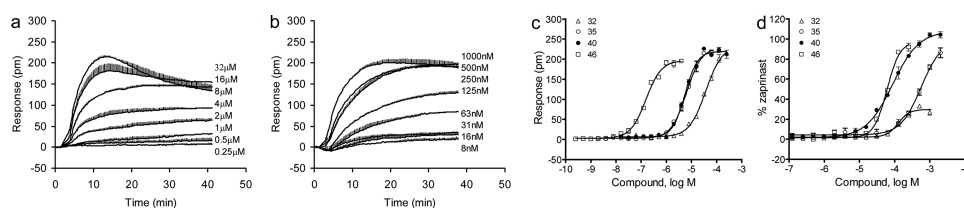


Figure 2. Dose responses of DHICA analogues. (a, b) Real time dose DMR responses of 35 and 46, respectively, in HT-29 cells. (c) DMR amplitudes of 32, 35, 40, and 46 as a function of their doses. The DMR amplitudes at 10 min poststimulation in HT-29 cells were calculated for all. (d) β -Arrestin translocation signals of 32, 35, 40, and 46 as a function of their doses. The Tango signals were normalized to the zaprinast maximal response, which was set to be 100%. The data represents mean ± SD from two independent measurements, each in duplicate (*n* = 4).

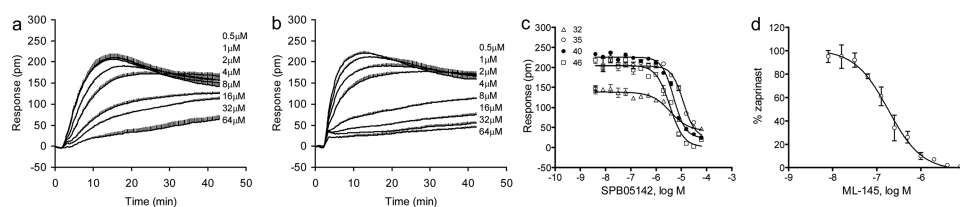


Figure 3. Dose-dependent inhibition of the DMR of DHICA analogues by GPR35 antagonists. (a, b) Real time DMR signals of compounds 35 and 40, respectively, in HT-29 cells in the presence of SPB05142 at different doses (indicated in graph). (c) The DMR amplitudes of 32, 35, 40, and 46 as a function of SPB05142 doses. The compound dose was fixed and was 64 μM , 32 μM , 32 μM , and 1 μM for compounds 32, 35, 40, and 46, respectively. The DMR amplitudes at 10 min poststimulation were calculated for all. (d) Tango signal of 100 μM 46 in the U2OS-GPR35-bla cell line as a function of ML-145 doses. The data represents mean \pm SD from two independent measurements, each in duplicate ($n = 4$).

that was higher than that of DHICA. Compound 40 displayed the highest efficacy among all the DHICA analogues; and its efficacy was even slightly higher than that of zaprinast (Table 1).

Next, we determined the specificity of the DMR signals of all DHICA analogues in HT-29 to the activation of endogenous GPR35. This DMR antagonist assay was performed using a recently discovered GPR35 antagonist, SPB05142.^{13,14} Compound 12 was excluded in this analysis, since it was inactive. Results showed that SPB05142 alone up to 64 μM did not trigger any obvious DMR. Furthermore, SPB05142 dose-dependently blocked the DMR of all other DHICA analogues, each at a fixed dose which was close to its EC_{50} . The SPB05142 dose inhibition of the DMR induced by representative compounds 32, 35, 40, and 46 was illustrated in Figure 3a–c, giving rise to an apparent IC_{50} of $3.6 \pm 0.4 \mu\text{M}$, $12.0 \pm 1.0 \mu\text{M}$, $5.0 \pm 0.4 \mu\text{M}$, and $3.6 \pm 0.3 \mu\text{M}$, respectively ($n = 4$). Similarly, ML-145, a potent GPR35 antagonist,¹⁵ also dose-dependently blocked the β -arrestin translocation signal of compound 46 in U2OS-GPR35-bla cell lines, yielding an IC_{50} of $0.17 \pm 0.01 \mu\text{M}$ (two independent measurements, $n = 4$) (Figure 3d). These results suggest that these DHICA analogues were GPR35 agonists.

Next, we performed correlation analysis between the results obtained using the two pharmacological assays. Results showed that only DHICA, 6, 7, and 23 displayed comparable potency in both assays; however, the rest compounds displayed lower potency in the Tango assay than those in the DMR assay (Figure 4a). Further, only compounds 26, 40, and 46 exhibited higher efficacy in the Tango assay than those in the DMR assay; in contrast, the remaining compounds exhibited lower efficacy in the Tango assay than those in the DMR assay (Figure 4b).

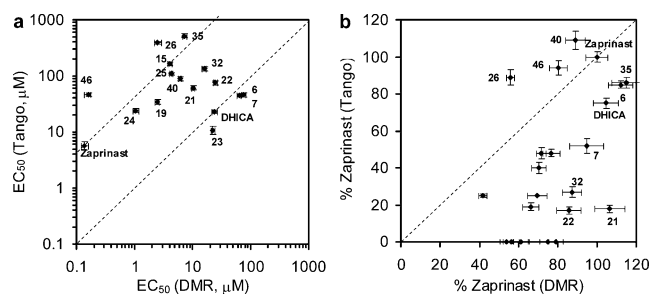


Figure 4. Correlation analyses between DMR and Tango assays. (a) The correlation plot between EC_{50} values of all agonists obtained using DMR and Tango assays. (b) The maximal responses of all agonists after normalization to the maximal response of zaprinast within the same plate. The data represents mean \pm SD from two independent measurements, each in duplicate ($n = 4$).

Such a cell line- and assay readout-dependent relative efficacy and potency indicates biased agonism of these compounds at the GPR35.¹⁶

In summary, we synthesized a series of DHICA analogues and found that several analogues display improved potency and efficacy, and many ligands displayed biased agonism. Further exploration of SAR may lead to the discovery of β -arrestin biased agonists for GPR35. Considering the importance of β -arrestin in regulating a diverse array of cell signaling pathway downstream GPCRs, such a class of GPR35 agonists may open the possibility to further elucidate the biology and physiology of these compounds at the GPR35.¹⁶

■ ASSOCIATED CONTENT

Supporting Information

Full names, structures, and NMR and mass spectroscopy characterization of all compounds, as well as detailed synthesis procedures of compounds 26 and 46. This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: 607-9747203. E-mail: fangy2@corning.com.

Notes

The authors declare the following competing financial interest(s): H.D. and Y.F. are employees and stockholders of Corning Inc.

■ ABBREVIATIONS

DHICA, 5,6-dihydroxyindole-2-carboxylic acid; DHFCA, dihydroxybenzofuran-2-carboxylic acid; DHTCA, dihydroxybenzo-*[b]*thiophene-2-carboxylic acid; DMR, dynamic mass redistribution; GPCR, G protein-coupled receptor; GPR35, G protein-coupled receptor-35; NBS, *N*-bromosuccinimide; SAR, structure activity relationship; YE210, 6-bromo-3-methylthieno-*[3,2-*b*]*thiophene-2-carboxylic acid

■ REFERENCES

- (1) MacKenzie, A. E.; Lappin, J. E.; Taylor, D. L.; Nicklin, S. A.; Milligan, G. GPR35 as a novel therapeutic target. *Front. Endocrin.* **2011**, 2, e68.
- (2) Yang, Y.; Lu, J. Y. L.; Wu, X.; Summer, S.; Whoriskey, J.; Saris, C.; Reagan, J. D. G-protein-coupled receptor 35 is a target of the asthma drugs cromolyn disodium and nedocromil sodium. *Pharmacology* **2010**, 86, 1–5.
- (3) Yang, Y.; Fu, A.; Wu, X.; Reagan, J. D. GPR35 is a target of the loop diuretic drugs bumetanide and furosemide. *Pharmacology* **2012**, 89, 13–17.
- (4) Deng, H.; Hu, H.; Fang, Y. Tyrphostin analogs are GPR35 agonists. *FEBS Lett.* **2011**, 585, 1957–1962.

- (5) Jenkins, L.; Brea, J.; Smith, N. J.; Hudson, B. D.; Reilly, G.; Bryant, N. J.; Castro, M.; Loza, M. I.; Milligan, G. Identification of novel species-selective agonists of the G-protein-coupled receptor GPR35 that promote recruitment of β -arrestin-2 and activate $G_{\alpha 13}$. *Biochem. J.* **2010**, 432, 451–459.
- (6) Deng, H.; Hu, H.; Ling, S.; Ferrie, A. M.; Fang, Y. Discovery of natural phenols as G protein-coupled receptor-35 (GPR35) agonists. *ACS Med. Chem. Lett.* **2012**, 3, 165–169.
- (7) Deng, H.; Fang, Y. Anti-inflammatory gallic acid and wedelolactone are G protein-coupled receptor-35 agonists. *Pharmacology* **2012**, 89, 211–219.
- (8) Wang, J.; Simonavicius, N.; Wu, X.; Swaminath, G.; Reagan, J.; Tian, H.; Ling, L. Kynurenic acid as a ligand for orphan G protein-coupled receptor GPR35. *J. Biol. Chem.* **2006**, 281, 22021–22028.
- (9) Oka, S.; Ota, R.; Shima, M.; Yamashita, A.; Sugiura, T. GPR35 is a novel lysophosphatidic acid receptor. *Biochem. Biophys. Res. Commun.* **2010**, 395, 232–237.
- (10) Deng, H.; Hu, H.; Fang, Y. Multiple tyrosine metabolites are GPR35 agonists. *Sci. Rep.* **2012**, 2, 373.
- (11) Fang, Y.; Ferrie, A. M.; Fontaine, N. H.; Mauro, J.; Balakrishnan, J. Resonant waveguide grating biosensor for living cell sensing. *Biophys. J.* **2006**, 91, 1925–1940.
- (12) Fang, Y. The development of label-free cellular assays for drug discovery. *Exp. Opin. Drug Discovery* **2011**, 6, 1285–1298.
- (13) Deng, H.; Hu, H.; He, M.; Hu, J.; Niu, W.; Ferrie, A. M.; Fang, Y. Discovery of 2-(4-methylfuran-2(SH)-ylidene)malononitrile and thieno[3,2-b]thiophene-2-carboxylic acid derivatives as G protein-coupled receptor-35 (GPR35) agonists. *J. Med. Chem.* **2011**, 54, 7385–7396.
- (14) Zhao, P.; Sharir, H.; Kapur, A.; Cowan, A.; Geller, E. B.; Adler, M. W.; Seltzman, H. H.; Reggio, P. H.; Heynen-Genel, S.; Sauer, M.; Chung, T. D. Y.; Bai, Y.; Chen, W.; Caron, M. G.; Barak, L. S.; Abood, M. E. Targeting of the orphan receptor GPR35 by pamoic acid: a potent activator of ERK and β -arrestin2, with antinociceptive activity. *Mol. Pharmacol.* **2010**, 78, 560–568.
- (15) Heynen-Genel, S.; Dahl, R.; Shi, S.; Sauer, M.; Hariharan, S.; Sergienko, E.; Dad, S.; Chung, T. D. Y.; Stonich, D.; Su, Y.; Caron, M.; Zhao, P.; Abood, M. E.; Barak, L. S. Antagonists for the orphan receptor GPR35. *Probe Reports from the Molecular Libraries Program* 2010; NBK5070.
- (16) Mailman, R. B. GPCR functional selectivity has therapeutic impact. *Trends Pharmacol. Sci.* **2007**, 28, 390–396.

■ NOTE ADDED AFTER ISSUE PUBLICATION

The footnote was missing in Table 1 in the version published on July 12, 2012. The revised version was published on the Web on May 21, 2014.