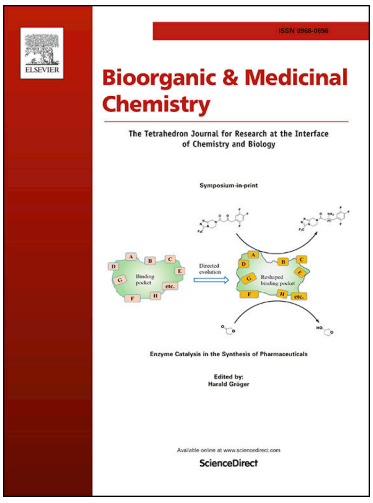
Journal Pre-proofs



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PII: S0968-0896(19)31029-6
DOI: https://doi.org/10.1016/j.bmc.2019.115232
Reference: BMC 115232

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 19 June 2019
Revised Date: 14 November 2019
Accepted Date: 20 November 2019

Please cite this article as: R.J. Hinklin, B.R. Baer, S.A. Boyd, M.D. Chicarelli, K.R. Condroski, W.E. DeWolf Jr., J. Fischer, M. Frank, G.P. Hingorani, P.A. Lee, N.A. Neitzel, S.A. Pratt, A. Singh, F.X. Sullivan, T. Turner, W.C. Voegtli, E.M. Wallace, L. Williams, T.D. Aicher, Discovery and Preclinical Development of AR453588 as an Anti-diabetic Glucokinase Activator, *Bioorganic & Medicinal Chemistry* (2019), doi: https://doi.org/10.1016/j.bmc.2019.115232

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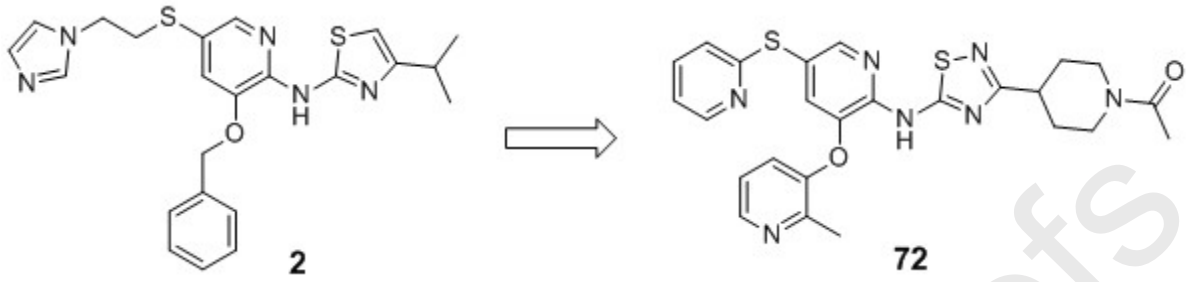
ABSTRACT

Glucose flux through glucokinase (GK) controls insulin release from the pancreas in response to high levels of glucose. Flux through GK is also responsible for reducing hepatic glucose output. Since many individuals with type 2 diabetes appear to have an inadequacy or defect in one or both of these processes, identifying compounds that can activate GK could provide a therapeutic benefit. Herein we report the further structure activity studies of a novel series of glucokinase activators (GKA). These studies led to the identification of pyridine **72** as a potent GKA that lowered post-prandial glucose in normal C57BL/6J mice, and after 14d dosing in *ob/ob* mice.

Graphical abstract



1. Introduction

Glucokinase (GK)[1] is a member of the hexokinase family of cellular enzymes responsible for the conversion of glucose to glucose-6-phosphate, the first step in glucose utilization. Its expression is restricted primarily to liver, pancreatic β-cells, enteroendocrine cells and the hypothalamus.[2,3] GK is the rate-limiting enzyme for glucose metabolism, which controls glucose-stimulated insulin secretion in the pancreas.[4] GK interconverts between inactive and active conformations in response to glucose concentration.[5] It effectively acts as the "glucose sensor" due to its substrate concentration at half-maximal velocity ($S_{0.5}$) of 7.5 mM glucose being in the physiological range for blood glucose and its positive cooperativity for glucose (Hill slope $h = 1.7$).[6,7]

Glucokinase activators (GKAs) control the blood glucose concentration by enhancing the ability of pancreatic β-cells to sense glucose and increase insulin secretion in a glucose-dependent manner. In addition, GKAs act in the liver to improve glucose disposal and decrease hepatic glucose output.[14] Each GKA has the potential to induce a unique conformation of the allosteric site of the active form of glucokinase, and the resulting complexes may exhibit unique enzymatic kinetic profiles. Scientists at Hoffman la Roche were the first to describe heterocyclic amide-based small molecule activators of glucokinase,[8] with subsequent disclosures by AstraZeneca,[9,10,11] Banyu (Merck),[12] and other companies[13,14,15,16] each containing variations on the heterocyclic amide core.

In recent years, a number of GKAs have progressed into clinical trials.[17,18] Reports from the trials have demonstrated the glucose lowering effect of activating GK. Depending on the

compound and dose, hypoglycemia is observed as a common adverse event. In clinical studies that last months, rather than weeks, the glucose lowering effect has not been durable. By activating GK at low glucose concentrations, insulin is released too often and further exacerbates peripheral insulin resistance. Identifying a compound with an improved kinetic profile should prevent insulin secretion from the β-cell at low blood glucose concentrations. The hypothesis is that better management of kinetics would both prevent hypoglycemia and lead to a more durable effect.

In a previous report, we disclosed our initial investigations into a new class of GKAs.[19] Utilizing structure-based design, a novel recognition motif was proposed and validated with pyridylthiazole **1**. (Figure 1) Subsequent structure-guided optimization resulted in the identification of **2** as an early *in vivo* proof-of-concept compound. The results of this early investigation served as the basis for a broad investigation into heteroaryl-aminopyridines as new cores for GKAs. Herein, we describe our further advances in the program to afford a compound with improved potency, pharmacokinetics and *in vivo* efficacy.

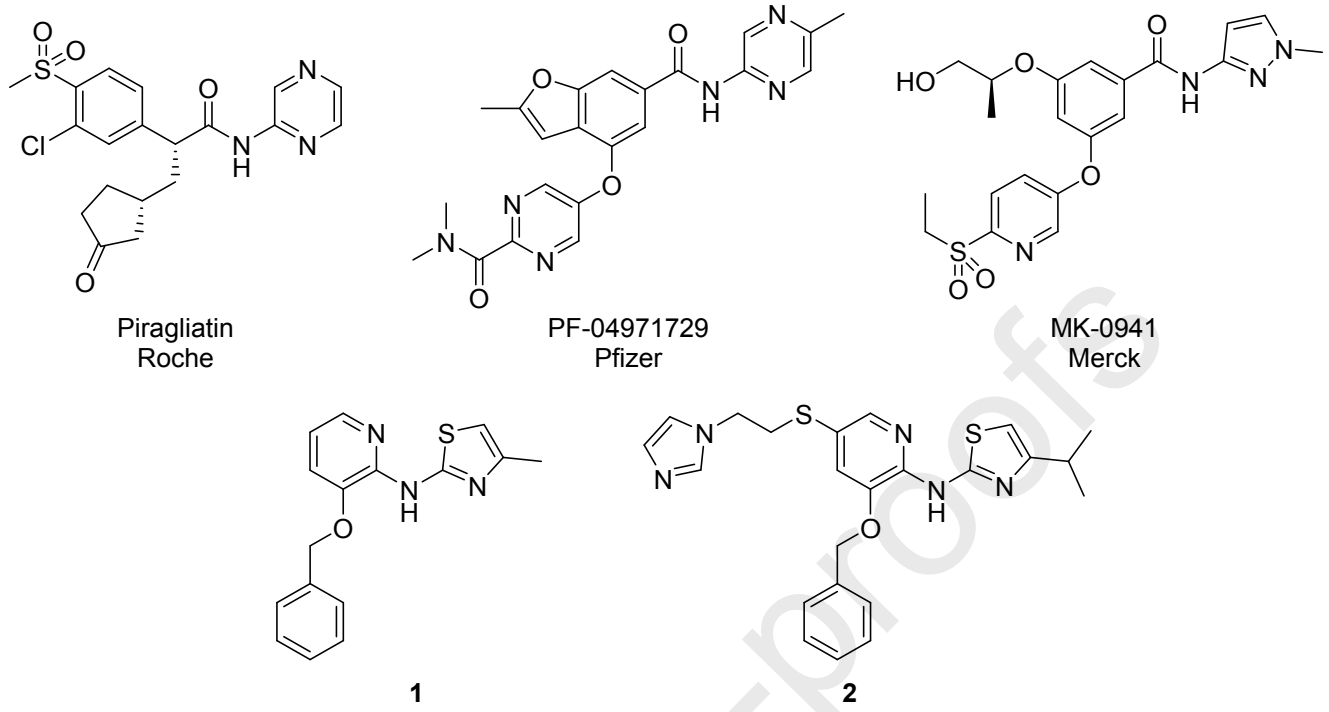
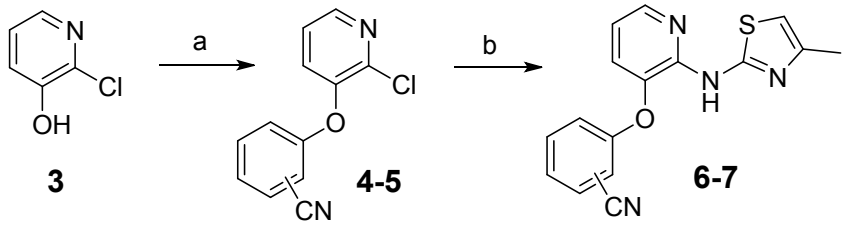


Figure 1. Representative glucokinase activators previously in clinical development and initial hit compound **1** and lead compound **2**.

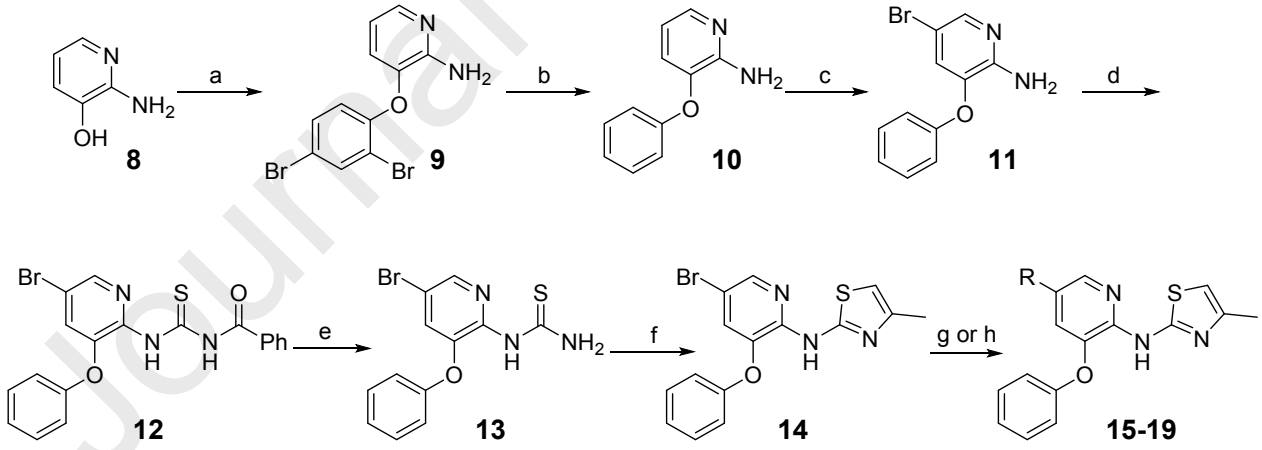
2. Chemistry

We designed methods to access 3-aryloxy-2-aminopyridines in an effort to elaborate on the scaffold of our initial hit compound **1**. The chemistry to access the initial analogs **6** and **7** utilized an S_NAr reaction of 3-hydroxy-2-chloropyridine, as shown in Scheme 1. Chloropyridine **3** was reacted with 2- or 4-fluorobenzonitrile to afford **4** and **5**, respectively. The resulting chloropyridines **4** and **5** were reacted with 4-methyl-2-aminothiazole under palladium-catalyzed conditions to afford target aminothiazoles **6** and **7**.[20]



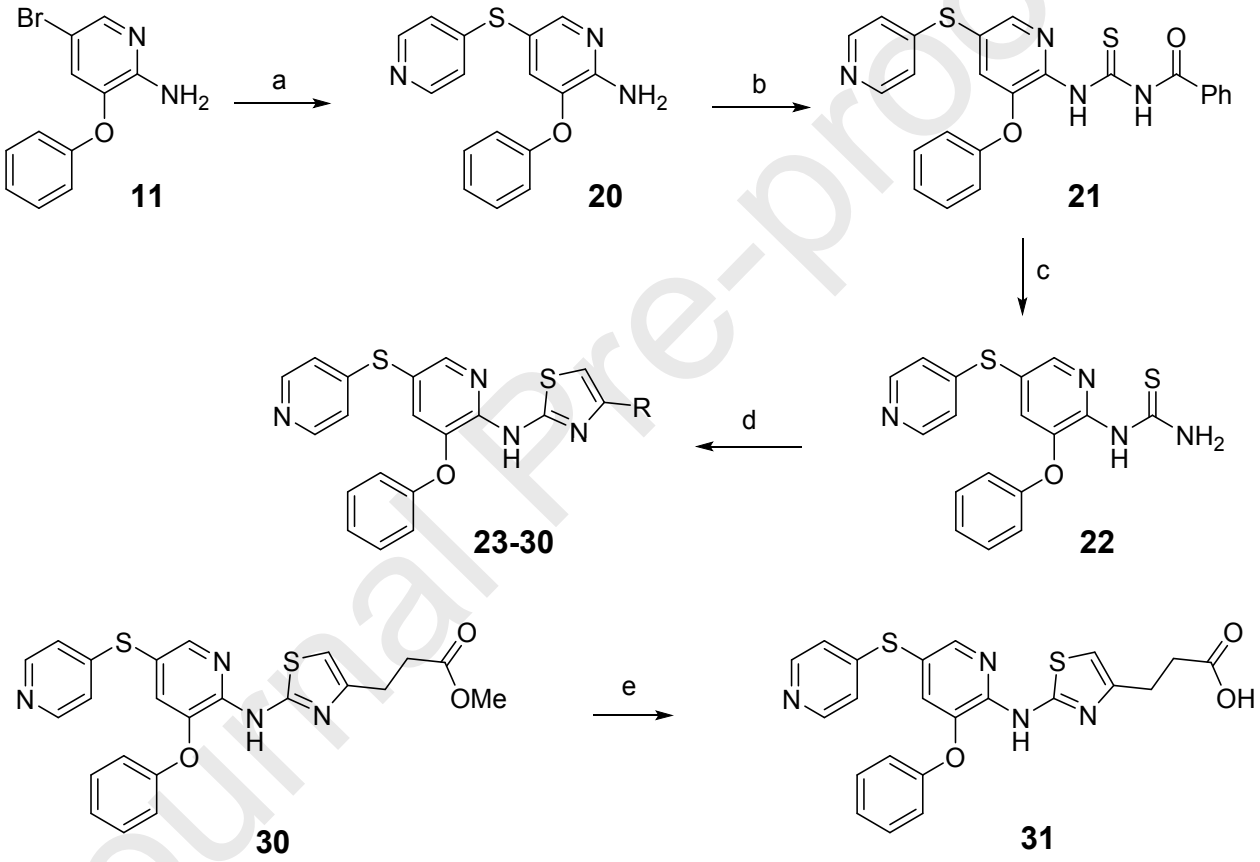
Scheme 1. Reagents and conditions: (a) K_2CO_3, 2- or 4-fluorobenzonitrile, DMF, 90 °C; (b) 4-methyl-2-aminothiazole, Pd_2dba_3, Xantphos, K_3PO_4, toluene:water, 90 °C.

3-Phenoxypyridines bearing 5-substituents were synthesized via the route in Scheme 2. S_NAr reaction of 2-aminopyridin-3-ol (**8**) with the electron-deficient 2,4-dibromo-1-fluorobenzene allowed for the introduction of the phenyl ether to afford **9**. Catalytic hydrogenolysis of the two remaining halogens afforded the phenyl ether **10**. The amino moiety allowed selective 5-bromination of the pyridyl ring of **10** to afford the bromide **11**. Condensation of the amino pyridine **11** with benzoylisothiocyanate afforded acylthiourea **12**. Cleavage to the benzoyl group of **12** followed by alkylation and cyclization with chloroacetone in a Hantzsch synthesis[21] afforded aminothiazole **14**. The 5-bromo substituent of **14** allowed the introduction of diverse functional groups. Initial reaction of **14** with MeLi to remove the acidic NH proton, followed by *n*-BuLi to facilitate a halogen-metal exchange, and finally quenching with water (to yield **15**) or a variety of disulfides afforded thioethers **17-19**. Alternatively, palladium-mediated coupling of **14** with benzylmercaptan directly afforded benzyl thioether **16**.[22]



Scheme 2. Reagents and conditions: (a) NaH, 2,4-dibromo-1-fluorobenzene, DMF, 90 °C; (b) H_2, 10% Pd/C; (c) Br_2; (d) PhC(O)NCS, THF; (e) NaOH, EtOH, 50 °C; (f) chloroacetone, Et_3N, EtOH, 70 °C; (g) i. MeLi, THF, -78 °C ii. BuLi, -78 °C iii. H_2O or RS-SR; (h) BnSH, Et_2N^iPr, Xantphos, Pd_2dba_3, dioxane, 95 °C.

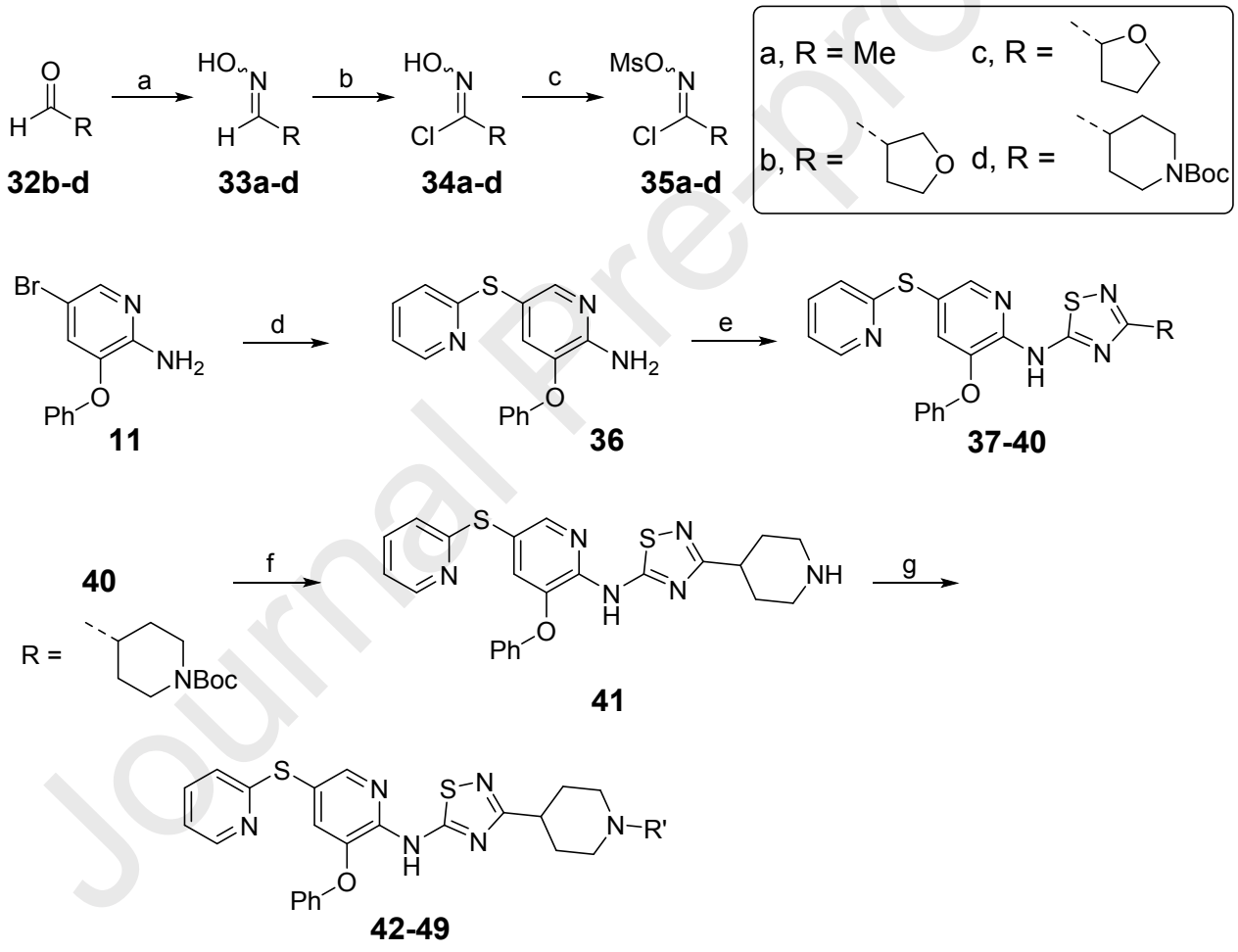
Utilizing similar chemistry in Scheme 2, but changing the order of introduction of functional groups, allowed the 4-substituent of the thiazole to be systematically changed via the route in Scheme 3. In this effort the 5-(4-pyridylthio) group was employed as a substituent on the pyridine core. Key thiourea intermediate **22** was obtained from bromide **11** by similar transformations as described above in Scheme 2. Reaction of the thiourea with a variety of alpha-halo ketones afforded thiazoles **23-30**. Hydrolysis of the ester in **30** afforded the acid **31**.



Scheme 3. Reagents and conditions: (a) i. MeLi, THF, -78 °C ii. n-BuLi, -78 °C iii. 4-pyridyl disulfide, RT; (b) PhC(O)NCS, THF; (c) NaOH, EtOH, 50 °C; (d) X-$CH_2C(O)R$, Et_3N, EtOH, 70 °C; (e) NaOH, THF, H_2O.

Replacement of the amino thiazole with an amino thiadiazole functionality was accomplished through the facile reaction of α-isothiocyano oxime *O*-sulfonate reagents with amines, as depicted in Scheme 4.[23] Oximes **33a-d** were cleanly converted to their corresponding α-chloro

oxime **34a-d** with *N*-chlorosuccinimde. Addition of triethylamine to a solution of **34a-d** and mesyl chloride in ether affords methylsulfonyloxy iminoyl chlorides **35a-d**. The intermediate **36** was synthesized in a similar fashion as **20**. Reaction of α-chloro oxime *O*-sulfonates **35a-d** in acetonitrile with NaNCS and pyridine at 45 °C forms an intermediate that is further reacted with **36** and cyclized at 60 °C to form the desired amino thiadiazoles **37-40**. Deprotection of the Boc-group in **40** with trifluoroacetic acid, followed by functionalization of the secondary amine afforded **42-49.**



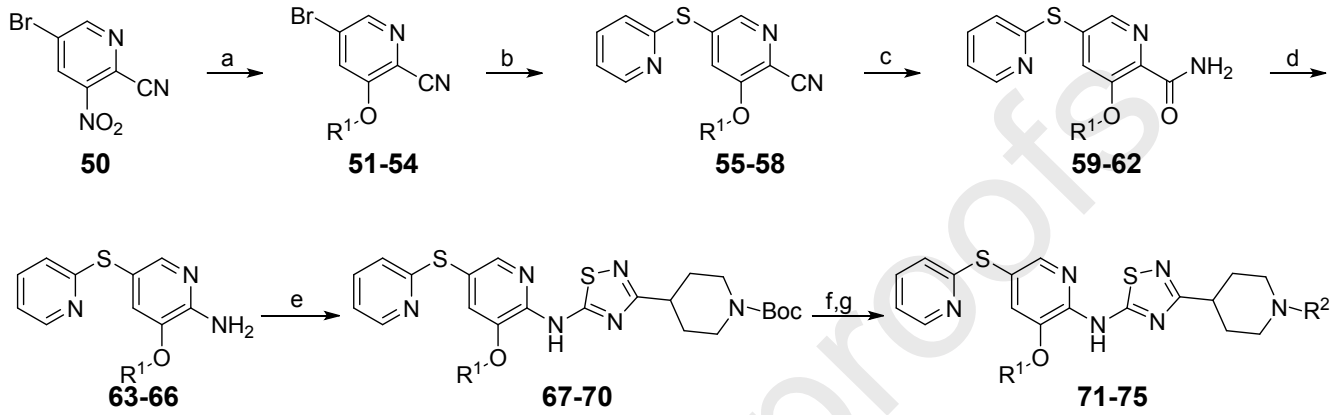
Scheme 4. Reagents and conditions: (a) $NH_2OH{\cdot}HCl$, Na_2CO_3, THF, H_2O; (b) NCS, DMF; (c) MsCl, Et_3N, Et_2O; (d) i. MeLi, THF, -78 °C ii. BuLi, -78 °C iii. 1,2-di(pyridin-2-yl)disulfane,

RT; (e) i. **35a-d**, NaNCS, pyridine, acetonitrile, 45 °C ii. **36**, 60 °C; (f) TFA; (g) R'X, DIEA or R'OH, EDCI, Et_3N.

We sought an efficient methodology for the installation of groups other than phenoxy at the 3-position of the pyridine core, and developed the S_NAr sequence shown in Scheme 5. An apparent strategy to achieve this is an S_NAr onto a 3-chloro-2-nitropyridyl compound. However, the nitro moiety can compete as a leaving group with the chloride. By changing to a 3-fluoro-2-nitropyridine, the desired fluoride displacement is the major product; however, the synthetic route to produce the starting material required an exothermic decomposition of 2-nitropyridine-3-diazonium tetrafluoroborate at 115 °C, which was not amenable to large scale synthesis.[24] Recognizing that nitro groups could be displaced in S_NAr reactions and that esters or nitriles are potential synthetic equivalents for an amino moiety via either a Curtius or Hoffman reaction, we investigated the 3-nitro-2-cyanopyridine as a synthetic intermediate. It was reported that nucleophiles (NaOMe and fluoride) selectively displaced the nitro moiety to produce 3-fluoropicolinonitrile and 3-methoxypicolinonitrile.[25] Indeed, as will be seen below, diverse hydroxyheterocycles displace the nitro moiety selectively.

For this initial SAR investigation of the range of tolerated groups at the 3-position, a set of analogs bearing the 3-(4-piperidinyl)-thiadiazole was chosen. Selective displacement of the nitro group from the commercially available cyanopyridine **50** with various phenols and heteroaryl alcohols afforded pyridyl ethers **51-54**.[26] Subsequent displacement of the 5-bromo group with pyridine-2-thiol afforded thioethers **55-58**. Partial hydrolysis of the nitrile to the corresponding primary amides **59-62** was accomplished with sulfuric acid under controlled conditions. Hofmann rearrangement of the resulting amides, using bromine in aqueous base, afforded the 2-aminopyridines **63-66**. The target thiadiazoles **67-70** were prepared as delineated in Scheme 4

above, using **35d** and sodium isothiocyanate. The piperidinyl group was further elaborated by acidic cleavage of the Boc-group, followed by acetylation or mesylation to generate amides **71-74**, and sulfonamide **75**.



Scheme 5. Reagents and conditions: (a) R^1OH, NaH, DMF; (b) 2-pyr-SH, NaH, DMF; (c) H_2SO_4; (d) Br_2, NaOH; (e) i. **35d**, NaNCS, pyridine, acetonitrile, 40 °C ii. **63-66**, 75 °C; (f) TFA; (g) Ac_2O, Et_3N, or MsCl, Et_3N.

3. Results and discussion

3.1 Initial hit and protein crystal structure

Upon examination of the crystal structure of **1**,[19] we hypothesized that the benzyloxy group could be replaced with an aryl ether to access similar space in the hydrophobic pocket of the enzyme. Such a modification would remove the benzylic carbon, a potential site of oxidative metabolism. Due to its straightforward synthesis, the first compound to test this hypothesis was the 2-cyanophenyl ether **6**. As can be seen in Table 1, its ability to promote GK activation was promising for a relatively unadorned compound. The V_{max} for **6**, as well as for the 4-cyano analog **7**, was below 100%. A compound which presents a lower $S_{0.5}$, but a lower V_{max} provides activation of glucose phosphoryolation at low glucose concentrations; however, it would be predicted to inhibit glucose phosphorylation at high blood glucose concentrations, leading to

prolonged postprandial hyperglycemia. (Figure 2) To identify activators with a V_{max} > 100% was an aim of the lead optimization for this series.

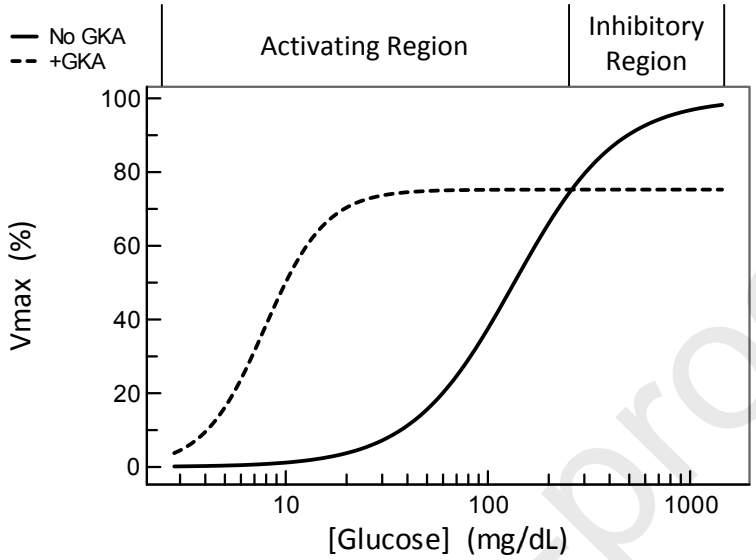


Figure 2. Graphical representation of a GKA with low $S_{0.5}$ and V_{max} of 75%. At blood glucose concentrations >250 mg/dL, the activity of GK bound to the GKA is lower than the native enzyme.

The co-crystal of glucokinase with glucose and **6** was used to elucidate the single crystal X-ray structure of the complex. (Figure 3) The biaryl ether occupied a similar pocket as the benzyl ether of **1**, however the nitrile group did not appear to make any favorable interactions with the enzyme.

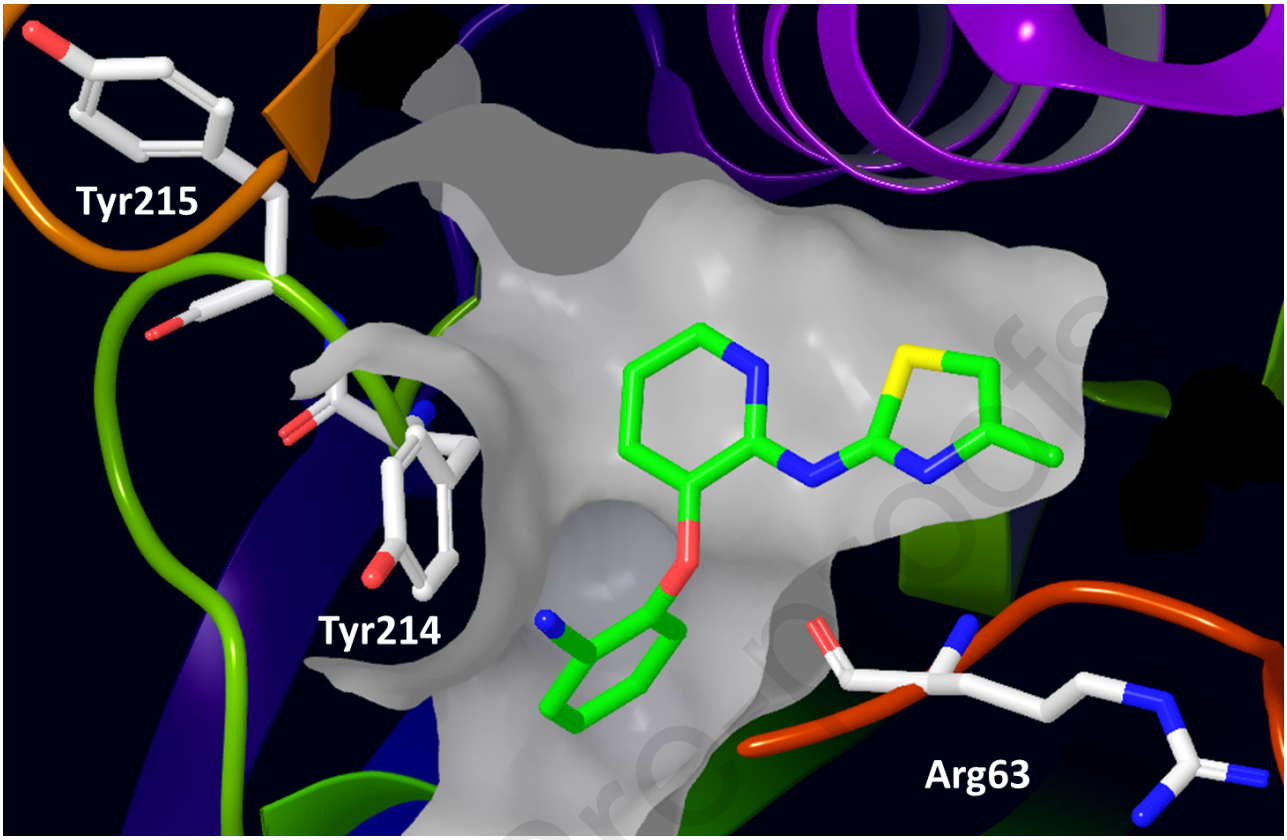


Figure 3. X-ray crystallographic structure of aryl ether **6** in complex with GK at 2.1 Å. (6E0E)

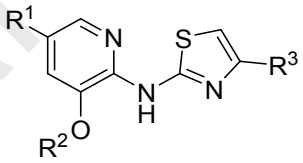
3.2. SAR of 3-O-Aryl GKAs.

An initial investigation of the SAR of analogs of **6** was undertaken to expand on this initial finding, (Table 1). Removal of the benzylic methylene of **1** to create the biaryl ether linkages led to improved potencies. Initially, the 2- and 4-nitriles on the phenyl ring were utilized to facilitate synthesis of the aryl ethers, as shown in Scheme 1, based on the facile S_NAr incorporation of the aryl group. Hence, alternative routes to the aryl ethers were identified, in order to rapidly gain access to a wider range of aryl ethers. After solving the cocrystal structure of **6** bound to GK, molecular modeling identified highly lipophilic areas surrounding the nitrile, which appeared to be a suboptimal substituent for this position on the aryl ring. A different route (Scheme 2) was utilized to prepare unsubstituted phenyl ethers, which were designed to better complement the

hydrophobic environment in this pocket. As anticipated, the EC_{50} of the phenyl ether **15** was greater than 5-fold more potent than **6**.

Previously, we reported that addition of groups at the 5-position of the pyridyl ring can confer dramatic improvements in potency.[19] The allosteric binding site for GKAs includes a number of flexible residues, which can adapt to a wide range of small molecule structures. Among these, Tyr215 can adopt two families of conformations, depending on the steric requirement of the activator. Surprisingly, in the structure of the GK-**6** complex, where **6** presents only a hydrogen along the vector facing Tyr215, the phenol group of Tyr215 is observed to be rotated away from the binding site, creating a larger hydrophobic pocket for activators bearing larger groups in this region. In the case of these 3-aryloxy GKAs, addition of a 5-bromo (**14**) resulted in a compound that was equipotent in terms of EC_{50} and $S_{0.5}$, but the V_{max} of the enzyme-activator complex was nearly doubled to 145%.

Table 1. Initial SAR of the pyridyl 3- and 5-substituents.



Compound	R^1	R^2	R^3	EC_{50} (nM)	$S_{0.5}$ (mM)	V_{max} (%)
1	H	Bn	Me	5800	3.2	79
2	$S(CH_2)_2$Imid	Bn	iPr	119	0.9	117
6	H	2-CN-Ph	Me	2359	1.4	83
7	H	4-CN-Ph	Me	3521	2.0	84
15	H	Ph	Me	419	0.9	80
14	Br	Ph	Me	400	1.0	145
16	BnS-	Ph	Me	157	1.1	127

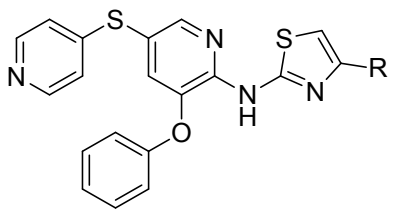
17	PhS-	Ph	Me	98	1.3	155
18	2-Pyr-S-	Ph	Me	72	0.5	151
19	4-Pyr-S-	Ph	Me	32	0.4	158

Based on the SAR from our previous work,[19] wherein thioethers were preferred 5-substituents, the bromide **14** was elaborated into a small set of thioethers (**16-19**). These compounds showed further improvements in the potency, with **19** having an EC_{50} of 32 nM. Relative to the previously published compound **2**, **19** was nearly 4-fold more potent with an improved V_{max}. However, with an $S_{0.5}$ value of 0.4 mM, the compound begins to activate GK below physiologically relevant glucose concentrations, which may result in severe hypoglycemia. In this series of compounds, potency and $S_{0.5}$ correlated, with the most potent compounds being the least glucose-sensitive activators. Subsequent efforts were to identify SAR where the correlation did not apply.

3.3. SAR of thiazole substituents

Based on the crystal structure of GK-**6**, the 4-methyl group on the thiazole points to a solvent-accessible channel. The SAR of this region are depicted in Table 2. While all of the compounds were potent, compounds such as **28** and **29** were especially intriguing. Both of these compounds displayed EC_{50} < 100 nM and V_{max} >100%, however the $S_{0.5}$ of the compounds was dramatically higher than many of the other compounds in the series. These data exemplify clear departures from earlier compounds that exhibited correlation between potency and $S_{0.5}$, thus demonstrating that judicious choice of substituents can have a dramatic influence on the kinetics of the enzyme-activator complexes.

Table 2. The SAR of 4-substituents of the aminothiazole



Compound	R	EC_{50} (nM)	$S_{0.5}$ (mM)	V_{max} (%)	ClogP
19	Me	32	0.4	158	5.5
23	Et	20	0.4	153	6.0
24	CF_3	213	2.1	115	5.9
25	cPr	103	1.0	150	5.9
26	iPr	58	0.7	135	6.4
27	iBu	112	1.1	151	6.9
28	cHex	92	2.4	117	7.6
29	$(CH_2)_2Ph$	47	1.9	150	7.4
31	$(CH_2)_2CO_2H$	22	0.4	131	4.3

3.4. In vivo correlation of $S_{0.5}$ and blood glucose levels

In order to determine how the *in vitro* enzyme kinetic parameters (particularly $S_{0.5}$ and V_{max}) affected anti-hyperglycemic activity, we evaluated many of the compounds in Table 2 in oral glucose tolerance tests (OGTT) in euglycemic C57BL/6 mice. The compounds were tested at 50 mg/kg in an attempt to determine their maximum blood-glucose-lowering efficacy as well as their potential to elicit hypoglycemia. Plasma drug concentrations were obtained at the 2.5 h time point of the OGTT studies, to provide evidence of adequate exposure of the compounds. (see Supplemental Information) The OGTT data for this set were compared, and an interesting trend emerged. The glucose concentration at the 2 h time point appeared to be correlated with $S_{0.5}$ (Figures 4 and 5). The hyperbolic curve fit of these two parameters was surprisingly tight (r^2 = 0.91). No such correlation was identified for V_{max}. From this data, the team hypothesized that an

$S_{0.5}$ of approximately 1.0 would confer a good balance of efficacy and safety from hypoglycemia (correlating with minimal blood glucose concentrations of ~80 mg/dL).

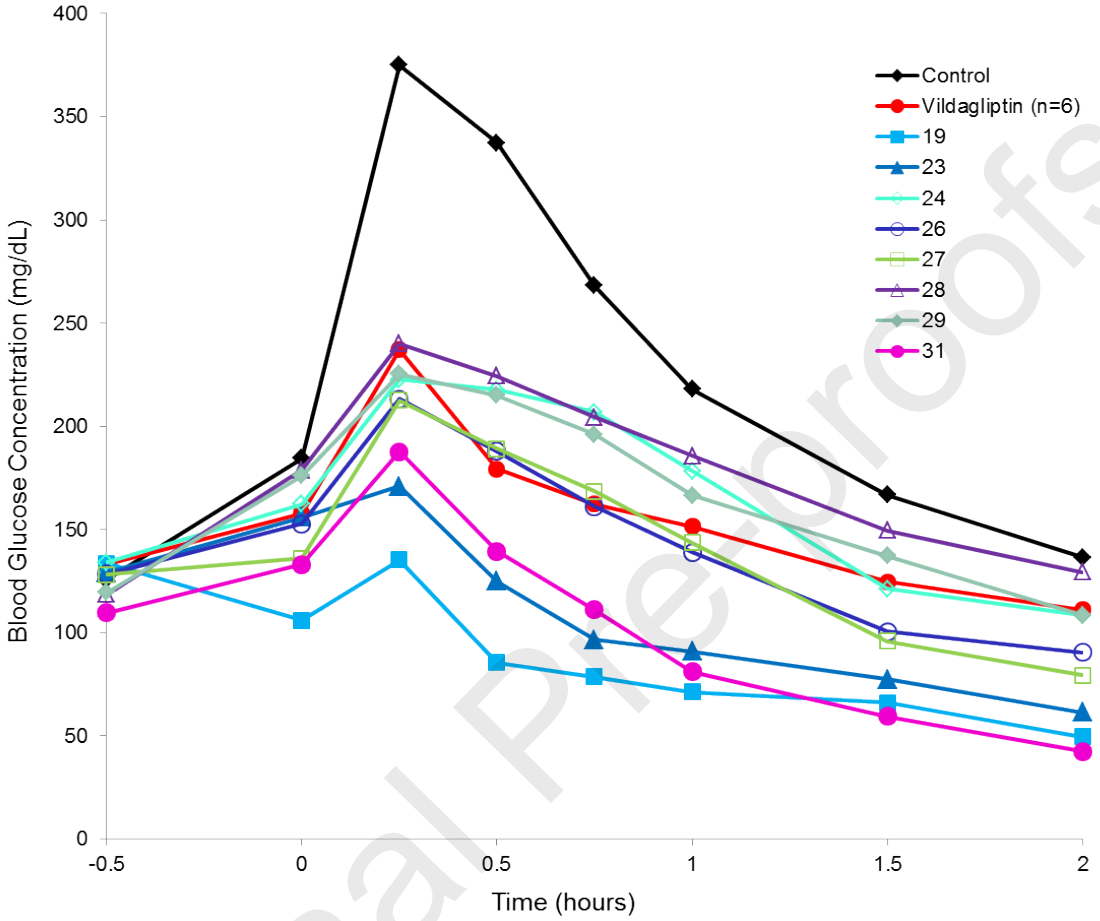


Figure 4. Overlay of OGTTs of compounds from Table 2 at 50 mg/kg PO (n=8 mice per time-point; values represent the arithmetic mean). The $glucose_{min}$ at 2 h appears to change depending on the compound.

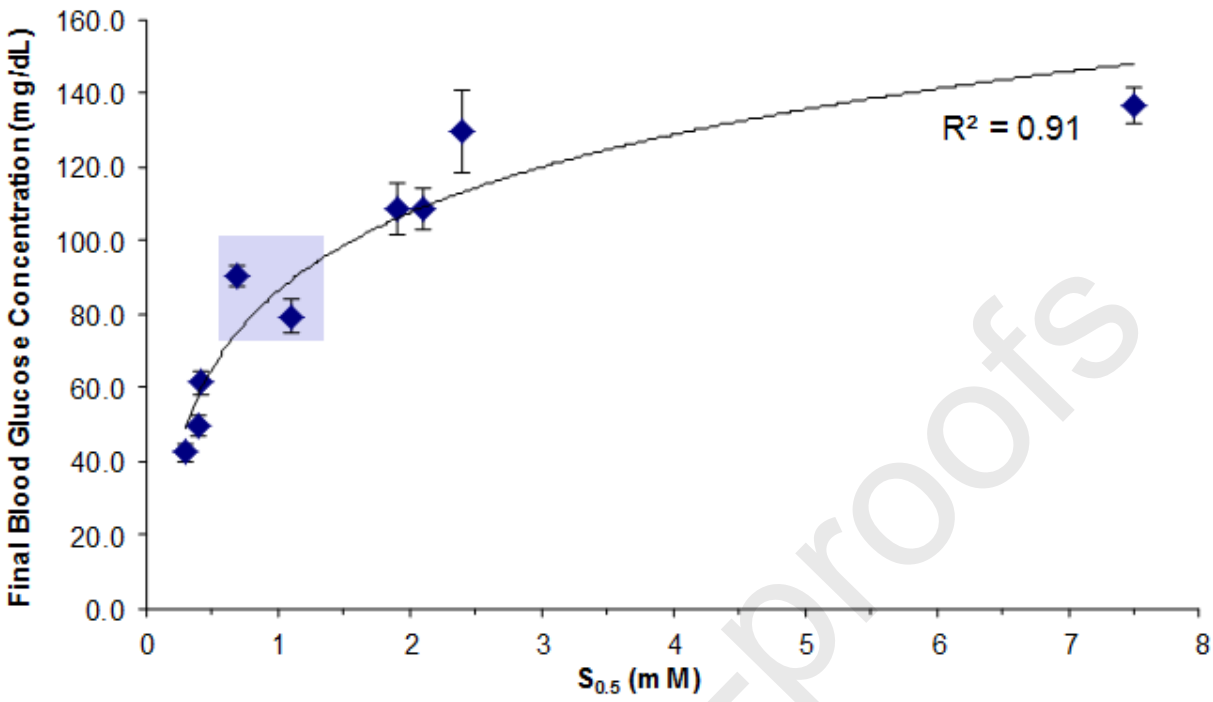


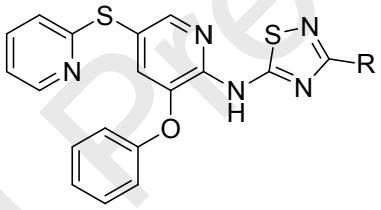
Figure 5. Correlation of the 2 h blood glucose concentration with the measured $S_{0.5}$ in the enzymatic assay. Highlighted area is the proposed range with best balance of efficacy and hypoglycemic risk.

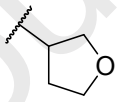
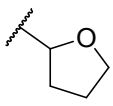
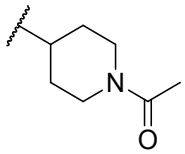
3.5. Thiadiazole SAR

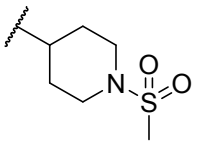
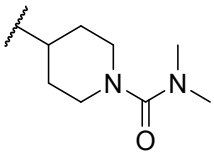
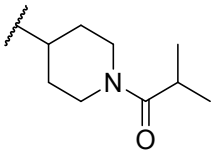
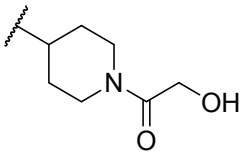
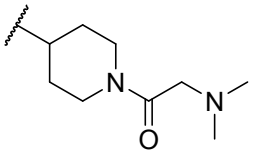
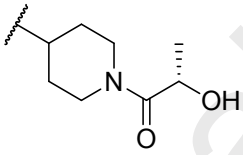
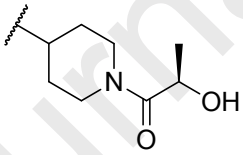
While the above SAR confirmed our hypothesis that there is a strong correlation between $S_{0.5}$ and hypoglycemic risk (blood glucose levels <60 mg/dL), electron-rich, unsubstituted 4-pyridines are not generally good drug candidates (high potential for drug-drug interactions via CYP inhibition). Because the potency, kinetics, and solubility are similar for compounds with either a S-4-pyridyl or a S-2-pyridyl group (**18** vs. **19**), we decided to utilize this group. We were also focused on lowering the ClogP of the series and felt that further investigation of the thiazole substituents was warranted. In addition, we were cognizant of the propensity for 2-amino thiazoles to be metabolized into potentially toxic thioureas.[27,28,29] By replacing the thiazole with thiadiazole, the potential for generation of thiourea metabolites is reduced.[30]

As shown in Table 3, inclusion of piperidinyl-derived substituents on the thiadiazole successfully lowered the ClogP of the series, while keeping the EC_{50} and V_{max} in a desirable range. One parameter that was difficult to predict was $S_{0.5}$. Small changes had little effect on EC_{50} and V_{max}, but dramatically changed the $S_{0.5}$. For example, changing the piperidinyl substituent from acetyl (**42**) to mesyl (**43**) kept the potency below 100 nM and the V_{max} well above 100%, however, the $S_{0.5}$ jumped from 0.61 mM to 2.8 mM. These compounds were still not ideal in terms of physical properties (3.9 < CLogP < 4.7), which correlated with medium to high predicted human hepatocyte clearance rates for all compounds except the acetyl and mesyl compounds, **42** and **43** respectively.

Table 3. *In vitro* data for initial set of substituted aminothiadiazoles.



Compound	R	EC_{50} (nM)	$S_{0.5}$ (mM)	V_{max} (%)	ClogP	Predicted Human Hepatocyte ER (%)
37	Me	78	0.44	148	5.5	69
38		21	0.55	138	4.6	47
39		116	0.73	160	4.6	63
42		49	0.61	144	3.9	30

43		70	2.8	134	4.5	16
44		21	0.56	151	5.2	72
45		29	0.77	147	4.7	65
46		36	0.71	143	4.1	66
47		20	0.48	139	4.6	52
48		37	0.51	133	4.4	51
49		69	0.57	138	4.4	62

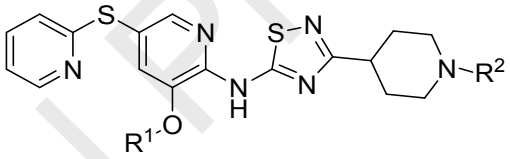
3.6. Replacement of 3-O-aryl with heterocycles

A potential problem for these compounds was the lipophilic unsubstituted 3-phenoxy group. An investigation as to whether the replacement of the phenyl with a polar heterocycle would improve the physical properties and metabolic stability while retaining the potency of the original lead was initiated. Table 4 lists the SAR of various heterocyclic ethers at the 3-position, keeping the thiadiazole and 5-position of the pyridine core constant. Unexpectedly, the higher

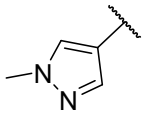
$S_{0.5}$ values of the phenoxy versions were generally not retained in the more potent compounds in the heterocyclic series.

One assay that was included after the start of the project was a human serum albumin-shifted EC_{50} assay. This was incorporated as a high-throughput method to estimate the plasma protein binding of the compounds.[31] This was a significant differentiating factor in our SAR investigation. By changing the phenyl group to a 2-methylpyridyl (**42** to **72**) the standard EC_{50} remained the same, but the EC_{50} in the presence of 4% human serum albumin dropped by 5-fold. Unfortunately, replacing the acetyl for mesyl in this series did not have the same beneficial effect on $S_{0.5}$ (**42** and **43** vs. **72** and **73**). Structural or physical properties that consistently had an effect on $S_{0.5}$ were not identified. From this work, **72** was chosen for further investigation.

Table 4. In vitro data for 3-(heteroaryloxy)pyridine analogs



Compound	R^1	R^2	EC_{50} (nM)	4% HSA EC_{50} (nM)	$S_{0.5}$ (mM)	V_{max} (%)	ClogP	Predicted Human Hepatocyte ER (%)
42	Ph	Ac	49	1640	0.61	144	3.9	30
43	Ph	Ms	70	3345	2.8	134	4.5	16
71	3-Pyr	Ac	233	2710	1.1	135	2.4	41
72	2-Me-3-Pyr	Ac	42	362	0.63	115	2.9	45
73	2-Me-3-Pyr	Ms	33	684	0.58	131	3.5	26
74	5-quinoline	Ac	9.3	504	0.42	109	3.8	NT

75		Ac	279	1967	0.97	126	2.0	32

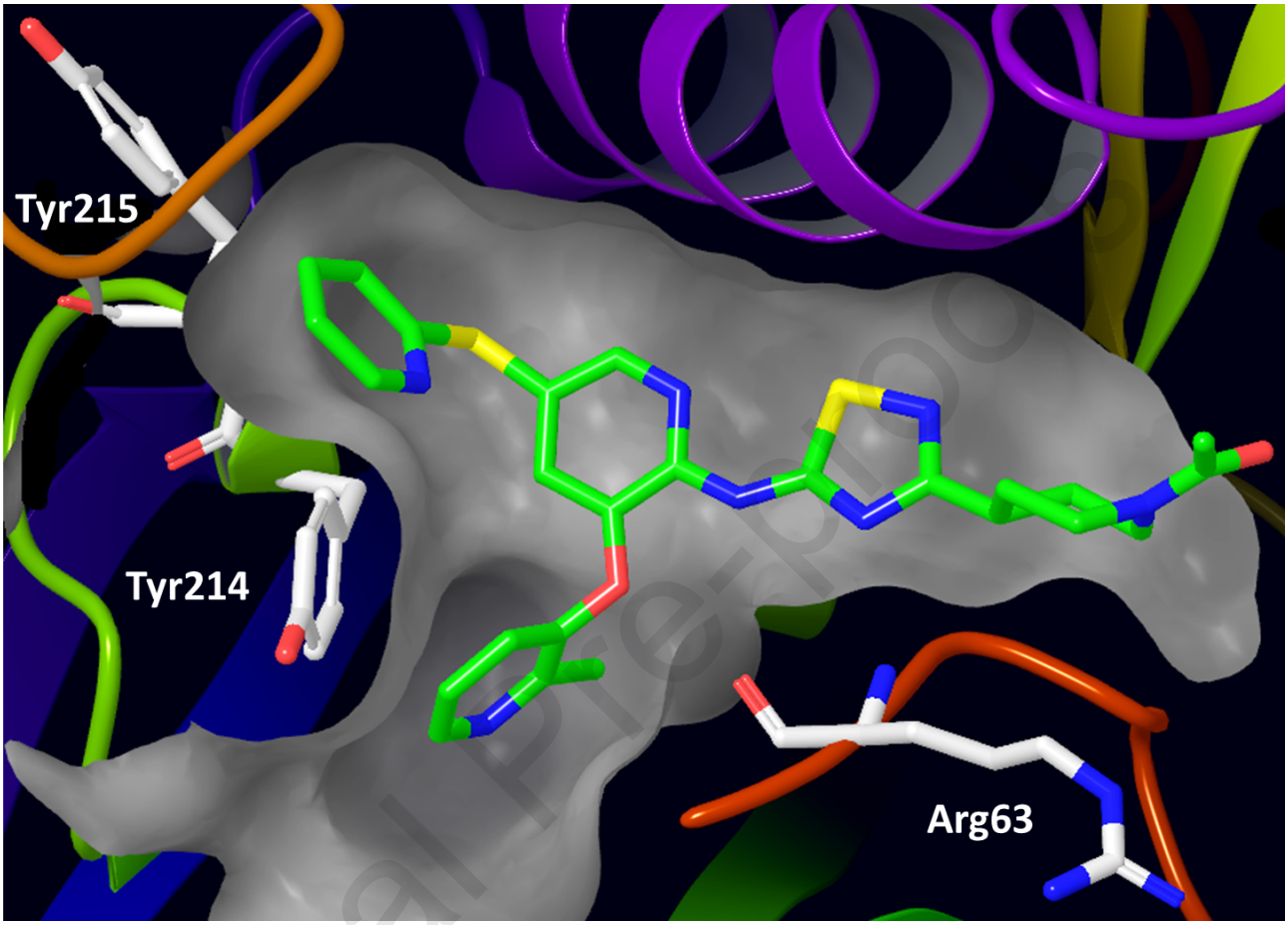


Figure 6. X-ray crystallographic structure of **72** bound to GK at 1.9 Å. (6E0I)

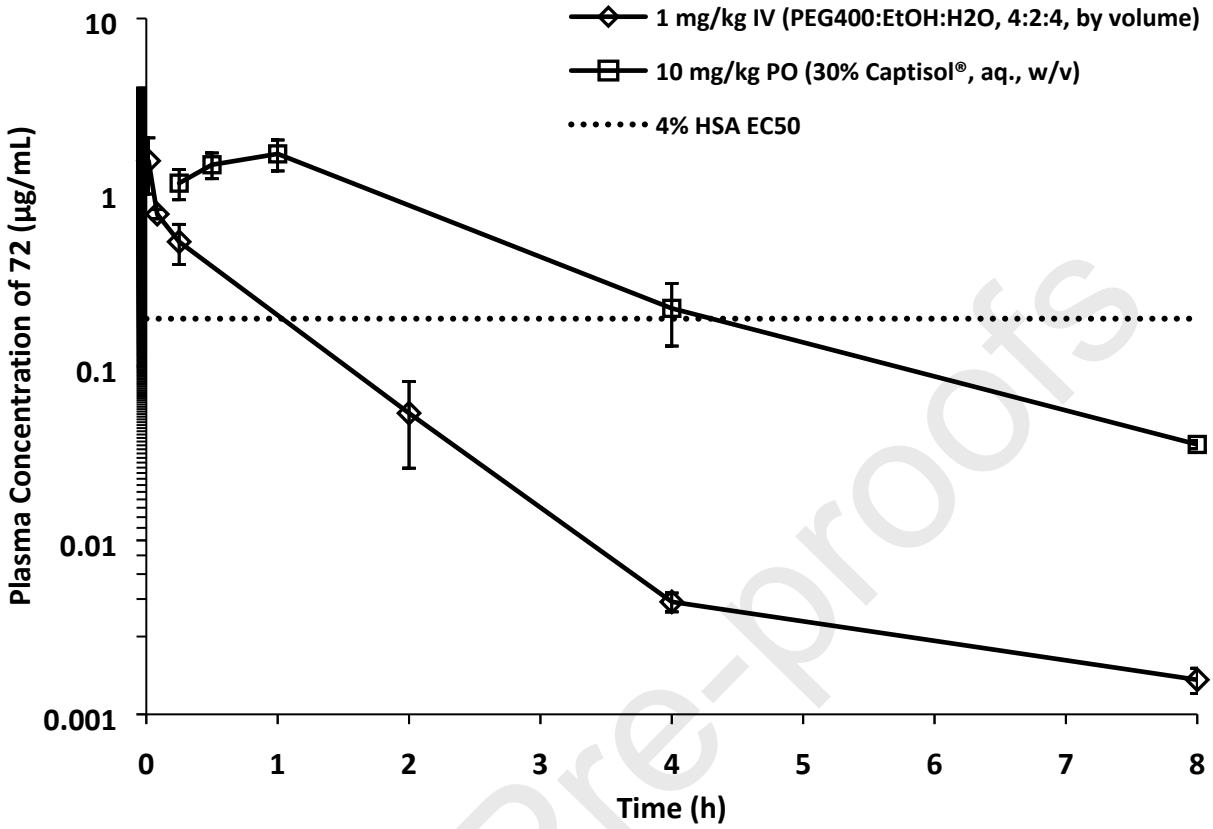
The structure of **72** bound to GK was solved by x-ray crystallography, confirming the predicted binding mode of the compound (Figure 6). The aminothiadiazole makes the typical dual hydrogen bonds with the main chain of Arg 63, while the 2-pyridyl thioether fills the pocket optionally occupied by the highly mobile side-chain of Tyr 215, making lipophilic contacts with both Tyr 214 and Tyr 215. The 2-methyl-3-pyridyl ether resides in the lower pocket as shown, forming a loose pi-stacking interaction with Tyr 214, while directing the 2-methyl group deep into a hydrophobic cleft in the protein. The acetyl piperidine substituent stretches through a

largely hydrophobic tunnel, directing the acetyl group toward a solvent-exposed opening on the protein surface.

Compound **72** was progressed into a set of secondary *in vitro* assays to determine its probability of *in vivo* success. The compound displayed acceptable stability in predictive microsomal and hepatocyte metabolism experiments (predicted mouse microsomal clearance: 41 mL/min/kg; predicted mouse hepatocyte clearance: 17 mL/min/kg). It was highly permeable in a Caco2 cell assay (A-B: 9.3 cm/s; pe ratio: 0.83) and did not display CYP_{450} inhibition up to 25 μM for 5 isoforms (1A2, 2C9, 2D6, 3A4, 2C19). These results provided the team with confidence to progress the compound into a set of *in vivo* pharmacology assays.

*3.7. Mouse PK of **72***

As shown in Figure 7, **72** showed acceptable pharmacokinetic properties in mouse, with an observed systemic clearance value in agreement with the predicted mouse hepatocyte clearance (21 vs. 17 mL/min/kg). Gratifylingly, compound **72** demonstrated a good bioavailability (60%) in mice and exposures that maintained plasma drug concentrations above the human serum albumin-shifted EC_{50} for approximately 4 hours.



Dose	AUC_{inf} (hr*µg/mL)	$t_{1/2}$ (hr)	CL (mL/min/kg)	V_{ss} (L/kg)
1 mg/kg IV	0.770	1.28	21.6	0.746
Dose	AUC_{inf} (hr*µg/mL)	C_{max} (µg/mL)	T_{max} (hr)	F (%)
10 mg/kg PO	4.65	1.67	1.00	60.3

Figure 7. Mean plasma concentration/time profiles of compound **72** after a single 1 mg/kg intravenous (IV; diamonds) or 10 mg/kg oral (PO; squares) dose of compound **72** in solution to male CD-1 mice (n=3 per time-point; values represent the arithmetic mean ± SD). The dashed midline represents the EC_{50} value for compound **72** as determined by the 4% HSA EC_{50} assay.

*3.8. Efficacy of **72** in mouse models of diabetes*

A dose-response oral glucose tolerance test (OGTT) study of compound **72** performed in C57BL/6 mice (Figure 8) indicated that 10 mg/kg effectively reduced the peak blood glucose at 15 minutes, and significantly reduced the total glucose area-under-the-curve ($AUC_{glucose}$) by 23%. The efficacy of **72** at 10 mg/kg was greater than the positive control sitagliptin at 30 mg/kg in this study. The 30 mg/kg dose dramatically blunted the glucose excursion, and the 2 h average blood glucose remained above 70 mg/dL, our threshold for overt hypoglycemia in these euglycemic mice. This compound was therefore determined to be effective with a relatively low potential for hypoglycemia and was advanced into a diabetic animal model.

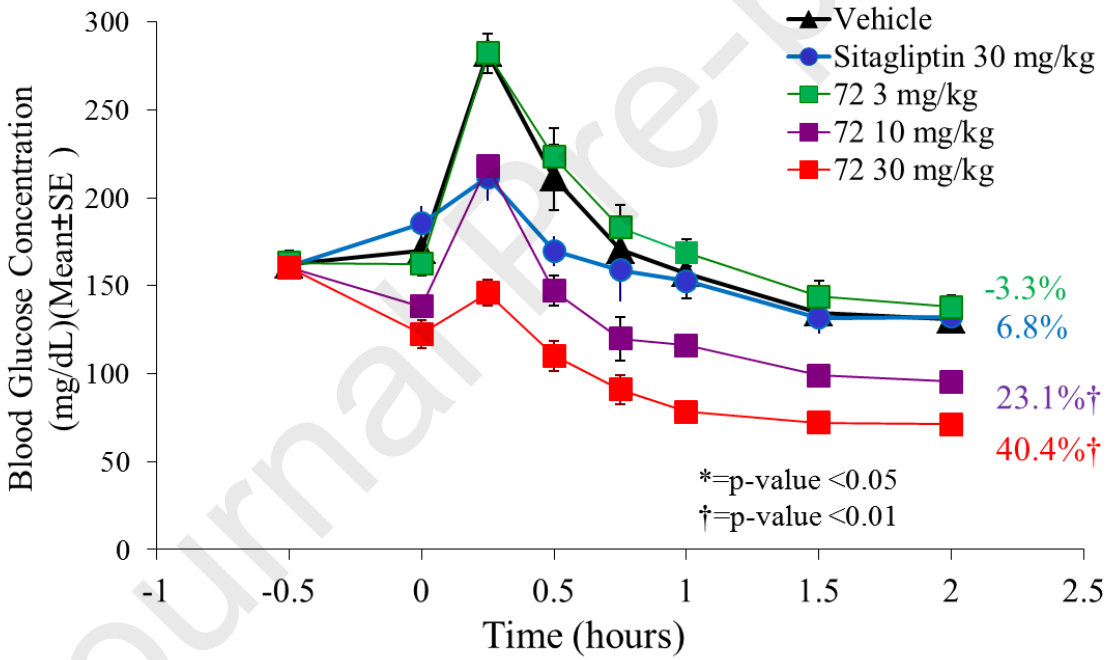


Figure 8. Dose response OGTT study of compound **72** in normoglycemic C57BL/6 mice (n=8 mice per time-point; values represent the arithmetic mean ± SD)

Compound **72** was studied in male diabetic *ob/ob* mice, dosed orally once-daily for 14 days, with OGTT performed on day 14 and non-fasted blood glucose measured over the course of the

study. There were three criteria in which we were interested: lowering of plasma glucose in the OGTTs; lowering of fasted plasma glucose before OGTT; and lowering of non-fasted plasma glucose throughout the study. As can be seen in Figures 9 and 10, the 10 and 30 mg/kg doses were effective at all three critical criteria. At 10 mg/kg, compound **72** lowered the fasted blood glucose from the control animals on day 14 (-0.5 hr time-point) as well as the AUC of the OGTT. Also shown in Figures 9 and 10 are the untreated non-diabetic control C57BL/6 mice. The 10 and 30 mg/kg doses controlled the fasted (t=-0.5 h in figure 9) and non-fasted blood glucose concentrations (Figure 10) to those of the non-diabetic controls. The 30 mg/kg dose had an average 2 h blood glucose level of 66 mg/dL, which was just below our 70 mg/dL threshold for hypoglycemia. However, no adverse effects were observed. Sitagliptin (30 mg/kg) was utilized as a positive control.

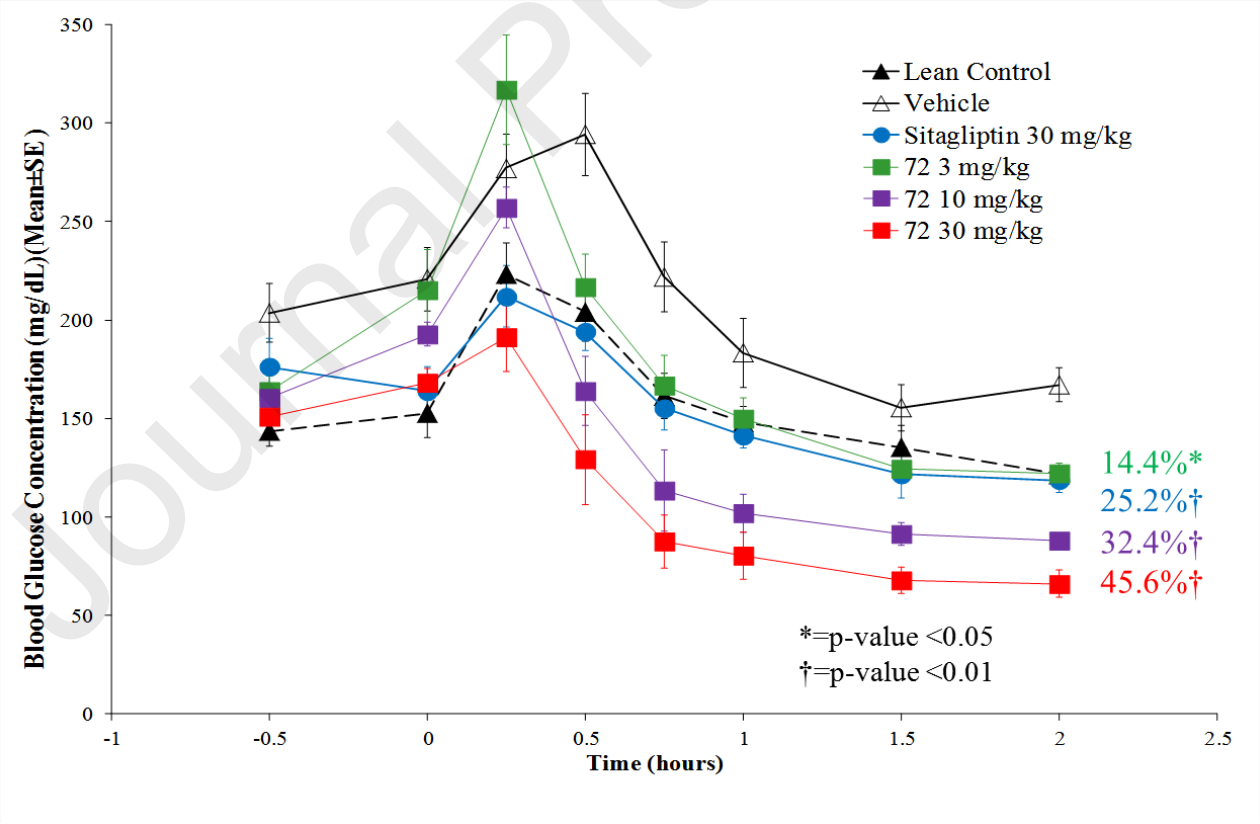


Figure 9. Compound 72 studied in *ob/ob* mouse, OGTT on day 14 (n=8 mice per time-point; values represent the arithmetic mean ± SD).

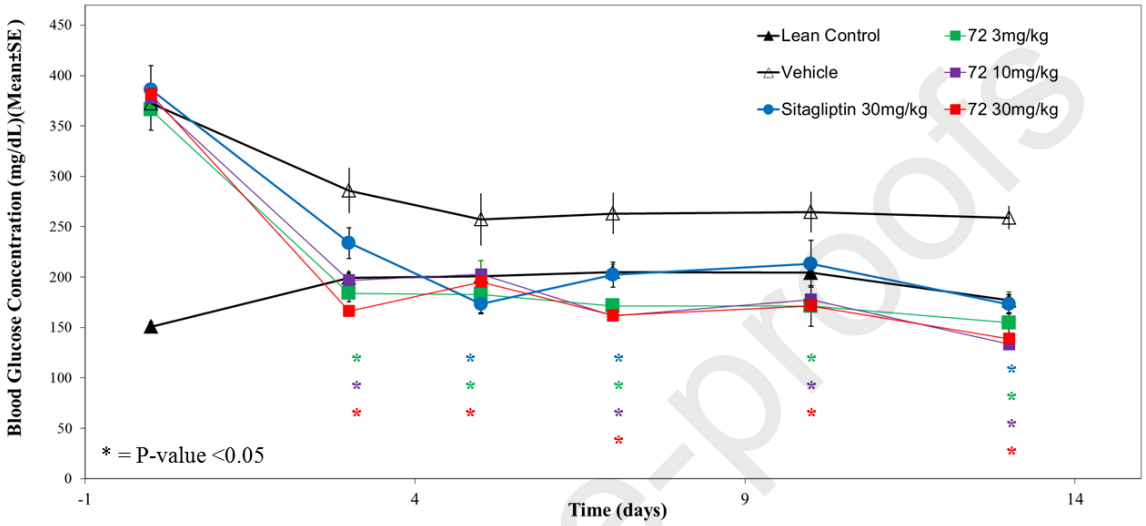


Figure 10. Non-fasted blood glucose values over the course of the 14d *ob/ob* study mice (n=8 per time-point; values represent the arithmetic mean ± SD). Day 0 blood glucose concentrations were high in *ob/ob* mice due to stress response from initial handling.

4. Conclusions

We have reported on the discovery and optimization of a new series of non-amide based GKAs, through iterative structure-based design. By changing the 3-substituent of the pyridine core from the original benzyl ether lead to an aryl ether, the potency and GK activation kinetics of the series were improved. Optimization of three different regions of the molecules led to the synthesis of acetylpiperidine **72**, which possessed a good balance of enzymatic, physicochemical, and pharmacokinetic properties. Compound **72** was progressed through the later stage assays of the project and demonstrated anti-hyperglycemic activity in a dose-ranging 14 day *ob/ob* mouse

study. Further optimization of this series to improve glucose-dependence and pharmacokinetics will be reported in future publications.

5. Experimental Section

5.1 Chemistry

Reagents were purchased from commercial sources and used without further purification. Nuclear magnetic resonance (NMR) spectra were measured in the indicated solvent with tetramethylsilane (TMS) or the residual solvent peak as the internal standard on a Varian 400 MHz spectrometer. Chemical shifts (δ) are in parts per million. LC-MS experiments were performed on an Agilent 1100 HPLC coupled with an Agilent MSD mass spectrometer using ESI as ionization source. Peaks were detected by UV absorbance at 220 and 254 nm, and MS full scan was applied to all experiments. All compounds disclosed in this paper were confirmed to be >95% purity via this method.

*5.1.1. 2-(2-(4-Methylthiazol-2-ylamino)pyridin-3-yloxy)benzonitrile (**6**)*

2-(2-Chloropyridin-3-yloxy)benzonitrile (0.667 g, 2.89 mmol), 4-methylthiazol-2-amine (0.300 g, 2.63 mmol) and potassium phosphate (0.614 g, 2.89 mmol) were dissolved in toluene (7 mL) and nitrogen bubbled through the solution for 5 minutes. Pd_2dba_3 (0.060 g, 0.0657 mmol) and 4,5-bis(diphenylphosphino)-9,9-dimethyl-9*H*-xanthene (0.041 g, 0.0723 mmol) were added and the reaction further bubbled through with nitrogen for 5 minutes. Degassed water (2 mL) was added and the reaction heated to 90 °C overnight under nitrogen. The reaction was cooled to room temperature and partitioned between water and ethyl acetate, the organic layer was dried over magnesium sulfate, filtered and concentrated. The residue was purified over silica gel (elution 10-40% EtOAc in hexanes) to afford 2-(2-(4-methylthiazol-2-ylamino)pyridin-3-yloxy)benzonitrile (0.385 g, 46.6% yield) as yellow solid. ^{1}H NMR ($CDCl_3$) δ ppm 8.23 (dd, *J* =

5.1, 1.6 Hz, 1H), 7.72 (dd, *J* = 7.6, 1.6 Hz, 1H), 7.53 (m, 1H), 7.24 (td, *J* = 7.6, 1.0 Hz, 1H), 7.20 (dd, *J* = 8.0, 1.6 Hz, 1H), 6.92-6.84 (m, 2H), 6.44 (q, *J* = 1.1 Hz, 1H), 2.33 (d, *J* = 1.1 Hz, 3H); MS (ES^+) *m/e* 309.0 $(M+H)^+$.

5.1.2. 4-(2-(4-Methylthiazol-2-ylamino)pyridin-3-yloxy)benzonitrile (7)

Synthesized via the same method as **6**. ^1H NMR ($CDCl_3$) δ ppm 8.23 (dd, *J* = 4.9, 1.6 Hz, 1H), 7.65 (m, 2H), 7.25 (dd, *J* = 7.8, 1.4 Hz, 1H), 7.05 (m, 2H), 6.92 (dd, *J* = 7.8, 4.9 Hz, 1H), 6.44 (q, *J* = 1.0 Hz, 1H), 2.32 (d, *J* = 1.0 Hz, 3H); MS (ES^+) *m/e* 309.0 $(M+H)^+$.

*5.1.3. 5-Bromo-N-(4-methylthiazol-2-yl)-3-phenoxypyridin-2-amine (**14**)*

A 250 mL round-bottomed flask was charged with 1-(5-bromo-3-phenoxypyridin-2-yl)thiourea (5.0 g, 15.42 mmol), 1-chloropropan-2-one (1.7 ml, 21.6 mmol), triethylamine (3.7 ml, 27.0 mmol), and EtOH (100 mL) and heated to 70 °C overnight. The reaction was cooled to room temperature, poured into water, extracted with CH_2Cl_2 (2 x 125 mL), dried over sodium sulfate, filtered and concentrated. The residue was purified over silica gel (elution 15% EtOAc in hexanes) to afford 5-bromo-*N*-(4-methylthiazol-2-yl)-3-phenoxypyridin-2-amine (5.0 g, 89.5% yield) as a white solid. ^1H NMR (d_6-DMSO) δ ppm 8.68 (br s, 1H), 8.13 (m, 1H), 7.42 (m, 2H), 7.24 (m, 1H), 7.12 (m, 1H), 7.07 (m, 1H), 7.04 (m, 1H), 6.44 (q, *J* = 1.1 Hz, 1H), 2.34 (d, *J* = 1.1 Hz, 3H); MS (apci) *m/e* 362.2, 364.2 $(M+H)^+$.

*5.1.4. N-(4-Methylthiazol-2-yl)-3-phenoxypyridin-2-amine hydrochloride (**15**)*

5-Bromo-*N*-(4-methylthiazol-2-yl)-3-phenoxypyridin-2-amine (0.125 g, 0.345 mmol) was dissolved in THF (30 mL) and cooled to -78 °C. 1.6M MeLi (0.270 ml, 0.431 mmol) was added slowly and stirred for 10 min. 2.5M butyllithium (0.022 g, 0.345 mmol) was added and stirred for 15 min. The reaction was quenched with aqueous ammonium chloride, extracted with CH_2Cl_2, dried over sodium sulfate, filtered and concentrated. The residue was purified over silica

gel (elution 10-20% EtOAc in hexanes) to afford freebase. The residue was dissolved in CH_2Cl_2 (2 mL) and 2M HCl (0.5 mL) in ether was added and concentrated to afford *N*-(4-methylthiazol-2-yl)-3-phenoxypyridin-2-amine hydrochloride (0.068 g, 61.6% yield). ^{1}H NMR (d_6-DMSO) δ ppm 8.20 (dd, *J* = 4.8, 1.2 Hz, 1H), 7.46 (t, *J* = 7.6 Hz, 2H), 7.40 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.23 (m, 1H), 7.15 (m, 3H), 6.89 (br s, 1H), 2.33 (s, 3H); MS (apci) *m/e* 284.2 $(M+H)^+$.

*5.1.5. 5-(Benzylthio)-N-(4-methylthiazol-2-yl)-3-phenoxypyridin-2-amine hydrochloride (**16**)*

A 10 mL round-bottomed flask was charged with 5-bromo-*N*-(4-methylthiazol-2-yl)-3-phenoxypyridin-2-amine (70 mg, 0.193 mmol), 4,5-bis(diphenylphosphino)-9,9-dimethyl-9H-xanthene (5.6 mg, 0.009 mmol), Pd_2dba_3 (4.4 mg, 0.005 mmol), *N*-ethyl-*N*-isopropylpropan-2-amine (0.067 ml, 0.39 mmol), phenylmethanethiol (0.025 ml, 0.21 mmol) and dioxane (2 mL). The reaction was purged with nitrogen and heated to 100 °C overnight. The solvent was removed and the residue was purified over silica gel (elution 15% EtOAc in hexanes) to afford free base. The residue was dissolved in CH_2Cl_2 (5 mL) and 2M HCl in ether (1 mL) was added and the solvent removed to afford 5-(benzylthio)-*N*-(4-methylthiazol-2-yl)-3-phenoxypyridin-2-amine hydrochloride (79.4 mg, 92.9% yield) as a white solid. ^{1}H NMR (d_6-DMSO) δ ppm 8.08 (m, 1H), 7.43 (m, 2H), 7.28-7.15 (m, 7H), 6.98 (m, 2H), 6.74 (br s, 1H), 4.12 (s, 2H), 2.26 (s, 3H); MS (apci) *m/e* 406.2 $(M+H)^+$.

*5.1.6. N-(4-Methylthiazol-2-yl)-3-phenoxy-5-(phenylthio)pyridin-2-amine hydrochloride (**17**)*

Synthesized via the same method as **15**. ^{1}H NMR (d_6-DMSO) δ ppm 8.20 (d, *J* = 2.0 Hz, 1H), 7.39 (m, 2H), 7.31 (m, 2H), 7.21 (m, 4H), 7.16 (m, 1H), 7.07 (m, 2H), 6.65 (s, 1H), 2.25 (s, 3H); MS (apci) *m/e* 392.2 $(M+H)^+$.

*5.1.7. N-(4-Methylthiazol-2-yl)-3-phenoxy-5-(pyridin-2-ylthio)pyridin-2-amine hydrochloride (**18**)*

Synthesized via the same method as **15**. ^{1}H NMR (d_6-DMSO) δ ppm 8.37 (m, 1H), 8.29 (m, 1H), 7.66 (m, 1H), 7.42 (t, *J* = 7.8 Hz, 2H), 7.38 (d, *J* = 1.8 Hz, 1H), 7.21-7.08 (m, 5H), 6.73 (s, 1H), 2.27 (s, 3H); MS (apci) *m/e* 393.2 $(M+H)^+$.

*5.1.8. N-(4-Methylthiazol-2-yl)-3-phenoxy-5-(pyridin-4-ylthio)pyridin-2-amine hydrochloride (**19**)*

Synthesized via the same method as **15**. ^{1}H NMR (d_6-DMSO) δ ppm 8.58 (d, *J* = 6.6 Hz, 2H), 8.38 (d, *J* = 1.6 Hz, 1H), 7.65 (d, *J* =6.8 Hz, 2H), 7.48 (d, *J* = 1.6 Hz, 1H), 7.42 (t, *J* = 7.8 Hz, 2H), 7.21-7.14 (m, 3H), 6.74 (s, 1H), 2.27 (s, 3H); MS (apci) *m/e* 393.2 $(M+H)^+$.

*5.1.9. N-(4-Ethylthiazol-2-yl)-3-phenoxy-5-(pyridin-4-ylthio)pyridin-2-amine hydrochloride (**23**)*

Synthesized via the same method as **14**. ^{1}H NMR (d_6-DMSO) δ ppm 8.58 (m, 2H), 8.38 (d, *J* = 2.0 Hz, 1H), 7.65 (m, 2H), 7.48 (d, *J* = 1.8 Hz, 1H), 7.42 (m, 2H), 7.21-7.14 (m, 3H), 6.74 (s, 1H), 2.63 (q, *J* = 7.6 Hz, 2H), 1.21 (t 7.6 Hz, 3H); MS (apci) *m/e* 407.3 $(M+H)^+$.

*5.1.10. 3-Phenoxy-5-(pyridin-4-ylthio)-N-(4-(trifluoromethyl)thiazol-2-yl)pyridin-2-amine hydrochloride (**24**)*

Synthesized via the same method as **14**. ^{1}H NMR (d_6-DMSO) δ ppm 12.08 (s, 1H), 8.87 (m, 2H), 8.44 (dd, *J* = 2.0, 1.2 Hz, 1H), 7.93 (s, 1H), 7.62 (m, 2H), 7.56 (dd, *J* = 2.0, 1.0 Hz, 1H), 7.43 (m, 2H), 7.23-7.15 (m, 3H); MS (apci) *m/e* 447.3 $(M+H)^+$.

*5.1.11. N-(4-Cyclopropylthiazol-2-yl)-3-phenoxy-5-(pyridin-4-ylthio)pyridin-2-amine hydrochloride (**25**)*

Synthesized via the same method as **14.** ^{1}H NMR (d_6-DMSO) δ ppm 8.52 (m, 2H), 8.34 (d, *J* = 1.8 Hz, 1H), 7.52 (m, 2H), 7.44-7.39 (m, 3H), 7.21-7.13 (m, 3H), 6.72 (s, 1H), 1.97 (m, 1H), 0.84 (m, 2H), 0.78 (m, 2H); MS (apci) *m/e* 419.3 $(M+H)^+$.

*5.1.12. N-(4-Isopropylthiazol-2-yl)-3-phenoxy-5-(pyridin-4-ylthio)pyridin-2-amine hydrochloride (**26**)*

Synthesized via the same method as **14**. ^{1}H NMR (d_6-DMSO) δ ppm 8.57 (m, 2H), 8.38 (d, *J* = 1.8 Hz, 1H), 7.63 (m, 2H), 7.47 (d, *J* = 1.8 Hz, 1H), 7.42 (m, 2H), 7.22-7.14 (m, 3H), 6.72 (s, 1H), 2.93 (m, 1H), 1.24 (d, *J* = 6.8 Hz, 6H); MS (apci) *m/e* 421.3 $(M+H)^+$.

*5.1.13. N-(4-Isobutylthiazol-2-yl)-3-phenoxy-5-(pyridin-4-ylthio)pyridin-2-amine hydrochloride (**27**)*

Synthesized via the same method as **14**. ^{1}H NMR (d_6-DMSO) δ ppm 8.57 (m, 2H), 8.38 (d, *J* = 2.0 Hz, 1H), 7.63 (m, 2H), 7.47 (d, *J* = 2.0 Hz, 1H), 7.42 (m, 2H), 7.21-7.14 (m, 3H), 6.74 (s, 1H), 2.47 (d, *J* = 7.2 Hz, 2H), 2.00 (m, 1H), 0.89 (d, *J* = 6.6 Hz, 6H); MS (apci) *m/e* 435.3 $(M+H)^+$.

*5.1.14. N-(4-Cyclohexylthiazol-2-yl)-3-phenoxy-5-(pyridin-4-ylthio)pyridin-2-amine hydrochloride (**28**)*

Synthesized via the same method as **14**. ^{1}H NMR (d_6-DMSO) δ ppm 8.57 (m, 2H), 8.33 (d, *J* = 2.0 Hz, 1H), 7.63 (m, 2H), 7.47 (d, *J* = 2.0 Hz, 1H), 7.42 (m, 2H), 7.21-7.14 (m, 3H), 6.71 (s, 1H), 2.59 (m, 1H), 1.97 (m, 2H), 1.76 (m, 2H), 1.68 (m, 1H), 1.46-1.27 (m, 4H), 1.21 (m, 1H); MS (apci) *m/e* 461.3 $(M+H)^+$.

*5.1.15. N-(4-Phenethylthiazol-2-yl)-3-phenoxy-5-(pyridin-4-ylthio)pyridin-2-amine hydrochloride (**29**)*

Synthesized via the same method as **14**. ^{1}H NMR (d_6-DMSO) δ ppm 8.58 (m, 2H), 8.38 (d, *J* = 2.0 Hz, 1H), 7.65 (m, 2H), 7.48 (d, *J* = 2.0 Hz, 1H), 7.42 (m, 2H), 7.31-7.15 (m, 8H, 6.75 (s, 1H), 3.00-2.86 (m, 4H); MS (apci) *m/e* 483.3 $(M+H)^+$.

*5.1.16. Methyl 3-(2-(3-phenoxy-5-(pyridin-4-ylthio)pyridin-2-ylamino)thiazol-4-yl)propanoate hydrochloride (**30**)*

Synthesized via the same method as **14**. ^{1}H NMR (d_6-DMSO) δ ppm 8.56 (m, 2H), 8.37 (d, *J* = 2.0 Hz, 1H), 7.62 (m, 2H), 7.46 (d, *J* = 2.0 Hz, 1H), 7.42 (m, 2H), 7.21-7.14 (m, 3H), 6.77 (s, 1H), 3.60 (s, 3H), 2.87 (t, *J* = 7.6 Hz, 2H), 2.70 (t, *J* = 7.6 Hz, 2H); MS (apci) *m/e* 465.2 $(M+H)^+$.

*5.1.17. 3-(2-(3-Phenoxy-5-(pyridin-4-ylthio)pyridin-2-ylamino)thiazol-4-yl)propanoic acid hydrochloride (**31**)*

Methyl 3-(2-(3-phenoxy-5-(pyridin-4-ylthio)pyridin-2-ylamino)thiazol-4-yl)propanoate (90 mg, 0.194 mmol) was dissolved in methanol (2 mL) and sodium hydroxide (77.5 mg, 1.94 mmol) was added and the reaction stirred at room temperature for 3 hr. The reaction was poured into water and pH adjusted to ~pH 2. The mixture was concentrated in vacuo and the residue was washed with EtOH. The EtOH layer was dried in vacuo to afford 3-(2-(3-phenoxy-5-(pyridin-4-ylthio)pyridin-2-ylamino)thiazol-4-yl)propanoic acid hydrochloride (50.7 mg, 50.0% yield) as a yellow solid. ^{1}H NMR (d_6-DMSO) δ ppm 8.58 (m, 2H), 8.38 (m, 1H), 7.64 (m, 2H), 7.48 (m, 1H), 7.43 (m, 2H), 7.22-7.15 (m, 3H), 6.78 (m, 1H), 2.71 (t, *J* = 7.4 Hz, 2H), 2.63 (t, *J* = 7.4 Hz, 2H); MS (apci) *m/e* 451.2 $(M+H)^+$.

*5.1.18. N-(3-Methyl-1,2,4-thiadiazol-5-yl)-3-phenoxy-5-(pyridin-2-ylthio)pyridin-2-amine hydrochloride (**37**)*

Synthesized via the same method as **39**. ^{1}H NMR (d_6-DMSO) δ ppm 12.30 (br s, 1H), 8.38 (m, 2H), 7.67 (m, 1H), 7.47-7.39 (m, 3H), 7.22-7.10 (m, 5H), 2.43 (s, 3H); MS (apci) *m/e* 394.2 $(M+H)^+$.

*5.1.19. 3-Phenoxy-5-(pyridin-2-ylthio)-N-(3-(tetrahydrofuran-3-yl)-1,2,4-thiadiazol-5-yl)pyridin-2-amine (**38**)*

A 20 mL vial was charged with **35b** (0.092 g, 0.41 mmol), pyridine (0.099 ml, 1.2 mmol), NaSCN (0.033 g, 0.41 mmol), and CH_3CN (4 mL) and heated to 45 °C for 45 min. 3-Phenoxy-5-(pyridin-2-ylthio)pyridin-2-amine (0.080 g, 0.27 mmol) was added and the reaction heated to 60 °C overnight. The reaction was cooled to room tempertaure and diluted with aqueous NH_4Cl and stirred for 5 min. The resultant solids were filtered and dried to afford 3-phenoxy-5-(pyridin-2-ylthio)-*N*-(3-(tetrahydrofuran-3-yl)-1,2,4-thiadiazol-5-yl)pyridin-2-amine (0.087 g, 71.5% yield). ^{1}H NMR (d_6-DMSO) δ ppm 12.40 (br s, 1H), 8.40 (d, *J* = 2.0 Hz, 1H), 8.37 (ddd, *J* = 4.9, 2.0, 1.0 Hz, 1H), 7.66 (ddd, *J* = 8.0, 7.4, 2.0 Hz, 1H), 7.48 (d, *J* = 1.8 Hz, 1H), 7.42 (m, 2H), 7.21-7.11 (m, 5H), 4.06 (t, *J* = 8.2 Hz, 1H), 3.90-3.75 (m, 3H), 3.60 (m, 1H), 2.26 (q, *J* = 7.6 Hz, 2H); MS (apci) *m/e* 450.1 $(M+H)^+$.

*5.1.20. N-(3-Phenoxy-5-(pyridin-2-ylthio)pyridin-2-yl)-3-(tetrahydrofuran-2-yl)-1,2,4-thiadiazol-5-amine hydrochloride (**39**)*

N-(Methylsulfonyloxy)tetrahydrofuran-2-carbimidoyl chloride (85.8 mg, 0.38 mmol) was dissolved in CH_3CN (2 mL). Sodium thiocyanate (24.4 mg, 0.30 mmol) and pyridine (60.7 µl, 0.75 mmol) were added and the reaction stirred at 45 ºC for 45 min. 3-Phenoxy-5-(pyridin-2-ylthio)pyridin-2-amine hydrochloride (50 mg, 0.15 mmol) was added and the reaction stirred at 60 ºC overnight. The reaction was partitioned between water and EtOAc, dried over sodium sulfate, filtered and concentrated. The residue was purified over silica gel (elution 40% EtOAc in hexanes) to afford the free base. The residue was dissolved in CH_2Cl_2 (5 mL) and 2M HCl in ether (0.5 mL) was added and concentrated to afford *N*-(3-phenoxy-5-(pyridin-2-ylthio)pyridin-2-yl)-3-(tetrahydrofuran-2-yl)-1,2,4-thiadiazol-5-amine hydrochloride (39.8 mg, 0.082 mmol, 54.3 % yield) as a tan solid. ^{1}H NMR (d_6-DMSO) δ ppm 12.43 (br s, 1H), 8.40 (d, *J* = 2.0 Hz, 1H), 8.37 (ddd, *J* = 4.9, 2.0, 1.0 Hz, 1H), 7.67 (ddd, *J* = 8.0, 7.4, 2.0 Hz, 1H), 7.49 (d, *J* = 2.0 Hz,

1H), 7.42 (m, 2H), 7.21-7.12 (m, 5H), 4.93 (dd, *J* = 7.4, 6.1 Hz, 1H), 3.93 (q, *J* = 7.2 Hz, 1H), 3.81 (td, *J* = 7.6, 5.7 Hz, 1H), 2.28-1.89 (m, 4H); MS (apci) *m/e* 450.2 $(M+H)^+$.

*5.1.21. Tert-butyl 4-(5-(3-phenoxy-5-(pyridin-2-ylthio)pyridin-2-ylamino)-1,2,4-thiadiazol-3-yl)piperidine-1-carboxylate (**40**)*

Synthesized via the same method as **39**. ^{1}H NMR (d_6-DMSO) δ ppm 12.33 (br s, 1H), 8.39 (d, *J* = 2.0 Hz, 1H), 8.37 (ddd, *J* = 4.9, 1.8, 0.8 Hz, 1H), 7.66 (ddd, *J* = 8.0, 7.4, 2.0 Hz, 1H), 7.47 (d, *J* = 1.8 Hz, 1H), 7.42 (m, 2H), 7.21-7.10 (m, 5H), 3.95 (m, 2H), 3.03-2.85 (m, 3H), 1.97 (m, 2H), 1.62 (m, 2H), 1.40 (s, 9H); MS (apci) *m/e* 563.0 $(M+H)^+$.

*5.1.22. 3-Phenoxy-N-(3-(piperidin-4-yl)-1,2,4-thiadiazol-5-yl)-5-(pyridin-2-ylthio)pyridin-2-amine (**41**)*

Tert-butyl 4-(5-(3-phenoxy-5-(pyridin-2-ylthio)pyridin-2-ylamino)-1,2,4-thiadiazol-3-yl)piperidine-1-carboxylate (9.1 g, 15 mmol) was dissolved in 1:1 CH_2Cl_2:TFA (50 mL) and stirred for 1 hr. The reaction was concentrated then redissolved in dichloromethane and aqueous $NaHCO_3$ was added and stirred for 15 minutes. The layers were separated, dried over sodium sulfate, filtered and concentrated to afford 3-phenoxy-*N*-(3-(piperidin-4-yl)-1,2,4-thiadiazol-5-yl)-5-(pyridin-2-ylthio)pyridin-2-amine (7.1 g, 100% yield). ^{1}H NMR (d_6-DMSO) δ ppm 12.39 (br s, 1H), 8.95 (m, 1H), 8.79 (m, 1H), 8.39 (d, *J* = 1.8 Hz, 1H), 8.37 (ddd, *J* = 4.9, 2.0, 1.0 Hz, 1H), 7.67 (ddd, *J* = 8.0, 7.6, 2.0 Hz, 1H), 7.47 (d, *J* = 1.8 Hz, 1H), 7.43 (m, 2H), 7.22-7.12 (m, 5H), 3.30 (m, 2H), 3.14 (tt, *J* = 10.7, 3.9 Hz, 1H), 3.03 (m, 2H), 2.17 (m, 2H), 1.97 (m, 2H); MS (apci) *m/e* 463.2 $(M+H)^+$.

*5.1.23. 1-(4-(5-(3-Phenoxy-5-(pyridin-2-ylthio)pyridin-2-ylamino)-1,2,4-thiadiazol-3-yl)piperidin-1-yl)ethanone (**42**)*

3-Phenoxy-*N*-(3-(piperidin-4-yl)-1,2,4-thiadiazol-5-yl)-5-(pyridin-2-ylthio)pyridin-2-amine (0.075 g, 0.16 mmol), triethylamine (0.09 ml, 0.65 mmol), and acetic anhydride (0.017 g, 0.16 mmol) were added to THF (15 mL) and stirred for 3 hr. The reaction was partitioned between water and dichloromethane, dried over sodium sulfate, filtered and concentrated. The residue was purified over silica gel (elution 5% MeOH in CH_2Cl_2) to afford 1-(4-(5-(3-phenoxy-5-(pyridin-2-ylthio)pyridin-2-ylamino)-1,2,4-thiadiazol-3-yl)piperidin-1-yl)ethanone (0.044 g, 54% yield). ^{1}H NMR (d_6-DMSO) δ ppm 12.34 (s, 1H), 8.39 (d, *J* = 1.8 Hz, 1H), 8.37 (ddd, *J* =4.9, 1.8, 0.8 Hz, 1H), 7.67 (ddd, *J* = 8.0, 7.6, 1.8 Hz, 1H), 7.47 (d, *J* = 1.8 Hz, 1H), 7.42 (m, 2H), 7.21-7.11 (m, 5H), 4.32 (m, 1H), 3.84 (m, 1H), 3.19 (m, 1H), 3.05 (tt, *J* = 10.7, 4.1 Hz, 1H), 2.76 (m, 1H), 2.07-1.95 (m, 5H), 1.73 (m, 1H), 1.59 (m, 1H); MS (apci) *m/e* 505.2 $(M+H)^+$.

*5.1.24. N-(3-(1-(Methylsulfonyl)piperidin-4-yl)-1,2,4-thiadiazol-5-yl)-3-phenoxy-5-(pyridin-2-ylthio)pyridin-2-amine (**43**)*

Synthesized via the same method as **42**. ^{1}H NMR (d_6-DMSO) δ ppm 12.36 (s, 1H), 8.40 (d, *J* = 1.8 Hz, 1H), 8.37 (ddd, *J* = 4.9, 1.8, 0.8 Hz, 1H), 7.67 (ddd, *J* = 8.2, 7.6, 2.0 Hz, 1H), 7.48 (d, *J* = 1.8 Hz, 1H), 7.42 (m, 2H), 7.21-7.11 (m, 5H), 3.59 (tt, *J* = 12.3, 3.7 Hz, 2H), 2.97-2.85 (m, 6H), 2.13 (m, 2H), 1.81 (m, 2H); MS (apci) *m/e* 541.2 $(M+H)^+$.

*5.1.25. N,N-Dimethyl-4-(5-(3-phenoxy-5-(pyridin-2-ylthio)pyridin-2-ylamino)-1,2,4-thiadiazol-3-yl)piperidine-1-carboxamide (**44**)*

Synthesized via the same method as **42**. ^{1}H NMR (d_6-DMSO) δ ppm 12.34 (s, 1H), 8.39 (d, *J* = 1.8 Hz, 1H), 8.37 (ddd, *J* = 4.9, 1.8, 0.8 Hz, 1H), 7.66 (td, *J* = 7.6, 1.8 Hz, 1H), 7.47 (d, *J* = 2.0 Hz, 1H), 7.42 (m, 2H), 7.21-7.11 (m, 5H), 3.58 (m, 2H), 2.97 (tt, *J* = 11.3, 3.7 Hz, 1H), 2.84 (m, 2H), 2.73 (s, 6H), 1.96 (m, 2H), 1.71 (m, 2H); MS (apci) *m/e* 534.1 $(M+H)^+$.

*5.1.26. 2-Methyl-1-(4-(5-(3-phenoxy-5-(pyridin-2-ylthio)pyridin-2-ylamino)-1,2,4-thiadiazol-3-yl)piperidin-1-yl)propan-1-one (**45**)*

Synthesized via the same method as **42**. ^{1}H NMR (d_6-DMSO) δ ppm 12.34 (s, 1H), 8.39 (d, *J* = 1.8 Hz, 1H), 8.37 (ddd, *J* = 4.9, 1.8, 0.8 Hz, 1H), 7.66 (ddd, *J* = 8.0, 7.4, 1.8 Hz, 1H), 7.47 (d, *J* = 1.8 Hz, 1H), 7.42 (m, 2H), 7.21-7.11 (m, 5H), 4.37 (d, *J* = 12.7 Hz, 1H), 3.98 (d, *J* = 12.9 Hz, 1H), 3.20 (t, *J* = 12.9 Hz, 1H), 3.07 (tt, *J* = 11.3, 3.9 Hz, 1H), 2.89 (sept, *J* = 6.6 Hz, 1H), 2.75 (t, *J* = 12.5 Hz, 1H), 2.02 (m, 2H), 1.76-1.51 (m, 2H), 1.00 (d, *J* = 6.6 Hz, 6H); MS (apci) *m/e* 533.2 $(M+H)^+$.

*5.1.27. 2-Hydroxy-1-(4-(5-(3-phenoxy-5-(pyridin-2-ylthio)pyridin-2-ylamino)-1,2,4-thiadiazol-3-yl)piperidin-1-yl)ethanone (**46**)*

3-Phenoxy-*N*-(3-(piperidin-4-yl)-1,2,4-thiadiazol-5-yl)-5-(pyridin-2-ylthio)pyridin-2-amine (0.075 g, 0.16 mmol), 2-chloro-2-oxoethyl acetate (0.022 g, 0.16 mmol), and triethylamine (0.016 g, 0.16 mmol) were added to THF (15 mL) and stirred for 1 hr. The reaction was partitioned between water and CH_2Cl_2, the organic layer was dried over sodium sulfate, filtered and concentrated. The residue was then refluxed with potassium carbonate (0.109 g, 0.79 mmol) in ethanol (25 mL) for 2 hr. The reaction was cooled to room temperature, filtered and concentrated. The residue was purified over silica gel (elution 1-2% MeOH in EtOAc) to afford 2-hydroxy-1-(4-(5-(3-phenoxy-5-(pyridin-2-ylthio)pyridin-2-ylamino)-1,2,4-thiadiazol-3-yl)piperidin-1-yl)ethanone (0.030 g, 36.4% yield). ^{1}H NMR (d_6-DMSO) δ ppm 12.34 (s, 1H), 8.39 (d, *J* = 1.8 Hz, 1H), 8.37 (ddd, *J* = 4.9, 2.0, 1.0 Hz, 1H), 7.66 (ddd, *J* = 8.0, 7.6, 2.0 Hz, 1H), 7.47 (d, *J* = 1.8 Hz, 1H), 7.42 (m, 2H), 7.21-7.11 (m, 5H), 4.49 (t, *J* = 5.5 Hz, 1H), 4.30 (d, *J* = 12.9 Hz, 1H), 4.10 (t, *J* = 4.7 Hz, 2H), 3.71 (d, *J* = 12.7 Hz, 1H), 3.17-3.04 (m, 2H), 2.86 (t, *J* = 12.3 Hz, 1H), 2.02 (m, 2H), 1.79-1.57 (m, 2H); MS (apci) *m/e* 521.2 $(M+H)^+$.

*5.1.28. 2-(Dimethylamino)-1-(4-(5-(3-phenoxy-5-(pyridin-2-ylthio)pyridin-2-ylamino)-1,2,4-thiadiazol-3-yl)piperidin-1-yl)ethanone dihydrochloride (**47**)*

Synthesized via the same method as **42**. ^{1}H NMR (d_6-DMSO) δ ppm 12.34 (s, 1H), 9.55 (br s, 1H), 8.39 (d, *J* = 2.0 Hz, 1H), 8.37 (ddd, *J* = 4.9, 1.8, 0.8 Hz, 1H), 7.67 (ddd, *J* = 8.0, 7.6, 1.8 Hz, 1H), 7.48 (d, *J* = 2.0 Hz, 1H), 7.43 (m, 2H), 7.22-7.12 (m, 5H), 4.31 (m, 3H), 3.65 (m, 2H), 3.27-3.10 (m, 2H), 2.96 (m, 1H), 2.81 (d, *J* = 4.5 Hz, 6H), 2.08 (m, 2H), 1.81 (m, 1H), 1.65 (m, 1H); MS (apci) *m/e* 548.2 $(M+H)^+$.

*5.1.29. (S)-2-Hydroxy-1-(4-(5-(3-phenoxy-5-(pyridin-2-ylthio)pyridin-2-ylamino)-1,2,4-thiadiazol-3-yl)piperidin-1-yl)propan-1-one (**48**)*

3-Phenoxy-*N*-(3-(piperidin-4-yl)-1,2,4-thiadiazol-5-yl)-5-(pyridin-2-ylthio)pyridin-2-amine (.075 g, 0.16 mmol), (*R*)-2-hydroxypropanoic acid (0.017 g, 0.20 mmol), *N*1-((ethylimino)methylene)-*N*3,*N*3-dimethylpropane-1,3-diamine hydrochloride (0.046 g, 0.24 mmol), and *N*,*N*-dimethylpyridin-4-amine (0.0020 g, 0.016 mmol) were added to CH_2Cl_2 (5 mL). Triethylamine (0.033 g, 0.32 mmol) was then added and the solution was stirred at room temperature for 18 hr. Water was added and extracted with CH_2Cl_2, dried over sodium sulfate, filtered and concentrated. The residue was purified by C18 reverse phase chromatography (elution 5 to 95% acetonitrile in water) to afford (*S*)-2-hydroxy-1-(4-(5-(3-phenoxy-5-(pyridin-2-ylthio)pyridin-2-ylamino)-1,2,4-thiadiazol-3-yl)piperidin-1-yl)propan-1-one (0.0252 g, 29.1% yield). ^{1}H NMR (d_6-DMSO) δ ppm 12.34 (s, 1H), 8.39 (d, *J* = 1.8 Hz, 1H), 8.37 (ddd, *J* = 4.7, 2.0, 1.0 Hz, 1H), 7.67 (td, *J* = 8.0, 2.0 Hz, 1H), 7.47 (d, *J* = 1.8 Hz, 1H), 7.42 (m, 2H), 7.21-7.11 (m, 5H), 4.82 (t, *J* = 6.8 Hz, 1H), 4.45 (m, 1H), 4.33 (m, 1H), 4.01 (m, 1H), 3.23-3.05 (m, 2H), 2.83 (m, 1H), 2.02 (m, 2H), 1.74 (m, 1H), 1.60 (m, 1H), 1.18 (d, *J* = 6.6 Hz, 3H); MS (apci) *m/e* 535.2 $(M+H)^+$.

*5.1.30. (R)-2-Hydroxy-1-(4-(5-(3-phenoxy-5-(pyridin-2-ylthio)pyridin-2-ylamino)-1,2,4-thiadiazol-3-yl)piperidin-1-yl)propan-1-one (**49**)*

Synthesized via the same method as **48**. ^{1}H NMR (d_6-DMSO) δ ppm 12.34 (s, 1H), 8.39 (d, *J* = 1.8 Hz, 1H), 8.37 (ddd, *J* = 4.7, 2.0, 1.0 Hz, 1H), 7.67 (td, *J* = 8.0, 2.0 Hz, 1H), 7.47 (d, *J* = 1.8 Hz, 1H), 7.42 (m, 2H), 7.21-7.11 (m, 5H), 4.82 (t, *J* = 6.8 Hz, 1H), 4.45 (m, 1H), 4.33 (m, 1H), 4.01 (m, 1H), 3.23-3.05 (m, 2H), 2.83 (m, 1H), 2.02 (m, 2H), 1.74 (m, 1H), 1.60 (m, 1H), 1.18 (d, *J* = 6.6 Hz, 3H); MS (apci) *m/e* 535.2 $(M+H)^+$.

*5.1.31. 1-(4-(5-(5-(Pyridin-2-ylthio)-3-(pyridin-3-yloxy)pyridin-2-ylamino)-1,2,4-thiadiazol-3-yl)piperidin-1-yl)ethanone hydrochloride (**71**)*

Synthesized via the same method as **72**. ^{1}H NMR (d_6-DMSO) δ ppm 8.80 (d, *J* = 2.7 Hz, 1H), 8.61 (dd, *J* = 5.3, 0.8 Hz, 1H), 8.55 (d, *J* = 1.8 Hz, 1H), 8.43 (ddd, *J* = 4.9, 2.0, 1.0 Hz, 1H), 8.11 (ddd, *J* = 8.6, 2.7, 1.0 Hz, 1H), 8.06 (d, *J* = 1.8 Hz, 1H), 7.91 (dd, *J* = 8.6, 5.3 Hz, 1H), 7.72 (ddd, *J* = 8.2, 7.6, 2.0 Hz, 1H), 7.24-7.17 (m, 2H), 4.31 (m, 1H), 3.83 (m, 1H), 3.18 (m, 1H), 3.05 (tt, *J* = 11.1, 3.9 Hz, 1H), 2.75 (m, 1H0, 2.05-1.93 (m, 5H), 1.72 (m, 1H), 1.57 (m, 1H); MS (apci) *m/e* 506.3 $(M+H)^+$.

*5.1.32. 1-(4-(5-(3-(2-Methylpyridin-3-yloxy)-5-(pyridin-2-ylthio)pyridin-2-ylamino)-1,2,4-thiadiazol-3-yl)piperidin-1-yl)ethanone hydrochloride (**72**)*

Tert-butyl 4-(5-(3-(2-methylpyridin-3-yloxy)-5-(pyridin-2-ylthio)pyridin-2-ylamino)-1,2,4-thiadiazol-3-yl)piperidine-1-carboxylate (546 mg, 0.945 mmol) was dissolved in CH_2Cl_2/MeOH (20 mL, 1:1) and 4 N HCl in dioxane (3 mL) was added and stirred for 1 hr and concentrated. *N*-(3-(2-methylpyridin-3-yloxy)-5-(pyridin-2-ylthio)pyridin-2-yl)-3-(piperidin-4-yl)-1,2,4-thiadiazol-5-amine dihydrochloride (60 mg, 0.109 mmol), Et_3N (76.0 µl, 0.545 mmol), and acetic anhydride (11.1 mg, 0.109 mmol) were added to THF (5 mL) and stirred for 45 min. The

reaction was partitioned between water and CH_2Cl_2, dried over sodium sulfate, filtered, concentrated and purified over silica gel (0-5% MeOH in EtOAc) to afford the freebase. The residue was dissolved in CH_2Cl_2 (5 mL) and 2N HCl in ether (0.5 mL) was added and concentrated to afford 1-(4-(5-(3-(2-methylpyridin-3-yloxy)-5-(pyridin-2-ylthio)pyridin-2-ylamino)-1,2,4-thiadiazol-3-yl)piperidin-1-yl)ethanone hydrochloride (41.8 mg, 0.0705 mmol, 64.7 % yield). ^{1}H NMR (d_6-DMSO) δ ppm 12.42 (s, 1H), 8.47 (d, *J* = 2.0 Hz, 1H), 8.39-8.34 (m, 2H), 7.70-7.64 (m, 2H), 7.55 (m, 1H), 7.45 (m, 1H), 7.19-7.13 (m, 2H), 4.32 (m, 1H), 3.84 (m, 1H), 3.19 (m, 1H), 3.06 (tt, *J* = 10.9, 3.9 Hz, 1H), 2.76 (m, 1H), 2.61 (s, 3H), 2.06-1.94 (m, 5H), 1.73 (m, 1H), 1.58 (m, 1H); MS (apci) *m/e* 520.2 $(M+H)^+$.

*5.1.33. N-(3-(2-Methylpyridin-3-yloxy)-5-(pyridin-2-ylthio)pyridin-2-yl)-3-(1-(methylsulfonyl)piperidin-4-yl)-1,2,4-thiadiazol-5-amine hydrochloride (**73**)*

Synthesized via the same method as **72**. ^{1}H NMR (d_6-DMSO) δ ppm 12.44 (s, 1H), 8.46 (d, *J* = 1.8 Hz, 1H), 8.37 (ddd, *J* = 4.7, 1.8, 1.0 Hz, 1H), 8.33 (m, 1H), 7.67 (ddd, *J* = 8.0, 7.6, 2.0 Hz, 1H), 7.61 (m, 1H), 7.48 (m, 1H), 7.38 (m, 1H), 7.19-7.13 (m, 2H), 3.58 (m, 2h), 2.95-2.85 (m, 6H), 2.58 (s, 3H), 2.13 (m, 2H), 1.81 (m, 2H); MS (apci) *m/e* 556.2 $(M+H)^+$.

*5.1.34. 1-(4-(5-(5-(Pyridin-2-ylthio)-3-(quinolin-5-yloxy)pyridin-2-ylamino)-1,2,4-thiadiazol-3-yl)piperidin-1-yl)ethanone (**74**)*

Synthesized via the same method as **72**. ^{1}H NMR ($CDCl_3$) δ ppm 9.54 (br s 1H), 8.96 (dd, *J* = 4.3, 1.8 Hz, 1H), 8.39 (ddd, *J* = 8.6, 1.8, 0.8 Hz, 1H), 8.36 (d, *J* = 1.8 Hz, 1H), 8.26 (ddd, *J* = 4.7, 1.8, 1.0 Hz, 1H), 7.97 (m, 1H), 7.64 (dd, *J* = 8.6, 7.8 Hz, 1H), 7.48-7.41 (m, 2H), 7.28 (d, *J* = 1.8 Hz, 1H), 7.14 (dd, *J* = 7.6, 0.8 Hz, 1H), 7.00-6.95 (m, 2H), 4.52 (m, 1H), 3.84 (m, 1H), 3.17 (m, 1H), 3.04 (tt, *J* = 10.9, 3.7 Hz, 1H), 2.78 (m, 1H), 2.12-2.03 (m, 5H), 1.90-1.69 (m, 2H); MS (apci) *m/e* 556.5 $(M+H)^+$.

*5.1.35. 1-(4-(5-(3-(1-Methyl-1H-pyrazol-4-yloxy)-5-(pyridin-2-ylthio)pyridin-2-ylamino)-1,2,4-thiadiazol-3-yl)piperidin-1-yl)ethanone (**75**)*

Synthesized via the same method as **72**. ^{1}H NMR ($CDCl_3$) δ ppm 9.11 (s, 1H), 8.38 (ddd, *J* = 4.8, 2.0, 1.0 Hz, 1H), 8.29 (m, 1H), 7.50 (m, 1H), 7.42 (m, 1H), 7.38 (s, 1H), 7.35 (s, 1H), 7.05-6.98 (m, 2H), 4.58 (m, 1H), 3.95-3.86 (m, 4H), 3.23 (m, 1H), 3.09 (tt, *J* = 10.9, 3.9 Hz, 1H), 2.84 (m, 1H), 2.18-2.07 (m, 5H), 1.97-1.76 (m, 2H); MS (apci) *m/e* 509.2 $(M+H)^+$.

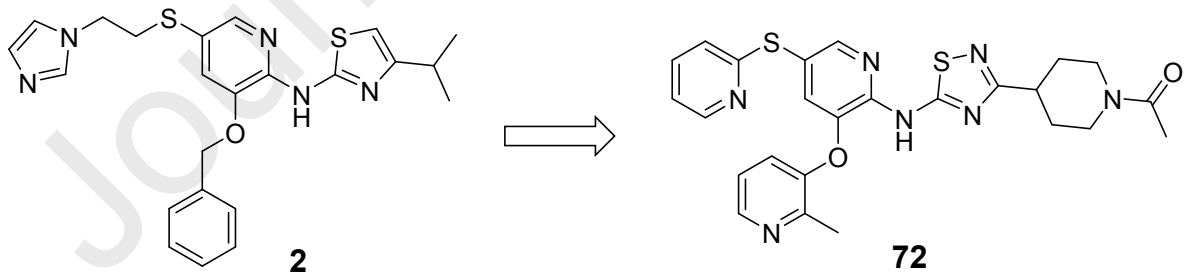
5.1.36 Accession Codes

PDB code for GK bound with **6** is 6E0E. PDB code for GK bound with **72** is 6E0I.

ABBREVIATIONS USED

AUC, area under the curve; GK, glucokinase; GKA, glucokinase activator; OGTT, oral glucose tolerance test; $S_{0.5}$, substrate concentration at half-maximal velocity; V_{max}, maximal enzymatic rate.

Graphical Abstract



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[31] Plasma protein binding was determined to be predominantly due to albumin.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: