Graphical Abstract

To create your abstract, type over the instructions in the template box below.
Fonts or abstract dimensions should not be changed or altered.

Discovery of new [1,4]dioxino[2,3-f]quinazoline-based inhibitors of EGFR including the T790M/L858R mutant

Leave this area blank for abstract info.

Xuemei Qin¹, Zhipeng Li¹, Leifu Yang², Peng Liu³, Liming Hu^{1*}, Chengchu Zeng¹, Zhiyong Pan²

¹College of Life Science and Bioengineering, Beijing University of Technology, Beijing, 100124, China

²Beijing Dalitai Pharmaceutical Technology Co., Ltd, Beijing, 100176, China

³Guangzhou Institute of Biomedicine and Health, Chinese Academy of Science, Guangzhou, 510530, China

Discovery of new [1,4]dioxino[2,3-f]quinazoline-based inhibitors of EGFR including the T790M/L858R mutant

Xuemei Qin¹, Zhipeng Li¹, Leifu Yang², Peng Liu³, Liming Hu^{1*}, Chengchu Zeng¹, Zhiyong Pan²

¹College of Life Science and Bioengineering, Beijing University of Technology, Beijing, 100124, China

²Beijing Dalitai Pharmaceutical Technology Co., Ltd, Beijing, 100176, China

³Guangzhou Institute of Biomedicine and Health, Chinese Academy of Science, Guangzhou, 510530, China

ARTICLE INFO

Article history:

Received

Received in revised form

Accepted

Available online

Keywords:

EGFR inhibitors

Lung cancer

T790M/L858R mutation

Quinazoline

ABSTRACT

A novel series of 2,3-dihydro-[1,4]dioxino[2,3-f]quinazoline derivatives were designed, synthesized and evaluated as reversible and noncovalent epidermal growth factor receptor (EGFR) inhibitors. Most of the compounds exhibited good potency against EGFR^{wt} and some showed moderate to excellent potency against EGFR^{T790M/L858R} mutant. The half-maximal inhibitory concentration (IC₅₀) values of twenty-one compounds against EGFR^{wt} were less than 50 nM, and those of six compounds were less than 10 nM. The IC₅₀ values of eleven compounds against EGFR^{T790M/L858R} were less than 100 nM. Among these, compound **b1** displayed the most potent inhibitory activity against EGFR^{wt} (IC₅₀ = 2.0 nM) and EGFR^{T790M/L858R} (IC₅₀ = 6.9 nM). Compounds with excellent inhibitory activities against EGFR^{wt} and EGFR^{T790M/L858R} kinase inhibitory activities showed good antiproliferative activities against H358 and A549 cells. Docking study was performed to position compound **b1** into the EGFR active pocket to determine the probable binding conformation.

2015 Elsevier Ltd. All rights reserved.

1. Introduction

The epidermal growth factor receptor (EGFR) is a member of the ErbB family,¹ which is an important mediator responsible for cell proliferation, survival, adhesion, migration, and differentiation through downstream signaling transduction by auto-phosphorylation of several tyrosine residues after EGFR dimerization.² Overexpression of EGFR has been observed in many human tumors such as breast, ovarian, head and neck cancers, colon and non-small-cell lung cancer (NSCLC), which had five-year survival of less than 15%.³ NSCLC has become the leading cause of cancer-related death worldwide,³ and accounts for approximately 80–85% of lung cancers.⁴ Genetic aberrations in the tyrosine kinase domain of EGFR have been identified as one of the key drivers of NSCLC progression.⁵ Thus, the EGFR has been emerging as one of the most effective and attractive therapeutic targets for NSCLC.^{5,6}

Gefitinib⁷ and erlotinib⁸ (Fig. 1), as first-generation ATP-competitive and reversible EGFR inhibitors, were approved by US Food and Drug Administration in 2002 and 2004, respectively, and have been shown to be particularly beneficial for NSCLC patients harboring somatic EGFR mutations L858R and delE746_A750, which account for 90% of all EGFR

mutations in NSCLC.⁹ However, emergence of acquired point mutations makes their efficacy diminish eventually, leading to drug resistance in roughly 50% NSCLC patients after treatment with gefitinib or erlotinib.^{10,11} Particularly, a single T790M point mutation (threonine⁷⁹⁰→methionine⁷⁹⁰) accounts for approximately 50% in clinically acquired resistant patients.^{12,13} In order to overcome the drug resistance caused by the T790M mutation, several second-generation EGFR inhibitors which can form a covalent bond with Cys797 have shown preclinical activity against EGFR with T790M mutation (such as canertinib and dacomitinib, shown in Figure 1).¹⁴ However, their clinical efficacy has been limited by associated with skin rash and gastrointestinal toxicity, possibly because of their potency against wild-type EGFR (EGFR^{wt}).^{15,16} Recently, third-generation covalent inhibitors such as AZD9291 and CO-1686 have been identified, which demonstrated selectivity for EGFR^{T790M} mutants over EGFR^{wt}. Early phase I data indicated promising efficacy and tolerability.^{17–21} While covalent inhibitors can't be displaced by ATP and are able to circumvent this issue, we concern about possible toxicity due to the prolonged off-target inhibition of EGFR^{wt} and pursue a non-covalent strategy.^{22–24} Herein, we describe our efforts to explore reversible and non-covalent inhibitors to tackle EGFR^{T790M/L858R} mutant.

* Corresponding author. Tel.: +86-10-67396211; fax: +86-10-67396211; e-mail: huliming@bjut.edu.cn (L. Hu)

reversible EGFR inhibitors

irreversible EGFR inhibitors

Figure 1. The chemical structures of epidermal growth factor receptor tyrosine kinase inhibitors.

To date, many studies have been targeted at finding new structures based on quinazoline EGFR inhibitors.²⁵⁻²⁷ The SARs of EGFR inhibitors revealed that the 4-anilinoquinazoline scaffold is crucial to EGFR inhibitory activity, and the 6- and 7-position side chains of 4-anilinoquinazoline scaffold mainly contribute to their physicochemical properties.²⁸ So far, the study of 5-substituent 4-anilinoquinazoline derivative is rarely reported. PD153035 (4-(3-bromoanilino)-6,7-dimethoxyquinazoline, Fig. 1) was the first reported high effective and selective EGFR inhibitor which competitively binds in the ATP site with the half maximal inhibition concentration IC_{50} of 0.025 nM resulting in the inhibition of the tyrosine kinase activity of the EGFR.²⁹ Though the research was stopped due to its poor water solubility, it was widely used as a lead compound for designing EGFR inhibitors.

Based on the lead compound, a novel series of EGFR inhibitors were designed through constructing a six-member ring at the 5- and 6-position of 4-anilinoquinazoline scaffold (Figure 2). From the public crystal structure,^{30,31} it is known that 4-arylamino fragment can well extend in the hydrophobic pocket in the back of the ATP-binding cleft, which provides the key hydrophobic interaction for achieving EGFR inhibition. So, the 4-position of quinazoline was substituted by different arylamino group, especially 3-ethynylphenylamino group and 3-chloro-4-fluoro phenylamino group, which have been widely utilized in designing many EGFR inhibitors, such as gefitinib and afatinib.

One consideration for using the cyclization at 5- and 6-position is that core cyclization makes the molecule small, thus makes it easier to tolerate the shift of the inhibitors inside the binding pocket due to the mutations, such as T790M. However,

when the cyclization is introduced at 5- and 6-position, the orientation of amino substitution could be affected by the potential stereo effect between the NH linker and the cyclic group. This would lead to the reduced EGFR inhibition since 4-arylamino group might no longer be favorably extended to the hydrophobic pocket in the back of the ATP-binding cleft. To keep the 4-arylamino group in the optimal binding position at the hydrophobic pocket, a dioxane group is introduced. As shown in Figure 2, the possible stereo effect between the cyclic group and NH linker could be avoided due to the H-bond interaction between the cyclic oxygen and the NH linker.

Based on these consideration and the known SAR of quinazoline EGFR inhibitors, we firstly installed primary carbon chain such as methoxyl, ethoxyl, methoxyethoxyl etc. and then a larger secondary carbon chain or cyclic group in 7-position of quinazoline. Herein, we present new 2,3-dihydro-[1,4]dioxino [2,3-*f*]quinazoline-10-amine derivatives as novel inhibitors for EGFR^{WT} and EGFR^{T790M/L858R}.

2. Results and discussion

2.1. Chemistry

The synthesis of compounds **a-j** followed the general pathway outlined in Scheme 1. The synthesis started with the commercial available 2,3,4-trihydroxybenzoic acid **1**, esterfying with iodomethane and $KHCO_3$, to give benzoate **2** in excellent yield. Three hydroxy were protected by benzyl and then ortho-position benzyl of the ester group was deprotected selectively by HAc/HCl (10:1) at 40 °C to give **3**, which reacted with 1-bromo-2-chloroethane to obtain **4**. The key intermediate **5** was achieved

Figure 2. Design strategy of novel EGFR inhibitors.

Scheme 1. Reagents and conditions: a) CH₃I, K₂CO₃, DMF, 40 °C; b) BnCl, K₂CO₃, KI, DMF, 60 °C; c) HCl/H₂O, HAc, 40 °C, 1 h; d) 1-Bromo-2-chloroethane, K₂CO₃, DMF, 70 °C; e) Pd/C, H₂, EtOH; f) K₂CO₃, DMF; g) R¹Br, K₂CO₃, DMF, 70 °C; h) HNO₃/HAc; i) Pd/C, H₂, EtOH; j) Formamidinium acetate, ethanol, reflux; k) POCl₃, reflux; l) Anilines, *i*-PrOH, reflux.

by intramolecular cyclization after **4** deprotecting. All of the four reactions had a yield over 80%. The intermediate **5** was reacted with different halogenoalkane to obtain **6a-6j**. Nitration of them gave **7a-7j** by fuming HNO₃/HAc (1:3). The next three step reactions followed the general approach. Subsequently, the intermediates **10a-10j** were reacted with different anilines to give the target compounds **a-j** over 50% yield.

2.2. Biological activity

2.2.1. Enzyme assay

All new compounds (**a-i**) were evaluated for their ability to inhibit the autophosphorylation of EGFR^{wt} and EGFR^{T790M/L858R} mutant kinase using Kinase-Glo luminescent kinase assays. The results were shown in Table 1. The studied compounds displayed inhibition to EGFR^{wt} with IC₅₀ values ranging from 400 nM to 2 nM, but **c5**, **e1**, **f1** and **f5** exhibited diminished inhibition compared to control gefitinib. Most compounds showed significant activity against EGFR^{T790M/L858R} with IC₅₀ values ranging from 323.6 nM to 6.9 nM.

To evaluate the relationship of the length of the carbon chain of R¹ of targeted compounds and their inhibitory activities against EGFR, compounds **a1-c9** were synthesized. The R¹ of **a1-c9** were substituted by primary carbon chain from short to long that showed well or moderate inhibitory activities against EGFR^{wt} (IC₅₀ = 2.0-387.8 nM). When R¹ was substituted by methyl, compounds **a1** and **a2** showed good activities against EGFR^{wt}, but no inhibitory activity against EGFR^{T790M/L858R} kinase. When the length of R¹ was two carbon atoms, compounds (**b1-b6**)

showed much better inhibitory activity against EGFR^{wt} (2.0-9.7 nM) and EGFR^{T790M/L858R} mutant (6.88-123.54 nM). Among the compounds, **b1** showed the highest inhibitory activity against EGFR^{wt} (IC₅₀ = 2.0 nM) and EGFR^{T790M/L858R} (IC₅₀ = 6.9 nM). When R¹ was replaced with a slightly larger group, such as methoxyethyl group, compounds (**c1** and **c2**) showed excellent activities against EGFR^{wt} (13.1 nM, 21.0 nM, respectively) and EGFR^{T790M/L858R} mutant (10.5 nM, 8.1 nM, respectively). When R² is electron-withdrawing group, nitro or cyano, compounds **c5-c9** showed weakened activities against EGFR^{wt} (IC₅₀ ≥ 36.2 nM) and no activities against EGFR^{T790M/L858R}.

To extensively explore the SARs of the novel scaffold, compounds (**e1-e8**, **f1-f5** and **g1-g2**) with R¹ being substituted by secondary carbon chain were synthesized. **e1-e6** and **f1-f5**, whose R² was electron-withdrawing group, such as nitro or cyano, showed 10 times less potency against EGFR^{wt} than **b1** and almost lost activity against EGFR^{T790M/L858R}. **e7**, **e8**, **g1** and **g2**, whose 4-position of quinazoline was substituted by 3-chloro-4-fluorophenylamino or 3-aminophenyl-acetylene, showed good inhibitory activities against EGFR^{wt} as well as EGFR^{T790M/L858R}.

Upon introduction of heterocyclic fragments in R¹, five-member heterocyclic compounds **d1** (IC₅₀ = 24.1 nM), **d2** (IC₅₀ = 27.2 nM), **h1** (IC₅₀ = 20.5 nM) and **h2** (IC₅₀ = 24.1 nM) showed 2-fold improvement in inhibitory activities against EGFR^{wt} compared to hexa-heterocyclic compounds **i1** (IC₅₀ = 58.8 nM) and **i2** (IC₅₀ = 57.8 nM). Unfortunately, all compounds with heterocyclic in R¹ did not show good inhibitory activities against EGFR^{T790M/L858R} mutation.

Table 1. In vitro enzymatic inhibitory activities of compounds **a1-i2** against EGFR^{wt} and EGFR^{T790M/L858R}

Compound	R ¹	R ²	R ³	EGFR ^{wt} IC ₅₀ (nM)	EGFR ^{T790M/L858R} IC ₅₀ (nM)
a1	Me	Cl	F	22.5	1000
a2	Me	ethynyl	H	10.9	> 1000
b1		Cl	F	2.0	6.9
b2		ethynyl	H	9.2	21.5
b3		MeO-	H	4.4	70.0
b4		Cl	Me	6.7	33.8
b5		NO ₂	H	9.7	71.0
b6		H	Me	3.6	123.5
c1		Cl	F	13.1	10.5
c2		ethynyl	H	21.0	8.1
c3		H	F	200.3	> 1000
c4		MeO-	H	159.9	109.1
c5		NO ₂	MeO-	> 1000	> 1000
c6		NO ₂	F	43.8	> 1000
c7		NO ₂	Me	74.6	> 1000
c8		NO ₂	Cl	36.2	> 1000
c9		CN	F	387.8	> 1000
d1		Cl	F	24.1	> 1000
d2		ethynyl	H	27.3	121.0
e1		NO ₂	MeO-	> 1000	> 1000
e2		NO ₂	F	82.5	> 1000
e3		NO ₂	Me	184.5	> 1000
e4		NO ₂	Cl	102.6	1303.2
e5		CN	F	313.6	>1000
e6		CN	Me	416.1	>1000
e7		Cl	F	29.1	46.28
e8		ethynyl	H	23.7	31.4

f1		NO ₂	MeO-	> 1000	> 1000
f2		NO ₂	F	232.3	270.6
f3		NO ₂	Me	363.3	> 1000
f4		CN	F	351.8	> 1000
f5		CN	Me	> 1000	> 1000
g1		Cl	F	247.2	89.2
g2		ethynyl	H	473.6	76.1
h1		Cl	F	20.5	150.9
h2		ethynyl	H	16.7	323.6
i1		Cl	F	58.8	> 1000
i2		ethynyl	H	57.8	> 1000
gefitinib				2.6	22.5

When 4-position of quinazoline scaffold are substituted by 3-chloro-4-fluorophenylamino (**a1**, **b1**, **c1**, **d1**, **e7**, **g1**, **h1**, **i1**) or 3-ethynylphenylamino (**a2**, **b2**, **c2**, **d2**, **e8**, **g2**, **h2**, **i2**), the compounds showed better inhibitory activity against EGFR^{wt} and EGFR^{T790M/L858R}, especially when R¹ is ethyl, methoxy ethyl or isopropyl. Therefore, it is worth mentioning that the inhibitory activity of the target compound against EGFR^{wt} and EGFR^{T790M/L858R} depends not only on the R¹, but also on the nature of the substituents of aniline.

From the crystal structure of gefitinib/EGFR or erlotinib/EGFR complex we knew the C-7 of the quinazoline part was to extend into the solvent region of EGFR kinase ATP binding

Table 2. EGFR inhibitory activity of compounds **j1-j5**

Compound	R ²	R ³	EGFR ^{wt} IC ₅₀ (nM)
j1	Cl	F	25.86
j2	Br	H	18.48
j3	Cl	H	21.95
j4	F	H	83.88
j5	CF ₃	H	97.18
gefitinib			17.98

pocket, so we introduced hydrophilic group morpholinopropoxy group as R¹ and synthesized five compounds to study their activities against EGFR. The results were shown in Table 2. Compared to gefitinib, compounds **j1**, **j2** and **j3** exhibited significant inhibition against EGFR wide-type. When R² is F or CF₃, compounds **j4** and **j5** showed 4-fold less inhibitory activities against EGFR than **j1**, **j2** and **j3**.

2.2.2. Antiproliferation assay

Seven compounds with excellent inhibitory activities against EGFR were chosen to evaluate their antiproliferative activities against H358 cell and A549 cell and the result was demonstrated in Table 3.

Table 3. Antiproliferative assay in vitro^a

Compound	H358 IC ₅₀ (μM)	A549 IC ₅₀ (μM)
b1	22.74	16.09
b2	8.89	10.54
b4	17.32	11.77
c1	5.93	13.88
c2	7.35	9.93
e7	6.92	11.62
e8	4.54	6.08
gefitinib	5.68	13.32
erlotinib	3.91	7.35

^a Values are means of at least two experiment.

The tested compounds possessed good antiproliferative activities with low IC₅₀ values against H358 and A549 cells

ppm (δ). Splitting patterns are designed as s, singlet; d, doublet; t, triplet; m, multiplet. ESI-MS spectra were obtained on an Esquire 6000 Mass Spectrometer. HRMS data were measured using a Bruker APEX IV Fourier transform ion cyclotron resonance mass spectrometer.

4.1.1. General procedure for the synthesis of methyl 6-nitro-2,3-dihydrobenzo[b][1,4]dioxine-5-carboxylate derivatives (**7a-7j**)

A solution of **6a-6j** (0.01 mol) in glacial acetic acid (10 mL) at 0 °C was treated dropwise with a 1:2 (v/v) mixture of glacial acetic acid/fuming nitric acid (10 mL). The resulting mixture was stirred at 0 °C for 90 min, then poured into ice water (150 mL) and the resultant precipitate was collected by filtration. The precipitate was washed with ice water three times to afford **7a-7j** as yellow or light-yellow solid.

4.1.2. General procedure for the synthesis of 2,3-dihydro-[1,4]dioxino[2,3-f]quinazolin-10(9H)-one derivatives (**9a-9j**)

A mixture of **8a-8j** (20 mmol) and formamidine acetate (24 mmol) in ethanol (60 mL) was heated at reflux for 5 h. The mixture was cooled and filtered. The precipitate was washed with ethanol and dried to afford **9a-9j**.

4.1.3. General procedure for the synthesis of 10-chloro-2,3-dihydro-[1,4]dioxino[2,3-f]quinazoline derivatives (**10a-10j**)

A mixture of intermediate **9a-9j** (7.0 mmol), and POCl₃ (15 mL) was heated at reflux temperature for 3 h. The solvent was removed under reduced pressure. The residue was dissolved in CH₂Cl₂ (50 mL) and the organic layer was washed with cold aqueous NaHCO₃ solution and brine, and dried over MgSO₄, filtered and evaporated to give the product **10a-10j**.

4.1.4. General procedure for the synthesis of 2,3-dihydro[1,4]-dioxino[2,3-f]quinazolin-10-amine derivatives (**a-j**)

A mixture of 4-chloroquinazolines derivatives **10a-10j** (5 mmol) and substituted anilines (6 mmol) in isopropanol (45 mL) was stirred at reflux for 3 h. The reaction mixture was cooled to room temperature and the resultant precipitate was collected by filtration. The solid was further dried in vacuum to give the compounds **a-j**.

4.1.4.1. *N*-(3-chloro-4-fluorophenyl)-5-methoxy-2,3-dihydro-[1,4]dioxino[2,3-f]quinazolin-10-amine (**a1**)

Yellow solid; yield: 50%; mp: 242-243 °C. ¹H NMR (500 MHz, CDCl₃ + CD₃OD, δ ppm): 4.11 (s, 3H), 4.55 (s, 2H), 4.69 (s, 2H), 7.26 (t, *J* = 8.7 Hz, 1H), 7.42 (s, 1H), 7.59-7.49 (m, 1H), 7.81 (d, *J* = 6.2 Hz, 1H), 8.59 (s, 1H); ¹³C NMR (125 MHz, CDCl₃ + CD₃OD, δ ppm): 57.39, 63.88, 65.79, 94.45, 99.24, 116.85, 117.02, 121.50, 123.97, 126.30, 132.38, 133.56, 135.10, 139.32, 148.51, 157.03, 158.27. HRMS (ESI) *m/z* calcd for C₁₇H₁₃ClFN₃O₃ [M+H]⁺, 362.0708; found, 362.0706.

4.1.4.2. *N*-(3-ethynylphenyl)-5-methoxy-2,3-dihydro[1,4]dioxino[2,3-f]quinazolin-10-amine (**a2**)

White solid; yield: 67%; mp: 244-246 °C. ¹H NMR (500 MHz, CDCl₃ + CD₃OD, δ ppm): 3.28 (s, 1H), 4.10 (s, 3H), 4.56 (dd, *J* = 4.8, 3.1 Hz, 2H), 4.72 (dd, *J* = 4.8, 3.1 Hz, 2H), 7.30 (s, 1H), 7.44 (d, *J* = 5.0 Hz, 2H), 7.68 (td, *J* = 4.6, 2.2 Hz, 1H), 7.80 (s, 1H), 8.56 (s, 1H). ¹³C NMR (125 MHz, CDCl₃ + CD₃OD, δ ppm): 57.24, 63.95, 65.86, 78.56, 82.37, 94.04, 99.34, 123.31, 124.38, 127.23, 129.23, 130.59, 133.57, 134.96, 135.95, 139.62, 148.41, 156.96, 158.23. HRMS (ESI) *m/z* calcd for C₁₉H₁₅N₃O₃ [M+H]⁺, 334.1192; found, 334.1189.

4.1.4.3. *N*-(3-chloro-4-fluorophenyl)-5-ethoxy-2,3-dihydro-[1,4]dioxino[2,3-f]quinazolin-10-amine (**b1**)

Yellow solid; yield: 40%; mp: 248-249 °C. ¹H NMR (500 MHz, DMSO-*d*₆, δ ppm): 1.43 (t, *J* = 7.0 Hz, 3H), 4.24 (q, *J* = 7.0 Hz, 2H), 4.44 (dd, *J* = 4.8, 3.1 Hz, 2H), 4.60 (dd, *J* = 4.8, 2.9 Hz, 2H), 6.99 (s, 1H), 7.54 (t, *J* = 9.0 Hz, 1H), 7.65 (ddd, *J* = 8.9, 4.3, 2.6 Hz, 1H), 7.95 (dd, *J* = 6.8, 2.5 Hz, 1H), 8.73 (s, 1H), 10.48 (s, 1H). ¹³C NMR (125 MHz, DMSO-*d*₆, δ ppm): 14.72, 64.06, 65.65, 65.83, 99.58, 117.20, 117.38, 119.59, 119.73, 126.52, 127.73, 132.91, 134.58, 140.38, 150.13, 154.73, 155.54, 158.58. HRMS (ESI) *m/z* calcd for C₁₈H₁₅ClFN₃O₃ [M+H]⁺, 376.0864; found, 376.0861.

4.1.4.4. 5-ethoxy-*N*-(3-ethynylphenyl)-2,3-dihydro-[1,4]dioxino[2,3-f]quinazolin-10-amine (**b2**)

Yellow solid; yield: 45%; mp: 248-250 °C. ¹H NMR (500 MHz, CDCl₃ + CD₃OD, δ ppm): 1.53 (t, *J* = 7.0 Hz, 3H), 3.24 (s, 1H), 4.29 (q, *J* = 7.0 Hz, 2H), 4.61-4.51 (m, 2H), 4.75-4.68 (m, 2H), 7.36 (s, 1H), 7.42 (d, *J* = 4.9 Hz, 2H), 7.73-7.66 (m, 1H), 7.81 (s, 1H), 8.52 (s, 1H). ¹³C NMR (125 MHz, CDCl₃ + CD₃OD, δ ppm): 14.12, 63.91, 65.83, 66.31, 78.57, 82.42, 94.62, 99.01, 123.24, 124.25, 127.04, 129.23, 130.50, 133.55, 134.84, 135.96, 139.43, 148.16, 156.33, 158.03. HRMS (ESI) *m/z* calcd for C₂₀H₁₇N₃O₃ [M+H]⁺, 348.1348; found, 348.1345.

4.1.4.5. 5-ethoxy-*N*-(3-methoxyphenyl)-2,3-dihydro-[1,4]dioxino[2,3-f]quinazolin-10-amine (**b3**)

Yellow solid; yield: 66%; mp: 249-251 °C. ¹H NMR (500 MHz, CDCl₃ + CD₃OD, δ ppm): 1.56 (t, *J* = 6.9 Hz, 3H), 3.87 (s, 3H), 4.35-4.28 (m, 2H), 4.56 (s, 2H), 4.71 (s, 2H), 6.90 (d, *J* = 7.7 Hz, 1H), 7.18 (d, *J* = 8.4 Hz, 2H), 7.33 (s, 1H), 7.38 (t, *J* = 8.1 Hz, 1H), 8.53 (s, 1H). ¹³C NMR (125 MHz, CDCl₃ + CD₃OD, δ ppm): 13.98, 55.32, 63.91, 65.84, 66.13, 94.12, 99.13, 110.23, 112.44, 116.21, 129.91, 133.61, 134.60, 136.85, 139.84, 148.26, 156.33, 158.19, 160.18. HRMS (ESI) *m/z* calcd for C₁₉H₁₉N₃O₄ [M+H]⁺, 354.1454; found, 354.1452.

4.1.4.6. *N*-(3-chloro-4-methylphenyl)-5-ethoxy-2,3-dihydro-[1,4]dioxino[2,3-f]quinazolin-10-amine (**b4**)

Yellow solid; yield: 52%; mp: 246-248 °C. ¹H NMR (500 MHz, CDCl₃ + CD₃OD, δ ppm): 1.55 (t, *J* = 7.0 Hz, 3H), 2.41 (s, 3H), 4.32 (q, *J* = 7.0 Hz, 2H), 4.55 (dd, *J* = 4.8, 3.0 Hz, 2H), 4.70 (dd, *J* = 4.8, 3.1 Hz, 2H), 7.30 (s, 1H), 7.33 (d, *J* = 8.2 Hz, 1H), 7.44 (dd, *J* = 8.2, 2.2 Hz, 1H), 7.74 (d, *J* = 2.2 Hz, 1H), 8.54 (s, 1H). ¹³C NMR (125 MHz, CDCl₃ + CD₃OD, δ ppm): 14.07, 19.50, 63.90, 65.81, 66.27, 94.50, 99.04, 122.17, 124.33, 131.20, 133.58, 134.51, 134.60, 134.77, 135.11, 139.54, 148.26, 156.36, 158.10. HRMS (ESI) *m/z* calcd for C₁₉H₁₈ClN₃O₃ [M+H]⁺, 372.1115; found, 372.1112.

4.1.4.7. 5-ethoxy-*N*-(3-nitrophenyl)-2,3-dihydro-[1,4]dioxino[2,3-f]quinazolin-10-amine (**b5**)

Yellow solid; yield: 42%; mp: 250-252 °C. ¹H NMR (500 MHz, CDCl₃ + CD₃OD, δ ppm): 1.56 (t, *J* = 7.0 Hz, 3H), 4.37 (q, *J* = 7.0 Hz, 2H), 4.57 (dd, *J* = 4.8, 3.1 Hz, 2H), 4.81-4.72 (m, 2H), 7.47 (s, 1H), 7.64 (s, 1H), 7.71 (t, *J* = 8.2 Hz, 1H), 8.21-8.12 (m, 2H), 8.65 (t, *J* = 2.1 Hz, 1H), 8.70 (s, 1H). ¹³C NMR (125 MHz, CDCl₃ + CD₃OD, δ ppm): 15.24, 65.22, 67.05, 67.40, 95.78, 100.79, 120.22, 122.29, 131.11, 135.02, 136.42, 138.74, 140.99, 149.60, 149.69, 157.86, 159.70. HRMS (ESI) *m/z* calcd for C₁₈H₁₆N₄O₅ [M+H]⁺, 369.1199; found, 369.1196.

4.1.4.8. 5-ethoxy-*N*-(*p*-tolyl)-2,3-dihydro-[1,4]dioxino[2,3-f]quinazolin-10-amine (**b6**)

Yellow solid; yield: 65%; mp: 254-256 °C. ¹H NMR (500 MHz, CDCl₃ + CD₃OD, δ ppm): 1.54 (t, *J* = 6.8 Hz, 3H), 2.40 (s, 3H), 4.38-4.22 (m, 2H), 4.55 (d, *J* = 2.1 Hz, 2H), 4.68 (s, 2H), 7.27 (d, *J* = 7.8 Hz, 2H), 7.39 (s, 1H), 7.51 (d, *J* = 7.9 Hz, 2H), 8.48 (s, 1H). ¹³C NMR (125 MHz, CDCl₃ + CD₃OD, δ ppm): 14.14, 20.98, 63.83, 65.72, 66.28, 94.73, 98.91, 123.85, 129.81, 133.07, 133.34, 134.79, 137.33, 139.42, 148.29, 156.08, 158.07. HRMS (ESI) *m/z* calcd for C₁₉H₁₉N₃O₃ [M+H]⁺, 338.1505; found, 338.1507.

4.1.4.9. *N*-(3-chloro-4-fluorophenyl)-5-(2-methoxyethoxy)-2,3-dihydro-[1,4]dioxino[2,3-*f*]quinazolin-10-amine (**c1**)

Yellow solid; yield: 40%; mp: 259-261 °C. ¹H NMR (400 MHz, CDCl₃ + CD₃OD, δ ppm): 3.47 (s, 3H), 3.83 (t, *J* = 4.0 Hz, 2H), 4.34 (t, *J* = 4.2 Hz, 2H), 4.56 (t, *J* = 2.0 Hz, 2H), 4.69 (t, *J* = 3.8 Hz, 2H), 7.26 (t, *J* = 7.6 Hz, 1H), 7.56-7.60 (m, 1H), 7.64 (s, 1H), 7.82 (q, *J* = 6.4 Hz, 1H), 8.64 (s, 1H), 10.00 (s, 1H). ¹³C NMR (125 MHz, CDCl₃ + CD₃OD, δ ppm): 59.27, 63.79, 65.82, 69.54, 69.99, 95.56, 99.36, 117.06, 121.80, 123.84, 126.19, 132.56, 133.75, 135.48, 139.09, 148.14, 155.51, 156.18, 157.51, 158.25. HRMS (ESI) *m/z* calcd for C₁₉H₁₇ClFN₃O₄ [M+H]⁺, 406.0970; found, 406.0969.

4.1.4.10. 5-ethoxy-*N*-(*p*-tolyl)-2,3-dihydro-[1,4]dioxino[2,3-*f*]quinazolin-10-amine (**c2**)

White solid; yield: 42%; mp: 228-229 °C. ¹H NMR (400 MHz, CDCl₃ + CD₃OD, δ ppm): 3.18 (s, 1H), 3.47 (s, 3H), 3.82 (t, *J* = 4.0 Hz, 2H), 4.34 (t, *J* = 4.0 Hz, 2H), 4.55 (s, 2H), 4.69 (s, 2H), 7.44 (d, *J* = 2.4 Hz, 2H), 7.64 (s, 1H), 7.74 (s, 1H), 7.79 (s, 1H), 8.47 (s, 1H), 10.06 (s, 1H). ¹³C NMR (125 MHz, CDCl₃ + CD₃OD, δ ppm): 58.73, 63.63, 65.58, 69.20, 69.99, 78.27, 82.18, 94.63, 99.37, 123.10, 124.19, 127.07, 128.96, 130.22, 133.48, 135.20, 135.95, 139.60, 148.47, 155.96, 158.01. HRMS (ESI) *m/z* calcd for C₂₁H₁₉N₃O₄ [M+H]⁺, 378.1454; found, 378.1450.

4.1.4.11. *N*-(4-fluorophenyl)-5-(2-methoxyethoxy)-2,3-dihydro-[1,4]dioxino[2,3-*f*]quinazolin-10-amine (**c3**)

Yellow solid; yield: 52%; mp: 243-245 °C. ¹H NMR (400 MHz, CDCl₃ + CD₃OD, δ ppm): 3.33 (s, 3H), 3.72 (t, *J* = 4.0 Hz, 2H), 4.24 (t, *J* = 4.0 Hz, 2H), 4.40 (t, *J* = 4.0 Hz, 2H), 4.58 (s, 2H), 6.88 (s, 1H), 7.21 (t, *J* = 8.8 Hz, 2H), 7.82 (t, *J* = 5.2 Hz, 2H), 8.36 (s, 1H), 9.55 (s, 1H). ¹³C NMR (125 MHz, CDCl₃ + CD₃OD, δ ppm): 59.23, 63.86, 65.31, 68.44, 70.53, 101.32, 101.61, 115.57, 115.75, 124.48, 128.91, 131.90, 134.39, 138.60, 145.81, 153.59, 157.20, 158.68, 160.62. HRMS (ESI) *m/z* calcd for C₁₉H₁₈FN₃O₄ [M+H]⁺, 372.1360; found, 372.1362.

4.1.4.12. 5-(2-methoxyethoxy)-*N*-(3-methoxyphenyl)-2,3-dihydro-[1,4]dioxino[2,3-*f*]quinazolin-10-amine (**c4**)

Yellow solid; yield: 44%; mp: 240-242 °C. ¹H NMR (400 MHz, CDCl₃ + CD₃OD, δ ppm): 3.47 (s, 3H), 3.83 (t, *J* = 4.0 Hz, 2H), 3.88 (s, 3H), 4.35 (t, *J* = 4.2 Hz, 2H), 4.55 (d, *J* = 2.0 Hz, 2H), 4.67 (d, *J* = 2.0 Hz, 2H), 6.88 (q, *J* = 8.4 Hz, 1H), 7.19 (d, *J* = 4.0 Hz, 1H), 7.33-7.41 (m, 2H), 8.47 (s, 1H), 10.05 (s, 1H). ¹³C NMR (125 MHz, CDCl₃ + CD₃OD, δ ppm): 49.37, 49.54, 49.71, 55.56, 59.18, 63.80, 65.81, 69.44, 70.03, 94.98, 99.19, 109.95, 112.35, 115.93, 130.00, 139.33, 148.05, 155.97, 157.95, 160.19. HRMS (ESI) *m/z* calcd for C₂₀H₂₁N₃O₅ [M+H]⁺, 384.1559; found, 384.1555.

4.1.4.13. *N*-(4-methoxy-3-nitrophenyl)-5-(2-methoxyethoxy)-2,3-dihydro-[1,4]dioxino[2,3-*f*]quinazolin-10-amine (**c5**)

Yellow solid; yield: 63%; mp: 237-245 °C. ¹H NMR (400 MHz, DMSO-*d*₆, δ ppm): 3.35 (s, 3H), 3.76 (t, *J* = 4.4 Hz, 2H), 3.87 (s, 1H), 4.13 (s, 3H), 4.29 (t, *J* = 4 Hz, 2H), 4.53 (s, 2H),

4.72 (s, 2H), 7.10 (s, 1H), 7.91 (s, 1H), 8.00 (d, *J* = 8.8 Hz, 1H), 8.91-8.88 (m, 2H), 11.01 (s, 1H). HRMS (ESI) *m/z* calcd for C₂₀H₂₀N₄O₇ [M+H]⁺, 429.1410; found, 429.1407.

4.1.4.14. *N*-(4-fluoro-3-nitrophenyl)-5-(2-methoxyethoxy)-2,3-dihydro-[1,4]dioxino[2,3-*f*]quinazolin-10-amine (**c6**)

Yellow solid; yield: 40%; mp: 234-235 °C. ¹H NMR (400 MHz, DMSO-*d*₆, δ ppm): 3.35 (s, 3H), 3.70 (s, 2H), 4.30 (s, 2H), 4.47 (s, 2H), 4.62 (s, 2H), 7.14 (s, 1H), 7.75-7.70 (m, 1H), 8.09 (d, *J* = 8.8 Hz, 1H), 8.54-8.52 (m, 1H), 8.76 (s, 1H), 10.58 (s, 1H). ¹³C NMR (125 MHz, CDCl₃ + CD₃OD, δ ppm): 59.05, 63.89, 65.85, 69.64, 70.09, 94.71, 99.36, 119.09, 121.70, 131.51, 132.50, 134.00, 134.83, 137.08, 139.57, 148.27, 154.47, 156.70, 158.36. HRMS (ESI) *m/z* calcd for C₁₉H₁₇FN₄O₆ [M+H]⁺, 417.1210; found, 417.1206.

4.1.4.15. 5-(2-methoxyethoxy)-*N*-(4-methyl-3-nitrophenyl)-2,3-dihydro-[1,4]dioxino[2,3-*f*]quinazolin-10-amine (**c7**)

Yellow solid; yield: 57%; mp: 251-254 °C. ¹H NMR (500 MHz, CDCl₃ + CD₃OD, δ ppm): 2.64 (s, 3H), 3.48 (s, 3H), 3.88 (s, 2H), 4.41 (s, 2H), 4.55 (s, 2H), 4.72 (s, 2H), 7.37 (s, 1H), 7.48 (d, *J* = 8.3 Hz, 1H), 7.88 (d, *J* = 7.2 Hz, 1H), 8.39 (s, 1H), 8.60 (s, 1H). ¹³C NMR (125 MHz, CDCl₃ + CD₃OD, δ ppm): 19.91, 59.03, 63.86, 65.87, 69.61, 70.10, 94.68, 99.40, 119.96, 128.43, 132.05, 133.38, 133.95, 134.75, 139.58, 148.27, 149.02, 156.57, 158.29. HRMS (ESI) *m/z* calcd for C₂₀H₂₀N₄O₆ [M+H]⁺, 413.1461; found, 413.1464.

4.1.4.16. *N*-(4-chloro-3-nitrophenyl)-5-(2-methoxyethoxy)-2,3-dihydro-[1,4]dioxino[2,3-*f*]quinazolin-10-amine (**c8**)

Yellow solid; yield: 44%; mp: 262-263 °C. ¹H NMR (500 MHz, CDCl₃ + CD₃OD, δ ppm): 3.34 (s, 6H), 3.49 (s, 2H), 3.89 (s, 2H), 4.41 (s, 2H), 4.55 (s, 2H), 7.08 (s, 1H), 7.63 (s, 1H), 7.71 (s, 1H), 7.94 (s, 1H), 8.49 (s, 1H), 8.66 (s, 1H). ¹³C NMR (125 MHz, CDCl₃ + CD₃OD, δ ppm): 58.75, 63.92, 65.81, 69.51, 70.22, 94.07, 99.71, 121.10, 123.98, 128.73, 132.01, 134.05, 134.86, 135.88, 140.07, 147.86, 148.57, 156.81, 158.38. HRMS (ESI) *m/z* calcd for C₁₉H₁₇ClN₄O₆ [M+H]⁺, 433.0915; found, 433.0919.

4.1.4.17. 2-fluoro-5-((5-(2-methoxyethoxy)-2,3-dihydro-[1,4]dioxino[2,3-*f*]quinazolin-10-yl)amino)benzonitrile (**c9**)

Yellow solid; yield: 53%; mp: 245-248 °C. ¹H NMR (500 MHz, CDCl₃ + CD₃OD, δ ppm): 3.48 (s, 3H), 3.88 (s, 2H), 4.41 (s, 2H), 4.54 (s, 2H), 4.71 (s, 2H), 7.33-7.26 (m, 1H), 7.39 (t, *J* = 8.7 Hz, 1H), 8.01-7.93 (m, 1H), 8.12 (s, 1H), 8.58 (s, 1H). ¹³C NMR (125 MHz, CDCl₃ + CD₃OD, δ ppm): 58.99, 63.90, 65.83, 69.60, 70.14, 94.48, 99.39, 101.64, 113.13, 117.10, 117.26, 129.11, 131.52, 132.96, 133.98, 134.74, 139.80, 148.30, 156.67, 158.43, 160.05, 162.12. HRMS (ESI) *m/z* calcd for C₂₀H₁₇FN₄O₄ [M+H]⁺, 397.1312; found, 397.1315.

4.1.4.18. *N*-(3-chloro-4-fluorophenyl)-5-((tetrahydrofuran-2-yl)-methoxy)-2,3-dihydro-[1,4]dioxino[2,3-*f*]quinazolin-10-amine (**d1**)

Yellow solid; yield: 49%; mp: 253-255 °C. ¹H NMR (400 MHz, DMSO-*d*₆, δ ppm): 1.72-2.04 (m, 4H), 3.70 (d, *J* = 4.0 Hz, 1H), 3.82 (d, *J* = 4.0 Hz, 1H), 4.11 (t, *J* = 4 Hz, 1H), 4.19 (t, *J* = 4 Hz, 1H), 4.24 (s, 1H), 4.46 (s, 2H), 4.61 (s, 2H), 7.08 (s, 1H), 7.54 (d, *J* = 8 Hz, 1H), 7.63-7.67 (m, 1H), 7.93-7.95 (m, 1H), 8.74 (s, 1H), 10.52 (s, 1H). HRMS (ESI) *m/z* calcd for C₂₁H₁₉ClFN₃O₄ [M+H]⁺, 432.1126; found, 432.1125.

4.1.4.19. *N*-(3-ethynylphenyl)-5-((tetrahydrofuran-2-yl)-methoxy)-2,3-dihydro-[1,4]dioxino[2,3-*f*]quinazolin-10-amine (**d2**)

Yellow solid; yield: 45%; mp: 209-212 °C. ¹H NMR (400 MHz, DMSO-*d*₆, δ ppm): 1.72-2.04 (m, 4H), 3.71 (t, *J* = 8.0 Hz, 1H), 3.82 (d, *J* = 4.0 Hz, 1H), 4.10 (s, 1H), 4.25 (s, 1H), 4.26-4.29 (m, 2H), 4.45 (s, 2H), 4.62 (s, 2H), 7.06 (s, 1H), 7.39-7.46 (m, 2H), 7.48-7.82 (m, 2H), 8.71 (s, 1H), 10.45 (s, 1H). ¹³C NMR (125 MHz, CDCl₃ + CD₃OD, δ ppm): 29.59, 31.76, 67.76, 69.79, 72.68, 76.33, 80.50, 82.53, 86.33, 98.55, 103.29, 127.25, 128.35, 131.19, 133.17, 134.48, 137.74, 138.71, 139.98, 143.64, 152.35, 160.27, 162.15. HRMS(ESI) *m/z* calcd for C₂₃H₂₁N₃O₄ [M+H]⁺, 404.1610; found, 404.1611.

4.1.4.20. 5-isopropoxy-*N*-(4-methoxy-3-nitrophenyl)-2,3-dihydro-[1,4]dioxino[2,3-*ff*]quinazolin-10-amine (**e1**)

Yellow solid; yield: 50%; mp: 216-219 °C. ¹H NMR (500 MHz, CDCl₃ + CD₃OD, δ ppm): 1.51 (d, *J* = 6.0 Hz, 6H), 4.16 (s, 3H), 4.62-4.54 (m, 2H), 4.77-4.68 (m, 2H), 4.94 (dt, *J* = 12.1, 6.0 Hz, 1H), 7.33 (s, 1H), 7.91 (d, *J* = 2.3 Hz, 1H), 8.02 (dd, *J* = 9.1, 2.4 Hz, 1H), 8.78 (s, 1H), 9.00 (d, *J* = 9.1 Hz, 1H). ¹³C NMR (125 MHz, CDCl₃ + CD₃OD, δ ppm): 21.31, 56.98, 63.87, 65.66, 73.58, 95.19, 99.99, 105.67, 117.19, 121.56, 132.29, 134.45, 135.19, 139.38, 144.62, 148.38, 149.45, 155.99, 157.41. HRMS (ESI) *m/z* calcd for C₂₀H₂₀N₄O₆ [M+H]⁺, 413.1461; found, 413.1453.

4.1.4.21. *N*-(4-fluoro-3-nitrophenyl)-5-isopropoxy-2,3-dihydro-[1,4]dioxino[2,3-*ff*]quinazolin-10-amine (**e2**)

Yellow solid; yield, 75%; mp: 223-226 °C. ¹H NMR (500 MHz, CDCl₃ + CD₃OD, δ ppm): 1.46 (d, *J* = 6.0 Hz, 6H), 4.56 (s, 2H), 4.74 (s, 2H), 4.90-4.82 (m, 1H), 7.41 (t, *J* = 9.5 Hz, 1H), 7.55 (s, 1H), 8.10 (s, 1H), 8.58-8.40 (m, 2H), 10.21 (s, 1H). HRMS (ESI) *m/z* calcd for C₁₉H₁₇FN₄O₅ [M+H]⁺, 401.1261; found, 401.1258.

4.1.4.22. 5-isopropoxy-*N*-(4-methyl-3-nitrophenyl)-2,3-dihydro-[1,4]dioxino[2,3-*ff*]quinazolin-10-amine (**e3**)

Yellow solid; yield: 75%; mp: 230-234 °C. ¹H NMR (500 MHz, CDCl₃ + CD₃OD, δ ppm): 1.47 (d, *J* = 6.0 Hz, 6H), 2.63 (s, 3H), 4.59-4.53 (m, 2H), 4.76-4.70 (m, 2H), 4.89 (dt, *J* = 12.1, 6.0 Hz, 1H), 7.45 (d, *J* = 8.3 Hz, 1H), 7.51 (s, 1H), 7.95-7.89 (m, 1H), 8.34 (t, *J* = 3.2 Hz, 1H), 8.54 (d, *J* = 3.3 Hz, 1H). ¹³C NMR (125 MHz, CDCl₃ + CD₃OD, δ ppm): 20.02, 21.45, 63.84, 65.77, 73.57, 95.55, 98.74, 119.51, 128.09, 131.83, 133.38, 134.11, 134.74, 135.12, 139.26, 148.06, 148.97, 155.75, 157.97. HRMS (ESI) *m/z* calcd for C₂₀H₂₀N₄O₅ [M+H]⁺, 397.1512; found, 397.1507.

4.1.4.23. *N*-(4-chloro-3-nitrophenyl)-5-isopropoxy-2,3-dihydro-[1,4]dioxino[2,3-*ff*]quinazolin-10-amine (**e4**)

Yellow solid; yield: 67%; mp: 229-232 °C. ¹H NMR (500 MHz, CDCl₃ + CD₃OD, δ ppm): 1.47 (d, *J* = 6.1 Hz, 6H), 4.56 (dd, *J* = 4.8, 3.0 Hz, 2H), 4.73 (dd, *J* = 4.7, 3.1 Hz, 2H), 4.89 (dt, *J* = 12.1, 6.0 Hz, 1H), 7.53 (s, 1H), 7.65-7.60 (m, 1H), 7.99 (dt, *J* = 8.7, 3.2 Hz, 1H), 8.36 (dd, *J* = 4.5, 2.6 Hz, 1H), 8.59 (s, 1H). ¹³C NMR (125 MHz, CDCl₃ + CD₃OD, δ ppm): 21.43, 63.84, 65.80, 73.72, 95.56, 98.87, 120.18, 124.11, 127.86, 132.28, 134.29, 135.24, 135.57, 139.13, 147.75, 147.99, 156.02, 157.81. HRMS (ESI) *m/z* calcd for C₁₉H₁₇ClN₄O₅ [M+H]⁺, 417.0966; found, 417.0963.

4.1.4.24. 2-fluoro-5-((5-isopropoxy-2,3-dihydro-[1,4]dioxino[2,3-*ff*]quinazolin-10-yl)amino)benzonitrile (**e5**)

Yellow solid; yield: 55%; mp: 250-263 °C. ¹H NMR (500 MHz, CDCl₃ + CD₃OD, δ ppm): 1.48 (d, *J* = 6.0 Hz, 6H), 4.55 (s, 2H), 4.71 (s, 2H), 4.90 (dt, *J* = 12.0, 5.9 Hz, 1H), 7.35 (dd, *J* = 10.4, 6.8 Hz, 1H), 7.46 (s, 1H), 8.02-7.94 (m, 1H), 8.07 (dd, *J*

= 5.2, 2.3 Hz, 1H), 8.56 (s, 1H). ¹³C NMR (125 MHz, CDCl₃ + CD₃OD, δ ppm): 21.43, 63.88, 65.77, 73.62, 95.37, 98.73, 101.84, 113.09, 117.14, 117.31, 128.62, 130.99, 132.86, 134.20, 135.05, 139.44, 148.12, 155.93, 158.12, 159.89, 161.96. HRMS (ESI) *m/z* calcd for C₂₀H₁₇FN₃O₄ [M+H]⁺, 381.1363; found, 381.1360.

4.1.4.25. 5-((5-isopropoxy-2,3-dihydro-[1,4]dioxino[2,3-*ff*]quinazolin-10-yl)amino)-2-methylbenzonitrile (**e6**)

Yellow solid; yield: 67%; mp: 229-236 °C. ¹H NMR (500 MHz, CDCl₃ + CD₃OD, δ ppm): 1.48 (d, *J* = 5.9 Hz, 6H), 2.59 (s, 3H), 4.54 (s, 2H), 4.68 (s, 2H), 4.92 (dt, *J* = 11.8, 5.8 Hz, 1H), 7.50-7.40 (m, 2H), 7.82 (d, *J* = 8.1 Hz, 1H), 7.99 (s, 1H), 8.58 (s, 1H). ¹³C NMR (125 MHz, CDCl₃ + CD₃OD, δ ppm): 19.95, 21.43, 63.83, 65.72, 73.53, 95.64, 98.84, 113.31, 117.20, 127.17, 128.11, 131.12, 134.07, 134.36, 135.34, 139.36, 140.38, 148.35, 155.71, 158.05. HRMS (ESI) *m/z* calcd for C₂₁H₂₀N₃O₄ [M+H]⁺, 401.1261; found, 401.1258.

4.1.4.26. *N*-(3-chloro-4-fluorophenyl)-5-isopropoxy-2,3-dihydro-[1,4]dioxino[2,3-*ff*]quinazolin-10-amine (**e7**)

Yellow solid; yield: 50%; mp: 250-253 °C. ¹H NMR (500 MHz, CDCl₃ + CD₃OD, δ ppm): 1.49 (d, *J* = 6.0 Hz, 6H), 4.57-4.51 (m, 2H), 4.71-4.64 (m, 2H), 4.92 (dt, *J* = 12.1, 6.0 Hz, 1H), 7.25 (t, *J* = 8.7 Hz, 1H), 7.43 (s, 1H), 7.57-7.50 (m, 1H), 7.81 (dd, *J* = 6.4, 2.5 Hz, 1H), 8.57 (s, 1H). ¹³C NMR (125 MHz, CDCl₃ + CD₃OD, δ ppm): 21.41, 63.82, 65.71, 73.53, 95.37, 98.69, 116.82, 117.00, 121.56, 123.95, 126.27, 132.44, 134.07, 134.94, 139.48, 148.28, 155.48, 155.73, 157.47, 158.18. HRMS (ESI) *m/z* calcd for C₁₉H₁₇ClFN₃O₃ [M+H]⁺, 390.1021; found, 390.1017.

4.1.4.27. *N*-(3-ethynylphenyl)-5-isopropoxy-2,3-dihydro-[1,4]dioxino[2,3-*ff*]quinazolin-10-amine (**e8**)

Light yellow solid; yield: 46%; mp: 203-206 °C. ¹H NMR (400 MHz, DMSO-*d*₆, δ ppm): 1.39 (d, *J* = 6 Hz, 6H), 4.27 (s, 1H), 4.44 (s, 2H), 4.61 (s, 2H), 4.79 (dd, *J* = 6 Hz, 1H), 7.11 (s, 1H), 7.38 (d, *J* = 8 Hz, 1H), 7.47 (t, *J* = 8 Hz, 1H), 7.73 (d, *J* = 8 Hz, 1H), 7.85 (s, 1H), 8.69 (s, 1H), 10.35 (s, 1H). ¹³C NMR (100 MHz, DMSO-*d*₆, δ ppm): 21.91, 63.96, 65.75, 72.28, 81.70, 83.46, 96.66, 99.66, 122.54, 125.46, 127.61, 129.32, 129.57, 133.12, 138.07, 140.18, 150.39, 154.02, 157.94. HRMS (ESI) *m/z* calcd for C₂₁H₁₉N₃O₃ [M+H]⁺, 362.1505; found, 362.1505.

4.1.4.28. 5-(1-ethoxyethoxy)-*N*-(4-methoxy-3-nitrophenyl)-2,3-dihydro-[1,4]dioxino[2,3-*ff*]quinazolin-10-amine (**f1**)

Yellow solid; yield: 69%; mp: 241-244 °C. ¹H NMR (400 MHz, DMSO-*d*₆, δ ppm): 1.33(d, *J* = 6 Hz, 3H), 3.32 (s, 3H), 3.63-3.56 (m, 2H), 4.13 (s, 3H), 4.52 (s, 2H), 4.71 (s, 2H), 4.82-4.83 (m, 1H), 7.21 (s, 1H), 7.91 (s, 1H), 8.02 (d, *J* = 9.2 Hz, 1H), 8.91-8.87 (m, 2H), 11.06 (s, 1H). ¹³C NMR (125 MHz, CDCl₃ + CD₃OD, δ ppm): 15.89, 57.02, 59.12, 63.82, 65.69, 75.17, 75.99, 95.56, 100.17, 105.67, 117.18, 121.58, 132.26, 134.57, 134.91, 139.41, 144.63, 148.41, 149.45, 156.15, 157.43. HRMS (ESI) *m/z* calcd for C₂₁H₂₂N₄O₇ [M+H]⁺, 443.1567; found, 443.1558.

4.1.4.29. 5-(1-ethoxyethoxy)-*N*-(4-fluoro-3-nitrophenyl)-2,3-dihydro-[1,4]dioxino[2,3-*ff*]quinazolin-10-amine (**f2**)

Yellow solid; yield: 44%; mp: 245-246 °C. ¹H NMR (400 MHz, DMSO-*d*₆, δ ppm): 1.33(d, *J* = 6 Hz, 3H), 3.32 (s, 3H), 3.62-3.56 (m, 2H), 4.46 (s, 2H), 4.61 (s, 2H), 4.82 (q, *J* = 6 Hz, 1H), 7.25 (s, 1H), 7.73 (d, *J* = 10 Hz, 1H), 8.09-8.07 (m, 1H), 8.53-8.51 (m, 1H), 8.77 (s, 1H), 10.60 (s, 1H). ¹³C NMR (125MHz, CDCl₃ + CD₃OD, δ ppm): 15.93, 59.12, 63.93, 65.81, 75.20, 75.91, 95.33, 99.18, 118.92, 121.84, 131.69, 132.66,

134.41, 134.83, 137.05, 139.98, 148.35, 152.34, 154.45, 156.14, 158.37. HRMS (ESI) m/z calcd for $C_{20}H_{19}FN_4O_6$ $[M+H]^+$, 431.1367; found, 431.1363.

4.1.4.30. 5-(1-ethoxyethoxy)-N-(4-methyl-3-nitrophenyl)-2,3-dihydro-[1,4]dioxino[2,3-f]quinazolin-10-amine (f3)

Yellow solid; yield: 57%; mp: 248-250 °C. 1H NMR (400 MHz, DMSO- d_6 , δ ppm): 1.33 (d, J = 6 Hz, 3H), 2.52 (s, 3H), 3.31 (s, 3H), 3.62-3.60 (m, 2H), 4.47 (s, 2H), 4.64 (s, 2H), 4.83-4.82 (m, 1H), 7.21 (s, 1H), 7.61 (d, J = 8.4 Hz, 1H), 7.91 (d, J = 8.4 Hz, 1H), 8.35 (s, 1H), 8.79 (s, 1H), 10.66 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD , δ ppm): 16.00, 20.13, 59.33, 63.82, 65.85, 74.98, 75.84, 95.98, 99.04, 119.55, 128.17, 132.00, 133.52, 134.32, 134.76, 135.05, 139.30, 148.27, 149.04, 155.97, 158.11. HRMS (ESI) m/z calcd for $C_{21}H_{22}N_4O_6$ $[M+H]^+$, 427.1618; found, 427.1612.

4.1.4.31. 5-(5-(1-ethoxyethoxy)-2,3-dihydro-[1,4]dioxino[2,3-f]quinazolin-10-ylamino)-2-fluorobenzonitrile (f4)

Yellow solid; yield: 57%; mp: 251-253 °C. 1H NMR (500 MHz, $CDCl_3$ + CD_3OD , δ ppm): 1.42 (d, J = 6.3 Hz, 3H), 3.41 (s, 3H), 3.65 (ddd, J = 14.2, 10.8, 4.9 Hz, 2H), 4.51 (dd, J = 4.8, 3.0 Hz, 2H), 4.71-4.67 (m, 2H), 4.91 (td, J = 6.3, 3.5 Hz, 1H), 7.28 (d, J = 7.9 Hz, 1H), 7.36 (t, J = 8.7 Hz, 1H), 7.95 (ddd, J = 8.9, 4.5, 2.8 Hz, 1H), 8.11 (dd, J = 5.4, 2.7 Hz, 1H), 8.56 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD , δ ppm): 15.94, 59.12, 63.91, 65.79, 75.21, 75.88, 95.33, 99.15, 101.58, 113.17, 117.16, 129.08, 131.53, 133.02, 134.37, 134.82, 140.00, 148.36, 156.07, 158.35, 160.01, 162.07. HRMS (ESI) m/z calcd for $C_{21}H_{19}FN_4O_4$ $[M+H]^+$, 411.1469; found, 411.1468.

4.1.4.32. 5-(5-(1-ethoxyethoxy)-2,3-dihydro-[1,4]dioxino[2,3-f]quinazolin-10-ylamino)-2-methylbenzonitrile (f5)

Yellow solid; yield: 40%; mp: 256-259 °C. 1H NMR (500 MHz, $CDCl_3$ + CD_3OD , δ ppm): 1.44 (d, J = 6.3 Hz, 3H), 2.58 (s, 3H), 3.42 (d, J = 4.7 Hz, 3H), 3.65 (ddd, J = 14.2, 10.9, 4.8 Hz, 2H), 4.58-4.51 (m, 2H), 4.72-4.65 (m, 2H), 4.99-4.91 (m, 1H), 7.43 (d, J = 8.4 Hz, 1H), 7.55 (s, 1H), 7.82 (dd, J = 8.4, 2.3 Hz, 1H), 7.97 (dd, J = 5.7, 2.3 Hz, 1H), 8.56 (d, J = 3.0 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD , δ ppm): 15.97, 20.05, 59.30, 63.81, 65.80, 74.99, 75.79, 95.80, 99.01, 113.39, 117.23, 127.22, 128.16, 131.17, 134.27, 134.90, 139.42, 140.47, 148.24, 155.88, 158.10. HRMS (ESI) m/z calcd for $C_{22}H_{22}N_4O_4$ $[M+H]^+$, 407.1719; found, 407.1723.

4.1.4.33. N-(3-chloro-4-fluorophenyl)-5-((1-methoxypropan-2-yl)oxy)-2,3-dihydro-[1,4]dioxino[2,3-f]quinazolin-10-amine (g1)

Light yellow solid; yield: 62%; mp: 255-257 °C. 1H NMR (500 MHz, $CDCl_3$ + CD_3OD , δ ppm): 2.30-2.14 (m, 1H), 2.48 (td, J = 14.4, 7.7 Hz, 1H), 3.94 (td, J = 8.3, 4.9 Hz, 1H), 4.15-4.02 (m, 4H), 4.52 (s, 2H), 4.70 (s, 2H), 5.28 (s, 1H), 7.35-7.23 (m, 2H), 7.58-7.51 (m, 1H), 7.88-7.80 (m, 1H), 8.57 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD , δ ppm): 32.67, 63.82, 65.82, 67.19, 72.57, 80.28, 95.36, 99.33, 116.85, 121.35, 124.31, 126.58, 132.50, 134.10, 134.62, 139.96, 148.37, 155.09, 155.56, 157.55, 158.34. HRMS (ESI) m/z calcd for $C_{20}H_{17}ClFN_3O_4$ $[M+H]^+$, 418.0970; found, 418.0971.

4.1.4.34. N-(3-ethynylphenyl)-5-((1-methoxypropan-2-yl)oxy)-2,3-dihydro-[1,4]dioxino[2,3-f]quinazolin-10-amine (g2)

Light yellow solid; yield: 59%; mp: 241-243 °C. 1H NMR (500 MHz, $CDCl_3$ + CD_3OD , δ ppm): 2.23 (dd, J = 13.0, 6.5 Hz, 1H), 2.48 (dd, J = 14.0, 6.2 Hz, 1H), 3.30 (d, J = 4.0 Hz, 1H), 3.94 (td, J = 8.5, 4.9 Hz, 1H), 4.16-4.01 (m, 4H), 4.53 (s, 2H), 4.70 (d, J = 2.2 Hz, 2H), 5.27 (s, 1H), 7.29 (s, 1H), 7.44 (d, J =

4.8 Hz, 2H), 7.70-7.64 (m, 1H), 7.81 (s, 1H), 8.56 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD , δ ppm): 32.67, 63.80, 65.83, 67.19, 72.58, 78.55, 80.21, 82.37, 95.53, 99.44, 123.32, 124.39, 127.26, 129.21, 130.54, 134.01, 134.87, 136.01, 139.93, 148.49, 154.91, 158.20. HRMS (ESI) m/z calcd for $C_{22}H_{19}N_3O_4$ $[M+H]^+$, 390.1453; found, 390.1457.

4.1.4.35. N-(3-chloro-4-fluorophenyl)-5-((1-methoxypropan-2-yl)oxy)-2,3-dihydro-[1,4]dioxino[2,3-f]quinazolin-10-amine (h1)

Light yellow solid; yield: 62%; mp: 256-258 °C. 1H NMR (400 MHz, DMSO- d_6 , δ ppm): 1.72-1.69 (m, 2H), 2.10-2.07 (m, 2H), 3.56-3.50 (m, 2H), 3.92-3.89 (m, 2H), 4.60 (br, 2H), 4.61 (br, 2H), 4.77 (dd, 1H, J = 4.4 Hz), 7.30 (s, 1H), 7.54 (t, 1H, J = 8.9 Hz), 7.65-7.64 (m, 1H), 7.95-7.92 (m, 1H), 8.75 (s, 1H), 10.51 (s, 1H). HRMS (ESI) m/z calcd for $C_{21}H_{19}ClFN_3O_4$ $[M+H]^+$, 432.1126; found, 432.1133.

4.1.4.36. N-(3-ethynylphenyl)-5-((1-methoxypropan-2-yl)oxy)-2,3-dihydro-[1,4]dioxino[2,3-f]quinazolin-10-amine (h2)

White solid; yield, 50%; mp: 252-254 °C. 1H NMR (400 MHz, DMSO- d_6 , δ ppm): 1.71-1.69 (m, 2H), 2.11-2.07 (m, 2H), 3.55-3.51 (m, 2H), 3.92-3.89 (m, 2H), 4.42 (s, 2H), 4.62 (s, 2H), 4.76 (dd, J = 4.4 Hz, 1H), 7.31 (s, 1H), 7.42 (d, J = 8 Hz, 1H), 7.49 (t, J = 8.0 Hz, 1H), 7.69 (d, J = 8 Hz, 1H), 7.79 (s, 1H), 8.75 (s, 1H), 10.53 (s, 1H). ^{13}C NMR (100MHz, DMSO- d_6 , δ ppm): 31.77, 64.08, 64.89, 65.86, 74.46, 81.92, 83.29, 95.51, 99.55, 122.62, 126.31, 128.52, 129.64, 130.09, 133.45, 135.53, 137.58, 140.77, 149.66, 153.87, 158.47. HRMS (ESI) m/z calcd for $C_{23}H_{21}N_3O_4$ $[M+H]^+$, 404.1610; found, 404.1612.

4.1.4.37. N-(3-chloro-4-fluorophenyl)-5-((1-methoxypropan-2-yl)oxy)-2,3-dihydro-[1,4]dioxino[2,3-f]quinazolin-10-amine (i1)

Light yellow solid; yield, 58%; mp: 262-265°C. 1H NMR (400 MHz, DMSO- d_6 , δ ppm): 1.33 (d, J = 6.4 Hz, 3H), 3.31 (s, 3H), 3.60-3.58 (m, 2H), 4.45 (br, 2H), 4.60 (br, 2H), 4.83-4.78 (m, 1H), 7.21 (s, 1H), 7.54 (t, J = 8.9 Hz, 1H), 7.66-7.63 (m, 1H), 7.95-7.92 (m, 1H), 8.75 (s, 1H), 10.52 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD , δ ppm): 16.00, 59.30, 63.79, 65.76, 75.02, 75.70, 96.02, 99.00, 116.97, 121.52, 123.91, 126.20, 132.49, 134.11, 135.23, 139.41, 148.54, 155.41, 155.69, 157.40, 158.18. HRMS (ESI) m/z calcd for $C_{20}H_{19}ClFN_3O_4$ $[M+H]^+$, 420.1126; found, 420.1123.

4.1.4.38. N-(3-ethynylphenyl)-5-((1-methoxypropan-2-yl)oxy)-2,3-dihydro-[1,4]dioxino[2,3-f]quinazolin-10-amine (i2)

Light yellow solid; yield, 55%; mp: 259-261 °C. 1H NMR (400 MHz, DMSO- d_6 , δ ppm): 1.29 (d, J = 6.4 Hz, 3H), 3.31 (s, 3H), 3.57-3.53 (m, 2H), 4.20 (s, 1H), 4.41 (br, 2H), 4.60 (br, 2H), 4.88-4.83 (m, 1H), 6.94 (s, 1H), 7.22-7.20 (m, 1H), 7.38 (t, J = 8 Hz, 1H), 7.89-7.87 (m, 1H), 8.07 (s, 1H), 8.43 (s, 1H), 9.65 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD , δ ppm): 16.44, 59.28, 63.70, 65.04, 74.05, 75.18, 83.44, 101.46, 102.93, 122.27, 122.63, 124.94, 127.51, 128.90, 132.20, 138.31, 138.77, 146.19, 152.40, 153.70, 156.56. HRMS (ESI) m/z calcd for $C_{22}H_{21}N_3O_4$ $[M+H]^+$, 392.1610; found, 392.1608.

4.1.4.39. N-(3-chloro-4-fluorophenyl)-5-(3-morpholinopropoxy)-2,3-dihydro-[1,4]dioxino[2,3-f]quinazolin-10-amine (j1)

White solid; yield: 65%; mp: 278-280 °C. 1H NMR (500 MHz, $CDCl_3$ + CD_3OD , δ ppm): 2.16-2.04 (m, 2H), 2.59 (dd, J = 26.1, 18.6 Hz, 6H), 3.74 (s, 4H), 4.22 (t, J = 6.2 Hz, 2H), 4.46 (s, 2H), 4.60 (s, 2H), 6.85 (s, 1H), 7.17 (t, J = 8.8 Hz, 1H), 7.52-7.48 (m, 1H), 7.93 (d, J = 6.4 Hz, 1H), 8.39 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD , δ ppm): 25.72, 53.47, 55.32, 63.75, 65.23,

66.60, 67.26, 100.86, 101.32, 116.36, 120.68, 122.21, 124.46, 131.99, 135.06, 138.44, 145.76, 153.23, 153.78, 155.78, 156.87. HRMS (ESI) m/z calcd for $C_{23}H_{24}ClFN_4O_4$ $[M+H]^+$, 475.1548; found, 475.1547.

4.1.4.40. *N*-(3-bromophenyl)-5-(3-morpholinopropoxy)-2,3-dihydro-[1,4]dioxino[2,3-*f*]quinazolin-10-amine (j2)

White solid; yield: 60%; mp: 282-283 °C. 1H NMR (500 MHz, $CDCl_3$ + CD_3OD , δ ppm): 2.15-2.07 (m, 2H), 2.59 (dd, J = 26.1, 18.5 Hz, 6H), 3.74 (t, J = 4.5 Hz, 4H), 4.22 (t, J = 6.3 Hz, 2H), 4.49-4.42 (m, 2H), 4.62-4.58 (m, 2H), 6.85 (s, 1H), 7.25 (d, J = 6.4 Hz, 2H), 7.60 (d, J = 6.6 Hz, 1H), 8.03 (s, 1H), 8.41 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD , δ ppm): 25.73, 53.48, 55.32, 63.75, 65.23, 66.61, 67.26, 100.88, 101.46, 120.55, 122.30, 124.80, 126.99, 130.08, 132.00, 138.39, 139.74, 145.80, 153.25, 153.75, 156.74. HRMS (ESI) m/z calcd for $C_{23}H_{25}BrN_4O_4$ $[M+H]^+$, 501.1137; found, 501.1138.

4.1.4.41. *N*-(3-chlorophenyl)-5-(3-morpholinopropoxy)-2,3-dihydro-[1,4]dioxino[2,3-*f*]quinazolin-10-amine (j3)

White solid; yield: 62%; mp: 280-282 °C. 1H NMR (500 MHz, $CDCl_3$ + CD_3OD , δ ppm): 2.16 - 2.04 (m, 2H), 2.59 (dd, J = 26.3, 18.6 Hz, 6H), 3.74 (t, J = 4.5 Hz, 4H), 4.22 (t, J = 6.3 Hz, 2H), 4.47 (dd, J = 4.7, 2.9 Hz, 2H), 4.60 (dd, J = 4.8, 2.9 Hz, 2H), 6.85 (s, 1H), 7.11 (dd, J = 8.0, 1.0 Hz, 1H), 7.31 (t, J = 8.1 Hz, 1H), 7.54-7.51 (m, 1H), 7.90 (t, J = 1.9 Hz, 1H), 8.41 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD , δ ppm): 25.72, 53.47, 55.32, 63.75, 65.23, 66.60, 67.25, 100.87, 101.47, 120.05, 121.95, 124.03, 129.78, 132.01, 134.36, 138.41, 139.61, 145.80, 153.24, 153.76, 156.77. HRMS (ESI) m/z calcd for $C_{23}H_{25}ClN_4O_4$ $[M+H]^+$, 457.1643; found, 457.1648.

4.1.4.42. *N*-(3-fluorophenyl)-5-(3-morpholinopropoxy)-2,3-dihydro-[1,4]dioxino[2,3-*f*]quinazolin-10-amine (j4)

White solid; yield: 60%; mp: 275-277 °C. 1H NMR (500 MHz, $CDCl_3$ + CD_3OD , δ ppm): 2.15-2.06 (m, 2H), 2.60 (dd, J = 26.3, 18.6 Hz, 6H), 3.74 (t, J = 4.6 Hz, 4H), 4.22 (t, J = 6.3 Hz, 2H), 4.47 (dd, J = 4.9, 3.0 Hz, 2H), 4.61 (dd, J = 4.8, 3.0 Hz, 2H), 6.89-6.77 (m, 2H), 7.33 (t, J = 5.3 Hz, 2H), 7.84-7.73 (m, 1H), 8.41 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD , δ ppm): 25.71, 53.47, 55.31, 63.74, 65.23, 66.57, 67.22, 100.00, 100.81, 101.47, 109.02, 109.23, 110.54, 117.19, 129.82, 132.02, 138.42, 140.02, 145.75, 153.19, 153.75, 156.75, 162.96. HRMS (ESI) m/z 441.1938 calcd for $C_{23}H_{25}FN_4O_4$ $[M+H]^+$, found, 441.1940.

4.1.4.43. 5-(3-morpholinopropoxy)-*N*-(3-(trifluoromethyl)phenyl)-2,3-dihydro-[1,4]dioxino[2,3-*f*]quinazolin-10-amine(j5)

White solid; yield: 56%; mp: 268-270 °C. 1H NMR (500 MHz, $CDCl_3$ + CD_3OD , δ ppm): 2.16-2.06 (m, 2H), 2.60 (dd, J = 26.6, 18.9 Hz, 6H), 3.81-3.68 (m, 4H), 4.22 (t, J = 6.3 Hz, 2H), 4.52-4.44 (m, 2H), 4.63-4.61 (m, 2H), 6.86 (s, 1H), 7.39 (d, J = 7.8 Hz, 1H), 7.52 (t, J = 8.0 Hz, 1H), 7.90 (d, J = 8.2 Hz, 1H), 8.13 (s, 1H), 8.42 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD , δ ppm): 25.71, 53.47, 55.31, 63.76, 65.26, 66.57, 67.23, 100.81, 101.44, 118.58, 120.41, 122.94, 124.02, 129.31, 131.11, 132.07, 138.41, 139.10, 145.81, 153.17, 153.83, 156.80. HRMS (ESI) m/z calcd for $C_{26}H_{25}F_3N_4O_4$ $[M+H]^+$, 491.1906; found, 491.1910.

4.2. EGFR inhibitory assay

Kinase-Glo luminescent kinase assay is a homogeneous non-radioactive method for determining the activity of purified kinases by quantifying the amount of ATP remaining in solution following a kinase reaction. Target compounds and positive compound gefitinib were dissolved in DMSO as 10 mM stock

solution, then dilute it to 100 μ M with DMSO and transferred to the dose plate. The compounds were serially diluted with DMSO in 5-fold. Then each concentration was diluted 10-fold with reaction buffer (containing 25 mM HEPES, 10 mM $MgCl_2$, 100 μ g/mL BSA, 0.01% TritonX-100, 2.5 mM DTT and adjusted pH to 7.4) to obtain a 10 $\times$ final concentration. Transfer compounds with its concentration ranging from 10 μ M to 0.0006 μ M to assay plate for EGFR activity test with a volume of 1 μ L/well. For HPE (hundred percent effect: No kinase and no compound, but containing ATP, substrate and 1% DMSO) and ZPE (zero percent effect: No compound but containing kinase, ATP, substrate and 1% DMSO) well, dilute 2 μ L DMSO 10-fold with reaction buffer to obtain 10% DMSO solution. Then transfer it to the assay plate, 1 μ L/well. Procedure for kinase reaction is: 1) Add 10 $\times$ compound to the assay plate in a 384-well plate layout, 1 μ L/well. For the HPE and ZPE wells, equal volume (1 μ L/well) of 10% DMSO was added to the 384-well assay plate; 2) Add 2.5 $\times$ kinase EGFR into the assay plate as 384-well plate layout, 4 μ L/well. For HPE wells, an equal volume (4 μ L/well) of assay buffer was added to the 384-well assay plate; 3) Centrifuge the assay plate with 1000 rpm for 1 min to mix them; 4) Pre-incubate the assay plate at 30 °C for 30 min; 5) Mix equal volume of 4 $\times$ ATP and 4 $\times$ substrate to obtain 2 $\times$ ATP-substrate mixtures; 6) Add 2 $\times$ ATP-substrate mixture to the assay plate, 5 μ L/well; 7) Centrifuge the assay plate at 1000 rpm for 1 min to mix; 8) Incubate the plate for one hour at 30 °C; 9) Kinase glo plus was added to each well (10 μ L/well), and then incubated the assay plate for 20 min at 27 °C; 10) Read luminescence signal with Envision. The raw data were analyzed by Prism 5.0 and the inhibitory rate was calculated by the following formula: Compound inhibitory rate = ("compound" reading-ZPE)/(HPE-ZPE)*100%.

4.3. Cell proliferative assay

The antiproliferative activity was determined using cell-counting kit-8 assay (Dojindo, Japan). H358 and A549 cells lines were seeded at a density of 8×10^3 cells/well in 96-well microtiter plates and were incubated at 37 °C overnight in a humidified incubator containing 5% CO_2 . Cells were dosed with compounds at final concentrations ranging from 0.025 μ M to 80 μ M in each well of the plate. After 48 h, 10 μ L of the CCK-8 solution was added and incubated for 1-4 h. Cell survival was determined by measuring the absorbance at 470 nm using a microplate reader. A calibration curve was prepared using the data obtained from the wells that contain known numbers of viable cells to determine the IC_{50} of the target compounds.

4.4. Docking study

The molecular docking of compound **b1** into the three-dimensional EGFR complex structure (PDB code: 1M17.pdb, downloaded from the PDB) was performed using CDocker. Unwanted water and ligands were removed by the DS4.0. The structures of the molecules were drawn by Gaussian03 software, then optimized the molecules to the minimum energy conformation used the semi-empirical AM1 method. Docking procedure was performed by AutoDock 4.0 software with the help of Autodock Tools.

Acknowledgments

The authors would like to acknowledge financial support from the National Science Foundation of China (21272020), the Project of Construction of Innovative Teams and Teacher Career Development for Universities and Colleges under Beijing Municipality (IDHT20140504), Beijing Key Laboratory for

Green Catalysis and Separation. Mingzhou Guo and Meiying Zhang (Chinese Peoples Liberat Army Gen Hosp) finished cytotoxicity test.

References

- Zhang, H.; Berezov, A.; Wang, Q.; Zhang, G.; Drebin, J.; Murali, R.; Greene, M. I. *J. Clin. Invest.* **2007**, *117*, 2051.
- Olayioye, M. A.; Neve, R. M.; Lane, H. A.; Hynes, N. E. *EMBO J.* **2000**, *19*, 3159.
- Siegel, R.; Naishadham, D.; Jemal, A. *Ca-Cancer J. Clin.* **2013**, *63*, 11.
- Herbst, R. S.; Heymach, J. V.; Lippman, S. M. *New Engl. J. Med.* **2008**, *359*, 1367.
- Sharma, S. V.; Bell, D. W.; Settleman, J.; Haber, D. A. *Nat. Rev. Cancer.* **2007**, *7*, 169.
- Sequist, L. V.; Martins, R. G.; Spigel, D.; Grunberg, S. M.; Spira, A.; Janne, P. A.; Joshi, V. A. *J. Clin. Oncol.* **2008**, *26*, 2442.
- Barker, A. J.; Gibson, K. H.; Grundy, W.; Godfrey, A. A.; Barlow, J. J.; Healy, M. P.; Woodburn, J. R.; Ashton, S. E.; Curry, B. J.; Sarlett, L.; Henthorn, L.; Richards, L. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1911.
- Moyer, J. D.; Barbacci, E. G.; Iwata, K. K.; Arnold, L.; Boman, B.; Cunningham, A.; Di Orio, C.; Doty, J.; Morin, M. J.; Moyer, M. P.; Neveu, M.; Pollack, V. A.; Pustilnick, L. R.; Reynolds, M. M.; Sloan, D.; Theleman, A.; Miller, P. *Caner Res.* **1997**, *57*, 4838.
- Ladanyi, M.; Pao, W. *Mod. Pathol.* **2008**, *21*, 16.
- Pao, W.; Miller, V. A.; Politi, K. A.; Riely, G. J.; Somwar, R.; Zakowski, M. F.; Kris, M. G.; Varmus, H. *PLoS Med.* **2005**, *2*, 73.
- Kobayashi, S.; Boggon, T. J.; Dayaram, T.; Janne, P. A.; Kocher, O.; Meyerson, M.; Johnson, B. E.; Eck, M. J.; Tenen, D. G.; Halmos, B. *New Engl. J. Med.* **2005**, *352*, 786.
- Pao, W.; Miller, V. A.; Politi, K. A.; Riely, G. J.; Somwar, R.; Zakowski, M. F.; Kris, M. G.; Varmus, H. *PLoS Med.* **2005**, *2*, 1.
- Gil, S.; Goetgheluck, J.; Paci, A.; Broutin, S.; Friard, S.; Couderc, L.J.; Ayoubi, J.M.; Picone, O.; Tcherakian, C. *Lung Cancer* **2014**, *85*, 481.
- Zhou, W.; Liu, X.; Tu, Z.; Ku, X.; Bai, F.; Zhao, Z.; Xu, Y.; Ding, K.; Li, H. *J. Med. Chem.* **2013**, *56*, 7821.
- Karikios, D. J.; Boyer, M. J. *J. Clin. Invest.* **2012**, *2*, 317.
- Ou, S. H. I. *Crit. Rev. Oncol. Hematol.* **2012**, *83*, 407.
- Ranson, M.; Pao, W.; Kim, D. W.; Kim, S. W.; Ohe, Y.; Felip, E.; Planchard, D.; Ghiorghiu, S.; Cantarini, M.; Janne, P. A. *Eur. J. Cancer* **2013**, *49*, 15.
- Sequist, L. V.; Soria, J. C.; Gadgeel, S.; Wakelee, H.; Camidge, D. R.; Varga, A.; Fidias, P.; Wozniak, A. J.; Neal, J. W.; Doebele, R. C.; Garon, E. B.; Jaw-Tsai, S.; Stern, J. C.; Allen, A.; Goldman, J. W. *J. Clin. Oncol.* **2013**, *31*, 2524.
- Cross, D. A.; Ashton, S. E.; Ghiorghiu, S.; Eberlein, C.; Nebhan, C. A.; Spitzler, P. J.; Orme, J. P.; Finlay, M. R.; Ward, R. A.; Mellor, M. J.; Hughes, G.; Rahi, A.; Jacobs, V. N.; Red Brewer, M.; Ichihara, E.; Sun, J.; Jin, H.; Ballard, P.; Al-Kadhimi, K.; Rowlinson, R.; Klinowska, T.; Richmond, G. H.; Cantarini, M.; Kim, D. W.; Ranson, M. R.; Pao, W. *Cancer Discov.* **2014**, *4*, 1046.
- Tjin Tham Sjin, R.; Lee, K.; Walter, A. O.; Dubrovskiy, A.; Sheets, M.; Martin, T. S.; Labenski, M. T.; Zhu, Z.; Tester, R.; Karp, R.; Medikonda, A.; Chaturvedi, P.; Ren, Y.; Haringsma, H.; Etter, J.; Raponi, M.; Simmons, A. D.; Harding, T. C.; Niu, D.; Nacht, M.; Westlin, W. F.; Petter, R. C.; Allen, A.; Singh, J. *Mol. Cancer Ther.* **2014**, *13*, 1468.
- Jänne, P. A.; Ramaligam, S. S.; Yang, J. C. H.; Ahn, M. J.; Kim, D.-W.; Kim, S. W.; Planchard, D.; Ohe, Y.; Felip, E.; Watkins, C.; Cantarini, M.; Ghiorghiu, S.; Ranson, M. *J. Clin. Oncol.* **2014**, *32*, 8009.
- Evans, D. C.; Watt, A. P.; Nicoll-Griffith, D. A.; Baillie, T. A. *Chem. Res. Toxicol.* **2004**, *17*, 3.
- Potashman, M. H.; Duggan, M. E. *J. Med. Chem.* **2009**, *52*, 1231.
- Kalgutkar, A. S.; Dalvie, D. K. *Expert Opin. Drug Dis.* **2012**, *7*, 561.
- Li, D.; Lv, P.; Zhang, H.; Zhang, H.; Hou, Y.; Liu, K.; Ye, Y.; Zhu, H. *Bioorg. Med. Chem.* **2011**, *19*, 5012.
- Ballard, P.; Barlaam, B.; Bradbury, R. H.; Dishington, A.; Hennequin, L. F.; Kickinson, D. M.; Hollinsworth, I. M.; Kettle, J. G.; Klinowska, T.; Ogilvie, D. J.; Pearson, S. E.; Scott, J. S.; Suleman, A.; Whittaker, R.; Williams, E. J.; Wood, R.; Wright, L. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6326.
- Barlaam, B.; Ballard, P.; Bradbury, R.H.; Ducray, R.; Germain, H.; Kickinson, D.M.; Hudson, K.; Kettle, J. G.; linowska, T. K.; Magnien, F.; Ogilvie, D. J.; Olivier, A.; Pearson, S. E.; Scott, J. S.; Suleman, A.; Trigwell, C. B.; Vautier, M.; Whittaker, R. D.; Wood, R. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 674.
- Cheng, W.; Yuan, Y.; Qiu, N.; Peng, P.; Sheng, R.; Hu, Y. *Bioorg. Med. Chem.* **2014**, *22*, 6796.
- Bridges, A. J.; Zhou, H.; Cody, D. R.; Rewcastle, G. W.; McMichael, A.; Showalter, H. D.; Fry, D. W.; Kraker, A. J.; Denny, W. A. *J. Med. Chem.* **1996**, *39*, 267.
- Yun, C. H.; Boggon, T. J.; Li, Y. Q.; Woo, M. S.; Greulich, H.; Meyerson, M.; Eck, M. J. *Cancer Cell* **2007**, *11*, 217.
- Stamos, J.; Sliwkowski, M.X.; Eigenbrot, C. *J. Biol. Chem.* **2002**, *277*, 46265.