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Letter

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Stimulation of Glucose-Dependent Insulin Secretion by a Potent, Selective sst_3 Antagonist

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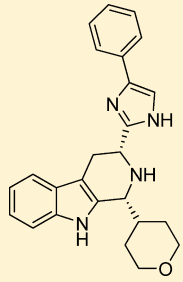
Supporting Information

ABSTRACT: This letter provides the first pharmacological proof of principle that the sst_3 receptor mediates glucose-stimulated insulin secretion (GSIS) from pancreatic β-cells. To enable these studies, we identified the selective sst_3 antagonist (1*R*,3*R*)-3-(5-phenyl-1*H*-imidazol-2-yl)-1-(tetrahydro-2*H*-pyran-4-yl)-2,3,4,9-tetrahydro-1*H*-β-carboline (**5a**), with improved ion channel selectivity and mouse pharmacokinetic properties as compared to previously described tetrahydro-β-carboline imidazole sst3 antagonists. We demonstrated that compound **5a** enhances GSIS in pancreatic β-cells and blocks glucose excursion induced by dextrose challenge in ipGTT and OGTT models in mice. Finally, we provided strong evidence that these effects are mechanism-based in an ipGTT study, showing reduction of glucose excursion in wild-type but not sst_3 knockout mice. Thus, we have shown that antagonism of sst_3 represents a new mechanism with potential in treating type 2 diabetes mellitus.



KEYWORDS: *somatostatin, type 2 diabetes, sst_3, glucose-stimulated insulin secretion*

Type 2 diabetes mellitus (T2DM) accounts for 90−95% of clinical diabetes and represents a major and growing health threat throughout the world. The overall estimated worldwide prevalence of diabetes in 2000 was 2.8% (171 million people), with that number projected to increase to 4.4% (366 million people) by 2030.[1] Within the United States, a 2001 estimate of the prevalence of diagnosed diabetes cases was even greater at 7.9% of the population (16.7 million people), with an even larger number of sufferers believed to be undiagnosed.[1] Because of the trend of increasing rates of obesity in the United States and developing world, the vast majority of newly diagnosed cases of diabetes are anticipated to fall within T2DM spectrum.[2] Diabetes mellitus carries a tremendous human suffering and economic burden as a consequence of the associated chronic complications (blindness, end-stage renal disease, limb amputations, heart disease, stroke, etc.) and increased morbidity and mortality. Unfortunately, despite numerous medication options for the treatment of T2DM, most patients fail to achieve long-term glycemic control, and new treatment options are urgently needed.[3]

A decline in glucose-stimulated insulin secretion (GSIS) from pancreatic β-cells is one of the primary defects associated with the development and progression of T2DM. Agents such as sulfonyl ureas and related K-ATP channel blockers that increase insulin secretion from β-cells are efficacious in lowering glucose, but because their effects are continuous and not dependent on plasma glucose levels, they carry the risk of evoking hypoglycemia and accelerating the loss of islet function. As such, identification of novel targets for stimulating GSIS has been a major strategy in the quest for new agents to treat T2DM;[4,5] DPP-4 inhibitors such as sitagliptin represent one recently introduced class of such agents. As part of our continuing efforts to identify novel GSIS targets, Zhou and coworkers recently determined that the somatostatin receptor subtype 3 (sst_3) is highly expressed in β-cells of human and rodent islets, and knocking down expression of sst_3 using siRNA technology enhances GSIS in INS-1 cells (a rat insulinoma cell line).[6] Somatostatin (somatotropin release-inhibiting factor, SRIF) is a cyclic tetradecapeptide existing in two biologically active forms, SRIF-14 and N-terminal extended SRIF-28, that acts as a neurotransmitter and hormone with broad inhibitory effects on both endocrine (growth hormone, insulin, glucagon, gastrin, cholecystokinin, etc.) and exocrine (gastric acid, pancreatic enzymes, etc.) secretion. These effects are mediated through interactions with five G-protein-coupled somatostatin receptor subtypes (sst_1, sst_2, sst_3, sst_4, and sst_5).[7] Of the five somatostatin receptor subtypes, the specific physiological functions of the individual receptor subtypes are best understood for sst_2 and sst_5.[8] In contrast to sst_2 and sst_5, relatively little is known about the function of sst_3. Expression of sst_3 has been described in brain, pituitary, stomach, pancreas, thymus, thyroid, prostate, vascular endothelial cells, and various human tumors. The sst_3 receptor has been linked to apoptotic signaling and proliferation and has been proposed as a possible target for cancer therapy.[9−11] To our knowledge, a role for the sst_3 receptor in regulating GSIS of pancreatic β-cells had not

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been previously described prior to this report. This letter describes the development of an sst_3 antagonist with suitable somatostatin receptor subtype and off target selectivity and rodent pharmacokinetic properties, to provide in vivo proof of principal that antagonism of sst_3 represents a viable novel strategy for stimulation of GSIS and, possibly, for treatment of T2DM.

Relatively few small molecule nonpeptide sst_3 antagonists have been described in the literature.[12] Several examples are shown in Figure 1. Compound **1** was described by Novartis scientists as a potent, selective silent competitive sst_3 antagonist (human sst_3 binding affinity <10 nM, selectivity against human sst_1, sst_2, sst_5 all >150-fold; selectivity against sst_4 21-fold; cAMP functional antagonist potency pK_B = 7.88).[13,14] Tetrahydro-β-carboline imidazole compounds **2** and **3** were described by Poitout, Thurieau, and co-workers at the Institut Henri Beaufour as having high binding affinities for sst_3 (0.64 and 1.7 nM, respectively), both with >1000-fold selectivity over the other somatostatin receptor subtypes.[15] In functional assays, these compounds behaved as competitive antagonists of sst_3. Novartis scientists described closely related β-carboline imidazole sst_3 antagonists such as **4** (sst_3 human pK_d 8.69, >400-fold selective against other receptor subtypes) in a recently issued patent, which claims these compounds as potential treatments for depression, anxiety, bipolar disorder, and cancer.[16] Unfortunately, in our hands, compounds **2**–**4** had poor pharmacokinetic properties in mice with high clearance rates (126, 95, and 31 mL/min/kg, respectively) and poor plasma exposures following oral dosing. This limited the usefulness of these compounds as tools for in vivo pharmacological assessment of sst_3 as a potential new GSIS target. Moreover, while not reported for these compounds, based on internal assays, compounds **2**–**4** had high affinities for several cardiac ion channels including the hERG channel (binding IC_{50} = 150, 77, and 176 nM, respectively) and/or the L type Ca^{2+} channel (binding IC_{50} = 128, 308, and 1526 nM, respectively). We chose to explore modifications of compounds related to **2**–**4** in an effort to identify novel analogs with improved pharmacokinetic and off-target profiles. On the basis of the slight improvement in Ca^{2+} channel selectivity observed upon incorporation of oxygen atoms into the *n*-butyl groups of **2** leading to **4**, we decided to combine features of compounds **3** and **4** by making 1-heterocycle substituted β-carboline analogues (**5**), a significant divergence from the original structure of the series reported in Novartis and Institut Henri Beaufour patents.

Analogues were readily prepared according to Scheme 1. Boc-D-Trp was treated with bromoacetophenone in the presence of cesium carbonate to provide ester **6**. The addition of toluene and ammonium acetate and removal of water via Dean–Stark trap at reflux afforded imidazole **7**. Removal of the Boc group (TFA) was followed by Pictet–Spengler cyclization with commercially available or routinely prepared aldehydes. Cyclization generally led to predominantly one isomeric product. Poitout and Thurieau observed the same for compound **3** and assumed that the major isomer had the *trans* stereochemistry; however, they did not offer any evidence to support this assignment.[15] On the basis of a strong NOE in the 1D NOE NMR spectrum between the two methine protons in major isomer **5a**, we have established that the relative stereochemistry must be *cis* instead. A second NOE study with a related analogue (not shown) also demonstrated that the major isomer is *cis*, giving us added confidence that, for these

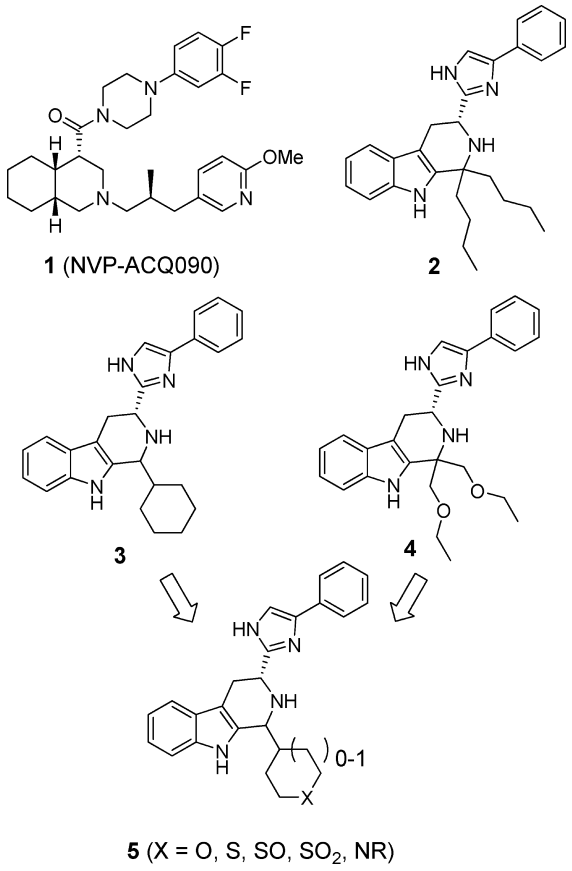
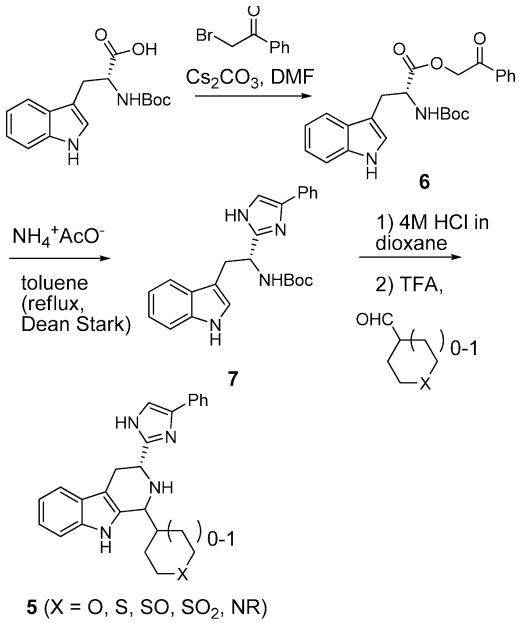


Figure 1. Previously described sst_3 selective antagonists.

Scheme 1. Synthesis of β-Carboline Analogues 5



analogues, the Pictet–Spengler reaction produces predominantly the *cis* isomers. In some cases, further elaboration was performed under routine conditions (for example, when the 1-substituent was Boc-piperidine, the Boc was removed, and further acylation or sulfonylation could be performed).

Compounds **5a**–**n** were evaluated in a sst_3 binding assay,[17] a cAMP assay measuring sst_3 functional antagonism potency

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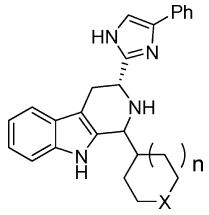
Table 1. Human sst_3 Binding and Functional Data and hERG Binding Data for 3 and 5a−n[a]



analogue (*n* is 1 except as noted)	X	IC_{50} (nM)		
		sst_3 binding	sst_3 cAMP	hERG binding[b]
3	CH_2	0.8 (5; SD 0.3)	26 (2)	77 (2)
5a (*cis*)	O	14 (425; SD 4.6)	22 (417; SD 17)	380 (2)
5b (*trans*)	O	390 (2)	1200 (1)	730 (2)
5c, *n* is 0 isomer 1	O	45 (2)	19 (1)	64 (2)
5d, *n* is 0 isomer 2	O	63 (2)	30 (1)	25 (2)
5e	S	1.7 (1)	10 (1)	120 (2)
5f isomer 1	SO	220 (1)	380 (2)	
5g isomer 2	SO	21 (1)	24 (2)	100 (2)
5h	SO_2	120 (2)	290 (2)	100 (2)
5i	NBoc	7 (2)	1000 (2)	
5j	NH	63 (1)	220 (2)	5100 (2)
5k	NAc	16 (2)	46 (2)	9800 (1)
5l	NSO_2Me	44 (2)		3500 (2)
5m	NCO_2Me	4 (2)	66 (2)	700 (2)
5n	NCONHMe	35 (2)	180 (2)	240 (2)

[a]Numbers in parentheses represent numbers of determinations and standard deviation (SD), when calculable. [b]hERG binding data were obtained by measuring displacement of ^{35}S-MK499 from HEK-293 cells stably expressing hERG.[19]

Table 2. Pharmacokinetic Properties of Compounds 3, 4, and 5a in C57BL/6N Mice[a]

no.	*F* %	AUCN po (μM h/mg)	C_{max} po (nM)	Vd_{ss} (L/kg)	Cl (mL/min/kg)	$t_{1/2}$ (h)
3	15	0.07	60	8.1	95	0.14
4	4	0.06	130	0.7	31	0.54
5a	45	1.3	840	1.6	15	1.6

[a]Mouse compound exposures were analyzed in blood; iv and po doses were 1 and 2 mg/kg, respectively.

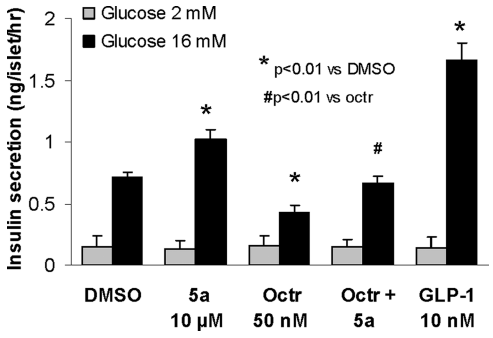


Figure 2. Effect of compound **5a** (10 μM) on GSIS in pancreatic β-cells from normal C57BL/6J mice (*n* = 4).

against SS-14,[18] and a hERG binding assay.[19] The resulting IC_{50} values for each assay are displayed in Table 1. Replacement of the cyclohexyl moiety in **3** with a variety of saturated heterocycles led, in general, to loss of binding potency (with the exception of compound **5e**, which had similar binding potency). Despite this, a number of analogues retained equivalent or better potency as compared to **3** in the assay measuring functional antagonism activity (analogues **5a**, **5c**, **5d**, **5e**, **5g**, and **5k**). Generally, the directional changes in sst3 binding affinity and functional potencies for each analogue track together, but that is not the case for all molecules. Structural changes among the series may affect ligand−receptor on-rates, off-rates, or both and, as such, can have differential effects on the binding affinities and functional antagonism potencies determined in the two different assay formats, wherein the binding assay is a competition readout at steady-state equilibrium, while the cAMP assay is an accumulation challenge assay following prebinding of the test molecule. Installation of a heterocycle, in some cases, also led to a modest to substantial reduction in binding affinity for the hERG channel (analogs **5a**, **5j**, **5k**, **5l**, and **5m**), demonstrating the feasibility of improving selectivity over hERG in the β-carboline sst_3 antagonist class through incorporation of heteroatoms into this fragment. As indicated above, Pictet−Spengler cyclization led to predominantly one stereoisomer product, which in the case of compound **5a**, we have established to be *cis*. Comparison of the potency of the isomerically pure *cis* (**5a**) and *trans* (**5b**) isomers indicated that the major *cis* isomer is more potent in both the sst_3 binding and the functional assays. On the basis of the comparable functional potency and 5-fold weaker hERG binding affinity (hERG IC_{50} = 380 nM) of compound **5a** as compared to **3**, we decided to further profile it. Compound **5a** was found to have good selectivity against the other human somatostatin receptor subtypes in binding assays (sst_1, sst_2, sst_4, and sst_5 IC_{50} values all >1 μM). Because our pharmacodynamic model was established in mice, we evaluated compound **5a** in the mouse sst_3 binding and cAMP-based functional assays (sst_3 binding IC_{50} = 8.9 nM, sst_3 cAMP IC_{50} = 16 nM), where it demonstrated equivalent potency to that observed on the human receptor. When screened against other mouse somatostatin receptor subtypes, it maintained good sst_3

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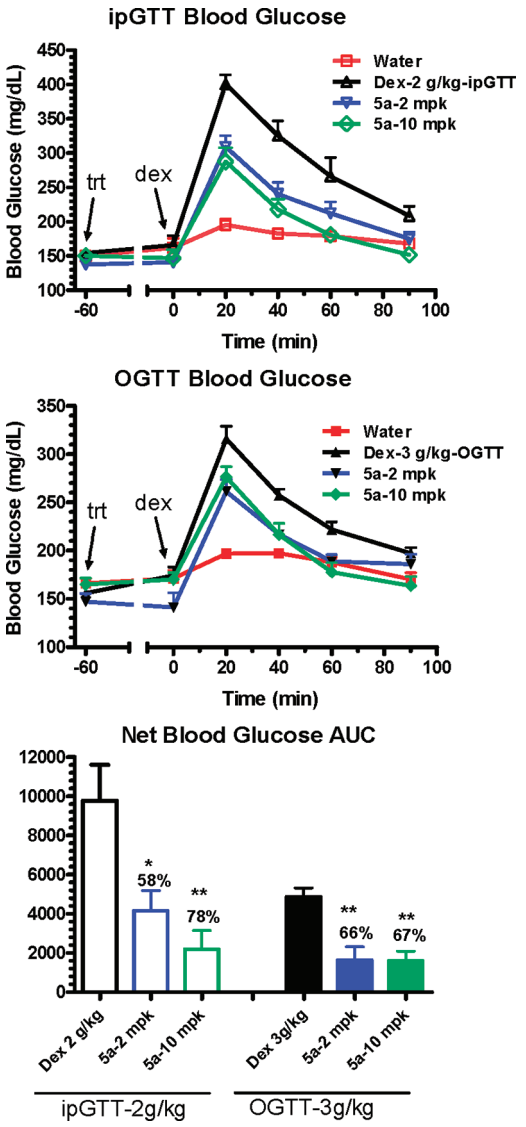

Figure 3. Evaluation of 2 and 10 mg/kg doses of compound **5a** in ipGTT and OGTT tests in C57BL/6N Tac mice following dextrose (dex) challenge. *p < 0.05, **p < 0.005 vs dextrose alone. Treatment = trt.

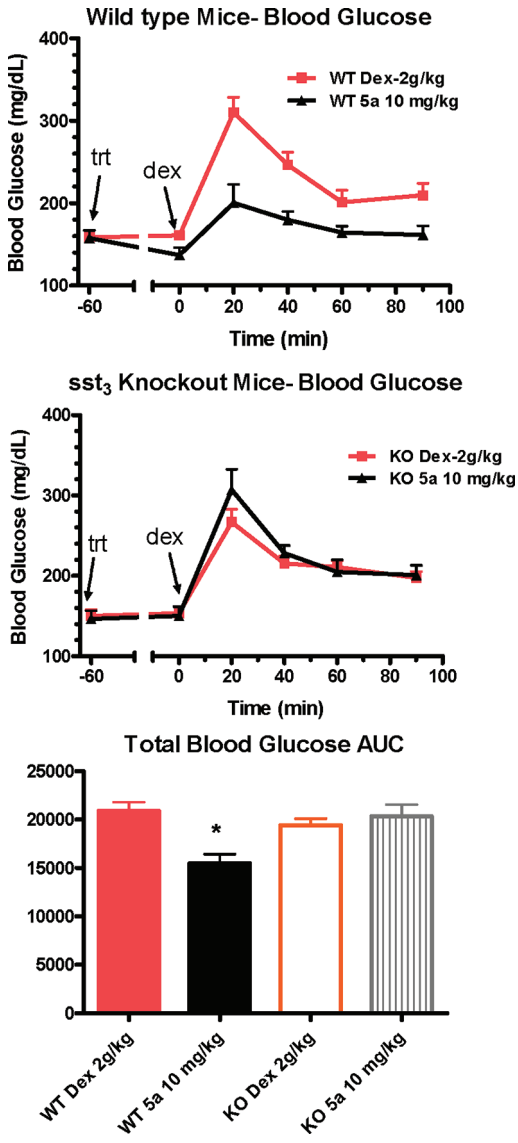

Figure 4. Evaluation of compound **5a** (10 mg/kg) in ipGTT studies in wild-type and sst_3 knockout mice challenged with 2 g/kg dextrose (dex). *p < 0.05 vs dextrose alone. Treatment = trt.

subtype selectivity (IC_{50} $msst_1$, $msst_2$, $msst_4$, and $msst_5$ all >10 μM). It demonstrated weaker potency as compared to compound **3** in the L type Ca^{2+} channel binding assay (Ca^{2+} IC_{50} = 1.9 μM). In a pharmacokinetic study in mice, compound **5a** had a reduced clearance rate, increased half-life, higher oral bioavailability, and significantly higher plasma exposures (po) as compared to parent compounds **3** and **4** (Table 2).

Compound **5a** was used to evaluate the role of sst_3 in GSIS from the β-cell (Figure 2). In this assay,[17] the effects of **5a** at 10 μM, octreotide (octr, a peptide somatostatin agonist at 50 nM), a combination of **5a** (10 μM) and octreotide at 50 nM, and negative (DMSO) and positive controls [glucagon-like peptide-1 (GLP-1) at 10 nM] on pancreatic islets isolated from normal C57BL/6J mice were determined in the presence of 2 and 16 mM glucose.[20] As shown, **5a** significantly enhances GSIS (at 16 mM glucose) relative to DMSO control (44% increase). On the other hand, it did not affect the insulin secretion at basal (2 mM) glucose in this assay. This result provides evidence that the sst_3 antagonist only enhances GSIS, not basal insulin secretion. GSIS is decreased significantly (by 39%) in the presence of the somatostatin agonist octreotide; however, **5a** (at 10 μM) can partially overcome the effect of 50 nM octreotide on GSIS. GLP-1 serving as the positive control (at 10 nM) elevates GSIS as expected.

On the basis of the favorable mouse in vitro sst_3 potency, the pharmacokinetic profile, and the effect on GSIS in isolated mouse islets of compound **5a**, we next sought to evaluate its ability to improve glucose tolerance in ip and po glucose tolerance test models (ipGTT and OGTT, respectively) in lean C57BL/6N Tac mice fasted for 5−6 h before dextrose challenge.[21] Figure 3 shows that dextrose challenge causes blood glucose excursion, which in both studies is ameliorated in a dose-dependent fashion by predosing (1 h prior to challenge) with compound **5a** (2 and 10 mg/kg). To demonstrate that these effects are mechanism-based, we evaluated compound **5a** in an ipGTT study simultaneously comparing effects in wild-type and sst_3 knockout mice. As shown in Figure 4, compound **5a** reduced glucose excursion resulting from dextrose challenge in the wild-type mice (26% reduction in total blood glucose

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AUC) but had no significant effect on glucose levels following dextrose challenge in sst_3 knockout mice. Blood insulin levels were not monitored in this and other typical GTT studies to avoid excessive stress to the animals and impacting glucose readout. However, in a parallel study in wild-type and knockout mice predosed at −60 min with 10 mg/kg of **5a**, a 50% elevation in plasma insulin levels was observed 10 min postdextrose challenge in wild-type mice with no effect on the knockout mice; however, because of variability, this effect did not reach statistical significance ($p = 0.12$, $n = 5$).

In summary, we have recently shown for the first time that the sst_3 receptor mediates GSIS from pancreatic β-cells. To provide pharmacological proof of principle for this observation, we developed β-carboline imidazole-based selective sst_3 antagonist **5a**, with improved ion channel selectivity and mouse pharmacokinetic characteristics as compared to previously described structurally related antagonists **2**−**4**. We demonstrated that compound **5a** enhances GSIS in pancreatic β-cells and blocks glucose excursion induced by dextrose challenge in ipGTT and OGTT models in mice. Moreover, we provided strong evidence that these effects are mechanism-based in an ipGTT study showing blockade of glucose excursion in wild-type but not sst_3 knockout mice. Thus, we have shown that antagonism of sst_3 represents a new GSIS target with potential in treating T2DM.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details for the synthesis and characterization of sst_3 antagonist **5a**, in vitro assays, GSIS assay with pancreatic islets, and GTTs. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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