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A Specific and Potent Inhibitor of Brassinosteroid Biosynthesis Possessing a Dioxolane Ring

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Screening for brassinosteroid biosynthesis inhibitors was performed to find azole derivatives that induced dwarfism, to resemble brassinosteroid-deficient mutants in *Arabidopsis,* and which could be rescued by brassinosteroid. Through this screening experiment, propiconazole fungicide was selected as a likely inhibitor of brassinosteroid biosynthesis and, thus, propiconazole derivatives with optimized activity and selectivity were synthesized. The biological activity of these compounds was evaluated by examining cress stem elongation. Among the compounds tested, 2*RS*,4*RS*-1-[2-(4-trifluoromethylphenyl)-4-*n*-propyl-1,3-dioxolan-2-ylmethyl]-1*H*-1,2,4-triazole (**12**) showed the most potent capability to retard cress stem elongation in the light. The compound-induced hypocotyl dwarfism was restored by the coapplication of 10 nM brassinolide but not by 1 μM gibberellin. These results suggest that **12** should affect brassinosteroid biosynthesis. The potency and specificity of **12** were greater than those of brassinazole, a previously reported brassinosteroid biosynthesis inhibitor.

KEYWORDS: Brassinosteroid; inhibitor; brassinosteroid biosynthesis; gibberellin biosynthesis; brassinazole

INTRODUCTION

The application of biologically active brassinosteroid homologues causes remarkable growth responses in plants, including stem elongation, pollen tube growth, leaf bending, leaf unrolling, root inhibition, proton pump activation (*1*), initiation of ethylene synthesis (*2*), tracheary element differentiation (*3*, *4*), and cell elongation (*5*). The functions of endogenous brassinosteroids have been revealed by identifying several brassinosteroid-deficient mutants of *Arabidopsis*, pea, tomato, and rice (*6*) and have revealed that brassinosteroids are essential for normal plant growth and development. Over 40 brassinosteroid analogues have been isolated from various plant species so far (*7*−*9*). Consequently, brassinosteroids have been recognized as a new class of phytohormone (*8*, *10*).

Specific biosynthesis inhibitors are useful in determining the physiological functions of endogenous substances. As shown in mode of action studies of gibberellins, gibberellin-deficient mutants and gibberellin biosynthesis inhibitors are both quite effective (*11*, *12*). Similarly, a specific inhibitor of brassinosteroid biosynthesis should provide a new and complementary approach to understanding the functions of brassinosteroids (*13*). Asami and Yoshida reported a brassinosteroid biosynthesis inhibitor, brassinazole, and showed that this compound was a useful tool for investigating the functions of brassinosteroids (*14*−*16*). Thus, the development of more specific and potent brassinosteroid biosynthesis inhibitors is worthwhile.

Many steps of brassinosteroid biosynthesis are thought to be catalyzed by cytochrome P450 enzymes. These steps include the production of 6α-hydroxycampestanol from campestanol, cathasterone from 6-oxocampestanol(*17*), teasterone from cathasterone (*18*), castasterone from typhasterole, and brassinolide from castasterone (*8*). Thus, the biosynthetic pathway of brassinosteroids includes several potential active sites for cytochrome P450 inhibitors. An important criterion for assessing the inhibition of cytochrome P450 in brassinosteroid biosynthesis by any compound is the nature of inhibition of other cytochrome P450 enzymes of physiological importance. Specific brassinosteroid biosynthesis inhibitors were developed from uniconazole, which is a triazole-type inhibitor targeting a P450 that catalyzes an early step of gibberellin biosynthesis and partly inhibits brassinosteroid biosynthesis (*19*). Chemical modification of uniconazole led to the isolation of brassinazole (*20*) and Brz2001 (*21*) (**Figure 1**). Pyrimidine derivatives of a P450-inhibiting fungicide were also found to inhibit brassinosteroid biosynthesis (*22*). Therefore, we have begun to screen known cytochrome P450 inhibitors to search for new potent inhibitors of brassinosteroid biosynthesis (Table 1). Among the tested compounds, propiconazole and some triazole derivatives inhibited brassinosteroid biosynthesis. We chose propiconazole as a

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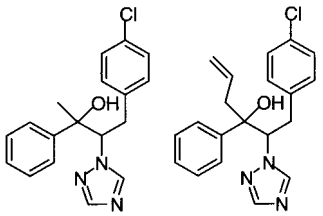


Brassinazole **Brz2001**

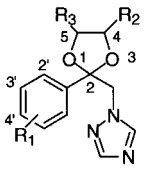
Figure 1. Structures of brassinazole and Brz2001.

Table 1. Retardation of Growth of Cress Seedlings by 1 μM Concentrations of Various Cytochrome P450 Inhibitors and Rescue from Their Effect by Brassinolide[a]

compd	hypocotyl length (cm)	
	without brassinolide	with brassiolide (10 nM)
control	2.55 ± 0.22	3.01 ± 0.23
brassinazole	1.10 ± 0.15	2.20 ± 0.25
bitertanol	2.60 ± 0.17	3.30 ± 0.27
pacrobutrazol	0.40 ± 0.10	0.90 ± 0.20
propiconazole	0.80 ± 0.10	2.45 ± 0.30
tebconazole	0.85 ± 0.17	1.70 ± 0.20
triadimefon	1.35 ± 0.10	2.30 ± 0.28
triadimenol	2.35 ± 0.18	3.15 ± 0.36

[a] Data are means ± SE obtained from 20 seedlings.

Table 2. Mono- and Disubstituted Dioxolanes Tested in This Study



no.	R_1	R_2	R_3	no.	R_1	R_2	R_3
P[a]	2′-Cl, 4′-Cl	*n*-C_3H_7	H	**9**	4′-Cl	C_2H_5	H
1	3′-Cl, 4′-Cl	*n*-C_3H_7	H	**10**	4′-Cl	*n*-C_4H_9	H
2	4′-Cl	*n*-C_3H_7	H	**11**	4′-Cl	CH_2OCH_3	H
3	2′-Cl	*n*-C_3H_7	H	**12**	4′-CF_3	*n*-C_3H_7	H
4	3′-Cl	*n*-C_3H_7	H	**13**	4′-Br	*n*-C_3H_7	H
5	H	*n*-C_3H_7	H	**14**	4′-C_6H_5	*n*-C_3H_7	H
6	4′-Cl	H	H	**15**	4′-CH_3	*n*-C_3H_7	H
7	4′-Cl	CH_3	CH_3	**16**	4′-NO_2	*n*-C_3H_7	H
8	4′-Cl	CH_3	H	**17**	4′-OCH_3	*n*-C_3H_7	H

[a] **P**, propiconazole.

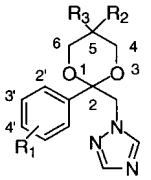
lead compound and have begun the development of new brassinosteroid biosynthesis inhibitors. The objective of this study was to synthesize and evaluate the biological activity of a novel group of potent brassinosteroid biosynthesis inhibitors.

MATERIALS AND METHODS

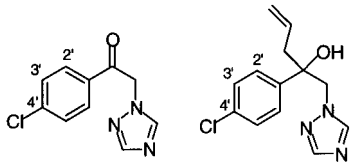
Chemicals. Chemicals for synthesis were purchased from Kanto Chemicals Co. Ltd. (Tokyo, Japan) and Tokyo Kasei Co. Ltd. (Tokyo, Japan). Triazole derivatives were purchased from Kanto Chemicals Co. Ltd. Melting points (mp) were determined on a Yanagimoto micromelting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded with tetramethylsilane (TMS) as an internal standard, using a Bruker 300 spectrometer operating at 300 MHz. Chromatographic separations were performed on a flash chromatograph (silica gel FL-60D, Fuji Silysia Chemical Ltd.).

Synthesis. The compounds in **Tables 2** and **3** and **Figure 2** were synthesized using previously described methods, as illustrated in **Figure 3** (*20*, *23*). Bromine was introduced to the α-position of a ketone to give an α-bromoketone. Ketalization of the α-bromoketone with the diols in toluene, in the presence of *p*-toluenesulfonic acid, gave diastereomixtures of the dioxolanes, which were converted to the corresponding diastereomixtures of triazoles by treating them with 1,2,4-triazole in DMSO. For the synthesis of compound **21**, α-bromoketone was coupled with triazole under basic conditions; subsequent reaction with allylmagnesium bromide resulted in a target compound.

Table 3. 1,3-Dioxanes Tested in This Study



no.	R_1	R_2	R_3
18	4′-Cl	H	H
19	4′-Cl	CH_3	CH_3



20 **21**

Figure 2. Ketone and allyl derivatives tested in this study.

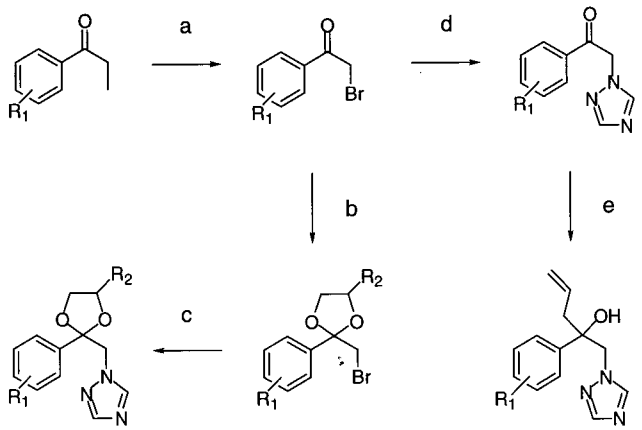


Figure 3. Synthetic pathway of triazole derivatives: (a) $AlCl_3$, Br_2, ether; (b) diol, *p*-toluenesulfonic acid, toluene; (c) 1,2,4-triazole, K_2CO_3, acetone; (d) allyl-MgBr, THF.

2-Bromo-1-(4-trifluoromethylphenyl)ethanone. A 10 mM solution of 1.88 g of 4′-(trifluoromethyl)acetophenone in 5 mL of pure anhydrous ether was cooled in an ice bath, 0.1 g of anhydrous aluminum chloride was introduced, and 1.59 g of 10 mM bromine was added gradually through a separatory funnel with continuous stirring. After the bromine had been added, the solvent and dissolved hydrogen bromide were removed at once under reduced pressure with a slight current of air. 4-(Trifluoromethyl)phenacyl bromide remained as a solid mass of brownish yellow crystals. The color was removed by shaking with a mixture of 10 mL of water and 10 mL of petroleum ether. The organic layer was dried with anhydrous sodium sulfate and concentrated at reduced pressure; the resulting residues were recrystallized from methanol. White crystals were obtained in 81% yield: mp 50−53 °C; ^{1}H NMR ($CDCl_3$) δ 4.48 (2H, s), 7.80 (2H, d, J = 8.2 Hz), 8.1 (2H, d, J = 8.2 Hz).

2RS,4RS-2-Bromomethyl-2-(4-trifluoromethylphenyl)-1,3-dioxolane. A solution of 2-bromo-1-(4-trifluoromethylphenyl)ethanone

(1.34 g, 5 mM), 1,2-pentanediol (0.60 mg, 5.8 mM), and *p*-toluenesulfonic acid (0.17 mg, 1 mM) in toluene (100 mL) was heated under reflux for 16 h. After cooling, diethyl ether (200 mL) was added. The resulting solution was washed with water, dried with sodium sulfate, filtered, and evaporated at reduced pressure. The residue was purified by chromatography over silica gel, using *n*-hexane as the eluent. A yellow oil was obtained in a 60% yield. ^{1}H NMR ($CDCl_3$) data of each diastereomer (a and b, tentatively designated) of 2*RS*,4*RS*-2-bromomethyl-2-(4-trifluoromethylphenyl)-1,3-dioxolane: (a) δ 0.98 (3H, t, J = 7.3 Hz), 1.29−1.89 (4H, m), 3.63 (2H, s) 3.73−3.81 (1H, m), 3.96−4.04 (2H, m), 7.66 (4H, s); (b) δ 0.93 (3H, t, J = 7.0 Hz), 1.29−1.89 (4H, m), 3.43 (1H, t, J = 8.1 Hz), 3.61 (1H, d, J = −14.8 Hz) 3.64 (1H, d, J = −14.8 Hz), 4.32 (1H, dd, J = 5.7, 8.1 Hz), 4.40−4.49 (1H, m), 7.66 (4H, s).

*2RS,4RS-1-[2-(4-Trifluoromethylphenyl)-4-n-propyl-1,3-dioxolan-2-ylmethyl]-1H-1,2,4-triazole (**12**).* To a solution of (0.44 g, 6.4 mM) 1,2,4-triazole in 10 mL of DMSO was added (0.41 g, 6 mM) 85% pure potassium hydroxide, and the mixture was stirred at 45 °C until it became a clear colorless solution. Then (1.37 g, 4 mM) 2*RS*,4*RS*-2-bromomethyl-2-(4-trifluoromethylphenyl)[1,3]dioxolane was added in 2 mL of DMSO. The mixture was stirred at 140 °C overnight. The yellow solution was cooled, and 100 mL of ice−water and 120 mL of diethyl ether were added. The water phase was separated, and the organic solution was washed with water, dried with sodium sulfate, filtered, and evaporated. The residue was purified by chromatography over silica gel, using ethyl acetate/*n*-hexane (7:3) as the eluent. White crystals were obtained in 40% yield: mp 62−65 °C; ^{1}H NMR ($CDCl_3$) data of each diastereomer (a and b, tentatively designated) of **12** (a) δ 0.92 (3H, t, J = 7.0 Hz), 1.26−1.45 (4H, m), 3.24 (1H, t, J = 6.6 Hz), 3.91 (2H, m), 4.50 (2H, s), 7.66 (4H, s), 7.94 (1H, s), 8.23 (1H, s); (b) δ 0.89 (3H, t, J = 7.0 Hz), 1.25−1.53 (4H, m), 3.34 (1H, t, J = 8.3 Hz), 3.76−3.82 (1H, m), 4.04 (1H, dd, J = 5.7, 8.3 Hz), 4.49 (1H, d, J = −14.6 Hz), 4.53 (1H, d, J = −14.6 Hz), 7.67 (4H, s), 8.04 (1H, s), 8.51 (1H, s). Anal. Calcd for $C_{16}H_{18}F_3N_3O_2$: C, 56.30; H, 5.32; N, 12.31; F, 16.70. Found: C, 56.14; H, 5.31; N, 11.95; F, 16.98.

Triazole derivatives **1**−**11** and **13**−**19** were prepared in a similar way.

*1-(4-Chlorophenyl)-2-(1,2,4-triazol-1-yl)ethanone (**20**).* A solution of (4.43 g, 20 mM) 4′-chlorophenacyl bromide, (1.38 g, 20 mM) 1,2,4-triazole, and (1.38 g, 10 mM) potassium carbonate in 100 mL of acetone was stirred for 12 h at room temperature. After removal of the potassium bromide by filtration, the solution was concentrated and 200 mL of water was added. The solution was extracted with ethyl acetate, then dried with sodium sulfate, filtered, and evaporated at reduced pressure. The residue was purified by chromatography over silica gel using ethyl acetate/*n*-hexane (1:1) as the eluent. White crystals were obtained in 81% yield: mp 149−150 °C; ^{1}H NMR (DMSO) δ 6.02 (2H, s), 7.71 (2H, d, J = 8.5 Hz), 8.06 (2H, d, J = 8.5 Hz), 8.10 (1H, s), 8.53 (1H, s).

*2RS-2-(4-Chlorophenyl)-1-(1,2,4-triazol-1-yl)pent-4-en-2-ol (**21**).* To solution of (0.22 g, 1 mM) 1-(4-chlorophenyl)-2-(1,2,4-triazol-1-yl)-ethanone (**20**) in dry THF (2.0 mL) under N_2 at −78 °C was added dropwise a 1 M solution of magnesium propenyl bromide (1.0 mL, 1.0 mM). The mixture was stirred for 1 h at −78 °C and for 1 h at room temperature. The solution was diluted with saturated aqueous ammonium chloride (10 mL), the phases were separated, and the aqueous phase was extracted with ethyl acetate (2 × 10 mL). The combined organic phases were dried ($MgSO_4$), filtered, and evaporated at reduced pressure. The residue was purified by chromatography over silica gel using ethyl acetate/*n*-hexane (4:1) as the eluent. A yellow oil was obtained in 30% yield: mp 104−107 °C; ^{1}H NMR ($CDCl_3$) δ 2.48 (1H, dd, J = 8.1, 14.0 Hz), 2.79 (1H, dd, J = 6.3, 14.0 Hz), 4.54 (1H, d, J = −14.0 Hz), 4.63 (1H, d, J = −14.0 Hz), 5.13 (1H, d, J = 6.3 Hz), 5.17 (1H, s) 5.61 (2H, d, J = 6.3 Hz), 7.32 (4H, s), 8.01 (1H, s), 8.71 (1H, s). Anal. Calcd for $C_{13}H_{14}ClN_3O$: C, 59.21; H, 5.35; N, 15.93; Cl, 13.44. Found: C, 58.85; H, 5.41; N, 15.15; Cl, 14.38.

Plant Materials and Growth Conditions. Cress seeds (*Lepidium sativum* L.) were purchased locally. The seeds used for the assay were sterilized in a 1% NaOCl solution for 20 min and washed with sterile distilled water five times. Cress seeds were sown in 0.8% agar-solidified medium containing half-strength Murashige and Skoog salts and 1.5% sucrose (w/v) in Agripots (Kirin Brewery Co., Ltd., Tokyo, Japan) with or without chemicals. These chemicals were dissolved and diluted with DMSO. DMSO alone was added in a control experiment. Cress seeds were grown in 16-h light (240 meinstein m^{-2} s^{-1}) and 8-h dark conditions in a growth chamber at 25 °C for 8 days. Hypocotyl length of 20 seedlings of cress was measured with a rular and each experiment was repeated three times.

RESULTS AND DISCUSSION

It has been demonstrated that cress (*L. sativum* L.) is very sensitive to an internal deficiency of brassinosteroids and is therefore a useful species for evaluating brassinosteroid biosynthesis inhibitors. For example, cress hypocotyl growth was retarded by the treatment of brassinazole or Brz2001, and this retardation was canceled by the coapplication of the most potent brassinosteroid, brassinolide, but not by other plant hormones (*20*, *21*).

Evaluation of Cytochrome P450 Inhibitors as New Lead Compounds for Brassinosteroid Biosynthesis Inhibitors. From studies using cytochrome P450 inhibitors, the azole moiety of the inhibitors is believed to act as a ligand to bind to the iron atom of the heme prosthetic group of the cytochrome P450 enzyme, forming a coordinated complex. In some cases, azole derivatives have multiple inhibition sites. For example, paclobutrazol, a gibberellin-biosynthesis inhibitor, retards the stem elongation of many plant species by blocking ent-kaurene oxidation and can also mildly affect other cytochrome P450 mono-oxygenases, such as the inhibition of sterol formation by blocking 14α-demethylation (*23*). Uniconazole, also a giberellin-biosynthesis inhibitor, was reported to be effective for reducing the level of brassinosteroids (*19*). Thus, chemical modification of azole derivatives with brassinosteroid biosynthesis inhibitory activity may produce specific brassinosteroid biosynthesis inhibitors. Brassinazole, the first specific brassinosteroid biosynthesis inhibitor, was developed on the basis of the chemical structures of both uniconazole and paclobutrazol. To find new lead compounds for brassinosteroid biosynthesis inhibitors, which target cytochrome P450 existing in the brassinosteroid biosynthesis pathway, we investigated the inhibitory potency of several azole derivatives that are available on the market. Among the azole derivatives tested, propiconazole was selected as the best lead compound (**Table 1**). Propiconazole is a fungicide that targets lanosterol 14α-demethylase in the ergosterol biosynthesis pathway (*23*). Moreover, this triazole is reported to show plant growth regulation activity by the inhibition of obtusifoliol 14α-demethylase (*24*, *25*). In our experiment, propiconazole-treated cress showed dwarfism that could be rescued considerably by brassinolide treatment. This implies that the morphological alteration of cress seedlings treated with propiconazole should be mainly due to the deficiency of brassinosteroid.

Biological Activity of the Propiconazole Derivatives. Although the synthesized compounds consist of four isomers, these compounds were subjected to biological testing without further purification. The results obtained are shown in **Figure 4**. To investigate which chemical substituent on the aromatic ring was responsible for the retardation of cress stem elongation, various substituents were introduced onto the aromatic ring of propiconazole (Table 2). The results were as follows: Among chloro-substituted phenyl compounds (**1**−**4**), the 4′-chloro derivative (**2**) exhibited activity as high as that of propiconazole (**P**), the 3′,4′-dichloro derivative (**1**) was the second highest, whereas the 2′-chloro and 3′-chloro analogues (**3**, **4**) showed little activity against the retardation of cress stem elongation. Considering that propiconazole has a chlorine atom on the 2′-

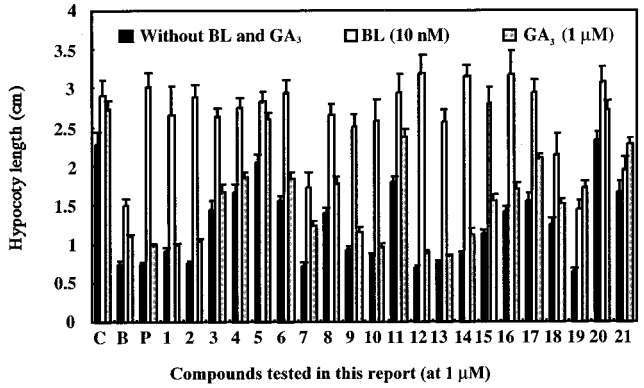


Figure 4. Retardation of growth of cress seedlings by triazole derivatives and rescue by BL and GA_3: C, control; B, brassinazole; P, propiconazole; BL, brassinolide; GA_3, gibberellic acid. Data are means ± SE obtained from 20 seedlings.

position of the phenyl ring but shows a good activity, the low activity of **3** or **4** can be due to the absence of a chlorine atom on the 4′-position of the phenyl ring. Further evidence comes from the fact that derivative **5**, lacking a substituent on the aromatic ring, exhibited little activity. Chemical variations of the substituents on the phenyl ring moiety in the synthesized series showed that the 4′-substitution of the phenyl ring is necessary. The key observation is the good recovery of cress growth from the **P**-, **1**-, and **2**-induced hypocotyl dwarfism by coapplication of brassinolide with these compounds, whereas coapplication of gibberellic acid was less effective for recovery. Compound **6**, which lacks the side chain at the 1,3-dioxolane moiety of the propiconazole derivatives, scarcely affected cress stem elongation. Consequently, the side-chain effects at 1,3-dioxolane, when the substituents on the phenyl group were fixed to the 4′-chlorine atom, were investigated. Compound **7**, methylated at both C4 and C5 of the 1,3-dioxolane moiety of the propiconazole derivatives, exhibited high activity. Compound **7**-induced hypocotyl dwarfism was noticeably rescued by coapplication of gibberellic acid, suggesting that the morphological changes in cress seedlings treated with **7** are at least partly due to gibberellin deficiency; similarly, uniconazole (a gibberellin-biosynthesis inhibitor) is known to reduce the level of gibberellin and induce dwarfism, which is almost completely rescued by the coapplication of gibberellin acid. For the optimal retardation of cress stem elongation, the C4-alkyl substituent *n*-propyl is required for **2**. Other alkyl groups are too short (methyl and ethyl; **8**, **9**) or too long (*n*-butyl; **10**). In this series, the degree of recovery from chemical-induced dwarfism of cress by brassinolide treatment appeared to increase with increasing inhibitory activity. Thus, the presence of an *n*-propyl group on the 1,3-dioxolane moiety of propiconazole derivatives is essential for the inhibitory activity of brassinosteroid biosynthesis. Introduction of an oxygen atom in the alkyl side chain on the 1,3-dioxolane caused a drastic loss of inhibition of cress hypocotyl elongation, which may indicate the presence of a subpocket in the inhibitor-binding site, which is lipophilic and has a limited size.

As the above results suggest that the chlorine atom at the 4′-position of the phenyl group was probably essential for the activity, other functional groups were then introduced into this position to investigate the factor for enhancing the activity. Some of the triazole derivatives having substituents other than chlorine (**12**−**17**) also maintained potent inhibitory activity of cress hypocotyl growth. The order of inhibitory activity of cress hypocotyl elongation was as follows: trifluoromethyl (**12**) > chlorine (**2**) = bromine (**13**) > phenyl (**14**) > methyl (**15**) > nitro (**16**) > methoxy (**17**). These results suggest the electron-withdrawing lipophilic substituents at the 4′-position of the phenyl group may be important for potent activity. The activities of **2**, **12**, and **13** were directly compared in a dose-dependent manner as in **Figure 5**. Among these chemicals, **12** was the most potent and **12**-treated cress showed the best recovery by the coapplication of brassinolide. As a result, it was suggested that the substituent at the 4′-position of phenyl rings should play an important role in the inhibition of brassinosteroid biosynthesis. A trifluoromethyl group at the 4′-position was the best substituent for the phenyl ring. The potency of inhibitory activity of stem elongation and degree of recovery by brassinolide treatment were well correlated.

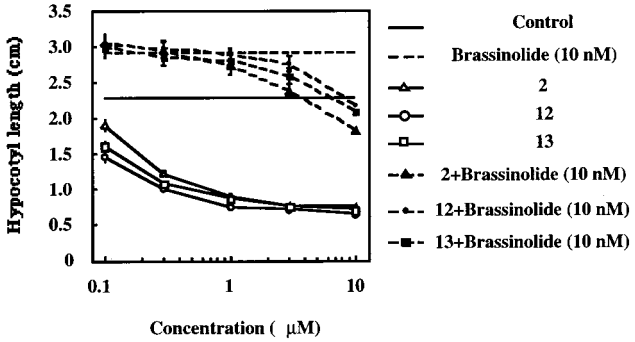


Figure 5. Hypocotyl length of light-grown, 8-day-old cress seedlings treated with various concentrations of **2**, **12**, and **13** from 0.1 to 10 μM: BL, brassinolide. Data are means ± SE obtained from 20 seedlings.

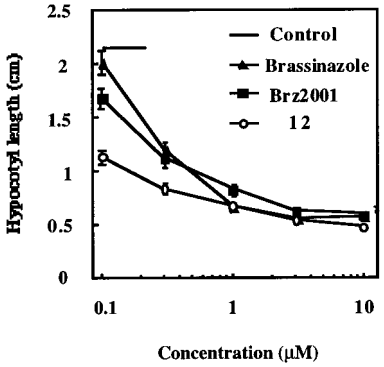


Figure 6. Hypocotyl length of light-grown, 8-day-old cress seedlings treated with various concentrations of brassinazole, Brz2001, and **12** from 0.1 to 10 μM. Data are means ± SE obtained from 20 seedlings.

The dioxane-type compounds with a 4′-chlorophenyl ring showed weaker activity than **12** in the hypocotyl elongation test (**Table 3**). Although compound **19**, with a dimethyl group on the dioxane ring, exhibited clear inhibition, **19**-treated dwarf cress showed good recovery by coapplication of 1 μM gibberellic acid and slight recovery by coapplication of 10 nM brassinolide, indicating that **19** affects both brassinosteroid and gibberellin biosynthesis. In **Figure 3**, chemicals that have no dioxolane ring are shown. As a result, ketone derivative (**20**) exhibited almost no activity. The hypocotyl length of cress seedlings treated with 1 μM allyl derivative (**21**) was 230% of that of **12**. Thus, it is demonstrated that the presence of the dioxolane moiety should be critical for the inhibition of cress hypocotyl elongation. As an overall result, **12** is the most potent inhibitor among the compounds in this study. The inhibitory activities of **12**, brassinazole, and Brz2001 were directly compared in a dose−response manner as is shown in **Figure 6**. At 0.1 μM or higher, **12** retarded the hypocotyl growth at the

level of half of the control, whereas at 0.1 μM Brz2001 or brassinazole exhibited little activity.

In conclusion, we have discovered a novel group of potential brassinosteroid biosynthesis inhibitors that are comparable to Brz2001 or brassinazole in their in vivo efficacy on cress. Inhibition of brassinosteroid biosynthesis by this new chemical series appears to be linked to the combination of the triazole moiety, the 4-*n*-propyl-1,3-dioxolane moiety, and the 4′-trifluoromethyphenyl moiety. Compound **12** consists of four isomers; current research will reveal the stereostructure−activity relationship of **12**, both in vivo and in vitro. This novel brassinosteroid biosynthesis inhibitor will play an important role in the investigation of the function of brassinosteroids, not only in other plants but also in tissues, organs, and biochemical processes. Moreover, this inhibitor will provide a way to reveal new brassinosteroid pathways or other novel mutants. In addition to its use in basic science, it may be possible to develop **12** as a commercial plant growth regulator.

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