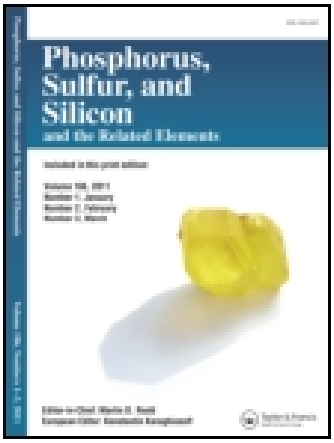

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Synthesis and Antibacterial Activity of Bisthioether Derivatives Containing a 1,3,4-Thiadiazoles Moiety

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SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF BISTHIOETHER DERIVATIVES CONTAINING A 1,3,4-THIADIAZOLES MOIETY

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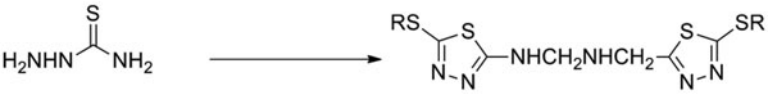
GRAPHICAL ABSTRACT



3(a-o)

Abstract *A series of bisthioether derivatives containing 1,3,4-thiadiazole moieties were designed and synthesized. All of the synthesized compounds were confirmed by ^{1}H-NMR, ^{13}C-NMR, IR, and elemental analysis. The antibacterial activities of bisthioether derivatives against Ralstonia solanacearum were evaluated in vivo; the results indicated that the synthesized compounds showed good antibacterial activity against R. solanacearum at 200 mg/L. In particular, the activity of compound* ***3m*** *was up to 73% at 100 mg/L.*

Supplemental materials are available for this article. Go to the publisher's online edition of Phosphorus, Sulfur, and Silicon and the Related Elements *to view the free supplemental file.*

Keywords Bisthioether compounds; 1,3,4-thiadiazoles moiety; synthesis; tobacco bacterial wilt; antibacterial activity

INTRODUCTION

Tobacco bacterial wilt, one of the primary diseases affecting tobacco production, is caused by *Ralstonia solanacearum* and is mainly spread through soil. Biological control of tobacco bacterial wilt is a promising approach. The result of numerous experiments showed that the biological control of tobacco disease can be greatly achieved in the laboratory; however, due to environment factors, biological control of the disease in the field is not

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as comparable. Controlling the disease requires continued pesticide use, but those utilized are mainly based on traditional organic pesticides, antibiotics, and copper compounds. The application of traditional organic pesticides is moderately effective, but high residue level and, consequently, negative environmental impact occur. Although antibiotics are highly efficient in tobacco bacterial wilt control, the emergence of resistant strains and the ban of using antibiotics in China and other countries complicate the management of the plant disease. Copper formulation, a commercial bactericide, is a type of inorganic pesticide that can enhance resistance of the host tobacco plant. Despite being useful in the treatment of plants affected by tobacco bacterial wilt, the use of copper formulation for field trials is largely limited by its phytotoxicity, strong alkalinity, and low mobility. Extensive research has been conducted to combat tobacco bacterial wilt, the most common methods being biological and chemical control. The chemical control method plays an important role in disease prevention because of its simple operation and economic advantages. Thus, the development of efficient, environmentally friendly antibacterial agents through chemical synthesis has become the core area of research for the eradication and/or prevention of tobacco bacterial wilt.[1]

1,3,4-Thiadiazoles derivatives have a wide range of biological activity in pesticides, including antibacterial,[2] antituberculosis,[3] antiviral,[4] antifungal,[5] anticancer,[6] anti-inflammatory,[7] and antiulcer properties.[8] One example of these derivatives is the 3-(5-nitrofuran-2-yl)-*N*-phenyl[1,2,4] triazolo[3,4-b][1, 3, 4]thiadiazol-6-amine prepared by Badr and Barwa .[9] This compound exhibits good antibacterial activity against *Staphylococcus aureus*, with the minimum inhibitory concentration of 25 μg/mL. Moreover, the N^2,N^4- bis(4-chlorophenyl)-6-((5-((3,5-dimethyl-1-phenyl-4,5-dihydro-1*H*-pyrazol-4-yl)amino)-1, 3, 4-thiadiazol-2-yl)thio)-1, 3, 5-triazine-2, 4-diamine reported by Dubey et al.[10] exhibits strong activity against *Pseudomonas aeruginosa* and *Bacillus cereus*, with the minimum inhibitory concentrations of 6.25 and 12.5 μg/mL, respectively. In the past decades, a large number of pesticides with potent bioactivities and containing 1,3,4-thiadiazoles such as fluthiamide,[11] fluthiacet-methyl[12] (Figure 1), bismerthiazol,[13] and thiodiazole-copper[14] have become commercial agents and have been widely used to control plant disease (Figure 1).

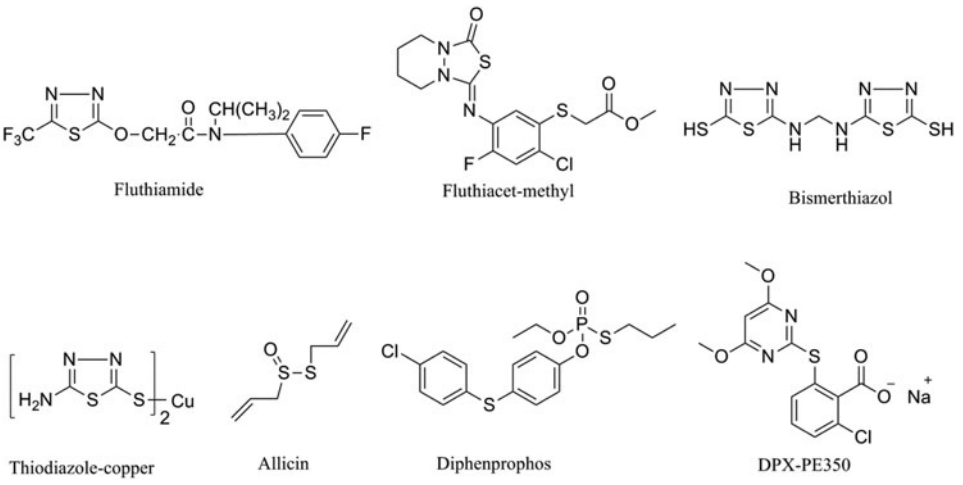

Figure 1 The commercialized pesticides of 1,3,4-thidiazole and thioether derivatives.

With the increasing applications mentioned above, research on the synthesis and bioactivity of 1,3,4-thiadiazoles derivatives has attracted growing attention from chemists and biologists in recent years.

Certain thioethers prepared by different groups have often displayed other interesting bioactivities, e.g., antibacterial,[15] antifungal,[16] antiviral,[17] and antitumor[18,19] properties. Some representative examples of these derivatives are commercialized as pesticides. Allicin, Diphenprophos, and DPX-PE350 are known for their ability to protect certain plants from severe diseases and pests (Figure 1). In our previous work,[20] a series of 2-substituted methylthio-5(2,4-dichlorophenyl)-1,3,4-thiadiazole were synthesized and evaluated for their antifungal activities, the results showed that these compounds exhibit moderate to good antifungal activity against *Rhizoctonia solani*.

To create efficient, environment friendly agents, we synthesized a series of bisthioether derivatives containing a 1,3,4-thidiazole moiety and evaluated them for antibacterial activity against *R. solanacearum*. Using the turbidity method and with *R. solanacearum* and *P. solanacearum* (*Pseuclomonas solanacearum*) as the test object, we conducted preliminary antibacterial activity testing on the synthesized compounds. The results showed that some compounds exhibited in vitro antibacterial activity against *R. solanacearum* at 200 mg/L. Among these compounds, **3e**, **3h**, **3i**, **3j**, **3k**, and **3m** showed better activity than the rest, especially **3k** and **3m**, with antibacterial activities of 81% and 88% at 200 mg/L, respectively. In addition, **3j** and **3m** displayed antibacterial activities of 52% and 73% at 100 mg/L, respectively. However, the synthesized compounds exhibited weak activity against *P. solanacearum*. The present work demonstrates that bisthioether derivatives containing a 1,3,4-thidiazole moiety can be used to develop potential agrochemicals. To the best of our knowledge, this is the first report on the antibacterial activity of bisthioether derivatives containing a 1,3,4-thidiazole moiety.

RESULTS AND DISCUSSION

Chemistry

The synthetic route of the title compounds is demonstrated in Figure 2. The intermediates 1 (5-amino-1,3,4-thiadiazole-2-thiol) was prepared by treating thiosemicarbazide and carbon disulfide using readily available starting materials and a procedure described in literature.[17] The subsequent reaction of intermediates 1 with 30% formaldehyde readily proceeded at 50 °C, resulting in the synthesis of intermediates 2. Subsequent treatment of intermediates 2 dissolved in KOH solution and then with different substituted benzyl chloride reactions at room temperature obtained the final compounds (**3a–3o**).

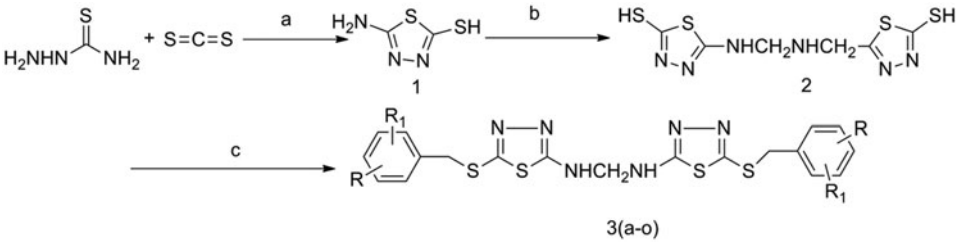

Figure 2 Schematic diagram for synthesis of final compounds (**3a–3o**).

Table 1 Yields of **3a** at different reaction conditions[a]

Entry	Base	Time (h)	Temperature (°C)	Solvent	Yield (%)
1	K_2CO_3	5	25	H_2O	22
2	NaOH	5	25	H_2O	30
3	KOH	5	25	H_2O	60
4	KOH	2.5	25	H_2O	28
5	KOH	5	25	H_2O	61
6	KOH	10	25	H_2O	60
7	KOH	5	12.5	H_2O	30
8	KOH	5	25	H_2O	59
9	KOH	5	50	H_2O	45
10	KOH	5	25	H_2O	62
11	KOH	5	25	C_2H_5OH	34
12	KOH	5	25	CH_3CN	23

[a]General reaction conditions: *n* (intermediates 2): *n* (KOH): *n* (benzyl bromide) = 1:2:2, *n* = 1 mmol; H_2O = 20 mL, 25 °C, 5 h.

To optimize the reaction conditions for the preparation of the title compounds, the compound **3a** was carried out under different conditions. The effects of different types of base, reaction time, reaction temperature, and solvents are summarized in Table 1. First, the effect of different types of base was investigated. Table 1 indicates that the reaction can yield 60% of the product when using KOH base (entry 3), whereas in the other base, the **3a** yield was significantly lower (entries 1 and 2). The reaction was carried out using various solvents in the presence of KOH at room temperature for 5 h. In H_2O, **3a** was obtained as a 62% yield (entry 10). Other solvents such as CH_3CN, and C_2H_5OH were used, with the **3a** yields being 23% and 34% respectively (entries 11 and 12). Furthermore, we examined the effect of reaction time on the thioetherification reaction of thiol to thioether. When the reaction time was prolonged from 2.5 to 5 h, **3a** yield increased from 28% to 61%. However, when the reaction time was further increased to 10 h, no improvement was observed (60%, entry 6). We also examined the effects of reaction temperature. When the reaction temperature was increased from 12.5°C to 25°C and then to 50°C, the yields obtained were 30%, 59%, and 45%, respectively (entries 7, 8, and 9). Based on these results, the optimal conditions for synthesis were selected in H_2O solvent with KOH base at a reaction temperature of 25°C and a reaction time of 5 h. However, the yields were moderate (52%–70%), may be due to the poor solubility of benzyl chloride in water, and part of benzyl chloride was turned into benzyl alcohol in percentage of KOH in H_2O. Therefore, 5,5′-(methylenebis(azanediyl))bis(1,3,4-thiadiazole-2-thiol) seemed excessive, and the excessive 5,5′-(methylenebis(azanediyl))bis(1,3,4-thiadiazole-2-thiol) become the corresponding potassium salt and dissolved in water, which then lost during the postprocessing.

All of the synthesized compounds (**3a**–**3o**) were confirmed by ^{1}H-NMR, ^{13}C-NMR, IR, and elemental analysis (Table 2). From the spectral data of compounds **3a** to **3o**, the IR spectrum of all the synthesized compounds showed broad absorption bands of around 3360 to 3220 cm^{-1} for NH, 3100 to 3020 cm^{-1} for Ar—H, and 2964 to 2914 cm^{-1} for CH_2, with the distinguishing benzene ring broad absorption peaks observed in the range of 1650 to 1612 cm^{-1} and C=N for amide in the range of 1575 to 1525 cm^{-1}. ^{1}H NMR spectrum showed the characteristic three peaks near δ 8.61 to 8.58 ppm for –NH- proton and near δ

Table 2 Physical and analytical data of the newly synthesized compounds **3a–3o**

					Elemental analysis					
					Found (%)			Calculated (%)		
Entry	Mol. Formula	Yield (%)	MW	MP (°C)	C	H	N	C	H	N
3a	$C_{19}H_{18}N_6S_4$	60	458.65	200–201	49.43	4.19	18.40	49.76	3.96	18.32
3b	$C_{19}H_{16}F_2N_6S_4$	61	494.63	147–148	46.44	3.67	16.93	46.14	3.26	16.99
3c	$C_{19}H_{16}F_2N_6S_4$	64	494.63	165–166	46.45	3.56	16.52	46.14	3.26	16.99
3d	$C_{19}H_{16}F_2N_6S_4$	70	494.63	153–154	46.49	3.47	16.75	46.14	3.26	16.99
3e	$C_{21}H_{22}N_6S_4$	62	486.70	148–149	51.56	4.82	17.10	51.82	4.56	17.27
3f	$C_{21}H_{22}N_6S_4$	62	486.70	145–147	52.05	5.01	17.21	51.82	4.56	17.27
3g	$C_{21}H_{22}N_6S_4$	64	486.70	137–138	51.94	4.86	17.53	51.82	4.56	17.27
3h	$C_{19}H_{16}Cl_2N_6S_4$	55	527.54	149–151	43.62	3.24	15.44	43.26	3.06	15.93
3i	$C_{19}H_{16}Cl_2N_6S_4$	66	527.54	152–154	43.34	3.36	15.58	43.26	3.06	15.93
3j	$C_{19}H_{16}Cl_2N_6S_4$	58	527.54	148–150	43.15	3.27	15.67	43.26	3.06	15.93
3k	$C_{19}H_{14}Cl_4N_6S_4$	52	596.43	142–143	38.47	2.35	14.15	38.26	2.37	14.09
3l	$C_{19}H_{14}Cl_4N_6S_4$	58	596.43	145–147	38.44	2.53	14.14	38.26	2.37	14.09
3m	$C_{21}H_{22}N_6O_2S_4$	68	518.70	145–147	48.86	4.33	16.62	48.63	4.28	16.20
3n	$C_{19}H_{16}N_8O_4S_4$	60	548.64	142–143	41.45	3.05	20.06	41.59	2.94	20.42
3o	$C_{19}H_{16}N_8O_4S_4$	64	548.64	146–148	41.88	2.83	19.96	41.59	2.94	20.42

4.73 to 4.71 ppm for $-NHCH_2NH-$ proton, with the characteristic singlet near δ 4.28 ppm for $-SCH_2Ar$ proton, appearing as a broad multiplet at δ 7.21 to 7.28 ppm for -ArH proton, with the methyl (Ar-CH_3) proton signals observed as a singlet near δ 2.24 to 2.20 ppm.

Antibacterial Activity

The herbicidal activities of title compounds against *R. solanacearum* and *P. solanacearum* were evaluated using the turbidimeter test ;[21] the results indicated that the synthesized compounds showed good antibacterial activities. In particular, the activity of compound **3m** *R. solanacearum* was up to 73% at 100 mg/L. The experimental details, table of activities (Table S 1), and the structure–activity relationship are presented in the online Supplemental Materials.

CONCLUSION

A new type of (bis)sulfide derivatives containing the 1,3,4-thiadiazoles moiety was designed and synthesized, and the antibacterial activities for compounds **3a** to **3o** against *R. solanacearum* and *P. solanacearum* were evaluated in vitro. Preliminary biological test results show that the synthesized compounds have weak to good antibacterial activity. In particular, the compound **3m** can inhibit *R. solanacearum* from reaching 73% at 100 mg/L. When the phenyl ring group had a 3-methoxyl substituent, the compound has significantly higher activity than the rest. To the best of our knowledge, this is the first report of synthesis and antibacterial activity of (bis)sulfide derivatives containing the 1,3,4-thiadiazoles moiety.

EXPERIMENTAL

Chemistry

Materials and Instrumentation. Substituted benzyl chloride was purchased from Aladdin and the Adamas. Unless otherwise stated, all the reagents and reactants were purchased from commercial suppliers, and melting points were uncorrected and determined on an XT-4 binocular microscope (Beijing Tech Instrument Co., China). The ^{1}H NMR and ^{13}C NMR spectra were recorded at room temperature on a JEOL ECX 500 NMR spectrometer operating at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR by using dimethyl sulfoxide (DMSO) as solvents and tetramethylsilane (TMS) as an internal standard. Infrared spectra were recorded in KBr on a Bruker VECTOR 22 spectrometer, and elemental analysis was performed on an Elemental Vario-III CHN analyzer. The course of the reactions was monitored by thin layer chromatography (TLC); analytical TLC was performed on silica gel GF 254. Intermediates 1 and 2 were prepared according to the reported methods[17,22] and used without further purifications. Sample ^{1}H and ^{13}C NMR spectra for **3a** and **3f** are presented in the online Supplemental Materials (Figures S 1–S 4).

Experimental Procedure for the Synthesis of Compounds

To a stirred solution of intermediate 2 (0.28 g, 1 mmol) in 20 mL 0.1 mol/L potassium hydroxide solution, substituted benzyl chloride (2 mmol) in ethanol (2 mL) was slowly added at room temperature. The mixture was stirred and heated at 50 °C for 8 h. The solvent was removed and the residue was purified by chromatography on silica gel (ethyl acetate/petroleum ether = v/v 1:1) to give the desired compound **3a** to **3o**.

Characterization of Final Compounds (3a–3o)

***N,N′*-Bis(5-(benzylthio)-1,3,4-thiadiazol-2-yl)methanediamine (3a).** IR (KBr, cm^{-1}): *v* 3360 (−NH str.), 3028 (ArH str.), 1525 (Ar-H str.), 1203 (C=N str.), 700 (S−C str.); ^{1}H NMR (DMSO-d_6, ppm): δ 8.64 (t, J = 5.7 Hz, 2H, NH), 7.37–7.21 (m, 10H, Ar-H), 4.77 (t, J = 5.75 Hz, 2H, CH_2), 4.28 (s, 4H, ArCH_2); ^{13}C NMR (DMSO-d_6, δppm): 169.0 (C=N), 151.5(C=N), 137.5 (Ar−C), 129.5 (Ar−C), 129.0 (Ar−C), 128.0 (Ar−C), 52.8 (C−N), 39.8 (Ar−CH_2).

***N,N′*-Bis(5-((2-fluorobenzyl)thio)-1,3,4-thiadiazol-2-yl)methane Diamine (3b).** IR (KBr, cm^{-1}): *v* 3259 (−NH str.), 3062 (ArH str.), 1489 (Ar−H str.), 1230 (C=N str.), 758 (S−C str.); ^{1}H NMR (DMSO-d_6, ppm): δ 8.70 (t, J = 5.75 Hz, 2H, NH), 7.40–7.31 (m, 4H, ArH), 7.21–7,13 (m, 4H, Ar−H), 4.78 (t, J = 5.75 Hz, 2H, CH_2), 4.28 (s, 4H, ArCH_2); ^{13}C NMR (DMSO-d_6, ppm): δ 169.3 (C=N), 161.8 (C=N), 159.8 (C=N), 150.7 (C=N), 131.9 (Ar−C), 130.4 (Ar−C), 125.0 (Ar−C), 124.5 (Ar−C), 124.7 (Ar−C), 116.0 (Ar−C), 115.9 (Ar−C), 53.0 (C−N), 34.3 (Ar−CH_2) .

***N,N′*-Bis(5-((3-fluorobenzyl)thio)-1,3,4-thiadiazol-2-yl)methanediamine (3c).** IR (KBr, cm^{-1}): *v* 3298.2 (−NH str.), 3084 (ArH str.), 1510 (Ar-H str.), 1259 (C=N str.), 723 (S−C str.); ^{1}H NMR (500 MHz, DMSO-d_6, ppm): δ 8.70 (t, J = 4.6 Hz, 2H, NH), 7.41–7.33 (m, 4H, Ar−H), 7.21–7.13 (m, 4H, Ar−H), 4.78 (t, J = 6.0 Hz, 2H, CH_2), 4.33 (s, 4H, ArCH_2); ^{13}C NMR (125 MHz, DMSO-d_6, ppm): δ 169.3 (C=N), 159.7 (C=N), 150.8 (C=N), 131.8 (Ar−C), 130.4 (Ar−C), 125.0 (Ar−C), 124.7 (Ar−C), 116.0 (Ar−C), 53.0 (C−N), 32.8 (Ar−CH_2).

***N,N′*-Bis(5-((4-fluorobenzyl)thio)-1,3,4-thiadiazol-2-yl)methanediamine (3d).** IR (KBr, cm^{-1}): *v* 3273 (−NH str.), 3076 (ArH str.), 1598 (Ar−H str.), 1217 (C=N str.), 723 (S−C str.); ^{1}H NMR (500 MHz, DMSO-d_6, ppm): δ 8.66 (t, J = 6.3 Hz, 2H, NH), 7.44–7.39 (m, 4H, Ar−H), 7.29–7.23 (m, 4H, Ar−H), 4.75 (t, J = 5.7 Hz, 2H, CH_2), 4.38 (s, 4H, ArCH_2); ^{13}C NMR (125 MHz, DMSO-d_6, ppm): δ 169.0 (C=N), 162.9 (C=N), 161.0 (C=N), 151.4 (C=N), 133.9 (Ar−C), 131.5 (Ar−C), 125.4 (Ar−C), 115.9 (Ar−C), 53.0 (C−N), 37.8 (Ar−CH_2).

***N,N′*-Bis(5-((2-methylbenzyl)thio)-1,3,4-thiadiazol-2-yl)methanediamine (3e).** IR (KBr, cm^{-1}): *v* 3259 (−NH str.), 3061 (ArH str.), 1639 (Ar−H str.), 1230 (C=N str.), 758 (S-C str.); ^{1}H NMR (500 MHz, DMSO-d_6, ppm): δ 8.63 (t, J = 5.7 Hz, 2H, NH), 7.21–7.12 (m, 6H, Ar−H), 7.08–7.06 (m, 2H, Ar−H), 4.77 (t, J = 5.75 Hz, 2H, CH_2), 4.28 (s, 4H, ArCH_2), 2.26 (s, 6H, ArCH_3); ^{13}C NMR (125 MHz, DMSO-d_6): δ 168.9 (C=N), 151.6 (C=N), 138.1 (Ar−C), 137.3 (Ar−C), 130.0 (Ar−C), 128.9 (Ar−C), 128.6 (Ar−C), 126.5 (Ar−C), 53.1 (C-N), 39.8 (Ar−CH_2), 21.4 (Ar−CH_3).

***N,N′*-Bis(5-((3-methylbenzyl)thio)-1,3,4-thiadiazol-2-yl)methanediamine (3f).** IR (KBr, cm^{-1}): *v* 3319 (−NH str.), 3101 (ArH str.), 1631 (Ar−H str.), 1217 (C=N str.), 723 (S-C str.); ^{1}H NMR (500 MHz, DMSO-d_6, ppm): δ 8.59 (t, J = 5.95 Hz, 2H, NH), 7.20–7.18 (m, 4H, Ar−H), 7.08–7.07(m, 4H, Ar−H), 4.73 (t, J = 5.7 Hz, 2H, CH_2), 4.24 (s, 4H, ArCH_2) , 2.22 (s, 6H, ArCH_3);^{13}C-NMR (125 MHz, DMSO-d_6, ppm): δ 168.9 (C=N), 151.6 (C=N), 138.1 (Ar−C), 137.3 (Ar−C), 130.0 (Ar−C), 128.9 (Ar−C), 128.6 (Ar−C), 126.5 (Ar−C), 53.0 (C-N), 32.1 (Ar−CH_2), 21.4 (Ar−CH_3).

***N,N′*-Bis(5-((4-methylbenzyl)thio)-1,3,4-thiadiazol-2-yl)methanediamine (3g).** IR (KBr, cm^{-1}): *v* 3257 (−NH str.), 3061 (ArH str.), 1639 (Ar−H str.), 1230 (C=N str.), 759 (S-C str.); ^{1}H NMR (500 MHz, DMSO-d_6, ppm): δ 8.65 (t, J = 5.7 Hz, 2H, NH), 7.21–7.13 (m, 6H, Ar−H), 7.08–7.06 (m, 2H, Ar−H), 4.77 (t, J = 5.15 Hz, 2H, CH_2), 4.28 (s, 4H, ArCH_2) , 2.26 (s, 6H, ArCH_3); ^{13}C NMR (125 MHz, DMSO-d_6, ppm): δ 168.9 (C=N), 151.6 (C=N), 138.1 (Ar−C), 137.3 (Ar−C), 130.0 (Ar−C), 128.9 (Ar−C), 128.6 (Ar−C), 126.5 (Ar−C), 53.0 (C-N), 38.9 (Ar−CH_2), 21.4 (Ar−CH_3).

***N,N′*-Bis(5-((2-chlorobenzyl)thio)-1,3,4-thiadiazol-2-yl)methanediamine (3h).** IR (KBr, cm^{-1}): *v* 3240 (−NH str.), 2976 (ArH str.), 1597 (Ar−H str.), 1210 (C=N str.), 775 (S-C str.); ^{1}H NMR (500 MHz, DMSO-d_6, ppm): δ 8.71 (t, J = 5.75 Hz, 2H, NH), 7.48–7.43 (m, 4H, Ar−H), 7.34–7.27 (m, 4H, Ar−H), 4.79 (t, J = 5.75 Hz, 2H, CH_2), 4.40 (s, 4H, ArCH_2); ^{13}C NMR (125 MHz, DMSO-d_6, ppm): δ 169.3 (C=N), 150.7 (C=N), 134.9 (Ar−C), 133.7 (Ar−C), 131.9 (Ar−C), 130.1 (Ar−C), 127.8 (Ar−C), 53.0 (C-N), 32.1 (Ar−CH_2).

***N,N′*-Bis(5-((3-chlorobenzyl)thio)-1,3,4-thiadiazol-2-yl)methanediamine (3i).** IR (KBr, cm^{-1}): *v* 3204 (−NH str.), 2976 (ArH str.), 1597 (Ar−H str.), 1201 (C=N str.), 719 (S-C str.); ^{1}H NMR (500 MHz, DMSO-d_6, ppm): δ 8.67 (t, J = 5.75 Hz, 2H, NH), 7.44 (s, 2H, Ar−H), 7.36–7.33 (m, 6H, Ar−H), 4.76 (t, J = 5.15 Hz, 2H, CH_2), 4.33 (s, 4H, ArCH_2); ^{13}C NMR (125 MHz, DMSO-d_6, ppm): δ 169.3 (C=N), 150.7 (C=N), 134.9 (Ar−C), 133.7 (Ar−C), 131.9 (Ar−C), 130.1 (Ar−C), 127.8 (Ar−C), 107.4 (Ar−C), 53.1 (C-N), 36.9 (Ar−CH_2).

***N,N′*-Bis(5-((4-chlorobenzyl)thio)-1,3,4-thiadiazol-2-yl)methanediamine (3j).** IR (KBr, cm^{-1}): *v* 3319 (−NH str.), 3086 (ArH str.), 1633 (Ar−H str.), 1132 (C=N str.), 839 (S-C str.); ^{1}H NMR (500 MHz, DMSO-d_6, ppm): δ 8.66 (t, J = 5.7 Hz, 2H, NH), 7.38–7.33 (m, 8H, Ar−H), 4.77 (t, J = 5.75 Hz, 2H, CH_2), 4.31 (s, 4H, ArCH_2); ^{13}C NMR (125 MHz, DMSO-d_6, ppm): δ 169.1 (C=N), 151.1 (C=N), 150.5 (C=N), 136.9 (Ar−C), 132.5 (Ar−C), 131.3 (Ar−C), 128.9 (Ar−C), 52.9 (C-N), 37.9 (Ar−CH_2).

***N,N′*-Bis(5-((3,4-dichlorobenzyl)thio)-1,3,4-thiadiazol-2-yl)methanediamine (3k).** IR (KBr, cm^{-1}): *v* 3304 (−NH str.), 2987 (ArH str.), 1558 (Ar−H str.), 1124 (C=N str.), 819 (S-C str.); ^{1}H NMR (500 MHz, DMSO-d_6, ppm): δ 8.66 (t, J = 5.7 Hz, 2H, NH), 7.62–7.56(s, 4H, Ar−H), 7.34–7.33 (m, 2H, Ar−H), 4.76 (t, J = 6.3 Hz, 2H, CH_2), 4.32 (s, 4H, $ArCH_2$); ^{13}C NMR (125 MHz, DMSO-d_6, ppm): δ 170.2 (C=N) 169.1 (C=N), 150.8 (C=N), 139.7 (Ar−C), 139.2 (Ar−C), 131.4 (Ar−C), 131.1 (Ar−C), 130.5 (Ar−C), 129.9 (Ar−C), 53.0 (C-N), 37.3 ($Ar-CH_2$).

***N,N′*-Bis(5-((2,6-dichlorobenzyl)thio)-1,3,4-thiadiazol-2-yl)methanediamine (3l).** IR (KBr, cm^{-1}): *v* 3280 (−NH str.), 3091 (ArH str.), 1558 (Ar−H str.), 1215 (C=N str.), 781 (S-C str.); ^{1}H NMR (500 MHz, DMSO-d_6, ppm): δ 8.78 (t, J = 5.75 Hz, 2H, NH), 7.49–7.48 (s, 4H, Ar−H), 7.37–7.34 (m, 2H, Ar−H), 4.82 (t, J = 5.7 Hz, 2H, CH_2), 4.48 (s, 4H, $ArCH_2$); ^{13}C NMR (125 MHz, DMSO-d_6, ppm): δ 168.9 (C=N), 151.6 (C=N), 138.1 (Ar−C), 137.3 (Ar−C), 130.0 (Ar−C), 128.9 (Ar−C), 128.6 (Ar−C), 126.5 (Ar−C), 53.1 (C-N), 38.8 ($Ar-CH_2$).

***N,N′*-Bis(5-((3-methoxybenzyl)thio)-1,3,4-thiadiazol-2-yl)methanediamine (3m).** IR (KBr, cm^{-1}): *v* 3242 (−NH str.), 2933 (ArH str.), 1602 (Ar−H str.), 1261 (C=N str.), 704 (S-C str.); ^{1}H NMR (500 MHz, DMSO-d_6, ppm): δ 8.66 (t, J = 6.3 Hz, 2H, NH), 7.24–7.21 (s, 2H, Ar−H), 6.91–6.90 (m, 4H, Ar−H), 6.84–6.80 (m, 2H, Ar−H), 4.77 (t, J = 6.0 Hz, 2H, CH_2), 4.31 (s, 4H, $ArCH_2$), 3.71 (s, 6H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6, ppm): δ 169.0 (C=N), 159.7 (C=N), 151.5 (C=N), 139.0 (Ar−C), 130.1 (Ar−C), 121.6 (Ar−C), 115.0 (Ar−C), 113.4 (Ar−C), 55.5 (OCH_3), 52.9 (C-N), 38.9 ($Ar-CH_2$).

***N,N′*-Bis(5-((3-nitrobenzyl)thio)-1,3,4-thiadiazol-2-yl)methanediamine (3n).** IR (KBr, cm^{-1}): *v* 3317 (−NH str.), 3122 (ArH str.), 1625 (Ar−H str.), 1236 (C=N str.), 692 (S-C str.); ^{1}H NMR (500 MHz, DMSO-d_6, ppm): δ 8.70 (t, J = 5.7 Hz, 2H, NH), 7.41–7.38 (m, 2H, Ar−H), 7.34–7.31 (m, 2H, Ar−H) 7.21–7.13 (m, 4H, Ar−H), 4.78 (t, J = 5.75 Hz, 2H, CH_2), 4.28 (s, 4H, $ArCH_2$); ^{13}C NMR (125 MHz, DMSO-d_6, δppm): 169.7 (C=N), 150.3 (C=N), 148.1 (C=N), 140.5 (C=N), 136.2 (Ar−C), 130.4 (Ar−C), 124.1 (Ar−C), 122.8 (Ar−C), 107.5 (Ar−C), 86.2 (Ar−C), 67.1 (C-N), 37.6 ($Ar-CH_2$).

***N,N′*-Bis(5-((4-nitrobenzyl)thio)-1,3,4-thiadiazol-2-yl)methanediamine (3o).** IR (KBr, cm^{-1}): *v* 3298 (−NH str.), 3122 (ArH str.), 1624 (Ar−H str.), 1298 (C=N str.), 692 (S-C str.); ^{1}H NMR (500 MHz, DMSO-d_6, ppm): δ 8.70 (t, J = 6.3 Hz, 2H, NH), 7.44–7.39 (m, 4H, Ar−H), 7.29–7.23 (m, 4H, Ar−H), 4.78 (t, J = 5.7 Hz, 2H, CH_2), 4.33 (s, 4H, $ArCH_2$); ^{13}C NMR (125 MHz, DMSO-d_6, ppm): δ 169.7 (C=N), 150.2 (C=N), 147.1 (C=N), 146.1 (C=N), 130.7 (Ar−C), 124.1 (Ar−C), 103.5 (Ar−C), 66.5 (C−N), 37.7 ($Ar-CH_2$).

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