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Natural products as sources of new fungicides (V): Design and synthesis of acetophenone derivatives against phytopathogenic fungi *in vitro* and *in vivo*

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ABSTRACT

A series of acetophenone derivatives (**10a–10i**, **11**, **12a–12g**, **13a–13g**, **14a–14d** and **15a–15l**) were designed, synthesized and evaluated for antifungal activities *in vitro* and *in vivo*. The antifungal activities of 53 compounds were tested against several plant pathogens, and their structure–activity relationship was summarized. Compounds **10a–10f** displayed better antifungal effects than two reference fungicides. Interestingly, the most potent compound **10d** exhibited antifungal properties against *Cytospora* sp., *Botrytis cinerea*, *Magnaporthe grisea*, with IC_{50} values of 6.0 to 22.6 μg/mL, especially *Cytospora* sp. (IC_{50} = 6.0 μg/mL). In the *in vivo* antifungal assays, **10d** displayed the significant protective efficacy of 55.3% to *Botrytis cinerea* and 73.1% to *Cytospora* sp. The findings indicated that **10d** may act as a potential pesticide lead compound that merits further investigation.

Keywords: Natural products; acetophenone derivatives; antifungal activity; structure–activity relationship

Pathogenic fungi, hard to control, are considered a great threat for farming and food production including livestock, poultry and crop.[1,2] Accordingly, >80% of the crops areas in the United States are treated with fungicides every year, and the benefit of fungicide use is estimated to boost farm income by nearly $13 billion annually. And in China, fungicides used as crop protection have also occupied a larger share of agricultural resources.[3] However, the emergence of fungicide-resistant strains has brought great challenges to normal production and life, which prompt us to develop new fungicides. Natural products with low mammalian toxicity, easy decomposition, friendly to environment, specific to targeted species, and unique mode of action, are an important source of antifungal agents.[4] 2,4-Dihydroxy-5-methylacetophenone (**1**), isolated from the cultures of the fungus *Polyporus picipes* (Figure 1), has been reported as a good antifungal agent against several phytopathogens in our patent.[5] Xanthoxylin (**2**), isolated from a medicinal plant *Melicope borbonica*, also exhibited good activities against *Penicillium expansum*.[6]

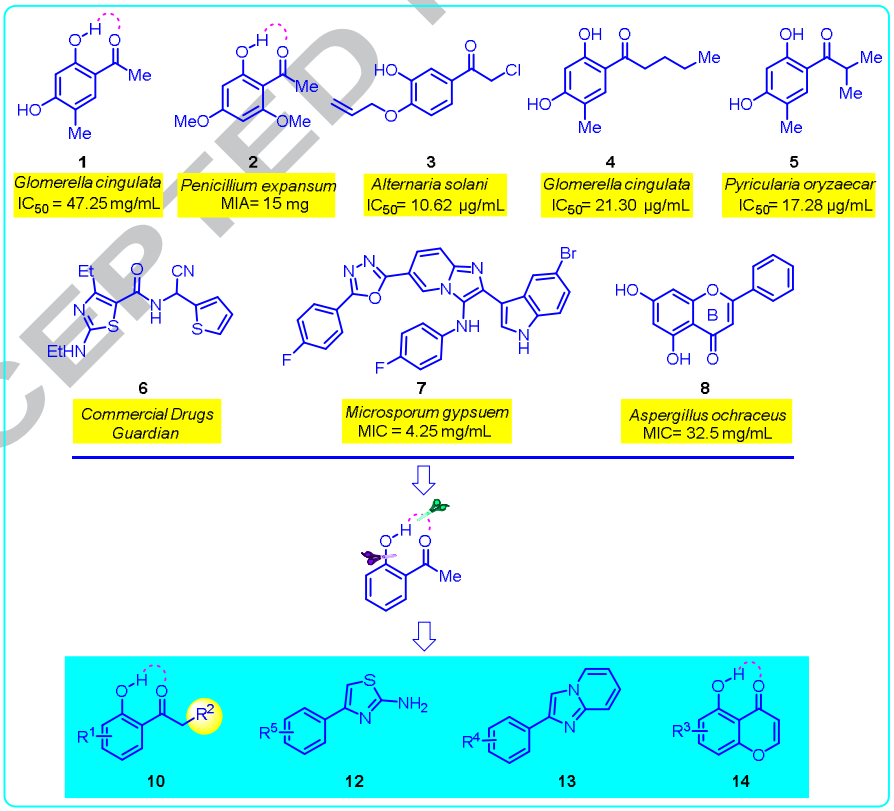
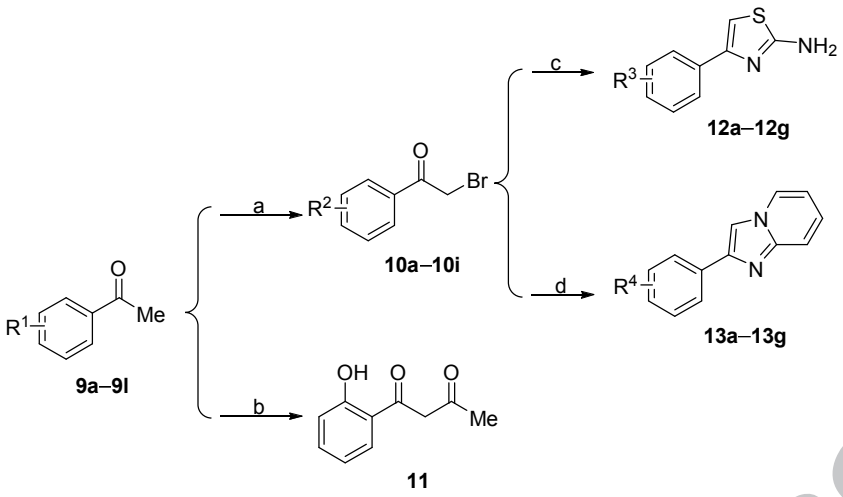


Figure 1. Design of the acetophenone derivatives (**10–14**).

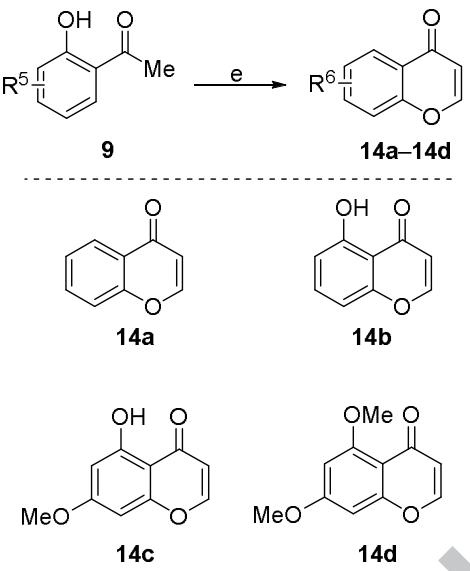
In our previous work, we assessed the antifungal activity of several series of acetophenone analogues (**3**–**5**) (Figure 1).[5,7,8] The SAR studies mainly focused on (1) alkyl or benzyl acetophenone ether derivatives on the acetophenone nuleus, which concluded that the coexistence of *m*-OH and *p*-lipophilic alkyl substituents were beneficial; (2) chlorine, alkyl or aromatic substitutions on α-position of ketone carbonyl, which illustrated that chlorine-substitution, the longer chain or multi-side chain are favorable. Interestingly, phenolic hydroxyl, which could form intramolecular hydrogen bond with ketone carbonyl groups, exhibited positive or negative efficiency in antifungal activity. To obtain higher antifungal acetophenone agents, some acetophenone analogues, especially bromoacetophenones (**10**), were synthesized. Considering the role of nitrogen-containing heterocycles (**6** and **7**) in antifungal agents, [9,10] we further synthesized their *N*-heterocyclic derivatives (**12**, **13**) based on bromoacetophenones. With the formation of B ring (**8**)[11], some chromen-4-one analogues (**14**) were also prepared (Figure 1). Inspired by these results, herein we report synthesis of several series of acetophenone derivatives (**10a**–**10i**, **11**, **12a**–**12g**, **13a**–**13g** and **14a**–**14d**) and their anti-phytopathogenic fungal activity *in vitro* and *in vivo*.



No.	R^1	No.	R^1	No.	R^2	No.	R^3	No.	R^4
9a	4-F	**9h**	2-OH	**10a**	4-F	**12a**	4-F	**13a**	4-F
9b	4-Cl	**9i**	2,6-OH-4-OMe	**10b**	4-Cl	**12b**	4-Cl	**13b**	4-Cl
9c	4-Br	**9j**	2,4-OMe-6-OH	**10c**	4-Br	**12c**	4-Br	**13c**	4-Br
9d	4-Me	**9k**	2,6-OH	**10d**	4-Me	**12d**	4-OMe	**13d**	4-Me
9e	4-OMe	**9l**	2-OH-4-OMe	**10e**	4-OMe	**12e**	4-SO_2Me	**13e**	4-OMe
9f	4-SO_2Me			**10f**	4-SO_2Me	**12f**	2-F	**13f**	4-SO_2Me
9g	2-F			**10g**	2-F	**12g**	2-OH	**13g**	2-OH
				10h	2-OH				
				10i	2-OH-4-OMe				

Scheme 1. Syhthesis of acetophenone derivatives **10a**–**10i**, **11**, **12a**–**12g** and **13a**–**13g**. Reagents and conditions (a) *p*-TSA, NBS, MeCN, 80 °C, 40%–89%; (b) NaH, EtOAc, THF, r.t., 25 min, 72%; (c) thiourea, I_2, 100 °C, DMF, 18–24 h, 57%–89%; (d) 2-aminopyridine, $NaHCO_3$, EtOH, reflux, 2 h, 51%–83%.

Synthetic routes to bromoacetophenones (**10a–10i**) and *N*-heterocyclic derivatives (**12a–12g** and **13a–13g**) are depicted in Scheme 1.[12–15] Bromoacetophenones were prepared from the corresponding commercial acetophenones by stirring the NBS with *p*-TSA in CH_3CN at 80 °C. Subsequently, compounds **12a–12g** were obtained in the presence of thiourea. Condensations of bromoacetophenones with 2-aminopyridine in the presence of sodium bicarbonate formed the *N*-heterocyclic derivatives **13a–13g**. Treatment of compound **9h** with NaH in ethyl acetate and THF gave compound **11**. Chromen-4-one analogues (**14a–14d**) were obtained from the corresponding *O*-hydroxyacetophenones as depicted in Scheme 2.[16]



Scheme 2. Synthesis of chromen-4-one analogues **14a**–**14d**. Reagents and conditions (e) NaH, HCOOEt/THF, aq. HCl, 56%–82%.

Preliminary screening of all compounds was performed for their *in vitro* antifungal activities against seven phytopathogenic fungi, *Fusarium solani*, *Cytospora* sp., *Colletotrichum gloeosporioides*, *Botrytis cinerea*, *Alternaria solani*, *Fusarium graminearum* and *Magnaporthe grisea* by using the mycelium growth rate method with thiophanate-methyl and hymexazol as the positive controls.[17] The results were listed in Table S1. To further evaluate the inhibitory potencies of the synthesized compounds, the half maximal inhibitory concentration (IC_{50}) values of the compounds with high inhibition rate (>70%) were determined. As listed in Table 1, eight of the tested compounds presented different fungicidal activity against the seven phytopathogens. Especially, some synthetic compounds **10a–10f** had more potent antifungal spectrum than the positive controls. Specifically, compound **10a**, with a fluorine atom at C-4', had antifungal activities against a broad panel of fungal species, *F. solani*, *Cytospora* sp., *C. gloeosporioides*, *A. solani*, *F. graminearum* and *M. grisea*, with IC_{50} values in range from 7.4 to 22.8 μg/mL. Interestingly, antifungal potency of **10c** with a 4'-bromine atom was 5.5−fold of **10a** against *Alternaria solani*, while **10b** with a 4'-chlorine atom was inactive, indicating the bromine substituent is the most sensitive to this pathogen. Compound **10a** presented the strongest inhibitory activities against *C. gloeosporioides* (IC_{50} = 10.9 μg/mL), and both **10a** and **10c** exerted the

stronger inhibition of *F. graminearum* growth with their IC_{50} values of 7.4 and 6.2 μg/mL, respectively, than the positive controls (Table 1), while **10b** had no effect. In addition, compounds **10a–10c** inhibited the growth of the rice blast fungus *Magnaporthe grisea*, a causal agent of the prevalent rice blast disease, with IC_{50} values of 11.3, 19.9, and 46.4 μg/mL, respectively, whose potencies were much more potent than these two positive drugs, indicating **10a** was the highest growth inhibitor. In case of compounds **10a–10f**, **12d** and **13b**, possessing a 4'-methyl substituent, displayed potent antifungal activities against two fungal pathogens, *Cytospora* sp. and *Magnaporthe grisea*, with IC_{50} values of 6.0 and 10.7 μg/mL, respectively, which gave much higher activity than the two control fungicides, but had a weak effect on *Botrytis cinerea* (IC_{50} = 22.6 μg/mL). These results indicated that 4'-methyl substituent seems to be the most sensitive to *Cytospora* sp. and *M. grisea.*

Table 1. Antifungal activity of compounds **10a**–**10f**, **12d** and **13b**.

Compd.	IC_{50} (μg/mL)						
	Fusarium solani	*Cytospora* sp.	*Colletotrichum gloeosporioides*	*Botrytis cinerea*	*Alternaria solani*	*Fusarium graminearum*	*Magnaporthe grisea*
10a	22.8±0.6	12.6±1.2	10.9±0.3	>100	48.7±0.9	7.4±0.4	11.3±0.9
10b	27.2±0.4	18.3±0.3	>100	>100	>100	>100	19.9±0.8
10c	25.8±0.9	34.7±0.7	25.6±0.4	73.4±0.6	8.8±0.6	6.2±1.2	46.4±2.2
10d	45.7±0.3	6.0±0.7	>100	22.6±0.4	42.7±0.3	57.7±0.9	10.7±0.9
10e	34.4±0.4	9.5±0.5	46.3±0.6	43.3±3.2	21.4±2.6	25.3±2.3	35.2±0.6
10f	77.8±1.9	26.5±0.6	33.3±0.6	71.7±0.8	25.0±0.4	37.6±0.8	61.9±0.9
12d	31.6±0.8	35.0±0.4	>100	>100	>100	47.7±1.4	>100
13b	>100	>100	>100	>100	>100	>100	38.4±1.2
Th.[a]	32.8±0.6	23.3±0.7	18.0±0.4	>100	>100	92.6±1.2	>100
Hy.[a]	>100	>100	>100	14.0±0.6	71.3±0.7	17.6±0.4	96.9±0.8

[a]Th. = Thiophanate-methyl, Hy. = Hymexazol.

Among the acetophenone derivatives, some compounds possessing α-bromo substitution presented distinct inhibition potency against all tested fungi, whereas the *N*-heterocyclic derivatives and chromen-4-one analogues were found to reduce the activity of the parent compounds, except for **12b** and **13d**. These findings suggested that the carbonyl of acetophenones was the most crucial for higher inhibitory effects and the cyclization of acyl chain was not favorable for improving the activity (i.e.,

12a–12g, **13a–13g**, and **14a–14d**). In comparison with *ortho*-fluoro substituted derivatives of α-bromoacetophenones, the *para*-substituted derivatives had more potent inhibition against tested fungi (**10a** *vs* **10g**) since the inhibition rate of the *ortho*-fluoro derivative **10g** at 50 μg/mL was less than 60% (Table S1).

As shown in Table 1, compounds **10a**, **10c** and **10d** exhibited good antifungal activity against different fungi. Specifically, compound **10d** showed a peak IC_{50} value of 6.0 μg/mL against *Cytospora* sp.. So we chose **10d** and *Cytospora* sp. as the templates to further explore the effect of acetophenone derivatives bearing various substituents at the alpha position of the carbonyl group. We synthesized 12 compounds derived from **10d** and evaluated their antifungal activity against the sensitive strain *Cytospora* sp. (Scheme S1 and Table S2). Under the test concentration (50 μg/mL), α-halogen substituted derivatives exhibited higher antifungal potential than others (excepting for α-fluoro substituent, the inhibition rates of **10d**, **15c** and **15b** over 90%); **15f**, **15h** and **15d** showed moderate potency (with their inhibition rates over 50%); **15j**, **15a**, **15i**, **15l**, **15g**, **15e** and **15k** exhibited low inhibitory to *Cytospora* sp. (as their inhibition rates lower than 50%), correspondingly. Further comparison of IC_{50} values, **10d** (Br, 6.0 μg/mL) > **15c** (I, 13.9 μg/mL) > **15b** (Cl, 15.4 μg/mL), confirmed the importance of bromine atom.

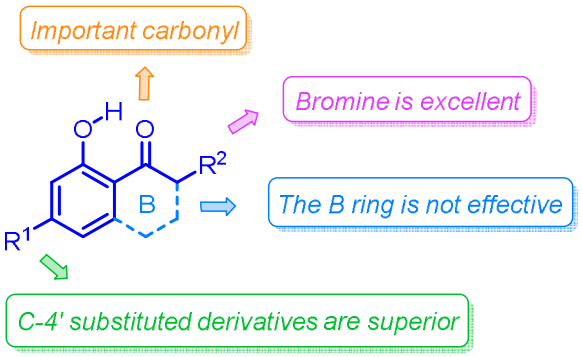


Figure 2. SAR of acetophenone derivatives.

Based on these results, it could be concluded that the structure-antifungal activity relationship was shown in Figure 2: 1) the α-position of acetophenone substituted with

bromine played a greater role for improving the antifungal activities; 2) for all α-bromine acetophenones, C-4' substituted acetophenones exhibited superior antifungal activity over C-2' substituted acetophenones; 3) considering the activity of *N*-heterocyclic derivatives, the carbonyl of acetophenone is a determinant; 4) the B-ring moiety is not effective.

Table 2. Calculated molecular properties of potential compounds.

Compd.	Log *P*[a]	TPSA[b]	nON[c]	nONNH[d]	MW[e]
10a	2.36	17.07	1	0	217.04
10b	2.88	17.07	1	0	233.49
10c	3.01	17.07	1	0	277.94
10d	2.25	26.30	2	0	213.07
10e	1.07	51.21	3	0	229.07
10f	2.31	17.07	1	0	277.13
12d	2.20	48.15	3	2	210.68
13b	3.65	17.31	2	0	208.26

[a]Log *P* = octanol–water partition coefficient, [b]TPSA = topologic polar surface area, [c]nON = number of hydrogen bond acceptors, [d]nONNH = number of hydrogen bond donors, [e]MW = molecular weight.

In order to further verify the "fungicide-likeness" of these compounds, their molecular physicochemical properties, including octanol-water partition coefficient, topologic polar surface area, the number of hydrogen bond acceptors, number of hydrogen bond donors and molecular weight, were calculated by using Molinspiration tool (as shown in Table 2).[21] For all preferred compounds, the Log *P* values are lower than 5, number of hydrogen bond acceptors are lower than 5 and number of hydrogen bond donors are lower than 2. Their physicochemical properties are corresponding to a screening rule of fungicides, which indicate their potential for candidate lead fungicides.[22]

Furthermore, the *in vivo* fungicidal activity (protective effect) of the more promising compound **10d** was tested against *B. cinerea* on tomato (Figure S1A) and *Cytospora* sp. on apple (Figure S1B) by utilizing reported method with a slight modification.[18-20] To our delight, compound **10d** exhibited excellent preventative effects against *B. cinerea* and *Cytospora* sp., with the protective efficacy of 55.3% and 73.1%, respectively. This result demonstrates the potential of the bromoacetophenone

analogues as novel candidates for protecting crop or stored agricultural products.

In conclusion, 53 compounds were prepared and their inhibition activities were evaluated against several plant-pathogenic fungi, and their structure–activity relationships were briefly summarized. Most of the compounds **10a–10f** had a good broad antifungal spectrum and good "fungicide-like" properties. Notably, the *in vivo* antifungal assays using *B. cinerea* and *Cytospora* sp. have demonstrated the potential of bromoacetophenone analogues as novel candidate lead pesticides.

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Supplementary data

Supplementary data associated with this article can be found in the online version.

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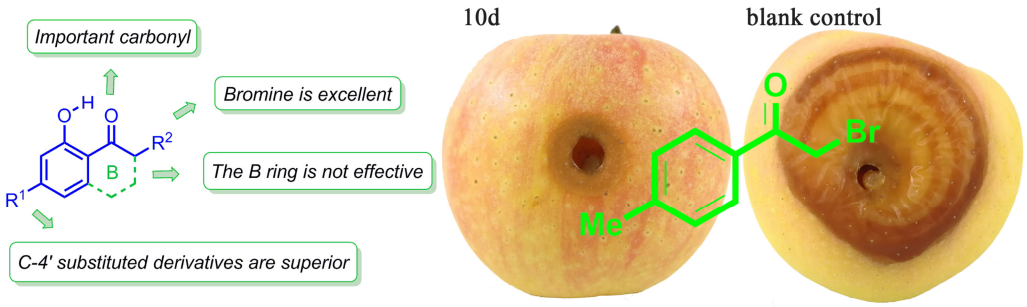

Highlights

- A series of acetophenone derivatives were synthesized and evaluated for antifungal activity.
- The structure–activity relationships were summarized.
- Compound **10d** exhibited excellent antifungal activity *in vitro* and *in vivo*.