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Synthesis and antifungal activities of 5-(3,4,5-trimethoxyphenyl)-2-sulfonyl-1,3,4-thiadiazole and 5-(3,4,5-trimethoxyphenyl)-2-sulfonyl-1,3,4-oxadiazole derivatives

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Abstract—Starting from the key intermediate 5-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazole-2-thiol (**4**) or the oxadiazole analogue (**5**), the title compounds **9** and **10** are synthesized by a two-step process. Thioetherification reaction of **4** or **5** with an organic halide catalyzed by indium or indium tribromide first affords appropriate sulfide **7** or **8**, which is then converted into title compounds **9** or **10** by hydrogen peroxide oxidation catalyzed by ammonium molybdate in ionic liquid [bmim]PF_6. The structures are unequivocally confirmed by spectroscopic (IR, ^1H and ^{13}C NMR) data and elemental analyses. The structures of **8d** and **10q** are further established by X-ray crystallographic diffraction analysis. The compounds have been shown to be fungicidally active. Title compounds **10i** and **10j** can inhibit mycelia growth by approximately 50% (EC_{50}) at 2.9–93.3 μg/mL in vitro against 10 kinds of fungi.

1. Introduction

Sulfone derivatives containing heterocyclic moiety are known for their interesting antifungal bioactivities and have attracted considerable attention in pesticide and medicinal formulation. A large number of reports on their synthesis and biological activities have appeared during the last three years.[1–8] Among them, thiadiazole and oxadiazole exhibit a wide range of biological especially antifungal activities.[9–11] While *p*,*p*′-bis[[[(2-aryl-sulfonamido)-1,3,4-oxadiazol-5-yl]methyl]amino] diphenyl sulfones are known for their moderate antifungal and antibacterial activities,[11] *p*,*p*′-bis(5-aryl-1,3,4-oxadiazole-2-yl-methylamino)diphenyl sulfones and *p*,*p*′-bis (2-aryl-1,3,4-oxadiazol-5-yl)diphenyl sulfones prepared by Meshkatalsadat et al.[12,13] exhibit medium inhibitory activity against *Candida albicans* and *Pseudomonas fluores*. Vikani reported *p*,*p*′-bis(2-substituted-benzalamino/benzoylamino/sulfon-amido-1,3,4-thiadiazol-5-yl-methyl amino)diphenyl sulfones displaying good antimicrobial bioactivities against Gram-positive bacteria, *B. mega* and *B. saphilis*, Gram-negative bacteria *Escherichia coli* and *P. fluores*, and fungus *Aspergillus niger*.[14] Kleefeld reported $KMnO_4$ oxidation of 5-(4-chlorophenyl)-2-methylthio-1,3,4-oxadiazole for the preparation of 1,3,4-oxadiazole sulfones having antifungal activity that could protect beans against artificial infection with *Botrytis* at the concentration of 100 mg/L.[15] Hu reported a series of bisoxadiazole sulfurether/sulfone derivatives and found part of the sulfone compounds exhibiting medium inhibitory activity against *E coli*.[16] However, the most common substituents that appear in the aryl(heterocycle) ring are nitro, halo, hydroxyl or mono methoxy groups and to the best of our knowledge, there is no report on antifungal activity of sulfones having trimethoxyphenyl group attached to the heterocyclic nucleus.

The incorporation of trimethoxyphenyl moiety in organic compounds, not limited to sulfones, of late has attracted considerable attention due to its naturally derived characterization and wide prevalence in pesticides and medicinal compounds.[17,18] In our previous work, many heterocyclic compounds containing trimethoxyphenyl moiety were reported with good fungicidal,

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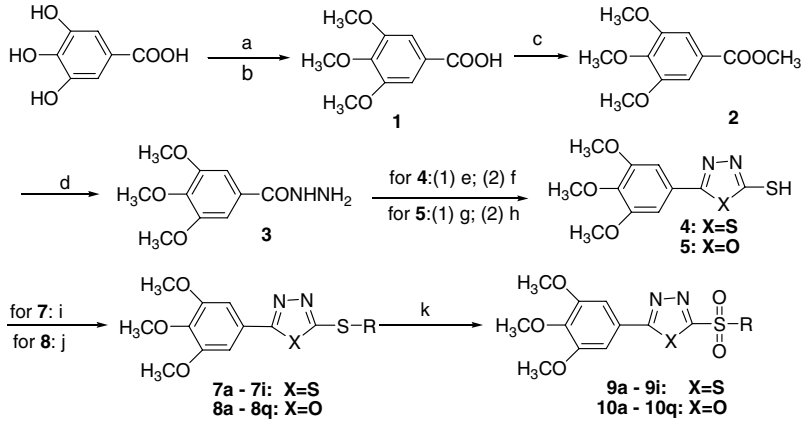
antiviral, and antitumor activities.[19–22] In continuation to extend our research on fungicidal compounds, we designed a series of new sulfones with 3,4,5-trimethoxyphenyl functionality present in the heterocyclic ring. Herein, we wish to report the synthesis and fungicidal activities of some novel sulfone derivatives containing trimethoxyphenyl substituted 1,3,4-thiadiazole and 1,3,4-oxadiazole moieties.

2. Chemistry

The synthetic route designed for the sulfone analogues **9** and **10** is summarized in Scheme 1. Following the reported method,[23] 5-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazole-2-thiol **4** was synthesized from gallic acid in five steps: etherification, esterification, hydrazidation, salt formation, and cyclization. 5-(3,4,5-Trimethoxy phenyl)-1,3,4-oxadiazole-2-thiol **5** was easily prepared by the reaction of 5-trimethoxy phenylhydride **3**, potassium hydroxide, and carbon disulfide in absolute ethanol under reflux condition. Then, 5-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazole-2-thiol **4** and the oxadiazole analogue **5** were converted to thioether derivatives containing thiadiazole **7** or oxadiazole moiety **8** by a thioetherification reaction with halide (RX) catalyzed by indium or indium tribromide. Treatment of sulfides **7** and **8** by H_2O_2 catalyzed by ammonium molybdate in ionic liquid afforded the heterocyclic sulfones **9** and **10**, with good yields.

In order to optimize the reaction conditions for the preparation of **9** and **10**, the synthesis of **10f** was carried out under several conditions. The effects of different catalysts, solvents, reaction times, reaction temperatures, and oxidant amounts on the reaction were investigated, and the results are summarized in Table 1. First, effect of two different catalysts was investigated in the presence of ionic liquid [bmim]PF_6. As it could be seen from Table 1, the reaction catalyzed by 1% equiv of ammonium molybdate yielded 94% **10f** in 2 h, while for $MnSO_4$, the yield of **10f** was only 37% for the same period (Table 1, entries 1 and 2). This proved superior catalytic activity of $(NH_4)_6Mo_7O_{24}$. When the catalyst amount was decreased to 0.5% and 0%, the reaction yield of **10f** declined to 90% and 71%, respectively (Table 1, entries 3 and 4). Hence 1% equiv $(NH_4)_6Mo_7O_{24}$ catalyst was chosen as ideal catalyst concentration. Effect of different solvents such as acetone, CH_3CN, DMF, ethanol, and acetic acid was also compared with



Scheme 1. Reagents and conditions: synthetic route to title compounds **9** and **10**. (a) $(CH_3)_2SO_4$, 10% NaOH; (b) 35% HCl; (c) CH_3OH, 98% H_2SO_4, reflux; (d) $NH_2NH_2{\cdot}H_2O$, CH_3OH, reflux for 5 h; (e) KOH, CS_2, C_2H_5OH , rt; (f) 98% H_2SO_4 , 0–5 °C; (g) KOH, CS_2, C_2H_5OH, reflux for 6 h ; (h) 5% HCl, ice-bath; (i) In, 3% NaOH, H_2O, RX(**6**), rt; (j) $InBr_3$, 3% NaOH, H_2O, RX(**6**), rt; (k) 30% H_2O_2, $(NH_4)_6Mo_7O_{24}$ (1 mol%), ionic liquid, 40 °C, 2 h.

Table 1. Synthesis of **10f** in ionic liquids under different reaction conditions

Entry	$(NH_4)_6Mo_7O_{24}$ (mol%)	H_2O_2 (equiv)	Solvent[a]	Time/h	Temperature/°C	**10f**[b]
1	1	5	[bmim]PF_6	2	40	94
2	1[c]	5	[bmim]PF_6	2	40	37
3	0.5	5	[bmim]PF_6	2	40	90
4	0	5	[bmim]PF_6	2	40	71
5	1	5	Acetone	2	40	44
6	1	5	CH_3CN	2	40	52
7	1	5	DMF	2	40	54
8	1	5	Ethanol	2	40	68
9	1	5	Acetic acid	2	40	64
10	1	5	[bmim]PF_4	2	40	87
11	1	5	[bmim]PF_6	1	40	84
12	1	5	[bmim]PF_6	2	20	79
13	1	5	[bmim]PF_6	2	50	90
14	1	3	[bmim]PF_6	10	40	76

[a] The amount of solvent was 12.8 equiv (with respect to the substrate **8f**) for the ionic liquid or 10 mL for the other solvents.
[b] Isolated yields after work-up.
[c] $MnSO_4{\cdot}H_2O$ (1 mol%) was used as catalyst.

the ionic solvent [bmim]PF_6 for the synthesis of **10f**. It was found that while oxidation could be easily achieved in [bmim]PF_6 in presence of catalyst $(NH_4)_6Mo_7O_{24}$ (Table 1, entry 1), the reaction afforded poor yield with other solvents (Table 1, entries 5–9). The other ionic liquid [bmim][PF_4] did not afford the same good result and the yield of **10f** decreased from 94% to 87% (Table 1, entries 1 and 10). Further, we also examined the effect of reaction time on the oxidation reaction of sulfide to sulfone. When the reaction time was prolonged from 1 h to 2 h, the yield of **10f** increased from 84% to 94% (Table 1, entries 1 and 11). As for the reaction temperature, it could be seen that the yield was relatively lower when the reaction was carried out at room temperature (Table 1, entry 12) than that at 40 °C (Table 1, entry 1). No substantial change was observed when the reaction system was heated to 50 °C (Table 1, entry 13). Hence, the temperature 40 °C was selected as the optimal one for our reaction. When lower amount of oxidant (3 equiv) was employed, only 76% yield of sulfone **10f** was obtained even with extended reaction time up to 10 h (Table 1, entry 14). Using the optimized condition, the best result was obtained when **8f** was reacted with 5 equiv H_2O_2 and 0.01 equiv $(NH_4)_6Mo_7O_{24}$ in 12.8 equiv of ionic solvent [bmim][PF_6] at 40 °C for 2 h.

Various substituted sulfones were obtained through such optimized ammonium molybdate catalyzed oxidation in ionic liquid. A wide range of aromatic, aliphatic. and heterocyclic sulfides with 1,3,4-oxadiazole or 1,3,4-thiadiazole moiety were employed in this procedure to synthesize the corresponding sulfones in good yields.

2.1. Crystal structure analysis

In compound **8d**, the bond lengths N(1)–C(11) (1.286(3) Å) and N(2)–C(10) (1.371(3) Å) are close to the C=N double bond distance (1.34 Å).[24] The bond length C(12)–S(1) [1.826(6) Å] is close to that of similar compound reported.[24] The bond length C(11)–S(1) [1.733(2)] is shorter than typical C–S (1.82 Å).[24] The S(1) atom and oxadiazole ring form a configuration in which the S(1) atom is sp^2 hybridized. The benzene ring [C(13), C(14), C(15), C(16), C(17), C(18)] and the adjacent carbon atom C(12) are fairly planar, and the deviation from the least-squares plane through the ring atoms is less than 0.0009(3) nm. The dihedral angle between the plane of oxadiazole group and the plane of the benzene is 82.76°. Also the trimethoxybenzene ring, the oxadiazole ring [C(1), C(2), C(3), C(4), C(5), C(6), C(10), C(11), N(1), N(2), O(4)], and the adjacent sulfur atom S(1) linked to the later are all fairly planar. The deviation from the least-squares plane through the ring atoms is less than 0.0457 nm. The dihedral angle between the plane of oxadiazole group and the plane of the trimethoxybenzene is 82.76°. As shown in the packing diagram of the title compound (Fig. 2), there exist no hydrogen bonds.

As for compound **10q**, the main structural characteristics are similar as that of compound **8d**. The bond lengths C(12)–S(1) and C(11)–S(1) are 1.798(5) Å and 1.786(3) Å, respectively, which are similar to that of the compound **8d**. The S(1) atom and oxadiazole ring form a configuration in which the S(1) atom is sp^2 hybridized. The benzene ring [C(13), C(14), C(15), C(16), C(17), C(18)] and the adjacent carbon atom C(12) are also fairly planar. The trimethoxybenzene ring and the oxadiazole ring [C(1), C(2), C(3), C(4), C(5), C(6), C(10), C(11), N(1), N(2), O(4)] as well as the sulfur atom S(1) linked to the later lie in a plane. As shown in the packing diagram of **10q** Figure 3(Fig. 4), there exist two intermolecular hydrogen bonds. One is C(12)–H(12B)...O(7), with the donor and acceptor distance 2.731 Å, C(12)–H(12B) = 0.97 Å, and H(2)...O(1) = 2.38 Å, 2.731(5) C(12)–H

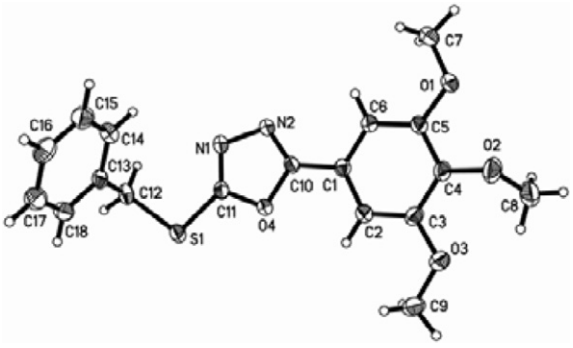


Figure 1. The molecular structure of compound **8d**.

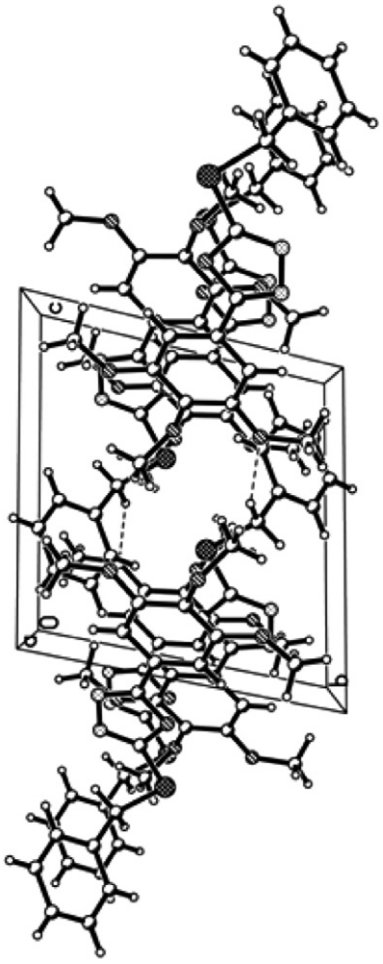


Figure 2. Packing diagram of the unit cell of compound **8d**.

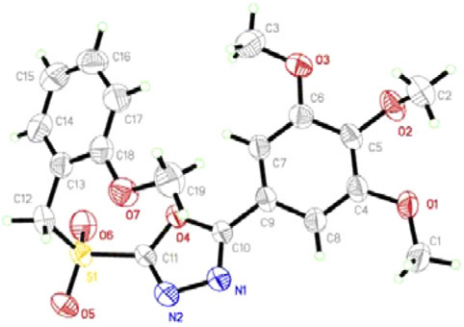


Figure 3. The molecular structure of compound **10q**.

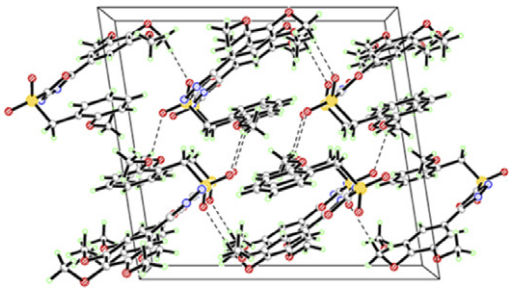


Figure 4. Packing diagram of the unit cell of compound **10q**.

(12B)...O(7) = 100.7°, the other being C(2)–H(2C)...O(6), with the donor–acceptor distance 3.462 Å, and C(2)–H(2C) = 0.96 Å, H(2C)...O(6) = 2.51 Å, C(2)–H(2C)...O(6) = 172.7°.

3. Antifungal activity

Inhibition effect of sulfone derivatives on phytopathogenic fungi was studied. The three fungi used in the fungicidal bioassay, *Gibberella zeae*, *Botrytis cinerea*, and *Sclerotinia sclerotiorum*, were collected and isolated from corresponding crops. The results of preliminary bioassays were compared with that of a commercial agricultural fungicide Hymexazol. As indicated in Table 2, oxadiazole sulfone compounds **10a**–**10e**, **10g**, **10i**, **10j**, and **10l** showed potent antifungal activities against all the tested fungi. However, significant lowering of activity was noticed with homologues of thiadiazole moiety (Table 2). It could be seen that there were two new title compounds, **10i** and **10j**, exhibiting promising antifungal activities even better than that of the commercial fungicide Hymexazol. At the concentration of 50 μg/mL, title compounds **10i** and **10j** inhibited growth of *G. zeae* at 55.8%, 100%, *B. cinerea* at 67.5%, 100%, and *S. sclerotiorum* at 100%, 100%, respectively, which are slightly better than that of Hymexazol (52.9% against *G. zeae*, and 77.5% against *S. sclerotiorum* at 50 μg/mL). Among the thiadiazole sulfone compounds, it could be seen that **9h** has higher antifungal activities against three phytopathogenic fungi than the other compounds **9a**–**9g** and **9i**. The inhibition effect of compounds **10i** and **10j** on mycelia growth in vitro at different concentrations (100, 50, 25, 12.5, and 6.25 μg/mL) is exhibited in Figure 5. Nearly complete inhibition of *G. zeae* mycelia growth was approached by **10j** at 100 μg/mL concentration, when compared with the complete growth in the control (Fig. 5).

Table 2. Fungicidal activities of sulfone derivatives **9** and **10**[a]

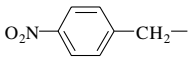
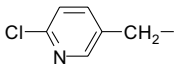
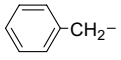
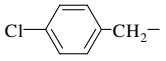
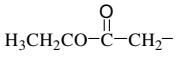
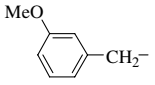
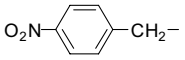
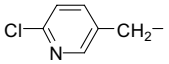
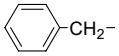
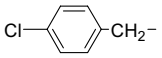
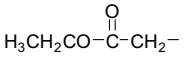
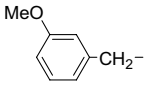
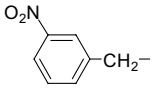
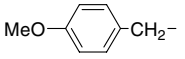
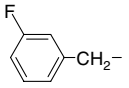
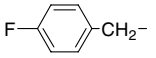
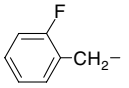
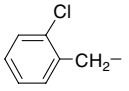
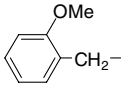
Compound	R	Concentration (μg/mL)	Inhibitory rate (%)		
			Gibberella zeae	*Botrytis cinerea*	*Sclerotinia sclerotiorum*
9a	$H_2C{=}CH{-}CH_2{-}$	50	14.0 ± 3.1	47.3* ± 9.0	40.5* ± 7.8
9b		50	−1.8	30.3* ± 7.1	20.0 ± 3.8
9c		50	−1.1	25.9 ± 4.2	21.3 ± 7.2
9d		50	8.3 ± 0.9	27.5 ± 7.1	22.9 ± 3.9
9e	$CH_3CH_2CH_2{-}$	50	13.5 ± 1.9	33.5* ± 4.3	38.1* ± 3.8
9f		50	5.5 ± 1.2	20.8 ± 2.1	20.3 ± 3.7
9g		50	21.1 ± 7.1	49.7* ± 10.0	49.4* ± 6.2
9h		50	43.2* ± 6.6	53.4* ± 7.0	74.5* ± 3.1
9i	$H_3C{-}$	50	11.6 ± 0.9	27.1* ± 3.1	40.7* ± 7.2

Table 2 (*continued*)

Compound	R	Concentration (μg/mL)	Inhibitory rate (%)		
			Gibberella zeae	*Botrytis cinerea*	*Sclerotinia sclerotiorum*
10a	$H_2C{=}CH{-}CH_2{-}$	50	26.4 ± 8.9	40.9* ± 2.2	40.9* ± 7.6
10b		50	42.1* ± 10.1	76.8* ± 5.0	62.2* ± 4.4
10c		50	38.9* ± 7.7	60.3* ± 8.0	67.9* ± 9.9
10d		50	54.9* ± 6.0	67.3* ± 5.5	71.2* ± 4.3
10e	$CH_3CH_2CH_2{-}$	50	59.0* ± 12.1	59.4* ± 8.8	64.0* ± 7.6
10f		50	23.3* ± 8.9	30.8* ± 11.2	35.5* ± 8.9
10g		50	52.0* ± 4.9	81.9** ± 3.2	79.9** ± 1.9
10h		50	5.7 ± 1.9	38.1* ± 7.1	40.6* ± 10.1
10i	$H_3C{-}$	50	55.8* ± 3.0	100** ± 3.1	100** ± 2.0
10j	$CH_3CH_2{-}$	50	67.5* ± 1.2	100** ± 0.9	100** ± 3.1
10k		50	11.8 ± 3.9	40.7* ± 4.1	38.3* ± 9.9
10l		50	33.9* ± 3.8	58.5* ± 5.5	58.5* ± 4.2
10m		50	9.3 ± 0.3	35.8* ± 1.9	38.3* ± 4.7
10n		50	28.1* ± 3.7	41.6* ± 2.2	62.7** ± 1.9
10o		50	18.2* ± 1.1	52.3* ± 2.2	33.4* ± 4.0
10p		50	11.9 ± 0.9	26.1* ± 2.2	39.6* ± 1.2
10q		50	23.7* ± 3.0	42.3* ± 4.9	37.2* ± 5.1
Hyme-xazol		50	52.9* ± 3.0	75.4** ± 4.1	77.5** ± 2.8

$n = 3$ for all groups.
[a] Growth inhibition expressed as a percentage of the control.
* $P < 0.05$.
** $P < 0.01$.

Further bioassays disclosed that compounds **10i** and **10j** had remarkable inhibitory effect on 10 kinds of plant pathogenic fungi under the laboratory condition (Table 3). The EC_{50} of **10j** on *G. zeae*, *Fusarium oxysporum*, *Cytospora mandshurica*, *Colletotrichum gloeosporioides*, *B. cinerea*, *S. sclerotiorum*, *Pyricularia grisea*, *Phytophthora infestans*, *Rhizoctonia solani*, and *Pyricularia oryzae* were 20.1 μg/mL, 18.0 μg/mL, 20.5 μg/mL, 26.3 μg/mL, 2.9 μg/mL, 4.3 μg/mL, 17.8 μg/mL, 51.4 μg/mL, 45.6 μg/mL, and 39.8 μg/mL, respectively. Compound **10i** was also

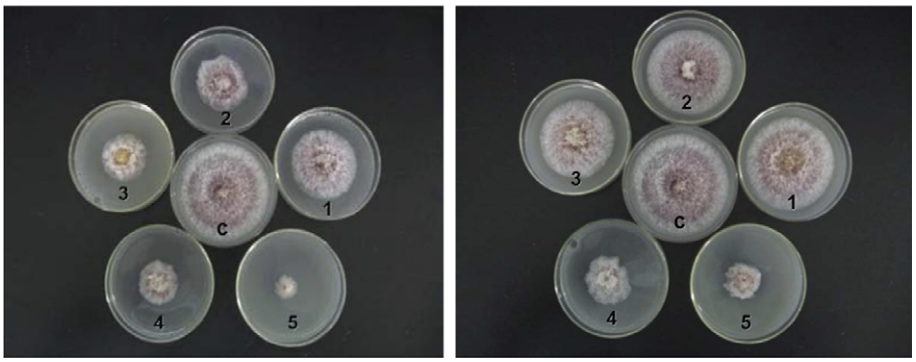


Figure 5. Effect of different concentrations of **10j** (Left) and **10i** (Right) on *the mycelial growth of Gibberella zeae* (6.25, 12.5, 25.0, 50.0, and 100 μg/mL). Key (μg/mL): 1, 6.25; 2, 12.5; 3, 25; 4, 50; 5,100; and C, control.

Table 3. Toxicity of **10i** and **10j** on 10 kinds of pathogenic fungi

Compound	Fungus	Toxic regression equation	EC_{50}[a] (μg/mL)	*r*
10j	*G. zeae*	$Y = 0.671x + 4.054$	20.1 ± 2.9	0.992
	P. grisea	$Y = 1.690x + 2.887$	17.8 ± 5.1	0.986
	C. gloeosporioides	$Y = 1.306x + 3.142$	26.3 ± 3.8	0.973
	B. cinerea	$Y = 1.710x + 4.218$	2.9 ± 2.0	0.951
	S. sclerotiorum	$Y = 0.867x + 4.452$	4.3 ± 3.1	0.985
	R. solani	$Y = 0.762x + 3.736$	45.6 ± 11.9	0.987
	P. oryzae	$Y = 0.953x + 3.465$	39.8 ± 9.7	0.959
	P. infestans	$Y = 1.640x + 2.193$	51.4 ± 13.7	0.986
	C. mandshurica	$Y = 1.107x + 2.364$	20.5 ± 1.1	0.970
	F. oxysporum	$Y = 0.997x + 2.466$	18.0 ± 3.1	0.989
10i	*G. zeae*	$Y = 0.809x + 3.816$	28.8 ± 10.1	0.991
	P. grisea	$Y = 2.530x + 1.233$	30.8 ± 15.0	0.932
	C. gloeosporioides	$Y = 2.505x + 1.644$	21.8 ± 5.9	0.839
	B. cinerea	$Y = 0.974x + 3.958$	11.7 ± 7.9	0.946
	S. sclerotiorum	$Y = 1.041x + 3.858$	12.5 ± 8.9	0.980
	R. solani	$Y = 0.667x + 3.686$	93.3 ± 4.4	0.966
	P. oryzae	$Y = 2.278x + 0.540$	90.7 ± 3.1	0.988
	P. infestans	$Y = 1.301x + 2.496$	83.2 ± 6.5	0.991
	C. mandshurica	$Y = 1.199x + 2.715$	80.5 ± 10.8	0.978
	F. oxysporum	$Y = 1.395x + 1.661$	24.5 ± 2.3	0.998
Hymexazol[b]	*R. solani*	$Y = 2.729x + 1.330$	52.1 ± 8.5[c]	0.995
Hymexazol[b]	*F. oxysporum*	$Y = 1.049x + 3.464$	29.1 ± 2.6	0.994

[a] EC_{50} concentrations needed to inhibit cell growth by 50% as determined from the dose–response curve. Determined in three separate experiments and each was performed in triplicate.
[b] The standard compound used for comparison of activity.
[c] The value was determined by using our assay protocol.

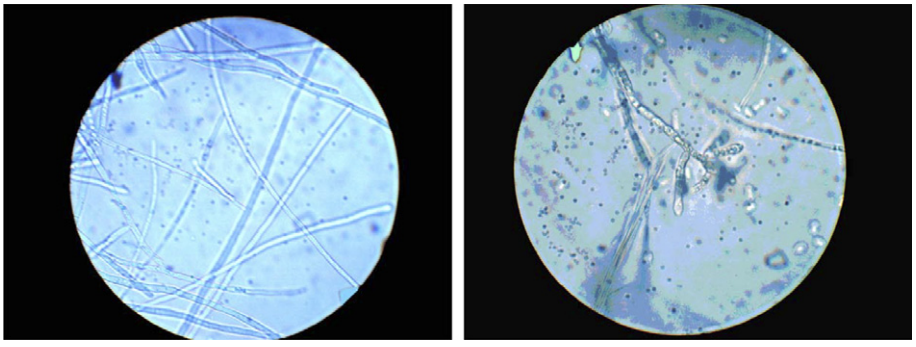


Figure 6. Microphotograph of hyphal morphology of *Gibberella zeae* under the microscope (800×) (left: control; right: treated with 100 μg/mL of **10j**).

highly effective against the ten kinds of fungi listed above and its EC_{50} values were 28.8 μg/mL, 24.5 μg/mL, 80.5 μg/mL, 21.8 μg/mL, 11.7 μg/mL, 12.5 μg/mL, 30.8 μg/mL, 83.2 μg/mL, 93.3 μg/mL, and 90.7 μg/mL, respectively. Compound **10j** is more active against *F. oxysporum* and *R. solani* than Hymexazol and its activity on *P. grisea* is similar to another commercial fungicide Myclobutanil.

The morphology changes of hypha were also studied. After three days of inocualtion, the colony diameter of *G. zeae* was 7.0 cm and its color was amaranth in the middle and white on the periphery. When treated with 100 μg/mL of compound **10j**, the hypha grew slowly with ramification, the amaranth color of mycelium in the middle became weaker.

In the beginning, the hypha of the control was slippy, vimineous and branched normally. The cytoplasm of the cell was homogeneous and limpid. But after treating with 100 μg/mL of compound **10j**, the hypha of *G. zeae* became coarse, malformed and branched at the tip. The cell of the hypha swelled and shortened. Its cytoplasm condensed and a few blanks were observed near the tip of the hypha (Fig. 6).

4. Conclusion

A series of novel sulfone derivatives containing 1,3,4-thiadiazole and 1,3,4-oxadiazole moieties were synthesized by the treatment of intermediate sulfides **7** and **8** with H_2O_2 in the presence of catalytic amount of ammonium molybdate in an ionic liquid. The method is easy, rapid and produces the title compounds **9** and **10** in good yield. The structures were verified by spectroscopic data. In the antifungal bioassay, the title compounds **9h**, **10a**–**10e**, **10g**, **10i**, **10j**, and **10l** were found to possess higher antifungal activities against three kinds of fungi in vitro. In particular, **10i** and **10j** had high inhibitory effects on the growth of *G. zeae*, *P. grisea*, *C. mandshurica*, *C. gloeosporioides*, *B. cinerea*, *S. sclerotiorum*, *P. oryzae*, *P. infestans*, *R. solani*, and *F. oxysporum*, with EC_{50} values ranging from 2.9 μg/mL to 93.3 μg/mL.

5. Experimental

5.1. Analysis and instruments

The title compounds were synthesized starting from gallic acid following a six-reaction step sequence. The melting points of the products were determined on a XT-4 binocular microscope (Beijing Tech Instrument Co., China) and were not corrected. The IR spectra were recorded on a Bruker VECTOR 22 spectrometer in KBr disk. ^1H and ^{13}C NMR (solvent $CDCl_3$) spectra were recorded on a JEOL-ECX 500 NMR spectrometer at room temperature using TMS as an internal standard. Elemental analysis was performed on an Elementar Vario-III CHN analyzer. Analytical TLC was performed on silica gel GF254. Column chromatographic purification was carried out using silica gel. All reagents were of analytical grade or chemically pure. All solvents were dried, deoxygenated, and redistilled before use. 3,4,5-Trimethoxyphenylhydrides **3** and 5-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazole-2-thiol **4** were prepared according to literature method as described.[23]

5.2. Preparation of 5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole-2-thiol (5)

A mixture of 0.56 g (10 mmol) of potassium hydroxide, 2.26 g (10 mmol) of compound **3**, and 1.14 g (15 mmol) of carbon disulfide in 50 mL of absolute ethanol was refluxed for 8 h. After the solvent was evaporated in vacuum, the residue was dissolved in ice-cold water and acidified with dilute hydrochloric acid. The precipitate was filtered off, washed with water, dried, and recrystallized from absolute ethanol to give compound **5**. The structure was confirmed by ^1H NMR, ^{13}C NMR, IR, and elemental analysis (see the Supporting information).

5.3. Preparation of 2-substituted methylthio-5-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazole (7a–7i)

To a 50 mL, three-necked, round-bottomed flask equipped with a magnetic stirrer were added 1.5 mmol of **4**, 20 mL of distilled water, and 2 mL (3%, w/w) of NaOH solution. The mixture was stirred at room temperature for 10 min. Then 1.5 mmol of halide **6** and 17.2 mg (0.15 mmol) of indium were added. The resulting mixture was stirred at room temperature for 4 h, filtered. The white solid resulted was washed with 5% Na_2CO_3 solution and distilled water, dried under vacuum, and recrystallized from ethanol to give compound **7**[23] (Characterization data are provided in the Supporting information).

5.4. Preparation of 2-substituted methylthio-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole (8a–8q)

A 50 mL round-bottomed flask equipped with a magnetic stirrer was charged with **5** (1.5 mmol) and 2 mL (3%, w/w) of sodium hydroxide solution. The mixture was dissolved in 20 mL of distilled water. The flask was stirred at room temperature for 10 min, and then halide **6** (1.5 mmol) and indium tribromide (0.15 mmol) were added to the reaction mixture and stirred at room temperature for 4 h. The mixture was filtered and the white solid obtained was washed with 5% Na_2CO_3 solution and distilled water, dried under vacuum, and recrystallized from ethanol to give compound **8** (see the Supporting information).

5.5. Preparation of 2-substituted sulfonyl-5-(3,4,5-trimethoxyphenyl)-1,3,4-thiadiazole (oxadiazole) (9a–9i, 10a–10q)

To a mixture of 1.1 mmol of compound **7** or **8**, 4000 mg (14 mmol) of [bmim]PF_6, and 140 mg (0.011 mmol) of $(NH_4)_6Mo_7O_{24}$ was added 560 mg (5.5 mmol) of 30% H_2O_2. The mixture was stirred at 40 °C for 2 h and then extracted with toluene (5 × 5 mL). The combined toluene phase was concentrated in vacuum. The crude prod-

uct was recrystallized (from anhydrous ethanol) to afford title compounds **9** and **10** (see the Supporting information).

5.5.1. 2-Methylsulfonyl-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole (10i). White needle, yield, 89%; mp 120 ~ 122 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.50 (s, 3 H, CH_3), 3.95 (s, 3H, MeO), 3.96 (s, 6H, 2 × MeO), 7.34 (d, 2H, ArH, J = 2.8 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 42.9, 56.4, 61.0, 104.8, 116.7, 142.3, 153.7, 161.8, 166.6; IR (KBr): 860, 1128, 1144, 1184, 1238, 1344, 1493, 1545, 1593, 2962, 3065 cm^{-1}; Anal. calcd for $C_{12}H_{14}N_2O_6S$ (314.3): C, 45.86; H, 4.49; N, 8.91. Found: C, 45.79; H, 4.30; N, 8.88.

5.5.2. 2-(Ethylsulfonyl)-5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazole (10j). White needle, yield, 97%; mp 109–111 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.56 (t, 3H, –CCH_3,J = 7.6 Hz), 3.64 (q, 2H, –CH_2C, J = 7.6 Hz), 3.95 (s, 3H, MeO), 3.96 (s, 6H, 2 × MeO), 7.35 (s, 2H, ArH). ^{13}C NMR (125 MHz, $CDCl_3$): δ 16.8, 50.0, 56.5, 61.1, 104.9, 116.9, 142.3, 153.8, 161.0, 166.6; IR (KBr): 841, 1130, 1145, 1173, 1240, 1350, 1495, 1551, 1595, 2922, 2972, 3080 cm^{-1}; Anal. calcd for $C_{13}H_{16}N_2O_6S$ (328.3): C, 47.55; H, 4.91; N, 8.53. Found: C, 47.75; H, 4.92; N, 8.52.

5.6. Crystal structure determination

A sample of size 0.30 × 0.24 × 0.20 mm^3 was selected for the crystallographic study. All diffraction measurements were performed at room temperature (293 K) using graphite monochromated MoKα radiation (λ = 0.71073 Å) and an Enraf–Nonius CAD-4 four-circle diffractometer. Accurate cell parameters and orientation matrix were obtained by least-squares refinement of the setting angles of 3049 reflections at the θ range of 2.09° < θ < 25.01° (for compound **8d**), and 3598 reflections at the θ range of 1.41° < θ < 26.00° (for compound **10q**). The systematic absences and intensity symmetries indicated the triclinic $P-1$ space group (for compound **8d**) and the monoclinic P21/c (for compound **10q**). A total of 4440 intensities with θ_{max} = 25° were collected in the $\omega/2\theta$ scan mode, as suggested by peak-shape analysis. The crystal and equipment stabilities were checked by the intensities of three standard reflections monitored every 2 h. No significant intensity decay was observed (2.0% variation). The intensities were corrected for Lorentz and polarization factors, but not for absorption effect (μ = 0.211 mm^{-1}). The structure was solved by direct methods using SHELXS-97.[25] The refinement (on F^2) was carried out by full-matrix least-squares method on the positional and anisotropic temperature parameters of the non-hydrogen atoms. The structure was refined to R = 0.0592 for the observed reflections and wR_2 = 0.1268 for all data (for compound **8d**). For compound **10q**, the structure was refined to R = 0.1785 for the observed reflections and wR_2 = 0.2011 for all data. The scattering factors were taken from SHELXL-97.[26] The CIF file has been deposited at the Cambridge Crystallographic Data Center as CCDC Nos. 282029 (for compound **8d**) and 619360 (for compound **10q**).

The molecular structures of the compounds **8d** and **10q** are shown in Figures 1 and 3, respectively. The packing diagrams of the unit cells of compounds **8d** and **10q** are shown in Figures 2 and 4, respectively.

5.7. Antifungal activity bioassay

Fungicidal activities of all the title compounds were bioassayed against three kinds of pathogenic fungi namely *G. zeae*, *B. cinerea*, *S. sclerotiorum*, using the mycelia growth rate test.[27] The compounds **10i** and **10j** were subjected to further evaluation against ten pathogenic fungi, namely, *G. zeae*, *P. grisea*, *C. mandshurica*, *C. gloeosporioides*, *B. cinerea*, *S. sclerotiorum*, *P. oryzae*, *P. infestans*, *R. solani*, and *F. oxysporum*. Experimental details[28] are provided in the Supporting information.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.04.014.

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