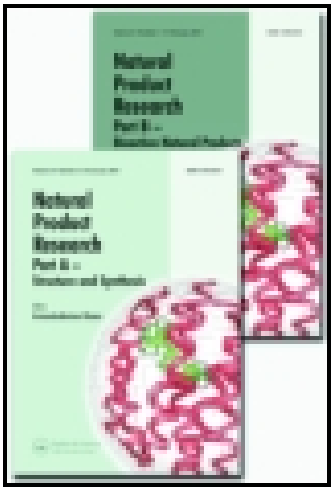

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Synthesis and antifungal activity of chalcone derivatives

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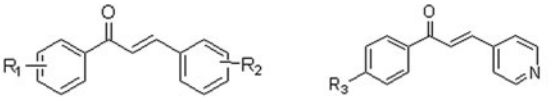

A series of its derivatives of chalcones were designed and synthesized. These compounds exhibited good anti-fungal activity of derivatives of chalcone against Selerotinia sclerotiorum and Helminthosprium maydis.

In the present study, using chalcone as a lead compound, a series of its derivatives (compounds **1**–**30**) were designed and synthesised. Their activity of anti-pathogenic fungi of plants has been evaluated. It is found that these compounds have good antifungal activity against *Sclerotinia sclerotiorum, Helminthosprium maydis, Botrytis cinerea, Rhizoctonia solani* and *Gibberella zeae.* Among them, the inhibition of growth for compound **30** against *S. sclerotiorum* showed 89.9%, with the median effective concentrations (EC_{50}) of 15.4 $\mu g\,mL^{-1}$. The inhibition of growth for compounds **28**, **29** and **30** at a concentration of 100 $\mu g\,mL^{-1}$ against *H. maydis* is 90.3%, 90.7% and 91.1%, with EC_{50} of 15.1, 18.3 and 18.1 $\mu g\,mL^{-1}$, respectively.

Keywords: antifungal activity; chalcone derivatives; inhibition of growth

1. Introduction

Chalcones represent an important group of natural compounds with a variety of biological activities including antibacterial and antifungal ones. They have numerous applications as pesticides, photo-protectors in plastics, food additives as well as anti-inflammatory and anticancer agents (Anto et al. 1995; Iwata et al. 1997; Dimmock et al. 1999; Go et al. 2005; Nowakowska 2007). Therefore, in the present study, chalcone derivatives were synthesised based on it. In the meantime, their antifungal activity has been evaluated in the laboratory to find new fungicides with high efficacy and low toxicity.

Sclerotinia sclerotiorum, Helminthosprium maydis, Botrytis cinerea, Rhizoctonia solani and *Gibberella zeae* are harmful pathogenic fungi of crops or vegetables (Li et al. 2007; Liu & Chen 2007; Chen et al. 2009). Over the past decades, synthetic fungicides including carbendazim have been used to prevent them. However, in recent years, they have developed resistance to the fungicides (Egashira et al. 2000; Ignatova et al. 2000; Guimarães et al. 2004; Ten Have et al. 2007). Moreover, their scope of resistance continues to expand and has already included many

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new fungicides (Finkers, Bai, et al. 2007; Finkers, van den Berg, et al. 2007; Finkers, et al. 2008). Therefore, new fungicides are continually required.

2. Results and discussion

All the synthesised derivatives in Table 1 of chalcone were screened for their activity against *S. sclerotiorum, H. maydis, B. cinerea, R. solani* and *G. zeae*. The results are presented in Table 2.

These compounds showed good antifungal activity (Tables 2–6) against *S. sclerotiorum, H. maydis, B. cinerea, R. solani* and *G. zeae*. Among them, the inhibition of growth for compound **30** against *S. sclerotiorum* exhibited 89.9%, with the median effective concentrations (EC_{50}) of 15.4 $\mu g\,mL^{-1}$. The inhibition of growth for compounds **28**, **29** and **30** at a concentration of 100 $\mu g\,mL^{-1}$ against *H. maydis* showed 90.3%, 90.7% and 91.1%, with EC_{50} of 15.1, 18.3 and 18.1 $\mu g\,mL^{-1}$, respectively. From Table 2, compounds **28**, **29** and **30** (having CH_3, OCH_3 and $CH_2CH_2CH_3$ substituents, respectively) with pyridine rings exhibit better activity than the others having the same substituents but without pyridine rings. It seems that pyridine rings may enhance biological activity of these compounds.

The preliminary results suggested that the design and synthesis of these compounds may be instructive to the investigations of the antifungal activity of derivatives of chalcone. It is also promising and beneficial to further studies in developing new and more effective fungicides in

Table 1. Structure of target compounds **1**–**30**.

Compounds	R_1	R_2	R_3	Yield (%)
1	4–F	2,4–Cl	–	75.0
2	4–OCH_3	2,4–Cl	–	60.2
3	4–CH_3	2,4–Cl	–	67.8
4	2–Cl	2,4–Cl	–	72.0
5	2–NO_2	2,4–Cl	–	79.1
6	2,4-Cl	2,4–Cl	–	68.4
7	H	4–OCH_3	–	73.0
8	4–Br	4–OCH_3	–	65.0
9	4–OCH_3	4–OCH_3	–	85.7
10	4–CH_3	4–OCH_3	–	86.0
11	4–Cl	4–OCH_3	–	88.3
12	3–Cl	4–OCH_3	–	84.5
13	3–Br	4–OCH_3	–	79.6
14	2,4–Cl	4–OCH_3	–	83.5
15	3,4–Cl	4–OCH_3	–	77.9
16	4–C_6H_5	4–OCH_3	–	72.8
17	2–C_4H_3O	4–OCH_3	–	56.5
18	4–F	3,4–(OCH_3)	–	68.4
19	4–Br	3,4–(OCH_3)	–	65.7
20	4–Cl	3,4–(OCH_3)	–	70.3
21	2–Cl	3,4–(OCH_3)	–	81.4
22	4–OCH_3	3,4–(OCH_3)	–	73.4
23	3–Cl	3,4–(OCH_3)	–	67.4
24	3–Br	3,4–(OCH_3)	–	71.6
25	3,4–Cl	3,4–(OCH_3)	–	69.0
26	–	–	4–Cl	84.7
27	–	–	4–Br	84.7
28	–	–	4–CH_3	83.3
29	–	–	4–OCH_3	76.3
30	–	–	4–$(CH_2)_2CH_3$	76.8

Table 2. Antifungal activity of compounds 1–30 against *S. sclerotiorum, H. maydis, B. cinerea, R. solani* and *G. zeae* at 100 μg mL^{-1}.

Compounds	Inhibition of growth (%)[a]				
	S. sclerotiorum	*H. maydis*	*B. cinerea*	*R. solani*	*G. zeae*
1	54.0 ± 0.01	48.9 ± 0.15	66.9 ± 0.05	44.3 ± 0.11	37.7 ± 0.02
2	19.2 ± 0.23	48.9 ± 0.04	36.9 ± 0.03	37.3 ± 0.07	48.2 ± 0.01
3	53.2 ± 0.15	59.1 ± 0.03	47.9 ± 0.13	52.6 ± 0.04	57.9 ± 1.01
4	59.1 ± 0.03	43.5 ± 0.21	40.6 ± 0.06	47.3 ± 0.01	56.7 ± 0.13
5	53.6 ± 0.02	52.1 ± 0.06	51.9 ± 0.02	18.1 ± 0.04	45.3 ± 0.31
6	23.7 ± 0.05	54.1 ± 0.08	54.3 ± 0.07	27.3 ± 0.04	18.1 ± 0.01
7	35.6 ± 0.02	58.6 ± 0.05	58.5 ± 0.41	18.3 ± 0.23	27.9 ± 0.13
8	38.5 ± 0.04	47.9 ± 0.12	46.7 ± 0.02	27.4 ± 0.33	48.9 ± 0.14
9	58.1 ± 0.34	23.1 ± 0.19	38.6 ± 0.25	31.8 ± 0.14	37.7 ± 0.37
10	35.3 ± 0.06	38.5 ± 0.38	42.4 ± 0.16	32.1 ± 0.27	39.6 ± 0.21
11	14.4 ± 0.52	40.1 ± 1.08	47.3 ± 0.62	43.8 ± 0.45	43.2 ± 0.44
12	33.3 ± 0.17	45.6 ± 0.32	58.6 ± 0.54	39.7 ± 0.03	21.6 ± 0.02
13	25.5 ± 0.21	52.3 ± 0.07	48.9 ± 0.49	51.2 ± 0.07	27.9 ± 0.14
14	38.4 ± 0.08	49.8 ± 0.02	47.7 ± 0.34	49.7 ± 0.22	31.5 ± 0.19
15	32.4 ± 0.06	54.8 ± 0.15	28.1 ± 1.04	54.3 ± 0.28	37.6 ± 0.36
16	34.3 ± 0.03	39.4 ± 0.33	34.6 ± 0.06	58.7 ± 0.05	39.3 ± 0.11
17	26.5 ± 0.01	52.3 ± 0.53	38.9 ± 0.02	55.6 ± 0.02	42.5 ± 0.27
18	19.3 ± 0.26	57.8 ± 0.05	41.3 ± 0.01	57.9 ± 0.13	38.8 ± 0.04
19	23.6 ± 0.06	54.3 ± 0.03	42.6 ± 0.08	61.2 ± 0.61	34.8 ± 0.54
20	24.4 ± 0.17	47.2 ± 0.23	49.3 ± 0.07	59.3 ± 0.47	51.2 ± 0.29
21	28.7 ± 0.03	48.3 ± 0.32	51.7 ± 0.63	51.4 ± 0.04	48.8 ± 0.13
22	30.1 ± 0.05	49.2 ± 0.03	56.6 ± 0.24	56.8 ± 0.01	40.7 ± 0.04
23	33.5 ± 0.13	51.6 ± 0.33	42.7 ± 0.27	49.1 ± 0.05	52.3 ± 0.03
24	36.7 ± 0.26	56.7 ± 0.13	48.3 ± 0.33	48.9 ± 0.09	41.9 ± 0.05
25	38.9 ± 0.03	44.2 ± 0.06	52.9 ± 0.05	57.6 ± 0.39	54.7 ± 0.28
26	68.8 ± 0.07	70.9 ± 0.03	46.4 ± 0.04	33.3 ± 0.45	70.1 ± 0.17
27	69.3 ± 0.13	62.9 ± 0.05	45.3 ± 0.08	25.5 ± 0.51	60.9 ± 0.37
28	63.9 ± 0.03	90.3 ± 0.18	42.3 ± 0.09	42.4 ± 0.43	63.9 ± 0.28
29	46.3 ± 0.06	90.7 ± 0.56	65.4 ± 0.81	40.4 ± 0.09	70.1 ± 0.38
30	89.9 ± 0.48	91.1 ± 0.03	43.6 ± 0.56	60.3 ± 0.04	63.5 ± 0.62
Carbendazim	100	87.5	91.3	100	100

[a]Based on the mean of triplicates.

the agricultural chemistry field. However, there is more work to be done. Of course, a number of derivatives of chalcone should be further synthesised for screening and surveying quantitative structure–activity relationships so as to find novel fungicides with high effect and low toxicity. Meanwhile, the mechanisms of activity of compounds **28**, **29** and **30**, and their safety to human being and non-target organisms also need to be investigated.

2.1. *Antifungal activity against* H. maydis

Compared with the efficient fungicide carbendazim, the synthesised compounds were submitted to laboratorial bioassay. The results are presented in Tables 3–5. They had good antifungal activity against *H. maydis*. The EC_{50} value of these compounds reached 15.1, 18.3 and 18.1 μg mL^{-1}, respectively. The results of regressive and correlative analyses indicated that the correlation was significant between concentration and efficacy. The correlative coefficient of compound **28** was 0.9020. The χ^2 test demonstrated that the results were reliable ($\chi^2 = 3.842$, df = 3, $p > 0.05$). The correlative coefficient of compound **29** was 0.9061. The χ^2 test demonstrated that the results were reliable ($\chi^2 = 4.367$, df = 3, $p > 0.05$). The correlative

Table 3. Antifungal activity of compound **28** against *H. maydis*.

	Compound **28**					Carbendazim				
Concentration ($\mu g\,mL^{-1}$)	200	100	50	25	12.5	100	50	25	12.5	6.3
Inhibition of growth[a] (%)	94.5	90.3	70.2	59.7	49.4	87.5	70.8	57.5	41.5	35.5
Regressive equation ($Y = aX + b$)	$Y = 1.3728\,X + 3.8382$					$Y = 1.2373\,X + 3.5189$				
EC_{50} ($\mu g\,mL^{-1}$)	15.1					15.7				
(95% CL)	(10.5–19.5)					(12.1– 19.6)				
Correlative coefficient (r)	0.9020					0.9808				
χ^2	3.842					2.862				

[a]Based on the mean of triplicates.

Table 4. Antifungal activity of compound **29** against *H. maydis*.

	Compound **29**					Carbendazim				
Concentration ($\mu g\,mL^{-1}$)	200	100	50	25	12.5	100	50	25	12.5	6.3
Inhibition of growth[a] (%)	97.6	90.7	70.3	56.7	43.6	87.5	70.8	57.5	41.5	35.5
Regressive equation ($Y = aX + b$)	$Y = 1.6684\,X + 2.8927$					$Y = 1.2373\,X + 3.5189$				
EC_{50} ($\mu g\,mL^{-1}$)	18.3					15.7				
(95% CL)	(14.2–22.4)					(12.1–19.6)				
Correlative coefficient (r)	0.9061					0.9808				
χ^2	4.367					2.862				

[a]Based on the mean of triplicates.

Table 5. Antifungal activity of compound **30** against *H. maydis*.

	Compound **30**					Carbendazim				
Concentration ($\mu g\,mL^{-1}$)	200	100	50	25	12.5	100	50	25	12.5	6.3
Inhibition of growth[a] (%)	97.4	91.1	71.2	55.1	44.4	87.5	70.8	57.5	41.5	35.5
Regressive equation ($Y = aX + b$)	$Y = 1.6654\,X + 2.9040$					$Y = 1.2373\,X + 3.5189$				
EC_{50} ($\mu g\,mL^{-1}$)	18.1					15.7				
(95% CL)	(13.9–22.2)					(12.1–19.6)				
Correlative coefficient (r)	0.9026					0.9808				
χ^2	4.318					2.862				

[a]Based on the mean of triplicates.

coefficient of compound **30** was 0.9026 and the χ^2 test demonstrated that the results were reliable ($\chi^2 = 4.318$, df = 3, $p > 0.05$).

2.2. *Antifungal activity against* S. sclerotiorum

As shown in Table 6, using the efficient fungicide carbendazim as the comparative standard, the synthesised compound was subjected to laboratorial bioassay. Its EC_{50} value reached 15.4 $\mu g\,mL^{-1}$. The results of regressive and correlative analyses revealed that the correlation was significant between concentration and efficacy. The correlative coefficient was 0.9090. As for the results of *S. sclerotiorum*, the χ^2 test also showed that the results were reliable ($\chi^2 = 3.522$, df = 3, $p > 0.05$).

Table 6. Antifungal activity of compound **30** against *S. sclerotiorum*.

	Compound **30**					Carbendazim				
Concentration ($\mu g\,mL^{-1}$)	200	100	50	25	12.5	100	50	25	12.5	6.3
Inhibition of growth[a] (%)	96.5	89.9	73.3	57.8	49.7	94.1	85.5	74.9	61.1	49.5
Regressive equation ($Y = aX + b$)	$Y = 1.4777\,X + 3.2434$					$Y = 1.2683\,X + 4.3132$				
EC_{50} ($\mu g\,mL^{-1}$ (95% CL)	22.4 (11.1–19.7)					3.5 (2.4–(4.6)				
Correlative coefficient (r)	0.9090					0.9614				
χ^2	3.522					0.605				

[a]Based on the mean of triplicates.

3. Experimental

3.1. *General*

S. sclerotiorum, H. maydis, B. cinerea, R. solani and *G. zeae* were obtained from the Chinese Academy of Agricultural Sciences. They were preserved at 4°C. All chemicals and solvents were purchased from commercial sources unless specified otherwise. IR spectra were recorded on a Thermofisher Nicolet-6700 spectrophotometer (Thermo Fisher Scientific, MA, USA). ^{1}H NMR spectra were taken on a Varian Unity Inova-400 instrument (Varian Medical Systems, Palo Alto, CA, USA) using deuteron-chloroform and DMSO-d6 as the solvent. The NMR data were provided in the supplementary materials.

3.2. *Synthesis of target compounds*

The target compounds were synthesised in accordance with the reaction shown in Figures 1 and 2. Appropriate aldehyde (0.01 mol) and acetophenone derivatives (0.01 mol) were dissolved in anhydrous ethanol (15 mL). The reaction mixture was stirred at 0°C for 8 h. Then, 10% NaOH (5 mL) was slowly added to the above mixture under stirring until the reaction was complete. The precipitate was filtered and washed with still water. The pure compounds were obtained by re-crystallisation in acetone and water.

3.3. *Assay of antifungal activity*

The antifungal activity of the synthesised compounds against *S. sclerotiorum, H. maydis, B. cinerea, R. solani* and *G. zeae* were determined using the plate growth rate method (Huang & Yang 2006).

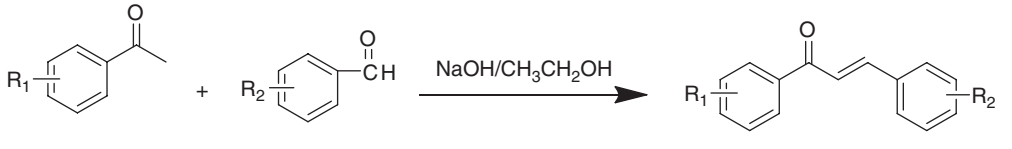

Figure 1. Synthetic method of target compounds **1**–**25**.

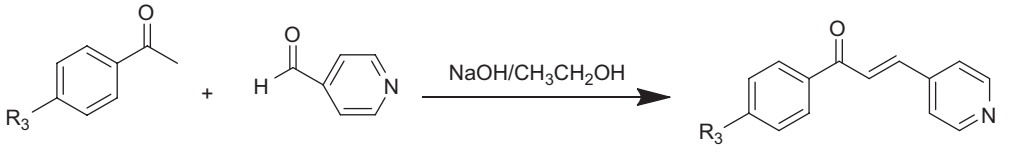

Figure 2. Synthetic method of target compounds **26**–**30**.

The synthesised compounds and carbendazim (purity 90%) were dissolved in dimethyl sulphoxide, respectively. They were added to the sterile culture medium (PDA) at 45°C, mixed to homogeneity and transferred to sterile Petri dishes to solidify. A mycelium agar disc (5 mm in diameter) of the target fungi was placed in the center of PDA plates. They were incubated at 28°C in the dark until the target fungi used as controls covered the surface of these plates. Control groups were treated with the corresponding solutions without the synthesised compounds or carbendazim. Each experiment was replicated three times. The diameter of the fungi in the cultures was measured and the inhibition of growth was calculated according to the formula of Abbott. EC_{50} values were calculated with the Statistics Package for the Social Sciences (SPSS) based on probit analysis.

4. Conclusions

A series of derivatives of chalcone have been successfully synthesised in this work, and were tested for their antifungal activity against *S. sclerotiorum, H. maydis, B. cinerea, R. solani* and *G. zeae* for the first time. It is found that these compounds have good antifungal activity against *S. sclerotiorum, H. maydis, B. cinerea, R. solani* and *G. zeae*. Among them, the inhibition of growth for compound **30** against *S. sclerotiorum* showed 89.9%, with the median effective concentrations (EC_{50}) of 15.4 $\mu g\,mL^{-1}$. The inhibition of growth for compounds **28**, **29** and **30** at a concentration of 100 $\mu g\,mL^{-1}$ against *H. maydis* exhibited 90.3%, 90.7% and 91.1%, with EC_{50} of 15.1, 18.3 and 18.1 $\mu g\,mL^{-1}$, respectively.

Supplementary material

Experimental details relating to this paper are available online, alongside Figures S1–S60.

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References

Anto R, Sukumaran K, Kuttan G, Rao M, Subbaraju V, Kuttan R. 1995. Anticancer and antioxidant activity of synthetic chalcones and related compounds. Cancer Lett. 97(1):33–37. doi:10.1016/0304-3835(95)03945-S.

Chen XJ, Cao MJ, Wang Y, Tong YH, Xu JY. 2009. Procymidone resistant mutagenesis of *Sclerotinia sclerotiorum* isolated from rapeseed stem. Chin J Oil Crop Sci. 31:503–508.

Dimmock JR, Elias DW, Beazely MA, Kandepu NM. 1999. Bioactivities of chalcones. Curr Med Chem. 6:1125–1149.

Egashira H, Kuwashima A, Ishiguro H, Fukushima K, Kaya T, Imanishi S. 2000. Screening of wild accessions resistant to gray mold (*Botrytis cinerea* Pers.) in lycopersicon. Acta Physiol Plant. 22(3):324–326. doi:10.1007/s11738-000-0046-x.

Finkers R, Bai Y, van den Berg P, van Berloo R, Meijer-Dekens F, ten Have A, van Kan J, Lindhout P, van Heusden A W. 2008. Quantitative resistance to *Botrytis cinerea* from *Solanum neorickii*. Euphytica. 159(1–2):83–92. doi:10.1007/s10681-007-9460-0.

Finkers R, van den Berg P, van Berloo R, ten Have A, van Heusden AW, van Kan JAL, Lindhout P. 2007. Three QTLs for *Botrytis cinerea* resistance in tomato. Theor Appl Genet. 114(4):585–593. doi:10.1007/s00122-006-0458-0.

Finkers R, Van Heusden AW, Meijer-Dekens F, van Kan JA, Maris P, Lindhout P. 2007. The construction of a *Solanum habrochaites* LYC4 introgression line population and the identification of QTLs for resistance to *Botrytis cinerea*. Theor Appl Genet. 114(6):1071–1080. doi:10.1007/s00122-006-0500-2.

Go ML, Wu X, Liu XL. 2005. Chalcones: an update on cytotoxic and chemoprotective properties. Curr Med Chem. 12(4):483–499. doi:10.2174/0929867053363153.

Guimarães RL, Chetelat RT, Stotz HU. 2004. Resistance to *Botrytis cinerea* in *Solanum lycopersicoides* is dominant in hybrids with tomato, and involves induced hyphal death. Eur J Plant Pathol. 110(1):13–23. doi:10.1023/B:EJPP.0000010133.62052.e4.

Huang W, Yang GF. 2006. Microwave-assisted, one-pot syntheses and fungicidal activity of polyfluorinated 2-benzylthiobenzothiazoles. Bioorg Med Chem. 14(24):8280–8285. doi:10.1016/j.bmc.2006.09.016.

Ignatova SI, Gorshkova NS, Tereshonkova TA. 2000. Resistance of tomato F1 hybrids to grey mold. Acta Physiol Plant. 22(3):326–328. doi:10.1007/s11738-000-0047-9.

Iwata S, Nishino T, Inoue H, Nagata N, Satomi Y, Nishino H, Shibata S. 1997. Antitumorigenic activities of chalcones (II): photo-isomerization of chalcones and the correlation with their biological activities. Biol Pharm Bull. 20(12):1266–1270. doi:10.1248/bpb.20.1266.

Li W, Zhou YJ, Chen HG. 2007. Sensitivity of *Sclerotinia sclerotiorum* isolates to carbendazim in Jiangsu province. Chin J Oil Crop Sci. 29:63–68.

Liu KY, Chen FX. 2007. Test of the toxin ability of agro-chemical in control of sclerotina. Anhui Agric Sci. 35:756–757.

Nowakowska Z. 2007. A review of anti-infective and anti-inflammatory chalcones. Eur J Med Chem. 42(2):125–137. doi:10.1016/j.ejmech.2006.09.019.

ten Have A, van Berloo R, Lindhout P, van Kan JAL. 2007. Partial stem and leaf resistance against the fungal pathogen *Botrytis cinerea* in wild relatives of tomato. Eur J Plant Pathol. 117(2):153–166. doi:10.1007/s10658-006-9081-9.