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Synthesis, SAR, and Evaluation of 4-[2,4-Difluoro-5-(cyclopropylcarbamoyl)phenylamino]pyrrolo [2,1-*f*][1,2,4]triazine-based VEGFR-2 kinase inhibitors

Zhen-wei Cai,* Donna Wei, Robert M. Borzilleri, Ligang Qian, Amrita Kamath, Steven Mortillo, Barri Wautlet, Benjamin J. Henley, Robert Jeyaseelan, Sr., John Tokarski, John T. Hunt, Rajeev S. Bhide, Joseph Fargnoli and Louis J. Lombardo

Bristol-Myers Squibb Research and Development, PO Box 4000, Princeton, NJ 08543-4000, USA

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Abstract—Introduction of the 2,4-difluoro-5-(cyclopropylcarbamoyl)phenylamino group at the C-4 position of the pyrrolo[2,1-*f*][1,2,4] triazine scaffold led to the discovery of a novel sub-series of inhibitors of VEGFR-2 kinase activity. Subsequent SAR studies on the 1,3,5-oxadiazole ring appended to the C-6 position of this new sub-family of pyrrolotriazines resulted in the identification of low nanomolar inhibitors of VEGFR-2. Antitumor efficacy was observed with compound **37** against L2987 human lung carcinoma xenografts in athymic mice.

Vascular endothelial growth factor receptor-2 (VEGFR-2) is a protein tyrosine kinase that drives angiogenesis, a process critical for tumor growth and metastasis.[1–3] Although angiogenesis is a complex and highly regulated process, and a substantial number of growth factors and cytokines have been identified in recent years that activate and maintain angiogenesis throughout tumorigenesis,[4,5] VEGFR-2 is the most dominant player. The signaling pathways triggered by binding of the VEGF ligand to its cognate receptor VEGFR-2 have been implicated in multiple steps of angiogenesis, including endothelial cell proliferation, survival, migration, differentiation, and vascular permeability.[6,7] Therefore, inhibition of VEGFR-2 kinase activity and subsequent interruption of VEGFR-2 downstream signaling pathways have been shown to be a very attractive strategy for inhibition of solid tumor progression.[8–10]

Recently, we described a class of hydroxamate-based pyrrolo[2,1-*f*][1,2,4]triazines **1**[11] and a series of aminothiazole-based derivatives **2**[12] as potent inhibitors of VEGFR-2 kinase activity (Fig. 1). In an effort to further expand the SAR of the pyrrolotriazine family of VEGFR-2 inhibitors, we postulated that the hydroxamate moiety could be replaced with *N*-cyclopropyl amide. The *N*-cyclopropyl amide and hydroxamate groups were believed to be engaged in similar interactions in specific region of ATP binding pocket.[12] Herein, we report subsequent SAR studies for this new sub-family of pyrrolotriazines **3** (Fig. 1) as VEGFR-2 kinase inhibitors, including further exploration of the substitutions (R) on the 1,3,4-oxadiazole ring.

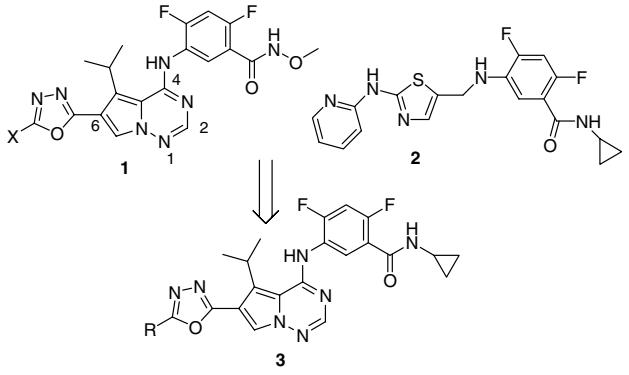

Figure 1. Structures of VEGFR-2 inhibitors.

Keywords: VEGFR-2; Kinase inhibitors; Pyrrolo[2,1-*f*][1,2,4]triazine.
* Corresponding author. Tel.: +1 609 252 5627; fax: +1 609 252 6601; e-mail: zhenwei.cai@bms.com

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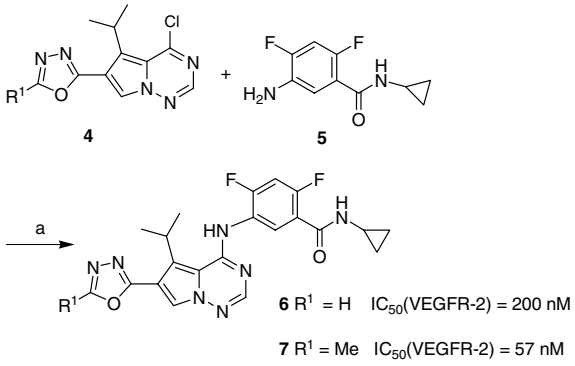
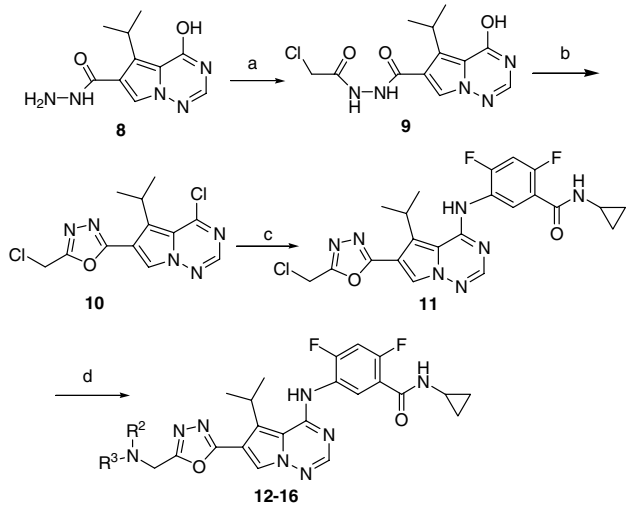
The *N*-cyclopropyl amide substituted pyrrolotriazine analogs **6** and **7** (Scheme 1) were prepared in excellent yields by condensation of chloroimidate **4**[11] with aniline **5**[12] in refluxing acetonitrile. Replacement of the methyl hydroxamate with *N*-cyclopropyl amide was well tolerated on the pyrrolotriazine scaffold. Compound **6** demonstrated moderate VEGFR-2 inhibitory activity (IC_{50} = 200 nM), while compound **7**, with methyl substitution on oxadiazole ring, exhibited almost a 4-fold increase in potency as compared to the unsubstituted compound **6**. Previous computer-assisted molecular modeling of **1** in the ATP binding site of VEGFR-2 kinase domain showed that methyl group on oxadiazole ring extended toward the protein surface.[11] Thus, terminal polar groups such as basic amino substituents were appended to oxadiazole ring to gain favorable interactions with aqueous environment and increase the aqueous solubility of the compounds, as shown in Scheme 2.

The synthesis of compounds **12**–**16** was accomplished by treatment of hydrazide **8**[11] with 2-chloroacetyl chloride and pyridine to furnish intermediate **9**. Heating of **9** in $POCl_3$ resulted in simultaneous oxadiazole ring closure as well as chloroimidate formation to afford **10**. Intermediate **11** was obtained from coupling of **10** with aniline **5** using similar chemistry as described for the preparation of compound **6**. Final compounds were prepared by displacement of chloride **11** with a set of amines R^2R^3NH (Table 1) under thermal conditions.



Scheme 1. Reagent and condition: (a) acetonitrile, reflux, 90%.

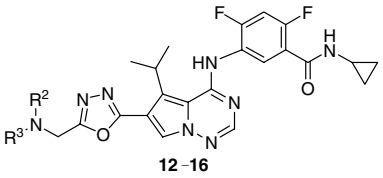


Scheme 2. Reagents and conditions: (a) chloroacetyl chloride, pyridine, THF/DMF (1:1); (b) neat $POCl_3$, 120 °C; (c) **5**, acetonitrile, reflux, 15% over 3 steps; (d) R^2R^3NH, dioxane, 40–50 °C, 45–70%.

The VEGFR-2 enzymatic activities of carbon-tethered oxadiazole compounds **12**–**16** are displayed in Table 1. Introducing structurally diverse amino substituents on the methyl group of compound **7** did not significantly influence the intrinsic activity and were approximately 2- to 4-fold less potent than the parent compound **7** in VEGFR-2 kinase assay.

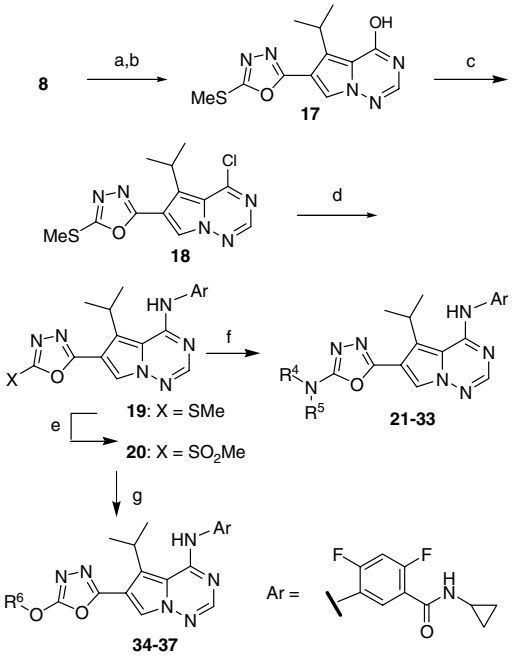
A novel synthetic approach was developed to attach polar group side chains directly to the oxadiazole ring (Scheme 3). The intermediate methyl thioether **17** was

Table 1. SAR of carbon tethered oxadiazole compounds[a]



Compound	NR^2R^3	VEGFR-2 IC_{50} (nM)
12	NH_2	110
13	NHMe	150
14	NMe_2	160
15	Morphlino	230
16	3-Hydroxypyrrolidino	200

[a] For assay conditions see Ref.12.

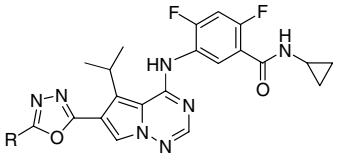


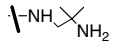
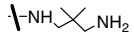
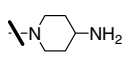
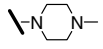
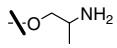
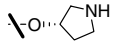
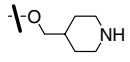
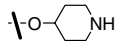
Scheme 3. Reagents and conditions: (a) 1,1′-thiocarbonyldiimidazole, DMF, rt; (b) MeI, rt, 91% over two steps; (c) neat $POCl_3$, 120 °C, 72%; (d) **5**, acetonitrile, reflux, 75%; (e) *m*-CPBA, CH_2Cl_2, 73%; (f) R^4R^5NH, dioxane, 70–100 °C, 45–90%; (g) R^6OH, NaH, THF, 65–85%.

prepared from treatment of hydrazide intermediate **8** with thiocarbonyldiimidazole, followed by alkylation of thiol intermediate using methyl iodide in a one pot reaction. Intermediate **17** was heated with $POCl_3$ to furnish chloroimidate **18**. The synthesis of intermediate **19** was carried out in a similar manner as outlined in Scheme 1. Oxidation of the thioether was accomplished upon treatment of *m*-CPBA to provide sulfone **20**, which provided an easy access for incorporating different tethered side chains by displacement of sulfone group with appropriate amines and alcohols (Table 2). The *N*-tethered oxadiazole analogs **21**–**33** were readily prepared by treatment of sulfone **20** with corresponding amines (R^4R^5NH) in dioxane at 70–100 °C. The *O*-tethered oxadiazole analogs were obtained in good yields by deprotonation of the alcohol (R^6OH) with NaH and treating resulting alkoxides with sulfone **20**. The protection of reagents containing an N—H group was not required during the synthesis of **34**–**37**,[13] and excellent regioselectivity was achieved in all cases.

The *N*- and *O*-tethered oxadiazole compounds **21**–**37** (Table 2) were initially screened for the inhibition of VEGFR-2 activity and human cytochrome CYP P450 isozyme activity, with an emphasis on inhibition of CYP3A4 activity. Analogs with potent inhibition of VEGFR-2 activity ($IC_{50} < 50$ nM) and weak inhibition of CYP3A4 activity ($IC_{50} > 4$ μM) were subsequently evaluated in a VEGF-stimulated HUVEC proliferation assay. The *N*-tethered analogs **21**–**24** with small alkyl side chains showed potent inhibition against VEGFR-2 activity and were 2- to 3- more potent than the methyl analog **7**. However, these compounds demonstrated significant inhibition of the CYP3A4 enzyme. The *N*-tethered hydroxyethyl analog **25** provided good VEGFR-2 inhibition, displayed moderate cellular potency in HUVEC proliferation assay, and reduced CYP3A4 activity as compared to **21**–**24**. Compounds **26** and **34** containing the acyclic amino side chains in which amino group being two carbon atoms away from *N*- or *O*-oxadiazole core were modestly potent VEGFR-2 inhibitors and displayed weak interactions with CYP3A4 isozyme. Introduction of heterocyclic amine side chain to the *N*- or *O*-oxadiazole core as shown in compounds **33**and**35** had a dramatic impact on the VEGFR-2 kinase activity, providing IC_{50} value of 21 and 36 nM,

Table 2. SAR of selected *N*- and *O*-tethered oxadiazole compounds[a]



Compound	R	IC_{50} (nM)		
		VEGFR-2	CYP3A4	HUVEC
21	NHMe	20	2100	nd[c]
22	NMe_2	38	800	nd
23	NH ^{n}Pr	17	400	nd
24	NH ^{i}Pr	21	400	nd
25	$NHCH_2CH_2OH$	32	5200	140
26		220	>40,000	nd
27		20	1400	nd
28[b]	$NHCH_2CH_2CH_2NH_2$	17	>40,000	110
29[b]	$NHCH_2CH_2CH_2NHMe$	16	>40,000	62
30	$NHCH_2CH_2CH_2NMe_2$	12	29,000	12
31[b]	NH^nBuNH_2	14	25,000	17
32[b]		10	300	nd
33		21	200	nd
34		300	>40,000	nd
35		36	4800	35
36		26	7600	25
37		11	5000	23

[a] For assay conditions see Ref.12.

[b] The final products were prepared by deprotection of the corresponding *tert*-butyloxycarbonyl (Boc) in 30% trifluoroacetic acid (TFA) in dichloromethane.

[c] nd, not determined.

respectively. Moreover, the ring constrained analog **35** was almost 8-fold more potent than conformation flexible analog **34** although the terminal amino groups in both compounds were two carbon atoms away from the *N*- or *O*-oxadiazole core. Compound **35** also displayed good cellular potency in HUVEC proliferation assay.

The preference of amino group being three carbons or more away from the *N*- or *O*-oxadiazole core with respect to activity was observed as in compounds **27**–**32** (with acyclic amino side chains), and **36**–**37** (with heterocyclic amine side chains), regardless of conformational nature of the amino group. All analogs demonstrated potent inhibitory activity in VEGFR-2 kinase assay as well as in cell proliferation assay except compounds **28** and **29**, which were moderate VEGFR-2 inhibitors in the cellular assay. Compounds **27** (branched alkyl amine side chain), **32** and **33** possessed strong inhibition with CYP3A4, while compounds with straight and long terminal side chains as **28**–**31** tended to be weak CYP3A4 inhibitors. The *O*-tethered oxadiazole analogs **36** and **37** exhibited moderate activity against CYP3A4.

Compounds that exhibited potent inhibition of HUVEC proliferation and a favorable CYP inhibition profile[14] were screened for in vitro metabolic stability studies using human (HLM) and mouse (MLM) liver microsomes (Table 3). Compounds **30**, **31**, and **37** demonstrated low metabolic rates in both HLM and MLM. Therefore, they were further evaluated in mouse oral exposure studies. After administration of a 50 mg/kg dose, **30** demonstrated moderate exposure levels of drug in plasma, and **31**provided extremely low oral adsorption, whereas **37** achieved high systemic exposure of drug with an AUC(0–4 h) of 53.2 μM h. On the basis of superior oral exposure profile of **37** in mouse, it was selected for evaluation in an in vivo efficacy study.

The in vivo anti-tumor activity of **37** was determined in a L2987 human lung carcinoma xenograft model in athymic mice. Following once daily oral administration of **37** at two doses for 14 days (in Fig. 2), tumor growth inhibition (%TGI) of 66% was achieved at high dose of 90 mg/kg. No adverse events were observed even at all doses. Compound **37** was not efficacious at a low dose of 30 mg/kg.

Table 3. Metabolic stability and mouse exposure data for **30**, **31**, and **37**

Compound	HLM/ MLM[a] nmol/min/mg protein	Mouse oral exposure[b,c]		
		C_{max} (μM)	T_{max} (h)	AUC (0–4 h) μM h
30	0.098/ 0.020	8.0	1.0	21.7
31	0.012/ 0.000	0.1	0.5	0.2
37	0.023/ 0.007	15.0	4.0	53.2

[a] Compounds at 3 μM concentration incubated in 10 mg of protein (HLM or MLM) for 10 min.
[b] Dosed at 50 mg/kg, all values are means of three mice.
[c] Vehicle: PEG400:water (1:1).

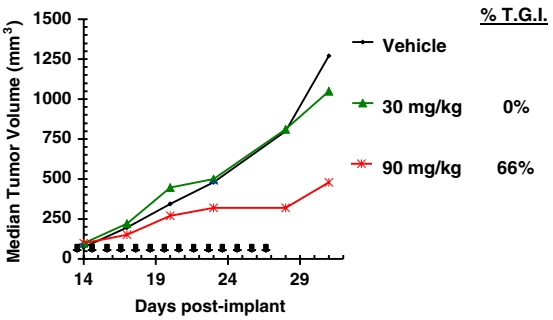


Figure 2. Antitumor activity of **37** versus L2987 human non-small cell lung carcinoma xenografts implanted in athymic mice. Arrows indicate dosing.

In summary, a series of *N*-cyclopropylamides was identified as a replacement for methylhydroxamate-substituted pyrrolotriazine VEGFR-2 kinase inhibitors. SAR studies of substituents on 1,3,4-oxadiazole provided a series of compounds with potent enzymatic and VEGF-stimulated HUVEC cellular inhibitory activity against VEGFR-2. Among them, compound **37** also demonstrated in vivo antitumor activity versus L2987 human lung carcinoma xenografts inplanted in athymic mice.

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13. Representative characterization data for compound **37**: *N*-cyclopropyl-2,4-difluoro-5-(5-isopropyl-6-(5-(piperidin-

4-yloxy)-1,3, 4-oxadiazol-2-yl)pyrrolo[2,1-*f*][1,2,4]triazin-4-ylamino)benzamide: HPLC R_t = 2.83 min, purity = 97.5% (Conditions: YMC ODS-A S5 4.6 × 50 mm column, 4 min gradient from 10% to 90% aqueous MeOH with 0.1% TFA); ^{1}H NMR (400 MHz, $CDCl_3$)δ 9.24 (t, 1H, J = 8.80 Hz), 8.04 (s, 1H), 7.99 (s, 1H), 7.48 (s, 1H), 7.02 (t, 1H, J = 10.52 Hz), 6.74 (d, 1H, J = 10.32 Hz), 5.02–5.15 (m, 1H), 4.05–4.25 (m, 1H), 3.10–3.25 (m, 2H), 2.90–3.00 (m, 1H), 2.70–2.85 (m, 2H), 2.20–2.30 (m, 2H), 1.80–1.95 (m, 2H), 1.56 (d, 6H, J = 7.36 Hz), 0.80–0.95 (m, 2H), 0.60–0.70 (m, 2H); MS (ESI$^+$) m/z 539.31 $(M + H)^+$.

14. Inhibition of human CYP isozymes by compounds **30**, **31**, and **37**

Compound	IC_{50} for CYP inhibition (μM)					
	1A2	2C9	2C19	2D6	3A4 (BFC)	3A4 (BzRes)
30	>40	39	>40	>40	37	29
31	>40	21	23	>40	27	25
37	>40	>40	38	>40	10	5