Bioorganic Chemical Investigations of Bioactive Compounds from Mushrooms

学 位 論 文 要 約

Summary of Doctoral Thesis

専　　攻：バイオサイエンス　　氏　名：アリフ　ヤヌアル　リヅワン
Course：Bioscience　　Name：Arif Yanuar Ridwan

論文題目：
Title of Thesis：Bioorganic Chemical Investigations of Bioactive Compounds From Mushrooms

論文要約：
Summary：

Fungi have been used as food and traditional medicines around the world for a long time, primarily in Asian countries. Furthermore, higher fungi, in particular Basidiomycetes have been identified as a promising source of bioactive metabolites with high structural diversity and a wide range of biological activity such as plant and fungal growth regulatory activity and cytotoxic secondary metabolites. Moreover, mushrooms contain secondary metabolites that exhibit a range of beneficial properties, such as antioxidant, antibacterial, antiviral, anticancer, and anti-inflammatory properties.

In recent years, our group has investigated for metabolites of mushroom-forming fungi with specialized activities, plants and fungi growth regulators. Erinaceolactones A to B, erinachromane A and B from the culture broth of *Hericium erinaceus*, 10-hydroxymelleolide, 13-hydroxymelleolide K from *Armillaria* sp. and fairy chemicals from *Lepista sordida* were reported as plant growth regulators[1–4]. Melleolide and armillarikin from the culture broth of *Armillaria* sp. were reported to regulate the growth of mycelia of *Coprinopsis cinerea* and *Flammulina velutipes*[1,3]. Our group also

reported plant growth regulators produced by other mushrooms, for example the edible mushroom *Russula vinosa*, *Leccinum extremiorientale*, *Stropharia rugosoannulata*, *Tricholoma flavovirens* and fairy ring-forming fungus *Lepista sordida* [5–9].

Nowadays, there are a number of studies that have been demonstrated that mushrooms have therapeutic potential against cancers. *Agaricus blazei*, *Armillaria mellea*, *Hericium erinaceus*, *Grifola frondosa*, and *Pleurotus ostreatus* were reported as promising anticancer sources[10–12]. Recently, our group has investigated the bioactive compounds which specifically suppressed the Axl and immune checkpoints molecules in the cancer microenvironment. Axl, a type of tyrosine kinase, has been reported to be a new epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKI) resistance mechanism[13]. Furthermore, the association between Axl and epithelial-mesenchymal transition (EMT) has been pointed out and involved in poor prognosis, tumor growth, and metastasis[14]. In spite of the role of Axl in cancer, it is crucial to develop the inhibitors targeting Axl. On the other hand, the programmed death-1 (PD-1) is a coinhibitory receptor expressed on the surface of activated T cells and B cells. PD-1 is mostly activated by its ligands, PD-L1 (programmed death ligand-1) and PD-L2 (programmed death ligand-2)[15]. PD-L1 is widely distributed in diverse cell types in lymphoid and nonlymphoid tissues, whereas PD-L2 is mainly expressed on dendritic cells (DCs) and some macrophages[16,17]. It is important to suppress the activity of PD-L1 and PD-L2 because the cancer cells provide a possible mechanism to avoid/escape immune surveillance by expressing PD-L1/PD-L2[18].

Pleurocybella porrigens is a species that belongs to Marasmiaceae family. This mushroom is distributed in temperate forests of the Northern hemisphere, including Japan. *P. porrigens* is known as the angel wing oyster mushroom or Sugihiratake in

Japanese, a white-rot wood-decay fungus on conifer wood. In the rainy season, *P. porrigens* is abundant and was a popular edible mushroom in Japan. However, in 2004, the wild mushroom *P. porrigens* poisoned 55 people who ate it. Among them, 17 people died due to acute encephalopathy. The case attracted several researchers to solve the relationship between mushroom intake and the disease. In recent years, our group has reported the isolation and characterization of a novel lectin and unusual amino acids from the mushroom. Our further investigation led to the discovery of a structurally-unique and unstable amino acid, pleurocybellaaziridine, as a toxic principle.[19] Studies by other groups also suggested the poisoning accident might involve vitamin D analogue and intoxication of cyanide and thiocyanate after ingestion.[20,21] Despite its toxic nature of *P. porrigens*, there are only a few reports of metabolites with physiological functions. On the other hand, apart from the elucidation of the molecular mechanism of the disease caused by the fungus, we are simply interested in biologically active secondary metabolites produced by the fungus, because it produces structurally unique compounds such as pleurocybellaaziridine.

Pholiota lubrica (Chanametsumutake in Japanese) is a wild edible mushroom that belongs to genus Pholiota of family Strophariaceae and has a widespread distribution, especially in temperate regions and frequently grows on wood in pine forest and its fruiting bodies occur in autumn. This mushroom has been reported as a source of the allelopathic compound against lettuce. In addition, other species from the genus Pholiota produced an antitumor substance, prebiotic, therapeutic agent, antihyperlipidemic and hepatoprotective substances.

Cortinarius caperatus (former name, Rozites caperata, Japanese name: Shogenji) is an edible mushroom and widely grows in the temperate zone of the northern

hemisphere. Additionally, this mushroom is a mild-flavored mushroom and its fruiting bodies have been eaten all over the world, particularly in Japan. This species has been reported to produce an antiviral compound, RC-183, towards herpes simplex virus. However, further studies on secondary metabolites and the biological activities of this mushroom have not been carried out.

The aforementioned evidences suggested mushrooms are a potential source of bioactive natural products. This study describes the isolation, structure elucidation, and evaluation of their biological activity from three mushrooms *P. porrigens*, *P. lubrica*, and *C. caperatus*.

1) Isolation and structural elucidation of bioactive compounds from the toxic mushroom *Pleurocybella porrigens*

Pleurocybella porrigens is a species belongs to Marasmiaceae family. This mushroom is distributed in temperate forests of the northern hemisphere, including Japan. *P. porrigens* is known as the angel's wing mushroom or Sugihiratake in Japanese, a white-rot wood-decay fungus on conifer wood. In the rainy season, *P. porrigens* is abundant and was a popular edible mushroom in Japan. However, in 2004, there was a poisoning outbreak due to the intake of this mushroom. The outbreak caused 55 people poisoned and among them 17 people died because of acute encephalopathy. The case led to the investigations of toxic substances from this mushroom. Our group has reported a novel lectin, unusual amino acids, and a structurally unique and unstable compound, pleurocybellaaziridine, which might be a toxic principle.

1.1) Fruiting bodies

A novel butenolide (1) along with three known ones (2–4) were isolated from the

fruiting bodies of *P. porrigens*. The chemical structure, including the absolute configuration of the novel compound (**1**) was determined based on the interpretation of 1D, 2D NMR, HRESIMS, specific rotation, and CD spectra. Compounds **1**–**3** reduced the expression of Axl, programmed death-ligand 1 (PD-L1), and programmed death-ligand 2 (PD-L2). Compounds **1** and **2** showed inhibition activity against mycelial growth of *Flammulina velutipes*.

1.2) Culture broth

One novel compound (**5**) and four known compounds (**6**–**9**) were isolated from the culture broth of *P. porrigens*. The planar structure of compound **5** was determined by extensive NMR and HRESIMS spectroscopic analysis. Compounds **7** and **9** showed the most potent inhibition activity against mycelial growth of *F. velutipes*.

2) Isolation and structural elucidation of bioactive compounds from the edible mushroom *Pholiota lubrica*.

Pholiota lubrica (Japanese name: Chanametsumutake) is an edible mushroom belongs to the Strophariaceae family, which is distributed in the temperate zone of the northern regions. This mushroom typically grows in the forest and its fruiting bodies occur in autumn. This mushroom has been reported to produce an allelopathy substance that inhibited the growth of lettuce. In addition, other species of the genus *Pholiota* produced an antitumor substance, prebiotic, therapeutic agent, antihyperlipidemic and hepatoprotective substances.

A bioassay-guided fractionation resulted in the isolation of a new cinnamamide, *N*-(1-cinnamoylpyrrolidin-2-yl)cinnamamide (**10**), along with eight known compounds (**11**–**18**). Their structures were determined by the interpretation of spectroscopic data. Compounds **10**, **12** and **18** exhibited the inhibitory activity against lettuce, while

compounds **11** and **16** promoted the growth of lettuce.

3) Isolation and structural elucidation of bioactive compounds from the edible mushroom *Cortinarius caperatus*

Cortinarius caperatus (Japanese name: Shogenji; English name: gypsy mushroom) is a mushroom that belongs to Cortinariaceae family, which grows widely in the temperate zone of the northern hemisphere. This mushroom has a mild taste and it has been eaten all over the world, particularly in Japan. This species has been reported to produce an antiviral substance against enveloped virus, the herpes simplex virus (HPV).

Nine compounds (**19**–**27**) were isolated from the fruiting bodies of *C. caperatus*. Their structures were identified by comparison of spectroscopic data to those of reported data. Compound **22** was first isolated from a natural source. Compounds **19**, **21**, **23**, and **24** inhibited growth of *F. velutipes*. Compounds **20**–**27** exhibited growth regulatory activities towards rice seedling, while compounds **22**–**25** and **27** regulated the growth of lettuce. In order to investigate their suppressing activity against Axl and immune checkpoint molecules, compounds **19**–**22** and **24** were examined toward lung cancer cells (A549). As a result, compound **22** effectively inhibited the Axl, (PD-L1) and PD-L2 gene expression, while compound **24** showed strong inhibition against PD-L2.

References

1. Wu, J. *et al.* Erinaceolactones A to C, from the culture broth of *Hericium erinaceus*. *J. Nat. Prod.* **78**, 155–158 (2015).
2. Wu, J. *et al.* Erinachromanes A and B and Erinaphenol A from the Culture Broth of *Hericium erinaceus*. *J. Agric. Food Chem.* **67**, 3134–3139 (2019).
3. Kobori, H. *et al.* Bioactive sesquiterpene aryl esters from the culture broth of

Armillaria sp. *J. Nat. Prod.* 163–167 (2015).

4. Choi, J. H. *et al.* Disclosure of the 'fairy' of fairy-ring-forming fungus *Lepista sordida*. *ChemBioChem* **11**, 1373 – 1377 (2010).
5. Matsuzaki, N. *et al.* Plant growth regulatory compounds from the mushroom *Russula vinosa*. *Mycoscience* **57**, 404–407 (2016).
6. Ito, A. *et al.* Plant growth regulators from the edible mushroom *Leccinum extremiorientale*. *Mycoscience* **58**, 383–386 (2017).
7. Ito, A. *et al.* Plant growth inhibitors from the culture broth of fairy ring-forming fungus *Lepista sordida*. *Mycoscience* **58**, 387–390 (2017).
8. Qiu, W. *et al.* A new compound from the mushroom *Tricholoma flavovirens*. *Biosci. Biotechnol. Biochem.* **78**, 754–756 (2017).
9. Wu, J. *et al.* Strophasterols A to D with an unprecedented steroid skeleton: From the mushroom *Stropharia rugosoannulata*. *Angew. Chemie - Int. Ed.* **51**, 10820–10822 (2012).
10. Hetland, G., Johnson, E., Lyberg, T. & Kvalheim, G. The mushroom Agaricus blazei murill elicits medicinal effects on tumor, infection, allergy, and inflammation through its modulation of innate immunity and amelioration of Th1/Th2 imbalance and inflammation. *Adv. Pharmacol. Sci.* **2011**, (2011).
11. Patel, S. & Goyal, A. Recent developments in mushrooms as anti-cancer therapeutics: a review. *3 Biotech* **2**, 1–15 (2012).
12. Li, G. *et al.* Anticancer potential of *Hericium erinaceus* extracts against human gastrointestinal cancers. *J. Ethnopharmacol.* **153**, 521–530 (2014).
13. Zhang, Z. *et al.* Activation of the AXL kinase causes resistance to EGFR-targeted therapy in lung cancer. *Nat. Genet.* **44**, 852–860 (2012).
14. Asiedu, M. K. *et al.* AXL induces epithelial-to-mesenchymal transition and regulates the function of breast cancer stem cells. *Oncogene* (2014). doi:10.1038/onc.2013.57
15. Freeman, B. G. J. *et al.* PD-1 Freeman JEM 2000. *J. Exp. Med.* **192**, 1028–1034 (2000).
16. Yamazaki, T. *et al.* Expression of Programmed Death 1 Ligands by Murine T Cells and APC. *J. Immunol.* **169**, 5538–5545 (2002).

17. Ishida, M. *et al.* Differential expression of PD-L1 and PD-L2, ligands for an inhibitory receptor PD-1, in the cells of lymphohematopoietic tissues. *Immunol. Lett.* **84**, 57–62 (2002).

18. Nishimura, H., Minato, N., Nakano, T. & Honjo, T. Immunological studies on PD-1-deficient mice: Implication of PD-1 as a negative regulator for B cell responses. *Int. Immunol.* **10**, 1563–1572 (1998).

19. Wakimoto, T. *et al.* Proof of the existence of an unstable amino acid: Pleurocybellaziridine in *Pleurocybella porrigens*. *Angew. Chemie - Int. Ed.* **50**, 1168–1170 (2011).

20. Sasaki, H. Sugihiratake mushroom (Angel's Wing Mushroom)-induced cryptogenic encephalopathy may involve vitamin D analogues. *Biol. Pharm. Bull.* **29**, 2514–2518 (2006).

21. Akiyama, H. . *et al.* Determination of cyanide and thiocyanate in Sugihiratake mushroom using HPLC method with fluorometric detection. *J. Heal. Sci.* **52**, 73–77 (2006).