Discovery and identification of 2-methoxy-1-naphthaldehyde as a novel strigolactone-signaling inhibitor

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Knowledge about strigolactone biosynthesis and signaling is increasing and the crystal structure of strigolactone receptor protein D14 has been resolved. Although a variety of strigolactone biosynthesis inhibitors and strigolactone agonists are known, no inhibitors of strigolactone signaling have been reported. Here, we conducted virtual screening in silico to identify chemical regulators that inhibit SL reception. We used LigandScout to analyze a pharmacophore model based on structural information about D14 protein and complex D14–D-OH (a hydrolysis product of strigolactone formed by D14). We identified a candidate compound, XM-47, and confirmed that it inhibits D14–SLR1 and D14–D53 interactions. A possible product of XM-47 hydrolysis, 2-methoxy-1-naphthaldehyde (2-MN), inhibits D14–SLR1 and D14–D53 interactions and restores the growth of rice tillering buds suppressed by strigolactone. © Pesticide Science Society of Japan

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Introduction

Strigolactones (SLs), terpenoid-derived lactones, are a class of plant hormones with multiple functions, including the suppression of shoot branching outgrowth, the regulation of root development, and the control of secondary growth and leaf senescence.¹–¹⁷ SLs also function as rhizosphere signals to induce the hyphal branching of arbuscular mycorrhizal fungi¹⁸ and trigger the germination of root parasitic weeds, such as Striga and Orobanche, which are among the most severe threats to agricultural production in sub-Saharan Africa.⁹–¹¹ All of these SL functions are closely related to agriculturally important plant traits, and SL regulators must be developed to improve crop production.

Two proteins, the α/β hydrolase family protein D14/AtD14/DAD2 (in rice/Arabidopsis/petunias, respectively) and the leucine-rich-repeat F-box protein D3/MAX2/PhMAX2 (in rice/Arabidopsis/petunias, respectively) are required for SL signaling.¹² D14, AtD14, and DAD2 bind and hydrolyze SL analogue GR24¹³–¹⁵; this hydrolytic activity is required for interaction between the D14–SL complex (DAD2–SL in petunias) and D3 (PhMAX2 in petunias).¹⁵–¹⁷ In rice, the D14–SL–D3 complex induces the ubiquitination and degradation of D53, which is probably a repressor of SL signaling, and transfers the SL signal.¹⁶,¹⁷

Several groups have resolved the crystal structure of SL receptors D14/AtD14/DAD2 (rice/Arabidopsis/petunias, respectively).¹³,¹⁵,¹⁸,¹⁹ Based on this structural information, we have suggested a model of SL reception by SL receptor D14 in rice, as follows. The hydrolysis of SL by D14 produces the hydroxy-D-ring, D-OH; D14 then forms a complex with D-OH. The presence of D-OH in the aperture area of the binding cavity of D14 triggers a change in the hydrophobicity around the surface of the binding pocket, which promotes interactions between D14 and other proteins.¹³ We assume that this model is applicable to the perception of SLs in Arabidopsis, petunias, and other plants.

Chemical tools for inhibiting SL signaling would be extremely valuable, not only for investigating the wide variety of roles played by SLs, particularly in plants for which genetic resources are not available, but also for agricultural use—to regulate the processes of plant growth, including branching, root growth, and senescence, and the seed germination of parasitic weeds. Although numerous SL agonists have been described,²⁰ no inhibitors of SL signaling have been reported.

Several chemical regulators of other plant hormones have been developed by using in silico drug design strategies. Auxinole, an auxin receptor inhibitor, was identified with virtual screening directed toward compounds with an indole struc-
In this study, we used virtual screening to identify SL-receptor inhibitors based on structural data for D-OH-bound D14 and identified one candidate SL antagonist, XM-47. Because XM-47 was predicted to be readily hydrolyzed to 2-methoxy-1-naphthaldehyde (2-MN) we focused on 2-MN. We evaluated the activity of 2-MN as an SL antagonist with a yeast two-hybrid system (Y2H) and by observing its effects on the tillering phenotype of rice.

Materials and Methods

1. Plant materials and rice tillering assay
An SL-deficient rice mutant, d10-2, of the Japonica-type cultivar (Oryza sativa L. cv. Nipponbare) and a d17-1 rice mutant of the Japonica-type cultivar (Oryza sativa L. cv. Shiokari) were used in this assay. The rice seeds were sterilized in a 2.5% sodium hypochlorite solution containing 0.02% Tween 20 for 20 min. The seeds were washed five times with sterilized water and then incubated in tubes filled with water at 25 °C for 24 hr. The germinated seeds were planted in a hydroponic culture solution with or without an experimental compound and incubated under the same conditions for 2 days. The plate assays (synthetic defined medium without histidine and adenine) were performed according to the manufacturer's protocol, except that the plate medium contained various combinations of SLs and test compounds.

2. Chemicals
GR24 was synthesized as described previously. A racemic mixture of two stereoisomers, (±)-(3R*,8bS*,2′R*)-GR24, of the four stereoisomers generated was used in the assays. The compounds used in the Y2H assay (1–61, listed in Supplemental Fig. S1) were purchased from the molecule library of Namiki Shoji Co., Ltd. (Tokyo, Japan); 2-MN (62), 2-methoxy-1-naphthoic acid (65), 1-naphthaldehyde (67), 2-hydroxy-1-naphthaldehyde (68), and 2-ethoxy-1-naphthaldehyde (69) were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan); and 2,3-dimethoxy-1-naphthaldehyde (64) and 1-(2-methoxy-naphthalen-1-yl)ethanone (66) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 6-Bromo-2-methoxy-1-naphthaldehyde (63) was synthesized as follows. The 6-Bromo-2-methoxy-1-naphthalene (2.39 g, 100 mmol), N-methylformanilide (8.0 mL, d = 1.10, 538 mmol), and phosphor yl chloride (8.7 mL, d = 1.64, 345 mmol) were dissolved in dry toluene (25 mL) in a 200 mL flask containing a magnetic stirrer. The mixture was refluxed and refluxed at 100 °C overnight, was allowed to cool to room temperature, and was then cooled on ice. A potassium acetate solution (5%) was added to neutralize the reaction solution and the aqueous layer was extracted three times with ethyl acetate (EtOAc; 20 mL). The organic layers were combined, washed with brine, and then dried over Na2SO4. The solvent was removed in vacuo and the resultant residue was purified with silica gel column chromatography (EtOAc/hexane = 1:2) to yield a solid, which was then recrystallized from EtOAc/hexane as a white solid. TLC (EtOAc/hexane = 1:2): Rf = 0.46. 1H NMR (500 MHz, CDCl3): δ 10.9 (s, 1H, –CHO), 9.17 (d, 1H, J = 9.16, ArH), 7.96 (d, 1H, J = 9.16, ArH), 7.92 (d, 1H, J = 2.29, ArH), 7.66 (dd, 1H, J = 9.16 and 2.29, ArH), 7.32 (d, 1H, J = 9.16, ArH), 4.05 (s, 3H, –OMe). HRMS (m/z): [M+H]+: Calcd. for C12H9O2Br, 264.9864; Found 264.9859.

3. Plasmid construction
For the Y2H assay, pGBK-T7 (Clontech, Mountain View, CA) and pGAD-T7 (Clontech) were used as the expression vectors. Plasmids pGBK–D14 and pGAD–SLR1 have been described previously. A fragment containing the D53 open reading frame was amplified from the total complementary DNA of rice seedlings with standard PCR, using primers D53-5 (5′-CAG TGA ATT CCA CCC GAT GCC CAC TCC GTG GGT GCC CCG-3′) and D53-3 (5′-TAT CGA TGC CCA CCC TCA ACA ATC TAG AAT TAT TC-3′), and was cloned into the SmaI site of the pGAD-T7 vector using the In-Fusion HD Cloning Kit (TakaraBio, Shiga, Japan) to construct pGAD-D53.

4. Y2H assay
The Matchmaker Two-Hybrid System (Clontech) was used for the Y2H assay. We used pGBK–D14 as the bait and pGAD–SLR1 or pGAD–D53 as the prey. The Saccharomyces cerevisiae AH109 strain was transformed with the bait and prey plasmids and grown in liquid medium for 2 days. The plate assays (synthetic defined medium without histidine and adenine) were performed according to the manufacturer’s protocol, except that the plate medium contained various combinations of SLs and test compounds.

5. Molecular docking simulation
The crystal structure of D14 at a resolution of 2.10 Å was obtained from the RCSB Protein Data Bank (ID: 3WIO), determined to be the co-crystal of D14 complexed with D-OH. For the docking simulation, H atoms were added to 2-MN, XM-47, and D14 using the AddH function in Chimera. The crystal structure of D14 at a resolution of 2.10 Å was obtained from the RCSB Protein Data Bank (ID: 3WIO), determined to be the co-crystal of D14 complexed with D-OH. For the docking simulation, H atoms were added to 2-MN, XM-47, and D14 using the AddH function in Chimera. The structure was minimized with the Molecular Modeling Toolkit using Amber99. The docking studies were performed with the automated docking tool AutoDock Vina.

6. Relative mRNA expression levels in rice
Nine-day-old rice seedlings, grown as described above, were transplanted to glass vials filled with 12 mL of sterilized hydroponic culture solution and incubated for 1 day at 25 °C under fluorescent light (70–100 µmol/m²/sec) with a 16-hr light/8-hr dark photoperiod. The compound solution to be tested was added to the hydroponic culture solution and the plants were incubated under the same conditions for 24 hr. The plants were harvested and cut into fragments of approximately 10 mm in
the border regions between the roots and leaves, which contained the apical meristems and the axillary meristems.

Total RNA was extracted with the Total RNA Extraction Kit (Plant) (RBC Bioscience, Taipei, Taiwan) and reverse transcribed with ReverTra Ace qPCR RT Master Mix (Toyobo, Tokyo, Japan). Quantitative real-time reverse transcription-PCR (qRT-PCR) was performed with primers specific for D10 (5′-CGT GGC GAT ATC GAT GGT-3′ and 5′-CGA CCT CCT CCA AGC TCT T-3′) and ubiquitin (5′-AAC CAG CTG AGG CCC AAG A-3′ and 5′-ACG ATT GAT TTA ACC AGT CCA TGA-3′) with Thermal Cycler Dice Real Time System TP800 (Takara Bio) and SYBR Premix Ex Taq (Takara Bio). The transcript levels of the D10 gene were normalized to those of ubiquitin.

7. Thermoinhibition assay

Arabidopsis seed thermoinhibition assay was performed according to the method described previously.29 Arabidopsis seeds were imbibed in 400 µL of distilled water with or without chemicals in a 24-well plate. Plates were incubated at 30°C under fluorescent light (35–40 µmol/m²/sec) with a 16-hr light/8-hr dark photoperiod for 2 days to count germination. Germination was scored for radicle protrusion and all germination tests were conducted with three replicates.

8. Striga germination assay

Striga seed germination assay was performed as described previously.30 Seeds of Striga hermonthica harvested in Sudan were kindly provided by Professor A. E. Babiker (Sudan University of Science and Technology) and imported with the permission of the Ministry of Agriculture, Forestry and Fisheries of Japan. Seeds of S. hermonthica were sterilized with a 1% sodium hypochlorite solution containing 0.01% Tween 20 for 5 min and washed five times with sterilized water. The seeds were then added to a 0.1% agar solution and dropped onto small, round, glass-fiber filters. Filters containing the seeds were arranged on a filter paper (70 mm diameter) in a Petri dish, and 1400 µL of sterilized water was added to the dish. The dishes were incubated at 30°C in the dark for 4 days. The small filters containing the seeds were transferred to a 96-well plate, and 10 µL of sterilized water or water including the appropriate chemical was added to each well. After incubation for 2 days under the same conditions, the number of germinated seeds was counted.

**Results**

1. In silico screening of SL-receptor inhibitors

In our previous crystallographic study, we showed that D-OH, a product of hydrolysis of SL by D14, binds to D14 at the aperture of its SL-binding cavity. In the structure of this complex, Val194, Ser270, and several aromatic amino acid residues of D14 surround D-OH and form favorable hydrophobic and van der Waals interactions with D-OH. Thus, D-OH functions as a plug for the catalytic cavity of D14, forming a hydrophilic region in the overall hydrophobic surface of the cap structure of D14.13 Although there are no significant differences between the overall structures of apo- and D-OH-bound D14 molecules, mutational analyses indicated that this D14–D-OH complex is important for SL signaling. Therefore, we used in silico screening, analyzing a pharmacophore model based on structural information for the D14 protein and the D14–D-OH complex, to identify lead compounds that inhibit SL receptors.

Screening was performed with LigandScout ver. 3.12 software31,32 by Affinity Science (Tokyo, Japan). First, we tried to generate a pharmacophore model from active conformation based on the position of D-OH in D14 (PDB ID: 3WIO); however, we could not obtain enough hydrogen-bond information for screening in this condition. Therefore, we set two conditions A and B. Condition A was set based on a structure-based pharmacophore model generated from the conformation of D14 (PDB: 4IHA). We employed this data set because of its good resolution (1.55 Å). Condition B was set by merging the previous pharmacophore model from 3WIO and a ligand-based pharmacophore model generated from the structure of D-OH.

More than 4.7 million compounds from the molecule library of Namiki Shoji Co., Ltd., were tested; 384 compounds were selected because they had eight matching features and they achieved high scores as calculated from those features that considered RMSs.31) We sorted them by scores and chose 61 commercially available compounds in order from the top (Supplemental Fig. S1, 1–61).

2. Inhibition by 2-MN of the D14–SLR1 and D14–D53 interactions induced by GR24 in a Y2H assay

To investigate whether the candidate compounds inhibited SL receptors, we used a Y2H assay to monitor SL-dependent D14–SLR1 and D14–D53 interactions. We tested 61 compounds and found that only one compound, XM-47 (1), inhibited both the D14–SLR1 and D14–D53 interactions induced by GR24 (Fig. 1a).

XM-47 (1) was expected to be a good candidate SL-receptor inhibitor; however, we anticipated that the imine bond in XM-47 (1) would be readily hydrolyzed in an aqueous solution to yield 2-MN (62) and 3,4-dihydroxybenzohydrazide. Therefore, we tested 2-MN in the same system. In a medium containing 2-MN (62), the interactions induced by GR24 were inhibited, even at a 2-MN concentration lower than that of XM-47 (1) (Fig. 1b). Of the 61 selected compounds tested (Supplemental Fig. S1), 15 had the 3,4-dihydroxybenzohydrazide-derived hydrazone moiety and were predicted to undergo hydrolysis to yield 3,4-dihydroxybenzohydrazide in an aqueous solution. However, in a medium containing these compounds, the interactions induced by GR24 were not inhibited. These results suggest that the activity of XM-47 (1) can be attributed to that of 2-MN (62).

3. 2-MN–D14 interaction as suggested by the docking simulation

To gain insight into the molecular mechanism underlying the inhibitory activities of these compounds, we simulated the bind-
The simulation showed that 2-MN localized to the site in the binding pocket of D14, overlapping the D-OH binding site (Fig. 2, right), and that the 2-MN moiety in XM-47 occupied the same place (Fig. 2, left). The polarity of the aldehyde group of 2-MN may affect its interaction with the indole ring of Trp205 in D14, which is reported to interact with the hydroxyl group of D-OH.13)

4. 2-MN inhibition of the suppressive effect of GR24 on second tiller outgrowth

The above data from in silico and Y2H analyses strongly indicate that 2-MN (62) is a good SL-receptor inhibitor candidate. To confirm whether 2-MN (62) inhibits SL signaling in plants, we tested several in planta assays. In hydroponically grown rice seedlings, the first and second tiller buds of SL-deficient mutants, such as d10 and d17, grow out, whereas those of wild-type plants remain dormant.24 Treatment of these mutants with
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SLs restores the dormant phenotype of the first and second tiller buds. Therefore, we performed a rice tillering assay to test whether 2-MN (62) inhibits the tiller-suppressing effects of SLs. When combinations of various concentrations of 2-MN (62) and 0.1 µM GR24 were applied to hydroponically grown d10-2 rice, 2-MN (62) combined with GR24 restored the growth of the second tiller bud that had been inhibited by GR24 (Fig. 3). The outgrowth of the second tiller bud of d10-2 plants treated with 20 µM 2-MN (62) and 0.1 µM GR24 was similar to that of corresponding untreated mutant plants (Fig. 3).

5. Effect of 2-MN on Arabidopsis seed thermoinhibition

The germination rate of Arabidopsis seeds decreases when they are exposed to high temperatures. GR24 rescues the germination of thermoinhibited wild-type seeds, but not that of a strigolactone-signaling mutant, max2-1.29) In our growth condition, the seed germination rate of Arabidopsis was ca. 15% at 30°C. GR24 increased the germination rate to approximately 62%. This alleviation of seed thermoinhibition by GR24 was inhibited when 2-MN (62) was co-treated with GR24, and 2-MN (62) showed a dose response that suppressed the effect of GR24 (Fig. 4).

6. Effects of 2-MN on the expression of an SL-synthesis gene

The D10 gene encodes carotenoid cleavage dioxygenase 7, which is involved in the SL biosynthetic pathway, and its expression is reported to be feedback-regulated by SL biosynthesis and SL signaling in rice.33) To investigate whether 2-MN (62) further inhibits SL signaling, we determined the effects of 2-MN treatment on the feedback regulation of D10 expression in d10-2 rice, in which the D10 gene is overexpressed.39) When 9-day-old rice seedlings grown in hydroponic culture were treated with 0.1 µM GR24, the expression of D10 was significantly suppressed (Fig. 5). When 20 µM 2-MN (62) was applied with 0.1 µM GR24, the expression of D10 was similar to that in mock-treated rice (Fig. 5).

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**Fig. 3.** Effect of 2-MN on the growth of rice second tiller buds. Upper panels: photographs of d10-2 mutant rice seedlings treated with the indicated chemicals. Arrows indicate second tillers. Lower graph: the length of the second tillers treated with the indicated chemicals. Seven-day-old d10-2 rice seedlings were treated with chemicals and grown for a further 7 days, and the lengths of the second tillers were measured. Error bars indicate SE of eight seedlings. Statistical differences between the groups were calculated with ANOVA followed by the Tukey–Kramer test. Bars with different letters are significantly different at \( p < 0.01 \).

**Fig. 4.** Suppression of GR24-stimulated germination of thermoinhibited Arabidopsis seeds by 2-MN. Arabidopsis seeds were imbibed in distilled water with or without chemicals and incubated at 30°C under fluorescent light with a 16-hr light/8-hr dark photoperiod for 2 days to count germination. All germination tests were conducted with three replicates in each. Error bars indicate the means±SE. Student’s t-test was used to determine the significance of differences relative to the germination rate of GR24-treated seeds without 2-MN treatment (\(* p < 0.05, ** p < 0.01\)).

**Fig. 5.** Effect of 2-MN on D10 expression. Relative transcript levels of D10 in d10-2 rice were analyzed with qRT-PCR. The data are the means±SD of three experiments. Student’s t test was used to determine the significance of differences relative to the transcript levels in mock-treated rice (\(** p < 0.01\)).
The structure–activity relationship of 2-MN derivatives

To investigate the relationship between the chemical structure of a compound and its SL-inhibiting activity and to identify inhibitors that are more efficient than 2-MN, we synthesized 2-MN derivatives (Fig. 6). The structure of 2-MN can be divided into the aldehyde group, the methoxy group, and the naphthalene ring. To change the electronic state of the naphthalene ring, we modified the ring with a halogen atom or a methoxy group to give 6-bromo-2-methoxy-1-naphthaldehyde (63) or 2,3-dimethoxy-1-naphthaldehyde (64), respectively. These derivatives were tested in Y2H assays. Compound 64 showed an inhibitory effect at 100 µM, whereas 63 showed no inhibitory effect in the Y2H assay (Fig. 7a). We then evaluated their activities in planta by co-applying them with 1 µM GR24 to SL-deficient rice. In this assay, we used a 10-fold higher concentration of GR24 than in the experiment shown in Fig. 3 to detect inhibitors stronger than 2-MN. The growth of second tiller buds of d17-1 rice co-treated with 20 µM 2-MN and 1 µM GR24 was similar to that in untreated d17-1 rice. Compounds 63 and 64 had effects similar to that of 2-MN (Fig. 7b). Because the aldehyde in 2-MN (62) was assumed to be oxidized to a carboxylic acid in the medium, we also tested 2-methoxy-1-naphthoic acid (65). However, this oxidized derivative showed significantly less inhibitory activity than 2-MN in both Y2H and rice tillering assays (Fig. 7). To investigate the importance of the aldehyde moiety as a carbonyl group, we prepared 1-(2-methoxynaphthalen-1-yl)-ethanone (66) (Supplemental Fig. S2a) and tested it in the Y2H assay; however, it showed no inhibitory activity (Supplemental Fig. S2b). To evaluate the contribution of the methoxy group of 62 to the receptor-inhibitor activity of 2-MN, we prepared 1-naphthaldehyde (67), 2-hydroxy-1-naphthaldehyde (68), and 2-ethoxy-1-naphthaldehyde (69) (Supplemental Fig. S2a); however, none of them showed inhibitory activity in the Y2H assay (Supplemental Fig. S2b). The result for 68 could be explained by its toxicity to yeast cell growth. Overall, the data collected in these structure–activity relationship studies suggest that the 2-methoxy group of 2-MN is essential for its inhibitory activity.

2-MN inhibition of SL-induced Striga seed germination

In addition to inhibiting branching, SLs also stimulate the seed germination of root parasitic weeds, such as Striga and Orobanche. Chemical regulators of SLs may be used to protect crops from damage by parasitic weeds. Recently, several groups have reported that S. hermonthica has a set of karrikin-receptor-like proteins (ShKA12s/ShHTLs), some of which bind and hydrolyze SLs. Therefore, we tested whether 2-MN inhibits the SL-induced seed germination of S. hermonthica.

After 4 days of conditioning, more than 90% of the seeds...
treated with 0.1 µM GR24 germinated; however, the germination percentage decreased when the seeds were co-treated with 100 µM 2-MN and 0.1 µM GR24. The co-application of 1 µM or 10 µM 2-MN with GR24 slightly but not significantly reduced the germination percentage (Fig. 8).

Discussion

Receptor inhibitors of plant hormones are excellent tools for various purposes, including agricultural use. Although many inhibitors of plant hormone receptors have been developed, no SL-receptor inhibitor has been reported. In this study, we performed virtual screening for inhibitors of SL receptors and identified a candidate compound, 2-MN (62). Further experiments using a Y2H assay and in planta assays suggested that 2-MN (62) inhibits the SL-induced interaction between SL-receptor D14 and its target proteins, D53 and SLR1, thus blocking SL signaling. Furthermore, docking simulation suggested that 2-MN (62) plugs the SL-binding cavity of D14, although the inhibitory effects of 2-MN (62) on the binding activity and enzymatic activity of D14 to SLs should be investigated to clarify the precise mode of action of 2-MN (62). The results of this study provide a good example of the application of virtual screening to the design of regulators of plant hormone signaling.

In this study, we set two conditions to create structure-based and ligand-based pharmacophore models and combined them for screening. We used only the D-OH to generate the ligand-based pharmacophore model. Using structural information of a greater variety of ligands, including 2-MN, will improve the pharmacophore model for more efficient screening of SL-receptor inhibitors.

We tested several 2-MN derivatives in our assays; however, we found no compound with stronger SL-inhibiting activity than 2-MN (62). This indicates that the aldehyde group and 2-methoxy group of 2-MN are essential for its SL-receptor inhibitory activity. We also tested compounds in which the naphthalene ring was modified with a 6-bromide or 3-methoxy group; however, both compounds showed almost the same activity as 2-MN (62). It is possible that further modification of the naphthalene ring, such as with a bulkier group or substitution with hetero rings, will provide better SL-receptor inhibitors. With virtual screening, we identified many candidate compounds with a 3,4-dihydroxybenzohydrazide moiety, which is present in XM-47. However, none of the compounds with this moiety, except XM-47, showed clear SL-inhibitory activity in our Y2H assay. Only 2-MN displayed SL-receptor inhibitory activity. Therefore, we concluded that this moiety is not essential for the inhibitory activity of XM-47, although we cannot exclude the possibility that this structure contributes to the binding affinity of XM-47 and the catalytic cavity of D14. Further research, such as analyzing the 2-MN–D14 co-crystal, might clarify the mechanism underlying the inhibitory effect of 2-MN and provide insights that allow the design of more efficient inhibitors of SL receptors.

There is crosstalk between many plant hormones. Because 2-MN has a similar structure to 1-naphthaleneacetic acid (NAA), an auxin, we suspected that 2-MN has auxin activity, thus affecting the tillering phenotype of rice. In a preliminary experiment, we compared the stability of the fluorescence of DII::VENUS before and after treatment with 2-MN in Arabidopsis; however, no change was observed (data not shown). We also applied NAA with GR24 to test its effect on rice shoot branching; however, no marked inhibitory effect was observed. Therefore, the SL-inhibiting activity of 2-MN is not explained by its auxin activity.

We also observed the inhibitory effect of 2-MN on the SL-induced germination of Striga seeds, although a higher concentration of 2-MN was required than for other assays. We do not think the inhibition of Striga seed germination by the high concentration of 2-MN was caused by its toxicity because the inhibitory effect was recovered by adding higher concentrations of GR24 (data not shown). Conn et al. reported that the stereospecificity of SL reception by ShKAI2s/ShHTLs differed from SL reception by D14.34 Differences between the SL-perception systems of rice D14 and ShKAI2 might cause the difference between the effects of 2-MN on rice tillering and Striga seed germination. We used the rice D14 structure to screen for SL-receptor inhibitors and for the docking simulation in this study; however, it would clearly be better to use information from Striga SL receptors to develop inhibitors of seed germination in Striga. Toh et al. recently reported the structural information of ShHTL5.36 We anticipate that it will be useful in designing of efficient inhibitors of Striga seed germination.

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