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含偕二甲基环丙烷结构的 1,3,4-噻二唑-脲化合物的合成、抑菌活性及分子对接研究

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摘要 为了寻找以天然可再生资源为基础的杀菌剂, 设计并合成了 18 个新型含偕二甲基环丙烷结构的 1,3,4-噻二唑-脲化合物. 在质量浓度为 50 mg/L 时, 初步测定了目标化合物对 6 种植物病原菌的抑菌活性. 结果表明, (+)-顺-1-(4-溴苯基)-3-{5-[(2,2-二甲基-3-丙基环丙基)甲基]-1,3,4-噻二唑-2-基}脲(**7i**)对西瓜炭疽病菌、玉米小斑病菌和番茄早疫病菌的抑制率分别为 91.2%、85.0%和 60.1%, 均高于阳性对照百菌清. 此外, 采用比较分子力场分析法(CoMFA)建立了一个合理有效的三维定量构效关系(3D-QSAR)模型(r^2=0.990, q^2=0.517); 通过分子对接探究了目标化合物与琥珀酸脱氢酶之间的作用模式, 并定量分析了其中关键的 Arg 残基与苯环之间的阳离子-π 相互作用; 前线分子轨道计算表明目标化合物中的偕二甲基环丙烷以及噻二唑-脲-苯结构可能对抑菌活性起主要贡献.

关键词 3-蒈烯; 偕二甲基环丙烷; 1,3,4-噻二唑-脲; 抑菌活性; 三维定量构效关系(3D-QSAR); 分子对接

Synthesis, Antifungal Activity and Molecular Docking Study of 1,3,4-Thiadiazole-Urea Compounds Containing *gem*-Dimethylcyclopropane Ring Structure

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Abstract In an attempt to search for natural renewable product-based antifungal agents, eighteen 1,3,4-thiadiazole-urea compounds containing *gem*-dimethylcyclopropane ring structure were designed and synthesized. The antifungal activity of the target compounds against six plant pathogens was preliminarily evaluated at the concentration of 50 mg/L. As a result, the inhibitory rates of (+)-*cis*-1-(4-bromophenyl)-3-(5-((2,2-dimethyl-3-propylcyclopropyl)methyl)-1,3,4-thiadiazol-2-yl) urea (**7i**) against *Colletotrichum orbicular*, *Bipolaris maydis* and *Alternaria solani* were 91.2%, 85.0% and 60.1%, respectively, which was better than that of the positive control chlorothalonil. Furthermore, a reasonable and effective 3D-quantitative structure-activity relationship (3D-QSAR) model (r^2=0.990, q^2=0.517) was established by the comparative molecular field analysis method (CoMFA). The binding mode between the target compounds and succinate dehydrogenase (SDH) was investigated by molecular docking, and the cation-π interaction between the key residue Arg and the benzene ring was quantitatively analyzed. The *gem*-dimethylcyclopropane and thiadiazole-urea-benzene moieties of the target compounds probably made major contributions to the antifungal activity by frontier molecular orbital calculation.

Keywords 3-carene; *gem*-dimethylcyclopropane; 1,3,4-thiadiazole-urea; antifungal activity; 3D-quantitative structure-activity relationship (3D-QSAR); molecular docking

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1 Introduction

3-Carene, a naturally occurring chiral bicyclic monoterpene, is a rare forest biomass resource containing three-membered small ring structure in nature. It is mainly found in the turpentine oils of several special pines, such as *Pinus longifolia*, *Pinus sylvestris L.* and *Pinus szemaoensis*, as well as in a variety of plant essential oils.[1] 3-Carene showed various biological activities, such as insecticidal,[2] antifungal,[3] anti-inflammatory[4] and acetylcholinesterase inhibitory[5] activities. And its derivatives also exhibited broad-spectrum pharmacological properties, such as antifungal,[6] anticancer,[7] sphingosine-1-phosphate ($S1P_1$) receptor agonistic[8] and tyrosyl-DNA phosphodiesterase 1 (TDP1) inhibitory[9] activities. Furthermore, the molecular structure of 3-carene can be modified to obtain bioactive derivatives bearing cyclopropyl group. Cyclopropane derivatives have important applications in the fields of agrochemical and medicine[10] due to their antifungal,[11] herbicidal,[12] anti-inflammatory,[13] anticancer,[14] antimicrobial[15] and antiviral activities.[16] Among them, isoflucypram, a succinate dehydrogenase inhibitor (SDHI) containing cyclopropane,[17] displayed high-effect antifungal activity, and was developed as a commercially available fungicide.

On the other hand, 1,3,4-thiadiazoles are a class of five-membered heterocyclic compounds. It is reported that 1,3,4-thiadiazole compounds exhibited diverse biological properties, including antifungal,[18] α-glucosidase inhibitory,[19] herbicidal,[20] insecticidal,[21] antibacterial,[22] and anticancer activities.[23] Furthermore, urea derivatives have important applications in the field of agrochemical and medicine. Compounds with urea skeleton hold widespread pharmacological activities such as antifungal,[24] herbicidal,[25] insecticidal,[26] antibacterial,[27] anti-inflammatory,[28] and anticancer activities.[29]

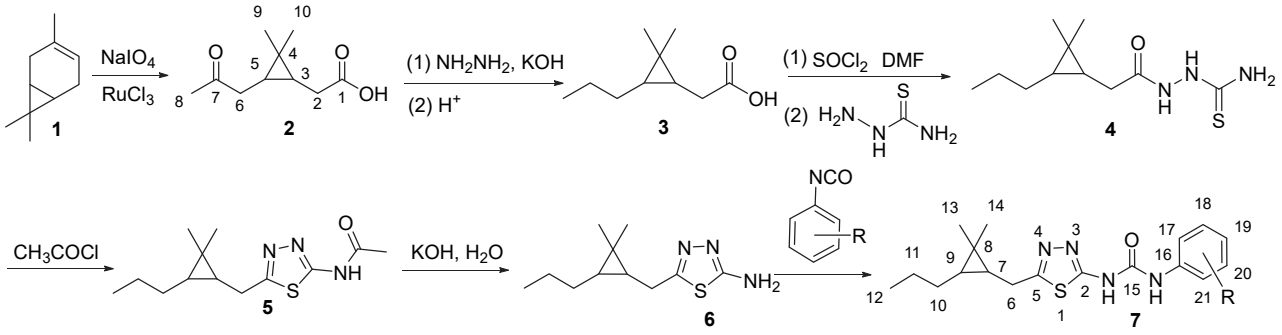
Based on the above clues, for continuing to explore high activity antifungal agents from renewable biomass resources,[30-33] a series of novel 1,3,4-thiadiazole-urea compounds containing *gem*-dimethylcyclopropane ring structure were designed and synthesized by combining bioactive 1,3,4-thiadiazole and urea moieties into the molecular skeleton of *gem*-dimethylcyclopropane converted from (+)-3-carene. Structural characterization, antifungal evaluation, 3D-quantitative structure-activity relationship (3D-QSAR), and the binding mode of the target compounds were carried out as well.

2 Results and discussion

2.1 Synthesis and characterization

The synthetic route of the target compounds **7a**～**7r** is shown in Scheme 1. The cyclohexene ring of (+)-3-carene was opened by the oxidative cleavage of the carbon-carbon double bond to give the keto acid compound **2** bearing a *gem*-dimethylcyclopropane. The ketone group of compound **2** was converted to the methylene group by the Wolff-Kishner-Huang reduction reaction to afford compound **3**. The carboxyl group of compound **3** was chloridized to form the acyl chloride, which reacted with thiosemicarbazide to prepare the acyl thiosemicarbazide compound **4**. Sequentially, 2-amino-1,3,4-thiadiazole compound **6** was formed by the ring-closing reaction using acetyl chloride as a dehydrant to avoid the ring-opening risk of the *gem*-dimethylcyclopropane moiety, followed by an alkaline hydrolysis reaction. Eighteen target 1,3,4-thiadiazole-urea compounds containing *gem*-dimethylcyclopropane ring structure **7a**～**7r** were synthesized by the nucleophilic addition reaction of compound **6** with different isocyanates.

All the structures of the target compounds were confirmed by FT-IR, NMR, ESI-MS, and elemental analysis. In the IR spectra, the characteristic absorption bands at 3399～3281 and 1724～1705 cm^{-1} were respectively attributed to the stretching vibrations of the N—H and C=O. The strong absorption bands at 1641～1584 and 1597～1510 cm^{-1} were assigned to the vibrations of the C=N and aromatic ring skeleton, respectively. In the ^1H NMR spectra, the



7a: R = *o*-Cl; **7b**: R = *m*-Cl; **7c**: R = *p*-Cl; **7d**: R = *o*-CH_3;
7e: R = *m*-CH_3; **7f**: R = *p*-CH_3; **7g**: R = *p*-OCH_3;
7h: R = o-Br; **7i**: R = *p*-Br; **7j**: R = *m*-F; **7k**: R = *p*-F;
7l: R = *o*,*p*-F_2; **7m**: R = *m*-CF_3; **7n**: R = *p*-CF_3;
7o: R = *p*-OCF_3; **7p**: R = *m*-CF_3-*p*-Cl;
7q: R = *m*,*m*-$(CF_3)_2$; **7r**: R = *p*-CH_3-*m*-Cl

Scheme 1 Synthesis of 1,3,4-thiadiazole-urea compounds containing *gem*-dimethylcyclopropane ring structure **7a**～**7r**

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signals at δ 8.24～6.81 were assigned to the protons of the benzene ring, and the characteristic peaks at about δ 12.04～8.18 were assigned to the amino protons of the urea moiety. The hydrogens in the *gem*-dimethylcyclopropane ring displayed signals in the region of δ 1.08～0.54. The ^{13}C NMR spectra of the target compounds showed peaks for carbon atoms at δ 155.7～151.3, 162.4～104.0 and 164.4～159.8 assigned to urea moiety, benzene ring, and 1,3,4-thiadiazole moiety, respectively. The signals at δ 28.7～13.9 were assigned to the *gem*-dimethylcyclopropane ring scaffold carbons. Their molecular weights and the N, C and H elemental ratios were consistent with the results of ESI-MS and elemental analysis, respectively.

2.2 *In vitro* antifungal activity

The antifungal activity of the target compounds **7a**～**7r** was preliminarily assayed by the *in vitro* method, using commercial fungicide chlorothalonil as positive control and six plant pathogens including tomato early blight (*Alternaria solani*), peanut speckle (*Cercospora arachidicola*), wheat scab (*Gibberella zeae*), cucumber fusarium wilt (*Fusarium oxysporum f. sp. cucumerinum*), watermelon anthracnose (*Colletotrichum orbiculare*), and corn southern leaf blight (*Bipolaris maydis*) as tested fungi. The results under the concentration of 50 mg/L were listed in Table 1.

It was found that most of the target compounds had a certain inhibitory effect on the tested plant pathogens, in which compound **7i** (R＝*p*-Br) with inhibitory rates of 91.2%, 85.0%, and 60.1% against *C. orbiculare*, *B. maydis*, and *A. solani*, respectively, exhibited better antifungal activity than that of the positive control chlorothalonil with the corresponding inhibitory rates of 75.0%, 81.8% and 45.0%. Besides, compound **7g** (R＝*p*-OCH_3) displayed a moderate but broad-spectrum antifungal effect against all the tested fungi. Almost all of the target compounds had inhibitory rates of more than 50% against *A. solani*, displaying a higher inhibitory rate than that of chlorothalonil with the 45.0% inhibitory rate. It was also found that, on the whole, the target compounds with the *p*-R on the benzene ring showed better inhibitory activity against the tested fungi than that of the others. For instance, the antifungal activity of compounds **7c** (R＝*p*-Cl) and **7f** (R＝*p*-CH_3) was better than that of compounds **7b** (R＝*m*-Cl), **7a** (R＝*o*-Cl), **7e** (R＝*m*-CH_3), and **7d** (R＝*o*-CH_3), respectively. Therefore, a 3D-QSAR study was subsequently performed.

2.3 3D-QSAR analysis

Because almost all of the target compounds had inhibitory rates of more than 50% against *A. solani*, displaying a higher inhibitory rate than that of chlorothalonil with the 45.0% inhibitory rate, they were used to build the 3D-QSAR model by the CoMFA method.[34] As presented in Table 2, fifteen target compounds were selected in the training set and three compounds in the test set. A 3D-QSAR model was then established. The value of correlation coefficient (r^2＝0.990), standard error of estimate (S ＝ 0.015), the Fischer ratio (F ＝ 125.963) and the cross-validated coefficient (q^2＝0.517) were listed in Table 3, implying that the built model was reasonable and effective. Meanwhile, the scatter plot of experimental AF values *vs* predicted AF[a] values was presented in Figure 1. All points were concentrated near the X＝Y line, illustrating that the 3D-QSAR model had a good predictive ability.

Table 1 Antifungal activity of the target compounds **7a**～**7r**

Compd.	Inhibitory rate/% under the concentration of 50 mg/L					
	A. solani	*C. arachidicola*	*G. zeae*	*F. oxysporum f. sp. cucumerinum*	*C. orbiculare*	*B. maydis*
7a	55.5	50.6	43.3	20.9	17.3	16.0
7b	56.3	60.0	60.0	26.4	17.3	24.0
7c	57.2	60.0	46.1	59.1	31.8	34.0
7d	48.0	60.0	39.2	22.7	24.5	20.0
7e	51.5	50.6	32.2	19.1	19.1	18.0
7f	66.1	60.0	53.1	22.7	17.3	18.0
7g	71.0	75.6	55.8	62.7	71.8	70.0
7h	57.8	53.8	57.2	26.4	17.3	26.0
7i	60.1	56.8	46.3	76.6	91.2	85.0
7j	56.3	60.0	53.1	28.2	24.5	22.0
7k	49.1	56.9	39.2	26.4	35.5	48.0
7l	56.3	60.0	44.7	26.4	17.3	20.0
7m	54.9	47.5	40.6	26.4	17.3	14.0
7n	58.8	60.0	46.1	26.4	26.4	26.0
7o	59.5	53.8	43.3	33.6	26.4	12.0
7p	56.8	60.0	46.1	19.1	13.6	18.0
7q	57.3	56.9	39.2	24.5	22.7	22.0
7r	67.1	23.0	46.4	14.5	12.3	14.8
Chlorothalonil[a]	45.0	94.4	58.3	91.7	75.0	81.8

[a] The commercial fungicide as a positive control.

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Table 2 AF values of experimental and predicted activities for the target compounds

Compd.	MW	AF	AF[a]	Residual
7a	378.92	−2.48	−2.45	−0.03
7b[b]	378.92	−2.47	−2.57	0.10
7c	378.92	−2.45	−2.53	0.08
7d[b]	358.50	−2.59	−2.28	−0.31
7e	358.50	−2.53	−2.19	−0.34
7f	358.50	−2.26	−2.49	0.23
7g	374.50	−2.18	−2.43	0.25
7h	423.37	−2.49	−2.44	−0.05
7i	423.37	−2.45	−2.57	0.12
7j	362.47	−2.45	−2.48	0.03
7k[b]	362.47	−2.57	−2.54	−0.03
7l	380.46	−2.47	−2.46	−0.01
7m	412.48	−2.53	−2.47	−0.06
7n	412.48	−2.46	−2.54	0.08
7o	428.47	−2.46	−2.54	0.08
7p	446.92	−2.53	−2.30	−0.23
7q	480.47	−2.55	−2.45	−0.10

[a] Predictive value. [b] Test-set compounds.

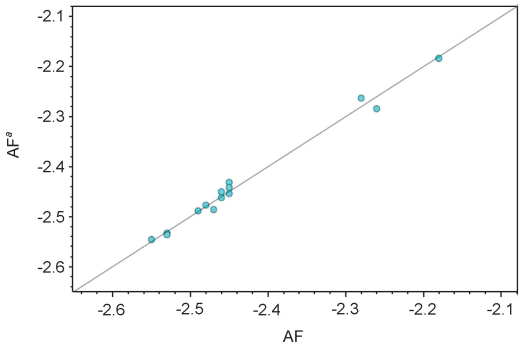


Figure 1 Scatter plot of AF values vs AF[a] values

The contribution ratios of the steric and the electrostatic fields were 66.9% and 33.1% (Table 3), respectively, indicating the steric field was the main factor affecting the inhibitory activity. As shown in Figure 2, the distinguishing colors represented different field contours. In the steric field contour map, the green region represented that introduction of large R groups was beneficial to increasing the antifungal activity, while the yellow defined the opposite. In the electrostatic field contour map, the blue region represented that the R group with electron-donating groups would improve antifungal activity, while the red defined the opposite. In Figure 2a, there was a green enclosed volume suspended on the 4-position of the benzene ring, which revealed that the introduction of bigger R groups at 4-position of the benzene ring would help to increase the bioactivity of these target compounds. For example, compounds **7g** (R=*p*-OCH_3), **7i** (R=*p*-Br) and **7o** (R=*p*-OCF_3) showed better bioactivity. Also, compound **7f** (R=*p*-CH_3) displayed higher antifungal activity than compounds **7e** (R=*m*-CH_3) and **7d** (R=*o*-CH_3). In Figure 2b, there was a blue enclosed volume suspended on the 4-position of the benzene ring. Therefore, an electron-donating R would favor the increase in their antifungal activity. For instance, compound **7g** (R=*p*-OCH_3) exhibited better antifungal activity than that of compounds **7f** (R=*p*-CH_3), **7i** (R=*p*-Br), **7c** (R=*p*-Cl) and **7k** (R=*p*-F).

Table 3 Summary of CoMFA analysis[a]

q^2	r^2	S	F	Contribution/%	
				Steric	Electrostatic
0.517	0.990	0.015	125.963	66.9	33.1

[a] q^2: cross-validated correlational coefficient, r^2: non-validated correlational coefficient, S: standard error of estimate, F: the Fischer ratio.

Furthermore, two novel compounds (Figure 3) were designed based on the 3D-QSAR analysis above, and their potential antifungal activities were predicted by the established model. As a result, compounds **3a** (AF[a]=−1.869) and **3b** (AF[a]=−1.909) had desirable antifungal activities with inhibitory rates of 86.7% and 86.8%, respectively, which deserved further study.

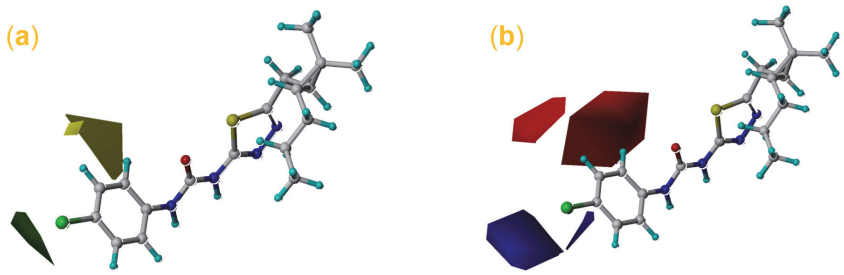
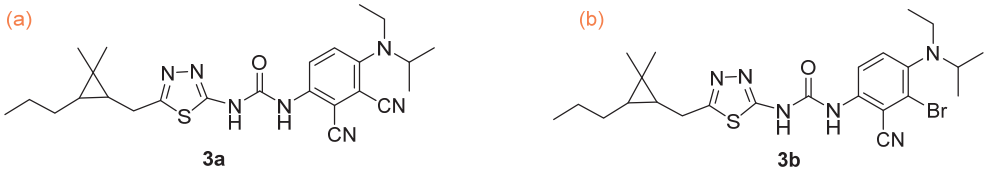


Figure 2 Contours of CoMFA

(a) Contours of steric contribution are represented in yellow and green; (b) contour of electrostatic contribution represented in red and blue



3a **3b**

Figure 3 Stuctures of compounds **3a** and **3b** designed based on the above 3D-QSAR analysis

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2.4 Molecular docking and theoretical calculation

We noticed that some kinds of carbohydrazide[35-36] and acyl urea[37-38] derivatives as potential SDH inhibitors were designed and synthesized. Besides, some compounds showed better inhibitory activities against plant pathogenic fungi and SDH. Because urea and carbohydrazide are bioisosterism of each other, and the molecular skeleton of urea is very similar to acyl urea. The binding mode of compound **7i** (R＝*p*-Br) with SDH was investigated by molecular docking (Figure 4), and compared with that of the commercial SDHI fungicide carboxin that is the ligand of the SDH-carboxin complex. It was found that compound **7i** (R＝*p*-Br) embedded completely in the ligand-binding domain. In Figure 4a, the O atom and N atom of urea moiety were interacted with the residues Tyr128 (D) and Trp206 (B) via the H-bonds with bond lengths of 0.279, 0.311, and 0.302 nm, respectively. In Figure 4b, the commercial SDHI carboxin with the residues Tyr128 (D) and Trp206 (B) formed two H-bonds with respective bond lengths of 0.273 and 0.297 nm. Besides, compound **7i** (R＝*p*-Br) and carboxin were surrounded by the same hydrophobic residues Ser77 (C), Ile78 (C), Arg81 (C), Pro202 (B), and His 249 (B). Therefore, compound **7i** (R＝*p*-Br) and carboxin shared the similar binding mode, implying that compound **7i** (R＝*p*-Br) was a potential SDH inhibitor.

To explore further the weak interaction of compound **7i** (R＝*p*-Br) with SDH residues, the PDB file produced by the above molecular docking was analyzed by the web program of Protein-Ligand Interaction Profiler (PLIP, https://plip-tool.biotec.tu-dresden.de/plip-web/plip/index).[39] The result was presented in Figure 5. As a result, besides the weak actions mentioned above, the cation-π interaction between the benzene ring of compound **7i** (R＝*p*-Br) and the residue Arg81 as well as the hydrophobic interaction between the *gem*-dimethylcyclopropane ring moiety and the residues Trp74, Phe65, Trp205 were found.

Furthermore, the cation-π interaction distances between the benzene rings of compounds **7i** (R＝*p*-Br), **7c** (R＝*p*-Cl), and **7k** (R＝*p*-F) and the Arg81 were 0.37, 0.39 and 0.41 nm (Figure 6), respectively, compatible with the descending rank of the antifungal activity against the tested fungi for three compounds.

The introduction of groups on the benzene ring can modulate the size of dipoles in π-system to influence the strength of cation-π interaction.[40] To explore further the relationship between cation-π interaction and antifungal activity, the calculation of charge distribution and electrostatic potentials (ESPs) distribution of benzene ring in compounds **7i** (R＝*p*-Br), **7c** (R＝*p*-Cl), and **7k** (R＝*p*-F) were subsequently performed by Gaussian 09, Multiwfn 3.7,[41-42] and VMD 1.9.3[43] software. The results were presented in Table 4 and Figure 7.

It was found that the positive charges in benzene ring of compounds **7i** (R＝*p*-Br), **7c** (R＝*p*-Cl) and **7k** (R＝*p*-F)

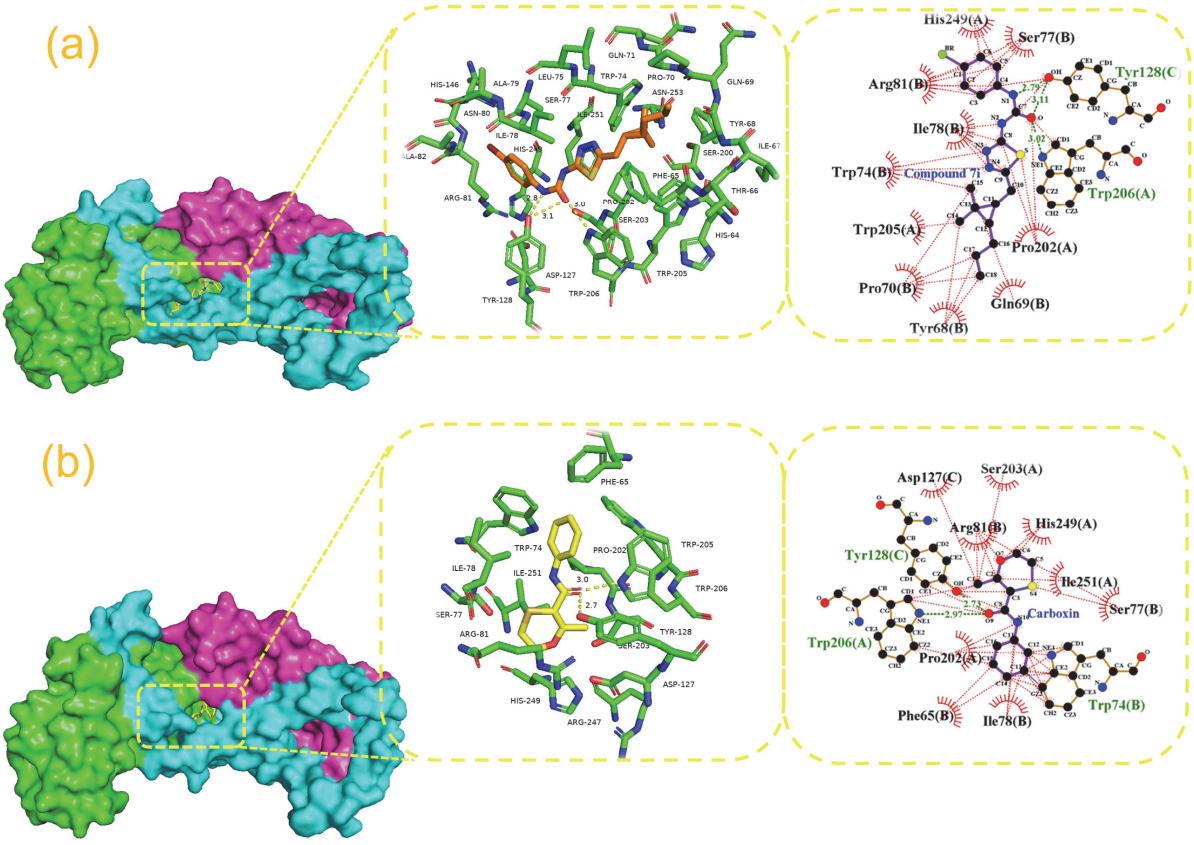

Figure 4 Binding mode and the interaction
(a) target compound 7i (R＝*p*-Br) with SDH residues; (b) commercial SDHI carboxin with SDH residues

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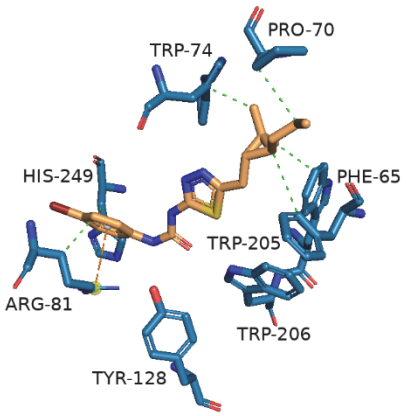


Figure 5 Benzene ring and *gem*-dimethylcyclopropane ring interaction with SDH residues

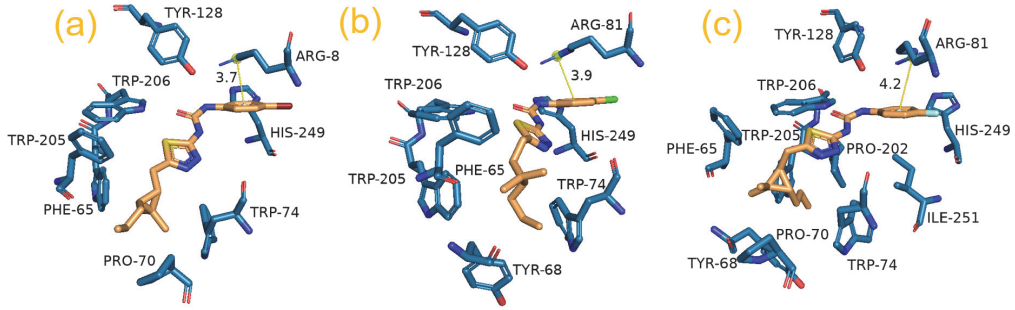
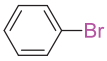
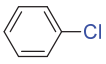
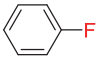


Figure 6 Cation-π interaction between Arg81 and the benzene rings of compounds **7i** (R=*p*-Br, **6a**), **7c** (R=*p*-Cl, **6b**) and **7k** (R=*p*-F, **6c**)

Table 4 Charge and population of the fragments in the compounds

Compd.	Fragment of target compound	Fragment charge	Fragment population
7i		−0.00545*e*	75.00
		0.02882*e*	39.97
7c		−0.00532*e*	57.00
		0.05794*e*	39.94
7k		0.01572*e*	48.98
		0.10539*e*	39.89

increased gradually, and the maximum ESPs points of benzene ring centers of these compounds were 13.5, 14.3, and 15.7 kJ/mol, respectively. With the enhancement of the electron-withdrawing ability, the benzene ring had more positive charges, and the cation-π effect was weakened, compatible with the descending rank of the antifungal activity against the tested fungi for three compounds. Taken together, it meant that the cation-π interaction between the benzene ring and the Arg was one of the important factors affecting the antifungal activity of the target compounds.

Besides, according to the frontier molecular orbital theory, the highest occupied molecular orbital (HOMO) has the priority to provide electrons and the lowest unoccupied molecular orbital (LUMO) easily accepts electrons, so the HOMO and LUMO are important factors that affect the bioactivity of compounds.[12] Therefore, the HOMOs and LUMOs of compounds **7i** (R=*p*-Br), **7c** (R=*p*-Cl) and **7k** (R=*p*-F) were calculated using Gaussian 09 on the high-performance computing platform of Guangxi University, and the results shown in Figure 8 were viewed on Multiwfn

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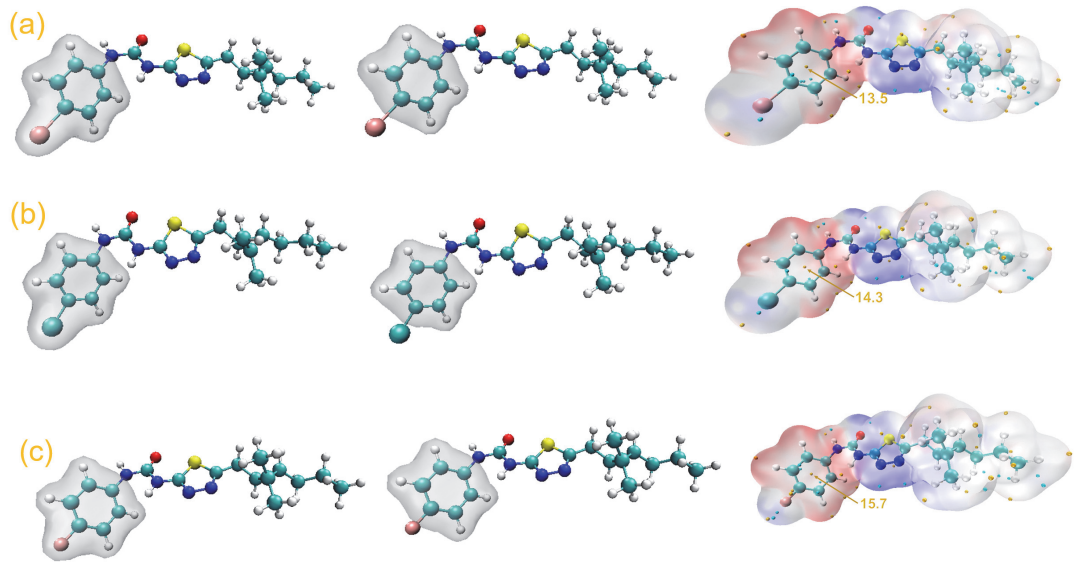

Figure 7 Fragment and ESPs of the compounds **7i** (R=*p*-Br, **7a**), **7c** (R=*p*-Cl, **7b**), and **7k** (R=*p*-F, **7c**)

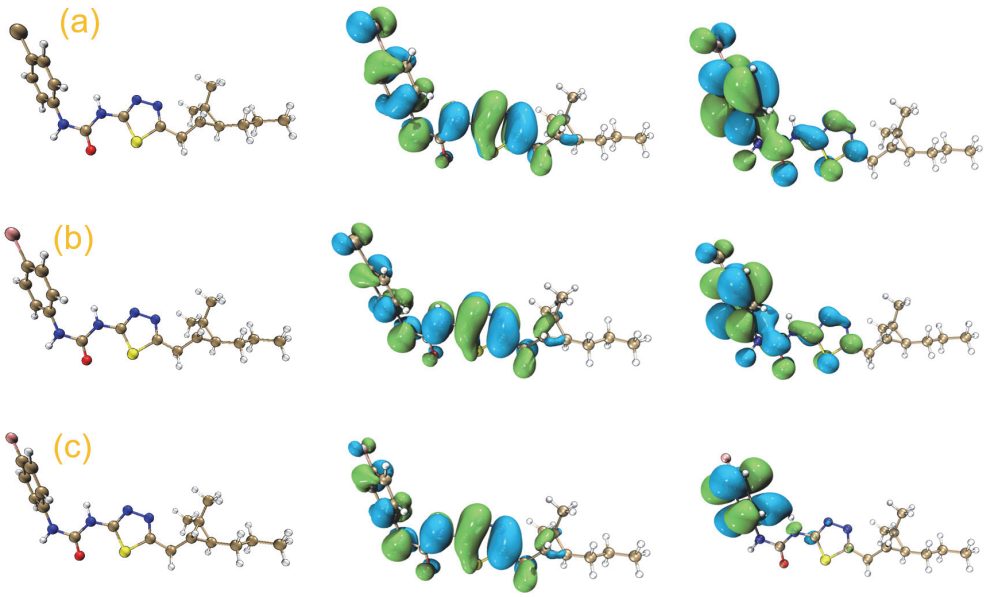

Figure 8 Frontier molecular orbitals of compounds **7i** (R=*p*-Br)
HOMO and LUMO (**8a**); **7c** (R=*p*-Cl): HOMO and LUMO (**8b**); **7k** (R=*p*-F): HOMO and LUMO (**8c**)

3.7 and VMD 1.9.3 software.

As shown in Figure 8, the HOMOs and LUMOs of these compounds were located on the urea group, the substituted benzene ring and the 1,3,4-thiadiazole ring. Also, it is worth noting that some HOMOs were distributed in the cyclopropane group as well, implying that these moieties probably made major contributions to the antifungal activity. These results would be helpful for further design and investigation of this new kind of compounds.

3 Conclusions

In summary, eighteen novel 1,3,4-thiadiazole-urea compounds containing *gem*-dimethylcyclopropane ring structure were designed and synthesized by using 3-carene as raw material, and their structures were characterized by FT-IR, NMR, ESI-MS and elemental analysis. The preliminary bioassay revealed that the inhibitory rates of compound **7i** (R=*p*-Br) against *Colletotrichum orbicular*, *Bipolaris maydis*, and *Alternaria solani* were 91.2%, 85.0% and 60.1%, respectively, which is better than that of the positive control chlorothalonil. Also, compound **7g** (R=*p*-OCH_3) with inhibitory rates of 56%～72% against all the tested plant pathogens showed broad-spectrum antifungal effect. Based on the preliminary bioassay results, a reasonable and effective 3D-QSAR model (r^2=0.990, q^2=0.517) was established, which was employed to propose two new compounds with potentially better activity. Furthermore, the cation-π interaction between the key residue Arg and the benzene ring in the target compounds was

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quantitatively investigated. Meanwhile, the *gem*-dimethylcyclopropane ring and thiadiazole-urea-benzene moieties of the target compounds probably made major contributions to the antifungal activity by frontier molecular orbital calculation. Overall, compounds **7i** (R＝*p*-Br) and **7g** (R＝*p*-OCH_3) deserved further study as lead compounds.

4 Experimental section

4.1 General Information

The gas chromatography (GC) analysis was conducted on an Agilent 6890 GC (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with column HP-1 (30 m, 0.530 mm, 0.88 μm) and FID. Melting points were recorded using an MP420 automatic melting point apparatus (Hanon Instruments Co., Ltd., Jinan, China) and were not corrected. High-pressure liquid chromatography (HPLC) and liquid chromatography (LC) analysis were conducted on Waters 1525 HPLC (2998 PDA detector and column C_{18} 5 μm (4.6 mm×150 mm), Waters Co., Ltd., Milford, MA, USA) and Wufeng LC-100 (chiral column IB, Shanghai Wufeng Scientific Instrument Co., Ltd., China) instruments, respectively. IR spectra were recorded on a Nicolet iS50 FT-IR spectrometer (Thermo Scientific Co., Ltd., Madison, WI, USA) by the KBr pellet method. ^{1}H NMR and ^{13}C NMR spectra were determined in DMSO-d_6 or $CDCl_3$ solvent on a Bruker Avance III HD 500 MHz/600 MHz spectrometer (Switzerland Bruker Co., Ltd., Zurich, Switzerland) with TMS as an internal standard. MS spectra were obtained employing the electrospray ionization (ESI) method on a TSQ Quantum Access MAX HPLC-MS instrument (Thermo Scientific Co., Ltd., Waltham, MA, USA). Elemental analyses were measured using a PE 2400 II elemental analyzer (Perkin-Elmer Instruments Co., Ltd., Waltham, MA, USA). CD spectra were determined in ethanol solvent on a MOS-450 circular dichroism spectrometer (Cbio-Logic Co., French). Optical rotation was measured on a WZZ-2S automatic polarimeter (Shanghai Precision Scientific Instrument Co., Ltd., China). (＋)-3-Carene [GC purity 98%, $[\alpha]_D^{25}$ ＋17.1 (*c* 0.67, CH_2Cl_2)] was provided by Wuzhou Pine Chemicals Co., Ltd., Wuzhou, Guangxi, China. Other reagents were purchased from commercial suppliers and used as received.

4.2 Synthesis of (－)-*cis*-2-(2,2-dimethyl-3-(2-oxopropyl)-cyclopropyl) acetic acid (**2**)

Compound **2** was prepared by the oxidation of 3-carene under weak alkaline condition.[44] (＋)-3-Carene (20 mL, 125.8 mmol) was dissolved in 100 mL of *tert*-butanol and 200 mL of water. To this mixture were added $NaIO_4$ (100.0 g, 0.6 mol) and $RuCl·3H_2O$ (0.44 g, 0.8 mmol). The mixture was stirred vigorously at 35 ℃ for 0.5 h, then raised to 45 ℃ for 3 h, and finally stirred at 55 ℃ for 1 h. Then, the mixture was cooled down to room temperature and filtered, and the filtrate was washed with ethyl acetate three times. Afterwards, the filtrate was extracted with ethyl acetate, and the organic phase was concentrated in vacuum, alkalized to pH 10 with NaOH aqueous solution. The aqueous phase was washed with ethyl acetate, and under an ice-water bath, the aqueous phase was slowly acidified to pH 3 by HCl, then extracted with ethyl acetate three times. The organic phase was dried over anhydrous $MgSO_4$ and concentrated to afford a brown oily liquid with yield of 75%. $[\alpha]_D^{25}$ －15.4 (*c* 1.02, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) *δ*: 2.44～2.36 (m, 2H, C_2-H), 2.32～2.22 (m, 2H, C_6-H), 2.18 (s, 3H, C_8-H), 1.13 (s, 3H, C_9-H or C_{10}-H), 0.98 (q, *J*＝8.2 Hz, 2H, C_3-H and C_5-H), 0.93 (s, 3H, C_9-H or C_{10}-H); ^{13}C NMR (150 MHz, $CDCl_3$) *δ*: 208.9, 179.2, 39.3, 30.1, 29.6, 28.4, 21.5, 21.1, 17.3, 15.0; IR (KBr) *ν*: 3500～3100 (COOH), 2955, 2870 (C—H), 1712 (C＝O) cm^{-1}; ESI-MS *m*/*z*: 185.05 ($M+H^+$). Anal. calcd for $C_{10}H_{16}O_3$: C 65.19, H 8.75; found C 65.18, H 8.73.

4.3 Synthesis of (＋)-*cis*-2-(2,2-dimethyl-3-propyl-cyclopropyl) acetic acid (**3**)

Compound **3** was prepared by the method reported in our previous work.[31] KOH (22.6 g, 403.6 mmol) was dissolved in ethylene glycol (85 mL) under stirring. To the mixture were added compound **2** (20.8 g, 133.0 mmol) and 65 mL of $N_2H_4·H_2O$ (*ω*＝80%). The mixture was heated and refluxed for 10 h. In this process, the water and hydrazine were continuously removed from the reaction system to accelerate the reaction. Upon completion, the mixture was cooled under an ice-water bath, acidified with aq. HCl (*ω*＝5%) to pH 3, and extracted with cyclohexane. The combined organic phase was washed with saturated NaCl aqueous solution, dried over anhydrous $MgSO_4$ and evaporated *in vacuo* to obtain a yellow oily liquid with yield of 60%. $[\alpha]_D^{25}$ ＋5.6 (*c* 0.51, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) *δ*: 2.34～2.28 (m, 2H, C_2-H), 1.35 (tq, *J*＝13.0, 6.5, 6.3 Hz, 2H, C_7-H), 1.26 (td, *J*＝14.2, 6.3 Hz, 1H, C_6-H_a), 1.18 (td, *J*＝14.0, 8.3 Hz, 1H, C_6-H_b), 1.08 (s, 3H, C_9-H or C_{10}-H), 0.93 (s, 3H, C_9-H or C_{10}-H), 0.91 (t, *J*＝7.5 Hz, 3H, C_8-H), 0.85～0.80 (m, 1H, C_3-H), 0.59～0.52 (m, 1H, C_5-H); ^{13}C NMR (150 MHz, $CDCl_3$) *δ*: 180.5, 29.9, 28.9, 26.5, 26.2, 23.1, 21.5, 17.1, 14.8, 14.1; IR (KBr) *ν*: 3500～3200 (COOH), 2962, 2867 (C—H), 1718 (C＝O) cm^{-1}; ESI-MS *m*/*z*: 169.03 ($M-H^+$). Anal. calcd for $C_{10}H_{18}O_2$: C 70.55, H 10.66; found C 70.53, H 10.64.

4.4 Synthesis of (＋)-*cis*-2-(2-(2,2-dimethyl-3-propyl-cyclopropyl)acetyl) hydrazine-1-carbothioamide (**4**)

Under an anhydrous atmosphere, three drops of *N*,*N*-dimethylformamide (DMF) were added to a solution of compound **3** (1.65 g, 10.0 mmol) in dichloromethane (DCM) (15 mL). Subsequently, a solution of $SOCl_2$ (2 mL) in DCM (10 mL) was dropwise added. The mixture was refluxed for 6 h under stirring. Afterwards, the crude product was obtained by distillation, with the DCM and the unreacted $SOCl_2$ removed. Without further purification, the crude product was dissolved in tetrahydrofuran (THF) (20 mL) and then added with thiosemicarbazide (1.8 g, 20 mmol) under an ice-water bath. Then the mixture was continuously stirred at room temperature for 12 h, and this reaction process was monitored by thin-layer chromatography (TLC). When the reaction was completed, saturated

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$NaHCO_3$ was added to neutralize the unreacted HCl, and the mixture was extracted with ethyl acetate for three times. The combined organic phase was dried over anhydrous $MgSO_4$ and evaporated in vacuum. The residue was purified by silica gel column chromatography (petrol ether/ethyl acetate, V∶V=2∶1) to obtain compound **4** as white solid with yield of 70%. m.p. 76.3～78.1 ℃; $[\alpha]_D^{25}$ +3.4 (*c* 0.50, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 10.13 (s, 1H, NH), 9.12 (s, 2H, NH), 8.82 (s, 1H, NH), 2.35～2.32 (m, 2H, C_2-H), 1.38～1.31 (m, 3H, C_7-H and C_6-H_a), 1.16 (m, 1H, C_6-H_b), 1.09 (s, 3H, C_9-H or C_{10}-H), 0.96 (s, 3H, C_9-H or C_{10}-H), 0.92～0.90 (m, 3H, C_8-H), 0.85～0.81 (s, 1H, C_3-H), 0.59～0.55 (m, 1H, C_5-H); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 30.5, 28.9, 26.5, 26.5, 23.1, 21.3, 17.6, 14.9, 14.1; IR (KBr) *v*: 3278, 3169 (N—H), 2958, 2930, 2861 (C—H), 1698 (C=O), 1625 (C=S) cm^{-1}; ESI-MS *m*/*z*: 244.10 ($M+H^+$). Anal. calcd for $C_{11}H_{21}N_3OS$: C 54.29, H 8.70, N 17.27; found C 54.27, H 8.71, N 17.25.

4.5 Synthesis of (+)-*cis*-*N*-(5-((2,2-dimethyl-3-propylcyclopropyl) methyl)-1,3,4-thiadiazol-2-yl) acetamide (**5**)

In a dry 50 mL flask, compound **4** (0.75 g, 3.1 mmol) and acetyl chloride (7 mL) were added, and then the mixture was continuously stirred at room temperature for 48 h. Upon completion, the saturated sodium bicarbonate solution was dropwise added under continuous stir until no gas was generated. Subsequently, the mixture was extracted with ethyl acetate and the organic layer was dried over anhydrous $MgSO_4$. Then, the ethyl acetate was removed in vacuo, and the crude product was further purified by silica gel column chromatography to obtain compound **5** as a colorless solid. Yield 85%. m.p. 105.1～107.3 ℃; $[\alpha]_D^{25}$ +7.5 (*c* 0.51, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 13.28 (s, 1H, NH), 3.05 (dd, J=16.3, 6.8 Hz, 1H, C_6-H_a), 2.93 (dd, J=16.3, 8.2 Hz, 1H, C_6-H_b), 2.49 (s, 3H, C_{13}-H), 1.43～1.35 (m, 3H, C_{11}-H and C_{10}-H_a), 1.29～1.27 (m, 1H, C_{10}-H_b), 1.12 (s, 3H, C_{13}-H or C_{14}-H), 1.04 (s, 3H, C_{13}-H or C_{14}-H), 0.95～0.89 (m, 4H, C_7-H and C_{12}-H), 0.71～0.63 (m, 1H, C_9-H); ^{13}C NMR (126 MHz, $CDCl_3$) δ: 168.9, 166.3, 160.6, 28.9, 27.0, 26.6, 26.1, 25.7, 23.2, 23.2, 18.0, 14.9, 14.1; IR (KBr) *v*: 3279 (N—H), 2957, 2918, 2795 (C—H), 1696 (C=O), 1573 (C=N) cm^{-1}; ESI-MS *m*/*z*: 268.09 ($M+H^+$); Anal. calcd for $C_{13}H_{21}N_3OS$: C 58.40, H 7.92, N 15.72; found C 58.39, H 7.94, N 15.70.

4.6 Synthesis of (+)-*cis*-5-((2,2-dimethyl-3-propylcyclopropyl) methyl)-1,3,4-thiadiazol-2-amine (**6**)

A solution of KOH (1.5 g, 4.8 mmol) in ethanol (10 mL) was added to a solution of compound **5** (0.7 g, 2.4 mmol) in ethanol (15 mL), and then the mixture was refluxed for 18 h under stirring. The reaction process was monitored by TLC. Upon completion, the mixture was cooled to room temperature, and filtered. The residue was washed with deionized water for three times to afford a white solid with yield of 65%. m.p. 132.3.1～132.8 ℃; $[\alpha]_D^{25}$ +11.5 (*c* 0.21, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.64 (s, 2H, NH), 2.94 (dd, J=16.2, 6.7 Hz, 1H, C_6-H_a), 2.79 (dd, J=16.2, 8.4 Hz, 1H, C_6-H_b), 1.44～1.31 (m, 3H, C_{11}-H and C_{10}-H_a), 1.29～1.21 (m, 1H, C_{10}-H_b), 1.09 (s, 3H, C_{13}-H or C_{14}-H), 1.00 (s, 3H, C_{13}-H or C_{14}-H), 0.93 (t, J=7.1 Hz, 3H, C_7-H), 0.86～0.79 (m, 1H, C_{12}-H), 0.64～0.59 (m, 1H, C_9-H); ^{13}C NMR (126 MHz, $CDCl_3$) δ: 179.8, 168.2, 162.9, 28.9, 26.9, 26.5, 26.2, 26.1, 23.2, 17.8, 14.9, 14.1; IR (KBr) *v*: 3316 (N—H), 2957, 2927, 2861 (C—H), 1633 (C=N) cm^{-1}; ESI-MS *m*/*z*: 226.09 ($M+H^+$). Anal. calcd for $C_{11}H_{19}N_3S$: C 58.63, H 8.50, N 18.65; found C 58.64, H 8.51, N 18.67.

4.7 General procedure for the synthesis of **7a**～**7r**

To a mixture of compound **6** (100 mg, 0.44 mmol) in dry acetonitrile (15 mL), substituted isocyanate (0.5 mmol) was added slowly under vigorous stirring at room temperature. Then, the reaction mixture was heated to 80 ℃ and stirred for 3～5 h. After the reaction was completed, the mixture was cooled to room temperature and filtrated to afford target compounds **7a**～**7r** in yields of 85%～95%.

(+)-*cis*-1-(2-Chlorophenyl)-3-(5-((2,2-dimethyl-3-propylcyclopropyl) methyl)-1,3,4-thiadiazol-2-yl) urea (**7a**): White solid, yield 88%. m.p. 140.0～142.7 ℃; $[\alpha]_D^{25}$ +15.0 (*c* 0.30, CH_3COCH_3); ^{1}H NMR (500 MHz, DMSO-d_6) δ: 11.47 (s, 1H, NH), 8.73 (s, 1H, NH), 8.12 (dd, J=8.3, 1.4 Hz, 1H, C_{21}-H), 7.49 (dd, J=8.0, 1.4 Hz, 1H, C_{18}-H), 7.36～7.30 (m, 1H, C_{20}-H), 7.11 (td, J=8.0, 1.43 Hz, 1H, C_{19}-H), 2.95 (dd, J=16.2, 6.8 Hz, 1H, C_6-H_a), 2.85 (dd, J=16.2, 8.3 Hz, 1H, C_6-H_b), 1.37～1.29 (m, 3H, C_{11}-H and C_{10}-H_a), 1.28～1.22 (m, 1H, C_{10}-H_b), 1.05 (s, 3H, C_{13}-H or C_{14}-H), 0.98 (s, 3H, C_{13}-H or C_{14}-H), 0.89 (t, J=7.1 Hz, 3H, C_{12}-H), 0.87～0.81 (m,1H, C_7-H), 0.60～0.55 (m, 1H, C_9-H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 164.4, 159.5, 151.3, 134.9, 129.4, 127.8, 124.5, 122.7, 121.7, 28.7, 26.3, 25.9, 25.8, 24.9, 22.7, 17.4, 14.8, 13.9; IR (KBr) *v*: 3360 (N—H), 2956, 2927, 2861 (C—H), 1707 (C=O), 1584 (C=N), 1536 (Ar—C=C) cm^{-1}; ESI-MS *m*/*z*: 379.12 ($M+H^+$). Anal. calcd for $C_{18}H_{23}ClN_4OS$: C 57.06, H 6.12, N 14.79; found C 57.05, H 6.10, N 14.77.

(+)-*cis*-1-(3-Chlorophenyl)-3-(5-((2,2-dimethyl-3-propylcyclopropyl) methyl)-1,3,4-thiadiazol-2-yl) urea (**7b**): White solid, yield 85%. m.p. 191.0～191.7 ℃; $[\alpha]_D^{25}$ +9.1 (*c* 0.31, CH_3COCH_3); ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.02 (s, 1H, NH), 9.28 (s, 1H, NH), 7.72 (s, 1H, C_{17}-H), 7.37 (d, J=8.1 Hz, 1H, C_{21}-H), 7.32 (t, J=8.0 Hz, 1H, C_{20}-H), 7.07 (d, J=7.1 Hz, 1H, C_{19}-H), 2.92 (dd, J=16.2, 6.8 Hz, 1H, C_6-H_a), 2.82 (dd, J=16.2, 8.3 Hz, 1H, C_6-H_b), 1.37～1.29 (m, 3H, C_{11}-H and C_{10}-H_a), 1.28～1.23 (m, 1H, C_{10}-H_b), 1.06 (s, 3H, C_{13}-H or C_{14}-H), 0.98 (s, 3H, C_{13}-H or C_{14}-H), 0.89 (t, J=7.1 Hz, 3H, C_{12}-H), 0.85～0.81 (m, 1H, C_7-H), 0.59～0.56 (m, 1H, C_9-H); ^{13}C NMR (151 MHz, DMSO-d_6) δ: 163.4, 161.0, 153.0, 140.4, 133.3, 130.5, 122.4, 118.1, 117.2, 28.7, 26.3, 25.9, 25.6, 25.1, 22.7, 17.4, 14.8, 13.9; IR (KBr) *v*: 3279 (N—H), 2957, 2928, 2864 (C—H), 1710 (C=O), 1615 (C=N), 1553 (Ar—C=C) cm^{-1}; ESI-MS *m*/*z*: 379.13 ($M+H^+$). Anal. calcd for $C_{18}H_{23}ClN_4OS$: C 57.06, H 9.36, N 14.79; found C 57.08, H 9.35, N 14.80.

(+)-*cis*-1-(4-Chlorophenyl)-3-(5-((2,2-dimethyl-3-pro-

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pylcyclopropyl)methyl)-1,3,4-thiadiazol-2-yl) urea (**7c**): White solid, yield 85%. m.p. 221.6～223.5 ℃; $[\alpha]_D^{25}$ +8.6 (*c* 0.45, CH_3COCH_3); ^1H NMR (500 MHz, DMSO-d_6) δ: 11.68 (s, 1H, NH), 9.93 (s, 1H, NH), 7.64 (d, *J*=8.3 Hz, 2H, C_{17}-H and C_{21}-H), 7.35～7.31 (m, 2H, C_{18}-H and C_{20}-H), 2.93 (dd, *J*=16.2, 6.7 Hz, 1H, C_6-H_a), 2.82 (dd, *J*=16.2, 8.3 Hz, 1H, C_6-H_b), 1.38～1.31 (m, 3H, C_{11}-H and C_{10}-H_a), 1.27～1.19 (m, 1H, C_{10}-H_b), 1.06 (s, 3H, C_{13}-H or C_{14}-H), 0.99 (s, 3H, C_{13}-H or C_{14}-H), 0.89 (t, *J*=7.1 Hz, 3H, C_{12}-H), 0.85～0.81 (m, 1H, C_7-H), 0.60～0.55 (m, 1H, C_9-H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 163.2, 159.8, 153.9, 138.4, 128.7, 126.0, 120.3, 28.8, 26.4, 26.1, 25.7, 25.2, 22.8, 17.4, 14.9, 14.0; IR (KBr) *v*: 3369 (N—H), 2956, 2929, 2862 (C—H), 1725 (C=O),1591 (C=N), 1544 (Ar—C=C) cm^{-1}; ESI-MS *m*/*z*: 379.13 ($M+H^+$). Anal. calcd for $C_{18}H_{23}ClN_4OS$: C 57.06, H 6.12, N 14.79; found C 57.05, H 6.10, N 14.80.

(−)-*cis*-1-(5-((2,2-Dimethyl-3-propylcyclopropyl)-methyl)-1,3,4-thiadiazol-2-yl)-3-(*o*-tolyl) urea (**7d**): White solid, yield 90%. m.p. 152.0～153.5 ℃; $[\alpha]_D^{25}$ −7.0 (*c* 0.31, CH_3COCH_3); ^1H NMR (500 MHz, DMSO-d_6) δ: 11.29 (s, 1H, NH), 8.54 (s, 1H, NH), 7.79 (d, *J*=8.0 Hz, 1H, C_{18}-H), 7.24～7.16 (m, 2H, C_{19}-H and C_{20}-H), 7.02 (t, *J*=7.3 Hz, 1H, C_{21}-H), 2.93 (dd, *J*=16.2, 6.8 Hz, 1H, C_6-H_a), 2.83 (dd, *J*=16.2, 8.3 Hz, 1H, C_6-H_b), 2.25 (s, 3H, C_{22}-H), 1.32～1.37 (m, 3H, C_{11}-H and C_{10}-H_a), 1.30～1.22 (m, 1H, C_{10}-H_b), 1.06 (s, 3H, C_{13}-H or C_{14}-H), 0.99 (s, 3H, C_{13}-H or C_{14}-H), 0.90 (t, *J*=6.9 Hz, 3H, C_{12}-H), 0.86～0.81 (m, 1H, C_7-H), 0.61～0.56 (m, 1H, C_9-H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 164.0, 160.3, 151.9, 136.4, 130.4, 128.5, 126.3, 123.8, 121.7, 28.8, 26.4, 26.0, 25.9, 25.0, 22.8, 17.9, 17.4, 14.9, 14.0; IR (KBr) *v*: 3340, 3305 (N—H), 2956, 2930, 2862 (C—H), 1712 (C=O), 1641 (C=N), 1587, 1537 (Ar—C=C) cm^{-1}; ESI-MS *m*/*z*: 359.16 ($M+H^+$). Anal. calcd for $C_{19}H_{26}N_4OS$: C 63.66, H 7.31, N 15.63; found C 63.65, H 7.30, N 15.64.

(+)-*cis*-1-(5-((2,2-Dimethyl-3-propylcyclopropyl)-methyl)-1,3,4-thiadiazol-2-yl)-3-(*m*-tolyl) urea (**7e**): White solid, yield 90%. m.p. 169.4～170.3 ℃; $[\alpha]_D^{25}$ +8.7 (*c* 0.30, CH_3COCH_3); ^1H NMR (600 MHz, DMSO-*d6*) δ: 11.19 (s, 1H, NH), 9.31 (s, 1H, NH), 7.39 (s, 1H, C_{17}-H), 7.31 (d, *J*=7.8 Hz, 1H, C_{21}-H), 7.18 (t, *J*=7.8 Hz, 1H, C_{20}-H), 6.84 (d, *J*=7.5 Hz, 1H, C_{19}-H), 2.93 (dd, *J*=16.2, 6.8 Hz, 1H, C_6-H_a), 2.83 (dd, *J*=16.2, 8.3 Hz, 1H, C_6-H_b), 2.29 (s, 3H, C_{22}-H), 1.37～1.30 (m, 3H, C_{11}-H and C_{10}-H_a), 1.29～1.24 (m, 1H, C_{10}-H_b), 1.06 (s, 3H, C_{13}-H or C_{14}-H), 0.99 (s, 3H, C_{13}-H or C_{14}-H), 0.89 (t, *J*=7.1 Hz, 3H, C_{12}-H), 0.86～0.82 (m, 1H, C_7-H), 0.60～0.56 (m, 1H, C_9-H); ^{13}C NMR (151 MHz, DMSO-d_6) δ: 163.5, 161.1, 152.4, 138.8, 138.1, 128.7, 123.4, 119.1, 115.8, 28.8, 26.3, 26.0, 25.7, 25.0, 22.8, 21.2, 17.3, 14.8, 13.9; IR (KBr) *v*: 3284 (N—H), 2957, 2927, 2867 (C—H), 1708 (C=O), 1623 (C=N), 1560 (Ar—C=C) cm^{-1}; ESI-MS *m*/*z*: 359.18 ($M+H^+$). Anal. calcd for $C_{19}H_{26}N_4OS$: C 63.66, H 7.31, N 15.63; found C 63.65, H 7.32, N 15.61.

(+)-*cis*-1-(5-((2,2-Dimethyl-3-propylcyclopropyl)-methyl)-1,3,4-thiadiazol-2-yl)-3-(*p*-tolyl) urea (**7f**): White solid, yield 92%. m.p. 174.5～175.7 ℃; $[\alpha]_D^{25}$ +7.7 (*c* 0.30, CH_3COCH_3); ^1H NMR (500 MHz, DMSO-d_6) δ: 12.04 (s, 1H, NH), 10.37 (s, 1H, NH), 7.57 (d, *J*=8.2 Hz, 2H, C_{17}-H and C_{21}-H), 7.08 (d, *J*=8.3 Hz, 2H, C_{18}-H and C_{20}-H), 2.94 (dd, *J*=16.1, 6.7 Hz, 1H, C_6-H_a), 2.83 (dd, *J*=16.1, 8.3 Hz, 1H, C_6-H_b), 2.25 (s, 3H, C_{22}-H), 1.41～1.33 (m, 3H, C_{11}-H and C_{10}-H_a), 1.32～1.25 (m, 1H, C_{10}-H_b), 1.08 (s, 3H, C_{13}-H or C_{14}-H), 1.01 (s, 3H, C_{13}-H or C_{14}-H), 0.91 (t, *J*=7.1 Hz, 3H, C_{12}-H), 0.88～0.83 (m, 1H, C_7-H), 0.62～0.55 (m, 1H, C_9-H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 163.4, 162.6, 154.1, 137.3, 130.8, 129.1, 118.5, 28.8, 26.3, 26.0, 25.8, 25.1, 22.8, 20.4, 17.3, 14.8, 14.0; IR (KBr) *v*: 3375, 3309 (N—H), 2956, 2925, 2862 (C—H), 1718(C=O), 1639 (C=N), 1597, 1541 (Ar—C=C) cm^{-1}; ESI-MS *m*/*z*: 359.16 ($M+H^+$). Anal. calcd for $C_{19}H_{26}N_4OS$: C 63.66, H 7.31, N 15.63; found C 63.67, H 7.31, N 15.63.

(+)-*cis*-1-(5-((2,2-Dimethyl-3-propylcyclopropyl)methyl)-1,3,4-thiadiazol-2-yl)-3-(4-methoxyphenyl) urea (**7g**): White solid, yield 95%. m.p. 176.7～177.4 ℃; $[\alpha]_D^{25}$ +6.0 (*c* 0.50, CH_3COCH_3); ^1H NMR (600 MHz, DMSO-d_6) δ: 10.74 (s, 1H, NH), 8.84 (s, 1H, NH), 7.39 (d, *J*=8.9 Hz, 2H, C_{17}-H and C_{21}-H), 6.93～6.81 (d, *J*=8.9 Hz, 2H, C_{18}-H and C_{20}-H), 3.73 (s, 3H, C_{22}-H), 2.93 (dd, *J*=16.2, 6.8 Hz, 1H, C_6-H_a), 2.84 (dd, *J*=16.2, 8.3 Hz, 1H, C_6-H_b), 1.38～1.29 (m, 3H, C_{11}-H and C_{10}-H_a), 1.28～1.23 (m, 1H, C_{10}-H_b), 1.06 (s, 3H, C_{13}-H or C_{14}-H), 0.98 (s, 3H, C_{13}-H or C_{14}-H), 0.89 (t, *J*=7.1 Hz, 3H, C_{12}-H), 0.82～0.86 (m, 1H, C_7-H), 0.60～0.55 (m, 1H, C_9-H); ^{13}C NMR (151 MHz, DMSO-d_6) δ: 164.2, 160.6, 155.7, 152.5, 131.9, 121.1, 114.5, 55.7, 29.2, 26.7, 26.4, 26.1, 25.4, 23.2, 17.8, 15.2, 14.4. IR (KBr) *v*: 3377 (N—H), 2957, 2942, 2864 (C—H), 1718 (C=O), 1602 (C=N), 1550, 1514 (Ar—C=C) cm^{-1}; ESI-MS *m*/*z*: 375.16 ($M+H^+$). Anal. calcd for $C_{19}H_{26}N_4O_2S$: C 60.94, H 7.00, N 14.96; found C 60.95, H 7.03, N 14.95.

(+)-*cis*-1-(2-Bromophenyl)-3-(5-((2,2-dimethyl-3-propylcyclopropyl)methyl)-1,3,4-thiadiazol-2-yl) urea (**7h**): White solid, yield 85%. m.p. 128.1～129.0 ℃; $[\alpha]_D^{25}$ +8.7 (*c* 0.31, CH_3COCH_3); ^1H NMR (500 MHz, DMSO-d_6) δ: 11.68 (s, 1H, NH), 8.62 (s, 1H, NH), 8.03 (dd, *J*=8.2, 1.3 Hz, 1H, C_{18}-H), 7.65 (dd, *J*=8.0, 0.9 Hz, 1H, C_{21}-H), 7.37 (td, *J*=8.3, 1.2 Hz, 1H, C_{20}-H), 7.06 (td, *J*=8.3, 1.2 Hz, 1H, C_{19}-H), 2.95 (dd, *J*=16.2, 6.8 Hz, 1H, C_6-H_a), 2.85 (dd, *J*=16.1, 8.3 Hz, 1H, C_6-H_b), 1.37～1.29 (m, 3H, C_{11}-H and C_{10}-H_a), 1.28～1.22 (m, 1H, C_{10}-H_b), 1.05 (s, 3H, C_{13}-H or C_{14}-H), 0.98 (s, 3H, C_{13}-H or C_{14}-H), 0.89 (t, *J*=7.0 Hz, 3H, C_{12}-H), 0.86～0.81 (m, 1H, C_7-H), 0.60～0.54 (m, 1H, C_9-H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 164.3, 159.7, 151.5, 136.0, 132.6, 128.2, 125.3, 122.7, 113.9, 28.7, 26.3, 25.9, 25.8, 24.9, 22.7, 17.3, 14.8, 13.9; IR (KBr) *v*: 3339, 3281 (N—H), 2957, 2927, 2858 (C—H), 1705 (C=O), 1583 (C=N), 1562, 1532 (Ar—C=C) cm^{-1}; ESI-MS *m*/*z*: 424.06 ($M+H^+$). Anal. calcd for $C_{18}H_{23}BrN_4OS$: C 51.07, H 5.48, N 13.23; found C 51.05, H 5.45, N 13.24.

(+)-*cis*-1-(4-Bromophenyl)-3-(5-((2,2-dimethyl-3-propylcyclopropyl)methyl)-1,3,4-thiadiazol-2-yl) urea (**7i**): White solid, yield 90%. m.p. 219.0～220.5 ℃; $[\alpha]_D^{25}$ +

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10.7 (*c* 0.51, CH_3COCH_3); ^1H NMR (600 MHz, DMSO-d_6) δ: 10.94 (s, 1H, NH), 9.21 (s, 1H, NH), 7.51～7.47 (m, 4H, C_{17}-H and C_{18}-H and C_{20}-H and C_{21}-H), 2.93 (dd, J=16.2, 6.8 Hz, 1H, C_6-H_a), 2.83 (dd, J=16.2, 8.3 Hz, 1H, C_6-H_b), 1.37～1.31 (m, 3H, C_{11}-H and C_{10}-H_a), 1.29～1.24 (m, 1H, C_{10}-H_b), 1.06 (s, 3H, C_{13}-H or C_{14}-H), 0.99 (s, 3H, C_{13}-H or C_{14}-H), 0.90 (t, J=7.1 Hz, 3H, C_{12}-H), 0.86～0.82 (m, 1H, C_7-H), 0.60～0.57 (m, 1H, C_9-H); ^{13}C NMR (151 MHz, DMSO-d_6) δ: 163.6, 160.7, 153.5, 138.3, 131.7, 120.8, 114.4, 28.8, 26.4, 26.0, 25.7, 25.2, 22.8, 17.4, 14.9, 14.0; IR (KBr) ν: 3368 (N—H), 2957, 2930, 2862 (C—H), 1724 (C=O) 1587 (C=N), 1542 (Ar—C=C) cm^{-1}; ESI-MS m/z: 424.07 ($M+H^+$). Anal. calcd for $C_{18}H_{23}BrN_4OS$: C 51.07, H 5.48, N 13.23; found C 51.05, H 5.47, N 13.20.

(+)-*cis*-1-(5-((2,2-Dimethyl-3-propylcyclopropyl)methyl)-1,3,4-thiadiazol-2-yl)-3-(3-fluorophenyl) urea (**7j**): White solid, yield 85%. m.p. 194.5～194.9 ℃; $[\alpha]_D^{25}$ +9.1 (*c* 0.31, CH_3COCH_3); ^1H NMR (600 MHz, DMSO-d_6) δ: 11.02 (s, 1H, NH), 9.29 (s, 1H, NH), 7.50 (d, J=11.7 Hz, 1H, C_{17}-H), 7.32 (dd, J=15.1, 8.1 Hz, 1H, C_{20}-H), 7.22 (d, J=8.0 Hz, 1H, C_{21}-H), 6.84 (td, J=8.4, 2.0 Hz, 1H, C_{19}-H), 2.92 (dd, J=16.2, 6.8 Hz, 1H, C_6-H_a), 2.82 (dd, J=16.2, 8.3 Hz, 1H, C_6-H_b), 1.37～1.29 (m, 3H, C_{11}-H and C_{10}-H_a), 1.28～1.23 (m, 1H, C_{10}-H_b), 1.05 (s, 3H, C_{13}-H or C_{14}-H), 0.98 (s, 3H, C_{13}-H or C_{14}-H), 0.89 (t, J=7.1 Hz, 3H, C_{12}-H), 0.86～0.81 (m, 1H, C_7-H), 0.59～0.55 (m, 1H, C_9-H); ^{13}C NMR (151 MHz, DMSO-d_6) δ: 163.6, 162.4 (d, J=241.04 Hz), 160.6, 153.0, 140.7, 130.4 (d, J=9.95 Hz), 114.5, 109.1 (d, J=21.01 Hz), 105.5 (d, J=26.54 Hz), 28.7, 26.3, 26.0, 25.6, 25.1, 22.7, 17.4, 14.8, 13.9; IR (KBr) ν: 3374, 3281 (N—H), 2958, 2932, 2863 (C—H), 1711 (C=O),1613 (C=N), 1556 (Ar—C=C) cm^{-1}; ESI-MS m/z: 363.11 ($M+H^+$). Anal. calcd for $C_{18}H_{23}FN_4OS$: C 59.65, H 6.40, N 15.46; found C 59.63, H 6.42, N 15.45.

(+)-*cis*-1-(5-((2,2-Dimethyl-3-propylcyclopropyl)methyl)-1,3,4-thiadiazol-2-yl)-3-(4-fluorophenyl) urea (**7k**): White solid, yield 88%. m.p. 193.0～193.6 ℃; $[\alpha]_D^{25}$ +9.6 (*c* 0.50, CH_3COCH_3); ^1H NMR (600 MHz, DMSO-d_6) δ: 10.87 (s, 1H, NH), 9.07 (s, 1H, NH), 7.53～7.48 (m, 2H, C_{17}-H and C_{21}-H), 7.17～7.12 (m, 2H, C_{18}-H and C_{20}-H), 2.92 (dd, J=16.2, 6.8 Hz, 1H, C_6-H_a), 2.82 (dd, J=16.2, 8.3 Hz, 1H, C_6-H_b), 1.37～1.30 (m, 3H, C_{11}-H and C_{10}-H_a), 1.28～1.23 (m, 1H, C_{10}-H_b), 1.05 (s, 3H, C_{13}-H or C_{14}-H), 0.98 (s, 3H, C_{13}-H or C_{14}-H), 0.89 (t, J=7.1 Hz, 3H, C_{12}-H), 0.85～0.81 (m, 1H, C_7-H), 0.59～0.55 (m, 1H, C_9-H); ^{13}C NMR (151 MHz, DMSO-d_6) δ: 163.6, 160.5, 157.9 (d, J=239.94 Hz), 152.5, 135.0, 120.7 (d, J=7.74 Hz), 115.4 (d, J=22.11 Hz), 28.7, 26.3, 26.0, 25.6, 25.0, 22.7, 17.3, 14.8, 13.9; IR (KBr) ν: 3375 (N—H), 2958, 2934, 2862 (C—H), 1721 (C=O), 1611 (C=N), 1553, 1510 (Ar—C=C) cm^{-1}; ESI-MS m/z: 363.14 ($M+H^+$). Anal. calcd for $C_{18}H_{23}FN_4OS$: C 59.65, H 6.40, N 15.46; found C 59.65, H 6.42, N 15.47.

(+)-*cis*-1-(2,4-Difluorophenyl)-3-(5-((2,2-dimethyl-3-propylcyclopropyl) methyl)-1,3,4-thiadiazol-2-yl) urea (**7l**): White solid, yield 85%. m.p. 163.3～163.9 ℃; $[\alpha]_D^{25}$ +7.2 (*c* 0.50, CH_3COCH_3); ^1H NMR (500 MHz, DMSO-d_6) δ: 11.45 (s, 1H, NH), 9.47 (s, 1H, NH), 7.95 (td, J=9.1, 6.1 Hz, 1H, C_{21}-H), 7.32 (ddd, J=11.5, 9.0, 2.9 Hz, C_{20}-H), 7.10～7.02 (m, 1H, C_{18}-H), 2.93 (dd, J=16.2, 6.8 Hz, 1H, C_6-H_a), 2.83 (dd, J=16.1, 8.4 Hz, 1H, C_6-H_b), 1.36～1.30 (m, 3H, C_{11}-H and C_{10}-H_a), 1.28～1.24 (m, 1H, C_{10}-H_b), 1.05 (s, 3H, C_{13}-H or C_{14}-H), 0.98 (s, 3H, C_{13}-H or C_{14}-H), 0.90 (d, J=7.0 Hz, 3H, C_{12}-H), 0.85～0.79 (m, 1H, C_7-H), 0.59～0.55 (m, 1H, C_9-H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 163.8, 161.0, 157.8 (dd, J=242.51, 11.81 Hz), 153.1 (d, J=247.05 Hz), 152.2, 123.2 (d, J=10.90 Hz), 123.2 (d, J=10.90 Hz), 111.2 (dd, J=21.80, 3.63 Hz), 104.0 (dd, J=26.79, 24.07 Hz), 28.7, 26.3, 26.0, 25.8, 25.0, 22.7, 17.3, 14.8, 13.9; IR (KBr) ν: 3378 (N—H), 2958, 2930, 2864 (C—H), 1717 (C=O),1600 (C=N), 1551 (Ar-C=C) cm^{-1}; ESI-MS m/z: 381.14 ($M+H^+$). Anal. calcd for $C_{18}H_{22}F_2N_4OS$: C 56.83, H 5.83, N 14.73; found C 56.80, H 5.80, N 14.71.

(+)-*cis*-1-(5-((2,2-Dimethyl-3-propylcyclopropyl)methyl)-1,3,4-thiadiazol-2-yl)-3-(3-(trifluoromethyl)phenyl) urea (**7m**): White solid, yield 85%. m.p. 183.7～185.7 ℃; $[\alpha]_D^{25}$ +9.8 (*c* 0.30, CH_3COCH_3); ^1H NMR (600 MHz, DMSO-d_6) δ: 11.21 (s, 1H, NH), 9.45 (s, 1H, NH), 8.02 (s, 1H, C_{17}-H), 7.69 (d, J=8.0 Hz, 1H, C_{21}-H), 7.53 (t, J=8.0 Hz, 1H, C_{20}-H), 7.36 (d, J=7.7 Hz, 1H, C_{19}-H), 2.91 (dd, J=16.2, 6.8 Hz, 1H, C_6-H_a), 2.82 (dd, J=16.2, 8.3 Hz, 1H, C_6-H_b), 1.37～1.29 (m, 3H, C_{11}-H and C_{10}-H_a), 1.28～1.22 (m, 1H, C_{10}-H_b), 1.05 (s, 3H, C_{13}-H or C_{14}-H), 0.98 (s, 3H, C_{13}-H or C_{14}-H), 0.88 (t, J=7.1 Hz, 3H, C_{12}-H), 0.86～0.80 (m, 1H, C_7-H), 0.61～0.55 (m, 1H, C_9-H); ^{13}C NMR (151 MHz, DMSO-d_6) δ: 163.2, 161.1, 153.4, 139.8, 130.0, 129.6 (d, J=31.04 Hz), 124.2 (q, J=271.91 Hz), 122.4, 119.0, 114.7, 28.7, 26.3, 25.9, 25.5, 25.1, 22.7, 17.4, 14.8, 13.9; IR (KBr) ν: 3288, 3264 (N—H), 2960, 2931, 2864 (C—H), 1711 (C=O), 1622 (C=N), 1567 (Ar—C=C) cm^{-1}; ESI-MS m/z: 413.15 ($M+H^+$). Anal. calcd for $C_{19}H_{23}F_3N_4OS$: C 55.33, H 5.62, N 13.58; found C 55.30, H 5.60, N 13.59.

(+)-*cis*-1-(5-((2,2-Dimethyl-3-propylcyclopropyl)methyl)-1,3,4-thiadiazol-2-yl)-3-(4-(trifluoromethyl)phenyl) urea (**7n**): White solid, yield 88%. m.p. 187.8～189.7 ℃; $[\alpha]_D^{25}$ +10.1 (*c* 0.30, CH_3COCH_3); ^1H NMR (500 MHz, DMSO-d_6) δ: 11.60 (s, 1H, NH), 9.90 (s, 1H, NH), 7.79 (d, J=8.5 Hz, 2H, C_{18}-H and C_{20}-H), 7.64 (d, J=8.7 Hz, 2H, C_{17}-H and C_{21}-H), 2.93 (dd, J=16.2, 6.8 Hz, 1H, C_6-H_a), 2.83 (dd, J=16.2, 8.3 Hz, 1H, C_6-H_b), 1.37～1.30 (m, 3H, C_{11}-H and C_{10}-H_a), 1.29～1.23 (m, 1H, C_{10}-H_b), 1.06 (s, 3H, C_{13}-H or C_{14}-H), 0.99 (s, 3H, C_{13}-H or C_{14}-H), 0.89 (t, J=7.1 Hz, 3H, C_{12}-H), 0.86～0.82 (m, 1H, C_7-H), 0.61～0.55 (m, 1H, C_9-H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 163.1, 162.1, 153.8, 143.0, 126.0 (q, J=3.63 Hz), 125.0 (q, J=270.96 Hz), 122.4 (q, J=32.32 Hz), 118.4, 28.7, 26.3, 26.0, 25.6, 25.1, 22.7, 17.3, 14.8, 13.9; IR (KBr) ν: 3378, 3206 (N—H), 2958, 2934, 2866 (C—H), 1713 (C=O), 1614 (C=N), 1560 (Ar—C=C) cm^{-1}; ESI-MS m/z: 413.16 ($M+H^+$). Anal. calcd for $C_{19}H_{23}F_3N_4OS$: C 55.33, H 5.62, N 13.58; found C 55.30, H 5.63, N 13.58.

(+)-*cis*-1-(5-((2,2-Dimethyl-3-propylcyclopropyl)me-

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thyl)-1,3,4-thiadiazol-2-yl)-3-(4-(trifluoromethoxy)phenyl) urea (**7o**): White solid, yield 95%. m.p. 186.4～185.7 ℃; $[\alpha]_D^{25}$ +8.3 (*c* 0.50, CH_3COCH_3); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.97 (s, 1H, NH), 9.29 (s, 1H, NH), 7.63 (d, J=9.0 Hz, 2H, C_{17}-H and C_{21}-H), 7.32 (d, J=8.7 Hz, 2H, C_{18}-H and C_{20}-H), 2.94 (dd, J=16.2, 6.8 Hz, 1H, C_6-H_a), 2.84 (dd, J=16.2, 8.3 Hz, 1H, , C_6-H_b), 1.37～1.31 (m, 3H, C_{11}-H and C_{10}-H_a), 1.29～1.25 (m, 1H, C_{10}-H_b), 1.07 (s, 3H, C_{13}-H or C_{14}-H), 0.99 (s, 3H, C_{13}-H or C_{14}-H), 0.90 (t, J=7.1 Hz, 3H, C_{12}-H), 0.87～0.83 (m, 1H, C_7-H), 0.61～0.57 (m, 1H, C_9-H); ^{13}C NMR (151 MHz, DMSO-d_6) δ: 163.6, 160.7, 152.5, 143.4, 138.2, 121.8, 120.3 (q, J=256.15 Hz), 120.2, 28.8, 26.4, 26.0, 25.7, 25.2, 22.8, 17.4, 14.9, 14.0; IR (KBr) ν: 3373, 3282 (N—H), 2960, 2939, 2873 (C—H), 1712 (C=O), 1614 (C=N), 1567, 1510 (Ar—C=C) cm^{-1}; ESI-MS m/z: 429.14 (M+H$^+$). Anal. calcd for $C_{19}H_{23}F_3N_4O_2S$: C 53.26, H 5.41, N 13.08; found C 53.28, H 5.40, N 13.05.

(+)-*cis*-1-(4-Chloro-3-(trifluoromethyl)phenyl)-3-(5-((2,2-dimethyl-3-propylcyclopropyl)methyl)-1,3,4-thiadiazol-2-yl) urea (**7p**): White solid, yield 85%. m.p. 198.6～199.3 ℃; $[\alpha]_D^{25}$ +7.7 (*c* 0.50, CH_3COCH_3); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 11.37 (s, 1H, NH), 9.59 (s, 1H, NH), 8.12 (d, J=2.2 Hz, 1H, C_{17}-H), 7.77 (dd, J=8.8, 2.0 Hz, 1H, C_{20}-H), 7.61 (d, J=8.8 Hz, 1H, C_{21}-H), 2.90 (dd, J=16.2, 6.9 Hz, 1H, C_6-H_a), 2.81 (dd, J=16.2, 8.3 Hz, 1H, C_6-H_b), 1.36～1.28 (m, 3H, C_{11}-H and C_{10}-H_a), 1.26～1.22 (m, 1H, C_{10}-H_b), 1.05 (s, 3H, C_{13}-H or C_{14}-H), 0.97 (s, 3H, C_{13}-H or C_{14}-H), 0.88 (t, J=7.1 Hz, 3H, C_{12}-H), 0.85～0.81 (m, 1H, C_7-H), 0.59～0.55 (m, 1H, C_9-H); ^{13}C NMR (151 MHz, DMSO-d_6) δ: 162.9, 162.0, 154.5, 138.8, 132.0, 126.8 (q, J=30.96 Hz), 123.6, 123.1, 122.8 (q, J=273.11 Hz), 117.3 (d, J=5.53 Hz), 28.7, 26.3, 25.9, 25.4, 25.2, 22.7, 17.3, 14.8, 13.9; IR (KBr) ν: 3274, 3199 (N—H), 2959, 2933, 2864 (C—H), 1711 (C=O), 1620 (C=N), 1570, 1556 (Ar—C=C) cm^{-1}; ESI-MS m/z: 447.12 (M+H$^+$). Anal. calcd for $C_{19}H_{22}ClF_3N_4OS$: C 51.06, H 4.96, N 12.54; found C 51.05, H 4.97, N 12.55.

(+)-*cis*-1-(3,5-Bis(trifluoromethyl)phenyl)-3-(5-((2,2-dimethyl-3-propylcyclopropyl)methyl)-1,3,4-thiadiazol-2-yl) urea (**7q**): White solid, yield 95%. m.p. 189.2～189.6 ℃; $[\alpha]_D^{25}$ +8.5 (*c* 0.51, CH_3COCH_3); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 11.71 (s, 1H, NH), 9.89 (s, 1H, NH), 8.24 (s, 2H, C_{17}-H and C_{21}-H), 7.65 (s, 1H, C_{19}-H), 2.89 (dd, J=16.3, 6.9 Hz, 1H, C_6-H_a), 2.81 (dd, J=16.3, 8.2 Hz, 1H, C_6-H_b), 1.36～1.28 (m, 3H, C_{11}-H and C_{10}-H_a), 1.27～1.21 (m, 1H, C_{10}-H_b), 1.05 (s, 3H, C_{13}-H or C_{14}-H), 0.97 (s, 3H, C_{13}-H or C_{14}-H), 0.88 (t, J=7.1 Hz, 3H, C_{12}-H), 0.85～0.81 (m, 1H, C_7-H), 0.59～0.55 (m, 1H, C_9-H); ^{13}C NMR (151 MHz, DMSO-d_6) δ: 163.2, 162.4, 155.6, 141.6, 130.7 (q, J=32.62 Hz), 122.2 (q, J=272.93 Hz), 118.3, 114.9, 28.7, 26.3, 25.9, 25.3, 25.3, 22.7, 17.4, 14.8, 13.9; IR (KBr) ν: 3372 (N—H), 2963, 2937, 2866 (C—H), 1712 (C=O), 1610 (C=N), 1549 (Ar—C=C) cm^{-1}; ESI-MS m/z: 481.14 (M+H$^+$). Anal. calcd for $C_{20}H_{22}F_6N_4OS$: C 50.00, H 4.62, N 11.66; found C 50.03, H 4.60, N 11.67.

(+)-*cis*-1-(3-Chloro-4-methylphenyl)-3-(5-((2,2-dimethyl-3-propylcyclopropyl)methyl)-1,3,4-thiadiazol-2-yl) urea (**7r**): White solid, yield 87%. m.p. 198.8～200.6 ℃; $[\alpha]_D^{25}$ +9.1 (*c* 0.50, CH_3COCH_3); ^{1}H NMR (600 MHz, DMSO- d_6) δ: 10.93 (s, 1H, NH), 9.14 (s, 1H, NH), 7.70 (s, 1H, C_{17}-H), 7.29～7.23 (m, 2H, C_{20}-H and C_{21}-H), 2.92 (dd, J=16.2, 6.8 Hz, 1H, C_6-H_a), 2.82 (dd, J=16.2, 8.3 Hz, 1H, C_6-H_b), 1.36～1.30 (m, 3H, C_{11}-H and C_{10}-H_a), 1.28～1.24 (m, 1H, C_{10}-H_b), 1.06 (s, 3H, C_{13}-H or C_{14}-H), 0.98 (s, 3H, C_{13}-H or C_{14}-H), 0.89 (t, J=7.1 Hz, 3H, C_{12}-H), 0.84～0.81 (m, 1H, C_7-H), 0.59～0.55 (m, 1H, C_9-H); ^{13}C NMR (151 MHz, DMSO-*d6*) δ: 163.3, 160.7, 152.8, 137.9, 133.2, 131.2, 129.3, 118.6, 117.5, 28.7, 26.3, 25.9, 25.6, 25.1, 22.7, 18.8, 17.3, 14.8, 13.9; IR (KBr) ν: 3368, 3182 (N—H), 2956, 2925, 2860 (C—H), 1707 (C=O), 1610 (C=N), 1568, 1545 (Ar—C=C) cm^{-1}; ESI-MS m/z: 394.25 ([M+H]$^+$). Anal. calcd for $C_{19}H_{25}ClN_4OS$: C 58.08, H 6.41, N 14.26; found C 58.05, H 6.40, N 14.27.

4.8 Antifungal activity test

The *in vitro* antifungal activities of all target compounds against six phytopathogenic fungi were tested by agar dilution method at the concentration of 50 mg/L, and chlorothalonil, a commercially available fungicide, was used as the positive control. With aseptic distilled water as a blank control, the plates containing the medicated solution were cultured in an incubator at (24±1) ℃ for 48 h, and then the mycelium diameter was measured. A respective value was obtained from the average for three replicates of the corresponding compounds when compared with the blank control. Finally, the inhibitory rates were calculated by the following formula:

Inhibitory rate (%)=(CK−PT)/CK×100%

where PT was the mycelium diameter of the tested compounds, and CK was the mycelium diameter of the blank control.

4.9 3D-QSAR analysis

3D-QSAR analysis of fifteen target compounds was carried out by using the CoMFA method on Sybyl-X 2.1.1 software, and the active factor (AF) was employed to express the antifungal activity of the target compounds against *A. solani*. The converted formula was as follows:

$$AF = \lg\{I/[(100 - I) \times M_w]\}$$

where I was relative inhibitory rate and M_w was the molecular weight.

The optimized conformations of compounds **7a**～**7r** were generated using the Tripos force field and Gasteiger-Hückel charges conjugate gradient method. Compound **7i** (R=*p*-Br) was selected as the template to superimpose other target molecules (Figure 9), and then the predicted AF values were calculated. Subsequently, the built 3D-QSAR model was subjected to Partial Least Squares (PLS) analysis for cross-validated coefficient (q^2), standard error of estimate (S), correlation coefficient (r^2), and Fisher ratio (F).

4.10 Molecular docking and theoretical calculation

Homology modeling was conducted according to the

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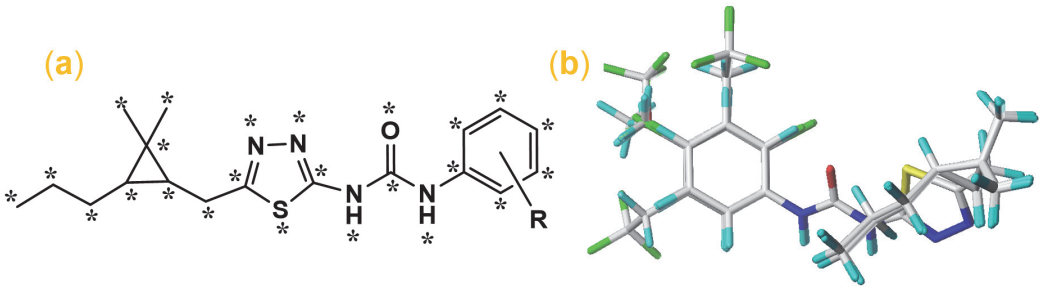

Figure 9 Asterisk skeleton of target compounds (a) and superposition modes of compounds (b)

published methods,[33,45] and carried out on the SWISS-MODEL web server (https://swissmodel.expasy.org/). The avian SDH (PDB ID: 2WQY) downloaded from RSCB Protein Data Bank (http://www1.rcsb.org/) was applied as the template, and target protein sequences available at NCBI website (https://www.ncbi.nlm.nih.gov/) were CEL 53558.1, CCO28606.1, and CEL58000.1, referred to subunit B, C, and D, respectively. Based on the established SDH structure, molecular docking was conducted on SYBYL-X 2.1.1 software, and the original ligand carboxin-binding site was chosen as the docking site in the process. Other operation details could adopt the default setting. The molecular docking analysis was operated on PLIP and PyMOL. Furthermore, the 2D interaction of compound **7i** (R=*p*-Br) and SDH was drawn by LigPlot^{+}v2.1.

Moreover, the theoretical calculation of the target compounds **7i** (R=*p*-Br), **7c** (R=*p*-Cl), and **7k** (R=*p*-F) was performed by Multiwfn 3.7, Gaussian 09 [DFT/B3LYP/6-31G (d, p)], and VMD 1.9.3 supported by the high-performance computing platform of Guangxi University, and the results were viewed on Multiwfn 3.7, VMD 1.9.3 and GaussView 5 software.

Supporting Information FT-IR, ^{1}H NMR, ^{13}C NMR and ESI-MS spectra for the synthesized compounds; model quality estimate of homology modeling (SWISS-MODEL web). The Supporting Information is available free of charge via the Internet at http://sioc-journal.cn.

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