e-ISSN:2320-1215
p-ISSN:2322-0112

Research and Reviews: Journal of Pharmacy and Pharmaceutical Sciences

A FGFR-2 Inhibitor, Ki23057, Evaluated as a Novel VEGFR-2 Kinase Inhibitor

Zong-Shan Ma[1], Qiang-wen Fan[1], Hong Yan[1]* and Da-Hai Yu[2]

[1]College of Life Science and Bio-engineering, Beijing University of Technology, Beijing, 100124, China
[2]Department of Oral and Maxillofacial Surgery, College of Stomatology, Guangxi Medical University, Shanghai, 530021, China

Research Article

Received date: 22/06/2016
Accepted date: 12/07/2016
Published date: 17/07/2016

***For Correspondence**

Hong Yan, College of Life Science and Bio-engineering, Beijing University of Technology, Beijing, 100124, China, Tel: +86-010-6739-6211; Fax: +86-010-6739-2001

E-mail: hongyan@bjut.edu.cn

Keywords: Ki23057, VEGFR-2 kinase inhibitor, FGFR-2 kinase inhibitor biological evaluation, Molecular docking

ABSTRACT

The aim of this study was to clarify the ability of a FGFR-2 inhibitor, Ki23057, to inhibit the VEGFR-2 signaling pathway which was a valuable approach in the treatment of cancers. An efficient and convenient synthetic route to Ki23057 has been developed utilizing a key one-pot method. Its biological activities as VEGFR-2 kinase inhibitors were evaluated by immunohistochemistry. The results exhibited that the Ki23057 has potent inhibitory activities against VEGFR-2 tyrosine kinase. Docking simulation was performed to demonstrate that Ki23057 is a potential agent for VEGFR-2 cancer therapy.

Angiogenesis, the formation of new capillary blood vessels from existing vasculature, plays an important role in the process of tumor growth and metastasis [1-3]. Among many factors involved in tumor angiogenesis, vascular endothelial growth factor (VEGF) and Fibroblast growth factors (FGF) have been identified as the most common regulators of tumor angiogenesis [4-7]. VEGFR-2 is a receptor tyrosine kinase that comprises blood vessels and can mediate endothelial cell proliferation, differentiation, and micro vascular permeability [8-10]. Blocking VEGFR-2 signaling pathway has become an attractive approach for the treatment of cancers [11,12]. Several successful strategies for the inhibition of VEGFR-2 have been effectively demonstrated in preclinical and clinical settings, such as Tivozanib, Vandetanib and Cabozantinib [13-15] **(Figure 1).**

Ki23057, a newly developed small-molecule-acting FGFR-2 inhibitor, competes with ATP for the binding site in the kinase and holds promise as a therapeutic agent in gastric cancer and colon cancer [16,17]. With chemical structure theory, Ki23057 approached the property similarity to above mentioned VEGFR-2 kinase inhibitors. The quinoline moiety skeleton of Ki23057 was same as that of Cabozantinib (blue), and two functional groups were similar to Tivozanib (red) and Vandetanib (green) respectively. So, we assumed Ki23057 will exhibit inhibitory activities against VEGFR-2. Ki23057 was synthesized and its inhibitory activities against VEGFR-2 were evaluated by immunohistochemistry. In addition, docking simulation was performed, and the structure-activity relationships and possible enzyme binding modes were also illustrated. Broadly used in modern drug design, molecular docking methods explore the ligand conformations adopted within the binding sites of macromolecular targets.

e-ISSN:2320-1215
p-ISSN:2322-0112

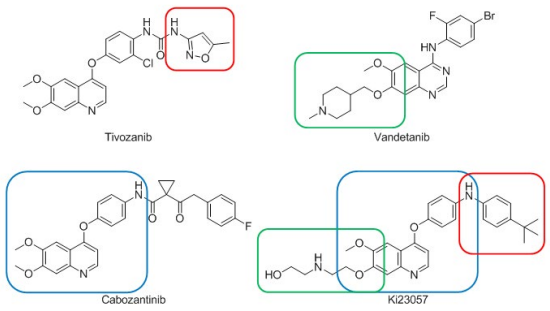


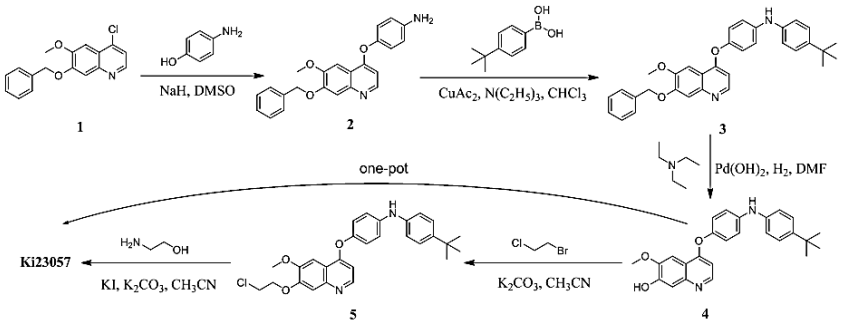
Figure 1. Representative VEGFR-2 kinase inhibitors and Ki23057.

This approach also estimates the ligand-receptor binding free energy by evaluating critical phenomena Involved in the intermolecular recognition process. The Auto Dock output results represented the docking scores as ΔG values. They were further converted to the predicted inhibition constants (Ki_{pred}). The Ki_{pred} values for analyzed docking poses were calculated from the ΔG parameters as follows:

$$\Delta G=RT(\ln Ki_{pred}) \quad (1)$$

$$Ki_{pred} = e^{\wedge}(\Delta G/RT) \quad (2)$$

The synthetic route of Ki23057 from 1 was outlined in **Scheme 1.** As previous approaches, 2 was synthesized from 1 with 4-aminophenol in the presence of sodium hydride in DMSO [18]. 2 were then attacked by 4-tert-butylphenylboronic acid to afford desired 3 with a catalytic amount of copper(II) acetate in dry $CHCl_3$. And 4 was obtained via a deprotection by $Pd(OH)_2$ in DMF. Then Ki23057 was prepared in two steps from 4 by a nucleophilic substitution (1-bromo-2-chloroethane) followed by a electrophilic substitution (ethanolamine) using K_2CO_3 in DMF [16]. We developed a one-pot method to synthesize Ki23057 from 4 by the change of solvent DMF to CH_3CN. This approach represents a noteworthy improvement in 73.5% overall yield and remarkably higher than the 8.6% yield of known method without separation and purification of 5 in shorter reaction time (10 h).



Scheme 1. Synthesis of Ki23057.

The antitumor activities of Ki23057 were evaluated against VEGFR-2 which was observed within the Tca8113 and HUVEC cells. The expressions of VEGFR-2 in Tca8113 and HUVEC cell (×200) were shown in **Figure 2.** As shown in **Figure 2**, the staining was strongest around the cell nucleus in blank control group in accord with the expression characteristic of VEGFR-2 **(Figure 2B and 2E).** The images showed the expression level of VEGFR-2 in Tca8113 was markedly decreased **(Figure 2C)**, and the expression of VEGFR-2 in HUVEC was blandly reduced after Ki23057 treatment **(Figure 2F).** The experimental results showed Ki23057 had relatively higher inhibitory activities for VEGFR-2 in Tca8113 with comparison to HUVEC. It could be concluded that Ki23057 was clearly beneficial for inhibiting the VEGFR-2 expression and showed specific selectivity for cell strain which needed further study.

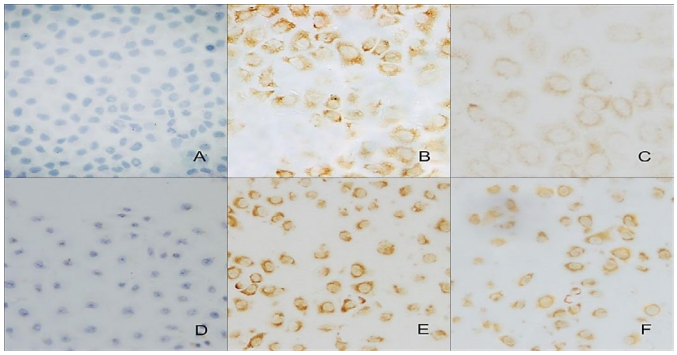


Figure 2. The expressions of VEGFR-2 in Tca8113 and HUVEC cell (×200). A, B, C were negative control group, blank control group and Ki23057 positive control group in Tca8113, respectively. D, E, F were negative control group, blank control group and Ki23057 positive control group in HUVEC, respectively. A and D were stained with hematoxylin, the others instead.

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Table 1. Molecular docking results of Ki23057 with VEGFR-2 and FGFR-2.

Compd	VEGFR-2		FGFR-2	
	$\triangle G^a$	K_i^b	$\triangle G^a$	K_i^b
Ki23057	-5.78	57.5	-3.39	3300
Original ligands	-6.82	9.65	-1.9	4028

[a] Binding free energy (kcal mol^{-1}). [b] Inhibition constant (μM).

Docking simulation was performed to demonstrate whether Ki23057 is a potential agent for VEGFR-2 cancer therapy. Ki23057 was docked into the target crystal structure of human VEGFR-2 kinase domain (PDB code: 1Y6A.pdb) [19] and FGFR-2 kinase domain (PDB code: 2PZR.pdb) [20], in complex with original ligands. Before performing docking calculations, the original ligand was extracted from the crystal structure, the structural water molecules were removed, and hydrogen atoms were added in standard geometry. For each compound, 100 docking experiments were initiated with randomized populations and solutions for individual runs were clustered if their final docked positions were within a tolerance of 2 Å RMSD. The grid size for the search of docking space was set at 60 × 60 × 60 distributed around the binding domain with a default grid spacing of 0.375 Å. The output files of AutoDock contained the final predicted conformations, the lowest energy docked and the estimated free energy of binding for each cluster and each individual docking. Binding affinities are reported as the binding free energies (ΔG) and inhibition constants (K_i), as shown in **Table 1.** Theoretical calculated results indicated that Ki23057 presented close binding affinities to that of original ligand of VEGFR-2 and FGFR-2 **(Table 1).** Obviously, Ki23057 presented relatively better binding affinities with VEGFR-2 (ΔG=-5.78 kcal mol^{-1}) than that of FGFR-2 (ΔG=-3.39 kcal mol^{-1}).

In order to understand the interaction between Ki23057 and VEGFR-2 kinase, the docking modeling of Ki23057 and original ligand are given to visualize the orientation and binding modes **(Figure 3)**. As shown in **Figure 3A**, Ki23057 showed high overlap ratios with original ligand in VEGFR-2 kinase, which was consistent with the biological activities results. Ki23057 is nicely bound to the ATP-binding cavity of VEGFR-2 via one π-π interaction and one hydrogen bond **(Figure 3B).** The phenyl ring at 4-position of quinoline forms a π-π interaction with the amino acid Phe916 (Ar-Ar, d=5.66 Å), which suggests that the quinoline moiety plays an important role in the combination of the receptor and ligand. In addition, the NH group of side chain forms a hydrogen bond with Pro837 (NH-O=C, d =2.43 Å), which indicated that the introduction of side chain at 7-position of quinoline might reinforce the combination of Ki23057 and the receptor, which might enhance the binding affinity. It was found that the active pocket was nicely occupied by Ki23057 and the t-Bu group on phenyl ring permitted deeper immersion into the bottom of the binding site **(Figure 3C and 3D).** All of these indicate that Ki23057 has better binding affinities with VEGFR-2.

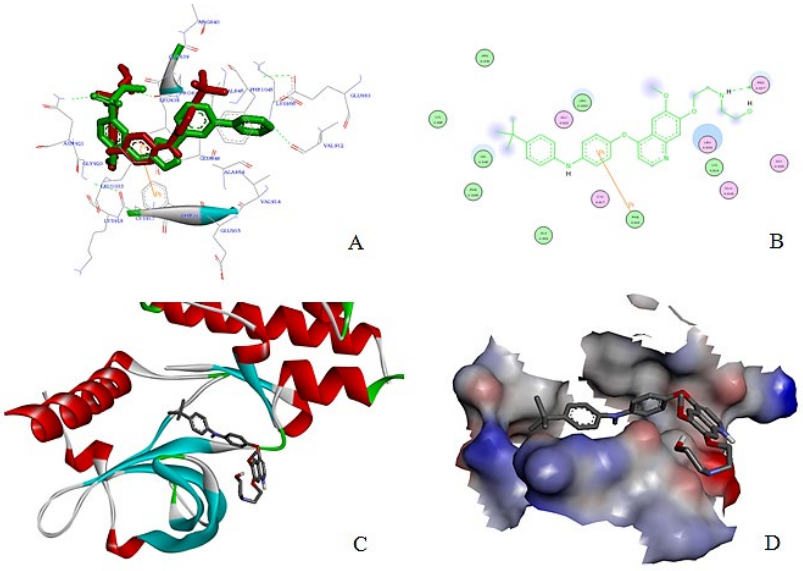

Figure 3. Docking of Ki23057 in VEGFR-2 kinase. (A) Interactions of Ki23057 (red) and original ligand (green) with VEGFR-2 kinase. (B) Binding interactions of Ki23057 with VEGFR-2 kinase. (C) 3D model of the interactions between Ki23057 with VEGFR-2 kinase. (D) Surface model of the interactions between Ki23057 with VEGFR-2 kinase

In this paper, Ki23057 was synthesized and evaluated for its inhibitory activities for VEGFR-2. We developed a one-pot method to synthesize Ki23057 from 4 with a noteworthy improvement in 73.5% overall yield and remarkably higher than the 8.6% yield. Its biological activities exhibited that the Ki23057 has potent inhibitory activities against VEGFR-2 tyrosine kinase in Tca8113 with comparison to HUVEC. Theoretical calculations presented that Ki23057 showed relatively better binding affinities with VEGFR-2 than that of FGFR-2. Binding models of Ki23057 indicated that a hydrogen bond and a π-π interaction with the protein residues in the ATP binding cavity might play a crucial role in VEGFR-2 inhibition. Good correlation of inhibitory activities between the immunohistochemistry and theoretical calculation supported that Ki23057 could be a promising and attractive candidate of anti-VEGFR-2 agents.

e-ISSN:2320-1215
p-ISSN:2322-0112

ACKNOWLEDGMENTS

This work was financially supported by the Key Projects in the Beijing Municipal Natural Science Foundation (No. KZ201510005007).

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