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Discovery and optimization of a novel series of GPR142 agonists for the treatment of type 2 diabetes mellitus

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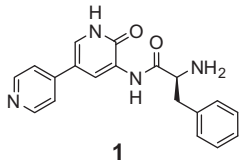
ABSTRACT

The discovery and initial optimization of a series of phenylalanine based agonists for GPR142 is described. The structure-activity-relationship around the major areas of the molecule was explored to give agonists 90 times more potent than the initial HTS hit in a human GPR142 inositol phosphate accumulation assay. Removal of CYP inhibition by exploration of the pyridine A-ring is also described.

An estimated 135 million people worldwide are affected by type 2 diabetes mellitus (T2DM), and the number of Americans affected is estimated to range between 11.6 million and 14 million people.[1] Therapies for treating T2DM and T2DM-related conditions are sought due to the increasing incidence of T2DM. Insulin secretagogue therapy is recognized as an appropriate therapy for T2DM management when diet and lifestyle modifications fail. Typically, secretagogue therapy augments circulating insulin levels in patients with a moderate degree of β-cell dysfunction. Sulfonylureas, which are prescribed as insulin secretagogues, reduce hyperglycemia when administered to patients with T2DM. However, the long-lasting effects of some sulfonylureas increase the risk of hypoglycemia; therefore new insulin secretagogues are sought to avoid this issue.[2]

With the recent sequencing of the human genome, several putative G Protein-coupled receptors (GPCRs) have been found in which the endogenous ligand is unknown (orphan GPCRs). Recently, it was reported that GPR142, highly expressed in pancreatic β-cells, is a receptor for aromatic amino acids with tryptophan being the most potent and efficacious.[3] It was found that (1) the receptor is coupled to the G_q signaling pathway, (2) tryptophan dose-dependently stimulates insulin secretion from isolated mouse islets in a glucose dependent manner, and (3) mice orally dosed with tryptophan show improved glucose tolerance when challenged with an oral glucose load. Based upon these findings, it was surmised that synthetic agonists to GPR142 might represent novel therapeutics for the treatment of T2DM. Because GPR142 will only stimulate insulin secretion under conditions of high blood glucose, it was hypothesized that GPR142 agonists could provide a benefit over existing therapies as the risk of hypoglycemia would be greatly reduced.

High throughput screening[4] (HTS) at Amgen identified several GPR142 agonists with moderate potency, including compound **1** (Fig. 1). Compound **1** had an EC_{50} of 4.8 μM in a human GPR142 inositol phosphate (IP) accumulation assay and 1.4 μM in a mouse GPR142 IP accumulation assay.[5] The high level of efficacy (E_{max} of 100%) for human GPR142 along with the low molecular weight (334.4) and low *C*log*P* (0.33) made this an attractive lead for optimization. Here, we describe the in vitro optimization of **1** resulting in several potent GPR142 agonists with significantly reduced inhibition of CYP 3A4 and 2D6.



1

Figure 1. Structure of GPR142 agonist from HTS.

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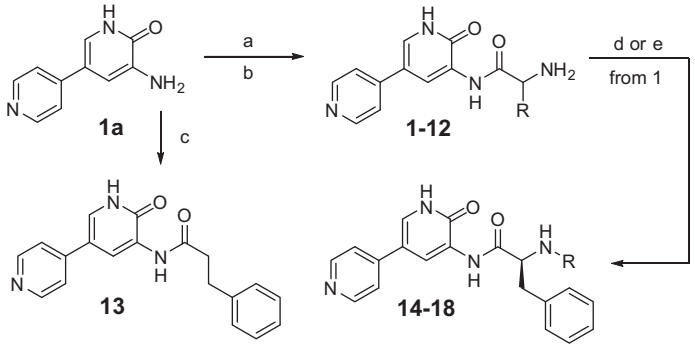
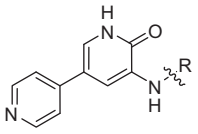
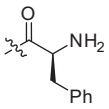
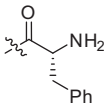
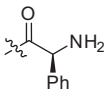
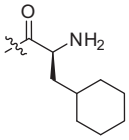
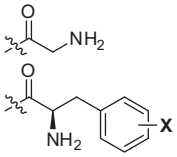


Scheme 1. Reagents and conditions: (a) HBTU (1.5 equiv), DIEA (3 equiv), DMF, RCH(NHBoc)CO_2H (1.2 equiv), 50 °C, 6 h, 55–77%; (b) TFA/DCM 1:1, rt, 2 h, 68–75%; (c) HBTU (1.5 equiv), DIEA (1.5 equiv), DMF, 3-phenylpropanoic acid (1.2 equiv), 50 °C, 18 h, 67%; (d, **14**) Acetic anhydride (5 equiv), DIEA (2 equiv), DCM, rt, 18 h, 79%; (e, **15–18**) RC(O)H (1.0 equiv), sodium triacetoxyborohydride, DCM, rt, 1 h, 25–87%.

Table 1
SAR exploration with phenylalanine derivatives

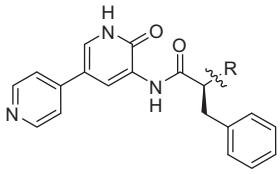


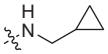
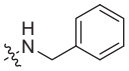
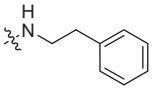
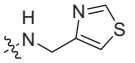
Compound	R	h-GPR142, IP EC_{50} (μM)[a, b]	E_{max}[c] (%)
1		4.8	115
2		13	93
3		>33	NA[d]
4		>33	30
5		>33	NA[d]
6	X = 2-Cl	>33	42
7	X = 3-Cl	>33	50
8	X = 4-Cl	3.8	105
9	X = 4-F	4.2	102
10	X = 4-CN	4.6	79
11	X = 4-Me	6.6	73
12	X = 4-OMe	25	40

[a] See Ref. 5 for assay protocol.
[b] Standard deviation for assay based on control compound was ±30%.
[c] %Fraction of maximal tryptophan response at 10 mM.
[d] No data available.

Exploration of the lead structure started with phenylalanine derivatives (Scheme 1). The synthesis of compounds **1**–**12** (Table 1) began by coupling the appropriate Boc protected amino acid with commercially available 5-amino-[3,4′-bipyridin]-6(1H)-one (**1a**) under standard HBTU coupling conditions. Deprotection of the Boc group with TFA in DCM gave the final primary amine products.

Table 2
SAR exploration of phenylalanine nitrogen



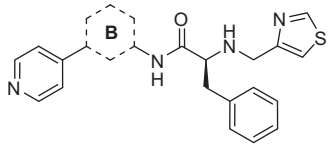
Compound	R	h-GPR142, IP EC_{50} (μM)[a,b]	E_{max}[c] (%)
1	NH_2	4.8	115
13	H	>33	NA[d]
14		>33	NA[d]
15		0.76	106
16		0.83	97
17		0.78	67
18		0.093	120

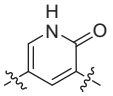
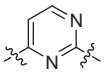
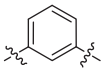
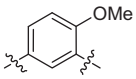
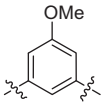
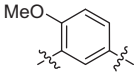
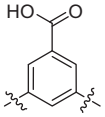
[a] Ref. 5 for assay protocol.
[b] Standard deviation for assay based on control compound was ±30%.
[c] %Fraction of maximal tryptophan response at 10 mM.
[d] No data available.

Compound **13** (Table 2) was synthesized by coupling 5-amino-[3,4′-bipyridin]-6(1H)-one (**1a**) with phenylpropanoic acid using HBTU under standard conditions. N-substituted phenylalanine derivatives **14**–**18** (Table 2) were synthesized, starting from **1**, by either acylation with acetic anhydride (**14**) or by reductive amination with the appropriate aldehyde under standard conditions using sodium triacetoxyborohydride (**15–18**).

Synthesis of B-ring derivatives **20–24** (Table 3) began by constructing the amino biaryl cores (**20b–24b**) via a Suzuki coupling of pyridine-4-boronic acid and the corresponding commercially available aryl bromides **20a–24a** followed by reduction of the nitro group using Pd on carbon under an atmosphere of hydrogen (Scheme 2). The amino biaryl intermediates **20b–24b** were coupled to Boc-L-phenylalanine under standard HBTU coupling conditions.[6] The target compounds **20–24** were completed by TFA mediated Boc deprotection of intermediates **20c–24c** followed by reductive amination using sodium triacetoxyborohydride with thi-

Table 3
B-ring SAR



Compound	B-ring	h-GPR142, IP EC_{50} (μM)[a,b]	Human serum EC_{50}[a,b] (μM)
18		0.093	0.50
19		0.36	0.29
20		0.088	0.97
21		0.089	1.0
22		0.053	0.81
23		0.24	0.98
24		0.11	0.48

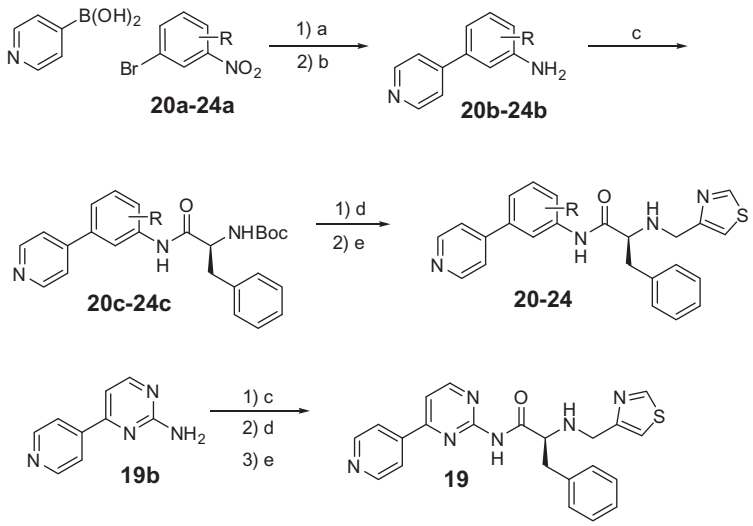
[a] Ref. 5 for assay protocol.
[b] Standard deviation for assay based on control compound was ±30%.

azole-4-carbaldehyde. Compound **19** was synthesized in an analogous fashion to compounds **20–24**, starting with 4-(pyridin-4-yl)pyrimidin-2-amine (**19b)** purchased from a commercial source.

The A-ring derivatives of compounds **18** and **20** (Table 4) were synthesized via one of two methods (Scheme 3). The first method began by constructing the amino biaryl cores (**26a**–**28a** & **33a**) via a Suzuki coupling of 3-bromoaniline (**Ar1**) or 5-bromo-2-methoxypyridin-3-amine (**Ar2**) with the appropriate boronic acid followed by HBTU-mediated coupling with Boc-L-phenylalanine to provide intermediates **26b**–**28b** & **33b**.

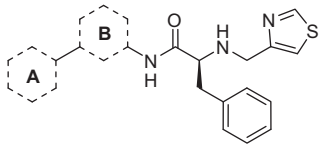
The second method started by first forming amide intermediates **25a** and **34a** by HBTU mediated coupling of Boc-L-phenylalanine with 3-bromoaniline (**Ar1**) or 5-bromo-2-methoxypyridin-3-amine (**Ar2**), followed by Suzuki coupling with the appropriate boronic acid to provide intermediates **30b**–**32b** and **34b**–**35b.** The intermediate, devoid of the A-ring (**25b**), was synthesized by hydrogenolysis of aryl bromide **25a** using Pd/C under an atmosphere of hydrogen gas. The pyrazole intermediate **29b** was constructed by reduction of the nitro group in commercially available 5-(3-nitrophenyl)-1H-pyrazole **(29a**) using Pd/C under an atmosphere of hydrogen gas, followed by standard HBTU coupling with Boc-L-phenylalanine as describe above. All the target compounds **25**–**35** were completed by deprotection of intermediates **25b**–**35b** under acidic conditions, followed by sodium triacetoxyborohydride mediated reductive amination with thiazole-4-carbaldehyde.

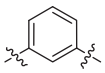
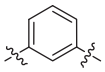
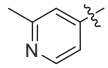
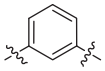
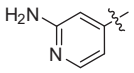
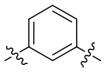
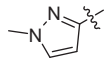
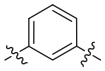
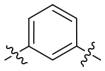
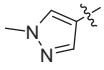
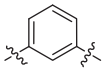
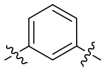
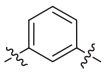
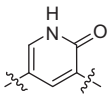
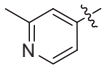
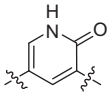
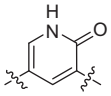
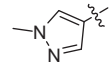
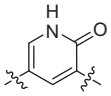
With the understanding that GPR142 is a receptor for aromatic amino acids, SAR exploration started with the L-phenylalanine portion of the molecule (Table 1). The unnatural D-phenylalanine **2** was only three times less active than **1**. However, other changes to the L-phenylalanine portion of the molecule lead to complete loss in activity (>33 μM), including the removal of the benzylic methylene (**3**), saturation of the aromatic ring (**4**), and the complete removal of the side-chain (**5**). Since the requirement for L-phenylalanine within this series was now demonstrated, a focused effort was made on exploring substituted L-phenylalanine derivatives. This exploration began with chloro L-phenylalanine derivatives. The 2-Cl (**6**) and the 3-Cl (**7**) derivatives had significantly diminished potency (>33 μM) relative to **1** whereas the potency of the 4-Cl derivative (**8**) was slightly improved. With this information, more 4-substituted L-phenylalanine derivatives were explored. Derivatives with an electron withdrawing substituent in the 4-position maintained their potency as exemplified by the 4-F derivative (**9**) and the 4-CN derivate (**10**). The lipophilic-electron rich 4-Me derivative (**11**) also maintained its potency. In contrast, the polar electron-rich 4-OMe derivative (**12**) had reduced potency. From this exploration it was determined that 4-Cl and 4-F L-phenylalanine could be used in future analogs instead of L-phenylalanine.



Scheme 2. Reagents and conditions: (a) pyridin-4-ylboronic acid (2 equiv), $Pd(PPh_3)_4$ (0.2 equiv), DMF/$NaHCO_3$ satd. aq. (4/1), Cs_2CO_3 (3.0 equiv), 70 °C, 16 h, 53–95%; (b) 10% Pd/C wet (0.5 equiv), H_2 (1 atm), MeOH, 16 h, 95–100%; (c) HBTU (1.5 equiv), DIEA (3 equiv), DMF, Boc-L-phenylalanine (1.2 equiv), 50 °C, 6 h 31–77%; (d) TFA/DCM 1:1, rt, 2 h, 65–78%; (e) thiazole-4-carbaldehyde (1.0 equiv), sodium triacetoxyborohydride, DCM, rt, 1 h, 36–87%.

Table 4
SAR exploration of A-ring



Compound	A–ring	B–ring	h-GPR142, IP EC_{50} [a,b] (μM)	Cyp Inh. (3 μM) 3A4/2D6 (%)
20			0.067	97/95
25	**H**		3.3	24/36
26			0.052	63/74
27			0.21	38/42
28			1.9	30/12
29			0.067	59/36
30			0.18	36/42
31			0.18	82/90
32			0.64	57/46
18			0.093	97/95
33			0.067	22/20
34			0.20	18/29
35			0.23	24/21

[a] Ref. 5 for assay protocol.
[b] Standard deviation for assay based on control compound was ±30%.

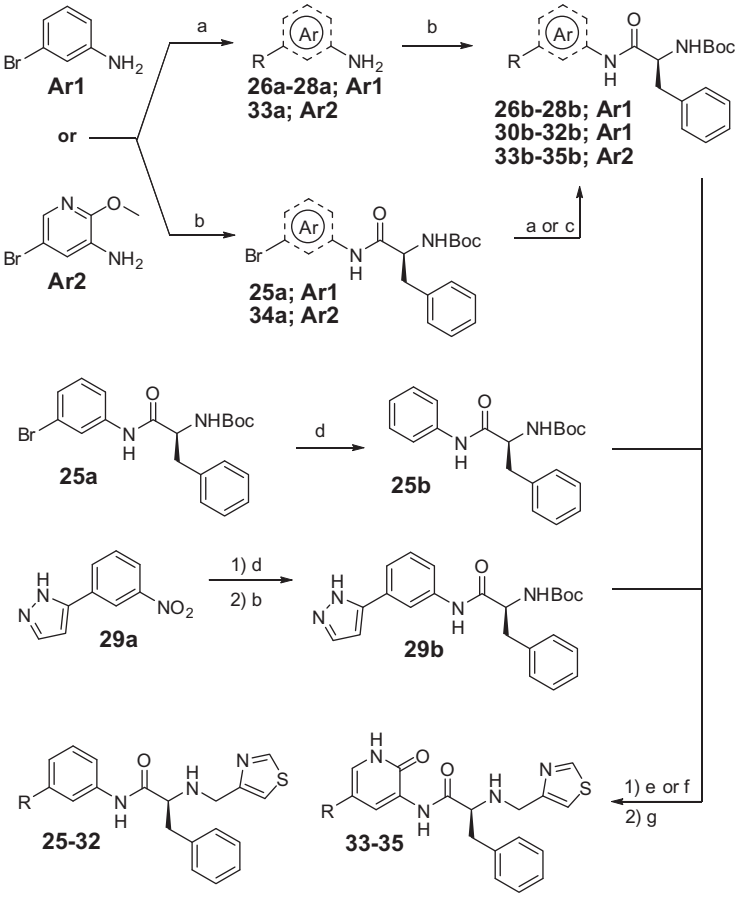
With the phenylalanine side chain now initially explored, attention was turned to the phenylalanine amine (Table 2). The importance of the phenylalanine amine was first explored by synthesis of the desamino derivative (**13**) and by eliminating the basicity of the amine via acetamide formation (**14**). Both of these changes lead to a complete loss of potency (>33 μM). It was pleasing to find that a mono alkyl substituent on the phenylalanine nitrogen gave a significant improvement in activity, including derivatives with cyclopropylmethyl (**15**, 0.76 μM), benzyl (**16**, 0.83 μM) and phenethyl (**17**, 0.78 μM). Along these lines, and with a desire to incorporate more polar substituents, *N*-methyl thiazole **18** was identified, which provided an h-GRP142 agonist with potency of 93 nM and an E_{max} of 120%. Compound **18** also had good potency in the presence of 100% human serum (502 nM, 100% E_{max}) and stimulated insulin secretion from isolated mouse islets (2.7 μM, 88% E_{max}).[7]

Next the influence of the pyridone ring on GPR142 activity in buffer and in the presence of 100% human serum was investigated (Table 3). Replacement of the pyridone with a pyrimidine ring (**19**) provided a compound with only a fourfold reduction in in vitro potency (360 nM). This indicated that the pyridone amide was not essential for potency and would allow manipulations in this area of the molecule. Next, the pyridone ring was replaced with a phenyl ring (**20**). Although the phenyl ring retained a good level of potency, it was observed that a reduction of polarity in this part of the molecule resulted in a compound with a greater shift in the presence of 100% human serum. Addition of polarity to compound **20** in an attempt to reduce the serum shift was next explored. The electron rich 6-methoxy phenyl **21** was equipotent to compound **20**; and the 5-methoxyphenyl **22** was slightly more potent than compound **20**, however both of these derivatives had a high serum shift when compared to compound **18**. A methoxy group at the 4 position of the phenyl ring (**23**) proved to be detrimental to the potency. Placing a carboxylic acid at the 5 position of the phenyl ring (**24**) slightly diminished the potency but kept the shift in human serum low, similar to that of compound **18**.

It is known that unencumbered basic nitrogens of heterocycles, in particular pyridines and imidazoles, bind to the heme portion of CYP enzymes, causing inhibition which can lead to undesirable drug-drug interactions.[8] As suspected, compounds **18** and **20**, which both contained a sterically unencumbered pyridine, had high levels of CYP inhibition (Table 4). With this in mind, it was decided to look for a replacement of the A-ring pyridine first with the synthetically more accessible phenyl B-ring and subsequently combine the most interesting A-rings with the pyridone B-ring (Table 4). To test the hypothesis that the pyridine ring was the major cause of the CYP inhibition, it was replaced with a phenyl ring (**32**) or completely removed (**25**). As predicted, compound **32** had a dramatic reduction in CYP inhibition when compared to compound **20**. Complete removal of the phenyl ring (**25**) reduced the CYP inhibition even further when compared to compounds **20**. While both changes successfully reduced CYP inhibition, the GPR142 activity was also significantly reduced.

Next, 2-substituted pyridines were investigated in the hope that the steric bulk would reduce the CYP inhibition. Indeed, the 2-methyl pyridine A-ring (**26**) not only improved the potency (52 nM), but also led to a moderate reduction in the CYP inhibition. The 2-amino pyridine A-ring **27** had a significant reduction in CYP inhibition but also had a slight loss in potency. Next, a series of compounds in which the pyridine A-ring was replaced with a pyrazole were evaluated. The 3-substituted 1-methyl-1*H*-pyrazole **28** had lower CYP inhibition, but was much less potent than **20**. On the other hand, the 5-substituted 1*H*-pyrazole **29** had a good balance of potency (67 nM) and reduced CYP inhibition as compared to compound **20**. The 4-substituted 1-methyl-1*H*-pyrazole **30** was three times less potent than **20**, and, much like 3-substituted 1-methyl-1*H*-pyrazole **28**, had lower CYP inhibition than **20**. The 4-substituted 1*H*-pyrazole **31** was also three times less potent than **20**, but in this case did not lower the CYP inhibition.

Next, the A-rings which gave the best balance of high potency and low CYP inhibition in the phenyl B-ring series were combined with the pyridone B-ring. It was gratifying to see that the 2-methyl pyridine A-ring (**33**) improved the potency slightly (67 nM) compared to compound **18**. As expected, this change dramatically reduced the level of CYP inhibition. More interestingly, compound **33** had a significantly lower level of CYP inhibition than the corresponding analogue with the phenyl B-ring (**26**). This is presumably due to an increase in the polarity of the pyridone ring (C log *P* of **33** = 1.39) versus the phenyl ring (C log *P* of **26** = 3.6). The pyrazole ring was also explored in combination with the pyridone B-ring,



Scheme 3. Reagents and conditions: (a) $ArB(OH)_2$ (2 equiv), $Pd(PPh_3)_4$ (0.2 equiv), DMF/$NaHCO_3$ satd aq (4/1), Cs_2CO_3 (3 equiv), 70 °C, 16 h, 40–95%; (b) HBTU (1.5 equiv), DIEA (3 equiv), DMF, Boc-L-phenylalanine (1.2 equiv), 50 °C, 6 h, 40–75%; (c) $ArB(OR)_2$ (1.2 equiv), $Pd_2(dba)_3$ (0.05 equiv), Xphos (0.2 equiv), *t*-AmylOH, K_3PO_4 (3.0 equiv), 100 °C, 16 h, 50%; (d) 10% Pd/C wet (0.5 equiv), H_2 (1 atm), MeOH, 16 h, 95–97%; (e, **25**–**32**) TFA/DCM 1:1, rt, 2 h, 65–83%; (f, **33**–**35**) 1 N HCl/dioxane 1:1, 80 °C, 2 h; (g) thiazole-4-carbaldehyde (1.0 equiv), sodium triacetoxyborohydride, DCM, rt, 1 h, 25–83%.

Table 5
Rat pharmacokinetic properties of key analogues

Compound	Cl (h/L/kg)[a]	Vss (L/kg)[a]	MRT (h)[a]	%F[b]	RLM[c] (%)
24[a,b]	2.9	0.5	0.2	0	74
33[ab]	2.6	0.75	0.3	19	11

[a] Compounds were dosed iv at 0.5 mg/kg.
[b] Compounds were dosed po at 2.0 mg/kg.
[c] See Ref. 10 for assay protocol.

but proved to be less effective. The 5-substituted 1*H*-pyrazole **34** and the 4-substituted 1-methyl-1*H*-pyrazole **35** both had lower CYP inhibition with the pyridone B-ring versus the phenyl B-ring, but none of these derivatives were as potent as the 2-methyl pyridine A-ring making their utility limited. These pyrazole derivatives also illustrate that the increase in polarity of the pyridone ring versus the phenyl ring leads to a reduction in CYP inhibition. It is interesting to note that the incorporation of the carboxylic acid on the B-ring (**24**, Table 3) was another method of lowering the CYP inhibition.[9]

From the early optimization of this lead, compound **33** emerged as the top compound in this series. This compound had good potency for h-GPR142 in both buffer (67 nM, 90% E_{max}) and in the presence of 100% human serum (290 nM, 97% E_{max}). The CYP inhibition IC_{50} was >30 μM for isoforms 3A4 and 2D6. A major issue with **33** was poor stability in human/rat liver microsomes (10%/11% remaining after 30 min incubation)[10] which translated to high in vivo clearance (Table 5). It should be noted that the issue of low stability in liver microsomes was addressed by the incorporation of a carboxylic acid in the B-ring (**24**, Table 3) but this compounds had very low bioavailability and high in vivo clearance due to glucuronidation of the carboxylic acid.[11]

In summary, a novel series of GPR142 agonists was identified. Initial efforts improved the potency of the HTS hit from 4.8 μM to 67 nM in a human GPR142 IP accumulation assay. Furthermore, the inhibition of CYP 3A4 and 2D6 was significantly reduced. Although low in vivo exposure has precluded the use of these compounds in proof-of-concept experiments, their promising in vitro properties has justified further exploration.[12]

References and notes

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5. Inositol Phosphate Accumulation Assay-HEK293 cells were dispensed into a poly-D-lysine tissue culture treated 96 well plate at a density of 25,000 cells per well. The next day, the cells (~80–90% confluent) were transfected with 100 ng receptor plasmid per well using Lipofectamine2000 according the manufacturer's instructions. Six hours after transfection the media was replaced with inositol free DMEM/10% dialyzed FCS supplemented with 1 μCi/mL tritiated inositol. After incubation overnight, the cells were washed

once in HBSS and then treated with the 100 μL HBSS/0.01% BSA containing various concentrations of test compounds (prepared as above in DMSO), 10 mM LiCl and incubated at 37 °C for 1 h. The media was aspirated and the cells were lysed with ice cold 20 mM formic acid. After incubation at 4 °C for 5 h, the lysate were added to yttrium silicate SPA beads, allowed to settle overnight and read on a Beckman TopCount scintillation counter. In measuring the EC_{50} with serum, HBSS/0.01% BSA was replaced with100% human serum.

6. Protection of the carboxylate in **24b** as the methyl ester was necessary to prevent homocoupling in the next step. The methyl ester in **24b** was deprotected by standard saponification conditions using LiOH in THF/water, after it was coupled to Boc-L-phenylalanine.
7. Mouse islets were run with 16.7 mM Glucose, 0.625% HAS and 1% DMSO. For details of assay conditions and mouse islet isolation see Lin, DC -H; Zhang, R.; Li, F.; Nguyen, K.; Chen, M.; Tran, T.; Lopez, E.; Lu, J. Y. L.; Li, X. N.; Tang, L.; Tonn, G. R.; Swaminath, G.; Reagan, J. D.; Tian, H.; Lin, Y.-J.; Houze, J. B.; Luo, J *PLoS ONE* **2011**, *6*, e27270. http://dx.doi.org/10.1371/journal.pone.0027270.
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9. IC_{50} >30 μM for all isoforms tested (3A4, 2D6 and 2C9).
10. Microsome stability were measured in a high throughput format by incubating inhibitors at 1 μm concentration with rat(r) or human (h) microsomes and the percentage of the parent compounds remaining were measured after 30 min of incubation by liquid chromatography/mass spectrometry analysis.
11. Measured clearance in rat hepatocytes was 18 (ml/min/10^{-6} cells) which equals a half-life of 39 min.
12. (a) Du, X.; Kim, Y-J; Lai, S-J.; Chen, X.; Lizarzaburu, M.; Turcotte, S.; Fu, Z.; Liu, Q.; Zhang, Y.; Motani, A.; Oda, K.; Okuyama, R.; Nara, F.; Murakoshi, M.; Fu, A.; Reagan, J.D.; Fan, P.; Xiong, Y.; Shen, W.; Li, L.; Houze, J.; Medina, J.C. *Bioorg. Med. Chem. Lett.* accepted for publication; (b) Yu, M.; Lizarzaburu, M.; Motani, A.; Fu, Z.; Du, X.; Liu, J.; Jiao, X.; Lai, S-J.; Fu, A.; Lin, D.; Liu, Q.; Murakoshi, M.; Nara, F.; Oda, K.; Okuyama, R.; Reagan, J.; Turcotte, S.; Zhang, Y.; Houze, J.; Medina, J.C.; Li, L. manuscript in preparation to *ACS Med. Chem. Lett.*