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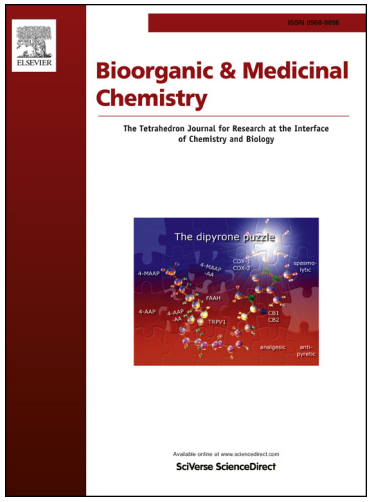

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Discovery of new [1,4]dioxino[2,3-f]quinazoline-based inhibitors of EGFR including the T790M/L858R mutant

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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 (IC_{50}) values of twenty-one compounds against $EGFR^{wt}$ were less than 50 nM, and those of six compounds were less than 10 nM. The IC_{50} values of eleven compounds against $EGFR^{T790M/L858R}$ were less than 100 nM. Among these, compound **b1** displayed the most potent inhibitory activity against $EGFR^{wt}$ (IC_{50} = 2.0 nM) and $EGFR^{T790M/L858R}$ (IC_{50} = 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.

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]

Gefitinib[7] and erlotinib[8] (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 (threonine^{790}→methionine^{790}) 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 $EGFR^{T790M/LR58R}$ mutant.

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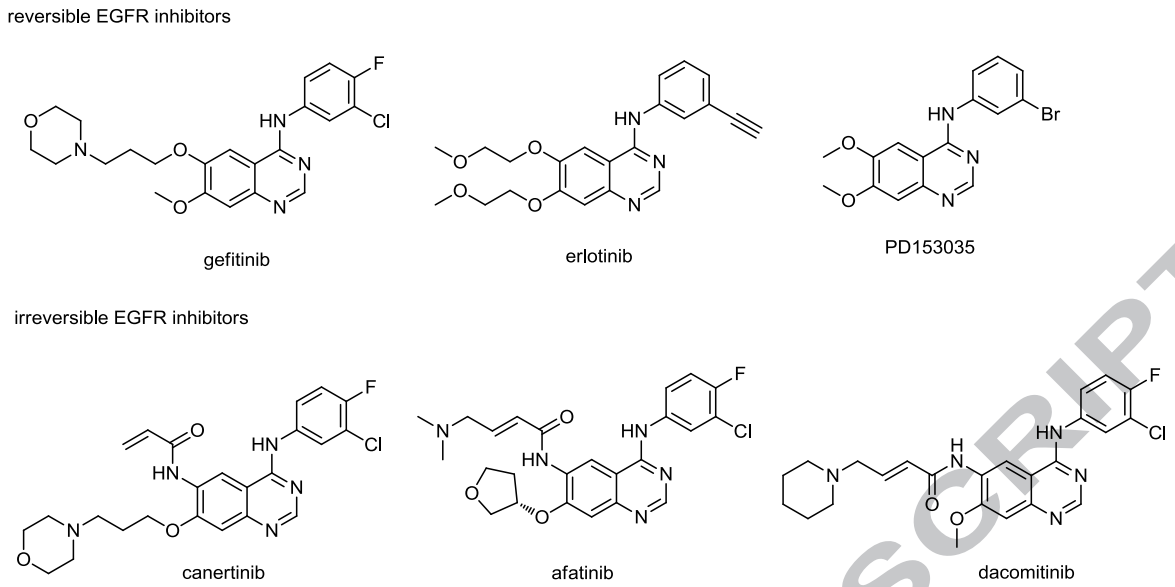


Figure 1. The chemical structures of epidermal growth factor receptor tyrosine kinase inhibitors.

To date, many studies have been targeted at finding new structures based on quinazolines 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, methoxylethoxyl etc. and then a larger secondary carbon chain or cyclic group in 7-position of quinazoline. Herein, we present new 2,3-dihidro-[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 ºC to give **3**, which reacted with 1-bromo-2-chloroethane to obtain **4**. The key intermediate **5** was achieved

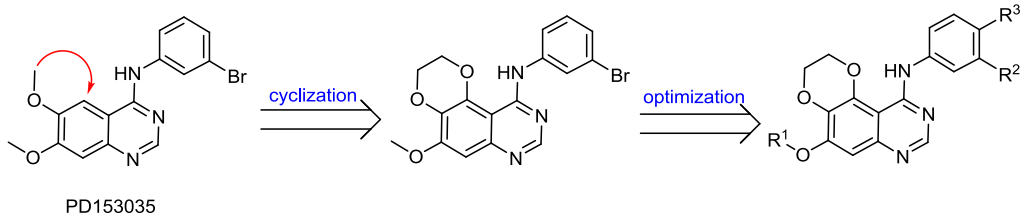
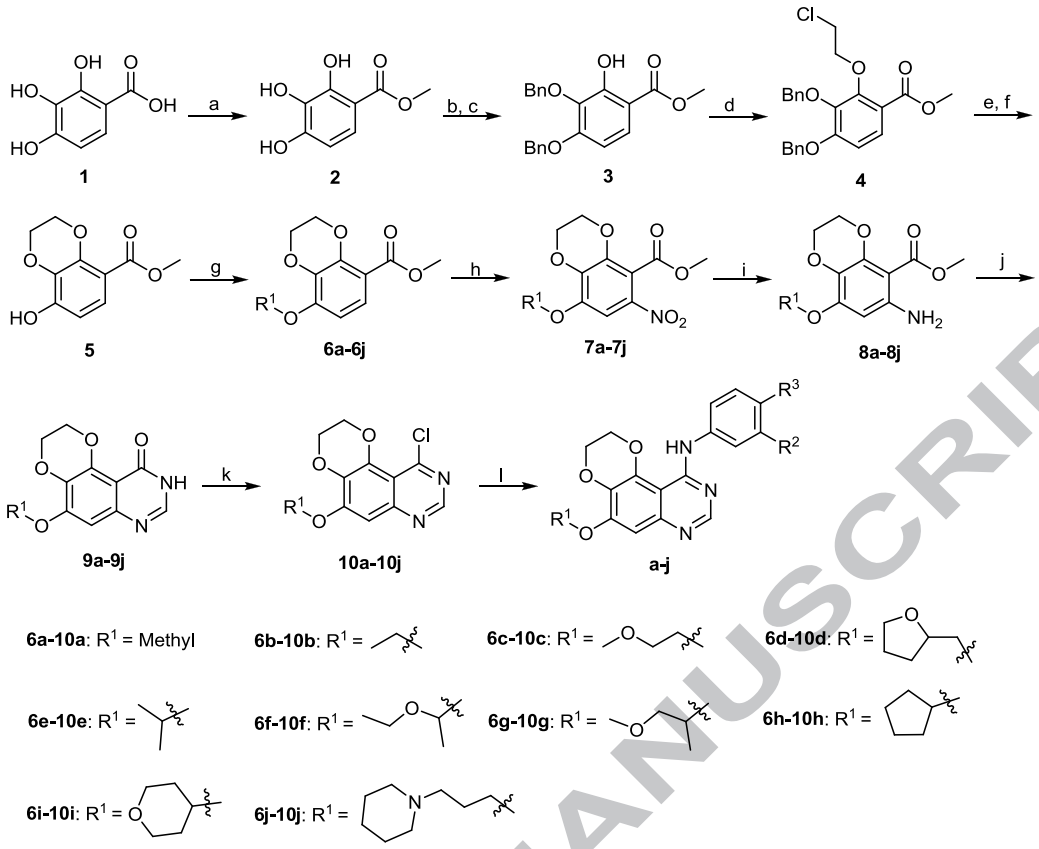


Figure 2. Design strategy of novel EGFR inhibitors.



Scheme 1. Reagants and conditions: a) CH_3I, $KHCO_3$, DMF, 40 ºC; b) BnCl, K_2CO_3, KI, DMF, 60 ºC; c) HCl/H_2O, HAc, 40 ºC, 1 h; d) 1-Bromo-2-chloroethane, K_2CO_3, DMF, 70 ºC; e) Pd/C, H_2, EtOH; f) K_2CO_3, DMF; g) R^1Br, K_2CO_3, DMF, 70 ºC; h) HNO_3/HAc; i) Pd/C, H_2, EtOH; j) Formamidine acetate, ethanol, reflux; k) $POCl_3$, 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 fumic HNO_3/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

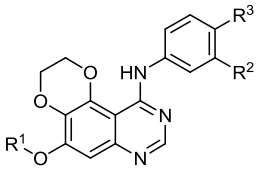
All new compounds (**a-i**) were evaluated for their ability to inhibit the autophosphorylation of $EGFR^{wt}$ and $EGFR^{T790M/L858R}$ mutant kinase using Kinase-Glo luminescent kinase assays. The results were shown in Table 1. The studied compounds displayed inhibition to $EGFR^{wt}$ with IC_{50} values 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 IC_{50} values ranging from 323.6 nM to 6.9 nM.

To evaluate the relationship of the length of the carbon chain of R^1 of targeted compounds and their inhibitory activities against EGFR, compounds **a1**-**c9** were synthesized. The R^1 of **a1**-**c9** were substituted by primary carbon chain from short to long that showed well or moderate inhibitory activities against $EGFR^{wt}$ (IC_{50} = 2.0-387.8 nM). When R^1 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 R^1 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 $EGFR^{wt}$ (IC_{50} = 2.0 nM) and $EGFR^{T790M/L858R}$ (IC_{50} = 6.9 nM). When R^1 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 R^2 is electron-withdrawing group, nitro or cyan, compounds **c5**-**c9** showed weakened activities against $EGFR^{wt}$ ($IC_{50} \geq$ 36.2 nM) and no activities against $EGFR^{T790M/L858R}$.

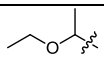
To extensively explore the SARs of the novel scaffold, compounds (**e1**-**e8**, **f1**-**f5** and **g1**-**g2**) with R^1 being substituted by secondary carbon chain were synthesized. **e1**-**e6** and **f1**-**f5**, whose R^2 was electron-withdrawing group, such as nitro or cyano, showed 10 times less 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-fluoro-phenylamino or 3-aminophenyl-acetylene, showed good inhibitory activities against $EGFR^{wt}$ as well as $EGFR^{T790M/L858R}$.

Upon introduction of heterocyclic fragments in R^1, five-member heterocyclic compounds **d1** (IC_{50} = 24.1 nM), **d2** (IC_{50} = 27.2 nM), **h1** (IC_{50} = 20.5 nM) and **h2** (IC_{50} = 24.1 nM) showed 2-fold improvement in inhibitory activities against $EGFR^{wt}$ compared to hexa-heterocyclic compounds **i1** (IC_{50} = 58.8 nM) and **i2** (IC_{50} = 57.8 nM). Unfortunately, all compounds with heterocyclic in R^1 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 $EGFR^{T790M/L858R}$



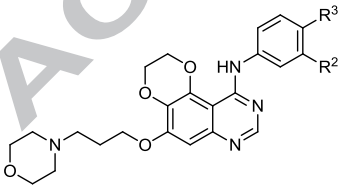
Compound	R^1	R^2	R^3	$EGFR^{wt}$ IC_{50} (nM)	$EGFR^{T790M/L858R}$ IC_{50} (nM)
a1	Me	Cl	F	22.5	1000
a2	Me	ethynyl	H	10.9	> 1000
b1		Cl	F	2.0	6.9
b2		ethynyl	H	9.2	21.5
b3		MeO-	H	4.4	70.0
b4		Cl	Me	6.7	33.8
b5		NO_2	H	9.7	71.0
b6		H	Me	3.6	123.5
c1		Cl	F	13.1	10.5
c2		ethynyl	H	21.0	8.1
c3		H	F	200.3	> 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 R^1 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 R^1, 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 pocket, so we introduced hydrophilic group morpholinopropoxy group as R^1 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 R^2 is F or CF_3, compounds **j4** and **j5** showed 4-fold less inhibitory activities against EGFR than **j1**, **j2** and **j3**.

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	CF3	H	97.18
gefitinib			17.98

2.2.2. Antiproliferation assay

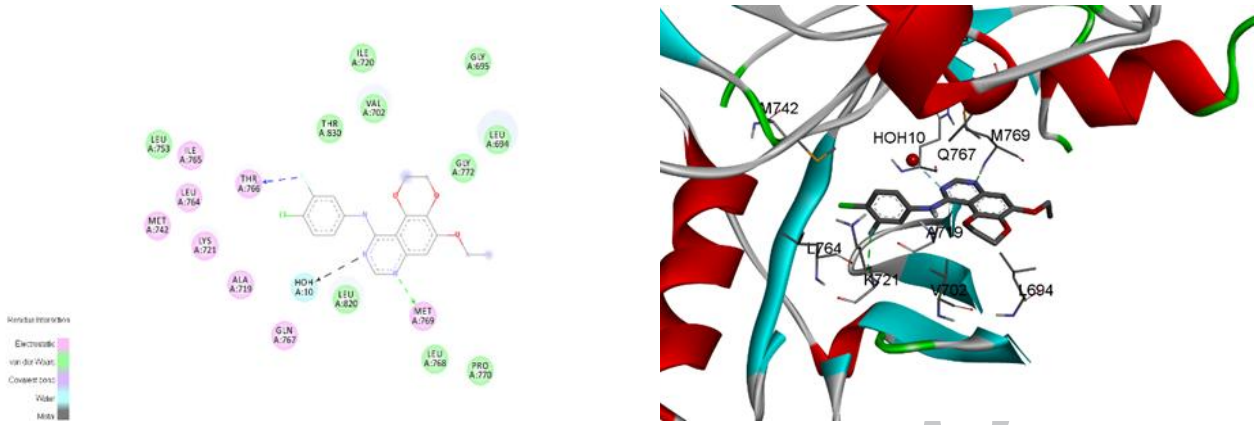
Seven compounds with excellent inhibitory activities against EGFR were chosen to evaluate their antiproliferative activities against H358 cell and A549 cell and the result was demonstrated in Table 3.

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 IC_{50} values against H358 and A549 cells



A B

Figure 3. 2D model of compound **b1** binding into the active site of EGFR (**A**); 3D model of compound **b1** binding into the active site of EGFR (**B**).

compared to the positive control gefitinib and erlotinib. However, compound **b1**, who displayed the most potent inhibitory activity against EGFR, did not show good antiproliferative activity to H358 and A549 cell. Compound **e8** exhibited the most potent antiproliferative activity against the H358 (IC_{50} = 4.54 μM) and A549 (IC_{50} = 6.08 μM) cell lines compared to positive controls. The reason may be explained that **e8** could entry the cell easier than **b1** do**.**

2.2.3. Cytotoxicity test

As shown in Table 4, compounds with good inhibitory activities against EGFR were evaluated for their toxicity against the human kidney epithelial 293T cell line using the MTT assay; these compounds were tested at multiple doses to study the viability of 293T cell. The median cytotoxic concentration (CC_{50}) showed that most of the tested compounds displayed almost no cytotoxicity in vitro against 293T cells.

Table 4. The median cytotoxic concentration (CC_{50}) data of texted compounds

compound	CC_{50}, μM
a2	11.73
b1	37.03
b2	28.22
b3	25.27
b5	42.00
b6	27.42
c2	22.09
c3	25.93
c4	20.81
d2	40.18
e8	22.14
erlotinib	28.22

2.2.4. Docking study

The most active compound **b1** was docked into the three dimensional EGFR active site (1M17.pdb) using Autodock software package (version 4.0) with the help of Autodock Tools. As shown in Figure 3, the amino acid residues which were modeled to be within the interaction distance between EGFR and compound **b1** were labeled. In the proposed binding mode, compound **b1** was nicely bound to the ATP binding pocket of EGFR through the hydrogen bond interaction and the hydrophobic interaction. The N-1 of the quinazoline forms an H-bond with the Met769 backbone nitrogen. The 3-chloro-4-fluoro phenylamino substituent extends into the hydrophobic pocket in the back of the ATP-binding cleft. A water (HOH-10) molecule-mediated hydrogen bonding interaction is observed between quinazoline nitrogen atom (N-3) and other amino acid. Fluoro atom forms H-bond with Thr766. This may explain why compounds with substituent 3-chloro-4-fluoro phenylamino showed the most effective inhibitory activity towards EGFR kinase.

3. Conclusion

A novel series of reversible and noncovalent 2,3-dihydro-[1,4]dioxino[2,3-f]quinazoline derivatives were designed, synthesized and evaluated as potential EGFR tyrosine kinase inhibitors. Seven compounds (**b1**, **b2**, **b4**, **c1**, **c2**, **e7** and **e8**) showed good inhibitory activities against both $EGFR^{wt}$ (IC_{50} < 30 nM) and $EGFR^{T790M/L858R}$ (IC_{50} < 50 nM), as well as significant antiproliferative activities against H358 and A549 cells. Most of the tested compounds displayed almost no cytotoxicity in vitro against 293T cell compared with the positive control erlotinib's CC_{50} values. Docking study showed that compound **b1** could be nicely bound to the ATP binding pocket of EGFR. The results have important implications for further design and development of more potent noncovalent reversible EGFR inhibitors. Further work based on these structures is in progress.

4. Experiments

4.1. Materials and methods

The reagents were purchased and used without further purification. Melting points were determined on a MP120 melting point apparatus (Hanon instruments Corp., Jinan, China) and are as read. The ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker 400 MHz Avance or on a Bruker 500 MHz Avance spectrometer with TMS and solvent signals allotted as internal standards. The chemical shifts are reported in

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 ºC 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 ºC 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 $POCl_3$ (15 mL) was heated at reflux temperature for 3 h. The solvent were removed under reduced pressure. The residue was dissolved in CH_2Cl_2 (50 ml) and the organic layer was washed with cold aqueous $NaHCO_3$ solution and brine, and dried over $MgSO_4$, 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 ºC. ^{1}H NMR (500 MHz, $CDCl_3$ + CD_3OD, 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); ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD, 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 $C_{17}H_{13}ClFN_3O_3$ $[M+H]^+$, 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 ºC. ^{1}H NMR (500 MHz, $CDCl_3$ + CD_3OD, 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). ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD, 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 $C_{19}H_{15}N_3O_3$ $[M+H]^+$, 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 ºC. ^{1}H 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). ^{13}C 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 $C_{18}H_{15}ClFN_3O_3$ $[M+H]^+$, 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 ºC. ^{1}H NMR (500 MHz, $CDCl_3$ + CD_3OD, 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). ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD, 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 $C_{20}H_{17}N_3O_3$ $[M+H]^+$, 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 ºC. ^{1}H NMR (500 MHz, $CDCl_3$ + CD_3OD, 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). ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD, 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 $C_{19}H_{19}N_3O_4$ $[M+H]^+$, 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 ºC. ^{1}H NMR (500 MHz, $CDCl_3$ + CD_3OD, 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). ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD, 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 $C_{19}H_{18}ClN_3O_3$ $[M+H]^+$, 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 ºC. ^{1}H NMR (500 MHz, $CDCl_3$ + CD_3OD, 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). ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD, 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 $C_{18}H_{16}N_4O_5$ $[M+H]^+$, 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 ºC. ^{1}H NMR (500 MHz, $CDCl_3$ + CD_3OD, 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). ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD, 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 $C_{19}H_{19}N_3O_3$ $[M+H]^+$, 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 ºC. ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD, 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). ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD, 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 $C_{19}H_{17}ClFN_3O_4$ $[M+H]^+$, 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 ºC. ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD, 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). ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD, 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 $C_{21}H_{19}N_3O_4$ $[M+H]^+$, 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 ºC. ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD, 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). ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD, 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 $C_{19}H_{18}FN_3O_4$ $[M+H]^+$, 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 ºC. ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD, 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). ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD, 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 $C_{20}H_{21}N_3O_5$ $[M+H]^+$, 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 ºC. ^{1}H 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 $C_{20}H_{20}N_4O_7$ $[M+H]^+$, 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 ºC. ^{1}H 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). ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD, 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 $C_{19}H_{17}FN_4O_6$ $[M+H]^+$, 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 ºC. ^{1}H NMR (500 MHz, $CDCl_3$ + CD_3OD, 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). ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD, 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 $C_{20}H_{20}N_4O_6$ $[M+H]^+$, 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 ºC. ^{1}H NMR (500 MHz, $CDCl_3$ + CD_3OD, 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). ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD, 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 $C_{19}H_{17}ClN_4O_6$ $[M+H]^+$, 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 ºC. ^{1}H NMR (500 MHz, $CDCl_3$ + CD_3OD, 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). ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD, 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 $C_{20}H_{17}FN_4O_4$ $[M+H]^+$, 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 ºC. ^{1}H 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 $C_{21}H_{19}ClFN_3O_4$ $[M+H]^+$, 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 ºC. ^{1}H 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). ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD, 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 $C_{23}H_{21}N_3O_4$ $[M+H]^+$, 404.1610; found, 404.1611.

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

Yellow solid; yield: 50%; mp: 216-219 ºC. ^{1}H NMR (500 MHz, $CDCl_3$ + CD_3OD, 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). ^{1}C NMR (125 MHz, $CDCl_3$ + CD_3OD, 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 $C_{20}H_{20}N_4O_6[M+H]^+$, 413.1461; found, 413.1453.

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

Yellow solid; yield, 75%; mp: 223-226 ºC. ^{1}H NMR (500 MHz, $CDCl_3$ + CD_3OD, 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 $C_{19}H_{17}FN_4O_5$ $[M+H]^+$, 401.1261; found, 401.1258.

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

Yellow solid; yield: 75%; mp: 230-234 ºC. ^{1}H NMR (500 MHz, $CDCl_3$ + CD_3OD, 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). ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD, 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 $C_{20}H_{20}N_4O_5$ $[M+H]^+$, 397.1512; found, 397.1507.

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

Yellow solid; yield: 67%; mp: 229-232 ºC. ^{1}H NMR (500 MHz, $CDCl_3$ + CD_3OD, 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). ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD, 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 $C_{19}H_{17}ClN_4O_5$ $[M+H]^+$, 417.0966; found, 417.0963.

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

Yellow solid; yield: 55%; mp: 250-263 ºC. ^{1}H NMR (500 MHz, $CDCl_3$ + CD_3OD, 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). ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD, 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 $C_{20}H_{17}FN_3O_4$ $[M+H]^+$, 381.1363; found, 381.1360.

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

Yellow solid; yield: 67%; mp: 229-236 ºC. ^{1}H NMR (500 MHz, $CDCl_3$ + CD_3OD, 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). ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD, 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 $C_{21}H_{20}N_3O_4$ $[M+H]^+$, 401.1261; found, 401.1258.

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

Yellow solid; yield: 50%; mp: 250-253 ºC. ^{1}H NMR (500 MHz, $CDCl_3$ + CD_3OD, 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). ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD, 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 $C_{19}H_{17}ClFN_3O_3$ $[M+H]^+$, 390.1021; found, 390.1017.

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

Light yellow solid; yield: 46%; mp: 203-206 ºC. ^{1}H 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). ^{13}C 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 $C_{21}H_{19}N_3O_3$ $[M+H]^+$, 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-f]quinazolin-10-amine (**f1**)*

Yellow solid; yield: 69%; mp: 241-244 ºC. ^{1}H 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). ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD, 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 $C_{21}H_{22}N_4O_7$ $[M+H]^+$, 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-f]quinazolin-10-amine (**f2**)*

Yellow solid; yield: 44%; mp: 245-246 ºC. ^{1}H 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). ^{13}C NMR (125MHz, $CDCl_3$ + CD_3OD, 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 ºC. ^{1}H 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 ºC. ^{1}H 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 ºC. ^{1}H 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-((tetrahydrofuran-3-yl)oxy)-2,3-dihydro-[1,4]dioxino[2,3-f]quinazolin-10-amine (**g1**)*

Light yellow solid; yield: 62%; mp: 255-257 ºC. ^{1}H 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-((tetrahydrofuran-3-yl)oxy)-2,3-dihydro-[1,4]dioxino[2,3-f]quinazolin-10-amine (**g2**)*

Light yellow solid; yield: 59%; mp: 241-243 ºC. ^{1}H 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-((tetrahydro-2H-pyran-4-yl)oxy)-2,3-dihydro-[1,4]dioxino[2,3-f]quinazolin-10-amine (**h1**)*

Light yellow solid; yield: 62%; mp: 256-258 ºC. ^{1}H 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-((tetrahydro-2H-pyran-4-yl)-oxy)-2,3-dihydro-[1,4]dioxino[2,3-f]quinazolin-10-amine (**h2**)*

White solid; yield, 50%; mp: 252-254 ºC. ^{1}H 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: 262-265ºC. ^{1}H 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 ºC. ^{1}H 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 ºC. ^{1}H 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 ºC. ^{1}H 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 ºC. ^{1}H 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 ºC. ^{1}H 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 ºC. ^{1}H 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 μM with DMSO and transferred to the dose plate. The compounds was 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 μg/mL BSA, 0.01% TritonX-100, 2.5 mM DTT and adjusted pH to 7.4) to obtain a 10× final concentration. Transfer compounds with its concentration ranging from 10 μM to 0.0006 μM to assay plate for EGFR activity test with a volume of 1 μL/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 μL DMSO 10-fold with reaction buffer to obtain 10% DMSO solution. Then transfer it to the assay plat, 1 μL/well. Procedure for kinase reaction is: 1) Add 10× compound to the assay plate in a 384-well plate layout, 1 μL/well. For the HPE and ZPE wells, equal volume (1 μL/well) of 10% DMSO was added to the 384-well assay plate; 2) Add 2.5× kinase EGFR into the assay plate as 384-well plate layout, 4 μL/well. For HPE wells, an equal volume (4 μL/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 ºC for 30 min; 5) Mix equal volume of 4× ATP and 4× substrate to obtain 2× ATP-substrate mixtures; 6) Add 2× ATP-substrate mixture to the assay plate, 5μL/well; 7) Centrifuge the assay plate at 1000 rpm for 1 min to mix; 8) Incubate the plate for one hour at 30 ºC; 9) Kinase glo plus was added to each well (10 μL/well), and then incubated the assay plate for 20 min at 27 ºC; 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 ºC overnight in a humidified incubator containing 5% CO_2. Cells were dosed with compounds at final concentrations ranging from 0.025 μM to 80 μM in each well of the plate. After 48 h, 10 μL 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.

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

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Graphical abstract

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

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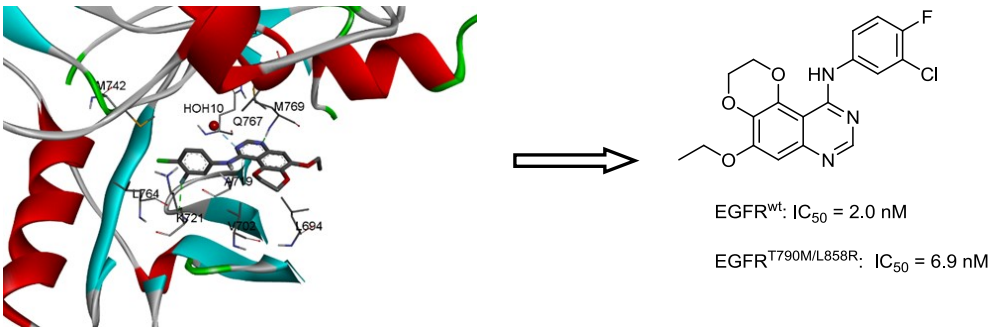

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