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SYNTHESIS AND *IN VITRO* ANTIFUNGAL ACTIVITIES OF *α, β*-UNSATURATED KETONES AS ANALOGUES OF KAKUOL

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Abstract – As a part of our ongoing program for the development of analogues of kakuol drugs, 27 *α, β*-unsaturated ketones compounds with a terminal C=C bond, were synthesized and characterized by spectroscopic analysis. Their antifungal activity was evaluated at the concentration of 50 *μ*g/mL against seven plant pathogenic fungi, and their structure-activity relationships (SAR) were also discussed. Especially compounds **9c**, **9g**, **10a** exhibited more potent antifungal activity against *A. solani* than that of thiabendazole (a positive control). SAR analysis demonstrated that the conjugated terminal C=C bond is necessary for improvement of the activity.

INTRODUCTION

Fungal plant diseases are one of the important concerns to agricultural production and food safety worldwide.[1] Phytopathogenic fungi are able to infect any tissue at any stage of plant growth, and result in severe yield losses and the quality decrease of agricultural products.[2] More severely, because of the increasing demand for higher quantity and quality of food, various plant fungicides are extensively used in current agriculture with the result that many of the currently used antifungal agents have the development of pathogen resistance.[3] Therefore, innovation in crop protection is essential for sustainability agriculture and global food protection.[4] Despite the availability of effective fungicides,[4] new antifungal chemicals are still needed to maintain ideal yields of crops and quality benefits, and to combat pathogens which show resistance or reduced sensitivity to existing antifungal compounds.

In 2005, Hwang and co-workers isolated kakuol (**Figure 1**) from the rhizomes of *Asarum sieboldii*,[5] a perennial herb belonging to the family of Aristolochiaceae, and demonstrated for the first time its antifungal activity against some important plant pathogens such as *Colletotrichum orbiculare*, *Rhizoctonia solani*, *Botrytis cinerea* and *Phytophthora capsici*.[4] Besides, some data related to antifungal activity of kakuol and methylkakuol have been reported in recent research.[4,6] Additionally, some acrylic ketone analogues also exhibited remarkable antifungal activity.[4] These research strongly indicated that the plant sourced kakuol possessed great potential to develop into novel antifungal drugs.

The above findings encouraged us to further extend the modification of kakuol analogues due to its structurally simple, chemically modifiable and biologically active nature, to find more potent antifungal agents. Thus, we designed and synthesized a series of kakuol derivatives containing an acrylic ketone moiety in our previous research.[7] However, these previous synthetic kakuol analogues (**Figure 2**, **a**) which possessed the structure bearing a C=C bond conjugated to the C=O group failed to exhibit antifungal activity.[7] This results made us realize that this might not be the most optimized structure, and some structural modifications should be made.

On the base of our previous work and SAR of other kakuol analogues, in the present research, we redesigned the structure of our target compounds, namely we adjusted the position of the C=C bond in the previous structure, placing it to the terminal and still conjugated to C=O bond. Our newly designed target coumpounds were a class of new 1-aryl acrylic ketones (**Figure 2**, **b**), and their antifungal activity against phytopathogenic fungi were assayed after synthetic work. So far, no systematic research on the synthesis, antifungal activity and SAR of these compounds was found.

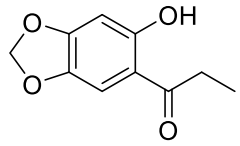


Figure 1. Chemical structure of kakuol

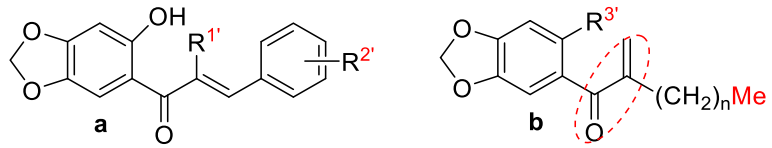
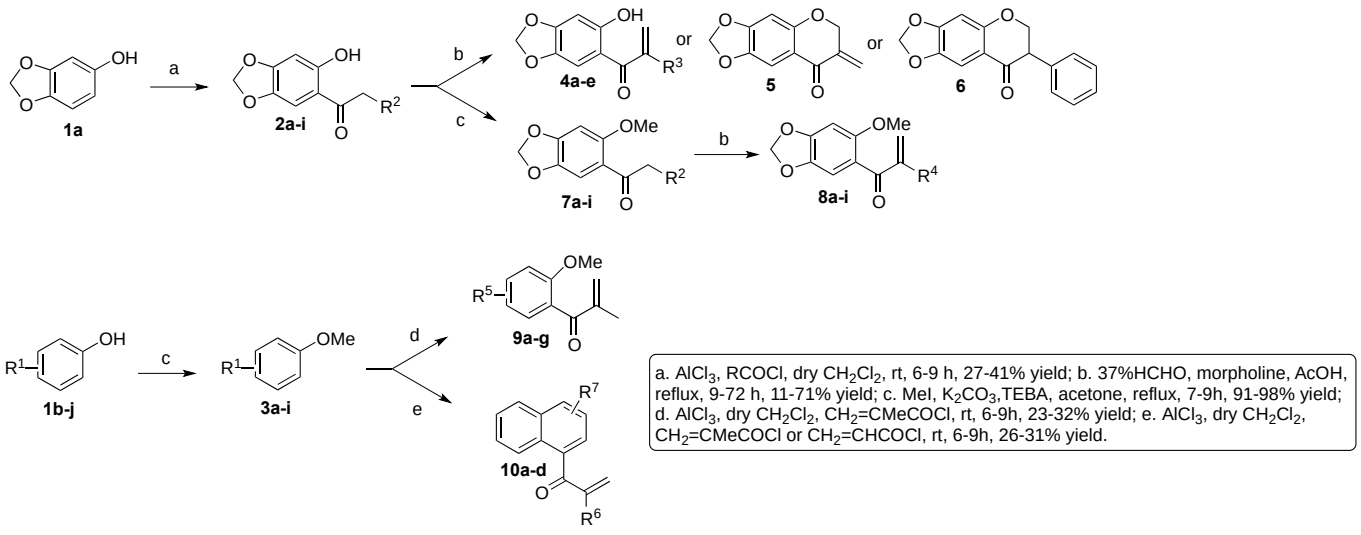


Figure 2. Chemical structure of previous synthetic analogues of kakuol (**a**) and current *α,β*-unsaturated ketones (**b**)

RESULTS AND DISCUSSION

The synthetic route of target compounds **4a**–**4e**, **5**, **6**, **8a**–**8i**, **9a**–**9g**, and **10a**–**10d** was outlined in **Scheme 1**. Commercially available sesamol (**1a**) dissolved in dichloromethane was used as a starting material to react with appropriate acyl chloride in the presence of anhydrous aluminum chloride to prepare compounds **2a-2i** in 27–41% yield. Because the yield was relatively low, we changed the way of reaction, and other commercially available phenols (**1b**–**1j**) were firstly reacted with methyl iodide in acetone under reflux to obtain compounds **3a**–**3i** in 91–98% yield. Next, the compounds **3a**–**3i** were reacted with

methylpropenoyl chloride or acryloyl chloride with the same reaction conditions as sesamol (**1a**) to obtain the desired target compounds **9a**–**9g**, and **10a**–**10d** in 23–32% yield. On the other hand, compounds **2a**–**2e** and **2i** dissolved in glacial acetic acid were reacted with 37% formaldehyde aqueous solution in the presence of morpholine (reaction catalyst) under reflux to afford target compounds **4a**–**4e**, **5** and **6** in 11–29% yield. It is also worth mentioning that when **2a** was used as a substrate, except for the desired target compound **4a**, we also obtained a unexpected cyclization product **5**. The above results showed that compound **2a** underwent intramolecular conjugate addition after initial α-methylenation to form 6,7-methylenedioxy-4-chromanone, followed by a second α-methylenation to afford adduct **5** under the current reaction conditions. And a similar cyclization reaction was also observed for reactant **2i** to yield compound **6**. At the same time, compounds **2a**–**2i** were also reacted with methyl iodide to obtain compounds **7a**–**7i** in 94–98% yield with the same reaction conditions as synthesis of compounds **3a–3i**. Finally, compounds **7a**–**7i** was treated with 37% formaldehyde aqueous solution to obtain the desired target compounds **8a**–**8i** in 46–71% yield with the same reaction conditions as synthesis of **4a**–**4e**. More interestingly, in the above reaction, compound **7a** was not only reacted with formaldehyde aqueous solution, but also treated with glacial acetic acid to afford the unexpected ester compound **8a**.



1b: R^1 = 4-Me;	2a: R^2 = H;	3a: R^1 = 4-Me;	4a: R^3 = H;	7e: R^2 = *n*-Bu;	8e: R^4 = *n*-Bu;	9e: R^5 = 3,5-diMe;
1c: R^1 = 4-Et;	2b: R^2 = Me;	3b: R^1 = 4-Et;	4b: R^3 = Me;	7f: R^2 = *n*-Pe;	8f: R^4 = *n*-Pe;	9f: R^5 = 4,5-diMe;
1d: R^1 = 4-*i*-Pr;	2c: R^2 = Et;	3c: R^1 = 4-*i*-Pr;	4c: R^3 = Et;	7g: R^2 = *n*-Hex;	8g: R^4 = *n*-Hex;	9g: R^5 = 4-Me-5-Cl;
1e: R^1 = 4-Cl;	2d: R^2 = *n*-Pr;	3d: R^1 = 4-Cl;	4d: R^3 = *n*-Pr;	7h: R^2 = *n*-Hep;	8h: R^4 = *n*-Hep;	10a: R^6 = Me, R^7 = 2-OMe;
1f: R^1 = 2,4-diMe;	2e: R^2 = *n*-Bu;	3e: R^1 = 2,4-diMe;	4e: R^3 = *n*-Bu;	7i: R^2 = Ph;	8i: R^4 = Ph;	10b: R^6 = H, R^7 = 2-OMe;
1g: R^1 = 3,4-diMe;	2f: R^2 = *n*-Pe;	3f: R^1 = 3,4-diMe;	7a: R^2 = H;	8a: R^4 = CH_2OAc;	9a: R^5 = 5-Me;	10c: R^6 = Me, R^7 = 4-OMe;
1h: R^1 = 3-Me-4-Cl;	2g: R^2 = *n*-Hex;	3g: R^1 = 3-Me-4-Cl;	7b: R^2 = Me;	8b: R^4 = Me;	9b: R^5 = 5-Et;	10d: R^6 = H, R^7 = 4-OMe.
1i: R^1 = 2,3-benzo;	2h: R^2 = *n*-Hep;	3h: R^1 = 2,3-benzo;	7c: R^2 = Et;	8c: R^4 = Et;	9c: R^5 = 5-*i*-Pr;	
1j: R^1 = 3,4-benzo;	2i: R^2 = Ph;	3i: R^1 = 3,4-benzo;	7d: R^2 = *n*-Pr;	8d: R^4 = *n*-Pr;	9d: R^5 = 5-Cl;	

Scheme 1. The synthetic route for preparation of **4a**–**e**, **5**, **6**, **8a**–**i**, **9a**–**g**, and **10a**–**d**

Antifungal Activity According to reported mycelium linear growth rate method,[8-11] the *in vitro* antifungal activities against seven plant pathogenic fungi (*A. solani*, *M. grisea*, *A. alternate*, *C. lunata*, *F. graminearum*, *F. solani*, *F. oxysporum*) of synthetic target compounds **4a**–**4e**, **5**, **6**, **8a**–**8i**, **9a**–**9g**, and **10a**–**10d** at the concentration of 50 μg/mL were assayed. Thiabendazole (≥99.1%), a commercial fungicides, was used as a positive control. Compound **4a** was regarded as the reference compound, whose antifungal activity was also assayed by the same method. The preliminary antifungal activity of each of target compounds against seven phytopathogenic fungi was summarized in **Table 1**.

Table 1. The preliminary antifungal activity of target compounds against seven tested fungi at 50 μg/mL (72 h)

compd.	Average inhibition rate ± S.D.(%) (n=3)						
No.	*M. g*	*A. s*	*C. l*	*F. s*	*A. a*	*F. o*	*F. g*
4a	40.3±3.5n	39.9±2.5k	18.0±2.3w	30.5±3.2qr	19.2±0.7s	41.0±1.2ij	39.1±1.1q
4b	54.6±2.4jk	-10.2±3.2r	62.6±1.6i	58.8±1.0ij	55.3±0.3hi	24.8±1.3n	31.2±0.8r
4c	41.5±2.2n	51.0±1.5i	62.1±0.2i	55.1±1.7k	46.1±1.6m	40.4±1.0ij	54.8±1.6n
4d	52.5±2.8jk	52.6±2.6i	56.7±1.1k	49.4±1.3mn	64.1±0.3f	46.6±2.1h	47.6±1.7p
4e	42.6±2.6mn	32.2±1.7l	51.5±1.3lm	46.5±1.2no	42.9±1.0n	41.2±0.8ij	61.6±1.3l
5	45.8±0.6lm	43.4±2.4jk	47.8±1.7no	66.4±1.2ef	51.2±1.3jk	45.6±2.0h	60.3±0.8l
6	11.5±0.2q	31.6±1.5l	34.1±2.2u	18.2±2.5t	14.3±1.5t	-3.7±0.1r	4.8±0.1u
8a	36.3±0.7o	45.5±3.7j	49.5±0.4mn	32.8±2.0pq	40.1±1.0op	30.2±1.3lm	36.2±1.8q
8b	61.8±0.8efgh	60.5±1.9h	59.7±1.7jk	75.2±1.5d	69.9±1.3d	**92.7±0.2b**	**88.2±1.2b**
8c	53.4±1.1k	68.4±1.5g	46.2±0.4op	58.4±1.8j	66.7±0.6ef	74.4±0.5c	79.7±1.4d
8d	60.7±0.6fgh	7.9±0.4p	56.6±1.5k	61.7±1.8hi	60.7±1.6g	46.3±1.9h	73.2±1.5gh
8e	62.6±1.1efg	31.6±1.5l	53.8±2.0l	60.7±1.7hij	66.1±0.6f	45.1±1.1h	71.6±1.6hi
8f	59.5±1.7ghi	47.4±2.2ij	41.5±0.8rs	53.3±2.3kl	53.3±0.8hi	-15.8±1.8s	65.2±1.1k
8g	56.5±0.6ijk	-7.9±0.4qr	50.6±2.4mn	43.9±1.8o	48.2±0.9lm	-29.3±0.6u	52.9±1.3no
8h	47.3±0.7l	46.0±0.2j	36.2±2.5tu	32.2±1.4pq	41.1±0.7no	-51.2±1.1v	50.8±1.4o
8i	49.7±1.9l	43.8±2.7jk	63.5±0.8i	51.5±2.4lm	53.7±2.0ij	35.7±1.2k	47.2±1.0p
9a	62.1±1.3efg	51.2±2.4i	**80.0±0.8ab**	74.6±0.3d	68.9±2.0de	58.8±1.0f	76.5±1.8ef
9b	71.1±1.0bc	**83.7±0.9b**	66.4±1.2h	75.9±1.4d	64.1±1.3f	57.1±0.9f	78.3±1.9de
9c	69.1±1.6cd	**86.1±0.8b**	74.9±0.4de	79.9±0.9c	69.4±2.7d	61.5±1.9e	**85.1±0.4c**
9d	60.5±2.2fgh	51.0±2.5i	34.5±2.1u	48.9±1.1mn	46.6±1.6m	31.7±1.3l	78.6±0.8de
9e	57.9±0.2hij	79.3±2.3c	37.0±2.6tu	49.3±0.6mn	49.5±1.8kl	23.3±2.0n	74.8±1.7fg
9f	65.7±3.0de	78.8±1.8cd	70.2±0.6g	63.5±0.8fgh	61.1±2.5g	41.9±1.6i	71.0±1.3hij
9g	73.1±2.6b	**86.5±0.7b**	73.2±1.0ef	65.7±2.6efg	77.2±2.5b	36.0±2.1k	74.5±0.5fg
10a	74.6±4.0b	**85.9±3.4b**	78.4±1.4bc	67.1±1.6e	73.1±1.7c	69.1±1.7d	**90.4±1.4b**
10b	55.1±2.9jk	57.1±2.6h	31.0±1.6v	34.9±0.8p	37.8±1.1pq	29.3±1.3m	52.0±2.4o

10c	53.9±1.6jk	58.8±2.7h	59.2±0.4jk	52.4±1.1kl	49.2±2.1kl	28.2±1.2m	51.2±2.1o
10d	47.4±1.6l	27.0±3.2m	42.3±2.7qr	25.0±1.6s	35.7±1.3q	16.6±1.4o	11.2±1.6t
TBZ	36.1±0.7o	75.2±1.9de	44.5±0.5pq	92.6±0.6b	42.9±1.0n	100±0.0a	97.9±0.3a

TBZ =Thiabendazole. The differences between data with the different lowercase letters within a column are significant for the tested fungus (p<0.05), which were carried out by Duncan's multiple comparison.

It was clearly seen from **Table 1** that almost all the target compounds except compound **6** displayed obvious activities against each fungus than that of the previous synthetic analogues of kakuol and reference compound **4a** at 50 μg/mL. Obviously, compound **8b** exhibited the highest activity in all the tested compounds against *F. oxysporum* with the inhibition rate of 92.7%. Especially, compounds **9b**, **9c**, **9g** and **10a** showed the excellent activity against *A. solani* with the inhibition rates of 83.7, 86.1, 86.5, and 85.9%, respectively, which were significantly higher than that of thiabendazole (75.2%) (P<0.05), a commercial fungicide. Furthermore, although almost all the target compounds showed poorer antifungal activity against *M. grisea*, most of them exceeded that of thiabendazole (36.1%) (P<0.05), and the similar cases were observed for *C. lunata* and *A. alternate.* It was worth mentioning that compounds **8b**, **9c**, and **10a** also exhibited the excellent activity against *F. graminearum* with the inhibition rates of 88.2, 85.1 and 90.4%, respectively. Compared with the other compounds, **8b**, **9a**–**9c**, **9f**, **9g** and **10a** showed inhibitory activities against almost all the tested plant pathogenic fungi with inhibition rates over 60%, which exhibited their broader antifungal spectrum.

Antifungal Toxicity The excellent activity of **8b**, **9c**, **9g** and **10a** (inhibition rates>85.0%) in **Table 1** encouraged us to further determine their median effective concentrations (EC_{50}) against corresponding three strains of fungi. Thiabendazole was used as the positive control. Toxicity regression equations for concentration-effect of the compounds and their corresponding EC_{50} values were listed in **Table 2**.

Table 2. Toxicity regression equations and EC_{50} values of compounds against three tested fungi

Fungus	Compd.	Regression equation[a)]	R^2	EC_{50} μg/mL	95%CI[b)]
F. o	**8b**	$y = 1.3229x + 3.2154$	0.9632	22.34	14.13–29.52
	TBZ [c)]	$y = 1.6983x + 4.4843$	0.9826	2.01	1.47–2.65
F. g	**8b**	$y = 0.9425x + 3.6650$	0.9471	26.09	15.70–31.27
	9c	$y = 1.1132x + 3.8744$	0.9968	10.26	9.19–11.56
	10a	$y = 1.5412x + 3.4456$	0.9949	10.20	8.90–11.89
	TBZ	$y = 1.5115x + 4.9387$	0.9877	1.10	0.68–1.59
A. s	**9c**	$y = 2.4468x + 2.3301$	0.9741	12.33	8.39–19.63
	9g	$y = 2.3272x + 2.7594$	0.9810	9.18	6.59–12.47
	10a	$y = 1.9627x + 3.3554$	0.9770	6.89	4.65–9.20

TBZ	y =2.1544x + 1.9935	0.9778	24.86	20.91–27.56

a) y: Probability of average inhibition rate; x: lg[concentration (μg/mL)]. b) Confidence interval of EC_{50} (μg/mL) at 95% probability. c) TBZ = Thiabendazole.

From **Table 2**, it was clearly seen that the tested compound **8b** displayed good activity against *F. oxysporum* with EC_{50} values of 22.34 μg/mL, although its activity was much lower than that of thiabendazole **(**EC_{50} = 2.01 μg/mL). As to the data against *F. graminearum*, **Table 2** indicated that all of the three tested compounds (**8b**, **9c**, **10a**) exhibited excellent activities, and their EC_{50} values were 10.20–26.09 μg/mL. Among them, the compound **10a** exhibited the most potent activity with an EC_{50} value of 10.20 μg/mL. Regarding *A. solani*, all tested compounds (**9c**, **9g**, **10a**) showed excellent activities in **Table 2**, and their EC_{50} values ranged from 6.89–12.33 μg/mL. Amazingly, the activities of the three tested compounds (**9c**, **9g**, **10a**) were all superior to thiabendazole (EC_{50} = 24.86 μg/mL), the positive control. The results described above indicated that all the tested compounds were obviously more active than the reference compound **4a**. These results were basically consistent with the screening results in **Table 1**.

Structure-Activity Relationship By comparing EC_{50} values in **Table 2**, along with inhibition rates of the compounds at 50 μg/mL in **Table 1**, it was obviously seen that almost all of the title compounds exhibited higher activity against each of the test fungi than the previous synthetic analogues of kakuol, which implied that it was effective to increase the antifungal activities of target compounds by adjusting the position of the C=C bond in the previous structure, namely placing it to the terminal and still conjugated to C=O bond. Furthermore, the results described above revealed that the methyl ethers compounds (**8b**–**8i**) exhibited the higher antifungal activities than the phenolic compounds (**4a**–**4e**) to some extent, but their antifungal activities decrease with increase of the length of carbon chain from **8b** to **8i**. Compounds **9a**–**9g** and **10a**–**10d** were designed and synthesized according to the structure of **8b**, the compound with shorter length of carbon chains but the better antifungal activities. Compared with compound **8b**, the introduction of electron-donating alkyl groups to the benzene ring (**9a**–**9c)** was beneficial for the improvement of the activities against almost all the tested fungi. On the contrary, the presence of chlorine atom (**9d)** remarkably decreased the activity. In addition, the effects of other bis-substituted groups (**9e**–**9g)** on the activity depended on their positions on benzene ring and the species of fungi. In order to understand the conditions more clearly, the structure-activity relationship for the antifungal activity was listed in (**Figure 3**).

Based on the antifungal data against *M. grisea*, the methyl ethers compounds (**8b**–**10a**) exhibited the higher antifungal activities than the phenolic compounds (**4a**–**4e**) and the two unexpected products (**5**, **6**) to some extent. Especially, while the introduction of 5-Et (**9b)**, 5-*i*-Pr (**9c)**, 4-Me-5-Cl (**9g)**, or naphthalene ring **(10a**) remarkably improved the activity, and the similar trends were observed in the

result against other tested fungi. Similarly, as to the result against *A. solani*, the presence of 5-Et (**9b)**, 5-*i*-Pr (**9c)**, 4-Me-5-Cl (**9g)**, or naphthalene ring **(10a**) also remarkably improved the activity, and compound **10a** possessed the highest activity (EC_{50} = 6.89 μg/mL), followed by **9g**, **9c** and **9b**. Regarding the data against *C. lunata*, *F. solani*, *A. alternate* and *F. oxysporum*, it was clearly seen that only the individual compounds exhibited the better activities from **Table 1.** Among them, especially compound **8b** showed the most potent activity against *F. oxysporum* with the inhibition rate of 92.7%, in most cases, the introduction of the above substituents had little effect on the activity against *F. oxysporum* compared with the corresponding result of reference compound **4a**. Finally, as far as the antifungal data against *F. graminearum*, most of tested compounds exhibited better antifungal activities, especially compounds **8b**, **9c** and **10a** showed the excellent activities with the EC_{50} values of 26.09, 10.26 and 10.20 μg/mL, respectively.

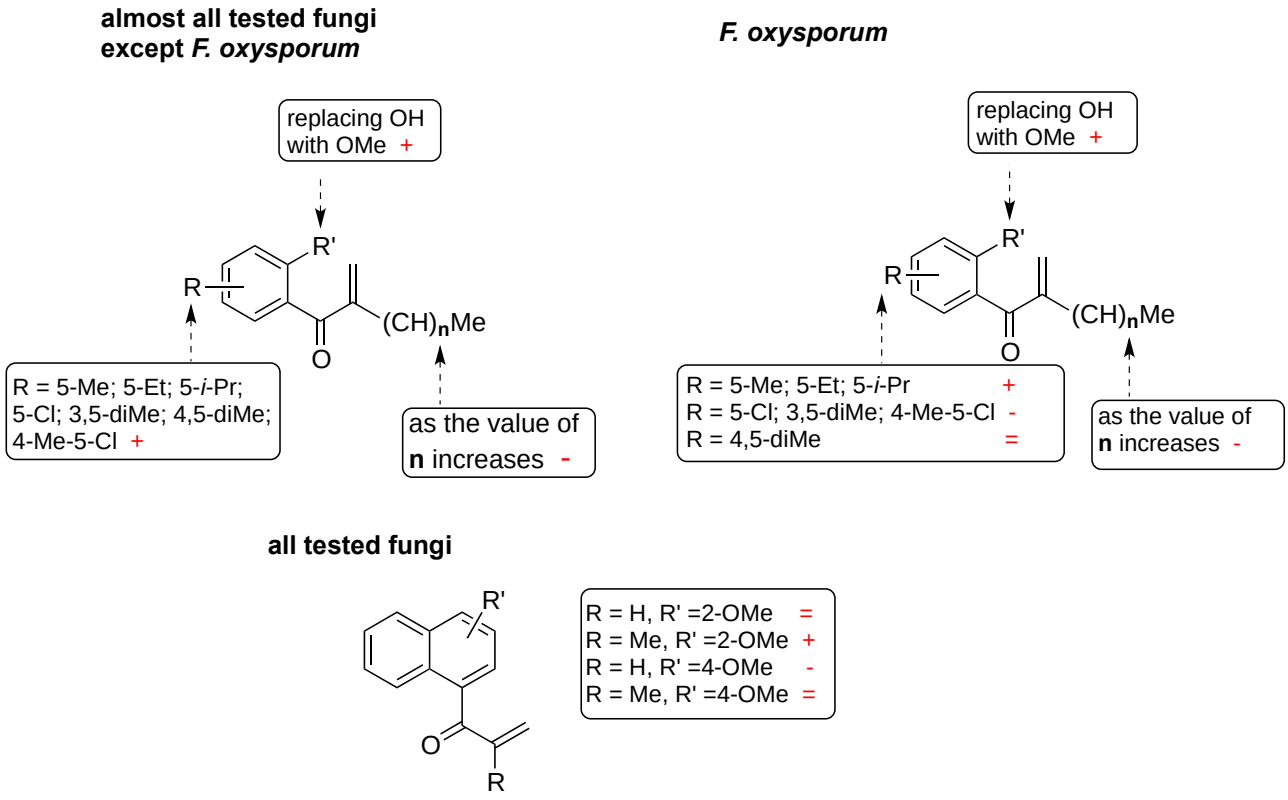


Figure 3. Structure-activity relationship of the main target compounds

In conclusion, we reported the synthesis of a series of α, *β*-unsaturated ketones analogues of kakuol containing terminal C=C bond, and their antifungal activity *in vitro* against seven plant pathogenic fungi was evaluated. Most of all the synthesized compounds displayed the obvious growth inhibition activity

against all the tested fungi, and were more active than the previous synthetic analogues of kakuol. Among all the tested compounds, target compounds **9c**, **9g** and **10a** displayed excellent activity against *A. solani* with EC_{50} values of 6.89–12.33 μg/mL, much lower than that of thiabendazole (EC_{50} = 24.86 μg/mL), a commercial fungicide. In addition, compounds **8b**, **9c** and **10a** also displayed higher antifungal activities against *F. graminearum* with their EC_{50} values of 10.20–26.09 μg/mL, likewise, compound **8b** exhibited excellent activity against *F. oxysporum* with an EC_{50} value of 22.34 μg/mL. SAR analysis demonstrated that the conjugated terminal C=C bond is necessary for improvement of the activity. Generally, both the length of the alkyl chain and substitution patterns of the benzene ring remarkably impact the activity for most of the test fungi. Therefore, the present results strongly suggest that the newly synthesized target compounds possess great potential to develop new antifungal agents for the effective control of plant fungal pathogens.

EXPERIMENTAL

All reagents and solvents were of reagent grade or purified according to standard methods before use. Thiabendazole (≥99.1%), a commercial fungicide, was purchased from Yi Fang Biotechnology Co.Ltd. (Zhejiang, China). Sesamol, the starting material, was purchased from Jiangsu Yancheng Chemical Factory (Jiangsu, China). The silica gel and GF_{254} silica gel of analytical thin-layer chromatography (TLC) were produced by the Qingdao Haiyang Chemical Co., Ltd., which we utilized during the experiment procedure. The plant pathogenic fungi, *Alternaria solani*, *Magnaporthe grisea*, *Alternaria alternata*, *Fusarium solani*, *Fusarium graminearum*, *Curvularia lunata* and *Fusarium oxysporum vasinfectum*, were provided by the Center of Pesticide Research, Northwest A&F University, China. These fungi were grown on potato dextrose agar (PDA) plates at 28 °C and maintained at 4 °C with periodic subculturing. Melting points of the compounds were determined on an mp 420 automatic melting point meter (Hanon Instrument, Beijing, China) and uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on a BrukerAvance III 500 MHz instrument. Chemical shift values (δ) were given in parts per million (ppm). Coupling constant values (J) were given in Hz. High resolution mass spectra (HR-MS) were carried out with a Micromass Auto Spec-3000 instrument. Low resolution mass spectra were carried out with a Thermo FisherLCQ Fleet instrument.

General procedure for the synthesis of compounds 2a–2i, 9a–9g, and 10a–10d

Adapted from reported procedure,[12] catalyst anhydrous aluminum chloride (1.20 g, 9 mmol) was dissolved in the dried CH_2Cl_2 (10 mL) with stirring in ice bath. Then appropriate acyl chloride (7 mmol) was dropwise added into the above mixture under nitrogen atmosphere. After a solution of compound **1a** or **3a**–**3i** (5 mmol) in dried CH_2Cl_2 (10 mL) was dropwise added into the above mixture under nitrogen atmosphere. The resulting solution was stirred for 10 min at 0 °C, and rise to room temperature until the

reaction was completed according to TLC detection. The organic reaction mixture was washed with 15% aqueous hydrochloric acid (3 × 20 mL), saturated sodium carbonate solution (3 × 20 mL), brine water (3 × 20 mL), and then was dried with anhydrous Na_2SO_4. After filtration, the solution was evaporated under vacuum to remove the solvent, and the residue was purified by column chromatography on silica gel using petroleum ether-EtOAc as eluent.

General procedure for the synthesis of compounds 3a–3i and 7a–7i

According to the reported method with modification,[13] compound **1b**–**1j** or **2a**–**2i** (5 mmol), anhydrous potassium carbonate (1.10 g, 8 mmol) and benzyltriethylammonium chloride (TEBA, 68 mg, 0.3 mmol) were dissolved in the acetone with stirring for 15 min at room temperature. Then the methyl iodide (0.8 mL, 9 mmol) was quickly added into the above mixture and heated slowly under reflux with stirring until the reaction was completed according to TLC detection. After removal of the reaction solvent under vacuum, the residue was dissolved in CH_2Cl_2 (30 mL). Then the mixture was washed with water (3 × 10 mL), and the organic phase was dried with anhydrous Na_2SO_4. After filtration and removal of the organic solvent, the crude intermediate **3a–3i** or **7a–7i** were obtained and directly used in the following step.

General procedure for the synthesis of target compounds 4a–4e, 5, 6 and 8a–8i

According to the reported method with modification,[14] the mixture of the appropriate ketone (**2a**–**2e**, **2i** or **7a**–**7i**, 2 mmol) and morpholine (0.26 mL, 3 mmol) in 20 mL of glacial acetic acid was heated under reflux and a 37% formaldehyde aqueous solution (5 mL, 62 mmol) was added dropwise over several hours. The progress of the reaction was monitored by TLC detected. Afterwards, completion of the reaction, acetic acid was stripped off under reduced pressure and the residue was diluted with EtOAc. The resulting solution was washed with 10% sodium bicarbonate aqueous solution (3 × 20 mL), 10% aqueous hydrochloric acid (2 × 20 mL), brine (2 × 20 mL) and water (2 × 20 mL). Then the remained organic mixture was dried with anhydrous Na_2SO_4. After filtration and removal of the solvent, the residue was purified by column chromatography on silica gel using petroleum ether-EtOAc as eluent to yield the products.

Characterization data of the target compounds

Compound 4a. CAS no. 1241783-13-0. Yield, 21%; yellow needle crystal; mp 117.3–118.0 °C (lit.[4] 117–118 °C). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 13.49 (s, 1H), 7.13 (s, 1H), 7.11–7.16 (m, 1H), 6.52 (dd, 1H, J = 16.8 Hz, 1.7 Hz), 6.48 (s, 1H), 5.99 (s, 2H), 5.90 (dd, 1H, J = 10.5 Hz, 1.7 Hz). Positive ESI-MS m/z: 193.04 $[M+H]^+$.

Compound 4b. CAS no. 1241783-14-1. Yield, 27%; yellow needle crystal; mp 78.6–79.2 °C (lit.[4] 78–79 °C). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 12.88 (s, 1H), 7.14 (s, 1H), 6.49 (s, 1H), 5.98 (s, 2H), 5.60 (t, 1H, J = 1.4 Hz), 5.32 (t, 1H, J = 0.9 Hz), 2.06 (t, 3H, J = 1.4 Hz). Positive ESI-MS *m/z*: 207.06 $[M+H]^+$.

Compound 4c. Yield, 24%; yellow needle crystal; mp 43.7–45.3 °C. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 12.95 (s, 1H), 7.12 (s, 1H), 6.49 (s, 1H), 5.98 (s, 2H), 5.53 (s, 1H), 5.27 (d, 1H, J = 0.6 Hz), 2.44 (q, 2H, J = 7.4 Hz), 1.11 (t, 3H, J = 7.4 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 202.1, 163.2, 154.7, 148.6, 140.2, 118.2, 111.4, 109.5, 101.9, 98.8, 26.6, 19.8. HR-MS $[M+H]^+$: Calcd for $C_{12}H_{13}O_4^+$ 221.0738. Found 221.0740.

Compound 4d. Yield, 27%; yellow needle crystal; mp 28.7–30.3 °C. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 12.97 (s, 1H), 7.13 (s, 1H), 6.48 (s, 1H), 5.98 (s, 2H), 5.55 (s, 1H), 5.29 (s, 1H), 2.41(t, 2H, J = 7.6Hz), 1.46–1.53 (m, 2H), 0.95 (t, 3H, J = 7.3 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 202.0, 163.3, 154.7, 147.3, 140.1, 119.5, 111.4, 109.5, 101.9, 98.8, 35.8, 21.1, 13.8. HR-MS $[M+H]^+$: Calcd for $C_{13}H_{15}O_4^+$ 235.0896. Found 235.0895.

Compound 4e. Yield, 22%; yellow oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 12.96 (s, 1H), 7.13 (s, 1H), 6.48 (s, 1H), 5.98 (s, 2H), 5.54 (s, 1H), 5.28 (s, 1H), 2.43 (t, 2H, J = 7.5 Hz), 1.42–1.48 (m, 2H), 1.33–1.40 (m, 2H), 0.91 (t, 3H, J = 7.3 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 202.0, 163.3, 154.7, 146.1, 140.1, 120.3, 111.4, 109.5, 101.9, 98.8, 31.2, 20.6, 17.4, 13.4. HR-MS $[M+H]^+$: Calcd for $C_{14}H_{17}O_4^+$ 249.1051. Found 249.1049.

Compound 5. Yield, 29%; yellow flaky crystal; mp 117.1–118.6 °C. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.34 (s, 1H), 6.42 (s, 1H), 6.26 (s, 1H), 6.00 (s, 2H), 5.52 (s, 1H), 4.95 (s, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 180.5, 160.2, 154.5, 143.6, 138.6, 121.6, 115.2, 104.7, 102.1, 98.5, 71.6. HR-MS $[M+H]^+$: Calcd for $C_{11}H_9O_4^+$ 205.0427. Found 205.0428.

Compound 6. Yield, 11%; yellow flaky crystal; mp 121.3–122.1 °C. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 7.25–7.35 (m, 5H), 7.16 (s, 1H), 6.72 (s, 1H), 6.11 (d, 2H, J = 6.3 Hz), 4.60–4.68 (m, 2H), 4.05–4.07 (m, 1H). ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 190.7, 159.7, 154.6, 143.4, 136.5, 129.2, 129.0, 127.8, 114.5, 103.8, 102.8, 98.8, 71.9, 51.1. HR-MS $[M+H]^+$: Calcd for $C_{16}H_{13}O_4^+$ 269.0739. Found 269.0739.

Compound 8a. Yield, 71%; yellow oil. ^{1}H-NMR (500MHz, $CDCl_3$) δ: 6.86 (s, 1H), 6.55 (s, 1H), 5.98 (s, 2H), 5.93 (s, 1H), 5.78 (s, 1H), 4.93 (s, 2H), 3.73 (s, 3H), 2.10 (s, 3H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 195.0, 170.5, 154.6, 151.1, 144.4, 141.3, 126.5, 120.4, 109.2, 101.9, 95.2, 62.8, 56.7, 20.9. HR-MS $[M+H]^+$: Calcd for $C_{14}H_{15}O_6^+$ 279.0795. Found 279.0797.

Compound 8b. Yield, 49%; brown oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 6.79(s, 1H), 6.55(s, 1H), 5.97 (s, 2H), 5.79 (t, 1H, J = 1.4Hz), 5.58 (s, 1H), 3.73 (s, 3H), 2.01 (s, 3H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ:

197.5, 153.9, 150.3, 145.5, 141.0, 126.8, 121.2, 108.9, 101.7, 95.3, 56.8, 17.6. HR-MS $[M+H]^+$: Calcd for $C_{12}H_{13}O_4^+$ 221.0736. Found 221.0735.

Compound 8c. Yield, 52%; yellow oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 6.81 (s, 1H), 6.54 (s, 1H), 5.97 (s, 2H), 5.71 (s, 1H), 5.57 (s, 1H), 3.73 (s, 3H), 2.43 (q, 2H, J = 7.4 Hz), 1.11 (t, 3H, J = 7.6 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 197.6, 154.1, 151.6, 150.4, 141.1, 124.2, 121.6, 109.1, 101.7, 95.3, 56.8, 24.1, 12.5. HR-MS $[M+H]^+$: Calcd for $C_{13}H_{15}O_4^+$ 235.0895. Found 235.0897.

Compound 8d. Yield, 54%; yellow oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 6.80 (s, 1H), 6.54 (s, 1H), 5.97 (s, 2H), 5.71(d, 1H, J = 1.2Hz), 5.58 (s, 1H), 3.73 (s, 3H), 2.39 (t, 2H, J = 7.3 Hz), 1.49–1.57 (m, 2H), 0.96 (t, 3H, J = 7.3Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 197.5, 154.1, 150.3, 150.0, 141.1, 125.4, 121.6, 109.1, 101.7, 95.2, 56.7, 33.2, 21.4, 13.8. HR-MS $[M+H]^+$: Calcd for $C_{14}H_{17}O_4^+$ 249.1051. Found 249.1050.

Compound 8e. Yield, 70%; yellow oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 6.80 (s, 1H), 6.53 (s, 1H), 5.97 (s, 2H), 5.71 (s, 1H), 5.57 (s, 1H), 3.72 (s, 3H), 2.41 (t, 2H, J = 7.4Hz), 1.44–1.50 (m, 2H), 1.34–1.41 (m, 2H), 0.93 (t, 3H, J = 7.3 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 197.5, 154.1, 150.3, 150.3, 141.1, 125.2, 121.6, 109.1, 101.7, 95.3, 56.8, 30.8, 30.4, 22.4, 14.0. HR-MS $[M+H]^+$: Calcd for $C_{15}H_{19}O_4^+$ 263.1209. Found 263.1206.

Compound 8f. Yield, 62%; yellow oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 6.80 (s, 1H), 6.54 (s, 1H), 5.97 (s, 2H), 5.71 (d, 1H, J = 1.1 Hz), 5.57 (s, 1H), 3.73 (s, 3H), 2.40 (t, 2H, J = 7.5 Hz), 1.46–1.50 (m, 2H), 1.32–1.35 (m, 4H), 0.90 (t, 3H, J = 7.0 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 197.6, 154.1, 150.3, 150.3, 141.0, 125.3, 121.6, 109.1, 101.7, 95.2, 56.7, 31.5, 31.1, 27.9, 22.6, 14.1. HR-MS $[M+H]^+$: Calcd for $C_{16}H_{21}O_4^+$ 277.1365. Found 277.1367.

Compound 8g. Yield, 53%; yellow oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 6.80 (s, 1H), 6.54 (s, 1H), 5.97 (s, 2H), 5.71 (d, 1H, J = 1.1 Hz), 5.57 (s, 1H), 3.72 (s, 3H), 2.40 (t, 2H, J = 7.5 Hz), 1.45–1.51 (m, 2H), 1.32–1.38 (m, 2H), 1.28–1.32 (m, 4H), 0.89 (t, 3H, J = 6.9 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 197.5, 154.1, 150.3, 150.3, 141.0, 125.3, 121.5, 109.1, 101.7, 95.2, 56.7, 31.7, 31.1, 29.0, 28.2, 22.7, 14.2. HR-MS $[M+H]^+$: Calcd for $C_{17}H_{23}O_4^+$ 291.1520. Found 291.1519.

Compound 8h. Yield, 46%; yellow oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 6.80 (s, 1H), 6.54 (s, 1H), 5.97 (s, 2H), 5.71 (d, 1H, J = 1.0 Hz), 5.57 (s, 1H), 3.72 (s, 3H), 2.40 (t, 2H, J = 7.5 Hz), 1.45–1.51 (m, 2H), 1.28–1.33 (m, 8H), 0.88 (t, 3H, J = 6.7 Hz).^{13}C-NMR (125 MHz, $CDCl_3$) δ: 197.5, 154.1, 150.3, 150.3, 141.1, 125.2, 121.6, 109.1, 101.7, 95.3, 56.7, 31.9, 31.1, 29.3, 29.2, 28.3, 22.7, 14.1. HR-MS $[M+H]^+$: Calcd for $C_{18}H_{25}O_4^+$ 305.1675. Found 305.1677.

Compound 8i. Yield, 50%; yellow oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.27–7.37 (m, 5H), 7.15 (s, 1H), 6.42 (s, 1H), 5.98 (s, 2H), 5.82 (s, 1H), 5.67 (s, 1H), 3.56 (s, 3H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 195.8, 155.8, 152.0, 151.3, 141.6, 137.5, 128.4, 128.1, 127.9, 127.3, 120.5, 109.6, 101.9, 94.8, 56.4. HR-MS

$[M+H]^+$: Calcd for $C_{17}H_{15}O_4^+$ 283.0896. Found 283.0895.

Compound 9a. CAS no. 1368398-08-6. Yield, 29%; yellow oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.16 (dd, 1H, J = 8.4 Hz, 2.0 Hz), 7.01 (d, 1H, J = 2.1 Hz), 6.82 (d, 1H, J = 8.5 Hz), 5.87 (t, 1H, J = 1.3 Hz), 5.61 (s, 1H), 3.75 (s, 3H), 2.28 (s, 3H), 2.02 (s, 3H). Positive ESI-MS *m/z*: 191.10 $[M+H]^+$.

Compound 9b. CAS no. 1368654-32-3. Yield, 31%; yellow oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.20 (dd, 1H, J = 8.5 Hz, 2.2 Hz), 7.04 (d, 1H, J = 2.2 Hz), 6.85 (d, 1H, J = 8.5 Hz), 5.88 (t, 1H, J = 1.3 Hz), 5.61 (s, 1H), 3.76 (s, 3H), 2.59 (q, 2H, J = 7.6 Hz), 2.02 (s, 3H), 1.21 (t, 3H, J = 7.6 Hz). Positive ESI-MS *m/z*: 205.12 $[M+H]^+$.

Compound 9c. CAS no. 1368964-71-9. Yield, 32%; orange oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.23 (dd, 1H, J = 8.5 Hz, 2.2 Hz), 7.07 (d, 1H, J = 2.3 Hz), 6.85 (d, 1H, J = 8.5 Hz), 5.88 (t, 1H, J = 1.3 Hz), 5.61 (s, 1H), 3.76 (s, 3H), 2.83–2.89 (m, 1H), 2.03 (s, 3H), 1.22 (d, 6H, J = 7.0 Hz). Positive ESI-MS *m/z*: 219.13 $[M+H]^+$.

Compound 9d. CAS no. 1352226-81-3. Yield, 24%; yellow oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.33 (dd, 1H, J = 8.8 Hz, 2.5 Hz), 7.18 (d, 1H, J = 2.6 Hz), 6.86 (d, 1H, J = 8.9 Hz), 5.93 (t, 1H, J = 1.3 Hz), 5.62 (s, 1H), 3.77 (s, 3H), 2.02 (s, 3H). Positive ESI-MS *m/z*: 211.04 $[M+H]^+$.

Compound 9e. CAS no. 1368630-71-0. Yield, 28%; colorless oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 6.96 (s, 1H), 6.70 (s, 1H), 5.94 (t, 1H, J = 1.3 Hz), 5.61 (s, 1H), 3.79 (s, 3H), 2.21 (s, 3H), 2.19 (s, 3H), 2.04 (s, 3H). Positive ESI-MS *m/z*: 205.12 $[M+H]^+$.

Compound 9f. CAS no. 1368439-22-8. Yield, 31%; yellow oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.00 (s, 1H), 6.72 (s, 1H), 5.84 (s, 1H), 5.61 (s, 1H), 3.75 (s, 3H), 2.28 (s, 3H), 2.19 (s, 3H), 2.01 (s, 3H). Positive ESI-MS *m/z*: 205.12 $[M+H]^+$.

Compound 9g. CAS no. 1368985-50-5. Yield, 23%; yellow oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.20 (s, 1H), 6.79 (s, 1H,), 5.90 (t, 1H, J = 1.3 Hz), 5.62 (s, 1H), 3.77 (s, 3H), 2.39 (s, 3H), 2.01 (s, 3H). Positive ESI-MS *m/z*: 225.06 $[M+H]^+$.

Compound 10a. Yield, 26%; yellow oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.88 (d, 1H, J = 9.1 Hz), 7.79 (d, 1H, J = 8.2 Hz), 7.46 (d, 1H, J = 8.5 Hz), 7.40–7.43 (m, 1H), 7.33–7.36 (m, 1H), 7.28 (d, 1H, J = 9.1 Hz), 5.90 (t, 1H, J = 1.3 Hz), 5.58 (s, 1H), 3.88 (s, 3H), 2.13 (s, 3H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 200.0, 153.7, 145.7, 131.8, 130.7, 129.4, 128.7, 128.0, 127.2, 124.0, 123.4, 122.9, 113.1, 56.6, 16.7. HR-MS $[M+H]^+$: Calcd for $C_{15}H_{15}O_2^+$ 227.0998. Found 227.0996.

Compound 10b. Yield, 31%; orange oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.90 (d, 1H, J = 9.1 Hz), 7.79 (d, 1H, J = 8.2 Hz), 7.63 (d, 1H, J = 8.8 Hz), 7.42–7.45 (m, 1H), 7.34–7.37 (m, 1H), 7.29 (d, 1H, J = 9.1 Hz), 6.72–6.77 (m, 1H), 5.98–6.03 (m, 2H), 3.90 (s, 3H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 197.9, 154.4, 138.4, 131.5, 131.4, 131.0, 128.8, 128.1, 127.5, 124.1, 123.9, 122.7, 113.1, 56.6. HR-MS $[M+H]^+$: Calcd for

$C_{14}H_{13}O_2^+$ 213.0839. Found 213.0840.

Compound 10c. Yield, 29%; yellow-green oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.31 (dd, 1H, J = 8.1 Hz, 1.2 Hz), 8.22 (d, 1H, J = 7.9 Hz), 7.58 (d, 1H, J = 8.0 Hz), 7.48–7.56 (m, 2H), 6.77 (d, 1H, J = 8.0 Hz), 5.90 (t, 1H, J = 1.3 Hz), 5.61 (s, 1H), 4.04 (s, 3H), 2.14 (s, 3H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 199.3, 157.8, 146.1, 132.4, 129.8, 128.4, 128.1, 127.7, 125.8, 125.7, 125.5, 122.2, 101.9, 55.7, 18.2. HR-MS $[M+H]^+$: Calcd for $C_{15}H_{15}O_2^+$ 227.0998. Found 227.0999.

Compound 10d. Yield, 26%; yellow oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.63 (d, 1H, J = 8.5 Hz), 8.32 (dd, 1H, J = 8.6 Hz, 0.9 Hz), 7.84 (d, 1H, J = 8.1 Hz), 7.58–7.62 (m, 1H), 7.51–7.54 (m, 1H), 6.99–7.05 (m, 1H), 6.79 (d, 1H, J = 8.1 Hz), 6.31 (dd, 1H, J = 17.3 Hz, 1.6 Hz), 5.95 (dd, 1H, J = 10.4 Hz, 1.6 Hz), 4.05 (s, 3H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 193.8, 158.8, 136.6, 132.2, 130.9, 129.6, 128.3, 127.8, 125.9, 125.8, 125.8, 122.2, 102.1, 55.8. HR-MS $[M+H]^+$: Calcd for $C_{14}H_{13}O_2^+$ 213.0839. Found 213.0841.

Antifungal Activity Assay

The *in vitro* antifungal activity of all target compounds agninst seven phytopathogenic fungi (*A. solani*, *M. grisea*, *A. alternate*, *C. lunata*, *F. graminearum*, *F. solani*, *F.oxysporum*) were investigated using the mycelium linear growth rate method.[15-18] The test fungi, provided by the Center of Pesticide Research, Northwest A&F University, China, were maintained on potato dextrose agar (PDA) medium slants were subcultured for 48 h in Petri dishes prior to testing and used for inoculation of fungal strains on PDA plates. All test compounds and the tebuconazole (the positive control) were completely dissolved in 0.5 mL DMSO and the solution was added to 9.5 mL of sterile water. The resulting solution was added to 90 mL of melted PDA medium at a temperature below 50 °C. After quickly and completely mixing, the medium containing the compounds at a concentration of 50 μg/mL was poured into sterilized Petri dishes for screening. The solution of DMSO without any compounds mixed with PDA served as the blank control. When the medium in the plate was partially solidified, a 5 mm thick and 4 mm diameter disc of fungus cut from beforehand subcultured Petri dishes was placed at the centre of semi-solid medium. The dishes were kept in an incubator at 28 °C for 72 h. Three replicates were performed for each experiment. The growth inhibitory rates were caculated according to the following formula and expressed as mean ± S.D. where d_o is the diameter of the fungus cut, d_c is the diameter of a fungal colony in the blank test, and d_s is the diameter of a fungal colony in the compound-treated test.

$$\text{inhibition rate (\%)} = [(d_c - d_o) - (d_s - d_o)]/(d_c - d_o) \times 100\%$$

Antifungal Toxicity Assay

Based on the above results of *in vitro* antifungal activity, the more active compounds **8b**, **9c, 9g** and **10a** were selected to determine their median effective concentration (EC_{50}) according to the same method described above. A stock solution was prepared by dissolving the tested compounds in DMSO, and then diluted by DMSO using serial two-fold dilution method to obtain a series of stock solutions. Each stock solution was respectively mixed with the autoclaved PDA medium to prepare a set of mediums containing 50, 25, 12.5, 6.25, 3.125, 1.5625, 0.78125 μg/mL of the tested compounds. Meanwhile, 0.5% DMSO in culture medium was used as a blank control. Each experiment was performed in triplicate. The concentration (μg/mL) of the compound was transformed to the corresponding logarithm value (logC). logC values for each compound and its corresponding probit values were used to establish toxicity regression equation by the linear least-square fitting method. EC_{50} values and their confidence interval at 95% probability (95% CI) were calculated from the toxicity regression equations by using PRISM software ver.5.0 (GraphPad Software Inc, San Diego, CA, USA).

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