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Synthesis and Antifungal Activity of Novel 5-Substituted 4-(1,3,4-Thiadiazol-2-yl) benzene-1,3-Diols

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ABSTRACT: *5-Substituted (amine, alkyl, aryl, heterocyclic) 4-(1,3,4-thiadiazol-2-yl)benzene-1,3-diols were synthesized, and their antifungal properties were examined. The compounds were obtained by the one-pot reaction of sulfinylbis((2,4-dihydroxyphenyl)methanethione) with hydrazides or thiosemicarbazides. Their structures were identified from elemental, IR, ^{1}H NMR, and MS spectra analyses. The activities of the derivatives against five phytopathogenic fungi in vitro were measured. Moderate fungicidal effect of the compounds under consideration was found.* © 2010 Wiley Periodicals, Inc. Heteroatom Chem 21:533–540, 2010; View this article online at wileyonlinelibrary.com. DOI 10.1002/hc.20645

INTRODUCTION

Differently substituted 1,3,4-thiadiazoles are known for their interesting antifungal bioactivities and have attracted considerable attention to pesticide and medicinal formulation. A large number of reports on their synthesis and biological activities have appeared during the past years [1–8]. Among them, 1,3,4-thiadiazoles with antifungal activity against phytopathogenic fungi are an interesting group of compounds [9–12]. For example reported 1,3,4-thiadiazol-2-ylureas exhibit excellent fungicidal activities against *Rhizoctonia solani* (*R. solani*), *Botrytis cinerea* (*B. cinerea*), and *Dothiorella gregaria* (*D. gregaria*) [11]. Especially various sulfone- and sulfoxide-1,3,4-thiadiazoles have been synthesized and evaluated for antifungal activities against several kinds of fungi [13–17]. For the other 1,3,4-thiadiazole derivatives, fungicidal activity under the in vivo conditions was confirmed [18–21]. The 3D-QSAR model of compounds gave good correlation between the activity and the steric-electrostatic properties [19].

The incorporation of dihydroxyphenyl moiety in organic compounds, not limited to 1,3,4-thiadiazole has attracted considerable attention due to its naturally derived characterization and wide prevalence in pesticides and medicinal compounds. In our previous work, many heterocyclic compounds containing 2,4-dihydroxyphenyl moiety were reported with good fungicidal, antibacterial, and antitumor activities [22–24]. This substituent seems to be crucial in the biological effects. It allows to reach a proper degree of hydrophobic–hydrophilic balance of the molecule that enables penetration of cell membranes by compounds. The presence of hydroxyl groups gives opportunity for the interactions with a potential molecular target by donor or acceptor hydrogen bonds [25,26]. Extending our research in the area of fungicidal compounds, we designed a series of new 1,3,4-thiadiazoles with 2,4-dihydroxyphenyl functionality present in the heterocyclic ring. Herein, we wish to report the synthesis and biological studies in this area of some novel 1,3,4-thiadiazole derivatives.

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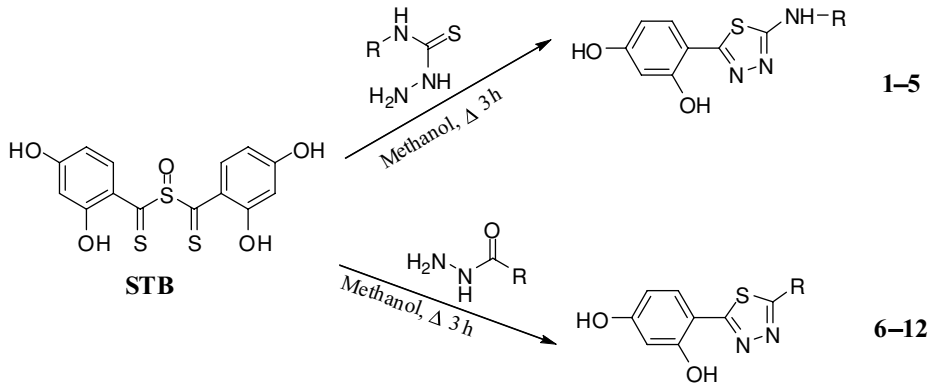


FIGURE 1 Synthesis scheme of 5-substituted 4-(1,3,4-thiadiazol-2-yl)benzene-1,3-diols.

RESULTS AND DISCUSSION

Chemistry

The 1,3,4-thiadiazole derivatives exemplified in this paper were prepared according to the route described in Figure 1 [27,28]. Treatment of sulfinyl-bis ((2,4-dihydroxyphenyl)methanethione) (STB) with appropriate 4-substituted-3-thiosemicarbazides in methanol afforded N-substituted 4-(5-amino-1,3,4-thiadiazol-2-yl)benzene-1,3-diols (**1–5**). Other analogues, without the amine group (**6–12**), were prepared from STB and hydrazides. STB as the starting reagent was synthesized from 2,4-dihydroxybenzenecarbodithioic acid and $SOCl_2$ in diethyl ether [27]. In the reaction of STB with nucleophiles, first the linear product of thioacyl derivative is formed, which transforms into the thiol form. Elimination of H_2S or H_2O molecule finally gives the 1,3,4-thiadiazole ring. So, electrophilic substrate STB also acts as an endogenous cyclizing reagent. Purity of compounds was monitored by the reversed-phase (RP-18) HPLC chromatography with methanol-water as a mobile phase (Table 1).

The structures of compounds and their physical and analytical data are presented in Table 1. The spectroscopic data of new derivatives are in agreement with the proposed structures and parameters characterizing other analogs [24,27]. In the ^{1}H NMR spectrum in the range of low fields as a rule, two signals are registered corresponding to protons of the OH group in the resorcinol moiety. The band about 9.5–9.9 ppm corresponds to the substituted amine group of compounds **1–4**. The resonance signals of C_5-H and C_2-H protons of resorcinol moiety appear as doublets in the range about 7.8–7.5 ppm with the coupling constant $J = 8.7$ Hz and in the range 6.5–6.4 with $J = 2.3$ Hz, respectively. However, C_6-H is registered in the doublet-doublets form at about 6.4–6.3 ppm of coupling constants $J = 8.5$ and 2.3 Hz. In IR spectrum, there is a strong band in the region about 1630 cm^{-1} corresponding to the vibration of C=N moiety and a weak one at 1050–1010 of N=C–S–C=N, characteristic of the 1,3,4-thiadiazole ring.

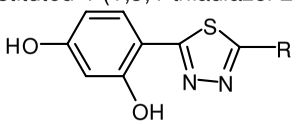
The mass spectra (EI-MS) of compounds gave molecular ion peaks, however, with different intensities. The major fragmentation pathway in most derivatives involved the cleavage of the S–C_2 and N–N bonds of 1,3,4-thiadiazole ring with formation of $(HO)_2C_6H_3CN^+$ (*m/z* 135) and *m/z* $[M - 135]^+$ ions [29]. The cleavage of C_2–N_3 and S–C_5 bonds directed toward $(HO)_2C_6H_3CS^+$ (*m/z* 153) fragmentation is also observed.

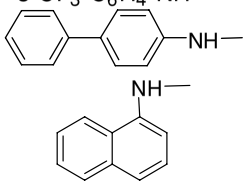
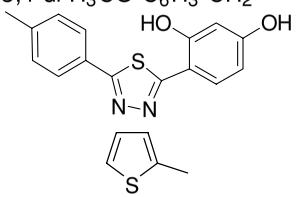
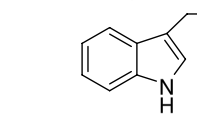
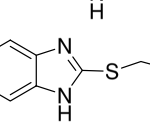
Biology

The results of in vitro screening against five strains of phytopathogenic fungi *Alternaria alternata*, *Botrytis cinerea*, *Rhizoctonia solani*, *Fusarium culmorum*, and *Phytophthora cactorum* are given in Table 2. The panel substituents of 1,3,4-thiadiazole ring include alkyls, aryls (differently modified) and heterocyclic rings. Activity of compounds is expressed as relative inhibition of growth (%) on the four degree scale with two different concentrations (200 and 20 μg mL^{-1}). Commonly applied fungicides studied under the same condition were used as the reference systems (Table 2).

The results presented in Table 2 show that antifungal properties of compounds under consideration are varied. In the laboratory studies at the concentration of 200 μg mL^{-1}, compound **7** reveals a significant fungistatic action (at the level 91–100%) against four fungi. Compounds **2, 3**, and **12** inhibit development of three or two pathogens but at a slightly lower level (50–90%). For the other compounds, weak or no growth-inhibitory activity was observed in vitro (Table 2). All compounds show a

TABLE 1 Structure, Physical, and Analytical Data of 5-Substituted 4-(1,3,4-thiadiazol-2-yl)benzene-1,3-diols



						Elemental Analysis (Calcd./Found)		
No.	*Substituent -R*	*Yield (%)*	*Mp (°C)*	*Mol. Formula/Mol. Wt.*	*log k (HPLC)*	*C*	*H*	*N*
1	4-CH_3-C_6H_4-NH-	89	237–239	$C_{15}H_{13}N_3O_2S$/299.34	0.115	60.18/60.12	4.38/4.39	14.04/14.00
2	3-CF_3-C_6H_4-NH-	84	236–237	$C_{15}H_{10}F_3N_3O_2S$/353.32	0.277	50.99/51.17	2.85/2.86	11.89/11.86
3		67	228–230	$C_{20}H_{15}N_3O_2S$/361.42	−0.246	66.46/66.70	4.18/4.17	11.63/11.60
4		75	243–245	$C_{18}H_{13}N_3O_2S$/335.38	0.118	64.46/64.63	3.91/3.92	12.53/12.50
5	$(CH_3)_2N$-	69	277–279	$C_{10}H_{11}N_3O_2S$/237.28	−0.466	50.62/50.81	4.67/4.68	17.71/17.69
6	$CH_3(CH_2)_6$-	73	160–162	$C_{15}H_{20}N_2O_2S$/292.40	0.630	61.62/61.74	6.89/6.90	9.58/9.60
7	3-CH_3O-4-HO-C_6H_3-	79	247–249	$C_{15}H_{12}N_2O_4S$/316.33	−0.260	56.95/57.14	3.82/3.83	8.86/8.88
8	3,4-di-H_3CO-C_6H_3-CH_2-	69	307–308	$C_{17}H_{16}N_2O_4S$/344.38	−0.355	59.29/59.41	4.68/4.69	8.13/8.11
9		67	124–126	$C_{22}H_{14}N_4O_4S_2$/462.50	0.217	57.13/56.92	3.05/3.06	12.11/12.09
10		65	248–249	$C_{12}H_8N_2O_2S_2$/276.33	−0.070	52.16/52.29	2.92/2.93	10.14/10.12
11		62	235–237	$C_{17}H_{13}N_3O_2S$/323.37	−0.156	63.14/63.38	4.05/4.04	12.99/13.02
12		64	272–273	$C_{16}H_{12}N_4O_2S_2$/356.42	0.930	53.92/54.18	3.39/3.40	15.72/15.70

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lower level of activity than the standard. With the compounds at 20 μg mL^{-1} concentration, only compound **7** inhibits growth of four pathogens at the level 20–50%. *F. culmorum* seems to be the most refractory but *R. solani* the most sensitive fungus in relation to the evaluated 1,3,4-thiadiazole derivatives.

The structure–activity analysis shows that from amino-1,3,4-thiadiazole group (**1–5**) the compound with CF_3-substituent acts more intense than with CH_3- substituent and 4-biphenyl derivative (**3**) than naphthalen-1-yl derivative (**4**). The compound with the tertiary amine group (**5**) exhibits the weakest antifungal properties. Among the compounds with other group (**6–12**), the most beneficial is the presence of the 4-hydroxy-3-methoxyphenyl substituent (**7**). The antifungal effect intensifies also additional heterocyclin ring in the form of benzo[d]imidazole (**12**) but decreases the second 1,3,4-thiadiazole ring (**9**) or thiophen-2-yl (**10**) moiety.

In conclusion, a series of novel 4-(1,3,4-thiadiazol-2-yl)benzene-1,3-diol derivatives was synthesized and evaluated for their fungicidal activity. The compounds under consideration show moderate fungicidal effect contrary to various heterocyclic derivatives with 2,4-dihydroxyphenyl moiety described previously [22,23].

EXPERIMENTAL

Analytical Studies

The melting point (mp) was determined using a Buchi B-540 (Switzerland) melting point apparatus. The elemental analysis was performed to determine C, H, and N contents (Perkin-Elmer 2400). The analyses were within ±0.4% of the theoretical values. The IR spectra were recorded with a Perkin-Elmer FT-IR 1725X spectrophotometer (in potassium bromide) or Varian 670-IR, FT-IR spectrometer (ATR). The spectra were made in the range of 600–4000 cm^{-1}. ^{1}H NMR spectra were recorded in DMSO-d_6 or $CDCl_3$ using a Varian Mercury 400 or a Bruker DRX 500 instrument. Chemical shifts (δ, ppm) were given in relation to tetramethylsilane (TMS). The spectra MS (EI, 70 eV) were recorded using the apparatus AMD-604.

The purity of the compounds was examined by a liquid chromatograph Knauer with a dual pump, a 20-μL simple injection valve and a UV–visible detector (330) nm. The Hypersil Gold C18 (1.9 μm, 100 × 2.1 mm) column was used as the stationary phase. The mobile phase included different contents of methanol and acetate buffer (pH 4, 20 nM) as the aqueous phase. The flow rate was 0.5 mL min^{-1} at room temperature. The retention time of an unretained solute (t_0) was determined by the injection of a small amount of acetone dissolved in water. Log k values for 70% of methanol (v/v) in the mobile phase are presented.

Preparation of Title Compounds

*4-(5-(p-Tolylamino)-1,3,4-thiadiazol-2-yl)benzene-1,3-diol (**1**).* A mixture of 4-(*p*-tolyl)-3-thiosemicarbazide (Alfa Aesar GmbH & Co. KG, Karlsruhe, Germany) (0.01 mol) and STB (0.075 mol) in methanol (50 mL) was refluxed for 3 h. The hot reaction mixture was filtered, and the filtrate was concentrated until to dry. The removed product was crystallized from methanol (2 × 50 mL).

^{1}H NMR (400 MHz, DMSO-d_6, δ): 10.85 (s, 1H, C_3-OH), 10.14 (s, 1H, C_1-OH), 9.87 (s, 1H, NH), 7.74 (d, J = 8.6 Hz, 1H, C_5-H), 7.51 (d, J = 8.5 Hz, 2H, C'_{AR}-H), 7.15 (d, J = 8.2 Hz, 2H, C'_{AR}-H), 6.42 (d, J = 2.3 Hz, 1H, C_2-H), 6.39 (dd, J = 8.5 and J = 2.3 Hz, 1H, C_6-H); IR (ATR, cm^{-1}): 3316, 3139 (OH, NH), 3033 (C_{AR}-H), 2917 (C-H), 2852 (C-H), 1620 (C=N), 1599, 1515, 1460 (C=C), 1454, 1426, 1321, 1250, 1226, 1181 (C-O), 1113, 1020, 988, 969, 872, 840, 815, 791, 760, 701, 678 (C-S-C); EI-MS (*m/z*, %): 299 (M^+, 100), 298 (11), 296 (4), 164 (18), 163 (7), 153 (4), 150 (3), 135 (3), 131 (4), 121 (2), 106 (4), 95 (2), 94 (6), 77 (3), 69 (2), 66 (4), 65 (5).

*4-(5-(3-(Trifluoromethyl)phenylamino)-1,3,4-thiadiazol-2-yl)benzene-1,3-diol (**2**).* A mixture of 4-[3-(trifluoromethyl)phenyl]-3-thiosemicarbazide (Alfa Aesar Chemie GmbH, Steinheim, Germany) (0.01 mol) and STB (0.075 mol) in methanol (50 mL) was refluxed for 3 h. The hot reaction mixture was filtered, and the filtrate was left at room temperature (24 h). The formed solid was filtered off and crystallized from methanol (2 × 25 mL).

^{1}H NMR (400 MHz, DMSO-d_6, δ): 11.12 (s, 1H, C_3-OH), 8.28–8.27 (s, 1H, C'_{AR}-H), 7.88–7.86 (d, J = 8.8 Hz, 1H, C_5-H), 7.85–7.82 (d, J = 8.2 Hz, 1H, C'_{AR}-H), 7.60–7.56 (t, J = 8.1 Hz, 1H, C'_{AR}-H), 7.33–7.31 (d, J = 8.2 Hz, 1H, C'_{AR}-H), 6.55–6.54 (d, J = 2.4 Hz, 1H, C_2-H), 6.45–6.42 (dd, J = 8.6 and J = 2.4 Hz, 1H, C_6-H); IR (KBr, cm^{-1}): 3498 (OH, NH), 3048 (C_{AR}-H), 1613 (C=N, C=C), 1579, 1498 (C=C), 1550, 1457, 1387, 1334 (CF_3), 1298, 1232, 1169 (C-O), 1128, 1073 (N=C–S–C=N), 982, 909, 842, 796, 762, 742, 695, 673 (C-S-C), 658, 603; EI-MS (*m/z*, %): 353 (M^+, 100), 352 (6), 334 (4), 324 (2), 219 (4), 218 (29), 204 (2), 198 (7), 191 (2), 186 (3), 167 (2), 153 (5), 150 (4), 145 (4), 136 (5), 135 (3), 95 (2), 94 (6), 69 (2), 66 (3).

*4-(5-(Biphenyl-4-ylamino)-1,3,4-thiadiazol-2-yl) benzene-1,3-diol (**3**).* A mixture of 4-(biphenyl-4-yl)-

TABLE 2 Growth-Inhibitory Activity of 5-Substituted 4-(1,3,4-Thiadiazol-2-yl)benzene-1,3-diols According to the 4-Point Scale[a] at the Concentrations of Compound 200 (20[b]) μg mL^{-1}

	Mycelia Growth Inhibition				
No.	*A. alternata*	*B. cinerea*	*F. culmorum*	*P. cactorum*	*R. solani*
1	1	1	0	1	1
2	2	2	0	2	2
3	2	2	0	1	1
4	0	0	0	1	1
5	1	0	0	0	1
6	1	1	0	1	0
7	2/1[b]	3/1[b]	3/0[b]	3/1[b]	3/1[b]
8	0	0	0	2	2
9	0	0	0	0	1
10	1	0	0	1	1
11	0	0	0	1	2
12	2	1	0	2	2
Prochloraz	3/3	3/3	3/3	–[c]	–[c]
Carbendazim	–[c]	–[c]	–[c]	3/3	3/3
Procymidon	2/2	3/3	–[c]	–[c]	–[c]

[a]The results are given in the four-degree scale determining the percentage of mycelium growth inhibition compared with the control: 0: <20% a lack of action, 1: 20–50% weak action, 2: 50–90% medium action, 3: >90% good action.
[b]Activity at the concentrations of compound 20 μg mL^{-1}.
[c] Test was not performed.

3-thiosemicarbazide (Aldrich Chemie GmbH, Steinheim, Germany) (0.01 mol) and STB (0.075 mol) in methanol (70 mL) was refluxed for 3 h. The formed solid was filtered off and crystallized from methanol (2 × 25 mL).

^{1}H NMR (400 MHz, DMSO-d_6, δ): 11.85 (s, 1H, C_3-OH), 10.20 (s, 1H, C_1-OH), 9.47 (s, 1H, NH), 8.08–8.06 (d, 1H, C_5-H), 7.49–7.29 (m, 7H, C'_{AR}-H), 7.36–7.31 (m, 2H, C'_{AR}-H), 6.39–6.38 (d, 1H, C_2-H), 6.37–6.36 (d, 1H, C_6-H); IR (ATR, cm^{-1}): 3372, 3243 (OH, NH), 2952 (C_{AR}-H), 1641 (C=N), 1617, 1592, 1565, 1488 (C=C), 1433, 1350, 1323, 1277, 1235, 1171 (C-O), 1125, 1048, 1012, 986, 951, 872, 854, 811, 758, 738, 700, 677 (C-S-C); EI-MS (*m/z*, %): 361 (M^+, 4), 345 (3), 329 (4), 302 (3), 228 (3), 256 (4), 225 (4), 211 (11), 210 (100), 170 (4), 169 (26), 168 (17), 167 (10), 153 (14), 150 (17), 149 (2), 137 (4), 135 (4), 121 (8), 108 (3), 97 (5), 95 (4), 94 (21), 93 (4), 84 (4), 81 (6), 80 (4), 79 (4), 77 (4), 75 (8), 69 (8), 67 (3), 66 (18), 65 (8), 63 (4), 53 (5), 52 (7), 51 (8), 45 (5), 44 (5), 40 (6), 39 (13).

*4-(5-(Naphthalen-1-ylamino)-1,3,4-thiadiazol-2-yl)benzene-1,3-diol (**4**).* A mixture of 4-(2-naphthyl)-3-thiosemicarbazide (Aldrich) (0.01 mol) and STB (0.075 mol) in methanol (50 mL) was refluxed for 3 h. The hot reaction mixture was filtered, and the filtrate was concentrated until to dry. The removed product was crystallized from methanol (2 × 50 mL).

^{1}H NMR (400 MHz, DMSO-d_6, δ): 10.88 (s, 1H, C_3-OH), 10.17 (s, 1H, C_1-OH), 9.92 (s, 1H, NH), 8.31–8.29 (m, 1H, C'_{AR}-H), 8.21–8.19 (d, J = 7.3 Hz, 1H, C'_{AR}-H), 7.97–7.93 (m, 1H, C'_{AR}-H), 7.81–7.78 (d, 1H, J = 8.4 Hz, C_5-H), 7.69–7.67 (d, J = 8.2 Hz, 1H, C'_{AR}-H), 7.61–7.51 (m, 3H, C'_{AR}-H), 6.45–6.43 (d, 1H, J = 2.4 Hz, C_2-H), 6.42–6.39 (dd, J = 8.6 and J = 2.4 Hz, 1H, C_6-H); IR (KBr, cm^{-1}): 3427, 3172 (OH, NH), 3058 (C_{AR}-H), 1635 (C=N), 1600, 1511, 1486 (C=C), 1465, 1431, 1401, 1341, 1296, 1220, 1187 (C-O), 1126, 1101, 1084 (N=C=S–C=N), 1047, 1022, 989, 971, 885, 835, 788, 736, 711, 678 (C-S-C), 640; EI-MS (*m/z*, %): 335 (M^+, 100), 334 (60), 201 (4), 200 (22), 199 (17), 185 (3), 173 (5), 172 (4), 168 (13), 167 (5), 153 (4), 150 (3), 141 (6), 140 (6), 135 (5), 128 (4), 127 (6), 126 (2), 121 (2), 115 (11), 94 (7), 77 (2), 66 (4), 63 (2), 51 (2), 39 (3).

*4-(5-(Dimethylamino)-1,3,4-thiadiazol-2-yl)benzene-1,3-diol (**5**).* A mixture of 4,4-dimethyl-3-thiosemicarbazide (Aldrich) (0.01 mol) and STB (0.075 mol) in methanol (50 mL) was refluxed for 3 h. The hot reaction mixture was filtered. The removed product was crystallized from methanol (2 × 30 mL).

^{1}H NMR (500 MHz, DMSO-d_6, δ): 11.09 (s, 1H, C_3-OH), 9.00 (s, 1H, C_1-OH), 6.69 (d, J = 8.6 Hz, 1H, C_5-H), 6.46 (d, J = 2.3 Hz, 1H, C_2-H), 6.39 (dd, J = 8.6 and J = 2.3 Hz, 1H, C_6-H), 3.17 (s, 6H, CH_3); IR (KBr, cm^{-1}): 3197 (OH), 2945 (CH), 2818 (CH),

1635 (C=N), 1598, 1572 (C=C), 1510, 1491, 1466 (C=C), 1419, 1321, 1286, 1221, 1181 (C-O), 1096, 1066 (N=C–S–C=N), 986, 969, 924, 863, 844, 814, 748, 691 (C-S-C), 640, 616; EI-MS (*m*/*z*, %): 237 (M^+, 100), 222 (5), 208 (18), 153 (19), 136 (7), 135 (22), 108 (6), 107 (5), 106 (2), 103 (3), 102 (26), 97 (8), 88 (28), 87 (13), 80 (4), 79 (3), 73 (4), 71 (4), 69 (9), 65 (4), 63 (4), 57 (5), 56 (5), 53 (5), 52 (8), 51 (7), 44 (14), 43 (17), 42 (17), 39 (8), 38 (5), 36 (14).

*4-(5-Heptyl-1,3,4-thiadiazol-2-yl)benzene-1,3-diol (**6**)*. A mixture of octanoic hydrazide (Aldrich) (0.01 mol) and STB (0.0075 mol) in methanol (50 mL) was refluxed for 3 h. The hot reaction mixture was filtered, and the filtrate was left at room temperature (48 h). The formed solid was filtered off and crystallized from methanol (2 × 30 mL).

^{1}H NMR (500 MHz, $CDCl_3$, δ): 10.91 (s, 1H, C_3-OH), 9.96 (s, 1H, C_1-OH), 7.93–7.91 (d, $J = 8.7$ Hz, 1H, C_5-H), 6.46–6.47 (d, $J = 2.3$ Hz, 1H, C_2-H), 6.43–6.41 (dd, $J = 8.7$ and 2.3 Hz, 1H, C_6-H), 3.06–3.03 (t, $J = 7.5$ Hz, 2H, CH_2), 1.76–1.70 (t, $J = 7.5$ Hz, 2H, CH_2), 1.36–1.23 (m, 8H, CH_2), 0.87–0.84 (t, 3H, CH_3); IR (KBr, cm^{-1}): 3401 (OH), 3045 (C_{AR}-H), 2953 (CH_3), 2923, 2953 (CH_2), 1604 (C=N), 1520, 1470 (C=C), 1420, 1394, 1322, 1284, 1245, 1210, 1180 (C-O), 1151, 1119, 1080, 1034 (N=C–S–C=N), 987, 971, 912, 866, 809, 726, 668 (C-S-C), 643, 620; EI-MS (*m*/*z*, %): 292 (M^+, 25), 264 (3), 263 (19), 245 (11), 235 (7), 223 (4), 222 (14), 221 (57), 210 (5), 209 (12), 208 (100), 168 (2), 167 (4), 153 (18), 147 (40), 136 (13), 135 (9), 115 (3), 108 (3), 107 (3), 106 (2), 97 (3), 80 (3), 69 (3), 55 (6), 54 (3), 53 (3), 52 (6), 51 (3), 45 (3), 43 (7), 41 (16), 39 (8).

*4-(5-(4-Hydroxy-3-methoxyphenyl)-1,3,4-thiadiazol-2-yl)benzene-1,3-diol (**7**)*. A mixture of 4-hydroxy-3-methoxybenzohydrazide (Alfa Aesar) (0.01 mol) and STB (0.0075 mol) in methanol (50 mL) was refluxed for 3 h. The hot reaction mixture was filtered, and water (50 mL) was added to the filtrate. The formed solid was filtered off and crystallized from methanol (30 mL).

^{1}H NMR (500 MHz, DMSO-d_6, δ): 11.03 (s, 1H, C_3-OH), 10.04 (s, 1H, C_1-OH), 9.70 (s, 1H, C'_4-OH), 8.02 (d, $J = 8.8$ Hz, 1H, C_5-H), 7.53 (d, $J = 2.3$ Hz, 1H, C'_1-H), 7.40 (dd, $J = 8.3$ and $J = 1.9$ Hz, 1H, C'_6-H), 6.92 (d, $J = 8.3$ Hz, 1H, C'_5-H), 6.51 (d, $J = 2.5$ Hz, 1H, C_2-H), 6.44 (dd, $J = 8.8$ and $J = 2.5$ Hz, 1H, C_6-H), 3.88 (s, 3H, CH_3); IR (KBr, cm^{-1}): 3295 (OH), 1606 (C=N, C=C), 1505 (C=C), 1326, 1269 (C-O-C), 1219, 1170 (C-O), 1121, 1027 (N=C–S–C=N), 979, 860, 648 (C-S-C); EI-MS (*m*/*z*, %): 316 (M^+, 100), 315 (12), 302 (5), 301 (7), 300 (33), 287 (5), 271 (3), 243 (4), 213 (3), 181 (8), 169 (3), 168 (11), 167 (51), 166 (19), 158 (4), 153 (15), 152 (9), 151 (53), 150 (10), 149 (17), 138 (13), 137 (34), 136 (13), 135 (15), 134 (11), 124 (5), 123 (10), 122 (5), 119 (6), 1108 (10), 107 (9), 106 (10), 97 (3), 81 (6), 80 (8), 79 (5), 77 (5), 69 (8), 65 (7), 64 (4), 63 (7), 55 (4).

*4-(5-(3,4-Dimethoxybenzyl)-1,3,4-thiadiazol-2-yl) benzene-1,3-diol (**8**)*. A mixture of 3,4-dimethoxyphenyl acetic acid hydrazide (Alfa Aesar) (0.01 mol) and STB (0.075 mol) in methanol (50 mL) was refluxed for 3 h. The hot reaction mixture was filtered, and the filtrate was concentrated until to dry. The removed product was crystallized from methanol (2 × 30 mL).

^{1}H NMR (400 MHz, DMSO-d_6, δ): 10.91 (s, 1H, C_3-OH), 10.01 (s, 1H, C_1-OH), 7.95 (d, $J = 8.6$ Hz, 1H, C_5-H), 6.97 (d, $J = 2.0$ Hz, 1H, C'_2-H), 6.92 (d, $J = 8.3$ Hz, 1H, C'_5-H), 6.87 (dd, $J = 8.3$ and 2.0 Hz, 1H, C'_6-H), 6.45 (d, $J = 2.4$ Hz, 1H, C_2-H), 6.41 (dd, $J = 8.6$ and $J = 2.4$ Hz, 1H, C_6-H), 4.35 (s, 2H, CH_2), 3.74 (s, 6H, CH_3); IR (ATR, cm^{-1}): 3315, 3175 (OH), 3075 (C_{AR}-H), 2961 (CH), 2890 (CH), 1641 (C=N), 1601, 1566, 1552 (C=C), 1512, 1468, 1445, 1433, 1388, 1367, 1342, 1311, 1244, 1222, 1206 (C-O), 1163, 1129, 1088, 1037, 1022, 956, 916, 883, 858, 810, 800, 752, 699 (C-S-C); EI-MS (*m*/*z*, %): 345 (M^+, 20), 344 (M^+, 100), 343 (8), 329 (13), 315 (3), 301 (4), 210 (4), 209 (31), 194 (15), 182 (6), 177 (2), 167 (2), 153 (3), 151 (9), 136 (2), 135 (6), 108 (3), 107 (6), 106 (3), 91 (2), 77 (2), 65 (3).

*4-(5-(4-(5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazol-2-yl)phenyl)-1,3,4-thiadiazol-2-yl) benzene-1,3-diol (**9**)*. A mixture of terephthalic dihydrazide (Alfa Aesar) (0.01 mol) and STB (0.0075 mol) in methanol (50 mL) was refluxed for 3 h. The hot reaction mixture was filtered, and the filtrate was left at room temperature (24 h). The formed solid was filtered off and crystallized from methanol (25 mL).

^{1}H NMR (400 MHz, DMSO-d_6, δ): 11.23 (s, 1H, OH), 11.04 (s, 1H, OH), 10.14 (s, 1H, OH), 10.00 (s, 1H, OH), 8.17 (m, 2H, C_{Ar}-H), 8.12 (m, 4H, C_{Ar}-H), 6.53 (m, 2H, C_{Ar}-H), 6.47 (m, 2H, C_{Ar}-H). IR (KBr, cm^{-1}): 3349 (OH), 1630 (C=N), 1598, 1510, 1461 (C=C), 1413, 1258, 1165 (C-O), 1112, 1025, 872, 850, 710, 679 (C-S-C); EI-MS (*m*/*z*, %): 462 (M^+, 2), 353 (2), 256 (5), 207 (7), 167 (5), 160 (3), 149 (8), 135 (4), 128 (4), 120 (4), 115 (3), 110 (6), 94 (7), 84 (4), 70 (4), 58 (22), 44 (100), 40 (66).

*4-(5-(Thiophen-2-yl)-1,3,4-thiadiazol-2-yl)benzene-1,3-diol (**10**)*. A mixture of thiophene-2-carboxylic hydrazide (Alfa Aesar) (0.01 mol) and STB (0.0075 mol) in methanol (50 mL) was refluxed for 3 h. The hot reaction mixture was filtered, and the

filtrate was concentrated until to dry. The formed solid was crystallized from methanol (2 × 30 mL).

^{1}H NMR (500 MHz, DMSO-d_6, δ): 11.14 (s, 1H, C_3-OH), 10.08 (s, 1H, C_1-OH), 8.07–8.05 (d, $J = 8.7$ Hz, 1H, C_5-H), 7.79–7.78 (dd, $J = 5.1$ and $J = 1.1$ Hz, 1H, $C_{tph(5)}$-H), 7.76–7.75 (dd, $J = 3.7$ and $J = 1.03$ Hz, 1H, $C_{tph(3)}$-H), 7.24–7.22 (m, 1H, $C_{tph(4)}$-H), 6.53–6.52 (d, $J = 2.3$ Hz, 1H, C_2-H), 6.48–6.46 (dd, $J = 8.7$ and $J = 2.3$ Hz. 1H, C_6-H); IR (KBr, cm^{-1}): 3107 (OH), 1631 (C=N), 1598, 1538, 1453 (C=C), 1416, 1329, 1257, 1234, 1175 (C-O), 1139, 1097, 1060 (N=C–S–C=N), 986, 970, 918, 850, 795, 777, 745, 727, 710, 651 (C–S–C), 624. EI-MS (*m/z*, *%*): 276 (M^+, 100), 247 (3), 168 (4), 167 (43), 153 (12), 143 (3), 142 (3), 141 (31), 138 (3), 135 (12), 127 (21), 124 (3), 119 (6), 114 (3), 112 (3), 111 (3), 110 (4), 109 (8), 108 (3), 107 (6), 106 (3), 97 (7), 96 (7), 95 (3), 83 (5), 82 (3), 80 (5), 71 (5), 70 (4), 69 (13), 65 (3), 63 (3), 58 (4), 53 (3), 52 (8), 51 (6), 45 (10), 39 (13).

*4-(5-((1H-Indol-2-yl)methyl)-1,3,4-thiadiazol-2-yl) benzene-1,3-diol (***11***).* A mixture of indole-3-acetic acid hydrazide (Aldrich) (0.01 mol) and STB (0.075 mol) in methanol (60 mL) was refluxed for 3 h. The hot reaction mixture was filtered. The removed product was crystallized from methanol (2 × 40 mL).

^{1}H NMR (400 MHz, DMSO-d_6, δ): 11.04 (s, 1H, C_3-OH, 10.84 (s, 1H, C_1-OH), 9.97 (s, 1H, NH), 7.92–7.90 (d, $J = 8.5$ Hz, 1H, C_5-H), 7.48–7.46 (m, $J = 2.3$ Hz, 1H, C'_{Ar}-H), 7.38–7.36 (m, 2H, C'_{Ar}-H), 7.11–7.07 (m, 1H, C'_{Ar}-H), 6.99–6.95 (m, 1H, C'_{Ar}-H), 6.41–6.40 (d, $J = 2.1$ Hz, 1H, C_2-H), 6.39–6.36 (dd, $J = 8.6$ and $J = 2.39$ Hz, 1H, C_6-H), 4.52 (s, 1H, CH_2); IR (KBr, cm^{-1}): 3426 (OH), 3111 (C_{AR}-H), 2923 (C-H), 1633 (C=N), 1529, 1460 (C=C), 1337, 1305, 1262, 1229, 1159, 1133, 1091 (N=C–S–C=N), 986, 964, 926, 843, 747, 673 (C-S-C); EI-MS (*m/z*, *%*): 323 (M^+, 100), 322 (8), 294 (3), 188 (7), 162 (2), 161 (14), 160 (3), 156 (3), 155 (9), 153 (2), 130 (26), 129 (3), 103 (3), 77 (3).

*4-(5-((1H-Benzo[d]imidazol-2-ylthio)methyl)-1, 3, 4- thiadiazol-2-yl)benzene-1,3-diol (***12***).* A mixture of 2-(1H-benzo[d]imidazol-2-ylthio) acetohydrazide (Alfa Aesar) (0.01 mol) and STB (0.0075 mol) in methanol (50 mL) was refluxed for 3 h. The hot reaction mixture was filtered, and water (50 mL) was added to the filtrate The formed solid was filtered off and crystallized from methanol (2 × 30 mL).

^{1}H NMR (500 MHz, DMSO-d_6, δ): 10.97 (s, 1H, C_3-OH), 10.02 (s, 1H, C_1-OH), 9.96–7.43 (d, $J = 8.7$ Hz, 1H, C_5-H), 7.52–7.48 (m, 2H, $C'_{4,7}$-H), 7.18–7.15 (m, 2H, $C'_{5,6}$-H), 6.46 (d, $J = 2.3$ Hz, 1H, C_2-H), 6.41–6.39 (dd, $J = 8.7$ and $J = 2.3$ Hz, 1H, C_6-H), 5.02 (s, 2H, CH_2); IR (KBr, cm^{-1}): 3615, 3397, 3147 (OH, NH), 3061 (C_{Ar}-H), 2968 (C-H), 2905 (C-H), 1611 (C=N), 1514, 1476 (C=C), 1420, 1348, 1319, 1272, 1226, 1187 (C-O), 1152, 1124, 1099 (N=C–S–C=N), 1036, 1009, 987, 931, 861, 811, 764, 745, 673 (C–S–C); MS (TS neg. Q1): 355 [M – H]$^-$.

Antifungal Activity Assays

The test in vitro estimating inhibition of mycelium in the agar culture medium caused by the compound under investigation was performed. Five strains of phytopathogenic fungi: *A. alternata, B. cinerea, R. solani, F. culmorum,* and *P. cactorum* were used. The solutions (suspensions) were prepared at the concentration making it possible to obtain 200 and 20 μg mL^{-1} of the studied substance after dilution with the potato dextrose agar. There were used Petri dishes into which the agar culture medium, and the studied substance were poured. When, the culture set, the infectious material of the tested fungus in the form of agar disk overgrown with mycelium was placed in three sites of its surface. After 3–5 days (temperature 22 ± 1) depending on the mycelium culture the linear growth of the mycelium was measured. The compound action was determined from the percentage of mycelium growth inhibition compared with the control using the equation:

$$J = \frac{(C - T)}{C} 100\%$$

where J is percentage of colony growth inhibition, C is a zone of fungus colony growth in the control combination (mm), and T is a zone of the fungus colony growth in the combination with the compound (mm).

As standards, the following fungicides tested under the same experimental conditions were used: carbendazim (Sarfun 500SC, Organica, Chemical Comp. S. A., Nowa Sarzyna, Poland); prochloraz (Mirage 450EC, Makhteshim Agan Industries Ltd., Airport City, Israel); procymidone (Sumilex 500SC, Sumitomo Chemical Comp. Ltd., Osaka, Japan). The results are given in the four-degree scale determining a percentage of mycelium growth inhibition compared with the control [30] (Table 2). Biological studies were made in the Institute of Industry Organic Chemistry in Warsaw with the SPR/BFF/01/b procedures (certificate GLP-OECD -1997).

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