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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design, synthesis and biological evaluation of novel 2,4,6-trisubstituted quinazoline derivatives as potential antitumor agents

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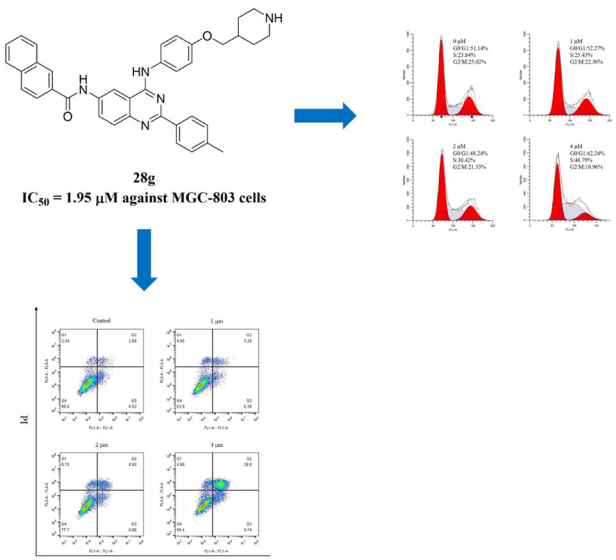
Abstract

In this study, a series of novel 2,4,6-trisubstituted quinazoline derivatives were designed, synthesized and biologically evaluated their antiproliferative activity against four human cancer cell lines (Eca-109, A549, PC-3 and MGC-803). The most of designed compounds showed considerable antiproliferative activity against the tested four cancer cell lines, while compound **28g** displayed the best antiproliferative activity with the IC_{50} values of 1.95 μM and 2.46 μM against MGC-803 cells and Eca-109 cells, respectively. Further mechanism studies indicated that **28g** significantly inhibited the cell migration and colony formation of MGC-803 cells. Besides, **28g** also dose-dependently induced cellular apoptosis and cell cycle arrest at S phase in MGC-803 cells. Overall, all these studies suggested that **28g** has the potential to act as a valuable lead compound for the development of antitumor agents.

Graphical Abstract



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Keywords Quinazoline · Synthesis · Antitumor · Apoptosis · Cell cycle analysis

Introduction

Cancer is one of the most lethal diseases all over the world nowadays and even in the future. It has resulted in 10 million cancer deaths which account for one-sixth of deaths over the world in 2020, according to the World Health Organization (WHO). Besides, the global cancer burden is expected to be increased from 19.3 million cases in 2020 to 28.4 million cases in 2040 [1], emphasizing the emergency of effectively controlling the cancer development and treating various kinds of cancer. Regarding the increasing rate of cancer prevalence, vast efforts have been taken in the cancer treatment and great successes were made, including surgery, chemotherapy and radiotherapy. Although many new cancer treatment strategies, such as immunotherapy [2–4] and stem cell therapy [5–7], have been developed and shown promising potential against cancer, the chemotherapy is still the main strategy widely applied to the treatment of cancer.

Quinazoline, an important nitrogen-containing heterocyclic compound, has been widely applied in the development of drug candidates due to the wide range of pharmacological effects its derivatives presented, including antitumor, antiviral, antibacterial, anti-inflammatory, and antimalarial activities [8–16]. To date, many quinazoline-based drugs have been approved for the treatment of hypertension (Doxazosin) [17], chronic graft-versus-host disease (Tucatinib) [18] and, most importantly, cancer (Gefitinib, Erlotinib, Afatinib and Idelalisib etc.) [19–21]. Among all of the quinazoline derivatives, 4-anilinoquinazoline plays an important role in the development of antitumor agents as seen in EGFR inhibitors Gefitinib, Erlotinib and Afatinib which are 4-aminoquinazoline derivatives. Besides, this skeleton also can be seen in HDAC inhibitors (**1**) [22], microtubule inhibitors (**2, 3**) [23–25], and aurora kinase inhibitors (**4, 5**) [26, 27]. Aliphatic amines, especially aliphatic nitrogen heterocycles, are well-known building blocks in drug design and play a key role in improving the pharmacologic properties of new chemical entities via altering their physicochemical and ADME properties [28]. Compounds **6** and **7** are potent aliphatic nitrogen heterocycles contained LSD1 inhibitors showed good antitumor activities both in vitro and in vivo [29, 30]. Furthermore, alkynyl groups as star building blocks also have been broadly exploited in the discovery and development of drugs due to the advantages of improving metabolic stability, affinity, selectivity and the antitumor activities of drug candidates, as showed in Erlotinib and LSD1 inhibitor **8** [31, 32]. Therefore, based on the molecule hybridization strategy, we introduced propargyl group and aliphatic amine moieties into the 2-position and 4-position of the 4-anilinoquinazoline core to design and synthesize a series of 2,4,6-trisubstituted quinazoline derivatives (Fig. 1) and evaluated their biological activities in vitro.

Results and discussion

Chemistry

The synthesis route of compounds **18a-18l** was shown in Scheme 1. The commercially available 4-nitrophenol (**9**) reacted with corresponding alcohol in the presence of triphenylphosphine and diisopropyl azodicarboxylate through Mitsunobu reaction to afforded compounds **10a-10l**, followed by a reduction reaction mediated by Fe powder to give compounds **11a-11l**. On the other hand, commercially available compound **12** reacted with thionyl chloride to obtain its acyl chloride derivative **13**, and then the latter performed a cyclization with the ammonium thiocyanate to form compound **14**. Compound **15** was obtained through a nucleophilic substitution between compound **14** and 3-bromopropyne in the presence of potassium hydroxide. After compound **15** underwent an acylation reaction, the acyl chloride derivative compound **16** was obtained. Then, the nucleophilic aromatic substitution between compound **16** and compounds **11a-11l** was performed to afford compounds **17a-17l**. Finally, the deprotection of compound **11a-11l** using trifluoroacetic acid in dichloromethane gave the target compound **18a-18l**.

As shown in Scheme 2, the synthesis route of compounds **21a-21w** is present. Compound **17l** was reduced by the Fe powder to afford its arylamine derivatives **19**, and then the latter performed an amidation reaction with appropriate carboxylic acid to give compounds **20a-20w**. Finally, target compounds **21a-21w** were obtained by removing the tert-butoxycarbonyl protecting group of compounds **20a-20w**.

As depicted in Scheme 3, compounds **28a-28h** were synthesized. After undergoing an acylation reaction, compound **13** was obtained. Then, compound **13** reacted with ammonium hydroxide in the tetrahydrofuran to form corresponding amide **21**. Further, a cyclization reaction between compound **22** and appropriate aldehyde was performed in the presence of sodium acetate and iodine to afford compounds **23a-23h**. Treatment of **23a-23h** with thionyl chloride gave the corresponding acyl chloride derivatives **24a-24h**, followed by the nucleophilic aromatic substituting with the amino of compound **11l** to afford compounds **25a-25h**. Subsequent reduction then gave the desired arylamine compounds **26a-26h**. Then, the amide condensation reaction was performed between compounds

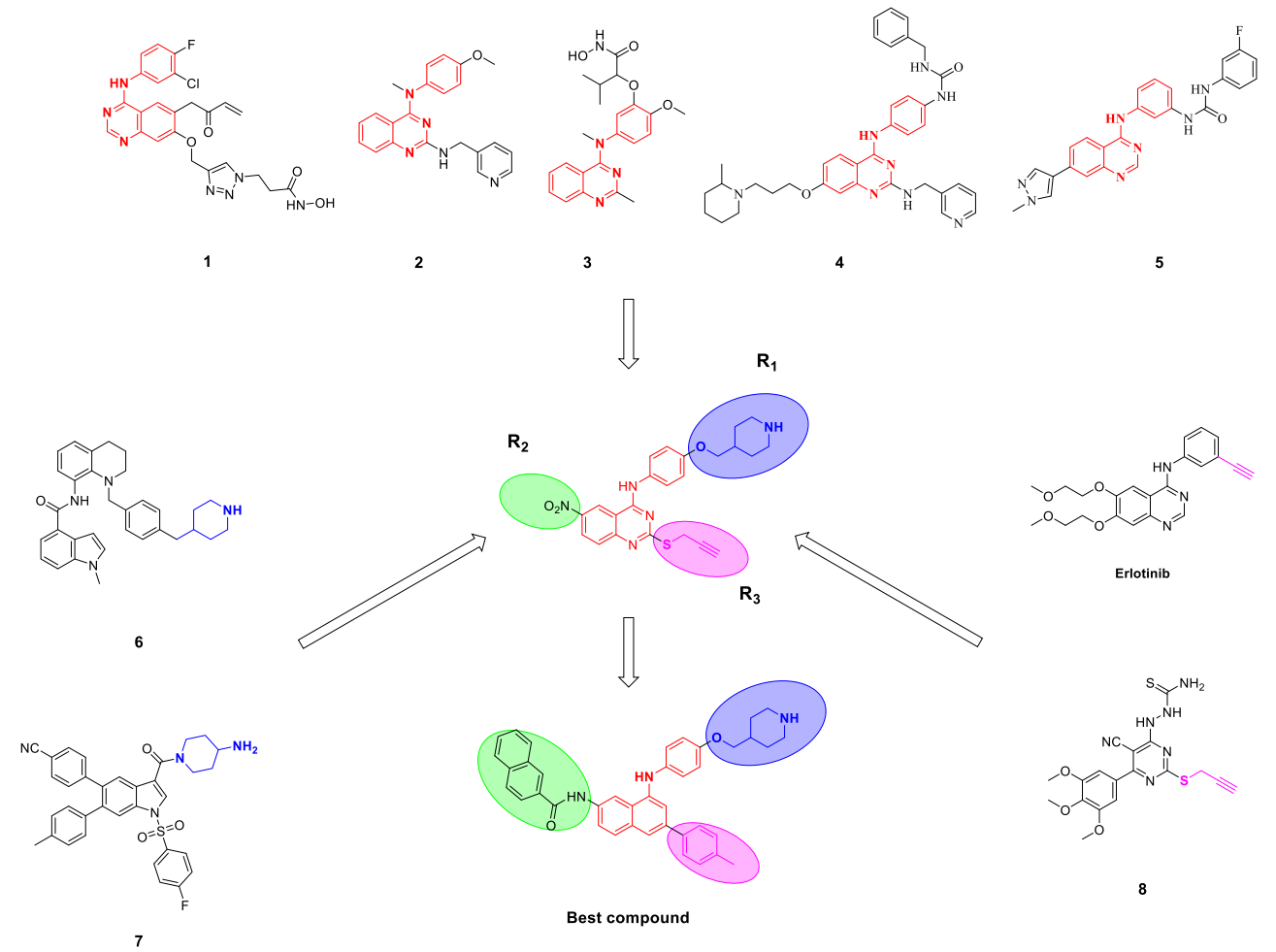


Fig. 1 The design strategy of 2,4,6-trisubstituted quinazoline derivatives reported in this paper

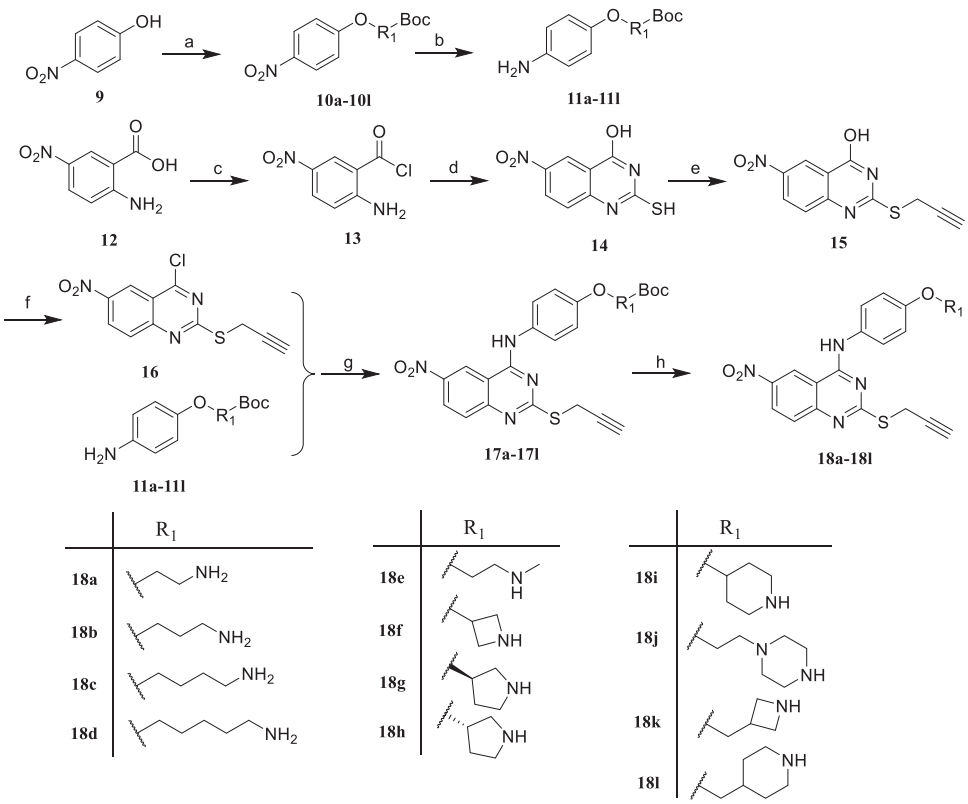
26a-**26h** and 2-naphthoic acid, which resulted in compounds **27a**-**27h**. Finally, the target compounds **28a**-**28h** were obtained by deprotecting the tert-butoxycarbonyl groups of **27a**-**27h**. The structures of all target compounds were confirmed by ^{1}H NMR, ^{13}C NMR and HRMS.

Biological activity

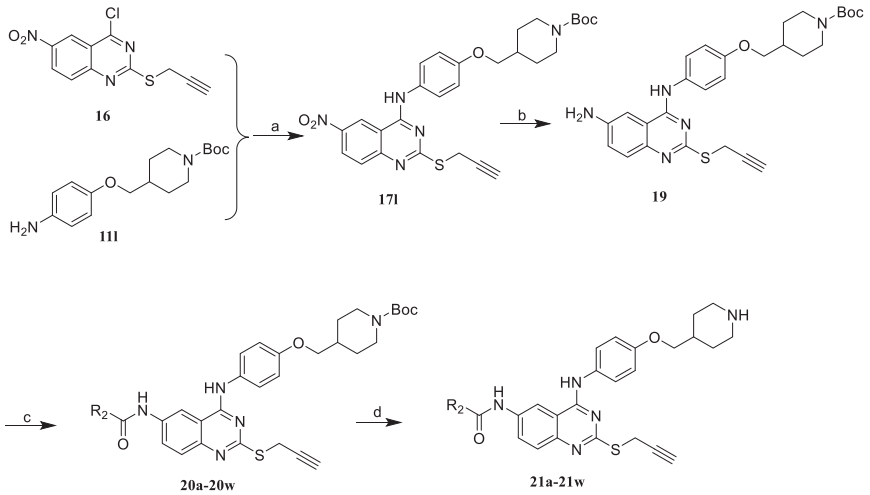
Antiproliferative activity

We tested the antiproliferative activity of all the target compounds against four human cancer cell lines, the Eca-109 (human esophageal epithelial cancer cell line), A549 (human non-small cell lung cancer cell line), PC-3 (human prostate cancer cell line) and MGC-803 (human gastric cancer cell line), by MTT assay and Gefitinib as well as the 5-Fluorouracil (5-FU) was used as the positive control. The results were summarized in Tables 1–3. To investigate the effects of aliphatic amine moiety of 4-position on the antiproliferative activity, compounds **18a**-**18l** were first designed and synthesized, and their antiproliferative activity results were shown in Table 1. Apart from **18c** and **18d**, all of the tested compounds showed medium to potent antiproliferative activity against these four human cancer cells. Generally, compared with straight-chain primary amine moieties, the compounds containing alicyclic amine moieties exhibited more potent antiproliferative activity. While the long chains are unfavorable for the bioactivity in the straight-chain primary amine moieties contained compounds (**18a**-**18d**), the bioactivity of big rings is superior to small rings in the alicyclic amine moieties contained compounds (**18f**-**18i, 18k**-**18l**). Besides, enhanced antiproliferative activities were observed when introduced a methyl into the terminal amino of compound **18a**, as shown in **18e**. Extending the piperidine-4yloxy moiety of compound **18i** to piperidine-4ylmethoxy moiety resulted in a more potent compound **18l**. Among these compounds, **18l** displayed the most potent antiproliferative activity against tested cancer cells and thus was chosen for further optimization.

For many quinazoline derivatives, the substitutions of the 6-and 7-position are essential for their bioactivities

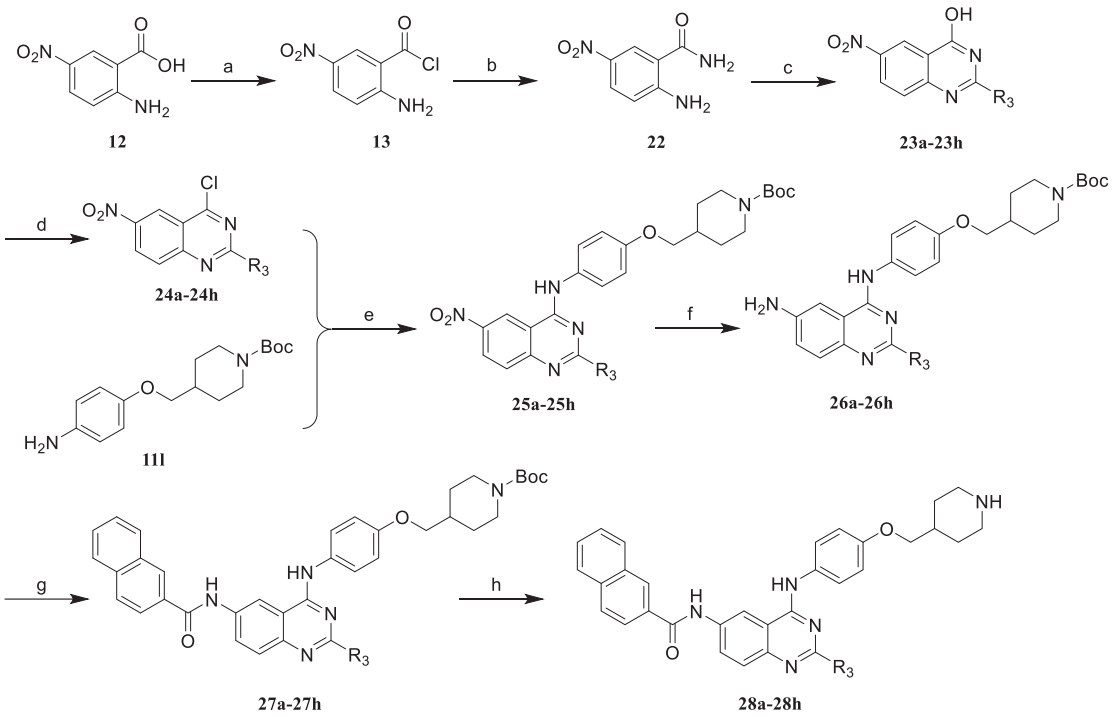


Scheme 1 Reaction conditions and reagents: a) corresponding alcohol, triphenylphosphine, diisopropyl azodicarboxylate, THF, 0 °C, 3 h; b) Fe, NH_4Cl, EtOH, H_2O, 85 °C, 4 h; c) $SOCl_2$, 75 °C, 2 h; d) ammonium thiocyanate, acetone, 0.5 h; e) 3-bromopropyne, KOH, H_2O, rt, 2 h; f) $SOCl_2$, DMF, 65 °C, 2 h; g) K_2CO_3, DMF, 80 °C, 2 h; h) THF, DCM, rt, 1 h



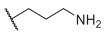
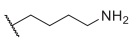
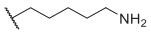
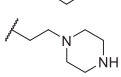
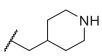
Scheme 2 Reaction conditions and reagents: a) K_2CO_3, DMF, 80 °C, 1 h; b) Fe, NH_4Cl, EtOH, H_2O, 85 °C, 4 h; c) corresponding carboxylic acid, HATU, DIPEA, DMF, rt, 6 h; d) THF, DCM, rt, 1 h

[8, 22, 33–35]. Thus, we hypothesized whether modifying the 6-position of **18l** could further improve its antiproliferative activity. To verify this hypothesis, we introduced various amide moieties into the 6-position of **18l** to design and synthesize compounds **21a**-**21w**. The antiproliferative activities of these compounds were shown in Table 2. Similarly, the alicyclic alkane moieties contained compounds also showed better activity, compared with those who had straight-chain alkane moieties in the 6-position (**21a**-**21f**). Then, diversely substituted benzamide were introduced to initially investigate the effects of aryl groups on the antiproliferative activities and resulted in **21g**-**21q**. It can be concluded that the electron-withdrawing groups, such as cyano and nitro groups (**21o**-**21q**) are unfavorable for the bioactivities, while electron-donating groups are tolerable (**21i**-**21m**). Further introducing heteroaromatic groups showed that nitrogen-contained heterocycles also are detrimental to antiproliferative activity (**21s**-**21t, 21v**-**21w**). Interestingly, the substitution of a naphthalene group at the 6-position resulted in the most potent compound **21u** which displayed enhanced activity against tested four



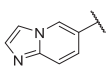
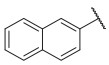
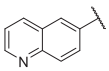
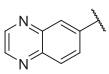
Scheme 3 Reaction conditions and reagents: a) $SOCl_2$, 75 °C, 2 h; b) NH_3•H_2O, THF, 0 °C, 0.5 h; c) corresponding aldehyde, C_2H_5ONa, iodine, DMF, 120 °C, 12 h; d) $SOCl_2$, 65 °C, 2 h; e) K_2CO_3, DMF, 80 °C, 1 h; f) Fe, NH_4Cl, EtOH, H_2O, 85 °C, 4 h; g) 2-naphthoic acid, HATU, DIPEA, DMF, rt, 6 h; h) THF, DCM, rt, 1 h

Table 1 In vitro antiproliferative activity of compounds **18a**-**18l** in MTT proliferation assay

Compd.	R_1	IC_{50} (μM)[a]			
		Eca-109	A549	PC-3	MGC-803
18a		9.30 ± 0.97	19.08 ± 1.28	10.92 ± 1.13	8.01 ± 0.28
18b		33.96 ± 1.53	>50	22.95 ± 1.44	23.36 ± 2.45
18c		>50	>50	>50	>50
18d		>50	>50	>50	>50
18e		6.87 ± 0.32	9.11 ± 0.96	9.42 ± 0.71	6.20 ± 0.79
18f		7.04 ± 0.85	20.21 ± 0.43	20.00 ± 0.62	13.54 ± 1.13
18g		6.07 ± 0.78	14.50 ± 1.65	7.78 ± 0.34	8.97 ± 0.84
18h		6.84 ± 0.84	13.93 ± 0.89	7.13 ± 0.51	8.91 ± 0.95
18i		5.79 ± 0.76	8.55 ± 1.08	5.14 ± 0.08	6.79 ± 0.89
18j		7.76 ± 0.30	15.49 ± 0.75	5.70 ± 0.16	7.82 ± 0.67
18k		12.54 ± 1.10	32.30 ± 2.33	12.62 ± 0.48	11.75 ± 1.66
18l		5.58 ± 0.75	7.96 ± 1.20	4.99 ± 0.01	4.75 ± 0.34
Gefitinib[b]	-	22.47 ± 0.43	7.26 ± 0.83	8.72 ± 0.41	9.17 ± 0.67
5-Fu[c]	-	7.91 ± 0.24	8.17 ± 0.26	9.35 ± 0.14	7.67 ± 0.54

Note: IC_{50} (μM)[a]: the concentration of compound required for cell activity to be suppressed by half; Gefitinib[b] and 5-FU[c]: Positive control

Table 2 In vitro antiproliferative activity of compounds **21a**-**21w** in MTT proliferation assay

Compd.	R_2	IC_{50} (μM)[a]			
		Eca-109	A549	PC-3	MGC-803
21a	$-CH_3$	26.81 ± 1.90	>50	31.41 ± 6.43	31.37 ± 3.32
21b	$-CH_2CH_3$	10.33 ± 0.25	14.50 ± 0.23	18.09 ± 1.45	15.70 ± 3.86
21c	$-CH_2CH_2CH_3$	11.27 ± 0.39	13.18 ± 0.14	21.24 ± 0.90	22.38 ± 1.44
21d		10.36 ± 0.50	17.89 ± 2.64	15.83 ± 2.27	14.57 ± 1.35
21e		7.79 ± 0.39	10.77 ± 1.14	10.23 ± 0.36	10.68 ± 1.54
21f		6.80 ± 0.30	6.55 ± 0.61	5.49 ± 0.36	10.38 ± 1.91
21g	$-C_6H_5$	6.30 ± 0.37	7.05 ± 0.76	4.91 ± 0.15	11.61 ± 0.07
21h	4-F-C_6H_5	8.15 ± 0.20	9.00 ± 0.75	5.89 ± 0.08	9.14 ± 0.60
21i	4-Cl-C_6H_5	5.17 ± 0.03	6.70 ± 0.37	5.38 ± 0.22	5.47 ± 0.18
21j	4-Br-C_6H_5	6.10 ± 0.29	8.10 ± 1.18	6.57 ± 0.02	6.95 ± 0.48
21k	4-CH_3-C_6H_5	5.07 ± 0.23	4.03 ± 0.14	4.81 ± 0.13	9.32 ± 0.37
21l	4-CH_3O-C_6H_5	9.40 ± 0.40	7.24 ± 0.53	3.90 ± 0.63	8.42 ± 0.28
21m	4-C_2H_5O-C_6H_5	7.15 ± 0.42	4.70 ± 0.11	3.69 ± 0.35	5.01 ± 0.22
21n	4-CF_3-C_6H_5	9.05 ± 0.44	8.36 ± 1.45	6.55 ± 0.09	9.66 ± 0.18
21o	4-CN-C_6H_5	16.85 ± 0.22	28.51 ± 1.54	11.97 ± 0.66	21.11 ± 2.02
21p	3-CN-C_6H_5	>50	>50	>50	>50
21q	4-NO_2-C_6H_5	>50	>50	34.46 ± 3.12	23.65 ± 0.92
21r		9.15 ± 0.52	7.58 ± 0.49	5.04 ± 0.48	9.60 ± 0.46
21s		36.70 ± 0.89	>50	34.20 ± 1.16	>50
21t		42.89 ± 0.95	12.13 ± 0.41	>50	>50
21u		4.91 ± 0.03	3.82 ± 0.06	3.62 ± 0.35	4.98 ± 0.70
21v		>50	>50	32.61 ± 2.30	>50
21w		>50	>50	33.14 ± 2.74	>50
Gefitinib[b]	-	22.47 ± 0.43	7.26 ± 0.83	8.72 ± 0.41	9.17 ± 0.67
5-Fu[c]	-	7.91 ± 0.24	8.17 ± 0.26	9.35 ± 0.14	7.67 ± 0.54

Note: IC_{50} (μM)[a]: the concentration of compound required for cell activity to be suppressed by half; Gefitinib[b] and 5-FU[c]: Positive control

cancer cell lines (IC_{50} = 4.91 μM, 3.82 μM, 3.62 μM and 4.98 μM against Eca-109, A549, PC-3 and MGC-803, respectively) compared with **18l**. However, this round of optimization still did not achieve our desired anti-proliferative activities.

To further improve the bioactivities, we then turned our attention to the 2-position of the quinazoline core which has not been modified yet. It has been reported that 2-arylquinazoline derivatives exhibited potent bioactivities in the cancer treatment and thus broadly applied in drug discovery [36–38]. Therefore, we designed a series of 2-arylquinazoline derivatives based on **21u** and evaluated their antiproliferative activity. As shown in Table 3, most of the compounds displayed improved activity compared with **21u**. It is worth noting that in the substituted phenyl groups, the *para*-substitution and *meta*-substitution exhibited better activity than the *ortho*-substitution (**28b**-**28f**). Particularly, *p*-tolyl contained compound **28g** displayed the most potent antiproliferative activity against Eca-109, A549,

Table 3 In vitro antiproliferative activity of compounds **28a**-**28h** in MTT proliferation assay

Compd.	R_3	IC_{50} (μM)[a]			
		Eca-109	A549	PC-3	MGC-803
28a	$-C_6H_5$	4.48 ± 0.13	4.19 ± 0.07	4.02 ± 0.40	2.22 ± 0.15
28b	2-F-C_6H_5	4.81 ± 0.03	5.93 ± 0.42	6.27 ± 0.10	4.83 ± 0.31
28c	3-F-C_6H_5	4.25 ± 0.15	4.37 ± 0.06	4.64 ± 0.10	4.51 ± 0.16
28d	4-F-C_6H_5	4.68 ± 0.25	5.30 ± 0.63	4.67 ± 0.16	3.64 ± 0.18
28e	2-Cl-C_6H_5	3.73 ± 0.07	3.15 ± 0.15	5.35 ± 0.53	3.45 ± 0.17
28f	2-CH_3-C_6H_5	3.85 ± 0.16	4.21 ± 0.09	4.76 ± 0.20	3.69 ± 0.34
28g	4-CH_3-C_6H_5	2.46 ± 0.13	3.21 ± 0.26	3.10 ± 0.35	1.95 ± 0.06
28h		3.25 ± 0.04	4.05 ± 0.13	3.70 ± 0.10	2.29 ± 0.13
Gefitinib[b]	-	22.47 ± 0.43	7.26 ± 0.83	8.72 ± 0.41	9.17 ± 0.67
5-Fu[c]	-	7.91 ± 0.24	8.17 ± 0.26	9.35 ± 0.14	7.67 ± 0.54

Note: IC_{50} (μM)[a]: the concentration of compound required for cell activity to be suppressed by half; Gefitinib[b] and 5-FU[c]: Positive control

PC-3 and MGC-803 cell lines with the IC_{50} values of 2.46 μM, 3.21 μM, 3.10 μM and 1.95 μM, respectively. Therefore, **28g** and MGC-803 cells were chosen for further investigating the underlying mechanism of these designed compounds.

Compound 28g inhibited the migration and colony formation of MGC-803 cells

The migration and invasion of tumour cells into the surrounding tissue and lymphatic and blood vessels is the initial step of cancer metastasis which is the leading cause of the cancer related death [39, 40]. Therefore, inhibition of the cancer cell metastasis is one of the effective strategies to treatment of cancer. Thus, we investigated the effects of compound **28g** on the migration of MGC-803 cells through Transwell assay. As shown in Fig. 2, the amount of migration cells was significantly decreased after incubating with **28g** with the increased concentration (0, 1, 2, 4 μM), which indicated that **28g** could dose-dependent inhibit the migration of MGC-803 cells. To further evaluate the antiproliferative ability of **28g** against MGC-803 cells, the colony formation assay was performed. As displayed in Fig. 2, after incubation of increased concentrations of compounds **28g** for 24 h, the cell colonies of MGC-803 cells were getting small and few compared with the control group (0 μM). It demonstrated that **28g** indeed could inhibit the colony formation of MGC-803 cells in a dose-dependent manner.

The effects of compound 28g on the cell cycle of MGC-803 cells

We then explored the effects of compound **28g** on the cell cycle distribution of MGC-803 cells using the flow cytometry assay. As depicted in Fig. 3, after the treatment of increasing concentrations of compound **28g** (1, 2, 4 μM) for 24 h, the percentage of MGC-803 cells in S phase was increased significantly (25.43% to 46.79%) compared with the control group (0 μM, 23.84%), which indicated that **28g** could arrest the cell cycle of MGC-803 cells at S phase in a dose-dependent manner.

The effects of compound 28g on the cell apoptosis of MGC-803 cells

Apoptosis is one kind of programmed cell death which orderly and effectively clears away the damaged cells and deregulation of apoptosis is the hallmark of cancer [41, 42]. Triggering apoptosis remains an efficient strategy for the cancer treatment [43]. To investigate whether compound **28g** could affect the apoptosis of MGC-803 cells, the DAPI staining assay and flow cytometry assay was performed. Before staining using DAPI, the cells were incubated with increasing concentrations of **28g** for 48 h. As shown in Fig. 4A, the number of cells with shrunken or broken nuclear was dramatically increased compared with the control group (0 μM). Further characterizing apoptosis effects of compound **28g** on MGC-803 cells was performed using Annexin V-FITC/PI double staining flow cytometry assay. As depicted in Fig. 4B, C, after incubating with **28g** with increasing concentrations for 48 h, the percentage of apoptotic MGC-803 cells was increased from 11.64% to 38.64% compared with the control group (0 μM, 9.21%). All these results demonstrated that compound **28g** could significantly induce the cell apoptosis of MGC-803 cells in a dose-dependent manner.

Conclusions

In summary, a series of 2, 4, and 6-trisubstituted quinazoline derivatives were designed, synthesized and evaluated for their

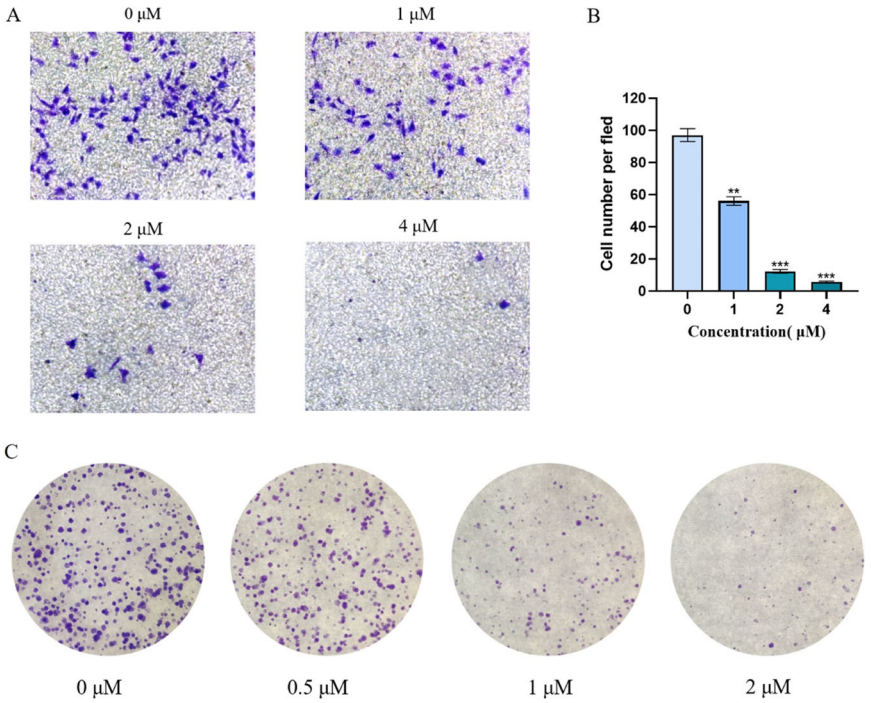


Fig. 2 **A** Compound **28g** inhibited migration of MGC-803 cells. **B** Quantification of the effects of **28g** on the migration of MGC-803 cells. **C** Compound **28g** inhibited the migration of MGC-803 cells. Compared with the control group, $^{**}P < 0.01$, $^{***}P < 0.001$

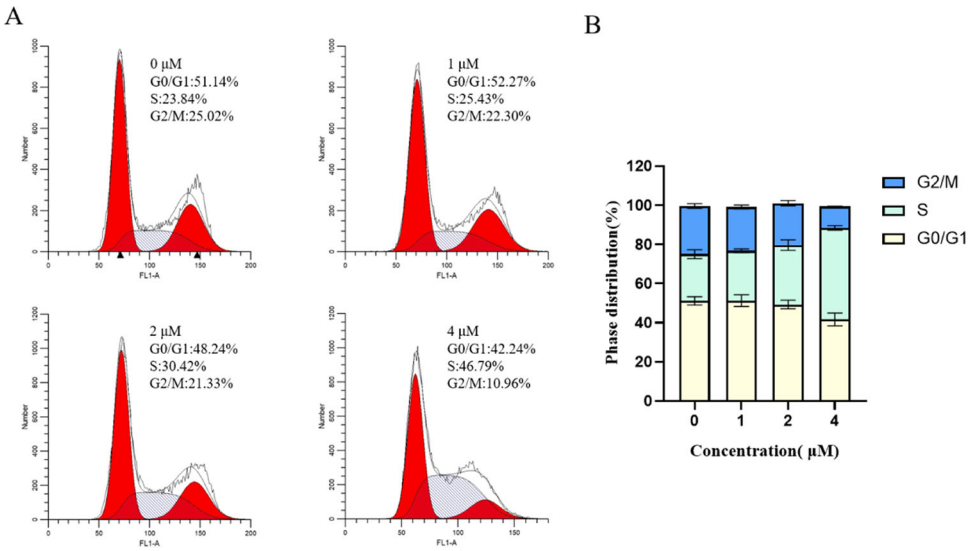


Fig. 3 **A** The effects of compound **28g** on the cell cycle of MGC-803 cells. **B** Quantitative analysis of the cell cycle distribution of MGC-803 cells

antiproliferative activity against four human cancer cell lines (Eca-109, A549, PC-3 and MGC-803) by MTT assay. The results manifested that most of the designed compounds exhibited moderate to excellent in vitro antiproliferative activity, while compound **28g** displayed the most potent antiproliferative activity against the tested cell lines Eca-109, A549, PC-3 and MGC-803 cell lines with the IC_{50} values of 2.46 μM, 3.21 μM, 3.10 μM and 1.95 μM, respectively. Further mechanism studies showed that **28g** could significantly inhibit the migration and colony formation of MGC-803 cells. In addition, DAPI staining assay and flow cytometry assay indicated that **28g** also dose-dependently induced cellular apoptosis and cell cycle arrest at S phase in MGC-803 cells. Taken together, all of these results suggested that **28g** has the potential to act as a valuable lead compound for the development of antitumor agents.

Experimental section

General procedure

All reagents and solvents were purchased from commercial sources and used without further purification. Reactions were

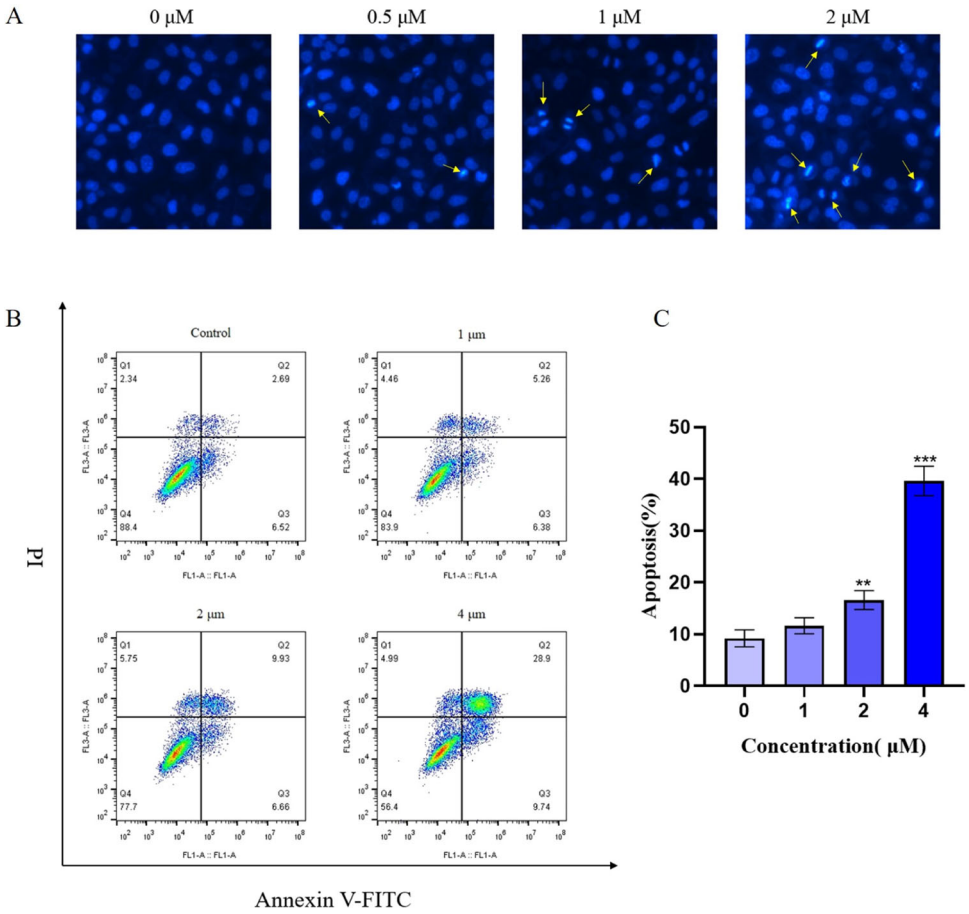


Fig. 4 **A** The effects of compound **28g** on the nuclear morphology of MGC-803 cells. The arrows in the figure indicate the cells with shrunken or broken nuclear. **B** The effects of compound **28g** on the apoptosis of MGC-803 cells. **C** Quantification of the effects of **28g** on the apoptosis of MGC-803 cells. Compared with the control group, $^{**}P < 0.01$, $^{***}P < 0.001$

monitored by thin-layer chromatography (TLC) on 0.25 mm silicagel plates (GF254) and visualized under UV light. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 400 MHz and 101 MHz spectrometer, respectively. High resolution mass spectra (HRMS) were recorded on a Waters Micromass Q-T of Micromass spectrometer by electrospray ionizaton (ESI). Melting points of target compounds were determined on an X-5 micromelting apparatus.

General procedure for the synthesis of compounds 11a-11l and 16

Compounds **11a**-**11l** and **16** were synthesized according to the published literature and the characterization data was consistent with these literature [44–46].

General procedure for the synthesis of compounds 17a-17l

Compound **16** (0.5 g, 1.80 mmol), compounds **11a**-**11l** (1.80 mmol) and K_2CO_3 (0.27 g, 1.98 mmol) were dissolved in 10 mL DMF, and then the reaction mixture was stirred at 90 °C for 1 h. After the reaction was completed, the mixture was treated with water and extracted with ethyl acetate. Then, the organic layer was dried with anhydrous Na_2SO_4 and concentrated in vacuo, followed by purifying through column chromatography to afford compounds **17a-17l** (yield: 79.4–90.2%).

General procedure for the synthesis of compounds 18a-18l

To a solution of compounds **17a**-**17l** (0.81 mmol) in DCM (10 mL), the TFA (2 mL) was added. After stirring at room temperature for 1 h, the resulting mixture was diluted with water and neutralized using Na_2CO_3, followed by extracting with ethyl acetate. Then, the organic layer was dried with anhydrous Na_2SO_4 and concentrated in vacuo. Finally, compounds **18a-18l** were obtained by column chromatography.

N-(4-(2-aminoethoxy)phenyl)-6-nitro-2-(prop-2-yn-1-ylthio)quinazolin-4-amine (**18a**)

Yellow solid, yield: 81.3%, mp: 198.5–199.3 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.52 (s, 1H), 8.45 (d, $J = 9.2$ Hz, 1H), 7.68 (d, $J = 8.5$ Hz, 3H), 7.00 (d, $J = 8.3$ Hz, 2H), 3.96 (d, $J = 6.9$ Hz, 4H), 3.16 (s, 1H), 2.92 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.43, 157.43, 155.81, 153.41, 143.21, 130.77, 127.51,

127.07, 124.56, 121.15, 114.20, 112.36, 80.41, 73.23, 70.03, 40.82, 18.63. HRMS (ESI) calcd for $C_{19}H_{18}N_5O_3S$ $[M + H]^+$: 396.1130, found: 396.1128.

N-(4-(3-aminopropoxy)phenyl)-6-nitro-2-(prop-2-yn-1-ylthio)quinazolin-4-amine (**18b**)

Faint yellow solid, yield: 80.8%, mp: 169.6–170.3 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.50 (s, 1H), 8.43 (d, $J = 9.2$ Hz, 1H), 7.66 (t, $J = 8.0$ Hz, 3H), 6.98 (d, $J = 8.5$ Hz, 2H), 4.06 (t, $J = 6.6$ Hz, 2H), 3.95 (s, 2H), 3.16 (s, 1H), 2.76 (t, $J = 6.9$ Hz, 2H), 1.84 (p, $J = 6.7$ Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.42, 157.34, 155.75, 153.46, 143.13, 131.00, 127.41, 126.99, 124.52, 121.16, 114.11, 112.54, 80.43, 73.21, 65.57, 38.07, 31.87, 18.61. HRMS (ESI) calcd for $C_{20}H_{19}N_5O_3S$ $[M + H]^+$: 410.1287, found: 410.1277.

N-(4-(4-aminobutoxy)phenyl)-6-nitro-2-(prop-2-yn-1-ylthio)quinazolin-4-amine (**18c**)

Faint yellow solid, yield: 83.6%, mp: 156.5–157.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.50 (s, 1H), 8.43 (d, $J = 9.1$ Hz, 1H), 7.65 (d, $J = 8.5$ Hz, 3H), 6.97 (d, $J = 8.4$ Hz, 2H), 4.02–3.97 (m, 2H), 3.95 (s, 2H), 3.15 (s, 1H), 2.64 (d, $J = 7.1$ Hz, 2H), 1.76 (s, 2H), 1.54 (t, $J = 8.1$ Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.42, 157.29, 155.68, 153.55, 143.08, 131.32, 127.34, 126.97, 124.55, 121.21, 114.11, 112.76, 80.47, 73.19, 67.54, 40.87, 28.72, 26.17, 18.59. HRMS (ESI) calcd for $C_{21}H_{21}N_5O_3S$ $[M + H]^+$: 424.1443, found: 424.1430.

N-(4-((5-aminopentyl)oxy)phenyl)-6-nitro-2-(prop-2-yn-1-ylthio)quinazolin-4-amine (**18d**)

Faint yellow solid, yield: 82.1%, mp: 130.4–131.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.54 (s, 1H), 8.46 (d, $J = 9.3$ Hz, 1H), 7.74–7.60 (m, 3H), 6.98 (d, $J = 8.4$ Hz, 2H), 4.00 (d, $J = 6.7$ Hz, 2H), 3.96 (s, 2H), 3.19–3.12 (m, 1H), 2.65 (t, $J = 6.0$ Hz, 2H), 1.80–1.67 (m, 2H), 1.47 (s, 4H).^{13}C NMR (101 MHz, DMSO-d_6) δ 169.43, 157.41, 155.77, 153.50, 143.19, 131.00, 127.48, 127.08, 124.59, 121.23, 114.14, 112.59, 80.45, 73.22, 67.53, 40.56, 30.82, 28.49, 22.82, 18.60. HRMS (ESI) calcd for $C_{22}H_{23}N_5O_3S$ $[M + H]^+$: 438.1600, found: 438.1592.

N-(4-(2-(methylamino)ethoxy)phenyl)-6-nitro-2-(prop-2-yn-1-ylthio)quinazolin-4-amine (**18e**)

Faint yellow solid, yield: 81.1%, mp: 91.8–92.4 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.52 (s, 1H), 8.45 (d, $J = 9.2$ Hz, 1H), 7.68 (d, $J = 8.6$ Hz, 3H), 7.00 (d, $J = 8.4$ Hz, 2H), 4.06 (s, 2H), 3.95 (s, 2H), 3.16 (s, 1H), 2.87 (d, $J = 6.5$ Hz, 2H), 2.37 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.44, 157.50, 155.76, 153.38, 143.22, 130.71, 127.54, 127.07, 124.54, 121.14, 114.19, 112.30, 80.39, 73.23, 67.19, 50.15, 35.91, 18.66. HRMS (ESI) calcd for $C_{20}H_{19}N_5O_3S$ $[M + H]^+$: 410.1287, found: 410.1282.

N-(4-(azetidin-3-yloxy)phenyl)-6-nitro-2-(prop-2-yn-1-ylthio)quinazolin-4-amine (**18f**)

Faint yellow solid, yield: 86.1%, mp: 207.9-208.6 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.56 (d, $J = 2.5$ Hz, 1H), 8.50 (dd, $J = 9.2$, 2.4 Hz, 1H), 7.73 (d, $J = 9.0$ Hz, 3H), 7.02–6.85 (m, 2H), 5.10 (p, $J = 5.7$ Hz, 1H), 4.36 (dd, $J = 11.3$, 6.6 Hz, 2H), 3.97 (d, $J = 2.5$ Hz, 2H), 3.97–3.92 (m, 2H), 3.16 (q, $J = 2.7$ Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.36, 157.47, 153.30, 152.81, 143.27, 131.94, 127.59, 127.12, 124.67, 121.13, 114.62, 112.26, 80.32, 73.27, 67.47, 52.45, 18.66. HRMS (ESI) calcd for $C_{20}H_{17}N_5O_3S$ $[M + H]^+$: 408.1130, found: 408.1121.

(R)-6-nitro-2-(prop-2-yn-1-ylthio)-N-(4-(pyrrolidin-3-yloxy)phenyl)quinazolin-4-amine (**18g**)

Faint yellow solid, yield: 85.5%, mp: 164.5–164.9 °C. ^{1}H NMR (400 MHz, DMSO-d6) δ 9.55 (s, 1H), 8.71–8.21 (m, 1H), 8.09–7.34 (m, 3H), 7.23–6.74 (m, 2H), 4.94 (s, 1H), 3.17 (s, 1H), 2.08 (s, 2H), 1.95 – 1.55 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.40, 157.48, 153.93, 153.37, 143.29, 131.12, 127.60, 127.15, 124.60, 121.15, 115.32, 112.29, 80.38, 76.78, 73.24, 51.24, 44.38, 31.57, 18.60. HRMS (ESI) calcd for $C_{21}H_{19}N_5O_3S$ $[M + H]^+$: 422.1287, found: 422.1271.

(S)-6-nitro-2-(prop-2-yn-1-ylthio)-N-(4-(pyrrolidin-3-yloxy)phenyl)quinazolin-4-amine (**18h**)

Faint yellow solid, yield: 85.5%, mp: 162.1–162.9 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.55 (s, 1H), 8.48 (d, $J = 9.3$ Hz, 1H), 7.70 (t, $J = 9.3$ Hz, 3H), 6.98 (d, $J = 8.5$ Hz, 2H), 4.92 (s, 1H), 3.96 (s, 2H), 3.14 (d, $J = 14.1$ Hz, 2H), 2.99 (d, $J = 9.8$ Hz, 2H), 2.89 (s, 1H), 2.13–1.81 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.40, 157.48, 153.93, 153.37, 143.29, 131.12, 127.60, 127.15, 124.60, 121.15, 115.32, 112.29, 80.38, 76.78, 73.24, 51.24, 44.38, 31.57, 18.60. HRMS (ESI) calcd for $C_{21}H_{19}N_5O_3S$ $[M + H]^+$: 422.1287, found: 422.1272.

6-nitro-N-(4-(piperidin-4-yloxy)phenyl)-2-(prop-2-yn-1-ylthio)quinazolin-4-amine (**18i**)

Faint yellow solid, yield: 89.4%, mp: 117.3–118.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.55 (s, 1H), 8.45 (d, $J = 9.3$ Hz, 1H), 7.80–7.62 (m, 3H), 7.04 (d, $J = 8.5$ Hz, 2H), 4.56 (dq, $J = 8.9$, 4.1 Hz, 1H), 3.96 (s, 2H), 3.26–3.16 (m, 1H), 3.12 (s, 2H), 2.87 (s, 2H), 2.04 (s, 2H), 1.70 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.40, 157.42, 153.89, 153.35, 143.24, 130.94, 127.54, 127.08, 124.55, 121.18, 115.64, 112.28, 80.36, 73.25, 71.16, 41.77, 29.37, 18.69. HRMS (ESI) calcd for $C_{22}H_{21}N_5O_3S$ $[M + H]^+$: 436.1443, found: 436.1428.

6-nitro-N-(4-(2-(piperazin-1-yl)ethoxy)phenyl)-2-(prop-2-yn-1-ylthio)quinazolin-4-amine (**18j**)

Faint yellow solid, yield: 89.4%, mp: 101.2–101.6 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.53 (s, 1H), 8.45 (d, $J = 9.2$ Hz, 1H), 7.67 (d, $J = 8.6$ Hz, 3H), 6.99 (d, $J = 8.4$ Hz, 2H), 4.10 (s, 2H), 3.95 (s, 2H),3.49 (s, 2H) 3.15 (s, 1H), 2.73 (d, $J = 27.1$ Hz, 4H), 2.44 (s, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.43, 157.43, 155.62, 153.39, 143.21, 130.76, 127.52, 127.07, 124.51, 121.16, 114.22, 112.33, 80.40, 73.22, 65.60, 57.08, 53.45, 45.24, 18.64. HRMS (ESI) calcd for $C_{23}H_{24}N_6O_3S$ $[M + H]^+$: 465.1709, found: 465.1690.

N-(4-(azetidin-3-ylmethoxy)phenyl)-6-nitro-2-(prop-2-yn-1-ylthio)quinazolin-4-amine (**18k**)

Faint yellow solid, yield: 86.7%, mp: 197.2–198.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.57 (s, 1H), 8.49 (d, J = 9.3 Hz, 1H), 7.72 (d, J = 8.6 Hz, 3H), 7.05 (d, J = 8.4 Hz, 2H), 4.17 (s, 2H), 4.04 (s, 2H), 3.97 (s, 2H), 3.82 (s, 2H), 3.39 (s, 1H), 3.20 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.41, 157.51, 155.45, 153.38, 143.28, 131.09, 127.58, 127.15, 124.59, 121.16, 114.37, 112.26, 80.42, 73.24, 67.57, 47.85, 31.02, 18.63. HRMS (ESI) calcd for $C_{21}H_{19}N_5O_3S$ $[M + H]^+$: 422.1287, found: 422.1272.

6-nitro-N-(4-(piperidin-4-ylmethoxy)phenyl)-2-(prop-2-yn-1-ylthio)quinazolin-4-amine (**18l**)

Faint yellow solid, yield: 81.2%, mp: 144.1–144.7 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.55 (s, 1H), 8.47 (d, J = 9.2 Hz, 1H), 7.70 (d, J = 8.7 Hz, 3H), 7.00 (d, J = 8.4 Hz, 2H), 3.97 (s, 2H), 3.90 (d, J = 6.1 Hz, 2H), 3.31 (d, J = 12.5 Hz, 2H), 3.16 (d, J = 2.9 Hz, 1H), 2.90 (t, J = 12.6 Hz, 2H), 2.06 (s, 1H), 1.93 (d, J = 13.8 Hz, 2H), 1.48 (q, J = 12.8 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.42, 157.50, 155.72, 153.38, 143.29, 130.78, 127.58, 127.14, 124.56, 121.17, 114.22, 112.30, 80.41, 73.25, 71.49, 43.07, 33.36, 25.61, 18.64. HRMS (ESI) calcd for $C_{19}H_{18}N_5O_3S$ $[M + H]^+$: 450.1600, found: 450.1589.

General procedure for the synthesis of compound 19

A mixture of compound **17l** (1.00 g, 1.82 mmol), Fe powder (0.71 g, 12.74 mmol) and NH_4Cl (0.29 g, 5.46 mmol), 5 mL H_2O and 15 mL EtOH in a flask was heated at 85 °C for 4 h. After the completion of the reaction, the residual Fe powder was removed under reduced pressure. Then the resulting liquid was extracted with ethyl acetate and water. Finally, the organic layer was dried with anhydrous Na_2SO_4 and concentrated in vacuo, followed by column chromatography to afford compound **19** (yield: 79.3%).

General procedure for the synthesis of compounds 20a-20w

A mixture of corresponding carboxylic acid (1.44 mmol), HATU (0.55 g, 1.44 mmol), 10 mL DMF and DIPEA (224 μL, 1.44 mmol) in 25 mL flask was stirred at room temperature for 10 min. Then, compound **19** (0.50 g, 0.96 mmol) was added and reacted at room temperature for an additional 6 h. After the consumption of starting materials, the resulting mixture was diluted with water and extracted with ethyl acetate. Then, the organic layer was dried with anhydrous Na_2SO_4 and evaporated under reduced pressure, followed by column chromatography to afford compounds **20a-20w** (yield: 81.2–90.3%).

General procedure for the synthesis of compounds 21a-21w

To a solution of compounds **20a**-**20w** (0.53 mmol) in DCM (10 mL), the TFA (2 mL) was added. After stirring at room temperature for 1 h, the resulting mixture was diluted with water and neutralized using Na_2CO_3, followed by extracting with ethyl acetate. Then, the organic layer was dried with anhydrous Na_2SO_4 and concentrated in vacuo. The crude product was purified by column chromatography to afford compounds **21a-21w**.

N-(4-((4-(piperidin-4-ylmethoxy)phenyl)amino)-2-(prop-2-yn-1-ylthio)quinazolin-6-yl)acetamide (**21a**)

Faint yellow solid, yield: 83.2%, mp: 143.5–144.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.25 (s, 1H), 9.88 (s, 1H), 8.60 (d, J = 2.2 Hz, 1H), 7.79 (dd, J = 9.0, 2.1 Hz, 1H), 7.69–7.63 (m, 2H), 7.57 (d, J = 8.9 Hz, 1H), 6.98–6.90 (m, 2H), 3.93 (d, J = 2.7 Hz, 2H), 3.82 (d, J = 6.3 Hz, 2H), 3.11 (t, J = 2.6 Hz, 1H), 3.02 (d, J = 11.9 Hz, 2H), 2.56 (t, J = 12.2 Hz, 2H), 2.11 (s, 3H), 1.79–1.93 (s, 1H), 1.74 (d, J = 13.2, Hz, 2H), 1.29–1.16 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.32, 162.99, 156.95, 155.27, 146.76, 135.87, 131.66, 127.13, 126.65, 124.19, 114.07, 113.18, 112.64, 80.92, 72.88, 72.23, 44.70, 35.29, 28.39, 23.77, 18.30. HRMS (ESI) calcd for $C_{25}H_{27}N_5O_2S$ $[M + H]^+$: 462.1964, found: 462.1949.

N-(4-((4-(piperidin-4-ylmethoxy)phenyl)amino)-2-(prop-2-yn-1-ylthio)quinazolin-6-yl)propion-amide (**21b**)

Faint yellow solid, yield: 84.6%, mp: 129.6–130.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.16 (s, 1H), 9.88 (s, 1H), 8.65 (d, J = 2.2 Hz, 1H), 7.77 (dd, J = 9.0, 2.2 Hz, 1H), 7.65 (d, J = 8.9 Hz, 2H), 7.57 (d, J = 8.9 Hz, 1H), 6.94 (d, J = 8.9 Hz, 2H), 3.92 (d, J = 2.7 Hz, 2H), 3.81 (d, J = 6.3 Hz, 2H), 3.11 (t, J = 2.6 Hz, 1H), 2.96 (dd, J = 11.9, 3.5 Hz, 2H), 2.46 (dd, J = 12.0, 2.5 Hz, 2H), 2.39 (q, J = 7.5 Hz, 2H), 1.85–1.70 (m, 1H), 1.70 (d, J = 13.3 Hz, 2H), 1.25–1.15 (m, 2H), 1.13 (d, J = 7.5 Hz, 3H).^{13}C NMR (101 MHz, DMSO-d_6) δ 171.98, 162.96, 156.97, 155.39, 146.79, 135.84, 131.55, 127.24, 126.68, 124.27, 114.06, 113.17, 112.52, 80.92, 72.86, 72.72, 45.68, 36.06, 29.70, 29.32, 18.29, 9.59. HRMS (ESI) calcd for $C_{26}H_{29}N_5O_2S$ $[M + H]^+$: 476.2120, found: 476.2107.

N-(4-((4-(piperidin-4-ylmethoxy)phenyl)amino)-2-(prop-2-yn-1-ylthio)quinazolin-6-yl)butyramide (**21c**)

Faint yellow solid, yield: 84.6%, mp: 148.7–149.3 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.16 (s, 1H), 9.88 (s, 1H), 8.65 (d, J = 2.2 Hz, 1H), 7.77 (dd, J = 9.0, 2.2 Hz, 1H), 7.65 (d, J = 8.9 Hz, 2H), 7.57 (d, J = 8.9 Hz, 1H), 6.94 (d, J = 8.9 Hz, 2H), 3.92 (d, J = 2.7 Hz, 2H), 3.81 (d, J = 6.3 Hz, 2H), 3.11 (t, J = 2.6 Hz, 1H), 2.96 (dd, J = 11.9, 3.5 Hz, 2H), 2.46 (dd, J = 12.0, 2.5 Hz, 2H), 2.39 (q, J = 7.5 Hz, 2H), 1.89–1.79 (m, 1H), 1.70 (dd, J = 13.3, 3.4 Hz, 2H), 1.25–1.15 (m, 2H), 1.13 (d, J = 7.5 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 171.22, 162.86, 156.94, 155.23, 146.56, 136.21, 131.80, 126.82, 126.48, 124.01, 114.04, 113.27, 112.58, 80.93, 72.87, 72.45, 54.85, 38.17, 35.86, 29.15, 18.54, 18.29, 13.66. HRMS (ESI) calcd for $C_{27}H_{31}N_5O_2S$ $[M + H]^+$: 490.2277, found: 490.2262.

N-(4-((4-(piperidin-4-ylmethoxy)phenyl)amino)-2-(prop-2-yn-1-ylthio)quinazolin-6-yl)cyclo-propanecarboxamide (**21d**)

Faint yellow solid, yield: 81.6%, mp: 136.1–138.7 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.63 (s, 1H), 9.93 (s, 1H), 8.71 (d, $J = 2.2$ Hz, 1H), 7.84 (dd, $J = 8.9$, 2.1 Hz, 1H), 7.67 (d, $J = 8.9$ Hz, 2H), 7.57 (d, $J = 8.9$ Hz, 1H), 7.00–6.89 (m, 2H), 3.93 (d, $J = 2.7$ Hz, 2H), 3.81 (d, $J = 6.3$ Hz, 2H), 3.11 (t, $J = 2.7$ Hz, 1H), 2.97 (s, 2H), 2.60–2.51 (m, 2H), 1.94–1.86 (m, 1H), 1.89-1.79 (m, 1H), 1.72 (d, $J = 12.6$ Hz, 2H), 1.19 (qd, $J = 12.1$, 4.1 Hz, 2H), 0.85 (t, $J = 5.2$ Hz, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.69, 162.92, 156.95, 155.29, 146.67, 136.00, 131.68, 126.98, 126.63, 124.13, 114.06, 113.23, 112.54, 80.93, 72.86, 72.50, 45.27, 35.78, 29.17, 18.29, 14.39, 7.17. HRMS (ESI) calcd for $C_{27}H_{29}N_5O_2S$ $[M + H]^+$: 488.2120, found: 488.2108.

N-(4-((4-(piperidin-4-ylmethoxy)phenyl)amino)-2-(prop-2-yn-1-ylthio)quinazolin-6-yl)cyclo-butanecarboxamide (**21e**)

Faint yellow solid, yield: 83.8%, mp: 133.5–134.3 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.06 (s, 1H), 9.92 (s, 1H), 8.67 (s, 1H), 7.78 (d, $J = 9.1$ Hz, 1H), 7.65 (d, $J = 8.4$ Hz, 2H), 7.57 (d, $J = 8.9$ Hz, 1H), 6.95 (d, $J = 8.5$ Hz, 2H), 3.93 (s, 2H), 3.81 (d, $J = 6.3$ Hz, 2H), 3.29 (d, $J = 8.3$ Hz, 2H), 3.14 (q, $J = 2.2$ Hz, 1H), 2.97 (d, $J = 11.9$ Hz, 2H), 2.47 (s, 1H), 2.29 (q, $J = 9.5$ Hz, 2H), 2.15 (q, $J = 10.5$, 9.9 Hz, 2H), 1.98 (q, $J = 9.2$ Hz, 1H), 1.84 (q, $J = 10.8$, 10.0 Hz, 2H), 1.71 (d, $J = 12.7$ Hz, 2H), 1.19 (tt, $J = 12.3$, 6.0 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.87, 162.98, 156.96, 155.38, 146.82, 135.81, 131.55, 127.35, 126.67, 124.27, 114.07, 113.15, 112.61, 80.91, 72.87, 72.69, 45.59, 39.42, 35.97, 29.56, 24.60, 18.29, 17.78. HRMS (ESI) calcd for $C_{28}H_{31}N_5O_2S$ $[M + H]^+$: 502.2277, found: 502.2263.

N-(4-((4-(piperidin-4-ylmethoxy)phenyl)amino)-2-(prop-2-yn-1-ylthio)quinazolin-6-yl)cyclo-pentanecarboxamide (**21f**)

Faint yellow solid, yield: 79.1%, mp: 142.7–143.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.89 (s, 1H), 8.68 (d, $J = 2.1$ Hz, 1H), 7.77 (dd, $J = 8.8$, 2.1 Hz, 1H), 7.65 (d, $J = 8.6$ Hz, 2H), 7.57 (d, $J = 8.9$ Hz, 1H), 6.95 (d, $J = 8.6$ Hz, 2H), 3.93 (d, $J = 2.6$ Hz, 2H), 3.85 (d, $J = 6.3$ Hz, 2H), 3.23–3.08 (m, 3H), 2.86 (p, $J = 7.9$ Hz, 1H), 2.78–2.65 (m, 2H), 1.92–1.67 (m, 9H), 1.58 (dt, $J = 8.5$, 3.9 Hz, 2H), 1.34 (qd, $J = 12.7$, 4.0 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.37, 162.89, 156.98, 155.19, 146.78, 135.98, 131.71, 127.33, 126.66, 124.34, 114.07, 113.16, 112.45, 80.91, 72.91, 71.89, 45.04, 43.99, 34.34, 30.06, 27.10, 25.70, 18.28. HRMS (ESI) calcd for $C_{29}H_{33}N_5O_2S$ $[M + H]^+$: 516.2433, found: 516.2419.

N-(4-((4-(piperidin-4-ylmethoxy)phenyl)amino)-2-(prop-2-yn-1-ylthio)quinazolin-6-yl)benzamide (**21g**)

Faint yellow solid, yield: 85.4%, mp: 131.0–131.9 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.70 (s, 1H), 10.05 (s, 1H), 8.89 (s, 1H), 8.07 (d, $J = 7.6$ Hz, 2H), 8.03 (d, $J = 9.0$ Hz, 1H), 7.70 (d, $J = 8.5$ Hz, 2H), 7.61 (dd, $J = 15.1$, 8.0 Hz, 2H), 7.53 (t, $J = 7.4$ Hz, 2H), 6.91 (d, $J = 8.5$ Hz, 2H), 3.95 (d, $J = 2.6$ Hz, 2H), 3.80 (d, $J = 6.3$ Hz, 2H), 3.15 (d, $J = 2.6$ Hz, 1H), 3.04 (d, $J = 12.1$ Hz, 2H), 2.64–2.54 (m, 2H), 1.92–1.82 (m, 1H), 1.75 (d, $J = 12.8$ Hz, 2H), 1.24 (qd, $J = 12.7$, 2.4 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.37, 163.34, 156.96, 155.22, 147.11, 135.70, 134.38, 131.66, 128.39, 128.31, 127.65, 126.49, 124.13, 114.48, 114.02, 113.12, 80.91, 72.94, 72.32, 44.91, 39.90, 35.25, 28.50, 18.32. HRMS (ESI) calcd for $C_{30}H_{29}N_5O_2S$ $[M + H]^+$: :524.2120, found: 524.2108.

4-fluoro-N-(4-((4-(piperidin-4-ylmethoxy)phenyl)amino)-2-(prop-2-yn-1-ylthio)quinazolin-6-yl)benzamide (**21h**)

Faint yellow solid, yield: 87.6%, mp: 191.6–192.4 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.62 (s, 1H), 9.96 (s, 1H), 8.82 (d, $J = 2.3$ Hz, 1H), 8.14 (dd, $J = 8.6$, 5.4 Hz, 2H), 7.94 (dd, $J = 9.0$, 2.1 Hz, 1H), 7.69 (d, $J = 8.6$ Hz, 2H), 7.64 (d, $J = 8.9$ Hz, 1H), 7.42 (t, $J = 8.7$ Hz, 2H), 6.98 (d, $J = 8.6$ Hz, 2H), 3.95 (d, $J = 2.7$ Hz, 2H), 3.88 (d, $J = 6.2$ Hz, 2H), 3.28 (d, $J = 12.3$ Hz, 2H), 3.15 (t, $J = 2.6$ Hz, 1H), 2.93–2.84 (m, 2H), 2.05 (s, 1H), 1.97–1.88 (m, 2H), 1.55–1.42 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.31, 163.44, 162.93(d, $J = 250.4$ Hz), 156.99, 155.15, 147.25, 135.46, 131.72, 130.82(d, $J = 2.8$ Hz), 130.43(d, $J = 9.1$ Hz), 128.63, 126.61, 124.25, 115.54(d, $J = 21.6$ Hz), 114.59, 114.14, 113.05, 80.90, 72.96, 71.47, 43.01, 33.43, 25.65, 18.32. HRMS (ESI) calcd for $C_{30}H_{28}FN_5O_2S$ $[M + H]^+$: 542.2026, found: 542.2012.

4-chloro-N-(4-((4-(piperidin-4-ylmethoxy)phenyl)amino)-2-(prop-2-yn-1-ylthio)quinazolin-6-yl)benzamide (**21i**)

Faint yellow solid, yield: 82.7%, mp: 144.5–145.3 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.65 (s, 1H), 9.95 (s, 1H), 8.81 (d, $J = 2.2$ Hz, 1H), 8.08 (d, $J = 8.3$ Hz, 2H), 7.93 (dd, $J = 8.9$, 2.1 Hz, 1H), 7.70–7.66 (m, 3H), 7.66–7.61 (m, 2H), 6.96 (d, $J = 8.6$ Hz, 2H), 3.95 (d, $J = 2.6$ Hz, 2H), 3.82 (d, $J = 6.3$ Hz, 2H), 3.15 (d, $J = 2.7$ Hz, 1H), 3.03 (dd, $J = 12.3$, 3.3 Hz, 2H), 2.57 (td, $J = 12.3$, 2.6 Hz, 2H), 1.93–1.83 (m, 1H), 1.75 (d, $J = 13.2$, Hz, 2H), 1.31–1.18 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.33, 163.54, 156.99, 155.39, 147.33, 136.63, 135.29, 133.11, 131.52, 129.56, 128.57, 126.66, 124.27, 114.63, 114.11, 113.05, 80.89, 72.92, 72.46, 45.13, 35.48, 28.82, 18.33. HRMS (ESI) calcd for $C_{30}H_{28}ClN_5O_2S$ $[M + H]^+$: 558.1730, found: 558.1716.

4-bromo-N-(4-((4-(piperidin-4-ylmethoxy)phenyl)amino)-2-(prop-2-yn-1-ylthio)quinazolin-6-yl)benzamide (**21j**)

Faint yellow solid, yield: 90.7%, mp: 156.7–157.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.97 (s, 1H), 10.18 (s, 1H), 8.94 (s, 1H), 8.18 (d, J = 9.1 Hz, 1H), 8.03 (d, J = 8.2 Hz, 2H), 7.75 (d, J = 8.5 Hz, 2H), 7.62 (dd, J = 12.7, 8.6 Hz, 3H), 6.85 (d, J = 8.6 Hz, 2H), 3.96 (d, J = 2.6 Hz, 2H), 3.80 (d, J = 6.2 Hz, 2H), 3.29 (d, J = 12.1 Hz, 2H), 3.15 (d, J = 2.7 Hz, 1H), 2.94–2.85 (m, 2H), 2.07–1.97 (s, 1H), 1.90 (d, J = 13.3 Hz, 2H), 1.48 (q, J = 12.5 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.31, 163.29, 156.84, 154.83, 146.93, 135.75, 133.35, 131.98, 131.27, 129.79, 126.35, 125.43, 124.49, 123.86, 114.38, 113.94, 113.18, 80.92, 72.95, 71.24, 42.84, 33.10, 25.22, 18.33. HRMS (ESI) calcd for $C_{30}H_{28}BrN_5O_2S$ $[M + H]^+$: 602.1225, found: 602.1209.

4-methyl-N-(4-((4-(piperidin-4-ylmethoxy)phenyl)amino)-2-(prop-2-yn-1-ylthio)quinazolin-6-yl)benzamide (**21k**)

Faint yellow solid, yield: 88.6%, mp: 126.7–127.4 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.60 (s, 1H), 10.04 (s, 1H), 8.88 (s, 1H), 7.99 (d, J = 7.8 Hz, 3H), 7.70 (d, J = 8.6 Hz, 2H), 7.63 (d, J = 8.9 Hz, 1H), 7.37 (d, J = 7.9 Hz, 2H), 6.96 (d, J = 8.7 Hz, 2H), 3.95 (d, J = 2.6 Hz, 2H), 3.82 (d, J = 6.3 Hz, 2H), 3.15 (s, 1H), 3.00 (d, J = 11.7 Hz, 2H), 2.55 (d, J = 10.9 Hz, 2H), 2.40 (s, 3H), 1.91–1.81 (m, 1H), 1.79–1.69 (m, 2H), 1.21 (qd, J = 11.2, 10.2, 3.6 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.21, 163.32, 156.98, 155.32, 147.10, 141.78, 135.74, 131.62, 131.53, 128.98, 128.42, 127.68, 126.51, 124.18, 114.44, 114.08, 113.10, 80.92, 72.92, 72.47, 45.12, 35.60, 28.95, 20.98, 18.28. HRMS (ESI) calcd for $C_{31}H_{31}N_5O_2S$ $[M + H]^+$: 538.2277, found: 538.2261.

4-methoxy-N-(4-((4-(piperidin-4-ylmethoxy)phenyl) amino)-2-(prop-2-yn-1-ylthio)quinazolin-6-yl)benzamide (**21l**)

Faint yellow solid, yield: 84.7%, mp: 137.6–138.4 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.45 (s, 1H), 9.95 (s, 1H), 8.82 (d, J = 2.3 Hz, 1H), 8.06 (d, J = 8.5 Hz, 2H), 7.95 (dd, J = 9.0, 2.1 Hz, 1H), 7.68 (d, J = 8.6 Hz, 2H), 7.63 (d, J = 8.9 Hz, 1H), 7.10 (d, J = 8.5 Hz, 2H), 6.95 (d, J = 8.6 Hz, 2H), 3.95 (d, J = 2.6 Hz, 2H), 3.86 (s, 3H), 3.82 (d, J = 6.4 Hz, 2H), 3.15 (t, J = 2.6 Hz, 1H), 3.00 (d, J = 11.9 Hz, 2H), 2.60–2.51 (m, 2H), 1.91–1.82 (m, 1H), 1.78–1.69 (m, 2H), 1.21 (qd, J = 12.2, 4.0 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.78, 163.27, 162.01, 156.97, 155.34, 147.09, 135.77, 131.57, 129.58, 128.57, 126.51, 126.41, 124.21, 114.39, 114.08, 113.68, 113.08, 80.92, 72.92, 72.53, 55.38, 45.28, 35.66, 29.08, 18.32. HRMS (ESI) calcd for $C_{31}H_{31}N_5O_3S$ $[M + H]^+$: 554.2226, found: 554.2210.

4-ethoxy-N-(4-((4-(piperidin-4-ylmethoxy)phenyl)amino)-2-(prop-2-yn-1-ylthio)quinazolin-6-yl)benzamide (**21m**)

Faint yellow solid, yield: 88.9%, mp: 138.7–139.6 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.45 (s, 1H), 9.96 (s, 1H), 8.88–8.80 (m, 1H), 8.04 (d, J = 8.4 Hz, 2H), 7.99–7.92 (m, 1H), 7.69 (d, J = 8.5 Hz, 2H), 7.62 (d, J = 8.9 Hz, 1H), 7.07 (d, J = 8.4 Hz, 2H), 6.95 (d, J = 8.5 Hz, 2H), 4.12 (q, J = 6.9 Hz, 2H), 3.95 (d, J = 2.7 Hz, 2H), 3.82 (d, J = 6.3 Hz, 2H), 3.15 (d, J = 2.7 Hz, 1H), 3.04 (d, J = 12.1 Hz, 2H), 2.64–2.53 (m, 2H), 1.92–1.82 (m, 1H), 1.76 (d, J = 12.7 Hz, 2H), 1.37 (t, J = 6.9 Hz, 3H), 1.25 (qd, J = 12.6, 2.5 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.78, 163.24, 161.30, 156.97, 155.28, 147.05, 135.84, 131.64, 129.59, 128.50, 126.49, 126.24, 124.18, 114.33, 114.17, 114.07, 113.09, 80.91, 72.93, 72.33, 63.38, 44.75, 35.24, 28.50, 18.32, 14.52. HRMS (ESI) calcd for $C_{32}H_{33}N_5O_3S$ $[M + H]^+$: 568.2382, found: 568.2368.

N-(4-((4-(piperidin-4-ylmethoxy)phenyl)amino)-2-(prop-2-yn-1-ylthio)quinazolin-6-yl)-4-(trifluoromethyl) benzamide (**21n**)

Faint yellow solid, yield: 87.8%, mp: 179.8–180.4 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.85 (s, 1H), 10.00 (s, 1H), 8.86 (s, 1H), 8.25 (d, J = 8.1 Hz, 2H), 7.97 (d, J = 7.7 Hz, 3H), 7.68 (dd, J = 15.7, 8.7 Hz, 3H), 6.98 (d, J = 8.6 Hz, 2H), 3.96 (s, 2H), 3.89 (s, 2H), 3.28 (d, J = 12.3 Hz, 2H), 3.17 (d, J = 3.0 Hz, 1H), 2.92–2.82 (m, 2H), 2.02–1.92 (m, 1H), 1.89 (d, J = 5.5 Hz, 2H), 1.45 (qd, J = 13.0, 4.0 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.24, 163.60, 156.99, 155.16, 147.36, 138.20, 135.22, 131.71, 131.36(d, J = 32.0 Hz), 128.55, 128.41, 126.70, 125.49(q, J = 3.6 Hz), 125.21(d, J = 273.5 Hz), 124.23, 114.61, 114.13, 113.07, 80.88, 72.95, 71.49, 43.18, 33.51, 25.83, 18.33. HRMS (ESI) calcd for $C_{31}H_{28}F_3N_5O_2S$ $[M + H]^+$: :592.1994, found: 592.1978.

4-cyano-N-(4-((4-(piperidin-4-ylmethoxy)phenyl)amino)-2-(prop-2-yn-1-ylthio)quinazolin-6-yl)benzamide (**21o**)

Faint yellow solid, yield: 89.4%, mp: 155.6-157.3 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.84 (s, 1H), 9.99 (s, 1H), 8.84 (s, 1H), 8.20 (d, J = 8.0 Hz, 2H), 8.08 (d, J = 8.1 Hz, 2H), 7.94 (d, J = 9.1 Hz, 1H), 7.66 (t, J = 8.8 Hz, 3H), 6.96 (d, J = 8.7 Hz, 2H), 3.95 (d, J = 2.6 Hz, 2H), 3.82 (d, J = 6.3 Hz, 2H), 3.15 (d, J = 2.7 Hz, 1H), 2.99 (d, J = 12.0 Hz, 2H), 2.55 (s, 2H), 1.87–1.77 (m, 1H), 1.73 (d, J = 12.7 Hz, 2H), 1.19 (qd, J = 12.3, 4.2 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.00, 163.67, 156.99, 155.40, 147.43, 138.41, 135.08, 132.54, 131.48, 128.47, 126.71, 124.27, 118.24, 114.70, 114.08, 114.03, 113.06, 80.88, 72.94, 72.57, 45.38, 35.75, 29.23, 18.34. HRMS (ESI) calcd for $C_{31}H_{28}N_6O_2S$ $[M + H]^+$: 549.2072, found: 549.2061.

3-cyano-N-(4-((4-(piperidin-4-ylmethoxy)phenyl)amino)-2-(prop-2-yn-1-ylthio)quinazolin-6-yl)benzamide (**21p**)

Faint yellow solid, yield: 87.5%, mp: 167.0-167.8 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.03 (s, 1H), 10.29 (s, 1H), 8.99 (s, 1H), 8.57 (d, J = 10.9 Hz, 1H), 8.37 (d, J = 7.9 Hz, 1H), 8.10 (d, J = 8.0 Hz, 2H), 7.79 (t, J = 7.8 Hz, 1H), 7.73 (d, J = 8.5 Hz, 2H), 7.63 (d, J = 9.0 Hz, 1H), 6.95 (d, J = 9.0 Hz, 2H), 3.96 (d, J = 2.6 Hz, 2H), 3.81 (d, J = 6.4 Hz, 2H), 3.13 (t, J = 2.7 Hz, 1H), 2.95 (dt, J = 12.1, 3.3 Hz, 2H), 2.46 (d, J = 12.0, Hz, 2H),1.87–1.77 (m, 1H),

1.70 (d, $J = 13.1$ Hz, 2H), 1.23–1.11 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.56, 163.46, 156.99, 155.35, 147.22, 135.48, 135.09, 132.52, 131.69, 131.27, 129.86, 127.95, 126.56, 124.10, 118.28, 114.60, 114.06, 113.22, 111.59, 80.92, 72.90, 72.77, 45.74, 36.12, 29.78, 18.34. HRMS (ESI) calcd for $C_{31}H_{28}N_6O_2S$ $[M + H]^+$: 549.2072, found: 549.2056.

4-nitro-N-(4-((4-(piperidin-4-ylmethoxy)phenyl)amino)-2-(prop-2-yn-1-ylthio)quinazolin-6-yl)benzamide (**21q**)

Faint yellow solid, yield: 82.3%, mp: 261.6-262.3 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.00 (s, 1H), 10.10 (s, 1H), 8.92 (s, 1H), 8.41 (d, $J = 8.4$ Hz, 2H), 8.30 (d, $J = 8.4$ Hz, 2H), 8.02 (d, $J = 8.9$ Hz, 1H), 7.70 (d, $J = 8.5$ Hz, 2H), 7.65 (d, $J = 8.9$ Hz, 1H), 6.96 (d, $J = 8.5$ Hz, 2H), 3.96 (d, $J = 2.6$ Hz, 2H), 3.81 (d, $J = 6.4$ Hz, 2H), 3.16 (d, $J = 2.9$ Hz, 1H), 3.01–2.92 (m, 2H), 2.50–2.43 (m, 2H), 1.86–1.78 (m, 1H), 1.71 (d, $J = 12.5$ Hz, 2H), 1.24–1.17 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.73, 163.69, 156.99, 155.43, 149.23, 147.40, 140.12, 135.20, 131.54, 129.17, 128.32, 126.67, 124.22, 123.60, 114.79, 114.08, 113.12, 80.88, 72.92, 72.77, 45.75, 36.12, 29.79, 18.33. HRMS (ESI) calcd for $C_{30}H_{28}N_6O_4S$ $[M + H]^+$: 569.1971, found: 569.1956.

N-(4-((4-(piperidin-4-ylmethoxy)phenyl)amino)-2-(prop-2-yn-1-ylthio)quinazolin-6-yl)furan-2-carboxamide (**21r**)

Faint yellow solid, yield: 86.3%, mp: 144.7–145.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.76–10.42 (m, 1H), 9.97 (s, 1H), 8.78 (s, 1H), 7.99 (s, 2H), 7.68 (d, $J = 8.5$ Hz, 2H), 7.62 (d, $J = 8.9$ Hz, 1H), 7.44 (d, $J = 3.5$ Hz, 1H), 6.96 (d, $J = 8.5$ Hz, 2H), 6.75 (dd, $J = 3.5$, 1.7 Hz, 1H), 3.95 (d, $J = 2.6$ Hz, 2H), 3.81 (d, $J = 6.3$ Hz, 2H), 3.15 (s, 1H), 2.97 (dd, $J = 9.4$, 6.2 Hz, 2H), 2.50 (s, 2H), 1.89–1.79 (m, 1H), 1.72 (d, $J = 11.8$ Hz, 2H), 1.18 (qd, $J = 12.2$, 4.1 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.48, 156.94, 156.24, 155.41, 147.32, 147.21, 145.91, 134.88, 131.51, 128.38, 126.61, 124.19, 114.87, 114.54, 114.11, 113.08, 112.19, 80.89, 72.91, 72.71, 45.60, 35.98, 29.59, 18.33. HRMS (ESI) calcd for $C_{28}H_{27}N_5O_3S$ $[M + H]^+$: 514.1913, found: 514.1901.

N-(4-((4-(piperidin-4-ylmethoxy)phenyl)amino)-2-(prop-2-yn-1-ylthio)quinazolin-6-yl)nicotinamide (**21s**)

Faint yellow solid, yield: 86.0%, mp: 165.4–166.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.09 (s, 1H), 10.15 (s, 1H), 9.24 (d, $J = 2.2$ Hz, 1H), 8.98 (s, 1H), 8.79 (d, $J = 4.8$ Hz, 1H), 8.46 (d, $J = 7.9$ Hz, 1H), 8.09 (d, $J = 9.1$ Hz, 1H), 7.73 (d, $J = 8.5$ Hz, 2H), 7.61 (dd, $J = 14.2$, 8.6 Hz, 2H), 6.95 (d, $J = 8.7$ Hz, 2H), 3.95 (d, $J = 2.6$ Hz, 2H), 3.82 (d, $J = 6.3$ Hz, 2H), 3.13 (s, 1H), 2.97 (d, $J = 12.4$ Hz, 2H), 1.89–1.79 (m, 1H), 1.72 (d, $J = 12.5$ Hz, 2H), 1.18 (qd, $J = 12.2$, 4.1 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.97, 163.58, 156.98, 155.14, 152.28, 148.67, 147.34, 135.37, 135.24, 131.73, 130.04, 128.37, 126.73, 124.23, 123.57, 114.45, 114.16, 113.07, 80.90, 72.94, 71.30, 42.82, 33.10, 25.22, 18.34. HRMS (ESI) calcd for $C_{29}H_{28}N_6O_2S$ $[M + H]^+$: 525.2072, found: 525.2060.

N-(4-((4-(piperidin-4-ylmethoxy)phenyl)amino)-2-(prop-2-yn-1-ylthio)quinazolin-6-yl)imidazo[1,2-a]pyridine-6-carboxamide (**21t**)

Faint yellow solid, yield: 88.5%, mp: 193.6–194.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.74 (s, 1H), 9.98 (s, 1H), 9.37 (s, 1H), 8.84 (d, $J = 2.2$ Hz, 1H), 8.14 (s, 1H), 7.96 (dd, $J = 8.9$, 2.1 Hz, 1H), 7.83 (dd, $J = 9.6$, 1.8 Hz, 1H), 7.76–7.70 (m, 3H), 7.70–7.62 (m, 2H), 6.98 (d, $J = 8.6$ Hz, 2H), 3.96 (d, $J = 2.7$ Hz, 2H), 3.89 (d, $J = 6.2$ Hz, 2H), 3.31 (s, 2H), 3.16 (t, $J = 2.7$ Hz, 1H), 2.98–2.87 (m, 2H), 2.13–2.03 (s, 1H), 1.98–1.90 (m, 2H), 1.50 (qd, $J = 11.0$, 3.2 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.50, 163.42, 156.97, 155.16, 147.26, 144.62, 135.36, 134.60, 131.73, 129.56, 129.01, 126.71, 124.26, 124.22, 123.04, 119.72, 116.27, 114.42, 114.15, 113.10, 80.90, 72.95, 71.46, 43.14, 33.48, 25.78, 18.33. HRMS (ESI) calcd for $C_{31}H_{29}N_7O_2S$ $[M + H]^+$: 564.2181, found: 564.2172.

N-(4-((4-(piperidin-4-ylmethoxy)phenyl)amino)-2-(prop-2-yn-1-ylthio)quinazolin-6-yl)-2-naphthamide (**21u**)

Faint yellow solid, yield: 89.8%, mp: 144.9–145.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.79 (s, 1H), 10.14–9.75 (m, 1H), 8.88 (s, 1H), 8.69 (s, 1H), 8.12 (d, $J = 8.1$ Hz, 3H), 8.02 (dd, $J = 13.3$, 8.3 Hz, 2H), 7.67 (q, $J = 7.1$, 5.5 Hz, 5H), 6.97 (d, $J = 8.5$ Hz, 2H), 3.97 (d, $J = 2.6$ Hz, 2H), 3.82 (d, $J = 6.3$ Hz, 2H), 3.17 (d, $J = 3.0$ Hz, 1H), 3.06–2.95 (m, 2H), 2.50 (s, 2H), 1.91–1.81 (q, $J = 7.6$ Hz, 1H), 1.74 (d, $J = 12.9$ Hz, 2H), 1.21 (qd, $J = 12.3$, 4.0 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.48, 163.45, 157.01, 155.36, 147.28, 135.61, 134.34, 132.08, 131.73, 131.57, 128.96, 128.62, 128.12, 128.08, 127.91, 127.66, 126.88, 126.65, 124.33, 124.27, 114.54, 114.10, 113.11, 80.93, 72.95, 72.43, 45.05, 35.43, 28.74, 18.36. HRMS (ESI) calcd for $C_{34}H_{31}N_5O_2S$ $[M + H]^+$: 574.2276, found: 574.2264.

N-(4-((4-(piperidin-4-ylmethoxy)phenyl)amino)-2-(prop-2-yn-1-ylthio)quinazolin-6-yl)quinoline-6-carboxamide (**21v**)

Faint yellow solid, yield: 85.4%, mp: 142.7–143.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.96 (s, 1H), 10.06 (s, 1H), 9.04 (d, $J = 4.2$ Hz, 1H), 8.93 (s, 1H), 8.77 (s, 1H), 8.56 (d, $J = 8.3$ Hz, 1H), 8.36 (d, $J = 8.8$ Hz, 1H), 8.18 (d, $J = 8.8$ Hz, 1H), 8.04 (d, $J = 8.9$ Hz, 1H), 7.83–7.58 (m, 4H), 6.97 (d, $J = 8.5$ Hz, 2H), 3.96 (d, $J = 2.6$ Hz, 2H), 3.84 (t, $J = 6.6$ Hz, 2H), 3.17 (d, $J = 2.8$ Hz, 1H), 3.04 (d, $J = 12.4$ Hz, 2H), 2.58 (t, $J = 12.2$ Hz, 2H), 1.89 (s, 1H), 1.76 (d, $J = 12.6$ Hz, 2H), 1.25 (q, 11.6 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.97, 163.48, 157.01, 155.32, 152.30, 148.81, 147.24, 137.17, 135.62, 132.23, 131.64,

129.18, 128.56, 128.35, 127.96, 127.08, 126.63, 124.20, 122.28, 114.55, 114.11, 113.15, 80.92, 72.93, 72.36, 44.84, 35.29, 28.53, 18.35. HRMS (ESI) calcd for $C_{33}H_{30}N_6O_2S$ $[M + H]^+$: 575.2229, found: 575.2213.

N-(4-((4-(piperidin-4-ylmethoxy)phenyl)amino)-2-(prop-2-yn-1-ylthio)quinazolin-6-yl)quinoxaline-6-carboxamide (**21w**)

Faint yellow solid, yield: 86.4%, mp: 143.7–144.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.03 (s, 1H), 10.03 (s, 1H), 9.09 (d, $J = 5.4$ Hz, 2H), 8.89 (d, $J = 19.9$ Hz, 2H), 8.49–8.41 (m, 1H), 8.28 (d, $J = 8.8$ Hz, 1H), 8.07–7.99 (m, 1H), 7.68 (t, $J = 9.5$ Hz, 3H), 6.96 (d, $J = 8.5$ Hz, 2H), 3.96 (d, $J = 2.6$ Hz, 2H), 3.82 (d, $J = 6.4$ Hz, 2H), 3.17 (d, $J = 2.5$ Hz, 1H), 3.00 (d, $J = 11.9$ Hz, 2H), 2.55 (d, $J = 11.5$ Hz, 2H), 1.91–1.81 (s, 1H), 1.74 (d, $J = 13.0$ Hz, 2H), 1.21 (qd, $J = 12.2$, 3.8 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.33, 163.59, 157.01, 155.37, 147.37, 147.18, 146.79, 143.53, 141.54, 135.44, 135.33, 131.54, 129.54, 128.85, 128.74, 128.50, 126.67, 124.26, 114.70, 114.09, 113.09, 80.91, 72.95, 72.51, 45.25, 35.64, 29.04, 18.34. HRMS (ESI) calcd for $C_{32}H_{29}N_7O_2S$ $[M + H]^+$: 576.2181, found: 576.2167.

General procedure for the synthesis of compound 22

Compound **13** (4.00 g, 20.00 mmol) was dissolved in THF (20 mL), and then the NH_3•H_2O was dropped slowly at 0 °C and stirred for an additional 30 min. The precipitate was separated by filtration, washed with water for three times and dried in the oven to afford compound **22** (yield: 89.3%).

General procedure for the synthesis of compounds 23a-23h

Compound **22** (3.00 g, 16.56 mmol), corresponding aldehyde (1.68 g, 16.56 mmol), C_2H_5ONa (1.09 g, 16.56 mmol) and iodine (5.25 g, 20.70 mmol) was dissolved in DMF (20 mL), and then the mixture was stirred at 120 °C for 12 h. After the consumption of starting materials, the resulting mixture was slowly dropped into a saturated sodium sulfite solution and stirred for 5 min. Then, the precipitate was separated by filtration, washed with water for three times and dried in the oven to afford compound **23a-23h** (yield: 40.2–55.6%).

General procedure for the synthesis of compounds 24a-24h, 25a-25h, 26a-26h and 27a-27h

The procedure for the synthesis of compounds **24a-24h, 25a-25h, 26a-26h** and **27a-27h** was similar with compounds **16, 17l, 19** and **20u**.

General procedure for the synthesis of compounds 28a-28h

To a solution of compounds **27a-27h** (0.53 mmol) in DCM (10 mL), the TFA (2 mL) was added. After stirring at room temperature for 1 h, the resulting mixture was diluted with water and neutralized using Na_2CO_3, followed by extracting with ethyl acetate. Then, the organic layer was dried with anhydrous Na_2SO_4 and concentrated in vacuo. The crude product was purified by column chromatography to afford compounds **28a-28h**.

N-(2-phenyl-4-((4-(piperidin-4-ylmethoxy)phenyl)amino)quinazolin-6-yl)-2-naphthamide (**28a**)

Faint yellow solid, yield: 89.2%, mp: 178.5–179.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.85 (s, 1H), 9.90 (s, 1H), 9.01–8.95 (m, 1H), 8.90 (s, 1H), 8.72 (s, 1H), 8.57 (s, 1H), 8.48–8.39 (m, 2H), 8.13 (q, $J = 10.4$, 7.2 Hz, 3H), 8.07–8.04 (m, 1H), 7.88 (dd, $J = 17.1$, 8.7 Hz, 3H), 7.71–7.64 (m, 2H), 7.51 (d, $J = 5.9$ Hz, 2H), 7.06 (d, $J = 8.5$ Hz, 2H), 3.93 (d, $J = 6.2$ Hz, 2H), 3.33 (d, $J = 10.9$ Hz, 2H), 2.93 (d, $J = 11.6$ Hz, 2H), 2.05–2.15 (m, 1H), 1.97 (d, $J = 13.8$ Hz, 2H), 1.53 (q, $J = 13.5$, 12.6 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.53, 158.11, 157.66, 154.81, 147.46, 138.44, 136.39, 134.36, 132.51, 132.10, 131.74, 129.99, 128.98, 128.36, 128.33, 128.32, 128.21, 128.13, 127.93, 127.74, 127.67, 126.89, 124.35, 123.76, 114.18, 114.06, 71.68, 43.40, 33.86, 26.26. HRMS (ESI) calcd for $C_{37}H_{33}N_5O_2$ $[M + H]^+$: 580.2713, found: 580.2700.

N-(2-(2-fluorophenyl)-4-((4-(piperidin-4-ylmethoxy)phenyl)amino)quinazolin-6-yl)-2-naphthamide (**28b**)

Faint yellow solid, yield: 87.8%, mp: 188.4–189.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.89 (s, 1H), 9.93 (s, 1H), 9.01 (s, 1H), 8.73 (s, 1H), 8.13 (s, 3H), 8.05 (d, $J = 7.0$ Hz, 2H), 7.83 (dd, $J = 31.1$, 8.6 Hz, 3H), 7.70 (d, $J = 19.1$ Hz, 3H), 7.51 (d, $J = 41.7$ Hz, 3H), 6.94 (d, $J = 8.4$ Hz, 2H), 3.82 (s, 2H), 3.10 (d, $J = 11.8$ Hz, 2H), 2.66 (t, $J = 12.3$ Hz, 2H), 1.96–1.86 (m, 1H), 1.79 (d, $J = 13.1$ Hz, 2H), 1.34–1.22 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.58, 159.72 (d, $J = 236.6$ Hz), 154.99, 147.06, 139.19, 136.83, 134.38, 132.19, 132.09, 131.71, 131.46, 131.36, 129.93, 129.86, 128.98, 128.30, 128.22, 128.15, 128.04, 127.96, 127.68, 126.91, 126.88, 124.37, 124.34, 123.87, 114.11, 113.86 (d, $J = 21.2$ Hz), 113.73, 72.04, 44.31, 34.62, 27.60. HRMS (ESI) calcd for $C_{37}H_{32}FN_5O_2$ $[M + H]^+$: 598.2618, found: 598.2604.

N-(2-(3-fluorophenyl)-4-((4-(piperidin-4-ylmethoxy)phenyl)amino)quinazolin-6-yl)-2-naphthamide (**28c**)

Faint yellow solid, yield: 89.2%, mp: 171.2–173.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.87 (s, 1H), 9.96 (s, 1H), 8.99 (s, 1H), 8.72 (s, 1H), 8.27 (d, $J = 7.8$ Hz, 1H), 8.12 (d, $J = 3.6$ Hz, 3H), 8.10 (s, 2H), 8.05 (d, $J = 7.6$ Hz, 1H), 7.91 (d, $J = 8.9$ Hz, 1H), 7.80 (d, $J = 8.5$ Hz, 2H), 7.68

(q, $J = 6.3$ Hz, 2H), 7.56 (q, $J = 7.4$ Hz, 1H), 7.36–7.30 (m, 1H), 7.04 (d, $J = 8.5$ Hz, 2H), 3.84 (d, $J = 6.3$ Hz, 2H), 3.00 (d, $J = 11.9$ Hz, 2H), 2.49 (s, 2H), 1.91–1.81 (m, 1H), 1.74 (d, $J = 12.7$ Hz, 2H), 1.28–1.19 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.55, 163.56(d, $J = 243.2$ Hz), 157.75, 156.90, 155.15, 147.25, 141.22(d, $J = 7.7$ Hz), 136.71, 134.37, 132.14, 132.10, 131.74, 130.35(d, $J = 8.0$ Hz), 128.96, 128.45, 128.21, 128.13, 128.05, 127.92, 127.67, 126.88, 124.35, 124.02, 123.66, 116.80 (d, $J = 21.4$ Hz), 114.24, 114.12, 113.92, 113.85(d, $J = 21.2$ Hz), 72.51, 45.23, 35.62, 29.00. HRMS (ESI) calcd for $C_{37}H_{32}FN_5O_2$ $[M + H]^+$: 598.2618, found: 598.2604.

N-(2-(4-fluorophenyl)-4-((4-(piperidin-4-ylmethoxy) phenyl)amino)quinazolin-6-yl)-2-naphthamide (**28d**)

Faint yellow solid, yield: 87.3%, mp: 190.2–190.8 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.86 (s, 1H), 9.91 (s, 1H), 8.97 (s, 1H), 8.73 (s, 1H), 8.46 (d, $J = 7.2$ Hz, 2H), 8.12 (q, $J = 8.3$, 7.8 Hz, 4H), 8.04 (d, $J = 7.5$ Hz, 1H), 7.88 (d, $J = 8.8$ Hz, 1H), 7.82 (d, $J = 8.5$ Hz, 2H), 7.66 (s, 2H), 7.33 (t, $J = 8.7$ Hz, 2H), 7.04 (d, $J = 8.4$ Hz, 2H), 3.87–3.84 (m, 2H), 3.11 (d, $J = 11.9$ Hz, 2H), 2.66 (t, $J = 12.1$ Hz, 2H), 1.98–1.88 (m, 1H), 1.81 (d, $J = 12.7$ Hz, 2H), 1.32 (d, $J = 13.1$ Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.54, 162.26 (d, $J = 248.2$ Hz), 157.71, 157.24, 154.96, 147.40, 136.38, 134.94 (d, $J = 2.5$ Hz), 134.37, 132.32, 132.09, 131.74, 129.95 (d, $J = 8.9$ Hz), 128.97, 128.29, 128.21, 128.13, 128.02, 127.94, 127.68, 126.90, 124.34, 123.91, 115.29 (d, $J = 21.6$ Hz), 114.19, 114.04, 113.95, 72.01, 44.11, 34.53, 27.31. HRMS (ESI) calcd for $C_{37}H_{32}FN_5O_2$ $[M + H]^+$: 598.2618, found: 598.2601.

N-(2-(2-chlorophenyl)-4-((4-(piperidin-4-ylmethoxy) phenyl)amino)quinazolin-6-yl)-2-naphthamide (**28e**)

Faint yellow solid, yield: 91.8%, mp: 185.3–186.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.93 (s, 1H), 9.01 (s, 1H), 8.73 (s, 1H), 8.13 (s, 3H), 8.05 (d, $J = 7.3$ Hz, 2H), 7.83 (dd, $J = 27.7$, 8.7 Hz, 3H), 7.70 (d, $J = 18.0$ Hz, 3H), 7.59–7.53 (m, 1H), 7.46 (s, 2H), 6.95 (d, $J = 8.5$ Hz, 2H), 3.89–3.83 (m, 2H), 3.25 (d, $J = 11.9$ Hz, 2H), 2.84 (t, $J = 12.3$ Hz, 2H), 2.06–1.96 (m, 1H), 1.89 (d, $J = 13.3$ Hz, 2H), 1.48–1.38 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.58, 159.71, 157.37, 154.86, 147.06, 139.20, 136.87, 134.38, 132.30, 132.09, 131.69, 131.46, 131.36, 129.85, 128.98, 128.98, 128.30, 128.22, 128.15, 127.97, 127.68, 126.91, 124.35, 123.86, 114.14, 113.84, 113.73, 71.49, 43.10, 33.45, 25.78. HRMS (ESI) calcd for $C_{37}H_{32}ClN_5O_2$ $[M + H]^+$: 614.2323, found: 614.2308.

N-(4-((4-(piperidin-4-ylmethoxy)phenyl)amino)-2-(o-tolyl)quinazolin-6-yl)-2-naphthamide (**28f**)

Faint yellow solid, yield: 85.2%, mp: 189.3–189.9 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.88 (s, 1H), 9.86 (s, 1H), 9.00 (s, 1H), 8.75 (s, 1H), 8.13 (d, $J = 10.2$ Hz, 3H), 8.05 (d, $J = 7.4$ Hz, 2H), 7.84 (dd, $J = 17.7$, 8.0 Hz, 2H), 7.79–7.61 (m, 4H), 7.30 (q, $J = 7.9$, 7.5 Hz, 3H), 6.97 (d, $J = 8.4$ Hz, 2H), 3.92–3.78 (m, 2H), 3.24–3.09 (m, 2H), 2.71 (t, $J = 12.6$ Hz, 2H), 2.52 (s, 3H), 2.01–1.91 (m, 1H), 1.82 (d, $J = 12.7$ Hz, 2H), 1.35 (dd, $J = 22.3$, 11.4 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.54, 161.46, 157.60, 155.01, 147.26, 139.33, 136.53, 136.46, 134.37, 132.41, 132.10, 131.74, 130.77, 130.03, 128.97, 128.44, 128.25, 128.12, 127.93, 127.67, 126.89, 125.45, 124.36, 124.24, 114.15, 113.92, 113.36, 71.86, 43.85, 34.25, 26.94, 21.14. HRMS (ESI) calcd for $C_{38}H_{35}N_5O_2$ $[M + H]^+$: 594.2869, found: 594.2856.

N-(4-((4-(piperidin-4-ylmethoxy)phenyl)amino)-2-(p-tolyl)quinazolin-6-yl)-2-naphthamide (**28g**)

Faint yellow solid, yield: 88.3%, mp: 200.4–200.9 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.84 (s, 1H), 9.86 (s, 1H), 8.95 (d, $J = 2.1$ Hz, 1H), 8.72 (s, 1H), 8.33 (d, $J = 7.9$ Hz, 2H), 8.17–8.11 (m, 3H), 8.10–8.04 (m, 2H), 7.87 (dd, $J = 9.0$, 3.7 Hz, 3H), 7.68 (q, $J = 6.6$ Hz, 2H), 7.31 (d, $J = 7.9$ Hz, 2H), 7.05 (d, $J = 8.5$ Hz, 2H), 3.92 (d, $J = 6.1$ Hz, 2H), 3.31 (d, $J = 12.5$ Hz, 2H), 2.91 (dd, $J = 13.6$, 10.7 Hz, 2H), 2.39 (s, 3H), 2.13–2.03 (m, 1H), 1.95 (d, $J = 13.7$ Hz, 2H), 1.59–1.44 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.52, 158.20, 157.57, 154.72, 147.53, 139.62, 136.17, 135.77, 134.36, 132.61, 132.10, 131.76, 128.97, 128.87, 128.28, 128.21, 128.14, 128.07, 127.94, 127.78, 127.68, 126.91, 124.34, 123.68, 114.19, 114.04, 113.96, 71.44, 42.95, 33.34, 25.51, 20.96. HRMS (ESI) calcd for $C_{38}H_{35}N_5O_2$ $[M + H]^+$: 594.2869, found: 594.2853.

N-(2-(furan-2-yl)-4-((4-(piperidin-4-ylmethoxy)phenyl) amino)quinazolin-6-yl)-2-naphthamide (**28h**)

Faint yellow solid, yield: 86.3%, mp: 192.2–193.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.82 (s, 1H), 9.84 (s, 1H), 8.93 (s, 1H), 8.71 (s, 1H), 8.12 (d, $J = 5.4$ Hz, 3H), 8.05 (t, $J = 7.9$ Hz, 2H), 7.92–7.87 (m, 3H), 7.85 (d, $J = 8.8$ Hz, 1H), 7.71–7.62 (m, 2H), 7.18 (d, $J = 3.3$ Hz, 1H), 7.02 (d, $J = 8.5$ Hz, 2H), 6.69–6.65 (m, 1H), 3.83 (d, $J = 6.3$ Hz, 2H), 2.99 (d, $J = 11.9$ Hz, 2H), 2.55 (s, 2H), 1.94–1.80 (m, 1H), 1.79–1.69 (m, 2H), 1.21 (qd, $J = 12.1$, 3.9 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.54, 157.29, 154.94, 152.96, 151.96, 147.21, 144.86, 136.26, 134.36, 132.40, 132.09, 131.75, 128.97, 128.34, 128.13, 128.11, 128.07, 127.92, 127.67, 126.89, 124.35, 123.46, 114.28, 114.13, 114.06, 112.48, 112.04, 72.57, 45.32, 35.70, 29.14. HRMS (ESI) calcd for $C_{35}H_{31}N_5O_3$ $[M + H]^+$: 570.2505, found: 570.2492.

Materials and methods

Materials

All tested compounds were dissolved in DMSO to make a 10 μM stock solution. RPMI-1640, DAPI and MTT were purchased from Solarbio. Annexin VFITC/PI Apoptosis

Detection Kit and Cell Cycle Detection Kit were purchased from Keygen Biotech. Fetal Bovine Serum (FBS) and Trypsin were purchased from Beyotime Biotechnology. All the other reagents used were of analytical grade.

Cell culture

Eca-109 (human esophageal epithelial cancer cell line), A549 (human non-small cell lung cancer cell line), PC-3 (human prostate cancer cell line)) and MGC-803 (human gastric cancer cell line) were purchased from American Type Culture Collection (ATCC, Shanghai, China). These four human cancer cell lines were maintained in RPMI-1640 medium which was supplemented with 10 % fetal bovine serum (FBS) and were cultured at 37 °C in a humidified atmosphere containing 5% CO_2.

MTT assay

The antiproliferative activity of individual compounds against four cancer cell lines was tested in vitro by MTT assay. Generally, the cells (1500–3300 cells/well) were cultured in 96-well plates for 24 h and then incubated in triplicate with different concentrations of individual compounds or DMSO contained medium (200 μL) for an additional 72 h. Then, 20 μL MTT solution (5%) was added to each well at 37 °C in a humidified atmosphere containing 5% CO_2 for 4 h. After that, the MTT-containing medium was removed and, following by adding DMSO (150 μL/well) to dissolve the formazan crystals.

The absorbance was measured by the microplate reader at the wave of 490 nm and the IC_{50} values were calculated using GraphPad Prism 8.0.1. The results were Mean ± SD of three independent experiments.

Colony formation assay

MGC-803 cells were seeded into the 6-well plate with a density of 800 cells/well and then the cells were cultures at 37 °C in a humidified atmosphere containing 5% CO_2 for 24 h. Next, the medium was replaced by different concentrations of **28g** contained medium. After incubating for 7 days, the culture medium was discarded and the plates were washed with PBS twice. Then, the cells were fixed with 4% paraformalclehyde for 20 min, followed by staining with 0.1% crystal violet. After that, the crystal violet solution was removed and carefully washed with water. After the plate is naturally dried, images were photographed and processed using Photoshop.

Transwell assay

MGC-803 cells were seeded into Corning®Costar® Transwell® cell culture chamber with porous membrane. The upper chambers were placed into a 24-well plate (the lower chambers). The FBS-free medium, different concentrations of **28g** and 6000-8000 MGC-803 cells were added in each upper chamber, while 500 μL 20% FBS contained medium was added into the lower chambers. After 24 h period of incubation at 37 °C, the medium was discarded and the migration cells were fixed with 4% paraformalclehyde for 20 min. Then, the cells were stained with 0.1% crystal violet solution, followed by carefully washed with water, naturally dried and photographed.

Cell cycle analysis

The distribution of cell cycle status was detected using the cell cycle detection kit (Keygen Biotech, China) and flow cytometer, according to the manufacturer's instructions. Briefly, the cells in the logarithmic growth phase were trypsinized, centrifuged, resuspended and seeded in the 6-well plate with a concentration of 7×10^4 cells/well. After 24 h period of incubation at 37 °C, the cells were harvested and treated with various concentrations of compound **28g** for another 24 h. Then, the cells were harvested, washed with PBS and incubated with 1 mL DNA staining solution and 10 μL permeabilization solution under dark conditions. Finally, the samples were subjected to the flow cytometer (BD, Biosciences, San Jose, CA, USA) for detecting the percentage of cell cycle phases. The data was analyzed using ModfitLT 5.

DAPI staining assay

The cells in the logarithmic growth phase were trypsinized, centrifuged, resuspended and seeded in the 6-well plate with a concentration of 7×10^4 cells/well. After 24 h period of incubation at 37 °C, the culture medium was replaced with indicated concentrations of **28g** for an additional 48 h. Then, the medium was removed and the plates were washed with PBS twice, followed by fixed with 1 mL 4% paraformalclehyde and stained with 1 mL DAPI solution (10 μg/mL) in the dark for 10 min. After discarding the DAPI solution, the cells were washed with PBS twice and photographed using a fluorescence microscope.

Cell apoptosis analysis

Cell apoptosis was detected by Annexin V-FITC/PI apoptosis detection kit (Keygen Biotech, China) and flow cytometer, according to the manufactory instructions. Briefly, MGC-803 cells were seeded in the 6-well plate with a concentration of 7×10^4 cells/well and cultured at 37 °C for 24 h. Then, the culture medium was replaced with indicated concentrations of **28g** for an additional 48 h. After that, the cells were harvested, washed with PBS and resuspended in

binding buffer (500 µL), followed by incubation with Annexin V-FITC (5 µL) and PI (5 µL) under dark conditions. Cell apoptosis at different stages was detected by flow cytometry and data was analyzed using Flowjo V10.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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