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Original Article

Discovery and characterization of novel small-molecule agonists of G protein-coupled receptor 119

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Aim: GPR119 is a G protein-coupled receptor (GPCR) that is highly expressed in pancreatic β-cells and intestinal L-cells and facilitates glucose-stimulated insulin secretion (GSIS). GPR119 may represent a novel target for the treatment of metabolic disorders. Here, we sought to identify novel small-molecule GPR119 agonists.
Methods: A cell-based high-throughput screening assay was established using HEK293 cells stably expressing GPR119 and pCRE-luc reporter plasmid (HEK293/GPR119/pCRE-luc). A compound library composed of 1440 compounds was screened. Mouse β-cell line MIN-6 and isolated mouse islets were used to evaluate the effects of candidate compounds on GSIS *in vitro*.
Results: Three compounds with novel structures (ZSY-04, -06, and -13) were found to activate GPR119-mediated signaling and to induce GPR119 desensitization. The EC_{50} values of ZSY-04, -06, and -13 in stimulating intracellular cAMP accumulation in HEK293/GPR119 cells were 2.758, 3.046, and 0.778 μmol/L, respectively. Furthermore, all three compounds displayed high selectivity for GPR119, and did not activate other 9 GPCRs tested. Moreover, all three compounds significantly increased GSIS in both MIN-6 mouse β-cells and isolated mouse islets at concentration of 10 μmol/L.
Conclusion: Three novel small-molecule GPR119 agonists (ZSY-04, -06, and -13) with high receptor selectivity and capacity to induce GSIS *in vitro* were discovered. These compounds are potential candidates to be structurally optimized into drugs for the treatment of type 2 diabetes.

Keywords: GPCR; GPR119; PSN632408; AR231453; high-throughput screening; cAMP; Ca^{2+}; β-cell; insulin; obesity; type 2 diabetes mellitus

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Introduction

Type 2 diabetes (T2D), which is characterized by insulin resistance, a reduction in insulin secretion by pancreatic β-cells, and impaired incretin response, is a global epidemic and is still increasing in association with an increase in obesity rates[1]. More than 300 million people worldwide have been diagnosed with T2D[2]. Inadequate T2D management is a leading cause of cardiovascular disease, blindness, kidney disease, amputations, and even death[3]. In 2004 alone, more than 3 million people died from diabetes[4]. The WHO estimates that diabetes will be the 7th leading cause of death by 2030[5]. The current treatment options for patients with T2D include lifestyle modifications, such as regular exercise and calorie restriction, and anti-diabetic medications.

Owing to the key role of β-cell dysfunction in the pathogenesis of T2D, major efforts have been taken to understand the factors or pathways involved in insulin secretion. A number of nutrients, hormones, and neurotransmitters have been found to influence glucose-stimulated insulin secretion (GSIS) via G protein-coupled receptors (GPCRs), including GLP-1R, GPR40, GPR120, and CB1/2[6–10]. Recently, the oleoylethanolamide (OEA)- and lysophosphatidylcholine (LPC)-activated GPR119 was shown to facilitate GSIS. GPR119 is highly expressed in pancreatic β-cells and intestinal L-cells[11–13]. The endogenous ligands LPC and OEA have been shown to promote GSIS directly by activating GPR119 on the β-cells and indirectly by activating GPR119 on the L-cells, which induces GLP-1 secretion[14–17]. A potent and selective GPR119 agonist discovered by Arena Pharmaceuticals, AR231453, has been shown to enhance GSIS in isolated mouse islets[12] and to promote GLP-1 secretion in mouse intestine L-cell lines[13]. AR231453 did not induce insulin secretion from islets isolated from GPR119-deficient mice, thereby confirming that its effects were mediated by GPR119[12]. Oral administration of AR231453 in rats significantly improved the circulating levels of insulin, GLP-1 and GIP, and lowered the blood glucose

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concentration in an oral glucose tolerance test[13]. Therefore, GPR119 represents a novel and attractive potential target for T2D therapy. A number of synthetic GPR119 agonists have advanced to clinical trials. Currently, several compounds have entered Phase 2 trials, including MBX-2982[18].

Here, we sought to identify novel GPR119 modulators. We established a high-throughput screening (HTS) assay based on the signal transduction of GPR119. Approximately 1440 compounds were screened, and several hits with novel chemical scaffolds were identified. Several hit compounds (ZSY-04, -06, and -13) were further characterized. These three compounds were all found to activate GPR119-mediated cAMP accumulation, calcium mobilization and ERK phosphorylation. They also induced desensitization of GPR119. These compounds displayed high selectivity towards GPR119, only activating GPR119 among the ten GPCRs tested. Most importantly, all three compounds induced GSIS in a β-cell line and in isolated mouse islets, indicating that they are potential candidates to be structurally optimized for the treatment of T2D.

Materials and methods

Reagents

Screening compounds were provided by the Chinese National Compound Resource Center. The hit compounds 4-ethyl-2-(3-(4-(4-methoxyphenyl)piperazine-1-carbonyl)piperidin-1-yl)pyrido[2,3-b]pyrazin-3(4*H*)-one (ZSY-04), 4-ethyl-2-(3-(4-phenylpiperazine-1-carbonyl)piperidin-1-yl)pyrido[2,3-b]pyrazin-3(4*H*)-one (ZSY-06), and 1-((4-(5-cyclopropyl-1,2,4-oxadiazol-3-yl)phenyl)sulfonyl)-*N*-((tetrahydrofuran-2-yl)methyl)piperidine-4-carboxamide (ZSY-13) were purchased from ChemDiv (San Diego, CA, USA) for further characterization. Sulfinpyrazone, TUG-424, TUG-891, CP55940, GLP-1, glucagon, isoproterenol, DPDPE, U50488, and DAGO were purchased from Sigma-Aldrich (St Louis, MO, USA). Fluo-4 AM was purchased from Invitrogen (Carlsbad, CA, USA). Mammalian expression vectors encoding human GPR119, GPR40, GPR120, CB2, GLP-1R, GCGR, β2 AR, DOR, KOR, MOR, and Gα16 were purchased from the UMR cDNA Resource Center (Rolla, MO, USA).

Cell culture and transfection

Human embryonic kidney (HEK) 293 cells and Chinese hamster ovary (CHO) cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 mg/L penicillin, and 100 mg/L streptomycin at 37 °C in a humidified atmosphere of 5% CO_2. For transient transfection, approximately 1×10^6 cells were mixed with 2 to 4 μg of plasmids in 200 μL of transfection buffer, and electroporation was performed with a Scientz-2C electroporation apparatus (Scientz Biotech, Ningbo, China). To generate cell lines stably expressing GPCRs in combination with Gα16 or a cAMP-responsive element-driven luciferase reporter (CRE-luc), the transfected cells were seeded into 10-cm dishes, and antibiotics (400 μg/mL G418 and/or 20 μg/mL blasticidin) were added to the culture medium the next day. The selection medium was changed every 3 d until the formation of colonies. Single colonies were selected, expanded, and tested for the expression of the transfected genes.

Reporter assay

Cells expressing GPR119 (or other GPCRs) and CRE-luc were plated at a density of 10 000 cells per well in a 96-well plate. After 24 h in culture, compounds at various concentrations were added. DMSO (1%) was used as a negative control. After an additional 24 h, luciferase activities were measured using the Steady-Glo luciferase assay system (Promega, Madison, WI, USA) and an EnVision (PerkinElmer, Waltham, MA, USA) microplate reader according to the manufacturer's instructions.

Cyclic-AMP (cAMP) assay

Cells were harvested with PBS and pelleted by centrifugation for 5 min at 1100 rounds per minute. The cells were then resuspended in an appropriate volume of assay buffer (PBS containing 500 μmol/L IBMX) to obtain a final cell count of 8×10^5 cells/mL. The cells were then plated in 384-well assay plates at 4000 cells/5 μL per well. Another 5 μL of buffer containing compounds at various concentrations was added to the assay plates and incubated for 30 min at room temperature. Intracellular cAMP measurement was performed with a HTRF Dynamic 2 cAMP kit (Cisbio, Cat No 62AM4PEJ) and an EnVision microplate reader according to the manufacturer's instructions.

Calcium mobilization assay

Cells expressing GPR119 (or other GPCRs) and Gα16 were seeded onto 96-well plates at a density of 3×10^4 cells/well and cultured overnight. The cells were then incubated with 2 μmol/L Fluo-4 AM in HBSS (5.4 mmol/L KCl, 0.3 mmol/L Na_2HPO_4, 0.4 mmol/L KH_2PO_4, 4.2 mmol/L $NaHCO_3$, 1.3 mmol/L $CaCl_2$, 0.5 mmol/L $MgCl_2$, 0.6 mmol/L $MgSO_4$, 137 mmol/L NaCl, 5.6 mmol/L *D*-glucose and 250 μmol/L sulfinpyrazone, pH 7.4) at 37 °C for 45 min. After a thorough washing, 50 μL of HBSS was added. After incubation at room temperature for 10 min, 25 μL of agonist (depending on the receptor) was dispensed into the well using a FlexStation III microplate reader (Molecular Devices), and the intracellular calcium change was recorded at an excitation wavelength of 485 nm and an emission wavelength of 525 nm.

Western blotting

Cells were serum starved for 2 h and then treated with compounds for the indicated duration at 37 °C. The cells were lysed, sonicated, and boiled at 95 °C for 5 min in sample buffer. Aliquots of protein were fractionated by SDS-PAGE on 10% polyacrylamide gels and transferred to PVDF membranes. The membranes were incubated first with blocking buffer (TBS with 0.05% Tween 20 and 5% nonfat milk) for 1 h at room temperature and then incubated overnight at 4 °C in buffer containing antibodies against GAPDH, ERK, or p-ERK.

The membranes were washed 3 times and incubated with secondary antibody for 1 h. After washing, immunostaining was visualized using Amersham ECL Plus Western blotting detection reagents (GE Healthcare).

RT-PCR

Total mRNA was isolated using TRIzol (Invitrogen), and 2 μg of RNA was used to synthesize cDNA using the PrimeScript™ RT reagent kit (Takara, DRR037A) according to the manufacturer's protocol. PCR was performed for 35 cycles at an annealing temperature of 57 °C, and PCR without the RNA template was used as the negative control. The products were analyzed by agarose gel electrophoresis and visualized with ethidium bromide.

Insulin secretion

MIN-6 β-cells were cultured in DMEM containing 25 mmol/L glucose and 10% FBS in a 96-well plate at 2.5×10^4 cells/well for 48 h. On the day of the experiment, the cell culture medium was aspirated and the plate was washed twice with Krebs-Ringer bicarbonate buffer (KRBB, containing $CaCl_2$, 1.26 mmol/L; KCl, 5.4 mmol/L; KH_2PO_4, 0.44 mmol/L; $MgCl_2$, 0.5 mmol/L; $MgSO_4$, 0.4 mmol/L; NaCl, 0.14 mol/L; $NaHCO_3$, 4.1 mmol/L; Na_2HPO_4, 0.34 mmol/L; 0.5% fatty acid-free BSA; HEPES, 20 mmol/L; pH 7.0). The cells were incubated at 37 °C for 2 h in KRBB containing 2.8 mmol/L glucose and then stimulated with various compounds in KRBB containing 2.8 or 16.8 mmol/L glucose for 2 h. The supernatants were collected, and the insulin concentrations were measured using a Cisbio HTRF Insulin kit (62RB3RDF) and an EnVision microplate reader according to the manufacturer's instructions.

Islets were isolated as previously described[19] from male C57BL/6 mice (8 weeks old) that had been anesthetized using an intraperitoneal injection of sodium pentobarbital (50 mg/kg). The abdomen was opened, and 5 mL of HBSS (GIBCO, 14185-052) with 0.25 mg/mL collagenase XI (Sigma, C7657) was injected into the pancreas via the bile duct. The distended pancreas was digested at 37 °C for 15 min, and the detached islets were collected and cultured overnight in RPMI-1640 supplemented with 11.1 mmol/L glucose and 10% FBS. The islets were washed with KRBB and 2.8 mmol/L glucose and then hand-selected under a stereomicroscope and transferred to 24-well plates. After preincubation for 30 min at 37 °C in the KRBB buffer (2.8 mmol/L glucose), the islets were incubated with various compounds for 2 h at 37 °C in KRBB buffers containing 2.8 or 16.8 mmol/L glucose. The supernatants were collected, and the insulin concentrations were measured.

Immunofluorescence staining

Sections (10 μm) of pancreas were blocked with 10% goat serum (GS) and 0.1% Triton-X-100 in PBS for 30 min and then incubated with a combination of rabbit anti-insulin and mouse anti-glucagon antibodies in PBS containing 0.1% Triton-X-100 and 1% GS at 4 °C overnight. After washing with PBS, the sections were incubated with a cocktail of secondary antibodies conjugated to Alexa Fluor 488 or Alexa Fluor 555 for 2 h at room temperature. Nuclei were counterstained with Hoechst 33342. Fluorescent images were obtained with an Olympus IX51 inverted fluorescent microscope.

Statistical analysis

Data were analyzed with GraphPad Prism software. Nonlinear regression analysis was performed to generate dose-response curves and to calculate the concentration for 50% of the maximal effect (EC_{50}). The data are presented as the mean±SEM. Two-tailed Student's *t*-tests were performed, and $P<0.05$ was considered to be statistically significant.

Results

Assay development and validation

To search for new GPR119 agonists, we developed several cell-based assays to measure GPR119 activity. As shown in Figure 1A, GPR119 is primarily distributed on the cell membrane when expressed in HEK293 cells. In HEK293 cells stably expressing GPR119 and the pCRE-luc reporter plasmid (HEK293/GPR119/pCRE-luc), the known agonists of GPR119 PSN632408 and AR231453 induced dose-dependent luciferase reporter expression with EC_{50} values of 4.892 μmol/L and 1.355 nmol/L, respectively (Figure 1B). In contrast, in HEK293 cells transfected with the control vector, only forskolin induced reporter gene expression (Figure 1C). Direct measurement of cAMP also confirmed that PSN632408 and AR231453 induced intracellular cAMP accumulation with EC_{50} values of 1.06 μmol/L and 1.121 nmol/L only in the cells expressing GPR119 (HEK293/GPR119) (Figure 1D), but not in control HEK293 cells (Figure 1E). Although GPR119 couples mainly to the Gαs/cAMP pathway, it could be forced to couple to the Gα16/calcium pathway in cells overexpressing Gα16, such as many other GPCRs[20]. As shown in Figure 1F, both PSN632408 and AR231453 induced a dose-dependent increase in calcium levels in HEK293/GPR119/Gα16 cells (Figure 1F). Interestingly, PSN632408 displayed similar EC_{50} values in all three assays, whereas AR231453 was much less potent in evoking a calcium response (EC_{50}=272.7 nmol/L), indicating that AR231453 is a biased ligand that favors the Gαs/cAMP pathway. Taken together, these results indicate that these cell-based assays are sensitive and stable and could be used to screen and evaluate GPR119 agonists.

High-throughput screening and discovery of novel GPR119 agonists

To accelerate the process, the initial screening was performed using a luciferase reporter assay in HEK293/GPR119/pCRE-luc cells. DMSO (1%) was used as a negative control, and PSN632408 (10 μmol/L) was included as the positive control. Compounds were tested in duplicate at a concentration of 10 μmol/L. The Z′ factor, the normalized 3×SD window between the negative and positive controls, was used to assess the robustness of the screening. As shown in Figure 2A, the Z′ value for the assay was 0.97, and the signal-to-background ratio was 2.73, indicating that the screen was adequately opti-

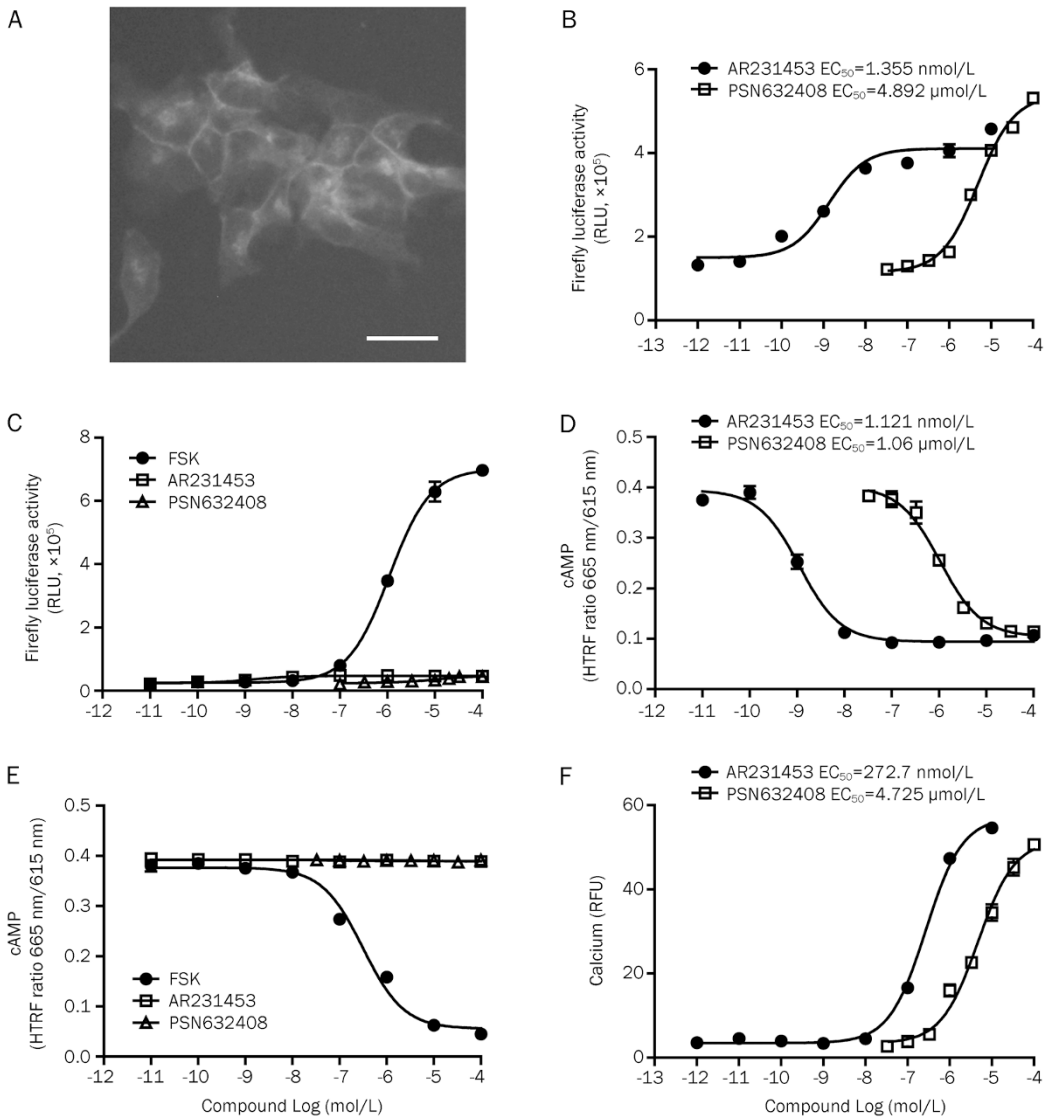

Figure 1. Development and validation of cell-based assays for the screening of GPR119 agonists. (A) Immunofluorescent image of HEK293 cells stably expressing GPR119-EGFP. Scale bars=20 μm. (B and C) Dose-response of PSN632408 and AR231453 in inducing luciferase expression in HEK293/GPR119/pCRE-luc cells (B) or the control HEK293/pCRE-luc cells (C). Forskolin (FSK) was used as a receptor-independent control. (D and E) Dose-response of PSN632408 and AR231453 in inducing intracellular cAMP accumulation in HEK293/GPR119 (D) and control HEK293 (E) cells. The lower the ratio, the higher the cAMP level. (F) Dose-response of PSN632408 and AR231453 in inducing a calcium response in HEK293/GPR119/Gα16 cells. Data are presented as the mean±SEM (*n*=3).

mized. A library composed of 1440 compounds was screened (Figure 2B), and compounds that induced a ≥2-fold increase in the luciferase activity were selected and further validated in triplicate (Figure 2C). Finally, three compounds, ZSY-04, -06, and -13 (Figure 2D–2F), displaying consistent agonist activity were further tested in a dose-dependent manner. These three compounds showed similar efficacy and potency as PSN632408 in the luciferase assay, with EC_{50} values at low micromolar levels (Figure 2G–2I). ZSY-04 and -06 are both pyridopyrazinone derivatives with the only difference at the phenyl substituent, whereas ZSY-13 contains a novel 3,6-disubstituted [1,2,4]triazolo[4,3-b]pyridazine core, which represents a new class of GPR119 agonists.

ZSY-04, -06, and -13 induce signal transduction and desensitization of GPR119

ZSY-04, -06, and -13 were further characterized using cAMP and calcium assays. As shown in Figure 3A-3C, ZSY-04, -06, and -13 induced dose-dependent intracellular cAMP accumulation in HEK293/GPR119 cells with EC_{50} values of 2.758 μmol/L, 3.046 μmol/L, and 0.778 μmol/L, respectively. In the cAMP assay, these compounds displayed similar potency as PSN632408, although the efficacy was not as high as PSN632408. ZSY-04, -06, and -13 also induced a dose-dependent calcium response in HEK293/GPR119/Gα16 cells with ZSY-04 and -06 displaying partial agonist activity and ZSY-13 showing full agonist activity (Figure 3D–3F).

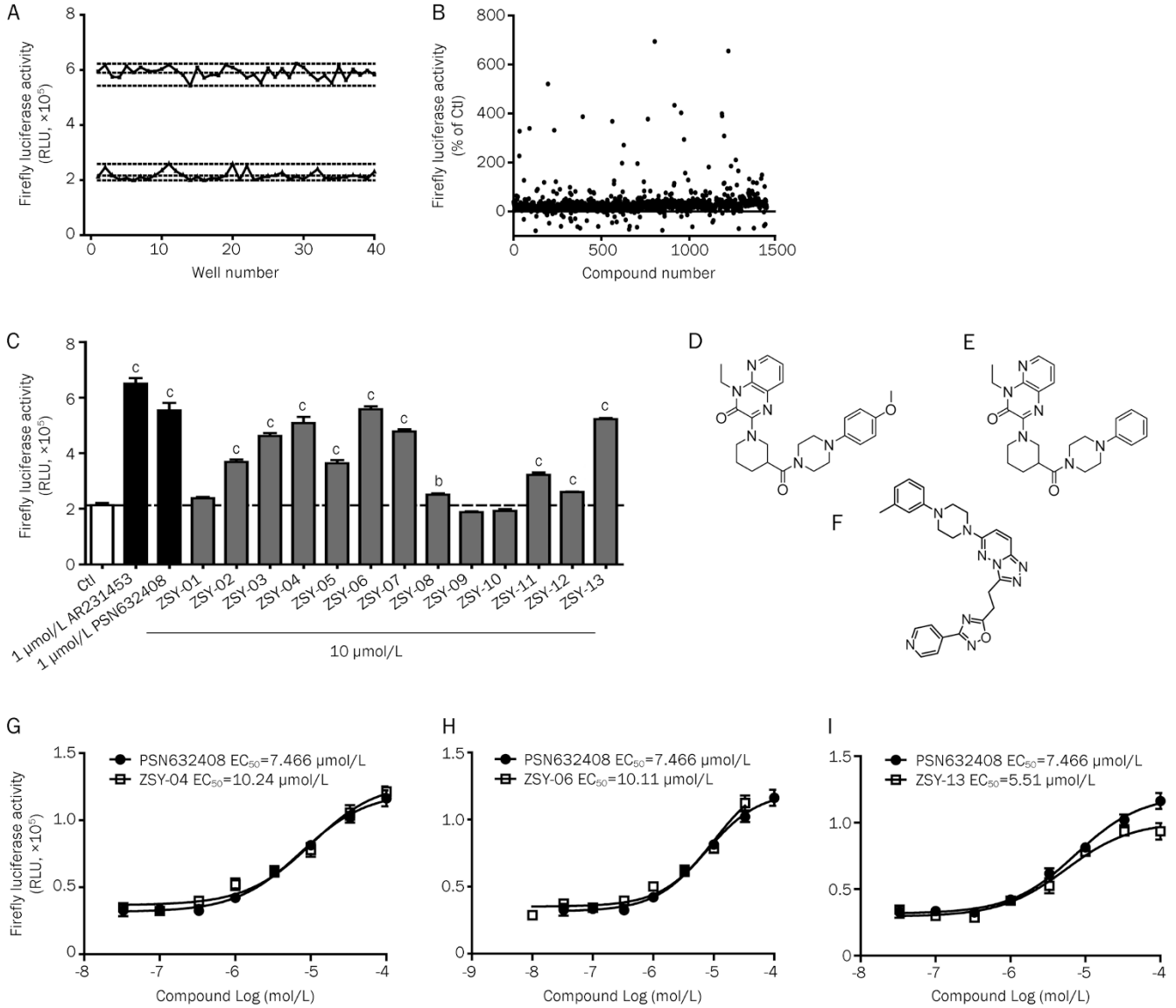

Figure 2. High-throughput screening and discovery of novel small molecule agonists of GPR119. (A) Z′ factor determination. Under the optimized conditions, replicates of positive and negative signals were studied. Dashed lines indicate the means±3SD of 40 data points. The Z′ value for the assay was 0.97, and the S/B ratio was 2.73. (B) Result of the primary screening of 1440 compounds using the luciferase assay. DMSO (1%) and PSN632408 (10 μmol/L) were used as the negative and positive controls, respectively. (C) Thirteen compounds that induced a greater than 2-fold increase in luciferase activity in the primary screening were further tested in triplicate (10 μmol/L). AR231453 (1 μmol/L) and PSN632408 (10 μmol/L) were used as the positive controls. Data are presented as the mean±SEM (n=3). ^{b}P<0.05, ^{c}P<0.01 *vs* vehicle control. (D–F) Chemical structures of ZSY-04, -06, and -13. (G–I) Dose-response curves of ZSY-04, -06, and -13 in the luciferase assay. Data are presented as the mean±SEM (n=3).

Many GPCRs couple to the extracellular signal-regulated protein kinase (ERK1/2) pathway. The activation of GPR119 with PSN632408 led to phosphorylation of ERK1/2 (Figure 3G). Similar to PSN632408, ZSY-04, -06, and -13 (10 μmol/L) also induced phosphorylation of ERK1/2 in HEK293 cells expressing GPR119. In contrast, in HEK293 cells transfected with empty vector, only the PKC activator PMA induced phosphorylation of ERK1/2, while the other compounds had no effect (Figure 3H). In addition, ZSY-04, -06, and -13 did not evoke a calcium response in HEK293 cells transfected with empty vector or other GPCRs, including GPR40, GPR120, CB2, GLP-1R, GCGR, β2AR, DOR, KOR, and MOR, at concentrations up to 100 μmol/L (Table 1). These results indicate that ZSY-04, -06, and -13 are highly selective GPR119 agonists.

Receptor desensitization is a common mechanism that leads to the loss of GPCR function after agonist stimulation. To assess whether ZSY-04, -06, and -13 induce GPR119 desensitization, HEK293/GPR119/Gα16 cells were first stimulated with the compounds (30 μmol/L) or 0.1% DMSO (Figure 4A, first arrow). PSN632408 and all the ZSY compounds induced robust calcium responses, confirming again that they are agonists of GPR119. After a 10 min incubation, the cells were

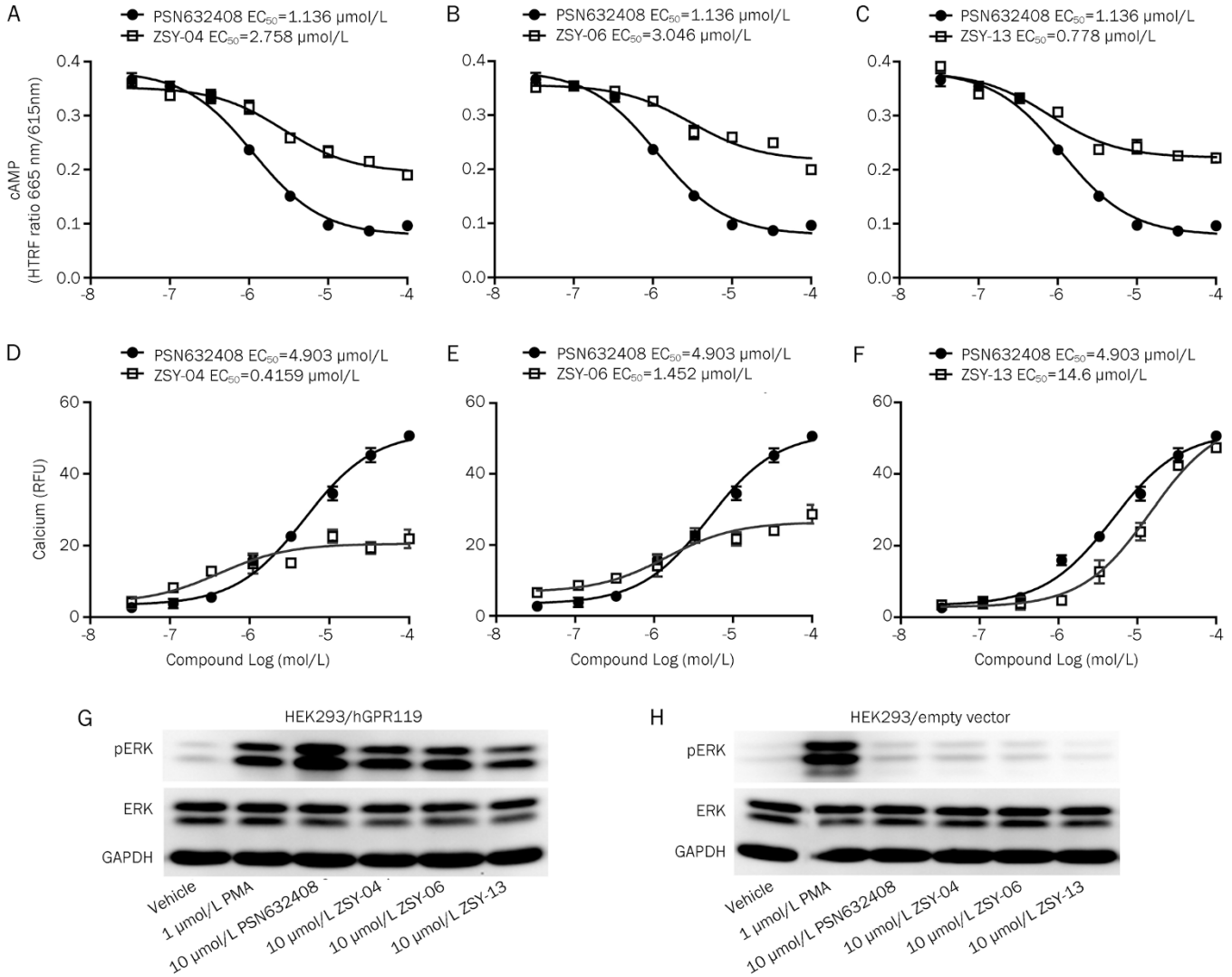

Figure 3. Activation of intracellular signal transduction in HEK293 cells expressing GPR119 by ZSY-04, -06, and -13. (A–C) Dose-response of ZSY-04 (A), -06 (B), and -13 (C) in inducing intracellular cAMP accumulation in HEK293/GPR119 cells. PSN632408 was used as a positive control. The ratio presented is inversely proportional to the cAMP level. (D–F) Dose-response of ZSY-04 (D), -06 (E), and -13 (F) in inducing a calcium response in HEK293/GPR119/Gα16 cells. PSN632408 was used as the positive control. Data are presented as the mean±SEM (n=3). (G and H) Representative Western blot analysis of ERK phosphorylation in HEK293/GPR119 cells (G) or HEK293 cells transfected with empty vectors (H) stimulated with ZSY-04, -06, -13, and PSN632408.

washed and restimulated with 30 μmol/L PSN632408 (Figure 4A, second arrow). Cells prestimulated with 0.1% DMSO responded normally to PSN632408 treatment, whereas prestimulation with PSN632408 or the ZSY compounds led to receptor desensitization, *ie*, a reduced calcium response at the second stimulation with PSN632408 (Figure 4).

ZSY-04, -06, and -13 induce insulin secretion in a mouse β-cell line and isolated islets

GPR119 is an insulinotropic receptor that is expressed in β-cells. Activation of GPR119 has been reported to enhance GSIS from β-cells. Thus, we examined the effects of ZSY-04, -06, and -13 on GSIS in a β-cell line and freshly isolated mouse islets. As shown in Figure 5A, GPR119 could be detected with RT-PCR analysis in two β-cell lines, MIN6 and HIT-T15. High-glucose (16.8 mmol/L) induced a significant increase in insulin release from MIN6 cells. Similar to GLP-1 (100 nmol/L), AR231453 (500 nmol/L) and PSN632488 (10 μmol/L), ZSY-04, -06, and -13 (tested at 1 and 10 μmol/L) significantly stimulated insulin secretion in high-glucose conditions from MIN6 cells (Figure 5B).

The freshly isolated mouse islets showed normal morphology (Figure 6B). Immunofluorescence staining showed that these islets contain both the insulin-expressing β-cells and the glucagon-expressing α-cells (Figure 6A). High-glucose (16.8 mmol/L) induced substantial insulin release from freshly isolated mouse islets (Figure 6C), while both GLP-1 (100 nmol/L) and AR231453 (500 nmol/L) significantly enhanced GSIS.

Table 1. Selectivity of ZSY-04, 06, and 13 on tested GPCRs.

Receptors	Calcium mobilization assay [EC_{50} (mol/L)]				
	Known agonists		ZSY-04	ZSY-06	ZSY-13
GPR119	PSN632408	4.903e-6	4.159e-7	1.452e-6	1.46e-5
GPR40	TUG-424	1.385e-7	NR	NR	NR
GPR120	TUG-891	5.133e-7	NR	NR	NR
CB2	CP55940	1.06e-9	NR	NR	NR
GLP-1R	GLP-1	1.982e-9	NR	NR	NR
GCGR	Glucagon	1.337e-8	NR	NR	NR
β2AR	Isoproterenol	5.702e-10	NR	NR	NR
DOR	DPDPE	1.328e-9	NR	NR	NR
KOR	U50488	2.456e-9	NR	NR	NR
MOR	DAGO	4.604e-9	NR	NR	NR

NR, No response at concentrations up to 100 μmol/L.

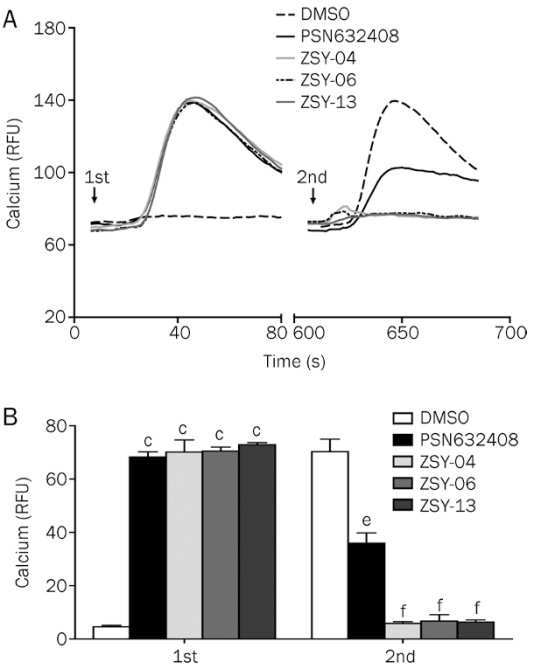

Figure 4. Desensitization of GPR119 induced by ZSY-04, -06, and -13. (A) HEK293/GPR119/Gα16 cells were loaded with Fluo-4 AM and stimulated (first arrow) with 30 μmol/L PSN632408 (positive control), ZSY-04, -06, -13, or DMSO (negative control). Intracellular calcium was measured. After a 10 min stimulation, cells were washed and restimulated (second arrow) with 30 μmol/L PSN632408. Intracellular calcium levels were re-measured. Representative calcium signals are presented. (B) Statistical analysis of the calcium peak value presented in (A). Data are presented as the mean±SEM (n=3). ^{c}P<0.01 *vs* control (first white bar). ^{e}P<0.05, ^{f}P<0.01 *vs* control (second white bar).

ZSY-04, -06, and -13 (tested at 1 and 10 μmol/L) also significantly stimulated insulin secretion in high-glucose conditions (Figure 6C). These stimulants were less effective or had no effect in low-glucose media. These results confirm that ZSY-04, -06, and -13 can induce insulin secretion via activation of GPR119.

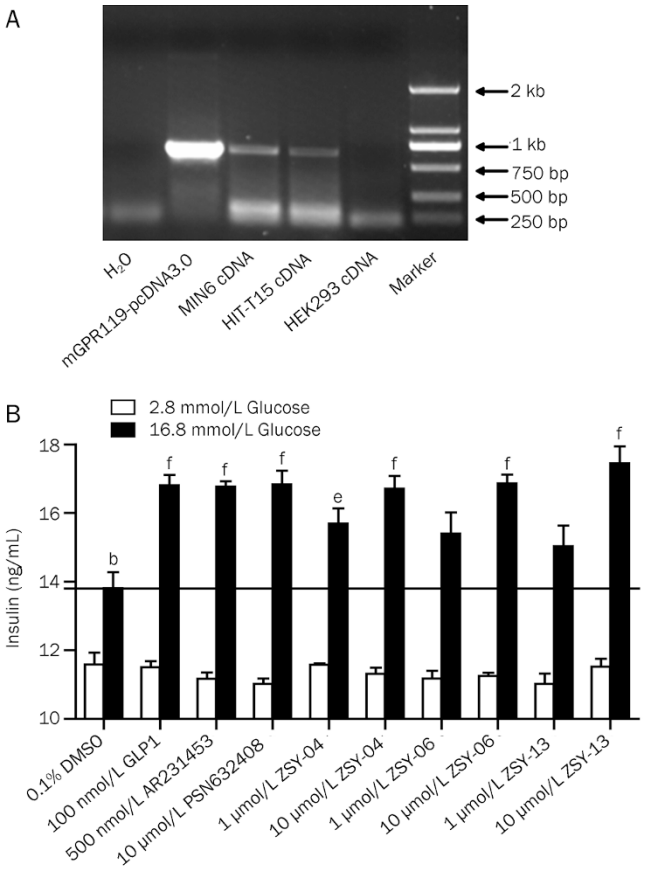

Figure 5. ZSY-04, -06, and -13 induced insulin release in mouse β-cell line MIN6. (A) RT-PCR analysis of GPR119 gene expressions in MIN6, HIT-T15 and HEK293 cells. (B) MIN6 cells were incubated in medium supplemented with 2.8 or 16.8 mmol/L glucose and the indicated compounds for 2 h and then insulin in the supernatants was measured. GLP-1 (100 nmol/L), AR231453 (500 nmol/L), and PSN632408 (10 μmol/L) were used as the positive controls. Data are presented as the mean±SEM (n=3). ^{b}P<0.05 *vs* DMSO control (2.8 mmol/L glucose). ^{e}P<0.05, ^{f}P<0.01 *vs* DMSO control (16.8 mmol/L glucose).

Discussion

Modulating GSIS in the pancreatic β-cells either directly by targeting specific GPCRs on β-cells or indirectly by increasing incretin levels through GLP-1 analogs and DPP4 inhibitors has become the focus of therapeutic strategies for T2D in recent years[21–24]. Although the latter approach has been quite successful in treating T2D, GLP-1 analogs such as exendin-4 are not orally administrable, while DDP4 inhibitors are limited by the endogenous level of GLP-1. GPR119 is primarily expressed in pancreatic β-cells and intestinal L-cells. GPR119 agonists may induce GSIS directly by activating GPR119 on β-cells or indirectly by activating the receptor on the intestinal L-cells, thereby inducing GLP-1 secretion, which in turn stimulates GSIS. GPR119 agonists may have therapeutic advantages over both DPP-4 inhibitors and GLP-1 analogs. GPR119 is therefore expected to function as a new therapeutic target for T2D[11–14].

The discovery of small-molecule agonists of GPR119 has

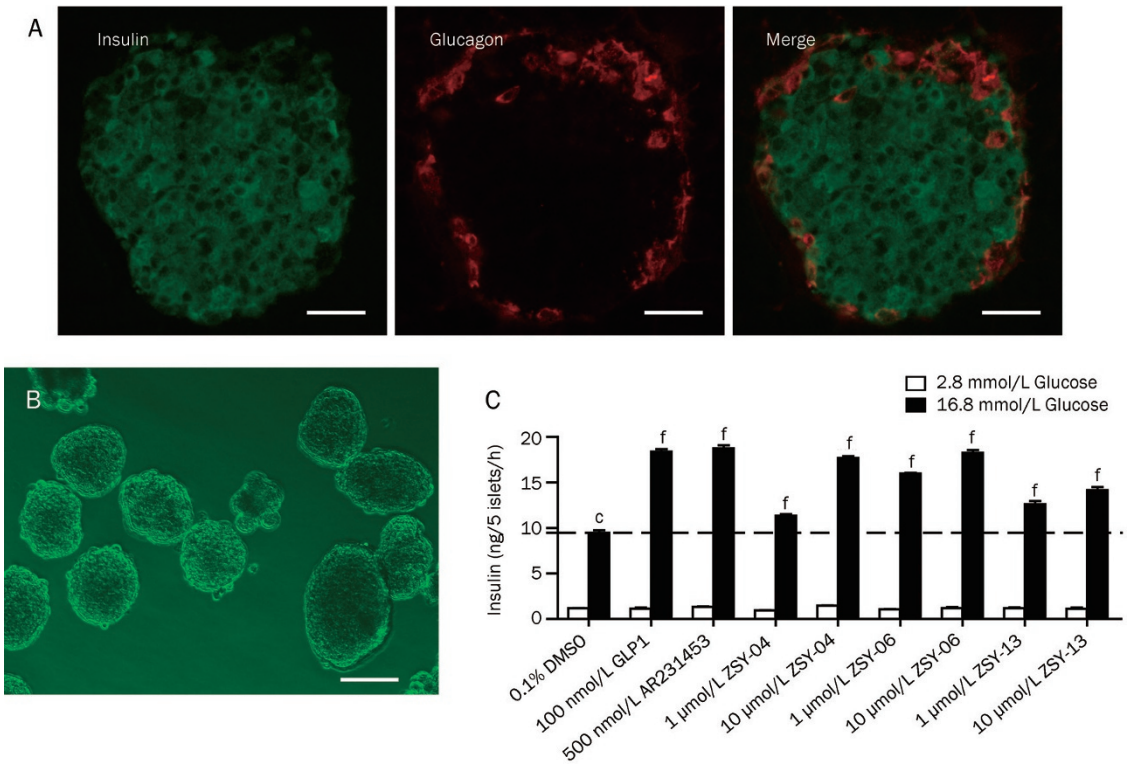

Figure 6. ZSY-04, -06, and -13 induced insulin release from isolated mouse islets. (A) Confocal images of immunofluorescent staining of insulin and glucagon in mouse islets. Scale bars=100 μm. (B) Phase-contrast image of freshly isolated mouse islets. Scale bars=200 μm. (C) Mouse islets were incubated in medium supplemented with 2.8 or 16.8 mmol/L glucose and the indicated compounds for 2 h, then insulin in the supernatants was measured. GLP-1 (100 nmol/L) and AR231453 (500 nmol/L) were used as the positive controls. Data are presented as the mean±SEM (n=3). ^{c}P<0.01 *vs* DMSO control (2.8 mmol/L glucose). ^{f}P<0.01 *vs* DMSO control (16.8 mmol/L glucose).

advanced our understanding of the functions of GPR119 in obesity and T2D. AR231453 is one of the early reported selective GPR119 agonists discovered by Arena Pharmaceuticals. It has been shown to enhance GSIS in isolated mouse islets[12] and to promote GLP-1 secretion in mouse intestine L-cell lines[13]. Oral administration of AR231453 in rats significantly improved the circulating levels of insulin, GLP-1 and GIP, and lowered the blood glucose concentration in an oral glucose tolerance test[13]. However, AR231453 has been suggested to exert toxic effects *in vivo* when administered chronically[25]. In addition to AR231453, a number of synthetic agonists have been developed for GPR119. It has been reported that PSN632408, a GPR119 agonist, suppressed food intake and reduced body weight gain in a rat model[26]. Yoshida *et al* have also reported three new GPR119 agonists: AS1269574, AS1907417, and AS1535907[27–30]. These GPR119 agonists induced GSIS from pancreatic β-cells only under high-glucose conditions and displayed *in vivo* activity. JNJ-38431055 from Johnson & Johnson progressed to clinical trials with positive results but was terminated shortly afterwards, presumably because of subpar performance in the trials[31]. GSK-1292263 by GlaxoSmithKline has also completed Phase 2 clinical trials and was reported to be well-tolerated in preliminary findings[32]. MBX-2982 from Metabolex Inc directly stimulated GSIS from isolated islets, enhanced insulin secretion during hyperglycemic clamps in rats, acutely lowered glucose excursion and increased plasma GLP-1 and GIP during OGTTs in mice and rats[33]. MBX-2982 has completed three Phase 1 studies and is currently in Phase 2 testing.

In the present study, we identified and characterized three novel GPR119 agonists: ZSY-04, -06, and -13. ZSY-04 and -06 are both pyridopyrazinone derivatives with the only difference at the phenyl substituent, whereas ZSY-13 contains a novel 3,6-disubstituted [1,2,4]triazolo[4,3-b]pyridazine core. All three compounds activated GPR119-mediated signaling and induced GPR119 desensitization. These compounds displayed high selectivity for GPR119 and did not activate the other nine GPCRs tested. More importantly, all three compounds were found to induce insulin secretion from isolated mouse islets. In conclusion, we have discovered three novel GPR119 agonists with high selectivity and normal cellular functions. These compounds are potential candidates to be structurally optimized for the treatment of T2D.

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Author contribution

Xin XIE and Shu-yong ZHANG designed the research; Shu-yong ZHANG performed the research; Shu-yong ZHANG, Jing LI, and Xin XIE analyzed the data; and Shu-yong ZHANG and Xin XIE wrote the paper.

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