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Phenylalanine derivatives as GPR142 agonists for the treatment of Type II diabetes

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ABSTRACT

GPR142 is a novel GPCR that is predominantly expressed in pancreatic β-cells. GPR142 agonists potentiate glucose-dependent insulin secretion, and therefore can reduce the risk of hypoglycemia. Optimization of our lead pyridinone-phenylalanine series led to a proof-of-concept compound **22**, which showed in vivo efficacy in mice with dose-dependent increase in insulin secretion and a decrease in glucose levels.

Type 2 diabetes mellitus (T2DM) is characterized by hyperglycemia as a result of insulin resistance combined with reduced insulin secretion from pancreatic β-cells. Uncontrolled hyperglycemia is a major risk factor for microvascular and macrovascular complications including retinopathy, nephropathy, neuropathy, and accelerated cardiovascular disease.[1] Insulin secretagogues such as sulfonylureas and meglitinides are widely used for the treatment of type 2 diabetes. However, their action is independent of extracellular blood glucose concentration and therefore can cause hypoglycemia.[2] This liability triggered considerable interest from the pharmaceutical industry to pursue glucose-stimulated insulin secretagogues to treat T2DM, such as DPP-IV inhibitors, analogs of glucagon-like peptide-1,[3] and GPR40 agonists.[4]

GPR142 is a novel G protein-coupled receptor (GPCR) which is predominantly expressed in pancreatic β-cells. It was found to be a receptor for aromatic amino acids with tryptophan representing one of the most potent ligands (EC_{50} of 0.2–1 mM).[5] It was also demonstrated that GPR142 agonists potentiate glucose-dependent insulin secretion from isolated pancreatic islets and in rodent models. Thus, a potent and selective GPR142 agonist could prove to be an effective anti-diabetic therapeutic with reduced risk of hypoglycemia.

We have previously reported our efforts in identifying a novel series of GPR142 agonists with a pyridinone-phenylalanine scaffold.[6] Previous lead optimization efforts discovered that addition of a methyl thiazole moiety (D-ring, Scheme 1) to the amino group of the phenylalanine motif in compound **1** boosted the potency of the agonists more than 50-fold. Compound **2** displayed an EC_{50} of 93 nM and a 120% E_{max} compared to tryptophan in our human inositol phosphate (IP) accumulation assay.[7] However, due to the lack of metabolic stability owing to *N*-dealkylation, compound **2** displayed high clearance (3.4 L/(h·kg)) after an i.v. administration of 0.5 mg/kg, and poor oral exposure (11 μg·h/L, 2% bioavailability with a 2 mg/kg oral dose) in rats. Therefore, it was not suitable as a tool compound for in vivo investigation.

Herein, we describe our identification of a tool compound **22** which demonstrated in vivo efficacy for this series of agonists.

The syntheses of the agonists are shown in Schemes 2–4. The syntheses of compounds **2**–**6**, **14**, **16**, **17**, **19**, **20** followed the synthetic route in our earlier work[6], applying reductive aminations with different heterocyclic aldehydes or ketones for the introduction of the D-ring. Compounds **7**, **18**, **21**–**24** and **27** were prepared through a synthetic route shown in Scheme 2. [8] Suzuki coupling of 4-pyridine boronic acid with 5-bromo-2-methoxypyridin-3-amine generated 6-methoxy-[3,4′-bipyridin]-5-amine **8**. (R)-2-acetoxy-3-

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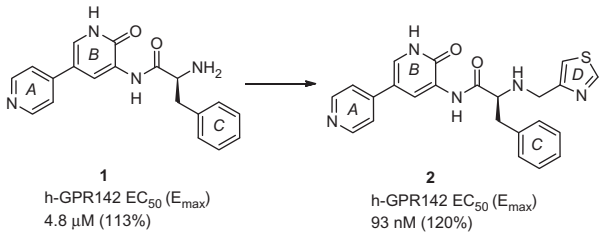


Scheme 1. Increased potency through *N*-substitution.

phenylpropanoic acid **9** was converted to the acid fluoride with cyanuric fluoride and coupled with compound **8** to form an amide bond. Subsequent deprotection of the acetate group generated (R)-2-hydroxy-*N*-(6-methoxy-[3,4′-bipyridin]-5-yl)-3-phenylpropanamide **10**. Compound **10** was converted to its nosylate upon treatment with 4-nitrobenzene-1-sulfonyl chloride (nosyl chloride). Nucleophilic displacement of the nosylate with various amines followed by deprotection under acidic conditions generated the final GPR142 agonists.

Compounds **25** and **26** were made through alkylation of the B-ring pyridinone nitrogen of compound **22** with either *tert*-butyl 2-bromoacetate or 2-chloro-*N*,*N*-dimethylethanamine (Scheme 3).

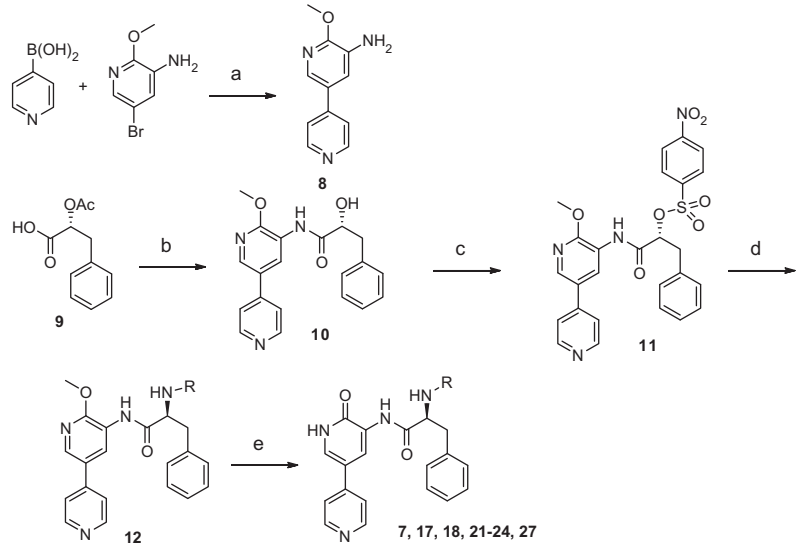
Synthesis of compound **35** followed the synthetic route shown in Scheme 4. 2-Chloroisonicotinic acid **28** was refluxed in thionyl chloride to generate the acid chloride and then coupled with hydrazinecarbothioamide to form 2-(2-chloroisonicotinoyl)hydrazinecarbothioamide **29**. Acidic cyclization of compound **29** using PPA generated thiadiazole **30**. The chloro group in compound **30** was then converted to a methyl amino group using a S_NAr reaction. The amino group connected to thiadiazole in compound **31** was coupled to Boc-protected phenylalanine to generate compound **32**. The Boc group in the resulting compound **32** was removed with TFA. Reductive amination of compound **33** with thiazole-4-carbaldehyde led to compound **35**. The other compounds in Table 5, i.e., **34**, **36**, and **37**, were prepared by the same route starting from the appropriate heterocyclic carboxylic acid.

Our initial lead optimization efforts aimed at exploring different heterocyclic D-rings to see whether the microsomal stabilities of the agonists could be improved while maintaining the potency and efficacy. The results are listed in Table 1. A variety of five- and six-membered heterocyclic D-rings were tolerated with good potency and efficacy, such as imidazole, pyrazole, pyridine and pyrimidine. However, all of them displayed high turnover in rat and human microsomal stability assays. [9]

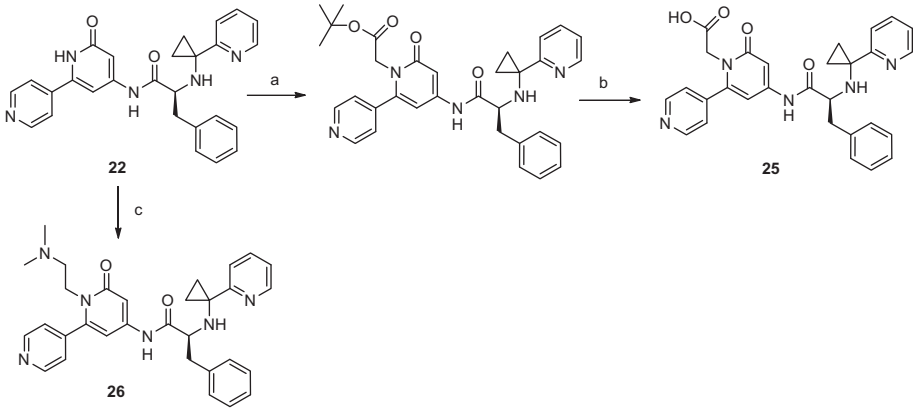
The benzyl-like nature of the carbon between the amino group and the heterocyclic D-ring may be one explanation as to why *N*-dealkylation was so facile. To mitigate this effect, the linker was elongated to two carbons (compound **13** in Table 2). The potency and efficacy were both significantly lowered, while no improvement in microsomal stability was seen. The heteroaromatic D-ring was also replaced with saturated heterocycles as in compounds **14**–**17**. Although the microsomal stabilities of compound **14** and **17** were improved, possibly due to the presence of polar groups such as a sulfone or amide, none of the compounds was sufficiently potent.

We then turned our attention to adding a substituent to the benzyl-like carbon to prevent the *N*-dealkylation. Methyl substitution in compound **18** (Table 3) was well tolerated with good efficacy and only a two-fold decrease in potency. For mono-substitution, the stereochemistry significantly influenced potency with one diastereomer being more potent as evident in the diastereomeric pair of compounds **18** and **19**. Compounds with ethyl substitution (**20**), and dimethyl substitution (**21**) [10] displayed a further decline in potency and significant lowering in efficacy, indicating that there is a steric limitation in this position. The exception was the cyclopropyl substitution on the benzylic position. Compound **22** not only was more potent than the parent pyridylmethyl compound **6**, but also displayed significantly improved stability (<10% turnover in human microsomes and 60% turnover in rat microsomes) as well as a great efficacy (127%). Replacing the pyridine D-ring in compound **22** by pyrimidine (**23**) or methyl thiazole (**24**) also resulted in a combination of good potency, efficacy and improved microsomal stability compared to compound **6**. However, compound **22** displayed the best overall profile. The dramatic difference between cyclopropyl- and dimethyl- substitution pattern could be due to the more planar nature of the cyclopropyl ring and reduced steric demand through distinct bond angles. As a result, the cyclopropyl ring can orient itself much differently from the dimethyl group.

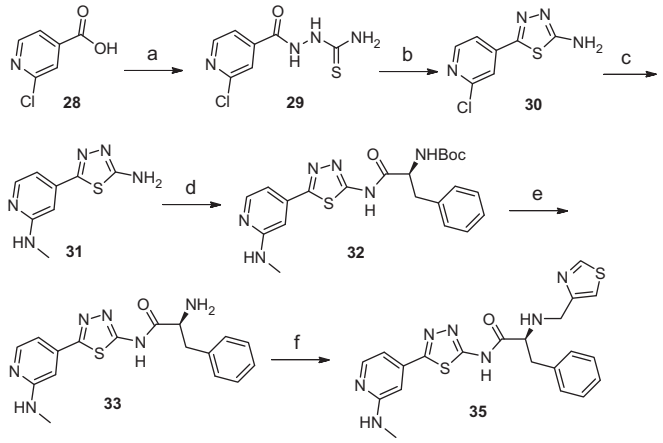
The pharmacokinetic profile of compound **22** was evaluated in rats (Table 6). Though compound **22** showed improved microsomal stability, it nonetheless displayed high in vivo clearance. The



Scheme 2. Synthesis of GPR142 agonists. a. $Pd_2(dba)_3$, X-Phos, K_3PO_4, BuOH, 3 h, 110 °C, 74%; b. (i) cyanuric fluoride, pyridine, DCM, −20 °C–10 °C; then **8**, DIPEA, DCM; (ii) K_2CO_3, 88% for 2 steps; c. 4-nitrobenzene-1-sulfonyl chloride, Et_3N, 69%; d. 1-(pyridin-2-yl)cyclopropanamine or other primary amines RNH_2, DMF, 100 °C, 30–59%; e. dioxane, water, HCl, 50 °C, 41–90%.

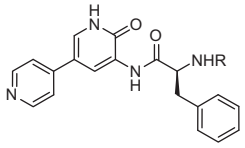


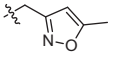
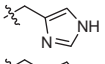
Scheme 3. Synthesis of GPR142 agonists **25** and **26**. a. *tert*-butyl 2-bromoacetate, Cs_2CO_3, DMF, 70 °C, 43%; b. TFA, 100%; c. 2-chloro-*N*,*N*-dimethylethanamine, Cs_2CO_3, DMF, 70 °C, 34%.



Scheme 4. Synthesis of GPR142 agonist **35**. a. (i) $SOCl_2$, reflux; (ii) hydrazinecarbothioamide, pyridine, 55%; b. PPA, 100 °C, 93%; c. $MeNH_2$ H_2O, 130 °C, 46%; d. Boc-phenylalanine, HBTU, 99%; e) TFA, 52%; f. $NaBH(OAc)_3$, 50 °C, 1 h, thiazole-4-carbaldehyde, 43%.

Table 1
Evaluation of various D-ring heterocycles



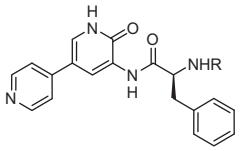
Compound	R	h-GPR142 IP		Microsome %Turnover[c] h/r
		EC_{50} (μM)[a]	E_{max}[a,b](%)	
2		0.93	120	68/99
3		2.2	93	78/95
4		0.11	107	60/96
5		0.21	92	90/100
6		0.095	155	95/95
7		0.44	95	90/99

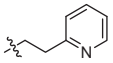
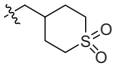
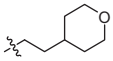
[a] Human inositol phosphate accumulation assay with HEK293 cells. See Ref. 7 for assay protocol.
[b] Percentage of maximal tryptophan response at 10 μM.
[c] % Turnover of parent compound after 30 min. of incubation with human or rat microsomes. See Ref. 9 for detailed conditions.

unusually high clearance could be due to a combination of first and second phase metabolism potentially including glucuronidation of the pyridinone. Modifications on the pyridinone B-ring moiety of compound **22** were made to prevent the potential glucuronidation from occurring (Table 4). While substitution on the pyridinone nitrogen with either a basic or an acidic group (compounds **25** and **26)** still resulted in high clearance in rats, compound **27** with a methoxy-pyridine B-ring had a much improved clearance of 1.25 L/(h·kg) in rat as well as good potency and efficacy. However, the potency of compound **27** was significantly shifted in the presence of serum (20-fold in 100% serum) which rendered it unsuitable for in vivo validation. Nevertheless, these changes demonstrated that B-ring modifications could influence the pharmacokinetic properties of the agonists significantly. We decided to next try additional heterocyclic B-rings to optimize the pharmacokinetic properties and serum shift.

At this point, it was also discovered that the cyclopropyl moiety in these compounds caused CYP 3A4 time-dependent inhibition. As this was considered undesirable even for a tool compound, we removed the cyclopropyl substitution at the D-ring while exploring further modifications on the B-ring. Previous studies[6] demonstrated that the B-ring tolerated polar heterocycles such as pyrimidine. The thiadiazole B-ring was among the first B-rings we explored in our optimization efforts and it combined a number of favorable attributes. Table 5 shows a number of agonists with thiadiazole as B-ring. It was encouraging that compound **34** showed equal potency and efficacy as compound **2**, with very good microsomal stability and low serum shift of only two-fold. Furthermore, consistent with the low microsomal turnover, compound **34** had low clearance of 0.44 L/(h·kg) in rat (Table 5). Even though its oral bioavailability was only 10%, it provided a good starting point. Subsequent work resulted in compounds **35–37** shown in Table 5. While compounds **36** and **37** exhibited higher in vivo clearance than compound **34**, the methylamino-substituted compound **35** showed improved in vivo clearance and bioavailability compared to compound **34** (Table 6). With a moderate microsomal stability, the lower in vivo clearance of **35** might be associated with higher protein-binding as evidenced in a higher six-fold serum shift of this compound. Though blessed with good efficacy at the human

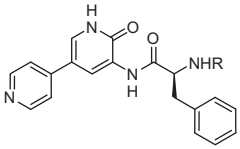
Table 2
D-ring modifications to improve microsomal stability



Compd	R	h-GPR142 IP		Microsome%Turnover[c] h/r
		EC_{50} (μM)[a]	E_{max}[a,b](%)	
2		0.093	120	68/99
13		1.32	76	100/100
14		20	91	21/58
15		0.39	78	83/98
16		1.06	84	66/80
17		2.23	78	19/67

[a] Human inositol phosphate accumulation assay with HEK293 cells. See Ref. 7 for assay protocol.
[b] Percentage of maximal tryptophan response at 10 μM.
[c] % Turnover of parent compound after 30 min. of incubation with human or rat microsomes. See Ref. 9 for detailed conditions.

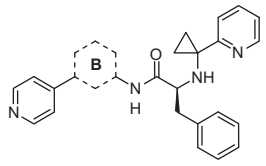
Table 3
D-ring α-benzylic substitutions to improve microsomal stability of the agonists

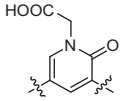
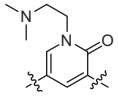
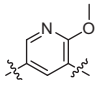


Compd	R	h-GPR142 IP		Microsome % Turnover[c] h/r
		EC_{50} (μM)[a]	E_{max}[a,b](%)	
6		0.095	155	95/95
18		0.22	107	100/99
19		6.0	60	95/95
20[d]		0.73	81	85/95
21		9.45	57	>95/100
22		0.054	127	<10/60
23		0.099	114	21/67
24		0.20	106	64/74

[a] Human inositol phosphate accumulation assay with HEK293 cells. See Ref. 7 for assay protocol.
[b] Percentage of maximal tryptophan response at 10 μM.
[c] % Turnover of parent compound after 30 min. of incubation with human or rat microsomes. See Ref. 9 for detailed conditions.
[d] Racemic.

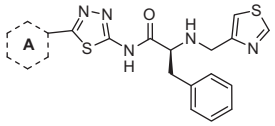
Table 4
Modifications on B-ring



Compd	R	h-GPR142 IP		Micorsome % Turnover[c] h/r	CL[d]
		EC_{50} (μM)[a]	E_{max}[a,b](%)		
25		0.26	83	10/12	5.0
26		0.28	118	19/22	3.1
27		0.35	123	15/54	1.3

[a] Human inositol phosphate accumulation assay with HEK293 cells. See Ref. 7 for assay protocol.
[b] Percentage of maximal tryptophan response at 10 μM.
[c] % Turnover of parent compound after 30 min. of incubation with human or rat microsomes. See Ref. 9 for detailed conditions.
[d] CL in L/(h·kg).

Table 5
Agonists with thiadiazole as B-ring and various A rings



Compd	R	h-GPR142 IP		Microsome%Turnover[c] h/r	CL/%F[d] (L/(h·kg))
		EC_{50} (μM)[a]	E_{max}[a,b](%)		
34		0.086	115	14/10	0.44/10
35		0.036	96	58/66	0.23/36
36		0.18	86	19/35	1.7/19
37		0.39	74	29/57	2.4/32

[a] Human inositol phosphate accumulation assay with HEK293 cells. See Ref. 7 for assay protocol.
[b] Percentage of maximal tryptophan response at 10 μM.
[c] % Turnover of parent compound after 30 min. of incubation with human or rat microsomes. See Ref. 9 for detailed conditions.
[d] PK done in rat. For iv in rat, 0.5 mg/kg for all compounds except 0.67 mg/kg for compound **35**, $n = 2$; for po in rat, 2 mg/kg for all compounds, $n = 3$.

GPR142, a further study of compound **35** indicated that it was a partial agonist (15 nM IC_{50}, 37% E_{max}) in HEK293 cells transfected with mouse GPR142. In addition, compound **35** was chemically not stable under weakly acidic condition. Both issues prevented this compound from being further used as a tool compound to demonstrate in vivo efficacy. Nevertheless, the modifications on the B-ring again indicated that this was a good site for improving the pharmacokinetic properties[11].

Compound **22** was therefore selected for in vivo evaluation in a glucose-challenged model in mice. Compound **22** was potent in both human and mouse GPR142 IP assay (mGPR142 EC_{50} = 1.8 nM, E_{max} = 81%).

Table 6
Detailed pharmacokinetic parameters of selected compounds

	Compound #	**22**	**34**	**35**
iv[a]	CL(L/(h·kg))	5.0	0.44	0.23
	AUCμg·h/L)	177	1235	3030
	Vdss (L/kg)	1.1	0.21	0.23
	T1/2 (h)	0.2	1.2	1.2
	Dose (mg/kg)	0.89	0.5	0.67
po[a]	AUCμg·h/L)	77	480	1730
	MRT (h)	1.79	1.93	2.36
	F (%)	20	10	36
	Dose (mg)	2.0	2.0	2.0

[a] PK done in rat. For iv in rat, 0.5 mg/kg for all compounds except 0.67 mg/kg for compound **35**, $n = 2$; for po in rat, 2 mg/kg for all compounds, $n = 3$.

B6D2F1 (Harlan) male mice were dosed subcutaneously with increasing doses of compound **22** or vehicle (milliQ water) at 10 mL/kg. 15 min later, mice were challenged with an oral glucose bolus (2 g/kg). 7.5 min after glucose challenge, blood samples were collected and assayed for blood glucose (AccuChek glucometer), serum insulin (ELISA, Alpco) and serum levels of compound **22**. The 7.5 min time point was based on the previously determined time to reach peak insulin levels in response to oral glucose challenge (T_{max}). The T_{max} was unaffected by treatment with compound **22**.

Dose-related increase in serum insulin levels (plasma EC_{50} = 0.29 μM; GraphPad Prism; non-linear fit, variable Hill slope) and decrease in blood glucose levels (EC_{50} = 0.85 μM) were observed in mice (Fig. 1). These results correlated very well with the observed dose-dependent increase in serum levels of compound **22**.

In conclusion, continued optimization of the phenylalanine series demonstrated that the D-ring tolerated a variety of heterocycles, and that B-ring variation could have significant impact on the pharmacokinetic properties of the agonists. The optimization also led to compound **22,** which showed in vivo efficacy in mouse

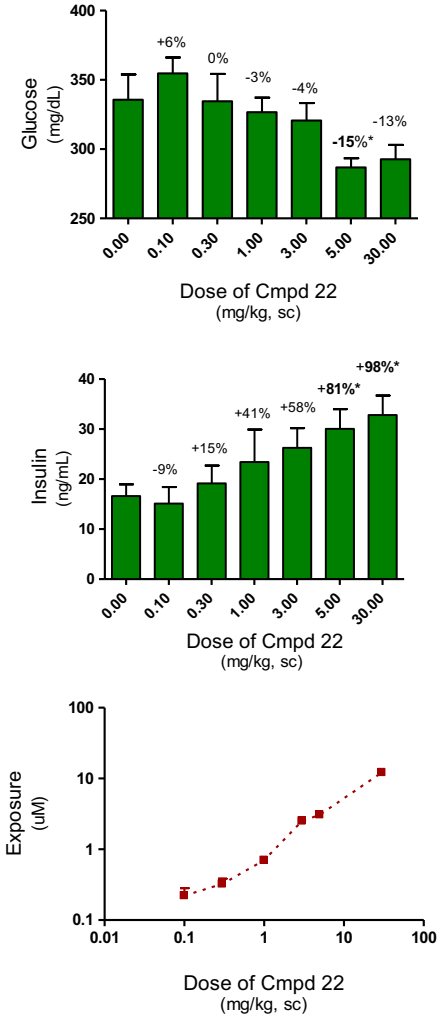


Fig. 1. The in vivo results of dosing compound **22** in a mice glucose-challenge model. Mice were dosed with compound **22** 15 min prior to oral glucose challenge. Blood samples were collected and assayed for blood glucose, serum insulin and exposure 7.5 min after oral glucose challenge. Data are shown as mean + sem, $n = 7$ mice per dose group. (a) Compound **22** lowered glucose in a dose-dependent manner. (b) Compound **22** stimulated the secretion of insulin in a dose-dependent manner. (c) Plasma exposure of compound **22** at different doses.

with dose-dependent increase in insulin secretion and decrease in glucose level. Further optimization on this series is ongoing and will be reported in due course.[11]

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7. *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. 6 h after transfection the media was replaced with inositol free DMEM/10% dialyzed FCS supplemented with 1μCi/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.
8. Compound **13** was prepared through a similar route as depicted in Scheme 2 with nosylate chemistry but relying on a slightly different sequence of steps. Compound **15** was also synthesized by a similar route with (R)-(tetrahydrofuran-2-yl)methanamine as nucleophile and triflate as leaving group (pyridinone as the B-ring) in 23% yield.
9. Microsome stability was 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. The% of parent compounds which was metabolized is reported as%turnover.
10. Compound **21** had an additional methyl group alpha to the A ring pyridine nitrogen for the purpose of improving CYP inhibition. The potency difference was usually within 2 fold between pyridine A ring and methyl pyridine A ring.
11. For further work in this series including more work in B-ring modifications, see Yu, M. et al. manuscript in preparation to ACS Med. Chem. Lett.