Full Paper

Synthesis of Substituted Benzylamino- and Heterocyclylmethylamino Carbodithioate Derivatives of 4-(3*H*)-Quinazolinone and their Cytotoxic Activity

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A new series of substituted benzylamino- and heterocyclylmethylamino carbodithioate derivatives of 4-(3*H*)-quinazolinone were synthesized *via* four steps starting from 2-amino-5-methylbenzoic acid and initially screened against A-549 (human non-small cell lung cancer), HCT-8 (human colon cancer), and Bel-7402 (human liver cancer) cell lines at the single concentration of 5 μg/mL using the colorimetric MTT assay. The IC_{50} values were determined for the compounds reaching ≥70% inhibition in primary screening by serial dilution. Among the newly synthesized compounds, **9n** exhibited potent *in vitro* cytotoxicity against A-549, HCT-8, and Bel-7402 cell lines with the IC_{50} values of 1.65, 0.93, and 1.43 μM, respectively.

Keywords: 4-(3*H*)-Qquinazolinone / Dithiocarbamate / Cytotoxicity / MTT assay

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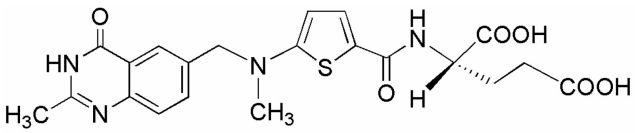
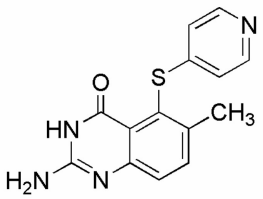
Introduction

Recently, classical antifolates such as raltitrexed **1** [1 – 3] (Fig. 1) and pemetrexed [4, 5], structurally analogous to natural folic acid, have been successfully used in cancer chemotherapy. However, many researchers are presently devoted to developing nonclassical antifolates in order to overcome the problems associated with classical antifolates [6 – 10]. One strategy commonly used is to replace the benzoyl L-glutamic acid moieties in classical antifolates with lipophilic side chains, the successful example being AG337 **2** [11 – 13] (Fig. 1) which is currently under clinical trials. In our research program on 4-(3*H*)-quinazolinone derivatives acting as antitumor agents, we have incorporated dithiocarbamate moieties with 4-(3*H*)-quinazolinone to synthesize a series of 4-(3*H*)-quinazolinone derivatives bearing various dithiocarbamate side chains.



1, Raltitrexed



2, AG337

Figure 1. Structures of Raltitrexed **1** and AG337 (**2**).

The *in vitro* evaluation of these compounds as inhibitors of the growth of K562 (human myelogenous leukaemia) cells showed that compound **3** (Fig. 2) containing a benzylamine moiety in the dithiocarbamate side chain was active with an IC_{50} value of 4.0 μM [14]. These results pro-

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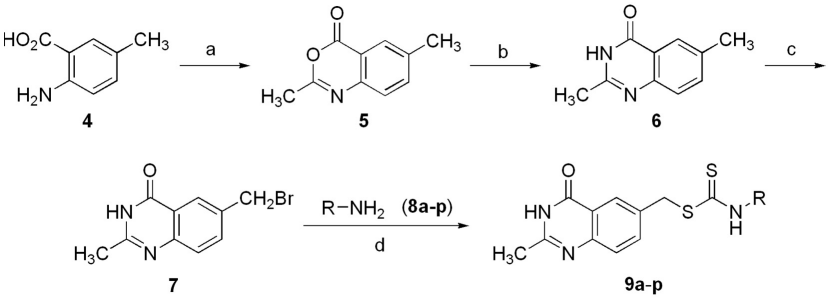
Table 1. *In vitro* cytotoxicity of compounds **9a–p** against A-549, HCT-8, and Bel-7402 cell lines.

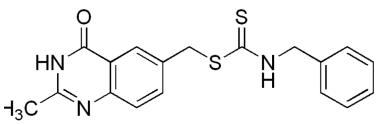
Compound	R	A-549		HCT-8		Bel-7402	
		% Inhibition at 5 μg/mL	IC_{50} (μM)	% Inhibition at 5 μg/mL	IC_{50} (μM)	% Inhibition at 5 μg/mL	IC_{50} (μM)
9a	$(Ph)_2CH$-	45.9	nt[a)]	53.0	nt	34.8	nt
9b	4-CH_3PhCH_2–	62.2	nt	88.5	6.60	86.9	2.81
9c	4-$(CH_3O)PhCH_2$–	71.5	2.83	82.7	>10	84.7	2.67
9d	2-$(CH_3O)PhCH_2$–	69.6	nt	75.9	>10	68.5[b)]	>10
9e	2,4-$(CH_3O)_2PhCH_2$–	−0.7	nt	22.3	nt	31.8	nt
9f	3,4,5-$(CH_3O)_3PhCH_2$–	7.5	nt	55.1	nt	35.9	nt
9g	3,4-methylenedioxy$PhCH_2$–	80.8	>10	88.4	>10	86.3	2.15
9h	4-F$PhCH_2$–	82.5	>10	89.6	>10	86.6	1.45
9i	2-F$PhCH_2$–	68.2	nt	88.5	>10	82.1	6.29
9j	4-Cl$PhCH_2$–	68.2	nt	87.1	>10	85.7	1.49
9k	2,4-Cl_2PhCH_2–	4.1	nt	11.1	nt	10.2	nt
9l	4-HOOC$PhCH_2$–	0	nt	11.9	nt	12.0	nt
9m	2-PyridinylCH_2–	−4.4	nt	−5. 5	nt	7.8	nt
9n	3-PyridinylCH_2–	81.8	1.65	88.6	0.93	86.6	1.43
9o	2-ThiophenylCH_2–	70.4	>10	88.6	>10	69.6[b)]	2.88
9p	2-TetrahydrofurylCH_2–	81.9	4.01	89.2	4.12	61.6[b)]	7.90
3	$PhCH_2$–	81.7	3.43	89.4	0.56	88.0	1.94
Raltitrexed **1**		71.0	0.044	78.4	0.20	74.7	0.13
5-Fluorouracil		76.7	1.77	76.3	2.61	84.4	2.61

[a)] nt – not tested.

[b)] For the Bel-7402 cell line, the IC_{50} values of the compounds effecting ≥60% inhibition at the concentration of 5 μg/mL were also tested.



Scheme 1. Synthetic route to compounds **9a–p**. a) Ac_2O, reflux 1.5 h; b) NH_3 / H_2O, rt, 48 h; c) *N*-bromosuccinimide, $(PhCO)_2O_2$, $CHCl_3$, reflux 3 h; d) CS_2, K_3PO_4, DMF, rt, 2 h.



3, IC_{50} = 4.0 μM for K562 cells

Figure 2. Structure of compound **3**.

moted us to prepare a new series of 4-(3*H*)-quinazolinone derivatives **9a–p** (Scheme 1 and Table 1), in which various substituted benzyl groups or heterocycles such as pyridine, thiophene or tetrahydrofuran were used to replace the benzyl group in compound **3**, to search for more effective antitumor agents acting as possible nonclassical antifolates and to investigate the structure-activity relationships of this type of compounds.

Results and discussion

Chemistry

The preparation of intermediate **6**, 2,6-dimethyl-4-(3*H*)-quinazolinone, by heating 2-amino-5-methylbenzoic acid **4** together with thioacetamide has already been reported [15]. Due to the high reaction temperature and being free of solvent, the product was frequently contaminated with a black resin-like substance and needed to be recrystallized repeatedly to obtain a pure product, usually leading to a lower yield. Therefore, an alternative method was utilized herein to overcome the above-mentioned problem. The starting compound **4** was cyclized with acetic anhydride to give 2,6-dimethylbenzoxazin-4-one **5**, which was converted into **6** in high yield by reaction with aqueous ammonia at room temperature. Bromina-

tion of **6** with *N*-bromosuccinimide (NBS) to yield 6-bromomethyl-2-methyl-4-(3*H*)-quinazolinone **7**, which was reacted with the mixture of carbon disulfide, anhydrous potassium phosphate, and various amines **8** according to a method described previously [14] to afford the desired compounds **9a–p**. The structures of the synthesized compounds were confirmed by ESI-MS, ^{1}H-MNR, and elemental analyses, and the data are given in Section 3, Experimental.

Assay for cytotoxic activities

The newly synthesized compounds **9a–p** were initially screened at the single concentration of 5 μg/mL using the colorimetric MTT (3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyl-tetrazolium bromide) assay to test their *in vitro* cytotoxicity against A-549 (human non-small cell lung cancer), HCT-8 (human colon cancer), and Bel-7402 (human liver cancer) cell lines. Compound **3**, the clinically used anticancer drug 5-fluorouracil, and raltitrexed **1**, which was synthesized according to our previously reported method [16], were used for comparative purposes. The cytotoxicity of tested compounds was estimated in terms of percent growth inhibition compared with untreated control cells, and the data are given in Table 1. Then, the compounds effecting ≥70% inhibition in one-dose prescreening were retested by serial dilution from 5 to 0.005 μg/mL. The results were expressed as IC_{50} (inhibitory concentration 50%), the concentration of the compound which inhibits the tumor cell growth by 50%, and are also reported in Table 1.

Based on the results assembled in Table 1, it can be seen that introduction of either the electron-donating groups (−CH_3, −OCH_3) or the electron-withdrawing groups (−F, −Cl, and −COOH) at the 4′-position of the phenyl ring did not cause the increase in cytotoxicity compared with the parent compound **3**. Moreover, the change of the substituent position from 4′ to 2′ (compare **9c**, **9d** and **9h**, **9i**) and the increase of the number of substituents (compare **9c**, **9e**, **9f** and **9j**, **9k**) led to the decrease in activity. Replacement of the phenyl group in compound **3** by 2-pyridinyl, 2-thiophenyl, and 2-tetrahydrofuryl, resulting in compounds **9m**, **9o**, and **9p**, also led to the decrease in activity. However, the cytotoxicity of compound **9n**, obtained by replacing of phenyl in compound **3** by 3-pyridinyl, was at least comparable to that of compound **3**. Although the IC_{50} values of compound **9n** were obviously higher than those of raltitrexed **1**, it exhibited a higher cytotoxicity than 5-fluorouracil against A-549, HCT-8, and Bel-7402 cell lines. Considering that **9n** can still be subjected to modification by converting it into its salts or quaternary ammonium salts, it is worthy of being investigated further.

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Experimental

Melting points were determined on a XT4A microscopic melting point apparatus (Keyi Electrooptical, Beijing, China) or a WRS-1B digital melting point apparatus (Precision Instruments, Shanghai, China) and are uncorrected. ^{1}H-NMR spectra were recorded on a Bruker AC-200P spectrometer at 200 MHz (Bruker, Zurich, Switzerland) or on a JNM-ECA300 spectrometer at 300 MHz (Jeol, Tokyo, Japan) using tetramethylsilane (TMS) as internal standard. Electrospray ionization (ESI) mass spectra were recorded on an Esquire-LC 00136 mass spectrometer (Bruker Daltonics, Bremen, Germany). Elemental analyses (C, H and N) were performed by the Institute of Chemistry, Chinese Academy of Science, on a Flash EA 1112 elemental analyzer (Thermo Electron, Waltham, MA, USA). Column chromatography was carried out on silica gel (200–300 mesh, Haiyang Chemical, Qingdao, China). 2-Amino-5-methylbenzoic acid **4** was prepared according to the reported method [15], and other reagents are commercial materials which were used without further purification.

Synthesis of the compounds

2,6-Dimethylbenzoxazin-4-one ***5***

A mixture of 1.5 g (0.01 mol) of 2-amino-5-methylbenzoic acid **4** and acetic anhydride 7.5 mL was heated at reflux under stirring for 1.5 h. After cooling in a refrigerator, the precipitate was collected by filtration and recrystallized from trichloromethane/cyclohexane (1/3) to give 1.6 g (92%) of **5** as white solid, m. p. 123.3–123.7°C; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 2.26 (s, 3H, CH_3), 2.48 (s, 3H, CH_3), 7.37 (dd, 1H, *J* = 8.6, 2.1 Hz, C_7–H), 7.92 (d, 1H, *J* = 2.1 Hz, C_5-H), 8.58 (d, 1H, *J* = 8.6 Hz, C_8-H). ESI-MS, m/z: 176.1 $[M+H]^+$. Anal. calcd. for $C_9H_9NO_2$: C, 68.57; H, 5.14; N, 8.00. Found: C, 68.50; H, 5.25; N, 7.97.

2,6-Dimethyl-4-(3H)-quinazolinone ***6***

2,6-Dimethylbenzoxazin-4-one (**5**) (0.88 g, 5.0 mmol) was dissolved in 15 mL of ethanol and 20 mL of 25% aqueous ammonia was added. The resulting mixture was stirred at room temperature for 48 h. After evaporation of ethanol, the aqueous solution was cooled in the refrigerator overnight. The separated solid was filtered off, washed with water, and recrystallized from acetic acid to afford 0.77 g (89%) of **6** as white needles, m. p. 249.9–250.0°C (m. p. 249.2–250.6°C [15]). All spectroscopic data were in full accordance with [15].

6-Bromomethyl-2-methyl-4-(3H)-quinazolinone ***7***

A mixture of 2,6-dimethyl-4-(3*H*)-quinazolinone (**6**) (2.44 g, 14.0 mmol), *N*-bromosuccinimide (2.75 g, 15.5 mmol), and benzoyl peroxide (0.4 g, 1.7 mmol) in 300 mL of trichloromethane

was stirred at reflux and under irradiation of a 100-W tungsten lamp for 3 h. After cooling to room temperature, the resulting precipitate was collected by filtration, washed with trichloromethane, and dried to give 2.69 g (76%) of **7** as white solid. m. p. >330°C (m.p. >330°C [15]). All spectroscopic data were in full accordance with [15].

General procedure for the preparation of compounds 9a–p

To a stirred mixture of amine **8** (1.5 mmol), anhydrous potassium phosphate (0.32 g, 1.5 mmol) in 15 mL of *N,N*-dimethylformamide, carbon disulfide (0.57 g, 7.5 mmol) was added. After stirring at room temperature for 30 min, 6-bromomethyl-2-methyl-4-(3*H*)-quinazolinone **7** (0.25 g, 1 mmol) was added. Stirring was continued at room temperature for 2 h, and the reaction mixture was poured into 100 mL of water. The separated precipitate was collected by filtration, and purified by column chromatography on silica gel using dichloromethane/methanol (95/5) as eluent to afford the desired compounds **9**.

9a: Yield 51%; m.p. 201.7–202.0°C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 2.34 (s, 3H, C_2–CH_3), 4.68 (s, 2H, CH_2S), 7.02 (s, 2H, $CHPh_2$), 7.25 – 7.40 (m, 10H, Ph-H), 7.51 (d, 1H, *J* = 8.4 Hz, quinazolinone 8-H), 7.75 (dd, 1H, *J* = 8.4, 2.1 Hz, quinazolinone 7-H), 8.08 (d, 1H, *J* = 2.1 Hz, quinazolinone 5-H), 10.94 (s, 1H, N*H*CH_2), 12.19 (s, 1H, NH). ESI-MS, m/z: 432.2 $[M+H]^+$. Anal. calcd. for $C_{24}H_{21}N_3OS_2 \cdot 1/4H_2O$: C, 66.13; H, 4.94; N, 9.64. Found: C, 65.96; H, 4.99; N, 9.48.

9b: Yield 74%; m.p. 208.6–209.6°C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 2.27 (s, 3H, Ph–CH_3), 2.34 (s, 3H, C_2–CH_3), 4.46 (s, 2H, CH_2S), 4.80 (s, 2H, CH_2Ph), 7.12, 7.17 (2d, *J* = 8.2 Hz, 4H, Ph-H), 7.52 (d, 1H, *J* = 8.3 Hz, quinazolinone 8-H), 7.75 (dd, 1H, *J* = 8.3, 2.1 Hz, quinazolinone 7-H), 8.07 (d, 1H, *J* = 2.1 Hz, quinazolinone 5-H). ESI-MS, m/z: 370.1 $[M+H]^+$. Anal. calcd. for $C_{19}H_{19}N_3OS_2$: C, 61.79; H, 5.14; N, 11.37. Found: C, 61.66; H, 5.22; N, 11.30.

9c: Yield 45%; m.p. 203.1–203.4°C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 2.34 (s, 3H, C_2–CH_3), 3.73 (s, 3H, OCH_3), 4.66 (s, 2H, CH_2S), 4.77 (s, 2H, CH_2Ph), 6.88 (d, 2H, *J* = 8.4 Hz, Ph-H), 7.21 (d, 2H, *J* = 8.4 Hz, Ph-H), 7.51 (d, 1H, *J* = 8.2 Hz, quinazolinone 8-H), 7.75 (d, 1H, *J* = 8.2 Hz, quinazolinone 7-H), 8.06 (s, 1H, quinazolinone 5-H), 10.44 (s, 1H, N*H*CH_2), 12.20 (brs, 1H, NH). ESI-MS, m/z: 386.2 $[M+H]^+$. Anal. calcd. for $C_{19}H_{19}N_3O_2S_2$: C, 59.20; H, 4.97; N, 10.90. Found: C, 58.84; H, 4.98; N, 10.58.

9d: Yield 66%; m.p. 198–199°C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 2.35 (s, 3H, C_2-CH_3), 3.80(s, 3H, OCH_3), 4.67 (s, 2H, CH_2S), 4.78 (s, 2H, CH_2Ph), 6.86 – 7.29 (m, 4H, Ph-H), 7.52 (d, 1H, *J* = 8.6 Hz, quinazolinone 8-H), 7.76 (dd, 1H, *J* = 8.6, 2.1 Hz, quinazolinone 7-H), 8.08 (d, 1H, *J* = 2.1 Hz, quinazolinone 5-H), 10.33 (s, 1H, N*H*CH_2), 12.20 (s, 1H, NH). ESI-MS, m/z: 386.3 $[M+H]^+$. Anal. calcd. for $C_{19}H_{19}N_3O_2S_2$: C, 59.20; H, 4.97; N, 10.90. Found: C, 58.91; H, 5.07; N, 10.70.

9e: Yield 51%; m.p. 195–197°C. ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 2.33 (s, 3H, C_2–CH_3), 3.74, 3.77 (2s, 6H, 2OCH_3), 4.65 (s, 2H, CH_2S), 4.68 (s, 2H, CH_2Ph), 6.45 (dd, 1H, *J* = 8.2, 2.0 Hz, Ph-H), 6.55 (d, 1H, *J* = 2.0 Hz, Ph-H), 7.04 (d, 1H, *J* = 8.2 Hz, Ph-H), 7.52 (d, 1H, *J* = 8.4 Hz, quinazolinone 8-H), 7.75 (dd, 1H, *J* = 8.4, 2.0 Hz, quinazolinone 7-H), 8.06 (d, 1H, *J* = 2.0 Hz, quinazolinone 5-H), 10.22 (s, 1H, N*H*CH_2), 12.20 (brs, 1H, NH). ESI-MS, m/z: 414.2 $[M-H]^-$. Anal. calcd. for $C_{20}H_{21}N_3O_3S_2$: C, 57.81; H, 5.09; N, 10.11. Found: C, 57.72; H, 5.24; N, 9.83.

9f: Yield 22%; m.p. 173.3–173.8°C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 2.34 (s, 3H, C_2–CH_3), 3.63 (s, 3H, OCH_3), 3.71 (s, 6H, 2OCH_3), 4.68 (s, 2H, CH_2S), 4.78 (s, 2H, CH_2Ph), 6.62 (s, 2H, Ph-H), 7.51 (d, 1H, *J* = 8.3 Hz, quinazolinone 8-H), 7.76 (dd, 1H, *J* = 8.3, 2.0 Hz, quinazolinone 7-H), 8.08 (d, 1H, *J* = 2.0 Hz, quinazolinone 5-H), 10.45 (s, 1H, N*H*CH_2), 12.21 (brs, 1H, NH). ESI-MS, m/z: 446.3 $[M+H]^+$. Anal. calcd. for $C_{21}H_{23}N_3O_4S_2$: C, 56.61; H, 5.20; N, 9.43. Found: C, 56.28; H, 5.28; N, 9.15.

9g: Yield 32%; m.p. 196.7–197.3°C. ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 2.34 (s, 3H, C_2–CH_3), 4.66 (s, 2H, CH_2S), 4.74 (s, 2H, CH_2Ph), 5.99 (s, 2H, OCH_2O), 6.81 (m, 3H, Ph-H), 7.51 (d, 1H, *J* = 8.4 Hz, quinazolinone 8-H), 7.76 (d, 1H, *J* = 8.4 Hz, quinazolinone 7-H), 8.07 (s, 1H, quinazolinone 5-H). ESI-MS, m/z: 400.1 $[M+H]^+$. Anal. calcd. for $C_{19}H_{17}N_3O_3S_2$: C, 57.12; H, 4.29; N, 10.52. Found: C, 56.89; H, 4.58; N, 10.22.

9h: Yield 82%; m.p. 193–194°C. ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 2.34 (s, 3H, C_2–CH_3), 4.47 (s, 2H, CH_2S), 4.83 (s, 2H, CH_2Ph), 7.11 – 7.37 (m, 4H, Ph-H), 7.52 (d, 1H, *J* = 8.4 Hz, quinazolinone 8-H), 7.75 (dd, 1H, *J* = 8.4, 2.1 Hz, quinazolinone 7-H), 8.07 (d, 1H, *J* = 2.1 Hz, quinazolinone 5-H) , 10.53 (s, 1H, N*H*CH_2), 12.23 (s, 1H, NH). ESI-MS, m/z: 374.2 $[M+H]^+$. Anal. calcd. for $C_{18}H_{16}FN_3OS_2$: C, 57.89; H, 4.32; N, 11.25. Found: C, 57.93; H, 4.38; N, 11.20.

9i: Yield 55%; m.p. 179–180°C. ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 2.33 (s, 3H, C_2–CH_3), 4.66 (s, 2H, CH_2S), 4.85 (s, 2H, CH_2Ph), 7.23 (m, 4H, Ph-H), 7.51 (d, 1H, *J* = 8.4 Hz, quinazolinone 8-H), 7.75 (d, 1H, *J* = 8.4 Hz, quinazolinone 7-H), 8.07 (s, 1H, quinazolinone 5-H), 10.50 (s, 1H, N*H*CH_2), 12.20 (s, 1H, NH). ESI-MS, m/z: 374.1 $[M+H]^+$. Anal. calcd. for $C_{18}H_{16}FN_3OS_2 \cdot CH_3OH$: C, 56.29; H, 4.94; N, 10.37. Found: C, 56.42; H, 4.74; N, 10.33.

9j: Yield 46%; m.p. 199.6–200.6°C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 2.34 (s, 3H, C_2–CH_3), 4.66 (s, 2H, CH_2S), 4.83 (s, 2H, CH_2Ph), 7.29 (d, 2H, *J* = 8.4 Hz, Ph-H), 7.39 (d, 2H, *J* = 8.4 Hz, Ph-H), 7.52 (d, 1H, *J* = 8.4 Hz, quinazolinone 8-H), 7.75 (dd, 1H, *J* = 8.4, 2.0 Hz, quinazolinone 7-H), 8.07 (d, 1H, *J* = 2.0 Hz, quinazolinone 5-H), 10.53 (s, 1H, N*H*CH_2), 12.21 (brs, 1H, NH). ESI-MS, m/z: 390.3 $[M+H]^+$. Anal. calcd. for $C_{18}H_{16}ClN_3OS_2$: C, 55.42; H, 4.11; N, 10.78. Found: C, 55.50; H, 4.15; N, 10.76.

9k: Yield 35%; m.p. 206–208°C. ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 2.35 (s, 3H, C_2–CH_3), 4.68 (s, 2H, CH_2S), 4.84 (s, 2H, CH_2Ph), 7.27 (d, 1H, *J* = 8.3 Hz, Ph 6-H), 7.43 (dd, 1H, *J* = 8.3, 1.9 Hz, Ph 5-H), 7.53 (d, 1H, *J* = 8.4 Hz, quinazolinone 8-H), 7.64 (d, 1H, *J* = 1.9 Hz, Ph 3-H), 7.75 (dd, 1H, *J* = 8.4, 1.8 Hz, quinazolinone 7-H), 8.08 (d, 1H, *J* = 1.8 Hz, quinazolinone 5-H), 10.53 (s, 1H, N*H*CH_2), 12.23 (s, 1H, NH). ESI-MS, m/z: 424.2 $[M+H]^+$. Anal. calcd. for $C_{18}H_{15}Cl_2N_3OS_2$: C, 50.94; H, 3.56; N, 9.90. Found: C, 50.77; H, 3.62; N, 9.78.

9l: Yield 30%; m.p. 196.5–198.8°C. ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 2.33 (s, 3H, C_2–CH_3), 4.67 (s, 2H, CH_2S), 4.92 (d, 2H, CH_2Ph), 7.35 (d, 2H, *J* = 8.2 Hz, Ph-H), 7.52 (d, 1H, *J* = 8.4, quinazolinone 8-H), 7.76 (dd, 1H, *J* = 8.4, 2.1 Hz, quinazolinone 7-H), 7.85 (d, 2H, *J* = 8.2 Hz, Ph-H), 8.08 (d, 1H, *J* = 2.1 Hz, quinazolinone 5-H), 10.58 (s, 1H, N*H*CH_2), 12.22 (brs, 1H, NH), 12.92 (brs, 1H, CO_2H). ESI-MS, m/z: 400.2 $[M+H]^+$. Anal. calcd. for $C_{19}H_{17}N_3O_3S_2 \cdot 1/2H_2O$: C, 55.86; H, 4.44; N, 10.29. Found: C, 55.92; H, 4.39; N, 10.03.

9m: Yield 58%; m.p. 214–215°C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 2.34 (s, 3H, C_2–CH_3), 4.66 (s, 2H, CH_2S), 4.77 (s, 2H, CH_2Pyr), 7.22 – 7.32 (m, 2H, Pyr-H), 7.52 (d, 1H, *J* = 8.6 Hz, quinazolinone 8-H), 7.72–7.81 (m, 2H, Pyr-H, quinazolinone 7-H), 8.08 (d, 1H, *J* = 2.0 Hz, quinazolinone 5-H), 8.52 (m, 1H, Pyr-H), 10.58 (s, 1H, N*H*CH_2), 12.20 (s, 1H, NH). ESI-MS, m/z: 357.2 $[M+H]^+$. Anal. calcd. for $C_{17}H_{16}N_4OS_2 \cdot 1/4\ H_2O$: C, 56.58; H, 4.58; N, 15.53. Found: C, 56.64; H, 4.54; N, 15.23.

9n: Yield 55%; m.p. 149–150°C. ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 2.34 (s, 3H, C_2–CH_3), 4.66 (s, 2H, CH_2S), 4.86 (d, 2H, CH_2Pyr), 7.36 (m, 1H, Pyr-H), 7.51 (d, 1H, *J* = 8.4 Hz, quinazolinone 8-H), 7.68

(m, 1H, Pyr-H), 7.75 (d, 1H, *J* = 8.4 Hz, quinazolinone 7-H), 8.06 (s, 1H, quinazolinone 5-H), 8.48 (m, 2H, Pyr-H), 10.55 (s, 1H, N*H*CH_2), 12.20 (s, 1H, NH). ESI-MS, m/z: 357.2 $[M+H]^+$. Anal. calcd. for $C_{17}H_{16}N_4OS_2 \cdot 5/4\ H_2O$: C, 53.88; H, 4.92; N, 14.78. Found: C, 54.16; H, 4.79; N, 14.38.

9o: Yield 49%; m.p. 194.2–195.3°C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 2.34 (s, 3H, C_2–CH_3), 4.66 (s, 2H, CH_2S), 5.01 (s, 2H, C*H*$_2$NH), 6.97 (dd, 1H, *J* = 4.8, 3.4 Hz, thiophene 4-H), 7.05 (d, *J* = 3.4 Hz, 1H, thiophene 3-H), 7.42 (d, 1H, *J* = 4.8 Hz, thiophene 5-H), 7.51(d, 1H, *J* = 8.6 Hz, quinazolinone 8-H), 7.75 (dd, 1H, *J* = 8.6, 2.1 Hz, quinazolinone 7-H), 8.06 (d, 1H, *J* = 2.1 Hz, quinazolinone 5-H), 10.55 (s, 1H, N*H*CH_2), 12.19 (brs, 1H, NH). ESI-MS, m/z: 362.3 $[M+H]^+$. Anal. calcd. for $C_{16}H_{14}N_3OS_3 \cdot 1/8\ H_2O$: C, 52.84; H, 3.92; N, 11.56. Found: C, 52.68; H, 4.25; N, 11.23.

9p: Yield 36%; m.p. 199–200°C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.5–1.90 (m, 4H, tetrahydrofuran-H), 2.33 (s, 3H, C_2–CH_3), 3.61–3.76 (m, 4H, NHC*H*$_2$, tetrahydrofuran-H), 4.07 (m, 1H, OCH), 4.64 (s, 2H, CH_2S), 7.51 (d, 1H, *J* = 8.6 Hz, quinazolinone 8-H), 7.74 (dd, 1H, *J* = 8.6, 2.1 Hz, quinazolinone 7-H), 8.05 (d, 1H, *J* = 2.1 Hz, quinazolinone 5-H), 10.16 (s, 1H, N*H*CH_2), 12.19 (s, 1H, NH). ESI-MS, m/z: 350.3 $[M+H]^+$. Anal. calcd. for $C_{16}H_{19}N_3O_2S_2$: C, 55.15; H, 5.21; N, 12.06. Found: C, 54.80; H, 5.39; N, 11.90.

Biological assay

The stock solutions of the tested compounds were prepared in DMSO and were used for serial dilutions in culture medium. A-549 (human non-small cell lung cancer), HCT-8 (human colon cancer), and Bel-7402 (human liver cancer) cell lines were grown in RPMI-1640 medium supplemented with 10% calf serum. For growth assays, exponentially growing cells were suspended in the above-mentioned medium at a density of 4×10^4 cells per mL, seeded onto 96-well plates well (200 μL/well), and incubated at 37°C in a humidified 5% CO_2 atmosphere for 24 h. After that, the cell medium in test wells was changed to new culture medium containing different concentrations of the tested compounds, while the cell medium in control wells was changed to new culture medium containing an equivalent volume of solvent. After incubation at 37°C in a humidified 5% CO_2 atmosphere for 3 d, 100 μL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, 0.5 mg/mL) in serum-free medium was added to each well and incubated at 37°C for an additional 4 h. Then, 200 μL of DMSO was added to each well and mixed thoroughly to dissolve the resulting formazan product. The cell viability was evaluated by measurement of the optical densities at 544 nm using a Microelisa Reader. The percentage of cell growth inhibition was calculated as follows:

$$\% \text{ Inhibition} = (\text{Mean OD}_{\text{control}} - \text{Mean OD}_{\text{test}})/\text{Mean OD}_{\text{control}} \times 100\%.$$

The dose-response curves of the compounds effecting ≥70% inhibition in one-dose prescreening for each cell line were measured with the concentrations of 5, 0.5, 0.05, and 0.005 μg/mL, and the concentration causing 50% cell growth inhibition (IC_{50}) was calculated. The results are given in Table 1.

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