Bioorganic & Medicinal Chemistry Letters 20 (2010) 6653–6656

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design, synthesis and biological evaluation of quinoline amide derivatives as novel VEGFR-2 inhibitors

Ying Yang †, Lei Shi †, Yang Zhou, Huan-Qiu Li, Zhen-Wei Zhu, Hai-Liang Zhu *

State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing 210093, People's Republic of China

ARTICLE INFO

Article history:
Received 27 March 2010
Revised 14 August 2010
Accepted 3 September 2010
Available online 11 October 2010

Keywords:
Quinoline amide
SAR
Docking
VEGFR-2 inhibitor

ABSTRACT

Vascular endothelial growth factor receptor-2 (VEGFR-2) plays a crucial role in the process of cancer angiogenesis. A series of quinoline amide derivatives were prepared and found to be good inhibitors of VEGFR-2. The inhibitory activities were investigated against VEGFR-2 kinase and human umbilical vein endothelial cells (HUVEC) in vitro. Compound **6** (5-chloro-2-hydroxy-*N*-(quinolin-8-yl)benzamide) exhibited the most potent inhibitory activity (IC_{50} = 3.8 and 5.5 nM for VEGFR-2 kinase and HUVEC, respectively). Docking simulation supported the initial pharmacophoric hypothesis and suggested a common mode of interaction at the ATP-binding site of VEGFR-2, which demonstrates that compound **6** is a potential agent for cancer therapy deserving further researching.

Angiogenesis, the formation of new blood vessels from existing vasculature, plays a major role in the progression of many human diseases, including cancer, where it is essential for the growth and survival of solid tumors.[1] The growth of new vessels supplies oxygen, nutrients, and growth factors to small tumors and removes the waste products of metabolism. Tumors that lack an adequate vasculature become necrotic or apoptotic and do not grow beyond a limited size. Thus, new means to retard angiogenesis have shown promise as potential cancer therapies and inhibition of the vascular endothelial growth factor (VEGF) signaling pathway has emerged as one of the most promising new approaches for cancer therapy.[2,3]

VEGF is secreted by tumors and induces a mitogenic response through its binding to one of three tyrosine kinase receptors (VEGFR-1–3) on nearby endothelial cells. Thus inhibition of this signaling pathway should block angiogenesis and subsequent tumor growth. There is much evidence that direct inhibition of the kinase activity of VEGFR-2 (also referred to as the kinase insert domain containing receptor (KDR)) will result in the reduction of angiogenesis and the suppression of tumor growth.[4,5] A number of small molecule structural classes, for example, indolin-2-ones,[6] phthalazines,[7] quinolinones,[8] imidazopyridines,[9] benzimidazoles,[10] pyridines,[11] and quinazolines,[12] have been disclosed as potent inhibitors of VEGFR-2 in vitro or have demonstrated antiangiogenic.

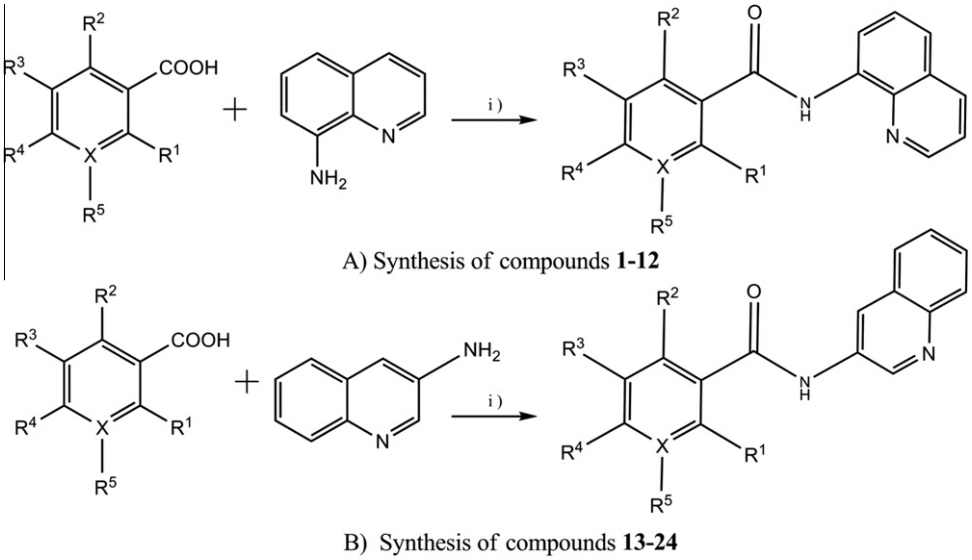
Because of the difficulties associated with the competitive inhibition of protein–protein interactions by small molecular weight compounds, modulating the activity of kinases by interfering either with ligand or substrate binding is difficult and targeting the catalytic site of kinases with ATP-competitive inhibitors is a more promising approach for drug intervention.[13] In view of this, our program has targeted the development of small-molecule inhibitors of VEGFR-2, based on molecular modeling and the investigation of SAR between new inhibitors and VEGFR-2. The designed quinoline amides could be interacting with the catalytic site of VEGFR-2 well. The functional groups of these amides could form hydrogen bonds with the catalytic site of VEGFR-2 as hydrogen donor or acceptor. All synthesized compounds were evaluated for their inhibitory activities against HUVEC and VEGFR-2 kinase.[14] Compound **6** displayed the most potential inhibitory activities against HUVEC and VEGFR-2 kinase. Docking simulations were performed using the X-ray crystallographic structure of the VEGFR-2 to explore the binding modes of compound **6** at the active site.[15,16]

The synthesis of quinoline amide derivatives followed the general reaction pathway outlined in Scheme 1. The compounds **1–24** (Table 1) were synthesized by coupling substituted quinoline amines with equimolar quantities of substituted benzoic acids, salicylic acids and niconacids, respectively, using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl) as condensing agent. The mixture was refluxed in anhydrous CH_2Cl_2 for 8–10 h.

The synthesized quinoline amides were evaluated for their abilities to inhibit the proliferation of HUVEC induced by either VEGF or basic fibroblast growth factor (bFGF) and VEGFR-2 kinase. Stimulation of HUVEC proliferation with bFGF was utilized as a measure of selectivity because this should be unaffected by a VEGFR-2 inhibitor. The effect of kinase inhibitors on cell proliferation was measured using 5-bromo-2-deoxyuridine (BrdU) incorporation method using commercially available kits. HUVEC was

* Corresponding author. Tel./fax: +86 25 8359 2672.
E-mail address: zhuhl@nju.edu.cn (H.-L. Zhu).
† Both authors contributed equally to this work.

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.09.014



Scheme 1. General synthesis of compounds **1**–**24**. Reagents and conditions: R: CH_2Cl_2; *T*: 40 °C; EDC 200 mg; reflux.

Table 1
Chemical structures of compounds **1**–**24**

Compd	R^1	R^2	R^3	R^4	R^5	X
1	H	H	H	H	H	C
2	H	H	H	NO_2	H	C
3	H	H	H	Cl	H	C
4	Cl	H	H	H	H	C
5	H	H	H	H	NO_2	C
6	OH	H	Cl	H	H	C
7	OH	H	H	CH_3	H	C
8	OH	H	H	H	CH_3	C
9	OH	H	Br	H	H	N
10	OH	H	CH_3	H	H	N
11	H	H	H	Cl	H	N
12	H	H	Br	H	H	N
13	H	H	H	H	H	C
14	Br	H	H	H	CH_3	C
15	H	H	Cl	H	CH_3	C
16	OH	H	Br	H	Br	C
17	OH	H	H	H	Cl	C
18	OH	H	Br	H	CH_3	C
19	OH	H	Br	H	CH_3	C
20	Br	H	Cl	H	H	N
21	Cl	H	CH_3	H	H	N
22	H	H	Br	H	H	N
23	H	H	Br	H	CH_3	C
24	OH	H	Cl	OH	H	N

Table 2
Inhibition (IC_{50}/nM) of VEGFR-2 kinase and inhibition (IC_{50}/nM) of HUVEC proliferation

Compd	VEGFR-2[a]	HUVEC[b] (VEGF)	HUVEC[b] (bFGF)
1	134.6	72.4	1160
2	112.7	46.7	450
3	124.6	15.2	220
4	23.6	122.7	1150
5	16.5	15.8	210
6	3.8	5.5	110
7	9.6	19.8	45
8	26.5	26.3	1140
9	5.5	12.9	560
10	13.5	13.6	1150
11	84.9	140.8	560
12	208.8	210.7	346
13	107.2	78.9	68
14	28.4	11.6	660
15	325.6	76.3	560
16	32.4	18.9	240
17	72.8	28.4	340
18	12.4	18.9	470
19	25.5	35.7	780
20	23.4	58.9	1240
21	102.1	77.6	885
22	88.5	59.5	1140
23	112.6	89.6	720
24	32.7	14.5	460

[a] IC_{50} values were averaged values determined by at least two independent experiments.
[b] Human umbilical vein endothelial cells. ND: not determined.

seeded in medium containing 5% fetal bovine serum (FBS) in type 1 collagen coated 96-well plates and incubated overnight at 37 °C, 5% CO_2. The medium was aspirated from the cells, and various concentrations of the inhibitor in serum-free medium were added to each well. After 30 min, either VEGF (10 ng/mL) or bFGF (0.3 ng/mL) was added to the wells. Cells were incubated for an additional 72 h and BrdU (10 μM) was added during the last 18–24 h of incubation. At the end of incubation, BrdU incorporation in cells was measured by ELISA according to manufacturer's instructions. Data were fitted with a curve described by the equation, $y = V_{max}(1 - (x/(K + x)))$, where K is equal to the IC_{50}. The results were summarized in Table 2. A number of given compounds displayed potent HUVEC and VEGFR-2 inhibitory activities. Compound **6** showed the greatest activities against HUVEC and VEGFR-2 kinase with IC_{50} of 3.8 nM and 5.5 nM, respectively, which was prepared by condensation of 5-chloro-2-hydroxybenzoic acid with quinolin-8-amine. Most of the synthesized compounds demonstrated significant selectivity profile against bFGF-induced proliferation of HUVEC. The IC_{50} against VEGF-induced HUVEC represented significant selectivity compared to bFGF.

As showed in Table 2, the IC_{50} values of all compounds against VEGFR-2 ranged broadly from 3.8 to 2010 nM. Compounds **1–12** were prepared by 8-amino quinoline, while compounds **13–24** were 3-amino quinoline derivatives. In general, compounds **1–12** showed greater inhibitory activities than compounds **13–24**. Compounds with hydroxyl or chlorine substituents at R^1 position showed much better inhibitory activity (with their IC_{50}s lower

than 100 nM) than the other compounds with hydrogen substituent at R^1 position. Furthermore, compounds **6**, **9**, **16** and **21** with chloro or bromo substituent at R^3 position exhibited better activity than compounds with hydrogen or methyl substituent at R^3 position. Structure–activity relationships in these quinoline amide derivatives demonstrated that compounds with hydroxyl or halogen groups displayed better inhibitory activities than those with hydrogen or methyl groups, which can be inferred from the IC_{50}s of compound **6**. We can infer that the hydroxyl group at R^1 and chlorine atom at R^3 may be responsible for the inhibitory activities.

Molecular docking of the most potent inhibitor **6** into ATP-binding site of VEGFR-2 kinase was performed on the binding model based on the VEGFR-2 complex structure (3B8R: PDB[17]). Figure 1 shows the binding modes of compound **6** into the ATP-binding cavity of VEGFR-2.

As illustrated in Figure 1, a large conformational change in the highly conserved Asp-Phe-Gly (DFG) loop, appeared in the docking mode with VEGFR-2. Inspection of these crystallographic structures, however, revealed a large conformational change in the highly conserved Asp-Phe-Gly (DFG) loop. This conformational change, opened up this extended hydrophobic pocket which cannot be accessed when the enzyme is in the 'active' conformation.

Visual inspection of the pose of **6** into the ATP-site revealed that compound 6 appears to be interact with the region through the protonated phenolic hydroxyl group projecting toward the oxygen of carboxyl group of Leu 840, forming a more optimal H-bond (O-H···O: 2.067 Å, 125.2°) interaction. The modeling also suggested that there were two π–π stacking interactions between quinoline ring and the benzene ring of Phe 918. The π–π interaction energies are of the same order of magnitude as hydrogen bonds and play an important role in stabilizing the three dimensional structure of the inhibitor–enzyme complex. The quinoline ring was accommodated in the mostly hydrophobic ATP-binding cleft. Also the quinoline ring projects into a hydrophobic region, which is comprised of the side chains of Ala 866, Phe 918, Cys 919, Leu 840 and Leu 1035. These residues influenced the accessibility of the hydrophobic pocket that flanks the ATP-binding site, and their size can be a key factor in controlling kinase selectivity. This potency increase was attributed to a key predicted hydrogen bond illustrated previously. In the other end of the ATP-binding pocket, the phenyl group with chloro substituent interacted with the residues Asp 1046, Glu 885 and Lys 868, which made the 3D structure more stable. The binding energy for compound **6** in the ATP-site is −10.54 kcal/mol with the estimated inhibition constant, K_i = 18.94 nM.

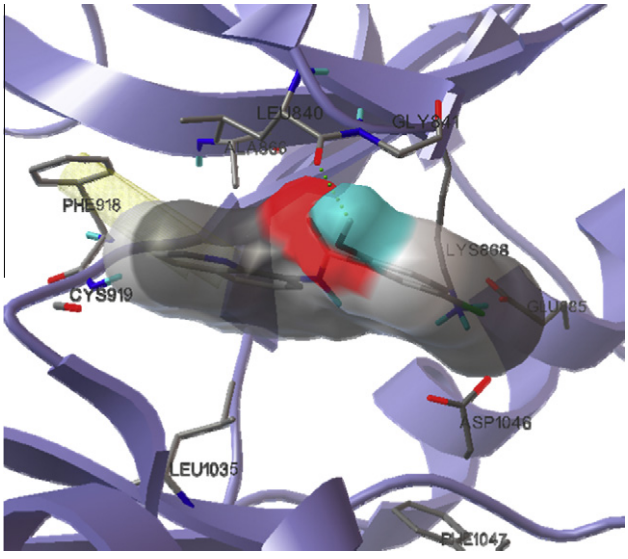


Figure 1. Molecular docking of compound **6** to the ATP-binding pocket of VEGFR-2. The molecules are colored by the atoms (red for oxygen, blue for nitrogen, gray for carbon and cyan for hydrogen). Hydrogen bonds are shown between the inhibitors and VEGFR-2 (green dotted lines).

In summary, two series of quinoline amide derivatives were synthesized and evaluated for their inhibitory activities against VEGFR-2 and HUVEC. Some of the prepared compounds displayed potent activities. The introduction of electron-withdrawing groups (like halogeno and hydroxyl group) at R^1 and R^3 position was favorable to the inhibitory activity. Compound **6** (5-chloro-2-hydroxy-*N*-(quinolin-8-yl)benzamide) exhibited the most potential activity with the IC_{50} = 3.8 nM for VEGFR-2 kinase and IC_{50} = 5.5 nM for HUVEC induced by VEGF. Docking simulation was performed to position compound **6** to the active site of VEGFR-2 kinase to determine the probable binding model. The results indicated that compound **6** was nicely bonded to VEGFR-2 with a hydrogen bond and two π–π stacking interactions, which demonstrated compound **6** would be a potential antitumor agent that deserves further research.

Acknowledgment

The work was financed by grants from National Natural Science Foundation of China (Project 30772627) and the Jiangsu National Science Foundation (No. BK2009239).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.09.014.

References and notes

1. Brown, J. M.; Giaccia, A. J. *Cancer Res.* **1998**, *58*, 1408.
2. 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. P.; Stafford, J. A. *J. Med. Chem.* **2008**, *51*, 4632.
3. (a) Hickey, K.; Grehan, D.; Reid, I. M.; O'Briain, S.; Walsh, T. N.; Hennessy, T. P. J. *Cancer* **1994**, *74*, 1693; (b) Kim, K. J.; Li, B.; Winer, J.; Armanini, M.; Gillett, N.; Phillips, H. S.; Ferrara, N. *Nature* **1993**, *362*, 841.
4. (a) Ferrara, N.; Gerber, H.-P.; LeCouter, J. *Nat. Med.* **2003**, *9*, 669; (b) Cross, M. J.; Claesson-Welsh, L. *Trends Pharmacol. Sci.* **2001**, *22*, 201; (c) McMahon, G. *Oncologist* **2000**, *5*, 3.
5. Prewett, M.; Huber, J.; Li, Y.; Santiago, A.; O'Connor, W.; King, K.; Overholser, J.; Hooper, A.; Pytowski, B.; Witte, L.; Bohlen, P.; Hicklin, D. J. *Cancer Res.* **1999**, *59*, 5209.
6. (a) Sun, L.; Tran, N.; Liang, C.; Tang, F.; Rice, A.; Schreck, R.; Waltz, K.; Shawver, L. K.; McMahon, G.; Tang, C. *J. Med. Chem.* **1999**, *42*, 5120; (b) Sun, L.; Tran, N.; Liang, C.; Hubbard, S.; Tang, F.; Lipson, K.; Schreck, R.; Zhou, Y.; McMahon, G.; Tang, C. *J. Med. Chem.* **2000**, *43*, 2655.
7. Bold, G.; Altmann, K. H.; Frei, J.; Lang, M.; Manley, P. W.; Traxler, P.; Wietfeld, B.; Bruggen, J.; Buchdunger, E.; Cozens, R.; Ferrari, S.; Furet, P.; Hofmann, F.; artiny-Baron, G.; Mestan, J.; Rosel, J.; Sills, M.; Stover, D.; Acemoglu, F.; Boss, E.; Emmenegger, R.; Lasser, L.; Masso, E.; Roth, R.; Schlachter, C.; Vetterli, W. *J. Med. Chem.* **2000**, *43*, 2310.
8. Fraley, M. E.; Arrington, K. L.; Buser, C. A.; Ciecko, P. A.; Coll, K. E.; Fernandes, C.; Hartman, G. D.; Hoffman, W. F.; Lynch, J. J.; McFall, R. C.; Rickert, K.; Singh, R.; Smith, S.; Thomas, K. A.; Wong, B. K. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 351.
9. Wu, Z.; Fraley, M. E.; Bilodeau, M. T.; Kaufman, M. L.; Tasber, E. S.; Balitza, A. E.; Hartman, G. D.; Coll, K. E.; Rickert, K.; Shipman, J.; Shi, B.; Sepp-Lorenzino, L.; Thomas, K. A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 909.
10. Bilodeau, M. T.; Cunningham, A. M.; Koester, T. J.; Ciecko, P. A.; Coll, K. E.; Huckle, W. R.; Hungate, R. W.; Kendall, R. L.; McFall, R. C.; Mao, X.; Rutledge, R. Z.; Thomas, K. A. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2485.
11. Bilodeau, M. T.; Rodman, L. D.; McGaughey, G. B.; Coll, K. E.; Koester, T. J.; Hoffman, W. F.; Hungate, R. W.; Kendall, R. L.; McFall, R. C.; Rickert, K. W.; Rutledge, R. Z.; Thomas, K. A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2941.
12. (a) Hennequin, L. F.; Thomas, A. P.; Johnstone, C.; Stokes, E. S.; Plé, P. A.; Lohmann, J. J.; Ogilvie, D. J.; Dukes, M.; Wedge, S. R.; Curwen, J. O.; Kendrew, J.; Lambert-van der Brempt, C. *J. Med. Chem.* **1999**, *42*, 5369; (b) Hennequin, L. F.; Stokes, E. S.; Thomas, A. P.; Johnstone, C.; Plé, P. A.; Ogilvie, D. J.; Dukes, M.; Wedge, S. R.; Kendrew, J.; Curwen, J. O. *J. Med. Chem.* **2002**, *45*, 1300.

13. (a) Cochran, A. G. *Chem. Biol.* **2000**, *7*, R85; (b) Manley, P. W.; Furet, P. In *Anticancer Agents: Frontiers in Cancer Chemotherapy*; Ojima, I., Vite, G., Altmann, K., Eds.; ACS Symposium Series 796; American Chemical Society: Washington, DC, 2001. p 282.
14. Polverino, A.; Coxon, A.; Starnes, C.; Diaz, Z.; DeMelfi, T.; Wang, L.; Bready, J.; Estrada, J.; Cattley, R.; Kaufman, S.; Chen, D.; Gan, Y.; Kumar, G.; Meyer, J.; Neervannan, S.; Alva, G.; Talvenheimo, J.; Montestruque, S.; Tasker, A.; Patel, V.; Radinsky, R.; Kendall, R. *Cancer Res.* **2006**, *66*, 8715.
15. Huey, R.; Morris, G. M.; Olson, A. J.; Goodsell, D. S. *J. Comput. Chem.* **2007**, *28*, 1145.
16. Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. *J. Comput. Chem.* **1998**, *19*, 1639.
17. Weiss, M. M.; Harmange, J.-C.; Polverino, A. J.; Bauer, D.; Berry, L.; Berry, V.; Borg, G.; Bready, J.; Chen, D. L.; Choquette, D.; Coxon, A.; DeMelfi, T.; Doerr, N.; Estrada, J.; Flynn, J.; Graceffa, R. F.; Harriman, S. P.; Aufman, S.; La, D. S.; Long, A.; Neervannan, S.; Patel, V. F.; Potashman, M.; Regal, K.; Roveto, P. M.; Schrag, M. L.; Starnes, C.; Tasker, A.; Teffera, Y.; Whittington, D. A.; Zanon, R. *J. Med. Chem.* **2008**, *51*, 1668.