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Arginase II Inhibitory Activity of Phenolic Compounds from *Caesalpinia sappan* L.

Dao C. To[1], Manh H. Tran[2], Phi H. Nguyen[3], Nguyen T. Ai Nhung[4], Tu T. T. Nguyen[5]*

[1]*Phenikaa University Nano Institute (PHENA), Phenikaa University, Yen Nghia, Ha Dong, Hanoi 12116, Vietnam*
[2] *School of Medicine & Pharmacy, The University of Danang, Hoa Quy, Ngu Hanh Son, Da Nang City 550000, Vietnam*
[3]*Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet, Cau Giay district, Hanoi 122100, Vietnam*
[4] *Department of Chemistry, University of Sciences, Hue University, Hue 530000, Vietnam*
[5] *Faculty of Traditional Medicine, Hanoi Medical University, 01 Ton That Tung Street, Dong Da District, Hanoi, Vietnam*

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ABSTRACT

Inhibition of arginase from natural compounds has been proposed as a therapeutic approach for the treatment of cardiovascular diseases. Therefore, this study aims to evaluate the arginase inhibitory activity of isolated compounds. Based on bioactivity-guided fractionation, the ethyl acetate fraction was further subjected to column chromatography to obtain seven compounds (**1**–**7**). The chemical structures of the isolated compounds were determined by analyzing NMR spectral data and referring to data from the literature. The arginase II inhibitory activity of compounds **1**–**7** were investigated *in vitro* using an enzyme solution prepared from the kidney of anesthetized C57BL/6 mice. Compounds **1**, **2**, and **7** potentially inhibit arginase II activity, with IC_{50} values ranging from 8.1 to 14.2 µM, whereas the remaining compounds were inactive. This study is the first to assess compounds isolated from *C. sappan* heartwood with regard to their arginase inhibitory activity.

Keywords: *Caesalpinia sappan*, Leguminosae, phenolic, arginase, C57BL/6 mice.

Introduction

Arginase (L-arginine urea hydrolase, or amidinohydrolase – EC 3.5.3.1) is a metalloenzyme first described in 1904 by Kossel and Dakin in mammalian liver samples.[1] Arginase is present in two isoforms – arginase I and arginase II,[2] – both of which have been expressed in the vascular endothelium and shown to reciprocally regulate NO production.[3] Arginase inhibition in hypercholesterolemic – ApoE(-/-) – mice or arginase II deletion – ArgII(-/-) – mice restores endothelial vasorelaxant function, reduces vascular stiffness, and markedly reduces atherosclerotic plaque burden. Furthermore, arginase activation contributes to vascular changes, including polyamine-dependent vascular smooth muscle cell proliferation and collagen synthesis. Collectively, arginase may play a key role in the prevention and treatment of atherosclerotic vascular disease.[4]

In reviewing the literature to identify arginase inhibitors derived from natural sources, we found that the ethyl acetate fraction of *Caesalpinia sappan* L. displays appreciable inhibitory activity.[5] *Caesalpinia sappan* L. belonged to the *Caesalpinia* genus, in which this genus possessed antimalarial and antioxidant,[6,7] cytotoxic, anti-inflammatory, and leishmanicidal.[6,8,9] Some reports have shown that this genus contained phytochemicals like polyphenols, glycosides, terpenoids, saponins, and flavonoids.[7,10] *Caesalpinia sappan* L. (Leguminosae) is distributed in Southeast Asia, and its heartwood, sappan lignum, is well known as a red dyestuff. In Vietnam, *C. sappan* is called "To Moc" and is scattered in low mountainous areas; it is both light demanding and drought tolerant.[11]

*Corresponding author. E mail: thanhtu@hmu.edu.vn
Tel: +84-902031192

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C. sappan has been used in traditional Vietnamese medicine for the treatment of menstrual and postpartum hematometra, blood stasis due to trauma, dizziness, and postpartum blood loss. In addition, it has been employed in the therapeutic treatment of bloody dysentery, enteralgia, intestinal hemorrhage, and infectious diarrhea and for cleaning wounds.[11] Several reports have shown that the main components in *C. sappan* are phenolics, alkaloids, steroids, flavonoids, terpenoids, tannins, saponins, and fatty acids.[10,12,13] *C. sappan* possesses biological activity, including anti-inflammatory,[12,13] neuroprotective,[14] anticancer,[15-18] anti-termite,[19] antioxidant,[20] and antimicrobial activities.[21-24] Despite the extensive literature,[5] no study have investigated the arginase inhibitory activity of compounds isolated from *C. sappan* heartwood. Therefore, this paper describes the isolation of *C. sappan* compounds and evaluates their arginase inhibitory activity.

Materials and Methods

General experimental procedures

The nuclear magnetic resonance (NMR) spectra were obtained using a Varian Unity Inova 400 MHz spectrometer. Silica gel (Merck, 63–200 µm particle size), RP-18 silica gel (Merck, 75 µm particle size), and Sephadex LH-20 (Sigma–Aldrich, MO, USA) were used for column chromatography (CC). Thin layer chromatography (TLC) was carried out using Merck silica gel 60 F_{254} and RP-18 F_{254} plates. The compounds were visualized under UV radiation (254, 365 nm) and by spraying the plates with 10% H_2SO_4 followed by heating with a heat gun.

Plant materials

The heartwood of *C. sappan* was collected at Dak Lak province, Vietnam in June 2020. Botanical identification was performed by Nguyen Quoc Binh. PhD., Vietnam National Museum of Nature, Vietnam Academy of Science and Technology (VAST). A voucher specimen (CS-L-0712) was deposited with the Natural Product Research and Development Lab, Phenikaa University, Vietnam.

Extraction and isolation

C. sappan heartwood (4.0 kg) was extracted in triplicate (3 h × 3 L) with methanol (MeOH) refluxing at 60°C. After removal of the solvent under reduced pressure, the residue was suspended in water and then

partitioned successively with *n*-hexane and ethyl acetate (EtOAc). The resulting fraction was concentrated under decreased pressure to give *n*-hexane (15 g; yield 0.375%) and EtOAc (180 g; yield 4.5%) fractions. Following activity-guided fractionation,[5] the EtOAc-soluble fraction (180 g) was separated using silica gel CC and a stepwise gradient of CH_2Cl_2–MeOH (100:1 to 0:1, each solution 3.0 L in volume) to yield 11 fractions (E1–E11) based on their TLC profiles. Fraction E4 (1.5 g) was subjected to silica gel CC and eluted with a gradient of *n*-hexane–acetone (5:1 to 1:1) to obtain five fractions (E4.1–E4.5). Further purification of subfraction E4.4 (400 mg) by Sephadex LH-20 CC, eluting with a gradient of MeOH–H_2O (2:1), yielded compounds **1** (11 mg) and **7** (13 mg). Fraction E7 (3.6 g) was subjected to silica gel CC, using CH_2Cl_2–acetone (10:1 to 1:1) as the eluent, to obtain seven subfractions (E7.1–E7.7). Further purification of E7.5 (600 mg) using RP-18 silica gel CC, eluting with a gradient of MeOH–H_2O (1:2 to 2:1), yielded compounds **2** (28 mg), **4** (35 mg), and **6** (32 mg). Finally, fraction E8 (2.1 g) was subjected to silica gel CC, using CH_2Cl_2–MeOH (10:1 to 1:1) as the eluent, to obtain five subfractions (E8.1–E8.5). Further purification of E8.3 (220 mg) using RP-18 silica gel CC, eluting with a gradient of MeOH–H_2O (1:3 to 2:1), yielded **3** (65 mg) and **5** (11 mg).

Arginase II activity assay

Arginase II solution was prepared from kidney lysates of anesthetized C57BL/6 mice. Kidney tissue lysates were prepared using a lysis buffer (50 mM Tris-HCl, pH 7.5, 0.1 mM EDTA, and protease inhibitors) by homogenization at 4°C followed by centrifugation for 20 min at 14,000 × g and 4°C. Briefly, aortic lysates were added to Tris-HCl. The hydrolysis of L-arginine by arginase was performed by incubating the mixture containing activated arginase and was stopped by adding an acid solution. For the calorimetric determination of urea, α-isonitrosopropiophenone was added, and the mixture was heated at 100°C for 45 min. After placing the sample in the dark for 10 min at room temperature, the urea concentration was determined spectrophotometrically by measuring the absorbance at 550 nm and 25°C using a spectrophotometer (Synergy HT BioTeck).[3] The level of arginase activity was expressed as relative to the "100% arginase activity". The percentage of arginase inhibitory activity and IC_{50} values were evaluated as next described. A stock solution (70 mM) was prepared in DMSO and stored at −26°C for each compound. These stock solutions were extemporaneously and successively diluted in ultrapure H_2O to afford final concentrations in the wells of 1000, 300, 100, 30, 10, 3, and 1 µM, respectively. For the first screening, compounds were tested at final concentrations of 10 and 100 µM. Each solution was incubated with arginase for 1 h, as described above. The percentage of arginase inhibition was calculated by conversion of the resulting absorbance (relative to the absorbance of controls with only solvent ("100% arginase activity")) and plotted on a semilogarithmic scale. The IC_{50} values were estimated by nonlinear sigmoidal curve-fitting by using Prism.

Statistical analysis

All treatments were conducted in triplicate and the results are presented as the mean ± standard deviation (S.D). The statistical significance of all treatment effects was evaluated by Student's t-test with a probability limit for the significance of $p < 0.05$, $p < 0.001$.

Results and Discussion

Chemical structure identification

The methanol extract was partitioned with EtOAc to yield the EtOAc fraction. Based on bioactivity-guided fractionation,[5] the EtOAc fraction was subjected to CC on silica gel, C18-RP silica gel, and Sephadex LH-20 to obtain seven compounds (**1**–**7**; Figure 1).

*Characterisation of compounds **1**–**7***

*Caesalpiniaphenol B (**1**):* colourless amorphous powder; IR ν_{max} (KBr): 3448, 2941, 1745, 1740, 1427, 1257, 1169 cm^{-1}; UV (MeOH) λ_{max} nm: 284, 270, 226; ^{1}H-NMR (400 MHz, Methanol-d_4) δ_H (ppm): 4.30 (1H, d, *J* = 11.6 Hz, H-2a), 4.19 (1H, d, *J* = 11.6 Hz, H-2b), 7.72 (1H, d, *J* = 8.8 Hz, H-5), 6.55 (1H, br d, *J* = 8.8 Hz, H-6), 6.35 (1H, br s, H-8), 2.82 (1H, d, *J* = 14.8 Hz, H-9a), 2.71 (1H, d, *J* = 14.8 Hz, H-9b), 7.51 (1H, br s, H-2′), 4.98 (2H, overlap); ^{13}C-NMR (100 MHz, Methanol-d_4) δ_C (ppm): 74.8 (C-2), 73.3 (C-3), 194.2 (C-4), 113.4 (C-4a), 113.4 (C-4a), 130.7 (C-5); 112.5 (C-6), 167.0 (C-7), 103.6 (C-8), 164.9 (C-8a), 30.9 (C-9), 128.3 (C-1′), 152.3 (C-2′), 72.4 (C-3′), 177.1 (C-5′).[12]

*3-(4′-hydroxybenzyl)-3,4-dihydro-2″,3″-dimethyl-3H-[1,3]dioxolo[4,5-c]chromen-7-ol (**2**):* yellow amorphous solid; UV (MeOH) λ_{max} nm: 283, 276; ^{1}H-NMR (400 MHz, Methanol-d_4) δ_H (ppm): 3.78 (1H, d, *J* = 10.4 Hz, H-2a), 3.63 (1H, d, *J* = 10.4 Hz, H-2b), 4.61 (1H, s, H-4), 7.15 (1H, d, *J* = 8.4 Hz, H-5), 6.51 (1H, br d, *J* = 8.4 Hz, H-6), 6.42 (1H, br s, H-8), 2.81 (1H, d, *J* = 14.4 Hz, H-9a), 2.75 (1H, d, *J* = 14.4 Hz, H-9b), 7.01 (2H, d, *J* = 8.0 Hz, H-2′/H-6′), 6.75 (2H, d, *J* = 8.0 Hz, H-3′/H-5′), 1.35 (3H, s, H-2″), 1.24 (3H, s, H-3″); ^{13}C-NMR (100 MHz, Methanol-d_4) δ_C (ppm): 69.0 (C-2), 78.6 (C-3), 75.2 (C-4), 111.7 (C-4a), 133.7 (C-5), 110.7 (C-6), 160.1 (C-7), 104.0 (C-8), 157.1 (C-8a), 39.0 (C-9), 128.0 (C-1′), 132.9 (C-2′/C-6′), 115.8 (C-3′/C-5′), 157.0 (C-4′), 109.6 (C-1″), 29.0 (C-2″), 27.3 (C-3″).

*Protosappanin A dimethyl acetal (**3**)*: yellow powder; IR ν_{max} (KBr): 3300, 1602, 1446, 1348 cm^{-1}; UV (MeOH) λ_{max} nm: 284, 262; ^{1}H-NMR (400 MHz, Pyridine-d_5) δ_H (ppm): 7.31 (1H, d, *J* = 8.4 Hz, H-1), 6.94 (1H, dd, *J* = 8.4, 2.4 Hz, H-2), 7.05 (1H, d, *J* = 2.4 Hz, H-4), 4.64 (1H, d, *J* = 12.8 Hz, H-6*β*), 3.98 (1H, d, *J* = 12.8 Hz, H-6*α*), 3.23 (1H, d, *J* = 13.6 Hz, H-7*β*), 3.02 (1H, d, *J* = 13.6 Hz, H-7*α*), 7.43 (1H, s, H-8), 7.33 (1H, s, H-11a), 3.28 (6H, s, 6a-OCH_3); ^{13}C-NMR (100 MHz, Pyridine-d_5) δ_C (ppm): 135.2 (C-1), 111.6 (C-2), 160.0 (C-3), 108.0 (C-4), 158.7 (C-4a), 68.4 (C-6); 100.6 (C-6a), 37.8 (C-7), 127.3 (C-7a), 118.7 (C-8), 146.8 (C-9), 146.6 (C-10), 119.9 (C-11), 132.6 (C-11a), 132.6 (C-12), 120.9 (C-12a), 48.7 (6a-OCH_3).

*Caesalpiniaphenol F (**4**):* colorless needles; UV (MeOH) λ_{max} nm: 282, 274; ^{1}H-NMR (400 MHz, Methanol-d_4) δ_H (ppm): 3.98 (1H, d, *J* = 10.4 Hz, H-2a), 3.67 (1H, d, *J* = 10.4 Hz, H-2b), 3.68 (1H, s, H-4), 7.01 (1H, d, *J* = 8.0 Hz, H-5), 6.31 (1H, br d, *J* = 8.0 Hz, H-6), 6.35 (1H, br s, H-8), 2.63 (2H, br s, H-9), 6.71 (1H, br s, H-2′), 6.70 (1H, d, *J* = 8.0 Hz, H-5′), 6.55 (1H, d, *J* = 8.0 Hz, H-6′), 3.80 (3H, s, 3′-OCH_3), 3.35 (3H, s, 4-OCH_3); ^{13}C-NMR (100 MHz, Methanol-d_4) δ_C (ppm): 68.3 (C-2), 71.3 (C-3), 79.8 (C-4), 113.0 (C-4a), 133.6 (C-5); 109.0 (C-6), 160.6 (C-7), 104.0 (C-8), 156.0 (C-8a), 41.2 (C-9), 128.9 (C-1′), 115.5 (C-2′), 148.3 (C-3′), 146.2 (C-4′), 115.7 (C-5′), 124.4 (C-6′), 57.1 (4-OCH_3), 56.3 (3′-OCH_3).[13]

*α,2′,4,4′-tetrahydroxydihydrochalcone (**5**):* reddish powder; ^{1}H-NMR (400 MHz, Methanol-d_4) δ_H (ppm): 6.30 (1H, s, H-3), 6.37 (1H, d, *J* = 8.8 Hz, H-5), 7.72 (1H, d, *J* = 8.8 Hz, H-6), 5.18 (1H, dd, *J* = 7.2, 4.8 Hz, H-8), 3.04 (1H, dd, *J* = 14.0, 4.8 Hz, H-9*β*), 3.04 (1H, dd, *J* = 14.0, 7.6 Hz, H-9*α*), 7.03 (2H, d, *J* = 8.4 Hz, H-2′/H-6′), 6.69 (2H, d, *J* = 8.4 Hz, H-3′/H-5′); ^{13}C-NMR (100 MHz, Methanol-d_4) δ_C (ppm): 112.3 (C-1), 169.9 (C-2), 103.9 (C-3), 169.9 (C-4), 109.5 (C-5), 133.7 (C-6); 205.2 (C-7), 74.5 (C-8), 42.3 (C-9), 129.4 (C-1′), 131.6 (C-2′/C-6′), 116.2 (C-3′/C-5′), 157.2 (C-4′).

*3-(3′-methoxy-4′-hydroxybenzyl)-3,4-dihydro-2″,3″-dimethyl-3H-[1,3]dioxolo[4,5-c]chromen-7-ol (**6**):* yellow amorphous solid; UV (MeOH) λ_{max} nm: 283, 276; 225; ^{1}H-NMR (400 MHz, Methanol-d_4) δ_H (ppm): 3.77 (1H, d, *J* = 10.8 Hz, H-2a), 3.63 (1H, d, *J* = 10.8 Hz, H-2b), 4.59 (1H, s, H-4), 7.11 (1H, d, *J* = 8.4 Hz, H-5), 6.46 (1H, dd, *J* = 8.4, 2.4 Hz, H-6), 6.33 (1H, d, *J* = 2.4 Hz, H-8), 2.83 (1H, d, *J* = 14.4 Hz, H-9a), 2.76 (1H, d, *J* = 14.4 Hz, H-9b), 6.74 (1H, d, *J* = 1.6 Hz, H-2′), 6.70 (1H, d, *J* = 8.0 Hz, H-5′), 6.57 (1H, dd, *J* = 8.0, 1.6 Hz, H-6′), 1.35 (3H, s, H-2″), 1.23 (3H, s, H-3″), 3.78 (3H, s, 3′-OCH_3); ^{13}C-NMR (100 MHz, Methanol-d_4) δ_C (ppm): 69.3 (C-2), 78.8 (C-3), 75.3 (C-4), 111.7 (C-4a), 133.8 (C-5), 110.8 (C-6), 160.5 (C-7), 104.1 (C-8), 157.3 (C-8a), 39.5 (C-9), 128.7 (C-1′), 115.7 (C-2′), 148.5 (C-3′), 146.5 (C-4′), 115.9 (C-5′), 124.5 (C-6′), 109.7 (C-1″), 29.1 (C-2″), 27.4 (C-3″), 56.4 (3′-OCH_3).

*3′-deoxy-4-O-methylepisappanol (**7**):* reddish powder; UV (MeOH) λ_{max} nm: 284, 282; 224; ^{1}H-NMR (400 MHz, Methanol-d_4) δ_H (ppm): 4.09 (1H, d, *J* = 11.6 Hz, H-2a), 3.79 (1H, br d, *J* = 11.6 Hz, H-2b), 3.56 (1H, s, H-4), 6.93 (1H, d, *J* = 8.4 Hz, H-5), 6.31 (1H, dd, *J* = 8.4, 2.0 Hz, H-6), 6.25 (1H, d, *J* = 2.0 Hz, H-8), 2.89 (1H, d, *J* = 14.4 Hz, H-9a), 2.68 (1H, d, *J* = 14.4 Hz, H-9b), 7.12 (2H, d, *J* = 8.4 Hz, H-2′/H-6′), 6.72 (2H, d, *J* = 8.4 Hz, H-3′/H-5′), 3.30 (3H, s, 4-OCH_3); ^{13}C-NMR

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(100 MHz, Methanol-d_4) δ_C (ppm): 78.1 (C-2), 71.5 (C-3), 77.9 (C-4), 112.7 (C-4a), 133.6 (C-5), 108.3 (C-6), 159.9 (C-7), 103.8 (C-8), 157.1 (C-8a), 40.0 (C-9), 133.0 (C-1′), 128.2 (C-2′/C-6′), 115.9 (C-3′/C-5′), 156.5 (C-4′), 56.2 (4-OCH_3).

The ^{1}H- and ^{13}C-NMR spectra of **1** show typical resonance splitting patterns, with a pair of doublets at δ_H 4.30 and 4.19 (each 1H, d, J = 11.6 Hz), a benzyl methylene signal (H-9) at δ_H 2.82 and 2.71 (each 1H, d, J = 14.8 Hz), a ketone at δ_C 194.2 (C-4), and a quaternary carbon at δ_C 74.3 (C-3), as shown in Figure 1, indicating that **1** is a 3-hydroxy-3-benzyl-4-chromanone-type homoisoflavonoid.[12,13] The resonances ascribed to H-5 at δ_H 7.72 (d, J = 8.8 Hz), H-6 at δ_H 6.55 (br d, J = 8.8 Hz), and H-8 at δ_H 6.35 (br s, H-8) are located on ring A. Diagnostic signals for the unsaturated furanone portion of **1** arise from the olefinic proton at δ_H 7.51 (br s, H-2′) and the methylene proton at δ_H 4.98 (2H, overlap) in the ^{1}H-NMR spectrum and from the olefinic carbons at δ_C 128.3 (C-1′) and 152.3 (C-2′), a methylene carbon at δ_C 72.4 (C-3′), and the ketone at δ_C 177.1 (C-5′) on ring B in the ^{13}C-NMR spectrum (Figure 1). By comparing the ^{1}H- and ^{13}C-NMR data of this compound with those published in the literature, compound **1** was identified as caesalpiniaphenol B.[12]

The ^{1}H-NMR spectra of compounds **2**, **4**, and **6** exhibit typical signals due to the aromatic protons of two benzene rings (rings A and B), methylene protons (H-2 and H-9), and oxygenated methine protons (H-4), while their ^{13}C-NMR spectra reveal the signals of 12 sp^2 carbons of two benzene rings, a methylene carbon (C-9), and three oxygenated carbons (C-2, C-3, and C-4), as shown in Figure 1, indicating that **2**, **4**, and **6** possess a chroman-4-one skeleton.[13,25] A comprehensive analysis of the ^{1}H- and ^{13}C-NMR spectra of **2** reveals that it gives rise to signals for seven aromatic protons, four oxygenated carbons (C-2, C-3, C-4, and C-8a), and a dioxodimethyl group (C-1″, C-2″, and C-3″; Figure 1). Similar to **2**, compound **6** possesses four oxygenated carbons and a dioxodimethyl group (C-1″, C-2″, and C-3″) but contains six aromatic protons and a methoxy carbon at C-3′. Compound **4** possesses six aromatic protons and four oxygenated carbons (C-2, C-3, C-4, and C-8a), similar to **6**, and also has two methoxy carbons at C-4 and C-3′ (Figure 1). By comparing the ^{1}H- and ^{13}C-NMR data of these compounds with those published in the literature, the structures of **2**, **4**, and **6** were identified as 3-(4′-hydroxybenzyl)-3,4-dihydro-2″,3″-dimethyl-3*H*-[1,3]dioxolo[4,5-c]chromen-7-ol,[25] caesalpiniaphenol F,[13] and 3-(3′-methoxy-4′-hydroxybenzyl)-3,4-dihydro-2″,3″-dimethyl-3*H*-[1,3]dioxolo[4,5-c]chromen-7-ol,[25] respectively.

Compound **3** was isolated as a yellow powder and its ^{1}H-NMR spectrum reveals signals for two trisubstituted benzene rings – δ_H 7.31 (H-1), 6.94 (H-2), 7.05 (H-4), 7.43 (H-8), and 7.33 (H-11a); two methylene groups – δ_H 4.64 (H-6β), 3.98 (H-6α), 3.23 (H-7β), and 3.02 (H-7α); and two methoxy groups – δ_H 3.28 (6H, s, 6a-OCH_3). The ^{13}C-NMR spectrum of **3** shows 17 carbon signals due to 12 sp^2 carbons of two benzene rings and signals for two methylene (C-6 and C-7) and two methoxy – δ_C 48.7 (6a-OCH_3) – carbons (Figure 1). According to this data, compound **3** was identified as protosappanin A dimethyl acetal after a comparison with reported data.[26]

Compound **5** was obtained as a reddish powder. The presence of protons and carbons at δ_H 5.18 (1H, dd, J = 7.2, 4.8 Hz, H-8)/δ_C 74.5 (C-8), δ_H 3.04 (1H, dd, J = 14.0, 4.8 Hz, H-9β), δ_H 3.04 (1H, dd, J = 14.0, 7.6 Hz, H-9α), and δ_C 42.3 (C-9) and a ketone carbon at δ_C 205.2 (C-9) in the ^{1}H- and ^{13}C-NMR spectra indicate that **5** is a dihydrochalcone (Figure 1).[27] A detailed analysis of the compound's spectra revealed a 1,2,4-trisubstituted benzene ring characterized by an ABX system, a 1,4-disubstituted benzene ring characterized by an A_2B_2 system, seven aromatic protons (H-3, H-5, H-6, H-2′/H-6′, and H-3′/H-5′), and 12 sp^2 carbons of two benzene rings (Figure 1). Thus, compound **5** was identified as α,2′,4,4′-tetrahydroxydihydrochalcone after comparing the NMR data with that in the literature.[27]

Compound **7** was isolated as a reddish powder, and its ^{1}H-NMR spectrum shows signals for a trisubstituted benzene ring – δ_H 6.93 (H-5), 6.31 (H-6), and 6.25 (H-8); an A_2B_2 spin system – δ_H 7.12 (H-2′/H-6′) and 6.72 (H-3′/H-5′); a methoxy group – δ_H 3.30 (4-OCH_3); and a methine proton – δ_H 3.56 (H-4), as depicted in Figure 1. The ^{13}C-NMR spectrum of **7** exhibits 16 carbon signals attributable to 12 sp^2 carbons of two benzene rings (rings A and B) and signals for two methylene carbons – δ_C 78.1 (C-2) and 40.0 (C-9); two oxygenated carbons – δ_C 71.5 (C-3) and 77.9 (C-4); and a methoxy carbon – δ_C 56.2 (4-OCH_3), as indicated in Figure 1. Based on this evidence and comparisons with published data, **7** was identified as 3′-deoxy-4-*O*-methylepisappanol.[28]

Anti-arginase II activity

Arginase II activity is upregulated in atherosclerosis-prone mice and is associated with impaired endothelial NO production; endothelial dysfunction; vascular stiffness; and, ultimately, aortic plaque development. Conversely, inhibiting endothelial arginase or deleting the arginase II gene enhances NO production, restores endothelial function and aortic compliance, and reduces plaque burden.

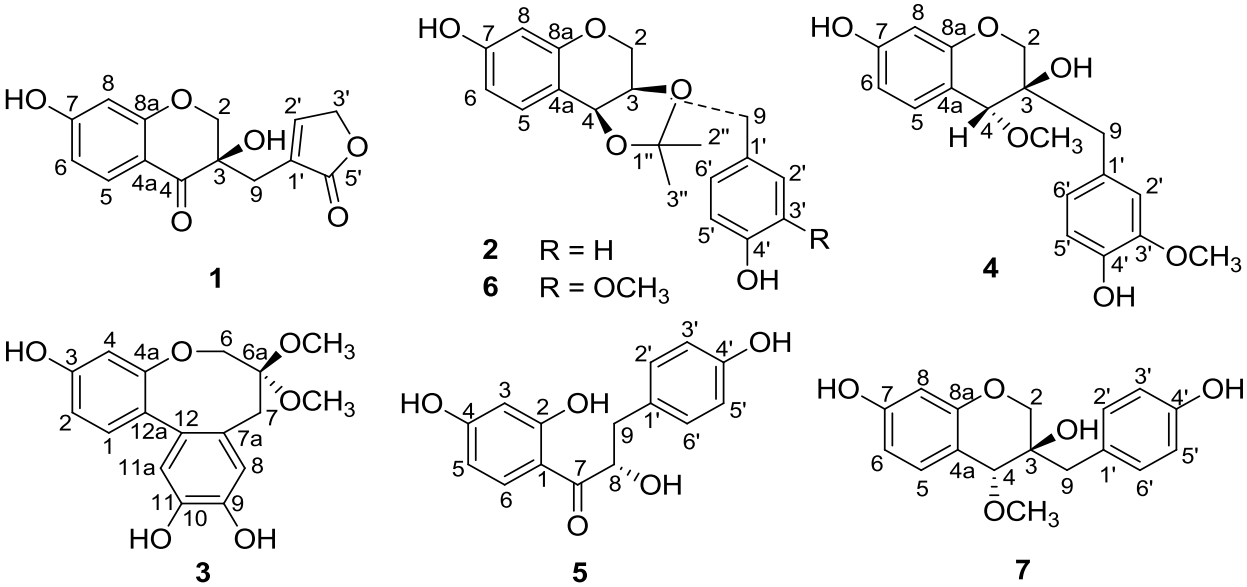

Figure 1: Chemical structures of isolated compounds (**1**–**7**)

Table 1: Arginase II inhibitory activity of isolated compounds (1–7).

Compounds	IC_{50} values (μM)[a]
1	14.2 ± 2.4
2	8.1 ± 1.3
3	> 100
4	> 100
5	> 100
6	> 100
7	12.6 ± 2.2
PG[b]	1.0 ± 0.1

[a] The inhibitory effects are represented as the molar concentration (μM) giving 50% inhibition (IC_{50}) relative to the vehicle control. These data represent the average values of three repeated experiments.
[b] Piceatannol-3′-*O*-*β*-D-glucopyranoside (PG) was used as positive control.

Therefore, arginase II represents a novel target for preventing and treating atherosclerotic vascular disease.[4] In the present study, we screened the isolated compounds for anti-arginase II activity. The results indicated that compounds **1**, **2**, and **7** significantly inhibits arginase II activity, with IC_{50} values of 14.2 ± 2.4, 8.1 ± 1.3, and 12.6 ± 2.2 μM, respectively, whereas the remaining compounds appear inactive. In this study, piceatannol-3′-O-*β*-D-glucopyranoside (PG), used as a positive control, had an IC_{50} value of 1.0 μM (Table 1).[4]
Several researchers have reported the inhibition of natural products in relation to arginase activity. For example, Lim *et al.* isolated 10 compounds from *Saururus chinensis* and screened the isolated compounds for anti-arginase II activity; compounds 7-hydroxysauchinone and sauchinone significantly inhibited arginase II activity, with IC_{50} values of 89.6 and 61.4 μM, respectively.[29] In another study, Kim *et al*. tested eight flavonoid-type substances isolated from a methanol extract of *Scutellaria indica* L. in relation to arginase II from mouse kidney homogenate; the results revealed that compounds (2*S*)-5,7-dihydroxy-8,2′-dimethoxyflavanone and (2*S*)-5,2′,5′-trihydroxy-7,8-dimethoxyflavanone significantly inhibited arginase II activity with IC_{50} values of 25.1 and 11.6 μM, respectively.[30] In addition, the administration of piceatannol-3′-*O*-*β*-D-glucopyranoside (~500 μg/mouse/day for 6 weeks) has also improved endothelial dysfunction in an animal model of hyperlipidemia via arginase inhibition.[31] *Bryophyllum pinnatum* is used in tropical Africa for the treatment of several diseases, such as kidney and urinary tract disorders. In another study, 25 mg/kg body weight aqueous extracts of *B. pinnatum* (AEBP) and 50 mg/kg body weight AEBP inhibited arginase II in addition to increasing the antioxidant status in CCl_4-intoxicated rats. These results suggest that *B. pinnatum* can protect the kidney against CCl_4-induced oxidative damage.[32] Arraki *et al.* also reported that the polyphenolic-enriched extracts from the two species *Cyperus glomeratus* and *Cyperus thunbergii*, together with five compounds (thunbergin A, *trans*-scirpusin A, aureusidin, *trans*-cyperusphenol A, and luteolin) isolated from these species and the methanol extract of *Morus alba* leaves (containing ellagic acid, luteolin-7-glucoside, luteolin-7-diglucoside, and luteolin), showed significant inhibition of arginase.[33,34] Another study demonstrated that *Syzygium cumini* leaves with the highest phenolic content and gallic acid concentration showed the highest arginase inhibitory and antioxidant activity.[35]
Arginase inhibitory activity has also been reported with regard to the genus *Caesalpinia*. Specifically, the ethyl acetate extract of *C. sappan* was evaluated in relation to arginase II from the kidney lysate of C57BL/6 mice and from HUVEC cells. The results indicated residual arginase II activity (31%) at the highest concentration of the extract used (50 μg/mL), and the calculated IC_{50} value was 36.82 μg/mL.[5] On the other hand, the methanol extract of the stem bark of *C. ferrea* (containing flavonoids, tannins, saponins, and steroids) showed 12.81% arginase inhibitory activity at a concentration of 100 μg/mL, indicating that *C. ferrea* extract does not serve as a potential substance to inhibit arginase.[36] Ethyl acetate and methanol stem bark extracts of *C. tortuosa* (containing saponins, tannins, and flavonoids) have been shown to inhibit the activity of arginase, with IC_{50} values of 33.81 and 11.58 μg/mL, respectively, with nor-NOHA acetate, as a standard drug, inhibiting arginase with an IC_{50} of 13.77 μg/mL.[37] In another study, the ethyl acetate and methanol bark extracts of *C. coriaria* displayed low arginase inhibitory activity at 14.43 and 33.59%, respectively, at a concentration of 100 μg/mL.[38] Although studies on the arginase inhibitory activity of the genus *Caesalpinia* have been conducted,[5,29-38] the anti-arginase II activity of compounds isolated from *C. sappan* has not been investigated to date. Our study shows that compounds **1**, **2**, and **7** potentially inhibit arginase II activity, with IC_{50} values ranging from 8.1 to 14.2 μM (Table 1).

Conclusion

Based on bioassay-guided extraction and isolation, seven natural compounds, including caesalpiniaphenol B (**1**), 3-(4′-hydroxybenzyl)-3,4-dihydro-2′′,3′′-dimethyl-3*H*-[1,3]dioxolo[4,5-c]chromen-7-ol (**2**), protosappanin A dimethyl acetal (**3**), caesalpiniaphenol F (**4**), α,2′,4,4′-tetrahydroxydihydrochalcone (**5**), 3-(3′-methoxy-4′-hydroxybenzyl)-3,4-dihydro-2′′,3′′-dimethyl-3*H*-[1,3]dioxolo[4,5-c]chromen-7-ol (**6**), and 3′-deoxy-4-*O*-methylepisappanol (**7**), were isolated from the heartwood of *C. sappan*. Compounds **1**, **2**, and **7** showed the most potent inhibitory activities against arginase II, with IC_{50} values of 14.2 ± 2.4, 8.1 ± 1.3, and 12.6 ± 2.2 μM, respectively, whereas the remaining compounds were inactive. These results suggest that the active constituents from *C. sappan* can prove useful in the research and development of anti-arginase agents.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Ivanenkov YA, Chufarova NV. Small-molecule arginase inhibitors. Pharm Pat Anal. 2014; 3(1):65-85.
2. Jenkinson CP, Grody WW, Cederbaum SD. Comparative properties of arginases. Comp Biochem Phys. 1996; 114(1):107-132.
3. White AR, Ryoo S, Li D, Champion HC, Steppan J, Wang D, Nyhan D, Shoukas AA, Hare JM, Berkowitz DE. Knockdown of arginase I restores NO signaling in the vasculature of old rats. Hypertension. 2006; 47(2):245-251.
4. Ryoo S, Bhunia A, Chang F, Shoukas A, Berkowitz DE, Romer LH. OxLDL-dependent activation of arginase II is dependent on the LOX-1 receptor and downstream RhoA signaling. Atherosclerosis. 2011; 214(2):279-287.
5. Shin W, Cuong TD, Lee JH, Min B, Jeon BH, Lim HK, Ryoo S. Arginase inhibition by ethylacetate extract of *Caesalpinia sappan* Lignum contributes to activation of endothelial nitric oxide synthase. Korean J Physiol Pharmacol. 2011; 15(3):123-128.
6. Pournaghi N, Khalighi-Sigaroodi F, Safari E, Hajiaghaee R. A review of the genus *Caesalpinia* L.: emphasis on the cassane and norcassane compounds and cytotoxicity effects. J Med Plants. 2020; 19(76):1-20.
7. Ogbeide O, Okhomina OK, Omoregie IG, Unuigbe CA, Augustine I, Isacc A, Iheanacho M, Akubuiro P, Solomon A, Irabor EEI, Owolabi JB, Falodun A. Antimalarial, ferric reducing antioxidant power and elemental analysis of *Caesalpinia pulcherrima* leaf extract. J Chem Soc Nigeria. 2020; 45(4):704 -711.

8. Erharuyi O, Adhikari A, Falodun A, Aimas J, Ahmmad M, Rehan I, Choudhary MI. Cytotoxic, anti-inflmmatory, and leishmanicidal activities of diterpenes isolated from the roots of *Caesalpinia pulcherrima*. Planta Med. 2017; 83(1-02):100-110.
9. Erharuyi O, Adhikari A, Falodun A, Imad R, Choudhary MI. Derivatization of cassane diterpenoids from *Caesalpinia pulcherrima* (L.) Sw. and evaluation of their cytotoxic and leishmanicidal activities. Tetrahedron Lett. 2016; 57(20):2201-2206.
10. Sadaf S, Erharuyi O, Falodun A, Choudhary MI, Sammer Y. Crystal structure of (3*S**,4*S**,4a*S**,5R*,6*R**,6a*S**,7*R**,11a*S**,11b*R**)-5,6-bis-(benzoyloxy)-3,4a-dihydroxy-4,7,11btrimethyl-1,2,3,4,4a,5,6,6a,7,11,11a,-11b-dodecahydrophenanthro[3,2-b]-furan-4-carboxylic acid methanol monosolvate. Acta Crystallogr. 2015; E71:739-740.
11. Bich DH. Selected Medicinal Plants in Vietnam. (1st ed.). Hanoi: Science and Technology Publishing House; 1999. 151-154 p.
12. Cuong TD, Hung TM, Kim JC, Kim EH, Woo MH, Choi JS, Lee JH, Min BS. Phenolic compounds from *Caesalpinia sappan* heartwood and their anti-inflammatory activity. J Nat Prod. 2012; 75(12):2069-2075.
13. Min BS, Cuong TD, Hung TM, Min BK, Shin BS, Woo MH. Compounds from the heartwood of *Caesalpinia sappan* and their anti-inflammatory activity. Bioor Med Chem Lett. 2012; 22(24):7436-7439.
14. Wan YJ, Xu L, Song WT, Liu YQ, Wang LC, Zhao MB, Jiang Y, Liu LY, Zeng KW, Tu PF. The ethanolic extract of *Caesalpinia sappan* heartwood inhibits cerebral ischemia/reperfusion injury in a rat model through a multi-targeted pharmacological mechanism. Front Pharmacol. 2019; 10:29.
15. Hung TM, Hai NX, Nhan NT, Quang TT, Quan TL, Cuong TD, Dang NH, Dat NT. Cytotoxic activity of new phenolics compounds from Vietnamese *Caesalpinia sappan*. Biosci Biotechnol Biochem. 2013; 77(12):2378-2382.
16. Widodo N, Puspitarini S, Widyananda MH, Alamsyah A, Wicaksono ST, Masruri M, Jatmiko DW. Anticancer activity of *Caesalpinia sappan* by downregulating mitochondrial genes in A549 lung cancer cell line. F1000Res. 2022; 11:169.
17. Bukke AN, Hadi FN, Babu KS, Shankar PC. In vitro studies data on anticancer activity of *Caesalpinia sappan* L. heartwood and leaf extracts on MCF7 and A549 cell lines. Data Brief. 2018; 19:868-877.
18. Astirin OP, Prayitno A, Artanti AN, Herawati E, Dewi AR, Aryani VR, Dewangga VS. Synergistic cytotoxic effect of *Anonna muricata* and *Caesalpinia sappan* nanoparticles via expression of BAD pro-apoptotic protein in HeLa cervical cancer cells. Trop J Nat Prod Res. 2021; 5(12):2108-2114.
19. Acero LH, Cruz MEP, Tuy FS. Termiticidal potential of sappan (*Caesalpinia sappan*) seeds. Int J Chem Eng Appl. 2018; 9(6):216-220.
20. Laksmiani NPL, Leliqia NPE, Armita PMN, Arijana NIGK, Saputra AABI, Prananingtyas KI. In-silico and In-vitro studies of antioxidant and sun protection activities of Sappan wood (*Caesalpinia sappan* L.). Trop J Nat Prod Res. 2020; 4(12):1072-1080.
21. Yim DG, Seo JK, Yum HW, Zahid MA, Park JY, Parvin R, Go J, Jin SK, Koo OK, Yang HS. Effects of *Caesalpinia sappan* L. extract on the color stability, antioxidant and antimicrobial activity in cooked pork sausages during cold storage. LWT Food Sci Technol. 2019; 112:108235.
22. Puttipan R, Chansakaow S, Khongkhunthian S, Okonogi S. *Caesalpinia sappan*: A promising natural source of antimicrobial agent for inhibition of cariogenic bacteria. Drug Discov Ther. 2018; 12(4):197-205.
23. Panthong S, Sakpakdeejaroen I, Kuropakornpong P, Jaicharoensub J, Itharat A. Antibacterial activity and stability evaluation of "Apo-taat" remedy extract for inhibiting diarrhoea-causing bacteria. Trop J Nat Prod Res. 2020; 4(12):1101-1107.
24. Pattananandecha T, Apichai S, Julsrigival J, Ogata F, Kawasaki N, Saenjum C. Antibacterial activity against foodborne pathogens and inhibitory effect on anti-inflammatory mediators production of brazilin-enriched extract from *Caesalpinia sappan* Linn. Plants (Basel). 2022; 11(13):1698.
25. Namikoshi M, Nakata H, Yamada H, Nagai M, Saitoh T. Homoisoflavonoids and related compounds: II. Isolation and absolute configurations of 3,4-dihydroxy lated homoisoflavans and brazilins from *Caesalpinia sappan* L. Chem Pharm Bull. 1987; 35(7):2761-2773.
26. Nguyen MTT, Awale S, Tezuka Y, Tran QL, Kadota S. Xanthine oxidase inhibitors from the heartwood of Vietnamese *Caesalpinia sappan*. Chem Pharm Bull. 2005; 53(8):984-988.
27. Li F, Awale S, Tezuka Y, Kadota S. Cytotoxic constituents from Brazilian red propolis and their structure–activity relationship. Bioorg Med Chem. 2008; 16(10):5434-5440.
28. Namikoshi M, Nakata H, Nuno M, Ozawa T, Saitoh T. Homoisoflavonoids and related compounds. III. Phenolic constituents of *Caesalpinia japonica* SIEB. et ZUCC. Chem Pharm Bull. 1987; 35(9):3568-3575.
29. Lim CJ, Cuong TD, Hung TM, Ryoo SW, Lee JH, Kim EH, Woo MH, Choi JS, Min BS. Arginase II inhibitory activity of phenolic compounds from *Saururus chinensis.* Bull Korean Chem Soc. 2012; 33(9):3079-3082.
30. Kim SW, Cuong TD, Hung TM, Ryoo S, Lee JH, Min BS. Arginase II inhibitory activity of flavonoid compounds from *Scutellaria indica*. Arch Pharm Res. 2013; 36(8):922-926.
31. Woo A, Shin W, Cuong TD, Min B, Lee JH, Jeon BH, Ryoo S. Arginase inhibition by piceatannol-3′-O-β-D-glucopyranoside improves endothelial dysfunction via activation of endothelial nitric oxide synthase in ApoE-null mice fed a high-cholesterol diet. Int J Mol Med. 2013; 31(4):801-810.
32. Anadozie SO, Akinyemi JA, Agunbiade S, Ajiboye BO, Adewale OB. *Bryophyllum pinnatum* inhibits arginase II activity and prevents oxidative damage occasioned by carbon tetrachloride (CCl4) in rats. Biomed Pharmacother. 2018; 101:8-13.
33. Arraki K, Totoson P, Decendit A, Zedet A, Maroilley J, Badoc A, Demougeot C, Girard C. Mammalian arginase inhibitory activity of methanolic extracts and isolated compounds from *Cyperus* species. Molecules. 2021; 26(6):1694.
34. Arraki K, Totoson P, Attia R, Zedet A, Pudlo M, Messaoud C, Demougeot C, Girard C. Arginase inhibitory properties of flavonoid compounds from the leaves of Mulberry (*Morus alba*, Moraceae). J Pharm Pharmacol. 2020; 72(9):1269-1277.
35. Hidayati AA, Sauriasari R, Elya B. Arginase inhibitory and antioxidant activities in *Syzygium cumini* (L.) Skeels leaves extracts collected from three different locations of java. Pharm Sci Asia. 2020; 47(1):65-73.
36. Indriani D, Elya B, Noviani A. Arginase inhibitory activity and total flavonoid content on *Caesalpinia ferrea* C. Mart stem bark extracts. Pharmacogn J. 2018; 10(6):1180-1183.
37. Najid A, Elya B, Noviani A. Arginase inhibitory activity of stem bark extracts of *Caesalpinia tortuosa* Roxb. Int J Appl Pharm. 2018; 10(1):130-132.
38. Wulansari A, Elya B, Noviani A. Arginase inhibitory and antioxidant activities of *Caesalpinia coriaria* (Jacq.) Willd. bark extract. Pharmacogn J. 2018; 10(6):1174-1179.