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Regular Article

Discovery of anti-phytopathogenic fungal activity of a new type of (*S*)-coumarin bearing a phenylpropanoid unit at the 3-position

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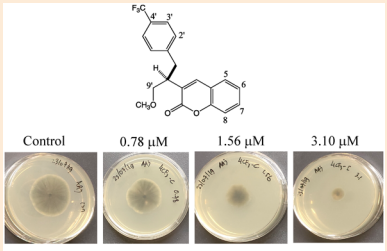
Supplementary material

The enantiospecific anti-phytopathogenic fungal activity of a new type of coumarin bearing a phenylpropanoid unit at the 3-position was found. (*S*)-3-[1-Methoxy-3-(4-methoxyphenyl)prop-2-yl]coumarin ((*S*)-**5**: EC_{50}=16.5 μM) was 30 times more effective than the (*R*)-form against the *Alternaria alternata* Japanese pear pathotype. Derivatives bearing different substituents on the 7′-aromatic ring and the coumarin ring were synthesized to discover the more potent compounds. The 3′-CF_3 and 4′-CF_3 derivatives, **39** and **40**, respectively, had the lowest EC_{50} values (1–2 μM) in this project, suggesting that the size of the electron-withdrawing and hydrophobic substituents at these positions gave an advantage. On the coumarin ring, the presence of the OCH_3 or CH_3 group at the 5-position accelerated the activity, as the (4′-OCH_3, 5-OCH_3) derivative **41** and (4′-OCH_3, 5-CH_3) derivative **45** were, respectively, 4–5 times more potent than the 4′-OCH_3 derivative (*S*)-**5**.



Alternaria alternata Japanese pear pathotype

Keywords: lignan, benzylidene lactone, antifungal activity, coumarin, lignano-9,9′-lactone.

Introduction

The development of novel pesticides based on dietary components is one of the important strategies in green chemistry. We have developed a stereoselective synthetic method to provide phytotoxic (*R*)-3-(1-aryl-3-hydroxyprop-2-yl)coumarin.[1)] As we found the nonenzymatic *trans–cis* isomerization of *o*-hydroxycinnamic acid in the biosynthesis of coumarin (Fig. 1),[2,3)] *E*-*β*-benzyl-*α*-(2-hydroxybenzylidene)-*γ*-butyrolactone **1a** (R_3=H) could be isomerized to 3-(1-aryl-3-hydroxyprop-2-yl)coumarin **2a** (R_3=OH) *via* *Z*-*β*-benzyl-*α*-(2-hydroxybenzylidene)-*γ*-butyrolactone in the plant body. In previous work, we carried out an antifungal study on (*R*)-*E*-*β*-benzyl-*α*-(2-methoxybenzylidene)-*γ*-butyrolactone **1b** (R_3=CH_3).[4)] Antifungal research on the (*R*/*S*)-3-(1-arylprop-2-yl)coumarin derivative **2a** (R_3=OH), **2b** (R_3=H), and **2c** (R_3=OCH_3) and the structural isomer of **2a** (**1a**: R_3=H) bearing phenolic benzylidene structure is a new project. The anti-phytopathogenic fungal activities and structure-function analyses of butane,[5)] tetrahydrofuran,[6)] benzylidene lactone type lignans,[7)] and neolignan[8)] have been reported. Research on coumarins as anti-phytopathogenic fungal reagents has also been continuing. Thus, the extraction,[9–13)] syntheses,[14–26)] identification of the mode of action as a DNA gyrase inhibitor,[27)] and the effect on peroxisomes[28)] have been reported. In this new study, we report on the antifungal evaluation of (*R*/*S*)-3-(1-arylprop-2-yl)coumarin, which has both lignan and coumarin structures. In the preliminary examination, the effect of the stereochemistry of phenolic benzylidene and the 9′-structurally arranged coumarin on the activity was evaluated. After screening to determine the stereochemistry of phenolic benzylidene and 9′-derivatives of coumarin showing higher activity, synthesized analogues bearing different substituents on the aromatic rings were applied to antifungal tests to clarify the effect of the substituent and position on the growth of fungi. The utilities of natural benzylidene and new type of coumarin lignans for pesticides would be shown in this research.

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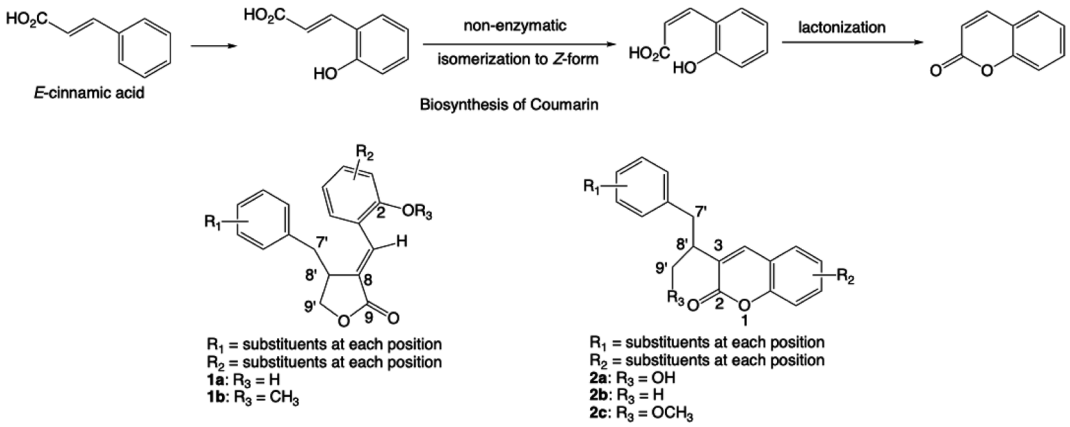


Fig. 1. Biosynthesis of coumarin and structures of *E*-2-hydroxybenzylidene lactone and coumarin bearing phenylpropanoid at 3-position

Materials and methods

Melting points (mp) data are uncorrected. The silica gel used was Wako gel C-300, FUJIFILM Co., INC. (Tokyo, Japan). Optical rotations were measured on a JASCO P-2100 instrument (JASCO Corporation, Japan). ^{1}H and ^{13}C NMR data were recorded on a JNM ECS400 spectrometer (JEOL, Tokyo, Japan). EIMS data were measured with ESI-JMS-MS700V (JEOL, Tokyo, Japan). Compounds (*S*)-**3**, (*R*)-**3**, (*S*)-**4**, (*R*)-**4**, (*R*)-**5**, and (*R*)-**6** have been synthesized in our previous experiment.[1,4] The compounds (*S*)-**5**, (*S*)-**6**, and **7**–**28** and 9′-hydroxychoumarin intermediates (**I-2**), which are enantiomers of previously synthesized compounds, were synthesized in this research (supporting information). The general synthetic methods of (*S*)-**5** and **29**–**50**, which were also synthesized in this project, from intermediate (**I-2**) and their chemical data are described in this section.

*1. General procedure for the syntheses of 9′-methoxycoumarins **29**–**50** from 9′-hydroxycoumarin intermediates* (***I-2***)

A reaction mixture of 9′-hydroxycoumarin intermediate **I-2**s (1.0 eq.), Ag_2O (2.5 eq.), and CH_3I (10 eq.) in DMF (substrate 0.8 mmol/1 mL) was stirred at room temperature for 16 hr before filtration with EtOAc. The filtrate was washed with brine. The organic solution was separated and dried (Na_2SO_4). Concentration followed by silica gel column chromatography (EtOAc/hexane=1/3 or 5% ether/hexane) gave 9′-OCH_3 compounds.

1.1. (S)-*3-[1-Methoxy-3-phenylprop-2-yl]-2H-chromen-2-one* (***29***)

14% yield, colorless oil; $[\alpha]_D^{25}$+39 (*c* 0.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.03 (2H, d, *J*=7.4 Hz), 3.30 (3H, s), 3.38 (1H, m), 3.54 (1H, dd, *J*=9.4, 4.4 Hz), 3.59 (1H, dd, *J*=9.4, 6.3 Hz), 7.14–7.25 (6H, m), 7.29 (1H, d, *J*=8.3 Hz), 7.40 (1H, d, *J*=7.7 Hz), 7.45 (1H, dd, *J*=7.9, 7.7 Hz), 7.50 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 36.2, 42.6, 59.0, 72.7, 116.4, 119.5, 124.3, 126.3, 127.7, 2×128.5, 2×129.2, 129.6, 130.9, 139.5, 140.0, 153.1, 161.4; MS (EI) *m*/*z* 294 (M^+, 24), 262 (100), 249 (58), 203 (97), 171 (49); HRMS (EI) *m*/*z* calcd for $C_{19}H_{18}O_3$ 294.1256, found 294.1264.

1.2. (S)-*3-[1-Methoxy-3-(2-methoxyphenyl)prop-2-yl]-2H-chromen-2-one* (***30***)

12% yield, colorless oil; $[\alpha]_D^{25}$+76 (*c* 0.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.05 (2H, d, *J*=7.2 Hz), 3.32 (3H, s), 3.45 (1H, m), 3.58 (1H, dd, *J*=9.4, 5.0 Hz), 3.68 (1H, dd, *J*=9.4, 7.1 Hz), 3.71 (3H, s), 6.76 (1H, d, *J*=8.2 Hz), 6.84 (1H, dd, *J*=7.4, 7.4 Hz), 7.13 (1H, dd, *J*=8.7, 8.1 Hz), 7.20 (1H, dd, *J*=8.1, 7.5 Hz), 7.26–7.30 (2H, m), 7.36 (1H, d, *J*=8.7 Hz), 7.38 (1H, s), 7.44 (1H, dd, *J*=8.2, 7.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.5, 41.6, 55.1, 58.8, 73.1, 110.1, 116.2, 119.5, 120.3, 124.1, 127.4, 2×127.6, 129.7, 130.5, 130.9, 139.2, 153.0, 157.5, 161.3; MS (EI) *m*/*z* 324 (M^+, 41), 292 (68), 121 (100); HRMS (EI) *m*/*z* calcd for $C_{20}H_{20}O_4$ 324.1362, found 324.1370.

1.3. (S)-*3-[1-Methoxy-3-(3-methoxyphenyl)prop-2-yl]-2H-chromen-2-one* (***31***)

27% yield, colorless oil; $[\alpha]_D^{25}$+56 (*c* 0.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.02 (2H, d, *J*=6.8 Hz), 3.32 (3H, s), 3.40 (1H, m), 3.55 (1H, dd, *J*=9.4, 4.4 Hz), 3.61 (1H, dd, *J*=9.4, 6.4 Hz), 3.76 (3H, s), 6.71–6.80 (3H, m), 7.17 (1H, dd, *J*=7.8, 7.8 Hz), 7.23 (1H, dd, *J*=7.6, 7.5 Hz), 7.30 (1H, d, *J*=8.2 Hz), 7.43 (1H, d, *J*=7.8 Hz), 7.46 (1H, dd, *J*=8.2, 7.8 Hz), 7.53 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 36.1, 42.4, 55.1, 58.9, 72.6, 111.7, 114.6, 116.3, 119.4, 121.5, 124.2, 127.6, 129.3, 129.5, 130.8, 139.9, 141.0, 153.0, 159.5, 161.3; MS (EI) *m*/*z* 324 (M^+, 74), 292 (100), 279 (55), 203 (93); HRMS (EI) *m*/*z* calcd for $C_{20}H_{20}O_4$ 324.1362, found 324.1365.

1.4. (S)-*3-[1-Methoxy-3-(4-methoxyphenyl)prop-2-yl]-2H-chromen-2-one* [(S)-***5***]

31% yield, colorless crystals, mp 95–96°C; $[\alpha]_D^{25}$+51(*c* 0.2, $CHCl_3$). NMR data agreed with previously synthesized (*R*)-form.

1.5. (S)-*3-[1-Methoxy-3-(2-methylphenyl)prop-2-yl]-2H-chromen-2-one* (***32***)

19% yield, colorless oil; $[\alpha]_D^{25}$+37 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.36 (3H, s), 2.98–3.08 (2H, m), 3.31 (3H, s), 3.36 (1H, m), 3.54 (1H, dd, *J*=9.3, 4.2 Hz), 3.62 (1H, dd, *J*=9.3, 6.3 Hz), 7.07–7.12 (4H, m), 7.23 (1H, dd, *J*=7.5, 7.5 Hz), 7.30 (1H, d, *J*=8.2 Hz), 7.42 (1H, d, *J*=8.3 Hz), 7.45 (1H, dd, *J*=7.5, 7.5 Hz), 7.57 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.5,

33.7, 41.6, 58.9, 72.7, 116.4, 119.5, 124.3, 125.9, 126.5, 127.7, 130.0, 130.1, 130.5, 130.9, 136.6, 137.7, 139.9, 153.2, 161.4; MS (EI) *m/z* 308 (M^+, 36), 276 (73), 203 (100), 171 (39), 105 (55); HRMS (EI) *m/z* calcd for $C_{20}H_{20}O_3$ 308.1412, found 308.1416.

1.6. (S)-*3*-[*1-Methoxy-3-*(*3-methylphenyl*)*prop-2-yl*]-*2*H-*chromen-2-one* (***33***)

17% yield, colorless oil; $[\alpha]_D^{25}$+35 (*c* 0.6, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.29 (3H, s), 2.98 (2H, d, *J*=7.4 Hz), 3.30 (3H, s), 3.38 (1H, m), 3.53 (1H, dd, *J*=9.3, 4.3 Hz), 3.59 (1H, dd, *J*=9.3, 6.5 Hz), 6.97–7.02 (3H, m), 7.13 (1H, dd, *J*=7.4, 7.4 Hz), 7.22 (1H, dd, *J*=8.5, 7.5 Hz), 7.29 (1H, d, *J*=8.2 Hz), 7.40–7.46 (2H, m), 7.52 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.5, 36.2, 42.5, 59.0, 72.7, 116.4, 119.5, 124.3, 126.3, 127.1, 127.7, 128.3, 129.8, 130.0, 130.8, 138.0, 139.4, 139.9, 153.1, 161.4; MS (EI) *m/z* 308 (M^+, 47), 276 (97), 263 (52), 203 (100), 171 (57); HRMS (EI) *m/z* calcd for $C_{20}H_{20}O_3$ 308.1412, found 308.1417.

1.7. (S)-*3*-[*1-Methoxy-3-*(*4-methylphenyl*)*prop-2-yl*]-*2*H-*chromen-2-one* (***34***)

24% yield, colorless crystals, mp 91–92°C (hexane); $[\alpha]_D^{25}$+46 (*c* 0.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.28 (3H, s), 2.98 (2H, d, *J*=7.4 Hz), 3.30 (3H, s), 3.36 (1H, m), 3.52 (1H, dd, *J*=9.5, 4.5 Hz), 3.59 (1H, dd, *J*=9.5, 6.4 Hz), 7.04 (2H, d, *J*=8.3 Hz), 7.07 (2H, d, *J*=8.3 Hz), 7.22 (1H, dd, *J*=7.7, 7.3 Hz), 7.29 (1H, d, *J*=8.2 Hz), 7.40 (1H, d, *J*=7.7 Hz), 7.44 (1H, dd, *J*=8.2, 7.3 Hz), 7.50 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.2, 35.8, 42.6, 59.0, 72.7, 116.4, 119.5, 124.3, 127.7, 2×129.1, 2×129.2, 129.8, 130.8, 135.8, 136.3, 139.9, 153.1, 161.4; MS (EI) *m/z* 308 (M^+, 41), 276 (100), 263 (52), 203 (68), 171 (52), 105 (83); HRMS (EI) *m/z* calcd for $C_{20}H_{20}O_3$ 308.1412, found 308.1419.

1.8. (S)-*3*-[*1-*(*2-Fluorophenyl*)*-3-methoxyprop-2-yl*]-*2*H-*chromen-2-one* (***35***)

29% yield, colorless oil; $[\alpha]_D^{25}$+75 (*c* 0.3, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.10 (2H, d, *J*=7.1 Hz), 3.32 (3H, s), 3.44 (1H, m), 3.58 (1H, dd, *J*=9.3, 4.9 Hz), 3.67 (1H, dd, *J*=9.3, 6.7 Hz), 6.95 (1H, d, *J*=9.0 Hz), 7.01 (1H, dd, *J*=9.1, 7.4 Hz), 7.12–7.26 (3H, m), 7.29 (1H, d, *J*=8.3 Hz), 7.40 (1H, d, *J*=7.7 Hz), 7.46 (1H, dd, *J*=8.1, 7.7 Hz), 7.50 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.3, 41.8, 58.9, 72.9, 115.3 (d, *J*=22.2 Hz), 116.3, 119.3, 124.0, 124.2, 126.2 (d, *J*=15.8 Hz), 127.6, 128.1 (d, *J*=8.0 Hz), 129.0, 130.8, 131.4 (d, *J*=4.7 Hz), 139.9, 153.1, 161.16, 161.18 (d, *J*=244.7 Hz); MS (EI) *m/z* 312 (M^+, 28), 280 (69), 267 (77), 203 (100), 171 (49); HRMS (EI) *m/z* calcd for $C_{19}H_{17}FO_3$ 312.1162, found 312.1168.

1.9. (S)-*3*-[*1-*(*3-Fluorophenyl*)*-3-methoxyprop-2-yl*]-*2*H-*chromen-2-one* (***36***)

30% yield, colorless oil; $[\alpha]_D^{25}$+50 (*c* 0.7, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.04 (2H, d, *J*=7.3 Hz), 3.32 (3H, s), 3.35 (1H, m), 3.54 (1H, dd, *J*=9.3, 4.3 Hz), 3.59 (1H, dd, *J*=9.3, 6.4 Hz), 6.85–6.93 (2H, m), 6.98 (1H, d, *J*=7.5 Hz), 7.18–7.26 (2H, m), 7.31 (1H, d, *J*=8.1 Hz), 7.42 (1H, d, *J*=7.6 Hz), 7.47 (1H, dd, *J*=8.1, 7.7 Hz), 7.53 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 35.8, 42.4, 58.9, 72.4, 113.2, 115.9, 116.3, 119.3, 124.2, 124.7, 127.6, 129.2, 129.8 (d, *J*=8.3 Hz), 130.9, 139.9, 142.0 (d, *J*=7.2 Hz), 153.0, 161.2, 162.8 (d, *J*=245.7 Hz); MS (EI) *m/z* 312 (M^+, 37), 280 (67), 267 (61), 203 (100), 171 (44); HRMS (EI) *m/z* calcd for $C_{19}H_{17}FO_3$ 312.1162, found 312.1170.

1.10. (S)-*3*-[*1-*(*4-Fluorophenyl*)*-3-methoxyprop-2-yl*]-*2*H-*chromen-2-one* (***37***)

21% yield, colorless crystals, mp 65–67°C; $[\alpha]_D^{25}$+46 (*c* 0.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.01 (2H, d, *J*=7.5 Hz), 3.32 (3H, s), 3.34 (1H, m), 3.54 (1H, dd, *J*=9.5, 4.2 Hz), 3.58 (1H, dd, *J*=9.5, 6.2 Hz), 6.94 (2H, m), 7.15 (2H, m), 7.24 (1H, dd, *J*=7.7, 7.6 Hz), 7.30 (1H, d, *J*=8.3 Hz), 7.42 (1H, d, *J*=7.6 Hz), 7.47 (1H, dd, *J*=8.3, 7.7 Hz), 7.50 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 35.3, 42.7, 58.9, 72.5, 2×115.2 (d, *J*=21.2 Hz), 116.4, 119.3, 124.2, 127.6, 129.4, 2×130.5 (d, *J*=8.0 Hz), 130.9, 135.1, 139.9, 153.0, 161.3, 161.4 (d, *J*=244.2 Hz); MS (EI) *m/z* 312 (M^+, 36), 280 (75), 267 (45), 203 (100), 171 (49); HRMS (EI) *m/z* calcd for $C_{19}H_{17}FO_3$ 312.1162, found 312.1167.

1.11. (S)-*3*-[*1-Methoxy-3-*(*2-trifluoromethylphenyl*)*prop-2-yl*]-*2*H-*chromen-2-one* (***38***)

18% yield, colorless oil; $[\alpha]_D^{25}$+78 (*c* 0.7, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.24 (2H, d, *J*=7.4 Hz), 3.31 (3H, s), 3.50 (1H, m), 3.56 (1H, dd, *J*=9.0, 4.5 Hz), 3.74 (1H, dd, *J*=9.0, 7.2 Hz), 7.21–7.31 (3H, m), 7.35–7.42 (3H, m), 7.46 (1H, dd, *J*=8.2, 7.5 Hz), 7.51 (1H, s), 7.62 (1H, d, *J*=7.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 32.6, 42.3, 58.9, 72.9, 116.3, 119.3, 124.3, 124.6 (q, *J*=273.8 Hz), 126.2 (q, *J*=5.7 Hz), 126.4, 127.6, 128.8 (q, *J*=29.5 Hz), 129.0, 130.9, 131.4, 131.7, 138.1, 140.3, 153.1, 161.2; MS (EI) *m/z* 362 (M^+, 8), 330 (58), 277 (80), 203 (100); HRMS (EI) *m/z* calcd for $C_{20}H_{17}F_3O_3$ 362.1130, found 362.1125.

1.12. (S)-*3*-[*1-Methoxy-3-*(*3-trifluoromethylphenyl*)*prop-2-yl*]-*2*H-*chromen-2-one* (***39***)

23% yield, colorless crystals, mp 108–111°C; $[\alpha]_D^{25}$+25 (*c* 1.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.10 (2H, d, *J*=7.5 Hz), 3.32 (3H, s), 3.38 (1H, m), 3.54 (2H, d, *J*=5.0 Hz), 7.25 (1H, dd, *J*=7.4, 6.3 Hz), 7.31 (1H, d, *J*=8.8 Hz), 7.36–7.50 (6H, m), 7.56 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 35.9, 42.2, 58.9, 72.2, 116.4, 119.3, 123.2 (q, *J*=3.8 Hz), 124.1 (q, *J*=272.2 Hz), 124.3, 125.9 (q, *J*=3.6 Hz), 127.6, 128.9, 129.2, 130.7 (q, *J*=32.0 Hz), 131.0, 132.5, 140.1, 140.4, 153.0, 161.3; MS (EI) *m/z* 362 (M^+, 16), 330 (63), 203 (100); HRMS (EI) *m/z* calcd for $C_{20}H_{17}F_3O_3$ 362.1130, found 362.1120.

1.13. (S)-*3*-[*1-Methoxy-3-*(*4-trifluoromethylphenyl*)*prop-2-yl*]-*2*H-*chromen-2-one* (***40***)

13% yield, colorless crystals, mp 84–86°C (hexane); $[\alpha]_D^{25}$+42 (*c* 0.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.09 (1H, dd, *J*=14.1, 8.5 Hz), 3.12 (1H, dd, *J*=14.1, 7.3 Hz), 3.33 (3H, s), 3.39 (1H, m), 3.54 (1H, dd, *J*=9.4, 4.6 Hz), 3.58 (1H, dd, *J*=9.4, 5.7 Hz), 7.23–7.26 (2H, m), 7.31–7.33 (3H, m), 7.43 (1H, d, *J*=7.6 Hz), 7.46–7.54 (3H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 35.9, 42.4, 58.9, 72.4, 116.4, 119.3, 124.2 (q, *J*=271.5 Hz), 124.3, 2×125.3 (q, *J*=3.6 Hz), 127.6, 128.6 (q, *J*=32.5 Hz), 129.1, 2×129.4, 131.0, 140.1, 143.7, 153.1, 161.2; MS (EI) *m/z* 362 (M^+, 26), 330 (59), 317 (26), 297 (31), 203 (100), 171 (31); HRMS (EI) *m/z* calcd for $C_{20}H_{17}F_3O_3$ 362.1130, found 362.1123.

1.14. (S)-*5-Methoxy-3-*[*1-methoxy-3-*(*4-methoxyphenyl*)-*prop-2-yl*]-*2*H-*chromen-2-one* (***41***)

42% yield, colorless oil, $[\alpha]_D^{25}$+55 (*c* 0.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 2.97 (2H, d, *J*=7.6 Hz), 3.31 (3H, s), 3.36 (1H, m), 3.52 (1H, dd, *J*=9.5, 4.7 Hz), 3.59 (1H, dd, *J*=9.5, 6.5 Hz), 3.77 (3H, s), 3.91 (3H, s), 6.67 (1H, d, *J*=8.2 Hz), 6.80 (2H, d, *J*=8.5 Hz), 6.89 (1H, d, *J*=8.3 Hz), 7.12 (2H, d, *J*=8.5 Hz), 7.37 (1H, dd, *J*=8.3, 8.2 Hz), 7.89 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 35.3, 42.7, 55.2, 55.9, 58.8, 72.8, 104.8, 108.7, 110.0, 2×113.7, 127.5, 2×130.0, 131.2, 131.5, 134.7, 154.0, 155.7, 157.8, 161.4; MS (EI) *m*/*z* 354 (M^+, 19), 322 (22), 233 (15), 121 (100); HRMS (EI) *m*/*z* calcd for $C_{21}H_{22}O_5$ 354.1467, found 354.1472.

1.15. (S)-*6-Methoxy-3-*[*1-methoxy-3-*(*4-methoxyphenyl*)-*prop-2-yl*]-*2*H-*chromen-2-one* (***42***)

82% yield, colorless crystals, mp 88–90°C (*iso*-Pr_2O-hexane), $[\alpha]_D^{25}$+27 (*c* 0.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 2.95 (1H, dd, *J*=13.9, 8.0 Hz), 2.99 (1H, dd, *J*=13.9, 6.9 Hz), 3.31 (3H, s), 3.34 (1H, m), 3.53 (1H, dd, *J*=9.4, 4.5 Hz), 3.59 (1H, dd, *J*=9.4, 6.3 Hz), 3.77 (3H, s), 3.83 (3H, s), 6.80 (2H, d, *J*=8.6 Hz), 6.85 (1H, d, *J*=2.8 Hz), 7.04 (1H, dd, *J*=9.0, 2.8 Hz), 7.10 (2H, d, *J*=8.6 Hz), 7.23 (1H, d, *J*=9.0 Hz), 7.45 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 35.2, 42.7, 55.2, 55.8, 58.9, 72.5, 109.6, 2×113.7, 117.3, 118.5, 119.7, 129.9, 2×130.1, 131.4, 139.7, 147.4, 155.9, 157.9, 161.5; MS (EI) *m*/*z* 354 (M^+, 64), 322 (58), 233 (18), 202 (22), 121 (100); HRMS (EI) *m*/*z* calcd for $C_{21}H_{22}O_5$ 354.1467, found 354.1475.

1.16. (S)-*7-Methoxy-3-*[*1-methoxy-3-*(*4-methoxyphenyl*)-*prop-2-yl*]-*2*H-*chromen-2-one* (***43***)

41% yield, colorless crystals, mp 83–84°C, $[\alpha]_D^{25}$+60 (*c* 0.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 2.69 (2H, d, *J*=7.3 Hz), 3.31 (3H, s), 3.52 (1H, dd, *J*=9.3, 4.6 Hz), 3.59 (1H, dd, *J*=9.3, 6.4 Hz), 3.76 (3H, s), 3.85 (3H, s), 6.78–6.81 (4H, m), 7.10 (2H, d, *J*=8.2 Hz), 7.30 (1H, d, *J*=8.3 Hz), 7.43 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 35.2, 42.5, 55.2, 55.7, 58.8, 72.7, 100.3, 112.3, 113.0, 2×113.7, 125.9, 128.4, 2×130.0, 131.5, 139.9, 154.7, 157.9, 161.6, 162.0; MS (EI) *m*/*z* 354 (M^+, 26), 322 (24), 233 (90), 202 (13), 121 (100); HRMS (EI) *m*/*z* calcd for $C_{21}H_{22}O_5$ 354.1467, found 354.1470.

1.17. (S)-*8-Methoxy-3-*[*1-methoxy-3-*(*4-methoxyphenyl*)-*prop-2-yl*]-*2*H-*chromen-2-one* (***44***)

23% yield, colorless oil, $[\alpha]_D^{25}$+51 (*c* 0.6, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 2.95 (1H, dd, *J*=13.9, 7.4 Hz), 2.99 (1H, dd, *J*=13.9, 7.2 Hz), 3.30 (3H, s), 3.34 (1H, m), 3.53 (1H, dd, *J*=9.3, 4.5 Hz), 3.60 (1H, dd, *J*=9.3, 6.7 Hz), 3.76 (3H, s), 3.95 (3H, s), 6.78 (2H, d, *J*=8.3 Hz), 6.97–7.02 (2H, m), 7.09 (2H, d, *J*=8.3 Hz), 7.15 (1H, dd, *J*=8.0, 8.0 Hz), 7.46 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 35.1, 42.8, 55.2, 56.2, 58.8, 72.5, 112.6, 2×113.7, 119.0, 120.0, 124.0, 129.8, 2×130.0, 131.3, 139.9, 142.6, 146.9, 157.9, 160.6; MS (EI) *m*/*z* 354 (M^+, 30), 322 (29), 121 (100); HRMS (EI) *m*/*z* calcd for $C_{21}H_{22}O_5$ 354.1467, found 354.1471.

1.18. (S)-*3-*[*1-Methoxy-3-*(*4-methoxyphenyl*)*prop-2-yl*]-*5-methyl-2*H-*chromen-2-one* (***45***)

70% yield, colorless oil, $[\alpha]_D^{25}$+51 (*c* 0.6, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 2.47 (3H, s), 2.99 (2H, d, *J*=7.5 Hz), 3.32 (3H, s), 3.36 (1H, m), 3.55 (1H, dd, *J*=9.4, 4.6 Hz), 3.63 (1H, dd, *J*=9.4, 6.4 Hz), 3.77 (3H, s), 6.80 (2H, d, *J*=8.6 Hz), 7.05 (1H, d, *J*=7.4 Hz), 7.11 (2H, d, *J*=8.6 Hz), 7.14 (1H, d, *J*=8.5 Hz), 7.33 (1H, dd, *J*=8.5, 7.4 Hz), 7.65 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 18.4, 35.3, 43.0, 55.2, 58.9, 72.7, 2×113.7, 114.3, 118.1, 125.4, 128.7, 2×130.1, 130.5, 131.4, 135.6, 136.9, 153.4, 157.9, 161.3; MS (EI) *m*/*z* 338 (M^+, 68), 306 (66), 127 (20), 185 (20), 121 (100); HRMS (EI) *m*/*z* calcd for $C_{21}H_{22}O_4$ 338.1518, found 338.1508.

1.19. (S)-*3-*[*1-Methoxy-3-*(*4-methoxyphenyl*)*prop-2-yl*]-*6-methyl-2*H-*chromen-2-one* (***46***)

25% yield, colorless crystals, mp 38–39°C (EtOH), $[\alpha]_D^{25}$+51 (*c* 0.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 2.37 (3H, s), 2.97 (2H, d, *J*=6.8 Hz), 3.31 (3H, s), 3.34 (1H, m), 3.53 (1H, dd, *J*=9.3, 4.5 Hz), 3.59 (1H, dd, *J*=9.3, 6.4 Hz), 3.76 (3H, s), 6.79 (2H, d, *J*=8.5 Hz), 7.10 (2H, d, *J*=8.6 Hz), 7.18 (1H, d, *J*=8.4 Hz), 7.19 (1H, s), 7.25 (1H, d, *J*=8.4 Hz), 7.44 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 20.7, 35.1, 42.6, 55.1, 58.8, 72.6, 2×113.6, 115.9, 119.1, 127.4, 129.3, 2×130.0, 131.4, 131.7, 133.7, 139.8, 151.0, 157.9, 161.5; MS (EI) *m*/*z* 338 (M^+, 73), 306 (80), 127 (21), 185 (21), 121 (100); HRMS (EI) *m*/*z* calcd for $C_{21}H_{22}O_4$ 338.1518, found 338.1511.

1.20. (S)-*3-*[*1-Methoxy-3-*(*4-methoxyphenyl*)*prop-2-yl*]-*7-methyl-2*H-*chromen-2-one* (***47***)

27% yield, colorless crystals, mp 127–128°C (EtOH), $[\alpha]_D^{25}$+55 (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 2.43 (3H, s), 2.96 (2H, d, *J*=6.6 Hz), 3.31 (3H, s), 3.34 (1H, m), 3.53 (1H, dd, *J*=9.4, 4.6 Hz), 3.59 (1H, dd, *J*=9.4, 6.3 Hz), 3.76 (3H, s), 6.79 (2H, d, *J*=8.4 Hz), 7.04 (1H, d, *J*=7.9 Hz), 7.099 (2H, d, *J*=8.4 Hz), 7.103 (1H, s), 7.29 (1H, d, *J*=7.9 Hz), 7.46 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 21.7, 35.2, 42.6, 55.2, 58.8, 72.6, 2×113.7, 116.4, 116.9, 125.3, 127.2, 128.2, 2×130.0, 131.4, 139.8, 141.8, 153.1, 157.9, 161.6; MS (EI) *m*/*z* 338 (M^+, 52), 306 (61), 217 (28), 152 (17), 121 (100); HRMS (EI) *m*/*z* calcd for $C_{21}H_{22}O_4$ 338.1518, found 338.1513.

1.21. (S)-*3-*[*1-Methoxy-3-*(*4-methoxyphenyl*)*prop-2-yl*]-*8-methyl-2*H-*chromen-2-one* (***48***)

47% yield, colorless oil, $[\alpha]_D^{25}$+42 (*c* 0.9, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 2.45 (3H, s), 2.98 (2H, d, *J*=7.0 Hz), 3.31 (3H, s), 3.36 (1H, m), 3.54 (1H, dd, *J*=9.4, 4.5 Hz), 3.60 (1H, dd, *J*=9.4, 6.4 Hz), 3.76 (3H, s), 6.79 (2H, d, *J*=8.6 Hz), 7.11 (2H, d, *J*=8.6 Hz), 7.12 (1H, dd, *J*=8.0, 7.3 Hz), 7.25 (1H, d, *J*=8.0 Hz), 7.30 (1H, d, *J*=7.3 Hz), 7.48 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 15.4, 35.1, 42.5, 55.1, 58.8, 72.6, 2×113.7, 119.0, 123.7, 125.2, 125.7, 129.1, 2×130.0, 131.4, 132.0, 140.2, 151.3, 157.9, 161.5; MS (EI) *m*/*z* 338 (M^+, 14), 306 (15), 121 (100); HRMS (EI) *m*/*z* calcd for $C_{21}H_{22}O_4$ 338.1518, found 338.1514.

1.22. (S)-*6-Fluoro-3-*[*1-methoxy-3-*(*4-methoxyphenyl*)-*prop-2-yl*]-*2*H-*chromen-2-one* (***49***)

43% yield, colorless oil, $[\alpha]_D^{25}$+14 (*c* 0.5, $CHCl_3$); ^{1}H NMR

(400 MHz, $CDCl_3$) δ 2.96 (2H, d, J=6.7 Hz), 3.31 (3H, s), 3.32 (1H, m), 3.53 (1H, dd, J=9.2, 4.2 Hz), 3.60 (1H, dd, J=9.2, 6.4 Hz), 3.77 (3H, s), 6.80 (2H, d, J=8.3 Hz), 7.10 (2H, d, J=8.3 Hz), 7.16–7.19 (2H, m), 7.29 (1H, m), 7.45 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 35.1, 42.7, 55.2, 58.9, 72.3, 112.8 (d, J=23.8 Hz), 2×113.7, 117.8 (d, J=8.5 Hz), 118.1 (d, J=24.8 Hz), 120.1 (d, J=9.2 Hz), 2×130.0, 130.9, 131.1, 138.9, 149.1, 158.0, 158.6 (d, J=243.5 Hz), 160.9; MS (EI) m/z 342 (M^+, 47), 310 (30), 206 (42), 137 (68), 121 (100); HRMS (EI) m/z calcd for $C_{20}H_{19}FO_4$ 342.1268, found 342.1276.

1.23. (S)-*7-Fluoro-3-[1-methoxy-3-(4-methoxyphenyl)-prop-2-yl]-2H-chromen-2-one* (***50***)

12% yield, colorless crystals, mp 117–119°C (EtOH), $[\alpha]_D^{25}$+37 (c 0.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.96 (2H, d, J=6.9 Hz), 3.29–3.33 (1H, overlapped), 3.31 (3H, s), 3.53 (1H, dd, J=9.4, 4.4 Hz), 3.59 (1H, dd, J=9.4, 6.4 Hz), 3.77 (3H, s), 6.80 (2H, d, J=8.5 Hz), 6.95–7.04 (2H, m), 7.10 (2H, d, J=8.5 Hz), 7.39 (1H, dd, J=8.6, 6.0 Hz), 7.47 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 35.2, 42.7, 55.2, 58.9, 72.4, 103.9 (d, J=25.6 Hz), 112.3 (d, J=22.9 Hz), 2×113.7, 116.1, 128.3, 129.0 (d, J=10.1 Hz), 2×130.0, 131.2, 139.3, 154.0 (d, J=12.7 Hz), 158.0, 160.9, 163.8 (d, J=252.1 Hz); MS (EI) m/z 342 (M^+, 70), 310 (47), 189 (15), 133 (18), 121 (100); HRMS (EI) m/z calcd for $C_{20}H_{19}FO_4$ 342.1268, found 342.1272.

2. *Evaluation of antifungal activity*

2.1. *Fungal strains*

The *Alternaria alternata* Japanese pear pathotype and *Colletotrichum lagenarium* employed were stored at Ehime University. Each fungal strain was cultured on potato dextrose agar (PDA, Sigma-Aldrich, Canada).

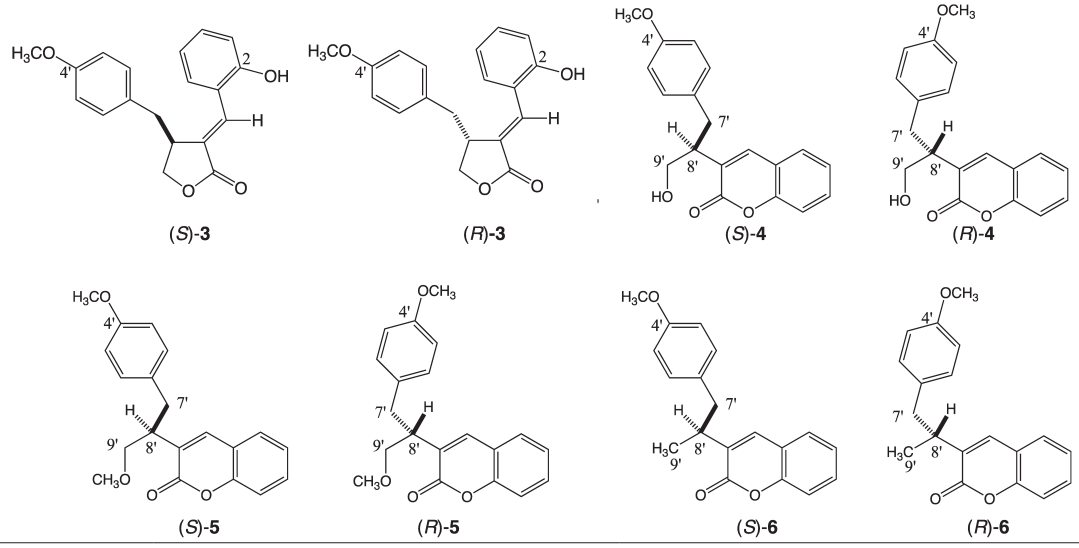
2.2. *Antifungal assay*

Thirty microliters of dimethyl sulfoxide solution containing each test compound was added to 3 mL of PDA at 50°C, followed by rapid mixing, and the resultant mixture was poured into a Petri dish (diameter 50 mm) to prepare the PDA agar plate containing the test compound. Dimethyl sulfoxide without any test compound served as the negative control. After inoculating each strain on the center of the PDA agar plate and incubation at 28°C for 3 days for *A. alternata* and for 5 days for *C. lagenarium*, respectively, the diameter of the mycelial colony was measured with a caliper. All assays were performed in triplicate.

2.3. *Calculation of the EC_{50} values*

The EC_{50} values were calculated using a standard dose–response curve by non-linear regression analysis fitting by employing PRISM software ver. 5.0 (GraphPad software Inc., San Diego, CA, U.S.A.). The antifungal activity data at six different concentrations of each compound were analyzed by this method. These analyses were performed in triplicate to obtain EC_{50} value of each compound.

Table 1. Comparison of Antifungal Activities between Enantiomers of *E*-2-hydroxybenzylidene lactone and coumarin compounds (EC_{50}±SD)



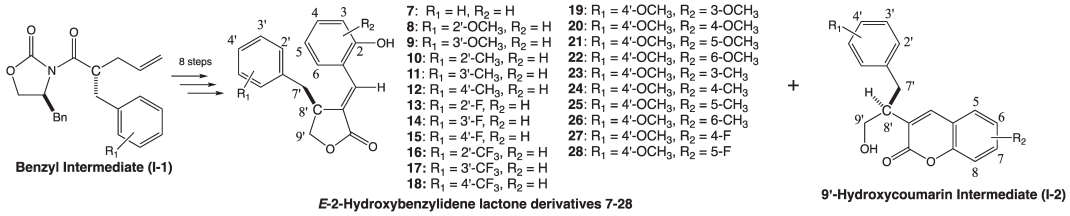
Compounds	*Alternaria alternata* Japanese pear pathotype	*Colletotricum lagenarium*
(*S*)-**3**	28.8±2.19 μM	77% growth at 500 μM
(*R*)-**3**	65% growth at 500 μM	72% growth at 500 μM
(*S*)-**4**	285±10.2 μM	51% growth at 500 μM
(*R*)-**4**	370±24.5 μM	426±29.5 μM
(*S*)-**5**	16.5±1.57 μM	86% growth at 500 μM
(*R*)-**5**	65% growth at 500 μM	90% growth at 500 μM
(*S*)-**6**	48.9±6.79 μM	77% growth at 500 μM
(*R*)-**6**	68.8±3.57 μM	72% growth at 500 μM

Results and discussion

We commenced with tests for the ability of coumarins bearing phenylpropanoids at the 3-position ((*S*)- and (*R*)-**4**, (*S*)- and (*R*)-**5**, (*S*)- and (*R*)-**6**) to inhibit the growth of phytopathogenic fungi. The results were compared with 2-hydroxybenzylidene-γ-butyrolactone ((*S*)- and (*R*)-**3**) (Table 1). In our previous study,[4] (*S*)-*E*-phenolic benzylidene lactone (*S*)-**3** was more susceptible than (*R*)-**3** against *A. alternata*. In this research, the antifungal activities of both coumarin enantiomer structures bearing the phenylpropanoid moiety **4**–**6** were estimated for the first time. In the case of coumarin compounds, which are assumed to be structures transformed from benzylidene lactone compounds (Fig. 1), (*R*)- and (*S*)-9′-OH, (*R*)- and (*S*)-9′-reductive coumarins **4**, **6**, and (*R*)-9′-OCH_3 derivative **5** showed lower activities than the (*S*)-9′-OCH_3 derivative **5** against *A. alternata*. The phytotoxic (*S*)- and (*R*)-9′-hydroxycoumarin **4**[1] were 17 to 22 times less potent than (*S*)-**5**, suggesting the disadvantage of the hydrophilic group at the 9′-position. Even though they have hydrophobic features, the (*S*)- and (*R*)-9′-reductive compounds **6** were 3 to 4 times less potent than (*S*)-9′-OCH_3 **5**. A greater difference was observed between (*S*)- and (*R*)-9′-OCH_3 **5**. Thus, the (*S*)-form was *ca.* 30 times more effective than the (*R*)-form. These results forced us to pursue our research further using the derivative **5** bearing the (*S*)-form and the 9′-OCH_3 group. A smaller difference between (*S*)- and (*R*)-form of **4** and **6** would be due to a different mode of action from **5**. Against *C. lagenarium*, only the (*R*)-9′-OH compound **4** showed lower activity (EC_{50}=426 μM).

To allow comparison of the effect of substituents on the 7′-aromatic ring, derivatives **29**–**50** were synthesized (Tables 2, 3). *E*-2-Hydroxybenzylidene lactone derivatives **7**–**28** and 9′-hydroxycoumarin intermediates (**I-2**) were prepared from the benzyl intermediate (**I-1**) obtained by stereoselective benzylation employing Evans' auxiliary (Table 2). The chiral centers of our desired derivatives were constructed by this stereoselec-

Table 2. Syntheses of (*S*)-*E*-2-Hydroxybenzylidene lactone derivatives **7**–**28** and Their Antifungal Activities (EC_{50}±SD)

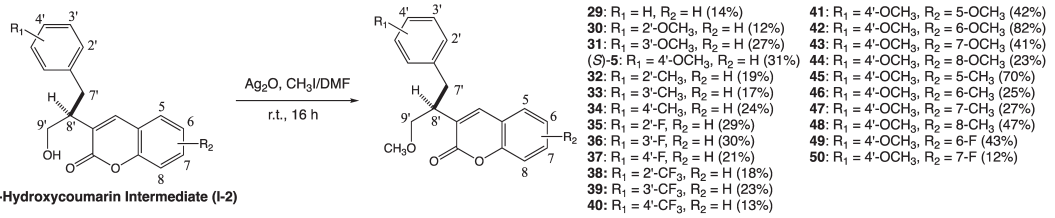


Compounds R_1, R_2	*Alternaria alternata* Japanese pear pathotype	*Colletotrichum lagenarium*
7: R_1=H, R_2=H	101±1.67 μM	55% growth at 500 μM
8: R_1=2′-OCH_3, R_2=H	122±21.0 μM	49% growth at 500 μM
9: R_1=3′-OCH_3, R_2=H	181±29.0 μM	65% growth at 500 μM
(*S*)-**3**: 4′-OCH_3, R_2=H (Table 1)	28.8±2.19 μM	77% growth at 500 μM
10: R_1=2′-CH_3, R_2=H	56.9±1.06 μM	60% growth at 500 μM
11: R_1=3′-CH_3, R_2=H	89.1±7.86 μM	173±0.97 μM
12: R_1=4′-CH_3, R_2=H	65.5±8.61 μM	76% growth at 500 μM
13: R_1=2′-F, R_2=H	99.2±6.60 μM	258±28.0 μM
14: R_1=3′-F, R_2=H	205±7.41 μM	372±72.9 μM
15: R_1=4′-F, R_2=H	218±37.3 μM	335±37.6 μM
16: R_1=2′-CF_3, R_2=H	52% growth at 500 μM	61% growth at 500 μM
17: R_1=3′-CF_3, R_2=H	97.1±11.5 μM	209±16.5 μM
18: R_1=4′-CF_3, R_2=H	141±7.84 μM	72% growth at 500 μM
19: R_1=4′-OCH_3, R_2=3-OCH_3	173±15.6 μM	50% growth at 500 μM
20: R_1=4′-OCH_3, R_2=4-OCH_3	235±9.73 μM	69% growth at 500 μM
21: R_1=4′-OCH_3, R_2=5-OCH_3	158±15.6 μM	62% growth at 500 μM
22: R_1=4′-OCH_3, R_2=6-OCH_3	56% growth at 500 μM	77% growth at 500 μM
23: R_1=4′-OCH_3, R_2=3-CH_3	55% growth at 500 μM	82% growth at 500 μM
24: R_1=4′-OCH_3, R_2=4-CH_3	88.1±17.0 μM	71% growth at 500 μM
25: R_1=4′-OCH_3, R_2=5-CH_3	53.6±12.4 μM	79% growth at 500 μM
26: R_1=4′-OCH_3, R_2=6-CH_3	95% growth at 500 μM	78% growth at 500 μM
27: R_1=4′-OCH_3, R_2=4-F	86.2±12.4 μM	326±31.8 μM
28: R_1=4′-OCH_3, R_2=5-F	126±9.44 μM	79% growth at 500 μM

tive benzylation. The final 9′-OCH_3 derivatives **29**–**50** were prepared from 9′-hydroxycoumarin intermediates (**I-2**) by methylation employing Ag_2O and CH_3I (Table 3). Mild reaction conditions were required for this methylation because of the production of *Z*-benzylidene lactone.

The activities of (*S*)-*E*-2-hydroxybenzylidene lactone derivatives **7**–**28** are illustrated in Table 2. Against *A. alternata*, the 4′-OCH_3 compound (*S*)-**3** showed the lowest EC_{50} value (29 μM). The activity of the non-substituted derivative **7** was 3.5 times less than that of the 4′-OCH_3 compound (*S*)-**3**. The 2′-, 3′-OCH_3 derivatives **8**, **9** were 4–6 times less potent than the 4′-OCH_3 compound ((*S*)-**3**). The 2′-, 3′-, and 4′-CH_3 derivatives **10**–**12** showed 2–3 times weaker activities. The derivatives bearing electron-withdrawing substituents, the F derivatives **13**–**15** and CF_3 derivatives **16**–**18**, exhibited 3–17 times less potent activities. Especially, the introduction of the CF_3 group to the 2′-position (derivative **16**) resulted in a strong drop in activity. The 4′-OCH_3 group, which is a higher electron-donating group, is indispensable for increasing the activity of (*S*)-2-hydroxybenzylidene lactone. The effects of substituents at the 3–6 positions were also checked by employing derivatives **19**–**28**. All the derivatives **19**–**28** were less active than the 4′-OCH_3 compound ((*S*)-**3**) against *A. alternata*, confirming the disadvantage of the presence of substituents at the 3–6 positions. Especially, a significant loss of activity in 6-OCH_3, 3-CH_3, and 6-CH_3 derivatives **22**, **23**, **26** was observed. The 2 to 8 times less potent activities of 3-OCH_3, 4-OCH_3, 5-OCH_3, 4-CH_3, 5-CH_3, 4-F, and 5-F derivatives **19**–**21**, **24**, **25**, **27**, **28** were shown. After screening the derivatives against *C. lagenarium*, we identified that the introduction of a small fluorine atom at each position from the 2′- to 4′-positions accelerated the activities, the derivatives **13**–**15** showing EC_{50} values of 258–372 μM. Although the 3′-OCH_3 derivative **9** was inactive, the presence of the larger hydrophobic substituent, CH_3 or CF_3 group, at the 3′-position was also advantageous. Thus, the EC_{50} values of the 3′-CH_3 derivative **11** and the 3′-CF_3 derivative **17** were 173 μM and 372 μM, respectively. Of the derivatives **19**–**28** bearing substituents at the 3–6-positions, only the (4′-OCH_3, 4-F) derivative **27**

Table 3. Syntheses of coumarin derivatives bearing phenylpropanoid unit at 3-position **29**–**50** and Their Antifungal Activities ($EC_{50}\pm SD$)



Compounds R_1, R_2	*Alternaria alternata* Japanese pear pathotype	*Colletotrichum lagenarium*
29: R_1=H, R_2=H	5.37±0.39 μM	51% growth at 500 μM
30: R_1=2′-OCH_3, R_2=H	392±63.4 μM	66% growth at 500 μM
31: R_1=3′-OCH_3, R_2=H	11.1±0.94 μM	55% growth at 500 μM
(*S*)-**5**: 4′-OCH_3, R_2=H (Table 1)	16.5±1.57 μM	86% growth at 500 μM
32: R_1=2′-CH_3, R_2=H	88.1±2.54 μM	55% growth at 500 μM
33: R_1=3′-CH_3, R_2=H	4.75±1.08 μM	59% growth at 500 μM
34: R_1=4′-CH_3, R_2=H	8.96±0.25 μM	87% growth at 500 μM
35: R_1=2′-F, R_2=H	94.6±15.2 μM	48% growth at 500 μM
36: R_1=3′-F, R_2=H	4.16±0.85 μM	345±47.6 μM
37: R_1=4′-F, R_2=H	3.44±0.45 μM	50% growth at 500 μM
38: R_1=2′-CF_3, R_2=H	193±7.28 μM	67% growth at 500 μM
39: R_1=3′-CF_3, R_2=H	1.41±0.06 μM	74% growth at 500 μM
40: R_1=4′-CF_3, R_2=H	1.71±0.26 μM	88% growth at 500 μM
41: R_1=4′-OCH_3, R_2=5-OCH_3	3.92±0.88 μM	56% growth at 500 μM
42: R_1=4′-OCH_3, R_2=6-OCH_3	75% growth at 500 μM	86% growth at 500 μM
43: R_1=4′-OCH_3, R_2=7-OCH_3	150±20.7 μM	60% growth at 500 μM
44: R_1=4′-OCH_3, R_2=8-OCH_3	129±15.9 μM	75% growth at 500 μM
45: R_1=4′-OCH_3, R_2=5-CH_3	2.92±0.35 μM	63% growth at 500 μM
46: R_1=4′-OCH_3, R_2=6-CH_3	29.3±0.73 μM	79% growth at 500 μM
47: R_1=4′-OCH_3, R_2=7-CH_3	89% growth at 500 μM	83% growth at 500 μM
48: R_1=4′-OCH_3, R_2=8-CH_3	27.7±2.17 μM	54% growth at 500 μM
49: R_1=4′-OCH_3, R_2=6-F	56% growth at 500 μM	90% growth at 500 μM
50: R_1=4′-OCH_3, R_2=7-F	74% growth at 500 μM	85% growth at 500 μM

showed weak activity (EC_{50}=326 μM). We designed more potent phenolic benzylidene lactone compounds **11**, **13**–**15**, **17**, and **27** against *C. lagenarium* than the lead compound, phenolic 4′-OCH_3 ((*S*)-**3**); however, their activities were weak.

The 22 analogues **29**–**50**, whose structural hallmarks are coumarin, were screened for antifungal activity (Table 3). Against *A. alternata*, significant losses of activity were observed in all the 2′-derivatives **30**, **32**, **35**, **38**, whose activities were 18–78 times less potent than the non-substituted derivative **29**, which had an EC_{50} value of 5 μM. Both electron-donating and electron-withdrawing substituents at the 2′-position reduced the activity. The activities of the 3′-substituted derivatives **31**, **33**, **36**, **39** were equipotent with the corresponding 4′-substituted derivatives (*S*)-**5**, **34**, **37**, **40**. Among them, the 3′-CF_3 and 4′-CF_3 derivatives **39**, **40** showed the lowest EC_{50} values (1.41 μM and 1.71 μM), which were 3 times more effective than the non-substituted derivative **29**. Although the activities of the 3′-CH_3, 4′-CH_3, 3′-F, and 4′-F derivatives **33**, **34**, **36**, **37** (EC_{50}=3.4–9.0 μM) were similar to the non-substituted derivative **29**, the 3′-OCH_3 and 4′-OCH_3 derivatives **31**, (*S*)-**5** were 2–3 times less effective than these compounds (**33**, **34**, **36**, **37**). A sizeable electron-withdrawing substituent at the 3′- or 4′-position would be necessary for higher activity. Substituents were introduced at the 5-position to the 8-position of the 4′-OCH_3 derivative (*S*)-**5**, to determine their influence on activity. Electron-donating OCH_3 and CH_3 groups at the 5-position accelerated the activity, the (4′-OCH_3, 5-OCH_3) derivative **41** and the (4′-OCH_3, 5-CH_3) derivative **45** being 4.2 and 5.6 times more potent, respectively, than (*S*)-**5**. Although the activities of the (4′-OCH_3, 6-CH_3) derivative **46** and the (4′-OCH_3, 8-CH_3) derivative **48** were almost equipotent with (*S*)-**5**, 8–9 times lower activities were observed in the (4′-OCH_3, 7-OCH_3) derivative **43** and the (4′-OCH_3, 8-OCH_3) derivative **44**. On the other hand, the (4′-OCH_3, 6-OCH_3) derivative **42** and the (4′-OCH_3, 7-CH_3) derivative **47** were inactive. The small electron-withdrawing fluorine derivatives, (4′-OCH_3, 6-F) derivative **49** and (4′-OCH_3, 7-F) derivative **50**, were also inactive. Against *C. lagenarium*, only the 3′-F derivative **36** was active, showing a high EC_{50} value (345 μM). Finally, we confirmed that coumarin without a phenylpropanoid unit at the 3-position did not show any antifungal activity.

Conclusion

In summary, access to coumarin derivatives bearing phenylpropanoid through syntheses enabled us to demonstrate the structure-function relationship of this new type of coumarin against *A. alternata*. It was shown that the (*S*)-configuration and the 9′-OCH_3 group are necessary for higher activity. As for the aromatic ring of the phenylpropanoid portion, the 3′-CF_3 derivative **39** and the 4′-CF_3 derivative **40** showed the highest activities, suggesting the importance of a higher electron-withdrawing group. In the coumarin ring, the electron donating group at the 5-position seemed to be effective, the (4′-OCH_3, 5-OCH_3) derivative **41** and (4′-OCH_3, 5-CH_3) derivative **45** showing the higher activities. Compared with *E*-benzylidene lactones bearing a phenolic 2-OH group, which is a precursor of the coumarin structures with the same stereochemistry, the coumarin derivatives bearing phenylpropanoid had higher activity against *A. alternata*. Although the mode of action was not studied, the results of the higher effect than *C. lagenarium* is also found in our previous lignan research.[1,4–6] The coumarin type lignan bearing phenylpropanoid at 3-position also showed species specific activity such as previously described antifungal lignans. Because of new coumarin compound bearing lignan structure, it is impossible to compare the mode of action with known coumarin compounds. Some *E*-benzylidene lactones bearing a phenolic 2-OH group were effective against *C. lagenarium*. These results will make an novel contribution to the development of novel agrochemicals based on both lignan and coumarin structures.

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Declarations of interest

None

Electronic supplementary materials

The online version of this article contains supplementary material, which is available at https://www.jstage.jst.go.jp/browse/jpestics/.

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