Design, synthesis, antifungal activity, and 3D-QSAR of coumarin derivatives

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In our research, a series of 8-substituted coumarin derivatives were synthesized, and their structures were confirmed by FT-IR, 1H-NMR, and MS (or HRMS). In activity screening, the synthesized compounds exhibited potent antifungal activity against 4 phytopathogenic fungi: Botrytis cinerea, Colletotrichum gloeosporioides, Fusarium oxysporum, and Valsa mali. Notably, 8-chloro coumarin and ethyl 8-chloro-coumarin-3-carboxylate showed the strongest fungus inhibition with EC50 of 0.085 and 0.078 mmol/L against V. mali. Furthermore, 3D-QSAR models (CoMFA and CoMSIA) of the title compounds against V. mali were established on the basis of their antifungal activities. The results indicated that the appropriate small, hydrophilic and electron-withdrawing groups on coumarin’s C-3 and C-8, respectively, could enhance the antifungal activity. The information obtained will be very helpful for designing new derivatives with high antifungal activities. © Pesticide Science Society of Japan

Keywords: 8-substituted coumarin, synthesis, antifungal activity, 3D-QSAR.

Introduction

The exploration and development of low-toxicity and high-performance fungicides have received considerable attention in recent years. Naturally occurring compounds are precious resources for new pesticide development. An important approach to optimizing the antifungal properties of organisms’ secondary metabolites has involved using them as the useful prototypes for further structure transformation and modification. Accordingly, strategies for designing and synthesizing botanical antifungal agents remain an ongoing, attractive research area.

Coumarins, which are abundant in higher plants, represent an important class of natural products with important applications. They exhibit a broad spectrum of biological activities, including anticancer, antioxidant, antiplasmodial, antimalarial, antivirus, antibacterial, and antifungal.1–7) The varied pharmacological activities and relatively simple structures of these compounds have spurred great interest in their possible therapeutic uses. In addition to coumarin’s use as the lead compound for the preparation of potent pharmacological molecules, it has also received considerable research attention for its interesting insecticidal, nematicidal, allelopathic, and antimicrobial activities in agriculture.8–13)

From the literature, we found that the antifungal activity of coumarins was certainly related to the nonpolar substituent at its 8-position.14) These findings have made coumarins a very attractive lead for further transformations at the 8-position with nonpolar substituents. In light of these interesting results and the continuation of our program aimed discovering natural products-based antifungal agents, a series of coumarin derivatives with a methyl, isopropyl, tert-butyl, allyl, phenyl, or chloro group on C-8 were designed, synthesized, and their antifungal activity against 4 phytopathogenic fungi were preliminarily evaluated. Based on the observed antifungal results, the three-dimensional quantitative structure-activity relationships (3D-QSAR) of the target compounds were also described.

Materials and Methods

1. General

All chemicals used were obtained from commercial sources and used without further purification. 2-Methyl-phenol, tert-butyl-phenol, 2-allyl-phenol, and 2-chloro-phenol were purchased from SA Chemical Technology Co., Ltd., Shanghai, China. 2-Phenyl-phenol was supplied by Xiya Reagents Co., Ltd.,
Chengdu, China. 2-i-Propyl-phenol was produced by Shanghai Demo Medical Tech. Co., Ltd., Shanghai, China. Acetic anhydride, benzeneacetonitrile, ethyl acetooacete, malonate paraformaldehyde, TBAB (tetrabutylammonium bromide), piperidine, triethylamine, and hydrous MgCl₂ were supplied by Sinopharm Chemical Reagent Co., Ltd., Shanghai, China. The inorganic bases and organic solvents were manufactured by Laiyang Kangde Chemical Co., Ltd., Laiyang, China. Thin-layer chromatography (TLC) was performed with silica gel plates using silica gel 60 GF254 (Qingdao Haiyang Chemical Co., Ltd., Qingdao, China). The melting points were measured using a WRS-1A digital melting point apparatus (Shanghai Precision Optical Instrument Co., Ltd., Shanghai, China) without calibration. IR spectra were recorded on a Nicolet 6700 infrared spectrophotometer (Thermo Electron, Madison, WI, USA) in KBr pellets. ¹H-NMR spectra were recorded on a Bruker Avance DRX-500 instrument (Bruker, Fällanden, Switzerland) in CDCl₃ solvent using tetramethylsilane as an internal standard. High-resolution mass spectra (HRMS) were recorded with a maXis Q-TOF instrument (Bruker, Karlsruhe, Germany).

### 2. Synthesis of target compounds

The synthetic routes of target compounds are outlined in Scheme 1. The intermediate 3-substituted salicylaldehydes were prepared by heating a mixture of the appropriate 2-substituted phenol, anhydrous MgCl₂, triethylamine, and paraformaldehyde under reflux in acetonitrile or tetrahydrofuran (THF) in accordance with the reported method. The 8-substituted coumarin derivatives (1a–1g) were synthesized in good yield (66–97%) using a base-catalyzed Perkin condensation reaction of appropriately 2-hydroxy-3-substituted benzaldehyde and acetic anhydride in K₂CO₃/TBAB. Under the optimized conditions, target compounds 2a–2g were obtained from benzeneacetonitrile and 2-hydroxy-3-substituted benzaldehyde by the Knoevenagel condensation reaction. In contrast to the traditional methodology of using an organic solvent, water used as the solvent in this reaction was free of pollution and environmentally safe. Subsequently, as shown in Scheme 1, a wide range of target compounds, 3a–3e and 4a–4e, were all efficiently furnished by the Knoevenagel reaction too. The synthesized target compounds were purified by silica gel column chromatography (CC), and their structures were characterized by melting point (mp), FT-IR, ¹H-NMR, MS, or HRMS (for new compounds only). To the best of our knowledge, compounds 2c (3-phenyl-8-isopropylcoumarin), 2e (3-phenyl-8-allylcoumarin), 2f (3,8-diphenylcoumarin), 3d (3-acetyl-8-phenylcoumarin), and 4d (ethyl 8-phenylcoumarin-3-carboxylate) were not reported in the literature.

#### 2.1. General procedure for the synthesis of compounds 1b–1g

The mixture of 3-methyl salicylaldehyde (2 mmol), acetic anhydride (2 mmol), anhydrous potassium carbonate (0.5 mmol), and tetrabutylammonium bromide (0.08 mmol) was stirred at 135–140°C for 1 hr. After that, a portion of the acetic acid generated in reaction was evaporated, and another portion of acetic anhydride (4 mmol) was added dropwise to the mixture, which continued to be stirred at 135–140°C for 6 hr. When TLC

### Scheme 1. Synthetic routes of target coumarin derivatives.

| Compd. | R  | Compd. | R  | Compd. | R  | Compd. | R  | Compd. | R  |
|--------|----|--------|----|--------|----|--------|----|--------|----|
| 1a     | H  | 2a     | H  | 3a     | H  | 4a     | H  |
| 1b     | CH₃| 2b     | CH₃| 3b     | CH₃| 4b     | CH₃|
| 1c     | (CH₂)₂CH | 2c | (CH₂)₂CH | 3c | CH₂=CHCH₂ | 4c | CH₂=CHCH₂ |
| 1d     | (CH₂)₃C | 2d | (CH₂)₃C | 3d | Ph     | 4d | Ph     |
| 1e     | CH₂=CHCH₂ | 2e | CH₂=CHCH₂ | 3e | Cl     | 4e | Cl     |
| 1f     | Ph  | 2f     | Ph  |        |     |        |     |
| 1g     | Cl  | 2g     | Cl  |        |     |        |     |
showed that the reaction was complete, the pH of the reaction mixture was adjusted to 8 with a saturated Na₂CO₃ solution. The reaction mixture was then extracted with ethyl acetate, and the organic phase was dried over anhydrous Na₂SO₄. The solvent was evaporated under reduced pressure to give crude 8-methylcoumarin (1b), which was purified by silica gel CC with petroleum ether and ethyl acetate (V : V = 10:1). Using the same procedure, 5 other 8-substituted coumarins were prepared by the reaction of acetic anhydride with different 3-substituted salicyldehydes.  

2.2. General procedure for synthesizing compounds 2a–2g  
Salicylaldehyde (10 mmol) and benzeneacetonitrile (15 mmol) were added to a solution of TBAB (1 mmol) in aqueous NaOH (50 mL, 0.05 N). After 12 hr of vigorous stirring at 90°C, the heterogeneous mixture was acidified with concentrated HCl (1.25 mL) to a pH of 3 at room temperature, then heated to 90°C and stirred for 5 hr. The solid was filtered after cooling, and then washed with cold water and dried to obtain crude 3-phenylcoumarin (2a), which was purified by silica gel CC with petroleum ether and ethyl acetate (V : V = 9:1). Using the same procedure, 6 other 3-phenyl-8-substituted coumarins were prepared by the reaction of benzeneacetonitrile with different 3-substituted salicyldehydes.  

2.2.1. 3-Phenyl-8-isopropylcoumarin (2c)  
Yellow solid; yield, 76.2%; mp, 119-121°C; IR (KBr, cm⁻¹) ν: 1698, 1598, 1445, 1354, 993, 931, 753; 1H-NMR (500 MHz, TMS/CDCl₃) δ: 7.81 (s, 1H, ArH), 7.72 (d, J = 7.5 Hz, 2H, ArH), 7.47–7.37 (m, 5H, ArH), 7.26 (t, J = 7.5 Hz, 1H, ArH), 3.74–3.65 (m, 1H, CH), 1.34 (d, J = 7.0 Hz, 6H, 2CH₃); HRMS: calculated for C₁₈H₁₇O₂ [M+H]+ 265.1229, found 265.1235.  

2.2.2. 3-Phenyl-8-allylcoumarin (2e)  
Yellow solid; yield, 81.4%; mp, 80–81°C; IR (KBr, cm⁻¹) ν: 1706, 1594, 1450, 1350, 993, 914, 777, 740; 1H-NMR (500 MHz, TMS/CDCl₃) δ: 7.81 (s, 1H, ArH), 7.72 (d, J = 7.5 Hz, 2H, ArH), 7.47–7.40 (m, 5H, ArH), 7.24 (t, J = 5.0 Hz, 1H, ArH), 6.10–6.01 (m, 1H, CH=C), 5.18–5.13 (m, 2H, C=CH₂), 3.68 (d, J = 7.0 Hz, 2H, –CH₂–); HRMS: calculated for C₁₈H₁₅O₂ [M+H]+ 263.1229, found 263.1235.  

2.2.3. 3,8-Diphenylcoumarin (2f)  
Yellow solid; yield, 90.5%; mp, 122–123°C; IR (KBr, cm⁻¹) ν: 1714, 1424, 960, 935, 757, 703; 1H-NMR (500 MHz, TMS/CDCl₃) δ: 7.87 (s, 1H, ArH), 7.73 (d, J = 7.5 Hz, 2H, ArH), 7.66 (d, J = 7.5 Hz, 2H, ArH), 7.59 (dd, J₁ = 7.5 Hz, J₂ = 1.0 Hz, 1H, ArH), 7.54 (d, J₁ = 7.5 Hz, 1H, ArH), 7.50 (t, J₁ = 7.5 Hz, 2H, ArH), 7.47–7.40 (m, 4H, ArH), 7.38 (t, J₁ = 7.5 Hz, 1H, ArH); HRMS: calculated for C₂₁H₁₇O₂ [M+H]+ 299.1072, found 299.1068.  

2.3. General procedure for synthesizing compounds 3a–3e  
Salicylaldehyde (2 mmol) and piperidine (1 mL) were added in turn to the solution of ethyl acetocetate (2 mmol) in EtOH (20 mL), and the mixture was then stirred for 24 hr at room temperature. After the reaction was complete, the precipitate was vacuum filtered and recrystallized from EtOH to give a yellow crystal of 3-acetylcoumarin (3a). Using the same procedure, 4 other 3-acetyl-8-substituted coumarins were prepared by the reaction of ethyl acetocetate with different 3-substituted salicyldehydes.  

2.3.1. 3-Acetyl-8-phenylcoumarin (3d)  
Yellow solid; yield, 97.4%; mp, 166–167°C; IR (KBr, cm⁻¹) ν: 1727, 1681, 1598, 1557, 1449, 1428, 1358, 977, 757, 694; 1H-NMR (500 MHz, TMS/CDCl₃) δ: 8.56 (s, 1H, ArH), 7.72 (d, J = 7.5 Hz, 1H, ArH), 7.65 (d, J = 7.5 Hz, 1H, ArH), 7.62 (d, J = 8.0 Hz, 2H, ArH), 7.51 (t, J = 7.5 Hz, 2H, ArH), 7.40–7.45 (m, 2H, ArH), 2.73 (s, 3H, CH₃C=O); HRMS: calculated for C₁₉H₁₇O₂ [M+H]+ 265.0865, found 265.0860.  

2.4. General procedure for synthesizing compounds 4a–4e  
Salicylaldehyde (2 mmol) was added to a mixture of malonate (2 mmol), piperidine (1 mL), and glacial acetic acid (3 drops) in EtOH (20 mL), and the mixture was then refluxed for 6 hr. After removing the solvent under reduced pressure, the crude product ethyl coumarin-3-carboxylate (4a) was purified by silica gel CC with petroleum ether and ethyl acetate (V : V = 3:1). Using the same procedure, 4 other ethyl 8-substituted coumarin-3-carboxylates were prepared by the reaction of malonate with different 3-substituted salicyldehydes.  

2.4.1. Ethyl 8-phenylcoumarin-3-carboxylate (4d)  
White solid; yield, 96.4%; mp, 123–125°C; IR (KBr, cm⁻¹) ν: 1752, 1594, 1462, 1304, 761, 698; 1H-NMR (500 MHz, TMS/CDCl₃) δ: 8.56 (s, 1H, ArH), 7.70 (dd, J₁ = 9.0 Hz, J₁₂ = 1.5 Hz, 1H, ArH), 7.61–7.59 (m, 3H, ArH), 7.49 (t, J = 8.0 Hz, 2H, ArH), 7.39–7.44 (m, 2H, ArH), 4.42 (q, J = 7.0 Hz, 2H, COOCH₂), 1.42 (t, J = 7.0 Hz, 3H, CH₃); HRMS: calculated for C₁₈H₁₆O₄ [M+H]+ 295.0970, found 295.0966.  

3. Procedure for antifungal activities  
The in vitro antifungal activities of the title compounds against the mycelium growth of B. cinerea, C. gloeosporioides, F. oxysporum, and V. mali were evaluated. Different proper concentrations of title compounds in acetone (1 mL) were mixed with liquid potato dextrose agar (PDA, 50°C, 100 mL) to obtain series concentrations of test compound mixed culture media. The mixed PDA was then poured into 12 petri dishes (d = 6 cm) and allowed to cool to room temperature to form a plate. After that, one cake of 4 mm plant fungii activated in advance was placed upside down on the mixed PDA plate and incubated at 28°C. The commercial fungicide Carbendazim was used as a positive control, and acetone was used as a blank. Each treatment was replicated three times. When the colony grew to 3/4 of the petri dish, its diameter was measured, and the average was calculated. Toxicity regression equations with a correlation coefficient and effective concentration that inhibited mycelium growth by 50% (EC₅₀) were expressed as the mean of values obtained using toxicity regression equation software.²⁰  

4. 3D-QSAR models  
4.1. Molecular modeling and alignment  
Molecular modeling was performed using Sybyl 7.3 (Tripos Inc., St. Louis, MO, USA) software. Initial 3D structures of all
molecules were built by the semiempirical method with default parameters using the Sketch Molecule function in Sybyl. Optimization of the structures was carried out using the Gasteiger–Hückel charge, Tripos force field, and Powell’s energy gradient algorithm with a convergence criterion of 0.021 kcal/mol.24)

4.2. Datasets for 3D-QSAR analysis
In this study, the EC<sub>50</sub> values of the title compounds—except 1a, 2a, 2f, and 4c, which showed no activity under setting concentrations (20 compounds total)—were selected for 3D-QSAR analysis. As listed in Table 2, the biological activities used in the study were expressed as pEC<sub>50</sub> (pEC<sub>50</sub> = −log EC<sub>50</sub>). Four typical active compounds (compounds 1d, 2b, 2g, and 3d) marked with an asterisk in Table 2 were selected as the testing set, while the remaining 16 compounds were used as the training set for CoMFA and CoMSIA.22,23) Molecule 4e, which had the best activity, served as a common template, and the 3D structures of the other 19 molecules were superposed on molecule 4e using the public skeleton method and the database alignment rule.24) The superposition of the title compounds is shown in Fig. 1. The public skeleton is represented by the red circles.

4.3. CoMFA/CoMSIA models and partial least squares analysis
The 3D-QSAR model of the title compounds was derived using CoMFA and CoMSIA modeling approaches, in which a ster, electrostatic, hydrophobic, hydrogen bond donor and hydrogen bond acceptor fields were calculated. 3D meshes of all molecules were constructed using the Create Automatically option in Sybyl with a SP<sup>3</sup> C<sup>+</sup> probe and 2.0 Å grid spacing.25) The van der Waals radius of the probe in CoMFA is 1.52 Å, while it is 1.00 Å in CoMSIA. The hydrophobic field of the probe in CoMSIA is +1.00, and the intensity of the hydrogen bond donor and hydrogen bond acceptor of the probe is +1. Steric and electrostatic fields were obtained using the Lennard–Jones potential and Coulomb’s law energy function with an effect threshold of 50 kcal/mol and 30 kcal/mol, respectively.

CoMFA and CoMSIA descriptors were used as independent variables, while experimental pEC<sub>50</sub> values were used as dependent variables. Linear regression analysis of the independent and dependent variables was carried out using partial least squares (PLS)26) regression. 3D-QSAR analysis was carried out in two steps. First, the performance of the models was evaluated by cross-validation, and the optimal number of components (ONC) was determined from the highest cross-validated correlation coefficient q<sup>2</sup> value.27) The non-cross-validated correlation coefficient r<sup>2</sup> value, standard error of estimate (SEE), and F-value were then calculated according to the definitions in the Sybyl 7.3 package. The contour maps and standard deviation values of the CoMFA and CoMSIA models were generated by the PLS coefficients.

The cross-validated correlation coefficient (q<sup>2</sup>) is given by28)

\[ q^2 = 1 - \frac{PRESS}{\sum_{i=1}^{N} (y_i - \bar{y})^2} \]  (1)

\[ PRESS = \sum_{i=1}^{N} (y_{pred} - y_i)^2 \]  (2)

In these expressions, \( y_i \) is the biological activity for training compounds, \( \bar{y} \) is the mean observed value corresponding to the average of the values for each cross-validation group, and \( y_{pred} \) is the predicted biological activity for \( y_i \).

Moreover, the predictive capacity of the models is conveyed by the predictive r<sup>2</sup> value, which is analogous to cross-validated r<sup>2</sup> (q<sup>2</sup>) and can be evaluated from29)

\[ r_{pred}^2 = \frac{(SD - PRESS)/SD}{SD} \]  (3)

where SD is the sum of the squared deviations between the biological activity of the training set compounds, and PRESS is the sum of the squared deviations between the observed and the predicted biological activity of the test compounds.

Results and Discussion

1. Antifungal activity
Antifungal activities of the title compounds against B. cinerea, C. gloeosporioides, F. oxysporum, and V. mali are summarized in Table 1. As illustrated, most of the synthesized compounds exhibited moderate to high antifungal activity against the 4 phytopathogenic fungi. However, it seems that selective toxicity to V. mali was the characteristic property of the 8-substituted coumarins. In particular, compounds 1b–1c, 1f–1g, 3d, 4d, and 4e showed stronger inhibition with EC<sub>50</sub> of <0.116 mmol/L against V. mali. Among them, 1g, 3d, and 4e possessed the strongest antifungal activity, with EC<sub>50</sub> of 0.085, 0.096 and 0.078 mmol/L against V. mali, respectively. In the series of 1a–1g, the introduction of an alkyl, aryl, or halogen group to the C-8 of coumarin significantly enhanced the antifungal activities of these compounds. A similar phenomenon appeared in the series of compounds 2a–2g except 2f. This suggested that the introduction of proper substituents to C-8 of coumarin was favorable to antifungal activity. In order to understand the detailed effects of substituents on antifungal activity, the 3D-QSAR of these compounds was further studied.
2. Study by 3D-QSAR models

Of the 4 tested fungi, *V. mali* seems to be the most sensitive to the title compounds. Therefore, the EC$_{50}$ data of the synthesized compounds against *V. mali* were selected for the further study by 3D-QSAR. The pEC$_{50}$ values of all compounds as predicted by the CoMFA and CoMSIA models are listed in Table 2, and correlations between the predicted and experimental pEC$_{50}$ values are presented in Fig. 2. The predicted pEC$_{50}$ values are very close to the corresponding experimental values for compounds in both the training set and the testing set, as shown in Table 2. The observation of mostly linear correlations in Fig. 2 demonstrates the high predictive power of these models.

![Fig. 2](image_url) Fitted predictions by CoMFA and CoMSIA models against experimental biological activities for compounds in the training and test sets; the solid lines express the linear regressions for the training and test set predictions. ▲, testing set; ◆, training set.

### Table 1. Antifungal activity of title compounds against four phytopathogens.$^a$

| Compd. | $B$. cinerea | $C$. gloeosporioides | $F$. oxysporum | $V$. mali |
|---|---|---|---|---|
| 1a (coumarin) | — | — | — | — |
| 1b | 0.228±0.009 | 0.366±0.011 | 0.225±0.007 | 0.108±0.007 |
| 1c | 0.307±0.013 | 0.144±0.006 | 0.245±0.010 | 0.116±0.008 |
| 1d | 0.287±0.011 | 0.198±0.008 | 0.237±0.013 | 0.152±0.005 |
| 1e | 0.259±0.006 | 0.254±0.011 | 0.264±0.011 | 0.174±0.007 |
| 1f | 0.476±0.016 | 0.503±0.020 | 0.385±0.016 | 0.113±0.006 |
| 1g | 0.171±0.005 | 0.456±0.019 | 0.241±0.006 | 0.085±0.002 |
| 2a | — | — | — | — |
| 2b | 0.227±0.007 | 0.296±0.018 | 0.314±0.015 | 0.296±0.013 |
| 2c | 0.435±0.018 | 0.371±0.020 | 0.320±0.009 | 0.163±0.007 |
| 2d | 0.347±0.014 | 0.613±0.024 | — | 0.282±0.013 |
| 2e | 0.202±0.006 | 0.321±0.011 | 0.281±0.016 | 0.405±0.019 |
| 2f | — | — | — | — |
| 2g | 0.275±0.011 | 0.724±0.027 | 0.506±0.023 | 0.420±0.023 |
| 3a | 0.659±0.021 | 0.457±0.019 | 0.243±0.009 | 0.154±0.008 |
| 3b | 0.560±0.019 | 0.589±0.021 | — | 0.546±0.027 |
| 3c | 0.271±0.014 | 0.294±0.012 | 0.360±0.020 | 0.520±0.022 |
| 3d | 0.509±0.023 | 1.330±0.038 | 0.605±0.034 | 0.096±0.003 |
| 3e | 0.185±0.008 | 0.155±0.009 | 0.361±0.017 | 0.207±0.014 |
| 4a | — | 0.361±0.013 | — | 0.255±0.011 |
| 4b | — | 0.313±0.016 | — | 0.177±0.009 |
| 4c | — | — | — | — |
| 4d | 0.170±0.009 | 0.174±0.006 | 0.291±0.007 | 0.113±0.007 |
| 4e | 0.206±0.007 | 0.133±0.002 | 0.150±0.009 | 0.078±0.003 |
| Carbendazim | 0.142±0.005 | 0.059±0.001 | 0.097±0.002 | 0.31±0.001 |

$^a$“—” means no remarkable antifungal activity.
The statistical parameters of the CoMFA and CoMSIA models are presented in Table 3. We can see that the optimal number of components (ONC) of the models are 4 and 5, respectively, whereas the corresponding non-cross-validated correlation coefficient \( q^2 \) are 0.651 and 0.733 (>0.5), respectively. The \( q^2 \) and \( r^2 \) values are commonly used to evaluate the predictive ability of a 3D-QSAR model, and the commonly accepted values for an excellent 3D-QSAR model are \( q^2 \geq 0.5 \) and \( r^2 > 0.8.30 \) Since the obtained \( q^2 \) and \( r^2 \) values in Table 3 meet the criteria, the 3D-QSAR models are reliable. The standard errors of estimate (SEE) for the models are 0.119 and 0.127, respectively, and the \( F \)-values are 182.6 and 215.4, respectively. The relative contributions of the steric and electrostatic fields of the CoMFA model are 62.7 and 37.3%, respectively, which indicates that the steric interactions mainly affect bioactivity. By comparison, the contributions of the steric, electrostatic, hydrophobic, hydrogen bond donor, and hydrogen bond acceptor of the CoMSIA model are 38.9, 21.5, 20.7, 5.1, and 13.8%, respectively; these suggest that steric and electrostatic features make major contributions to the bioactivity of the title compounds.

CoMFA and CoMSIA contour maps for compound 4e are shown in Fig. 3 and Fig. 4, respectively. Regarding the CoMFA

| Table 2. | Experimental, predicted data and residuals obtained by the CoMFA and CoMSIA models for the compounds’ activities (pEC_{50}) in the training and test sets. |
| Compd<sup>a</sup> | R<sub>1</sub> | R<sub>2</sub> | EC<sub>50</sub> (mmol/L) | pEC<sub>50</sub> | Residual |
| --- | --- | --- | --- | --- | --- |
| 1a | H | H | — | — | — |
| 1b | H | CH<sub>3</sub> | 0.108 | 3.968 | 3.933 | 0.035 | 0.031 |
| 1c | H | CH(CH<sub>3</sub>)<sub>2</sub> | 0.116 | 3.937 | 3.927 | 0.010 | 0.016 |
| 1d* | H | C(CH<sub>3</sub>)<sub>3</sub> | 0.152 | 3.818 | 3.810 | 0.008 | 0.011 |
| 1e | H | CH<sub>2</sub>CH=CH<sub>2</sub> | 0.174 | 3.760 | 3.799 | 0.039 | 0.003 |
| 1f | H | Ph | 0.113 | 3.946 | 3.966 | 0.021 | 0.033 |
| 1g | H | Cl | 0.085 | 4.072 | 4.066 | 0.007 | 0.008 |
| 2a | Ph | H | — | — | — |
| 2b* | Ph | CH<sub>3</sub> | 0.296 | 3.529 | 3.576 | 0.047 | 0.062 |
| 2c | Ph | CH(CH<sub>3</sub>)<sub>2</sub> | 0.163 | 3.787 | 3.756 | 0.031 | 0.036 |
| 2d | Ph | C(CH<sub>3</sub>)<sub>3</sub> | 0.282 | 3.549 | 3.562 | 0.013 | 0.008 |
| 2e | Ph | CH<sub>2</sub>CH=CH<sub>2</sub> | 0.405 | 3.392 | 3.420 | 0.028 | 0.031 |
| 2f | Ph | Ph | — | — | — |
| 2g* | Ph | Cl | 0.420 | 3.377 | 3.396 | 0.019 | 0.069 |
| 3a | CH<sub>3</sub>CO | H | 0.154 | 3.813 | 3.852 | 0.039 | 0.020 |
| 3b | CH<sub>3</sub>CO | CH<sub>3</sub> | 0.546 | 3.263 | 3.314 | 0.052 | 0.014 |
| 3c | CH<sub>3</sub>CO | CH<sub>2</sub>CH=CH<sub>2</sub> | 0.520 | 3.284 | 3.275 | 0.009 | 0.008 |
| 3d* | CH<sub>3</sub>CO | Ph | 0.096 | 4.018 | 4.036 | 0.011 | 0.034 |
| 3e | CH<sub>3</sub>CO | Cl | 0.207 | 3.684 | 3.681 | 0.002 | 0.010 |
| 4a | COOEt | H | 0.255 | 3.593 | 3.646 | 0.053 | 0.060 |
| 4b | COOEt | CH<sub>3</sub> | 0.177 | 3.753 | 3.718 | 0.036 | 0.004 |
| 4c | COOEt | CH<sub>2</sub>CH=CH<sub>2</sub> | — | — | — |
| 4d | COOEt | Ph | 0.113 | 3.948 | 3.916 | 0.031 | 0.027 |
| 4e | COOEt | Cl | 0.078 | 4.106 | 4.088 | 0.017 | 0.014 |

<sup>a</sup> * means representative compounds

The statistical parameters of the CoMFA and CoMSIA models are presented in Table 3. We can see that the optimal number of components (ONC) of the models are 4 and 5, respectively, whereas the corresponding non-cross-validated correlation coefficient \( q^2 \) of the highly predictive CoMFA and CoMSIA models are 0.651 and 0.733 (>0.5), respectively. The \( q^2 \) and \( r^2 \) values are commonly used to evaluate the predictive ability of a 3D-QSAR model, and the commonly accepted values for an excellent 3D-QSAR model are \( q^2 ≥ 0.5 \) and \( r^2 > 0.8.30 \) Since the obtained \( q^2 \) and \( r^2 \) values in Table 3 meet the criteria, the 3D-QSAR models are reliable. The standard errors of estimate (SEE) for the models are 0.119 and 0.127, respectively, and the \( F \)-values are 182.6 and 215.4, respectively. The relative contributions of the steric and electrostatic fields of the CoMFA model are 62.7 and 37.3%, respectively, which indicates that the steric interactions mainly affect bioactivity. By comparison, the contributions of the steric, electrostatic, hydrophobic, hydrogen bond donor, and hydrogen bond acceptor of the CoMSIA model are 38.9, 21.5, 20.7, 5.1, and 13.8%, respectively; these suggest that steric and electrostatic features make major contributions to the bioactivity of the title compounds.

CoMFA and CoMSIA contour maps for compound 4e are shown in Fig. 3 and Fig. 4, respectively. Regarding the CoMFA
contour maps (Fig. 3), polyhedra in green and yellow (Fig. 3A) and blue and red (Fig. 3B) represent the steric and electrostatic fields, respectively. More explicitly, polyhedra in green and blue symbolize the increase of the volume or electropositivity of substituents in this region, which will be favorable for the enhancement of biological activity of compounds, while polyhedra in yellow and red signify the decrease of steric hindrance or the presence of electronnegative substituents in this area, which might facilitate the reinforcement of biological activity of the chemicals. Large yellow polyhedra appear near C-3 and C-8 (Fig. 3A), which indicates that bulky substitutes in these areas are unfavorable for antifungal activity. This could be supported by the activity of 1b [8-CH₃] and 1g [8-Cl]>1d [8-(CH₂)₂], 1e [8-CH₂CH=CH₂] and 1f [8-Ph]; 2c [8-(CH₂)₃, 3-Ph]>2d [8-C(CH₃)₃, 3-Ph] and 2e [8-CH₂CH=CH₂, 3-Ph]; 3a [8-H, 3-CH₃CO]>3b [8-CH₃, 3-CH₃CO] and 3c [8-CH₂CH=CH₂, 3-CH₃CO]; 1b–1g (except 1e)>2b–2e and 2g. The contour map of the electrostatic field in the CoMFA model is presented in Fig. 3B. The larger red polyhedra near C-8 indicate that an electronwithdrawing group is of benefit to the activity in this region. In fact, compounds with –Cl at the C-8 position had more potential than those with -CH₃ at the same position, in most cases; this finding is elucidated by activity in the order of 1g>1b, 3c>3b, and 4c>4b. Moreover, there are also large red polyhedral near C-3, which indicates that the electronegative groups may benefit activity. This could be supported by the fact that 4e [3-COOC₃H₇, 8-Cl] has the highest activity among 1g [3-H, 8-Cl], 2g [3-Phe, 8-Cl], and 3e [3-COCH₃, 8-Cl].

The steric, electrostatic, hydrophobic, hydrogen bond donor and hydrogen bond acceptor fields contour maps of CoMSIA are shown in Fig. 4. The colored polyhedral in the steric and electrostatic fields map of CoMSIA in Fig. 4A have the same meaning as those of CoMFA in Fig. 3. The steric and electrostatic contour maps of the CoMSIA model were discovered to be ostensibly similar to polyhedra in color concluded from the CoMFA model, which demonstrated that both computational models are in agreement and trustworthy in these circumstances. CoMSIA hydrophobic contour maps are displayed in Fig. 4B. The yellow polyhedral mean that hydrophobic groups are favored, while the gray blocks indicate that hydrophilic groups are favored. A large gray region was found around the C-8 position, which may suggest that hydrophilic groups should be introduced here. In fact, the yellow regions in panel (A) and the gray regions in panel (B) of Fig. 4 are overlapped. Since bulkiness and hydrophobicity for alkyl groups correlate, we can conclude that it should be better to introduce a relatively small and hydrophilic group, such as a smaller alkyl group or smaller Cl in the C-8 position. Moreover, a large gray polyhedron appears near the C-3 position, which indicates that the hydrophilic group should be better in this area. CoMSIA contour maps of the hydrogen bond donor and acceptor fields are shown in Fig. 4C. Cyan and purple polyhedral indicate regions where hydrogen bond donor groups are favored and unfavored, respectively, whereas magenta and red polyhedral indicate regions where hydrogen bond acceptor groups are favored and unfavored, respectively. There is a small cyan polyhedron near the C-8 position, but since the hydrogen bond donor groups on C-8 are not included in the compound set, it may be an artifact. The magenta polyhedron near the C-3 position suggest that hydrogen bond acceptor groups seem to be preferable here. The activity orders of 4e [3-COOEt, 8-Cl] and 3e [3-COCH₃, 8-Cl]>3f [3-Phe, 8-Cl], 4d [3-COOEt, 8-Phe] and 3d [3-COCH₃, 8-Phe]>2f [3-Phe, 8-Phe], and 4a [3-COOEt, 8-H] and 3a [3-COCH₃, 8-H]>2a [3-Phe, 8-H] could be evidence.

Coumarin derivatives, as a class of pesticide candidates that can produce good antifungal activities, have been involved in the study of structure activity relationship (SAR) in previous studies. A study by de Araújo et al. showed that the presence of a short aliphatic chain or electron-withdrawing group can favor the activity of coumarin derivatives. Meanwhile, Song et al. emphasized that the introduction of a bromine substituent at an appointed position can hardly result in higher antifungal activities, and the introduction of different substituents at different positions could cause different effects on the antifungal activity. The colored polyhedral in the steric and electrostatic fields map of CoMSIA in Fig. 4A have the same meaning as those of CoMFA in Fig. 3. The steric and electrostatic contour maps of the CoMSIA model were discovered to be ostensibly similar to polyhedra in color concluded from the CoMFA model, which demonstrated that both computational models are in agreement and trustworthy in these circumstances. CoMSIA hydrophobic contour maps are displayed in Fig. 4B. The yellow polyhedral mean that hydrophobic groups are favored, while the gray blocks indicate that hydrophilic groups are favored. A large gray region was found around the C-8 position, which may suggest that hydrophilic groups should be introduced here. In fact, the yellow regions in panel (A) and the gray regions in panel (B) of Fig. 4 are overlapped. Since bulkiness and hydrophobicity for alkyl groups correlate, we can conclude that it should be better to introduce a relatively small and hydrophilic group, such as a smaller alkyl group or smaller Cl in the C-8 position. Moreover, a large gray polyhedron appears near the C-3 position, which indicates that the hydrophilic group should be better in this area. CoMSIA contour maps of the hydrogen bond donor and acceptor fields are shown in Fig. 4C. Cyan and purple polyhedral indicate regions where hydrogen bond donor groups are favored and unfavored, respectively, whereas magenta and red polyhedral indicate regions where hydrogen bond acceptor groups are favored and unfavored, respectively. There is a small cyan polyhedron near the C-8 position, but since the hydrogen bond donor groups on C-8 are not included in the compound set, it may be an artifact. The magenta polyhedron near the C-3 position suggest that hydrogen bond acceptor groups seem to be preferable here. The activity orders of 4e [3-COOEt, 8-Cl] and 3e [3-COCH₃, 8-Cl]>3f [3-Phe, 8-Cl], 4d [3-COOEt, 8-Phe] and 3d [3-COCH₃, 8-Phe]>2f [3-Phe, 8-Phe], and 4a [3-COOEt, 8-H] and 3a [3-COCH₃, 8-H]>2a [3-Phe, 8-H] could be evidence.
activities of angular furanocoumarins. Based on the previous study, we first constructed a 3D-QSAR model for the antifungal activities of coumarin derivatives. Our 3D-QSAR study showed that small, hydrophilic and electron-withdrawing substituents such as COOC₂H₅ seem to be better at the 3 position, whereas it is obvious that small electron-withdrawing substituents are favorable at the 8 position. From the above information, we can conclude that the small electron withdrawing groups on coumarin's phenyl ring favor the activity, and hydrophilic electron-donating groups on coumarin's pyrone ring enhance the activity. It is helpful to quantify the model results and give more details for the optimization of coumarin derivatives.

**Conclusion**

In summary, a series of coumarin derivatives were designed, synthesized, and evaluated for antifungal activities against 4 plant pathogenic fungi: *B. cinerea*, *C. gloeosporioides*, *F. oxysporum*, and *V. mali*. The results showed that most of these compounds exhibited moderate to potent activities. Among them, 4e and 1g possessed the strongest antifungal activity, with EC₅₀ values of 0.085 and 0.078 mmol/L against *V. mali*, respectively; therefore, they have been selected as leading molecules for further development. Furthermore, CoMFA and CoMSIA models were established on the basis of their antifungal activities against *V. mali*, with cross-validated q² values of 0.651 and 0.733, respectively, and non-cross-validated conventional r² values of 0.918 and 0.949, respectively. The results revealed that the appropriate small, electron-withdrawing and hydrophilic group on C-3 might help enhance antifungal activity, while the small and electron-withdrawing group on C-8 would be favored for antifungal activity. Further studies are underway to verify and improve the activity profiles of our leading scaffolds.

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