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Discovery and preclinical studies of 5-isopropyl-6-(5-methyl-1,3,4-oxadiazol-2-yl)-*N*-(2-methyl-1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)pyrrolo[2,1-*f*][1,2,4]triazin-4-amine (BMS-645737), an in vivo active potent VEGFR-2 inhibitor

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Abstract—We report herein a series of substituted *N*-(1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)pyrrolo[2,1-*f*][1,2,4]triazin-4-amines as inhibitors of vascular endothelial growth factor receptor-2 tyrosine kinase. Through structure–activity relationship studies, biochemical potency, pharmacokinetics, and kinase selectivity were optimized to afford BMS-645737 (**13**), a compound with good preclinical in vivo activity against human tumor xenograft models.

Angiogenesis, the formation of new blood vessels from preexisting microvasculature, is a complex process that normally occurs during wound healing, organ regeneration, and the female reproductive cycle.[1] It can also occur in cancer whereby the newly created capillaries supply growing tumors with nutrients and allow waste removal.[2] Vascular endothelial growth factor (VEGF) is a key pro-angiogenic cytokine released by many tumors and the angiogenic activity of the VEGF family of proteins is mediated by three VEGFR receptors (VEGFR-1, VEGFR-2, and VEGFR-3). The VEGFR-2 receptor, the principal kinase involved in multiple processes of angiogenesis, has therefore become an attractive cancer target for which many small molecule inhibitors have shown promising results in preclinical and clinical studies.[3]

We recently disclosed a series of potent indole-based VEGFR-2 kinase inhibitors from which emerged BMS-540215, a compound with excellent enzymatic potency against VEGFR-2, a good kinase selectivity profile, an acceptable safety profile, and robust preclinical in vivo activity against a variety of human tumor xenograft models.[4] Our clinical prodrug candidate BMS-582664 was subsequently developed to improve the pharmaceutic and pharmacokinetic properties of the parent indole BMS-540215 (**1**).[4] This report now describes the synthesis, structure–activity relationship (SAR), and antitumor activity of the corresponding 7-azaindoles.

We first investigated a series of 7-azaindoles bearing C-6 ether side-chains. The 7-azaindole analog **2** and BMS-540215 (**1**) were essentially equipotent against

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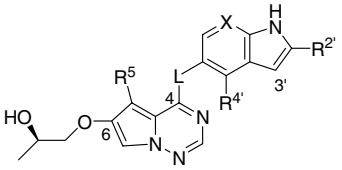
Table 1. SAR at the $R^{2'}$, $R^{4'}$, R^5, X and L positions of (*R*)-1-(4-(1*H*-pyrrolo[2,3-*b*]pyridin-5-yloxy)-5-alkyllpyrrolo[2,1-*f*][1,2,4]triazin-6-yloxy)propan-2-ol and (*R*)-1-(4-(1*H*-pyrrolo[2,3-*b*]pyridin-5-ylamino)-5-alkyllpyrrolo[2,1-*f*][1,2,4]triazin-6-yloxy)propan-2-ol[a]



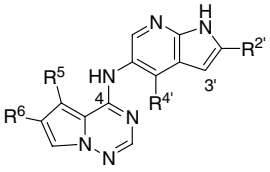
Compound	X	L	$R^{2'}$	$R^{4'}$	R^5	VEGFR-2, IC_{50}[a] (μM)	Mouse 4-h oral exposure AUC[c] (μM h)
1	CH	O	Me	F	Me	0.025	136.0
2	N	O	Me	F	Me	0.019	29.6
3	N	O	H	F	Me	0.027[b]	11.1
4	N	O	Me	H	Me	0.122	ND[d]
5	N	NH	Me	H	Me	0.223	ND
6	N	NH	H	H	*i*-Pr	0.165	ND

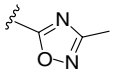
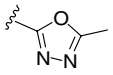
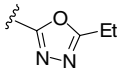
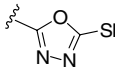
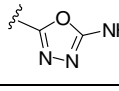
[a] IC_{50} values are reported as the mean of at least three individual determinations. Variability around the mean was <20%. For assay conditions see Ref. 5a.
[b] Value reported from two experiments (var. <10%).
[c] Compounds were evaluated in 4-h exposure study in mice at 50 mg/kg and formulated as solutions of 1:1 PEG(poly(ethylene glycol))400:water.
[d] ND, data not determined.

VEGFR-2 but the azaindole analog showed decreased plasma exposure upon oral administration in mice (Table 1). Removing the $R^{2'}$ methyl substituent retained high biochemical potency but **3** also showed poor plasma exposure. A number of other structural modifications including: (i) removal of the $R^{4'}$ fluorine, (ii) the oxygen to amine linker replacement, and (iii) the R^5 isopropyl substitution, provided compounds **4**–**6** respectively, which showed 6- to 12-fold decreases in potency against VEGFR-2 kinase.

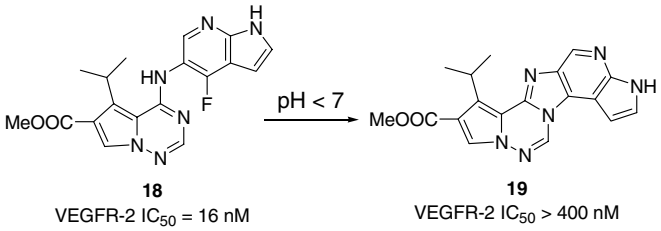
Related 4-phenylaminopyrrolo[2,1-*f*][1,2,4]triazine-based VEGFR-2 kinase inhibitors bearing C-6 esters, ester isosteres and amides have been studied previously.[5] This study showed that, with respect to biochemical potency, an isopropyl group was the optimum C-5 alkyl substitution with pyrrolotriazines bearing C-4 amino substitution. Consistent with these findings, introduction of a R^5 isopropyl group in ester **8** indeed gave a 10-fold increase in potency against VEGFR-2 compared to the R^5methyl analog **7** (Table 2). Compounds **9**–**17** described in Table 2 represent a selection of various ester isosteres studied in this series. Oxadiazole **9** was a more potent (~4-fold) inhibitor of VEGFR-2 (IC_{50} = 30 nM) than the corresponding *des*-fluoro analogs **10** and **11**. However, the chemical stability of fluoro analogs related to **9** was a concern since we had observed ring forming reactions leading to pentacyclic intermediates under acidic conditions. For example, ester **18** gave rise to the pentacyclic compound **19** when treated with acid (Scheme 1). Likewise, the potential for the formation of a pentacyclic intermediate derived from **14** precluded any further investigation on this and related fluoro analogs. The *des*-fluoro analogs **12** and **13** bearing regioisomeric oxadiazole rings, in turn, were found to be more potent than **10** and **11**. Finally, oxadiazole analogs **15**–**17** were prepared and tested against VEGFR-2. In essence, these analogs bearing various C-2 substituents off the oxadiazole ring were equipotent to **13** except for the thiomethyl analog **16** which showed about 5-fold decrease in potency. An activated sulfoxide intermediate was prepared from **16** and used to introduce various

Table 2. SAR at the R^6, R^5, $R^{2'}$ and $R^{4'}$ positions of *N*-(1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)pyrrolo[2,1-*f*][1,2,4]triazin-4-amine



Compound	R^6	R^5	$R^{2'}$	$R^{4'}$	VEGFR-2, IC_{50}[a] (μM)
7	COOMe	Me	H	H	0.121
8		*i*-Pr	H	H	0.017
9		*i*-Pr	H	F	0.030
10		*i*-Pr	H	H	0.096
11		*i*-Pr	Me	H	0.113
12		*i*-Pr	H	H	0.031
13		*i*-Pr	Me	H	0.025
14		*i*-Pr	Me	F	0.013[b]
15		*i*-Pr	Me	H	0.036
16		*i*-Pr	Me	H	0.150
17		*i*-Pr	Me	H	0.025

[a] IC_{50} values are reported as the mean of at least three individual determinations. Variability around the mean was <35%. For assay conditions see Ref. 5a.
[b] Value reported from one experiment.



18
VEGFR-2 IC_{50} = 16 nM

19
VEGFR-2 IC_{50} > 400 nM

Scheme 1. Formation of pentacyclic intermediate **19** from 4-fluoro-5-amino-7-azaindole precursor **18** under acidic conditions.

nucleophiles into this region of the inhibitor which should be solvent exposed according to molecular modeling.[5] Attempts to modulate the properties of the compound using this strategy afforded various analogs from which the aminomethyl derivative **17** stood out, although **13** was still a superior compound overall based on additional profiling provided below.

Additional profiling data of compounds **12**, **13**, and **17** are provided in Tables 3–5. These potent VEGFR-2 inhibitors were further profiled with respect to CYP3A4 and hERG patch-clamp inhibit,ion (Table 3).[6] Increased inhibition of CYP3A4 activity was observed with the $R^{2'}$-Me analogs **13** and **17**. However, although substitution on azaindole ring indeed resulted in higher CYP3A4 inhibition (**13** vs **12**), it also afforded compounds with improved kinase selectivity profile (vide infra). Likewise, the *des*-methyl azaindole analog **12** was further differentiated by its lower activity in hERG patch-clamp assay.

Compounds **12**, **13**, and **17** were also evaluated in mouse pharmacokinetic studies[6] (Table 4). The unsubstituted azaindole **12** showed decreased exposure, higher clearance and lower bioavailability in comparison with the $R^{2'}$-Me analogs **13** and **17**. When **13** was administered at 30 mg/kg orally as a suspension in 0.5% methocel and at 5 mg/kg intravenously in PEG400:0.01 N HCl (1:1), the compound was rapidly absorbed with a T_{max} of 0.5 h, a favorable half-life of 2.9 h, and mean residence time (MRT) of 2.7 h. The measured oral bioavailability (F_{po}) in this experiment was high (>100%) and **13** had low clearance (Cl) and a moderate volume of distribution (V_{ss}). In vivo exposure from solid dosage formulation was assessed in the rat as well. At a similar dose (30 mg/kg) the measured oral bioavailability in the rat was 68% and AUC was 287 μM h for a crystalline suspension of **13** in 0.5% methocel. Aqueous solubility for compound **13** was found to be low at pH 6.5 (2.8 μg/mL) and increased with a decrease in pH to 400 μg/mL at pH 2; however despite low solubility and poten-

Table 3. CYP3A4 and hERG Inhibition of compounds **12**, **13**, and **17**[a]

Compound	CYP 3A4 IC_{50}[b] (μM)	hERG inhibition[b]
12	>40	29% at 10 μM
13	11.5	64% at 10 μM (IC_{50} = 6.6 μM)
17	6.4	64% at 10 μM

[a] IC_{50} values are reported as the mean of at least three individual determinations. Variability around the mean was <50%.
[b] For assay details, see Ref. 5a.

Table 5. Kinase selectivity profile of compounds **12** and **13**

Enzyme	**12** IC_{50}[a] (nM)	**13** IC_{50} (nM)
VEGFR-2 (human)	31	25
Flk-1 (mouse)	ND[b]	53
VEGFR-1	ND	268
FGFR-1	61	78
PDGFR-β	389	>2000
EGFR	150	>1000
HER-2	500	>5000
LCK	185	>2000
PKCα[c]	>50,000	>50,000
JAK-3[c]	>50,000	>50,000

[a] IC_{50} values are reported as the mean of at least three individual determinations. Variability around the mean was <20%.
[b] ND, data not determined.
[c] Value reported from one experiment.

Table 4. Parameters for **12**, **13**, and **17** in a 24-h PK study in mice[a,b]

Compound	Dose (mg/kg)	AUC_{tot} (μM h)	$t_{1/2}$ (h)	MRT (h)	Cl (ml/min/kg)	V_{ss} (L/kg)	F_{po} (%)
12	iv, 5	12.4	1.2	1.7	18	1.9	29
	po, 30	21.7	4.5				
13	iv, 5	25	2.9	2.7	8.5	1.4	>100
	po, 30	182	2.5				
17	iv, 5	46	2.8	4.3	4.5	1.2	99
	po, 30	272	2.8				

[a] Data taken from a composite serum-concentration profile of nine mice (male, Balb-c). For assay details see Ref. 5a.
[b] Vehicle: iv: **12**: PEG400:sodium phosphate buffer, 0.1 M, pH 2 (70:30). Compounds **13** and **17**: PEG400:0.01N HCl (1:1). po: **12**: 100% Capmul MCM. **13** and **17**: 0.5% methocel.

Table 6. Cell proliferation data of compound **13**

Cell	**13** IC_{50}[a] (nM)
VEGF-HUVEC[b]	13
bFGF-HUVEC	138
L-2987 tumor	>2500
N87 tumor	>10,000
H1993 tumor	>10,000
GTL-16 tumor	>10,000

[a] IC_{50} values are reported as the mean of at least three individual determinations. Variability around the mean was <20%.
[b] Human umbilical vein endothelial cells stimulated by VEGF.

tial for pH-dependent absorption, rodents consistently showed satisfactory exposure.

By comparison with the *des*-methyl analog **12**, the $R_{2'}$ methyl analog **13** showed a better kinase selectivity profile. The substituted analog **13** indeed showed decreased activity versus EGF, HER-2, LCK, and PDGFR-β kinases (Table 5). Furthermore, **13** had moderate potency against VEGFR-1 and FGFR-1, two other kinases implicated in angiogenesis. It also showed good potency against Flk-1, the mouse homolog of VEGFR-2.

The inhibition of proliferation of HUVEC cells (human umbilical vein endothelial cells) by **13** was measured (Table 6). The cellular potencies of **13** against VEGF- and bFGF-stimulated HUVECs were high (IC_{50} = 13 and 138 nM respectively) but **13** showed low anti-proliferative potency against a panel of tumor cell lines. In particular, activity of **13** against L2987 human lung carcinoma cell line used in the in vivo tumor xenograft mouse efficacy model was low (IC_{50} > 2.5 μM).

Compound **13** was therefore tested for anti-tumor activity in vivo against the human L2987 xenograft solid tumor in athymic mice at two dose levels (7.5 and 15 mg/kg) (Fig. 1). The treatment and control group size consisted of 9–10 mice. Once daily oral administration of **13** in Capmul for 14 days (day 21 to day 34 post-implant) inhibited tumor growth in a dose-dependent manner. Tumor growth inhibition was indeed observed at both doses over the dosing period, with a slightly greater delay in tumor growth observed at the higher dose. No overt toxicity (weight loss, morbidity) was observed at either dose. Pharmacokinetic analysis using multiple time points during a 24-h-period after the last dose (i.e., at day 15) revealed a C_{max} of 1.03 μM and AUC = 11 μM h at the minimum efficacious dose level of 7.5 mg/kg. The higher dose level resulted in a slightly higher than proportional increase in these blood levels (C_{max} of 2.64 μM and AUC = 30 μM h). Antitumor activity was evident at both doses as demonstrated by ⩾50% tumor growth inhibition over one tumor volume doubling time which was 4 days in this study. Rather than directly affecting the tumor, the robust in vivo activity of **13** is likely driven by its antiangiogenic properties based on the high cellular potencies of VEGF- and FGF- stimulated HUVECs combined with weak cellular potency in multiple tumor cell lines, most notably L2987 (IC_{50} > 2.5 μM). The activity of **13** in this tumor xenograft model (MED = 7.5 mg/kg) was therefore significantly higher than the first generation inhibitor BMS-540215 (**1**) for which the minimum efficacious dose was 60 mg/kg using the same model and dosing regimen. Other tumor xenografts of different histological origins were also used to assess the broad spectrum in vivo efficacy of **13**. The latter was found active in all models used (e.g., H3396, HCT-116VM, and colo-205) and systematically showed good in vivo activity at modest dose. Plasma protein binding, which was found to be 97.2% for **13** in human sera, did not represent a problem with these inhibitors.

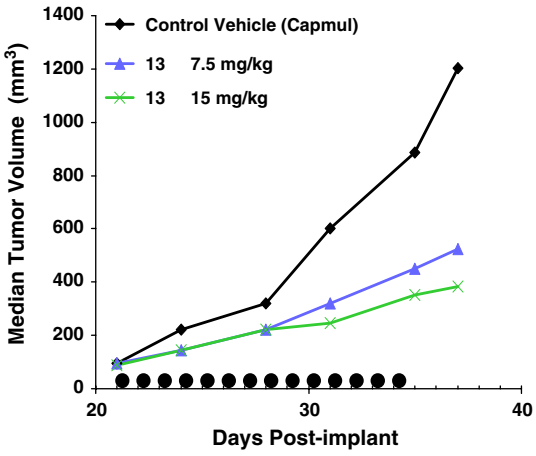
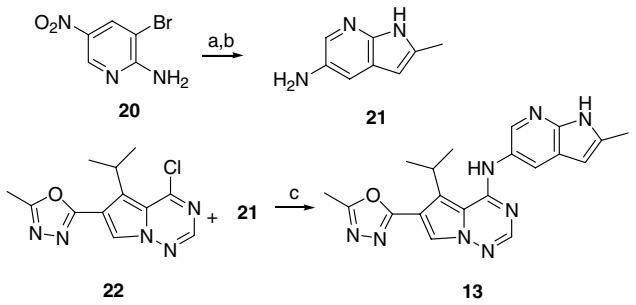


Figure 1. In vivo antitumor activity of **13** versus L-2987 human xenograft in athymic mice with dosing for 14 days from day 21 to day 35 post-implant (shown by black dots).

The synthesis of **13** outlined in Scheme 2 starts with the palladium-catalyzed reaction between commercially available aminopyridine **20** and isopropenyl acetate which, after catalytic hydrogenation, provided azaindole **21**. Intermediate **21** was then coupled with chloroimidate **22**[5] in DMF in the presence of diisopropylethylamine to provide **13** in 93% yield.[7]

In summary, a novel series of *N*-(1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)pyrrolo[2,1-*f*][1,2,4]triazin-4-amine VEGFR-2 inhibitors was identified. Biochemical potency and kinase selectivity optimization afforded 5-isopropyl-6-(5-



Scheme 2. Synthesis of **13**. Reagents and conditions: (a) isopropenyl acetate, Pd_2dba_3, Bu_3SnOMe, 2-dicyclohexylphosphino-2′-4′-6′-triisopropyl biphenyl, toluene, sealed tube, 135 °C; (b) H_2, Pd/C, ethanol, 89%, two steps; (c) $(i\text{-Pr})_2NEt$, DMF, 93%.

methyl-1,3,4-oxadiazol-2-yl)-*N*-(2-methyl-1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)pyrrolo[2,1-*f*][1,2,4]triazin-4-amine (**13**) as an orally active compound in the L2987 xenograft model of human lung carcinoma. This compound demonstrated tumor growth inhibition during the dosing period and was considered for further development based on its favorable pharmacokinetic, potency and kinase selectivity profile.

Acknowledgments

We thank Dr. Robert Kramer for scientific advice and the Discovery Analytical Sciences (Bristol-Myers Squibb) for compound characterization.

Supplementary data

Full experimental details and characterization data for compounds **2**–**19** are available. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.03.057.

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6. For the assay conditions of in vitro kinase, cellular proliferation, metabolic stability, cytochrome P450 and pharmacokinetics, see Ref. 5a. See Supporting information for hERG assay conditions.
7. The coupling reactions yielding other analogs disclosed in Tables 1 and 2 proceeded similarly using alternate syntheses of chloroimidates (see Refs.5a and 5b) and azaindoles (see Refs.7a and 7b): (a) Thibault, C.; L'Heureux, A.; Bhide, R. S.; Ruel, R. *Org. Lett.* **2003**, *5*, 5023; (b) L'Heureux, A.; Thibault, C.; Ruel, R. *Tetrahedron Lett.* **2004**, *45*, 2317.