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Synthesis and biological activities of 2-oxocycloalkylsulfonamides

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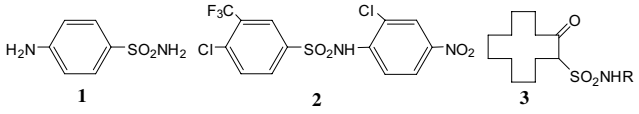
Abstract—A series of novel 2-oxocycloalkylsulfonamides (**4**) were synthesized and their structures confirmed by IR, ^{1}H NMR, and elemental analysis. The bioassay showed that they have fair to excellent fungicidal activities against *Botrytis cinerea Pers* and *Sclerotinia sclerotiorum*. Among them, compounds $\mathbf{4A}_{10}$, $\mathbf{4A}_{11}$, $\mathbf{4A}_{12}$, $\mathbf{4B}_2$, and $\mathbf{4B}_3$, the EC_{50} values of which were 2.12, 3.66, 3.96, 2.38, and 2.43 μg/mL, respectively, displayed excellent fungicidal activity against *B. cinerea Pers*, and are comparable with commercial fungicide procymidone (the EC_{50} value is 2.45 μg/mL). 3D QSAR against *B. cinerea Pers* was studied, a statistically significant and chemically meaningful CoMFA model was developed and some compounds which have a high predicted activity were forecasted. In addition, the bioassay also showed that the compounds have good inhibitory activities against human tumor cells HL-60, BGC-823, Bel-7402 and KB. It is interesting to point out that the antitumor activities of compounds **4** are in accordance with their fungicidal activity to a great extent: compounds having relatively best antitumor activities ($\mathbf{4A}_{10}$, $\mathbf{4A}_{11}$, $\mathbf{4A}_{12}$, and $\mathbf{4B}_3$) also displayed excellent fungicidal activity.

1. Introduction

It is common knowledge that sulfanilamide (**1**) was the first synthetic antibacterial agent active against a wide range of infections. Further developments led to a range of sulfonamides which proved effective against Gram-positive organisms, especially *pneumococci* and *meningococci*.[1] Sulfonamides are also used as agricultural fungicides. For example, flusulfamide (**2**) is used as a soil fungicide for the control of *Plasmodiophora brassicae* on the Chinese cabbage.[2] On the other hand, we found that several 2-oxocyclododecylsulfonamides (**3**) have some fungicidal activity.[3]



1 **2** **3**

Based on our discovery and the fact that all of the sulfonamide drugs and fungicides are benzene sulfonamide derivatives, we decided to explore whether latter's cycloalkane analogues (cyclohexane and cycloheptane derivatives) have a similar biological activity. Thus, a series of 2-oxocyclohexylsulfonamides (**4A**) and 2-oxocycloheptylsulfonamides (**4B**) were synthesized, and their fungicidal activity against some economically important fungus species (*Botrytis cinerea Pers* and *Sclerotinia sclerotiorum*) was evaluated. Then 3D QSAR against *B. cinerea Pers* was studied by CoMFA (Comparative Molecular Field Analysis) and a statistically significant and chemically meaningful CoMFA model was developed. According to the model, five new 2-oxocycloalkylsulfonamides ($\mathbf{4P}_1$–$\mathbf{4P}_5$) were designed and synthesized to check their predictive ability. In addition, antitumor

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activities of compounds **4A** and **4B** were also evaluated. In this paper, we would like to report their synthesis and biological activities.

2. Results and discussion

2.1. Chemical synthesis

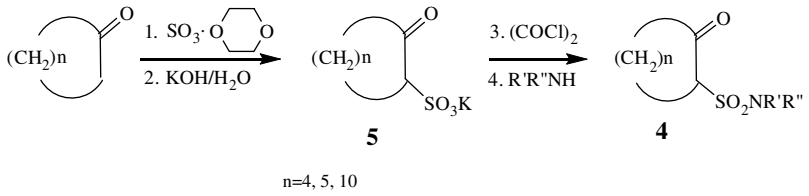
The synthetic route of compounds **4** is shown in Scheme 1. Potassium 2-oxocycloalkylsulfonates (**5**), prepared from readily available cycloalkanes by sulfonation with a sulfur trioxide–dioxane adduct and neutralization with potassium hydroxide,[4] were allowed to react with oxalyl chloride to give the corresponding sulfonyl chloride, which were converted into title compounds (**4**) by amination with amines.[3]

The yields of compounds **4** from potassium 2-oxocycloalkylsulfonamides are good (60–88%). The structures of compounds **4** were confirmed by IR, ^{1}H NMR, and elemental analysis. In the ^{1}H NMR spectra, the α-hydrogen of most of 2-oxocyclohexylsulfonamides displayed a ddd at δ 3.72–3.92 due to the coupling with two hydrogens of β-methylene (an axial hydrogen and an equatorial hydrogen) and the long-range coupling with α′-equatorial hydrogen, respectively.

2.2. Fungicidal activity

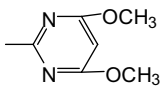
The fungicidal activities of compounds **4** against phytopathogen *B. cinerea Pers* and *S. sclerotiorum* were evaluated using commercial fungicides procymidone or chlorothalonil as the controls. The results are shown in Table 1.

From Table 1, we can see that compounds **4** have fair to excellent fungicidal activities against *B. cinerea Pers*. Among them, compounds $\mathbf{4A}_{10}$, $\mathbf{4A}_{11}$, $\mathbf{4A}_{12}$, $\mathbf{4B}_2$, and $\mathbf{4B}_3$, the EC_{50} values of which were 2.12, 3.66, 3.96, 2.38, and 2.43 μg/mL, respectively, displayed excellent fungicidal activity, and are comparable with that of procymidone (the EC_{50} value is 2.45 μg/mL). In general, the structure–activity relationship is as follows: the compounds with two substituents, especially two electron-withdrawing groups such as trifluoromethyl and chlorine atom on the benzene ring have good fungicidal activity against *B. cinerea Pers*. In addition, compounds **4** have fair to good fungicidal activity against *S. sclerotiorum*. Among them, compound $\mathbf{4B}_3$ has excellent fun-



Scheme 1.

Table 1. The fungicidal activities of compounds **4** against *Botrytis cinerea Pers* and *Sclerotinia sclerotiorum*

Compound				*B. cinerea*			*S. sclerotiorum*		
No.	*n*	R′	R″	Regression eq	*r*	EC_{50} (μg/mL)	Regression eq	*r*	EC_{50} (μg/mL)
$\mathbf{4A}_1$	4	*p*-MeC_6H_4	H	$Y = 3.33 + 1.26x$	0.9179	21.22	$Y = 0.18 + 2.33x$	0.9982	115.94
$\mathbf{4A}_2$	4	*o*-ClC_6H_4	H	$Y = 1.63 + 2.18x$	0.9966	35.21	$Y = 0.20 + 2.61x$	0.9999	69.41
$\mathbf{4A}_3$	4	*p*-ClC_6H_4	H	$Y = 3.09 + 1.55x$	0.9669	16.97	$Y = 1.01 + 2.31x$	0.8748	53.44
$\mathbf{4A}_4$	4	*o*-BrC_6H_4	H	$Y = 0.03 + 2.92x$	0.9988	50.58	$Y = 1.98 + 1.80x$	0.8801	47.83
$\mathbf{4A}_5$	4	*m*-$CF_3C_6H_4$	H	$Y = 3.57 + 0.74x$	0.9867	85.24	$Y = 0.90 + 1.90x$	0.9686	143.88
$\mathbf{4A}_6$	4	2,3-$Me_2C_6H_3$	H	$Y = 2.93 + 1.50x$	0.9527	24.19	$Y = 2.37 + 1.48x$	0.9637	59.34
$\mathbf{4A}_7$	4	2,4-$Me_2C_6H_3$	H	$Y = 3.09 + 1.55x$	0.9669	16.97	$Y = -0.41 + 2.64x$	0.9907	111.82
$\mathbf{4A}_8$	4	2-Me-4-FC_6H_3	H	$Y = 2.59 + 1.53x$	0.9773	37.67	$Y = 2.82 + 1.02x$	0.9645	136.88
$\mathbf{4A}_9$	4	3,4-$Cl_2C_6H_3$	H	$Y = 3.40 + 1.86x$	0.9796	7.23	$Y = 2.48 + 1.82x$	0.9633	24.24
$\mathbf{4A}_{10}$	4	2-CF_3-4-ClC_6H_3	H	$Y = 4.53 + 1.44x$	0.9941	2.12	$Y = 1.45 + 2.12x$	0.9897	47.50
$\mathbf{4A}_{11}$	4	2,5-$(CF_3)_2C_6H_3$	H	$Y = 4.01 + 1.75x$	0.9832	3.66	$Y = 2.16 + 1.54x$	0.9974	70.52
$\mathbf{4A}_{12}$	4	3,5-$(CF_3)_2C_6H_3$	H	$Y = 3.98 + 1.71x$	0.9985	3.96	$Y = 1.56 + 2.49x$	0.9934	24.15
$\mathbf{4A}_{13}$	4	$CH_2CH_2OCH_2CH_2$		$Y = 3.57 + 0.74x$	0.9867	85.24	$Y = 1.54 + 1.37x$	0.9989	331.16
$\mathbf{4A}_{14}$	4		H	$Y = 3.57 + 0.98x$	0.9538	28.63	$Y = 1.20 + 1.66x$	0.9886	197.42
$\mathbf{4B}_1$	5	2,3-$Me_2C_6H_3$	H	$Y = 3.25 + 1.64x$	0.9865	11.62	$Y = 2.52 + 1.48x$	0.9946	47.56
$\mathbf{4B}_2$	5	3,4-$Cl_2C_6H_3$	H	$Y = 4.41 + 1.56x$	0.9701	2.38	$Y = 2.59 + 1.98x$	0.9867	16.37
$\mathbf{4B}_3$	5	3,5-$(CF_3)_2C_6H_3$	H	$Y = 4.51 + 1.28x$	0.9930	2.43	$Y = 3.29 + 1.89x$	0.9992	8.05
Procymidone				$Y = 3.46x + 3.66$	0.9920	2.45			
Chlorothalonil							$Y = 4.08 + 1.08x$	0.9924	7.16

gicidal activity (the EC_{50} value is 8.05 μg/mL), and is comparable with that of chlorothalonil (the EC_{50} value is 7.16 μg/mL).

2.3. 3D QSAR of compounds 4 against *B. cinerea Pers*

Statistically significant and chemically meaningful CoMFA models were developed using the data of fungicidal activity of 14 compounds **4** against *B. cinerea Pers*. The results of the CoMFA studies are summarized in Table 2.

The model M1, in which the q^2 values are 0.000 (leave-one-out) and 0.149 (cross-validate), respectively, was bad. $4A_{13}$ and $4A_{14}$ were deleted to develop a better model (model M4) which showed a predictive ability with a leave-one-out q^2 value of 0.552 and a cross-validate q^2 value of 0.572. The non-cross-validated r^2 value was 0.969, $F = 42.229$, standard error of estimate was 4.646. The contributions of the steric and the electrostatic field to the activity were 55.8% and 44.2%, respectively, by PLS analysis. Figure 1 shows the distribution of the steric and electrostatic fields of the model M4. Yellow regions nearly surrounding the positions 4 and 5 of the benzene ring indicate that small groups will improve the activity. Yellow and green regions near position 2 of the benzene ring, according to the proportion of two types of regions, indicate that suitable small groups tend to increase the activity. A yellow region pointed to the parent ring shows that the activity will be increased, if the bulk of the ring is enlarged suitably. Red and blue regions near positions 2 and 5, according to the proportion of two types of regions, indicate that suitable electronegative groups are favorable to the increase of the activity. Five new compounds (**4P**$_1$–**4P**$_5$) were designed, synthesized, and evaluated based on the CoMFA result (Table 3).

The results showed that the predicated values (the EC_{50} values are 31.34, 4.52, 16.93, 31.63, and 18.31 μg/mL, respectively) are in accordance with the experimental values (the EC_{50} values are 41.98, 6.05, 6.44, 29.45 and 28.90 μg/mL, respectively) in the order of magnitude. In the other words, the CoMFA model 4 has a better predicative ability. The results also showed that α-oxocyclododecyl group may be too large to obtain the deriv-

Table 2. CoMFA models of compounds **4**

Models	Deleted compounds	q^2		No validation		
		Leave-one-out	Cross-validate	Standard error of estimate	r^2	*F* values
M1	0	0.000	0.149			
M2	**4A**$_{13}$	0.447	0.422			
M3	**4A**$_{14}$	−0.019	−0.039			
M4	**4A**$_{13}$, **4A**$_{14}$	0.552	0.572	4.646	0.969	42.229

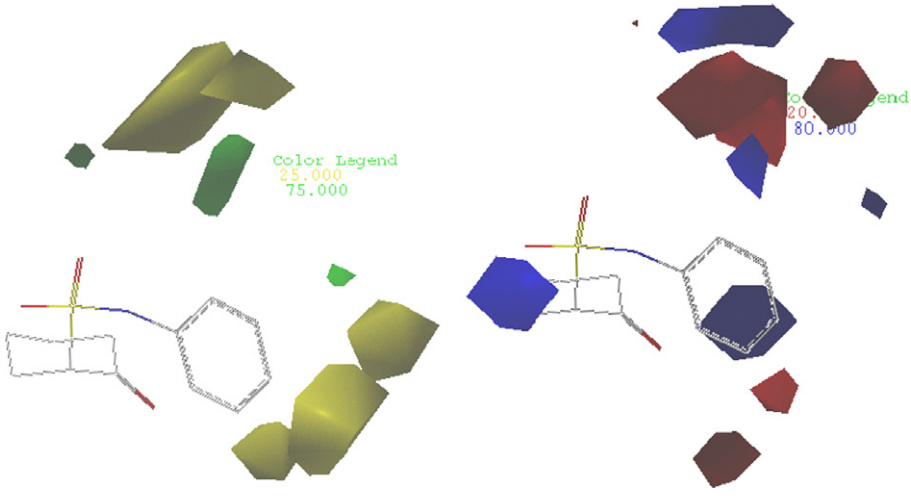


Figure 1. Steric (left) and electrostatic (right) contour map of CoMFA M4 represented by the skeleton of α-oxocyclohexylsulfonamide.

Table 3. The predicted and experimental EC_{50} values of designed compounds

Compound				Predicted value (EC_{50}, μg/mL)	Experimental value (EC_{50}, μg/mL)		
No	*n*	R′	R″		Regression eq	*r*	EC_{50} (μg/mL)
4P$_1$	4	4-FC_6H_4	H	31.34	$Y = 2.60 + 1.48x$	0.9712	41.98
4P$_2$	4	2,5-$Cl_2C_6H_3$	H	4.52	$Y = 3.55 + 1.85x$	0.9852	6.05
4P$_3$	10	2,3-$Me_2C_6H_3$	H	11.31	$Y = 4.37 + 0.78x$	0.9279	6.44
4P$_4$	10	3,4-$Cl_2C_6H_3$	H	16.93	$Y = 3.95 + 0.72x$	0.9410	29.45
4P$_5$	10	3,5-$(CF_3)_2C_6H_3$	H	31.63	$Y = 3.57 + 0.98x$	0.9229	28.90

atives possessing a high activity, and only two substituents on the benzene ring are not enough to obtain the compounds possessing a high activity. Based on the above results, the second group of compounds in which the parent rings are cyclohexyl and cycloheptal and phenyl bearing two or three substituents on the side chain were designed and their fungicidal activity (EC_{50} values) was predicated (Table 4).

The predicated results showed that the seven-membered ring derivatives are more active than the six-membered ring derivatives for the same substituted pattern, and the derivatives bearing three substituents on the benzene ring are more active than those bearing two substituents on the benzene ring. Among the designed compounds, $\mathbf{4P}_{15}$ and $\mathbf{4P}_{16}$ have the best predicated activity against *B. cinerea Pers* (the predicted EC_{50} values of $\mathbf{4P}_{15}$ and $\mathbf{4P}_{16}$ are 0.50 and 0.12, respectively). Therefore, the compounds possessing a still better activity may be obtained through structural modification. Further work has been planned.

2.4. Antitumor activity

Compounds **4** (**4A** and **4B**) were evaluated for their ability to inhibit human tumor cells HL-60, BGC-823, Bel-7402, and KB (Table 5). Among them, compounds $\mathbf{4A}_{11}$, $\mathbf{4A}_{12}$, and $\mathbf{4B}_3$ have high inhibitory activity against HL-60 (the inhibition rate exceeds 90% at 100 μg/mL) and a good inhibitory activity against BGC-823, Bel-7402, and KB (the inhibition rates exceed 50% except $\mathbf{4B}_3$ against Bel-7402). In addition, compound $\mathbf{4A}_{10}$ has a good inhibitory activity against HL-60 (inhibition rate is 78.61% at 100 μg/mL). It is interesting to point out that the antitumor activities of compounds **4** are in accordance with their fungicidal activity to a great extent.

Trifluoromethyl group on the benzene ring may play a more important role in the antitumor activity than in the fungicidal activity: compounds $\mathbf{4A}_{11}$, $\mathbf{4A}_{12}$, and $\mathbf{4B}_3$ bearing two trifluoromethyls on the benzene ring have good activities against four human tumor cells and plant pathogen *B. cinerea Pers*; however, $\mathbf{4A}_{10}$ bearing one trifluoromethyl on the benzene ring has a good fungicidal activity against *B. cinerea Pers* and a good antitumor activity only against HL-60; and $\mathbf{4B}_1$ and $\mathbf{4B}_2$ having no trifluoromethyl on the benzene ring have a good fungicidal activity and a low antitumor activity.

Table 4. The predicted EC_{50} values of second group of designed compounds

Compound				Predicted EC_{50} values (μg/mL)
No	*n*	R′	R″	
$\mathbf{4P}_6$	4	2-CF_3-4-FC_6H_3	H	3.64
$\mathbf{4P}_7$	4	2-CF_3-4-BrC_6H_3	H	1.20
$\mathbf{4P}_8$	4	2-CF_3-4-OHC_6H_3	H	1.47
$\mathbf{4P}_9$	4	2-CF_3 -4,5-$Cl_2C_6H_2$	H	0.83
$\mathbf{4P}_{10}$	4	2-CF_3-4-Cl-5-BrC_6H_2	H	0.76
$\mathbf{4P}_{11}$	5	2-CF_3-4-FC_6H_3	H	1.10
$\mathbf{4P}_{12}$	5	2-CF_3-4-ClC_6H_3	H	1.90
$\mathbf{4P}_{13}$	5	2-CF_3-4-BrC_6H_3	H	0.51
$\mathbf{4P}_{14}$	5	2-CF_3-4-OHC_6H_3	H	1.29
$\mathbf{4P}_{15}$	5	2-CF_3-4,5-$Cl_2C_6H_2$	H	0.50
$\mathbf{4P}_{16}$	5	2-CF_3-4-Cl-5-BrC_6H_2	H	0.12

Table 5. Inhibition of compounds **4** to human tumor cell lines at 100 μg/mL

Compound	Inhibition rate (%)			
	HL-60	BGC-823	Bel-7402	KB
$\mathbf{4A}_1$	−3.55	32.05	27.46	−22.21
$\mathbf{4A}_2$	0.29	29.18	1.98	−10.06
$\mathbf{4A}_3$	−12.96	16.03	4.47	−3.83
$\mathbf{4A}_4$	15.35	29.58	22.13	−14.39
$\mathbf{4A}_5$	16.98	21.65	11.40	−6.30
$\mathbf{4A}_6$	9.38	24.47	21.76	−20.62
$\mathbf{4A}_7$	12.15	15.63	0.52	7.97
$\mathbf{4A}_8$	−0.99	28.68	3.22	−3.72
$\mathbf{4A}_9$	13.47	5.54	1.39	17.75
$\mathbf{4A}_{10}$	78.61	36.35	20.95	11.22
$\mathbf{4A}_{11}$	95.67	57.72	68.31	68.61
$\mathbf{4A}_{12}$	95.88	70.76	66.93	75.95
$\mathbf{4A}_{13}$	10.97	21.32	6.01	−4.35
$\mathbf{4A}_{14}$	2.62	26.41	39.26	2.73
$\mathbf{4B}_1$	22.88	23.84	12.09	2.01
$\mathbf{4B}_2$	15.04	28.88	4.03	−6.30
$\mathbf{4B}_3$	95.79	56.95	45.27	58.97

3. Conclusion

2-Oxocycloalkylsulfonamides (**4A** and **4B**) obtained by the replacement of the benzene ring of traditional sulfonamide fungicide with saturated six- and seven-membered rings have fair to excellent fungicidal activities against *B. cinerea Pers* and *S. sclerotiorum*. Among them, compounds $\mathbf{4A}_{10}$, $\mathbf{4A}_{11}$, $\mathbf{4A}_{12}$, $\mathbf{4B}_2$, and $\mathbf{4B}_3$ displayed excellent fungicidal activity against *B. cinerea Pers*, and are comparable with commercial fungicide procymidone. 3D QSAR study indicated that their fungicidal activity may be further improved through structural modification. The fact that the antitumor activity of compounds **4A** and **4B** against four human tumor cell lines in accordance with their fungicidal activity to a great extent lays a foundation for further research.

4. Materials and methods

4.1. Chemical synthesis

4.1.1. General. Infrared spectra were recorded in potassium bromide discs on a Shimadzu IR-435 spectrophotometer; NMR spectra were recorded in $CDCl_3$ with a Bruker DPX300 spectrometer, using TMS as an internal standard; elemental analysis was performed by the analytical center in the Institute of Chemistry (Beijing), Chinese Academy of Science; melting points were measured on a Yanagimoto melting point apparatus and are uncorrected. The solvents and reagents were used as received or were dried prior to use as needed.

4.1.2. Potassium 2-oxocycloalkylsulfonamides (5). Compounds **5A**, **5B**, and **5C** were prepared according to the method given in Ref. 4.

4.1.3. General synthetic procedure for *N*-aryl-2-oxocycloalkylsulfonamides (4). To the slurry of **5** (0.009 mol) and DMF (0.05 mL) in methylene chloride (15 mL) was added oxalyl chloride (0.80 mL,

0.009 mol); the mixture was stirred at 5–15 °C for 1 h. After the slurry had been cooled in ice-water bath, it was filtered at reduced pressure. The filtrate was added to the solution of arylamine (0.008 mol) and triethylamine in methylene chloride (10 mL) at 5–10 °C. After being stirred at rt for 2 h, water (10 mL) was poured into the mixture. The organic layer was separated, washed with 6 mol HCl (2× 5 mL) and water (2× 5 mL), and dried over sodium sulfate. After evaporating the solvent in vacuum, the crude product was recrystallized from the acetone/petroleum ether to give pure **4**.

4.1.3.1. *N*-(4-Methylphenyl)-α-oxocyclohexylsulfonamide ($4A_1$). White crystals, mp 103–104 °C; yield, 68%; IR (KBr, cm^{-1}) ν_{max}: 3200, 2950, 2880, 1700, 1590, 1340, 1160, 815; ^1H NMR, δ: 1.58–2.16 (m, 5H), 2.20–2.67 (m, 3H), 2.32 (s, 3H, CH_3), 3.72 (ddd, 1H, J = 10.41, 5.94, 0.85 Hz), 6.79 (s, 1H, N–H), 7.11 (s, 4H). Anal. Calcd for $C_{13}H_{17}NO_3S$: C, 58.40; H, 6.41; N, 5.24. Found: C, 58.56; H, 6.46; N, 5.11.

4.1.3.2. *N*-(2-Chlorophenyl)-α-oxocyclohexylsulfonamide ($4A_2$). White crystals, mp 112–113 °C; yield, 84%; IR (KBr, cm^{-1}) ν_{max}: 3250, 2920, 2850, 1705, 1590, 1330, 1160; ^1H NMR, δ: 1.66–1.84 (m, 2H), 2.00–2.16 (m, 3H), 2.36–2.40 (m, 1H), 2.46–2.49 (m, 1H), 2.62–2.68 (m, 1H), 3.92 (ddd, 1H, J = 10.64, 5.74, 0.92 Hz), 7.08–7.13 (m, 1H, Ar–H), 7.23–7.28 (m, 1H, Ar–H), 7.35 (s, 1H, NH), 7.37 (dd, 1H, J = 8.04, 1.41 Hz), 7.67 (dd, 1H, J = 8.17, 1.37 Hz). Anal. Calcd for $C_{12}H_{14}ClNO_3S$: C, 50.59; H, 4.90; N, 4.87. Found: C, 50.10; H, 4.94; N, 4.81.

4.1.3.3. *N*-(4-Chlorophenyl)-α-oxocyclohexylsulfonamide ($4A_3$). White crystals, mp 81–83 °C; yield, 70%; IR (KBr, cm^{-1}) ν_{max}: 3250, 2870, 2950, 1710, 1600, 1315, 1160, 810; ^1H NMR, δ: 1.61–1.83 (m, 2H), 1.98–2.08 (m, 2H), 2.11–2.17 (m, 1H), 2.32–2.36 (m, 1H), 2.43–2.45 (m, 1H), 2.60–2.67 (m, 1H), 3.72 (ddd, 1H, J = 10.52, 5.92, 0.97 Hz), 6.95 (s, 1H, NH), 7.15–7.19 (m, 2H, CH), 7.26–7.31 (m, 2H). Anal. Calcd for $C_{12}H_{14}ClNO_3S$: C, 50.59; H, 4.90; N, 4.87. Found: C, 50.11; H, 4.91; N, 4.80.

4.1.3.4. *N*-(2-Bromophenyl)-α-oxocyclohexylsulfonamide ($4A_4$). White crystals, mp 75–76 °C; yield, 77%; IR (KBr, cm^{-1}) ν_{max}: 3300, 2920, 2850, 1710, 1590, 1330, 1160; ^1H NMR, δ: 1.66–1.85 (m, 2H), 2.00–2.18 (m, 3H), 2.34–2.40 (m, 1H), 2.47–2.53 (m, 1H), 2.61–2.68 (m, 1H), 3.90 (dd, 1H, J = 10.82, 5.74 Hz), 7.00–7.06 (m, 1H, Ar–H), 7.27–7.32 (m, 1H, Ar–H), 7.35 (s, 1H, NH), 7.54 (dd, 1H, J = 8.04, 1.44 Hz), 7.67 (dd, 1H, J = 8.22, 1.51 Hz). Anal. Calcd for $C_{12}H_{14}BrNO_3S$: C, 43.38; H, 4.25; N, 4.22. Found: C, 42.84; H, 4.18; N, 4.22.

4.1.3.5. *N*-(3-Trifluoromethylphenyl)-α-oxocyclohexylsulfonamide ($4A_5$). White crystals, mp 103–104 °C; yield, 73%; IR (KBr, cm^{-1}) ν_{max}: 3300, 2950, 2880, 1710, 1600, 1310, 1160; ^1H NMR, δ: 1.65–1.84 (m, 2H), 1.99–2.18 (m, 3H), 2.34–2.39 (m, 1H), 2.46–2.49 (m, 1H), 2.62–2.69 (m, 1H), 3.76 (ddd, 1H, J = 10.51, 5.96, 0.81 Hz), 7.19 (s, 1H, NH), 7.40–7.50 (m, 4H, Ar–H). Anal. Calcd for $C_{13}H_{14}F_3NO_3S$: C, 48.59; H, 4.39; N, 4.36. Found: C, 48.76; H, 4.48; N, 4.78.

4.1.3.6. *N*-(2,3-Dimethylphenyl)-α-oxocyclohexylsulfonamide ($4A_6$). White crystals, mp 71–72 °C; yield, 80%; IR (KBr, cm^{-1}) ν_{max}: 3200, 2920, 2850, 1690, 1580, 1310, 1160; ^1H NMR, δ: 1.62–1.69 (m, 1H), 1.86–1.87 (m, 1H), 1.98–2.06 (m, 2H), 2.25 (s, 3H), 2.29 (s, 3H), 2.20–2.47 (m, 3H), 2.65–2.72 (m, 1H), 3.90 (ddd, 1H, J = 10.11, 5.96, 1.04 Hz), 6.67 (s, 1H, N–H), 7.03–7.09 (m, 2H), 7.16–7.20 (m, 1H). Anal. Calcd for $C_{14}H_{19}NO_3S$: C, 59.76; H, 6.81; N, 4.98. Found: C, 59.80; H, 6.80; N, 4.88.

4.1.3.7. *N*-(2,4-Dimethylphenyl)-α-oxocyclohexylsulfonamide ($4A_7$). Yellow crystals, mp 84–86 °C; yield, 60%; IR (KBr, cm^{-1}) ν_{max}: 3300, 2950, 2880, 1700, 1610, 1315, 1160, 800; ^1H NMR, δ: 1.61–1.69 (m, 1H), 1.85–1.87 (m, 1H), 1.97–2.08 (m, 2H), 2.18–2.45 (m, 9H), 2.66–2.71 (m, 1H), 3.89 (ddd, 1H, J = 10.02, 5.94, 1.04 Hz), 6.58 (s, 1H, N–H), 6.95–7.01 (m, 2H), 7.24–7.27 (m, 1H). Anal. Calcd for $C_{14}H_{19}NO_3S$: C, 59.76; H, 6.81; N, 4.98. Found: C, 59.89; H, 6.86; N, 4.94.

4.1.3.8. *N*-(4-Fluoro-2-methylphenyl)-α-oxocyclohexylsulfonamide ($4A_8$). White crystals, mp 107–108 °C; yield, 76%; IR (KBr, cm^{-1}) ν_{max}: 3200, 2920, 2860, 1690, 1610, 1310, 1140; ^1H NMR, δ: 1.69–1.85 (m, 2H), 2.02–2.19 (m, 3H), 2.37–2.40 (m, 1H), 2.53–2.70 (m, 2H), 3.81 (ddd, 1H, J = 10.67, 5.92, 0.81 Hz), 7.46 (s, 1H, NH), 7.69 (s, 3H). Anal. Calcd for $C_{13}H_{16}FNO_3S$: C, 54.72; H, 5.65; N, 4.91. Found: C, 54.87; H, 5.66; N, 5.08.

4.1.3.9. *N*-(3,4-Dichlorophenyl)-α-oxocyclohexylsulfonamide ($4A_9$). White crystals, mp 109–110 °C; yield, 75%; IR (KBr, cm^{-1}) ν_{max}: 3300, 2950, 1700, 1595, 1320, 1150, 820; ^1H NMR, δ: 1.61–1.84 (m, 2H), 1.99–2.18 (m, 3H), 2.34–2.37 (m, 1H), 2.47–2.51 (m, 1H), 2.61–2.68 (m, 1H), 3.75 (ddd, 1H, J = 10.68, 5.95, 0.86 Hz), 6.99 (s, 1H, N–H), 7.09 (dd, 1H, J = 8.70, 2.52 Hz), 7.37 (d, 1H, J = 2.60 Hz), 7.38 (d, 1H, J = 8.60 Hz). Anal. Calcd for $C_{12}H_{13}Cl_2NO_3S$: C, 44.73; H, 4.07; N, 4.35. Found: C, 44.92; H, 4.13; N, 4.27.

4.1.3.10. *N*-(4-Chloro-2-trifluoromethylphenyl)-α-oxocyclohexylsulfonamide ($4A_{10}$). White crystals, mp 119–120 °C; yield, 78%; IR (KBr, cm^{-1}) ν_{max}: 3330, 2950, 2880, 1710, 1600, 1310, 1120; ^1H NMR, δ: 1.67–1.81 (m, 2H), 2.04–2.11 (m, 3H), 2.30–2.38 (m, 1H), 2.54–2.66 (m, 2H), 3.76 (dd, 1H, J = 12.45, 6.38 Hz), 7.30 (s, 1H, NH), 7.51 (dd, 1H, J = 8.80, 2.45 Hz), 7.59 (d, 1H, J = 2.45 Hz), 7.70 (d, 1H, J = 8.80 Hz). Anal. Calcd for $C_{13}H_{13}ClF_3NO_3S$: C, 43.89; H, 3.68; N, 3.94. Found: C, 43.74; H, 3.64; N, 3.99.

4.1.3.11. *N*-[2,5-Di(trifluoromethyl)phenyl]-α-oxocyclohexylsulfonamide ($4A_{11}$). White crystals, mp 107–108 °C; yield, 83%; IR (KBr, cm^{-1}) ν_{max}: 3330, 2950, 2880, 1710, 1600, 1310, 1120; ^1H NMR, δ: 1.69–1.82 (m, 2H), 2.07–2.17 (m, 3H), 2.31–2.42 (m, 1H), 2.59–2.67 (m, 2H), 3.99 (dd, 1H, J = 11.91, 5.58 Hz), 7.51 (s, 1H), 7.54 (s, 1H, NH), 7.75 (d, 1H, J = 8.25 Hz), 8.05 (s, 1H). Anal. Calcd

for $C_{14}H_{13}F_6NO_3S$: C, 43.19; H, 3.37; N, 3.60. Found: C, 43.19; H, 3.52; N, 4.11.

4.1.3.12. *N*-[3,5-Di(trifluoromethyl)phenyl]-α-oxocyclohexylsulfonamide ($4A_{12}$). White crystals, mp 91–92 °C; yield, 80%; IR (KBr, cm^{-1}) ν_{max}: 3200, 2920, 2860, 1690, 1610, 1310, 1140; ^1H NMR, δ: 1.69–1.85 (m, 2H), 2.02–2.19 (m, 3H), 2.37–2.40 (m, 1H), 2.53–2.70 (m, 2H), 3.81 (ddd, 1H, J = 10.67, 5.92, 0.81 Hz), 7.46 (s, 1H, NH), 7.69 (s, 3H). Anal. Calcd for $C_{14}H_{13}F_6NO_3S$: C, 43.19; H, 3.37; N, 3.60. Found: C, 43.32; H, 3.36; N, 3.75.

4.1.3.13. *N*-(α-Oxocyclohexylsulfonyl)morpholine ($4A_{13}$). White crystals, mp 90 °C; yield, 80%; IR (KBr, cm^{-1}) ν_{max}: 3400, 2920, 2850, 1700, 1590, 1330, 1160; ^1H NMR, δ: 1.71–1.80 (m, 2H), 2.03–2.20 (m, 3H), 2.43–2.55 (m, 2H), 2.74–2.85 (m, 1H), 3.30–3.33 (m, 4H), 3.71 (t, 4H, J = 4.7)3.72–3.81 (m, 1H, J = 4.20, 1.20 Hz). Anal. Calcd for $C_{10}H_{17}NO_4S$: C, 48.57; H, 6.93; N, 5.66. Found: C, 48.12; H, 6.81; N, 5.81.

4.1.3.14. *N*-(4,6-Dimethoxy-2-pyrimidyl)-α-oxocyclohexylsulfonamide ($4A_{14}$). White crystals, mp 129–130 °C; yield, 72%; IR (KBr, cm^{-1}) ν_{max}: 3250, 2950, 1720, 1600, 1330, 1160; ^1H NMR, δ: 1.70–1.80 (m, 1H), 1.89–2.00 (m, 2H), 2.10–2.19 (m, 1H), 2.38–2.54 (m, 3H), 2.70–2.80 (m, 1H), 3.88 (s, 6H, OCH_3), 4.77 (t, 1H, J = 6.74 Hz), 5.75 (s, 1H), 7.28 (s, 1H, N–H). Anal. Calcd for $C_{12}H_{17}N_3O_3S$: C, 45.70; H, 5.43; N, 13.33. Found: C, 45.87; H, 5.48; N, 13.04.

4.1.3.15. *N*-(2,3-Dimethylphenyl)-α-oxocycloheptylsulfonamide ($4B_1$). White crystals, mp 142–143 °C; yield, 75%; IR (KBr, cm^{-1}) ν_{max}: 3200, 2920, 2850, 1700, 1580, 1320, 1150; ^1H NMR, δ: 1.28–1.51 (m, 3H), 1.88–2.11 (m, 4H), 2.30 (s, 6H), 2.33–2.39 (m, 1H), 2.58–2.65 (m, 1H), 2.79–2.88 (m, 1H), 3.98 (dd, 1H, J = 11.08, 4.51 Hz), 6.56 (s, 1H, NH), 7.04–7.11 (m, 2H), 7.36–7.39 (m, 1H). Anal. Calcd for $C_{15}H_{21}NO_3S$: C, 60.99; H, 7.17; N, 4.74. Found: C, 60.87; H, 7.04; N, 4.86.

4.1.3.16. *N*-(3,4-Dichlorophenyl)-α-oxocycloheptylsulfonamide ($4B_2$). White crystals, mp 122–123 °C; yield, 80%; IR (KBr, cm^{-1}) ν_{max}: 3200, 2920, 2850, 1680, 1590, 1330, 1160; ^1H NMR, δ: 1.30–1.51 (m, 3H), 1.86–2.11 (m, 4H), 2.31–2.39 (m, 1H), 2.60–2.67 (m, 1H), 2.75–2.84 (m, 1H), 3.81 (dd, 1H, J = 11.11, 4.24 Hz), 7.05 (s, 1H, NH), 7.21 (dd, 1H, J = 8.66, 2.53 Hz), 7.41 (d, 1H, J = 8.67 Hz), 7.50 (d, 1H, J = 2.52 Hz). Anal. Calcd for $C_{13}H_{15}Cl_2NO_3S$: C, 46.44; H, 4.50; N, 4.17. Found: C, 46.07; H, 4.47; N, 4.19.

4.1.3.17. *N*-[3,5-Di(trifluoromethyl)phenyl]-α-oxocycloheptylsulfonamide ($4B_3$). White crystals, mp 86–87 °C; yield, 88%; IR (KBr, cm^{-1}) ν_{max}: 3250, 2920, 2850, 1690, 1610, 1330, 1140; ^1H NMR, δ: 1.26–1.53 (m, 3H), 1.88–2.12 (m, 4H), 2.35–2.42 (m, 1H), 2.63–2.69 (m, 1H), 2.76–2.86 (m, 1H), 3.82 (dd, 1H, J = 11.04, 4.08 Hz), 7.31 (s, 1H, NH), 7.71 (s, 1H), 7.79 (s, 2H). Anal. Calcd for $C_{15}H_{15}F_6NO_3S$: C, 44.67; H, 3.75; N, 3.47. Found: C, 44.78; H, 3.74; N, 3.55.

4.1.3.18. *N*-(4-Fluorophenyl)-α-oxocyclohexylsulfonamide ($4P_1$). White crystals, mp 115–116 °C; yield, 71%; IR (KBr, cm^{-1}) ν_{max}: 3300, 2950, 2880, 1700, 1600, 1310, 1160; ^1H NMR, δ: 1.63–1.67 (m, 1H), 1.81–1.84 (m, 1H), 1.97–2.05 (m, 2H), 2.09–2.18 (m, 1H), 2.34–2.45 (m, 2H), 2.62–2.69 (m, 1H), 3.72 (ddd, 1H, J = 10.18, 5.99, 0.95 Hz), 6.93 (s, 1H, N–H), 6.98–7.06 (m, 2H), 7.18–7.26 (m, 2H) Anal. Calcd for $C_{12}H_{14}FNO_3S$: C, 53.52; H, 5.20; N, 5.16. Found: C, 53.26; H, 5.24; N, 4.21.

4.1.3.19. *N*-(2,5-Dichlorophenyl)-α-oxocyclohexylsulfonamide ($4P_2$). Yellow crystals, mp 125–126 °C; yield, 69%; IR (KBr, cm^{-1}) ν_{max}: 3300, 2950, 2870, 1710, 1585, 1340, 1160, 850; ^1H NMR, δ: 1.67–1.84 (m, 2H), 2.03–2.16 (m, 3H), 2.35–2.39 (m, 1H), 2.53–2.68 (m, 2H), 3.92 (ddd, 1H, J = 10.92, 5.73, 0.93 Hz), 7.07 (dd, 1H, J = 8.58, 2.4 Hz), 7.30 (d, 1H, J = 8.61 Hz), 7.40 (s, 1H, N–H), 7.71 (d, 1H, J = 2.4 Hz). Anal. Calcd for $C_{12}H_{13}Cl_2NO_3S$: C, 44.73; H, 4.07; N, 4.35. Found: C, 44.87; H, 4.07; N, 4.21.

4.1.3.20. *N*-(2,3-Dimethylphenyl)-α-oxocyclododecylsulfonamide ($4P_3$). White crystals, mp 116–117 °C; yield, 80%; IR (KBr, cm^{-1}) ν_{max}: 3250, 2920, 2850, 1700, 1580, 1320, 1150; ^1H NMR, δ: 1.27–1.42 (m, 14H), 1.56–1.63 (m, 1H), 1.79–1.96 (m, 2H), 2.25 (s, 3H), 2.30 (s, 3H), 2.71–2.89 (m, 2H), 4.22 (dd, 1H, J = 11.78, 2.88 Hz), 6.25 (s, 1H, NH), 7.05–7.12 (m, 2H), 7.29–7.32 (m, 1H). Anal. Calcd for $C_{20}H_{31}NO_3S$: C, 65.72; H, 8.55; N, 3.83. Found: C, 65.61; H, 8.42; N, 3.70.

4.1.3.21. *N*-(3,4-Dichlorophenyl)-α-oxocyclododecylsulfonamide ($4P_4$). White crystals, mp 144–145 °C; yield, 74%; IR (KBr, cm^{-1}) ν_{max}: 3250, 2920, 2850, 1710, 1590, 1320, 1140; ^1H NMR, δ: 1.28–1.42 (m, 14H), 1.72–1.90 (m, 3H), 2.15–2.25 (m, 1H), 2.67–2.77 (m, 1H), 2.83–2.92 (m, 1H), 4.31 (dd, 1H, J = 11.52, 3.03 Hz), 6.92 (s, 1H, NH), 7.14 (dd, 1H, J = 8.67, 2.58 Hz), 7.38–7.42 (m, 2H). Anal. Calcd for $C_{18}H_{25}Cl_2NO_3S$: C, 53.20; H, 6.20; N, 3.45. Found: C, 52.88; H, 6.11; N, 3.43.

4.1.3.22. *N*-[3,5-Di(trifluoromethyl)phenyl]-α-oxocyclododecylsulfonamide ($4P_5$). White crystals, mp 85–86 °C; yield, 71%; IR (KBr, cm^{-1}) ν_{max}: 3200, 2920, 2850, 1690, 1610, 1340, 1140; ^1H NMR, δ: 1.30–1.42 (m, 14H), 1.76–1.93 (m, 3H), 2.13–2.21 (m, 1H), 2.65–2.74 (m, 1H), 2.90–2.99 (m, 1H), 4.39 (dd, 1H, J = 11.32, 3.16 Hz), 6.99 (s, 1H, NH), 7.68 (s, 1H), 7.73 (s, 2H). Anal. Calcd for $C_{20}H_{25}F_6NO_3S$: C, 50.73; H, 5.32; N, 2.96. Found: C, 50.54; H, 5.51; N, 2.88.

4.2. Fungicidal activities of compounds 4

4.2.1. Method. Fungicidal activities of the title compounds against *B. cinerea Pers* and *S. sclerotiorum* were evaluated in vitro using the method given in Ref. 4.

4.2.2. Fungicidal activities. The inhibition rates of compounds **4** against the above-mentioned two fungi were determined at concentrations of 100, 50, 25, 12.5, and 6.25 μg/mL, respectively. EC_{50} values were estimated

using logit analysis.[5] The results are shown in Table 1. Commercial fungicides procymidone or chlorothalonil were used as control in the above bioassay.

4.3. 3D QSAR of compounds 4 against *B. cinerea Pers*

4.3.1. Method and software. CoMFA (Comparative Molecular Field Analysis)[6] was used and all molecular modeling calculations utilized the software of Sybyl 7.0 (Tripos Inc., USA) running on the Silicon Graphics Fuel Visual workstation (Silicon Graphics Inc., USA).

4.3.2. Optimization. Minimum energy conformations of all the structures were calculated using the Minimize module of Sybyl 7.0. The force field was MMFF94[7] with an 8 Å cutoff for non-bonded interactions, and the atomic point charges were also calculated by MMFF94. The minimizations were implemented with the steepest descent method for the first 100 steps, followed by the BFGS method[8] until the RMS of gradient was less than 0.005 kcal/(mol Å).

4.3.3. CoMFA model. The alignment procedure was calculated with the Align Database module.[9] The best active compound $\mathbf{4A}_{10}$ was selected as the template, $C(O)CS(O_2)N$ was chosen as the common substructure, and all of the compounds ($\mathbf{4A}_1$–$\mathbf{4A}_{14}$, $\mathbf{4B}_1$–$\mathbf{4B}_3$) were aligned to a new database.

All CoMFA calculations were done with the Tripos CoMFA module.[9] For the CoMFA calculation, the alignment molecules were placed in a 3D cubic lattice with 2 Å spacing. The default sp^3 carbon atom with +1 charge was selected as the probe atom for the calculation of the steric (Lennard-Jones 6–12 potential) and electrostatic fields (Coulombic potential) around the aligned molecules with a distance-dependent dielectric constant at all lattice points. The column filtering was set to 1.0 kcal/mol. Values of steric and electrostatic energies were truncated at 30 kcal/mol.

The regression analysis was carried out using the partial least squares (PLS) method.[10] To check the statistical significance of the models, leave-one-out method and cross-validate method were done with SAMPLS method.[11] The optimum number of components to be used in the conventional analysis was defined as that which yielded the highest cross-validated q^2. The results of the CoMFA studies are summarized in Table 2.

4.3.4. Design of new compounds and prediction of fungicidal activity. Five new compounds ($\mathbf{4P_1}$-$\mathbf{4P_5}$) were designed and synthesized based on the CoMFA results. For their physical and elemental data, see Table 1 and the ^{1}H NMR and IR data, see Table 2. Their fungicidal activity (EC_{50}) against *B. cinerea Pers* was evaluated using the method given in Section 2.3 and the predicted EC_{50} values were calculated by the predict property method in QSAR module. The results are shown in Table 3.

4.4. Antitumor activity of compounds 4

Human promyelocytic leukemic cell line (HL 60), human gastric carcinoma cell line (BGC-823), human hepatocellular carcinoma cell line (Bel-7402), and human nasopharyngeal carcinoma cell line (KB) were grown and maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum, penicillin (10 U mL^{-1}), and streptomycin (100 μg mL^{-1}) at 37 °C in a humidified incubator in an atmosphere of 5% CO_2. All the experiments were performed on exponentially growing cancer cells.

HL-60 cell line was assayed by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazoliumbromide] method (Roche Molecular Biochemicals, 1465-007). BGC-823, Bel-7402 and KB were assayed by SRB method.[12] The results are listed in Table 5.

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