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Discovery of a nortropanol derivative as a potent and orally active GPR119 agonist for type 2 diabetes

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ABSTRACT

The lead optimization studies of a series of GPR119 agonists incorporating a nortropanol scaffold are described. Extensive structure–activity relationship (SAR) studies of the lead compound **20f** led to the identification of compound **36j** as a potent, single digit nanomolar GPR119 agonist with high agonist activity. Compound **36j** was orally active in lowering blood glucose levels in a mouse oral glucose tolerance test and increased plasma insulin levels in a rat hyperglycemic model. It showed good to excellent pharmacokinetic properties in rats and monkeys and no untoward activities in counter-screen assays. Compound **36j** demonstrated an attractive in vitro and in vivo profile for further development.

Diabetes is a serious illness that affects millions of people worldwide. In the United States alone, 23.6 million children and adults—7.8% of the population—have diabetes. Worldwide, the total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030.[1] As people's living conditions improve this number is ironically on the rise.[2] Diabetes is divided into type 1 diabetes (insulin-dependent diabetes mellitus or juvenile diabetes) and type 2 diabetes (noninsulin-dependent diabetes mellitus or adult-onset diabetes) with the latter one being more common and affecting >90% of diabetic patients. Current therapeutic treatment for type 1 diabetes includes insulin injections or an insulin pump. For type 2 diabetes, more therapeutic treatments are available, especially when the disease is in the early stage. They include glucose-independent insulin secretagogues (sulfonylureas), insulin sensitizers (biguanides (metformin) and thiazoladinediones (Avandia, Actos)), insulin, and a relatively new class of agents, the glucose-dependent insulin secretagogues such as GLP-1 mimetics (Byetta, Victoza) and DPP-4 inhibitors (Januvia). Novel diabetes medications, especially oral medications that do not have the side effects of older ones (e.g., hypoglycemia, weight gain) are active areas of research both in industry and in academics to meet the needs of diabetic patients.

G protein-coupled receptor 119 (GPR119) agonists have emerged as a promising therapeutic approach for the treatment of type 2 diabetes.[3] GPR119 was discovered recently by several groups and has been referred to by different names such as RUP3 or glucose-dependent insulinotropic receptor (GDIR).[3c] It is a G protein-coupled receptor which is selectively expressed on pancreatic beta cells and intestinal enteroendocrine cells. Agonists to GPR119 stimulate glucose-dependent insulin secretion in vitro and lower an elevated blood glucose level in vivo.[4] Additionally, they have been demonstrated to stimulate the release of the incretins (glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP)).[5] Numerous patents describing GPR119 agonists have been disclosed,[3] and several companies have advanced GPR119 agonists into the clinic for the treatment of type 2 diabetes (Ortho-McNeil/Arena (APD-668 and APD-597; both discontinued), Sanofi-Aventis/Metabolex (SAR-260093/MBX-2982; Ph II), GlaxoSmithKline (GSK-1292263; Ph II), Astellas/Prosidion (PSN-821; Ph II) and Bristol-Meyers Squibb (Ph I)).[3d]

In our continued effort to develop GPR119 agonists for the treatment of diabetes,[6] we became interested in the pyrimidine derivative **1** as a lead structure (Fig. 1).[7] In particular, we were interested in bicyclic scaffolds **2**–**7** as replacements for the piperidinol scaffold in **1** as shown in Figure 1. Here we describe the outcome of this endeavor and the discovery of a nortropanol derivative **36j** which is a potent agonist of the GPR119 receptor in vitro and orally active in lowering blood glucose levels in rodents in vivo.

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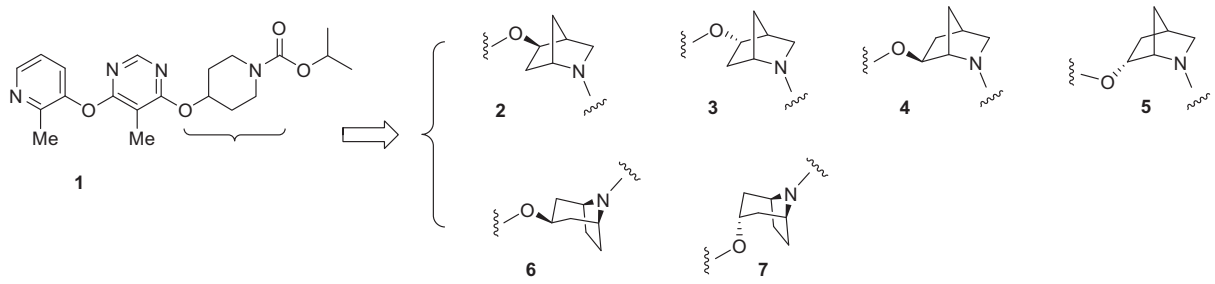
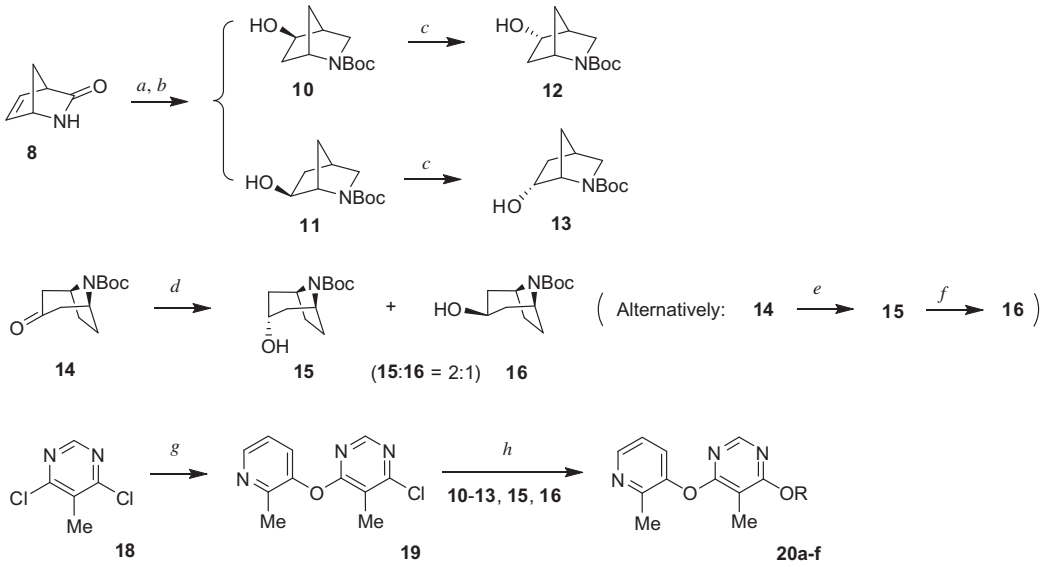


Figure 1.



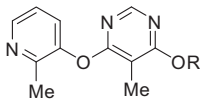
Scheme 1. (a) (i) $LiAlH_4$, (ii) Boc_2O, DIEA; (b) $Hg(OAc)_2$, H_2O then $NaBH_4$; (c) (i) DMSO, $(COCl)_2$ then NEt_3, (ii) L-Selectride; (d) $NaBH_4$; (e) L-Selectride; (f) (i) *p*-NO_2PhCO_2H, DEAD, Ph_3P, (ii) NaOH, H_2O; (g) 2-Methylpyridin-3-ol, K_2CO_3; (h) KO-*t*-Bu.

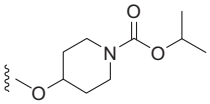
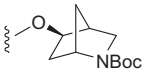
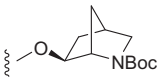
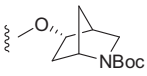
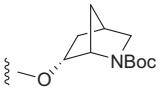
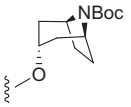
Scheme 1 illustrates the synthesis of compounds **20a–f** which incorporates target scaffolds **2–7**. We used a *t*-butyl carbamate group for compounds **20a–f** instead of the isopropyl carbamate in **1** for our initial screening. Alcohols **10** and **11** were prepared according to the published patent procedures as shown in Scheme 1.[8] Then they were transformed to alcohols **12** and **13** by a two-step sequence: a Swern oxidation followed by an L-Selectride reduction.[9] Alcohols **15** and **16** were prepared in one step from the nortropinone **14** by $NaBH_4$ reduction.[10] Alternatively, alcohol **15** was obtained exclusively from **14** by an L-Selectride reduction. Then **15** was converted to the alcohol **16** in high yield by the Mitsunobu protocol. Alcohols **10–13**, **15**, and **16** were incorporated into the final targets **20e–f** as shown in Scheme 1 according to published procedures.[7]

Compounds **20a–f** were evaluated for their abilities to activate the human GPR119 receptor in a cell-based cAMP assay.[11] The results expressed in EC_{50} values are shown in Table 1. Of the scaffolds evaluated, those that maintain a 4-hydroxypiperidine structure instead of 3-hydroxypiperidine gave better GPR119 activation activities (**20a**, **20c**, **20e**, and **20f** vs **20b** and **20d**). Compound **20f**, incorporating a 3β-nortropanol scaffold, gave the best EC_{50} value which is equivalent to the EC_{50} value of compound **1**. Presumably, compound **20f** best mimics the active conformation of **1**.[3e]

Encouraged by the potency of **20f** we further explored the optimization of in vitro GPR119 agonist activities based on this nortropanol scaffold. Various N-derivatizations of the bicyclic motif were examined first. The Boc group in compound **20f** was removed by treatment with trifluoroacetic acid. Libraries of carbamates, sulfonamides, amides, ureas, and amines were prepared from the result-

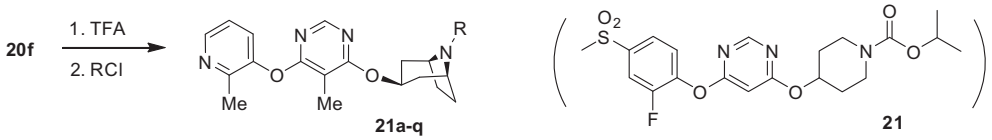
Table 1

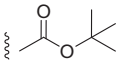
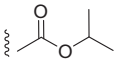
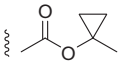
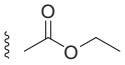
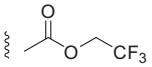
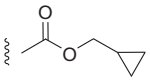
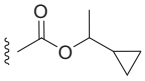
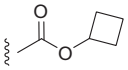
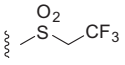
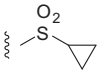
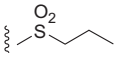
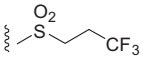
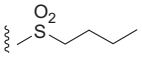
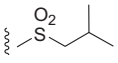
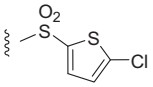
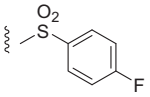


Compound no.	-OR	EC_{50} (nM)[a]
1		15
20a		558
20b		2580
20c		860
20d		>20,000
20e		176
20f		18

[a] The standard deviations are <50%.

Table 2[a]



Compound no.	-R	*h*EC$_{50}$ (nM)	*h*% max	*m*EC$_{50}$ (nM)	*m*% max
21		82	100%	403	100%
20f		18	31%	300	20%
21a		24	72%	55	20%
21b		18	40%	>30,000	12%
21c		131	53%	324	25%
21d		61	47%	10,357	20%
21e		37	44%	7,573	33%
21f		38	31%	59	31%
21g		30	35%		
21h		330	35%		
21i		112	56%	660	62%
21j		>5000	9%		
21k		24	57%	63	76%
21l		32	41%	53	49%
21m		112	68%	250	62%
21n		30	36%	26	29%
21o		15	64%	70	69%
21p		25	10%	145	77%
21q		42	10%	350	78%

[a] *h*EC$_{50}$: EC$_{50}$ for human GPR119; *m*EC$_{50}$: EC$_{50}$ for mouse GPR119; *h*% max: magnitude of cAMP stimulation for human GPR119 expressed in% compared to the reference GPR119 agonist **21**[12] which is defined to have 100% cAMP stimulation; *m*% max: magnitude of cAMP stimulation for mouse GPR119 expressed in% compared the reference GPR119 agonist **21**[12] which is defined to have 100% cAMP stimulation. The standard deviations are <50% for EC$_{50}$ and <10% for % max.

ing amine and tested for GPR119 agonist activities. We found that carbamates and sulfonamides were more active in general than amides, ureas, and amines. Representative examples of carbamates and sulfonamides are shown in Table 2. The in vitro agonistic activities were evaluated by two measures: the potency measure as expressed in EC$_{50}$ values in the cAMP assay and the magnitude of agonist activity measure as expressed in % max which is a comparison of the test compound to a reference GPR119 agonist **21**[12]

Table 3

Compound no.	oGTT@3 mpk (% of vehicle)	$C_{1\ h}$ (nM)	Rat $AUC_{0-6\ h}$ (10 mpk, nM/h)
21	78%[a,c]	480[a]	20,672
21a	100%[d]	193	175
21b	95%[d]	130	2841
21k	87%[c]	2,330	879
21o	89%[b,c]	160[b]	0

[a] @1 mpk.
[b] @10 mpk.
[c] $p \leqslant 0.05$.
[d] $p > 0.05$.

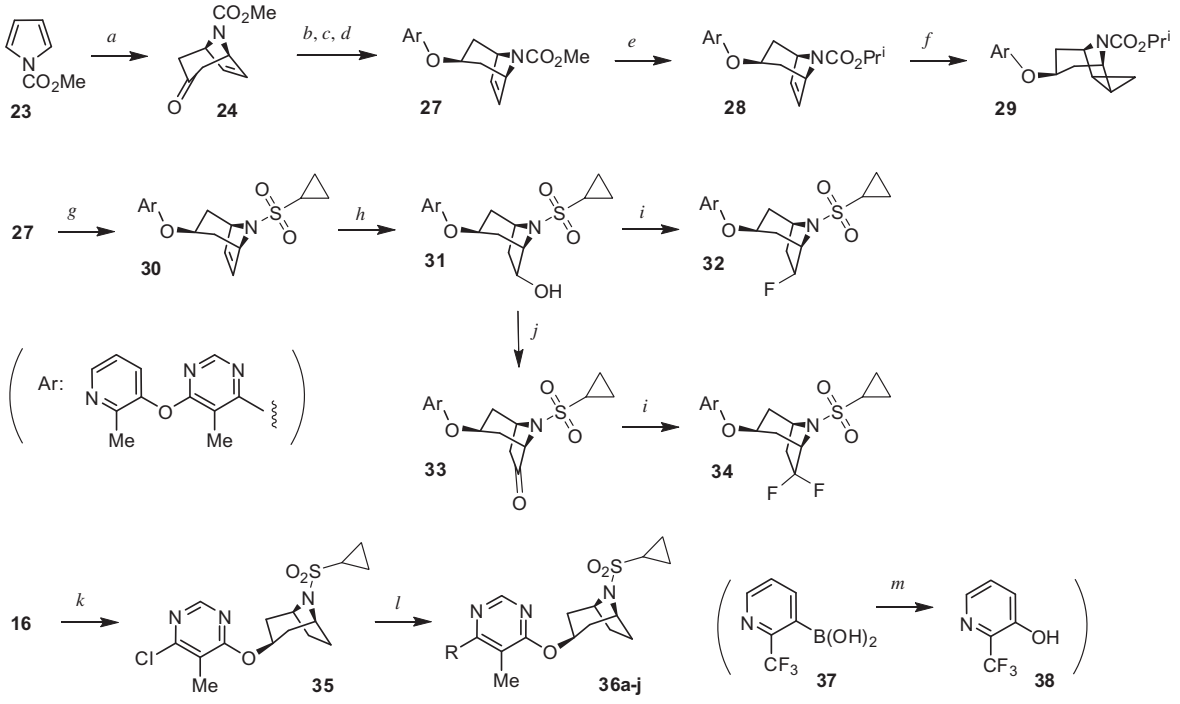
Table 4[a]

Compound no.	hEC$_{50}$ (nM)	h% max	mEC$_{50}$ (nM)	m% max
28	18	14%	52	48%
29	>30,000			
30	38	81%	240	78%
31	1150			
32	130	29%	710	74%
33	918			
34	62	45%	220	52%

[a] The standard deviations are <50% for EC_{50} and <10% for % max.

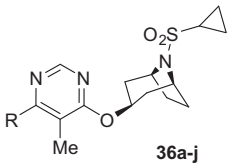
(defined as 100% activation). The in vitro activities were evaluated in two species as expressed in hEC$_{50}$ and h% max for human and mEC$_{50}$ and m% max for mouse. Of the carbamates evaluated, good potencies were observed in general as exemplified by the hEC$_{50}$s of compounds **20f**, **21a**, and **21b**. However, the carbamates appear to be partial agonists (except **21a**) as shown by their relatively low h% max. Moreover, they have low agonist activity for mouse GPR119 receptor as shown by their high mEC$_{50}$ values or low m% max. This made their evaluation in the mouse oGTT model difficult. The alkyl sulfonamides, on the other hand, seem to have better agonist activity. For example, sulfonamides **21k** and **21o** have low EC_{50} values and relatively high % max in both human and mouse GPR119 assays. The aromatic sulfonamides **21p** and **21q** have the desired low hEC$_{50}$s. However, their agonist activities were rather low as indicated by their low h% max.

Compounds **21a**, **21b**, **21k** and **21o** were evaluated in vivo in our mouse model of oral glucose tolerance test (oGTT). Test compound or vehicle was orally administered to overnight-fasted animals at 3 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. The glucose level of mice dosed with test compound was expressed as a percentage of the glucose level in vehicle treated mice (defined as 100%). Typically, highly efficacious compounds in this assay gave glucose levels of 80% or lower (e.g., compound **21** in Table 3). Compounds producing glucose levels of 95% or higher are usually not efficacious in a statistically significant way. Mouse plasma samples of drug-treated animals were collected at 1 h post dosing and analyzed for drug concentrations to get an estimate of the correlation between pharmacokinetics and pharmacodynamics of test compounds. Selected compounds were also evaluated in our rat pharmacokinetics assay[13] to assess their oral bioavailability and potential for further development. As shown in Table 3, carbamates **21a** and **21b** did not lower blood glucose level significantly in the mouse oGTT assay consistent with their low agonist activity (low m% max) for the mouse GPR119 receptor. The sulfonamide **21k** on the other hand was orally active in lowering blood glucose (87% of vehicle at 3 mpk). This is consistent with the potent EC_{50} value (63 nM) and high agonist activity (76% max) of **21k** in mouse. It is also noteworthy that compound **21k** had a high plasma level in mouse ($C_{1\ h}$ = 2.3 μM), much higher than its EC_{50} value in mouse. Compound **21k** was evaluated in a rat pharmacokinetics assay and it

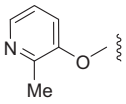
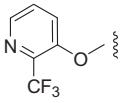
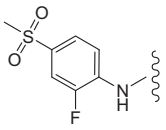
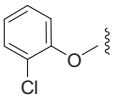
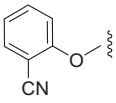
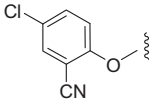
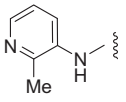
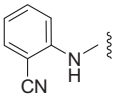
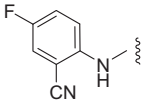
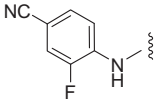
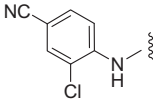


Scheme 2. (a) (i) 1,1,3,3-Tetrabromopropan-2-one, $ZnEt_2$, (ii) Zn–Cu;[15] (b) L-Selectride; (c) (i) p-NO_2PhCO_2H, DEAD, Ph_3P, (ii) NaOH, H_2O; (d) **19**, KO-t-Bu; (e) (i) TMSI, (ii) i-PrOCOCl and NEt_3; (f) $Pd(OAc)_2$, diazomethane; (g) (i) TMSI, (ii) c-$PrSO_2Cl$ and NEt_3; (h) (i) 9-BBN, (ii) H_2O_2; (i) DAST, heat; (j) DMSO, $(COCl)_2$ then NEt_3; (k) (i) **18**, NaH, (ii) TFA, (iii) c-$PrSO_2Cl$ and NEt_3; (l) ArOH, K_2CO_3, or $ArNH_2$, NaH, or $ArNH_2$, NaO-t-Bu, $Pd(dba)_2$, BINAP; (m) H_2O_2.

Table 5[a]



36a-j

Compound no.	R	*h*EC$_{50}$ (nM)	*h*% max	*m*EC$_{50}$ (nM)	*m*% max	oGTT[b]	$C_{1\ h}$ (nM)	Rat AUC$_{0-6\ h}$[c]
21k		24	57%	63	76%	87%[d]	2330	879
36a		2120	54%	NA	NA			
36b		166	89%	640	88%			
36c		44	81%	82	58%			
36d		7	58%	68	74%	88%[d]	290	461
36e		50	66%	290	44%			
36f		54	61%	2,000	110%			
36g		86	59%	180	80%			
36h		34	82%	820	110%			
36i		15	81%	220	84%	95%[e]	0	
36j		3	96%	140	110%	91%[d]	275	2170

[a] The standard deviations are <50% for EC$_{50}$ and <10% for % max.
[b] @3 mpk, po (% of vehicle).
[c] @10 mpk, po (nM/h).
[d] $p \leqslant 0.05$.
[e] $p > 0.05$.

showed moderate plasma level with an area-under-the-curve (AUC) from 0 to 6 h of 879 nM. The sulfoxide **21o** was orally active at a higher dose of 10 mpk (blood glucose 89% of control). Its plasma level at 1 hour time point was more than two times of its EC$_{50}$ value in mouse. However, in the rat pharmacokinetics assay, it did not give any appreciable plasma level.

We further explored the optimization of potency and oral bioavailability based on the sulfonamide **21k**. The two-carbon bridge of the nortropanol scaffold and the methylpyridinol substituent on the pyrimidine ring were modified as shown in Scheme 2 and Tables 4 and 5. For the structural changes on the two-carbon bridge of the nortropanol scaffold, they were not well tolerated.

Table 6

P450 enzyme inhibition IC_{50}	>20 μM for 3A4, 2D6, and 2C9 isozymes
PXR	0.09 @ 10 μM
hERG ionworks	9% @ 10 μM
GPCR-1	<30% @ 10 μM
Monkey PK, 3 mpk, 20%HPBCD	$AUC_{0-24\ h}$ = 16,548 nM/h; C_{max} = 997 nM; T_{max} = 4.5 h
Ames	Negative

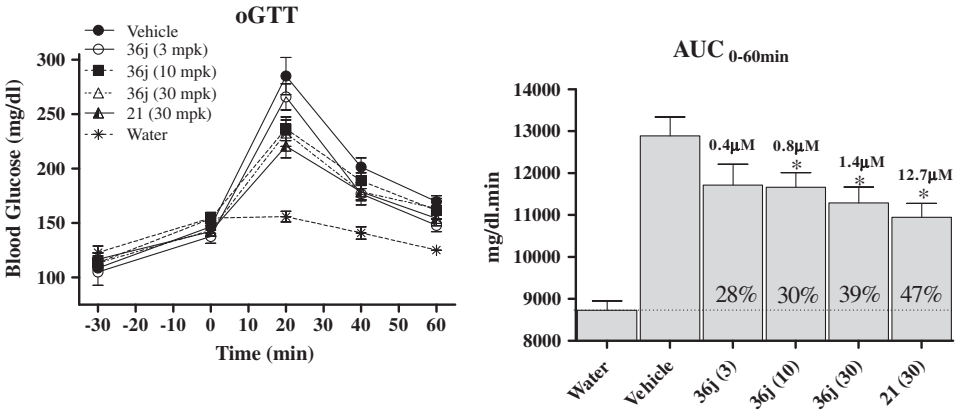


Figure 2. oGTT PK/PD (dose response) of compound **36j** in lean mice (10% NMP, 5% cremophor, and 20% HPβCD vehicle).

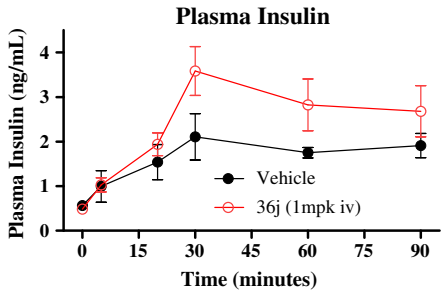


Figure 3. Compound **36j** increases insulin release during hyperglycemia (300 mg/dl) in lean, anesthetized rats.

As shown in Table 4, of the analogs synthesized (compounds **28**–**34**), only the unsaturated-bridge compound (**30**) gave good activity (low EC_{50}s and high % max). All of the other derivatives produced moderate to drastic reduction of activity. Changes to the methylpyridinol portion of **21k** were much more tolerated. These compounds (**36a**–**j**) were prepared from intermediate **35** by displacement reactions under a variety of conditions as shown in Scheme 2. The phenyl or pyridylhydroxy or amine reagents required for step *l* in Scheme 2 are mostly commercially available except the trifluoromethylpyridinol reagent **38** which was prepared by H_2O_2 oxidation of the corresponding boronic acid **37**.[14] Compounds **36a**–**j** showed excellent improvement in in vitro activities as exemplified by compounds **36d**, **36i**, and **36j**. Compound **36j** in particular showed a potent human GPR119 agonist activity with a 3 nM EC_{50} and 96% max. It was orally active at 3 mpk with a 1 h plasma concentration roughly twice its mouse EC_{50} value. It also had good plasma level in the rat pharmacokinetic assay. Compound **36d** also displayed a single digit nM *h*EC_{50} and orally active at 3 mpk. However its plasma level in the rat pharmacokinetic assay was rather low. Compound **36i** did not lower blood glucose level significantly orally in the mouse oGTT assay presumably due to its low exposure in mice ($C_{1\ h}$ = 0).

Compound **36j** was further evaluated in a number of in vitro and in vivo assays to assess its potential for further development as shown in Table 6 and Figures 2 and 3. It did not produce undesired activities in our standard enzyme inhibition, enzyme induction (PXR),[16] and hERG[17] assays. It is highly selective for the GPR119 receptor and inactive for ca. 40 other GPCRs in our in-house GPCR counter-screen assay. Compound **36j** showed excellent monkey pharmacokinetics when dosed at 3 mpk orally with a 0–24 h AUC of 16.5 μM and T_{max} of 4.5 h. In the Ames assay for carcinogenicity, compound **36j** turned out to be negative.

In a pharmacokinetic and pharmacodynamic dose-response study in lean mice (Fig. 2), compound **36j** showed a dose dependent lowering of blood glucose excursion (as represented by the excursion AUC from 0 to 60 min) with statistically significant lowering of 30% and 39% at 10 and 30 mpk, respectively. The plasma concentrations of **36j** increased approximately linearly (0.4, 0.8, and 1.4 μM) at 3, 10, and 30 mpk although not proportionally. Since GPR119 agonists have been reported to stimulate glucose-dependent insulin secretion,[4] we further studied the in vivo effect of compound **36j** on insulin in a rat (anesthetized) hyperglycemic clamp model (Fig. 3). The animals were anesthetized and glucose infusion was initiated at time 0 min. Once glucose levels reached the target concentration of 300 mg/dl (20 min), compound **36j** or vehicle was administered intravenously (1 mpk). As shown in Figure 3, the insulin level in the compound **36j** treated arm was significantly higher than the vehicle arm. Glucose was maintained at 300 mg/dl throughout the study; no change in glucose infusion rate was observed (data not shown), probably because hyperglycemia was clamped at a high concentration.

In summary, we have discovered a potent and orally active GPR119 agonist **36j** incorporating a nortropanol scaffold. Compound **36j** is a single digit nM GPR119 agonist with high magnitude of agonist activity. It is orally active in lowering blood glucose levels and was shown to increase plasma insulin levels in a hyperglycemic model. Compound **36j** also showed good to excellent pharmacokinetic properties in rats and monkeys and no untoward activities such as enzyme inhibition or enzyme induction. Compound **36j** demonstrated an attractive in vitro and in vivo profile for further development. The follow-up studies and results will be reported in due course.

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