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Original article

Synthesis and cytotoxic activity of *N*-((2-methyl-4(3*H*)-quinazolinon-6-yl)methyl) dithiocarbamates

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ABSTRACT

A series of *N*-((2-methyl-4(3*H*)-quinazolinon-6-yl)methyl)dithiocarbamates **5a**–**w** were synthesized and evaluated for their cytotoxic activity against five human cancer cell lines. We found that compound **5k** inhibited proliferation of A549, MCF-7, HeLa, HT29 and HCT-116 cells with IC_{50} values of 5.44, 7.15, 12.16, 10.35 and 11.44 μM, respectively. Compound **5i** was the most potent with an IC_{50} value of 3.65 μM against proliferation of MCF-7 cells, while **5n** was the most potent with an IC_{50} value of 5.09 μM against proliferation of A549 cells. Cell cycle analysis showed that both **5i** and **5k** arrested A549 cells at S and G2/M phases, suggesting that these compounds act through mechanisms different from 5-Fluorouracil, which arrests cells at S phase only.

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1. Introduction

Incorporation of lipophilic side chains into the benzene ring of quinazoline has led to the emergence of many effective anticancer drugs such as AG337 and Trimetrexate (Fig. 1) [1–7]. In the search for quinazoline-based anticancer agents at our laboratory, dithiocarbamate moiety occurring in phytoalexins Brassinin (Fig. 1), which possesses cancer chemopreventive activity and cytotoxic effect [8–12], had been introduced into the C6 position of 2-methyl-4(3*H*)-quinazolinone. Among the resulting compounds, **1a** (Fig. 1) derived from benzylamine displayed obviously in vitro inhibitory activity against K562 (human myelogenous leukemia) and HeLa (human cervical carcinoma) cells [13,14]. Thereafter, another series of derivatives of 2-methyl-4(3*H*)-quinazolinone had been synthesized from benzylamines bearing different substituents in the benzene ring or heterocyclomethylamines, of which compound **1b** (Fig. 1) containing (pyridin-3-yl)methylamine moiety inhibited the growth of A549 (human lung cancer), HCT-8 (human colorectal cancer) and Bel-7402 (human liver cancer) cell lines with IC_{50} values within micromolar concentrations [15]. For 4(3*H*)-quinazolinones bearing other alkyl than methyl, aryl, or heteroaryl group at the C2 position, however, their dithiocarbamate derivatives hardly showed any inhibitory activity against the growth of A549, HCT-8 and Bel-7402 cell lines [16]. Therefore, it seems that a methyl group at the C2 position is crucial for this type of compounds to generate cytotoxic effects.

Our previous work on the synthesis and cytotoxic evaluation of dithiocarbamate derivatives of 2-methyl-4(3*H*)-quinazolinone indicated that the connection of amino group with benzyl or heterocyclomethyl group is favorable to the cytotoxic activity [13–15]. As to the structure of Brassinin, it is noteworthy that the amino group of dithiocarbamate moiety is also linked with indole ring by methylene. In this context, the structurally reversed analogs of compounds **1a** and **1b** would be synthesized, so that 4(3*H*)-quinazolinone moiety previously served as ester component would be converted into amino component. Herein, we report the synthesis of a new series of *N*-((2-methyl-4(3*H*)-quinazolinon-6-yl)methyl)dithiocarbamates (Scheme 1 and Table 1) and evaluation of these compounds as potential anticancer agents.

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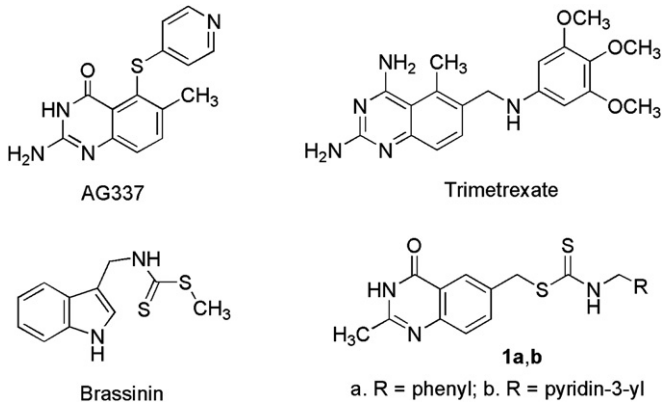


Fig. 1. Structures of AG337, Trimetrexate, Brassinin, compounds **1a** and **1b**.

2. Chemistry

As shown in Scheme 1, two methods, namely Gabriel method and Delepine method, were exploited to prepare the key intermediate 6-(aminomethyl)-2-methyl-4(3*H*)-quinazolinone dihydrochloride (**4**) required for the synthesis of the target molecules. In the case of Gabriel method, the reaction of 6-(bromomethyl)-2-methyl-4(3*H*)-quinazolinone (**2**) with potassium phthalimide gave 2-((2-methyl-4(3*H*)-quinazolinon-6-yl)methyl)isoindoline-1,3-dione (**3**), which was converted into **4** by hydrazinolysis, and then acidification with dilute hydrochloric acid. Alternatively, intermediate **4** was obtained by acid treatment of the crude product from heating **2** with hexamethylenetetramine. In comparison with Gabriel method, Delepine method possessed advantages such as shorter reaction time, higher yield and no isolation and purification of intermediate required, thus, it was adopted for the preparation of **4** in this study. Eventually, treating **4** and carbon disulfide with different halohydrocarbon in the presence of anhydrous potassium phosphate generated the target compounds **5a**–**w**.

3. Pharmacology

3.1. Cytotoxic evaluation

The MTT [3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyl-tetrazolium bromide] cell proliferation assay was used to evaluate cytotoxic activity of the synthesized compounds against five human cancer cell lines including A549 (lung cancer), MCF-7 (breast adenocarcinoma), HeLa (cervical carcinoma), HT29 and HCT-116 (colorectal cancer) cell lines. The inhibition of cell proliferation was determined 72 h after cells were exposed to the tested compounds at a concentration of 10 μg/mL. The compounds with 50% or more inhibition compared with vehicle-treated cells were considered active. Inhibition of cell proliferation by these active compounds at various concentrations was further measured, and their IC_{50} (the concentration that causes 50% of cell growth inhibition) values were determined and summarized in Table 1. The previously synthesized compounds **1a**, **1b** [13,14] and the anticancer drug 5-Fluorouracil were used as positive controls.

Table 1
Cytotoxic activity (IC_{50}, μM) of compounds **5a**–**w** against five human cancer cell lines.

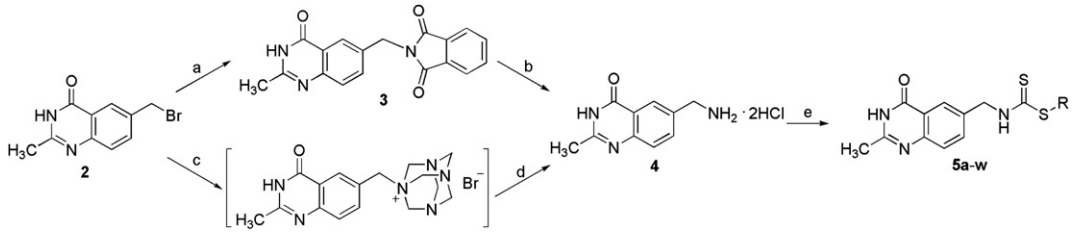
Compound	R	IC_{50} (μM)				
		A549	MCF-7	HeLa	HT29	HCT-116
5a	CH_3–	n.t.[a]	15.03	n.t.	n.t.	n.t.
5b	CH_3CH_2–	n.t.	n.t.	n.t.	n.t.	n.t.
5c	$CH_3CH_2CH_2$–	n.t.	15.61	n.t.	n.t.	n.t.
5d	$(CH_3)_2CH$–	n.t.	12.33	n.t.	n.t.	n.t.
5e	$CH_3(CH_2)_2CH_2$–	n.t.	19.41	n.t.	n.t.	n.t.
5f	$CH_3(CH_2)_3CH_2$–	n.t.	12.52	n.t.	n.t.	n.t.
5g	$CH_3(CH_2)_4CH_2$–	n.t.	n.t.	n.t.	n.t.	n.t.
5h	CyclohexylCH_2–	12.78	13.22	n.t.	10.35	n.t.
5i	$Ph_2C(CN)CH_2CH_2$–	5.49	3.65	n.t.	n.t.	n.t.
5j	CH_2=$CHCH_2$–	20.66	29.80	n.t.	n.t.	n.t.
5k	HC≡CCH_2–	5.44	7.15	12.16	10.35	11.44
5l	$PhCH_2$–	n.t.	n.t.	n.t.	n.t.	n.t.
5m	4-CH_3PhCH_2–	8.39	8.85	n.t.	12.58	15.64
5n	4-$FPhCH_2$–	5.09	7.07	13.73	13.68	11.57
5o	2,4-F_2PhCH_2–	5.49	8.43	n.t.	12.21	n.t.
5p	F_5PhCH_2–	17.20	6.65	20.45	12.26	11.92
5q	4-$ClPhCH_2$–	9.49	8.69	15.34	15.18	15.77
5r	4-$BrPhCH_2$–	7.73	7.15	13.38	14.88	13.98
5s	4-NO_2PhCH_2–	6.54	7.74	8.54	13.51	11.26
5t	4-$CNPhCH_2$–	n.t.	n.t.	n.t.	n.t.	n.t.
5u	4-HO_2CPhCH_2–	10.69	9.94	n.t.	n.t.	n.t.
5v	4-EtO_2CPhCH_2–	7.25	6.41	20.07	16.19	11.56
5w	Ph_2CH–	18.00	19.16	n.t.	n.t.	n.t.
1a		11.70	25.06	18.40	10.91	18.26
1b		5.58	12.65	13.66	4.71	3.65
5-Fluorouracil		6.76	7.99	98.54	8.46	6.00

[a] n.t.: Cell growth inhibition was lower than 50% at the concentration of 10 μg/mL and the IC_{50} value was not tested.

Among the tested compounds, **5i**, **5k** and **5s** presented prominent activity against the growth of A549 with IC_{50} values of 5.49, 5.44, and 6.54 μM, respectively. Therefore, they were selected for further investigation in A549 cell line. The tested compounds with concentrations of 12.0, 6.0 and 3.0 μM in medium, were incubated with the cells for 4, 8, 12, 24, 36 and 48 h, and cell proliferation was monitored at each timepoint using MTT assay. Identical amount of DMSO in medium served as the vehicle control. The growth curves of A549 cells treated with **5i**, **5k**, **5s** or 5-Fluorouracil were illustrated in Fig. 2.

3.2. Cell cycle analysis

To gain further insight into the mechanisms of action of these new and active compounds, we examined if treatment with these compounds in A549 cells would lead to cell cycle arrest. A549 cells were treated with **5i**, **5k**, **5s**, or 5-Fluorouracil at a concentration equivalent to its IC_{50} for 24 h. Cells were harvested for cell cycle



Scheme 1. Synthetic pathway for compounds **5a**–**w**. Reagents and conditions: a. potassium phthalimide, DMF, reflux 8 h; b. (i) 80% hydrazine hydrate, ethanol, reflux 5 h; (ii) 2 mol/L HCl, 60–90 °C 3 h; c. hexamethylenetetramine, trichloromethane, reflux 4 h; d. concentrated HCl, methanol, reflux 2 h; e. CS_2, K_3PO_4, RX, DMF, rt, 2–12 h.

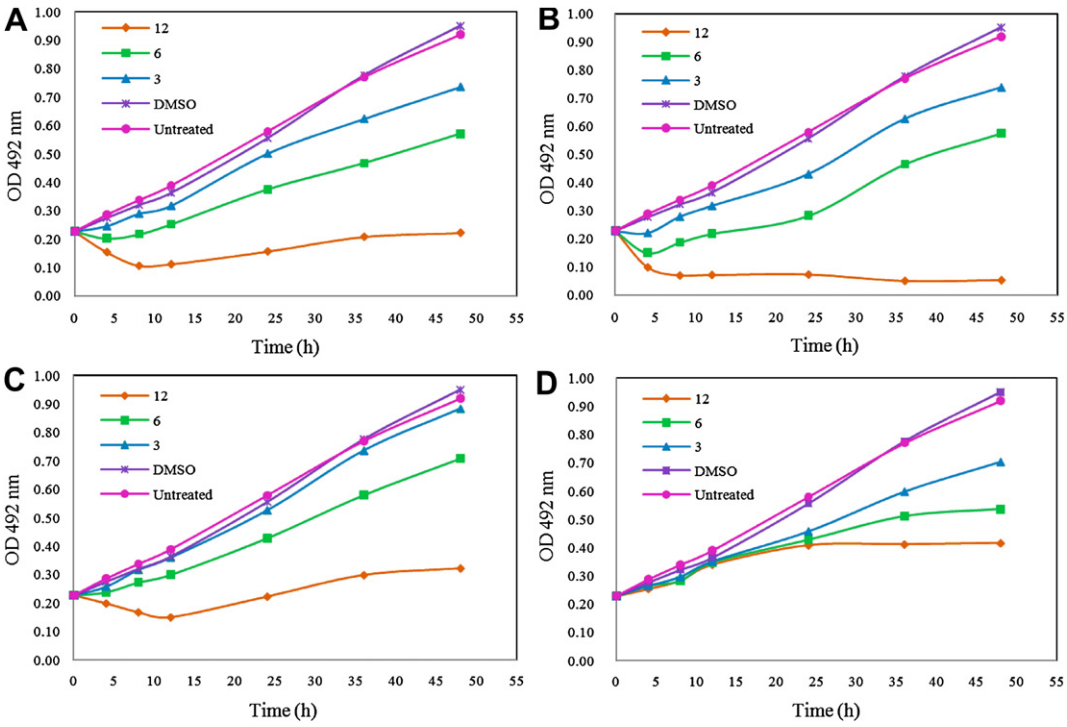


Fig. 2. Cytotoxic effects of **5i** (A), **5k** (B), **5s** (C) and 5-Fluorouracil (D) on A549 cells. Adherent cells proliferated in 96-well plates (7.5×10^3 cells/well) were incubated with a serial of concentrations (12.0, 6.0 and 3.0 μM) and determined by MTT assay at various time intervals.

analysis by a flow cytometry. Three independent experiments were performed, and data from one representative experiment were shown in Fig. 3 and Table 2.

4. Results and discussion

To search for more efficient anticancer agents and to investigate the contribution of dithiocarbamate moiety in target molecules to the cytotoxic activity, alkyl, substituted alkyl, alkenyl and alkynyl groups were chosen to serve as R substituents for compounds **5a**–**k**. Meanwhile, different benzyl groups were incorporated in compounds **5l**–**w** to examine the electronic or steric effect of substituents attached to the benzyl ring on the cytotoxic activity.

Of the compounds listed in Table 1, **5k** containing propargyl group exhibited inhibitory activity against proliferation of all five cell lines with IC_{50} values in the range of 5.44–12.16 μM, whereas **5j** containing allyl group only inhibited proliferation of two cell lines (A549 and MCF-7) with IC_{50} values of 20.66 and 29.80, respectively. Among the compounds **5a**–**g** in which R is a different alkyl group, five compounds (**5a** and **5c**–**f**) showed moderate activity against MCF-7 cells only. Introduction of cyano and phenyl or cyclohexyl groups enhanced the inhibitory activity, in which **5h** had IC_{50} values of 12.78, 13.22 and 10.35 μM against A549, MCF-7 and HT29 cells, and **5i** 5.49 and 3.65 μM against A549 and MCF-7, respectively. These results indicated that the R substituents containing electron-rich groups, such as allyl, propargyl or phenyl, were favorable to the cytotoxic activity.

Unlike lead compound **1a** in which 4(3*H*)-quinazolinone moiety served as ester component, its counterpart **5l** was inactive against all five cell lines. Introduction of a halogen atom in the position 4 of benzyl group in **5l** led to a substantial increase of cytotoxic activity, and fluorine atom was more favorable than chlorine or bromine atom. Thus, in the resulting compounds, **5n** showed lower IC_{50} values against five cell lines than those of **5q** or **5r**. However, introduction of the second or more fluorine atoms could not further enhance the activity (**5o** and **5p**), in comparison to compound **5n**. Interestingly, either electron-donating group (methyl in **5m**) or electron-withdrawing group (nitro in **5s**, carboxyl in **5u** or ethyloxycarbonyl in **5v**) in the position 4 of benzyl group was favorable to the cytotoxic activity, with the exception of cyano group retaining the activity of **5t** with the unsubstituted compound **5l**. These results suggested that the electronic effect of substituents attached to the benzyl ring was not a critical determinant of activity, while the steric effect would play a role in generating cytotoxicity for this type of compounds. Consequently, by comparing **5l** with **5m**, **5s** with **5t**, and **5u** with **5v**, it could be found that the bulky groups at the 4-position of the benzene rings were more beneficial to the cytotoxicity over the small ones.

It also can be seen from Table 1 that the cytotoxic activity of this type of compounds against A549 and MCF-7 cells was in general more potent than that against HeLa, HT29 and HCT-116 cells. Among them, compound **5i** was the most effective with an IC_{50} value of 3.65 μM against MCF-7 cells, while **5n** was the most effective with an IC_{50} value of 5.09 μM against A549 cells. It is worth noting that IC_{50} values of both **5n** and **5i** against A549 and MCF-7 cells were lower than those of the positive control 5-Fluorouracil. Moreover, the active compounds (**5k**, **5n**, **5p**–**s** and **5v**) against HeLa cells were more potent than 5-Fluorouracil (IC_{50}, 98.54 μM), the most effective compound **5s** had an IC_{50} value of 8.54 μM. However, the active compounds (**5k**, **5m**–**s** and **5v**) against HT29 and HCT-116 cells were less potent than 5-Fluorouracil which possessed the IC_{50} values lower than 10 μM. In addition, these active compounds exhibited cytotoxicity against A549, MCF-7 and HeLa cell lines similar to compound **1b**, but were less potent than **1b** against HT29 and HCT-116 cells.

The growth curves indicated that all three representative compounds **5i**, **5k** and **5s** can effectively suppress cell proliferation in a dose-dependent manner (Fig. 2). Among the three tested compounds, **5k** was the most effective, which inhibited almost 100% of cell proliferation at 12 μM within 8 h of treatment, about

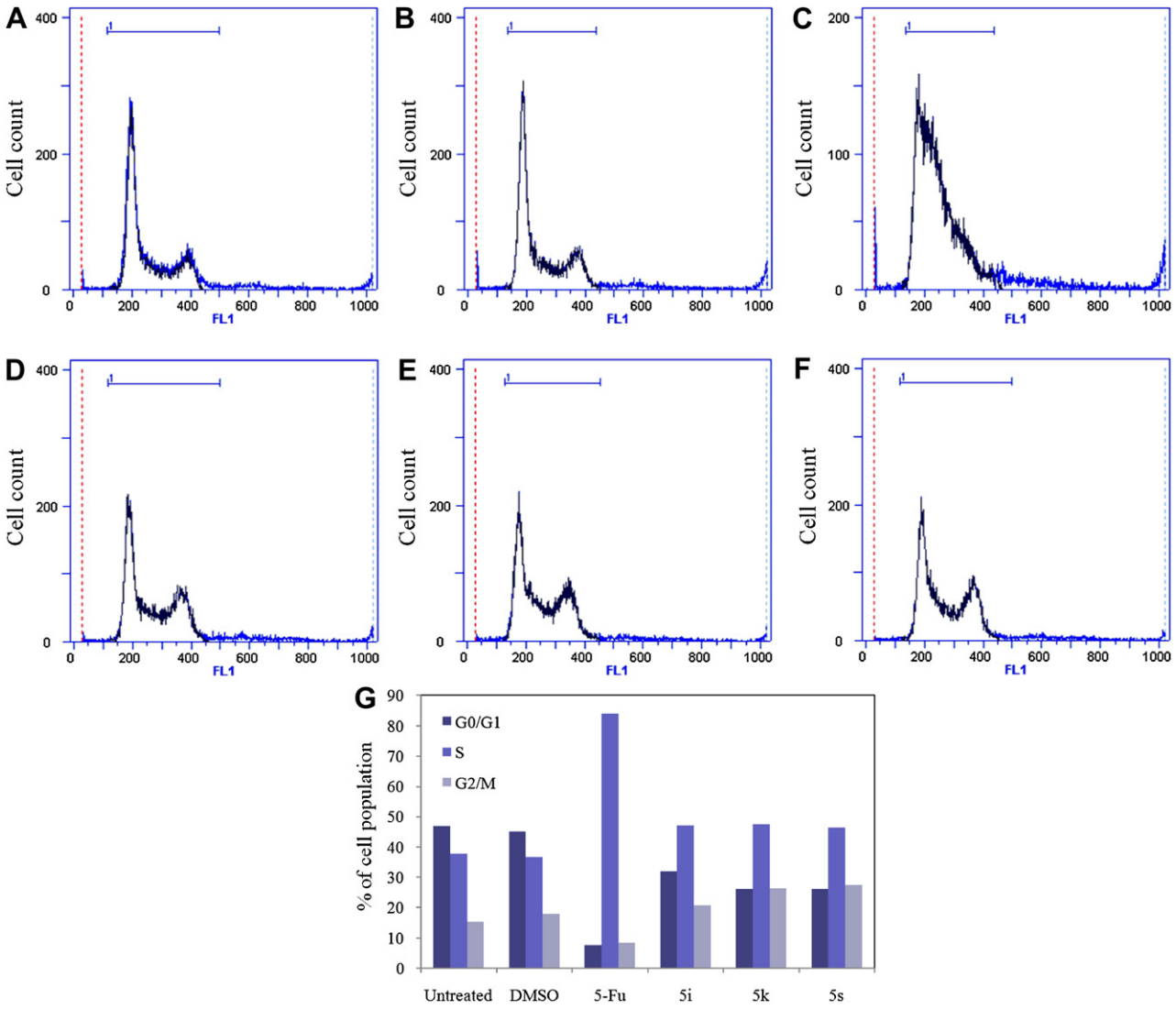


Fig. 3. Effects of compounds **5i**, **5k** and **5s** on cell cycle distribution. A549 cells were treated with **5i** (D), **5k** (E), **5s** (F), or 5-Fluorouracil (C) at a concentration equivalent to its IC_{50} for 24 h.

40% at 6 μM, and 25% at 3 μM after 48 h of treatment. Moreover, as compared with the effect of 5-Fluorouracil at the concentration of 12 μM, **5k** showed more rapid and efficient inhibitory activity against A549 cells. Particularly, **5k** at a final concentration of 12 μM was killing almost 100% of cells within 8 h of treatment, whereas 5-Fluorouracil at the same concentration only about 12% within the same duration, and even after 48 h treatment 5-Fluorouracil merely killed 55% cells. These results were also consistent with IC_{50} values we determined in this study.

5-Fluorouracil is a well known inhibitor of thymidylate synthase, thus blocks DNA replication. As expected, treatment of A549 cells with 5-Fluorouracil almost completely arrested the cell cycle at S phase. Treatment of A549 cells with **5i**, **5k**, or **5s** led to a moderate arrest at both S phase and G2/M phase (Fig. 3 and Table 2). Our results suggest that these new compounds may target both at DNA replication and mitosis progression. Further investigation of their mechanisms is warranted.

Table 2
Effects of compounds **5i**, **5k** and **5s** on A549 cell cycle progression.

Compound	Cell cycle distribution (%)		
	G0/G1	S	G2/M
Untreated	46.9	37.6	15.3
DMSO	45.3	36.8	17.9
5-Fluorouracil	7.5	84.2	8.4
5i	32.1	47.2	20.7
5k	26.1	47.7	26.3
5s	26.1	46.5	27.4

5. Conclusion

A number of dithiocarbamates in which 4(3*H*)-quinazolinone moiety served as amino component were synthesized and evaluated for their cytotoxic activity against five human cancer cell lines. Our results indicated that compound **5k** inhibited the growth of five cell lines with lower IC_{50} values than those of lead compound **1a** for each cell line. Compound **5i** and **5n** selectively inhibited proliferation of MCF-7 or A549 cells with IC_{50} values of 3.65 μM and 5.09 μM, respectively, and both of them were more potent than lead compound **1a** and the positive control 5-Fluorouracil. These results suggest that the structurally reversed modification of lead compound **1a** is a fairly efficient approach to enhance its cytotoxic activity. Cell cycle profiling suggests that these compounds target the cell cycle at both DNA replication and mitosis progression.

6. Experimental

Melting points were determined on an X-6 microscopic melting point apparatus and are uncorrected. IR spectra were recorded on a Bruker Tensor 27 spectrometer. ^{1}H NMR spectra were recorded on a Bruker AC-200P spectrometer at 200 MHz using tetramethylsilane (TMS) as internal standard. Low-resolution electrospray ionization (ESI) mass spectra were recorded on a Bruker Daltonics Esquire-LC 00136 mass spectrometer, and high-resolution electrospray ionization (HR-ESI) mass spectra were recorded on an Agilent LC/SMD TOF mass spectrometer. Elemental analyses were performed by Institute of Chemistry, Chinese Academy of Science, on a Flash EA 1112 elemental analyzer. Column chromatography was carried out on silica gel (200–300 mesh). 6-Bromomethyl-2-methyl-4(3*H*)-quinazolinone (**2**) was prepared according to the reported method [15], and other commercially available reagents were used without further purification. Compounds **5a** and **5d** were prepared with iodomethane and 2-iodopropane respectively, while the other compounds were prepared with the corresponding bromohydrocarbons.

Human breast cancer cell line MCF-7, human lung cancer cell line A549, human cervical carcinoma cell cline HeLa and human colon carcinoma cell lines HT29 and HCT-116 were kindly provided by Laboratory of Cancer Biology, Capital Normal University. All cancer cells were grown in high glucose DMEM supplemented with 10% fetal bovine serum, 2 mM glutamine, 50 units/mL penicillin, and 50 mg/mL streptomycin. Cell proliferation assay was determined using the CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay (Promega).

6.1. Synthesis of 6-(aminomethyl)-2-methyl-4(3H)-quinazolinone dihydrochloride (*4*) through Gabriel method

6.1.1. Preparation of 2-((2-methyl-4(3H)-quinazolinon-6-yl) methyl)isoindoline-1,3-dione (*3*)

A solution of 6-(bromomethyl)-2-methyl-4(3*H*)-quinazolinone (**2**) (2.20 g, 8.7 mmol) and potassium phthalimide (2.09 g, 11.3 mmol) in *N*,*N*-dimethylformamide (DMF) (30 mL) was refluxed for 8 h. After stirring at room temperature overnight, the reaction mixture was poured into water (280 mL). The precipitate formed was collected by filtration and recrystallized from DMF/H_2O (3:2) to yield 1.93 g (70%) as a white solid, mp 297–299 °C. IR (KBr, cm^{-1}) ν: 3165, 3026, 2948, 2871, 1707, 1679, 1617, 1426, 1395, 1097, 948, 845, 752, 712. ^{1}H NMR ($CDCl_3$) δ: 2.57 (s, 3H, C_2–CH_3), 4.97 (s, 2H, NCH_2), 7.63 (d, 1H, $J = 8.4$ Hz, quinazolinone 8-H), 7.70–7.89 (m, 5H, Ar–H), 8.29 (s, 1H, quinazolinone 5-H), 11.76 (br s, 1H, NH). ESI-MS *m*/*z*: 342 $[M + Na]^+$. Anal. calcd for $C_{18}H_{13}N_3O_3 \cdot 1/5H_2O$: C, 66.95; H, 4.18; N, 13.01. Found: C, 67.17; H, 4.21; N, 13.23.

6.1.2. Preparation of 6-(aminomethyl)-2-methyl-4(3H)-quinazolinone dihydrochloride (*4*)

To a heated solution of 2-((2-methyl-4(3*H*)-quinazolinon-6-yl) methyl)isoindoline-1,3-dione (**3**) (1.65 g, 5.2 mmol) in ethanol (75 mL), 80% hydrazine hydrate (0.48 g, 7.8 mmol) and ethanol (8 mL) were added and the mixture was refluxed for 5 h. The solvents were evaporated under reduced pressure to dryness and the residue dissolved in 2 mol/L HCl (72 mL). The mixture was heated for 2 h at 60 °C, and then for an additional hour at 90 °C, followed by addition of water (28 mL). After cooling to room temperature, the precipitate was filtered off and the filtrate was evaporated under reduced pressure to dryness. The crude product was purified by recrystallization from acetone/water (5:2) to yield 1.02 g (75%) as a white solid, mp > 320 °C. IR (KBr, cm^{-1}) ν: 3424, 2903, 2696, 1718, 1652, 1596, 1567, 1499, 1302, 1095, 895, 782. ^{1}H NMR (DMSO-d_6) δ: 2.61 (s, 3H, C_2–CH_3), 4.21 (s, 2H, CH_2N), 7.83 (d, 1H, $J = 8.4$ Hz, quinazolinone 8-H), 8.05 (d, 1H, $J = 8.4$ Hz, quinazolinone 7-H), 8.31 (s, 1H, quinazolinone 5-H). ESI-MS *m*/*z*: 190 $[M\text{-}2HCl + H]^+$. Anal. calcd for $C_{10}H_{13}Cl_2N_3O \cdot 0.3H_2O$: C, 44.89; H, 5.12; N, 15.71. Found: C, 44.82; H, 4.98; N, 15.72.

6.2. Procedure for the preparation of 6-(aminomethyl)-2-methyl-4(3H)-quinazolinone dihydrochloride (*4*) through Delepine method

A mixture of 6-(bromomethyl)-2-methyl-4(3*H*)-quinazolinone (**2**) (1.20 g, 4.6 mmol) and hexamethylenetetramine (0.66 g, 4.6 mmol) in trichloromethane (83 mL) was refluxed for 4 h. After cooling to room temperature, the separated solid was collected by filtration and dried in air. The solid was dissolved in methanol (83 mL) under reflux, and then concentrated hydrochloric acid (6 mL) was added. The mixture was refluxed for 2 h, and then cooled to room temperature. The precipitate was collected by filtration and purified by recrystallization from acetone/water (5:2) to yield 0.85 g (71%) as a white solid, mp > 320 °C. All the spectroscopic data were in accordance with those of compound **4** obtained through Gabriel method.

6.3. General procedure for the preparation of compounds *5a*–*w*

To a solution of 6-(aminomethyl)-2-methyl-4(3*H*)-quinazolinone dihydrochloride (**4**) (0.52 g, 2.0 mmol) in DMF (25 mL), finely powered anhydrous potassium phosphate (0.51 g, 2.4 mmol) and carbon disulfide (0.8 mL, 13.3 mmol) were added. After stirring for 1.5 h at room temperature, halohydrocarbon (3 mmol) was added and the stirring was continued till completion of the reaction (monitored by TLC). The reaction mixture was poured into water (150 mL), and the crude product was obtained by filtration if there was solid separated off. Otherwise, the solution was extracted with dichloromethane (20 mL × 3) and the combined organic phases were dried over anhydrous sodium sulfate, evaporated under reduced pressure to dryness. The crude product was purified by column chromatography (CC) on silica gel or recrystallization from appropriate solvent to give compounds **5a**–**w**.

6.3.1. Methyl N-((2-methyl-4(3H)-quinazolinon-6-yl)methyl) dithiocarbamate (*5a*)

Yield 40%, white solid, mp 224–225 °C (CC, eluent: dichloromethane/methanol = 97:3). IR (KBr, cm^{-1}) ν: 3163, 3029, 2817, 1719, 1655, 1568, 1509, 1421, 1395, 1304, 1096, 950, 888. ^{1}H NMR (DMSO-d_6) δ: 2.56 (s, 3H, CH_3), 2.61 (s, 3H, CH_3), 4.98 (s, 2H, CH_2NH), 7.75 (d, 1H, $J = 8.4$ Hz, quinazolinone 8-H), 7.89 (d, 1H, $J = 8.4$ Hz, quinazolinone 7-H), 8.04 (s, 1H, quinazolinone 5-H). ESI-MS *m*/*z*: 280 $[M + H]^+$. ESI-HRMS *m*/*z*: calcd for $C_{12}H_{14}N_3OS_2$ ($[M + H]^+$): 280.0578; found: 280.0564.

6.3.2. Ethyl N-((2-methyl-4(3H)-quinazolinon-6-yl)methyl) dithiocarbamate (*5b*)

Yield 42%, off-white solid, mp 215–217 °C (CC, eluent: dichloromethane/methanol = 97:3). IR (KBr, cm^{-1}) ν: 3241, 3020, 2924, 1658, 1620, 1488, 1384, 1097, 805, 837. ^{1}H NMR (DMSO-d_6) δ: 1.24 (t, 3H, $J = 7.3$ Hz, CH_2CH_3), 2.34 (s, 3H, C_2–CH_3), 3.18 (q, 2H, $J = 7.3$ Hz, CH_2CH_3), 4.94 (s, 2H, CH_2NH), 7.54 (d, 1H, $J = 8.3$ Hz, quinazolinone 8-H), 7.68 (d, 1H, $J = 8.3$ Hz, quinazolinone 7-H), 7.97 (s, 1H, quinazolinone 5-H). ESI-MS *m*/*z*: 294 $[M + H]^+$. Anal. calcd for $C_{13}H_{15}N_3OS_2$: C, 53.22; H, 5.15; N, 14.32. Found: C, 53.22; H, 5.34; N, 13.98.

6.3.3. Propyl N-((2-methyl-4(3H)-quinazolinon-6-yl)methyl) dithiocarbamate (*5c*)

Yield 36%, yellowish solid, mp 200–202 °C (CC, eluent: dichloromethane/methanol = 9:1). IR (KBr, cm^{-1}) ν: 3204, 2960,

2923, 1678, 1619, 1488, 1376, 1309, 940. ^{1}H NMR ($CDCl_3$) δ: 1.02 (t, 3H, J = 7.2 Hz, CH_2CH_3), 1.76 (m, 2H, $CH_2CH_2CH_3$), 2.56 (s, 2H, C_2–CH_3), 3.30 (t, 2H, J = 6.8 Hz, SCH_2CH_2), 5.03 (s, 2H, CH_2NH), 7.51 (d, 1H, J = 8.3 Hz, quinazolinone 8-H), 7.68 (d, 1H, J = 8.3 Hz, quinazolinone 7-H), 8.09 (s, 1H, quinazolinone 5-H). ESI-MS m/z: 308 $[M+H]^+$. Anal. calcd for $C_{14}H_{17}N_3OS_2$: C, 54.69; H, 5.57; N, 13.67. Found: C, 54.65; H, 5.71; N, 13.68.

6.3.4. Isopropyl N-((2-methyl-4(3H)-quinazolinon-6-yl)methyl) dithiocarbamate (**5d**)

Yield 55%, white solid, mp 193.0–193.8 °C (CC, eluent: dichloromethane/methanol = 9:1). IR (KBr, cm^{-1}) ν: 3240, 3156, 2957, 1652, 1618, 1487, 1383, 1320, 1092, 946, 852. ^{1}H NMR (DMSO-d_6) δ: 1.32 (d, 6H, J = 6.8 Hz, $CH(CH_3)_2$), 2.33 (s, 3H, C_2–CH_3), 3.90 (m, 1H, SCH), 4.92 (s, 2H, CH_2NH), 7.53 (d, 1H, J = 8.4 Hz, quinazolinone 8-H), 7.67 (d, 1H, J = 8.4 Hz, quinazolinone 7-H), 7.95 (s, 1H, quinazolinone 5-H). ESI-MS m/z: 308 $[M+H]^+$. Anal. calcd for $C_{14}H_{17}N_3OS_2$: C, 54.69; H, 5.57; N, 13.67. Found: C, 54.76; H, 5.66; N, 13.71.

6.3.5. Butyl N-((2-methyl-4(3H)-quinazolinon-6-yl)methyl) dithiocarbamate (**5e**)

Yield 34%, yellow solid, mp 198–199 °C (from DMF/H_2O = 1:1.2). IR (KBr, cm^{-1}) ν: 3211, 3003, 2957, 2927, 1679, 1620, 1488, 1376, 1308, 1080, 939, 830. ^{1}H NMR (DMSO-d_6) δ: 0.88 (t, 3H, J = 7.2 Hz, CH_2CH_3), 1.36 (m, 2H, $CH_2CH_2CH_3$), 1.59 (m, 2H, $CH_2CH_2CH_3$), 2.34 (s, 3H, C_2–CH_3), 3.20 (t, 2H, J = 7.2 Hz, SCH_2CH_2), 4.94 (s, 2H, CH_2NH), 7.54 (d, 1H, J = 8.4 Hz, quinazolinone 8-H), 7.68 (d, 1H, J = 8.4 Hz, quinazolinone 7-H), 7.96 (s, 1H, quinazolinone 5-H). ESI-MS m/z: 322 $[M+H]^+$. Anal. calcd for $C_{15}H_{19}N_3OS_2$: C, 56.04; H, 5.96; N, 13.07. Found: C, 55.91; H, 5.97; N, 13.07.

6.3.6. Pentyl N-((2-methyl-4(3H)-quinazolinon-6-yl)methyl) dithiocarbamate (**5f**)

Yield 64%, white solid, mp 180.5–182.1 °C (CC, eluent: dichloromethane/methanol = 96:4). IR (KBr, cm^{-1}) ν: 3216, 2921, 1679, 1621, 1526, 1486, 1375, 1308, 1074, 938, 849. ^{1}H NMR (DMSO-d_6) δ: 0.86 (t, 3H, J = 6.6 Hz, CH_2CH_3), 1.30 (m, 4H, $CH_2CH_2CH_3$), 1.60 (m, 2H, $SCH_2CH_2CH_2$), 2.34 (s, 3H C_2–CH_3), 3.19 (t, 2H, J = 7.3 Hz, SCH_2CH_2), 4.94 (s, 2H, CH_2NH), 7.54 (d, 1H, J = 8.4 Hz, quinazolinone 8-H), 7.69 (dd, 1H, J = 8.4 and 2.0 Hz, quinazolinone 7-H), 7.96 (d, 1H, J = 2.0 Hz, quinazolinone 5-H). ESI-MS m/z: 336 $[M+H]^+$. Anal. calcd for $C_{16}H_{21}N_3OS_2$: C, 57.28; H, 6.31; N, 12.53. Found: C, 57.09; H, 6.40; N, 12.32.

6.3.7. Hexyl N-((2-methyl-4(3H)-quinazolinon-6-yl)methyl) dithiocarbamate (**5g**)

Yield 67%, yellow solid, mp 195–197 °C (from DMF/H_2O = 0.8:1). IR (KBr, cm^{-1}) ν: 3216, 2955, 2926, 2854, 1680, 1621, 1487, 1376, 1309, 1075, 939, 830. ^{1}H NMR (DMSO-d_6) δ: 0.84 (t, 3H, J = 6.6 Hz, CH_2CH_3), 1.27 (m, 6H, $CH_2 \times 3$), 1.58 (m, 2H, CH_2), 2.33 (s, 3H, C_2–CH_3), 3.18 (t, 2H, J = 6.1 Hz, SCH_2CH_2), 4.92 (s, 2H, CH_2NH), 7.53 (d, 1H, J = 8.4 Hz, quinazolinone 8-H), 7.67 (d, 1H, J = 8.4 Hz, quinazolinone 7-H), 7.95 (s, 1H, quinazolinone 5-H). ESI-MS m/z: 350 $[M+H]^+$. Anal. calcd for $C_{17}H_{23}N_3OS_2$: C, 58.42; H, 6.63; N, 12.02. Found: C, 58.34; H, 6.62; N, 12.08.

6.3.8. Cyclohexylmethyl N-((2-methyl-4(3H)-quinazolinon-6-yl) methyl)dithiocarbamate (**5h**)

Yield 65%, white solid, mp 191.4–192.1 °C (CC, eluent: dichloromethane/methanol = 95:5). IR (KBr, cm^{-1}) ν: 3445, 3227, 2921, 2851, 1661, 1617, 1485, 1371, 1322, 1099, 945, 854. ^{1}H NMR (DMSO-d_6) δ: 1.10 (m, 5H, cyclohexane-H), 1.72 (m, 6H, cyclohexane-H), 2.35 (s, 3H, C_2–CH_3), 3.13 (d, 2H, J = 6.7 Hz, SCH_2), 4.94 (s, 2H, CH_2NH), 7.55 (d, 1H, J = 8.3 Hz, quinazolinone 8-H), 7.69 (d, 1H, J = 8.3 Hz, quinazolinone 7-H), 7.96 (s, 1H, quinazolinone 5-H). ESI-MS m/z: 362 $[M+H]^+$. Anal. calcd for $C_{18}H_{23}N_3OS_2$: C, 59.80; H, 6.41; N, 11.62. Found: C, 59.57; H, 6.37; N, 11.61.

6.3.9. 3-Cyano-3,3-diphenylpropyl N-((2-methyl-4(3H)-quinazolinon-6-yl)methyl)dithiocarbamate (**5i**)

Yield 55%, white solid, mp 165.5–167.4 °C (CC, eluent: dichloromethane/methanol = 96:4). IR (KBr, cm^{-1}) ν: 3442, 3168, 2932, 1656, 1620, 1490, 1384, 1313, 1086, 933, 697. ^{1}H NMR (DMSO-d_6) δ: 2.33 (s, 3H, C_2–CH_3), 2.84 (m, 2H, CH_2), 3.17 (m, 2H, CH_2), 4.91 (s, 2H, CH_2NH), 7.39 (m, 10H, Ph–H), 7.53 (d, 1H, J = 8.4 Hz, quinazolinone 8-H), 7.67 (d, 1H, J = 8.4 Hz, quinazolinone 7-H), 7.95 (s, 1H, quinazolinone 5-H). ESI-MS m/z: 485 $[M+H]^+$. Anal. calcd for $C_{27}H_{24}N_4OS_2 \cdot 1/2H_2O$: C, 65.69; H, 5.10; N, 11.35. Found: C, 65.74; H, 5.04; N, 11.39.

6.3.10. Allyl N-((2-methyl-4(3H)-quinazolinon-6-yl)methyl) dithiocarbamate (**5j**)

Yield 53%, white solid, mp 189.4–189.9 °C (CC, eluent: dichloromethane/methanol = 96:4). IR (KBr, cm^{-1}) ν: 3425, 1680, 1618, 1488, 1384, 1314, 1090, 939, 833. ^{1}H NMR (DMSO-d_6) δ: 2.35 (s, 3H, C_2–CH_3), 3.92 (d, 2H, J = 6.8 Hz, SCH_2CH), 4.94 (s, 2H, CH_2NH), 5.12 (d, 1H, J = 9.9 Hz, $=CH_2$), 5.29 (d, 1H, J = 16.9 Hz, $=CH_2$), 5.84 (m, 1H, $CH=CH_2$), 7.55 (d, 1H, J = 8.4 Hz, quinazolinone 8-H), 7.69 (d, 1H, J = 8.4 Hz, quinazolinone 7-H), 7.97 (s, 1H, quinazolinone 5-H). ESI-MS m/z: 306 $[M+H]^+$. Anal. calcd for $C_{14}H_{15}N_3OS_2 \cdot 1/2H_2O$: C, 53.48; H, 5.13; N, 13.36. Found: C, 53.42; H, 5.14; N, 13.29.

6.3.11. Prop-2-ynyl N-((2-methyl-4(3H)-quinazolinon-6-yl)methyl) dithiocarbamate (**5k**)

Yield 79%, white solid, mp 188.7–189.7 °C (CC, eluent: dichloromethane/methanol = 95:5). IR (KBr, cm^{-1}) ν: 3443, 3294, 3240, 2922, 1660, 1619, 1487, 1384, 1093, 944, 854. ^{1}H NMR (DMSO-d_6) δ: 2.35 (s, 3H, C_2–CH_3), 3.17 (t, 2H, J = 2.6 Hz, C≡CH), 4.09 (d, 2H, J = 2.6 Hz, $CH_2C\equiv$), 4.95 (s, 2H, CH_2NH), 7.56 (d, 1H, J = 8.4 Hz, quinazolinone 8-H), 7.71 (d, 1H, J = 8.4 Hz, quinazolinone 7-H), 7.99 (s, 1H, quinazolinone 5-H). ESI-MS m/z: 304 $[M+H]^+$. Anal. calcd for $C_{14}H_{13}N_3OS_2$: C, 55.42; H, 4.32; N, 13.85. Found: C, 55.02; H, 4.38; N, 13.59.

6.3.12. Benzyl N-((2-methyl-4(3H)-quinazolinon-6-yl)methyl) dithiocarbamate (**5l**)

Yield 32%, white solid, mp 186.6–188 °C (CC, eluent: dichloromethane/methanol = 95:5). IR (KBr, cm^{-1}) ν: 3441, 3229, 3024, 2925, 1661, 1618, 1489, 1383, 1322, 1088, 946, 854, 702. ^{1}H NMR (DMSO-d_6) δ: 2.35 (s, 3H, C_2–CH_3), 4.53 (s, 2H, SCH_2), 4.96 (s, 2H, CH_2NH), 7.29 (m, 5H, Ph–H), 7.55 (d, 1H, J = 8.3 Hz, quinazolinone 8-H), 7.70 (d, 1H, J = 8.3 Hz, quinazolinone 7-H), 7.99 (s, 1H, quinazolinone 5-H). ESI-MS m/z: 356 $[M+H]^+$. Anal. calcd for $C_{18}H_{17}N_3OS_2 \cdot H_2O$: C, 57.88; H, 5.13; N, 11.25. Found: C, 58.09; H, 4.87; N, 10.90.

6.3.13. 4-Methylbenzyl N-((2-methyl-4(3H)-quinazolinon-6-yl) methyl)dithiocarbamate (**5m**)

Yield 72%, white solid, mp 199.2–200.2 °C (CC, eluent: dichloromethane/methanol = 95:5). IR (KBr, cm^{-1}) ν: 3449, 3234, 3022, 2927, 1654, 1618, 1487, 1317, 1090, 955, 853. ^{1}H NMR (DMSO-d_6) δ: 2.27 (s, 3H, Ph–CH_3), 2.35 (s, 3H, C_2–CH_3), 4.47 (s, 2H, SCH_2), 4.95 (s, 2H, CH_2NH), 7.12 (d, 1H, J = 7.9 Hz, Ph–H), 7.26 (d, 1H, J = 7.9 Hz, Ph–H), 7.55 (d, 1H, J = 8.4 Hz, quinazolinone 8-H), 7.69 (dd, 1H, J = 8.4, 2.0 Hz, quinazolinone 7-H), 7.98 (d, 1H, J = 2.0 Hz, quinazolinone 5-H). ESI-MS m/z: 370 $[M+H]^+$. Anal. calcd for $C_{19}H_{19}N_3OS_2 \cdot 1/2CH_3OH$: C, 60.75; H, 5.49; N, 10.90. Found: C, 60.71; H, 5.14; N, 10.82.

6.3.14. 4-Fluorobenzyl N-((2-methyl-4(3H)-quinazolinon-6-yl) methyl)dithiocarbamate (**5n**)

Yield 78%, white solid, mp 198.7–199.2 °C (CC, eluent: dichloromethane/methanol = 95:5). IR (KBr, cm^{-1}) ν: 3442, 3228,

3022, 2958, 1663, 1621, 1508, 1384, 1227, 1085, 947, 842 ^{1}H NMR (DMSO-d_6) δ: 2.35 (s, 3H, C_2—CH_3), 4.53 (s, 2H, SCH_2), 4.95 (s, 2H, CH_2NH), 7.14 (m, 2H, Ph—H), 7.42 (m, 2H, Ph—H), 7.55 (d, 1H, J = 8.4 Hz, quinazolinone 8-H), 7.69 (d, 1H, J = 8.4 Hz, quinazolinone 7-H), 7.98 (s, 1H, quinazolinone 5-H). ESI-MS m/z: 374 $[M+H]^+$. Anal. calcd for $C_{18}H_{16}FN_3OS_2 \cdot 2/3H_2O$: C, 56.08; H, 4.53; N, 10.90. Found: C, 55.99; H, 4.38; N, 10.90.

*6.3.15. 2,4-Difluorobenzyl N-((2-methyl-4(3H)-quinazolinon-6-yl) methyl)dithiocarbamate (**5o**)*

Yield 63%, white solid, mp 180.9—182.0 °C (CC, eluent: dichloromethane/methanol = 95:5). IR (KBr, cm^{-1}) ν: 3422, 2925, 1657, 1618, 1503, 1384, 1088, 967, 847. ^{1}H NMR (DMSO-d_6) δ: 2.35 (s, 3H, C_2—CH_3), 4.52 (s, 2H, SCH_2), 4.94 (s, 2H, CH_2NH), 7.06 (m, 1H, Ph—H), 7.23 (m, 1H, Ph—H), 7.53 (s, 1H, Ph—H), 7.55 (d, 1H, J = 8.4 Hz, quinazolinone 8-H), 7.69 (d, 1H, J = 8.4 Hz, quinazolinone 7-H), 7.97 (s, 1H, quinazolinone 5-H). ESI-MS m/z: 392 $[M+H]^+$. Anal. calcd for $C_{18}H_{15}F_2N_3OS_2$: C, 55.23; H, 3.86; N, 10.73. Found: C, 55.30; H, 3.87; N, 10.55.

*6.3.16. 2,3,4,5,6-Pentafluorobenzyl N-((2-methyl-4(3H)-quinazolinon-6-yl)methyl)dithiocarbamate (**5p**)*

Yield 85%, white solid, mp 214.3—215.5 °C (CC, eluent: dichloromethane/methanol = 95:5). IR (KBr, cm^{-1}) ν: 3435, 3231, 2958, 1623, 1619, 1503, 1384, 1127, 988, 855. ^{1}H NMR (DMSO-d_6) δ: 2.34 (s, 3H, C_2—CH_3), 4.65 (s, 2H, SCH_2), 4.94 (s, 2H, CH_2NH), 7.55 (d, 1H, J = 8.4 Hz, quinazolinone 8-H), 7.69 (d, 1H, J = 8.4 Hz, quinazolinone 7-H), 7.95 (s, 1H, quinazolinone 5-H). ESI-MS m/z: 466 $[M+H]^+$. Anal. calcd for $C_{18}H_{12}F_5N_3OS_2 \cdot 1/2H_2O$: C, 47.57; H, 2.88; N, 9.25. Found: C, 47.83; H, 3.02; N, 9.03.

*6.3.17. 4-Chlorobenzyl N-((2-methyl-4(3H)-quinazolinon-6-yl) methyl)dithiocarbamate (**5q**)*

Yield 78%, white solid, mp 185.7—186.6 °C (CC, eluent: dichloromethane/methanol = 95:5). IR (KBr, cm^{-1}) ν: 3440, 3230, 2953, 1662, 1619, 1490, 1384, 1323, 1086, 946, 855. ^{1}H NMR (DMSO-d_6) δ: 2.35 (s, 3H, C_2—CH_3), 4.54 (s, 2H, SCH_2), 4.96 (s, 2H, CH_2NH), 7.40 (m, 4H, Ph—H), 7.55 (d, 1H, J = 8.3 Hz, quinazolinone 8-H), 7.69 (d, 1H, J = 8.3 Hz, quinazolinone 7-H), 7.98 (s, 1H, quinazolinone 5-H). ESI-MS m/z: 390 $[M+H]^+$. Anal. calcd for $C_{18}H_{16}ClN_3OS_2 \cdot H_2O$: C, 53.00; H, 4.45; N, 10.30. Found: C, 52.87; H, 4.19; N, 9.99.

*6.3.18. 4-Bromobenzyl N-((2-methyl-4(3H)-quinazolinon-6-yl) methyl)dithiocarbamate (**5r**)*

Yield 58%, white solid, mp 186.7—188.5 °C (CC, eluent: dichloromethane/methanol = 95:5). IR (KBr, cm^{-1}) ν: 3442, 3165, 2904, 1673, 1620, 1486, 1383, 1309, 1071, 933, 831. ^{1}H NMR (DMSO-d_6) δ: 2.35 (s, 3H, C_2—CH_3), 4.52 (s, 2H, SCH_2), 4.95 (s, 2H, CH_2NH), 7.36—7.66 (m, 6H, Ph—H, quinazolinone 8-H and 7-H), 7.97 (s, 1H, quinazolinone 5-H). ESI-MS m/z: 434 $[M+H]^+$. Anal. calcd for $C_{18}H_{16}BrN_3OS_2$: C, 49.77; H, 3.71; N, 9.67. Found: C, 50.03; H, 3.86; N, 9.60.

*6.3.19. 4-Nitrobenzyl N-((2-methyl-4(3H)-quinazolinon-6-yl) methyl)dithiocarbamate (**5s**)*

Yield 57%, white solid, mp 190—192 °C (CC, eluent: dichloromethane/methanol = 98:2). IR (KBr, cm^{-1}) ν: 3445, 3157, 2854, 1668, 1619, 1515, 1382, 1342, 1074, 932, 830. ^{1}H NMR (DMSO-d_6) δ: 2.33 (s, 3H, C_2—CH_3), 4.68 (s, 2H, SCH_2), 4.94 (s, 2H, CH_2NH), 7.53(d, 1H, J = 8.4 Hz, quinazolinone 8-H), 7.65 (m, 3H, quinazolinone 7-H and Ph—H), 7.95 (s, 1H, quinazolinone 5-H), 8.16 (d, 2H, J = 8.7 Hz, Ph—H). ESI-MS m/z: 401 $[M+H]^+$. Anal. calcd for $C_{18}H_{16}N_4O_3S_2 \cdot 1/2H_2O$: C, 52.80; H, 4.18; N, 13.68. Found: C, 52.92; H, 4.17; N, 13.44.

*6.3.20. 4-Cyanobenzyl N-((2-methyl-4(3H)-quinazolinon-6-yl) methyl)dithiocarbamate (**5t**)*

Yield 22%, white solid, mp 179.4—180.4 °C (CC, eluent: dichloromethane/methanol = 95:5). IR (KBr, cm^{-1}) ν: 3440, 3157, 2877, 2232, 1671, 1619, 1488, 1384, 1313, 1074, 933, 830 ^{1}H NMR (DMSO-d_6) δ: 2.34 (s, 3H, C_2—CH_3), 4.63 (s, 2H, SCH_2), 4.95 (s, 2H, CH_2NH), 7.56 (m, 3H, quinazolinone 8-H and Ph—H), 7.73 (m, 3H, quinazolinone 7-H and Ph—H), 7.96 (s, 1H, quinazolinone 5-H). ESI-MS m/z: 381 $[M+H]^+$. Anal. calcd for $C_{19}H_{16}N_4OS_2 \cdot 1/2H_2O$: C, 58.59; H, 4.40; N, 14.38. Found: C, 58.78; H, 4.37; N, 14.34.

*6.3.21. 4-Carboxybenzyl N-((2-methyl-4(3H)-quinazolinon-6-yl) methyl)dithiocarbamate (**5u**)*

Yield 49%, white solid, mp 221.9—223.5 °C (from dichloromethane/methanol = 3:1). IR (KBr, cm^{-1}) ν: 3429, 2924, 1680, 1617, 1493, 1384, 1315, 1088, 939, 834. ^{1}H NMR (DMSO-d_6) δ: 2.35 (s, 3H, C_2—CH_3), 4.62 (s, 2H, SCH_2), 4.96 (s, 2H, CH_2NH), 7.52 (m, 3H, quinazolinone 8-H and Ph—H), 7.69 (d, 1H, J = 8.4 Hz, quinazolinone 7-H), 7.89 (d, 2H, J = 8.2 Hz, Ph—H), 7.98 (s, 1H, quinazolinone 5-H). ESI-MS m/z: 400 $[M+H]^+$. Anal. calcd for $C_{19}H_{17}N_3O_3S_2 \cdot 1/2CH_2Cl_2$: C, 52.99; H, 4.11; N, 9.51. Found: C, 52.90; H, 4.32; N, 9.51.

*6.3.22. 4-Ethoxycarbonylbenzyl N-((2-methyl-4(3H)-quinazolinon-6-yl)methyl)dithiocarbamate (**5v**)*

Yield 48%, white solid, mp 202.9—203.2 °C (CC, eluent: dichloromethane/methanol = 97:3). IR (KBr, cm^{-1}) ν: 3450, 3244, 2985, 1717, 16593, 1616, 1383, 1279, 1102, 948, 853, 711. ^{1}H NMR (DMSO-d_6) δ: 1.31 (t, 3H, J = 7.1 Hz, CH_2CH_3), 2.35 (s, 3H, C_2—CH_3), 4.30 (q, 2H, J = 7.1 Hz, OCH_2CH_3), 4.62 (s, 2H, SCH_2), 4.95 (s, 2H, CH_2NH), 7.53 (m, 3H, quinazolinone 8-H and Ph—H), 7.68 (d, 1H, J = 8.4 Hz, quinazolinone 7-H), 7.90 (d, 2H, J = 8.2 Hz, Ph—H), 7.98 (s, 1H, quinazolinone 5-H). ESI-MS m/z: 428 $[M+H]^+$. Anal. calcd for $C_{21}H_{21}N_3O_3S_2$: C, 58.99; H, 4.95; N, 9.83. Found: C, 59.42; H, 4.93; N, 9.86.

*6.3.23. Benzhydryl N-((2-methyl-4(3H)-quinazolinon-6-yl)methyl) dithiocarbamate (**5w**)*

Yield 47%, white solid, mp 207.9—208.2 °C (CC, eluent: dichloromethane/methanol = 95:5). IR (KBr, cm^{-1}) ν: 3445, 2928, 1674, 1620, 1509, 1387, 1324, 934, 704. ^{1}H NMR (DMSO-d_6) δ: 2.33 (s, 3H, C_2—CH_3), 4.89 (s, 3H, CH_2NH), 5.41 (s, 1H, SCH), 7.31 (m, 10H, Ph—H), 7.51 (m, 2H, quinazolinone 8-H and 7-H), 7.93 (s, 1H, quinazolinone 5-H). ESI-MS m/z: 432 $[M+H]^+$. Anal. calcd for $C_{24}H_{21}N_3OS_2$: C, 66.79; H, 4.90; N, 9.74. Found: C, 66.64; H, 4.97; N, 9.78.

6.4. MTT assay

Cytotoxic activity of the synthesized compounds was determined by MTT assay on the following cell lines: A549, MCF-7, HeLa, HT29 and HCT-116. The tested compounds were prepared in DMSO as stock solutions and stored at −20 °C. After thawing, the stock solutions were freshly diluted to a series of concentrations in medium just prior to the assay. Final concentration of DMSO in medium was 0.2%.

Cells in logarithmic growth were plated in 96-well plate at following densities: 7.5×10^3 cells/well for A549, 5×10^3 cells/well for MCF-7, 1×10^4 cells/well for HeLa, 5×10^3 cells/well for HT29, and 5×10^3 cells/well for HCT-116. 24 h after seeding, 10 μL of 2% DMSO (vehicle control) or solution of the tested compounds was added into each well and incubated for 72 h, 20 μL of MTT solution was then added into each well and incubated for an additional 2 h, and absorbance at 492 nm was measured on a microplate reader. Cell proliferation inhibition was given by the expression:

$$\text{Cell proliferation inhibition (\%)} = \left[1 - \left(\text{OD}_{\text{cells+test compound}} - \text{OD}_{\text{blank}}\right) \Big/ \left(\text{OD}_{\text{cells+DMSO}} - \text{OD}_{\text{blank}}\right)\right] \times 100$$

where OD means the absorbance at 492 nm in the test ($OD_{cells + test\ compound}$), control ($OD_{cells + DMSO}$) or blank ($OD_{blank}$) wells. Each value was the means of three independent experiments. The concentration causing 50% proliferation inhibition (IC_{50}) was determined from the sigmoidal curve obtained by plotting percent cell proliferation inhibition versus concentration using SPSS 16.0 for Windows. 5-Fluorouracil, a positive control, was tested in the same way. Timepoint experiments using active compounds we synthesized and 5-Fluorouracil as a positive control were performed in A549 cells.

6.5. Cell cycle profiling

A549 cells were incubated with active compounds or 5-Fluorouracil for 24 h at 37 °C. Cells were harvested by trypsinization, washed with PBS once, and fixed in ethanol (75%) at 4 °C overnight. Fixed cells were pelleted by centrifugation, washed once with PBST, and resuspended in DAPI (1 mg/mL)-containing PBS. The resuspended cells were ready for cell cycle analysis by a flow cytometry (Cell Quanta SC, Beckman Coulter, USA).

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