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Discovery of fused bicyclic agonists of the orphan G-protein coupled receptor GPR119 with in vivo activity in rodent models of glucose control

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ABSTRACT

We herein outline the design of a new series of agonists of the pancreatic and GI-expressed orphan G-protein coupled receptor GPR119, a target that has been of significant recent interest in the field of metabolism, starting from our prototypical agonist AR231453. A number of key parameters were improved first by incorporation of a pyrazolopyrimidine core to create a new structural series and secondly by the introduction of a piperidine ether group capped with a carbamate. Chronic treatment with one compound from the series, **3k**, showed for the first time that blood glucose and glycated hemoglobin (HbA1c) levels could be significantly reduced in Zucker Diabetic Fatty (ZDF) rats over several weeks of dosing. As a result of these and other data described here, **3k** (APD668, JNJ-28630368) was the first compound with this mechanism of action to be progressed into clinical development for the treatment of diabetes.

We have recently described the identification of GPR119 (also termed glucose-dependent insulinotropic receptor, or GDIR) as a constitutively active, pancreatic β-cell-expressed G-protein coupled receptor (GPCR) whose activation can elevate intracellular cAMP levels in both transfected CHO cells and pancreatic β-cell lines.[1] Such activity would be expected to stimulate glucose-dependent insulin release from β-cells. In addition, we showed that GPR119 could stimulate incretin hormone release from cells localized in the GI tract and thus the receptor might be capable of regulating glucose homeostasis by multiple mechanisms.[2] These early target validation studies suggested that GPR119 may be an interesting target for the treatment of type 2 diabetes that may be amenable to the discovery of orally acting agents.

GPR119 can be activated by phospholipids and lipid amides in vitro,[3,4] but it remains unclear as to whether these are physiologically important ligands. A role for lipid amides in the activation of GPR119 could be implied by its phylogenetic proximity to cannabinoid receptors.[5] However, the potency and efficacy of these putative ligands at the GPR119 receptor is modest and some examples are also known to modulate other biological targets, including PPARα nuclear receptors and TRPV1 channels.[6] Lipid amides might also be expected to possess poor stability in vivo. Hence, the discovery of AR231453 (**1a**, Fig. 1) was essential for initial investigations into the role of this target in plasma glucose control.[7] However, whereas **1a** was an excellent tool for acute studies in mouse, it had a number of drawbacks. The relatively poor exposure in rat and the toxicity observed after multiple doses of **1a** in mouse (which may in part have been a consequence of the vehicle mixtures it was necessary to use as a result of poor solubility) meant that it was not suitable for examining the effect of GPR119 modulation in any of the known and well characterized chronic models of diabetes in rodents. Hence we undertook to look for alternative structures to demonstrate activity in proof of concept experiments of a chronic or sub-chronic nature, with the long term goal of identifying a compound suitable for clinical testing.

Starting from our prototypical GPR119 agonist **1a**, we were keen to investigate the effect of removing both the undesirable aniline and nitro portions of the molecule simultaneously by introducing a simple 6,5-fused ring system. We envisioned that such a modification would maintain several of the hydrogen bond acceptor interactions that we believed may be important for activity, such as the two nitrogens in the pyrimidine ring and the sulfone portion, and these would be held in a similar position to those in **1a** by introduction of this constraint to a key dihedral angle.[8] The resulting compound (**2**), which could be prepared in four

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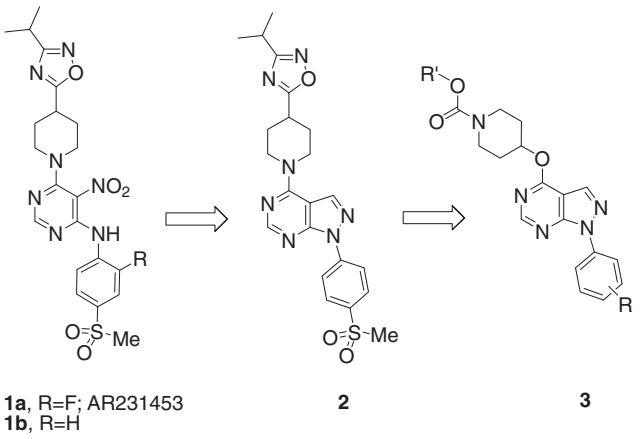
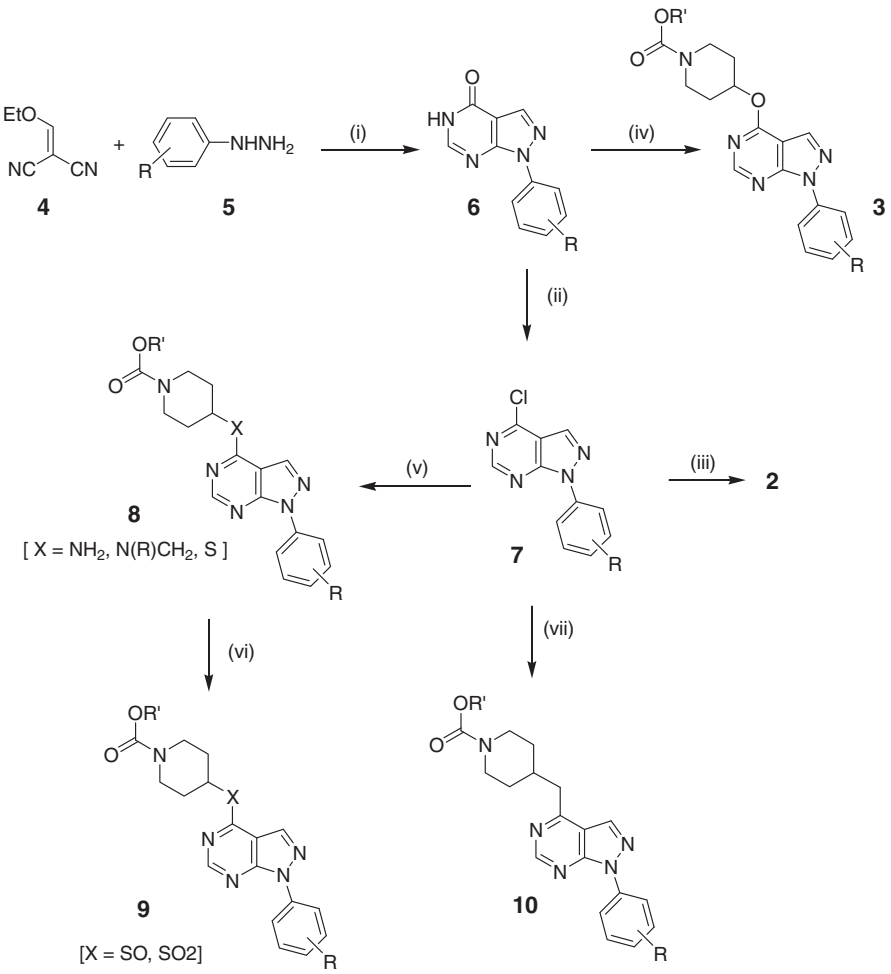


Figure 1. Design of bicyclic GPR119 analogues starting from AR231453.

steps by the route shown in Scheme 1, did retain significant agonist activity at the human GPR119 receptor (EC_{50} = 42 nM; n = 2 in the melanophore assay[7]) but was around 50-fold less potent than **1a** in our hands. Encouraged by this, we incorporated a second modification which we had previously shown to be a useful replacement for the oxadiazole piperidine portion of the monocyclic pyrimidine series into this pyrazolopyrimidine scaffold.[9] The reversed piperidine carbamate ether modification could be prepared from the same intermediate used to prepare **2** (Scheme 1).[10] Thus, **6** was reacted with the *tert*-butyl carbamate (Boc) derivative of 4-hydroxy piperidine under Mitsonobu conditions to furnish **3a** in reasonable yield. In contrast to **2**, **3a** was more similar in activity to **1a** (EC_{50} = 0.65 nM; n = 304) and the des-fluoro analogue **1b** (EC_{50} = 5.1 nM; n = 4) in our melanophore assay (Table 1). In addition, we could discern no significant difference in apparent efficacy between the compounds in this assay platform. Hence we set about exploring some of the aspects of the SAR around our new bicyclic scaffold GPR119 agonist.

We assumed in the first instance that the new series of compounds would be binding to the receptor in a similar orientation to the monocyclic series as implied by our scaffold design described above. Thus for our first investigation we maintained the 4-methylsulfone or 2-fluoro-4-methylsulfone substitution pattern on the southern aromatic ring portion and examined the effect of changes to the carbamate or linker portion (X in compound **8**). Compounds **3b–k** (Table 1) were prepared in the same manner as **3a** via Mitsonobu reaction of **6** with the appropriate carbamate derivative of 4-hydroxy piperidine, or alternatively could be prepared by removal of the Boc group from **3a** and reaction of the



Scheme 1. Synthesis of pyrazolopyrimidine analogues. Reagents and conditions: (i) (a) MeOH, reflux, (b) H_2O, HCO_2H, reflux; 70–85%; (ii) $POCl_3$, $PhNMe_2$, reflux, 30–70%; (iii) 4-(3-isopropyl-1,2,4-oxadiazol-5-yl)piperidine, NaH, THF. rt 65%; (iv) 4-hydroxypiperidine carbamates, DEAD, Ph_3P, THF rt, reverse addition; 40–60%; (v) 4-X-substituted piperidine carbamates, NaH, THF, rt; 40–80%; (vi) *m*CPBA, DCM, 75%; (vii) (a) *tert*-butyl 4-methylenepiperidine-1-carboxylate (prepared in 60% yield over two-steps from piperidine-4-one by standard Boc protection followed by Wittig reaction (CH_3PPh_3Br, ^nBuLi, 0 °C, 4 h)), 9-BBN, THF reflux; mixture added to **7** then (b) $PdCl_2(dppf)_2$, $AsPh_3$, K_2CO_3, DMF, H_2O, microwave, 150 °C, 20 min, 20%.

resultant secondary amine with the requisite chloroformate reagent to prepare the desired carbamate. Minor changes to the alkyl group of the carbamate did not result in significant changes in the agonist activity, and in some cases apparent efficacy, of the resulting molecules. However, it should be noted that secondary and primary alcohol carbamates were significantly more stable under acidic conditions than the Boc derivatives, making them much more suitable for dosing via the oral route. When the size of the alkyl group was further increased, however, (**3f**) a 3–10-fold decrease in potency was observed. Furthermore, the incorporation of heteroatoms into this portion of the molecule in an effort to improve water solubility, resulted in a decrease in both activity and efficacy (e.g., **3g**) and all activity was lost when we attempted to introduce basic groups into this region (e.g., **3i**). In contrast to the monocyclic series described previously, where we had seen a significant improvement in potency by introducing a fluoro substituent onto the southern aromatic ring,[7] the same modification in this bicyclic series where X=O were generally of similar potency and apparent efficacy to the des-fluoro analogue (e.g., **3j** and **3k**).

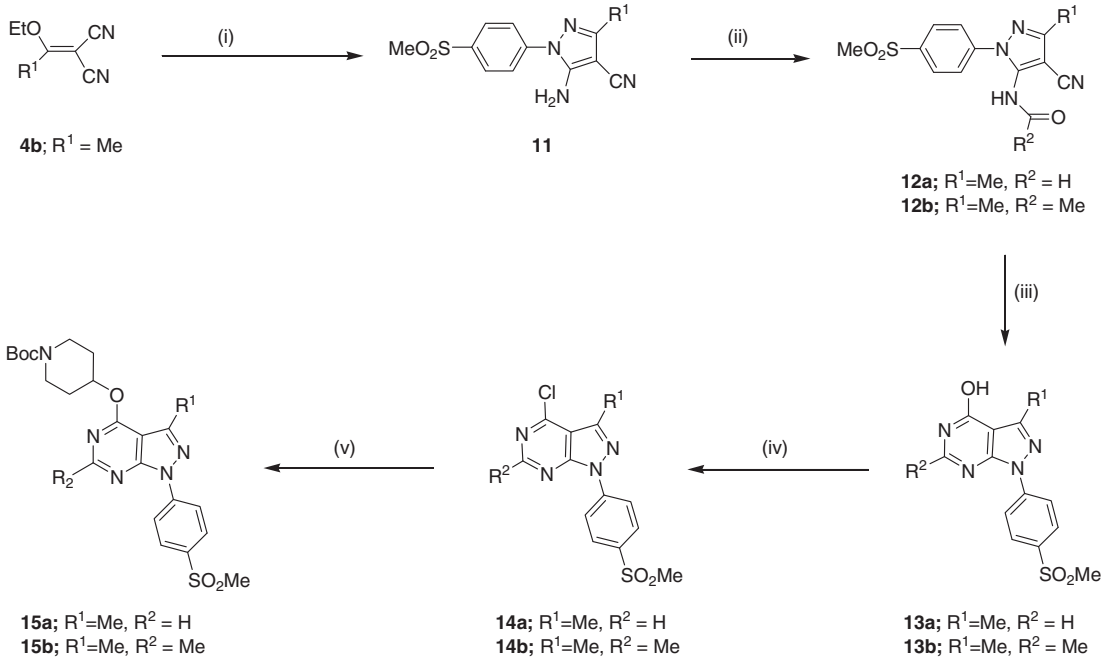
We next investigated the result of changing the linker atom or atoms between the bicyclic scaffold and the appended piperidine ring. Such compounds could be prepared by nucleophilic displacement of the 4-chloro substituent from the pyrazolopyrmidine **7** with a piperidine derivative containing the appropriate linker portion XH. The sulfoxide and sulfone linker derivatives were in turn prepared by oxidation of the thioether linked analogues (Scheme 1). The incorporation of a simple secondary amine or aminomethylene linker (**8a** and **8b**) was not well tolerated. However, in the case of the aminomethylene linker, the presence of an additional alkyl group on the nitrogen to provide the tertiary amine analogues (**8c–e**), again resulted in compounds with single digit nanomolar EC_{50} values. However, in contrast to the ether linked series, compounds of this type were subject to rapid metabolism both in vitro and in vivo and as a result, were not pursued further.

In a somewhat more conservative change, switching from the ether linker to the thioether had little effect on activity (**8f** and **8g**). However, oxidation of the thioether to either the sulfoxide (**9a**) or the sulfone (**9b**) linked analogues resulted in a significant decrease in activity.

Finally we examined the effect of replacing the ether linker with a methylene group. Compounds **10a** and **10b** were prepared in moderate yield from the intermediates **7** (R = 4-SO_2Me and 2-F, 4-SO_2Me, respectively) via a palladium catalyzed cross-coupling ($PdCl_2(dppf)_2$ in the presence of triphenyl arsine ligand) with a borane reagent prepared from the Wittig product of Boc-piperidin-4-one and methyltriphenylphosphonium bromide. Again, little difference in the activity between **10a** and **10b** and their ether linked counterparts was observed and as this series was significantly more difficult to prepare, it was not pursued further.

For the next stage of our SAR exploration, we examined a number of modifications to the bicyclic core portion of the molecule. Initially, we tried to apply highly conservative changes, such as the introduction of methyl groups onto the pyrazolopyrimidine core. Generally, such compounds could be prepared using very similar routes to those outlined earlier, but in most cases each of the intermediates was isolated, as shown in Scheme 2. For the preparation of the 3-methyl derivative **15a**, the pyrazole intermediate **11** (R^1 = Me) was prepared as shown and acylated with formic acid and the intermediate cyclized without isolation to provide **13a** in good yield. Similarly, for the preparation of **15b**, the acylation was carried out with acetyl chloride following which the intermediate **12b** (which was isolated) was subsequently cyclized to provide **13b**. Each of these intermediates could be converted to the test compounds by the usual sequence via reaction with phosphoryl chloride to give the aromatic chlorides (**14a** and **14b**) which underwent nucleophilic displacement with Boc-4-hydroxy piperidine under microwave conditions to provide **15a** and **15b**. However, each addition of a methyl group, first to the 3-position and then to the 6-position, resulted in an order of magnitude reduction in the agonist potency compared to **3a**.

We next prepared some alternative 6,5-fused bicyclic heterocycles to assess how more extensive changes to the core may affect activity at the receptor. The preparation of the first three examples from a common intermediate **17** is shown in Scheme 3.



4b; R^1 = Me

11

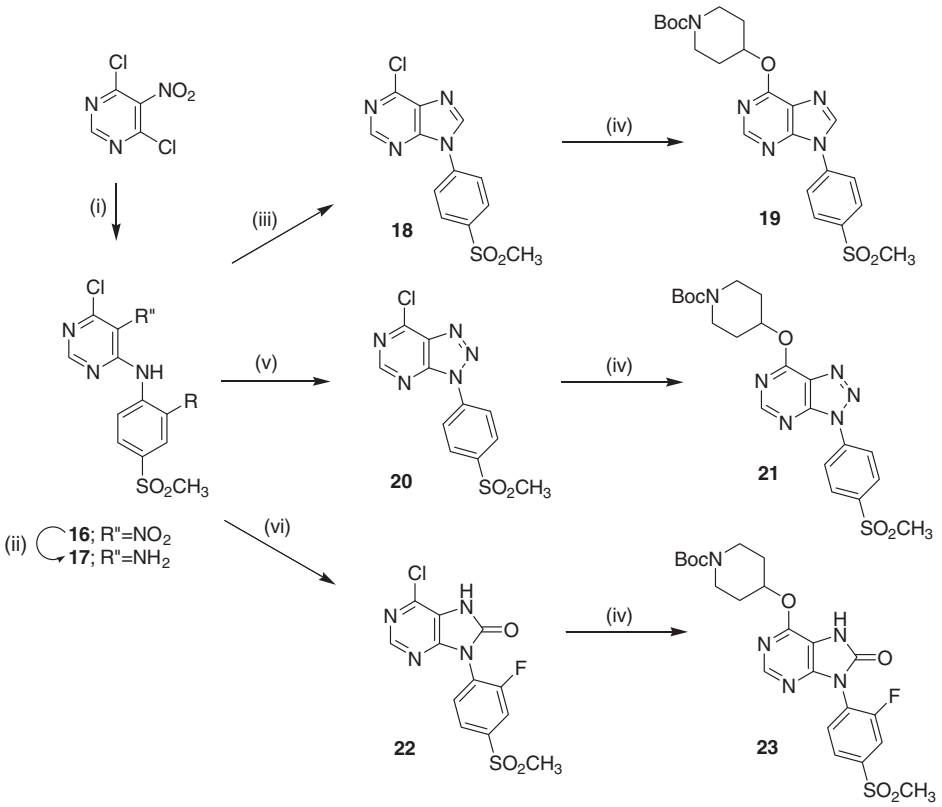
12a; R^1=Me, R^2 = H
12b; R^1=Me, R^2 = Me

13a; R^1=Me, R^2 = H
13b; R^1=Me, R^2 = Me

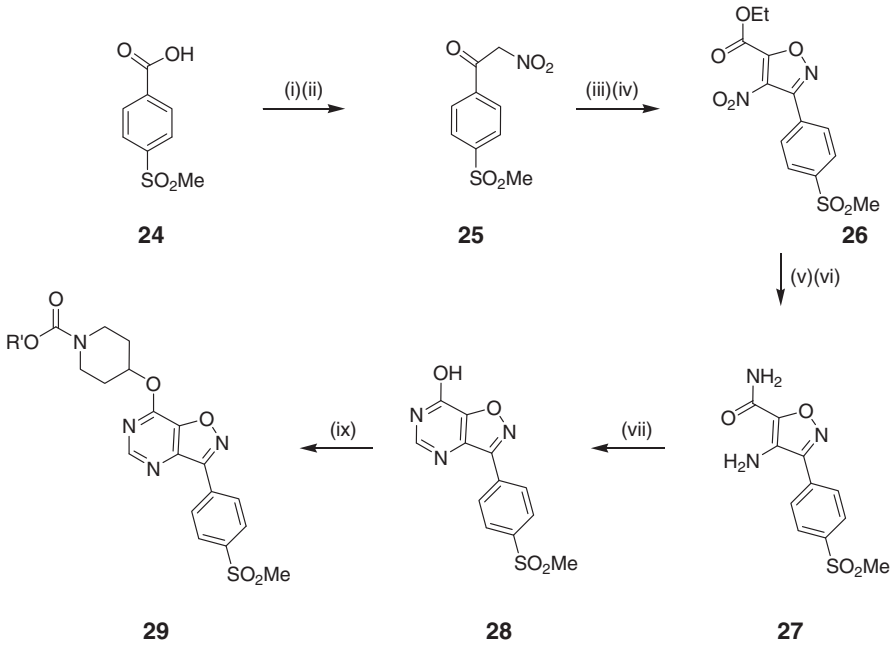
14a; R^1=Me, R^2 = H
14b; R^1=Me, R^2 = Me

15a; R^1=Me, R^2 = H
15b; R^1=Me, R^2 = Me

Scheme 2. Synthesis of substituted pyrazolopyrimidine analogues. Reagents and conditions: (i) (a) 4-$MeSO_2$-$PhNHNH_2$, NaOMe, MeOH, reflux, 70–90% yield; (ii) either AcCl (R^2 = Me) or HCO_2H (R^2 = H), (intermediate **12a** not isolated); (iii) H_2O, KOH, reflux; 30–50% yield two-steps; (iv) $POCl_3$, $PhNMe_2$, reflux; 50–60% yield; (v) 4-hydroxypiperidine carbamates, K_2CO_3, DMF 100 °C, microwave; 40–60% yield.



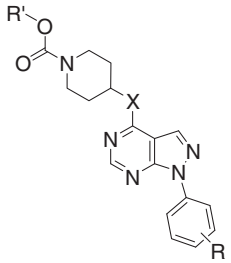
Scheme 3. Preparation of alternative nitrogen containing bicyclic analogues. Reagents and conditions: (i) 4-(methylsulfonyl)aniline (or 2-fluoro analogue), 0 °C, 90%; (ii) EtOAc, H_2, 10% Pd/C, rt, 85%; (iii) $C(OEt)_3$, Ac_2O, reflux, 45%; (iv) THF, NaH, 4-hydroxypiperidine carboxylic acid ^{t}Bu ester, rt 25–60%; (v) AcOH, $NaNO_2$, rt, 25%; (vi) $COCl_2$, 30%.



Scheme 4. Preparation of isoxazolopyrimidine analogues. Reagents and conditions: (i) (a) $COCl_2$, DCM, DMF 0 °C–rt; (b) phenol, Et_3N, 0 °C–rt; (ii) KO^{t}Bu, DMSO/CH_3NO_2, 0 °C–rt; (iii) EtOH, NH_2OH·HCl, AcOH, reflux; (iv) Et_2O, THF; EtOCOCOCl, rt; (v) satd NH_4Cl, Zn, rt; (vi) NH_4OH (aq), MeOH, THF, rt; (vii) $CH(OEt)_3$, Ac_2O, reflux; (viii) $POCl_3$, reflux; (ix) THF, NaH, and either, 4-hydroxypiperidine-1-carboxylic acid ^{t}Bu ester (to prepare **29a**) or 4-hydroxypiperidine-1-carboxylic acid ^{i}Pr ester (to prepare **29b**).

4,6-Dichloro-5-nitro-pyrimidine could be selectively reacted with 4-sulfone substituted anilines to provide **16** in good yield. Reduction of the nitro group by catalytic hydrogenation provided **17** in essentially quantitative yield. In practical terms, **17** had to be reacted immediately in the next step to avoid decomposition but the construction of three alternative heterocycles was possible form this starting point. Firstly, reaction with triethyl orthoformate in the presence of acetic anhydride provided the purine intermedi-

Table 1
SAR around the pyrazolopyrimidine core



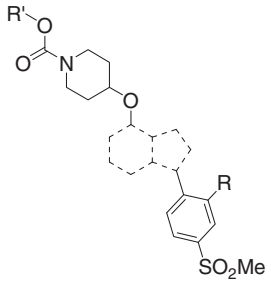
	R	X	R′	hGPR119 EC_{50}[a] (nM)	% E_{max}[b]	*n*	rGPR119 EC_{50}[a] (nM)	*n*
3a	4-SO_2Me	O	^{t}Bu	1.1	96	14	9.8	2
3b	4-SO_2Me	O	Et	3.8	91	3	53	3
3c	4-SO_2Me	O	^{i}Pr	3.5	83	6	n.d.	—
3d	4-SO_2Me	O	^{n}Bu	7.5	79	6	n.d.	—
3e	4-SO_2Me	O	^{i}Bu	4.2	99	5	n.d.	—
3f	4-SO_2Me	O	^{c}Pent	14.6	80	13	21	2
3g	4-SO_2Me	O	3-THF	435	71	3	n.d.	—
3h	4-SO_2Me	O	$CH_2{}^t$Bu	8.1	105	5	65	3
3i	4-SO_2Me	O	$CH_2CH_2NMe_2$	>10,000	—	3	n.d.	—
3j	2-F,4-SO_2Me	O	^{t}Bu	0.9	79	9	19.6	4
3k	2-F,4-SO_2Me	O	^{i}Pr	2.7	89	109	33	18
8a	4-SO_2Me	NH	^{t}Bu	250	101	3	>10,000	1
8b	4-SO_2Me	$NHCH_2$	^{t}Bu	265	87	3	n.d.	—
8c	4-SO_2Me	N(Me)CH_2	^{t}Bu	11.6	89	4	n.d.	—
8d	4-SO_2Me	N(Et)CH_2	^{t}Bu	6.3	67	7	n.d.	—
8e	2-F,4-SO_2Me	N(Et)CH_2	^{i}Pr	3.4	82	4	264	3
8f	4-SO_2Me	S	^{t}Bu	5.6	104	4	15.5	3
8g	2-F,4-SO_2Me	S	^{t}Bu	0.5	98	7	7.3	4
9a	4-SO_2Me	SO	^{t}Bu	2400	—	2	>10,000	1
9b	4-SO_2Me	SO_2	^{t}Bu	>10,000	—	6	>10,000	2
10a	4-SO_2Me	CH_2	^{t}Bu	7.3	91	15	120	5
10b	2-F,4-SO_2Me	CH_2	^{t}Bu	3.4	72	3	88	3

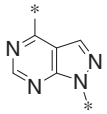
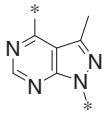
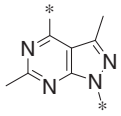
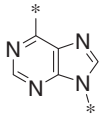
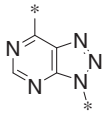
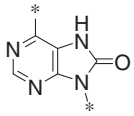
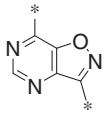
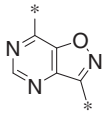
[a] Mean EC_{50} from multiple determinations (*n*) in melanophores transfected with either the human (hGPR119) or rat (rGPR119) sequence of the receptor.
[b] E_{max} = apparent maximum efficacy in the melanophore assay when the curve height was normalized to the positive control (AR231453; defined as a full agonist, that is, 100% efficacy).

ate **18** which could be reacted with Boc-4-hydroxypiperidine, under similar conditions to those used previously in the pyrazolopyrimidine series, to prepare **19**. An alternate cyclization of the diamine **17** could be effected with sodium nitrite under acidic conditions to provide the triazolopyrimidine **20**, which again could be converted to the test compound by reaction with Boc-4-hydroxypiperidine. Finally, the ring closure to form a five-membered ring could also be carried out with phosgene from the fluorinated intermediate (**17**, R = F) to provide the purinone **23** by way of the intermediate **22** (R = F). Each of these test compounds retained significant agonist activity at human GPR119 (Table 2), although **19** appeared to be somewhat less potent than either **21** or **23** or indeed the parent compound, **3a**. We hypothesized at this stage, that the bicyclic core was essentially interchangeable as long as it could display the key binding motifs (i.e., the hydrogen bond acceptor of the sulfone group on the southern aromatic ring and the piperidine carbamate in the northern portion) in the appropriate positions for interaction with the receptor. This was later confirmed to some extent, when it was reported that a very close analogue in which the bicyclic portion was a dihydropyrrolopyrimidine also retained significant activity at the receptor.[11] We also suspected however, that the core itself was making key binding interactions with the receptor. The somewhat reduced activity of the purine **19** suggested to us that a hydrogen bond acceptor functionality in the 2-position of the bicyclic ring system, as present in **3a**, **21** and **23**, was advantageous. This led us to propose the next target for synthesis, the isoxazolpyrimidine **29a** in which such a group was retained. The synthesis of this core was considerably more complex than for the other series and is outlined in Scheme 4. The key step in the preparation of **29a** was the acylation and subsequent cyclization of the oxime, prepared from **25**, with ethyl 2-chloro-2-oxoacetate to provide **26**. Reduction of the nitro group and conversion of the ester to the primary amide provided the intermediate **27** which could be converted to the bicyclic intermediate **28** with triethyl orthoformate in a similar manner to the ring closure reaction used for the pyrazolopyrimidine series. The sequence was completed by conversion of **28** to the chloro-heterocycle by treatment with $POCl_3$ and finally nucleophilic displacement with the appropriate carbamate protected 4-hydroxy piperidine. Again, the new heterocyclic core template provided compounds that retained significant activity at the human GPR119 receptor (Table 2).

At this stage, we wanted to show that the non-acid labile compounds (i.e., compounds with carbamates other than Boc on the piperidine group) that appeared to have activity in the low nanomolar range or below in our in vitro assay, would show similar acute activity in the oral glucose tolerance test (oGTT) to the prototypical GPR119 agonist, AR231453. So as to be able to correlate the in vivo data with receptor activity, we first tested a selected number of compounds, including several Boc derivatives, using the rat sequence of GPR119, again using the melanophore platform. In each case there was a significant 3–20-fold rightward shift in the rat receptor dose–response curves relative to the human receptor (Tables 1 and 2), in line with what was previously observed for AR231453.[7] Indeed, across our whole program in this area, we have consistently observed such a difference with very few significant outliers from this trend regardless of the core scaffold. The efficacy of the compounds (measured relative to **1a**) was however, similar between species in this assay platform. Despite

Table 2
Effect of core changes on GPR119 activity



	Core	R	R′	hGPR119 EC_{50}[a] (nM)	% E_{max}[b]	*n*	rGPR119 EC_{50}[a] (nM)	*n*
3a		H	^{t}Bu	1.1	96	14	9.8	2
15a		H	^{t}Bu	15	103	5	42	2
15b		H	^{t}Bu	116	90	7	n.d.	—
19		H	^{t}Bu	33.4	95	3	n.d.	—
21		H	^{t}Bu	7.4	103	4	42	2
23		F	^{t}Bu	0.6	64	4	n.d.	—
29a		H	^{t}Bu	0.7	100	7	17	5
29b		H	^{i}Pr	14	65	7	40	2

[a] Mean EC_{50} from multiple determinations (*n*) in melanophores transfected with either the human (hGPR119) or rat (rGPR119) sequence of the receptor.
[b] E_{max} = apparent maximum efficacy in the melanophore assay when the curve height was normalized to the positive control (AR231453; defined as a full agonist, that is, 100% efficacy).

this apparent species difference in agonist potency, **3k**, the most potent isopropyl carbamate in the pyrazolopyrimidine series and its isoxazole analogue **29b**, were both shown to have in vivo activity in both the acute mouse and rat oGTT after oral administration.[12] Compound **3k** appeared to be somewhat more active in this assay compared to **29b**, with a minimum efficacious dose of around 3 mg/kg (mouse) or 10 mg/kg (rat, Fig. 2). We speculated that this difference may be due to differences in compound adsorption through the gut, based on the observation that in our hands **3k** was more soluble in aqueous solutions then the isoxazolopyrimidine analogue. In any event, this greater in vivo potency coupled with the significantly simpler route needed to prepare **3k** led us to profile it more extensively to determine whether it could be a suitable tool for a broader examination of how GPR119 was involved in glucose control both acutely and under more chronic conditions.

We first confirmed in alternative in vitro platforms that **3k** was a potent and selective agonist of the receptor of interest. Thus, **3k** was shown to increase adenylate cyclase activation in HEK293 cells transfected with human GPR119 (but not in non-transfected

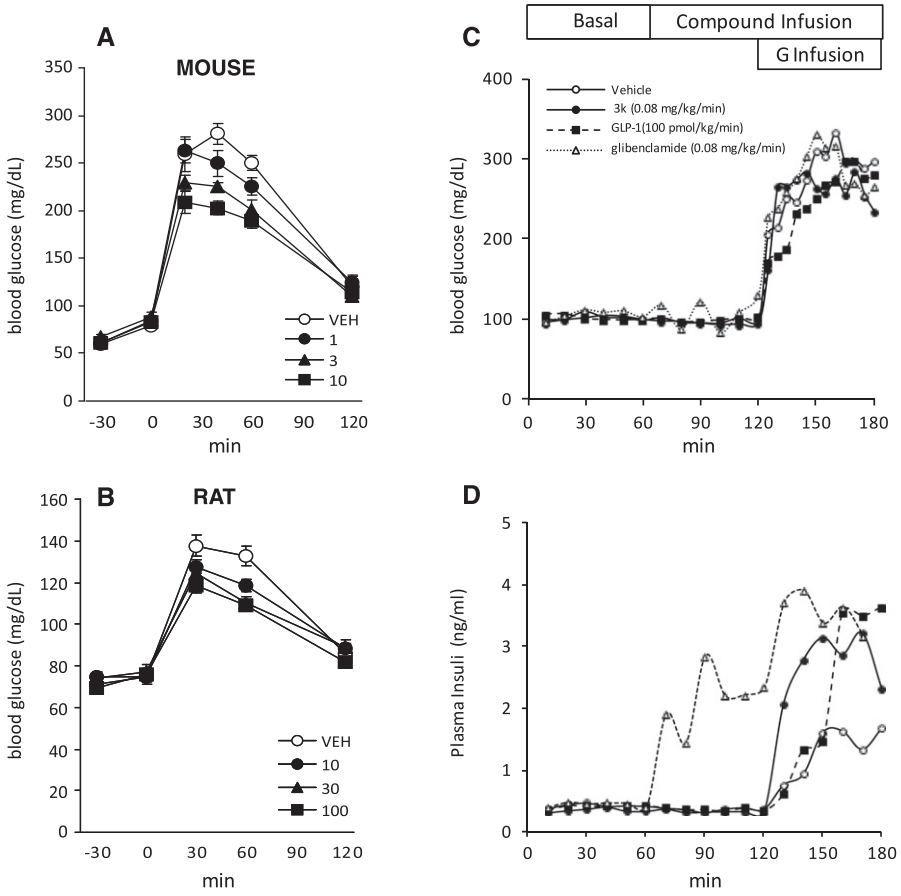


Figure 2. Effect of **3k** on oral glucose tolerance in c57/bl6 Mouse (Panel A) and Sprague–Dawley rat (Panel B), as well as hyperglycemic clamp in Sprague–Dawley rat (Panel C & D). Compound **3k** markedly reduced blood glucose levels during oral glucose tolerance test in a dose-dependent manner in both mouse and rat. In hyperglycemic clamp, **3k** showed no effect during euglycemic condition, but significantly stimulated insulin release when blood glucose levels were raised to approximately 300 mg/dl.

cells) in a concentration-dependent manner with an EC_{50} of 23 nM. Compound **3k** also enhanced insulin release from both rat and human isolated pancreatic islets in a glucose-dependent manner as previously observed for **1a**.[1] In a standard panel of around 80 known receptors and ion channels, **3k** did not show any binding in excess of 50% of control to any other proteins at concentrations up to 10 μM.

Compound **3k** was highly bound to plasma proteins of male and female cynomolgus monkeys and humans ($\geqslant$99%), but was less extensively bound to male (93.0%) and female (96.6%) rats. In human hepatic microsomes, **3k** showed no significant inhibition of any of the five major CYP isoforms with the exception of CYP2C9 (K_i = 0.1 μM). In addition, **3k** was a poor substrate of P-glycoprotein and was highly permeable across Caco-2 cell monolayers. Compound **3k** was not genotoxic in the in vitro bacterial/microsomal activation, mouse lymphoma, or the in vivo mouse micronucleus assays but did show moderate inhibition of the hERG channel in a patch clamp assay (IC_{50} = 3 μM).

In pharmacokinetic assessments across multiple species using single oral doses of **3k**, absorption was rapid to moderate ($t_{max} \leqslant$2 h) in mice, Sprague–Dawley (SD) rats, and monkeys, but slower in dogs (t_{max} = 6 h) and showed a dose-dependent increase in rats and monkeys. In general, exposure was dose-dependent at lower doses and appeared to plateau at doses greater than 300 mg/kg. Exposure was greater in female rats compared with males. Absolute oral bioavailability was moderate to good in mice, rats, and monkeys (44–79%), but was lower in dogs (22%). The volume of distribution (Vd_{ss}) values were somewhat variable ranging from 0.1 L/kg in monkey to 2.6 L/kg in rats. Elimination, based on mean $t_{1/2}$ after intravenous (iv) dosing, was rapid to moderate in mice, rats, dogs, and monkeys (0.8–3.9 h). The data from rat were used to confirm that good exposure was observed at the doses shown to have activity in the oGTT. Interestingly, the pharmacokinetic profile of **3k** in Zucker fa/fa rats was somewhat different from that in SD rats. After oral administration, the t_{max} and $t_{1/2}$ were longer, and the AUC and the oral bioavailability were greater in the Zucker compared with the SD rats. Following iv administration, the Zucker rats also had larger AUC values, longer $t_{1/2}$ values and greater Vd_{ss} values. In animal models under euglycemic conditions, **3k** had no effect on plasma insulin and blood glucose levels at doses up to 30 mg/kg. However, **3k** significantly improved blood glucose handling during glucose challenge in several diabetic and non-diabetic rodent models. In addition **3k** (0.08 mg/kg/min iv) showed a clear glucose-dependent effect on insulin release in a hyperglycemic clamp model in the Sprague–Dawley rat (Fig. 2).[13]

With these data in hand, we examined the effect of chronic treatment with **3k**, which had not been possible with previous receptor ligands, in Zucker Diabetic Fatty (ZDF) rats. Compound **3k** significantly reduced blood glucose and glycated hemoglobin (HbA1c) levels over eight weeks of treatment (QD), with no desensitization of the acute drug response over this period at 30 mg/kg po.[14] Taken together, these profiling, pharmacokinetic and pharmacology data showed for the first time that chronic activation of the GPR119 receptor with a selective agonist could have a positive effect on glycemic parameters and at least with **3k**, was not subject to any major concerns regarding tachyphylaxis that has been associated with prolonged activation of other GPCRs. These observations, coupled with the acute effect observed in an oGTT in cynomolgus monkeys (10 mg/kg po),[15] led us to progress **3k** (APD668, JNJ-28630368) into clinical studies as a first-in-class

GPR119 agonist to examine the effects of modulation of this receptor in humans. The clinical data will be reported elsewhere in due course.

References and notes

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12. Animals were grouped (rats 2/cage, mice 4/cage) in regular cages with bedding under normal light conditions (lights on 6:30 am–6:30 pm). They were given ad libitum access to food and water. Animals were allowed to acclimate to the facility for several days before handling.
    Procedures:
    Mouse: Following two handling sessions, animals were fasted for 3–16 h.
    The oral glucose tolerance test (OGTT) was executed as follows: Compound was administered 0–30 min prior to first blood sample. At time 0, a tail nick and glucose test was performed with a hand held glucometer, then a bolus of glucose (2 mg/kg, po) was administered. Glucose was again tested at 20, 40, 60, and 120 min post glucose administration. During the entire oGTT, a total volume of approximately 10 drops of blood was collected. After the sample at 120 min, the animals were euthanized.
    Rat: Animals are fasted 3–16 h.
    The oral glucose tolerance test (OGTT) was executed as follows: At time −30 min, blood was collected via a tail nick and a glucose test performed with a hand held glucometer, and compound was then administered. At time 0, blood was again collected for glucose reading and then a bolus of glucose (3 g/kg po, 6 ml/kg) administered. Blood glucose levels were further tested at 30, 60, and 120 min post glucose administration. During the entire oGTT, a total volume of approximately eight drops of blood was collected. Rats were used twice with a one week lapse between experiments. After the second test, the animals are euthanized.
13. *Hyperglycemic clamp method*: Male SD rats were fasted overnight before the clamp experiment. After 1 h of baseline blood glucose and plasma insulin level data collection, vehicle or compound was infused into the jugular vein for 2 h. GLP-1 or glybenclamide were infused in different groups of rats as the positive control. Blood glucose levels were maintained at 100 mg/dl for the first and second hour of clamp study. For the third hour of clamp study, the blood glucose levels were increased to, and maintained, at 300 mg/dl via a bolus of 30% glucose injection followed by constant glucose infusion with various infusion rates.
14. Male ZDF rats (6 weeks old, baseline body weight of 200–250 g) were divided into 3 treatment groups based upon fed body weight and blood glucose levels. Blood glucose and HbA1c levels were determined on Days 1, 31, and 56 of vehicle (100% PEG400) or **3k** treatment. Blood glucose and HbA1c levels in rats treated with **3k** at 30 mg/kg/day were significantly decreased, thus these rats did not develop diabetes, whereas, the vehicle treated rats did.

Effect of chronic 56-day administration of **3k** on blood glucose and hba1c levels in ZDF rats

	Blood glucose (mg/dL, Day 56)	HbA1c (%)			Body weight gain (g)
		Day 1	Day 31	Day 56	
Vehicle, (100% PEG400) (n = 5)	408.0 ± 46.4	3.6 ± 0.2	7.3 ± 0.4	7.4 ± 0.8	206.8 ± 15.4
3k, 10 mg/kg (n = 6)	359.3 ± 49.3	3.5 ± 0.1	7.3 ± 0.6	8.2 ± 1.0	224.9 ± 4.1
3k, 30 mg/kg (n = 7)	105.2 ± 4.9[a]	3.4 ± 0.1	5.2 ± 0.1[a]	4.7 ± 0.1[a]	204.2 ± 3.5

Data represent the mean ± standard error of eight values.
HbA1c = glycated hemoglobin; n = number of subjects.
[a] Statistically different compared with that in vehicle treated group (T-test, $P < 0.05$).

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