Journal of Natural Medicines (2023) 77:891–897
https://doi.org/10.1007/s11418-023-01731-9

ORIGINAL PAPER

# Arginase inhibitory activities of guaiane sesquiterpenoids from *Curcuma comosa* rhizomes

**Nhat Nam Hoang[1] · Takeshi Kodama[1] · Yu Nakashima[1] · Kiep Minh Do[1] · Saw Yu Yu Hnin[1] · Yuan-E Lee[1] · Prema[2] · Naotaka Ikumi[3] · Hiroyuki Morita[1]**

Received: 30 May 2023 / Accepted: 9 July 2023 / Published online: 18 July 2023


## Abstract

Arginases are bimanganese enzymes involved in many human illnesses, and thus are targets for disease treatments. The screening of traditional medicinal plants demonstrated that an ethanol extract of *Curcuma comosa* rhizomes showed significant human arginase I and II inhibitory activity, and further fractionation led to the isolation of three known guaiane sesquiterpenoids, alismoxide (**1**), 7α,10α-epoxyguaiane-4α,11-diol (**2**) and guaidiol (**3**). Tests of their inhibitory activities on human arginases I and II revealed that **1** exhibited selective and potent competitive inhibition for human arginase I ($IC_{50}$ = 30.2 μM), whereas the other compounds lacked inhibitory activities against human arginases. To the best of our knowledge, this is the first demonstration of human arginase I inhibitory activity by a sesquiterpenoid. Thus, **1** is a primary and specific inhibitory molecule against human arginase I.

**Keywords** Sesquiterpenoids · Arginase inhibitory activity · Guaiane sesquiterpenoids · *Curcuma comosa*

## Introduction

Arginases are trimeric metalloenzymes that catalyze the hydrolysis of L-arginine to form L-ornithine and urea, and play a key role in the urea cycle and the regulation of nitric oxide (NO) homeostasis in mammals. Arginases are present in two isoforms: arginase I, a cytosolic enzyme with highest abundance in the liver, and arginase II, a mitochondrial enzyme [1, 2]. However, the physiological functions of these two differently localized isoforms remain unknown [3]. Inhibition of human arginases has been proposed as a potential therapy for various illnesses, such as cardiovascular [4], anti-inflammatory [5], autoimmune [6], oncological [7], and infectious [8] diseases, characterized by abnormally high arginase activity or abnormally low NO synthase activity.

✉ Hiroyuki Morita
hmorita@inm.u-toyama.ac.jp

[1] Institute of Natural Medicine, University of Toyama, 2630-Sugitani, Toyama 930-0194, Japan

[2] Department of Chemistry, University of Yangon, Yangon 11041, Myanmar

[3] Japan Preventive Medical Laboratory Company, Ltd., 3-6-36 Toyoda, Suruga-ku, Shizuoka 422-8027, Japan

Several synthetic human arginase competitive inhibitors for clinical usage, such as L-*nor*-*N*-hydroxyarginine (*nor*-NOHA) [9, 10] and 2(*S*)-amino-6-boronohexanoic acid (ABH) [11], as well as CB-1158 (numidargistat), an ABH homologue, have been developed from L-*N*-hydroxyarginine (NOHA) [9, 10], an intermediate in NO synthesis [3]. NOHA [9], *nor*-NOHA [12], and ABH [13] exert competitive inhibition by binding to the substrate as well as the bimanganese-binding sites, at the active center of arginases [3]. Currently, CB-1158 is in Phase I/II clinical trials as a drug candidate for cancer therapy [14]. However, although such recognition mechanisms may be required for powerful arginase inhibitory activity, mechanistically distinct inhibitor(s) might also be necessary to develop anti-arginase drug(s) that may be used as drug therapies with fewer side effects.

Several arginase inhibitors from natural resources have been described. Piceatannol, a stilbene, was reported as a selective natural inhibitor for human arginase I [15]. Apart from human arginase inhibitors, flavonoids such as quercetin, fisetin, and kaempferol were reported as inhibitors of bovine liver and *Leishmania* arginases [3, 16]. In addition, obacunone [17], a limonoid, sauchinone [18], a lignan, and salvianolic acid B [19], a caffeic acid derivative, were reported to inhibit murine liver lysate arginases I and II.

However, these natural inhibitors have not been successfully forwarded to clinical settings, due to their poor pharmacokinetics profiles [20]. In the course of our search for a natural arginase inhibitor, we found that a 70% ethanol extract of *Curcuma comosa* rhizomes from Myanmar exhibited this activity. *C. comosa* is an herb belonging to the Zingiberaceae family and is distributed in tropical and subtropical areas of Asia, such as Thailand, Indonesia, Malaysia, and Myanmar. The rhizome of *C. comosa*, known as Sa-nwinga in Myanmar, is used in Myanmar traditional medicine for treating headaches, diabetes mellitus, and hypertension [21]. The *C. comosa* rhizome extracts reportedly showed estrogenic [22], antioxidant [23], antiallergic [24], and anti-inflammatory [25] activities. Previous phytochemical investigations indicated that many interesting chemical constituents, including sesquiterpenes [26], labdane diterpenes [27], flavonoid glycosides [28], and diarylheptanoids [29], are produced by this plant. Assay-guided fractionation of the 70% ethanol extract of the *C. comosa* rhizomes led to the isolation of three known guaiane sesquiterpenoids, alismoxide (**1**), with selective arginase I inhibitory activity, as well as 7α,10α-epoxyguaiane-4α,11-diol (**2**) and guaidiol (**3**) (Fig. 1). Herein, we report the isolation and structural elucidations of **1**−**3**, as well as their inhibitory activities against human arginase.

## Materials and methods

### Chemicals and reagents

*Nor*-NOHA was purchased from Bachem AG (Bubendorf, Switzerland). Unless otherwise specified, all chemical reagents were purchased from Fujifilm Wako Pure Chemical (Osaka, Japan).

### General experimental procedures

NMR spectra were recorded on an ECX400P spectrometer (JEOL, Tokyo, Japan). Chemical shift values are expressed in $\delta$ (ppm), based on the $\delta$ residuals of $CDCl_3$ at 7.26 for $^1H$ NMR and 77.0 for $^{13}C$ NMR. HR–ESI–MS data were obtained with an LC–MS–IT–TOF spectrometer (Shimadzu, Kyoto, Japan). Open column chromatography was performed with normal-phase silica gel (silica gel 60N, spherical, neutral, 40–50 µm) (Kanto Chemical, Tokyo, Japan) and reverse phase silica gel (Cosmosil $75C_{18}$-OPN) (Nacalai Tesque, Kyoto, Japan). Analytical TLC was performed on pre-coated silica gel 60 $F_{254}$ plates and RP-18 $F_{254}$ plates (Merck, Billerica, MA, USA), with spot detection by visualization with a UV lamp (254 and 365 nm), as well as spraying with a *p*-anisaldehyde stain solution and heating at 120 °C for 10 min. An SH-1200 microplate reader (Corona Electric, Hitachinaka, Japan) was used to measure the absorbance of the urea produced in the arginase inhibition activity assay. Specific optical rotations were measured on a P2100 polarimeter (JASCO, Tokyo, Japan). A MiniAmp thermal cycler (Thermo Fisher, Waltham, MA, USA) was used to incubate protein assays.

### Plant materials

The *C. comosa* rhizomes were collected from Maubin Township, Ayeyarwady Division, Myanmar, in 2019 and positively identified by Dr. New Ni Tun, a lecturer at the Department of Botany, University of Yangon (Myanmar). A voucher specimen (TMPW 31707) was deposited in the Museum for Materia Medica, Analytical Research Center for Ethnomedicines, Institute of Natural Medicine, University of Toyama, Japan.

### Extraction and isolation

The dried *C. comosa* rhizomes (1.6 kg) were extracted with 70% aqueous EtOH (3.0 L × 5) by sonication (90 min each) to afford the extract. The extract (252.5 g) was evaporated under vacuum, and the remaining aqueous residue was successively partitioned into *n*-hexane (31.0 g), $CHCl_3$ (67.0 g), MeOH (28.0 g), and $H_2O$ extracts (126.5 g). The *n*-hexane extract was separated on a Cosmosil $75C_{18}$-OPN column, using a $H_2O$/MeOH (2:1, 1:1, 1:3, 1:5, and 1:7) solvent system, to give five fractions: $F_1$ (5.2 g), $F_2$ (8.7 g), $F_3$ (2.7 g), $F_4$ (11.0 g), and $F_5$ (3.3 g). The $F_1$ fraction was subjected to normal phase silica gel open column chromatography, using a $CHCl_3$/MeOH solvent system (9.9:0.1, 4.9:0.1, 9.5:0.5, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, and 0:10) with increasing polarity to afford **1** (14 mg). Fraction $F_2$ was chromatographed on an open silica gel column, which was eluted with a hexane/

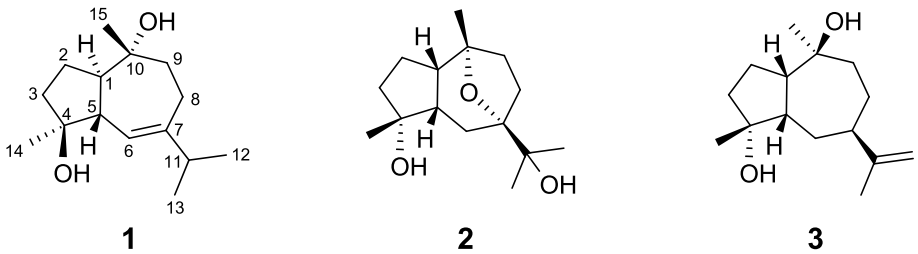



**Fig. 1** Structures of **1**−**3** isolated from *C. comosa* rhizomes

AcOEt solvent system (9.5:0.5, 7:3, 5:5, 3:7, and 1:9) to obtain **2** (8 mg). Fraction $F_3$ was chromatographed on a Cosmosil 75$C_{18}$-OPN with a $H_2O$/MeOH (2:1, 1:1, 1:3, 1:5, 1:7, and 1:9) solvent system to afford **3** (35 mg).

### Construction of expression plasmids, and expression and purification of human arginases I and II

The cDNAs encoding human arginases I (chromosome 6q23) and II (chromosome 14q24) [30, 31] were purchased from Integrated DNA Technologies (Coralville, IA, USA). The open reading frames (ORFs) of the human arginase I and II cDNAs were amplified by PCR, using the primer pairs shown in Table S3 (*BamH*I and *Sal*I sites are underlined and bolded, respectively). Each resultant PCR amplification product was gel-purified and then inserted into the *BamH*I/*Sal*I sites of the expression vector pQE-80L for production as an *N*-terminal hexahistidine-tag fusion protein, using an In-Fusion cloning kit (Takara Bio, Otsu, Japan). After sequence confirmation, the arginase I and II expression plasmids were each transformed into *Escherichia coli* M15 (pREP4) competent cells. The *E. coli* M15 (pREP4) cells harboring arginases I and II were grown at 37 °C in Luria–Bertani (LB) medium, supplemented with 100 μg $mL^{-1}$ ampicillin and 25 μg $mL^{-1}$ kanamycin. When the $OD_{600}$ reached 0.6, recombinant protein expression was induced by adding isopropyl D-thiogalactopyranoside to a final concentration of 0.5 mM, and then each culture was incubated overnight at 16 °C. The cell pellets were collected by centrifugation at 6500 rpm for 15 min at 4 °C and stored at − 80 °C. The pellets were resuspended in 50 mM Tris–HCl (pH 8.0), containing 100 mM NaCl and 5% (v/v) glycerol (buffer A), placed on ice, and sonicated intermittently every 10 s, for a total of 30 min. The lysates were centrifuged at 9000 rpm for 20 min. The supernatants were collected and then loaded on a Ni Sepharose 6 Fast Flow open column (Cytiva, Tokyo, Japan) equilibrated with buffer A. The column was washed with buffer A containing 50 mM imidazole, and arginases I and II were eluted with buffer A containing 500 mM imidazole. The collected proteins were concentrated and then purified by gel filtration chromatography on a HiLoad 16/60 Superdex 200 (Cytiva) column. Finally, the purified enzymes were concentrated to 10 mg $mL^{-1}$ in 20 mM Tris–HCl (pH 8.0), containing 100 mM NaCl, 5% (v/v) glycerol, and 1 mM DTT.

### Arginase inhibitory activity

Arginase inhibitory activities were evaluated using a colorimetric assay to quantify urea production, according to the previously described method with slight modifications [32]. Compounds **1**−**3** were dissolved in DMSO. In brief, arginase I and arginase II were incubated at 55 °C for 10 min for activation, and then 0.2 ng of arginase I or II was added into a final volume of 35 μL reaction buffer (28 mM Tris–HCl, pH 7.5) containing 4.3 mM $MnCl_2$, 14.3 mM L-arginine, 0.015% bovine serum albumin (for arginase I), and inhibitors **1**−**3** or DMSO as a blank. After an incubation for 30 min at 37 °C, the reaction was stopped by adding 60 μL of $H_2SO_4$/$H_3PO_4$/$H_2O$ (1:3:7) on ice, and then 5 μL of α-isonitrosopropiophenone (5% in absolute ethanol) was added. Subsequently, the resultant reaction mixture was heated at 95 °C for 45 min in the dark, and then centrifuged for 10 min to remove the precipitates. The supernatants were transferred to a 96-well plate, and after the microplate was shaken for 2 min, the absorbance at 550 nm was read. Arginase inhibitory activity is expressed as the percentage of inhibition relative to "100% arginase activity". A Dixon plot analysis was performed to determine the type of inhibition of **1** against arginase I. Data were collected in triplicate, at concentrations ranging from 7.5 to 120 μM for **1** and 2.5 to 10 mM for L-arginine.

### Docking simulation

The protein molecule used in the docking simulation was arginase I, PDB ID: 6Q9P. The three-dimensional model of **1** was generated using the Avogadro 1.2 program, and the compound was docked into the arginase I structure by Autodock Vina 1.0.2 [33]. The side chains of the Arg21, Asn130, Ser137, His141, Asp181, Asp183, Glu186, Thr246, and Glu277 residues were set as flexible. The binding affinity of **1** was calculated to be − 6.9 kcal $mol^{-1}$.

## Results and discussion

The 70% ethanol extract of *C. comosa* rhizomes showed inhibitory activities against human arginase I with an $IC_{50}$ value of 61.4 μg $mL^{-1}$ and arginase II with an $IC_{50}$ value of 80.9 μg $mL^{-1}$. Subsequently, the *n*-hexane-soluble fraction of *C. comosa* rhizomes was subjected to normal and reverse phase silica gel open chromatography to yield three guaiane sesquiterpenoids, **1**−**3**. Comparisons of spectroscopic data of **1**−**3** (Tables S1 and S2 and Figs. S1−S9) with those reported in the literature unambiguously identified the structure of **1** as alismoxide (**1**) with a *trans*-fused ring system [34, 35], **2** as 7α,10α-epoxyguaiane-4α,11-diol (**2**) [36], and **3** as guaidiol (**3**) [37] with a *cis*-fused ring system. Furthermore, the absolute configuration of **1** was determined as 1*R*,4*S*,5*R*,10*R*-**1**, based on the well-matched optical rotation value of **1** {$[\alpha]_D^{25}$ + 5.0° (*c* 0.9, MeOH)} with the previously reported values of 1*R*,4*S*,5*R*,10*R*-**1** in the literature {$[\alpha]_D^{25}$ + 5.2° (*c* 0.5, MeOH)} [34, 38].

**Table 1** Inhibitory activities of **1**–**3** against human arginases I and II

| Sample | $IC_{50}$ (μM)[b] | |
|---|---|---|
| | Arginase I | Arginase II |
| **1** | 30.2 ± 2.7[c] | > 300 |
| **2** | > 300 | > 300 |
| **3** | > 300 | > 300 |
| *nor*-NOHA[a] | 8.7 ± 0.6[c] | 12.8 ± 1.3[c] |

[a]Positive control

[b]$IC_{50}$, the half-maximal inhibitory concentration

[c]Data are presented as mean ± SD (standard deviations, $n = 3$)

According to the previous report, the inhibitory activities of **1**–**3** against human arginases I and II were assessed by a colorimetric method using α-isonitrosopropiophenone [32]. *Nor*-NOHA was used as the positive control, and showed $IC_{50}$ values of 8.7 ± 0.6 μM and 12.8 ± 1.3 μM against human arginases I and II, respectively, consistent with the previously reported values of *nor*-NOHA in the literature [human arginase I ($IC_{50}$ values 1.4–6.0 μM) and human arginase II ($IC_{50}$ value: 1.3 μM)] [12, 39]. Compound **1** inhibited human arginase I with an $IC_{50}$ value of 30.2 ± 2.7 μM (Table 1), which was 3–20-fold weaker than *nor*-NOHA ($IC_{50}$ value of 8.7 μM) and ABH ($IC_{50}$ value of 1.45 μM) [11], while it was 2.3 times stronger than the natural product inhibitor, piceatannol ($IC_{50}$ value: 69 μM) [15]. In contrast, neither **2** nor **3** was active against human arginase I, even at a 300 μM concentration, suggesting that **1** could be a valuable natural human arginase I inhibitor as compared with the previously reported ones. Further assays revealed no activities of **1**–**3** against human arginase II, even at 300 μM concentrations, suggesting that **1** could be a selective inhibitor of human arginase I.

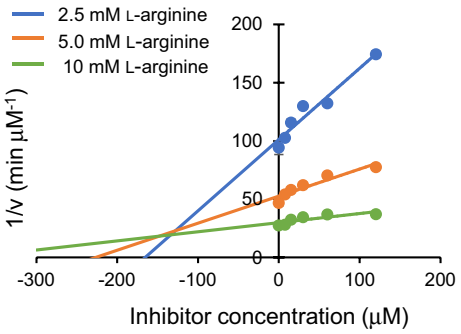
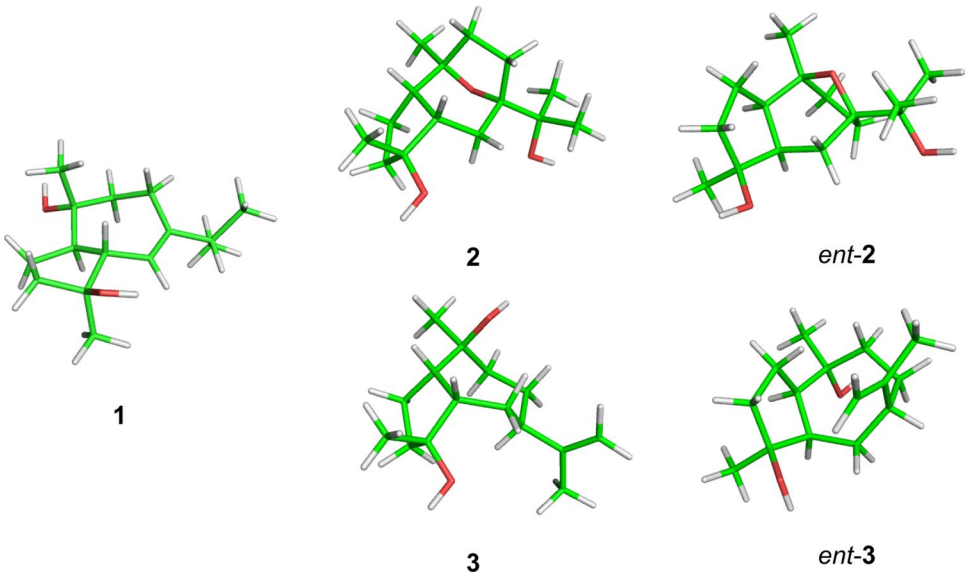



**Fig. 2** Dixon plot analysis of the binding mode of **1** with arginase I

To the best of our knowledge, this is the first demonstration of human arginase I inhibitory activity by a sesquiterpenoid. To clarify the mode of inhibition by **1**, we performed kinetic studies of human arginase I. First, we checked whether the human arginase I used in this experiment showed activity at a level consistent with previous reports. The Michaelis–Menten plot analysis revealed $K_M$, $k_{cat}$, and $k_{cat}/K_M$ values of 11.6 mM, 20.9 $min^{-1}$, and $1.8 \times 10^3$ $M^{-1}$ $min^{-1}$ against human arginase I with respect to L-arginine (Fig. S10), which were comparable to the previously reported kinetics parameters of human arginase I heterologously expressed in *E. coli* ($K_M = 2.3$ mM, $k_{cat} = 5.0$ $min^{-1}$, and $k_{cat}/K_M = 2.1 \times 10^3$ $M^{-1}$ $min^{-1}$) [40]. Subsequently, the Dixon plot analysis revealed that **1** is a competitive inhibitor of L-arginine with a $K_I$ value of 136.5 μM, which is roughly 85-fold higher than the substrate affinity (11.6 mM), suggesting that **1** selectively inhibited human arginase I by binding to the L-arginine binding site (Fig. 2). The $K_I$ value of **1** was lower than those of the synthetic inhibitors, *nor*-NOHA ($K_I = 0.50$ μM for arginase I) and ABH ($K_I = 0.11$ μM for arginase I) [11], while it was

**Fig. 3** Three-dimensional structures of **1**, **2**, **3**, *ent*-**2**, and *ent*-**3**



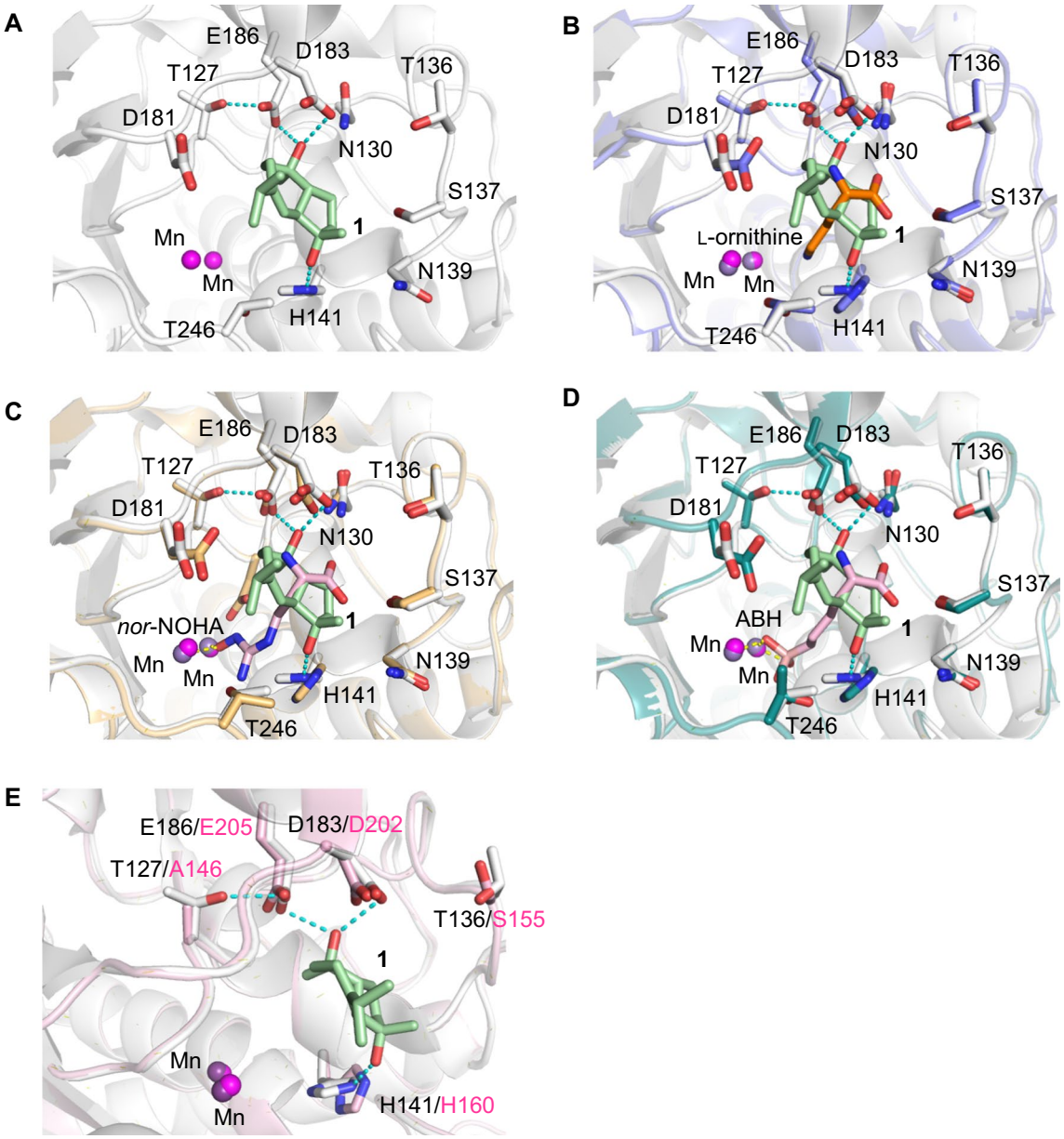



**Fig. 4** Docking model of alismoxide (**1**) to human arginase I (PDB ID: 6Q9P). Close-up view of the substrate binding site of human arginase I complexed with **A 1**, **B 1** and L-ornithine (PDB ID: 3GMZ), **C 1** and *nor*-NOHA (PDB ID: 3KV2), and **D 1** and ABH. Compound **1** is shown as a green stick model. **E** Superimposition of human arginase I complexed with **1** and human arginase II (PDB ID: 4HZE). Arginases I and II are highlighted with white and pink colors, respectively

similar to that of the natural product inhibitor, piceatannol ($K_I = 136$ μM for arginase I) [15].

A structural feature of the guaiane sesquiterpenoid is the presence of a bicyclo[5.3.0]decane skeleton. Comparisons of the structures of **1**−**3** suggested that the significantly different functionalities between the active compound **1** and the non-active compounds **2** and **3** are the presence of the 7-isopropyl moieties attached to the olefinic groups at C-6 and C-7 in **1**. These moieties in **1** may account for the acquisition of the selective inhibitory activity of **1** against human arginase I. However, comparisons of the three-dimensional structures of **1**−**3** and enantiomers of **2** and **3** indicated that **1** possessed a flatter structure across the board, as compared with those of **2** and **3** and their *ent*-forms, due to the *trans*-fused ring system in **1** (Fig. 3). Thus, rather than the differences of the functionalities between **1** and **2**/**3**, the totally different three-dimensional structure of **1** might be the major factor, leading to the selectively competitive inhibition against human arginase I in a competitive manner with respect to L-arginine.

To further characterize the interactions between human arginase I and **1** in the L-arginine-binding site, we performed a docking simulation for **1** and human arginase I (PDB ID: 6Q9P) [14]. The docking simulation predicted that **1** is accommodated in an L-arginine-binding site by hydrogen bonds between the side chains of His141, Asp183, and Glu186 in human arginase I, with a binding affinity of − 6.9 kcal $mol^{-1}$ (Fig. 4A, B). However, **1** did not indicate any interaction with manganese, in contrast with *nor*-NOHA and ABH, which exhibit potent inhibitory activities by coordinating with not only L-arginine, but also the two manganese atoms in the active center (Fig. 4C, D) [3]. The lower inhibitory activity of **1** than the synthetic inhibitors such as *nor*-NOHA and ABH would be caused by fewer interactions at the catalytic center, as compared to those of the synthetic inhibitors. On the other hand, the docking studies of **2** and **3** to human arginase I are not available, since these compounds were non-active for arginase I, which indicates that both compounds lack any interaction with this enzyme. However, considering the docking result of **1** and the three-dimensional structures of **2** and **3** (Fig. 3), totally different three-dimensional structures of **2** and **3** compared with that of **1** would prevent not only the hydrogen-bond formations with His141, Asp183, and Glu186 observed in the docking study between **1** and arginase I, but also their binding to the active-site cavity of human arginase I.

One of the interesting points is that **1** showed tenfold stronger inhibitory activity against human arginase I ($30.2 \pm 2.7$ μM) than human arginase II (> 300 μM). The detailed catalytic mechanisms for the distinct catalytic properties between arginases I and II, such as kinetic parameters, have not yet been determined. However, considering that the different residues in the active-sites between human arginases I and II are only Thr127 and Thr136 in human arginase I and Ala146 and Ser155 in human arginase II [41, 42], the selective inhibitory activity of **1** for human arginase I may be derived from the C-10 hydroxyl group of **1** and Glu186, which form a hydrogen bond network with Thr127 and Asp183 in human arginase I (Fig. 4E). However, to fully understand the distinct catalytic properties between arginases I and II, further investigations are still needed to elucidate the selective inhibitory mode of **1** against human arginase I.

In conclusion, we isolated three guaiane sesquiterpenoids, alismoxide (**1**), 7α,10α-epoxyguaiane-4α,11-diol (**2**), and guaidiol (**3**) from *C. comosa* rhizomes. Our study revealed that **1** is a selective arginase I inhibitor in a competitive manner with respect to L-arginine. Thus, this study provided new insights into a naturally occurring arginase I inhibitor.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s11418-023-01731-9.

**Author contributions** NNH: performed all experiments. KMD and TK: supported the structure elucidation of compounds. SYYH: established arginases I and II expression system and prepared these proteins. P collected the plant sample. YN: performed the docking simulation. HM: designed this study. TK, YN, YL, NI, and HM: wrote the manuscript. All authors commented on the manuscript. All authors read and approved the final manuscript.

**Funding** This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan (JSPS KAKENHI Grants 22H02777 to H.M., 23K06179 to T.K., and JP22K15303 to Y.N.).

## Declarations

**Conflict of interest** The authors declare no competing final interest.

## References

1. Detroja TS, Samson AO (2022) Virtual screening for FDA-approved drugs that selectively inhibit arginase type 1 and 2. Molecules 27:5134
2. Caldwell RB, Toque HA, Narayanan SP, Caldwell RW (2015) Arginase: an old enzyme with new tricks. Trends Pharmacol Sci 36:395–405
3. Pudlo M, Demougeot C, Girard-Thernier C (2017) Arginase inhibitors: a rational approach over one century. Med Res Rev 37:475–513
4. Pernow J, Jung C (2013) Arginase as a potential target in the treatment of cardiovascular disease: reversal of arginine steal? Cardiovasc Res 98:334–343
5. Munder M (2009) Arginase: an emerging key player in the mammalian immune system. Br J Pharmacol 158:638–651
6. Wegner A, Verhagen J, Wraith DC (2018) Myeloid-derived suppressor cells mediate tolerance induction in autoimmune disease. Immunology 151:26–42
7. Andersen MH (2018) The balance players of the adaptive immune system. Cancer Res 78:1379–1382
8. Ilari A, Fiorillo A, Baiocco P, Poser E, Angiulli G, Colotti G (2015) Targeting polyamine metabolism for finding new drugs against leishmaniasis: a review. Mini Rev Med Chem 15:243–252
9. Di Costanzo L, Ilies M, Thorn KJ, Christianson DW (2010) Inhibition of human arginase I by substrate and product analogues. Arch Biochem Biophys 496:101–108
10. Colleluori DM, Ash DE (2001) Classical and slow-binding inhibitors of human type II arginase. Biochemistry 40:9356–9362
11. Collet S, Carreaux F, Boucher JL, Pethe S, Lepoivre M, Danion-Bougot R, Danion D (2000) Synthesis and evaluation of ω-borono-α-amino acids as active-site probes of arginase and nitric oxide synthases. J Chem Soc, Perkin Trans 1(2):177–182
12. Cox JD, Cama E, Colleluori DM, Pethe S, Boucher JL, Mansuy D, Ash DE, Christianson DW (2001) Mechanistic and metabolic inferences from the binding of substrate analogues and products to arginase. Biochemistry 40:2689–2701
13. Di Costanzo L, Sabio G, Mora A, Rodriguez PC, Ochoa AC, Centeno F, Christianson DW (2005) Crystal structure of human arginase I at 1.29-Å resolution and exploration of inhibition in the immune response. Proc Natl Acad Sci U S A 102:13058–13063

14. Grobben Y, Uitdehaag JCM, Willemsen-Seegers N, Tabak WWA, de Man J, Buijsman RC, Zaman GJR (2019) Structural insights into human arginase-1 pH dependence and its inhibition by the small molecule inhibitor CB-1158. J Struct Biol X 4:100014
15. Muller J, Cardey B, Zedet A, Desingle C, Grzybowski M, Pomper P, Foley S, Harakat D, Ramseyer C, Girard C, Pudlo M (2020) Synthesis, evaluation and molecular modelling of piceatannol analogues as arginase inhibitors. RSC Med Chem 11:559–568
16. Bordage S, Pham TN, Zedet A, Gugglielmetti AS, Nappey M, Demougeot C, Girard-Thernier C (2017) Investigation of mammal arginase inhibitory properties of natural ubiquitous polyphenols by using an optimized colorimetric microplate assay. Planta Med 83:647–653
17. Yoon J, Park M, Lee JH, Min BS, Ryoo S (2014) Endothelial nitric oxide synthase activation through obacunone-dependent arginase inhibition restored impaired endothelial function in ApoE-null mice. Vascul Pharmacol 60:102–109
18. Lim CJ, Cuong TD, Hung TM, Ryoo SW, Lee JH, Kim EH, Woo MH, Choi JS, Min BS (2012) Arginase II inhibitory activity of phenolic compounds from *Saururus chinensis*. Bull Korean Chem Soc 33:3079–3082
19. Joe Y, Zheng M, Kim HJ, Kim S, Uddin MJ, Park C, Ryu DG, Kang SS, Ryoo S, Ryter SW, Chang KC, Chung HT (2012) Salvianolic acid B exerts vasoprotective effects through the modulation of heme oxygenase-1 and arginase activities. J Pharmacol Exp Ther 341:850–858
20. Gao K, Lunev S, van den Berg MPM, Al-Dahmani ZM, Evans S, Mertens DALJ, Meurs H, Gosens R, Groves MR (2021) A synthetic peptide as an allosteric inhibitor of human arginase I and II. Mol Biol Rep 48:1959–1966
21. Tun KN, Aminah NS, Kristanti AN, Aung HT, Takaya Y (2020) Sesquiterpene from Myanmar medicinal plant (*Curcuma comosa*). In: Perveen S, Al-Taweel A (eds) Terpenes and terpenoids-recent advances. IntechOpen, London, pp 77–90
22. Winuthayanon W, Suksen K, Boonchird C, Chuncharunee A, Ponglikitmongkol M, Suksamrarn A, Piyachaturawat P (2009) Estrogenic activity of diarylheptanoids from *Curcuma comosa* Roxb. requires metabolic activation. J Agric Food Chem 57:840–845
23. Boonmee A, Srisomsap C, Karnchanatat A, Sangvanich P (2011) An antioxidant protein in *Curcuma comosa* Roxb. rhizomes. Food Chem 124:476–480
24. Matsumoto T, Nakamura S, Fujimoto K, Ohta T, Ogawa K, Yoshikawa M, Onishi E, Fukaya M, Matsuda H (2015) Structure of diarylheptanoids with antiallergic activity from the rhizomes of *Curcuma comosa*. J Nat Med 69:142–147
25. Thampithak A, Jaisin Y, Meesarapee B, Chongthammakun S, Piyachaturawat P, Govitrapong P, Supavilai P, Sanvarinda Y (2009) Transcriptional regulation of iNOS and COX-2 by a novel compound from *Curcuma comosa* in lipopolysaccharide-induced microglial activation. Neurosci Lett 462:171–175
26. Qu Y, Xu F, Nakamura S, Matsuda H, Pongpiriyadacha Y, Wu L, Yoshikawa M (2009) Sesquiterpenes from *Curcuma comosa*. J Nat Med 63:102–104
27. Chokchaisiri R, Chaneiam N, Svasti S, Fucharoen S, Vadolas J, Suksamrarn A (2010) Labdane diterpenes from the aerial parts of *Curcuma comosa* enhance fetal hemoglobin production in an erythroid cell line. J Nat Prod 73:724–728
28. Chokchaisiri R, Innok P, Suksamrarn A (2012) Flavonoid glycosides from the aerial parts of *Curcuma comosa*. Phytochem Lett 5:361–366
29. Suksamrarn A, Ponglikitmongkol M, Wongkrajang K, Chindaduang A, Kittidanairak S, Jankam A, Yingyongnarongkul BE, Kittipanumat N, Chokchaisiri R, Khetkam P, Piyachaturawat P (2008) Diarylheptanoids, new phytoestrogens from the rhizomes of *Curcuma comosa*: isolation, chemical modification and estrogenic activity evaluation. Bioorg Med Chem 16:6891–6902
30. Jenkinson CP, Grody WW, Cederbaum SD (1996) Comparative properties of arginases. Comp Biochem Physiol B, Biochem Mol 114:107–132
31. Wu G, Morris SM Jr (1998) Arginine metabolism: nitric oxide and beyond. Biochem J 336:1–17
32. Pham TN, Bordage S, Pudlo M, Demougeot C, Thai KM, Girard-Thernier C (2016) Cinnamide derivatives as mammalian arginase inhibitors: synthesis, biological evaluation and molecular docking. Int J Mol Sci 17:1656
33. Trott O, Olson AJ (2010) AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem 31:455–461
34. Peng GP, Tian G, Huang XF, Lou FC (2003) Guaiane-type sesquiterpenoids from *Alisma orientalis*. Phytochemistry 63:877–881
35. Blay G, García B, Molina E, Pedro JR (2006) Syntheses of (+)-alismoxide and (+)-4-epi-alismoxide. J Org Chem 71:7866–7869
36. Wang HX, Liu CM, Liu Q, Gao K (2008) Three types of sesquiterpenes from rhizomes of *Atractylodes lancea*. Phytochemistry 69:2088–2094
37. Wang XN, Fan CQ, Yin S, Lin LP, Ding J, Yue JM (2008) Cytotoxic terpenoids from *Turraea pubescens*. Helv Chim Acta 91:510–519
38. Yoshikawa M, Hatakeyama S, Tanaka N, Matsuoka T, Yamahara J, Murakami N (1993) Crude drugs from aquatic plants. II. On the constituents of the rhizome of *Alisma orientale* Juzep. Originating from Japan, Taiwan, and China. Absolute stereostructures of 11-deoxyalisols B and B 23-acetate. Chem Pharm Bull 41:2109–2112
39. Van Zandt MC, Jagdmann GE, Whitehouse DL, Ji M, Savoy J, Potapova O, Cousido-Siah A, Mitschler A, Howard EI, Pyle AM, Podjarny AD (2019) Discovery of *N*-substituted 3-amino-4-(3-boronopropyl)pyrrolidine-3-carboxylic acids as highly potent third-generation inhibitors of human arginase I and II. J Med Chem 62:8164–8177
40. Stone EM, Glazer ES, Chantranupong L, Cherukuri P, Breece RM, Tierney DL, Curley SA, Iverson BL, Georgiou G (2010) Replacing $Mn^{2+}$ with $Co^{2+}$ in human arginase I enhances cytotoxicity toward L-arginine auxotrophic cancer cell lines. ACS Chem Biol 5:333–342
41. Cama E, Colleluori DM, Emig FA, Shin H, Kim SW, Kim NN, Traish AM, Ash DE, Christianson DW (2003) Human arginase II: crystal structure and physiological role in male and female sexual arousal. Biochemistry 42:8445–8451
42. Ilies M, Di Costanzo L, Dowling DP, Thorn KJ, Christianson DW (2011) Binding of α, α-disubstituted amino acids to arginase suggests new avenues for inhibitor design. J Med Chem 54:5432–5443