Isolation and characterization of the plant immune-priming compounds Imprimatin B3 and -B4, potentiators of disease resistance in Arabidopsis thaliana

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Plant activators are chemical crop protectants that fortify the immune system in plants. Unlike pesticides that target pathogens, plant activators provide durable effects against a broad spectrum of diseases, which have not been overcome by pathogenic microbes. Plant activators are not only useful agrochemicals, but can also help to elucidate the details of the plant immune system. Using an established high-throughput screening procedure, we previously identified 5 compounds, designated as Imprimatins, which prime plant immune response. These compounds increased disease resistance against pathogenic Pseudomonas bacteria in Arabidopsis plants by inhibiting 2 salicylic acid (SA) glucosyltransferases (SAGTs), resulting in accumulation of the phytohormone SA. Here, we report the isolation of 2 additional Imprimatins, B3 and B4, which are structurally similar to Imprimatin B1 and B2. Because these compounds did not have strong inhibitory effects on SAGTs in vitro, they may exert their function after metabolic conversion in vivo.

Plant activators are compounds that protect plants from pathogens by activating their immune system. In contrast to commonly used pesticides that directly target pathogens, plant activators are not pathogen specific; thus, pathogens have not developed resistance to these agents, and these activators have been proved to be durable in the field. A commonly used plant activator, benzo-thiadiazole (BTH), is a synthetic analog of salicylic acid (SA), a phytohormone essential for resistance against biotrophic pathogens. Probenazole is another plant activator that has been used for more than 30 y to protect paddy-field rice in East Asia from blast fungus and bacterial leaf blight. Probenazole does not have the SA analog activity, but is known to increase disease resistance in plants.

Although many bioactive compounds that prime plant immunity have been found thus far, only few practical plant activators have been developed. In order to explore a wide variety of molecules with the aim of obtaining more effective compounds that are applicable over a broad range of crops, several high-throughput chemical screening procedures have been developed. These approaches use Arabidopsis seedlings in combination with a promoter reporter system for defense genes as markers of activity. However, the compounds that constitutively activate defense responses are often associated with arrested growth and reduction in yield. To avoid this dilemma, new screening approaches need to be developed.

We have previously established a novel screening system for chemicals that prime immunity, but that do not induce hypersensitive cell death, using a model plant-microbe interaction system that involves Arabidopsis suspension-cultured cells and a bacterial pathogen, Pseudomonas syringae pv tomato DC3000 avrRpm1 (Pst-avrRpm1). After screening a commercial library of 10,000 structurally diversified small organic molecules, we isolated 5 plant immune-priming compounds. These immune-inducing compounds were classified into groups A and B, according to their molecular structure, and were designated Imprimatin A1, A2, A3, B1, and B2 (Fig. 1A). We demonstrated that these compounds could inhibit both a known and a previously unknown SA glucosyltransferase (SAGT) in vitro in a SA-competitive manner, and enhance disease resistance in Arabidopsis plants to both virulent and avirulent strains of Pst by increasing SA accumulation during pathogen infection.

Here, we identified another plant immune-priming compound and further obtained a bioactive molecule from derivative analysis of commercially available compounds (Fig. 1A).
These were designated as Imprimatin B3 and B4, respectively, because they shared a similar structure as Imprimatin B1 and B2. Imprimatin B3 and B4 upregulated \textit{Pst-avrRpm1}-induced cell death in Arabidopsis suspension-cultured cells in a concentration-dependent manner, as did SA and tiadinil (Fig. 1B). These compounds also enhanced the disease resistance of Arabidopsis seedlings against both avirulent and virulent \textit{Pst} strains when hydroponically grown seedlings were inoculated with \textit{Pst-avrRpm1}.
treated with 100 μM of Imprimatin compound by the root- drenching method for 3 d before inoculation (Fig. 1C). Unlike previously identified Imprimatin A and B compounds, which target SAGTs in Arabidopsis, Imprimatin B3 and B4 had a very weak inhibitory effect on SAGT activity in vitro compared with other Imprimatin compounds (Table 1). The IC50 values of Imprimatin B3 and B4 for SAGT inhibition are apparently higher than their respective concentrations for cell death potentiation as revealed in Figure 1B. Considering the molecular similarity among Imprimatin B compounds and their ability to prime immune responses in Arabidopsis, Imprimatin B3 and B4 may be converted to more effective molecules for SAGT inhibition inside the plants, through an as-yet unknown metabolic pathway(s).

We also evaluated the effect of the Imprimatin compounds on plant growth (Fig. 1D). Arabidopsis seeds were germinated and grown in liquid MS media containing Imprimatins at a concentration of 50 or 100 μM. In contrast to tiadinil, which markedly inhibited seedling growth, Imprimatin A2 and group-B Imprimatin compounds showed only moderate growth inhibitory effects, in a concentration-dependent manner, similar to that observed with SA. Imprimatin A1 and A3 did not affect growth at the concentration range effective for immune priming. These results suggest that further exploration of derivatives of these Imprimatin compounds may lead to isolation of appropriate compounds as practical plant activators, which target SA metabolism to enhance disease resistance.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Table 1. IC50 values of Imprimatin B3 and B4 for SAGTs in Arabidopsis

|    | Imprimatin UGT74F1 |    | Imprimatin UGT76B1 |
|----|-------------------|----|-------------------|
| B3 | 155.3 ± 12.6 | B4 | 163.4 ± 32.0 |
| B4 | 84.8 ± 11.7 |     | 136.7 ± 22.0 |

Inhibitory effects of Imprimatins on UGT74F1 and UGT76B1 were determined from 4 experiments. Data are expressed as mean (SD).

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