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# Desmoschinensisflavones A and B, two rare flavones having a hybrid benzyl benzoate ester-flavone structural framework from *Desmos chinensis* Lour†

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Two rare flavones having a hybrid benzyl benzoate ester-flavone structural framework, desmoschinensisflavones A and B (**1** and **2**), together with 12 known compounds (**3–14**) were isolated from the fruit, leaf, and twig extracts of *Desmos chinensis* (red flower). The new structures were characterized by UV, IR, NMR, and HRESITOFMS data. Desmoschinensisflavones A and B have a distinctive skeleton of benzoate ester-flavones with a C-4'' and C-6 and C-8 connection via a methylene group, respectively. Plausible biosynthesis pathways to compounds **1** and **2** are proposed based on an intermolecular nucleophilic 1,4-addition to *ortho*-quinone intermediates. Compounds **6–8** and **12** showed weakly antioxidant inhibition with IC<sub>50</sub> values in the range of 65.4–74.6 μM.

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## Introduction

The genus *Desmos* belongs to the Annonaceae family. These plants are a rich source of flavonoids, alkaloids, benzoate esters, chalcones, and oxepinones.<sup>1–4</sup> Hybrid-flavonoids have rarely been reported from this genus, such as biflavones from *D. chinensis*<sup>1</sup> and flavan-chalcones from *D. cochinchinensis*.<sup>5</sup> Several species of this genus have been used in Chinese folk medicines.<sup>4</sup> For example, the roots of *D. cochinchinensis* have been used as an antimalarial,<sup>6</sup> while the stem and root of *D. dumosus* have mainly been used to treat muscle pain in Thai traditional medicine.<sup>7</sup> *Desmos chinensis* Lour. is a large climbing shrub, known in the local name (Thailand) as 'Sai-Yud', which is distributed widely in Southern and South East Asian countries.<sup>1</sup> The root part of *D. chinensis* has been popularly used as treatment of various

conditions in Southern and South East Asia. For example, the decoction of *D. chinensis* roots has been used for the treatment of malaria.<sup>8</sup> In India, Malaysia, and Thailand, the roots of *D. chinensis* have been used to treat diarrhea, dysentery, fever, parturition vertigo, and postpartum pot herbs.<sup>1,9</sup> Previous phytochemical studies on *D. chinensis* produced several types of compounds, including biflavones,<sup>1</sup> benzyl benzoate esters,<sup>10</sup> C-benzylated chalcones,<sup>9</sup> oxoaporphine alkaloids,<sup>10</sup> and flavonoids.<sup>11</sup> Some of these compounds showed antimicrobial,<sup>12</sup> antifungal,<sup>11</sup> antitumor, and acute toxic activities.<sup>13</sup>

Of the three different flowering species of *D. chinensis* growing at Mae Fah Luang University, the red, yellow, and giant flowering forms, herein, we report the isolation and structure elucidation of two new hybrid benzoate ester-flavones (**1** and **2**) together with 12 known compounds (**3–14**) isolated from the fruit, leaf, and twig extracts of the red flowering species of *D. chinensis*. This is the first report on the isolation of natural products having a hybrid benzyl benzoate ester-flavone skeleton from the Annonaceae family to the best of our knowledge. The antioxidant and α-glucosidase inhibitory properties of some of the compounds are also reported.

## Results and discussion

Fourteen compounds, including the hybrid benzyl benzoate ester-flavones **1** and **2**, flavonoids (**3–8**), benzyl benzoate esters (**9–12**), chalcone (**13**), and alkaloid (**14**) were isolated and characterized from the extract of the fruits, leaves, and twigs of *D. chinensis*. Compounds **1** and **2** are new hybrids of a benzyl benzoate ester and a flavone. The known compounds were identified as

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isounonal (3),<sup>14</sup> unonal (4),<sup>14</sup> quercetin (5),<sup>15</sup> kaempferol (6),<sup>15</sup> desmal (7),<sup>8</sup> 6-formyl-2,5,7-trihydroxy-8-methylflavanone (8),<sup>16</sup> benzyl benzoate (9),<sup>17</sup> benzyl-2-hydroxybenzoate (10),<sup>17</sup> benzyl-2,3-dihydroxy benzoate (11),<sup>18</sup> benzyl-2,6-dihydroxybenzoate (12),<sup>17</sup> desmosdumotin D (13),<sup>19</sup> and lauterine (14)<sup>20</sup> by comparisons with the literature reported spectroscopic data (Fig. 1).

Compound **1** was obtained as a yellow powder (mp. 180–183 °C) from the leaf extract. The HR-ESI mass spectrum of **1** showed a negative  $[M - H]^-$  ion peak at  $m/z$  523.1381 (calcd for 523.1393), corresponding to a molecular formula of  $C_{31}H_{24}O_8$  with 20 degrees of unsaturation. The UV absorption maxima at  $\lambda_{max}$  252, 279, and 331 nm suggested a flavone skeleton,<sup>1</sup> while the IR spectrum displayed the characteristics of hydroxy absorption bands at 3442 and 3375  $cm^{-1}$  and carbonyl absorption bands at 1727 (ester functionality) and 1659 (conjugate ketone functionality)  $cm^{-1}$ . The <sup>13</sup>C and DEPT 135 NMR spectroscopic data of **1** (Table 1) indicated 31 carbons, including two carbonyls, thirteen methines, two methylenes, one methyl, and thirteen quaternary carbons.

Analysis of the <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data of **1** (Table 1) suggested that this compound had a hybrid structure of a benzyl benzoate ester and a flavone. <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data of the flavone unit (Table 1) displayed resonances for hydroxy protons at  $\delta_H$  8.39 (1H, s, OH-7) and 13.10 (1H, s, OH-5), a monosubstituted aromatic ring at  $\delta_H$  7.90 (2H, dd,  $J = 7.5, 1.9$  Hz, H-2',6')/ $\delta_C$  126.2 and 7.48–7.56 (3H, m, H-3',5'/H-4')/ $\delta_C$  129.2/131.8, isolated aliphatic protons at  $\delta_H$  3.93 (2H, s, H- $\alpha$ )/ $\delta_C$  21.1, an olefinic proton at  $\delta_H$  6.65 (2H, s, H-2)/ $\delta_C$  105.1, and a methyl group at  $\delta_H$  2.32 (3H, s, CH<sub>3</sub>-8)/ $\delta_C$  8.1.

The <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data of the benzyl benzoate ester unit (Table 1) displayed resonances for a monosubstituted aromatic ring at  $\delta_H$  7.40–7.48 (5H, m, H-3''',7'''/H-4''',6'''/H-5''')/ $\delta_C$  128.8/129.1/129.4, *ortho*-coupled aromatic protons at  $\delta_H$  6.51 (1H, d,  $J = 8.6$  Hz, H-6'')/ $\delta_C$  109.7 and 7.77 (1H, d,  $J = 8.6$  Hz, H-5'')/ $\delta_C$  139.5 and a set of isolated aliphatic protons at  $\delta_H$  5.52 (2H, s, H-1''')/ $\delta_C$  68.6.

The HMBC correlations (Fig. 2) of the flavone unit indicated that the methyl group was located at C-8 from the HMBC correlations of the methyl proton CH<sub>3</sub>-8 ( $\delta_H$  2.32) to  $\delta_C$  158.9 (C-7), 103.1 (C-8), and 153.7 (C-8a). The hydroxy group was located at C-7 from the HMBC correlations of the hydroxy proton OH-7 ( $\delta_H$  8.39) to  $\delta_C$  158.9 (C-7) and 103.1 (C-8). The hydrogen-bonded hydroxy proton was located at C-5 from the HMBC correlations of OH-5 ( $\delta_H$  13.10) to  $\delta_C$  105.0 (C-4a), 157.1 (C-5) and 110.4 (C-6) (Fig. 2). Furthermore, the *ortho*-coupled aromatic protons of the benzoate ester unit were assigned by the HMBC correlations of H-5'' ( $\delta_H$  7.77) to  $\delta_C$  21.1 (C- $\alpha$ ), 156.1 (C-3''), and 159.0 (C-7''). However, the key HMBC correlation of H- $\alpha$  ( $\delta_H$  3.93) to  $\delta_C$  157.1 (C-5), 110.4 (C-6), 158.9 (C-7) 118.8 (C-4''), and 139.5 (C-5'') clearly indicated the linkage between the benzyl benzoate ester and the flavone moieties. The NOESY correlations between OH-5 ( $\delta_H$  13.10), OH-7 ( $\delta_H$  8.39), H-5'' ( $\delta_H$  7.77) to methylene protons (H- $\alpha$ ,  $\delta_H$  3.93) further confirmed the C- $\alpha$  linkage between these two structural moieties. Therefore, compound **1** was named as desmoschinensisflavone A.

Compound **2** was obtained as a yellow powder (mp. 179–181 °C) from the leaf extract and had the molecular ion peak at  $m/z$  523.1402  $[M - H]^-$  (calcd  $m/z$  for  $C_{31}H_{23}O_8$ , 523.1393) based on the HRESITOFMS and <sup>13</sup>C NMR spectroscopic data. The <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data of **2** (Table 1) were similar to those of **1**, except for the replacement of the methyl group at C-8 by a benzyl benzoate ester unit, and attachment of the methyl group at C-6. The HMBC correlations from CH<sub>3</sub>-6 at  $\delta_H$  2.15 (3H, s) to  $\delta_C$  157.7 (C-5), 106.0 (C-6), and 159.2 (C-7), indicated that the methyl group was attached to the C-6 position. Moreover, the location of benzyl benzoate ester unit was determined to be at the C-8 position based on the HMBC correlations between H- $\alpha$  at  $\delta_H$  4.13 (2H, s) to  $\delta_C$  159.2 (C-7), 104.7 (C-8), 152.8 (C-8a), 154.5 (C-3''), 118.2 (C-4''), and 138.0 (C-5'') (Fig. 2). Thus, structure **2** was identified as a desmoschinensisflavone B.

Putative biogenetic pathways to compounds **1** and **2** are shown in Scheme 1. The reduction of the isolated compounds **3** and **4** followed by dehydration would produce the *ortho*-

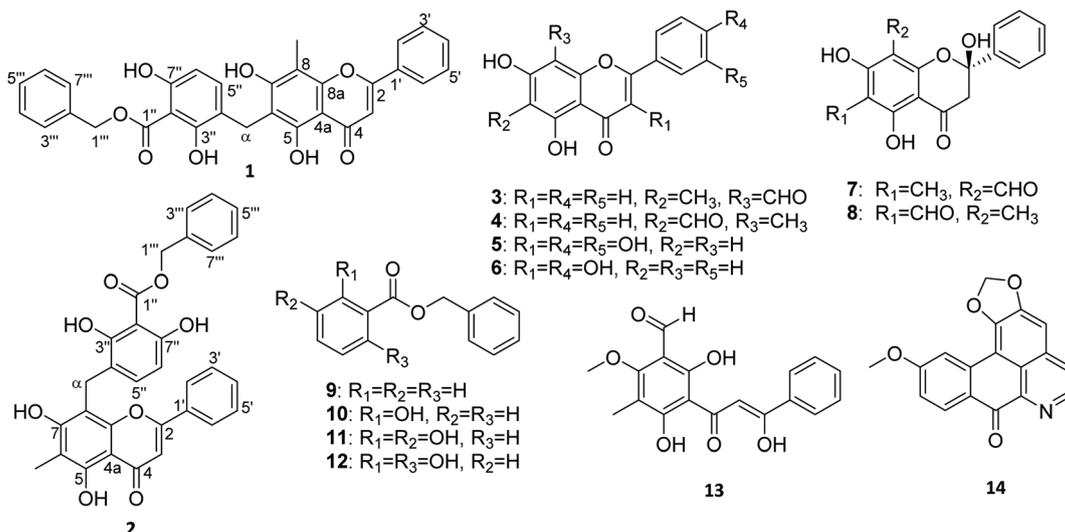
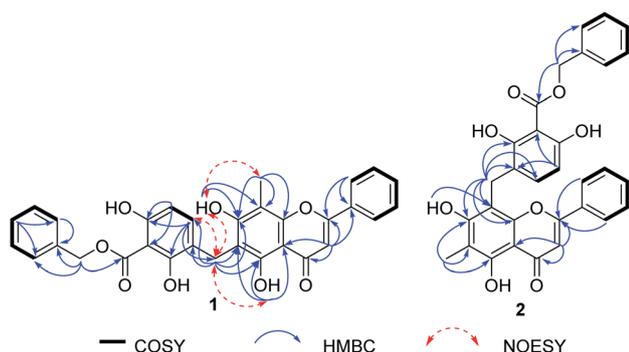


Fig. 1 Isolated compounds (1–14) from the fruit, leaf, and twig extracts of *D. chinensis*.



Table 1 NMR Spectroscopic data of compounds 1 and 2 (400 MHz in CDCl<sub>3</sub>)

Position	1			2		
	$\delta_C$	$\delta_H$ (mult $J$ in Hz)	HMBC ( $^1H \rightarrow ^{13}C$ )	$\delta_C$	$\delta_H$ (mult $J$ in Hz)	HMBC ( $^1H \rightarrow ^{13}C$ )
<b>Flavone skeleton</b>						
2	163.4			163.3		
3	105.1	6.65 (s)	2, 4, 4a, 1'	104.1	6.70 (s)	2, 4, 4a
4	182.9			182.7		
4a	105.0			105.0		
5	157.1			157.7		
6	110.4			106.0		
7	158.9			159.2		
8	103.1			104.7		
8a	153.7			152.8		
$\alpha$	21.1	3.93 (s)	5, 6, 7, 4'', 5''	29.7	4.13 (s)	7, 8, 8a, 3'', 4'', 5''
1'	131.6			131.7		
2'	126.2	7.90 (dd, 7.5, 1.9)	2, 1'	126.2	7.91 (dd, 7.4, 2.0)	2
3'	129.2	7.48–7.56 (m)	2', 4'	129.1	7.58–7.59 (m)	4'
4'	131.8	7.48–7.56 (m)	2', 3', 5'	131.8	7.58–7.59 (m)	
5'	129.2	7.48–7.56 (m)	2', 4'	129.1	7.58–7.59 (m)	4'
6'	126.2	7.90 (dd, 7.5, 1.9)	2, 1'	126.2	7.91 (dd, 7.4, 2.0)	2
<b>Benzyl benzoate-ester skeleton</b>						
1''	169.7			169.6		
2''	99.5			99.8		
3''	156.1			154.5		
4''	118.8			118.2		
5''	139.5	7.77 (d, 8.6)	$\alpha$ , 3'', 7''	138.0	7.43–7.45 (m)	
6''	109.7	6.51 (d, 8.6)	2'', 7''	109.7	6.39 (d, 8.6)	2'', 4''
7''	159.0			158.1		
1'''	68.6	5.52 (s)	1'', 2''', 3'''	68.8	5.54 (s)	1'', 2''', 3'''
2'''	133.6			133.3		
3'''	128.8	7.40–7.48 (m)	2'''	128.9	7.43–7.45 (m)	
4'''	129.1	7.40–7.48 (m)		129.3	7.43–7.45 (m)	
5'''	129.4	7.40–7.48 (m)	3''', 7'''	129.5	7.43–7.45 (m)	
6'''	129.1	7.40–7.48 (m)		129.3	7.43–7.45 (m)	
7'''	128.8	7.40–7.48 (m)	2'''	128.9	7.43–7.45 (m)	
OH-5		13.10 (s)	4a, 5, 6		12.94 (s)	
CH <sub>3</sub> -6				7.7	2.15 (s)	5, 6, 7
OH-7		8.39 (s)	7, 8		8.19 (s)	8
CH <sub>3</sub> -8	8.1	2.32 (s)	7, 8, 8a			

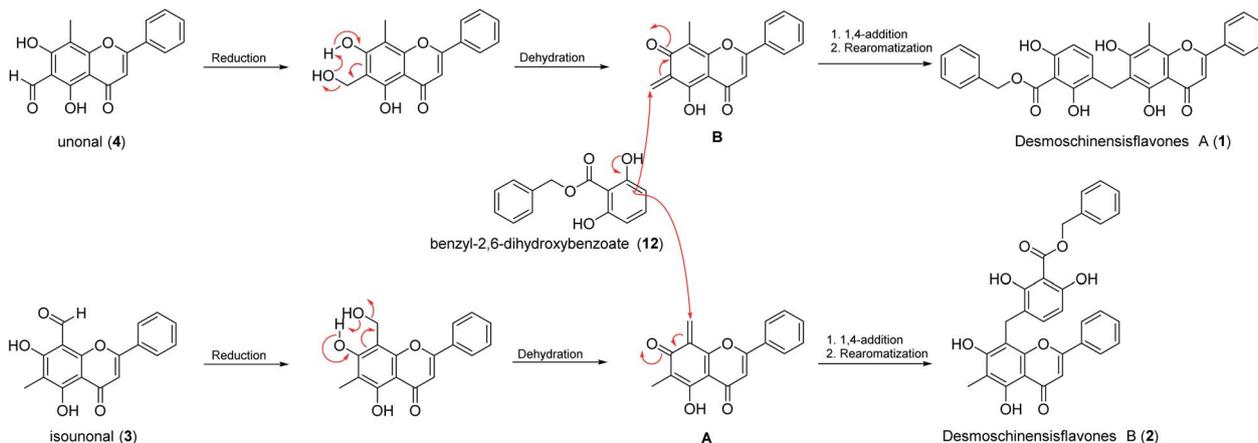
Fig. 2 COSY, selected HMBC ( $^1H \rightarrow ^{13}C$ ), and NOESY ( $^1H \rightarrow ^1H$ ) correlations of compounds 1 and 2.

quinone methide intermediates **A** and **B**, respectively. Desmoschinensisflavone **A** (**1**) could be derived from intermediate **B** through coupling with benzyl benzoate **12** via a 1,4-addition

reaction followed by rearomatization, whereas desmoschinensisflavone **B** (**2**) could be obtained from intermediate **A** by coupling with benzyl benzoate **12**. The isolation of compounds **3**, **4**, and **12** in this study would support the proposed putative biogenetic pathways of compounds **1** and **2**.

Only compounds isolated in sufficient amounts and that were stable (compounds **1**, **3**, **4**, **6–8**, **12**, and **13**) were evaluated for their antioxidant activities against DPPH and ABTS and  $\alpha$ -glucosidase inhibitory activity. Compounds **6**, a mixture of **7** and **8**, and **12** in the DPPH radical scavenging assay had modest IC<sub>50</sub> values of 74.6, 65.4, and 65.5  $\mu$ M, respectively. While the mixture **7** and **8** in the ABTS<sup>•+</sup> scavenging assay showed weak antioxidant activity with an IC<sub>50</sub> value of 259.3  $\mu$ M, while compounds **6** and **12** were inactive in this assay. All tested compounds showed weaker antioxidant activities than ascorbic acid which was used as the positive control (IC<sub>50</sub> = 15.9 (DPPH) and 8.2 (ABTS)  $\mu$ M). None of the compounds **1**, **3**, **4**, **6–8**, **12**, and **13** showed inhibitory activities against  $\alpha$ -glucosidase at 200  $\mu$ g mL<sup>-1</sup>.





Scheme 1 Plausible biosynthetic pathway for 1 and 2.

## Conclusions

The *Desmos* genus (Annonaceae) is well known as a rich source of flavonoids. Some minor compounds such as chalcones, benzyl benzoate esters, and alkaloids have also been identified. In this study, eight flavones (1–8), four benzyl benzoate esters (9–12), one chalcone (13), and one alkaloid (14) were isolated and identified from the red flowering species of *D. chinensis*. To the best of our knowledge, this is the first discovery of unique hybrids of a benzyl benzoate ester and a flavone (1 and 2) from the Annonaceae family. The putative biogenetic pathways of compounds 1 and 2 is proposed via two *ortho*-quinone methide intermediates of the precursor 3 or 4 coupling with benzyl benzoate ester 12. Some of the isolated compounds from this study were also evaluated for their antioxidant activities and their  $\alpha$ -glucosidase inhibitory activities. Unfortunately, none of them showed significant activities.

## Materials and methods

### General experimental procedures

The IR spectra were recorded using a PerkinElmer FTS FT-IR spectrometer and SHIMADSU spectrophotometer. UV-spectra has been recorded with a PerkinElmer or Varian Cary 5000 UV-vis NIR spectrophotometer. The melting point was determined using a Gallenkamp melting point apparatus. The NMR spectra were recorded using a 400 MHz Bruker AM400 spectrometer and 400 MHz Bruker FTNMR Ultra Shield with tetramethylsilane as the internal standard. HRESIMS mass spectra measured on a Bruker micro TOF mass spectrometer. Quick column chromatography (QCC) and column chromatography (CC) were carried out on silica gel 60 (5–40  $\mu\text{m}$ , SiliCycle® Inc.) and silica gel 100 (63–200  $\mu\text{m}$ , SiliCycle® Inc.), respectively. Sephadex LH-20, when indicated, was also used for CC. Pre-coated thin-layer chromatography (TLC) was performed using silica gel 60 F<sub>254</sub> plates (Merck, USA).

### Plant material

The fruit, leaves, and twigs of *D. chinensis* were collected from Mae Fah Luang University Botanical Garden, Chiang Rai,

Thailand (N: 20.0397°, E: 99.8938°) in June 2017. Plant authentication was verified by Assoc. Prof. Dr Surat Laphookhieo, and a voucher specimen (No. MFU-NPR0162) was deposited at the Natural Products Research Laboratory, School of Science, Mae Fah Luang University.

### Extraction and isolation

Air-dried leaves of *D. chinensis* (1.2 kg) were extracted with EtOAc for 3 days at room temperature and concentrated under reduced pressure to give an EtOAc extract (161.2 g). The leaf extract (161.2 g) was subjected to quick column chromatography (QCC) over silica gel (100% hexanes to 100% EtOAc) to give seven fractions (1LA–1LG). Fraction 1LB (24.7 g) was subjected to CC over Sephadex LH-20 (100% MeOH) to give six subfractions (2LA–2LF). Separation of subfraction 2LC (5.53 g) using CC (1 : 9 v/v, acetone–hexanes) gave six subfractions (3LA–3LF). Subfraction 3LA (20.8 mg) was further purified by CC (1 : 4 v/v, acetone–hexanes) yielding compound 10 (13.3 mg), while subfraction 3LC (127.1 mg) was subjected to CC (1 : 1 v/v, CH<sub>2</sub>Cl<sub>2</sub>–hexanes) to give compound 11 (15.2 mg) together with five subfractions (4LA–4LE). Subfraction 4LC (19.1 mg) was further purified by CC (7 : 3 v/v, CH<sub>2</sub>Cl<sub>2</sub>–hexanes) to provide compound 13 (9.9 mg). Fraction 3LF (781.8 mg) was further separated by CC (100% CH<sub>2</sub>Cl<sub>2</sub>) to afford three subfractions (5LA–5LC). Subfraction 5LC (209.1 mg) was further separated by CC (1 : 4 v/v, acetone–hexanes) to give compound 3 (26.1 mg). Fraction 2LD (7.15 g) was subjected to QCC (1 : 9 v/v, EtOAc–hexanes) to give six subfractions (6LA–6LF). Subfraction 6LB (529.8 mg) was chromatographed by CC (1 : 99 v/v, MeOH–CH<sub>2</sub>Cl<sub>2</sub>) to provide compound 4 (5.1 mg) together with five subfractions (7LA–7LE). A mixture of compounds 7 and 8 (23.8 mg) was obtained from 7LE (205.4 mg) by CC (2 : 3 v/v, EtOAc–hexanes). Fraction 6LD (116.1 mg) was chromatographed over silica gel CC (3 : 2 v/v, CH<sub>2</sub>Cl<sub>2</sub>–hexanes) to give compound 1 (4.1 mg) together with five subfractions (8LA–8LE), while compound 2 (1.8 mg) was obtained from 8LB (16.1 mg) by CC (4 : 1 v/v, CH<sub>2</sub>Cl<sub>2</sub>–hexanes). Fraction 1LE (1.3 g) was isolated by CC over Sephadex LH-20 to give compound 5 (1.1 mg) together with eight subfractions (9LA–9LH). Subfraction 9LE (80.9 mg) was



further separated by CC (1 : 1 v/v, acetone–hexanes) to give compound **6** (2.7 mg). Subfraction 1LG (5.3 g) was chromatographed by CC (3 : 7 v/v, acetone–hexanes) to afford ten subfractions (10LA–10LJ). After that, compound **14** (1.3 mg) was obtained from 10LB (4.2 mg) by CC (1 : 9 v/v, CH<sub>2</sub>Cl<sub>2</sub>–hexanes).

Air-dried fruit of *D. chinensis* (97.6 kg) were extracted with CH<sub>2</sub>Cl<sub>2</sub> over a period of 3 days at room temperature and concentrated under reduced pressure to give an CH<sub>2</sub>Cl<sub>2</sub> extract (18.0 g). The fruit extract (18.0 g) was subjected to CC over silica gel (3 : 7 v/v, acetone–hexanes) to give seven subfractions (1FA–1FG). Fraction 1FC (1.5 g) was purified by CC over Sephadex LH-20 (100% MeOH) to afford compound **11** (3.4 mg), compound **12** (9.2 mg) together with five subfractions (2FA–2FE). While compound **7** (1.3 mg) was obtained from 2FC (14.8 mg) by HPLC RP C<sub>18</sub> (2 : 3 v/v, ACN–H<sub>2</sub>O with 0.05% TFA, 2 mL min<sup>-1</sup>). Subfraction 1FE (1.5 g) was separated by CC (1 : 9 v/v, acetone–hexanes) to give eight subfractions (3FA–3FH). Compounds **3** (3.0 mg) and **4** (4.1 mg), were obtained from 3FF (439.2 mg) by CC (1 : 9 v/v, acetone–hexanes).

Air-dried twigs of *D. chinensis* (1.39 kg) were extracted with EtOAc for 3 days at room temperature and concentrated under reduced pressure to give an EtOAc extract (29.9 g). The twig extract (29.9 g) was subjected to QCC over silica gel (100% hexanes to 100% acetone) to afford six subfractions (1TA–1TF). Fraction 1TB (2.6 g) was subjected to CC (7 : 3 v/v, CH<sub>2</sub>Cl<sub>2</sub>–hexanes) to give six subfractions (2TA–2TF). Fraction 2TB (198.1 mg) was purified by CC (7 : 3 v/v, CH<sub>2</sub>Cl<sub>2</sub>–hexanes) to afford compound **11** (3.9 mg) together with four subfractions (3TA–3TD). Purification of subfraction 3TC (37.1 mg) yielded compound **4** (2.1 mg) by CC (4 : 1 v/v, CH<sub>2</sub>Cl<sub>2</sub>–hexanes), while subfraction 3TD (170.9 mg) was separated by CC (100% CH<sub>2</sub>Cl<sub>2</sub>) to provide compound **3** (2.7 mg).

Desmoschinensisflavone A (**1**) yellow powder; mp. 180–183 °C; UV (MeOH) λ<sub>max</sub> (log ε) 252 (3.10), 280 (3.06), and 331 (2.96) nm; IR (neat) ν<sub>max</sub> 3442, 3375, 2923, 2852, 1779, 1727, 1659, and 1129 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data, see Table 1; HRESITOFMS *m/z* 523.1381 [M – H]<sup>-</sup> (calcd *m/z* for C<sub>31</sub>H<sub>23</sub>O<sub>8</sub>, 523.1393).

Desmoschinensisflavone B (**2**) yellow powder; mp. 179–181 °C; UV (MeOH) λ<sub>max</sub> (log ε) 255 (3.00), 277 (3.01), and 329 (2.92) nm; IR (neat) ν<sub>max</sub> 3442, 2955, 2923, 2852, 1787, 1728, 1660, and 1112 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data, see Table 1; HRESITOFMS *m/z* 523.1402 [M – H]<sup>-</sup> (calcd *m/z* for C<sub>31</sub>H<sub>23</sub>O<sub>8</sub>, 523.1393).

## Conflicts of interest

There are no conflicts to declare.

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