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Decursinol chloroacrylates useful as fungicides

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Abstract

Natural products decursin and decursinol angelate were recently reported as benign fungicides for controlling rice blast. Inspired by the structural similarity of the cumarin compounds and gained hint from the skeletal motifs, we designed and prepared synthetic compounds to increase the natural product efficacy and evaluated their antifungal activities against various plant disease pathogens in vitro. Synthetically prepared compound **4** and **5** indeed suppressed the mycelial growth of *B. cinerea*, *F. oxysporum*, *P. italicum*, and *R. quercus-mongolicae*. Additionally, compound **5** effectively prevents the growth of *C. coccodes* and *C. parasitica*. Furthermore, both **4** and **5** possess better inhibitory activities on spore germination of *F. oxysporum and M. oryzae* than the natural product decursin and commercial pesticide Iprodione. These results suggest that the effect of the lead compound for plant disease protection can be improved by tuning the structure of the original natural product and decursinol chloroacrylates **4** and **5** are candidates for the control of *F. oxysporum* and *M. oryzae*.

Keywords: Natural products, Semisynthesis, Antifungal activity, Decursin derivatives, Fusarium oxysporum, Magnaporthe oryzae, Organic Synthesis

Introduction

Plant diseases have damaged the quantity and the quality of crop production, causing massive economic problems and further threatening global food safety. Plant disease symptoms vary in type and severity, and can even lead to the death of animals that feed on. For example, rice blast disease caused by *M. Oryzae* now spreads in 85 rice-cultivating countries. It causes leaf, neck rot, panicle, node blast, and collar rot, and an annual loss in global rice production is about 10 to 30% [1]. Also, *B. cinerea*, which causes soft rot and gray mold, has a wide host range of more than 200, including grapefruit and vegetables, resulting in significant economic losses. Although several chemical reagents are available to prevent this pathogen, the genomic plasticity of *B. cinereal* makes the control difficult [2]. To date, chemical fungicides are one of the most widely utilized means of controlling plant diseases worldwide. However, long-term use of them can increase the resistance of pathogens and cause fatal damage to aquatic ecosystems [3, 4]. Therefore, it is urgent to develop new fungicides that are fatal to fungi but harmless to the environment and animals.

Since the discovery of penicillin, there has been continuous research on developing drugs and crop protecting reagents through chemical modification and simplification of natural bioactive compounds. Natural substances have biocompatibility, high selectivity, and low environmental impact so that similar effects can be expected on their derivatives [5]. For instance, Canagliflozin, sold under the name Invokana to treat type 2 diabetes, is a derivative of phlorizin, a natural substance found in the root bark of unripe apples. The drug, which acts as an inhibitor of SGLT2, has developed into analogues with increased selectivity, such as dapagliflozin and canagliflozin [6]. Natural products Strobilurin A and B were first

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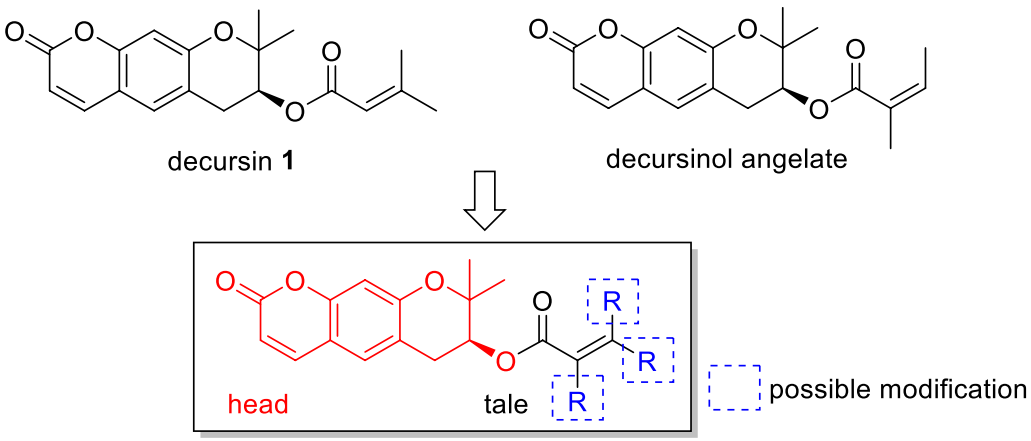


Scheme 1 A rationale for modification

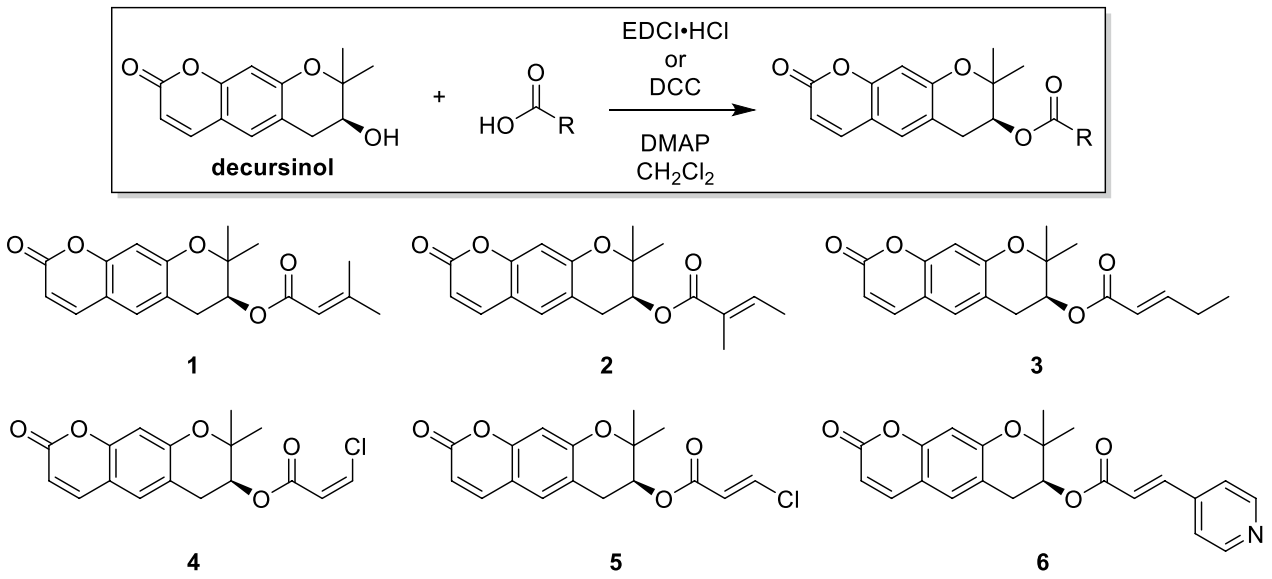
isolated from *S. tenacellus* in 1977 and found that they have inhibitory activity against various fungi. Although strobilurins rapidly inhibit spore germination fungi and do not cause harm to terrestrial animals, they result in critical damage to aquatic animals. In the past 20 years, numerous synthetic strobilurin fungicides such as pyraclostrobin, fluoxastrobin and orysastrobin have been developed and used [7].

Decursin **1** is one of the coumarin compounds in *Angelica gigas*, which is used as a medicinal agent in Oriental Medicine. In 1966, the substance was first isolated, and its biochemical activity has since been studied [8]. Decursin has (1) inhibitory effects on several cancer cell lines, including breast cancer, and (2) potential as the medicine of inflammatory diseases caused by macrophages [9, 10]. On the other side, the antimicrobial effects of decursin have rarely been studied in the field of crop protection. Interestingly, Kim and coworkers disclosed decursin and its constitutional isomer decursinol angelate (B) inhibit spore germination and mycelial growth of *M. oryzae*, proving decursin useful in plant disease control, especially rice blast [11, 12].

Decursin and decursinol angelate have the same molecular formula but different substitution patterns at the unsaturated ester part (Scheme 1). The skeletal difference is subtle, but the inhibition rate was noticeably different [12]. We envisioned that changing substituents in the unsaturated ester part could increase or decrease the inhibition rate and eventually develop a benign candidate for rice blast. With the question in mind, we designed several unsaturated ester tales, focusing on the possible modification sites (Scheme 2). Based on the rationale, derivatives 2–6 of decursin were synthesized from decursinol by Steglich esterification conditions and were tested their activities on various plant pathogens (Table 1).

Materials and methods (including Safety information)

Unless otherwise noted, all reactions were carried out under Ar in flamed-dried glassware using anhydrous solvents. Anhydrous solvents were prepared by distillation over the indicated drying agents prior to use and were transferred under Ar: THF, Et_2O (Mg/anthracene), toluene (Na/K), CH_2Cl_2, MeOH (Mg); DMF and Et_3N were dried by an adsorption solvent purification system based on molecular sieves. Thin layer chromatography (TLC): Macherey–Nagel precoated plates (POLYGRAM®SIL/UV254). Flash chromatography: Merck silica gel 60 (40−63 μm) with technical grade solvents. NMR: Spectra were recorded on Bruker AV VIII 400 or 600 spectrometers in the solvents indicated. The solvent signals were used as references, and the chemical shifts were converted to the TMS scale ($CDCl_3$: $\delta_C = 77.0$ ppm; residual $CHCl_3$ in $CDCl_3$: $\delta_H = 7.26$ ppm; CD_3OD: $\delta_C = 49.0$ ppm; residual CHD_2OD in CD_3OD: $\delta_H = 3.31$ ppm; CD_2Cl_2: $\delta_C = 54.0$ ppm; residual $CHDCl_2$ in CD_2Cl_2: $\delta_H = 5.32$ ppm). FT-IR spectra were obtained on Thermo Scientific Nicolet 6700 and reported in frequency of the absorption (cm^{-1}). High resolution mass spectra (HRMS) were recorded on an AB SCIEX Q-TOF 5600 mass spectrometer. Optical rotation ($[\alpha]_D^{20}$ and $[\alpha]_D^{25}$): Krüss P8000-T, 10 cm/1 mL cell. Unless otherwise noted, all commercially available compounds (Acros, Aldrich, Alfa Aesar, TCI) were used as received. Melting points were determined on a A. KRÜSS OPTRONIC M3000.



Scheme 2 A general method for the synthesis of decursin (**1**) and its derivatives

Decursin (compound 1)

N-(3-Dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (0.392 g, 2.00 mmol, 2 equiv) and DMAP (0.0489 g, 0.0400 mmol, 0.4 equiv) were added to a stirred mixture of 3-methyl crotonic acid (0.120 g, 1.20 mmol, 1.2 equiv) and decursinol (0.246 g, 1.00 mmol) in CH_2Cl_2 (8 mL) at room temperature. After stirring overnight, the reaction was quenched with H_2O. After phase separation, the aqueous layer was rinsed with ethyl acetate. The organic extracts were combined, dried over Na_2SO_4, and concentrated *in vacuo*. Purification of the crude product by flash chromatography (hexane:EtOAc, 7:3) gave the title compound as a white solid (0.199 g, 60.6%). R_f – 0.80 (50% EtOAc: 50% Hexane); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.57 (d, *J* = 9.4 Hz, 1H), 7.14 (s, 1H), 6.78 (s, 1H), 6.23 (d, *J* = 9.4 Hz, 1H), 5.66 (s, 1H), 5.07 (app.t, *J* = 4.9 Hz, 1H), 3.18 (dd, *J* = 17.1, 4.8 Hz, 1H), 2.85 (dd, *J* = 17.1, 4.8 Hz, 1H), 2.13 (s, 3H), 1.87 (s, 3H), 1.37 (s, 3H), 1.35 (s, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 165.9, 161.4, 158.6, 156.6, 154.3, 143.3, 128.8, 116.1, 115.7, 113.4, 112.9, 104.8, 76.9, 69.2, 28.0, 27.6, 25.1, 23.3, 20.5; HR-MS (ESI): *m*/*z* calcd for $C_{19}H_{21}O_5^+$ $[M+H]^+$: 329.1384, found 329.1384

Spectral characteristics were identical to those previously reported [13].

(S)-8,8-Dimethyl-2-oxo-7,8-dihydro-2H,6H-pyrano[3,2-g] chromen-7-yl (E)-2-methylbut-2-enoate (compound 2)

N-(3-Dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (0.636 g, 3.24 mmol, 2 equiv) and DMAP (0.0792 g, 0.648 mmol, 0.4 equiv) were added to a stirred mixture of Tiglic acid (0.195 g, 1.95 mmol, 1.2 equiv) and decursinol (0.400 g, 1.62 mmol) in CH_2Cl_2 (8 mL) at room temperature. After being stirred at this temperature overnight, the reaction was quenched with H_2O. After phase separation, the aqueous layer was rinsed with ethyl acetate. The organic extracts were combined, dried over $MgSO_4$, and concentrated *in vacuo*. Purification of the crude product by flash chromatography (hexane:EtOAc, 7:3) gave the title compound as transparent oil (0.465 g, 87.5%). R_f – 0.69 (50% EtOAc: 50% Hexane); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.58 (d, *J* = 9.5 Hz, 1H), 7.15 (s, 1H), 6.84 – 6.78 (m, 2H), 6.23 (d, *J* = 9.5 Hz, 1H), 5.08 (t, *J* = 5.0 Hz, 1H), 3.25 – 3.15 (m, 1H), 2.88 (dd, *J* = 17.2, 5.3 Hz, 1H), 1.82 – 1.78 (m, 3H), 1.76 (m, 3H), 1.39 (s, 3H), 1.37 (s, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 167.3, 161.4, 156.6, 154.4, 143.3, 138.6, 128.8, 128.3, 116.0, 113.5, 113.0, 104.8, 76.9, 70.3, 27.9, 25.2, 23.3, 14.6, 12.2; HR-MS (ESI): *m*/*z* calcd for $C_{19}H_{21}O_5^+$ $[M+H]^+$: 329.1384, found 329.1383.

(S)-8,8-dimethyl-2-oxo-7,8-dihydro-2H,6H-pyrano[3,2-g] chromen-7-yl (E)-pent-2-enoate (compound 3)

A mixture of (*S*)-(+)-decurinol (0.144 g, 0.583 mmol, 1 equiv), *N,N'*-Dicyclohexylcarbodiimide (0.181 g, 0.875 mmol, 1.5 equiv), and 4-(dimethylamino)pyridine (0.0285 g, 0.233 mmol, 0.4 equiv) was dissolved in anhydrous dichloromethane. Then, trans-2-pentenoic acid (0.064 ml, 0.641 mmol, 1.1 equiv) was added, and the reaction mixture was stirred at room temperature overnight. The reaction mixture was then filtered through a pad of Celite with CH_2Cl_2, and the filtrate was

Table 1 In vitro antifungal activity of decursin and it's derivatives (MIC[b], μg/ml)

Compd	Fungi														Oomycetes		
	A.a. [a]	*A.n*	*B.c*	*B.d*	*C.c*	*C.p*	*F.g*	*F.o*	*F.s*	*M.o*	*O.u*	*P.i*	*S.h*	*R.s*	*R.q*	*P.ca*	*P.ci*
Decursin (**1**)	> 200	> 200	> 200	> 200	> 200	> 200	> 200	> 200	> 200	25	> 200	> 200	> 200	> 200	> 200	> 200	> 200
Compound **2**	> 200	> 200	200	> 200	> 200	> 200	> 200	> 200	> 200	25	> 200	> 200	> 200	> 200	> 200	> 200	> 200
Compound **3**	> 200	> 200	> 200	> 200	> 200	> 200	> 200	> 200	> 200	25	> 200	> 200	> 200	> 200	> 200	> 200	> 200
Compound **4**	> 200	> 200	200	> 200	> 200	> 200	> 200	50	> 200	12.5	> 200	100	> 200	> 200	50	> 200	> 200
Compound **5**	> 200	> 200	200	> 200	50	100	> 200	12.5	> 200	3.125	200	> 200	> 200	> 200	> 200	> 200	> 200
Compound **6**	> 200	> 200	200	> 200	> 200	> 200	> 200	> 200	> 200	> 200	> 200	> 200	> 200	> 200	> 200	> 200	> 200
Decursinol	> 200	> 200	> 200	> 200	> 200	> 200	> 200	> 200	> 200	200	> 200	> 200	> 200	> 200	> 200	> 200	> 200
Iprodione	3.125	> 200	1.563	> 200	> 200	> 200	> 200	> 200	> 100	25	> 100	6.25	12.5	6.25	12.5	3.125	3.125
Azoxystrobin	0.05	100	> 100	> 200	> 200	> 200	> 200	> 200	> 100	0.05	> 100	0.05	12.5	> 200	> 100	> 100	> 100

[a] A.a., Alternaria alternata; A.n., Aspergillus niger; B.c., Botrytis cinerea; B.d., Botryosphaeria dothidea; C.c., Colletotrichum coccodes; C.p., Cryphonectria parasitica; F.g., Fusarium graminearum; F.o., Fusarium oxysporum; F.s., Fusarium solani; M.o., Magnaporthe oryzae. O.u., Ophiostoma ulmi; P.i., Penicillium italicum; S.h., Sclerotinia homoeocarpa; R.s., Rhizoctonia solani; R.q., Raffaelea quercus-mongolicae; P.ca., Phytophthora cactorum; P.ci., Phytophthora cinnamomic. All values were generated based on three replicates

[b] MIC: Minimum inhibitory concentration

concentrated *in vacuo*. Purification of the crude product by flash chromatography (hexane:EtOAc, 7:3) gave the title compound as a white solid (0.168 g, 88.3%). R_f – 0.68 (50% EtOAc: 50% Hexane); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.58 (d, *J* = 9.5 Hz, 1H), 7.15 (s, 1H), 7.04 (dt, *J* = 15.6, 6.3 Hz, 1H), 6.81 (s, 1H), 6.23 (d, *J* = 9.5 Hz, 1H), 5.80 (dt, *J* = 15.7, 1.7 Hz, 1H), 5.11 (app.t, *J* = 4.9 Hz, 1H), 3.20 (dd, *J* = 18.2, 4.9 Hz, 1H), 2.88 (dd, *J* = 17.2, 4.8 Hz, 1H), 2.27 – 2.15 (m, 2H), 1.39 (s, 3H), 1.36 (s, 3H), 1.05 (t, *J* = 7.4 Hz, 3H); HR-MS (ESI): *m*/*z* calcd for $C_{19}H_{21}O_5^+$ $[M+H]^+$: 329.1384, found 329.1383. Spectral characteristics were identical to those previously reported [14].

(S)-8,8-dimethyl-2-oxo-7,8-dihydro-2H,6H-pyrano[3,2-g] chromen-7-yl (Z)-3-chloroacrylate (compound 4)

A mixture of (*S*)-(+)-decurinol (0.202 g, 0.82 mmol, 1.0 equiv), *N,N'*-Dicyclohexylcarbodiimide (0.254 g, 1.23 mmol, 1.5 equiv), and 4-(dimethylamino)pyridine (0.040 g, 0.33 mmol, 0.4 equiv) was dissolved in anhydrous dichloromethane. Then, cis-chloro acrylic acid (0.096 g, 0.90 mmol, 1.1 equiv) was added, and the resulting mixture was stirred at room temperature overnight. The reaction mixture was then filtered through a pad of Celite with CH_2Cl_2, and the filtrate was concentrated *in vacuo*. Purification of the crude product by flash chromatography (hexane:EtOAc, 8:2) gave the title compound as a white solid (126.4 mg, 46.0%). %). R_f – 0.52 (50% EtOAc: 50% Hexane); M. p. 97.9–100.6 ℃;$[\alpha]_D^{20}$: + 195.8 (c = 0.1, $CHCl_3$); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.58 (d, *J* = 9.2 Hz, 1H), 7.16 (s, 1H), 6.80 (s, 1H), 6.75 (d, *J* = 8.3 Hz, 1H), 6.24 (d, *J* = 9.5 Hz, 1H), 6.18 (d, *J* = 8.3 Hz, 1H), 5.15 (app.t, *J* = 4.9 Hz, 1H), 3.24 (ddd, *J* = 17.1, 4.8, 1.1 Hz, 1H), 2.92 (dd, *J* = 17.7, 5.0 Hz, 1H), 1.40 (s, 3H), 1.38 (s, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 162.8, 161.3, 156.4, 154.3, 143.3, 134.1, 128.8, 120.9, 115.6, 113.5, 113.0, 104.8, 76.5, 70.8, 27.8, 25.1, 23.2; IR(neat): 3100, 3046, 2982, 2921, 2849, 1721, 1625, 1564, 1516 cm^{-1} HR-MS (ESI): *m*/*z* calcd for $C_{17}H_{16}ClO_5^+$ $[M+H]^+$: 335.0681, found: 335.0681.

(S)-8,8-dimethyl-2-oxo-7,8-dihydro-2H,6H-pyrano[3,2-g] chromen-7-yl (E)-3-chloroacrylate (compound 5)

To a solution of (*S*)-(+)-decurinol (0.207 g, 0.84 mmol, 1 equiv), *N,N'*-Dicyclohexylcarbodiimide (0.347 g, 1.68 mmol, 1.5 equiv), and 4-(dimethylamino)pyridine (0.0410 g, 0.356 mmol, 0.4 equiv) in anhydrous CH_2Cl_2 was added trans-chloro acrylic acid (0.0984 g, 0.924 mmol, 1.1 equiv). The reaction mixture was stirred at room temperature overnight. The reaction mixture was then filtered through a pad of Celite with CH_2Cl_2, and the filtrate was concentrated *in vacuo*. Purification of the crude product by flash chromatography (hexane:EtOAc, 8:2) gave the title compound as a white solid (69.8 g, 24.8%). R_f – 0.69 (50% EtOAc: 50% Hexane); M.p. 150.7–153.3 ℃;$[\alpha]_D^{20}$: + 55.0 (c = 0.2, $CHCl_3$); ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.58 (d, *J* = 9.5 Hz, 1H), 7.35 (d, *J* = 13.5 Hz, 1H), 7.15 (s, 1H), 6.79 (s, 1H), 6.27–6.19 (m, 2H), 5.13 (app.t, *J* = 4.7 Hz, 1H), 3.21 (dd, *J* = 17.3, 4.8 Hz, 1H), 2.89 (dd, *J* = 17.3, 4.6 Hz, 1H), 1.38 (s, 3H), 1.36 (s, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 163.5, 161.3, 156.3, 154.4, 143.2, 139.0, 128.8, 124.4, 115.4, 113.6, 113.1, 105.0, 76.5, 70.9, 27.9, 25.0, 23.4; IR(neat): 3020, 3085, 3005, 2927, 2851, 1714, 1622, 1604, 1560 cm^{-1}; HR-MS (ESI) *m*/*z* calcd for $C_{17}H_{16}ClO_5^+$ $[M+H]^+$: 335.0689, found: 335.0681.

(S)-8,8-dimethyl-2-oxo-7,8-dihydro-2H,6H-pyrano[3,2-g] chromen-7-yl (E)-3-(pyridin-4-yl)acrylate (compound 6)

N-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (0.108 g, 0.55 mmol, 1.1 equiv) and DMAP (0.0061 g, 0.05 mmol, 0.1 equiv) were added to a stirred mixture of (*E*)-3-(pyridin-4-yl)acrylic acid (0.082 g, 0.55 mmol, 1.1 equiv) and decursinol (0.123 g, 0.50 mmol) in CH_2Cl_2 (5 mL) at room temperature. After being stirring at this temperature for 24 h, the reaction was quenched with H_2O. After phase separation, the aqueous layer was rinsed with CH_2Cl_2. The organic extracts were combined, dried over Na_2SO_4, and concentrated *in vacuo*. Purification of the crude product by flash chromatography (hexane:EtOAc, 3:7) gave the title compound as a white solid (0.169 g, 89.6%). R_f – 0.32 (70% EtOAc: 30% Hexane); M.p. 211–215 °C;$[\alpha]_D^{20}$ = + 53.9 (c = 0.25, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.72–8.59 (m, 2H), 7.79–7.53 (m, 2H), 7.36–7.30 (m, 2H), 7.18 (s, 1H), 6.83 (s, 1H), 6.58 (d, *J* = 16.0 Hz, 1H), 6.24 (d, *J* = 9.4 Hz, 1H), 5.21 (app.t, *J* = 4.6 Hz, 1H), 3.26 (ddd, *J* = 17.2, 4.8, 1.2 Hz, 1H), 3.00–2.89 (m, 1H), 1.44 (s, 3H), 1.39 (s, 3H); ^{13}C NMR ($CDCl_3$, 151 MHz): δ 165.3, 161.3, 156.4, 154.4, 149.6, 143.2, 142.6, 142.5, 128.8, 123.1, 122.4, 115.4, 113.7, 113.2, 105.0, 76.6, 71.0, 28.0, 25.0, 23.6; IR (film): 3067, 2981, 2931, 2852, 1724, 1626, 1562, 1515, 1135 cm^{-1}; HRMS: Calcd for $C_{22}H_{20}NO_5^+$ $[M+H]^+$ 378.1336, found 378.1342.

Experimental procedures

Fungal strains and media

Phytopathogenic fungi and oomycetes (Table 1) were used to test the antifungal activity of decursin and its derivatives. These strains were maintained on potato dextrose agar [15]. All fungal and oomycete strains were cryogenically stored in 20% glycerol at – 80 ℃ before use.

In vitro antifungal activity of decursin and its derivatives

The antifungal activity of decursin and its derivatives was evaluated by a serial broth dilution method as described previously [16]. Phytophathogenic fungi and oomycetes

listed in Table 1 were used in this study. Decursin and its derivatives was dissolved in dimethylsulfoxide (DMSO) at a concentration of 20 mg/m1 as a stock solution, which was used to determine the minimal inhibitory concentration (MIC) value against mycelial growth. Decursin and its derivatives were treated in a range of 0.048–200 μg/ml and the final concentration of DMSO was 1% v/v, and potato dextrose broth treated with DMSO was used as a control. All plates were incubated for 4–5 days at 25 ℃, and MIC values were measured. The experiment was repeated three times in triplicate against each fungal and oomycete pathogen [16].

Spore germination assay

For spore germination assay, *F. oxysporum* strains were cultured in 5 ml of carboxymethylcellulose medium (CMC) for five days at 25 °C on a rotary shaker (200 rpm) (Leslie and Summerell, 2006). To obtain *M. oryzae* spores, *M. oryzae* strains were incubated on PDA at 25 °C for 10 days. The percentage of growth inhibition (mean ± standard deviation) was calculated from mean values as:

$$\%\text{inhibition} = 100[(A—B)/A],$$

where A: mycelial growth in control and B: mycelial growth in sample.

Results/discussion

Chemistry

Decursin derivatives could be synthesized by Steglich esterification using carbodiimide reagents such as 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride(EDCI) reagent or N,N'-Dicyclohexylcarbodiimide (DCC) reagent, respectively. However, due to its low toxicity and easily removable byproducts, we preferred EDCI when preparing most substrates. In the case of decursinol chloroacrylates, the use of EDCI reagent did not give the desired products at all, whereas DCC reagent provided desired products in moderate yield (compound **4** and **5**). Chloroacrylic acid is thought to be incompatible with EDCI·HCl because a spot of the acid disappeared on TLC plates, and no ester product was formed. When EDCI·HCl was used to synthesize derivative **3**, isolation of the product from impurities was tedious during the purification process. On the contrary, compound **6** with an aromatic ring was obtained in higher yields using EDCI·HCl.

In vitro antifungal activity of decursin and its derivatives against various plant pathogens

We investigated in vitro antimicrobial activity of decursin and its derivatives against various plant pathogenic fungi and oomycetes (Table 1). Most tested strains showed relatively strong resistance against decursin and derivatives except *M. oryzae* (MIC, 25 μg/ml) (Table 1). Whereas most derivatives of decursin showed similar antimicrobial activity against plant pathogens, 4 or 5 efficiently inhibited mycelial growth of some fungal strains (*Botrytis cinerea*, *F. oxysporum*, *Penicillium italicum*, and *Raffaelea quercus-mongolicae*). Among tested compounds, only **5** strongly inhibited the mycelial growth of *Colletotrichum coccodes* and *Cryphonectria parasitica*. In contrast, MIC values of 4 were 100 μg/m1 and 50 μg/m1 against *P. italicum* and *R. quercus-mongolicae*, respectively; MIC of 5 was over 200 μg/m1 (Table 1). We also compared the antifungal activity of the derivatives with commercial fungicide Iprodione and Azoxystrobin to gauge any possible commercial value. With respect to *Magnaporthe oryzae,* compound 4 and 5 are more effective than Iprodione (MIC, 12.5, 3.125, and 25 μg/ml, respectively), at least in the in vitro assay. The presence of halogen atoms within compounds 4 and 5 could contribute to the higher inhibitory effects as halogen atoms can change the electron density of molecules and provide a site for possible hydrogen bonding.

Decursin and the synthetic derivatives would be degraded into alcohols and carboxylic acids by enzymatic hydrolysis. We tested decursinol and cis-3-chloroacrylic acid to identify active components and found both are inactive. These results indicate that the head and tale component should be linked together to show the antifungal activities.

Spore germination and viability assay

The effects of decursin, 4, and 5 on the spore germination of *F. oxysporum* and *M. oryzae* were further investigated (Table 2). Decursin slightly inhibited germination of *F. oxysporum* spores regardless of tested concentrations. However, compounds 4 or 5 significantly decreased

Table 2 Effect of decursin, 4 and 5 on spore germination of *F. oxysporum* and *M. oryzae*

Compd	Conc (μg/ml)	*F. oxysporum*	*M. oryzae*
		Spore germination (%, mean ± SD, n = 3)	Spore germination (%, mean ± SD, n = 3)
Control		82.3 ± 6.8	93.5 ± 2.0
Decursin	200	62.5 ± 5.4	22.5 ± 2.6
	50	69.7 ± 0.7	33.3 ± 2.5
4	200	15.1 ± 1.8	1.2 ± 0.1
	50	30.4 ± 3.2	2.0 ± 0.3
5	200	12.4 ± 2.1	0.8 ± 0.7
	50	17.6 ± 1.8	1.4 ± 0.4

[a] Each value represents the mean ± standard deviation of three replicates

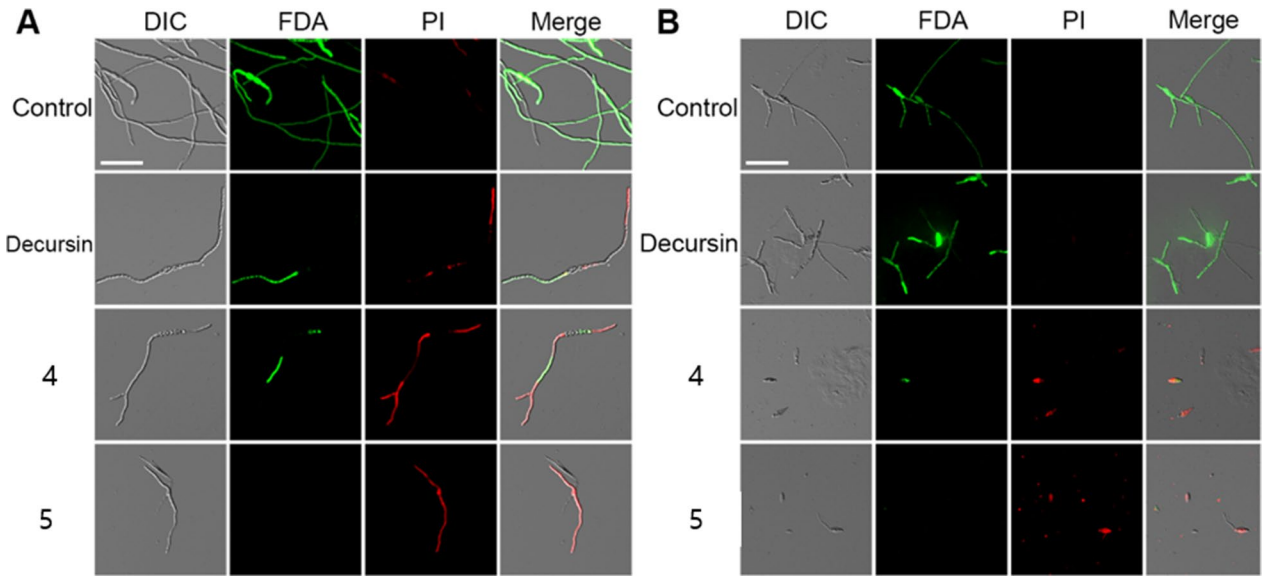


Fig. 1 Inhibitory effects of decursin and compound 4 and 5 against spore germination of **A** *F. oxysporum* and **B** *M. oryzae*. [Spores were stained with FDA and PI for fluorescence-based live-dead assays. The concentration of decursin, 4, and 5 was 200 mg/m1. Photographs were taken 24 h after spore germination. Scale bar = 40 μm]

germination rates of *F. oxysporum* spores. In accordance with MIC values, most spores of *M. oryzae* failed to germinate when of 4 or 5 were supplemented over 50 μg/m1.

To evaluate the viability of germinated spores, we performed FDA and PI double-staining assay. In living cells, the FDA changes from non-fluorescent FDA to the green fluorescent metabolite fluorescein. Contrastively, the nucleus staining dye PI cannot pass through a viable cell membrane. As shown in Fig. 1, spores of *F. oxysporum* and *M. oryzae* successfully germinated with strong green fluorescence (Fig. 1). When decursin was treated, however, most of *F. oxysporum* spores germinated, but germinated spores showed abnormal and short length of germ tube. Moreover, red fluorescence in mycelial cells were easily observed, indicating that some cells of germinated spores are inviable. In treatment with 4 or 5, most mycelial cells died 24 h after germination (Fig. 1A). In *M. oryzae*, most spores failed to germinate became inviable after treatment of 4 and 5 (Fig. 1B).

Mycelial growth inhibition test

We further investigated the inhibitory effects of decursin, 4, and 5 on vegetative growth of *F. oxysporum* and *M. oryzae* (Table 3 and Fig. 2). As expected, decursin, 4, and 5 significantly reduced mycelial growth of both fungal strains and 5 showed more potent inhibitory activity than 4. Intriguingly, decursin more effectively inhibited mycelial growth of *F. oxysporum* than 4 or 5 (Table 3 and Fig. 2). In contrast, spore germination and initial mycelial growth were much highly inhibited by 4 or 5 compared to decursin.

Table 3 Inhibitory activity of decursin, 4, and 5 against mycelial growth of *F. oxysporum* and *M. oryzae*

Compd	Conc (μg/ml)	Mycelial growth inhibition (%)[a]	
		F. oxysporum[b]	*M. oryzae*[c]
Control		0 ± 0	0 ± 0
DMSO	125 μl	18.1 ± 2.5	3.5 ± 2.7
Decursin	50	77.5 ± 1.4	22.4 ± 2.3
	12.5	73.9 ± 1.4	10.5 ± 2.2
4	50	55.4 ± 0	100 ± 0
	12.5	26.1 ± 2.9	51.8 ± 2.3
5	50	64.7 ± 1.4	100 ± 0
	12.5	40.5 ± 1.4	100 ± 0

[a] Each value represents the mean ± standard deviation of three replicates

[b] Radial growth was measured 3 days after inoculation

[c] Radial growth was measured 5 days after inoculation

In summary, a series of decursin-like compounds were synthesized through steglich esterification and tested for antifungal activities. In the bioassay, decursinol chloroacrylates selectively inhibited the mycelial growth of several fungi, while other derivatives showed no antifungal effects or similar effects to decursin. In particular, the chloroacrylates showed improved spore germination inhibition of *F. oxysporum* and *M. oryzae*. As

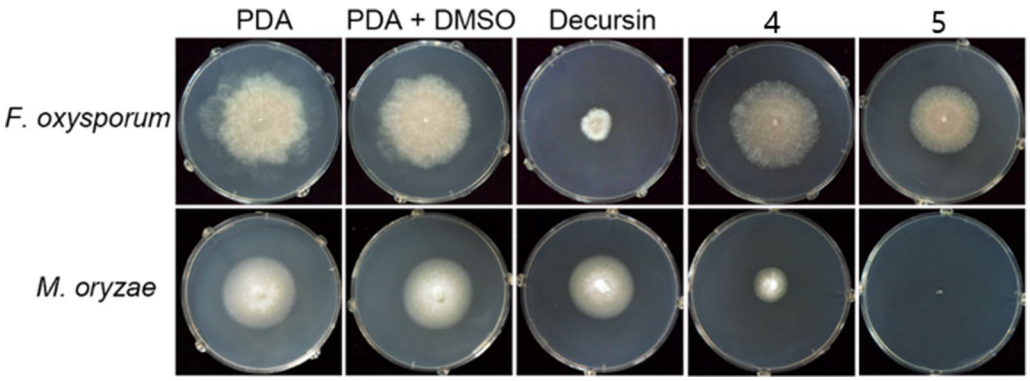


Fig. 2 Mycelial growth of *F. oxysporum* and *M. oryzae* strains on PDA and PDA supplemented with decursin, 4, and 5. [Concentration of decursin, 4, and 5 was 12.5 mg/m1. Photographs were taken 3 days after inoculation in *F. oxysporum*, and 5 days after inoculation in *M. oryzae*]

the chloroacrylates are easily degraded in environments, and their decomposed byproducts are found to be inactive, they would be promising candidates for the control of *F. oxysporum* and *M. oryzae*. This work suggests that the effect of the lead compound in the development of fungicides for plant disease protection can be improved by modifying the structure of the original natural product. Further studies are underway in our laboratory to evaluate in vivo antifungal activities of the chloroacrylates and will be disclosed in due course.

Abbreviations

DCC: N,N′-Dicyclohexylcarbodiimide; EDCI·HCl: 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide Hydrochloride; DMAP: 4-Dimethylaminopyridine.

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Author contributions

All authors equally contributed to the study conception and design. Y-JS and HJ synthesized and characterized materials, and JS carried out the biological assays. HS and YK prepared the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

All authors declare no conflict of interest.

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