

## 化学応用分野

## Division of Natural Products Chemistry

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### ◇研究目的

和漢薬や天然薬物（特にアジアの伝統薬物）から、膵臓癌、糖尿病、抗マラリア、骨粗鬆症、痛風などに有用な医薬シーズを探索すること、ならびに、和漢薬や天然薬物が薬物代謝酵素に及ぼす影響を解明することを目的とする。

### ◇研究概要

#### I) 伝統薬物から栄養飢餓状態で殺細胞作用を有する物質の探索

国立がんセンターとの共同研究で、膵臓癌 PANC-1 細胞株を用い、低栄養状態 (IMEM) で PANC-1 に対する殺細胞活性を示し、通常培地 (DMEM) では細胞の成育に活性を示さないような薬物を天然資源より探索している。その結果、ミャンマー産薬用植物 *Kayea assamica* より PANC-1 細胞株に対して選択的な細胞毒性を有する新化合物を見出し、報告した。

#### II) 天然薬物から酵母 $Ca^{2+}$ シグナル伝達阻害物質の探索と医薬への応用

広島大学で開発したポジティブスクリーニング法を用いて、新規医薬シーズ開発を目的に研究を行っている。中国及び東南アジア産生薬によるスクリーニングを行い、これまで約 1000 サンプルの試験を終え、25 検体に阻害活性を見い出している。その中で、ベトナム産薬用植物 *Eurycoma longifolia* の成分を検討中である。

#### III) 天然薬物の薬物代謝酵素阻害に関する研究

通常、和漢薬を始めとする天然薬物は合成医薬品と併用されている事から、天然薬物が“薬物代謝酵素 (シトクローム P450, CYP)”に及ぼす影響 (薬物間相互作用) を系統的に検証しておく事が、天然薬物の有効利用の上で必要とされる。我々は、漢方生薬及びインドネシア・ジャムウ生薬について CYP3A4 及び CYP2D6 阻害活性を検索し、強い阻害活性を示した生薬について、その活性成分の解明を行っている。本年は、CYP3A4 に対する mechanism-based inhibition を示したジャムウ生薬 “*Cinnamomum burmani*” 及び CYP2D6 阻害活性を示したジャムウ生薬 “*Lunasia amara*” の活性成分について検討した。

#### IV) 骨粗鬆症に有効な天然薬物成分の開発研究

抗骨粗鬆活性が見い出された紅豆杉のリグナンと類縁のリグナンについて、破骨細胞類似の細胞を用いたアッセイ系で活性を検討中である。

#### V) ブラジル産プロポリスの生物活性成分の研究

ブラジル産プロポリスの生物活性成分研究の一環として、レッドプロポリスが膵臓癌 PANC-1 細胞株に対して低栄養状態で選択的殺細胞活性を示す事を見だし、その活性成分の研究を行っている。

上記の研究課題によって得られた本年度の成果 (原著及び学会報告) は下記の通りである。

## ◇著書

- 1) Awale S., Hamazaki T., Kadowaki M., Saiki I., Tezuka Y., and Kadota S.: Chapter IV. Commonly Encountered Diseases in Traditional Medicine, In Myanmar Traditional Medicine Handbook, by Department of Traditional Medicine, Ministry of Health, and Japan International Cooperation Agency (JICA), 89–120, 2008.

## ◇原著論文

- 1) Awale S., Li F., Onozuka H., Esumi H., Tezuka Y., and Kadota S.: Constituents of Brazilian Red Propolis and their Preferential Cytotoxic Activity against Human Pancreatic Cancer PANC-1 Cell Line in Nutrient Deprived Condition. *Bioorg. Med. Chem.*, **16**: 181–189, 2008.

**Abstract:** Human pancreatic cancer cells such as PANC-1 are known to exhibit marked tolerance to nutrition starvation that enables them to survive for prolonged period of time even under extremely nutrient-deprived conditions. Thus, elimination of this tolerance to nutrition starvation is regarded as a novel approach in anticancer drug development. In this study, the MeOH soluble extract of Brazilian red propolis was found to kill 100% PANC-1 cells preferentially in the nutrient-deprived condition at the concentration of 10 µg/mL. Further phytochemical investigation led to the isolation of 43 compounds including three new compounds, (6a*S*,11a*S*)-6a-ethoxymedicarpan (**1**), 2-(2',4'-dihydroxyphenyl)-3-methyl-6-methoxybenzofuran (**2**), and 2,6-dihydroxy-2-[(4-hydroxyphenyl)methyl]-3-benzofuranone (**3**). Among them, (6a*R*,11a*R*)-3,8-dihydroxy-9-methoxypterocarpan (**21**, DMPC) displayed the most potent 100% preferential cytotoxicity (PC<sub>100</sub>) at the concentration of 12.5 µM. Further study on the mode of cell death induced by DMPC against PANC-1 cells indicated that killing process was not accompanied by DNA fragmentation, rather through a nonapoptotic pathway accompanied by necrotic-type morphological changes.

- 2) Toyooka N., Zhou D., Kobayashi S., Tsuneki H., Wada T., Sakai H., Nemoto H., Sasaoka T., Tezuka Y., Subehan, Kadota S., Garraffo H. M., Spande T. F., and Daly J. W.: Synthesis of the Proposed Structures of Poison-Frog Alkaloids 179 and 207E and Their Inhibitory Effects on Neuronal Nicotinic Acetylcholine Receptors. *Synlett*, 61–64, 2008.

- 3) Win N. N., Awale S., Esumi H., Tezuka Y., and Kadota S.: Panduratin D—I, Novel Secondary Metabolites from Rhizomes of *Boesenbergia pandurata*. *Chem. Pharm. Bull.*, **56**: 491–496, 2008.

**Abstract:** Investigation of the non-polar fraction of *Boesenbergia pandurata* of Myanmar led to the identification of six novel secondary metabolites, panduratin D–I (**1–6**), together with known diastereomers, panduratin B1 (**7**) and B2 (**8**). Their structures were determined based on extensive spectroscopic analysis. The in vitro preferential cytotoxicity of all isolates was examined against human pancreatic PANC-1 cancer cells under nutrient-deprived conditions. All exhibited a mild activity.

- 4) Yin J., Han N., Xu X., Liu Z., Zhang B., and Kadota S.: Inhibitory activity of the ethyl acetate fraction from *Viscum coloratum* on bone resorption. *Planta Med.*, **74**: 120–125, 2008.

- 5) Li F., Awale S., Tezuka Y., and Kadota S.: Cytotoxic constituents from Brazilian red propolis and their structure–activity relationship. *Bioorg. Med. Chem.*, **16**: 5434–5440, 2008.

**Abstract:** Several classes of flavonoids [flavanoids (**1–10**), flavonol (**11**), isoflavones (**12–18**), isoflavanones (**19–22**), isoflavans (**23–26**), chalcones (**27–30**), auronol (**31**), pterocarpan (**32–37**), 2-arylbenzofuran (**38**), and neoflavonoid (**39**)] and lignans (**40–42**) isolated from the MeOH extract of Brazilian red propolis were investigated for their cytotoxic activity against a panel of six different cancer cell lines including murine colon 26-L5 carcinoma, murine B16-BL6 melanoma, murine Lewis lung carcinoma, human lung A549 adenocarcinoma, human cervix HeLa adenocarcinoma, and human HT-1080 fibrosarcoma cell lines. Based on the observed results, structure–activity relationships were discussed. Among the tested compounds, 7-hydroxy-6-methoxyflavanone (**3**) exhibited the most potent activity against B16-BL6 (IC<sub>50</sub>, 6.66 µM), LLC (IC<sub>50</sub>, 9.29 µM), A549 (IC<sub>50</sub>, 8.63 µM), and HT-1080

(IC<sub>50</sub>, 7.94 μM) cancer cell lines, and mucronulatol (**26**) against LLC (IC<sub>50</sub>, 8.38 μM) and A549 (IC<sub>50</sub>, 9.9 μM) cancer cell lines. These activity data were comparable to those of the clinically used anticancer drugs, 5-fluorouracil and doxorubicin, against the tested cell lines, suggesting that **3** and **26** are the good candidates for future anticancer drug development.

6) **Yin J., Han N., Liu Z., Xu X., Zhang B., and Kadota S.: The *in vitro* anti-osteoporotic activity of some diarylheptanoids and lignans from the rhizomes of *Dioscorea spongiosa*. *Planta Med.*, 74: 1451–1453, 2008.**

7) **Win N. N., Awale S., Esumi H., Tezuka Y., and Kadota S.: Novel anticancer agents, kayeassamins A and B from the flower of *Kayea assamica* of Myanmar. *Bioorg. Med. Chem. Lett.*, 18: 4688–4691, 2008.**

**Abstract:** The CHCl<sub>3</sub>-soluble fraction of 70% EtOH extract of the flower of *Kayea assamica* completely killed human pancreatic PANC-1 cancer cells preferentially under nutrient-deprived conditions at 1 μg/mL. Bioassay-guided fractionation and isolation afforded two novel compounds, kayeassamins A (**1**) and B (**2**). Their structures were elucidated using extensive spectroscopic methods and the modified Mosher method. Each compound showed 100% preferential cytotoxicity (PC<sub>100</sub>) against PANC-1 cells under nutrient-deprived conditions at 1 μM. Furthermore, both compounds inhibited the migration of PANC-1 cells in the wound closure assay.

8) **Toyooka N., Zhou D., Nemoto H., Tezuka Y., Kadota S., Jones T. H., Garraffo H. M., Spande T. F., and Daly J. W.: First Enantioselective Synthesis of a Hydroxyindolizidine Alkaloid from the Ant *Myrmecaria melanogaster*. *Synlett*, 1894–1896, 2008.**

9) **Win N. N., Awale S., Esumi H., Tezuka Y., and Kadota S.: Novel anticancer agents, kayeassamins C–I from the flower of *Kayea assamica* of Myanmar. *Bioorg. Med. Chem.*, 16: 8653–8660, 2008.**

**Abstract:** A CHCl<sub>3</sub>-soluble fraction of 70% EtOH extract of the flower of *Kayea assamica* from Myanmar exhibited 100% preferential cytotoxicity (PC<sub>100</sub>) against human pancreatic cancer PANC-1 cells under nutrient-deprived conditions at 1 μg/mL. Bioassay-guided fractionation and isolation afforded nine new coumarins, kayeassamins A (**8**), B (**9**), and C–I (**1–7**), together with nine known coumarins (**10–18**). The structures of these compounds were identified by extensive spectroscopic techniques as well as by comparison with published data. Absolute configuration at C-1' of **1** was established as *S*-configuration by the modified Mosher method. All the isolates were evaluated for their *in vitro* preferential cytotoxicity using novel anti-austerity strategy. Among them, the novel coumarins, kayeassamins A (**8**), B (**9**), D (**2**), E (**3**), and G (**5**) exhibited the most potent preferential cytotoxicity (PC<sub>100</sub> 1 μM) in a concentration- and time-dependent manner and induced apoptosis-like morphological changes of PANC-1 cells within 24 h of treatment. Based on the observed cytotoxicity, structure-activity relationships have been established.

10) **Subehan, Kadota S., and Tezuka Y.: *In Vitro* Mechanism-based Inactivation of Cytochrome P450 3A4 by a New Constituent of *Cinnamomum burmani*. *Planta Med.*, 74: 1474–1480, 2008.**

**Abstract:** Cytochrome P450 enzymes play an important role in drug metabolism. Various studies have reported the potential inhibition of these enzymes by natural compounds, leading to possible drug–herb interaction. One study reported that the MeOH extract of *Cinnamomum burmani* inhibited CYP3A4 in a mechanism-based mode. Further phytochemical investigation on this plant led to the isolation of 17 compounds including 2 new compounds: cinnamic aldehyde cyclic syringylglycerol 1,3-acetal (**1**) and 5'-hydroxy-5-hydroxymethyl-4",5"-methylenedioxy-1,2,3,4-dibenzo-1,3,5-cycloheptatriene (**2**). The isolated constituents were tested for their preincubation time-dependent inhibition of CYP3A4 at 0 and 20 min. Only the new compound **2** showed an increase in the inhibitory activity to >50 % after 20-min preincubation. Further investigations of the inactivation by **2** displayed characteristics of a mechanism-based inactivator, with K<sub>i</sub> and k<sub>inact</sub> values of 7.7 μM and 0.04 min<sup>-1</sup>, respectively.

## ◇学会報告 (\*: 特別講演, シンポジウム, ワークショップ等)

- 1) Subehan, 門田重利, 手塚康弘 : Mechanism-based inactivation of cytochrome P450 3A4 by constituents of *Cinnamomum burmani*. 日本薬学会第 128 年会, 2008, 3, 26-28, 横浜.
- 2) 高橋直人, Subehan, 門田重利, 手塚康弘 : インドネシア生薬 *Lunasia amara* BLANCO の薬物代謝酵素 CYP2D6 阻害活性成分. 日本薬学会第 128 年会, 2008, 3, 26-28, 横浜.
- 3) Nwet Nwet Win, Suresh Awale, Hiroyasu Esumi, Yasuhiro Tezuka, Shigetoshi Kadota : Panduratin D—I, Novel Secondary Metabolites from Rhizomes of *Boesenbergia pandurata* of Myanmar. 日本薬学会第 128 年会, 2008, 3, 26-28, 横浜.
- 4) 豊岡尚樹, 周 徳軍, 小林創史, 恒枝宏史, 和田 努, 酒井秀紀, 根本英雄, 笹岡利安, 手塚康弘, Subehan, 門田重利, H. Martin Garraffo, Thomas F. Spande, John W. Daly : 毒ガエルアルカロイド 179, 207E の合成. 日本薬学会第 128 年会, 2008, 3, 26-28, 横浜.
- 5) Feng Li, Suresh Awale, Yasuhiro Tezuka, Shigetoshi Kadota : Cytotoxic constituents from Brazilian red propolis and their structure-activity relationship. 日本薬学会北陸支部第 118 回例会, 2008, 7, 8, 富山.
- 6) 三宅克典, 手塚康弘, 門田重利 : ベトナム生薬 *Euricoma longifolia* Jack の成分研究. 日本生薬学会第 55 回年会, 2008, 9, 19-20, 長崎.
- 7) Nwet Nwet Win, Suresh Awale, Hiroyasu Esumi, Yasuhiro Tezuka, Shigetoshi Kadota : Novel anti-cancer agents from the flower of *Kayea assamica* of Myanmar. 日本生薬学会第 55 回年会, 2008, 9, 19-20, 長崎.
- 8) 宮本竜也, Suresh Awale, 李 峰, Nwet Nwet Win, 手塚康弘, 門田重利 : ミャンマーの薬用植物 Dant-da-ku-ni から得られた新規抗癌活性成分. 日本生薬学会第 55 回年会, 2008, 9, 19-20, 長崎.
- \* 9) 門田重利, Suresh Awale, Nwet Nwet Win, 手塚康弘, 三好千香, 江角浩安 : 伝統薬からの栄養飢餓耐性制御薬の開発 (Discovering natural anti-cancer agents that retards the cancer cells tolerance to nutrition starvation). 第 67 回日本癌学会学術総会, 「シンポジウム 18 低酸素環境と血管新生抑制療法」, 2008, 10, 28-30, 名古屋.

## ◇海外調査

- 1) 門田重利 : 国際協力機構 (JICA) に係わるミャンマー-伝統医療プロジェクト, 運営指導調査, 2008, 2/5-2/10, ミャンマー.
- 2) 門田重利 : 国際協力機構 (JICA) に係わるミャンマー-伝統医療プロジェクト, 終了時評価調査, 2008, 12/10-12/20, ミャンマー.
- 3) 手塚康弘, Suresh Awale : 国際協力機構 (JICA) に係わるミャンマー-伝統医療プロジェクト, 2008, 7/18-7/26, ミャンマー.

## ◇共同研究

### 国内

- 1) 江角浩安 : 国立がんセンター-研究所支所, 「がん生物学に基づく新しい治療法に関する研究」, 2003, 4~
- 2) 宮川都吉 : 広島大学大学院・先端物質科学研究科, 「酵母  $\text{Ca}^{2+}$  シグナルによる細胞周期制御に関する総合的研究」, 2004, 4~
- 3) 信川高寛 : 金沢医科大学, 「紅豆杉の生物活性物質の探索」, 2004, 4~

### 海外

- 1) Dejair Message, Alfredo A. G. Fuertas : ブラジル・ヴィソサ大学, 「プロポリスの品質評価に関する研究」, 1996, 10~
- 2) 殷 軍 : 中国・瀋陽薬科大学, 「漢方方剤の抗骨粗鬆症活性成分に関する研究」, 2004, 10~

- 3) 李 建新：中国・南京大学化学工学院・薬物化学研究所, 「抗骨粗鬆症に有効な薬物の開発研究」, 2004, 4～
- 4) Tran Le Quan：ベトナム・国立ホーチミン市大学, 「ベトナム産薬用植物の科学的評価に関する研究」, 2003, 4～

#### ◇研究費取得状況

- 1) 厚生労働省がん研究助成（分担：門田重利）「がん生物学に基づく新しい治療法の開発に関する研究」

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大学院博士1年：信川真智子

大学院博士2年：李 峰, 三宅克典, 守川耕平（10月入学）

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外国人研究生：張 紅燕（2007, 12/23～2008, 3/31）

#### ◇学位（修士，博士）取得者

博士論文：

Subehan：Active Indonesian Medicinal Plants and Their Mechanism-based Inactivators on Inhibition of Cytochrome P450 2D6 and 3A4

Nwet Nwet Win：Chemical Constituents of *Boesenbergia pandurata* and *Kayea assamica* of Myanmar and Their Preferential Cytotoxicity against PANC-1 Cell Line in Nutrient-Deprived Conditions