Discovery and Evaluation of *N*-Cyclopropyl-2,4-difluoro-5-((2-(pyridin-2-ylamino)thiazol-5-ylmethyl)amino)benzamide (BMS-605541), a Selective and Orally Efficacious Inhibitor of Vascular Endothelial Growth Factor Receptor-2

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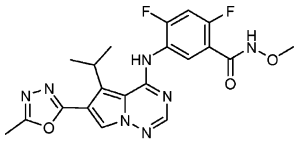
Abstract: Substituted 3-((2-(pyridin-2-ylamino)thiazol-5-ylmethyl)-amino)benzamides were identified as potent and selective inhibitors of vascular endothelial growth factor receptor-2 (VEGFR-2) kinase activity. The enzyme kinetics associated with the VEGFR-2 inhibition of **14** (K_i = 49 ± 9 nM) confirmed that the aminothiazole-based analogues are competitive with ATP. Analogue **14** demonstrated excellent kinase selectivity, favorable pharmacokinetic properties in multiple species, and robust in vivo efficacy in human lung and colon carcinoma xenograft models.

Tumor-induced angiogenesis, or the formation of new capillary networks between neoplastic cells and endothelial cells of the host,[1] is required for solid tumor growth and metastases.[2] VEGF,[a] a key stimulator of tumor angiogenesis, promotes endothelial cell proliferation, survival, migration, invasion, and differentiation.[3] The intrinsic activity responsible for these cellular events is dependent on the highly specific binding of the VEGF ligands to their respective cell surface expressed tyrosine kinase receptors VEGFR-1 (Flt-1), VEGFR-2 (KDR, Flk-1), and VEGFR-3 (Flt-4).[4] For more than a decade, the VEGF signaling pathway has been the target of extensive drug discovery research aimed at developing therapies that control the abnormal vascular development operative in cancer progression.[5] To this end, the chimeric anti-VEGF monoclonal antibody bevacizumab (Genentech) was recently approved to treat patients with metastatic colorectal cancer when combined with standard chemotherapy (IFL/Saltz regimen).[6] In addition to biological-based antiangiogenesis approaches, several small-molecule inhibitors of VEGFR-2 kinase activity have progressed to various stages of preclinical and clinical development.[5]

We have recently disclosed the structure−activity relationships (SARs) of various ATP-competitive VEGFR-2 inhibitors assembled from the pyrrolo[2,1-*f*][1,2,4]triazine scaffold.[7] Four-point pharmacophore[8a] and atom pairs[8b] similarity searches conducted with the potent *N*-methoxybenzamide-substituted pyrrolo[2,1-*f*][1,2,4]triazine inhibitor **1** (VEGFR-2 IC_{50} = 18 nM)[7b] identified several *N*-acyl-2-aminothiazole-based cyclin-dependent kinase (CDK2) inhibitors[9] from the Bristol-Myers Squibb compound collection. In general, these compounds displayed weak to moderate inhibition of VEGFR-2 kinase activity. For example, *N*-(5-((5-*tert*-butyloxazol-2-yl)methylthio)thiazol-2-yl)-3,4-dimethoxybenzamide (**2**), which demonstrated an IC_{50} of 50 nM against CDK2, was subsequently found to be a 1900 nM inhibitor of VEGFR-2.



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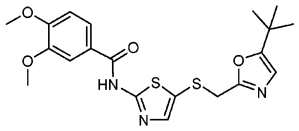

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A comparison of the relative binding modes of **1** and **2** in the VEGFR-2 ATP-binding site is provided in Figure 1.[7b,10] In this model, the pyrrolo[2,1-*f*][1,2,4]triazine and aminothiazole cores are anchored to the hinge region of the adenine binding pocket via a highly conserved hydrogen-bond interaction with the backbone amide-NH of Cys919. The hydroxamate of **1** is believed to be engaged in hydrogen-bond interactions with Asp1046 and Glu885. The overlay of **1** and **2** in this model suggested that attachment of the *N*-methoxybenzamide onto the aminothiazole template may lead to improved potency relative to the initial lead **2**. Herein, we describe the optimization and preclinical assessment of these selective aminothiazole-based VEGFR-2 inhibitors.

To evaluate the aforementioned hypothesis, *N*-acyl-2-aminothiazole and the *N*-2-pyridinylaminothiazole moiety used to prepare potent pan-Src kinase inhibitors[11] and structurally related VEGFR-2 inhibitors[12] were appended via a thiomethyl linker to the *N*-methoxybenzamide. As depicted in Table 1, the initial *N*-2-pyridinylaminothiazole analogue **3** displayed potent inhibition of VEGFR-2 kinase activity and VEGF-stimulated human umbilical vein endothelial cell (HUVEC) proliferation. Contrary to the SAR observed in the pyrrolo[2,1-*f*][1,2,4]triazine series,[7b] the simple *N*-methylamide analogue **4** retained activity in the biochemical assay. Because of the potential metabolic instability associated with the thiomethyl group, variations in the linker region (X−Y) were pursued. The methylamine analogue **5** was approximately 3-fold less potent than **4** in the VEGFR-2 assay. The ethyl- and *n*-propylbenzamide analogues provided similar biochemical potency relative to **5** (data not shown). Consistent with earlier observations,[7b] incorporation of a fluorine atom at R^1 of the benzamide ring (i.e., **6**) provided a 10-fold increase in intrinsic activity relative to **5**. Compound **6** also demonstrated significant antiproliferative activity in the VEGF-driven HUVEC assay. Since the 5-*tert*-butyloxazolylmethylthio moiety was previously shown to be critical for potent CDK2 inhibition in the aminothiazole series,[9] we evaluated and confirmed the selectivity of the current series for VEGFR-2 versus CDK2 (compound **6**, IC_{50} = 5100 nM). Replacement of the methyl group of amide **6** with a cyclopropyl substituent was well tolerated with the amine (Y = NH) and ethyl (Y = CH_2) linkers (i.e., **7** and **8**, respectively). Compound **8** was found to be the most potent analogue in the biochemical and cellular

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[a] Abbreviations: VEGF, vascular endothelial growth factor; IFL, irinotecan-5-fluorouracil-leucovorin; HUVEC, human umbilical vein endothelial cell; PK, pharmacokinetics; TFA, trifluoroacetic acid; AUC, area under the curve; V_{ss}, steady-state volume of distribution; Cl, clearance; MRT, mean residence time; TGI, tumor growth inhibition; hERG, human ether-a-go-go-related gene; HEK, human embryonic kidney cells.

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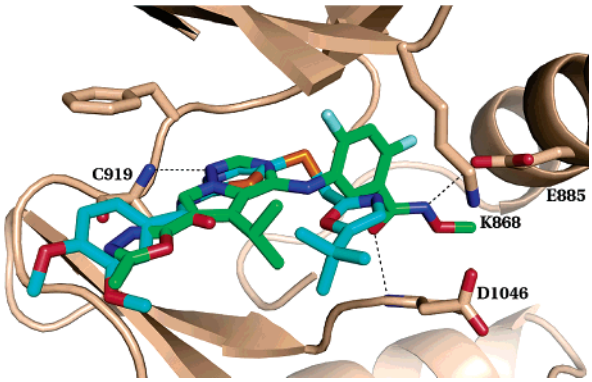
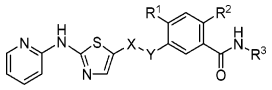


Figure 1. Proposed binding model of **1** and **2** in the ATP-binding site of VEGFR-2 kinase.

Table 1. Enzymatic and Cellular Activities[a] of *N*-2-Pyridinylaminothiazole Analogues



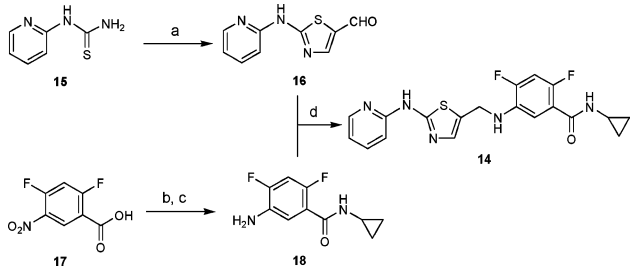
compd	X	Y	R^1	R^2	R^3	IC$_{50}$, nM: VEGFR-2 kinase inhibition[b]	IC$_{50}$, nM: growth inhibition of HUVECs[c] driven by VEGF[d]
3	S	CH_2	H	H	OCH_3	74[e]	110
4	S	CH_2	H	H	CH_3	88[e]	ND[f]
5	CH_2	NH	H	H	CH_3	280[e]	ND[f]
6	CH_2	NH	F	H	CH_3	30	20
7	CH_2	NH	F	H	cyclopropyl	29	14
8	CH_2	CH_2	F	H	cyclopropyl	14	5
9	CH_2	NH	F	H	cyclopentyl	110	ND[f]
10	CH_2	NH	F	H	benzyl	17	120
11	CH_2	NH	F	H	CH_2(2-pyridyl)	64	53
12	CH_2	NH	F	H	$(CH_2)_2$-*N*-morpholine	85	ND[f]
13	CH_2	NH	F	F	CH_3	71	52
14	CH_2	NH	F	F	cyclopropyl	23	25

[a] See ref 7b for a description of the assay conditions. [b] IC$_{50}$ values are reported as the mean of at least three independent determinations. Variability around the mean value was <50%. [c] Human umbilical vein endothelial cells. [d] All compounds evaluated in the HUVEC assay demonstrated an IC$_{50}$ > 2500 nM in the unstimulated growth of L2987 human lung carcinoma cells. [e] Single determination. [f] ND = not determined.

proliferation assays. However, **8** exhibited poor in vitro metabolic stability[13a] and provided relatively low systemic exposures in mouse 4 h oral PK studies.[13b] Additional amide analogues, such as the cyclopentyl derivative **9** and **11**−**12**, which contain terminal polar groups, were 2- to 4-fold less potent than **7** in the VEGFR-2 kinase assay. The benzylamide **10** demonstrated significant enzymatic activity but was nearly one order of magnitude less potent than **7** in the HUVEC proliferation assay.

Additional profiling revealed that the 4-fluorobenzamide derivative **7** possessed low chemical stability under aqueous acidic conditions.[14a] Thus, a second fluorine atom was introduced on the ring at R^2 in an effort to increase the electron-deficient nature of the benzamide ring. It was postulated that the resulting decrease in basicity of the aniline nitrogen would minimize the potential for acid-catalyzed hydrolysis. Although the corresponding methylamide **13** was slightly less potent in the biochemical assay, cyclopropylamide **14** (BMS-605541) retained excellent in vitro potency and displayed enhanced solution stability at low pH.[14b] On the basis of its improved chemical stability and impressive in vitro profile, **14** was selected for further characterization in kinase selectivity, PK, and in vivo efficacy studies.

Scheme 1[a]



[a] Reagents and conditions: (a) 2-bromo-3-hydroxypropenal, NaOAc, acetone, reflux, 2 h, 66%; (b) (1) thionyl chloride, reflux, 3 h; (2) CH_2Cl_2, cyclopropylamine, Et_3N, −40 °C to room temperature, 80% overall; (c) H_2, 10% Pd/C, EtOAc−EtOH (1:1), 2 h, 97%; (d) Et_3SiH, CH_2Cl_2−TFA (1:1), 1 h, 74%.

Table 2. Kinase Selectivity Profile of **14**

kinase	enzyme IC$_{50}$, nM	kinase	enzyme IC$_{50}$, nM
VEGFR-2	23	IKK	>10000
Flk-1	40	SRPK1	>10000
VEGFR-1	400	IGF-1R	>25000
PDGFR-β	200	PKC α, δ, τ, ζ	>40000
c-kit	370	Her1	>50000
FGFR-1	>5000	Her2	>50000
GSK-3	>5000	Lck	>50000
MEK	>5000	Itk	>50000
AKT	>5000	Syk	>50000
p38	>5000	JAK-3	>50000
Src	>10000	CaMKII	>50000
CDK2	>10000	MAPKAP K2	>50000

Compound **14** was prepared in a straightforward manner according to the synthetic sequence outlined in Scheme 1. Thus, condensation of thiourea **15**[15] with 2-bromo-3-hydroxypropenal[16] in the presence of sodium acetate in refluxing acetone provided key aldehyde intermediate **16**. 5-Amino-*N*-cyclopropyl-2,4-difluorobenzamide (**18**) was obtained from 2,4-difluoro-5-nitrobenzoic acid **17**[17] using a two-step reaction sequence involving cyclopropylamide bond formation and subsequent reduction of the nitro group. The reductive amination of aldehyde **16** with **18** was accomplished with triethylsilane to afford analogue **14** in 74% yield.

To better understand the enzyme inhibitory properties of **14**, the compound was evaluated in an in-house kinase selectivity panel and the K_i was determined for VEGFR-2 kinase. Analogue **14** was confirmed to be an ATP-competitive inhibitor of VEGFR-2, with a K_i of 49 ± 9 nM. As illustrated in Table 2, **14** was found to be a potent inhibitor of the mouse homologue of VEGFR-2 (Flk-1) and demonstrated moderate activity against VEGFR-1, PDGFR-β, and c-kit. Greater than 200-fold selectivity was observed for all the other receptor and nonreceptor kinase targets found in the panel.

The pharmacokinetic parameters obtained for **14** in mouse, rat, and cynomolgus (cyno) monkey are summarized in Table 3. In each case, **14** demonstrated a moderate steady-state volume of distribution (V_{ss}) and low systemic clearance (Cl) compared to the hepatic blood flow for the respective species. The compound was well absorbed following oral administration of drug from solution formulations at the doses denoted in Table 3. Favorable half-lifes ($t_{1/2}$) and mean residence times (MRT) were observed across species with **14**, albeit these values were slightly higher in cyno. The measured oral bioavailabilities (F_{po}) were 100% for mouse and rat and 52% in monkey.

The encouraging pharmacokinetic profile exhibited by **14** supported additional characterization of the compound in in vivo efficacy studies. Thus, the aminothiazole analogue was evaluated in the L2987 human lung and HCT-116 human colon carcinoma

Table 3. Pharmacokinetic Parameters for **14** in Mouse, Rat, and Cyno

parameter	mouse[a]	rat[b]	cyno[b]
po dose[c] (mg kg^{-1})	90	50	5
iv dose[d] (mg kg^{-1})	10	10	1[e]
C_{max} (μM), po	148	44.0(6.0)	8.0(4.0)
T_{max} (h), po	0.5	2.0(0)	0.75(0)
AUC (μM·h), po	649[f]	202[g](44)	28.5[g](13)
$t_{1/2}$ (h), po	1.7	2.2(1.2)	7.1(1.5)
MRT (h), po	3.4	3.5(0.4)	7.9(0.3)
Cl (mL min^{-1} kg^{-1}), iv	11.8	13.6(2.1)	3.9(0.6)
V_{ss} (L kg^{-1}), iv	1.7	1.1(0.1)	1.6(0.3)
F_{po} (%)	100	100	52

[a] Composition serum concentration−time profiles were constructed for the PK analysis. [b] Data reported as an average of three animals with associated standard deviations in parentheses. [c] Vehicle: PEG400/Tween-80/water (40:10:50). [d] Vehicle: PEG400/Tween-80/water (20:5:75). [e] Vehicle: 30:70 PEG400/water. [f] $AUC_{(0-24h)}$. [g] AUC_{total}.

Table 4. In Vivo Antitumor Activity of **14** against Established L2987 and HCT-116 Xenografts Implanted Subcutaneously in Athymic Mice

tumor model	dose, mg kg^{-1}	schedule	TGI,[a] %
L2987[b]	60	1qd × 14	16
	90		55
	180		67
L2987[c]	25	2qd × 14	23
	50		67
HCT-116[c]	12.5	2qd × 14	21
	25		51
	50		64

[a] Maximum percent tumor growth inhibition over one tumor volume doubling time. [b] Vehicle: PEG400/water (70:30). [c] Vehicle: 100% PEG400.

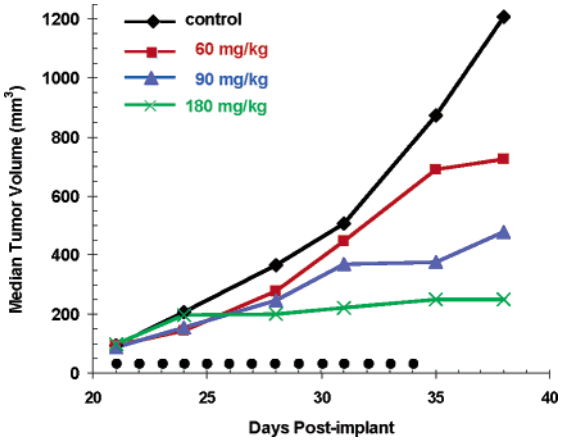


Figure 2. In vivo efficacy of **14** versus L2987 human tumor xenografts in athymic mice. Circles indicate dosing (1qd × 14).

xenograft models, and the results are summarized in Table 4.[18] In the L2987 study, **14** displayed antitumor activity when administered at doses of 90 and 180 mg/kg once daily for 14 consecutive days (Table 4). Figure 2 illustrates the dose-dependent growth inhibition of **14** in the L2987 tumor model. Although considerable tumor growth was noted at the first measurement (day 24), nearly complete tumor stasis was observed at the 180 mg/kg dose level throughout the remainder of the dosing regimen. In an effort to minimize the peak drug concentrations achieved during these studies, a second L2987 tumor xenograft study was conducted at lower doses on a 2qd × 14 schedule. Thus, twice daily administration of **14** at 50 mg/kg provided an identical TGI as the 180 mg/kg dose level on the once daily schedule (Table 4). Similar TGI values were obtained with **14** in the more sensitive HCT-116 tumor model at doses of 50 and 25 mg/kg on the twice daily dosing regimen. No overt toxicity as measured by weight loss or morbidity was observed at any of the dose levels tested in these experiments.

Structurally related aminothiazole analogues[12] have demonstrated high affinity for the hERG-encoded rapidly activating delayed rectifier potassium channel (I_{Kr}).[19] Since potent inhibition of the hERG channel and blockade of the I_{Kr} current have been linked to drug-induced (acquired) QT-interval prolongation and ultimately life threatening ventricular arrhythmias including torsades de pointes, **14** was evaluated in preliminary in vitro cardiovascular safety studies. Compound **14** was found to have an IC_{50} of 16.6 μM against the hERG channel expressed in HEK-293 cells.[19] The activity observed in the electrophysiology assay suggests a low to moderate risk for prolongation of the QT interval in vivo.

In conclusion, novel 3-((2-(pyridin-2-ylamino)thiazol-5-ylmethyl)amino)benzamides with potent VEGFR-2 kinase inhibitory activity were identified from the *N*-methoxybenzamide-substituted pyrrolo[2,1-*f*][1,2,4]triazines using computer-assisted drug design techniques. Optimization of the series SAR led to the identification of **14**, which was orally active in the human lung (L2987) and colon (HCT-116) carcinoma xenograft models at multiple dose levels. On the basis of its favorable in vitro pharmacology, in vivo efficacy, and pharmacokinetic profile, **14** was advanced into preclinical in vivo safety studies.

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Supporting Information Available: Experimental procedures and characterization data for **3**−**14**, table of combustion analysis and HPLC analysis data for key compounds, and detailed description of pharmacokinetic assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) (a) The rates of metabolism in mouse and human liver microsomes were both 0.16 nmol min^{-1} mg $protein^{-1}$ after a 10 min incubation at 10 μM. (b) The drug concentrations obtained during a mouse (male Balb/C) coarse oral PK study after a single 50 mg/kg dose using 70% PEG400-H_2O as the vehicle were 13.4 μM at 1 h and 6.2 μM at 4 h, $AUC_{0-4h} = 36$ μM·h. For assay conditions, see ref 7b.

(14) (a) 50% degradation observed at pH 1 (0.1 N HCl) after 14 h. (b) 12% degradation observed at pH 1 (0.1 N HCl) after 14 h.

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