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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and biological evaluation of novel disulfides incorporating 1,3,4-thiadiazole scaffold as promising antitumor agents

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Abstract

In the present study, fourteen 2,5-disubstituted 1,3,4-thiadiazole derivatives containing disulfide group were prepared. The resulting compounds **7a**–**7n** were identified by IR, NMR, MS, and elemental analysis. Their antiproliferative properties in vitro were studied employing standard CCK-8 assay against SMMC-7721, MCF-7, and A549 lines. Bioassay indicated that some compounds showed stronger antitumor effects than reference drugs PX-12 and 5-fluorouracil. Among these screened compounds, compound **7h** showed excellent biological activities in inhibiting SMMC-7721 cell proliferation with IC_{50} at 1.93 ± 0.08 μM. Compounds **7k** and **7i** manifested highly effective growth inhibitory activity versus MCF-7 cells, with IC_{50} at 3.04 ± 0.09 and 3.54 ± 0.17 μM, respectively. For A549 cells, compound **7m** was found to have the highest antitumor potency with IC_{50} at 3.67 ± 0.13 μM.

Keywords Disulfides · 1,3,4-Thiadiazole · Synthesis · Antitumor activity

Introduction

Malignant tumor is a major disease characterized by abnormal proliferation and metastasis of cells. Its high incidence rate and low survival rate have become one of the greatest threats to human health today. In order to alleviate the suffering of cancer patients, improve the life quality of patients, and prolong the lives of patients, medical scientists from all over the world have made unremitting efforts, but they have not yet fundamentally solved this thorny problem which human face. In recent years, targeted treatment has become an important means of cancer treatment, and has aroused the attention of medical experts around the world. Thioredoxin (Trx) is a kind of multifunctional protein that is generally expressed in various biological tissues. The Trx system has become a hot spot in the research of tumor molecular biology, and it is a new target of tumor therapy with important clinical significance (Tonissen and Trapani 2009; Lu and Holmgren 2014; Karlenius and Tonissen 2010). Over the last decades, there have been several anticancer drugs targeting Trx system in clinical trials (Hashemy et al. 2006; Mehta et al. 2009; Tan et al. 2010). Among them, PX-12 (1-methylpropyl 2-imidazolyl disulfde) is a novel small molecule inhibitor of Trx, which has antitumor effects for a variety of tumor cells. It is also the first disulfide compound which has been reported to enter clinical trials as anticancer drug (Ramanathan et al. 2011; Diraimondo et al. 2013; Baker et al. 2013) (Fig. 1). Therefore, the role of disulfides in the antitumor field has been paid more and more attention (Saiz et al. 2014; Zhu et al. 2015), and this has also stimulated people's interest in the study of disulfide antitumor drugs.

Five-membered heterocyclic compounds, such as 1,3,4-oxadiazoles, 1,3,4-thiadiazoles, and 1,2,4-triazoles, have a very wide range of applications in industry, agriculture, and medicine. In recent years, 1,3,4-thiadiazoles occupies an important position in research and development in the field of medicine, which has aroused widespread interest in the pharmaceutical industry and has conducted a large number of studies owing to their remarkable biological properties, including antitumor (Shi et al. 2013; Bhatt et al. 2018;

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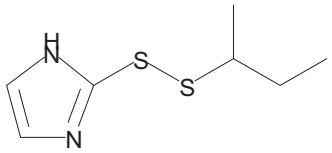


Fig. 1 Structure of PX-12

Polkam et al. 2015; Rezki et al. 2015), antimicrobial (Shetty et al. 2016; Muğlu et al. 2018), anticholinesterase (Skrzypek et al. 2013; Altintop et al. 2013), antidiabetic (Pattn et al. 2011; Lee et al. 2010), antidepressant (Clerici et al. 2001; Khan et al. 2016), Analgesic (Schenone et al. 2006; Yavuz et al. 2013), anticonvulsant (Luszczki et al. 2015; Karakus et al. 2009), antiinflammatory (Dekhane et al. 2011; Shkair et al. 2016), antimalarial (Huang et al. 2012), antitubercular (Patel et al. 2017), antileishmanial (Alipour et al. 2011; Poorrajab et al. 2009; Foroumadi et al. 2005), antiglaucoma (Casini et al. 2003), and acetylcholinesterase inhibitory properties (Xue et al. 2014). Consequently, people increasingly pay close attention to the development value and potential application prospects of 1,3,4-thiadiazole compounds in the field of medicine. Based on these scientific facts, in order to screen out new antitumor lead compound-bearing disulfide core with high efficiency and low toxicity, hybrid compounds possessing disulfide and 1,3,4-thiadiazole moieties will be formed employing certain chemical means, and some tumor cells growth inhibitory effects will be examined in our research.

Material and methods

Synthesis

All chemical reagents were purchased from Energy Chemical Co. and Tianjin Hengshan Chemical Technology Co., Ltd., and they were used as received from commercial supplies without further purification unless otherwise specified. The bioassay reagents, including RPMI-1640 and PBS, were getted from Tianjin Jun Yao Biological Technology Co., Ltd. Melting points (m.p., uncorrected) were determined by a X-4 microscope melting point apparatus. Infrared spectra (IR) were obtained in KBr pellets on a Nicolet Avatar 370 spectrometer. Both ^{1}H NMR and ^{13}C NMR (400 and 100 MHz, respectively) spectra of the synthesized compounds were acquired on a Bruker Avance III 400 MHz spectrometer using DMSO-d_6 as solvent and tetramethylsilane as the internal standard. ESI mass spectra were recorded on a Waters Xevo G2 QTof (ESI) mass spectrometer. Elemental analyses (C, H, N) were performed on a Flash EA1112 analyzer. The reaction progress of some intermediates, for example **5a–5n** and **6a–6n**, was monitored by thin layer chromatography (TLC).

General method for the synthesis of 2-substituted phenyl-5-substituted disulfanyl-1,3,4-thiadiazoles (7a–7n)

To the solution of intermediate **2** and 2-substituted phenyl-5-mercapto-1,3,4-thiadiazoles **6a–6n** (3.0 mmol) in methanol (15 ml) was added the aqueous solution of $NaHCO_3$ (0.40 g/4.7 mmol in 25 ml water) at ambient temperature with vigorous stirring. The reaction mixture continued to stir for 3 h, then it was cooled at 5–10 °C for 12 h. The precipitated solid was separated, followed by recrystallization from mixture of ethanol and water to yield the desired products.

2-Phenyl-5-(n-butyldisulfanyl)-1,3,4-thiadiazole (7a)

Yellow oil; Yield 60%; IR (cm^{-1}): 3145, 2959, 2939, 2871, 2361, 2342, 1966, 1907, 1694, 1458, 1401, 1281, 1176, 1001, 979, 762, 621, 573; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.95 (2H, d, $J = 7.8$ Hz, Ph-H), 7.58 (2H, t, $J = 7.3$ Hz, Ph-H), 7.50 (1H, t, $J = 7.6$ Hz, Ph-H), 3.06 (2H, t, $J = 6.8$ Hz, S-S-CH_2), 1.65–1.72 (2H, m, S-S-CH$_2$CH_2), 1.36–1.44 (2H, m, CH_2CH$_3$), 0.88 (3H, t, $J = 7.2$ Hz, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 168.56, 167.89, 131.75 (2C), 129.69 (2C), 128.77, 127.59, 38.99, 30.95, 21.44, 13.81; MS (ESI, *m/z*): 283.4 $[M + H]^+$; Calculated for $C_{12}H_{14}N_2S_3$: C, 51.03; H, 5.00; N, 9.92. Found: C, 51.12; H, 4.97; N, 9.97.

2-(4-Aminophenyl)-5-(n-butyldisulfanyl)-1,3,4-thiadiazole (7b)

Yellow solid; m.p.: 73.4–75.3 °C; Yield 69%; IR (cm^{-1}): 3132, 2361, 1635, 1401, 1177, 1107, 991, 826, 619; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.60 (2H, d, $J = 8.56$ Hz, Ph-H), 6.64 (2H, d, $J = 8.5$ Hz, Ph-H), 5.92 (2H, s, NH_2), 3.02 (2H, t, $J = 7.2$ Hz, S-S-CH_2); 1.64–1.72 (2H, m, S-S-CH$_2$CH_2), 1.35–1.44 (2H, m, CH_2CH$_3$), 0.97 (3H, t, $J = 7.2$ Hz, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 170.75, 168.12, 152.67, 129.47 (2C), 116.73, 114.18 (2C), 38.89, 30.85, 21.34, 13.87; MS (ESI, *m/z*): 298.5 $[M + H]^+$; Calculated for $C_{12}H_{15}N_3S_3$: C, 48.45; H, 5.08; N, 14.13. Found: C, 48.35; H, 5.11; N, 14.19.

2-(4-Methylphenyl)-5-(n-butyldisulfanyl)-1,3,4-thiadiazole (7c)

Yellow oil; Yield 62%; IR (cm^{-1}): 3145, 2962, 2872, 2360, 2342, 2075, 1635, 1398, 1183, 1120, 1077, 976, 817, 760,

710, 620, 572; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.81 (2H, d, $J = 8.0$ Hz, Ph-H), 7.35 (2H, d, $J = 8.0$ Hz, Ph-H), 3.03 (2H, t, $J = 7.2$ Hz, S-S-CH_2); 2.36 (3H, s, Ph-CH_3), 1.61–1.70 (2H, m, S-S-CH$_2$CH_2), 1.33–1.42 (2H, m, CH_2CH$_3$), 0.87(3H, t, $J = 4.4$ Hz, CH$_2$CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.18, 169.92, 142.11, 130.39 (2C), 127.86 (2C), 127.00, 38.86, 30.92, 21.47, 21.36, 13.85; MS (ESI, *m/z*): 297.5 $[M + H]^+$; Calculated for $C_{13}H_{16}N_2S_3$: C, 52.67; H, 5.44; N, 9.45. Found: C, 52.79; H, 5.47; N, 9.38.

2-(4-Methoxyphenyl)-5-(n-butyldisulfanyl)-1,3,4-thiadiazole (7d)

Yellow solid; m.p.: 55.1–57.0 °C; Yield 73%, IR (cm^{-1}): 3443, 3134, 2360, 2342, 1636, 1401, 1307, 1254, 1131, 1108, 1033, 993, 823, 807, 639, 618; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.90 (2H, d, $J = 8.6$ Hz, Ph-H), 7.10 (2H, d, $J = 8.7$ Hz, Ph-H), 3.84 (3H, s, Ph-OCH_3), 3.06 (2H, t, $J = 7.2$ Hz, S-S-CH_2); 1.65–1.73 (2H, m, S-S-CH$_2$CH_2), 1.35–1.45 (2H, m, CH_2CH$_3$), 0.87 (3H, t, $J = 7.2$ Hz, CH$_2$CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 170.59, 169.57, 162.25, 129.63 (2C), 122.29, 115.27 (2C), 55.94, 38.85, 30.89, 21.33, 13.85; MS (ESI, *m/z*): 313.5 $[M + H]^+$; Calculated for $C_{13}H_{16}N_2OS_3$: C, 49.97; H, 5.16; N, 8.97. Found: C, 50.11; H, 5.13; N 8.92.

2-(3,4,5-Trimethoxyphenyl)-5-(n-butyldisulfanyl)-1,3,4-thiadiazole (7e)

White solid; m.p.: 64.0–66.1 °C; Yield 76%; IR (cm^{-1}): 3447, 3145, 2361, 2343, 2075, 1637, 1509, 1401, 1331, 1192, 1114, 991, 845, 620; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.22 (2H, s, Ph-H), 3.88 (6H, s, Ph-OCH_3), 3.74 (3H, s, Ph-OCH_3), 3.07 (2H, t, $J = 7.2$ Hz, S-S-CH_2), 1.67–1.74 (2H, m, S-S-CH$_2$CH_2), 1.36–1.47 (2H, m, CH_2CH$_3$), 0.90 (3H, t, $J = 7.6$ Hz, CH$_2$CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 184.28, 163.35, 153.85 (2C), 140.74, 124.96, 105.29 (2C), 60.61, 56.62 (2C), 38.77, 30.90, 21.33, 13.84; MS (ESI, *m/z*): 373.5 $[M + H]^+$; Calculated for $C_{15}H_{20}N_2O_3S_3$: C, 48.36; H, 5.41; N, 7.52. Found: C, 48.26; H, 5.43; N, 7.46.

2-(4-Chlorophenyl)-5-(n-butyldisulfanyl)-1,3,4-thiadiazole (7f)

Yellow solid; m.p.: 37.2–39.1 °C; Yield 77%; IR (cm^{-1}): 3443, 3134, 2361, 2341, 1636, 1401, 1132, 1108, 837, 815, 639, 616, 569; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.98 (2 H, d, $J = 8.5$ Hz, Ph-H), 7.63 (2H, d, $J = 8.5$ Hz, Ph-H), 3.07 (2H, t, $J = 7.2$ Hz, S-S-CH_2);1.66–1.73 (2H, m, S-S-CH$_2$CH_2), 1.34–1.45 (2H, m, CH_2CH$_3$), 0.90 (3H, t, $J = 7.2$ Hz, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 173.93, 167.55, 136.57, 129.86 (2C), 129.44 (2C), 128.54, 38.83, 30.94, 21.37, 13.85; MS (ESI, *m/z*): 317.4 $[M + H]^+$; Calculated for $C_{12}H_{13}ClN_2S_3$: C, 45.48; H, 4.13; N, 8.84. Found: C, 45.59; H, 4.10; N 8.89.

2-(4-Nitrophenyl)-5-(n-butyldisulfanyl)-1,3,4-thiadiazole (7g)

Yellow solid; m.p.: 94.2–96.1 °C; Yield 70%; IR (cm^{-1}): 3134, 2360, 2074, 1635, 1517, 1401, 1346, 1107, 991, 864, 853, 752, 687, 619; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.38 (2H, d, $J = 8.6$ Hz, Ph-H), 8.24 (2H, d, $J = 8.6$ Hz, Ph-H), 3.10 (2H, t, $J = 7.2$ Hz, S-S-CH_2), 1.67–1.74 (2H, m, S-S-CH$_2$CH_2), 1.37–1.46 (2H, m, CH_2CH$_3$), 0.89 (3H, t, $J = 7.2$ Hz, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 174.69, 167.45, 149.18, 135.23, 129.16 (2C), 124.93 (2C), 38.76, 30.97, 21.35, 13.85; MS (ESI, *m/z*): 328.4 $[M + H]^+$; Calculated for $C_{12}H_{13}N_3O_2S_3$: C, 44.02; H, 4.00; N, 12.83. Found: C, 43.91; H, 3.97; N, 12.90.

2-Phenyl-5-(2-butyldisulfanyl)-1,3,4-thiadiazole (7h)

Yellow oil; Yield 70%; IR (cm^{-1}): 3134, 2968, 2930, 2874, 2360, 2342, 1967 1889, 1636, 1457, 1289, 1108, 1001, 978, 762, 689, 622; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.95 (2H, d, $J = 6.7$ Hz, Ph-H), 7.58 (2H, t, $J = 7.1$ Hz, Ph-H), 7.49 (1H, t, $J = 7.2$ Hz, Ph-H), 3.18–3.25 (1H, m, S-S-CH), 1.63–1.75 (1H, m, CH$_2$-Ha), 1.54–1.63 (1H, m, CH$_2$-Hb), 1.32 (3H, d, $J = 6.8$ Hz, CH$_3$CH), 0.95 (3H, t, $J = 7.2$ Hz, CH$_2$CH$_3$); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 172.51, 169.65, 131.87, 129.81 (2C), 129.75, 127.95 (2C), 49.35, 28.83, 20.10, 11.56; MS (ESI, *m/z*): 283.4 $[M + H]^+$; Calculated for $C_{12}H_{14}N_2S_3$: C, 51.03; H, 5.00; N, 9.92. Found: C, 51.15; H, 5.05; N, 9.86.

2-(4-Aminophenyl)-5-(2-butyldisulfanyl)-1,3,4-thiadiazole (7i)

Yellow solid; m.p.: 99.4–101.3 °C; Yield 75%; IR (cm^{-1}): 3151, 2074, 1635, 1401, 1107, 994, 823, 617; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.61 (2H, d, $J = 8.6$ Hz, Ph-H), 6.64 (2H, d, $J = 8.6$ Hz, Ph-H), 5.92 (2H, s, NH$_2$), 3.15–3.23 (1H, m, S-S-CH); 1.67–1.76 (1H, m, CH$_2$-Ha), 1.55–1.66 (1H, m, CH$_2$-Hb), 1.33 (3H, d, $J = 6.8$ Hz, CH$_3$CH), 0.95 (3H, t, $J = 7.2$ Hz, CH$_2$CH$_3$); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 170.93, 168.23, 152.70, 129.51 (2C), 116.67, 114.15 (2C), 49.28, 28.74, 20.14, 11.59; MS (ESI, *m/z*): 298.5 $[M + H]^+$; Calculated for $C_{12}H_{15}N_3S_3$: C, 48.45; H, 5.08; N, 14.13. Found: C, 48.32; H, 5.13; N, 14.07.

2-(4-Methylphenyl)-5-(2-butyldisulfanyl)-1,3,4-thiadiazole (7j)

Yellow oil; Yield 70%; IR (cm^{-1}): 3134, 2360, 1635, 1507, 1401, 1312, 1289, 1260, 1215, 1183, 1110, 996, 976, 817,

760, 710, 620; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.83 (2H, d, $J = 8.0$ Hz, Ph-H), 7.36 (2H, d, $J = 8.0$ Hz, Ph-H), 3.18–3.26 (1H, m, S-S-CH), 2.37 (3H, s, Ph-CH_3), 1.67–1.76 (1H, m, CH_2-Ha), 1.55–1.65 (1H, m, CH_2-Hb), 1.34 (3H, d, $J = 6.8$ Hz, CH_3CH), 0.95 (3H, t, $J = 7.2$ Hz, CH_2CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 172.24, 169.52, 142.01, 130.38 (2C), 127.84 (2C), 127.07, 49.32, 28.79, 21.47, 20.11, 11.56; MS (ESI, *m/z*): 297.5 $[M + H]^+$; Calculated for $C_{13}H_{16}N_2S_3$: C, 52.67; H, 5.44; N, 9.45. Found: C, 52.53; H, 5.49; N, 9.39.

2-(4-Methoxyphenyl)-5-(2-butyldisulfanyl)-1,3,4-thiadiazole (7k)

White solid; m.p.: 42.6–44.2 °C; Yield 75%; IR (cm^{-1}): 3145, 2075, 1635, 1401, 1309, 1256, 1109, 992, 837, 811, 620; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.90 (2H, d, $J =$ 8.1 Hz, Ph-H), 7.10 (2H, d, $J = 8.0$ Hz, Ph-H), 3.84 (3H, s, Ph-OCH_3), 3.21–3.27 (1H, m, S-S-CH), 1.66–1.73 (1H, m, CH_2-Ha), 1.58–1.65 (1H, m, CH_2-Hb), 1.35 (3H, d, $J =$ 6.4 Hz, CH_3CH), 0.96 (3H, t, $J = 7.2$ Hz, CH_2CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 171.15, 169.59, 162.28, 129.69 (2C), 122.26, 115.30 (2C), 55.97, 49.31, 28.77, 20.13, 11.58; MS (ESI, *m/z*): 313.5 $[M + H]^+$; Calculated for $C_{13}H_{16}N_2OS_3$: C, 49.97; H, 5.16; N, 8.97. Found: C, 49.82; H, 5.21; N, 8.90.

2-(3,4,5-Trimethoxyphenyl)-5-(2-butyldisulfanyl)-1,3,4-thiadiazole (7l)

Yellow oil; Yield 69%; IR (cm^{-1}): 3135, 2085, 1634, 1586, 1509, 1403, 1330, 1240, 1129, 998, 845, 821, 759, 732, 618; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.22 (2H, s, Ph-H), 3.88 (3H, s, Ph-OCH_3), 3.87 (3H, s, Ph-OCH_3), 3.74 (3H, s, Ph-OCH_3), 3.20–3.29 (1H, m, S-S-CH), 1.68–1.78 (1H, m, CH_2-Ha), 1.57–1.67 (1H, m, CH_2-Hb), 1.36 (3H, d, $J =$ 6.8 Hz, CH_3CH), 0.96 (3H, t, $J = 7.6$ Hz, CH_2CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 172.13, 169.62, 153.84 (2C), 140.85 (2C), 124.54, 105.44, 60.60, 56.59 (2C), 49.27, 28.79, 20.01, 11.53; MS (ESI, *m/z*): 373.5 $[M + H]^+$; Calculated for $C_{15}H_{20}N_2O_3S_3$: C, 48.36; H, 5.41; N, 7.52. Found: C, 48.48; H, 5.37; N, 7.58.

2-(4-Chlorophenyl)-5-(2-butyldisulfanyl)-1,3,4-thiadiazole (7m)

White solid; m.p.: 52.1–53.9 °C; Yield 72%; IR (cm^{-1}): 3423, 3134, 1634, 1401, 1133, 1108, 996, 851, 821, 639, 615; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.99 (d, $J = 8.4$ Hz, 2H, Ph-H), 7.63 (2H, d, $J = 8.5$ Hz, Ph-H), 3.22–3.30 (1H, m, S-S-CH), 1.69–1.78 (1H, m, CH_2-Ha), 1.57–1.67 (1H, m, CH_2-Hb), 1.36 (3H, d, $J = 6.8$ Hz, CH_3CH), 0.97 (3H, t, $J = 7.2$ Hz, CH_2CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 166.98, 154.52, 136.65, 129.92 (2C), 129.59 (2C), 128.51, 49.34, 28.81, 20.12, 11.58; MS (ESI, *m/z*): 317.4 $[M + H]^+$; Calculated for $C_{12}H_{13}ClN_2S_3$: C, 45.48; H, 4.13; N, 8.84. Found: C, 45.40; H, 4.16; N, 8.79.

2-(4-Nitrophenyl)-5-(2-butyldisulfanyl)-1,3,4-thiadiazole (7n)

White solid; m.p.: 114.3–116.5 °C; Yield 74%; IR (cm^{-1}): 3443, 3133, 2359, 1635, 1519, 1401, 1343, 1108, 985, 856, 752, 688, 639, 619; ^{1}H NMR (400 MHz, DMSO-d_6,) δ: 8.37 (2H, d, $J = 8.0$ Hz, Ph-H), 8.24 (2H, d, $J = 7.9$ Hz, Ph-H), 3.26–3.32 (1H, m, S-S-CH), 1.68–1.75 (1H, m, CH_2-Ha), 1.59–1.68 (1H, m, CH_2-Hb), 1.36 (3H, d, $J = 6.4$ Hz, CH_3CH), 0.98 (3H, t, $J = 7.2$ Hz, CH_2CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 175.41, 167.38, 149.23, 135.27, 129.22 (2C), 124.96 (2C), 49.39, 28.84, 20.13, 11.59; MS (ESI, *m/z*): 328.4 $[M + H]^+$; calculated for $C_{12}H_{13}N_3O_2S_3$: C, 44.02; H, 4.00; N, 12.83. Found: C, 44.11; H, 4.04; N, 12.78.

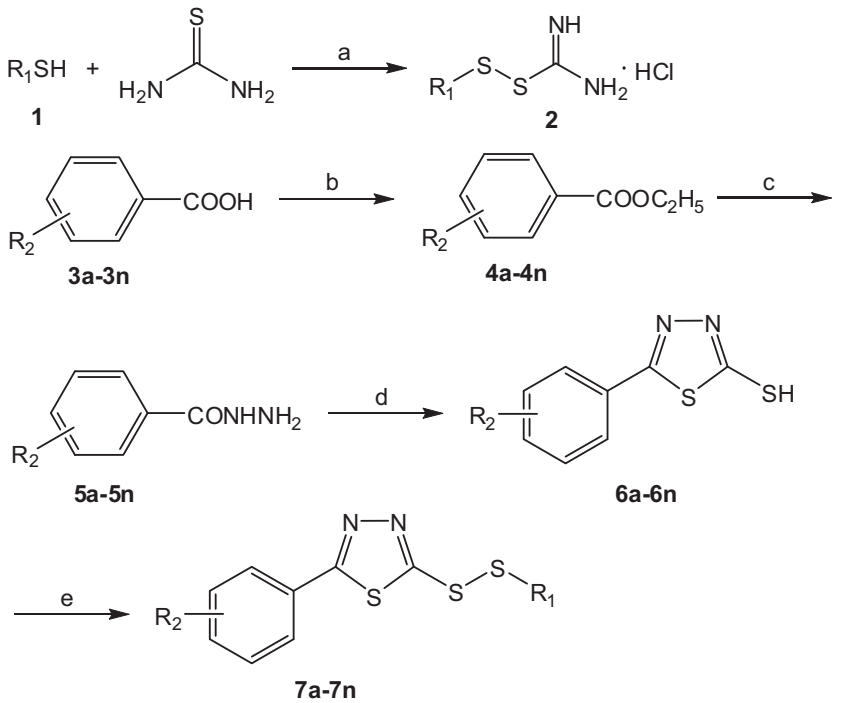
Tumor cell growth inhibitory assay

The cell lines (SMMC-7721, MCF-7, and A549) were cultured in proper medium in a 5% CO_2 at 37 °C during the experiment. The inhibition (IC_{50}) of the selected tumor cells proliferation by target compounds **7a–7n** and reference drugs was measured by our previous method as described in literature (Wang et al. 2017).

Results and discussion

Synthesis

The synthesis pathway of 2-substituted phenyl-5-substituted disulfanyl-1,3,4-thiadiazoles **7a–7n** is shown in Scheme 1, all of the fourteen 1,3,4-thiadiazole derivatives are unknown compounds. The reaction of thiourea and *n*-butylthiol, 2-butylthiol yielded S-alkyl-thioisothiourea hydrochloride **2** following the literature method (Sirakawa et al. 1970), which was directly used for the next step reaction without being purified. The synthesis of 2-substituted phenyl-5-mercapto-1,3,4-thiadi-azoles **6a–6n** was commenced with benzoic acid by four steps in total yield 45.1–65.0% (Jha et al. 2010; Li et al. 2013). First, condensation of appropriate benzoic acid **3a–3n** with ethanol gave ester **4a–4n** (79.3–88.4%). Hydrazidation of ester **4a–4n** delivered the benzohydrazide **5a–5n** (80.1–91.4%), which reacted with CS_2 and KOH produced compounds **6a–6n** (71.0–80.5%). Finally, nucleophilic replacement of **6a–6n** with **2** in the presence of $NaHCO_3$ yielded 2-substituted phenyl-5-substituted disulfanyl-1,3,4-thiadiazoles **7a–7n** in moderate



Scheme 1 Synthetic pathway of the title compounds **7a**–**7n**. Reaction conditions and reagents: **a** Concd. HCl, H_2O_2 (30%), 0–5 °C, 3 h; **b** EtOH, concd. H_2SO_4, reflux, 8–12 h; **c** $NH_2NH_2{\cdot}H_2O$, EtOH, reflux, 5–**8** h; **d** (i) KOH, CS_2, EtOH, 0–5 °C, 2 h; (ii) Concd. H_2SO_4, 0–5 °C, 2 h; **e** Compound **2**, MeOH, $NaHCO_3/H_2O$, rt, 3 h

R_2 (R_1 = n-C_4H_9): H (**7a**), 4-NH_2 (**7b**), 4-CH_3 (**7C**), 4-OCH_3 (**7d**), 3, 4, 5-$(OCH_3)_3$ (**7e**), 4-Cl (**7f**), 4-NO_2 (**7g**); R_2 (R_1 = 2-C_4H_9): H (**7h**), 4-NH_2 (**7i**), 4-CH_3 (**7j**), 4-OCH_3 (**7k**), 3, 4, 5-$(OCH_3)_3$ (**7l**), 4-Cl (**7m**), 4-NO_2 (**7n**)

yields. The pure form of the compounds **7a**–**7n** was obtained by recrystallization from the mixture of ethanol and water.

Pharmacology evaluation

The title compounds consist of two series, which were known as **7a–7g** and **7h–7n**, respectively. Compounds **7a–7g** all possess a *n*-butyl group linked with disulfide bond, whereas, compounds **7h–7n** all carry 2-butyl group bonded to disulfide. Evaluation of the antitumor activity in vitro for 2-substituted phenyl-5-substituted disulfanyl-1,3,4-thiadiazoles **7a–7n** was carried out by utilizing CCK-8 assay versus three types of tumor cell lines, namely, SMMC-7721, MCF-7, and A549. The inhibitory activities IC_{50} (μM) are shown in Table 1.

We found from the results that all tested compounds **7a–7n** displayed admissible antiproliferative activities for selected tumor cell lines. For SMMC-7721 cells, **7m** showed moderate antitumor effect ($IC_{50} = 17.83 \pm 0.68$ μM), whereas, the rest of tested compounds exhibited good inhibitory efficacy ($IC_{50} = 1.93$–8.60 μM). Meanwhile, most title compounds were found to possess higher antiproliferative activity in comparison with 5-fluorouracil ($IC_{50} = 5.62 \pm 0.28$ μM). Among them, six compounds, including **7d**, **7e**, **7h**, **7i**, **7j**, and **7l** revealed higher antitumor effect than PX-12 ($IC_{50} = 4.05 \pm 0.32$ μM). By comparing **7a** with **7h**, although they have the same phenyl substituent in position 2 of 1,3,4-thiadiazole, we found that the difference is obvious in their antitumor efficacy. Compound **7h** having 2-butyl group linked to disulfide, showed

Table 1 Inhibition (IC_{50}) of SMMC-7721, MCF-7, and A549 cells proliferation by compounds **7a–7n**

Compound	IC_{50} (μM)		
	SMMC-7721	MCF-7	A549
7a	4.64 ± 0.18	8.60 ± 0.38	32.77 ± 1.98
7b	8.60 ± 0.33	25.95 ± 1.47	7.80 ± 0.33
7c	4.39 ± 0.11	9.66 ± 0.45	9.85 ± 0.42
7d	2.94 ± 0.09	8.00 ± 0.37	6.95 ± 0.22
7e	2.28 ± 0.12	7.80 ± 0.12	33.37 ± 2.17
7f	7.90 ± 0.32	6.74 ± 0.31	8.80 ± 0.29
7g	5.14 ± 0.24	8.20 ± 0.33	14.37 ± 0.56
7h	1.93 ± 0.08	8.20 ± 0.21	18.73 ± 0.51
7i	3.69 ± 0.26	3.54 ± 0.17	8.00 ± 0.18
7j	3.89 ± 0.15	4.49 ± 0.16	6.35 ± 0.39
7k	6.15 ± 0.31	3.04 ± 0.09	33.37 ± 1.88
7l	2.94 ± 0.13	8.25 ± 0.25	5.89 ± 0.24
7m	17.83 ± 0.68	6.35 ± 0.18	3.67 ± 0.13
7n	6.89 ± 0.29	9.10 ± 0.40	35.63 ± 2.28
PX-12	4.05 ± 0.32	5.12 ± 0.21	10.51 ± 0.30
5-FU	5.62 ± 0.28	14.26 ± 0.66	8.13 ± 0.34

better activity than **7a**. Similarly, **7b** and **7i**, **7d** and **7k**, **7f** and **7m** also emerged obvious differences in antitumor potency. Especially noteworthy is compound **7h** displayed excellent growth inhibitory activity ($IC_{50} = 1.93 \pm 0.08\,\mu M$). We also found substitutes at phenyl ring, have obvious effects on biological activity, but further research and discussion are needed. For MCF-7 cells, compound **7b** carrying 4-amino group at the phenyl ring, showed moderate antiproliferative activity ($IC_{50} = 25.95 \pm 1.47\,\mu M$). The other screened compounds emerged good cytotoxic efficacy ($IC_{50} < 10\,\mu M$), and displayed higher activities than 5-fluorouracil ($IC_{50} = 14.26 \pm 0.66\,\mu M$). But most compounds did not show obvious advantage in comparison with PX-12 ($IC_{50} = 5.12 \pm 0.21\,\mu M$). **7a** and **7h**, **7e** and **7l**, **7f** and **7m**, **7g** and **7n** showed similar activity against MCF-7 cells, respectively. but **7b** and **7i**, **7c** and **7j**, **7d** and **7k** revealed obvious differences in antitumor potency, respectively. Compounds **7k** and **7i** were manifested promising growth inhibitory activity with IC_{50} at 3.04 ± 0.09 and $3.54 \pm 0.17\,\mu M$, respectively. **7i**, **7j**, **7k**, and **7l** carrying electron-donating groups, including 4-amino, 4-methyl, 4-methoxy and 3,4,5-trimethoxy, respectively, were superior to **7h** in antitumor efficacy. For A549 cells, six compounds, including **7a**, **7e**, **7g**, **7h**, **7k**, and **7n** revealed moderate antiproliferative activities ($IC_{50} = 14.37–35.63\,\mu M$), whereas, the rest of compounds displayed good potency ($IC_{50} = 3.67–9.85\,\mu M$), and some compounds showed higher effects than PX-12 ($IC_{50} = 10.51 \pm 0.30\,\mu M$) and 5-fluorouracil ($IC_{50} = 8.13 \pm 0.34\,\mu M$). Especially, 4-chloro-substituted derivative, compound **7m** was found to have the strongest cytotoxic efficacy ($IC_{50} = 3.67 \pm 0.13\,\mu M$). Except **7e** ($IC_{50} = 35.63 \pm 2.28\,\mu M$), the other compounds carrying various substituents all showed highly enhanced potency against A549 cells compared to compound **7a**. In addition, we found that the tested compounds displayed preferential growth inhibitory activity to SMMC-7721 compared to A549 cells on the whole. Therefore, we believe that the above findings are useful for further studies on structure–activity relationship.

Conclusion

The present work comprises the synthesis of some new 2-substituted phenyl-5-substituted disulfanyl-1,3,4-thiadiazoles **7a–7n** and screening for their antitumor activities against SMMC-7721, MCF-7, and A549 lines via CCK-8 assay. Our preliminary investigation revealed that the tested compounds have potency of inhibition for three tumor cell lines, and the effects of substituents on anticancer efficacy have been observed. Interestingly, some compounds showed stronger antitumor effects than reference drugs PX-12 and 5-fluorouracil, and several screened compounds, such as **7h**, **7i**, **7k**, and **7m** displayed promising biological activities. Therefore, the pharmacological results could be helpful for the development of new anticancer agents.

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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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