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Synthesis of Carlactone Derivatives to Develop a Novel Inhibitor of Strigolactone Biosynthesis

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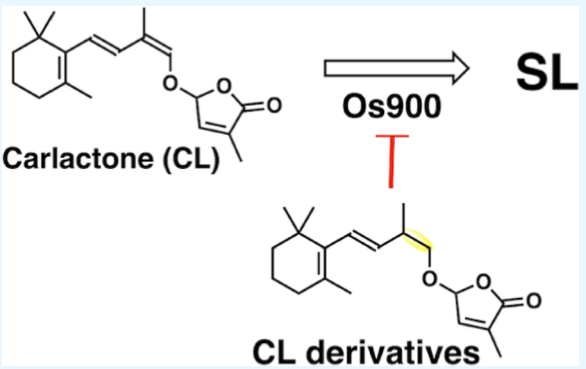
ABSTRACT: Strigolactones (SLs), phytohormones that inhibit shoot branching in plants, promote the germination of root-parasitic plants, such as *Striga* spp. and *Orobanche* spp., which drastically reduces the crop yield. Therefore, reducing SL production via chemical treatment may increase the crop yield. To design specific inhibitors, it is valid to utilize the substrate structure of the target proteins as lead compounds. In this study, we focused on Os900, a rice enzyme that oxidizes the SL precursor carlactone (CL) to 4-deoxyorobanchol (4DO), and synthesized 10 CL derivatives. The effects of the synthesized CL derivatives on SL biosynthesis were evaluated by the Os900 enzyme assay in vitro and by measuring 4DO levels in rice root exudates. We identified some CL derivatives that inhibited SL biosynthesis in vitro and in vivo.



INTRODUCTION

Orobanche and *Striga* species are root-parasitic plants, which infest major crops, such as pea, tomato, sorghum, and rice.[1−3] In particular, *Striga* causes enormous crop damage, with annual losses of US$7 billion in Sub-Saharan Africa alone.[2] In 1966, strigol was first discovered as a germination stimulant for *Striga lutea*.[1] After the discovery of strigol, some derivatives have been found in various plant species, and strigol and its derivatives are now called strigolactones (SLs).[4] In addition, SLs have been identified as inducers of hyphal branching in arbuscular mycorrhizal fungi.[5] In 2008, SLs were found to regulate the number of shoot branches as a novel class of phytohormones.[6,7]

SLs are synthesized from all-*trans*-β-carotene via an important SL precursor called carlactone (CL).[8,9] CL is synthesized by the D27 carotenoid isomerase and two carotenoid cleavage dioxygenases (CCD7 and CCD8). In *Arabidopsis*, tomato, and cowpea, CYP711A shows catalytic activity only for converting CL to CLA.[10,11] In contrast, CYP711As in rice and sorghum catalyze multiple reactions. In rice, after Os900 (CYP711A2) and Os1400 (CYP711A3) synthesize carlactonoic acid (CLA) from CL, CLA is oxidized and cyclized to 4-deoxyorobanchol (4DO), a major SL, by Os900. Furthermore, the synthesized 4DO was converted into orobanchol by Os1400.[11] Although the activities of CYP711As vary among plant species, all known CYP711As commonly exhibit enzymatic activities of oxidation from CL to CLA.

Because the root exudates of SL biosynthesis mutant plants exhibit less germination-stimulating activity than wild-type plants for root-parasitic plants, SL biosynthesis inhibitors may help control the damage to root-parasitic plants. Until now, several compounds have been reported as SL biosynthesis inhibitors (Figure 1). B2 and D6 with hydroxamic acid inhibit D27 and CCD7/8, respectively, and these compounds induce morphological changes like SL-deficient mutant in *Arabidopsis*.[12,13] In our previous work, we screened for CYP711A inhibitors and identified TIS13, tebuconazole, and triflumizole as lead compounds with azoles.[14−16] In addition, we performed a structure−activity relationship study based on the structure of TIS13 and developed TIS108 and KK5 as potent and specific SL biosynthesis inhibitors.[17,18] In addition, treatment with TIS108, which is a specific CYP711A inhibitor, in rice reduced the infestation of *Striga*, without affecting SL function as a phytohormone.[19] This finding indicates that the specific inhibition of CYP711A can limit root-parasitic weed damage.

To develop P450 inhibitors, it is useful to utilize azole-containing chemicals because the azole moiety coordinates with the heme iron, which is essential for P450 activity. However, azole-containing chemicals may also inhibit the activities of other P450 enzymes.[20] Thus, the synthesis of substrate analogues is a useful approach for the development of specific P450 inhibitors. For instance, (+)-6-nor-abscisic acid, an abscisic acid (ABA) analogue, inhibits CYP707As, which

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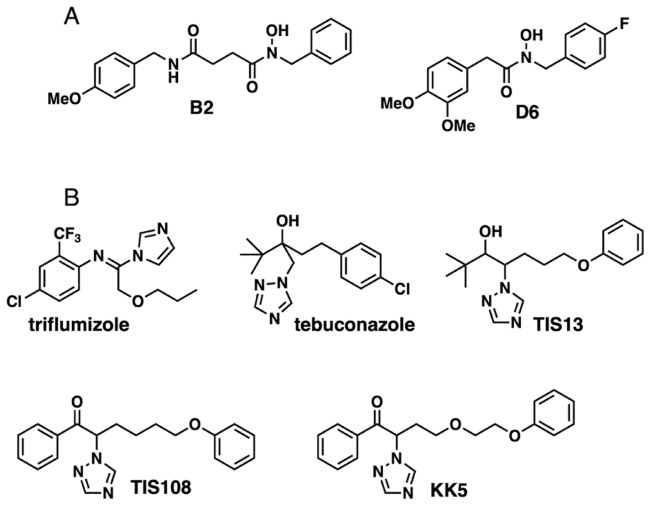

Figure 1. SL biosynthesis inhibitors. (A) Hydroxamic acid derivatives-type inhibitors. (B) Inhibitors with an azole moiety.

are involved in ABA catabolism.[21] In addition, galeterone, a pregnenolone analogue containing a benzimidazole moiety, inhibits steroid 17-α-hydroxylase (CYP17A).[22] Although various SL biosynthesis inhibitors have been reported, few attempts have been made to develop substrate analogues of SL biosynthesis inhibitors. Thus, we synthesized CL derivatives and evaluated their inhibitory activities on CYP711A in rice.

RESULTS AND DISCUSSION

Synthesis of CL Derivatives. To synthesize CL analogues, several problems need to be resolved, although CL can be synthesized in a small number of reaction steps. The coupling reaction of aldehyde with 3-methyl-2(5*H*)-furanone (butenolide) has a very low yield (approximately 1%).[23] This might be because the enol-ether bridge of SLs is unstable under base conditions.[24] Low yields have disadvantages in the development of inhibitors. To synthesize stable compounds, we focused on the four parts of the CL structure (A: cyclohexene group, B: methyl group, C: diene, D: butenolide) (Figure 2A) and synthesized 10 CL derivatives with reference to the CL synthesis method (Figure 2B).

Except for KCL7, in which the starting material was *trans*-benzalacetone, all KCLs were synthesized via 1−6 steps from β-ionone as the starting material (Schemes 1 and 2). The coupling reaction of each alcohol with butenolide, ester, or other heterocycles gave CL derivatives in a 21−58% yield. It is suggested that the yield was improved through changing the enol-ether bridge of CL because the yield of the coupling reaction with butenolide was 27% in the synthesis of KCL2. Although the diastereomers of KCL5 and KCL7 were isolated by silica gel column chromatography, the diastereomers of KCL2 and KCL3 could not be separated. Therefore, we used the diastereomixtures of KCL2 and KCL3 to evaluate the biological assays.

Evaluation of Germination Activity of CL Derivatives in Striga. Approximately 40 and 60% of CL-treated *Striga* seeds germinated, at the concentrations of 33 and 330 μM CL, respectively.[8] Thus, we first checked the germination-stimulating activity of synthetic chemicals for *Striga*. When we treated *Striga* seeds with 10 or 100 μM CL derivatives, none of the CL derivatives promoted *Striga* germination in comparison with the water-treated control (Table 1). In addition, we performed a yeast three-hybrid assay to evaluate SL activity of CL derivatives. CL derivatives did not show binding activity to ShHTL7, a highly sensitive SL receptor in *Striga* (Figure S1). While GR24 promoted the growth of ShHTL7- and ShMAX2-expressed yeast, even KCL2, which has the most similar structure to CL, did not affect the growth of the yeast, suggesting that the enol-ether bond is essential for *Striga* germination activity, as reported previously.[8]

Effect of CL Derivatives on the Enzymatic Activity of Os900. To evaluate the inhibitory activity of CL derivatives on Os900, we performed an Os900 inhibitory assay. With reference to our previous work,[16] we collected microsomal fractions including Os900 from heterologously expressed yeast, as reported by Yoneyama et al.[11] Microsomal fractions containing CL and 50 μM NADPH were incubated with or without the CL derivatives at 28 °C. Os900 converts CL to CLA and CLA to 4DO. As 4DO levels were correlated with CLA levels in the Os900 inhibitory assay,[16] we evaluated Os900 inhibitory activity by measuring CLA levels in the reaction mixture using liquid chromatography-tandem mass

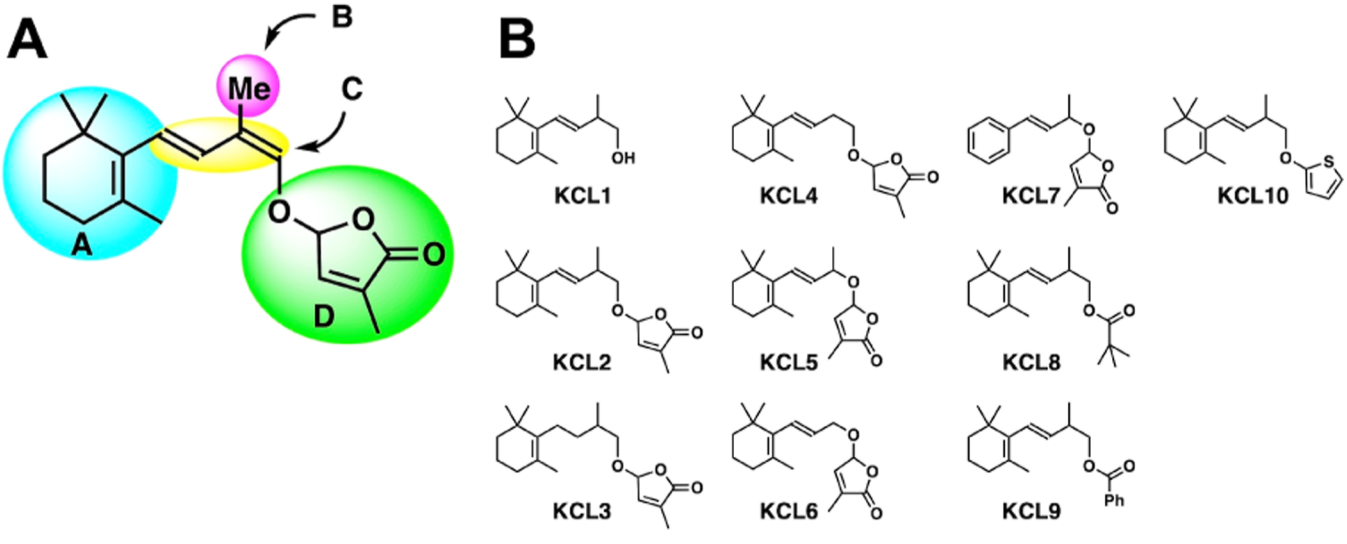

Figure 2. Structures of CL derivatives. (A) CL structure, with focus on four parts. (B) Structures of synthesized CL derivatives.

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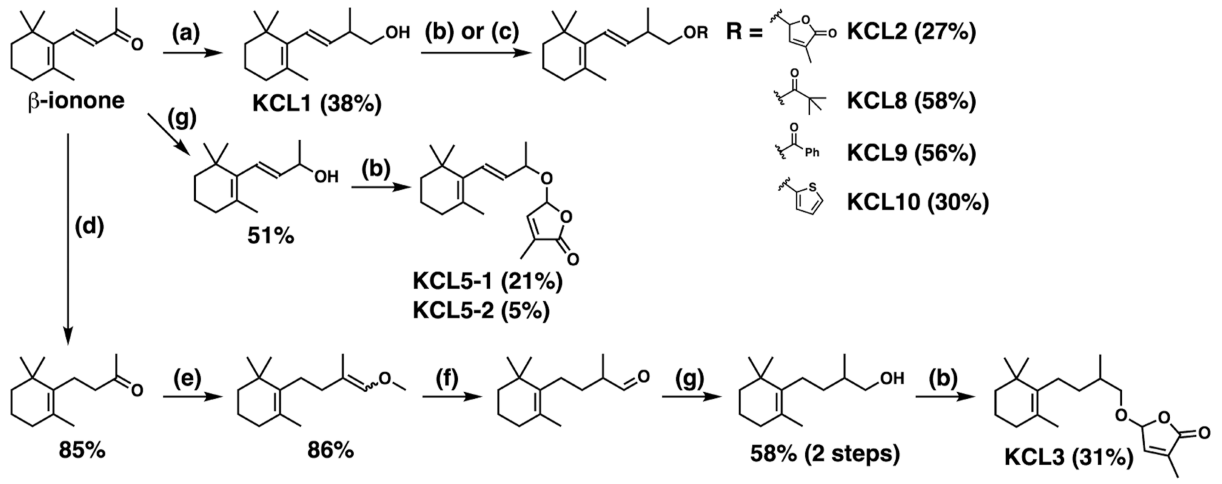
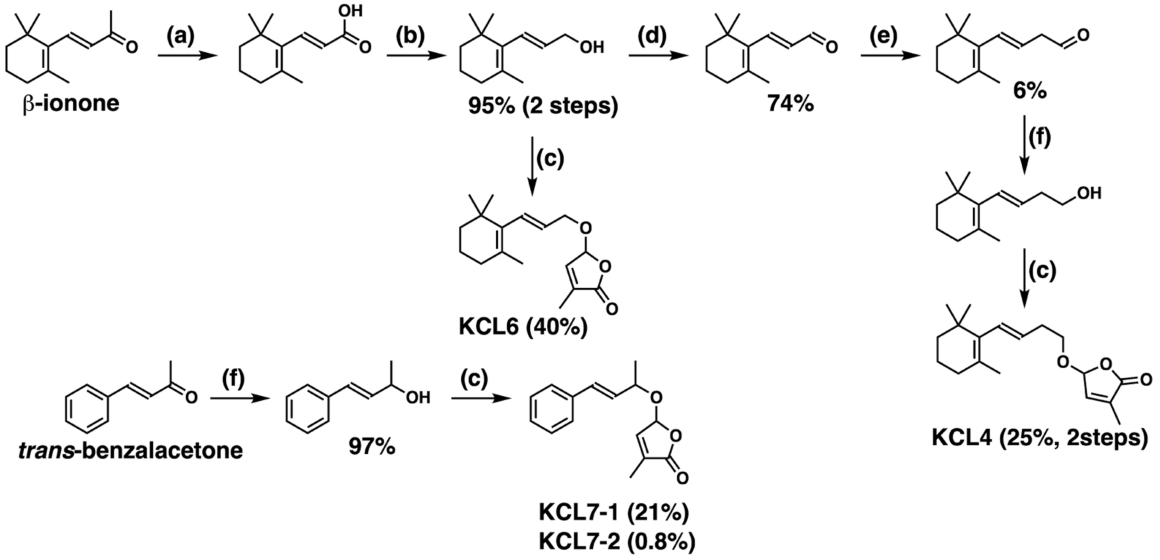
Scheme 1. Synthesis of KCL1, 2, 3, 5, 8, 9, and 10; (a) (1) $C_4H_7ClO_2$, Phenothiazine, NaH, Pyridine, −15 to 10 °C, 3.5 h (2) NaOH, MeOH, 0 °C, 1 h (3) $NaBH_4$, MeOH, 0 °C to rt, Overnight; (b) R−Br or R−COCl, *n*-BuLi, THF, 0 °C to rt, Overnight; (c) Thiophen-2(5*H*)-one or *tert*-Butyl (4-Methylthiazol-2-yl)carbamate, DEAD, PPh_3, Toluene, 0 °C to rt, 2 h or Overnight; (d) (1) Et_3SiH, $RhCl(PPh_3)_3$, 55 °C, 5 h (2) K_2CO_3, MeOH, 0 °C to rt, Overnight; (e) $Ph_3P^+CH_2OCH_3Cl^-$, *n*-BuLi, THF, −78 to 0 °C, 30 min; rt, 24 h; (f) TsOH, Acetone/H_2O = 3:1, Reflux, 3 h; (g) $NaBH_4$, MeOH, 0 °C to rt, Overnight



Scheme 2. Synthesis of KCL4, 6, and 7; (a) NaClO, MeOH, 0 °C, 1 h; rt, 24 h; (b) Red-Al, THF, reflux, 1 h; (c) 5-Bromo-3-methyl-2(5*H*)-furanone, *n*-BuLi, THF, 0 °C to rt, 3 h; (d) MnO_2, CH_2Cl_2, rt, 1 h; (e) (1) $C_4H_7ClO_2$, Phenothiazine, NaH, Pyridine, −15 to 10 °C, 3.5 h (2) NaOH, MeOH, 0 °C, 1 h (3) AcOH, 0 °C, 10 min; (f) $NaBH_4$, EtOH, rt, 2 or 5 h



spectrometry (LC−MS/MS). CLA levels were determined when 100 μM CL derivatives were added to Os900-containing microsomal proteins. Nine compounds significantly reduced CLA levels at 100 μM (Figure 3A). In contrast, the other three compounds (KCL1, KCL7-1, and KCL7-2) did not show the inhibitory activity of Os900. This led to a reduction in the inhibitory activity against Os900, in which the CL structure was converted from cyclohexene to benzene and butenolide to alcohol. Additionally, no remarkable change in the inhibitory activity was observed among the diastereomers of KCL5. Next, to evaluate the Os900 inhibitory activity of CL derivatives in more detail, an Os900 inhibitory assay was performed using 10, 30, and 100 μM of the eight compounds, which showed a significant reduction in CLA levels at 100 μM. As the eight compounds reduced CLA levels in a dose-dependent manner, CL derivatives were recognized as substrates of Os900 (Figure 3B).

Especially, KCL2 showed a comparatively good inhibitory activity against Os900. Interestingly, KCL8, which lacks a butenolide structure, showed an inhibitory activity of Os900 at the concentration of 10 μM, suggesting that the butenolide structure, which is highly conserved in active SLs, is not essential for inhibiting Os900 activity.

Effect of CL Derivatives on 4DO Levels in Rice Root Exudates. Next, we evaluated the effect of KCL2, 4, 8, and 10 on the endogenous 4DO levels in planta because the selected chemicals had different characteristic structures: the conversion of CL from an enol-ether bond to an ether bond for KCL2, the deletion of the methyl group of KCL2 for KCL4, the conversion of KCL2 from butenolide to *tert*-butyl ester for

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Table 1. Evaluation of *Striga* Germination Activity Affected by CL Derivatives[a]

	Striga germination (%)	
entry	10 μM	100 μM
KCL1	0	0.6 ± 1.1
KCL2	0	0.9 ± 1.7
KCL3	1.0 ± 1.2	0.4 ± 0.9
KCL4	0	0
KCL5	0	0.5 ± 0.9
KCL6	0	0
KCL7	0	1.3 ± 1.6
KCL8	0.4 ± 0.8	0.5 ± 1.1
KCL9	0	1.1 ± 1.2
KCL10	0	1.4 ± 1.8
control	0.9 ± 1.1	
epi-GR24 (1 μM)	60 ± 11**	

[a]1 μM *epi*-GR24 (synthetic SL analogue) was used as positive control. The data are mean ± S.D. (n = 4). ** denotes statistically significant difference from germination rate in DMSO diluted to 2% with water (control) (Dunnett's test; $p < 0.01$).

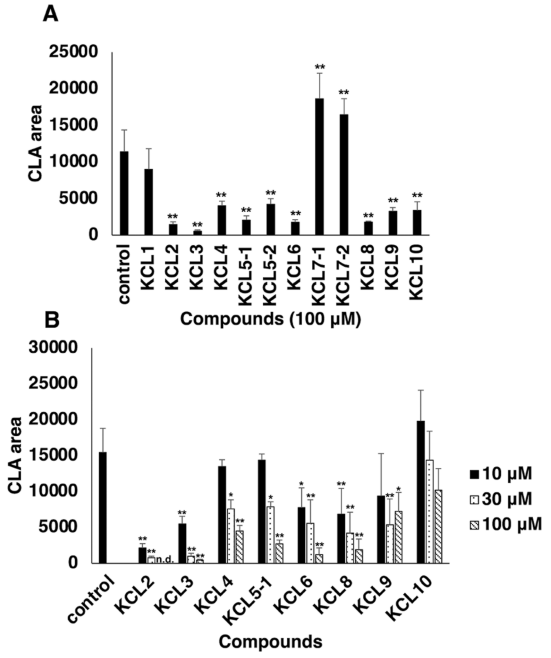

Figure 3. Effects of CL derivatives on Os900. CLA levels in case of (A) addition of 100 μM and (B) addition of 10−100 μM of each CL derivative to microsomal proteins, including Os900. CLA levels were determined by LC−MS/MS. "n.d." = not detected. The data are mean ± S.D. (n = 3). * and ** denote statistically significant differences from the level for non-addition of CL derivatives (control) (Dunnett's test; $0.01 < p < 0.05$ and $p < 0.01$ respectively).

KCL8, and the conversion of KCL2 from butenolide to thiophene for KCL10 (Figure 2). Levels of 4DO in root exudates correlate with the endogenous level of 4DO in roots.[17] To estimate the effect of CL derivatives on the production of 4DO in planta, we measured 4DO levels in rice root exudates using LC−MS/MS. We treated two-week-old rice seedlings grown on a phosphate-starvation culture solution with CL derivatives, since the endogenous level of SL in rice grown under phosphate-starvation conditions are increased, and consequently SL analyses become easy.[25] As a result of quantification of 4DO levels in root exudates, KCL 2, 4, and 8 displayed a significant reduction in 4DO levels (around 1−10 pg/mL) at 100 μM (Figure 4A,B).

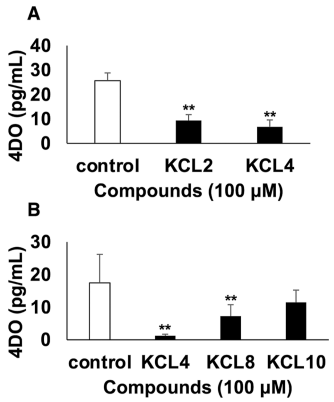

Figure 4. Effects of CL derivatives on the production of 4DO in planta at the concentration of 100 μM. (A) Comparison of KCL2 with KCL4, (B) KCL4 with KCL8 and KCL10. 4DO levels were determined by LC−MS/MS. The data are the means ± S.D. (n = 3 in A, n = 5 in B). ** denotes statistically significant difference from the 4DO level in the control (Dunnett's test; $p < 0.01$).

These results suggest that CL derivatives inhibit SL biosynthesis in planta by inhibiting Os900. In the Os900 inhibitory assay, KCL2, which has the most similar structure to CL among the 10 CL derivatives, showed the strongest inhibition activity of Os900. In contrast, KCL4, which eliminates the methyl group to be oxidized by Os900, showed similar 4DO levels to KCL2 in rice. This result suggests that KCL4 could not be metabolized by Os900, resulting in strong inhibitory effects on 4DO levels in rice. On the other hand, KCL7 increased CLA levels in Os900 inhibitory assay. It is possible that KCL7 showed an allosteric effect against Os900. However, since the details are not uncovered, we need to confirm the action mechanism(s) of KCL7 against Os900. Among the synthesized CL derivatives, the butenolide compounds KCL2 and KCL4 significantly reduced CLA and 4DO levels in vitro and in planta, respectively. These results indicate that the butenolide moiety is an important functional group for the substrate recognition of Os900. However, KCL8, with a *tert*-butyl ester group, also reduced 4DO levels in root exudates, albeit with a slightly lower inhibitory activity. This suggests that the lack of a butenolide moiety does not induce a dramatic decrease in the inhibitory effect on SL biosynthesis. The absence of butenolide is one of the advantages of the development of SL biosynthesis inhibitors because butanolide is an expensive moiety. Therefore, KCL2, KCL4, and KCL8 can be useful as lead compounds in SL biosynthesis inhibitors because of their simpler structures to synthesize than CL.

The downstream pathway of SL biosynthesis differs from that of CL among plants, but all reported SLs have been proposed to be synthesized via CL.[26−28] Therefore, a CL mimic could be a SL biosynthesis inhibitor in various plants. Recently, it was uncovered that the accumulation of a novel non-canonical SL (CL + 30) arose in TIS108-treated rice roots and root exudates. Additionally, despite the fact that TIS108 shows inhibitory activity against Os900 and Os1400, the treatment of TIS108 did not show the significant change of the endogenous methyl carlactonoate (MeCLA) level, one of the non-canonical SL.[19] It remains unknown how these non-canonical SLs are biosynthesized. However, CL derivatives

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might help to reveal the biosynthesis pathway of non-canonical SLs because the production of non-canonical SLs such as CL +30 and MeCLA is expected to be involved in the enzymes which convert CL as substrate (Figure S2).

CONCLUSIONS

In this study, we demonstrated that CL derivatives inhibit Os900 and reduce 4DO levels in rice. However, known SL biosynthesis inhibitors show inhibitory activity at concentrations less than 10 μM. Compared to these inhibitors, CL derivatives showed very weak inhibitory activity against SL biosynthesis. Nevertheless, this is the first study to report a substrate analogue type of SL biosynthesis inhibitor and the use of CYP711A inhibitors without azole. In the near future, by introducing heterocycles, such as imidazole and triazole, into the CL derivatives found in this study, it is expected that a specific and potent SL biosynthesis inhibitor can be developed.

EXPERIMENTAL SECTION

Germination Assay of *Striga* Seeds. We performed the *Striga* germination assay as previously described.[29] *Striga* seeds were conditioned for 7 days at 30 °C. After diluting 5 mM compound dissolved in DMSO with distilled water, 10 μL of an aqueous solution containing 10 or 100 μM of each compound was added to a 96-well plate. *Striga* seeds were incubated at 30 °C, and their germination was evaluated microscopically after 1 day.

Yeast Three-Hybrid Experiment. We performed a yeast three-hybrid assay as previously described.[30] The AH109 yeast strains, which was co-transformed with pGAD-ShHTL7 and pBridge- ShMAX2-ASK1, were grown in SD lacking leucine, tryptophan, and methionine aqueous medium at 30 °C for 3 days. Then, the co-transformed yeast was transferred onto SD lacking leucine, tryptophan, histidine, adenine, and methionine plates containing 100 μM CL derivatives or 1 μM GR24 and incubated at 30 °C for 6 days.

Conditions of the Os900 Assay. We performed the Os900 inhibitory assay as in our previous work.[16] A mixture of rac-CL, 50 μM NADPH, and Os900-containing microsomal proteins was incubated with or without CL derivatives at 28 °C for 40 min. The mixture was quenched with 50 μL of H_2O and 200 μL of ethyl acetate and extracted three times with 200 μL of ethyl acetate. After the combined organic layers were concentrated in vacuo to obtain a concentrate, the concentrate was dissolved in 200 μL of ethyl acetate. The concentrate was then transferred to glass inserts, concentrated in vacuo, and dissolved in acetonitrile. The solution was analyzed by LC−MS/MS.

Plant Materials and Growth Conditions. Rice seedlings were grown as described previously,[7,16] and we used "Nipponbare" as the wild-type rice. After rice seeds were sterilized with 2.5% sodium hypochlorite, it was incubated in sterilized water at 25 °C in dark for 2 days. The germinated rice was seeded into a phosphate-deficient hydroponic culture medium solidified with 0.7% agar and grown under fluorescent white light with a 14 h light/10 h dark photoperiod for 7 d. Each rice seedling was transferred to a brown glass vial containing 12 mL phosphate-deficient hydroponic culture medium. After the seedlings were grown under the same conditions for six days, they were transferred to a new brown glass vial containing 12 mL of hydroponic culture medium under phosphate-deficient conditions with or without 100 μM CL derivatives and grown for one day. To analyze the 4DO levels in the root exudates, the culture media were corrected.

Quantification of the CLA Level. LC−MS/MS analysis was performed using a QTOF X500R system (SCIEX, Framingham, MA, USA) and an ultrahigh-performance liquid chromatograph (UPLC) equipped with a reversed-phase column (CORTECS UPLC phenyl 1.6 μm, 2.1 × 75 mm; Waters).

To separate CLA by UPLC, we used water (solvent A) and acetonitrile (solvent B) containing 0.05% (v/v) acetic acid. The mobile phase was changed to linear gradient of 20 to 80% solvent B (0−5 min), 80% solvent B (5−7 min). The parent ion (m/z) was 331.1 CLA. The samples were quantified using fragment ions 113.02 for CLA in a negative mode.

Quantification of the 4DO Level in Rice Root Exudates. To analyze 4DO levels in rice root exudates, the hydroponic culture medium was extracted twice with ethyl acetate and deuterium-labeled 5-deoxystrigol (d_6-5DS) as the internal standard.[31] The combined organic layers were concentrated in vacuo and dissolved in 1 mL of 15% (v/v) ethyl acetate in hexane. The solution was loaded onto Sep-Pak Vac 1 cc (100 mg) silica cartridges (Waters), washed with 2 mL of 15% (v/v) ethyl acetate in hexane, after then eluted three times with 35% (v/v) ethyl acetate in hexane, and concentrated in vacuo. The solution was dissolved in 50% acetonitrile in water and subjected to LC−MS/MS.

LC−MS/MS analysis was performed using a TripleTOF 5600 system (SCIEX, Framingham, MA, USA) and a UPLC system (Nexera; Shimadzu, Kyoto, Japan) equipped with a reversed-phase column (Acquity UPLC BEH-C18, 2.1 × 50 mm, 1.7 μm; Waters).[32]

For separation by UPLC, we used water (solvent A) and acetonitrile (solvent B), both of which contained 0.1% (v/v) formic acid. The mobile phase had a flow rate of 0.3 mL/min and was altered with a linear gradient of 20 to 40% solvent B (0−4 min), 40 to 70% solvent B (4−7 min), 70 to 99% solvent B (7−9 min), 99% solvent B (9−11 min), and 99 to 20% solvent B (11−11.10 min). The parent ion (m/z) was 331.1 4DO. The samples were quantified using fragment ions 216.1 for 4DO in a positive mode.

Chemicals. CL derivatives were synthesized as described in the Supporting Information.

(3E)-2-Methyl-4-(2,6,6-trimethylcyclohex-1-enyl)but-3-enol (KCL1). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.95 (dq, J = 15.8, 0.9 Hz, 1H), 5.22 (dd, J = 15.8, 8.3 Hz, 1H), 3.53 (dd, J = 10.3, 5.7 Hz, 1H), 3.43 (dd, J = 10.3, 7.7 Hz, 1H), 2.47−2.38 (m, 1H), 1.99−1.95 (m, 2H), 1.67 (d, J = 0.9 Hz, 3H), 1.62−1.57 (m, 2H), 1.46−1.43 (m, 2H), 1.05 (d, J = 6.6 Hz, 3H), 0.99 (s, 3H), and 0.98 (s, 3H).

^{13}C NMR ($CDCl_3$, 126 MHz): δ 137.3, 136.1, 129.1, 128.3, 67.4, 40.6, 39.3, 33.8, 32.6, 28.8, 28.7, 21.5, 19.2, and 16.7.

IR (neat): 3335, 2959, 2925, 2866, 2827, 1456, 1373, 1359, 1258, 1205, 1031, 970, and 939 cm^{-1}.

HRMS (DART) calcd for $C_{14}H_{25}O$ $[M + H]^+$: 209.1900; found, 209.1910.

3-Methyl-5-((3E)-2-methyl-4-(2,6,6-trimethylcyclohex-1-enyl)but-3-enyloxy)furan-2(5H)-one (KCL2) as a 53:47 Diastereomeric Mixture. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.80−6.77 (m, 1H), 5.89 (br d, J = 16.0 Hz, 1H), 5.81−5.78 (m, 1H), 5.24 (dd, J = 16.0, 7.7 Hz, 1H), [(3.79 (dd, J = 9.2, 6.3 Hz), 3.75 (dd, J = 9.2, 6.6 Hz)), 1H], [(3.54 (dd, J = 9.2, 7.2 Hz), 3.51 (dd, J = 9.2, 7.2 Hz)), 1H], 2.61−2.51 (m, 1H), 1.98−1.93 (m, 2H), 1.94 (dd, J = 1.4, 1.4 Hz, 3H), [(1.65 (br

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d, J = 0.9 Hz), 1.63 (br d, J = 0.9 Hz)), 3H], 1.62−1.56 (m, 2H), 1.45−1.42 (m, 2H), [(1.07 (br d, J = 6.6 Hz), 1.06 (br d, J = 6.9 Hz)), 3H], 0.964 (br d, J = 1.4 Hz, 3H), and 0.957 (br d, J = 1.4 Hz, 3H).

^{13}C NMR ($CDCl_3$, 126 MHz): δ 171.9, 142.9, 142.8, 137.4, 135.7, 135.5, 134.0, 128.1, 128.0, 101.7, 101.6, 75.1, 74.9, 39.3, 37.6, 37.4, 33.9, 32.6, 28.6, 21.3, 19.3, 17.1, and 10.6.

IR (neat): 3087, 2956, 2926, 2864, 2828, 1769, 1669, 1455, 1372, 1344, 1205, 1143, 1090, 1022, 970, and 930 cm^{-1}.

HRMS (ESI) calcd for $C_{19}H_{29}O_3$ [M + H]$^+$: 305.2111; found, 305.2110.

3-Methyl-5-(2-methyl-4-(2,6,6-trimethylcyclohex-1-enyl)-butyloxy)furan-2(5H)-one (KCL3) as a 51:49 Diastereomeric Mixture. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.81−6.79 (m, 1H), 5.79−5.78 (m, 1H), [(3.78 (dd, J = 9.2, 5.7 Hz), 3.70 (dd, J = 9.2, 6.6 Hz)), 1H], [(3.52 (dd, J = 9.2, 6.0 Hz), 3.44 (dd, J = 9.2, 6.9 Hz)), 1H], 2.06−1.99 (m, 1H), 1.95 (dd, J = 1.4, 1.4 Hz, 3H), 1.93−1.88 (m, 3H), 1.80−1.73 (m, 1H), 1.61−1.53 (m, 5H), 1.50−1.39 (m, 3H), 1.27−1.18 (m, 1H), and 0.98−0.96 (m, 9H).

^{13}C NMR ($CDCl_3$, 126 MHz): δ 172.0, 142.9, 137.2, 134.0, 126.8, 101.83, 101.75, 75.3, 75.2, 39.8, 34.9, 34.20, 34.17, 33.7, 32.7, 28.6, 25.92, 25.88, 19.8, 19.5, 16.9, 16.8, and 10.6.

IR (neat): 3089, 2927, 2868, 1768, 1668, 1454, 1377, 1344, 1205, 1138, 1090, 1022, and 930 cm^{-1}.

HRMS (ESI) calcd for $C_{19}H_{30}O_3Na$ [M + Na]$^+$: 329.2087; found, 329.2075.

3-Methyl-5-((3E)-4-(2,6,6-trimethylcyclohex-1-enyl)but-3-enyloxy)furan-2(5H)-one (KCL4). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.80−6.79 (m, 1H), 5.93 (br d, J = 15.8 Hz, 1H), 5.81−5.80 (m, 1H), 5.33(ddd, J = 15.8, 7.2, 6.9 Hz, 1H), 3.93 (ddd, J = 9.2, 7.2, 6.9 Hz, 1H), 3.72 (ddd, J = 9.2, 7.2, 6.9 Hz, 1H), 2.44 (q, J = 6.9 Hz, 2H), 1.98−1.94 (m, 5H), 1.64 (br s, 3H), 1.61−1.56 (m, 2H), 1.46−1.42 (m, 2H), and 0.96 (s, 6H).

^{13}C NMR ($CDCl_3$, 126 MHz): δ 171.9, 142.8, 137.3, 134.0, 130.4, 129.1, 128.2, 101.5, 70.0, 39.3, 33.8, 33.3, 32.6, 28.6 (2C), 21.4, 19.2, and 10.6.

IR (neat): 3088, 2925, 2864, 2827, 1769, 1668, 1445, 1344, 1205, 1140, 1089, 1016, 969, and 932 cm^{-1}.

HRMS (ESI) calcd for $C_{18}H_{26}O_3Na$ [M + Na]$^+$: 313.1774; found, 313.1778.

3-Metahyl-5-((3E)-4-(2,6,6-trimethylcyclohex-1-enyl)but-3-en-2-yloxy)furan-2(5H)-one (KCL5). KCL5-1. ^{1}H NMR (CD_3OD, 600 MHz): δ 6.96−6.95 (m, 1H), 6.20 (br d, J = 16.5 Hz, 1H), 5.98−5.97 (m, 1H), 5.33 (dd, J = 16.5, 8.3 Hz, 1H), 4.46−4.40 (m, 1H), 2.01 (br t, J = 6.2 Hz, 2H), 1.89 (dd, J = 1.4, 1.4 Hz, 3H), 1.69 (s, 3H), 1.66−1.62 (m, 2H), 1.49−1.47 (m, 2H), 1.33 (d, J = 6.2 Hz, 3H), 1.02 (s, 3H), and 1.01 (s, 3H). ^{13}C NMR (CD_3OD, 150 MHz): δ 174.0, 145.7, 137.8, 134.9, 134.3, 133.4, 130.5, 100.6, 78.5, 40.5, 34.8, 33.6, 29.3, 29.1, 22.2, 21.7, 20.3, and 10.5.

IR (neat): 3090, 2959, 2926, 2904, 2865, 2828, 1769, 1445, 1362, 1339, 1314, 1204, 1155, 1133, 1087, 1015, 975, and 935 cm^{-1}.

HRMS (ESI) calcd for $C_{18}H_{26}O_3Na$ [M + Na]$^+$: 313.1774; found, 313.1772.

KCL5-2. ^{1}H NMR (CD_3OD, 600 MHz): δ 6.97−6.96 (m, 1H), 6.11 (br d, J = 15.8 Hz, 1H), 6.02−6.01 (m, 1H), 5.44 (dd, J = 15.8, 7.6 Hz, 1H), 4.43−4.38 (m, 1H), 2.00 (br t, J = 6.2 Hz, 2H), 1.89 (dd, J = 1.4, 1.4 Hz, 3H), 1.69 (s, 3H), 1.65−1.61 (m, 2H), 1.48−1.46 (m, 2H), 1.34 (d, J = 6.9 Hz, 3H), 1.02 (s, 3H), and 1.00 (s, 3H). ^{13}C NMR (CD_3OD, 150 MHz): δ 174.1, 145.9, 137.9, 136.4, 134.1, 131.0, 130.1, 102.6, 79.6, 40.6, 35.0, 33.6, 29.2 (2C), 21.9, 21.6, 20.3, and 10.4.

IR (neat): 3089, 2927, 2866, 1766, 1446, 1375, 1335, 1205, 1134, 1088, 1012, and 939 cm^{-1}.

HRMS (ESI) calcd for $C_{18}H_{26}O_3Na$ [M + Na]$^+$: 313.1774; found, 313.1775.

3-Methyl-5-((E)-3-(2,6,6-trimethylcyclohex-1-enyl)-allyloxy)furan-2(5H)-one (KCL6). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.83−6.82 (m, 1H), 6.19 (br d, J = 15.8 Hz, 1H), 5.90−5.88 (m, 1H), 5.53 (ddd, J = 15.8, 7.5, 5.7 Hz, 1H), 4.42 (ddd, J = 11.7, 5.7, 1.4 Hz, 1H), 4.27 (ddd, J = 11.7, 7.5, 1.2 Hz, 1H), 1.99 (br t, J = 6.0 Hz, 2H), 1.95 (dd, J = 1.7, 1.4 Hz, 3H), 1.68 (d, J = 0.9 Hz, 3H), 1.63−1.58 (m, 2H), 1.46−1.44 (m, 2H), 1.00 (s, 3H), and 0.99 (s, 3H).

^{13}C NMR ($CDCl_3$, 126 MHz): δ 172.0, 143.1, 136.4, 134.0, 133.6, 129.9, 127.5, 100.1, 71.0, 39.3, 33.8, 32.7, 28.7, 28.6, 21.4, 19.1, and 10.6.

IR (neat): 3090, 2924, 2864, 2827, 1768, 1667, 1448, 1375, 1359, 1339, 1205, 1136, 1089, 1021, 998, 971, and 930 cm^{-1}.

HRMS (ESI) calcd for $C_{17}H_{24}O_3Na$ [M + Na]$^+$: 299.1618; found, 299.1632.

3-Methyl-5-((3E)-4-phenylbut-3-en-2-yloxy)furan-2(5H)-one (KCL7). KCL7-1. ^{1}H NMR (CD_3OD, 600 MHz): δ 7.45 (d, J = 8.3 Hz, 2H), 7.32 (dd, J = 8.3, 6.9 Hz, 2H), 7.26−7.23 (m, 1H), 6.97−6.96 (m, 1H), 6.67 (dd, 15.8 Hz, 3.4 Hz, 1H), 6.16 (ddd, J = 15.8, 8.3, 2.8 Hz, 1H), 5.97−5.95 (m, 1H), 4.57−4.52 (m, 1H), 1.89 (m, 3H), and 1.38 (br d, J = 6.0 Hz, 3H). ^{13}C NMR (CD_3OD, 150 MHz): δ 174.1, 145.8, 137.6, 134.7, 134.3, 130.3, 129.7 (2C), 129.1, 127.7 (2C), 100.9, 78.1, 22.1, and 10.5.

IR (neat): 3084, 3059, 3028, 2979, 2929, 1763, 1666, 1493, 1446, 1338, 1205, 1149, 1134, 1088, 1014, and 931 cm^{-1}.

HRMS (ESI) calcd for $C_{15}H_{16}O_3Na$ [M + Na]$^+$: 267.0992; found, 267.1001.

KCL7-2. ^{1}H NMR (CD_3OD, 600 MHz): δ 7.42−7.40 (m 2H), 7.32−7.29 (m, 2H), 7.25−7.21 (m, 1H), 6.99−6.98 (m, 1H), 6.60 (br d, 15.8 Hz, 1H), 6.26(dd, J = 15.8, 6.9 Hz, 1H), 6.06−6.05 (m, 1H), 4.56−4.51 (m, 1H), 1.89 (dd, J = 2.1, 1.4 Hz, 3H), and 1.40 (br d, J = 6.2 Hz, 3H). ^{13}C NMR (CD_3OD, 150 MHz): δ 174.2, 146.0, 138.1, 134.2, 132.5, 131.9, 129.6 (2C), 128.8, 127.6 (2C), 102.5, 78.6, 21.5, and 10.4.

IR (neat): 3084, 3026, 2978, 2927, 1763, 1666, 1493, 1446, 1377, 1331, 1207, 1134, 1088, 1012, 968, and 935 cm^{-1}.

HRMS (ESI) calcd for $C_{15}H_{16}O_3Na$ [M + Na]$^+$: 267.0992; found, 267.0986.

(3E)-2-Methyl-4-(2,6,6-trimethylcyclohex-1-enyl)but-3-enyl Pivalate (KCL8). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.90 (d, J = 15.8 Hz, 1H), 5.28 (dd, J = 15.8, 7.7 Hz, 1H), 4.01−3.93 (m, 2H), 2.63−2.54 (m, 1H), 1.96 (br t, J = 6.3 Hz, 2H), 1.66 (br s, 3H), 1.62−1.57 (m, 2H), 1.45−1.42 (m, 2H), 1.20 (s, 9H), 1.08 (d, J = 6.9 Hz, 3H), and 0.97 (br d, J = 1.2 Hz, 6H).

^{13}C NMR ($CDCl_3$, 126 MHz): δ 178.6, 137.4, 135.6, 128.0, 127.9, 68.8, 39.3, 38.8, 36.8, 33.9, 32.6, 28.7, 28.6, 27.2 (3C), 21.4, 19.3, and 17.2.

IR (neat): 2960, 2927, 2904, 2869, 2828, 1731, 1480, 1459, 1397, 1361, 1282, 1261, 1150, 1133, 1087, and 970 cm^{-1}.

HRMS (ESI) calcd for $C_{19}H_{32}O_2Na$ [M + Na]$^+$: 315.2295; found, 315.2293.

(3E)-2-Methyl-4-(2,6,6-trimethylcyclohex-1-enyl)but-3-enyl Benzoate (KCL9). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.07−8.04 (m, 2H), 7.56−7.52 (m, 1H), 7.42 (t, J = 7.7 Hz, 2H), 5.96 (br d, J = 16.0 Hz, 1H), 5.34 (dd, J = 16.0, 7.7 Hz, 1H), 4.27−4.20 (m, 2H), 2.77−2.70 (m, 1H), 1.95 (br t, J = 6.3 Hz,

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2H), 1.64 (s, 3H), 1.61−1.55 (m, 2H), 1.44−1.41 (m, 2H), 1.16 (d, J = 6.6 Hz, 3H), 0.96 (s, 3H), and 0.95 (s, 3H).

^{13}C NMR ($CDCl_3$, 126 MHz): δ 166.5, 137.4, 135.5, 132.8, 130.4, 129.6 (2C), 128.3, 128.2 (2C), 128.1, 69.2, 39.3, 37.0, 33.8, 32.5, 28.6 (2C), 21.3, 19.3, and 17.3.

IR (neat): 3062, 2962, 2925, 2865, 2827, 1719, 1451, 1268, 1109, 1069, 1026, and 973 cm^{-1}.

HRMS (ESI) calcd for $C_{21}H_{28}O_2Na$ $[M + Na]^+$: 335.1982; found, 335.1988.

5-((3E)-2-Methyl-4-(2,6,6-trimethylcyclohex-1-enyl)but-3-enyloxy)thiophene (KCL10). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.70 (dd, J = 5.7, 4.0 Hz, 1H), 6.53 (dd, J = 5.7, 1.4 Hz, 1H), 6.20 (dd, J = 3.7, 1.4 Hz, 1H), 5.94 (br d, J = 15.8 Hz, 1H), 5.31 (dd, J = 15.8, 7.7 Hz, 1H), 3.93 (dd, J = 8.9, 6.6 Hz, 1H), 3.88 (dd, J = 8.9, 6.9 Hz, 1H), 2.78−2.69 (m, 1H), 1.96 (br t, J = 5.7 Hz, 2H), 1.66 (br d, J = 0.9 Hz, 3H), 1.62−1.57 (m, 2H), 1.45−1.42 (m, 2H), 1.15 (d, J = 6.6 Hz, 3H), and 0.98 (s, 6H).

^{13}C NMR ($CDCl_3$, 126 MHz): δ 165.8, 137.4, 135.2, 128.3, 128.1, 124.6, 111.8, 104.7, 78.4, 39.3, 37.3, 33.9, 32.6, 28.7 (2C), 21.4, 19.3, and 17.1.

IR (neat): 3111, 3075, 2957, 2924, 2863, 2827, 1537, 1455, 1382, 1358, 1193, 1083, and 970 cm^{-1}.

HRMS (ESI) calcd for $C_{18}H_{26}OSNa$ $[M + Na]^+$: 313.1597; found, 313.1583.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c00098.

Experimental procedures and NMR spectral data of KCLs (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Cook, C. E.; Whichard, L. P.; Turner, B.; Wall, M. E.; Egley, G. H. Germination of Witchweed (Striga lutea Lour.): Isolation and Properties of a Potent Stimulant. *Science* **1966**, *154*, 1189−1190.

(2) Ejeta, G. Breeding for Striga Resistance in Sorghum: Exploitation of an Intricate Host−Parasite Biology. *Crop Sci.* **2007**, *47*, S216−S227.

(3) Parker, C. Observations on the current status of Orobanche and Striga problems worldwide. *Pest Manage. Sci.* **2009**, *65*, 453−459.

(4) Butler, L. G. *"Allelopathy, Organisms, Processes and Applications"*; Inderjit, K. M., Dakshini, M.; Enhelling, F. A., Eds.; American Chemical Society. 1995; pp 158−166.

(5) Akiyama, K.; Matsuzaki, K. i.; Hayashi, H. Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature* **2005**, *435*, 824−827.

(6) Gomez-Roldan, V.; Fermas, S.; Brewer, P. B.; Puech-Pagès, V.; Dun, E. A.; Pillot, J. P.; Letisse, F.; Matusova, R.; Danoun, S.; Portais, J. C.; et al. Strigolactone inhibition of shoot branching. *Nature* **2008**, *455*, 189−194.

(7) Umehara, M.; Hanada, A.; Yoshida, S.; Akiyama, K.; Arite, T.; Takeda-Kamiya, N.; Magome, H.; Kamiya, Y.; Shirasu, K.; Yoneyama, K.; et al. Inhibition of shoot branching by new terpenoid plant hormones. *Nature* **2008**, *455*, 195−200.

(8) Alder, A.; Jamil, M.; Marzorati, M.; Bruno, M.; Vermathen, M.; Bigler, P.; Ghisla, S.; Bouwmeester, H.; Beyer, P.; Al-Babili, S. The path from β-carotene to carlactone, a strigolactone-like plant hormone. *Science* **2012**, *335*, 1348−1351.

(9) Seto, Y.; Sado, A.; Asami, K.; Hanada, A.; Umehara, M.; Akiyama, K.; Yamaguchi, S. Carlactone is an endogenous biosynthetic precursor for strigolactones. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111*, 1640−1645.

(10) Zhang, Y.; Cheng, X.; Wang, Y.; Díez-Simón, C.; Flokova, K.; Bimbo, A.; Bouwmeester, H. J.; Ruyter-Spira, C. The tomato MAX1 homolog, SlMAX1, is involved in the biosynthesis of tomato strigolactones from carlactone. *New Phytol.* **2018**, *219*, 297−309.

(11) Yoneyama, K.; Mori, N.; Sato, T.; Yoda, A.; Xie, X.; Okamoto, M.; Iwanaga, M.; Ohnishi, T.; Nishiwaki, H.; Asami, T.; et al. Conversion of carlactone to carlactonoic acid is a conserved function of MAX1 homologs in strigolactone biosynthesis. *New Phytol.* **2018**, *218*, 1522−1533.

(12) Harrison, P. J.; Newgas, S. A.; Descombes, F.; Shepherd, S. A.; Thompson, A. J.; Bugg, T. D. H. Biochemical characterization and selective inhibition of β-carotene cis-trans isomerase D27 and carotenoid cleavage dioxygenase CCD8 on the strigolactone biosynthetic pathway. *FEBS J.* **2015**, *282*, 3986−4000.

(13) Sergeant, M. J.; Li, J. J.; Fox, C.; Brookbank, N.; Rea, D.; Bugg, T. D.; Thompson, A. J. Selective inhibition of carotenoid cleavage dioxygenases: phenotypic effects on shoot branching. *J. Biol. Chem.* **2009**, *284*, 5257−5264.

(14) Ito, S.; Kitahata, N.; Umehara, M.; Hanada, A.; Kato, A.; Ueno, K.; Mashiguchi, K.; Kyozuka, J.; Yoneyama, K.; Yamaguchi, S.; et al. A new lead chemical for strigolactone biosynthesis inhibitors. *Plant Cell Physiol.* **2010**, *51*, 1143−1150.
(15) Ito, S.; Umehara, M.; Hanada, A.; Yamaguchi, S.; Asami, T. Tebuconazole derivatives are potent inhibitors of strigolactone biosynthesis. *J. Pestic. Sci.* **2013**, *38*, 147−151.
(16) Kawada, K.; Uchida, Y.; Takahashi, I.; Nomura, T.; Sasaki, Y.; Asami, T.; Yajima, S.; Ito, S. Triflumizole as a Novel Lead Compound for Strigolactone Biosynthesis Inhibitor. *Molecules* **2020**, *25*, 5525.
(17) Ito, S.; Umehara, M.; Hanada, A.; Kitahata, N.; Hayase, H.; Yamaguchi, S.; Asami, T. Effects of triazole derivatives on strigolactone levels and growth retardation in rice. *PLoS One* **2011**, *6*, No. e21723.
(18) Kawada, K.; Takahashi, I.; Arai, M.; Sasaki, Y.; Asami, T.; Yajima, S.; Ito, S. Synthesis and Biological Evaluation of Novel Triazole Derivatives as Strigolactone Biosynthesis Inhibitors. *J. Agric. Food Chem.* **2019**, *67*, 6143−6149.
(19) Ito, S.; Braguy, J.; Wang, J. Y.; Yoda, A.; Fiorilli, V.; Takahashi, I.; Jamil, M.; Felemban, A.; Miyazaki, S.; Mazzarella, T.; et al. Canonical strigolactones are not the major determinant of tillering but important rhizospheric signals in rice. *Sci. Adv.* **2022**, *8*, No. eadd1278.
(20) Okazaki, M.; Kittikorn, M.; Ueno, K.; Mizutani, M.; Hirai, N.; Kondo, S.; Ohnishi, T.; Todoroki, Y. Abscinazole-E2B, a practical and selective inhibitor of ABA 8'-hydroxylase CYP707A. *Bioorg. Med. Chem.* **2012**, *20*, 3162−3172.
(21) Ueno, K.; Araki, Y.; Hirai, N.; Saito, S.; Mizutani, M.; Sakata, K.; Todoroki, Y. Differences between the structural requirements for ABA 8'-hydroxylase inhibition and for ABA activity. *Bioorg. Med. Chem.* **2005**, *13*, 3359−3370.
(22) Njar, V. C. O.; Brodie, A. M. H. Discovery and development of Galeterone (TOK-001 or VN/124-1) for the treatment of all stages of prostate cancer. *J. Med. Chem.* **2015**, *58*, 2077−2087.
(23) Scaffidi, A.; Waters, M. T.; Ghisalberti, E. L.; Dixon, K. W.; Flematti, G. R.; Smith, S. M. Carlactone-independent seedling morphogenesis in Arabidopsis. *Plant J.* **2013**, *76*, 1−9.
(24) Uraguchi, D.; Kuwata, K.; Hijikata, Y.; Yamaguchi, R.; Imaizumi, H.; Am, S.; Rakers, C.; Mori, N.; Akiyama, K.; Irle, S.; et al. A femtomolar-range suicide germination stimulant for the parasitic plant Striga hermonthica. *Science* **2018**, *362*, 1301−1305.
(25) Yoneyama, K.; Xie, X.; Kusumoto, D.; Sekimoto, H.; Sugimoto, Y.; Takeuchi, Y.; Yoneyama, K. Nitrogen deficiency as well as phosphorus deficiency in sorghum promotes the production and exudation of 5-deoxystrigol, the host recognition signal for arbuscular mycorrhizal fungi and root parasites. *Planta* **2007**, *227*, 125−132.
(26) Wakabayashi, T.; Hamana, M.; Mori, A.; Akiyama, R.; Ueno, K.; Osakabe, K.; Osakabe, Y.; Suzuki, H.; Takikawa, H.; Mizutani, M.; et al. Direct conversion of carlactonoic acid to orobanchol by cytochrome P450 CYP722C in strigolactone biosynthesis. *Sci. Adv.* **2019**, *5*, No. eaax9067.
(27) Wakabayashi, T.; Shida, K.; Kitano, Y.; Takikawa, H.; Mizutani, M.; Sugimoto, Y. CYP722C from Gossypium arboreum catalyzes the conversion of carlactonoic acid to 5-deoxystrigol. *Planta* **2020**, *251*, 97.
(28) Yoda, A.; Mori, N.; Akiyama, K.; Kikuchi, M.; Xie, X.; Miura, K.; Yoneyama, K.; Sato-Izawa, K.; Yamaguchi, S.; Yoneyama, K.; et al. Strigolactone biosynthesis catalyzed by cytochrome P450 and sulfotransferase in sorghum. *New Phytol.* **2021**, *232*, 1999−2010.
(29) Sugimoto, Y.; Ueyama, T. Production of (+)-5-deoxystrigol by Lotus japonicus root culture. *Phytochemistry* **2008**, *69*, 212−217.
(30) Takahashi, I.; Fukui, K.; Asami, T. On improving strigolactone mimics for induction of suicidal germination of the root parasitic plant Striga hermonthica. *Abiotech* **2021**, *2*, 1−13.
(31) Ueno, K.; Hanada, A.; Yamaguchi, S.; Asami, T. Preparation of multideuterated 5-deoxystrigol for use as an internal standard for quantitative LC/MS. *J. Labelled Compd. Radiopharm.* **2010**, *53*, 763−766.
(32) Ito, S.; Yamagami, D.; Umehara, M.; Hanada, A.; Yoshida, S.; Sasaki, Y.; Yajima, S.; Kyozuka, J.; Ueguchi-Tanaka, M.; Matsuoka, M.; et al. Regulation of Strigolactone Biosynthesis by Gibberellin Signaling. *Plant Physiol.* **2017**, *174*, 1250−1259.

https://doi.org/10.1021/acsomega.3c00098
ACS Omega 2023, 8, 13855−13862