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ORIGINAL RESEARCH

Design, synthesis, antiproliferative and antibacterial evaluation of quinazolinone derivatives

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Abstract

A series of novel quinazolinone derivatives bearing a disulfide bond were designed and synthesized. Their in vitro antiproliferative activities were evaluated using CCK-8 assay against SMMC-7721, Hela, A549 and MCF-7 human cancer cell lines and normal cell lines L929. The preliminary bioassay results demonstrated that all compounds **7a**–**7h**, **8a**–**8h** and **9a**–**9h** exhibited antiproliferation with various degrees, and some compounds showed better effects than positive control 5-fluorouracil against different cancer cell lines. Among these compounds, **8c** and **9**f showed significant antiproliferative activity against SMMC-7721 cells with IC_{50} values of 2.88 and 2.56 μM, respectively. In Hela cells, compounds **9c** and **9d** showed highly effective biological activity with IC_{50} values of 3.16 and 2.68 μM, respectively. Compounds **7a** and **9a** exhibited good inhibitory effect against A549 cells with IC_{50} values of 3.53 and 3.54 μM, respectively. In MCF-7 cells, compounds **7e**, **8e** and **9e** displayed excellent activity with IC_{50} values of 1.26, 1.12 and 1.85 μM, respectively. Besides, most of the tested compounds showed low cytotoxic effect against the normal cell lines L929. Biological evaluation indicated that all the tested compounds possessed antibacterial activity with certain degrees.

Keywords Quinazolinone · Disulfides · Antiproliferative and antibacterial activities

Introduction

Cancer is one of the major causes of death, and one of the main reasons to which high mortality can be attributed is the failure of the current treatment options (Vale et al. 2017). Despite significant progress has been achieved in anticancer therapy, the management of malignancies in humans still constitutes one of the most intractable worldwide health problems. Therefore, it is important to identify effective drugs and new targets for the treatment of cancer (Alegaon et al. 2017). Quinazolinone derivatives constitute an important class of biologically active compounds (Poudapally et al. 2017). The quinazolinone core is found in both natural products and in pharmaceuticals, representing a privileged scaffold given the range of activities such as antidiabetic (Wei et al. 2017), antibacterial (Pandey et al. 2009; Wang et al. 2014), antiinflammatory (Abdel-Aziz et al. 2016; Nanthakumar et al. 2014), anticoagulant (Xing et al. 2017), antioxidant (Mohamed and Rao 2017; Haghighijoo et al. 2017), antiproliferative (Xuan et al. 2015; Venkatesh et al. 2016), anticonvulsant (Amir et al. 2014; Al-Salem et al. 2015), antifibrotic (Marzaro et al. 2016) and antituberculosis (Lu et al. 2015). The potent anticancer activities of luotonin and camptothecin family natural alkaloids have drawn extreme interest worldwide (Fig. 1). Their parent representative 2-substituted-quinazolin-4(3*H*)-one analog acts as an excellent lead drug in anticancer research.

Meanwhile, disulfide derivatives are also known to display a wide spectrum of biological activities because the disulfide group makes up the core structure of numerous biologically active compounds with many types of biological activities including antibacterial (Turos et al. 2008),

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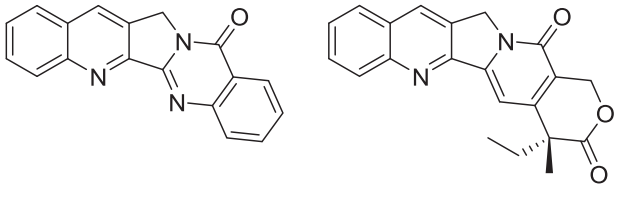


Fig. 1 Chemical structures of luotonin A and camptothecin

antitumor (Yi and Su 2013; Gaowa et al. 2015), antioxidant (Lakes et al. 2013), and anti-SARS (Wang et al. 2017). Consequently, the importance of disulfide derivatives in the field of medicinal chemistry has received much attention in the past few decades (Fig. 2).

These mentioned facts encouraged us to carry on our investigation on quinazolinone derivatives bearing a disulfide bond, in the pursuit of novel compounds with antiproliferative activity having the potential of becoming new drugs. On the other hand, treatment of bacterial infections still remains an important and challenging therapeutic problem, due to the emergence of bacterial resistance to current therapeutic agents. We are also interested in exploring if these new compounds have antibacterial activity.

Results and discussion

Chemistry

As depicted in Scheme 1, the preparation method of *S*-alkylthioisothiourea hydrochloride **3** was obtained from a reported method (Sirakawa et al. 1970). Compound **5** was prepared from compound **4** using a reported synthetic route (Hroch et al. 2017; Mandapati et al. 2017). Intermediates **6a–6h** were obtained by a reported literature method. Finally, the target compounds **7a–7h, 8a–8h** and **9a–9h** were obtained by the reaction of compound **3** and intermediates **6**a–**6**h in the presence of $NaHCO_3$ in methanol and water at room temperature. All new synthesized compounds **7a–7h**, **8a–8h** and **9a–9h** were purified by silica gel column chromatography and their structures were characterized by IR, ^{1}H NMR, ^{13}C NMR and high resolution-electrospray ionization mass spectrometer (HR-ESI-MS).

Pharmacology and discussion

The synthesized compounds were evaluated for their in vitro antiproliferative activity against SMMC-7721, Hela, A549 and MCF-7 human cancer cell lines and L929 normal cell lines by CCK-8[2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium monosodium salt] assay. Inhibition of cell proliferation by these active compounds at various concentrations was measured, and their IC_{50} (the concentration that causes a 50% cell proliferation inhibition) values were calculated and are summarized in Table 1. Moreover, antimicrobial activities of all compounds were evaluated against *Escherichia coli* and *Staphylococcus aureus* strains. The results are presented in Table 2.

Antiproliferative activity

As shown in Table 1, the tested compounds **7a–7h**, **8a–8h** and **9a–9h** exhibited antiproliferation with different degrees, and some compounds showed better effects than positive control 5-fluorouracil against various cancer cell lines. This class of the compounds exhibited antiproliferation against SMMC-7721 cells with IC_{50} values range from 2.56 to 33.72 μM. As compared with compound **7a**, which has no substituent at the phenyl ring while R_1 is 2-butyl group, except compound **7f**, the other compounds showed reduced antiproliferative activities. Compound **7f** electron-donating group substituted showed high activity with an IC_{50} value of 3.04 μM. As compared with compound **8a**, which has no substituent at the phenyl ring while R_1 is *n*-butyl group, compound **8c** electron-donating group substituted displayed better activity with an IC_{50} value of 2.88 μM. As compared with compound **9a**, which has no substituent at the phenyl ring while R_1 is *i*-butyl group, except compound **9f**, the other compounds showed reduced antiproliferative activities. Compound **9f** electron-donating group substituted showed significant activity with an IC_{50} value of 2.56 μM. Especially, while R_1 is the same substituent, electron-donating group substituted derivatives, including **7f**, **8c** and **9f**, displayed better activities than other compounds. In Hela cells, compounds **9c** and **9d** carrying 4-methyl and 4-trifluoromethyl substituents, while R_1 is *i*-butyl group, displayed highly effective biological activities with IC_{50} values of 3.16 and 2.68 μM, respectively. With the exception of compounds **9c** and **9d**, the other compounds exhibited better-to-moderate antiproliferative activities with IC_{50} values ranging from 4.06 to 29.32 μM. As compared with compound **7a**, which has no substituent at the phenyl ring while R_1 is 2-butyl group, the other compounds showed enhanced antiproliferative activities. As compared with compound **8a**, which has no substituent at the phenyl ring while R_1 is *n*-butyl group, except compound **8e**, the other compounds showed enhanced antiproliferative activities. As compared with compound **9a**, which has no substituent at the phenyl ring while R_1 is *i*-butyl group, except compounds **9e**, **9g** and **9h**, the other compounds showed enhanced antiproliferative activities. The electronic effects of substituents at the phenyl ring on antiproliferative activity against Hela cells did not show apparent regularity. Especially, while R_2 is the same substituent, 2-butyl group

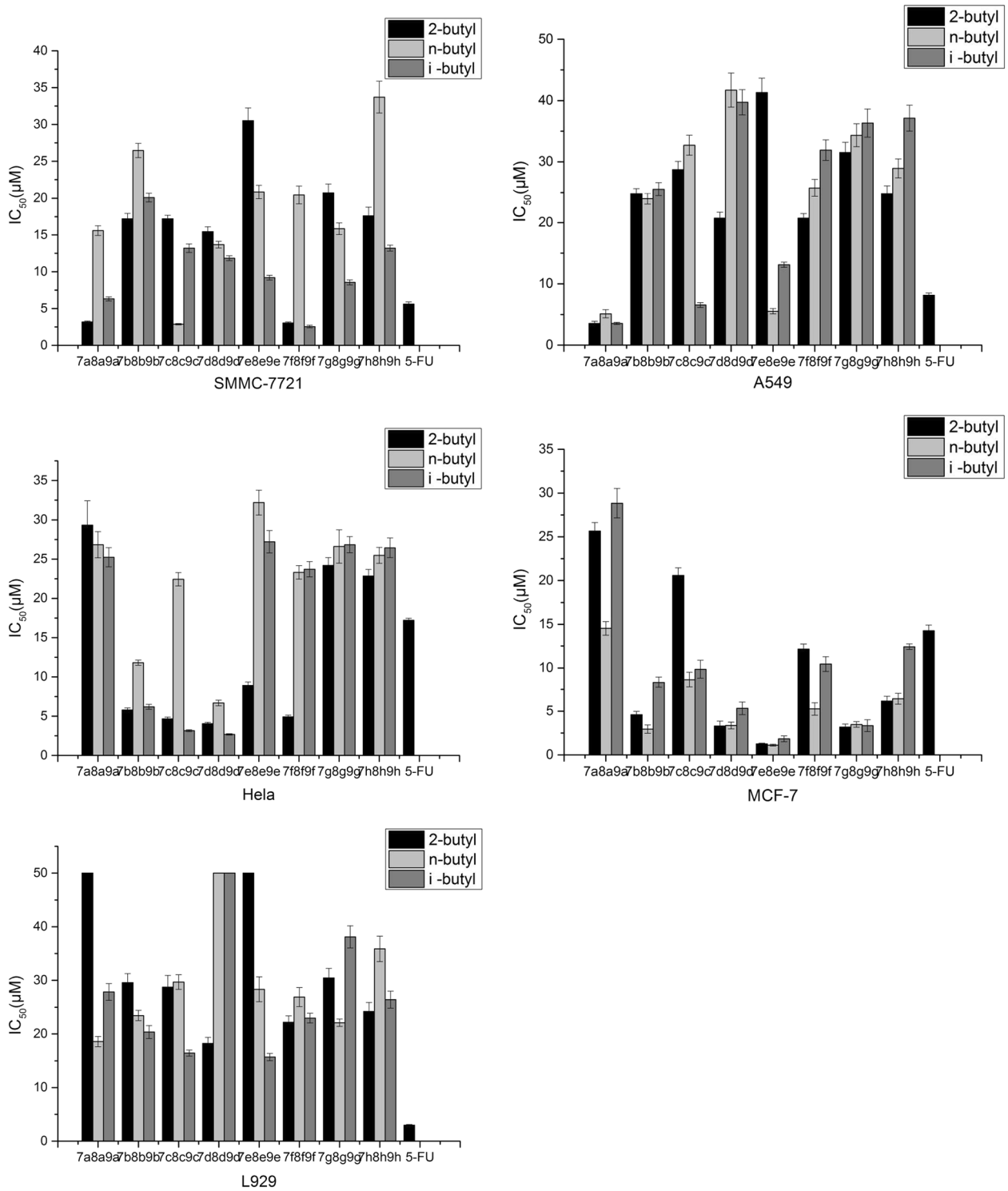
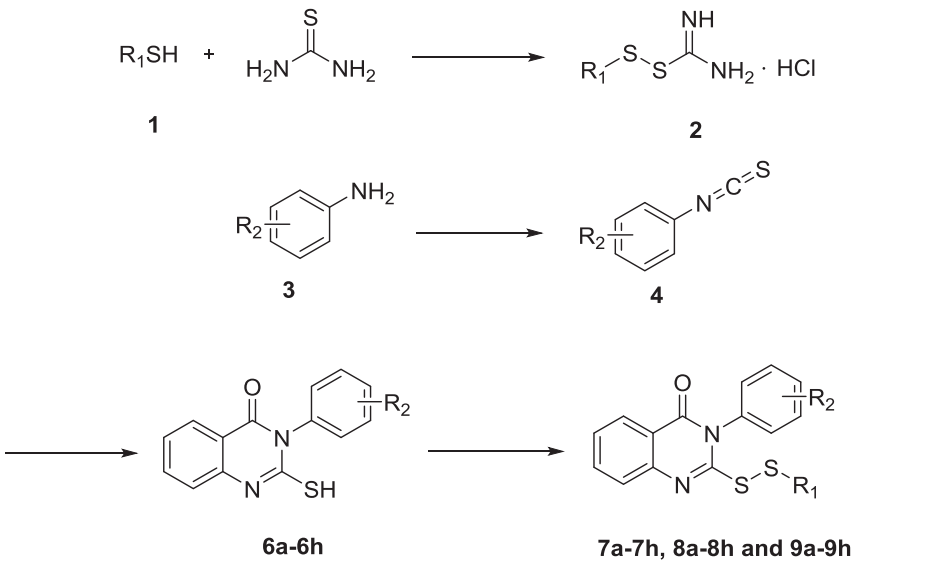


Fig. 2 IC_{50} of compounds **7a**–**7h**, **8a**–**8h** and **9a**–**9h** against SMMC-7721, Hela, A549, MCF-7 and L929 cells

derivatives displayed higher activities than *n*-butyl group derivatives. In A549 cells, the majority of compounds exhibited moderate antiproliferative activity. Among them, compounds **7a** and **9a**, which have no substituent at the phenyl ring while R_1 are 2-butyl groups and *i*-butyl groups, revealed high antitumor activities with IC_{50} values of 3.53 and 3.54 μM, respectively. Especially, while compounds has no substituent at the phenyl ring displayed higher activities than other compounds. In MCF-7 cells, most compounds showed better effects than positive control

Scheme 1 Synthesis of target compounds **7a–7h**, **8a–8h** and **9a–9h**



R2 (R1=2–C4H9): H(7a), 4–Cl(7b), 4–CH3(7c), 4–CF3(7d), 4–NO2(7e), 4–OCH3(7f), 3–Cl(7g), 3–CH3(7h)
R2 (R1=n–C4H9): H(8a), 4–Cl(8b), 4–CH3(8c), 4–CF3(8d), 4–NO2(8e), 4–OCH3(8f), 3–Cl(8g), 3–CH3(8h)
R2 (R1= i–C4H9): H(9a), 4–Cl(9b), 4–CH3(9c), 4–CF3(9d), 4–NO2(9e), 4–OCH3(9f), 3–Cl(9g), 3–CH3(9h)

Reagents and conditions: (a) concd HCl, H2O2(30%), 0–5 °C, 3 h; (b) CS2, Et3N, EtOH/H2O (2:1), catalyst, rt, 3 h;

(c) anthranilic acid, ethanol, reflux, 6 h; (d) compound 2, ethanol, NaHCO3/H2O, rt, 3 h.

5-fluorouracil. While compounds with no substituent at the phenyl ring displayed lower activities than other compounds. Among these compounds, all carrying an electron-drawing group substituted derivatives revealed higher activities than the electron-donating group substituted derivatives. Especially, while R_2 is the same substituent, 4-nitro substituted derivatives **7e**, **8e** and **9e** displayed higher activities with IC_{50} values of 1.12, 1.12 and 1.85 μM, respectively, than other compounds. Furthermore, most of the compounds **7a**–**7h**, **8a**–**8h** and **9a**–**9h** exhibited a weak cytotoxic activity against L929 cells, and all compounds showed highly lower cytotoxic effect than 5-fluorouracil. These results indicated that no matter R_1 is a 2-butyl, *n*-butyl or *i*-butyl group, the electronic effects of the substituents at the phenyl ring on the antiproliferative activity against SMMC-7721, Hela, A549 and MCF-7 cells did not show apparent regularity. Therefore, it is necessary that the further investigation is carried out through structural transformation for improving the potency and selectivity of this class of compounds.

Antibacterial activity

As shown in Table 2, all the tested compounds **7a–7h**, **8a–8h** and **9a–9h** exhibited antibacterial activity with different degrees, but none of them showed better effects than the positive control gentamicin against Gram-negative bacteria *E. coli* and Gram-positive bacteria *S. aureus*. The results obtained on Gram-negative bacteria *E. coli* showed that compounds **7d**, **7f–7h**, **8e**, **8h**, **9b–9d** and **9g** exhibited same activities with a minimum inhibitory concentration (MIC) value of 64 μg/mL. The results obtained on Gram-positive bacteria *S. aureus* showed that compounds **7a–7b**, **7d**, **7f**, **7h**, **8d**, **8f–8h** and **9e–9h** displayed same activities against *S. aureus* with an MIC value of 64 μg/mL. Especially noteworthy is that the compounds **7e** and **8a** showed a promising antibacterial activity against *E. coli* and *S. aureus* with an MIC value of 32 μg/mL. Other compounds showed moderate activities with a same MIC value of 128 μg/mL.

Conclusion

In summary, 24 novel quinazolinone derivatives bearing a disulfide bond were designed and synthesized and gave satisfactory analytical and spectroscopic data, which were in full accordance with their depicted structures. All of the synthetic compounds were evaluated for their in vitro antiproliferative activities against SMMC-7721, Hela, A549 and MCF-7 human cancer cell lines by CCK-8 assay. In

Table 1 In vitro antiproliferative activities of target compounds against various cell lines

Compound	IC_{50}[a] (μM)				
	SMMC-7721	Hela	A549	MCF-7	L929
7a	3.18 ± 0.12	29.32 ± 3.11	3.53 ± 0.38	25.66 ± 0.96	>50
7b	17.20 ± 0.74	5.80 ± 0.27	24.72 ± 0.91	4.60 ± 0.38	29.60 ± 1.68
7c	17.20 ± 0.48	4.67 ± 0.22	28.72 ± 1.37	20.59 ± 0.87	28.76 ± 2.17
7d	15.44 ± 0.67	4.06 ± 0.16	20.72 ± 0.98	3.30 ± 0.56	18.23 ± 1.13
7e	30.52 ± 1.72	8.92 ± 0.43	41.32 ± 2.32	1.26 ± 0.08	>50
7f	3.04 ± 0.15	4.92 ± 0.21	20.72 ± 0.77	12.16 ± 0.58	22.17 ± 1.24
7g	20.72 ± 1.12	24.20 ± 0.99	31.53 ± 1.66	3.18 ± 0.36	30.48 ± 1.76
7h	17.60 ± 1.18	22.84 ± 0.86	24.72 ± 1.36	6.16 ± 0.56	24.22 ± 1.66
8a	15.60 ± 0.66	26.84 ± 1.67	5.12 ± 0.66	14.53 ± 0.78	18.58 ± 0.96
8b	26.48 ± 0.97	11.80 ± 0.33	23.92 ± 0.87	2.96 ± 0.47	23.44 ± 0.95
8c	2.88 ± 0.09	22.44 ± 0.85	32.72 ± 1.63	8.65 ± 0.87	29.69 ± 1.37
8d	13.68 ± 0.45	6.68 ± 0.35	41.71 ± 2.78	3.37 ± 0.39	>50
8e	20.84 ± 0.89	32.20 ± 1.58	5.53 ± 0.43	1.12 ± 0.10	28.33 ± 2.32
8f	20.44 ± 1.21	23.32 ± 0.86	25.72 ± 1.41	5.26 ± 0.71	26.89 ± 1.77
8g	15.84 ± 0.78	26.60 ± 2.12	34.33 ± 1.87	3.48 ± 0.32	22.08 ± 0.69
8h	33.72 ± 2.15	25.48 ± 1.01	28.92 ± 1.55	6.42 ± 0.62	35.88 ± 2.37
9a	6.32 ± 0.28	25.24 ± 1.22	3.54 ± 0.17	28.84 ± 1.68	27.84 ± 1.55
9b	20.08 ± 0.59	6.20 ± 0.31	25.53 ± 1.09	8.35 ± 0.61	20.36 ± 1.22
9c	13.20 ± 0.57	3.16 ± 0.12	6.54 ± 0.39	9.85 ± 1.02	16.43 ± 0.56
9d	11.84 ± 0.33	2.68 ± 0.08	39.71 ± 2.06	5.33 ± 0.72	>50
9e	9.20 ± 0.32	27.21 ± 1.43	13.12 ± 0.41	1.85 ± 0.33	15.69 ± 0.68
9f	2.56 ± 0.17	23.72 ± 0.97	31.91 ± 1.69	10.45 ± 0.85	22.96 ± 0.92
9g	8.56 ± 0.34	26.84 ± 1.03	36.33 ± 2.31	3.35 ± 0.68	38.11 ± 2.08
9h	13.20 ± 0.40	26.44 ± 1.25	37.13 ± 2.11	12.43 ± 0.32	26.40 ± 1.59
5-FU[b]	5.62 ± 0.28	17.21 ± 0.27	8.13 ± 0.37	14.26 ± 0.66	2.98 ± 0.15

[a]The concentration that causes a 50% cell proliferation inhibition

[b]Used as a positive control

particularly, compounds **8**c and **9**f showed significant antiproliferative activity against SMMC-7721 cells with IC_{50} values of 2.88 and 2.56 μM, respectively. In Hela cells, compounds **9**c and **9**d showed highly effective biological activity against with IC_{50} values of 3.16 and 2.68 μM, respectively. In A549 cells, compounds **7**a and **9**a exhibited good inhibitory effect with IC_{50} values of 3.53 and 3.54 μM, respectively. In MCF-7 cells, compounds **7**e, **8**e and **9**e displayed excellent activity with IC_{50} values of 1.26, 1.12 and 1.85 μM, respectively. In addition, many of the tested compounds showed low cytotoxic effect against the normal cell lines L929. Therefore, the results will be significant in the development of potent antitumor agents. Otherwise, biological evaluation indicated that all the tested compounds possessed antibacterial activity with certain degrees. Therefore, the results will be significant in the development of potent antitumor agents. Meanwhile, all the compounds **7a–7h**, **8a–8h** and **9a–9h** exhibited variable inhibitory effects on the growth of the bacterial *E. coli* strains and *S. aureus* strains.

Experimental

Chemistry

Reactions were monitored by TLC performed on glass packed silica gel GF254 plates. Melting points were determined by an X-6 microscope melting point apparatus and are uncorrected. Infrared spectra were recorded in KBr pellets on a Nicolet Avatar 370 spectrometer. ^{1}H NMR and ^{13}C NMR spectra were collected at resonance frequencies of 400 MHz and 100 MHz, respectively. NMR spectra were performed on a Bruker Avance III 400 MHz spectrometer using DMSO-d_6 as a solvent and tetramethylsilane as an internal standard. The chemical shifts for ^{1}H NMR are

Table 2 In vitro antibacterial activities of compounds **6a–6h**, **7a–7h** and **8a–8h** against two bacterial strains

Compound	Gram-negative bacteria *Escherichia coli* MIC[a] (μg/mL)	Gram-positive bacteria *Staphylococcus aureus* MIC (μg/mL)
7a	128	64
7b	128	64
7c	128	128
7d	64	64
7e	32	32
7f	64	64
7g	64	128
7h	64	64
8a	32	32
8b	128	128
8c	128	128
8d	128	64
8e	64	128
8f	128	64
8g	128	64
8h	64	64
9a	128	128
9b	64	128
9c	64	128
9d	64	128
9e	128	64
9f	128	64
9g	64	64
9h	128	64
Gentamicin[b]	2	2

[a]Minimum inhibitory concentration

[b]Used as a positive control

reported in ppm from tetramethylsilane (0 ppm) or referenced to the solvent (DMSO-d_6 2.50) on the δ scale. Chemical shifts (δ) for ^{13}C NMR spectra are referenced to the signals for residual deuterated solvents (DMSO-d_6 39.5). Multiplicities are reported by the following abbreviations: s (singlet), d (doublet), t (triplet), m (multiplet), *J* (coupling constants in hertz). High-resolution mass data were taken with a Waters Xevo G2 QT of mass spectrometer. The products were purified by flash column chromatography using silica gel (200–300 mesh) with indicated solvents.

General procedure for the synthesis of intermediates 6a–6h

A mixture of substituted anthranilic acid (10 mmol) and (substituted-phenyl)-phenylisothiocyanate (10 mmol) in 30 ml of absolute ethanol containing triethylamine (11 mmol) was heated under reflux for 6 h. The reaction mixture was filtered while hot, the solvent was removed under reduced pressure and the solid obtained was dried and recrystallized from ethanol.

2-Sulfhydryl-3-phenyl-quinazolin-4(3*H*)-one (6a)

White solid; yield 48.6%; m.p.: 296.5–298.7 °C; IR (KBr) cm^{-1}: 2516, 1662, 1531, 1453, 988; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.28 (d, $J = 8.0$ Hz, 2H, Ar–H), 7.34–7.50 (m, 6H, Ar–H), 7.80 (t, $J = 8.0$ Hz, 1H, Ar–H), 7.96 (d, $J = 8.0$ Hz, 1H, Ar–H), 13.07 (s, 1H, SH); MS-ESI (*m/z*): $C_{14}H_{10}N_2OS$ $[M+H]^+$ 255.1.

2-Sulfhydryl-3-(4-chlorophenyl)-quinazolin-4(3*H*)-one (6b)

White solid; Yield 55.4%; m.p.: 286.5–288.3 °C; IR (KBr) cm^{-1}: 2516, 1662, 1531, 1453, 988; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.36 (t, $J = 8.0$ Hz, 3H, Ar–H), 7.45 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.55 (d, $J = 8.0$ Hz, 2H, Ar–H), 7.80 (t, $J = 8.0$ Hz, 1H, Ar–H), 7.96 (d, $J = 8.0$ Hz, 1H, Ar–H), 13.10 (s, 1H, SH); MS-ESI (*m/z*): $C_{14}H_9ClN_2OS$ $[M+H]^+$ 289.0.

2-Sulfhydryl-3-(4-methylphenyl)-quinazolin-4(3*H*)-one (6c)

White solid; yield 62.1%; m.p.: 306.5–307.1 °C; IR (KBr) cm^{-1}: 2966, 2516, 1662, 1531, 1453, 988; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.38 (s, 3H, CH_3), 7.14 (d, $J = 8.0$ Hz, 2H, Ar–H), 7.28 (d, $J = 12.0$ Hz, H, Ar–H), 7.35 (t, $J = 8.0$ Hz, 1H, Ar–H), 7.45 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.79 (t, $J = 8.0$ Hz, 1H,Ar–H), 7.95 (d, $J = 8.0$ Hz, 1H, Ar–H), 13.04 (s, 1H, SH); MS-ESI (*m/z*): $C_{15}H_{12}N_2OS$ $[M+H]^+$ 270.1.

2-Sulfhydryl-3-(4-trifluoromethylphenyl)-quinazolin-4(3*H*)-one (6d)

White solid; yield 56.3%; m.p.: 276.5–277.7 °C; IR (KBr) cm^{-1}: 2966, 2516, 1662, 1531, 1453, 988; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.37 (t, $J = 8.0$ Hz, 1H, Ar–H), 7.47 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.59 (d, $J = 8.0$ Hz, 2H, Ar–H), 7.81 (t, $J = 8.0$ Hz, 1H, Ar–H), 7.88 (d, $J = 8.0$ Hz, 2H, Ar–H), 7.97 (d, $J = 8.0$ Hz, 1H, Ar–H), 13.15 (s, 1H, SH); MS-ESI (*m/z*): $C_{15}H_9F_3N_2O_3S$ $[M+H]^+$323.0.

2-Sulfhydryl-3-(4-nitrophenyl)-quinazolin-4(3*H*)-one (6e)

White solid; yield 45.8%; m.p.: 316.5–317.5 °C; IR (KBr) cm^{-1}: 2516, 1662, 1531, 1453, 988; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.38 (t, $J = 8.0$ Hz, 1H, Ar–H), 7.47 (d,

$J = 8.0$ Hz, 1H, Ar–H), 7.67 (d, $J = 8.0$ Hz, 2H, Ar–H), 7.82 (t, $J = 8.0$ Hz, 1H, Ar–H), 7.98 (d, $J = 8.0$ Hz, 1H, Ar–H), 8.36 (d, $J = 8.0$ Hz, 2H, Ar–H), 13.19 (s, 1H, SH); MS-ESI (*m/z*): $C_{14}H_9N_3OS$ $[M+H]^+$ 300.0.

2-Sulfhydryl-3-(4-methoxyphenyl)-quinazolin-4(3*H*)-one (6f)

White solid; yield 65.9%; m.p.: 301.8–302.9 °C; IR (KBr) cm^{-1}: 2966, 2516, 1662, 1531, 1453, 988; ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.36 (s, 3H, CH_3), 7.01 (d, $J =$ 8.0 Hz, 2H, Ar–H), 7.18 (d, $J = 8.0$ Hz, 2H, Ar–H), 7.35 (t, $J = 8.0$ Hz, 1H, Ar–H), 7.45 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.78 (t, $J = 4.0$ Hz, 1H, Ar–H), 7.95 (d, $J = 8.0$ Hz, 1H, Ar–H), 13.02 (s, 1H, SH); MS-ESI (*m/z*): $C_{15}H_{12}N_2O_2S$ $[M+H]^+$285.1

2-Sulfhydryl-3-(3-chlorophenyl)-quinazolin-4(3*H*)-one (6g)

White solid; yield 63.7%; m.p.: 289.3–290.6 °C; IR (KBr) cm^{-1} : 2516, 1662, 1531, 1453, 988; ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.31 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.36 (t, $J = 8.0$ Hz, 1H, Ar–H), 7.45–7.50 (m, 4H, Ar–H), 7.80 (t, $J = 8.0$ Hz, 1H, Ar–H), 7.96 (d, $J = 4.0$ Hz, 1H, Ar–H), 13.11 (s, 1H, SH); MS-ESI (*m/z*): $C_{14}H_9ClN_2OS$ $[M+H]^+$289.0.

2-Sulfhydryl-3-(3-methylphenyl)-quinazolin-4(3*H*)-one (6h)

White solid; yield 57.1%; m.p.: 275.6–277.4 °C; IR (KBr) cm^{-1}: 2966, 2516, 1662, 1531, 1453, 988; ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.35 (s, 3H, CH_3), 7.09 (s, 2H, Ar–H), 7.22 (d, $J = 4.0$ Hz, H, Ar–H), 7.36 (s, 2H, Ar–H), 7.45(d, $J = 8.0$ Hz, 1H, Ar–H), 7.79 (t, $J = 8.0$ Hz, 1H, Ar–H), 7.95 (d, $J = 8.0$ Hz, 1H, Ar–H), 13.04 (s, 1H, SH); MS-ESI (*m/z*): $C_{15}H_{12}N_2OS$ $[M+H]^+$270.0.

General procedure for the synthesis of compounds 7a–7h, 8a–8h and 9a–9h

S-Alkyl-thioisothiourea hydrochloride **2** (2.2 mmol) and 2-sulfhydryl-3-(4-substituted-phenyl)-quinazolin-4(3*H*)-one **6a–6h** (2.0 mmol) were dissolved in 5 mL of water and 15 mL of ethanol. A solution of $NaHCO_3$ (3.0 mmol) in 4 mL of water was added dropwise with vigorous stirring at room temperature. The mixture was stirred for additional 6 h. The insoluble solid was collected and purified by silica gel column chromatography with petroleum ether/ethyl acetate (10: 1, volume ratio) as an eluent to afford the desired products. The newly synthesized compounds **7a–7h, 8a–8h** and **9a–9h** were purified by silica gel column chromatography and their structures were characterized by IR, ^1H NMR,^{13}C NMR and HR-ESI-MS.

2-(2-Butyldisulfanyl)-3-phenyl-quinazolin-4(3*H*)-one (7a)

White solid; yield 53.6%; m.p.: 124.3–125.6 °C; ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.01 (t, $J = 8.0$ Hz, 3H, CH_3), 1.27 (d, $J = 8.0$ Hz, 3H, CH_3), 1.49–1.68 (m, 2H, CH_2), 3.08–3.16 (m, 1H, CH), 7.52–7.55 (m, 3H, Ar–H), 7.58–7.61 (m, 3H, Ar–H), 7.68 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.88 (t, $J = 8.0$ Hz, 1H, Ar–H), 8.11 (d, $J = 8.0$ Hz, 1H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 11.72, 20.36, 28.84, 48.25, 120.38, 126.84, 126.98, 127.14, 130.01 (2C), 130.05 (2C), 130.61, 135.47, 136.10, 147.61, 156.22, 161.27; IR (KBr, cm^{-1}): 1687, 1576, 1552, 769, 500 cm^{-1}; HR-MS (ESI): *m/z* 343.0957 $[M+H]^+$ (calcd. for $C_{18}H_{19}N_2OS_2$: 343.0939).

2-(2-Butyldisulfanyl)-3-(4-chlorophenyl)-quinazolin-4(3*H*)-one (7b)

White solid; yield 47.3%; m.p.: 142.2–143.6 °C; ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.01 (t, $J = 8.0$ Hz, 3H, CH_3), 1.27 (d, $J = 8.0$ Hz, 3H, CH_3), 1.49–1.68 (m, 2H, CH_2), 3.08–3.17 (m, 1H, CH), 7.53 (t, $J = 8.0$ Hz, 1H, Ar–H), 7.61 (d, $J = 8.0$ Hz, 2H, Ar–H), 7.68 (d, $J = 8.0$ Hz, 3H, Ar–H), 7.88 (t, $J = 8.0$ Hz, 1H, Ar–H) 8.10 (d, $J = 8.0$ Hz, 1H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 11.73, 20.36, 28.83, 48.31, 120.32, 126.86, 127.06, 127.15, 130.18 (2C), 132.03 (2C), 135.01, 135.40, 135.57, 147.57, 155.84, 161.29; IR (KBr, cm^{-1}): 1694, 1577, 1556, 771, 509 cm^{-1}; HR-MS (ESI): *m/z* 377.0563 $[M+H]^+$ (calcd. for $C_{18}H_{18}N_2OS_2Cl$: 377.0549).

2-(2-Butyldisulfanyl)-3-(4-methylphenyl)-quinazolin-4(3*H*)-one (7c)

White solid; yield 63.9%; m.p.: 123.3–124.8 °C; ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.01 (t, $J = 8.0$ Hz, 3H, CH_3), 1.26 (d, $J = 8.0$ Hz, 3H, CH_3), 1.49–1.65 (m, 2H, CH_2), 2.42 (s, 3H, CH_3), 3.07–3.15 (m, 1H, CH), 7.39 (s, 4H, Ar–H), 7.53 (t, $J = 8.0$ Hz, 1H, Ar–H), 7.67 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.87 (t, $J = 8.0$ Hz, 1H, Ar–H), 8.10 (d, $J =$ 8.0 Hz, 1H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 11.73, 20.37, 28.84, 48.17, 55.96, 115.19, 120.34, 126.82, 126.92, 127.15, 128.41 (2C), 131.25 (2C), 135.43, 147.63, 156.87, 160.74, 161.50; IR (KBr, cm^{-1}): 1688, 1576, 1553, 769, 516 cm^{-1}; HR-MS (ESI): *m/z* 357.1113 $[M+H]^+$ (calcd. for $C_{19}H_{21}N_2OS_2$: 357.1095).

2-(2-Butyldisulfanyl)-3-(4-trifluoromethylphenyl)-quinazolin-4(3*H*)-one (7d)

White solid; yield 43.9%; m.p.: 133.2–135.1 °C; ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.01 (t, $J = 8.0$ Hz, 3H,

CH_3), 1.27 (d, $J = 8.0$ Hz, 3H, CH_3), 1.49–1.68 (m, 2H, CH_2), 3.09–3.18 (m, 1H, CH), 7.54 (t, $J = 8.0$ Hz, 1H, Ar–H), 7.69 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.85 (d, $J = 8.0$ Hz, 2H, Ar–H), 7.89 (t, $J = 8.0$ Hz, 1H, Ar–H), 8.00 (d, $J = 8.0$ Hz, 1H, Ar–H), 8.11 (d, $J = 8.0$ Hz, 1H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm):11.71, 20.33, 28.83, 48.37, 120.37, 124.31($J = 271.0$ Hz), 126.90, 127.10, 127.17 (2C, $J = 5.0$ Hz), 127.15, 130.91 (2C, $J = 32.0$ Hz), 131.35, 135.61, 139.90, 147.57, 155.34, 161.25; IR (KBr, cm^{-1}): 1688, 1578, 1556, 770, 509 cm^{-1}; HR-MS (ESI): *m/z* 411.0835 $[M+H]^+$ (calcd. for $C_{19}H_{18}N_2OS_2F_3$: 411.0813).

2-(2-Butyldisulfanyl)-3-(4-nitrophenyl)-quinazolin-4(3*H*)-one (7e)

White solid; yield 65.2%; m.p.: 193.3–194.5 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.01 (t, $J = 8.0$ Hz, 3H, CH_3), 1.27 (d, $J = 4.0$ Hz, 3H, CH_3), 1.50–1.68 (m, 2H, CH_2), 3.10–3.18 (m, 1H, CH), 7.55 (t, $J = 8.0$ Hz, 1H, Ar–H), 7.70 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.88–7.94 (m, 3H, Ar–H), 8.12 (d, $J = 8.0$ Hz, 1H, Ar–H), 8.45 (d, $J = 8.0$ Hz, 2H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 11.74, 20.38, 28.83, 48.43, 120.36, 125.28 (2C), 126.92, 127.16, 127.19, 131.92 (2C), 135.71, 141.91, 147.53, 148.83, 154.98, 161.19; IR (KBr, cm^{-1}): 1690, 1578, 1558, 778, 525 cm^{-1}; HR-MS (ESI): *m/z* 388.0806 $[M+H]^+$ (calcd. for $C_{18}H_{18}N_3O_3S_2$: 388.0789).

2-(2-Butyldisulfanyl)-3-(4-methoxyphenyl)-quinazolin-4(3*H*)-one (7f)

White solid; yield 56.3%; m.p.: 130.2–131.6 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.01 (t, $J = 4.0$ Hz, 3H, CH_3), 1.26 (d, $J = 8.0$ Hz, 3H, CH_3), 1.48–1.67 (m, 2H, CH_2), 3.06–3.15 (m, 1H, CH), 3.85 (s, 3H, CH_3), 7.12 (d, $J = 12.0$ Hz, 2H, Ar–H), 7.43 (d, $J = 8.0$ Hz, 2H, Ar–H), 7.51 (t, $J = 8.0$ Hz, 1H, Ar–H), 7.66 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.86 (t, $J = 8.0$ Hz, 1H, Ar–H), 8.10 (d, $J = 4.0$ Hz, 1H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 11.74, 20.38, 28.86, 48.16, 55.95, 115.18 (2C), 120.37, 126.81, 126.87, 127.15, 128.42, 131.25 (2C), 135.38, 147.64, 156.88, 160.74, 161.48; IR (KBr, cm^{-1}): 1686, 1578, 1558, 773, 528 cm^{-1}; HR-MS (ESI): *m/z* 373.1061 $[M+H]^+$ (calcd. for $C_{19}H_{21}N_2O_2S_2$: 373.1044).

2-(2-Butyldisulfanyl)-3-(3-chlorophenyl)-quinazolin-4(3*H*)-one (7g)

White solid; yield 42.6%; m.p.: 133.3–135.6 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.01 (t, $J = 8.0$ Hz, 3H, CH_3), 1.27 (d, $J = 8.0$ Hz, 3H, CH_3), 1.49–1.68 (m, 2H, CH_2), 3.09–3.17 (m, 1H, CH), 7.51–7.58 (m, 2H, Ar–H), 7.61–7.69 (m, 3H, Ar–H), 7.79 (s, 1H, Ar–H), 7.88 (t, $J = 8.0$ Hz, 1H, Ar–H) 8.10 (d, $J = 8.0$ Hz, 1H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 11.73, 20.36, 28.85, 48.34, 120.38, 126.87, 127.06, 127.15, 129.06, 130.17, 130.76, 131.61, 134.07, 135.57, 137.48, 147.56, 155.69, 161.22; IR (KBr, cm^{-1}): 1692, 1577, 1556, 768, 529 cm^{-1}; HR-MS (ESI): *m/z* 377.0558 $[M+H]^+$ (calcd. for $C_{18}H_{18}N_2OS_2Cl$: 377.0549).

2-(2-Butyldisulfanyl)-3-(3-methylphenyl)-quinazolin-4(3*H*)-one (7h)

White solid; yield 49.1%; m.p.: 121–122.6 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.00 (t, $J = 4.0$ Hz, 3H, CH_3), 1.25 (d, $J = 8.0$ Hz, 3H, CH_3), 1.45–1.66 (m, 2H, CH_2), 2.38 (s, 3H, CH_3), 3.06–3.14 (m, 1H, CH), 7.31 (d, $J = 8.0$ Hz, 2H, Ar–H), 7.39 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.45–7.53 (m, 2H, Ar–H), 7.67 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.86 (t, $J = 8.0$ Hz, 1H, Ar–H), 8.09 (d, $J = 8.0$ Hz, 1H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 11.65, 20.28, 21.20, 28.82, 48.25, 120.20, 126.80, 126.85, 126.97, 127.08, 129.86, 130.10, 131.28, 135.44, 135.86, 139.76, 147.54, 156.24, 161.27; IR (KBr, cm^{-1}): 1695, 1577, 1556, 770, 536 cm^{-1}; HR-MS (ESI): *m/z* 357.1109 $[M+H]^+$ (calcd. for $C_{19}H_{21}N_2OS_2$: 357.1095).

2-(*n*-Butyldisulfanyl)-3-phenyl-quinazolin-4(3*H*)-one (8a)

White solid; yield 50.4%; m.p.: 92.9–93.5 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.87 (t, $J = 8.0$ Hz, 3H, CH_3), 1.38–1.47 (m, 2H, CH_2), 1.57–1.64 (m, 2H, CH_2), 2.94 (t, $J = 8.0$ Hz, 2H, CH_2), 7.51–7.60 (m, 6H, Ar–H), 7.70 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.89 (t, $J = 8.0$ Hz, 1H, Ar–H), 8.11 (d, $J = 8.0$ Hz, 1H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm):13.92, 21.30, 30.56, 37.80, 120.38, 126.89, 126.98, 127.13, 130.01 (2C), 130.04 (2C), 130.62, 135.45, 136.01, 147.68, 156.11, 161.25; IR (KBr, cm^{-1}): 1685, 1578, 1556, 768, 498 cm^{-1}; HR-MS (ESI): *m/z* 343.0955 $[M+H]^+$ (calcd. for $C_{18}H_{19}N_2OS_2$: 343.0939).

2-(*n*-Butyldisulfanyl)-3-(4-chlorophenyl)-quinazolin-4(3*H*)-one (8b)

White solid; yield 57.8%; m.p.: 104.9–106.5 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.87 (t, $J = 8.0$ Hz, 3H, CH_3), 1.35–1.47 (m, 2H, CH_2), 1.57–1.64 (m, 2H, CH_2), 2.94 (t, $J = 8.0$ Hz, 2H, CH_2), 7.54 (t, $J = 8.0$ Hz, 1H, Ar–H), 7.60 (d, $J = 8.0$ Hz, 2H, Ar–H), 7.68 (t, $J = 8.0$ Hz, 3H, Ar–H), 7.89 (t, $J = 8.0$ Hz, 1H, Ar–H), 8.11 (d, $J = 8.0$ Hz, 1H, Ar–H); ^{13}C NMR(100 MHz, DMSO-d_6) δ (ppm): 13.92, 21.31, 30.57, 37.83, 120.35, 126.90, 127.01, 127.13, 130.14 (2C), 132.02 (2C), 134.94, 135.40, 135.50, 147.65,

155.76, 161.24; IR (KBr, cm^{-1}): 1682, 1578, 1556, 770, 510 cm^{-1}; HR-MS (ESI): *m/z* 377.0566 $[M+H]^+$ (calcd. for $C_{18}H_{18}N_2OS_2Cl$: 377.0549).

2-(*n*-Butyldisulfanyl)-3-(4-methylphenyl)-quinazolin-4(3*H*)-one (8c)

White solid; yield 64.8%; m.p.: 81.7–83.4 °C; ^{1}1H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.86 (t, $J = 4.0$ Hz, 3H, CH_3), 1.36–1.45 (m, 2H, CH_2), 1.55–1.63 (m, 2H, CH_2), 2.42 (s, 3H, CH_3), 2.92 (t, $J = 4.0$ Hz, 2H, CH_2), 7.37 (s, 4H, Ar–H), 7.52 (t, $J = 8.0$ Hz, 1H, Ar–H), 7.68 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.87 (t, $J = 8.0$ Hz, 1H, Ar–H), 8.10 (d, $J = 8.0$ Hz, 1H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 13.92, 21.28, 21.34, 30.54, 37.73, 120.33, 126.89, 126.97, 127.14, 129.71 (2C), 130.56 (2C), 133.33, 135.45, 140.41, 147.68, 156.36, 161.33; IR (KBr, cm^{-1}): 1687, 1578, 1556, 764, 514 cm^{-1}; HR-MS (ESI): *m/z* 357.1112 $[M+H]^+$ (calcd. for $C_{19}H_{21}N_2OS_2$: 357.1095).

2-(*n*-Butyldisulfanyl)-3-(4-trifluoromethylphenyl)-quinazolin-4(3*H*)-one (8d)

White solid; yield 57.6%; m.p.: 122.3–124.6 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.87 (t, $J = 8.0$ Hz, 3H, CH_3), 1.38–1.47 (m, 2H, CH_2), 1.58–1.65 (m, 2H, CH_2), 2.95 (t, $J = 8.0$ Hz, 2H, CH_2), 7.55 (t, $J = 8.0$ Hz, 1H, Ar–H), 7.71 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.83 (d, $J = 8.0$ Hz, 2H, Ar–H), 7.90 (t, $J = 8.0$ Hz, 1H, Ar–H), 8.00 (d, $J = 8.0$ Hz, 1H, Ar–H), 8.12 (d, $J = 8.0$ Hz, 1H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 13.90, 21.30, 30.55, 37.85, 120.37, 126.29 ($J = 271.0$ Hz), 126.95, 127.11, 127.16 (2C, $J = 4.0$ Hz), 127.15, 130.92 (2C, $J = 32.0$ Hz), 131.34, 135.60, 139.82, 147.64, 155.26, 161.24; IR (KBr, cm^{-1}): 1683, 1578, 1557, 770, 507 cm^{-1}; HR-MS (ESI): *m/z* 411.0832 $[M+H]^+$ (calcd. for $C_{19}H_{18}N_2OS_2F_3$: 411.0813).

2-(*n*-Butyldisulfanyl)-3-(4-nitrophenyl)-quinazolin-4(3*H*)-one (8e)

White solid; yield 42.7%; m.p.: 159–160.5 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.87 (t, $J = 8.0$ Hz, 3H, CH_3), 1.38–1.47 (m, 2H, CH_2), 1.58–1.65 (m, 2H, CH_2), 2.96 (t, $J = 8.0$ Hz, 2H, CH_2), 7.56 (t, $J = 8.0$ Hz, 1H, Ar–H), 7.71 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.91 (d, $J = 8.0$ Hz, 3H, Ar–H), 8.12 (d, $J = 8.0$ Hz, 1H, Ar–H), 8.44 (d, $J = 8.0$ Hz, 2H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 13.92, 21.29, 30.55, 37.88, 120.36, 125.27 (2C), 126.97, 127.16, 127.18, 131.92 (2C), 135.68, 141.83, 147.60, 148.81, 154.90, 161.17; IR (KBr, cm^{-1}): 1686, 1576, 1558, 767, 521 cm^{-1}; HR-MS (ESI): *m/z* 388.0808 $[M+H]^+$ (calcd. for $C_{18}H_{18}N_3O_3S_2$: 388.0789).

2-(*n*-Butyldisulfanyl)-3-(4-methoxyphenyl)-quinazolin-4(3*H*)-one (8f)

White solid; yield 66.5%; m.p.: 140.8–141.9 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.87 (t, $J = 8.0$ Hz, 3H, CH_3), 1.37–1.47 (m, 2H, CH_2), 1.57–1.64 (m, 2H, CH_2), 2.93 (t, $J = 4.0$ Hz, 2H, CH_2), 3.85 (s, 3H, CH_3), 7.11 (d, $J = 8.0$ Hz, 2H, Ar–H), 7.42 (d, $J = 8.0$ Hz, 2H, Ar–H), 7.53 (t, $J = 8.0$ Hz, 1H, Ar–H), 7.68 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.87 (t, $J = 8.0$ Hz, 1H, Ar–H), 8.10 (d, $J = 8.0$ Hz, 1H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 13.94, 21.30, 30.56, 37.70, 55.96, 115.18 (2C), 120.37, 126.87, 126.90, 127.15, 128.33, 131.26 (2C), 135.39, 147.71, 156.78, 160.75, 161.47; IR (KBr, cm^{-1}): 1685, 1575, 1553, 775, 528 cm^{-1}; HR-MS (ESI): *m/z* 373.1060 $[M+H]^+$ (calcd. for $C_{19}H_{21}N_2O_2S_2$: 373.1044).

2-(*n*-Butyldisulfanyl)-3-(3-chlorophenyl)-quinazolin-4(3*H*)-one (8g)

White solid; yield 53.7%; m.p.: 94.7–95.8 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.87 (t, $J = 4.0$ Hz, 3H, CH_3), 1.38–1.46 (m, 2H, CH_2), 1.57–1.64 (m, 2H, CH_2), 2.94 (t, $J = 8.0$ Hz, 2H, CH_2), 7.51–7.56 (m, 2H, Ar–H), 7.60–7.70 (m, 3H, Ar–H), 7.77 (s, 1H, Ar–H), 7.88 (t, $J = 8.0$ Hz, 1H, Ar–H), 8.10 (d, $J = 8.0$ Hz, 1H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 13.94, 21.32, 30.59, 37.87, 120.39, 126.93, 127.07, 127.14, 129.06, 130.17, 130.77, 131.60, 134.07, 135.56, 137.41, 147.63, 155.61, 161.21; IR (KBr, cm^{-1}): 1693, 1577, 1554, 771, 520 cm^{-1}; HR-MS (ESI): *m/z* 377.0560 $[M+H]^+$ (calcd. for $C_{18}H_{18}N_2OS_2Cl$: 377.0549).

2-(*n*-Butyldisulfanyl)-3-(3-methylphenyl)-quinazolin-4(3*H*)-one (8h)

White solid; yield 46.3%; m.p.: 119.9–121.7 °C; ^{1}1H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.83 (t, $J = 4.0$ Hz, 3H, CH_3), 1.34–1.43 (m, 2H, CH_2), 1.53–1.61 (m, 2H, CH_2), 2.36 (s, 3H, CH_3), 2.90 (t, $J = 4.0$ Hz, 2H, CH_2), 7.29 (d, $J = 8.0$ Hz, 2H, Ar–H), 7.37 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.43–7.50 (m, 2H, Ar–H), 7.68 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.83 (t, $J = 8.0$ Hz, 1H, Ar–H), 8.09 (d, $J = 8.0$ Hz, 1H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6) δ(ppm): 13.92, 21.25, 21.37, 30.63, 37.88, 120.33, 126.88 (2C), 126.95, 127.11, 129.81, 130.20, 131.23, 135.32, 135.87, 139.69, 147.68, 156.20, 161.19; IR (KBr, cm^{-1}): 1695, 1577, 1556, 768, 536 cm^{-1}; HR-MS (ESI): *m/z* 357.1112 $[M+H]^+$ (calcd. for $C_{19}H_{21}N_2OS_2$: 357.1095).

2-(*i*-Butyldisulfanyl)-3-phenyl-quinazolin-4(3*H*)-one (9a)

White solid; yield 53.1%; m.p.: 90.5–91.6 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.99 (d, $J = 4.0$ Hz, 6H,

CH_3), 1.83–1.93 (m, 1H, CH), 2.81 (d, $J = 4.0$ Hz, 2H, CH_2), 7.50–7.60 (m, 6H, Ar–H), 7.69 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.89 (t, $J = 8.0$ Hz, 1H, Ar–H), 8.11 (d, $J = 8.0$ Hz, 1H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 21.95 (2C), 27.91, 47.34, 120.38, 126.88, 126.98, 127.14, 130.02 (2C), 130.03 (2C), 130.62, 135.47, 135.99, 147.68, 156.11, 161.26; IR (KBr, cm^{-1}): 1687, 1577, 1555, 770, 496 cm^{-1}; HR-MS (ESI): *m/z* 343.0955 $[M+H]^+$ (calcd. for $C_{18}H_{19}N_2OS_2$: 343.0939).

2-(*i*-Butyldisulfanyl)-3-(4-chlorophenyl)-quinazolin-4(3*H*)-one (9b)

White solid; yield 50.3%; m.p.: 100.8–102.1 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.99 (d, $J = 4.0$ Hz, 6H, CH_3), 1.84–1.94 (m, 1H, CH), 2.82 (d, $J = 8.0$ Hz, 2H, CH_2), 7.54 (t, $J = 8.0$ Hz, 1H, Ar–H), 7.59 (d, $J = 8.0$Hz, 2H, Ar–H), 7.68 (t, $J = 8.0$ Hz, 3H, Ar–H), 7.89 (t, $J = 8.0$ Hz, 1H, Ar–H), 8.11 (d, $J = 8.0$ Hz, 1H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 21.94 (2C), 27.89, 47.33, 120.36, 126.90, 127.01, 127.14, 130.14 (2C), 132.04 (2C), 134.94, 135.39, 135.52, 147.65, 155.75, 161.25; IR (KBr, cm^{-1}): 1696, 1579, 1555, 765, 508 cm^{-1}; HR-MS (ESI): *m/z* 377.0566 $[M+H]^+$ (calcd. for $C_{18}H_{18}N_2OS_2Cl$: 377.0549).

2-(*i*-Butyldisulfanyl)-3-(4-methylphenyl)-quinazolin-4(3*H*)-one (9c)

White solid; yield 54.1%; m.p.: 107.7–109.1 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.98 (d, $J = 8.0$ Hz, 6H, CH_3), 1.82–1.90 (m, 1H, CH), 2.41 (s, 3H, CH_3), 2.79 (d, $J = 8.0$ Hz, 2H, CH_2), 7.37 (s, 4H, Ar–H), 7.51 (t, $J = 8.0$ Hz, 1H, Ar–H), 7.68 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.86 (t, $J = 8.0$ Hz, 1H, Ar–H), 8.10 (d, $J = 8.0$ Hz, 1H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 21.35, 21.94 (2C), 27.89, 47.29, 120.33, 126.87, 126.93, 127.15, 129.71 (2C), 130.56 (2C), 133.32, 135.42, 140.38, 147.69, 156.37, 161.32; IR (KBr, cm^{-1}): 1689, 1577, 1555, 770, 514 cm^{-1}; HR-MS (ESI): *m/z* 357.1110 $[M+H]^+$ (calcd. for $C_{19}H_{21}N_2OS_2$: 357.1095).

2-(*i*-Butyldisulfanyl)-3-(4-trifluoromethylphenyl)-quinazolin-4(3*H*)-one (9d)

White solid; yield 59.1%; m.p.: 126.8–127.9 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.00 (d, $J = 8.0$ Hz, 6H, CH_3), 1.85–1.95 (m, 1H, CH), 2.83 (d, $J = 8.0$ Hz, 2H, CH_2), 7.55 (t, $J = 8.0$ Hz, 1H, Ar–H), 7.71 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.83 (d, $J = 8.0$ Hz, 2H, Ar–H), 7.90 (t, $J = 8.0$ Hz, 1H, Ar–H), 7.99 (d, $J = 8.0$ Hz, 2H, Ar–H), 8.12 (d, $J = 8.0$ Hz, 1H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 21.93 (2C), 27.86, 47.36, 120.39, 124.30($J = 271.0$ Hz), 126.95, 127.10, 127.22 (2C, $J = 6.0$ Hz), 127.16, 130.93 (2C, $J = 32.0$ Hz), 131.36, 135.62, 139.83, 147.65, 155.26, 161.25; IR (KBr, cm^{-1}): 1687, 1577, 1555, 774, 508 cm^{-1}; HR-MS (ESI): *m/z* 411.0832 $[M+H]^+$ (calcd. for $C_{19}H_{18}N_2OS_2F_3$: 411.0813).

2-(*i*-Butyldisulfanyl)-3-(4-nitrophenyl)-quinazolin-4(3*H*)-one (9e)

White solid; yield 55.6%; m.p.: 143.3–144.6 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.00 (d, $J = 8.0$ Hz, 6H, CH_3), 1.87–1.95 (m, 1H, CH), 2.84 (d, $J = 8.0$ Hz, 2H, CH_2), 7.56 (t, $J = 8.0$ Hz, 1H, Ar–H), 7.71 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.90 (d, $J = 8.0$ Hz, 3H, Ar–H), 8.12 (d, $J = 4.0$ Hz, 1H, Ar–H), 8.44 (d, $J = 8.0$ Hz, 2H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 21.92(2C), 27.84, 47.29, 120.37, 125.29 (2C), 126.97, 127.17, 127.19, 131.93 (2C), 135.72, 141.83, 147.61, 148.83, 154.90, 161.19; IR (KBr, cm^{-1}): 1688, 1577, 1557, 766, 524 cm^{-1}; HR-MS (ESI): *m/z* 388.0807 $[M+H]^+$ (calcd. for $C_{18}H_{18}N_3O_3S_2$: 388.0789).

2-(*i*-Butyldisulfanyl)-3-(4-methoxyphenyl)-quinazolin-4(3*H*)-one (9f)

White solid; yield 43.9%; m.p.: 143.9–145.1 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.99 (d, $J = 4.0$ Hz, 6H, CH_3), 1.83–1.93 (m, 1H, CH), 2.81 (d, $J = 8.0$ Hz, 2H, CH_2), 3.85 (s, 3H, CH_3), 7.11 (d, $J = 8.0$ Hz, 2H, Ar–H), 7.42 (d, $J = 8.0$ Hz, 2H, Ar–H), 7.53(t, $J = 8.0$ Hz, 1H, Ar–H), 7.68 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.88 (t, $J = 8.0$ Hz, 1H, Ar–H), 8.10 (d, $J = 8.0$ Hz, 1H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 21.96 (2C), 27.89, 47.24, 55.96, 115.17 (2C), 120.37, 126.86, 126.89, 127.16, 128.32, 131.27 (2C), 135.40, 147.72, 156.78, 160.75, 161.48; IR (KBr, cm^{-1}): 1690, 1576, 1552, 779, 529 cm^{-1}; HR-MS (ESI): *m/z* 373.1057 $[M+H]^+$ (calcd. for $C_{19}H_{21}N_2O_2S_2$: 373.1044).

2-(*i*-Butyldisulfanyl)-3-(3-chlorophenyl)-quinazolin-4(3*H*)-one (9g)

White solid; yield 57.6%; m.p.: 114.4–115.3 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 1.00 (d, $J = 8.0$ Hz, 6H, CH_3), 1.83–1.94 (m, 1H, CH), 2.82 (d, $J = 4.0$ Hz, 2H, CH_2), 7.52–7.56 (m, 2H, Ar–H), 7.60–7.70 (m, 3H, Ar–H), 7.77 (s, 1H, Ar–H), 7.89 (t, $J = 8.0$ Hz, 1H, Ar–H), 8.11 (d, $J = 8.0$ Hz, 1H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm):21.95, 21.98, 27.92, 47.37, 120.38, 126.92, 127.06, 127.15, 129.07, 130.17, 130.77, 131.60, 134.05, 135.58, 137.39, 147.63, 155.61, 161.22; IR (KBr, cm^{-1}): 1694, 1577, 1555, 769, 520 cm^{-1}; HR-MS (ESI): *m/z* 377.0560 $[M+H]^+$ (calcd. for $C_{18}H_{18}N_2OS_2Cl$: 377.0549).

2-(*i*-Butyldisulfanyl)-3-(3-methylphenyl)-quinazolin-4(3*H*)-one (9h)

White solid; yield 66.7%; m.p.: 120.3–121.3 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 0.99 (d, $J = 4.0$ Hz, 6H, CH_3), 1.83–1.93 (m, 1H, CH), 2.38 (s, 3H, CH_3), 2.81 (d, $J = 8.0$ Hz, 2H, CH_2), 7.29 (d, $J = 8.0$ Hz, 2H, Ar–H), 7.39 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.44–7.55 (m, 2H, Ar–H), 7.68 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.88 (t, $J = 8.0$ Hz, 1H, Ar–H), 8.10 (d, $J = 8.0$ Hz, 1H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 21.24, 21.94, 21.98, 27.94, 47.35, 120.34, 126.89, 126.98 (2C), 127.13, 129.83, 130.20, 131.28, 135.45, 135.86, 139.69, 147.67, 156.19, 161.24; IR (KBr, cm^{-1}): 1698, 1577, 1558, 788, 545 cm^{-1}; HR-MS (ESI): *m/z* 357.1111 $[M+H]^+$ (calcd. for $C_{19}H_{21}N_2OS_2$: 357.1095).

Cell culture

Compounds **7a**–**7h**, **8a**–**8h** and **9a**–**9h**, 5-fluorouracil was dissolved in DMSO to make stock solutions at a concentration of 1.0×10^{-2} mol/L. During the experiment, cell culture medium RPMI-1640 was used to dilute the stock solution to the desired concentration. Cells in the exponential phase were seeded in 96-well culture plates at the confluence of 1×10^4 cells/well, kept in a 37 °C, 5% CO_2 incubator for 24 h. The medium containing different concentrations of compounds was replaced with a fresh medium, and kept in a 37 °C, 5% CO_2 incubator for 48 h. Then, 90 μL of fresh medium and 10 μL of CCK-8 were added, kept at 37 °C and 5% CO_2 for 1 h. The sample cell was added into 96-well microplate reader and read at 450 nm, and the absorbance value (optical density (OD)) was recorded. Cell viability was calculated from the mean values for three wells using the following formula: Relative cell viability = (OD value for the test group-blank OD) / (control OD value-blank OD value) × 100%.

Bacterial culture

Gram-negative bacteria *E. coli* and Gram-positive bacteria *S. aureus* were employed as the representative bacteria for testing the antibacterial activities of the constructed systems. In brief, the bacteria were cultured in liquid Luria–Bertani (LB) medium (5 mL) on a shaking incubator (37 °C, 170 rpm) overnight. Herein, Gram-negative bacteria *E. coli* and Gram-positive bacteria *S. aureus* were referred to the corresponding resistant variants of the *E. coli* and *S. aureus*, respectively. The concentration of the bacteria was quantified on the basis of the measurement of OD at $\lambda = 600$ nm (note that OD600 of 2.0 was determined to have a concentration of 109 colony-forming units per milliliter (CFU/mL) based on calibration).

Determination of MIC

Aiming to determine the MIC, a stock solution of Gram-negative bacteria *E. coli* and Gram-positive bacteria *S. aureus* was prepared. Briefly, Gram-negative bacteria *E. coli* and Gram-positive bacteria *S. aureus* were cultured in 5 mL of liquid LB (supplemented with 50 μg/mL) broth at 37 °C on a constant temperature shaker at 170 rpm. After incubation overnight, the concentration of the bacterial suspension was diluted with pure nutrient broth to ~10^5 CFU/mL and measured with a UV-3900. A sample of compounds **7a**–**7h**, **8a**–**8h** and **9a**–**9h** was dissolved in deionized water and diluted from 512 to 2 μg/mL by a series of twofold dilutions using the nutrient broth. The diluted bacterial solution (100 μL) and the samples (1 mL) with varying concentration were incubated at 37 °C in glass culture tubes, respectively. After incubation for 16 h, the viability of bacteria was determined by measuring OD600 on a UV-3390 instrument. In comparison to a positive control, the MIC was defined as the lowest polymer concentration that inhibited more than 90% of the germ growth.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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