ChemComm

# Development of potent inhibitors for strigolactone receptor DWARF 14



| | |
|---|---|
| Journal: | *ChemComm* |
| Manuscript ID | CC-COM-03-2020-001989.R1 |
| Article Type: | Communication |
| | |

COMMUNICATION

# Development of potent inhibitors for strigolactone receptor DWARF 14

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

Masahiko Yoshimura, [a†] Sojung F. Kim, [b,c†] Ryosuke Takise, [a†] Shuhei Kusano,[d] Sakuya Nakamura, [d] Masanori Izumi, [d] Akiko Yagi, [a] Kenichiro Itami [a,c] and Shinya Hagihara *[c,d]

**Strigolactones (SLs) are plant hormones that suppress shoot branching through the perception by their receptor protein DWARF 14 (D14). The artificial regulation of SL signaling has been considered a potent agricultural technique because plant architecture is strongly related to crop yield. In this paper, we describe the development of a small-molecule D14 inhibitor that functions at sub-micromolar levels. This potent inhibitor may be a lead compound for a first-in-class plant growth regulator.**

Strigolactones (SLs) are sesquiterpene lactones that function as plant hormones to suppress shoot branching.[1] SLs are composed of a tricyclic lactone (ABC-rings) and a butenolide (D-ring), which are connected via an enol ether bond.[2] The biological activity of SLs in plants is triggered by their perception by a member of the $\alpha/\beta$ hydrolase fold family, DWARF 14 (D14).[3] D14 was initially identified in rice, and its orthologues have been discovered in Arabidopsis (AtD14), petunia (DAD2), and pea (RMS3). Because D14 family conserves hydrolase activity, SLs are hydrolyzed upon the perception.[4] In addition to shoot branching, SLs are implicated in the regulation of root system architecture,[5] secondary stem growth,[6] and leaf senescence.[7] Moreover, SLs play important roles in the rhizosphere, as SLs exudated from the roots attract arbuscular mycorrhizal (AM) fungi that supply water and nutrients to the plant and parasitic plants that cause huge agricultural damage.[8]

Since all these biological effects of SLs are associated with crop yield, precise control of SL signaling could be an innovative technology for enhancement of crop production.[9] Especially in highly fertilized fields, dwarf and highly branched structures are desirable traits for increasing biomass.[10] To this end, we previously developed DL1, a small-molecule antagonist for AtD14.[11] DL1 was identified via chemical screening for inhibitors of D14-mediated hydrolysis of fluorescence turn-on strigolactone analog, Yoshimulactone Green (YLG).[12] DL1 inhibits the expression of SL-inducible genes *in planta* and enhances shoot branching in *Arabidopsis* and rice. So far, regulation of plant trait has been achieved mainly by breeding or genetic modification, which irreversibly alters the trait. On the other hand, the use of a D14 inhibitor would be a chemical-based approach that enables the adjustable control of plant architecture in desired situations without any permanent genetic modifications. In addition, DL1 is anticipated to enhance symbiosis between plants and AM fungi because the *d14* mutant shows higher AM fungal colonization.[13] However, to realize the practical applications of a D14 inhibitor, compounds with higher affinity to D14 than DL1 are required.[14] In this paper, we describe the development of DL1 analogs with higher potency discovered through a structure-activity relationship study.

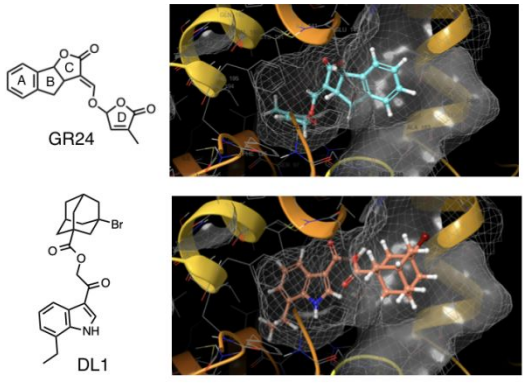
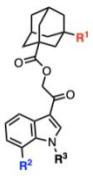



Figure 1. Structure of D14 ligands. DL1 was docked into the SL binding pocket which was created by removing GR24 from D14-GR24 complex (PDB: 5DJ5).

[a.] *Graduate School of Science Nagoya University, Chikusa, Nagoya, 464-8602, Japan.*
[b.] *Department of Chemistry, The Pennsylvania State University, University Park, PA 16802, USA*
[c.] *Institute of Transformative Bio-Molecules (WPI-ITbM), Nagoya University, Chikusa, Nagoya, 464-8601, Japan.*
[d.] *Center for Sustainable Resource Science, RIKEN, 2-1 Hirosawa, Wako city, Saitama, 351-0198, Japan.*
† These authors contributed equally to this work.
Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

First, we created a docking model of the D14-DL1 complex based on the reported crystal structure of D14 in complex with synthetic SL GR24.[15] The binding pocket of D14 was created by removing GR24 from the D14-GR24 complex. Computationally generated conformational isomers of DL1 were docked into the binding pocket, and energy minimization was performed on each structure. The most stable conformation obtained through the docking study is shown in Figure 1 — the ethyl indole moiety sits in the inner binding pocket instead of the SL D-ring, and the bromoadamantane moiety in the outer pocket forms a hydrogen bond with W155 of D14. To validate this model binding structure, we replaced various substituents on DL1 with hydrogen atom, then quantified the inhibitory activity of the DL1 derivatives using $IC_{50}$ values for YLG hydrolysis by AtD14 (Table 1 and Figure S5). Although the ethyl group of DL1 was expected to be important for the binding to AtD14, its replacement with hydrogen atom resulted in only a slight decrease in activity. The retention of activity may be explained by the docking model of DL1d, in which the indole ring is accommodated in a reversed orientation (Fig S2). On the other hand, an additional methyl group on the indole nitrogen significantly decreased affinity, suggesting that the methyl group of DL1e causes steric repulsion with the structurally rigid conserved catalytic site of D14 (Figure S3). The bromine atom is crucial for binding, indicating that its hydrogen bonding ability plays an important role in the DL1-D14 interaction.[16] These data all support the plausibility of the docking model.

Table 1. Structure activity relationship of DL1 derivatives.

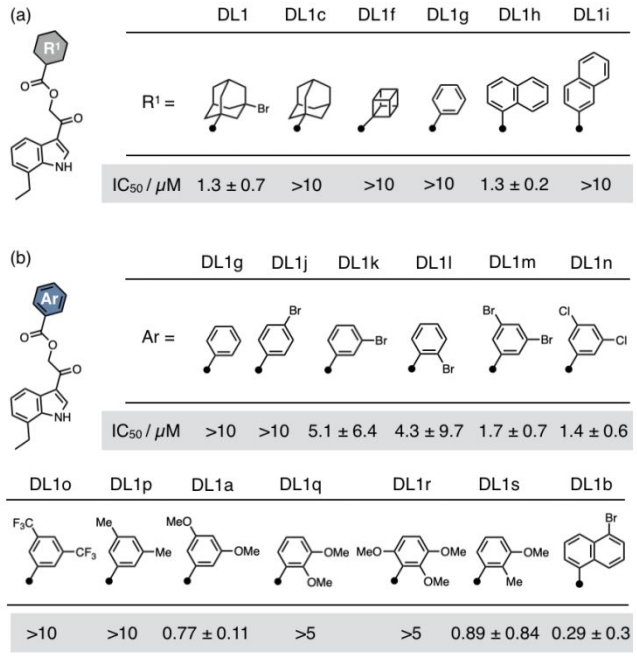


| | DL1 | DL1c | DL1d | DL1e |
|---|---|---|---|---|
| $R^1$ = | Br | H | Br | Br |
| $R^2$ = | Et | Et | H | Et |
| $R^3$ = | H | H | H | Me |
| $IC_{50}$ / μM | 1.3 ± 0.7 | >10 | 1.7 ± 1.5 | >20 |

Next, we substituted the adamantane moiety of DL1 with other groups that would allow for efficient derivatization. Among the carbon frameworks tested, only the 1-naphthyl group was found to be comparable to bromoadamantane (Table 2a). Although swapping in a simple phenyl ring yielded a poor inhibitor, the addition of an ortho- or meta- bromine significantly improved inhibitory activity, and meta-dibromo substitution afforded even higher activity (Table 2b). These data indicate that having a hydrogen bond acceptor at these positions is important for the interaction between D14 and DL1 analogs. Correspondingly, the meta-dimethoxy analog DL1a showed even higher inhibitory activity than DL1m, whereas the dimethyl analog DL1p and di(trifluoromethyl) analog DL1o had negligible activity. The addition of a substituent larger than a methyl group at the ortho-position (DL1q and DL1r) may cause steric repulsion with the binding pocket. Consequently, DL1a was found to be the most potent D14 inhibitor with a benzene scaffold.

The effect of halogen substitution was further corroborated with naphthyl derivatives of DL1. The inhibitory activity of 2-naphthyl derivative DL1h was increased by an order of magnitude with bromine substitution. The bromonaphthalene derivative DL1b exhibited the highest inhibitory activity ($IC_{50}$ = 0.29 μM) among the compounds tested in this study.

Table 2. $IC_{50}$ values of DL1 derivatives.




The actual shoot branching enhancement caused by DL1a and DL1b, which both exhibited high inhibitory activity against D14 in YLG assay, is shown in Figure 2. *Arabidopsis thaliana* seeds were cultured in the presence of DL1, DL1a, or DL1b for 30 days, then the number of primary rosette leaf branches longer than 5 mm were counted (Fig. 2, Fig. S2). In the absence of compound, the rosette leaf branch rarely appeared (two single-branching plants out of six plants), whereas some cauline leaf branches were observed in all plants. In contrast, it is visually apparent that D14 inhibitors enhanced rosette bud outgrowth. In the previous report, we demonstrated that DL1 suppresses the SL-induced expression of BRC1, which functions locally within the bud to prevent rosette branch outgrowth.[11] The results of this present study are consistent with the conclusion that BRC1 downregulation is necessary to allow branches to develop.[17] The parent compound DL1 caused on average one branch in each plant. DL1a, which showed higher *in vitro* D14 inhibitory activity than DL1, enhanced branching more than DL1 (1.6 branches on average), and the strongest inhibitor DL1b caused on average two branches per plant. Comparable number of branching was observed even in the presence of 0.1 μM DL1b, reflecting the high affinity of DL1b to AtD14 (Fig. S6). On the other hand, we observed less branching at 10 μM DL1b. This is likely due to the low solubility of DL1b, as the medium turned cloudy when the compound was added to the medium at 10 μM.

The solubility of the compound should be the next problem to be solved.

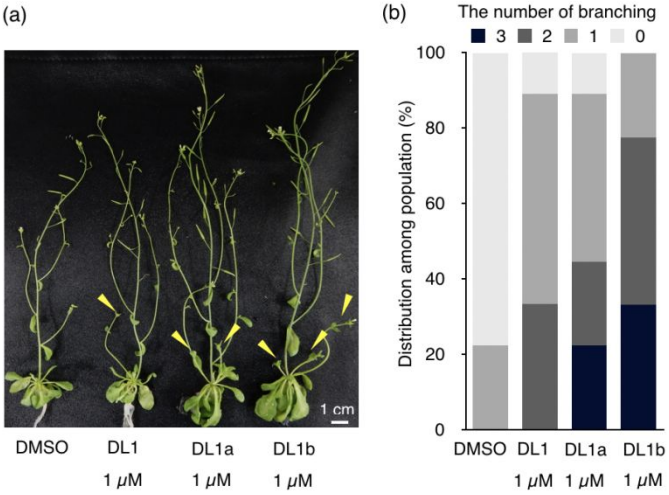



Figure 2. Shoot branching enhancement caused by DL1 analogs. (a) 30-day-old wild-type Arabidopsis plants treated with DMSO or 1 μM DL1 analogues. The yellow arrowheads indicate primary rosette buds. (b) Distribution diagram of plants with the indicated number of primary rosette leaf branches (n = 9).

## Conclusions

We have conducted a structure-activity relationship study of the D14 inhibitor DL1. The data support the plausibility of the docking model in which the ethyl indole moiety of DL1 is accommodated proximately to the D14 catalytic site. The substitution of bromoadamantane with bromonaphthalene resulted in significant enhancement of D14 inhibitory activity. The compound DL1b demonstrates high potency for *in vivo* branching induction and should be a lead compound for developing a first-in-class plant growth regulator.

This work was funded by a Grant-in-Aid for Scientific Research on Innovative Areas "Chemistry for Multimolecular Crowding Biosystems" (JSPS KAKENHI Grant No. JP17H06350), JSPS KAKENHI (19K15537), PRESTO program from Japan Science and Technology Agency (JST, JPMJPR15Q9), CREST program from JST (JPMJCR19R1), and the VICHF-IREU (NSF Award Number: 1205646) from the Center for Selective C-H Functionalization.

## Conflicts of interest

There are no conflicts to declare.

## Notes and references

1 M. Umehara, A. Hanada, S. Yoshida, K. Akiyama, T. Arite, N. Takeda-Kamiya, H. Magome, Y. Kamiya, K. Shirasu, K. Yoneyama, J. Kyozuka and S. Yamaguchi, *Nature*, 2008, **455**, 195–200; V. Gomez-Roldan, S. Fermas, P. B. Brewer, V. Puech-Pagès, E. A. Dun, J. P. Pillot, F. Letisse, R. Matusova, S. Danoun, J. C. Portais, H. Bouwmeester, G. Bécard, C. A. Beveridge, C. Rameau and S. F. Rochange, *Nature*, 2008, **455**, 189–194.
2 B. Zwanenburg and T. Pospíšil, *Mol. Plant*, 2013, **6**, 38–62.
3 T. Arite, M. Umehara, S. Ishikawa, A. Hanada, M. Maekawa, S. Yamaguchi and J. Kyozuka, *Plant Cell Physiol.*, 2009, **50**, 1416–1424; 1 D. C. Nelson, A. Scaffidi, E. A. Dun, M. T. Waters, G. R. Flematti, K. W. Dixon, C. A. Beveridge, E. L. Ghisalberti and S. M. Smith, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**, 8897–8902.
4 C. Hamiaux, R. S. M. Drummond, B. J. Janssen, S. E. Ledger, J. M. Cooney, R. D. Newcomb and K. C. Snowden, *Curr. Biol.*, 2012, **22**, 2032–2036.
5 H. Sun, J. Tao, S. Liu, S. Huang, S. Chen, X. Xie, K. Yoneyama, Y. Zhang and G. Xu, *J. Exp. Bot.*, 2014, **65**, 6735–6746.
6 J. Agusti, S. Herold, M. Schwarz, P. Sanchez, K. Ljung, E. A. Dun, P. B. Brewer, C. A. Beveridge, T. Sieberer, E. M. Sehr and T. Greb, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**, 20242–20247.
7 H. Ueda and M. Kusaba, *Plant Physiol.*, 2015, **169**, 138–147.
8 K. Akiyama, K. I. Matsuzaki and H. Hayashi, *Nature*, 2005, **435**, 824–827.
9 H. J. Bouwmeester, R. Fonne-Pfister, C. Screpanti, A. De Mesmaeker, *Angew. Chem. Int. Ed.* 2019, **55**, 12778-12786; C. Screpanti, R. Fonne-Pfister, A. Lumbroso, S. Rendine, M. Lachia, A. DeMesmaeker *Bioorg. Med. Chem. Let***.** 2016, **26**, 2392-2400.
10 Y. Wang and J. Li, *Curr. Opin. Plant Biol.*, 2011, **14**, 94–99.
11 M. Yoshimura, A. Sato, K. Kuwata, Y. Inukai, T. Kinoshita, K. Itami, Y. Tsuchiya and S. Hagihara, *ACS Cent. Sci.*, 2018, **4**, 230–234.
12 Y. Tsuchiya, M. Yoshimura, Y. Sato, K. Kuwata, S. Toh, D. Holbrook-Smith, H. Zhang, P. McCourt, K. Itami, T. Kinoshita and S. Hagihara, *Science*, 2015, **349**, 864–868.
13 S. Yoshida, H. Kameoka, M. Tempo, K. Akiyama, M. Umehara, S. Yamaguchi, H. Hayashi, J. Kyozuka and K. Shirasu, *New Phytol.*, 2012, **196**, 1208–1216.
14 O. Mashita, H. Koishihara, K. Fukui, H. Nakamura and T. Asami, *J. Pestic. Sci.*, 2016, **41**, 71–78.
15 L. H. Zhao, X. E. Zhou, W. Yi, Z. Wu, Y. Liu, Y. Kang, L. Hou, P. W. De Waal, S. Li, Y. Jiang, A. Scaffidi, G. R. Flematti, S. M. Smith, V. Q. Lam, P. R. Griffin, Y. Wang, J. Li, K. Melcher and H. E. Xu, *Cell Res.*, 2015, **25**, 1219–1236.
16 F. Y. Lin and A. D. Mackerell, *J. Phys. Chem. B*, 2017, **121**, 6813–6821.
17 J. A. Aguilar-Martínez, C. Poza-Carrión and P. Cubas, *Plant Cell*, 2007, **19**, 458–472.