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A value-added use of volatile turpentine: antifungal activity and QSAR study of *β*-pinene derivatives against three agriculture fungi

Jian Li,[&a] Xiangrong Tian,[&b] Yanqing Gao,[*b] Shibin Shang,[c] Juntao Feng,[b] and Xing Zhang[b]

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Abstract

In consideration of turpentine and its analogues possess some agricultural biological activity, persistent efforts to take advantage of renewable, abundant natural resource have been made. Three series of derivatives from *β*-pinene were synthesized and the fungicidal activities against *Rhizoctonia solani*, *Fusarium graminearum*, and *Botrytis cinerea* were investigated. Most of synthesized compounds exhibited moderate to significant fungicidal activity. Among them, acylthiourea derivatives from *β*-pinene showed more promising results than the other compounds. It was worth noting that compounds 7b and 7d displayed excellent fungicidal activity against *Rhizoctonia solani*, with values of IC_{50} 2.439 and 1.857 μg/mL, which was close to or even better than the control tiadimenol (1.945 μg/mL, a commercial fungicide). The structure-activity relationship (SAR) analysis indicated that the compounds with more net positive charge possessed better fungicidal activity. The quantitative structure -activity relationship (QSAR) model (R^2 = 0.9879, F = 348.41, S^2 = 0.0047) was obtained through the best multi-linear regression. The built model revealed a strong

correlation of fungicidal activity against *Rhizoctonia solani* with molecular features of the title compounds. Additionally, the SAR and QSAR studies showed that the introduction of the electron-withdrawing group, which can increase positive charge, was in favor of the fungicidal activity. These encouraging results may enlighten with an alternative, promising use of *β*-pinene as the design and exploration eco-friendly fungicides with low toxicity and high efficiency.

Keywords: *turpentine, β-pinene, fungicidal activity, QSAR*

1. Introduction

In the development of modern crop production, ensuring both of food safety and disease prevention is a key issue. In traditional agriculture, chemical fungicides were used to protect crops from plant diseases. However, the abuse of single synthesized fungicide has led to the development of resistance as well as increased side effects to environment and harmless creatures.[1-5] This is also one of the main reasons why efficient and eco-friendly fungicides need to be explored.

As a remarkable alternative mean to classic agrochemicals, botanical fungicides have played an increasingly important role in integrated and ecological disease management.[6] During the long-standing interaction between plants and environment, some secondary metabolites have been produced, which endow plants the ability to withstand adversity. Namely, plant secondary metabolites can protect plants from pest and diseases attacks. Currently, a range of secondary metabolites from natural sources such as avonoids, alkaloids and terpenoids have been developed as the lead compounds for the preparation of potent fungicides of less or slower resistance and

lower pollution.[7, 8]

Turpentine is a renewable and abundant natural resource commodity in China. As a secondary metabolite secreted from some species of pine, turpentine have been proposed to have potential activity.[9] As shown in **Scheme 1**, *β*-pinene is an important component of turpentine. Based on this promising reality, *β*-pinene can be used as lead active material in some fields, such as natural resource in pharmaceutical industry, insecticides and fungicide in crop protection, repellents in health care, polyterpine-resin in polymer materials, gum arabic food, and solvent in paint and varnish.[10, 11] *β*-pinene analogues have been reported to have broad-spectrum bioactivity, such as antibacterial,[12-15] antifeedant,[16-23] repellent,[24-27] and antifungal.[28] Among these analogues, cumic acid is a transformant of *β*-pinene in plants and it has good antimicrobial activity.[29] Encouragingly, the dehydrocumic acid, which almost has the same molecular structure as cumic acid, can be chemically synthesized from *β*-pinene.[30] It was expected to develop some eco-friendly fungicide of high efficiency and selectivity from dehydrocumic acid.

In order to obtain novel natural product-based fungicides, three series of derivatives from *β*-pinene were synthesized on the basis of molecular similarity. The fungicidal activities of the title compounds against three important agricultural pathogens *Rhizoctonia solani* (*R. solani*), *Fusarium graminearum* (*F. graminearum*) and *Botrytis cinerea* (*B. cinerea)* were investigated. Moreover, the QSAR was also performed on all of the title compounds using the Gaussian and CODESSA sofware packages, which can account for the structural features responsible for the fungicidal

activity. This exploration was expected to improve the added value of *β*-pinene as botanical fungicides in organic agriculture.

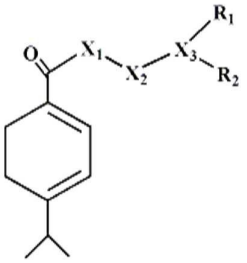
2. Experimental sections

2.1. Synthetic procedures and identification

β-pinene (1) was obtained from a commercial source (Wu Zhou Pine Chemicals Ltd., Guangxi, China) and used without further purification. All other chemicals used were of reagent grade. The Fourier transform infrared (FT-IR) spectra of dried compounds were measured on a Nicolet IS10 (Nicolet, Madison, USA) spectrophotometer, using a KBr pellets technique. The ^{1}H NMR spectra were recorded on a Bruker AV-300 (Bruker, Karlsruhe, Germany) nuclear magnetic resonance spectrometer with $CDCl_3$ or DMSO-d_6 as the solvent and TMS as an internal standard. The MS spectra were taken on an Agilent-5973 (Agilent, Santa Clara, USA) spectrophotometer. ESI mass spectral data were obtained on a Bruker Q-TOF mass spectrometer, equipped with electrospray ionization source, operated in positive ion mode. The elemental analysis (C, H, and N) was carried out on a Vario EL-III (Elementar, Hanau, Germany) elemental analyzer and the results were in good agreement with the calculated values. X-ray intensity data were collected on a Bruker/Enraf-Nonius CAD-4 (Bruker, Karlsruhe, Germany) diffractometer. Melting points were taken on XT-5 (Saiao, Beijing, China) and uncorrected. All reactions were traced by thin-layer chromatography (TLC) analysis, which was performed using Merck silica gel 60 GF254 plates, visualized under 254 nm UV light and eluted with a gradient of petroleum ether/acetone (v/v = 8:1). The preparation of title compounds was shown in

Scheme 2, and the substituted groups of *β*-pinene derivatives were shown in **Table 1.** The pathogenic fungi were provided by research & development center of biorational pesticide, Northwest A & F University.

Table 1 The substituted groups of *β*-pinene derivatives



Compd.	X_1	X_2	X_3	R_1	R_2	Compd.	X_1	X_2	X_3	R_1	R_2
5a	N	-	-	C_6H_5	-	**7f**	C=S	N	-	2,4- methylphenyl	-
5b	N	-	-	*o*-$CH_3C_6H_4$	-	**8a**	O	N	C	CH_3	funan-2-yl
5c	N	-	-	*m*-$CH_3C_6H_4$	-	**8b**	O	N	C	CH_3	thiophene-2-yl
5d	N	-	-	*p*-$CH_3C_6H_4$	-	**8c**	O	N	C	CH_3	4-ClC_6H_4
5e	N	-	-	2,4- methylphenyl	-	**8d**	O	N	C	CH_3	4-$CH_3C_6H_4$
5f	N	-	-	3,5- methylphenyl	-	**8e**	O	N	C	cyclohexyl	cyclohexyl
7a	C=S	N	-	C_6H_5	-	**8f**	O	N	C	H	pyridine
7b	C=S	N	-	benzyl	-	**8g**	O	N	C	CH_3	CH_3
7c	C=S	N	-	*o*-$CH_3C_6H_4$	-	**8h**	O	N	C	CH_3	CH_2CH_3
7d	C=S	N	-	*m*-$CH_3C_6H_4$	-	**8i**	O	N	C	H	C_6H_5
7e	C=S	N	-	*p*-$CH_3C_6H_4$	-	**8j**	O	N	C	CH_3	C_6H_5

2.1.1. Synthesis of 2-hydroxy-6, 6-dimethylbicyclo [3.1.1] heptane-2-carboxylic acid (2). *β*-pinene was oxidized by basic potassium permanganate in the homogeneous solution water/*tert*-butanol (v/v = 1:2). $KMnO_4$ (12.0 g, 0.076 mol) and NaOH (1.5 g, 0.038 mol) were dissolved in the mixture of water (100 mL) and *t*-BuOH (50 mL). While stirring vigorously, *β*-pinene (5.2 g, 0.038 mol) was added. The reaction was maintained at 15-25 °C for 0.5 h, and then was heated to 80 °C. After hot filtration, cooling, acidification, and vacuum drying, the crude compound was precipitated. [31] The target chemical 2 was obtained by recrystallization with

ethanol/toluene to give colorless crystals (0.30 mm × 0.20 mm × 0.10 mm in size) suitable for X-ray single crystal diffraction. The crystal structure was solved by direct methods and refined on F^2 using all data by full-matrix least squares procedures with SHELXS 97.

2.1.2. Synthesis of 4-isopropylcyclohexa-1, 3-dienecarboxylic acid (3). A solution of 40 g concentrated sulfuric acid in 100 mL water was added dropwise into a solution of compound 2 (10.0 g, 0.054 mol) at room temperature. Then the reaction was heated to 75 °C. After reacting for 6 h, the mixture was recrystallized with water/ethanol (v/v = 1:1) to give a white flaky solid 3.

2.1.3. Synthesis of 4-isopropylcyclohexa-1, 3-dienecarbonyl chloride (4). In a 250 mL flask with water-cooled condenser, thermometer, drying tube and dropping funnel, compound 3 (5.0 g, 0.027 mol) was dissolved in CH_2Cl_2 (50 mL). Thionyl chloride (0.082 mmol) was added dropwise within 1h and refluxed for 4 h at 65 °C. The chloride (4) was obtained after removing the solvent and excess thionyl chloride.

2.1.4. Synthesis of 4-isopropylcyclohexa-1, 3-dienecarboxamides (5a-f). A solution of the above chloride (4) in 10 mL of CH_2Cl_2 was added dropwise to a solution of 40 mmol of amine and 40 mmol of triethylamine in 40 mL of CH_2Cl_2 within 30 minutes at room temperature. After reacting for 12 h, the residue was dried with anhydrous $MgSO_4$ and purified by ethanol crystallization to give the six resulting compounds 5a-f.[13]

2.1.5. Synthesis of 4-isopropylcyclohexa-1, 3-dienecarboxthioureas (7a-f). A solution of KSCN (3.0 g, 0.03 mol) and anhydrous 100 mL CH_2CN was added to a

250 mL flask equipped with water-cooled condenser, thermometer, drying tube, and dropping funnel. After reacting for 24 h at 70 °C, the intermediate compound 6 was collected by filtration and used without further purification. Then the arylamine (0.03 mol) was added and the resulting mixture was refluxed for 2 h. The six resulting compounds 7a-f were obtained after drying with anhydrous $MgSO_4$ and purification by silica gel chromatography [ethyl acetate / petroleum ether (v/v = 1:10)].[32]

2.1.6. Synthesis of oximyl 4-isopropylcyclohexa-1, 3-dienecarboxylates (8a-j). A solution of dihydrocumyl chloride in 50 mL of CH_2Cl_2 was added dropwise to a solution of 0.030 mol of oxime and 0.040 mol of triethylamine in 40 mL of CH_2Cl_2 within 30 minutes at 15-25 °C. After that, the reaction mixture was kept at room temperature for 2 h and washed with distilled water. The residue was dried with anhydrous $MgSO_4$ and purified by silica gel chromatography [ethyl acetate/petroleum ether (v/v = 1:10)] to give the ten resulting compounds 8a-j.[30]

2.2. Biological assay

The fungicidal effect was determined by inhibition of mycelia radial growth. The test chemicals were dissolved in DMSO at various concentrations (2560 μg/mL, 1280 μg/mL, 640 μg/mL, 320 μg/mL, 160 μg/mL). The solutions were diluted into one tenth with Potato-Dextrose Agar (PDA) at 50 °C to give the required drug-containing medium at series of concentrations (256 μg/mL, 128 μg/mL, 64 μg/mL, 32 μg/mL, 16 μg/mL). Evaluations were based on a percentage scale of 0-100, where 0 = no activity and 100 = complete eradication. The standard deviations of the tested biological values were ± 5%. The IC_{50} values were calculated by probit analysis. For

comparative purposes, the commercial product thiadimenol was tested under the same conditions.

Petri dishes containing 10 mL of the drug-containing agar medium were inoculated by placing 6 mm fungus-coated discs upside down on the agar surface. The petri dishes containing 10 mL DMSO were set as the negative control. Plates were incubated at 30 °C and the mycelia radial growth was measured after three days. The inhibition rate was calculated by/(%) = $(T_{zone}-C_{zone})/T_{zone} \times 100$, where T_{zone} was the inhibition zone of test compounds, and C_{zone} was the inhibition zone of the negative control. The IC_{50} values of compounds were calculated with SPSS Statistic program version 17.0.[33]

2.3 Building and validation of the QSAR model

The general procedure of QSAR model building and validation was shown in **Fig. 1.** Firstly, the optimal conformers with the lowest energy of the title compounds were performed at the DFT/6-31G (d) level using a Gaussian 03W package of programs.[34] Secondly, the calculated results were changed into a form compatible with CODESSA 2.7.15 using Ampac 9.1.3. Thirdly, all the molecular descriptors involved in these compounds were calculated by CODESSA 2.7.15. In order to find out which structural features play an important role in fungicidal activity against *R. solani*, the best multiple regression analysis was selected to generate the QSAR model. In this model, the statistical criteria were indicated by the squared correction coefficient (R^2), the squared standard error of the estimates (S^2), and the Fisher significance ratio (F). The tested IC_{50} values were converted into the corresponding log IC_{50} and used as

dependent variables in the QSAR studies. The quality of the final model was determined using both an internal validation and the "leave-one-out" cross-validation methods.

3. Results and discussion

3.1. Synthesis

Polymerization reaction easily took place due to inappropriate temperature, time, and concentration of H_2SO_4 in the synthesis of compound 3. During the reaction process, too high temperature led to the appearance of yellow and globular material; too long reaction time led to the dark product. The white flake solid (compound 3) was obtained with the optimum reaction condition: 80 °C, 20% H_2SO_4, and 6 h. Infrared spectrum data showed that the absorption peak of hydroxyl (3100 cm^{-1}) disappeared, and carbon-carbon double bond (1572 cm^{-1}) appeared.

The oil-soluble acyl chloride and water-soluble KSCN could not react homogeneously. The anhydrous CH_2CN was chosen to make a homogeneous reaction. The intermediate isothiocyanate needed not to be purified and used directly in the synthesis of acylthiourea derivatives from *β*-pinene (7a-f).

The preparation of oximes played an important role in the synthesis of title compounds 8a-j. According to the literatures reported, the yield and compatibility of the reaction were affected by the solvent and base.[35] At present work, the ethanol and sodium carbonate were selected as the suitable solvent and base in preparing compounds (8a-j).

3.2. Crystal structure analysis

The target chemical 2 was obtained by recrystallization with ethanol/toluene to give colorless crystals suitable for X-ray single crystal diffraction, and the XRD peaks observed in the value of 2θ range of 10-13 °C, θ_{max} = 25.3 °C, θ_{min} = 1.6 °C. The crystallographic parameters was shown as: a = 26.796 (5) Å, b = 6.6560 (13) Å, c = 12.250 (3) Å, β = 112.23 (3)º, V = 2022.5 (9) Å^3, Z = 8, μ = 0.009 mm^{-1}, T = 293 K, R [$F^2 > 2\sigma(F^2)$] = 0.056, wR (F^2) = 0.153, S = 1.00, λ = 0.71073 Å, F_{000} = 800.

3.3. Fungicidal activity and structure-activity relationships (SAR)

3.3.1. Fungicidal activity of title compounds against *R. solani*. The results of fungicidal activity against *R. solani* were summarized in **Table 2**, from which it can be seen that the title compounds exhibited certain fungicidal activity against *R. solani*. The introduction of acyl thiourea group increased fungicidal activity against *R. solani* significantly. Compounds 7a-f exhibited about 90% inhibition rate against *R. solani* at 64 μg/mL. It was notable that the IC_{50} values of 7b and 7d were similar to or lower than thiadimenol, a commercialized fungicide. Compared with compound 3, all the derivatives had more significant fungicidal activity. There were several postulations about mechanisms elucidating the antimicrobial activity.[36] The most acceptable mechanism is the interaction between positively charged molecules and negatively charged microbial cell membrane. The compounds having protonated groups can generate electrostatic forces with the electrostatic negative charges on the microbial cell surfaces.[37] Based on this electrostatic interaction mechanism, the analogues with more positive charge would possess higher antimicrobial activity. The introduction of amide, acyl thiourea, and oxime groups into compound 3 increased its cationic centers

and its solubility in organic and aqueous media, which strengthened the net positive charge and leaded to a better fungicidal activity. In regard to different kinds of derivatives, the fungicidal activity level was acyl thiourea (7a-f) > oxime ester (8a-j) > amide (5a-f), which complied with above mechanism; for the same kind of derivatives, the activity results also met the mechanism well. Take oxime derivatives from *β*-pinene as example, compounds 8c, 8i, 8j, and 8d, with electron-withdrawing group has better activity than other compounds with electron-donating groups.

Table 2 Fungicidal activity of compounds against *R. solani*

		Fungicidal activity (%) at a concentration of (μg/mL)								
NO.	Compd.	256	128	64	32	16	IC_{50}	y=*a*x+*b*	R^2	log IC_{50}
1	**3**	100	67	40	20	0	92.712	y = 0.236 x - 2.722	0.977	1.967
2	**5a**	100	70	53	31	24	69.777	y = 0.175 x - 1.445	0.952	1.844
3	**5b**	100	70	50	30	23	72.485	y = 0.177 x - 1.528	0.971	1.860
4	**5c**	100	72	53	30	25	68.481	y = 0.177 x - 1.456	0.964	1.836
5	**5d**	100	70	50	30	23	72.485	y = 0.177 x - 1.528	0.971	1.860
6	**5e**	100	70	48	30	24	73.144	y = 0.177 x - 1.525	0.986	1.864
7	**5f**	100	72	54	33	25	66.345	y = 0.174 x - 1.382	0.961	1.822
8	**7a**	100	98	89	76	58	3.017	y = 0.235x - 0.102	0.990	0.480
9	**7b**	100	98	89	77	58	2.439	y = 0.236 x - 0.083	0.987	0.387
10	**7c**	100	97	87	73	57	3.284	y = 0.221 x - 0.099	0.990	0.516
11	**7d**	100	98	89	78	58	1.857	y = 0.237 x - 0.063	0.983	0.269
12	**7e**	100	97	87	73	57	3.284	y = 0.221 x - 0.099	0.990	0.516
13	**7f**	100	97	86	72	57	3.770	y = 0.218 x - 0.112	0.994	0.576
14	**8a**	100	95	75	53	33	32.604	y = 0.206 x - 1.089	0.991	1.513
15	**8b**	100	95	76	55	33	31.371	y = 0.207 x - 1.051	0.985	1.497
16	**8c**	100	95	80	61	33	27.470	y = 0.211 x - 0.948	0.957	1.439
17	**8d**	100	95	76	57	33	30.444	y = 0.206 x - 1.011	0.980	1.484
18	**8e**	100	93	70	50	30	36.840	y = 0.200 x - 1.155	0.984	1.567
19	**8f**	100	95	75	54	33	32.146	y = 0.206 x - 1.069	0.989	1.507
20	**8g**	100	95	74	52	32	33.902	y = 0.207 x - 1.138	0.992	1.530
21	**8h**	100	94	72	51	32	35.174	y = 0.201 x - 1.114	0.992	1.546
22	**8i**	100	95	79	60	32	28.749	y = 0.211 x - 0.995	0.960	1.459
23	**8j**	100	95	77	58	32	30.230	y = 0.208 x - 1.026	0.972	1.480
24	**Thiadimenol**	100	98	89	76	59	1.945	y = 0.235 x - 0.065	0.992	0.289

3.3.2. Fungicidal activity of title compounds against *F. graminearum*. The results

of fungicidal activity of the title compounds against *F. graminearum* were listed in **Table 3**, from which it was shown that compounds 3, 5a-f, 7a-f and 8a-j exhibited low fungicidal activity against *F. graminearum*. The death rate of most compounds was about 30-40% at 256 μg/mL, which was less effective than fungicidal activity against *R. solani*.

Table 3 Fungicidal activity of compounds against *F. graminearum*

Compd.	Fungicidal activity (%) at a concentration of (μg/mL)			Compd.	Fungicidal activity (%) at a concentration of (μg/mL)		
	256	128	64		256	128	64
3	20	0	-	**7f**	43	20	0
5a	30	10	0	**8a**	38	20	0
5b	30	10	0	**8b**	40	20	0
5c	30	10	0	**8c**	40	20	0
5d	30	10	0	**8d**	40	20	0
5e	30	10	0	**8e**	40	20	0
5f	30	10	0	**8f**	40	20	0
7a	44	20	0	**8g**	37	20	0
7b	45	20	0	**8h**	36	20	0
7c	43	20	0	**8i**	40	20	0
7d	45	20	0	**8j**	40	20	0
7e	30	20	0	**Thiadimenol**	100	100	89

3.3.3. Fungicidal activity of title compounds against *B. cinerea*. The results of fungicidal activity of the title compounds against *B. cinerea* were listed in **Table 4**, from which it can be seen that all of the test compounds exhibited low fungicidal activity against *B. cinerea*. These compounds have lowest fungicidal activity against *B. cinerea*, and the death rate was about 20-30% at 256 μg/mL.

Table 4 Fungicidal activity of compounds against *B. cinerea*

Compd.	Fungicidal activity (%) at a concentration of (μg/mL)			Compd.	Fungicidal activity (%) at a concentration of (μg/mL)		
	256	128	64		256	128	64
3	10	0	-	**7f**	33	20	0
5a	20	10	0	**8a**	38	10	0
5b	20	10	0	**8b**	30	10	0
5c	20	10	0	**8c**	30	10	0
5d	20	10	0	**8d**	30	10	0
5e	20	10	0	**8e**	30	10	0
5f	20	10	0	**8f**	30	10	0
7a	34	20	0	**8g**	27	10	0
7b	35	20	0	**8h**	26	10	0
7c	33	20	0	**8i**	30	10	0
7d	35	20	0	**8j**	30	10	0
7e	30	20	0	**Thiadimenol**	100	100	89

3.4 QSAR study on fungicidal activity against *R. solani*

In QSAR study, the selection of structure descriptors was an important step to determine molecular features correlating the activity. The descriptors can be categorized as constitutional, topological, geometrical, electrostatic, quantum chemical, and thermodynamic. In this research, the most important factors were geometry and charge distribution, which involved some descriptors, such as HOMO energy (the energy of the highest occupied molecular orbital), LUMO energy (the energy of the lowest occupied molecular orbital), dipole moment, etc. Many regression approaches, such as best multi-linear, multi-linear regression, principal component analysis, partial least square regression, and heuristic regression can be available in CODESSA 2.7.15 software. In view of number of samples and descriptors involved in this work, the best multi-linear regression was chosen.

To determine the number of descriptors is an another important step. A simple

rule called “breaking point” was used in the improvement of the statistical quality of the model. As shown in **Fig. 2**, the R^2 value of the best multi-linear regression had a dramatic increase before the number of the descriptors was up to 4. The significance of the descriptors within the model was reflected in the *t*-test. Descriptors with high *t* values were accepted and those with low *t* values were rejected. After the number of the descriptors reached a certain value, the improvement of the regression model became less insignificant ($\Delta R^2 < 0.02\text{-}0.04$). Furthermore, another point worth noting was that the number of the descriptors conformed to the linear regression given by Eq. (1)

$$N \geq 3\,(k + 1) \qquad (1)$$

Where N is the number of samples and k is the number of descriptors. Consequently, the final QSAR model with four descriptors was selected as the best model. The values of four descriptors of sample compounds were listed in **Table 5.**

The best statistical model for the log IC_{50} data had the following statistical characteristic: $R^2 = 0.9879$, $F = 348.41$, $S^2 = 0.0047$. This model included four descriptors in descending order according to their statistical significance, which were shown in **Table 6**, and the regression coefficients X and their standard errors ΔX were also listed.

The values of experimental and predicted log IC_{50} were listed in **Table 7**, and the plot of the comparision between the predicted and experimental values was shown in **Fig. 3.** The four-descriptor QSAR model equation was described in the following Eq. (2)

$$\log IC_{50} = - 8.3866 - 39.598 \times HOMO - 19.346 \times DM + 8.8104 \times q^{O}_{max} + 30.670 \times q_{min} \quad (2)$$

$$N = 22,\ R^2 = 0.9879,\ F = 348.41,\ S^2 = 0.0047$$

Table 5 Fungicidal activity and structural descriptors of the title compounds

No.	Compd.	log IC_{50}	Structure descriptors			
			HOMO	DM	q^{O}_{max}	q_{min}
1	**5a**	1.844	-0.3512	-0.2067	0.3524	-0.3512
2	**5b**	1.860	-0.3534	-0.2067	0.3523	-0.3534
3	**5c**	1.836	-0.3523	-0.2067	0.3524	-0.3523
4	**5d**	1.860	-0.3525	-0.2067	0.3520	-0.3525
5	**5e**	1.864	-0.3546	-0.2066	0.3519	-0.3546
6	**5f**	1.822	-0.3534	-0.2067	0.3521	-0.3534
7	**7a**	0.480	-0.2713	-0.2072	0.2748	-0.2713
8	**7b**	0.387	-0.2710	-0.2070	0.2522	-0.2768
9	**7c**	0.516	-0.2725	-0.2072	0.2745	-0.2725
10	**7d**	0.269	-0.2718	-0.2072	0.2748	-0.2718
11	**7e**	0.516	-0.2718	-0.2072	0.2749	-0.2718
12	**7f**	0.576	-0.2731	-0.2072	0.2746	-0.2731
13	**8a**	1.513	-0.2896	-0.2095	0.3705	-0.2896
14	**8b**	1.497	-0.2917	-0.4339	0.3685	-0.4339
15	**8c**	1.439	-0.2922	-0.2071	0.3706	-0.2922
16	**8d**	1.484	-0.2950	-0.2070	0.3696	-0.2950
17	**8e**	1.567	-0.2963	-0.2070	0.3697	-0.2963
18	**8f**	1.507	-0.2873	-0.2073	0.3706	-0.2873
19	**8g**	1.530	-0.2945	-0.2086	0.3698	-0.2945
20	**8h**	1.546	-0.2970	-0.2071	0.3691	-0.2970
21	**8i**	1.459	-0.2907	-0.2072	0.3697	-0.2907
22	**8j**	1.480	-0.2940	-0.2071	0.3697	-0.2940

Table 6 The best four-descriptor model

Descriptor No.	*X*	±Δ*X*	*t*-Test	Descriptor
0	-8.3866	1.5216	-5.5119	Intercept
1	-3.9598×10^{1}	1.1968×10^{1}	-3.3086	HOMO[a]
2	-1.9346×10^{1}	7.4859	-2.5843	DM[b]
3	8.8104	4.5244×10^{-1}	19.4733	q^{O}_{max}[c]
4	3.0670×10^{1}	1.1916×10^{1}	2.5738	q_{min}[d]

[a] Energy of the highest occupied molecular orbit in atomatic units. [b] Dipole moment. [c] Max net atomic charge for a O atom. [d] Min net atomic charge.

Table 7 The difference between the experimental log IC_{50} and predicted log IC_{50}

NO.	Compd.	Calc. log IC_{50}	Exp. log IC_{50}	Difference	NO.	Compd.	Calc. log IC_{50}	Exp. log IC_{50}	Difference
1	**5a**	1.8522	1.9670	-0.1148	**12**	**7f**	0.4792	0.4792	0.5760
2	**5b**	1.8710	1.8480	0.0270	**13**	**8a**	1.5159	1.5159	1.5130
3	**5c**	1.8620	1.8600	0.0020	**14**	**8b**	1.4970	1.4970	1.4970
4	**5d**	1.8603	1.8360	0.0243	**15**	**8c**	1.4936	1.4936	1.4390
5	**5e**	1.8762	1.8640	0.0122	**16**	**8d**	1.5078	1.5078	1.4840
6	**5f**	1.8692	1.8220	0.0472	**17**	**8e**	1.5203	1.5203	1.5670
7	**7a**	0.4649	0.4800	-0.0151	**18**	**8f**	1.4537	1.4537	1.5070
8	**7b**	0.3879	0.3870	0.0009	**19**	**8g**	1.5361	1.5361	1.5300
9	**7c**	0.4730	0.5160	-0.0430	**20**	**8h**	1.5232	1.5232	1.5460
10	**7d**	0.4694	0.2690	0.2004	**21**	**8i**	1.4742	1.4742	1.4590
11	**7e**	0.4702	0.5160	-0.0458	**22**	**8j**	1.5017	1.5017	1.4800

The developed QSAR model was validated by both internal validation and "leave-one-out" cross-validation methods. In the internal validation, the compounds were divided into three subsets A, B, and C. The compounds **1, 4, 7, 10,** etc., belonged to subset A; **2, 5, 8, 11**, etc., belonged to subset B; and **3, 6, 9, 12,** etc., belonged to subset C. Two subsets, (A and B), (B and C), or (A and C) were selected as the training set, and the remaining subset was treated as the test set. The correlation equation, obtained from each of the training sets using the same descriptors, was used to predicted values of the corresponding test sets. Internal validation results were listed in **Table 8.**

Table 8 Internal validation of the QSAR model

Training set	*N*	R^2	*F*	S^2	Test set	*N*	R^2	*F*	S^2
A+B	15	0.9800	340.78	0.0043	C	7	0.9843	345.90	0.0045
B+C	14	0.9711	339.58	0.0041	A	8	0.9814	343.25	0.0044
A+C	15	0.9724	339.63	0.0050	B	7	0.9793	341.47	0.0043
Average		0.9745	340.00	0.0045	Average		0.9817	343.54	0.0044

[a] Compounds A: **1, 4, 7, 10, 13, 16, 19, 22,** Compounds B: **2, 5, 8, 11, 14, 17, 20,** Compounds C: **3, 6, 9, 12, 15, 18, 21.**

The difference between $R_{Training}^2$ and R_{Test}^2 were within 5% for the three sets, and the average values of $R_{Training}^2$ = 0.9745 and R_{Test}^2 = 0.9817 were very similar to the integrated R^2 value, which signified the obtained model possessed the predictive power of three-fold cross-validation. In a similar way to the internal validation, the "leave-one-out" method can be implemented. Every fourth compounds **1, 5, 9, 13, 17, 21** were put into an external test set, and the remaining compounds were left in the training set. With the same four descriptors, the R^2 of training set was 0.9640, and the R^2 of the test set was 0.9533, which also indicated that the obtained QSAR model was of satisfactory.

Some structural features could make a difference to fungicidal activity by interpreting the descriptors involved in the QSAR model. The first important descriptor was the HOMO energy, which is directly related to the ionization potential of the compounds.[38, 39] In **Fig. 4**, the HOMO energy maps for compounds 7b and 7d were shown. In the Eq. (2), the HOMO energy and activity was negatively correlated, which suggested that the electron withdrawing substitution groups of derivatives are beneficial for the fungicidal activity against *R. solani*. The consequence obtained from QSAR study partially met the above SAR study result.

The second important descriptor was dipole moment, which is a measure of the polarization between the positive and negative electrical charges in a system.[40, 41] The C=O, C=S, and N-H group exhibited permanent polarization because of a significant electronegative difference between atoms. Among the title compounds, acylthiourea derivatives from *β*-pinene displayed the better fungicidal activity, which illustrated the

dipole moment played a critical role in modulating the activity.

The 3rd and 4th important descriptors were max net atomic charge for an O atom and min net atomic charge. Both of these two descriptors can give an expression on the features of the charge distribution in the molecules. In Eq. (2), appearance with a positive sign in the model indicated that molecule with higher descriptor value had a higher log IC_{50}. In contrary, a negative sign in the model indicated that molecule with lower descriptor value had a higher log IC_{50}.

In summary, both the SAR and QSAR studies indicated that the electron withdrawing substitution groups of β-pinene derivatives had positive effect for the fungicidal activity. In the light of these results, the further research on the correlation of SAR and QSAR is expected to carry on, and the design and exploration of potential efficient fungicides would be implemented.

4. Conclusions

In purpose of increasing added value of turpentine，three series of β-pinene analogues were prepared and their fungicidal activity against three common agricultural fungi were determined. The acylthiourea derivatives from β-pinene 7a-f displayed more significant activity than the other derivatives. It was worth noting that compounds 7b and 7d exhibited excellent activity. Both the SAR and QSAR studies indicated the structural features had apparent influence on the fungicidal activity. From these satisfying consequences, some insight into the exploitation of eco-friendly fungicides from natural products with low toxicity and high efficiency can be gained.

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Notes and references

[a] *College of Forestry, Northwest Agriculture and Forestry University, Yangling, Shaanxi 712100, People's Republic of China.*

[b] *Research & Development Center of Biorational Pesticide, College of Plant Protection, Northwest Agriculture and Forestry University, Yangling, Shaanxi 712100, People's Republic of China.*

[c] *Institute of Chemical Industry of Forest Products, Chinese Academy of Forestry, Nanjing, Jiangsu 210042, People's Republic of China.*

[&]The authors contributed equally to this work and should be considered co-first authors.

[*]Corresponding author: Telephone: +86-29-87091977. Fax: +86-29-87082392. E-mail: gaoyanqinggc@nwsuaf.edu.cn.

† Electronic Supplementary Information (ESI) available: IR, ^{1}H NMR, MS, and elemental analysis data for the target compounds. See DOI:10.1039/b000000x/

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Figure captions

Scheme 1 Chemical structures of major constitutes of turpentine

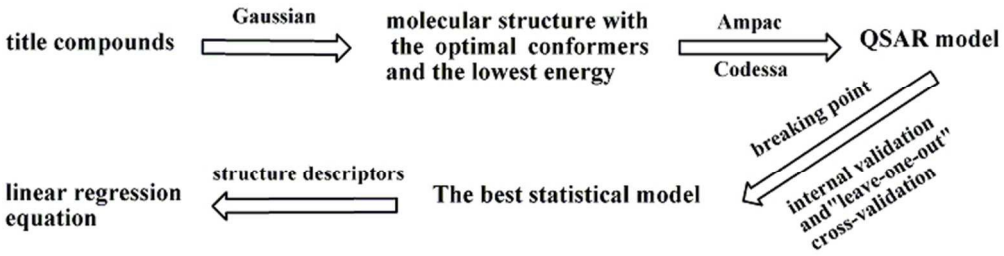
Scheme 2 Preparation of title compounds

Fig. 1 The general procedure of QSAR model building and validation

Fig. 2 The "breaking point" rule results

Fig. 3 Experimental log IC_{50} versus predicted log IC_{50}

Fig. 4 Optimized structures and HOMO energy maps for compounds 7b and 7d from DFT calculation of Gaussian 03W. The green parts represent positive molecular orbitals, and the red parts represent negative molecular orbitals



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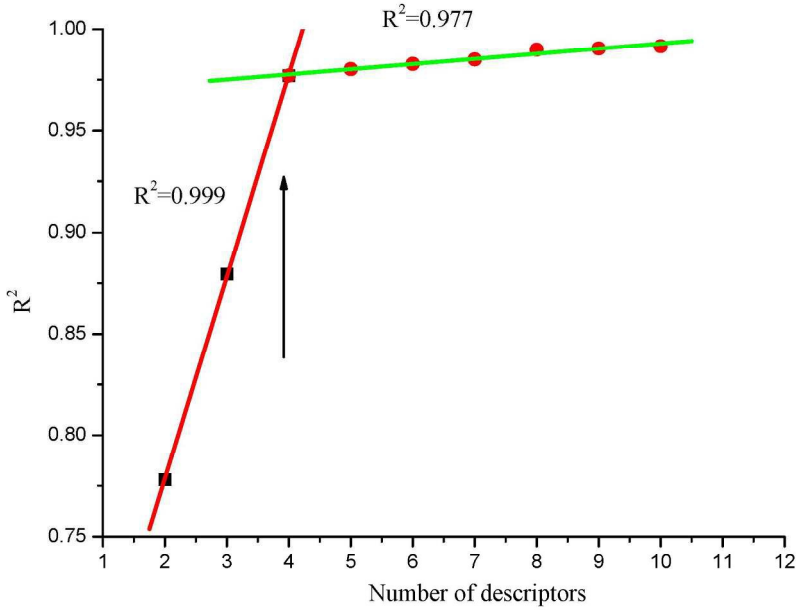

The breaking point" rule results
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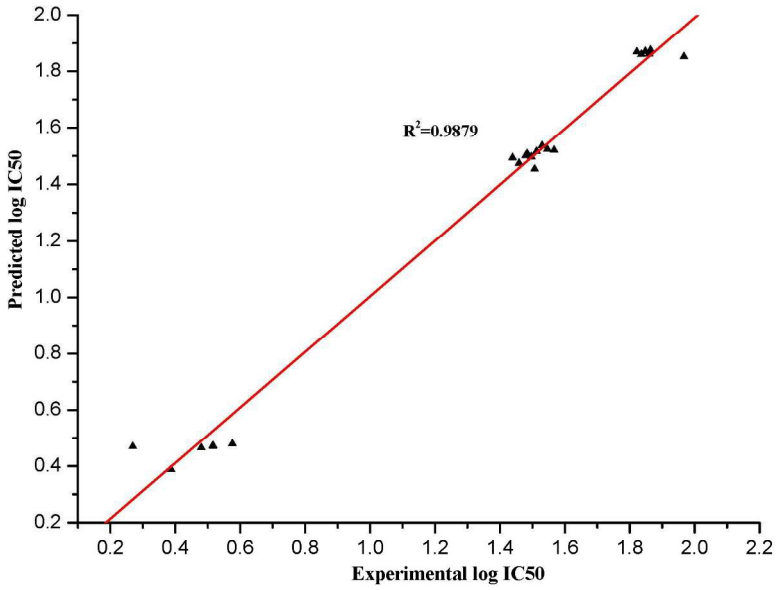

Experimental log IC50 versus predicted log IC50
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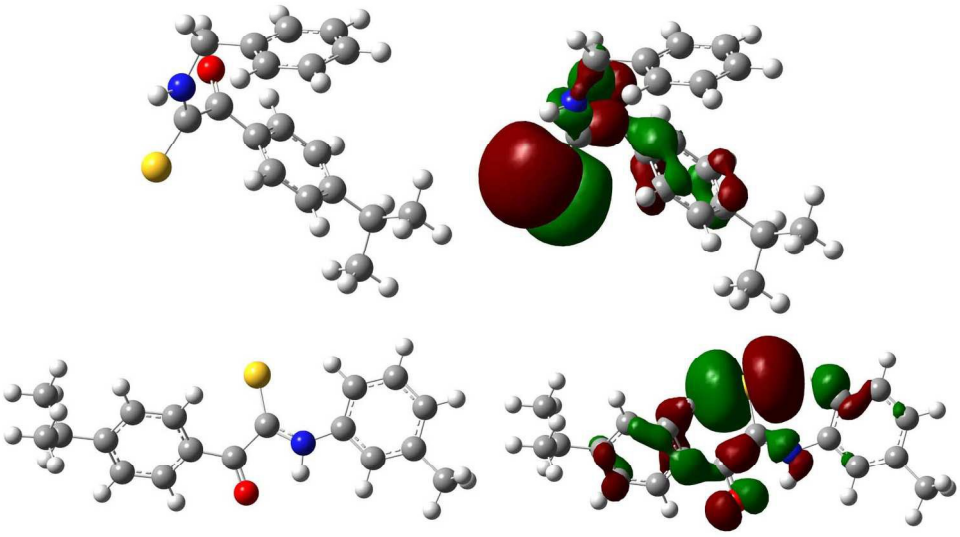
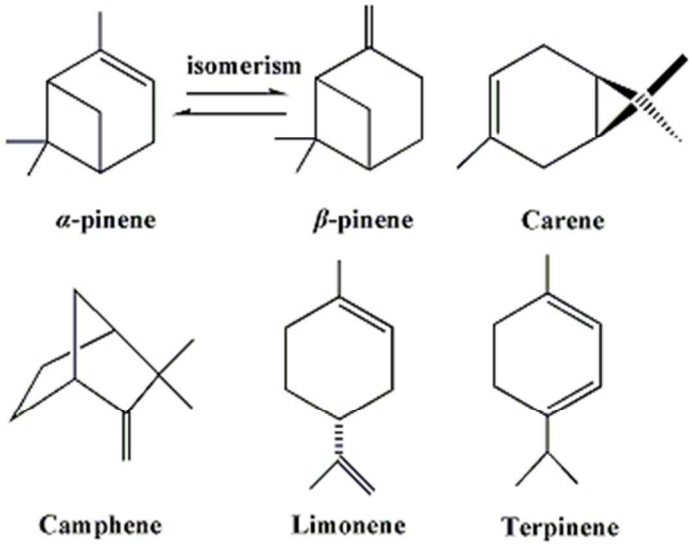


Optimized structures and HOMO energy maps for compounds 7b and 7d from DFT calculation of Gaussian 03W. The green parts represent positive molecular orbitals, and the red parts represent negative molecular orbitals
254x190mm (220 x 220 DPI)



Chemical structures of major constitutes of turpentine
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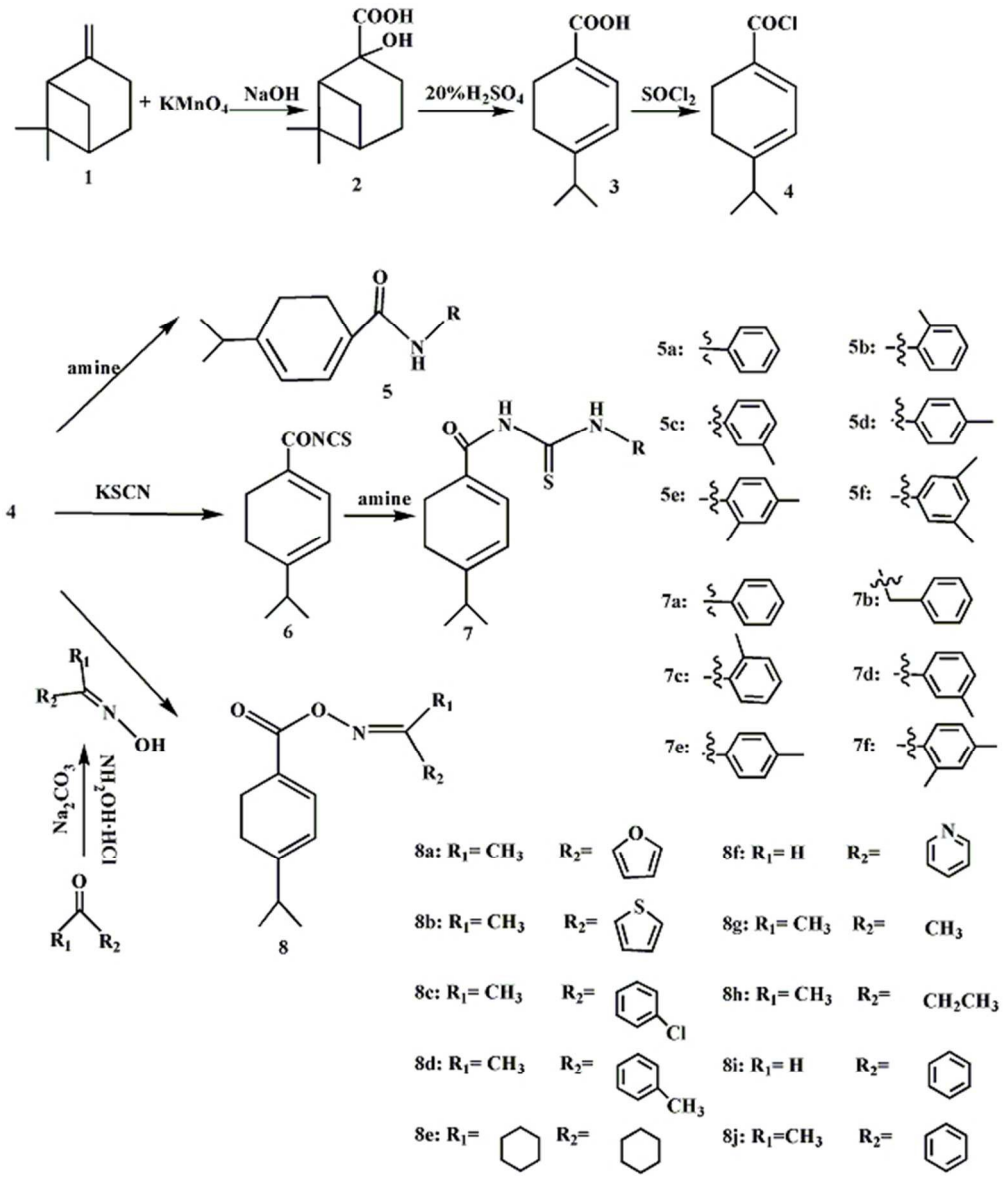

Preparation of title compounds
174x204mm (96 x 96 DPI)