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Research paper

# Design, synthesis and biological evaluation of novel EGFR/HER2 dual inhibitors bearing a oxazolo[4,5-g]quinazolin-2(1H)-one scaffold

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#### A R T I C L E I N F O

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#### ABSTRACT

For the purpose of developing novel EGFR/HER2 tyrosine kinases inhibitors with high inhibition activity and low toxicity, two novel series of oxazolo[4,5-g]quinazolin-2(1H)-one derivatives as EGFR/HER2 dual inhibitors introducing two electrophiles 2-(2-bromoacetyl)ethyl and 2-(2-chloroacetoxy)ethyl group as side-chain at 1-position respectively and evaluated their EGFR and HER2 inhibition activity and toxicity comparing with Lapatinib. All these compounds were evaluated by EGFR and HER2 kinase inhibition and two anti-proliferation assays *in vitro*. Most of the designed compounds exhibited moderate to high inhibition activity against EGFR and HER2. Especially, compounds **110**, **11p**, **12e** and **12f** presented high inhibition against EGFR and HER2. Furthermore, compounds **11p** and **12f** also had well exhibition to excellent anti-proliferation activity against human lung adenocarcinoma cell line (A549) and human breast cancer cell line (SK-Br3), and **12f** also exhibited the lowest toxicity against human embryonic lung fibroblast cell line (HELF) cell. Finally, compound **12f** presented remarkably higher inhibition efficacy towards tumour growth than Lapatinib in a mouse lewis lung cancer (LLC) xenograft model.

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#### 1. Introduction

EGFR family contains four structurally related receptors, EGFR (ErbB1/HER1), ErbB2 (HER2), ErbB3 (HER3) and ErbB4 (HER4), for which a variety of different ligands have been characterized. EGFR has become one of the targets in anticancer drug research because of its wide distribution and essential role in cell proliferation, differentiation and migration [1,2]. Overexpression of the EGFR and HER2 is frequently observed in various solid tumors, and coexpression of EGFR and HER2 has been found in different tumors such as breast, ovarian, prostate and colon cancer [3]. Therefore, EGFR and HER2 have been the most well studied targets in current drug research, and EGFR/HER2 dual inhibitors can be more effective than just EGFR or HER2 inhibitors because of HER2 binding either EGFR or HER2 to form a hetero- or homodimer for the signal transduction of EGFR signal cascade [4,5]. In recent years, several EGFR/HER2 dual inhibitors have been approved for cancer by U.S. Food and

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Drug Administration (FDA), such as the first generation EGFR/HER2 inhibitor Lapatinib (Tykerb) [6] and the second generation EGFR/ HER2 inhibitor Afatinib (Gilotrif) [7]. As shown in Fig. 1, Lapatinib is a reversible EGFR/HER2 dual inhibitor while Afatinib is an irreversible EGFR/HER2 dual inhibitor. Quinoline moiety in Lapatinib and Afatinib is an important pharmacophore for anticancer activity and represents an attractive scaffold for EGFR inhibitors [8], and Afatinib has higher inhibition activity than Lapatinib because Afatinib is an irreversible inhibitor which contains a Michael addition receptor moiety which can form a covalent bind with the conserved cysteine residue present in the lip of the EGFR ATP binding site (Cys797) to achieve occupancy greater than the reversible inhibitor Lapatinib [9]. Unfortunately, due to toxicity-related dose-limitation, Afatinib only displayed 7% response rate for gefitinib-resistant nonsmall cell lung cancer (NSCLC) patients in a phase III clinical trial [10]

For the purpose of developing novel EGFR/HER2 tyrosine kinases inhibitors with high inhibition activity and low toxicity, we designed two novel series of oxazolo[4,5-g]quinazolin-2(1H)-one derivatives as EGFR/HER2 dual inhibitors introducing two electrophiles 2-(2-bromoacetyl)ethyl and 2-(2-chloroacetoxy)ethyl group as side-chain at 1-position respectively and evaluated their EGFR





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Fig. 1. The structures of Lapatinib and Afatinib.

and HER2 inhibition activity and toxicity comparing with Lapatinib (Fig. 2). Eventually, compounds **110**, **11p**, **12e** and **12f** presented higher inhibition against EGFR and HER2 than Lapatinib. Moreover, compounds **11p** and **12f** also had well exhibition to excellent antiproliferation activity against human lung adenocarcinoma cell line (A549) and human breast cancer cell line (SK-Br3), and among which **12f** showed the lowest toxicity. Furthermore, compound **12f** presented remarkably higher inhibition efficacy towards tumour growth than Lapatinib in a mouse lewis lung cancer (LLC) xenograft model (P < 0.05). Therefore, we reported compound **12f** as a promising candidate for clinical development as novel EGFR/HER2 dual inhibitors.

#### 2. Results and discussion

#### 2.1. Rational and design

Based on our earlier work with oxazolo[4,5-g]quinazolin-2(1H)one inhibitors [11,12], we planned to develop EGFR/HER2 dual inhibitors with high inhibition activity and low toxicity by exploring the different electrophiles at 1-position. 2-(2-bromoacetyl)ethyl and 2-(2-chloroacetoxy)ethyl were expected to be more promiscuous because of their (expectedly) high reactivity [13,14]. These two side-chains in target compounds were responsible for the covalent binding to an active site cysteine residue.

With respect to the high inhibition activity, 2-(2-bromoacetyl) ethyl group was adopted at 1-position due to its high electrophilic reactivity and nucleophilic displacement with aniline at 8-position to give series I compounds. Then, 2-(2-chloroacetoxy)ethyl group which was less reactive [15] compared with 2-(2-bromoacetyl) ethyl group was introduced at 1-position, and aniline was displaced at 8-position according to the inhibition activity results of series I compounds to give series II compounds (Fig. 2).

#### 2.2. Chemistry

The synthetic method for target compounds was showed in



Series II compoun

Fig. 2. The design dual EGFR/HER2 inhibitors.

Schemes 1 and 2. Compounds **2~6** were synthesized in as described in our earlier research [12].

The series I compounds were obtained by alkylation of compound **6** with 2-bromoethan-1-ol, cyclization with formamidine acetate in EtOH, esterification with bromoacetyl bromide, chlorination with thionyl chloride using DMF as catalyst, and nucleophilic displacement with aniline (Scheme 1). Likewise, the series II compounds were synthesized in as described in Scheme 2.

#### 2.3. Biological activities

### 2.3.1. Kinase inhibitory activity and cancer cell proliferation inhibitory activity

Our principle behind the designed compounds is to improve the inhibition activity and reduce toxicity. Therefore, EGFR and HER2 tyrosine kinase inhibition assay and cancer cell proliferation inhibition assay were used to assess the in vitro antitumor activities of designed compounds. EGFR and HER2 kinase inhibition assay was performed according to the instructions of the manufacturer, and human lung adenocarcinoma cell line (A549) [16] which overexpresses EGFRWT and human breast cancer cell line (SK-Br3) [17] overexpressing HER2 were selected as cell models to evaluate the anti-proliferation of the designed compounds. As shown in Table 1, most of the designed compounds exhibited moderate to high inhibition activity against EGFR and HER2, which revealed that introducing 2-(2-bromoacetyl)ethyl at 1-position of oxazolo[4,5-g] quinazolin-2(1H)-one scaffold contributed to the activity. As indicated by the results that compounds containing some functional groups (such as halogens, alkoxyl groups etc.) at 8-position caused an increase in EGFR and HER2 inhibition with the exception of compounds 11a, 11b, 11d, 11i, 11o and 11p. Among these, the compounds which contained polar groups (such as piperazinyl, morpholino etc.) would also demonstrate increased inhibition activities towards EGFR and HER kinase like 110 and 11p. However, compounds containing some big functional groups would demonstrate decreased inhibition activities like 11e, 11f, 11g and **11h.** This may be due to their relatively large structures, which sterically hindered their interaction with the active site on kinase. Above all, compounds 110 and 11p exhibited promising activities with IC\_{50} values of 0.008  $\mu$ M and 0.0010  $\mu$ M on EGFR, 0.033  $\mu$ M and 0.021 µM on HER2, respectively. This was a significant improvement when compared to Lapatinib which had IC<sub>50</sub> values of 0.026 µM on EGFR and 0.017 µM on HER2 (Table 1).

The cellular anti-proliferative effects were mainly coincident with kinase inhibition activity, most compounds exhibited moderate to high inhibition activities and cytotoxicities to A549 and SK-Br3 cell. Compounds **110** and **11p** exhibited high activities with IC<sub>50</sub> values of 2.03  $\mu$ M and 3.60  $\mu$ M against A549, 12.50  $\mu$ M and 2.30  $\mu$ M against SK-Br3, respectively. And, Lapatinib had IC<sub>50</sub> values of 6.74  $\mu$ M on EGFR and 0.47  $\mu$ M against SK-Br3 (Table 1).

According the inhibition activity result of **11a–11q**, 2-(2chloroacetoxy)ethyl group was introduced at 1-position, and aniline was displaced at 8-position to give compounds **12a–12g**. As shown in Table 1, almost all of compounds had potent activity on EGFR and HER2 as well as A549 and SK-Br3 cells except compound **12d**. The EGFR and HER2 kinase inhibition activities of compounds **12a–12g** had a slight decrease compared with compounds **11a–11q** when they had the same aniline displaced at 8-position, such as **11d** vs. **12c**, **11i** vs. **12b**, **11p** vs. **12f**, etc.. The cancer cell proliferation inhibitory activities of compounds **12a–12g** were also mainly coincident with kinase inhibition activity, most compounds exhibited moderate to high inhibition activities and cytotoxicities to A549 and SK-Br3 cell. We were excited finding that compound **12f** exhibited equivalent inhibition activity towards EGFR and HER2 kinase as well as A549 and SK-Br3 cells (Table 1).



Scheme 1. Reagents and reaction condition: (a) fuming HNO<sub>3</sub>, acetic acid, DCM, -15 °C, 1 h; (b) SnCl<sub>2</sub>·2H<sub>2</sub>O, conc. HCl, 45 °C, 7 h; (c) CDI, anhydrous THF, 25 °C, 2 h; (d) conc. HNO<sub>3</sub>, 60 °C, 8 h; (e) reduced iron powder, acetic acid, MeOH, reflux, 3 h; (f) 2-bromoethanol, acetonitrile, K<sub>2</sub>CO<sub>3</sub>, MeCN/DMF, 60 °C, 3 h; (g) formamidine acetate, ethanol, reflux, 5 h; (h) 2-bromoacetyl bromide, DMF, r.t.; (i) SOCl<sub>2</sub>, DMF(cat.), reflux, 16 h; (j) aniline, i-PrOH, reflux, 6–24 h.



Scheme 2. Reagents and reaction condition: (a) 2- chloroacetyl chloride, DMF, r.t.; (b) SOCl<sub>2</sub>, DMF(cat.), reflux, 16 h; (c) aniline, i-PrOH, reflux, 6–24 h.

#### 2.3.2. Normal cell cytotoxicity

As further evaluation for the selective cytotoxicity of the most promising compounds (**11p** and **12f**), they have been tested over normal human embryonic lung fibroblast cell line (HELF) [18] using MTT assay. As illustrated in Table 2, compounds **11p** and **12f** had lower cytotoxicity to HELF cell compared with Lapatinib, and

compound **12f** exhibited the lowest cytotoxicity to normal human lung cell and normal human breast cell.

#### 2.3.3. In vivo antitumor activity

The xenograft mouse lewis lung cancer (LLC) tumor mice model [12] was used to investigate the *in vivo* antitumor activity of

#### Table 1

The IC<sub>50</sub> values of the designed compounds toward EGFR and HER2 kinase as well as A549 and SK-Br3 cells.







Comp.	R <sub>1</sub>	R <sub>2</sub>	Enzymatic. IC <sub>50</sub> <sup>a</sup> (µM)		Cell inhibition $IC_{50}^{a}(\mu M)$	
			EGFR	HER2	A549 <sup>b</sup>	SK-Br3 <sup>c</sup>
11a 11b	Cl Cl	F O	$\begin{array}{c} 0.46 \pm 0.07 \\ 0.077 \pm 0.013 \end{array}$	1.1 ± 0.2 0.18 ± 0.03	21.28 ± 3.54 2.17 ± 0.93	$\frac{11.44 \pm 0.97}{2.44 \pm 0.15}$
11c 11d	H Cl	t-Bu	$\begin{array}{c} 0.87 \pm 0.17 \\ 0.16 \pm 0.04 \end{array}$	$6.2 \pm 1.1$ $0.12 \pm 0.01$	>50 11.91 ± 2.45	>50 7.68 ± 3.61
11e	Н		ND	ND	>50	ND
11f	Н	o ∽ <sup>N</sup> ↓ <sup>N</sup> ↓∕	ND	ND	ND	ND
11g	Н		9.3 ± 2.4	>10	>50	ND
11h	Н		8.5 ± 1.5	$4.7\pm0.7$	ND	>50
11i 11j	F H	F O	0.30 ± 0.06 >10	1.3 ± 0.2 ND	>50 >50	1.17 ± 0.16 >50
11k	Н		$6.4 \pm 0.5$	>10	>50	>50
11l 11m	H H	-COOEt	ND >10	>10 3.9 ± 0.6	ND ND	ND 17.94 ± 4.30
11n 11o	– <b>C≡CH</b> Cl	H N	$\begin{array}{c} 5.2 \pm 0.9 \\ 0.008 \pm 0.004 \end{array}$	ND 0.033 ± 0.010	ND 2.03 ± 0.54	ND 12.50 ± 2.41
11p	Cl		0.010 ± 0.002	0.021 ± 0.007	3.60 ± 0.89	$2.30\pm0.37$

(continued on next page)

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Comp.	R <sub>1</sub>	R <sub>2</sub>	Enzymatic. IC <sub>50</sub> <sup>a</sup> (μM)		Cell inhibition $IC_{50}^{a}(\mu M)$	
			EGFR	HER2	A549 <sup>b</sup>	SK-Br3 <sup>c</sup>
11q	Cl	N	$0.184 \pm 0.027$	>10	15.07 ± 1.06	>50
12a 12b 12c	Cl F Cl	F F O CF3	$\begin{array}{c} 0.33 \pm 0.09 \\ 4.7 \pm 1.1 \\ 0.51 \pm 0.14 \end{array}$	$\begin{array}{c} 1.6 \pm 0.2 \\ 3.2 \pm 0.7 \\ 0.23 \pm 0.03 \end{array}$	$\begin{array}{c} 5.64 \pm 1.51 \\ 10.46 \pm 0.79 \\ 24.72 \pm 3.67 \end{array}$	$\begin{array}{l} 33.04 \pm 6.83 \\ >50 \\ 2.05 \pm 0.53 \end{array}$
12d	Cl	,0, F	>10	>10	ND	ND
12e	Cl		0.020 ± 0.011	0.009 ± 0.010	$1.22 \pm 0.60$	25.1 ± 8.54
12f	Cl	N	0.019 ± 0.010	0.035 ± 0.008	$4.49 \pm 2.68$	0.47 ± 0.35
12g	Cl	N	$0.24\pm0.06$	0.077 ± 0.023	ND	46.88 ± 1.62
Lapatinib			$0.026\pm0.012$	$0.017\pm0.010$	6.74 ± 1.33	$0.49 \pm 0.04$

<sup>a</sup> The values represent the mean  $\pm$  SD of at least three independent experiments.

<sup>b</sup> A549 is a human lung cancer cell line which overexpresses EGFR<sup>WT</sup>.

<sup>c</sup> SK-Br3 is a HER2 overexpressing human breast cancer cell line.

Table 2	
The cytotoxicity IC <sub>50</sub> values of <b>11p</b> and	12f over HELF normal cell line.

Comp	Cell inhibition $IC_{50}^{a}$ (µM) HELF <sup>b</sup>
11p	54.30 ± 7.15
12f	>100
Lapatinib	$12.10 \pm 2.31$

 $^{\rm a}$  The values represent the mean  $\pm$  SD of at least three independent experiments.

<sup>b</sup> HELF is a human embryonic lung fibroblast cell line.

designed compound **12f**. As shown in Fig. 3, compound **12f** and Lapatinib were well tolerated and displayed significant *in vivo* antitumor efficacy when compared with water as vehicle control, and compound **12f** presented remarkably higher inhibition efficacy towards tumour growth than Lapatinib (P < 0.05) (Fig. 3A). The images of the tumor harvested from the mice visually showed the greatest tumor shrinkage after treatment with compound **12f** (Fig. 3B), and no significant change of the body weight during the

#### study.

#### 2.4. Structure-activity relationships

As illustrated in our earlier research, N<sub>1</sub>, N<sub>3</sub> atoms in pyrimidine ring are ATP competitive binding sites, which is pharmacophore of EGFR inhibitors. And also, the proton in aniline at 8-position is essential. As shown in Fig. 4, introducing electrophiles side-chain, such as 2-(2-bromoacetyl)ethyl and 2-(2-chloroacetoxy)ethyl group can achieve medium to high inhibition activity, and the EGFR and HER2 kinase inhibition activities had a slight decrease when X = Cl compared with X = Br when they had the same aniline displaced at 8-position because 2-(2-chloroacetoxy)ethyl was less reactive compared with 2-(2-bromoacetyl)ethyl group, such as **11d** vs. **12c**, **11i** vs. **12b**, **11p** vs. **12f**, etc.. Halogen introduced in phenyl amines can improve the inhibition activity (Cl > F > H), such as compounds **11a**, **11i**, **11k**, and morpholinyl, 4-methylpiperazin-1-y introduced in phenyl amines may increase the compound inhibition activity, such as compounds **11o**, **11p**, **12e**, **12f**. However, tert-



**Fig. 3.** (A) In vivo antitumor effect of Compound **12f** in a LLC xenograft mouse model. Eighteen mice were weighed and randomly divided into three groups (n = 6): 1) Control; 2) Lapatinib; 3) Compound **12f**. Mice bearing established LLC tumor xenografts were dosed with Compound **12f** (100 mg/kg.qd.po) over a 14 day period, and Lapatinib (100 mg/kg.qd.po) was employed as reference drug. Tumor size was measured every other day. Data are shown as the mean  $\pm$  SD (n = 6). Statistical significance (p < 0.05) for antitumor efficacy, based upon tumor growth relative to the controls. (B) Representative images of the tumor harvested from the mice after treatment with vehicle, Lapatinib and compound **12f**.

Table 1 (continued)



Fig. 4. Structure-activity relationships identified of designed compounds.

butyl and some big functional groups such as 4-(morpholine-4carboxamido)phenyl, 4-(3-cyclopentylureido)phenyl, 4-(piperidine-1-carboxamido)phenyl, 4-(3-cyclohexyl ureido)phenyl groups introduced in phenyl amines would decreased inhibition activities like **11c**, **11e**, **11f**, **11g** and **11h**. This may be due to their relatively large structures, which sterically hindered their interaction with the active site on kinase (Fig. 4 and Table 1).

#### 2.5. Molecular docking study

Docking study using GOLD 4.1 was performed. GOLD is a genetic algorithm based docking program that allows a wide range of flexibility for the ligand and the protein [19]. It is used to predict the binding modes and orientation of the synthesized compounds at the active site of the ATP binding site of EGFR-TK. The crystal structure of the enzyme with Lapatinib (ID: 1XKK) was obtained from protein data bank (PDB). The kinase or catalytic domain includes an N-terminal lobe, which consists mainly of  $\beta$ -strands but contains one  $\alpha$ -helix and C helix. The C-terminal lobe is mainly  $\alpha$ helical, and a short strand termed the hinge region connects the two lobes [20]. The docking studies were progressed by positioning the compound **12f** in the Lapatinib binding site, and the results had revealed that the oxazolo[4,5-g]quinazolin-2(1H)-one ring bounded to a narrow hydrophobic pocket in the N-terminal domain of EGFR active site where N5 of the oxazolo[4,5-g]quinazolin-2(1H)one core interacted with the backbone NH of Met793 via a hydrogen bond. A water molecule-mediated hydrogen bonding interaction between the N7 and the Thr854 side chain was observed which was similar to Lapatinib. These interactions underscored the importance of nitrogen atoms for the binding and the subsequent inhibitory capacity. The aniline moiety at the C4 lay in a deep hydrophobic pocket. The morpholinyl at the C4 of aniline moiety lay in the same site of the 3-fluorobenzyl)oxy)phenyl moiety of Lapatinib. It was glad to found a covalent bonding interaction existing between the 2-(2-chloroacetoxy)ethyl sidechains and the Cys797 cysteine residue which revealed that 12f could form the covalent binding to an active site cysteine residue of EGFR (Fig. 5).

#### 3. Conclusion

In summary, a series of novel oxazolo[4,5-g]quinazolin-2(1H)one derivatives as EGFR/HER2 dual inhibitors were synthesized and subjected to pharmacological evaluation. The results showed that most of oxazolo[4,5-g]quinazolin-2(1H)-one derivatives exhibited moderate to high EGFR inhibition activities. Protein tyrosine kinase inhibitions assay indicated that many of these derivatives exerted potent activity on both EGFR and HER2 kinases. Moreover, these derivatives demonstrated high anti-proliferation potencies against A549 and SK-Br3 cells. Among them two designed compounds **11p** and **12f** exhibited more potent inhibition activities than the



**Fig. 5.** Binding model of Compound **12f** (brown) in complex with EGFR (PDB ID: 1XKK) and Lapatinib (bright blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

reference compounds Lapatinib which merited further evaluation. Especially, **12f** also exhibited the lowest toxicity against HELF cell compared with Lapatinib and **11p**. Furthermore, compound **12f** presented remarkably higher inhibition efficacy towards tumour growth than Lapatinib. In conclusion, the findings presented herein showed that introducing electrophiles (such as 2-(2-bromoacetyl) ethyl and 2-(2-chloroacetoxy)ethyl) at 1-position of oxazolo[4,5-g] quinazolin-2(1H)-one scaffold is an excellent strategy for the development of EGFR/HER2 dual inhibitors.

#### 4. Experimental sections

#### 4.1. Chemistry experiment

#### 4.1.1. Chemistry: general procedures

All commercially available starting materials, reagents and solvents were used without further purification unless otherwise stated. Melting points were determined with an Electro thermal melting point apparatus, and are uncorrected. High-resolution mass spectra (HRMS) were recorded on QSTAR XL Hybrid MS/MS mass spectrometer. <sup>1</sup>H -NMR and <sup>13</sup>C NMR spectra on a Bruker AV 300300 or 500 MHz spectrometer were recorded in DMSO-d<sub>6</sub>. Chemical shifts are reported in d (ppm) units relative to the internal standard tetramethylsilane (TMS). The reaction conditions were not optimized for reaction yields. All oxygen-sensitive or moisturesensitive reactions were run under nitrogen atmosphere. All the reactions were monitored by thin layer chromatography (TLC) on pre-coated silica gel G plates at 254 nm under a UV laMp using ethyl petroleum ether/acetate or DCM/MeOH as eluent. Column chromatography separations were obtained on silica gel (200-300 mesh).

#### 4.1.2. Purity analysis

The purity of the synthesized compounds were measured by high performance liquid chromatography (HPLC, Shimadzu LC-2010 system, Kyoto, Japan) equipped with a Diamonsil C18 column (5  $\mu$ m particle size, 250 mm × 4.6 mm). The mobile phase consisted of acetonitrile and water with a flow rate of 1.0 mL/min. The detection wavelength was 344 nm and sample injected volume was 20  $\mu$ L. All compounds evaluated for EGFR/HER2 inhibitory potency had a purity of  $\geq$  95%.

### 4.1.3. Ethyl 6-amino-3-(2-hydroxyethyl)-2-oxo-2,3-dihydrobenzo [d]oxazole-5-carboxylate (**7**)

Compound **6** (13.2 g, 60.0 mmol) was dissolved in 75 mL DMF, and MeCN 150 mL, 2-bromoethan-1-ol (22.5 g, 180 mmol) and K<sub>2</sub>CO<sub>3</sub> (24.8 g, 180 mmol) were successively added, and the mixture was stirred at 60 °C for 4 h. After cooled to room temperature, ice water (500 mL) was added and the aqueous phase was extracted with AcOEt (4 × 200 mL). The organic layers were combined, dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated to give the crude product. The crude product was purified by silica-gel column chromatography (PE/EA, 4/1), R<sub>f</sub> = 0.25. Drying gave compound **7** (12.8 g, yield, 80.1%); mp: 159–161 °C; <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>)  $\delta$  10.37 (1H, s), 7.65 (1H, s), 6.75 (2H, s), 6.68 (1H, s), 4.37 (2H, t, *J* = 7.2 Hz), 4.21 (2H, q, *J* = 6.9 Hz), 3.77 (2H, t, *J* = 7.2 Hz), 1.27 (3H, t, *J* = 6.9 Hz).

#### 4.1.4. 1-(2- hydroxyethyl) oxazolo[4,5-g]quinazoline-2,8(1H,7H)dione (**8**)

Compound **7** (12.0 g, 45.0 mmol) and formamidine acetate (5.15 g, 49.5 mmol) and ethanol (120 mL) was successively added and the mixture was refluxed for 24 h under the protection of N<sub>2</sub>. The mixture was cooled to room temperature and filtered, and the solid was washed with cold EtOH (50 mL) and water (50 mL), and dried to give compound as white power (9.2 g, yield, 82.9%); mp: 237–239 °C; <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>)  $\delta$  12.27 (1H, br s), 8.04 (1H, s), 7.91 (1H, s), 7.57 (1H, s), 4.96 (1H, t, *J* = 5.7 Hz), 3.95 (2H, t, *J* = 5.4 Hz), 3.70 (2H, t, *J* = 5.4 Hz).

### 4.1.5. General procedure for the synthesis of 1-(2-(2-bromoacetyl) ethyl) oxazolo[4,5-g]quinazolin-2(1H)-one derivatives (**11a-q**)

4.1.5.1. 1-(2-(2-bromoacetyl)ethyl)oxazolo[4,5-g]quinazoline-2,8(1H,7H)-dione (**9a**). Compound **8** (3.0 g, 12.1 mmol) was dissolved in 50 mL DMF, and bromoacetyl bromide (7.3 g, 36.4 mmol) was added dropwise at 0 °C. The reaction mixture was stirred at room temperature for 30 min, and then the mixture was poured into ice water (200 mL) and stirred for 15 min, followed by filtering and distilling under a reduced pressure. The white product was purified by silica-gel column chromatography (DCM/MeOH, 100/1), R<sub>f</sub> = 0.15. Drying give compound **9a** (3.8 g, yield, 85.4%); mp: 245–246 °C; <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>)  $\delta$  12.27 (1H, br s), 8.05 (1H, s), 7.98 (1H, s), 7.57 (1H, s), 4.46 (2H, t, *J* = 4.6 Hz), 4.24–4.23 (4H, m).

4.1.5.2. 1-(2-(2-bromoacetyl)ethyl)-8-Chlorooxazolo[4,5-g]quinazolin-2(1H)-one (**10a**). Compound **9a** (3.7 g, 10 mmol), thionyl chloride (40 mL) and DMF (4 drops) was successively added and the mixture was refluxed for 24 h. After cooled to room temperature, most of the excess of thionyl chloride and DMF was removed under reduced pressure to give the yellow residue. The crude product was purified by silica-gel column chromatography (DCM), R<sub>f</sub> = 0.21. Drying gave compound **10a** (3.3 g, yield, 85.4%); mp: 184–185 °C; <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>)  $\delta$  8.43 (1H, s), 8.02 (1H, s), 7.65 (1H, s), 4.43 (2H, t, *J* = 4.4 Hz), 4.24–4.22 (4H, m).

4.1.5.2.1.  $1-(2-(2-bromoacetyl)ethyl)-8-(3-Chloro-4-fluorophenylamino)oxazolo[4,5-g]quinazolin-2(1H)-one (11a). A mixture of compound 10a (155 mg, 0.4 mmol) and 3-chloro-4-fluoroaniline (70 mg, 0.48 mmol) in isopropanol (6 mL) was stirred for 24 h. After cooled to room temperature, the mixture was filtered and washed with chill isopropanol (3 mL) and the residue was treated with aqueous NaHCO<sub>3</sub> (10 mL) and extracted with EtOAc/MeOH (20:1, 20 mL). The organic layer was washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated under reduced pressure. Purified by silica-gel column chromatography (DCM/MeOH, 100/1), <math>R_f = 0.23$ . Drying gave 163 mg (yield, 82.3%) of the title compound as white solid; mp: 229–231 °C; HPLC purity: 98%; HRMS, ESI<sup>+</sup>, *m/z*: Calcd for C<sub>19</sub>H<sub>14</sub>BrClFN<sub>4</sub>O<sub>4</sub> (M + H)<sup>+</sup>, 494.9881;

found, 494.9879; <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>)  $\delta$  11.60 (1H, br s), 9.01 (1H, s), 8.90 (1H, s), 8.08 (1H, dd, J = 6.8, 2.5 Hz), 7.82–7.78 (2H, m), 7.54 (1H, t, J = 9.0 Hz), 4.57 (2H, t, J = 4.7 Hz), 4.29 (2H, s), 4.20 (2H, t, J = 4.7 Hz); <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>)  $\delta$  167.06, 159.15, 153.10, 149.82, 147.69, 136.44, 134.13 (d, J = 2.7 Hz), 132.49, 126.28, 125.04 (d, J = 7.3 Hz), 116.85 (d, J = 21.8 Hz), 110.37, 103.58, 99.88, 62.05, 41.88, 25.61.

4.1.5.2.2. 1-(2-(2-bromoacetyl)ethyl)-8-((3-chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)oxazolo[4,5-g]quinazolin-2(1H)-one (**11b**). Purified by silica-gel column chromatography (DCM/MeOH, 100/1), R<sub>f</sub> = 0.20. Light yellow solid, 200 mg, yield, 83.3%; mp: 252–254 °C; HPLC purity: 97%; HRMS, ESI+,*m/z* $: Calcd for C<sub>26</sub>H<sub>20</sub>BrClFN<sub>4</sub>O<sub>5</sub> (M + H)<sup>+</sup>, 601.0289; found, 601.0285; <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>) <math>\delta$  11.71 (1H, br s), 9.16 (1H, s), 8.91 (1H, s), 7.75–7.65 (m, 1H), 7.50–7.45 (m, 1H), 7.38–7.28 (m, 2H), 7.24–7.10 (m, 1H), 5.30 (s, 2H), 4.60 (2H, t, *J* = 4.6 Hz), 4.33 (2H, s), 4.24 (2H, t, *J* = 4.6 Hz); <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>)  $\delta$  167.06, 163.80, 160.58, 158.92, 153.12, 151.54, 149.81, 147.57, 136.28, 132.39, 130.61 (d, *J* = 7.5 Hz), 126.05 (d, *J* = 3.2 Hz), 124.37, 123.32 (d, *J* = 2.6 Hz), 121.10, 114.75 (d, *J* = 7.7 Hz), 114.14 (d, *J* = 8.3 Hz), 113.91, 110.29, 103.46, 99.89, 69.38, 62.05, 41.84, 25.62.

4.1.5.2.3. 1-(2-(2-bromoacetyl)ethyl)-8-((3-chloro-4-(tert-butyl) phenyl)amino) oxazolo[4,5-g]quinazolin-2(1H)-one (**11c**). Purified by silica-gel column chromatography (DCM/MeOH, 200/1),  $R_f = 0.22$ . Light yellow solid, 172 mg, yield, 86.1%; mp: 224–226 °C; HPLC purity: 97%; HRMS, ESI<sup>+</sup>, *m*/*z*: Calcd for C<sub>23</sub>H<sub>24</sub>BrN<sub>4</sub>O<sub>4</sub> (M + H)<sup>+</sup>, 499.0981; found, 499.0978; <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>)  $\delta$  11.64 (1H, br s), 9.01 (1H, s), 8.86 (1H, s), 7.86 (1H, s), 7.66 (2H, d, *J* = 8.6 Hz), 7.51 (2H, d, *J* = 8.6 Hz), 4.60 (2H, t, *J* = 4.6 Hz), 4.33 (2H, s), 4.24 (2H, t, *J* = 4.6 Hz), 1.32 (9H, s); <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>)  $\delta$  167.04, 158.66, 155.04, 153.08, 149.57, 147.42, 138.90, 135.88, 132.28, 130.40, 124.60, 119.40, 110.16, 103.40, 99.70, 62.00, 41.80, 34.67, 31.35, 25.63.

4.1.5.2.4. 1-(2-(2-bromoacetyl)ethyl)-8-((3-chloro-4-(3-(tri-fluoromethyl)phenoxy)phenyl)amino)oxazolo[4,5-g]quinazolin-2(1H)-one (**11d**). Purified by silica-gel column chromatography (DCM/MeOH, 70/1), R<sub>f</sub> = 0.23. Light yellow solid, 214 mg, yield, 83.9%; mp: 257–259 °C; HPLC purity: 98%; HRMS, ESI<sup>+</sup>,*m/z* $: Calcd for C<sub>26</sub>H<sub>18</sub>BrClF<sub>3</sub>N<sub>4</sub>O<sub>5</sub> (M + H)<sup>+</sup>, 637.0001; found, 636.9997; <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>) <math>\delta$  11.76 (1H, br s), 9.18 (1H, s), 8.96 (1H, s), 8.20 (1H, d, *J* = 2.5 Hz), 7.90 (1H, dd, *J* = 8.8, 2.5 Hz), 7.86 (1H, s), 7.64 (1H, t, *J* = 7.8 Hz), 7.51 (1H, d, *J* = 7.8 Hz), 7.38 (1H, d, *J* = 8.8 Hz), 7.32–7.20 (2H, m), 4.59 (2H, t, *J* = 4.6 Hz), 4.31 (2H, s), 4.23 (2H, t, *J* = 4.6 Hz); <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>)  $\delta$  167.04, 159.01, 157.07, 153.10, 149.88, 147.85 (d, *J* = 24.8 Hz), 136.69, 134.85, 132.50, 131.55, 126.58, 126.22, 124.79, 122.02, 120.76, 119.96 (d, *J* = 3.0 Hz), 119.50, 113.47 (d, *J* = 4.5 Hz), 110.47, 103.52, 100.04, 62.02, 41.86, 25.64.

4.1.5.2.5. 1-(2-(2-bromoacetyl)ethyl)-8-((4-(morpholine-4-carboxamido)phenyl)amino)oxazolo[4,5-g]quinazolin-2(1H)-one (**11e**). Purified by silica-gel column chromatography (DCM/MeOH, 50/1), R<sub>f</sub> = 0.22. Light yellow solid, 197 mg, yield, 86.0%; mp: 263–265 °C; HPLC purity: 98%; HRMS, ESI<sup>+</sup>,*m/z* $: Calcd for C<sub>24</sub>H<sub>24</sub>BrN<sub>6</sub>O<sub>6</sub> (M + H)<sup>+</sup>, 571.0940; found, 571.0933; <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>) <math>\delta$  11.52 (1H, br s), 9.02 (1H, s), 8.88 (1H, s), 8.78 (1H, s), 7.86 (1H, s), 7.65–7.58 (4H, m), 4.62 (2H, t, *J* = 4.6 Hz), 4.34 (2H, s), 4.25 (2H, t, *J* = 4.6 Hz), 3.66–3.63 (4H, m), 3.49–3.46 (4H, m); <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>)  $\delta$  167.06, 158.69, 155.06, 153.10, 149.59, 147.44, 138.91, 135.90, 132.30, 130.42, 124.61, 119.41, 110.18, 103.41, 99.68, 65.98, 62.00, 44.19, 41.80, 25.63.

4.1.5.2.6. 1-(2-(2-bromoacetyl)ethyl)-8-((4-(3-cyclopentylureido) phenyl)amino)oxazolo[4,5-g]quinazolin-2(1H)-one (11f). Purified by silica-gel column chromatography (DCM/MeOH, 50/1),  $R_f = 0.21$ . Light yellow solid, 189 mg, yield, 83.2%; mp: 266–268 °C; HPLC purity: 96%; HRMS, ESI<sup>+</sup>, *m/z*: Calcd for C<sub>25</sub>H<sub>26</sub>BrN<sub>6</sub>O<sub>5</sub> (M + H)<sup>+</sup>, 569.1132; found, 569.1128; <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>) δ 11.60 (1H, br s), 9.02 (1H, s), 8.86 (1H, s), 8.76 (1H, s), 7.86 (1H, s), 7.65–7.58 (4H, m), 6.24 (1H, bs), 4.60 (2H, t, J = 4.6 Hz), 4.33 (2H, s), 4.23 (2H, t, J = 4.6 Hz), 1.78–1.69 (2H, m), 1.67–1.56 (2H, m), 1.54–1.42 (1H, m), 1.30–1.03 (4H, m); <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>) δ 167.07, 159.01, 156.89, 154.30, 153.12, 149.90, 149.41, 147.69, 142.44, 136.53, 133.63, 132.51, 129.97, 126.20, 124.63, 124.28, 121.09, 118.66, 112.40, 110.41, 109.70, 106.07, 103.38, 100.00, 89.40, 62.03, 47.43, 41.85, 32.86, 25.61, 22.43.

4.1.5.2.7. 1-(2-(2-bromoacetyl)ethyl)-8-((4-(piperidine-1-carboxamido)phenyl)amino)oxazolo[4,5-g]quinazolin-2(1H)-one (**11g**). Purified by silica-gel column chromatography (DCM/MeOH, 50/1), R<sub>f</sub> = 0.20. Light yellow solid, 185 mg, yield, 81.1%; mp: 259–261 °C; HPLC purity: 97%; HRMS, ESI<sup>+</sup>,*m/z* $: Calcd for C<sub>25</sub>H<sub>26</sub>BrN<sub>6</sub>O<sub>5</sub> (M + H)<sup>+</sup>, 569.1148; found, 569.1143; <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>) <math>\delta$  11.39 (1H, br s), 8.89 (1H, s), 8.86 (1H, s), 8.63 (1H, s), 7.84 (1H, s), 7.62–7.54 (4H, m), 4.61 (2H, t, *J* = 4.6 Hz), 4.32 (2H, s), 4.24 (2H, t, *J* = 4.6 Hz), 3.47–3.43 (4H, m), 1.59–1.51 (6H, m); <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>)  $\delta$  167.05, 158.74, 154.78, 153.13, 149.66, 147.48, 139.39, 135.96, 132.35, 130.13, 124.63, 119.40, 110.20, 103.31, 99.76, 62.02, 44.68, 41.81, 25.62, 25.51, 24.08.

4.1.5.2.8. 1-(2-(2-bromoacetyl)ethyl)-8-((4-(3-cyclohexylureido) phenyl)amino)oxazolo[4,5-g]quinazolin-2(1H)-one (11h). Purified by silica-gel column chromatography (DCM/MeOH, 50/1),  $R_f = 0.21$ . Light yellow solid, 192 mg, yield, 82.4%; mp: 267–268 °C; HPLC purity: 97%; HRMS, ESI+, *m/z*: Calcd for C<sub>26</sub>H<sub>28</sub>BrN<sub>6</sub>O<sub>5</sub> (M + H)<sup>+</sup>, 583.1204; found, 583.1200; <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>)  $\delta$  11.45 (1H, br s), 8.92 (1H, s), 8.86 (1H, s), 8.80 (1H, s), 7.84 (1H, s), 7.59–7.48 (4H, m), 6.36 (1H, br s), 4.60 (2H, t, *J* = 4.6 Hz), 4.33 (2H, s), 4.22 (2H, t, *J* = 4.6 Hz), 1.88–1.46 (5H, m), 1.40–1.08 (6H, m); <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>)  $\delta$  167.02, 158.64, 154.43, 153.10, 149.60, 147.46, 139.32, 132.33, 129.40, 125.05, 117.20, 110.15, 103.25, 99.68, 61.99, 47.48, 41.79, 25.63, 25.25, 24.24.

4.1.5.2.9. 1-(2-(2-bromoacetyl)ethyl)-8-((3,4-difluorophenyl) amino)oxazolo[4,5-g]quinazolin-2(1H)-one (**11**i). Purified by silicagel column chromatography (DCM/MeOH, 100/1),  $R_f = 0.24$ . White solid, 159 mg, yield, 82.8%; mp: 228–230 °C; HPLC purity: 98%; HRMS, ESI+, *m/z*: Calcd for C<sub>19</sub>H<sub>14</sub>BrF<sub>2</sub>N<sub>4</sub>O<sub>4</sub> (M + H)<sup>+</sup>, 479.0166; found, 479.0164; <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>)  $\delta$  11.71 (1H, br s), 9.14 (1H,s), 8.95 (1H,s), 8.03 (1H, ddd, *J* = 12.5, 7.5, 2.5 Hz), 7.87 (1H, s), 7.70 (1H, d, *J* = 9.0 Hz), 7.58 (1H, dd, *J* = 19.4, 9.0 Hz), 4.61 (2H, t, *J* = 4.8 Hz), 4.32 (2H, s), 4.25 (2H, t, *J* = 4.8 Hz); <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>)  $\delta$  167.05, 159.11, 153.11, 149.93, 147.72, 136.74, 132.53, 121.19 (q, *J* = 3.0 Hz), 117.42 (d, *J* = 18.0 Hz), 114.90 (d, *J* = 21.0 Hz), 110.37, 103.25, 100.16, 62.02, 41.84, 25.62.

4.1.5.2.10. 1-(2-(2-bromoacetyl)ethyl)-8-((4-(benzyloxy)phenyl) amino)oxazolo[4,5-g]quinazolin-2(1H)-one (**11***j*). Purified by silicagel column chromatography (DCM/MeOH, 70/1),  $R_f = 0.25$ . Light yellow solid, 182 mg, yield, 82.7%; mp: 250–251 °C; HPLC purity: 97%; HRMS, ESI+, *m*/*z*: Calcd for  $C_{26}H_{22}BrN_4O_5$  (M + H)<sup>+</sup>, 549.0773; found, 549.0771; <sup>1</sup>H NMR (300 MHz, DMSO-d\_6)  $\delta$  11. 48 (1H, br s), 8.97 (1H, s), 8.83 (1H, s), 7.65 (1H, s), 7.62 (1H, s), 7.48–7.33 (5H, m), 7.14 (1H, s), 7.11 (1H, s), 5.15 (2H, s), 4.59 (2H, t, *J* = 4.9 Hz), 4.34 (2H, s), 4.22 (2H, t, *J* = 4.9 Hz); <sup>13</sup>C NMR (75 MHz, DMSO-d\_6)  $\delta$  167.02, 158.79, 156.62, 153.12, 149.82, 147.41, 136.92, 132.24, 129.72, 128.43, 127.85, 127.67, 125.96, 114.84, 110.22, 103.29, 100.02, 69.46, 62.03, 41.80, 25.61.

4.1.5.2.11. 1-(2-(2-bromoacetyl)ethyl)-8-((4-((4-chlorobenzyl) oxy)phenyl)amino)oxazolo[4,5-g]quinazolin-2(1H)-one (11k). Purified by silica-gel column chromatography (DCM/MeOH, 70/1), Rf = 0.25. Light yellow solid, 187 mg, yield, 79.9%; mp: 251–252 °C; HPLC purity: 98%; HRMS, ESI+,*m/z* $: Calcd for C<sub>26</sub>H<sub>21</sub>BrClN<sub>4</sub>O<sub>5</sub> (M + H)<sup>+</sup>, 583.0333; found, 583.0331; <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>) <math>\delta$  11.58 (1H, br s), 9.10 (1H, s), 8.97 (1H, s), 8.14–8.05 (4H, m), 7.90 (1H, s), 7.57–7.48 (4H, m), 4.63 (2H, t, *J* = 4.9 Hz); <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>)  $\delta$  167.02,

164.92, 158.98, 153.08, 149.81, 147.69, 141.73, 137.14, 135.17, 132.70, 132.48, 129.84, 129.76, 128.49, 126.33, 123.74, 110.70, 103.49, 100.19, 65.35, 62.04, 41.84, 25.62.

4.1.5.2.12. Ethyl-4-((1-(2-(2-bromoacetoxy)ethyl)-2-oxo-1,2dihydrooxazolo[4, 5-g]quinazolin-8-yl)amino)benzoate (111). Purified by silica-gel column chromatography (DCM/MeOH, 100/1),  $R_f = 0.20$ . Light yellow solid, 175 mg, yield, 85.0%; mp: 227–229 °C; HPLC purity: 96%; HRMS, ESI+, *m*/*z*: Calcd for C<sub>22</sub>H<sub>20</sub>BrN<sub>4</sub>O<sub>6</sub> (M + H)<sup>+</sup>, 515.0566; found, 515.0567; <sup>1</sup>H NMR (300 MHz, DMSOd<sub>6</sub>)  $\delta$  11.63 (1H, br s), 9.15 (1H, s), 8.95 (1H, s), 8.03 (4H, s), 7.88 (1H, s), 4.61 (2H, t, *J* = 4.8 Hz), 4.36–4.30 (4H, m), 4.25 (2H, t, *J* = 4.8 Hz), 1.34 (3H, t, *J* = 7.1 Hz); <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>)  $\delta$  167.05, 165.14, 158.97, 153.10, 149.92, 147.72, 141.44, 137.20, 132.51, 129.70, 126.85, 123.71, 110.67, 103.29, 100.28, 62.05, 60.74, 41.82, 25.63, 14.17.

4.1.5.2.13. 1-(2-(2-bromoacetyl)ethyl)-8-((4-((4-methoxybenzyl) oxy)phenyl)amino)oxazolo[4,5-g]quinazolin-2(1H)-one (11m). Purified by silica-gel column chromatography (DCM/MeOH, 70/1),  $R_f = 0.23$ . Light yellow solid, 190 mg, yield, 81.9%; mp: 256–258 °C; HPLC purity: 97%; HRMS, ESI+, *m/z*: Calcd for C<sub>27</sub>H<sub>24</sub>BrN<sub>4</sub>O<sub>6</sub> (M + H)<sup>+</sup>, 579.0879; found, 579.0873; <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>)  $\delta$  11.55 (1H, br s), 9.02 (1H, s), 8.86 (1H, s), 7.87 (1H, s), 7.66 (2H, d, *J* = 9.0 Hz), 7.43 (2H, d, *J* = 9.0 Hz), 7.13 (2H, d, *J* = 9.0 Hz), 6.97 (2H, d, *J* = 9.0 Hz), 5.10 (2H, s), 4.62 (2H, t, *J* = 5.0 Hz), 4.34 (2H, s), 4.25 (2H, t, *J* = 5.0 Hz), 3.79 (3H, s); <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>)  $\delta$  167.08, 167.25, 159.01, 158.89, 156.79, 153.12, 149.73, 147.50, 135.99, 132.37, 129.52, 128.73, 126.06, 114.89, 113.82, 110.14, 103.22, 99.80, 69.21, 62.03, 61.30, 55.08, 41.81, 25.63.

4.1.5.2.14. 1-(2-(2-bromoacetyl)ethyl)-8-((3-ethynylphenyl)amino)oxazolo[4,5-g]quinazolin-2(1H)-one (**11n**). Purified by silicagel column chromatography (DCM/MeOH, 200/1), R<sub>f</sub> = 0.25. Light yellow solid, 158 mg, yield, 84.5%; mp: 230–231 °C; HPLC purity: 98%; HRMS, ESI<sup>+</sup>, m/z: Calcd for C<sub>21</sub>H<sub>16</sub>BrFN<sub>4</sub>O<sub>4</sub> (M + H)<sup>+</sup>, 467.0355; found, 467.0357; <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>)  $\delta$  11.71 (1H, br s), 9.17 (1H, s), 8.95 (1H, s), 7.96 (1H, s), 7.88 (1H, s), 7.85 (1H, s), 7.52 (1H, t, *J* = 7.8 Hz), 7.43 (1H, d, *J* = 7.8 Hz), 4.61 (2H, t, *J* = 4.6 Hz), 4.33 (2H, s), 4.30 (1H, s), 4.26 (2H, t, *J* = 4.6 Hz); <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>)  $\delta$  167.04, 159.18, 153.10, 149.82, 147.69, 137.15, 136.42, 132.51, 129.47, 129.20, 127.41, 125.10, 122.06, 110.37, 103.40, 99.91, 82.84, 81.39, 62.05, 41.84, 25.62.

4.1.5.2.15. 1-(2-(2-bromoacetyl)ethyl)-8-((3-chloro-4-(4-methylpiperazin-1-yl)phenyl)amino)oxazolo[4,5-g]quinazolin-2(1H)-one (**110**). Purified by silica-gel column chromatography (DCM/MeOH, 30/1), R<sub>f</sub> = 0.23. Yellow solid, 184 mg, yield, 80.0%; mp: 272–275 °C; HPLC purity: 97%; HRMS, ESI+,*m/z* $: Calcd for C<sub>24</sub>H<sub>25</sub>BrClN<sub>6</sub>O<sub>4</sub> (M + H)<sup>+</sup>, 575.0709; found, 575.0710; <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>) <math>\delta$  9.88 (1H, br s), 8.57 (1H, s), 8.53 (1H, s), 8.09 (1H, d, *J* = 2.4 Hz), 7.86 (1H,dd, *J* = 8.8, 2.4 Hz), 7.69(1H, s), 7.28 (1H, d, *J* = 8.8 Hz), 4.60 (2H, t, *J* = 4.6 Hz), 4.34 (2H, s), 4.25 (2H, t, *J* = 4.6 Hz), 3.22–3.05 (4H, m), 2.96 (3H, s), 2.56–2.52 (4H, m); <sup>13</sup>C NMR (126 MHz, DMSO-d<sub>6</sub>)  $\delta$  167.04, 159.04, 153.13, 149.72, 147.67, 132.51, 127.04, 126.29, 124.19, 120.58, 110.36, 103.61, 99.76, 62.08, 57.04, 52.31, 46.64, 41.92, 25.62.

4.1.5.2.16. 1 - (2 - (2 - bromoacetyl)ethyl) - 8 - ((3 - chloro - 4 - morpholinophenyl)amino)oxazolo[4,5-g]quinazolin-2(1H)-one (**11p**). Purified by silica-gel column chromatography (DCM/MeOH, 40/1), R<sub>f</sub> = 0.22. Yellow solid, 190 mg, yield, 84.4%; mp: 275–277 °C; HPLC purity: 98%; HRMS, ESI+,*m/z* $: Calcd for C<sub>23</sub>H<sub>22</sub>BrClN<sub>5</sub>O<sub>5</sub> (M + H)<sup>+</sup>, 562.0493; found, 562.0492; <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>) <math>\delta$  11.63 (1H, br s), 9.10 (1H, s), 8.94 (1H, s), 7.95 (1H, d, *J* = 2.4 Hz), 7.87 (1H, s), 7.76 (1H, dd, *J* = 8.8, 2.4 Hz), 7.30 (1H, d, *J* = 8.8 Hz), 4.60 (2H, t, *J* = 4.6 Hz), 4.34 (2H, s), 4.25 (2H, t, *J* = 4.6 Hz), 3.86–3.70 (4H, m), 3.10–2.98 (4H, m); <sup>13</sup>C NMR (126 MHz, DMSO-d<sub>6</sub>)  $\delta$  167.03, 159.02, 153.15, 149.74, 147.69, 132.53, 127.06, 126.31, 124.21, 120.60, 110.38, 103.63, 99.77, 66.35, 62.10, 54.93, 41.92, 25.61.

4.1.5.2.17. 1-(2-(2-bromoacetyl)ethyl)-8-((3-chloro-4-(piperidin-1-yl)phenyl)amino)oxazolo[4,5-g]quinazolin-2(1H)-one (**11q**). Purified by silica-gel column chromatography (DCM/MeOH, 35/1),  $R_f = 0.22$ . Yellow solid, 181 mg, yield, 80.8%; mp: 271–273 °C; HPLC purity: 97%; HRMS, ESI+, *m/z*: Calcd for C<sub>24</sub>H<sub>24</sub>BrClN<sub>5</sub>O<sub>4</sub> (M + H)<sup>+</sup>, 560.0600; found, 560.0599; <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>)  $\delta$  9.61 (1H, br s), 8.55 (1H, s), 8.23 (1H, s), 7.96 (1H, d, *J* = 2.4 Hz), 7.75 (1H,dd, *J* = 8.8, 2.4 Hz), 7.64 (1H, s), 7.20 (1H, d, *J* = 8.8 Hz), 4.60 (2H, t, *J* = 4.6 Hz), 4.33 (2H, s), 4.24 (2H, t, *J* = 4.6 Hz), 3.01–2.85 (4H, m), 1.79–1.64 (4H, m), 1.63–1.48 (2H, m); <sup>13</sup>C NMR (126 MHz, DMSO-d<sub>6</sub>)  $\delta$  167.05, 159.02, 153.15, 149.74, 147.69, 132.53, 127.06, 126.31, 124.21, 120.60, 110.38, 103.63, 99.77, 66.35, 62.10, 49.52, 41.92, 26.13, 25.62, 24.74.

### 4.1.6. General procedure for the synthesis of 1-(2-(2-chloroacetoxy) ethyl) oxazolo[4.5-g]quinazolin-2(1H)-one derivatives (**12a**–**g**)

4.1.6.1 1-(2-(2-chloroacetoxy)ethyl) oxazolo[4,5-g]quinazoline-2, 8(1H,7H)-dione (**9b**). Compound **8** (2.0 g, 8.1 mmol) was dissolved in 50 mL DMF, and bromoacetyl bromide (2.9 g, 24.3 mmol) was added dropwise at 0 °C. The reaction mixture was spured at room temperature for 30 min, and then the mixture was poured into ice water (200 mL) and stirred for 15 min, followed by filtering and distilling under a reduced pressure. The white product was purified by silica-gel column chromatography (DCM/MeOH, 100/1), R<sub>f</sub> = 0.17. Drying give compound **9b** (2.3 g, yield, 87.8%); mp: 233–234 °C; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>)  $\delta$  12.27 (1H, br s), 8.05 (1H, s), 7.98 (1H, s), 7.57 (1H, s), 4.60 (2H, t, *J* = 4.6 Hz), 4.32 (2H, s), 4.24 (2H, t, *J* = 4.6 Hz).

4.1.6.2. 1-(2-(2-chloroacetoxy)ethyl)-8-Chlorooxazolo[4,5-g]quinazolin-2(1H)-one (**10b**). Compound **9a** (2.0 g, 6.1 mmol), thionyl chloride (25 mL) and DMF (4 drops) was successively added and the mixture was refluxed for 24 h. After cooled to room temperature, most of the excess of thionyl chloride and DMF was removed under reduced pressure to give the yellow residue. The crude product was purified by silica-gel column chromatography (DCM), R<sub>f</sub> = 0.23. Drying gave compound **10b** (1.7 g, yield, 81.5%); mp: 182–184 °C; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>)  $\delta$  8.43 (1H, s), 8.02 (1H, s), 7.65 (1H, s), 4.60 (2H, t, *J* = 4.6 Hz), 4.32 (2H, s), 4.24 (2H, t, *J* = 4.6 Hz).

4.1.6.2.1. 1-(2-(2-chloroacetoxy)ethyl)-8-(3-Chloro-4fluorophenylamino)oxazolo[4,5-g]quinazolin-2(1H)-one (12a). A mixture of compound 10b (137 mg, 0.4 mmol) and 3-chloro-4fluoroaniline (70 mg, 0.48 mmol) in isopropanol (6 mL) was stirred for 12 h. After cooled to room temperature, the mixture was filtered and washed with chill isopropanol (3 mL) and the residue was treated with aqueous NaHCO<sub>3</sub> (10 mL) and extracted with EtOAc/MeOH (20:1, 20 mL). The organic layer was washed with brine, dried over Na2SO4, and concentrated under reduced pressure. Purified by silica-gel column chromatography (DCM/MeOH, 100/1), R<sub>f</sub> = 0.23. Drying gave 154 mg (yield, 85.6%) of the title compound as white solid; mp: 228-230 °C; HPLC purity: 98%; HRMS, ESI+, m/z: Calcd for C<sub>19</sub>H<sub>14</sub>Cl<sub>2</sub>FN<sub>4</sub>O<sub>4</sub> (M + H)<sup>+</sup>, 451.0371; found, 451.0381; <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ 11.90 (1H, br s), 9.26 (1H, s), 8.96 (1H, s), 8.13 (1H, dd, J = 6.8, 2.6 Hz), 7.90-7.84 (2H, m), 7.56 (1H, t, J = 9.1 Hz), 4.60 (2H, t, J = 4.6 Hz), 4.32 (2H, s), 4.24 (2H, t, J = 4.6 Hz); <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>)  $\delta$  167.24, 159.18, 156.73, 153.10, 149.85, 147.71, 136.56, 134.14 (d, J = 2.6 Hz), 132.51, 126.32, 125.05 (d, J = 7.5 Hz), 116.86 (d, J = 21.8 Hz), 114.01, 110.39, 103.51, 99.97, 62.02, 41.87, 40.99.

4.1.6.2.2. 1-(2-(2-chloroacetoxy)ethyl)-8-((3,4-difluorophenyl) amino)oxazolo[4,5-g]quinazolin-2(1H)-one (**12b**). Purified by silicagel column chromatography (DCM/MeOH, 100/1),  $R_f = 0.24$ . White solid, 144 mg, yield, 82.8%; mp: 227–228 °C; HPLC purity: 96%; HRMS, ESI+, *m/z*: Calcd for C<sub>19</sub>H<sub>14</sub>ClF<sub>2</sub>N<sub>4</sub>O<sub>4</sub> (M + H)<sup>+</sup>, 435.0666; found, 435.0674; <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>)  $\delta$  11.83 (1H, br s),

9.24 (1H, s), 8.96 (1H, s), 8.08–8.01 (1H, m), 7.88 (1H, s), 7.73–7.70 (1H, m), 7.63–7.53 (1H, m), 4.61 (2H, t, J = 4.6 Hz), 4.33 (2H, s), 4.25 (2H, t, J = 4.6 Hz); <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>)  $\delta$  167.21, 159.07, 153.08, 149.85, 147.67, 136.73, 132.47, 121.11 (q, J = 3.0 Hz), 117.33 (d, J = 18.0 Hz), 113.80 (d, J = 20.0 Hz), 110.38, 103.39, 100.10, 62.00, 41.83, 40.95.

4.1.6.2.3. 1-(2-(2-chloroacetoxy)ethyl)-8-((3-chloro-4-(3-(tri-fluoromethyl)phenoxy)phenyl)amino)oxazolo[4,5-g]quinazolin-2(1H)-one (**12c**). Purified by silica-gel column chromatography (DCM/MeOH, 100/1),  $R_f = 0.24$ . Light yellow solid, 208 mg, yield, 87.8%; mp: 255–256 °C; HPLC purity: 97%; HRMS, ESI<sup>+</sup>, *m/z*: Calcd for C<sub>26</sub>H<sub>18</sub>Cl<sub>2</sub>F<sub>3</sub>N<sub>4</sub>O<sub>5</sub> (M + H)<sup>+</sup>, 593.0601; found, 593.0603; <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>)  $\delta$  11.82 (1H, br s), 9.23 (1H, s), 8.98 (1H, s), 8.22 (1H, d, J = 1.9 Hz), 7.94–7.89 (2H, m), 7.66 (1H, t, J = 7.9 Hz), 7.53 (1H, d, J = 7.4 Hz), 7.40 (1H, d, J = 8.9 Hz), 7.34–7.23 (2H, m), 4.60 (2H, t, J = 4.6 Hz), 4.32 (2H, s), 4.23 (2H, t, J = 4.6 Hz); <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>)  $\delta$  167.29, 159.01, 157.11, 153.17, 150.08, 148.08, 147.86 (d, J = 16.5 Hz), 134.90, 132.55, 131.62, 126.28, 124.83, 122.12, 120.85, 120.03 (d, J = 4.1 Hz), 113.47 (d, J = 3.8 Hz), 110.53, 103.18, 100.43, 62.07, 41.87, 40.99.

4.1.6.2.4. 1-(2-(2-chloroacetoxy)ethyl)-8-((3-chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)oxazolo[4,5-g]quinazolin-2(1H)-one (**12d**). Purified by silica-gel column chromatography (DCM/MeOH, 100/1), R<sub>f</sub> = 0.25. Light yellow solid, 191 mg, yield, 85.7%; mp: 251–252 °C; HPLC purity: 98%; HRMS, ESI+,*m/z* $: Calcd for C<sub>26</sub>H<sub>20</sub>Cl<sub>2</sub>FN<sub>4</sub>O<sub>5</sub> (M + H)<sup>+</sup>, 557.0789; found, 557.0797; <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>) <math>\delta$  11.88 (1H, br s), 9.23 (1H, s), 8.94 (1H, s), 7.97 (1H, d, *J* = 2.4 Hz), 7.89 (1H, s), 7.75–7.71 (1H, m), 7.52–7.43 (1H, m), 7.37–7.30 (2H, m), 7.22–7.15 (1H, m), 4.60 (2H, t, *J* = 4.6 Hz), 4.33 (2H, s), 4.24 (2H, t, *J* = 4.6 Hz); <sup>13</sup>C NMR (75 MHz, DMSO-d<sub>6</sub>)  $\delta$  167.26, 163.81, 160.60, 158.92, 153.12, 151.54, 149.82, 147.57, 136.28, 132.40, 130.61 (d, *J* = 7.5 Hz), 126.11, 124.37, 121.10, 114.75 (d, *J* = 17.3 Hz), 114.14 (d, *J* = 8.3 Hz), 113.93, 110.29, 103.46, 99.92, 69.40, 62.04, 41.86, 40.98.

4.1.6.2.5. 1-(2-(2-chloroacetoxy)ethyl)-8-((3-chloro-4-(4-methylpiperazin-1-yl)phenyl)amino)oxazolo[4,5-g]quinazolin-2(1H)one (**12e**). Purified by silica-gel column chromatography (DCM/MeOH, 30/1), R<sub>f</sub> = 0.24. Yellow solid, 176 mg, yield, 82.6%; mp:265–266 °C; HPLC purity: 98%; HRMS, ESI<sup>+</sup>,*m/z*: Calcd forC<sub>24</sub>H<sub>25</sub>Cl<sub>2</sub>N<sub>6</sub>O<sub>4</sub> (M + H)<sup>+</sup>, 531.1309; found, 531.1314; <sup>1</sup>H NMR $(300 MHz, DMSO-d<sub>6</sub>) <math>\delta$  9.87 (1H, br s), 8.59 (1H, s), 8.54 (1H, s), 8.09 (1H, d, *J* = 2.4 Hz), 7.86 (1H,dd, *J* = 8.8, 2.4 Hz), 7.69(1H, s), 7.28 (1H, d, *J* = 8.8 Hz), 4.62 (2H, t, *J* = 4.6 Hz), 4.33 (2H, s), 4.20 (2H, t, *J* = 4.6 Hz), 3.21–3.06 (4H, m), 2.95 (3H, s), 2.57–2.54 (4H, m); <sup>13</sup>C NMR (126 MHz, DMSO-d<sub>6</sub>)  $\delta$  167.24, 159.00, 153.13, 149.72, 147.67, 132.51, 127.04, 126.29, 124.19, 120.58, 110.36, 103.61, 99.76, 62.08, 57.04, 52.31, 46.63, 41.92, 41.02.

4.1.6.2.6. 1-(2-(2-chloroacetoxy)ethyl)-8-((3-chloro-4-morpholinophenyl)amino)oxazolo[4,5-g]quinazolin-2(1H)-one (**12f**). Purified by silica-gel column chromatography (DCM/MeOH, 40/1), R<sub>f</sub> = 0.22. Yellow solid, 167 mg, yield, 80.7%; mp: 269–271 °C; HPLC purity: 98%; HRMS, ESI+,*m/z* $: Calcd for C<sub>23</sub>H<sub>22</sub>Cl<sub>2</sub>N<sub>5</sub>O<sub>5</sub> (M + H)<sup>+</sup>, 518.0993; found, 518.0990; <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>) <math>\delta$  11.86 (1H, br s), 9.25 (1H, s), 8.94 (1H, s), 7.94 (1H, d, *J* = 2.2 Hz), 7.88 (1H, s), 7.75 (1H,dd, *J* = 8.6, 2.2 Hz), 7.27 (1H, d, *J* = 8.6 Hz), 4.60 (2H, t, *J* = 4.6 Hz), 4.33 (2H, s), 4.24 (2H, t, *J* = 4.6 Hz), 3.79–3.73 (4H, m), 3.05–2.2.99 (4H, m); <sup>13</sup>C NMR (126 MHz, DMSO-d<sub>6</sub>)  $\delta$  167.28, 159.02, 153.15, 149.74, 147.69, 132.53, 127.06, 126.31, 124.21, 120.60, 110.38, 103.63, 99.77, 66.35, 62.10, 54.93, 41.92, 41.02.

4.1.6.2.7. 1-(2-(2-chloroacetoxy)ethyl)-8-((3-chloro-4-(piperidin-1-yl)phenyl)amino)oxazolo[4,5-g]quinazolin-2(1H)-one (**12g**). Purified by silica-gel column chromatography (DCM/MeOH, 35/1),  $R_f = 0.21$ . Yellow solid, 185 mg, yield, 89.4%; mp: 270–272 °C; HPLC purity: 98%; HRMS, ESI+, *m/z*: Calcd for C<sub>24</sub>H<sub>24</sub>Cl<sub>2</sub>N<sub>5</sub>O<sub>4</sub> (M + H)+,516.1200; found, 516.1206; <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>) δ 9.64 (1H, br s), 8.54 (1H, s), 8.26 (1H, s), 7.95 (1H, d, J = 2.4 Hz), 7.74 (1H,dd, J = 8.8, 2.4 Hz), 7.66 (1H, s), 7.20 (1H, d, J = 8.8 Hz), 4.60 (2H, t, J = 4.6 Hz), 4.33 (2H, s), 4.24 (2H, t, J = 4.6 Hz), 2.97–2.88 (4H, m), 1.75–1.62 (4H, m), 1.61–1.50 (2H, m); <sup>13</sup>C NMR (126 MHz, DMSO-d<sub>6</sub>) δ 167.28, 159.02, 153.15, 149.74, 147.69, 132.53, 127.06, 126.31, 124.21, 120.60, 110.38, 103.63, 99.77, 66.35, 62.10, 49.52, 41.92, 41.02, 26.13, 24.74.

#### 4.2. Biological evaluation

#### 4.2.1. In vitro enzymatic activity assay

EGFR<sup>WT</sup>, HER2 and the Z'-Lyte Kinase Kit were purchased from Invitrogen. The experiments were performed according to the instructions of the manufacturer. Briefly, the respective final concentrations of different kinases were determined as follows: EGFR (PV3872, Invitrogen), 0.200 µg/mL; HER2 (PV3366, Invitrogen), 0.192 µg/mL. Six concentration (0.0001–10 µM) gradients were set for all the tested compounds in DMSO, and a  $4 \times$  compound solution was prepared. An ATP solution and a kinase/peptide mixture were prepared right before use. The 10 µL kinase reaction consisted of 2.5 µL of compound solution, 5 µL of kinase/peptide mixture, and 2.5 µL of ATP solution. A 100% phosphorylation control was provided by 5 µL of phosphopeptide solution instead of the kinase/ peptide mixture. A 100% inhibition control was prepared using  $2.5 \ \mu L$  of kinase buffer instead of the ATP solution, and  $2.5 \ \mu L$  of 4%DMSO instead of the compound solution was used as a 0% inhibition control. The solutions on the plate were mixed thoroughly, and the plate was incubated for 1 h at room temperature. After 5 µL of development solution was added to each well, the plate was incubated for 1 h at room temperature; the non-phosphopeptides were cleaved in this time. Finally, 5 µL of stop reagent was added to stop the reaction. The activity was measured with an Infinite M100 Pro multilabel reader. Curve fitting and data presentations were performed using Graph Pad Prism 5.0. Every experiment was repeated at least three times. Data represented as means  $\pm$  SD from three independent experiments.

## 4.2.2. Cancer cell proliferation inhibition and normal cell cytotoxicity assay

Human lung adenocarcinoma cell line (A549), human breast cancer cell line (SK-Br3), human embryonic lung fibroblast cell line (HELF) were provided by Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. A549 cell was cultured in RPMI 1640 medium supplemented with 10% FBS, and Penicillin 100 U/mL and Streptomycin 100 U/mL were added. Cell cultures were maintained in a humidified atmosphere of 5% CO<sub>2</sub> at 37 °C. Cells were seeded at respective density  $(2-4 \times 10^4/mL)$  in 96-well plates in a volume of 180 µL per well. After seeding 24 h, the medium was removed. The test compounds were dissolved in DMSO and diluted with culture medium to different concentrations (the final concentration of DMSO was 0.1%). 20 µL of the test compound solution was added in duplicates, and incubation continued for 48 h in a humidified atmosphere of 5% CO<sub>2</sub> at 37 °C. Remove the medium, methylthiazolyldiphenyl-tetrazolium bromide (MTT) 20 µL was added to each well and incubated for additional 3-4 h. The medium was replaced by 150 mL DMSO to solubilize the purple formazan crystals produced and the absorbance was measured on a microplate reader at 570 nm. The compound IC<sub>50</sub> values were calculated using Graph Pad Prism 5.0. Data represented as means  $\pm$  SD from three independent experiments. The cell proliferation inhibition assay of SK-Br3 cell was the same as A549 cell. HELF cell in the normal cell cytotoxicity assay were cultured the same as A549 cell except cultured in DMEM medium.

#### 4.2.3. In vivo antitumor activity assay

Six week old C57BL/6 mice were weighed and randomly divided into three groups 1) Control; 2) Lapatinib; 3) Compound **12f** (n = 6 mice/group). LLC xenografts were initiated by subcutaneous implantation of 2 × 10<sup>6</sup> cells in the right flank. Upon reaching an average tumor volume of 100 mm<sup>3</sup> (7 days post implantation), each group was dosed orally for 14 days with either vehicle (MCT (medium-chain triglycerides): ethanol (9:1)) only or with Lapatinib (100 mg/kg prepared in MCT: ethanol = 9:1) or compound **12f** (100 mg/kg prepared in MCT: ethanol = 9:1) daily. The doses were in a volume of 0.1 mL/20 g of the animal body weight. Tumor volumes were measured every other day using vernier calipers, and volumes were calculated using the following formula: tumor volume (mm<sup>3</sup>) = W<sup>2</sup>(L/2), where W = width and L = length in mm. The volumes of each group were carried out with the use of the one-way analysis of ANOVA for values of P < 0.05.

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#### Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2016.04.072.

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