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Discovery of MK-8282 as a Potent G-Protein-Coupled Receptor 119 Agonist for the Treatment of Type 2 Diabetes

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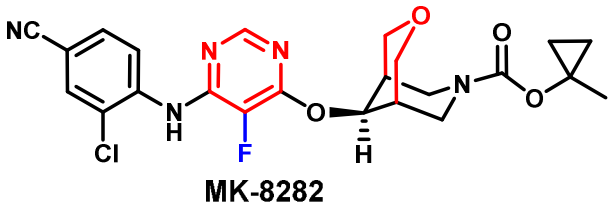
KEYWORDS: GPR119, Type 2 Diabetes, oGTT, sitagliptin.

ABSTRACT: The ever-growing prevalence of Type-2 diabetes in the world has necessitated an urgent need for multiple orally effective agents that can regulate glucose homeostasis with a concurrent reduction in body weight. G-Protein coupled receptor 119 (GPR119) is a GPCR target at which agonists have demonstrated glucose-dependent insulin secretion and shows beneficial effects on glycemic control. Herein, we describe our efforts leading to the identification of a potent, oral GPR-119 agonist, MK-8282, which shows improved glucose tolerance in multiple animal models and has excellent off-target profile. The key design elements in the compounds involved a combination of a fluoro-pryimidine and a conformationally constrained bridged piperidine to impart good potency and efficacy.

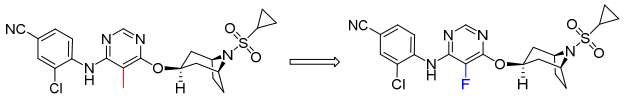


MK-8282

Diabetes Mellitus (DM) is a chronic metabolic disorder and in 2016 was reported as the 7th leading cause of death in the United States.[1] An estimated 422 million individuals were living with diabetes in 2014, a number which is expected to significantly increase by 2030.[2] Type 2 diabetes comprises 90% of people with diabetes around the world and is characterized by hepatic glucose overproduction, insulin resistance and insulin-producing pancreatic β-cell dysfunction. Multiple treatments aimed toward glycemic control are now available either as immunotherapies or combinations with hypoglycemia and weight-gain risks being the major limitations.[3] In this regard, several deorphanized G protein receptors (GPCRs) including GPR119, have attracted attention as potential targets for Type 2 Diabetes (T2D) to provide glucose-dependent insulin secretion.[4]

GPR119 is a class A (rhodopsin-like) GPCR which is primarily expressed in the pancreas, fetal liver and GI tract in humans.[5] Multiple synthetic agonists of GPR119 have been reported by us and others that show efficacy in rodent models of diabetes and obesity.[6-8] However, there have also been reports of GPR119 agonists that entered clinic and have been discontinued at phase 1 and 2 stages. Compounds from Arena, Prosidion (now Astellas) and GSK have all entered clinical trials and have been discontinued except Metabolex.[9-10]

Since the initial reports from Jones et al of an optimized GPR119 agonist, multiple pharmaceutical companies including us have prepared GPR119 agonists inspired by the original Arena compounds.[9] Herein, we will report our optimization efforts that led to the identification of the preclinical candidate MK-8282.



Compound **1**
hEC_{50} = 3 nM (96% max)
mEC_{50} = 135 nM (105% max)
mouse oGTT: 9% lowering @ 10 mpk
Poor exposures in rat / monkey
ClogP = 4.31

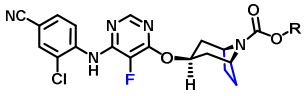
Compound **2**
hEC_{50} = 6 nM (83% max)
mEC_{50} = 29 nM (91% max)
mouse oGTT : 19% lowering @ 10mpk
Poor exposure in rats
ClogP = 3.7

Figure 1: Changing methyl (**1**) to fluoro (**2**) pyrimidine improves oGTT efficacy

Our interest in GPR119 agonists with bridged piperidines have previously been reported (Fig. 1).[7] Although these compounds had good in vitro activity in a cAMP assay, they did not show robust efficacy in our acute oGTT (oral glucose tolerance test) screen.[11] These compounds were highly lipophilic with poor solubility and were also characterized by poor plasma exposures (low AUC) in rat and rhesus. We hypothesized that if we could reduce the lipophilicity of compound **1,** by changing the methyl group in the pyrimidine core to fluorine, it would provide better physiochemical properties and may also improve the in vivo activity. As can be seen from Figure 1, compound **2** had a ClogP value of 3.7 compared to Compound **1** which had a ClogP of 4.31 (ClogP values were measured using ChemDraw). Though the plasma exposures in rat (AUC) of compound **2** did not significantly improve with

this change, we were very excited to see the dramatic improvement in glucose lowering, in the oGTT screening assay with the unbound exposures at the time of measurement (the oGTT values in Figure 1, Tables 1 and 2 were derived from a screen which measured glucose at a single 20 minute time point post glucose administration).[11] Encouraged by this fact, we did a quick SAR by changing the sulfonamide to carbamate in Compound **2** and looking at multiple carbamate variations as in Table 1. Unfortunately most of these carbamates also exhibited poor rat plasma exposures. However we were pleased to identify compound **4** which showed good potency and activity in oGTT at 3 mpk. Unlike other carbamates this compound showed measurable unbound plasma levels in rat PK studies albeit with very low concentrations.

Table 1: Comparison of carbmate SAR



R	Compound #	hEC_{50} (nM)	mEC_{50} (nM)	oGTT
	**3**	**2.6** % max = 81	**21** % max = 44	Inactive
	**4**	**3.5** % max = 79	**67** % max = 42	**17% lowering at 3mpk**
	**5**	**5.1** % max = 77	**42** % max = 18	Inactive
	**6**	**8** % max = 87	**46** % max = 29	Inactive

We reasoned that if we could make this bridge less lipophilic and convert the ethylene bridge on the piperidine on the right hand side to an oxa-bicyclo ring system, it could result in improved physiochemical properties that could translate into improved exposures (Figure 2). We hypothesized that as most of the functionalities in the core were maintained, the activity would not be affected. However, nearly all these compounds were much weaker in the in vitro assays, though it was gratifying to see that, as predicted, these compounds had excellent solubility and higher unbound fractions. The best from this class, compound **7,** is shown in Figure 2.[12] As can be seen the bridge modification reduces the lipophilicity ClogP by 1.3 units which translated into improved properties. Note also that unlike the ethylene bridged rings which had the anti-orientation with pyrimidine ethers we could only make the syn-version here as all our efforts to make the anti-compounds with this oxygen bridge failed. We believe the morpholine ring system (compound **7**) provides a steric constraint for reduction of the ketone from the same side and hence only the syn alcohol is obtained.

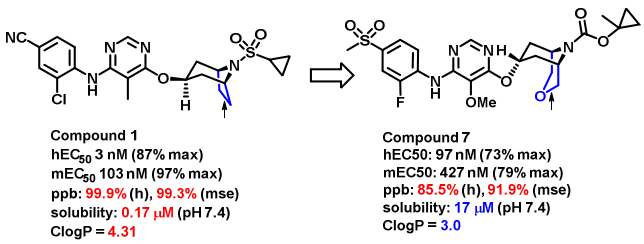
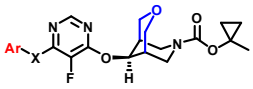


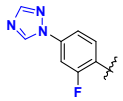
Figure 2. Modification of the bridge improves solubility

Generally, tying up the piperidine by introducing a conformational lock could work favorably, if it is on the bioactive conformation. We wondered if the bridge in Compound **7** is at the ideal position, based on the weaker activity and decided to move it away from the carbamate nitrogen. We reasoned that this would also enable the carbamate some flexibility in its orientation. We thus moved the bridge one carbon away from the functionalized nitrogen and ended up with the type of compounds highlighted in Table 2. We have recently reported on this type of oxazabicyclo[3.3.1]nonanes with a methylpyrimidine ring system.[13] However, the fluoropyrimidine core consistently showed superior oGTT activity in our hands and so this novel bridge was combined with the fluoro core and we made compounds shown in Table 2. In this case, we also found that we could generate both syn and anti-ethers with the bridged ring system and syn was more preferred (Pfizer has also identified similar bridged ring systems).[14]

Compounds in Table 2, highlight the aryl group SAR on the left hand side while keeping the new bridged piperidine ring and the preferred cyclopropyl-methyl group intact on the right side of the molecule. As can be seen, for the first time, we were able to generate a good balance of potent in vitro numbers and low ClogP values into the compounds and this resulted in multiple compounds with good activity, in vitro and in vivo.[15] Specifically, compounds **8**, **10**, **12** and **14,** showed excellent efficacy in our oGTT screening assay at 3 mpk with good overall pharmacokinetic properties. Bis-ethers, generally, did not show consistent in vivo efficacy, however, compound **13** was an exception. This core did not tolerate major additions to the aryl group and most of the effort involved fine tuning SAR with smaller groups.

Table 2: Comparison of Aryl SAR with oxa-bicyclo ring and pharmacokinetic profile



Compound#	Ar	X	hEC_{50} (nM)	mEC_{50} (nM)	oGTT@ 3mpk
8		NH	6 (%max= 78)	25 (%max= 76)	21%
9		NH	13 (%max= 78)	113 (%max= 108)	31%
10		NH	15 (%max= 94)	44 (%max= 73)	27%
11		NH	17 (%max= 63)	58 (%max= 84)	26%
12		NH	19 (%max= 73)	278 (%max= 83)	27%
13		O	26 (%max= 114)	75 (%max= 136)	20%
14		NH	10 (%max= 92)	58 (%max= 99)	29%

The 4-chloro-cyano aryl in **14** had best overall properties and so to generate carbamate SAR on the right hand side we picked this as our preferred group. Figure 3 shows a small selection of the best compounds from this effort and as can be seen multiple groups were tolerated but none showed activity superior to **14**. We also looked at several carbamate isosteres (ureas, amides and sulfonamides), however, the pyrimidyl group as in compound **20**, which also had the fluoro changed to a cyclopropyl, was the best of the lot. Based on the overall profile we then decided to select compound **14** for further follow-up.

The synthesis of compound **14** is shown in Scheme 1. The commercially available fluoro-malonate was treated with formamidine, to generate the dihydroxy fluoro-pyrimidine **21** which was then converted to the bis-chloropyrimidine **22**. A single chloride could be displaced by treatment with the corresponding aniline, in the presence of sodium hydride, in good yield to give the key intermediate **23**. The bridged piperidine alcohol **26** was made by previously reported procedures.[16-17] Displacement of aryl chloride **23** with the alcohol **26** then resulted in the syn and anti-versions of the pyrimidyl ether which were separated through column chromatography. The syn version which was the more active and preferred compound was then deprotected and coupled with activated methyl-cyclopropyl carbonate to provide compound **14**. This was a very convergent method to generate this compound and multi-gram quantities of the same, was prepared for follow-up studies.

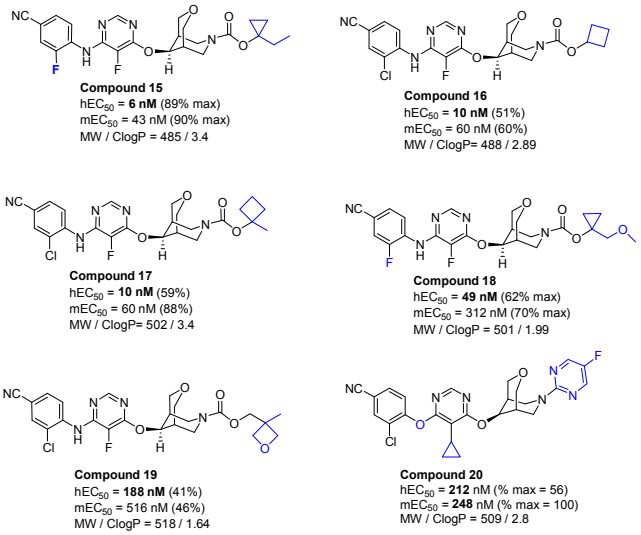
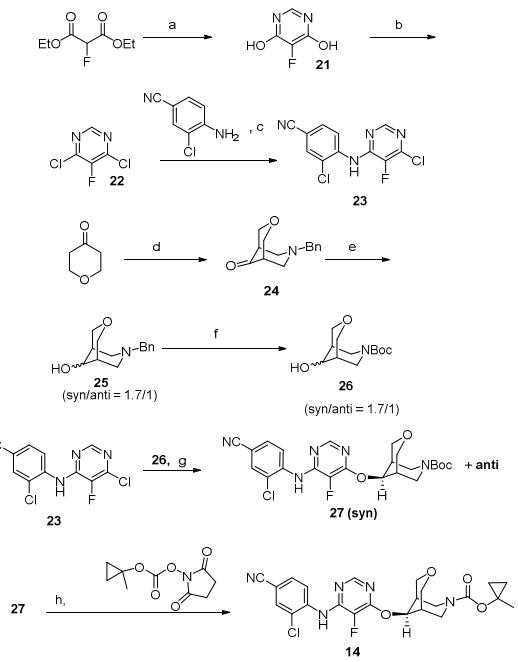


Figure 3. Carbamate SAR in oxa-bicyclo ring system

Scheme 1



[a]Reagents: (a) $NHCHNH_2.HCl$, MeOH, 100%; (b) $POCl_3$, PhNEt2, 96%; (c) NaH, THF, 76%; (d) $BnNH_2$, $(HCHO)_n$, AcOH, iPrOH, 50%; (e) NaBH4, MeOH, 60% (f) Pd/C, H2, MeOH, (Boc)2O, 83%; (g) NaH, THF, 54%; (h) 4N HCl, Dioxane, Et3N, CH2Cl2, 90% over 2 steps.

Compound **14** showed >150 fold selectivity over other receptors (Eurofin panlabs assay), transporters and ion channels. The pharmacokinetic profile of compound **14** was then tested in rat, monkey and dog. It was gratifying to see that it had excellent oral bioavailability (>35%) and half-life in all three species (Table 3).

Table 3: Comparison of compound 14 PK across species

Species	IV/PO (mpk)	CL_p (mL/min/kg)	Vd_{ss} (L/kg)	t½ (h)	po AUC (μM•h)	F (%)
Rat	1/30	7.3	5.8	8.8	63	35
Monkey	1/3	3.1	2.6	12	13	38
Dog	1/10	0.77	4.4	70	131	43 (est)

Compound **14** was then evaluated in an oGTT in lean C57Bl/6 mice. Unlike the acute screening assay, we monitored blood glucose for 60 minutes after oral administration. The vehicle or drug in this study was administered 30 minutes prior to the challenge; data are presented as area under the curve (AUC) and/or a graph. As can be seen in Figure 4, compound **14** reduced glucose excursion in a dose down experiment from 1 to 0.03 mpk. At doses above 1 upto 30 mpk the reduction in glucose excursion were similar at ~50%. The Cmax for the lowest dose which achieved significant excursion (0.3 mPk, t = 1h), was 60 nM (Figure 4).[18]

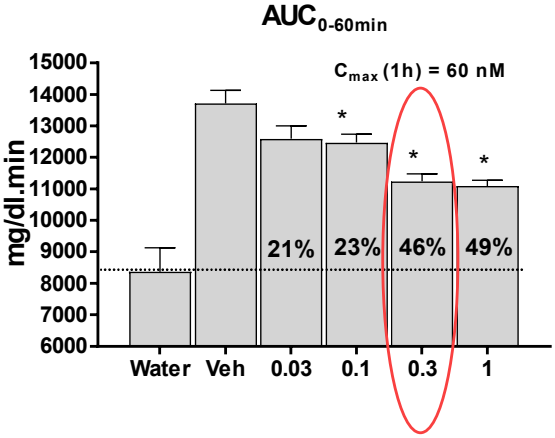


Figure 4: oGTT dose down of Compound **14**

Next we took compound **14** into two known diabetic disease models, namely, DIO and *Lepr*$^{db/db}$ mice. Here again we were able to show improvement in an oGTT, with a reduction in glucose excursion that was comparable to that observed in lean mice albeit at higher doses (Figure 5).

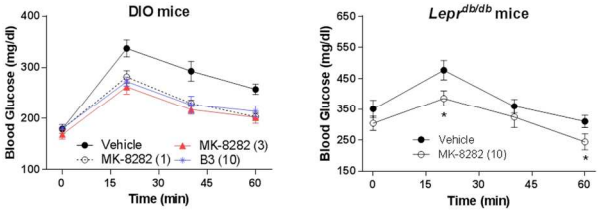


Figure 5: oGTT in DIO and db/db mice with compound **14**

To ensure that the glucose dependent mechanism is indeed mediated through GPR119 we also tested **14** in GPR119 knockout mice. As expected **14** did not improve glucose tolerance in the GPR119 KO mice and reduced the levels only in wild type animals (Figure 6). As prior work demonstrated that GPR119 agonists increase incretin release from enteroendocrine cells, we evaluated the effect of **14** on GLP-1 release in vivo and the impact of co-administration with DPP-IV inhibition on plasma GLP-1.[19] For this purpose we monitored plasma GLP-1(7-36) amide levels in response to a glucose administration in lean mice treated with sitagliptin, compound **14**, or a combination of both agents.

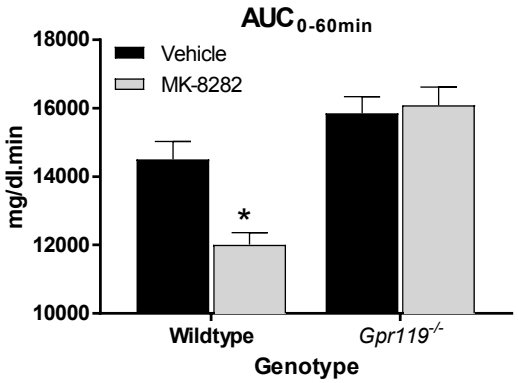


Figure 6: oGTT in WT and GPR119-/- mice with **14**

Sitagliptin increased GLP-1 levels after a glucose challenge, whereas administration of compound **14** alone did not impact plasma GLP-1. However, when both sitagliptin and GPR119 agonist **14** were dosed together, we saw a significant increase in GLP-1 levels prior to and 10 minutes after glucose administration (Figure 7).

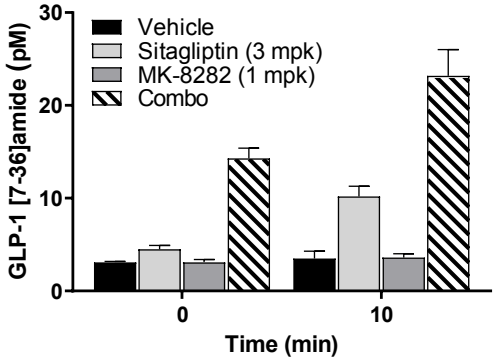


Figure 7: Plasma GLP-1[7-36] amide levels in response to oral glucose administration in lean mice.

The impact of co-administration of sitagliptin and compound **14** on plasma glucose after an oGTT was also evaluated in Sprague-Dawley rats. As shown in Figure 8, both sitagliptin (1 mpk) and compound **14 (3** mpk**)** alone**,** reduced glucose excursion to similar levels, however, the combination of agents produced an apparent additive effect on this excursion.

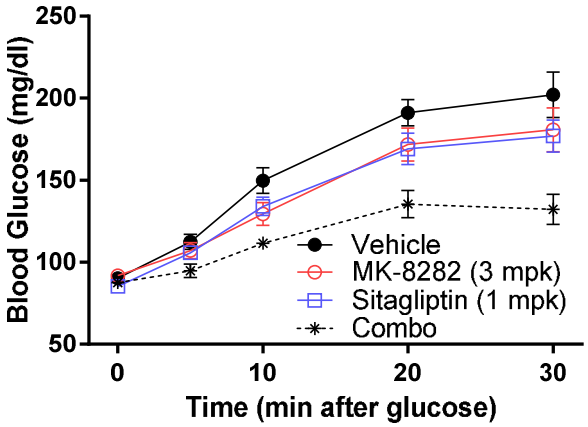


Figure 8: GPR 119 agonism and DPP-IV inhibition display additivity on oGTT in lean rats.

In summary we have identified a novel, potent GPR119 agonist by coupling a fluoro-pyrimidine to a constrained bridged piperidine ring system. Fine-tuning the SAR by balancing lipophilicity with potency, we discovered compound **14**, which showed robust glucose lowering in lean as well as DIO and diabetic mice. We also showed the lack of efficacy in GPR119 knockouts to prove that the glucose lowering mechanism is mediated through this receptor. We were also able to demonstrate cooperativity with DPP-IV inhibition in lowering blood glucose levels. Based on the excellent potency, clean ancillary target profile, good oral bioavailability and exposures and promising in vivo data in disease models, we selected compound **14** as our preclinical candidate MK-8282. Further follow-up of GPR119 agonists and additional SAR will be disclosed in the near future.

ASSOCIATED CONTENT

Supporting Information

Synthetic experimental details including ^{1}H NMR and mass spectral data for selected compounds and description of primary biological assays. 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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ABBREVIATIONS

GPR119, G-protein coupled receptor 119; SAR, structure activity relationships; oGTT, oral glucose tolerance test; DIO, diet induced obese; AUC, area under the curve; GLP-1, glucagon like peptide 1; GPCR, G protein coupled receptors; DM, diabetes mellitus.

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(11) In this oGTT screen, test compound or vehicle was orally administered to overnight-fasted animals at 3 to 10 mg/kg. Glucose was administered to the animals 30 min post-dosing (3 g/kg po), and blood glucose levels were measured using a hand-held glucometer (Bayer Breeze 2) prior to compound or vehicle dosing, prior to glucose administration, and 20 min after glucose administration. Efficacy in this assay was expressed as % blood glucose lowering at 20 minute post glucose treatment. This was defined as [(1-(20 min glucose of test compound treated mice/20 min glucose of vehicle treated mice))x100]. Typically, highly efficacious compounds in this assay gave glucose lowering values of 20% or greater.

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(15) A table of ClogP values for compounds in Table 2 is provided in the supporting information.

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(18) A table with plasma protein binding values in rat, mouse, monkey, dog and human, is provided in the supporting information. In rat, these molecules were highly protein bound with only 1% free levels observed.

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