IPGSA

THE 23RD INTERNATIONAL CONFERENCE ON PLANT GROWTH SUBSTANCES

JUNE 25-29 2019

ABSTRACT BOOK

CHEMICAL AND SYNTHETIC BIOLOGY

PARIS / France

www.ipgsa2019.com

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DEVELOPMENT OF STRIGOLACTONE AGONISTS POSSESSING SELECTIVITY FOR DWARF14 RECEPTOR

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ABSTRACT

Strigolactones (SLs) are plant hormone identified as shoot branching inhibitors and function as rhizosphere communication chemicals. We previously reported that a series of phenoxyfuranone compounds, designated ‘debranones’, mimic SL activity. When docking into SL receptor DWARF14 (D14), as with natural SLs, debranones are hydrolyzed by D14 and the hydrolytic product is then covalently linked with D14. Recent study showed that intact SL induce active signaling state of D14 and hydrolysis of SLs is a deactivation step. In this work, we prepared several analogs of debranones which are not cleaved by D14. We evaluated the biological activities of these compounds with rice tillering assays using SL-biosynthesis mutant. These compounds showed tiller inhibition activity more potently than GR24, a widely used SL analog. We demonstrated that these compounds bind to the rice D14 receptor and induce the interaction between D14 and repressor protein D53. As SLs also show activity by inducing seed germination of root parasitic plants, the induction activity of these derivatives was also evaluated. These compounds had no significant effect on seed germination of root parasitic plant Striga hermonthica. These results suggest that these compounds are SL agonists with selectivity for plant hormonal activity.

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NEW MOLECULAR COMPONENTS REGULATING APICAL HOOK DEVELOPMENT IN ARABIDOPSIS REVEALED BY CHEMICAL GENOMICS

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ABSTRACT

Phytohormone-mediated differential growth imparts the morphological adaptability and plasticity to plants in response to environmental stimuli. Understanding the cellular and molecular mechanisms underlying such process, which is one of the common targets for genetic manipulation, is critical for the successful improvement of crop performance by biotechnology. Apical hook is an ideal model for studying differential growth. In the current study, we applied the chemical biology strategy and identified a novel small molecule compound named Hit-45 primarily regulating the opening process during apical hook development. Our data showed that Hit-45 functions through modulating the crosstalk of phytohormone signaling. Moreover, we successfully identified a WD40-repeat protein named Delay of Apical Hook Opening in *axr1-30* (DAPIA) as the direct target of Hit-45 by a combination of biochemical, genetic and biophysical approaches. We believe that the compound Hit-45 may be a powerful chemical tool to unravel unknown molecular components involved in the phytohormone crosstalk regulating the apical hook development, improving our understanding of the mechanisms governing differential cell growth.

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SYNTHESES OF TRITIUM-LABELED CYTOKININS AND AUXINS WITH HIGH MOLAR RADIOACTIVITY

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ABSTRACT

In studies of the interactions of small molecules with receptors, enzymes and other complex biological molecules and systems, compounds labeled with tritium are indispensable because they can be detected and quantified at the nanomolar level of concentration. Our department has more than 60 years of experience in radiochemical syntheses and is one of a few laboratories capable of providing labeled plant hormones for research purposes in the world.

Out of existing methods for preparation of tritium-labeled compounds, we utilize methods based on:
1) heterogenous exchange catalyzed by platinum metals in a solution with tritium gas - this approach has been applied for tritiation of aromatic cytokinins and aromatic cytokinin ribosides;
2) catalytic tritiation of multiple bonds – the first choice for preparation of labeled dihydrozeatin;
3) tritiodehalogenation - suitable for preparation of labeled auxins and precursors for cytokinins bearing a double bond in the N^6-side chain. The procedure for purine type compounds starts with an appropriate 2-chloropurine derivative. Tritium label is introduced into the position 2 of the purine core. Selected N^6-side chain is inserted in the next step. Thus, cytokinins such as [2-^{3}H]isopentenyladenine, [2-^{3}H]zeatins, and their ribosides, *N*-glucosides, and *O*-glucosides can be prepared.

Raw radioactive products are purified by HPLC. Our procedures provide labeled tracers of top-level radiochemical purity and very high molar radioactivity (>1 TBq/mmol). Some deuterium labeled cytokinins have been prepared in the analogical way as standards for mass spectromerty.

Financial support was provided by the projects 17-14007S and 19-12262S from the Czech Science Foundation.

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DEVELOPMENT OF THE NEW SYNTHETIC AGONIST OF HTL/KAI2 RECEPTOR

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ABSTRACT

HTL/KAI2 hydrolase protein was initially identified as a signaling component involved in hypocotyl elongation under red and far red light condition, and HTL/KAI2 was now recognized as a receptor for karrikins, a group of smoke-derived compound. Karrikins induce seed germination of various plants and affect photomorphogenesis. However, endogenous ligand, physiological role and signaling pathway of HTL/KAI2 remain largely unclear. In the case of D14 hydrolase protein, a paralogue of HTL/KAI2, GR24 and a lot of chemical tools advanced the research of biological function and molecular mechanism of D14. On the other hand, few chemical tools for HTL/KAI2 were available. Karrikins and (-)-GR24 can be used as agonists for HTL/KAI2, although the limited supply of these synthetic substances would hinder large-scale experiment like mutant screening in HTL/KAI2 pathway. Therefore, we newly designed simple molecules with HTL/KAI2 agonistic activity, and assessed these agonists in several biological assays of HTL/KAI2 responses. Herein, we demonstrated our new HTL/KAI2 agnonist would be promising chemical tools for HTL/KAI2 signaling.

POSTER PRESENTATION

P14

THE FUNGAL SESQUITERPENOID PYRENOPHORIC ACID B USES THE PLANT ABA BIOSYNTHETIC PATHWAY TO INHIBIT SEED GERMINATION.

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ABSTRACT

Pyrenophoric acid (P-Acid) and pyrenophoric acids B and C (P-Acid B and P-Acid C) are three phytotoxic sesquiterpenoids produced by the ascomycete seed pathogen Pyrenophora semeniperda, a fungus proposed as a mycoherbicide for biocontrol of cheatgrass, an extremely invasive weed. When tested in cheatgrass bioassays these metabolites were able to delay seed germination, with P-Acid B being the most active compound. Here, we have investigated the cross-kingdom activity of P-Acid B and its mode of action and found that it activates the ABA signaling pathway in order to inhibit seedling establishment. P-Acid B inhibits seedling establishment in wild-type Arabidopsis thaliana while several mutants affected in the early perception as well as in downstream ABA signaling components were insensitive to the fungal compound. However, in spite of structural similarities between ABA and P-Acid B, the later is not able to bind to the PYR/PYL family of ABA receptors. Instead, we have found that P-Acid B activates the ABA biosynthesis pathway at the level of alcohol dehydrogenase ABA2. We propose that the fungi Pyrenophora semeniperda manipulates plant ABA biosynthesis as a strategy to reduce seed germination, increasing its ability to cause seed mortality and thereby increase its fitness through higher reproductive success.

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A CHEMICAL SCREENING IDENTIFIED A CANDIDATE THAT TARGETS YUCCCA

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ABSTRACT

Chemical regulators are useful in both basic research and application in agriculture studies. To identify new regulators in root growth, a phenotype-directed chemical screening was performed by using Arabidopsis mutants *eto1-2* and *ctr1-1*, which are the dominantly active in ethylene signal and display constitutive “short root” phenotype. A chemical was identified to recover the root elongation in *eto1-2* and *ctr1-1*. Further studies revealed that the chemical displays impact on auxin related responses rather than the ethylene signal. Genetics and biochemical investigation demonstrated that chemical inhibited the enzyme activity of the flavin-containing monooxygenase (FMOs) YUCCA, a key enzyme in indole-3-pyruvic acid (IPA) branch of auxin biosynthesis pathway. Taken together, our study identified an inhibitor of YUCCA that is useful for dissecting different steps in auxin biosynthesis and in the regulation of root elongation.

JIANG Kai

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COVALENT RECEPTOR INHIBITORS OF STRIGOLACTONES THAT AFFECT PLANT ARCHITECTURE AND STRIGA GERMINATION

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ABSTRACT

Strigolactones, a class of plant hormones with multiple functions, mediate plant-plant and plant-microorganism communications in the rhizosphere. In this study, we developed potent strigolactone antagonists, which covalently bind to the strigolactone receptor D14, by preparing an array of triazole urea compounds. Using yeast two-hybrid and rice-tillering assays, we identified a triazole urea compound KK094 as a potent inhibitor of strigolactone receptors. Liquid chromatography-tandem mass spectrometry analysis and X-ray crystallography revealed that KK094 was hydrolyzed by D14, and that a reaction product of this degradation covalently binds to the Ser residue of the catalytic triad of D14. Furthermore, we identified two triazole urea compounds KK052 and KK073, whose effects on D14-D53/D14-SLR1 complex formation were opposite due to the absence (KK052) or presence (KK073) of a trifluoromethyl group on their phenyl ring. These results demonstrate that triazole urea compounds are potentially powerful tools for agricultural application and may be useful for the elucidation of the complicated mechanism underlying strigolactone perception.

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FPX IS A NOVEL CHEMICAL INDUCER THAT PROMOTES CALLUS FORMATION AND SHOOT REGENERATION IN PLANTS

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ABSTRACT

Auxin and cytokinin controls callus formation from developed plant organs as well as shoot regeneration from callus. Dedifferentiation and regeneration of plant cells by auxin and cytokinin stimulation are considered to be caused by the reprograming of callus cells, but this hypothesis is still argued to this day. Although an elucidation of the regulatory mechanisms of callus formation and shoot regeneration has helped advance plant biotechnology research, many plant species are intractable to transformation because of difficulties with callus formation. In this study, we identified Fipexide (FPX) as a useful regulatory compound through a chemical biology-based screening. FPX was shown to act as a chemical inducer in callus formation, shoot regeneration, and Agrobacteriuminfection. With regards to morphology, the cellular organization of FPX-induced calli differed from those produced under auxin/cytokinin conditions. Microarray analysis revealed that the expression of approximately 971 genes were up-regulated two-fold after a two-day FPX treatment compared with non-treated plants. Among these 971 genes, 598 genes were also induced by auxin/cytokinin, whereas 373 genes were specifically expressed upon FPX treatment only. FPX can promote callus formations in rice, poplar, soybean, tomato and cucumber, and thus can be considered a useful tool for revealing the mechanisms of plant development and for use in plant transformation technologies.

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SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL TRIAZOLE DERIVATIVES AS STRIGOLACTONE BIOSYNTHESIS INHIBITORS

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ABSTRACT

Strigolactones (SLs) are one of the plant hormones that control several important agronomic traits, such as shoot branching, leaf senescence and stress tolerance. In addition, SLs are known as seed germination compounds in root parasitic plants, *Orobanche* spp. and *Striga* spp., which are harmful plants for crops. Manipulation of the SL biosynthesis can reduce their damages. We previously reported that triazole derivative, TIS108, inhibits SL biosynthesis. In this study, we synthesized a number of novel TIS108 derivatives. Structure-activity relationship studies reveals that 4-(2-phenoxyethoxy)-1-phenyl-2-(1*H*-1,2,4-triazol-1-yl)butan-1-one (KK5) inhibits the level of 4-deoxyorobanchol in root stronger than TIS108. We further found that KK5-treated Arabidopsis showed increased branching phenotype with the up-regulated gene expression of *AtMAX3* and *AtMAX4*. Furthermore, *Striga* germination assay showed that KK5 could inhibit the seed germination. These results indicate that KK5 is a specific SL biosynthesis inhibitor in rice and arabidopsis.

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NEW KINETIN-LIKE DERIVATIVES WITH UVA/UVB PHOTOPROTECTIVE AND ANTIOXIDANT PROPERTIES

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ABSTRACT

Kinetin, a compound that belongs among aromatic cytokinins, has been described as a multiactive molecule with various activities both in animal and plant cells. It is able to delay leaf senescence in plants and concurrently possess various antioxidant properties. Furthermore, this small molecule is able to delay age-related characteristics in human fibroblasts. Nowadays, Kinetin and its THP derivative 6-(furfurylamino)-9-(tetrahydropyran-2-yl)purine (Pyratine) are utilized in cosmetics as these compounds were proven to possess positive effect on human skin.

New kinetin-like derivatives with modified furfuryl moiety, including ring saturation or oxygen atom substitution with sulphur, were prepared. New derivatives were also modified at N9 atom by THF (tetrahydrofuran-2-yl) and THP (tetrahydropyran-2-yl). These protective groups were previously found to reduce cytokinin toxicity as well as enhance their transport in plants.

Prepared compounds were screened in three cytokinin bioassays, Amaranthus caudatus betacyanin test, Tobacco callus biotest and wheat leaf senescence bioassay. Selected compounds were tested for Nrf2 gene activation and ability to absorb oxygen radicals in ORAC assay as well as in Caenorhabditis elegans in-vivo model to examine their antioxidant properties. Once cytotoxicity on human skin cells was excluded, selected compounds were tested on human dermal fibroblasts (NHDF) and immortalised human keratinocyte cell line (HaCaT) to exclude phototoxicity and reveal possible UVA and UVB photoprotective effect. Proven photoprotective properties were connected with ability to supress ROS generation and inhibit glutathione depletion.

The work was supported from ERDF project "Development of pre-applied research in nanotechnogy and biotechnology" (No. CZ.02.1.01/0.0/0.0/17_048/0007323).

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STRUCTURAL INFLUENCE OF PURINE MOIETY SUBSTITUTIONS ON THE ANTISENESCENCENT AND PROTECTIVE PROPERTIES OF CYTOKININ DERIVATIVES

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ABSTRACT

Cytokinins as well as some of their purine based derivatives are able to modulate a number of important developmental processes including leaf senescence, which is associated with chlorophyll breakdown and oxidative damage. Several derivatives of cytokinins were proven to protect plant as well as human skin cells against stress factors. Structural changes via particular substitutions of particular cytokinin atoms can dramatically change resulting biological activities related to senescence and cell protection. Some recently prepared derivatives showed to be photoprotective for human skin fibroblasts against UVA and UVB radiation and others showed to be active against human skin aging. Thus, we focused on controlled structural support of protective responses against oxidative damage. The main goal of this study is to show how the antisenescent activity of cytokinin derivatives may be related to their chemical structure. Several cytokinin derivative structures obtained using rtg diffraction methods will be shown and their physical properties before and after derivatization will be discussed. Structural motifs in cytokinin derivatives that may be responsible for their significant regulatory and protective activity should be taken into account while designing new functionalized molecules that should bring prospect to plant or animal cells.

The work was supported from ERDF project "Development of pre-applied research in nanotechnogy and biotechnology" (No. CZ.02.1.01/0.0/0.0/17_048/0007323).

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