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# Synthesis, Fungicidal Activity, and Structure−Activity Relationship of 2-Oxo- and 2-Hydroxycycloalkylsulfonamides

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To explore new potential fungicides, a series of novel compounds, including 11 2-oxocycloalkylsulfonamide (**3**) and 21 2-hydroxycycloalkylsulfonamide (**4**) derivatives, were synthesized and their structures were confirmed by $^1$H nuclear magnetic resonance (NMR), infrared (IR), and elemental analysis. The results of the bioassay showed that the compounds **3** and **4** possessed excellent fungicidal activity against *Botrytis cinerea* Pers. both *in vitro* and *in vivo*. The fungicidal activity of the compounds with 7- or 8-membered rings is better than those with 5-, 6-, or 12-membered rings. According to the results of the mycelium growth rate test, the $EC_{50}$ values of the compounds **3C**, **4C**, **3D**, and **4D** were 0.80, 0.85, 1.22, and 1.09 $\mu$g/mL, respectively, and similar to or better than commercial fungicide procymidone. The bioassay results of spore germination indicated that most of the compounds exhibited obvious inhibitory effects against *B. cinerea* and the inhibition rates of 2-oxocycloalkylsulfonamides were higher than 2-hydroxycycloalkylsulfonamides, among them. The $EC_{50}$ values of compounds **3A**, **3B$_{17}$**, **3E**, and **4A** were 4.21, 4.21 3.24, and 5.29 $\mu$g/mL, respectively. Those compounds containing 5- or 6-membered rings showed better activity than those containing 7-, 8-, or 12-membered rings. Furthermore, the results of the pot culture test showed that almost all of the compounds had effective control activity *in vivo* and 2-hydroxycycloalkylsulfonamides were obviously superior to 2-oxocycloalkylsulfonamides. The compounds **3E**, **4C** and **4D** presented higher control efficacy than procymidone and pyrimethanil against gray mold disease on cucumber plants.

**KEYWORDS: 2-Hydroxycycloalkylsulfonamides; synthesis; *Botrytis cinerea*; fungicidal activity; structure−activity relationship**

## INTRODUCTION

Sulfanilamide, the first synthetic antibacterial agent active against a wide range of infections, is also used as an agricultural fungicide, and the representative products include flusulfamide (*1*) (**A**, **Figure 1**), tolnifanide (*2*) (**B**, **Figure 1**), cyazofamid (*3*, *4*) (**C**, **Figure 1**), and amisulbrom (*5*) (**D**, **Figure 1**). They all have an excellent control effect toward the diseases caused by oomycetes. Beucheta and co-workers found that the compounds of sulfonamide, which encompass the structure of thiazolidine (**E**, **Figure 1**), had good fungicidal activity (*6*). The fungicidal activity of 1,2,4-triazole sulfonamide (**F**, **Figure 1**) and coumarin sulfonamide (**G**, **Figure 1**) had been reported in the literature (*7*, *8*). Besides, the structure−activity relationship between *N*-aryl benzene sulfonamide (**H**, **Figure 1**) and *Botrytis cinerea* Pers. was studied by Saíz-Urra et al. (*9*). Accordingly, we can conclude that they all containing the structure of arylsulfonamide.

Furthermore, the compounds 2-oxocyclododecylsulfonamides (**I**, **Figure 2**) had been proven to have excellent fungicidal activity against *Gibberella zeae* and *Venturia nashicola* (*10*). Further research showed that 2-oxocycloalkylsulfonamides (**J**, **Figure 2**) have excellent activity against *B. cinerea* Pers. (*11*). In our present work, the 2-position carbonyl was converted to the corresponding hydroxyl (**K**, **Figure 2**) and the size of the rings changed simultaneously. Many new derivatives were designed and synthesized for the purpose of novel and effective pesticide discovery. The fungicidal activities of those new compounds against *B. cinerea* were evaluated using the mycelium growth rate tests, spore germination tests, and pot culture tests. The relationships were obtained between the size of the ring and the difference of the substituent on benzene to the fungicidal activity, by comparing of the influence of the 2-position carbonyl and the 2-position hydroxyl on the fungicidal effect.

The synthetic route of compounds **4** is shown in **Scheme 1**.

## MATERIALS AND METHODS

**General.** Infrared (IR) spectra were recorded in potassium bromide disks on a Bruker IFS 55 spectrophotometer. Nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$ unless indicated otherwise with a Bruker 300 MHz spectrometer, using tetramethylsilane (TMS) as the internal standard. Elemental analysis was performed by the analytical center at the Institute of Chemistry, Chinese Academy of Sciences, Beijing, China. Melting points were measured on a X-5 melting-point apparatus,

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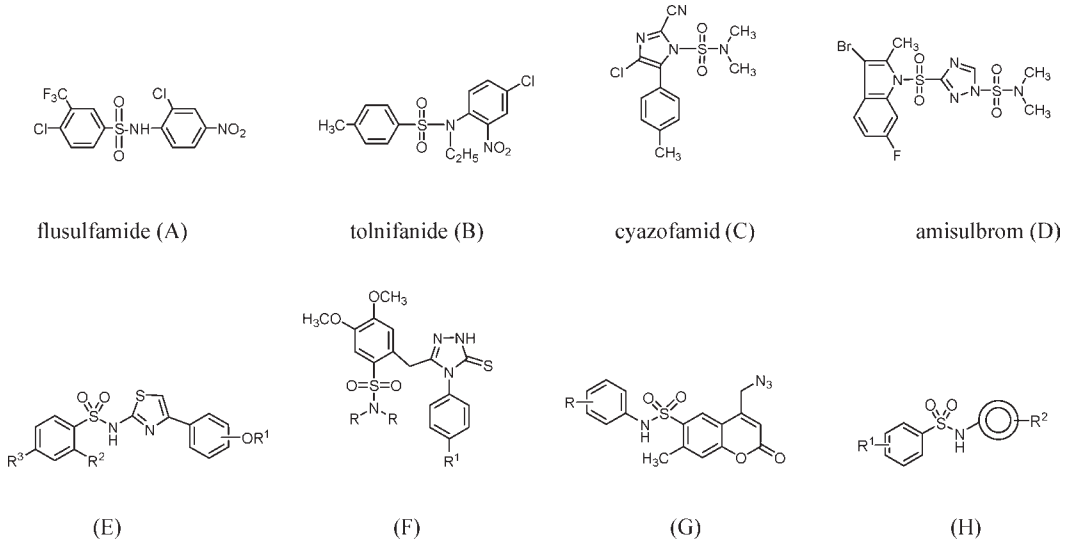
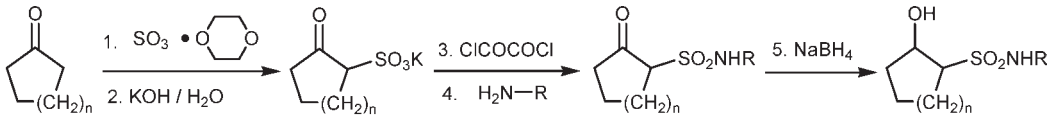



**Figure 1.** Structures of compounds **A**−**H**.

**Scheme 1.** Synthetic Route of Compounds **4**




A, n=1; B, n=2; C, n=3; D, n=4; E, n=8

and the thermometer was uncorrected. The solvents and reagents were used as received or were dried prior to use as needed.

**Chemical Synthesis.** Synthesis of compounds followed the outline in **Scheme 1**.

*Synthesis of Compounds 2*. Compounds **2** were synthesized from cyclanones according to the method given in ref *12*. Potassium 2-oxocyclopentylsulfonate (**2A**) and potassium 2-oxocyclooctylsulfonate (**2D**) are new compounds. Their physical data were shown as follows. **2A**: white solid, with a yield of 73%. mp 214−216 °C. $^1$H NMR ($D_2O$) $\delta$: 1.76−1.85 (m, 1H), 1.99−2.08 (m, 1H), 2.16−2.42 (m, 4H), 3.65 (t, 1H, $J$ = 8.41 Hz). IR ($\nu$, cm$^{-1}$): 3450, 2920, 2850, 1700. **2D**: white solid, with a yield of 82%. mp 235−238 °C. $^1$H NMR ($D_2O$) $\delta$: 1.01−1.10 (m, 1H), 1.30−1.44 (m, 3H), 1.69−1.84 (m, 4H), 2.10−2.18 (m, 2H), 2.33−2.40 (m, 1H), 2.64−2.74 (m, 1H), 3.86 (dd, 1H, $J$ = 8.72, 6.41 Hz). IR ($\nu$, cm$^{-1}$): 3450, 2920, 2850, 1700.

*Synthesis of Compounds 3*. Compounds **3** were synthesized from compounds **2** as previously described (*11*). Foregone compounds, *N*-(4-methylphenyl)-2-oxocyclohexylsulfonamide (**3B$_2$**), *N*-(2,4-dimethylphenyl)-2-oxocyclohexylsulfonamide (**3B$_3$**), *N*-(2-methyl-4-fluorophenyl)-2-oxocyclohexylsulfonamide (**3B$_6$**), *N*-(2-chlorophenyl)-2-oxocyclohexylsulfonamide (**3B$_8$**), *N*-(2-bromophenyl)-2-oxocyclohexylsulfonamide (**3B$_9$**), *N*-(4-chlorophenyl)-2-oxocyclohexylsulfonamide (**3B$_{10}$**), *N*-(3,4-dichlorophenyl)-2-oxocyclohexylsulfonamide (**3B$_{14}$**), *N*-[2,5-di(trifluoromethyl)phenyl]-2-oxocyclohexylsulfonamide (**3B$_{15}$**), *N*-[3,5-di(trifluoromethyl)phenyl]-2-oxocyclohexylsulfonamide (**3B$_{16}$**), and *N*-(2-trifluoromethyl-4-chlorophenyl)-2-oxocyclohexylsulfonamide (**3B$_{17}$**), were synthesized in ref *11*.

The physical and elemental data of new compounds **3A**, **3B$_1$**, **3B$_4$**, **3B$_5$**, **3B$_7$**, **3B$_{11}$**, **3B$_{12}$**, **3B$_{13}$**, **3C**, **3D**, and **3E** are listed in **Table 1**, and the $^1$H NMR and IR data are listed in **Table 2**.

*Synthesis of Compounds 4*. To the solution of compound **3** (0.01 mol) in methanol (30 mL) at 0−5 °C, sodium borohydride solution (0.016 mol of $NaBH_4$ + 8 mL of 1% NaOH + 8 mL of $CH_3OH$) was added dropwise, and the mixture was stirred at 5−15 °C for 1 h. After the methanol was evaporated in vacuum, the residue was dissolved in ethyl acetate (80 mL), washed with 5% HCl (30 mL) and water (20 mL), and dried over sodium sulfate. After the solvent was evaporated in vacuum, the crude product was recrystallized from the acetone/petroleum ether to give pure compound **4**. The physical and elemental data of the title compounds are listed in **Table 1**, and the $^1$H NMR and IR data are listed in **Table 2**.

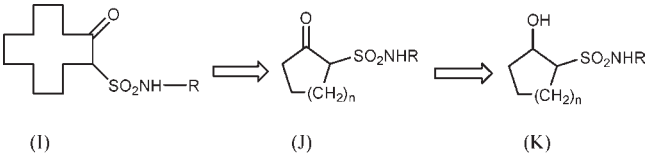



**Figure 2.** Structures of compounds **I**−**K**.

**Bioassay of Fungicidal Activities.** *Effect of the New Compounds on the Mycelial Growth of B. cinerea in Solid Media*. The compounds **3A**, **3B$_{17}$**, **3C**, **3D**, **3E**, **4A**, **4B$_1$**−**4B$_{17}$**, **4C**, **4D**, and **4E** were dissolved in acetone and mixed with sterile molten potato dextrose agar (PDA) to obtain final concentrations of 50, 25, 12.5, 6.25, 3.13, 1.56, and 0.78 $\mu$g/mL. The commercial fungicide procymidone (with a percentage composition of 96%) was used as the positive control. Fungicidal activities of the compounds against *B. cinerea* were evaluated *in vitro* using the method given in ref *12*. The inhibition rate was calculated according to eq 1

$$I_1 = (\overline{D}_1 - \overline{D}_0)/\overline{D}_1 \times 100\% \quad (1)$$

where $I_1$ is the inhibition rate, $\overline{D}_1$ is the average diameter of mycelia in the blank test, and $\overline{D}_0$ is the average diameter of mycelia in the presence of compounds. The $EC_{50}$ and $EC_{80}$ values were estimated using logit analysis (*13*), and the results are given in **Table 3**.

*Effect on the Germination of B. cinerea*. The spore germination method was given in ref *14*. Conidial germination assays were carried out on microscope slides. The conidial suspensions were prepared by seeding about $2\times10^5$ spores/mL conidia in a 0.05% Tween 80 solution, and the acetone solution of compounds (5000 $\mu$g/mL) was diluted with conidial suspension to obtain five different final concentrations of 200, 50, 12.5, 3.12, and 0.78 $\mu$g/mL. Then, 30 $\mu$L of these cultures was taken to inoculate in a concave slide and incubated in a humid chamber with 23 ± 1 °C and 100% relative humidity. Three replicates were performed, and the commercial fungicide procymidone was used as the positive control. After 8 h, conidial germination was determined directly on the slides by counting the number of germinated conidia in five microscope fields each containing

**Table 1.** Physical and Elemental Data of Compounds **3** and **4**

| compound | | | | | elemental analysis (%) | | |
|---|---|---|---|---|---|---|---|
| number | *n* | R | mp (°C) | yield (%) | C (calcd) | H (calcd) | N (calcd) |
| **3A** | 1 | 2-$CF_3$-4-$ClC_6H_3$ | 89−91 | 61 | 42.34 (42.18) | 3.15 (3.24) | 4.26 (4.10) |
| **$3B_1$** | 2 | $C_6H_5$ | 105−106 | 86 | 56.71 (56.90) | 6.12 (5.97) | 5.47 (5.53) |
| **$3B_4$** | 2 | 2-Me-6-Et$C_6H_3$ | 83−85 | 81 | 61.33 (60.99) | 7.16 (7.17) | 4.58 (4.74) |
| **$3B_5$** | 2 | 2,5-$(OMe)_2C_6H_3$ | 76−78 | 68 | 53.96 (53.66) | 6.02 (6.11) | 4.28 (4.47) |
| **$3B_7$** | 2 | 2-$FC_6H_4$ | 143−144 | 83 | 53.45 (53.12) | 5.12 (5.20) | 5.19 (5.16) |
| **$3B_{11}$** | 2 | 4-$BrC_6H_4$ | 81−82 | 80 | 43.14 (43.38) | 4.36 (4.25) | 4.40 (4.22) |
| **$3B_{12}$** | 2 | 4-$CF_3C_6H_4$ | 89−90 | 77 | 48.69 (48.59) | 4.50 (4.39) | 4.23 (4.36) |
| **$3B_{13}$** | 2 | 2,4-$Cl_2C_6H_3$ | 103−104 | 85 | 44.98 (44.73) | 4.01 (4.07) | 4.30 (4.35) |
| **3C** | 3 | 2-$CF_3$-4-$ClC_6H_3$ | 64−66 | 78 | 45.76 (45.47) | 3.93 (4.09) | 3.91 (3.79) |
| **3D** | 4 | 2-$CF_3$-4-$ClC_6H_3$ | 79−80 | 76 | 46.67 (46.94) | 4.59 (4.46) | 3.70 (3.65) |
| **3E** | 8 | 2-$CF_3$-4-$ClC_6H_3$ | 96−98 | 73 | 51.49 (51.87) | 5.56 (5.73) | 3.23 (3.18) |
| **4A** | 1 | 2-$CF_3$-4-$ClC_6H_3$ | 93−95 | 78 | 42.12 (41.93) | 3.56 (3.81) | 4.15 (4.07) |
| **$4B_1$** | 2 | $C_6H_5$ | 87−88 | 88 | 56.67 (56.45) | 6.78 (6.71) | 5.31 (5.49) |
| **$4B_2$** | 2 | 4-$MeC_6H_4$ | 82−83 | 80 | 58.05 (57.97) | 7.31 (7.11) | 5.08 (5.20) |
| **$4B_3$** | 2 | 2,4-$Me_2C_6H_3$ | 100−102 | 86 | 59.08 (59.34) | 7.40 (7.47) | 4.99 (4.94) |
| **$4B_4$** | 2 | 2-Me-6-Et$C_6H_3$ | 103−105 | 83 | 60.52 (60.58) | 7.86 (7.79) | 4.58 (4.71) |
| **$4B_5$** | 2 | 2,5-$(OMe)_2C_6H_3$ | 104−105 | 76 | 53.61 (53.32) | 6.53 (6.71) | 4.65 (4.44) |
| **$4B_6$** | 2 | 2-Me-4-$FC_6H_3$ | 115−117 | 90 | 49.54 (49.26) | 5.01 (5.13) | 4.79 (4.60) |
| **$4B_7$** | 2 | 2-$FC_6H_4$ | 102−104 | 89 | 52.64 (52.73) | 5.98 (5.90) | 5.22 (5.12) |
| **$4B_8$** | 2 | 2-$ClC_6H_4$ | 94−95 | 85 | 49.61 (49.74) | 5.76 (5.57) | 4.80 (4.83) |
| **$4B_9$** | 2 | 2-$BrC_6H_4$ | 80−82 | 88 | 43.46 (43.12) | 4.69 (4.83) | 4.33 (4.19) |
| **$4B_{10}$** | 2 | 4-$ClC_6H_4$ | 95−97 | 91 | 50.02 (49.74) | 5.48 (5.57) | 4.87 (4.83) |
| **$4B_{11}$** | 2 | 4-$BrC_6H_4$ | 96−97 | 82 | 43.04 (43.12) | 4.76 (4.83) | 4.23 (4.19) |
| **$4B_{12}$** | 2 | 4-$CF_3C_6H_4$ | 127−128 | 78 | 48.56 (48.29) | 4.78 (4.99) | 4.50 (4.33) |
| **$4B_{13}$** | 2 | 2,4-$Cl_2C_6H_3$ | 104−105 | 87 | 44.67 (44.45) | 4.54 (4.66) | 4.26 (4.32) |
| **$4B_{14}$** | 2 | 3,4-$Cl_2C_6H_3$ | 125−127 | 86 | 44.60 (44.45) | 4.48 (4.66) | 4.48 (4.32) |
| **$4B_{15}$** | 2 | 2,5-$(CF_3)_2C_6H_3$ | 90−91 | 89 | 42.78 (42.97) | 4.02 (3.86) | 3.67 (3.58) |
| **$4B_{16}$** | 2 | 3,5-$(CF_3)_2C_6H_3$ | 112−113 | 86 | 43.15 (42.97) | 3.80 (3.86) | 3.67 (3.58) |
| **$4B_{17}$** | 2 | 2-$CF_3$-4-$ClC_6H_3$ | 109−110 | 87 | 43.90 (43.64) | 4.12 (4.23) | 3.86 (3.91) |
| **4C** | 3 | 2-$CF_3$-4-$ClC_6H_3$ | 74−75 | 71 | 45.51 (45.23) | 4.43 (4.61) | 3.69 (3.77) |
| **4D** | 4 | 2-$CF_3$-4-$ClC_6H_3$ | 117−118 | 59 | 46.43 (46.69) | 5.17 (4.96) | 3.48 (3.63) |
| **4E** | 8 | 2-$CF_3$-4-$ClC_6H_3$ | 142−143 | 55 | 51.76 (51.64) | 6.23 (6.16) | 3.02 (3.17) |

approximately 60 conidia, respectively. The germination inhibition rate was calculated according to eq 2

$$I_2 = (\overline{G}_1 - \overline{G}_0)/\overline{G}_1 \times 100\% \quad (2)$$

where $I_2$ is the adjusted germination inhibition rate, $\overline{G}_1$ is the average germination rate of spores in the blank test, and $\overline{G}_0$ is the average germination rate of spores in the presence of compounds. The $EC_{50}$ and $EC_{80}$ values were estimated using logit analysis (*13*), and the results are given in **Table 4**.

*Effect on the Ability of B. cinerea To Colonize Cucumber Leaves (15).* The cucumber (*Cucumis sarivus* L.) seedlings at 2−3 leaf stages were used to assay the fungicidal activity against *B. cinerea*. The compounds were confected to 2.5% EC formulations, in which pesticide emulsifier 500 (0.375%) and pesticide emulsifier 600 (2.125%) were the additives, methanol (5%) was the co-solvent, and xylene was the solvent. The formulation was diluted to a series of concentrations at 500, 125, and 31.25 μg/mL with water to obtain the solutions that were spread on the surface of the cucumber leaves. After air drying, the upper sides of the leaves were inoculated with 6 mm plugs of *B. cinerea* Pers., which was maintained on PDA. This procedure was repeated 3 times, and 9 replicates were performed. The commercial fungicides procymidone and pyrimethanil (with a percentage composition of 95%) were used as the positive control. The inhibition rate was calculated according to eq 1, and the results were shown in **Table 5**.

## RESULTS AND DISCUSSION

**Synthesis of Compounds 4.** There are advantages of high yields, short reaction times, easy preparation, and mild reaction conditions when the compounds **3** are being reduced. In general, the compounds with a small ring were easy to reduce, but with the increase of the rine size, both the reaction time and temperature were somewhat increased, accompanying the decrease of the yields. The yields of compounds **4** from **3** are fair too good (55−91%), as shown in **Table 1**. The structures of compounds **4** were confirmed by elemental analysis (**Table 1**), $^1$H NMR, and IR (**Table 2**).

**Fungicidal Activity and Structure−Activity Relationship (Mycelial Growth Rate Method).** As shown in **Table 3**, the compounds **4** exhibited good fungicidal activity against *B. cinerea*. Among them, compounds **$4B_{15}$**, **$4B_{16}$**, **$4B_{17}$**, **4C**, and **4D**, with $EC_{50}$ values of 2.53, 4.44, 3.37, 0.85, and 1.09 μg/mL, repectively, exhibited excellent fungicidal activity against *B. cinerea*.

*Structure−Activity Relationship 1.* The substituent of the benzene ring might exert a greater influence on the activity. The activity was significantly decreased with the benzene ring containing electron-donating groups (Me, Et, and MeO). The compounds with a halogen atom in the *para* postiton of the benzene ring showed higher activities than in the adjacent position. For example, the compounds **$4B_8$**, **$4B_9$**, and **$4B_{13}$** (with $EC_{50}$ values of 75.65, 10.98, and 53.24 μg/mL, respectively) had lower fungicidal activity than the compounds **$4B_{10}$**, **$4B_{11}$**, and **$4B_{14}$** (with $EC_{50}$ values of 7.38, 8.79, and 7.80 μg/mL, respectively). Besides, all of the compounds with a trifluoromethyl group on the benzene ring presented higher fungicidal activity, particularly the one with bis-(trifluoromethyl).

*Structure−Activity Relationship 2.* The fungicidal activity was affected obviously by the size of cycloalkane and was consistent with the structure−activity relationship of 2-hydroxycycloalkylsulfonamides **4** and 2-oxocycloalkylsulfonamides **3** (**Figure 3**). The contribution of a 7-membered ring for fungicidal activity was the biggest according to the $EC_{50}$ values. The $EC_{50}$

**Table 2.** $^1$H NMR and IR Data of Compounds **3** and **4**

| compound number | $^1$H NMR ($CDCl_3$, $\delta$) | IR ($\nu$, $cm^{-1}$) |
|---|---|---|
| **3A** | 1.87−2.57 (m, 6H, $3CH_2$), 3.84 (t, 1H, CH, $J$ = 8.85 Hz), 7.24 (br, 1H, NH), 7.52 (dd, 1H, $J$ = 8.85, 2.25 Hz), 7.63 (d, 1H, $J$ = 2.25 Hz), 7.71 (d, 1H, $J$ = 8.85 Hz) | 3299, 2977, 2919, 1753 |
| **3B$_1$** | 1.62−2.66 (m, 8H, $4CH_2$), 3.72 (dd, 1H, CH, $J$ = 10.80, 6.00 Hz), 6.87 (br, 1H, NH), 7.19−7.34 (m, 5H, $C_6H_5$) | 3248, 2951, 2861, 1705 |
| **3B$_4$** | 1.23 (t, 3H, $CH_3$), 1.71−2.81 (m, 8H, $4CH_2$), 2.39 (s, 3H, $CH_3$), 4.02 (dd, 1H, CH, $J$ = 11.70, 5.70 Hz), 6.54 (br, 1H, NH), 7.07−7.19 (m, 3H, $C_6H_3$) | 3298, 2952, 2860, 1708 |
| **3B$_5$** | 1.63−2.59 (m, 8H, $4CH_2$), 3.82 (s, 3H, $OCH_3$), 3.86 (s, 3H, $OCH_3$), 3.69 (dd, 1H, CH, $J$ = 11.70, 5.70 Hz), 6.96 (br, 1H, NH), 6.25 (dd, 1H, Ph-H, $J$ = 9.00, 3.00 Hz), 7.84 (d, 1H, Ph-H, $J$ = 9.00 Hz), 7.12 (d, 1H, Ph-H, $J$ = 3.00 Hz) | 3308, 2954, 2859, 1708 |
| **3B$_7$** | 1.69−2.68 (m, 8H, $4CH_2$), 3.86 (dd, 1H, CH, $J$ = 11.20, 5.60 Hz), 7.06−7.60 (m, 5H, $C_6H_4$ + NH) | 3238, 2948, 2865, 1705 |
| **3B$_{11}$** | 1.61−2.67 (m, 8H, $4CH_2$), 3.72 (dd, 1H, CH, $J$ = 10.52, 5.92 Hz), 6.95 (s, 1H, NH), 7.12−7.17 (m, 2H, Ph-H), 7.23−7.29 (m, 2H, Ph-H) | 3267, 2943, 2865, 1724 |
| **3B$_{12}$** | 1.66−2.67 (m, 8H, $4CH_2$), 3.76 (dd, 1H, $J$ = 10.52, 5.96 Hz), 7.12 (s, 1H, NH), 7.21−7.50 (m, 4H, $C_6H_4$) | 3262, 2957, 2870, 1724 |
| **3B$_{13}$** | 1.64−2.67 (m, 8H, $4CH_2$), 3.85 (dd, 1H, CH, $J$ = 11.20, 5.70 Hz), 7.12 (br, 1H, NH), 7.26−7.83 (m, 3H, $C_6H_3$) | 3340, 2950, 2869, 1714 |
| **3C** | 1.40−2.73 (m, 10H, $5CH_2$), 4.11 (dd, 1H, CH−$SO_2$, $J$ = 10.5, 4.2 Hz), 7.24 (br, 1H, NH), 7.51 (dd, 1H, $J$ = 8.70, 2.10 Hz, Ph-H), 7.61 (d, 1H, $J$ = 2.10 Hz, Ph-H), 7.74 (d, 1H, $J$ = 8.70 Hz, Ph-H) | 3359, 2928, 2858, 1708 |
| **3D** | 1.19−2.67 (m, 12H, $6CH_2$), 4.24 (dd, 1H, CH−$SO_2$, $J$ = 11.40, 3.30 Hz), 7.17 (br, 1H, NH), 7.51 (dd, 1H, $J$ = 9.00, 2.10 Hz, Ph-H), 7.61 (d, 1H, $J$ = 2.10 Hz, Ph-H), 7.77 (d, 1H, $J$ = 9.00 Hz, Ph-H) | 3198, 2929, 2870, 1675 |
| **3E** | 1.28−1.42 (m, 14H), 1.61−1.67 (m, 1H), 1.82−1.92 (m, 2H), 2.29−2.37 (s, 1H), 2.73−2.91 (m, 2H), 4.18 (dd, 1H, $J$ = 11.70, 3.00 Hz), 6.74 (br, 1H, NH), 7.53 (dd, 1H, $J$ = 8.82, 2.28 Hz, Ph-H), 7.62 (d, 1H, $J$ = 2.28 Hz, Ph-H), 7.78 (d, 1H, $J$ = 8.82 Hz, Ph-H) | 3267, 2924, 2870, 1708 |
| **4A** | 1.63−2.37 (m, 6H, $3CH_2$), 2.39 (br, 1H, OH), 3.55 (m, 1H, CH−O), 4.58 (dd, 1H, $J$ = 9.30, 4.80 Hz, CH−$SO_2$), 7.14 (br, 1H, NH), 7.50 (dd, 1H, $J$ = 8.70, 2.10 Hz, Ph-H), 7.61 (d, 1H, $J$ = 2.10 Hz, Ph-H), 7.81 (d, 1H, $J$ = 8.70 Hz, Ph-H) | 3504, 3141, 2962, 2856 |
| **4B$_1$** | 1.20−2.06 (m, 8H, $4CH_2$), 2.86 (s, 1H, OH), 3.08 (ddd, 1H, $J$ = 12.00, 3.90, 2.10 Hz, CH−O), 4.53 (s, 1H, CH−$SO_2$), 6.83 (s, 1H, NH), 7.14−7.33 (m, 5H, $C_6H_5$) | 3453, 3168, 2945, 2859 |
| **4B$_2$** | 1.22−2.06 (m, 8H, $4CH_2$), 2.32 (s, 3H, $CH_3$), 2.62 (br, 1H, OH), 3.02−3.08 (dd, 1H, $J$ = 12.15, 4.12, 1.89 Hz, CH−O), 4.52 (s, 1H, CH−$SO_2$), 6.79 (s, 1H, NH), 7.08−7.13 (m, 4H, $C_6H_4$) | 3504, 3244, 2938, 2856 |
| **4B$_3$** | 1.23−2.06 (m, 8H, $4CH_2$), 2.29 (s, 3H, $CH_3$), 2.32 (s, 3H, $CH_3$), 2.41 (br, 1H, OH), 3.01−3.08 (ddd, 1H, $J$ = 12.09, 4.32, 1.89 Hz, CH−O), 4.53 (s, 1H, CH−$SO_2$), 6.59 (s, 1H, NH), 6.90−7.01 (m, 2H, Ph-H), 7.21−7.24 (m, 1H, Ph-H) | 3465, 3228, 2943, 2856 |
| **4B$_4$** | 1.25 (t, 3H, $CH_3$), 1.69−2.76 (m, 9H, $4CH_2$ + OH), 2.36 (s, 3H, $CH_3$), 3.01−309 (m, 1H, $J$ = 12.18, 4.47, 1.82 Hz, CH−O), 4.50 (s, 1H, CH−$SO_2$), 6.97 (br, 1H, NH), 7.06−7.13 (m, 3H, $C_6H_3$) | 3493, 3242, 2934, 2850 |
| **4B$_5$** | 1.23−2.09 (m, 9H, $4CH_2$ + OH), 2.99 (m, 1H, CH−O), 3.77 (s, 3H, $OCH_3$), 3.86 (s, 3H, $OCH_3$), 4.46 (br, 1H, CH−$SO_2$), 6.91 (br, 1H, NH), 6.23 (dd, 1H, $J$ = 9.00, 3.00 Hz, Ph-H), 7.83 (d, 1H, $J$ = 9.00 Hz, Ph-H), 7.18 (d, 1H, $J$ = 3.00 Hz, Ph-H) | 3426, 3325, 2952, 2850 |
| **4B$_6$** | 1.23−2.12 (m, 8H, $4CH_2$), 2.25 (s, 3H, $CH_3$), 3.01−3.07 (ddd, 1H, $J$ = 11.41, 4.42, 1.86 Hz, CH−O), 4.52 (s, 1H, CH−$SO_2$), 6.73−6.79 (m, 2H, NH + Ph-H), 7.08−7.13 (m, 1H, Ph-H), 7.23−7.28 (m, 1H, Ph-H) | 3504, 3262, 2943, 2861 |
| **4B$_7$** | 1.26−2.05 (m, 8H, $4CH_2$), 2.90 (br, 1H, OH), 3.03−3.09 (ddd, 1H, $J$ = 12.11, 4.04, 1.89 Hz, CH−O), 4.55 (br, 1H, CH−$SO_2$), 7.05 (br, 1H, NH), 7.07−7.63 (m, 4H, $C_6H_4$) | 3458, 3121, 2978, 2859 |
| **4B$_8$** | 1.23−2.12 (m, 8H, $4CH_2$), 2.98 (br, 1H, OH), 3.09−3.15 (ddd, 1H, $J$ = 11.52, 4.40, 1.92 Hz, CH−O), 4.55 (br, 1H, CH−$SO_2$), 7.08−7.13 (m, 1H, Ph-H), 7.23−7.28 (m, 1H, Ph-H), 7.35 (s, 1H, NH), 7.37 (m, 1H, Ph-H), 7.67 (m, 1H, Ph-H) | 3516, 3251, 2939, 2853 |
| **4B$_9$** | 1.25−2.08 (m, 8H, $4CH_2$), 2.94 (br, 1H, OH), 3.05−3.11 (ddd, 1H, $J$ = 11.62, 4.34, 1.82 Hz, CH−O), 4.50 (br, 1H, CH−$SO_2$), 6.96−7.02 (m, 1H, Ph-H), 7.24−7.30 (m, 1H, Ph-H), 7.33 (s, 1H, NH), 7.52 (m, 1H, Ph-H), 7.63 (m, 1H, Ph-H) | 3508, 3253, 2939, 2859 |
| **4B$_{10}$** | 1.24−2.00 (m, 8H, $4CH_2$), 2.96 (br, 1H, OH), 3.04−3.10 (ddd, 1H, $J$ = 11.67, 4.37, 1.84 Hz, CH−O), 4.52 (br, 1H, CH−$SO_2$), 7.34 (br, 1H, NH), 7.19−7.30 (m, 4H, $C_6H_4$) | 3429, 3136, 2939, 2859 |
| **4B$_{11}$** | 1.23−2.02 (m, 8H, $4CH_2$), 2.97 (br, 1H, OH), 3.03−3.08 (ddd, 1H, $J$ = 12.10, 4.12, 1.83 Hz, CH−O), 4.51 (br, 1H, CH−$SO_2$), 7.31 (br, 1H, NH), 7.10−7.22 (m, 4H, $C_6H_4$) | 3428, 3138, 2939, 2859 |
| **4B$_{12}$** | 1.22−2.18 (m, 8H, $4CH_2$), 2.79 (br, 1H, OH), 3.11−3.17 (ddd, 1H, $J$ = 11.03, 5.06, 2.01 Hz, CH−O), 4.56 (br, 1H, CH−$SO_2$), 7.39 (br, 1H, NH), 7.34 (d, 2H, $J$ = 8.51 Hz, Ph-H), 7.57 (d, 2H, $J$ = 8.51 Hz, Ph-H) | 3454, 3150, 2946, 2902 |
| **4B$_{13}$** | 1.24−2.07 (m, 8H, $4CH_2$), 2.64 (br, 1H, OH), 3.02−3.07 (ddd, 1H, $J$ = 12.19, 3.83, 2.03 Hz, CH−O), 4.55 (br, 1H, CH−$SO_2$), 7.00 (br, 1H, NH), 7.24−7.70 (m, 3H, $C_6H_3$) | 3479, 3281, 2936, 2866 |
| **4B$_{14}$** | 1.22−2.06 (m, 8H, $4CH_2$), 2.56 (br, 1H, OH), 3.04−3.10 (ddd, 1H, $J$ = 12.08, 4.06, 2.01 Hz, CH−O), 4.54 (s, 1H, CH−$SO_2$), 6.99 (br, 1H, NH), 7.09 (dd, 1H, $J$ = 8.7, 2.52 Hz, Ph-H), 7.37 (d, 1H, $J$ = 2.60 Hz, Ph-H), 7.38 (d, 1H, $J$ = 8.60 Hz, Ph-H) | 3442, 3158, 2944, 2854 |
| **4B$_{15}$** | 1.26−2.06 (m, 8H, $4CH_2$), 2.35 (br, 1H, OH), 3.10−3.17 (m, 1H, CH−O), 4.60 (br, 1H, CH−$SO_2$), 7.29 (br, 1H, NH), 7.49 (d, 1H, $J$ = 8.22 Hz, Ph-H), 7.76 (d, 1H, $J$ = 8.22 Hz, Ph-H), 8.13 (s, 1H, Ph-H) | 3483, 3278, 2939, 2869 |
| **4B$_{16}$** | 1.23−2.09 (m, 8H, $4CH_2$), 2.40 (br, 1H, OH), 3.14−3.20 (m, 1H, CH−O), 4.63 (br, 1H, CH−$SO_2$), 7.31 (br, 1H, NH), 7.63 (s, 1H), 7.70 (s, 2H) | 3466, 3263, 2940, 2859 |
| **4B$_{17}$** | 1.25−2.09 (m, 8H, $4CH_2$), 2.45 (br, 1H, OH), 3.07−3.13 (ddd, 1H, $J$ = 12.08, 4.10, 1.99 Hz, CH−O), 4.52 (br, 1H, CH), 6.98 (br, 1H, NH), 7.48−7.80 (m, 3H, $C_6H_3$) | 3477, 3235, 2957, 2855 |
| **4C** | 1.40−2.18 (m, 10H, $5CH_2$), 2.40 (br, 1H, OH), 3.15−3.20 (m, 1H, CH−O), 4.68−4.70 (m, 1H, CH−$SO_2$), 7.05 (br, 1H, NH), 7.49−7.80 (m, 3H, $C_6H_3$) | 3527, 3233, 2934, 2855 |
| **4D** | 1.40−2.14 (m, 10H, $5CH_2$), 2.41 (br, 1H, OH), 3.33−3.37 (m, 1H, CH−O), 4.52−4.55 (m, 1H, CH−$SO_2$), 7.19 (br, 1H, NH), 7.48−7.80 (m, 3H, $C_6H_3$) | 3498, 3228, 2930, 2864 |
| **4E** | 1.24−1.39 (m, 14H), 1.47−1.56 (m, 2H), 1.74 (br, 1H, OH), 1.83−2.76 (m, 4H), 3.29−3.31 (m, 1H, CH−OH), 4.19 (s, 1H, CH−$SO_2$), 6.94 (br, 1H, NH), 7.52−7.77 (m, 3H, $C_6H_3$) | 3508, 3183, 2934, 2865 |

values of compounds **4C** and **3C** were 0.85 and 0.80 $\mu$g/mL, which were better than procymidone (with an $EC_{50}$ value of 0.99 $\mu$g/mL), respectively. According to the $EC_{80}$ values, the contribution of a 8-membered ring for fungicidal activity became the biggest. The $EC_{80}$ values of compounds **4D** and **3D** were 2.50 and 2.88 $\mu$g/mL, which were 1.18 and 1.03 times that of procymidone, respectively. Therefore, regardless of the size of the ring reducing or increasing, the fungicidal activity will significantly decrease.

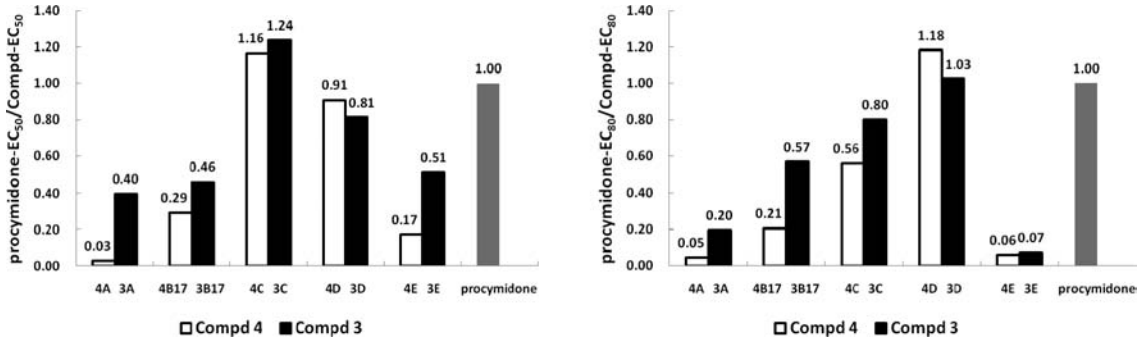



**Figure 3.** Relationship between the size of the ring and the $EC_{50}$ and $EC_{80}$ values of the compounds **4** and **3**. The ordinate is the ratio of $EC_{50}$ and $EC_{80}$ values of procymidone to the $EC_{50}$ and $EC_{80}$ values of the compounds.

**Table 3.** Fungicidal Activities of Compounds **4** and **3** against *B. cinerea* (Mycelial Growth Rate Method)

| compound number | $EC_{50}$ ($\mu$g/mL) | $EC_{80}$ ($\mu$g/mL) |
|---|---|---|
| **$4B_1$** | 22.90 | 443.24 |
| **$4B_2$** | 18.01 | 65.37 |
| **$4B_3$** | 21.49 | 332.24 |
| **$4B_4$** | 104.68 | $>10^4$ |
| **$4B_5$** | 22.30 | 181.29 |
| **$4B_6$** | 34.06 | 310.15 |
| **$4B_7$** | 23.99 | 84.16 |
| **$4B_8$** | 75.65 | $>10^4$ |
| **$4B_9$** | 10.98 | 250.23 |
| **$4B_{10}$** | 7.38 | 86.49 |
| **$4B_{11}$** | 7.07 | 21.40 |
| **$4B_{12}$** | 8.79 | 65.86 |
| **$4B_{13}$** | 53.24 | 517.71 |
| **$4B_{14}$** | 7.80 | 25.47 |
| **$4B_{15}$** | 2.53 | 16.02 |
| **$4B_{16}$** | 4.44 | 14.64 |
| **4A** | 36.38 | 64.39 |
| **$4B_{17}$** | 3.37 | 14.26 |
| **4C** | 0.85 | 5.27 |
| **4D** | 1.09 | 2.50 |
| **4E** | 5.72 | 49.36 |
| **3A** | 2.50 | 15.08 |
| **$3B_{17}$** | 2.16 | 5.20 |
| **3C** | 0.80 | 3.70 |
| **3D** | 1.22 | 2.88 |
| **3E** | 1.94 | 39.83 |
| procymidone | 0.99 | 2.96 |

**Table 4.** Fungicidal Activity of Compounds **3A**−**3E** and **4A**−**4E** against the Spores of *B. cinerea*

| compound number | $EC_{50}$ ($\mu$g/mL) | $EC_{80}$ ($\mu$g/mL) |
|---|---|---|
| **3A** | 4.21 | 226.64 |
| **$3B_{17}$** | 4.21 | 124.27 |
| **3C** | 18.07 | 387.82 |
| **3D** | 18.96 | 549.44 |
| **3E** | 3.24 | 114.57 |
| **4A** | 5.29 | 43.03 |
| **$4B_{17}$** | 40.09 | 260.18 |
| **4C** | 76.96 | $>10^3$ |
| **4D** | 23.62 | 693.34 |
| **4E** | 419.99 | $>10^3$ |
| procymidone | 0.98 | 3.18 |

*Structure*−*Activity Relationship 3*. The fungicidal activity of 2-hydroxycycloalkylsulfonamides **4** against *B. cinerea* was similar to that of 2-oxocycloalkylsulfonamides **3**. We can infer that the two series of compounds have a similar mode of action.

**Table 5.** Control Efficiency of Compounds **3A**−**3E** and **4A**−**4E** against *B. cinerea* Pers. (Leaf Method)

| compound number | concentration ($\mu$g/mL) | average diameter of the spot (cm) | control efficiency (%) |
|---|---|---|---|
| | 500 | 0.36 | 74.77 ± 11.02 def |
| **3A** | 125 | 0.39 | 72.42 ± 13.25 defg |
| | 31.25 | 0.56 | 60.68 ± 12.18 hi |
| | 500 | 1.00 | 28.99 ± 11.02 k |
| **$3B_{17}$** | 125 | 1.13 | 20.78 ± 9.56 klm |
| | 31.25 | 1.19 | 16.08 ± 12.90 lmn |
| | 500 | 0.72 | 49.53 ± 11.95 j |
| **3C** | 125 | 1.06 | 21.95 ± 15.15 klm |
| | 31.25 | 1.25 | 11.97 ± 16.85 mn |
| | 500 | 0.46 | 67.72 ± 18.86 efgh |
| **3D** | 125 | 0.47 | 67.14 ± 12.86 efgh |
| | 31.25 | 0.50 | 64.79 ± 9.50 fgh |
| | 500 | 0.04 | 97.07 ± 3.62 a |
| **3E** | 125 | 0.06 | 95.60 ± 6.90 a |
| | 31.25 | 0.24 | 82.98 ± 8.20 bcd |
| | 500 | 0.27 | 81.12 ± 18.83 cd |
| **4A** | 125 | 0.44 | 68.90 ± 11.81 efgh |
| | 31.25 | 0.53 | 62.44 ± 8.67 ghi |
| | 500 | 0.09 | 93.54 ± 9.59 ab |
| **$4B_{17}$** | 125 | 0.39 | 72.42 ± 16.30 defg |
| | 31.25 | 0.66 | 53.05 ± 11.76 ij |
| | 500 | 0.07 | 95.01 ± 7.27 a |
| **4C** | 125 | 0.10 | 92.67 ± 7.72 ab |
| | 31.25 | 0.36 | 74.77 ± 11.81 def |
| | 500 | 0.08 | 94.42 ± 6.27 a |
| **4D** | 125 | 0.10 | 93.55 ± 7.63 ab |
| | 31.25 | 0.12 | 91.49 ± 6.96 abc |
| | 500 | 1.05 | 26.06 ± 18.39 kl |
| **4E** | 125 | 1.20 | 15.49 ± 18.51 lmn |
| | 31.25 | 1.30 | 8.45 ± 9.96 n |
| | 500 | 0.10 | 91.78 ± 8.93 abc |
| procymidone | 125 | 0.38 | 73.59 ± 14.75 def |
| | 31.25 | 0.55 | 61.27 ± 9.73 hi |
| | 500 | 0.00 | 100.00 ± 0 a |
| pyrimethanil | 125 | 0.24 | 82.98 ± 10.17 bcd |
| | 31.25 | 0.33 | 76.53 ± 9.17 de |
| CK | 0 | 1.42 | |

**Results of the Spore Germination Test.** As shown in **Table 4**, those two types of compounds restrained spore germination at low concentrations, and they could inhibit the germ tube elongation of *B. cinerea* under certain concentrations. This result is consistent with the mycelium growth rate method. Most of the compounds (except the compounds **4C** and **4E**) had fair to good inhibitory activity. Furthermore, the activitiy of 2-oxocycloalkylsulfonamides **3** showed better than 2-hydroxycycloalkylsulfonamides **4**, and there was no regularity relationship between the

size of the ring and their activity. Overall, the compounds containing a 5-membered ring had a preferable activity compared to other compounds.

**Results of the *in Vivo* Test.** *In vivo* experiments showed that the blank control group had a bad attack on the third day after inoculating the fungus. Inoculating the pathogen had caused the disease spots on the seedling leaves, which were extended at faster speed; meanwhile, the plant showed a retarded growth rate. The growth rate was not significantly affected in treatment groups, except for the leaves caused by the inoculating pathogen with different degree disease spots. The control group stand degraded and withered after the 7 day, and the plants that were sprayed by the better protective effect compounds grew normally. As shown in **Table 5**, most compounds (except the compounds **$3B_{17}$**, **3C**, and **4E**) showed excellent fungicidal activities *in vivo* against the *B. cinerea*. The variance analysis using SPSS 18.0 software was carried out for analysis of the control efficiency. Particularly, compounds **3E**, **4C**, and **4D** showed better activities than the control fungicides procymidone and pyrimethanil on cucumber plants.

The control efficiency of 2-hydroxycycloalkylsulfonamides **4** was significantly higher than that of 2-oxocycloalkylsulfonamides **3** *in vivo*. The result was opposite that *in vitro*, which should be related to the absorption and conduction in the plant tissue. Besides, 2-hydroxycycloalkylsulfonamides **4** may have better affinity to the plant tissue. This may be similar to the relationship between triadimenol and triadimefon (*16*). Accordingly, carbonyl compounds were converted into the corresponding hydroxyl compounds in plants, as the effective substances acting on the target. Further studies about this mechanism have been planned.

### Note Added after ASAP Publication

Figure 1 was modified in the version of this paper published ASAP October 7, 2010. The correct version published October 15, 2010.

### LITERATURE CITED


(1) Tanaka, S.; Kochi, S. I.; Kunita, H.; Ito, S. I.; Kameya-Iwaki, M. Biological mode of action of the fungicide, flusulfamide, against *Plasmodiophora brassicae* (clubroot). *Eur. J. Plant Pathol.* **1999**, *105* (6), 577−584.

(2) Tabuchi, T.; Yamamoto, T.; Nakayama, M. Sulfonamide derivatives. WO Patent 0,065,913, 2000.

(3) Shigeru, M.; Satoshi, A.; Tomona, Y.; Yasuko, T.; Takeshi, O.; Norifusa, M. Fungicidal activity of the novel fungicide cyazofamid against *Phytophthora infestans* and other plant pathogenic fungi in vitro. *Pestic. Biochem. Physiol.* **2001**, *71* (2), 92−99.

(4) Shigeru, M.; Satoshi, A.; Yasuko, T.; Takeshi, O.; Norifusa, M.; Hideto, M. The biochemical mode of action of the novel selective fungicide cyazofamid: Specific inhibition of mitochondrial complex III in *Phythium spinosum*. *Pestic. Biochem. Physiol.* **2001**, *71* (2), 107−115.

(5) Yang, J. C.; Wu, Q.; Liu, R. L.; Ma, S. C.; Liu, C. L. Recent advances on the development of agricultural fungicides. *Agrochemicals* **2008**, *47* (6), 402−405.

(6) Beucheta, P.; Varache-Lembègea, M.; Neveua, A.; Légerb, J.-M.; Vercauterena, J.; Larrouturea, S.; Deffieuxa, G.; Nuhrich, A. New 2-sulfonamidothiazoles substituted at C-4: Synthesis of polyoxy genated aryl derivatives and in vitro evaluation of antifungal activity. *Eur. J. Med. Chem.* **1999**, *34*, 773−779.

(7) Ezabadi, I. R.; Camoutsis, C.; Zoumpoulakis, P.; Geronikaki, A.; Soković, M.; Glamocilija, J.; Cirić, A. Sulfonamide-1,2,4-triazole derivatives as antifungal and antibacterial agents: Synthesis, biological evaluation, lipophilicity, and conformational studies. *Bioorg. Med. Chem.* **2008**, *16* (3), 1150−1161.

(8) Basanagouda, M.; Shivashankar, K.; Kulkarni, M. V.; Rasal, V. P.; Patel, H.; Mutha, S. S.; Mohite, A. A. Synthesis and antimicrobial studies on novel sulfonamides containing 4-azidomethyl coumarin. *Eur. J. Med. Chem.* **2010**, *45*, 1151−1157.

(9) Saíz-Urra, L.; Pérez González, M.; Collado, I. G.; Hernández-Galán, R. Quantitative structure−activity relationship studies for the prediction of antifungal activity of *N*-arylbenzenesulfonamides against *Botrytis cinerea*. *J. Mol. Graphics Modell.* **2007**, *25* (5), 680−690.

(10) Wang, X. P.; Wang, D. Q. Synthesis and antifungal activity of N-substituted-2-oxocyclododecylsulphonamides. *Chem. J. Chin. Univ.* **1997**, *18* (6), 889−893.

(11) Li, X. H.; Yang, X. L.; Liang, X. M.; Kai, Z. P.; Yuan, H. Z.; Yuan, D. K.; Zhang, J. J.; Wang, R. Q.; Ran, F. X.; Qi, S. H.; Ling, Y.; Chen, F. H.; Wang, D. Q. Synthesis and biological activities of 2-oxocycloalkylsulfonamides. *Bioorg. Med. Chem.* **2008**, *16* (8), 4538−4544.

(12) Li, X. H.; Yang, X. L.; Ling, Y.; Fan, Z. J.; Wang, D. Q.; Li, Z. M. The synthesis and fungicidal activity of novel α-oxocycloalkylsulfonylureas. *J. Agric. Food Chem.* **2005**, *53*, 2202−2206.

(13) Berkson, J. Astatistically and precise and relatively simple method of estimating the bioassay with quantal response, based on the logistic function. *J. Am. Stat. Assoc.* **1953**, *48*, 565−599.

(14) Vivek, K. B.; Savita, S.; Sun, C. K. Chemical composition and antifungal activity of essential oil and various extract of *Silene armeria* L. *Bioresour. Technol.* **2008**, *99*, 8903−8908.

(15) Shen, D.; Li, B. J.; Li, X. X.; Song, J. P.; Shi, Y. X.; Wang, H. P. Pathogenicity analysis of different *Botrytis cinerea* strains in cucumber. *China Veg.* **2009**, *18*, 25−28.

(16) Jiang, S. R.; Cao, G. Y. Research on metabolism of $^{14}$C-triadimefon in cucumber seedlings. *Acta Agric. Nucl. Sin.* **1991**, *5* (3), 189−192.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