Convenient Synthesis and Biological Activity of Mono and Diacyl 2,5-Dimercapto-1,3,4-thiadiazole Derivatives

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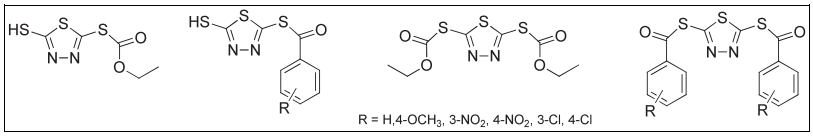

New derivatives of 2,5-dimercapto-1,3,4-thiadiazole substituted both at one or two exocyclic sulfur atoms with a series of aroyl or ethoxycarbonyl groups were synthesized in reactions of 2,5-dimercapto-1,3,4-thiadiazole salts with appropriate acid chlorides or ethyl chloroformate in mild conditions. The products were characterized by spectroscopy (^{1}H NMR, ^{13}C NMR, IR, and HRMS). Some from the synthesized compounds were screened *in vitro* and *in vivo* for antibacterial and antifungal activities against a panel of reference strains of microorganisms. The study revealed that ethyl *S*-(5-mercapto-1,3,4-thiadiazol-2-yl) carbonothioate seems to be the most active and versatile compound against Gram-positive bacteria, Gram-negative bacteria, and plant pathogenic fungi.

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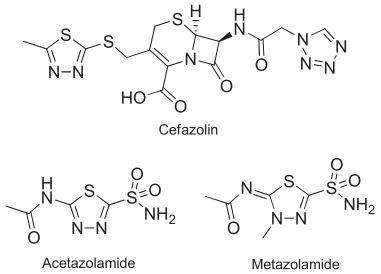
INTRODUCTION

Thiadiazoles belong to the group of five-membered heterocyclic arrangements containing one sulfur and two nitrogen atoms in the structure and were first described in literature in 1882 by Fischer [1–4]. Among several thiadiazole isomers, 1,3,4-thiadiazoles are the objects of the highest interest and intensive study [5,6]. Such arrangements exhibit a broad spectrum of biological activity and are used widely in medicine due to their precious antibacterial [7,8], antifungal [9,10], antituberculosis [11,12], anti-inflammatory [13,14], anticonvulsant [15,16], anticancer [17,18], and antiparasitic [19,20] activities. 1,3,4-Thiadiazoles also serve as adenosine/histamine receptor antagonist and antidepressant agents [21,22]. It is worth mentioning that some compounds of this group such as cefazolin [23]—a thiadiazole analogue of antibacterial cephalosporanic acid, acetazolamide, and metazolamide [24]—anhydrase inhibitors of the sulphonamide nature, belong to commercial drugs and are successfully applied in treatment (Scheme 1). Thiadiazoles are also used in agriculture as herbicides, fungicides, insecticides, and plant-growth regulators [25–30], and in material industry. The latter area comprises lubricants, corrosion inhibitors, thermoplastic resins, and dyes [31–34].

The most commonly reported methodology for the synthesis of this group of compounds uses *N,N′*-diacylhydrazines which treated with diphosphorus pentasulfide [35–37] or Lawesson's reagent [38–40] undergo cyclization forming the desired heterocyclic arrangement. Other sources describe also exchange of oxygen atom in 1,3,4-oxadiazoles to sulfur using thiourea [41–43], the cyclization of bithioureas [44,45], or thiosemicarbazides with another compounds containing a carbonyl group [46–48].

One of the representatives of thiadiazole family is 2,5-dimercaptothiadiazole (DMTD **1**), prepared efficiently in a simple manner from easy accessible reagents: carbon disulfide and hydrazine hydrate [49]. This compound played a role of the leading unit in our study on mono- and diacylation reactions of heterothiols by means of aromatic acid chlorides. Bearing in mind a versatile nature and broad range of biological interactions of thiadiazole derivatives, we have decided to synthesize a series of heterocyclic molecules containing this moiety. All the synthesized compounds were screened *in vitro* and *in vivo* for their antimicrobial activity against different fungal and bacterial pathogenic strains in order to find new agents for the treatment of microbial infection. Therefore, the main purpose of these studies is to investigate the antimicrobial potential of the obtained 1,3,4-thiadiazoles and identify compounds with a wide spectrum of activity, which could find an application in agriculture and medicine.

Scheme 1. Structure of the commercially available drug including 1,3,4-thiadiazole unit.



RESULTS AND DISCUSSION

2,5-Dimercapto-1,3,4-thiadiazole (DMTD **1**), prepared from hydrazine hydrate and carbon disulfide according to the methodology presented in literature [20], ethyl chloroformate, and the selected aromatic acid chlorides (**2a–f**), derived from commercially available acids and thionyl chloride $SOCl_2$, were the key reagents in our synthesis (Scheme 2). First trials of *S*-acylation, aiming at searching for the best conditions, were conducted by means of DMTD **1** and ethyl chloroformate in chloroform as a solvent, and in the presence of triethyl amine (TEA), as the agent responsible for binding of acidic hydrogen from the mercapto group.

The reactions proceeded smoothly at low temperatures (~0°C) in a relatively short time (1 h for **3** and 5 h for **4**), and the type of product depended mainly on the molar ratio of DMTD and acylation agent. Monoacyl derivative of DMTD **3** was obtained in high yield using equimolar amount of ethyl chloroformate, while its diacyl derivative **4** needed long reaction time and a slight excess of ethyl chloroformate (2.2 equiv; Table 1). It was also found that the better yield of thiadiazole **3** might be achieved when the solution of acylation agent is gradually introduced into DMTD **1**–TEA mixture. Otherwise, monoacylation product **3** is usually accompanied with diacylated derivative **4**.

With the optimized reaction conditions in hand, the key DMTD **1** was treated with a range of aromatic acid chlorides (**2a–f**) to give a series of *S*-monosubstituted (**5a–f**) and *S,S′*-disubstituted (**6a–f**) derivatives of DMTD. The yields of 5-mercapto-1,3,4-thiadiazol-2-yl benzothioates (**5a–f**) and 1,3,4-thiadiazole-2,5-diyl dibenzothioates (**6a–f**) are listed in Table 1. Comparing the two series of DMTD derivatives, it has to be noticed that the yields of *S,S′*-dibenzoyl derivatives (**4**, **6a–f**, 40–72%) are lower than their monosubstituted counterparts (**3**, **5a–f**, 61–83%). This might be attributed to the formation of side product, small amounts of monoacyl derivative, during the synthesis of disubstituted arrangement. The structure of new products was confirmed by means of elemental analysis, ^{1}H, ^{13}C NMR spectroscopy, high resolution mass spectrometry, and IR spectrophotometry. Products of both groups are crystallic solids, hardly soluble in many solvents. It was noticed that melting points of diacyl derivatives (**6a–f,** Table 1) are generally lower in contrast to their monoacylated counterparts (**5a–f,** Table 1), which may be caused by the existence of hydrogen bonding in the latter group. The results of elemental analysis remain in a good agreement with the calculated values. In the ^{1}H NMR spectra of 5-mercapto-1,3,4-thiadiazol-2-yl alkanethioates (**5a–f**), one can observe the characteristic peak of the proton adjacent to sulfur atom at the position 5 of the ring or to the corresponding tautomeric NH form, which appears as a broad singlet in the range from 10.23 to 12.70 ppm. Such signal disappears in the series of disubstituted 1,3,4-thiadiazole-2,5-diyl dialkanethioates (**6a–f**). The presence of carbonyl groups introduced during the acylation at the mercapto function of the starting DMTD **1** may be traced by means of ^{13}C NMR spectroscopy, where the characteristic C═O peak appears in the range between 184 and 192 ppm.

Scheme 2. Synthesis of 5-mercapto-1,3,4-thiadiazol-2-yl benzothioates (**5a–f**) and 1,3,4-thiadiazole-2,5-diyl dibenzothioates (**6a–f**). Reagents and conditions: (*i*) NaOH, H_2SO_4, reflux; (*ii*) $SOCl_2$, toluene, reflux, 2–8 h; (*i′*) TEA, 2-butanol, rt; (*ii′*) TEA, $CHCl_3$, rt.

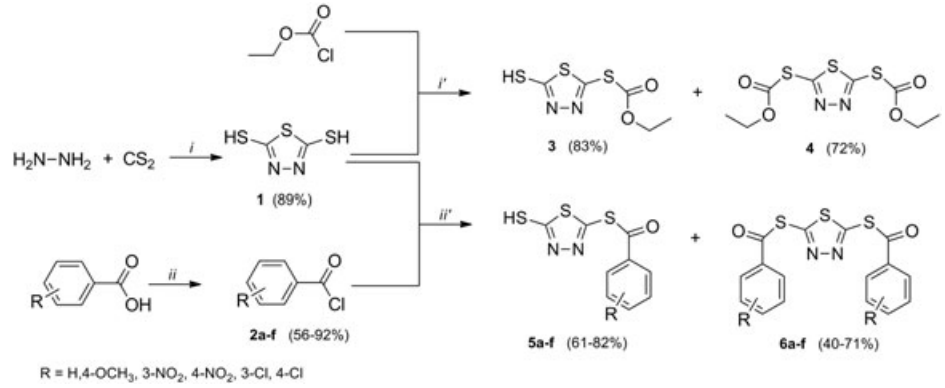


Table 1

Products of the reaction of DMTD **1** with ethyl chloroformate and aromatic acid chlorides.

Monosubstituted DMTD **3, 5a–f**			Disubstituted DMTD **4, 6a–f**		
Product	Yield, %	Mp, °C	Product	Yield, %	Mp, °C
3	83	101–103	**4**	72	82–84
5a (R^1 = H)	78	227–229	**6a** (R^1 = H)	68	192–194
5b (R^1 = 4-CH_3O)	82	217–219	**6b** (R^1 = 4-CH_3O)	71	214–216
5c (R^1 = 3-NO_2)	73	173–175	**6c** (R^1 = 3-NO_2)	57	116–118
5d (R^1 = 4-NO_2)	61	210–212	**6d** (R^1 = 4-NO_2)	52	138–140
5e (R^1 = 3-Cl)	65	191–193	**6e** (R^1 = 3-Cl)	40	171–173
5f (R^1 = 4-Cl)	74	188–190	**6f** (R^1 = 4-Cl)	54	168–170

The complementary IR analysis also indicates to the presence of the carbonyl group in both series of monosubstituted (**3**, **5a–f**) and disubstituted (**4**, **6a–f**) derivatives of DMTD forming a strong band in the range from 1650 to 1690 cm^{-1}. In order to confirm the structure of new products, a high-resolution mass spectrometric analysis was also performed. The measured mass of the $[M + H]^+$ ion is consistent with the expected mass for all compounds.

Some from the synthesized monosubstituted derivatives of DMTD **1** such as **3**, **5a**, **5b**, **5d**, and **5f** were screened *in vitro* for antibacterial and antifungal activities using the broth microdilution method according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) [50] and Clinical and Laboratory Standards Institute guidelines [51] against a panel of reference and clinical or saprophytic strains of microorganisms, including Gram-positive bacteria, Gram-negative bacteria, and fungi. The microorganisms belonging to ATCC originate from American Type Culture Collection, routinely used for the evaluation of antimicrobials. The inhibition of microbial growth was assessed by comparison with a control culture prepared without any sample tested [52]. Ciprofloxacin (Cip) or fluconazole (Flu) were used as reference antibacterial or antifungal compounds, respectively.

The results of antimicrobial screening revealed, that two among the studied monosubstituted DMTD derivatives: **5a**, bearing the benzoyl group at the mercapto substituent and **5d** with 4-nitrobenzoyl group, showed no inhibitory effect on the growth of all reference strains of bacteria and fungi (Table 2). The other compounds (**3**, **5b**, **5f**) exhibited a different bacteriostatic or bactericidal activity against tested microorganisms (MIC = 62.5–1000 μg/mL, MBC = 125–2000 μg/mL) or were just inactive against these bacteria. The widest spectrum of antimicrobial activity against both of bacteria and fungi exhibited ethyl *S*-(5-mercapto-1,3,4-thiadiazol-2-yl) carbonothioate (**3**). This compound showed good activity with bactericidal effect (MBC/MIC = 1–2) towards Gram-positive bacteria. The minimum concentrations, which inhibited the growth of these microorganisms, ranged from 62.5 μg/mL to 125 μg/mL (MBC = 62.5–250 μg/mL). The reference bacteria belonging to *Bacillus* spp. were the most susceptible to this substance (MIC = MBC = 62.5 μg/mL). The compound **3** indicated also moderate or mild activity towards some of Gram-negative bacteria (MIC = MBC = 250–1000 μg/mL), and also moderate antifungal activity against *Candida albicans spp. ATTC* with MIC = 250–500 μg/mL and MFC = 500 μg/mL. It is worth mentioning that all reference Gram-positive bacteria were also sensitive to compounds containing 4-methoxybenzoyl substituent **5b** and 4-chlorobenzoyl substituent **5f** (MIC = 250–1000 μg/mL and MBC = 250–1000 μg/mL). The remaining **5a** and **5d** showed lower activity against some of the tested Gram-positive and Gram-negative bacteria with MIC = 500–1000 μg/mL, mainly 1000 μg/mL (MBC ≥ 1000 μg/mL) or had no inhibitory effect on the growth of reference microorganisms. The study also revealed that these derivatives were generally inactive against the examined fungi.

We have also studied influence of some synthesized monosubstituted DMTD derivatives (**3**, **4**, **5a–f**) on the selected plant pathogenic fungi (Table 3).

The biological experiments (*in vitro* and *in vivo*) were carried out according to European Plant Protection Organization (EPPO) Standards [53] with the use of the following strains of fungi: *Botrytis cinerea*, *Phythophtora cactorum*, *Rhizoctonia solani*, *Phoma betae*, *Fusarium culmorum*, *Fusarium oxysporum*, *Alternaria alternate*, *Blumeria graminis*, *Phytophtora infestans* and mold fungi*: Penicillium ochrochloron,* which play the key role in biodeterioration of technical materials (attacks plastic and textiles). The fungicidal activity of the tested compounds was expressed as percentage of mycelial growth inhibition of fungi (*in vitro* assays) or percentage of wheat leaf area infected (*in vivo* assays) with respect to the control. Table 3 presents the results of both antifungal tests.

Table 2

The activity data expressed as MIC (MBC) [μg/mL] against the reference strains of bacteria and fungi for the selected monosubstituted DMTD derivatives (**3**, **5a**, **5b**, **5d**, **5f**).

Species	MIC (MBC or MFC) [μg/mL] of the tested compounds					
	3	5a	5b	5d	5f	Positive control
		Gram-positive bacteria				Cip[a]
Staphylococcus aureus ATCC 25923	62.5 (125)	2000 (2000)	500 (2000)	1000 (>2000)	500 (1000)	0.488
Staphylococcus aureus ATCC 6538	125 (250)	2000 (2000)	1000 (2000)	1000 (>2000)	1000 (1000)	0.244
Staphylococcus aureus ATCC 43300	125 (125)	1000 (2000)	1000 (1000)	1000 (>2000)	1000 (1000)	0.244
Staphylococcus epidermidis ATCC 12228	125 (125)	2000 (2000)	1000 (2000)	1000 (>2000)	500 (1000)	0.122
Micrococcus luteus ATCC 10240	62.5 (125)	1000 (1000)	500 (000)	1000 (>2000)	500 (1000)	0.976
Bacillus subtilis ATCC 6633	62.5 (62.5)	1000 (2000)	250 (500)	1000 (>2000)	250 (250)	0.031
Bacillus cereus ATCC 10876	62.5 (62.5)	500 (1000)	500 (500)	2000 (>2000)	500 (500)	0.061
		Gram-negative bacteria				Cip[a]
Bordetella bronchiseptica ATCC 4617	250 (250)	1000 (2000)	2000 (2000)	1000 (>2000)	1000 (2000)	0.976
Escherichia coli ATCC25922	1000 (1000)	2000 (2000)	2000 (>2000)	2000 (>2000)	2000 (2000)	0.004
Klebsiella pneumoniae ATCC 13883	2000 (2000)	2000 (>2000)	—	—	—	0.122
Proteus mirabilis ATCC 12453	500 (500)	2000 (2000)	2000 (>2000)	2000 (>2000)	2000 (2000)	0.030
Salmonella typhimurium ATCC1 4028	1000 (1000)	—	—	—	—	0.061
Pseudomonas aeruginosa ATCC 9027	2000 (>2000)	—	—	—	—	0.488
		Fungi				Flu[b]
Candida albicans ATCC 2091	500 (500)	2000 (>2000)	2000 (2000)	2000 (>2000)	2000 (2000)	0.244
Candida albicans ATCC 10231	250 (500)	—	2000 (2000)	2000 (>2000)	2000 (2000)	0.976
Candida parapsilosis ATCC 22019	1000 (1000)	2000 (>2000)	2000 (>2000)	2000 (>2000)	2000 (2000)	1.953

[a]The standard antibiotic for Gram-positive and Gram-negative bacteria—ciprofloxacin (**Cip**) used as positive control.
[b]The standard antibiotic for fungi—fluconazole (**Flu**) used as positive control.
[c]Bioactivity ranges: no bioactivity when MIC >1000 μg/mL; mild bioactivity for MIC 501–1000 μg/mL; moderate bioactivity for MIC 126–500 μg/mL; good bioactivity for MIC 26–125 μg/mL; strong bioactivity for MIC 10–25 μg/mL; very strong bioactivity when MIC <10 μg/mL; ATCC—American Type Culture Collection.

Most of the tested compounds showed week to moderate antifungal activity against plant pathogens. The best results were observed for one compound: ethyl *S*-(5-mercapto-1,3,4-thiadiazol-2-yl) carbonothioate (**3**) which caused the complete growth inhibition of five strains at the concentration of 200 mg/L. It is worth noting, that this compound was still active even after lowering the concentration to 100 mg/L. The most sensitive species to the compound **3** was *Phytophtora cactorum* for which at the concentration of 100 mg/L the mycelial growth was not observed up to 7 days on PDA medium. In addition, disubstituted DMTD derivative **4** showed moderate antifungal effect in the *in vivo* test against wheat powdery mildew on winter wheat seedlings.

CONCLUSION

Thus, we have elaborated the easy and efficient methodology for the preparation of new *S*-monoacylated and symmetrically substituted *S,S′*-diacylated 2,5-dimercapto-1,3,4-thiadiazole derivatives basing on 2,5-dimercapto-1,3,4-thiadiazole salts and aromatic acid chlorides or chloroformates. It was shown experimentally that compounds of these groups exhibit antibacterial and antifungal activities. Among them in particular ethyl *S*-(5-mercapto-1,3,4-thiadiazol-2-yl), carbonothioate seems to be the most active and versatile compound against Gram-positive bacteria, Gram-negative bacteria, and plant pathogenic fungi.

Table 3

Antifungal activity of monosubstituted DMTD derivatives (**3**, **4**, **5a–f**) against various strains of plant pathogenic fungi—percentage of mycelial growth inhibition and percentage of wheat leaf area infected.

Species	3	3	4	5a	5b	5c	5d	5e	5f
In vitro C [mg/L]	200	100	200	200	200	200	200	200	200
Alternaria alternata	48	—	44	42	0	0	4	14	0
Botrytis cinerea	100	80	80	60	28	20	28	30	16
Fusarium culmorum	100	29	46.6	16.6	13.3	16.6	16.6	16.6	16.6
Phytophtora cactorum	100	100	18.5	0	0	0	0	0	0
Rhizoctonia solani	60	56	56	0	0	0	0	0	0
Phytophtora infestans	100	15	36	0	0	0	0	0	0
Fusarium oxysporum	100	19	56	12	0	7.6	0	0	0
Penicillium ochrochloron	33.3	—	37	0	0	0	0	0	0
In vivo C [mg/L]	1000	—	1000	1000	1000	1000	1000	1000	1000
Blumeria graminis	16	—	44.4	4.9	30.8	12.3	11.1	7.4	7.4

EXPERIMENTAL

All solvents and reagents were purchased from commercial sources and used without further purification. Melting points were determined on a Stuart SMP3 melting point apparatus without corrections. ^{1}H and ^{13}C NMR spectra were recorded on an Agilent 400-NMR spectrometer using DMSO-d_6, $CDCl_3$ or CD_3CN as the solvent and TMS as the internal standard. Elemental analyses were performed with a VarioEL analyzer. FT-IR spectra were recorded between 4000 and 650 cm^{-1} on an FT-IR Nicolet 6700 apparatus with a Smart iTR accessory. High-resolution mass spectra were obtained by means of a Waters ACQUITY UPLC/Xevo G2QT instrument. Thin-layer chromatography was performed on silica gel 60 F_{254} (Merck) TLC plates using benzene/EtOAc (3:1 v/v) as the mobile phase.

2,5-Dimercapto-1,3,4-thiadiazole (DMTD, 1). was synthesized based on the methodology described in literature from commercially available hydrazine hydrate and carbon disulfide yielding 87% of white solid, mp 163–165°C (mp 164–170°C [20]).

Carboxylic acid chlorides (2a–f). were prepared by refluxing the selected carboxylic acids (0.10 mol) with thionyl chloride (15 mL) in dry toluene (100 mL) until the acids were fully consumed (TLC, 2–8 h). The crude acid chlorides obtained in high yields (**2a–f**, 68–92%) were purified by distillation under reduced pressure or crystallization and used in the subsequent reactions with DMTD **1**.

Benzoyl chloride (2a). Yield 92%, colorless liquid, BP 195–198°C (BP 195–197°C [54]).

4-Methoxybenzoyl chloride (2b). Yield 85%, colorless liquid, BP 140–145°C (25 mmHg) [BP 142°C (25 mmHg) [55]].

3-Nitrobenzoyl chloride (2c). Yield 68%, yellow solid, mp 32–34°C (mp 32–33°C [56]).

4-Nitrobenzoyl chloride (2d). Yield 70%, yellow solid, mp 71–73°C (mp 72–73°C [56]).

3-Chlorobenzoyl chloride (2e). Yield 74%, colorless liquid, BP 100–105°C (15 mmHg) [BP 103–104°C (14 mmHg) [57]].

4-Chlorobenzoyl chloride (2f). Yield 90%, colorless liquid, BP 105–110°C (15 mmHg) [BP 107–108°C (17 mmHg) [58]].

Synthesis of ethyl* S-*(5-mercapto-1,3,4-thiadiazol-2-yl) carbonothioate (3). The solution of ethyl chloroformate (2.1 mL, 0.022 mol) in 10 mL of 2-butanol was added dropwise to a cooled (0°C) solution of DMTD **1** (3.00 g, 0.020 mol) and TEA (2.8 mL, 0.020 mol) in 30 mL of 2-butanol. The mixture was agitated at 0°C for 3 h. Then, 20 mL of water was introduced to wash the organic layer, and the aqueous layer was discarded. The organic layer was washed with 20% H_2SO_4 solution (20 mL), again with water (20 mL) and dried over anhydrous $MgSO_4$. The solvent was evaporated, and the crude yellow product **3** was crystallized from toluene.

Ethyl* S-*(5-mercapto-1,3,4-thiadiazol-2-yl) carbono-thioate (3). Yield 83%, gray solid, mp 101–103°C, IR (ATR) ν, 2965, 2883, 2846, 2553, 2162 (S—H), 1721 (C═O), 1497, 1463, 1441, 1328, 1267, 1165, 1123, 1110, 1063, 1004, 844, 772, 663 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ: 1.36 (3H, t, J = 6.8 Hz, CH_3); 4.41 (2H, q, J = 6.8 Hz, OCH_2); 11.86 (1H, br s, SH). ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ: 14.1 (CH_3); 66.4 (OCH_2); 161.6; 164.4; 190.4 (CO). *Anal.* Calcd. for $C_5H_6N_2O_2S_3$: C, 27.01; H, 2.72; N, 12.60. Found: C, 27.12; H, 2.78; N, 12.62. HRMS (ES): 222.9660 $[M + H]^+$.

Synthesis of* S,S′-*(1,3,4-thiadiazole-2,5-diyl) dicarbono-thioate (4). The solution of ethyl chloroformate (4.2 mL, 0.044 mol) in 40 mL of chloroform was added dropwise to a cooled (0°C) solution of DMTD **1** (3.00 g, 0.020 mol) and TEA (5.6 mL, 0.040 mol) in 40 mL of chloroform. The mixture was agitated at 0°C for 5 h. Then, 30 mL of water was introduced to wash the organic layer, and the aqueous layer was discarded. The organic layer was washed with 20% H_2SO_4 solution

(30 mL), again with water (30 mL) and dried over anhydrous $MgSO_4$. The solvent was evaporated, and the crude yellow product **4** was purified by means of column chromatography (Al_2O_3, benzene/EtOAc, 1:5 v/v).

Diethyl S,S′-(1,3,4-thiadiazole-2,5-diyl) dicarbono-thioate (4). Yield 72%, yellow solid, mp 82–84°C (mp 86°C [59]), IR (ATR) ν, 2960, 2885, 2846, 2551, 2162, 1721 (C═O), 1471, 1457, 1447, 1389, 1375, 1331, 1181, 1150, 1117, 1010, 1073, 1003, 864, 805, 663 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$, ppm) δ: 1,38 (6H, t, J = 7.2 Hz, CH_3); 4.42 (4H, q, J = 7.2 Hz, OCH_2). ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ: 14.2 (CH_3); 66.1 (OCH_2); 161.4; 165.2; 183.5; 183.7 (CO). *Anal.* Calcd. for $C_8H_{10}N_2O_4S_3$: C, 32.64; H, 3.42; N, 9.52. Found: C, 32.60; H, 3.40; N, 9.55. HRMS (ES): 294.9878 $[M + H]^+$.

General procedure for the synthesis of *S*-(5-mercapto-1,3,4-thiadiazol-2-yl) benzothioates 5a–f. In 20 mL of chloroform, 1.50 g (0.010 mol) of DMTD **1** and 1.4 mL (0.010 mol) of TEA were dissolved. The resulted clear orange solution was added dropwise to a stirring solution of 0.010 mol of acyl chloride $R^1C_6H_4COCl$ (**2**) in 10 mL of chloroform and agitated at room temperature for the next 24 h. The crude precipitated product was washed with water (2 × 25 mL), dried on air, and crystallized from the appropriate solvent (*i*-PrOH, hexane, or acetone).

S-*(5-Mercapto-1,3,4-thiadiazol-2-yl) benzothioate (5a).* Yield 78%, white solid, mp 227–229°C, IR (ATR) ν, 3084, 2965, 2890, 2847, 2553 (S—H), 2164, 1667 (C═O), 1607, 1578, 1489, 1446, 1431, 1269, 1210, 1179, 1126, 1064, 903, 767, 722, 677 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$, ppm) δ: 7.25–7.55 (5H, m, Ph); 12.11 (1H, br s, SH). ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm) δ: 127.9; 129.3; 134.9; 135.1 (Ph); 160.3; 166.1; 185.5 (CO). *Anal.* Calcd. for $C_9H_6N_2OS_3$: C, 42.50; H, 2.38; N, 11.01. Found: C, 42.53; H, 2.39; N, 11.03. HRMS (ES): 254.9714 $[M + H]^+$.

S-*(5-Mercapto-1,3,4-thiadiazol-2-yl) 4-methoxybenzo-thioate (5b).* Yield 82%, yellow solid, mp 217–219°C, IR (ATR) ν, 3044, 2952, 2866, 2843, 2766, 2551 (S—H), 2162, 1652 (C═O), 1596, 1569, 1496, 1424, 1263, 1224, 1172, 1118, 1058, 1018, 894, 840, 724 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6, ppm) δ: 3.81 (3H, s, OCH_3); 7.00 (2H, d, J = 8.0 Hz, C_6H_4); 7.87 (2H, d, J = 8.0 Hz, C_6H_4), 12.62 (1H, br s, SH). ^{13}C NMR (100.6 MHz, DMSO-d_6, ppm) δ: 55.4 (OCH_3); 113.8; 122.9; 128.6; 166.9 (C_6H_4); 162.8; 165.3; 190.9 (CO). *Anal.* Calcd. for $C_{10}H_8N_2O_2S_3$: C, 42.24; H, 2.84; N, 9.85. Found: C, 42.28; H, 2.85; N, 9.87. HRMS (ES): 284.9826 $[M + H]^+$.

S-*(5-Mercapto-1,3,4-thiadiazol-2-yl) 3-nitrobenzo-thioate (5c).* Yield 73%, yellow solid, mp 173–175°C, IR (ATR) ν, 3041, 2952, 2867, 2831, 2556 (S—H), 2165, 1688 (C═O), 1610, 1530, 1495, 1473, 1433, 1344, 1322, 1264, 1207, 1130, 1063, 935, 856, 720 cm^{-1}. ^1H NMR (400 MHz, CD_3CN, ppm) δ: 7.83 (1H, t, J = 8.0 Hz, C_6H_4); 8.44 (2H, d, J = 8.0 Hz, C_6H_4); 8.69 (1H, s, C_6H_4); 12.70 (1H, br s, SH). ^{13}C NMR (100.6 MHz, CD_3CN, ppm) δ: 118.3; 123.3; 130.3; 132.1; 134.4; 146.8 (C_6H_4); 161.7; 166.3; 186.2 (CO). *Anal.* Calcd. for $C_9H_5N_3O_3S_3$: C, 36.11; H, 1.68; N, 14.04. Found: C, 36.09; H, 1.66; N, 14.03. HRMS (ES): 299.9563 $[M + H]^+$.

S-*(5-Mercapto-1,3,4-thiadiazol-2-yl) 4-nitrobenzo-thioate (5d).* Yield 61%, yellow solid, mp 210–212°C, IR (ATR) ν, 3043, 2954, 2869, 2872, 2558 (S—H), 2167, 1671 (C═O), 1607, 1527, 1471, 1405, 1346, 1322, 1260, 1198, 1182, 1112, 1052, 912, 861, 714 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6, ppm) δ: 8.16 (2H, d, J = 8.0 Hz, C_6H_4); 8.32 (2H, d, J = 8.0 Hz, C_6H_4); 10.26 (1H, br s, SH). ^{13}C NMR (100.6 MHz, DMSO-d_6, ppm) δ: 123.7; 130.6; 136.4; 149.9 (C_6H_4); 162.0; 165.8; 186.0 (CO). *Anal.* Calcd. for $C_9H_5N_3O_3S_3$: C, 36.11; H, 1.68; N, 14.04. Found: C, 36.15; H, 1.70; N, 14.07. HRMS (ES): 299.9569 $[M + H]^+$.

S-*(5-Mercapto-1,3,4-thiadiazol-2-yl) 3-chlorobenzo-thioate (5e).* Yield 65%, white solid, mp 191–193°C, IR (ATR) ν, 3059, 2957, 2874, 2841, 2543 (S—H), 2162, 1683 (C═O), 1586, 1570, 1488, 1418, 1268, 1195, 1123, 1061, 945, 786, 723 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6, ppm) δ: 7.86 (1H, s, C_6H_4); 7.92 (1H, d, J = 8.0 Hz, C_6H_4); 8.08 (1H, d, J = 8.0 Hz, C_6H_4); 8.12 (1H, t, J = 8.0 Hz, C_6H_4); 11.62 (1H, br s, SH). ^{13}C NMR (100.6 MHz, DMSO-d_6, ppm) δ: 127.9; 128.8; 130.6; 132.6; 133.3; 137.6 (C_6H_4); 161.7; 165.9; 184.2 (CO). *Anal.* Calcd. for $C_9H_5ClN_2OS_3$: C, 37.43; H, 1.75; N, 9.70. Found: C, 37.46; H, 1.73; N, 9.68. HRMS (ES): 288.9329 $[M + H]^+$.

S-*(5-Mercapto-1,3,4-thiadiazol-2-yl) 4-chlorobenzo-thioate (5f).* Yield 74%, white solid, mp 188–190°C, IR (ATR) ν, 3056, 2953, 2875, 2843, 2496 (S—H), 2162, 1673 (C═O), 1584, 1569, 1485, 1434, 1399, 1261, 1200, 1128, 1110, 890, 832, 724 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6, ppm) δ: 7.57 (2H, d, J = 8.0 Hz, C_6H_4); 7.95 (2H, d, J = 8.0 Hz, C_6H_4); 10.23 (1H, br s, SH). ^{13}C NMR (100.6 MHz, DMSO-d_6, ppm) δ: 128.7; 129.6; 131.1; 137.7 (C_6H_4); 162.8; 166.4; 186.3 (CO). *Anal.* Calcd. for $C_9H_5ClN_2OS_3$: C, 37.43; H, 1.75; N, 9.70. Found: C, 37.46; H, 1.77; N, 9.72. HRMS (ES): 288.9326 $[M + H]^+$.

General procedure for the synthesis of *S,S′*-(1,3,4-tiadiazole-2,5-diyl) bis(R^1-substituted benzothioates 6a–f. In 20 mL of chloroform, 1.50 g (0.010 mol) of DMTD **1** and 2.8 mL (0.020 mol) of TEA were dissolved. The resulted solution was added dropwise to a stirring solution of 0.022 mol of acyl chloride $R^1C_6H_4COCl$ (**2**) in 10 mL of chloroform and agitated at room temperature for 24 h. The crude precipitated product was washed with water (25 mL), 5% $NaHCO_3$ aqueous solution (25 mL), and again with water (25 mL). The solid was dried on air and crystallized or washed with the appropriate solvent (*i*-PrOH, hexane or acetone).

S,S′-*(1,3,4-Thiadiazole-2,5-diyl) dibenzothioate (6a)*. Yield 68%, white solid, mp 192–194°C (mp 185°C [60]), IR (ATR) ν, 3047, 2165, 1668 (C═O), 1591, 1578, 1490, 1444, 1276, 1207, 1175, 1069, 898, 770, 682 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6, ppm) δ: 7.56–7.68 (6H, m, 2 × Ph); 7.82 (4H, m, 2 × Ph). ^{13}C NMR (100.6 MHz, DMSO-d_6, ppm) δ: 128.1; 129.2; 134.7; 135.0 (Ph); 161.2; 165.9; 185.5; 187.1 (CO). *Anal.* Calcd. for $C_{16}H_{10}N_2O_2S_3$: C, 53.61; H, 2.81; N, 7.81. Found: C, 53.59; H, 2.80; N, 7.83. HRMS (ES): 358.9979 $[M + H]^+$.

S,S′-*(1,3,4-Tiadiazole-2,5-diyl) bis(4-methoxybenzo-thioate) (6b)*. Yield 71%, white solid, mp 214–216°C, IR (ATR) ν, 3101, 2841, 2163, 1664 (C═O), 1597, 1574, 1512, 1456, 1278, 1261, 1223, 1168, 1117, 1072, 1016, 903, 830, 792 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6, ppm) δ: 3.81 (6H, s, 2 × OCH_3); 7.16 (4H, d, J = 8.0 Hz, 2 × C_6H_4); 7.90 (4H, d, J = 8.0 Hz, 2 × C_6H_4). ^{13}C NMR (100.6 MHz, DMSO-d_6, ppm) δ: 55.4 (OCH_3); 113.9; 123.9; 128.7; 167.0 (C_6H_4); 162.7; 165.5; 189.6; 190.4 (CO). *Anal.* Calcd. for $C_{18}H_{14}N_2O_4S_3$: C, 51.66; H, 3.37; N, 6.69. Found: C, 51.68; H, 3.40; N, 6.71. HRMS (ES): 419.0191 $[M + H]^+$.

S,S′-*(1,3,4-Tiadiazole-2,5-diyl) bis(3-nitrobenzothioate) (6c)*. Yield 57%, yellow solid, mp 116–118°C, IR (ATR) ν, 3090, 2164, 1691 (C═O), 1617, 1526, 1478, 1442, 1401, 1348, 1324, 1265, 1192, 1133, 1071, 921, 821, 777, 716 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6, ppm) δ: 7.85 (2H, t, J = 8.0 Hz, 2 × C_6H_4); 8.30 (2H, d, J = 8.0 Hz, 2 × C_6H_4); 8.56–8.62 (4H, m, 2 × C_6H_4). ^{13}C NMR (100.6 MHz, DMSO-d_6, ppm) δ: 119.8; 121.1; 130.5; 133.9; 134.2; 147.1 (Ph); 162.3; 165.9; 187.5; 188.0 (CO). *Anal.* Calcd. for $C_{16}H_8N_4O_6S_3$: C, 42.85; H, 1.80; N, 12.49. Found: C, 42.81; H, 1.82; N, 12.49. HRMS (ES): 448.9682 $[M + H]^+$.

S,S′-*(1,3,4-Tiadiazole-2,5-diyl) bis(4-nitrobenzothioate) (6d)*. Yield 52%, yellow solid, mp 138–140°C, IR (ATR) ν, 3101, 2165, 1670 (C═O), 1607, 1526, 1472, 1410, 1346, 1322, 1261, 1197, 1182, 1115, 1052, 902, 846, 714 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6, ppm) δ: 8.16–8.36 (8H, m, 2 × C_6H_4). ^{13}C NMR (100.6 MHz, DMSO-d_6, ppm) δ: 123.8; 130.5; 134.9; 137.7 (C_6H_4); 162.9; 165.9; 187.4; 187.9 (CO). *Anal.* Calcd. for $C_{16}H_8N_4O_6S_3$: C, 42.85; H, 1.80; N, 12.49. Found: C, 42.86; H, 1.81; N, 12.47. HRMS (ES): 448.9677 $[M + H]^+$.

S,S′-*(1,3,4-Tiadiazole-2,5-diyl) bis(3-chlorobenzo-thioate) (6e)*. Yield 40%, yellow solid, mp 171–173°C, IR (ATR) ν, 3071, 2164, 1690 (C═O), 1597, 1575, 1475, 1417, 1301, 1261, 1231, 1172, 1138, 1035, 897, 850, 748, 719 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6, ppm) δ: 6.49 (2H, s, 2 × C_6H_4); 6.65 (2H, t, J = 8.0 Hz, 2 × C_6H_4); 6.71 (2H, d, J = 8.0 Hz, 2 × C_6H_4); 7.31 (2H, d, J = 8.0 Hz, 2 × C_6H_4). ^{13}C NMR (100.6 MHz, DMSO-d_6, ppm) δ: 125.8; 127.7; 128.2; 130.3; 130.5; 134.4 (C_6H_4); 162.9; 165.8; 191.6; 192.0 (CO). *Anal.* Calcd. for $C_{16}H_8Cl_2N_2O_2S_3$: C, 44.97; H, 1.89; N, 6.56. Found: C, 44.96; H, 1.91; N, 6.57. HRMS (ES): 426.9199 $[M + H]^+$.

S,S′-*(1,3,4-Tiadiazole-2,5-diyl) bis(4-chlorobenzo-thioate) (6f)*. Yield 54%, white solid, mp 168–170°C, IR (ATR) ν, 3086, 2163, 1673 (C═O), 1584, 1570, 1485, 1399, 1372, 1275, 1198, 1174, 1070, 893, 830, 738, 716 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6, ppm) δ: 7.65 (4H, d, J = 8.0 Hz, 2 × C_6H_4); 8.02 (4H, d, J = 8.0 Hz, 2 × C_6H_4). ^{13}C NMR (100.6 MHz, DMSO-d_6, ppm) δ: 128.9; 129.3; 131.1; 137.7 (C_6H_4); 162.3; 165.4; 187.1; 187.5 (CO). *Anal.* Calcd. for $C_{16}H_8Cl_2N_2O_2S_3$: C, 44.97; H, 1.89; N, 6.56. Found: C, 44.99; H, 1.87; N, 6.54. HRMS (ES): 426.9193 $[M + H]^+$.

Microbiology. In vitro *antimicrobial screening with the use of American Type Culture Collection ATCC.* Compounds **3, 5a, 5b, 5d,** and **5f** were screened *in vitro* for antibacterial and antifungal activities using the broth microdilution method according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) [50] and Clinical and Laboratory Standards Institute guidelines [51] against a panel of reference and clinical or saprophytic strains of microorganisms, including Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 43300, *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 10876, *Micrococcus luteus* ATCC 10240), Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Klebsiellapneumoniae* ATCC 13883, *Proteus mirabilis* ATCC 12453, *Bordetella bronchiseptica* ATCC 4617, *Salmonella typhimurium* ATCC 14028, *Pseudomonas aeruginosa* ATCC 9027), and fungi (*Candida albicans* ATCC 2091, *Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC 22019). The microorganisms belonging to ATCC originate from American Type Culture Collection and are usually used for the evaluation of antimicrobials. Microbial cultures were subcultured on nutrient agar or Sabouraud agar at 35°C for 18–24 h or 30°C for 24–48 h for bacteria and fungi, respectively. The surface of adequate agar (for bacteria: Mueller-Hinton agar and for fungi: RPMI 1640 with MOPS) was inoculated with the suspension of the analyzed species. Microbial suspensions were prepared in sterile saline (0.85% NaCl) with an optical density of McFarland standard scale 0.5—approximately 1.5×10^8 CFU (colony forming units)/mL for bacteria and 0.5 McFarland standard scale—approximately 5×10^5 CFU/mL for fungi. Each examined compound was dissolved in 1 mL of DMSO. Both bacterial and fungal suspensions were placed on Petri dishes with solid media containing 2 mg/mL of the tested compound and incubated at 37°C for 24 h and 30°C for 48 h for bacteria and fungi, respectively. The inhibition of microbial growth was assessed by comparison with a control

culture prepared without any sample tested. Ciprofloxacin or fluconazole was used as a reference antibacterial or antifungal compound, respectively. Minimal inhibitory concentration (MIC) for the examined compounds was evaluated by the microdilution broth method [52], using the twofold dilutions in Mueller-Hinton broth (for bacteria) and RPMI 1640 broth with MOPS (for fungi) prepared in 96-well polystyrene plates. Final concentrations of the compounds ranged from 1000 to 0.488 μg/mL. Microbial suspensions were prepared in sterile saline (0.85% NaCl) with an optical density of 0.5 McFarland standard. Next, 2 μL of each bacterial or fungal suspension was added per each well containing 200-μL broth and various concentrations of the examined compounds. After incubation (37°C for 24 h for bacteria and 30°C for 48 h for fungi), the MIC was assessed by spectrophotometric method as the lowest concentration of the compound exhibiting complete bacterial or fungal growth inhibition. Appropriate DMSO, growth and sterile controls were carried out. The medium with no tested substances was used as control. The minimal bactericidal concentration (MBC) or minimal fungicidal concentration (MFC), presenting the lowest concentration of the compound required to kill a particular bacteria or fungi, was determined by removing 20 μL of the culture used for MIC determinations from each well and spotting onto appropriate agar medium. The plates were incubated for 37°C for 24 h and 30°C for 48 h for bacteria and fungi, respectively. The lowest concentration of the compound with no visible growth observed was chosen as a MBC or MFC concentration. Each experiment was repeated three times. Bioactivity ranges were defined as follows: no bioactivity when MIC >1000 μg/mL; mild bioactivity for MIC 501–1000 μg/mL; moderate bioactivity for MIC 126–500 μg/mL; good bioactivity for MIC 26–125 μg/mL; strong bioactivity for MIC 10–25 μg/mL; very strong bioactivity when MIC <10 μg/mL. The MBC/MIC or MFC/MIC ratios were calculated in order to determine bactericidal/fungicidal (MBC/MIC ≤4, MFC/MIC ≤4) or bacteriostatic/fungistatic (MBC/MIC >4, MFC/MIC >4) effect of the tested compounds.

In vitro and in vivo antimicrobial screening against plant pathogenic fungi. The biological experiments on DMTD derivatives (**3**, **4**, **5a–f**) were carried out according to European Plant Protection Organization (EPPO) Standards [53] with the use of the following strains of fungi: *B. cinerea*, *P. cactorum*, *R. solani*, *P. betae*, *F. culmorum*, *F. oxysporum*, *A. alternate*, *B. graminis*, *P. infestans*, and mold fungi: *P. ochrochloron.*

In vitro fungicidal bioassay. Fungitoxicity of compounds was assessed *in vitro* using agar growth medium poison technique against seven phytopathogenic fungi and one mold species. Sterile potato dextrose agar (PDA) medium in 100-mm Petri dishes containing synthesized compounds at the concentration of 200 mg/L was infected with agar discs with thin mycelium of fungi taken from the margin of young vigorously growing 7-day-old culture. Linear growth of each colony was determined after incubation for 7 days at 25 ± 2°C. The fungicidal activity of the tested compounds was expressed as a percentage inhibition of mycelium compared to the control combination. Percentage inhibition was calculated as $(1 - A / B) \times 100\%$, where A represents a colony diameter in Petri dishes with tested compounds and B is the mean colony diameter in control dishes without compounds. Each measurement consisted of at least three replicates.

In vivo fungicidal bioassay. Wheat plants (*Triticum aestivum*, L.) winter cultivar Kobra were grown under normal glasshouse propagation conditions (temperature, 20–25°C; lighting, 14-h photoperiod of daylight supplemented by lamps 400 W) when plants had two expanded leaves. Seedlings were sprayed with acetone solution of the tested compounds at the concentration of 1000 mg/L containing 0.0125% Tween-20 as surfactant. Two hours after spraying, they were inoculated with powdery mildew (*B. graminis)* using dry inoculums from diseased wheat plants. After inoculation, plants were maintained in the growth chamber for approximately 8 days (at 20°C, 65% relative humidity and under 12 h dark/12 h light with 200 L × mol/m^2 × s photon flux density supplied by high output white fluorescent tubes). Macroscopic assessment of the percentage area covered by powdery mildew on the inoculated leaf was scored according to assessment keys presented in the EPPO Standards [53]. Disease scores were converted into relative values, expressed as a percentage of the reading on the control, and efficacy was calculated according to the Abbott's formula.

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