Synthesis, Molecular Modeling and Biological Evaluation of 4-Alkoxyquinazoline Derivatives as Novel Inhibitors of VEGFR2

Liang Lu,a, Ting-Ting Zhao,a, Tian-Bao Liu,a Wen-Xue Sun,a Chen Xu,a Dong-Dong Li,b* and Hai-Liang Zhu*b,a

State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University; Nanjing 210093, People’s Republic of China; and College of Chemical Engineering, Nanjing Forestry University; Nanjing 210073, People’s Republic of China.

Received May 10, 2016; accepted June 18, 2016; advance publication released online August 26, 2016

A series of novel quinazoline derivatives have been designed and synthesized, and their inhibitory activities have also been tested against A549 (carcinomic human alveolar basal epithelial cell), MCF-7 (breast cancer) and HeLa (cervical cancer cell). Of these compounds, compound 4t showed the most potent inhibitory activity (IC50 = 0.22 µg/mL for HeLa, IC50 = 0.15 µg/mL for A549 and IC50 = 0.24 µg/mL for MCF-7). Docking simulation had been performed to position compound 4t into the vascular endothelial growth factor receptor (VEGFR) active site to determine the probable binding model. These results suggested that compound 4t with potent inhibitory activity in tumor growth inhibition may be a potential anticancer agent.

Key words quinazoline; Schiff’s base; vascular endothelial growth factor receptor 2; anticancer

In the late 20th century, the impact of angiogenesis on tumor development and progression has attracted many researchers shifting their focus on this field. Angiogenesis, the formation of new blood vessels sprouting from the pre-existing vasculature, is essential for the rapid expansion of a tumor mass and is a critical process for the formation of metastases. The importance of neovascularization implies the existence of multiple controls, which are coming into focus, making up of positive and negative signals. Targeting angiogenic regulatory factors will be promising for cancer treatment. Vascular endothelial growth factor (VEGF) family consists of VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placenta growth factor (PIGF). VEGFR2 is a potent negative signal of physiological angiogenesis during tumor’s proliferation and migration. The biological functions of VEGF are mediated by its binding to the VEGF receptor family. In mammals, the VEGFR family is transmembrane tyrosine kinase and consists of three members, VEGFR1, VEGFR2 and VEGFR3. VEGFR2 is the major regulator of VEGF-driven responses in endothelial cells and can mediate proliferation, differentiation, and micro-vascular permeability. Moreover, it has been proven to be a prerequisite signal transducer in both physiologic and pathologic angiogenesis. Therefore, as a drug development target, inhibitors of the activation of VEGFR2 might be beneficial to treatment of tumor. Recently, it was reported that a number of compounds have been confirmed as potent inhibitors of VEGFR2 in vitro or possessed antiangiogenic activity (Fig. 1), such as sunitinib (Su-11248), Sorafenib (BAY-43-9006), Tivozanib(10) and ZD4190. As shown in Fig. 1, the 4-anilino-quinazoline and 4-ol-quinoline scaffolds employed by ZD4190 and Tivozanib respectively, are the best scaffolds for the development of VEGFR2 inhibitors, mainly because N1, N4-nitrogen or O-oxigen of these two compounds could interact with the kinase domain via the gatekeeper Cys919 and Phe918 of VEGFR. Based on this, we chose the 4-ol-quinazoline core as the scaffold for the design of a series of novel VEGFR2 inhibitors. Quinazoline is a heterocyclic structure matrix with extensive biological activity. The introduction of 4-aryloxy group into quinazoline framework can afford a series of quinazoline derivatives possessing anticancer activity. Recently, Ravez et al. synthesized a series of 4-aryloxy-6,7-dimethoxyquinazolines as the inhibitors of VEGFR2 used for the therapy of cancer. In order to search for novel inhibitors of VEGFR2, we designed and synthesized a series of quinazoline analogs based on Schiff’s base scaffold. We chose Schiff’s base moiety as it constitutes an important class of biologically active drug molecules due to their wide range of pharmacological properties such as antibacterial, antitumor, DNA-binding activities and so on. However, to our knowledge, few reports have been dedicated to the VEGFR2 activities of quinazoline derivatives owing Schiff’s base. Herein, to keep on research on antitumor compounds with VEGFR2 inhibitory activity, we described the synthesis and structure–activity relationship (SAR) of a new series of quinazoline derivatives owing Schiff’s base group and studied their antitumor activities against A549 (carcinomic human alveolar basal epithelial cell), MCF-7 (breast cancer) and HeLa (cervical cancer cell). Molecular docking was performed by fitting these quinazoline-based analogs and the reference compound (Tivozanib) into the ATP binding site of VEGFR2 (PDB code: 4ASE).

Results and Discussion

Chemistry Compound 3 was synthesized by the routes outlined in Chart 1 according to Mirzaei et al.’s method. Compound 3 was prepared by the reaction of 4-hydroxyquinazoline in excess ethyl-bromacetate and then hydrazinolysis of ethyl ester group by hydrazine hydrate with yield of 78%. 2-(Quinazolin-4-yloxy) acetoxyhydrazide was then reacted with substituted benzaldehyde to prepare the corresponding quinazoline derivatives 4a–y. The chemical structures of these quinazoline derivatives were summarized in Table 1. All these

*To whom correspondence should be addressed. e-mail: lidon@njfu.edu.cn; zhuhl@nju.edu.cn

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Fig. 1. Various VEGFR Tyrosine Kinase Inhibitors

Table 1. Structure of Compounds 4a-y
compounds gave satisfactory elementary analyses (±0.4%). 1H-NMR and electrospray ionization (ESI)-MS spectra data were consistent with the assigned structures.

**Biological Activity**

**Antiproliferative Effects against Cancer Cells**

To test the anticancer activities of the synthesized compounds, we evaluated the target compounds by *in vitro* anti-proliferation assays against three human cancer cell lines A549 (carcinomic human alveolar basal epithelial cell), MCF-7 (breast cancer, with VEGFR protein over expression) and HeLa (cervical cancer cell). The results are summarized in Table 2. With a few exceptions, the active analogs showed a remarkable potent antitumor activity, suggesting that 4-alkoxyquinazoline derivatives could significantly enhance anticancer potency. Among these compounds, 4t showed the most potent biological activity (IC50=0.22µg/mL for HeLa, IC50=0.15µg/mL for A549 and IC50=0.34µg/mL for MCF-7), comparable to the positive control tivozanib (IC50=0.34µg/mL for HeLa, IC50=0.19µg/mL for A549 and IC50=0.22µg/mL for MCF-7).

According to the data presented in Table 2, we could arrive at the conclusion that the activity of the tested compounds may be correlated to the variation and modifications of the structure. Among the 25 synthetic new 4-alkoxyquinazoline derivatives, compounds 4r–y, whose IC50 values ranged from 0.15 to 36.72µg/mL, displayed higher antitumor potencies than compounds 4a–q, with IC50 values ranging from 0.56 to 86.67µg/mL, which demonstrate the dissubstituted compound showed more potent anticancer activity than those with one substituent group. We also observed that electron-donating groups substituent improved the VEGFR2 inhibitory activity compared to electron-withdrawing groups substituent and the order was (NO2,CF3)>(OMe). Moreover, a comparison of the *ortho*-position substitution on benzene ring demonstrated that an *ortho* halogen group (4n–p) have more slightly improved VEGFR2 inhibitory activity and the potency order is F<Cl<Br. Last but not least, compounds with substitution at

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**Table 2. Inhibition of Cancer Cells Proliferation by Compounds 4a–y**

| Compounds | IC50±S.D. (µg/mL) |
|-----------|------------------|
|           | HeLa             | A549             | MCF-7             |
| 4a        | 11.60±1.22       | 1.13±0.23        | 1.94±0.47         |
| 4b        | 2.48±0.28        | 1.86±0.39        | 12.70±0.38        |
| 4c        | 5.90±0.58        | 2.02±0.32        | 0.42±0.04         |
| 4d        | 4.53±0.46        | 7.74±0.78        | 6.85±0.56         |
| 4e        | 13.85±1.96       | 7.74±1.38        | 88.60±2.34        |
| 4f        | 62.30±2.75       | 3.36±0.75        | 67.20±1.97        |
| 4g        | 9.39±0.97        | 8.60±0.91        | 6.75±0.69         |
| 4h        | 1.54±0.19        | 6.77±0.78        | 58.10±1.33        |
| 4i        | 57.79±1.34       | 4.46±0.36        | 4.17±0.26         |
| 4j        | 7.29±1.23        | 10.89±0.94       | 37.79±0.64        |
| 4k        | 1.78±0.09        | 7.19±0.25        | 5.06±0.22         |
| 4l        | 1.85±0.16        | 5.80±0.41        | 1.50±0.19         |
| 4m        | 12.49±1.04       | 11.82±0.70       | 16.7±0.46         |
| 4n        | 5.85±0.35        | 8.02±0.97        | 9.19±0.84         |
| 4o        | 24.66±1.35       | 13.82±1.03       | 10.00±0.58        |
| 4p        | 46.67±1.95       | 15.87±0.55       | 14.75±0.24        |
| 4q        | 0.56±0.04        | 11.20±1.23       | 4.46±0.36         |
| 4r        | 43.40±1.58       | 63.63±2.34       | 91.79±2.23        |
| 4s        | 0.68±0.23        | 0.96±0.35        | 1.54±0.14         |
| 4t        | 0.22±0.04        | 0.15±0.03        | 0.24±0.02         |
| 4u        | 3.38±1.96        | 4.43±0.24        | 0.90±0.23         |
| 4v        | 18.69±1.05       | 9.67±0.69        | 11.30±1.33        |
| 4w        | 22.80±1.84       | 14.20±0.94       | 10.64±0.86        |
| 4x        | 8.16±0.75        | 3.12±0.26        | 0.96±0.05         |
| 4y        | 22.34±0.79       | 36.72±2.89       | 31.20±1.45        |
| Tivozanib | 0.34±0.02        | 0.19±0.04        | 0.25±0.03         |

*a* Inhibition of the growth of HeLa cell lines. *b* Inhibition of the growth of A549 cell lines. *c* Inhibition of the growth of MCF-7 cell lines.
the para- or meta-position showed more potent activities than those with substitution at the ortho-position. For example, the inhibitory activity of 4-alkoxyquinazoline derivatives (4c, d, 4n, o) with different substituents increases in the following order: 2-Br<2-Cl<H<4-Cl<4-Br. The results suggest that stronger electro negativity groups in the 4-position and 3-position and weaker groups in the 2-position afford better inhibitory activity. On the basis of the conclusion obtained, we designed and evaluated the compounds with 3,4-dimethoxy (4t) and 2,4-dichloro (4s) group substitution, the activity has been significantly enhanced up to 0.15 and 0.68 µg/mL, respectively.

**Kinase Selectivity**

In order to validate the anti-proliferative effect produced by interaction of VEGFR protein and synthesized compounds, all compounds of the series were subjected to *in vitro* VEGFR2, epidermal growth factor receptor (EGFR), basic fibroblast growth factor (bFGF) and platelet-derived growth factor receptor (PDGFR) kinase inhibitory assays. As shown in Table 3, all synthesized compounds exhibited much more higher inhibitory activity for VEGFR2 than for EGFR, bFGF and PDGFR, like reference tivozanib. Additionally, good agreement had been found between the IC₅₀ values of these compounds and their relevant IC₅₀ values in the anti-proliferative assay. Hence, a further study comparing the anti-proliferative activity against the HeLa cell line with the VEGFR2 inhibitory activity of the top 10 compounds (4t, q, s, h, k, l, b, u, d, n) was performed and the result revealed that there is a moderate correlation between VEGFR2 inhibition and the inhibition of cancer cellular proliferation, as evidenced in Fig. 2, with a correlation coefficient of 5.032 and an R² value of 0.9627. In conclusion, the synthesized compounds can inhibit

**Table 4. Cytotoxicity of Selected Compounds**

| Compounds | CC₅₀ (µM) |
|-----------|-----------|
| 4a        | 276.33    |
| 4b        | 283.11    |
| 4c        | 245.36    |
| 4s        | 265.27    |
| 4t        | 299.13    |
| Tivozanib | 233.66    |

*a* Values are the average of two independent experiments run in triplicate.

**Table 5. The Docking Calculation of the Synthesized Compounds (4a-y)**

| Compounds | CDOCKER INTERACTION ENERGY (kcal/mol) | Compounds | CDOCKER INTERACTION ENERGY (kcal/mol) |
|-----------|----------------------------------------|-----------|----------------------------------------|
| 4a        | −47.3466                               | 4n        | −45.7967                               |
| 4b        | −49.2058                               | 4o        | −45.4098                               |
| 4c        | −49.0136                               | 4p        | −43.9412                               |
| 4d        | −48.5115                               | 4q        | −45.3549                               |
| 4e        | −47.9316                               | 4r        | −44.8755                               |
| 4f        | −49.3442                               | 4s        | −46.2903                               |
| 4g        | −45.5025                               | 4t        | −54.5336                               |
| 4h        | −48.9949                               | 4u        | −49.4427                               |
| 4i        | −50.6367                               | 4v        | −49.3532                               |
| 4j        | −47.8716                               | 4w        | −51.5396                               |
| 4k        | −46.8227                               | 4x        | −49.3153                               |
| 4l        | −47.9512                               | 4y        | −45.7061                               |
| 4m        | −47.3670                               |           |                                        |

Fig. 2. Correlation between the Anti-proliferative Activity against HeLa and the VEGFR2 Inhibitory Activity, $R^2=0.9627$, Which Indicated That There Was a Moderate Correlation between VEGFR2 Inhibition and Inhibition of Cellular Proliferation

Fig. 3. Two Dimensional Molecular Docking Model of Compound 4t with VEGFR2 (Entry 4ASE in the Protein Data Bank)
the function of VEGFR2 and the anti-proliferative effect was produced partly by the interaction between VEGFR2 protein and the molecule inhibitors.

Cytotoxicity Test

Five of the top active compounds (4a–c, s, t) were chosen to evaluate for their toxicity against human macrophage for further study on pharmacological activities with the median cytotoxic concentration (CC50) data of tested compounds. These compounds were tested at multiple doses to study the viability of macrophage.

As showed in Table 4, the top five active compounds exhibited lower cytotoxic than Tivozanib, and the compound 4t has the cytotoxic with CC50 values of 299.13 µM, which was better than that of Tivozanib (233.66 µM). The results demonstrated that compound 4t could be a potential inhibitor as anticancer.

Molecular Docking

In order to gain more understanding of the SARs observed at the VEGFR2, molecular docking of the most potent inhibitor 4t into the active center of the epidermal growth factor family (PDB code: 4ASE).

All docking runs were applied Lig and Fit Dock protocol of Discovery Studio 3.5. The docking calculation of the synthesized compounds was showed in Table 5. The interaction energy of the compounds and their antibacterial activity showed the corresponding results. Among the docking calculation of the synthesized compounds, compound 4t showed the lowest interaction energy. The binding model of compound 4t and VEGFR2 is depicted in Figs. 3 and 4. Figures 3 and 4 showed the binding mode of compound 4t interacting with VEGFR2 protein and the docking results revealed that three amino acids Phe918, Cys919 and His1026 located in the binding pocket of the protein played vital roles in the conformation with compound 4t, which were stabilized by two π-bonds and a hydrogen bond that are shown in the 2D and 3D diagrams. One π–π bond with 5.7743 was formed between Phe918 and the quinazoline ring while the other π–sigma bond with 5.98083 involved Asp1046. The hydrogen bond with 2.2 Å was formed between Cys919 and the quinazoline ring. The enzyme assay data and the molecular docking results demonstrated that compound 4t is a potential inhibitor of VEGFR2.

Conclusion

In summary, a series of 4-alkoxyquinazoline derivatives have been designed and synthesized, and their inhibitory activities were also tested against A549, MCF-7 and HeLa cell lines. Compound 4t exhibited the most potent inhibitory activity (IC50=0.22 µg/mL for HeLa, IC50=0.15 µg/mL for A549 and IC50=0.24 µg/mL for MCF-7), which was compared with the positive control tivozanib. Preliminary SARs and molecular modeling study provided further insight into interactions between the enzyme and its ligand. Analysis of the compound 4t’s binding conformation in active site displayed that the compound 4t has been stabilized by the interactions with Phe918, Cys919 and His1026. The result provided valuable information for the design of VEGFR2 inhibitors as antitumor agents.

Acknowledgments This work was supported by the Jiangsu National Science Foundation (No. BK2009239) and the Fundamental Research Fund for the Central Universities (No. 1092020804).

Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

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