Potent *N*-(1,3-Thiazol-2-yl)pyridin-2-amine Vascular Endothelial Growth Factor Receptor Tyrosine Kinase Inhibitors with Excellent Pharmacokinetics and Low Affinity for the hERG Ion Channel

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A series of *N*-(1,3-thiazol-2-yl)pyridin-2-amine KDR kinase inhibitors have been developed that possess optimal properties. Compounds have been discovered that exhibit excellent in vivo potency. The particular challenges of overcoming hERG binding activity and QTc increases in vivo in addition to achieving good pharmacokinetics have been acomplished by discovering a unique class of amine substituents. These compounds have a favorable kinase selectivity profile that can be accentuated with appropriate substitution.

Introduction

Vascular endothelial growth factor (VEGF) is a selective mediator of endothelial cell mitogenesis and chemotaxis that has been identified as a principal factor in the induction of angiogenesis.[1] It has been implicated in a variety of pathological conditions that are angiogenesis-dependent including cancer, arthritis, diabetic retinopathy, and some inflammatory diseases. VEGF has been a particular focus of antiangiogenic therapies against malignant disease. Its expression has been shown to be up-regulated in a variety of tumors and has been reported to be a prognostic indicator of tumor progression and/or decreased survival.[2] VEGF activation of the transmembrane receptor tyrosine kinase-linked KDR (VEGFR-2) induces vascular endothelial cell mitosis and migration.[3] Therapeutic approaches that target VEGF or KDR have shown positive results in preclinical tumor models and have been actively undergoing clinical evaluation. Bevacizumab, a VEGF antibody was reported to be efficacious in Phase III clinical trials.[4] Clinical evaluations have also been undertaken with an anti-KDR antibody, a soluble VEGF decoy-receptor, as well as small molecule inhibitors of KDR kinase activity.[5]

We have previously disclosed *N*-(1,3-thiazol-2-yl)-pyridin-2-amines as a potent lead class of KDR kinase inhibitors.[6] In this paper, we present the development of this series toward compounds with ideal drug characteristics. The initial lead compound **1** (Figure 1), while potent in the KDR enzyme assay, had poor potency in an endothelial cell mitogenesis assay.[7] Following a strategy that was successful in other lead series, we sought to increase cell potency by the incorporation of a solubility-enhancing amine substituent. A benzylic pyrrolidine was thus incorporated at the 5-pyridyl position to produce **2** (Figure 1) with excellent potency in the cell-based assay. Compound **2**, however, had poor pharmacokinetics and was a potent binder to the potassium channel encoded by the human ether-a-go-go-related gene (hERG) which is an inward rectifying potassium channel involved in ventricular repolarization. This channel underlies the rapid component of the delayed rectifier K+ current (IKr), and blockade of IKr may lead to prolongation of the QT interval and to potentially fatal ventricular arrhythmias including torsades de pointes.[8] Many marketed drugs are known to block IKr.[9] In several cases this has led to regulatory intervention and withdrawal of drugs from the market. This paper will address the optimization to decrease affinity for the hERG channel and to achieve compounds with excellent in vivo potency and excellent pharmacokinetics in three species.

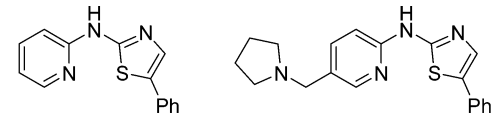

Figure 1. Lead structures.

Chemistry. (2-Chloropyridin-4-yl)methanol (**3**, Scheme 1)[10] was treated with *tert*-butyldimethylsilyl chloride and imidazole to provide **4**. Compound **4** was aminated with benzophenone imine, and the crude material was hydrolyzed with hydroxylamine to provide **5**.[11] The aminopyridine was then reacted with NaH and a 2-chlorothiazole (R^2 = CN or Ph) to provide the *N*-(1,3-thiazol-2-yl)pyridin-2-amine **6**. The resulting coupled products **6** (R^1 = H) were desilylated with HF−pyridine

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Scheme 1

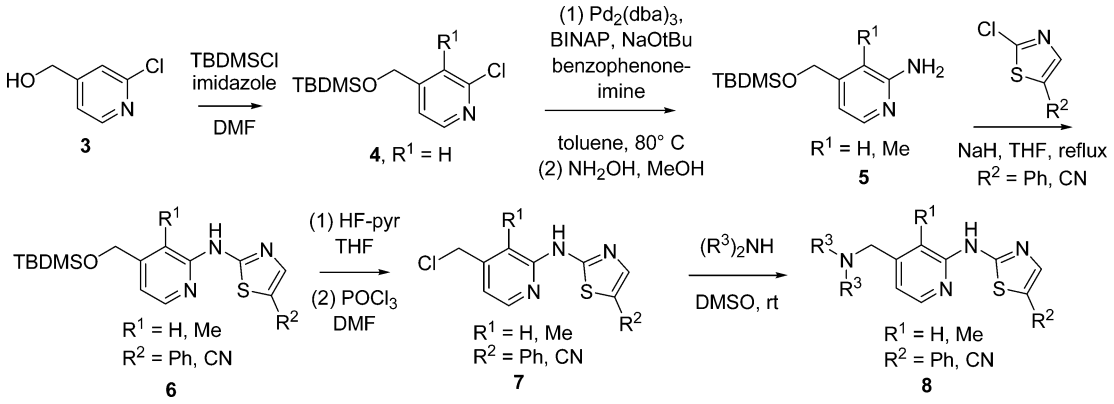
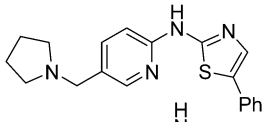
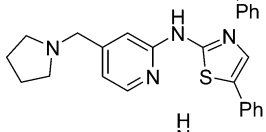
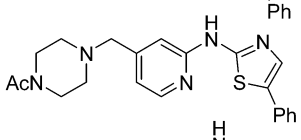
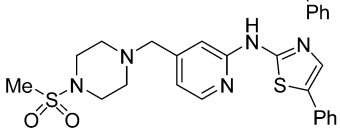
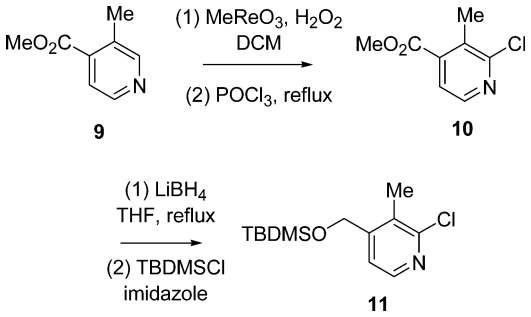


Table 1.

Cmpd	Structure	KDR IC_{50}[a] (nM)	Endo. Mit.[a] IC_{50} (nM)	KDR Phos. IC_{50} (nM)	hERG binding IP (nM)	Dog pharmacokinetics[b] t1/2 (h)	Cl (mL/min/kg)
2		6 ± 2	28	24	8	1.7 (2)	>100 (2)
12[c]		3 ± 0.4	24	15	22	nd	nd
13[c]		8 ± 1	28 ± 2	43	240	nd	nd
14		2 ± 0.6	17 ± 0	29	130	2.0 (2)	13.4 (2)

[a] For determinations where n > 2, the standard deviation is given. For determinations where $n = 2$ the number is shown in parentheses and in these cases the determinations were within ± 25% of the mean. The remainder of the data is from single determinations. [b] Average of two dogs after iv dosing. [c] Compounds **12** and **13** were produced as part of a HPLC-purified library and were characterized by exact mass measurement.

Scheme 2



and converted to the chloromethylpyridines **7** (R^1 = H) with $POCl_3$ and DMF. Reaction of the chloride with a variety of amines at ambient temperature in DMSO resulted in the final products **8**.

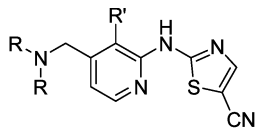
The synthesis of a key intermediate for the case where R^1 = Me is shown in Scheme 2. Methyl 3-methylisonicotinate (**9**) was oxidized with $MeReO_3$ and hydrogen peroxide,[12] and the resulting pyridine *N*-oxide was treated with $POCl_3$ to afford methyl 2-chloro-3-methylisonicotinate **10** (produced as a 2:1 ratio with the regioisomeric methyl 2-chloro-5-methylisonicotinate). Reduction of the ester with $LiBH_4$ and silylation as above resulted in **11**. Intermediate **11** was then converted according to the method employed for **4** (R^1 = H) to ultimately provide the products **8** (R^1 = Me).

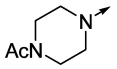
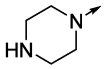
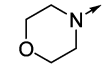
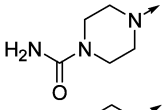
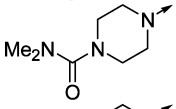
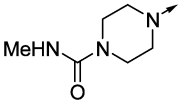
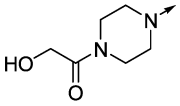
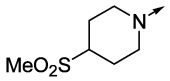
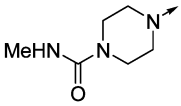
Results and Discussion

The lead compound **2** was potent against KDR (Table 1) as well as potent in two cell assay formats. The two cell-based formats[7] are an endothelial cell mitogenesis assay (Endo. Mit.) measuring inhibition of VEGF-induced DNA synthesis and a cell-based KDR autophosphorylation inhibition assay (KDR Phos.) in HEK293 cells stably transfected to express high levels of full-length human KDR. However, **2** was found to be very potent in an hERG binding assay and exhibited poor pharmacokinetics in dog with a clearance well above hepatic blood flow (Table 1). The lipophilicity of **2** was high (log $P = 3.59$) and was considered to be a contributing factor to its hERG binding and poor pharmacokinetics. By employing the pyridine substitution pattern outlined in Scheme 1, we initially sought to explore whether variation of the amine substituent would favorably impact the properties of the series.

The first clue that the undesirable properties of **2** could be altered by choice of pendant amine came from a small library of analogues. This library produced 40 compounds related to **12** (Table 1) where the amine substituent was varied. All changes of this 4-(amino-

Table 2.



Cmpd	R_2N-	R'	KDR IC_{50}[a] (nM)	Endo. Mit.[a] IC_{50} (nM)	KDR Phos.[a] IC_{50} (nM)	hERG binding IP (nM)	LogP	Human Prot. Bnd. (%)
15		H	13 ± 3	36 ± 11	41 ± 14	10,600 (2)	2.05	89.4
16		H	15 ± 10	24	13	3,770 (2)	0.72	30.6
17		H	8 ± 1	28 ± 2	43	1,670	nd	90.9
18		H	8 ± 0	47 ± 1	24	24,720	1.91	90.8
19		H	10 ± 4	28 ± 8	40 ± 21	3,510 (2)	2.74	95.9
20		H	12 ± 3	31 ± 8	27 ± 6	9,280	2.06	87.4
21		H	9 ± 5	39 ± 14	35 ± 8	9,871[b]	1.68	73.8
22		H	7 ± 2	48 ± 7	31 ± 14	>10,000 (2)	2.06	65.5
23		Me	12 ± 5	38 ± 11	16 ± 1	3,830[b]	3.27	91.3

[a] For determinations where $n > 2$, the standard deviation is given. For determinations where $n = 2$, the number is shown in parentheses and the individual determinations are within ±25% of the mean unless noted otherwise. The remainder of the data is from single determinations. [b] The two determinations are within ±50% of the mean

methyl)pyridine moiety did not effect potency or kinase selectivity which is consistent with our postulated binding mode of these compounds where the amine extends out of the ATP-binding cleft and is exposed to solvent.[6] However, this library provided an insight into modifying both hERG binding activity and metabolism. Compound **12** is representative of all but one amine in the library, with excellent enzyme and cell-based potency but, like **2** displayed very potent hERG binding (IP = 22 nM). This compound was also metabolized very rapidly in a rat liver microsomal incubation ($t_{1/2}$ = 8 min). The one exception to these observations that was found in this library was **13** (Table 1). This compound, bearing an *N*-acetylpiperazine moiety displayed significantly lower, albeit still potent, hERG binding (IP = 240 nM) and a greater resistance to metabolism ($t_{1/2}$ = 251 min). A subsequent analogue of **13** bearing a methanesulfonamide moiety (**14**, Table 1) showed a similar potency profile to **13** and was additionally found to have a moderate clearance in dog, indicating the significant improvement these unique substituted piperazine endgroups provide relative to **2**.

This discovery was extended to produce compounds with excellent pharmacokinetics and very low affinity for hERG. We focused on changes to the lipophilic thiazoyl-5-phenyl moiety which was postulated to be a key contributor to both the hERG binding and the poor pharmacokinetics. In initial SAR studies it was found that the phenyl moiety could be replaced with a cyano moiety with only a moderate loss of potency.[6] When a nitrile was employed to replace the phenyl in **13**, a potent inhibitor was produced (Table 2, **15**). This compound had excellent enzyme potency and cell-based activity in the two assay formats. Moreover, this molecule displayed significantly lower affinity for hERG and good physical properties, with a low log *P* of 2.06 and a significant free fraction in human plasma (89.4% plasma bound). Compound **15** also showed excellent dog pharmacokinetics (Table 3), with a long half-life, low clearance and an excellent oral bioavailability. This optimal profile is dependent on the acetamide as either an unsubstituted piperazine (**16**) or a morpholine (**17**) produced compounds that were still potent but had slightly greater affinity for hERG and significantly poorer dog pharmacokinetics (Cl = 24.2 and 12.4 mL/

Table 3. Dog Pharmacokinetic Data for Selected Compounds[a]

compd	$t_{1/2}$ (h)	Cl (mL/min/kg)	V_{dss} (L/kg)	F (%)
15	7.2	1.2	0.72	87
16	7.9[b]	24.2[b]	21.9[b]	100
17	2.5	12.4	1.0	nd
18	2.9	2.6	0.34	nd
19	2.0	7.4	0.88	nd
20	17.4	0.51	0.72	99
21	6.6	0.88	0.47	61
22	6.3	1.8	0.96	95
23	7.0	2.0	1.2	75

[a] Average of two dogs dosed iv (0.25 mg/kg for **17**, **18**, and **19** or 1 mg/kg for all others) as a solution in DMSO and po (1 mpk) as a solution in acidified methocel or 0.05 M citric acid (aq). The individual determinations are within ±25% of the mean unless noted otherwise. [b] The two determinations are within ±50% of the mean.

min/kg, respectively, Table 3). The acetamide however could be replaced by other polar carbonyl-containing moieties to maintain or enhance the profile. The terminal urea **18** was potent and had very low affinity for hERG with still good dog pharmacokinetics albeit with a diminished $t_{1/2}$ (2.9 h). The *N,N*-dimethylurea analogue **19** had significantly increased affinity for hERG and an even higher clearance. Interestingly, a metabolite of **19**, the monomethyl urea **20**, displayed a lower affinity than **19** for hERG and resulted in exceptional dog pharmacokinetics, with a $t_{1/2}$ of 17.4 h, very low clearance of 0.51 mL/min/kg, and outstanding bioavailability (99%). Other polar piperazine amides proved to have excellent properties, such as the hydroxyacetamide **21**, and the optimal properties were not limited to piperazine derivatives. The piperidine **22**, with a polar methylsulfonyl moiety also displayed low hERG affinity and excellent pharmacokinetic behavior in dogs.

In one example of additional substitution of the pyridine ring, we appended a methyl group to the 3-position of **20**, resulting in **23**. This compound was very similar in potency to **20**, with a slightly greater affinity for hERG. It also had excellent pharmacokinetics in dogs (Table 3) albeit with a slightly increased clearance and lower $t_{1/2}$. The introduction of this methyl group lead to a different profile than **20** with respect to both pharmacokinetics in other species and to kinase selectivity (vide infra).

While hERG binding is an imperfect predictor for in vivo QT prolongation, it is one of the few available high throughput assay techniques.[13] As a critical follow-up to binding measurements, several of these compounds were evaluated for their ability to prolong QTc in vivo in anesthetized dogs. While **2** caused a 10% increase in the QTc interval at a plasma concentration of 0.59 μM, the optimized compounds with greatly diminished hERG binding showed a significantly reduced effect in vivo. For **15** a 10% increase in QTc was not observed when dosed up to 60 μM plasma levels. Additionally, compound **23** did not show a 10% increase in QTc up to 22 μM plasma levels. Thus, these optimized compounds are expected to present little risk of QT prolongation in vivo.

The pharmacokinetic behavior of **20** was profiled in three species (Table 4). As mentioned above, the dog pharmacokinetics for **20** were excellent, with a long half-life, low clearance, and high oral bioavailability. Somewhat poorer pharmacokinetics were observed for **20** in both rat and rhesus. Following iv administration moderate clearances and half-lives close to 2 h were observed (Table 4). After oral administration, **20** was well absorbed in both rat and rhesus. Compound **20** was not a potent reversible inhibitor of the major human CYP isozymes; IC_{50} values for CYP 2C9, 2D6 and 3A4 were all greater than 50 μM.

Table 4. Pharmacokinetic Data for **20**[a]

PK parameter	dog[b]	rat[c]	Rhesus[d]
$t_{1/2}$ (h)	17.4	1.9 ± 0.35	1.7
Cl (mL/min/kg)	0.51	13.3 ± 1.0	17.2
V_{dss} (L/kg)	0.72	1.5 ± 0.19	1.3
F (%)	99	49	43

[a] Dosed iv as a DMSO solution and po as a solution in 0.05 M citric acid (aq). [b] Average of two dogs dosed at each of 1 mg/kg iv and 1 mg/kg po. The individual determinations are within ±25% of the mean. [c] Average of three rats (with standard deviation) dosed at each of 2 mg/kg iv and 10 mg/kg po. [d] Average of two rhesus dosed at each of 1 mg/kg iv and 1 mg/kg po. The individual determinations are within ±25% of the mean.

Table 5. Pharmacokinetic Data for **23**[a]

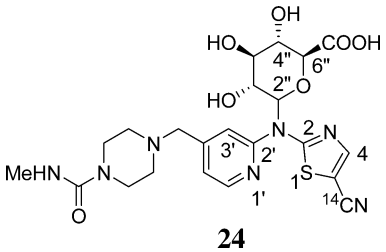
PK parameter	dog[b]	rat[c]	Rhesus[d]
$t_{1/2}$ (h)	7.0	2.4 ± 0.72	8.8
Cl (mL/min/kg)	2.0	3.0 ± 0.45	1.7
V_{dss} (L/kg)	1.2	0.6 ± 0.06	1.3
F (%)	75	73	92

[a] Dosed iv as a DMSO solution and po as a solution in 0.05 M citric acid (aq). [b] Average of two dogs dosed at each of 1 mg/kg iv and 1 mg/kg po. [c] Average of three rats dosed at each of 2 mg/kg iv and 10 mg/kg po. [d] Average of two rhesus dosed at each of 1 mg/kg iv and 1 mg/kg po.

Compound **23**, the 3-methylpyridine analogue of **20**, had slightly diminished but still excellent pharmacokinetic behavior in dog, with a long half-life of 7.0 h and low clearance (2.0 mL/min/kg) (Table 5). Interestingly the introduction of the 3-methyl group significantly enhanced pharmacokinetics in the rat and rhesus relative to **20**. Following an iv dose, the mean plasma clearance was low in both the rat and rhesus (3.0 and 1.7 mL/min/kg, respectively), and the $t_{1/2}$ was slightly improved in the rat (2.4 h) and significantly improved in the rhesus (8.8 h). High bioavailabilities were observed in both rat and rhesus relative to **20**. It is also not a potent inhibitor of the major CYP isozymes; IC_{50} values for CYP 2C9, 2D6, and 3A4 were all greater than 100 μM.

To profile the in vivo metabolism and elimination of **20**, a ^{14}C-labeled compound was dosed iv to rats, dogs, and rhesus. Biotransformation was found to be the major route of elimination in all of these species; the fraction of the dose excreted unchanged in bile and urine was <16%. *N*-Glucuronidation of the parent compound was identified as the major route of metabolism in both rat and rhesus (37.6 and 63.3% of iv dose, respectively) but was a minor component in dogs. Mass spectrometric and NMR analysis has shown the structure of the *N*-glucuronide metabolite to be **24** (Figure 2), where glucuronidation has occurred on the central nitrogen. Other minor metabolites in rats and monkeys were products of oxidation and *N*-demethylation. In dogs, **20** was eliminated in the form of several oxidative metabolites, of which *N*-demethylated products were the most abundant.

Consistent with the in vivo observations, studies conducted with hepatocytes also showed species-dependent metabolism of [^{14}C]**20**. *N*-Glucuronidation was



24

Figure 2. *N*-Glucuronide of [^{14}C]**20**.

Table 6. Kinase Selectivity Expressed as the Ratio between the IC_{50} for the Given Kinase and the IC_{50} for KDR

kinase	**20**	**23**
Flk-1	4	3
Flt-1	25	81
Flt-3	28	83
Flt-4	4.2	3.5
PDGFRβ	18	14
c-kit	45	40
c-fms	84	81
FGFR1	104	581
FGFR2	41	271
EGFR	>1600	>1600
insulinR	560	734
Src	84	474
Lyn	nd	869
Lck	950	2670
FynT	850	3728
CDK4	61	185
CDK2	328	1140

the major pathway observed in rat and monkey hepatocyte incubations, with oxidation and *N*-demethylation also observed in these species. However, in dog and human hepatocytes, *N*-demethylation and oxidation appeared to be the dominant metabolic routes while the *N*-glucuronide conjugate of **20** was not detected in dog and human hepatocyte preparations.

The rate of metabolism in hepatocytes followed the of the rank order monkey > rat ≫ dog. This agreed well with the rank order of in vivo plasma clearance: monkeys (17 mL/min/kg) > rats (13 mL/min/kg) ≫ dog (0.51 mL/min/kg). The rate of **20** metabolism in human hepatocytes from three different donors was similar to that in dogs. In addition, plasma protein binding is similar across all of these species. It is expected that **20** would be a low clearance compound in humans as it is in dogs.

Given the observation of significant glucuronidation of **20** in rat and rhesus, it is interesting to note that **23**, with the 3-methylpyridine substituent, has significantly improved pharmacokinetics in these species relative to **20**. It is likely that the 3-methylpyridine substitution sterically blocks the glucuronidation event and leads to the observed improvement in pharmacokinetics.

Kinase Selectivity. The kinase selectivity profiles for **20** and **23** are shown in Table 6. The values in the table are expressed as a ratio of the given kinase IC_{50} to the KDR kinase IC_{50}. Compound **20** shows a slight degree of selectivity against Flt-4 (VEGFR-3), a receptor homologue primarily expressed on lymphatic endothelial cells, and Flk-1 (the mouse isoform of KDR), both of which are highly homologous to human KDR. It was found to have moderate selectivity against the highly homologous c-kit, Flt-1, Flt-3, c-fms, and PDGFRβ enzymes. It also shows moderate to good selectivity against the less homologous FGFR-1 and FGFR-2 receptor tyrosine kinases, the c-Src nonreceptor tyrosine kinase, and one of the cyclin-dependent kinases, CDK4. A very high degree of selectivity is displayed against the EGFR, IGF-1R, and insulin receptor tyrosine kinases, other Src family nonreceptor tyrosine kinases, Lyn and FynT, and the other cyclin-dependent kinase tested (CDK2).

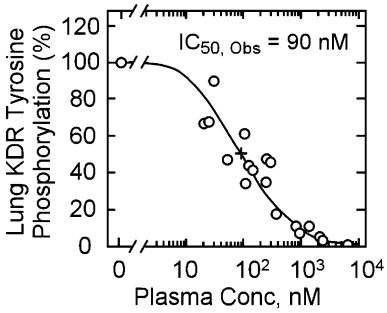


Figure 3. Determination of in vivo IC_{50} for the inhibition of mouse lung KDR phosphorylation by **20**.

In our initial SAR studies we observed that substitution of the pyridine ring can enhance selectivity.[6] The most consistent observation of this effect has occurred with the introduction of the 3-methyl substituent on the pyridine ring. When such a substituent was introduced into **20**, resulting in **23**, an enhanced level of selectivity was observed. Compound **23** maintained comparable selectivity to **20** versus Flk-1, Flt-4, PDGFRβ, and c-kit. However, moderate improvements in selectivity were seen against Flt-1, Flt-3, and the cyclin dependent kinases tested. Most notably, selectivity was significantly enhanced against FGFR-1, FGFR-2, and Src.

In Vivo Activity. A whole animal pharmacodynamic assay was used to evaluate the in vivo activity of **20**.[7] The assay measures the inhibition of VEGF-induced tyrosine phosphorylation of mouse lung KDR as a function of compound dose and time after dosing. This method produces an in vivo IC_{50} value for a KDR inhibitor as a function of compound plasma concentration. A curve was generated comparing phosphorylated KDR levels in the lung between compound treated and untreated lungs after VEGF-stimulation by tail vein injection of human $VEGF_{165}$. Compound **20** inhibits VEGF-stimulated mouse lung KDR autophosphorylation in a dose-dependent manner with an IC_{50} value of 90 ± 20 nM as shown in Figure 3. This value is indicative of a high level of in vivo activity of the compound, particularly when taking into consideration the 4-fold shift in activity that was observed between KDR kinase activity and Flk-1 (the mouse isoform of KDR, Table 6).

Conclusion

A series of *N*-(1,3-thiazol-2-yl)pyridin-2-amines has been optimized to provide KDR kinase inhibitors with optimal properties. Initial problems encountered with cell-potent compounds in this series involved poor pharmacokinetics and potent hERG binding activity. The problems associated with hERG binding and pharmacokinetics were resolved by the variation of two key moieties in the molecules. The discovery of a unique series of amine substituents, coupled with the replacement of a lipophilic phenyl with a cyano substituent, lead directly to the solution of these challenges. The

most highly optimized compound **20** was shown to have low affinity for hERG, excellent pharmacokinetics, and good activity in an in vivo assay of KDR kinase inhibition. Detailed pharmacokinetic analysis suggested that **20** is likely to have a low clearance in humans.

Experimental Section

General Methods. General Experimental Information. Proton and carbon NMR spectra were recorded on Varian Unity 400 or VRX-400 spectrometers (400 MHz) or Varian Unity or Varian Plus (500 MHz) spectrometers. Chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane as an internal standard. High-resolution mass spectra were recorded on a Bruker 3T Fourier transform ion cyclotron resonance mass spectrometer equipped with electrospray ionization. The MH^+ signal of the analyte of interest is mass measured against the internal calibrant, polypropylene glycol (average mass (425). Solvents and reagents were obtained from commercial sources and used without further purification. The reported yields are the actual isolated yields of purified material and are not optimized. Flash column chromatography was performed on an Isco Combiflash system employing prepacked silica columns (Isco Redi-Sep columns or Applied Separations Speed cartridges). Preparative reverse phase HPLC was performed on an automated Gilson system using a gradient of 5% MeCN to 95% MeCN (0.1% TFA) over 20 min on a Supelcosil ABZ-Plus column (10 cm × 21.2 mm, 5 μM). Analytical HPLC method A: Thermoseparation Products HPLC with a gradient from 10% MeCN to 100% MeCN (0.1% H_3PO_4) in 3 min using a YMC-Pack ProC18 4.6 × 50 mm column. Analytical HPLC method B: Waters' LC/MS comprised of a 2695 LC with a gradient from 7% MeCN to 100% MeCN (0.05% TFA) in 3 min, using a YMC PRO LC 3 × 50 mm column. UV analysis was performed at 215 nM for both methods.

4-({[*tert*-Butyl(dimethyl)silyl]oxy}methyl)-2-chloropyridine (4). To a solution of (2-chloropyridin-4-yl)methanol[10] (16.83 g, 117 mmol) in dry THF (300 mL) were added imidazole (9.58 g, 140.7 mmol) and TBDMSCl (21.2 g, 140.7 mmol) at RT. After 6 h imidazole (2.39 g, 35.1 mmol) and TBDMSCl (5.3 g, 35.1 mmol) were added and stirring continued. After 18 h the mixture was filtered and concentrated. Flash column chromatography (7% EtOAc/hexanes) gave the title compound (29.35 g, 97%) as a clear oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.31 (d, J = 5.13 Hz, 1 H), 7.31 (s, 1 H), 7.15 (m, 1 H), 4.73 (s, 2 H), 0.96 (s, 9 H), 0.12 (s, 6 H).

4-({[*tert*-Butyl(dimethyl)silyl]oxy}methyl)pyridin-2-amine (5, R^1 = H). To a solution of 4-({[*tert*-butyl(dimethyl)-silyl]oxy}methyl)-2-chloropyridine (29.35 g, 113.8 mmol) in dry toluene (250 mL) were added NaOtBu (15.3 g, 159.4 mmol), racemic-BINAP (2.13 g, 3.41 mmol), $Pd_2(dba)_3$ (1.04 g, 1.14 mmol), and benzophenone imine (23 mL, 136.6 mmol). The mixture was degassed (3 × pump/N_2) then heated to 80 °C. After 5 h the mixture was cooled to RT, diluted with Et_2O (1 L), and filtered through a pad of Celite. The filtrate was concentrated. The residue was taken up in 250 mL of MeOH, and hydroxylamine (15 mL of 50% solution in H_2O) was added at RT. After 18 h the mixture was filtered through a pad of Celite and concentrated. The residue was taken up in Et_2O, cooled to 0 °C, and filtered. The filtrate was concentrated. Flash column (gradient, 50−100% EtOAc/hexanes) gave 4-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)pyridin-2-amine (17.89 g, 66%) as a light yellow solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.99 (d, J = 5.13 Hz, 1 H), 6.57 (m, 1 H), 6.51(d, J = 0.74 Hz, 1 H), 4.64 (s, 2 H), 4.39 (bs, 2 H), 0.95 (s, 9 H), 0.11 (s, 6 H).

[4-(*tert*-Butyldimethylsilanyloxymethyl)pyridin-2-yl]-(5-phenylthiazol-2-yl)amine (6, R^1 = H, R^2 = Ph). 4-(*tert*-Butyldimethylsilanyloxymethyl)pyridin-2-ylamine (1.00 g, 4.19 mmol) was dissolved in 20 mL of anhydrous THF at room temp, and NaH (60% dispersion, 0.670 g, 16.8 mmol) was added. When the bubbling stopped, 2-chloro-5-phenylthiazole[16] 0.739 g (3.78 mmol) was added, and the reaction was heated to reflux. After 2 h the THF was removed in vacuo, and the resulting solution was taken to neutral pH with 1 M HCl (aq) and filtered. The residue was purified by flash column chromatography using 20% EtOAc in hexane to provide 1.31 g (79%) of the pure title compound. ^{1}H NMR ($CDCl_3$): δ 9.09 (bs, 1H), 8.32 (d, 1H, J = 5.2 Hz), 7.62 (s, 1H), 7.56 (d, 2H, J = 7.4 Hz), 7.38 (t, 2H, J = 7.6 Hz), 7.26 (overlapping with $CHCl_3$, 1H), 6.90 (s, 1H), 6.82 (d, 1H, J = 5.2 Hz), 4.75 (s, 2H), 0.96 (s, 9H), 0.14 (s, 6H). mp 207 °C.

[2-(5-Phenylthiazol-2-ylamino)pyridin-4-yl]methanol. [4-(*tert*-Butyldimethylsilanyloxymethyl)pyridin-2-yl](5-phenylthiazol-2-yl)amine (0.805 g, 2.03 mmol) was dissolved in 10 mL of THF, and the resulting solution was cooled to 0°C. Hydrogen fluoride−pyridine (Aldrich, HF ~70%, pyridine ~30%) 1.20 mL was added. After 1 h the reaction was allowed to warm to RT. The THF was removed in vacuo, and the residue was diluted with sat Na_2CO_3 (aq). The resulting precipitate was filtered to provide the pure title compound as a white solid (0.560 g, 98% yield). ^{1}H NMR (DMSO-d_6): δ 11.35 (bs, 1H), 8.25 (d, 1H, J = 5.2 Hz), 7.79 (s, 1H), 7.59 (d, 2H, J = 7.4 Hz), 7.39 (t, 2H, J = 7.6 Hz), 7.25 (t, 1H, J = 7.3 Hz), 7.08 (s, 1H), 6.86 (d, 1H, J = 5.2 Hz), 5.42 (bs, 1H), 4.51 (s, 2H). Mp 236−237 °C.

(4-Chloromethylpyridin-2-yl)(5-phenylthiazol-2-yl)-amine (7). [2-(5-Phenylthiazol-2-ylamino)pyridin-4-yl]methanol (0.500 g, 1.77 mmol) was stirred in anhydrous CH_2Cl_2 (5 mL) under N_2. *N,N*-Dimethylformamide (0.137 mL, 1.76 mmol) was added followed by the addition of phosphorus oxychloride (0.165 mL, 1.76 mmol). After 1.5 h the reaction was concentrated and quenched by the addition of saturated $NaHCO_3$ (aq). A precipitate formed which was filtered and washed with water to provide the title compound as a tan solid (0.482 g, 90% yield). ^{1}H NMR (DMSO-d_6) δ 11.49 (bs, 1H), 8.34 (d, 1H, J = 5.2 Hz), 7.81 (s, 1H), 7.60 (d, 2H, J = 7.7 Hz), 7.39 (t, 2H, J = 7.6 Hz), 7.26 (t, 1H, J = 7.0 Hz), 7.13 (s, 1H), 6.99 (d, 1H, J = 5.3 Hz), 4.77 (s, 2H).

(5-Phenylthiazol-2-yl)(4-pyrrolidin-1-ylmethylpyridin-2-yl)amine (12). Prepared in a library format: Pyrrolidine (0.011 g, 0.15 mmol) was dissolved in 0.1 mL of DMSO. (4-Chloromethylpyridin-2-yl)(5-phenyl-thiazol-2-yl)amine **7** (0.0095 g, 0.031 mmol) dissolved in 0.2 mL of DMSO was added to the pyrrolidine solution. After 16 h the reaction was directly purified by reverse phase preparative chromatography. The resulting product as a TFA salt was characterized by HRMS $[M + H]^+$ calcd: 337.1487, found: 337.1511. HPLC (method A): 98.0% purity.

1-{4-[2-(5-Phenylthiazol-2-ylamino)pyridin-4-ylmethyl]piperazin-1-yl}ethanone (13). Prepared in a library format according to preparation of **12**. HRMS $[M + H]^+$ calcd: 394.1702, found: 394.1697. HPLC (method A): 98.5% purity.

4-{[4-(Methylsulfonyl)piperazin-1-yl]methyl}-*N*-(5-phenyl-1,3-thiazol-2-yl)pyridin-2-amine (14). (4-Chloromethylpyridin-2-yl)(5-phenylthiazol-2-yl)amine **7** (0.053 g, 0.176 mmol) was dissolved in 1 mL of DMSO. Et_3N (0.122 mL, 0.88 mmol) was added followed by addition of 1-(methylsulfonyl) hydrochloride (0.042 g, 0.21 mmol), and the reaction was stirred at RT. After 30 min the reaction was warmed to 45 °C. After 2 h the reaction was diluted with water, and the resulting solid was filtered and washed with water. The solid was purified by reverse phase chromatography to provide 27 mg (28% yield) of the title compound as a trifluoroacetate salt. ^{1}H NMR (DMSO-d_6) δ 11.49 (bs, 1H), 8.51 (d, 1H, J = 5.5 Hz), 7.79 (s, 1H), 7.60 (d, 2H, J = 7.8 Hz), 7.44 (t, 2H, J = 7.3 Hz), 7.37 (m, 1H), 7.32 (bs, 1H), 7.25 (d, 1H, J = 5.5 Hz), 4.31 (s, 2H), 3.50 (bs, 4H), 3.32 (bs, 4H), 2.95 (s, 3H). mp = 183−184 °C. HRMS $[M + H]^+$ calcd: 430.1731, found: 430.1722. HPLC (method A): 98.5% purity. HPLC (method B): 98.9% purity.

2-Chlorothiazole-5-carbonitrile. A flame-dried round-bottom flask under N_2 was charged with 150 mL of anhydrous MeCN. $CuCl_2$ (12.9 g, 95.9 mmol, 1.2 equiv) was added, and the reaction was maintained in a room-temperature bath. *tert*-Butyl nitrite (14.3 mL, 120 mmol, 1.5 equiv) was added gradually over 10 min. After 10 min, 2-aminothiazole-5-carbonitrile[17] (10.0 g, 79.9 mmol) was added as a solid gradually. The reaction was stirred at room temp for 4h. The

reaction was poured into 400 mL of 0.5 M HCl (aq). The mixture was extracted 3× with EtOAc. The organic phases were dried over Na_2SO_4, filtered and concentrated to afford pure desired product (8.40 g, 73% yield). ^{1}H NMR ($CDCl_3$) δ 8.04 (s).

2-[4-(*tert*-Butyldimethylsilanyloxymethyl)pyridin-2-ylamino]thiazole-5-carbonitrile (6, R^1 = H, R^2 = CN). 4-(*tert*-Butyldimethylsilanyloxymethyl)pyridin-2-ylamine (16.1 g, 67.5 mmol) was dissolved in 200 mL of anhydrous THF under N_2. NaH (60% suspension, 8.09 g, 202 mmol, 3 equiv) was added (vigorous bubbling occurs), and the resulting mixture was stirred for 15 min. 2-Chlorothiazole-5-carbonitrile (10.1 g, 81.0 mmol) was added, and the reaction was heated to reflux. After 2 h the reaction was cooled and was quenched by the addition of water. The THF was removed in vacuo, and the resulting aqueous solution was adjusted to pH = 7 by the addition of 1 M HCl (aq). The resulting precipitate was filtered and washed with water to provide the desired product in moderate purity (23.4 g, 101% yield). ^{1}H NMR ($CDCl_3$) δ 10.32 (bs, 1H), 8.33 (d, 1H, J = 5.3 Hz), 7.99 (s, 1H), 6.96 (s, 1H), 6.91 (d, 1H, J = 5.3 Hz), 4.78 (s, 2H), 0.98 (s, 9H), 0.16 (s, 6H).

2-(4-Hydroxymethylpyridin-2-ylamino)thiazole-5-carbonitrile. 2-[4-(*tert*-Butyldimethylsilanyloxymethyl)pyridin-2-ylamino]thiazole-5-carbonitrile (23.4 g, 67.5 mmol) was dissolved in 130 mL of anhydrous THF. Hydrogen fluoride-pyridine (HF ~70% pyridine ~30%, 70 mL) was added, and the reaction was stirred for 1 h. A precipitate formed and was filtered and washed with THF. The filtrate was diluted with 1 L of water and filtered. The solid was air-dried and combined with the solid filtered from the reaction to afford 16.0 g (101%) of the title compound. ^{1}H NMR (DMSO-d_6) δ 12.23 (bs, 1H), 8.30 (d, 1H, J = 5.3 Hz), 8.26 (s, 1H), 7.15 (s, 1H), 6.99 (d, 1H, J = 5.3 Hz), 5.49 (t, 1H, J = 5.7 Hz) 4.54 (d, 2H, J = 5.7 Hz).

2-(4-Chloromethylpyridin-2-ylamino)thiazole-5-carbonitrile (7, R^1 = H, R^2 = CN). 2-(4-Hydroxymethylpyridin-2-ylamino)thiazole-5-carbonitrile (16.0 g, 68.8 mmol) was stirred in anhydrous CH_2Cl_2 (140 mL) under N_2. Dimethylformamide (5.32 mL, 68.8 mmol) was added followed by the addition of phosphorus oxychloride (6.41 mL, 68.8 mmol). After 4 h the reaction was quenched by the addition of 200 mL of saturated aqueous $NaHCO_3$. A precipitate formed which was filtered and washed with water to provide 15.5 g (90% yield) of the title compound. ^{1}H NMR (DMSO-d_6) δ 12.35 (bs, 1H), 8.40 (d, 1H, J = 5.3 Hz), 8.28 (s, 1H), 7.20 (s, 1H), 7.12 (d, 1H, J = 5.3 Hz), 4.82 (s, 2H).

2-[4-(4-Acetylpiperazin-1-ylmethyl)pyridin-2-ylamino]-thiazole-5-carbonitrile (15). 1-Acetylpiperazine (0.460 g, 3.59 mmol) was dissolved in 2 mL of anhydrous DMSO. 2-(4-Chloromethylpyridin-2-ylamino)thiazole-5-carbonitrile (0.300 g, 1.20 mmol) was added, and the solution was stirred for 30 min. The reaction solution was purified by preparative reverse phase HPLC. The fractions containing the desired compound were concentrated to dryness to afford the TFA salt (0.260 g, 47.5% yield). ^{1}H NMR (free base, $CDCl_3$) δ 9.94 (bs, 1H), 8.35 (d, 1H, J = 5.1 Hz), 7.99 (s, 1H), 7.00 (d, 1H, J = 5.4 Hz), 6.95 (s, 1), 3.66 (t, 2H, 4.8 Hz), 3.56 (s, 2H), 3.52 (t, 2H, J = 4.9 Hz), 2.50 (t, 2H, J = 5.0 Hz), 2.45 (t, 2H, J = 5.0 hz), 2.11 (s, 3H). dec 241−245 °C. Anal. ($C_{18}H_{19}N_6O_3S$) C, H, N.

2-(4-Piperazin-1-ylmethylpyridin-2-ylamino)thiazole-5-carbonitrile (16). The procedure was followed as for **15** but employing *tert*-butyl piperazine-1-carboxylate (0.373 g, 2.00 mmol) in 1 mL of anhydrous DMSO and 2-(4-chloromethylpyridin-2-ylamino)thiazole-5-carbonitrile (0.100 g, 0.401 mmol). The reaction solution was purified by preparative reverse phase HPLC. The fractions containing the desired compound were concentrated to dryness to afford the TFA salt (0.052 g, 25% yield). A pure sample of this product (0.365 g, 0.708 mmol) was stirred in 15 mL of EtOAc, and the mixture was cooled to 0 °C. Hydrogen chloride (g) was bubbled into the solution for 30 min. The reaction was allowed to warm to ambient temperature and after 2 h was concentrated to dryness. The residue was purified by preparative reverse phase HPLC. The resulting TFA salt was stirred in saturated aqueous $NaHCO_3$, and the resulting precipitate was filtered and washed with water and dried. This provided 0.130 g (62% yield) of the pure title compound. ^{1}H NMR (DMSO-d_6) δ 12.58 (bs, 1H), 10.18 (bs, 1H), 8.34 (d, 1H, J = 5.1 Hz), 8.27 (s, 1H), 7.11 (s, 1H), 7.04 (d, 1H, J = 5.1 Hz), 3.57 (s, 2H), 3.32 (m, 4H), 3.01 (m, 4H). HRMS [M + H]$^+$ calcd: 301.1235, found: 301.1197. Anal. ($C_{14}H_{16}N_6S$) C, H, N.

2-(4-Morpholin-4-ylmethylpyridin-2-ylamino)thiazole-5-carbonitrile (17). The procedure was followed as for **15** but employing morpholine (0.087 g, 1.00 mmol), 0.8 mL of DMSO, and 2-(4-chloromethylpyridin-2-ylamino)thiazole-5-carbonitrile (0.050 g, 0.20 mmol). After preparative reverse phase HPLC the title compound was obtained as the TFA salt (0.017 g, 21% yield). ^{1}H NMR (DMSO-d_6) δ 12.58 (bs, 1H), 10.18 (bs, 1H), 8.50 (bs, 1H), 8.31 (s, 1H), 7.20 (s, 2H), 4.4 (bs, 2H), 4.0 (bs, 2H), 3.4 (bs, 2H), 3.2 (bs, 2H). HRMS [M + H]$^+$ calcd: 302.1076, found: 302.1103. HPLC (method A): 99% purity. HPLC (method B): 98.5% purity.

4-({2-[(5-Cyano-1,3-thiazol-2-yl)amino]-4-pyridinyl}-methyl)-1-piperazinecarboxamide (18). The procedure was followed as for **15** but employing 1-piperazinecarboxamide (0.144 g, 1.12 mmol), 1 mL of DMSO, and 2-(4-chloromethylpyridin-2-ylamino)thiazole-5-carbonitrile (0.070 g, 0.28 mmol). After preparative reverse phase HPLC, the title compound was obtained as the TFA salt (0.0249 g, 18% yield). ^{1}H NMR (CD_3-OD) δ 8.49 (d, 1H, J = 5.0 Hz), 8.06 (s, 1H), 7.15 (m, 2H), 4.25 (s, 2H), 3.64 (bs, 4H), 3.15 (s, 4H). HRMS [M + H]$^+$ calcd: 344.1293, found: 344.1250. HPLC (method A): 97.9% purity. HPLC (method B): 97.5% purity.

4-({2-[(5-Cyano-1,3-thiazol-2-yl)amino]-4-pyridinyl}-methyl)-*N,N*-dimethyl-1-piperazinecarboxamide (19). The procedure was followed as for **15** but employing *N,N*-dimethyl-1-piperazinecarboxamide (0.298 g, 1.90 mmol), 1 mL of DMSO, and 2-(4-chloromethylpyridin-2-ylamino)thiazole-5-carbonitrile (0.119 g, 0.47 mmol). The reaction was diluted with water and the resulting precipitate collected by filtration. The solid was washed with water and hexanes and then air-dried overnight to afford the free base (0.0913 g, 49% yield). Free base: ^{1}H NMR (CD_3OD) δ 8.33 (d, 1H, J = 5.0 Hz), 8.03 (s, 1H), 7.08 (s, 1H), 7.04 (d, 1H, J = 5.0 Hz), 3.58 (s, 1H), 3.29 (t, 4H, J = 6.0 Hz), 2.84 (s, 6H), 2.49 (t, 4H, J = Hz). HRMS [M + H]$^+$ calcd: 372.1607, found: 372.1611. HPLC (method A): 95.2% purity. HPLC (method B): 98% purity.

4-[2-(5-Cyanothiazol-2-ylamino)pyridin-4-ylmethyl]piperazine-1-carboxylic Acid Methylamide (20). 2-{[4-(Chloromethyl)pyridin-2-yl]amino}-1,3-thiazole-5-carbonitrile (8.00 g, 31.9 mmol) was stirred in 60 mL of DMSO. 1-[(Methylamino)carbonyl]piperazin-4-ium chloride (11.5 g, 63.8 mmol) was added, followed by addition of triethylamine (13.34 mL, 95.7 mmol). The reaction was allowed to stir at room temperature for 15 h, at which time an additional 2.00 g piperazine hydrochloride (11.1 mmol) and 6.6 mL of Et_3N (48 mmol) were added. After 1 h the reaction was diluted with 300 mL of water. The resulting precipitate was filtered, washed with water, and air-dried. The solid was purified by flash chromatography (eluted with 92:8 DCM/MeOH), and the resulting still somewhat impure product was purified by reverse phase preparative HPLC. The resulting TFA salt was dissolved in water, and the resulting solution was adjusted to pH 7 with saturated aqueous $NaHCO_3$. The resulting precipitate was filtered and air-dried to afford 9.40 g of the title compound (82% yield). ^{1}H NMR (DMSO-*d*6) δ 12.20 (bs, 1H), 8.32 (d, 1H, J = 5.49 Hz), 8.26 (s, 1H), 7.13 (s, 1H), 7.03 (d, 1H, J = 5.19 Hz), 6.42 (bd, 1H, J = 4.27 Hz), 3.52 (s, 2H), 3.29 (m, 4H), 2.51 (d, 3H, J = 4.27 Hz), 2.33 (m, 4H). [M + H]$^+$ = 358.1443. Anal. ($C_{16}H_{19}N_7$-OS) C, H, N.

2-{4-[4-(2-Hydroxyethanoyl)piperazin-1-ylmethyl]pyridin-2-ylamino}thiazole-5-carbonitrile (21). The procedure was followed as for **15** but employing 1-glycoloylpiperazine hydrochloride (259 mg, 1.44 mmol), 2 mL of DMSO, 2-(4-chloromethylpyridin-2-ylamino)thiazole-5-carbonitrile (180 mg, 0.72 mmol), and diisopropylethylamine (0.38 mL, 2.15 mmol). After 3 h the mixture was diluted with H_2O and extracted with EtOAc (3×). The combined organic layers were dried (MgSO4),

filtered, and concentrated. Flash column chromatography (gradient, 5−15% EtOH/EtOAc then 5−10% MeOH/$CHCl_3$) gave the title compound as a pale yellow solid 86 mg, 33%: ^{1}H NMR (DMSO-d_6) δ12.19 (s, 1 H), 8.33 (d, 1 H, J = 5.1 Hz), 8.32 (s, 1 H), 7.14 (s, 1 H), 7.04 (d, 1 H, J = 5.2 Hz), 4.54 (t, 1 H, J = 5.6 Hz), 4.08 (d, 2 H, J = 5.6 Hz), 3.55 (s, 2 H), 3.49 (s, 2 H), 3.36 (s, 2 H), 2.38 (s, 4 H); HRMS $[M + H]^+$ calcd: 359.1290, found: 359.1285. HPLC (method A): 97.2% purity. HPLC (method B): 98.3% purity.

2-[(4-{[4-(Methylsulfonyl)piperidin-1-yl]methyl}pyridin-2-yl)amino]-1,3-thiazole-5-carbonitrile (22). 2-{[4-(Chloromethyl)pyridin-2-yl]amino}-1,3-thiazole-5-carbonitrile (3.490 g, 13.92 mmol) was dissolved in 20 mL of DMSO. 4-(Methylsulfonyl)piperidine (3.409 g, 20.88 mmol) and *N*-ethyl-*N*,*N*-diisopropylamine (9.70 mL, 55.68 mmol) were added, and the solution was stirred for 17 h. The reaction was diluted with water, and the resulting precipitate was filtered and washed with water. The precipitate was then purified by flash column chromatography (gradient, 3−10% MeOH/CH_2Cl_2). The fractions containing the desired compound were concentrated to dryness to afford 1.82 g of the title compound (35% yield). ^{1}H NMR (DMSO-d_6) δ 12.18 (bs, 1H), 8.32 (d, 1H, J = 5.19 Hz), 8.26 (s, 1H), 7.15 (s, 1H), 7.01 (d, 1H, J = 5.19 Hz), 3.54 (s, 2H), 3.14−3.09 (m, 1H), 2.93 (s overlapping m, 5H), 2.08−1.98 (m, 4H), 1.68−1.55 (m, 2H). HRMS $[M + H]^+$ calcd: 378.1058, found: 378.1057. HPLC (method A): 100% purity. HPLC (method B): 100% purity.

3-Methyl-1-oxyisonicotinic Acid Ethyl Ester. 3-Methylisonicotinic acid ethyl ester[18] (11.2 g, 67.8 mmol) was dissolved in 24 mL of dichloromethane. Methyl trioxorhenium (84.5 mg, 0.34 mmol) was added followed by the addition of hydrogen peroxide (30% aqueous, 13.8 mL, 135 mmol). The reaction was allowed to stir overnight. Additional methyl trioxorhenium (84.5 mg, 0.34 mmol) and hydrogen peroxide (30% aqueous, 13.8 mL, 135 mmol) were added. After 7 h, 25 mg of MnO_2 was added to the reaction (vigorous bubbling occurred). After bubbling had subsided (30 min), the reaction was diluted with water and extracted 3× with dichloromethane. The combined extracts were dried over Na_2SO_4, filtered, and concentrated to afford 11.1 g (90% yield) of the title compound as a white solid. ^{1}H NMR ($CDCl_3$) δ 8.06 (s overlapping with d 2H), 7.82 (d, 1H), 4.37 (q, 2H, J = 7.0 Hz), 2.55 (s, 3H), 1.40 (t, 3H, J = 7.0 Hz).

(2-Chloro-3-methylpyridin-4-yl)methanol. 3-Methyl-1-oxyisonicotinic acid ethyl ester (10.0 g, 55.3 mmol) was stirred in $POCl_3$ (25.8 mL, 42.4 g, 277 mmol) in a flask equipped with a reflux condenser and a drying tube. The resulting mixture was heated to reflux. After 3 h the reaction was cooled to room temperature. The excess $POCl_3$ was removed in vacuo. The residue was diluted with dichloromethane and washed with aqueous $NaHCO_3$ (sat). The aqueous phase was extracted 2× with dichloromethane, dried over Na_2SO_4, filtered, and concentrated. This afforded two isomeric products as a 2.5:1 mixture as a tan solid (9.76 g, 88% yield). A portion of this mixture (4.81 g, 24.1 mmol) was dissolved in 70 mL of anhydrous THF in an oven-dried flask under N_2. A reflux condenser was added, and a solution of $LiBH_4$ in THF (2 M, 14.5 mL, 28.9 mmol) was added. The reaction was heated to reflux for 2 h, allowed to cooled to room temperature, and was then quenched by the addition of 1 M HCl (aq). The solution was extracted 3× with dichloromethane. The combined extracts were dried over Na_2SO_4, filtered and concentrated. The resulting residue was purified by flash column chromatography (dissolved sample in DCM, eluted with 4:1 DCM/EtOAc) which afforded 0.77 g (20% yield) of the undesired (2-chloro-5-methylpyridin-4-yl)-methanol and 1.82 g (48% yield) of the desired title compound. ^{1}H NMR ($CDCl_3$) δ 8.22 (d, 1H, J = 4.9 Hz), 7.44 (d, 1H, J = 4.9 Hz), 5.53 (t, 1H, J = 5.4 Hz), 4.56 (d, 2H, J = 6.1 Hz), 2.23 (s, 3H).

4-(*tert*-Butyldimethylsilanyloxymethyl)-2-chloro-3-methylpyridine (11). (2-Chloro-3-methylpyridin-4-yl)-methanol (6.71 g, 42.6 mmol), *tert*-butyldimethylsilyl chloride (7.06 g, 46.8 mmol), and imidazole (3.48 g, 51.1) were dissolved in 80 mL of anhydrous DMF in an oven-dried flask under N_2 and stirred overnight. The bulk of the DMF was removed in vacuo, and the residue was diluted with saturated aqueous $NaHCO_3$. The mixture was extracted 4 × 100 mL of DCM, and the combined extracts were dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography (eluting with 93:7 hexanes/EtOAc) to afford 8.89 g of the title compound as a white solid. ^{1}H NMR ($CDCl_3$) δ 8.23 (d, 1H, J = 4.9 Hz), 7.40 (d, 1H, J = 4.9 Hz), 4.69 (s, 2H), 2.27 (s, 3H), 0.96 (s, 9H), 0.13 (s, 6H).

4-(*tert*-Butyldimethylsilanyloxymethyl)-3-methylpyridin-2-ylamine (5, R^1 = Me). An oven-dried flask under N_2 was charged with 4-(*tert*-butyldimethylsilanyloxymethyl)-2-chloro-3-methylpyridine (8.89 g, 32.7 mmol), NaOtBu (4.40 g, 45.8 mmol), tris(dibenzylideneacetone)dipalladium(0) (0.599 g, 0.65 mmol), racemic-2,2′-bis(diphenylphosphino)-1,1′-binaphthyl (1.22 g, 1.96 mmol), and anhydrous toluene (50 mL). Benzophenone imine (6.58 mL, 39.2 mmol) was added, and the reaction was heated to 80 °C. After 3 h the reaction was allowed to cool to room temperature and was diluted with 50 mL of diethyl ether. The resulting mixture was filtered through Celite, washing with ether. The filtrate was concentrated and redissolved in 10 mL of MeOH, and hydroxylamine (50% aqueous, 3.00 mL, 98.1 mmol) was added. After stirring overnight, an additional 0.680 mL of hydroxylamine solution was added. After 17 h the solution was concentrated in vacuo. The residue was purified by flash column chromatography (hexane/EtOAc) to afford 6.83 g (83% yield) of the title product. ^{1}H NMR ($CDCl_3$) δ 7.80 (d, 1H), 6.85 (d, 1H), 4.65 (s, 2H), 4.35 (bs, 2H), 2.00 (s, 3H), 0.95 (s, 9H), 0.15 (s, 6H).

2-[4-(*tert*-Butyldimethylsilanyloxymethyl)-3-methylpyridin-2-ylamino]thiazole-5-carbonitrile (6, R^1 = Me, R^2 = CN). An oven-dried flask under N_2 was charged with NaH (60% dispersion, 2.60 g, 108 mmol). Anhydrous THF (50 mL) was added followed by 4-(*tert*-butyldimethylsilanyloxymethyl)-3-methylpyridin-2-ylamine (6.83 g, 27.1 mmol) and 2-chloro-5-cyanothiazole (4.69 g, 32.5 mmol). The resulting solution was heated to reflux. After 3 h the reaction was allowed to cool to room temperature and diluted with water. The pH was adjusted to 7 with 1 M HCl. The resulting precipitate was filtered and washed with water to afford 10.5 g (107% yield) of the title compound. ^{1}H NMR ($CDCl_3$) δ 8.41 (bs, 1H), 8.27 (d, 1H, J = 5.2 Hz), 7.94 (s, 1H), 7.19 (d, 1H, J = 5.2 Hz), 4.74 (s, 2H), 2.21 (s, 3H), 0.96 (s, 9H), 0.13 (s, 6H).

2-(4-Hydroxymethyl-3-methylpyridin-2-ylamino)thiazole-5-carbonitrile. 2-[4-(*tert*-Butyldimethylsilanyloxymethyl)-3-methylpyridin-2-ylamino]thiazole-5-carbonitrile (10.5 g, 29.1 mmol) was dissolved in 60 mL of THF. HF−pyridine (∼70% HF, ∼30% pyridine, 29 mL) was added, and the reaction was stirred at room temperature. After 4 h the resulting precipitate was filtered and washed with THF to afford 4.39 g (61% yield) of the pure title compound. ^{1}H NMR (DMSO-d_6) δ 11.40 (s, 1H), 8.30 (s, 1H), 8.26 (d, 1H, J = 5.5 Hz), 7.21 (d, 1H, J = 5.2 Hz), 4.56 (s, 2H), 2.24 (s, 3H). MS $[M + H]^+$ = 247.1.

2-(4-Chloromethyl-3-methylpyridin-2-ylamino)thiazole-5-carbonitrile (7, R^1 = Me, R^2 = CN). 2-(4-Hydroxymethyl-3-methylpyridin-2-ylamino)thiazole-5-carbonitrile (4.39 g, 17.8 mmol) was stirred in 40 mL of anhydrous DCM. Anhydrous *N*,*N*-dimethylformamide (1.38 mL, 17.8 mmol) was added followed by phosphorus oxychloride (1.66 mL, 17.8 mmol). After 2 h the reaction was concentrated in vacuo and quenched with saturated $NaHCO_3$ (aq). The resulting orange solid was filtered, washed with water, and dried to afford 3.98 g (84% yield) of the title compound. ^{1}H NMR (DMSO-d_6) δ 11.54 (bs, 1H), 8.33 (s, 1H), 8.29 (d, 1H, J = 5.1 Hz), 7.19 (d, 1H, J = 5.1 Hz), 4.84 (s, 2H), 2.39 (s, 3H).

4-[2-(5-Cyanothiazol-2-ylamino)-3-methylpyridin-4-ylmethyl]piperazine-1-carboxylic Acid Methylamide (23). 2-(4-Chloromethyl-3-methylpyridin-2-ylamino)thiazole-5-carbonitrile (0.328 g, 1.24 mmol) and 4-methylcarbamoyl-piperazin-1-ium chloride (0.445 g, 2.48 mmol) were dissolved in 3 mL of DMSO. Diisopropylethylamine (0.863 mL, 4.96 mmol) was added, and the reaction was stirred for 5 h. The reaction mixture was then directly loaded onto a reverse phase purification system, which afforded the title compound as the TFA

salt (0.345 g, 57% yield). ^{1}H NMR (DMSO-d_6) δ 11.60 (s, 1H), 9.77 (bs, 1H), 8.36 (s overlapping with d, 2H), 7.23 (d, 1H, J = 5.2 Hz), 6.69 (s, 1H), 4.44 (s, 2H), 4.03 (s, 2H), 3.36 (s, 2H), 3.06 (s, 4H), 2.59 (s, 3H), 2.42 (s, 3H). HRMS $[M + H]^+$ calcd: 372.1606, found: 372.1597. HPLC (method A): 100%, HPLC (method B): 100% purity.

***N*-(5-Cyano-1,3-thiazol-2-yl)-*N*-[4-({4-[(methylamino)-carbonyl]piperazin-1-yl}methyl)pyridin-2-yl]-D-glucopyranuronosylamine (24).** NMR data for the N-glucuronide conjugate was acquired on a 500 MHz Inova spectrometer (Varian Inc. Palo Alto, CA) equipped with a 3-mm MIDG probe (Varian Inc.) at 25 °C. The N-glucuronide conjugate, isolated from Rhesus urine, was dissolved in 160 μL of CD_3OD and transferred to a 3-mm NMR tube. The site of glucuronide conjugation was inferred from the 1D ^{1}H, TOCSY, gHMQC, gHMBC data sets. All chemical shifts are referenced to the CD_2HOD resonance set at δ_H 3.33 ppm/δ_C 48.0 ppm.

NMR data on the N-glucuronide conjugate showed the presence of all peaks expected from **20** and also those from the glucuronide moiety. The glucuronide anomeric CH (2″) appeared at δ_H 6.41 ppm (d, 7.4 Hz) and at δ_C 73.5 ppm. Pyridine C-2′ and cyanothiazole C-2 appeared at δ_C 137.6 and 153.6 ppm, respectively. gHMBC correlations were observed from H2″ to both C-2′ and C-2. gHMBC correlations were also observed from pyridine H-6′ (8.48 ppm) to C-2′ and from cyanothiazole H-1 (8.06 ppm) to C-2. The combined NMR data placed the N-glucuronide at the bridge nitrogen connecting the pyridine and the cyanothiazole rings.

N-glucuronide conjugate NMR data, listed as position, δ_H (ppm), δ_C (ppm). Thiazole: C-2, 153.6; CH-1, 8.06, 133.4; C-5, 84.2; 5-CN, 99.3. Pyridine: C-2′, 137.6; CH-3′, 7.89, 100.9; C-4′, 136.5; CH-5′, 7.27, 105.9; CH-6′, 8.48, 130.9. Piperazine: CH_2, 3.49, 28.2; CH_2, 2.73, 11.4. Methylamide: C=O, 145.6; N-Me, 2.73, 11.4. Glucuronide: CH-1″, 6.41, 73.5; CH-2″, 4.02, 54.1; CH-3″, 3.69, 56.9; CH-4″, 3.59, 62.5; CH-5″, 4.08, 63.1.

Biological Methods. Enzyme, cell-based, and in vivo kinase assays were performed according to previously described procedures.[7]

The affinity of compounds for the IKr channel was evaluated in radioligand competition experiments analogous to [^{3}H]-dofetilide binding assays.[19] The human ERG potassium channel was stably expressed in 293 embryonic kidney cells,[20] and plasma membrane fractions prepared from these cells were used for competition experiments with [^{35}S]-MK499.[21] Results are reported as inflection points (IP) for the test compounds to inhibit binding of the radioligand. Most determinations were carried out at least twice, and the repeats were generally within 2-fold of the original value. Standard deviations from the mean value are shown for IP values determined three or more times.

Class III electrophysiologic activity was assessed in anesthetized dogs with the compound of interest being administered by intravenous infusion in an escalating-dose protocol. Plasma levels of compound and electrocardiogram data were determined before and during the infusions. The effects on the QT_c interval (the duration from the start of the QRS complex to the end of the T wave, corrected for heart rate) were correlated with drug concentrations in plasma.

For rat pharmacokinetics, fasted male Sprague−Dawley rats were given a 10 mg/kg dose by oral gavage and a 2 mg/kg dose by bolus intravenous injection. Oral bioavailability was determined using a solution of the mesylate salt in 0.05 M citric acid and the iv dose was administered as a DMSO solution. The bioavailability was assessed in fasted beagle dogs as a solution in 0.05 M citric acid or HCl-acidified methocel. For iv study a 1-mg/kg dose was administered intravenously as a DMSO solution. The bioavailability of a 1 mg/kg oral dose was assessed in fasted Rhesus monkeys using a solution in 0.05 M citric acid. In a crossover design, a 1 mg/kg dose was administered intravenously as a DMSO solution on a separate occasion.

The disposition of [^{14}C]**20** was assessed in bile-duct cannulated rats. Following iv administration of a 4-mg/kg dose (solution in DMSO), bile and urine were collected over ice for 48 h. In a preliminary assessment of the excretion and metabolic pathways, bile and urine were collected from one dogs for 72 and 120 h, respectively, after iv administration of a 2.4-mg/kg dose (solution in DMSO) of [^{14}C]**20**. In a preliminary assessment of the routes of excretion and metabolic pathways of **20**, urine and feces were collected from one monkey for 96 h and bile for 72 h after iv administration of a 1.6-mg/kg dose (solution in DMSO) of [^{14}C]**20**.

The effect of **20** and **23** on the metabolism of probe substrates for CYP 1A2 (substrate: phenacetin), 2C8 (paclitaxel), 2C9 (diclofenac), 2C19 (*S*-mephenytoin), 2D6 (bufuralol), and 3A4 (testosterone) was measured in human liver microsomes.

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Supporting Information Available: Table of elemental analysis data. This material is available free of charge via the Internet at http://pubs.acs.org.

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