Bioorganic & Medicinal Chemistry Letters 28 (2018) 2465–2470

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Characterization of a helminthosporic acid analog that is a selective agonist of gibberellin receptor

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ARTICLE INFO

Keywords:
Terpenoid
GA-like
Natural product
GID1
Plant hormone
Selectivity

ABSTRACT

Helminthosporol, a natural growth regulator isolated from a fungus, stimulates hypocotyl growth and seed germination, similar to gibberellin (GA). We recently reported that helminthosporic acid (H-acid), a synthetic analog of helminthosporol, acts as an agonist of GA receptor. In this study, we showed that a H-acid analog, in which the hydroxymethyl group at the C-8 position of H-acid was converted to a keto group, acts as a selective GA receptor agonist. 1) This analog shows higher hypocotyl elongation activity in *Arabidopsis* than H-acid does, and induces the degradation of DELLA protein and 2) leads to the formation of the GID1-DELLA complex and 3) regulates the expression of GA-related genes. In addition, 4) its hypocotyl elongation activity was not observed in a *atgid1a* single mutant, and 5) this analog could promote only the interaction between specific GA receptors and DELLA proteins *in vitro*. Taken together, our results strongly suggest that the selectivity of the reported H-acid analog depends on the specificity of its GA receptor binding activity.

Gibberellins (GAs) are tetracyclic diterpenoids that function as growth regulators and are involved in a wide range of bioprocesses throughout the life cycle, from seed germination to flowering. Of the more than 100 known GA compounds in found nature, only a few, including GA_1 and GA_4, have biological activity. Bioactive GAs bind to the GA receptor, GIBBERELLIN INSENSITIVE DWARF1 (GID1), leading to a conformation change that allows DELLA protein, which acts as a repressor of GA responses by affecting gene expression repressors, to bind to the GID1-GA complex. Then, the DELLA protein is degraded through the ubiquitin-proteasome pathway.[1–3] Although rice contains one GID1 protein and one DELLA protein, *Arabidopsis thaliana* has three GID1 orthologs (AtGID1a, b, and c) and five DELLA proteins (RGA, GAI, RGL1, RGL2, and RGL3).[4,5] Degradation of DELLA relieves repression and facilitates GA responses, including induction of α-amylase synthesis in aleurone cells, stem elongation, dormancy, flowering, and fruit senescence.

After a GA was identified in a fungal pathogen of rice, *Gibberella fujikuroi* many other species were searched for GA or GA-like substances.[6] One such GA-like substance, helminthosporol, was identified in the culture medium of the fungus *Helminthosporium sativum*, a pathogenic ascomycete that causes seedling blight and root rot.[7] Helminthosporol is a sesquiterpenoid compound that shows GA-like bioactivity and promotes the growth of the second leaf sheath of rice.[8] To understand the structure-activity relationships of its GA-like activity, helminthosporol-related analogs, including helminthosporic acid (H-acid), were synthesized (**1**, Fig. 1).[9–12] Although it has not been chemically characterized, our group recently reported that H-acid acts as an agonist of GA receptor.[13] Thus, H-acid binds to GID1, promotes formation of the GID1-DELLA complex, induces DELLA protein degradation, and regulates the expression of GA-related genes in the same manner as GAs.

Although we reported the mode of function of H-acid,[13] some synthesized H-acid-related analogs were still uncharacterized.[14–17] Kim et al. focused on the hydroxymethyl group of H-acid.[18] Their work showed higher amylase-induction activity for H-acid analogs in which the hydroxymethyl group at the C-8 position was replaced with a hydroxy group (**2** and **3**, Fig. 1) or a keto group (**4** and **5**, Fig. 1). However, the mode of action of these analogs has not been clarified.

In this study, we investigated the physiological activity of these H-acid analogs. One analog (**5**, Fig. 1), which has a keto group at the C-8 position, has strong elongation-promoting activity not only in *Arabidopsis* but also in other agricultural plants. Similar to GA, this analog

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https://doi.org/10.1016/j.bmcl.2018.06.005
Received 10 April 2018; Received in revised form 30 May 2018; Accepted 1 June 2018
Available online 05 June 2018
0960-894X/

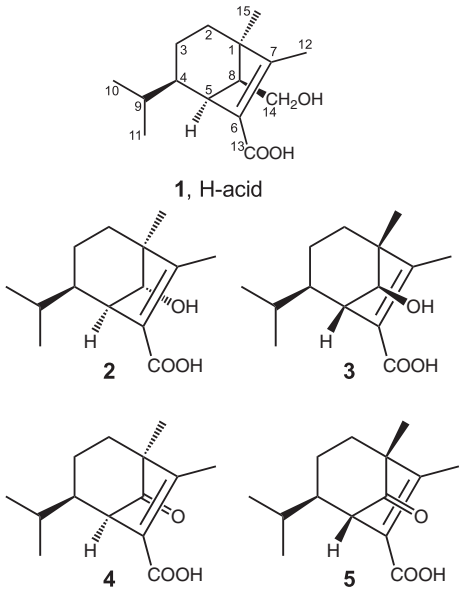

Fig. 1. Chemical structures of helminthosporic acid (**1**) and its analogs (**2–5**).

induces the formation of the GID1-DELLA complex and degradation of DELLA protein and regulates the expression GA-related genes. We further demonstrated that the higher GA-like activities of analog (**5**) were due to the its preferential binding to the AtGID1a subtype receptor. Our results suggest that the selectivity of analog (**5**) depends on its GA receptor binding specificity. Thus, our results showed that this H-acid analog may be a useful tool for the development of a selective GA receptor agonist.

We first determined whether the test H-acid analogs (**2–5**, Fig. 1) have GA activity in *Arabidopsis*. It is known that GA promotes seed germination in imbibed seeds and hypocotyl elongation, and that paclobutrazol (PAC), a GA biosynthesis inhibitor, suppresses these activities. PAC-inhibited seed germination was restored by the application of 1 μM GA_4 or 10 μM H-acid (**1**); however, the tested H-acid analogs (**2–5**; 10 μM) could not promote seed germination (Fig. 2A). For the hypocotyl elongation assay, the germinated seeds were transferred to new half MS medium containing the test compounds. In contrast to the results in the seed germination assay, PAC-inhibited hypocotyl growth was restored by the application of analog (**5**), in a dose-dependent manner (Fig. 2B).

To elucidate the biological effects of the H-acid-related analogs on GA-like activity in the hypocotyls of other agricultural plants, GA-deficient mutant rice Tan-ginbozu, radish, turnip, and cucumber were used. The results showed that analog (**5**), at 10 μM, significantly promoted the second leaf sheath or increased the hypocotyl length of all tested plants (Fig. S1). This demonstrates that analog (**5**) has the potential for wide applications as a GA-like compound in agriculture.

Since analog (**5**) showed higher elongation activity than H-acid (**1**), especially in *Arabidopsis*, we investigated whether it shares the same GA signaling pathway *in planta* as GA. GA binding allows the GA receptor GID1 to interact with DELLA, which functions as a repressor of GA responses by affecting GA-biosynthetic gene expression. GA-dependent degradation of DELLA protein is essential for GA-induced growth and development in plants. Thus, to clarify whether analog (**5**) promotes the degradation of DELLA, a *pRGA::GFP-RGA* transgenic line (with RGA fused to green fluorescent protein [GFP]) was used. Four-day-old *pRGA::GFP-RGA* seedlings were inoculated in liquid half MS medium containing GA_4 or analog (**5**). Similar to treatment with GA_4, treatment with analog (**5**) led to the disappearance of fluorescence caused by degradation of the RGA-fused GFP in the primary roots of the seedlings (Fig. 3A). This clearly shows that analog (**5**) induces the degradation of DELLA *in planta* by acting as a GID1 agonist, probably by forming a GID1-(**5**)-DELLA complex.

Next, we investigated the effect of analog (**5**) on GA-related gene expression. In higher plants, GA concentrations are limited by the expression levels of GA-catabolic and GA-biosynthetic genes.[19] To determine whether these genes similarly respond to analog (**5**) as they do

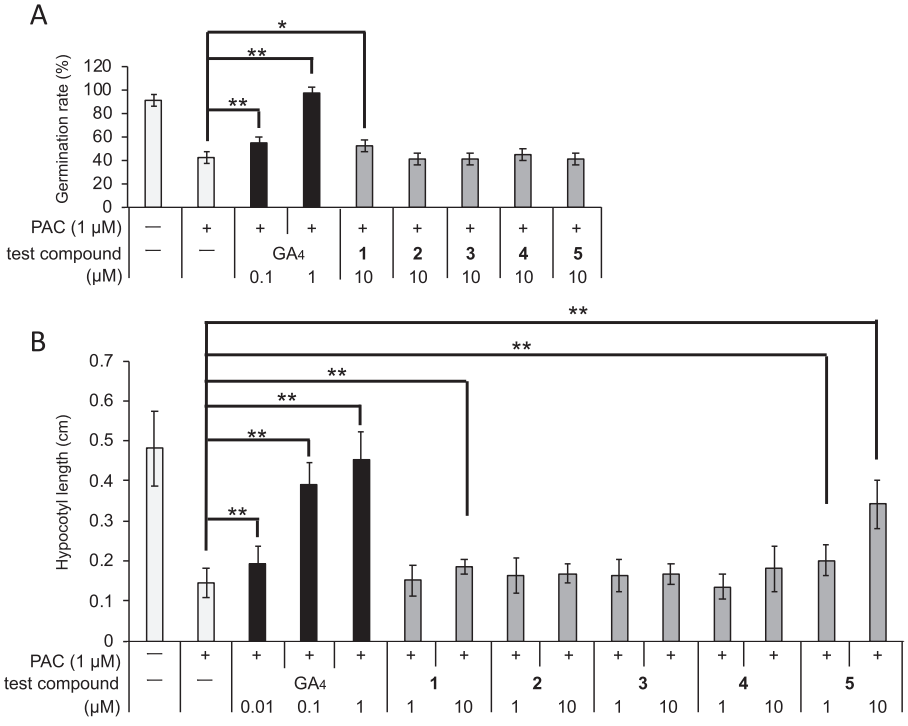

Fig. 2. Gibberellin (GA)-like activity of H-acid analogs in *Arabidopsis*. (A) Germination rate of *Arabidopsis*. WT *Arabidopsis* (Columbia) seeds were plated on half MS containing test compounds and 0.1% DMSO or Paclobutrazol (PAC; control). Error bars indicate the SD of three replicates, $n \geq 25$. (B) Hypocotyl growth of *Arabidopsis* WT. The germinated seeds were transferred onto new half MS medium containing test compounds and 0.1% DMSO or PAC as a control. Two-day-old seedlings were scanned and measured with ImageJ. Error bars indicate the SE, $n \geq 20$. Asterisks indicate significant differences versus the control (Student's *t*-test; $^{**}p < 0.01$, $^{*}p < 0.05$).