Regular Article

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Design, Synthesis and Antiproliferative Evaluation of Novel Disulfides Containing 1,3,4-Thiadiazole Moiety

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A series of novel disulfides containing 1,3,4-thiadiazole moiety were designed, synthesized, and the structures of all products were identified by spectral data (IR, NMR, and high resolution (HR)-MS). Their *in vitro* antiproliferative activities were evaluated using 2-(2-methoxy-4-nitro-phenyl)-3-(4-nitro-phenyl)-5-(2,4-disulfopheyl)-2*H*-tetrazolium monosodium salt (CCK-8) assay against human cancer cell lines, A549 (human lung cancer cell), HeLa (human cervical cancer cell), SMMC-7721 (human liver cancer cell) and normal cell lines L929. The bioassay results indicated that most of the tested compounds 6a–k, 7a–k and 8a–k exhibited antiproliferation with different degrees, and some compounds showed better effects than positive control 5-fluorouracil (5-FU) against various cancer cell lines. Among these compounds, compound 6e exhibited the most potent inhibitory activity against A549 cells with IC_{50} value of 3.62 μM. Compounds 6i, 7a, 7g, 8a and 8b showed significantly antiproliferative activities against HeLa cells with IC_{50} values of 3.88, 3.76, 3.59, 3.38 and 3.12 μM, respectively. Compounds 6a, 7a and 8a owned high antiproliferative activities against SMMC-7721 cells with IC_{50} values of 2.54, 2.69 and 2.31 μM, respectively. Furthermore, all of the tested compounds showed weak cytotoxic effect against the normal cell lines L929. Based on the preliminary results, the substituent groups are vital for improving the potency and selectivity of this class of compounds.

Key words 1,3,4-thiadiazole; disulfide; antiproliferative activity

Cancer has become one of the most terrible diseases around the world because of its low cure and high mortality.[1,2] It is predicted that the number of new cases could further rise to 19.3 million by 2025.[3,4] Despite significant progress has been achieved in anticancer therapy, identification of safe and effective anticancer agents are still one of the challenges in the field of drug discovery.

1,3,4-Thiadiazoles are an important class of heterocyclic bioactive compounds with a broad range of biological activities such as antioxidant,[5,6] antibacterial,[7,8] antidepressant,[9,10] antidiabetic,[11,12] antifungal,[13,14] anticonvulsant,[15,16] anti-inflammatory,[17,18] anticholinesterase,[19] antileishmanial,[20] inhibition of human protoporphyrinogen oxidase.[21] In particular, a large number of 1,3,4-thiadiazole derivatives display interesting antitumour activities *in vitro* and *in vivo* conditions.[22–26] Therefore, the importance of 1,3,4-thiadiazole in the fields of medicinal chemistry has received much attention in the past few decades.[27] On the other hand, disulfide derivatives are also acknowledged to possess a wide spectrum of bioactivities because the disulfide group makes up the core structure of numerous biologically active compounds with many types of biological activities including antibacterial,[28,29] anti-human immunodeficiency virus (HIV)-1,[30] antitumor,[31–33] and herbicidal properties.[34,35]

Particularly, the antitumor character of disulfide derivatives attracts great interests from medicinal chemists in recent years. Moreover, the metabolic evaluation of the most promising compound is further performed to reveal that disulfide bond can be stable in human plasma over 8 h, indicating a good prospect of these compounds for *in vitro* antitumor activities.[36]

We previously reported the synthesis of a series of disulfides bearing 1,3,4-oxadiazole moiety and their *in vitro* antiproliferative activities were evaluated.[37] We observed that some compounds showed better effects than positive control 5-fluorouracil against various cancer cell lines. Due to their attractive pharmacological property, in the paper we report the synthesis of disulfides containing 1,3,4-thiadiazole moiety. These compounds were evaluated for their antiproliferative activities against three human cancer cell lines, A549 (human lung cancer cell), HeLa (human cervical cancer cell), SMMC-7721 (human liver cancer cell) and one normal cell line L929 (Mouse fibroblasts) with the ultimate aim of developing novel potent antitumor agents.

Results and Discussion

Chemistry As depicted in Chart 1, thirty three disulfides containing 1,3,4-thiadiazole moiety were synthesized and reported for the first time. The preparation of *S*-alkyl-thioisothiourea hydrochloride **2** was carried out by reported literature method.[38] The preparation of 2-chloro-*N*-(substituted-phenyl)-acetamide **4** was carried out by reported literature method.[39] The reaction of commercially available 2,5-dimercapto-1,3,4-thiadiazole with compound **4** in the presence of NaOH in water and ethanol at room temperature yielded the desired intermediates **5a**–**k** in 70–81% yields. Finally, the target compounds **6a**–**k**, **7a**–**k** and **8a**–**k** were successfully obtained by the reaction of intermediates **5a**–**k** and compound **2** in the presence of $NaHCO_3$ in methanol and water at room temperature. All of the novel synthesized compounds **6a**–**k**, **7a**–**k** and **8a**–**k** 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 (HR-ESI)-MS.

Biological Activity The antiproliferative activities of

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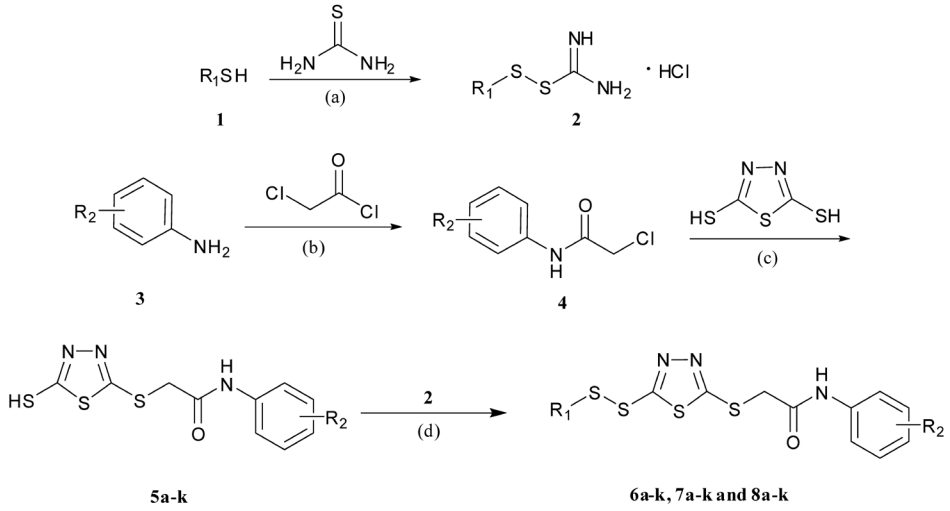

R_2 (R_1=2-C_4H_9): H (**6a**), 2-Cl (**6b**), 3-Cl (**6c**), 4-Cl (**6d**), 3-NO_2 (**6e**), 4-NO_2 (**6f**), 2-OCH_3 (**6g**), 4-OCH_3 (**6h**), 4-F (**6i**), 4-CF_3 (**6j**), 4-CH_3 (**6k**);
R_2 (R_1=n-C_4H_9): H (**7a**), 2-Cl (**7b**), 3-Cl (**7c**), 4-Cl (**7d**), 3-NO_2 (**7e**), 4-NO_2 (**7f**), 2-OCH_3 (**7g**), 4-OCH_3 (**7h**), 4-F (**7i**), 4-CF_3 (**7j**), 4-CH_3 (**7k**);
R_2 (R_1=i-C_4H_9): H (**8a**), 2-Cl (**8b**), 3-Cl (**8c**), 4-Cl (**8d**), 3-NO_2 (**8e**), 4-NO_2 (**8f**), 2-OCH_3 (**8g**), 4-OCH_3 (**8h**), 4-F (**8i**), 4-CF_3 (**8j**), 4-CH_3 (**8k**).

Reagents and conditions: (a) conc. HCl, H_2O_2 (30%), 0–5°C, 6 h; (b) CH_2Cl_2–NaOH, r.t. 5–6 h; (c) ethanol, NaOH–H_2O, r.t. 4–5 h; (d) ethanol, $NaHCO_3$–H_2O, r.t. 6 h.

Chart 1. Synthesis of the Target Compounds **6a**–**k**, **7a**–**k** and **8a**–**k**

target compounds **6a**–**k**, **7a**–**k** and **8a**–**k** against three human cancer cell lines, A549, HeLa, SMMC-7721 and one normal cell line L929 were evaluated by 2-(2-methoxy-4-nitrophenyl)-3-(4-nitro-phenyl)-5-(2,4-disulfopheyl)-2*H*-tetrazolium monosodium salt (CCK-8) assay *in vitro*, and their IC_{50} values were calculated and are presented in Table 1. 5-Fluorouracil (5-FU) was used as a positive control.

As shown in Table 1, some of the tested compounds revealed stronger antiproliferative effect than previous reported compounds against various cancer cell lines.[37] All compounds **6a**–**k**, **7a**–**k** and **8a**–**k** showed antiproliferation against four cell lines with certain degrees, and the substituent groups of phenyl ring played important roles in the potency of biologically active compounds. In A549 cells, some compounds exhibited better activities than positive control 5-FU. In particular, compound **6e** exhibited the most potent inhibitory activity against A549 cells with IC_{50} value of 3.62 μM. As compared with compound **6a** which has no substituent at the phenyl ring while R_1 is 2-butyl group, except compounds **6f**, **j** and **k**, the other compounds showed enhanced antiproliferative activities. As compared with compound **7a** which has no substituent at the phenyl ring while R_1 is *n*-butyl group, compounds **7g**, **h** and **k** carrying electron-donating substituents exhibited increased activities. As compared with compound **8a** which has no substituent at the phenyl ring while R_1 is *i*-butyl group, except compound **8e**, electron-donating group substituted derivatives, including **8g**, **h** and **k** displayed better activities than compounds **8b**–**f**, **8i** and **j** carrying electron-drawing substituents. Especially, while R_1 is the same substituent, 3-nitro substituted derivatives **6e**, **7e** and **8e** displayed higher activities than other compounds. These results indicated that the influence of substituents on antiproliferative activity against A549 cells is complex and unexplained at present. In HeLa cells, except compounds **6b** and **7f** showed moderate antiproliferative activity with IC_{50} values of 20.89 and 27.36 μM, respectively, the other compounds showed good antiproliferative effect, and displayed higher activities than positive control 5-FU. Among them, compounds **6i**, **7a**, **g**, **8a** and **b** showed significantly biological activities with IC_{50} values of 3.88, 3.76, 3.59, 3.38 and 3.12 μM, respectively. As compared with compounds **6a**, **7a** and **8a** which have no substituent at the phenyl ring, except compounds **6i**, **7g**, **h** and **8b**, the other compounds **6b**–**k**, **7b**–**k** and **8b**–**k** all resulted in a decrease in activity. But, no matter R_1 is 2-butyl, *n*-butyl or *i*-butyl group, the electronic effects of substituents at the phenyl ring on antiproliferative activity against HeLa cells did not show apparent regularity. In SMMC-7721 cells, compounds **6a**, **e**–**i**, **7a**, **b**, **d**, **e**, **8a** and **e** displayed good proliferation inhibitory activities, the other compounds showed lower antiproliferative effect than positive control 5-FU. As compared with **6a**, **7a** and **8a** which have no substituent at phenyl ring and owned high antiproliferative activities with IC_{50} values of 2.54, 2.69 and 2.31 μM respectively, the other compounds bearing electron-donating or electron-withdrawing groups, including **6b**–**k**, **7b**–**k** and **8b**–**k**, all exhibited decreased antitumor effects. However, the bioassay results also indicated that the electronic effects of substituents on antitumor activity against SMMC-7721 cells did not show apparent regularity. In L929 cells, all compounds exhibited weak cytotoxic activity, and showed lower cytotoxic effect than positive control 5-FU. Especially noteworthy are compounds **6g**, **i**, **7b** and **8a**, which showed significant antiproliferative activities against A549, HeLa and SMMC-7721 cells, exhibited weak cytotoxicities against L929 cells. The results suggested that compounds **6g**, **i**, **7b** and **8a** displayed superior selectivity against the tested cancer cell lines. Moreover, we also found from the results that the 2-butyl substituted derivatives showed better biological activities than *n*-butyl and *i*-butyl substituted derivatives on the whole. 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.

Table 1. *In Vitro* Antiproliferative Activities of Compounds **6a**–**k**, **7a**–**k** and **8a**–**k** against Various Cell Lines

Compound	IC_{50} (μM)[a]			
	A549	HeLa	SMMC-7721	L929
6a	12.65±0.35	4.77±0.06	2.54±0.10	18.28±1.26
6b	9.50±0.24	20.89±0.17	8.21±0.30	62.51±2.85
6c	8.02±0.19	6.11±0.12	18.71±1.28	55.17±3.08
6d	8.26±0.20	5.48±0.08	6.08±0.57	7.81±0.03
6e	3.62±0.06	8.16±0.23	3.74±0.12	5.44±0.12
6f	17.63±0.51	8.07±0.45	4.21±0.22	6.72±0.17
6g	8.69±0.27	6.86±0.37	3.00±0.04	9.91±0.37
6h	8.00±0.38	8.23±0.51	4.49±0.34	33.08±2.17
6i	6.53±0.15	3.88±0.11	3.53±0.26	23.50±0.59
6j	25.86±0.81	4.91±0.07	9.78±0.39	34.60±1.20
6k	19.90±0.26	8.04±0.30	6.50±0.45	18.13±0.38
7a	12.76±1.06	3.76±0.13	2.69±0.22	68.79±2.45
7b	8.71±0.28	6.17±0.02	5.26±0.81	69.76±1.95
7c	8.51±0.32	9.62±0.39	15.43±0.73	67.88±2.06
7d	23.27±0.78	8.93±0.45	4.34±0.35	29.77±1.92
7e	7.70±0.13	6.76±0.24	4.32±0.29	13.69±0.56
7f	22.12±0.57	27.36±1.58	18.06±0.18	18.54±1.78
7g	8.17±0.25	3.59±0.12	6.40±0.25	29.14±0.76
7h	7.84±0.30	4.70±0.15	7.29±0.34	38.65±1.68
7i	25.90±1.08	6.85±0.76	7.55±0.13	31.74±0.97
7j	28.49±1.23	16.10±0.81	26.33±1.67	36.51±2.07
7k	12.19±0.36	12.33±1.02	25.40±0.76	38.17±1.76
8a	8.56±0.21	3.38±0.05	2.31±0.27	57.20±2.58
8b	24.42±0.57	3.12±0.32	7.85±0.36	63.51±1.89
8c	26.72±1.08	9.23±0.23	27.09±1.34	29.32±2.67
8d	29.52±0.89	9.82±0.17	9.23±0.32	8.06±0.33
8e	7.71±0.01	5.80±0.14	3.92±0.05	8.70±0.04
8f	25.47±0.91	11.52±0.25	11.19±0.51	7.41±0.12
8g	9.80±0.38	7.22±0.53	22.87±0.47	10.60±0.27
8h	8.71±0.29	6.19±0.18	7.32±0.55	26.70±0.36
8i	17.29±0.21	10.42±0.28	9.29±0.78	32.41±1.73
8j	20.02±0.17	8.44±0.12	25.17±1.23	29.45±0.67
8k	9.31±0.23	8.36±0.32	12.82±0.04	6.99±0.02
5-FU[b]	8.13±0.34	17.21±0.67	5.62±0.28	2.98±0.15

a) IC_{50} is the drug concentration effective in inhibiting 50% of the cell growth measured by CCK-8 assay. *b*) Used as a positive control.

Conclusion

We successfully prepared a series of novel disulfides containing 1,3,4-thiadiazole moiety, and the newly synthesized compounds were evaluated for their *in vitro* antiproliferative activities against A549, HeLa, and SMMC-7721 human cancer cell lines and L929 normal cell lines by CCK-8 assay. Some of the compounds inhibited the proliferation better than positive control 5-FU. In particular, compound **6e** exhibited the most potent inhibitory activity against A549 cells with IC_{50} value of 3.62 μM. Compounds **6i**, **7a**, **g**, **8a** and **b** displayed significantly antiproliferative activities against HeLa cells with IC_{50} values of 3.88, 3.76, 3.59, 3.38 and 3.12 μM respectively. Compounds **6a**, **7a** and **8a** owned high antiproliferative activities against SMMC-7721 cells with IC_{50} values of 2.54, 2.69 and 2.31 μM respectively. In addition, all the tested compounds showed low cytotoxic effect against the normal cell lines L929. Further structural optimization and mechanism studies on compounds **6a**–**k**, **7a**–**k** and **8a**–**k** are ongoing.

Experimental

Chemistry All reagents were obtained from commercial suppliers and used without further purification, unless specified. All 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- 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 dimethyl sulfoxide (DMSO)-d_6 as solvent and tetramethylsilane as internal standard. The chemical shifts for ^{1}H-NMR are 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), dd (doublet of doublet), *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 5a–k A solution of sodium hydroxide (6 mmol) in water (20 mL) was added to a mixture of 2,5-dimercapto-1,3,4-thiadiazole (6 mmol), appropriate 2-chloro-*N*-(substituted-phenyl)-acetamide **4** (5 mmol) and ethanol (10 mL). The mixture was stirred for 4–5 h at room temperature (the end of reaction was monitored by TLC), after completion, the mixture was poured into water. The resulting precipitate was collected by filtration, washed well with water and further purified by recrystallization from ethanol to afford target compounds.

2-Sulfhydryl-5-[(phenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**5a**)

White solid; Yield 76.7%; mp 136.5–138.7°C; IR (KBr) cm^{-1}: 3273, 2529, 1663, 1538, 1441, 757; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 14.59 (s, 1H, SH), 10.34 (s, 1H, NH), 7.57 (d, 2H, Ar-H, J=8.1 Hz), 7.33 (t, 2H, Ar-H, J=7.6 Hz), 7.08 (t, 1H, Ar-H, J=7.3 Hz), 4.17 (s, 2H, CH_2); MS-ESI (m/z): $C_{10}H_{10}N_3OS_3$ $[M+H]^+$ 284.0.

2-Sulfhydryl-5-[(2-chlorophenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**5b**)

White solid; Yield 75.9%; mp 132.7–134.6°C; IR (KBr) cm^{-1}: 3339, 2546, 1653, 1542, 1442, 752; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 14.63 (s, 1H, SH), 9.91 (s, 1H, NH), 7.72 (d, 1H, Ar-H, J=7.9 Hz), 7.51 (d, 1H, Ar-H, J=7.9 Hz), 7.35 (t, 1H, Ar-H, J=7.5 Hz), 7.22 (t, 1H, Ar-H, J=7.5 Hz), 4.22 (s, 2H, CH_2); MS-ESI (m/z): $C_{10}H_9N_3OS_3Cl$ $[M+H]^+$ 318.3.

2-Sulfhydryl-5-[(3-chlorophenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**5c**)

White solid; Yield 73.2%; mp 157.7–159.8°C; IR (KBr) cm^{-1}: 3362, 2529, 1645, 1549, 1429, 778; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 14.60 (s, 1H, SH), 10.61 (s, 1H, NH), 7.78 (s, 1H, Ar-H), 7.44 (d, 1H, Ar-H, J=8.4 Hz), 7.36 (t, 1H, Ar-H, J=8.0 Hz), 7.15 (d, 1H, Ar-H, J=7.8 Hz), 4.18 (s, 2H, CH_2); MS-ESI (m/z): $C_{10}H_9N_3OS_3Cl$ $[M+H]^+$ 318.3.

2-Sulfhydryl-5-[(4-chlorophenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**5d**)

White solid; Yield 80.7%; mp 164.8–166.4°C; IR (KBr) cm^{-1}: 3242, 2522, 1658, 1542, 1490, 744; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 14.59 (s, 1H, SH), 10.49 (s, 1H, NH), 7.60 (d, 2H, Ar-H, J=8.8 Hz), 7.38 (t, 2H, Ar-H, J=8.8 Hz), 4.18 (s, 2H, CH_2); MS-ESI (m/z): $C_{10}H_9N_3OS_3Cl$ $[M+H]^+$ 318.3.

2-Sulfhydryl-5-[(3-nitro-phenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**5e**)

Yellow solid; Yield 70.3%; mp 180.3–182.6°C; IR (KBr) cm^{-1}: 3339, 2548, 1681, 1548, 1432, 736; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 14.60 (s, 1H, SH), 10.85 (s, 1H, NH), 8.59 (s, 1H, Ar-H), 7.95 (d, 1H, Ar-H, J=7.9 Hz), 7.89 (d, 1H, Ar-H, J=8.2 Hz), 7.64 (t, 1H, Ar-H, J=8.2 Hz), 4.22 (s, 2H, CH_2); MS-ESI (m/z): $C_{10}H_9N_4O_3S_3$ $[M+H]^+$ 329.0.

2-Sulfhydryl-5-[(4-nitro-phenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**5f**)

Yellow solid; Yield 73.8%; mp 194.8–196.8°C; IR (KBr) cm^{-1}: 3323, 2541, 1696, 156, 1497, 751; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 14.61 (s, 1H, SH), 10.97 (s, 1H, NH), 8.24 (d, 2H, Ar-H, J=8.9 Hz), 7.82 (d, 2H, Ar-H, J=8.9 Hz), 4.22 (s, 2H, CH_2); MS-ESI (m/z): $C_{10}H_9N_4O_3S_3$ $[M+H]^+$ 329.0.

2-Sulfhydryl-5-[(2-methoxyphenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**5g**)

White solid; Yield 78.9%; mp 145.2–146.7°C; IR (KBr) cm^{-1}: 3302, 2540, 1668, 1543, 1489, 741; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 14.61 (s, 1H, SH), 9.58 (s, 1H, NH), 7.94 (d, 1H, Ar-H, J=8.0 Hz), 7.05–7.12 (m, 2H, Ar-H), 6.92 (t, 1H, Ar-H, J=7.1 Hz), 4.23 (s, 2H, CH_2), 3.84 (s, 3H, OCH_3); MS-ESI (m/z): $C_{11}H_{12}N_3O_2S_3$ $[M+H]^+$ 314.0.

2-Sulfhydryl-5-[(4-methoxyphenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**5h**)

White solid; Yield 74.5%; mp 157.2–158.5°C; IR (KBr) cm^{-1}: 3296, 2549, 1662, 1545, 1489, 721; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 14.60 (s, 1H, SH), 10.20 (s, 1H, NH), 7.47 (d, 2H, Ar-H, J=9.0 Hz), 6.90 (d, 2H, Ar-H, J=9.0 Hz), 3.84 (s, 2H, CH_2), 3.72 (s, 3H, OCH_3); MS-ESI (m/z): $C_{10}H_9N_4O_3S_3$ $[M+H]^+$ 314.0.

2-Sulfhydryl-5-[(4-fluorophenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**5i**)

White solid; Yield 72.1%; mp 170.2–172.1°C; IR (KBr) cm^{-1}: 3249, 2524, 1658, 1553, 1510, 715; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 14.60 (s, 1H, SH), 10.40 (s, 1H, NH), 7.57–7.60 (d, 2H, Ar-H, J=9.0 Hz), 7.17 (t, 2H, Ar-H, J=8.8 Hz), 4.16 (s, 2H, CH_2); MS-ESI (m/z): $C_{10}H_9N_3OS_3F$ $[M+H]^+$ 302.0.

2-Sulfhydryl-5-[(4-trifluoromethylphenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**5j**)

White solid; Yield 70.8%; mp 171.5–172.2°C; IR (KBr) cm^{-1}: 3440, 2539, 1670, 1546, 1489, 715; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 14.60 (s, 1H, SH), 10.71 (s, 1H, NH), 7.78 (d, 2H, Ar-H, J=8.4 Hz), 7.70 (d, 2H, Ar-H, J=8.4 Hz), 4.23 (s, 2H, CH_2); MS-ESI (m/z): $C_{11}H_9N_3OS_3F_3$ $[M+H]^+$ 352.0.

2-Sulfhydryl-5-[(4-methylphenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**5k**)

White solid; Yield 77.6%; mp 178.5–180.2°C; IR (KBr) cm^{-1}: 3268, 2532, 1654, 1538, 1495, 719; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 14.59 (s, 1H, SH), 10.25 (s, 1H, NH), 7.45 (d, 2H, Ar-H, J=8.3 Hz), 7.13 (d, 2H, Ar-H, J=8.2 Hz), 4.14 (s, 2H, CH_2), 2.26 (s, 3H, CH_3); MS-ESI (m/z): $C_{11}H_{12}N_3OS_3$ $[M+H]^+$ 298.0.

General Procedure for the Synthesis of Compounds 6a–k, 7a–k and 8a–k *S*-Alkyl-thioisothiourea hydrochloride **2** (2.2 mmol) and 2-sulfhydryl-5-(arylaminoformoxyl methylthio)-1,3,4-thiadiazol **5a–k** (2.0 mmol) were dissolved in 2 mL water and 15 mL ethanol. A solution of $NaHCO_3$ (3.0 mmol) in 4 mL water was added dropwise with vigorous stirring at room temperature. The mixture was stirred for additional 6h. The insoluble solid was collected and purified by silica gel column chromatography with petroleum ether–ethyl acetate (10 : 1, volume ratio) as eluent to afford the desired products.

2-(2-Butyldisulfanyl)-5-[(phenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**6a**)

Yellow oily liquid; Yield 75.6%; IR (KBr) cm^{-1}: 3446, 1684, 1558, 1507, 755, 503; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 10.37 (s, 1H, NH), 7.57 (d, 2H, Ar-H, J=7.8 Hz), 7.33 (t, 2H, Ar-H, J=7.7 Hz), 7.08 (t, 1H, Ar-H, J=7.4 Hz), 4.29 (s, 2H, CH_2), 3.12–3.21 (m, 1H, CH), 1.51–1.71 (m, 2H, CH_2), 1.29 (d, 3H, CH_3, J=6.8 Hz), 0.92 (t, 3H, CH_3, J=7.3 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 172.27, 167.01, 165.49, 139.13, 129.28 (2C), 124.12, 119.66 (2C), 49.37, 38.82, 28.75, 20.13, 11.59; HR-MS (ESI): m/z 438.2515 $[M+Na]^+$ (Calcd for $C_{14}H_{17}N_3OS_4Na$ 438.2505).

2-(2-Butyldisulfanyl)-5-[(2-chlorophenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**6b**)

Yellow oily liquid; Yield 72.8%; IR (KBr) cm^{-1}: 3447, 1699, 1588, 1523, 753, 526; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 9.95 (s, 1H, NH), 7.74 (d, 1H, Ar-H, J=8.0 Hz), 7.51 (d, 1H, Ar-H, J=8.0 Hz), 7.34 (t, 1H, Ar-H, J=8.4 Hz), 7.21 (t, 1H, Ar-H, J=7.5 Hz), 4.36 (s, 2H, CH_2), 3.13–3.21 (m, 1H, CH), 1.52–1.72 (m, 2H, CH_2), 1.30 (d, 3H, CH_3, J=6.8 Hz), 0.93 (t, 3H, CH_3, J=7.4 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 172.45, 166.85, 166.23, 134.97, 129.96, 127.91, 126.85, 126.43, 125.86, 49.40, 38.34, 28.79, 20.12, 11.59; HR-MS (ESI): m/z 427.9773 $[M+Na]^+$ (Calcd for $C_{14}H_{16}N_3OS_4NaCl$ 427.9763).

2-(2-Butyldisulfanyl)-5-[(3-chlorophenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**6c**)

Yellow oily liquid; Yield 76.7%; IR (KBr) cm^{-1}: 3447, 1684, 1595, 1541, 779, 526; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 10.57 (s, 1H, NH), 7.79 (s, 1H, Ar-H), 7.43 (d, 1H, Ar-H, J=8.3 Hz), 7.36 (t, 1H, Ar-H, J=7.9 Hz), 7.14 (d, 1H, Ar-H, J=7.8 Hz), 4.29 (s, 2H, CH_2), 3.12–3.20 (m, 1H, CH), 1.51–1.71 (m, 2H, CH_2), 1.29 (d, 3H, CH_3, J=6.8 Hz), 0.93 (t, 3H, CH_3, J=7.4 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 172.40, 166.74, 165.92, 140.55, 133.69, 130.91, 123.81, 119.16, 118.01, 49.39, 38.76, 28.77, 20.11, 11.57; HR-MS (ESI): m/z 427.9766 $[M+Na]^+$ (Calcd for $C_{14}H_{16}N_3OS_4NaCl$ 427.9763).

2-(2-Butyldisulfanyl)-5-[(4-chlorophenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**6d**)

White solid; Yield 74.8%; mp 65.1–67.6°C; IR (KBr) cm^{-1}: 3447, 1667, 1541, 1490, 746, 503; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 10.53 (s, 1H, NH), 7.62 (d, 2H, Ar-H, J=8.7 Hz), 7.33 (d, 2H, Ar-H, J=8.7 Hz), 4.30 (s, 2H, CH_2), 3.04–3.12 (m, 1H, CH), 1.46–1.67 (m, 2H, CH_2), 1.25 (d, 3H, CH_3, J=6.7 Hz), 0.88 (t, 3H, CH_3, J=7.3 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 172.41, 166.72, 165.62, 138.08, 129.11 (2C), 127.79, 121.14 (2C), 49.38, 38.78, 28.78, 20.09, 11.59; HR-MS (ESI): m/z 405.9958 $[M+H]^+$ (Calcd for $C_{14}H_{17}N_3OS_4Cl$ 405.9943).

2-(2-Butyldisulfanyl)-5-[(3-nitro-phenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**6e**)

White solid; Yield 71.7%; mp 93.1–94.5°C; IR (KBr) cm^{-1}: 3439, 1694, 1605, 1525, 741, 509; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 10.90 (s, 1H, NH), 8.61 (s, 1H, Ar-H), 7.94 (d, 1H, Ar-H, J=8.1 Hz), 7.88 (t, 1H, Ar-H, J=8.0 Hz), 7.63 (d, 1H, Ar-H, J=8.2 Hz), 4.34 (s, 2H, CH_2), 3.11–3.19 (m, 1H, CH), 1.50–1.70 (m, 2H, CH_2), 1.28 (d, 3H, CH_3, J=6.7 Hz), 0.91 (t, 3H, CH_3, J=7.4 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 172.43, 166.61, 166.31, 148.40, 140.23, 130.64, 125.51, 118.53, 113.71, 49.38, 38.68, 28.75, 20.07, 11.53; HR-MS (ESI): m/z 417.0198 $[M+H]^+$ (Calcd for $C_{14}H_{17}N_4O_3S_4$ 417.0183).

2-(2-Butyldisulfanyl)-5-[(4-nitro-phenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**6f**)

White solid; Yield 75.4%; mp 107.0–108.9°C; IR (KBr) cm^{-1}: 3441, 1680, 1563, 1511, 751, 497; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 10.98 (s, 1H, NH), 8.24 (d, 2H, Ar-H, J=9.2 Hz), 7.83 (d, 2H, Ar-H, J=9.2 Hz), 4.37 (s, 2H, CH_2), 3.11–3.19 (m, 1H, CH), 1.50–1.70 (m, 2H, CH_2), 1.29 (d, 3H, CH_3, J=6.8 Hz), 0.91 (t, 3H, CH_3, J=7.4 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 172.44, 166.47, 165.11, 145.18, 142.88, 125.28 (2C), 119.28 (2C), 49.39, 38.85, 28.77, 20.04, 11.53; HR-MS (ESI): m/z 417.0197 $[M+H]^+$ (Calcd for $C_{14}H_{17}N_4O_3S_4$ 417.0183).

2-(2-Butyldisulfanyl)-5-[(2-methoxyphenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**6g**)

Yellow oily liquid; Yield 70.1%; IR (KBr) cm^{-1}: 3364, 1687, 1602, 1536, 749, 512; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 9.62 (s, 1H, NH), 7.97 (d, 1H, Ar-H, J=8.0 Hz), 7.04–7.12 (m, 2H, Ar-H), 6.91 (t, 1H, Ar-H, J=8.1 Hz), 4.35 (s, 2H, CH_2), 3.84 (s, 3H, OCH_3), 3.12–3.21 (m, 1H, CH), 1.51–1.72 (m, 2H, CH_2), 1.30 (d, 3H, CH_3, J=6.8 Hz), 0.92 (t, 3H, CH_3, J=7.4 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 172.33, 167.16, 165.85, 149.80, 127.42, 125.12, 121.76, 120.77, 111.69, 56.20, 49.38, 38.63, 28.75, 20.12, 11.57; HR-MS (ESI): m/z 402.0448 $[M+H]^+$ (Calcd for $C_{15}H_{20}N_3O_2S_4$ 402.0438).

2-(2-Butyldisulfanyl)-5-[(4-methoxyphenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**6h**)

Yellow oily liquid; Yield 74.8%; IR (KBr) cm^{-1}: 3437, 1665, 1549, 1512, 745, 521; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 10.25 (s, 1H, NH), 7.48 (d, 2H, Ar-H, J=8.8 Hz), 6.89 (d, 2H, Ar-H, J=8.9 Hz), 4.25 (s, 2H, CH_2), 3.72 (s, 3H, OCH_3), 3.12–3.20 (m, 1H, CH), 1.51–1.71 (m, 2H, CH_2), 1.29 (d, 3H, CH_3, J=6.7 Hz), 0.92 (t, 3H, CH_3, J=7.3 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 172.25, 167.08, 164.96, 155.98, 132.27, 121.21 (2C), 114.41 (2C), 55.64, 49.36, 38.74, 28.75, 20.12, 11.58; HR-MS (ESI): m/z 402.0440 $[M+H]^+$ (Calcd for $C_{15}H_{20}N_3O_2S_4$ 402.0438).

2-(2-Butyldisulfanyl)-5-[(4-fluorophenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**6i**)

White solid; Yield 73.6%; mp 67.6–68.6°C; IR (KBr) cm^{-1}: 3444, 1666, 1558, 1508, 745, 515; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 10.44 (s, 1H, NH), 7.59 (dd, 2H, Ar-H, J_1=5.0 Hz, J_2=9.0 Hz), 7.17 (d, 2H, Ar-H, J=8.8 Hz), 4.28 (s, 2H, CH_2), 3.12–3.21 (m, 1H, CH), 1.51–1.74 (m, 2H, CH_2), 1.29 (d, 3H, CH_3, J=6.7 Hz), 0.92 (t, 3H, CH_3, J=7.32 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 172.33, 166.85, 165.40, 158.68 (d, J_{C-F}=239.1 Hz), 135.53 (d, J_{C-F}=2.3 Hz), 121.43 (d, 2C, J_{C-F}=7.8 Hz), 115.79 (d, 2C, J_{C-F}=22.1 Hz), 49.38, 38.73, 28.76, 20.08, 11.56; HR-MS (ESI): m/z 390.0245 $[M+H]^+$ (Calcd for $C_{14}H_{17}N_3OS_4F$ 390.0238).

2-(2-Butyldisulfanyl)-5-[(4-trifluoromethylphenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**6j**)

White solid; Yield 74.5%; mp 82.7–84.1°C; IR (KBr) cm^{-1}: 3446, 1676, 1606, 1543, 709, 506; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 10.75 (s, 1H, NH), 7.79 (d, 2H, Ar-H, J=8.5 Hz), 7.69 (d, 2H, Ar-H, J=8.6 Hz), 4.34 (s, 2H, CH_2), 3.10–3.19 (m, 1H, CH), 1.49–1.70 (m, 2H, CH_2), 1.28 (d, 3H, CH_3, J=6.7 Hz), 0.91 (t, 3H, CH_3, J=7.4 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 172.41, 166.64, 166.16, 142.66, 126.45 (q, 2C, J_{C-F}=3.5 Hz), 124.70 (q, J_{C-F}=269.8 Hz), 124.24 (q, 2C, J_{C-F}=31.9 Hz), 119.52, 49.35, 38.82, 28.74, 19.99, 11.45; HR-MS (ESI): m/z 440.0216 $[M+H]^+$ (Calcd for $C_{15}H_{17}N_3OS_4F_3$ 440.0207).

2-(2-Butyldisulfanyl)-5-[(4-methylphenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**6k**)

White solid; Yield 72.1%; mp 82.7–84.1°C; IR (KBr) cm^{-1}: 3442, 1668, 1610, 1542, 747, 510; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 10.30 (s, 1H, NH), 7.45 (d, 2H, Ar-H, J=8.2 Hz), 7.13 (d, 2H, Ar-H, J=8.1 Hz), 4.27 (s, 2H, CH_2), 3.12–3.20 (m, 1H, CH), 2.25 (s, 3H, CH_3), 1.51–1.71 (m, 2H, CH_2), 1.29 (d, 3H, CH_3, J=6.8 Hz), 0.92 (t, 3H, CH_3, J=7.4 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 172.26, 167.01, 165.20, 136.66, 133.06, 129.62 (2C), 119.67 (2C), 49.38, 38.85, 28.77, 20.92, 20.11, 11.58; HR-MS (ESI): m/z 386.0498 $[M+H]^+$ (Calcd for

$C_{15}H_{20}N_3OS_4$ 386.0489).

2-(*n*-Butyldisulfanyl)-5-[(phenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**7a**)

White solid; Yield 72.3%; mp 89.7–91.5°C; IR (KBr) cm^{-1}: 3447, 1681, 1600, 1542, 753, 502; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 10.38 (s, 1H, NH), 7.57 (d, 2H, Ar-H, J=7.72 Hz), 7.33 (t, 2H, Ar-H, J=7.6 Hz), 7.08 (t, 1H, Ar-H, J=7.4 Hz), 4.29 (s, 2H, CH_2), 3.0 (t, 2H, CH_2, J=7.2 Hz), 1.61–1.68 (m, 2H, CH_2), 1.32–1.41 (m, 2H, CH_2), 0.86 (t, 3H, CH_3, J=7.4 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 171.65, 167.05, 165.46, 139.18, 129.21 (2C), 124.06, 119.67 (2C), 38.93, 38.90, 30.90, 21.37, 13.87; HR-MS (ESI): m/z 372.0346 $[M+H]^+$ (Calcd for $C_{14}H_{18}N_3OS_4$ 372.0333).

2-(*n*-Butyldisulfanyl)-5-[(2-chlorophenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**7b**)

White solid; Yield 71.9%; mp 68.8–69.7°C; IR (KBr) cm^{-1}: 3446, 1684, 1558, 1507, 757, 503; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 9.95 (s, 1H, NH), 7.74 (d, 1H, Ar-H, J=7.1 Hz), 7.51 (d, 1H, Ar-H, J=8.0 Hz), 7.35 (t, 1H, Ar-H, J=8.6 Hz), 7.22 (t, 1H, Ar-H, J=6.6 Hz), 4.36 (s, 2H, CH_2), 3.01 (t, 2H, CH_2, J=7.2 Hz), 1.61–1.69 (m, 2H, CH_2), 1.32–1.41 (m, 2H, CH_2), 0.87 (t, 3H, CH_3, J=7.4 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 171.81, 167.02, 166.26, 134.95, 129.99, 127.95, 126.95, 126.53, 126.00, 38.85, 38.31, 30.87, 21.31, 13.86; HR-MS (ESI): m/z 405.9955 $[M+H]^+$ (Calcd for $C_{14}H_{17}N_3OS_4Cl$ 405.9943).

2-(*n*-Butyldisulfanyl)-5-[(3-chlorophenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**7c**)

White solid; Yield 73.7%; mp 80.6–81.8°C; IR (KBr) cm^{-1}: 3446, 1683, 1594, 1541, 779, 503; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 10.57 (s, 1H, NH), 7.79 (s, 1H, Ar-H), 7.43 (d, 1H, Ar-H, J=8.3 Hz), 7.36 (t, 1H, Ar-H, J=7.9 Hz), 7.15 (d, 1H, Ar-H, J=7.8 Hz), 4.30 (s, 2H, CH_2), 3.0 (t, 2H, CH_2, J=7.2 Hz), 1.61–1.68 (m, 2H, CH_2), 1.32–1.41 (m, 2H, CH_2), 0.86 (t, 3H, CH_3, J=7.3 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 171.72, 166.95, 165.97, 140.55, 133.66, 130.99, 123.84, 119.14, 118.04, 38.85, 38.72, 30.86, 21.29, 13.85; HR-MS (ESI): m/z 405.9955 $[M+H]^+$ (Calcd for $C_{14}H_{17}N_3OS_4Cl$ 405.9943).

2-(*n*-Butyldisulfanyl)-5-[(4-chlorophenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**7d**)

White solid; Yield 73.6%; mp 102.7–103.4°C; IR (KBr) cm^{-1}: 3447, 1663, 1569, 1541, 745, 510; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 10.51 (s, 1H, NH), 7.61 (d, 2H, Ar-H, J=8.8 Hz), 7.39 (d, 2H, Ar-H, J=8.8 Hz), 4.29 (s, 2H, CH_2), 3.0 (t, 2H, CH_2, J=7.2 Hz), 1.61–1.68 (m, 2H, CH_2), 1.32–1.41 (m, 2H, CH_2), 0.86 (t, 3H, CH_3, J=7.4 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 171.70, 166.91, 165.64, 138.07, 129.13 (2C), 127.80, 121.18 (2C), 38.91, 38.80, 30.88, 21.35, 13.85; HR-MS (ESI): m/z 405.9955 $[M+H]^+$ (Calcd for $C_{14}H_{17}N_3OS_4Cl$ 405.9943).

2-(*n*-Butyldisulfanyl)-5-[(3-nitro-phenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**7e**)

White solid; Yield 72.8%; mp 44.9–46.3°C; IR (KBr) cm^{-1}: 3426, 1683, 1614, 1527, 737, 511; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 10.89 (s, 1H, NH), 8.61 (s, 1H, Ar-H), 7.94 (d, 1H, Ar-H, J=8.0 Hz), 7.89 (d, 1H, Ar-H, J=8.1 Hz), 7.63 (t, 1H, Ar-H, J=8.2 Hz), 4.34 (s, 2H, CH_2), 2.98 (t, 2H, CH_2, J=7.2 Hz), 1.59–1.66 (m, 2H, CH_2), 1.30–1.39 (m, 2H, CH_2), 0.85 (t, 3H, CH_3, J=7.4 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 166.41, 166.35, 165.23, 148.40, 140.22, 130.67, 125.54, 118.56, 113.72, 38.88, 38.61, 30.85, 21.29, 13.80; HR-MS (ESI): m/z 417.0197 $[M+H]^+$ (Calcd for $C_{14}H_{17}N_4O_3S_4$ 417.0183).

2-(*n*-Butyldisulfanyl)-5-[(4-nitro-phenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**7f**)

White solid; Yield 73.2%; mp 118.9–121.7°C; IR (KBr) cm^{-1}: 3442, 1686, 1566, 1504, 751, 498; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 11.0 (s, 1H, NH), 8.25 (d, 2H, Ar-H, J=9.2 Hz), 7.83 (d, 2H, Ar-H, J=9.2 Hz), 4.37 (s, 2H, CH_2), 3.0 (t, 2H, CH_2, J=7.2 Hz), 1.60–1.67 (m, 2H, CH_2), 1.31–1.40 (m, 2H, CH_2), 0.86 (t, 3H, CH_3, J=7.3 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 171.77, 166.71, 166.57, 145.21, 142.92, 125.40 (2C), 119.34 (2C), 38.87, 38.81, 30.86, 21.31, 13.82; HR-MS (ESI): m/z 417.0194 $[M+H]^+$ (Calcd for $C_{14}H_{17}N_4O_3S_4$ 417.0183).

2-(*n*-Butyldisulfanyl)-5-[(2-methoxyphenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**7g**)

Yellow oily liquid; Yield 72.6%; IR (KBr) cm^{-1}: 3402, 1687, 1602, 1536, 749, 513; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 9.63 (s, 1H, NH), 7.97 (d, 1H, Ar-H, J=8.0 Hz), 7.04–7.12 (m, 2H, Ar-H), 6.91 (t, 1H, Ar-H, J=8.1 Hz), 4.35 (s, 2H, CH_2), 3.84 (s, 3H, OCH_3), 3.0 (t, 2H, CH_2, J=7.2 Hz), 1.61–1.68 (m, 2H, CH_2), 1.32–1.41 (m, 2H, CH_2), 0.86 (t, 3H, CH_3, J=7.4 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 171.69, 167.28, 165.87, 149.79, 127.44, 125.10, 121.75, 120.76, 111.67, 56.19, 38.84, 38.63, 30.86, 21.30, 13.85; HR-MS (ESI): m/z 402.0454 $[M+H]^+$ (Calcd for $C_{15}H_{20}N_3O_2S_4$ 402.0438).

2-(*n*-Butyldisulfanyl)-5-[(4-methoxyphenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**7h**)

White solid; Yield 69.7%; mp 92.0–93.6°C; IR (KBr) cm^{-1}: 3430, 1674, 1602, 1512, 828, 521; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 10.25 (s, 1H, NH), 7.48 (d, 2H, Ar-H, J=8.9 Hz), 6.90 (t, 2H, Ar-H, J=8.9 Hz), 4.25 (s, 2H, CH_2), 3.72 (s, 3H, OCH_3), 3.0 (t, 2H, CH_2, J=7.2 Hz), 1.60–1.68 (m, 2H, CH_2), 1.31–1.41 (m, 2H, CH_2), 0.86 (t, 3H, CH_3, J=7.4 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 171.64, 167.12, 164.94, 155.99, 132.30, 121.23 (2C), 114.34 (2C), 55.59, 38.92, 38.81, 30.90, 21.37, 13.85; HR-MS (ESI): m/z 402.0452 $[M+H]^+$ (Calcd for $C_{15}H_{20}N_3O_2S_4$ 402.0438).

2-(*n*-Butyldisulfanyl)-5-[(4-fluorophenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**7i**)

White solid; Yield 74.3%; mp 101.7–103.0°C; IR (KBr) cm^{-1}: 3430, 1664, 1558, 1509, 838, 518; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 10.45 (s, 1H, NH), 7.59 (dd, 2H, Ar-H, J_1=5.0 Hz, J_2=9.0 Hz), 7.17 (d, 2H, Ar-H, J=8.8 Hz), 4.28 (s, 2H, CH_2), 3.0 (t, 2H, CH_2, J=7.2 Hz), 1.60–1.68 (m, 2H, CH_2), 1.31–1.40 (m, 2H, CH_2), 0.86 (t, 3H, CH_3, J=7.4 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 171.70, 166.94, 165.38, 158.70 (d, J_{C-F}=239.2 Hz), 135.51 (d, J_{C-F}=2.0 Hz), 121.43 (d, 2C, J_{C-F}=7.7 Hz), 115.74 (d, 2C, J_{C-F}=22.2 Hz), 38.92, 38.75, 30.88, 21.35, 13.81; HR-MS (ESI): m/z 390.0252 $[M+H]^+$ (Calcd for $C_{14}H_{17}N_3OS_4F$ 390.0238).

2-(*n*-Butyldisulfanyl)-5-[(4-trifluoromethylphenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**7j**)

White solid; Yield 71.2%; mp 105.9–107.0°C; IR (KBr) cm^{-1}: 344, 1684, 1608, 1543, 840, 506; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 10.76 (s, 1H, NH), 7.79 (d, 2H, Ar-H, J=8.6 Hz), 7.7 (d, 2H, Ar-H, J=8.6 Hz), 4.34 (s, 2H, CH_2), 3.0 (t, 2H, CH_2, J=7.2 Hz), 1.60–1.67 (m, 2H, CH_2), 1.31–1.40 (m, 2H, CH_2), 0.85 (t, 3H, CH_3, J=7.4 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 171.72, 166.91, 166.23, 142.67, 126.57 (q, 2C, J_{C-F}=3.6 Hz), 124.74 (q, J_{C-F}=269.7 Hz), 124.18 (q, 2C, J_{C-F}=31.8 Hz), 119.56, 38.83, 38.78, 30.84, 21.27, 13.78; HR-MS (ESI): m/z 440.0217 $[M+H]^+$ (Calcd for $C_{15}H_{17}N_3OS_4F_3$

440.0207).

2-(*n*-Butyldisulfanyl)-5-[(4-methylphenylcarbamoyl)-methylhio]-1,3,4-thiadiazole (**7k**)

White solid; Yield 70.7%; mp 79.7–82.0°C; IR (KBr) cm^{-1}: 3450, 1677, 1605, 1544, 817, 506, 7.46 (d, 2H, Ar-H, J=8.3 Hz), 7.13 (d, 2H, Ar-H, J=8.2 Hz), 4.27 (s, 2H, CH_2), 3.0 (t, 2H, CH_2, J=7.2 Hz), 2.25 (s, 3H, CH_3), 1.60–1.68 (m, 2H, CH_2), 1.31–1.41 (m, 2H, CH_2), 0.86 (t, 3H, CH_3, J=7.3 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 171.60, 167.14, 165.21, 136.67, 133.06, 129.62 (2C), 119.66 (2C), 39.01, 38.87, 30.88, 21.33, 20.91, 13.86; HR-MS (ESI): m/z 386.0498 $[M+H]^+$ (Calcd for $C_{15}H_{20}N_3OS_4$ 386.0489).

2-(*i*-Butyldisulfanyl)-5-[(phenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**8a**)

White solid; Yield 73.6%; mp 90.8–92.1°C; IR (KBr) cm^{-1}: 3447, 1682, 1600, 1541, 751, 503; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 10.38 (s, 1H, NH), 7.57 (d, 2H, Ar-H, J=8.0 Hz), 7.33 (t, 2H, Ar-H, J=7.6 Hz), 7.08 (t, 1H, Ar-H, J=7.3 Hz), 4.30 (s, 2H, CH_2), 2.91 (d, 2H, CH_2, J=6.8 Hz), 1.85–1.98 (m, 1H, CH), 0.96 (d, 6H, CH_3, J=6.6 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 171.44, 167.16, 165.48, 139.16, 129.25 (2C), 124.09, 119.68 (2C), 48.22, 38.87, 28.16, 21.68 (2C); HR-MS (ESI): m/z 372.0349 $[M+H]^+$ (Calcd for $C_{14}H_{18}N_3OS_4$ 372.0333).

2-(*i*-Butyldisulfanyl)-5-[(2-chlorophenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**8b**)

White solid; Yield 70.4%; mp 90.7–91.7°C; IR (KBr) cm^{-1}: 3447, 1685, 1590, 1524, 757, 503; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 9.95 (s, 1H, NH), 7.75 (d, 1H, Ar-H, J=7.8 Hz), 7.50 (d, 1H, Ar-H, J=7.4 Hz), 7.34 (t, 1H, Ar-H, J=7.3 Hz), 7.21 (t, 1H, Ar-H, J=7.3 Hz), 4.37 (s, 2H, CH_2), 2.91 (d, 2H, CH_2, J=6.8 Hz), 1.85–1.99 (m, 1H, CH), 0.96 (d, 6H, CH_3, J=6.7 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 171.65, 166.98, 166.21, 135.00, 129.93, 127.87, 126.74, 126.29, 125.70, 48.30, 38.40, 28.20, 21.70 (2C); HR-MS (ESI): m/z 405.9941 $[M+H]^+$ (Calcd for $C_{14}H_{17}N_3OS_4Cl$ 405.9943).

2-(*n*-Butyldisulfanyl)-5-[(3-chlorophenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**8c**)

White solid; Yield 68.7%; mp 62.8–64.5°C; IR (KBr) cm^{-1}: 3447, 1684, 1593, 1541, 779, 503; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 10.57 (s, 1H, NH), 7.79 (s, 1H, Ar-H), 7.42 (d, 1H, Ar-H, J=8.3 Hz), 7.36 (t, 1H, Ar-H, J=8.0 Hz), 7.14 (d, 1H, Ar-H, J=7.9 Hz), 4.30 (s, 2H, CH_2), 2.91 (d, 2H, CH_2, J=6.8 Hz), 1.85–1.98 (m, 1H, CH), 0.96 (d, 6H, CH_3, J=6.7 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 171.54, 166.98, 165.95, 140.55, 133.67, 130.97, 123.83, 119.15, 118.04, 48.20, 38.74, 28.15, 21.67 (2C); HR-MS (ESI): m/z 405.9958 $[M+H]^+$ (Calcd for $C_{14}H_{17}N_3OS_4Cl$ 405.9943).

2-(*i*-Butyldisulfanyl)-5-[(4-chlorophenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**8d**)

White solid; Yield 69.8%; mp 101.6–103.5°C; IR (KBr) cm^{-1}: 3447, 1663, 1558, 1541, 836, 510; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 10.54 (s, 1H, NH), 7.61 (d, 2H, Ar-H, J=8.8 Hz), 7.38 (d, 2H, Ar-H, J=8.8 Hz), 4.30 (s, 2H, CH_2), 2.89 (d, 2H, CH_2, J=6.8 Hz), 1.83–1.97 (m, 1H, CH), 0.95 (d, 6H, CH_3, J=6.6 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 171.55, 167.06, 165.72, 138.09, 129.23 (2C), 127.71, 121.17 (2C), 48.10, 38.71, 28.13, 21.67 (2C); HR-MS (ESI): m/z 405.9950 $[M+H]^+$ (Calcd for $C_{14}H_{17}N_3OS_4Cl$ 405.9943).

2-(*i*-Butyldisulfanyl)-5-[(3-nitro-phenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**8e**)

White solid; Yield 65%; mp 44.7–46.5°C; IR (KBr) cm^{-1}: 3431, 1692, 1620, 1528, 737, 514; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 10.89 (s, 1H, NH), 8.61 (s, 1H, Ar-H), 7.95 (d, 1H, Ar-H, J=8.0 Hz), 7.89 (d, 1H, Ar-H, J=7.8 Hz), 7.64 (t, 1H, Ar-H, J=8.2 Hz), 4.35 (s, 2H, CH_2), 2.91 (d, 2H, CH_2, J=6.8 Hz), 1.84–1.98 (m, 1H, CH), 0.95 (d, 6H, CH_3, J=6.6 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 171.61, 166.84, 166.34, 148.41, 140.22, 130.68, 125.54, 118.56, 113.72, 48.21, 38.65, 28.14, 21.62 (2C); HR-MS (ESI): m/z 417.0195 $[M+H]^+$ (Calcd for $C_{14}H_{17}N_4O_3S_4$ 417.0183).

2-(*i*-Butyldisulfanyl)-5-[(4-nitro-phenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**8f**)

White solid; Yield 71.8%; mp 120.9–122.7°C; IR (KBr) cm^{-1}: 3441, 1687, 1615, 1504, 751, 497; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 10.98 (s, 1H, NH), 8.24 (d, 2H, Ar-H, J=9.2 Hz), 7.83 (d, 2H, Ar-H, J=9.2 Hz), 4.37 (s, 2H, CH_2), 2.90 (d, 2H, CH_2, J=6.8 Hz), 1.84–1.97 (m, 1H, CH), 0.95 (d, 6H, CH_3, J=6.6 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 171.60, 166.75, 166.54, 145.20, 142.91, 125.39 (2C), 119.34 (2C), 48.21, 38.81, 28.15, 21.63 (2C); HR-MS (ESI): m/z 417.0187 $[M+H]^+$ (Calcd for $C_{14}H_{17}N_4O_3S_4$ 417.0183).

2-(*i*-Butyldisulfanyl)-5-[(2-methoxyphenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**8g**)

White solid; Yield 65.3%; mp 51.1–53.0°C; IR (KBr) cm^{-1}: 3374, 1675, 1602, 1529, 746, 512; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 9.62 (s, 1H, NH), 7.97 (d, 1H, Ar-H, J=7.32 Hz), 7.05–7.12 (m, 2H, Ar-H), 6.91 (t, 1H, Ar-H, J=8.1 Hz), 4.35 (s, 2H, CH_2), 3.84 (s, 3H, OCH_3), 2.92 (d, 2H, CH_2, J=6.8 Hz), 1.86–1.99 (m, 1H, CH), 0.96 (d, 6H, CH_3, J=6.7 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 171.52, 167.28, 165.83, 149.70, 127.51, 125.02, 121.64, 120.76, 111.60, 56.17, 48.24, 38.68, 28.17, 21.67 (2C); HR-MS (ESI): m/z 402.0449 $[M+H]^+$ (Calcd for $C_{15}H_{20}N_3O_2S_4$ 402.0438).

2-(*i*-Butyldisulfanyl)-5-[(4-methoxyphenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**8h**)

White solid; Yield 70.5%; mp 95.0–95.9°C; IR (KBr) cm^{-1}: 3431, 1673, 1603, 1511, 829, 522; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 10.25 (s, 1H, NH), 7.48 (d, 2H, Ar-H, J=8.8 Hz), 6.90 (t, 2H, Ar-H, J=8.9 Hz), 4.26 (s, 2H, CH_2), 3.72 (s, 3H, OCH_3), 2.91 (d, 2H, CH_2, J=6.8 Hz), 1.85–1.98 (m, 1H, CH), 0.96 (d, 6H, CH_3, J=6.6 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 171.14, 167.19, 164.94, 155.98, 132.30, 121.23 (2C), 114.37 (2C), 55.61, 48.23, 38.80, 28.17, 21.67 (2C); HR-MS (ESI): m/z 402.0443 $[M+H]^+$ (Calcd for $C_{15}H_{20}N_3O_2S_4$ 402.0438).

2-(*i*-Butyldisulfanyl)-5-[(4-fluorophenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**8i**)

White solid; Yield 75.6%; mp 89.9–91.3°C; IR (KBr) cm^{-1}: 3431, 1680, 1557, 1500, 834, 515; ^{1}H-NMR 400 MHz, DMSO-d_6): δ (ppm): 10.45 (s, 1H, NH), 7.59 (dd, 2H, Ar-H, J_1=5.0 Hz, J_2=9.2 Hz), 7.17 (d, 2H, Ar-H, J=8.8 Hz), 4.29 (s, 2H, CH_2), 2.91 (d, 2H, CH_2, J=6.8 Hz), 1.85–1.98 (m, 1H, CH), 0.96 (d, 6H, CH_3, J=6.6 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6): δ (ppm): 171.50, 167.04, 165.40, 158.68 (d, J_{C-F}=239.0 Hz), 135.53 (d, J_{C-F}=2.1 Hz), 121.44 (d, 2C, J_{C-F}=7.7 Hz), 115.79 (d, 2C, J_{C-F}=22.1 Hz), 48.22, 38.74, 28.16, 21.65 (2C); HR-MS (ESI): m/z 390.0246 $[M+H]^+$ (Calcd for $C_{14}H_{17}N_3OS_4F$ 390.0238).

2-(*i*-Butyldisulfanyl)-5-[(4-trifluoromethylphenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**8j**)

White solid; Yield 73.6%; mp 94.3–96.3°C; IR (KBr) cm^{-1}: 3429, 1685, 1606, 1544, 840, 506; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 10.76 (s, 1H, NH), 7.79 (d, 2H, Ar-H, J=8.6 Hz), 7.7 (d, 2H, Ar-H, J=8.6 Hz), 4.34 (s, 2H, CH_2), 2.90 (d, 2H, CH_2, J=6.8 Hz), 1.84–1.97 (m, 1H, CH), 0.96 (d, 6H, CH_3, J=6.6 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 171.55, 166.97, 166.25, 142.67, 126.61 (q, 2C, J_{C-F}=3.7 Hz), 124.75 (q, J_{C-F}=269.7 Hz), 124.16 (q, 2C, J_{C-F}=31.9 Hz), 119.56, 48.15, 38.77, 28.13, 21.63 (2C); HR-MS (ESI): m/z 440.0204 $[M+H]^+$ (Calcd for $C_{15}H_{17}N_3OS_4F_3$ 440.0207).

2-(*i*-Butyldisulfanyl)-5-[(4-methylphenylcarbamoyl)-methylthio]-1,3,4-thiadiazole (**8k**)

White solid; Yield 70.9%; mp 72.5–74.3°C; IR (KBr) cm^{-1}: 3440, 1677, 1602, 1542, 817, 507; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 10.30 (s, 1H, NH), 7.46 (d, 2H, Ar-H, J=8.2 Hz), 7.12 (d, 2H, Ar-H, J=8.2 Hz), 4.27 (s, 2H, CH_2), 2.90 (d, 2H, CH_2, J=6.8 Hz), 2.25 (s, 3H, CH_3), 1.85–1.98 (m, 1H, CH), 0.96 (d, 6H, CH_3, J=6.6 Hz); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 171.42, 167.19, 165.20, 136.67, 133.06, 129.62 (2C), 119.67 (2C), 48.21, 38.86, 28.16, 21.67 (2C); HR-MS (ESI): m/z 386.0496 $[M+H]^+$ (Calcd for $C_{15}H_{20}N_3OS_4$ 386.0489).

Cell Culture and CCK-8 Bioassay The following established *in vitro* human cancer cell lines and normal cell lines were used: A549, HeLa, SMMC-7721 and L929, which were obtained from Tumor Cell Resources Bank, Chinese Academy of Medical Sciences. Cell counting kit-8 was from Dojindo (Japan).

Compounds **6a**–**k**, **7a**–**k** and **8a**–**k**, and 5-FU were respectively 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 37°C, 5% CO_2 incubator for 24 h. Replaced the medium containing different concentrations of compounds in fresh medium, at 37°C, 5% CO_2 incubator for 48 h. Then added 90 μL of fresh medium and 10 μL of CCK-8 into culture plates and cultivated at the same conditions for 1h. The sample cell was added into 96-well microplate reader and read the plate at 450 nm, recorded the absorbance value (optical density (OD)). Cell viability was calculated from the mean values for three wells using the following formula:

$$\text{Relative cell viability} = \frac{\text{OD value for the test group} - \text{blank OD}}{\text{control OD value} - \text{blank OD value}} \times 100\%$$

The IC_{50} value is defined as the concentration that causes a 50% cell proliferation inhibition.

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Conflict of Interest The authors declare no conflict of interest.

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