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Asymmetric synthesis and effect of absolute stereochemistry of YCZ-2013, a brassinosteroid biosynthesis inhibitor

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ABSTRACT

The four stereoisomers of 2*RS*,4*RS*-1-[[2-(2,4-dichlorophenyl)-4-(2-(2-propenyloxy)phenoxymethyl)-1,3-dioxolan-2-yl]methyl]-1*H*-1,2,4-triazole (**YCZ-2013**), a novel brassinosteroid biosynthesis inhibitor, were prepared. The diastereomers of 2*RS*,4*R*-**5** and 2*RS*,4*S*-**5** were prepared by using the corresponding optically pure *R* and *S* toluene-4-sulfonic acid 2,3-dihydroxypropyl ester (*R*-**4**,*S*-**4**). The enatiomerically and diastereomerically pure acetonide (**5**) was obtained by a method involving diastereoselective crystallisation of the tosylate salt, followed by re-equilibration with the mother liquor and chromatography. The optical purity of four target compounds (**YCZ-2013**) was confirmed by chiral high-performance liquid chromatography (HPLC) and NMR. The effects of these stereoisomers on *Arabidopsis* stem elongation indicated that the *cis* isomers of 2*S*,4*R*-**YCZ-2013** and 2*R*,4*S*-**YCZ-2013** exhibited potent inhibitory activity with IC_{50} values of approximately 24 ± 3 and 24 ± 2 nM, respectively. The IC_{50} values of the *trans* isomers of 2*S*,4*S*-**YCZ-2013** and 2*R*,4*R*-**YCZ-2013** are approximately 1510 ± 50 and 3900 ± 332 nM, respectively. Co-application of brassinolide (10 nM), the most potent BR, and GA_3 (1 μM) to *Arabidopsis* seedlings grown in the dark with 2*R*,4*S*-**YCZ-2013** and 2*S*,4*R*-**YCZ-2013** revealed that brassinolide recovered the induced dwarfism of *Arabidopsis* seedlings, whereas GA_3 showed no effect.

Brassinosteroids (BRs) are a group of naturally occurring steroidal plant hormones that serve as important signal mediators with well-defined functions, including stem elongation, pollen tube growth, leaf bending, leaf unrolling, root inhibition, proton pump activation.[1–3] Because BRs control several important agronomic traits such as flowering, plant architecture, seed yield, and stress tolerance,[2,3] efforts have been made to control BR levels in plant tissues using genetic approaches. Overexpression of *DWARF4*, an enzyme that catalyses a rate-limiting step in BR biosynthesis, enhances plant growth and seed yield in *Arabidopsis*.[4] Similarly, transgenic rice plants overexpressing a sterol C-22 hydroxylase that catalyses a key step in BR biosynthesis displayed increased biomass and seed yields,[5] and available evidence indicates that mutations in BR biosynthesis may be a means to improve biomass production.[6]

Specific inhibitors targeting BR biosynthesis are quite useful for manipulating BR levels in plant tissues. In fact, it is thought that the use of inhibitors to control BR levels has advantages over genetic mutants because inhibitors can be used at different stages of plant growth and development.[7] Moreover, they can be applied to different plant species with great ease. Consequently, we carried out a systematic search for specific inhibitors of BR biosynthesis. Recently, we discovered a new class of highly potent and selective BR biosynthesis inhibitors (**YCZ**, the general structure is shown in Fig. 1) of the triazole type.[8–10] Our previous effort was limited to the synthesis and determination of the inhibition activity of *Arabidopsis* stem elongation of epimeric mixtures of **YCZ**. Because these compounds contain two chiral centres on the 1,3-dioxolane ring (C-2 and C-4, see Fig. 1), each compound gives rise to a total of four stereoisomers. In some cases, stereoisomers of azole compounds have different biological activity.[11,12] Thus, the study of the effect of absolute stereochemistry at each chiral centre of **YCZ** on BR biosynthesis inhibition represents a straightforward approach to determine 3D structural information of the inhibitor of BR biosynthesis inhibition.

To explore the stereochemistry-activity relationships of **YCZ** on BR biosynthesis, we chose 2*RS*,4*RS*-1-[[2-(2,4-dichlorophenyl)-4-(2-(2-propenyloxy)phenoxymethyl)-1,3-dioxolan-2-yl]methyl]-1*H*-1,2,4-triazole (**YCZ-2013**, the chemical structure is shown in Fig. 1) as the target compound in this study. This choice was based on the following reasons: First of all, we investigated the instrumental analysis data of related compounds in the literature and found that the stereochemical properties of the itraconazole intermediate (see Fig. 1), which shares a common chemical structure with an intermediate of the target compound **YCZ-2013** in the process of chemical synthesis, have been studied in considerable detail.[13] These instrumental analysis data are quite useful regarding the identification of the absolute stereochemical configuration of each isomer of **YCZ-2013**. Secondly, our previous work has also

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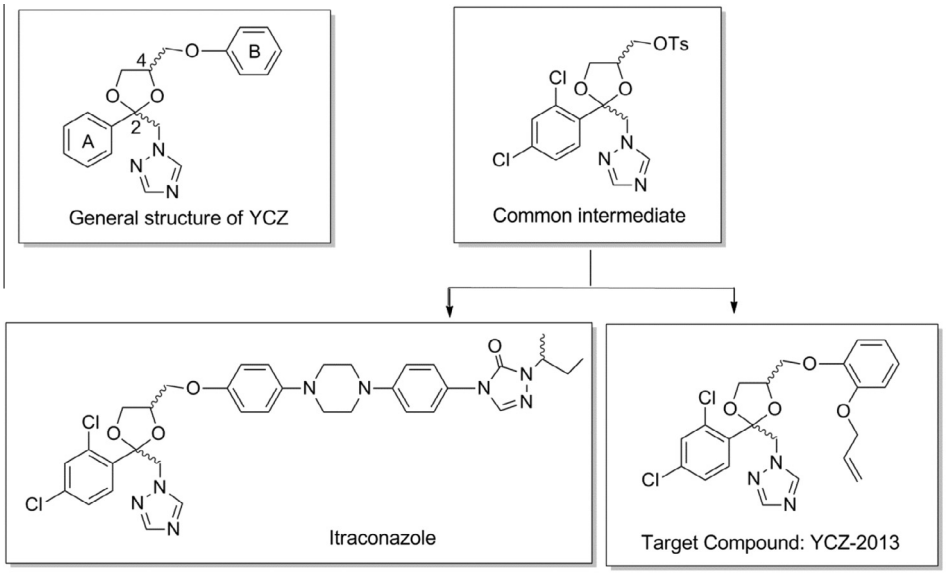
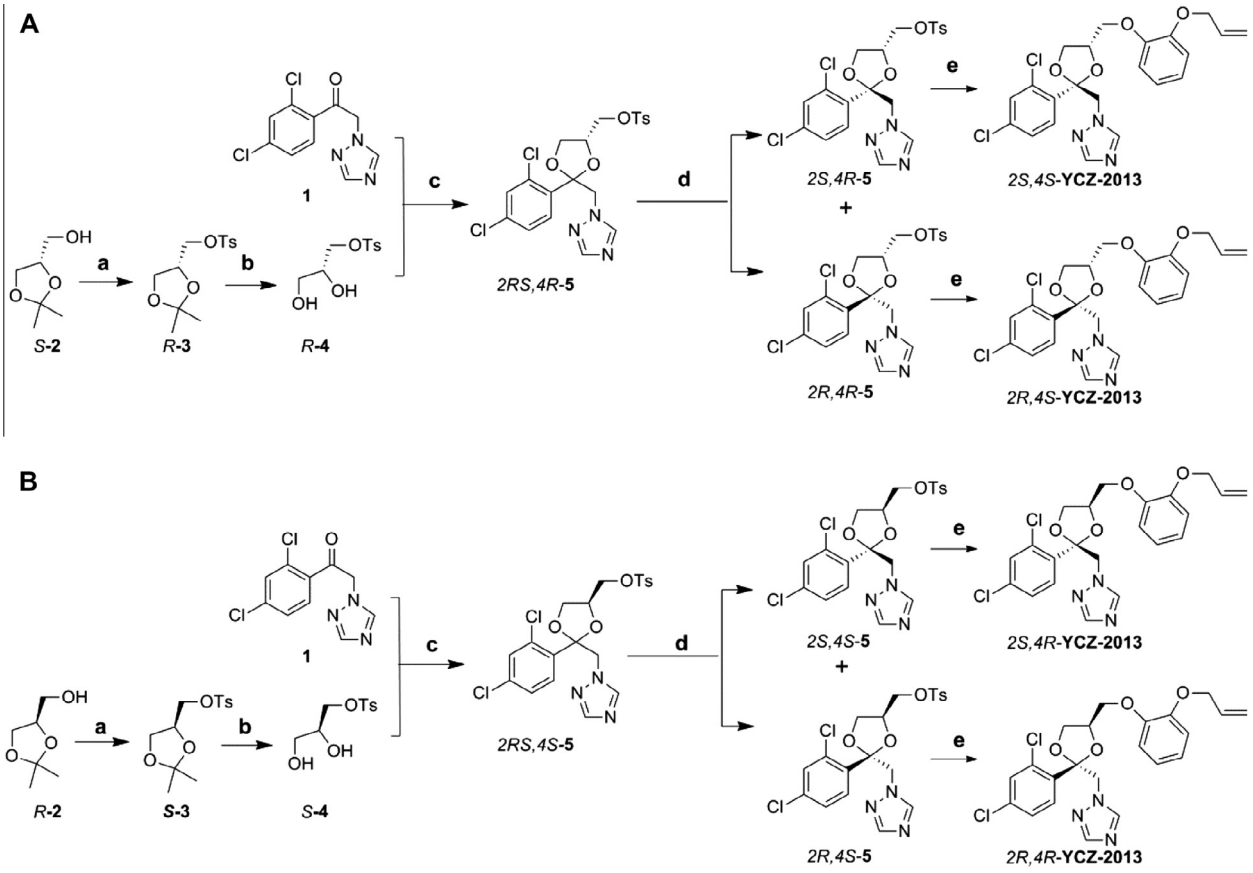


Figure 1. Chemical structures of **YCZ**s and their related compounds.

demonstrated that an analogue with an allyloxy substitution at position 2 of ring B (see general structure of **YCZ** in Fig. 1) exhibited excellent inhibitory activity on BR biosynthesis.[10]

The development of a synthetic route for the preparation of diastereomeric and enantiomeric pure key intermediates 2*S*,4*R*-**5** and 2*R*,4*R*-**5** are outlined in Scheme 1A. Compound **1** was prepared by coupling 1,2,4-triazole with 2,4-dichlorophenacyl bromide using a method previously described.[14] The key intermediate of the diastereomer mixture of 2*RS*,4*R*-**5** was prepared based on the following steps: Tosylation of commercially available optically pure isopropylidene glycerol *S*-**2** was achieved by a standard protocol (tosyl chloride in pyridine at 0 °C) to produce the corresponding *R*-**3**.



Scheme 1. General route for chemical synthesis. Reagents and conditions: (a) TsCl, pyridine, 0 °C, acetone; (b) HCl, Reflux, 6 h; (c) 3 equiv TfOH, toluene, rt, 60 h; (d) recrystallisation and PTLC purification; (e) phenol, KOH, DMF, 50 °C, 12 h.

Hydrolysis of *R*-**3** with 1 M HCl in MeOH yielded the corresponding toluene-4-sulfonic acid 2,3-dihydroxypropyl ester (*R*-**4**). Then, a diastereoselective ketalisation of **1** with optically pure *R*-**4** was carried out using 3 equivalents of trifluoromethanesulfonic acid (TfOH) in toluene at room temperature for 60 h to generate the corresponding crude diastereomeric mixture of 2*RS*,4*R*-**5** as we previously described.[8] At this point, the stereochemistry at C-4 in 2*RS*,4*R*-**5** emanates from the chiral starting material *R*-**4**, while C-2 is a new chiral centre that was created during ketalisation. After neutralisation and work-up of the reaction mixture, the crude product was dissolved in *iso*-butyl methyl ketone and treated with tosic acid. Crystals from the crude solution are mainly tosylate salts of 2*R*,4*R*-**5**. The diastereomeric highly pure 2*R*,4*R*-**5** was obtained by recrystallisation from acetonitrile using a method described previously.[13,15] On the other hand, the 2*S*,4*R*-**5** predominantly remained in the mother liquid during the purification process. To isolate the diastereomerically pure 2*S*,4*R*-**5**, the mother liquid was concentrated under reduced pressure. The remaining light yellow oil was subjected to PTLC and column chromatography (3:7, ethyl acetate:hexane). With these purification processes, 2*S*,4*R*-**5** was obtained at a high purity that was sufficient for NMR characterisation. The ^{1}H NMR spectra illustrate the obvious differences not only in the aromatic region (7–8.5 ppm) but also in the aliphatic region (3.4–4.8 ppm), indicating different 1,3-dioxolane ring configurations in the *cis* and *trans* diastereomers (data not shown). Our ^{1}H NMR data for each isomer were in good agreement with those of literature.[13,15] Preparation of diastereomerically pure 2*S*,4*S*-**5** and 2*R*,4*S*-**5** was carried out in a similar way by using *R*-**2** as the starting material (Scheme 1B). In this case, diastereomerically pure 2*S*,4*S*-**5** was obtained in a similar way to 2*R*,4*R*-**5**. Meanwhile, diastereomerically pure 2*R*,4*S*-**5** was obtained from the mother liquid in the processes of the recrystallisation of 2*S*,4*S*-**5** followed by PTLC and column chromatography as described above.

The optical purity of stereoisomers of 2*S*,4*R*-**5**, 2*R*,4*R*-**5**, 2*S*,4*S*-**5** and 2*R*,4*S*-**5** were confirmed by chiral HPLC (Daicel Chem. Ltd, CHIRALPAK OJ, ϕ4.6 mm, 250 mm) under previously reported conditions.[16] The retention times of each stereoisomers are listed in Table 1. We found that the optical purities of each purified stereoisomer were greater than 99% (data not shown). Thus, we have successfully purified the four stereo-isomers by separating the diastereomer mixture of 2*RS*,4*R*-**5** to afford pure 2*R*,4*R*-**5**, and 2*S*,4*R*-**5**. By the same strategy, diastereomerically pure 2*R*,4*S*-**5** and 2*S*,4*S*-**5** were obtained. At this stage, although the diastereomeric purity of these stereoisomers has been well established, their enantiomeric purity is still undetermined. To confirm the enantiomeric purity of these stereoisomers, we determined the retention time of the enantiomeric mixture by mixing 2*S*,4*S*-**5** with 2*R*,4*R*-**5**, and 2*S*,4*R*-**5** with 2*R*,4*S*-**5**. The mixture was subjected to chiral HPLC analysis. As shown in Table 1, both mixtures were separated by chiral HPLC. This result clearly established the enantiomeric purity of all the stereoisomers. Next, we compared the rotation of the prepared stereoisomers with the data previously reported. As shown in Table 1, we found that the properties of 2*R*,4*R*-**5** and 2*S*,4*S*-**5** are in good agreement with the data previously reported.[13,15] However, to the best of our knowledge, the specific rotations of 2*R*,4*S*-**5** and 2*S*,4*R*-**5** have not been reported in the literature.

With the building blocks of key intermediates in hand, the displacement of the *O*-tosyl group in compounds **5** with the phenolic oxygen in 2-allyloxyphenol under basic conditions afforded the final products **YCZ-2013**.[8,17] The optical purity of each purified stereoisomer were determined by using chiral HPLC, and their specific rotations were determined. As shown in Table 1, the retention times of the four stereoisomers of **YCZ-2013** are 11.57, 19.16, 13.40, and 13.79 min. Enantiomers prepared by mixing 2*R*,4*S*-**YCZ-2013** with 2*S*,4*R*-**YCZ-2013** and 2*S*,4*S*-**YCZ-2013** with 2*R*,4*R*-**YCZ-2013** can be separated by chiral HPLC (Table 1). This result clearly established the stereochemical purity of the four stereoisomers of **YCZ-2013**.

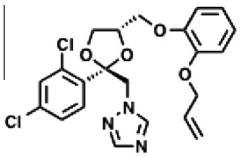
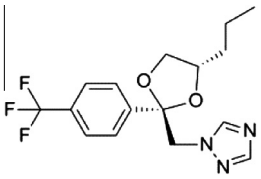
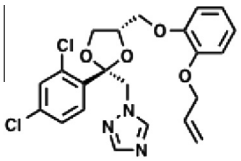
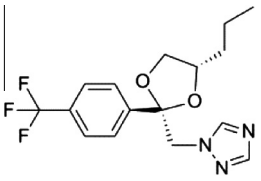
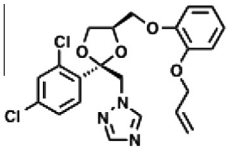
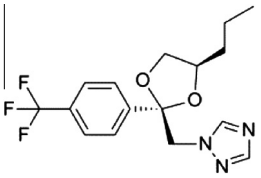
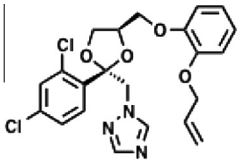
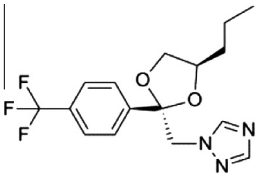
To evaluate the biological activity of the synthesised compounds on inhibition of BR biosynthesis, an assay system using *Arabidopsis* seedlings grown in the dark was used.[11] In the dark, the hypocotyls and roots of *Arabidopsis* seedlings grow significantly, and the apical hook of the cotyledon is maintained without opening. However, BR synthesis-deficient *Arabidopsis* mutants such as *dwarf 1* show remarkable dwarfism and the opening of the apical hook of cotyledons in the dark.[18] This unique phenotype of de-etiolation in the dark has been used in screens for BR biosynthesis inhibitors.[8] In the present study, we adopted this assay to determine the effects of test compounds on the hypocotyl elongation of *Arabidopsis* seedlings grown in the dark.

The chemical structures of the compounds used for biological studies are shown in Table 2. The test compounds were used at concentrations of 0, 0.001, 0.005, 0.01, 0.05, 1, 10, and 100 μM, and the IC_{50} values were calculated accordingly. As shown in Table 2, the *cis* isomers of 2*R*,4*S*-**YCZ-2013** and 2*S*,4*R*-**YCZ-2013** exhibited potent inhibitory activity, retarding the hypocotyl elongation of *Arabidopsis* seedling grown in the dark, with IC_{50} values of 24 ± 3 and 24 ± 2 nM, respectively. In contrast, the *trans* isomers of 2*S*,4*S*-**YCZ-2013** and 2*R*,4*R*-**YCZ-2013** exhibited relatively weak inhibitory activity with IC_{50} values of 1510 ± 50 and 3900 ± 332 nM, respectively. This result clearly indicated that the inhibitory activity of the *cis*-isomers (2*R*,4*S*-**YCZ-2013** and 2*S*,4*R*-**YCZ-2013**) is more potent those that of the *trans*-isomers (2*S*,4*S*-**YCZ-2013** and 2*R*,4*R*-**YCZ-2013**). Sekimata and his co-workers reported the stereochemical structure–activity relationship of Brz220 on the retardation of *Arabidopsis* hypocotyls growth.[11] Previously reported data for Brz220 are listed in Table 2 as a reference for discussion. The IC_{50} values of the four stereoisomers of **YCZ-2013** range from 24 to 3900 nM, while isomers of Brz220

Table 1
Specific rotations and retention times of **5s** and **YCZ-2013** in chiral HPLC

Compound	$[\alpha]_D$	$[\alpha]_D$ (reference)	rt (min)
2*S*,4*R*-**5**	−6.84 ± 0.19 (*c* = 1, 25.0 °C, $CHCl_3$)	—	18.61
2*R*,4*R*-**5**	+15.42 ± 0.24 (*c* = 1, 24.6 °C, $CHCl_3$)	+16.44 (*c* = 1%, EtOH)*	15.69
2*S*,4*S*-**5**	−16.30 ± 0.25 (*c* = 1, 24.6 °C, $CHCl_3$)	−16.37 (*c* = 1%, MeOH)*	16.28
2*R*,4*S*-**5**	+4.66 ± 0.39 (*c* = 1, 25.3 °C, $CHCl_3$)	—	12.99
2*S*,4*S*-**5**+2*R*,4*R*-**5**	—	—	15.34, 16.70
2*S*,4*R*-**5**+2*R*,4*S*-**5**	—	—	12.65, 18.66
2*S*,4*S*-**YCZ-2013**	+10.47 ± 0.51 (*c* = 1, 22.2 °C, $CHCl_3$)	—	19.16
2*R*,4*S*-**YCZ-2013**	+10.93 ± 0.64 (*c* = 1, 22.1 °C, $CHCl_3$)	—	11.57
2*S*,4*R*-**YCZ-2013**	−14.60 ± 1.80 (*c* = 1, 22.3 °C, $CHCl_3$)	—	13.40
2*R*,4*R*-**YCZ-2013**	−17.58 ± 0.61 (*c* = 1, 22.0 °C, $CHCl_3$)	—	13.79
2*S*,4*S*-**YCZ-2013**+2*R*,4*R*-**YCZ-2013**	—	—	13.53, 18.81
2*S*,4*R*-**YCZ-2013**+2*R*,4*S*-**YCZ-2013**	—	—	11.54, 13.16

Table 2
IC_{50} Values obtained from *Arabidopsis* hypocotyl growth

Compound	Structure	IC_{50} (nM)	Compound	Structure	IC_{50} (nM)
2*S*,4*S*-**YCZ-2013**		1510 ± 50	Brz22022 (2*S*,4*S*)		2330
2*R*,4*S*-**YCZ-2013**		24 ± 2	Brz22011 (2*R*,4*S*)		6950
2*S*,4*R*-**YCZ-2013**		24 ± 3	Brz22012 (2*S*,4*R*)		1210
2*R*,4*R*-**YCZ-2013**		3900 ± 332	Brz22021 (2*R*,4*R*)		18700

Data for Brz220 are adopted from Ref. 11.

range from 1210 to 18700 nM. This result clearly indicates that the inhibitory potency of **YCZ-2013** is greater than Brz220. The rate of the IC_{50} values of the active form of **YCZ-2013** with Brz220 is approximately 1/50, which is in agreement with our previous observations when using epimeric mixtures.[10]

The establishment of the biosynthetic pathways of BRs and functional analysis of BR biosynthesis enzymes provided evidence that several steps in BR biosynthesis are catalysed by P450 enzymes.[19] CYP90s are involved in C22 and C23 side-chain hydroxylation of BRs.[20] CYP92A6 catalyses the biochemical conversion of castasterone from typhasterol,[21] and CYP85A2 catalyses the lactonisation of castasterone to brassinolide.[22] Furthermore, the inhibition mechanisms of cytochrome P450 have been studied in considerable detail.[23] It has been demonstrated that triazole derivatives have a widespread ability to inhibit P450s due to the intrinsic affinity of the nitrogen electron pair in heterocyclic molecules for the prosthetic heme iron. The triazoles thus bind not only to lipophilic regions of the protein but also simultaneously to the prosthetic heme iron.[24] In this context, one factor that affects the binding affinity of the inhibitor to the target enzyme is the structure of the lipophilic moiety of the inhibitor. The *cis* and/or *trans* isomer of the inhibitor is the most significant factor involved in the 3D configuration. Data obtained in the present work provide evidence that the *cis* isoforms of the **YCZ-2013** are more potent than those of *trans* isoforms, indicating that the structural requirements for the lipophilic binding of the inhibitor with the target enzyme are at the opposite site of the 1,3-dioxolane moiety. It is worth mentioning that the main difference in chemical structures of **YCZ-2013** and Brz220 are 2-(2-propenyloxy)phenoxymethyl moiety (for **YCZ-2013**) and propyl moiety (for Brz-220). Regarding the inhibitory potency of **YCZ-2013** is greater than Brz220, it is reasonable to estimate that the binding pocket is relatively fit well to 2-(2-propenyloxy)phenoxymethyl moiety than propyl moiety. Additionally, the IC_{50} of the inactive form of Brz220 (2*R*,4*R*-Brz220, IC_{50} = 18700 nM) which is about 15 times to that of active form (2*S*,4*R*-Brz220, IC_{50} = 1210 nM). In contrast, the IC_{50} value of the inactive form of **YCZ-2013** (2*R*,4*R*-**YCZ-2013**) is 3900 nM which is approximately 60 times to that of active form (2*R*,4*S*-**YCZ-2013**, IC_{50} = 24 nM; 2*S*,4*R*-**YCZ-2013**, IC_{50} = 24 nM). This observation indicated that the propyl moiety of Brz220 may not fit well to the binding pocket which allows Brz220 to be more flexible.

Another factor that greatly influences the usage of **YCZ** in manipulating the BR level in plants is its selectivity. The assay method used in the present work is based on the determination of the inhibitory activity of **YCZ-2013** on the retardation of stem elongation of *Arabidopsis* seedlings. To determine the selectivity of **YCZ-2013** on BR biosynthesis inhibition, other factors involved in stem elongation of *Arabidopsis* need to be determined. GA biosynthesis inhibitors such as paclobutrazol retard the stem elongation of many plant species by blocking *ent*-kaurene oxidation and also mildly affect other cytochrome P450 monooxygenases.[25] This retardation can be rescued by the application of GA. To rule out the possibility of GA biosynthesis inhibition by our analogues, we tested the effects of brassinolide (BL), the most biologically active BR, and GA_3 on the recovery of chemically induced dwarfism of *Arabidopsis* seedlings grown in the dark. The *cis* isomers of 2*R*,4*S*-**YCZ-2013** and 2*S*,4*R*-**YCZ-2013** were subjected to the bioassay at a concentration of 0.5 μM, and *Arabidopsis* seedlings were grown in the presence or absence of BL (10 nM) or GA_3 (1 μM) for 5 days in the dark. The data shown in Table 3 are expressed as a percentage relative to the untreated control. As shown in Table 3, in the presence of BL (10 nM) or GA_3 (1 μM), the average hypocotyl length of *Arabidopsis* seedlings was approximately 108.4 ± 8.1% and 105.2 ± 6.8%, respectively. This result indicates that BL and GA_3 stimulate the hypocotyl elongation of *Arabidopsis* seedlings. We found that compounds 2*R*,4*S*-**YCZ-2013** and 2*S*,4*R*-**YCZ-2013**

Table 3
Retardation of *Arabidopsis* seedling growth by **YCZ-2013s** and rescue of growth with BL and GA_3

No.	Hypocotyl length relative to untreated *Arabidopsis* seedlings (%)		
	Chem.*	Chem. + BL (10 nM)	Chem. + GA_3 (1 μM)
Control	100	108.4 ± 8.1	105.2 ± 6.8
2*R*,4*S*-**YCZ-2013**	21.8 ± 0.7	100.5 ± 2.2	24.1 ± 0.4
2*S*,4*R*-**YCZ-2013**	22.6 ± 0.8	97.4 ± 1.7	25.0 ± 0.1
2*RS*,4*RS*-**YCZ-2013**	24.9 ± 1.8	102.6 ± 2.9	25.4 ± 1.0

* The final concentration of the chemicals was 0.5 μM.

exhibited high inhibitory activity on *Arabidopsis* seedling elongation. The hypocotyl length of the chemically treated or untreated *Arabidopsis* seedlings was approximately 21.8 ± 0.7% and 22.6 ± 0.8%, respectively. The racemic mixture of **YCZ-2013** (0.5 μM) retarded the stem elongation of *Arabidopsis* seedlings in the dark by approximately 24.9 ± 1.8%. Co-application of BL (10 nM) showed significant recovery; 2*R*,4*S*-**YCZ-2013** co-applied with BL reversed the hypocotyl lengths to 100.5 ± 2.2%, and in the case of 2*S*,4*R*-**YCZ-2013** and BL treatment, it was reversed to 97.4 ± 1.7%. A similar recovery rate can be observed with the treatment of a racemic mixture of **YCZ-2013** (Table 3). However, co-application of GA_3 (1 μM) instead of BL did not show a significant recovery of the chemical induced dwarfism of *Arabidopsis* seedlings (see Table 3). These results clearly indicate that the dwarfism of *Arabidopsis* seedlings induced by 2*R*,4*S*-**YCZ-2013** and 2*S*,4*R*-**YCZ-2013** can be recovered by the application of BL (10 nM) but not by GA (1 μM).

In conclusion, we conducted stereochemically restricted synthesis of four stereo-isomers of **YCZ-2013**. Stereochemical structure–activity relationship studies indicate that the *cis* isomers (2*R*,4*S*-**YCZ-2013** and 2*S*,4*R*-**YCZ-2013**) exhibited potent inhibition, retarding the stem elongation of *Arabidopsis* seedlings grown in the dark. The IC_{50} values of the *cis* isomers are smaller than 1/60 that of *trans* isomers, indicating that the configuration of the *cis* isomers may fit to the binding site of the target enzymes better than the *trans*. 2*R*,4*S*-**YCZ-2013** and 2*S*,4*R*-**YCZ-2013** are the most potent inhibitors of BR biosynthesis at present. We expect that further application studies of this synthetic series may lead to the establishment of new technology to regulate plant growth and development.

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17. *2R,4S-1-[4-(2-Allyloxyphenoxymethyl)-2-(2,4-dichlorophenyl)-[1,3]-dioxolan-2-ylmethyl]-1H-[1,2,4]-triazole (2R,4S-**YCZ-2013**)*: Yield: 22%, mp: 71.8–73.3 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.68 (dd, *J* = 6.3, 10.0 Hz, 1H), 3.95–4.00 (m, 3H), 4.43–4.48 (m, 1H), 4.53–4.56 (m, 2H), 4.80–4.91 (m, 2H), 5.22–5.26 (m, 1H), 5.35–5.40 (m, 1H), 5.98–6.07 (m, 1H), 6.86–6.98 (m, 4H), 7.22 (dd, *J* = 2.3, 8.5 Hz, 1H), 7.47 (d, *J* = 2.1 Hz, 1H), 7.50 (d, *J* = 8.5 Hz, 1H), 7.85 (s, 1H), 8.18 (s, 1H). The HRMS-ESI calcd for $C_{22}H_{21}Cl_2N_3O_4Na$ $[M+Na]^+$ was 484.0801, with 484.0808 determined experimentally.
*2S,4R-1-[4-(2-Allyloxyphenoxymethyl)-2-(2,4-dichlorophenyl)-[1,3]-dioxolan-2-ylmethyl]-1H-[1,2,4]-triazole (2S,4R-**YCZ-2013**)*: Yield: 26%, mp: 71.7–72.7 °C ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.68 (dd, *J* = 6.3, 10.0 Hz, 1H), 3.95–4.00 (m, 3H), 4.43–4.48 (m, 1H), 4.53–4.56 (m, 2H), 4.80–4.91 (m, 2H), 5.22–5.26 (m, 1H), 5.35–5.40 (m, 1H), 5.98–6.07 (m, 1H), 6.86–6.98 (m, 4H), 7.22 (dd, *J* = 2.3, 8.5 Hz, 1H), 7.47 (d, *J* = 2.1 Hz, 1H), 7.50 (d, *J* = 8.5 Hz, 1H), 7.85 (s, 1H), 8.18 (s, 1H). The HRMS-ESI calcd for $C_{22}H_{21}Cl_2N_3O_4Na$ $[M+Na]^+$ was 484.0801, with 484.0805 determined experimentally.
2S,4S-1-[4-(2-Allyloxyphenoxymethyl)-2-(2,4-dichlorophenyl)-[1,3]-dioxolan-2-ylmethyl]-1H-[1,2,4]-triazole: Yield: 9% (2*S*,4*S*-**YCZ-2013**), mp: 112.9–113.9 °C ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.84–3.88 (m, 2H), 4.01–4.06 (m, 2H), 4.23–4.29 (m, 1H), 4.46–4.48 (m, 2H), 4.71–4.80 (m, 2H), 5.23–5.26 (m, 1H), 5.30–5.36 (m, 1H), 5.93–6.02 (m, 1H), 6.79–6.95 (m, 4H), 7.17 (dd, *J* = 2.3, 8.5 Hz, 1H), 7.43 (d, *J* = 2.1 Hz, 1H), 7.58 (d, *J* = 8.5 Hz, 1H), 7.91 (s, 1H), 8.18 (s, 1H). The HRMS-ESI calcd for $C_{22}H_{21}Cl_2N_3O_4Na$ $[M+Na]^+$ was 484.0801, with 484.0808 determined experimentally.
2R,4R-1-[4-(2-Allyloxyphenoxymethyl)-2-(2,4-dichlorophenyl)-[1,3]-dioxolan-2-ylmethyl]-1H-[1,2,4]-triazole: Yield: 6% (2*R*,4*R*-**YCZ-2013**), mp: 116.1–117.9 °C ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.84–3.88 (m, 2H), 4.01–4.06 (m, 2H), 4.23–4.29 (m, 1H), 4.46–4.48 (m, 2H), 4.71–4.80 (m, 2H), 5.23–5.26 (m, 1H), 5.30–5.36 (m, 1H), 5.93–6.02 (m, 1H), 6.79–6.95 (m, 4H), 7.17 (dd, *J* = 2.3, 8.5 Hz, 1H), 7.43 (d, *J* = 2.1 Hz, 1H), 7.58 (d, *J* = 8.5 Hz, 1H), 7.91 (s, 1H), 8.18 (s, 1H). The HRMS-ESI calcd for $C_{22}H_{21}Cl_2N_3O_4Na$ $[M+Na]^+$ was 484.0801, with 484.0809 determined experimentally.
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