Bioorganic & Medicinal Chemistry Letters 22 (2012) 942–944

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Discovery and molecular docking of quinolyl-thienyl chalcones as anti-angiogenic agents targeting VEGFR-2 tyrosine kinase

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ARTICLE INFO

Article history:
Received 4 September 2011
Revised 2 November 2011
Accepted 3 December 2011
Available online 8 December 2011

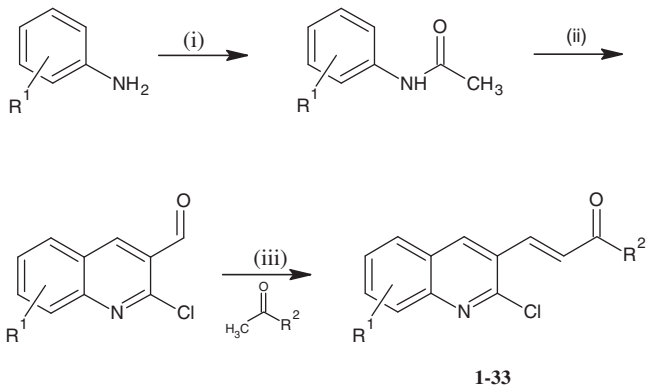
Keywords:
Quinolyl-thienyl chalcones
Angiogenesis
VEGFR-2 kinase
Docking

ABSTRACT

Vascular endothelial growth factor Receptor-2 (VEGFR-2) kinase inhibition is one of the well established strategies to promptly tackle tumor growth by suppression of angiogenesis. In the current study, structure-based virtual screening methodology of a series of quinolyl-thienyl chalcones indicated their strong potential as VEGFR-2 kinase inhibitors. In vitro VEGFR-2 kinase inhibitory activity was found to be significant (compound **19**, IC_{50}: 73.41 nM). All compounds showed significant inhibition of human umbilical vein endothelial cells (HUVEC) proliferation (compound **19**, IC_{50}: 21.78 nM). Molecular interactions of the compounds were studied using molecular docking studies.

Cancer is one of challenging field for medicinal chemists to discover effective yet safer chemotherapeutic agents targeting various biochemical processes involved in progression of cancers.[1] Among these targets, angiogenesis is one of the critical processes affecting growth and development of cancerous cells. Angiogenesis refers to generation of new blood vessels from existing vasculature. It is the key factor in development and progression of various human diseases, including cancer, where it is necessary for the growth, spread and survival of tumors.[2] Angiogenesis is involved in metastasis (uncontrolled spread of tumor cells) by supplying oxygen, nutrients, and related growth factors to small tumors and removing the waste products of metabolism. Tumors that lack an adequate vasculature become necrotic or apoptotic leading to their limited growth. 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 chemotherapy of cancers.[3,4]

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.[5] There is much evidence that direct inhibition of the kinase activity of VEGFR-2 will result in the reduction of angiogenesis and the suppression of tumor growth.[1] In literature, several structural classes of small molecule inhibitors for example, indolin-2-ones,[6] phthalazines,[7] quinolinones,[8] imidazopyridines,[9] benzimidazoles,[10] quinoline amides[1] and pyridines,[11] and quinazolines,[12] have been reported as potent inhibitors of VEGFR-2 and angiogenesis. In the



1-33

Scheme 1. Reagents and conditions: Reaction protocol for the synthesis of chalcones (**1**–**33**): (i) AcOH, H_3PO_4, reflux, 4–6 h; (ii) $POCl_3$, DMF, 80 °C; (iii) acetylthiophene, NaOH, rt, 2 h.

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doi:10.1016/j.bmcl.2011.12.017

current investigation, we have discovered quinolyl-thienyl chalcones as a new class of small-molecule inhibitors of VEGFR-2.

The synthesis of compounds **1**–**11** and **12**–**33** (Scheme 1) has been already described.[13,14] In order to evaluate the synthesized compounds for their in vitro VEGFR-2 kinase inhibitory profile[15] and anti-angiogenic effects via cellular proliferation assays using HUVEC. Cell proliferation assay was determined employing 5-bromo-2-deoxyuridine (BrdU) method based on commercially available kits (Roche Diagnostics, USA). HUVEC were cultured in a medium comprising 5% FBS in Collagen-coated 96-well plates (type 1) and were incubated overnight at 37 °C and 5% CO_2. The medium was aspirated from the cells, and various concentrations of the compounds (inhibitors) in serum-free medium were added to each well. After 30 min, VEGF (10 ng ml^{-1}) was added. Cells were further incubated for an additional 72 h and BrdU (10 M) was added during the last 18–24 h of incubation. 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} valve (Dev et al., 2004).

All compounds of the series were subjected to in vitro VEGFR-2 kinase inhibitory assay followed by cell proliferation assay using HUVEC. Interestingly, compound **19** showed most potent activity against VEGFR-2 kinase (IC_{50}: 73.41 nM) and HUVEC (IC_{50}: 21.78 nM) among the series (Table 1). Detailed analysis showed the fact that derivatives having 2,5-dichloro thienyl ring were the most active especially when methyl group is located at position 7 of quinoline ring. However, derivatives 2,4-dimethylthienyl groups were least active because of less polar and more bulkier methyl groups in comparison to dichloro groups. Among other derivatives, compounds having 3-halothien-2-yl groups especially in the case of 3-chlorothien-2-yl group were also active but lesser than compounds with 2,4-dichlorothienyl ring. Compounds having 5-iodo thienyl groups were least active among halogen, which could be attributed to larger atomic size of iodine as in comparison to the size of deep pocket inside ATP-binding site of VEGFR-2 kinase.

Structural coordinates of VEGFR-2 kinase shows remarkable conformational changes between hydrophobic ATP-binding pocket of active and inactive enzyme states.[1] In the current investigation, molecular docking studies were conducted using a complex

Table 1
Inhibitory effects as IC_{50} values of quinolyl-thienyl chalcones for VEGFR-2 kinase inhibition assay and HUVEC proliferation assay

Compound	R^1	R^2	IC_{50}[a] (nM)	
			VEGFR-2	HUVEC
1	6-Methyl	Thien-3-yl	287.28	106.94
2	6-Methyl	3-Methylthien-2-yl	184.53	127.39
3	6-Methyl	4-Methylthien-2-yl	169.17	106.41
4	6-Methyl	5-Methylthien-2-yl	176.24	127.59
5	6-Methyl	2,5-Dimethylthien-3-yl	453.41	228.57
6	6-Methyl	3-Chlorothien-2-yl	87.11	37.49
7	6-Methyl	5-Chlorothien-2-yl	107.23	59.41
8	6-Methyl	2,5-Dichlorothien-3-yl	81.43	26.19
9	6-Methyl	3-Bromothien-2-yl	129.26	61.91
10	6-Methyl	5-Bromothien-2-yl	112.52	88.43
11	6-Methyl	5-Iodothien-2-yl	154.27	129.28.
12	7-Methyl	Thien-3-yl	207.51	106.94
13	7-Methyl	3-Methylthien-2-yl	157.21	124.11
14	7-Methyl	4-Methylthien-2-yl	139.36	98.93
15	7-Methyl	5-Methylthien-2-yl	129.54	102.29
16	7-Methyl	2,5-Dimethylthien-3-yl	561.29	293.78
17	7-Methyl	3-Chlorothien-2-yl	82.71	34.02
18	7-Methyl	5-Chlorothien-2-yl	92.23	49.51
19	7-Methyl	2,5-Dichlorothien-3-yl	73.41	21.78
20	7-Methyl	3-Bromothien-2-yl	112.26	61.91
21	7-Methyl	5-Bromothien-2-yl	103.82	83.61
22	7-Methyl	5-Iodothien-2-yl	148.31	134.76
23	8-Methyl	Thien-3-yl	389.16	291.21
24	8-Methyl	3-Methylthien-2-yl	267.27	188.96
25	8-Methyl	4-Methylthien-2-yl	232.78	152.63
26	8-Methyl	5-Methylthien-2-yl	261.88	144.72
27	8-Methyl	2,5-Dimethylthien-3-yl	>1000	ND[b]
28	8-Methyl	3-Chlorothien-2-yl	129.56	91.49
29	8-Methyl	5-Chlorothien-2-yl	189.07	116.49
30	8-Methyl	2,5-Dichlorothien-3-yl	357.81	294.56
31	8-Methyl	3-Bromothien-2-yl	312.98	187.21
32	8-Methyl	5-Bromothien-2-yl	367.32	243.08
33	8-Methyl	5-Iodothien-2-yl	481.97	283.57.

[a] IC_{50} values were averaged values determined by at least three experiments.
[b] ND: not determined.

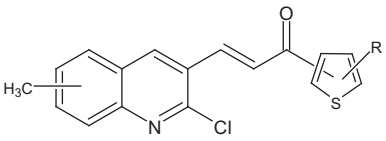

Figure 1. General structure representing series of synthesized quinolyl-thienyl chalcones.

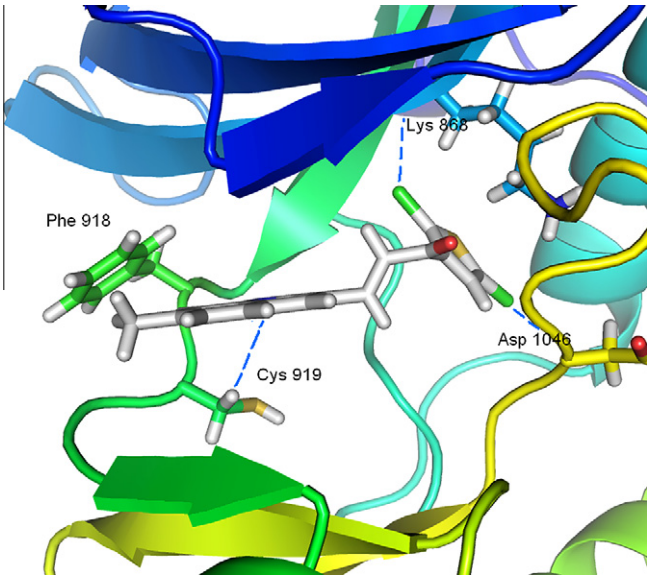

Figure 2. Binding mode of the most active compound (**19**) docked inside ATP-binding pocket of VEGFR-2 tyrosine kinase. Hydrogen bonds are visible (blue dotted lines). Aromatic nitrogen of quinolyl ring and both chlorine atoms are interacting with Cys 919, Asp 1046 and Lys 868, respectively.

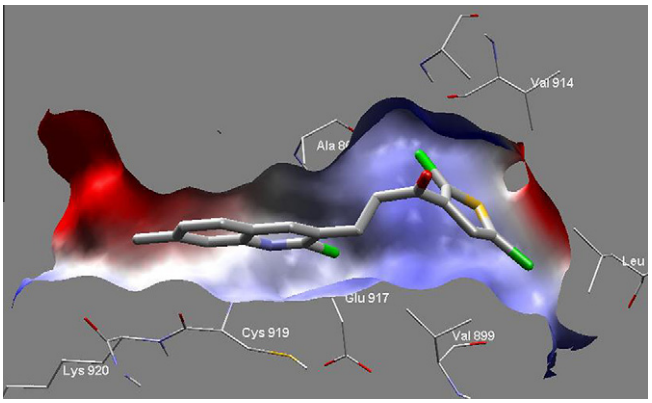

Figure 3. A 3D cut-view surface of ATP-binding pocket of VEGFR-2 tyrosine kinase showing electrostatic and steric interactions of the most active compound (**19**). [Color encoding (White area: hydrophobic region, red area: area with aggregated negative electrostatic potential, and blue area: area with aggregated positive electrostatic potential). Hydrogen atoms 9 except polar ones) were omitted for clarity.

structure (PDB: 2XIR) at a resolution of 1.5 Å. ATP-binding pocket of VEGFR-2 kinase posses the conserved Asp-Phe-Gly (DFG) loop which plays a crucial role in designing and/or virtual screening of new compounds targeting VEGFR-2 tyrosine kinase domain. Figures 2 and 3 shows binding mode of the most potent compound (**19**).

Molecular insights based on molecular docking[16] indicated favorable binding interactions of quinolyl-thienyl chalcones with ATP-binding pocket of VEGFR-2 kinase including Asp-Phe-Gly (DFG). Structural analysis revealed the fact that quinolyl moiety was favorably penetrated into flat but slightly polar pocket surrounded by Cys 919, Phe 918, Leu 840, and Lys 920 (Fig. 1). Compound **19** showed significant binding interaction with amino group of Cys 919 via hydrogen bonding, which was further reinforced by favorable electrostatic interaction of Chlorine atom at position 2 of quinoline moiety with aggregated positively charged pocket (light blue color in Fig. 3). On the other hand, 5-chloro moiety of thienyl ring was identified to be involved in hydrogen bonding with amino group of Asp 1046. Furthermore, 2-chloro group of the same ring was found to be interacting with Lys 868 thus further supporting the favorable binding interactions of compound **19** with ATP-binding pocket of tyrosine kinase domain of VEGFR-2 kinase. Thienyl moiety along 2,5-dichloro moiety penetrated deeply with sling turning owing to its better potency. Deeper pocket favorably matched thienyl ring based on its shape, electrostatic environment (Fig. 2) and hydrogen bonding. However, all compounds with methyl group at position 8 of quinoline ring showed very low activity which could be attributed to its minor steric clash with outer pocket of the ATP-binding pocket of VEGFR-2 kinase.

In conclusion, a series of quinolyl-thienyl chalcones showed significant anti-angiogenic potential. These compounds have strong potential to be further developed as a new class of VEGFR-2 kinase inhibitory with promising anti-angiogenic potential. Further chemical modification via fragment modifications guided by structure and ligand-based computational methodologies can lead to discover better agents as potential clinical candidates.

References and notes

1. (a) Yang, Y.; Shi, L.; Zhou, Y.; Li, H.-Q.; Zhu, Z.-W.; Zhu, H.-L. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6653; (b) 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.; 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. Kim, K. J.; Li, B.; Winer, J.; Armanini, M.; Gillett, N.; Phillips, H. S.; Ferrara, N. *Nature* **1993**, *362*, 841.
4. Cross, M. J.; Claesson-Welsh, L. *Trends Pharmacol. Sci.* **2001**, *22*, 201.
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. 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.; Artinybaron, 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. 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. Rizvi, S. U. F.; Siddiqui, H. L.; Parvez, M.; Ahmad, M.; Siddiqui, W. A. *Chem. Pharm. Bull.* **2010**, *58*, 301.
14. Rizvi, S. U. F.; Siddiqui, H. L.; Ahmad, M. N.; Ahmad, M.; Bukhari, M. H. *Med. Chem. Res.* doi:10.1007/s00044-011-9647-8.
15. Briefly, VEGFR kinase protein (Upstate Biotechnology, Lake Placid, USA). The enzyme selectivity screen was performed with a tyrosine kinase assay kit (Invitrogen, PanVera Co., USA) based on fluorescence polarization detection. Reactions were performed in 96-well polystyrene round-bottomed plates in a final volume of 100 mL. Reaction mixtures contained 20 mM HEPES (pH 7.4), 5 mM$MgCl_2$, 2 mM $MnCl_2$, 50 mM Na_3VO_4, 200 ng/mL enzyme, 20 mM ATP, and 1 ng/mL poly(Glu,Tyr) 4:1. One hundred compounds at concentrations of 10 and 1 mM were tested. After 1 h of incubation, the reactions were terminated by adding a 6 mM EDTA solution; anti-phosphotyrosine antibody, PTK green tracer, and FP dilution buffer mixtures were then added. The fluorescence polarization values were measured after 30 min at room temperature, using a fluorescence reader (BioTek, USA). Kinase inhibition analysis was performed by using Prism 5.0 (GraphPad Software Inc., USA).
16. (a) Khan, I.; Nisar, M.; Shah, M. R.; Shah, H.; Khan, A. Z.; Khan, H.; Samiullah; Gilani, S. N.; Gul, F.; Khann.; Ullah, M. *Fitoter.* **2011**, *82*, 1003; (b) Khan, I.; Nisar, M.; Ahmad, M.; Shah, H.; Saeed, M.; Halimi, S. M. A.; Kaleem, W. A.; Qayum, M.; Aman, A.; Abdullah, S. M. *Fitoter.* **2010**, *82*, 276.