PHASE TRANSFER CATALYSTS PROMOTING THE ONE-POT SYNTHESIS UNDER ULTRASONIC IRRADIATION AND BIOLOGICAL ACTIVITY OF N-(5-SUBSTITUTED-1,3,4-THIADIAZOLE-2-YL)-N'-(5-METHYLISOXAZOYL) -THIOUREA DERIVATIVES

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Abstract: Reaction of 2-amino-5-substitute-1,3,4-thiadiazoles with 5-methylisoxazoyl chloride and ammonium thiocyanate under the condition of solid–liquid phase-transfer catalysis using polyethylene glycol-600 (PEG-600) as the catalyst under ultrasonic irradiation yielded N-(5-substituted-1,3,4-thiadiazole-2-yl)-N'-(5-methylisoxazoyl)-thiourea derivatives **3a-l** in good-to-excellent yield. The chemical structure of all compounds was established by ^{1}H NMR, FTIR, MS, and elemental analysis studies. Some of the compounds were investigated for fungicidal activity. The bioassay results indicated that some of these compounds exhibit moderate fungicidal activities.

Keywords: thiourea, 5-methylisoxazole, 1,3,4-thidiazole derivatives; one-pot synthesis; phase transfer catalysis; ultrasonic irradiation; fungicidal activity.

Introduction

Research on the synthesis and biological activity of heterocyclic compounds is an important developmental oriental in pesticide and medicine chemistry [1-4]. 5-Methylisoxazole-4-carboxylic acid is the mediate of Leflunomide which is a drug used for the treatment of rheumatoid arthritis, an illness that affects soft tissues and bones and can cause irreversible joint deformities and functional impairment[5]. It was shown that 2-amino-5-substitute-1,3,4-thiadiazoles are very useful starting materials for the synthesis of various bioactive molecules.[6-7]

Phase transfer catalysis (PTC) is a powerful technique accomplishing a variety of reactions under mild conditions and efficient way. A logical combination is when phase transfer catalyzed reactions are further promoted by ultrasound irradiation. This technique has been widely recognized as an efficient synthetic tool and attracted much attention [8-9].

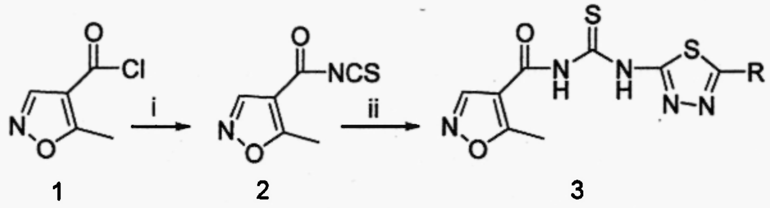
In view of these facts, herein we report a one-pot facile, efficient, and high-yield method for the synthesis of N-(5-substituted-1,3,4-thidiazole-2-yl)-N'-(5-methylisoxazoyl)-thiourea under the condition of solid-liquid phase transfer catalysis using polyethylene glycol-600 (PEG-600). (Scheme-1)

Experimental Section

Melting points were determined using a Yanaco MP-241 apparatus and are uncorrected. Infrared spectra were recorded on a Bruker Equinox55 spectrophotometer as KBr tablets. ^{1}H

NMR spectra were measured on a Bruker AC-P500 instrument (300MHz) using TMS as an internal standard and $CDCl_3$ as solvent. Elemental analyses were performed on a Yanaco MT-3CHN elemental analyzer.

All starting materials are commercial products of chemical or analytic grade purity. Sulfuric chloride was distillated and ammonium thiocyanate was baked before use. Analytical TLC was performed on silica gel GF_{254}.



Scheme-1. Reagents and conditions: (i) NH_4NCS, PEG-600, r.t. ultrasonic irradiation; (ii) $ArNH_2$, r.t. ultrasonic irradiation.

General Process

N-(5-substituted-1,3,4-thiadiazole-2-yl) -N'-(5-methylisoxazoyl)-thiourea

Powdered ammonium thiocyanate (1.14 g, 15 mmol), 5-methylisoxazole-4-carbonyl chloride (1.41 g, 10 mmol), PEG-600 (0.18 g, 3% with respect to ammonium thiocyanate) and methylene dichloride (25 ml) were placed in a dried round-bottomed flask containing a magnetic stirrer bar and stirred under ultrasonic irradiation at room temperature for 20 min. Then 2-amino-5-substituted-1,3,4-thiadiazole (4.5 mmol) in methylene dichloride (10 ml) were dropwised over 0.5 h, and the mixture was stirred for 0.5 h under ultrasonic irradiation. The corresponding N-(5-substituted-1,3,4-thiadiazole-2-yl)-N'-(5-methylisoxazoyl)-thiourea precipitated immediately. The product was filtered, washed with water to remove inorganic salts, dried, and recrystallized from DMF-EtOH-H2O to give **3a-3j**.

N-(1,3,4-thiadiazole-2-yl) -N'-(5-methylisoxazoyl)-thiourea 3a

Yield: 82.6 %; m.p.: 171-172 □; ^{1}H NMR: 9.32 (s, 1H, H-isoxazole), 2.85 (s, 3H, CH_3); IR (KBr) vcm^{-1}: 3168 (N-H), 1688 (C=O), 1302 (C=S); ESI-MS: 268 (M-1) ; Anal. calcd. For $C_8H_7N_5O_2S_2$: C 35.68, H 2.62, N 26.01; found: C 35.56, H 2.71, N 26.23.

N-(5-methyl-1,3,4-thiadiazole-2-yl) -N'-(5-methylisoxazoyl)-thiourea 3b

Yield: 83.6 %; m.p.: >230 □; ^{1}H NMR: 3.65 (s, 1H, H-isoxazole), 2.74 (s, 3H, CH_3), 2.47 (s, 3H, CH_3); IR (KBr) vcm^{-1}: 3161 (N-H), 1687 (C=O), 1298 (C=S);ESI-MS: 282 (M-1) ; Anal. calcd. For $C_9H_9N_5O_2S_2$: C 38.15, H 3.20, N 24.72; found: C 38.10, H 3.41, N 24.62.

N-(5-ethyl-1,3,4-thiadiazole-2-yl) -N'-(5-methylisoxazoyl)-thiourea 3c

Yield: 85.8 %; m.p.: >230 □; ^{1}H NMR: 9.51 (s, 1H, NH), 3.72 (s, 1H, H-isoxazole), 3.15 (q, 2H, CH_2), 2.83 (s, 3H, CH_3), 1.60 (s, 3H, CH_3); IR (KBr) vcm^{-1}: 3165 (N-H), 1680 (C=O), 1304 (C=S);ESI-MS: 296 (M-1) ; Anal. calcd. For $C_{10}H_{11}N_5O_2S_2$: C 40.39, H 3.73, N 23.55; found: C 40.10, H 3.81, N 23.76.

N-(5-cyclopropyl-1,3,4-thiadiazole-2-yl) -N'-(5-methylisoxazoyl)-thiourea 3d

Yield: 84.6 %; m.p.: 169-170 □; ^{1}H NMR: 9.49 (s, 1H, H-isoxazole), 2.83 (s, 3H, CH_3), 1.65 (s, H, CH), 1.16-1.29 (m, 4H, cycloprpyl-H); IR (KBr) vcm^{-1}: 3170 (N-H), 1687 (C=O), 1310 (C=S);ESI-MS: 308 (M-1) ; Anal. calcd. For $C_{11}H_{11}N_5O_2S_2$: C 42.71, H 3.58, N 22.64; found: C 42.65, H 3.68, N 22.66.

N-(5-phenyl-1,3,4-thiadiazole-2-yl) -N'-(5-methylisoxazoyl)-thiourea 3e

Yield: 90.1 %; m.p.: 157-158 □; ^{1}H NMR: 9.62 (s, 1H, H-isoxazole), 7.45-7.81 (m, 5H, C_6H_5), 2.87 (s, 3H, CH_3); IR (KBr) vcm ^{1}: 3165 (N-H), 1680 (C=O), 1303 (C=S) ;ESI-MS: 344 (M-1) ; Anal. calcd. For $C_{14}H_{11}N_5O_2S_2$: C 48.68, H 3.21, N 20.28; found: C 48.91, H 3.56, N 20.44.

N-(5-o-tolyl-1,3,4-thiadiazole-2-yl) -N'-(5-methylisoxazoyl)-thiourea 3f

Yield: 84.2 %; m.p.: 188-190 □; ^{1}H NMR: 9.69 (s, 1H, H-isoxazole), 7.29-7.79 (m, 4H, C_6H_4), 2.86 (s, 3H, CH_3), 2.68 (s, 3H, CH_3); IR (KBr) vcm^{-1}: 3161 (N-H), 1686 (C=O), 1289 (C=S);ESI-MS: 358 (M-1) ; Anal. calcd. For $C_{15}H_{13}N_5O_2S_2$: C 50.12, H 3.65, N 19.48; found: C 50.23, H 3.55, N 19.65.

N-(5-(2-fluorophenyl)-1,3,4-thiadiazole-2-yl) -N'-(5-methylisoxazoyl)-thiourea 3g

Yield: 83.5 %; m.p.: 173-174 □; ^{1}H NMR: 9.48 (s, 1H, H-isoxazole), 7.29-8.21 (m, 4H, C_6H_4), 2.87 (s, 3H, CH_3); IR (KBr) vcm ^{1}: 3255 (N-H), 1689 (C=O), 1297 (C=S) ;ESI-MS: 362 (M-1) ; Anal. calcd. For $C_{14}H_{10}FN_5O_2S_2$: C 46.27, H 2.77, N 19.27; found: C 46.54, H 2.98, N 19.53.

N-(5-(2-chlorophenyl)-1,3,4-thiadiazole-2-yl) -N'-(5-methylisoxazoyl)-thiourea 3h

Yield: 88.4 %; m.p.: 117-120 □; ^{1}H NMR: 9.36 (s, 1H, H-isoxazole), 7.39-8.15 (m, 4H, C_6H_4), 2.86 (s, 3H, CH_3); IR (KBr) vcm^{-1}: 3154 (N-H), 16801 (C=O), 1315 (C=S);ESI-MS: 378 (M-1) ; Anal. calcd. For $C_{14}H_{10}ClN_5O_2S_2$: C 44.27, H 2.65, N 18.44; found: C 44.02, H 2.56, N 18.66.

N-(5-(4-chlorophenyl)-1,3,4-thiadiazole-2-yl) -N'-(5-methylisoxazoyl)-thiourea 3i

Yield: 87.6 %; m.p.: 165-168 □; ^{1}H NMR: 9.34 (s, 1H, H-isoxazole), 7.38-8.04 (m, 4H, C_6H_4), 2.85 (s, 3H, CH_3); IR (KBr) vcm ^{1}: 3167 (N-H), 1682 (C=O), 1296 (C=S);ESI-MS: 378 (M-1) ; Anal. calcd. For $C_{14}H_{10}ClN_5O_2S_2$: C 44.27, H 2.65, N 18.44; found: C 44.44, H 2.36, N 18.46.

N-(5-(4-methoxyphenyl)-1,3,4-thiadiazole-2-yl) -N'-(5-methylisoxazoyl)-thiourea 3j

Yield: 84.6 %; m.p.: 155-157 □; ^{1}H NMR: 9.65 (s, 1H, H-isoxazole), 7.24-7.94 (m, 4H, C_6H_4), 3.89 (s, 3H, CH_3), 2.86 (s, 3H, CH_3); IR (KBr) vcm ^{1}: 3161 (N-H), 1697 (C=O), 1302 (C=S);ESI-MS: 374 (M-1) ; Anal. calcd. For $C_{15}H_{13}N_5O_3S_2$: C 47.99, H 3.49, N 18.65; found: C 47.89, H 3.58, N 18.56.

N-(5-m-tolyl-1,3,4-thiadiazole-2-yl) -N'-(5-methylisoxazoyl)-thiourea 3k

Yield: 85.6 %; m.p.: 158-159 □; ^{1}H NMR: 9.67 (s, 1H, H-isoxazole), 7.25-7.81 (m, 4H, C_6H_4), 3.65 (s, 3H, CH_3), 2.85 (s, 3H, CH_3); IR (KBr) vcm^{-1}: 3169 (N-H), 1684 (C=O), 1304 (C=S); ESI-MS: 358 (M-1) ; Anal. calcd. For $C_{15}H_{13}N_5O_2S_2$: C 50.12, H 3.65, N 19.48; found: C 49.98, H 3.78, N 19.67.

N-(5-(4-nitrophenyl)-1,3,4-thiadiazole-2-yl) -N'-(5-methylisoxazoyl)-thiourea 3l
Yield: 88.8 %; m.p.: 168-169 □; ^{1}H NMR: 9.42 (s, 1H, H-isoxazole), 7.34-8.55 (m, 4H, C_6H_4), 2.88 (s, 3H, CH_3); IR (KBr) $v\mathrm{cm}^{-1}$: 3198 (N-H), 1687 (C=O), 1301 (C=S); ESI-MS: 389 (M-1); Anal. calcd. For $C_{14}H_{10}N_6O_4S_2$: C 43.07, H 2.58, N 21.53; found: C 43.21, H 2.59, N 21.56.

Biological Activities

The method for testing the primary biological activities was performed in an isolated culture. Under a sterile condition, 1 mL of sample was added to the culture plates, followed by the addition of 9 mL of culture medium. The final mass concentration was 50 µg/mL. The blank assay was performed with 1 mL of sterile water. Circle mycelium with a diameter of 4 mm was cut using a drill. The culture plates were cultivated at (24±1)□. The extended diameters of the circle mycelium were measured after 72 h. The relative inhibition rate of the circle mycelium compared to blank assay was calculated via the following equation:

$$\text{Relative inhibition rate (\%)} = \frac{d_{ex} - d_{ex}'}{d_{ex}} \times 100\%$$

Where d_{ex} is the extended diameter of the circle mycelium during the blank assay; and d_{ex}', is the extended diameter of the circle mycelium during testing.

Results and Discussion

Synthesis

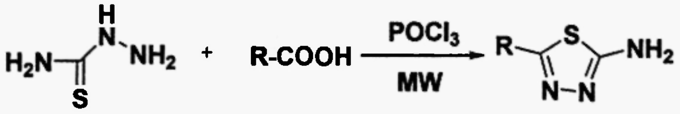
The same reaction under ultrasound required only 0.5 h to complete with excellent yields (Table-1). Ultrasonic irradiation was carried out with KQ-218 ultrasonic cleaner 20 kHz/50W. Compared with the conventional method, a shorter time, cleaner reaction accompained with higher yields were observed (from 77–81% to 82-90% compared with conventional method at r.t.). Generally, the reaction proceeded quickly with excellent yield as shown in Table 1.

Table-1 : Effect of different reaction conditions

Compounds	Reaction Time		Yield (%)	
	r.t.	Ultrasonic	r.t.	Ultrasonic
3a	3 h	0.5 h	83.4	85.8
3b	3 h	0.5 h	81.5	84.7
3c	3 h	0.5 h	83.5	90.1

On this note, we have conducted our reaction using PEG-600 as solid-liquid phase transfer catalyst under ultrasonic irradiation, this is a facile and convenient method for the synthesis of N-(5-substituted-1,3,4-thidiazole-2-yl) -N'-(5-methylisoxazoyl)-thiourea derivatives (**Scheme-1**), PEG-600 as a phase transfer catalyst is indispensable for these reactions. In addition, the ultrasonic irradiation method distinctly improves the efficiency of the synthetic process and shorten there action time. The catalyst PEG-600 is inexpensive, relatively nontoxic, highly stable and easily available.

In addition, we also studied the synthesis method of 2-amino-5-substituted-1,3,4-thiadiazole. Several procedures are available for the one-step synthesis of 2-amino-5-substituted-1,3,4-thiadiazole derivatives. Yet the reaction of aryl and alkyl-substituted acid with thiosemicarbazide in the presence of dehydrating agent $POCl_3$, affords a series of 2-amino-5-aryl-1,3,4-thiadiazoles under microwave irradiation(MWI) (**Scheme-2**). The 2-amino-5-substituted-1,3,4-thiadiazole intermediates of the last step were prepared in this way.



R=H, CH_3, C_2H_5, ph, o-CH_3ph, m-CH_3ph,p-Clph, o-Clph, o-Fph, p-OCH_3ph, p-NO_2ph, cyclopropyl

Scheme-2. Microwave irradiation synthesis of 2-amino-5-substituted-1,3,4-thiadiazoles

Biological Activities

Fungicidal activities of compounds **7a, 7c, 7e** against *G. zeae* Petch, *Phytophthora infestans* (Mont.) de Bary, *Botryosphaeria berengeriana* f. sp. *piricola* (Nose) koganezawa et Sakuma, *Fusarium oxysporum* f.sp. *cucumerinum*, and *Cercospora arachidicola*. The bioassay results showed that most of them had rather moderate fungicidal activities which against *G. zeae* Petch, *Phytophthora infestans* (Mont.) de Bary, *Botryosphaeria berengeriana* f. sp. *piricola* (Nose) koganezawa et Sakuma, *Fusarium oxysporum* f.sp. *cucumerinum*, and *Cercospora arachidicola* were 52.7%, 33.6%, 35.8%.

In Conclusion

A new series of acyl thiourea derivatives were synthesized using polyethylene glycol-600 (PEG-600) as the catalyst under ultrasonic irradiation. The method offers several advantages: an easy, rapid, one-pot reaction, environmental-friendliness and good yields. Biological activity of were evaluated and they showed certain activity.

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