



Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: <http://www.elsevier.com/locate/ejmech>

Research paper

Design, synthesis and biological activities of novel oxazolo[4,5-g]quinazolin-2(1H)-one derivatives as EGFR inhibitors

Siyuan Yin, Liliang Zhou, Jinsheng Lin, Lingjing Xue^{**}, Can Zhang^{*}

State Key Laboratory of Natural Medicines and Jiangsu Key Laboratory of Drug Discovery for Metabolic Diseases, Center of Drug Discovery, China Pharmaceutical University, 24 Tong Jia Xiang, Nanjing 210009, China

ARTICLE INFO

Article history:

Received 16 March 2015

Received in revised form

3 July 2015

Accepted 6 July 2015

Available online 10 July 2015

Keywords:

EGFR inhibitor

Oxazolo[4,5-g]quinazolin-2(1H)-one scaffold

Protein tyrosine kinases (PTK)

Docking

SAR

ABSTRACT

A series of oxazolo[4,5-g]quinazolin-2(1H)-one derivatives employing Erlotinib as lead compound were synthesized and evaluated for their EGFR inhibition activity. These compounds having variation at the 1 and 8-position, included ether and esters hydrophilic side-chain and aromatic head fragment, respectively. All these compounds were evaluated by EGFR inhibition and two anti-proliferation assays *in vitro*. Four compounds were found more potent than Erlotinib in EGFR-TK assay. Furthermore, compounds **18**, **42** and **50** also had good to excellent anti-proliferation activity against human epidermoid cancer cell line (KB) and renal cell carcinoma cell line (A498). Finally, compound **50** presented remarkably higher inhibition efficacy towards tumor growth than Erlotinib in a mouse lewis lung cancer (LLC) xenograft model. Furthermore, compound **50** displayed the most distinguished effect on extending the survival period of the tumor-bearing mice.

© 2015 Elsevier Masson SAS. All rights reserved.

1. Introduction

Epidermal growth factor receptor (EGFR) is a tyrosine kinase receptor which plays an essential role in normal cell growth and differentiation, and is involved in tumor proliferation and survival [1,2]. EGFR over-expression is a common feature in many human solid malignancies including non-small-cell lung cancer, ovarian cancer, breast cancer, etc., which is associated with poor clinical prognosis [3–6].

Quinazoline-containing derivatives form an important class of synthetic products and represent an attractive scaffold for EGFR inhibitors. Huge interest has been attracted over the past years because of their varied biological activities, notably as kinase inhibitors [7–10]. The 4-anilinoquinazoline scaffold (Fig. 1) has led to the development and the marketing of a series of anti-tumor agents such as Gefitinib [11], Erlotinib [12], and Lapatinib [13].

Recently, we developed oxazolo[4,5-g]quinazolin-2(1H)-one derivatives, using Gefitinib as a lead compound. In that work, we found the potential of building oxazolo[4,5-g]quinazolin-2(1H)-

one scaffold and adopting the hydrophilic side-chain at its 1-position for EGFR small molecule inhibitors [14]. So based on this hypothesis that oxazolo[4,5-g]quinazolin-2(1H)-one scaffold is useful as a template for creation of new EGFR small molecule inhibitors, we synthesized a novel series of oxazolo[4,5-g]quinazolin-2(1H)-one derivatives adopting 2-methoxyethoxyl and 4-ethyloxy-4-oxobutyl group as side-chain at 1-position respectively and evaluated their EGFR inhibition activity comparing with Erlotinib (Fig. 2). Eventually, compounds **18**, **42** and **50** were discovered as promising inhibitors against EGFR. Therefore, we reported compounds **18**, **42** and **50** as promising candidates for clinical development as novel EGFR kinase inhibitors.

2. Results and discussion

2.1. Chemistry

The synthetic method for the target compounds is showed in Scheme 1 and Scheme 2. Nitration of the starting material ethyl 4-hydroxybenzoate (**4**) led to ethyl 4-hydroxy-3-nitrobenzoate (**5**), followed by reduction with tin (II) chloride dihydrate to give ethyl 3-amino-4-hydroxybenzoate (**6**), which was cyclocondensed with di-(1H-imidazol-1-yl)-methanone (CDI) in anhydrous THF to afford ethyl 2-oxo-2,3-dihydrobenzo-oxazole-5-carboxylate (**7**). Nitration

^{*} Corresponding author.^{**} Corresponding author.E-mail addresses: xuelingjing65@163.com (L. Xue), zhangcan@cpu.edu.cn (C. Zhang).

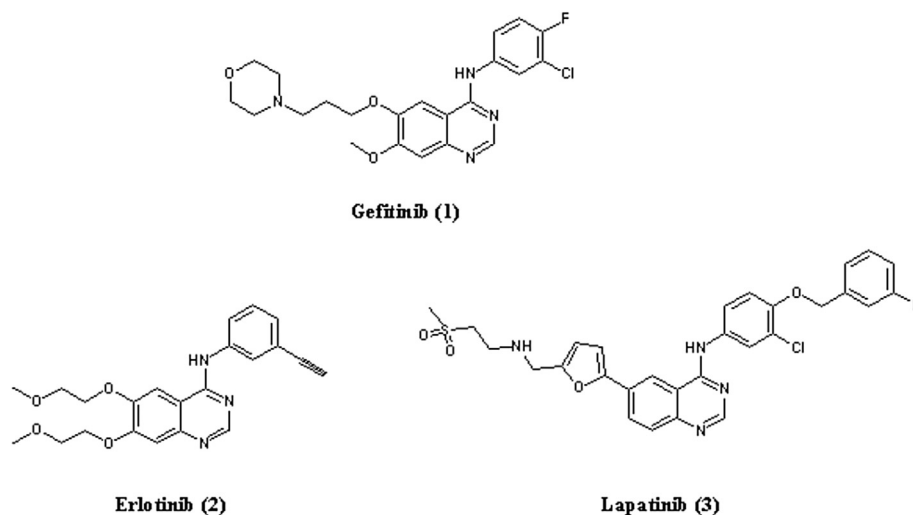


Fig. 1. The structures of Gefitinib, Erlotinib and Lapatinib.

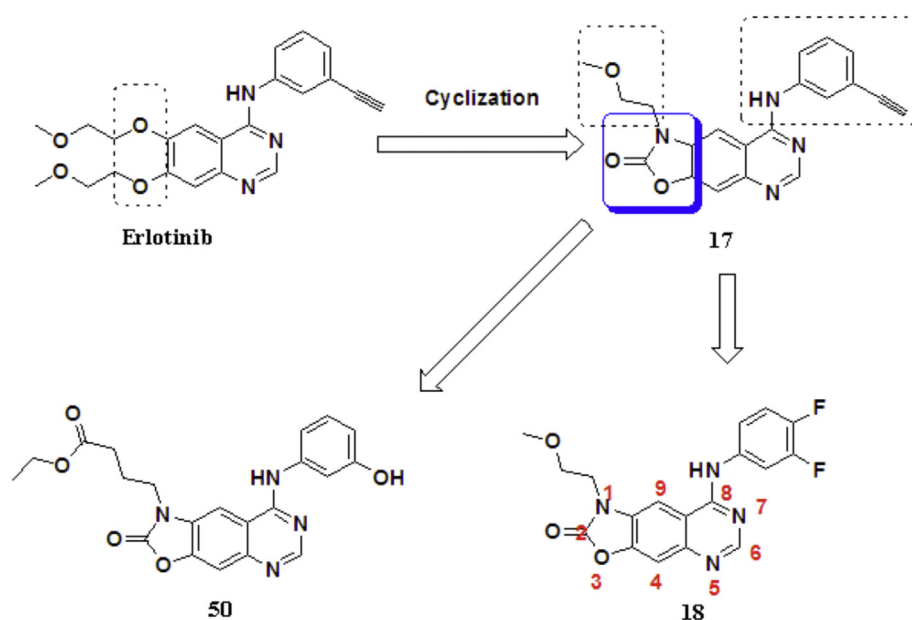


Fig. 2. The design inhibitors of EGFR.

of compound **7** with concentrated HNO_3 , and subsequent purification via column chromatography, yielded compound **8** which was then reduced with iron powder to produce compound **9**.

The series I compounds were obtained by alkylation of compound **9** with 1-chloro-2-methoxyethane, cyclization with formamidine acetate in EtOH, chlorination with thionyl chloride using DMF as catalyst, and nucleophilic displacement with aniline (Scheme 1). Likewise, the series II compounds were synthesized as described in Scheme 2.

2.2. Biological activities

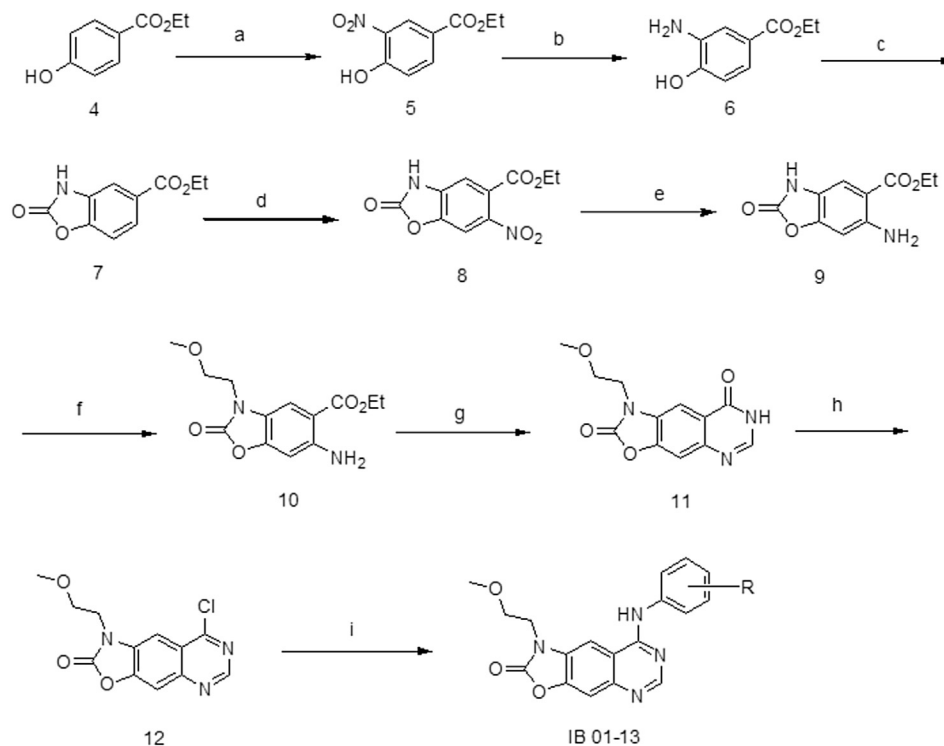
2.2.1. Kinase inhibitory activity

Kinase assay was carried out to test the inhibitory activity of the designed compounds at the concentration of 0.04 mg/mL. As shown in Table 1, most of compounds inhibited EGFR dramatically, whereas compounds **21**, **22**, **36**, and **44** had no obvious inhibitory activity. The IC_{50} values of EGFR inhibition were further tested using

Erlotinib as the positive control.

Most compounds exhibited moderate to high inhibition activities with EGFR kinase. These results revealed that introducing the moderate hydrophilic groups to the 1-position contributed to the activity. As indicated by the results of compounds **49**, **50**, **52** on compared to compounds **32**, **38**, **39**, aniline moieties directly conjugated to the 8-position increased EGFR inhibitory activities as compared to benzyl amino groups. Compounds containing some functional groups at 8-position, such as halogens, hydroxyl groups, alkoxy groups etc., caused an increase in EGFR inhibition with the exception of compounds **36**, **44**, **48** and **22**. This is due in part to their relatively large structures, which sterically hindered their interaction with the active site of the kinase. Additionally, the compounds which contained functional groups such as *t*-butyl, sulfanilamido group, etc., also demonstrated decreased inhibition activities like compounds **37**, **52** and **20**.

The IC_{50} values of compounds **18**, **42** and **50** were 0.026 μM , 0.0073 μM and 0.0087 μM respectively. This is a significant



Scheme 1. Reagents and reaction condition: (a) fuming HNO_3 , acetic acid, DCM, -15°C , 1 h; (b) $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$, conc. HCl , 45°C , 7 h; (c) CDI, anhydrous THF, 25°C , 2 h; (d) conc. HNO_3 , 60°C , 8 h; (e) reduced iron powder, acetic acid, MeOH, reflux, 3 h; (f) 1-chloro-2-methoxyethane, DMF, K_2CO_3 , KI, 70°C , 12 h; (g) formamidine acetate, ethanol, reflux, 5 h; (h) SOCl_2 , DMF(cat.), reflux, 16 h; (i) phenyl aniline, *i*-PrOH, reflux, 6–24 h.

improvement when compared to Erlotinib which has an IC_{50} value of $0.035 \mu\text{M}$ (Table 1).

2.2.2. Anti-proliferative effects on KB and A498 cells

In our study, KB cells and A498 cells overexpressing EGFR [17,18] were selected as cell models to evaluate the anti-proliferation of the synthesized compounds. The cellular anti-proliferative effects were mainly coincident with kinase inhibition activity, most compounds exhibited moderate to high inhibition activities and cytotoxicities to KB and A498 cells. In addition, compounds with benzoheterocycle introduced to the 8-position also showed moderate cytotoxicity for KB and A498 cells. For example, compound **45** was found to be effective at concentration of $38.4 \mu\text{M}$ and $26.8 \mu\text{M}$, respectively, and exhibited high inhibition of EGFR with IC_{50} of $0.13 \mu\text{M}$. Interestingly, the compounds **26** and **54** only showed moderate activity against EGFR yet exhibited promising anti-proliferative effects for KB and A498 cells, and this result indicated that compounds **26** and **54** may work through multiple molecular mechanisms (Table 2).

2.2.3. In vivo antitumor activity

For the *in vivo* antitumor activity, the xenograft mouse lewis lung cancer (LLC) tumor mice model was used. LLC cells also overexpress EGFR [19]. Compound **50** was further evaluated for its *in vivo* antitumor efficacy in the LLC xenograft mouse model. As shown in Fig. 3, compound **50** and Erlotinib were well tolerated and displayed significant *in vivo* antitumor efficacy when compared with water as vehicle control, and compound **50** presented remarkably higher inhibition efficacy towards tumor growth than Erlotinib ($P < 0.001$) (Fig. 3a). Furthermore, compound **50** displayed the most distinguished effect on extending the survival period of the tumor-bearing mice (Fig. 3b), and no significant change of the body weight during the study was seen.

2.3. Structure–activity relationships

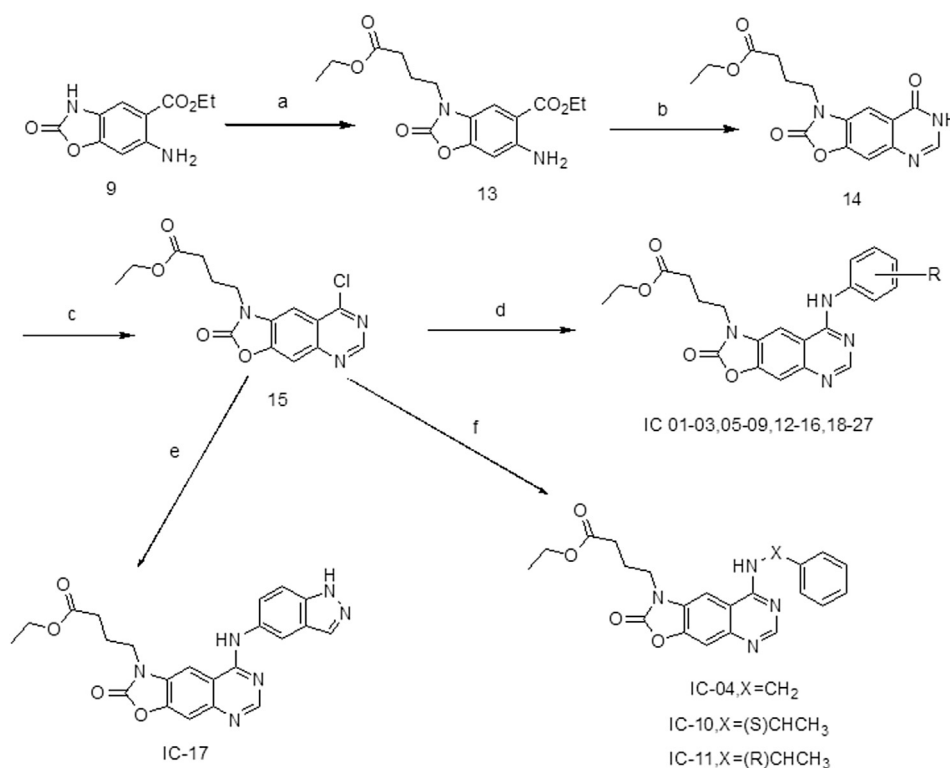
As shown in Fig. 4, N_1 , N_3 atoms in pyrimidine ring are crucial for compound binding. Introducing moderate hydrophilic side-chain, such as 2-methoxyethoxyl and 4-ethoxy-4-oxobutyl group can achieve medium to good inhibition activity. The proton in Fragment B is essential, and if phenyl amines are replaced by benzylaminos, the inhibition activities will be reduced, such as compound **38** and compound **39**. Halogen, hydroxy, alkyl and benzyloxy groups introduced in the phenyl amines improved the inhibition activity, such as compound **18**, **22**, **40**, **49**, **50**. However, tert-butyl and sulfonamide groups introduced in phenyl amines may reduce the compound inhibition activity, such as compounds **20**, **37**, **52** (Fig. 4 and Table 1).

2.4. Molecular docking study

Docking study was carried out for the target compounds into EGFR using Glide version 7.3, Tripos Inc and Molegro virtual docker version 2007. The crystal structure of the enzyme with Erlotinib (ID: 1M17) was obtained from protein data bank (PDB).

It is found that Gefitinib mimic ATP and the ATP binding site is sandwiched between the lobes where ATP forms critical hydrogen bonding interactions and the hinge region. The binding of ATP itself involves two important hydrogen bonding interactions with the amino acids Gln767 and Met769 in the kinase backbone, and the quinazoline ring of Erlotinib contains two nitrogen atoms that act as hydrogen bond acceptors like ATP. The kinase or catalytic domain includes an N-terminal lobe, which consists mainly of β -strands but contains one α -helix and C helix. The C-terminal lobe is mainly α -helical, and a short strand termed the hinge region connects the two lobes [15].

The docking studies were progressed by positioning the



Scheme 2. Reagents and reaction condition: (a) ethyl 4-bromobutanoate, acetonitrile, K_2CO_3 , KI, reflux, 4 h; (b) formamidine acetate, ethanol, reflux, 24 h; (c) $SOCl_2$, DMF(cat.), reflux, 7 h; (d) phenyl aniline, *i*-PrOH, reflux, 6–24 h; (e) 1H-indazol-5-amine, *i*-PrOH, reflux, 13 h; (f) phenylmethanamine, *i*-PrOH, TEA, 50 °C, 24 h.

compounds in the Erlotinib binding site in accordance with the published crystal structures of quinazoline core derivatives binding to EGFR [16], 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 Met769 via a hydrogen bond. A water molecule-mediated hydrogen bonding interaction between the N7 and the Thr830 side chain was observed which was similar to Erlotinib. 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 3'-hydroxyphenyl group at the C4 of aniline moiety lay in the same site of the 3'-ethynylbenzyl moiety of Erlotinib. It was glad to found a hydrogen bonding interaction existing between the 3'-OH oxygen atom and the backbone OH of Thr766 or NH of Leu764. A hydrogen bonding interaction between 3'-OH hydrogen atom and the backbone carbonyl oxygen of Ala719 could also be noticed in Fig. 5.

3. Conclusion

In summary, a series of novel oxazolo[4,5-*g*]quinazolin-2(1H)-one derivatives were synthesized and subjected to pharmacological evaluation. The results showed that most of the oxazolo[4,5-*g*]quinazolin-2(1H)-one derivatives possessed moderate to high EGFR inhibition activities. Protein tyrosine kinase inhibitions assay indicated that many of these derivatives were good inhibitors of EGFR with IC_{50} of 1.0–50 μ M. Furthermore, these derivatives demonstrated high anti-proliferation potencies against KB and A498 cells. Among them three compounds **18**, **42**, and **50** exhibited more potent inhibition activities than the reference Erlotinib which merited further evaluation. Especially, **50** substantially inhibited

tumor growth in a LLC xenograft mouse model and resulted in prolonged survival time. In conclusion, the findings presented herein showed that using the oxazolo[4,5-*g*]quinazolin-2(1H)-one scaffold decorated with 2-methoxyethoxyl or 4-ethoxy-4-oxobutyl group in position 1 is an excellent strategy for the development of EGFR inhibitors.

4. Experimental sections

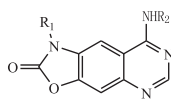
4.1. Chemistry experiment

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. Infrared (IR) spectra were recorded on Thermo FTIR spectrometer (KBr disks). 1H NMR and ^{13}C NMR spectra on a Bruker AV 300 or 500 MHz spectrometer were recorded in DMSO- d_6 or $CDCl_3$. Chemical shifts are reported in δ (ppm) units relative to the internal standard tetramethylsilane (TMS). The reaction conditions were not optimized for reaction yields. All oxygen-sensitive or moisture-sensitive 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).

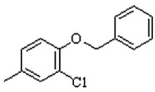
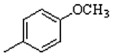
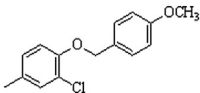
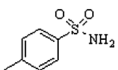


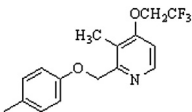
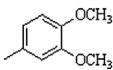
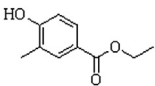

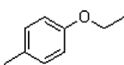
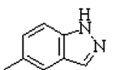
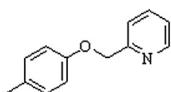

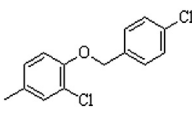
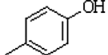
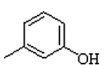
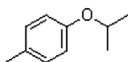
4.1.2. Purity analysis

The purity of the synthesized compounds were measured by

Table 1The inhibition and IC₅₀ values of the designed compounds toward EGFR kinase.

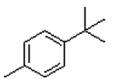
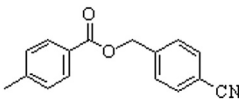
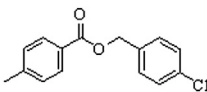
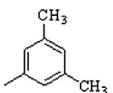
Comp.	R ₁	R ₂	Inhibition ^a /%	Enzymatic, IC ₅₀ ^b (μM)
16	–CH ₂ CH ₂ OCH ₃		88	0.47 ± 0.18
17	–CH ₂ CH ₂ OCH ₃		92	0.19 ± 0.03
18	–CH ₂ CH ₂ OCH ₃		97	0.026 ± 0.017*
19	–CH ₂ CH ₂ OCH ₃		91	5.1 ± 1.3
20	–CH ₂ CH ₂ OCH ₃		99	>10
21	–CH ₂ CH ₂ OCH ₃		78	>10
22	–CH ₂ CH ₂ OCH ₃		40	0.34 ± 0.08
23	–CH ₂ CH ₂ OCH ₃		81	>10
24	–CH ₂ CH ₂ OCH ₃		78	>10
25	–CH ₂ CH ₂ OCH ₃		92	0.56 ± 0.08
26	–CH ₂ CH ₂ OCH ₃		98	1.3 ± 0.1
27	–CH ₂ CH ₂ OCH ₃		94	5.5 ± 0.5
28	–CH ₂ CH ₂ OCH ₃		98	0.44 ± 0.09
29	–CH ₂ (CH ₂) ₂ COOC ₂ H ₅		93	7.8 ± 1.4
30	–CH ₂ (CH ₂) ₂ COOC ₂ H ₅		96	5.7 ± 1.3
31	–CH ₂ (CH ₂) ₂ COOC ₂ H ₅		92	>10
32	–CH ₂ (CH ₂) ₂ COOC ₂ H ₅		93	>10
33	–CH ₂ (CH ₂) ₂ COOC ₂ H ₅		96	1.2 ± 0.5

Table 1 (continued)

Comp.	R ₁	R ₂	Inhibition ^a /%	Enzymatic. IC ₅₀ ^b (μM)
34	–CH ₂ (CH ₂) ₂ COOC ₂ H ₅		92	>10
35	–CH ₂ (CH ₂) ₂ COOC ₂ H ₅		93	7.5 ± 2.1
36	–CH ₂ (CH ₂) ₂ COOC ₂ H ₅		97	>10
37	–CH ₂ (CH ₂) ₂ COOC ₂ H ₅		92	>10
38	–CH ₂ (CH ₂) ₂ COOC ₂ H ₅		88	>10
39	–CH ₂ (CH ₂) ₂ COOC ₂ H ₅		90	>10
40	–CH ₂ (CH ₂) ₂ COOC ₂ H ₅		97	0.78 ± 0.23
41	–CH ₂ (CH ₂) ₂ COOC ₂ H ₅		96	>10
42	–CH ₂ (CH ₂) ₂ COOC ₂ H ₅		99	0.0073 ± 0.0021*
43	–CH ₂ (CH ₂) ₂ COOC ₂ H ₅		99	>10
44	–CH ₂ (CH ₂) ₂ COOC ₂ H ₅		61	>10
45	–CH ₂ (CH ₂) ₂ COOC ₂ H ₅		99	0.13 ± 0.02
46	–CH ₂ (CH ₂) ₂ COOC ₂ H ₅		86	2.3 ± 0.13
47	–CH ₂ (CH ₂) ₂ COOC ₂ H ₅		99	0.14 ± 0.01
48	–CH ₂ (CH ₂) ₂ COOC ₂ H ₅		78	0.053 ± 0.019
49	–CH ₂ (CH ₂) ₂ COOC ₂ H ₅		99	0.020 ± 0.014*
50	–CH ₂ (CH ₂) ₂ COOC ₂ H ₅		100	0.0087 ± 0.0179*
51	–CH ₂ (CH ₂) ₂ COOC ₂ H ₅		99	0.16 ± 0.02

(continued on next page)

Table 1 (continued)

Comp.	R ₁	R ₂	Inhibition ^a /%	Enzymatic IC ₅₀ ^b (μM)
52	–CH ₂ (CH ₂) ₂ COOC ₂ H ₅		82	>10
53	–CH ₂ (CH ₂) ₂ COOC ₂ H ₅		82	>10
54	–CH ₂ (CH ₂) ₂ COOC ₂ H ₅		85	0.66 ± 0.05
55	–CH ₂ (CH ₂) ₂ COOC ₂ H ₅		98	1.5 ± 0.2
Erlotinib			100	0.035 ± 0.012

^a Inhibition of EGFR kinase of compounds under the concentration of 0.04 mg/mL.

^b Data represent mean ± SD, n = 3, *P < 0.05 vs. Erlotinib.

Table 2

Cellular anti-proliferative data for some of the designed compounds toward KB and A498 cells.

Comp.	Cell inhibition IC ₅₀ ^a (μM)		Comp.	Cell inhibition IC ₅₀ ^a (μM)	
	KB ^b	A498 ^c		KB ^b	A498 ^c
18	1.1 ± 0.3*	17.6 ± 4.2	19	11.7 ± 2.7	47.0 ± 15.6
26	6.1 ± 1.9*	6.2 ± 1.7*	28	33.5 ± 13.3	11.1 ± 2.3*
37	18.7 ± 4.1	8.0 ± 1.5*	42	6.3 ± 1.1*	15.2 ± 1.6
45	38.4 ± 5.8	26.8 ± 4.2	46	17.1 ± 3.7	18.6 ± 3.4
47	17.9 ± 2.9	36.4 ± 5.8	49	19.5 ± 4.1	41.1 ± 7.4
50	3.7 ± 0.3*	7.6 ± 1.1*	51	61.6 ± 17.6	5.0 ± 0.7*
54	5.5 ± 0.6*	8.7 ± 1.3*	Erlotinib	16.6 ± 4.3	25.6 ± 5.1

^a Data represent mean ± SD, n = 3, *P < 0.05 vs. Erlotinib.

^b KB cells are a cell line derived from a human carcinoma of the nasopharynx that overexpresses both EGFR and Src.

^c A498 cells are a renal cell carcinoma (RCC) cell line that overexpresses both EGFR and Src.

high performance liquid chromatography (HPLC, Shimadzu LC-2010 system, Kyoto, Japan) equipped with a Diamonsil C18 column (5 μm 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 340 nm and sample injected volume was 20 μL. All compounds evaluated for EGFR inhibitory potency had a purity of ≥95%.

4.1.3. Ethyl 6-nitro-2-oxo-2, 3-dihydrobenzo[d]oxazole-5-carboxylate (8)

Ethyl 4-hydroxybenzoate **4** (100 g, 0.602 mol) was dissolved in the mixture of DCM (400 mL) and glacial acetic acid (150 mL) and cooled to –15 °C. Fuming nitric acid (70 mL) was slowly added and the reaction was stirred at –15 °C for 1 h. When the starting material was completely consumed, the solvent was slowly poured to 400 mL cold water and extracted with DCM (3 × 300 mL). The organic layer was washed with brine, dried over anhydrous Na₂SO₄ and evaporated. The crude product compound **5** (123 g, yield, 97%) was obtained.

Compound **5** (123 g, 0.58 mol) was added to conc. HCl (370 mL), and stannous chloride dihydrate (395 g, 1.75 mol) was portionwise added to the mixture at 0 °C, then heated to 45 °C and stirred for 7 h dramatically. The mixture was cooled to room temperature and filtered, and the solid was washed with acetate (200 mL), then dissolved in 500 mL water and basified with saturate NaHCO₃ until a pH of 9–10 was obtained. The resulting precipitate was filtered and washed with water, over anhydrous Na₂SO₄ and evaporated to give compound **6** as pale yellow solid (95.2 g, yield, 90%).

1, 1'-Carbonyl-diimidazole (CDI, 89.5 g, 0.552 mol) was added to a solution of compound **6** (95.2 g, 0.53 mol) in anhydrous THF (400 mL) at room temperature and stirred for 2 h at the same temperature. Diluted aqueous HCl solution (50 mL) was added to the mixture and the aqueous phase was extracted with EtOAc. The

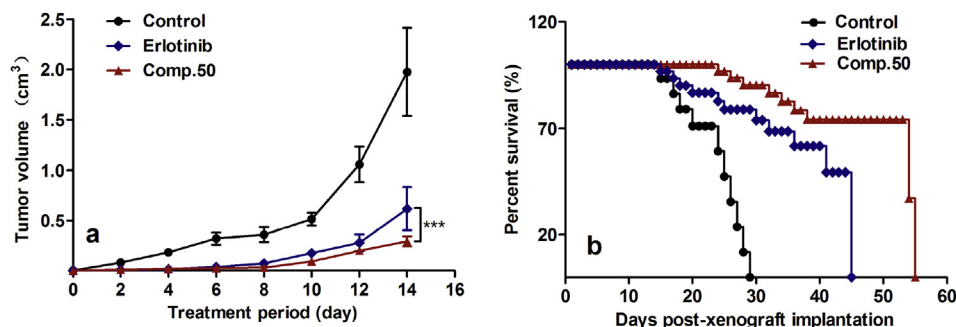


Fig. 3. (a) *In vivo* antitumor effect of compound **50** in a LLC xenograft mouse model. Eighteen mice were weighed and randomly divided into three groups (n = 6): 1) Control; 2) Erlotinib; 3) Compound **50**. Mice bearing established LLC tumor xenografts were dosed with compound **50** (100 mg/kg.qd.po) over a 14 day period. Erlotinib (100 mg/kg.qd.po) was employed as reference drug. Tumor size was measured every other day. Data are shown as the mean ± SD (n = 6). Statistical significance (p < 0.001) for antitumor efficacy, based upon tumor growth relative to the saline controls. (b) Survival curves of LLC tumor bearing mice received different treatments.

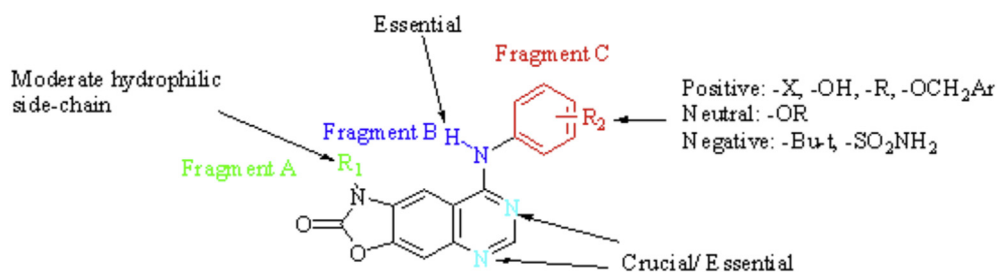


Fig. 4. Structure–activity relationships identified in this study.

organic solution was washed with saturated aqueous NaCl solution, dried over anhydrous Na₂SO₄, and evaporated. The resulting solid compound **7** (103.5 g, yield, 95%) was essentially pure and could be used to the following reaction.

Compound **7** (103.5 g, 0.500 mol) was added portionwise to 500 mL conc. HNO₃ at the temperature of 0–5 °C. The mixture was heated to 60 °C and stirred for 8 h at this temperature, after the starting material was completely consumed, the solution was poured to 2.5 L cold water, the resulting precipitate was filtered and washed with chill water, dried, the crude product was purified by silica gel column chromatography with petroleum ether/acetate (4/1, v/v) as mobile phase to give compound **8** (88.3 g, yield, 70%) as a pale yellow solid. Mp: 162–164 °C, ¹H NMR (300 MHz, DMSO-*d*₆) δ: 12.60 (1H, bs), 8.16 (1H, s), 4.30 (2H, q, *J* = 6.9 Hz), 1.29 (3H, t, *J* = 6.9 Hz); IR (film, cm⁻¹): 3289, 3062, 2990, 1788, 1712, 1626, 1540, 1477, 1372, 1292, 1247, 1104, 929, 893, 651.

4.1.4. Ethyl 6-amino-2-oxo-2,3-dihydrobenzo[d]oxazole-5-carboxylate (**9**)

A mixture of compound **8** (88.3 g, 0.35 mol), reduced iron power (60.8 g, 1.08 mol), acetic acid (600 mL) in 2 L CH₃OH was mechanically stirred at reflux for 3 h. To this mixture was added aqueous ammonia until the pH was adjusted to 10. The boiling mixture was filtered and the solid was washed with hot CH₃OH (500 mL). The solvent was removed from filtrate and the resulting solid was extracted with boiling acetone (600 mL) and filtered. The acetone extracts were concentrated in vacuo. The residue was recrystallized from EtOAc (300 mL) to 71.5 g (yield, 92%) of compound **9** as light brown solid. The compound was used without further purification: Mp: 173–175 °C, ¹H NMR (300 MHz, CDCl₃) δ: 8.63 (1H, bs), 7.57 (1H, s), 6.53 (1H, s), 4.35 (2H, q, *J* = 7.0 Hz), 1.39 (3H, t, *J* = 7.0 Hz); IR (film, cm⁻¹): 3459, 3351, 3255, 2991, 1775, 1665, 1583, 1426, 1279, 1229, 1126, 1077, 929, 866, 706.

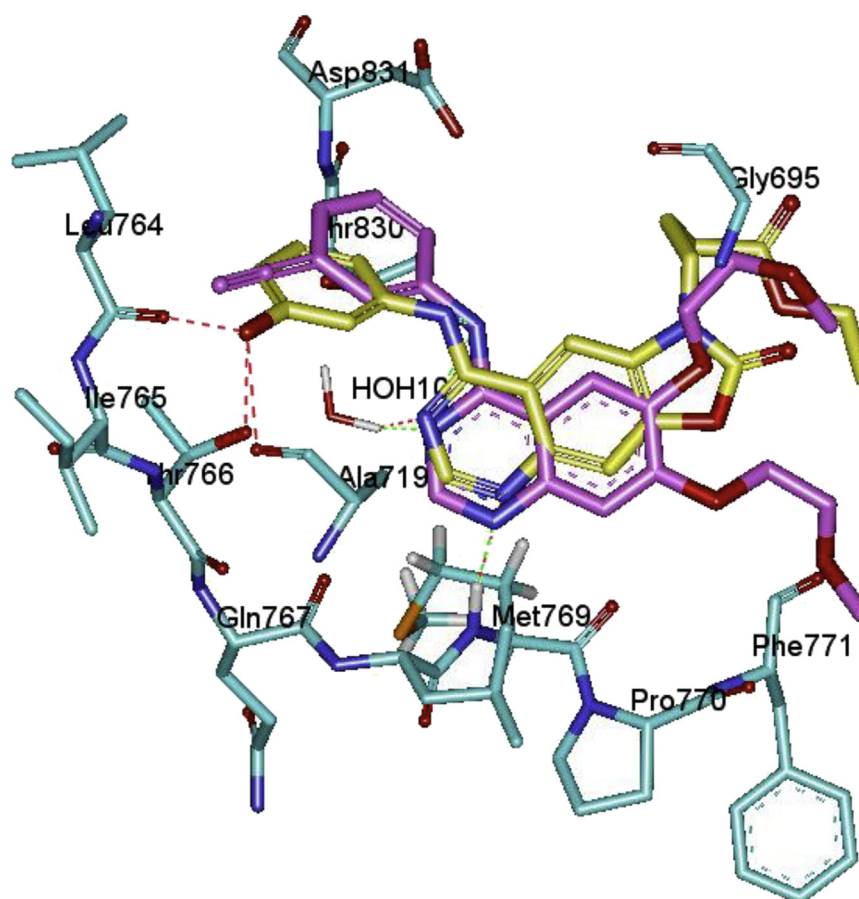


Fig. 5. Binding model of compound **50** (yellow) in complex with EGFR (PDB ID: 1M17) and Erlotinib (purple). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4.1.5. General procedure for the synthesis of 1-(2-methoxyethyl)oxazolo[4,5-g]quinazolin-2(1H)-one derivatives (**16–28**)

4.1.5.1. Ethyl 6-amino-3-(2-methoxyethyl)-2-oxo-2,3-dihydrobenzo[d]oxazole-5-carboxylate (10**).** K_2CO_3 (24.8 g, 180 mmol), KI (0.75 g, 4.5 mmol) and 1-chloro-2-methoxyethane (17.0 g, 180 mmol) were added successively to a solution of the compound **9** (20 g, 90.0 mmol) in DMF (120 mL) and the mixture was stirred at 70 °C for 5 h. The mixture was poured to water (500 mL) and the aqueous phase was extracted with AcOEt (3 × 400 mL). The organic solution was washed with water followed by saturated aqueous NaCl solution, dried over anhydrous Na_2SO_4 , and evaporated to afford the compound **10** (24.0 g, yield, 95%), which was used to the next reaction without further purification. Mp: 187–189 °C; ^1H NMR (500 MHz, CDCl_3) δ : 7.52 (1H, s), 6.51 (1H, s), 5.77 (2H, bs), 4.35 (2H, q, $J = 7.1$ Hz), 3.94 (2H, t, $J = 5.4$ Hz), 3.69 (2H, t, $J = 5.4$ Hz), 3.36 (3H, s), 1.40 (3H, t, $J = 7.1$ Hz); IR (film, cm^{-1}): 3468, 3358, 2987, 2891, 1780, 1691, 1584, 1489, 1432, 1370, 1328, 1280, 1226, 1111, 1064, 954, 832, 789, 746, 660.

4.1.5.2. 1-(2-Methoxyethyl)oxazolo[4,5-g]quinazolin-2,8(1H,7H)-dione (11**).** A solution of compound **10** (24.0 g, 85.5 mmol) and formamidine acetate (10.7 g, 102.6 mmol) in ethanol (120 mL) was refluxed for 24 h under the protection of N_2 . The mixture was cooled to room temperature and filtered, and the solid was washed with EtOH (100 mL) and water (50 mL), and dried to give compound **11** as white powder (17.9 g, yield, 80%); Mp: 218–219 °C; ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ : 12.27 (1H, bs), 8.06 (1H, s), 7.91 (1H, s), 7.58 (1H, s), 4.10 (2H, t, $J = 5.0$ Hz), 3.67 (2H, t, $J = 5.0$ Hz), 3.25 (3H, s); IR (film, cm^{-1}): 3439, 3167, 3012, 2881, 1773, 1663, 1587, 1482, 1366, 1279, 1111, 975, 909, 751, 681.

4.1.5.3. 8-Chloro-1-(2-methoxyethyl)oxazolo[4,5-g]quinazolin-2(1H)-one (12**).** Compound **11** (17.9 g, 68.4 mmol) was added to thionyl chloride (200 mL) with magnetic stirring, then DMF (1.0 mL) was slowly added dropwise and the reaction flask was refluxed for 6 h. Most of the excess of thionyl chloride and DMF was then removed under reduced pressure and the yellow residue was purified by silica-gel column chromatography (DCM/MeOH, 40/1), $R_f = 0.25$. Drying gave compound **12** (17.6 g, yield, 92%); Mp: 150–152 °C; ^1H NMR (500 MHz, CDCl_3) δ : 8.97 (1H, s), 7.85 (1H, s), 7.80 (1H, s), 4.16 (2H, t, $J = 4.9$ Hz), 3.79 (2H, t, $J = 4.9$ Hz), 3.37 (3H, s); IR (film, cm^{-1}): 3425, 3064, 2951, 2856, 2784, 1792, 1603, 1566, 1484, 1439, 1343, 1260, 1112, 951, 863, 806, 738, 685.

4.1.5.3.1. 8-(3-Chloro-4-fluorophenylamino)-1-(2-methoxyethyl)oxazolo[4,5-g]quinazolin-2(1H)-one (16**).** 3-Chloro-4-fluoroaniline (172 mg, 1.18 mmol) was added to a solution of compound **12** (300 mg, 1.07 mmol) in isopropanol (10 mL) and refluxed for 8 h. The mixture was cooled to room temperature and filtered, the solid was washed with chill isopropanol (5 mL), the residue was treated with aqueous NaHCO_3 (10 mL) and extracted with EtOAc/MeOH (20:1, 30 mL). The organic layer was washed with brine, dried over Na_2SO_4 , and concentrated. Purified by silica-gel column chromatography (DCM/MeOH, 40/1), $R_f = 0.25$. Drying gave 358 mg (yield, 86%) of the title compound as white solid: Mp: 217–220 °C; HPLC purity: 98%; HRMS, ESI^+ , m/z : Calcd for $\text{C}_{18}\text{H}_{15}\text{ClF}_2\text{N}_4\text{O}_3$ ($\text{M}+\text{H}^+$), 389.0811; found, 389.0809; ^1H NMR ($\text{DMSO}-d_6$) δ : 9.64 (1H, bs), 8.55 (1H, s), 8.19 (1H, s), 8.10 (1H, d, $J = 4.8$ Hz), 7.79 (1H, m), 7.63 (1H, s), 7.45 (1H, t, $J = 9$ Hz), 4.06 (2H, m), 3.79 (2H, m), 3.30 (3H, s); ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$) δ : 156.78, 154.28, 153.40, 152.85, 152.35, 147.11, 146.23, 136.35, 131.00, 123.61, 122.40 (d, $J = 6.3$ Hz), 118.79 (d, $J = 15.0$ Hz), 116.45 (d, $J = 21.2$ Hz), 111.55, 106.58, 100.31, 67.69, 58.11, 41.95.

Compounds **17–28** were synthesized according to the same procedure as compound **16**.

4.1.5.3.2. 8-(3-Ethynylphenylamino)-1-(2-methoxyethyl)oxazolo[4,5-g]quinazolin-2(1H)-one (17**).** Purified by silica-gel column chromatography (DCM/MeOH, 50/1), $R_f = 0.29$. White solid, 324 mg, yield, 84%; Mp: 215–217 °C; HPLC purity: 97%; HRMS, ESI^+ , m/z : Calcd for $\text{C}_{20}\text{H}_{17}\text{N}_4\text{O}_3$ ($\text{M}+\text{H}^+$), 361.1295; found, 361.1298; ^1H NMR ($\text{DMSO}-d_6$) δ : 9.64 (1H, bs), 8.53 (1H, s), 8.26 (1H, s), 7.96 (1H, s), 7.86 (1H, d, $J = 8.1$ Hz), 7.63 (1H, s), 7.39 (1H, dd, $J = 8.1$ Hz, $J = 7.5$ Hz), 7.20 (1H, d, $J = 7.5$ Hz), 4.16 (1H, s), 4.05 (2H, t, $J = 5.3$ Hz), 3.76 (2H, t, $J = 5.3$ Hz), 3.26 (3H, s); ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) δ : 156.94, 153.47, 152.98, 147.19, 146.21, 139.53, 130.95, 128.87, 126.65, 124.91, 123.29, 122.69, 121.76, 111.76, 106.58, 100.61, 83.40, 80.51, 58.12, 41.94.

4.1.5.3.3. 8-(3,4-Difluorophenylamino)-1-(2-methoxyethyl)oxazolo[4,5-g]quinazolin-2(1H)-one (18**).** Purified by silica-gel column chromatography (DCM/MeOH, 50/1), $R_f = 0.28$. White solid, 313 mg, yield, 87%; Mp: 220–224 °C; HPLC purity: 98%; HRMS, ESI^+ , m/z : Calcd for $\text{C}_{18}\text{H}_{15}\text{F}_2\text{N}_4\text{O}_3$ ($\text{M}+\text{H}^+$), 373.1107; found, 373.1111; ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ : 9.76 (1H, bs), 8.27 (1H, s), 8.06 (1H, m), 7.71 (1H, s), 7.69 (1H, s), 7.59 (1H, m), 7.48 (1H, q, $J = 9.2$ Hz), 4.08 (2H, t, $J = 5.5$ Hz), 3.79 (2H, t, $J = 5.5$ Hz), 3.29 (3H, s); ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) δ : 157.76, 153.39, 152.81, 150.31 (d, $J = 12.8$ Hz), 147.18, 147.12, 147.01, 146.21, 143.90 (d, $J = 12.8$ Hz), 136.18 (d, $J = 3.0$ Hz), 136.06 (d, $J = 3.0$ Hz), 118.38 (d, $J = 3.8$ Hz), 118.30 (d, $J = 3.8$ Hz), 117.03, 116.79, 111.53, 111.36, 111.08, 106.57, 100.27, 67.70, 58.10, 48.52, 41.94.

4.1.5.3.4. 1-(2-Methoxyethyl)-8-(4-methoxyphenylamino)oxazolo[4,5-g]quinazolin-2(1H)-one (19**).** Purified by silica-gel column chromatography (DCM/MeOH, 50/1), $R_f = 0.22$. White solid, yield, 354 mg, 90%; Mp: 206–209 °C; HPLC purity: 97%; HRMS, ESI^+ , m/z : Calcd for $\text{C}_{19}\text{H}_{19}\text{N}_4\text{O}_4$ ($\text{M}+\text{H}^+$), 367.1401; found, 367.1398; ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ : 9.68 (1H, bs), 8.54 (1H, s), 8.28 (1H, s), 7.87 (2H, d, $J = 6.2$ Hz), 7.66 (1H, s), 7.42 (2H, d, $J = 6.2$ Hz), 4.07 (2H, m), 3.79 (5H, m), 3.78 (3H, s), 3.29 (3H, s); ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) δ : 157.38, 155.92, 153.52, 153.35, 146.97, 131.74, 130.74, 124.51, 113.73, 111.58, 106.49, 100.60, 67.67, 58.11, 55.22, 41.93.

4.1.5.3.5. 8-(4-tert-Butylphenylamino)-1-(2-methoxyethyl)oxazolo[4,5-g]quinazolin-2(1H)-one (20**).** Purified by silica-gel column chromatography (DCM/MeOH, 90/1), $R_f = 0.27$. White solid, yield, 423 mg, 86%; Mp: 241–243 °C; HPLC purity: 96%; HRMS, ESI^+ , m/z : Calcd for $\text{C}_{22}\text{H}_{25}\text{N}_4\text{O}_3$ ($\text{M}+\text{H}^+$), 393.1921; found, 393.1925; ^1H NMR (125 MHz, $\text{DMSO}-d_6$) δ : 9.57 (1H, bs), 8.49 (1H, s), 8.28 (1H, s), 7.69 (2H, d, $J = 8.0$ Hz), 7.62 (1H, s), 7.42 (1H, d, $J = 8.0$ Hz), 4.07 (2H, m), 3.79 (2H, m), 3.29 (3H, s), 1.32 (9H, s); ^{13}C NMR (300 MHz, $\text{DMSO}-d_6$) δ : 157.21, 153.44, 153.18, 147.09, 146.15, 146.07, 136.31, 130.77, 124.98, 122.29, 111.65, 106.47, 100.54, 67.67, 58.05, 41.88, 33.99, 31.12.

4.1.5.3.6. 8-(4-Chlorophenylamino)-1-(2-methoxyethyl)oxazolo[4,5-g]quinazolin-2(1H)-one (21**).** Purified by silica-gel column chromatography (DCM/MeOH, 60/1), $R_f = 0.29$. White solid, yield, 332 mg, 84%; Mp: 203–206 °C; HPLC purity: 98%; HRMS, ESI^+ , m/z : Calcd for $\text{C}_{18}\text{H}_{16}\text{ClN}_4\text{O}_3$ ($\text{M}+\text{H}^+$), 371.0905; found, 371.0909; ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ : 9.56 (1H, bs), 8.45 (1H, s), 8.28 (1H, s), 7.65 (2H, d, $J = 9.0$ Hz), 7.63 (1H, s), 6.99 (2H, d, $J = 9.0$ Hz), 4.07 (2H, m), 3.79 (2H, m), 3.29 (3H, s); ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) δ : 156.91, 153.39, 152.90, 147.16, 146.21, 138.03, 130.97, 128.23, 127.22, 123.76, 111.67, 106.54, 100.45, 67.66, 58.05, 41.92.

4.1.5.3.7. 1-(2-Methoxyethyl)-8-(4-((3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl)methoxy)phenylamino)oxazolo[4,5-g]quinazolin-2(1H)-one (22**).** Purified by silica-gel column chromatography (DCM/MeOH, 40/1), $R_f = 0.29$. White solid, yield, 517 mg, 87%; Mp: 322–325 °C; HPLC purity: 97%; HRMS, ESI^+ , m/z : Calcd for $\text{C}_{27}\text{H}_{25}\text{F}_3\text{N}_5\text{O}_5$ ($\text{M}+\text{H}^+$), 556.1802; found, 556.1797; ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ : 9.56 (1H, bs), 8.44 (1H, s), 8.36 (1H, m), 8.27 (1H, s), 7.66 (2H, d, $J = 6.2$ Hz), 7.63 (1H, s), 7.15 (1H, m), 7.08 (2H, d, $J = 6.2$ Hz), 5.20 (2H, s), 4.92 (2H, m), 4.06 (2H, m), 3.79 (2H, m),

3.28 (3H, s), 2.24 (3H, s); ^{13}C NMR (75 MHz, DMSO- d_6) δ : 161.36, 157.28, 155.39, 154.99, 153.43 (d, J = 13.5 Hz), 147.63, 147.05, 146.09, 132.11, 130.73, 124.40 (q, J = 245.0 Hz), 121.38, 114.50 (d, J = 16.5 Hz), 111.58 (d, J = 3.0 Hz), 107.57, 106.40, 100.65, 70.45, 67.69, 64.90, 64.44, 58.10, 41.93, 9.93.

4.1.5.3.8. 8-(3-Isopropoxyphenylamino)-1-(2-methoxyethyl)oxazolo[4,5-g]quinazolin-2(1H)-one (23). Purified by silica-gel column chromatography (DCM/MeOH, 80/1), R_f = 0.26. White solid, yield, 332 mg, 83%; Mp: 245–247 °C; HPLC purity: 98%; HRMS, ESI $^+$, m/z : Calcd for $\text{C}_{21}\text{H}_{23}\text{N}_4\text{O}_4$ (M+H) $^+$, 395.1714; found, 395.1715; ^1H NMR (500 MHz, DMSO- d_6) δ : 9.53 (1H, bs), 8.54 (1H, s), 8.28 (1H, s), 7.64 (2H, d, J = 6.2 Hz), 7.50 (1H, s), 7.35 (1H, d, J = 7.5 Hz), 7.29 (1H, m), 6.71 (1H, d, J = 7.5 Hz), 4.62 (1H, m), 4.08 (2H, m), 3.79 (2H, m), 3.29 (3H, s), 1.31 (9H, d, J = 5.7 Hz); ^{13}C NMR (125 MHz, DMSO- d_6) δ : 157.54, 157.04, 153.41, 153.04, 147.21, 146.12, 140.23, 130.86, 129.04, 114.36, 111.74, 110.67, 110.07, 106.50, 100.48, 69.23, 67.66, 58.04, 41.88, 21.79.

4.1.5.3.9. 4-(1-(2-Methoxyethyl)-2-oxo-1,2-dihydrooxazolo[4,5-g]quinazolin-8-ylamino) benzenesulfonamide (24). Purified by silica-gel column chromatography (DCM/MeOH, 35/1), R_f = 0.25. White solid, yield, 355 mg, 80%; Mp: 285–288 °C; HPLC purity: 97%; HRMS, ESI $^+$, m/z : Calcd for $\text{C}_{18}\text{H}_{18}\text{N}_5\text{O}_5\text{S}$ (M+H) $^+$, 416.1023; found, 416.1028; ^1H NMR (125 MHz, DMSO- d_6) δ : 8.9–10.2 (1H, bs), 8.58 (1H, s), 8.31 (1H, s), 8.04 (2H, d, J = 8.2 Hz), 7.85 (2H, d, J = 8.2 Hz), 7.66 (1H, s), 6.8–7.4 (2H, bs), 4.07 (2H, m), 3.78 (2H, m), 3.27 (3H, s); ^{13}C NMR (500 MHz, DMSO- d_6) δ : 156.88, 153.45, 152.84, 147.34, 146.38, 142.38, 138.36, 131.16, 126.28, 121.48, 111.90, 106.63, 100.62, 67.73, 58.11, 41.99.

4.1.5.3.10. 8-(3-Acetylphenylamino)-1-(2-methoxyethyl)oxazolo[4,5-g]quinazolin-2(1H)-one (25). Purified by silica-gel column chromatography (DCM/MeOH, 50/1), R_f = 0.30. White solid, yield, 303 mg, 75%; Mp: 259–261 °C; HPLC purity: 98%; HRMS, ESI $^+$, m/z : Calcd for $\text{C}_{20}\text{H}_{19}\text{N}_4\text{O}_4$ (M+H) $^+$, 379.1041; found, 379.1399; ^1H NMR (125 MHz, DMSO- d_6) δ : 9.83 (1H, bs), 8.55 (1H, s), 8.33 (1H, s), 8.31 (1H, s), 8.22 (1H, d, J = 7.4 Hz), 7.75 (1H, d, J = 7.4 Hz), 7.64 (1H, s), 7.55 (1H, t, J = 7.8 Hz), 4.08 (2H, m), 3.80 (2H, m), 3.29 (3H, s), 2.62 (3H, s); ^{13}C NMR (500 MHz, DMSO- d_6) δ : 197.66, 157.07, 153.46, 153.00, 147.21, 146.22, 139.82, 137.15, 130.93, 128.75, 126.79, 123.53, 121.18, 111.85, 106.54, 100.66, 67.71, 58.09, 41.94, 26.72.

4.1.5.3.11. 8-(4-(4-Chlorobenzoyloxy)-3-chlorophenylamino)-1-(2-methoxyethyl) oxazolo[4,5-g]quinazolin-2(1H)-one (26). Purified by silica-gel column chromatography (DCM/MeOH, 45/1), R_f = 0.26. White solid, yield, 475 mg, 87%; Mp: 305–306 °C; HPLC purity: 98%; HRMS, ESI $^+$, m/z : Calcd for $\text{C}_{25}\text{H}_{21}\text{Cl}_2\text{N}_4\text{O}_4$ (M+H) $^+$, 511.0934; found, 511.0932; ^1H NMR (500 MHz, DMSO- d_6) δ : 10.37 (1H, bs), 8.67 (2H, m), 8.50 (1H, s), 7.93 (1H, m), 7.70 (2H, d, J = 9.8 Hz), 7.49 (4H, m), 7.30 (1H, d, J = 7.8 Hz), 5.24 (2H, s), 4.08 (2H, m), 3.79 (2H, m), 3.29 (3H, s); ^{13}C NMR (125 MHz, DMSO- d_6) δ : 157.00, 153.59, 152.99, 149.69, 147.01, 146.35, 135.68, 133.07, 132.47, 131.09, 129.24, 128.43, 124.08, 122.26, 121.07, 114.36, 111.52, 106.46, 100.22, 67.72, 58.13, 41.98.

4.1.5.3.12. 8-(3, 4-Dimethoxyphenylamino)-1-(2-methoxyethyl) oxazolo[4,5-g] quinazolin-2(1H)-one (27). Purified by silica-gel column chromatography (DCM/MeOH, 50/1), R_f = 0.31. White solid, yield, 343 mg, 90%; Mp: 235–238 °C; HPLC purity: 97%; HRMS, ESI $^+$, m/z : Calcd for $\text{C}_{20}\text{H}_{21}\text{N}_4\text{O}_5$ (M+H) $^+$, 397.1508; found, 397.1511; ^1H NMR (500 MHz, DMSO- d_6) δ : 9.50 (1H, bs), 8.46 (1H, s), 8.37 (1H, s), 8.25 (1H, s), 7.60 (1H, s), 7.38 (1H, s), 7.30 (1H, d, J = 4.9 Hz), 7.01 (1H, d, J = 4.9 Hz), 4.05 (2H, m), 3.77–3.79 (8H, m), 3.29 (3H, s); ^{13}C NMR (125 MHz, DMSO- d_6) δ : 157.34, 153.50, 148.46, 147.02, 146.04, 145.58, 132.27, 130.71, 115.19, 111.84, 111.66, 108.71, 106.49, 100.57, 67.66, 58.08, 55.74, 55.60, 41.90.

4.1.5.3.13. Ethyl 4-hydroxy-3-(1-(2-methoxyethyl)-2-oxo-1, 2-dihydrooxazolo[4,5-g] quinazolin-8-ylamino)benzoate (28). Purified by silica-gel column chromatography (DCM/MeOH, 70/1),

R_f = 0.27. White solid, yield, 390 mg, 86%; Mp: 277–279 °C; HPLC purity: 96%; HRMS, ESI $^+$, m/z : Calcd for $\text{C}_{21}\text{H}_{21}\text{N}_4\text{O}_6$ (M+H) $^+$, 425.1456; found, 425.1461; ^1H NMR (500 MHz, DMSO- d_6) δ : 9.40 (1H, bs), 8.45 (1H, s), 8.28 (1H, s), 8.16 (1H, s), 7.76 (1H, d, J = 6.4 Hz), 7.65 (1H, s), 7.06 (1H, d, J = 6.4 Hz), 4.30 (2H, m), 4.08 (2H, m), 3.80 (2H, m), 3.31 (3H, s), 1.32 (3H, m); ^{13}C NMR (125 MHz, DMSO- d_6) δ : 165.38, 157.97, 156.48, 153.52, 146.93, 146.19, 130.88, 128.17, 128.01, 126.10, 120.14, 116.23, 111.69, 106.42, 100.83, 67.76, 60.14, 58.05, 41.94, 14.19.

4.1.6. General procedure for the synthesis of 1-(4-ethoxy-4-oxobutyl) oxazolo[4,5-g]quinazolin-2(1H)-one derivatives (29–55)

4.1.6.1. Ethyl 6-amino-3-(4-ethoxy-4-oxobutyl)-2-oxo-2,3-dihydrobenzo[d]oxazole-5-carboxylate (13). K_2CO_3 (24.8 g, 180 mmol), KI (0.75 g, 4.5 mmol) and ethyl 4-bromobutanoate (19.3 g, 99 mmol) were added successively to a solution of the compound **9** (20 g, 90.0 mmol) in acetonitrile (220 mL) and the mixture was refluxed for 3 h. The mixture was poured to water (500 mL) and the aqueous phase was extracted with AcOEt (3 \times 400 mL). The organic solution was washed with brine, dried over anhydrous Na_2SO_4 , and the solvent was evaporated to afford the compound **10** (29.4 g, yield, 97%), which was used to the next reaction without further purification. Mp: 76–78 °C; ^1H NMR (500 MHz, CDCl_3) δ : 7.45 (1H, s), 6.52 (1H, s), 5.0–6.0 (2H, bs), 4.36 (2H, q, J = 7.1 Hz), 4.14 (2H, q, J = 7.0 Hz), 3.84 (2H, t, J = 7.0 Hz), 2.46 (2H, t, J = 7.2 Hz), 2.11 (2H, p, J = 7.0 Hz), 1.41 (2H, t, J = 7.1 Hz), 1.26 (2H, t, J = 7.2 Hz), 2.11 (2H, t, J = 7.2 Hz); IR (film, cm^{-1}): 3462, 3354, 2985, 1779, 1715, 1680, 1587, 1485, 1372, 1285, 1230, 1181, 1103, 1061, 953, 875, 786, 749, 677.

4.1.6.2. Ethyl 4-(2,8-dioxo-7,8-dihydrooxazolo[4,5-g]quinazolin-1(2H)-yl)butanoate (14). A solution of compound **13** (29.4 g, 87.3 mmol) and formamidine acetate (10.9 g, 104.8 mmol) in ethanol (120 mL) was refluxed for 24 h under the protection of N_2 . The mixture was cooled to room temperature and filtered, the solid was washed with EtOH (100 mL) and water (50 mL), and dried to give compound **14** as white power (22.7 g, yield, 82%); Mp 187–189 °C; ^1H NMR (500 MHz, CDCl_3) δ : 12.28 (1H, bs), 8.06 (1H, s), 7.90 (1H, s), 7.58 (1H, s), 3.97 (4H, m), 2.45 (2H, t, J = 7.2 Hz), 1.98 (2H, p, J = 7.0 Hz), 1.13 (2H, t, J = 7.2 Hz); IR (film, cm^{-1}): 3437, 3108, 2942, 1794, 1723, 1677, 1590, 1489, 1369, 1267, 1206, 1063, 959, 914, 751.

4.1.6.3. Ethyl 4-(8-chloro-2-oxoxazolo[4,5-g]quinazolin-1(2H)-yl)butanoate (15). Compound **14** (22.7 g, 71.6 mmol) was added to thionyl chloride (150 mL) with magnetic stirring, then DMF (1.0 mL) was slowly added dropwise and the reaction flask was heated to reflux for 6 h. Most of the excess of thionyl chloride and DMF was then removed under reduced pressure and the yellow residue was purified by silica gel column chromatography with DCM/MeOH (100/1, R_f = 0.30) as mobile phase to give compound **15** (21.6 g, yield, 90%); Mp: 187–189 °C; ^1H NMR (500 MHz, CDCl_3) δ : 8.81 (1H, s), 8.02 (1H, s), 7.99 (1H, s), 3.97 (4H, m), 2.45 (2H, t, J = 7.2 Hz), 1.98 (2H, m), 1.12 (2H, t, J = 7.0 Hz); IR (film, cm^{-1}): 3420, 2986, 2870, 1809, 1727, 1606, 1563, 1484, 1356, 1258, 1180, 1065, 961, 861, 745, 692.

4.1.6.3.1. Ethyl 4-(8-(3-Chloro-4-fluorophenylamino)-2-oxoxazolo[4,5-g]quinazolin-1(2H)-yl)butanoate (29). 3-Chloro-4-fluoroaniline (130 mg, 0.89 mmol) was added to a solution of compound **15** (250 mg, 0.74 mmol) in isopropanol (10 mL) and stirred at reflux for 7 h. The mixture was cooled to room temperature and filtered, the solid was washed with chill isopropanol (5 mL), the residue was treated with aqueous NaHCO_3 (10 mL) and extracted with EtOAc/MeOH (20:1, 30 mL). The organic layer was washed with brine, dried over Na_2SO_4 , and concentrated.

Purified by silica-gel column chromatography (DCM/MeOH, 50/1), $R_f = 0.30$, gave 286 mg (yield, 87%) of the title compound; Mp: 245–247 °C; HPLC purity: 98%; HRMS, ESI⁺, m/z : Calcd for $C_{21}H_{19}ClFN_4O_4$ (M+H)⁺, 445.1073; found, 445.1081; ¹H NMR (500 MHz, DMSO-*d*₆) δ : 9.72 (1H, bs), 8.58 (1H, s), 8.20 (1H, s), 8.12 (1H, s), 7.80 (1H, m), 7.68 (1H, s), 7.48 (1H, t, $J = 9.1$ Hz), 3.97 (4H, m), 2.47 (1H, t, $J = 7.3$ Hz), 2.13 (2H, p, $J = 6.9$ Hz), 1.10 (3H, t, $J = 7.1$ Hz); ¹³C NMR (75 MHz, DMSO-*d*₆) δ : 172.14, 156.81, 154.91, 153.55, 152.82, 151.69, 147.17, 146.44, 136.52 (d, $J = 3.0$ Hz), 136.43, 130.97, 123.68, 122.50 (d, $J = 6.8$ Hz), 118.91, 118.61, 116.48 (d, $J = 21.0$), 111.61, 106.50, 100.07, 59.68, 41.65, 30.66, 22.18, 13.89.

4.1.6.3.2. Ethyl 4-(8-(3-ethynylphenylamino)-2-oxooxazolo[4,5-*g*]quinazolin-1(2H)-yl)butanoate (30). Purified by silica-gel column chromatography (DCM/MeOH, 60/1), $R_f = 0.28$. White solid, 256 mg, yield, 83%; Mp: 240–243 °C; HPLC purity: 97%; HRMS, ESI⁺, m/z : Calcd for $C_{23}H_{21}N_4O_4$ (M+H)⁺, 417.1557; found, 417.1566; ¹H NMR (500 MHz, DMSO-*d*₆) δ : 9.66 (1H, bs), 8.59 (1H, s), 8.24 (1H, s), 8.00 (1H, s), 7.92 (1H, d, $J = 8.0$ Hz), 7.45 (1H, t, $J = 7.8$ Hz), 7.27 (1H, d, $J = 7.6$ Hz), 4.21 (1H, s), 3.98 (4H, m), 2.49 (2H, t, $J = 7.2$ Hz), 2.14 (2H, p, $J = 6.9$ Hz), 1.10 (3H, t, $J = 7.0$ Hz); ¹³C NMR (75 MHz, DMSO-*d*₆) δ : 172.14, 156.81, 154.91, 153.55, 152.82, 151.69, 147.17, 146.44, 136.54, 136.43, 130.97, 123.68, 122.54, 122.45, 118.91, 118.61, 116.62, 116.34, 111.61, 106.50, 100.07, 59.68, 41.65, 30.66, 22.18, 13.89.

4.1.6.3.3. Ethyl 4-(2-oxo-8-(phenylamino)oxazolo[4,5-*g*]quinazolin-1(2H)-yl) butanoate (31). Purified by silica-gel column chromatography (DCM/MeOH, 50/1), $R_f = 0.29$. White solid, 250 mg, yield, 86%; Mp: 213–217 °C; HPLC purity: 98%; HRMS, ESI⁺, m/z : Calcd for $C_{21}H_{21}N_4O_4$ (M+H)⁺, 393.1557; found, 393.1563; ¹H NMR (500 MHz, DMSO-*d*₆) δ : 10.20 (1H, bs), 8.61 (1H, s), 8.50 (1H, s), 7.80 (1H, d, $J = 7.7$ Hz), 7.69 (1H, s), 7.44 (2H, t, $J = 7.7$ Hz), 7.21 (1H, d, $J = 7.4$ Hz), 3.96 (4H, m), 2.49 (2H, t, $J = 7.4$ Hz), 2.11 (2H, p, $J = 7.1$ Hz), 1.10 (3H, t, $J = 7.2$ Hz); ¹³C NMR (125 MHz, DMSO-*d*₆) δ : 172.11, 157.18, 153.58, 153.07, 147.12, 146.37, 138.97, 130.91, 128.41, 123.77, 122.62, 111.64, 106.42, 100.23, 59.82, 41.65, 30.68, 22.17, 13.86.

4.1.6.3.4. Ethyl 4-(8-(4-chlorophenylamino)-2-oxooxazolo[4,5-*g*]quinazolin-1(2H)-yl) butanoate (33). Purified by silica-gel column chromatography (DCM/MeOH, 50/1), $R_f = 0.30$. White solid, 265 mg, yield, 84%; Mp: 225–227 °C; HPLC purity: 98%; HRMS, ESI⁺, m/z : Calcd for $C_{21}H_{20}ClN_4O_4$ (M+H)⁺, 427.1168; found, 427.1173; ¹H NMR (500 MHz, DMSO-*d*₆) δ : 9.93 (1H, bs), 8.58 (1H, s), 8.34 (1H, s), 7.87 (1H, d, $J = 8.6$ Hz), 7.66 (1H, s), 7.47 (2H, t, $J = 8.6$ Hz), 3.96 (4H, m), 2.48 (2H, t, $J = 7.0$ Hz), 2.11 (2H, p, $J = 6.9$ Hz), 1.10 (3H, t, $J = 7.0$ Hz); ¹³C NMR (125 MHz, DMSO-*d*₆) δ : 172.10, 157.05, 153.51, 152.66, 146.52, 146.46, 137.93, 131.10, 128.28, 127.44, 123.98, 111.55, 106.03, 100.35, 59.81, 41.67, 30.65, 22.17, 13.86.

4.1.6.3.5. Ethyl 4-(8-(4-(benzyloxy)phenylamino)-2-oxooxazolo[4,5-*g*]quinazolin-1(2H)-yl)butanoate (34). Purified by silica-gel column chromatography (DCM/MeOH, 50/1), $R_f = 0.31$. White solid, 335 mg, yield, 91%; Mp: 276–276 °C; HPLC purity: 97%; HRMS, ESI⁺, m/z : Calcd for $C_{28}H_{27}ClN_4O_5$ (M+H)⁺, 499.1976; found, 499.1977; ¹H NMR (500 MHz, DMSO-*d*₆) δ : 9.56 (1H, bs), 8.45 (1H, s), 8.21 (1H, s), 7.64 (1H, s), 7.61 (1H, d, $J = 9.2$ Hz), 7.48 (2H, d, $J = 8.0$ Hz), 7.39 (2H, t, $J = 6.5$ Hz), 7.34 (1H, t, $J = 7.2$ Hz), 7.07 (2H, d, $J = 9.0$ Hz), 5.13 (2H, s), 3.96 (4H, m), 2.47 (2H, t, $J = 7.4$ Hz), 2.11 (2H, p, $J = 6.8$ Hz), 1.10 (3H, t, $J = 7.2$ Hz); ¹³C NMR (75 MHz, DMSO-*d*₆) δ : 172.15, 157.15, 154.99, 153.62, 153.30, 147.06, 146.26, 137.13, 131.96, 130.74, 128.37, 127.74, 127.62, 124.62, 114.67, 111.53, 106.43, 100.21, 69.38, 59.86, 41.65, 30.69, 22.17, 13.90.

4.1.6.3.6. Ethyl 4-(8-(4-methoxyphenylamino)-2-oxooxazolo[4,5-*g*]quinazolin-1(2H)-yl)butanoate (35). Purified by silica-gel column chromatography (DCM/MeOH, 50/1), $R_f = 0.25$. White solid, 281 mg, yield, 90%; Mp: 226–229 °C; HPLC purity: 98%; HRMS,

ESI⁺, m/z : Calcd for $C_{22}H_{23}N_4O_5$ (M+H)⁺, 423.1663; found, 423.1670; ¹H NMR (500 MHz, DMSO-*d*₆) δ : 9.54 (1H, bs), 8.45 (1H, s), 8.21 (1H, s), 7.64 (1H, s), 7.62 (2H, d, $J = 8.8$ Hz), 7.00 (2H, d, $J = 8.8$ Hz), 3.96 (4H, m), 3.79 (3H, s), 2.47 (2H, t, $J = 7.3$ Hz), 2.12 (2H, p, $J = 6.8$ Hz), 1.10 (3H, t, $J = 7.1$ Hz); ¹³C NMR (75 MHz, DMSO-*d*₆) δ : 172.10, 157.40, 155.95, 153.59, 153.28, 147.04, 146.23, 131.72, 130.71, 124.64, 111.67, 111.51, 106.40, 100.17, 59.82, 55.17, 41.63, 30.68, 22.14, 13.86.

4.1.6.3.7. Ethyl 4-(8-(4-(4-methoxybenzyloxy)phenylamino)-2-oxooxazolo[4,5-*g*] quinazolin-1(2H)-yl)butanoate (36). Purified by silica-gel column chromatography (DCM/MeOH, 45/1), $R_f = 0.26$. White solid, 348 mg, yield, 89%; Mp: 310–312 °C; HPLC purity: 98%; HRMS, ESI⁺, m/z : Calcd for $C_{29}H_{29}N_4O_6$ (M+H)⁺, 529.2082; found, 529.2092; ¹H NMR (500 MHz, DMSO-*d*₆) δ : 9.51 (1H, bs), 8.43 (1H, s), 8.20 (1H, s), 7.62 (1H, s), 7.60 (2H, d, $J = 9.0$ Hz), 7.38 (2H, d, $J = 8.8$ Hz), 7.04 (2H, d, $J = 9.0$ Hz), 6.94 (2H, d, $J = 8.8$ Hz), 5.03 (2H, s), 3.96 (4H, m), 3.75 (3H, s), 2.45 (2H, t, $J = 7.2$ Hz), 2.10 (2H, p, $J = 6.8$ Hz), 1.08 (3H, t, $J = 7.1$ Hz); ¹³C NMR (75 MHz, DMSO-*d*₆) δ : 172.11, 158.93, 157.38, 155.06, 153.60, 153.68, 147.04, 146.26, 131.82, 130.74, 129.37, 128.97, 124.60, 114.70, 113.75, 111.51, 106.42, 100.16, 69.15, 59.83, 55.03, 41.64, 30.68, 22.15, 13.88.

4.1.6.3.8. 4-(1-(4-ethoxy-4-oxobutyl)-2-oxo-1,2-dihydrooxazolo[4,5-*g*]quinazolin-8-ylamino) benzenesulfonamide (37). Purified by silica-gel column chromatography (DCM/MeOH, 40/1), $R_f = 0.24$. White solid, 289 mg, yield, 83%; Mp: 281–284 °C; HPLC purity: 98%; HRMS, ESI⁺, m/z : Calcd for $C_{21}H_{22}N_5O_6S$ (M+H)⁺, 472.1285; found, 472.1290; ¹H NMR (300 MHz, DMSO-*d*₆) δ : 9.54 (1H, bs), 8.61 (1H, s), 8.29 (1H, s), 8.04 (2H, d, $J = 8.4$ Hz), 7.86 (2H, d, $J = 8.4$ Hz), 7.71 (1H, s), 7.29 (2H, bs), 3.98 (4H, m), 2.27 (2H, t, $J = 7.3$ Hz), 2.12 (2H, p, $J = 6.7$ Hz), 1.17 (3H, t, $J = 7.1$ Hz); ¹³C NMR (75 MHz, DMSO-*d*₆) δ : 172.17, 156.88, 153.59, 152.81, 147.40, 146.62, 142.39, 138.39, 131.21, 126.32, 121.53, 111.87, 106.59, 100.17, 59.87, 41.69, 30.68, 22.20, 13.90.

4.1.6.3.9. Ethyl 4-(8-(4-((3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl)methoxy) phenylamino)-2-oxooxazolo[4,5-*g*]quinazolin-1(2H)-yl)butanoate (40). Purified by silica-gel column chromatography (DCM/MeOH, 40/1), $R_f = 0.27$. White solid, 398 mg, yield, 88%; Mp: 340–342 °C; HPLC purity: 97%; HRMS, ESI⁺, m/z : Calcd for $C_{30}H_{29}F_3N_5O_6$ (M+H)⁺, 612.2064; found, 612.2074; ¹H NMR (500 MHz, DMSO-*d*₆) δ : 9.55 (1H, bs), 8.44 (1H, s), 8.37 (1H, d, $J = 5.2$ Hz), 8.23 (1H, s), 7.63 (1H, s), 7.61 (2H, m), 7.16 (1H, d, $J = 5.3$ Hz), 7.09 (2H, d, $J = 8.4$ Hz), 5.21 (2H, s), 4.92 (2H, q, $J = 8.4$ Hz), 3.97 (4H, m), 2.47 (2H, t, $J = 7.0$ Hz), 2.25 (3H, s), 2.12 (2H, m), 1.10 (3H, t, $J = 7.0$ Hz); ¹³C NMR (125 MHz, DMSO-*d*₆) δ : 172.12, 161.35, 157.41, 155.38, 155.03, 153.61, 153.27, 147.60, 147.06, 146.27, 132.03, 130.76, 124.62, 123.48 (q, $J = 240.0$ Hz), 121.35, 114.62, 111.52, 107.56, 106.42, 100.22, 70.44, 64.71 (q, $J = 34.3$ Hz), 41.65, 30.68, 22.15, 13.87, 9.91.

4.1.6.3.10. Ethyl 4-(8-(3,4-dimethoxyphenylamino)-2-oxooxazolo[4,5-*g*]quinazolin-1(2H)-yl)butanoate (41). Purified by silica-gel column chromatography (DCM/MeOH, 45/1), $R_f = 0.28$. White solid, 301 mg, yield, 90%; Mp: 243–245 °C; HPLC purity: 98%; HRMS, ESI⁺, m/z : Calcd for $C_{23}H_{25}N_4O_6$ (M+H)⁺, 453.1769; found, 453.1776; ¹H NMR (500 MHz, DMSO-*d*₆) δ : 9.55 (1H, bs), 8.47 (1H, s), 8.37 (1H, s), 8.22 (1H, s), 7.63 (1H, s), 7.36 (1H, s), 7.30 (1H, d, $J = 4.9$ Hz), 7.01 (1H, d, $J = 4.9$ Hz), 3.95 (4H, m), 3.79 (6H, s), 2.47 (2H, t, $J = 7.0$ Hz), 2.12 (2H, m), 1.10 (3H, t, $J = 7.0$ Hz); ¹³C NMR (125 MHz, DMSO-*d*₆) δ : 172.12, 157.39, 153.27, 148.48, 147.07, 146.26, 145.66, 132.15, 130.74, 115.38, 111.82, 111.56, 108.32, 106.44, 100.16, 59.84, 55.73, 55.60, 41.64, 30.69, 22.17, 13.88.

4.1.6.3.11. Ethyl 3-(1-(4-ethoxy-4-oxobutyl)-2-oxo-1,2-dihydrooxazolo[4,5-*g*] quinazolin-8-ylamino)-4-hydroxybenzoate (42). Purified by silica-gel column chromatography (DCM/MeOH, 50/1), $R_f = 0.26$. White solid, 297 mg, yield, 84%; Mp: 312–313 °C; HPLC purity: 96%; HRMS, ESI⁺, m/z : Calcd for $C_{24}H_{25}N_4O_7$ (M+H)⁺,

481.1718; found, 481.1721; ^1H NMR (300 MHz, DMSO- d_6) δ : 10.75 (1H, bs), 9.41 (1H, bs), 8.68 (1H, s), 8.26 (1H, s), 8.08 (1H, s), 7.79 (1H, d, $J = 7.6$ Hz), 7.66 (1H, s), 7.08 (1H, d, $J = 7.6$ Hz), 4.30 (2H, m), 3.98 (4H, m), 3.26 (2H, m), 2.13 (2H, m), 1.32 (3H, t, $J = 7.0$ Hz), 1.14 (3H, t, $J = 6.7$ Hz); ^{13}C NMR (125 MHz, DMSO- d_6) δ : 172.14, 165.32, 158.38, 153.62, 153.12, 147.01, 146.42, 130.85, 128.76, 128.22, 125.92, 120.60, 116.29, 111.60, 106.38, 100.51, 60.20, 59.84, 41.67, 30.96, 30.69, 22.14, 21.43, 14.19, 13.90.

4.1.6.3.12. Ethyl 4-(8-(*p*-toluidino)-2-oxooxazol[4,5-*g*]quinazolin-1(2H)-yl)butanoate (43). Purified by silica-gel column chromatography (DCM/MeOH, 50/1), $R_f = 0.31$. White solid, 261 mg, yield, 87%; Mp: 215–218 °C; HPLC purity: 98%; HRMS, ESI $^+$, m/z : Calcd for $\text{C}_{22}\text{H}_{23}\text{N}_4\text{O}_4$ ($\text{M}+\text{H}$) $^+$, 407.1714; found, 407.1718; ^1H NMR (300 MHz, DMSO- d_6) δ : 9.53 (1H, bs), 8.48 (1H, s), 8.22 (1H, s), 7.61 (2H, t, $J = 8.8$ Hz), 7.23 (2H, t, $J = 8.8$ Hz), 3.96 (4H, m), 2.33 (3H, s), 2.12 (2H, m), 1.10 (3H, m); ^{13}C NMR (125 MHz, DMSO- d_6) δ : 172.10, 157.20, 153.16, 147.10, 146.26, 136.34, 132.87, 130.77, 128.83, 122.73, 111.58, 106.42, 100.15, 59.82, 41.62, 30.67, 22.16, 20.44, 13.86.

4.1.6.3.13. Ethyl 4-(8-(4-ethoxyphenylamino)-2-oxooxazol[4,5-*g*]quinazolin-1(2H)-yl)butanoate (44). Purified by silica-gel column chromatography (DCM/MeOH, 50/1), $R_f = 0.30$. White solid, 291 mg, yield, 90%; Mp: 230–233 °C; HPLC purity: 97%; HRMS, ESI $^+$, m/z : Calcd for $\text{C}_{23}\text{H}_{25}\text{N}_4\text{O}_5$ ($\text{M}+\text{H}$) $^+$, 437.1819; found, 437.1829; ^1H NMR (300 MHz, DMSO- d_6) δ : 9.63 (1H, bs), 8.44 (1H, s), 8.28 (1H, s), 7.65 (1H, s), 7.61 (2H, t, $J = 8.3$ Hz), 6.97 (2H, t, $J = 8.3$ Hz), 3.99 (6H, m), 2.48 (2H, t, $J = 6.7$ Hz), 2.11 (2H, t, $J = 6.9$ Hz), 1.35 (3H, t, $J = 6.5$ Hz), 1.10 (3H, t, $J = 6.8$ Hz); ^{13}C NMR (125 MHz, DMSO- d_6) δ : 172.13, 157.39, 155.16, 153.60, 153.29, 147.04, 146.22, 131.69, 130.70, 124.57, 114.17, 111.56, 106.37, 100.36, 59.82, 41.63, 30.68, 22.17, 14.64, 13.87.

4.1.6.3.14. Ethyl 4-(2-oxo-8-(4-(pyridin-2-ylmethoxy)phenylamino)oxazol[4,5-*g*]quinazolin-1(2H)-yl)butanoate (46). Purified by silica-gel column chromatography (DCM/MeOH, 40/1), $R_f = 0.26$. White solid, 325 mg, yield, 88%; Mp: 275–277 °C; HPLC purity: 98%; HRMS, ESI $^+$, m/z : Calcd for $\text{C}_{27}\text{H}_{26}\text{N}_5\text{O}_5$ ($\text{M}+\text{H}$) $^+$, 500.1928; found, 500.1933; ^1H NMR (300 MHz, DMSO- d_6) δ : 9.56 (1H, bs), 8.60 (1H, s), 8.22 (1H, s), 8.06 (1H, m), 7.89 (2H, m), 7.57–7.66 (3H, m), 7.36 (1H, m), 7.10 (2H, m), 5.21 (2H, s), 3.96 (4H, m), 2.45 (2H, t, $J = 6.7$ Hz), 2.09 (2H, m), 1.15 (3H, t, $J = 6.8$ Hz); ^{13}C NMR (125 MHz, DMSO- d_6) δ : 172.20, 160.24, 157.35, 153.46, 153.25, 149.02, 146.78, 145.50, 144.11, 136.87, 130.80, 124.62, 122.85, 121.56, 114.64, 106.97, 106.42, 104.05, 67.14, 59.82, 42.96, 30.70, 21.42, 13.90.

4.1.6.3.15. Ethyl 4-(8-(3,4-difluorophenylamino)-2-oxooxazol[4,5-*g*]quinazolin-1(2H)-yl)butanoate (47). Purified by silica-gel column chromatography (DCM/MeOH, 50/1), $R_f = 0.31$. White solid, 266 mg, yield, 84%; Mp: 255–256 °C; HPLC purity: 97%; HRMS, ESI $^+$, m/z : Calcd for $\text{C}_{21}\text{H}_{19}\text{F}_2\text{N}_4\text{O}_4$ ($\text{M}+\text{H}$) $^+$, 429.1369; found, 429.1377; ^1H NMR (300 MHz, DMSO- d_6) δ : 9.72 (1H, bs), 8.56 (1H, s), 8.18 (1H, s), 8.04 (1H, m), 7.64 (1H, s), 7.48 (1H, m), 3.96 (4H, m), 2.48 (2H, t, $J = 7.2$ Hz), 2.13 (2H, m), 1.10 (3H, t, $J = 7.0$ Hz); ^{13}C NMR (75 MHz, DMSO- d_6) δ : 171.12, 158.40, 153.10, 150.28, 150.08, 148.37 (d, $J = 11.3$ Hz), 147.38, 147.02, 146.85, 145.14, 144.97, 139.01, 134.54 (d, $J = 2.3$ Hz), 134.41 (d, $J = 1.5$ Hz), 132.30, 120.33 (d, $J = 2.3$ Hz), 117.14, 116.90, 113.13, 112.85, 110.56, 102.64, 101.29, 59.74, 41.91, 30.56, 22.24, 21.39, 13.87.

4.1.6.3.16. Ethyl 4-(8-(4-(4-chlorobenzoyloxy)-3-chlorophenylamino)-2-oxooxazol[4,5-*g*]quinazolin-1(2H)-yl)butanoate (48). Purified by silica-gel column chromatography (DCM/MeOH, 50/1), $R_f = 0.31$. White solid, 385 mg, yield, 92%; Mp: 322–325 °C; HPLC purity: 96%; HRMS, ESI $^+$, m/z : Calcd for $\text{C}_{28}\text{H}_{25}\text{Cl}_2\text{N}_4\text{O}_5$ ($\text{M}+\text{H}$) $^+$, 567.1197; found, 567.1198; ^1H NMR (300 MHz, DMSO- d_6) δ : 10.53 (1H, bs), 8.67 (2H, m), 7.98 (1H, m), 7.70 (2H, d, $J = 9.8$ Hz), 7.50 (4H, m), 7.29 (1H, d, $J = 7.8$ Hz), 5.24 (2H, s), 3.97 (4H, m), 2.48 (2H, t, $J = 7.2$ Hz), 2.11 (2H, m), 1.11 (3H, t,

$J = 7.0$ Hz); ^{13}C NMR (125 MHz, DMSO- d_6) δ : 172.15, 157.84, 153.35, 151.49, 150.53, 147.00, 135.58, 132.50, 131.91, 131.71, 129.24, 121.44, 124.98, 123.22, 121.06, 114.22, 110.89, 103.39, 101.46, 69.44, 59.82, 41.83, 30.64, 22.21, 13.90.

4.1.6.3.17. Ethyl 4-(8-(4-hydroxyphenylamino)-2-oxooxazol[4,5-*g*]quinazolin-1(2H)-yl)butanoate (49). Purified by silica-gel column chromatography (DCM/MeOH, 40/1), $R_f = 0.25$. White solid, 272 mg, yield, 90%; Mp: 238–241 °C; HPLC purity: 97%; HRMS, ESI $^+$, m/z : Calcd for $\text{C}_{21}\text{H}_{21}\text{N}_4\text{O}_5$ ($\text{M}+\text{H}$) $^+$, 409.1506; found, 409.1514; ^1H NMR (500 MHz, DMSO- d_6) δ : 9.45 (1H, bs), 9.32 (1H, bs), 8.42 (1H, s), 8.20 (1H, s), 7.59 (1H, s), 7.47 (2H, d, $J = 8.7$ Hz), 6.82 (2H, d, $J = 8.7$ Hz), 3.98 (2H, t, $J = 7.1$ Hz), 3.93 (2H, t, $J = 6.6$ Hz), 2.47 (2H, t, $J = 7.2$ Hz), 2.12 (2H, p, $J = 6.8$ Hz), 1.10 (3H, t, $J = 7.1$ Hz); ^{13}C NMR (125 MHz, DMSO- d_6) δ : 172.18, 157.54, 154.27, 153.42, 146.98, 146.21, 130.66, 130.09, 125.08, 115.51, 111.51, 106.38, 100.21, 59.89, 41.66, 30.72, 22.18, 13.92.

4.1.6.3.18. Ethyl 4-(8-(3-hydroxyphenylamino)-2-oxooxazol[4,5-*g*]quinazolin-1(2H)-yl)butanoate (50). Purified by silica-gel column chromatography (DCM/MeOH, 40/1), $R_f = 0.25$. White solid, 266 mg, yield, 88%; Mp: 237–239 °C; HPLC purity: 98%; HRMS, ESI $^+$, m/z : Calcd for $\text{C}_{21}\text{H}_{21}\text{N}_4\text{O}_5$ ($\text{M}+\text{H}$) $^+$, 409.1506; found, 409.1507; ^1H NMR (500 MHz, DMSO- d_6) δ : 9.46 (1H, bs), 9.42 (1H, bs), 8.52 (1H, s), 8.25 (1H, s), 7.64 (1H, s), 7.34 (1H, s), 7.18 (2H, m), 6.56 (1H, m), 3.96 (4H, m), 2.47 (2H, t, $J = 7.2$ Hz), 2.11 (2H, p, $J = 7.0$ Hz), 1.10 (3H, t, $J = 7.1$ Hz); ^{13}C NMR (75 MHz, DMSO- d_6) δ : 172.18, 157.45, 157.16, 153.65, 153.14, 147.21, 146.40, 140.00, 130.98, 129.07, 113.26, 111.72, 100.97, 106.52, 100.25, 59.87, 41.68, 30.70, 22.19, 13.90.

4.1.6.3.19. Ethyl 4-(8-(4-isopropoxyphenylamino)-2-oxooxazol[4,5-*g*]quinazolin-1(2H)-yl)butanoate (51). Purified by silica-gel column chromatography (DCM/MeOH, 50/1), $R_f = 0.28$. White solid, 303 mg, yield, 91%; Mp: 233–235 °C; HPLC purity: 98%; HRMS, ESI $^+$, m/z : Calcd for $\text{C}_{24}\text{H}_{27}\text{N}_4\text{O}_5$ ($\text{M}+\text{H}$) $^+$, 451.1976; found, 451.1979; ^1H NMR (300 MHz, DMSO- d_6) δ : 9.60 (1H, bs), 8.46 (1H, s), 8.25 (1H, s), 7.63 (1H, s), 7.59 (2H, d, $J = 8.8$ Hz), 6.98 (2H, d, $J = 8.8$ Hz), 4.61 (1H, m), 3.96 (4H, m), 2.42 (2H, t, $J = 7.5$ Hz), 2.12 (2H, m), 1.29 (6H, d, $J = 6.0$ Hz), 1.10 (3H, t, $J = 6.6$ Hz); ^{13}C NMR (125 MHz, DMSO- d_6) δ : 172.10, 157.39, 154.14, 153.51, 153.14, 146.64, 146.28, 131.46, 130.78, 124.65, 115.55, 111.46, 106.16, 100.27, 69.37, 59.81, 41.64, 30.67, 22.14, 21.80, 13.86.

4.1.6.3.20. Ethyl 4-(8-(4-tert-butylphenylamino)-2-oxooxazol[4,5-*g*]quinazolin-1(2H)-yl)butanoate (52). Purified by silica-gel column chromatography (DCM/MeOH, 70/1), $R_f = 0.26$. White solid, 282 mg, yield, 85%; Mp: 236–239 °C; HPLC purity: 98%; HRMS, ESI $^+$, m/z : Calcd for $\text{C}_{25}\text{H}_{29}\text{N}_4\text{O}_4$ ($\text{M}+\text{H}$) $^+$, 449.2183; found, 449.2188; ^1H NMR (500 MHz, DMSO- d_6) δ : 9.57 (1H, bs), 8.49 (1H, s), 8.24 (1H, s), 7.68 (2H, t, $J = 8.7$ Hz), 7.64 (1H, s), 7.43 (2H, t, $J = 8.7$ Hz), 3.96 (4H, m), 2.47 (2H, t, $J = 7.2$ Hz), 2.12 (2H, m), 1.32 (9H, s), 1.10 (3H, t, $J = 7.1$ Hz); ^{13}C NMR (75 MHz, DMSO- d_6) δ : 172.17, 157.30, 153.66, 153.23, 147.18, 146.38, 136.31, 130.92, 125.51, 125.11, 122.50, 111.64, 106.51, 100.21, 59.86, 41.68, 34.09, 31.70, 22.18, 13.90.

4.1.6.3.21. 4-Cyanobenzyl 4-(1-(4-ethoxy-4-oxobutyl)-2-oxo-1,2-dihydrooxazol[4,5-*g*]quinazolin-8-ylamino)benzoate (53). Purified by silica-gel column chromatography (DCM/MeOH, 50/1), $R_f = 0.24$. White solid, 330 mg, yield, 81%; Mp: 340–342 °C; HPLC purity: 97%; HRMS, ESI $^+$, m/z : Calcd for $\text{C}_{30}\text{H}_{26}\text{N}_5\text{O}_6$ ($\text{M}+\text{H}$) $^+$, 552.1878; found, 552.1884; ^1H NMR (500 MHz, DMSO- d_6) δ : 9.85 (1H, bs), 8.64 (1H, s), 8.27 (1H, s), 8.08 (4H, s), 7.89 (2H, d, $J = 8.2$ Hz), 7.70 (1H, s), 7.69 (2H, d, $J = 8.2$ Hz), 5.45 (2H, s), 4.00–3.95 (4H, m), 2.48 (2H, t, $J = 7.2$ Hz), 2.14 (2H, p, $J = 7.0$ Hz), 1.10 (3H, t, $J = 7.2$ Hz); ^{13}C NMR (75 MHz, DMSO- d_6) δ : 172.17, 165.06, 156.71, 153.58, 152.74, 147.48, 146.65, 144.18, 142.01, 132.43, 131.27, 130.10, 128.23, 123.35, 120.98, 118.64, 114.22, 111.93, 110.65, 106.62, 100.12, 64.97, 59.87, 41.69, 30.67, 22.20, 13.90.

4.1.6.3.22. 4-Chlorobenzyl 4-(1-(4-ethoxy-4-oxobutyl)-2-oxo-1,2-dihydrooxazolo[4,5-g]quinazolin-8-ylamino)benzoate (54).

Purified by silica-gel column chromatography (DCM/MeOH, 50/1), $R_f = 0.25$. White solid, 323 mg, yield, 78%; Mp: 334–337 °C; HPLC purity: 98%; HRMS, ESI^+ , m/z : Calcd for $C_{29}H_{26}ClN_4O_6$ ($M+H$) $^+$, 561.1535; found, 561.1546; 1H NMR (500 MHz, DMSO- d_6) δ : 9.88 (1H, bs), 8.64 (1H, s), 8.29 (1H, s), 8.06 (4H, m), 7.71 (1H, s), 7.52 (2H, d, $J = 8.6$ Hz), 7.48 (2H, d, $J = 8.6$ Hz), 5.35 (2H, s), 3.97 (4H, m), 2.48 (2H, t, $J = 7.2$ Hz), 2.13 (2H, p, $J = 6.9$ Hz), 1.10 (3H, t, $J = 7.1$ Hz); ^{13}C NMR (75 MHz, DMSO- d_6) δ : 172.17, 165.78, 153.58, 152.70, 147.26, 146.69, 144.04, 135.32, 132.66, 131.32, 130.03, 129.81, 128.48, 123.64, 121.05, 111.91, 111.91, 106.49, 100.22, 65.09, 59.86, 41.69, 30.66, 22.20, 13.90.

4.1.6.3.23. Ethyl 4-(8-(3,5-dimethylphenylamino)-2-oxooxazolo[4,5-g]quinazolin-1(2H)-yl)butanoate (55). Purified by silica-gel column chromatography (DCM/MeOH, 50/1), $R_f = 0.30$. White solid, 261 mg, yield, 84%; Mp: 224–226 °C; HPLC purity: 97%; HRMS, ESI^+ , m/z : Calcd for $C_{23}H_{25}N_4O_4$ ($M+H$) $^+$, 421.1870; found, 421.1877; 1H NMR (500 MHz, DMSO- d_6) δ : 9.45 (1H, bs), 8.52 (1H, s), 8.25 (1H, s), 7.64 (1H, s), 7.44 (2H, s), 6.80 (1H, s), 3.96 (4H, m), 2.47 (2H, t, $J = 7.2$ Hz), 2.32 (6H, s), 2.12 (2H, m), 1.32 (9H, s), 1.10 (3H, t, $J = 7.2$ Hz); ^{13}C NMR (75 MHz, DMSO- d_6) δ : 172.16, 157.16, 153.62, 153.20, 147.15, 146.35, 138.82, 137.44, 130.92, 125.36, 120.21, 111.65, 106.51, 100.16, 59.84, 41.66, 30.69, 22.17, 21.02, 13.89.

4.1.6.3.24. Ethyl 4-(8-(benzylamino)-2-oxooxazolo[4,5-g]quinazolin-1(2H)-yl)butanoate (32). Phenylmethanamine (95.4 mg, 0.89 mmol) and TEA (150 mg, 1.48 mmol) was added to a solution of compound **12** (250 mg, 0.74 mmol) in isopropanol (10 mL) and stirred at 50 °C for 24 h. The mixture was cooled to room temperature and extracted in vacuo, the residue was treated with aqueous $NaHCO_3$ (10 mL) and extracted with EtOAc/MeOH (20:1, 30 mL). The organic layer was washed with brine, dried over anhydrous Na_2SO_4 , and concentrated. Purified by silica-gel column chromatography (DCM/MeOH, 90/1), $R_f = 0.23$. Drying gave 210 mg (yield, 70%) of the title compound as pale yellow solid: Mp: 220–223 °C; HPLC purity: 96%; HRMS, ESI^+ , m/z : Calcd for $C_{22}H_{23}N_4O_4$ ($M+H$) $^+$, 407.1714; found, 407.1721; 1H NMR (500 MHz, DMSO- d_6) δ : 8.65 (1H, t, $J = 5.7$ Hz), 8.41 (1H, s), 8.07 (1H, s), 7.55 (1H, s), 7.38 (2H, d, $J = 7.2$ Hz), 7.33 (2H, t, $J = 7.7$ Hz), 7.25 (2H, t, $J = 7.2$ Hz), 4.83 (2H, d, $J = 5.7$ Hz), 3.94 (2H, q, $J = 7.1$ Hz), 3.89 (2H, t, $J = 7.2$ Hz), 2.44 (2H, t, $J = 7.2$ Hz), 2.07 (2H, p, $J = 6.9$ Hz), 1.08 (3H, t, $J = 7.1$ Hz); ^{13}C NMR (125 MHz, DMSO- d_6) δ : 172.08, 158.97, 153.59, 146.14, 145.84, 139.27, 130.47, 128.25, 127.20, 126.77, 111.24, 105.84, 100.45, 59.79, 43.58, 41.62, 30.68, 22.11, 13.86.

4.1.6.3.25. (S)-ethyl 4-(2-oxo-8-(1-phenylethylamino)oxazolo[4,5-g]quinazolin-1(2H)-yl)butanoate (38). Purified by silica-gel column chromatography (DCM/MeOH, 50/1), $R_f = 0.33$. Pale yellow solid, 202 mg, yield, 65%; Mp: 255–257 °C; HPLC purity: 97%; HRMS, ESI^+ , m/z : Calcd for $C_{23}H_{25}N_4O_4$ ($M+H$) $^+$, 421.1870; found, 421.1869; 1H NMR (500 MHz, DMSO- d_6) δ : 8.36 (1H, s), 8.24 (1H, d, $J = 7.2$ Hz), 8.19 (1H, s), 7.54 (1H, s), 7.44 (2H, d, $J = 7.6$ Hz), 7.33 (2H, t, $J = 7.8$ Hz), 7.22 (2H, d, $J = 7.4$ Hz), 5.65 (1H, p, $J = 7.2$ Hz), 4.79 (1H, p, $J = 5.7$ Hz), 3.95 (3H, m), 2.44 (2H, t, $J = 7.2$ Hz), 2.12 (2H, m), 1.63 (3H, d, $J = 7.0$ Hz), 1.10 (3H, t, $J = 7.1$ Hz); ^{13}C NMR (125 MHz, DMSO- d_6) δ : 171.56, 158.10, 153.61, 153.54, 146.52, 146.03, 144.47, 130.38, 128.14, 126.51, 125.94, 111.23, 106.19, 100.25, 67.10, 59.76, 49.04, 41.59, 30.92, 22.14, 21.35.

4.1.6.3.26. (R)-ethyl 4-(2-oxo-8-(1-phenylethylamino)oxazolo[4,5-g]quinazolin-1(2H)-yl)butanoate (39). Purified by silica-gel column chromatography (DCM/MeOH, 50/1), $R_f = 0.33$. Pale yellow solid, 208 mg, yield, 67%; Mp: 252–255 °C; HPLC purity: 97%; HRMS, ESI^+ , m/z : Calcd for $C_{23}H_{25}N_4O_4$ ($M+H$) $^+$, 421.1870; found, 421.1869; 1H NMR (500 MHz, DMSO- d_6) δ : 8.36 (1H, s), 8.23 (1H, d, $J = 7.2$ Hz), 8.19 (1H, s), 7.53 (1H, s), 7.45 (2H, d, $J = 7.6$ Hz), 7.33 (2H, t, $J = 7.8$ Hz), 7.23 (2H, d, $J = 7.4$ Hz), 5.66 (1H, p, $J = 7.2$ Hz), 4.79 (1H,

p, $J = 5.7$ Hz), 3.95 (3H, m), 2.44 (2H, t, $J = 7.2$ Hz), 2.11 (2H, m), 1.63 (3H, d, $J = 7.0$ Hz), 1.10 (3H, m); ^{13}C NMR (125 MHz, DMSO- d_6) δ : 171.56, 158.10, 153.61, 153.54, 146.53, 146.02, 144.47, 130.36, 128.13, 126.51, 125.94, 111.23, 106.19, 100.25, 67.09, 59.76, 49.04, 41.59, 30.92, 22.13, 21.34.

4.1.6.3.27. Ethyl 4-(8-(1H-indazol-5-ylamino)-2-oxooxazolo[4,5-g]quinazolin-1(2H)-yl)butanoate (45). 1H-Indazol-5-amine (118 mg, 0.89 mmol) was added to a solution of compound **15** (250 mg, 0.74 mmol) in isopropanol (10 mL) and stirred at reflux for 13 h. The mixture was cooled to room temperature and filtered, the solid was washed with chill isopropanol (5 mL), the residue was treated with aqueous $NaHCO_3$ (10 mL) and extracted with EtOAc/MeOH (20:1, 30 mL). The organic layer was washed with brine, dried over Na_2SO_4 , and concentrated. Purified by silica-gel column chromatography (DCM/MeOH, 70/1), $R_f = 0.21$. Drying gave 238 mg (yield, 75%) of the title compound as white solid: Mp: 287–289 °C; HPLC purity: 96%; HRMS, ESI^+ , m/z : Calcd for $C_{18}H_{15}F_2N_4O_3$ ($M+H$) $^+$, 373.1107; found, 373.1111; 1H NMR (300 MHz, DMSO- d_6) δ : 13.10 (1H, s), 9.76 (1H, bs), 8.47 (1H, s), 8.33 (1H, s), 8.15 (1H, s), 8.10 (1H, s), 8.06 (1H, d, $J = 6.7$ Hz), 7.91 (1H, s), 7.64 (1H, s), 7.60 (1H, s), 7.58 (1H, d, $J = 6.7$ Hz), 3.95 (4H, m), 2.48 (2H, t, $J = 7.2$ Hz), 2.13 (2H, p, $J = 6.9$ Hz), 1.10 (3H, t, $J = 7.1$ Hz); ^{13}C NMR (125 MHz, DMSO- d_6) δ : 171.63, 157.66, 153.61, 153.32, 147.04, 146.28, 144.11, 131.60, 130.68, 123.90, 114.13, 111.57, 109.87, 106.98, 106.24, 104.05, 100.18, 67.16, 41.68, 30.29, 22.41, 22.13.

4.2. Biological evaluation

4.2.1. EGFR tyrosine kinase enzyme inhibition assay

EGFR tyrosine kinase activity was determined by an enzyme-linked-immuno-sorbent assay (ELISA) in 96-well plates pre-coated in a 10 μ L reaction volume including 4 μ L diluted test compounds, 4 μ L substrate and 2 μ L ATP. After incubation for 1 h at 37 °C, the reactions were stopped with 10 μ L 2% (v/v) H_3PO_4 . The plates were then aspirated and washed twice with 200 μ L 0.9% (w/v) NaCl, and incorporation of ^{33}P i was determined with a micro-plate scintillation counter. The residual kinase activities for each concentration of compound and the compound IC_{50} values were calculated using GraphPad Prism 5.0. Comparisons between all groups were made using a two-tailed Student's *t* test (SPSS version 11.5 software). Data represented as means \pm SD from three independent experiments, and differences between groups were considered statistically significant at $P < 0.05$.

4.2.2. Cancer cell proliferation inhibition assay

Human epidermoid cancer cell line (KB) and renal cell carcinoma cell line (A498) were provided by Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. KB cell was cultured in DMEM 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_2 at 37 °C. Cells were seeded at respective density ($2-4 \times 10^4$ /mL) in 96-well plates in a volume of 180 μ L 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 μ L of the test compound solution was added in duplicates, and incubation continued for 48 h in a humidified atmosphere of 5% CO_2 at 37 °C. Remove the medium, and cells were fixed with 10% trichloroacetic acid (50 μ L) for 1 h at 37 °C, and then washed 5 times with tap water and air-dried. Cells that survived were stained with 0.4% (w/v) SRB dye solution (100 μ L) for 20 min at room temperature. Then, remove the SRB dye solution, and the plate was washed with tap water and dried. 150 μ L of Trizma base (10 mM) was added to each well, and the absorbance was measured at 570 nm using a

microplate reader. The compound IC₅₀ values were calculated using GraphPad Prism 5.0. Comparisons between all groups were made using a two-tailed Student's *t* test (SPSS version 11.5 software). Data represented as means \pm SD from three independent experiments, and differences between groups were considered statistically significant at *P* < 0.05. The cell proliferation inhibition assay of A498 cell was the same as KB cell except cultured in RPMI 1640 medium.

4.2.3. *In vivo* antitumor activity assay

Six week old SCID mice were weighed and randomly divided into three groups 1) Control; 2) Erlotinib; 3) Compound **50** (*n* = 6 mice/group). LLC xenografts were initiated by subcutaneous implantation of 2×10^6 cells in the right flank. Upon reaching an average tumor volume of 100 mm³ (7 days post implantation), Each group was dosed orally for 14 days with either vehicle only or with Erlotinib at 100 mg/kg or compound **50** at 100 mg/kg 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 (mm³) = $W^2(L/2)$, 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.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (81273468, 81473153), National Basic Research Program of China (2015CB755500), Natural Science Foundation of Jiangsu (BK20140672), and 111 Project from the Ministry of Education of China and the State Administration of Foreign Expert Affairs of China (No. 111-2-07). We greatly appreciated Professor Tao Lu and Ms. Yanmin Zhang at China Pharmaceutical University for docking study.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.ejmech.2015.07.008>.

References

- [1] A. Citri, Y. Yarden, EGF–ERBB signalling: towards the systems level, *Nat. Rev. Mol. Cell. Biol.* 7 (2006) 505–516.
- [2] M.L. Barbosa, L.M. Lima, R. Tesch, C.R. Sant'Anna, F. Totzke, M.G. Kubbutat, C. Schächtele, S.A. Laufer, E.J. Barreiro, Novel 2-chloro-4-anilino-quinazoline derivatives as EGFR and VEGFR-2 dual inhibitors, *Eur. J. Med. Chem.* 71 (2014) 1–14.
- [3] P.M. Ellis, W. Morzycki, B. Melosky, C. Butts, V. Hirsh, F. Krasnoshtein, N. Murray, F.A. Shepherd, D. Soulieres, M.S. Tsao, G. Goss, The role of the epidermal growth factor receptor tyrosine kinase inhibitors as therapy for advanced, metastatic, and recurrent non-small-cell lung cancer: a Canadian national consensus statement, *Curr. Oncol.* 16 (2009) 27–48.
- [4] H. Aktas, H. Cai, G.M. Cooper, Ras links growth factor signaling to the cell cycle machinery via regulation of cyclin D1 and the Cdk inhibitor p27KIP1, *Mol. Cell. Biol.* 7 (1997) 3850–3857.
- [5] S. Bugge, S.J. Kaspersen, S. Larsen, U. Nonstad, G. Bjørkøy, E. Sundby, B.H. Hoff, Structure-activity study leading to identification of a highly active thienopyrimidine based EGFR inhibitor, *Eur. J. Med. Chem.* 75 (2014) 354–374.
- [6] T.E. Balus, R.C. Rizzo, Quantitative prediction of fold resistance for inhibitors of EGFR, *Biochemistry* 48 (2009) 8435–8448.
- [7] T. Holbro, N.E. Hynes, ErbB receptors: directing key signaling networks throughout life, *Annu. Rev. Pharmacol. Toxicol.* 44 (2004) 195–217.
- [8] C. Garcia-Echeverria, D. Fabbro, Therapeutically targeted anticancer agents: inhibitors of receptor tyrosine kinases, *Mini Rev. Med. Chem.* 4 (2004) 273–283.
- [9] L. Zhang, C.W. Fan, Z.R. Guo, Y. Li, S.Y. Zhao, S.B. Yang, Y.Y. Yang, J.R. Zhu, D. Lin, Discovery of a potent dual EGFR/HER-2 inhibitor L-2 (selatinib) for the treatment of cancer, *Eur. J. Med. Chem.* 69 (2013) 833–841.
- [10] J.R. Woodburn, The epidermal growth factor receptor and its inhibition in cancer therapy, *Pharmacol. Ther.* 82 (1999) 241–250.
- [11] M. Ranson, L.A. Hammond, D. Ferry, M. Kris, A. Tullo, P.I. Murray, V. Miller, S. Averbuch, J. Ochs, C. Morris, A. Feyereislova, H. Swaisland, E.K. Rowinsky, ZD1839, a selective oral epidermal growth factor receptor–tyrosine kinase inhibitor, is well tolerated and active in patients with solid, malignant tumors: results of a phase I trial, *J. Clin. Oncol.* 20 (2002) 2240–2250.
- [12] Q. Luo, Y.H. Gu, W. Zheng, X.X. Wu, F.Y. Gong, L.Y. Gu, Y. Sun, Q. Xu, Erlotinib inhibits T-cell-mediated immune response via down-regulation of the c-Raf/ERK cascade and Akt signaling pathway, *Toxicol. Appl. Pharmacol.* 251 (2011) 130–136.
- [13] D. Collins, A.K. Hill, L. Young, Lapatinib: a competitor or companion to trastuzumab? *Cancer Treat. Rev.* 35 (2009) 574–581.
- [14] J.S. Lin, W. Shen, J.W. Xue, J. Sun, X. Zhang, C. Zhang, Novel oxazolo[4,5-*g*]quinazolin-2(1H)-ones: dual inhibitors of EGFR and Src protein tyrosine kinases, *Eur. J. Med. Chem.* 55 (2012) 39–48.
- [15] J.J. Liao, Molecular recognition of protein kinase binding pockets for design of potent and selective kinase inhibitors, *J. Med. Chem.* 50 (2007) 409–424.
- [16] J.A. Bikker, N. Brooijmans, A. Wissner, T.S. Mansour, Kinase domain mutations in cancer: implications for small molecule drug design strategies, *J. Med. Chem.* 52 (2009) 1493–1509.
- [17] S. Stiirker, T. Reichert, K.A. Reiffen, W. Wagner, EGFR and PCNA expression in oral squamous cell carcinomas—a valuable tool in estimating the patient's prognosis, *Oral Oncol. Eur. J. Cancer* 298 (1993) 273–277.
- [18] S. Mowafy, N.A. Farag, K.A. Abouzid, Design, synthesis and *in vitro* anti-proliferative activity of 4,6-quinazolinodiamines as potent EGFR-TK inhibitors, *Eur. J. Med. Chem.* 61 (2013) 132–145.
- [19] T. Ishikawa, M. Seto, H. Banno, Y. Kawakita, M. Oorui, T. Taniguchi, Y. Ohta, T. Tamura, A. Nakayama, H. Miki, H. Kamiguchi, T. Tanaka, N. Habuka, S. Sogabe, J. Yano, K. Aertgeerts, K. Kamiyama, Design and synthesis of novel human epidermal growth factor receptor 2 (HER2)/Epidermal growth factor receptor (EGFR) dual inhibitors bearing a pyrrolo[3,2-*d*]pyrimidine scaffold, *J. Med. Chem.* 54 (2011) 8030–8050.