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Synthesis and biological evaluation of novel azole derivatives as selective potent inhibitors of brassinosteroid biosynthesis

Kazuhiro Yamada, Osamu Yajima, Yuko Yoshizawa, Keimei Oh *

Department of Biotechnology, Faculty of Bioresource Sciences, Akita Prefectural University, 241-438, Shimoshinjo Nakano, Akita 010-0195, Japan

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ABSTRACT

Brassinosteroids (BRs) are phytohormones that control several important agronomic traits, such as flowering, plant architecture, seed yield, and stress tolerance. To manipulate the BR levels in plant tissues using specific inhibitors of BR biosynthesis, a series of novel azole derivatives were synthesized and their inhibitory activity on BR biosynthesis was investigated. Structure–activity relationship studies revealed that 2*RS*, 4*RS*-1-[4-(2-allyloxyphenoxymethyl)-2-(4-chlorophenyl)-[1,3]dioxolan-2-ylmethyl]-1*H*-[1,2,4]triazole (**G_2**) is a highly selective inhibitor of BR biosynthesis, with an IC_{50} value of approximately 46 ± 2 nM, which is the most potent BR biosynthesis inhibitor observed to date. Use of gibberellin (GA) biosynthesis mutants and BR signaling mutants to analyze the mechanism of action of this synthetic series indicated that the primary site of action is BR biosynthesis. Experiments feeding BR biosynthesis intermediates to chemically treated *Arabidopsis* seedlings suggested that the target sites of this synthetic series are CYP90s, which are responsible for the C-22 and/or C-23 hydroxylation of campesterol.

1. Introduction

Plant responses to internal and external stimuli through the activation of gene expression are regulated by signal transduction pathways. Brassinosteroids (BRs) serve as important signal mediators that are involved in plant growth and development. Molecular genetic analysis established the role of BRs as endogenous plant hormones with well-defined functions including extreme dwarfism, delayed senescence, male sterility, and constitutive photomorphogenesis in the dark.[1–3] Exogenous application of BR has been shown to promote cell expansion and division, regulating leaf senescence, pollen development, fruit ripening, and to modulate the plant response to numerous environmental cues.[4] Because BRs control several important agronomic traits such as flowering, plant architecture, seed yield, and stress tolerance,[5,6] various efforts have been made to control BR levels in plant tissues using genetic approaches. Using transgenic techniques to manipulate endogenous BR content has a remarkable effect on plant growth. Overexpression of DWARF4, an enzyme that catalyzes a rate-limiting step in BR biosynthesis, enhances plant growth and seed yield in *Arabidopsis*.[7] Similarly, transgenic rice plants over expressing a sterol C-22 hydroxylase that catalyzes a key step in BR biosynthesis increased biomass and seed yields,[8] and available evidence indicates that mutations in BR biosynthesis may be a means to improve biomass production.[9]

An alternative method to manipulate the BR levels in plant tissues is the use of specific inhibitors that target the enzymes involved in BR biosynthesis. Asami and Yoshida reported the discovery of brassinazole (Brz, chemical structure shown in Fig. 1), the first class of synthetic BR biosynthesis inhibitors.[10,11] Studies on the mode of actions of Brz have shown that the target site(s) of Brz is DWARF4.[12] Brz has been used not only to reveal the role of BR in various plant species,[13] but also for the isolation and characterization of genes that function in BR signaling.[14,15] The mutants, BZR1 (Brassinazole Resistant 1)[16] and BZR2 (Brassinazole Resistant 2),[17] also known as BES1, were identified as nuclear transcription factors that are dominant gain-of-function mutants resistant to Brz. BZR1 and BZR2 are components located downstream of the BR receptors in BR signal transduction pathways. The signal transduction of BR is thought to function through BR binding to the extracellular domain of the leucine-rich-repeat receptor-like kinase (LRR–RLK) BRI1 (Brassinosteroid Insensitive 1)[18] and BAK1, a coreceptor of BRI1,[19] which convey the BR signal to downstream components including BIN2 (BR-Insensitive 2)[20] and BSU1 (bri1 Suppressor 1).[21] Subsequent phosphorylation of BZR1 and BZR2/BES1 inhibits their activity through multiple mechanisms.[22] BR-induced dephosphorylation activates the BZR1 and BZR2/BES1 proteins, which directly regulate the transcription of

Abbreviation: BR, brassinosteroid; BL, brassinolide; GA, gibberellin; Brz, brassinazole; DMF, dimethylformamide; mp, melting point; rt, room temperature.

* Corresponding author. Tel.: +81 18 872 1590; fax: +81 18 872 1676.
E-mail address: jmwang@akita-pu.ac.jp (K. Oh).

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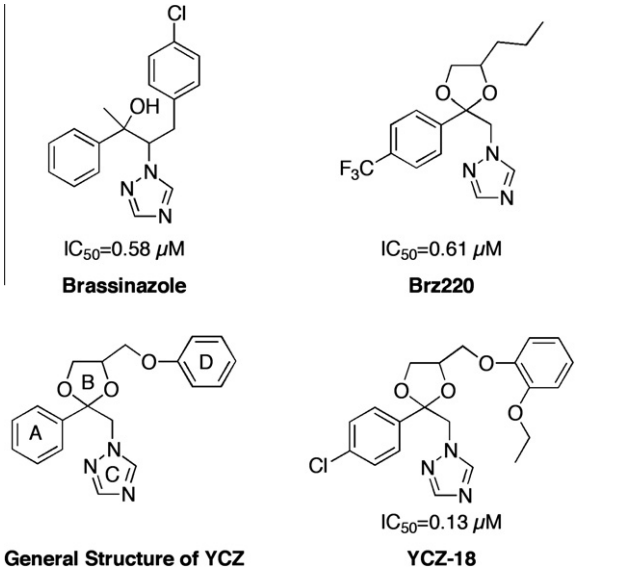


Figure 1. Chemical structures of brassinosteroid biosynthesis inhibitors and general structure of chemicals in this study.

BR-responsive genes. Consequently, inhibitors of BR biosynthesis are useful tool for searching for components of the BR signal transduction pathway. This method has advantages over mutant screens using BR-deficient mutants as a background because it can be used at different stages of plant growth and development.[16] Moreover, it can be applied to different plant species with great ease. In this context, searching for selective and potent inhibitors of BR biosynthesis represents a useful approach to dissecting the functions of BRs as well as their signal transduction pathways.

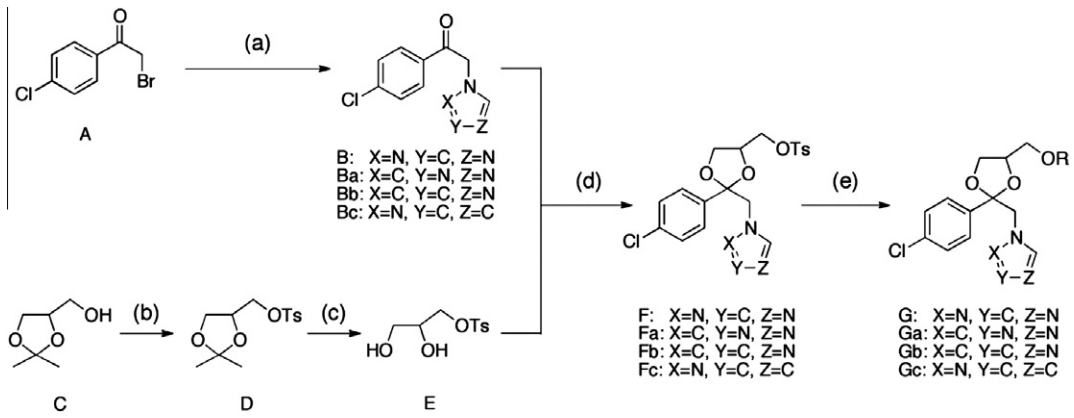
Our research interest is the design and synthesis of novel plant hormone biosynthesis inhibitors.[23–27] In the course of this work, we carried out a systemic search for novel BR biosynthesis inhibitors based on the following observations. First, molecular analysis of enzymes involved in BR biosynthesis provided a broad hint for the design of new inhibitors. Several steps in BR biosynthesis proceed through the action of P450 enzymes. CYP90s are involved in C-22 and C-23 side-chain hydroxylation of BR,[28] CYP92A6 catalyzes the biochemical conversion of castasterone from typhasterol,[29] and CYP85A2 catalyzes the lactonization of castasterone to brassinolide.[30] Accordingly, strategies for designing P450 inhibitors can be attributed to the identification of BR synthesis inhibitors. Second, cytochrome P450 inhibition mechanisms have been studied in detail.[31] Azole derivatives have been demonstrated to have widespread ability as inhibitors of P450s, due to the intrinsic affinity of the nitrogen electron pair in heterocyclic molecules for the prosthetic heme iron. The azoles bind not only to lipophilic regions of the protein but also simultaneously to the prosthetic heme iron. Therefore, we carried out a biorational approach using ketoconazole as a molecular scaffold, which lead to the discovery of a new series of BR biosynthesis inhibitors (**YCZ** series, general chemical structure shown in Fig. 1).[32]

Initial structure–activity relationship studies indicated that the introduction of a mono substitution of a chlorine atom at position 4 or a double substitution of chlorine atoms at positions 2 and 4 on ring A (Fig. 1) exhibited potent inhibition of BR biosynthesis.[33] We also found that substitutions on ring D (as shown in Fig. 1) dramatically affected the inhibitory potency against BR biosynthesis of this synthetic series.[32] To study further the structure–activity relationships of this synthetic series and determine their mode of action, we synthesized new compounds by the chemical optimization of ring D and ring C. Subsequently, we used GA-deficient mutants, BR signaling mutants and BR biosynthetic intermediates to determine the target site of the inhibitors.

2. Results and discussion

2.1. Chemistry

Preparation of target compounds **G–Gc** were carried out by a method described previously (Scheme 1).[32] This method consisted of the following four steps: (1) formation of 1-(4-chlorophenyl)-2-azoleyl ethanone **B–Bc**; (2) tosylation of isopropylideneglycerol **C**; (3) deprotection of isopropylidene ketal **D**; and (4) ketal formation to generate **F–Fc**. Compounds **B–Bc** were prepared by reacting α-bromoketone **A** with different azoles in DMF using a method that we described previously.[34] When using 1,2,4-triazole as a reagent to prepare compound **B**, compound **Ba** is obtained as a by-product and separated by column chromatography. The tosylation of isopropylidene glycerol **C** was achieved using a standard protocol (tosyl chloride in pyridine at 0 °C), and hydrolysis of **C** with 1 M HCl in MeOH yielded glyceryl tosylate **E**. Ketal formation to generate **F–Fc** was carried out using 3 equiv of trifluoromethanesulfonic acid (TfOH) in toluene at room temperature for 60 h, according to a previously described method.[35] To generate compounds **Ga–Gc**, compounds **Fa–Fc** were reacted with 2-ethoxyphenol in a basic condition, as described previously.[36] Other compounds were prepared by reacting compound **F** with corresponding phenols or naphthols by the same method. All of the compounds synthesized



Scheme 1. Reagents and conditions: (a) azoles, triethylamine, DMF, −10 °C, 1 h, rt, 3 h; (b) TsCl, pyridine, 0 °C; (c) MeOH, HCl, reflux, 6 h; (d) 3 equiv TfOH, toluene, rt, 60 h; and (e) phenols, KOH, DMF, 50 °C, 12 h.