Bioorganic & Medicinal Chemistry xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Design, synthesis, and docking studies of afatinib analogs bearing cinnamamide moiety as potent EGFR inhibitors

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ARTICLE INFO

Article history:
Received 7 January 2016
Revised 6 February 2016
Accepted 9 February 2016
Available online xxxx

Keywords:
Afatinib
Cinnamamide
Synthesis
Docking
EGFR inhibitors
Antitumor activity

ABSTRACT

Two series of afatinib derivatives bearing cinnamamide moiety (**10a–n** and **11a–h**) were designed, synthesized and evaluated for the IC_{50} values against four cancer cell lines (A549, PC-3, MCF-7 and Hela). Two selected compounds (**10e**, **10k**) were further evaluated for the inhibitory activity against EGFR and VEGFR2/KDR kinases. Seven of the compounds showed excellent cytotoxicity activity and selectivity with the IC_{50} values in single-digit μM to nanomole range. Three of them are equal to more active than positive control afatinib against one or more cell lines. The most promising compound **10k** showed the best activity against A549, PC-3, MCF-7 and Hela cancer cell lines and EGFR kinase, with the IC_{50} values of 0.07 ± 0.02 μM, 7.67 ± 0.97 μM, 4.65 ± 0.90 μM and 4.83 ± 1.28 μM, which were equal to more active than afatinib (0.05 ± 0.01 μM, 4.1 ± 2.47 μM, 5.83 ± 1.89 μM and 6.81 ± 1.77 μM), respectively. Activity of compounds **10e** (IC_{50} 9.1 nM) and **10k** (IC_{50} 3.6 nM) against EGFR kinase were equal to the reference compound afatinib (IC_{50} 1.6 nM). Structure–activity relationships (SARs) and docking studies indicated that replacement of the aqueous solubility 4-(dimethylamino)but-2-enamide group by cinnamamide moiety didn't decrease the antitumor activity. The results suggested that methoxy substitution had a significant impact on the activity and methoxy substituted on C-4 or C-2,3,4 position was benefit for the activity.

1. Introduction

The epidermal growth factor receptor (EGFR) family plays a critical role in vital cellular processes and in various cancers, including proliferation, differentiation, migration, apoptosis, and angiogenesis. Therefore, developing EGFR inhibitors is one of the research hotspots in molecular targeted therapy for the treatment of human cancer.[1,2]

In recent years, many quinazoline derivatives were reported as EGFR signal transduction pathway inhibitors, such as gefinitib,[3,4] erlotinib,[5] afatinib (BIBW-2992),[6–8] dacomitinib[9,10] and LP-7[11] (the structures of them are shown in Fig. 1). Among them, afatinib, which is a powerful, irreversible tyrosine kinase inhibition of EGFR, with IC_{50} value (half-maximal inhibitory concentration) of 0.5 nM, exhibits potent anti-tumor activity against non small-cell lung cancer (NSCLC), and was approved by the FDA for the treatment of patients with metastatic NSCLC in 2013.[12]

In this study, we devoted to synthesize novel EGFR inhibitors bearing a common pharmacophore quinazoline moiety. LP-7 is a excellent quinazoline EGFR kinase inhibitor but with disappointing activity against NSCLC cell line A549. We thought that the –CN group of LP-7 was not favorable to the activity against A549. In order to investigate the effect to the activity of the aqueous solubility chains and inspired by LP-7, we focused on the replacement of the aqueous solubility 4-(dimethylamino)but-2-enamide group by cinnamamide moiety and removed the –CN group of LP-7 to afford the first series compounds. Moreover, inspired by dacomitinib, the small molecule tetrahydrofuran were substituted with methyl group to obtain the second series compounds. In order to screen compounds with excellent in vitro/in vivo anti-tumor activity as well as improved pharmacokinetic, further studies on analogous of afatinib were carried out in this research to study the effect of variety, number, and position of substituents of aryl ring on the antitumor activity. As a result, two novel quinazoline derivatives bearing different substituents at aryl ring (**10a–n** and **11a–h**) were

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http://dx.doi.org/10.1016/j.bmc.2016.02.017
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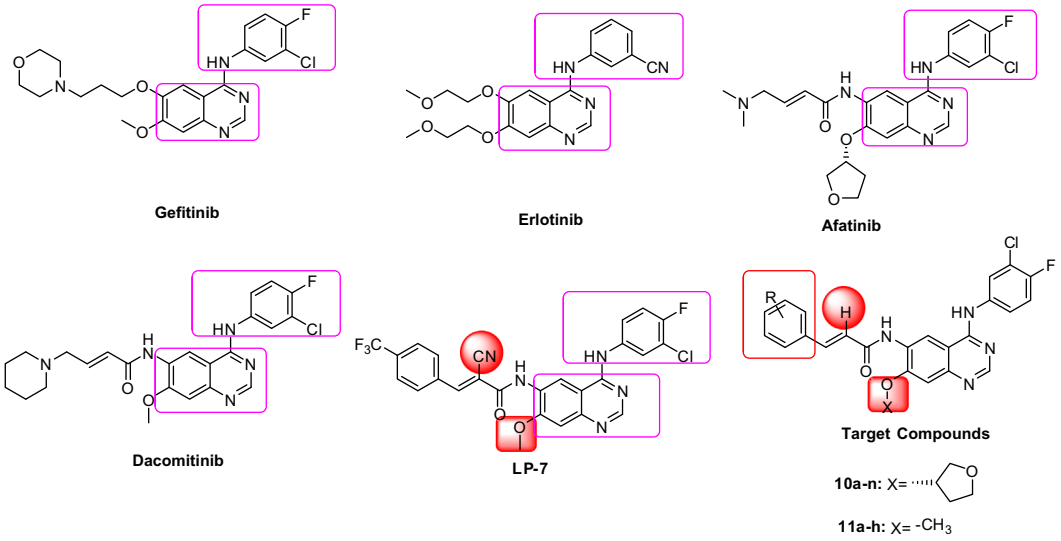


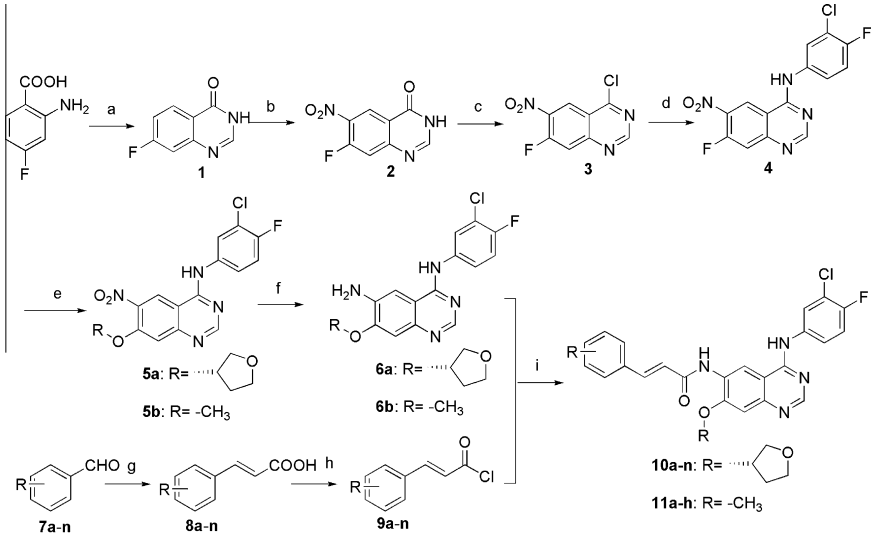
Figure 1. Structures of small-molecule epidermal growth factor receptor (EGFR) inhibitors based on the quinazoline scaffold and the target compounds **10a–n** and **11a–h**.

designed and synthesized. The structures of target compounds are shown in Figure 1.

Herein we disclosed the design, synthesis and antitumor activity against A549 (human lung cancer), PC-3 (human prostate cancer), MCF-7 (human breast cancer) and Hela (human cervical cancer) cancer cell lines, EGFR and VEGFR2/KDR kinases of novel afatinib analogs. Moreover, docking studies were presented in this paper as well.

2. Chemistry

The structures and preparation of target compounds **10a–n** and **11a–h** are described in Scheme 1. Compounds **10a–n** and **11a–h** were synthesized from commercially available 2-amino-4-fluorobenzoic acid through seven steps. Cyclization reaction of 2-amino-4-fluorobenzoic acid in the present of formamidine acetate afforded compound **1** as a white solid. Subsequently, compound **1** was nitrified with concentrated sulfuric acid and fuming nitric acid refluxed for 2 h to give 7-fluoro-6-nitroquinazolin-4(3*H*)-one (**2**). Chlorination of **2** with $SOCl_2$ and DMF (cat.) for 4 h at 90 °C to achieve **3** as light yellow solid. Nucleophilic displacement of the compound **3** with phenylamine gave access to the intermediate **4**. Compound **4** condensed with (*S*)-tetrahydrofuran-3-ol in the present of potassium *tert*-butoxide obtained **5a**. On the other hand, compound **4** treated with methanol and 50% sodium hydroxide to furnish **5b**. **5a** and **5b** were then followed by nitro-hydrogenation reaction with $NH_2NH_2{\cdot}H_2O$ (80%), $FeCl_3$ and activated carbon to generate key intermediate **6a** and **6b**, respectively. Finally, intermediates **6a** and



7a,8a,9a,10a,11a: R=4-cyano
7b,8b,9b,10b,11b: R=2-nitro
7c,8c,9c,10c,11c: R=2-chloro-4-fluoro
7d,8d,9d,10d,11d: R=2,5-dimethoxy
7e,8e,9e,10e,11e: R=4-methoxy
7f,8f,9f,10f,11f: R=2,4-dimethoxy
7g,8g,9g,10g,11g: R=3,5-dimethoxy
7h,8h,9h,10h,11h: R=3,4,5-trimethoxy
7i,8i,9i,10i: R=4-nitro
7j,8j,9j,10j: R=2,3-dichloro
7k,8k,9k,10k: R=2,3,4,-trimethoxy
7l,8l,9l,10l: R=3,5-dibromo-4-hydroxy
7m,8m,9m,10m: R=4-hydroxy-3,5-dimethoxy
7n,8n,9n,10n: R=4-bromo

Scheme 1. Synthetic route of target compounds. Reagents and conditions: (a) EtOH, 24 h; (b) Concd H_2SO_4, fuming HNO_3, 2 h; (c) $SOCl_2$, DMF (cat.), 4 h; (d) isopropanol, triethylamine, 1.5 h; (e) potassium *tert*-butoxide, THF, 3 h; CH_3OH, NaOH; (f) $N_2H_4{\cdot}H_2O$, $FeCl_3$, activated carbon, EtOH, 1 h; (g) pyridine, piperidine (cat.), 20 h; (h) $(COCl)_2$, DMF (cat.), CH_2Cl_2, 15 min; (i) DIPEA, CH_2Cl_2, 1 h.

6b condensed with the corresponding cinnamoyl chloride **9a**–**n** to afford target compounds **10a**–**n** and **11a**–**h**, respectively.

Cinnamoyl chloride **9a**–**n** were prepared from corresponding benzaldehyde **7a**–**n** condensed with propanedioic acid in the present of pyridine and piperidine refluxed for 20 h, followed by chlorinationto with oxalyl chloridefurnish and DMF (cat.) to furnish compounds **9a**–**n**, respectively.

3. Results and discussion

3.1. Biological evaluation

Taking afatinib or/and staurosporine as reference compounds, the target compounds (**10a**–**n** and **11a**–**h**) were evaluated for the cytotoxicity against four cancer cell lines A549, PC-3, MCF-7, and Hela by 3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) cell proliferation assay. Compounds **10e** and **10k** were further evaluated for the EGFR and VEGFR2/KDR kinase inhibitory activity. The results expressed as IC_{50} values were summarized in Tables 1 and 2 and the values are the average of at least two independent experiments.

As illustrated in Table 1, nine of the synthesized compounds showed moderate to excellent cytotoxicity activity against the different cancer cells. Three of them are equal to more active than positive control afatinib against one or more cell lines. We could easily see that the first series of the target compounds (**10a**–**n**) were much more active than the second series (**11a**–**h**) against the four tested cancer cell lines. For the first series, the compounds **10b**–**f**, **10h** and **10k** showed equal to excellent cytotoxicity activity against A549 and PC-3 cancer cell lines to reference compound afatinib. It's suggested that the target compounds shown well selectivity against A549 and PC-3 to the other two cancer cell lines.

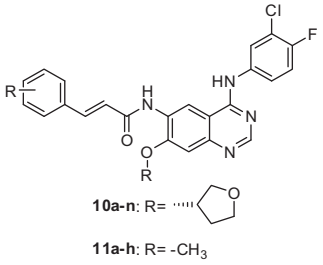
The inhibitory activities of target compounds were increased in varying degrees because of the different substituents of aryl moiety. It was noticeable that compounds with methoxy group substitution (**10d**, **10e**, **10f**, **10h** and **10k**) had a significant impact on the activity. Especially, methoxy group substituted at C-4 position is more preferred, such as compounds **10e**, **10f**, **10h** and **10k**. What's more, compound **10k** with 2,3,4-trimethoxy showed the best

Table 2
EGFR and VEGFR2/KDR kinase inhibitory activity of compounds **10e**, **10k**, afatinib and staurosporine

Compounds no.	R	IC_{50}[a] (nM)	
		EGFR	VEGFR2/KDR
10e	4-Methoxy	9.1	886
10k	2,3,4-Trimethoxy	3.6	478
Afatinib[b]	—	1.6	—
Staurosporine[b]	—	48	—

[a] The values are an average of two separate determinations.
[b] Used as a positive controls.

Table 1
Structures and activity of target compounds **10a**–**n** and **11a**–**h**



Compounds no.	R	IC_{50}[a] (μM)			
		A549	PC-3	MCf-7	Hela
10a	4-Cyano	NA[c]	36.55 ± 0.24	9.827	ND[d]
10b	2-Nitro	8.82 ± 1.22	7.76 ± 1.01	28.40 ± 1.96	29.60 ± 1.54
10c	2-Chloro-4-fluoro	5.19 ± 1.72	7.15 ± 0.91	26.19 ± 1.53	37.49 ± 1.40
10d	2,5-Dimethoxy	7.30 ± 1.24	8.73 ± 0.84	36.28 ± 1.52	26.76 ± 1.26
10e	4-Methoxy	3.05 ± 1.58	2.24 ± 0.91	5.07 ± 0.93	34.62 ± 1.74
10f	2,4-Dimethoxy	9.35 ± 0.95	2.76 ± 0.86	28.56 ± 2.08	26.45 ± 1.64
10g	3,5-Dimethoxy	10.80 ± 1.00	25.60 ± 1.14	30.26 ± 1.54	34.25 ± 1.54
10h	3,4,5-Trimethoxy	4.15 ± 0.59	20.50 ± 0.24	28.35 ± 1.22	24.50 ± 1.00
10i	4-Nitro	12.04 ± 1.53	22.79 ± 0.13	26.68 ± 1.42	27.35 ± 2.24
10j	2,3-Dichloro	23.22 ± 1.30	27.50 ± 0.23	30.05 ± 1.24	30.50 ± 1.22
10k	2,3,4,-Trimethoxy	0.07 ± 0.02	7.67 ± 0.97	4.65 ± 0.90	4.83 ± 1.28
10l	3,5-Dibromo-4-hydroxy	28.80 ± 1.61	25.50 ± 1.14	30.62 ± 1.41	27.68 ± 5.27
10m	4-Hydroxy-3,5-dimethoxy	26.95 ± 0.27	26.50 ± 3.14	30.50 ± 2.04	27.43 ± 2.24
10n	4-Bromo	35.32 ± 1.56	28.15 ± 2.24	32.35 ± 2.74	27.45 ± 2.54
11a	4-Cyano	NA	NA	NA	ND
11b	2-Nitro	33.25 ± 2.25	14.26 ± 1.09	36.52 ± 1.54	ND
11c	2-Chloro-4-fluoro	NA	32.68 ± 1.41	NA	ND
11d	2,5-Dimethoxy	11.19 ± 1.04	28.56 ± 3.24	34.25 ± 2.14	ND
11e	4-Methoxy	NA	NA	NA	ND
11f	2,4-Dimethoxy	33.25 ± 1.24	38.50 ± 2.26	36.57 ± 1.54	ND
11g	3,5-Dimethoxy	NA	NA	38.45 ± 1.32	ND
11h	3,4,5-Trimethoxy	NA	38.24 ± 2.28	NA	ND
Afatinib[b]	—	0.05 ± 0.01	4.10 ± 2.47	5.83 ± 1.89	6.81 ± 1.77

[a] The values are an average of two separate determinations.
[b] Used as a positive controls.
[c] NA: not active.
[d] ND: Not determined.

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activity against A549, PC-3, MCF-7 and Hela cancer cell lines, with the IC_{50} values of 0.07 ± 0.02 μM, 7.67 ± 0.97 μM, 4.65 ± 0.90 μM and 4.83 ± 1.28 μM, which were equal to more active than afatinib (0.05 ± 0.01 μM, 4.10 ± 2.47 μM, 5.83 ± 1.89 μM and 6.81 ± 1.77 μM), respectively. Usually, electron withdrawing groups are unfavourable for the activity, that is, why compounds **10a–c**, **10i–j** and **10n** exhibited unsatisfied antitumor activity.

In view of above, the results suggested that replacement of the aqueous solubility 4-(dimethylamino)but-2-enamide group by cinnamamide moiety didn't decrease the antitumor activity and variations in substitutions of the aryl moiety had a significant impact on the activity. Moreover, 2,3,4-trimethoxy substitution of aryl moiety is beneficial for the activity. The activity toward EGFR kinase of compound **10e** and **10k** is equal to the reference compound afatinib, with the IC_{50} values of 3.6 nM. The inhibitory activity against EGFR and VEGFR2/KDR kinases of compounds **10e** and **10k** shown that these series of compounds were potent selective EGFR inhibitors. However, exact action mechanism is not quite clear right now. Further study will be carried out to identify the target in near future.

3.2. Molecular docking study

To explore the binding modes of target compounds with the active site of EGFR, molecular docking simulation studies were carried out by using SURFLEX-DOCK module of SYBYL package version. Based on the in vitro inhibition results, we selected compound **10k**, our best EGFR inhibitor in this study, as ligand example, and the structure of EGFR was selected as the docking model (PDB ID code:4G5J[13]).

The binding modes of compound **10k** and lead compound afatinib were shown in Figure 2a and b. As depicted in Figure 2b, compound **10k** and lead compound can nearly overlap in the binding model and quinazoline moiety and amide group formed two hydrogen bonds with residues MET793 and CYS797, respectively. Analysis of compound **10k**'s binding mode in the active binding site demonstrated that the docking mode of the **10k** is similar to the lead compound with the same H-bond between quinazoline group, amide group and MET793, CYS797. The results of Figure 2b showed that **10k** and lead compound can bind to the ATP-binding pocket of the EGFR protein. The two hydrogen bonds and combine of ATP-binding pocket really play an important role in the inhibitory potency of afatinib analogs bearing cinnamamide moiety against EGFR kinase according to the docking results. Furthermore, the docking results also give us a new direction to design new EGFR inhibitors. The above-mentioned results of SARs analysis and molecular docking study may allow the rational design of more potent EGFR inhibitors.

4. Conclusions

In summary, we designed and synthesized two series of afatinib derivatives bearing cinnamamide moiety and evaluated for the IC_{50} values against four cancer cell lines. Two selected compounds

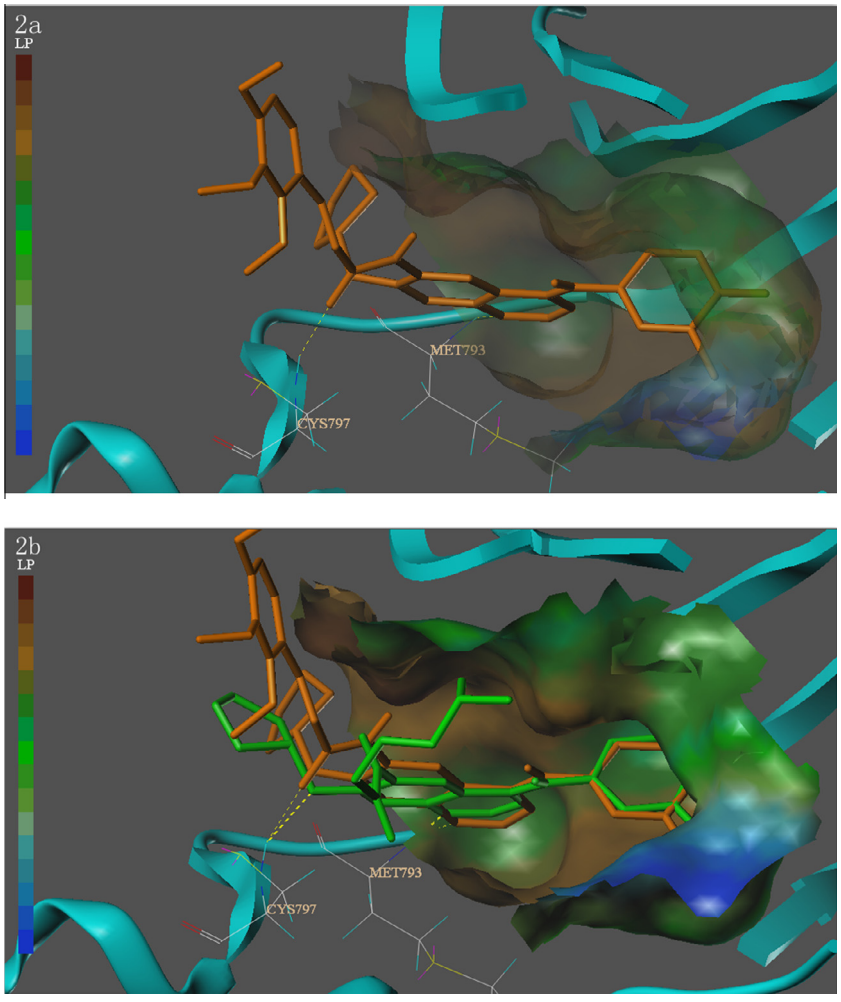

Figure 2. (a–b) Binding poses of compound **10k** with EGFR. The proteins were displayed by cyan ribbon. Compound **10k** and lead compound were displayed by orange and green sticks, respectively. H-bonding interactions between the **10k**, lead compound and EGFR were indicated with dashed lines in yellow.

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(**10e**, **10k**) were further evaluated for the inhibitory activity against EGFR and VEGFR2/KDR kinases. Seven of the compounds showed excellent cytotoxicity activity with the IC_{50} valuables in single-digit μM to nanomole range. The results noticeable that methoxy (**10d**, **10e**, **10f**, **10h** and **10k**) substitution had a significant impact on the activity. Especially, methoxy substituted at C-4 position is more preferred, such as compounds **10e**, **10f**, **10h** and **10k**. Moreover, the most promising compound **10k** with 2,3,4-trimethoxy group showed the best activity against A549, PC-3, MCF-7 and Hela cancer cell lines and EGFR kinase, with the IC_{50} values of 0.07 ± 0.02 μM, 7.67 ± 0.97 μM, 4.65 ± 0.90 μM and 4.83 ± 1.28 μM, 3.6 nM, which were equal to more active than afatinib (0.05 ± 0.01 μM, 4.10 ± 2.47 μM, 5.83 ± 1.89 μM and 6.81 ± 1.77 μM, 1.6 nM), respectively. Structure–activity relationships (SARs) and docking studies indicated that replacement of the aqueous solubility 4-(dimethylamino)but-2-enamide group by cinnamamide moiety didn't decrease the antitumor activity. The results suggested that methoxy substitution had a significant impact on the activity and substituted on C-4 and C-2,3,4 position was benefit for the activity. Further study will be carried out to identify the exact action mechanism in near future.

5. Experimental

5.1. Chemistry

All melting points were obtained on a Büchi Melting Point B-540 apparatus (Büchi Labortechnik, Flawil, Switzerland) and were uncorrected. NMR spectra were performed using Bruker 400 MHz spectrometers (Bruker Bioscience, Billerica, MA, USA) with TMS as an internal standard. Mass spectra (MS) were taken in ESI mode on Agilent 1100 LC–MS (Agilent, Palo Alto, CA, USA). All the materials were obtained from commercial suppliers and used without purification, unless otherwise specified. Yields were not optimized. TLC analysis was carried out on silica gel plates GF254 (Qindao Haiyang Chemical, China). All the materials were obtained from commercial suppliers and used without purification, unless otherwise specified. Yields were not optimized.

5.2. Preparation of 7-fluoroquinazolin-4(3*H*)-one (1)

A mixture of 2-amino-4-fluorobenzoic acid (50.0 g, 0.323 mol), formamidine acetate (67.0 g, 0.644 mol) and ethanol (300 mL) was heated and refluxed for 24 h and the reaction was monitored by thin-layer chromatography (TLC). The reaction mixture was poured into ice-water, then filtered and the filter cake was washed with 50 percent ethanol, dried to obtained **1** as white solid[14] (51.0 g, 96.4%) and was used for next step without further purification. Mp 260.1–261.0 °C. ESI-MS *m*/*z*: $[M-H]^-$163.1. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.35 (s, 1H), 8.17 (d, *J* = 6.8 Hz, 1H), 8.14 (d, *J* = 7.2 Hz, 1H), 7.43 (d, *J* = 9.8 Hz, 1H), 7.37 (t, *J* = 8.8 Hz, 1H).

5.3. Preparation of 7-fluoro-6-nitroquinazolin-4(3*H*)-one (2)

To the mixture of compound **1** (50.0 g, 0.239 mol), concentrated sulfuric acid (103 mL), fuming nitric acid (105 mL) was slowly added at 70 °C, the reaction mixture then was stirred at 110 °C for 2 h. After completion of reaction as indicated by TLC, the reaction mixture was cooled to room temperature and the reaction mixture was slowly added to ice/water with vigorous stirring yielding a precipitate. The mixture was then filtered, dried to furnish 7-fluoro-6-nitroquinazolin-4(3*H*)-one **2** as light yellow solid[15] (48.5 g, 76.1%). Mp 277.3–278.5 °C. ^{1}H NMR (400 MHz, DMSO) δ 12.77 (s, 1H), 8.68 (dd, *J* = 8.2, 2.7 Hz, 1H), 8.28 (s, 1H), 7.73 (dd, *J* = 12.2, 2.8 Hz, 1H).

5.4. Preparation of 4-chloro-7-fluoro-6-nitroquinazoline (3)

A solution of compound **2** (48.0 g,0.230 mol), thionyl chloride (407 mL) and *N*,*N*-dimethylformamide (2 mL) was heated to 100 °C until the mixture melted transparently, and then stirred for 4 h. After completed, the reaction mixture was cooled to room temperature, and the solution was concentrated under a reduced pressure to remove the solvent. Toluene thereto (400 mL) was added to the residue and concentrated again, dried to obtain 4-chloro-7-fluoro-6-nitroquinazoline **3** as yellow solid[15] (50.7 g, 97.0%). Mp 118.2–119.3 °C. ^{1}H NMR (400 MHz, DMSO) δ 8.66 (dd, *J* = 8.2, 1.2 Hz, 1H), 8.41 (s, 1H), 7.75 (d, *J* = 12.2 Hz, 1H).

5.5. Preparation of *N*-(3-chloro-4-fluorophenyl)-7-fluoro-6-nitroquinazolin-4-amine (4)

To the solution of compound **3** (50.0 g, 0.220 mol), isopropanol (400 mL), triethylamine (34 mL), 3-choro-4-fluoroaniline was added at room temperature. The reaction mixture then was stirred at room temperature for 1.5 h. After completion of reaction as indicated by TLC, the mixture was then filtered, washed with isopropanol and water, dried to yield *N*-(3-chloro-4-fluorophenyl)-7-fluoro-6-nitroquinazolin-4-amine **4** as orange solid[16] (65.0 g, 87.8%). Mp 261.4–262.5 °C. ^{1}H NMR (400 MHz, DMSO) δ 10.43 (s, 1H), 9.51 (d, *J* = 7.8 Hz, 1H), 8.68 (s, 1H), 8.08 (d, *J* = 6.4 Hz, 1H), 7.79 (d, *J* = 5.2 Hz, 1H), 7.76 (s, 1H), 7.45 (t, *J* = 9.0 Hz, 1H).

5.6. Preparation of (*S*)-*N*-(3-chloro-4-fluorophenyl)-6-nitro-7-((tetrahydrofuran-3-yl)oxy)quinazolin-4-amine (5a)

To the mixture of potassium tert-butoxide (15.2 g, 0.136 mol), anhydrous THF (400 mL), (*S*)-tetrahydrofuran-3-ol (10 mL,0.124 mol) was added drop-wise at room temperature. The mixture was stirred at room temperature for 1 h. a solution of compound **4** (20.0 g, 0.060 mol) in anhydrous THF (200 mL) was added via a dropping funnel over 1 h (the dropping funnel was rinsed with15 mL of THF) at room temperature and the reaction mixture was stirred at room temperature for 2 h, the reaction was monitored by TLC. The solution was concentrated under a reduced pressure and the residue was poured into water with stirring yielding a precipitate, then the mixture was filtered and washed with ice-water and ethanol, dried to afford (*S*)-*N*-(3-chloro-4-fluorophenyl)-6-nitro-7-((tetrahydrofuran-3-yl)oxy) quinazolin-4-amine **5a** as yellow solid (23.9 g, 99.5%). Mp 210.1–211.0 °C. ^{1}H NMR (400 MHz, DMSO) δ 10.10 (d, *J* = 11.8 Hz, 1H), 9.16 (d, *J* = 13.2 Hz, 1H), 8.62 (d, *J* = 13.4 Hz, 1H), 8.13 (d, *J* = 6.8 Hz, 1H), 7.75 (s, 1H), 7.45–7.35 (m, 2H), 5.40 (s, 1H), 3.99–3.79 (m, 4H), 2.34 (dd, *J* = 13.8, 6.2 Hz, 1H), 2.04 (d, *J* = 5.6 Hz, 1H).

5.7. Preparation of *N*-(3-chloro-4-fluorophenyl)-7-methoxy-6-nitroquinazolin-4-amine (5b)

To the mixture of compound **4** (11.37 g, 0.334 mol), methanol (227 mL), 50 percent sodium hydroxide (15 mL) was added at room temperature. The reaction mixture then was refluxed for 1 h and monitored by TLC. The reaction mixture was cooled to room temperature and was poured into sodium bicarbonate solution yielding a precipitate, the mixture then was separated by filtration and washed with water, dried to afford *N*-(3-chloro-4-fluorophenyl)-7-methoxy-6-nitroquinazolin-4-amine **5b** as yellow solid[17] (11.2 g, 94.9%). Mp 292.3–293.0 °C. ESI-MS *m*/*z*: $[M+H]^+$349.1. ^{1}H NMR (400 MHz, DMSO) δ 10.13 (s, 1H), 9.20 (s, 1H), 8.66 (s, 1H), 8.20–8.10 (m, 1H), 7.84–7.73 (m, 1H), 7.51–7.41 (m, 2H), 4.05 (s, 3H).

5.8. Preparation of (*S*)-*N*-(3-chloro-4-fluorophenyl)-7-((tetrahydrofuran-3-yl)oxy)quinazoline-4,6-diamine (6a)

The solution of compound **5a** (21.4 g, 0.053 mol) and ethanol (700 mL) was stirred at 60 °C, an appropriate amount of activated carbon (7.0 g) and ferric chloride (1.8 g) were added at the temperature, the mixture was heated to 80 °C and 80 percent hydrazine hydrate (25 mL) was added to the solution. The reaction mixture then was refluxed for 1.5 h and monitored by TLC. The mixture was filtered and the precipitate was washed with ethanol. The filtrate was concentrated under reduce pressure and the residue was poured into water with stirred for 30 min. The precipitate was filtered and dried to furnish (*S*)-*N*-(3-chloro-4-fluorophenyl)-7-((tetrahydrofuran-3-yl)oxy)quinazoline-4,6-diamine **6a** as light yellow solid (17.3 g, 87.3%). Mp 139.1–140.1 °C. ^{1}H NMR (400 MHz, DMSO) δ 9.41 (s, 1H), 8.38 (s, 1H), 8.17 (t, J = 12.6 Hz, 1H), 7.88–7.73 (m, 1H), 7.38 (dd, J = 17.2, 8.2 Hz, 2H), 7.05 (s, 1H), 5.41 (s, 2H), 5.20 (s, 1H), 4.00–3.87 (m, 3H), 3.77 (dd, J = 12.6, 7.8 Hz, 1H), 2.29 (td, J = 14.2, 7.2 Hz, 1H), 2.19–2.06 (m, 1H).

5.9. Preparation of *N*-(3-chloro-4-fluorophenyl)-7-methoxyquinazoline-4,6-diamine (6b)

The synthesis of compound **8** was similar to the compound 7. The solution of compound **5b** (11.2 g, 0.032 mol) and ethanol (370 mL) was stirred at 60 °C, an appropriate amount of activated carbon (3.5 g) and ferric chloride (1.3 g) were added at the temperature, the mixture was heated to 80 °C and 80 percent hydrazine hydrate (16 mL) was added to the solution. The reaction mixture then was refluxed for 1.5 h and monitored by TLC. The mixture was filtered and the precipitate was washed with ethanol. The filtrate was concentrated under reduce pressure and the residue was poured into water with stirred for 30 min. The precipitate was filtered and dried to obtain *N*-(3-chloro-4-fluorophenyl)-7-methoxyquinazoline-4,6-diamine **6b** as yellow solid (8.8 g, 85.9%). Mp 258.4–259.7 °C. ^{1}H NMR (400 MHz, DMSO) δ 9.39 (s, 1H), 8.37 (s, 1H), 8.18 (dd, J = 6.8, 2.5 Hz, 1H), 7.84–7.76 (m, 1H), 7.44–7.34 (m, 2H), 7.10 (s, 1H), 5.38 (s, 2H), 3.96 (s, 3H).

5.10. General procedure for the preparation of compounds 8a–n

To the mixture of substituted benzaldehydes **7a**–**n** (2.5 mmol), pyridine (20.0 mL), piperidine (0.2 mL), propanedioic acid (5.0 mmol) was added at room temperature and the mixture was heated and refluxed for 20 h and monitored by TLC. The reaction mixture was cooled to room temperature and the solution was acidified to pH = 5 to yielding a precipitate. The mixture was filtered and washed with water to furnish the compounds **8a**–**n**.

5.11. General procedure for the preparation of compounds 9a–n

Oxalyl chloride (2.0 mmol) was added drop-wise to a stirred mixture of compounds **8a**–**n** (1.0 mmol) and DMF (0.02 mmol) in dichloromethane (16 mL) in room temperature for 10 min, and the mixture was distilled and dissolved in dichloromethane (16 mL) immediately. The solution was used for the next step without further purification.

5.12. General procedure for the preparation of compounds 10a–n and 11a–h

A solution of compound **9a**–**n** (1.0 mmol) in dichloromethane (16 mL) was added drop-wise to a solution of aniline compounds **6a** or **6b** (0.4 mmol) and diisopropylethylamine (0.4 mmol) in dichloromethane (15 mL) in an ice bath. Upon completion of the addition, the reaction mixture was removed from the ice bath and placed in room temperature for 30 min and monitored by TLC. The mixture was washed with 10% K_2CO_3 (50 mL ×3) followed by brine (50 mL ×1), and the organic phase was separated, dried, and evaporated to yield **12a**–**n** and **13a**–**h** which were purified by dichloromethane.

5.12.1. (*S*)-*N*-(4-((3-Chloro-4-fluorophenyl)amino)-7-((tetrahydrofuran-3-yl)oxy)quinazolin-6-yl)-3-(4-cyanophenyl) acrylamide (10a)

This compound was obtained as yellow solid in 80% yield. Mp 267.5–268.9 °C. ESI-MS m/z: $[M+H]^+$530.1. ^{1}H NMR (400 MHz, DMSO) δ 9.78 (s, 1H), 9.18 (s, 1H), 8.75 (s, 1H), 8.04 (d, J = 6.4 Hz, 1H), 7.93 (d, J = 8.2 Hz, 2H), 7.87 (d, J = 8.2 Hz, 2H), 7.70 (d, J = 15.8 Hz, 2H), 7.60–7.40 (m, 4H), 5.30 (s, 1H), 4.10–3.93 (m, 3H), 3.80 (d, J = 5.4 Hz, 1H), 2.45–2.34 (m, 1H), 2.23 (s, 1H).

5.12.2. (*S*)-*N*-(4-((3-Chloro-4-fluorophenyl)amino)-7-((tetrahydrofuran-3-yl)oxy)quinazolin-6-yl)-3-(2-nitrophenyl) acrylamide (10b)

This compound was obtained as yellow solid in 68% yield. Mp 176.5–177.1 °C. ESI-MS m/z: $[M+H]^+$550.1. ^{1}H NMR (400 MHz, DMSO) δ 9.84 (s, 1H), 9.27 (s, 1H), 8.83 (s, 1H), 8.12 (dd, J = 8.2, 1.0 Hz, 1H), 8.03–7.94 (m, 2H), 7.93–7.80 (m, 3H), 7.73–7.67 (m, 2H), 7.53 (t, J = 9.2 Hz, 1H), 7.42 (s, 1H), 7.28 (d, J = 15.6 Hz, 1H), 5.32 (d, J = 6.2 Hz, 1H), 4.12 (d, J = 9.8 Hz, 1H), 4.05–3.97 (m, 2H), 3.81 (td, J = 8.2, 5.4 Hz, 1H), 2.40 (dt, J = 14.2, 7.1 Hz, 1H), 2.26–2.18 (m, 1H). ^{13}C NMR (101 MHz, DMSO) δ 163.83, 158.60, 156.28, 154.69, 153.84, 151.17, 148.75, 136.52, 135.33, 134.34, 131.09, 130.32, 129.37, 129.21, 127.06, 126.29, 125.21, 125.05, 117.23, 117.02, 116.58, 107.85, 103.32, 80.37, 72.35, 67.16, 32.86. ESI-HRMS m/z: calcd for $C_{27}H_{21}ClFN_5O_5$ $[M+H]^+$: 550.1293; found 550.1253.

5.12.3. (*S*)-3-(2-Chloro-4-fluorophenyl)-*N*-(4-((3-chloro-4-fluorophenyl)amino)-7-((tetrahydrofuran-3-yl)oxy)quinazolin-6-yl)acrylamide (10c)

This compound was obtained as yellow solid in 65% yield. Mp 179.6–181.5 °C. ESI-MS m/z: $[M+H]^+$558.1. ^{1}H NMR (400 MHz, DMSO) δ 9.62 (s, 1H), 9.12 (s, 1H), 8.61 (s, 1H), 8.09 (d, J = 4.4 Hz, 1H), 7.98–7.85 (m, 2H), 7.77 (s, 1H), 7.58 (d, J = 8.8 Hz, 1H), 7.41 (dt, J = 15.4, 7.8 Hz, 2H), 7.33–7.20 (m, 2H), 5.33 (s, 1H), 4.11–3.90 (m, 4H), 3.80 (d, J = 5.4 Hz, 1H), 2.39 (dd, J = 14.2, 7.0 Hz, 1H), 2.21 (s, 1H).

5.12.4. (*S*)-*N*-(4-((3-Chloro-4-fluorophenyl)amino)-7-((tetrahydrofuran-3-yl)oxy)quinazolin-6-yl)-3-(2,5-dimethoxyphenyl)-acrylamide (10d)

This compound was obtained as yellow solid in 72% yield. Mp 278.9–280.3 °C. ESI-MS m/z: $[M+H]^+$565.1. ^{1}H NMR (400 MHz, DMSO) δ 9.72 (s, 1H), 9.28 (s, 1H), 8.89 (s, 1H), 7.98 (dd, J = 6.8, 2.6 Hz, 1H), 7.92 (d, J = 15.8 Hz, 1H), 7.68 (ddd, J = 8.8, 4.3, 2.6 Hz, 1H), 7.55 (t, J = 9.0 Hz, 1H), 7.49 (s, 1H), 7.24 (t, J = 9.6 Hz, 2H), 7.06 (d, J = 9.0 Hz, 1H), 7.02 (dd, J = 9.0, 2.8 Hz, 1H), 5.30 (t, J = 5.6 Hz, 1H), 4.13 (d, J = 9.8 Hz, 1H), 4.01 (dt, J = 15.6, 6.1 Hz, 2H), 3.83 (d, J = 4.4 Hz, 3H), 3.80 (dd, J = 7.0, 4.2 Hz, 1H), 3.78 (s, 3H), 2.41 (dt, J = 14.4, 7.1 Hz, 1H), 2.27–2.19 (m, 1H).

5.12.5. (*S*)-*N*-(4-((3-Chloro-4-fluorophenyl)amino)-7-((tetrahydrofuran-3-yl)oxy)quinazolin-6-yl)-3-(4-methoxyphenyl)-acrylamide (10e)

This compound was obtained as yellow solid in 63% yield. Mp 240.9–242.3 °C. ESI-MS m/z: $[M+H]^+$535.1. ^{1}H NMR: (400 MHz, DMSO) δ 9.43 (s, 1H), 9.08 (s, 1H), 8.59 (s, 1H), 8.10 (dt, J = 13.0, 6.5 Hz, 1H), 7.78 (ddd, J = 9.0, 4.4, 2.8 Hz, 1H), 7.63 (d, J = 8.6 Hz, 2H), 7.59 (s, 1H), 7.44 (t, J = 9.2 Hz, 1H), 7.28 (s, 1H), 7.09 (d,

J = 15.8 Hz, 1H), 7.04 (s, 1H), 7.01 (s, 1H), 5.32 (dd, *J* = 6.2, 4.8 Hz, 1H), 4.02 (ddd, *J* = 10.8, 10.4, 5.8 Hz, 2H), 3.95 (dd, *J* = 15.4, 7.8 Hz, 1H), 3.81 (d, *J* = 4.2 Hz, 3H), 3.78 (dd, *J* = 8.2, 5.2 Hz, 1H), 2.37 (dt, *J* = 14.2, 7.1 Hz, 1H), 2.24–2.15 (m, 1H). ^{13}C NMR (101 MHz, DMSO) *δ* 164.80, 161.21, 157.56, 155.21, 153.68, 153.28, 146.58, 141.18, 136.78, 130.09(2C), 128.64, 127.76, 124.57, 123.40, 119.99, 117.01, 116.80, 115.86, 114.89(2C), 109.09, 106.94, 79.60, 72.45, 67.11, 55.77, 32.87. ESI-HRMS *m*/*z*: calcd for $C_{28}H_{24}ClFN_4O_4$ $[M+H]^+$: 535.1548; found 535.1518.

5.12.6. (*S*)-*N*-(4-((3-Chloro-4-fluorophenyl)amino)-7-((tetrahydrofuran-3-yl)oxy)quinazolin-6-yl)-3-(2,4-dimethoxyphenyl)-acrylamide (10f)

This compound was obtained as yellow solid in 80% yield. Mp 295.5–296.3 °C. ESI-MS *m*/*z*: $[M+H]^+$565.2. HRMS: 564.1575. ^{1}H NMR (400 MHz, DMSO) *δ* 9.61 (s, 1H), 9.26 (s, 1H), 8.86 (s, 1H), 7.99 (dd, *J* = 6.8, 2.6 Hz, 1H), 7.88 (d, *J* = 15.8 Hz, 1H), 7.69 (ddd, *J* = 8.8, 4.4, 2.6 Hz, 1H), 7.60 (d, *J* = 9.2 Hz, 1H), 7.54 (t, *J* = 9.2 Hz, 1H), 7.47 (s, 1H), 7.10 (d, *J* = 15.8 Hz, 1H), 6.67–6.61 (m, 2H), 5.29 (t, *J* = 5.6 Hz, 1H), 4.12 (d, *J* = 10.0 Hz, 1H), 4.05–3.94 (m, 2H), 3.89 (s, 3H), 3.83 (s, 3H), 3.82–3.77 (m, 1H), 2.40 (dt, *J* = 14.2, 7.0 Hz, 1H), 2.26–2.17 (m, 1H). ^{13}C NMR (101 MHz, DMSO) *δ* 165.37, 162.86, 159.68, 158.87, 156.46, 154.99, 150.58, 150.42, 150.30, 136.56, 135.01, 130.05, 129.68, 126.71, 125.45, 119.65, 119.47, 117.32, 117.10, 116.37, 107.81, 106.63, 98.90, 80.46, 72.33, 67.16, 56.28, 55.95, 32.83. ESI-HRMS *m*/*z*: calcd for $C_{29}H_{26}ClFN_4O_5$ $[M+H]^+$: 565.1654; found 565.1624.

5.12.7. (*S*)-*N*-(4-((3-Chloro-4-fluorophenyl)amino)-7-((tetrahydrofuran-3-yl)oxy)quinazolin-6-yl)-3-(3,5-dimethoxyphenyl) acrylamide (10g)

This compound was obtained as yellow solid in 58% yield. Mp 181.0–182.3 °C. ESI-MS *m*/*z*: $[M+H]^+$565.1. ^{1}H NMR (400 MHz, DMSO) *δ* 9.55 (s, 1H), 9.20 (s, 1H), 8.78 (s, 1H), 8.02 (d, *J* = 5.6 Hz, 1H), 7.87 (d, *J* = 15.8 Hz, 1H), 7.71 (s, 1H), 7.60 (d, *J* = 8.2 Hz, 1H), 7.50 (t, *J* = 9.0 Hz, 1H), 7.43 (s, 1H), 7.09 (d, *J* = 15.8 Hz, 1H), 6.64 (d, *J* = 7.4 Hz, 2H), 5.29 (s, 1H), 4.10 (d, *J* = 10.2 Hz, 1H), 4.06–3.92 (m, 2H), 3.89 (s, 3H), 3.83 (s, 3H), 3.80–3.75 (m, 1H), 2.40 (dd, *J* = 13.6, 6.6 Hz, 1H), 2.28–2.15 (m, 1H).

5.12.8. (*S*)-*N*-(4-((3-Chloro-4-fluorophenyl)amino)-7-((tetrahydrofuran-3-yl)oxy)quinazolin-6-yl)-3-(3,4,5-trimethoxyphenyl)acrylamide (10h)

This compound was obtained as yellow solid in 58% yield. Mp 143.8–144.5 °C. ESI-MS *m*/*z*: $[M+H]^+$596.1. ^{1}H NMR (400 MHz, DMSO) *δ* 9.86 (s, 1H), 9.46 (s, 1H), 9.03 (s, 1H), 8.54 (s, 1H), 8.14 (dd, *J* = 6.8, 2.6 Hz, 1H), 7.81 (ddd, *J* = 9.0, 4.2, 2.8 Hz, 1H), 7.61 (d, *J* = 15.6 Hz, 1H), 7.43 (t, *J* = 9.2 Hz, 1H), 7.27 (s, 1H), 7.13 (d, *J* = 15.6 Hz, 1H), 7.02 (s, 2H), 5.33 (s, 1H), 4.08–4.01 (m, 2H), 4.00–3.95 (m, 1H), 3.86 (s, 6H), 3.82–3.77 (m, 1H), 3.71 (d, *J* = 4.8 Hz, 3H), 2.38 (dt, *J* = 14.4, 7.2 Hz, 1H), 2.23–2.15 (m, 1H).

5.12.9. (*S*)-*N*-(4-((3-Chloro-4-fluorophenyl)amino)-7-((tetrahydrofuran-3-yl)oxy)quinazolin-6-yl)-3-(4-nitrophenyl)acrylamide (10i)

This compound was obtained as yellow solid in 78% yield. Mp 245.7–246.8 °C. ESI-MS *m*/*z*: $[M+H]^+$550.1. ^{1}H NMR (400 MHz, DMSO) *δ* 9.69 (s, 1H), 9.07 (s, 1H), 8.58 (s, 1H), 8.31 (d, *J* = 8.4 Hz, 2H), 8.11 (d, *J* = 6.4 Hz, 1H), 7.95 (d, *J* = 5.6 Hz, 1H), 7.93 (s, 1H), 7.77 (s, 1H), 7.73 (s, 1H), 7.44 (m, 2H), 7.30 (s, 1H), 5.32 (s, 1H), 4.09–3.87 (m, 4H), 3.79 (d, *J* = 6.0 Hz, 1H), 2.38 (dd, *J* = 13.4, 6.9 Hz, 1H), 2.20 (d, *J* = 6.4 Hz, 1H).

5.12.10. (*S*)-*N*-(4-((3-Chloro-4-fluorophenyl)amino)-7-((tetrahydrofuran-3-yl)oxy)quinazolin-6-yl)-3-(2,3-dichlorophenyl)acrylamide (10j)

This compound was obtained as yellow solid in 65% yield. Mp 273.5–274.8 °C. ESI-MS *m*/*z*: $[M+H]^+$574.1. ^{1}H NMR (400 MHz, DMSO) *δ* 9.90 (s, 1H), 9.30 (s, 1H), 8.89 (s, 1H), 8.02–7.98 (m, 1H), 7.97 (d, *J* = 3.0 Hz, 1H), 7.83 (dd, *J* = 7.8, 1.2 Hz, 1H), 7.74 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.69 (ddd, *J* = 8.8, 4.3, 2.6 Hz, 1H), 7.59–7.46 (m, 3H), 7.37 (d, *J* = 15.6 Hz, 1H), 5.31 (s, 1H), 4.13 (d, *J* = 10.4 Hz, 1H), 4.05–3.95 (m, 2H), 3.81 (td, *J* = 8.2, 5.4 Hz, 1H), 2.42 (dt, *J* = 14.2, 7.4 Hz, 1H), 2.27–2.18 (m, 1H).

5.12.11. (*S*)-*N*-(4-((3-Chloro-4-fluorophenyl)amino)-7-((tetrahydrofuran-3-yl)oxy)quinazolin-6-yl)-3-(2,3,4-trimethoxyphenyl)acrylamide (10k)

This compound was obtained as yellow solid in 48% yield. Mp 244.0–245.8 °C.ESI-MS *m*/*z*: $[M+H]^+$596.1. ^{1}H NMR (400 MHz, DMSO) *δ* 9.62 (s, 1H), 9.25 (s, 1H), 8.80 (s, 1H), 8.02 (dd, *J* = 6.8, 2.6 Hz, 1H), 7.82 (d, *J* = 15.8 Hz, 1H), 7.71 (ddd, *J* = 8.9, 4.4, 2.6 Hz, 1H), 7.52 (t, *J* = 9.2 Hz, 1H), 7.45 (d, *J* = 8.8 Hz, 1H), 7.37 (s, 1H), 7.15 (d, *J* = 15.8 Hz, 1H), 6.95 (d, *J* = 8.8 Hz, 1H), 5.32 (d, *J* = 6.4 Hz, 1H), 4.11 (d, *J* = 10.6 Hz, 1H), 4.05–3.94 (m, 3H), 3.86 (s, 3H), 3.84 (s, 3H), 3.81 (dd, *J* = 8.2, 2.8 Hz, 1H), 3.78 (s, 3H), 2.40 (dt, *J* = 14.2, 7.2 Hz, 1H), 2.26–2.16 (m, 1H). ^{13}C NMR (101 MHz, DMSO) *δ* 165.23, 165.07, 158.76, 155.66, 154.79, 152.95, 151.03, 142.44, 140.49, 136.26, 135.37, 129.77, 126.48, 125.21, 122.97, 121.55, 120.82, 117.33, 117.12, 116.30, 109.11, 108.07, 103.18, 80.49, 72.36, 66.99, 61.87, 60.81, 56.54, 32.84. ESI-HRMS *m*/*z*: calcd for $C_{30}H_{28}ClFN_4O_6$ $[M+H]^+$: 595.1760; found 595.1718.

5.12.12. (*S*)-*N*-(4-((3-Chloro-4-fluorophenyl)amino)-7-((tetrahydrofuran-3-yl)oxy)quinazolin-6-yl)-3-(3,5-dibromo-4-hydroxyphenyl)acrylamide (10l)

This compound was obtained as yellow solid in 78% yield. Mp 279.6–281.3 °C. ESI-MS *m*/*z*: $[M+H]^+$679.2. ^{1}H NMR (400 MHz, DMSO) *δ* 9.91 (s, 1H), 9.35 (s, 1H), 9.08 (s, 1H), 8.55 (s, 1H), 8.21–8.09 (m, 2H), 7.90 (d, *J* = 13.8 Hz, 2H), 7.53 (d, *J* = 15.6 Hz, 1H), 7.43 (dd, *J* = 16.0, 7.0 Hz, 1H), 7.27 (s, 1H), 7.17 (d, *J* = 15.6 Hz, 1H), 5.34 (s, 1H), 4.05 (s, 1H), 3.96 (dd, *J* = 15.2, 9.5 Hz, 2H), 3.80 (dd, *J* = 12.8, 7.5 Hz, 2H), 2.45–2.31 (m, 1H), 2.26–2.14 (m, 1H).

5.12.13. (*S*)-*N*-(4-((3-Chloro-4-fluorophenyl)amino)-7-((tetrahydrofuran-3-yl)oxy)quinazolin-6-yl)-3-(4-hydroxy-3,5-dimethoxyphenyl)acrylamide (10m)

This compound was obtained as yellow solid in 63% yield. Mp 184.3–186.2 °C. ESI-MS *m*/*z*: $[M+H]^+$581.2. ^{1}H NMR (400 MHz, DMSO) *δ* 9.85 (s, 1H), 9.35 (s, 1H), 9.03 (s, 1H), 8.52 (s, 1H), 8.13 (dd, *J* = 6.8, 2.6 Hz, 1H), 7.80 (dd, *J* = 8.4, 3.4 Hz, 1H), 7.56 (d, *J* = 15.6 Hz, 1H), 7.42 (t, *J* = 9.2 Hz, 1H), 7.25 (s, 1H), 7.01 (d, *J* = 15.6 Hz, 1H), 6.96 (s, 2H), 5.32 (s, 1H), 4.08–3.93 (m, 3H), 3.86–3.79 (m, 6H), 3.80–3.75 (m, 1H), 2.38 (td, *J* = 14.2, 7.6 Hz, 1H), 2.22–2.13 (m, 1H).

5.12.14. (*S*)-3-(3-Bromophenyl)-*N*-(4-((3-chloro-4-fluorophenyl) amino)-7-((tetrahydrofuran-3-yl)oxy)quinazolin-6-yl) acrylamide (10n)

This compound was obtained as yellow solid in 58% yield. Mp 277.1–282.6 °C. ESI-MS *m*/*z*: $[M+H]^+$584.1. ^{1}H NMR (400 MHz, DMSO) *δ* 9.74 (s, 1H), 9.29 (s, 1H), 8.89 (s, 1H), 7.98 (dd, *J* = 6.8, 2.4 Hz, 1H), 7.91 (s, 1H), 7.73–7.59 (m, 4H), 7.54 (t, *J* = 9.0 Hz, 2H), 7.48–7.33 (m, 2H), 5.75 (s, 1H), 5.29 (s, 1H), 4.14 (d, *J* = 10.4 Hz, 1H), 4.01 (m, 2H), 3.81 (dd, *J* = 13.6, 8.0 Hz, 1H), 2.41 (dt, *J* = 14.2, 7.0 Hz, 1H), 2.23 (dd, *J* = 12.8, 6.4 Hz, 1H).

5.12.15. *N*-(4-((3-Chloro-4-fluorophenyl)amino)-7-methoxyquinazolin-6-yl)-3-(4-cyanophenyl)acrylamide (11a)

This compound was obtained as yellow solid in 55% yield. Mp 240.1–242.0 °C. ESI-MS *m*/*z*: $[M+H]^+$474.1. ^{1}H NMR (400 MHz, DMSO) δ 9.94 (s, 1H), 9.14 (s, 1H), 8.70 (s, 1H), 8.09–8.02 (m, 1H), 7.94 (s, 1H), 7.92 (s, 1H), 7.85 (s, 1H), 7.83 (s, 1H), 7.79–7.72 (m, 1H), 7.69 (d, *J* = 15.8 Hz, 1H), 7.46 (dd, *J* = 15.6, 6.0 Hz, 2H), 7.39 (d, *J* = 4.8 Hz, 1H), 4.08 (s, 3H).

5.12.16. *N*-(4-((3-Chloro-4-fluorophenyl)amino)-7-methoxyquinazolin-6-yl)-3-(2-nitrophenyl)acrylamide (11b)

This compound was obtained as yellow solid in 62% yield. Mp 278.9–281.3 °C. ESI-MS *m*/*z*: $[M+H]^+$494.1. ^{1}H NMR (400 MHz, DMSO) δ 9.94 (s, 1H), 9.86 (s, 1H), 9.06 (s, 1H), 8.54 (s, 1H), 8.14 (dd, *J* = 6.8, 2.2 Hz, 1H), 8.10 (d, *J* = 8.2 Hz, 1H), 7.92 (t, *J* = 11.2 Hz, 1H), 7.90–7.83 (m, 2H), 7.83–7.78 (m, 1H), 7.69 (t, *J* = 7.6 Hz, 1H), 7.42 (t, *J* = 9.2 Hz, 1H), 7.31 (d, *J* = 11.0 Hz, 1H), 7.26 (d, *J* = 15.4 Hz, 1H), 4.06 (s, 3H).

5.12.17. 3-(2-Chloro-4-fluorophenyl)-*N*-(4-((3-chloro-4-fluorophenyl)amino)-7-methoxyquinazolin-6-yl)acrylamide (11c)

This compound was obtained as yellow solid in 58% yield. Mp 256.5–257.9 °C. ESI-MS *m*/*z*: $[M+H]^+$502.1. ^{1}H NMR (400 MHz, DMSO) δ 9.86 (s, 1H), 9.85 (s, 1H), 9.07 (s, 1H), 8.55 (s, 1H), 8.13 (d, *J* = 5.6 Hz, 1H), 7.90 (d, *J* = 12.8 Hz, 1H), 7.88–7.84 (m, 1H), 7.81 (d, *J* = 8.2 Hz, 1H), 7.59 (d, *J* = 8.6 Hz, 1H), 7.43 (t, *J* = 7.0 Hz, 1H), 7.41–7.35 (m, 1H), 7.31 (d, *J* = 6.8 Hz, 1H), 7.28 (d, *J* = 15.6 Hz, 1H), 4.06 (s, 3H).

5.12.18. *N*-(4-((3-Chloro-4-fluorophenyl)amino)-7-methoxyquinazolin-6-yl)-3-(2,5-dimethoxyphenyl)acrylamide (11d)

This compound was obtained as yellow solid in 50% yield. Mp 272.1–273.0 °C. ESI-MS *m*/*z*: $[M+H]^+$509.1. ^{1}H NMR (400 MHz, DMSO) δ 9.93 (s, 1H), 9.27 (s, 1H), 8.86 (s, 1H), 7.99 (dd, *J* = 6.2, 2.3 Hz, 1H), 7.90 (d, *J* = 15.8 Hz, 1H), 7.70 (dd, *J* = 8.2, 3.5 Hz, 1H), 7.54 (t, *J* = 9.2 Hz, 1H), 7.44 (s, 1H), 7.27 (d, *J* = 15.8 Hz, 1H), 7.19 (d, *J* = 2.6 Hz, 1H), 7.06 (d, *J* = 9.2 Hz, 1H), 7.01 (dd, *J* = 9.0, 2.8 Hz, 1H), 4.11 (s, 3H), 3.83 (s, 3H), 3.78 (s, 3H).

5.12.19. *N*-(4-((3-Chloro-4-fluorophenyl)amino)-7-methoxyquinazolin-6-yl)-3-(4-methoxyphenyl)acrylamide (11e)

This compound was obtained as yellow solid in 60% yield. Mp 253.4–254.7 °C. ESI-MS *m*/*z*: $[M+H]^+$479.1. ^{1}H NMR (400 MHz, DMSO) δ 9.66 (s, 1H), 9.05 (s, 1H), 8.56 (s, 1H), 8.12 (dd, *J* = 6.8, 2.2 Hz, 1H), 7.84–7.76 (m, 1H), 7.62 (s, 1H), 7.60 (s, 1H), 7.57 (s, 1H), 7.43 (t, *J* = 9.2 Hz, 1H), 7.31 (s, 1H), 7.10 (d, *J* = 15.8 Hz, 1H), 7.04 (s, 1H), 7.01 (s, 1H), 4.05 (s, 3H), 3.81 (s, 3H).

5.12.20. *N*-(4-((3-Chloro-4-fluorophenyl)amino)-7-methoxyquinazolin-6-yl)-3-(2,4-dimethoxyphenyl)acrylamide (11f)

This compound was obtained as yellow solid in 56% yield. Mp 247.1–248.3 °C. ESI-MS *m*/*z*: $[M+H]^+$509.1. ^{1}H NMR (400 MHz, DMSO) δ 9.87 (s, 1H), 9.28 (s, 1H), 8.88 (s, 1H), 8.01–7.93 (m, 1H), 7.84 (d, *J* = 15.8 Hz, 1H), 7.72–7.65 (m, 1H), 7.57 (s, 1H), 7.54 (d, *J* = 7.2 Hz, 1H), 7.50 (s, 1H), 7.14 (d, *J* = 15.8 Hz, 1H), 6.65 (s, 1H), 6.62 (d, *J* = 8.8 Hz, 1H), 4.10 (s, 3H), 3.89 (s, 3H), 3.82 (d, *J* = 6.8 Hz, 3H).

5.12.21. *N*-(4-((3-Chloro-4-fluorophenyl)amino)-7-methoxyquinazolin-6-yl)-3-(3,5-dimethoxyphenyl)acrylamide (11g)

This compound was obtained as light yellow solid in 65% yield. Mp 253.2–254.3 °C. ESI-MS *m*/*z*: $[M+H]^+$509.1. ^{1}H NMR (400 MHz, DMSO) δ 9.88 (s, 1H), 9.28 (s, 1H), 8.89 (s, 1H), 8.03–7.96 (m, 1H), 7.85 (d, *J* = 15.8 Hz, 1H), 7.73–7.65 (m, 1H), 7.57 (d, *J* = 5.8 Hz, 1H), 7.55 (s, 1H), 7.52 (d, *J* = 4.8 Hz, 1H), 7.15 (d, *J* = 15.8 Hz, 1H), 6.65 (d, *J* = 6.4 Hz, 2H), 4.10 (s, 3H), 3.88 (d, *J* = 10.2 Hz, 3H), 3.83 (d, *J* = 8.8 Hz, 3H).

5.12.22. *N*-(4-((3-Chloro-4-fluorophenyl)amino)-7-methoxyquinazolin-6-yl)-3-(3,4,5-trimethoxyphenyl)acrylamide (11h)

This compound was obtained as yellow solid in 73% yield. Mp 179.6–181.2 °C. ESI-MS *m*/*z*: $[M+H]^+$539.2.^{1}H NMR (400 MHz, DMSO) δ 9.88 (s, 1H), 9.65 (s, 1H), 9.06 (s, 1H), 8.54 (s, 1H), 8.13 (d, *J* = 5.2 Hz, 1H), 7.80 (d, *J* = 8.2 Hz, 1H), 7.58 (d, *J* = 15.6 Hz, 1H), 7.43 (t, *J* = 9.2 Hz, 1H), 7.32 (s, 1H), 7.21 (d, *J* = 15.6 Hz, 1H), 7.00 (s, 2H), 4.06 (s, 3H), 3.86 (s, 6H), 3.70 (d, *J* = 10.0 Hz, 3H).

5.13. Cytotoxicity assay in vitro

The cytotoxic activities of target compounds (**10a**–**n** and **11a**–**h**) were evaluated with A549, PC-3, MCF-7 and Hela cell lines by the standard MTT assay in vitro, with compounds EGFR inhibitors afatinib as positive control. The cancer cell lines were cultured in minimum essential medium (MEM) supplement with 10% fetal bovine serum (FBS). Approximately 4×10^3 cells, suspended in MEM medium, were plated onto each well of a 96-well plate and incubated in 5% CO_2 at 37 °C for 24 h. The test compounds at indicated final concentrations were added to the culture medium and the cell cultures were continued for 72 h. Fresh MTT was added to each well at a terminal concentration of 5 μg/mL and incubated with cells at 37 °C for 4 h. The formazan crystals were dissolved in 100 μL DMSO each well, and the absorbency at 492 nm (for absorbance of MTT formazan) and 630 nm (for the reference wavelength) was measured with the ELISA reader. All of the compounds were tested three times in each of the cell lines. The results expressed as inhibition rates or IC_{50} (half-maximal inhibitory concentration) were the averages of two determinations and calculated by using the Bacus Laboratories Incorporated Slide Scanner (Bliss) software.

5.14. EGFR and VEGFR2/KDR kinases assay in vitro

The target compounds **10e** and **10k** are tested for their activity against EGFR or VEGFR2/KDR kinases through the mobility shift assay. All kinases assays were performed in 96-well plates in a 50 μL reaction volume. The kinase buffer contains 50 mM HEPES, pH 7.5, 10 mM $MgCl_2$, 0.0015% Brij-35 and 2 mM DTT. The stop buffer contains 100 mM HEPES, pH 7.5, 0.015% Brij-35, 0.2% Coating Reagent #3 and 50 mM EDTA. Dilute the compounds to 500 μM by 100% DMSO, then transfer 10 μL of compound to a new 96-well plate as the intermediate plate, add 90 μL kinase buffer to each well. Transfer 5 μL of each well of the intermediate plate to 384-well plates. The following amounts of enzyme and substrate were used per well: kinase base buffer, FAM-labeled peptide, ATP and enzyme solution. Wells containing the substrate, enzyme, DMSO without compound were used as DMSO control. Wells containing just the substrate without enzyme were used as low control. Incubate at room temperature for 10 min. Add 10 μL peptide solution to each well. Incubate at 28 °C for specified period of time and stop reaction by 25 μL stop buffer. At last collect data on Caliper program and convert conversion values to inhibition values. Percent inhibition = (max − conversion)/(max − min) × 100. 'max' stands for DMSO control; 'min' stands for low control.

5.15. Docking studies

For docking purposes, the three-dimensional structure of the EGFR (PDB code: 4G5J) was obtained from RCSB Protein Data Bank.[13] Hydrogen atoms were added to the structure allowing for appropriate ionization at physiological pH. The protonated state of several important residue were adjusted by using SYBYL6.9.1

(Tripos, St. Louis, USA) in favor of forming reasonable hydrogen bond with the ligand. Molecular docking analysis was carried out by the SURFLEX-DOCK module of SYBYL 6.9.1 package to explore the binding model for the active site of c-Met with its ligand. All atoms located within the range of 5.0 Å from any atom of the cofactor were selected into the active site, and the corresponding amino acid residue was, therefore, involved into the active site if only one of its atoms was selected. Other default parameters were adopted in the SURFLEX-DOCK calculations. All calculations were performed on Silicon Graphics workstation.

Acknowledgments

We gratefully acknowledge the generous support provided by The National Natural Science Funds (No. 81460527), Science and Technology Project Founded by the Education Department of Jiangxi Province, Top-notch talent project of Jiangxi Science & Technology Normal University (2015QNBJRC001), Program of Key Laboratory of Drug Design and Optimization, Jiangxi Science & Technology Normal University (300098010306), College Students' Science and Technology Innovation Project of Jiangxi Science & Technology Normal University (20150904040) and the Innovation Fund Designated for Graduate Students of Jiangxi Province (YC2015-X28).

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