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SAR Studies of *N*-[2-(1*H*-tetrazol-5-yl)-phenyl]-benzamide Derivatives as Potent G Protein-Coupled Receptor-35 Agonists

Lai Wei[†,‡,∥], Tao Hou[†,∥], Chang Lu[†,‡], Jixia Wang[†], Xiuli Zhang[*,†,§], Ye Fang[∥], Yaopeng Zhao[†], Jiatao Feng[†], Jiaqi Li[†,‡], Lala Qu[†,‡], Hailong Piao[†] and Xinmiao Liang[*,†,§]

†Key Lab of Separation Science for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China

‡University of Chinese Academy of Sciences, Beijing, 100049, China

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

§Co-innovation Center of Neuroregeneration, Nantong University, Nantong 226019, China

*Corresponding authors: Xiuli Zhang, Xinmiao Liang

KEYWORDS: GPR35, dynamic mass redistribution, *N*-[2-(1*H*-tetrazol-5-yl)-phenyl]-benzamide derivatives, Lipinski's Rule

ABSTRACT: G protein-coupled receptor-35 (GPR35) has emerged as a potential target in the treatment of pain, inflammatory and metabolic diseases. We have discovered a series of potent GPR35 agonists based on a coumarin scaffold and found that the introduction of a 1*H*-tetrazol-5-yl group significantly increased their potency. We designed and synthesized a new series of *N*-[2-(1*H*-tetrazol-5-yl)-phenyl]-benzamide derivatives through a two-step synthetic approach, and characterized their agonistic activities against GPR35 using a dynamic mass redistribution (DMR) assay. *N*-(5-bromo-2-(1*H*-tetrazol-5-yl)phenyl)-4-methoxybenzamide (**56**) and *N*-(5-bromo-2-(1*H*-tetrazol-5-yl)phenyl)-2-fluoro-4-methoxybenzamide **(63)** displayed the highest agonistic potency agonist GPR35 with an EC_{50} of 0.059 μM and 0.041 μM, respectively. The physicochemical properties of selected compounds were calculated to evaluate their druglikeness, suggesting that compounds **56** and **63** have good drug-like properties. Together, N-[2-(1H-tetrazol-5-yl)-phenyl]-benzamide derivatives are potentially great candidates for developing potent GPR35 agonists.

G protein-coupled receptors (GPCRs) as the most successful druggable receptor family have an important position in drug development.[1] However, the physiological functions of many GPCRs, in particular orphan receptors,[2] are far from clear. Discovering potent probe molecules for these orphan receptors has great significance in understanding their physiological functions and for drug development. The orphan G protein-coupled receptor 35 (GPR35) was first discovered in 1998, and later has been implicated in a variety of diseases,[3] such as cardiovascular diseases,[4] gastric cancer,[3, 5] artery disease[6] and type 2 diabetes.[7]

In the past decade, several endogenous ligands have been discovered to activate GPR35; these ligands include kynurenic acid,[5] lysophosphatidic acid,[8] multiple tyrosine metabolites[9] and the mucosal chemokine CXCL17,[10] but with variable potency. Several classes of synthetic agonists including the phosphodiesterase 5 inhibitor zaprinast (**1**),[11] the antiasthma drugs doxantrazole (**2**) and pemirolast (**3**)[12] have also been reported to display GPR35 agonistic activity. Furthermore, zaprinast is the most widely used reference agonist to elucidate the biological functions of GPR35. Many more GPR35 agonists were discovered through high-throughput screening,[13, 14] and only few structure-activity relationship (SAR) studies have been reported in the literature so far.[15, 16]

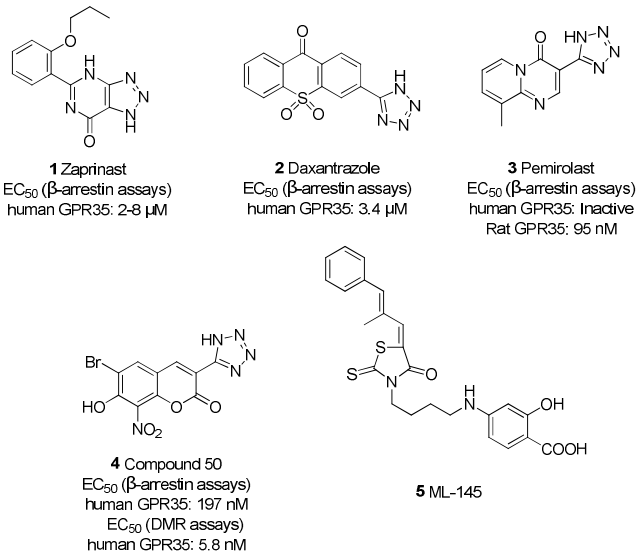
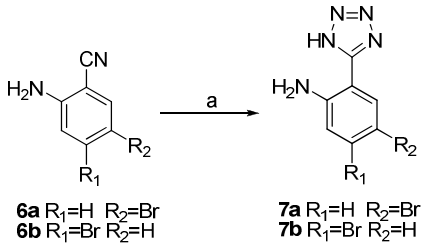


Figure 1. Selected GPR35 agonists with potencies at GPR35 (**1**-**4**) and antagonist (**5**).

Our previous studies have shown that tetrazole **4** possessed significant agonism at GPR35[17]. Several other studies have shown that halogen atom substitution also increased the agonistic activity in certain positions.[14, 15, 16] The introduction of lipophilic residues and hydrogen bond accepting groups at

specific positions in the molecule could probably enhance the activity of compunds.[15, 16] Inspired by these findings, a variety of *N*-[2-(1*H*-tetrazol-5-yl)-phenyl]-amido derivatives with different halogen atoms at the 4 or 5-positions were synthesized. The compounds were prepared starting from **6a-6c** and **8**.

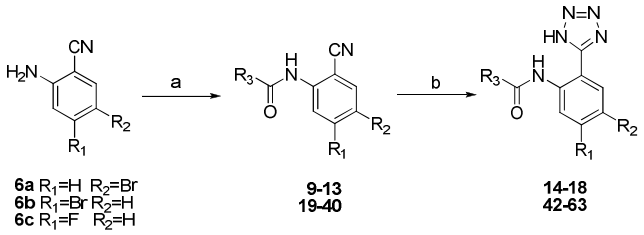
Scheme 1. Synthesis of Compound **7a** and **7b**[a]



[a]Reagents and conditions: (a) NaN_3, $AlCl_3$, THF, 90°C, 5h, yield 81-86%.

The 2-(1*H*-tetrazol-5-yl)aniline derivatives **7a** and **7b** were obtained from **6a** and **6b** via the [3+2] cycloaddition of nitriles and sodium azide catalyzed by aluminum chloride at 90°C with excellent yields (84% and 91%, respectively) (**Scheme 1**).

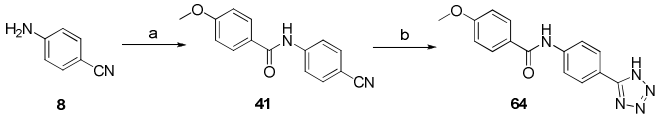
Scheme 2. Synthesis of Compound **14** and its Derivatives[a]



[a]Reagents and conditions: (a) R_3COCl, pyridine, overnight, yield 68-87%. (b) NaN_3, $AlCl_3$, THF, 90°C, 5h, yield 69-91%

The amines **6a-6c**, **8** were reacted with various acid chlorides in pyridine as the base and solvent at room temperature, yielding a series of *N*-(2-cyanophenyl)amido derivatives (**19-41**) (**Scheme 2, Scheme 3**). The *N*-[2-(1*H*-tetrazol-5-yl)-phenyl]-benzamide derivatives (**42-64**) were synthesized from *N*-(2-cyanophenyl)benzamide derivatives (**19-41**) via a [3+2] cycloaddition, also with good yields (69% ~ 91%) (**Scheme 2, Scheme 3**).

Scheme 3. Synthesis of Compound **41** and **64**[a]



[a]Reagents and conditions: (a) 4-Methoxybenzoyl chloride, pyridine, overnight. (b) NaN_3, $AlCl_3$, THF, 90°C, 5h.

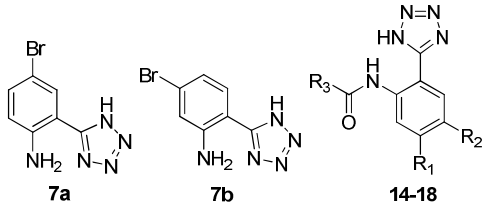
All *N*-[2-(1*H*-tetrazol-5-yl)-phenyl]-amido compounds obtained were tested using a dynamic mass redistribution (DMR) assay[18] using HT-29, a cell line endogenously expressing GPR35.[19] The reference agonist zaprinast was also used to perform a DMR desensitization assay.[19] Results showed that all *N*-[2-(1*H*-tetrazol-5-yl)-phenyl]-amido compounds obtained not only gave rise to a dose-dependent DMR in HT-29, but also desensitized the cells to subsequent stimulation with 1 μM zaprinast. Notably, the potency to trigger DMR for all compounds was found to be almost equal to that to desensitize the zaprinast response (**Table 1, Table 2**), suggesting that these compounds are GPR35 agonists.

The DMR antagonist assay further showed that the known GPR35 antagonist ML-145 (**5, Fig. 1**) dose-dependently and completely blocked the DMR arising from all *N*-[2-(1*H*-tetrazol-5-yl)-phenyl]-amido derivatives, each at its respective EC_{80} to EC_{100} concentration.[20] This suggests that the DMR of these *N*-[2-(1*H*-tetrazol-5-yl)-phenyl]-amido derivatives were specific to the activation of GPR35.

Inspired by our previous findings, a tetrazolyl group was firstly introduced into the simplest compounds **6a** and **6b**, yielding compounds **7a** and **7b**. Compared to the inactive compounds **6a** and **6b**, **7a** and **7b** both showed moderate potency with an EC_{50} of 29.99 μM and 44.06 μM, respectively. This result suggests that the introduction of the tetrazolyl to the molecule significantly improves the agonistic activity.

Next, an amide linker was introduced into the compound **7b**, given that lipophilic residues and hydrogen bond-accepting groups may also play an important role in activating GPR35.[15] The propanamide (**14,** EC_{50} 0.62μM) showed more than 50-fold increase in potency compared to **7b.** The introduction of the bulkier alkyl substituents further improved potency. For instance, isopropyl (**15,** EC_{50} 0.38 μM) and cyclohexyl (**16,** EC_{50} 0.44 μM) substituted compounds displayed more than 100-fold increased potency compared to **7b**. Compounds with an aromatic group in this position also had relatively higher potency, such as furyl (**17,** EC_{50} 1.06 μM), thienyl (**18,** EC_{50} 0.52μM) and phenyl (**42,** EC_{50} 0.87 μM).

Table 1. Potency of 2-Substituted 2-(1*H*-tetrazol-5-yl)aniline Derivatives in DMR Assays



compd	R_1	R_2	R_3	EC_{50}[a] (μM)	desensitization IC_{50}[b] (μM)	antagonist IC_{50}[c] (μM)
7a				29.99±2.80	35.56±2.54	0.67±0.19
7b				44.06±4.06	75.34±9.31	0.67±0.25
14	Br	H	ethyl	0.62±0.06	0.70±0.07	0.47±0.11
15	Br	H	isopropyl	0.38±0.02	0.28±0.02	0.58±0.06
16	Br	H	cyclohexyl	0.44±0.03	0.20±0.01	0.43±0.08
17	Br	H	furyl	1.06±0.13	0.44±0.03	0.29±0.08
18	Br	H	thienyl	0.51±0.02	0.26±0.03	0.50±0.05

[a]EC_{50} to trigger DMR.[b]IC_{50} to desensitize upon cells repeated stimulation with 1 μM zaprinast. [c]IC_{50} of known GPR35 antagonist 5 to block the agonism. The data respresent mean ± sd from two independent measurements, each with four replicates (n = 8).

Since the benzoic acid derivatives are easy to obtain, we chose the phenyl-substituted derivative **42** (*N*-(5-bromo-2-(1*H*-tetrazol-5-yl)phenyl)benzamide) for further modification. *N*-(5-bromo-2-(1*H*-tetrazol-5-yl)phenyl)benzamide derivatives with different substituents in the *o-, m-,* and *p*-position were investigated (mono- or di-substitutions).

For the mono-substitutions, the same substituent in different positions of the benzene ring could significantly change the activity. The following rank order of potency among compounds with the same substituent in different positions (*o-, m-,* and *p*-position) was observed: *m*-methyl (**44,** EC_{50} 0.86 μM) ≈ *p*-methyl (**45,** EC_{50} 1.33 μM) > *o*-methyl (**43,** EC_{50} 2.05 μM) (**Figure S1a**); *p*-chloro (**48,** EC_{50} 0.52 μM) > *o*-chloro (**46,** EC_{50} 0.87 μM) > *m*-chloro (**47,** EC_{50} 2.18 μM) (**Figure S1b**); *p*-fluoro (**52,** EC_{50} 0.45 μM) ≈ *o*-fluoro (**50,** EC_{50} 0.35μM) > *m*-fluoro (**51,** EC_{50} 0.61 μM) (**Figure S1c**); *p*-methoxyl (**56,** EC_{50} 0.059 μM) > *o*-methoxyl (**54,** EC_{50} 0.86 μM) > *m*-methoxyl (**55,** EC_{50} 1.94 μM) (**Figure S1d**); *p*-trifluoromethyl (**54,** EC_{50} 3.65 μM) > *m*-trifluoromethyl (**55,** EC_{50} 4.60μM). These results suggest that substituents in *para*-position led to a considerable increase in potency except for the methyl-derivatives with better tolerance in *meta*-position. For *N*-(5-bromo-2-(1*H*-tetrazol-5-yl)phenyl)benzamide derivatives with varying substituents in the *para*-position, the rank order of potency was as follows: methoxyl (**56,** EC_{50} 0.059 μM) > fluoro (**52,** EC_{50} 0.45 μM) > chloro (**48,** EC_{50} 0.52 μM) > methyl (**45,** EC_{50} 1.33 μM) > trifluoromethyl (**54,** EC_{50} 3.65 μM), suggesting that methoxyl in the *para*-position was favorable for the agonistic activity.

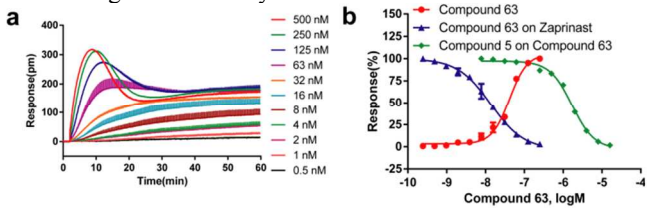
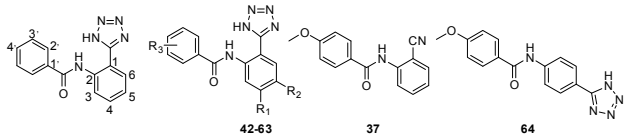


Figure 2. (a) Real time kinetic responses of **63** at different doses in HT-29 cells. (b) DMR amplitudes of compound **63** as a function of doses compared with the dose-dependent desensitization of zaprinast DMR by **63**, and the dose-dependent inhibition of the DMR of 100 nM compound **63** by compound **5**. The data represents mean ± sd from two independent measurement, each with four replicates (n=8).

For the di-substitution of the benzamide ring, the rank order of potency of *o,p*-dichloro (**49**), *o,p*-difluoro (**53**), *m,p*-dimethoxy (**57**) was as follows: *o,p*-difluoro (**53,** EC_{50} 0.13 μM) > *o,p*-dichloro (**49,** EC_{50} 0.31 μM) > *m,p*-dimethoxy (**57,** EC_{50} 0.83μM), suggesting that the *o,p*-positions led to a visible increase in activity compared to the *o*- or *p*-mono-substituted compounds. However, the di-methoxy in *m,p*-positions (**57**) led to a large reduction in activity compared to compound **56**, suggesting that bulky *m,p*-substituted compounds are not well tolerated, but *o,p*-di-substitutions are.
Compounds **50** and **56** showed the highest agonistic potency among the derivatives. Therefore, we devised the synthesis of compound **63**, which was substituted with a fluoro in *o*-position and a methoxy in the *p*-position. As expected, compound **63** displayed further improved potency (EC_{50} 0.041μM, **Figure 2**).

Table 2. Potency of N-(2-(1*H*-tetrazol-5-yl)phenyl)benzamide Derivatives in DMR Assay



compd	R_1	R_2	R_3			EC_{50}[a] (μM)	desensitization IC_{50}[b] (μM)	antagonist IC_{50}[c] (μM)
			ortho	*meta*	*para*			
37						inactive	——	——
42	Br	H	H	H	H	0.87±0.09	0.30±0.03	0.47±0.06
43	Br	H	CH_3	H	H	2.05±0.11	0.81±0.05	0.19±0.02
44	Br	H	H	CH_3	H	0.86±0.04	0.36±0.02	0.12±0.03
45	Br	H	H	H	CH_3	1.33±0.13	0.51±0.03	0.13±0.04
46	Br	H	Cl	H	H	0.87±0.10	0.41±0.03	0.77±0.08
47	Br	H	H	Cl	H	2.18±0.37	0.48±0.03	0.42±0.11
48	Br	H	H	H	Cl	0.52±0.03	0.21±0.01	0.52±0.05
49	Br	H	Cl	H	Cl	0.31±0.03	0.11±0.01	1.11±0.42
50	Br	H	F	H	H	0.35±0.02	0.17±0.01	0.52±0.07
51	Br	H	H	F	H	0.61±0.07	0.21±0.02	0.57±0.07
52	Br	H	H	H	F	0.45±0.02	0.16±0.01	0.18±0.06
53	Br	H	F	H	F	0.13±0.01	0.06±0.01	0.22±0.03
54	Br	H	OCH_3	H	H	0.86±0.06	0.40±0.03	0.28±0.05
55	Br	H	H	OCH_3	H	1.94±0.08	0.81±0.07	0.56±0.08
56	Br	H	H	H	OCH_3	0.059±0.007	0.026±0.003	0.20±0.09
57	Br	H	H	OCH_3	OCH_3	0.83±0.07	0.26±0.02	1.00±0.16
58	H	Br	H	H	OCH_3	0.30±0.03	0.11±0.01	1.38±0.15
59	F	H	H	H	OCH_3	0.20±0.01	0.082±0.005	0.34±0.08
60	H	H	H	H	OCH_3	0.65±0.05	0.28±0.02	1.00±0.22
61	Br	H	H	CF_3	H	4.60±0.46	1.28±0.08	0.84±0.06
62	Br	H	H	H	CF_3	3.65±0.19	1.36±0.16	0.25±0.05
63	Br	H	F	H	OCH_3	0.041±0.005	0.014±0.002	1.69±0.16
64					OCH_3	17.58±3.30	8.36±0.99	0.63±0.19

[a]EC_{50} to trigger DMR.[b]IC_{50} to desensitize cells upon cells repeated stimulation with 1μM zaprinast. [c]IC_{50} of known GPR35 antagonist **5** to block the agonist-induced DMR. The data represents mean ± sd from two independent measurements, each with four replicates (n = 8).

There is evidence that the halogen atom bromine is very important for the retention of compound activity agonist GPR35.[14-17, 21] In order to study the effect of the change of the bromine atom on the activity of the compound, we chose the compound **56** for further study. Results showed that transforming the substitution position of bromine atom (**58**, EC_{50} 0.30μM), replacing the bromine with a fluorine (**59**, EC_{50} 0.20μM), or removing the bromine (**60**, EC_{50} 0.65μM) all decreased potency.

Compound **37**, a synthetic precursor of **60** was also tested and found to be devoid of activity, demonstrating the necessity of the tetrazole. The activity was completely lost when the

tetrazolyl was replaced by cyano. (**Figure S1e**). In addition, changing the position of the tetrazoyl also significantly reduced the activity of the compound (**64**, EC_{50} 17.58 μM).

We further examined the activity of compounds **53** and **56** through the ERK phosphorylation. Compound **53** and compound **56** increased ERK phosphorylation (**Figure. 3a**). As control, the known GPR35 agonist zaprinast also triggered ERK phosphorylation (**Figure. 3b**). Moreover, the GPR35 antagonist ML-145 attenuated ERK phosphorylation induced by these compounds (**Figure. 3b**). These results suggested that compounds **53** and **56** induved the phosphorylation of ERK via the activation of GPR35.

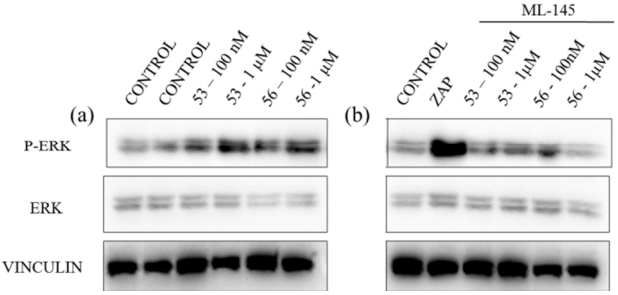


Figure 3. (a) Western blot of ERK1/2 and p-ERK1/2 after treatment with compounds **53** and **56** for 15 minutes at concentrations of 100 nM, 1 μM respectively. (b) Western blot of ERK1/2 and p-ERK1/2 after treatment with the known GPR35 agonist Zaprinast (Zap) at 1 μM and western blot of ERK1/2 and p-ERK1/2 after treatment with compounds for 10 minutes at the same concentrations as in (a) in the presence of ML-145 (25 μM) for 5 minutes.

In order to evaluate the druglikeness of a compound, several physiochemical properties such as partition coefficient (clogP), the ligand efficiency (LE) and the ligand-lipophilicity efficiency (LLE) were introduced.[22-24] These parameters were calculated for the compounds with relatively high potency, including compounds **14-16, 18, 42, 44, 46, 48-54, 56-60** and **63** (**Table 3**). Lipinski's Rule of Five considered that the clogP value of a druglike compound should be lower than 5.[25, 26] The clogP values of all these selected compounds were found to be within this range. In fact, most of these compounds exhibited a clogP value between 2 and 3, which is favorable for orally administered drugs.

The LE combines physiochemical with pharmacological properties, and represents the binding force between each atom and receptors and calculates as follows: LE = pEC_{50}/N (N = non-hydrogen atoms). The LE of most selected compounds were found to be about 0.3, except for compounds **54** and **57**.

Table 3. Physicochemical Properties of Selected Compounds

compd	pEC_{50}	clogP[a]	LE	LLE
14	6.21	1.27	0.37	4.94
15	6.42	1.58	0.36	4.84
16	6.36	2.78	0.30	3.58
18	6.29	1.98	0.31	4.31
42	6.06	2.23	0.29	3.83
44	6.06	2.73	0.28	3.33
46	6.06	2.19	0.28	3.87
48	6.28	3.02	0.29	3.26
49	6.50	2.93	0.28	3.57
50	6.46	2.04	0.29	4.42
51	6.21	2.45	0.28	3.76
52	6.35	2.45	0.29	3.90
53	6.88	2.21	0.30	4.67
54	6.07	2.29	0.26	3.78
56	7.23	2.36	0.31	4.87
57	6.08	2.01	0.24	4.07
58	6.52	2.36	0.28	4.16
59	6.70	1.64	0.29	5.06
60	6.19	1.31	0.28	4.88
63	**7.38**	**2.15**	**0.32**	**5.23**

[a]Calculated by the Chembiodraw Ultra 11.0.

LLE (LEE = pEC_{50} - clogP), another useful parameter, combines the potency and lipophilicity. Among these compounds, **14**, **15**, **56**, **59**, **60** and **63** showed a LLE value of 5,. A suitable drug candidate should have a LE > 0.3 and LLE > 5. In summary, the new compounds **56** and **63** with potent potency showed a very good clogP, and suitable LE and LLE values.

In conclusion, a series of *N*-[2-(1*H*-tetrazol-5-yl)-phenyl]-amido derivatives were synthesized as potent GPR35 agonists through a two-step synthesis method. SAR analysis showed that a bromine substituent in the 5-position and a *p*-methoxybenzamide in the 2-position would significantly improve the activity (**56,** EC_{50} 0.059 μM). The *o,p*-distribution such as *o,p*-dichloro (**49**) and *o,p*-difluoro (**53**) could also improve the potency when compared with the *o*- or *p*-monosubstituted analogs. Combining these findings, compound **63** was synthesized and found to display the highest potency (EC_{50} 0.041 μM) and good physicochemical properties. Together, this study provides a new series of potent GPR35 agonists, such as **56** and **63**, which may become useful leading ligands to further elucidate the physiological roles of GPR35.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Experimental details and characterization data for the reported compounds; NMR spectra; and biological assays.

AUTHOR INFORMATION

Corresponding Author

*For Xiuli Zhang: phone, +86 411 84379519; E-mail, zhangxiuli@dicp.ac.cn.

*For Xinmiao Liang: phone, +86 411 84379519; E-mail, liangxm@dicp.ac.cn,

Author Contributions

‖These authors contributed equally to this work.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

GPR35, G protein-coupled receptor 35; GPCR, G protein-coupled receptor; SARs, structure activity relationships; DMR, dynamic mass redistribution; THF, tetrahydrofuran; LE, ligand efficiency; LLE, ligand-lipophilicity efficiency.

REFERENCES

(1) Rask-Andersen, M.; Almen, M. S.; Schioth, H. B. Trends in the exploitation of novel drug targets. *Nat. Rev. Drug Discov.* **2011,** 10, 579-590.

(2) Chung, S.; Funakoshi, T.; Civelli, O. Orphan GPCR research. *Br. J. Pharmacol.* **2008,** 153, S339-S346.

(3) O'Dowd, B. F.; Nguyen, T.; Marchese, A.; Cheng, R.; Lynch, K. R.; Heng, H. H. Q.; Kolakowski, L. F.; George, S. R. Discovery of three novel G-protein-coupled receptor genes. *Genomics* **1998,** 47, 310-313.

(4) Min, K. D.; Asakura, M.; Liao, Y. L.; Nakamaru, K.; Okazaki, H.; Takahashi, T.; Fujimoto, K.; Ito, S.; Takahashi, A.; Asanuma, H.; Yamazaki, S.; Minamino, T.; Sanada, S.; Seguchi, O.; Nakano, A.; Ando, Y.; Otsuka, T.; Furukawa, H.; Isomura, T.; Takashima, S.; Mochizuki, N.; Kitakaze, M. Identification of genes related to heart failure using global gene expression profiling of human failing myocardium. *Biochem. Biophys. Res. Commun.* **2010,** 393, 55-60.

(5) Wang, J. H.; Simonavicius, N.; Wu, X. S.; 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.

(6) Sun, Y. V.; Bielak, L. E.; Peyser, P. A.; Turner, S. T.; Sheedy, P. E.; Boerwinkle, E.; Kardia, S. L. R. Application of machine learning algorithms to predict coronary artery calcification with a sibship-based design. *Genet. Epidemiol.* **2008,** 32, 350-360.

(7) Costa, V.; Federico, A.; Pollastro, C.; Ziviello, C.; Cataldi, S.; Formisano, P.; Ciccodicola, A. Computational Analysis of Single Nucleotide Polymorphisms Associated with Altered Drug Responsiveness in Type 2 Diabetes. *Int. J. Mol. Sci.* **2016,** 17, 14.

(8) 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.

(9) Deng, H. Y.; Hu, H. B.; Fang, Y. Multiple tyrosine metabolites are GPR35 agonists. *Sci. Rep.* **2012,** 2, 12.

(10) Maravillas-Montero, J. L.; Burkhardt, A. M.; Hevezi, P. A.; Carnevale, C. D.; Smit, M. J.; Zlotnik, A. Cutting Edge: GPR35/CXCR8 Is the Receptor of the Mucosal Chemokine CXCL17. *J. Immunol.* **2015,** 194, 29-33.

(11) Taniguchi, Y.; Tonai-Kachi, H.; Shinjo, K. Zaprinast, a well-known cyclic guanosine monophosphate-specific phosphodiesterase inhibitor, is an agonist for GPR35. *FEBS Lett.* **2006,** 580, 5003-5008.

(12) MacKenzie, A. E.; Caltabiano, G.; Kent, T. C.; Jenkins, L.; McCallum, J. E.; Hudson, B. D.; Nicklin, S. A.; Fawcett, L.; Markwick, R.; Charlton, S. J.; Milligan, G. The antiallergic mast cell stabilizers lodoxamide and bufrolin as the first high and equipotent agonists of human and rat GPR35. *Mol Pharmacol* **2014,** 85, 91-104.

(13) Deng, H. Y.; Hu, H. B.; Ling, S. Z.; 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.

(14) Deng, H. Y.; Hu, H. B.; He, M. Q.; Hu, J. Y.; Niu, W. J.; Ferrie, A. M.; Fang, Y. Discovery of 2-(4-Methylfuran-2(5H)-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.

(15) Funke, M.; Thimm, D.; Schiedel, A. C.; Muller, C. E. 8-Benzamidochromen-4-one-2-carboxylic Acids: Potent and Selective Agonists for the Orphan G Protein-Coupled Receptor GPR35. *J. Med. Chem.* **2013,** 56, 5182-5197.

(16) Thimm, D.; Funke, M.; Meyer, A.; Muller, C. E. 6-Bromo-8-(4-[(3)H]methoxybenzamido)-4-oxo-4H-chromene-2-carboxylic Acid: a powerful tool for studying orphan G protein-coupled receptor GPR35. *J Med Chem* **2013,** 56, 7084-99.

(17) Wei, L.; Wang, J. X.; Zhang, X. L.; Wang, P.; Zhao, Y. P.; Li, J. Q.; Hou, T.; Qu, L. L.; Shi, L. Y.; Liang, X. M.; Fang, Y. Discovery of 2H-Chromen-2-one Derivatives as G Protein-Coupled Receptor-35 Agonists. *J. Med. Chem.* **2017,** 60, 362-372.

(18) Ferrie, A. M.; Wu, Q.; Fang, Y. Resonant waveguide grating imager for live cell sensing. *Appl. Phys. Lett.* **2010,** 97, 3.

(19) Deng, H. Y.; Hu, H. B.; Fang, Y. Tyrphostin analogs are GPR35 agonists. *FEBS Lett.* **2011,** 585, 1957-1962.

(20) Deng, H. Y.; Fang, Y. Discovery of nitrophenols as GPR35 agonists. *Medchemcomm* **2012,** 3, 1270-1274.

(21) Deng, H. Y.; Fang, Y. Synthesis and Agonistic Activity at the GPR35 of 5,6-Dihydroxyindole-2-carboxylic Acid Analogues. *ACS Med. Chem. Lett.* **2012,** 3, 550-554.

(22) Edwards, M. P.; Price, D. A. Role of physicochemical properties and ligand lipophilicity efficiency in addressing drug safety risks. *Annu. Rep. Med. Chem.* **2010**, *45*, 380-391.

(23) Ertl, P.; Rohde, B.; Selzer, P. Fast calculation of molecular polar surface area as a sum of fragment-based contributions and its application to the prediction of drug transport properties. *J. Med. Chem.* **2000,** 43, 3714-3717.

(24) Kuntz, I. D.; Chen, K.; Sharp, K. A.; Kollman, P. A. The maximal affinity of ligands. *Proc. Natl. Acad. Sci. U. S. A.* **1999,** 96, 9997-10002.

(25) Leeson, P. D.; Springthorpe, B. The influence of drug-like concepts on decision-making in medicinal chemistry. *Nat. Rev. Drug Discov.* **2007,** 6, 881-890.

(26) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.* **2012,** 64, 4-17.

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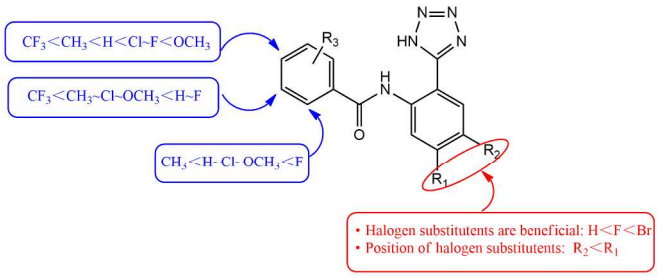

• The substitute position of R_3: *meta* ≤ *ortho* < *para*