Bioorganic & Medicinal Chemistry Letters xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Evaluation of anti-diabetic effect and gall bladder function with 2-thio-5-thiomethyl substituted imidazoles as TGR5 receptor agonists

Xuqing Zhang *, Zhihua Sui, Jack Kauffman, Cuifen Hou, Cailin Chen, Fuyong Du, Thomas Kirchner, Yin Liang, Dana Johnson, William V. Murray, Keith Demarest

Cardiovascular and Metabolic Research, Janssen Research & Development, LLC, Welsh & McKean Roads, Box 776, Spring House, PA 19477, United States

ARTICLE INFO

Article history:
Received 15 December 2016
Revised 23 February 2017
Accepted 26 February 2017
Available online xxxx

Keywords:
TGR5
Imidazole
OGTT
Gall bladder

ABSTRACT

A novel series of 2-thio-5-thiomethyl substituted imidazoles was discovered to be potent TGR5 agonists that possessed glucose-lowering effects while inhibiting gall bladder emptying in mice.

Takeda G-protein-coupled receptor 5 (TGR5), also known as GPR 131, GPBAR1, or M-BAR is G protein-coupled receptor activated by bile acids (BAs).[1] It is broadly expressed in human tissues, such as the GI tract, gall bladder, spleen, lung, brown adipose tissue and placenta.[2–4] Bile acids induce glucagon like peptide-1 (GLP-1) secretion from primary intestinal enteroendocrine cells by signal transduction through G_s protein-mediated cyclic adenosine monophosphate (cAMP) accumulation via TGR5. The process could regulate homeostasis of blood glucose through a variety of mechanism including promoting glucose-induced insulin secretion, suppressing glucagon release, delaying gastric emptying, promoting satiety, and increasing glucose disposal in the peripheral tissues.[5–8] Moreover, activation of TGR5 in brown adipose tissue has been proposed to increase energy expenditure through the induction of type 2 iodothyronine deiodinase (D2). Therefore, TGR5 activation provides a promising strategy for treatment of type 2 diabetes mellitus and associated metabolic disorders. In particular, a potent and selective TGR5 agonist may be beneficial for the treatment of type 2 diabetes and obesity.

There have been several reports in the literature of both selective steroidal TGR5 agonists and synthetic, non-steroidal TGR5 agonists.[9–23] While both chemo-types exhibited potent TGR5 agonist activity in cell-based functional assays and were orally efficacious in lowering glucose levels in rodents, some adverse side effects, of which mostly in gall bladder, have been observed through activation of the TGR5 receptor in the epithelium of gall bladder by administration of either steroidal or non-steroidal TGR5 agonists. To alleviate or eliminate the strong side effect on the gall bladder, increasing efforts have been made to develop gut-restricted TGR5 agonists specifically targeting the GI tract while avoiding systemic exposure.[22,23] Therefore, GI restricted TGR5 agonists are a potential anti-diabetes mellitus strategy to target the intestine locally to minimize this unwanted side effect. However, it is still highly challenging to pursue adequate anti-diabetic effect by solely stimulating the production of GLP-1 secretion from the gut without all other benefits on increasing energy homeostasis, enhancing insulin sensitivity and improving glucose tolerance through systemic exposure approach.

Herein, we report our effort to develop novel and selective TGR5 agonists for the treatment of metabolic diseases.[24,25] The goal of the program was to identify an orally efficacious TGR5 agonist from this series to enable evaluation on both anti-diabetic effect and gall bladder function in disease-relevant preclinical models. Examination of non-steroidal TGR5 agonist chemotypes in the literature[9–15] led us to postulate that a common pharmacophore **1** may participate in ligand-receptor binding (Fig. 1). This prompted us to synthesize a few small chemical libraries based on template 1 by modifying the core heteroaryl ring as well as the linkers A–E for preliminary screening. A hit scaffold **2** was discovered to display moderate TGR5 agonistic activity in murine enteroendocrine cells (STC-1 cells) expressing TGR5. To further explore **2**, we then conducted detail SAR studies to improve TGR5 activity in STC-1 cells.

* Corresponding author.
E-mail address: xzhang5@its.jnj.com (X. Zhang).

http://dx.doi.org/10.1016/j.bmcl.2017.02.069
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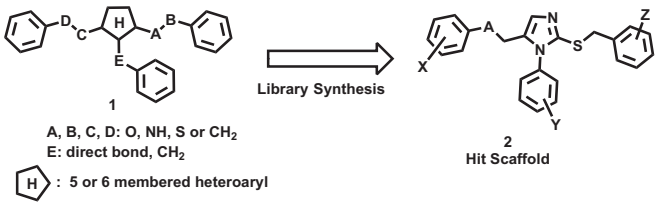
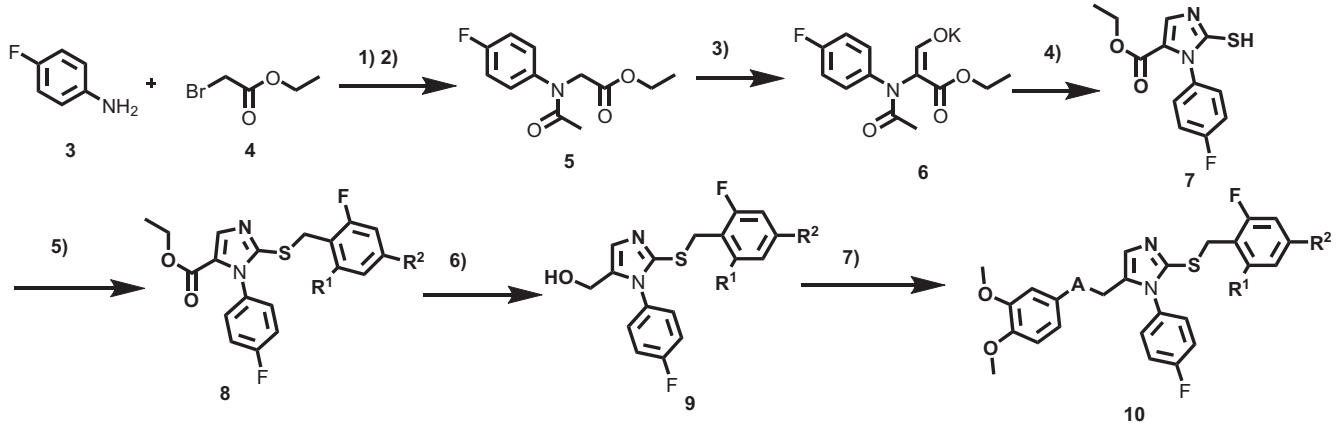


Fig. 1. Pharmacophore-based design of novel TGR5 agonists.

To explore SAR on this scaffold, an efficient synthesis was developed and utilized (Scheme 1). Aniline **3** was coupled with ethyl 2-bromoacetate **4** under weakly basic conditions followed by acylation of the aniline adduct to give ethyl *N*-acetyl-*N*-(4-fluorophenyl)glycinate **5** in 68% yield over two steps. Intermediate **5** was then treated with potassium ethoxide and ethyl formate to afford the adduct **6** in 65% yield, this underwent acid catalyzed cyclization by the treatment with KSCN to yield imidazole **7** in 71% yield. Alkylations of thiol **7** with various benzyl halides or mesylates in the presence of potassium carbonate followed by DIBAL reduction gave the corresponding imidazolylmethanols **9**. Compounds **9** were coupled with 3,4-di-methoxy-phenol, 3,4-di-methoxy-thiophenol or 3,4-dimethoxy-aniline via the corresponding mesylates to afford the target compounds **10**.

We began our SAR exploration on the left side of the structure (Table 1). Consistent with the SAR of a structurally similar series discovered in our group earlier,[24] modification on the left side aromatic ring identified 3,4-di-methoxy as optimal for TGR5 agonistic activity (data not shown). Polar groups (A) on the linker were poorly tolerated while lipophilic S containing linker displayed potent TGR5 agonistic activity. As evidenced by **11** with EC_{50} of 160 nM in STC-1 cells and EC_{50} of 5.68 μM in NCI H716 cells, increasing polarity by replacing the S with a O or a NH group resulted in dramatic loss or abolishment on TGR5 agonistic potency (EC_{50} of 5.68 μM for **12** and EC_{50} of >50 μM for **13** in STC-1 cells). For the *in vitro* potency evaluation, both **11** and **12** showed a preference for mouse TGR5 in STC-1 cells over human TGR5 in NCI H76 cells. One potential reason is due to low amino acid sequence identity of the two receptors.[3] However, expression level of TGR5 in different cell lines would also play a critical role on the potency evaluation. It is possible that rather lower expression of human TGR5 attributed to the lower human TGR5 potency observed in NCI-716 cells. Masking of the polar and basic free amino group of **13** with a formyl group revived TGR5 agonistic activity with EC_{50} returning to sub μM range in STC-1 cells for **14**. Unfortunately, introduction of a slightly bigger acetyl substitution on the basic aniline abolished TGR5 activity as evidenced by **15** with EC_{50} >50 μM in both STC-1 and NCI-716 cells. We then shifted our effort on the right-side of the structure. In contrast to the low tolerance for modification at the left-side linker, 4-substitution on the right-side phenyl ring was tolerated for modification as illustrated by 4-CN substituted phenyl analogue **16** with EC_{50} of 290 nM in STC-1 cells. Furthermore, incorporation of more polar carboxylic acid (**17**) or tetrazole group (**19**) still maintained low μM of EC_{50} in STC-1 cells as compared to **16**, providing an efficient path to improve compound solubility with maintaining moderate *in vitro* TGR5 agonistic potency. Unfortunately, further increasing polarity by oxidation of the 2-S group on the imidazole ring into sulfoxide resulted in 6–7-fold of drop in TGR5 agonistic potency in STC-1 cells (EC_{50} of 44.9 μM in STC-cells for **18**), suggesting polarity was not tolerated on the central imidazole ring. Given the moderate TGR5 potency achieved by the right-side phenyl ring substituted with a polar group such as carboxylic acid or tetrazole at the 4-position, other polar amide and sulfonamide analogues **20**–**26** were explored. To our delight, compared with the carboxylic acid analogue **17**, the corresponding amides with terminal sulfonic acid (**20**), terminal quaternary ammonium salt (**21**) or D-arginine group displayed 2–15-fold EC_{50} boost in STC-1 cells. While significantly less potent in STC-1 cells, **21** was interesting since its quaternary ammonium salt structure could limit its intestinal permeability. Such a GI restricted agent for targeting the intestine locally might minimize unwanted side effect on the gall bladder. We then introduced a more polar sulfonamide moiety into the side chain on the phenyl ring. As expected, **23** bearing a small sulfonamide group maintained good TGR5 agonistic potency with EC_{50} of 590 nM in STC-1 cells. However, combination of polar sulfonamide and quaternary ammonium salt group into the side chain failed to maintain good TGR5 agonistic potency in both STC-1 and NCI H716 cells. As exemplified by **24** and **25**, substitution of one quaternary ammonium salt group dramatically reduced TGR5 agonistic potency (**24**, EC_{50} of 9.36 μM; **25**, EC_{50} of 11.75 μM) in STC-1 cells. Furthermore, a bis quaternary ammonium salt group on the side chain abolished activity (**26**). To evaluate both anti-diabetic effects and gall bladder function, **19** was selected as the tool compound for *in vivo* studies to help us decide whether a TGR5 agonist devoid of undesired gall bladder effects could be achieved.

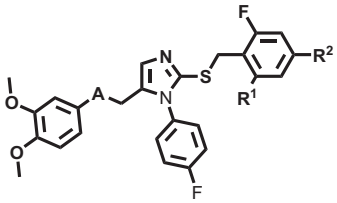
Compound **19** was evaluated for its *in vivo* glucose lowering activity with an oral glucose tolerance test (OGTT) in C57 BL/6 mice



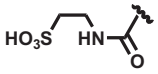
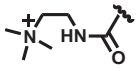
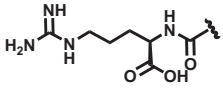
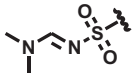
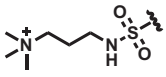
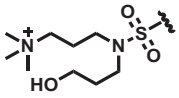
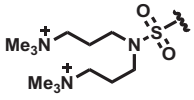
Scheme 1. Reagents and conditions: 1) AcONa, EtOH, 2 h, 80 °C (80%); 2) AcCl, TEA, 3 h, 0–30 °C (85%); 3) EtOK, HCO_2Et, toluene, overnight, 30 °C (65%); 4) KSCN, conc. HCl, 2 h, 90 °C (71%); 5) K_2CO_3, acetone, 1 h, 30 °C (75–92%); 6) DIBAL, 2 h, 0–30 °C (45–70%); 7) $SOCl_2$, overnight, 0–25 °C then Cs_2CO_3, 3,4-di-MeO-Ph-AH (A = O, S or NH), 2 h, 25 °C (50–75%).

Please cite this article in press as: Zhang X., et al. Bioorg. Med. Chem. Lett. (2017), http://dx.doi.org/10.1016/j.bmcl.2017.02.069

Table 1
SAR of R^1, R^2 and A of 2-benzylthio substituted imidazoles **10**.



10

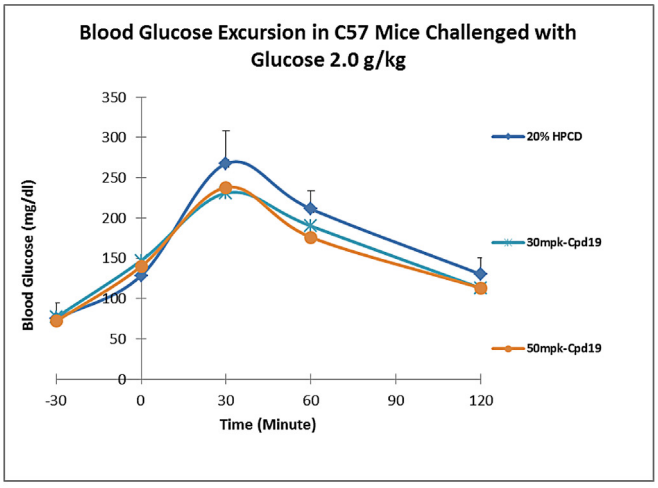
ID	R^1	R^2	A	STC-1 (mouse)[a] EC_{50} (μM)	NCI H716 (human)[b] EC_{50} (μM)
11	Cl	H	S	0.16	5.68
12	Cl	H	O	5.68	17.8
13	Cl	H	NH	>50	>50
14	Cl	H	NCHO	0.99	21.75
15	Cl	H	NAc	>50	>50
16	F	CN	S	0.29	4.94
17	F	CO_2H	S	6.08	>50
18	F	CO_2H	SO	44.9	>50
19	F		S	2.06	42.62
20	F		S	0.38	8.26
21	F		S	2.3	>50
22	F		S	0.76	36.8
23	F		S	0.59	16.63
24	F		S	9.36	29.55
25	F		S	11.75	20.75
26	F		S	>50	>50

[a] STC-1: murine enteroendocrine cell lines expressing TGR5.
[b] NCI-H716: human enteroendocrine cell lines expressing TGR5.

(Fig. 2). The area under the curve $AUC_{0-120\ min}$ (absolute $AUC_{0-120\ min}$) for glucose levels versus time were calculated after oral administrations of **19** at 30 mg/kg and 50 mg/kg in 20% 2-hydroxypropyl-β-cyclodextrin. It was found to cause 9.8% and 12.0% reduction in blood glucose absolute $AUC_{0-120\ min}$ at 30 mg/kg and 50 mg/kg respectively compared with the vehicle control group (Fig. 2). A pharmacokinetic profile *in vivo* revealed the absorption of **19**. The plasma drug exposure was measured as 1.18 μM 45 min post oral dose at 50 mg/kg. While moderately absorbed,[26] **19** displayed an acute glucose-lowering effect and improved glucose tolerance in C57 BL/6 mice induced by a relatively high drug dose. In the context of reported TGR5 agonist driven gall bladder toxicity via systemic administration, peripherally biased **19** was selected for further evaluation of its gall bladder filling effect in multiple rodent models.

First, **19** was evaluated for its ability to inhibit on the egg yolk-induced reduction of gall bladder size in CD-1 mice (Fig. 3).[27] The mice were dosed orally at 5 ml/kg 20% HPBCD or a suspension of **19** in the same vehicle at 50 mg/kg. One hour later, saline or 30% egg yolk suspension was orally administrated at 20 ml/kg. Gall bladder emptying was assessed by comparing gall bladder weights from control and egg yolk administered mice. It was clear that contraction and emptying of the gall bladder was induced by egg yolk compared with the vehicle/saline group. Pleasingly, **19** at 50 mg/kg did not block the egg yolk-induced gall bladder emptying effect at 15 min. It exhibited relatively low systemic exposure in CD-1 mice plasma, with 0.02 μM at 1.25 h, which might explain that it did not significantly inhibit the gall bladder emptying effect.

Next, we sought to further explore the effect of TGR5 agonist **19** on egg yolk-induced gall bladder emptying in fasted C57 BL/6 mice



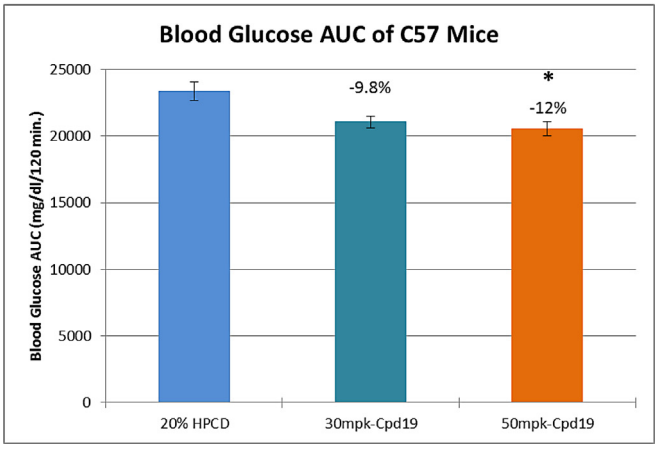
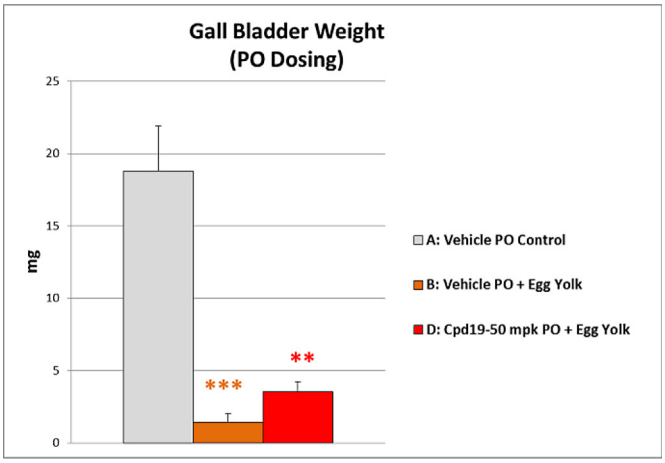


Fig. 2. Compound **19** reduced AUC of blood glucose in C57 BL/6 mice (OGTT): One-way ANOVA Analysis ($^{*}P < 0.05$ vs Vehicle).



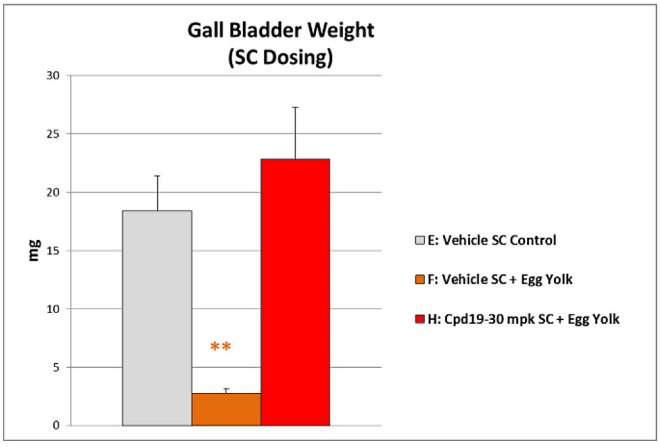


Fig. 4. Effect of **19** on gall bladder function in fasted C57 BL/6 mice, 50 mg/kg po vs. 30 mg/kg sc: On-way ANOVA Analysis, $^{**}P < 0.01$ vs Vehicle control; $^{***}P < 0.001$ vs Vehicle control.

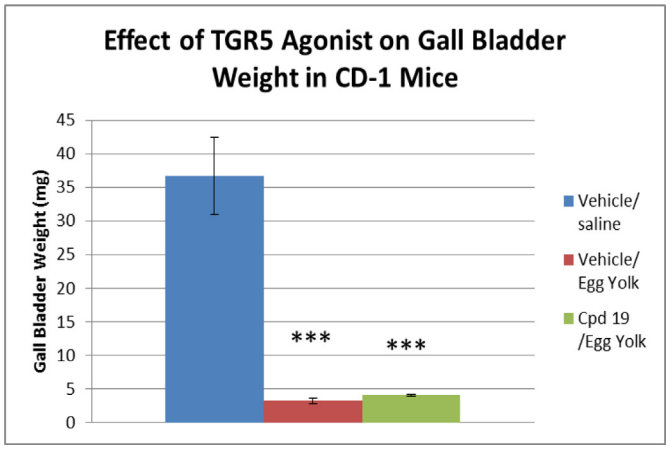
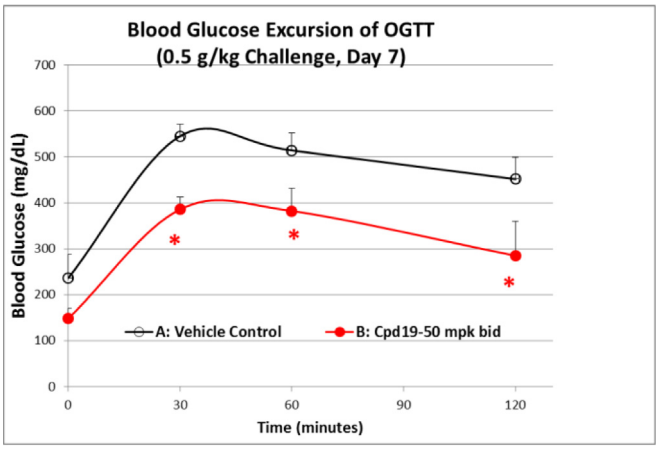


Fig. 3. Effects of **19** on gall bladder weight in CD-1 mice at 50 mg/kg PO: One-way ANOVA-Dunnett's Analysis $^{***}P < 0.001$ vs Vehicle/Saline group.

(Fig. 4). To investigate whether systemic exposure could affect the gall bladder emptying effect, we decided to run head-to-head comparison of gall bladder function between oral administration (PO) and subcutaneous injection (SC) of **19**. Fasted C57 mice received **19** at an oral dose of 50 mg/kg or subcutaneous injection of 30 mg/kg followed by oral administration of egg yolk 30 min later. Then the animals were sacrificed and the gall bladders were weighed 15 min later. When given 50 mg/kg PO, **19** had no significant effect on the gall bladder weight. However, **19** dramatically inhibited the gall bladder emptying when given 30 mg/kg subcutaneously compared to the vehicle control. The plasma exposure levels were also measured 45 min post dosing of **19**. It exhibited 15.8 μM of systemic exposure via subcutaneous injection compared with 1.03 μM via oral administration, suggesting that a gut restricted TGR5 agonist could maintain its therapeutic benefit while reducing the potential for toxic effects on gall bladder emptying.

Given its glucose-lowering effect without a significant gall bladder filling effect via oral administration in the acute models using C57 BL/6 mice, **19** was evaluated further in a 2 week chronic study using db/db mice. After twice a day oral dose (bid) of **19** (50 mg/kg) to db/db mice for one week, **19** decreased the fed blood glucose level prior to a fasted OGTT study on day 7 without a significant effect on the body weight. It also displayed significant glucose-lowering effect and improved glucose homeostasis in an OGTT study on day 7 (Fig. 5). However, two of eight mice in this study expired on day 11. Upon the necropsy after the study, it was found that **19** impaired the gall bladder function in the db/db mice. The gall bladder weight increased dramatically compared with that of the control group; moreover, the gall bladders turned dark red containing bile which was not visibly clear, indicating the severe gall bladder filling effect. This data convincingly demonstrated that **19** as a TGR5 agonist inhibited the gall bladder contraction and impaired the gall bladder emptying upon chronic oral administration. While compound **19** had low systemic exposure, it was still



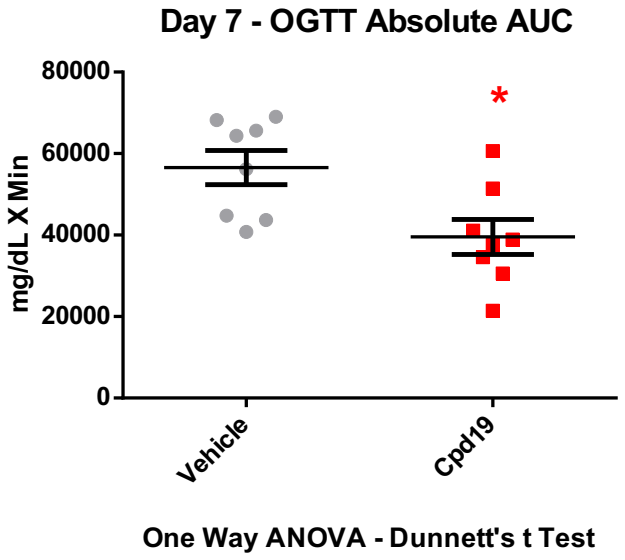


Fig. 5. Effects of **19** on glycemic control and gall bladder function in a 2 week study in db/db mice (50 mg/kg PO bid): One-way ANOVA analysis $^{*}P < 0.05$ vs Vehicle group.

sufficient to cause gall bladder filling at this dose/exposure probably due to the drug accumulation in the gall. Overall, the anti-diabetic benefit for a low absorbed TGR5 agonist likely could not be separated from concurrent pharmacology in the gallbladder.

In summary, we designed and synthesized a series of 2-thio-5-thiomethyl substituted imidazoles as TGR5 receptor agonists culminating in the identification of compound **19** as a suitable tool to assess *in vivo*. This molecule displayed moderate *in vitro* activity in both murine TGR5 expressing STC-1 cells and human TGR5 expressing NCI H716 cells. It was chosen as the tool compound for evaluation on both anti-diabetic effect and gall bladder function. Compound **19** with low oral exposure significantly reduced blood glucose excursion during an OGTT study in C57 BL/6 mice. It had no significant effect on egg yolk-induced gall bladder emptying over 15 min in CD-1 mice. Assessed by another acute study, **19** exhibited no significant effect on the gall bladder emptying at oral administration of 50 mg/kg but significantly inhibited the gall bladder emptying when given subcutaneously at 30 mg/kg. In a 2 week chronic study using db/db mice, **19** significantly improved glucose homeostasis in an OGTT study on day 7 but severely blocked the gallbladder emptying.

Acknowledgments

The authors thank ADME/PK and Lead Generation Biology teams at Janssen R&D for their technical assistance. The authors gratefully acknowledge Dr. James Lanter for scientific discussion and revising the paper.

A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2017.02.069.

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