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Discovery of *N*-arylpyrroles as agonists of GPR120 for the treatment of type II diabetes

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ABSTRACT

The discovery of a novel series of *N*-arylpyrroles as agonists of GPR120 (FFAR4) is discussed. One lead compound is a potent GPR120 agonist, has good selectivity for related receptor GPR40 (FFAR1), has acceptable PK properties, and is active in 2 models of Type 2 Diabetes in mice.

The prevalence of type 2 diabetes is increasing and has become a public health crisis in much of the world.[1] The disorder is characterized by hyperglycemia, insulin-secreting β-cell dysfunction, and insulin resistance.[1] Furthermore, many of the co-morbidities associated with diabetes, such as cardiovascular disease, kidney disease, neuropathy, and retinopathy, are mediated by inflammatory processes that are common in diabetics.[2]

GPR120 (Free Fatty Acid Receptor 4) is a 7-transmembrane GPCR that is activated by omega-3 fatty acids and is mainly expressed in the intestine, adipose, and macrophages.[3] The activation of GPR120 has been implicated in a number of processes such as release of gastrointestinal peptides including glucagon-like peptide-1 (GLP-1), adipogenesis, lipogenesis, and glucose tolerance.[3] Additionally, GPR120 is postulated to mediate anti-inflammatory and insulin-sensitizing effects in the adipose and macrophages.[3] These benefits suggest that GPR120 agonists have the potential to be an effective treatment for type 2 diabetes.

Thiophene **1** and isothiazole **2** were existing leads from our GPR120 agonist program (Fig. 1).[4] While continuing investigations of those series, we were probing various substitutions of the central 5-membered heterocyclic ring to see if changing the heterocycle would give an increase in potency over **1** and **2**, or improved ADME or pharmacokinetic properties compared to the existing leads. In this vein, we decided to explore *N*-arylpyrroles **3** as GPR120 agonists.[5]

The initial SAR from the isothiazole series indicated that electron-withdrawing substituents on the pyrrole ring at the 3-position, in particular CF_3, were favored for GPR120 agonist activity.[4] In addition, concerns about the instability of the electron-rich pyrrole ring compel the incorporation of electron-withdrawing groups as well.[6] Synthesis of the 3-trifluoromethylpyrrole analogs starts with the bromination of *N*-phenylsulfonylpyrrole **4** at the 3-position followed by installation of the 2-carbomethoxy to give **5** (Scheme 1).[5,7] Treatment with methyl 2,2-difluoro-2-(fluorosulfonyl)acetate in the presence of copper iodide gives an inseparable mixture of **5** and **6**.[8] Reduction of the bromine of **5** with hydrogen and Pd/C enables the isolation of **6** from the undesired reduced product. Finally, deprotection of the phenylsulfonyl with TBAF gives the desired methyl 3-(trifluoromethyl)-1H-pyrrole-2-carboxylate **7.** Treatment with an arylboronic acid in the presence of copper acetate and pyridine gives the *N*-arylpyrrole **8**.[9] Reduction of the ester with LAH followed by Mitsunobu reaction gives **10**. Hydrolysis of the ester gives the desired acid **11**, while reduction of the ester with LAH gives the alcohol **12**.

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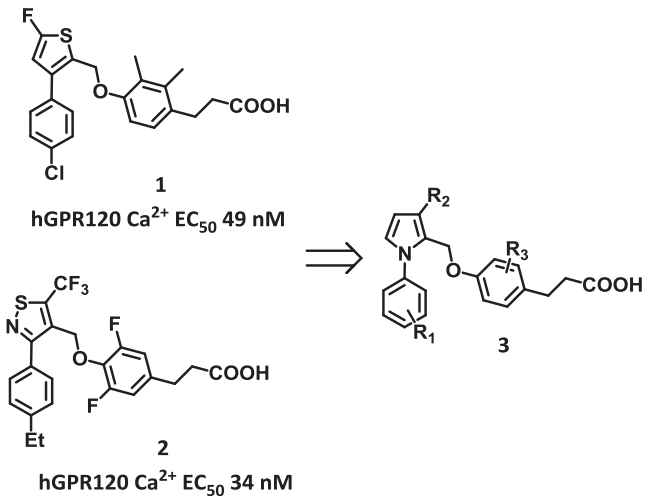
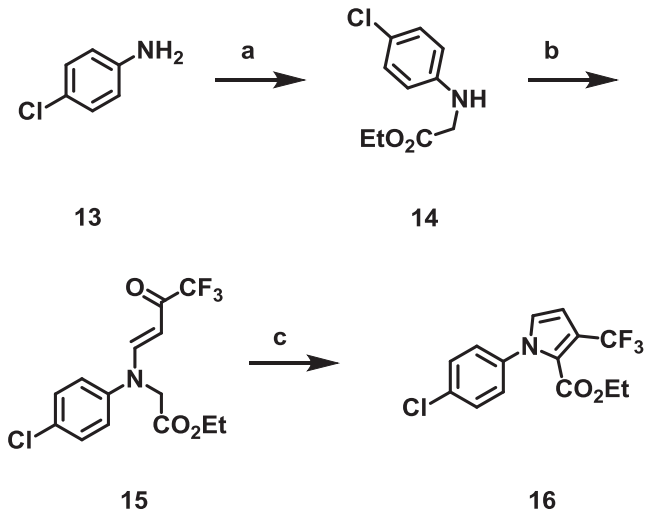


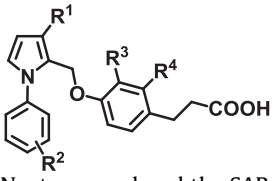
Fig. 1. Heterocyclic GPR120 Agonists.



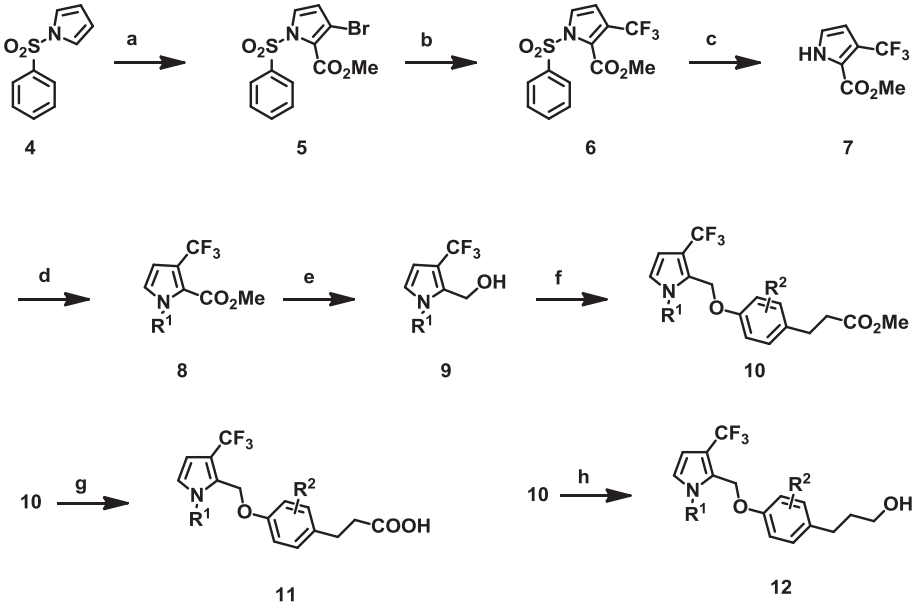
Scheme 2. Reagents and Conditions: (a) ICH_2CO_2Et, NaOAc; (b) $EtOCHCHCOCF_3$, DCM, 40 °C; (c) DBU, EtOAc, 120 °C, sealed tube.

Because the trifluoromethylation step from the synthetic scheme above was not amenable to scale-up, an improved synthesis of intermediate **16** was developed (Scheme 2). Condensation of 4-chloroaniline **13** with ethyl 2-iodoacetate gives **14**. Addition to 4-ethoxy-1,1,1-trifluorobut-3-en-2-one gives **15**, which is cyclized with DBU in moderate yield to give the desired pyrrole **16**.[10] This new route enabled the multi-gram synthesis of lead compounds for *in vivo* studies.

We first made a selection of compounds with substitution at the 3-position of the pyrrole to explore the SAR of this position (Table 1). At this early stage, we only made compounds with the 2,3-dimethyl- or 2,3-difluoro- substitution on the phenylpropionic acid moiety because these groups were known to afford good potency in other series. The unsubstituted pyrrole **17** was active in the calcium mobilization assay, but discolored over time indicating air oxidation of this compound. Installing bromo **18** and chloro **19** at this position gave a similar level of potency, with the added benefit of air stability. The cyano group **20** gave a large loss in potency while the CF_3 group **21** and **22** gave a 2-fold boost in hGPR120 potency and was air stable. This established the CF_3 as an optimal functional group at the 3-position of the pyrrole ring and was used in further analogs.



Next, we explored the SAR around the aryl ring attached to the pyrrole nitrogen in the presence of the 3-CF_3 and the 2,6-difluorophenylpropionic acid sidechain (Table 2). The EC_{50} value of the unsubstituted phenyl **23** was about 100 nM. The 4-methyl **24** was 2-fold more potent, however ethyl **25** and bromo **26** did not show improvement over the phenyl. 4-chloro **27** was a slight improvement, but most other substitutions on the ring resulted in a loss of potency in the calcium assay. Addition of a pyridyl nitrogen at the 3-position also gave a loss in activity compared to the phenyl.



Scheme 1. Reagents and Conditions: (a) (i) Br_2, HOAc; (ii) LDA, MeOCOCl; (b) (i) $FSO_2CF_2CO_2CH_3$, CuI, NMP, 80 °C; (ii) H_2, 10% Pd/C, EtOH; (c) TBAF, THF; (d) $R^1B(OH)_2$, $Cu(OAc)_2$, pyridine, DCM; (e) LAH, THF, 0 °C; (f) R^2-phenol, ADDP, Bu_3P, toluene, 60 °C; (g) LiOH, THF, MeOH, water; (h) LAH, THF, 0 °C.

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Table 1
SAR of the 3-position of the pyrrole.

Cpd. No.	R^1	R^2	R^3	R^4	hGPR120 Ca^{2+} EC_{50} (nM)[a]
17	H	4-Br	Me	Me	164
18	Br	4-Cl	F	F	161
19	Cl	4-Cl	Me	Me	173
20	CN	4-Cl	F	F	1370
21	CF_3	4-Cl	Me	Me	88
22	CF_3	4-Cl	F	F	91

[a] hGPR120 Ca^{2+} assay in HEK-293 cells (n > 2, average values, SEM < ±25%).

Table 2
SAR of the *N*-aryl/heteroaryl group.

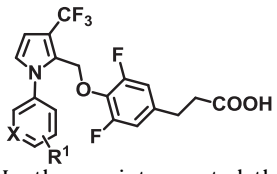
Cpd. No.	R^1	X	hGPR120 Ca^{2+} EC_{50} (nM)[a]
23	H	CH	101
24	4-Me	CH	50
25	4-Et	CH	117
26	4-Br	CH	112
27	4-Cl	CH	80
28	4-F	CH	134
29	4-OMe	CH	184
30	3-F	CH	448
31	3-F-4-Cl	CH	368
32	3-F-4-Me	CH	262
33	2-F-4-Cl	CH	179
34	2-F-4-Me	CH	118
35	2,4-di-F	CH	156
36	3,4-di-F	CH	175
37	4-Me	N	289
38	4-Et	N	877
39	4-MeO	N	197

[a] hGPR120 Ca^{2+} assay in HEK-293 cells (n > 2, average values, SEM < ±25%).

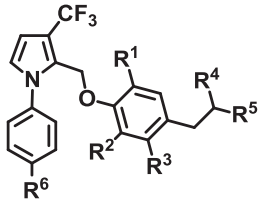
Table 3
SAR of the phenylpropionic acid sidechain.

Cpd. No.	R^1	R^2	R^3	R^4	R^5	R^6	hGPR120 Ca^{2+} EC_{50} (nM)[a]
27	F	F	H	H	COOH	Cl	80
40	F	F	H	H	CH_2OH	Cl	43
21	H	Me	Me	H	COOH	Cl	88
41	H	Me	Me	H	CH_2OH	Cl	533
22	H	F	F	H	COOH	Cl	91
42	H	F	F	H	CH_2OH	Cl	162
43	H	F	F	H	COOH	Et	40
44	F	F	H	Me	COOH	Cl	290
45	F	F	H	Me	COOH	F	547
28	F	F	H	H	COOH	F	134
46	Me	H	H	H	COOH	F	280
47	H	Me	Me	H	COOH	F	346
48	H	H	CF_3	H	COOH	F	834
49	H	H	Cl	H	COOH	F	923
50	F	F	H	H	COOMe	Cl	>5000

[a] hGPR120 Ca^{2+} assay in HEK-293 cells (n > 2, average values, SEM < ±25%).



Lastly, we interrogated the SAR of the phenylpropionic acid sidechain (Table 3). The EC_{50} value of the 2,6-difluoropropionic acid **27** was 80 nM, while reduction to the propanol sidechain **40** gave a boost in activity to 43 nM. Conversely, when the substitution on the phenyl was modified to 2,3-dimethyl **21** or 2,3-difluro **22**, reduction to the propanol **41** and **42** led to a decline in potency over the parent acid. The combination of 2,3-difluoro substitution with the introduction of an ethyl group at R^6 **43** gave an EC_{50} of 40 nM, the most potent compound in the series. Methyl substitution was introduced on the carbon α- to the acid **44** to try to improve metabolic stability, but disappointingly gave a 3-fold loss in potency. When R^6 is F **28**, the 2,6-difluorophenylpropionic acid derivative had an EC_{50} of 134 nM. Changing the 2,6-di-F to 2-methyl **46** or 2,3-di-methyl **47** gave a 2- to 3-fold loss in activity. Substitution at the 3-position of the central aryl ring with CF_3 **48** or Cl **49** afforded analogs with poor potency, while the methyl ester **50** resulted in a complete loss of hGPR120 activity.



Early ADME data was used to try to prioritize the more potent compounds for pharmacokinetic studies and ultimately *in vivo* glucose-lowering experiments. Three of the more potent compounds (**24**, **27** and **43**) from the primary screen were profiled for their cytochrome P450 inhibition (Cyp), their microsomal stability (Intr. Cl $t_{1/2}$) and their plasma protein binding (PPB) (Table 4). All 3 compounds had low cytochrome P450 inhibition with slight activity against 2C9 and 2C19, and all were highly plasma protein bound in rat, mouse, and human. The one distinguishing factor for

Table 4
Cyp inhibition, microsomal stability and PPB for selected compounds.

Cpd. No.	hGPR120 Ca^{2+} EC_{50} (nM)	Cyps 3A4, 2D6, 1A2 (μM)	Cyp 2C9 (μM)	Cyp 2C19 (μM)	Intr. CL human $t_{1/2}$ (min)	Intr. CL mouse $t_{1/2}$ (min)	Intr. CL rat $t_{1/2}$ (min)	PPB human (% bound)	PPB mouse (% bound)	PPB rat (% bound)
24	50	>10	4.2	>10	7	85	<4	99.92	99.59	99.85
27	80	>10	7	10	51	155	132	99.92	99.63	99.83
43	40	>10	7	9	15	7	<4	99.98	99.86	99.92

Table 5
PK of **27** in mouse, rat and dog.

Species	IV dose (mg/kg)	IV $t_{1/2}$ (h)	IV CL (mL/min/kg)	IV V_{ss} (L/kg)	PO dose (mg/kg)	PO $t_{1/2}$ (h)	PO C_{max} (ng/mL)	PO AUC_{last} (h*ng/mL)	F (%)
Mouse	1.15	4.14	4.9	1.14	14.6	3.45	18,667	81,714	166
Rat	2	1.64	3.2	0.42	10	2.87	11,632	60,877	116
Dog	1	4.72	2.45	0.52	5	4.37	8923	47,897	145

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compound **27** was its much lower intrinsic clearance in human, mouse and rat liver microsomes versus compounds **24** and **43**, most likely due to the oxidation of the 4-alkyl substituents. Metabolite ID studies of **27** showed that the major metabolites were cleavage of the central ether linkage and β -oxidation of the parent propionic acid sidechain, while the acyl glucuronide of the parent was detected in minor amounts. Due to its suitable metabolic profile, compound **27** was chosen for further studies.

The *in vitro* activity of **27** was assessed in multiple species and cell lines to determine if it was suitable for *in vivo* experiments. The human GPR120 Ca^{2+} EC_{50} in the primary high-expressing HEK-293 transfected cell line was 80 nM, while the potencies for rat and mouse were 713 nM and 193 nM respectively. The human β -arrestin EC_{50} in a CHO-K1 cell line was 69 nM, while the human Ca^{2+} EC_{50} in HT-29 cells, a low-expressing endogenous cell line, was 137 nM. Together, this data indicated that **27** should be sufficiently potent to see *in vivo* effects in mice, but perhaps not in rats due to its high rEC_{50}.

We were also interested in the selectivity of **27** for GPR120 over the related GPR40 (FFAR1) receptor.[11] In humans, the selectivity was found to be about 42-fold, with an hGPR40 EC_{50} of 3340 nM, while the mouse GPR40 EC_{50} was found to be 3520 nM, 18-fold selectivity. Although **27** maintains some potency for GPR40, we believed that this low level of activity would not compromise our efforts to observe clear effects of GPR120 agonism *in vivo*. To evaluate selectivity in a more general sense, **27** was submitted for testing against 50 GPCR's, ion channels, and transporters. All were below 50% inhibition except for 5HT2b (62% inhibition), 5HT5a (58%), norepinephrine transporter (51%) and Cl^- channel (66%) at 10 μM concentration.

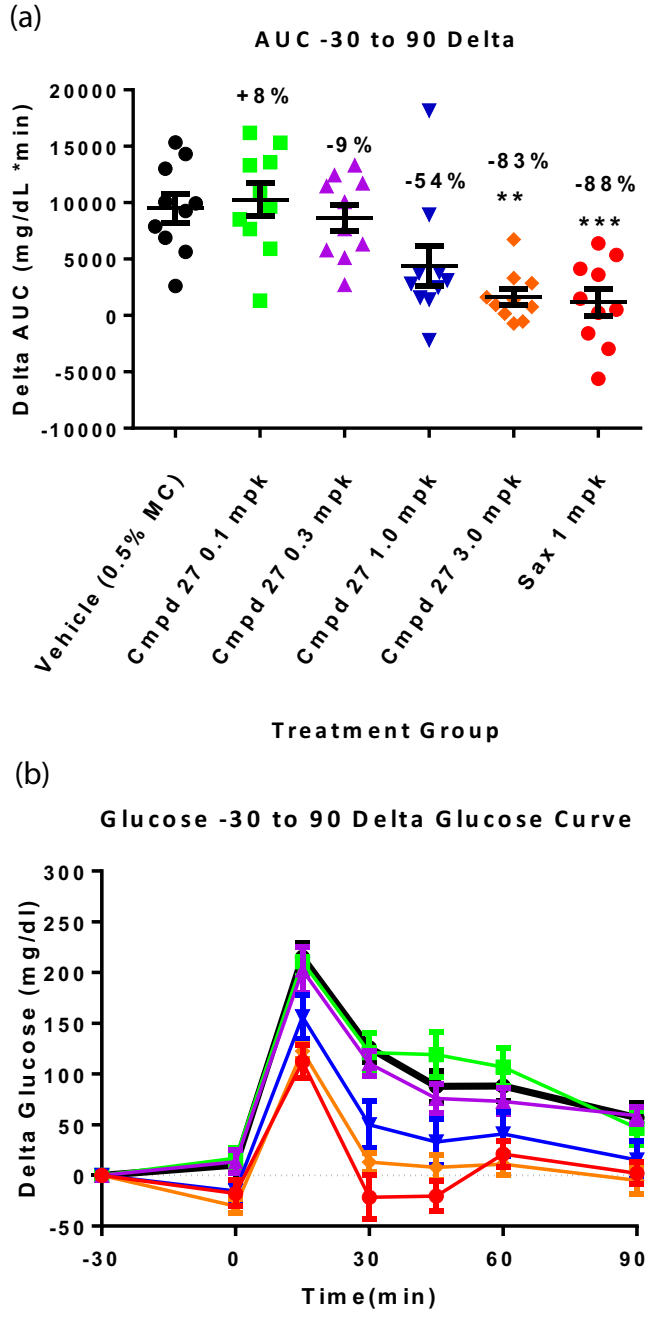

Fig. 2. Acute oGTT of **27** in DIO mice (*** = P < 0.001, ** = P < 0.01 1 Way ANOVA w/Tukey post test).

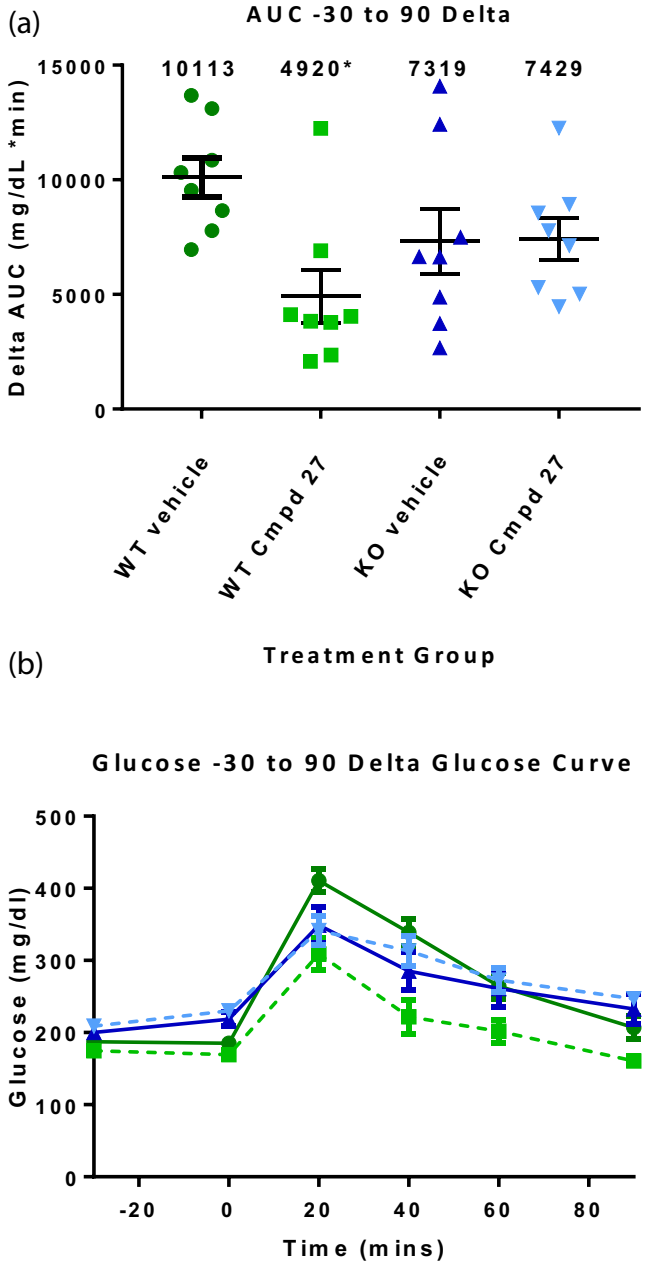

Fig. 3. Acute ipGTT in wild-type and GPR120 knockout mice (* = P < 0.05 1 Way ANOVA w/Tukey post test).

Table 6
27 in a 2-week DIO mouse model of type 2 diabetes.

Group	Number of mice	Glucose baseline: mean mg/dL (*SD*)	Glucose final: mean mg/dL (*SD*)	Glucose vehicle-subtracted LS mean Change (*95% CI*)	Insulin baseline: Mean pg/mL (SD)	Insulin final: mean pg/mL (SD)	Insulin vehicle-subtracted LS mean change (95% CI)
Vehicle	11	187 (*24*)	184 (*30*)	–	16,959 (*4943*)	7304 (*2967*)	–
27 10 mg/kg QD	11	202 (*35*)	163 (*13*)	−23.7 (*−48.5; 1.1*)	17,395 (*5930*)	7756 (*3357*)	+457 (*−2498; 3412*)
27 10 mg/kg BID	10	203 (*35*)	170 (*27*)	−16.4 (*−41.9; 9.1*)	16,966 (*7312*)	6225 (*2744*)	−1079 (*−4106; 1948*)
27 30 mg/kg QD	10	201 (*36*)	151 (*16*)	**−35.6**[a] (**−61.0; −10.2**)	17,067 (*6144*)	3856 (*1133*)	**−3447**[a] (**−6474; −420**)
Rosi 3 mg/kg	11	188 (*29*)	148 (*12*)	**−36.6**[a] (**−61.1; −12.0**)	15,956 (*6091*)	1348 (*25 0*)	**−5969**[a] (**−8928; −3011**)

[a] Significantly different from vehicle.

The pharmacokinetics of **27** was obtained in mouse, rat and dog to determine if it had a suitable profile to investigate the *in vivo* effects of the GPR120 mechanism (Table 5). **27** had an adequate half-life, low clearance in all 3 species, and high blood levels and bioavailability. The low volume of distribution (V_{ss}) indicates that **27** mainly resides in the plasma compartment, most likely due to its high plasma protein binding. Although not ideal, this profile was thought to be sufficient for *in vivo* studies in rodents.

An anesthetized guinea pig study was performed to gauge the cardiovascular safety of **27**. There was no effect on the PQ, QRS, QT or QTcB intervals, heart rate, body temperature or ECG morphology, however **27** did significantly decrease mean arterial blood pressure by 10% relative to vehicle at 22.4 mg/kg (C_{max} 105,000 ng/mL), the highest concentration tested.[12]

Next, compound **27** was evaluated in an oral glucose tolerance test (oGTT) in diet-induced obese (DIO) mice to assess the consequences of GPR120 agonism in an acute model of type 2 diabetes (Fig. 2). **27** was dosed orally at doses from 0.1 to 3.0 mg/kg 30 min (time-30) prior to the oral glucose administration (time 0), and glucose levels were measured from 30 min pre-glucose to 90 min post-glucose. **27** lowered glucose in a dose-dependent manner, significantly lowering glucose at 1 and 3 mg/kg. The 3 mg/kg dose lowered glucose to an extent similar to saxagliptin, a DPP-4 inhibitor positive control, at 1 mg/kg.[13]

To confirm that the glucose-lowering effect of compound **27** was indeed on target, an intra-peritoneal GTT was performed at 10 mg/kg in GPR120 knockout mice and in wild-type mice (Fig. 3).[4a] While **27** lowered glucose to a significant extent in the wild-type mice, it did not show any appreciable difference in acute glucose-lowering in the GPR120 KO mice compared to vehicle thus confirming that the glucose-lowering effect is due to agonism of GPR120, and not an off-target effect from GPR40, for example.

Next, compound **27** was assessed in a 2-week DIO mouse model of type 2 diabetes to determine its effects on metabolic parameters after repeat dosing (Table 6).[14] Compound **27** was delivered at 10 mg/kg QD, 10 mg/kg BID, and 30 mg/kg QD along with a positive control, rosiglitazone at 3 mg/kg QD, for 15 days. Both rosiglitazone and the 30 mg/kg dose of **27** significantly reduced both fasting glucose and insulin compared to vehicle. **27** at 30 mg/kg also significantly lowered body weight 4.6% relative to vehicle in this study. These effects on both fasting glucose and insulin at 30 mg/kg QD were confirmed in a second 15-day DIO assay conducted in the same manner, and give credence to the insulin-sensitizing effects of GPR120 agonism.[15]

In summary, we have discovered a new class of heterocyclic GPR120 agonists with good *in vitro* potency and acceptable pharmacokinetic properties that show positive effects in both acute and chronic rodent models of type 2 diabetes. We hope to report on further development of this series in the future.

Acknowledgements

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bmcl.2018.02.013.

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5. Z. Sui, M.P. Winters, All synthetic protocols for each of the compounds described in this paper, and all biological assay protocols not described here, are included in the following patent, US Pat. WO 2015134038 A1 20150911 or in ref. 4 (a).
6. Synthesis of methyl 3-chloro-1H-pyrrole-2-carboxylate was conducted following the procedure in WO 2008154271 and then employed in an analogous fashion as in Scheme 1. The 3-Br compound was synthesized from intermediate 5 following Scheme 1. The 3-CN compound was made by cyanation of the 3-Br product of the Mitsunobu reaction with Zn(CN)2/Pd(DPPF)2.
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12. This finding is most likely due to the high dose of 27 and not the GPR120 mechanism because other series did not show this effect. No further cardiovascular safety studies were performed with 27.
13. The plasma exposure from the 3 mg/kg dose was predicted to be 3974 ng/mL from the mouse PK study which corresponds to 8.64 μM 27. Factoring in the high plasma protein binding in the mouse (99.63%) gives an effective concentration of 27 of 32 nM, lower than the 193 nM EC50 in mouse, but

not so low that it would be inconceivable to see an effect. See Smith DA, Kerns EH. *Nature Rev. Drug Disc.*. 2010;9:929. for a discussion on the limitations of using plasma protein binding in drug discovery.

14. Chronic study methods: Diet-induced obese mice from an internal colony of C57Bl/6 mice that were at least 21 weeks of age and had been on a high-fat diet for at least 15 weeks were sorted based on plasma glucose levels and body weight. The mice weighing 52–54g at study start, were sham-dosed for 5 days and then administered the indicated dose in 0.5% methylcellulose for 15 days. On Day 15, animals were fasted for 6 hours prior bleeding for determination of glucose and insulin levels. Statistical significance of changes in glucose and insulin from baseline were determined by ANCOVA. It is noted that the modest decrease in insulin levels and the slight decline in body weight in the vehicle group on day 15 relative to baseline may have been due to stress from the twice-daily handling of the animals.
15. In a duplicate 15-day DIO mouse study, 30 mg/kg 27 lowered fasting glucose 28.5% compared to vehicle and lowered fasting insulin 4759 pg/mL.