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Design, Synthesis and Antifungal Activity of Novel Thioureas Containing 1,3,4-Thiadiazole and Thioether Skeleton

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Abstract To find new lead compounds with high antifungal activity, a series of new thiourea derivatives containing 1,3,4-thiadiazole and thioether skeleton was designed *via* linking the active sub-structures. The target compounds were prepared *via* three steps from the commercially available thiosemicarbazide. Their structures were characterized by means of HRMS, ^{1}H NMR, ^{13}C NMR and IR spectroscopy. The preliminary results indicate that the title compounds show various antifungal activity against the tested fungi. Compounds **4**c, **4**g, **4**h, **4**k, **4**n, **4**o, **4**p, **4**q and **4**r display excellent antifungal activities against one or more tested fungi with inhibitory efficiencies of 90%—100% at 200 μg/mL. Especially, compound **4**o shows the best inhibitory effect against *Curvularia lunata, Cotton Fusarium Wilt, P. P. var nicotianae* and *Fusarium spp.* with the EC_{50} values of 28.12, 30.41, 15.2 and 6.22 μg/mL, respectively, which are even superior to those of triadimefon(98.73, 96.58, 105.37 and 102.18 μg/mL). The preliminary structure-activity relationship indicates that allyl and aromatic groups are favorable to their antifungal activities.

Keywords Thiourea; 2-Amino-5-alkylthio-1,3,4-thiadiazole; Antifungal activity

1 Introduction

Approximately 70%—80% of plant diseases are caused by plant pathogenic fungi. Plant fungous diseases negatively affect a large number of important flowers, fruits and vegetables, and limit crop production worldwide, especially in developing countries. For example, *Fusarium Wilt* can cause stem blight of cotton and lead to yield loss[1]; the premature withered leaves of corn caused by *Curvularia lunata* can result in a yield loss of 20%—30%, and the yield reduction will be more than 50% in seriously damaged fields，or even the whole field fails to harvest[2]. The loss caused by plant pathogenic fungi has attracted wide attention. Although many new antifungal agents have been reported in recent years, the emergence of drug resistance has become an obstacle. Thus, more effective classes of such agents are desired and the search for new antifungal agents with high activity remains a significant task for the current pesticide research[3,4].

Thiourea derivatives are the subject of hot topics for their broad-spectrum biological activities based on the virtue group of —NHCSNH—. Various thiourea derivatives have been synthesized as potential antimicrobial[5—7], antiviral[8,9], anticancer[10], antitumor[11], antimalarial[12], herbicidal[13], insecticidal[14] agents and plant growth regulators[15]. Some thioureas, such as thiophanate, thiophanate methyl and thiophamine have been used worldwidely as antifungal agents(Fig.1).

On the other hand, 1,3,4-thiadiazoles are organic heterocyclic compounds and have been reported to have a wide application in pharmaceuticals and pesticides due to their good and extensive biological activities[16—20]. 1,3,4-Thiadiazole nucleus constitutes the active part of several biologically active compounds including antifungal agents, such as bismerthiazol and 2,5-dimercapto-1,3,4-thiadiazole zinc salt[21]. Introduction of a thiadiazole ring into the thiourea may improve the biological activities. In addition, thioether compounds also exhibit various biological activities[22] and thioether incorporated 1,3,4-thiadiazole scaffold is an important pharmacophore[23].

Considering the biological significance of the aforementioned active groups and in continuation to our previous work on the synthesis of various bioactive molecules[24—27], we reported herein the design, synthesis and antifungal activity of some new thiourea derivatives bearing 1,3,4-thiadiazole and thioether skeleton. The target compounds were designed as shown in Fig.2 *via* linking the active sub-structures and new lead compounds with favorable biological activities were expected to be found.

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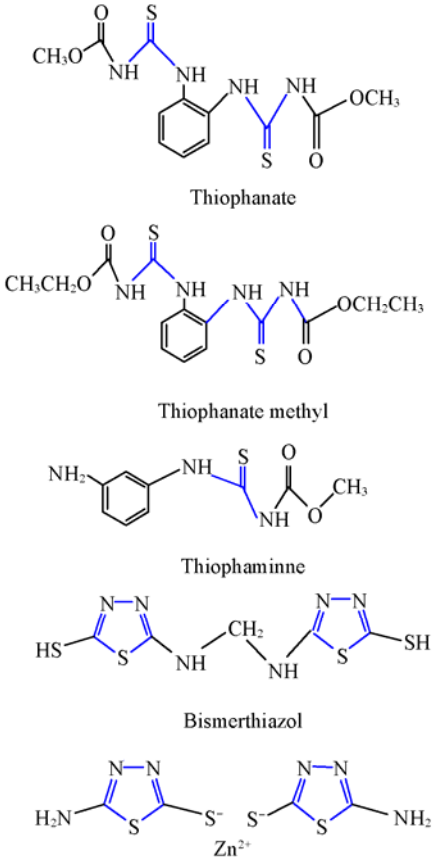


Fig.1 Antifungal agents with thiourea group or 1,3,4-thiadiazole ring

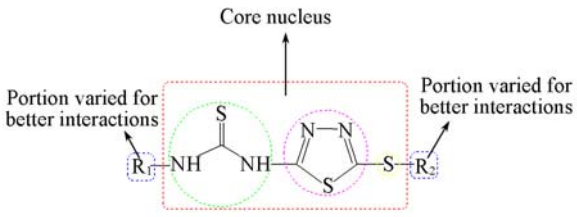


Fig.2 Design strategy for target compounds

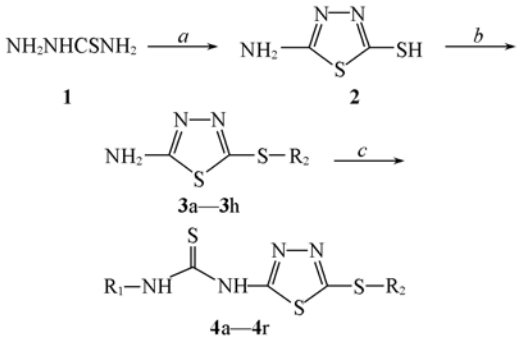
2 Experimental

2.1 Reagents and Instruments

All reagents were of analytical grade or were chemically pure and used without further treatment unless otherwise noted. The melting points were determined on an X-5 digital microscopic melting-point apparatus(Beijing Tech. Instruments Co., Beijing, China) and were not corrected. The infrared spectra were measured on a Nicolet IS10 Fourier transform infrared spectrophotometer(4000—400 cm^{-1}, KBr pellets). ^1H NMR and ^{13}C NMR spectra were obtained on a Bruker DPX-400 or Advance 300 spectrometer in $CDCl_3$ or DMSO-d_6 solutions, respectively. The chemical shifts were recorded in parts per million(δ) downfield from TMS[$(CH_3)_4Si$]. The high-resolution mass spectrometry(HRMS) data were recorded on a Waters Q-Tof micro^{TM} instrument with an electrospray ionization source(ESIMS).

2.2 Preparation of Target Compounds 4a—4r

The target compounds **4**a—**4**r were prepared in this paper according to Scheme 1.



4a: R_1=Et, R_2=Et; **4**b: R_1=Et, R_2=*n*-Pr; **4**c: R_1=Et, R_2=allyl; **4**d: R_1=Et, R_2=*n*-Bu; **4**e: R_1=Et, R_2=*n*-hexyl; **4**f: R_1=Et, R_2=benzyl; 4g: R_1=Et, R_2=2-F-6-Cl-benzyl; 4h: R_1=Et, R_2=2-Cl-5-methylenepyridine; 4i: R_1=allyl, R_2=Et; 4j: R_1=allyl, R_2=*n*-Pr; **4**k: R_1=allyl, R_2=allyl; **4**l: R_1=allyl, R_2=*n*-Bu; **4**m: R_1=allyl, R_2=*n*-hexyl; **4**n: R_1=allyl, R_2=benzyl; **4**o: R_1=allyl, R_2=2-F-6-Cl-benzyl; **4**p: R_1=allyl, R_2=2-Cl-5-methylenepyridine; **4**q: R_1=4-CF_3-phenyl, R_2=*n*-Pr; **4**r: R_1=4-CF_3-phenyl, R_2=allyl.

Scheme 1 Synthetic routes of target compounds 4a—4r

Reagents and conditions: *a*. CS_2, DMF, reflux, 4 h; *b*. R_2—Cl(**3**a—**3**h: R_2=Et, *n*-Pr, allyl, *n*-Bu, *n*-hexyl, benzyl, 2-F-6-Cl-benzyl, 2-Cl-5-methylenepyridine), NaOH, CH_3OH, stirring, 5 h; *c*. R_1—NCS(R_1=Et, allyl, 4-CF_3-phenyl), DMF:CH_3CN(1:1, volume ratio), reflux, 10 h.

2.2.1 Syntheses of 2-Amino-5-alkylthio-1,3,4-thiadiazoles(3a—3h)

2-Amino-5-alkylthio-1,3,4-thiadiazoles(**3**a—**3**h) were prepared *via* two steps according to the reported method[28,29], which began with thiosemicarbazide(**1**).

2.2.2 Preparation of Target Compounds 4a—4r

Compounds **3**a—**3**h(2.0 mmol) were dissolved in dry DMF(3.0 mL) in a round bottom flask. Subsequently, a solution of isothiocyanate(R_1—Cl, 2.5 mmol) in dry acetontrile(3.0 mL) was added dropwise through funnel. The mixture was stirred for 10 h under reflux with TLC monitoring using ethyl acetate:ethylene dichloride(1:2, volume ratio) as eluent. After the completion of the reaction, the solvent was removed by reduced pressure distillation. The residue was purified by recrystalization in ethanol or mixed solvents(ethanol and DMF) to afford pure compounds(**4**a—**4**r). Yields of the products varied between 81%—94%.

N-Ethyl-*N*′-[5-(ethylthio)-1,3,4-thiadiazol-2-yl]-thiourea (**4**a): yield 92%, a light yellow solid, m. p. 189.4—190.5 °C. IR(KBr), $\tilde{\nu}$/cm^{-1}: 3326(N—H), 1338(C═S), 1528, 1455, 1378, 1259, 1058, 852(thiadiazole ring), 645(C—S—C). ^1H NMR (400 MHz, $CDCl_3$), δ: 1.36(t, *J*=8.0 Hz, 3H, CH_3), 1.48(t, *J*=8.0 Hz, 3H, CH_3), 3.25(q, *J*=8.0 Hz, 2H, SCH_2), 3.74(q, *J*=8.0 Hz, 2H, NCH_2), 7.54（br, 2H, NH). ^{13}C NMR(100 MHz, $CDCl_3$), δ: 13.83(CH_3), 14.78(CH_3), 28.52(SCH_2), 40.37(NCH_2), 158.67, 164.42(thiadiazole ring, C_2, C_5), 178.53(C═S). HRMS(ESI): *m/z*[$C_7H_{12}N_4S_3$+H]$^+$, cacld. 249.0302; found 249.0299.

N-Ethyl-*N*′-[5-(propylthio)-1,3,4-thiadiazol-2-yl]-thiourea (**4**b): yield 87%, a white solid, m. p. 185.5—187.2 °C. IR(KBr), $\tilde{\nu}$/cm^{-1}: 3327(N—H), 1336(C═S), 1531, 1458, 1380,

1264, 1056, 864(thiadiazole ring), 647(C—S—C). ^{1}H NMR (400 MHz, $CDCl_3$), δ: 1.07(t, J=8.0 Hz, 3H, CH_3), 1.34(t, J=8.0 Hz, 3H, CH_3), 1.76—1.86(m, 2H, CH_2CH_3), 3.18(t, J=8.0 Hz, 2H, SCH_2), 3.71(q, J=8.0 Hz, 2H, NCH_2), 7.46(br, 2H，NH). ^{13}C NMR(100 MHz, $CDCl_3$), δ: 13.25(CH_3), 13.86(CH_3), 22.76(CH_2), 36.17(SCH_2), 40.33(NCH_2), 158.97, 164.40 (thiadiazole ring, C_2, C_5), 178.22(C═S). HRMS(ESI): *m/z* $[C_8H_{14}N_4S_3+H]^+$, calcd. 263.0459; found 263.0456.

N-Ethyl-*N*′-[5-(allylthio)-1,3,4-thiadiazol-2-yl]-thiourea (**4**c): yield 94%, a white solid, m. p. 164.8—166.6 °C. IR(KBr), $\tilde{\nu}$/cm^{-1}: 3325(N—H), 1326(C═S), 1572, 1459, 1375, 1252, 1044, 826(thiadiazole ring), 641(C—S—C). ^{1}H NMR (400 MHz, $CDCl_3$), δ: 1.38(t, J=8.0 Hz, 3H, CH_3), 3.76(q, J=8.0 Hz, 2H, NCH_2), 3.86(d, J=16.0 Hz, 2H, SCH_2), 5.21—5.39(m, 2H, ═CH_2), 5.95—6.04(m, 1H, ═CH), 7.54(br, 1H, NH), 14.02(br, 1H, NH). ^{13}C NMR(100 MHz, $CDCl_3$), δ: 13.87(CH_3), 37.05(SCH_2), 40.37(NCH_2), 119.5(═CH_2), 132.05(═CH), 157.72, 165.01(thiadiazole ring, C_2, C_5), 178.26(C═S). HRMS(ESI): *m/z*$[C_8H_{12}N_4S_3+H]^+$, calcd. 261.0302, found 261.0299.

N-Ethyl-*N*′-[5-(butylthio)-1,3,4-thiadiazol-2-yl]-thiourea (**4**d): yield 83%, a light yellow solid, m. p. 165.9—166.8 °C. IR(KBr), $\tilde{\nu}$/cm^{-1}: 3326(N—H), 1336(C═S), 1528, 1459, 1375, 1259, 1056, 852(thiadiazole ring), 645(C—S—C). ^{1}H NMR (400 MHz, $CDCl_3$), δ: 0.98(t, J=8.0 Hz, 3H, CH_3), 1.36(t, J=8.0 Hz, 3H, CH_3), 1.46—1.53(m, 2H, CH_2), 1.74—1.82(m, 2H, CH_2), 3.24(t, J=6.0 Hz, 2H, SCH_2), 3.74(q, J=8.0 Hz, 2H, NCH_2), 7.54(br, 2H, NH). ^{13}C NMR(100 MHz, $CDCl_3$), δ: 13.57(CH_3), 13.88(CH_3), 21.84(CH_2), 31.29(CH_2), 33.97 (SCH_2), 40.34(NCH_2), 159.02, 164.41(thiadiazole ring, C_2, C_5), 178.31(C═S). HRMS(ESI): *m/z*$[C_9H_{16}N_4S_3+H]^+$, calcd. 277.0615, found 277.0611.

N-Ethyl-*N*′-[5-(hexylthio)-1,3,4-thiadiazol-2-yl]-thiourea (**4**e): yield 94%, a white solid, m. p. 149.1—151.2 °C. IR(KBr), $\tilde{\nu}$/cm^{-1}: 3323(N—H), 1338(C═S), 1538, 1455, 1378, 1265, 1058, 854(thiadiazole ring), 646(C—S—C). ^{1}H NMR (400 MHz, $CDCl_3$), δ: 0.92(t, J=8.0 Hz, 3H, CH_3), 1.31—1.34(m, 4H, CH_2 CH_2), 1.36(t, J=8.0 Hz, 3H, CH_3), 1.43—1.51(m, 2H, CH_2), 1.75—1.80(m, 2H, CH_2), 3.23(t, J=8.0 Hz, 2H, SCH_2), 3.73(q, J=6.0 Hz, 2H, NCH_2), 7.41(br, 1H, NH), 13.83(br, 1H, NH). ^{13}C NMR(100 MHz, $CDCl_3$), δ: 13.88(CH_3), 13.99(CH_3), 22.51(CH_2), 28.36(CH_2), 29.22(CH_2), 31.25(CH_2), 34.29(SCH_2), 40.34(NCH_2), 159.14, 164.39 (thiadiazole ring, C_2, C_5), 178.31(C═S). HRMS(ESI): *m/z* $[C_{11}H_{20}N_4S_3+H]^+$, calcd. 305.0928, found 305.0924.

N-Ethyl-*N*′-[5-(benzylthio)-1,3,4-thiadiazol-2-yl]-thiourea (**4**f): yield 89%, a white solid, m. p. 199.1—201.7 °C. IR(KBr), $\tilde{\nu}$/cm^{-1}: 3341(N—H), 1342(C═S), 1525, 1454, 1389, 1256, 1054, 827(thiadiazole ring), 650(C—S—C). ^{1}H NMR (400 MHz, $CDCl_3$), δ: 1.33(t, J=8.0 Hz, 3H, CH_3), 3.70(q, J=8.0 Hz, 2H, NCH_2), 4.44(s, 2H, SCH_2), 7.32—7.40(m, 5H, ArH), 7.54(br, 2H, NH). ^{13}C NMR(100 MHz, $CDCl_3$), δ: 13.86(CH_3), 38.61(SCH_2), 40.35(NCH_2), 128.05(CH), 128.86 (CH), 128.96(CH), 135.68(C), 158.03, 165.04(thiadiazole ring, C_2, C_5), 178.41(C═S). HRMS(ESI): *m/z*$[C_{12}H_{14}N_4S_3+H]^+$, calcd. 311.0459, found 311.0460.

N-Ethyl-*N*′-[5-(2-fluoro-6-chlorobenzylthio)-1,3,4-thiadiazol-2-yl]-thiourea(**4**g): yield 81%, a light yellow solid, m. p. 195.6—197.3 °C. IR(KBr), $\tilde{\nu}$ /cm^{-1}: 3333(N—H), 1339(C═S), 1529, 1451, 1384, 1243, 1047, 834(thiadiazole ring), 651(C—S—C). ^{1}H NMR(400 MHz, $CDCl_3$), δ: 1.34(t, J=8.0 Hz, 3H, CH_3), 3.73(q, J=6.0 Hz, 2H, NCH_2), 4.58(s, 2H, SCH_2), 7.01(m, 1H, ArH), 7.25(m, 2H, ArH), 7.54(br, 2H, NH). ^{13}C NMR(100 MHz, $CDCl_3$), δ: 13.06(CH_3), 29.19(SCH_2), 39.37(NCH_2), 113.66(CH), 117.43(CH), 122.14(C), 125.08 (CH), 128.83(CCl), 130.28(CF), 159.05, 161.98(thiadiazole ring, C_2, C_5), 178.37(C═S). HRMS(ESI): *m/z* $[C_{12}H_{12}ClFN_4S_3+H]^+$, calcd. 362.9975, found 362.9977.

N-Ethyl-*N*′-[5-(6-chloro-pyridin-3-ylmethylthio)-1,3,4-thiadiazol-2-yl]-thiourea(**4**h): yield 84%, a light yellow solid, m. p. 199.5—201.3 °C. IR(KBr), $\tilde{\nu}$ /cm^{-1}: 3335(N—H), 1339(C═S), 1527, 1457, 1375, 1257, 1050, 845(thiadiazole ring), 643(C—S—C). ^{1}H NMR(400 MHz, $CDCl_3$), δ: 1.14(t, J=8.0 Hz, 3H, CH_3), 3.48(q, J=8.0 Hz, 2H, NCH_2), 4.46(s, 2H, SCH_2), 7.51(d, J=8.0 Hz, 1H, pyridine-H), 7.88(m, 1H, pyridine-H), 8.39(d, J=4.0 Hz, 1H, pyridine-H), 8.45(br, 1H, NH), 11.45(br, 1H, NH). ^{13}C NMR(100 MHz, $CDCl_3$), δ: 14.08(CH_3), 34.0(SCH_2), 39.37(NCH_2), 124.65(CH), 133.46(C), 140.70 (CH), 149.66(CCl), 150.50(HCN), 157.71, 162.30(thiadiazole ring, C_2, C_5), 179.22(C═S). HRMS(ESI): *m/z* $[C_{11}H_{12}ClN_5S_3+H]^+$, calcd. 346.0022, found 346.0020.

N-Allyl-*N*′-[5-(ethylthio)-1,3,4-thiadiazol-2-yl]-thiourea (**4**i): yield 83%, a white solid, m. p. 165.9—167.3 °C. IR(KBr), $\tilde{\nu}$/cm^{-1}: 3318(N—H), 1332(C═S), 1532, 1452, 1371, 1256, 1067, 861(thiadiazole ring), 649(C—S—C). ^{1}H NMR (400 MHz, $CDCl_3$), δ: 1.46(t, J=8.0 Hz, 3H, CH_3), 3.24(q, J=8.0 Hz, 2H, SCH_2), 4.37(s, 2H, NCH_2), 5.27(d, J=8.0 Hz, 1H, ═CH_2), 5.38(d, J=16.0 Hz, 1H, ═CH_2), 5.95—6.03(m, 1H, ═CH), 7.54(br, 1H, NH), 14.06(br, 1H, NH). ^{13}C NMR(100 MHz, $CDCl_3$), δ: 13.65(CH_3), 27.35(SCH_2), 46.79(NCH_2), 116.68(═CH_2), 131.57(═CH), 157.85, 163.37(thiadiazole ring, C_2, C_5), 177.53(C═S). HRMS(ESI): *m/z* $[C_8H_{12}N_4S_3+H]^+$, calcd. 261.0302, found 261.0300.

N-Allyl-*N*′-[5-(propylthio)-1,3,4-thiadiazol-2-yl]-thiourea (**4**j): yield 82%, a light yellow solid, m. p. 159.5—160.1 °C. IR(KBr), $\tilde{\nu}$ /cm^{-1}: 3320(N—H), 3085(═CH), 1331(C═S), 1534, 1458, 1372, 1256, 1053, 856(thiadiazole ring), 648(C—S—C). ^{1}H NMR(400 MHz, $CDCl_3$), δ: 1.08(t, J=8.0 Hz, 3H, CH_3), 1.77—1.86(m, 2H, CH_2), 3.20(t, J=8.0 Hz, 2H, SCH_2), 4.37(s, 2H, NCH_2), 5.28(d, J=12.0 Hz, 1H, ═CH_2), 5.39(d, J=16.0 Hz, 1H, ═CH_2), 5.95—6.04(m, 1H, ═CH), 7.54(br, 2H, NH). ^{13}C NMR(100 MHz, $CDCl_3$), δ: 13.26(CH_3), 22.69(CH_2), 36.05(SCH_2), 47.74(NCH_2), 117.64(═CH_2), 132.53(═CH), 159.15, 162.09(thiadiazole ring, C_2, C_5), 178.99(C═S). HRMS(ESI): *m/z*$[C_9H_{14}N_4S_3+H]^+$, calcd. 275.0459, found 275.0455.

N-Allyl-*N*′-[5-(allylthio)-1,3,4-thiadiazol-2-yl]-thiourea (**4**k): yield 84%, a yellow solid, m. p. 144.9—146.5 °C. IR(KBr), $\tilde{\nu}$/cm^{-1}: 3324(N—H), 1332(C═S), 1529, 1457, 1374, 1255, 1051, 857(thiadiazole ring), 648(C—S—C). ^{1}H NMR (400 MHz, $CDCl_3$), δ: 3.84(d, J=8.0 Hz, 2H, SCH_2), 4.37(s, 2H, NCH_2), 5.21—5.41(m, 4H, ═CH_2), 5.96—6.02(m, 2H, ═CH), 7.56(br, 2H, NH). ^{13}C NMR(100 MHz, $CDCl_3$), δ: 36.85(SCH_2),

47.80(NCH_2), 117.9(=CH_2), 119.51(=CH_2), 132.04(=CH), 132.48(=CH), 158.00, 164.47(thiadiazole ring, C_2, C_5), 178.59(C=S). HRMS(ESI): *m/z*[$C_9H_{12}N_4S_3$+H]$^+$, calcd. 273.0302, found 273.0299.

N-Allyl-*N*′-[5-(butylthio)-1,3,4-thiadiazol-2-yl]-thiourea (**4**l): yield 84%, a yellow solid, m. p. 145.6—147.1 °C. IR(KBr), $\tilde{\nu}$/cm^{-1}: 3325(N—H), 1333(C=S), 1533, 1459, 1373, 1258, 1056, 853(thiadiazole ring), 649(C—S—C). ^{1}H NMR (400 MHz, $CDCl_3$), δ: 0.98(t, *J*=8.0 Hz, 3H, CH_3), 1.47—1.53(m, 2H, CH_2), 1.73—1.80(m, 2H, CH_2), 3.23(t, *J*=8.0 Hz, 2H, SCH_2), 4.37(s, 2H, NCH_2), 5.27(d, *J*=8.0 Hz, 1H, =CH_2), 5.38(d, *J*=16.0 Hz, 1H, =CH_2), 5.94—6.03(m, 1H, =CH), 7.54(br, 2H, NH). ^{13}C NMR(100 MHz, $CDCl_3$), δ: 13.59(CH_3), 18.42(CH_2), 31.22(CH_2), 33.92(SCH_2), 58.44 (NCH_2), 117.56(=CH_2), 132.55(=CH), 159.27, 164.63 (thiadiazole ring, C_2, C_5), 178.64(C=S). HRMS(ESI): *m/z* [$C_{10}H_{16}N_4S_3$+H]$^+$, calcd. 289.0615, found 289.0613.

N-Allyl-*N*′-[5-(hexylthio)-1,3,4-thiadiazol-2-yl]-thiourea (**4**m): yield 81%, a light yellow solid, m. p. 135.2—137.8 °C. IR(KBr), $\tilde{\nu}$/cm^{-1}: 3324(N—H), 1331(C=S), 1534, 1458, 1372, 1256, 1056, 850(thiadiazole ring), 648(C—S—C). ^{1}H NMR (400 MHz, $CDCl_3$), δ: 0.92(t, *J*=8.0 Hz, 3H, CH_3), 1.31—1.35(m, 4H, CH_2CH_2), 1.43—1.50(m, 2H, CH_2), 1.73—1.80(m, 2H, CH_2), 3.22(t, *J*=8.0 Hz, 2H, SCH_2), 4.36(s, 2H, NCH_2), 5.27(d, *J*=8.0 Hz, 1H, =CH_2), 5.38(d, *J*=16.0 Hz, 1H, =CH_2), 5.94—6.04(m, 1H, =CH), 7.54(br, 2H, NH). ^{13}C NMR(100 MHz, $CDCl_3$), δ: 13.99(CH_3), 22.51(CH_2), 28.34(CH_2), 29.15(CH_2), 31.26(CH_2), 34.18(SCH_2), 47.73 (NCH_2), 117.61(=CH_2), 132.54(=CH), 159.29, 164.40 (thiadiazole ring, C_2, C_5), 178.69(C=S). HRMS(ESI): *m/z* [$C_{12}H_{20}N_4S_3$+H]$^+$, calcd. 317.0928, found 317.0925.

N-Allyl-*N*′-[5-(benzylthio)-1,3,4-thiadiazol-2-yl]-thiourea (**4**n): yield 85%, a light yellow solid, m. p. 183.1—184.2 °C. IR(KBr), $\tilde{\nu}$/cm^{-1}: 3337(N—H), 1327(C=S), 1526, 1450, 1379, 1250, 1060, 838(thiadiazole ring), 651(C—S—C). ^{1}H NMR (400 MHz, $CDCl_3$), δ: 4.34(s, 2H, NCH_2), 4.44(s, 2H, SCH_2), 5.22(d, *J*=8.0 Hz, 1H, =CH_2), 5.36(d, *J*=8.0 Hz, 1H, =CH_2), 5.92—6.02(m, 1H, =CH), 7.31—7.40(m, 5H, ArH), 7.56(br, 2H, NH). ^{13}C NMR(100 MHz, $CDCl_3$), δ: 38.38(SCH_2), 47.79(NCH_2), 117.88(=CH_2), 128.05(CH), 128.86(CH), 129.00(CH), 132.50(=CH), 135.64(C), 158.30, 164.64 (thiadiazole ring, C_2, C_5), 178.77(C=S). HRMS(ESI): *m/z* [$C_{13}H_{14}N_4S_3$+H]$^+$, calcd. 323.0459, found 323.0455.

N-Allyl-*N*′-[5-(2-fluoro-6-chlorobenzylthio)-1,3,4-thiadiazol-2-yl]-thiourea(**4**o): yield 92%, a yellow solid, m. p. 206.9—208.1 °C. IR(KBr), $\tilde{\nu}$/cm^{-1}: 3336(N—H), 1331(C=S), 1537, 1461, 1376, 1247, 1054, 843(thiadiazole ring), 652(C—S—C). ^{1}H NMR(400 MHz, $CDCl_3$), δ: 4.36(s, 2H, NCH_2), 4.58(s, 2H, SCH_2), 5.23(d, *J*=12.0 Hz, 1H, =CH_2), 5.36(d, *J*=16.0 Hz, 1H, =CH_2), 5.95—6.02(m, 1H, =CH), 7.03—7.29(m, 3H, ArH), 7.54(br, 2H, NH). ^{13}C NMR(100 MHz, $CDCl_3$), δ: 18.41(SCH_2), 58.67(NCH_2), 114.22(CH), 114.45(CH), 117.72(=CH_2), 125.75(C), 132.48(CH), 138.49(=CH), 142.91(CCl), 150.98(CF), 157.04, 163.50(thiadiazole ring, C_2, C_5), 178.13(C=S). HRMS(ESI): *m/z* [$C_{13}H_{12}ClFN_4S_3$+H]$^+$, calcd. 374.9975, found 374.9977.

N-Allyl-*N*′-[5-(6-chloro-pyridin-3-ylmethylthio)-1,3,4-thiadiazol-2-yl]-thiourea(**4**p): yield 91%, a yellow solid, m. p. 195.2—196.7 °C. IR(KBr), $\tilde{\nu}$/cm^{-1}: 3325(N—H), 1328(C=S), 1524, 1462, 1370, 1260, 1053, 842(thiadiazole ring), 649 (C—S—C). ^{1}H NMR(400 MHz, $CDCl_3$), δ: 4.36(s, 2H, NCH_2), 4.39(s, 2H, SCH_2), 5.23(d, *J*=8.0 Hz, 1H, =CH_2), 5.35(d, *J*=20.0 Hz, 1H, =CH_2), 5.93—6.03(m, 1H, =CH), 7.33(d, *J*=8.0 Hz, 2H, pyridine-H), 7.69—7.72(m, 1H, pyridine-H), 7.58(br, 1H, NH), 8.40(s, 1H, NH). ^{13}C NMR(100 MHz, $CDCl_3$), δ: 34.40(SCH_2), 47.78(NCH_2), 117.79(=CH_2), 124.44 (CH), 131.0(C), 132.55(=CH), 139.11(CH), 149.80(CCl), 151.11(HCN), 154.31, 159.80(thiadiazole ring, C_2, C_5), 179.00(C=S). HRMS(ESI): *m/z*[$C_{12}H_{12}ClN_5S_3$+H]$^+$, calcd. 358.0022, found 358.0019.

N-(4-Trifluoromethylphenyl)-*N*′-[5-(propylthio)-1,3,4-thiadiazol-2-yl]-thiourea(**4**q): yield 89%, a white solid, m. p. 209.7—211.1 °C. IR(KBr), $\tilde{\nu}$/cm^{-1}: 3207(N—H), 1332(C=S), 1539, 1491, 1400, 1256, 1049, 857(thiadiazole ring), 659(C—S—C). ^{1}H NMR(400 MHz, DMSO-d_6), δ: 1.00(t, *J*=8.0 Hz, 3H, CH_3), 1.64—1.75(m, 2H, CH_2), 3.19(t, *J*=8.0 Hz, 2H, SCH_2), 7.67(d, *J*=8.0 Hz, 2H, ArH), 7.96(d, *J*=8.0 Hz, 2H, ArH), 10.82(s, H, NH), 14.59(br, 1H, NH). ^{13}C NMR(100 MHz, DMSO-d_6), δ: 13.40(CH_3), 22.93(CH_2), 35.19(SCH_2), 119.11 (CF_3), 122.26(CH), 126.06(CCF_3), 126.22(CH), 143.63(CN), 154.78, 160.51(thiadiazole ring, C_2, C_5), 175.16(C=S). HRMS(ESI): *m/z*[$C_{13}H_{13}F_3N_4S_3$ +H]$^+$, calcd. 379.0333, found 379.0331.

N-(4-Trifluoromethylphenyl)-*N*′-[5-(allylthio)-1,3,4-thiadiazol-2-yl]-thiourea(**4**r): yield 90%, a white solid, m. p. 207.7—209.1 °C. IR(KBr), $\tilde{\nu}$/cm^{-1}: 3207(N—H), 1332(C=S), 1539, 1491, 1400, 1256, 1049, 853(thiadiazole ring), 659(C—S—C). ^{1}H NMR(400 MHz, DMSO-d_6), δ: 3.87(s, 2H, SCH_2), 5.19(d, *J*=8.0 Hz, 1H, =CH_2), 5.31(d, *J*=16.0 Hz, 1H, =CH_2), 5.90—5.98(m, 1H, =CH), 7.67(d, *J*=8.0 Hz, 2H, ArH), 7.96(d, *J*=8.0 Hz, 2H, ArH), 10.83(s, H, NH), 13.86(br, 1H, NH). ^{13}C NMR(100 MHz, DMSO-d_6), δ: 37.16(SCH_2), 119.14(=CH_2), 119.16(CF_3), 122.31(CH), 126.06(CH), 126.66 (CCF_3), 133.34(=CH), 143.63(CN), 154.10, 159.82(thiadiazole ring, C_2, C_5), 179.22(C=S). HRMS(ESI): *m/z* [$C_{13}H_{11}F_3N_4S_3$ +H]$^+$, calcd. 377.0176, found 377.0169.

2.3 Antifungal Activity Assays

The antifungal activities for compounds **4**a—**4**r were investigated preliminarily using mycelium growth rate method. Compounds **4**a—**4**r were dissolved in dimethylsulfoxide(DMSO) at 1.0 mg/mL and were diluted to obtain the required concentrations of 200, 100, 50, 25 and 12.50 μg/mL. The solutions of compounds **4**a—**4**r(1.0 mL) with different concentrations were added to the culture medium(3.0 mL) in a conical beaker at 50 °C. The mixture was poured into a Petri-dish(Φ=9 mm). After the mixture was cooled, the solidified plates were incubated with 6.0 mm mycelium disk at 28 °C for 6 d. The mixed medium was used as the blank control. A commercial antifungal agent, triadimefon, was employed as a standard and tested under the same conditions. The mycelia elongation radius(mm) of the fungi settlements was measured after 6 d of culture. All the experiments were carried out in triplicate.

The growth inhibitory rates were calculated with the following equation:

$$I(\%)=[(C-T)/C]\times 100\%$$

where I is the growth inhibitory rate(%), C is the control settlement radius(mm), and T is the treatment group fungi settlement radius(mm). The grades of efficacy were Grade A(inhibitory rate⩾90%), Grade B(inhibitory rate 70%—89%), Grade C(inhibitory rate 50%—69%) and Grade D(inhibitory rate<50%).

EC_{50} here meant the 50% inhibitory concentration for mycelia growth rate and was calculated on the basis of toxic regression equation.

3 Results and Discussions

3.1 Chemistry

Compounds(**4**a—**4**r) were synthesized as outlined in Scheme 1. To optimize the reaction conditions of the third step, the synthesis of compound **4**a, which was obtained by the reaction of ethyl isothiocyanate with compound **3**a(2-amino-5-ethylthio-1,3,4-thiadiazole), was studied as a model reaction(Table 1). The best result could be obtained when ethyl isothiocyanate and compound **3**a were dissolved at a ratio of 1.2—1.3:1 in a mixed solvent of DMF and acetonitrile(1:1, volume ratio) and refluxed for 10 h to afford **4**a, a light-yellow solid, yield 85%. This product was further purified by recrystallization in a mixed solvent of *V*(DMF):*V*(ethanol)=1:1.

Compounds **4**a—**4**r were confirmed by means of IR, ^{1}H NMR, ^{13}C NMR and HRMS. The IR spectra of compounds **4**a—**4**r showed N—H absorption peaks in the range of 3207 to 3341 cm^{-1}, intensive absorption bands in the range of 1327 to 1342 cm^{-1} that were attributed to the C═S in the —NHCSNH— groups, and a thioether bond(C—S—C) absorption at approximately 641—659 cm^{-1}. In the ^{13}C NMR spectra of compounds **4**a—**4**r, the prominent signals of C═S(δ) appeared in the range of δ 178.13 to 179.0. The signals that corresponded to the carbons of the 1,3,4-thiadiazole ring(C_2, C_5) appeared at approximately δ 154.31—159.14 and δ 159.80—165.04, respectively. All the compounds(**4**a—**4**r) gave rise to the molecular-ion peak $[M+H]^+$ in the high-resolution mass spectrum(HRMS), which agreed with the calculated data.

Table 1 Effect of various conditions on productivity of compound 4a

Entry	Solvent	Reflux time/h	Yield[a](%)
1	DMF	6	37
2	DMF	10	46
3	DMF	14	55
4	Ethanol	10	NR[b]
5	Acetonitrile	10	NR[b]
6	DMF/acetonitrile(volume ratio, 1:1)	6	60
7	DMF/acetonitrile(volume ratio, 1:1)	10	85
8	DMF/acetonitrile(volume ratio, 1:1)	14	86

a. Isolated yield; *b*. no reaction.

3.2 Antifungal Activity

The inhibitory effects of compounds **4**a—**4**r on phytopathogenic fungi were studied. Four representative fungi in Chinese agroecosystem, *Curvularia lunata, Cotton Fusarium wilt, P. P. var. nicotianae* and *Fusarium spp.*, were chosen for fungicide screening. The commercial antifungal agent, triadimefon[30,31], was employed as a standard. The results are summarized in Table 2.

The results shown in Table 2 demonstrate that most of the compounds(**4**a—**4**r) exhibit good antifungal activities, except compounds **4**a, **4**b, **4**d, **4**e, **4**l and **4**m; however, all the compounds show different inhibitory activities against different

Table 2 Antifungal activities of compounds 4a—**4**r

Compd.	*Curvularia lunata*		*Cotton Fusarium Wilt*		*P. P. var nicotianae*		*Fusarium spp.*	
	I^a(%)	EC_{50}/(μg·mL^{-1})	I^a(%)	EC_{50}/(μg·mL^{-1})	I^a(%)	EC_{50}/(μg·mL^{-1})	I^a(%)	EC_{50}/(μg·mL^{-1})
4a	78.65±2.31[b]	101.28	75.73±5.37[b]	117.25	82.90±1.56[b]	**99.39**[d]	83.69±0.69[b]	**99.85**[d]
4b	76.64±4.52	105.48	66.34±4.26	126.11	81.60±1.17	**91.14**	80.31±0.87	**100.9**
4c	**100±0.81**[c]	**79.05**[d]	76.34±4.78	102.94	84.94±1.25	**89.48**	86.23±0.98	**95.17**
4d	56.62±5.31	139.61	69.54±3.56	110.22	58.75±4.32	135.31	53.56±4.56	138.6
4e	42.14±4.02	250.08	49.67±5.69	220.74	52.55±2.65	142.34	54.57±5.06	142.58
4f	84.66±2.56	**95.31**	88.93±3.47	109.06	67.14±4.76	121.42	87.78±0.76	**95.44**
4g	**100±0.92**	**72.83**	**100±0.98**[c]	**68.22**[d]	86.94±1.28	**96.08**	88.6±0.64	**96.06**
4h	**100±0.52**	**86.81**	**91.33±1.36**	**90.09**	**90.34±0.98**[c]	**93.10**	**91.69±0.38**[c]	**95.56**
4i	87.63±2.47	**94.49**	76.95±3.97	119.37	84.14±1.37	**102.0**	85.70±0.97	**96.76**
4j	83.15±3.42	**92.20**	77.94±5.23	120.07	85.54±1.08	**97.40**	82.75±1.02	**88.82**
4k	**100±1.12**	**34.20**	**100±1.48**	**35.15**	**100±0.32**	**38.08**	85.31±0.91	**95.46**
4l	85.65±3.35	**96.65**	78.62±2.69	102.25	68.20±1.98	135.96	72.85±1.35	106.22
4m	81.65±2.41	108.48	70.14±1.31	115.71	75.34±2.01	119.92	76.45±1.73	103.12
4n	**100±0.96**	**30.84**	**100±0.79**	**39.11**	88.53±0.65	**96.33**	86.61±0.72	**97.32**
4o	**100±0.36**	**28.12**	**100±0.65**	**30.41**	**100±0.46**	**15.2**	**100±0.49**	**6.22**
4p	**100±1.04**	**40.14**	87.43±1.32	**92.21**	**100±0.37**	**31.5**	**100±0.72**	**29.39**
4q	**95.76±1.01**	**85.20**	86.53±2.14	**93.88**	**91.73±0.59**	**69.25**	86.23±0.47	**91.72**
4r	**100±0.63**	**34.80**	**100±0.63**	**23.71**	**100±0.45**	**20.61**	**100±0.38**	**17.54**
Triadimefon	83.05±1.35	98.73	75.73±2.19	96.58	84 .34±1.01	105.37	79.87±2.01	102.18

a. I(%) represents the growth inhibitory rate(%) at c=200 μg/mL; *b*. each value is the mean±standard deviation(n=3) in this table; *c*. bold numbers represent the efficacies reaching the scope of Grade A(inhibitory rate 90%—100%) in this column; *d*. bold numbers represent the EC_{50} value being lower than that of triadimefon in this column.

fungi strains. Compounds **4**c, **4**g, **4**h, **4**k, **4**n, **4**o, **4**p, **4**q and **4**r show very good inhibitory activities against *Curvularia lunata,* and the growth inhibitory rates of these compounds achieve Grade A(inhibitory rate: 90%—100%). Compounds **4**g, **4**h, **4**k, **4**n, **4**o and **4**r exhibit significant inhibitory effects against *Cotton Fusarium wilt.* Compounds **4**h, **4**k, **4**o, **4**p, **4**q and **4**r exhibit excellent antifungal activities against *P. P. var. nicotianae.* Whereas compounds **4**h, **4**o, **4**p and **4**r exhibit excellent inhibitory activities against *Fusarium spp.* at 200 μg/mL. In addition, compounds **4**g, **4**h, **4**k, **4**n, **4**o, **4**p, **4**q and **4**r show broad spectrum antifungal activities. Among all the compounds, compound **4**o shows the best inhibitory effects with the EC_{50} values of 28.12, 30.41, 15.2 and 6.22 μg/mL against *Curvularia lunata*, *Cotton Fusarium Wilt*, *P. P. var nicotianae and Fusarium spp.*, respectively, which are even superior to those of triadimefon(98.73, 96.58, 105.37 and 102.18 μg/mL). For example, compound **4**o was found to be sixteen times more active against *Fusarium spp.* than triadimefon by the EC_{50} value.

The antifungal activities of compounds **4**a—**4**r are quite different, but their structures are similar. Thus, their activities can be influenced somewhat by the nature of the substituents(R_1, R_2) in their molecules. Compounds **4**c, **4**g, **4**h, **4**k, **4**n, **4**o, **4**p, **4**q and **4**r with an allyl group or aromatic ring(R_2=allyl, benzyl, 2-fluoro-6-chlorobenzyl, 2-chloro-5-methylene pyridine) exhibit better inhibitory activities against *Curvularia lunata*. However, compounds **4**a, **4**b, **4**d and **4**e with alkyl(R_1=Et; R_2=Et, *n*-Pr, *n*-Bu, *n*-hexyl) substituents show lower activity. As such, compound **4**o(R_1=allyl, R_2= 2-fluoro-6-chlorobenzyl) with allyl group and aromatic ring in a single molecule exhibits the best inhibitory activities.

4 Conclusions

Thiourea derivatives containing a 1,3,4-thiadiazole and thioether skeleton(**4**a—**4**r) were synthesized *via* three steps from commercially available thiosemicarbazide. The preliminary results indicated that the thiourea derivatives **4**a—**4**r showed various antifungal activities. Among them, compounds **4**c, **4**g, **4**h, **4**k, **4**n, **4**o, **4**p, **4**q and **4**r displayed excellent antifungal activities against one or more tested fungi. Compound **4**o particularly exhibited the best inhibitory effects on the four tested fungi. Therefore the results have built a foundation for further improving the potency and hold great promise towards the pursuit to discover novel plant antifungal agents.

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