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Research Article

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Synthesis and Antitumor Activity Evaluation in Vitro of 4-aminoquinazoline Derivatives Containing 1,3,4-thiadiazole

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Abstract

In order to search for new antitumor drugs with high efficiency and low toxicity, a series of novel 4-aminoquinazoline derivatives containing 1,3,4-thiadiazole group were designed, synthesized and evaluated for antiproliferative activity against four human cancer cell lines (H1975, PC-3, MCF-7 and HGC-27) using MTT assay in vitro. Among them, compound N-(5-((3,5-dichlorobenzyl)thio)-1,3,4-thiadiazol-2-yl)-2-((4-(phenylamino)quinazolin-2-yl)thio)acetamide (15o) showed good anti-tumor proliferation activity against four tested cancer cell lines, with IC\textsubscript{50} values of 1.96±0.15μM to PC-3 cell. The antitumor activity was significantly better than that of gefitinib. Further mechanism studies showed that compound 15o inhibited the migration ability of PC-3 tumor cells in a concentration dependent and time-dependent manner, and blocked the cell cycle in S phase. At the same time, cell cloning experiment further proved that compound 15o significantly inhibited the colony formation of PC-3 cells, The inhibition rate was as high as 92% at 2.0 μM.

Graphical Abstract

Keywords: Quinazoline derivatives, 1,3,4-thiadiazole, antitumor activity, synthesis

Introduction
Malignant tumor is a tissue with abnormal physiological function formed by excessive proliferation or abnormal differentiation of tumor cells. At present, the clinical treatment methods of malignant tumors include surgical treatment, radiotherapy, chemotherapy, immunotherapy and targeted therapy, among which chemotherapy is still an effective method for the treatment of malignant tumors. [1-4] However, the adverse side effect and the development of resistance to traditional anticancer drugs call for an urgent exploration of new anticancer agents. [5-6]

Quinazoline derivatives with a wide range of pharmacological activities[7], such as anti-tumor[8-9], anti-plasmodium[10-11], anti-virus[12-14] and anti-convulsant[15] etc. In addition, many anticancer drugs containing the 4-anilinoquinazolin scaffold had been approved by US Food and Drug Administration (FDA) for clinical use, such as Gefitinib[16], Erlotinib[17], Afatinib[18], Lapatinib[19], and so forth (Fig. 1). Thus, the 4-aminoquinazoline was selected as the scaffold to study.

Meanwhile, it has been reported that compounds (5-7)[20-22] (Fig. 1) which contain 1,3,4-thiadiazole group exhibited significant antiproliferative activity against cancer cells. Therefore, we introduced the biologically pharmacophore 1,3,4-thiadiazole into quinazoline ring by using the combination principles to synthesize a series of new hybrid molecules and evaluate their

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**Fig. 1 Design of target compounds**
antiproliferative activity in vitro.

Results and discussion

Chemistry

Fig. 2 Synthetic routes of target compounds 15a-o. Reaction conditions and reagents: (a) KOH, substituted benzyl chloride, r.t, 8 h. (b) chloroacetyl chloride, KOH, 1,4-Dioxane, r.t, 3.5 h. (c) Et₃N, CS₂, Acetone, r.t, 2 h. (d) Chloroform, Triphosgene, r.t, 0.5 h. (e) Anthranilonitrile, Pyridine, 100 °C, 8 h; (f) KOH, 1,4-Dioxane, 70 °C, 2 h.

The synthetic strategy to prepare the target compounds was depicted in Fig. 2. Firstly, After 5-amino-1,3,4-thiadiazole-2-thiol and KOH are dissolved in water, a substituted benzyl compounds is added for 8 h to obtained compounds (9a-o). Then, compound (10a-o) were acquired from the reaction of compound (9a-o) with chloroacetyl chloride. Next, Compound (12) was prepared by the reaction of aniline, carbon disulfide and triethylamine in acetone for 2 h. Then, compound (13) was acquired from the reaction of compound (12) with triphosgene in chloroform for 0.5 h, that was further reacted with anthranilonitrile in pyridine for 8 h to get compound (14). Finally, compounds (10a-o) were
reacted respectively with compound (14) under basic condition in the mixture of H₂O and 1,4-dioxane for 2 h to produce compounds (15a-o). The structure of target compounds were confirmed by ¹H NMR, ¹³C NMR and HRMS.

**Evaluation of antitumor activity**

**MTT assay**

The in vitro antiproliferative activities of all the prepared compounds (15a-o) were evaluated against H1975 (Human lung cancer cell line), PC-3 (Human prostate cancer cell line), MCF-7 (Human breast cancer cell line) and HGC-27 (Human gastric carcinoma cell line) by MTT assay. Gefitinib was selected as positive reference drugs. The IC₅₀ values (concentration required to achieve 50% inhibition of the tumor cell proliferation) of the tested compounds for each cell line are presented in Table 1.

As shown in Table 1, the majority of the compounds exhibited moderate to potent antiproliferative activity against four human cancer cells. In general, compound (15a-o) had better bioactivity against H1975 and PC-3 than MCF-7 and HGC-27 cells. Comparing compounds (15b), (15e), (15h) with (15c), (15f), (15i). we concluded that compounds with halogen element at benzene, whether in the ortho, meta, or para position of benzene, the order of antiproliferative activity was F>Cl. As far as the H1975 cell line was concerned, from the bioactivity data of compounds (15h-m), the electron donating group at 4-position was better than the electron withdrawing group. Moreover, compound (15l) has better activity than compound (15m), it means that a small volume of electron-donating group at 4-position may be beneficial to bioactivity. Among all the target compounds, compound (15o) showed the most potent anti-proliferative activity against H1975, PC-3, MCF-7 and
HGC-27 cell lines with IC$_{50}$ values of 2.35±0.12μM, 1.96±0.15μM, 2.85±0.21μM, and 3.46±0.15μM, respectively, which were potent than Gefitinib.

Table 1 IC$_{50}$ values of target compounds against four cancer cell lines

| Compounds | R     | IC$_{50}$ (μM) $^a$ | H1975 | PC-3 | MCF-7 | HGC-27 |
|-----------|-------|---------------------|-------|------|-------|--------|
| (15a)     | -     | >50                 | 18.55±0.45 | >50  | 15.15±0.28 |
| (15b)     | 2-F   | 12.80±0.70          | 19.06±0.63 | 28.32±0.82 | 28.45±0.67 |
| (15c)     | 2-Cl  | >50                 | 20.61±0.75 | 32.45±0.85 | 18.32±0.45 |
| (15d)     | 2-CF$_3$ | 15.77±0.49       | 16.40±0.92 | >50  | 13.65±0.57 |
| (15e)     | 3-F   | 11.02±0.63          | 14.82±0.61 | 29.75±0.56 | 21.38±0.47 |
| (15f)     | 3-Cl  | >50                 | 16.75±0.83 | 11.38±0.27 | 16.21±0.79 |
| (15g)     | 3-CH$_3$ | 21.38±0.47       | 11.09±0.70 | >50  | 25.69±0.73 |
| (15h)     | 4-F   | 14.75±0.67          | 13.02±0.46 | 20.79±1.21 | >50    |
| (15i)     | 4-Cl  | >50                 | 13.63±0.34 | 24.28±0.65 | 13.38±0.83 |
| (15j)     | 4-CF$_3$ | 29.34±0.91       | 25.67±0.83 | >50  | 28.15±0.28 |
| (15k)     | 4-CN  | >50                 | >50    | >50  | 38.15±0.91 |
| (15l)     | 4-CH$_3$ | 9.93±0.58          | 28.13±0.95 | 41.52±1.45 | 18.15±0.36 |
| (15m)     | 4-OCH$_3$ | 13.56±0.52       | 16.68±0.74 | 49.85±0.58 | >50    |
| (15n)     | 3,4-diCl | 23.90±0.65        | 14.74±0.23 | 15.47±1.15 | 28.37±0.28 |
| (15o)     | 3,5-diCl | 2.35±0.12         | 1.96±0.15  | 2.85±0.21  | 3.46±0.15  |
| Gefitinib $^b$ | -     | 9.20±0.76          | 8.92±0.41  | 8.15±0.28  | 9.56±0.88  |

$^a$ Antitumor activity was assayed by exposure for 72 h to substances and expressed as concentration required to inhibit tumor cell proliferation by 50% (IC$_{50}$). Data are presented as the mean ± SDs of three independent experiments. $^b$ Positive control.

Cell cycle arrest by compound 15o

Compound 15o was chosen to further evaluate its possible anticancer mechanism of action against PC-3 cell based on the above results. After 24 h treatment with compound 15o, PC-3 cells were arrested in S phase (Fig.3). With the increase of compound 15o concentration, the percentage of PC-3 cells in S phase increased significantly, which were 21.41%, 30.62%, 35.22% and 44.38% respectively, indicating that compound 15o blocked the cell cycle of PC-3 in S phase.
Fig. 3 (A) Cells were treated with different concentrations (0, 0.5, 1.0, 2μmol/L) for 24 h. Then analyze DNA content by flow cytometry. (B) Quantitative analysis of the cell cycle distribution of PC-3 cells for 24 h.

**Cell migration inhibition arrest by compound 15o**

As shown in **Fig. 4**, with the extension of time and the increase of compound 15o concentration, the cell migration area gradually decreases, indicating that the migration ability of PC-3 cells decreases, and compound 15o inhibits the migration of PC-3 cells in a time-dependent and concentration-dependent manner.

Fig. 4 (C) Compound 15o inhibits migration of PC-3 cell. (D) Quantitative analysis chart
of cell migration rate.

**Cell clone arrest by compound 15o**

As shown in Fig. 5, compared with the control group, with the increase of compound 15o concentration, the number of cell clone formation decreased and the cell community decreased significantly, indicating that compound 15o significantly inhibited the colony formation of PC-3 cells, The inhibition rate was as high as 92% at 2.0 μM.

![Diagram showing cell clone formation](image)

**Fig.5** (A) Compound 15o inhibits colony formation of PC-3 cell. (B) Quantitative analysis chart of cell colony formation inhibition rate.

**Conclusion**

In this work, a series of novel 4-aminoquinazoline derivatives bearing 1,3,4-thiadiazole group were designed, synthesized and evaluated for antiproliferative activity against four human cancer cell lines (H1975, PC-3, MCF-7, and HGC-27). The results suggested that most compounds had potent anti-proliferative activity. Especially, the compound (15o) exhibited the best antiproliferative activity
against H1975, PC-3, MCF-7, and HGC-27 cell lines. Further studies showed that compound 15o could inhibit the cloning and migration of PC-3 cell and block the cell cycle in S phase. The above facts illustrated that this work would have remarkable implications for further design and development of antitumor agent.

**Materials and methods**

**Materials**

Reagents and solvents were purchased from commercial sources and were used without further purification. High resolution mass spectra (HRMS) were recorded on a Waters Micromass Q-T of Micromass spectrometer by electrospray ionizaton (ESI). \(^1\)H NMR and \(^13\)C NMR spectra were recorded on a Bruker 400MHz and 101MHz spectrometer respectively. Melting points were determined on an X-5 micromelting apparatus. Column chromatography was carried out on 200-300 mesh silicagel (Qingdao Haiyang Chemical, China). Reactions were monitored by thin-layer chromatography (TLC) on 0.25mm silicagel plates (GF254).

**Synthesis of Compounds**

Compounds (9-10) [23-24]and compounds (11-14) [25] were synthesized according to the published literature.

General procedure for synthesis of derivatives(15a-o). Potassium hydroxide (1.18 mmol) was dissolved in water (20 mL), then compound (14) (0.79 mmol) was added. When the system became transparent, compounds (10a-o) (0.75 mmol) were dissolved in dioxane (3 mL) and added to the reaction system, the mixture was heated to 70 ℃ for 2 h, a large amount of white precipitate was precipitated out. After the reaction was completed, filtered (the filter cake was washed with water and dioxane for three times), dried and then crude products were purified by column chromatography to obtain compounds (15a-o).
N-(5-(benzylthio)-1,3,4-thiadiazol-2-yl)-2-((4-(phenylamino)quinazolin-2-yl)thio)acetamide (15a). white solid, yield 82.3%, mp 201-202 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) δ (ppm) 12.92 (s, 1H, O=C-NH), 9.91 (s, 1H, NH), 8.49-7.13 (m, 14H, Ar-H), 4.46 (s, 2H, CH$_2$), 4.19 (s, 2H, CH$_2$). $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ (ppm) 167.94 (C=O), 165.14, 159.51, 157.72, 157.12, 149.94, 138.54, 136.71, 133.54, 128.91, 128.47, 128.43, 127.50, 126.17, 125.10, 124.10, 123.30, 122.50, 113.11 (Ar-C, thiadiazole-C), 37.60 (CH$_2$-C=O), 34.28 (CH$_2$). HRMS (ESI) calcd for C$_{25}$H$_{21}$N$_6$OS$_3$ [M+H]$^+$ : 517.0939, found: 517.0940.

N-(5-((2-fluorobenzyl)thio)-1,3,4-thiadiazol-2-yl)-2-((4-(phenylamino)quinazolin-2-yl)thio)acetamide (15b). white solid, yield 77.9%, mp 208-209 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) δ (ppm) 12.93 (s, 1H, O=C-NH), 9.91 (s, 1H, NH), 8.51–7.09 (m, 13H, Ar-H), 4.47 (s, 2H, CH$_2$), 4.19 (s, 2H, CH$_2$). $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ (ppm) 168.01 (C=O), 165.13, 161.55, 159.90, 157.12, 156.92, 149.93, 138.53, 133.54, 131.28, 129.94, 128.42, 126.16, 125.10, 124.48, 124.10, 123.86, 123.31, 122.50, 115.52, 113.10 (Ar-C and thiadiazole-C), 34.28 (CH$_2$-C=O), 31.45 (CH$_2$). HRMS (ESI) calcd for C$_{25}$H$_{20}$FN$_6$OS$_3$ [M+H]$^+$ : 535.0845, found: 535.0846.

N-(5-((2-chlorobenzyl)thio)-1,3,4-thiadiazol-2-yl)-2-((4-(phenylamino)quinazolin-2-yl)thio)acetamide (15c). white solid, yield 72.6%, mp 237-238 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) δ (ppm) 12.93 (s, 1H, O=C-NH), 9.90 (s, 1H, NH), 8.48-7.13 (m, 13H, Ar-H), 4.53 (s, 2H, CH$_2$), 4.19 (s, 2H, CH$_2$). $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ (ppm) 168.08 (C=O), 165.15, 160.05, 157.11, 156.81, 149.93, 138.54, 134.18, 133.54, 133.25, 131.33, 129.62, 129.54, 128.43, 127.32, 126.17, 125.10, 124.10, 123.30, 122.49, 113.10 (Ar-C and thiadiazole-C), 35.81 (CH$_2$-C=O), 34.34 (CH$_2$). HRMS (ESI) calcd for C$_{25}$H$_{20}$ClN$_6$OS$_3$ [M+H]$^+$ : 551.0549, found: 551.0551.

N-(5-((2-(trifluoromethyl)benzyl)thio)-1,3,4-thiadiazol-2-yl)-2-((4-(phenylamino)quinazolin-2-yl)thio)acetamide (15d). white solid, yield 79.4%, mp 228-229 °C. $^1$H NMR (400 MHz,
DMSO-$d_6$ δ (ppm) 13.00 (s, 1H, O=C-NH), 9.90 (s, 1H, NH), 8.49-7.13 (m, 13H, Ar-H), 4.62 (s, 2H, CH$_2$), 4.20 (s, 2H, CH$_2$). $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ (ppm) 168.06 (C=O), 165.12, 157.11, 156.75, 149.93, 138.54, 134.76, 133.53, 132.84, 131.90, 128.42, 128.39, 127.25, 126.96, 126.27, 126.21, 126.16, 125.10, 124.09, 123.31, 122.49, 113.10 (Ar-C, thiadiazole-C and CF$_3$), 34.54 (CH$_2$-C=O), 34.28 (CH$_2$). HRMS (ESI) calcd for C$_{26}$H$_{20}$F$_3$N$_6$O$_3$ [M+H]$^+$ : 585.0813, found: 585.0814.

N-(5-((3-fluorobenzyl)thio)-1,3,4-thiadiazol-2-yl)-2-((4-(phenylamino)quinazolin-2-yl)thio)acetamide (15e). white solid, yield 83.6%, mp 204-205 ℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ (ppm) 12.92 (s, 1H, O=C-NH), 9.91 (s, 1H, NH), 8.51–7.05 (m, 13H, Ar-H), 4.49 (s, 2H, CH$_2$), 4.19 (s, 2H, CH$_2$). $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ (ppm) 167.97 (C=O), 165.13, 160.75, 159.65, 157.34, 157.12, 149.93, 139.84, 138.53, 133.53, 130.42, 128.42, 126.16, 125.09, 125.05, 124.10, 123.30, 122.50, 115.76, 114.43, 113.10 (Ar-C and thiadiazole-C), 36.86 (CH$_2$-C=O), 34.28 (CH$_2$). HRMS (ESI) calcd for C$_{25}$H$_{20}$F$_3$N$_6$O$_3$ [M+H]$^+$ : 535.0845, found: 535.0847.

N-(5-((3-chlorobenzyl)thio)-1,3,4-thiadiazol-2-yl)-2-((4-(phenylamino)quinazolin-2-yl)thio)acetamide (15f). white solid, yield 78.9%, mp 214-215 ℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ (ppm) 12.90 (s, 1H, O=C-NH), 9.90 (s, 1H, NH), 8.51–7.09 (m, 13H, Ar-H), 4.47 (s, 2H, CH$_2$), 4.19 (s, 2H, CH$_2$). $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ (ppm) 168.03 (C=O), 165.15, 159.78, 157.20, 157.11, 149.94, 139.57, 138.53, 133.53, 132.95, 130.27, 128.74, 128.42, 127.60, 127.42, 126.16, 125.09, 124.10, 123.30, 122.50, 113.10 (Ar-C and thiadiazole-C), 36.70 (CH$_2$-C=O), 34.33 (CH$_2$). HRMS (ESI) calcd for C$_{25}$H$_{20}$ClN$_6$O$_3$ [M+H]$^+$ : 551.0549, found: 551.0550.

N-(5-((3-methylbenzyl)thio)-1,3,4-thiadiazol-2-yl)-2-((4-(phenylamino)quinazolin-2-yl)thio)acetamide (15g). white solid, yield 68.5%, mp 206-207 ℃. $^1$H NMR (400 MHz, DMSO-$d_6$) δ (ppm) 12.89 (s, 1H, O=C-NH), 9.90 (s, 1H, NH), 8.51–7.06 (m, 13H, Ar-H), 4.42 (s, 2H, CH$_2$), 4.19
\(1^3\)C NMR (101 MHz, DMSO-\(d_6\)) \(\delta\) (ppm) 168.00 (C=O), 165.16, 159.60, 157.73, 157.11, 149.94, 138.54, 137.65, 136.49, 133.53, 129.49, 128.42, 128.36, 128.17, 126.17, 125.99, 125.09, 124.09, 123.30, 122.49, 113.10 (Ar-C and thiadiazole-C), 37.59 (CH\(_2\)-C=O), 34.33 (CH\(_2\)), 20.86 (CH\(_3\)).

HRMS (ESI) calcd for C\(_{26}\)H\(_{23}\)N\(_6\)OS\(_3\) [M+H]\(^+\) : 531.1095, found: 531.1097.

N-(5-((4-fluorobenzyl)thio)-1,3,4-thiadiazol-2-yl)-2-((4-(phenylamino)quinazolin-2-y1)thio)acetamide (15h). white solid, yield 82.9%, mp 207-208 °C. \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) (ppm) 12.93 (s, 1H, O=C-NH), 9.90 (s, 1H, NH), 8.49–7.09 (m, 13H, Ar-H), 4.46 (s, 2H, CH\(_2\)), 4.19 (s, 2H, CH\(_2\)). \(^13\)C NMR (101 MHz, DMSO-\(d_6\)) \(\delta\) (ppm) 167.94 (C=O), 165.13, 162.65, 159.53, 157.50, 157.12, 149.93, 138.54, 133.54, 133.09, 131.00, 128.42, 126.16, 125.10, 124.10, 123.31, 122.50, 115.36, 113.10 (Ar-C and thiadiazole-C), 36.73 (CH\(_2\)-C=O), 34.26 (CH\(_2\)).

HRMS (ESI) calcd for C\(_{25}\)H\(_{20}\)FN\(_6\)OS\(_3\) [M+H]\(^+\) : 535.0845, found: 535.0847.

N-(5-((4-chlorobenzyl)thio)-1,3,4-thiadiazol-2-yl)-2-((4-(phenylamino)quinazolin-2-y1)thio)acetamide (15i). white solid, yield 79.1%, mp 229-230 °C. \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) (ppm) 12.86 (s, 1H, O=C-NH), 9.89 (s, 1H, NH), 8.49-7.12 (m, 13H, Ar-H), 4.46 (s, 2H, CH\(_2\)), 4.17 (s, 2H, CH\(_2\)). \(^13\)C NMR (101 MHz, DMSO-\(d_6\)) \(\delta\) (ppm) 168.33 (C=O), 165.29, 160.49, 157.08, 156.78, 149.96, 138.57, 136.12, 133.51, 132.04, 130.75, 128.42, 128.40, 126.17, 125.05, 124.06, 123.30, 122.46, 113.09 (Ar-C and thiadiazole-C), 36.74 (CH\(_2\)-C=O), 34.59 (CH\(_2\)).

HRMS (ESI) calcd for C\(_{25}\)H\(_{20}\)ClN\(_6\)OS\(_3\) [M+H]\(^+\) : 551.0549, found: 551.0549.

N-(5-((4-(trifluoromethyl)benzyl)thio)-1,3,4-thiadiazol-2-yl)-2-((4-(phenylamino)quinazolin-2-y1)thio)acetamide (15j). white solid, yield 81.7%, mp 243-244 °C. \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) (ppm) 12.91 (s, 1H, O=C-NH), 9.90 (s, 1H, NH), 8.51–7.09 (m, 13H, Ar-H), 4.56 (s, 2H, CH\(_2\)), 4.18 (s, 2H, CH\(_2\)). \(^13\)C NMR (101 MHz, DMSO-\(d_6\)) \(\delta\) (ppm) 168.05 (C=O), 165.16, 157.11,
N-(5-((4-cyanobenzyl)thio)-1,3,4-thiadiazol-2-yl)-2-((4-(phenylamino)quiazolin-2-yl)thio)acetamide (15k). white solid, yield 68.9%, mp 201-202 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ (ppm) 12.89 (s, 1H, O=C-NH), 9.90 (s, 1H, NH), 8.49-7.12 (m, 13H, Ar-H), 4.54 (s, 2H, CH$_2$), 4.18 (s, 2H, CH$_2$). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ (ppm) 168.10 (C=O), 165.17, 159.97, 157.10, 156.82, 149.93, 143.08, 138.54, 133.53, 132.33, 129.88, 128.42, 126.15, 125.09, 124.09, 123.31, 122.49, 118.61, 113.09 (Ar-C, thiadiazole-C and CN), 36.90 (CH$_2$-C=O), 34.35 (CH$_2$). HRMS (ESI) calcd for C$_{26}$H$_{20}$F$_3$N$_6$OS$_3$ [M+H]$^+$: 585.0813, found: 585.0813.

N-(5-((4-methylbenzyl)thio)-1,3,4-thiadiazol-2-yl)-2-((4-(phenylamino)quinazolin-2-yl)thio)acetamide (15l). white solid, yield 84.2%, mp 213-214 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ (ppm) 12.79 (s, 1H, O=C-NH), 9.90 (s, 1H, NH), 8.50–7.09 (m, 13H, Ar-H), 4.40 (s, 2H, CH$_2$), 4.18 (s, 2H, CH$_2$), 2.24 (s, 3H, CH$_3$). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ (ppm) 168.06 (C=O), 165.19, 159.78, 157.62, 157.10, 149.95, 138.55, 136.72, 133.58, 133.54, 129.03, 128.81, 128.42, 126.17, 125.08, 124.09, 123.30, 122.48, 113.10 (Ar-C and thiadiazole-C), 37.46 (CH$_2$-C=O), 34.38 (CH$_2$), 20.64(CH$_3$). HRMS (ESI) calcd for C$_{26}$H$_{23}$N$_6$OS$_3$ [M+H]$^+$: 542.0892, found: 542.0892.

N-(5-((4-methoxybenzyl)thio)-1,3,4-thiadiazol-2-yl)-2-((4-(phenylamino)quinazolin-2-yl)thio)acetamide (15m). white solid, yield 80.6%, mp 238-239 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ (ppm) 12.90 (s, 1H, O=C-NH), 9.90 (s, 1H, NH), 8.51–6.83 (m, 13H, Ar-H), 4.40 (s, 2H, CH$_2$), 4.19 (s, 2H, CH$_2$), 3.70 (s, 3H, OCH$_3$). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ (ppm) 167.89 (C=O), 165.13, 159.36, 158.64, 157.89, 157.12, 149.93, 138.54, 133.55, 130.18, 128.43, 128.36, 126.17, 125.10,
124.11, 123.31, 122.50, 113.88, 113.10 (Ar-C and thiadiazole-C), 55.00 (OCH$_3$), 37.27 (CH$_2$-C=O),
34.24 (CH$_2$). HRMS (ESI) calcd for C$_{26}$H$_{23}$N$_6$O$_2$S$_3$ [M+H]$^+$ : 569.0864, found: 569.0863.

N-(5-((3,4-dichlorobenzyl)thio)-1,3,4-thiadiazol-2-yl)-2-((4-(phenylamino)quinazolin-2-yl)thio)acetamide (15n). white solid, yield 71.3%, mp 217-218 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) δ (ppm) 12.87 (s, 1H, O=C-NH), 9.82 (s, 1H, NH), 8.48-7.13 (m, 12H, Ar-H), 4.48 (s, 2H, CH$_2$), 4.19 (s, 2H, CH$_2$). $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ (ppm) 172.29 (C=O), 170.10, 166.97, 156.65, 150.61, 150.46, 150.28, 139.45, 138.86, 133.25, 130.71, 130.38, 129.61, 129.14, 128.41, 126.28, 124.55, 123.68, 123.18, 122.17, 112.98 (Ar-C and thiadiazole-C), 38.02 (CH$_2$-C=O), 36.10 (CH$_2$). HRMS (ESI) calcd for C$_{25}$H$_{19}$Cl$_2$N$_6$OS$_3$ [M+H]$^+$ : 585.0160, found: 585.0159.

N-(5-((3,5-dichlorobenzyl)thio)-1,3,4-thiadiazol-2-yl)-2-((4-(phenylamino)quinazolin-2-yl)thio)acetamide (15o). white solid, yield 66.1%, mp 244-245 °C. $^1$H NMR (400 MHz, DMSO-$d_6$) δ (ppm) 12.94 (s, 1H, O=C-NH), 9.89 (s, 1H, NH), 8.48-7.11 (m, 12H, Ar-H), 4.48 (s, 2H, CH$_2$), 4.19 (s, 2H, CH$_2$). $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ (ppm) 167.90 (C=O), 165.08, 159.55, 157.13, 157.03, 149.92, 141.56, 138.51, 133.85, 133.52, 128.40, 127.72, 127.08, 126.15, 125.10, 124.10, 123.30, 122.52, 113.10 (Ar-C and thiadiazole-C), 36.05 (CH$_2$-C=O), 34.20 (CH$_2$). HRMS (ESI) calcd for C$_{25}$H$_{19}$Cl$_2$N$_6$OS$_3$ [M+H]$^+$ : 585.0160, found: 585.0159.

**Evaluation of antiproliferative activity**

Cells in the logarithmic growth phase cells at 3000-5000 cells/well were seeded in 96-well plates for 24 h. Then the cells were treated in triplicate with various concentrations of each compound at 37 °C in the humidified 5% CO2 atmosphere for 72h. And added MTT to each well at a final concentration of 5mg/ml. After incubated for 4 h in 37°C, the medium was aspirated. Add 150 μL DMSO to each well to dissolve formazan and the plate was shaken on a shaker for 10min. The OD vaules was measured by
microplate reader at a wavelength of 490 nm and the IC50 values were obtained using SPSS 20.0 software. Results were Mean ± SD of three independent experiments.

**Cell cycle distribution assay**

The PC-3 cells in logarithmic growth phase were divided into 2 × 10^5 / well were inoculated in 6-well plates and treated overnight at 37 °C and 5% CO2. The concentrations were 0 (blank control group), 0.5μM, 1.0μM and 2.0μM. PC-3 cells were treated with compound 15o and treated for 24 hours at 37 °C and 5% CO2. Then cells were harvested and fixed with 70% ethanol for 8 h at 4 °C. The fixed cells were washed and resuspended using phosphate buffer saline (PBS) containing 50 mg/mL propidium iodide (PI) and 10 mg/mL RNaseA. Then cell suspension was incubated for 40 min in a dark place at room temperature. After that, samples were analyzed for DNA content with flow cytometry. (Becton, Dickinson and Company, NJ)

**Cell migration experiment**

The PC-3 cells in logarithmic growth phase were divided into 4 × 10^5 / well were inoculated in 6-well plates and treated overnight at 37 °C and 5% CO2. When the cell density reached 85%, The 200 μL gun head evenly marks the "cross" at the bottom of the 6-well plates. The cells were treated with concentrations were 0 (blank control group), 0.5μM, 1.0 μM and 2.0μM, and photographed at the same position of each well with a microscope at 0h, 12h and 24h to record the trend of cell migration.

**Cell clone arrest**

PC-3 cells in logarithmic growth stage were inoculated in 6-well plates at 1000 / well, and cultured overnight at 37 °C and 5% CO2. PC-3 cells were treated with compound 15o at concentrations of 0 (blank control group), 0.5μM, 1.0μM and 2.0μM under the condition of 37 °C and 5% CO2 for 8 days. When the cell community visible to the naked eye appeared at the bottom of the 6-well plate, the
culture was terminated. The cells were fixed at 4 °C with 4% paraformaldehyde for 0.5h, and stained with 0.1% crystal violet for 20min.

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Compliance with ethical standards

Conflict of interest All authors declare no competing interests.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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Reference

1. Bixel K, Vetter M, Davidson B, et al. Intraperitoneal chemotherapy following neoadjuvant chemotherapy and optimal interval tumor reductive surgery for advanced ovarian cancer. Gynecol Oncol. 2020; 156: 530-534. https://doi.org/10.1016/j.ygyno.2019.12.016.

2. Bonet M, Garcia V, Farre N, et al. Radiation therapy for bone-only metastases in breast cancer patients: A Goco survey of current clinical practice. Rep Pract Oncol Radiother. 2020; 25: 113-116. https://doi.org/10.1016/j.rpor.2019.12.019.

3. Fu R G, Sun Y, Sheng W B, et al. Designing multi-targeted agents: An emerging anticancer drug discovery paradigm. Eur J Med Chem. 2017; 136: 195-211. https://doi.org/10.1016/j.ejmech.2017.05.016.

4. Hagemans J, Van Rees J M, Alberda W J, et al. Locally recurrent rectal cancer : long-term outcome of curative surgical and non-surgical treatment of 447 consecutive patients in a tertiary referral centre. Eur J Surg Oncol. 2020; 46: 448-454. https://doi.org/10.1016/j.ejso.2019.10.037.

5. Rettenmaier M A, Micha J P, Bohart R, et al. A retrospective study comparing the efficacy of dose-dense chemotherapy, intraperitoneal chemotherapy and dose-dense chemotherapy with hyperthermic intraperitoneal chemotherapy in the treatment of advanced stage ovarian carcinoma. Eur J Obstet Gynecol Reprod Biol. 2020; 244: 101-105. https://doi.org/10.1016/j.ejogrb.2019.10.047.
6. Nurgali, K., Jagoe, R. T., Abalo, R. Editorial: Adverse Effects of Cancer Chemotherapy: Anything New to Improve Tolerance and Reduce Sequelae?. Front. Pharmacol. 2018; 9: 245. https://doi.org/10.3389/fphar.2018.00245.

7. Alagarsamy, V., Chitra, K., Saravanan, G., Solomon, V.R., Sulthana, M.T., Narendhar, B. An overview of quinazolines: Pharmacological significance and recent developments. European Journal of Medicinal Chemistry. 2018; 151: 628-685. https://doi.org/10.1016/j.ejmech.2018.03.076.

8. Maestri, V., Tarozzi, A., Simoni, E., Cilia, A., Poggesi, E., Naldi, M., Nicolini, B., Pruccoli, L., Rosini, M., Minarini, A. Quinazoline based α1-adrenoreceptor antagonists with potent antiproliferative activity in human prostate cancer cell lines. European Journal of Medicinal Chemistry. 2017; 136: 259-269. https://doi.org/10.1016/j.ejmech.2017.05.003.

9. Zhang, Y., Chen, L., Xu, H., Li, X., Zhao, L., Wang, W., Li, B., Zhang, X. 6,7-Dimorpholinooalkoxy quinazoline derivatives as potent EGFR inhibitors with enhanced antiproliferative activities against tumor cells. European Journal of Medicinal Chemistry. 2018; 147: 77-89. https://doi.org/10.1016/j.ejmech.2018.01.090.

10. Desroches, J., Kieffer, C., Primas, N., Hutter, S., Gellis, A., El-Kashef, H., Rathelot, P., Verhaeghe, P., Azas, N., Vanelle, P. Discovery of new hit-molecules targeting Plasmodium falciparum through a global SAR study of the 4-substituted-2-trichloromethylquinazoline antiplasmodial scaffold. European Journal of Medicinal Chemistry. 2017; 125: 68-86. https://doi.org/10.1016/j.ejmech.2016.09.029.

11. Mendoza-Martinez, C., Correa-Basurto, J., Nieto-Meneses, R., Marquez-Navarro, A., Aguilar-Suarez, R., Dinora Montero-Cortes, M., Nogueda-Torres, B., Suarez-Contreras, E., Galindo-Sevilla, N., Rojas-Rojas, A., Rodriguez-Lezama, A., Hernandez-Luis. Design, synthesis and biological evaluation of quinazoline derivatives as anti-trypanosomatid and anti-plasmodial agents. European Journal of Medicinal Chemistry. 2015; 96: 296-307. https://doi.org/10.1016/j.ejmech.2015.04.028.

12. Nurgali, K., Jagoe, R. T., Abalo, R. Editorial: Adverse Effects of Cancer Chemotherapy: Anything New to Improve Tolerance and Reduce Sequelae?. Front. Pharmacol. 2018; 9, 245. https://doi.org/10.1016/j.antiviral.2016.08.005.

13. Kumar, K.S., Ganguly, S., Veerasamy, R., De Clercq, E. Synthesis, antiviral activity and cytotoxicity evaluation of Schiff bases of some 2-phenyl quinazoline-4(3)H-ones. European Journal of Medicinal Chemistry. 2010; 45: 5474-5479. https://doi.org/10.1016/j.ejmech.2010.07.058.

14. Piotrowska, D.G., Andrei, G., Schols, D., Snoeck, R., Lysakowska, M. Synthesis, anti-varicella-zoster virus and anti-cytomegalovirus activity of quinazoline-2,4-diones containing isoxazolidine and phosphonate substructures. European Journal of Medicinal Chemistry. 2017; 126: 84-100. https://doi.org/10.1016/j.ejmech.2016.10.002.

15. Jatav, V., Mishra, P., Kashaw, S., Stables, J.P. CNS depressant and anticonvulsant activities of some novel 3-[5-substituted 1,3,4-thiadiazole-2-yl]-2-styryl quinazoline-4(3H)-ones. European Journal of Medicinal Chemistry. 2008; 43: 1945-1954. https://doi.org/10.1016/j.ejmech.2007.12.003.

16. Argiris, A., Mittal, N. Gefitinib as first-line, compassionate use therapy in patients with advanced non-small-cell lung cancer. Lung Cancer. 2004; 43: 31. https://doi.org/10.1016/j.lungcan.2003.10.010.
17. Lin, P., An, F., Xu, X., Zhao, L., Liu, L., Liu, N., Wang, P., Liu, J., Wang, L., Li, M. Chronopharmacodynamics and mechanisms of antitumor effect induced by erlotinib in xenograft-bearing nude mice. Biochemical and Biophysical Research Communications. 2015; 460: 362-367. https://doi.org/10.1016/j.bbrc.2015.03.039.

18. Imai, H., Kaira, K., Suzuki, K., Anzai, M., Tsuda, T., Ishizuka, T., Kuwako, T., Naruse, I., Nemoto, K., Uchino, J., Morozumi, N., Ishihara, S., Minato, K., Hisada, T. A phase II study of afatinib treatment for elderly patients with previously untreated advanced non-small-cell lung cancer harboring EGFR mutations. Lung Cancer. 2018; 126: 41-47. https://doi.org/10.1016/j.lungcan.2018.10.014.

19. Gomez, H.L., Doval, D.C., Chavez, M.A., Ang, P.C.S., Aziz, Z., Nag, S., Ng, C., Franco, S.X., Chow, L.W.C., Arbushites, M.C., Casey, M.A., Berger, M.S., Stein, S.H., Sledge, G.W. Efficacy and Safety of Lapatinib As First-Line Therapy for ErbB2-Amplified Locally Advanced or Metastatic Breast Cancer. Journal of Clinical Oncology. 2008; 26: 2999-3005. https://doi.org/10.1200/jco.2007.14.0590.

20. Yadagiri, B., Gurrala, S., Bantu, R., Nagarapu, L., Polepalli, S., Srujana, G., Jain, N. Synthesis and evaluation of benzosuberone embedded with 1, 3, 4-oxadiazole, 1, 3, 4-thiadiazole and 1, 2, 4-triazole moiety as new potential anti proliferative agents. Bioorganic & Medicinal Chemistry Letters. 2015; 25: 2220-2224. https://doi.org/10.1016/j.bmcl.2015.03.032.

21. Gomha, S.M., Kheder, N.A., Abdelaziz, M.R., Mabkhot, Y.N., Alhajoj, A.M. A facile synthesis and anticancer activity of some novel thiazoles carrying 1,3,4-thiadiazole moiety. Chemistry Central Journal. 2017; 11: 25. https://doi.org/10.1186/s13065-017-0255-7.

22. Shukla, K., Ferraris, D.V., Thomas, A.G., Stathis, M., Duvall, B., Delahanty, G., Alt, J., Rais, R., Rojas, C., Gao, P., Xiang, Y., Dang, C.V., Slusher, B.S., Tsukamoto, T. Design, synthesis, and pharmacological evaluation of bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl)ethyl sulfide (BPTES) analogs as glutaminase inhibitors. Journal of Medicinal Chemistry. 2012; 55: 10551-10563. https://doi.org/10.1021/jm301191p.

23. Shen, L.-H., Li, H.-Y., Shang, H.-X., Tian, S.-T., Lai, Y.-S., Liu, L.-J. Synthesis and cytotoxic evaluation of new colchicine derivatives bearing 1,3,4-thiadiazole moiety. Chinese Chemical Letters. 2013; 24: 299-302. https://doi.org/10.1016/j.cclet.2013.01.052.

24. Mao, R., Shao, J., Zhu, K., Zhang, Y., Ding, H., Zhang, C., Shi, Z., Jiang, H., Sun, D., Duan, W., Luo, C. A Potent, Selective and Cell Active Protein Arginine Methyltransferase 5 (PRMT5) Inhibitor Developed by Structure-based Virtual Screening and Hit Optimization. Journal of Medicinal Chemistry. 2017; 60: 6289-6304. https://doi.org/10.1021/acs.jmedchem.7b00587.

25. Rocco, S.A., Barbarini, J.E., Rittner, R. Syntheses of Some 4-Anilinoquinazoline Derivatives. Synthesis-Stuttgart.2004; 3: 429-435. https://doi.org/10.1055/s-2004-815949.
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