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Letters

Discovery and Preclinical Studies of (*R*)-1-(4-(4-Fluoro-2-methyl-1*H*-indol-5-yloxy)-5-methylpyrrolo[2,1-*f*][1,2,4]triazin-6-yloxy)propan-2-ol (BMS-540215), an In Vivo Active Potent VEGFR-2 Inhibitor

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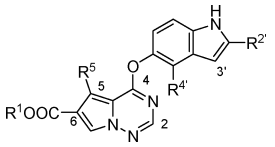
Abstract: A series of substituted 4-(4-fluoro-1*H*-indol-5-yloxy)pyrrolo-[2,1-*f*][1,2,4]triazine-based inhibitors of vascular endothelial growth factor receptor-2 kinase is reported. Structure−activity relationship studies revealed that a methyl group at the 5-position and a substituted alkoxy group at the 6-position of the pyrrolo[2,1-*f*][1,2,4]triazine core gave potent compounds. Biochemical potency, kinase selectivity, and pharmacokinetics of the series were optimized and in vitro safety liabilities were minimized to afford BMS-540215 (**12**), which demonstrated robust preclinical in vivo activity in human tumor xenograft models. The L-alanine prodrug of **12**, BMS-582664 (**21**), is currently under evaluation in clinical trials for the treatment of solid tumors.

Inhibition of tumor angiogenesis as a means of tumor growth arrest has been an area of intense research interest over the past decade.[1] Vascular endothelial growth factor (VEGF) isoforms and their cognate tyrosine kinase receptors (VEGFRs) have been especially attractive targets for the inhibition of angiogenesis.[2,3] VEGFR-2 is of particular interest because it is the principal kinase involved in multiple processes of angiogenesis, including vascular permeability, endothelial cell (EC) proliferation, migration, and survival.[4] As a result, VEGFR-2 has become a compelling target for small molecule kinase inhibitors.[5] Recently, clinical validation of the VEGF signaling pathway has been provided by the demonstration of an overall survival benefit in colorectal cancer patients treated with bevacizumab, a monoclonal antibody to the ligand VEGF-A, in combination with standard regimens (IFL or Saltz).[6] Many small molecule inhibitors of the VEGFR-2 kinase domain have shown promising results in preclinical studies and are currently under clinical evaluation.[7] Herein, we describe the identification of a selective inhibitor of VEGFR-2 kinase with robust preclinical in vivo activity and a safety profile that is suitable for chronic dosing.

Recently, we reported that phenol-substituted pyrrolo[2,1-*f*]-[1,2,4]triazines afforded potent inhibitors of VEGFR-2 kinase but lacked good oral bioavailability.[8] Our search for compounds with better pharmacokinetic properties led to the discovery of 4-(4-fluoro-1*H*-indol-5-yloxy)pyrrolo[2,1-*f*][1,2,4]triazines shown in Table 1. This report describes the synthesis, structure−activity relationships, and antitumor activity of this indole-based series of compounds. Initial structure−activity relationship studies (SAR) in the series were investigated using analogues with an ester group at the 6-position. Similar to earlier observations,[8] incorporation of a methyl group at the 5-position (R^5), as in compound **2**, gave optimal potency against the VEGFR-2 enzyme. Although the introduction of a methyl group at the

Table 1. SAR at the R^5, $R^{2'}$, and $R^{4'}$ Positions of 4-(1*H*-Indol-5-yloxy)pyrrolo[2,1-*f*][1,2,4]triazine[a]



compd	R^1	$R^{2'}$	$R^{4'}$	R^5	VEGFR-2, IC_{50}, μM
1	Me	H	H	H	0.21[b]
2	Et	H	H	Me	0.087
3	Et	H	H	Et	0.31
4	Et	H	H	*i*-Pr	0.47
5	Me	Me	H	Me	0.078
6	Me	Me	F	Me	0.017

[a] IC_{50} values are reported as the mean of at least three individual determinations. Variability around the mean value was <50%. [b] value reported from one experiment.

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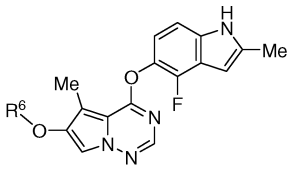
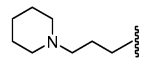
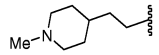
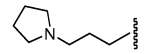
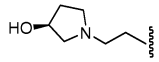
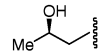
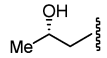
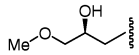
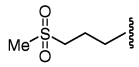
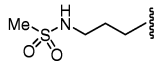
Table 2. SAR at R^6 Linker



compd	R^6	VEGFR-2,[a] IC_{50} (μM)	mouse 4 hour exposure[b] AUC (μM*hr)	mouse 4 hour exposure[b] Cmax, (μM)	CYP3A4,[c] IC_{50} (μM)	hERG[e] inhibition
7	H	0.024	0.2	0.2	ND[d]	ND
8		0.017	14.0	4.2	1.0	70% @ 1μM
9		0.020	2.2	0.7	0.7	ND
10		0.020	20.5	5.9	3.4	66% @ 1μM
11		0.024	9.9	3.0	10.0	38% @ 1μM
12		0.025	136.0	41.0	18.0	IC_{50} = 18 μM
13		0.040	146.0	56.0	4.0	ND
14		0.042	128.0	41.0	1.0	IC_{50} = 18 μM
15		0.070	185.0	57.0	17.0	IC_{50} = 20 μM
16		0.066	96.0	27.0	1.0	ND

[a] IC_{50} values are reported as the mean of at least three individual determinations or as individual IC_{50} values in the case of less than three measurements. Variability around the mean value was <50%. For assay conditions see ref 9. [b] Compounds were evaluated in 4 h exposure study in mice at 50 mg/kg and formulated as solutions in 7:1:2 poly(ethylene glycol)400:ethanol:water. [c] For CYP inhibition assay details see ref 9. [d] ND, data not determined; [e] See Supporting Information.

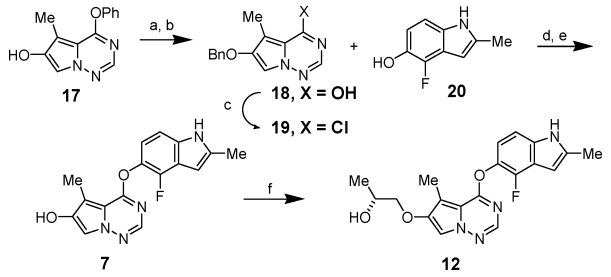
2-position of the indole ring ($R^{2'}$) did not affect the potency against VEGFR-2 (**2**, **5**), introduction of a fluorine group at the 4-position of indole ring ($R^{4'}$) gave a 4-fold increase in potency against VEGFR-2 (**6**).

On the basis of these data, we chose to continue our SAR studies using analogues with methyl groups at R^5 and $R^{2'}$ and with fluorine at $R^{4'}$. It was observed that the compounds with a 6-hydroxy substituent showed good enzyme potency. Therefore, our subsequent SAR studies were conducted using analogues with a 6-position ether. The ester at the 6-position was converted to a hydroxyl group (**7**) via a reduction, oxidation, and Baeyer−Villiger rearrangement sequence.[8] Although phenol **7** was a potent kinase inhibitor, it showed poor plasma exposure upon oral administration. Therefore, the phenol was alkylated with different groups to afford the series of 6-position ethers described in Table 2. Molecular modeling suggested that these compounds would bind in the ATP binding site[8,9] and that the linkers at the 6-position would be solvent exposed. Consistent with this hypothesis, substitution at R^6 affected kinase activity only marginally, but allowed modulation of aqueous solubility, pharmacokinetic properties, and the in vitro safety profile. Compounds containing an amine, alcohol, or ether group on the R^6 tether (Table 2) were potent against the VEGFR-2 enzyme, whereas analogues containing a sulfone (**15**) or a sulfonamide (**16**) exhibited moderate potency. In 4 h oral exposure studies in mice, amines generally had modest in vivo exposures (**8**−**11**), whereas compounds with an alcohol, sulfone, or sulfonamide group (**12** to **16**) showed high oral exposures.

Compounds in Table 2 were further differentiated by their activity in hERG patch-clamp and CYP3A4 liability assays. Compounds with an amino group on the side chain (**8**, **10**) exhibited potent hERG ion channel activity and CYP3A4 inhibition, which was reduced in the 3-hydroxypyrrolidine derivative **11**. Compounds without an amino group on the side chain (**12**, **14**, and **15**) possessed reduced potency against the hERG ion channel and a range of activity against a panel of cytochrome P450 (CYP) isozymes.[11] Among the compounds tested, **11**, **12**, and **15** showed reduced inhibition of CYP3A4 activity compared to compounds **13** and **14**. On the basis of its superior enzyme potency against VEGFR-2, acceptable plasma protein binding (human 98.1%), and good exposure profile in the mouse, compound **12** was chosen for additional kinase selectivity, pharmacokinetic, and in vivo efficacy studies.

The reaction sequence for the preparation of **12** is detailed in Scheme 1. The 6-position phenol **17**[8] was first protected as a benzyl ether by treatment with benzyl bromide and K_2CO_3 in acetonitrile. The resulting intermediate was heated in aqueous HCl at 80 °C to remove the phenyl ether protecting group at the 4-position to obtain **18** in 64% yield over two steps. The 4-oxo group of **18** was converted to chloroimidate **19** by treatment with phosphorus oxychloride at 110 °C. Reaction of 4-fluoro-5-hydroxy-2-methylindole[10] **20** with crude **19** in DMF in the presence of NaH and hydrogenolysis of the resulting 6-benzylated intermediate with ammonium formate and 10% palladium on carbon afforded the key compound **7** in 76% overall yield. Treatment of **7** with (*R*)-(+)-propylene oxide in ethanol at 70 °C in the presence of triethylamine afforded **12** with high optical purity (99.5% ee).

Scheme 1. Synthesis of **12**[a]



[a] Reagents and reaction conditions: (a) benzyl bromide, K_2CO_3, 70 °C; (b) 1 N HCl, 80 °C, 64%, two steps; (c) $POCl_3$, 110 °C; (d) DMF, NaH, 76%, two steps; (e) 10% Pd/C, $HCOONH_4$, DMF, 86%; (f) (*R*)-(+)-propylene oxide, Et_3N, EtOH, 70 °C, 53%.

Table 3. Kinase Selectivity Profile of **12**

enzyme	IC_{50} (nM)	K_i (nM)
VEGFR-2 (human)	25	26
Flk-1 (mouse)	89	28
VEGFR-1	380	60
FGFR-1	148	
PDGFR-β	>6000	
EGFR	>1900	
LCK	>2500	
PKCα	>25000	
JAK-3	>50000	

Table 4. Parameters for**12** in a 24 Hour PK Study in Mice[a]

dose, mg/kg	AUC_{tot}, μM·h	$t_{1/2}$, h	MRT, h	Cl, mL/min/kg	V_{ss}, L/kg	F_{po}, %
iv, 10	49	2.7	3.6	9.3	2.0	
po, 60	224	3.6				88

[a] Data taken from an average of three animals. For assay details see ref 9.

The enzyme kinetics and kinase selectivity profile of **12** were examined (Table 3). Compound **12** was found to be an ATP competitive inhibitor of human VEGFR-2, with a K_i of 26 nM. It had moderate potency against two of three other kinases implicated in angiogenesis, VEGFR-1 and FGFR-1, but it exhibited good selectivity against PDGFR-β.[11] Since compound **12** targets the mouse endothelial cells rather than the human tumor cells in the preclinical in vivo mouse models, it was also tested and found to be good inhibitor of Flk-1, the mouse homologue of VEGFR-2. Compound **12** showed good selectivity against a variety of other receptor and nonreceptor kinases as shown in Table 3. It should be noted that AZD2171[7f] which also contains fluoroindole group **20** on a quinazoline nucleus, exhibits potent activity against PDGFR-β and c-Kit kinases.

Inhibition of proliferation of human umbilical vein endothelial cells (HUVEC) by **12** was investigated. Following stimulation by growth factors VEGF-A or FGF, the inhibition of proliferation of HUVEC cells was measured. A good correlation was observed between cellular potency and activity against the isolated enzymes with potency against VEGF-stimulated HUVECs (40 nM) being higher than against FGF-stimulated HUVECs (276 nM).[11] When tested against a panel of human tumor cell lines, **12** showed lower antiproliferative potency (IC_{50} > 2 μM). In particular, activity was low against the cell line used in the in vivo tumor xenograft mouse efficacy model (human breast carcinoma H3396), supporting the concept that the efficacy of the compound is driven by its antiangiogenic properties.

In a mouse (male Balb-c) pharmacokinetic study[11] (Table 4) **12** was administered at 60 mg/kg orally as a solution in PEG400:Tween80 (75:25) and at 10 mg/kg intravenously in PEG400:water (3:2). The compound was rapidly absorbed with T_{max} of 1 h, a favorable half-life ($t_{1/2}$) of 2.7 h, and mean residence time (MRT) of 3.6 h. The measured oral bioavailability (F_{po}) in this experiment was 88%. The steady-state volume of distribution (V_{ss}) was high, indicating extensive extravascular distribution, and systemic clearance (Cl) was much lower than the hepatic blood flow.

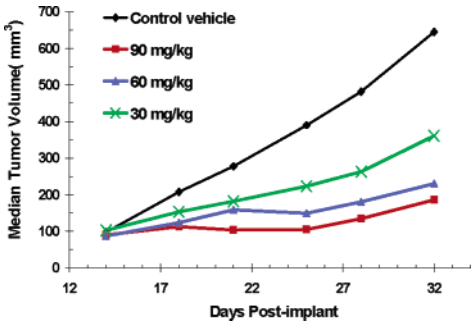


Figure 1. In vivo antitumor activity of **12** versus H3396 xenografts in athymic mice with dosing once a day for 10 days from day 14 to day 23 postimplant.

Table 5. Rat 24 Hour PK Study of **12**[a]

	solution	micronized suspension		
dose (mg/kg)	25	25	100	200
C_{max} (μM)	6.4	0.55	2.05	1.71
AUC_{tot} (μM·h)	13.4	3.35	8.43	7.61

[a] Vehicle for solution study was PEG400:ethanol:water (7:1:2) and for suspension study was water with 0.1% tween 80.

The in vivo efficacy of **12** was evaluated against H3396 xenografts in athymic mice (Figure 1). Once daily oral administration of **12** as a solution in PEG 400:water (7:3) for 10 days (days 14 to 23 post implant) inhibited the growth of established tumors (staged to 80−120 mm^3 size) in a dose dependent manner. At 60 and 90 mg/kg doses, nearly complete inhibition of growth was observed throughout the dosing period, with tumor growth inhibition[11] of 85% and 97%, respectively. No overt toxicity as measured by weight loss and morbidity was observed in either cohort of animals, suggesting good safety.

Solid dosage formulations of compound **12** were assessed in the rat to support advancement of this compound as a development candidate. Rats were dosed with micronized **12** as a suspension in water to mimic the solid dosage formulation which would be used in the clinic. At 25 mg/kg, micronized suspensions afforded significantly lower systemic exposures than those obtained from solution formulations (Table 5). Additionally, drug exposure did not increase proportionally with dose, suggesting that nonlinear pharmacokinetics in humans could present a development issue.

Low aqueous solubility at equilibrium (<1 μg/mL at pH 6.5) and low pK_a (2.8) were believed to be contributing to the observed poor absorption from solid dosage form. Prodrug formation was recognized as an effective means to improve the pharmaceutic and pharmacokinetic properties of this otherwise attractive development candidate. The successful outcome of this strategy will be reported on shortly in a subsequent manuscript, which will describe the prodrug **21** (Figure 2) which is currently in clinical development.

In summary, a novel series of 4-(4-fluoro-1*H*-indol-5-yloxy)-pyrrolo[2,1-*f*][1,2,4]triazines with potent VEGFR-2 activity was identified. Structure−activity relationship studies led to the discovery of (*R*)-1-(4-(4-fluoro-2-methyl-1*H*-indol-5-yloxy)-5-methylpyrrolo[2,1-*f*][1,2,4]triazin-6-yloxy)propan-2-ol (**12**) as

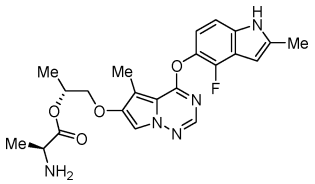


Figure 2. Structure of **21**.

an orally active compound in the H3396 xenograft model of human breast carcinoma, demonstrating almost complete tumor growth stasis during the dosing period. On the basis of its excellent enzymatic potency against VEGFR-2, good kinase selectivity profile, and acceptable safety profile, compound **12** was considered as a candidate for further development and its prodrug formation was considered to improve pharmaceutics properties.

Acknowledgment. We thank George Trainor and Robert Kramer for scientific advice, and the Discovery analytical sciences for compound characterization.

Supporting Information Available: Characterization data for compounds **1**−**16**. Full experimental details and the characterization data for compound **12**. This material is available free of charge on the web at http://pubs.acs.org.

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