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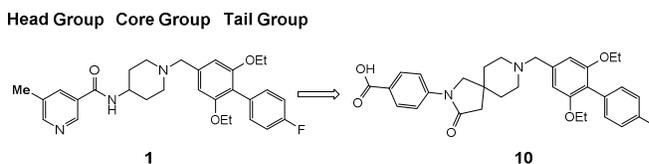
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# Discovery and pharmacology of a novel somatostatin subtype 5 (SSTR<sub>5</sub>) antagonist: Synergy with DPP-4 inhibition

Weiguo Liu\*, Pengcheng P. Shao, Gui-Bai Liang, John Bawiec, Jiafang He, Susan D. Aster, Margaret Wu, Garry Chicchi, John Wang, Kwei-Lan Tsao, Jin Shang, Gino Salituro, Yun-Ping Zhou, Cai Li, Taro E. Akiyama, Daniel E. Metzger, Beth Ann Murphy, Andrew D. Howard, Ann E. Weber, Joseph L. Duffy

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**ABSTRACT:** We report new SSTR<sub>5</sub> antagonists with enhanced potency, subtype selectivity, and minimal off-target activities as compared to previously reported compounds. Starting from the reported SSTR<sub>5</sub> antagonist **1**, we systematically surveyed changes in the central core and head piece while maintaining the diphenyl tail group constant. From this study the azaspirodecanone **10** emerged as a new highly potent and selective SSTR<sub>5</sub> antagonist. Compound **10** lowered glucose excursion by 94% in an oral glucose tolerance test (OGTT) in mice following a 3 mg/kg oral dose. The compound increased both total and active circulating incretin hormone GLP-1 levels in mice at a dose of 10 mg/kg. A synergistic effect was also demonstrated when compound **10** was coadministered with a DPP-4 inhibitor, substantially increasing circulating active GLP-1[7-36] amide and insulin in response to a glucose challenge.



**KEYWORDS:** Somatostatin, SSTR<sub>5</sub> antagonists, Type 2 diabetes mellitus (T2DM), Glucose-dependent insulin secretion (GDIS)

Somatostatin receptor subtype 5 (SSTR<sub>5</sub>) is one of five G-protein coupled receptors that sense the peptide hormone somatostatin (SST), also called somatotropin release-inhibiting factor. SSTR1-5 are widely distributed throughout the body.<sup>1</sup> Their endogenous ligands exist in two isoforms with 14 and 28 amino acids (SST-14 and SST-28), respectively.<sup>2</sup> While SST-14 is mainly secreted from pancreatic  $\delta$  cells, neurons, and the stomach, SST-28 mostly exists in the enteroendocrine  $\delta$  cells in the small intestine, colon, and pancreas.<sup>3</sup> The SST-mediated biological effects involve a broad range of functional regulation and hormonal secretion control, including inhibiting the secretion of both insulin and the insulinotropic glucagon-like peptide 1 (GLP-1), which have been the subject of numerous studies and publications.<sup>4</sup>

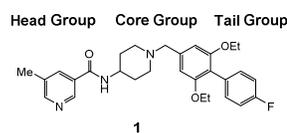
Selective antagonism of endogenous SST effects on SSTR<sub>5</sub> should be beneficial for maintaining glucose homeostasis. The target SSTR<sub>5</sub> is prominently expressed in pancreatic islet  $\beta$  cells as well as some  $\delta$  and  $\alpha$  cells.<sup>5</sup> It is also highly expressed in enteroendocrine cells of the gastrointestinal (GI) tract. SST inhibits GLP-1 secretion via primarily SSTR<sub>5</sub>,<sup>4</sup> while its inhibitory effect on insulin secretion is mediated by multiple SST receptors, including SSTR<sub>5</sub>.<sup>6</sup>

Evidence for the role of SSTR<sub>5</sub> in glucose homeostasis comes from SSTR<sub>5</sub> knockout (KO) mice. Increased glucose-dependent insulin secretion (GDIS) was demonstrated in the

perfused pancreas of SSTR<sub>5</sub> KO mice as compared with wild type (WT) mice.<sup>7</sup> Pancreatic islets isolated from SSTR<sub>5</sub> KO mice displayed increased total insulin content as compared with islets obtained from WT mice. As a result, the KO mice exhibited decreased blood glucose and plasma insulin levels and increased leptin and glucagon concentrations compared with WT mice. In addition, the SSTR<sub>5</sub> KO mice displayed decreased susceptibility to high fat diet (HFD)-induced insulin resistance.<sup>8</sup>

SSTR<sub>5</sub> selective small molecule antagonists have been reported to lower glucose and insulin excursion during an oral glucose tolerance test (OGTT) in both mice and rats.<sup>9,10,11,12</sup> Based on these cellular and preclinical pharmacology studies, SSTR<sub>5</sub> is an attractive investigational target for treatment of type 2 diabetes mellitus (T2DM). Although a range of therapeutic agents are available for the treatment of T2DM, such as sulfonylureas, metformin, PPAR $\gamma$ -selective agonists, DPP-4 inhibitors, and recently SGLT2 inhibitors, a high proportion of diabetic patients still fail to achieve or maintain glycemic targets due to limitations and/or liabilities associated with these agents.<sup>13</sup> An SSTR<sub>5</sub> antagonist offers the possible dual effects of increasing GLP-1 release from the GI tract and promoting insulin secretion from the pancreatic islets in response to changes in blood glucose levels. Therefore, an SSTR<sub>5</sub> antagonist could serve as monotherapy for the treatment of T2DM,

and may also be synergistic in combination with antidiabetic GLP-1 protective agents such as DPP-4 inhibitors. To this end, we have investigated the small molecule SSTR5 selective antagonists as therapeutic agents for treatment of T2DM and their potential for synergistic effects with a DPP-4 inhibitor. This study involved optimization of a previously reported SSTR5 selective antagonist, and full preclinical pharmacology characterization of the optimized compound.



**Table 1.** Lead Series of SSTR5 Antagonists

Structure	SSTR5 <sup>a</sup> binding <i>h/m</i> <sup>b</sup> IC <sub>50</sub> (nM)	SSTR5 cAMP <i>h/m</i> <sup>b</sup> IC <sub>50</sub> (nM)	hERG <sup>c</sup> IC <sub>50</sub> (μM)	OGTT <sup>d</sup> (%) Dose (mg/kg)
	1.3/1.0	3.1/2.7	1.69	87% 10
	2.5/16	4.5/6.1	15	56% 30
	7.6/2.7	247/98	0.26	
	13/N.D.	26/N.D.	0.21	
	0.7/0.8	8.6/28	0.22	60% 30
	2.9/0.6	67.8/7.1	0.21	
	21/13	73/N.D.	N.D.	
	1.6/2.5	67/N.D.	0.95	
	83/32	110/7.4	25	54% 30
	1.2/2.1	1.1/0.9	45	94% 3

<sup>a</sup> All IC<sub>50</sub> values calculated are the mean of at least duplicate experiments within a maximal threefold range. N.D. = not determined.

<sup>b</sup> Potency against human (h) and mouse (m) SSTR5.

<sup>c</sup> hERG inhibition measured by competitive binding with MK-499, a known hERG blocker.

<sup>d</sup> Decrease in oral glucose AUC vs vehicle in male C57BL/6 mice fed with HFD (D12492) for 21 days, n=3, t=0-120 min, compound dosed 1h before glucose.

Literature reports of small molecule SSTR5 selective antagonists are relatively few. Martin reported the first nonpeptidic

selective SSTR5 antagonists,<sup>14</sup> which was followed by additional reports detailing the structure-activity relationship (SAR) around the aminopiperidine based core structures.<sup>15,16</sup> These SSTR5 selective antagonists, such as **1** (Table 1), generally consist of three components; a heterocyclic head group, an aminopiperidine core group, and a substituted aromatic tail group. Recently, additional reports have appeared showing variation is tolerated in all three regions of this lead series, notably with the discovery of a spirocyclic core subunit.<sup>10,11,12</sup> These accounts prompted us to detail our optimization of this lead series, and report our finding of preclinical pharmacologic synergy in promoting circulation of active GLP-1 by oral coadministration of an SSTR5 antagonist with a DPP-4 inhibitor.

In our studies, **1** is a potent SSTR5 antagonist with good efficacy in our acute mouse oral glucose tolerance test (OGTT) with 87% lowering of plasma glucose following a 10 mg/kg oral dose with good residual whole blood exposure (C<sub>2,5h</sub> = 0.11 μM). We modified the class of SSTR5 antagonists represented by **1** in an attempt to identify new lead structures with diminished potency against cardiac ion-channels such as hERG, while maintaining SSTR5 antagonist potency and SSTR subtype selectivity. Compounds were tested in an SSTR5 filtration binding assay, which measures the competitive displacement of radiolabeled SST-28 from cell membranes expressing the human or mouse SSTR5 receptor. Potent compounds were also tested in a functional assay in Chinese hamster ovary (CHO-K1) cells expressing the human or mouse SSTR5 receptor. This whole cell assay measures the compound's ability to inhibit the SST-28 mediated reduction of cAMP accumulation induced by forskolin. Potent compounds were also counter screened against the related membrane binding assays for SSTR1-4.

For this optimization, we maintained the reported biaryl tail piece (R) in **1**, and synthesized clusters of individual analogs to survey changes in the central core and head piece. The design principle centered on replacing the 4-aminopiperidine core in **1** with alternative scaffolds while conserving the overall architecture for activity. Representative lead compounds from each of these clusters are listed in Table 1, and additional SAR tables within each series and physicochemical data for **1-10** are provided in the supporting information. A small set of analogs with fused bicyclic core structures represented by **2** were prepared. The fused cyclopentylpyrrole ring system yielded potent SSTR5 antagonists. After a secondary SAR screen of different head pieces (supplementary material Table S1), compound **2** was found to be optimal and was selected for further profiling (cis-racemic, more potent C-4 diastereomer characterized as described in the supplementary material). The compound showed reasonable oral bioavailability in a mouse PK experiment (F = 48%, t<sub>1/2</sub> = 1.7 h), and modest efficacy in the OGTT assay with 56% lowering of plasma glucose excursion following a 30 mg/kg oral dose with high residual whole blood exposure (C<sub>2,5h</sub> = 0.76 μM). The compound was devoid of significant off-target activity except for modest potencies on hERG (MK-499 binding IC<sub>50</sub> = 15 μM) and weak SSTR1 binding activity (IC<sub>50</sub> = 6 μM).

The carbon-linked head group series represented by **3** was also explored. A variety of 2-aryl substituents provided similar potency among the 2,5-disubstituted imidazole derivatives such as **3**, however a significant loss of potency was observed

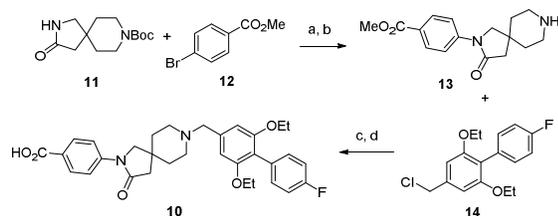
for the corresponding 3-oxadiazole derivative (Table S1). Compound **3** is a potent inhibitor of hERG (MK-499 binding  $IC_{50} = 0.29 \mu\text{M}$ ) and also maintained potent SSTR3 antagonist activity ( $IC_{50} = 119 \text{ nM}$ ). This ancillary activity was persistent across the entire carbon-linked imidazole core series.

The sulfonamide series represented by **4** and **5** was derived from extending the 4-aminopiperidine core in **1** to spiro- or biperidines and replacing the head piece with a sulfonamide. This series of sulfonamides is comprised of highly potent and highly selective SSTR5 antagonists with moderate bioavailability but with excellent in vivo efficacy in OGTT assays. Compound **5** (mouse PK,  $F = 13\%$ ) lowered glucose excursion in OGTT by 60% following an oral dose of 30 mg/kg despite moderate residual whole blood exposure ( $C_{2.5h} = 0.092 \mu\text{M}$ ). However, similar to the arylimidazole series characterized by **3**, this class of analogs was consistently potent in inhibiting hERG. Sustained SAR investigation efforts failed to mitigate this off-target activity.

The ether linked series, such as **6** and **7**, was designed by replacing the connecting NH in **1** with an O or  $\text{CH}_2\text{O}$  group. Similar to the sulfonamide series, all of the ether-linked compounds afforded persistent inhibition of hERG. Although these compounds were highly selective SSTR5 antagonists, their potencies were generally moderate without significant changes in ion channel activities.

Finally, replacing the 4-aminopiperidine in **1** with spiroheterocycle variants afforded series represented by **8** and **9**, which showed promise in improving both potency and off-target selectivity. Compound **8**, tested as a racemic mixture, is one of the most potent binders to the SSTR5 receptor while **9** afforded high selectivity over ion channel activities, with MK-499  $IC_{50} = 25 \mu\text{M}$ . Compound **9** displayed good oral bioavailability and reasonable in vivo efficacy of 54% glucose lowering in OGTT assay at a 30 mg/kg oral dose with good whole blood exposure ( $C_{2.5h} = 0.46 \mu\text{M}$ ), and despite its moderate mSSTR5 binding potency ( $IC_{50} = 32 \text{ nM}$ ) and mSSTR5 functional potency ( $IC_{50} = 7.4 \text{ nM}$ ). Examination of the emerging SAR indicated that significant SSTR5 potency was gained by the spiroheterocycle core group as in **8**, whereas significant selectivity over hERG was gained by the carboxyl-containing head group as in **2**. Combining these features (including supporting information compounds S25 – S30) led to the optimal **10**, which provided the desired profile of potency and selectivity. This compound was chosen for more extensive pharmacological profiling.

#### Scheme 1. Synthesis of spiro-piperidine analog **10**



Reagents and conditions: (a)  $\text{K}_3\text{PO}_4$ ,  $\text{Pd}_2(\text{dba})_3$ , dioxane,  $100^\circ\text{C}$ , 88%; (b) HCl, dioxane, EtOAc, RT, 95%; (c)  $\text{Cs}_2\text{CO}_3$ , DMF, RT, 86%; (d) LiOH, MeOH,  $50^\circ\text{C}$ , 79%. For synthesis of intermediate **14**, see supporting information.

The synthesis of compound **10** is outlined in Scheme 1. Starting with Boc protected spiro-piperidine intermediate **11**,

Buchwald-Hartwig C-N coupling with 4-bromobenzoic methyl ester **12** followed by deprotection of the Boc group afforded **13**. This intermediate was then alkylated with **14** followed by hydrolysis of the ester to give **10**. The compounds discussed in this communication were synthesized by similar sequences with starting materials that are either commercially available or can be made with simple and well-established steps.

Compound **10** is an advanced tool for the investigation of SSTR5 pharmacology.<sup>17</sup> It is a very potent and highly selective SSTR5 antagonist with hSSTR5 binding ( $IC_{50} = 1.2 \text{ nM}$ ) and cAMP antagonist ( $IC_{50} = 1.1 \text{ nM}$ ). It is inactive in the binding assay against hSSTR1-4 ( $IC_{50} > 10 \mu\text{M}$ ), and this represents improved subtype selectivity as compared to **1** (hSSTR1  $IC_{50} < 300 \text{ nM}$ ). At higher concentrations, **10** exhibited partial agonist activity in the hSSTR5 cAMP assay, achieving 44% maximal activation at  $8.3 \mu\text{M}$  with an inflection point of  $2.7 \mu\text{M}$ . The antagonist **10** is highly selective over hERG (MK-499 binding  $IC_{50} = 45 \mu\text{M}$ ) and other cardiovascular ion channels ( $\text{IK}_s$ ,  $\text{Ca}_v1.2$ ,  $\text{Na}_v1.5$   $IC_{50} > 15 \mu\text{M}$ ). Analysis of **10** in over 160 ancillary target assays (Eurofins Panlabs screen) revealed only moderate binding activity against the prostanoid DP receptor ( $IC_{50} = 3 \mu\text{M}$ ).

The pharmacokinetic properties of **10** indicated that it is a suitable tool for in vivo preclinical testing. The oral bioavailability of **10** in rat, dog, and rhesus ranges from 40%-72% with unbound free fractions of 2.2%-5.7% across species (Table 2). The compound shows no appreciable inhibition of human P450 enzymes (CYP3A4, 2D6, 2C9  $IC_{50} > 50 \mu\text{M}$ ) and no appreciable PXR activation. The solubility of **10** was modest in neutral aqueous solution (0.3 mg/mL), but it was more soluble in a 10% Tween formulation (5.3 mg/mL). This formulation was used for pharmacokinetic studies.

**Table 2.** Pharmacokinetic Properties of compound **10**

Species	Dose <sup>a</sup> (iv/po)	$F_u$ <sup>b</sup> (%)	$Cl^c$	Vd (K/kg)	$t_{1/2}$ (h)	F (%)
Rat	1 / 2	5.7	15.2	2.38	1.9	41
Dog	0.25 / 1	4.4	2.2	2.82	16.5	72
Rhesus	0.25 / 1	5.4	11.8	1.39	2.0	40

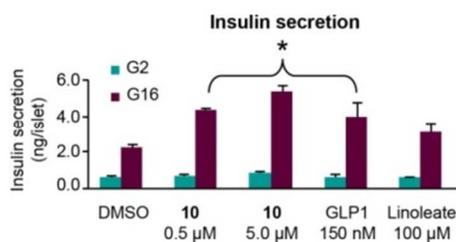
<sup>a</sup>Dosed in mg/kg

<sup>b</sup>Measured at 0.1  $\mu\text{M}$  concentration with [ $^3\text{H}$ -**10**];  $F_u$  (%) for human and mouse are 2.2 and 4.6 respectively.

<sup>c</sup>Measured in mL/kg/min.

The pharmacology of **10** was investigated to more fully profile the opportunity provided by selective antagonism of SSTR5. Ex vivo, **10** was incubated with isolated human pancreatic islets from non-diabetic donors using a method we have previously reported (Figure 1).<sup>18</sup> The compound potentiated insulin secretion in a glucose-dependent manner. In the presence of high glucose concentration (16 mM, labeled G16), insulin secretion in human islets was significantly enhanced by **10** at both 5  $\mu\text{M}$  and 0.5  $\mu\text{M}$  compound concentration, presumably by blocking the inhibitory effect of endogenous SST-14 and SST-28 secreted from these islets on insulin secretion. However, insulin secretion at low glucose concentration (2 mM, labelled as G2) was not affected by **10**, consistent with the GDIS mechanism. The effect of **10** was similar to 50 nM of

the GDIS peptide GLP-1, as well as linoleate, a small molecule GPR40 agonist used as a positive control.<sup>19</sup> The secretion of SST-14 and glucagon in these islets was similarly monitored in this experiment, and was unaffected by **10** (data not shown).



**Figure 1.** Compound **10** increased glucose-dependent insulin secretion in isolated human islets with 2 mM and 16 mM of glucose (G2, G16). \* $P \leq 0.05$  vs DMSO.

Compound **10** also lowered glucose excursions in vivo in a dose-dependent manner during OGTT in HFD mice (Table 3). Oral administration of 0.03 to 3 mg/kg of **10** dose-dependently reduced the net glucose area under the curve (AUC) in OGTT mouse ranged from 23% to 84% as compared to vehicle. Plasma exposures of the compound were measured 3 hours post dose. From nine related OGTT experiments in HFD mice, we calculate the minimal exposure for maximal efficacy ( $EC_{90}$ ) = 484 nM. Furthermore, the exposure required for significant efficacy ( $\geq 50\%$  reduction) may be calculated ( $EC_{50} \geq 54$  nM). Taking into consideration the measured free fraction in mouse plasma ( $F_u\% = 4.6$ ), the unbound concentration of **10** affording  $EC_{50}$  is 2.5 nM. This is roughly consistent with the mouse SSTR5  $IC_{50}$  (0.9 nM) in the cell-based cAMP assay. The OGTT efficacy of **10** was entirely ablated in SSTR5 KO mice (supporting information), validating SSTR5 as the biological target.

**Table 3.** Compound **10** dose titration in HFD mice OGTT

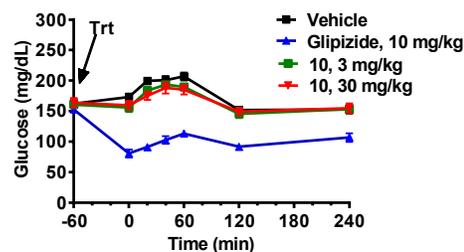
Oral dose (mg/kg)	Blood glu AUC (change %) <sup>a</sup>	Plasma exposure (nM @ 3h)	
		whole blood	unbound
3	-84%**	351	16.1
1	-74%*	131	6.0
0.3	-59%*	34	1.6
0.1 (MED) <sup>b</sup>	-58%*	11	0.5
0.03	-23%	4	0.2

<sup>a</sup>Reduction in blood glucose AUC as compared to vehicle, n = 8.

\* $P < 0.01$  vs veh; \*\* $P < 0.001$  vs veh.

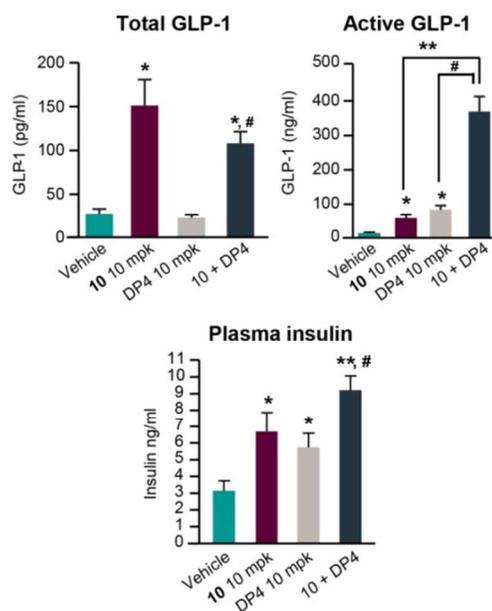
<sup>b</sup>MED = minimal dose for significant efficacy.

As a glucose-responsive mechanism, administration of an SSTR5 antagonist is expected to afford minimal risk of hypoglycemia. This risk was assessed in 4-hr fasted lean C57BL/6N mice (Figure 2). An efficacious oral dose of **10** (3 mg/kg) and a supra-efficacious dose (30 mg/kg) administered to the mice did not decrease basal glucose levels significantly over the subsequent 5 hours. Glipizide, a marketed sulfonylurea K<sup>+</sup>-ATP blocker, was utilized as a positive control in this experiment.<sup>20</sup> Glipizide dosed orally at 10 mg/kg caused significant decreases of basal blood glucose levels starting at 60 minutes after dosing.



**Figure 2.** Blood glucose in 4-hr fasted lean mice following administration of **10** or glipizide.

Inhibition of the DPP-4 enzyme, which is responsible for the rapid degradation of active GLP-1[7-36] amide to inactive GLP-1[9-36] amide in vivo, is a proven therapy for the treatment of T2DM. Given that a selective SSTR5 antagonist potentiates glucose-induced secretion of GLP-1, we anticipated that coadministration of a DPP-4 inhibitor and an SSTR5 antagonist should have synergistic effects on circulating active GLP-1[7-36] and therefore GDIS. As shown in the OGTT assay results in Figure 3, administration of the SSTR5 antagonist **10** to lean C57/B6N mice afforded a substantial increase in circulating total GLP-1 and plasma insulin levels, and a measurable increase in circulating active GLP-1 (blood measurements taken 5 minutes post glucose challenge). The combination of **10** with the DPP-4 inhibitor des-fluoro sitagliptin<sup>21</sup> resulted in significantly increased circulating active GLP-1 and further increased plasma insulin levels, compared with either **10** or the DPP-4 inhibitor alone. Similar synergistic efficacy between SSTR5 antagonists and DPP-4 inhibitors were recently independently reported by researchers using healthy mice, DIO mice, ZDF rats, and several GDIS receptor KO mouse strains.<sup>12,22</sup>



**Figure 3.** Combination of **10** with DPP-4 inhibitor des-F-sitagliptin (DP4) (10+10 mpk) in HFD mouse OGTT studies. \* $P < 0.05$  vs veh; \*\* $P < 0.01$  vs veh; # $P < 0.001$  vs 10.

In summary, we have identified multiple new lead SSTR5 antagonists through systematic lead hopping efforts based on the existing literature compound **1**. Medicinal chemistry iteration and SAR optimization led to the discovery of azaspi-

rodecanones, a novel series of highly selective and potent SSTR5 antagonists with diverse structural features and minimal off-target activity profiles regarding hERG, PXR, and CYP inhibition. We demonstrated that the exemplar SSTR5 antagonist **10** significantly lowers glucose excursions in a dose-dependent manner in a rodent diabetic model without risk of hypoglycemia. The compound increased pancreatic insulin secretion as well as total and active GLP-1 release, and demonstrates synergistic effects in combination with DPP-4 inhibitors. These results indicate that selective SSTR5 antagonists could serve as therapeutic agents for the treatment of type 2 diabetes as either monotherapy or in combination with existing anti-diabetic drugs such as sitagliptin. Further optimization of this lead compound to achieve desirable physical chemical and pharmacokinetic properties are discussed in the following manuscript in this issue.

## ASSOCIATED CONTENT

### Supporting Information

Supporting information is available free of charge on the ACS Publications website at <http://pubs.acs.org>. Experimental procedures for the preparation of compounds **2-10**. SAR table of additional representative analogs. In vitro and in vivo assay protocols. SSTR5 WT/KO OGTT study results (PDF).

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### Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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## ABBREVIATIONS

SSTR5, somatostatin receptor subtype 5; SST, somatostatin; GLP-1, glucagon-like peptide 1; GI, gastrointestinal; KO, genetic knockout; GDIS, glucose-dependent insulin secretion; WT, genetic wild-type; HFD, high fat diet; OGTT, oral glucose tolerance test; T2DM, type 2 diabetes mellitus; AUC, area under the curve; DPP-4, dipeptidyl peptidase 4; SGLT2, sodium-glucose co-transporter 2; PK, pharmacokinetic; mpk, milligram per kilogram; SAR, structure activity relationship; CHO, Chinese hamster ovary.

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## Discovery and pharmacology of a novel somatostatin subtype 5 (SSTR5) antagonist: Synergy with DPP-4 inhibition

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