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Synthesis, antifungal activity and 3D-QSAR study of novel nopol-based 1,3,4-thiadiazole–thioether compounds

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Abstract

A series of novel nopol derivatives containing 1,3,4-thiadiazole–thioether moiety were synthesized from *β*-pinene, which is a natural, abundant and renewable biomass resource. Their structures were characterized by FT-IR, ^{1}H NMR, ^{13}C NMR, ESI–MS and elemental analysis. In vitro antifungal activity of the target compounds was preliminarily evaluated against eight tested plant pathogens, including *Fusarium oxysporum* f. sp. *cucumerinum*, *Cercospora arachidicola*, *Physalospora piricola*, *Alternaria solani*, *Gibberella zeae*, *Rhizoeotnia solani*, *Bipolaris maydis* and *Colleterichum orbicalare*. The bioassay results revealed that, at the concentration of 50 μg/mL, all the target compounds showed certain inhibition activity against the eight tested fungi. Compounds **5f** (R = *m*–OCH_3), **5i** (R = *m*–F) and **5r** (R = *m*–I) had excellent inhibition rates of 77.8%, 88.9% and 77.8%, respectively, against *P. piricola*, showing much better antifungal activity than that of the positive control chlorothalonil. Meanwhile, compound **5 m** (R = *p*–Cl) displayed antifungal activity of 80.7% against *R. solani*. Furthermore, the analysis of three-dimensional quantitative structure–activity relationship (3D-QSAR) was performed for the relationship between the structures of the target compounds and their antifungal activity against *P. piricola* by CoMFA method. A reasonable CoMFA model ($n = 6$; $q^2 = 0.597$; $r^2 = 0.985$) was established.

Keywords *β*-pinene · nopol · 1,3,4-thiadiazole–thioether · antifungal activity · 3D-QSAR

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Introduction

Turpentine oil is a terpenic non-wood forest product, as well as a natural, abundant and renewable biomass resource in nature. It is obtained by the steam distillation of the oleoresin exudate from living pine trees, and its major constituents are α-pinene and β-pinene [1]. The content of β-pinene in turpentine oil of *Pinus elliottii* can be as high as 25–35% [2], but compared with α-pinene, the study on the development and utilization of β-pinene is relatively fewer, indicating that conversion of β-pinene into high value-added fine chemicals is worthy of further investigation.

Nopol, 2-(6,6-dimethyl-2-bicyclo [3.1.1] hept-2-enyl) ethanol, contains three reactive functional groups, namely a hydroxyl group, a carbon–carbon double bond and a four-membered ring. It can be readily prepared by the Prins reaction of β-pinene with paraformaldehyde [3, 4]. Nopol and its derivatives exhibited a broad spectrum of biological activities, such as antifungal [5–9], antifeedant [10], repellent [11] and treatment of gastrointestinal irritable syndrome activities [12]. Thus, nopol deserves further study for agrochemical or pharmaceutical use based on its bioactive property and chemical reactivity.

1,3,4-Thiadiazole, as the representative of five-membered unsaturated heterocycles, showed a great deal of biological activities, including antifungal [13–15], antibacterial [16, 17], anticancer [18, 19], herbicidal [20–22], insecticidal [23, 24], antiviral [25] and anti-inflammatory activities [26]. Bismerthiazol and thiodiazole-copper are an important class of fungicides containing 1,3,4-thiadiazole moiety, which show lower toxicity, less residue, safer to the environment and stronger bioactive properties [27–30]. In recent years, our research group reported several classes of natural product-based 1,3,4-thiadiazole derivatives and found that some of the target compounds exhibited excellent to good antifungal and herbicidal activities (Fig. 1) [31–34].

In continuation of our interest in the investigation of natural product-based bioactive compounds [35–40], a series of nopol-based

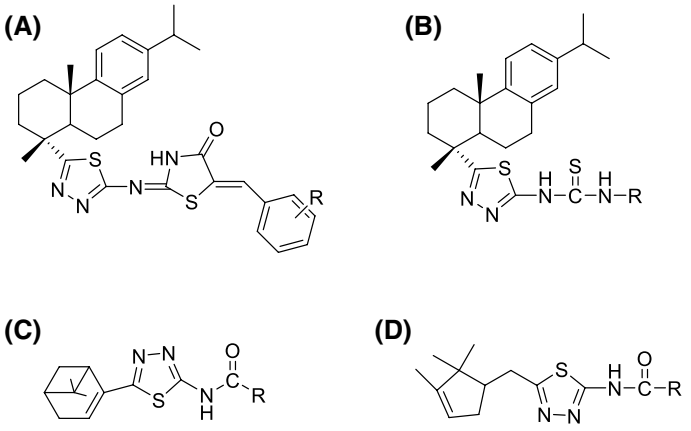


Fig. 1 Structures of the 1,3,4-thiadiazole derivatives reported by our research group

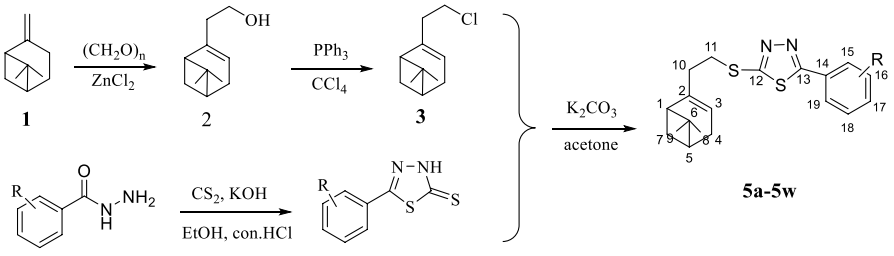
1,3,4-thiadiazole–thioether compounds were designed and synthesized by introducing 1,3,4-thiadiazole–thioether moiety into the skeleton of nopol converted from *β*-pinene. Structural characterization, antifungal evaluation and 3D-QSAR study were carried out as well.

Results and discussion

Synthesis and characterization

The synthetic route of nopol-based 1,3,4-thiadiazole–thioether compounds **5a-5w** is illustrated in Scheme 1. At first, nopol (**2**) was prepared by Prins reaction of *β*-pinene (**1**) with paraformaldehyde in the presence of $ZnCl_2$ as a catalyst [41]. Then, nopyl chloride (**3**) was prepared by Appel reaction of nopol (**2**) in a good yield [42], followed by nucleophilic substitution reaction of nopyl chloride (**3**) with self-prepared 5-substituted phenyl-1,3,4-thiadiazole-2-thiones (**4**) to afford a series of novel nopol-based 1,3,4-thiadiazole–thioether compounds **5a-5w** [43].

The structures of all the title compounds and the key intermediates (**2**) and (**3**) were confirmed by FT-IR, ^{1}H NMR, ^{13}C NMR, ESI–MS and elemental analysis, and the related spectra can be found in Supporting Information. In the IR spectra of the target compounds, the weak absorption bands at about 3025 cm^{-1} were attributed to the stretching vibrations of the unsaturated C-H in the nopol moiety. The weak absorption bands at 1585–1614 cm^{-1} and the strong absorption bands at 1429–1477 cm^{-1} were assigned to the vibrations of C=C in the nopol moiety and C=N in 1,3,4-thiadiazole moiety, respectively. Also, the absorption bands at 605–712 cm^{-1} revealed the presence of C-S-C in thioether moiety. In the ^{1}H-NMR spectra, the olefinic protons of nopol scaffold showed signals at about 5.37 ppm, and the other protons bonded to the saturated carbons of the nopol moiety displayed signals in the range of 0.87–2.51 ppm. The protons on the saturated carbon bonded to the S atom displayed the signals at about 3.40 ppm. The ^{13}C NMR spectra of all the



5a R=H; **5b** R=*o*-CH_3; **5c** R=*m*-CH_3; **5d** R=*p*-CH_3; **5e** R=*o*-OCH_3; **5f** R=*m*-OCH_3; **5g** R=*p*-OCH_3; **5h** R=*o*-F; **5i** R=*m*-F; **5j** R=*p*-F; **5k** R=*o*-Cl; **5l** R=*m*-Cl; **5m** R=*p*-Cl; **5n** R=*o*-Br; **5o** R=*m*-Br;**5p** R=*p*-Br; **5q** R=*m*-I; **5r** R=*p*-I; **5s** R=*m*, *p*-OCH_3; **5t** R=*m*, *m*-OCH_3; **5u** R=*m*-CF_3; **5v** R=*p*-$C(CH_3)_3$; **5w** R=*o*-Cl-*p*-F

Scheme 1 Synthetic route of nopol-based 1,3,4-thiadiazole–thioether compounds **5a-5w**

target compounds showed peaks for the two olefinic carbons of the nopol moiety at about 145 ppm and 118 ppm, respectively, and the unsaturated carbons in the thiadiazole heterocycle and the benzene ring showed signals at 165–168 ppm and 95–160 ppm, respectively. The saturated carbon bonded to the S atom displayed the signals at 30–32 ppm. Their molecular weights were confirmed by ESI–MS.

Antifungal activity

Antifungal activity of the target compounds **5a-5w** was evaluated preliminarily by in vitro method against *Fusarium oxysporum* f. sp. *cucumerinum, Cercospora arachidicola, Physalospora piricola, Alternaria solani, Gibberella zeae, Rhizoeotnia solani, Bipolaris maydis* and *Colleterichum orbicalare* at 50 μg/mL. The results are listed in Table 1. Compounds **5a-5w** were found to exhibit certain antifungal activity against the eight tested fungi, especially against *P. piricola*. Compounds **5i** (R = *m*–F), **5f** (R = *m*–OCH_3) and **5q** (R = *m*–I) had excellent inhibition rates of 88.9%, 77.8% and 77.8%, respectively, against *P. piricola*, showing much better than that of the positive control chlorothalonil with inhibition rate of 75%. In addition, compound **5 m** (R = *p*–Cl) displayed antifungal activity of 80.7% against *R. solani*.

CoMFA analysis

The CoMFA method has become one of the important computer simulation methods in drug design and medicinal chemistry. In this work, CoMFA was employed to study the structure–activity relationship of the synthesized compounds against *P. piricola*. The partial least squares (PLS) relevant results of the CoMFA models are listed in Table 2. The cross-validated coefficient q^2 of 0.597 ($q^2 > 0.5$) with six optimal number of component (ONC), non-cross-validated correlation coefficient (r^2) between the experimental and predicted activities was 0.985($r^2 > 0.8$), and standard error of estimate (SEE) and Fisher ratio (F) value were calculated as 0.046 and 100.925, respectively. As shown in Table 3, the experimental ED values were in good agreement with the predicted ED values, and the scatter diagram is shown in Fig. 2. The data signified the generated CoMFA model was reliable.

The CoMFA steric and electrostatic contour maps are shown in Fig. 3. The results of the CoMFA model also included contributions of steric and electrostatic fields of 72.6% and 27.4%, respectively. Based on these field contributions, the steric field was the most important in the CoMFA model. In the steric map of CoMFA (Fig. 3A), the green contour near the 3-position of the benzene ring indicated that the introduction of bulky groups in these positions was favorable to increase the antifungal activity. For instance, compounds **5a-5w** bearing methyl (**5c**), methoxy (**5f**) or trifluoromethyl (**5u**) substituents at the 3-position of phenyl ring had better antifungal activity. In contrast, the yellow blocks (Fig. 3A), nearby the 2-position of the phenyl ring, indicated that steric bulky substituents in these positions were associated with worse antifungal activities. For example, the compounds **5b** bearing substituents at the 2-position of the phenyl ring showed worse antifungal activity than the unsubstituted compound **5a**. In the electrostatic map

Table 1 Antifungal activity of nopol-based 1,3,4-thiadiazole–thioether compounds **5a-5w** at 50 μg/mL

Compounds	Relative inhibition rate (%) against the fungi							
	F. oxysporum f. sp. *cucumerinum*	*C. arachidicola*	*P. piricola*	*A. solani*	*G. zeae*	*R. solani*	*B. maydis*	*C.orbicalare*
5a (R = H)	62.2	54.2	59.3	40.9	23.8	50.6	31.6	21.6
5b (R = *o*–CH_3)	8.1	20.8	33.3	0	23.8	50.6	31.6	29.7
5c (R = *m*–CH_3)	29.7	25.0	59.3	22.7	33.3	44.6	28.9	21.6
5d (R = *p*–CH_3)	45.9	54.2	40.7	36.4	14.3	62.7	57.9	21.6
5e (R = *o*–OCH_3)	21.6	29.2	59.3	9.1	23.8	53.0	34.2	35.1
5f (R = *m*–OCH_3)	54.1	54.2	77.8	63.6	33.3	32.5	23.7	16.2
5 g (R = *p*–OCH_3)	51.4	50.0	29.6	18.2	33.3	53.0	31.6	27.0
5 h (R = *o*–F)	43.2	45.8	59.3	45.5	19.0	44.6	34.2	32.4
5i (R = *m*–F)	37.8	33.3	88.9	27.3	38.1	8.4	13.2	29.7
5j (R = *p*–F)	24.3	25.0	51.9	4.5	28.6	8.4	18.4	18.9
5 k (R = *o*–Cl)	29.7	33.3	25.9	22.7	33.3	38.6	18.4	21.6
5 l (R = *m*–Cl)	21.6	54.2	59.3	0	14.3	14.5	42.1	21.6
5 m (R = *p*–Cl)	16.2	25.0	22.2	0	52.4	80.7	60.5	62.2
5n (R = *o*–Br)	40.5	41.7	28.5	40.9	38.1	38.6	31.6	40.5
5o (R = *m*–Br)	56.8	54.2	29.6	27.3	19.0	62.7	39.5	45.9
5p (R = *p*–Br)	56.8	54.2	33.3	40.9	47.6	68.7	50.0	35.1
5q (R = *m*–I)	54.1	62.5	77.8	50.0	23.8	36.1	21.1	10.8
5r (R = *p*–I)	10.8	16.7	40.7	9.1	47.6	60.2	36.8	29.7
5 s (R = *m*, *p*–OCH_3)	27.1	35.7	50.1	24.1	22.8	16.8	24.9	46.7
5t (R = *m*,*m*–OCH_3)	28.6	51.6	47.3	28.6	27.4	46.2	39.5	44.3
5u (R = *m*–CF_3)	18.9	45.8	59.3	0	23.8	26.5	21.1	10.8
5v (R = *p*–$C(CH_3)_3$)	51.4	54.2	29.6	50.0	47.6	62.7	52.6	29.7
5w (R = *o*–Cl–*p*–F)	43.2	66.7	31.1	45.5	33.3	62.7	34.2	29.7

Table 1 (continued)

Compounds	Relative inhibition rate (%) against the fungi							
	F. oxysporum f. sp. *cucumerinum*	*C. arachidicola*	*P. piricola*	*A. solani*	*G. zeae*	*R. solani*	*B. maydis*	*C.orbicalare*
Chlorothalonil	100	73.3	75.0	73.9	73.1	96.1	90.4	91.3

Table 2 Experimental and predictive activities of CoMFA

Statistical parameters	CoMFA
q^{2a}	0.597
ONC[b]	6
r^{2c}	0.985
SE[d]	0.046
F-value[e]	100.925
Field contribution (%)	
Steric	72.6
Electrostatic	27.4

[a]Cross-validated correlation coefficient after the leave-one-out procedure

[b]Optiml number of components

[c]Non-cross-validated correlation coefficient

[d]Standard error of estimate

[e]F-test value

Table 3 The ED values of experimental and predicted activities

Compound	ED	ED"	Residue
5c	− 2.339	− 2.368	0.029
5d	− 2.666	− 2.723	0.057
5f	− 1.979	− 2.001	0.022
5 g	− 2.9	− 2.919	0.019
5 h	− 2.345	− 2.378	0.033
5j	− 2.496	− 2.445	− 0.051
5 k	− 2.986	− 2.924	− 0.062
5 l	− 2.421	− 2.393	− 0.028
5n	− 2.942	− 2.996	0.054
5p	− 2.845	− 2.799	− 0.046
5r	− 2.834	− 2.826	− 0.008
5 s	− 2.603	− 2.558	− 0.045
5t	− 2.651	− 2.658	0.007
5u	− 2.449	− 2.449	0
5v	− 2.976	− 3	0.024
5w	− 2.941	− 2.937	− 0.004

ED = experimental value, ED″ = predictive value of ED

of CoMFA (Fig. 3B), red block meant that an increase in the negative charge will lead to increase the antifungal activity, while the blue contour defines the opposite. In fact, the electron-donating group substituted at the 3-position of the phenyl

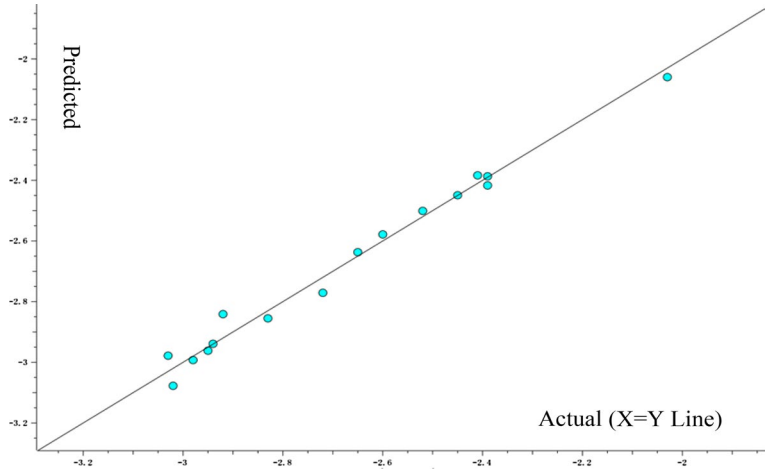
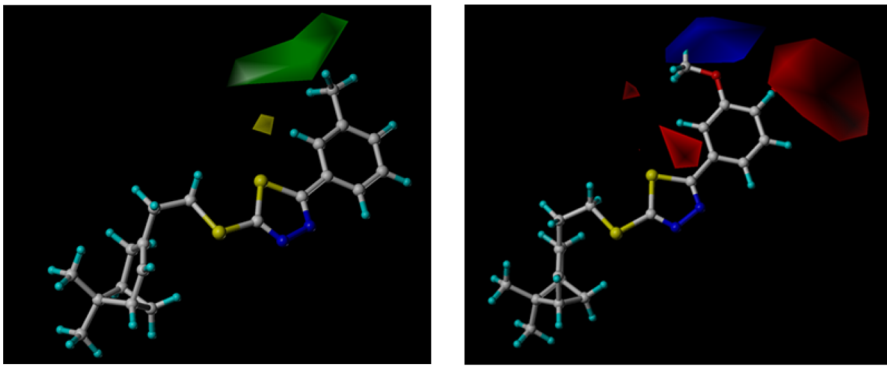


Fig. 2 Scatter plot of predicted ED vs experimental ED for CoMFA model



(A) Contours of steric contribution: green contour favors steric or bulky group, yellow contour denotes disfavored region.

(B) Contours of electrostatic contribution: blue contour indicates electropositive charge, red contour electronegative charge.

Fig. 3 **A** Contours of steric contribution: green contour favors steric or bulky group, and yellow contour denotes disfavored region. **B** Contours of electrostatic contribution: blue contour indicates electropositive charge and red contour electronegative charge. (Color figure online)

ring, such as the antifungal activity of **5c** (R = *m*–CH_3) and **5f** (R = *m*–OCH_3), had better than or equal to that of the unsubstituted compound **5a** (R = H).

Experimental

Materials and methods

The GC analysis was performed on an Agilent 6890 GC (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with an HP-1 (30 m, 0.530 mm, 0.88 μm) column. The ^{1}H NMR and ^{13}C NMR spectra were recorded using tetramethylsilane (TMS) as the internal standard on a Bruker Avance III HD spectrometer at 600 and 150 MHz, respectively. The IR spectra were recorded by employing a Nicolet iS50 FT-IR spectrometer (Thermo Scientific Co., Ltd., Madison, WI, USA) using the KBr pellet method. Mass spectra were obtained by means of the electrospray ionization (ESI) method on 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 (PerkinElmer Instruments Co., Ltd., USA). *β*-Pinene (GC purity 98%) was provided by Jiangxi Xuesong Natural Medicinal Oil Co., Ltd. (Ji'an, Jiangxi, China). All other materials and reagents were purchased from commercial suppliers and used as received. 5-Substituted phenyl-1,3,4-thiadiazole-2-thiones (**4**) were prepared by our laboratory according to the literature [44].

General procedure for the synthesis of nopol *(2)*

A mixture of paraformaldehyde (85.68 g, 0.95 mol), *β*-pinene (300 mL, 1.90 mol) was magnetically stirred under solvent-free condition. When the mixture was heated to 75 ℃, a catalytic quantity of anhydrous $ZnCl_2$ was added to the reaction system. Afterward, the reaction mixture was stirred for 1 h at 75 ℃ and then continuously heated to 110 ℃ for 10 h. When the reaction was completed, the reaction mixture was cooled to room temperature. Then, the organic layer was separated, washed three times with deionized water and dried over anhydrous Na_2SO_4. The crude product was further purified by vacuum distillation to obtain nopol (**2**) as a colorless transparent liquid at 60–70 ℃/1333 Pa (GC purity 94.5%). Yield 70%. IR (KBr, ν/cm^{-1}): 3370 (s, OH), 3025 (w, C=C–H), 2991, 2914, 2831 (s, C–H), 1468 (m, CH_2), 1381, 1368 (m,CMe_2), 1046 (s, C–O); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 5.36–5.35 (m, 1H, H-3), 3.62–3.58 (m, 2H, H-11), 2.40 (dt, J = 8.6, 5.6 Hz, 1H, H-1), 2.32–2.21 (m, 4H, H-7, H-10), 2.14–2.10 (m, 1H, H-5), 2.05 (td, J = 5.6, 1.4 Hz, 1H, H-4), 1.51 (s, 1H, H-12), 1.29 (s, 3H, H-8), 1.16 (d, J = 8.6 Hz, 1H, H-4), 0.86 (s, 3H, H-9); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 144.72, 119.38, 59.98, 45.61, 40.72, 40.22, 37.90, 31.76, 31.39, 26.25, 21.19.

General procedure for the synthesis of nopyl chloride *(3)*

Under stirring, a solution of triphenylphosphine (25.3 g, 0.09 mol) and nopol (14.6 g, 15 mL, 0.09 mol) dissolved in carbon tetrachloride (52 mL) was refluxed for 3 h. After cooling to room temperature, a white precipitate was removed by filtration. The filtrate was evaporated to give a yellowish oil, which was subsequently

washed with petroleum ether and further purified by vacuum distillation to obtain nopyl chloride (**3**) as a pale yellow liquid at 80–90 ℃/1333 Pa (GC purity 94.0%). Yield 80%, IR (KBr, ν/cm^{-1}): 3029(w, C=C–H), 2987,2916, 2981, 2831(s, C–H), 1470(m, CH_2), 1383, 1368(m,CMe_2),741(w, C–Cl); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 5.38–5.33 (m, 1H, H-3), 3.59–3.44 (m, 2H, H-11), 2.47–2.37 (m, 3H, H-1, H-10), 2.32–2.20 (m, 2H, H-7), 2.11–2.10 (m, 1H, H-5), 2.05 (td, J = 4.8, 2.3 Hz, 1H, H-4), 1.30 (s, 3H, H-9), 1.19 (d, J = 8.6 Hz, 1H, H-4), 0.86 (s, 3H, H-8); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 144.41, 119.27, 45.57, 42.64, 40.71, 40.16, 38.06, 31.64, 31.32, 26.27, 21.21.

General procedure of 2-((2-(6,6-dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethyl) thio)-1,3,4-thiadiazole derivatives *(5a-5w)*

Nopyl chloride (**3**) was added dropwise to a solution of compounds (**4**) (3 mmol), K_2CO_3 (0.41 g, 3 mmol) and PEG-400 (0.34 g, 0.5 mmol) in acetone (20 mL), and the reaction mixture was refluxed for 6 h. Upon completion of the reaction (monitored by TLC), the mixture was concentrated in vacuum. Then, the crude product was poured into saturated sodium bicarbonate solution, and the mixture was extracted with ethyl acetate (3 × 20 mL). The combined organic layer was washed with saturated salt water three times, dried over anhydrous sodium sulfate and purified by silica gel column chromatography (petroleum ether/ethyl acetate = 20:1 ~ 10:1, v/v) to afford the target compounds (**5a-5w**).

Spectral data of all the target compounds

2-((2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethyl) thio)-5-phenyl-1,3,4-thiadiazole *(5a)*

Pale yellow liquid, yield: 61.2%; IR (KBr, ν/cm^{-1}): 3027 (w, = C–H), 2985, 2912, 2831 (s, C–H), 1462,1433 (s, C=N), 687 (s, C–S–C); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 7.93–7.88 (m, 2H, Ar–H), 7.51–7.46 (m, 3H, Ar–H), 5.37 (dq, J = 4.3, 1.4 Hz, 1H, H-3), 3.45–3.39 (m, 2H, H-11), 2.50 (td, J = 7.5, 1.4 Hz, 2H, H-10), 2.41 (dt, J = 8.6, 5.6 Hz, 1H, H-1), 2.34–2.21 (m, 2H, H-7), 2.12 (d, J = 5.6 Hz, 2H, H-4, H-5), 1.31 (s, 3H, H-9), 1.20 (d, J = 8.6 Hz, 1H, H-4), 0.88 (s, 3H, H-8); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 168.12, 165.66, 145.58, 130.95, 130.02, 129.17, 127.70, 118.71, 45.54, 40.76, 38.08, 36.34, 32.23, 31.72, 31.32, 26.28, 21.27; ESI–MS *m/z*: Calcd. for $C_{19}H_{22}N_2S_2$ $[M+H]^+$ 343.12, found 343.01 $[M+H]^+$. Anal. Calcd. for $C_{19}H_{22}N_2S_2$: C, 66.63; H, 6.47; N, 8.18; Found: C, 66.62; H, 6.45; N, 8.16.

2-((2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethyl) thio)-5-(2-methylphenyl)-1,3,4-thiadiazole *(5b)*

Pale yellow liquid, yield: 66.3%; IR (KBr, v/cm^{-1}): 3022 (w, =C–H), 2983, 2916, 2831 (s, C–H), 1468, 1431 (s, C=N), 718 (m, C–S–C); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 7.63 (d, J = 7.7 Hz, 1H, Ar–H), 7.39 (td, J = 7.5, 1.1 Hz, 1H, Ar–H), 7.35 (d, J = 7.1 Hz, 1H, Ar–H), 7.30 (t, J = 7.7 Hz, 1H, Ar–H), 5.38 (dd, J = 2.7, 1.3 Hz, 1H, H-3), 3.48–3.42 (m, 2H, H-11), 2.60 (s, 3H, H-20), 2.50 (td, J = 7.5, 1.4 Hz, 2H, H-10), 2.41 (dt, J = 8.6, 5.6 Hz, 1H, H-1), 2.32–2.23 (m, 2H, H-7), 2.12 (d, J = 5.6 Hz, 2H, H-4, H-5), 1.31 (s, 3H, H-9), 1.20 (d, J = 8.6 Hz, 1H, H-4), 0.88 (s, 3H, H-8); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 167.34, 166.17, 145.58, 137.22, 131.61, 130.62, 130.30, 129.02, 126.26, 118.70, 45.53, 40.75, 38.08, 36.33, 32.03, 31.73, 31.32, 26.29, 21.58, 21.27; ESI–MS *m/z*: Calcd. for $C_{20}H_{24}N_2S_2$ $[M+H]^+$ 357.14, found 357.02 $[M+H]^+$. Anal. Calcd. for $C_{20}H_{24}N_2S_2$: C, 67.37; H, 6.79; N, 7.86; Found: C, 67.36; H, 6.78; N, 7.85.

2-((2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethyl) thio)-5-(3-methylphenyl)-1,3,4-thiadiazole *(5c)*

Pale yellow liquid, yield: 71.9%; IR (KBr, v/cm-1): 3025 (w, =C–H), 2983, 2916, 2879, 2831 (s, C–H), 1470, 1445 (s, C=N), 691 (s, C–S–C); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 7.74 (s, 1H, Ar–H), 7.68 (d, J = 7.7 Hz, 1H, Ar–H), 7.36 (t, J = 7.6 Hz, 1H, Ar–H), 7.30 (d, J = 7.6 Hz, 1H, Ar–H), 5.37 (dq, J = 4.3, 1.4 Hz, 1H, H-3), 3.45–3.37 (m, 2H, H-11), 2.50 (td, J = 7.6, 1.3 Hz, 2H, H-10), 2.44 (s, 3H, H-20), 2.41 (dt, J = 8.6, 5.6 Hz, 1H, H-1), 2.32–2.22 (m, 2H, H-7), 2.12 (d, J = 5.6 Hz, 2H, H-4, H-5), 1.31 (s, 3H, H-9), 1.20 (d, J = 8.6 Hz, 1H, H-4), 0.88 (s, 3H, H-8); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 168.35, 165.46, 145.60, 139.02, 131.77, 129.89, 129.05, 128.20, 124.95, 118.69, 45.52, 40.75, 38.08, 36.35, 32.23, 31.72, 31.32, 26.28, 21.34, 21.27; ESI–MS *m/z*: Calcd. for $C_{20}H_{24}N_2S_2$ $[M+H]^+$ 357.14, found 357.01 [M + H] +. Anal. Calcd. for $C_{20}H_{24}N_2S_2$: C, 67.37; H, 6.79; N, 7.86; Found: C, 67.35; H, 6.78; N, 7.84.

2-((2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethyl) thio)-5-(4-methylphenyl)-1,3,4-thiadiazole *(5d)*

Pale yellow liquid, yield: 72.2%; IR (KBr, v/cm^{-1}): 3020 (w, =C–H), 2981, 2918, 2879, 2833 (s, C–H), 1456 (s, C=N), 712 (w, C–S–C); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 7.79 (d, J = 8.1 Hz, 2H, Ar–H), 7.30–7.26 (m, 2H, Ar–H), 5.37 (dq, J = 4.3, 1.4 Hz, 1H, H-3), 3.45–3.36 (m, 2H, H-11), 2.50 (td, J = 7.6, 1.3 Hz, 2H, H-10), 2.42 (s, 3H, H-20), 2.41 (dt, J = 8.6, 5.6 Hz, 1H, H-1), 2.32–2.22 (m, 2H, H-7), 2.12 (d, J = 5.5 Hz, 2H, H-4, H-5), 1.31 (s, 3H, H-9), 1.20 (d, J = 8.6 Hz, 1H, H-4), 0.88 (s, 3H, H-8); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 168.29, 165.11, 145.61, 141.41, 129.84, 127.62, 127.30, 118.67, 45.52, 40.75, 38.08, 36.36, 32.21, 31.72, 31.31, 26.28, 21.51, 21.27; ESI–MS *m/z*: Calcd. for $C_{20}H_{24}N_2S_2$ $[M+H]^+$ 357.14, found 357.01 $[M+H]^+$. Anal. Calcd. for $C_{20}H_{24}N_2S_2$: C, 67.37; H, 6.79; N, 7.86; Found: C, 67.35; H, 6.77; N, 7.85.

2-((2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethyl) thio)-5-(2-methoxyphenyl)-1,3,4-thiadiazole *(5e)*

Pale yellow liquid, yield: 69.3%; IR (KBr, v/cm^{-1}): 3022 (w, =C–H), 2981, 2916, 2875, 2831 (s, C–H), 1600, 1500(s, C=C), 1466 (s, C=N), 681 (s, C–S–C); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 8.45 (dd, J = 7.9, 1.6 Hz, 1H, Ar–H), 7.50–7.44 (m, 1H, Ar–H), 7.12 (t, J = 7.6 Hz, 1H, Ar–H), 7.04 (d, J = 8.3 Hz, 1H, Ar–H), 5.37 (dq, J = 4.3, 1.4 Hz, 1H, H-3), 4.01 (s, 3H, H-20), 3.44–3.36 (m, 2H, H-11), 2.50 (td, J = 7.6, 1.3 Hz, 2H, H-10), 2.40 (dt, J = 8.5, 5.6 Hz, 1H, H-1), 2.32–2.22 (m, 2H, H-7), 2.12 (d, J = 5.5 Hz, 2H, H-4, H-5), 1.30 (s, 3H, H-9), 1.20 (d, J = 8.6 Hz, 1H, H-4), 0.88 (s, 3H, H-8); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 166.32, 161.97, 155.62, 145.75, 131.92, 128.27, 121.34, 119.17, 118.52, 111.33, 55.71, 45.54, 40.76, 38.07, 36.49, 32.00, 31.72, 31.32, 26.29, 21.27; ESI–MS *m/z*: Calcd. for $C_{20}H_{24}N_2OS_2$ $[M+H]^+$ 373.13, found 373.01 $[M+H]^+$. Anal. Calcd. for $C_{20}H_{24}N_2OS_2$: C, 64.48; H, 6.49; N, 7.52; Found: C, 64.47; H, 6.48; N, 7.51.

2-((2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethyl) thio)-5-(3-methoxyphenyl)-1,3,4-thiadiazole *(5f)*

Pale yellow liquid, yield: 72.6%; IR (KBr, v/cm^{-1}):3029 (w, =C–H), 2987, 2914, 2877, 2831 (s, C–H), 1604(s, C=C), 1477 (s, C=N), 687 (s, C–S–C); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 7.54–7.51 (m, 1H, Ar–H), 7.42–7.35 (m, 2H, Ar–H), 7.03 (ddd, J = 8.0, 2.5, 1.1 Hz, 1H, Ar–H), 5.37 (dq, J = 4.3, 1.4 Hz, 1H, H-3), 3.89 (s, 3H, H-20), 3.45–3.37 (m, 2H, H-11), 2.50 (td, J = 7.5, 1.2 Hz, 2H, H-10), 2.41 (dt, J = 8.6, 5.6 Hz, 1H, H-1), 2.41–2.22 (m, 2H, H-7), 2.12 (d, J = 5.5 Hz, 2H, H-4, H-5), 1.31 (s, 3H, H-9), 1.20 (d, J = 8.6 Hz, 1H, H-4), 0.88 (s, 3H, H-8); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 168.01, 165.76, 160.05, 145.58, 131.16, 130.20, 120.48, 118.71, 117.35, 111.99, 55.49, 45.52, 40.74, 38.08, 36.35, 32.21, 31.72, 31.32, 26.28, 21.27; ESI–MS *m/z*: Calcd. for $C_{20}H_{24}N_2OS_2$ $[M+H]^+$ 373.13, found 373.02 $[M+H]^+$. Anal. Calcd. for $C_{20}H_{24}N_2OS_2$: C, 64.48; H, 6.49; N, 7.52; Found: C, 64.47; H, 6.47; N, 7.51.

2-((2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethyl) thio)-5-(4-methoxyphenyl)-1,3,4-thiadiazole *(5 g)*

Pale yellow liquid, yield: 81.4%; IR (KBr, v/cm^{-1}): 3024 (w, =C–H), 2985, 2916, 2880, 2832 (s, C–H), 1603, 1520(s, C=C), 1453 (s, C=N), 662 (m, C–S–C); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 7.85–7.82 (m, 2H, Ar–H), 6.98 (d, J = 8.8 Hz, 2H, Ar–H), 5.37 (dq, J = 4.3, 1.4 Hz, 1H, H-3), 3.88 (s, 3H, H-20), 3.43–3.35 (m, 2H), 2.50 (td, J = 7.5, 1.2 Hz, 2H, H-10), 2.40 (dt, J = 8.6, 5.6 Hz, 1H, H-1), 2.31–2.22 (m, 2H, H-7), 2.11 (d, J = 5.5 Hz, 2H, H-4, H-5), 1.30 (s, 3H, H-9), 1.19 (d, J = 8.6 Hz, 1H, H-4), 0.87 (s, 3H, H-8); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 167.96, 164.56, 161.79, 145.62, 129.23, 122.71, 118.65, 114.54, 55.46, 45.52, 40.75, 38.07, 36.38, 32.23, 31.71, 31.31, 26.28, 21.27; ESI–MS *m/z*: Calcd. for $C_{20}H_{24}N_2OS_2$ $[M+H]^+$ 373.13, found 373.01 $[M+H]^+$. Anal. Calcd. for $C_{20}H_{24}N_2OS_2$: C, 64.48; H, 6.49; N, 7.52; Found: C, 64.46; H, 6.48; N, 7.50.

2-((2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethyl) thio)-5-(2-fluorophenyl)-1,3,4-thiadiazole *(5 h)*

Pale yellow liquid, yield: 80.8%; IR (KBr, v/cm^{-1}): 3025 (w, =C–H), 2983, 2916, 2879, 2831 (s, C–H), 1614, 1583 (m, C=C), 1458 (s, C=N), 670 (s, C–S–C); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 8.36 (td, J = 7.6, 1.7 Hz, 1H, Ar–H), 7.49 (ddd, J = 15.5, 5.4, 1.7 Hz, 1H, Ar–H), 7.34–7.29 (m, 1H, Ar–H), 7.26- 7.19 (m, 1H, Ar–H), 5.37 (dq, J = 4.3, 1.4 Hz, 1H, H-3), 3.47–3.39 (m, 2H, H-11), 2.50 (td, J = 7.5, 1.3 Hz, 2H, H-10), 2.41 (dt, J = 8.6, 5.6 Hz, 1H, H-1), 2.32–2.22 (m, 2H, H-7), 2.12 (d, J = 5.6 Hz, 2H, H-4, H-5), 1.31 (s, 3H, H-9), 1.20 (d, J = 8.6 Hz, 1H, H-4), 0.88 (s, 3H, H-8); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 167.36, 160.25, 159.32, 145.55, 132.37, 128.66, 124.92, 118.73, 118.17, 116.14, 45.52, 40.74, 38.08, 36.35, 32.17, 31.72, 31.31, 26.28, 21.27; ESI–MS *m/z*: Calcd. for $C_{19}H_{21}FN_2S_2$ $[M+H]^+$ 361.11, found 360.99 $[M+H]^+$. Anal. Calcd. for $C_{19}H_{21}FN_2S_2$: C, 63.30; H, 5.87; N, 7.77; Found: C, 63.29; H, 5.86; N, 7.75.

2-((2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethyl) thio)-5-(3-fluorophenyl)-1,3,4-thiadiazole *(5i)*

Pale yellow liquid, yield: 65.8%; IR (KBr, v/cm^{-1}): 3025 (w, =C–H), 2983, 2916, 2879, 2829 (s, C–H), 1610, 1591(s, C=C), 1472 (s, C=N), 683 (s, C–S–C); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 7.66 (dt, J = 17.0, 4.8 Hz, 2H, Ar–H), 7.45 (td, J = 8.1, 5.8 Hz, 1H, Ar–H), 7.22–7.17 (m, 1H, Ar–H), 5.37 (dq, J = 4.3, 1.4 Hz, 1H, H-3), 3.47–3.39 (m, 2H, H-11), 2.50 (td, J = 7.5, 1.3 Hz, 2H, H-10), 2.41 (dt, J = 8.6, 5.6 Hz, 1H, H-1), 2.32–2.22 (m, 2H, H-7), 2.12 (dd, J = 5.5, 1.0 Hz, 2H, H-4, H-5), 1.31 (s, 3H, H-9), 1.20 (d, J = 8.6 Hz, 1H, H-4), 0.88 (s, 3H, H-8); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 166.66, 166.39, 162.9, 145.49, 131.93, 130.84, 123.56, 118.79, 117.88, 114.41, 45.51, 40.74, 38.08, 36.28, 32.23, 31.72, 31.31, 26.27, 21.27; ESI–MS *m/z*: Calcd. for $C_{19}H_{21}FN_2S_2$ $[M+H]^+$ 361.11, found 361.00 $[M+H]^+$. Anal. Calcd. for $C_{19}H_{21}FN_2S_2$: C, 63.30; H, 5.87; N, 7.77; Found: C, 63.28; H, 5.86; N, 7.76.

2-((2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethyl) thio)-5-(4-fluorophenyl)-1,3,4-thiadiazole *(5j)*

Pale yellow liquid, yield: 68.5%; IR (KBr, v/cm^{-1}): 3027 (w, =C–H), 2989, 2918, 2879, 2833 (s, C–H), 1602, 1516(s, C=C), 1454 (s, C=N), 664 (m, C–S–C); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 7.93–7.87 (m, 2H, Ar–H), 7.21–7.14 (m, 2H, Ar–H), 5.37 (dq, J = 4.3, 1.4 Hz, 1H, H-3), 3.46–3.37 (m, 2H, H-11), 2.50 (dd, J = 15.0, 1.0 Hz, 2H, H-10), 2.40 (dt, J = 8.6, 5.6 Hz, 1H, H-1), 2.32–2.22 (m, 2H, H-7), 2.12 (dd, J = 5.5, 1.0 Hz, 2H, H-4, H-5), 1.31 (s, 3H, H-9), 1.20 (d, J = 8.6 Hz, 1H, H-4), 0.87 (s, 3H, H-8); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 166.88, 165.74, 164.29, 145.53, 129.67, 126.32, 118.75, 116.46, 116.31, 45.51, 40.74, 38.08, 36.31, 32.23, 31.71, 31.31, 26.27, 21.26; ESI–MS *m/z*: Calcd. for $C_{19}H_{21}FN_2S_2$ $[M+H]^+$ 361.11, found 360.99 $[M+H]^+$. Anal. Calcd. for $C_{19}H_{21}FN_2S_2$: C, 63.30; H, 5.87; N, 7.77; Found: C, 63.29; H, 5.85; N, 7.76.

2-((2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethyl) thio)-5-(2-chlorophenyl)-1,3,4-thiadiazole *(5 k)*

Pale yellow liquid, yield: 74.2%; IR (KBr, v/cm^{-1}): 3029 (w, =C–H), 2983, 2914, 2879, 2831 (s, C–H), 1433 (s, C=N), 662 (w, C–S–C); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 8.31- 8.26 (m, 1H, Ar–H), 7.55–7.51 (m, 1H, Ar–H), 7.46–7.40 (m, 2H, Ar–H), 5.37 (dq, J = 4.3, 1.4 Hz, 1H, H-3), 3.47–3.41 (m, 2H, H-11), 2.51 (td, J = 7.5, 1.2 Hz, 2H, H-10), 2.41 (dt, J = 8.6, 5.6 Hz, 1H, H-1), 2.32–2.22 (m, 2H, H-7), 2.12 (d, J = 5.6 Hz, 2H, H-4, H-5), 1.31 (s, 3H, H-9), 1.20 (d, J = 8.6 Hz, 1H, H-4), 0.88 (s, 3H, H-8); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 167.57, 163.41, 145.55, 132.38, 131.49, 130.83, 130.55, 128.94, 127.37, 118.74, 45.52, 40.75, 38.08, 36.36, 32.15, 31.72, 31.32, 26.28, 21.27; ESI–MS m/z: Calcd. for $C_{19}H_{21}ClN_2S_2$ $[M+H]^+$ 377.08, found 376.97 $[M+H]^+$. Anal. Calcd. for $C_{19}H_{21}ClN_2S_2$: C, 60.54; H, 5.62; N, 7.43; Found: C, 60.53; H, 5.61; N, 7.42.

2-((2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethyl) thio)-5-(3-chlorophenyl)-1,3,4-thiadiazole *(5 l)*

Pale yellow liquid, yield: 74.7%; IR (KBr, v/cm^{-1}): 3027 (w, =C–H), 2985, 2916, 2877, 2829 (s, C–H),1595, 1572(s, C=C), 1458, 1427 (s, C=N), 681 (s, C–S–C); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 7.91 (t, J = 1.7 Hz, 1H, Ar–H), 7.77 (dd, J = 7.6, 1.2 Hz, 1H, Ar–H), 7.48–7.44 (m, 1H, Ar–H), 7.41 (t, J = 7.8 Hz, 1H, Ar–H), 5.37 (dq, J = 4.3, 1.4 Hz, 1H, H-3), 3.47–3.38 (m, 2H, H-11), 2.50 (td, J = 7.5, 1.1 Hz, 2H, H-10), 2.41 (dt, J = 8.6, 5.6 Hz, 1H, H-1), 2.32–2.22 (m, 2H, H-7), 2.12 (d, J = 5.3 Hz, 2H, H-4, H-5), 1.31 (s, 3H, H-9), 1.20 (d, J = 8.6 Hz, 1H, H-4), 0.88 (s, 3H, H-8); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 166.49, 166.45, 145.48, 135.22, 131.60, 130.89, 130.43, 127.52, 125.81, 118.80, 45.51, 40.74, 38.08, 36.28, 32.26, 31.72, 31.31, 26.28, 21.27; ESI–MS m/z: Calcd. for $C_{19}H_{21}ClN_2S_2$ $[M+H]^+$ 377.08, found 376.98 $[M+H]^+$. Anal. Calcd. for $C_{19}H_{21}ClN_2S_2$: C, 60.54; H, 5.62; N, 7.43; Found: C, 60.53; H, 5.60; N, 7.41.

2-((2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethyl) thio)-5-(4-chlorophenyl)-1,3,4-thiadiazole *(5 m)*

Pale yellow liquid, yield: 70.8%; IR (KBr, v/cm^{-1}): 3022 (w, =C–H), 2987, 2935, 2912, 2833 (s, C–H), 1595 (s, C=C), 1450 (s, C=N), 652 (s, C–S–C); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 7.84 (d, J = 8.5 Hz, 2H, Ar–H), 7.46 (d, J = 8.5 Hz, 2H, Ar–H), 5.37 (dq, J = 4.3, 1.4 Hz, 1H, H-3), 3.46–3.37 (m, 2H, H-11), 2.50 (td, J = 7.5, 1.1 Hz, 2H, H-10), 2.40 (dt, J = 8.6, 5.6 Hz, 1H, H-1), 2.32–2.22 (m, 2H, H-7), 2.11 (d, J = 4.9 Hz, 2H, H-4, H-5), 1.30 (s, 3H, H-9), 1.19 (d, J = 8.6 Hz, 1H, H-4), 0.87 (s, 3H, H-8); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 166.82, 166.08, 145.51, 137.03, 129.46, 128.83, 128.49, 118.77, 45.51, 40.73, 38.08, 36.29, 32.24, 31.72, 31.31, 26.28, 21.27; ESI–MS m/z: Calcd. for $C_{19}H_{21}ClN_2S_2$ $[M+H]^+$ 377.08, found 376.96 $[M+H]^+$. Anal. Calcd. for $C_{19}H_{21}ClN_2S_2$: C, 60.54; H, 5.62; N, 7.43; Found: C, 60.52; H, 5.61; N, 7.41.

2-((2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethyl) thio)-5-(2-bromophenyl)-1,3,4-thiadiazole *(5n)*

Pale yellow liquid, yield: 65.8%; IR (KBr, v/cm^{-1}): 3025 (w, =C–H), 2985, 2916, 2879, 2831 (s, C–H), 1431 (s, C=N), 637 (w, C–S–C); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 8.13 (dd, J = 7.9, 1.6 Hz, 1H, Ar–H), 7.73 (dd, J = 8.0, 0.9 Hz, 1H, Ar–H), 7.46 (td, J = 7.8, 1.1 Hz, 1H, Ar–H), 7.35 (td, J = 7.9, 1.7 Hz, 1H, Ar–H), 5.37 (dq, J = 4.3, 1.4 Hz, 1H, H-3), 3.48–3.40 (m, 2H, H-11), 2.51 (dd, J = 15.0, 1.0 Hz, 2H, H-10), 2.41 (dt, J = 8.6, 5.6 Hz, 1H, H-1), 2.32–2.22 (m, 2H, H-7), 2.12 (d, J = 5.5 Hz, 2H, H-4, H-5), 1.31 (s, 3H, H-9), 1.20 (d, J = 8.6 Hz, 1H, H-4), 0.88 (s, 3H, H-8); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 167.51, 164.83, 145.54, 133.97, 131.63, 130.96, 127.85, 122.30, 118.75, 45.52, 40.75, 38.09, 36.35, 32.17, 31.72, 31.32, 26.29, 21.27; ESI–MS *m/z*: Calcd. for $C_{19}H_{21}BrN_2S_2$ $[M+H]^+$ 421.03, found 420.94 $[M+H]^+$. Anal. Calcd. for $C_{19}H_{21}BrN_2S_2$: C, 54.15; H, 5.02; N, 6.65; Found: C, 54.14; H, 5.01; N, 6.64.

2-((2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethyl) thio)-5-(3-bromophenyl)-1,3,4-thiadiazole *(5o)*

Pale yellow liquid, yield: 67.4%; IR (KBr, v/cm^{-1}): 3027 (w, =C–H), 2983, 2914, 2875, 2831 (s, C–H), 1595, 1566 (m, C=C), 1427 (s, C=N), 681 (s, C–S–C); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 8.07 (t, J = 1.7 Hz, 1H, Ar–H), 7.82 (d, J = 7.8 Hz, 1H, Ar–H), 7.62 (dd, J = 8.0, 0.8 Hz, 1H, Ar–H), 7.36 (t, J = 7.9 Hz, 1H, Ar–H), 5.37 (dq, J = 4.3, 1.4 Hz, 1H, H-3), 3.47–3.38 (m, 2H, H-11), 2.50 (td, J = 7.5, 1.2 Hz, 2H, H-10), 2.41 (dt, J = 8.6, 5.6 Hz, 1H, H-1), 2.32–2.22 (m, 2H, H-7), 2.12 (d, J = 4.8 Hz, 2H, H-4, H-5), 1.31 (s, 3H, H-9), 1.20 (d, J = 8.6 Hz, 1H, H-4), 0.88 (s, 3H, H-8); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 166.41, 166.35, 145.48, 133.81, 131.82, 130.66, 130.41, 126.25, 123.19, 118.81, 45.51, 40.74, 38.09, 36.28, 32.27, 31.72, 31.31, 26.28, 21.27; ESI–MS *m/z*: Calcd. for $C_{19}H_{21}BrN_2S_2$ $[M+H]^+$ 421.03, found 420.93 $[M+H]^+$. Anal. Calcd. for $C_{19}H_{21}BrN_2S_2$: C, 54.15; H, 5.02; N, 6.65; Found: C, 54.13; H, 5.01; N, 6.64.

2-((2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethyl) thio)-5-(4-bromophenyl)-1,3,4-thiadiazole *(5p)*

Pale yellow liquid, yield: 71.6%; IR (KBr, v/cm^{-1}): 3022 (w, =C–H), 2981, 2918, 2879, 2831 (s, C–H), 1585(m, C=C), 1445 (s, C=N), 658 (s, C–S–C); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 7.79–7.75 (m, 2H, Ar–H), 7.63–7.60 (m, 2H, Ar–H), 5.37 (dq, J = 4.3, 1.4 Hz, 1H, H-3), 3.45–3.39 (m, 2H, H-11), 2.50 (td, J = 7.5, 1.3 Hz, 2H, H-10), 2.40 (dt, J = 8.6, 5.6 Hz, 1H, H-1), 2.32–2.22 (m, 2H, H-7), 2.11 (d, J = 5.6 Hz, 2H, H-4, H-5), 1.31 (s, 3H, H-9), 1.19 (d, J = 8.6 Hz, 1H, H-4), 0.87 (s, 3H, H-8); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 166.90, 166.13, 145.50, 132.42, 129.01, 128.93, 125.36, 118.78, 45.51, 40.73, 38.08, 36.29, 32.24, 31.72, 31.31, 26.28, 21.27; ESI–MS *m/z*: Calcd. for $C_{19}H_{21}BrN_2S_2$ $[M+H]^+$ 421.03, found 420.94 $[M+H]^+$. Anal. Calcd. for $C_{19}H_{21}BrN_2S_2$: C, 54.15; H, 5.02; N, 6.65; Found: C, 54.13; H, 5.01; N, 6.63.

2-((2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethyl) thio)-5-(3-iodophenyl)-1,3,4-thiadiazole *(5q)*

Pale yellow liquid, yield: 70.5%; IR (KBr, v/cm^{-1}): 3029 (w, =C–H), 2983, 2914, 2877, 2829 (s, C–H), 1589,1558 (s, C=C), 1433 (s, C=N), 683 (s, C–S–C); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 8.27 (t, J = 1.7 Hz, 1H, Ar–H), 7.85 (dd, J = 7.8, 1.1 Hz, 1H, Ar–H), 7.83–7.79 (m, 1H, Ar–H), 7.21 (t, J = 7.9 Hz, 1H, Ar–H), 5.37 (dq, J = 4.3, 1.4 Hz, 1H, H-3), 3.45–3.38 (m, 2H, H-11), 2.50 (td, J = 7.5, 1.4 Hz, 2H, H-10), 2.41 (dt, J = 8.6, 5.6 Hz, 1H, H-1), 2.32–2.22 (m, 2H, H-7), 2.12 (d, J = 6.6 Hz, 2H, H-4, H-5), 1.31 (s, 3H, H-9), 1.20 (d, J = 8.6 Hz, 1H, H-4), 0.88 (s, 3H, H-8); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 166.41, 166.19, 145.49, 139.75, 136.21, 131.83, 130.72, 126.85, 118.80, 94.64, 45.51, 40.74, 38.09, 36.28, 32.28, 31.72, 31.32, 26.28, 21.28; ESI–MS m/z: Calcd. for $C_{19}H_{21}IN_2S_2$ $[M+H]^+$ 469.02, found 468.95 $[M+H]^+$. Anal. Calcd. for $C_{19}H_{21}IN_2S_2$: C, 48.72; H, 4.52; N, 5.98; Found: C, 48.70; H, 4.51; N, 5.97.

2-((2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethyl) thio)-5-(4-iodophenyl)-1,3,4-thiadiazole *(5r)*

Pale yellow liquid, yield: 68.1%; IR (KBr, v/cm^{-1}): 3027 (w, =C–H), 2983, 2935, 2918, 2831 (s, C–H), 1585(s, C=C), 1445 (s, C=N), 656 (s, C–S–C); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 7.84–7.81 (m, 2H, Ar–H), 7.64–7.61 (m, 2H, Ar–H), 5.37 (dq, J = 4.3, 1.4 Hz, 1H, H-3), 3.46–3.37 (m, 2H, H-11), 2.50 (td, J = 7.5, 1.3 Hz, 2H, H-10), 2.40 (dt, J = 8.6, 5.6 Hz, 1H, H-1), 2.32–2.22 (m, 2H, H-7), 2.12 (d, J = 6.6 Hz, 2H, H-4, H-5), 1.31 (s, 3H, H-9), 1.19 (d, J = 8.6 Hz, 1H, H-4), 0.87 (s, 3H, H-8); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 167.08, 166.14, 145.50, 138.36, 129.47, 129.02, 118.78, 97.34, 45.51, 40.74, 38.08, 36.29, 32.24, 31.72, 31.32, 26.28, 21.28; ESI–MS m/z: Calcd. for $C_{19}H_{21}IN_2S_2$ $[M+H]^+$469.02, found 468.94 $[M+H]^+$. Anal. Calcd. for $C_{19}H_{21}IN_2S_2$: C, 48.72; H, 4.52; N, 5.98; Found: C, 48.71; H, 4.50; N, 5.97.

2-((2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethyl) thio)-5-(3,4-dimethoxyphenyl)-1,3,4-thiadiazole *(5 s)*

Pale yellow liquid, yield: 64.7%; IR (KBr, v/cm^{-1}): 3026 (w, =C–H), 2981, 2912, 2877, 2831 (s, C–H), 1603, 1521(s, C=C), 1433 (s, C=N), 644 (m, C–S–C); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 7.59 (d, J = 2.0 Hz, 1H, Ar–H), 7.33 (dd, J = 8.3, 2.0 Hz, 1H, Ar–H), 6.92 (d, J = 8.3 Hz, 1H, Ar–H), 5.36 (dq, J = 4.3, 1.4 Hz, 1H, H-3), 3.96 (d, J = 13.9 Hz, 6H), 3.40–3.37 (m, 2H, H-11), 2.50 (dd, J = 10.9, 4.3 Hz, 2H, H-10), 2.40 (dt, J = 8.6, 5.6 Hz, 1H, H-1), 2.32–2.22 (m, 2H, H-7), 2.11 (d, J = 5.5 Hz, 2H, H-4, H-5), 1.30 (s, 3H, H-9), 1.19 (d, J = 8.6 Hz, 1H, H-4), 0.87 (s, 3H, H-8); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 168.05, 164.74, 151.48, 149.42, 145.62, 122.91, 121.52, 118.66, 111.11, 109.57, 56.10, 56.05, 45.52, 40.74, 38.07, 36.39, 32.24, 31.71, 31.31, 26.28, 21.27; ESI–MS m/z: Calcd. for $C_{21}H_{26}N_2O_2S_2$ $[M+H]^+$403.14, found 403.05 $[M+H]^+$. Anal. Calcd. for $C_{21}H_{26}N_2O_2S_2$: C, 62.66; H, 6.51; N, 6.96; Found: C, 62.65; H, 6.50; N, 6.94.

2-((2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethyl) thio)-5-(3,5-dimethoxyphenyl)-1,3,4-thiadiazole *(5t)*

Pale yellow liquid, yield: 73.6%; IR (KBr, ν/cm^{-1}): 2983, 2917, 2876, 2831 (s, C–H), 1591(s, C=C), 1465 (s, C=N), 1347 (s, C=S), 680 (s, C–S–C); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 7.16 (d, J = 2.3 Hz, 2H, Ar–H), 6.62 (t, J = 2.3 Hz, 1H, Ar–H), 5.38 (dq, J = 4.3, 1.4 Hz, 1H, H-3), 3.87 (s, 6H), 3.35- 3.33 (m, 2H, H-11), 2.51 (td, J = 7.5, 1.3 Hz, 2H, H-10), 2.41 (dt, J = 8.6, 5.6 Hz, 1H, H-1), 2.32–2.22 (m, 2H, H-7), 2.12 (d, J = 6.4 Hz, 2H, H-4, H-5), 1.31 (s, 3H, H-9), 1.20 (d, J = 8.6 Hz, 1H, H-4), 0.88 (s, 3H, H-8); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 165.61, 164.67, 161.16, 145.28, 125.20, 119.00, 104.38, 104.31, 55.64, 45.46, 40.72, 38.08, 36.40, 31.70, 31.31, 30.68, 26.26, 21.25; ESI–MS *m*/*z*: Calcd. for $C_{21}H_{26}N_2O_2S_2$ $[M+H]^+$ 403.14, found 403.04 $[M+H]^+$. Anal. Calcd. for $C_{21}H_{26}N_2O_2S_2$: C, 62.66; H, 6.51; N, 6.96; Found: C, 62.64; H, 6.49; N, 6.95.

2-((2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethyl)thio)-5-(3-(trifluoromethyl) phenyl)-1,3,4-thiadiazole *(5u)*

Pale yellow liquid, yield: 74.8%; IR (KBr, ν/cm^{-1}): 3025 (w, =C–H), 2987, 2918, 2877, 2831 (s, C–H), 1472 (s, C=N), 695 (s, C–S–C); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 8.17 (s, 1H, Ar–H), 8.08 (d, J = 7.8 Hz, 1H, Ar–H), 7.75 (d, J = 7.8 Hz, 1H, Ar–H), 7.63 (t, J = 7.8 Hz, 1H, Ar–H), 5.38 (dq, J = 4.3, 1.4 Hz, 1H, H-3), 3.48–3.41 (m, 2H, H-11), 2.51 (td, J = 7.5, 1.3 Hz, 2H, H-10), 2.41 (dt, J = 8.6, 5.6 Hz, 1H, H-1), 2.32–2.22 (m, 2H, H-7), 2.12 (d, J = 5.5 Hz, 2H, H-4, H-5), 1.31 (s, 3H, H-9), 1.20 (d, J = 8.6 Hz, 1H, H-4), 0.88 (s, 3H, H-8); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 166.76, 166.38, 145.46, 131.90, 131.68, 130.76, 129.80, 127.42, 124.41, 122.68, 118.84, 45.51, 40.73, 38.09, 36.26, 32.29, 31.72, 31.31, 26.27, 21.26; ESI–MS *m*/*z*: Calcd. for $C_{20}H_{21}F_3N_2S_2$ $[M+H]^+$ 411.11, found 411.01 $[M+H]^+$. Anal. Calcd. for $C_{20}H_{21}F_3N_2S_2$: C, 58.52; H, 5.16; N, 6.82; Found: C, 58.51; H, 5.15; N, 6.80.

2-((2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethyl)thio)-5-(4-(tert-butyl) phenyl)-1,3,4-thiadiazole *(5v)*

Pale yellow liquid, yield: 75.9%; IR (KBr, ν/cm^{-1}): 3026 (w, =C–H), 2964, 2916, 2874, 2832 (s, C–H), 1607(w, C=C), 1455 (s, C=N), 695 (s, C–S–C); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 7.83 (d, J = 8.4 Hz, 2H, Ar–H), 7.50 (d, J = 8.4 Hz, 2H, Ar–H), 5.37 (dq, J = 4.3, 1.4 Hz, 1H, H-3), 3.43–3.37 (m, 2H, H-11), 2.50 (dd, J = 10.9, 4.3 Hz, 2H, H-10), 2.41 (dt, J = 8.6, 5.6 Hz, 1H, H-1), 2.32–2.22 (m, 2H, H-7), 2.12 (d, J = 5.5 Hz, 2H, H-4, H-5), 1.37 (s, 9H), 1.31 (s, 3H, H-9), 1.20 (d, J = 8.6 Hz, 1H, H-4), 0.88 (s, 3H, H-8); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 168.17, 165.14, 154.53, 145.62, 127.49, 127.24, 126.13, 118.67, 45.52, 40.75, 38.08, 36.37, 34.99, 32.20, 31.72, 31.32, 31.15, 26.28, 21.27; ESI–MS *m*/*z*: Calcd. for $C_{23}H_{30}N_2S_2$ $[M+H]^+$ 399.19, found 399.05 $[M+H]^+$. Anal. Calcd. for $C_{23}H_{30}N_2S_2$: C, 69.30; H, 7.59; N, 7.03; Found: C, 69.28; H, 7.58; N, 7.02.

2-((2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethyl) thio)-5-(2-chloro-4-fluorophenyl)-1,3,4-thiadiazole *(5w)*

Pale yellow liquid, yield: 60.9%; IR (KBr, v/cm^{-1}): 3027 (w, =C–H), 2983, 2916, 2879, 2831 (s, C–H),1600, 1500(s, C=C), 664 (w, C–S–C); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 8.30 (dd, J=8.9, 6.1 Hz, 1H, Ar–H), 7.30–7.26 (m, 1H, Ar–H), 7.16 (ddd, J=8.9, 7.6, 2.6 Hz, 1H, Ar–H), 5.37 (dq, J=4.3, 1.4 Hz, 1H, H-3), 3.47–3.40 (m, 2H, H-11), 2.50 (td, J=7.5, 1.3 Hz, 2H, H-10), 2.41 (dt, J=8.6, 5.6 Hz, 1H, H-1), 2.32–2.22 (m, 2H, H-7), 2.12 (d, J=5.6 Hz, 2H, H-4, H-5), 1.31 (s, 3H, H-9), 1.20 (d, J=8.6 Hz, 1H, H-4), 0.88 (s, 3H, H-8); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 167.55, 163.43, 162.50, 145.51, 133.37, 132.41, 125.47, 118.77, 117.82, 115.18, 45.52, 40.74, 38.08, 36.34, 32.16, 31.72, 31.31, 26.28, 21.27; ESI–MS m/z: Calcd. for $C_{19}H_{20}ClFN_2S_2$ $[M+H]^+$ 395.07, found 394.95 $[M+H]^+$. Anal. Calcd. for $C_{19}H_{20}ClFN_2S_2$: C, 57.78; H, 5.10; N, 7.09; Found: C, 57.76; H, 5.09; N, 7.07.

In vitro antifungal activity test

The biological activity of the nopol-based 1,3,4-thiadiazole–thioether compounds was evaluated in vitro through the agar dilution method according to the literature [45]. The strains were incubated in petri dishes with potato–sugar–agar (PSA) culture medium to culture new mycelia for the antifungal assay. The tested compound was dissolved in acetone. As an emulsifier, Sorporl-144 (200 mg/L) was added to dilute the solution of each sample to 500 mg/L. Then, 50 mg/L of the test compounds were made by mixing 1 mL 500 mg/L stock solution with 9 mL of PSA culture medium. A bacterium tray of 5-mm-diameter cut along the external edge of the mycelium was transferred to the flat containing the tested compound and put in equilateral triangular style in triplicate. After culturing 48 h at (24 ± 1) ℃ in the incubator, the expanded colony diameters of strains were measured and compared with that treated with aseptic distilled water. Aseptic distilled water without the test samples was served as a blank control, and the commercial protective fungicide chlorothalonil was used as a positive control. All the experiments were performed in three replicates to calculate the relative inhibition percentage.

3D-QSAR analysis

3D-QSAR model was performed to find the correlation between the structure and activity of the target molecules according to previously reported procedures [46]. All the molecular modeling calculations were constructed using SYBYL-X 2.0. According

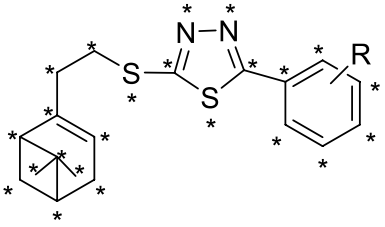


Fig. 4 Asterisk skeleton of the target compounds

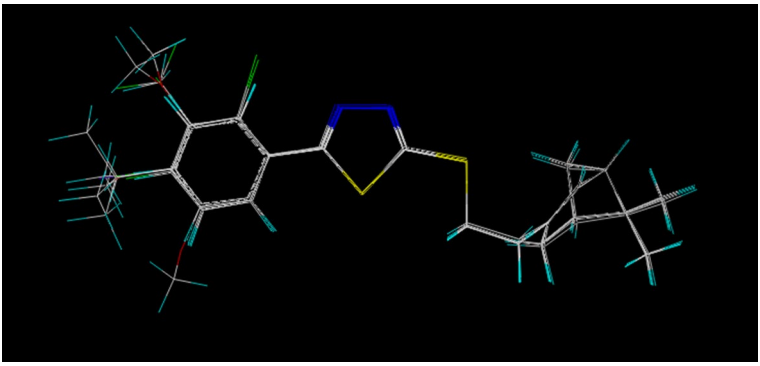


Fig. 5 Alignment superposition of molecules based on compound **5f**

to reported literature [47], ED value of the antifungal activity against *P. piricola* was expressed by the formula:

$$\mathrm{ED} = \log\{I/[(100 - I) \times \mathrm{MW}]\}$$

where I is the percent inhibition at 50 μg/mL and MW is the molecular weight of the target compounds. Complete conformational optimization of each structure was performed using a conjugate gradient procedure based on the Tripos force field with termination convergence energy of 0.005 kcal/(mol*Å) and a maximum of 1000 iterations and Gasteiger–Hückel charges. The steric and electrostatic fields were calculated using a sp^3 hybridized carbon atom as a probe with + 1.0 charge. Compound **5f** was selected as the template, in which the atoms marked with an asterisk constituted the common superimposed skeleton (Fig. 4), and sixteen test compounds as training set were superimposed (Fig. 5). CoMFA descriptors are independent variables, and ED values are dependent variables. The cross-validation with the leave-one-out method was carried out to obtain the cross-validated q^2 and the optimal number of components. Then, a non-cross-validation analysis under the optimal number of components was performed. The modeling capability was indicated by the correlation coefficient squared r^2, and the prediction capability was judged by the r^2 and q^2.

Conclusion

In conclusion, twenty-three novel nopol-based 1,3,4-thiadiazole–thioether compounds were synthesized, characterized and evaluated for their antifungal activity by using the natural product β-pinene as starting material. It was found by preliminary bioassay that, at the concentration of 50 μg/mL, some of the target compounds exhibited excellent antifungal activity against *P. piricola*, in which compound **5i** (R = *m*–F) held the highest inhibition rate of 88.9% against *P. piricola*, better than that of the commercial fungicide chlorothalonil (75%). Besides, compound **5 m**

(R = *p*–Cl) showed antifungal activity of 80.7% against *R. solani*. In order to design more effective antifungal molecules against *P. piricola*, 3D-QSAR analysis was performed by CoMFA method. A reasonable 3D-QSAR model ($q^2 = 0.597$; $r^2 = 0.985$) was established.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11164-021-04510-x.

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