5th KRIBB-RIKEN

Chemical Biology Joint Symposium

Seminar Room, International Conference Building,

Ochang Branch Institute, KRIBB, Korea

October 17, 2023

***Organized by**

Chemical Biology Research Center,
Korea Research Institute of Bioscience and Biotechnology (KRIBB), Korea

Natural Product Biosynthesis Research Unit,
RIKEN Center for Sustainable Resource Science (CSRS), Japan

***Supported by**

KRIBB Research Initiative Program, Korea
RIKEN Symposium, Japan

PROGRAM

Oct 16 (MON)

Arrive at Gimpo Airport (10:45 or 11:20)
Move to Ochang (GEE Hotel, ~ 15:00)
Welcome Dinner (Ochang, 18:00~20:00)

Oct 17 (TUE)

09:00 ~ 09:30 **Registration**

09:30 ~ 09:35 **Opening Address**
Dr. Jae-Hyuk Jang (Associate Director, Chemical Biology Research Center, KRIBB)
Dr. Shunji Takahashi (Unit Leader, Natural Product Biosynthesis Research Unit, RIKEN)

09:35 ~ 09:45 **Congratulatory Remark**
Jang Seong Kim (President, KRIBB)
Daisuke NAMIOKA (Minister for Economic Affairs/Head of Chancery, Embassy of Japan in Korea)
(Read by Tetsuyuki TAMURA, First Secretary/Science Officer, Embassy of Japan in Korea)

09:45 ~ 09:50 **Group Photo**

Special Presentation 1

Chair
Dr. Bo Yeon Kim (KRIBB)

09:50 ~ 10:10 **Combating drug-resistant malaria using fungal metabolites**
Dr. Hiroyuki Osada (RIKEN)

10:10 ~ 10:30 **Structural Motif-Based Discovery of Bioactive Bacterial Secondary Metabolites**
Prof. Dong-Chan Oh (Seoul Nat. Univ.)

10:30 ~ 10:50 **Screening system of *Saccharomyces cerevisiae* is a useful tool for the discovery of natural compound**
Prof. Yukihiro Asami (Kitasato Univ.)

10:50 ~ 11:10 **Coffee Break**

Session 1 Discovery of Novel Natural Products

Chairs
Prof. Kazuro Shiomi (Institute of Microbial Chemistry) / Dr. Won Gon Kim (KRIBB)

11:10 ~ 11:25 **Discovery of Plant and Microorganism-derived Natural Products with Antimicrobial and Stem-cell Growth Stimulation Activity**
Prof. Hyukjae Choi (Yeungnam Univ.)

11:25 ~ 11:40 **Microbial Natural Products Discovery: Rapid Isolation and Identification of Biologically Active Secondary metabolites from the CBC Fraction Library**
Dr. Jun-Pil Jang (KRIBB)

11:40 ~ 11:55 **New tetrahydrofuran-fused decalin metabolite isolated from a fungus**
Dr. Toshihiko Nogawa (RIKEN)

11:55 ~ 12:10 **Isolation of new kinanthraquinones and analysis of biosynthetic mechanism using a heterologous expression system**
Dr. Katsuyuki Sakai (RIKEN)

Young Researchers Presentation

Chair
Dr. Jun-Pil Jang (KRIBB)

12:10 ~ 12:50 **Poster Briefing (2 min per head)**

P1 **Discovery of Novel Microbial Secondary Metabolites by Zinc-regulated Culture of Actinomycete**
Dr. Gwi Ja Hwang (KRIBB)

P2 **Discovery of Novel Pluramycin Derivatives from *Streptomyces* sp. W2061 under Phosphate Depletion Medium Using LCMS Pattern Analysis**
Byeongsan Lee (KRIBB)

P3 ***Ent*-Peniciherqueinone Suppresses Acetaldehyde-Induced Cytotoxicity and Oxidative Stress by Inducing ALDH and Suppressing MAPK Signaling**
Taehoon Oh (KRIBB)

P4 **New Spiroacetal Polyketides Isolated from *Streptomyces* sp.**
Min Hee Kim (KRIBB)

P5 **De Novo Biosynthesis of Zingerone in *E. coli* Using a Feruloyl-CoA-Preferred Benzalacetone Synthase**
Juhee Won (KRIBB)

P6 **Isolation of Novel Rifamycin Derivatives from a Microbial Metabolites Fraction Library of *Amycolatopsis* sp. 22MC006**
Ji-Eun Lee (KRIBB)

P7 **Isolation of Novel Migrastatin Derivatives from a Microbial Metabolites Fraction Library of *Streptomyces* sp. 20A130**
Jihoon Park (KRIBB)

P8 **Protective effect of HGC against corticosterone-induced toxicity and oxidative stress mediated via autophagy and MAPK pathway**
Jongtae Roh (KRIBB)

P9 **STK-X, a unique tubulin target agent, enhances binding affinity between tubulin dimers and ETS family proteins**
Dr. Ho Jin Han (KRIBB)

P10 **Expression of SYO_1.56 SARP Regulator Unveils Unprecedented Elasnin Derivatives with Remarkable Antibacterial Activity**
Dr. Islam A. Abdelhakim (Egypt) (RIKEN)

P11 **Discovery and characterization of novel bifunctional sesquiterpene synthases catalyzing drimenol synthesis in marine bacteria**
Dr. Nhu Ngoc Quynh Vo (Vietnam) (RIKEN)

P12 **Engineering of Cytochrome P450 for Synthesis of Novel Reveromycin Derivatives**
Ya Fen Yong (Malaysia) (RIKEN)

P13 **Biosynthesis of verticilactams produced by *Streptomyces spiroverticillatus* JC-8444**
Dr. Yu Zheng (China) (RIKEN)

P14 **AI-based screening system for antifungal substances by monitoring morphological changes of *Aspergillus oryzae***
Harumi Aono (RIKEN)

P15 **A Combination Strategy of Multidrug-Sensitive Budding Yeast and Chemical Modifications Enabling to Find a New Overlooked Antifungal Compound, Sakurafusariene, from In-House Fractionated Library**
Prof. Aoi Kimishima (Kitasato Univ.)

P16 **Synthesis and Activity Evaluation of Borrelidin Analogs Focusing on Threonyl tRNA Synthetase Inhibitory Activity as a New Insecticide Target**
Naozumi Kondo (Kitasato Univ.)

12:50 ~ 14:00 **Lunch (Institute Dining Hall)**

14:00 ~ 14:30 **Poster Presentation (Standing)**

Session 2 Evaluation of Biological Activity

Chair
Dr. Sung-Kyun Ko (KRIBB) / Prof. Yukihiro Asami (Kitasato Univ.)

14:30 ~ 14:45 **Analysis of regulatory mechanisms of acrolein-induced lysosomal retrograde transport by chemical biological approach**
Prof. Yukiko Sasazawa (Juntendo Univ.)

14:45 ~ 15:00 **A novel inhibitor of fungal cell wall synthesis that targets RHO1 small GTPase**
Dr. Yushi Futamura (RIKEN)

15:00 ~ 15:15 **Synergistic effect of anticancer drug resistance and Wnt3a on primary ciliogenesis**
Dr. Kyung Ho Lee (KRIBB)

15:15 ~ 15:30 **MO-2097, a nature-inspired benzofuran, is a potent HIF-1 α inhibitor and a promising anticancer agent**
Dr. Nak-Kyun Soung (KRIBB)

Session 3 Biosynthesis of Bioactive Molecules

Chairs
Dr. Young-Soo Hong (KRIBB)

15:30 ~ 15:45 **Production of natural products by heterologous gene expression**
Dr. Shunji Takahashi (RIKEN)

15:45 ~ 16:00 **β-NAD as a building block in natural product biosynthesis**
Dr. Takayoshi Awakawa (RIKEN)

16:00 ~ 16:15 **Structure-activity relationship of decalin-containing tetramic acids collected from genetically engineered fungi**
Prof. Naoki Kato (Setsunan Univ.)

16:15 ~ 16:30 **Discovery of new Natural Products though Genome Mining of BGC-rich Streptomyces lineage**
Prof. Hahk-Soo Kang (Konkuk Univ.)

16:30 ~ 16:50 **Coffee Break**

Special Presentation 2

Chair
Prof. Masaya Imoto (Juntendo Univ.)

16:50 ~ 17:10 **Role of c-Myc on the cytotoxicity of ROS inducers in cancer cells**
Dr. Nobumoto Watanabe (RIKEN)

17:10 ~ 17:30 **Open Drug Discovery Platform, Pharmaco-Net: Synergistic effect of applying artificial intelligence (AI) to the field of natural products research**
Dr. Park, Young Bin (CSO, Calici, US/Korea)

17:30 ~ 17:35 **Introduction to the Journal of Antibiotics**
Prof. Kazuro Shiomi (Institute of Microbial Chemistry)

17:35 ~ 17:45 **Awards Ceremony (Poster Presentation)**

17:45 ~ 17:50 **Closing Remark**
Dr. Jong Seog Ahn (KRIBB)
Dr. Hiroyuki Osada (RIKEN)

18:00 ~ **Dinner Meeting**

Oct 18 (WED)

09:00 ~ 12:00	**Free Discussion**
	Advisory comments from Dr. K. Shiomi and Dr. M. Imoto
12:00 ~ 13:00	**Lunch**
13:00 ~ 16:00	**Lab. Tour and Staff Meeting**
16:00 ~ 18:30	**Move to Seoul**

Oct 19 (THU)

12:00	**Departure from Gimpo Airport**

Special Presentation 1

Chair

Dr. Bo Yeon Kim (KRIBB)

Combating drug-resistant malaria using fungal metabolites

Prof. Dr. Hiroyuki Osada

Unit Leader
Chemical Resource Development Research Unit
RIKEN Center for Sustainable Resource Science

Abstract

It is problem that the causal parasite of malaria, *Plasmodium falciparum*, became drug-resistant. To combat the drug-resistant malaria, we have explored the compounds that active against the drug-resistant parasite from diverse microbial fermentation broth. During the screening, we identified a fungal strain identified as *Fusarium sp*. RK 97-94, has potent antimalarial activity. The active principal was identified to be lucilactaene which was previously identified as a lead compound of antitumor compound by us.

We have activated the production of lucilactaene derivatives by the following two methods; one is the treatment of the producer strain with a compound activating the secondary metabolism and the other one is the gene manipulation of the biosynthetic genes. By these methods, six new lucilactaene derivatives were isolated. Among these compounds, dihydrolucilactaene (DHLC) displayed exceptionally strong antimalarial activity with the almost same concentration (IC_{50} value of 1.5 nM) against 3D7 (chloroquine-sensitive) and K1 (chloroquine-resistant) strains. Then, we examined the mode of action of DHLC. DHLC mainly targets trophozoites of *P. falciparum*, followed by schizont and ring stage. It has a different stage-specific inhibitory profile than CQ and artemisinin, implying a distinct mode of action.

References

1) Abdelhakim IA, Bin Mahmud F, Motoyama T, Futamura Y, Takahashi S & Osada H: Dihydrolucilactaene, a potent antimalarial compound from *Fusarium sp*. RK97-94. *J. Nat. Prod.* **85**: 63-69 (2022)
2) Kato S, Motoyama T, Futamura Y, Uramoto M, Nogawa T, Hayashi T, Hirota H, Tanaka A, Takahashi-Ando N, Kamakura T & Osada H: Biosynthetic gene cluster identification and biological activity of lucilactaene from *Fusarium sp*. RK97-94 *Biosci. Biotechnol. Biochem.* **86**: 1303-1307 (2020)

Curriculum Vitae

NAME: Hiroyuki OSADA

CURRENT POSITION:
1) Unit Leader, Chemical Resource Development Research Unit,
   RIKEN Center for Sustainable Resource Science
2) Professor, Department of Pharmaceutical Sciences,
   University of Shizuoka

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EDUCATION:

1974-1978: The University of Tokyo,
Department of Agricultural Chemistry
1978-1983: Doctor Course, The University of Tokyo, Faculty of Agriculture
Dr. Agriculture (1983) The University of Tokyo

APPOINTMENTS:

1983-1991: Scientist, Antibiotics Laboratory, RIKEN
1985-1986: Fogarty Fellow, National Cancer Institute, NIH, USA.
1992- 2015: Chief Scientist, Antibiotics Laboratory, RIKEN
1999- 2020: Visiting Professor, Saitama University
2013- 2022: Director of Chemical Biology Research Group, RIKEN CSRS
2022- Current position

ACADEMIC ACTIVITIES:

President of the Society for Actinomycetes Japan (2012-2015)
President of the Japanese Association for Molecular Target Therapy of Cancer (2015-2018)
President of the Japanese Society for Chemical Biology (2018-present)
President of the Federation of Microbiological Societies of Japan (2020-present)
Board member of the International Chemical Biology Society (2018-present)

EDITORIAL BOARD MEMBER OF JOURNALS:

Assay and Drug Development Technology
Cancer Science
Journal of Antibiotics
Journal of Microbiology and Biotechnology
Oncology Research

ADVISORY BOARD MEMBER OF JOURNALS:

ACS Chemical Biology

AWARDS & HONOR:

Research Promotion Award of Agricultural Chemical Society of Japan (1991)
Sumiki-Umezawa Memorial Award from Japan Antibiotic Research Association (1996)
Award of the Society for Actinomycetes Japan (2000)

Award of the Minister of Education, Culture, Sports, Science and Technology (2001)
Award of the Bioindustry Association (2007)
Award of Agricultural Chemical Society of Japan (2009)
Significant Achievement Award (S) RIKEN (2010)
Inhoffen Award in Germany (2015)
Special Award of Agricultural Chemical Society of Japan (2016)
Medal with Purple Ribbon, Government of Japan (2021)
Changbai Mountain Friendship Award, People's Government of Jilin Province (2021)

Structural Motif-Based Discovery of Bioactive Bacterial Secondary Metabolites

Dong-Chan Oh[1,*]

Professor
[1]*Natural Products Research Institute, College of Pharmacy, Seoul National University, Korea*

Abstract

A targeted and logical discovery method was devised for natural products containing piperazic acid (Piz), which is biosynthesized from ornithine by L-ornithine N-hydroxylase (KtzI) and N−N bond formation enzyme (KtzT). Genomic signature-based screening of a bacterial DNA library (2020 strains) using polymerase chain reaction (PCR) primers targeting ktzT identified 62 strains (3.1%). The PCR amplicons of KtzT-encoding genes were phylogenetically analyzed to classify the 23 clades into two monophyletic groups, I and II. Cultivating hit strains in media supplemented with $^{15}NH_4Cl$ and applying $^{1}H–^{15}N$ heteronuclear multiple bond correlation (HMBC) along with $^{1}H–^{15}N$ heteronuclear single quantum coherence (HSQC) and $^{1}H–^{15}N$ HSQC-total correlation spectroscopy (HSQC-TOCSY) NMR experiments detected the spectroscopic signatures of Piz and modified Piz. Chemical investigation of the hit strains prioritized by genomic and spectroscopic signatures led to the identification of a new azinothricin congener, polyoxyperuin B seco acid (1), previously reported chloptosin (2) in group I, depsidomycin D (3) incorporating two dehydropiperazic acids (Dpz), and lenziamides A and B (4 and 5), structurally novel 31-membered cyclic decapeptides in group II. By consolidating the phylogenetic and chemical analyses, clade−structure relationships were elucidated for 19 of the 23 clades. Lenziamide A (4) inhibited STAT3 activation and induced G2/M cell cycle arrest, apoptotic cell death, and tumor growth suppression in human colorectal cancer cells. Moreover, lenziamide A (4) resensitized 5-fluorouracil (5-FU) activity in both in vitro cell cultures and the in vivo 5-FU-resistant tumor xenograft mouse model. This work demonstrates that the genomic and spectroscopic signature-based searches provide an efficient and general strategy for new bioactive natural products containing specific structural motifs.

Reference

1) Shin, D., Byun, W. S., Kang, S., ... Lee, S. K., Oh, D.-C. *J. Am. Chem. Soc.* ASAP (2010)

Curriculum Vitae

Name : Dong-Chan Oh

Affiliation:
Director and Professor, Natural Products Research Institute, College of Pharmacy, Seoul National University, Korea

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Education:
2001-2006: Ph.D. Marine Natural Products Chemistry, Scripps Institution of Oceanography, University of California-San Diego, USA
1996-1998: M.S. Marine Chemistry, Seoul National University, Korea
1992-1996: B.S. Oceanography (Minor in Chemistry), Seoul National University, Korea

Previous Appointments:
2018-: Professor, College of Pharmacy, Seoul National University, Korea

2013-2018: Associate Professor, College of Pharmacy, Seoul National University, Korea

2012-2017: Howard Hughes Medical Institute International Early Career Scientist, HHMI, USA

2009-2013: Assistant Professor, College of Pharmacy, Seoul National University, Korea

2008-2009: Instructor, Dept. of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, USA

2006-2008: Postdoctoral Research Fellow, Dept. of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, USA

Research Interest

Discovery and structure eludciation of microbial natural products

Screening system of *Saccharomyces cerevisiae* is a useful tool for the discovery of natural compound

Yukihiro Asami

Professor
Laboratory of Applied Microbial Chemistry, Department of Drug Discovery Sciences, Ōmura Satoshi Memorial Institute, Kitasato University, Japan

Abstract

Exploring Functional Inhibitors of the Mitochondrial ADP/ATP Carrier Protein (AAC) Based on Interspecies Structural Differences Using Budding Yeast.

AAC is a membrane transporter weighing roughly 30 kDa. It facilitates the exchange between ADP and ATP on the mitochondrial inner membrane, transporting matrix-generated ATP to the cytosol. This research is based on the structural disparities of AACs found in pests. We worked with transformants that expressed AACs from the *Acyrthosiphon pisum*, *Tribolium castaneum*, or humans within the inner membrane of the mitochondria of the *aacΔ* budding yeast, which inherently lacks the endogenous AAC.

A significant advantage of utilizing budding yeast is its ability to growth in both aerobic and anaerobic environments. Consequently, the mitochondria's respiratory function is not essential for budding yeast survival. By adjusting culture conditions, we observed growth variations attributing from differing ATP production mechanisms. Specifically, in glucose-rich media, ATP is synthesized both in the cytosol and mitochondria. However, in glycerol-based media, ATP production is predominantly mitochondria-dependent. Thus, in the presence of compounds inhibiting mitochondrial function, budding yeast fails to survive.

During screening, this characteristic helped us identify positive microbial culture samples that flourished in glucose media but faltered in glycerol media. Concurrently, we compared growth in budding yeast expressing human AAC to gauge selectivity.

We employed a paper disk method for screening. After identifying positive samples based on the presence or absence of a growth inhibition circle diameter in each budding yeast, the selected strains were cultured to isolate active compounds that suppress mitochondrial function. This was followed by a chemical structure analysis. Consequently, we discovered two new analogues of ascosterosides with exomethylene groups on the lanostane skeleton and a novel analogue of peptaibols[1-3]. While the inhibitory effect of ascosterosides on 1,3-β-D-glucan synthase has been documented, there is no reported inhibitory effect of ascosterosides on mitochondrial function. In contrast, peptaibols are known to impede mitochondrial respiratory function, hinting at a potentially similar mechanism of action.

Exploring Mitochondrial Function Inhibitors Using Drug-Sensitive Budding Yeast

During their research, we recognized the need to refine the screening system, aiming to enhance the detection rate of positive samples and filter out recurrent hit compounds. Building upon their initial concept, which centered on the structural discrepancies in mitochondrial AAC between humans and insect pests, we pursued bioactive compounds that could inhibit mitochondrial function in budding yeast, a fungus particularly sensitive to drugs. Inhibitors

targeting mitochondrial function are prospective lead compounds for pesticides against pests and plant-pathogenic fungi.

Budding yeast, a model organism, is extensively utilized in fundamental biology and applied microbiology. Within the realms of natural product chemistry and chemical biology, it serves as a prominent tool for compound screening, target molecule identification and quantification, and the validation of bioactive entities. However, budding yeast's pronounced drug resistance complicates the discovery of compounds and their action analysis. This resistance is attributed to factors like drug efflux pumps and the presence of ergosterol in the cell membrane. With this knowledge, we anticipated that we could uncover mitochondrial function inhibitors not previously identified by employing strains with compromised drug efflux pumps or those where ergosterol expression is controllable.

In our investigation, we utilized two types of drug-sensitive budding yeasts. The screening methodology remained consistent with previous approaches. Initially, the *S. cerevisiae* strain BY25929 from the NBRP — with the ABC transporter expression genes *yrs1Δ, yrr1Δ, pdr1Δ,* and *pdr3Δ* disrupted — was employed. Subsequently, the *S. cerevisiae* strain 12geneΔ0HSR-iERG6 from Prof. Takeo Usui was used[4]. This strain has twelve ABC transporter expression genes disrupted, namely: *yor1Δ, aus1Δ, pdr5Δ, pdr10Δ, pdr11Δ, pdr12Δ, pdr15Δ, snq2Δ, pdr1Δ, pdr3Δ, pdr8Δ,* and *yrr1Δ*. Additionally, it has regulated *erg6* expression. The results were promising: screening hit rates surged to 5.3% for *S. cerevisiae* BY25929 and 8.4% for 12geneΔ0HSR-iERG6, compared to budding yeast expressing the pest AAC (1.4% hit ratio).

In the screening process utilizing the *S. cerevisiae* strain BY25929, a new compound, decatamariic acid, was identified from the culture of the *Aspergillus tamarii* strain. This compound demonstrated ATP efflux inhibitory activity when assessed with isolated mitochondria. The absolute configuration of its distinctive decalin skeleton was also ascertained computationally[5]. During the screening with the 12geneΔ0HSR-iERG6 strain, several compounds emerged from the culture of the *Fusarium concentricum* strain, including a new compound named fusaramin featuring a tetramic acid structure, and the novel compounds traminines A and B, both characterized by a unique side chain structure[6,7]. Fusaramin's inhibitory action on mitochondrial function primarily stems from the impairment of VDAC1. Additionally, fusaramin was observed to suppress FoF1-ATP synthase and complex III, especially at higher concentrations than those needed for its VDAC1 inhibitory effects[8]. In contrast, traminine A exhibited inhibitory actions on complex III and FoF1-ATP synthase. Traminine B, meanwhile, acts by binding to the Qo site of complex III, suggesting its binding manner deviates somewhat from that of myxothiazol[7]. Additionally, in screenings using the 12geneΔ0HSR-iERG6 strain aimed at identifying antifungal agents, we discovered several new compounds that did not present mitochondrial function inhibitory activity[9-11].

Through the aforementioned screening system, we identified compounds from microbial culture samples that demonstrate inhibitory activity on mitochondrial function. While enhanced screening processes might elevate the acquisition rate of biologically active compounds with unique chemical structures, we understand that continuous research is crucial. For instance, these findings need to be translated into novel pesticide seed compounds suitable for practical application. Specifically, a comprehensive assessment of the biological activities of these discovered compounds against a spectrum of pests and plant-pathogenic fungi is vital. Subsequent research will underscore the potential benefits of these compounds.

To this end, we emphasize the significance of collaborative research. Beyond our own team, we advocate partnerships with universities and corporations. Such collaboration aims to

elucidate the bioactivity of these compounds against pests and plant-pathogenic fungi, fostering research that champions plant protection.

References

1) Suga T, Asami Y, Hashimoto S, Nonaka K, Iwatsuki M, Nakashima T, Sugahara R, Shiotsuki T, Yamamoto T, Shinohara Y, Ichimaru N, Murai M, Miyoshi H, Ōmura S, Shiomi K. Ascosteroside C, a new mitochondrial respiration inhibitor discovered by pesticidal screening using recombinant *Saccharomyces cerevisiae*. *J. Antibiot.* **2015**, 68, 649-652.
2) Watanabe Y, Suga T, Narusawa S, Iwatsuki M, Nonaka K, Nakashima T, Shinohara Y, Shiotsuki T, Ichimaru N, Miyoshi H, Asami Y, Ōmura S, Shiomi K. Decatamariic acid, a new mitochondrial respiration inhibitor discovered by pesticidal screening using drug-sensitive *Saccharomyces cerevisiae*. *J. Antibiot.* **2017**, 70, 395-399.
3) Suga T, Asami Y, Hashimoto S, Nonaka K, Iwatsuki M, Nakashima T, Watanabe Y, Sugahara R, Shiotsuki T, Yamamoto T, Shinohara Y, Ichimaru N, Murai M, Miyoshi H, Ōmura S, Shiomi K. Trichopolyn VI: a new peptaibol insecticidal compound discovered using a recombinant *Saccharomyces cerevisiae* screening system. *J. Gen. Appl. Microbiol.* **2015**, 61, 82-87.
4) Chinen T, Nagumo Y, Usui T. Construction of a genetic analysis-available multidrug sensitive yeast strain by disruption of the drug efflux system and conditional repression of the membrane barrier system. *J. Gen. Appl. Microbiol.* **2014**, 60,160-162.
5) Watanabe Y, Suga T, Narusawa S, Iwatsuki M, Nonaka K, Nakashima T, Shinohara Y, Shiotsuki T, Ichimaru N, Miyoshi H, Asami Y, Ōmura S, Shiomi K. Decatamariic acid, a new mitochondrial respiration inhibitor discovered by pesticidal screening using drug-sensitive *Saccharomyces cerevisiae*. *J. Antibiot.* **2017**, 70, 395-399.
6) Sakai K, Unten Y, Iwatsuki M, Matsuo H, Fukasawa W, Hirose T, Chinen T, Nonaka K, Nakashima T, Sunazuka T, Usui T, Murai M, Miyoshi H, Asami Y, Ōmura S, Shiomi K. Fusaramin, an antimitochondrial compound produced by *Fusarium* sp., discovered using multidrug-sensitive *Saccharomyces cerevisiae*. *J. Antibiot.* **2019**, 72, 645-652.
7) Sakai K, Unten Y, Kimishima A, Nonaka K, Chinen T, Sakai K, Usui T, Shiomi K, Iwatsuki M, Murai M, Miyoshi H, Asami Y, Ōmura S. Traminines A and B, produced by *Fusarium concentricum*, inhibit oxidative phosphorylation in *Saccharomyces cerevisiae* mitochondria. *J. Ind. Microbiol. Biotechnol.* **2021**, 48, kuab051.
8) Unten Y, Murai M, Sakai K, Asami Y, Yamamoto T, Masuya T, Miyoshi H. Natural tetramic acids elicit multiple inhibitory actions against mitochondrial machineries presiding over oxidative phosphorylation. *Biosci. Biotechnol. Biochem.* **2021**, 85, 2368-2377.
9) Sakai K, Suga T, Iwatsuki M, Chinen T, Nonaka K, Usui T, Asami Y, Ōmura S, Shiomi K. Pestiocandin, a new papulacandin class antibiotic isolated from *Pestalotiopsis humus*. *J. Antibiot.* **2018**, 71, 1031-1035.
10) Sakai K, Hirose T, Iwatsuki M, Chinen T, Kimura T, Suga T, Nonaka K, Nakashima T, Sunazuka T, Usui T, Asami Y, Ōmura S, Shiomi K. Pestynol, an antifungal compound discovered using a *Saccharomyces cerevisiae* 12geneΔ0HSR-iERG6-based assay. *J. Nat. Prod.* **2018**, 81, 1604-1609.
11) Kimishima A, Ono Y, Sakai K, Sakai K, Honsho M, Naher K, Tokiwa T, Kojima H, Higo M, Nonaka K, Iwatsuki M, Fuji SI, Chinen T, Usui T, Asami Y. A combination strategy

of multidrug-sensitive budding yeast and chemical modifications enabling to find a new overlooked antifungal compound, sakurafusariene, from in-house fractionated library. *J. Agric. Food. Chem.* **2023**, 71, 3219-3224.

Curriculum Vitae

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Education:

2004 - Doctor of Science (Saitama University)

Previous Appointments:

2001 - 2004	RIKEN Trainee, Antibiotics Laboratory, Discovery Research Institute, RIKEN
2004 - 2006	RIKEN Special postdoctoral researcher, Antibiotics Laboratory, Discovery Research Institute, RIKEN
2007 - 2009	Research fellow, Laboratory of Microbiology, Graduate School of Pharmaceutical Sciences, The University of Tokyo
2009 - 2012	RIKEN Visiting Scientist, Antibiotics Laboratory, Discovery Research Institute, RIKEN
2009 - 2012	Visiting Scientist, Chemical Biology Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB)
2012 - 2020	Project Assist. Prof. (2012-2017), Project Lecturer (2017-2018), Project Assoc. Prof. (2018-2020), Laboratory of Biological Functions and Laboratory of Applied Microbial Chemistry, Department of Drug Discovery Sciences, Kitasato Institute for Life Sciences, Kitasato University
2018 - Present	RIKEN Visiting Scientist, Natural Product Biosynthesis Research Unit, Chemical Biology Research Group, Center for Sustainable Resource Science, RIKEN
2020 - Present	Professor, Laboratory of Applied Microbial Chemistry, Department of Drug Discovery Sciences, Ōmura Satoshi Memorial Institute, Kitasato University
2023 - Present	Department head, Department of Drug Discovery Sciences, Ōmura Satoshi Memorial Institute, Kitasato University

Research Interest

Investigation of new bioactive compounds and examination of their mechanisms of action through model organisms

Session 1

Discovery of Novel Natural Products

Chair

Prof. Kazuro Shiomi (Institute of Microbial Chemistry)

Dr. Won Gon Kim (KRIBB)

Discovery of Plant and Microorganism-derived Natural Products with Antimicrobial and Stem-cell Growth Stimulation Activity

Geum Jin Kim[1,2], Jimin Moon[1], Hunmin Lee[1], Junhyeung Park[3], Yo Han Seo[4], So-Ri Son[5], Jun-Pil Jang[4], Sang-Jip Nam[6], Dae-Sik Jang[5], Jee-Heon Jeong[3], Jae-Hyuk Jang[4], Hyukjae Choi[1,2,*]

Professor

[1]*College of Pharmacy, Yeungnam University, Korea*

[2]*Cell Culture Institute, Yeungnam University, Korea*

[3]*Department of Precision Medicine, School of Medicine, Sungkyunkwan University, Korea*

[4]*Chemical Biology Research Center, KRIBB, Korea*

[5]*Department of Biomedical and Pharmaceutical Sciences, Kyung Hee University, Korea*

[6]*Department of Chemistry and Nanoscience, Ewha Womans University, Korea*

Abstract

Natural product has been accepted as a promising source of lead compounds in drug discovery. Although the rediscovery rate of known natural product increases rapidly, natural products are the prolific sources of novel chemical scaffolds and pharmacophores. On these regards, we have tried to discover new bioactive natural products by investigating less-studied sources and applying new analytical techniques. In this presentation, new natural products with antimicrobial, stem-cell growth stimulating and antioxidants activities from two terrestrial plants and a fungal strain will be discussed.[1]

Reference

1) Son, S.-R., Kim, G.J., Choi, Y.J., Shim, S.H., Nam, J.-W., Lee, S., Jang, D.S., Choi, H. *Org. Chem. Front.* **10**: 4320 (2023)

Curriculum Vitae

Name : Hyukjae Choi

Affiliation:
College of Pharmacy, Yeungnam University

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Education:
BS in Oceanography, Seoul National University (1999)
MS in Marine Natural Products Chemistry, Seoul National University (2001)
Ph.D. in Marine Natural Products Chemistry, Seoul National University (2009)

Previous Appointments:
Postdoctoral Researcher, Scripps Institution of Oceanography (2009-2012)

Research Interest

- Discovery of Bioactive Natural Products
- Structure Elucidation of Natural Products
- Absolute Configuration Determination

Microbial Natural Products Discovery: Rapid Isolation and Identification of Biologically Active Secondary metabolites from the CBC Fraction Library

Jun-Pil Jang[1] and Jae-Hyuk Jang[1]

Research scientist
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Abstract

A microbial fraction library has been constructed as a part of research program for advancement of central microbial resource center of the Ministry of Science and ICT. The fraction library made by a methodical separation method based on basic chromatographic techniques. Chemical screening, all active fractions are analyzed by LCMS and the active principle structural classes are elucidated. In the proof-of-concept study, we show the processes involved in generating the subfractions, the throughput of the structural elucidation work, as well as the ability to rapidly isolate and identify new and biologically active compounds.

Reference

1) Grkovic T., et al. *ACS Chem Biol.* 2020. 15, 1104-1114.
2) Harvey A L., Edrada-Ebel R., Quinn R J. *Nat. Rev. Drug Discovery* 2015. 14, 111-129.

Curriculum Vitae

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Education:
B.S. (2005), M.S. (2007), and Ph.D. (2012) degrees from Chungnam National University, Department of Food and Nutrition.

Previous Appointments:
KRIBB as a postdoctora researcher (2012-2013).

RIKEN (Dr. Hiroyuki Osada Lab.) as a visiting scientist of RIKEN-KRIBB joint research unit (2013-2017).

Research Interest

Discovery of novel bioactive metabolites from natural products and molecular target identification of these compounds.

New tetrahydrofuran-fused decalin metabolite isolated from a fungus

Toshihiko Nogawa[1,2], Naoki Kato[3,4], Takeshi Shimizu[2], Akiko Okano[2], Yushi Futamura[2], Shunji Takahashi[4], Hiroyuki Koshino[1], Hiroyuki Osada[2]

Technical Scientist

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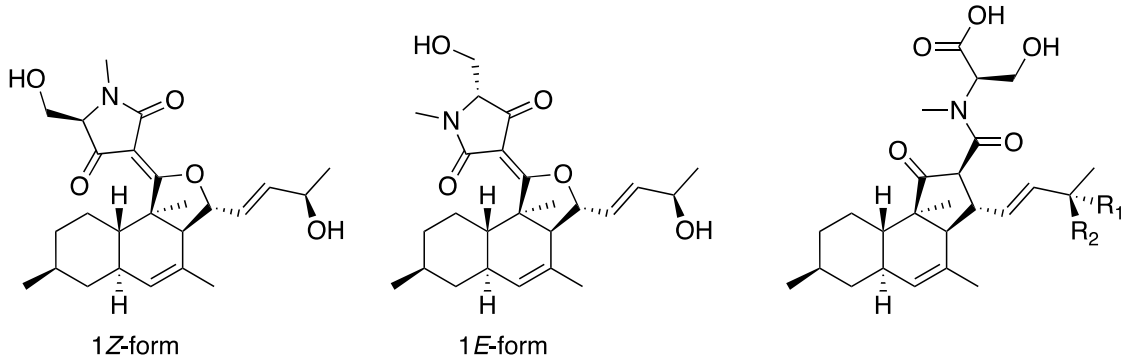
Abstract

Previously we had isolated new cyclopentane-fused decalin metabolites, wakodecalines A and B from a fungus *Pyrenochaetopsis* sp. RK10-F058 by a combination of screenings of structural properties and biological activities [1)]. Our continuous search for new secondary metabolites revealed that the same fungal extract had contained structurally related decalin compounds. One of which was isolated and identified as a new decalin metabolite designated as wakodecaline C [2)]. The structure including the absolute configuration was determined by a combination of spectroscopic methods including NMR and mass spectrometry, chemical reaction, and calculation of ECD spectra. Wakodecaline C had unique structural feature containing a tetrahydrofuran-fused decalin skeleton and tetramic acid moiety, which was connected through a double bond. In this presentation, we are going to show the detail of isolation, structural elucidation, and biological activities.



wakodecaline C

Reference

1) Nogawa, T., Kato, N., Shimizu, T., Okano, A., Futamura, Y., Takahashi S., Osada, H. *J. Antibiot.* **71**: 123-128 (2018)

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Curriculum Vitae

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1990-1994 Kanagawa University, Faculty of Science
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Research Interest

Isolation and structure elucidation of secondary metabolites from natural sources

Mass spectrometry, especially direct ionization method applicable for MS imaging and ion mobility separation

Separation by Supercritical fluid chromatography

Isolation of new kinanthraquinones and analysis of biosynthetic mechanism using a heterologous expression system

Katsuyuki Sakai[1], Yushi Futamura[1], Hiroyuki Koshino[1], Hiroyuki Osada[1], Shunji Takahashi[1]

Postdoctoral researcher

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Abstract

Natural products especially secondary metabolites of actinomycetes, produce biological activities with a wide variety of chemical structures and play a very important role in drug discovery research [1,2]. Anthraquinone compounds are one of the important natural products obtained from actinomycetes and other sources, and these compounds are known to have a wide variety of chemical structures and biological activity such as antitumor, antibacterial and antimalarial activities.

Anthraquinone compound, kinanthraquinone (KQ), was isolated from *Streptomyces* sp. SN-593. The compound contains a carboxamide group in the molecule and is rare class in natural products obtained from microorganisms [3]. Our group was interested in the biological activity and biosynthetic pathway of KQ with the rare substituent and identified the KQ gene cluster [4,5]. The aim of this study was to elucidate the biosynthetic mechanism of KQ and to obtain new anthraquinone compounds using a heterologous expression system.

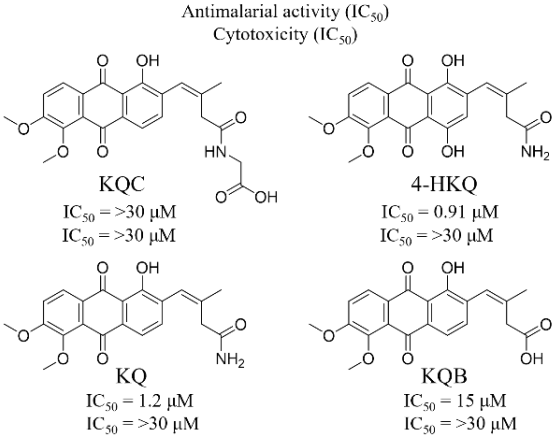

Figure 1. Chemical structure of KQs.

The heterologous expression strain *S. lividans* TK23/pKU592*aac(3)IV*::*kiq2*/pTYM19::*sav2794p-kiqA* was cultured to obtain the known compounds KQ and KQB, and the new compounds KQC and 4-hydroxy KQ (4-HKQ) (Figure 1). The chemical structure of each compound was determined by MS and NMR. The biological activity of the compounds was examined and found IC_{50} values of 0.91-15 μM against *Plasmodium falciparum* without cytotoxycity. To confirm how new compounds were involved in the biosynthetic pathway of KQ, we performed feeding experiments with KQ and KQB. We revealed that KQC and 4-HKQ are biosynthesized from KQB and KQ, respectively.

Reference

1) Newman, D. J., and Cragg G. M. *J. Nat. Prod.* **83**(3): 770–803 (2020)
2) Bérdy, J. *J. Antibiot.*, **58**(1): 1–26 (2005)
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5) Sakai, K., *et al*., *J. Antibiot*. **74**(9): 593–595 (2021)

Curriculum Vitae

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Bachelor of Science (March 31, 2015)
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Previous Appointments:

April 1, 2020 - Natural Product Biosynthesis Research Unit, REKEN CSRS (Postdoctoral Researcher)

Research Interest

Isolation and structural analysis of natural products.

I particularly love isolation of new compounds.

Young Researchers Presentation/ Poster Presentation (Standing)

Chair

Dr. Jun-Pil Jang (KRIBB)

Discovery of Novel Microbial Secondary Metabolites by Zinc-regulated Culture of Actinomycete

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Post-doctoral researcher
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Abstract

Microorganisms are major composers for ecosystems, and their secondary metabolites have been used in medicine and lead compounds. Among the microorganisms, actinomycetes are the largest group for producing the diverse secondary metabolites.[1] A recent bioinformatics analysis of the genomic sequences of actinomycetes indicated that they are able to produce more secondary metabolites than expected.[2] Despite their potency, many biosynthetic pathways are silent in the absence of specific culture conditions or chemical cues.[3] The various strategies have been developed to access cryptic metabolism, and controlling the metal ion composition of the culture media is a promising strategy.[4] Metal exposure to actinomycetes is known to affect growth and biosynthetic metabolic pathways through regulation of cluster-specific transcription factors contained in the actinomycetes biosynthetic gene clusters, and metal ions could be a trigger for the production of secondary metabolites.[5]

Therefore, in this study, *Streptomyces* sp. 13F051 was cultured with or without zinc metal ion, and their metabolic profiles were investigated by LC-MS. These efforts resulted in the production of three different classes of compounds in the culture medium with Zn^{2+} which are not recognized or rarely produced in the absence of Zn^{2+}. Based on the NMR, UV, and MS data, the induced compounds were identified as two lipopeptides (**1** and **2**), two carbazole analogues (**3** and **4**), and several siderophore analogues. Bioactivity evaluation indicated that compounds **1** and **2** had moderate antibacterial effect against several human and fish pathogenic bacteria.

Reference

1) Genilloud, O. *Nat. Prod. Rep*. **34**(10): 1203–1232 (2017)
2) Medema, M. H., de Rond, T., Moore, B. S. *Nat. Rev. Genet*. **22**(9): 553–571 (2021)
3) Ikeda, H., Kazuo, S. Y., Omura, S. J. *Ind. Microbiol. Biotechnol*. **41**(2): 233−250 (2014)
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5) Dubey, M. K.; Meena, M.; Aamir, M.; Zehra, A.; Upadhyay, R. S. R. *New and Future Developments in Microbial Biotechnology and Bioengineering*; Elsevier: 259–277 (2019)

Discovery of Novel Pluramycin Derivatives from *Streptomyces* sp. W2061 under Phosphate Depletion Medium Using LCMS Pattern Analysis

Byeongsan Lee[1, 2], Gwi Ja Hwang[1], Kyung Taek Heo[1], Jae Kyoung Lee[1], Jun-Pil Jang[1], Bang Yeon Hwang[2], Yong-Yeon Cho[3], Jae-Hyuk Jang[1,4,*], and Young-Soo Hong[1,3,*]

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Abstract

Actinomycetes are producers of pluramycins, including kidamycins, altromycins, hedamycins and rubiflavins, which possess potent antitumor properties. Pluramycins consist of an angucycline aglycone, which has diversely branched acyl chains at positions C-2 and can display various deoxy-amino sugars at positions C-8 and C-10. Actinomycetes are known to produce various secondary metabolites depending on the nutritional factors. Furthermore, altering carbon, nitrogen, and phosphate concentrations in microbial culture media can affect metabolite production. The supplemented of $MgCO_3$ in the culture medium induced free phosphate depletion, which stimulates different metabolite production pattern. With this principle, *Streptomyces* sp. W2061 produce collismycins in culture media without $MgCO_3$, but several known kidamycins have been identified under cultured in media supplemented with $MgCO_3$. In this study, we purified three new pluramycin compounds through LCMS analysis of crude extract from *Streptomyces* sp. W2061 cultured under phosphate-limiting conditions. The compounds were identified as kidamycin derivatives and their bioactivities were investigated.

Reference

1) Byeongsan L., Ga-Eun L., Gwi Ja H., Kyung Taek H., Jae Kyoung L., Jun-Pil J., Bang Yeon H., Jae-Hyuk J., Yong-Yeon C. & Young-Soo H. *J Antibiot* **76**, 585–591 (2023)
2) Yoshitake T., Satoshi Ō. The Search for Bioactive Compounds from Microorganisms. *Springer* **16**, 303–326 (1986)
3) Byeongsan L., Kyung Taek H., Jae-Hyuk J., Young-Soo H. *Front Bioeng Biotechnol* **10**, (2023)

Ent-Peniciherqueinone Suppresses Acetaldehyde-Induced Cytotoxicity and Oxidative Stress by Inducing ALDH and Suppressing MAPK Signaling

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Abstract

Alcohol consumption has increased globally, and it causes a variety of physical side effects and social problems. Ethanol enters the bloodstream via absorption in the stomach and small intestine, after which it is distributed across all body organs . Until this phase, alcohol dehydrogenase (ADH) in the liver cells rapidly converts alcohol to acetaldehyde. However, until acetaldehyde is metabolized by aldehyde dehydrogenases (ALDHs) and converted into water and acetic acid, it circulates in the bloodstream and strongly induces cytotoxicity and oxidative stress in organs such as the liver and brain. Moreover, acute acetaldehyde exposure causes nausea, vomiting, dizziness, and muscle pain and chronic acetaldehyde exposure cause severe liver and brain cell damage. ALDH is the main enzyme involved in the detoxification and metabolism of acetaldehyde to acetate. ALDH is present in various types of tissues and efficiently metabolizes acetaldehyde. Therefore, preserving the levels of ALDH is an important mechanism for protecting tissues from the effects of acetaldehyde.

Reference

1) Oh, T., et al. Pharmaceutics. **12**(12): 1229 (2020)

New Spiroacetal Polyketides Isolated from *Streptomyces* sp.

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Abstract

Actinomycetes are an important source of natural products with novel scaffolds and diverse biological activities that can be used for therapeutic agents. *Streptomyces* is well-known for its ability to produce various bioactive compounds. However, despite the genomic diversity of *Streptomyces* and their capacity for producing a number of secondary metabolites, many biosynthetic gene clusters remain silent. To discover novel secondary metabolites, we applied OSMAC approach to *Streptomyces* sp. Therefore, two known pteridic acid D and E (1–2) as well as three new polyketides, compounds 3–5, were isolated. The planar structures of these compounds were determined to be 6, 6-spiroacetal polyketides based on 1D/2D NMR and HRESIMS analysis. In particular, the signals for acetal quaternary carbon atoms with chemical shift of nearly 100 ppm in the 13C NMR spectrum of compounds 3–5 suggested that they have spiroacetal core structures. The relative configurations of compounds 3–5 were assigned by coupling constants, ROESY correlations and acetonide derivatization. The absolute configurations were established using a modified Mosher's method. All compounds (1–5) were evaluated for their cytotoxicities against several cell lines. However, none of the compounds showed any cytotoxicity.

Reference

1) Atanasov, A.G., Zotchev, S.B., Dirsch, V.M. *Nat. Rev. Drug Discov.* **20**: 200–216 (2021)
2) Nogawa, T., Takahashi, S., Okano, A., Kawatani, M., Uramoto, M., Saito, T., & Osada, H. *J. Antibiot.* **65**: 123–128 (2012)
3) Nong, XH., Wei, XY. & Qi, SH. *J. Antibiot.* **70**: 1047–1052 (2017)
4) I. R. G. Thistlethwaite, F. M. Bull, C. Cui, P. D. Walker, S. S. Gao, L. Wang, Z. Song, J. Masschelein, R. Lavigne, M. P. Crump, P. R. Race, T. J. Simpson and C. L. Willis. *Chem. Sci.* **8**: 6196–6201 (2017)

De Novo Biosynthesis of Zingerone in *E. coli* Using a Feruloyl-CoA-Preferred Benzalacetone Synthase

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Abstract

Zingerone, also known as vanillylacetone or 4-hydroxy-3-methoxyphenylethyl methyl ketone, is a key component responsible for the characteristic tingling sensation associated with ginger (*Zingiber officinale*). It offers a wide range of potential bioactivity including anti-inflammatory, antioxidant, anticancer, antihyperlipidemic, and antibacterial properties and effectiveness in addressing age-related neurological disorders.[1] All these things considered, zingerone is valuable for not only flavoring agent but also pharmaceutical applications. Microbial synthesis would be a more sustainable and attractive option for zingerone production, although it could be obtained from natural plant extraction or chemical synthesis. Our research identified a novel gene from *Piper methysticum*, which encodes a feruloyl-CoA-preferred benzalacetone synthase (BAS) for zingerone biosynthesis. Based on this discovery, we have constructed a de novo zingerone biosynthesis pathway in *Escherichia coli* by integrating six heterologous genes including tyrosine ammonia-lyase (*optal*), cinnamate-4-hydroxylase (*sam5*), caffeic acid O-methyltransferase (*com*), coumarate CoA ligase (*4cl2nt*), BAS (*pmpks*), and benzalacetone reductase (*rzs1*). The engineered L-tyrosine overproducing *E. coli* ΔCOS4 strain, excelled by yielding zingerone concentrations exceeding 24.03 ± 2.53 mg/L through a complete de novo synthesis.[3] [Supported by grants the NRF fund (NRF2020R1I1A2068713)]

Reference

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Isolation of Novel Rifamycin Derivatives from a Microbial Metabolites Fraction Library of *Amycolatopsis* sp. 22MC006

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Graduate student in the master course

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Abstract

Four new rifamycin derivatives (**1**-**4**), together with two known compound (**5** and **6**) were isolated from a microbial metabolites fraction library of *Amycolatopsis* sp. 22MC006 based on Global Natural Product Social Molecular Networking platform (GNPS) analysis. Their chemical structures were determined by detailed NMR and MS spectroscopic analyses. They belong to a class of rifamycin antibiotics family having unique carbon skeletons and dioxabicyclo[3.2.1]octan ring. Their cytotoxic effects were tested against mouse neuroblasts N2a, human cervical cancer HeLa, human gastric cancer AGS, human prostate cancer PC3, and murine melanoma B16F10 cells. Compound **3** displayed cytotoxic activity against AGS cell.

Reference

1) Yanrong S., Juanli Z., Xiuyu T., Xingkang W., Tianhong L., Chunhua L., and Yuemao S., *Organic Letters.* 2019. 21 (4), 900-903.
2) Seoung Rak L., Felix S., Jan W. S., Huijuan G., Jae Sik Y., Moonyoung S., Won Hee J., Z. Wilhelm de B., Christine B., and Ki Hyun K., *Chemistry A European Journal*. 2022. 28, 1-9.

Isolation of Novel Migrastatin Derivatives from a Microbial Metabolites Fraction Library of *Streptomyces* sp. 20A130

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Abstract

Actinomycetes, especially those from the genus *Streptomyces*, are known to be a prolific source of bioactive secondary metabolites. To discover and isolate such valuable metabolites, we have constructed a microbial metabolites fraction library and a spectral database based on the photodiode array detector attached LC/MS analysis. In our search for novel metabolites by chemical screening of a microbial metabolites fraction library of *Streptomyces* sp. 20A130, led to the isolation and characterization of five new (1–5) and a known (6) migrastatin derivatives. The structures of these compounds (1–5) were elucidated by detailed NMR and MS spectroscopic analyses. The relative and absolute configuration were determined using the magnitudes of coupling constants, ROESY correlations, and chemical means (Mosher's and acetonide derivatization). Migrastatin is a non-cytotoxic glutarimide-containing compound possessing a 14- membered ring macrolide, which has been reported to inhibit *in vitro* migration of human esophageal cancer EC17 cells and mouse melanoma B16 cells. On the basis of these findings, compounds (1–5) were evaluated for their inhibitory activities against cancer cell migration (mouse melanoma B16 cell and human breast cancer MDA-MB-231 cell lines).

Reference

1) Zhou W., Posri P., Liu X., Ju Z., Lan W., Mahmud T. *J. Nat. Prod.* 2021, 84, 9, 2411–2419

Protective effect of HGC against corticosterone-indcued toxicity and oxidative stress mediated via autophagy and MAPK pathway

Jongtae Roh[1,2], Jun-Pil Jang[1], Jae-Hyuk Jang[1,2], and Sung-Kyun Ko[1,2*]

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Abstract

Excessive stress, a major problem in modern societies, affects people of all ages worldwide. Corticosterone is one of the most abundant hormones secreted during stressful conditions and depression (1). In this study, we used a corticosterone-induced cellular stress model to investigate the protective effects of a novel agent against apoptosis and oxidative stress. We analyzed the effects of hygrolansamycin (HGC) on autophagy and mitogen-activated protein kinase (MAPK) phosphorylation-related genes in PC12 cells. HGC isolated from *Streptomyces sp*. exerted protective effects against corticosterone-induced injury. Additionally, HGC decreased cellular oxidative stress caused by corticosterone. At the protein level, HGC inhibited the expression of autophagy-related genes induced by corticosterone. HGC effectively reduced phosphorylation of c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinase (ERK), and p38 activation. Thus, our study demonstrates that HGC has protective effects against corticosterone-induced apoptosis and oxidative stress through inhibition of autophagy and the MAPK pathway.

Reference

1) Oswald, Lynn M., et al. (2006). *Neuropsychopharmacology*, *31*(7), 1583-1591.

STK-X, a unique tubulin target agent, enhances binding affinity between tubulin dimers and ETS family proteins

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Abstract

Cancer remains a leading global cause of death despite various treatment approaches, including surgery, radiation, chemotherapy, and microtubule-targeting agents.[1] Although many tubulin-targeting agents have been developed and used, they frequently present side effects, such as toxicity and drug resistance, which limit their application.[2] To improve therapeutic outcomes for cancer patients, developing safer and more effective microtubule-targeting agents is needed. Our study introduces STK-X, a novel agent that addresses the shortcomings of existing tubulin-targeting agents.

STK-X exhibits an enhanced binding affinity between tubulin and ETS family proteins, a feature not observed in other tubulin targeting agents. This unique property of STK-X offers the opportunity for combination treatment with other tubulin depolymerizing agents, such as vinblastine and colchicine, potentially enhancing their activity. The efficacy of this combination therapy was confirmed using cell line and cancer organoid models.

Reference

1) Miller KD, Nogueira L, Mariotto AB, Rowland JH, Yabroff KR, Alfano CM, *et al.*, 2019. CA Cancer J Clin **2019**;69(5):363-85 doi 10.3322/caac.21565.
2) Yusuf RZ, Duan Z, Lamendola DE, Penson RT, Seiden MV. Curr Cancer Drug Targets **2003**;3(1):1-19 doi 10.2174/1568009033333754.

Expression of SYO_1.56 SARP Regulator Unveils Unprecedented Elasnin Derivatives with Remarkable Antibacterial Activity

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Visiting researcher

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Abstract

Actinomycetes are prolific producers of natural products, particularly antibiotics. However, a significant proportion of its biosynthetic gene clusters (BGCs) remain silent under typical laboratory conditions [1)]. Activating these silent BGCs represents a promising strategy to discover novel antibiotics with unique structures that could play a vital role in combating the rapidly evolving antimicrobial drug resistance. *Streptomyces* Antibiotic Regulatory Proteins (SARPs) have extensively been found in actinomycetes, where they typically act as cluster-specific activators [2)]. Expression of these regulators has the potential to activate not only the gene clusters they are located in but also clusters distributed on the entire genome, implying a good tool for the activation of silent BGCs and the discovery of new antibiotics [3, 4)].

Herein, The *syo_1.56* gene encoding for a SARP family regulator was identified in *Streptomyces* sp. RK18-A0406 by TBLASTN search using Fur22 from *Streptomyces* sp. SN-593 as a query sequence [5)]. This gene is located 1.7 kb upstream of a previously uncharacterized typeII PKS gene cluster. The *syo_1.56* gene was PCR-amplified, cloned into an integrative pKU460 vector harboring *sav2794* promoter, and transformed into the parent strain. The resulting transformant and wild-type strains were cultured, extracted, and comparative LC-MS analysis was performed for the detection of induced metabolites. Scale-up culture of transformant strain was prepared and the induced metabolites were isolated, identified, and their biological activities were evaluated.

Expression of *syo_1.56* gene, in *Streptomyces* sp. RK18-A0406 resulted in a remarkable increase in the production of known antimycins and the discovery of twelve elasnin derivatives, with ten of them being new. All the isolated elasnins exhibited potent antibacterial activity, selectively against a wide panel of methicillin-resistant *Staphylococcus aureus* (IC_{50} 6.25 – 50 µM); whereas some of them also displayed strong antimalarial activity. Unexpectedly, no typeII PKS products were detected in the transformant, and no homologous SARP regulators were identified near the antimycin and putative elasnin gene clusters. This data indicate the Syo_1.56 potential to coregulate two distant and distinct pathways, implying its pleiotropic nature rather than the well-recognized pathway-specificity.

Reference

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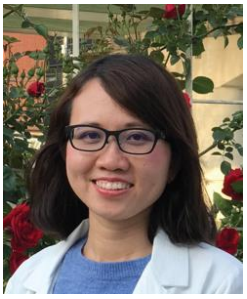
5) Panthee S. *et al*. *J Antibiot*. **64**: 509-513 (2011)

Discovery and characterization of novel bifunctional sesquiterpene synthases catalyzing drimenol synthesis in marine bacteria



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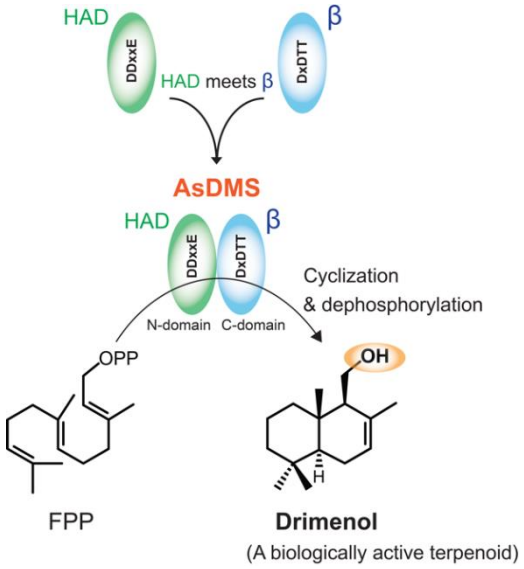
Abstract

Terpenoids are the largest known class of natural products enriched with structurally diverse 'bioactive' compounds. This class of compounds and their biosynthesis are predominantly characterized in plants and fungi; however, terpenoids derived from bacteria are much less studied. In particular, marine bacteria hold a yet untapped biosynthetic potential of terpenoids. Indeed, there were no reports of sesquiterpene synthases catalyzing the biosynthesis of drimane-type sesquiterpenes (C_{15} terpenoids) in bacteria, including marine bacteria. Herein, using genome-based mining and biochemical validation, we discover novel sesquiterpene synthases responsible for drimenol synthesis from five different marine bacteria species; all of which belong to the haloacid dehalogenase (HAD)-like hydrolase superfamily and conserve both DDxxE (N-domain) and DxDTT (C-domain) motifs typical of class I and II terpene synthases, respectively. Using *Aquimarina spongiae* drimenol synthase ("AsDMS"), we characterized the drimenol biosynthesis mechanism, in which the AsDMS enzyme exploits the two motifs for its bifunctional reactivity, i.e., cyclization of farnesyl pyrophosphate (FPP) into drimenyl pyrophosphate and dephosphorylation of drimenyl pyrophosphate into drimenol.

Remarkably, we demonstrated a novel and unique assembly of both N-domain (HAD-like domain) and C-domain (terpene synthase β domain) of AsDMS, each evolved from different evolutionary origins. Moreover, by genetic and biochemical analyses, we successfully verified the existence of a genetically encoded production pathway of drimenol in marine bacteria. This work highlights the first examples of the bacterial drimane sesquiterpene synthases while also indicating the divergence of terpene synthases' structures and functions, thus further shedding light on the role of bacteria, particularly marine bacteria, in the terpenoid production.



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Engineering of Cytochrome P450 for Synthesis of Novel Reveromycin Derivatives

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Abstract

[Purpose] Reveromycin A (RM-A), an inhibitor of eukaryotic isoleucyl-tRNA synthetase [1)] displays various biological activities [2-4)] with induction of osteoclast apoptosis as the most prominent bioactivity [5)]. The presence of hemisuccinate moiety at carbon-18 position is postulated as the main factor contributing to the isomerization of RM-A (6,6-spiroacetal ring) to RM-B (5,6-spiroacetal ring) under acidic condition [6)]. Diminished biological activities were reported in RM-B and 5,6-spiroacetal reveromycin derivatives [7)] highlighting the importance of stable 6,6-spiroacetal ring. Cytochrome P450revI (P450revI) catalyzes C18-hydroxylation of reveromycin T (RM-T), which is essential for subsequent hemisuccinate formation in RM-A biosynthesis. The rationale of this study is therefore to create stable reveromycin derivatives via altering the regiospecificity of P450revI.

[Method] Amino acid residues that could influence the positioning of RM-T in P450revI active site were determined from the crystal structure. P450revI variants were created via site-directed mutagenesis. Recombinant mutant proteins were subjected to *in vitro* enzyme assays with RM-T as substrate. DNA sequence of the most promising variant was introduced into *Streptomyces* sp. SN-593 with disrupted *revI* gene. The mutant culture was extracted and was then subjected to UPLC-PDA-MS analysis.

[Results & Discussion] Based on the UPLC-PDA-MS analysis, the detected new peak has the same maximum absorbance (λ_{max}) and mass-to-charge ratio (*m/z*) as RM-T1, indicating the production of a new hydroxylated product of RM-T. A new RM-A derivative was detected in *Streptomyces* sp. SN-593/P450revI variant.

[Conclusions] Taken together, through controlling the regiospecificity of P450revI, a novel hydroxylated product of RM-T is produced and is accepted as a substrate for the enzymes involved in subsequent hemisuccinylation.

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Biosynthesis of verticilactams produced by *Streptomyces spiroverticillatus* JC-8444

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Abstract

Verticilactam (**1**) is a polycyclic macrolactam isolated from *Streptomyces spiroverticillatus* JC-8444. It is characterized with a β-ketoamide unit in a 16-membered polyketide macrolactam conjugated with an octalin skeleton. Previously, heterologous expression of the *vtl* biosynthetic gene cluster in *Streptomyces avermitilis* SUKA17 strain led to two new isolated geometric isomers, verticilactams B (**2**) and C (**3**). Verticilactams (**1**-**3**) are proposed to be generated by mono-hydroxylation, transannular cyclization, and Michael addition reactions in post-PKS modification. Like other polycyclic macrolactams, the molecular mechanism of these reactions remains unclear. Therefore, the purpose of this study is to investigate the cryptical mechanistic mystery in verticilactams biosynthesis.

First, we performed in-frame deletion of the *vtlG* (P450) gene in BAC vector pKU503*vtl*. The unmethylated form of pKU503*vtl*::Δ*vtlG* plasmid was isolated and subsequently introduced into the *S. avermitilis* SUKA17 host. The pKU492*aac(3)IV-sav2794p-vtlR* plasmid containing a LuxR-family transcriptional regulator *vtlR* gene under the control of *sav2794* promoter, was also introduced into the same strain. Metabolites of the transformed strain was analyzed by UPLC-MS. To examine the function of VtlG, a DNA fragment containing the *vtlG* gene was PCR-amplified from pKU503*vtl* plasmid and ligated into the pET-28b(+) vector. For protein expression, the resultant pET-28b(+)::*vtlG* was transformed into *Escherichia coli* BL21 Star™ (DE3). The recombinant VtlG was expressed at 20°C in Terrific broth (TB) medium supplemented with kanamycin and 5-aminolevulinic acid. Protein purification was conducted using Ni-NTA agarose column chromatography. *In vitro* assay was conducted at 30°C in 50 mM Tris-HCl (pH 7.5) buffer. The reaction products were extracted with ethyl acetate twice and subjected to UPLC-MS analysis.

The Δ*vtlG* strain abolished the production of **1** but accumulated pentaene macrolactam compound **4**. We also isolated a cyclized compound **5**, which was converted from **4** during purification from the culture broth of *vtlG* disruptant. In the *in vitro* assay, VtlG (P450) rapidly converted **4** into hydroxylatd pentaene macrolactam compound **6**, whereas compound **5** was not recognized as a substrate. Notably, **6** was converted into **1** in the long term VtlG reaction, therefore confirmed compounds **4** and **6** as the on-pathway biosynthetic intermediate of **1**. The detailed results and discussion will be presented in the symopsium.

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AI-based screening system for antifungal substances by monitoring morphological changes of *Aspergillus oryzae*

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Abstract

Recently, antimicrobial resistance of pathogenic fungi has become a problem, thus it is necessary to develop novel antifungal agents with new mode of action. We have so far discovered novel anticancer and antifungal agents based on the morphological change database, MorphoBase[1, 2]. To accelerate this approach, we aimed to construct AI-based morphological database (2G-MorphoBase) for natural products screening. In this study, we used *Aspergillus oryzae* as a test strain, which is has many drug efflux-pumps than other fungi[3], and rarely used for antifungal drug screening.

First, we investigated the growth inhibitory and morphological changes of about 120 well-characterized compounds including typical antifungal drugs. About a half of the compounds had significant antifungal activity. Then, a representative compound for each pharmaceutical class was selected, and the morphological changes were collected at various concentrations. 2G-MorphoBase was constructed by NVIDIA DIGITS software using millions of fungi images, that enabled us to predict pharmacological actions with more than 80% probability.

In the course of screening from 6,418 cultured broth extracts, we found that some broth extracts had antifungal activity along with unique morphological changes.

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A Combination Strategy of Multidrug-Sensitive Budding Yeast and Chemical Modifications Enabling to Find a New Overlooked Antifungal Compound, Sakurafusariene, from In-House Fractionated Library

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Abstract

The *Fusarium* sp. FKI-7550 strain enabled us to discover three characteristic compounds, fusaramin [1)] and traminines A and B[2)]. We envisioned that *Fusarium* sp. FKI-7550 strain would produce more novel compounds and decided to create a fractionated library to discover overlooked compounds. In this symposium, we disclose our discovery of a new antifungal natural product, sakurafusariene [3)], from the in-house fractionated library of the culture broth of *Fusarium sp*. FKI-7550 strain by using a combination strategy of multidrug-sensitive yeast [4,5)] and chemical modification. Throughout our investigation, we encountered challenges in the isolation of the natural product. A chemical modification strategy via alkylation of sakurafusariene allowed for removal of the impurities enabling us to elucidate the structure of sakurafusariene. Furthermore, we synthesized ester derivatives using a method inspired by the isolation study, which gave us invaluable information to verify a preliminary structure−activity relationship study against a plant pathogenic fungus, *Pyricularia oryzae* growth inhibitory activity.

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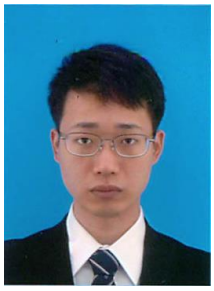
Recent publications:

1. **Kimishima A,** et al, Re-evaluation and a Structure–Activity Relationship Study for the Selective Anti-anaerobic Bacterial Activity of Luminamicin toward Target Identification. *ACS Infect. Dis*. **9**, 1602-1609 (2023)

Synthesis and Activity Evaluation of Borrelidin Analogs Focusing on Threonyl tRNA Synthetase Inhibitory Activity as a New Insecticide Target



Naozumi Kondo,[1] Aoi Kimishima,[1,2] Serino Maruyama,[1] Sota Honma,[1,2] Atsuka Nishitomi,[1] Masako Honsho,[1,2] Yoshihiro Watanabe,[1,2] Masato Iwatsuki,[1,2] and Yukihiro Asami[1,2]

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Abstract

Moths are at particularly high risk of acquiring insecticide resistance among agricultural pests.[1)] Therefore, insecticide seeds with novel mechanisms of action that are effective in controlling moth pests are in demand. However, moth pests have low sensitivity to insecticides, making it difficult to find new insecticidal compounds. Silkworms, a model insect for moths, is known to show highly insecticide sensitivity and their insecticide selectivity correlates with moth pests.[2,3)] Therefore, we have constructed and described a high sensitive ditection system for insecticide candidates using silkworm first instar larvae.[3)]

In this symposium, we report on borrelidin,[4)] which was re-identified as an insecticidal compound from the Ōmura Natural Compound library[5)] via the silkworm assay system. Although borrelidin is known as an inhibitor of the protein synthesis pathway (threonyl-tRNA synthetase)[4)] in various organisms, there is no precedent for the development of insecticides with this mechanism of action.[6)] This motivated us to synthesize borrelidin derivatives. As a result, we found new derivatives with improved their selective toxicity against human cells compared to natural products.[7)] We are currently conducting experiments to determine the enzymatic activity of borrelidin derivatives against threonyl-t RNA synthetase.

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Session 2
Evaluation of Biological Activity

Chair

Dr. Sung-Kyun Ko (KRIBB)

Prof. Yukihiro Asami (Kitasato Univ.)

Analysis of regulatory mechanisms of acrolein-induced lysosomal retrograde transport by chemical biological approach

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Abstract

Parkinson's disease (PD) is a progressive neurodegenerative disorder caused by dopaminergic neuronal loss in substantia nigra. To identify biomarkers for PD diagnosis, we performed metabolomic analysis and found that oxidative stress inducer acrolein was increased in PD patients' serum. Furthermore, we found that acrolein induced autophagy through lysosomal clustering around microtubule organizing center (MTOC) in human neuroblastoma SH-SY5Y cells. Lysosomal retrograde transport has been recognized as one of the regulators of autophagy; however, its regulatory mechanisms have not yet been fully elucidated. In this study, we aimed to identify the mechanism of lysosomal retrograde transport using acrolein.

We found that acrolein-induced lysosomal retrograde transport was regulated by not only TRPML1/ALG2 complex but also JIP4, whose detailed mechanisms have been unelucidated. We found that JIP4 was phosphorylated by acrolein which may play an important role in lysosomal retrograde transport mediated by TRPML1-ALG2. To elucidate the importance of JIP4 phosphorylation, we tried to identify a JIP4 kinase by chemical biological approach. We screened for the kinase inhibitor which inhibited acrolein-induced lysosomal retrograde transport using kinase inhibitor library and found Jak3 inhibitor VI and Gö6976 as the hits. However, their target kinases, Jak3 and PKC were not involved in acrolein-induced lysosomal retrograde transport, suggesting that their off-target kinases may include the true JIP4 kinase. By cross-referencing the previous study profiling the activity of 178 commercially available kinase inhibitors against a panel of 300 recombinant protein kinases [1)], we successfully identified Ca^{2+}/calmodulin-dependent protein kinase II (CaMK2) as a kinase responsible for JIP4 phosphorylation. We demonstrated a novel mechanism for lysosomal retrograde transport by which Ca^{2+} released from TRPML1 by acrolein-derived oxidative stress activates CaMK2G, and then activated CaMK2G phosphorylates JIP4 to interact with the TRPML1/ALG2 complex [2)].

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Curriculum Vitae

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Research Interest

Parkinson’s disease; autophagy; lysosomal trafficking; drug discovery

A novel inhibitor of fungal cell wall synthesis that targets RHO1 small GTPase

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Abstract

Candida albicans is an opportunistic fungal pathogen that causes candidiasis in human hosts. Although several effective antifungal drugs, such as candins, have been developed, the number of drugs is not enough and some of them have problems with antimicrobial spectrum and side effects. Furthermore, the multi-drug resistant strains, such as *Candida auris*, has been emerging. Thus, the development of new drugs is urgently needed. We have so far explored the bioactive compounds based on two phenotypic screenings: iHOPE (in-house phenotypic evaluation)[1)], based on dead/alive of various organisms and MorphoBase, based on cell-morphological changes of mammalian cells[2)] and rice blast fungi[3)]. In this study, we aimed to search for anticandidal compounds in combination with these screening platforms.

In the course of screening of anticandidal compounds from a chemical library of RIKEN NPDepo, we found that tetrakis(pentafluorophenytl)borate (TPPB) had a potent antifungal activity against *C. albicans* (IC_{50} = 0.012 μM). Next, we investigated the mode of action of TPPB by two phenotypic profiling methods; one is based on cell morphological changes induced by the compound and another one is the change of the compound sensitivity to the set of haploinsufficiency strains of *C. albicans*. TPPB showed swelling phenotype in *C. albicans* similar to micafungin (MCFG), an inhibitor of the 1,3-β-D-glucan synthase FKS1. Haploinsufficiency profiling using the Merck *Candida albicans* Double Barcoded strain library revealed that small GTPase $RHO1^{-/+}$ mutant strain is highly sensitive to TPPB, not $FKS1^{-/+}$ mutant strain. RHO1 is known to regulate the cell wall synthesis via FKS1. Aniline blue staining showed that TPPB inhibited cell wall synthesis as expected. These data suggested that TPPB is a unique antifungal compound, targeting RHO1.

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Curriculum Vitae

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Research Interest

Chemical biology of natural products

Synergistic effect of anticancer drug resistance and Wnt3a on primary ciliogenesis

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Abstract

Primary cilia, antenna-like cellular sensor structures, are generated from the mother centriole in the G0/G1 cell-cycle phase under control by cellular signaling pathways involving Wnt, hedgehog, and platelet-derived growth factor. Although primary ciliary dynamics have been reported to be closely related to ciliopathy and tumorigenesis, the molecular basis for the role of primary cilia in human disease is lacking. To clarify how Wnt3a affects primary ciliogenesis in anticancer drug-resistant cells, we derived specific drug-resistant subcell lines from A549 human lung cancer cells using anticancer drugs doxorubicin, dasatinib, and paclitaxel (A549/Dox, A549/Das, and A549/Pac, respectively). The primary cilia-containing cell population and primary cilia length increased in the A549/Dox and A549/Pac subcell lines under increased MDR1 expression, when compared to those in the parental A549 cells. In the A549/Das subcell line, primary cilia length increased but the cell population was not affected. In addition, Wnt3a increased primary cilia-containing cell population and primary cilia length in A549/Dox, A549/Das, and A549/Pac cells, without change of cell growth. Abnormal shapes of primary cilia were frequently observed by anticancer drug resistance and Wnt3a stimulation. Taken together, our results indicate that anticancer drug resistance and Wnt3a affect primary ciliogenesis synergistically, suggesting a potential new strategy for overcoming anticancer drug resistance.

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Curriculum Vitae

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Research Interest:

Primary cilia, Wnt signaling pathway, Cytokinesis, Anticancer drug resistance

MO-2097, a nature-inspired benzofuran, is a potent HIF-1 α inhibitor and a promising anticancer agent

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Abstract

Hypoxia-inducible factor 1 (HIF-1), the transcriptional activator that mediates adaptive responses to hypoxia, is up-regulated in the tumor microenvironment and is recognized as a significant cancer drug target. In an ongoing pursuit of novel HIF-1 inhibitors, a series of nature-inspired benzofurans with modifications on the chiral rings of moracins O and P were synthesized and evaluated for their inhibitory activity. This study identified a potent compound MO-X featuring reduced structural complexity and increased efficiency. Furthermore, MO-X exhibited inhibitory effects on hypoxia-induced HIF-1α accumulation in HeLa CCL2 cells via inhibition of the hnRNPs protein, whose binding affinities were confirmed by ITC analysis. In addition, MO-X demonstrated *in vivo* efficacy, without toxicity, in a xenograft model using Balb/C mice. Remarkably, the immunohistochemistry staining of MO-X treated tissues showed significantly decreased expression of HIF-1α and increased levels of the apoptosis marker cleaved caspase 3, confirming *in vivo* efficacy. Since there is an increasing demand for individually customized anticancer drugs, and organoids represent one of the most suitable platforms for testing potential drugs on patient-specific tumors, MO-X was tested in this system with significant anticancer results.

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Curriculum Vitae

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Chungbuk Nat. University, College of Medicine 2002, master degree
Chungbuk Nat. University, College of Medicine 2005, doctoral degree

Previous Appointments:
NCI/NIH, USA (2005-2010) Post-Doc

KRIBB (2010-Present) Senior/Principal Research Scientist

Research Interest

My research team is dedicated to investigating the mechanisms of action underlying small molecular compounds sourced from natural products. We place a strong emphasis on validating the significance of RNA-binding proteins, particularly in relation to their anti-cancer potential. Our approach involves the utilization of both 2D cell cultures and more complex 3D spheroids and cancer organoids to assess anti-cancer efficacy. Notably, the integration of cancer organoids into our studies provides insights into the potential drug responses that could manifest uniquely among individual patients. As such, we envision our team's endeavors as fundamental research poised to pave the way for the eventual realization of personalized medicine

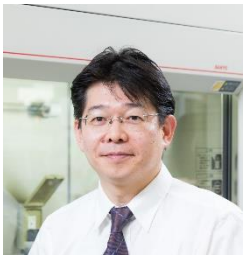
Session 3
Biosynthesis of Bioactive Molecules

Chairs

Dr. Young-Soo Hong (KRIBB)

Production of natural products by heterologous gene expression

Shunji Takahashi



Unit Leader
Natural Product Biosynthesis Research Unit
RIKEN Center for Sustainable Resource Science, Japan

Abstract

Microorganisms such as actinomycetes and filamentous fungi are a rich repository of valuable secondary metabolites. The understanding of biosynthetic mechanisms is important to utilize microbial metabolites efficiently. Therefore, we elucidate a key reaction of biosynthetic pathways by genetic and biochemical approaches. We diversify microbial metabolites by modifying gene clusters and pathway engineering[1-4]. In addition to utilizing transcriptional regulators, we develop novel methods to activate biosynthetic gene clusters by small molecules and create natural products[5-6]. We are constructing microbial biosynthetic platforms and efficiently produce valuable natural products using genetic resources from nature. In this presentation I will focus on heterologous gene expression of natural products using BAC vector[7-9] and elucidation of biosynthetic mechanisms.

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Curriculum Vitae

Name: Shunji Takahashi

Affiliation:
Natural Product Biosynthesis Research Unit, REKEN CSRS

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Education:
1997: D.Sci., Chiba University, Japan

Previous Appointments:
1997-1998: Postdoctoral Scholar, University of Tokyo
1998-2002: Research Associate, Graduate School of Medicine, Chiba University
2002-2005: Postdoctoral Scholar, University of Kentucky, USA
2005-2007: Postdoctoral Researcher, Antibiotics Laboratory, RIKEN
2007-2015: Senior Research Scientist, Antibiotics Laboratory, RIKEN
2011-2013: Team Head, Cheminformatics and Compound Creation Team
2012-present: Guest Professor, Tokyo Denki University
2013-present: Unit Leader, Natural Product Biosynthesis Research Unit, RIKEN CSRS
2013-2017: Unit Leader, Global Research Cluster, RIKEN-KRIBB Joint Research Unit
2015-2021: Associate Professor, University of Tokyo
2017-2022: Unit Leader, CSRS, RIKEN-KRIBB Joint Research Unit
2020-present: Professor, Saitama University

Research Interest

Microorganisms such as actinomycetes and filamentous fungi are a rich repository of valuable secondary metabolites. The understanding of biosynthetic mechanisms is important to utilize microbial metabolites efficiently. For this reason we elucidate a key reactions of biosynthetic pathways by genetic and biochemical methods. We diversify microbial metabolites by modifying gene clusters and pathway engineering. In addition to utilizing transcriptional regulators, we develop novel methods to activate biosynthetic gene clusters by small molecules and create natural products. We are constructing microbial biosynthetic platforms and efficiently produce valuable natural products using genetic resources from nature.

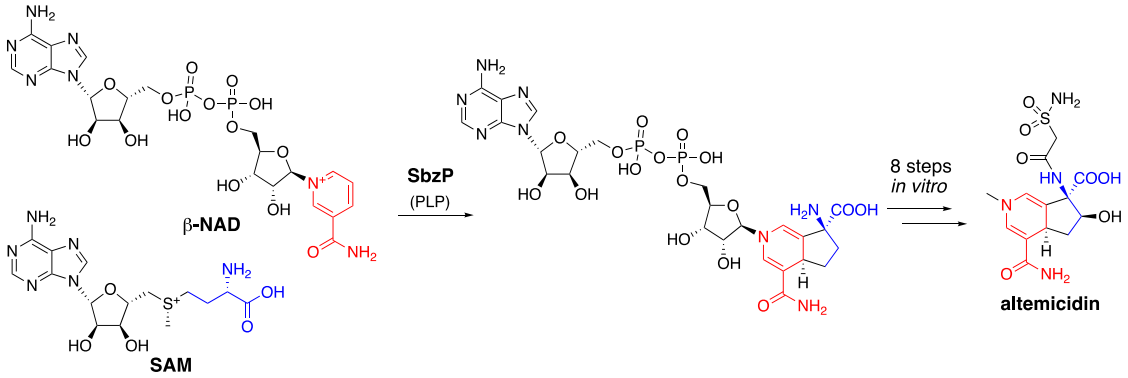
β-NAD as a building block in natural product biosynthesis

Takayoshi Awakawa

Team Leader
RIKEN Center for Sustainable Resource Science

Abstract

β-Nicotinamide adenine dinucleotide (β-NAD) is a pivotal metabolite for all living organisms and functions as a primary electron acceptor and carrier during central catabolic processes[1]. We demonstrated the first example that β-NAD functions as a building block for the assembly of potent and structurally intriguing bacterial secondary metabolites, such as the anti-cancer compound altemicidin[2] and the Ile-tRNA inhibitor SB-203208 from *Streptomyces*. Our biochemical analyses identified a PLP-dependent enzyme (SbzP) as a novel family of enzyme catalyzing the scaffold formation *via* [2+3]-annulation reaction at the pyridinium moiety of β-NAD, utilizing *S*-adenosyl methionine as a co-substrate (**Figure**). Reconstitution of the complete downstream biosynthetic pathway revealed the function of several new dinucleotide-tailoring enzymes, leading to altemicidin scaffold. Intriguingly, SbzP homologs are distriubted among various genomes of Gram-positive and -negative bacterial phyla, such as actino-, chloroflexi-, proteo- and cyanobacteria, and β-NAD-utilization was demonstrated for eight representative proteins by heterologous expression. The findings of this study fundamentally expand our understanding of the bacterial secondary metabolic repertoire and will enable the discovery and exploitation of a novel class of β-NAD-derived natural products[3,4]. In this presentation, the structural basis and reaction mechanism of the [2+3]-annulation reaction between β-NAD and β,γ-unsaturated quinonoid derived from SAM will be discussed in more detail.



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Curriculum Vitae

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Education:

B.S. 2006/3 The University of Tokyo, Agricultural and Life Sciences
M.S. 2008/3 The University of Tokyo, Agricultural and Life Sciences
Ph.D. 2011/3 The University of Tokyo, Agricultural and Life Sciences

Previous Appointments:

2011/4 Assistant Professor, The University of Tokyo, Graduate School of Pharmaceutical Sciences

2014/4 Visiting Scholar, UC San Diego, Scripps Institute of Oceanography (Prof. Bradley Moore)

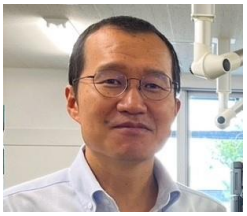
2016/4 Assistant Professor, The University of Tokyo, Graduate School of Pharmaceutical Sciences

2019/7 Associate Professor, The University of Tokyo, Graduate School of Pharmaceutical Sciences

Research Interest

His current research interest mainly focuses on the engineering

Structure-activity relationship of decalin-containing tetramic acids collected from genetically engineered fungi

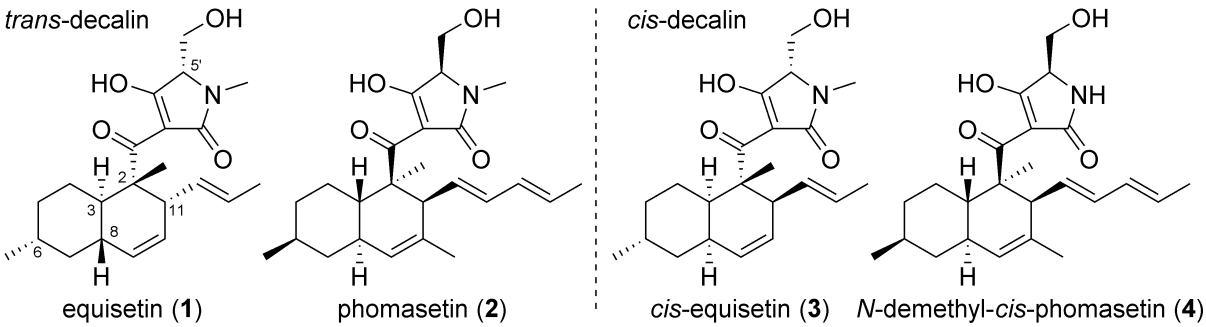


Naoki Kato

Associate Professor
Faculty of Agriculture, Setsunan University

Abstract

Decalin-containing tetramic acid is a bioactive scaffold primarily produced by filamentous fungi. The structural diversity of this group of compounds is generated by characteristic enzymes of fungal biosynthetic pathways, including decalin synthase responsible for the stereoselective decalin formation via the intramolecular Diels–Alder reaction. HIV-1 integrase inhibitors, equisetin (**1**) and phomasetin (**2**), and thier derivatives **3** and **4** that differ in the decalin configuration were collected from genetically engineered mutants derived from producing fungi[1,2] and used for a structure-activity relationship study. Our evaluation of biological activities, such as cytotoxicity against several cancer cell lines and antibacterial, antifungal, antimalarial, and mitochondrial inhibitory activities, demonstrated a relationship between decalin configuration and biological activity. In addition to these known biological activities, we revealed that the compounds showed inhibitory activity against the insect steroidogenic glutathione *S*-transferase Noppera-bo[3]. Engineering the decalin configurations would be useful not only to find derivatives with better biological activities but also to discover overlooked biological activities.



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3) Kato N., *et al*. *PLOS ONE* 10.1371/journal.pone.0290851 (2023)

Curriculum Vitae

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Education:

1999-2002 Graduate School of Bioagricultural Sciences, Nagoya University
1997-1999 Graduate School of Agriculture, Hokkaido University
1993-1997 Faculty of Agriculture, Hokkaido University

Previous Appointments:

2002-2003 Research associate, Dep. of Biological Sciences, Northern Illinois University

2004-2005 NEDO Research fellow, Forestry Research Institute, Oji Paper Company Ltd.

2005-2007 Postdoctoral Researcher, Antibiotics Laboratory, RIKEN

2007-2010 Special Postdoctoral Researcher, Antibiotics Laboratory, RIKEN

2010-2013 ASI Research Scientist, Chemical Biology Core Facility, RIKEN ASI

2010-2011 Visiting Scientist, Max-Planck-Institute of Molecular Physiology

2013-2020 Research Scientist, Natural Product Biosynthesis Research Unit, , RIKEN Center for Sustainable Resource Science

2015, 2016 Visiting Scientist, Korea Research Institute of Bioscience and Biotechnology

2020-present Associate Professor, Faculty of Agriculture, Setsunan University

Research Interest

Biosynthesis of fungal secondary metabolites

Catalytic mechanism of Diels-Alderase

Discovery of new natural products through genome mining of BGC-rich *Streptomyces* lineage

Hiyoung Kim[1] and Hahk-Soo Kang[1*]

Associate Professor
[1]Department of Biomedical Science and Engineering, Konkuk University, Seoul 05029, Korea

Abstract

Streptomyces has been known to be a rich source of biosynthetic gene clusters (BGCs), many of which are still yet to be characterized. Through comparative analysis of 158 phylogenetically diverse Streptomyces genome sequences, we have identified a specific Streptomyces phylogenetic lineage highly enriched with BGCs (on average 50 BGCs/genome). A genetic similarity network analysis of BGCs identified from this phylogenetic lineage indicated that the majority of them are cryptic and possess unique genotypes. Due to their potential to produce novel metabolites, we cloned several cryptic BGCs, which are prioritized on the basis of uniqueness of BGC genotypes, and successfully expressed them in a heterologous host *S. albus* J1074. One of the BGCs belonging to the family of reducing type II PKS (polyketide synthase) produced novel metabolites with a previously unknown carbon skeleton. Here, we present the structures and biosynthesis of new metabolites discovered from the BGC-rich Streptomyces phylogenetic lineage. This study highlights that comparative genomics combined with BGC similarity network analysis provides a powerful platform to identify cryptic BGCs with unique genotypes that could potentially lead to the discovery of novel natural products.

Reference

1) Chung, Y.H., Kim, H., Ji, C.H., Je, H.W., Lee, D., Shim, S.H., Joo, H.S., Kang, H.S. *mSystems* **6**(4): e00489-21 (2021)

Curriculum Vitae

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Associate Professor
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Education:
2012, Ph.D in Natural Products Chemistry, University of Illinois at Chicago, USA
2003, BS in Biotechnology, Chung-Ang University, South Korea

Previous Appointments:
2022 Administrative staff, The Korean Society of Pharmacognosy

2020 Newsletter editor, The Korean Society for Microbiology and Biotechnology

2016-2019 Assistant Professor, Department of Biomedical Science and Engineering, Konkuk University, South Korea

2012-2016 Postdoctoral Associate, The Rockefeller University, USA

Research Interest

The main focus of our lab is to develop synthetic biology tools for natural product BGC refactoring and their application to drug discovery. These synthetic biology tools developed in-house are used to create high-titer strains of clinically important natural products for industrial application and also to activate silent biosynthetic gene clusters that could lead to the discovery of new biologically active natural products.

Special Presentation 2

Chair

Prof. Masaya Imoto (Juntendo Univ.)

Role of c-Myc on the cytotoxicity of ROS inducers in cancer cells

Nobumoto Watanabe

Temporary Employee
Chemical Resource Development Research Unit, RIKEN CSRS
Visiting Professor
Guangzhou Medical University, Guangdong, China

Abstract

c-Myc is a critical regulator of cell proliferation and growth. Elevated levels of c-Myc cause transcriptional amplification, leading to various types of cancers. Small molecules that specifically inhibit c-Myc-dependent regulation are potentially invaluable for anticancer therapy. Because c-Myc does not have enzymatic activity or targetable pockets, researchers have attempted to obtain small molecules that inhibit c-Myc cofactors, activate c-Myc repressors, or target epigenetic modifications to regulate the chromatin of c-Myc-addicted cancer without any clinical success. In this study, we screened for c-Myc inhibitors using a cell-dependent assay system in which the expression of c-Myc and its transcriptional activity can be inferred from monomeric Keima and enhanced GFP fluorescence, respectively. We identified several mitochondrial inhibitors, as hit compounds. The compounds enhanced the c-Myc phosphorylation of threonine-58, consequently increasing the proteasome-mediated c-Myc degradation. The mechanistic analysis of these compounds revealed that they enhanced the degradation of c-Myc protein through the activation of glycogen synthetic kinase 3 by reactive oxygen species (ROS) from damaged mitochondria. Furthermore, we found that the inhibition of cell growth by these compounds was caused by both ROS-dependent and ROS-independent pathways. Interestingly, ROS-dependent growth inhibition occurred only in the presence of c-Myc, which may reflect the representative features of cancer cells. Consistently, the ROS inducer sensitivity of cells was correlated to the endogenous c-Myc levels in various cancer cells. Overall, our study provides an effective strategy for identifying c-Myc inhibitors and proposes a novel concept for utilizing ROS inducers for cancer therapy.

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Curriculum Vitae

Name: Nobumoto Watanabe

Affiliation:
Chemical Resource Development Research Unit, RIKEN CSRS
E-mail: nwatanab@riken.jp

Education:

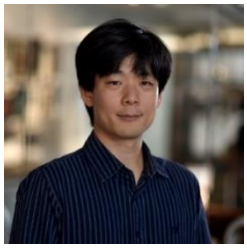
1987	Ph.D. (Dr. of Science), Tokyo University, Tokyo, Japan
1984	M.S., Tokyo University, Tokyo, Japan
1982	B.S., Tokyo University, Tokyo, Japan

Previous Appointments:

2014-2023	Affiliated Professor RIKEN Molecular and Chemical Somatology Tokyo Medical and Dental University
2013-2022	Unit Leader Bioprobe Application Research Unit RIKEN Center for Sustainable Resource Science
2009-2013	Team Head Chemical Library Validation Team RIKEN Advanced Science Institute
2006-2018	Visiting Professor, the School of Biological Sciences Universiti Sains Malaysia, Malaysia
2001-2015	Senior Research Scientist Antibiotics laboratory RIKEN Advanced Science Institute (Discovery Research Institute)
1996-2001	Senior Research Scientist Cell Science and Gene Banking Division RIKEN Tsukuba Institute (Tsukuba Life Science Center)
1992-1994	Research Associate Molecular Biology and Virology Laboratory The Salk Institute, La Jolla, CA, USA
1989-1995	Research Scientist Gene Bank, RIKEN Tsukuba Life Science Center
1987-1989	Postdoctoral Fellow Laboratory of Molecular Oncology RIKEN Tsukuba Life Science Center

Research Interests:
Analyses of mechanism of action of bioactive compounds
Mechanism of cell cycle regulation
Isolation and analyses of compounds for cancer therapy

Open drug discovery platform, pharmaco-net: Synergistic effect of applying artificial intelligence (AI) to the field of natural products research



Young Bin Park

CSO
Calici Co., USA & Korea

Abstract

In a situation where natural product research is attracting attention again, the research method used in a single compound between new drug/functional materials research in natural product research is increasing in importance. In this situation, the reality is that the importance of MoA (Mode of Action) confirmation of the single compound of the natural product is being emphasized. Recently, the task of increasing efficiency and accuracy by introducing artificial intelligence (AI) used in all fields to the development of new drugs and functional substances is becoming one of the basics of research and development. Therefore, supporting natural product research using AI can significantly accelerate the discovery and development of novel compounds from natural sources. In consequence, using Pharmaco-Net's integrated diverse AI algorithms platform can help increase the efficiency and accuracy of natural product research. Pharmaco-Net will employ AI-driven virtual screening techniques to analyze and prioritize natural product compounds for further experimental testing. Perform molecular docking and molecular dynamics (MD) simulations to predict the binding affinity of natural compounds with specific drug targets of protein on Pharmaco-Net. Virtual screening is a computational technique that uses AI and molecular modeling to analyze and prioritize chemical compounds for their potential to interact with specific biological targets, such as proteins or enzymes. In the context of natural product research, Pharmaco-Net can help identify promising bioactive compounds from single compounds, complex mixtures or libraries of natural products based on protein structure without the need for labor-intensive experimental assays.

Reference

1) https://www.calici.co/
2) https://pharmaco-net.org/

Curriculum Vitae

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Education:
Seoul National University, Ph.D., 2007 - 2012

Previous Appointments:
National Institute of Infectious Diseases(NIID, Tokyo), Research Officer, 2011 - 2016
Baylor College of Medicine(BCM, Houston), Postdoc Research Associate, 2016 - 2018

Research Interest

Drug/Functional material development, Natural product research, Web-application platform based on artificial intelligence