Journal Pre-proof

Design, synthesis, and *in vitro* and *in vivo* anti-angiogenesis study of a novel vascular endothelial growth factor receptor-2 (VEGFR-2) inhibitor based on 1,2,3-triazole scaffold

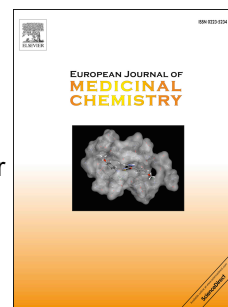De-pu Wang, Kai-li Liu, Xin-yang Li, Guo-qing Lu, Wen-han Xue, Xin-hua Qian, Kamara Mohamed O, Fan-hao Meng
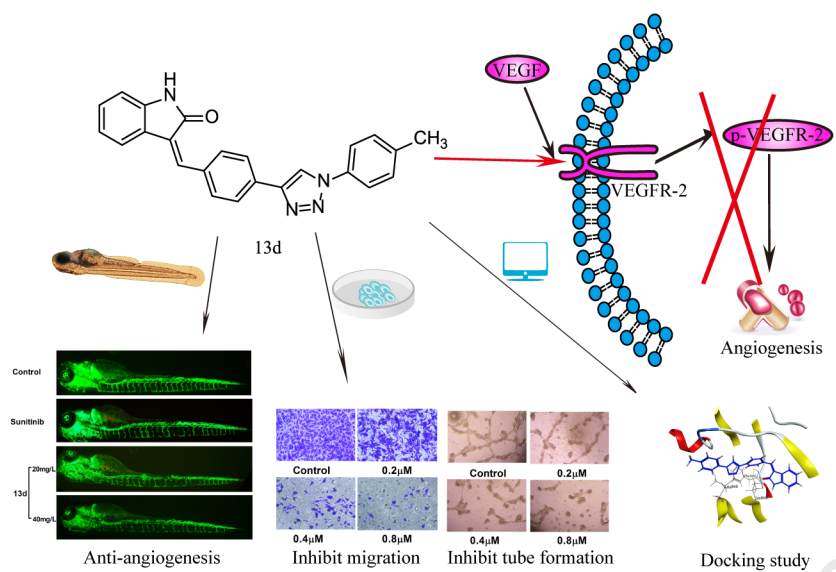
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13d

Anti-angiogenesis

Inhibit migration

Inhibit tube formation

Docking study

VEGFR-2

p-VEGFR-2

Angiogenesis

VEGF

CH₃

Control

Sunitinib

13d

20mg/L

40mg/L

Control

0.2μM

0.4μM

0.8μM

Control

0.2μM

0.4μM

0.8μM

Design, synthesis, and *in vitro* and *in vivo* anti-angiogenesis study of a novel vascular endothelial growth factor receptor-2 (VEGFR-2) inhibitor based on 1,2,3-triazole scaffold

De-pu Wang[a,1], Kai-li Liu[a,1], Xin-yang Li[a, b], Guo-qing Lu[a], Wen-han Xue[a], Xin-hua Qian[a], Kamara Mohamed O[a], Fan-hao Meng[a,*]

[a] School of Pharmacy, China Medical University, Shenyang, 110122, China.

[b] Department of Pharmacy, Shengjing Hospital of China Medical University, Shenyang, 110004, China.

[*] Correspondence: Professor Fan-hao Meng, School of pharmacy, China Medical University, Shenyang, 77 Puhe Road, 110122, P. R. China.

E-mail: fhmeng@cmu.edu.cn Fax: +86-24-31939448

[1] The contributions of these authors are equal to this work.

1

2

Abstract

In the past five years, our team had been committed to click chemistry research, exploring the biological activity of 1,2,3-triazole by synthesizing different target inhibitors. In this study, a series of novel indole-2-one derivatives based on 1,2,3-triazole scaffolds were synthesized for the first time, and their inhibitory activity on vascular endothelial growth factor receptor-2 (VEGFR-2) was tested. Most of the compounds had shown promising activity in the VEGFR-2 kinase assay and had low toxicity to human umbilical vein endothelial cells (HUVECs). The compound **13d** (IC_{50} = 26.38 nM) had better kinase activity inhibition ability than sunitinib (IC_{50} = 83.20 nM) and was less toxic to HUVECs. Moreover, it had an excellent inhibitory effect on HT-29 and MKN-45 cells. On the one hand, by tube formation assay, transwell, and western blot analysis, compound **13d** could inhibit VEGFR-2 protein phosphorylate on HUVECs, thereby inhibiting HUVECs migration and tube formation. *In vivo* study, the zebrafish model with VEGFR-2 labeling also verified that compound **13d** had more anti-angiogenesis ability than sunitinib. On the other hand, molecular docking and molecular dynamics (MD) simulation results showed that compound **13d** could stably bind to the active site of VEGFR-2. Based on

the above findings, compound **13d** could be considered an effective

anti-angiogenesis drug and has more development value than sunitinib.

Keywords: 1,2,3-Triazole; Anti-angiogenesis; VEGFR-2; Zebrafish.

1. Introduction

Angiogenesis is the formation of new blood vessels by sprouting or

splitting from pre-existing blood vessels [1-4]. It plays a critical role in

the pathogenesis of various disorders, most notably growth and metastasis

of solid tumors [5]. The growth and metastasis of tumors require new

blood vessels to transport nutrients and oxygen [6]. Vascular endothelial

growth factor (VEGF) is a highly specific vascular endothelial growth

factor that is overexpressed in most solid tumors [7]. Excessive VEGF in

solid tumors promotes the angiogenesis of adjacent blood vessels, and the

excessive production of blood vessels can cause the imbalance of the

metabolic microenvironment and accelerate tumor invasion and

metastasis [6,8,9]. Therefore, anti-angiogenesis is an essential form of

inhibiting tumor growth and metastasis [10-12].

Vascular endothelial growth factor receptor-2 (VEGFR-2)，as the vital

receptor of VEGF, which activated by VEGF initiates downstream signal

transduction, ultimately leads to angiogenesis, tumor proliferation, and

migration [7,13]. Therefore, a long-held concept that inhibition of

VEGFR-2 would cause efficient anti-angiogenesis and antitumor

response [14,15]. At present, the small molecule VEGFR-2 inhibitors

1 have emerged as promising anti-angiogenesis agents against a wide

2 variety of cancers, such as sorafenib, cabozantinib, and sunitinib, and

3 applied in clinical cancer therapy (**Fig. 1**) [16-18]. However,

4 chemotherapy drugs can cause specific adverse side effects and affect

5 patients' health [19]. Therefore, it is necessary to find low toxicity

6 VEGFR-2 inhibitors with a novel structure to treat cancer and enrich

7 VEGFR-2 inhibitors [7].

8   In the course of identifying various chemical fragments that could

9 serve as a scaffold for novel anti-angiogenesis agents, our programs

10 began with the indolin-2-one derivative sunitinib, a potent multitargeted

11 kinase inhibitor of VEGFR-2, PDGFR, and c-KIT kinases [20-22]. The

12 privileged indolin-2-one scaffold (red dashed rectangle in **Fig. 1**) was

13 regarded as the most promising pharmacophore binding to the active site

14 of VEGFR-2 kinase [23,24]. Moreover, it should be pointed out that

15 numerous indolin-2-one derivatives have been reported as potent

16 antitumor agents [23,25-28]. Therefore, the indolin-2-one scaffold was

17 selected as a nucleus to develop a new VEGFR-2 inhibitor. The

18 combination principle of multiple pharmacophores for developing a

19 single molecule usually may enhance biological activity and improve

20 bioavailability [29]. In our previous work, inspired by various biological

21 properties of 1,2,3-triazole (blue dashed rectangle in **Fig. 1**), which could

22 bind with biomolecular targets to fulfill its envisaged role as potential

pharmacophore [30-32], two series of 1,2,3-triazole derivatives (**TS-13n, B-10n** in **Fig. 1**) had been independently reported as potent antitumor agents in our laboratory [33-35].

Based on the findings mentioned earlier, we have been inspired to design and synthesize a series of novel derivatives derived by insertion of a 1,4-diphenyl-1*H*-1,2,3-triazole moiety on the active nucleus of indolin-2-one through an ethylene bridge (**Fig. 1**). Herein, twenty-eight novel indolin-2-one derivatives bearing 1,2,3-triazole moiety and their VEGFR-2 kinase inhibitory activity were reported. These derivatives exhibited potent inhibition of VEGFR-2 tyrosine kinase activity and efficiently inhibited angiogenesis with less toxicity *in vitro* and *in vivo*, suggesting that these derivatives could be considered promising lead compounds for further development of novel anti-angiogenesis agents.
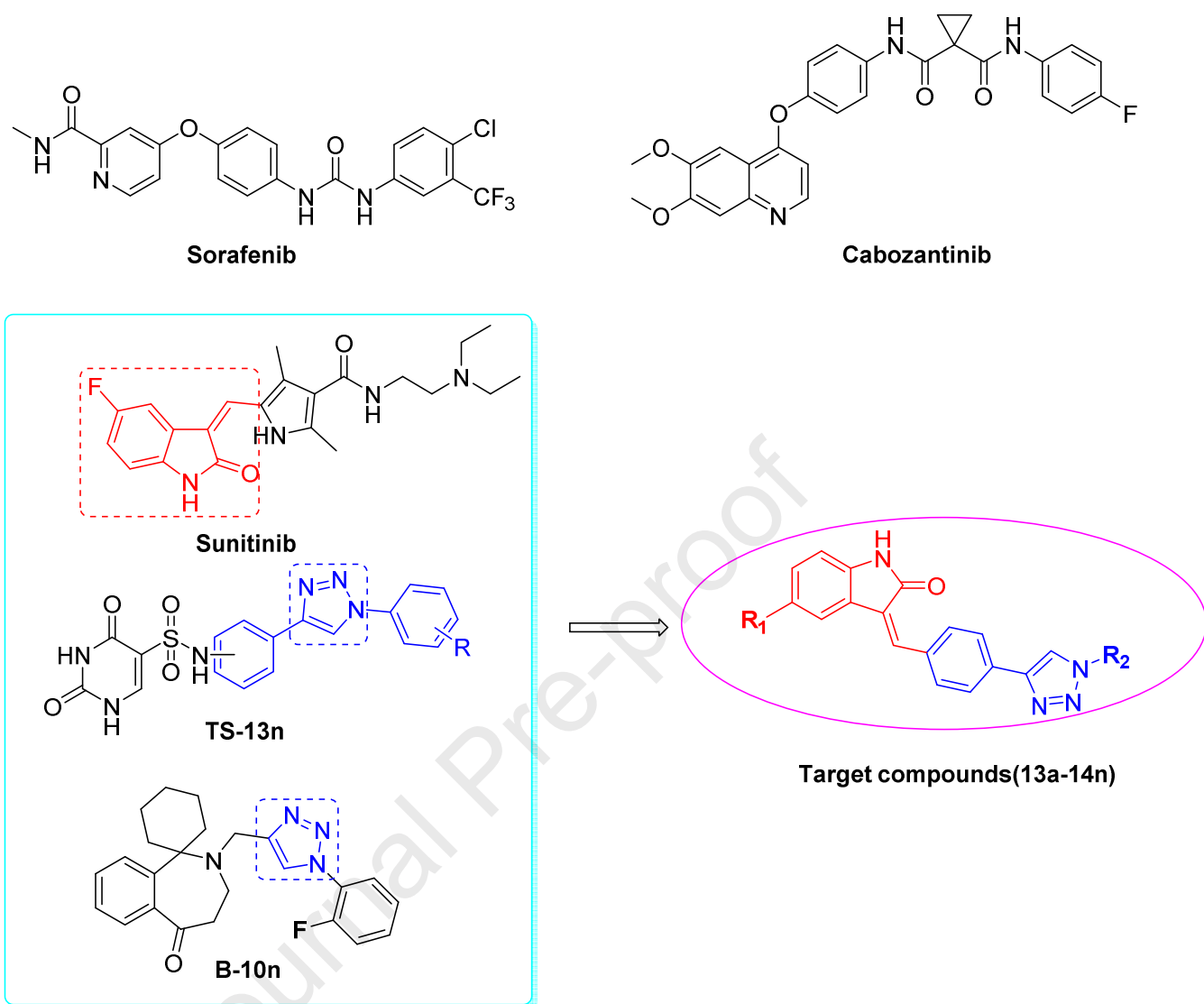
Fig. 1. Structures of sorafenib, cabozantinib, sunitinib, TS-13n, B-10n and design of the target compounds.

2. Results and discussion

2.1. Chemistry

The synthetic pathway adopted to prepare novel indole-2-one-1,2,3-triazole derivatives (**13a**-**14n**) was depicted in **Scheme 1**. First, the preparation of different substituted azide benzene (**2a**-**2n**) involved diazo-reaction and displacement reaction with sodium

1 azide (NaN$_3$) (synthetic detail procedure is given in the Experimental

2 section) [35]. Click chemistry is a practical approach for generating a

3 1,2,3-triazole unit [34]. The novel intermediates (**4a**-**4n**) were obtained in

4 high yields via Cu (I)-catalyzed azide-alkyne cycloaddition (CuAAC)

5 between aryl-azides (**2a**-**2n**) and commercial 4-ethynylbenzaldehyde (**3**)

6 in the presence of sodium ascorbate and CuSO$_4$·5H$_2$O as a catalyst in

7 DMF and water mixture as the solvent system [35]. The compounds **9-10**

8 were prepared from compounds **5-6** via Sandmeyer's method described in

9 the literature [37,38], reduced which using hydrazine hydrate to give

10 compound **11-12** [39]. Finally, the title compounds (**13a-14n**) were

11 accomplished by employing Claisen-Schmidt condensation reaction

12 between indolin-2-ones (**11-12**) and various 1,2,3-triazole aromatic

13 aldehydes (**4a-4n**) with a catalytic amount of piperidine as a base [40,

14 41].
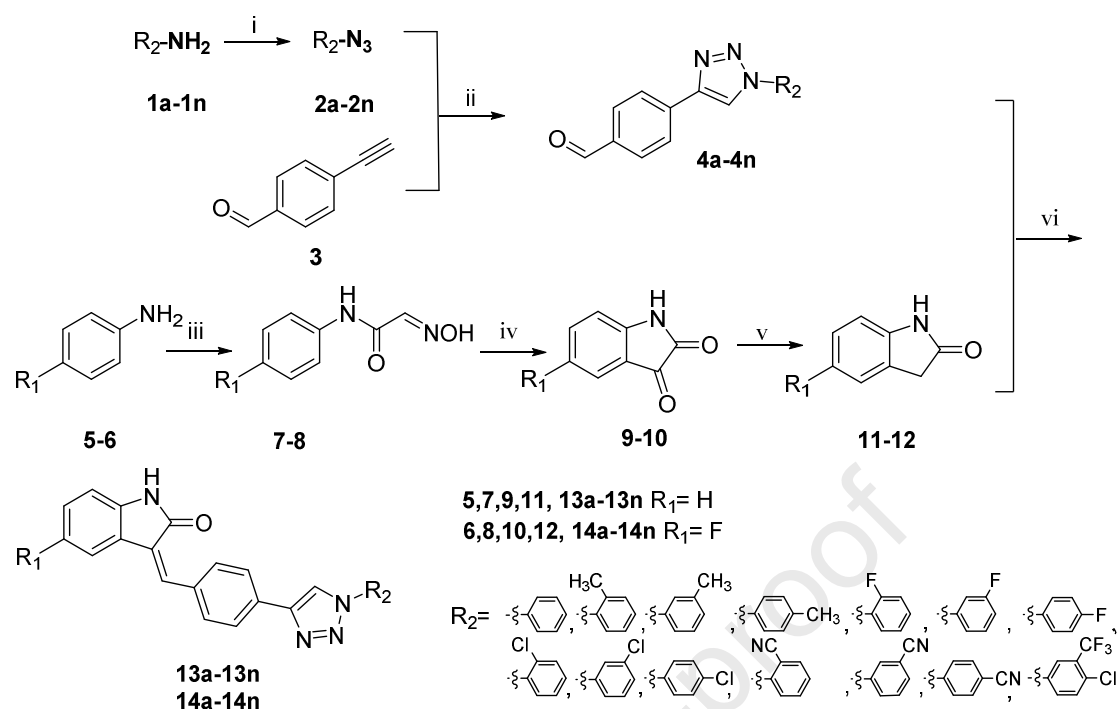
Scheme 1. Reagents and conditions: (i) $NaNO_2$, HCl, NaN_3, CH_2Cl_2, H_2O, 0-5°C, 3-5 h; (ii) $CuSO_4 \cdot 5H_2O$, ascorbic acid, KI, DMF, H_2O, 50°C, 6-10 h; (iii) chloral hydrate, Na_2SO_4, $NH_2OH \cdot$ HCl, HCl, H_2O, 85°C, 3 h; (iv) conc H_2SO_4, 60°C, 0.5 h, 90°C 1.5 h; (v) $N_2H_4 \cdot H_2O$, EtOH, H_2O, 100°C, 10 h; (vi) EtOH, piperidine, 80°C, 4-8 h.

The known intermediates **9-12** and novel intermediates **4a-4n** were characterized by melting points and spectroscopic techniques (^{1}H NMR, ESI-MS), and the target compounds (**13a-14n**) also were characterized by melting points and spectroscopic techniques (^{1}H NMR, ^{13}C NMR, HRMS, and FT-IR). The melting points of intermediates **9-12** approximately matched with melting points data in existing literatures [42,43].

Since the indole-2-one derivative has an exocyclic double bond, the

1 target compounds could exist in the form of *E* or *Z* isomers. As for

2 compound **13m**, **14a**, **14b**, **14j**, **14k**, and **14m**, the ^{1}H NMR spectra

3 revealed the existence of their inseparable *E*/*Z* mixtures, even the *E*/*Z*

4 ratios using the corresponding chemical shifts and integrals. However,

5 because we could not separate them from the silica gel column

6 chromatography or conventional method, the ^{13}C NMR spectra of these

7 mixtures were not shown.

8 Although several documents had reported that the analogs of title

9 compounds obtained in the similar synthetic method were

10 *Z*-configurations [44,45], confirmation of the stereochemistry of

11 indole-2-one-1,2,3-triazole derivatives still be requisite. Thus, the most

12 potency compound **13d**, as the representative compound, was chosen to

13 undergo the NOESY experiment. The NOE correlation (**Fig. 2**) between

14 H$_6$ (δ=7.73 ppm) and H$_{11}$ (δ=7.85 ppm) suggested that the isomer

15 obtained correspond to *Z*-configuration (*Z*-**13d**), which should not exist in

16 *E*-configuration (*E*-**13d**). This result also illustrated that other compounds

17 (except **13m**, **14a**, **14b**, **14j**, **14k,** and **14m**) also were *Z* isomers.
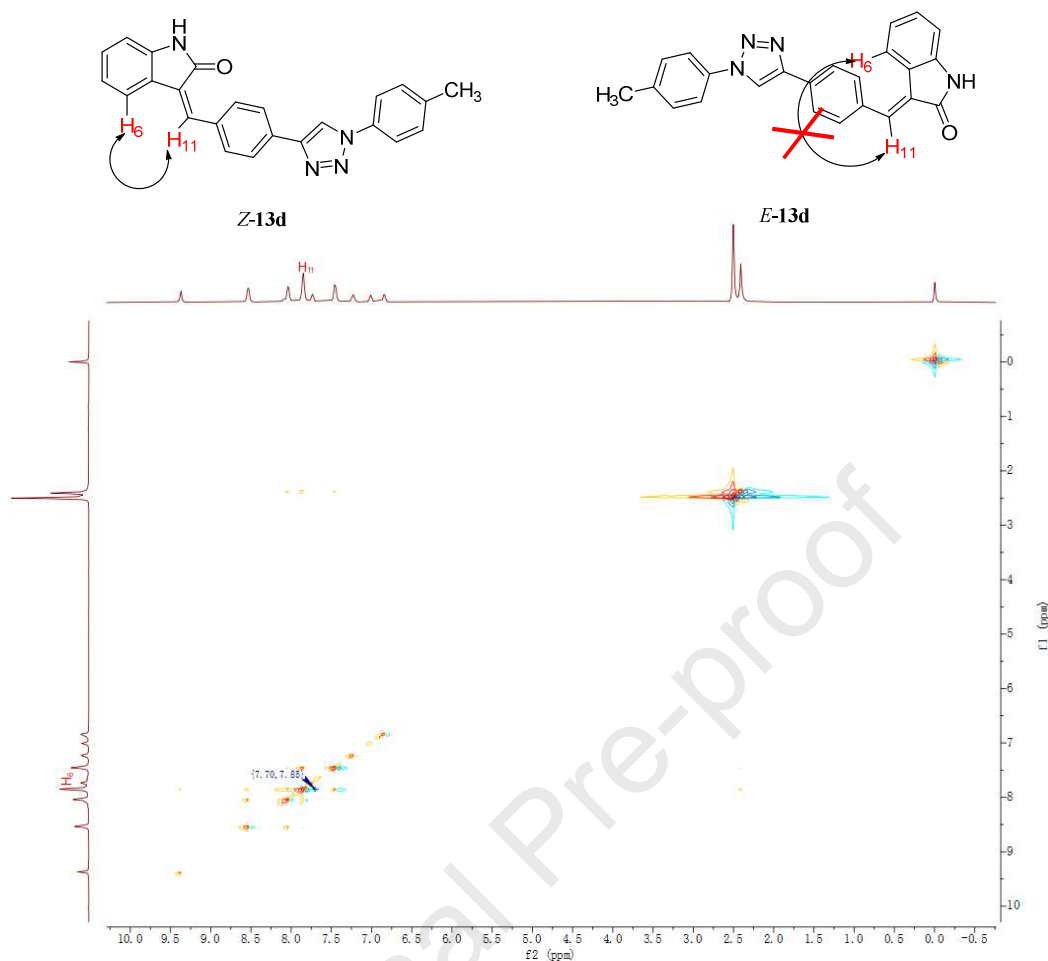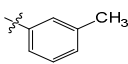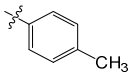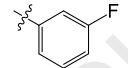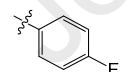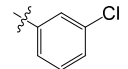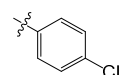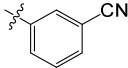
Fig. 2. NOESY of the representative compound **13d.**
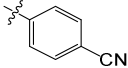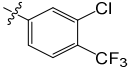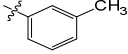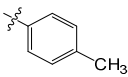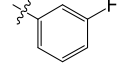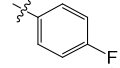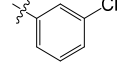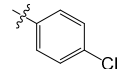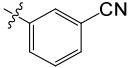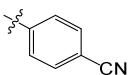
2.2. Biological evaluation
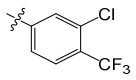
2.2.1. VEGFR-2 inhibitory assay in vitro and structure-activity relationship study (SAR)

To evaluate the inhibitory effects of sunitinib and all indole-2-one derivatives based on 1,2,3-triazole scaffolds (**13a-14n**) on VEGFR-2 kinase activity, VEGFR-2 inhibitory assay was carried out and the results were summarized in **Table 1**. Based on the VEGFR-2 kinase activity evaluation, the preliminary structure-activity relationship (SAR) in this work was summarized.

1   **Table 1**. The IC_{50} value of compounds (**13a-14n**) and sunitinib against

2   VEGFR-2, HT-29 cells, MKN-45 cells, and HUVECs.

Compd NO.	R^1	R^2	IC_{50}[a]			
			VEGFR-2 (nM)	HT-29 (μM)	MKN-45(μM)	HUVECs (μM)
13a	H		93.11±1.95	7.07±1.10	11.59±0.81	12.68±1.69
13b	H		110.60±2.37	8.71±0.48	9.77±0.86	14.69±0.93
13c	H		44.67±0.94	3.94±0.38	4.69±0.24	13.70±0.13
13d	H		**26.38±1.09**	1.61±0.45	1.92±0.37	7.94±0.36
13e	H		115.42±2.37	10.53±0.79	12.54±0.97	19.26±1.07
13f	H		>200	>20	>20	>20
13g	H		198.54±1.37	17.73±3.80	16.70±0.82	14.71±0.23
13h	H		>200	>20	>20	>20
13i	H		>200	>20	>20	>20
13j	H		162.81±2.36	13.99±0.41	16.21±0.99	18.24±0.58
13k	H		169.42±1.45	14.39±1.44	14.63±0.53	19.72±1.02
13l	H		>200	>20	>20	>20

13m	H		178.91±1.36	15.78±2.55	>20	>20
13n	H		108.36±0.88	9.67±0.39	8.89±0.42	>20
14a	F		99.67±0.73	8.12±1.29	11.13±0.17	13.70±0.13
14b	F		71.55±0.16	5.87±0.32	7.21±1.90	8.63±0.27
14c	F		89.16±0.63	7.32±0.64	6.81±1.49	9.88±0.76
14d	F		36.90±0.54	3.98±0.61	34.62±0.34	13.83±0.42
14e	F		139.50±1.65	8.98±0.42	8.51±0.54	14.36±1.99
14f	F		171.12±1.09	12.97±0.72	8.19±1.30	17.25±1.22
14g	F		192.51±0.47	>20	>20	>20
14h	F		119.93±1.36	5.95±0.27	7.54±0.55	18.24±0.58
14i	F		186.44±1.02	10.46±0.43	14.08±0.88	19.42±0.59
14j	F		170.18±2.19	11.24±0.86	15.81±0.52	19.58±2.14
14k	F		187.24±2.88	13.98±0.43	15.21±0.73	>20
14l	F		193.41±0.97	>20	>20	>20
14m	F		190.07±1.98	>20	>20	>20

14n	F		168.89±2.34	16.52±0.77	>20	>20
sunitinib	-	-	83.20±1.36	10.34±0.96	9.25±0.77	6.37±0.59

1   a：IC_{50} values are presented as mean values of at least three independent
2   determinations.

3   We found that compared to the positive drug (sunitinib IC_{50} = 83.20

4   nM), most compounds exhibited excellent inhibitory activity against

5   VEGFR-2. The semi-inhibitory concentration IC_{50} value of seven

6   compounds (**13a**, **13c**, **13d**, **14a**-**14d**) was less than 100 nM, which all

7   showed excellent inhibition to VEGFR- 2. Furthermore, **13d** (IC_{50} =

8   26.38 nM) and **14d** (IC_{50} = 36.9 nM) exhibited the best activities among

9   them.

10   Moreover, we analyzed the effect of two substitutions of the

11   indolin-2-one, **13a-13n** (R_1 = H) and **14a-14n** (R_1 = F) possessed similar

12   IC_{50} values for each comparison. It showed that the substitution effect of

13   the indole-2-one part might not have too obvious value on the

14   structure-activity relationship. Then, concerning the activity of

15   derivatives with an unsubstituted or substituted phenyl group attached to

16   triazole scaffold, incorporation of unsubstituted phenyl group or phenyl

17   substituted by electron-donating groups led to compounds **13a-13d** and

18   **14a-14d** with the better inhibitory action on VEGFR-2 relative to the

19   electron-withdrawing groups substituted analogs (**13e-13n** and **14e-14n**).

20   Furthermore, the activity of tolyl substituted and unsubstituted derivatives

13

was 4-CH$_3$>3-CH$_3$>2-CH$_3$>H. Besides, there was no significant difference in the inhibitory activity between electron-withdrawing groups.

The results showed that the phenyl side groups substituted with electron-donor groups might significantly influence indole 1,2,3-triazole scaffolds' increased potency. In contrast, electron-withdrawing groups might have a common effect or decrease the inhibitory activities of scaffolds.

2.2.2. HT-29 cell lines, MKN-45 cell lines, HUVECs proliferation assay in vitro

CCK-8 assay was used to explore the effects of target compounds on the cell proliferation of human colon cancer (HT-29) cell lines, human gastric cancer (MKN-45) cell lines, and human umbilical vein endothelial cells (HUVECs). The positive control sunitinib was also conducted to assess the inhibitory abilities of cell proliferation of all compounds, shown as IC$_{50}$ values, and summarized in **Table 1**. Including the most potency compound **13d** verified by the VEGFR-2 kinase inhibition assay, the target compounds' overall toxicity to HUVECs was lower than that of sunitinib. Furthermore, compound **13d** had a good effect on inhibiting cell viability for HT-29 and MKN-45 cells. Interestingly, combined with the VEGFR-2 kinase inhibition assay results, the compound **13d** could inhibit the kinase activity of VEGFR-2 more effectively than sunitinib *in vitro* and simultaneously had less toxic to HUVECs.

14

1 *2.2.3. Anti-angiogenesis effect in vitro*

2 Based on the above experimental results, compound **13d** had the most

3 potential to become a VEGFR-2 inhibitor. Therefore, the inhibitory effect

4 of compound **13d** on angiogenesis was further explored. Transwell was

5 used to explore the inhibitory ability of compound **13d** on HUVEC cell

6 migration, and the results were shown in **Fig. 3A**. As the compound

7 concentration increased, the migration ability of HUVECs decreased,

8 signified that the changes have occurred in a concentration-dependent

9 manner. Since compound **13d** was less toxic to HUVECs at low

10 concentrations, the facts excluded the migratory inhibition of compound

11 **13d** caused by toxic effects. At the same time, HUVECs were treated

12 with the same concentration of compound **13d** to explore its effect on

13 HUVECs' ability to form tubules, and the results were shown in **Fig. 3B**.

14 Similarly, the tube formative length decreased with the concentration of

15 **13d** increased.

16 These data indicated that compound **13d** could inhibit HUVECs

17 migration and tube formation *in vitro*, which was an essential step in

18 inhibiting angiogenesis.

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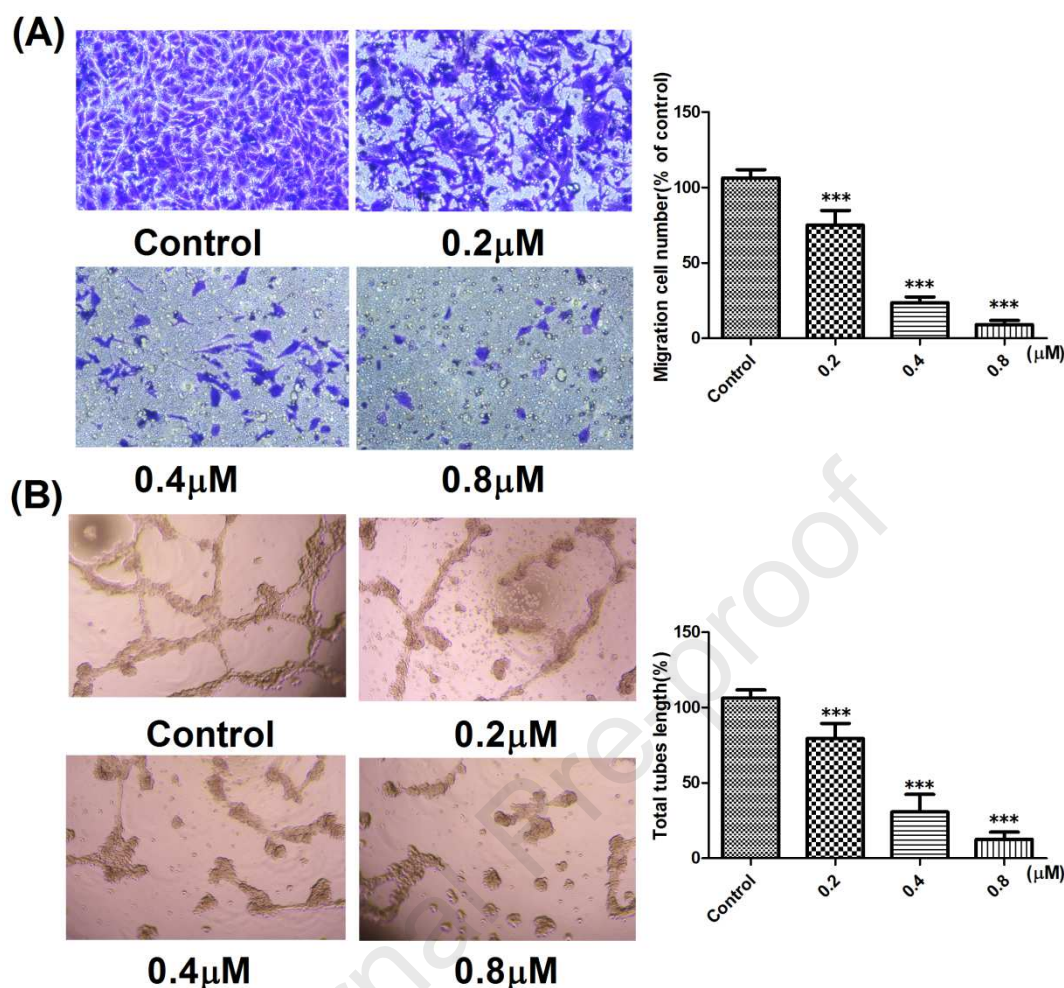
Fig. 3. Compound **13d** inhibited HUVECs migration and the tube

formation. (**A**) Transwell analyzed the inhibitory effects of compound

13d on HUVECs migration. (**B**) Effects of compound **13d** on the tube

formation of HUVECs *in vitro*.

2.2.4. Western blot analysis and anti-angiogenesis research in vivo.

Furthermore, Western blot was carried out to analyze VEGFR-2

protein's phosphorylation on HUVECs cell membranes at different

compound concentrations. The results were shown in **Figure 4A**. As the

concentration of compound **13d** increased, the phosphorylation of

VEGFR-2 in HUVECs decreased. That is, the activation of VEGFR-2

decreased. Simultaneously, to verify the effect of compound **13d** on inhibiting angiogenesis *in vivo*, four groups of blood vessel-specific fluorescent transgenic zebrafish, in a total of 40, were used for anti-angiogenesis research. Zebrafish is currently the ideal vascular biology research and anti-tumor angiogenesis drug evaluation model, which can intuitively observe angiogenesis. The results were shown in **Fig. 4B**. At a 40 mg/L concentration, compound **13d** had a better inhibitory effect on zebrafish internode vascular (ISV) angiogenesis than sunitinib. Also, compound **13d** even effectively inhibited zebrafish ISV regeneration at a lower concentration (20 mg/L).

In general, compound **13d** could effectively inhibit the phosphorylation of VEGFR-2 with better anti-angiogenesis ability than sunitinib *in vivo*. These results were significantly different.

(A)

(B)

Ratio of intact zebrafish ISV roots (% of Control)

1
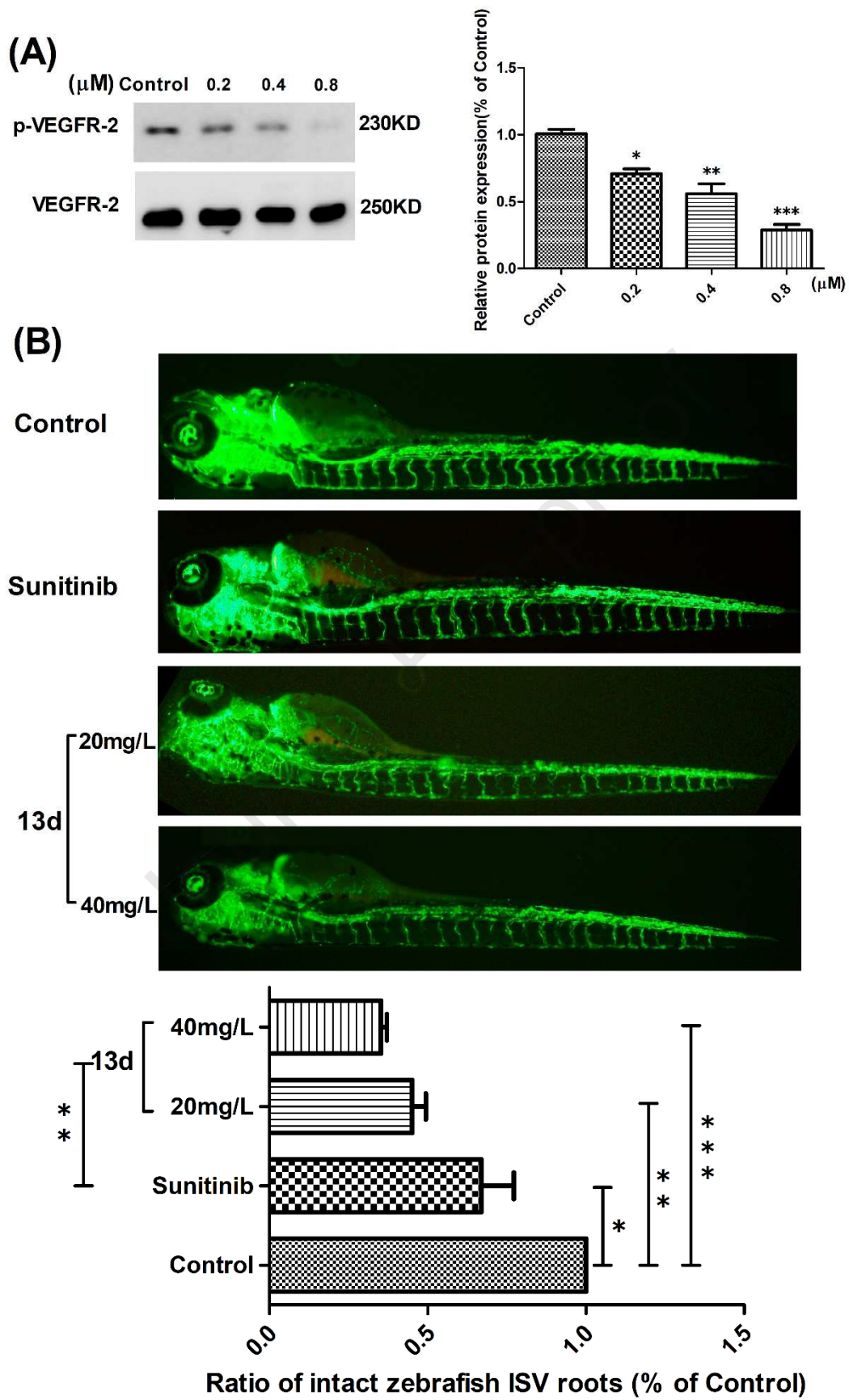
Fig. 4. The compound **13d** could inhibit the phosphorylation of VEGFR-2

and the angiogenesis of zebrafish microvessels. (**A**) Western blot analysis

of the phosphorylation changes of VEGFR-2 with increasing compound

13d concentration. (**B**) The effects of compound **13d** and sunitinib on the

neovascularization of zebrafish *in vivo*.

2.3. Molecular modeling

2.3.1. Molecular docking study

Molecular docking simulations were performed using MOE (Molecular

Operating Environment, version 2016.08, Chemical Computing Group

Inc., Canada) software to study the possible binding pattern of target

compounds in the active site of VEGFR-2. Moreover, the crystal structure

of VEGFR-2 in complex with sunitinib (PDB ID: 4AGD) was adopted in

the docking calculations. The best docking conformation of the potent

compound **13d** based on the compound activity against VEGFR-2 kinase

was selected as the most probable binding conformation. The positive

drug sunitinib, as the co-crystallized ligand, was re-docked using the

same procedure as **13d**.

The re-docking result demonstrated that sunitinib (**Fig. 5B**) could stack

well with the original ligand (**Fig. 5A**), two H-bonds predicted by the

simulation of sunitinib were exactly the same to those existed in the

crystal structure, and the Arene-H conjugates were roughly the same.

These data strongly certified the feasibility of the simulation and

1   rationalized the predictive interactions of the compound **13d** with

2   VEGFR-2 (**Fig. 5C**).

3   The docking conformation of compound **13d** in the binding site

4   showed that carbonyl on the indole fragment formed a strong H-bond

5   with Phe1047, the lateral phenyl and the phenyl group linked to the

6   indole formed Arene-H conjugates with Val848 and Leu840, respectively.

7   By contrast, either H-bonds or the Arene-H interactions of **13d** were less

8   than sunitinib. Simultaneously, as a whole molecule, **13d** spatially

9   extended more in-depth into the pocket of protein; this provided a

10   reasonable explanation that compound **13d** had better kinase activity

11   inhibition ability than sunitinib, which was confirmed by the VEGFR-2

12   kinase assay.

1
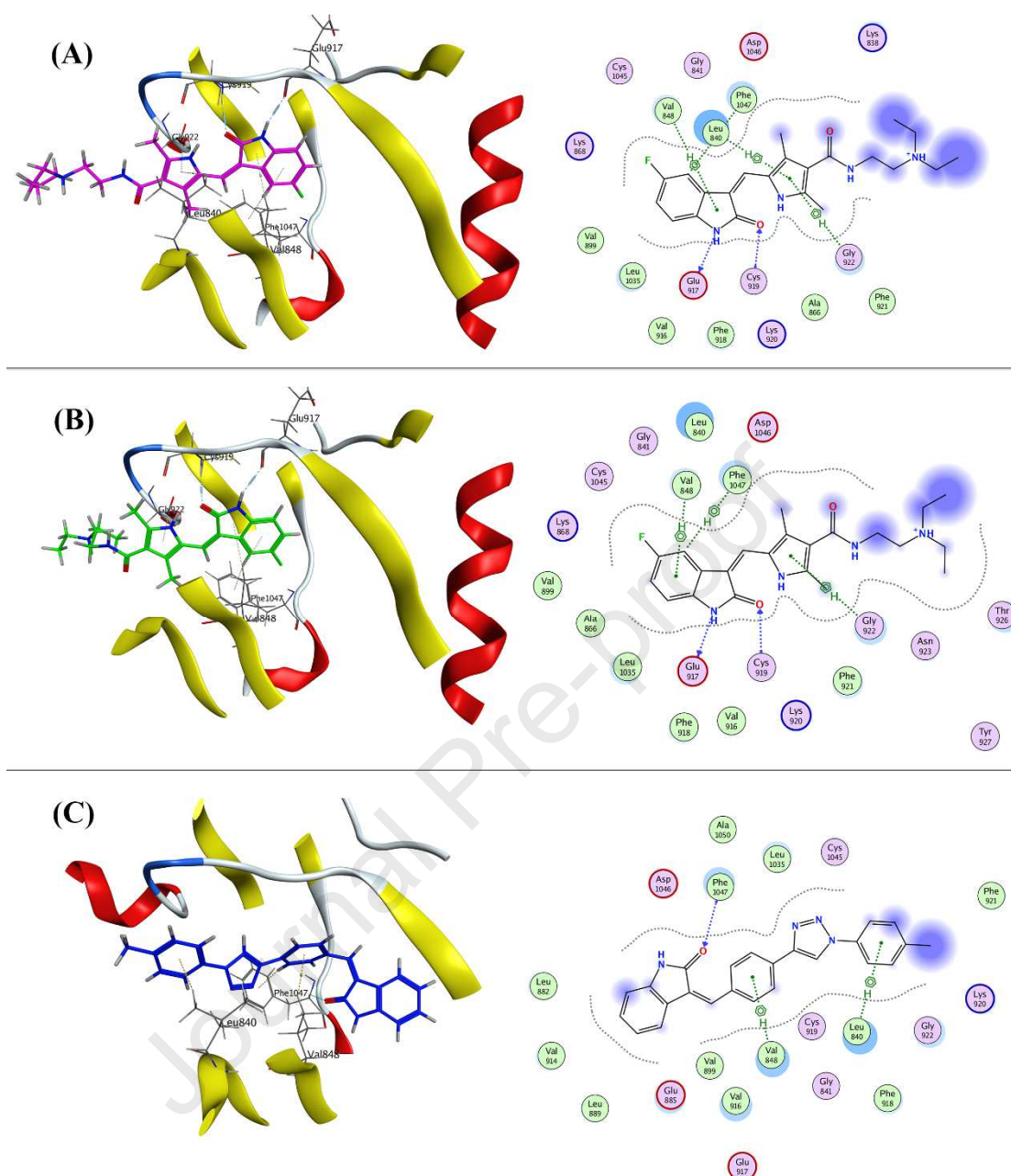
Fig. 5. The 2D diagram and 3D representation of original ligand (**A**),

sunitinib (**B**) and compound **13d** (**C**) showing their interactions with the

VEGFR-2 active site (PDB code: 4AGD).

2.3.2. Molecular dynamics (MD) simulation

Based on the compound activity against VEGFR-2 kinase, the docking

conformation of the potent compound **13d** obtained in MOE (Molecular

Operating Environment) was selected to conduct molecular dynamics (MD) simulation in-depth the binding pose. MD simulation was then carried out in explicit aqueous solution for 10 ns. For comparison, MD simulation of positive control sunitinib in complex with VEGFR-2 was also performed.

The stability of two systems under simulation was evaluated by the root-mean-square deviation (RMSD) of the backbone atoms related to the starting structures (**Fig. 6**). As can be seen in the plots, all systems were stable during the 10-ns MD simulation; the values of RMSD of compound **13d** remained between 2 and 2.5 Å after reaching the summit, which demonstrated that the hit compound **13d** was stabilized in the active site.
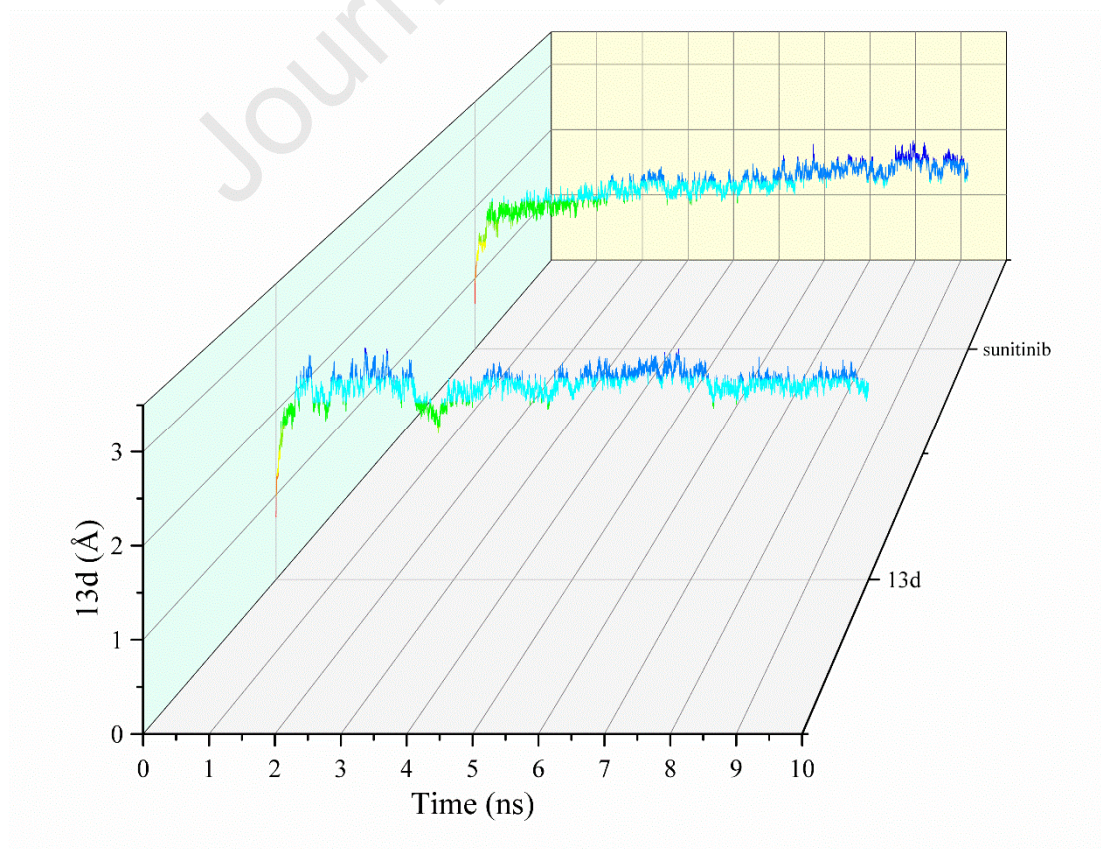
1 **Fig. 6.** RMSD tendency of two systems (**13d** and sunitinib) versus time in

2 the 10 ns MD simulation.

3 *2.4. Toxicity prediction*

4 **Table 2**. The pharmacokinetic studies of **13d** and sunitinib.

Compound NO.	Pharmacokinetic studies			
	Water solubility [b]	Plasma protei binding [c]	Acute Oral Toxicity [d]	Tetrahymena pyriformis [e]
13d	-3.051	0.831	1.773	1.299
Sunitinib	-3.217	0.914	2.683	1.653

5 [b] unit: logS; [c] unit: 100%; [d] unit: kg/mol; [e] unit: $pIGC_{50}$ (ug/L).

6 As a comprehensive and open-source tool, admetSAR was used to

7 predict **13d** and sunitinib's toxicity, and the results were shown in **Table 2**

8 [46]. Since the acute oral toxicity units and Tetrahymena pyriformis are

9 kg/mol and $pIGC_{50}$ (ug/L), respectively (the lower value represents the

10 lower toxicity), compound **13d** had low toxicity than sunitinib in both

11 toxicity prediction assays, which inconsistent with the result of HUVECs

12 proliferation assay.

13 **3. Conclusion**

14 The newly synthesized indolin-2-one derivatives based on

15 1,2,3-triazole scaffolds, evaluated as VEGFR-2 inhibitors, showed

16 promising activity in the VEGFR-2 kinase inhibition assay. Among them,

17 compound **13d** showed better activity inhibition on VEGFR-2 and

18 lowered toxicity to HUVECs, and had an excellent inhibitory effect on

19 HT-29 and MKN-45 cells than sunitinib. Transwell and tube formation

20 experiments showed that compound **13d** inhibited HUVECs migration

and tube formation ability in a concentration-dependent manner. Furthermore, western blot analysis manifested compound **13d** could decrease the phosphorylation of VEGFR-2 in HUVECs. At the same time, by using VEGFR-2 specific fluorescent transgenic zebrafish, the inhibitory effect of compound **13d** on angiogenesis was verified *in vivo*. Besides, the rationality and scientificity of molecular design were verified by docking research and molecular dynamics simulation.

In conclusion, we designed and synthesized compound **13d** as a novel VEGFR-2 inhibitor that is more effective, less toxic than sunitinib. Compound **13d** provided a better choice for drug research and the development of new VEGFR-2 inhibitors.

4. Experimental

4.1. Chemistry

4.1.1. General methods

Unless otherwise stated, all chemical reagents and solvents were purchased from commercial sources and can be used without further purification. Melting points were measured on a capillary electrothermal melting point apparatus without calibration. By thin layer chromatography (TLC), using GF254 silica gel from Qingdao Ocean Chemical Company (Qingdao, China), the reaction progress was monitored with a fluorescent indicator on a 254nm glass plate and visualized by ultraviolet light. Column chromatography was performed

1 using silica gel (200-300 mesh) from Qingdao Ocean Chemical Company

2 (Qingdao, China), recorded ^{1}H NMR and ^{13}C NMR spectra on a Bruker

3 AMX500 (^{1}H at 500 MHz, ^{13}C at 126 MHz) magnetic resonance

4 spectrometer at ambient temperature. All NMR spectra were recorded

5 using DMSO-d_6 as a solvent, and chemical shifts were reported in ppm

6 (parts per million) relative to tetramethylsilane (TMS) as an internal

7 standard.

8 *4.1.2. The synthesis of substituted azido benzenes (2a-2n)*

9 Compound **2a** was synthesized by using the following procedure: To a

10 stirred solution of aniline (**1a**) (3.00 g, 32.21 mmol) in dichloromethane

11 (120 mL) were added 30 mL of aqueous $NaNO_2$ (2.67 g, 38.66 mmol)

12 solution and 37% concentrated hydrochloric acid (HCl) (3 mL)

13 successively. After 0.5 h of stirring at 0°C, a solution of sodium azide

14 (NaN_3) (2.93 g, 45.10 mmol) dissolved in water (20 mL) was added

15 dropwise, and the reaction mixture was kept stirring at $0\text{-}5^\circ$C for 3-5 h.

16 The progress of the reaction was monitored through the TLC test. After

17 the reaction was completed, the organic phase was separated and washed

18 with brine, dried over anhydrous Na_2SO_4, filtered, and the solvent was

19 removed under reduced pressure. The residue was purified by column

20 chromatography on silica gel using petroleum ether to afford the title

21 compound as pale yellow oil liquid.

22 Compounds **2b-2n** were prepared using the identical synthetic

procedure as **2a**.

*4.1.3. The synthesis of substituted azido benzenes (**4a-4n**)*

Compound **4a** was synthesized by using the following procedure: Reaction of commercially 4-ethynylbenzaldehyde (**3**) (1.00 g, 7.68 mmol) with azidobenzene (**2a**) (1.01 g, 8.45 mmol) in 15 mL of DMF proceeded at 50°C for 5 h in the presence of 4 mL of aqueous copper sulfate pentahydrate (CuSO$_4$•5H$_2$O) (0.15 g, 0.60 mmol), ascorbic acid (0.15 g, 0.85 mmol) and a catalytic amount of KI. The progress of the reaction was monitored through the TLC test. After the reaction was completed, the reaction was added 100 mL of water and extracted with ethyl acetate (3×100 mL). The combined extracts were washed with brine, dried over anhydrous Na$_2$SO$_4$, filtered, and the solvent was removed under reduced pressure. The crude residue was purified by column chromatography on silica gel using ethyl acetate/petroleum ether to afford the title compound as pale yellow solid.

Compounds **4b-4n** were prepared using the identical synthetic procedure as **4a**.

*4.1.3.1. 4-(1-phenyl-1H-1,2,3-triazol-4-yl)benzaldehyde (**4a**)*

Light yellow solid, yield: 49.52%, mp: 187.3°C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.04 (s, 1H, CHO), 9.50 (s, 1H, H-triazole), 8.18 (d, J = 7.6 Hz, 2H, Ar-H), 8.05 (d, J = 7.5 Hz, 2H, Ar-H), 7.97 (d, J = 7.8 Hz, 2H, Ar-H), 7.65 (t, J = 7.4 Hz, 2H, Ar-H), 7.54 (t, J = 7.3 Hz, 1H, Ar-H).

ESI-MS [M+H]$^+$ m/z: 250.13.

*4.1.3.2. 4-[1-(o-tolyl)-1H-1,2,3-triazol-4-yl]benzaldehyde (**4b**)*

Light yellow solid, yield: 45.15%, mp: 119.4°C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.04 (s, 1H, CHO), 9.16 (d, J = 1.5 Hz, 1H, H-triazole), 8.18 (s, 2H, Ar-H), 8.04 (d, J = 3.1 Hz, 2H, Ar-H), 7.52 (s, 3H, Ar-H), 7.46 (s, 1H, Ar-H), 2.23 (s, 3H, CH$_3$). ESI-MS [M+H]$^+$ m/z: 264.15.

*4.1.3.3. 4-[1-(m-tolyl)-1H-1,2,3-triazol-4-yl]benzaldehyde (**4c**)*

Light brown solid, yield: 44.12%, mp: 174.4°C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.04 (s, 1H, CHO), 9.48 (s, 1H, H-triazole), 8.17 (d, J = 7.7 Hz, 2H, Ar-H), 8.04 (d, J = 7.7 Hz, 2H, Ar-H), 7.80 (s, 1H, Ar-H), 7.75 (d, J = 7.5 Hz, 1H, Ar-H), 7.52 (t, J = 7.7 Hz, 1H, Ar-H), 7.35 (d, J = 7.0 Hz, 1H, Ar-H), 2.44 (s, 3H, CH$_3$). ESI-MS [M+H]$^+$ m/z: 264.15.

*4.1.3.4. 4-[1-(p-tolyl)-1H-1,2,3-triazol-4-yl]benzaldehyde (**4d**)*

Light brown solid, yield: 46.14%, mp: 189.4-193.3°C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.04 (s, 1H, CHO), 9.46 (d, J = 1.8 Hz, 1H, H-triazole), 8.17 (d, J = 6.4 Hz, 2H, Ar-H), 8.04 (d, J = 6.4 Hz, 2H, Ar-H), 7.84 (d, J = 6.4 Hz, 2H, Ar-H), 7.45 (d, J = 6.6 Hz, 2H, Ar-H), 2.40 (s, 3H, CH$_3$). ESI-MS [M+H]$^+$ m/z: 264.16.

*4.1.3.5. 4-[1-(2-fluorophenyl)-1H-1,2,3-triazol-4-yl]benzaldehyde (**4e**)*

Light yellow solid, yield: 46.26%, mp: 126.8°C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.05 (s, 1H, CHO), 9.30 (s, 1H, H-triazole), 8.20 (d, J = 7.1 Hz, 2H, Ar-H), 8.04 (d, J = 7.2 Hz, 2H, Ar-H), 7.93 (t, J = 7.6 Hz, 1H,

Ar-H), 7.64 (d, J = 10.4 Hz, 2H, Ar-H), 7.49 (t, J = 6.8 Hz, 1H, Ar-H).

ESI-MS [M+H]$^+$ m/z: 268.10.

*4.1.3.6. 4-[1-(3-fluorophenyl)-1H-1,2,3-triazol-4-yl]benzaldehyde (**4f**)*

Light brown solid, yield: 44.25%, mp: 179.9°C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.03 (s, 1H, CHO), 9.53 (s, 1H, H-triazole), 8.15 (d, J = 7.8 Hz, 2H, Ar-H), 8.04 (d, J = 7.8 Hz, 2H, Ar-H), 7.90 – 7.83 (m, 2H, Ar-H), 7.72 – 7.66 (m, 1H, Ar-H), 7.39 (t, J = 8.4 Hz, 1H, Ar-H). ESI-MS [M+H]$^+$ m/z: 268.09.

*4.1.3.7. 4-[1-(4-fluorophenyl)-1H-1,2,3-triazol-4-yl]benzaldehyde (**4g**)*

Yellow solid, yield: 46.54%, mp: 203.5°C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.04 (s, 1H, CHO), 9.47 (s, 1H, H-triazole), 8.16 (d, J = 6.8 Hz, 2H, Ar-H), 8.07 – 7.94 (m, 4H, Ar-H), 7.51 (dd, J = 9.1, 4.8 Hz, 2H, Ar-H). ESI-MS [M+H]$^+$ m/z: 268.10.

*4.1.3.8. 4-[1-(2-chlorophenyl)-1H-1,2,3-triazol-4-yl]benzaldehyde (**4h**)*

Light yellow solid, yield: 42.05%, mp: 121.3°C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.04 (s, 1H, CHO), 9.26 (s, 1H, H-triazole), 8.19 (d, J = 7.8 Hz, 2H, Ar-H), 8.04 (d, J = 7.8 Hz, 2H, Ar-H), 7.82 (t, J = 8.0 Hz, 2H, Ar-H), 7.68 (t, J = 7.6 Hz, 1H, Ar-H), 7.63 (t, J = 7.5 Hz, 1H, Ar-H). ESI-MS [M+H]$^+$ m/z: 284.03.

*4.1.3.9. 4-[1-(3-chlorophenyl)-1H-1,2,3-triazol-4-yl]benzaldehyde (**4i**)*

Light yellow solid, yield: 46.15%, mp: 191.4°C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.04 (s, 1H, CHO), 9.57 (s, 1H, H-triazole), 8.15 (d, J = 6.1

Hz, 2H, Ar-H), 8.09 (s, 1H, Ar-H), 8.04 (d, $J = 4.7$ Hz, 2H, Ar-H), 7.98 (d,

$J = 6.3$ Hz, 1H, Ar-H), 7.68 (t, $J = 6.2$ Hz, 1H, Ar-H), 7.61 (d, $J = 6.1$ Hz,

1H, Ar-H). ESI-MS [M+H]$^+$ m/z: 284.13.

4.1.3.10. 4-[1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl]benzaldehyde (4j)

Light brown solid, yield: 38.95%, mp: 202.5°C. ^{1}H NMR (500 MHz,

DMSO-d_6) δ 10.04 (s, 1H, CHO), 9.53 (s, 1H, H-triazole), 8.16 (d, $J = 8.1$

Hz, 2H, Ar-H), 8.04 (d, $J = 8.2$ Hz, 2H, Ar-H), 8.00 (d, $J = 8.8$ Hz, 2H,

Ar-H), 7.73 (d, $J = 8.8$ Hz, 2H, Ar-H). ESI-MS [M+H]$^+$ m/z: 284.11.

4.1.3.11. 2-[4-(4-formylphenyl)-1H-1,2,3-triazol-1-yl]benzonitrile (4k)

Light brown solid, yield: 49.42%, mp: 217.5°C. ^{1}H NMR (500 MHz,

DMSO-d_6) δ 10.06 (s, 1H, CHO), 9.44 (s, 1H, H-triazole), 8.20 (d, $J = 7.7$

Hz, 3H, Ar-H), 8.07 (d, $J = 7.8$ Hz, 2H, Ar-H), 8.01 (dt, $J = 16.1, 7.9$ Hz,

2H, Ar-H), 7.82 (t, $J = 7.5$ Hz, 1H, Ar-H). ESI-MS [M+H]$^+$ m/z: 275.12.

4.1.3.12. 3-[4-(4-formylphenyl)-1H-1,2,3-triazol-1-yl]benzonitrile (4l)

Light brown solid; yield: 43.19%, mp: 102.3°C. ^{1}H NMR (500 MHz,

DMSO-d_6) δ 10.04 (s, 1H, CHO), 9.60 (s, 1H, H-triazole), 8.48 (s, 1H,

Ar-H), 8.34 (d, $J = 7.4$ Hz, 1H, Ar-H), 8.14 (d, $J = 7.4$ Hz, 2H, Ar-H),

8.03 (dd, $J = 21.2, 6.6$ Hz, 3H, Ar-H), 7.86 (t, $J = 7.8$ Hz, 1H, Ar-H).

ESI-MS [M+H]$^+$ m/z: 275.13.

4.1.3.13. 4-[4-(4-formylphenyl)-1H-1,2,3-triazol-1-yl]benzonitrile (4m)

Light brown solid, yield: 44.05%, mp: 113.5°C. ^{1}H NMR (500 MHz,

DMSO-d_6) δ 10.04 (s, 1H, CHO), 9.64 (s, 1H, H-triazole), 8.19 (d, $J = 8.8$

Hz, 2H, Ar-H), 8.15 (d, J = 6.7 Hz, 4H, Ar-H), 8.04 (d, J = 8.1 Hz, 2H,

Ar-H). ESI-MS [M+H]$^+$ m/z: 275.10.

4.1.3.14.

4-{1-[4-chloro-3-(trifluoromethyl)phenyl]-1H-1,2,3-triazol-4-yl}benzalde

hyde (4n)

Light yellow solid, yield: 45.64%, mp: 209.4°C. ^{1}H NMR (500 MHz,

DMSO-d_6) δ 10.03 (s, 1H, CHO), 9.66 (s, 1H, H-triazole), 8.40 (s, 1H,

Ar-H), 8.30 (d, J = 8.7 Hz, 1H, Ar-H), 8.13 (d, J = 7.9 Hz, 2H, Ar-H),

8.03 (t, J = 8.6 Hz, 3H, Ar-H). ESI-MS [M+H]$^+$ m/z: 352.10.

4.1.4. The synthesis of substituted 2-(hydroxyimino)- N- phenylacetamide

(7-8)

Compound **7** was synthesized by using the following procedure: To a

stirred solution of anhydrous sodium sulfate (40.00 g, 281.62 mmol) in

water (150 mL), chloral hydrate (10.00 g, 60.46 mmol) was added. Then

the commercially aniline (**5**) (3.00 g, 32.21 mmol), 37% concentrated

hydrochloric acid (3.6 mL), and hydroxylamine hydrochloride (8.30 g,

119.45 mmol) were sequentially added. The mixture was stirred at 80°C

for 3 h. After the reaction was completed, the reaction system was cooled

to room temperature, filtered and dried to obtain a crude product as a

brown solid, which was uesd in the next step without purification.

Compound **8** were prepared using the identical synthetic procedure as

7.

*4.1.5. The synthesis of substituted indoline-2,3-dione (**9-10**)*

Compound **9** was synthesized by using the following procedure: A round bottom flask was charged with compound **7** (2.00 g, 12.18 mmol) and concentrated sulfuric acid H_2SO_4 (30 mL). After 0.5 h of stirring at $60^{\circ}C$, the temperature of the reaction mixture was raised to $90^{\circ}C$ for 1.5 h. After the reaction was completed, the reaction system was cooled to room temperature and poured into ice water (80 mL). The solid precipitate was filtered with suction, washed with water (4×50 mL) and dried. The crude residue was purified by column chromatography on silica gel using ethyl acetate/petroleum ether to obtain an orange solid (mp: $194^{\circ}C$).

Compound **10** (mp: $225^{\circ}C$) were prepared using the identical synthetic procedure as **9**.

*4.1.6. The synthesis of substituted indolin-2-one (**11-12**)*

Compound **11** was synthesized by using the following procedure: To a solution of compound **9** (2.00 g, 14.00 mmol) in ethanol (50 mL) was added 80% hydrazine hydrate (12.78 g, 203.90 mmol) and water (30 mL). The reaction mixture was stirred at $100^{\circ}C$ for 10 h. The progress of the reaction was monitored through the TLC test. After the reaction was completed, the solvent was removed under reduced pressure. The crude residue was purified by column chromatography on silica gel using ethyl acetate/petroleum ether to afford the title compound as pale yellow solid.

Compound **12** were prepared using the identical synthetic procedure as

11.

4.1.6.1. indolin-2-one (11)

Light yellow solid, yield: 72.06%, mp: 122.4°C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.38 (s, 1H, N-H), 7.22 – 7.11 (m, 2H, Ar-H), 6.91 (t, J = 7.4 Hz, 1H, Ar-H), 6.81 (d, J = 7.7 Hz, 1H, Ar-H), 3.45 (s, 2H, CH$_2$). ESI-MS [M+H]$^+$ *m/z*: 134.03.

4.1.6.2. 5-fluoroindolin-2-one (12)

Light yellow solid, yield: 62.14%, mp: 142.4°C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.35 (s, 1H, N-H), 7.08 (d, J = 8.4 Hz, 1H, Ar-H), 6.97 (t, J = 9.4 Hz, 1H, Ar-H), 6.77 (d, J = 4.1 Hz, 1H, Ar-H), 3.48 (s, 2H, CH$_2$). ESI-MS [M+H]$^+$ *m/z*: 152.06.

4.1.7. The synthesis of target compounds (13a-14n)

Compound **13a** was synthesized by using the following procedure: Reaction of indolin-2-ones (**11**) (1.00 g, 7.51mmol) with compound **4a** (2.06g, 8.26 mmol) in ethanol (35 mL) was proceeded at 80°C for 4-8 h in the presence of catalytic amount of piperidine. The progress of the reaction was monitored through the TLC test. After the reaction was completed, the reaction system was cooled to room temperature. The precipitate thus formed were collected by filtration, washed with ethanol (2×15 mL), and dried to yield pure target compound **13a** in moderate yield.

Compound **13b-14n** were prepared in moderate to good yields using

1 the identical synthetic procedure as **13a.**

2 *4.1.7.1.*

3 *(Z)-3-[4-(1-phenyl-1H-1,2,3-triazol-4-yl)benzylidene]indolin-2-one (**13a**)*

4 Yellow powder, yield: 62.44%, mp: 243°C. FI-IR (KBr, v_{max} cm^{-1}):

5 3431 (NH), 1704 (C=O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.64 (s, 1H,

6 N-H), 9.43 (s, 1H, H-triazole), 8.10 (d, *J* = 7.8 Hz, 2H, Ar-H), 7.98 (d, *J* =

7 7.7 Hz, 2H, Ar-H), 7.86 (d, *J* = 7.7 Hz, 2H, H-vinylic, Ar-H), 7.68 – 7.62

8 (m, 4H, Ar-H), 7.54 (t, *J* = 7.2 Hz, 1H, Ar-H), 7.25 (t, *J* = 7.5 Hz, 1H,

9 Ar-H), 6.88 (t, *J* = 7.8 Hz, 2H, Ar-H). ^{13}C NMR (126 MHz, DMSO-d_6) δ

10 168.48 (C=O), 146.59 (C-triazole), 142.88, 136.45 (C-vinylic), 135.06,

11 134.09, 131.24 (C-triazole), 130.07, 130.03 (2C), 129.82 (2C), 128.67,

12 127.57, 125.37 (2C), 122.34, 121.03, 120.74, 120.13 (C-vinylic), 119.92

13 (2C), 110.02. ESI-HRMS calcd for $C_{23}H_{17}N_4O$ [M+H]$^+$365.1324, found:

14 365.1398.

15 *4.1.7.2.*

16 *(Z)-3-{4-[1-(o-tolyl)-1H-1,2,3-triazol-4-yl]benzylidene}indolin-2-one*

17 *(**13b**)*

18 Orange powder, yield: 53.42%, mp: 239°C. FI-IR (KBr, v_{max} cm^{-1}):

19 3441 (NH), 1711 (C=O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.65 (s, 1H,

20 N-H), 9.08 (s, 1H, H-triazole), 8.11 (d, *J* = 7.8 Hz, 2H, Ar-H), 7.85 (d, *J* =

21 7.7 Hz, 2H, H-vinylic, Ar-H), 7.67 (s, 1H, Ar-H), 7.63 (d, *J* = 7.6 Hz, 1H,

22 Ar-H), 7.56 – 7.50 (m, 3H, Ar-H), 7.46 (s, 1H, Ar-H), 7.24 (t, *J* = 7.6 Hz,

1H, Ar-H), 6.94 – 6.84 (m, 2H, Ar-H), 2.24 (s, 3H, CH$_3$). ^{13}C NMR (126

MHz, DMSO-d_6) δ 168.50 (C=O), 145.77 (C-triazole), 142.88, 136.07

(C-vinylic), 135.11, 133.97, 132.91 (C-triazole), 132.53, 131.29, 129.99

(2C), 129.78, 127.55, 126.90, 125.83, 125.35 (2C), 124.76, 123.52,

122.33, 121.01 (C-vinylic), 120.77, 110.01, 17.31 (CH$_3$). ESI-HRMS

calcd for C$_{24}$H$_{19}$N$_4$O [M+H]$^+$ 379.1481, found: 379.1560.

4.1.7.3.

(Z)-3-{4-[1-(m-tolyl)-1H-1,2,3-triazol-4-yl]benzylidene}indolin-2-one

(13c)

Brown powder, yield: 64.61%, mp: 240°C. FI-IR (KBr, ν_{max} cm^{-1}): 3405

(NH), 1700 (C=O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.64 (s, 1H, N-H),

9.39 (s, 1H, H-triazole), 8.09 (d, J = 7.2 Hz, 2H, Ar-H), 7.85 (d, J = 7.3

Hz, 2H, H-vinylic, Ar-H), 7.81 (s, 1H, Ar-H), 7.76 (d, J = 7.5 Hz, 1H,

Ar-H), 7.66 (s, 1H, Ar-H), 7.63 (d, J = 7.5 Hz, 1H, Ar-H), 7.51 (t, J = 7.4

Hz, 1H, Ar-H), 7.33 (d, J = 6.9 Hz, 1H, Ar-H), 7.24 (t, J = 7.2 Hz, 1H,

Ar-H), 6.88 (t, J = 8.3 Hz, 2H, Ar-H), 2.44 (s, 3H, CH$_3$). ^{13}C NMR (126

MHz, DMSO-d_6) δ 168.49 (C=O), 146.51 (C-triazole), 142.88, 139.55

(C-vinylic), 136.40, 135.06, 134.06, 131.28 (C-triazole), 130.04, 130.01

(2C), 129.58, 129.22, 127.56, 125.34 (2C), 122.33, 121.01, 120.75,

120.30, 120.04 (C-vinylic), 116.98, 110.01, 20.79 (CH$_3$). ESI-HRMS

calcd for C$_{24}$H$_{19}$N$_4$O [M+H]$^+$ 379.1481, found: 379.1550.

4.1.7.4.

1. *(Z)-3-{4-[1-(p-tolyl)-1H-1,2,3-triazol-4-yl]benzylidene}indolin-2-one*

2. *(13d)*

3. Yellow powder, yield: 51.64%, mp: 248°C. FI-IR (KBr, ν_{max} cm^{-1}):

4. 3427 (NH), 1692 (C=O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.63 (s, 1H,

5. N-H), 9.35 (s, 1H, H-triazole), 8.53 (d, J = 8.2 Hz, 2H, Ar-H), 8.03 (d, J =

6. 8.2 Hz, 2H, Ar-H), 7.85 (d, J = 7.3 Hz, 3H, H-vinylic, Ar-H), 7.73 (d, J =

7. 7.4 Hz, 1H, Ar-H), 7.45 (d, J = 7.9 Hz, 2H, Ar-H), 7.23 (t, J = 7.6 Hz, 1H,

8. Ar-H), 7.01 (t, J = 7.5 Hz, 1H, Ar-H), 6.84 (d, J = 7.7 Hz, 1H, Ar-H), 2.41

9. (s, 3H, CH$_3$). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.99 (C=O), 146.58

10. (C-triazole), 140.67, 138.34 (C-vinylic), 135.86, 134.23, 133.65, 132.50

11. (2C), 131.75 (C-triazole), 130.13 (2C), 128.85, 126.72, 125.37, 124.76

12. (2C), 120.93, 120.11 (C-vinylic), 119.85 (2C), 119.65, 109.23, 20.44

13. (CH$_3$). ESI-HRMS calcd for C$_{24}$H$_{19}$N$_4$O [M+H]$^{+}$ 379.1481, found:

14. 379.1550.

15. *4.1.7.5. (Z)-3-{4-[1-(2-fluorophenyl)-1H-1,2,3-triazol-4-yl]benzylidene}*

16. *indolin-2-one (13e)*

17. Yellow powder, yield: 57.81%, mp: 234°C. FI-IR (KBr, ν_{max} cm^{-1}):

18. 3504 (NH), 1693 (C=O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.63 (s, 1H,

19. N-H), 9.19 (s, 1H, H-triazole), 8.12 (d, J = 7.9 Hz, 2H, Ar-H), 7.93 (t, J =

20. 7.8 Hz, 1H, Ar-H), 7.85 (d, J = 8.0 Hz, 2H, H-vinylic, Ar-H), 7.68 – 7.59

21. (m, 4H, Ar-H), 7.48 (t, J = 7.6 Hz, 1H, Ar-H), 7.24 (t, J = 7.7 Hz, 1H,

22. Ar-H), 6.88 (dd, J = 13.5, 7.3 Hz, 2H, Ar-H). ^{13}C NMR (126 MHz,

DMSO-d_6) δ 168.47 (C=O), 154.69, 152.70, 146.21 (C-triazole), 142.88,

135.02 (C-vinylic), 134.17, 132.50 (C-triazole), 130.99, 130.01 (d, $J =$

6.0 Hz, 2C, C-fluorobenzene), 127.60, 125.83 (d, $J =$ 3.3 Hz,

C-fluorobenzene), 125.44 (d, $J =$ 4.6 Hz, 2C, C-fluorobenzene), 124.86

(C-vinylic), 123.25 (d, $J =$ 4.1 Hz, C-fluorobenzene), 122.33, 121.00,

120.74, 117.11, 116.96, 110.00. ESI-HRMS calcd for $C_{23}H_{16}FN_4O$

$[M+H]^+$ 383.1230, found: 383.1307.

4.1.7.6.   (Z)-3-{4-[1-(3-fluorophenyl)-1H-1,2,3-triazol-4-yl]benzylidene}

indolin-2-one (13f)

Yellow powder, yield: 63.46%, mp: 246°C. FI-IR (KBr, ν_{max} cm^{-1}):

3286 (NH), 1687 (C=O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.66 (s, 1H,

N-H), 9.45 (s, 1H, H-triazole), 8.53 (d, $J =$ 8.0 Hz, 2H, Ar-H), 8.02 (d, $J =$

7.9 Hz, 2H, Ar-H), 7.90 – 7.81 (m, 3H, H-vinylic, Ar-H), 7.70 (dd, $J =$

19.4, 7.4 Hz, 2H, Ar-H), 7.38 (t, $J =$ 8.3 Hz, 1H, Ar-H), 7.22 (t, $J =$ 7.4 Hz,

1H, Ar-H), 7.00 (t, $J =$ 7.4 Hz, 1H, Ar-H), 6.83 (d, $J =$ 7.6 Hz, 1H, Ar-H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 166.98 (C=O), 163.28, 161.33, 146.80

(C-triazole), 140.69, 135.78 (C-vinylic), 133.79 (C-triazole), 132.52 (2C),

131.75 (d, $J =$ 9.4 Hz, C-fluorobenzene), 131.41, 130.05 (d, $J =$ 4.1 Hz,

C-fluorobenzene), 128.87, 126.81, 125.39, 124.78 (2C), 120.92, 120.38

(C-vinylic), 119.66, 115.83 (d, $J =$ 3.4 Hz, C-fluorobenzene), 109.23,

107.29 (d, $J =$ 3.3 Hz, C-fluorobenzene). ESI-HRMS calcd for

$C_{23}H_{16}FN_4O$ $[M+H]^+$ 383.1230, found: 383.1298.

1 *4.1.7.7.   (Z)-3-{4-[1-(4-fluorophenyl)-1H-1,2,3-triazol-4-yl]benzylidene}*

2 *indolin-2-one (13g)*

3 Yellow powder, yield: 63.91%, mp: 238°C. FI-IR (KBr, ν_{max} cm^{-1}):

4 3448 (NH), 1686 (C=O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.66 (s, 1H,

5 N-H), 9.35 (s, 1H, H-triazole), 8.52 (d, J = 7.7 Hz, 2H, Ar-H), 8.01 (d, J =

6 7.6 Hz, 4H, Ar-H), 7.81 (s, 1H, H-vinylic), 7.71 (d, J = 7.1 Hz, 1H, Ar-H),

7 7.49 (t, J = 8.1 Hz, 2H, Ar-H), 7.21 (t, J = 7.1 Hz, 1H, Ar-H), 6.99 (t, J =

8 7.1 Hz, 1H, Ar-H), 6.83 (d, J = 7.3 Hz, 1H, Ar-H). ^{13}C NMR (126 MHz,

9 DMSO-d_6) δ 166.96 (C=O), 162.55, 160.60, 146.69 (C-triazole), 140.66,

10 135.82 (C-vinylic), 133.70 (C-triazole), 133.00 (d, J = 3.0 Hz,

11 C-fluorobenzene), 132.51 (2C), 131.58, 130.02, 128.86, 126.74, 125.35,

12 124.76 (d, J = 3.2 Hz, 2C, C-fluorobenzene), 122.32 (d, J = 8.9 Hz),

13 120.92 (C-vinylic), 120.51, 119.65, 116.65 (d, J = 23.3 Hz,

14 C-fluorobenzene), 109.21. ESI-HRMS calcd for $C_{23}H_{16}FN_4O$ [M+H]$^+$

15 383.1230, found: 383.1313.

16 *4.1.7.8.   (Z)-3-{4-[1-(2-chlorophenyl)-1H-1,2,3-triazol-4-yl]benzylidene}*

17 *indolin-2-one (13h)*

18 Yellow powder, yield: 64.14%, mp: 229°C. FI-IR (KBr, ν_{max} cm^{-1}):

19 3431 (NH), 1710 (C=O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.63 (s, 1H,

20 N-H), 9.17 (s, 1H, H-triazole), 8.11 (d, J = 7.9 Hz, 2H, Ar-H), 7.89 – 7.78

21 (m, 4H, H-vinylic, Ar-H), 7.71 – 7.60 (m, 4H, Ar-H), 7.24 (t, J = 7.7 Hz,

22 1H, Ar-H), 6.88 (t, J = 9.0 Hz, 2H, Ar-H). ^{13}C NMR (126 MHz,

DMSO-d_6) δ 168.47 (C=O), 145.81 (C-triazole), 142.89, 135.86 (C-vinylic), 135.05, 134.32, 132.55, 131.70, 131.11 (C-triazole), 130.47, 130.02 (2C), 128.39, 128.29, 127.63, 125.41 (2C), 124.80, 124.08, 122.35, 121.03 (C-vinylic), 119.66, 110.02. ESI-HRMS calcd for $C_{23}H_{16}ClN_4O$ [M+H]$^+$ 399.0934, found: 399.1011.

4.1.7.9. (Z)-3-{4-[1-(3-chlorophenyl)-1H-1,2,3-triazol-4-yl]benzylidene} indolin-2-one (13i)

Orange powder, yield: 60.19%, mp: 227°C. FI-IR (KBr, ν_{max} cm^{-1}): 3430 (NH), 1685 (C=O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.66 (s, 1H, N-H), 9.48 (s, 1H, H-triazole), 8.53 (d, J = 8.0 Hz, 2H, Ar-H), 8.10 (s, 1H, Ar-H), 8.00 (dd, J = 18.4, 8.0 Hz, 3H, Ar-H), 7.84 (s, 1H, H-vinylic), 7.72 (d, J = 7.6 Hz, 1H, Ar-H), 7.68 (t, J = 8.1 Hz, 1H, Ar-H), 7.60 (d, J = 8.0 Hz, 1H, Ar-H), 7.22 (t, J = 7.5 Hz, 1H, Ar-H), 7.00 (t, J = 7.3 Hz, 1H, Ar-H), 6.84 (d, J = 7.6 Hz, 1H, Ar-H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.97 (C=O), 146.80 (C-triazole), 140.68, 137.46 (C-vinylic), 135.80, 134.10, 133.79, 132.53 (2C), 131.55, 131.42 (C-triazole), 128.89, 128.44, 126.80, 124.77 (3C), 120.93, 120.42 (C-vinylic), 119.71, 119.67, 118.50, 109.23. ESI-HRMS calcd for $C_{23}H_{16}ClN_4O$ [M+H]$^+$ 399.0934, found: 399.1000.

4.1.7.10. (Z)-3-{4-[1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl]benzylidene} indolin-2-one (13j)

Yellow powder, yield: 59.06%, mp: 235°C. FI-IR (KBr, ν_{max} cm^{-1}):

3432 (NH), 1689 (C=O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.66 (s, 1H,

N-H), 9.45 (s, 1H, H-triazole), 8.53 (d, J = 7.9 Hz, 2H, Ar-H), 8.02 (t, J =

8.4 Hz, 4H, Ar-H), 7.84 (s, 1H, H-vinylic), 7.74 (d, J = 7.7 Hz, 3H, Ar-H),

7.22 (t, J = 7.4 Hz, 1H, Ar-H), 7.01 (t, J = 7.4 Hz, 1H, Ar-H), 6.84 (d, J =

7.6 Hz, 1H, Ar-H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.98 (C=O),

146.81 (C-triazole), 140.67, 135.83 (C-vinylic), 135.23, 133.76, 132.95,

132.53 (2C), 131.49 (C-triazole), 129.80 (2C), 128.89, 126.78, 125.61,

124.78 (2C), 121.61 (2C), 120.94, 120.33 (C-vinylic), 119.68, 109.23.

ESI-HRMS calcd for $C_{23}H_{16}ClN_4O$ [M+H]$^+$ 399.0934, found: 399.1018.

4.1.7.11.

(Z)-2-(4-{4-[(2-oxoindolin-3-ylidene)methyl]phenyl}-1H-1,2,3-triazol-1-y

l)benzonitrile (13k)

Brown powder, yield: 49.58%, mp: 239$^°$C. FI-IR (KBr, ν_{max} cm^{-1}): 3432

(NH), 1693 (C=O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.65 (s, 1H, N-H),

9.36 (s, 1H, H-triazole), 8.20 (d, J = 7.8 Hz, 1H, Ar-H), 8.12 (d, J = 7.7

Hz, 2H, Ar-H), 8.06 – 7.96 (m, 2H, Ar-H), 7.88 (d, J = 7.8 Hz, 2H, Ar-H),

7.82 (t, J = 7.5 Hz, 1H, H-vinylic), 7.67 (s, 1H, Ar-H), 7.62 (d, J = 7.7 Hz,

1H, Ar-H), 7.25 (t, J = 7.6 Hz, 1H, Ar-H), 6.88 (t, J = 7.7 Hz, 2H, Ar-H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 168.47 (C=O), 146.49 (C-triazole),

142.91, 137.65 (C-vinylic), 134.95, 134.74, 134.65, 134.39, 132.55

(C-triazole), 130.76, 130.29, 130.06 (2C), 127.73, 125.56, 125.52 (2C),

122.90, 122.38, 121.04, 120.74 (C-vinylic), 115.62, 110.02, 106.97.

1 ESI-HRMS calcd for $C_{24}H_{16}N_5O$ [M+H]$^+$ 390.1277, found: 390.1349.

2 *4.1.7.12.*

3 *(Z)-3-(4-{4-[(2-oxoindolin-3-ylidene)methyl]phenyl}-1H-1,2,3-triazol-1-y*

4 *l)benzonitrile (**13l**)*

5 Yellow powder, yield: 60.12%, mp: 240°C. FI-IR (KBr, v_{max} cm^{-1}):

6 3433 (NH), 1696 (C=O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.62 (s, 1H,

7 N-H), 9.52 (s, 1H, H-triazole), 8.49 (s, 1H, Ar-H), 8.35 (d, J = 8.2 Hz, 1H,

8 Ar-H), 8.07 (d, J = 8.0 Hz, 2H, Ar-H), 8.01 (d, J = 7.7 Hz, 1H, Ar-H),

9 7.87 (d, J = 7.5 Hz, 3H, H-vinylic, Ar-H), 7.66 (s, 1H, Ar-H), 7.62 (d, J =

10 7.7 Hz, 1H, Ar-H), 7.25 (t, J = 7.7 Hz, 1H, Ar-H), 6.88 (t, J = 7.9 Hz, 2H,

11 Ar-H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.46 (C=O), 146.85

12 (C-triazole), 142.92, 136.87 (C-vinylic), 134.93, 134.33, 132.53, 132.18,

13 131.24 (C-triazole), 130.06 (2C), 127.71, 125.41 (2C), 124.79, 124.49,

14 123.26, 122.36, 121.02, 120.74, 120.38 (C-vinylic), 117.62, 112.74,

15 110.02. ESI-HRMS calcd for $C_{24}H_{16}N_5O$ [M+H]$^+$ 390.1277, found:

16 390.1355.

17 *4.1.7.13.*

18 *(Z/E)-4-(4-{4-[(2-oxoindolin-3-ylidene)methyl]phenyl}-1H-1,2,3-triazol-1*

19 *-yl)benzonitrile (**13m**)*

20 Yellow powder, yield: 53.41%, mp: 241°C. FI-IR (KBr, v_{max} cm^{-1}):

21 3430 (NH), 1684 (C=O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.63 (s, 1H,

22 N-H), 9.56 (s, 0.3H, H-triazole), 9.53 (s, 0.7H, H-triazole), 8.52 (d, J =

7.8 Hz, 1.3H, Ar-H), 8.20 (d, *J* = 7.7 Hz, 2H, Ar-H), 8.14 (d, *J* = 8.3 Hz,

2H, Ar-H), 8.08 (d, *J* = 7.6 Hz, 0.7H, Ar-H), 8.02 (d, *J* = 7.9 Hz, 1.3H,

Ar-H), 7.86 (d, *J* = 7.7 Hz, 0.6H, H-vinylic, Ar-H), 7.82 (s, 0.7H,

H-vinylic), 7.71 (d, *J* = 7.4 Hz, 0.7H, Ar-H), 7.65 (s, 0.3H, Ar-H), 7.61 (d,

J = 7.7 Hz, 0.3H, Ar-H), 7.23 (q, *J* = 9.2, 7.6 Hz, 1H, Ar-H), 7.00 (t, *J* =

7.4 Hz, 0.7H, Ar-H), 6.89 (d, *J* = 8.0 Hz, 0.7H, Ar-H), 6.84 (d, *J* = 7.7 Hz,

0.7H, Ar-H). ESI-HRMS calcd for $C_{24}H_{16}N_5O$ [M+H]$^+$ 390.1277, found:

390.1349.

4.1.7.14.

(Z)-3-(4-{1-[4-chloro-3-(trifluoromethyl)phenyl]-1H-1,2,3-triazol-4-yl}be

*nzylidene)indolin-2-one (**13n**)*

Yellow powder, yield: 63.92%, mp: 219$^°$C. FI-IR (KBr, ν_{max} cm^{-1}):

3432 (NH), 1677(C=O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.66 (s, 1H,

N-H), 9.59 (d, *J* = 2.1 Hz, 1H, H-triazole), 8.53 (d, *J* = 6.6 Hz, 2H, Ar-H),

8.43 (s, 1H, Ar-H), 8.32 (d, *J* = 8.4 Hz, 1H, Ar-H), 8.03 (t, *J* = 7.5 Hz, 3H,

Ar-H), 7.84 (s, 1H, H-vinylic), 7.72 (d, *J* = 6.5 Hz, 1H, Ar-H), 7.22 (t, *J* =

6.5 Hz, 1H, Ar-H), 7.00 (t, *J* = 6.4 Hz, 1H, Ar-H), 6.83 (d, *J* = 6.1 Hz, 1H,

Ar-H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.94 (C=O), 146.98

(C-triazole), 140.67, 135.73 (C-vinylic), 135.37, 133.86, 133.26, 132.53

(2C), 131.23 (C-triazole), 130.06, 128.89, 127.81 (d, *J* = 31.6 Hz,

C-trifluoromethylphenyl), 126.84, 125.38, 125.06, 124.75 (d, *J* = 3.6 Hz,

2C, C-trifluoromethylphenyl, Ar-C), 123.23, 120.92, 120.64 (C-vinylic),

119.67, 119.15 (d, J = 5.2 Hz, C-trifluoromethylphenyl), 109.22.

ESI-HRMS calcd for $C_{24}H_{15}ClF_3N_4O$ [M+H]$^+$ 467.0808, found: 467.0887.

4.1.7.15. (Z/E)-5-fluoro-3-[4-(1-phenyl-1H-1,2,3-triazol-4-yl)benzylidene] indolin-2-one (14a)

Orange powder, yield: 69.92%, mp: 229$^{°}$C. FI-IR (KBr, v_{max} cm^{-1}): 3432 (NH), 1687 (C=O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.67 (s, 1H, N-H), 9.44 (s, 0.5H, H-triazole), 9.42 (s, 0.5H, H-triazole), 8.54 (d, J = 7.8 Hz, 1H, Ar-H), 8.12 (d, J = 8.0 Hz, 1H, Ar-H), 8.06 (d, J = 7.9 Hz, 1H, Ar-H), 7.97 (d, J = 7.6 Hz, 2H, Ar-H), 7.92 (s, 0.5H, Ar-H), 7.86 (d, J = 7.7 Hz, 1H, H-vinylic), 7.74 (s, 0.5H, Ar-H), 7.65 (t, J = 7.7 Hz, 2.5H, Ar-H), 7.53 (t, J = 7.4 Hz, 1H, Ar-H), 7.33 (d, J = 9.0 Hz, 0.5H, Ar-H), 7.11 (t, J = 9.0 Hz, 0.5H, Ar-H), 7.05 (t, J = 9.1 Hz, 0.5H, Ar-H), 6.88 (dd, J = 8.4, 4.5 Hz, 0.5H, Ar-H), 6.81 (dd, J = 8.3, 4.3 Hz, 0.5H, Ar-H). ESI-HRMS calcd for $C_{23}H_{16}FN_4O$ [M+H]$^+$ 383.1230, found: 383.1313.

4.1.7.16. (Z/E)-5-fluoro-3-{4-[1-(o-tolyl)-1H-1,2,3-triazol-4-yl] benzylidene} indolin-2-one (14b)

Yellow powder, yield: 55.15%, mp: 221$^{°}$C. FI-IR (KBr, v_{max} cm^{-1}): 3432 (NH), 1701 (C=O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.68 (s, 1H, N-H), 9.09 (d, J = 1.1 Hz, 1H, H-triazole), 8.54 (d, J = 8.2 Hz, 1H, Ar-H), 8.12 (d, J = 8.0 Hz, 1H, Ar-H), 8.06 (d, J = 8.2 Hz, 1H, Ar-H), 7.93 (s, 0.4H, Ar-H), 7.85 (d, J = 8.0 Hz, 1H, H-vinylic), 7.74 (s, 0.6H, Ar-H),

7.67 (d, J = 9.0 Hz, 0.4H, Ar-H), 7.56 – 7.51 (m, 3H, Ar-H), 7.48 – 7.43

(m, 1H, Ar-H), 7.32 (d, J = 9.2 Hz, 0.6H, Ar-H), 7.10 (t, J = 8.9 Hz, 0.6H,

Ar-H), 7.04 (t, J = 9.0 Hz, 0.4H, Ar-H), 6.88 (dd, J = 8.0, 4.7 Hz, 0.6H,

Ar-H), 6.81 (dd, J = 7.8, 4.4 Hz, 0.4H, Ar-H), 2.24 (s, 3H, CH$_3$).

ESI-HRMS calcd for C$_{24}$H$_{18}$FN$_4$O [M+H]$^+$ 397.1386, found: 397.1463.

4.1.7.17. (Z)-5-fluoro-3-{4-[1-(m-tolyl)-1H-1,2,3-triazol-4-yl]benzylidene}

indolin-2-one (14c)

Orange powder, yield: 65.29%, mp: 217°C. FI-IR (KBr, v_{max} cm^{-1}):

3432 (NH), 1686 (C=O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.66 (s, 1H,

N-H), 9.39 (s, 1H, H-triazole), 8.54 (d, J = 8.0 Hz, 2H, Ar-H), 8.05 (d, J =

8.0 Hz, 2H, Ar-H), 7.92 (s, 1H, Ar-H), 7.81 (s, 1H, H-vinylic), 7.75 (d, J

= 8.2 Hz, 1H, Ar-H), 7.66 (d, J = 9.3 Hz, 1H, Ar-H), 7.52 (t, J = 7.8 Hz,

1H, Ar-H), 7.34 (d, J = 7.6 Hz, 1H, Ar-H), 7.05 (t, J = 9.4 Hz, 1H, Ar-H),

6.81 (dd, J = 8.6, 4.4 Hz, 1H, Ar-H), 2.45 (s, 3H, CH$_3$). ^{13}C NMR (126

MHz, DMSO-d_6) δ 166.99 (C=O), 158.74, 156.87, 146.52 (C-triazole),

139.55, 137.61 (C-vinylic), 136.85, 136.37, 133.39, 132.75 (2C), 132.08

(C-triazole), 129.58, 129.25, 126.31 (d, J = 8.6 Hz, C-fluorobenzene),

124.79 (2C, C-vinylic, Ar-C), 120.30 (d, J = 6.3 Hz, C-fluorobenzene),

117.03, 114.98 (d, J = 24.0 Hz, C-fluorobenzene), 109.94 (d, J = 8.3 Hz,

C-fluorobenzene), 107.20, 107.00, 20.79 (CH$_3$). ESI-HRMS calcd for

C$_{24}$H$_{18}$FN$_4$O [M+H]$^+$ 397.1386, found: 397.1460.

4.1.7.18. (Z)-5-fluoro-3-{4-[1-(p-tolyl)-1H-1,2,3-triazol-4-yl]benzylidene}

1 *indolin-2-one (**14d**)*

2 Orange powder, yield: 56.25%, mp: 204°C. FI-IR (KBr, ν_{max} cm^{-1}):

3 3433 (NH), 1683 (C=O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.67 (s, 1H,

4 N-H), 9.36 (s, 1H, H-triazole), 8.54 (d, J = 7.9 Hz, 2H, Ar-H), 8.05 (d, J =

5 7.8 Hz, 2H, Ar-H), 7.92 (s, 1H, Ar-H), 7.84 (d, J = 7.6 Hz, 2H, H-vinylic,

6 Ar-H), 7.67 (d, J = 9.0 Hz, 1H, Ar-H), 7.45 (d, J = 7.8 Hz, 2H, Ar-H),

7 7.05 (t, J = 9.1 Hz, 1H, Ar-H), 6.81 (dd, J = 7.8, 4.2 Hz, 1H, Ar-H), 2.40

8 (s, 3H, CH$_3$). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.01 (C=O), 158.76,

9 156.89, 146.50 (C-triazole), 138.35, 137.65, 136.86 (C-vinylic), 134.19,

10 133.38, 132.76 (2C), 132.13 (C-triazole), 130.14 (2C), 126.35 (d, J = 3.4

11 Hz, C-fluorobenzene), 124.80 (2C), 120.22 (C-vinylic), 119.82 (2C),

12 114.99 (d, J = 24.1 Hz, C-fluorobenzene), 109.96 (d, J = 8.1 Hz,

13 C-fluorobenzene), 107.12 (d, J = 25.4 Hz, C-fluorobenzene), 20.44 (CH$_3$).

14 ESI-HRMS calcd for C$_{24}$H$_{18}$FN$_4$O [M+H]$^{+}$ 397.1386, found: 397.1464.

15 *4.1.7.19.*

16 *(Z)-5-fluoro-3-{4-[1-(2-fluorophenyl)-1H-1,2,3-triazol-4-yl]benzylidene}i*

17 *ndolin-2-one (**14e**)*

18 Yellow powder, yield: 64.18%, mp: 248°C. FI-IR (KBr, ν_{max} cm^{-1}):

19 3432 (NH), 1699 (C=O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.66 (s, 1H,

20 N-H), 9.21 (s, 1H, H-triazole), 8.14 (d, J = 7.9 Hz, 2H, Ar-H), 7.93 (t, J =

21 7.6 Hz, 1H, Ar-H), 7.84 (d, J = 7.8 Hz, 2H, H-vinylic, Ar-H), 7.73 (s, 1H,

22 Ar-H), 7.68 – 7.58 (m, 2H, Ar-H), 7.48 (t, J = 7.4 Hz, 1H, Ar-H), 7.31 (d,

J = 9.0 Hz, 1H, Ar-H), 7.09 (t, J = 8.6 Hz, 1H, Ar-H), 6.90 – 6.84 (m, 1H,

Ar-H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.44 (C=O), 157.95, 156.08,

154.71, 152.71, 146.14 (C-triazole), 139.21, 136.73 (C-vinylic), 133.72,

131.34 (C-triazole), 130.03 (2C), 127.32 (d, J = 2.7 Hz, C-fluorobenzene),

125.83, 125.57 (2C, C-vinylic, Ar-C), 125.45 (d, J = 3.5 Hz,

C-fluorobenzene), 123.38 (d, J = 4.2 Hz, C-fluorobenzene), 121.68 (d, J

= 8.7 Hz, C-fluorobenzene), 117.06 (d, J = 19.4 Hz, C-fluorobenzene),

116.41, 110.68 (d, J = 8.4 Hz, C-fluorobenzene), 109.23. ESI-HRMS

calcd for $C_{23}H_{15}F_2N_4O$ [M+H]$^+$ 401.1136, found: 401.1201.

4.1.7.20.

(Z)-5-fluoro-3-{4-[1-(3-fluorophenyl)-1H-1,2,3-triazol-4-yl]benzylidene}i

ndolin-2-one (14f)

Orange powder, yield: 51.56%, mp: 241°C. FI-IR (KBr, v_{max} cm^{-1}):

3432 (NH), 1685 (C=O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.65 (s, 1H,

N-H), 9.45 (s, 1H, H-triazole), 8.53 (d, J = 8.1 Hz, 2H, Ar-H), 8.03 (d, J =

8.2 Hz, 2H, Ar-H), 7.93 – 7.82 (m, 3H, H-vinylic, Ar-H), 7.74 – 7.62 (m,

2H, Ar-H), 7.39 (t, J = 8.5 Hz, 1H, Ar-H), 7.04 (t, J = 8.9 Hz, 1H, Ar-H),

6.81 (dd, J = 8.1, 4.4 Hz, 1H, Ar-H). ^{13}C NMR (126 MHz, DMSO-d_6) δ

168.43 (C=O), 163.27, 161.32, 156.08, 146.62 (C-triazole), 139.23,

137.63 (d, J = 10.4 Hz, C-fluorobenzene), 136.70 (C-vinylic), 133.76,

132.75, 131.78 (d, J = 9.3 Hz, C-fluorobenzene), 131.34 (C-triazole),

130.08 (2C), 127.35 (d, J = 2.8 Hz, C-fluorobenzene), 125.47 (2C),

124.83 (C-vinylic), 120.46 (d, J = 8.8 Hz, C-fluorobenzene), 116.34 (d, J

= 23.6 Hz, C-fluorobenzene), 115.80 (d, J = 2.9 Hz, C-fluorobenzene),

110.70 (d, J = 8.1 Hz, C-fluorobenzene), 107.50 (d, J = 3.8 Hz,

C-fluorobenzene). ESI-HRMS calcd for $C_{23}H_{15}F_2N_4O$ [M+H]$^+$ 401.1136,

found: 401.1212.

4.1.7.21.

(Z)-5-fluoro-3-{4-[1-(4-fluorophenyl)-1H-1,2,3-triazol-4-yl]benzylidene}i

ndolin-2-one (14g)

Orange powder, yield: 66.81%, mp: 252$^°$C. FI-IR (KBr, v_{max} cm^{-1}):

3440 (NH), 1689 (C=O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.66 (s, 1H,

N-H), 9.36 (s, 1H, H-triazole), 8.52 (d, J = 8.1 Hz, 2H, Ar-H), 8.04 – 7.97

(m, 4H, Ar-H), 7.88 (s, 1H, H-vinylic), 7.64 (d, J = 8.5 Hz, 1H, Ar-H),

7.49 (t, J = 8.5 Hz, 2H, Ar-H), 7.03 (t, J = 8.8 Hz, 1H, Ar-H), 6.80 (dd, J

= 8.2, 4.3 Hz, 1H, Ar-H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.00

(C=O), 162.54, 160.59, 158.74, 156.87, 146.62 (C-triazole), 137.56,

136.84 (C-vinylic), 133.42, 132.96 (d, J = 2.8 Hz, C-fluorobenzene),

132.74 (2C), 131.95 (C-triazole), 126.36 (d, J = 3.7 Hz, C-fluorobenzene),

124.78 (2C), 122.25 (d, J = 9.0 Hz, C-fluorobenzene), 120.53 (C-vinylic),

116.62 (d, J = 23.1 Hz, 2C, C-fluorobenzene), 114.95 (d, J = 23.8 Hz,

C-fluorobenzene), 109.92 (d, J = 8.3 Hz, C-fluorobenzene), 107.09 (d, J

= 25.4 Hz, C-fluorobenzene). ESI-HRMS calcd for $C_{23}H_{15}F_2N_4O$ [M+H]$^+$

401.1208, found: 401.1215.

4.1.7.22.

(Z)-3-{4-[1-(2-chlorophenyl)-1H-1,2,3-triazol-4-yl]benzylidene}-5-fluoroi

*ndolin-2-one (**14h**)*

Yellow powder, yield 57.36%, mp: 235°C. FI-IR (KBr, ν_{max} cm^{-1}): 3432 (NH), 1708 (C=O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.68 (s, 1H, N-H), 9.19 (s, 1H, H-triazole), 8.13 (d, *J* = 7.8 Hz, 2H, Ar-H), 7.89 – 7.78 (m, 4H, H-vinylic, Ar-H), 7.74 (s, 1H, Ar-H), 7.71 – 7.61 (m, 2H, Ar-H), 7.32 (d, *J* = 9.1 Hz, 1H, Ar-H), 7.10 (t, *J* = 8.7 Hz, 1H, Ar-H), 6.88 (dd, *J* = 8.0, 4.5 Hz, 1H, Ar-H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.44 (C=O), 157.96, 156.08, 145.73 (C-triazole), 139.21, 136.77 (C-vinylic), 134.29, 133.67, 131.70, 131.45, 130.46 (C-triazole), 130.07 (2C), 128.47, 128.33 (d, *J* = 14.0 Hz, C-fluorobenzene), 127.32 (d, *J* = 3.0 Hz, C-fluorobenzene), 125.49 (2C), 124.20 (C-vinylic), 121.69 (d, *J* = 8.7 Hz, C-fluorobenzene), 116.34 (d, *J* = 23.8 Hz, C-fluorobenzene), 110.70 (d, *J* = 8.4 Hz, C-fluorobenzene), 109.33 (d, *J* = 25.7 Hz, C-fluorobenzene). ESI-HRMS calcd for $C_{23}H_{15}ClFN_4O$ [M+H]$^+$ 417.0840, found: 417.0921.

4.1.7.23.

(Z)-3-{4-[1-(3-chlorophenyl)-1H-1,2,3-triazol-4-yl]benzylidene}-5-fluoroi

*ndolin-2-one (**14i**)*

Orange powder, yield: 64.52%, mp: 248°C. FI-IR (KBr, ν_{max} cm^{-1}): 3432 (NH), 1685 (C=O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.66 (s, 1H, N-H), 9.47 (s, 1H, H-triazole), 8.53 (d, *J* = 8.0 Hz, 2H, Ar-H), 8.08 (s, 1H,

Ar-H), 8.02 (d, J = 8.0 Hz, 2H, Ar-H), 7.97 (d, J = 8.1 Hz, 1H, Ar-H),

7.90 (s, 1H, H-vinylic), 7.66 (q, J = 7.9 Hz, 2H, Ar-H), 7.59 (d, J = 8.0

Hz, 1H, Ar-H), 7.04 (t, J = 9.0 Hz, 1H, Ar-H), 6.80 (dd, J = 8.2, 4.3 Hz,

1H, Ar-H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.99 (C=O), 158.74,

156.88, 146.72 (C-triazole), 137.49 (d, J = 14.2 Hz, C-fluorobenzene),

136.87 (C-vinylic), 134.10, 133.52, 132.76 (2C), 131.79, 131.53

(C-triazole), 128.44, 126.44 (d, J = 2.9 Hz, C-fluorobenzene), 126.29 (d,

J = 8.9 Hz, C-fluorobenzene), 124.81 (2C), 120.49 (C-vinylic), 119.68,

118.47, 115.00 (d, J = 23.9 Hz, C-fluorobenzene), 109.95 (d, J = 8.1 Hz,

C-fluorobenzene), 107.12 (d, J = 25.4 Hz, C-fluorobenzene). ESI-HRMS

calcd for $C_{23}H_{15}ClFN_4O$ [M+H]$^+$ 417.0840, found: 417.0914.

4.1.7.24.

(Z/E)-3-{4-[1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl]benzylidene}-5-fluo

roindolin-2-one (14j)

Orange powder, yield: 48.67%, mp: 233°C. FI-IR (KBr, v_{max} cm^{-1}):

3439 (NH), 1687 (C=O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.67 (s, 1H,

N-H), 9.45 (d, J = 11.2 Hz, 1H, H-triazole), 8.53 (d, J = 8.3 Hz, 1H,

Ar-H), 8.10 (d, J = 8.6 Hz, 1H, Ar-H), 8.02 (dd, J = 14.0, 8.5 Hz, 3H,

Ar-H), 7.91 (s, 0.5H, Ar-H), 7.86 (d, J = 8.0 Hz, 1H, H-vinylic), 7.73 (d, J

= 7.4 Hz, 2.5H, Ar-H), 7.66 (d, J = 9.0 Hz, 0.5H, Ar-H), 7.31 (d, J = 9.2

Hz, 0.5H, Ar-H), 7.11 (t, J = 8.8 Hz, 0.5H, Ar-H), 7.04 (t, J = 8.9 Hz,

0.5H, Ar-H), 6.88 (dd, J = 8.4, 4.6 Hz, 0.5H, Ar-H), 6.81 (dd, J = 8.3, 4.3

Hz, 0.5H, Ar-H). ESI-HRMS calcd for $C_{23}H_{15}ClFN_4O$ [M+H]$^+$ 417.0840, found: 417.0909.

4.1.7.25.

(Z/E)-2-(4-{4-[(5-fluoro-2-oxoindolin-3-ylidene)methyl]phenyl}-1H-1,2,3 -triazol-1-yl)benzonitrile (14k)

Brown powder, yield: 58.26%, mp: 218$^{°}$C. FI-IR (KBr, v_{max} cm^{-1}): 3439 (NH), 1690 (C=O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.68 (s, 1H, N-H), 9.36 (s, 1H, H-triazole), 8.55 (d, J = 7.7 Hz, 1.7H, Ar-H), 8.19 (d, J = 7.2 Hz, 1H, Ar-H), 8.14 (d, J = 7.2 Hz, 0.3H, Ar-H), 8.07 (d, J = 7.6 Hz, 1.7H, Ar-H), 7.99 (t, J = 9.3 Hz, 2H, Ar-H), 7.93 (s, 0.8H, Ar-H), 7.88 (d, J = 6.8 Hz, 0.3H, Ar-H), 7.81 (t, J = 6.7 Hz, 1H, H-vinylic), 7.74 (s, 0.2H, Ar-H), 7.67 (d, J = 8.4 Hz, 0.8H, Ar-H), 7.32 (d, J = 8.8 Hz, 0.2H, Ar-H), 7.10 (d, J = 8.7 Hz, 0.2H, Ar-H), 7.05 (t, J = 8.8 Hz, 0.8H, Ar-H), 6.88 (s, 0.2H, Ar-H), 6.81 (d, J = 4.6 Hz, 0.8H, Ar-H). ESI-HRMS calcd for $C_{24}H_{15}FN_5O$ [M+H]$^+$ 408.1182, found: 408.1260.

4.1.7.26.

(Z)-3-(4-{4-[(5-fluoro-2-oxoindolin-3-ylidene)methyl]phenyl}-1H-1,2,3-tr iazol-1-yl)benzonitrile (14l)

Yellow powder, yield: 63.43%, mp: 246$^{°}$C. FI-IR (KBr, v_{max} cm^{-1}): 3430 (NH), 1679 (C=O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.68 (s, 1H, N-H), 9.53 (s, 1H, H-triazole), 8.54 (d, J = 7.9 Hz, 2H, Ar-H), 8.49 (s, 1H, Ar-H), 8.35 (d, J = 8.3 Hz, 1H, Ar-H), 8.02 (t, J = 7.6 Hz, 3H, Ar-H), 7.92

(s, 1H, Ar-H), 7.87 (t, J = 8.0 Hz, 1H, H-vinylic), 7.67 (d, J = 8.8 Hz, 1H,

Ar-H), 7.05 (t, J = 9.0 Hz, 1H, Ar-H), 6.81 (dd, J = 8.3, 4.3 Hz, 1H, Ar-H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 166.99 (C=O), 158.75, 156.88, 146.85

(C-triazole), 137.52, 136.83 (C-vinylic), 133.61, 132.79 (2C), 132.23,

131.68, 131.25 (C-triazole), 130.27, 126.52 (d, J = 3.2 Hz,

C-fluorobenzene), 124.84 (2C), 124.55, 123.31, 120.62 (C-vinylic),

117.64, 115.05 (d, J = 24.3 Hz, C-fluorobenzene), 112.71, 109.98 (d, J =

8.3 Hz, C-fluorobenzene), 107.16 (d, J = 25.4 Hz, C-fluorobenzene).

ESI-HRMS calcd for $C_{24}H_{15}FN_5O$ [M+H]$^+$ 408.1182, found: 408.1265.

4.1.7.27.

(Z/E)-4-(4-{4-[(5-fluoro-2-oxoindolin-3-ylidene)methyl]phenyl}-1H-1,2,3

-triazol-1-yl)benzonitrile (14m)

Yellow powder, yield: 59.91%, mp: 215°C. FI-IR (KBr, v_{max} cm^{-1}):

3432 (NH), 1684 (C=O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.68 (s, 1H,

N-H), 9.10 (s, 1H, H-triazole), 8.54 (d, J = 8.2 Hz, 1H, Ar-H), 8.12 (d, J =

8.0 Hz, 1H, Ar-H), 8.06 (d, J = 8.2 Hz, 1H, Ar-H), 7.93 (s, 0.4H, Ar-H),

7.85 (d, J = 7.9 Hz, 1H, H-vinylic), 7.74 (s, 0.6H, Ar-H), 7.67 (d, J = 8.8

Hz, 0.4H, Ar-H), 7.56 – 7.50 (m, 3H, Ar-H), 7.46 (s, 1H, Ar-H), 7.32 (d, J

= 9.2 Hz, 0.6H, Ar-H), 7.11 (t, J = 8.8 Hz, 0.6H, Ar-H), 7.05 (t, J = 8.9

Hz, 0.4H, Ar-H), 6.88 (dd, J = 8.3, 4.6 Hz, 0.6H, Ar-H), 6.81 (dd, J = 8.3,

4.3 Hz, 0.4H, Ar-H). ESI-HRMS calcd for $C_{24}H_{15}FN_5O$ [M+H]$^+$ 408.1182,

found: 408.1259.

1 *4.1.7.28.*

2 *(Z)-3-(4-{1-[4-chloro-3-(trifluoromethyl)phenyl]-1H-1,2,3-triazol-4-yl}be*

3 *nzylidene)-5-fluoroindolin-2-one (**14n**)*

4   Orange powder, yield: 47.50%, mp: 251°C. FI-IR (KBr, v_{max} cm^{-1}):

5 3432 (NH), 1684 (C=O). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.66 (s, 1H,

6 N-H), 9.58 (s, 1H, H-triazole), 8.52 (d, J = 7.5 Hz, 2H, Ar-H), 8.41 (s, 1H,

7 Ar-H), 8.31 (d, J = 8.7 Hz, 1H, Ar-H), 8.01 (d, J = 6.9 Hz, 3H, Ar-H),

8 7.89 (s, 1H, H-vinylic), 7.64 (d, J = 8.7 Hz, 1H, Ar-H), 7.03 (t, J = 8.8 Hz,

9 1H, Ar-H), 6.80 (s, 1H, Ar-H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.96

10 (C=O), 158.73, 156.86, 146.91 (C-triazole), 137.48, 136.87 (C-vinylic),

11 135.35, 133.60, 133.01 (d, J = 64.2 Hz, 2C, C- trifluoromethylphenyl),

12 131.61 (C-triazole), 130.30, 127.81 (d, J = 31.7 Hz, C-

13 trifluoromethylphenyl), 126.49 (d, J = 3.0 Hz, C- trifluoromethylphenyl),

14 126.26 (d, J = 8.8 Hz, C- trifluoromethylphenyl), 124.94 (d, J = 29.6 Hz,

15 2C, C-fluorobenzene, C- trifluoromethylphenyl), 123.23, 121.06, 120.73

16 (C-vinylic), 119.15 (d, J = 5.5 Hz, C-fluorobenzene), 115.03 (d, J = 24.0

17 Hz, C-fluorobenzene), 109.96 (d, J = 8.4 Hz, C-fluorobenzene), 107.13 (d,

18 J = 25.1 Hz, C-fluorobenzene). ESI-HRMS calcd for $C_{24}H_{14}ClF_4N_4O$

19 [M+H]$^{+}$ 485.0787, found: 485.0793.

20 *4.2. VEGFR-2 kinase assay in vitro*

21   The ADP-Glo kinase assay kit (Promega, Madison) was used for

22 VEGFR-2 kinase analysis. According to manufacturer's instructions, The

general procedure is as follows: 20 µL mix VEGFR-2 kinase (Invitrogen, USA), 30 µL substrate (ADP-Glo, 50 µM), 30 µL ATP (50 µM) and 20 µL different concentrations (0 nM, 25 nM, 50 nM, 100 nM, 200 nM) of test compounds, and the total volume in a 96-well light-proof microtiter plate are 100µL of final buffer Incubate in liquid. Wells containing substrate and compound-free kinase were used as overall reaction controls. The assay plate was incubated at 37°C for 30 min in the dark. Use a full-wavelength microplate reader to perform detection at dual wavelengths to obtain fluorescence values, which are further used to calculate IC_{50} values. The obtained data were compared with sunitinib as a standard inhibitor for VEGFR-2.

4.3. Cell culture and CCK-8 assay in vitro

Human colon cancer (HT-29) cells, human gastric cancer (MKN-45) cells, human umbilical vein endothelial cells (HUVECs) were purchased from the China Cell Resource Bank and recorded as F0 generation cells. Standardized training according to the protocol provided by the supplier dilute fibronectin to 1 mg/mL with sterile water. Add 25 mL Fetal Bovine Serum (FBS), 5 mL Endothelial Cell Growth Supplement (ECGS), and 5 mL P/S solution to 500 mL Endothelial Cell Medium (ECM) (ScienCell, SC-1001). These operations are protected from light. All compounds were dissolved in DMSO as a 1 mM stock solution. Further dilutions of all compounds were performed in DMEM (GIBCO, 21127-022) incomplete

52

1   medium. The compound was serially diluted to the final concentration:

2   1.25 μM, 2.5 μM, 5 μM, 10 μM, 20 μM. The concentration of DMSO is

3   0.5% [47].

4   The antiproliferative activity was determined using the CCK-8 assay

5   [48]. HT-29, MKN-45, and HUVECs of F4 generation were cultured in

6   culture flasks. When the number of cells reached 80%, the cells were

7   harvested and transferred to a 96-well plate (treated with fibronectin at a

8   concentration of 2 mg/cm^2) and cultured at 3000 cells per well overnight.

9   ECM complete medium was aspirated in DMEM complete medium, and

10   50 ng/mL VEGF was added. 100 μL of the diluted compound was added

11   to the corresponding experimental wells. The mixture was incubated for

12   24 h, and 10 mL of CCK-8 reagent was added to each well and incubated

13   for 4h. The optical density (OD) of each well was detected at 490 nm.

14   *4.4. Transwell assay*

15   A transwell chamber (pore size 8 μm; Corning Costar, Cambridge,

16   Massachusetts, USA) was used to measure the migration capacity of

17   HUVEC. For transwell analysis, HUVECs were treated with serum-free

18   DMEM medium containing different concentrations of compound **13d** (0

19   μM, 0.2 μM, 0.4 μM or 0.8 μM) for 24 hours, and then collected and

20   counted. 1×10^4 HUVECs were inoculated into the upper chamber, and the

21   lower chamber contained DMEM medium supplemented with 20% serum.

22   After 24 hours of incubation, the filter was fixed in methanol and stained

1 with 0.1% crystal violet. Gently wipe the upper surface of the filter,

2 image under the microscope, and count the total number of HUVECs that

3 have migrated through the filter's lower surface [49].

4 *4.5. Tube formation*

5 After treated with different concentrations (0 μM, 0.2 μM, 0.4 μM or

6 0.8 μM) of compound **13d**, the HUVECs were collected. 1×10^4 cells

7 HUVECs were seeded on Matrigel's surface (#354234, Corning, USA),

8 then cultured for 6 hours. The tube formation was then observed and

9 photographed with a microscope. Use image pro plus software to

10 calculate the total length of tubes.

11 *4.6 Western blot analysis*

12 HUVECs were treated with different concentrations (0 μM, 0.2 μM,

13 0.4 μM or 0.8 μM) of compound **13d** for 24 hours. Primary antibody (100

14 μL/cm2): VEGFR-2 (26415-1-AP, Proteintech Group), p-VEGFR-2

15 (Tyr951) (#2471, Cell Signaling Technology). The cellular level of

16 protein was determined by standard western blotting.

17 *4.7. Zebrafish labeling and culture*

18 The research group cooperated with China Zebrafish Resource Center

19 (CZRC) to construct a blood vessel marker zebrafish (Tg(kdrl:EGFP))

20 with a genotype of s843Tg/+, which was recorded as the F0 generation.

21 Collect the fish eggs of the F2 generation, and add different

22 concentrations of target compounds to the culture solution after 24 hours

1   to make the final concentration of the culture solution 40 mg/L. After 24

2   hours of action, the zebrafish were anesthetized *in vivo*, and the internode

3   angiogenesis and sprouting were observed under a confocal microscope.

4   *4.8. Molecular docking study*

5   Molecular docking study was carried out with MOE (Molecular

6   Operating Environment, version 2016.08, Chemical Computing Group

7   Inc., Canada) [50]. Two mol2 format files of compound **13d** and sunitinib

8   were subjected to energy minimization with Amber10: EHT force-field in

9   MOE. The crystal structure of VEGFR-2 in complex with sunitinib (PDB

10   ID: 4AGD) was downloaded from Protein Data Bank

11   (https://www.rcsb.org/) and successively optimized with Structure

12   Preparation and Protonate 3D [51]. The ligand atoms were chosen as the

13   docking site in the docking procedure, which was conducted with default

14   parameters.

15   *4.9. Molecular dynamics (MD) simulations*

16   MD simulations of two systems in PDB format were conducted using

17   NAMD software (version 2.14), and configuration files were generated in

18   VMD (visual molecular dynamics) [52]. Energy minimization and

19   equilibration with Gasteiger–Huckel charges used Boltzmann's initial

20   velocity in CHARMM 22 force field file, which performed in a 15 Å3

21   size water box. Ultimately, 10 ns MD simulations for three proteins at

22   constant temperature (300 K) and pressure (1 atm) were carried out to

1 analyze three systems' binding affinity and stability.

2 **Acknowledgments**

12

13

14

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- A novel VEGFR-2 inhibitor based on 1,2,3-triazole scaffold was identified.

- Compound **13d** had better kinase activity inhibition ability than sunitinib.

- Compound **13d** could inhibit angiogenesis more effectively than sunitinib.

Conflict of Interest

The authors declared that they have no conflicts of interest in this work.

We declared that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.