Catechol-O-Methyltransferase Inhibitors Isolated From Thai Propolis

Ryo Miyata1, Tomoharu Motoyama1, Shogo Nakano1, Sohei Ito1, Kazuma Mukaide1, Boonyadist Vongsak2, and Shigenori Kumazawa1

Abstract

Propolis is an aggregate of functional components found in plant resins and has been reported to exhibit a variety of valuable biological activities. This study investigated the inhibitory properties of propolis from Thailand toward human catechol-O-methyltransferase (COMT), a key neurotransmitter involved in Parkinson’s disease and depression. Samples collected from Chanthaburi and Chiang Mai exhibited relatively high inhibitory activity against COMT. γ-Mangostin (1) and 6-prenyleriodictyol (3) were identified as COMT inhibitors with IC50 values of 62 and 75 μM, respectively. In an enzyme inhibition assay, 1 exhibited mixed inhibition toward COMT. The results suggest that both 1 and propolis have potential applications in the prevention and treatment of psychological illness.

Keywords

propolis, COMT, Parkinson’s disease, xanthone, mangostin, prenylflavonoid, mangosteen, Thailand

Introduction

Catechol-O-methyltransferase (COMT, EC 2.1.1.6) catalyzes the transfer of the methyl group from S-adenosyl-L-methionine (SAM) to one of the hydroxy groups of a catechol-bearing substrate in the presence of magnesium ions (Mg2+). COMT plays a dominant role in the inactivation of endogenous catecholamine neurotransmitters, including dopamine, noradrenaline, and adrenaline; therefore, it is seen as a target for the treatment of Parkinson’s disease, schizophrenia, and depression. Tolcapone, entacapone, and opicapone are COMT inhibitors used to treat Parkinson’s disease. However, tolcapone exhibits severe hepatotoxicity, and the toxicity of opicapone has not yet been evaluated in detail. Therefore, COMT inhibitors with low toxicity and a good safety profile are required.

Natural sources of pharmaceuticals may offer many potentially safe COMT inhibitors because of their low toxicity. To date, various compounds that can act as COMT inhibitors, such as caffeic acid and rutin, have been isolated from medicinal plants. In this context, we have focused on propolis in searching for new COMT inhibitor candidates. Propolis is a natural resinous substance collected from the buds and exudates of certain trees and plants by honeybees. Notably, it has been reported to display a variety of valuable biological activities and has been used as a folk medicine in many regions of the world. Therefore, propolis is a candidate in the search for new medicinal agents. We recently reported on the components of Thai propolis. For example, xanthone derivatives, including α- and γ-mangostin, are the major components found in propolis from the Chanthaburi region. This kind of propolis originates from resin on the surface of mangosteen fruits. On the other hand, propolis from Chiang Mai contains prenylflavonoid derivatives.

Although the components of Thai propolis are potentially valuable pharmaceutical resources, they are not used effectively and are almost entirely wasted. This study investigated the COMT inhibitory activity of Thai propolis and its major compounds to further assess the pharmaceutical value and efficacy of these resources.

1Graduate School of Integrated Pharmaceutical and Nutritional Sciences, University of Shizuoka, Shizuoka, Japan
2Pharmaceutical Innovations of Natural Products Unit (PhInNat), Faculty of Pharmaceutical Sciences, Burapha University, ChonBuri, Thailand

Corresponding Author:
Shigenori Kumazawa, Graduate School of Integrated Pharmaceutical and Nutritional Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Shizuoka, Japan.
Email: kumazawa@u-shizuoka-ken.ac.jp

Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (https://creativecommons.org/licenses/by-nc/4.0/) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access page (https://us.sagepub.com/en-us/nam/open-access-at-sage).
Results and Discussion

To identify any effective COMT inhibitors in propolis, three propolis samples were collected from different apiaries (Chanthaburi, Chiang Mai, and Phatthalung) in Thailand (Figure 1 and Table 1), and the COMT inhibitory activity of ethanol extracts from the samples were evaluated by using HPLC.

The extracts obtained from Chanthaburi and Chiang Mai propolis exhibited strong COMT inhibitory activity at a concentration of 0.5 mg/mL (Table 1). By contrast, the extracts from Phatthalung propolis did not significantly inhibit COMT. To identify the COMT inhibitors in propolis, the major compounds found in the samples (Figure 2) were tested individually by using the same COMT inhibitory assay.

The major compounds in Chanthaburi propolis are γ- and α-mangostins (1 and 2), which have a xanthone skeleton. The other compounds are prenylated flavanones 3 to 7, which are the major constituents of Chiang Mai propolis. Table 2 shows that 1 and 6-prenyleriodictyol (3) exhibited COMT inhibitory activity with IC_{50} values of 62 and 75 μM, respectively. Tested compounds other than 1 and 3 did not inhibit COMT. Further analysis identified 1 and 3 as the COMT inhibitors present in the propolis from Chanthaburi and Chiang Mai, respectively.

We subsequently investigated the type of inhibition exhibited by 1, which had the highest COMT inhibitory activity among the tested compounds. The Lineweaver–Burk plot in Figure 3 indicates the mixed inhibition of 1 towards COMT.

COMT has two substrate-binding sites: one for the methyl donor in the SAM substrate and the other for various methyl-accepting catechol substrates, such as 2-hydroxyestradiol, catechins, and dihydroxybenzoic acid. Many small-molecule inhibitors are known to bind to the methyl-acceptor pocket of COMT.\(^{12}\) Compound 1 exhibits mixed inhibition, indicating that it binds to the methyl-acceptor pocket of COMT and another site outside the substrate-binding pocket.

The amount of 3 isolated from the Chiang Mai propolis was very small; therefore, a kinetic study could not be conducted for this compound. Flavonoids such as luteolin and quercetin seem to bind to the methyl-acceptor pocket of COMT.\(^{13}\) Further, myricetin has shown mixed inhibition towards COMT.\(^{14}\) These reports suggest that 3 may exhibit mixed inhibition similar to that exhibited by the flavonoid derivatives.

Conclusions

In this study, we investigated the COMT inhibitory activity of Thai propolis and found that 1 exhibits mixed inhibition toward COMT. Compound 1 was isolated from various parts of mangosteen trees.\(^{15,17}\) This suggests that not only mangosteen propolis but also the components of the mangosteen tree itself may be valuable sources of COMT inhibitors for the prevention and treatment of Parkinson’s disease and depression.

Materials and Methods

Biological Materials

The Chanthaburi and Chiang Mai propolis were the same as those used in previous studies.\(^{10,11}\) The Phatthalung sample was collected from Ban Na Pa Kho Sub-district, Bang Kaeo District, Phatthalung Province, Thailand in May 2017. A voucher sample (no. BV20170501) of the Phatthalung propolis evaluated in this study has been deposited at the Faculty of Pharmaceutical Sciences, Burapha University, Thailand.

Table 1. Sample Information for Each Type of Propolis and the COMT Inhibitory Activity of its Ethanol Extract.

| Collection site | Bee species          | COMT inhibitory rate (%)\(^{a}\) |
|-----------------|----------------------|----------------------------------|
| Chanthaburi     | Tetragonula pagdeni  | 92.1 ± 0.4                       |
| Chiang Mai      | Apis mellifera       | 70.0 ± 0.6                       |
| Phatthalung     | Unknown              | −0.2 ± 0.9                       |

\(^{a}\)Sample concentration: 0.5 mg/mL.
Tested Compounds

γ- And α-mangostins (1 and 2) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Compounds 3 to 7 were isolated from Chiang Mai propolis as previously described.11

Expression and Purification of Human Recombinant COMT

A plasmid sample of human COMT (PMID: NP_009294.1) with C33S, C95S, and C188S mutations in the Novagen pET22b(+) vector via NdeI and HindIII, which contained a C-terminal histidine tag, was purchased from GenScript (Piscataway, NJ, USA). The plasmid was transformed into SHuffle T7. The strain was cultivated in lysogeny broth at 30 °C. When the OD600 value reached 0.6 to 0.8, 0.5 mM isopropyl-β-D-thiogalactopyranoside was added, and the cells were grown for ∼15 h at 16 °C. The cells were collected by centrifugation at 5000 g for 10 min and then suspended in buffer A (100 mM Tris-HCl [pH 8.0], 300 mM NaCl, 5 mM MgCl2, 1 mM EDTA, 10% glycerol, 5 mM β-mercaptoethanol, and 10 μM phenylmethylsulfonyl fluoride). After sonication of the cells, the supernatant was collected by centrifugation at 11 000 g for 30 min. The supernatant was loaded onto a 5 mL HisTrap HP column (GE Healthcare, Uppsala, Sweden) equilibrated with buffer B (100 mM Tris-HCl [pH 7.5], 100 mM NaCl, 5 mM MgCl2, and 5 mM β-mercaptoethanol) and then washed with 50 mL of buffer B. The column was washed with buffer B containing 100 mM imidazole, and the samples were then eluted with 30 mL of buffer B containing 300 mM imidazole. After concentrating the eluted samples, the samples were loaded onto a 5 mL HiTrap desalting column (GE Healthcare) to replace buffer C (50 mM Tris-HCl [pH 7.5] and 1.5 mM MgCl2). The purity of the COMT was confirmed by SDS-PAGE (Figure S1). The concentrations of the COMT samples were estimated by measuring the absorption at 280 nm with a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Protein samples were stored at −30 °C.

COMT Inhibitory Assays

3′,4′-Dihydroxyacetophenone, 4′-hydroxy-3′-methoxyacetophenone, and tolcapone were purchased from Tokyo Chemical Industry Co. (Tokyo, Japan). 3′-Hydroxy-4′-methoxyacetophenone was purchased from Sigma–Aldrich. COMT inhibitory assays were performed by following a previously reported method with a slight modification.18 The assay buffer (50 mM Tris-HCl [pH 7.5], 1.5 mM MgCl2, 20 μM 3,4-dihydroxyacetophenone, 200 μM SAM, and 0-200 μM inhibitor) was preincubated at 37 °C for 5 min. COMT samples were added to the buffer solution with a final

Table 2. COMT Inhibitory Activity of Test Compounds (1-7).

| Compound | IC50 (μM) |
|----------|-----------|
| 1        | 62        |
| 2        | >200      |
| 3        | 75        |
| 4        | >200      |
| 5        | >200      |
| 6        | >200      |
| 7        | >200      |
| tolcapone| 0.38      |

Figure 2. Major compounds in Chanthaburi (1 and 2) and Chiang Mai (3-7) propolis.

Figure 3. Lineweaver–Burk plot of γ-mangostin (1) mixed inhibition toward COMT.
concentration of 1 μM (total volume: 200 μL), and the reaction was started. After incubation of the mixture at 37 °C for 10 min, 3% aqueous HClO₄ (50 μL) was added to terminate the reaction. In the reaction, 4′-hydroxy-3′-methoxycetophenone and 3′-hydroxy-4′-methoxycetophenone were produced from 3,4-dihydroxyacetophenone by COMT. To quantify the amount of both acetophenone products produced, an aliquot (20 μL) of the solution was injected onto an HPLC column under the following conditions: column, 5 μm, 2.1 × 150 mm, DAICEL CHIRALPAK® IA; flow rate, 0.4 mL/min; eluent, 0.1% trifluoroacetic acid in H₂O–acetonitrile (85:15, v/v); detection, 280 nm. Standard curves for each enzyme product were plotted from the concentrations related to the integrated areas of the HPLC chromatograms. The percentage inhibition was calculated according to the following equation: Inhibition = [(concentration of enzyme products in the control experiment) − (concentration of enzyme products in the sample experiment)] × 100/(concentration of enzyme products in the control experiment). For analytical HPLC, a PU-4180 RHPLC pump (Jasco, Tokyo, Japan), MD-4017 photodiode array detector (Jasco), and AS-4050 HPLC autosampler (Jasco) were used. Data were analyzed with ChromNAV software (v.2, Jasco).

Kinetic Study of γ-Mangostin (1) for COMT Inhibition
A kinetic study of 1 for COMT inhibition was performed by following a previously reported method with a slight modification for COMT. This assay was performed with various concentrations of 3′,4′-dihydroxyacetophenone (30–75 μM) either with or without 1. The reaction velocity was estimated by measuring the concentrations of 4′-hydroxy-3′-methoxycetophenone and 3′-hydroxy-4′-methoxycetophenone 7 min after the start of the reaction. A Lineweaver–Burk plot was used to determine the type of inhibition exhibited by 1.

Acknowledgments
The authors thank Pharmaceutical Innovations of Natural Products Unit (PhInNat) for coordinating with beekeepers and Philip Hawke of the University of Shizuoka Scientific English program for his comments on the English in the manuscript.

Author Contributions
Ryo Miyata contributed toward the expression and assay of COMT, and the preparation of the manuscript. Tomoharu Motoyama contributed toward the expression of COMT. Shogo Nakano and Sohei Ito supervised the experiments and checked the descriptions in the manuscript. Kazuma Mukaide isolated the compounds from Chiang Mai propolis. Boonyadist Vongsak collected propolis samples. Shigenori Kumazawa supervised all the processes in the experiments and the manuscript preparation. All authors have read and approved the final manuscript.

Declaration of Conflicting Interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding
The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the Japan Society for the Promotion of Science (grant number KAKENHI JP18KK0165, KAKENHI JP20J23632).

Ethical Approval
This study was approved by the Committee of Genetic Recombination Experiment, the University of Shizuoka, Japan. This article does not contain any studies with animal or human subjects.

Informed Consent
Not applicable, because this article does not contain any studies with human or animal subjects.

Trial Registration
Not applicable, because this article does not contain any clinical trials.

ORCID iDs
Sohei Ito https://orcid.org/0000-0002-9937-3100
Shigenori Kumazawa https://orcid.org/0000-0001-9687-9619

Supplemental Material
Supplemental material for this article is available online.

References
1. Axelrod J, Tomchick R. Enzymic O-methylation of adrenaline and other catechols. J Bio Chem. 1958;233(3):702-705. doi:10.1016/S0021-9258(18)64731-3
2. Tunbridge EM, Harrison PJ, Weinberger DR. Catechol-O-methyltransferase, cognition, and psychosis: Val158Met and beyond. Biol Psychiatry. 2006;60(2):141-151. doi:10.1016/j.biopsych.2005.10.024
3. Bildér RM, Volavka J, Lachman HM, et al. The catechol-O-methyltransferase polymorphism: relations to the tonic-phasic dopamine hypothesis and neuropsychiatric phenotypes. Neuropharmacology. 2004;29(11):1943-1961. doi:10.1016/j.neuropharm.2003.10.038
4. Silva TB, Borges F, Serrao MP, et al. Liver says no: the ongoing search for safe catechol O-methyltransferase inhibitors to replace tolcapone. Drug Discov Tod. 2020;25(10):1846-1854. doi:10.1016/j.drudis.2020.07.015
5. Olanow CW. Tolcapone and hepatotoxic effects. Arch Neurol. 2000;57(2):263-267. doi:10.1001/archneur.57.2.263
6. Fabbri M, Ferreira JJ, Lees A, et al. Opicapone for the treatment of Parkinson’s disease: a review of a new licensed medicine. Mov Disord. 2018;33(10):1528-1539. doi:10.1002/mds.27475
7. Jatana N, Apoorva N, Malik S, et al. Inhibitors of catechol-O-methyltransferase in the treatment of neurological disorders. *Cent Nerv Syst Agents Med Chem.* 2013;13(3):166-194. doi:10.2174/1871524913666140109113341
8. Engelbrecht I, Petzer JP, Petzer A. Evaluation of selected natural compounds as dual inhibitors of catechol-O-methyltransferase and monoamine oxidase. *Cent Nerv Syst Agents Med Chem.* 2019;19(2):133-145. doi:10.2174/1871524919666190619090852
9. Salantino A, Fernandes-Silva CC, Righi AA, et al. Propolis research and the chemistry of plant products. *Nat Prod Rep.* 2011;28(5):925-936. doi:10.1039/c0np00072h
10. Ishizu E, Honda S, Vongsak B, et al. Identification of plant origin of propolis from Thailand stingless bees by comparative analysis. *Nat Prod Comm.* 2018;13(8):973-975. doi:10.1177/1934578x1801300813
11. Mukaide K, Honda S, Vongsak B, et al. Prenylflavonoids from propolis collected in Chiang Mai, Thailand. *Phytochem Lett.* 2021;43:88-93. doi:10.1016/j.phytol.2021.03.015
12. Ikeda M, Iijima H, Shinoda I, et al. Inhibitory effect of bovine lactoferrin on catechol-O-methyltransferase. *Molecules.* 2017;22(8):1373-1384. doi:10.3390/molecules22081373
13. Cao Y, Chen ZJ, Jiang HD, et al. Computational studies of the regioselectivities of COMT-catalyzed meta-/para-O methylations of luteolin and quercetin. *J Phys Chem B.* 2014;118(2):470-481. doi:10.1021/jp410296s
14. Zhao DF, Fan YF, Yu HN, et al. Discovery and characterization of flavonoids in vine tea as catechol-O-methyltransferase inhibitors. *Fitoterapia.* 2021;152:104913. doi:10.1016/j.fitote.2021.104913
15. Jinsart W, Ternai B, Buddhasukh D, et al. Inhibition of wheat embryo calcium-dependent protein kinase and other kinases by mangostin and γ-mangostin. *Phytochemistry.* 1992;31(11):3711-3713. doi:10.1016/s0031-9422(00)97514-9
16. Mohd Ghazali SAIS, Lian GEC, Abd Ghani KD. Chemical constituent from roots of *Garcinia mangostana* (Linn.). *Int J Chem.* 2010;2(1):134-142. doi:10.5539/ijc.v2n1p134
17. Ryu HW, Curtis-Long MJ, Jung S, et al. Xanthones with neuraminidase inhibitory activity from the seedcases of *Garcinia mangostana*. *Bioorg Med Chem.* 2010;18(17):6258-6264. doi:10.1016/j.bmc.2010.07.033
18. Cotton NJH, Stoddard B, Parson WW. Oxidative inhibition of human soluble catechol-O-methyltransferase. *J Biol Chem.* 2004;279(22):23710-23718. doi:10.1074/jbc.m401086200
19. Honda S, Fukuyama Y, Nishiwaki H, et al. Conversion to purpuragallicin, a key step in the mechanism of the potent xanthine oxidase inhibitory activity of pyrogallol. *Free Radic Biol Med.* 2017;106(5):228-235. doi:10.1016/j.freeradbiomed.2017.02.037