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Original Article

Chemical constituents from heartwoods of *Caesalpinia sappan* with antiplatelet aggregation activities

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ABSTRACT

Objective: To clarify the active constituents of the heartwoods of *Caesalpinia sappan*, a traditional Chinese medicine with the functions of promoting blood circulation (Huoxue in Chinese) and removing blood stasis (Quyu in Chinese).
Methods: The chemical constituents were isolated and purified by combination of silica gel and Sephadex LH-20 column chromatography, along with semipreparative HPLC. Their chemical structures were established by multiple spectroscopic methods and comparison with literature data. The *in vitro* antiplatelet aggregation activities were evaluated using mouse platelet induced by AYPGKF-NH_2, a gold agonist of protease-activated receptor 4 (PAR4).
Results: Two new phenols, methyl 2-(4,4′,5′-trihydroxy-2′-(methoxymethyl) biphenyl-2-yloxy) acetate (**1**) and 1′-methylcaesalpin J (**2**), together with 24 known compounds (**3**–**26**), were isolated from the heartwoods of *C. sappan*. Among them, sappanchalcone (**16**) and brazilin (**20**) showed inhibitory activities against mouse platelet aggregation with IC_{50} values of 114.8 μmol/L and 100.8 μmol/L, respectively.
Conclusion: Antiplatelet compounds from *C. sappan* targeting at PAR4 are reported for the first time.

1. Introduction

Thrombotic disorders are a major cause of morbidity and mortality throughout the world (McFadyen, Schaff & Peter, 2018). Antiplatelet therapies are an essential tool against thrombotic diseases. However, mechanism-based bleeding events have restricted the use of higher doses of standard antiplatelet agents (Wong et al., 2017). Emerging evidence shows that protease-activated receptor 4 (PAR4) is a promising target for antiplatelet therapy (Rudinga, Khan & Kong, 2018). For example, a PAR4 inhibitor, BMS-986120, showed a low bleeding liability and a markedly wider therapeutic window compared to clopidogrel tested in the same nonhuman primate model (Wong et al., 2017).

Traditional Chinese medicines (TCM) with functions of promoting blood circulation (Huoxue in Chinese) and removing blood stasis (Huayu or Quyu in Chinese) are claimed to be useful in antiplatelet therapies and the treatment of thrombotic diseases (Xia et al., 2018; Xia, Wang & Wang, 2016; Zhuo et al., 2016). The dried heartwoods of *Caesalpinia sappan* L. (Leguminosae), known as Sumu in Chinese with the functions of Huoxue and Quyu, is recorded in the Pharmacopoeia of the People's Republic of China (The Pharmacopoeia Commission, 2015). *C. sappan* is cultivated in Fujian, Guangdong, Guangxi, Guizhou, Hainan, Sichuan, Taiwan, and Yunnan of China. It is also found in Cambodia, India, Laos, Malaysia, Myanmar, Sri Lanka, and Vietnam of Asia, along with Africa and America (Chen, Zhang & Ding, 2010). It contains several kinds of phenolic compounds, such as xanthones, coumarins, chalcones, flavones, homoisoflavonoids, and brazilins (Nirmal, Rajput, Prasad & Ahmad, 2015). In a screening for antiplatelet agents from TCM, the 90% ethanolic extracts of *C. sappan* heartwoods showed an inhibitory activity against mouse platelet aggregation induced by 100 mol/L of AYPGKF-NH_2 (H–Ala–Tyr–Pro–Gly–Lys–Phe–NH_2), with an inhibition of 36% at a concentration of 150 μg/mL. AYPGKF-NH_2 is thought to be a gold agonist of PAR4. To clarify the bioactive constituents from the heartwoods of *C. sappan*, two new phenols (**1** and **2**), along with 24 known compounds (**3** – **26**) (Fig. 1), were isolated and identified the ethanolic extracts

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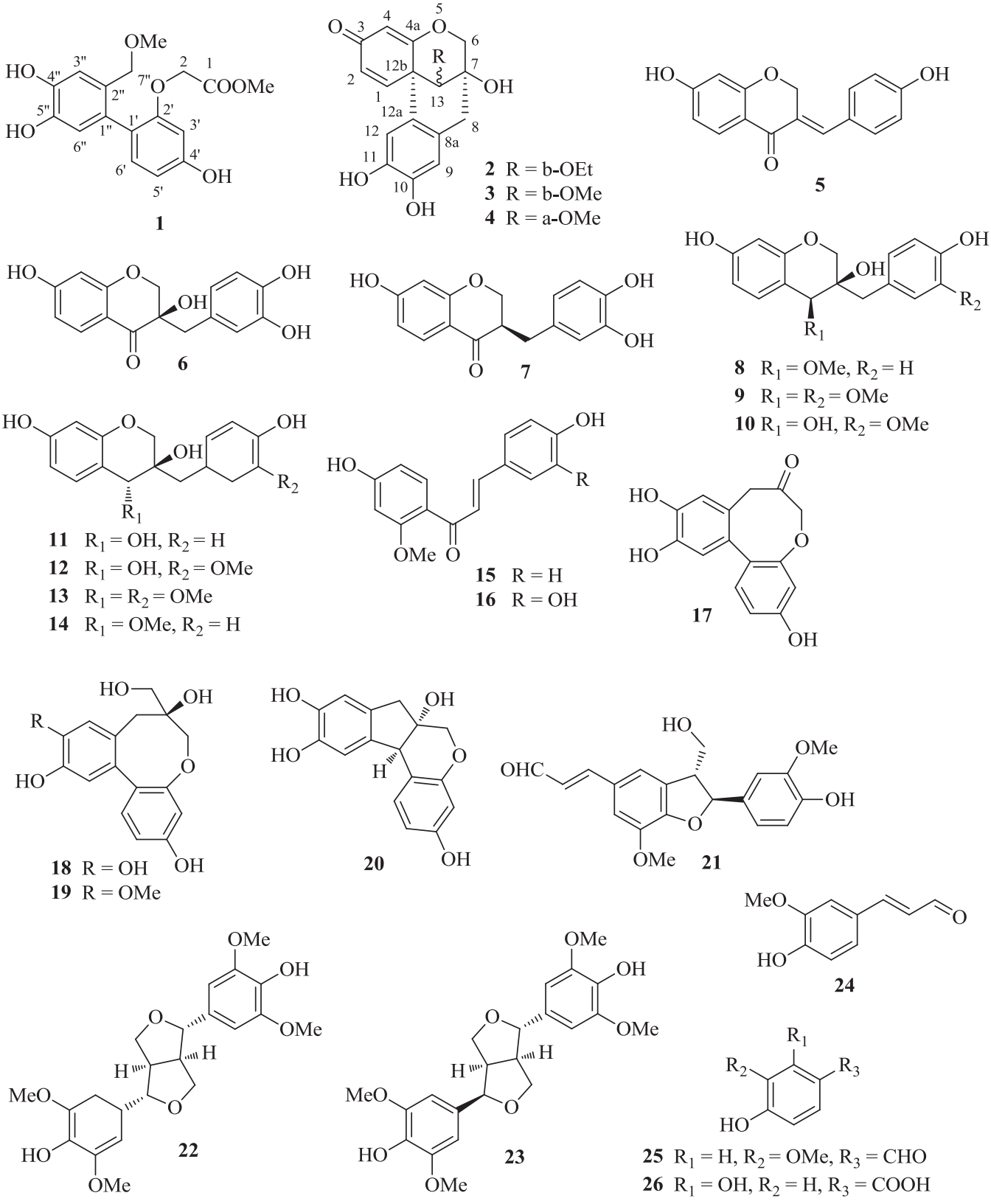

Fig. 1. Chemical structures of compounds **1–26** from *C. sappan*.

of the plant. The structural elucidation of these new compounds and the results of antiplatelet aggregation bioassay are reported.

2. Materials and methods

2.1. General

Melting points were determined using a WRX-4 Melting-point Apparatus (Shanghai YiCe Apparatus & Equipment Co., Ltd, uncorrected). UV spectra were taken on a Shimadzu UV-2401PC spectrophotometer (Shimadzu, Kyoto, Japan). Electronic circular dichroism (ECD) spectra were recorded on a Chirascan CD spectrometer (Applied Photophysics Ltd., Leatherhead, UK). IR spectra were measured on a Bruker Tensor 27 FTIR Spectrometer (Bruker Corp., Ettlingen, Germany) with KBr disks. ^{1}H and ^{13}C NMR spectra were collected on a Bruker AM-400, DRX-500 and Avance III-600 spectrometers (Bruker Bio-Spin GmbH, Rheinstetten, Germany) with TMS as an internal standard. ESI-MS and HR-ESI-MS analyses were performed on an API QSTAR Pulsar 1 spectrometer (Applied Biosystems/MDS Sciex, Foster City, CA, USA.). Silica gel G (Qingdao Meigao Chemical Co., Ltd., Qingdao, China), C_{18} silica gel (Fuji Silysia Chemical Ltd., Aichi, Japan), and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) were used for column chromatography, and silica gel GF_{254} (Qingdao Meigao Chemical Co.) on precoated plates was used for thin layer chromatography. Semipreparative HPLC was performed on an Agilent 1200 series pump (Agilent Technologies, Santa Clara, USA.) equipped with a diode array detector and an Agilent Zorbax SB-C_{18} column (5.0 μm, ϕ 9.4 × 250 mm) or a Welch Ultimate AQ-C_{18} column (5 μm, ϕ 4.6 × 300 mm, Welch Materials Inc., Shanghai, China).

2.2. Plant material

The heartwoods of *C. sappan* were collected from Jingxi County, Guangxi, China, in June 2008, and were authenticated by Prof. Chun-Lin Long at Minzu University of China, Beijing, China. The voucher specimens (jx080610) were deposited at the Key Laboratory of Economic Plants and Biotechnology, Kunming Institute of Botany, Chinese Academy of Sciences.

2.3. Extraction and isolation

The dried, powdered heartwoods of *C. sappan* (12.2 kg) were ultrasonically extracted for 30 min with 90% EtOH at 60 °C. The solvent was concentrated to provide the crude extracts (1.06 kg), which were suspended in water and then successively extracted with petroleum ether, ethyl acetate, and *n*-butanol.

The EtOAc-soluble part (935.7 g) was subjected to silica gel column chromatography (CH_2Cl_2-MeOH, 1:0→0:1) to yield fractions 1–12. Fr. 2 (914.4 mg) was separated on an RP-18 silica gel column eluted with MeOH–H_2O (50%→100%), Sephadex LH-20 column chromatography (MeOH), and semipreparative HPLC [Agilent Zorbax SB-C_{18}, MeOH–H_2O (contains 0.5% TFA), 30:70, 2 mL/min] to yield **25** (59.5 mg, t_R = 20.857 min) and **24** (6.9 mg, t_R = 38.676 min).

Fr. 4 (20.9 g) was separated on an RP-18 silica gel column eluted with MeOH–H_2O (10%→100%) to yield 12 subfractions (Fr. 4.1–Fr. 4.12). Fr. 4.2 (1.3 g) was recrystallized from MeOH to yield **17** (384.0 mg). Fr. 4.4 (3.1 g) was separated on Sephadex LH-20 column chromatography (MeOH) to yield five further fractions (Fr. 4.4.1–Fr. 4.4.5). Fr. 4.4.3 (201.5 mg) was purified by silica gel column chromatography (CH_2Cl_2-acetone, 30:1) and semipreparative HPLC (Agilent Zorbax SB-C_{18}, CH_3CN-H_2O, 16:84, 2 mL/min) to yield **2** (20.2 mg, t_R = 26.460 min) and **1** (16.9 mg, t_R = 49.278 min). Fr. 4.4.4 (138.7 mg) was purified by silica gel column chromatography (CH_2Cl_2–MeOH, 10:1) and semipreparative HPLC (Welch Ultimate AQ-C_{18}, CH_3CN–H_2O, 30:70, 1 mL/min) to yield **8** (6.3 mg, t_R = 15.492 min). Fr. 4.4.5 (300.9 mg) was purified by silica gel column chromatography (CH_2Cl_2–MeOH, 100:1) and semipreparative HPLC [Welch Ultimate AQ-C_{18}, MeOH–H_2O (contains 0.5% TFA), 30:70, 1 mL/min] to yield **6** (36.7 mg, t_R = 17.474 min). Fr. 4.5 (2.5 g) was purified by silica gel column chromatography (CH_2Cl_2-acetone, 30:1) and recrystallized from MeOH to yield **3** (43.9 mg). Fr. 4.7 (5.9 g) was separated on silica gel column chromatography (petroleum ether-acetone, 8:1) to yield 13 further fractions (Fr. 4.7.1-Fr. 4.7.13). Fr. 4.7.4 (83.2 mg) was purified by semipreparative HPLC (Agilent Zorbax SB-C_{18}, MeOH–H_2O, 44:56, 2 mL/min) to yield **9** (21.6 mg, t_R = 31.258 min). Fr. 4.7.9 (45.4 mg) was recrystallized from acetone to yield **7** (12.4 mg). Fr. 4.7.13 (1.4 g) was separated on Sephadex LH-20 column chromatography (MeOH) and silica gel column chromatography (CH_2Cl_2-ethyl acetate, 40:1) to yield five fractions (Fr. 4.7.13.1–Fr. 4.7.13.5). Fr. 4.7.13.4 (163.8 mg) was recrystallized from MeOH to yield **4** (35.4 mg). Fr. 4.7.13.5 (46.8 mg) was purified by semipreparative HPLC (Agilent Zorbax SB-C_{18}, CH_3CN–H_2O, 20:80, 2 mL/min) to yield **11** (7.1 mg, t_R = 10.470 min) and **12** (3.3 mg, t_R = 11.827 min). Fr. 4.7.13.3 (20 mg) was purified by semipreparative HPLC (Agilent Zorbax SB-C_{18}, CH_3CN–H_2O, 28:72, 2 mL/min) to yield **10** (2.2 mg, t_R = 9.544 min).

Fr. 6 (370.0 g) were subjected to silica gel column chromatography (CH_2Cl_2-acetone, 20:1→0:1) to yield 14 subfractions (Fr. 6.1–Fr. 6.14). Fr. 6.8 (4.2 g) was separated on an RP-18 silica gel column eluted with MeOH–H_2O (10%→100%) to yield nine further fractions (Fr. 6.8.1–Fr. 6.8.9). Fr. 6.8.6 (193.9 mg) was purified by silica gel column chromatography (CH_2Cl_2-ethyl acetate, 10:1; petroleum ether-acetone, 5:1) to give **14** (13.8 mg). Fr. 6.8.7 (108.9 mg) was separated on silica gel column chromatography (CH_2Cl_2-ethyl acetate, 20:1) to yield three fractions (Fr. 6.8.7.1–Fr. 6.8.7.3). Fr. 6.8.7.1 (10.5 mg) was purified by semipreparative HPLC (Agilent Zorbax SB-C_{18}, CH_3CN–H_2O, 35:65, 2 mL/min) to yield **22** (5.8 mg, t_R = 12.866 min). Fr. 6.8.7.2 (25.2 mg) was purified by semipreparative HPLC (Agilent Zorbax SB-C_{18}, CH_3CN–H_2O, 30:70, 2 mL/min) to yield **13** (10.6 mg, t_R = 21.165 min) and a mixture (2.2 mg), which was purified by semipreparative HPLC (Agilent Zorbax SB-C_{18}, MeOH/H_2O, 55:45, 2 mL/min) to yield **21** (1.7 mg, t_R = 10.487 min). Fr. 6.8.8 (151.7 mg) was separated on silica gel column chromatography (CH_2Cl_2-ethyl acetate, 20:1) to yield three fractions (Fr. 6.8.8.1–Fr. 6.8.8.3). Fr. 6.8.8.1 (18 mg) was purified by semipreparative HPLC (Agilent Zorbax SB-C_{18}, MeOH–H_2O, 52:48, 2 mL/min) to yield **23** (1.4 mg, t_R = 28.224 min), **15** (11 mg, t_R = 38.626 min), and **5** (0.7 mg, t_R = 52.085 min). Fr. 6.12 (52.7 g) were subjected to silica gel column chromatography (CH_2Cl_2-ethyl acetate, 15:1→0:1) to yield 14 further fractions (Fr. 6.12.1–Fr. 6.12.14). Fr. 6.12.8 (8.2 g) was separated on an RP-18 silica gel column eluted with MeOH–H_2O (5%→100%) to yield 15 fractions (Fr. 6.12.8.1–Fr. 6.12.15). Fr. 6.12.8.1 (24.7 mg) was purified by semipreparative HPLC (Welch Ultimate AQ-C_{18}, CH_3CN–H_2O, 8:92, 1 mL/min) to yield **26** (4.8 mg, t_R = 54.165 min). Fr. 6.12.8.2 (3.7 g) was purified by silica gel column chromatography (CH_2Cl_2–MeOH, 50:1) to give **20** (2.1 g). Fr. 6.12.8.15 (127.7 mg) was purified by silica gel column chromatography (CH_2Cl_2–MeOH, 100:1) and recrystallized from MeOH to yield **16** (9.1 mg). Fr. 6.12.14 (9.1 g) was separated on an RP-18 silica gel column eluted with MeOH–H_2O (5%→100%) to yield six fractions (Fr. 6.12.14.1–Fr. 6.12.14.6). Fr. 6.12.14.6 (280.8 mg) was purified by silica gel column chromatography (CH_2Cl_2–MeOH, 50:1) and preparative thin-layer chromatography (CH_2Cl_2–MeOH, 8:1) to yield **19** (46.5 mg). Fr. 6.14 (24.25 g) was separated on an RP-18 silica gel column eluted with MeOH–H_2O (5%→100%) to yield six further fractions (Fr. 6.14.1–Fr. 6.14.6). Fr. 6.14.2 (6.18 g) was purified by silica gel column chromatography (CH_2Cl_2–MeOH, 35:1) to yield **18** (2.5 g).

2.3.1. Methyl 2-(4,4′,5′-trihydroxy-2′- (methoxymethyl)biphenyl-2-yloxy)acetate (1)

Brown solid; UV (MeOH) λ_{max} (log ε) 285 (3.77), 247 (3.93), 206 (4.60) nm; IR ν_{max} (KBr) 3432, 1739, 1614, 1504, 1447, 1173, 1120, 1082 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) δ_H 6.93 (1H, d, J = 8.2 Hz, H-6′), 6.86 (1H, s, H-3″), 6.62 (1H, s, H-6″), 6.45 (1H, dd, J = 8.2, 2.2 Hz, H-5′), 6.32 (1H, d, J = 2.2 Hz, H-3′), 4.54 (2H, s, H_2-2), 4.19 (2H, m, H_2-7″), 3.73 (3H, s, 1-OMe), 3.18 (3H, s, 7″-OMe); ^{13}C NMR (CD_3OD, 126 MHz) δ_C 171.3 (C, C-1), 159.0 (C, C-4′), 157.3 (C, C-2′), 145.4 (C, C-4″), 145.3 (C, C-5″), 133.2 (CH, C-6′), 131.1 (C, C-1″), 129.5 (C, C-2″), 122.6 (C, C-1′), 118.7 (CH, C-6″), 116.4 (CH, C-3″), 109.0 (CH, C-5′), 101.1 (CH, C-3′), 73.5 (CH_2, C-7″), 66.2 (CH_2, C-2), 57.9 (CH_3, 1-OMe), 52.5 (CH_3, 7″-OMe); ESI-MS m/z 357 $[M+Na]^+$; HR-ESI-MS m/z 357.0947 $[M+Na]^+$ (calcd for $C_{17}H_{18}NaO_7$, 357.0950).

2.3.2. 1′-Methylcaesalpin J (2)

Yellow solid; $[\alpha]_D^{22}$ +408.2 (c 0.29, MeOH); UV (MeOH) λ_{max} (log ε) 449 (2.22), 376 (2.61), 286 (3.81), 213 (4.43) nm; ECD (c 0.0065, MeOH) λ_{max} ($\Delta\varepsilon$) 315 (+2.50), 278 (+2.06), 248 (+1.58), 205 (−8.74) nm; IR ν_{max} (KBr) 3425, 1657, 1624, 1594, 1523, 1448, 1384, 1352, 1274, 1234, 1176, 1120, 1094, 1052, 999, 874 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) δ_H 6.97 (1H, d, J = 9.8 Hz, H-1), 6.58 (1H, s, H-9), 6.45 (1H, dd, J = 9.8, 1.5 Hz, H-2), 6.36 (1H, s, H-12), 5.55 (1H, d, J = 1.5 Hz, H-4), 4.16 (1H, dd, J = 10.5, 1.9 Hz, H-6β), 4.07 (1H, d, J = 10.5 Hz, H-6α), 3.91 (1H, s, H-13), 3.81 (1H, m, H-1′a), 3.64 (1H, m, H-1′b), 3.20 (1H, br d, J = 16.7 Hz, H-8a), 3.12 (1H, d, J = 16.7 Hz, H-8b), 1.12 (3H, t, J = 7.0 Hz, H_3-2′); ^{13}C NMR (CD_3OD, 126 MHz) δ_C 191.9 (C, C-3), 178.9 (C, C-4a), 150.6 (CH, C-1), 147.0 (C, C-10), 145.3 (C, C-11), 130.8 (CH, C-2), 127.4 (C, C-8a), 125.2 (C, C-12a), 116.3 (CH, C-9), 113.9 (CH, C-12), 109.9 (CH, C-4), 83.9 (CH, C-13), 76.8 (CH_2, C-6), 70.4 (CH_2, C-1′), 69.4 (C, C-7), 54.4 (C, C-12b), 43.5 (CH_2, C-8), 15.8 (CH_3, C-2′); ESI-MS m/z 353 $[M+Na]^+$; HR-ESI-MS m/z 353.0998 $[M+Na]^+$ (calcd for $C_{18}H_{18}NaO_6$, 353.1001).

2.3.3. Caesalpin J (3)

Colorless needles (MeOH); mp 263–265 °C; $[\alpha]_D^{21}$ +283.1 (c 0.12, MeOH); ECD (c 0.008, MeOH) λ_{max} ($\Delta\varepsilon$) 313 (+1.88), 277 (+1.57), 248 (+1.19), 205 (−6.47) nm; ESI-MS m/z 339 $[M+Na]^+$.

2.3.4. Epicaesalpin J (4)

Yellow oil (MeOH); $[\alpha]_D^{20}$ +353.8 (c 0.15, MeOH); ECD (c 0.008, MeOH) λ_{max} ($\Delta\varepsilon$) 313 (+1.30), 274 (+1.77), 252 (+1.69), 205 (−6.73) nm; ESI-MS m/z: 339 $[M+Na]^+$.

2.4. Antiplatelet aggregation activities assay

Institute of Cancer Research (ICR) mice (male, 30–40 g) were obtained from Nanjing Qinglongshan Animal Center (Nanjing, Jiangsu, China). The animals were acclimatized for at least 1 d prior to use. Mouse gel-filtered platelets were prepared as described previously (Su et al., 2016). Briefly, the mice were anesthetized using chloral hydrate, blood was taken out from the abdominal aorta of mice by using 2.5 mL syringe. The blood was centrifugated at 200× g for 10 min to get platelet-rich plasma (PRP). The PRP was filtered by using the column packed with Sepharose 2B beads and eluted with Tyrode's buffer (5.56 mmol/L glucose, 137 mmol/L NaCl, 2.7 mmol/L KCl, 2.56 mmol/L NaH_2PO_4•$2H_2O$, 20 mmol/L HEPES, 137 mmol/L $MgCl_2$•$6H_2O$, 0.1% BSA, pH = 7.4) 1.5 mL tubes. The high concentration gel-filtered platelets were collected and pooled, and adjusted to 2.5×10^8 platelets/mL using Tyrode's buffer.

In vitro platelet aggregation was tested using a four-channel aggregometer (LBY-NJ4, Pulisheng Instrument Co., Ltd., Beijing, China) according to the turbidimetric method as previously described (Geraghty et al., 2011; Maione et al., 2014). Gel-filtered platelets with samples or vehicles were preincubated for 5 min at 37 °C. After this, platelet aggregation was induced by AYPGKF-NH_2. The maximum aggregation rate was measured within 5 min with continuous stirring. The percentage (%) of inhibition of platelet aggregation was calculated by the formula: $[(X-Y)/X] \times 100\%$. X was the maximum aggregation rate of vehicle-treated gel-filtered platelets; Y was the maximum aggregation rate of sample-treated gel-filtered platelets.

3. Results and discussion

3.1. Structure elucidation

The HR-ESI-MS of **1** exhibited a ion peak at m/z 357.0947 $[M+Na]^+$ (calcd for $C_{17}H_{18}NaO_7$, 357.0950) responding to a molecular formula $C_{17}H_{18}O_7$ with nine degrees of unsaturation. Its IR spectrum displayed the presence of hydroxy (3432 cm^{-1}), carbonyl (1739 cm^{-1}), and aromatic (1614, 1504, and 1447 cm^{-1}) groups. The NMR spectra of **1** showed signals for one carbonyl group [δ_C 171.3 (C, C-1)], one 1,2,4-trisubstituted phenyl ring [δ_H 6.93 (d, J = 8.2 Hz, H-6′), 6.45 (dd, J = 8.2, 2.2 Hz, H-5′), and 6.32 (d, J = 2.2 Hz, H-3′); ring A], one tetrasubstituted phenyl ring [δ_H 6.86 (s, H-3″) and 6.62 (s, H-6″); ring B], two methoxy groups [δ_H 3.73 (3H, s, 1-OMe) and 3.18 (3H, s, 7″-OMe)], and two oxygenated methylene groups [δ_C 73.5 (CH_2, C-7″) and 66.2 (CH_2, C-2)]. These two phenyl rings were linked through C-1′-C-1″ based on the HMBC correlations from H-6′ to C-1″ and from H-6″ to C-1′, along with the ROESY correlation of H-6′/H-6″ (Fig. 2), implying compound **1** to be a biphenyl. Based on the HMBC correlations from 1-OMe to C-2, H_2-2 to C-2′ and the ROESY correlations of H_2-2/H-3′, a 2-methoxy-2-oxoethoxy was located at C-2′ of ring A. A methoxymethyl group was attached to C-2″ of ring B was deduced by the HMBC correlations from 7″-OMe to C-7″ and from H-3″ to C-7″, along with the ROESY correlations of H-3″/H-7″. Based on the HMBC correlations from H-6′ to C-4′, from H-3″ to C-5″, and H-6″ to C-4″, the remaining three hydroxy groups

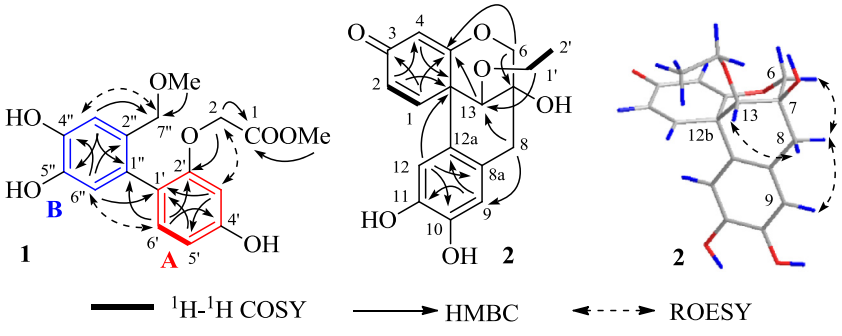

Fig. 2. Key 2D NMR correlations of compounds **1** and **2**.

were located at C-4′, C-4″, and C-5″, respectively. Therefore, the structure of **1** was determined to be methyl 2-(4,4′,5′-trihydroxy-2′-(methoxymethyl)biphenyl-2-yloxy)acetate.

Compound **2** was obtained as a yellow solid. The molecular formula, $C_{18}H_{18}O_6$, was inferred from its NMR data and HR-ESI-MS, which showed an ion peas at *m/z* 353.0998 $[M+Na]^+$ (calcd for $C_{18}H_{18}NaO_6$, 353.1001). The IR spectrum exhibited absorptions due to an α,β-unsaturated ketone at 1657 and 1624 cm^{-1}, an aromatic ring at 1594, 1523, and 1448 cm^{-1}, and hydroxy groups at 3425 cm^{-1}. The NMR data of **2** were very similar to those of caesalpin J (**3**) also from *C. sappan* (Shimokawa, Kinjo, Yamahara, Yamasaki & Nohara, 1985), except that compound **2** had signals for an ethoxy group [δ_H 3.81 (m, H-1′a), 3.64 (m, H-1′b), and 1.12 (3H, t, J = 7.0 Hz, H_3-2′); δ_C 70.4 (CH_2, C-1′) and 15.8 (CH_3, C-2′)], rather than the signals for a methoxy group in caesalpin J (**3**). Based on the HMBC correlations from H-1′a and H-1′b to C-13 (Fig. 2), the ethoxy group was located at C-13. The other connections in the structure of **2** were deduced the same as those of caesalpin J (**3**) by 2D NMR correlations (Fig. 2).

The relative configuration of **2** was elucidated by its ROESY spectrum. The key correlations of H-13/H-8a and H-8b/H-6α indicated that H-13, the C-12b-C-12a bond, and the C-7-C-8 bond should be in α-orientation. The ECD spectrum of **2** was very similar to those of caesalpin J (**3**) and epicaesalpin J (**4**) (Fig. 3), implying that the absolute configuration of **2** was 7*R*,12b*S*,13*S*. Therefore, the structure of **2** was determined to be 1′-methylcaesalpin J.

By comparison of spectroscopic data with those reported in literature, the known compounds were identified as caesalpin J (**3**) (Shimokawa et al., 1985), epicaesalpin J (**4**) (Zhao et al., 2014b), (*E*)-7-hydroxy-3-(4-hydroxybenzylidene)chroman-4-one (**5**) (Namikoshi, Nakata & Saitoh, 1987b), sappanone B (**6**) (Fu et al., 2008), (-)-3-deoxysappanone B (**7**) (Fu et al., 2008; Saitoh et al., 1986), 3′-deoxy-4-*O*-methylsappanol (**8**) (Namikoshi, Nakata, Nuno, Ozawa & Saitoh, 1987a), caesalpiniaphenol F (**9**) (Min et al., 2012), 3′-*O*-methylesappanol (**10**) (Namikoshi, Nakata, Yamada, Nagai & Saitoh, 1987c), 3′-deoxyepisappanol (**11**) (Lai et al., 2011; Namikoshi et al., 1987b), 3′-*O*-methylepisappanol (**12**) (Namikoshi et al., 1987c), 3′,4-di-*O*-methylepisappanol (**13**) (Zhao, Wang, Li, Koike & Bai, 2014a), 3′-deoxy-4-*O*-methylepisappanol (**14**) (Moon, Chung, Seo & Kang, 2010), 4,4′-dihydroxy-2′-methoxychalcone (**15**) (Ogunlana et al., 2015), sappanchalcone (**16**) (Baek et al., 2000), protosappanin A (**17**) (Nagai, Nagumo, Lee, Eguchi & Kawai, 1986), (-)-protosappanin B (**18**) (Zhao, Cai, Tu & Tang, 2016), 10-*O*-methyprotosappanin B (**19**) (Zhao et al., 2016), brazilin (**20**) (Nirmal et al., 2015), (-)-balanophonin (**21**) (Sy & Brown, 1999), (-)-syringaresinol (**22**) (Panyo, Matsunami & Panichayupakaranant, 2016), (-)-episyringaresinol (**23**) (Liu et al., 2015), coniferyl aldehyde (**24**) (Zhao, Luo, Li, Yi & Zhou, 2008), 4–hydroxy-3-methoxybenzaldehyde (**25**) (Ito et al., 2001), and 2,4-dihydroxybenzoic acid (**26**) (Scott, 1972).

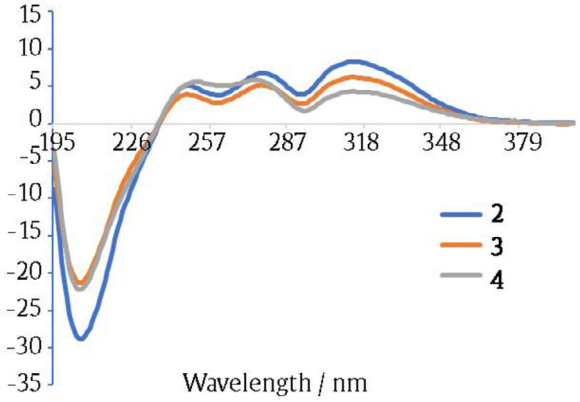

Fig. 3. ECD spectra of compounds **2–4**.

3.2. Antiplatelet aggregation activity

All isolated compounds, except for compounds **4, 10, 12–14, 18**, and **19**, were evaluated for inhibitory activity against mouse platelet aggregation induced by AYPGKF-NH_2. As shown in Table 1, at a concentration of 100 μmol/L, (-)-3-deoxysappanone B (**7**) and (-)-episyringaresinol (**23**) possessed weak inhibitory effects on aggregation of mouse platelet induced by AYPGKF-NH_2 with inhibitions of 28.8% and 24.4%, respectively. Sappanchalcone (**16**) and brazilin (**20**) possessed moderate inhibitory activities with IC_{50} values of 114.8 μmol/L and 100.8 μmol/L, respectively. Other tested compounds were inactive.

A number of researches showed that PAR4 was a promising target for safer antiplatelet drug development (Rudinga et al., 2018). TCM is an excellent source for new candidates or drugs discovery. But until now, only one compound YD-3 (Wu et al., 2000), a PAR4 inhibitor, was reported from TCM. In the present study, we reported several PAR4 inhibitors, which may server as candidates for further structural modification to develop medicines.

Table 1
Effects of compounds from *C. sappan* on aggregation of mouse platelet induced by AYPGKF-NH_2[a].

Compounds	Concentration/(μmol·L^{-1})	Inhibition/%
(−)-3-deoxysappanone B (**7**)	100	28.8
sappanchalcone (**16**)[b]	150	68.6
	130	53.9
	100	46.7
	80	24.9
	50	7.3
brazilin (**20**)[b]	150	73.2
	130	55.5
	100	51.5
	80	40.1
	50	19.1
(−)-episyringaresinol (**23**)	100	24.4

[a] Inhibitions of compounds **1**−**3, 5, 6, 8, 9, 11, 15, 17, 21, 22**, and **24**−**26** were less than 20% at concentration of 100 μmol/L.
[b] IC_{50} values of compounds **16** and **20** were 114.8 μmol/L and 100.8 μmol/L, respectively.

4. Conclusion

Twenty-six compounds including two new phenols were isolated from the heartwoods of *C. sappan*, a traditional Chinese medicine with functions of promoting blood circulation and removing blood stasis. Two compounds showed moderate inhibitory activities against mouse platelet aggregation induced by AYPGKF-NH_2, a gold agonist of PAR4. Antiplatelet compounds from *C. sappan* targeting at PAR4 are reported for the first time.

Declaration of Competing Interest

The authors declare no conflict of interest.

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References

Baek, N. I., Jeon, S. G., Ahn, E. M., Hahn, J. T., Bahn, J. H., Jang, J. S., et al. (2000). Anticonvulsant compounds from the wood of *Caesalpinia sappan* L. *Archives of Pharmacal Research, 23*(4), 344–348.

Chen, D. Z., Zhang, D. X., & Ding, H. (2010). Fabaceae: *Caesalpinia*. In Z. Y. Wu, P. H. Raven, & D. Y. Hong (Eds.). In *Flora of China: 10* (pp. 41–47). St. Louis: Science Press, Beijing, and Missouri Botanical Garden Press.

Fu, L. C., Huang, X. A., Lai, Z. Y., Hu, Y. J., Liu, H. J., & Cai, X. L. (2008). A new 3-benzylchroman derivative from *Sappan Lignum* (*Caesalpinia sappan*). *Molecules, 13*(8), 1923–1930.

Geraghty, D. P., Ahuja, K. D., Pittaway, J., Shing, C., Jacobson, G. A., Jager, N., et al. (2011). *In vitro* antioxidant, antiplatelet and anti-inflammatory activity of *Carpobrotus rossii* (pigface) extract. *Journal of Ethnopharmacology, 134*(1), 97–103.

Ito, J., Chang, F. R., Wang, H. K., Park, Y. K., Ikegaki, M., Kilgore, N., et al. (2001). Anti-AIDS agents. 48. Anti-Hiv activity of moronic acid derivatives and the new melliferone-related triterpenoid isolated from *Brazilian propolis*. *Journal of Natural Products, 64*(10), 1278–1281.

Lai, W. C., Wang, H. C., Chen, G. Y., Yang, J. C., Korinek, M., Hsieh, C. J., et al. (2011). Using the pER8: Gus reporter system to screen for phytoestrogens from *Caesalpinia sappan*. *Journal of Natural Products, 74*(8), 1698–1706.

Liu, Y. P., Wang, X. C., Li, X. B., Li, K. K., Huang, L. G., Wen, C. Q., et al. (2015). Studies on non-alkaloid constituents from *Ochrosia elliptica*. *China Journal of Chinese Materia Medica, 40*(8), 1508–1513.

Maione, F., De Feo, V., Caiazzo, E., De Martino, L., Cicala, C., & Mascolo, N. (2014). Tanshinone IIA, a major component of *Salvia milthorriza* Bunge, inhibits platelet activation via ERK-2 signaling pathway. *Journal of Ethnopharmacology, 155*(2), 1236–1242.

McFadyen, J. D., Schaff, M., & Peter, K. (2018). Current and future antiplatelet therapies: Emphasis on preserving haemostasis. *Nature Reviews Cardiology, 15*(3), 181.

Min, B. S., Cuong, T. D., Hung, T. M., Min, B. K., Shin, B. S., & Woo, M. H. (2012). Compounds from the heartwood of *Caesalpinia sappan* and their anti-inflammatory activity. *Bioorganic & Medicinal Chemistry Letters, 22*(24), 7436–7439.

Moon, H. I., Chung, I. M., Seo, S. H., & Kang, E. Y. (2010). Protective effects of 3′-deoxy-4-*O*-methylepisappanol from *Caesalpinia sappan* against glutamate-induced neurotoxicity in primary cultured rat cortical cells. *Phytotherapy Research, 24*(3), 463–465.

Nagai, M., Nagumo, S., Lee, S. M., Eguchi, I., & Kawai, K. I. (1986). Protosappanin A, a novel biphenyl compound from Sappan Lignum. *Chemical and Pharmaceutical Bulletin, 34*(1), 1–6.

Namikoshi, M., Nakata, H., Nuno, M., Ozawa, T., & Saitoh, T. (1987a). Homoisoflavonoids and related compounds. III. Phenolic constituents of *Caesalpinia japonica* Sieb. et Zucc. *Chemical and Pharmaceutical Bulletin, 35*(9), 3568–3575.

Namikoshi, M., Nakata, H., & Saitoh, T. (1987b). Homoisoflavonoids from *Caesalpinia sappan*. *Phytochemistry, 26*(6), 1831–1833.

Namikoshi, M., Nakata, H., Yamada, H., Nagai, M., & Saitoh, T. (1987c). Homoisoflavonoids and related compounds. II. Isolation and absolute configurations of 3, 4-dihydroxylated homoisoflavans and brazilins from *Caesalpinia sappan* L. *Chemical and Pharmaceutical Bulletin, 35*(7), 2761–2773.

Nirmal, N. P., Rajput, M. S., Prasad, R. G., & Ahmad, M. (2015). Brazilin from *Caesalpinia sappan* heartwood and its pharmacological activities: A review. *Asian Pacific Journal of Tropical Medicine, 8*(6), 421–430.

Ogunlana, O. O., Kim, H. S., Wataya, Y., Olagunju, J. O., Akindahunsi, A. A., & Tan, N. H. (2015). Antiplasmodial flavonoid from young twigs and leaves of *Caesalpinia bonduc* (Linn) Roxb. *Journal of Chemical and Pharmaceutical Research, 7*(1), 931–937.

Panyo, J., Matsunami, K., & Panichayupakaranant, P. (2016). Bioassay-guided isolation and evaluation of antimicrobial compounds from *Ixora megalophylla* against some oral pathogens. *Pharmaceutical Biology, 54*(9), 1522–1527.

Rudinga, G. R., Khan, G. J., & Kong, Y. (2018). Protease-activated receptor 4 (PAR4): A promising target for antiplatelet therapy. *International Journal of Molecular Sciences, 19*(2), 573.

Saitoh, T., Sakashita, S., Nakata, H., Shimokawa, T., Kinjo, J.-. E., Yamahara, J., et al. (1986). 3-Benzylchroman derivatives related to Brazilin from *Sappan Lignum*. *Chemical and Pharmaceutical Bulletin, 34*(6), 2506–2511.

Scott, K. N. (1972). Carbon-13 nuclear magnetic resonance of biologically important aromatic acids. I. Chemical shifts of benzoic acid and derivatives. *Journal of the American Chemical Society, 94*(24), 8564–8568.

Shimokawa, T., Kinjo, J., Yamahara, J., Yamasaki, M., & Nohara, T. (1985). Two novel aromatic compounds from *Caesalpinia sappan*. *Chemical and Pharmaceutical Bulletin, 33*(8), 3545–3547.

Su, X. L., Su, W., Wang, Y., Wang, Y. H., Ming, X., & Kong, Y. (2016). The pyrrolidinoindoline alkaloid PSM2 inhibits platelet aggregation and thrombus formation by affecting PI3K/Akt signaling. *Acta Pharmacologica Sinica, 37*(9), 1208–1217.

Sy, L. K., & Brown, G. D. (1999). Coniferaldehyde derivatives from tissue culture of *Artemisia annua* and *Tanacetum parthenium*. *Phytochemistry, 50*(5), 781–785.

The Pharmacopoeia Commission of the People's Republic of China (2015). In *Pharmacopoeia of the People's Republic of China: 1* (p. 164). Beijing: China Medical Science Press.

Wong, P. C., Seiffert, D., Bird, J. E., Watson, C. A., Bostwick, J. S., Giancarli, M., et al. (2017). Blockade of protease-activated receptor-4 (PAR4) provides robust antithrombotic activity with low bleeding. *Science Translational Medicine, 9*(371) eaaf5294.

Wu, C. C., Huang, S. W., Hwang, T. L., Kuo, S. C., Lee, F. Y., & Teng, C. M. (2000). YD-3, a novel inhibitor of protease-induced platelet activation. *British Journal of Pharmacology, 130*(6), 1289–1296.

Xia, M. Y., Wang, L., & Wang, Y. H. (2016). Antiplatelet and antithrombotic activities of *Piper* plants. *Natural Product Research and Development, 28*(10), 1676–1685.

Xia, M. Y., Yang, J., Zhang, P. H., Li, X. N., Luo, J. F., Long, C. L., et al. (2018). Amides, isoquinoline alkaloids and dipeptides from the aerial parts of *Piper mullesua*. *Natural Products and Bioprospecting, 8*(6), 419–430.

Zhao, H., Wang, X., Li, W., Koike, K., & Bai, H. (2014a). A new minor homoisoflavonoid from *Caesalpinia sappan*. *Natural Product Research, 28*(2), 102–105.

Zhao, M. B., Cai, C. Q., Tu, P. F., & Tang, L. (2016). Conformational analysis and NMR assignments of dibenzoxocins from *Sappan Lignum*. *Magnetic Resonance in Chemistry, 54*(7), 601–605.

Zhao, M. B., Li, J., Shi, S. P., Cai, C. Q., Tu, P. F., Tang, L., et al. (2014b). Two new phenolic compounds from the heartwood of *Caesalpinia sappan* L. *Molecules, 19*(1), 1–8.

Zhao, Y. X., Luo, J. R., Li, C. S., Yi, T. M., & Zhou, J. (2008). Palaeophytochemical constituents from the Miocene-Fossil wood of *Picea likiangensis* in Xun-Dian of Yunnan, China. *Bulletin of the Korean Chemical Society, 29*(8), 1613–1616.

Zhuo, J. X., Wang, Y. H., Su, X. L., Mei, R. Q., Yang, J., Kong, Y., et al. (2016). Neolignans from *Selaginella moellendorffii*. *Natural Products and Bioprospecting, 6*(3), 161–166.