Synthesis, Antifungal Activity and Molecular Dynamics Study of Novel Aromatic Geranyl Sulfonamide Compounds as Potential Complex III Inhibitors

Xinning Song
China Agricultural University
Jianing Li
China Agricultural University
Xingxing Lu
China Agricultural University
Zhiyang Jiang
China Agricultural University
Qing Han
China Agricultural University
Kai Zhu
China Agricultural University
Huilin Li
China Agricultural University
Yun Ling
China Agricultural University
Hongxia Duan (hxduan@cau.edu.cn)
China Agricultural University https://orcid.org/0000-0003-2516-854X

Research Article

Keywords: Essential oil, Aromatic geranyl sulfonamide derivatives, Fungicidal activity, Structure-activity relationship, Molecular mechanism

Posted Date: October 21st, 2021

DOI: https://doi.org/10.21203/rs.3.rs-966411/v1

Version of Record: A version of this preprint was published at Medicinal Chemistry Research on March 2nd, 2022. See the published version at https://doi.org/10.1007/s00044-022-02864-4.

Abstract

Essential oils (EOs), as unique natural products, are promising resources for the discovery of green agrochemicals. Geraniol was found to exhibit substantial fungicidal activities within citronella oil in this study. A series of novel geranyl benzenesulfonamide compounds were synthesized and found to display considerable fungicidal activities. Some aromatic geranyl sulfonamide derivatives were further optimized and then evaluated the bioactivities against six phytopathogenic fungi. Geranyl thiofuran-sulfonamide compounds **4d-1** (median effective concentration (EC_{50}) against *Rhizoctonia solani*: 24.97 mg/L and EC_{50} against *Sclerotinia sclerotiorum*: 27.26 mg/L), **4d-2** (EC_{50} against *S. sclerotiorum*: 18.53 mg/L) and Geranyl pyridine-sulfonamide compound **4e-2** (EC_{50} against *R. solani*: 29.31 mg/L and EC_{50} against *S. sclerotiorum*: 29.98 mg/L) were screened as “star molecules” with highly efficient fungicidal activities. The structure-activity relationship (SAR) study revealed that the electron-rich thiofuran ring and chlorine-substituted piperidine played remarkable roles on increasing the fungicidal activities. The molecular mechanisms of the “star molecules” were also clarified by performing molecular docking and molecular dynamics (MD) simulations, and these geranyl sulfonamide compounds were identified as potential Complex III inhibitors. The main component originating from the natural plant EOs ought to be studied to discover novel pathogenic fungicidal candidates used to control agricultural plant diseases.

Introduction

In recent years, plant diseases have received increasing attention because of their negative effects not only on the yields but also on the quality of crops worldwide[1]. Numerous fungicides are continuously used to control various plant diseases, causing the development of extraordinary pathogen resistance to known fungicides[2]. Therefore, the discovery of novel, highly efficient fungicides for use in modern plant disease control is particularly crucial and valuable.

Plant essential oils (EOs) are natural aromatic and volatile oily liquids extracted from the flowers, leaves, roots, barks, fruits, seeds and resins of herbaceous plants[3]. EOs have been shown to display sufficient biosafety in humans when applied in cosmetics and as antimicrobials[4, 5]. On account of their special ingredients and combinations, many pharmacological properties of EOs have long been recognized and reported, such as insecticidal activities[6, 7], antibacterial activities[8, 9], and antifungal activities as well[10, 11]. Citronella oil, a well-known EO, extracted from citronella plants has been reported to function not only as a mosquito repellent[12] but also as a promising insecticide against *Myzus persicae*[13]. In addition, citronella oil exhibited excellent antibacterial activity against *Propionibacterium acnes*[14]. Notably, citronella oil has been recognized as an outstanding antifungal agent against *Aspergillus niger*[15] and *A. flavus*[16] in the area of medicine. Besides, both geraniol and citronellol, two major components of citronella oil, have also been reported to possess valuable antifungal property against *Trichophyton rubrum*, one of the most common medicinal fungi that causes chronic dermatophytosis in humans[17]. On the other hand, geraniol sensitizes fungi to some commercial antifungal agents, such as fluconazole, by increasing the exudation of fungal cellular substances and cytomembrane mobility[18]. However, to

date, studies on citronella oil as an agricultural fungicide are still limited. As a consequence, the use of natural citronella oil or its main components in the development of new green antifungal agents in agriculture remains prospective.

The cytochrome bc1 complex (Complex III) is a vital protein in fungal organisms that comprises the middle part of the mitochondrial respiratory chain[19]. Complex III catalyses reversible electron transferring from ubiquinone to cytochrome c, coupled with proton translocation across the inner mitochondrial membrane through the “Q-cycle”. Inhibitors bind to the ubiquinone reaction site in Complex III and modulate the Q-cycle to further alter their antifungal activities[20]. Antimycin (Scheme 1) is the first known commercial fungicide that binds to the ubiquinone oxidation site (Qo site) of Complex III blocking the mitochondrial electron transfer between cytochrome b and c[21]. Moreover, some commercial sulfonamide fungicides, such as cyazofamid and amisulbrom (Scheme 1), were identified as excellent inhibitors binding at the ubiquinone reduction site (Qi site) of Complex III, and whose sulfonamide azolyl pharmacophores may be responsible for their efficient activities[22]. Additionally, benzene sulfonamide compounds have been reported to display a higher binding affinity for Complex III than triazole sulfonamides. Hence, the structure of benzenesulfonamides appears to be a better choice for the construction of new Complex III inhibitors owning to their simple fragments and better bioactivities[23].

Consequently, natural citronella oil and its main ingredients were selected for fungicidal activities evaluation in this study. A variety of aromatic geranyl sulfonamide compounds were designed, synthesized and subsequently evaluated for their fungicidal activities. Some “star molecules” with considerable fungicidal activities were screened successfully in our study. Molecular docking and MD simulations were performed to clarify the binding modes between these compounds and the potential target, Complex III. Conclusively, those aromatic geranyl sulfonamide compounds are potential fungicidal candidates and Complex III inhibitors for use in agricultural plant diseases control.

Results And Discussion

Screening the Fungicidal Activities of Citronella Oil

Citronella oil was chosen as the basic material in our study owing to its previously reported multiple excellent bioactivities compared to other EOs. The fungicidal activities of the entire original citronella oil and its three main components, geraniol, citronellol and citronellal (Scheme 2), were evaluated against two plant pathogenic fungi, *R. solani* and *Gibberella saubinetii*, at a concentration of 50 mg/L in this study. The fungicidal activities are demonstrated in **Table S1** and Fig. 1. The entire original citronella oil displayed satisfactory activities against *R. solani* (inhibition rate greater than 30%) and *G. saubinetii* (inhibition rate greater than 20%); these results were better than the previous report describing citronella oil, with an approximately 15% inhibitory rate against *R. solani*[24]. Remarkably, geraniol exhibited the most excellent fungicidal activities aginst *R. solani* and *G.saubinetii* (inhibition rate approximately 50% and 20% separately) when compared to the other two main ingredients of citronella oil, namely citronellol

and citronellal, which were even better than the corresponding activities of citronella oil itself. In conclusion, geraniol was proposed to be the main source of the fungicidal activities of citronella oil in our study. The more electron-rich vinyl group in geraniol compared to the structures of citronellol and citronellal was proposed to contribute to its superior antifungal activities in the present study. Consequently, geraniol, the main fungicidal component of citronella oil, was chosen as a suitable lead to explore novel, highly efficient antifungal agents in subsequent experiments.

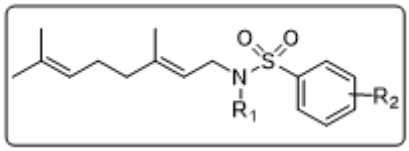
Synthesis and Fungicidal Activities Screening of Aromatic Geranyl sulfonamide Compounds

Sulfonamide compounds have aroused increasing interests in recent years due to their extensive antifungal activities[25, 26]. Therefore, a series of benzenesulfonamide compounds based on the natural product geraniol as the lead compound were designed and synthesized firstly in our study. The synthesized procedures were shown in Scheme 3. Geraniol was selected as the original material **1** of this described reaction scheme. Intermediate **2** was obtained from original material **1** and phthalimide via the Mitsunobu reaction (DIAD and PPh_3 served as the acid-binding agent and catalyst, respectively). The dry reaction environment was vital for the high yields of intermediate **2**. Subsequently, intermediate **2** was reduced to obtain the key intermediate **3**, geranylamine, catalysed by hydrazine hydrate. All target compounds **4a** were prepared from geranylamine through a condensation reaction utilizing the corresponding aromatic sulfonyl chloride analogues with trimethylamine as the acid-binding agent. Finally, compounds **4a** were methylated to generate **5a** under the circumstances of sodium ethoxide and iodomethane. The structures of target compounds are shown in **Scheme 4**. All structures of the synthesized geranyl benzenesulfonamide compounds **4a** and **5a** were confirmed by recording ^{1}H-NMR, ^{13}C-NMR and ESI-MS spectrums, which were shown in **Fig S2-S27** in supplementary information.

The bioactivities of thirteen firstly synthesized geranyl benzenesulfonamide compounds were evaluated against two plant pathogenic fungi, *R. solani* and *G. saubinetii*, at the concentration of 50 mg/L in the present study. The results of the fungicidal activities are presented in Table 1 and **Fig S1**. Most of the geranyl benzenesulfonamide compounds appeared to exhibit considerable antifungal activities against *R. solani* and *G. saubinetii*, with inhibitory rates ranging from 40–60%, which were generally better than the lead compound geraniol, whose inhibitory rates of approximately 50% and 20% against the corresponding fungi. Among the synthesized geranyl benzenesulfonamides, four compounds, **4a-1**, **4a-3**, **4a-4**, and **4a-5**, showed an approximately 50% inhibitory rate against *R. solani*. Two compounds, **4a-3** and **4a-5**, also showed greater than or approximately 50% inhibitory rates against *G. saubinetii*. Nevertheless, compound **5a-1**, **5a-2**, and **5a-3**, in which the sulfonamides were substituted with methyl groups, exhibited substantially decreased antifungal activities, with only 10-30% inhibitory rate. Hence, it was boldly inferred that the aromatic sulfonamides with the fragment of geraniol might have considerable fungicidal activities, and were of worth for further optimization as fungicidal agents. Consequently, some heterocyclic groups were introduced to design and synthesize novel aromatic sulfonamide analogues **4b-**

4e (**Scheme 4**) using the three-step reaction[27] mentioned above (Scheme 3) in order to further improve the fungicidal activities of the aromatic geranyl sulfonamide compounds in the present study. All structures of the synthesized compounds **4b-4e** were confirmed by recording ^{1}H-NMR, ^{13}C-NMR and ESI-MS spectrums, which were shown in **Fig S28-S49** in supplementary information.

Table 1 *In vitro* inhibitory rate for the fungicidal activity (%) of geranyl benzenesulfonamide compounds at a concentration of 50 mg/L



Compound	R_1	R_2	*R. solani*	*G. saubinetii*
4a-1	H	2-CH_3	49.49±0.25	47.12±1.11
4a-2	H	3-CH_3	46.88±1.19	46.83±1.78
4a-3	H	4-CH_3	49.49±0.85	48.12±0.00
4a-4	H	2-CF_3	55.61±0.25	33.51±1.11
4a-5	H	4-NO_2	56.63±0.75	54.31±0.25
4a-6	H	2,6-Cl	29.59±1.04	26.18±0.25
4a-7	H	4-CN	44.39±0.48	47.12±0.75
4a-8	H	4-OCH_3	42.86±0.41	41.88±1.60
4a-9	H	3,4-OCH_3	37.76±1.50	39.88±1.31
4a-10	H	4-OCF_3	43.37±0.25	16.23±0.41
5a-1	CH_3	4-CH_3	32.14±1.93	0±0.65
5a-2	CH_3	4-NO_2	25.00±0.48	16.75±1.89
5a-3	CH_3	4-OCH_3	22.45±0.41	14.66±0.85
Pyraclostrobin	-	-	95.41±0.25	72.77±0.00

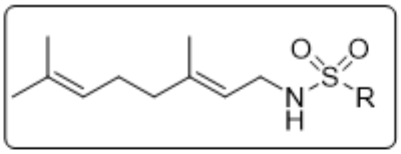
Fungicidal Activities Assays of Newly Synthesized Aromatic Geranyl Sulfonamide Compounds

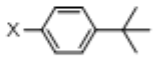
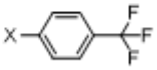
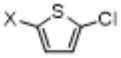
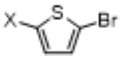
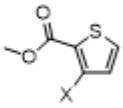
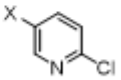
The bioactivities of the newly synthesized target compounds and their lead, geraniol, were evaluated against six plant pathogenic fungi: *R. solani, G. saubinetii, S. sclerotiorum*, *Valsa mali*, *Gibberella fujikuroi*,

and Magnaporthe oryzae at the concentration of 50 mg/L. The fungicidal activities are demonstrated in **Table S2** and Fig. 2. The commercial fungicide pyraclostrobin was served as the positive control. As shown in Fig. 2, almost all of the synthesized compounds displayed considerably higher fungicidal activities contrasted with the lead compound, geraniol. Besides, most of the newly synthesized aromatic geranyl sulfonamide compounds appeared to overall exhibit better fungicidal activities against *R. solani, G. saubinetii*, and *S. sclerotiorum*, than against the other fungi tested (*V. mali*, *G. fujikuroi* and *M. oryzae*). Furthermore, it was surprising that the majority of newly synthesized aromatic geranyl sulphonamide compouds, for instance compound **4b-1**, **4d-1**, **4d-2**, **4d-3**, **4d-4** and **4e-2** (with inhibition rates higher than 90%) displayed good and specific fungicidal activities against *S. sclerotiorum*. Especially, **4d-2** and **4e-2** displayed inhibition rates of 99% and 100% against *S. sclerotiorum*, respectively, whose fungicidal activities were similar to the commercial fungicide pyraclostrobin (100%).

The EC_{50} values of highly effective synthesized compounds were determined in this study (compounds with inhibition rates greater than 60% at 50 mg/L). The results are shown in Table 2. The EC_{50} values of compounds **4d-1** and **4e-2** against *R. solani* were 24.97 and 27.16 mg/L, respectively, which further confirming that they possessed good fungicidal activities. Satisfactorily, the entire seven synthesized aromatic geranyl sulfonamide compounds possessed EC_{50} values lower than 50 mg/L against *S. sclerotiorum*, particularly compound **4d-2**, which exhibited the lowest EC_{50} value of only 18.53 mg/L, however, it exhibited no bioactivities against *S. sclerotiorum*. Moreover, compound **4d-1** displayed good fungicidal activities against not only *S. sclerotiorum* but also *R. solani* with the EC_{50} values of 27.26 mg/L and 24.97 mg/L, respectively. Compound **4e-2**, whose fungicidal activities were slightly inferior to the activities of **4e-1**, possessing EC_{50} values of 29.98 and 29.13 mg/L, respectively. As the EC_{50} values mentioned above, the aforementioned compounds, **4d-1, 4d-2** and **4e-2**, were designated as “star molecules” in this study, whose structures are shown in Scheme 5.

Table 2 EC_{50} values of some aromatic geranyl sulfonamide analogues



Compounds	R	EC_{50} (mg/L)	
		S. sclerotiorum	*R. solani*
4b-1		44.86	N.T.[a]
4b-3		34.46	N.T.
4d-1		**27.26**	**24.97**
4d-2		**18.53**	N.T.
4d-3		41.40	N.T.
4d-4		41.97	N.T.
4e-2		**29.98**	**29.31**
Pyraclostrobin	-	0.12	0.01

[a] N.T. represents not tested.

Structure-Activity Relationship (SAR) of Novel Aromatic Geranyl Sulfonamide Compounds

The SARs of the 24 synthesized aromatic geranyl sulfonamide compounds and their bioactivities against *R. solani*, *G. saubinetii*, *S. sclerotiorum* were discussed in this study. Among the compounds with electron-donating substituted groups, compounds **4a-1**, **4a-2** and **4a-3**, whose benzene rings were substituted by methyl displayed moderate fungicidal activities regardless of the positions of the substituent group on the benzene rings. Once the methyl was displaced by more negative groups such as methoxy and tert-buty, the fungicidal activities of corresponding compounds (**4a-8** and **4b-1**) were still maintained in medium level or slightly decreased, however, the introduction of multiple negative groups resulted in decrease in the bioactivities. For instance, the fungicidal activities against *S. sclerotiorum* of **4b-2** with three substituted tert-buty almost disappeared. One the other hand, synthesized compounds whose benzene rings were substituted by electron-withdrawing groups generally exhibited better fungicidal activities than those benzene rings substituted by electron-donating groups. For example, **4a-4** and **4a-5** with strong electron-withdrawing groups trifluoromethyl and nitro substituted both exhibited the highest inhibitory rates against *R. solani* at values of 55.61±0.25% and 56.63±0.75% respectively. Interestingly,

the introduction of electron-withdrawing group containing trifluoromethyl usually lead to low fungicidal activities against *G. saubinetii*, besides, the introduction of multiple chlorine substituents (**4a-6**) also resulted in disappearance of fungicidal activities against three phytopathogenic fungi. Notably, if the hydrogen atom of the amine group in geranylamine was replaced, such as in compounds **5a-1**, **5a-2**, and **5a-3**, the fungicidal activities were decreased substantially compared to their corresponding unsubstituted compounds **4a-3**, **4a-5**, and **4a-8**, indicating that increasing in steric hindrance of geranylamine might generate bad influence of fungicidal activities.

Moreover, in case that the benzene ring of synthesized compounds was substituted by a more electron-rich and rigid naphthalene ring, the fungicidal activities of those compounds were substantially cut down, such as compound **4c-1** and **4c-2**. It was worth noting that almost all of the compounds with thiofuran groups substituted exhibited good fungicidal activities against *G. saubinetii* and *S. sclerotiorum*, especially **4d-1**, whose inhibition rate against *S. sclerotiorum* was at a value of 97.38±1.11%. Nevertheless, the fungicidal activities of the synthesized aromatic geranyl sulfonamide compounds such as compound **4e-1** were significantly weakened for the introduction of naked piperidine rings, unexpectedly, those compounds containing the chlorine-substituted piperidine displayed best fungicidal activities among the whole synthesized compounds. All in all, we can infer that it may be a beneficial strategy to introduce various substituted heterocycles to improve fungicidal activities of aromatic geranyl sulfonamide compounds.

Potential Molecular Mechanisms of Novel Aromatic Geranyl Sulfonamide Compounds

The sulfonamide was regarded as the important pharmacodynamic characteristic of fungicides inhibiting complex III. As a consequence, the potential target of the newly synthesized aromatic geranyl sulfonamide compounds in this study was predicted as complex III, which was for the purpose of exploring the molecular mechanisms of these compounds. Firstly, the “star molecules” **4d-1**, **4d-2**, **4e-2** and commercial sulfonamide fungicide amisulbrom were docked separately into the binding site of Complex III (PDB ID: 1PPJ) to obtain reasonable initial conformations for MD simulations. Then, four independent 50 ns MD simulations were performed in this study. The root-mean-square deviation (RMSD) of protein Cα atom backbone was calculated to illustrate the conformational changes. As shown in **Fig S50**, the RMSD values of these four systems were finally convergent at approximately 2.0-4.0 Å, consequently, the last 2 ns trajectories were sampled to probe the interaction mechanisms and to calculate the binding free energies.

The averaged conformations of these four complex systems were extracted from the last 2 ns, and the binding modes were presented in Fig. 3. The triazole ring of amisulbrom was buried inside the binding site, interacting with PHE4 and PHE206 through π-π stacking. In addition, the indole ring of amisulbrom which was solvent-oriented formed π-π stacking interaction with PHE4.

As shown in Fig. 3, the newly synthesized aromatic geranyl sulfonamide compounds exhibited similar interaction mechanisms with Complex III, whereas were distinct from the mechanism of amisulbrom.

Evidently, compound **4d-1**, **4d-2** and **4e-2** bound tightly to Complex III, whose aromatic rings extended into a hydrophobic pocket composed of ALA3, ILE5, LEU7, PRO8, GLY24 inside the binding cavity, and the aliphatic chains towards outside. The sulfonamide moieties of **4d-1**, **4d-2** and **4e-2** were tightly anchored in the binding cavity by hydrogen bonds. For instance, the sulfonamide of **4d-2** can only formed one hydrogen bond with ARG86 at a distance of 2.6 Å; two hydrogen bonds were observed between the sulfonamide of **4e-2** and ASN1, GLY20 at a distance of 2.6 and 2.5 Å, respectively; however, the sulfonamide moiety of **4d-1** can form hydrogen bonds with GLY20 and ARG86 at a distance of 1.8 and 2.7 Å, respectively. Apart from hydrophobic interactions and hydrogen bonds, the π interactions existing between the novel aromatic geranyl sulfonamides and TRP17, PHE206, TRY210 also contributed a lot to their binding. To sum up, some of the amino acid residues mentioned above, such as TRP17, GLY20, ARG86 and PHE 206 were predicted as the crucial residues involved in the processes of the novel aromatic geranyl sulfonamides binding to complex III, which was further confirmed by decomposition binding free energies calculation (**Fig S51**).

The binding free energies of Complex III with **4d-1**, **4d-2**, **4e-2** and amisulbrom were finally calculated, and the results were presented in Table 3. Generally, the total binding free energies of **4d-1**, **4d-2** and **4e-2** with complex III (the ΔG_{total} value of -48.98, -41.24 and -47.75 kcal/mol) were much lower the energy of amisulbrom (the ΔG_{total} value of -35.49 kcal/mol), indicating there were stronger binding affinities existing between the novel aromatic sulfonamide compounds and complex III, when compared with amisulbrom. As shown in Table 3, among the three synthesized compounds, the binding free energies of **4d-1** and **4e-2** were similar and slightly lower than **4c-2**, which was mainly due to the difference of the electrostatic contributions (the E_{el} values of **4d-1**, **4e-2** and **4d-2** were -17.74, -17.17 and -9.45, respectively). Consequently, from the perspective of molecular simulation, it was be concluded that the novel aromatic geranyl sulfonamide compounds can not only interacted with their potential target, complex III in brand new mechanisms, but also exhibited high binding affinities, which made them becoming potential inhibitors of complex III.

Table 3
The binding free energy of Complex III with the novel aromatic geranyl sulfonamide compounds and commercial fungicide amisulbrom calculated using the MM/GBSA method

Compounds	Predicted Energy (kcal/mol)						
	E_{vdw}	E_{el}	E_{gb}	E_{surf}	ΔG_{gas}	ΔG_{solv}	ΔG_{total}
4d-1	-43.36	-17.74	18.08	-5.96	-61.11	12.13	-48.98
4d-2	-41.44	-9.45	15.30	-5.66	-50.89	9.65	-41.24
4e-2	-45.92	-17.17	21.21	-5.88	-63.09	15.33	-47.75
Amisulbrom	-46.67	-2.67	19.09	-5.23	-49.34	13.86	-35.49

Conclusions

Geraniol, as one of three main components of citronella oil, exhibited best fungicidal activities against two plant pathogenic fungi: *R. solani* and *G. saubinetii*, even compared with the entire citronella oil. Thus, 24 novel aromatic geranyl sulfonamide derivatives were synthesized based on geraniol as lead and their fungicidal activities against *R. solani, G. saubinetii, S. sclerotiorum*, *V. mali*, *G. fujikuroi*, *and M. oryzae* were evaluated in this study. Some of the synthesized aromatic geranyl sulfonamide compounds exhibited good fungicidal activities with inhibitory rates greater than 50% against *R. solani, G. saubinetii* and *S. sclerotiorum*. The SAR analysis suggested that the introduction of an electron-rich thiofuran ring and chlorine-substituted piperidine into geranylamine substantially improved the fungicidal activities against some phytopathogenic fungi, particularly *S. sclerotiorum* and *R. solani*. The thiofuran-sulfonamide geranyl compounds **4d-1** and **4d-2** showed excellent antifungal activity against *R. solani* with an EC_{50} value of 24.97 mg/L and against *S. sclerotiorum* with an EC_{50} value of 18.53 mg/L, respectively. Another compound **4e-2**, which contained a chlorine-substituted piperidine, also displayed good fungicidal activities against *R. solani* with an EC_{50} value of 29.98 mg/L and against *S. sclerotiorum* with an EC_{50} value of 29.31 mg/L. Furthermore, the potential molecular mechanism of these aromatic geranyl sulfonamide compounds was proposed to be possibly competitively bound to the binding site of Complex III via hydrogen bonds and multiple π stacking interactions, and some vital residues TRP17, GLY20, ARG86 and PHE 206. Their binding affinities to Complex III were also calculated to be better than that of amisulbrom but will be confirmed in a future study. Therefore, three aromatic geranyl sulfonamide compounds, **4d-1**, **4d-2** and **4e-2**, were screened as “star molecules” with fungicidal activities and potential Complex III inhibitors, which were worthy of investigation in a follow-up study of novel fungicidal candidates.

Experimental

Instruments and Chemicals

Citronella oil was purchased from AFU (Beijing, China) as a mixture without purification. Geraniol, citronellol and citronellal were purchased from MACKLIN (China). Pyraclostrobin was purchased from J&K (China). For all reactions, the solvents and chemical reagents, obtained from China National Medicines Co., Ltd, Beijing, China, were of analytical or synthetic grade and were used without purification. Column chromatography purification was performed using silica gel. NMR spectra were obtained using a Bruker Avance DPX300 spectrometer with tetramethylsilane as the internal standard. Mass spectra were obtained with an Agilent 1100 LC-MSD-Trap mass spectrometer equipped with a standard electrospray ionization (ESI) source.

Synthetic Procedures

General Synthetic Procedure for Intermediate 2

Geraniol (original material **1**, 32.41 mmol, 500 mg), phthalimide (35.65 mmol, 525 mg) and triphenylphosphine (PPh_3, 35.65 mmol, 9.35 mg) were dissolved in tetrahydrofuran (THF, 30 mL) in a 100

mL round-bottom flask. Diisopropyl azodicarboxylate (DIAD 35.65 mg, 7.21 mmol) was slowly added dropwise (1 mL/min) to the mixture in an ice bath. The mixture was incubated at 25°C for 6 h. Intermediate **2** was purified using column chromatography (petroleum ether/ethyl acetate = 10/1) in yields of 44.9%.

General Synthesis Procedure for Intermediate 3

Intermediate **2** (1.76 mmol, 50 mg) and hydrazine hydrate (110 mg, 2.12 mmol) were dissolved in ethyl alcohol (EtOH, 30 mL) in a 100 mL round-bottom flask. This solution was refluxed for 5 h. The reaction mixture was concentrated under reduced pressure to remove the EtOH. Finally, intermediate **3** was purified using column chromatography (petroleum ether/ethyl acetate = 10/1) in yields of 29.4%.

General Synthesis Procedure for Target Compounds 4a *and* 4b-4e

The plant germ-resisting compounds **4a-4d** were prepared via a crosslinking reaction with different combinations of intermediate **3** and acyl chloride compounds or sulfonyl chloride compounds. Taking the synthetic method of compound **4a-1** as an example, intermediate **3** (700 mg, 4.57 mmol) and triethylamine (920 mg, 9.14 mmol) were dissolved in dichloromethane (DCM, 30 mL), and 2-methylbenzenesulfonyl chloride (1045 mg, 5.48 mmol) was slowly added dropwise (1 mL/min) to the mixture in an ice bath. The mixture was incubated at 25°C for 15 h. Compound **4a-1** was purified using column chromatography (petroleum ether/ethyl acetate = 10/1) to give a yield of 63.2%. All of the other target compounds were prepared using the similar procedures.

Date for 4a-1

Yellow liquid; yield = 51.4%; ^{1}H NMR (300 MHz, Chloroform-*d*) δ 8.00 (dd, *J* = 8.2, 1.5 Hz, 1H), 7.47 (td, *J* = 7.4, 1.4 Hz, 1H), 7.38 – 7.29 (m, 2H), 5.16 – 4.92 (m, 2H), 4.35 (s, 1H), 3.57 (t, *J* = 6.4 Hz, 2H), 2.66 (s, 3H), 2.08 – 1.84 (m, 4H), 1.68 (s, 3H), 1.59 (s, 3H), 1.54 (s, 3H); ^{13}C NMR (75 MHz, Chloroform-*d*) δ 140.83, 137.67, 136.65, 132.34, 132.10, 131.50, 129.29, 125.75, 123.27, 118.19, 40.42, 38.96, 25.85, 25.30, 19.94, 17.32, 15.81; ESI-MS: $[M+NH_4]^+$: observed: 325.1937; expected: 325.1944.

Date for 4a-2

Yellow liquid; yield = 43.2%; ^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.74–7.65 (m, 2H), 7.43-7.37 (m, 2H), 5.12–4.98 (m, 2H), 4.33 (d, *J* = 6.3Hz, 1H), 3.59 (t, *J* = 6.4Hz, 2H), 2.44 (s, 3H), 2.00-1.92 (m, 4H), 1.68 (s, 3H), 1.58 (s, 3H), 1.55 (s, 3H); ^{13}C NMR (75 MHz, Chloroform-*d*) δ 140.79, 139.57, 138.87, 133.01, 131.52, 128.55, 127.16, 123.91, 123.26, 118.23, 40.68, 38.97, 25.86, 25.30, 20.98, 17.32, 15.87; ESI-MS: $[M-H]^-$: observed: 306.1537; expected: 306.1533.

Date for 4a-3

Yellow liquid; yield = 49.8%; ^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.75-7.69 (m, 2H), 7.36–7.27 (m, 2H), 5.01 (dd, *J* = 11.1, 6.3Hz, 2H), 4.45 (t, *J* = 5.8Hz, 1H), 3.55 (t, *J* = 6.5Hz, 2H), 2.42 (s, 3H), 1.94 (dt, *J* = 11.1,

6.0Hz, 4H), 1.65 (s, 3H), 1.56 (s, 3H), 1.52 (s, 3H). ^{13}C NMR (75 MHz, Chloroform-*d*) δ 142.92, 140.53, 136.84, 131.40, 129.26, 126.85, 123.34, 118.33, 40.64, 38.96, 25.86, 25.28, 21.13, 17.29, 15.86; ESI-MS: $[M-H]^-$: observed: 306.1534; expected: 306.1533.

Date for 4a-4

Yellow liquid; yield = 63.8%; ^{1}H NMR (300 MHz, Chloroform-*d*) δ 8.28-8.20 (m, 1H), 7.90-7.85 (m, 1H), 7.78–7.67 (m, 2H), 5.07–4.96 (m, 2H), 4.57 (s, 1H), 3.63 (t, *J* = 6.4Hz, 2H), 1.94 (dt, *J* = 12.6, 6.9Hz, 4H), 1.68 (s, 3H), 1.58 (s, 3H), 1.55 (s, 3H); ^{13}C NMR (75 MHz, Chloroform-*d*) δ 140.93, 138.60, 132.28, 131.98, 131.41, 131.33, 128.16, 128.08, 127.99, 127.91, 127.25, 126.81, 123.20, 120.88, 117.91, 40.81, 38.88, 25.78, 25.20, 17.22, 15.75; ESI-MS: $[M-H]^-$: observed: 360.1520; expected: 360.1521.

Date for 4a-5

White solid; yield = 42.1%; ^{1}H NMR (300 MHz, Chloroform-*d*) δ 8.41–8.30 (m, 2H), 8.14–8.00 (m, 2H), 5.00 (dt, *J* = 13.1, 6.9Hz, 2H), 4.73 (t, *J* = 6.4Hz, 1H), 3.65 (t, *J* = 6.4Hz, 2H), 2.03–1.84 (m, 4H), 1.65 (s, 3H), 1.55 (d, *J* = 2.9Hz, 6H); ^{13}C NMR (75 MHz, Chloroform-*d*) δ 149.69, 145.95, 141.58, 131.68, 128.01, 123.94, 123.03, 117.66, 40.78, 38.95, 25.81, 25.27, 17.28, 15.9; ESI-MS: $[M+H]^-$: observed: 339.1368; expected: 339.1373.

Date for 4a-6

Yellow liquid; yield = 56.7%; ^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.49-7.46 (m, 2H), 7.37-7.32 (m, 1H), 5.32 (t, *J* = 5.7Hz, 1H), 5.08–4.95 (m, 2H), 3.71 (t, *J* = 6.6Hz, 2H), 2.03–1.80 (m, 4H), 1.67 (s, 3H), 1.58 (s, 6H); ^{13}C NMR (75 MHz, Chloroform-*d*) δ 141.46, 135.64, 134.58, 131.89, 131.54, 131.01, 123.20, 117.35, 40.91, 38.96, 25.79, 25.31, 17.34, 15.91; ESI-MS: $[M-H]^-$: observed: 360.0599; expected: 360.0597.

Date for 4a-7

Yellow liquid; yield = 45.7%; ^{1}H NMR (300 MHz, Chloroform-d) δ 8.07–7.95 (m, 2H), 7.90-7.75 (m, 2H), 5.03 (q, *J* = 7.0Hz, 2H), 4.64 (t, *J* = 5.8Hz, 1H), 3.64 (t, *J* = 6.4Hz, 2H), 1.95 (qd, *J* = 8.6, 7.7, 4.5Hz, 4H), 1.68 (s, 3H), 1.58 (s, 3H), 1.57 (s, 3H); ^{13}C NMR (75 MHz, Chloroform-d) δ 144.40, 141.41, 132.50, 131.65, 127.40, 123.07, 117.73, 115.93, 40.72, 38.95, 25.82, 25.28, 17.32, 15.91; ESI-MS: $[M-H]^-$: observed: 317.1327; expected: 317.1329.

Date for 4a-8

Yellow liquid; yield = 55.9%; ^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.91–7.69 (m, 2H), 7.11–6.83 (m, 2H), 5.10–4.95 (m, 2H), 3.85 (s, 3H), 4.48 (s, 1H), 3.53 (t, *J* = 6.4Hz, 2H), 2.02–1.85 (m, 4H), 1.65 (s, 3H), 1.55 (s, 3H), 1.52 (s, 3H); ^{13}C NMR (75 MHz, Chloroform-*d*) δ 162.47, 140.56, 131.44, 131.35, 128.95, 123.31, 118.33, 113.82, 55.25, 40.61, 38.98, 25.87, 25.29, 17.31, 15.88; ESI-MS: $[M+H]^-$: observed: 324.1623; expected: 324.1628.

Date for 4a-9

Yellow liquid; yield = 46.9%; ^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.48 (dd, *J* = 8.4, 2.2Hz, 1H), 7.34 (d, *J* = 2.2Hz, 1H), 6.93 (d, *J* = 8.4Hz, 1H), 5.11–4.95 (m, 2H), 4.11 (d, *J* = 7.1Hz, 1H), 3.92 (d, *J* = 5.7Hz, 6H), 3.54 (t, *J* = 6.4Hz, 2H), 1.97-1.91 (m, 4H), 1.65 (s, 3H), 1.56 (s, 3H), 1.53 (s, 3H); ^{13}C NMR (75 MHz, Chloroform-*d*) δ 152.16, 148.80, 140.67, 131.47, 123.26, 120.77, 118.27, 110.17, 109.42, 69.69, 55.89, 40.66, 39.00, 25.89, 25.27, 21.58, 17.29, 15.88; ESI-MS: $[M-H]^-$: observed: 352.1592; expected: 352.1588.

Date for 4a-10

Yellow liquid; yield = 61.0%; ^{1}H NMR (300 MHz, Chloroform-*d*) δ 8.05–7.86 (m, 2H), 7.43–7.31 (m, 2H), 5.14–4.95 (m, 2H), 4.41 (t, *J* = 5.7Hz, 1H), 3.62 (t, *J* = 6.4Hz, 2H), 1.96 (dt, 4H), 1.68 (s, 3H), 1.58 (s, 3H), 1.56 (s, 3H); ^{13}C NMR (75 MHz, Chloroform-*d*) δ 151.74, 141.20, 138.34, 131.60, 128.93, 123.14, 120.57, 117.94, 40.67, 38.96, 25.82, 25.28, 17.28, 15.88; ESI-MS: $[M-H]^-$: observed: 376.1202; expected: 376.1200.

Date for 4b-1

Yellow solid; yield = 63.2%; ^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.81 - 7.49 (m, 4H), 5.08 - 4.98 (m, 2H), 4.44 (t, *J* = 5.73, 5.73 Hz, 1H), 3.58 (t, *J* = 6.93, 7.19 Hz, 2H), 1.99 – 1.87 (m, 4H), 1.66 (s, 3H), 1.56 (s, 3H), 1.53 (s, 3H), 1.24 (s, 9H); ^{13}C NMR (75 MHz, Chloroform-*d*) δ 155.31, 139.91, 136.08, 130.77, 126.03, 126.03, 124.98, 124.98, 122.65, 117.70, 39.99, 38.32, 34.10, 30.07, 30.07, 30.07, 25.20, 24.63, 16.66, 15.20; ESI-MS: $[M + Na^+]$: observed: 372.1971; expected: 372.1968.

Date for 4b-2

Yellow solid; yield = 55.3%; ^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.17 (s, 2H), 5.15-4.99 (m, 2H), 4.22-4.12 (m, 1H), 3.58 (t, *J* = 7.02, 5.94 Hz, 2H), 2.00 – 1.91 (m, 4H), 1.66 (s, 3H), 1.56 (s, 3H),1.53 (s, 3H), 1.28-1.24(q, *J* = 6.22, 5.92 Hz, 18H), 1.15-1.13 (d, *J* = 6.74 Hz, 3H); ^{13}C NMR (75 MHz, Chloroform-*d*) δ 168.13, 140.62, 133.80, 132.37, 131.70, 123.82, 123.14, 118.04, 39.50, 35.82, 30.92, 26.33, 25.63, 25.63, 17.66, 17.66, 17.66, 17.66, 17.66, 17.66, 16.37, 16.37, 16.37; ESI-MS: $[M + Na^+]$: observed: 442.2752; expected: 442.2750.

Date for 4b-3

Yellow solid; yield = 39.8%; ^{1}H NMR (300 MHz, Chloroform-*d*) δ 8.03 - 7.77 (m, 4H), 5.06-4.98 (m, 2H), 4.74 (t, *J* = 5.96, 5.53 Hz, 1H), 3.62 (t, *J* = 6.22, 5.92 Hz, 2H), 1.99 – 1.87 (m, 4H), 1.66 (s, 3H), 1.56 (s, 6H); ^{13}C NMR (75 MHz, Chloroform-*d*) δ 144.00, 141.65, 131.98, 127.69, 126.29, 126.24, 126.19, 126.14, 123.51, 118.22, 41.08, 39.32, 26.19, 25.64, 17.64, 16.27; ESI-MS: $[M + Na^+]$: observed: 384.1219 ; expected: 384.1216.

Date for 4c-1

Yellow solid; yield = 44.5%; ^{1}H NMR (300 MHz, Chloroform-*d*) δ 9.16 – 8.21 (m, 7H), 5.21 (t, *J* = 7.08, 1.21 Hz, 1H), 5.02 – 4.94 (m, 2H), 3.27 (m, 2H), 2.17 – 2.05 (m, 4H), 1.68 (s, 3H),1.25 (s, 3H) 1.27 (s, 3H); ^{13}C NMR (75 MHz, Chloroform-*d*) δ 141.15, 140.85, 131.83, 131.53, 131.41, 126.98, 123.29, 117.90, 40.93, 39.01, 25.88, 25.29, 17.32, 15.88; ESI-MS: [M + Na^{+}]: observed: 366.1498; expected: 366.1498.

Date for 4c-2

Yellow solid; yield = 47.2%; ^{1}H NMR (300 MHz, Chloroform-*d*) δ 8.56– 7.49 (m, 6H), 4.96 – 4.87 (m, 2H), 4.56 (d, *J* = 5.49, 5.67 Hz, 1H), 3.51 (t, *J* = 6.42, 6.48 Hz, 2H), 2.88 (s, 6H), 1.90 – 1.75 (m, 4H), 1.64 (s, 3H), 1.52 (s, 3H), 1.39 (s, 3H); ^{13}C NMR (75 MHz, Chloroform-*d*) δ 152.05, 140.91, 134.93, 131.73, 130.39, 129.93, 129.75, 128.35, 128.35, 123.65, 123.17, 118.81, 118.57, 115.16, 45.40, 41.13, 39.20, 30.90, 26.10, 25.62, 17.64, 16.0; ESI-MS: [M + Na^{+}]: observed: 387.2103; expected: 387.2101.

Date for 4d-1

Yellow solid; yield = 37.4%; ^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.61 – 7.07 (m, 3H), 5.09 – 5.07 (m, 1H), 5.03 - 5.01 (m, 1H), 4.58 (d, J = 7.1 Hz, 1H), 3.65 (m, 2H), 1.98 – 1.94 (m, 4H), 1.65 (s, 3H), 1.55 (s, 6H); ^{13}C NMR (75 MHz, Chloroform-*d*) δ 141.15, 140.85, 131.83, 131.53, 131.41, 126.98, 123.29, 117.90, 40.93, 39.01, 25.88, 25.29, 17.32, 15.88; ESI-MS: [M + Na^{+}]: observed: 322.0910; expected: 322.0906.

Date for 4d-2

Yellow solid; yield = 51.6%; ^{1}H NMR (300 MHz, Chloroform-*d*): δ 7.40 - 6.92 (m, 2H), 5.11 (t, *J* = 7.1, 1.2 Hz, 1H), 5.03 (d, *J* = 6.7, 4.0 Hz, 1H), 4.70 (t, *J* = 5.6, 5.6 Hz, 1H), 3.66 (t, *J* = 6.4, 6.4 Hz, 2H), 2.08 – 1.92 (m, 4H), 1.67 (d, *J* = 0.8 Hz, 3H), 1.59 (s, 6H); ^{13}C NMR (75 MHz, Chloroform-*d*) δ 142.37, 139.45, 136.67, 132.70, 130.99, 126.22, 124.67, 117.56, 40.91, 39.11, 25.88, 25.30, 17.32, 15.94; ESI-MS: [M + Na^{+}]: observed: 356.0517; expected: 356.0516.

Date for 4d-3

Yellow solid; yield = 47.7%; ^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.36 - 7.04 (m, 2H), 5.09 (t, *J* = 7.0, 1.1 Hz, 1H), 5.02 (m, *J* = 5.8 Hz, 2H), 3.64 (t, *J* = 6.4 Hz, 2H), 2.06 – 1.89 (m, 4H), 1.65 (s, 3H), 1.57 (s, 6H); ^{13}C NMR (75 MHz, Chloroform-*d*) δ 141.83, 141.11, 131.86, 131.47, 130.02, 123.33, 119.25, 117.80, 40.91, 39.01, 25.90, 25.31, 17.34, 15.95; ESI-MS: [M + Na^{+}]: observed: 400.0014; expected: 400.0011.

Date for 4d-4

Yellow solid; yield = 55.5%; ^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.59 - 7.53 (m, 2H), 6.19 (t, *J* = 5.84, 5.94 Hz, 1H), 5.03-4.98 (m, 2H), 3.94 (s, 3H), 3.64 (t, *J* = 7.14, 6.78 Hz, 2H), 1.97 – 1.82 (m, 4H), 1.66 (s, 3H), 1.58 (s, 3H),1.53 (s, 3H); ^{13}C NMR (75 MHz, Chloroform-*d*) δ 161.14, 146.05, 140.96, 131.31, 131.15, 130.53, 130.20, 123.65, 118.39, 53.14, 52.92, 41.39, 39.33, 25.65, 17.67, 16.19; ESI-MS: [M + Na^{+}]: observed: 356.0998; expected: 356.0996.

Date for 4e-1

Yellow solid; yield = 43.7%; ^{1}H NMR (300 MHz, Chloroform-*d*) δ 8.69 – 7.32 (m, 4H), 5.08 – 5.01 (m, 2H), 4.88 (s, 1H), 3.57 (t, *J* = 6.39, 6.42 Hz, 2H), 2.15 – 2.07 (m, 4H), 1.69 (s, 3H), 1.53 (s, 6H); ^{13}C NMR (75 MHz, Chloroform-*d*) δ 155.88, 142.25, 141.16, 131.03, 130.94, 130.90, 130.87, 127.57, 127.41, 40.09, 38.30, 25.27, 24.90, 16.98, 15.31; ESI-MS: [M + Na^{+}]: observed: 295.1471; expected: 295.1475.

Date for 4e-2

Yellow solid; yield = 40.6%; ^{1}H NMR (300 MHz, Chloroform-*d*) δ 8.86 – 7.47 (m, 3H), 5.06 – 4.99 (m, 2H), 4.92 (s, 1H, NH), 3.66 (t, *J* = 6.39, 6.42 Hz, 2H), 2.01 – 1.90 (m, 4H), 1.67 (s, 3H), 1.58 (s, 6H); ^{13}C NMR (75 MHz, Chloroform-*d*) δ 154.32, 147.45, 140.86, 136.34, 135.15, 130.99, 123.61, 122.45, 117.02, 40.04, 38.30, 25.16, 24.64, 16.67, 15.31; ESI-MS: [M + Na^{+}]: observed: 329.1083; expected: 329.1085.

General Synthesis Procedure for Target Compounds **5a**.

Taking the synthetic method of compound **5a-1** as an example. **4a-1** (300 mg, 0.98 mmol) was dissolved in alcohol (10 mL) followed by adding sodium ethoxide (20% *v/v*, 180 mg, 1.50 mmol) and then the mixture was stirred for 0.5 h at room temperature. Iodomethane (1 mL) was dripped into the mixture and then it was stirred for 3 h at room temperature. After the reaction was completed, the mixture was concentrated under vacuum and was extracted using ethyl acetate and saturated NaCl solution. Finally, the organic phase was washed by Na_2SO_4, and then was concentrated to give pure **5a-1** in a yield of 73.4%. All of the other target compounds were prepared using the similar procedures.

Date for 5a-1

Yellow liquid; yield = 73.4%; ^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.77–7.63 (m, 2H), 7.32-7.28 (m, 2H), 5.06 (dd, *J* = 14.7, 7.3Hz, 2H), 3.61 (d, *J* = 7.1Hz, 2H), 2.62 (s, 3H), 2.43 (s, 3H), 2.04-1.98 (m, 4H), 1.66 (s, 3H), 1.57 (s, 6H); ^{13}C NMR (75 MHz, Methylene Chloride-d_2) δ 140.90, 138.93, 132.36, 129.49, 127.31, 125.27, 124.92, 121.47, 115.95, 45.33, 37.27, 31.60, 23.93, 23.40, 19.22, 15.39, 13.87; ESI-MS: $[M+H]^{-}$: observed: 322.1834; expected: 322.1835.

Date for 5a-2

Yellow solid; yield = 78.1%; ^{1}H NMR (300 MHz, Chloroform-*d*) δ 8.54–8.24 (m, 2H), 7.99-7.95 (m, 2H), 5.15–4.91 (m, 2H), 3.70 (d, *J* = 7.2Hz, 2H), 2.71 (s, 3H), 2.04-1.96 (m, 4H), 1.65 (s, 3H), 1.60 (s, 3H), 1.56 (s, 3H); ^{13}C NMR (75 MHz, Methylene Chloride-d_2) δ 147.72, 141.73,141.73, 139.92, 129.67, 126.29, 121.99, 121.24, 115.05, 45.36, 37.25, 31.55, 23.88, 23.39, 15.37, 13.89; ESI-MS: $[M+H]^{-}$: observed: 353.1533; expected: 353.1530.

Date for 5a-3

Yellow liquid; yield = 69.2%; ^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.75–7.66 (m, 2H), 7.03–6.92 (m, 2H), 5.11–4.97 (m, 2H), 3.85 (s, 3H), 3.58 (d, *J* = 7.1Hz, 2H), 2.60 (s, 3H), 2.03-1.95 (m, 4H), 1.64 (s, 3H), 1.56 (d, *J* = 3.7Hz, 6H); ^{13}C NMR (75 MHz, Chloroform-*d*) δ 162.43, 140.81, 131.39, 129.22, 128.87, 123.39, 117.90, 113.79, 55.22, 47.25, 39.20, 33.52, 25.85, 25.31, 17.30, 15.78; ESI-MS: $[M+H]^-$: observed: 338.1784; expected: 338.1784.

Fungicidal Activity Assay

The fungicidal activities of tested compounds against *G. saubinetii, R. solani, G. fujikuroi, V. mali, S. sclerotiorum* and *M. oryzae* were screened *in vitro* at the concentration of 50 mg/L through performing the previously reported method[28]. The fungi were provided by the College of Plant Protection, China Agricultural University (Beijing, China). The commercial fungicide pyraclostrobin was selected as the positive control because of its broad-spectrum antifungal property, and the pure DMSO solvent was applied as the blank control. The antifungal rates of tested compounds were calculated using the following formula.

$$\text{Antifungal Rate (\%)} = [(D_{blank} - D_{compound}) / (D_{blank} - D_{Disk})] \times 100\%$$

Where the D_{blank} represents the average diameter of fungal growth on untreated PDA, $D_{compound}$ represents the average diameter of fungal growth on treated PDA and the D_{Disk} represents the diameter of mycelia disks inoculated in the center of the Petri dishes.

In the precision antifungal test, the median effective concentration (EC_{50}) of compounds with good inhibition activities over 60% were evaluated by three parallel repeats according to previously reported procedure[28]. The values of each EC_{50} were determined with a standard procedure using SPSS 15.0 software.

Molecular Docking

The crystal structure of Complex III co-crystallized with antimycin (PDB ID: 1PPJ) was obtained from Protein Data Bank (PDB) as the docking receptor. The 3D structures of four sulfonamide compounds, including the commercial sulfonamide fungicide amisulbrom and newly synthesized aromatic sulfonamide molecules **4d-1, 4d-2**, and **4e-2**, were constructed as ligands in Sketch mode and optimized using Tripos force field and the Gasteiger-Huckel charge in commercial SYBYL 7.3 software. Then, these optimized ligands were docked into the binding site of the receptor utilizing Surflex-Dock mode with all parameters set as default in the SYBYL 7.3 software, through which the complexes between four sulfonamide compounds and complex III protein were finally generated.

MD Simulation and Binding Free Energy Calculation

MD simulations starting from four complexes generated through docking strategy were performed with AMBER 14 package. The AMBER ff99SB force field[29] and the GAFF force field[30] were exerted to receptor and ligands respectively. All of the four complexes were solvated in a 12 Å cubic box[31] with

TIP3P explicit water models and the total number of net charges were balanced to neutral by adding Na^+ cations. The steepest descent method and the conjugate gradient method were applied to minimize the energy of the complexes. Then the temperature of the complexes was heated slowly from 0K to 300 K, followed by a 50 ns equilibration of the whole system in the NPT ensemble. For each complex, the generated trajectory of the last 2 ns was used to calculate the binding free energy between receptor and ligand by using the MM/GBSA approach.

$\Delta G_{bind} = G_{complex} - G_{protein} - G_{ligand}$ (1)

For each component, the value of G was calculated using the following formulas:

$G = G_{gas} + G_{solv}$ (2)

$G_{gas} = E_{vdw} + E_{ele}$ (3)

$G_{solv} = E_{gb} + E_{surf}$ (4)

where the G_{gas} is the total gas-phase free energy, the G_{solv} is the solvation free energy, the E_{ele} is the electrostatic energy, the G_{vdw} is the van der Waals energy, the E_{gb} is the polar solvation energy, and the E_{surf} is the nonpolar solvation energy.

Declarations

Conflicts of Interest

The authors have no conflicts of interest to declare.

Acknowledgements

This study was financially supported by the National Natural Science Foundation of China (no. 31772207), a grant from the National Key Research and Development Plan (no. 2017YFD0200504) and the State Key Laboratory for Biology of Plant Diseases and Insect Pests (no. SKLOF201902).

References

1. Wodnicka A, Huzar E, Krawczyk M, Kwiecien H. Synthesis and antifungal activity of new salicylic acid derivatives. Pol J Chem Technol. 2017;19(1):143-8.
2. Widawsky D, Rozelle S, Jin S, Huang J. Pesticide productivity, host-plant resistance and productivity in China. Agr Econ. 1998;19(1-2):203-17.
3. Burt S. Essential oils: their antibacterial properties and potential applications in foods — a review. Int J Food Microbiol. 2004;94(3):223-53.

4. Rodríguez A, Batlle R, Nerín C. The use of natural essential oils as antimicrobial solutions in paper packaging. Part II. Prog Org Coat. 2007;60(1):33-8.
5. Walker CH. The significance of pesticide residues in the environment. Outlook Agr. 1976;9(1):16-20.
6. Kordali S, Cakir A, Ozer H, Cakmakci R, Kesdek M, Mete E. Antifungal, phytotoxic and insecticidal properties of essential oil isolated from *Turkish Origanum* acutidens and its three components, carvacrol, thymol and p-cymene. Bioresource Technol. 2008;99(18):8788-95.
7. Silva W, Dória G, Maia R, Nunes R, Carvalho G, Blank A, et al. Effects of essential oils on *Aedes aegypti* larvae: alternatives to environmentally safe insecticides. Bioresource Technol. 2008;99(8):3251-5.
8. Prudent D, Perineau F, Bessiere J, Michel G, Baccou J. Analysis of the essential oil of wild oregano from Martinique (*Coleus aromaticus* Benth.) — evaluation of its bacteriostatic and fungistatic properties. J Essent Oil Res. 1995;7(2):165-73.
9. Hammer KA, Carson CF, Riley TV. Antimicrobial activity of essential oils and other plant extracts. J Appl Microbiol. 1999;86(6):985-90.
10. Hammer K, Carson C, Riley T. Antifungal activity of the components of *Melaleuca alternifolia* (tea tree) oil. J Appl Microbiol. 2003;95(4):853-60.
11. Xing Y, Li X, Xu Q, Yun J, Lu Y. Original article: Antifungal activities of cinnamon oil against *Rhizopus nigricans*, *Aspergillus flavus* and *Penicillium expansum* in vitro and in vivo fruit test. Food Sci Tech. 2010;45(9):1837-42.
12. Specos MMM, García JJ, Tornesello J, Marino P, Vecchia MD, Tesoriero MVD, et al. *Microencapsulated citronella* oil for mosquito repellent finishing of cotton textiles. T Roy Soc Trop Med H. 2010;104(10):653-8.
13. Pinheiro PF, Queiroz VTd, Rondelli VM, Costa AV, Marcelino TdP, Pratissoli D. Insecticidal activity of citronella grass essential oil on *Frankliniella schultzei* and *Myzus persicae*. Cienc Agrotec 2013;37(2):138-44.
14. Lertsatitthanakorn P, Taweechaisupapong S, Aromdee C, Khunkitti W. Antibacterial activity of citronella oil solid lipid particles in oleogel against *Propionibacterium acnes* and its chemical stability. Int J Hydrogen Energy. 2008;2(4):167-71.
15. Li WR, Shi QS, Ouyang YS, Chen YB, Duan SS. Antifungal effects of citronella oil against *Aspergillus niger* ATCC 16404. Appl Microbiol Biot. 2013;97(16):7483-92.
16. Jantamas S, Matan N, Aewsiri T. Improvement of antifungal activity of citronella oil against Aspergillus flavus on rubberwood (*Hevea brasiliensis*) using heat curing. J Trop For Sci. 2016;28(1):39-47.
17. Pereira FdO, Mendes JM, Lima IO, Mota KSdL, Oliveira WAd, Lima EdO. Antifungal activity of geraniol and citronellol, two monoterpenes alcohols, against *Trichophyton rubrum* involves inhibition of ergosterol biosynthesis. Pharm Biol. 2015;53(2):228-34.
18. Singh S, Fatima Z, Ahmad K, Hameed S. Fungicidal action of geraniol against *Candida albicans* is potentiated by abrogated CaCdr1p drug efflux and fluconazole synergism. Plos One. 2018;13(8):

e0203079/1-e0203079/26.

19. Huang LS, Cobessi D, Tung EY, Berry EA. Binding of the respiratory chain inhibitor antimycin to the mitochondrial bc(1) complex: A new crystal structure reveals an altered intramolecular hydrogen-bonding pattern. J Mol Biol. 2005;351(3):573-97.
20. Gisi U, Sierotzki H, Cook A, McCaffery A. Mechanisms influencing the evolution of resistance to Qo inhibitor fungicides. Pest Manag Sci. 2002;58(9):859-67.
21. Vonjagow G, Engel WD. Complete inhibition of electron transfer from ubiquinol to cytochrome b by the combined action of antimycin and myxothiazol. Febs Lett. 1981;136(1):19-24.
22. Chen C, Wu QY, Shan LY, Zhang B, Verpoort F, Yang GF. Discovery of cytochrome bc(1) complex inhibitors inspired by the natural product karrikinolide. Rsc Adv. 2016;6(100):97580-6.
23. Cheng H, Liu HF, Yang L, Zhang R, Chen C, Wu Y, et al. N-(3,5-Dichloro-4-(2,4,6-trichlorophenoxy)phenyl)benzenesulfonamide: A new dual-target inhibitor of mitochondrial complex II and complex III via structural simplification. Bioorg Med Chem. 2020;28(5):115299.
24. Ali EOM, Shakil NA, Rana VS, Sarkar DJ, Majumder S, Kaushik P, et al. Antifungal activity of nano emulsions of neem and citronella oils against phytopathogenic fungi, *Rhizoctonia solani* and *Sclerotium rolfsii*. Ind crop prod 2017;108:379-87.
25. Priya BS, Nanjunda SS, Tejesvi MV, Basappa SG, Gaonkar SL, Naveen S, Shashidhara PJ, Rangappa, KS. Synthesis, characterization, antimicrobial and single crystal X-ray crystallographic studies of some new sulfonyl, 4-chloro phenoxy benzene and dibenzoazepine substituted benzamides. Eur J Med Chem. 2006;41(11):1262-70.
26. Syamaiah K, Mallikarjuna Reddy G, Padmavathi V, Padmaja A. Synthesis and antimicrobial activity of some new amido / sulfonamido-linked 3,4-disubstituted pyrroles. Medicinal Chemistry Research. 2014;23(7):3287-97.
27. Nobuta T, Kawabata T. Catalyst-controlled site-selective asymmetric epoxidation of nerylamine and geranylamine derivatives. Chem Commun. 2017;53(67):9320-3.
28. Zhang X, Lei P, Sun T, Jin X, Yang X, Ling Y. Design, synthesis, and fungicidal activity of novel thiosemicarbazide derivatives containing piperidine fragments. Molecules. 2017;22(12):2085.
29. Duan Y, Wu C, Chowdhury S, Lee MC, Xiong GM, Zhang W, et al. A point-charge force field for molecular mechanics simulations of proteins based on condensed-phase quantum mechanical calculations. J Comput Chem. 2003;24(16):1999-2012.
30. Wang JM, Wolf RM, Caldwell JW, Kollman PA, Case DA. Development and testing of a general amber force field. J Comput Chem. 2004;25(9):1157-74.
31. Jorgensen WL, Chandrasekhar J, Madura JD, Impey RW, Klein ML. Comparison of simple potential functions for simulating liquid water. J Chem Phys. 1983;79(2):926-35.

Figures

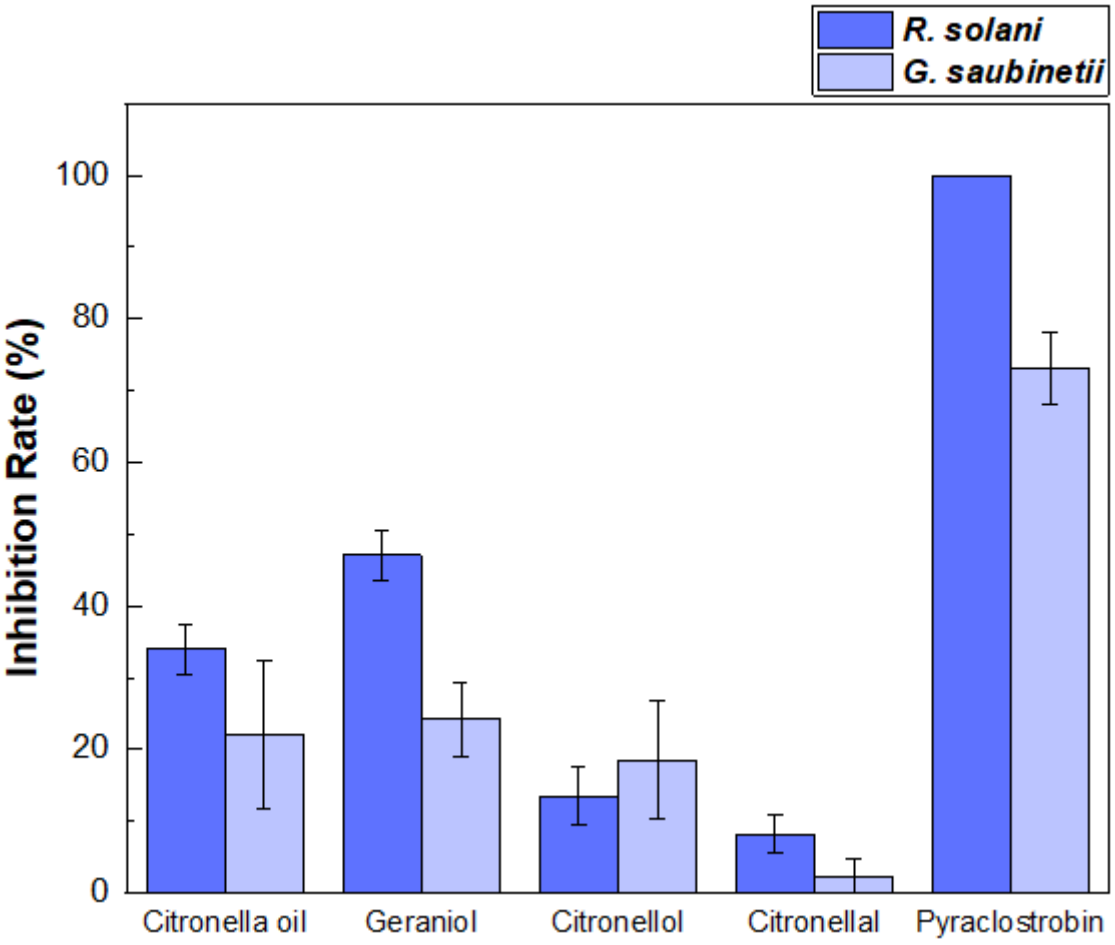


Figure 1

The in vitro fungicidal activities of the entire original citronella oil and the three main components: geraniol, citronellol and citronellal

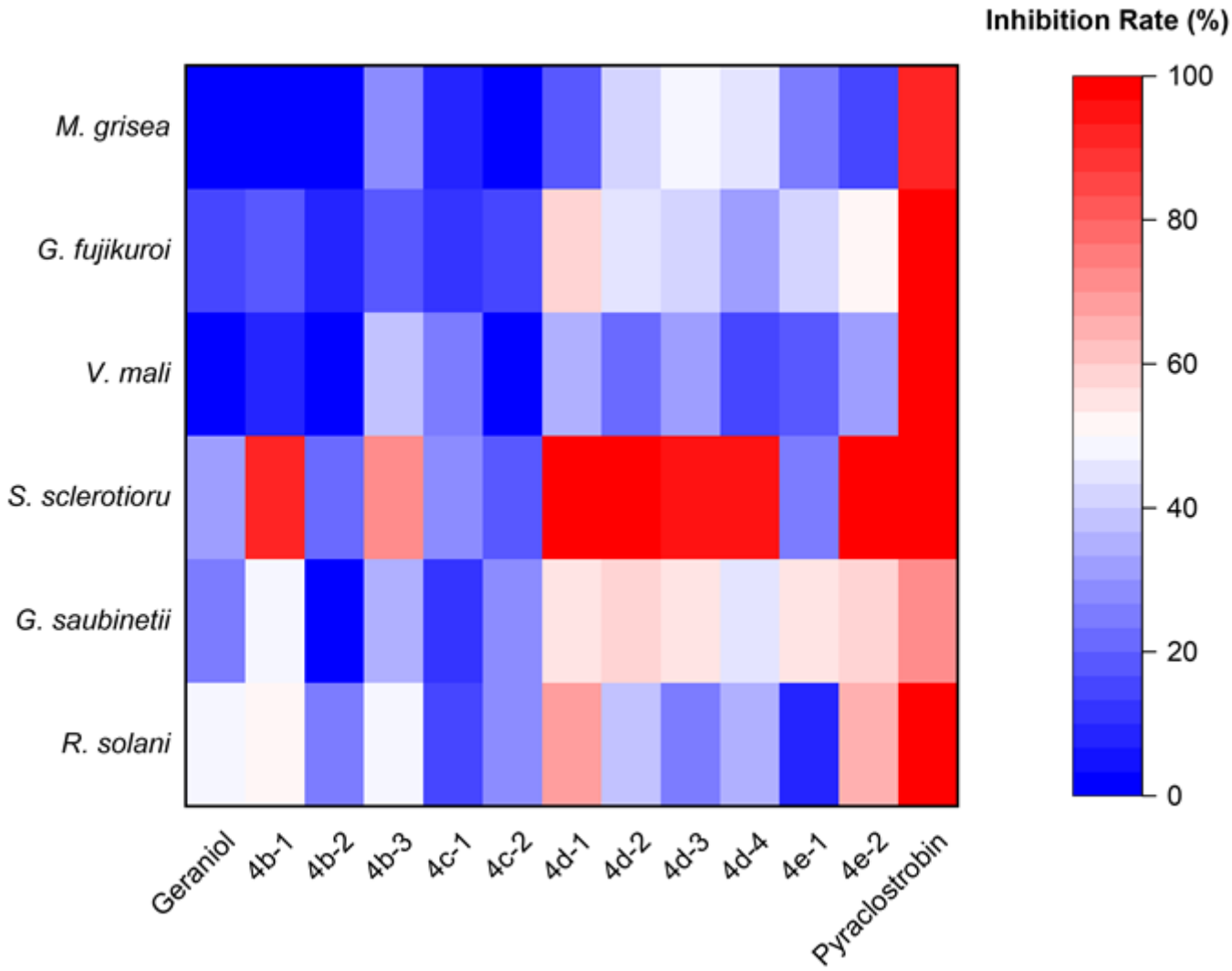


Figure 2

The in vitro fungicidal activities of the target compounds 4b-4e

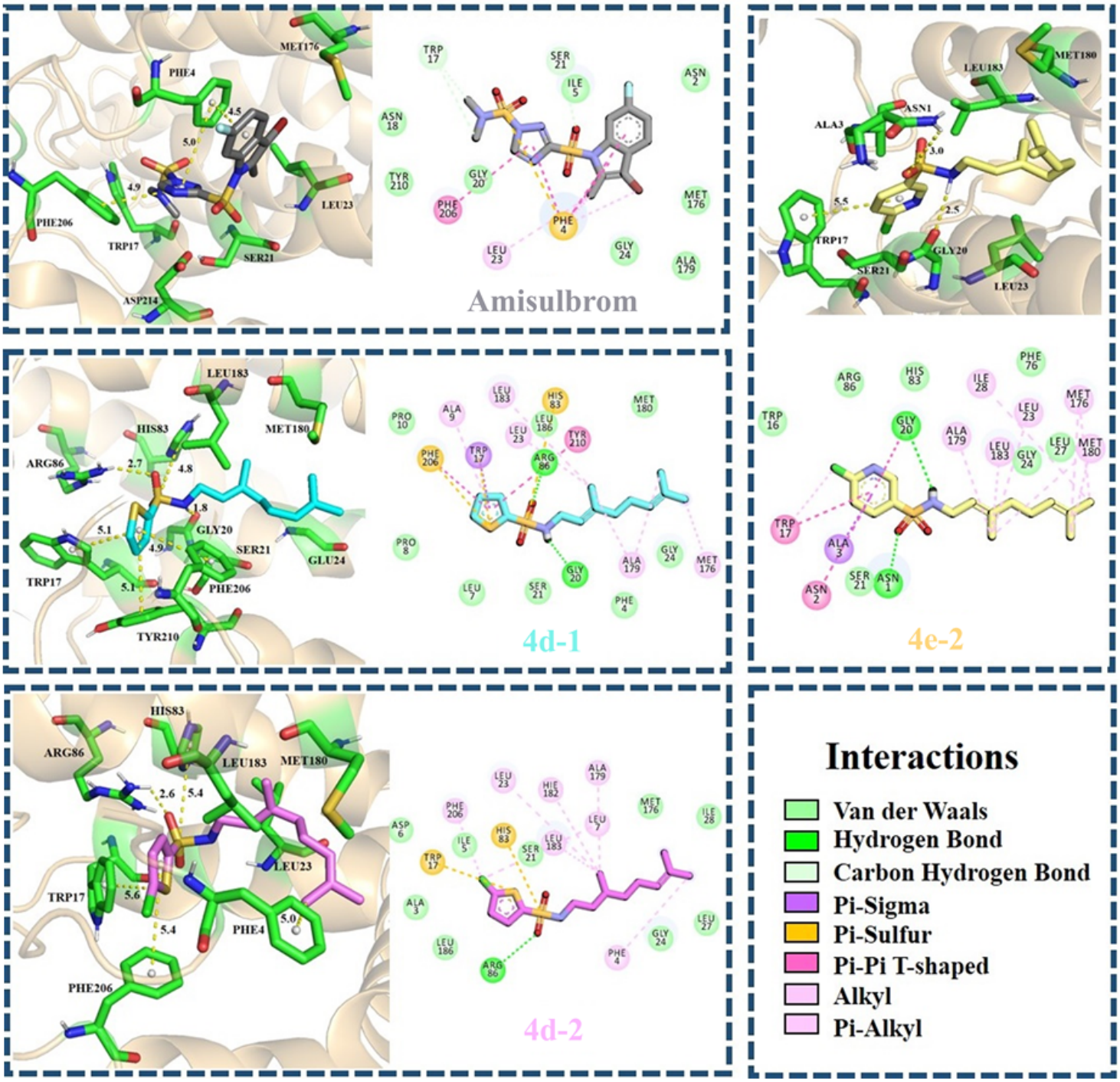


Figure 3

Molecular interactions of the commercial sulfonamide fungicide amisulbrom

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryInformation.doc

- floatimage1.png
- scheme1.png
- scheme2.png
- scheme3.png
- scheme4.png
- scheme5.png