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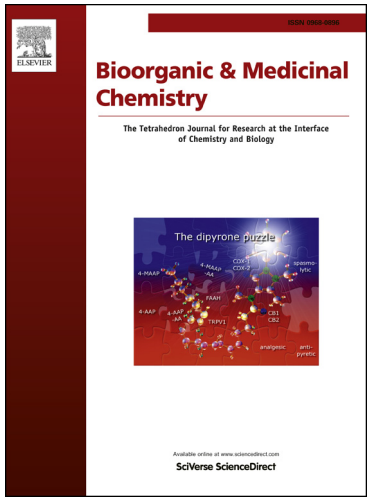

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Combination of 4-anilinoquinazoline, arylurea and tertiary amine moiety to discover novel anticancer agents

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Abstract

In present study, 4-anilinoquinazolines scaffold, arylurea and tertiary amine moiety were combined to design, synthesize gefitinib analogues and discover novel anticancer agents. A series of 4-anilinoquinazoline derivatives (**1**, **2**, **3** and **4**) bearing arylurea and tertiary amine moiety at its 6-position were synthesized. Their antiproliferative activities *in vitro* were evaluated via MTT assay against A431 cell and A549 cell. The SAR of the title compounds was discussed. The compounds **2d**, **2i** and **2j** with potent antiproliferative activities were evaluated their inhibitory activity against EGFR-TK. Compound **2j** displayed potent inhibitory activity against EGFR-TK. In addition, compound **2j**, at 50 mg/kg, can completely inhibit cancer growth in established nude mouse A549 xenograft model *in vivo*. These results suggest that the 4-anilinoquinazoline derivatives bearing diarylurea and tertiary amino moiety at its 6-position can serve as anticancer agents and EGFR inhibitors.

Keywords gefitinib * diaryl urea * tertiary amine * EGFR-TK inhibitor * anticancer agent

Introduction

The epidermal growth factor receptor (EGFR) belongs to the family of protein-tyrosine kinase receptor (RTK), which plays an essential role in the regulation of cell growth, differentiation, and survival.[1] RTKs have been observed over-expression and/or constitutive activation in numerous types of human tumor, including colon, breast, ovarian, head and neck, and non-small cell lung cancers. Among the known RTKs, EGFR and human epidermal growth factor receptor (HER2) have been extensively studied and clinically validated as targets for chemical therapies.[2, 3] Thousands of small organic molecule inhibitors against EGFR or both EGFR and HER2 were synthesized and evaluated. Gefitinib, erlotinib and lapatinib were launched successfully to treat cancer in clinic (Figure 1).[4, 5] The shared structure of gefitinib, erlotinib and lapatinib is 4-anilinoquinazoline, whose structure activity relationship has been elucidated.[6]

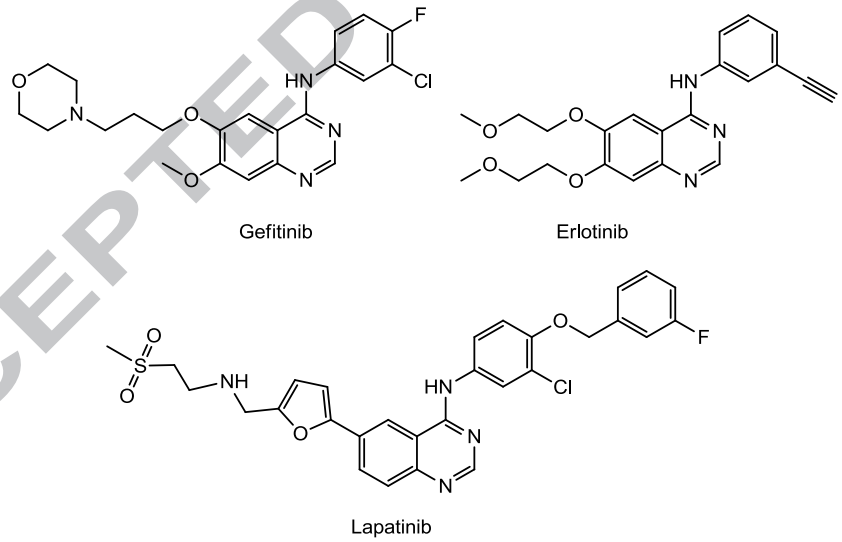


Figure 1. The structures of gefitinib, erlotinib and lapatinib

However, the efficacy of small organic molecules inhibitors such as gefitinib, etc. is restricted to a small subset of patients due to molecular heterogeneity among and within tumors.[7, 8] The drug resistance caused by receptor mutation is another issue needed to pay attention.[9-11] Numbers of compounds with different structures were discovered as EGFR or multi-target inhibitors.[12-16] The essential pharmacophore for

4-anilinoquinazolines is the two nitrogen atoms at 1-posotion, 3-position and the N-aryl at 4-position as well.[17] The morpholine moiety in gefitinib is not involved in any interaction with EGFR and is randomly ordered due to its lower electron density. Consequently, the modification of the 6-substituted group in 4-anilinoquinazoline with the group bearing active group provide the inhibitors with improved potency.[18-20] Similarly, the replacement of 6-substituted group in gefitinib with acrylamido group produced irreversible EGFR inhibitors[21] and EGFR-T790M inhibitors.[22]

There are two hydrogen-bond donors and one hydrogen-bond acceptor in the urea moiety. Aryl urea moiety is present in many classes of biologically active compounds.[23-25] Recent years, some kinase inhibitors with aryl urea moiety were launched for the treatment of cancers or tested clinically. For example, linifanib, developed by Abbott, is used for the treatment of colorectal cancer.[26] Sorafenib, developed by Bayer, is used for the treatment of advanced renal cell carcinoma.[27] Meanwhile, urea moiety and 4-anilinoquinazolines scaffold were combined to search for new compounds with antimalarial activity.[28] The *N*-mustard pharmacophore was attached to the 6-position of the 4-anilinoquinazolines via a urea linker to discover dual functional antitumor agents.[29] In addition, urea moiety consists in PI3K and mTOR dual inhibitors PKI-179,[30] PKI-587[31] and PKI-402,[32] which were developed by Wyeth in clinical trial for the treatment of solid tumors.

Tertiary amine group is considered as a water soluble moiety in drug discovery. *N*-methylpiperazinyl, pyrrolidyl and diethylamino can be found in drug structure.[33] What's more, the pharmacokinetic property and water solubility of a drug could be significantly improved after a tertiary amine moiety was attached.[34]

Given that tertiary amine and urea are important moieties in drug design, we intend to combine tertiary amine group, urea moiety and 4-anilinoquinazoline scaffold into one molecule to discover novel anticancer agents and EGFR-TK inhibitors (Figure 2). Herein, we report the synthesis and biological activities evaluation of thirty new compounds.

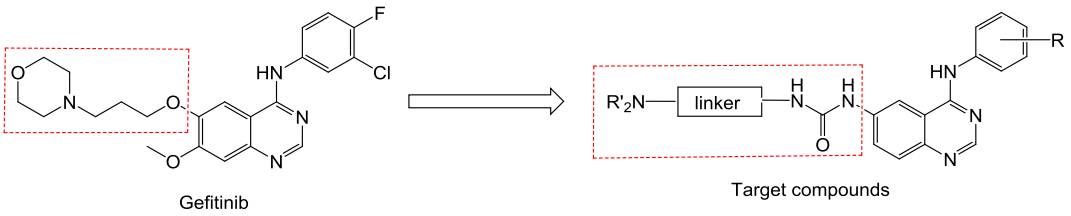
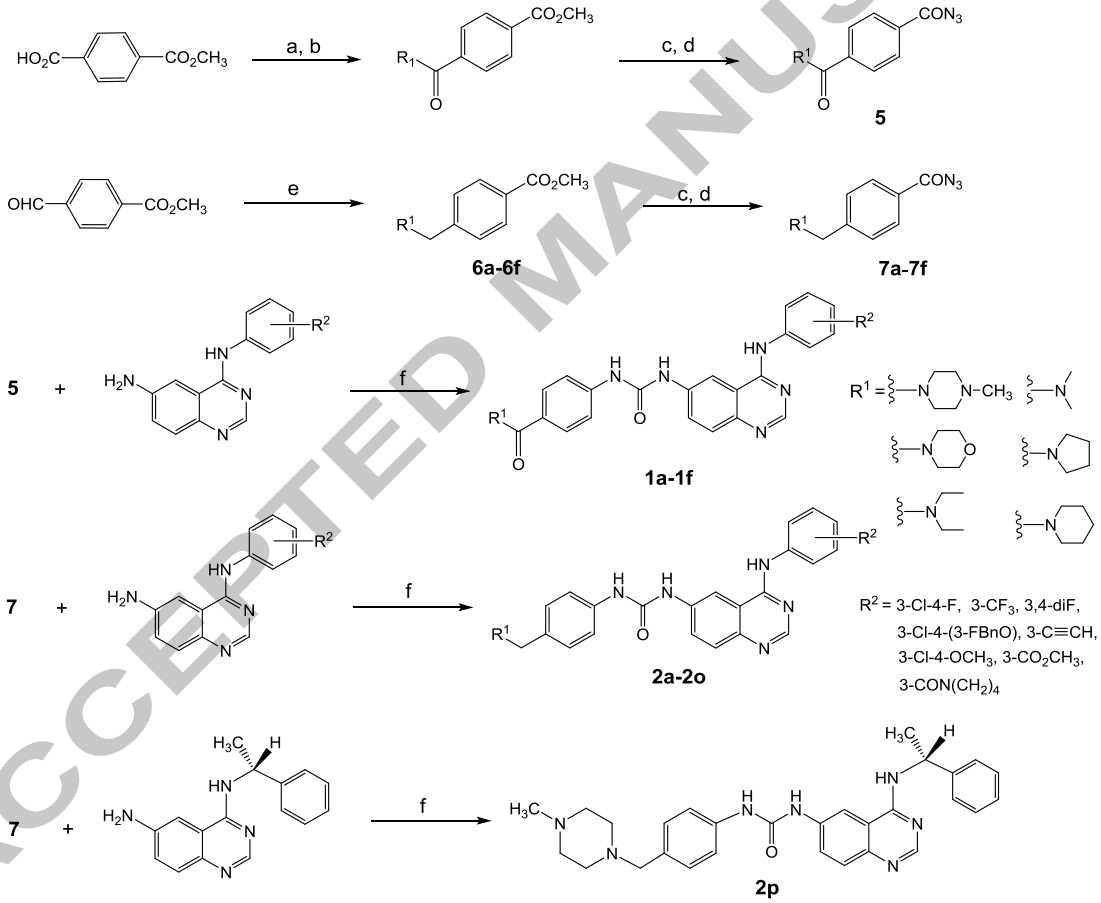


Figure 2. The design of target compounds

2. Results and discussion

2.1. Chemistry

The synthetic routes for the title compounds **1**, **2** are outlined in Scheme1.

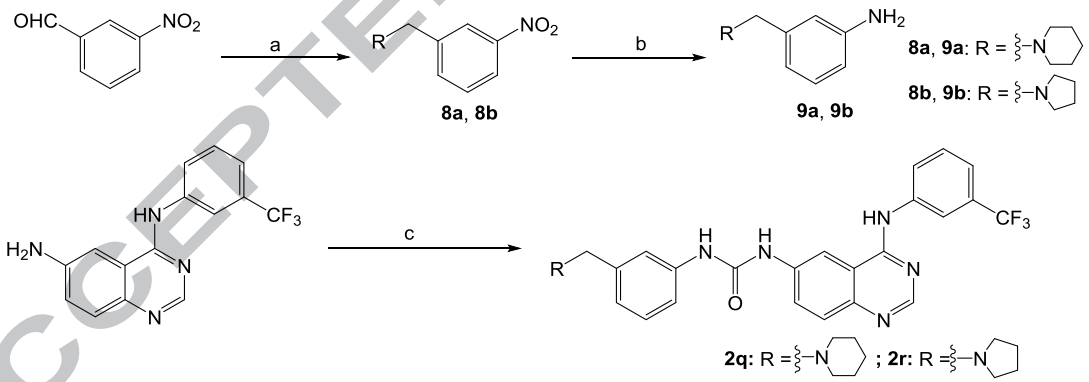


Scheme 1. Reagents and conditions: (a) $(COCl)_2$, CH_2Cl_2, rt, 2 h; (b) secondary amine, THF, 0°C to rt, 3 h; (c) 2 M NaOH, MeOH, rt, 4 h; (d) i: $SOCl_2$, DMF, reflux, 2 h; ii: NaN_3, THF/H_2O, DIPEA, rt, 4 h; (e) secondary amine, CH_2Cl_2, $NaBH(OAc)_3$, rt, 5 h; (f) toluene, reflux, 2 h.

Commercially available 4-methoxycarbonylbenzoic acid was used as starting material to prepare intermediates **5**. The details were previously described in our work.[25] Intermediate **6** was prepared by reductive amination of methyl 4-formylbenzoate and secondary amine. The obtained **6** was hydrolyzed and

subsequently acidified to produce corresponding acid, which is an amino acid and difficult to purify. So the crude product acid reacted with thionyl chloride to give acid chloride, the latter reacted with sodium azide afforded acyl azide **7**. 6-Amino-4-anilinoquinazolines were prepared according to the reported steps.[35] Heating the acyl azide **5** in toluene produced isocyanate, which was not isolated and reacted with 6-amino-4-anilinoquinazoline to produce targeted compounds **1a-1e**. Alternatively, refluxing the mixture of acyl azide **7**, 6-amino-4-anilinoquinazolines and toluene yielded compounds **2a**-**2o**. Also, compound **2p** was prepared by the refluxing of **7** and (R)-6-amino-4-(1-phenylethylamino)quinazoline in toluene.

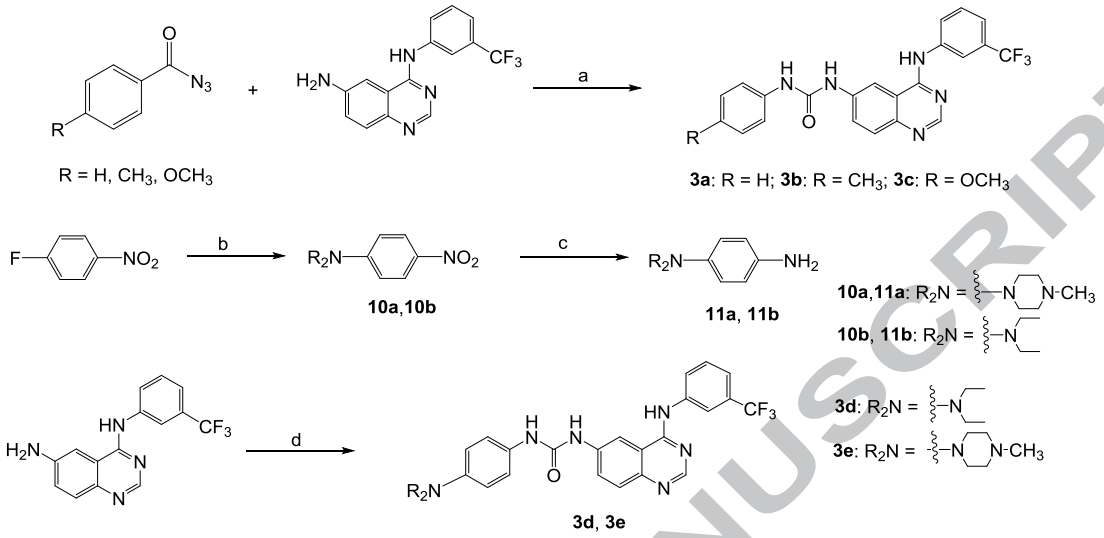
To probe the structure-activity relationship (SAR) of compounds with a *tertiary*-amino substituted group at the *m*-position of phenyl urea moiety, compound **2q** and **2r** were designed and synthesized (Scheme 2). Intermediate **9** was prepared from 3-nitrobenzaldehyde according to the reported steps.[36] 4-(3-Trifluoromethyl phenylamino)quinoline-6-amine successively reacted with carbonyldimidazole (CDI) and **9** to produce compound **2q** and **2r**.



Scheme 2. Reagents and conditions**:** (a) piperidine or pyrrolidine, CH_2Cl_2, $NaBH(OAc)_3$, rt, 5 h; (b) iron powder, NH_4Cl, $MeOH/H_2O$, reflux, 1 h; (c) i: CDI, MeCN, rt, 8 h; ii: **9**, reflux, 4 h.

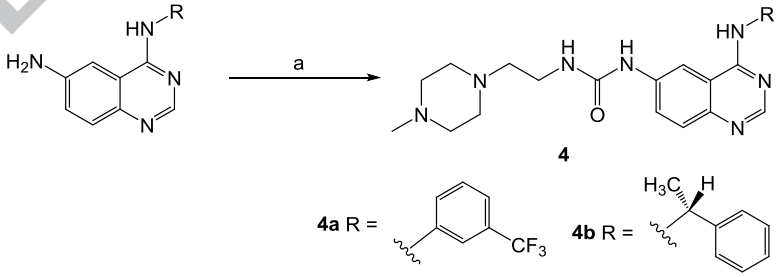
To further discuss the activity of the compounds with a substituted group at the *p*-position of urea moiety, compounds **3a**-**3c** were synthesized according to the same steps in the preparation of **2a**. The nucleophilic substitution of a secondary amine with *p*-fluoronitrobenzene yielded compound **10**, which was reduced by catalytic hydrogenation to produce amine **11**. The successive reaction of

4-(3-trifluoromethylphenylamino)quinozoline-6-amine with CDI and **11** produced compounds **3d** and **3e** (Scheme 3).



Scheme 3. Reagents and conditions: (a) toluene, reflux, 2 h. (b) secondary amine, DMF, K_2CO_3, 50 °C, 1 h; (c) Pd-C, H_2, EtOH, rt, 4 h; (d) i: CDI, acetonitrile, rt, 8 h; ii: **11**, reflux, 4 h.

To boost the structural diversity of the design compounds, the 6-position of 4-anilinoquinazoline was combined with 2-(4-methylpiperazin-1-yl)ethyl urea to yield compounds **4**. The 4-(3-trifluoromethylphenylamino) quinolinyl-6-amine successively reacted with CDI and 2-(4-methylpiperazin-1-yl)ethylamine to produce compound **4a.** Likewise, (R)-4-(1-phenylethylamino) quinazolinyl-6-amine was used as starting material to prepared **4b**. The synthetic route is depicted in Scheme 3.



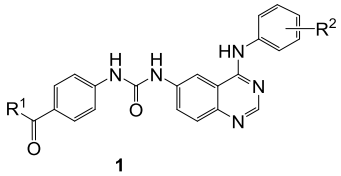
Scheme 4. Reagents and conditions: (a) i: CDI, acetonitrile, rt, 8 h; ii: 2-(4-methylpiperazin-1-yl)ethylamine, rt, to reflux, 4 h.

All the newly synthesized compounds were characterized by ^{1}H-NMR and HRMS.

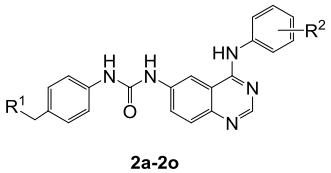
2.2. The antiproliferative activity *in vitro*

The antiproliferative activities of synthesized compounds were evaluated against human epithelial carcinoma cell line (A431) and lung adenocarcinoma epithelial cell line (A549) by applying the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-*2H*-tetrazolium bromide (MTT) colorimetric assay. The EGFR-TK inhibitor gefitinib was used as the positive control. The results are summarized in Table 1.

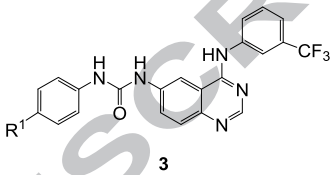
Table 1 Antiproliferative activities of compounds against two cancer cell lines ($\bar{x} \pm s$, n = 3)



1



2a-2o



3

compds	R^1	R^2	IC_{50} (μM)	
			A431	A549
1a	$CH_3N(CH_2CH_2)_2N$-	3-Cl-4-F	>10	>10
1b	$CH_3N(CH_2CH_2)_2N$-	3-C≡CH	>10	>10
1c	$CH_3N(CH_2CH_2)_2N$-	3-CF_3	>10	8.31±1.21
1d	$CH_3N(CH_2CH_2)_2N$-	3-Cl-4-(3-FBnO)	>10	7.39±0.52
1e	$O(CH_2CH_2)_2N$-	3-Cl-4-F	>10	>10
2a	$CH_3N(CH_2CH_2)_2N$-	3-Cl-4-F	4.00±0.20	3.48±0.12
2b	$CH_3N(CH_2CH_2)_2N$-	3-C≡CH	2.81±0.05	5.17±0.70
2c	$CH_3N(CH_2CH_2)_2N$-	3,4-diF	>10	>10
2d	$CH_3N(CH_2CH_2)_2N$-	3-Cl-4-(3-FBnO)	0.64±0.04	0.90±0.15
2e	$CH_3N(CH_2CH_2)_2N$-	3-Cl-4-OCH_3	2.01±0.10	3.57±0.33
2f	$CH_3N(CH_2CH_2)_2N$-	3-CF_3	2.41±0.19	1.65±0.09
2g	$CH_3N(CH_2CH_2)_2N$-	3-CO_2CH_3	5.63±1.01	>10
2h	$CH_3N(CH_2CH_2)_2N$-	3-$CON(CH_2)_4$	>10	>10
2i	$(CH_2)_5N$-	3-CF_3	1.55±0.14	1.05±0.07
2j	$(CH_2)_4N$-	3-CF_3	1.36±0.04	0.83±0.11
2k	$O(CH_2CH_2)_2N$-	3-CF_3	8.49±0.78	4.03±0.43
2l	$(CH_3CH_2)_2N$-	3-CF_3	3.98±0.39	2.07±0.20
2m	$(CH_3)_2N$-	3-CF_3	1.03±0.20	1.12±0.20
2n	$O(CH_2CH_2)_2N$-	3-C≡CH	>10	>10
2o	$(CH_3CH_2)_2N$-	3-C≡CH	1.24±0.17	2.25±0.10
2p			>10	>10
2q			1.33±0.09	1.06±0.19
2r			1.23±0.08	0.86±0.20
3a	H-		>10	>10

3b	CH_3-	>10	4.14±0.58
3c	CH_3O-	4.95±0.85	9.47±1.40
3d	$(CH_3CH_2)_2N$-	6.46±0.77	3.52±0.39
3e	$CH_3N(CH_2CH_2)_2N$-	2.20±0.23	2.76±0.12
4a		>10	>10
4b		>10	>10
gefitinib		3.74±0.41	5.76±0.42

As the data listed in table 1, most designed compounds displayed significant antiproliferative activities against two human cancer cell lines, some of which were more potent than that of gefitinib. The amine moiety was combined by a benzoyl group in compounds **1**, which showed poor antiproliferative activities. While in the structure of compounds **2**, the tertiary amine moiety was attached by a benzyl group. The antiproliferative activity of compounds **2** is higher than that of compounds **1** against the two cancer cell lines. In order to discover compounds with potent antiproliferative activity and explore structure-activity relationship of compounds **2**, we initially focused on the variation of substituents at 4-phenylamino group attached to the 4-position of quinazoline. Except compounds **2c**, **2g** and **2h,** compounds **2a**, **2b**, **2d**, **2e** and **2f** displayed potent antiproliferative activities against the two cancer cell lines. When 4-phenylamino group was replaced by the 1-phenylethylamino group (compound **2p**), the activity dropped dramatically. Compound **2d** displayed significant antiproliferative activities against the two cancer cell lines and its activity against A431 is 5.8-fold to gefitinib. Changing 3-fluorobenzyloxy group in **2d** to methoxy (compound **2e**) resulted in a decrease in activity. Compound **2f** with a 3-trifluoromethyl group also showed potent antiproliferative activities against the two cancer cell lines. These results suggest that the activity of compounds **2** is closely related to the substituted group at the 4-position of quinazoline. Taking molecular weight into consideration, we chose compound **2f** to carry on the further SAR probe. Next the 3-trifluoromethyl group was remained, and the *N*-methylpiperazin-1-yl group was replaced by tertiary amino substituents, such as piperidyl, pyrrolidinyl, 4-morpholinyl, diethylamino, and dimethylamino to prepare compounds **2i**-**2m**. The

data in Table 1 showed that these five compounds displayed potent antiproliferative activities against the two cancer cell lines. Moreover, the activity of compound **2j** increased 3 to 5-fold to gefitinib in cell-based activity. The fact that the activity of compounds **2k** and **2n** is weaker than **2l** and **2o** indicates that morpholinyl moiety is an unfavorable substituent group in design compound. These results may be related to the poor cell permeability of morpholinyl moiety.[37] After the tertiary amino moiety was moved from the *p*-position of phenyl urea moiety to the *m*-position, compound **2q** and **2r** displayed the potent antiproliferative activities which is similar to that of compound **2i** and **2j**.

To probe the effect of tertiary amino moiety on activity, compounds **3** were synthesized. Without tertiary amino group, the activity of compounds **3a**, **3b** and **3c** against two cancer lines dropped significantly. Compounds **3d** and **3e** bearing an amino substituents at the *p*-position of the phenylurea moiety exhibited an improvement activity compared with compounds **3a**, **3b** and **3c**, but a decline in activity compared to compounds **2l** and **2f**. These results reveal that the tertiary amino moiety attached by a benzyl group is favorable for the antiproliferative activity. Furthermore, the replacement of phenylurea with 2-(4-methylpiperazin-1-yl)ethylurea (compounds **4a**, **4b**) resulted in a total loss in antiproliferative activities. Building on the discussions above, we can reveal a SAR in which diarylurea and tertiary amino moiety are beneficial for the antiproliferative activities of gefitinib analogues. These results accord with our design idea.

2.3. EGFR inhibitory activity assay

Table 2 The inhibitory activities of selected compounds against kinase

compds	IC_{50} (nM)		
	$EGFR^{wt}$	$EGFR^{L858R}$	VEGFR-2
2d	8.4	nt	>1000
2i	5.0	10.5	nt
2j	4.1	11.5	565
gefitinib	1.3	2.6	nt
sorafenib	nt	nt	1.2

nt = not tested.

Next, to elucidate the mechanism of antiproliferative activities of title compounds, three compounds were selected to evaluate their inhibitory activity against EGFR and VEGFR-2 by performing an ATP depletion assay.[38] Gefitinib and sorafenib were used as the positive drugs. The data are summarized in Table 2.

The data in Table 2 indicate that the selected compounds **2d**, **2i** and **2j** display remarkable potency against $EGFR^{wt}$, compounds **2i** and **2j** exhibit potent inhibitory activity against EGFR(L858R) and compound **2j** displays weak activity against VEGFR-2. These results suggest that the tested compounds are EGFR inhibitors.

We take into consideration both the antiproliferative activities and EGFR inhibitory activities of compounds **2d**, **2i**, **2j** and gefitinib together. Compounds **2d**, **2i** and **2j** exhibited more potent activities than gefitinib in cell based-activity, but weaker activities than gefitinib in enzymatic-based activities. These results may relate to other property of compounds **2d**, **2i** and **2j**, such as pharmacokinetics.[37]

2.4. Anticancer effects in established nude mouse A549 xenograft model *in vivo*

Compound **2j** displayed potent antiproliferative activity against A549 cell and inhibitory activity against EGFR. Thus, we evaluated whether compound **2j** could inhibit cancer growth in an established nude mouce A549 xenograft model *in vivo*. Gefitinib (50 mg/kg) was used as positive drug. **2j** was dissolved and dosed orally at 50 mg/kg once a day for 12 days. The control group was administrated orally the solvent only.

The change in the cancer volumes was depicted in Figure 3. In this model, the growth of cancer in both treatment group and positive group can be inhibited completely compared to the control group ($P < 0.001$). The curves in Figure 3 indicate that compound **2j** displays a little stronger anticancer effect than gefitinib. These results suggest that compound **2j** is an effective anticancer agent *in vivo*. In addition, the significant weight loss of tested animals was not observed during the experimental period.

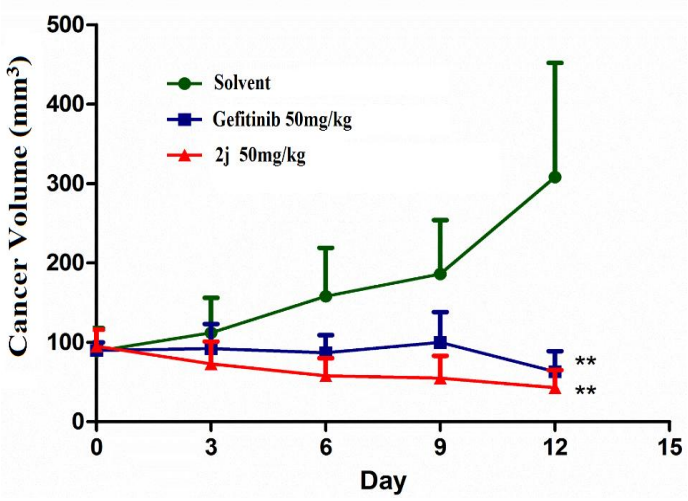


Figure 3 The anticancer effect of compound **2j** in established xenograft A549 lung adenocarcinoma models (n = 5). Mice bearing cancers were orally administered solvent, compound **2j** and gefitinib once daily for 12 days. $^{**}P < 0.001$ *vs* solvent.

2.5. Docking study

To further explain the potent activities of compound **2j** and **2r**, we performed a docking analysis utilizing the C-DOCKER program within Discovery Studio 2.5 software package. Docking simulations were performed on the kinase domain of $EGFR^{wt}$ (PDB code 1M17)[39] and $EGFR^{L858R}$ (PDB code 4LQM)[40]. From the docking results of compound **2j** and **2r** with $EGFR^{wt}$ (Figure 4, A and B), we observed that: (1) both compounds **2j** and **2r** formed hydrogen bonds with hinge residues Met769 and Gln767 by oxygen atom and hydrogen atom of the urea moiety; (2) the *N*1 of the quinazoline moiety in compound **2j** and **2r** formed two hydrogen bonds with Thr830 and Asp831, and the *N*3 of the quinazoline moiety formed two hydrogen bonds with Lys721.

From the docking results of compound **2j** and **2r** with $EGFR^{L858R}$ (Figure 4, C and D), we observed that: (1) the two compounds formed two hydrogen bonds with the hinge residue Met793 by the urea moiety; (2) the 4-amino moiety of compound **2j** formed hydrogen bond with Asp800, while the trifluoromethyl moiety of compound **2r** formed two hydrogen bonds with Ser720.

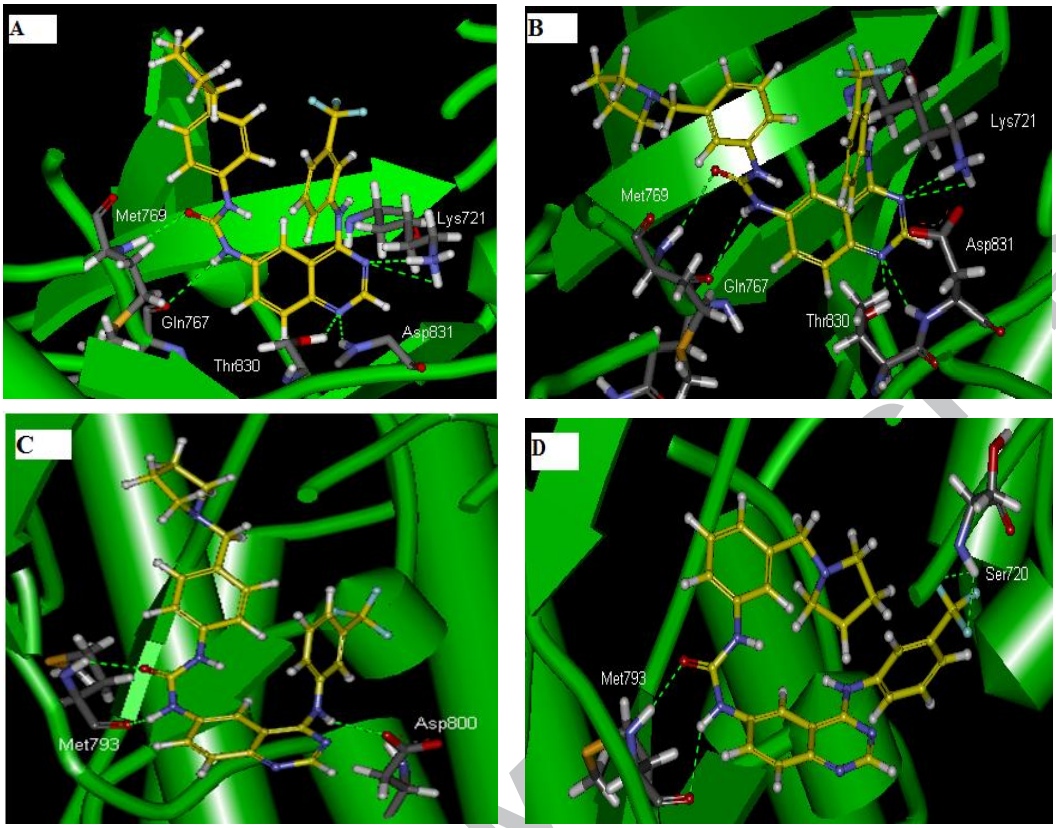


Figure 4. Docking modes of compounds **2j** and **2r** with $EGFR^{wt}$ (A and B); compounds **2j** and **2r** with $EGFR^{L858R}$ (C and D). Selected residues Lys721, Gln767, Met769, Thr830, Asp831 in $EGFR^{wt}$ and Ser720, Met793, Asp800 in $EGFR^{L858R}$ are shown. Green dashed lines indicate hydrogen bonds.

3. Conclusion

In present study, a series of 4-anilinoquinazoline derivatives bearing diarylurea and *tertiary*-amine moiety at its 6-position were synthesized and characterized. The designed compounds displayed significant antiproliferative activities against A431 cell and A549 cell. The SAR of the tested compounds was discussed. The compounds **2i** and **2j** with potent antiproliferative activities exhibited potent inhibitory activity against $EGFR^{wt}$ and $EGFR^{L858R}$. Compound **2j** can suppress the cancer growth in vivo as well. These results suggest that 4-anilinoquinazoline derivatives bearing diarylurea and tertiary amino moiety at its 6-position can serve as anticancer agents and EGFR inhibitors.

4. Experimental

4.1. Chemistry

Unless specified otherwise, all starting materials, reagents and solvents were commercially available. All reactions were monitored by thin-layer chromatography on silica gel plates (GF-254) and visualized with UV light. All the melting points were determined on a Beijing micro melting-point apparatus and thermometer was uncorrected. ^{1}H-NMR spectra were recorded on a 400 Bruker NMR spectrometer with tetramethylsilane (TMS) as an internal reference. All chemical shifts are reported in parts per million (ppm). High-resolution mass measurements were performed using electrospray ionization (positive mode) on a quadrupole time-of-flight (QTOF) mass spectrometer (Maxis Q-TOF, Bruker Inc.).

4.1.1. General procedures for the synthesis of compound 1a-1e

The intermediate **5** was prepared from 4-(methoxycarbonyl) benzoic acid as the steps we previously reported.[25] 6-Amino-4-anilinoquinazoline was prepared as reported approach.[35]

The solution of compound **5** (0.27 g, 0.99 mmol) in toluene (5.0 mL) was refluxed for 1 h under N_2 and cooled to about 30 °C. 6-Amino-4-anilinoquinazoline (0.99 mmol) was added. Then the mixture was stirred at 30°C for another 2 h. The solvent was removed and the residue was purified by flash column chromatograph over silica gel (chloroform/methanol = 10:1, v/v) to give **1a**–**1e** as white solids.

4.1.2. 1-(4-((3-Chloro-4-fluorophenyl)amino)quinazolin-6-yl)-3-(4-(4-methyl piperazin-1-carbonyl)phenyl)urea (1a)

Yield 45.4%. mp: 183.5-184.5 °C. ^{1}H-NMR (DMSO-d_6): δ 9.89 (s, 1H, N-H), 9.13 (s, 1H, N-H), 9.07 (s, 1H, N-H), 8.55 (s, 1H, Ar-H), 8.51 (d, 1H, Ar-H), 8.04 (s, 1H, Ar-H), 7.90 (d, 2H, Ar-H, J = 8.8 Hz), 7.78 (d, 1H, Ar-H), 7.58 (d, 2H, Ar-H, J = 8.4 Hz), 7.40 (t, 1H, Ar-H), 7.37 (s, 1H, Ar-H), 7.35 (s, 1H, Ar-H), 7.22 (d, 1H, Ar-H), 4.22 (s, 1H, C≡CH), 3.48 (br, 4H, 2×N-CH_2), 2.32 (br, 4H, 2×N-CH_2), 2.20 (s, 3H, N-CH_3). ^{13}C-NMR (DMSO-d_6): δ 169.4, 157.5, 153.7 (d, $J_{C\text{-}F}$ = 241 Hz), 153.0, 152.9, 146.4, 141.3, 138.0, 137.2, 129.5, 129.0, 128.7 (2×CH), 127.1, 124.1, 123.0, 119.2, 118.1 (2×CH), 116.9, 116.0, 110.6, 54.9, 46.0 (4×CH_2). ESI-HRMS *m/z*: calc'd for $C_{27}H_{25}ClFN_7O_2$ $[M+H]^+$: 534.1821; found: 534.1817.

4.1.3. 1-(4-((3-Ethynylphenyl)amino)quinazolin-6-yl)-3-(4-(4-methylpiperazin-

1-carbonyl)phenyl)urea (1b)

Yield 42.5%. mp: 168.0-168.5 °C. ^{1}H-NMR (DMSO-d_6): δ 9.83 (s, 1H, N-H), 9.13 (s, 1H, N-H), 9.05 (s, 1H, N-H), 8.55 (s, 1H, Ar-H), 8.51 (d, 1H, Ar-H), 8.04 (s, 1H, Ar-H), 7.90 (d, 2H, Ar-H, *J* = 8.8 Hz), 7.78 (d, 1H, Ar-H), 7.58 (d, 2H, Ar-H, *J* = 8.4 Hz), 7.40 (t, 1H, Ar-H), 7.37 (s, 1H, Ar-H), 7.35 (s, 1H, Ar-H), 7.22 (d, 1H, Ar-H), 4.22 (s, 1H, C≡CH), 3.48 (br, 4H, 2×N-CH_2), 2.32 (br, 4H, 2×N-CH_2), 2.20 (s, 3H, N-CH_3). ^{13}C-NMR (DMSO-d_6): δ 169.4, 157.5, 153.1, 152.9, 146.3, 140.9, 139.2, 138.0, 129.6, 129.4, 129.0, 128.8 (2×CH), 127.1, 126.7, 125.4, 123.0, 122.1, 118.3 (2×CH), 116.1, 110.2, 84.1, 81.0, 54.6, 48.0 (2×CH_2), 45.4 (2×CH_2). ESI-HRMS *m/z*: calc'd for $C_{29}H_{27}N_7O_2$ $[M+H]^+$: 506.2304; found: 506.2301.

4.1.4. 1-(4-((3-Trifluoromethylphenyl)amino)quinazolin-6-yl)-3-(4-(4-methyl piperazin-1-carbonyl)phenyl)urea (1c)

Yield 54.3%. mp: 190.0~192.0 °C . ^{1}H-NMR (DMSO-d_6): δ 10.03 (s, 1H, N-H), 9.19 (s, 1H, N-H), 9.15 (s, 1H, N-H), 8.58 (s, 2H, Ar-H), 8.30 (s, 1H, Ar-H), 8.22 (d, 1H, Ar-H, *J* = 8.4 Hz), 7.89 (d, 1H, Ar-H, *J* = 9.2 Hz), 7.79 (d, 1H, Ar-H, *J* = 9.2 Hz), 7.63 (t, 1H, Ar-H), 7.59 (d, 2H, Ar-H, *J* = 8.8 Hz), 7.45 (d, 1H, Ar-H, *J* = 7.6 Hz), 7.36 (d, 2H, Ar-H, *J* = 8.8 Hz), 3.48 (br, 4H, 2×N-CH_2), 2.31 (br, 4H, 2×N-CH_2), 2.20 (s, 3H, N-CH_3). ^{13}C-NMR (DMSO-d_6): δ 169.4, 157.5, 153.2, 152.9, 146.4, 141.0, 138.6, 138.2, 130.3, 130.0, 129.5, 129.1, 128.8 (2×CH), 127.1, 126.1, 124.7 (d, J_{C-F} = 271 Hz), 119.9, 118.5, 118.0 (2×CH), 116.1, 110.2, 55.6, 48.4 (4×CH_2). ESI-HRMS *m/z*: calc'd for $C_{28}H_{26}F_3N_7O_2$ $[M+H]^+$: 550.2178; found: 550.2173.

4.1.5. 1-(4-((3-Chloro-4-(3-fluorophenylmethoxy)phenyl)amino)quinazolin-6-yl)-3-(4-(4-methylpiperazin-1-carbonyl)phenyl)urea (1d)

Yield 34.7%. mp: 166.0~167.5 °C . ^{1}H-NMR (DMSO-d_6): δ 9.78 (s, 1H, N-H), 9.14 (s, 1H, N-H), 9.05 (s, 1H, N-H), 8.50 (s, 1H, Ar-H), 8.48 (d, 1H, Ar-H), 8.00 (d, 1H, Ar-H), 7.87 (d, 1H, Ar-H, *J* = 9.2 Hz), 7.75 (d, 1H, Ar-H, *J* = 8.8 Hz), 7.71 (d, 1H, Ar-H, *J* = 9.2 Hz), 7.57 (d, 2H, Ar-H), 7.44-7.51 (m, 1H, Ar-H), 7.31-7.37 (m, 4H, Ar-H), 7.26 (d, 1H, Ar-H, *J* = 8.8 Hz), 7.20 (m, 1H, Ar-H), 5.26 (s, 2H, ArCH_2-O), 3.44 (br, 4H, 2×N-CH_2), 2.33 (br, 4H, 2×N-CH_2), 2.20 (s, 3H, N-CH_3). ^{13}C-NMR (DMSO-d_6): δ 169.4, 162.7 (d, J_{C-F} = 242 Hz), 157.6, 153.1, 153.0, 150.2, 146.4,

140.1, 140.0, 138.8, 138.2, 132.2, 131.2, 130.4, 128.9, 128.6 (2×CH), 126.9, 126.8, 124.5, 123.8, 123.0, 121.6, 118.9, 118.0 (2×CH), 116.0, 110.4, 56.0, 48.2 (4×CH_2). ESI-HRMS *m/z*: calc'd for $C_{34}H_{31}ClFN_7O_3$ $[M+H]^+$: 640.2239; found: 640.2237.

4.1.6. 1-(4-((3-Chloro-4-fluorophenyl)amino)quinazolin-6-yl)-3-(4-morpholine-4-carbonyl)phenyl)urea (1e)

Yield 37.4%. mp: 173.0~174.5 °C . ^{1}H-NMR (DMSO-d_6): δ 9.92 (s, 1H, N-H), 9.15 (s, 1H, Ar-H), 9.08 (s, 1H, N-H), 8.55 (s, 2H, Ar-H), 8.52 (s, 1H, Ar-H), 8.17 (d, 1H, Ar-H), 7.88 (d, 1H, Ar-H), 7.77-7.83 (m, 2H, Ar-H), 7.59 (d, 2H, Ar-H, *J* = 8.0 Hz), 7.45 (t, 1H, Ar-H), 7.40 (d, 2H, Ar-H, *J* = 8.0 Hz), 3.60 (br, 4H, 2×O-CH_2), 3.51 (br, 4H, 2×N-CH_2). ^{13}C-NMR (DMSO-d_6): δ 169.4, 157.5, 154.1 (d, J_{C-F} = 241 Hz), 153.2, 152.9, 146.4, 139.0, 138.3, 137.8, 129.5, 129.1, 128.8 (2×CH), 127.0, 124.0, 123.2, 119.8, 118.2 (2×CH), 116.8, 116.0, 110.4, 56.6 (2×CH_2), 50.8 (2×CH_2). ESI-HRMS *m/z*: calc'd for $C_{26}H_{22}ClFN_6O_3$ $[M+H]^+$: 521.1504; found: 521.1528.

4.1.7. General procedures for the synthesis of compounds 6a-6f

Compounds **6a-6f** were prepared according to the reported method.[41]

4.1.8. Methyl 4-((4-methylpiperazin-1-yl)methyl)benzoate (6a)

Yield 86.5%. ^{1}H-NMR ($CDCl_3$): δ 8.00 (d, 2H, Ar-H, *J* = 8.4 Hz), 7.42 (d, 2H, Ar-H, *J* = 8.4 Hz), 3.92 (s, 3H, OCH_3), 3.57 (s, 2H, Ar-CH_2-N), 2.49 (br, 8H, 4×N-CH_2), 2.31 (s, 3H, N-CH_3).

4.1.9. Methyl 4-(morpholinomethyl)benzoate (6b)

Yield 85.0%. ^{1}H-NMR ($CDCl_3$): δ 8.00 (d, 2H, Ar-H, *J* = 8.0 Hz), 7.42 (d, 2H, Ar-H, *J* = 8.4 Hz), 3.92 (s, 3H, OCH_3), 3.72 (t, 4H, 2×O-CH_2, *J* = 4.4 Hz), 3.55 (s, 2H, Ar-CH_2-N), 2.45 (t, 4H, 2×N-CH_2, *J* = 4.4 Hz).

4.1.10. Methyl 4-((diethylamino)methyl)benzoate (6c)

Yield 83.3%. ^{1}H-NMR ($CDCl_3$): δ 7.99 (d, 2H, Ar-H, *J* = 8.0 Hz), 7.43 (d, 2H, Ar-H, *J* = 8.0 Hz), 3.91 (s, 3H, OCH_3), 3.63 (s, 2H, Ar-CH_2-N), 2.54 (q, 4H, 2×N-CH_2, *J* = 7.2 Hz), 1.06 (t, 6H, 2×CH_3, *J* = 7.2 Hz).

4.1.11. Methyl 4-(piperidin-1-ylmethyl)benzoate (6d)

Yield 90.2%. ^{1}H-NMR ($CDCl_3$): δ 8.00 (d, 2H, Ar-H, *J* = 8.4 Hz), 7.42 (d, 2H, Ar-H, *J* = 8.4 Hz), 3.94 (s, 3H, OCH_3), 3.54 (s, 2H, Ar-CH_2-N), 2.40 (br, 4H,

2×N-CH_2), 1.57-1.63 (m, 4H, 2×CH_2), 1.41-1.49 (m, 2H, CH_2).

4.1.12. Methyl 4-(pyrrolidin-1-ylmethyl)benzoate (6e)

Yield 87.3%. ^{1}H-NMR ($CDCl_3$): δ 8.08 (d, 2H, Ar-H, *J* = 8.4 Hz), 7.66 (d, 2H, Ar-H, *J* = 8.4 Hz), 4.10 (s, 2H, Ar-CH_2-N), 3.94 (s, 3H, OCH_3), 3.04 (br, 4H, 2×N-CH_2), 2.02-2.09 (m, 4H, 2×CH_2).

4.1.13. Methyl 4-((dimethylamino)methyl)benzoate (6f)

Yield 86.4%. ^{1}H-NMR ($CDCl_3$): δ 8.06 (d, 2H, Ar-H, *J* = 8.0 Hz), 7.54 (d, 2H, Ar-H, *J* = 8.0 Hz), 3.94 (s, 3H, OCH_3), 3.76 (s, 2H, Ar-CH_2-N), 2.46 (s, 6H, 2×N-CH_3).

4.1.14. General procedures for the synthesis of compounds 7a-7f

The mixture containing compound **6 (**2.51 mmol**)**, methanol (5.0 mL), 2 M NaOH (1.6 mL) was stirred at room temperature for 4 h, concentrated under reduced pressure and acidified with concentrated hydrochloric acid to pH 2. The aqueous phase was concentrated to dryness under reduced pressure to afford the crude 4-aminomethylbenzoic acid (containing sodium chloride) which was carried on the next step without further purification. The 4-aminomethylbenzoic acid (2.51 mmol) and 2 drops of DMF were added to thionyl chloride (10 mL). The mixture was refluxed for 2 h. The volatile was removed under reduced pressure to give a pale yellow solid which was then dissolved in anhydrous THF (10 mL) and was added dropwise to a suspension of sodium azide (0.24 g, 3.77 mmol) in 10 mL of THF/H_2O (4:1, v/v) at 0-5 °C. Then the mixture was stirred at room temperature overnight and THF was removed under reduced pressure. The aqueous phase was extracted with dichloromethane (20 mL × 3). The organic layer was combined, washed with brine (20 mL), dried (Na_2SO_4), filtered and concentrated under reduced pressure to produce compounds **7a-7f** as yellow or brown oil.

4.1.15. 4-((4-Methylpiperazin-1-yl)methyl)benzoyl azide (7a)

Yield 76.3%. ^{1}H-NMR ($CDCl_3$): δ 7.99 (d, 2H, Ar-H, *J* = 8.0 Hz), 7.44 (d, 2H, Ar-H, *J* = 8.0 Hz), 3.58 (s, 2H, Ar-CH_2-N), 2.48 (br, 8H, 4×N-CH_2), 2.31 (s, 3H, N-CH_3). MS: 260 $[M+H]^+$.

4.1.16. 4-(Morpholinomethyl)benzoyl azide (7b)

Yield 81.0%. ^{1}H-NMR ($CDCl_3$): δ 8.00 (d, 2H, Ar-H, *J* = 8.4 Hz), 7.46 (d, 2H, Ar-H, *J* = 8.4 Hz), 3.74 (t, 4H, 2×O-CH_2, *J* = 4.4 Hz), 3.58 (s, 2H, Ar-CH_2-N), 2.47 (t, 4H, 2×N-CH_2, *J* = 4.0 Hz). MS: 247 $[M+H]^+$.

4.1.17. 4-((Diethylamino)methyl)benzoyl azide (7c)

Yield 70.5%. ^{1}H-NMR ($CDCl_3$): δ 8.06 (br, 2H, Ar-H), 7.60 (br, 2H, Ar-H), 2.93 (s, 2H, Ar-CH_2-N), 1.70 (br, 4H, 2×N-CH_2), 1.28 (br, 6H, 2×CH_3). MS: 232 $[M+H]^+$.

4.1.18. 4-(Piperidin-1-ylmethyl)benzoyl azide (7d)

Yield 69.3%. ^{1}H-NMR ($CDCl_3$): δ 7.98 (d, 2H, Ar-H, *J* = 8.0 Hz), 7.46 (d, 2H, Ar-H, *J* = 8.0 Hz), 3.59 (s, 2H, Ar-CH_2-N), 2.44 (br, 4H, 2×N-CH_2), 1.65-1.59 (m, 4H, 2×CH_2), 1.53-1.56 (m, 2H, CH_2). MS: 244 $[M+H]^+$.

4.1.19. 4-(Pyrrolidin-1-ylmethyl)benzoyl azide (7e)

Yield 78.5%. ^{1}H-NMR ($CDCl_3$): δ 7.90 (d, 2H, Ar-H, *J* = 8.0 Hz), 7.43 (d, 2H, Ar-H, *J* = 8.0 Hz), 3.68 (s, 2H, Ar-CH_2-N), 2.52 (br, 4H, 2×N-CH_2), 1.79 (br, 4H, 2×CH_2). MS: 230 $[M+H]^+$.

4.1.20. 4-((Dimethylamino)methyl)benzoyl azide (7f)

Yield 76.4%. ^{1}H-NMR ($CDCl_3$): δ 8.03 (d, 2H, Ar-H, *J* = 8.0 Hz), 7.51 (d, 2H, Ar-H, *J* = 8.0 Hz), 3.65 (s, 2H, Ar-CH_2-N), 2.37 (s, 6H, 2×N-CH_3). MS: 204 $[M+H]^+$.

4.1.21. General procedures for the synthesis of compounds 2a-2p and 3a-3c

Compound **7** or benzoyl azide or 4-methylbenzoyl azide (0.77 mmol) was added to toluene (5 mL). The mixture was stirred, added 4-anilinoquinazoline-6-amine or (R)-6-amino-4-(1-phenylethylamino)quinazoline (0.62 mmol), refluxed for 2 h, cooled to room temperature. The precipitate was filtered and purified by column chromatography on silica gel (dichloromethane /methanol = 30:1, v/v) to produce compounds **2a-2p** or **3a-3c** as yellow or off-white powder.

4.1.22. 1-(4-((3-Chloro-4-fluorophenyl)amino)quinazolin-6-yl)-3-(4-((4-methyl piperazin-1-yl)methyl)phenyl)urea (2a)

Yield 68.5%. mp: 191.5-192.0 °C. ^{1}H-NMR (DMSO-d_6): δ 9.90 (s, 1H, N-H), 9.02 (s, 1H, N-H), 8.92 (s, 1H, N-H), 8.54 (s, 1H, Ar-H), 8.50 (s, 1H, Ar-H), 8.16-8.19 (m, 1H, Ar-H), 7.88 (dd, 1H, Ar-H, J_1 = 1.6 Hz, J_2 = 8.8 Hz), 7.80-7.85 (m, 1H, Ar-H), 7.77 (d, 1H, Ar-H, *J* = 8.8 Hz), 7.47 (s, 1H, Ar-H), 7.44 (d, 2H, Ar-H, *J* = 8.0 Hz),

7.21 (d, 2H, Ar-H, J = 8.0 Hz), 3.46 (s, 2H, Ar-CH_2-N), 2.34 (br, 8H, 4×N-CH_2), 2.17 (s, 3H, N-CH_3). ^{13}C-NMR (DMSO-d_6): δ 157.5, 153.7 (d, J_{C-F} = 241 Hz), 153.1, 152.9, 146.2, 138.8, 138.3, 137.2, 132.2, 129.9 (2×CH), 129.0, 127.0, 124.1, 123.0, 119.2, 118.6 (2×CH), 116.9, 116.0, 110.2, 62.0, 55.1 (2×CH_2), 52.7 (2×CH_2), 46.0. ESI-HRMS *m/z*: calc'd for $C_{27}H_{28}ClFN_7O$ $[M+H]^+$: 520.2028; found: 520.2022.

4.1.23. 1-(4-((3-Ethynylphenyl)amino)quinazolin-6-yl)-3-(4-((4-methyl piperazin-1-yl)methyl)phenyl)urea (2b)

Yield 64.5%. mp: 129.5-131.0 °C. ^{1}H-NMR (DMSO-d_6): δ 9.86 (s, 1H, N-H), 9.32 (s, 1H, N-H), 9.28 (s, 1H, N-H), 8.54 (s, 1H, Ar-H), 8.51 (s, 1H, Ar-H), 8.04 (s, 1H, Ar-H), 7.90 (d, 2H, Ar-H, J = 8.4 Hz), 7.76 (d, 1H, Ar-H, J = 8.0 Hz), 7.61 (d, 2H, Ar-H, J = 8.4 Hz), 7.40 (t, 1H, Ar-H, J = 8.0 Hz), 7.20-7.25 (m, 3H, Ar-H), 4.23 (s, 1H, C≡CH), 3.46 (s, 2H, Ar-CH_2-N), 3.01-3.09 (m, 4H, 2×N-CH_2), 2.65 (s, 3H, N-CH_3), 2.37 (s, 4H, 2×N-CH_2). ^{13}C-NMR (DMSO-d_6): δ 157.6, 153.2, 152.9, 146.2, 140.3, 139.2, 138.4, 131.3, 130.0 (2×CH), 129.3, 128.8, 127.0, 126.8, 125.4, 123.3, 122.1, 118.4 (2×CH), 116.2, 110.2, 84.1, 81.0, 61.9, 54.2 (2×CH_2), 51.6 (2×CH_2), 44.8. ESI-HRMS *m/z*: calc'd for $C_{29}H_{30}N_7O$ $[M+H]^+$: 492.2512; found: 492.2506.

4.1.24. 1-(4-((3,4-Difluorophenyl)amino)quinazolin-6-yl)-3-(4-((4-methyl piperazin-1-yl)methyl)phenyl)urea (2c)

Yield 42.3%. mp: 190.6-192.0 °C. ^{1}H-NMR (DMSO-d_6): δ 9.90 (s, 1H, N-H), 9.02 (s, 1H, N-H), 8.92 (s, 1H, N-H), 8.55 (s, 1H, Ar-H), 8.50 (s, 1H, Ar-H), 8.05-8.12 (m, 1H, Ar-H), 7.89 (dd, 1H, Ar-H, J_1 = 1.6 Hz, J_2 = 8.8 Hz), 7.78 (d, 1H, Ar-H, J = 8.8 Hz), 7.64 (d, 1H, Ar-H, J = 8.4 Hz), 7.41-7.50 (m, 3H, Ar-H), 7.21 (d, 2H, Ar-H, J = 8.0 Hz), 3.43 (s, 2H, Ar-CH_2-N), 2.34 (br, 8H, 4×N-CH_2), 2.16 (s, 3H, N-CH_3). ^{13}C-NMR (DMSO-d_6): δ 157.5, 153.1, 152.9, 149.2 (d, J_{C-F} = 228 Hz), 146.3 (d, J_{C-F} = 228 Hz), 146.2, 138.8, 138.3, 137.1, 132.2, 129.9 (2×CH), 129.0, 127.0, 118.9, 118.6 (2×CH), 117.4, 116.0, 111.6, 110.3, 62.1, 55.1 (2×CH_2), 52.8 (2×CH_2), 46.1. ESI-HRMS *m/z*: calc'd for $C_{27}H_{28}F_2N_7O$ $[M+H]^+$: 504.2323; found: 504.2329.

4.1.25. 1-(4-((3-Chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)quinazolin-6-yl)-3-(4-((4-methylpiperazin-1-yl)methyl)phenyl)urea (2d)

Yield 54.6%. mp: 197.3-198.7 °C. ^{1}H-NMR (DMSO-d_6): δ 9.76 (s, 1H, N-H), 8.97 (s, 1H, N-H), 8.90 (s, 1H, N-H), 8.49 (s, 1H, Ar-H), 8.46 (d, 1H, Ar-H, J = 2.0 Hz), 8.01 (d, 1H, Ar-H, J = 2.0 Hz), 7.87 (dd, 1H, Ar-H, J_1 = 2.0 Hz, J_2 = 9.2 Hz), 7.75 (d, 1H, Ar-H, J = 9.2 Hz), 7.71 (d, 1H, Ar-H, J = 2.0 Hz), 7.44-7.52 (m, 3H, Ar-H), 7.30-7.36 (m, 2H, Ar-H), 7.26 (d, 1H, Ar-H, J = 8.8 Hz), 7.21 (d, 2H, Ar-H, J = 8.8 Hz), 7.15-7.19 (m, 1H, Ar-H), 5.26 (s, 2H, Ar-CH_2-O), 3.39 (s, 2H, Ar-CH_2-N), 2.33 (br, 8H, 4×N-CH_2), 2.15 (s, 3H, N-CH_3). ^{13}C-NMR (DMSO-d_6): δ 162.7 (d, $J_{C\text{-}F}$ = 242 Hz), 157.6, 153.1, 153.0, 150.0, 146.2, 140.2, 140.1, 138.8, 138.1, 134.0, 132.2, 131.0, 129.8 (2×CH), 128.9, 126.8, 124.6, 123.8, 122.8, 121.4, 118.6 (2×CH), 116.0, 115.2, 114.5, 110.4, 69.8, 62.1, 55.1 (2×CH_2), 52.8 (2×CH_2), 46.1. ESI-HRMS *m/z*: calc'd for $C_{34}H_{34}ClFN_7O_2$ $[M+H]^+$: 626.2447; found: 626.2441.

4.1.26. 1-(4-((3-Chloro-4-methoxyphenyl)amino)quinazolin-6-yl)-3-(4-((4-methylpiperazin-1-yl)methyl)phenyl)urea (2e)

Yield 61.2%. mp: 198.0-201.0 °C. ^{1}H-NMR (DMSO-d_6): δ 9.74 (s, 1H, N-H), 8.98 (s, 1H, N-H), 8.91 (s, 1H, N-H), 8.49 (s, 1H, Ar-H), 8.46 (d, 1H, Ar-H, J = 2.0 Hz), 7.98 (d, 1H, Ar-H, J = 2.8 Hz), 7.87 (dd, 1H, Ar-H, J_1 = 2.0 Hz, J_2 = 8.8 Hz), 7.71-7.76 (m, 2H, Ar-H), 7.46 (d, 2H, Ar-H, J = 8.4 Hz), 7.18-7.23 (m, 3H, Ar-H), 3.88 (s, 3H, O-CH_3), 3.39 (s, 2H, Ar-CH_2-N), 2.35 (br, 8H, 4×N-CH_2), 2.17 (s, 3H, N-CH_3). ^{13}C-NMR (DMSO-d_6): δ 157.6, 153.2, 153.1, 151.2, 146.1, 138.8, 138.1, 133.5, 132, 129.9 (2×CH), 128.9, 126.8, 124.6, 122.9, 120.7, 118.6 (2×CH), 116.0, 113.0, 110.4, 62.1, 56.7, 55.1 (2×CH_2), 52.8 (2×CH_2), 46.0. ESI-HRMS *m/z*: calc'd for $C_{28}H_{31}ClN_7O_2$ $[M+H]^+$: 532.2228; found: 532.2222.

4.1.27. 1-(4-((4-Methylpiperazin-1-yl)methyl)phenyl)-3-(4-((3-(trifluoromethyl) phenyl)amino)quinazolin-6-yl)urea (2f)

Yield 23.8%. mp: 183.0-184.5 °C. ^{1}H-NMR (DMSO-d_6): δ 10.02 (s, 1H, N-H), 9.04 (s, 1H, N-H), 8.93 (s, 1H, N-H), 8.57 (s, 1H, Ar-H), 8.55 (d, 1H, Ar-H, J = 2.0 Hz), 8.31 (s, 1H, Ar-H), 8.23 (d, 1H, Ar-H, J = 8.0 Hz), 7.89 (dd, 1H, Ar-H, J_1 = 2.0 Hz, J_2 = 8.8 Hz), 7.79 (d, 1H, Ar-H, J = 8.8 Hz), 7.63 (t, 1H, Ar-H, J = 8.0 Hz), 7.44-7.48 (m, 3H, Ar-H), 7.22 (d, 2H, Ar-H, J = 8.4 Hz), 3.39 (s, 2H, Ar-CH_2-N), 2.35 (br, 8H, 4×N-CH_2), 2.17 (s, 3H, N-CH_3). ^{13}C-NMR (DMSO-d_6): δ 157.5, 153.1,

152.9, 146.3, 140.9, 138.8, 138.4, 132.2, 130.0, 129.9 (2×CH), 129.8, 129.0, 127.1, 126.1, 124.7 (d, J_{C-F} = 266 Hz), 119.9, 118.7 (2×CH), 118.5, 116.1, 110.3, 62.0, 55.0 (2×CH_2), 52.7 (2×CH_2), 46.0. ESI-HRMS *m/z*: calc'd for $C_{28}H_{29}F_3N_7O$ $[M+H]^+$: 536.2386; found: 536.2380.

4.1.28. Methyl 3-((6-(3-(4-((4-methylpiperazin-1-yl)methyl)phenyl)ureido) quinazolin-4-yl)amino)benzoate (2g)

Yield 22.5%. mp: 144.5-147.0 °C. ^{1}H-NMR (DMSO-d_6): δ 10.07 (s, 1H, N-H), 9.81 (s, 1H, N-H), 9.52 (s, 1H, N-H), 8.56 (s, 1H, Ar-H), 8.55 (s, 1H, Ar-H), 8.46 (s, 1H, Ar-H), 8.23 (d, 1H, Ar-H, *J* = 7.6 Hz), 7.92 (d, 1H, Ar-H, *J* = 8.4 Hz), 7.78 (d, 1H, Ar-H, *J* = 8.8 Hz), 7.71 (d, 1H, Ar-H, *J* = 7.6 Hz), 7.50-7.59 (m, 3H, Ar-H), 7.26 (br, 2H, Ar-H), 3.89 (s, 3H, O-CH_3), 3.51 (s, 2H, Ar-CH_2-N), 2.95 (br, 8H, 4×N-CH_2), 2.72 (s, 3H, N-CH_3). ^{13}C-NMR (DMSO-d_6): δ 166.6, 158.0, 153.2, 152.3, 144.2, 140.1, 139.6, 138.8, 130.6, 130.5, 130.4, 129.4 (2×CH), 127.7, 127.5, 127.2, 124.9, 123.6, 118.4 (2×CH), 116.0, 110.3, 60.6, 52.7 (2×CH_2), 49.5, 45.9 (2×CH_2), 42.8. ESI-HRMS *m/z*: calc'd for $C_{29}H_{32}N_7O_3$ $[M+H]^+$: 526.2567; found: 526.2561.

4.1.29. 1-(4-((4-Methylpiperazin-1-yl)methyl)phenyl)-3-(4-((3-(pyrrolidin-1-yl carbonyl)phenyl)amino)quinazolin-6-yl)urea (2h)

Yield 20.6%. mp: 161.5-163.0 °C. ^{1}H-NMR (DMSO-d_6): δ 9.87 (s, 1H, N-H), 9.04 (s, 1H, N-H), 8.98 (s, 1H, N-H), 8.53 (s, 1H, Ar-H), 8.51 (s, 1H, Ar-H), 8.04 (s, 1H, Ar-H), 7.95 (d, 1H, Ar-H, *J* = 8.4 Hz), 7.91 (d, 1H, Ar-H, *J* = 8.8 Hz), 7.77 (d, 1H, Ar-H, *J* = 8.8 Hz), 7.48 (s, 1H, Ar-H), 7.45 (d, 2H, Ar-H, *J* = 6.0 Hz), 7.26 (s, 1H, Ar-H), 7.22 (d, 2H, Ar-H, *J* = 8.4 Hz), 3.45-3.51 (m, 4H, 2×CH_2), 3.40 (s, 2H, Ar-CH_2-N), 2.39 (br, 8H, 4×N-CH_2), 2.26 (s, 3H, N-CH_3), 1.81-1.93 (m, 4H, 2×CH_2). ^{13}C-NMR (DMSO-d_6): δ 168.6, 157.6, 153.2, 153.0, 146.2, 139.8, 139.0, 138.3, 137.7, 131.7, 130.0 (2×CH), 128.9, 128.8, 126.9, 123.7, 122.4, 121.2, 118.6 (2×CH), 116.2, 110.4, 61.8, 54.7 (2×CH_2), 52.2 (2×CH_2), 49.5 (2×CH_2), 46.4, 26.5 (2×CH_2). ESI-HRMS *m/z*: calc'd for $C_{32}H_{37}N_8O_2$ $[M+H]^+$: 565.3039; found: 565.3034.

4.1.30. 1-(4-((Piperidin-1-yl)methyl)phenyl)-3-(4-((3-(trifluoromethyl)phenyl) amino)quinazolin-6-yl)urea (2i)

Yield 20.3%. mp: 156.0-158.0 °C. ^{1}H-NMR (DMSO-d_6): δ 10.02 (s, 1H, N-H),

9.06 (s, 1H, N-H), 8.97 (s, 1H, N-H), 8.58 (s, 1H, Ar-H), 8.55 (d, 1H, Ar-H, J = 1.6 Hz), 8.30 (s, 1H, Ar-H), 8.23 (d, 1H, Ar-H, J = 8.0 Hz), 7.90 (dd, 1H, Ar-H, J_1 = 1.6 Hz, J_2 = 8.8 Hz), 7.80 (d, 1H, Ar-H, J = 8.8 Hz), 7.63 (t, 1H, Ar-H, J = 8.0 Hz), 7.49 (d, 2H, Ar-H, J = 8.0 Hz), 7.46 (d, 1H, Ar-H, J = 8.0 Hz), 7.26 (d, 2H, Ar-H, J = 8.0 Hz), 3.56 (s, 2H, Ar-CH_2-N), 2.45 (br, 4H, 2×N-CH_2), 1.54 (br, 4H, 2×CH_2), 1.42 (br, 2H, CH_2). ^{13}C-NMR (DMSO-d_6): δ 157.5, 153.1, 152.9, 146.3, 140.9, 138.7, 138.4, 132.5, 130.0, 129.8 (2×CH), 129.5, 129.0, 127.1, 126.0, 124.7 (d, $J_{C\text{-}F}$ = 270 Hz), 119.9, 118.6 (2×CH), 118.4, 116.1, 110.3, 62.9, 54.2 (2×CH_2), 26.0 (2×CH_2), 24.5. ESI-HRMS *m/z*: calc'd for $C_{28}H_{28}F_3N_6O$ $[M+H]^+$: 521.2277; found: 521.2271.

4.1.31. 1-(4-(Pyrrolidin-1-yl-methyl)phenyl)-3-(4-((3-(trifluoromethyl)phenyl) amino)quinazolin-6-yl)urea (2j)

Yield 21.5%. mp: 176.1-179.2 °C. ^{1}H-NMR (DMSO-d_6): δ 10.02 (s, 1H, N-H), 9.03 (s, 1H, N-H), 8.91 (s, 1H, N-H), 8.57 (s, 1H, Ar-H), 8.56 (d, 1H, Ar-H, J = 2.0 Hz), 8.31 (s, 1H, Ar-H), 8.23 (d, 1H, Ar-H, J = 8.0 Hz), 7.89 (dd, 1H, Ar-H, J_1 = 2.0 Hz, J_2 = 8.8 Hz), 7.79 (d, 1H, Ar-H, J = 9.2 Hz), 7.63 (t, 1H, Ar-H, J = 8.0 Hz), 7.40-7.50 (m, 3H, Ar-H), 7.24 (d, 2H, Ar-H, J = 8.0 Hz), 3.53 (s, 2H, Ar-CH_2-N), 2.44 (br, 4H, 2×N-CH_2), 1.65-1.75 (m, 4H, 2×CH_2). ^{13}C-NMR (DMSO-d_6): δ 157.5, 153.1, 152.9, 146.3, 140.9, 138.6, 138.3, 133.5, 130.0, 129.5, 129.4 (2×CH), 129.0, 127.1, 126.0, 124.7 (d, $J_{C\text{-}F}$ = 270 Hz), 118.7 (2×CH), 119.9, 118.4, 116.1, 110.3, 59.6, 53.9 (2×CH_2), 23.5 (2×CH_2). ESI-HRMS *m/z*: calc'd for $C_{27}H_{26}F_3N_6O$ $[M+H]^+$: 507.2120; found: 507.2115.

4.1.32. 1-(4-(Morpholinomethyl)phenyl)-3-(4-((3-(trifluoromethyl)phenyl) amino)quinazolin-6-yl)urea (2k)

Yield 44.6%. mp: 186.0-188.0°C. ^{1}H-NMR (DMSO-d_6): δ 10.02 (s, 1H, N-H), 9.00 (s, 1H, N-H), 8.90 (s, 1H, N-H), 8.57 (s, 1H, Ar-H), 8.55 (d, 1H, Ar-H, J = 2.4 Hz), 8.30 (s, 1H, Ar-H), 8.23 (d, 1H, Ar-H, J = 8.0 Hz), 7.89 (dd, 1H, Ar-H, J_1 = 2.4 Hz, J_2 = 8.8 Hz), 7.79 (d, 1H, Ar-H, J = 8.8 Hz), 7.63 (t, 1H, Ar-H, J = 8.0 Hz), 7.43-7.50 (m, 3H, Ar-H), 7.24 (d, 2H, Ar-H, J = 8.4 Hz), 3.57 (t, 4H, 2×O-CH_2, J = 4.0 Hz), 3.41 (s, 2H, Ar-CH_2-N), 2.34 (br, 4H, 2×N-CH_2). ^{13}C-NMR (DMSO-d_6): δ 157.5, 153.1, 152.9, 146.3, 140.9, 138.9, 138.3, 131.7, 130.0 (2×CH), 129.8, 129.5,

129.0, 127.1, 126.1, 124.7 (d, J_{C-F} = 271 Hz), 119.9, 118.7 (2×CH), 118.5, 116.1, 110.3, 66.6 (2×CH_2), 62.5 (CH_2), 53.5 (2×CH_2). ESI-HRMS *m/z*: calc'd for $C_{27}H_{26}F_3N_6O_2$ $[M+H]^+$: 523.2069; found: 523.2064.

4.1.33. 1-(4-(Diethylaminomethyl)phenyl)-3-(4-((3-(trifluoromethyl)phenyl) amino)quinazolin-6-yl)urea (2l)

Yield 25.4%. mp: 134.8-137.0 °C. ^{1}H-NMR (DMSO-d_6): δ 10.03 (s, 1H, N-H), 9.17 (br, 2H, 2×N-H), 8.58 (s, 1H, Ar-H), 8.55 (d, 1H, Ar-H, *J* = 1.6 Hz), 8.30 (s, 1H, Ar-H), 8.23 (d, 1H, Ar-H, *J* = 8.0 Hz), 7.90 (dd, 1H, Ar-H, J_1 = 2.0 Hz, J_2 = 8.8 Hz), 7.80 (d, 1H, Ar-H, *J* = 9.2 Hz), 7.63 (t, 1H, Ar-H, *J* = 8.0 Hz), 7.41-7.57 (m, 3H, Ar-H), 7.22-7.39 (m, 2H, Ar-H), 3.53 (s, 2H, Ar-CH_2-N), 2.56 (br, 4H, 2×N-CH_2), 0.92-1.14 (m, 6H, 2×CH_3). ^{13}C-NMR (DMSO-d_6): δ 157.5, 153.1, 152.9, 146.3, 140.9, 138.9, 138.3, 130.9, 130.0 (2×CH), 129.8, 129.5, 129.0, 127.1, 126.1, 124.7 (d, J_{C-F} = 271 Hz), 119.9, 118.6 (2×CH), 118.5, 116.1, 110.3, 56.0, 46.3 (2×CH_2), 21.6 (2×CH_3). ESI-HRMS *m/z*: calc'd for $C_{27}H_{28}F_3N_6O$ $[M+H]^+$: 509.2277; found: 509.2271.

4.1.34. 1-(4-((Dimethylamino)methyl)phenyl)-3-(4-((3-(trifluoromethyl) phenyl)amino)quinazolin-6-yl)urea (2m)

Yield 34.8%. mp: 152.3-154.0 °C. ^{1}H-NMR (DMSO-d_6): δ 10.06 (s, 1H, N-H), 9.46 (s, 1H, N-H), 9.43 (s, 1H, N-H), 8.57 (s, 1H, Ar-H), 8.56 (s, 1H, Ar-H), 8.31 (s, 1H, Ar-H), 8.23 (d, 1H, Ar-H, *J* = 8.4 Hz), 7.91 (d, 1H, Ar-H, *J* = 8.8 Hz), 7.80 (d, 1H, Ar-H, *J* = 8.8 Hz), 7.63 (t, 1H, Ar-H, *J* = 8.0 Hz), 7.56 (d, 2H, Ar-H, *J* = 8.0 Hz), 7.45 (d, 1H, Ar-H, *J* = 7.6 Hz), 7.36 (d, 2H, Ar-H, *J* = 8.0 Hz), 3.86 (s, 2H, Ar-CH_2-N), 2.48 (s, 6H, 2×N-CH_3). ^{13}C-NMR (DMSO-d_6): δ 157.6, 153.2, 152.9, 146.3, 140.9, 140.4, 138.3, 131.3, 130.0 (2×CH), 129.8, 129.5, 129.0, 127.1, 126.1, 124.7 (d, J_{C-F} = 271 Hz), 119.9, 118.5, 118.4 (2×CH), 116.2, 110.3, 61.1, 43.1 (2×CH_3). ESI-HRMS *m/z*: calc'd for $C_{25}H_{24}F_3N_6O$ $[M+H]^+$: 481.1964; found: 481.1958.

4.1.35. 1-(4-((3-Ethynylphenyl)amino)quinazolin-6-yl)-3-(4-(morpholino methyl)phenyl)urea (2n)

Yield 46.6%. mp: 204.6-206.0 °C. ^{1}H-NMR (DMSO-d_6): δ 9.83 (s, 1H, N-H), 9.02 (s, 1H, N-H), 8.93 (s, 1H, N-H), 8.55 (s, 1H, Ar-H), 8.50 (s, 1H, Ar-H), 8.05 (s, 1H, Ar-H), 7.92 (s, 1H, Ar-H), 7.90 (s, 1H, Ar-H), 7.77 (d, 1H, Ar-H, *J* = 8.8 Hz),

7.45 (d, 2H, Ar-H, *J* = 8.0 Hz), 7.40 (t, 1H, Ar-H, *J* = 8.0 Hz), 7.20-7.26 (m, 3H, Ar-H), 4.22 (s, 1H, C≡CH), 3.55-3.59 (m, 4H, 2×O-CH_2), 3.37 (s, 2H, Ar-CH_2-N), 2.34 (br, 4H, 2×N-CH_2). ^{13}C-NMR (DMSO-d_6): δ 157.6, 153.1, 153.0, 146.3, 140.3, 138.9, 138.2, 131.6, 130.0 (2×CH), 129.3, 128.9, 127.1, 127.0, 125.4, 123.3, 122.2, 118.6 (2×CH), 116.1, 110.4, 84.0, 81.0, 66.6 (2×CH_2), 62.4, 53.5 (2×CH_2). ESI-HRMS *m/z*: calc'd for $C_{28}H_{27}N_6O_2$ $[M+H]^+$: 479.2195; found: 479.2190.

4.1.36. 1-(4-((Diethylamino)methyl)phenyl)-3-(4-((3-ethynylphenyl)amino) quinazolin-6-yl)urea (2o)

Yield 32.5%. mp: 181.0-182.0 °C. ^{1}H-NMR (DMSO-d_6): δ 9.83 (s, 1H, N-H), 9.00 (s, 1H, N-H), 8.90 (s, 1H, N-H), 8.54 (s, 1H, Ar-H), 8.49 (s, 1H, Ar-H), 8.04 (s, 1H, Ar-H), 7.92 (s, 1H, Ar-H), 7.89 (s, 1H, Ar-H), 7.77 (d, 1H, Ar-H, *J* = 9.2 Hz), 7.46 (d, 2H, Ar-H, *J* = 8.0 Hz), 7.41 (t, 1H, Ar-H, *J* = 8.0 Hz), 7.25 (s, 1H, Ar-H), 7.22 (d, 2H, Ar-H, *J* = 7.2 Hz), 4.22 (s, 1H, C≡CH), 3.48 (s, 2H, Ar-CH_2-N), 2.46 (br, 4H, 2×N-CH_2), 0.98 (t, 6H, 2×CH_3, *J* = 6.8 Hz). ^{13}C-NMR (DMSO-d_6): δ 157.6, 153.1, 153.0, 146.3, 140.3, 138.7, 138.3, 133.1, 129.7 (2×CH), 129.3, 128.9, 127.1, 127.0, 125.4, 123.3, 122.2, 118.7 (2×CH), 116.1, 110.4, 84.0, 81.0, 56.7, 46.4 (2×CH_2), 11.9 (2×CH_3). ESI-HRMS *m/z*: calc'd for $C_{28}H_{29}N_6O$ $[M+H]^+$: 465.2403; found: 465.2357.

4.1.37. (R)-1-(4-((4-Methylpiperazin-1-yl)methyl)phenyl)-3-(4-((1-phenylethyl) amino)quinazolin-6-yl)urea (2p)

Yield 25.6%. mp: 130.2-133.8 °C. 1H-NMR (DMSO-d_6): δ 8.94 (s, 1H, N-H), 8.92 (s, 1H, N-H), 8.39 (d, 1H, N-H, *J* = 8.4 Hz), 8.37 (s, 1H, Ar-H), 8.32 (s, 1H, Ar-H), 7.80 (d, 1H, Ar-H, *J* = 8.4 Hz), 7.63 (d, 1H, Ar-H, *J* = 8.8 Hz), 7.46 (d, 4H, Ar-H, *J* = 7.6 Hz), 7.31 (t, 2H, Ar-H, *J* = 7.2 Hz), 7.15-7.25 (m, 3H, Ar-H), 5.61 (t, 1H, Ar-CH-N, *J* = 6.8 Hz), 3.57 (s, 2H, Ar-CH_2-N), 2.38 (br, 8H, 4×N-CH_2), 2.21 (s, 3H, N-CH_3), 1.60 (d, 3H, CH_3, *J* = 7.2 Hz). ^{13}C-NMR (DMSO-d_6): δ 158.6, 153.8, 153.2, 145.7, 145.3, 138.9, 137.4, 131.9, 129.9 (2×CH), 128.6 (2×CH), 128.4, 127.0, 126.7 (2×CH), 126.5, 118.6 (2×CH), 115.6, 110.0, 62.0, 54.9 (2×CH_2), 52.6, 49.7 (2×CH_2), 45.8, 22.6. ESI-HRMS *m/z*: calc'd for $C_{29}H_{34}N_7O$ $[M+H]^+$: 496.2825; found: 496.2819.

4.1.38. 1-Phenyl-3-(4-((3-(trifluoromethyl)phenyl)amino)quinazolin-6-yl)urea (3a)

Yield 65.7% mp: 237.0-238.2 °C. ^{1}H-NMR (DMSO-d_6): δ 10.02 (s, 1H, N-H), 9.02 (s, 1H, N-H), 8.92 (s, 1H, N-H), 8.58 (s, 1H, Ar-H), 8.56 (d, 1H, Ar-H, J = 2.0 Hz), 8.30 (s, 1H, Ar-H), 8.23 (d, 1H, Ar-H, J = 8.0 Hz), 7.89 (dd, 1H, Ar-H, J_1 = 2.0 Hz, J_2 = 8.8 Hz), 7.80 (d, 1H, Ar-H, J = 9.2 Hz), 7.63 (t, 1H, Ar-H, J = 8.0 Hz), 7.53 (d, 2H, Ar-H, J = 8.0 Hz), 7.46 (d, 1H, Ar-H, J = 8.0 Hz), 7.32 (t, 2H, Ar-H, J = 8.0 Hz), 7.01 (t, 1H, Ar-H, J = 7.6 Hz). ^{13}C-NMR (DMSO-d_6): δ 157.5, 153.1, 152.9, 146.3, 140.9, 140.0, 138.3, 130.0, 129.8, 129.5, 129.3 (2×CH), 129.0, 127.1, 126.1, 124.7 (d, J_{C-F} = 270 Hz), 119.9, 118.8 (2×CH), 118.5, 116.1, 110.4. ESI-HRMS *m/z*: calc'd for $C_{22}H_{17}F_3N_5O$ $[M+H]^+$: 424.1385; found: 424.1380.

4.1.39. 1-(4-Methylphenyl)-3-(4-((3-(trifluoromethyl)phenyl)amino)quinazolin-6-yl)urea (3b)

Yield 68.1%. mp: 239.5-241.0 °C. ^{1}H-NMR (DMSO-d_6): δ 10.02 (s, 1H, N-H), 8.99 (s, 1H, N-H), 8.82 (s, 1H, N-H), 8.57 (s, 1H, Ar-H), 8.55 (d, 1H, Ar-H, J = 2.0 Hz), 8.30 (s, 1H, Ar-H), 8.22 (d, 1H, Ar-H, J = 8.0 Hz), 7.88 (dd, 1H, Ar-H, J_1 = 2.0 Hz, J_2 = 8.8 Hz), 7.80 (d, 1H, Ar-H, J = 8.8 Hz), 7.63 (t, 1H, Ar-H, J = 8.0 Hz), 7.45 (d, 1H, Ar-H, J = 8.0 Hz), 7.41 (d, 2H, Ar-H, J = 8.4 Hz), 7.12 (d, 2H, Ar-H, J = 8.4 Hz), 2.26 (s, 3H, CH_3). ^{13}C-NMR (DMSO-d_6): δ 157.5, 153.1, 152.8, 146.2, 140.9, 138.4, 137.4, 131.4, 130.0, 129.8, 129.7 (2×CH), 128.9, 127.1, 126.1, 124.7 (d, J_{C-F} = 270 Hz), 119.9, 118.9 (2×CH), 118.5, 116.1, 110.3, 20.8. ESI-HRMS *m/z*: calc'd for $C_{23}H_{19}F_3N_5O$ $[M+H]^+$: 438.1542; found: 438.1536.

4.1.40. 1-(4-methoxyphenyl)-3-(4-((3-(trifluoromethyl)phenyl)amino) quinazolin-6-yl)urea (3c)

Yield 61.7%. mp: 242.0-243.2 °C. ^{1}H-NMR (DMSO-d_6): δ 10.02 (s, 1H, N-H), 8.97 (s, 1H, N-H), 8.76 (s, 1H, N-H), 8.57 (s, 1H, Ar-H), 8.54 (s, 1H, Ar-H), 8.30 (s, 1H, Ar-H), 8.22 (d, 1H, Ar-H, J = 8.0 Hz), 7.89 (d, 1H, Ar-H, J = 8.0 Hz), 7.79 (d, 1H, Ar-H, J = 8.8 Hz), 7.63 (t, 1H, Ar-H, J = 8.0 Hz), 7.42-7.46 (m, 3H, Ar-H), 6.90 (d, 2H, Ar-H, J = 8.8 Hz), 3.73 (s, 3H, OCH_3). ^{13}C-NMR (DMSO-d_6): δ 157.5, 155.1, 153.3, 152.7, 146.1, 140.9, 138.5, 133.0, 130.0, 129.8, 128.8, 127.1, 126.1, 124.7 (d,

$J_{C\text{-}F}$ = 266 Hz), 120.7 (2×CH), 120.0, 118.5, 116.1, 114.5 (2×CH), 110.2, 55.6. ESI-HRMS *m/z*: calc'd for $C_{23}H_{19}F_3N_5O_2$ $[M+H]^+$: 454.1491; found: 454.1485.

4.1.41. General procedures for the synthesis of compounds 8a, 8b

The solution of 3-nitrobenzaldehyde (1.0 g, 6.62 mmol) and piperidine (or pyrrolidine) (7.95 mmol) in dichloromethane (30 mL) was stirred at room temperature for 2 h, then added portionwise triacetoxyborohydride (4.21 g, 19.86 mmol). The mixture was stirred for another 3 h, added water (20 mL). The organic phase was separated. The water phase was extracted with dichloromethane (20 mL × 2). The organic layer was combined, washed with brine (20 mL), dried (Na_2SO_4), filtered and concentrated to produce compounds **8a**, **8b** as yellow oil.

4.1.42. 3-(Piperidin-1-ylmethyl)nitrobenzene (8a)

Yield 98.9%. ^{1}H-NMR ($CDCl_3$): δ 8.20 (s, 1H, Ar-H), 8.10 (d, 1H, Ar-H, *J* = 8.0 Hz), 7.69 (d, 1H, Ar-H, *J* = 8.0 Hz), 7.48 (t, 1H, Ar-H, *J* = 8.0 Hz), 3.55 (s, 2H, Ar-CH_2-N), 2.39 (br, 4H, 2×N-CH_2), 1.56-1.62 (m, 4H, 2×CH_2), 1.42-1.48 (m, 2H, CH_2).

4.1.43. 3-(Pyrrolidin-1-ylmethyl)nitrobenzene (8b)

Yield 98.0%. ^{1}H-NMR ($CDCl_3$): δ 8.20 (s, 1H, Ar-H), 8.10 (d, 1H, Ar-H, *J* = 8.0 Hz), 7.69 (d, 1H, Ar-H, *J* = 8.0 Hz), 7.48 (t, 1H, Ar-H, *J* = 8.0 Hz), 3.71 (s, 2H, Ar-CH_2-N), 2.51-2.54 (m, 4H, 2×N-CH_2), 1.79-1.82 (m, 4H, 2×CH_2).

4.1.44. General procedures for the synthesis of compounds 9a, 9b

Compounds **9a**, **9b** were prepared according to the reported method.[33]

4.1.45. 3-(Piperidin-1-ylmethyl)aniline (9a)

Yield 93.0%. mp: 96.7-97.8 °C. ^{1}H-NMR ($CDCl_3$): δ 7.11 (t, 1H, Ar-H, *J* = 7.6 Hz), 6.73 (s, 1H, Ar-H), 6.72 (d, 1H, Ar-H, *J* = 8.0 Hz), 6.60 (d, 1H, Ar-H, *J* = 7.2 Hz), 3.66 (s, 2H, Ar-CH_2-N), 2.41 (br, 4H, 2×N-CH_2), 1.58-1.64 (m, 4H, 2×CH_2), 1.42-1.48 (m, 2H, CH_2).

4.1.46. 3-(Pyrrolidin -1-ylmethyl)aniline (9b)

Yield 93.2%. mp: 61.8-63.0 °C. ^{1}H-NMR ($CDCl_3$): δ 7.11 (t, 1H, Ar-H, *J* = 7.6 Hz), 6.74 (s, 1H, Ar-H), 6.73 (d, 1H, Ar-H, *J* = 7.2 Hz), 6.60 (d, 1H, Ar-H, *J* = 8.0 Hz), 3.56 (s, 2H, Ar-CH_2-N), 2.53-2.56 (m, 4H, 2×N-CH_2), 1.80-1.83 (m, 4H, 2×CH_2).

4.1.47. General procedures for the synthesis of compounds **2q** and **2r**

To a solution of 4-(3-trifluoromethylphenylamino)quinazolin-6-amine (0.12 g, 0.39 mmol) in acetonitrile (5 mL), CDI (0.24 g, 1.51 mmol) was added. The reaction mixture was stirred at room temperature for 8 h. To this suspension, compound **9** in 5 mL of acetonitrile was added and stirred at room temperature for 2 h. The mixture was then refluxed for another 2 h and cooled to room temperature. The off-white precipitate was filtered and purified by chromatography on silica gel (dichloromethane/methanol = 20:1, v/v) to give compound **2q** or **2r** as off-white powder.

4.1.48. 1-(3-((Piperidin-1-yl)methyl)phenyl)-3-(4-((3-(trifluoromethyl)phenyl) amino)quinazolin-6-yl)urea (2q)

Yield 31.3%. mp: 207.1-209.0 °C. ^{1}H-NMR (DMSO-d_6): δ 10.02 (s, 1H, N-H), 8.97 (s, 1H, N-H), 8.93 (s, 1H, N-H), 8.58 (s, 1H, Ar-H), 8.56 (d, 1H, Ar-H, J = 2.4 Hz), 8.30 (s, 1H, Ar-H), 8.22 (d, 1H, Ar-H, J = 8.0 Hz), 7.89 (dd, 1H, Ar-H, J_1 = 2.0 Hz, J_2 = 8.8 Hz), 7.79 (d, 1H, Ar-H, J = 9.2 Hz), 7.63 (t, 1H, Ar-H, J = 8.0 Hz), 7.50 (s, 1H, Ar-H), 7.46 (d, 1H, Ar-H, J = 8.0 Hz), 7.38 (d, 1H, Ar-H, J = 8.0 Hz), 7.24 (t, 1H, Ar-H, J = 8.0 Hz), 6.93 (d, 1H, Ar-H, J = 8.0 Hz), 3.40 (s, 2H, Ar-CH_2-N), 2.33 (br, 4H, 2×N-CH_2), 1.51 (br, 4H, 2×CH_2), 1.40 (br, 2H, CH_2). ^{13}C-NMR (DMSO-d_6): δ 157.5, 153.1, 152.9, 146.3, 140.9, 139.9, 138.3, 130.0, 129.8, 129.5, 129.1, 128.9, 127.1, 126.1, 124.7 (d, $J_{C\text{-}F}$ = 271 Hz), 123.1, 119.9, 119.1, 118.5, 117.4, 116.2, 110.3, 63.4, 54.4 (2×CH_2), 25.9 (2×CH_2), 24.4. ESI-HRMS *m/z*: calc'd for $C_{28}H_{28}F_3N_6O$ $[M+H]^+$: 521.2277; found: 521.2271.

4.1.49. 1-(3-((Pyrrolidin-1-yl)methyl)phenyl)-3-(4-((3-(trifluoromethyl)phenyl) amino)quinazolin-6-yl)urea (2r)

Yield 34.0%. mp: 204.0-206.5 °C. ^{1}H-NMR (DMSO-d_6): δ 10.02 (s, 1H, N-H), 9.01 (s, 1H, N-H), 8.95 (s, 1H, N-H), 8.57 (s, 1H, Ar-H), 8.56 (d, 1H, Ar-H, J = 2.0 Hz), 8.30 (s, 1H, Ar-H), 8.22 (d, 1H, Ar-H, J = 8.0 Hz), 7.89 (dd, 1H, Ar-H, J_1 = 2.0 Hz, J_2 = 8.8 Hz), 7.79 (d, 1H, Ar-H, J = 9.2 Hz), 7.63 (t, 1H, Ar-H, J = 8.0 Hz), 7.56 (s, 1H, Ar-H), 7.46 (d, 1H, Ar-H, J = 8.0 Hz), 7.37 (d, 1H, Ar-H, J = 8.0 Hz), 7.25 (t, 1H, Ar-H, J = 8.0 Hz), 6.95 (d, 1H, Ar-H, J = 7.6 Hz), 3.59 (s, 2H, Ar-CH_2-N), 2.49

(br, 4H, 2×N-CH_2), 1.72 (br, 4H, 2×CH_2). ^{13}C-NMR (DMSO-d_6): δ 157.5, 153.1, 152.9, 146.3, 140.9, 140.0, 138.3, 130.0, 129.8, 129.5, 129.1, 129.0, 127.1, 126.1, 124.7 (d, J_{C-F} = 271 Hz), 122.8, 119.9, 118.9, 118.6, 117.4, 116.1, 110.3, 60.0, 53.9 (2×CH_2), 23.5 (2×CH_2). ESI-HRMS *m/z*: calc'd for $C_{27}H_{26}F_3N_6O$ $[M+H]^+$: 507.2120; found: 507.2115.

4.1.50. General procedures for the synthesis of compounds 10a, **10b**

Compounds **10a** and **10b** were prepared according to the reported process.[42]

4.1.51. 4-(4-Methylpiperazin-1-yl)nitrobenzene (10a)

Yield 92.7%. mp: 95.2-95.6 °C. ^{1}H-NMR ($CDCl_3$): δ 8.15 (d, 2H, Ar-H, *J* = 9.2 Hz), 6.85 (d, 2H, Ar-H, *J* = 9.2 Hz), 3.48 (t, 4H, 2×N-CH_2, *J* = 7.2 Hz), 1.25 (t, 4H, 2×N-CH_2, *J* = 7.2 Hz), 2.40 (s, 3H, N-CH_3).

4.1.52. 4-(Diethylamino)nitrobenzene (10b)

Yield 94.2%. mp: 64.0-64.8 °C. ^{1}H-NMR ($CDCl_3$): δ 8.13 (d, 2H, Ar-H, *J* = 9.2 Hz), 6.61 (d, 2H, Ar-H, *J* = 9.2 Hz), 3.48 (q, 4H, 2×N-CH_2, *J* = 4.8 Hz), 2.60 (t, 6H, 2×CH_3, *J* = 4.8 Hz).

4.1.53. General procedures for the synthesis of compounds 11a and 11b

Compounds **11a** and **11b** were prepared according to the reported method.[43]

4.1.54. 4-(4-Methylpiperazin-1-yl)aniline (11a)

Yield 89.7%. mp: 72.0-73.4 °C. ^{1}H-NMR ($CDCl_3$): δ 6.84 (d, 2H, Ar-H, *J* = 8.8 Hz), 6.78 (d, 2H, Ar-H, *J* = 8.8 Hz), 3.11 (t, 4H, 2×N-CH_2, *J* = 4.8 Hz), 2.63 (t, 4H, 2×N-CH_2, *J* = 4.8 Hz), 2.39 (s, 3H, N-CH_3).

4.1.55. 4-(Diethylamino)aniline (11b)

Yield 85.2%. mp: 55.3-57.0 °C. ^{1}H-NMR ($CDCl_3$): δ 7.80 (d, 2H, Ar-H, *J* = 9.2 Hz), 6.74 (d, 2H, Ar-H, *J* = 9.2 Hz), 3.45 (q, 4H, 2×N-CH_2, *J* = 7.2 Hz), 1.10 (t, 6H, 2×CH_3, *J* = 7.2 Hz).

4.1.56. General procedures for the synthesis of compounds 3d, **3e**

Compounds **3d** and **3e** were synthesized by the same process with compound **2q**.

4.1.57. 1-(4-(Diethylamino)phenyl)-3-(4-((3-(trifluoromethyl)phenyl)amino) quinazolin-6-yl)urea (3d)

Yield 33.4%. mp: 182.0-184.0 °C. ^{1}H-NMR (DMSO-d_6): δ 10.00 (s, 1H, N-H),

8.86 (s, 1H, N-H), 8.56 (s, 1H, N-H), 8.51 (s, 1H, Ar-H), 8.49 (s, 1H, Ar-H), 8.30 (s, 1H, Ar-H), 8.22 (d, 1H, Ar-H, J = 7.6 Hz), 7.89 (d, 1H, Ar-H, J = 8.4 Hz), 7.78 (d, 1H, Ar-H, J = 8.8 Hz), 7.63 (t, 1H, Ar-H, J = 8.0 Hz), 7.45 (d, 1H, Ar-H, J = 7.2 Hz), 7.29 (d, 2H, Ar-H, J = 8.0 Hz), 6.65 (d, 2H, Ar-H, J = 8.0 Hz), 3.29 (q, 4H, 2×N-CH_2, J = 6.8 Hz), 1.07 (t, 6H, 2×CH_3, J = 6.4 Hz). ^{13}C-NMR (DMSO-d_6): δ 157.5, 153.4, 152.7, 146.1, 144.1, 140.9, 138.8, 130.0, 129.8, 128.9, 128.5, 127.0, 126.0, 124.7 (d, J_{C-F} = 271 Hz), 121.5 (2×CH), 119.8, 118.5, 116.2, 112.9 (2×CH), 109.9, 44.3 (2×CH_2), 12.8 (2×CH_3). ESI-HRMS *m/z*: calc'd for $C_{26}H_{26}F_3N_6O$ $[M+H]^+$: 495.2120; found: 495.2115.

4.1.58. 1-(4-(4-Methylpiperazin-1-yl)phenyl)-3-(4-((3-(trifluoromethyl)phenyl) amino)quinazolin-6-yl)urea (3e)

Yield 37.5%. mp: 215.2-216.4 °C. ^{1}H-NMR (DMSO-d_6): δ 10.00 (s, 1H, N-H), 8.91 (s, 1H, N-H), 8.65 (s, 1H, N-H), 8.56 (s, 1H, Ar-H), 8.53 (d, 1H, Ar-H, J = 2.0 Hz), 8.30 (s, 1H, Ar-H), 8.22 (d, 1H, Ar-H, J = 7.6 Hz), 7.88 (dd, 1H, Ar-H, J_1 = 2.0 Hz, J_2 = 8.8 Hz), 7.78 (d, 1H, Ar-H, J = 8.8 Hz), 7.63 (t, 1H, Ar-H, J = 8.0 Hz), 7.45 (d, 1H, Ar-H, J = 7.6 Hz), 7.36 (d, 2H, Ar-H, J = 8.8 Hz), 6.91 (d, 2H, Ar-H, J = 8.8 Hz), 3.07 (t, 4H, 2×N-CH_2, J = 4.8 Hz), 2.46 (t, 4H, 2×N-CH_2, J = 4.8 Hz), 2.23 (s, 3H, N-CH_3). ^{13}C-NMR (DMSO-d_6): δ 157.5, 153.2, 152.8, 147.1, 146.2, 140.9, 138.6, 131.9, 130.0, 129.5, 129.0, 127.0, 126.1, 124.7 (d, J_{C-F} = 271 Hz), 120.3 (2×CH), 119.9, 118.5, 116.6 (2×CH), 116.2, 110.1, 55.1 (2×CH_2), 49.2 (2×CH_2), 46.1. ESI-HRMS *m/z*: calc'd for $C_{27}H_{27}F_3N_7O$ $[M+H]^+$: 522.2229; found: 522.2224.

4.1.59. General procedures for the synthesis of compounds 4a and 4b

To a solution of 6-amino-4-anilinoquinazoline or 4-(1-phenylethyl)quinazoline-4,6-diamine (0.23 mmol) in acetonitrile (5 mL), CDI (0.11 g, 0.69 mmol) was added. The reaction mixture was stirred at room temperature for 8 h when lots of solid produced. To this suspension, the solution of 2-(4-methylpiperazin-1-yl)ethylamine in 5 mL of acetonitrile was added. Then, the mixture was stirred at room temperature for 2 h, refluxed for another 2 h, cooled to room temperature and concentrated under reduced pressure. The residue was added water (20 mL) and the mixture was extracted with dichloromethane (20 mL × 3). The organic layer was combined,

washed with brine (20 mL), dried (Na_2SO_4), concentrated under reduced pressure and purified by chromatography on silica gel (dichloromethane/methanol = 15:1, v/v) to give compounds **4a** or **4b** as yellow powder.

4.1.60. 1-(2-(4-Methylpiperazin-1-yl)ethyl)-3-(4-((3-(trifluoromethyl)phenyl) amino)quinazolin-6-yl)urea (4a)

Yield 45.5%. mp: 169.8-170.5 °C. ^{1}H-NMR (DMSO-d_6): δ 9.96 (s, 1H, N-H), 9.03 (s, 1H, N-H), 8.54 (s, 1H, N-H), 8.46 (d, 1H, Ar-H, J = 2.0 Hz), 8.29 (s, 1H, Ar-H), 8.21 (d, 1H, Ar-H, J = 8.0 Hz), 7.83 (dd, 1H, Ar-H, J_1 = 2.0 Hz, J_2 = 8.8 Hz), 7.74 (d, 1H, Ar-H, J = 8.8 Hz), 7.62 (t, 1H, Ar-H, J = 8.0 Hz), 7.44 (d, 1H, Ar-H, J = 7.6 Hz), 6.35 (t, 1H, Ar-H, J = 5.2 Hz), 3.20-3.29 (m, 4H, 2×CH_2), 2.41-2.49 (m, 8H, 4×N-CH_2), 2.27 (s, 3H, N-CH_3). ^{13}C-NMR (DMSO-d_6): δ 157.4, 155.6, 152.5, 145.9, 141.0, 139.2, 130.0, 129.5, 128.8, 126.7, 124.7 (d, J_{C-F} = 261 Hz), 126.0, 119.8, 118.4, 116.2, 109.3, 57.6, 54.7 (2×CH_2), 52.4 (2×CH_2), 45.5, 36.9. ESI-HRMS *m/z*: calc'd for $C_{23}H_{27}F_3N_7O$ $[M+H]^+$: 474.2229; found: 474.2224.

4.1.61. (R)-1-(2-(4-Methylpiperazin-1-yl)ethyl)-3-(4-((1-phenylethyl)amino) quinazolin-6-yl)urea (4b)

Yield 33.6%. mp: 101.3-103.5 °C. ^{1}H-NMR (DMSO-d_6): δ 8.89 (s, 1H, N-H), 8.30 (s, 1H, N-H), 8.28 (s, 1H, N-H), 8.26 (d, 1H, Ar-H, J = 2.0 Hz), 7.74 (dd, 1H, Ar-H, J_1 = 2.0 Hz, J_2 = 8.8 Hz), 7.58 (d, 1H, Ar-H, J = 8.8 Hz), 7.45 (d, 2H, Ar-H, J = 7.2 Hz), 7.31 (t, 2H, Ar-H, J = 8.0 Hz), 7.21 (t, 1H, Ar-H, J = 7.2 Hz), 6.29 (t, 1H, Ar-H, J = 4.2 Hz), 5.50-5.64 (m, 1H, Ar-CH-N), 3.21-3.30 (m, 4H, 2×CH_2), 2.36-2.46 (m, 8H, 4×N-CH_2), 2.23 (s, 3H, N-CH_3), 1.59 (d, 3H, CH_3, J = 7.2 Hz). ^{13}C-NMR (DMSO-d_6): δ 158.5, 155.7, 153.4, 145.4, 145.2, 138.3, 128.6 (2×CH), 128.3, 127.0, 126.7 (2×CH), 126.1, 115.7, 109.9, 57.7, 54.9 (2×CH_2), 52.7 (2×CH_2), 49.7, 45.8, 36.9, 22.6. ESI-HRMS *m/z*: calc'd for $C_{24}H_{32}N_7O$ $[M+H]^+$: 434.2668; found: 434.2663.

4.2. Biological materials and methods

4.2.1. Antiproliferative assay

The *in vitro* antiproliferative activities of compounds were determined by MTT (3-[4, 5-dimethyl-2-thiazolyl]-2, 5-diphenyl-*2H*-tetrazolium bromide) assay. A431 or

A549 cells (1500-4000 cells per well) were seeded into 96-well plates in 200 μL medium and incubated for 24 h. A series of concentrations of synthesized compounds and gefitinib were added to the wells with DMSO as vehicle control. The mixture was incubated at 37˚C, with a final concentration of 1% DMSO. After 72 h of incubation, 20 μL of MTT solution (5 mg/mL in PBS) was added to each well and incubated at 37˚C for 4 h. The supernatant of each well was removed and the formed blue formazan crystals were dissolved in 200 μL of DMSO. The optical density at 490 nm wavelength was determined by Varioskan Flash Multimode Reader (Thermo scientific). Three separate experiments with triplicate data were performed to obtain mean cell viability. The IC_{50} value, that is, the concentration (μM) of a compound was able to cause 50% cell death with respect to the control culture, were calculated by means of GraphPad Prism 5 Software.

4.2.2. ELISA-based EGFR-TK assay

Theses assays were carried out as described previously.[38] All of the enzymatic reactions were conducted at 30 °C for 40 min. The 50 μL reaction mixture contains 40 mM Tris, pH 7.4, 10 mM $MgCl_2$, 0.1 mg/ml BSA, 1 mM DTT, 10 μM ATP, EGFR and the substrate. The compounds were diluted in 10% DMSO and 5 μL of the dilution was added to a 50 μL reactor so that the final concentration of DMSO is 1% in all of reactions. The assay was performed using Kinase-Glo Plus luminescence kinase assay kit. It measures kinase activity by quantitating the amount of ATP remaining in solution following a kinase reaction. The luminescent signal from the assay is correlated with the amount of ATP present and is inversely correlated with the amount of kinase activity. The IC_{50} values were calculated using nonlinear regression with normalized dose−response fit using Prism GraphPad sofeware.

4.2.3. Anticancer effects in established nude mouse A549 xenograft model *in vivo*

Mice (BALB/C, SPF grade, male, 18-20 g) were purchased from Shanghai Slakey Laboratory Animal Co., LTD and fed at Experiment Animal Center of Xi′an Jiaotong University Health Science Center. The experimental protocol was approved by Ethic Committee of Xi′an Jiaotong University.

A549 cells at 3×10^5 were injected subcutaneously into the flank. Once the tumor xenografts reached 100 mm^3, all tumor-bearing nude mice were randomly divided into three groups, with 5 mice in one group. Gefitinib and **2j** were dissolved in DMSO/PEG400/H_2O (1:7:2, V/V/V), respectively, and dosed orally at 50 mg/kg once a day for 12 days. In the control group, the same volume of solvent was administered orally. Tumor volumes and body weights were recorded at intervals of 3 days. Tumor volume was calculated as length × width × width ÷ 2 and is reported in mm^3. Results are expressed as the mean ± standard error.

5. Molecular modeling

Protein coordinates obtained from the crystal structure of erlotinib binding site (1M17) or PD168393 binding site (4LQM) was used to dock the compounds. The molecular docking procedure was performed by using C-DOCKER protocol within Discovery Studio 2.5. For enzyme preparation, the hydrogen atoms were added. The whole EGFR enzyme was defined as a receptor and the site sphere was selected on the basis of the ligand binding location of erlotinib or PD168393. Erlotinib or PD168393 was removed and compound **2j** or **2r** was placed. After end of molecular docking, ten docking poses were scored and selected based on calculated C-DOCKER energy.

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Figure and scheme Legends

Figure 1. The structures of gefitinib, erlotinib and lapatinib

Figure 2. The design of target compounds

Figure 3. The anticancer effect of compound **2j** in established xenograft A549 lung adenocarcinoma models (n = 5). Mice bearing cancers were orally administered solvent, compound **2j** and gefitinib once daily for 12 days. $^{**}P < 0.001$ *vs* solvent.

Figure 4. Docking modes of compounds **2j** and **2r** with $EGFR^{wt}$ (A and B); compounds **2j** and **2r** with $EGFR^{L858R}$ (C and D). Selected residues Lys721, Gln767, Met769, Thr830, Asp831 in $EGFR^{wt}$ and Ser720, Met793, Asp800 in $EGFR^{L858R}$ are shown. Green dashed lines indicate hydrogen bonds.

Scheme 1. Reagents and conditions: (a) $(COCl)_2$, CH_2Cl_2, rt, 2 h; (b) secondary amine, THF, 0°C to rt, 3 h; (c) 2 M NaOH, MeOH, rt, 4 h; (d) i: $SOCl_2$, DMF, reflux, 2 h; ii: NaN_3, THF/H_2O, DIPEA, rt, 4 h; (e) secondary amine, CH_2Cl_2, $NaBH(OAc)_3$, rt, 5 h; (f) toluene, reflux, 2 h.

Scheme 2. Reagents and conditions**:** (a) piperidine or pyrrolidine, CH_2Cl_2, $NaBH(OAc)_3$, rt, 5 h; (b) iron powder, NH_4Cl, MeOH/H_2O, reflux, 1 h; (c) i: CDI, MeCN, rt, 8 h; ii: **9**, reflux, 4 h.

Scheme 3. Reagents and conditions: (a) toluene, reflux, 2 h. (b) secondary amine, DMF, K_2CO_3, 50 °C, 1 h; (c) Pd-C, H_2, EtOH, rt, 4 h; (d) i: CDI, acetonitrile, rt, 8 h; ii: **11**, reflux, 4 h.

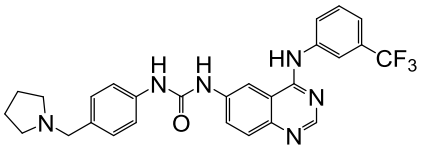
Scheme 4. Reagents and conditions: (a) i: CDI, acetonitrile, rt, 8 h; ii: 2-(4-methylpiperazin-1-yl)ethylamine, rt, to reflux, 4 h.

Table 1 Antiproliferative activities of compounds against two cancer cell lines ($\bar{x} \pm s$, n = 3)

Table 2 The inhibitory activities of selected compounds against kinase

Graphical abstract

	IC_{50}
A431	1.36 μM
A549	0.83 μM
$EGFR^{wt}$	4.1 nM



2j