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Research paper

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

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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.



## 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

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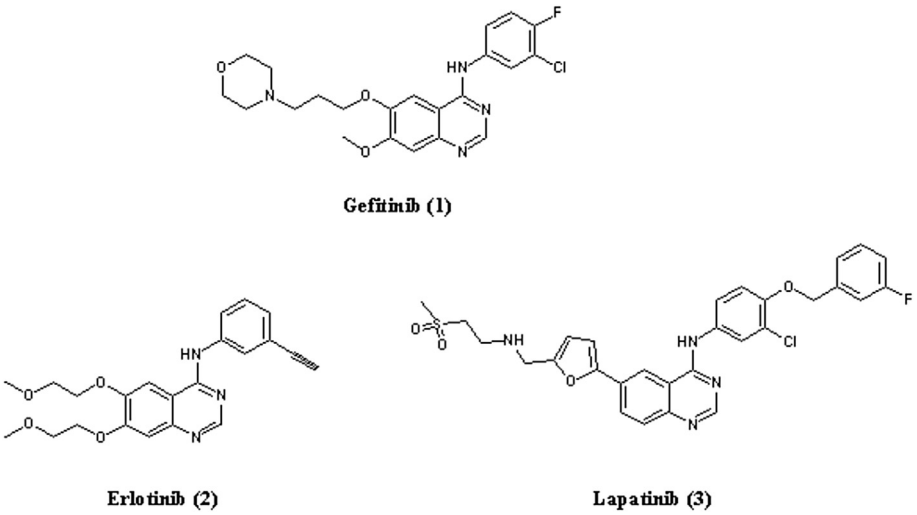



**Fig. 1.** The structures of Gefitinib, Erlotinib and Lapatinib.

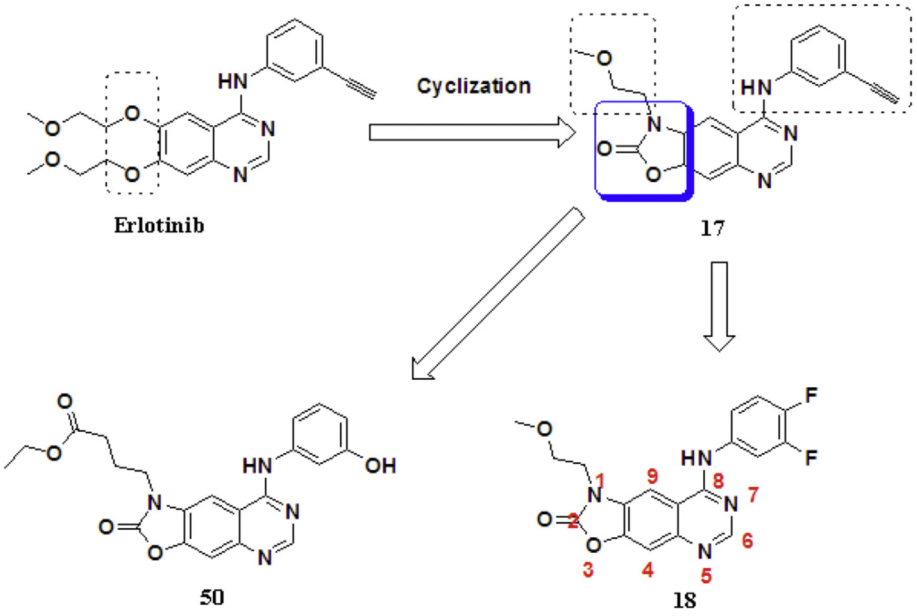



**Fig. 2.** The design inhibitors of EGFR.

of compound **7** with concentrated $HNO_3$, and subsequent purification via column chromatography, yielded compound **8** which was then reduced with iron power 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.

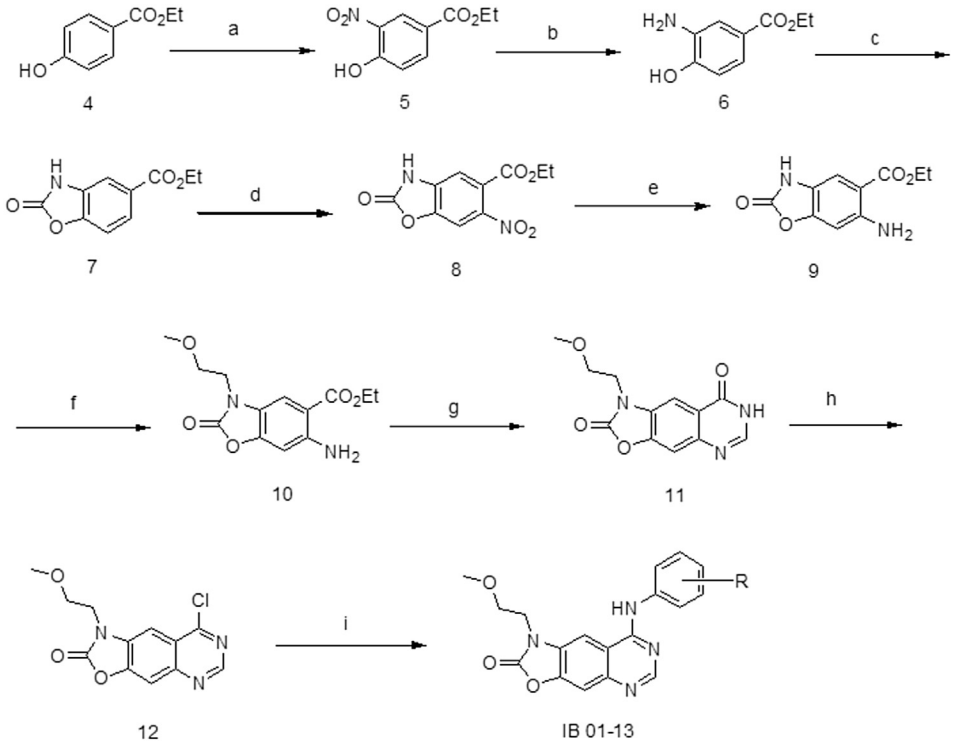
### 2.2. Biological activities

#### 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 conjuncted 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, alkoxyl 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) $SnCl_2 \cdot 2H_2O$, 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 μM (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 μM and 26.8 μM, respectively, and exhibited high inhibition of EGFR with $IC_{50}$ of 0.13 μM. 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

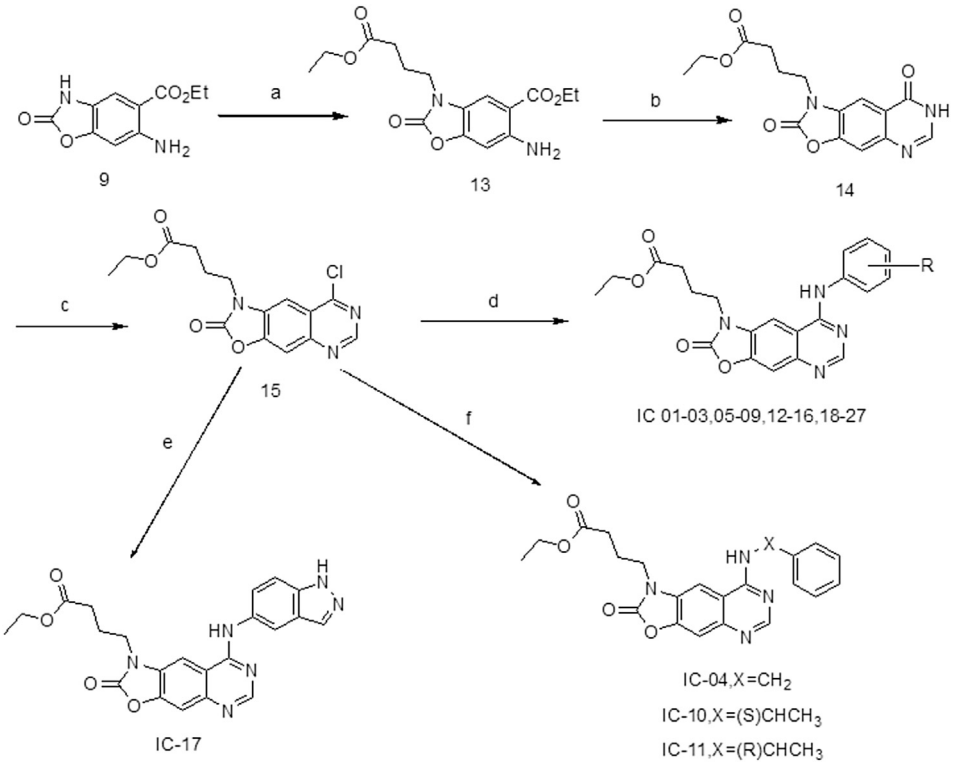
As shown in Fig. 4, $N_1$, $N_3$ atoms in pyrimidine ring are crucial for compound binding. Introducing moderate hydrophilic side-chain, such as 2-methoxyethoxyl and 4-ethyloxy-4-oxobutyl group can achieve medium to good inhibition activity. The proton in Fragment B is essential, and if phenyl amines 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′-ethynyllbenzyl 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 μM. 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-ethyloxy-4-oxobutyl group in position 1 is an excellent strategy for the development of EGFR inhibitors.

## 4. Experimental sections

### 4.1. Chemistry experiment

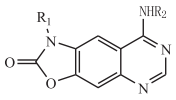
#### 4.1.1. Chemistry: general procedures

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).$^1$H 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 d (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 1**
The inhibition and $IC_{50}$ values of the designed compounds toward EGFR kinase.



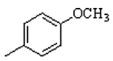
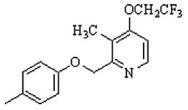
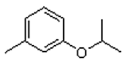
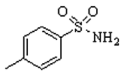
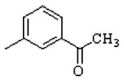
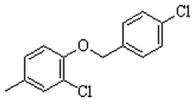
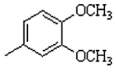
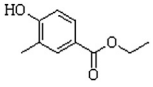
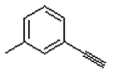
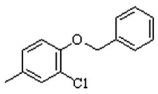
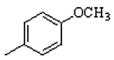
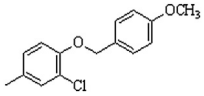
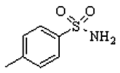
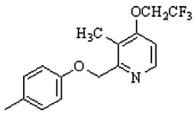
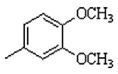
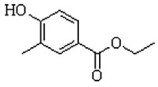
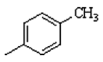
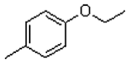
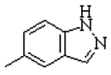
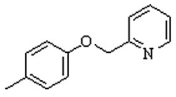
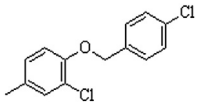
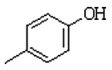
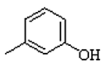
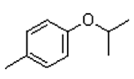
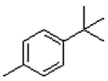
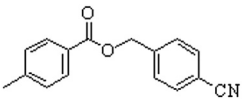
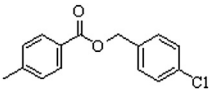
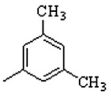
| Comp. | $R_1$ | $R_2$ | Inhibition[a]/% | Enzymatic. $IC_{50}$[b](μM) |
|---|---|---|---|---|
| **16** | $-CH_2CH_2OCH_3$ |  | 88 | 0.47 ± 0.18 |
| **17** | $-CH_2CH_2OCH_3$ |  | 92 | 0.19 ± 0.03 |
| **18** | $-CH_2CH_2OCH_3$ |  | 97 | 0.026 ± 0.017* |
| **19** | $-CH_2CH_2OCH_3$ |  | 91 | 5.1 ± 1.3 |
| **20** | $-CH_2CH_2OCH_3$ |  | 99 | >10 |
| **21** | $-CH_2CH_2OCH_3$ |  | 78 | >10 |
| **22** | $-CH_2CH_2OCH_3$ |  | 40 | 0.34 ± 0.08 |
| **23** | $-CH_2CH_2OCH_3$ |  | 81 | >10 |
| **24** | $-CH_2CH_2OCH_3$ |  | 78 | >10 |
| **25** | $-CH_2CH_2OCH_3$ |  | 92 | 0.56 ± 0.08 |
| **26** | $-CH_2CH_2OCH_3$ |  | 98 | 1.3 ± 0.1 |
| **27** | $-CH_2CH_2OCH_3$ |  | 94 | 5.5 ± 0.5 |
| **28** | $-CH_2CH_2OCH_3$ |  | 98 | 0.44 ± 0.09 |
| **29** | $-CH_2(CH_2)_2COOC_2H_5$ |  | 93 | 7.8 ± 1.4 |
| **30** | $-CH_2(CH_2)_2COOC_2H_5$ |  | 96 | 5.7 ± 1.3 |
| **31** | $-CH_2(CH_2)_2COOC_2H_5$ |  | 92 | >10 |
| **32** | $-CH_2(CH_2)_2COOC_2H_5$ |  | 93 | >10 |
| **33** | $-CH_2(CH_2)_2COOC_2H_5$ |  | 96 | 1.2 ± 0.5 |

**Table 1** (*continued*)

| Comp. | $R_1$ | $R_2$ | Inhibition[a]/% | Enzymatic. $IC_{50}$[b](μM) |
|---|---|---|---|---|
| **34** | $-CH_2(CH_2)_2COOC_2H_5$ |  | 92 | >10 |
| **35** | $-CH_2(CH_2)_2COOC_2H_5$ |  | 93 | 7.5 ± 2.1 |
| **36** | $-CH_2(CH_2)_2COOC_2H_5$ |  | 97 | >10 |
| **37** | $-CH_2(CH_2)_2COOC_2H_5$ |  | 92 | >10 |
| **38** | $-CH_2(CH_2)_2COOC_2H_5$ |  | 88 | >10 |
| **39** | $-CH_2(CH_2)_2COOC_2H_5$ |  | 90 | >10 |
| **40** | $-CH_2(CH_2)_2COOC_2H_5$ |  | 97 | 0.78 ± 0.23 |
| **41** | $-CH_2(CH_2)_2COOC_2H_5$ |  | 96 | >10 |
| **42** | $-CH_2(CH_2)_2COOC_2H_5$ |  | 99 | 0.0073 ± 0.0021* |
| **43** | $-CH_2(CH_2)_2COOC_2H_5$ |  | 99 | >10 |
| **44** | $-CH_2(CH_2)_2COOC_2H_5$ |  | 61 | >10 |
| **45** | $-CH_2(CH_2)_2COOC_2H_5$ |  | 99 | 0.13 ± 0.02 |
| **46** | $-CH_2(CH_2)_2COOC_2H_5$ |  | 86 | 2.3 ± 0.13 |
| **47** | $-CH_2(CH_2)_2COOC_2H_5$ |  | 99 | 0.14 ± 0.01 |
| **48** | $-CH_2(CH_2)_2COOC_2H_5$ |  | 78 | 0.053 ± 0.019 |
| **49** | $-CH_2(CH_2)_2COOC_2H_5$ |  | 99 | 0.020 ± 0.014* |
| **50** | $-CH_2(CH_2)_2COOC_2H_5$ |  | 100 | 0.0087 ± 0.0179* |
| **51** | $-CH_2(CH_2)_2COOC_2H_5$ |  | 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** | $-CH_2(CH_2)_2COOC_2H_5$ |  | 82 | >10 |
| **53** | $-CH_2(CH_2)_2COOC_2H_5$ |  | 82 | >10 |
| **54** | $-CH_2(CH_2)_2COOC_2H_5$ |  | 85 | 0.66 ± 0.05 |
| **55** | $-CH_2(CH_2)_2COOC_2H_5$ |  | 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 $Na_2SO_4$ 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 $NaHCO_3$ until a pH of 9–10 was obtained. The resulting precipitate was filtered and washed with water, over anhydrous $Na_2SO_4$ 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

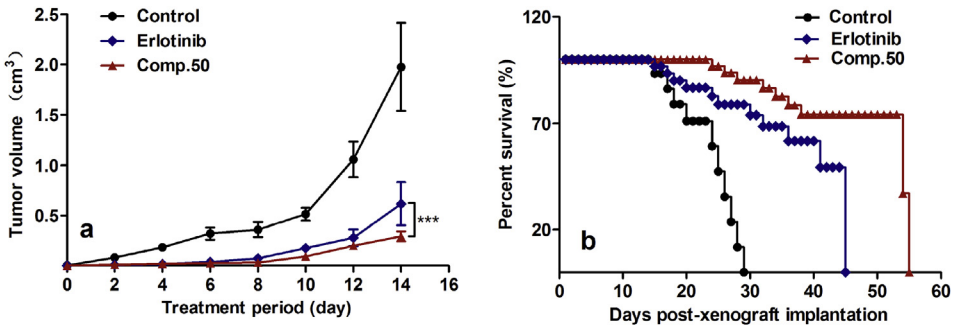



**Fig. 3.** (a) *In vivo* antitumor effect of compound **50** in a LLC xenograft mouse model. Eighteen mice were weighed and randomly divided into three groups (n = 6): 1) Control; 2) Erlotinib; 3) Compound **50**. Mice bearing established LLC tumor xenografts were dosed with compound **50** (100 mg/kg.qd.po) 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) Survial curves of LLC tumor bearing mice received different treatments.

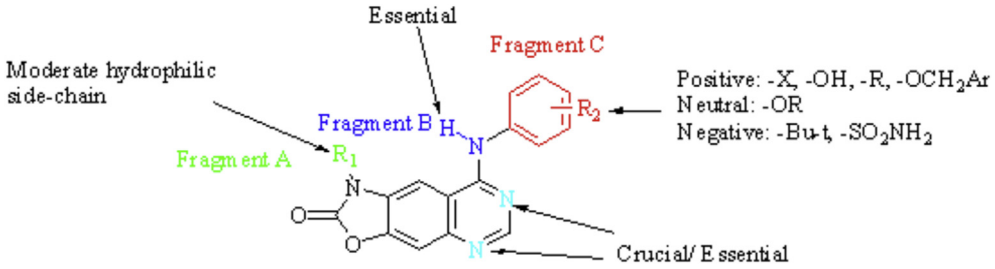



**Fig. 4.** Structure–activity relationships identified in this study.

organic solution was washed with saturated aqueous NaCl solution, dried over anhydrous $Na_2SO_4$, 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. $HNO_3$ 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, $^1$H 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 $CH_3OH$ 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 $CH_3OH$ (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, $^1$H NMR (300 MHz, $CDCl_3$) 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.

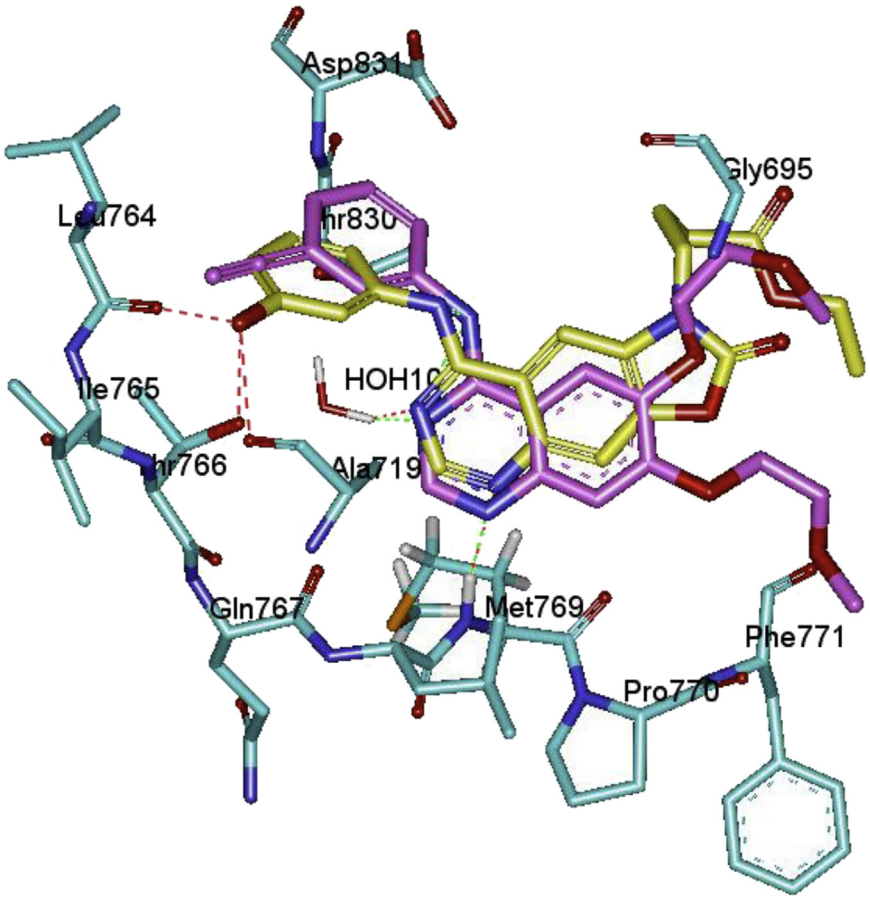



**Fig. 5.** Binding model of compound **50** (yellow) in complex with EGFR (PDB ID: 1M17) and Erlotinib (purple). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

*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 follow 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; $^1$H 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]quinazoline-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 power (17.9 g, yield, 80%); Mp: 218–219 °C; $^1$H NMR (500 MHz, 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 give compound **12** (17.6 g, yield, 92%); Mp: 150–152 °C; $^1$H 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 $C_{18}H_{15}ClFN_4O_3$ $(M+H)^+$, 389.0811; found, 389.0809; $^1$H NMR (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, 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 $C_{20}H_{17}N_4O_3$ $(M+H)^+$, 361.1295; found, 361.1298; $^1$H NMR (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, 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 $C_{18}H_{15}F_2N_4O_3$ $(M+H)^+$, 373.1107; found, 373.1111; $^1$H NMR (500 MHz, 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, 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 $C_{19}H_{19}N_4O_4$ $(M+H)^+$, 367.1401; found, 367.1398; $^1$H NMR (500 MHz, 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, 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 $C_{22}H_{25}N_4O_3$ $(M+H)^+$, 393.1921; found, 393.1925; $^1$H NMR (125 MHz, 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, 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 $C_{18}H_{16}$ Cl $N_4O_3$ $(M+H)^+$, 371.0905; found, 371.0909; $^1$H NMR (500 MHz, 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, 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 $C_{27}H_{25}$ $F_3N_5O_5$ $(M+H)^+$, 556.1802; found, 556.1797; $^1$H NMR (500 MHz, 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 $C_{21}H_{23}N_4O_4$ $(M+H)^+$, 395.1714; found, 395.1715; $^1$H 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 $C_{18}H_{18}N_5O_5S$ $(M+H)^+$, 416.1023; found, 416.1028; $^1$H 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 $C_{20}H_{19}N_4O_4$ $(M+H)^+$, 379.1041; found, 379.1399; $^1$H 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-Chlorobenzyloxy)-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 $C_{25}H_{21}$ $Cl_2N_4O_4$ $(M+H)^+$, 511.0934; found, 511.0932; $^1$H 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 $C_{20}H_{21}N_4O_5$ $(M+H)^+$, 397.1508; found, 397.1511; $^1$H 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 $C_{21}H_{21}N_4O_6$ $(M+H)^+$, 425.1456; found, 425.1461; $^1$H 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 × 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; $^1$H 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. Ethyl4-(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; $^1$H 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-oxooxazolo[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; $^1$H 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-oxooxazolo[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); $^{13}C$ 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); $^{13}C$ 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), Rf = 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); $^{13}C$ 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); $^{13}C$ 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); $^{13}C$ 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); $^{13}C$ 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); $^{13}C$ 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); $^{13}C$ 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); $^{13}C$ 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); $^{13}C$ 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; $^1$H 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-oxooxazolo[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 $C_{22}H_{23}N_4O_4$ (M+H)$^+$, 407.1714; found, 407.1718; $^1$H 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-oxooxazolo[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 $C_{23}H_{25}N_4O_5$ (M+H)$^+$, 437.1819; found, 437.1829; $^1$H 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)oxazolo[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 $C_{27}H_{26}N_5O_5$ (M+H)$^+$, 500.1928; found, 500.1933; $^1$H 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-oxooxazolo[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 $C_{21}H_{19}F_2N_4O_4$ (M+H)$^+$, 429.1369; found, 429.1377; $^1$H 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-chlorobenzyloxy)-3-chlorophenylamino)-2-oxooxazolo [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 $C_{28}H_{25}Cl_2N_4O_5$ (M+H)$^+$, 567.1197; found, 567.1198; $^1$H 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-oxooxazolo[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 $C_{21}H_{21}N_4O_5$ (M+H)$^+$, 409.1506; found, 409.1514; $^1$H 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-oxooxazolo[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 $C_{21}H_{21}N_4O_5$ (M+H)$^+$, 409.1506; found, 409.1507; $^1$H 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-oxooxazolo [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 $C_{24}H_{27}N_4O_5$ (M+H)$^+$, 451.1976; found, 451.1979; $^1$H 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-oxooxazolo [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 $C_{25}H_{29}N_4O_4$ (M+H)$^+$, 449.2183; found, 449.2188; $^1$H 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-dihydrooxazolo[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 $C_{30}H_{26}N_5O_6$ (M+H)$^+$, 552.1878; found, 552.1884; $^1$H 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; $^1$H 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; $^1$H 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 concentrated 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; $^1$H 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; $^1$H 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; $^1$H 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; $^1$H 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 μL reaction volume including 4 μL diluted test compounds, 4 μL substrate and 2 μL ATP. After incubation for 1 h at 37 °C, the reactions were stopped with 10 μL 2% (v/v) $H_3PO_4$. The plates were then aspirated and washed twice with 200 mL 0.9% (w/v) NaCl, and incorporation of $^{33}$Pi was determined with a microplate 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 version11.5 software). Data represented as means ± 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 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% $CO_2$ at 37 °C. Remove the medium, and cells were fixed with 10% trichloroacetic acid (50 μL) 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 μL) for 20 min at room temperature. Then, remove the SRB dye solution, and the plate was washed with tap water and dried. 150 μL of Trizma base (10 mM) was added to each well, and the absorbance was measured at 570 nm using a

microplate reader. 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 version11.5 software). Data represented as means ± 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 $2 \times 10^6$ cells in the right flank. Upon reaching an average tumor volume of 100 $mm^3$ (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 ($mm^3$) = $W^2(L/2)$, 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 $P < 0.05$.

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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.2015.07.008.

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