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Discovery of phenyl acetamides as potent and selective GPR119 agonists

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ABSTRACT

The paper describes the SAR/SPR studies that led to the discovery of phenoxy cyclopropyl phenyl acetamide derivatives as potent and selective GPR119 agonists. Based on a *cis* cyclopropane scaffold discovered previously, phenyl acetamides such as compound **17** were found to have excellent GPR119 potency and improved physicochemical properties. Pharmacokinetic data of compound **17** in rat, dog and rhesus will be described. Compound **17** was suitable for QD dosing based on its predicted human half-life, and its projected human dose was much lower than that of the recently reported structurally-related benzyloxy compound **2**. Compound **17** was selected as a tool compound candidate for NHP (Non-Human Primate) efficacy studies.

Diabetes can cause serious health complications such as cardiovascular disease, blindness and kidney failure. It is becoming more common in the United States and worldwide. For example, from 1980 through 2014, the number of Americans with diagnosed diabetes has increased fourfold (from 5.5 million to 22.0 million).[1] Among diabetic patients, a majority (approximately 90–95%) have type 2 diabetes (T2D). Therefore, more effective and safer therapies for type 2 diabetes are needed. G protein-coupled receptor 119 (GPR119) agonists have emerged as a potential new approach for the treatment of type 2 diabetes.[2]

GPR119 is present primarily in the pancreas and the intestine. Activation of GPR119 increases insulin, GLP-1, GIP and PYY secretion.[2b] GPR119 agonists stimulate insulin release in a glucose-dependent manner.[3] The glucose dependent insulin secretion (GIDS) mechanism makes GPR119 an attractive target for the treatment of type 2 diabetes with low risk for hypoglycemia. Consequently, GPR119 agonists have been studied extensively and several compounds have entered clinical trials for the treatment of diabetes. However, despite the promising effects in preclinical species (especially in rodents) and enormous efforts by both academia and many pharmaceutical companies, it is still a challenge to demonstrate robust glycemic control efficacy in humans.[4] So far, no compound has been reported to progress beyond phase II clinic trials.[5]

To increase the probability of success in glycemic control efficacy, we were interested in developing a fixed-dose combination (FDC) of a GPR119 agonist with a DPP4 inhibitor such as sitagliptin.[6] It has been reported that in rat models, such combinations provide greater glucose lowering effects than either the GPR119 agonist or the DPP4 inhibitor alone.[7,8] Our previous preclinical candidate 1 (Fig. 1) which had excellent potency, selectivity and *in vivo* efficacy,[9] was unsuitable for a fixed dose combination with sitagliptin (QD) due to, (a) a very long half-life in humans and, (b) poor solubility in, for example, fasted-state simulated intestinal fluid (FaSSIF)[10] which would result in a high likelihood of a need for the use of enabled formulations in a FDC setting with sitagliptin. Structure-activity relationship (SAR) and structure-property relationship (SPR)[11] efforts that were focused on reducing the predicted human $t_{1/2}$ and improving FaSSIF solubility, while maintaining good potency at activation of GPR119 led to the discovery of benzyloxy compound **2** (Fig. 1). Compound **2** possessed excellent GPR119 potency, selectivity, and significantly improved physicochemical properties compared to its precursor **1**.[6] The

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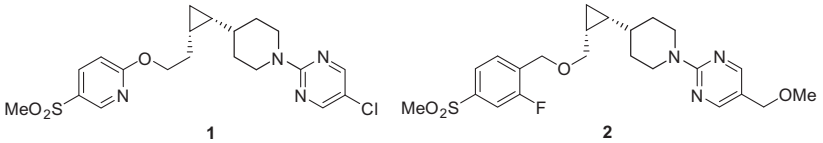


1

h GPR119 EC_{50} 3.3 nM (69%)[a]
rat PK: $t_{1/2}$ 14.7 h
Cl_{int} 1446 mL/min/kg
Vd_u 1725 L/kg
FaSSIF solubility: 21 μM
LogD 4.9[b]

2

h GPR119 EC_{50} 2.1 nM (88%)[a]
rat PK: $t_{1/2}$ 1.0 h
Cl_{int} 1000 mL/min/kg
Vd_u 54 L/kg
FaSSIF solubility: 161 μM
LogD 3.5[b]

Fig. 1. Structure of our GPR119 agonists.[6] (a) The percentage in parentheses: % control at max dose. (b) HPLC logD at pH 7.

projected human half-life for compound **2** was ~15 h which was in the target range for a QD dosing. Its projected human dose (QD), however, was ~300 mg which was suboptimal as it would need to be combined with a 200 mg once daily dose of sitagliptin. Herein we report our continued efforts in this area to improve the GPR119 potency and to further improve the overall pharmacokinetic profiles to reduce the projected human dose within this series of GPR119 agonists.

Compound **2** is a benzyloxy analogue while compound **1** is a phenoxy derivative. As we reported before,[6] changing from a phenoxy analogue to the corresponding benzyloxy group resulted in a favorable drop in lipophilicity and decreased unbound volume of distribution (Vd_u). For example in rat, compound **1** had Vd_u 1725, while compound **2** had Vd_u = 54 L/kg which helped reduce its rat $t_{1/2}$ (1.0 h vs 14.7 h). However, high intrinsic clearance Cl_{int} was observed for many benzyloxy analogues due to extensive benzylic oxidative metabolism. Half-life can be shortened by increasing Cl_{int} and/or by decreasing Vd_u of a compound. However, increasing Cl_{int} will also lead to an increased QD dose projection and potentially higher levels of metabolites. Therefore, although the benzyloxy series was promising in reducing $t_{1/2}$ and improving FaSSIF solubility, SAR studies on the original phenoxy lead were revisited in an effort to keep the Cl_{int} and projected human dose in the targeted range (*i.e.* <200 mg projected human QD dose).

As we had already demonstrated that, the *cis* cyclopropyl piperidine scaffold provides several advantages such as improved GPR119 potency and selectivity,[9] we elected to keep this core intact. Extensive SAR studies were first performed on the left hand side of the molecule by replacing the pyridinyl sulfone group of **1** with different substituted aromatic rings. Among the many structurally diverse derivatives synthesized, we were pleased to find that phenyl acetamides provided some promising new directions.

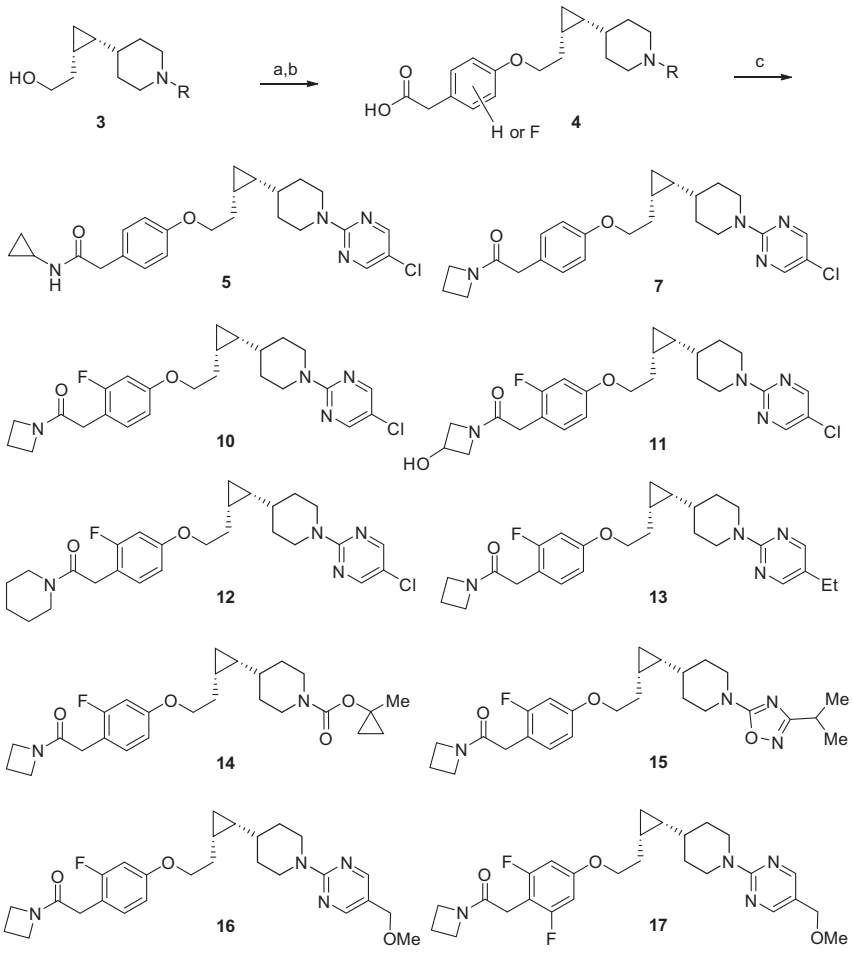
The representative synthesis of the phenyl acetamides is illustrated in Scheme 1. (1*S*,2*R*) *cis* piperidinyl cyclopropyl alcohol **3** was converted to phenylacetic acid **4** via a Mitsunobu reaction with methyl 2-(4-hydroxyphenyl)acetate (or the corresponding F substituted phenol) followed by methyl ester hydrolysis. The detailed syntheses of intermediate **3** with different piperidine capping groups have been described in Ref. 12. Finally, amide bond formation with EDC, HOBt or HATU afforded a variety of phenyl acetamide final products.

Because of the commercial availability of some acetamide phenol starting materials, a few acetamide final products (such as **6** and **9**, Scheme 2) were directly synthesized via a Mitsunobu reaction with chloropyrimidine *cis* piperidinyl cyclopropyl alcohol **3**. The 3-pyridinyl analogue **8** was synthesized by a nucleophilic aromatic substitution reaction between chloropyridinyl azetidine acetamide and chloropyrimidine *cis* piperidinyl cyclopropyl alcohol **3**.

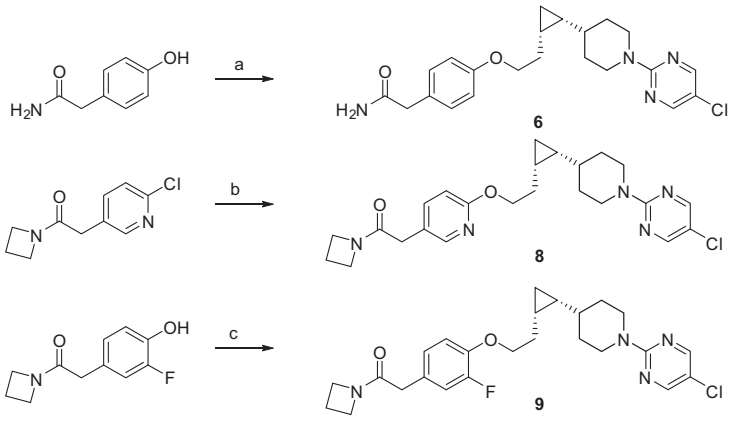
Another alternative synthesis was described in Scheme 3. Readily available bromo phenol **18** was coupled with MOM pyrimidine *cis* piperidinyl cyclopropyl alcohol **3** via a Mitsunobu reaction. Then a Negishi reaction of bromide **19** with (2-(*tert*-butoxy)-2-oxoethyl)zinc(II) bromide followed by *tert*-butyl ester deprotection gave the MOM pyrimidine phenylacetic acid **4**. The final product **17** was synthesized by a routine HATU amide coupling reaction.

SAR and SPR results of acetamides synthesized in Schemes 1 and 2 are summarized in Table 1. Our previous preclinical candidate **1** is also shown for comparison. We were pleased to find that secondary amide **5** had excellent GPR119 potency (hGPR119 EC_{50} = 1.8 nM). However, its FaSSIF solubility (22 μM) remained as poor as that of compound **1** (21 μM). In addition to **5**, other secondary amides we synthesized all exhibited poor FaSSIF solubility. Primary amide **6** (Scheme 2) had reduced GPR119 potency. In contrast to primary amide **6** and secondary amide **5**, tertiary amide **7** had improved FaSSIF solubility (97 μM) and maintained excellent GPR119 potency (hGPR119 EC_{50} = 0.8 nM). Furthermore we were pleased to find that in rat PK studies, $t_{1/2}$ of tertiary amide **7** became 4.7 h compared to a very long $t_{1/2}$ (15 h) of compound **1**. The 3-pyridinyl acetamide **8** (Scheme 2) which has a pyridine moiety as compound **1**, was less active than phenyl **7**. 3-F substituted **9** (Scheme 2) was also less active (hGPR119 EC_{50} = 2.2 nM) than **7**. However, 2-F substituted **10** was more potent (hGPR119 EC_{50} = 0.2 nM) than unsubstituted **7** (hGPR119 EC_{50} = 0.8 nM). Compound **10** also had good FaSSIF solubility (137 μM). SAR studies from an amide library synthesis revealed that while a variety of amines were tolerated for GPR119 potency, azetidine amide **10** was one of the most potent analogues (for example, compared with hydroxyl azetidine **11** and piperidine amide **12**). However with a long projected human half-life (~46 h based on allometric scaling from rat), compound **10** was still not ideally matched for a QD fixed-dose combination (FDC) partner with sitagliptin. Although hydroxyl azetidine **11** had a reduced rat $t_{1/2}$ (3.5 h), its hERG activity (hERG IC_{50} = 5.3 μM) remained a concern. Chloropyrimidine compounds **7**–**12** all had moderate hERG inhibition. Many historical GPR119 agonists have struggled with hERG inhibition as well as solubility issues,[14] and some even had CNS side effects, potentially due to off-target activities that accompany their highly lipophilicic features.[5,8b]

We next turned our attention to piperidine capping groups on the right-hand side (RHS) of the molecule such as ethylpyrimidine, oxadiazole and carbamates which are structure moieties of some clinical GPR119 agonists.[5] Ethylpyrimidine **13** maintained excellent potency (hGPR119 EC_{50} = 0.4 nM), however, with no improvement in hERG selectivity. Methylcyclopropyl carbamate **14** was less active in an IKr binding assay (hERG IC_{50} = 23 μM), however, it was also less potent at GPR119 (hGPR119 EC_{50} = 3.3 nM). Moreover, in a cardiac ion channel blockade activity assay,[15] functional hERG inhibition for **14** was determined to be more potent (hERG IC_{50} = 8.6 μM) than in IKr binding assay. Isopropyl oxadiazole (**15**) also reduced potency (hGPR119 EC_{50} = 2.0 nM) relative to the chloropyrimidine **10**, and hERG selectivity was still suboptimal



Scheme 1. Representative synthesis of the acetamides. Reagents and conditions: (a) methyl 2-(4-hydroxyphenyl)acetate, PPh_3, DIAD or di-*tert*-butyl azodicarboxylate, DCM, rt; (b) LiOH, THF/MeOH/H_2O, rt; (c) amine, EDC or HATU, $^iPr_2NEt$, DMF or DCM, rt.



Scheme 2. Synthesis of acetamide **6**, **8** and **9**. Reagents and conditions: (a) chloropyrimidine intermediate **3**, di-*tert*-butyl azodicarboxylate, DCM, rt, 33%; (b) chloropyrimidine intermediate **3**, NaHMDS, DMF, 150 °C, 4%; (c) chloropyrimidine intermediate **3**, di-*tert*-butyl azodicarboxylate, DCM, rt, 71%.

(hERG IC_{50} = 7.0 μM). Furthermore, the projected human half-lifes of several isopropyl oxadiazole phenylacetamides including **15** were generally projected to be too short to support a QD human dose less than 200 mg.

We previously reported that a MOM pyrimidine improved solubility in the benzyloxy series relative to the corresponding chloropyrimidine RHS moiety.[6] We were pleased to discover that the corresponding MOM pyrimidine compound **16** not only had excellent FaSSIF solubility (152 μM) and GPR119 potency (hGPR119 EC_{50} = 0.7 nM), but also reduced hERG activity (hERG IC_{50} = 19 μM). Since 2-F substitution improved GPR119 potency (compound **10** vs **7**), the 2,6-difluoro analogue **17** was synthesized. Compound **17** now had hGPR119 EC_{50} = 0.2 nM. In IKr binding assay, its hERG IC_{50} = 9.2 μM. In cardiac ion channel blockade activ-

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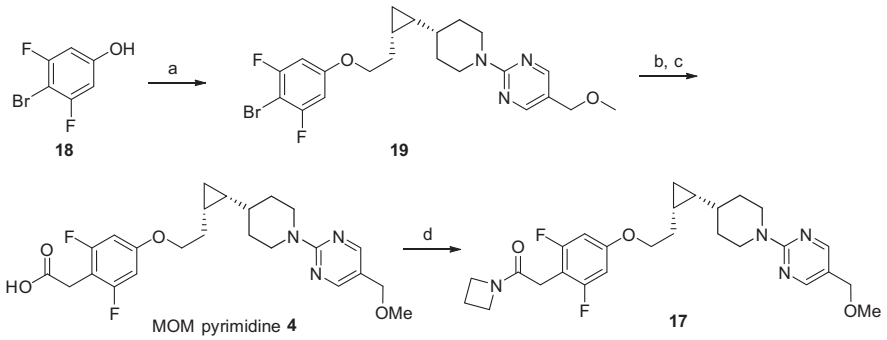

Scheme 3. An alternative synthesis of compound **17**. Reagents and conditions: (a) MOM pyrimidine intermediate **3**, di-*tert*-butyl azodicarboxylate, DCM, rt; (b) (2-(*tert*-butoxy)-2-oxoethyl)zinc(II) bromide, X-Phos Pre-catalyst, 65 °C; (c) TFA, DCM, 53% over three steps; (d) azetidine, HATU, $^{i}Pr_2NEt$, DMF, rt, 72%.

Table 1
SAR results of the acetamides synthesized in Schemes 1 and 2 (nd = not determined).

Comps	hGPR119[a] EC_{50} (nM)	mGPR119[a] EC_{50} (nM)	FaSSIF[b] (μM)	hERG IC_{50}[c] (μM)	rat $t_{1/2}$ (h)
1	3.3 (69%)	6.5 (95%)	21	9.9	15
5	1.8 (104%)	1.5 (91%)	22	>60	nd
6	67 (85%)	16 (68%)	nd	nd	nd
7	0.8 (82%)	0.5 (93%)	97	7.4	4.7
8	9.4 (112%)	5.1 (133%)	83	2.1	nd
9	2.2 (106%)	1.6 (91%)	65	3.5	nd
10	0.2 (106%)	0.4 (146%)	137	3.3	6.9
11	0.8 (89%)	6.7 (110%)	144	5.3	3.5
12	1.5 (116%)	2.2 (92%)	124	3.3	nd
13	0.4 (103%)	0.84 (111%)	140	3.2	nd
14	3.3 (127%)	6.6 (115%)	138	23	1.6
15	2.0 (78%)	6.7 (102%)	126	7.0	1.8
16	0.7 (82%)	1.6 (90%)	152	19	1.9
17	0.2 (91%)	0.8 (116%)	139	9.1	3.5

[a] Human and mouse GPR119 EC_{50} values are means of at least 2 independent experiments except compound **6** and **8**. The percentage in parentheses, % control at max dose; magnitude of cAMP stimulation expressed in % compared to an internal agonist control; the control was defined to have 100% cAMP stimulation.
[b] Kinetic solubility in fasted-state simulated intestinal fluid (FaSSIF) at pH 6.5.
[c] IKr binding assay, Ref. 13.

ity assay,[15] functional hERG inhibition was confirmed to be mild (hERG IC_{50} = 12 μM). Compound **17** also had a favorable rat $t_{1/2}$ (3.5 h).

Because of its excellent GPR119 potency, good hERG selectivity and favorable rat $t_{1/2}$, compound **17** was scaled-up and further profiled. The synthetic protocols described in Schemes 1 and 3 were both suitable for a multi-gram scale synthesis of compound **17**. An off-target screen was performed against an extensive panel of 168 receptors, ion channels, and enzymes, and no off-target activities were found with IC_{50}s < 10 μM. Compound **17** was also selective over CYP3A4 (IC_{50} > 50 μM) and hPXR (EC_{50} > 30 μM).[16] In equilibration solubility studies, crystalline **17** had FaSSIF solubility 0.051 mg/mL (vs 0.009 mg/mL of compound **1**), which confirmed the improvements observed in the kinetic FaSSIF solubility data (139 μM vs 21 μM) and verified that compound **17** indeed exhibited improved FaSSIF solubility compared to compound **1**.

In liver microsomal stability studies, the *in vitro* intrinsic clearance (Cl_{int}) of compound **17** was determined to be 189, 39, 44, and 39 mL/min/kg in rat, dog, rhesus and human liver microsomes, respectively. Compared with the previous benzyloxy compound **2** (Cl_{int} was 1104, 60, 319, 42 mL/min/kg in rat, dog, rhesus and human liver microsomes), compound **17** was significantly more stable in rat and rhesus liver microsomes. As a result, compound **17** had improved pharmacokinetic profiles in rat and rhesus monkey, which are shown in Table 2.

In rat PK studies, compound **17** possessed lower *in vivo* unbound clearance than compound **2** (430 vs 1000 mL/min/kg). Previously, compound **2** had low bioavailability in rat and rhesus (27% and 5%), likely due to first-pass metabolism. In contrast, the bioavailability of compound **17** was improved to 80% (rat) and 44% (rhesus). In mouse oral glucose tolerance test (oGTT) dose titration PK/PD studies,[17] the MED_{max} (minimal efficacious dose for maximal efficacy) for **17** was determined to be 0.1 mpk compared to the MED_{max} of compound **2** at 3 mpk. The mouse oGTT efficacy was ablated in GPR119$^{-/-}$ mice at a suprapharmacological dose of 3 mpk, which confirmed that the efficacy was GPR119-mediated. Compound **17** also had low rat oGTT MED_{max} that was 0.3 mpk. Based on mouse oGTT MED_{max} which was 0.1 mpk, and the blood concentration of **17** at 90 min post-dosing at 0.1 mpk was 10 nM in mice, the target plasma trough in humans for acute

Table 2
Pharmacokinetic profiles of compound **17**.

Species	PPB[a] (% unbound)	Cl_p (mL/min/kg)	Vd (L/kg)	$t_{1/2}$ (h)	F (%)
Rat	6.7%	29	4.8	3.5	80
Dog	5.5%	2.0	4.3	29	55
Rhesus	8.0%	14	3.5	7.6	44

[a] Human PPB (% unbound): 3.8%. PPB data reported in Ref. 6 were screening assay data. For comparison reason, PPB data reported herein were also screening assay data.

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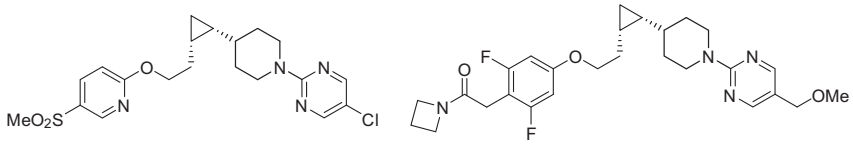

1

h GPR119 EC_{50} 3.3 nM (69%)[a]
rat PK: $t_{1/2}$ 14.7 h
Cl_{int} 1446 mL/min/kg
Vd_u 1725 L/kg
FaSSIF solubility: 21 μM
LogD 4.9[b]

17

h GPR119 EC_{50} 0.2 nM (91%)[a]
rat PK: $t_{1/2}$ 3.5 h
Cl_{int} 430 mL/min/kg
Vd_u 72 L/kg
FaSSIF solubility: 139 μM
LogD 4.1[b]

Fig. 2. From compound **1**–**17**. (a) The percentage in parentheses: % control at max dose. (b) HPLC logD at pH 7.

efficacy was estimated to be ~6 nM.[18] The predicted human half-life of compound **17** was ~12 h, suitable for QD. The projected human dose (QD) was <10 mg, which is much lower than ~300 mg of compound **2**.[19] The predicted PK of **2** and **17** are very similar, with the decreased dose resulting from the significantly increased potency and thus reduced plasma trough target of **17**.

One major issue for the development of GPR119 agonists for the treatment of type 2 diabetes is the poor translation from rodent *in vivo* efficacy models to human clinical trials.[4,5] Species differences in pharmacology between mouse and human has been reported.[20] Consequently, we were also interested in identification of a suitable tool compound for NHP (non-human primate) studies to further support PD translation of GPR119 pharmacology to humans. Compound **17** possesses excellent rhesus GPR119 potency (rhesus GPR119 EC_{50} = 0.1 nM) and an excellent rhesus PK profile, and was therefore selected for further profiling in rhesus glycemic efficacy studies that will be reported elsewhere in due course.

In summary, starting from our previous preclinical candidate **1**, left-hand side SAR/SPR studies led to the discovery of a phenyl acetamide lead with improved physical properties such as FaSSIF solubility (Fig. 2). Incorporation of 2,6 di-F substitution and an azetidine amide were found to be optimal LHS modifications, which further improved GPR119 potency. The RHS piperidine capping group optimization which focused on solubility, hERG selectivity, and rat $t_{1/2}$ subsequently, led to the discovery of compound **17**. Compared with compound **1**, compound **17** had reduced Vd_u (from 1725 to 72 L/kg), which contributed to its reduced rat $t_{1/2}$. The predicted human half-life of compound **17** was suitable for QD dosing. Because of high GPR119 potency, its projected human dose was much lower than that of compound **2**. Compound **17** was a candidate for NHP (Non-Human Primate) efficacy studies. Results of those *in vivo* pharmacodynamic studies in rhesus will be reported in due course.

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References

1. Center for Disease Control and Prevention, Diabetes Public Health Resource; 2015. <http://www.cdc.gov/diabetes/statistics/prev/national/figpersons.htm>.
2. (a) Jones RM, Leonard JN, Buzard DJ, Lehmann J. *Expert Opin Ther Pat*. 2009;19:1339;
(b) Hansen HS, Rosenkilde MM, Holst JJ, Schwartz TW. *Trends Pharmacol Sci*. 2012;33:374;
(c) Ohishi T, Yoshida S. *Expert Opin Invest Drugs*. 2012;21:321.
3. Chu ZL, Jones RM, He H, et al. *Endocrinology*. 2007;148:2601.
4. (a) Polli JW, Hussey E, Bush M, et al. *Xenobiotica*. 2013;43:498;
(b) Katz LB, Gambale JJ, Rothenberg PL, et al. *Diabetes Obes Metab*. 2012;14:709.
5. For an excellent review, see: Ritter K, Buning C, Halland N, Pöverlein C, Schwink L. *J Med Chem*. 2016;59:3579.
6. Liu P, Hu Z, DuBois BG, et al. *ACS Med Chem Lett*. 2015;6:936.
7. (a) Chu Z, Carroll C, Alfonso J, et al. *Endocrinology*. 2008;149:2038;
(b) Buzard DJ, Kim SH, Lehmann J, et al. *Bioorg Med Chem Lett*. 2014;24:4332.
8. (a) Wacker DA, Wang Y, Broekema M, et al. *J Med Chem*. 2014;57:7499;
(b) Scott JS, Bowker SS, Brocklehurst KJ, et al. *J Med Chem*. 2014;57:8984.
9. Szewczyk JW, Acton J, Adams AD, et al. *Bioorg Med Chem Lett*. 2011;21:2665.
10. (a) Galia E, Nicolaides E, Horter D, Lobenberg R, Reppas C, Dressman JB. *Pharm Res*. 1998;15:698;
(b) Dressman JB, Reppas C. *Eur J Pharm Sci*. 2000;11:S73.
11. (a) Kerns EH, Di L. *Drug Discovery Today*. 2003;8:316;
(b) Meanwell NA. *Chem Res Toxicol*. 2011;24:1420.
12. (a) Wood HB, Szewczyk JW, Huang Y, Adams AD. WO 2009129036 A1; 2009.(b) Edmondson S, Hu Z, Liu P, et al. WO2013074388A1; 2013.
13. Lynch JJ, Wallace AA, Stupienski RF, et al. *J Pharmacol Exp Ther*. 1994;269:541.
14. For an excellent example to address solubility issue of GPR119 agonists, see: Scott JS, Birch AM, Brocklehurst KJ, et al. *J Med Chem*. 2012;55:5361.
15. Measured by PatchXpress® systems. For a review on ion channels, see: Aidley PR, Stanfield PR, eds. *Ion Channels: Molecules in Action*. Cambridge University Press; 1996.
16. Pregnane X receptor (PXR) is a nuclear receptor known to regulate expression of drug-metabolizing enzymes including cytochrome P450 (CYP450), see: Chu V et al. *Drug Metab Dispos*. 2009;37:1339–1354.
17. Compounds were tested *in vivo* using an oral glucose tolerance test (oGTT) in eleven-week old, lean C57BL/6 Tac mice (n = 8/group). Overnight fasted mice were administered compound by oral gavage; vehicle: PEG400/23.5% HPBCD (15/85 v/v). 30 min later, they received an oral glucose challenge (3 g/kg). Blood glucose was measured via tail nick using a glucometer at 0, 20, 40, and 60 minutes post-glucose challenge. The area under the curve (AUC) for the glucose response was calculated for each mouse. The glucose AUC of compound treated mice was compared to that of vehicle-treated mice using an unpaired Student's t-test or One-Way ANOVA where appropriate. The data is expressed as the % change of the Glucose AUC relative to vehicle.
18. The human plasma trough target was estimated from the mouse blood concentration at 90 min (10 nM) using a blood/plasma ratio of 0.55 and adjustment for potency and plasma protein binding differences between mice and humans.
19. Using microsomal stability data across species, the predicted human plasma clearance was ~4 mL/min/kg. The Vd (~4.2 L/kg) and bioavailability (~60%) were estimated from preclinical species.
20. Scott JS, Brocklehurst KJ, Brown HS, et al. *Bioorg Med Chem Lett*. 2013;23:3175.