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4-Aminopyrimidine-5-carbaldehyde oximes as potent VEGFR-2 inhibitors. Part II

Shenlin Huang *, Ronghua Li, Kenneth R. LaMontagne, Lee M. Greenberger, Peter J. Connolly

Johnson & Johnson Pharmaceutical Research & Development L.L.C., 1000 Route 202, Raritan, NJ 08869, USA

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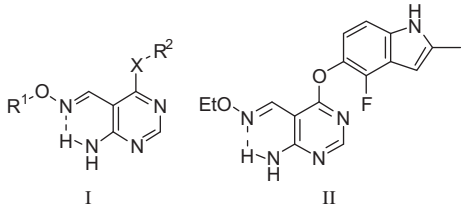
ABSTRACT

A series of 4-aminopyrimidine-5-carbaldehyde oxime was discovered to have potent VEGFR-2 inhibitory activity. Described here are the chemistry for analogue synthesis and SAR study results. The PK properties, kinase profiling, and in vivo efficacy study for compound **4b** are also discussed.

Angiogenesis, the formation of new capillaries from the endothelium of an existing vascular network, plays a crucial role in tumor growth. Solid tumors cannot grow beyond several cubic millimeters until they establish a blood supply because cells must be within 100–200 μm of a blood vessel to survive.[1] Many factors have been shown to affect endothelial cell function resulting in angiogenesis in vivo, including FGF (fibroblast growth factor), angiopoietins, PDGF (platelet-derived growth factor), EGF (epidermal growth factor), and VEGF (vascular endothelial growth factor). Of these factors, VEGF is a major initiator of angiogenesis.[2] It acts as a potent proangiogenic factor by promoting endothelial sprouting, migration, proliferation, and survival both in vitro and in vivo.[3] These effects are primarily mediated by the receptor tyrosine kinase, VEGF receptor 2 (VEGFR-2).[4] There has been a tremendous interest in pharmacologically targeting VEGF and VEGFR-2 in oncology.[5] Inhibition of VEGF or VEGFR-2 kinase activity has been shown to suppress tumor angiogenesis and tumor growth in various models. This research has led to the development of an FDA-approved anti-VEGF antibody, bevacizumab (Avastin),[6] as well as three small molecule inhibitors of VEGFR-2 kinase, sorefenib (BAY-43-9006),[7] sunitinib (Su-11248)[8] and pazopanib.[9] Numerous other small molecule targeting agents and monoclonal antibodies are currently in clinical development with the intention of inhibiting this critical pathway in angiogenesis.[10,11]

In our research efforts to develop small molecule kinase inhibitors for cancer therapy, a novel series of 4-aminopyrimidine-5-carbaldehyde oxime was discovered to be potent not only for VEGFR-2 but also for several other kinase targets.[12] This scaffold (**I**, shown in Fig. 1) is considered as an open form of quinazoline since there is a *pseudo* six-membered ring formed via the intramolecular H-bond. We previously reported a related subseries of antiproliferative VEGFR-2 inhibitors, exemplified by compound **II**. Here a new subseries of VEGFR-2 inhibitors with different properties is described.[13]



I II

Figure 1. 4-Aminopyrimidine-5-carbaldehyde.

In an effort to modulate biological properties of **II**, monocyclic alternatives to the indol-5-yloxy group were investigated, particularly N-substituted 1-amino-4-phenoxy. Initial SAR focused on urea derivatives where the methyl oxime was maintained and 2-chloro-4-phenoxy was used as a starting point. Our previous work showed that *O*-alkyl oximes gave optimal potency. It was also reported by Kubo et al.[14] that a Cl atom at the 2-position of the phenyl ring was beneficial for VEGFR-2 potency. The chemistry is shown in Scheme 1. Treating 4-amino-6-chloropyrimidine-5-carbaldehyde[15] (**1**) with methoxyamine hydrochloride in acetic acid generated oxime **2**. Displacing Cl of compound **2** with 4-amino-3-chlorophenol in DMSO in the presence of Cs_2CO_3 provided intermediate **3**, which was then reacted with various isocyanates to afford urea analogues. Alternatively, intermediate **3** was reacted with 4-nitrophenyl

* Corresponding author. Tel.: +1 858 332 4684; fax: +1 858 812 1684.
E-mail address: shenlinhuang@hotmail.com (S. Huang).

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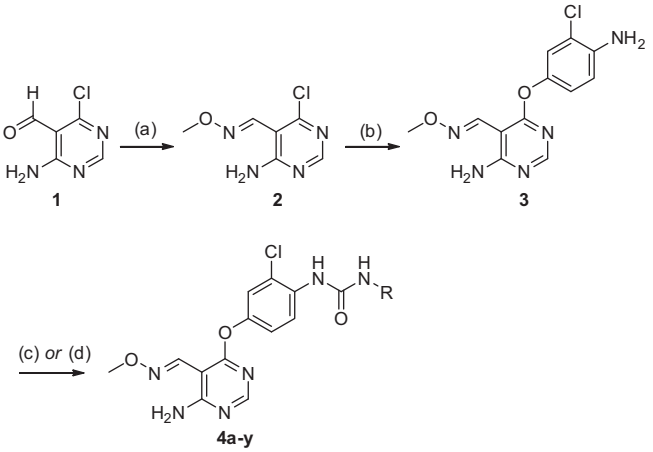


Scheme 1. Synthesis of compounds **4a**–**y**. Reagents and conditions: (a) methoxyamine hydrochloride, acetic acid, 95%; (b) 4-amino-3-chlorophenol, Cs_2CO_3, DMSO, 94%; (c) RNCO, CH_2Cl_2, 60–90%; (d) 4-$NO_2C_6H_4OC(O)Cl$, THF; then RNH_2

chloroformate to give the 4-nitrophenyl carbamate, which was then treated with amines to generate urea compounds.

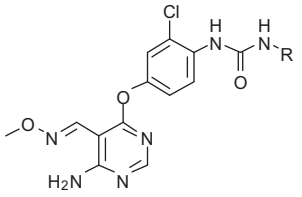
These compounds were tested for VEGFR-2 kinase activity. The results are shown in Table 1. For alkyl ureas, Et group gave higher potency than Me and Pr groups. It was also more potent than *i*-Pr and cyclopropyl groups. A hydrophilic group, for example, hydroxyl or amino, reduced potency. For the three pyridyl isomers, pyridin-2-yl was three-fold less potent than both pyridin-3-yl and pyridin-4-yl. On the other hand, all three five-membered heteroaryls gave similar potencies. An unsubstituted phenyl group was also potent, but adding Cl, F, or MeO to the 2-position of the phenyl ring had little impact on potency. 2-Me, however, led to a slight decrease in potency. Moving 2-F to the 3- or 4-position also barely affected potency. 2,4-Difluorophenyl substitution conferred the same potency as mono fluorophenyl. Therefore, for aryl ureas, high potency with single-digit nM IC_{50}s could be achieved with many variations. This potency was reduced three-fold with insertion of a CH_2 linkage between phenyl ring and urea group. Interestingly, cyclohexyl was only twofold less potent than phenyl.

Next, the urea group was fixed at Me or Et for a follow-up SAR study due to favorable physiochemical properties, for example, lower molecular weight and better solubility. Similar chemistry was used to modify the urea functionality and 2-chlorophenoxy group. The results are shown in Table 2. Changing urea NHC(O)NHEt to amide NHC(O)Me or reversed amide C(O)NHMe resulted in a loss of potency (amide) or complete loss of activity (reversed amide). While replacing urea with carbamate NHC(O)OMe only led to a 10-fold potency loss, the reversed carbamate OC(O)NHEt was inactive. Replacing NH with CH_2 was also highly detrimental to activity. These data demonstrate that the urea functionality is crucial for target inhibition. Replacing 2-Cl on the phenoxy ring with 2-F, 2-Me, 2-Br or H all resulted in potency loss, especially with 2-Br substitution, which led to complete loss of activity. Shifting 2-Cl to the 3-position gave a less potent analogue. As stated above, 2-Cl plays a key role for activity.

To assess kinase selectivity of this series, compound **4b** (JNJ-38158471) was selected for screening against a panel of 184 kinases. At a single concentration of 1 μM with 2 μM of ATP used, only a few kinases including VEGFR-2, FMS, KIT, and RET were inhibited more than 50%. IC_{50} values for these and several other kinases of interest were further determined in a 10-point titration IC_{50} determination assay. The data are shown in Table 3 and reveal that compound **4b** is a highly selective, potent VEGFR-2 inhibitor. It is interesting to note that **4b** did not potently inhibit closely related VEGF receptors (VEGFR-1 = 4.4 μM and VEGFR-3 = 1.1 μM). When tested in vitro in tumor cell proliferation assays, **4b** showed no antiproliferative effects on human cancer cell lines, A375 and HCT116, even at the highest concentration (100 μM) assayed. This is in contrast to the previously reported antiproliferative series.

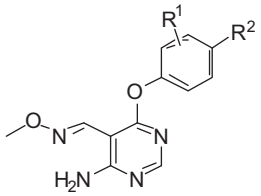
Pharmacokinetic parameters for compound **4b** were measured in both Balb/c mice and Sprague–Dawley rats. As shown in Table 4, **4b** exhibited good bioavailability in rats (30%) though its mouse

Table 1
VEGFR-2 potencies for compounds **4a–y**



Compds	R	VEGFR-2 inhibition IC_{50} (nM)
4a	Methyl	25
4b	Ethyl	11
4c	Propyl	28
4d	Isopropyl	73
4e	Cyclopropyl	29
4f	2-Hydroxyethyl	168
4g	3-Hydroxypropyl	400
4h	*N,N*-Dimethylaminoethyl	285
4i	4-(Pyrrolidin-1-yl)-butyl	759
4j	Pyridin-2-yl	34
4k	Pyridin-3-yl	10
4l	Pyridin-4-yl	11
4m	Thiazol-2-yl	3.4
4n	4,5-Dimethylthiazol-2-yl	2.0
4o	5-Methylisoxazol-3-yl	2.0
4p	Phenyl	3.0
4q	2-Chlorophenyl	2.6
4r	2-Methylphenyl	7.9
4s	2-Methoxyphenyl	2.5
4t	2-Fluorophenyl	3.3
4u	3-Fluorophenyl	1.7
4v	4-Fluorophenyl	2.8
4w	2,4-Difluorophenyl	3.0
4x	2,4-Difluorophenylmethyl	10.3
4y	Cyclohexyl	6.5

Table 2
VEGFR-2 potencies for compounds **5a–j**



Compds	R^1	R^2	VEGFR-2 inhibition IC_{50} (μM)
5a	2-Cl	NHC(O)Me	2.21
5b	H	C(O)NHMe	>100
5c	2-Cl	NHC(O)OMe	0.152
5d	H	OC(O)NHEt	>100
5e	H	$CH_2C(O)NHMe$	19.7
5f	2-F	NHC(O)NHMe	0.142
5g	2-Me	NHC(O)NHEt	0.180
5h	2-Br	NHC(O)NHMe	>100
5i	3-Cl	NHC(O)NHEt	0.179
5j	H	NHC(O)NHEt	0.142

Table 3
Kinase selectivity profile for compound **4b**

Kinase tested	IC_{50} (nM)
CSF1R (FMS)	622
FLT1 (VEGFR-1)	4420
FLT3	4800
FLT4(VEGFR-3)	1100
KDR (VEGFR-2)	42
KIT	513
PDGFRA (PDGFR alpha)	1110
RET	183

Table 4
Pharmacokinetic properties of compound **4b**

Species	Balb/c mouse	Rat
Parameters (IV)		
Dose (mg/kg)	2	2
C_{max} (ng/mL)	2987	5401
AUC (ng h/mL)	6114	4799
V_{ss} (L/kg)	0.7	0.4
$t_{1/2}$ (h)	2.8	0.7
Clearance (mL/min/kg)	5.6	7.4
Parameters (PO)		
Dose (mg/kg)	10	10
C_{max} (ng/mL)	2786	1034
AUC (ng h/mL)	12305	7304
$t_{1/2}$ (h)	2.1	10.0
Oral bioavailability (%)	16	30

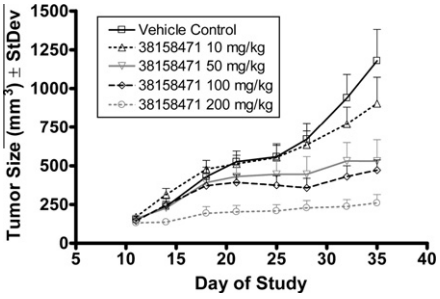


Figure 2. A431 tumor xenograft study.

bioavailability (16%) was less desirable. Compound **4b** showed relatively slow clearance in both mice and rats. The volume of distribution in mice was nearly two-fold higher than in rats. In the mouse PK study, its IV half-life was similar to that for PO dose. On the other hand, its oral half-life in rats was much longer than that for IV dose. This difference could be due to a slow absorption.

The in vivo efficacy of compound **4b** was evaluated in several well-established, diverse human tumor xenograft models in nude mice. The compound was formulated as a suspension in 0.5% methylcellulose and dosed PO once daily. Before daily treatment, tumor cells were implanted and allowed to establish growth for 10–14 days. In the A431 epidermoid carcinoma and HCT116 colorectal carcinoma models, compound **4b** showed dose-dependent antitumor activity (Figs. 2 and 3, respectively). Optimum efficacy with 90% growth inhibition was achieved at doses ranging from 100 to 200 mg/kg daily. The treatment was well tolerated; following continuous dosing for 24 days, animal body weights were comparable with control animals. In the A375 skin melanoma model, statistically significant efficacy was obtained with daily doses of 100 mg/kg of **4b**, a 90% inhibition of growth (Fig. 4). It is more interesting to note that tumors did not rapidly regrow upon discontinuation of treatment in the study. This tumor growth delay

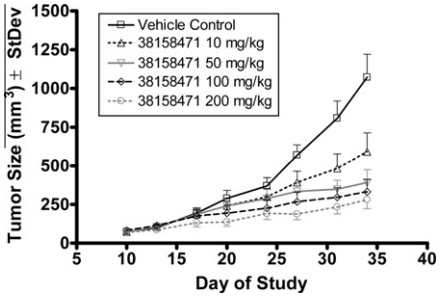


Figure 3. HCT116 tumor xenograft study.

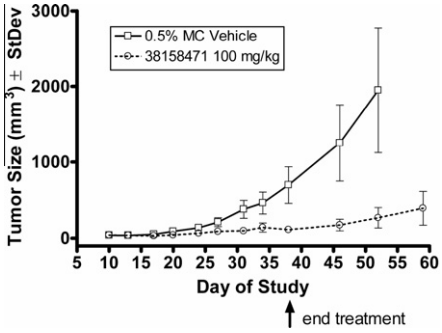


Figure 4. A375 tumor xenograft study.

activity may be correlated with the compound's ability to inhibit the dynamics of tumor blood vessel turnover, similar to SU11248 maintenance therapy.[16]

In summary, compounds belonging to a novel series of 4-aminopyrimide-5-carbaldehyde oxime were discovered to be potent and selective VEGFR-2 inhibitors. An efficient chemistry was developed for analogue synthesis. This series showed reasonable PK properties in both mice and rats. Compound **4b** exhibited activity against a variety of solid tumor types when administered alone. It represents an exciting new angiogenesis inhibitor for clinical development in areas in which dysregulated vascularization occurs, including rheumatoid arthritis, diabetic retinopathy, and tumor growth.

Acknowledgments

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References and notes

1. (a) Carmeliet, P.; Jain, R. K. *Nature* **2000**, *407*, 249; (b) Folkman, J. *Nat. Med.* **1995**, *1*, 27.
2. Ferrara, N.; Gerber, H. P.; LeCouter, J. *Nat. Med.* **2003**, *9*, 669.
3. Ferrara, N. *J. Mol. Med.* **1997**, *77*, 527.
4. Millauer, B.; Wizigmann-Voos, S.; Schnurch, H.; Martinez, R.; Moller, N. P. H.; Risau, W.; Ullrich, A. *Cell* **1993**, *72*, 835.
5. Liu, B.; Earl, H. M.; Baban, D. *Biochem. Biophys. Res. Commun.* **1995**, *217*, 721.
6. Ferrara, N.; Hillan, K. J.; Gerber, H.-P.; Novotny, W. *Nat. Rev. Drug Discovery* **2004**, *3*, 391.
7. Strumberg, D. *Drugs Today* **2005**, *41*, 773.
8. Motzer, R. J.; Michaelson, M. D.; Redman, B. G.; Hudes, G. R.; Wilding, G.; Filglin, R. A.; Ginsberg, M. S.; Kim, S. T.; Baum, C. M.; DePrimo, S. E.; Li, J. Z.; Bello, C. L.; Theuer, C. P.; George, D. J.; Rini, B. I. *J. Clin. Oncol.* **2006**, *24*, 16.
9. Harris, P. A.; Boloor, A.; Cheung, M.; Kumar, R.; Crosby, R. M.; Davis-Ward, R. G.; Epperly, A. H.; Hinkle, K. W.; Hunter, R. N. III; Johnson, J. H.; Knick, V. B.; Laudeman, C. P.; Luttrell, D. K.; Mook, R. A.; Nolte, R. T.; Rudolph, S. K.; Szewczyk, J. R.; Truesdale, A. T.; Veal, J. M.; Wang, L.; Stafford, J. A. *J. Med. Chem.* **2008**, *51*, 4632.

10. (a) Connell, R. D. *Expert Opin. Ther. Patent* **2002**, *12*, 1763; (b) Boyer, S. J. *Curr. Top. Med. Chem.* **2002**, *2*, 973.
11. Traxler, P. *Expert. Opin. Ther. Targets* **2003**, *2*, 215.
12. (a) Huang, S.; Li, R.; Connolly, P. J.; Xu, G.; Gaul, M. D.; Emanuel, S. L.; LaMontagne, K. R.; Greenberger, L. M. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 6063; (b) Gaul, M. D.; Xu, G.; Kirkpatrick, J.; Ott, H.; Baumann, C. A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4861; (c) Xu, G.; Searle, L. L.; Huges, T. V.; Beck, A. K.; Connolly, P. J.; Abad, M. C.; Neeper, M. P.; Struble, G. T.; Springer, B. A.; Emanuel, S. L.; Gruninger, R. H.; Pandy, N.; Adams, M.; Moreno-Mazza, S.; Fuentes-Pesquera, A. R.; Middleton, S. A.; Greenberger, L. M. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3495; (d) Xu, G.; Abad, M. C.; Connolly, P. J.; Neeper, M. P.; Struble, G. T.; Springer, B. A.; Emanuel, S. L.; Pandey, N.; Gruninger, R. H.; Adams, M.; Moreno-Mazza, S.; Fuentes-Pesquera, A. R.; Middleton, S. A. *Bioorg. Med. Chem.* **2008**, *18*, 4615.
13. LaMontagne, K. R.; Butler, J.; Borowski, V. B.; Fuentes-Pesquera, A. R.; Blevitt, J. M.; Huang, S.; Connolly, P. J.; Greenberger, L. M. *Angiogenesis* **2009**, *12*, 287.
14. Kubo, K.; Shimizu, T.; Ohyama, S.; Murooka, H.; Iwai, A.; Nakamura, K.; Hasegawa, K.; Kobayashi, Y.; Takahashi, N.; Takahashi, K.; Kato, S.; Izawa, T.; Isoe, T. *J. Med. Chem.* **2005**, *48*, 1359.
15. Gomtsyan, A.; Didomenico, S.; Lee, C. H.; Matulenko, M. A.; Kim, K.; Kowaluk, E. A.; Wismer, C. T.; Mikusa, J.; Yu, H.; Kohlhaas, K.; Jarvis, M. F.; Bhagwat, S. S. *J. Med. Chem.* **2002**, *45*, 3639.
16. Schueneman, A. J.; Himmelfarb, E.; Geng, L.; Tan, J.; Donnelly, E.; Mendel, D.; McMahon, G.; Hallahan, D. *Cancer Res.* **2003**, *63*, 4009.