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Structural modification of olibergin A, an isoflavonoid, from *Dalbergia stipulacea* Roxb. and its cytotoxicity†

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Fifteen derivatives were synthesized from olibergin A, a major isoflavonoid isolated from the stems of *Dalbergia stipulacea* Roxb. All compounds were evaluated for cytotoxicity against HCT-116, HT-29, MCF-7 and vero cell lines using MTT assay. Cytotoxicity results showed 5-hydroxy-7,2',4',5'-tetramethoxyisoflavone (**5**) was the most active with IC₅₀ values of 19.03 ± 0.70, 10.83 ± 1.65, 12.53 ± 0.70 and 13.53 ± 0.84 μM against HCT-116, HT-29, MCF-7 and vero cell lines, respectively. It should be noted that 5-hydroxy-7,2',4',5'-tetramethoxyisoflavone (**5**) showed two times less toxicity against vero cells than the cisplatin standard (IC₅₀ = 6.55 ± 0.81 μM) while **5** and cisplatin exhibited nearly equal cytotoxicity against the MCF-7 cell line. 5,7,2',4',5'-Pentamethoxyisoflavanone (**10**) showed an IC₅₀ value of 30.34 ± 1.15 μM against the HCT-116 cell line and exhibited weak cytotoxicity against normal cells, the vero cell line. In addition, 5,7,4'-trihydroxy-2',5'-dimethoxyisoflavan oxime (**13**) demonstrated cytotoxicity against HT-29 cells with an IC₅₀ value of 31.41 ± 1.38 μM and displayed weak activity toward the vero cell line. The information revealed that these compounds were suitable for development to anticancer agents against HCT-116, HT-29 and MCF-7 cell lines.

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1. Introduction

Isoflavonoids are widely distributed in the Fabaceae family, especially in the *Dalbergia* genus such as *D. parviflora*, *D. olivari*, *D. verlutina* and *D. stipulacea*. Many isoflavonoids show various biological activities, for example anti-tumor promoting activity on TPA-induced EBV-EA activation,¹ cytotoxicity against T-47D and BT20 human breast cancer cell lines,² human epidermoid carcinoma of the oral cavity (KB), breast adenocarcinoma (MCF-7), human small cell lung cancer (NCI-H187) cell lines^{3,4} and also estrogenicity.⁵

Dalbergia stipulacea Roxb. belongs to the family Fabaceae. It is a climbing shrub found throughout southern China, eastern India, Myanmar, Laos, Thailand and Vietnam.⁶ It is believed that this plant has been used as an emmenagogue and for abortion when taken in moderate amounts. It can be used for gonorrhoea, syphilis and mouth ulcer.⁶ Furthermore, the roots of this plant are fish poison.⁷ Luteolin 4'-rutoside and luteolin

were isolated from the leaves of this plant.⁸ The chemical constituents from the stems and the roots of this plant are chalcones, isoflavonoids, flavonoids, pterocarpan, diterpenes and coumarins.^{9–11} *R,R*-Velucarpin A, a pterocarpan, from the roots of this plant showed cytotoxicity against HeLa cells with an IC₅₀ value of 10.9 ± 0.42 μM (Posri *et al.*, 2021).⁹ (–)-12 α -hydroxyrotenone from the methanol extract of *D. stipulacea* roots displayed cytotoxicity against HepG2 cell line with an IC₅₀ value of 18.01 ± 1.51 mM.¹⁰ It was found that (–)-vestitol from the stems of this plant displayed the strongest antifungal activity against *Pythium insidiosum* and had higher activity than the amphotericin-B standard.¹¹

Olibergin A, a major isoflavonoid, was isolated from the stems of *D. stipulacea*.¹¹ In previous studies, this compound has been evaluated for biological activities such as the inhibitory effects on TPA-induced EVB-EA activation and on nitric oxide production in RAW 264.7 cells, antiplasmodial, antifungal activity against *Pythium insidiosum* and cytotoxicity against KB, HeLa S-3, MCF-7, HepG2, HT-29 and MRC-5 cell lines.^{11–14} It was reported that olibergin A showed cytotoxicity against KB, HeLa and MRC-5 cell lines with IC₅₀ values of 48.06, 59.82 and 25.03 μM, respectively.^{13,15} In addition, from our screening evaluation on the cytotoxicity of olibergin A against HCT-116, HT-29, MCF-7 and vero cell lines, this compound exhibited moderate to weak activity by showing IC₅₀ values of 79.23 ± 4.78, 32.18 ± 1.73, 72.12 ± 1.12 and 88.83 ± 4.33 μM, respectively. In order to take advantage of this major compound and

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continuing our research on new anticancer agents with high efficacy and low toxicity from natural products, we planned to modify the chemical structure of olibergin A using simple organic reactions and examine the cytotoxicity of all derivatives against four cell lines including HCT-116, HT-29, MCF-7 and vero cell lines. Fifteen derivatives (2–16) from olibergin A were synthesized including methylation, nitration, acetylation, mesylation, hydrogenation, hydrazone and oxime formation reactions.

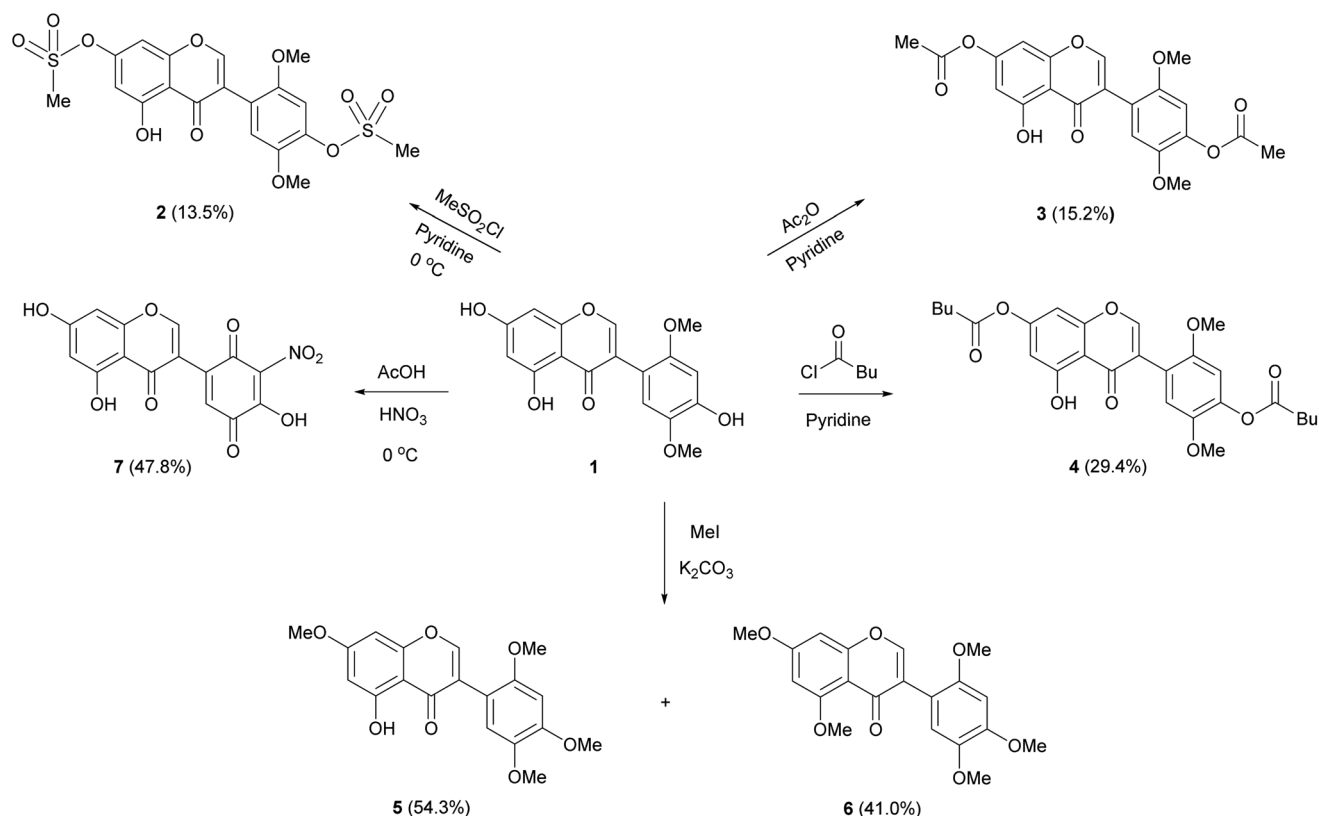
2. Results and discussion

Chemistry

The structural modification of isoflavonoid derivatives started from olibergin A (**1**), a major product from the stems of *D. stipulacea*.¹¹ In our cytotoxicity screening, this compound showed cytotoxicity against HCT-116, HT-29, MCF-7 and vero cell lines, with IC₅₀ values of 79.23 ± 4.78, 32.18 ± 1.73, 72.12 ± 1.12 and 88.83 ± 4.33 μM, respectively. All derivatives were synthesized into three parts, as here described. For the first part, derivatization of hydroxyl groups at C-5, C-7 and C-4' were performed to obtain five derivatives (2–6) and also a nitration reaction to provide **7** as shown in Scheme 1. The second part was examined under catalytic hydrogenation leading to reduction at the C-2/C-3 positions and yielded **8–11** as shown in Scheme 2, and the third part was the conversion of the carbonyl group at the C-4 position to hydrazone and oxime derivatives (**12–16**) as shown in Scheme 3.

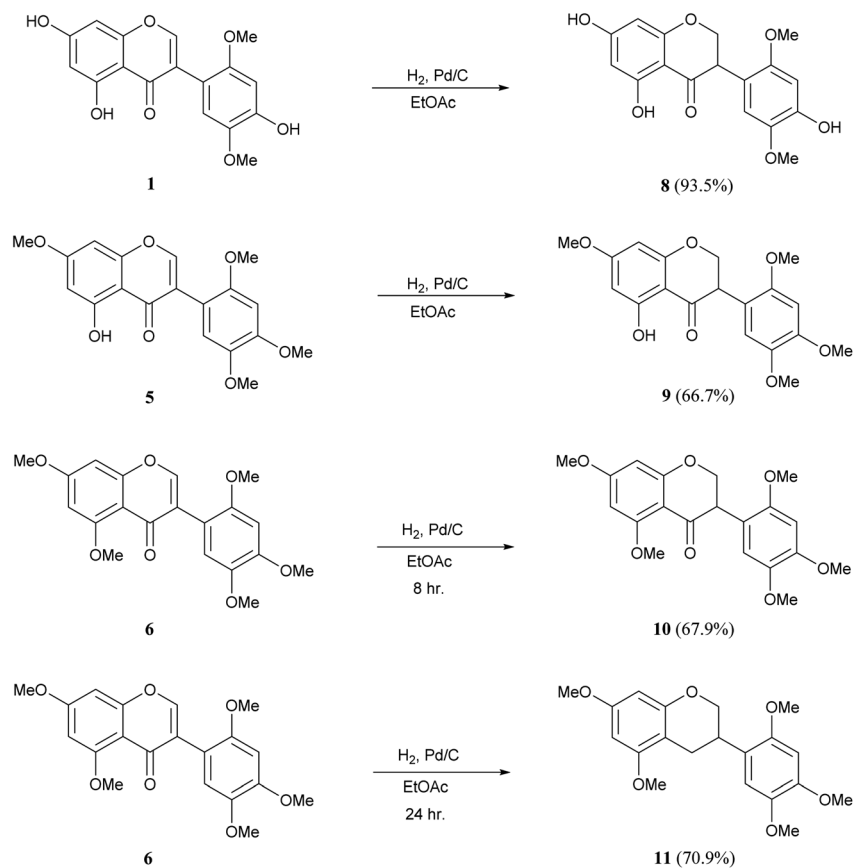
Treatment of **1** with methanesulfonyl chloride, acetic anhydride and valeroyl chloride in the presence of pyridine provided isoflavone derivatives **2** (13.5%), **3** (15.2%) and **4** (29.4%), respectively (Scheme 1).¹⁶ The ¹H NMR data of **2** showed a singlet signal of two methyl groups at δ_H 3.24 corresponding to mesyl groups, while the signals at δ_H 2.34 of **3** represented two additional acetoxy groups. In addition, the ¹³C NMR spectrum of **3** and **4** displayed carbonyl signals at δ_C 180.7 (compound **3**) and at δ_C 181.3 (compound **4**). In the case of **4**, the proton signals of *n*-butyl groups showed at δ_H 2.60, 1.76, 1.47 and 0.98. The methylation of **1** was carried out using CH₃I/K₂CO₃ to obtain the isoflavone derivatives **5** and **6** in 54.3 and 41.0% yield, respectively.¹⁷ The ¹H NMR spectrum of **5** exhibited four methoxy groups while those of **6** showed five methoxy groups and no intramolecular H-bonding signal around δ_H 12.8. Compound **1** was treated with a mixture of nitric acid and acetic acid at 0 °C to furnish derivative **7** in 47.8% yield.¹⁸ The disappearance of two methoxy protons in the ¹H NMR spectral data of the B ring was observed and a singlet proton at δ_H 6.77 (H-6') was found. The ¹³C NMR spectral data showed two signals of α,β-unsaturated ketone groups of a quinone moiety at δ_C 185.2 (C-2') and δ_C 171.3 (C-5') in the B ring. The HMBC correlations between the proton at δ_H 6.77 (H-6') and carbons at δ_C 116.6 (C-3), δ_C 171.3 (C-5') and δ_C 163.2 (C-4') confirmed the assignment of compound **7**.

Catalytic hydrogenation of **1**, **5** and **6** was performed under hydrogen gas, using palladium on charcoal as a catalyst for 8 hours to furnish isoflavanones **8** (93.5%), **9** (66.7%) and **10**

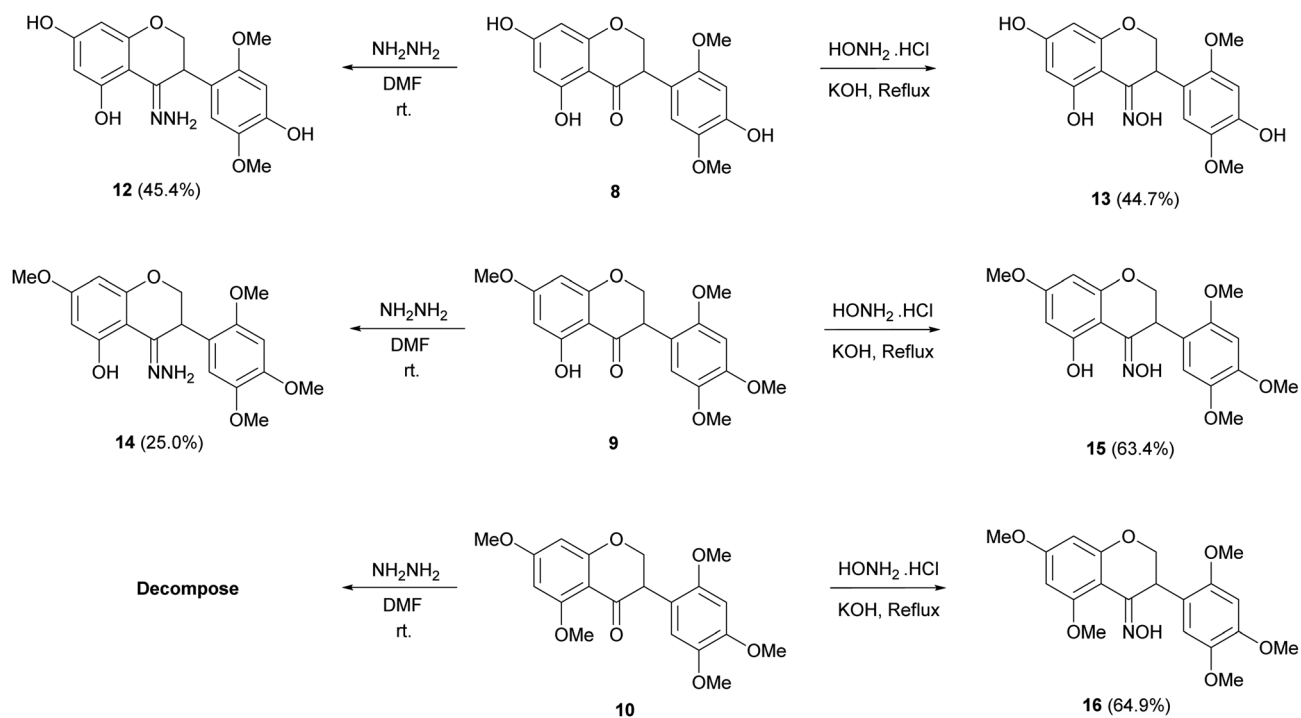


Scheme 1 Derivatives of Olibergin A (**1**).

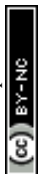




Scheme 2 Derivatives of hydrogenation.



Scheme 3 Hydrazone and oxime derivatives.



(67.9%).¹⁹ Isoflavan **11** was obtained from hydrogenation of **6** for a longer time (24 hours) in 70.9% yield (Scheme 2). It should be noted that the reduction reactions of **1** and **5** for a long time gave no isoflavan derivative. This may be due to the chelating effect between the hydroxy group at C-5 and the carbonyl group at C-4 position. The disappearance of the olefinic proton at δ_{H} 7.77 (H-2) of **1** and the presence of ABX signals at δ_{H} 4.53 (H-2), 4.40 (H-2) and 4.29 (H-3) confirmed the isoflavanone core structure of **8**. The isoflavanone **8** was identified as a racemic mixture product, which was confirmed by the ECD spectrum and optical rotation by showing the lack of optical rotation and no obviously observed Cotton effect in the experimental ECD spectrum. This evidence was observed in **9** and **10** as well. In the case of **11**, the ¹H NMR data showed five alicyclic protons at δ_{H} 4.26 and 3.98 (H-2), 3.54 (H-3), 2.87 and 2.69 (H-4) and the absence of a carbonyl carbon signal at the C-4 position.

Hydrazone derivatives **12** (45.4%) and **14** (25.0%) were synthesized by the condensation between compounds **8** and **9** with hydrazine hydrate in DMF as a solvent at room temperature, respectively (Scheme 3).²⁰ In contrast, it was attempted to construct a hydrazone derivative of compound **10** but the product decomposed immediately after purification. The ¹³C NMR spectra of C-4 in hydrazone derivatives, **12** and **14**, showed upfield shift (δ_{C} 158.1 and 163.0) while the signals of carbonyl compounds, **8** and **9**, showed at δ_{C} 197.3 and 197.4. The isoflavanones **8**, **9** and **10** were further treated with hydroxylamine hydrochloride in KOH and gave oxime derivatives **13**, **15** and **16** in 44.7, 63.4 and 64.9% yield, respectively.²⁰ In the same manner as **12** and **14**, oxime carbons (C-4) of these compounds showed upfield shift to δ_{C} 160.3–167.3 in the ¹³C-NMR spectra.

Biological activity

All compounds were evaluated for cytotoxicity against two human colon cancer cell lines, HCT-116 and HT-29, and breast cancer cells (MCF-7) as well as normal cells, vero cells (kidney

African Green Monkey cells), using MTT assay. The cytotoxicity results are shown in Table 1.

Cytotoxicity of isoflavonoid, olibergin A (**1**), showed weak activity against three cancer cell lines and vero cells as mentioned above (Table 1). Fortunately, some synthesized olibergin A derivatives exhibited stronger activity than the starting material, especially compound **5** which displayed IC₅₀ values of 19.03 ± 0.70, 10.83 ± 1.65 and 12.53 ± 0.70 μM against HCT-116, HT-29 and MCF-7 cancer cell lines, respectively. In the cases of cytotoxicity against HCT-116 cell line, compound **5** showed four times stronger cytotoxicity than **1**; it seems that the methoxy groups at C-7 and C-4' and the hydroxyl group at C-5 in the isoflavone core structure may play an important role for the activity. Similar results were detected for cytotoxicity against MCF-7 cells, which exhibited six times stronger than **1**. In addition, this derivative demonstrated three times stronger than the starting material against the HT-29 cell line. It is interesting that **5** showed activity against MCF-7 (IC₅₀ = 12.53 ± 0.70 μM) nearly equal to the cisplatin standard (IC₅₀ = 10.42 ± 0.85 μM), but **5** displayed cytotoxicity against normal cells with an IC₅₀ value of 13.53 ± 0.84 μM, while the cisplatin standard (IC₅₀ = 6.55 ± 0.81 μM) displayed more toxic than **5**. This information may be useful for further study on the anticancer agent. In comparison between **5** and **6**, compound **6** showed weak activity to all cell lines. The results indicate that the hydroxyl group at the C-5 position of the isoflavone derivative was necessary for the activity. Cytotoxicity results of **2**, **3** and **4** exhibited inactivity against all tested cell lines, which means mesyl and ester functional groups at C-7 and C-4' positions dramatically decrease the activity.

In order to know the effect of the double bond at C-2/C-3, isoflavones were reduced to be isoflavanones **8**, **9** and **10** and also isoflavan **11**. It was found that **9** and **11** displayed stronger activity against all cell lines than olibergin A (**1**) by showing IC₅₀ values around 19–37 μM. This information confirmed that presence of a saturated moiety on the C ring may be important

Table 1 Cytotoxicity of all compounds (IC₅₀, μM) at 72 hours^a

Compound	HCT-116	HT-29	MCF-7	Vero cell
1	79.23 ± 4.78	32.18 ± 1.73	72.12 ± 1.12	88.83 ± 4.33
2	Inactive	Inactive	Inactive	Inactive
3	Inactive	Inactive	Inactive	111.61 ± 12.26
4	Inactive	Inactive	Inactive	Inactive
5	19.03 ± 0.70	10.83 ± 1.65	12.53 ± 0.70	13.53 ± 0.84
6	177.62 ± 02.23	154.47 ± 12.08	98.53 ± 15.36	>268.55
7	57.18 ± 3.56	Inactive	45.91 ± 3.27	24.59 ± 3.79
8	92.69 ± 1.84	69.92 ± 6.47	147.87 ± 1.96	93.63 ± 10.23
9	37.07 ± 1.28	19.20 ± 1.86	30.97 ± 1.89	33.52 ± 1.25
10	30.34 ± 1.15	Inactive	73.75 ± 0.77	116.38 ± 20.19
11	28.94 ± 3.19	29.38 ± 0.08	21.06 ± 2.61	31.13 ± 4.99
12	126.32 ± 0.03	97.77 ± 1.73	165.59 ± 4.79	133.92 ± 1.56
13	82.60 ± 7.89	31.41 ± 1.38	109.55 ± 2.48	103.71 ± 24.96
14	Inactive	Inactive	Inactive	Inactive
15	26.40 ± 0.05	38.55 ± 2.77	28.93 ± 3.94	29.78 ± 4.85
16	137.80 ± 0.36	114.82 ± 2.62	110.40 ± 4.52	158.70 ± 8.29
Cisplatin	4.93 ± 0.77	5.30 ± 0.53	10.42 ± 0.85	6.55 ± 0.81

^a Inactive at >200 μM.



for the activity. However, isoflavanone **8** demonstrated weak cytotoxicity ($IC_{50} \cong 69\text{--}147 \mu\text{M}$). Comparing the activity between **8** and **9**, it seems that methoxy groups at C-7 and C-4' may be necessary for the activity. It is interesting that **10** showed strong activity against the HCT-116 cell line with an IC_{50} value of $30.34 \pm 1.15 \mu\text{M}$, but displayed weak activity against vero cells ($IC_{50} = 116.38 \pm 20.19 \mu\text{M}$). This information indicates that this compound is suitable for the development of an anticancer agent against HCT-116 cells.

In the cases of oximes **13** and **15**, compound **13** displayed cytotoxicity against HT-29 ($IC_{50} = 31.41 \pm 1.38 \mu\text{M}$) but showed weak cytotoxicity against vero cells ($IC_{50} = 103.71 \pm 24.96 \mu\text{M}$). There was good evidence for the development of this compound. Compound **15** displayed IC_{50} around 26–38 μM toward all cell lines. Comparing between **13** and **15**, it seems the methoxy groups at C-7 and C-4' may be important for the cytotoxicity. In the cases of **15** and **16**, compound **16** exhibited weaker cytotoxicity against all cell lines. It seems the hydroxy group at the C-5 position is more important for the cytotoxicity than the methoxy group in the same manner as isoflavones **5** and **6**.

Hydrazones **12** and **14** showed weak and inactive against four cell lines; these results suggest that this functional group is not suitable for cytotoxicity.

3. Experimental part

General experimental procedures

Melting points were determined on a SANYO Gallenkamp (Leicester, UK) melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO P-1020 digital polarimeter (Japan). UV spectra were measured on an Agilent 8453 UV-Visible spectrophotometer (Waldbronn, Germany). IR spectra were recorded with diamond dust, using an Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) Bruker TENSOR 27 (Germany). NMR spectra were collected at 400 MHz (^1H) and at 100 MHz (^{13}C) using a Bruker AVANCE NEO. The LC-QTOF-MS/MS analysis was performed on an Agilent HPLC 1260 series coupled with a QTOF 6540 UHD accurate mass (Agilent Technologies, Waldbronn, Germany). Circular dichroism spectroscopy is determined using a Jasco J-815 CD Spectrometer. Thin layer chromatography (TLC) was carried out on MERCK silica gel 60 F_{254} TLC aluminium sheets. Column chromatography separations were carried out on silica gel less than 0.063 mm, 0.063–0.200 mm (Darmstadt, Germany). Preparative thin layer chromatography (PLC) was carried out on glass supported silica gel plates using silica gel 60 PF_{254} for preparative layer chromatography (Darmstadt, Germany). All solvents were routinely distilled prior to use.

Plant material

The stems of *D. stipulacea* were collected in February 2018 at Ban Na Phaeng village, Wiang Kao district, Khon Kaen province, Thailand ($16^\circ 41' 53.5''\text{N}$, $102^\circ 20' 24.1''\text{E}$). Plant material (voucher specimen KKU012018) was identified by Assoc. Prof. Suppachai Tiwawanant, Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand.

Preparation of isoflavone derivatives

General procedure to prepare 2, 3 and 4 (ref. 16). To a solution of compound **1** (52.8 mg, 0.1599 mmol) in pyridine (0.5 mL) was added dropwise MsCl (excess) at 0 °C, and the reaction mixture was stirred at room temperature for 30 min. The entire reaction mixture was poured into cold water and extracted with EtOAc ($3 \times 20 \text{ mL}$). The organic layer were combined, washed with water, saturated NaCl, dried over anhydrous Na_2SO_4 and evaporated to give a crude oil, which was purified by PLC (20% EtOAc:hexane) to give **2** (10.5 mg, 13.5%).

The reaction of **1** with acetic anhydride and valerory chloride at room temperature were examined in the same procedure as described above to construct **3** (15.2%) and **4** (29.4%), respectively.

Methylation to prepare 5 (ref. 17 and 21–23) and 6 (ref. 17, 22 and 24). A mixture of **1** (121.4 mg, 0.3757 mmol), anhydrous potassium carbonate (550.5 mg) and methyl iodide (0.25 mL) in dry acetone (5 mL) was refluxed overnight. The reaction mixture was evaporated, work up with water and extracted with EtOAc ($3 \times 20 \text{ mL}$), washed with water and saturated NaCl, dried over anhydrous Na_2SO_4 , filtered and evaporated to dryness. The residue was purified by column chromatography (30% EtOAc:hexane) to give **5** (73.1 mg, 54.3%) and **6** (57.3 mg, 41.0%).

Nitration reaction to prepare 7 (ref. 18). Compound **1** (15.2 mg, 0.0460 mmol) was treated with a mixture of nitric acid and acetic acid (0.7 : 1, 0.25 mL) at 0 °C with stirring for 1 h. The reaction mixture was poured into ice cold water, extracted with EtOAc ($3 \times 20 \text{ mL}$), washed with water and saturated NaCl, dried over anhydrous Na_2SO_4 , filtered and evaporated to dryness. The residue was recrystallized to give nitroisoflavone **7** (6.8 mg, 47.8% yield).

Hydrogenation to prepare 8, 9, 10 and 11 (ref. 19). To a solution of **1** (60.0 mg, 0.1805 mmol) in EtOAc (5 mL) was added a catalytic amount of palladium on charcoal. The solution was stirred under hydrogen gas at room temperature for 8 h. The mixture was filtered and evaporated *in vacuo* to provide isoflavanone **8** (56.1 mg, 93.5%).

The hydrogenations of **5** and **6** were examined in the same procedure as described above to furnish isoflavanones **9** (62.2 mg, 66.7%) and **10** (32.7 mg, 67.9%), respectively. Moreover, the hydrogenation of **6** was done in the same procedure as described above but took 24 hours to give isoflavan **11** (55.7 mg, 88.6%).

General procedure for the preparation of oximes 13, 15 and 16 (ref. 20). To a solution of flavanones **8** (0.1508 mmol) in EtOH (2 mL) was added $\text{NH}_2\text{OH} \cdot \text{HCl}$ (0.75 mmol) and 20% aqueous KOH (1 mL). The reaction mixture was refluxed for 3 hours and then poured into the cold 10% HCl. The mixture was extracted with EtOAc ($3 \times 20 \text{ mL}$), washed with water, saturated NaCl, dried over anhydrous Na_2SO_4 and evaporated to dryness. The crude solid was purified by PLC (40% EtOAc:hexane) to yield oxime derivative **13** (23.4 mg, 44.7%).

The reactions of **9** (0.0916 mmol) and **10** (0.1349 mmol) with $\text{NH}_2\text{OH} \cdot \text{HCl}$ (2.76 and 0.67 mmol) were examined in the same procedure as described above and then **15** (21.8 mg, 63.4%) and **16** (34.1 mg, 64.9%), respectively, were obtained.



General procedure for the preparation of hydrazones 12 and 14 (ref. 20). To a solution of $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$ (excess) in DMF (1 mL) was added compound **8** (0.0451 mmol) at room temperature and was stirred for 24 hours. The entire reaction mixture was poured into cold water and extracted with EtOAc (3×20 mL). The organic layers were combined, washed with water, saturated NaCl, dried over anhydrous Na_2SO_4 and evaporated to dryness. The crude solid was purified by PLC (40% EtOAc:hexane) to yield hydrazone derivative **12** (7.1 mg, 45.4%).

The preparation of hydrazone derivatives from **9** (0.1249 mmol) and **10** (0.1130 mmol) with $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$ was examined in the same procedure as described above and then **14** (21.8 mg, 63.4%) was obtained. Unfortunately, the hydrazone derivative from **10** decomposed.

Spectroscopic data

Olibergin A (**1**) yellow solid; mp 240–242 °C; FT-IR (neat) ν_{max} cm^{-1} : 3254, 2963, 2940, 1655, 1577, 1620, 1455, 1309, 1177, 1037, 861; ^1H NMR (400 MHz, CDCl_3): δ 12.87 (1H, s, OH-5), 7.77 (1H, s, H-2), 6.78 (1H, s, H-6'), 6.56 (1H, s, H-3'), 6.30 (1H, d, $J = 2.1$ Hz, H-8), 6.21 (1H, d, $J = 2.1$ Hz, H-6), 3.78 (3H, s, OMe-5'), 3.66 (3H, s, OMe-2') ppm; ^{13}C NMR (100 MHz, CDCl_3): 180.7 (C-4), 163.8 (C-7), 161.9 (C-5), 158.1 (C-8a), 154.7 (C-2), 152.2 (C-2'), 147 (C-4'), 140.7 (C-5'), 120.2 (C-3), 114.9 (C-1'), 109.8 (C-6'), 105.4 (C-4a), 100.3 (C-6), 99.1 (C-3'), 94.1 (C-8), 56.6 (OMe-5'), 56.2 (OMe-2') ppm; HRESI-MS m/z 331.0818 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{22}\text{H}_{23}\text{O}_7$, 331.0815).

5-Hydroxy-7,4'-dimesyl-2',5'-dimethoxyisoflavone (**2**) white powder; mp 218–220 °C; FT-IR (neat) ν_{max} cm^{-1} : 2945, 2843, 1644, 1590, 1508, 1461, 1354, 1273, 1212, 1172, 1110, 967, 891; ^1H NMR (400 MHz, CDCl_3): δ 12.82 (1H, s, OH-5), 8.04 (1H, s, H-2), 6.75 (1H, d, $J = 2.2$ Hz, H-6), 6.96 (1H, d, $J = 2.2$ Hz, H-8), 7.00 (1H, s, H-3'), 7.07 (1H, s, H-6'), 3.78 (3H, s, OMe-2'), 3.88 (3H, s, OMe-5'), 3.24 (6H, s, $\text{MeSO}_2\text{O-7,4}'$) ppm; ^{13}C NMR (100 MHz, CDCl_3): 181.1 (C-4), 163.2 (C-5), 157.3 (C-7), 156.7 (C-8a), 154.2 (C-2), 151.8 (C-2'), 145.5 (C-4'), 139.2 (C-5'), 120.7 (C-3), 118.4 (C-6'), 117 (C-1'), 110.8 (C-4a), 109.5 (C-3'), 105.8 (C-6), 101.7 (C-8), 57.2 (OMe-2'), 57.0 (OMe-5), 38.6, 38.9 ($\text{MeSO}_2\text{O-7,4}'$) ppm; HRESI-MS m/z 487.0366 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{19}\text{H}_{19}\text{O}_{11}\text{S}_2$, 487.0369).

7,4'-Diacetoxy-5-hydroxy-2',5'-dimethoxyisoflavone (**3**) white solid; mp 242–244 °C; FT-IR (neat) ν_{max} cm^{-1} : 2968, 2935, 1772, 1752, 1649, 1509, 1453, 1193, 1168, 1129, 1031, 882; ^1H NMR (400 MHz, CDCl_3): δ 12.78 (1H, s, OH-5), 7.97 (1H, s, H-2), 6.59 (1H, d, $J = 2.0$ Hz, H-6), 6.77 (1H, d, $J = 2.0$ Hz, H-8), 7.75 (1H, s, H-3'), 7.00 (1H, s, H-6'), 3.74 (3H, s, OMe-2'), 3.82 (3H, s, OMe-5'), 2.34 (3H, s, MeCOO-7), 2.34 (3H, s, $\text{MeCOO-4}'$) ppm; ^{13}C NMR (100 MHz, CDCl_3): 180.6 (C-4), 168.8 (Mecmb.b.lineCOO-7), 168 ($\text{Mecmb.b.lineCOO-4}'$), 162.1 (C-5), 156.5 (C-7), 155.6 (C-8a), 155.5 (C-2), 151.2 (C-2'), 144.1 (C-4'), 140.3 (C-5'), 120.4 (C-3), 116.6 (C-6'), 116 (C-1'), 109.3 (C-4a), 107.3 (C-3'), 105.3 (C-6), 100.7 (C-8), 56.4 (OMe-2'), 56.3 (OMe-5'), 21 (MeCOO-7), 20.5 ($\text{MeCOO-4}'$) ppm; HRESI-MS m/z 415.1033 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{21}\text{H}_{19}\text{O}_9$, 415.1029).

7,4'-Dibutoxy-5-hydroxy-2',5'-dimethoxyisoflavone (**4**) yellow powder; mp 135–137 °C; FT-IR (neat) ν_{max} cm^{-1} : 2951, 2868,

1765, 1646, 1589, 1511, 1462, 1277, 1218, 1039; ^1H NMR (400 MHz, CDCl_3): δ 12.78 (1H, s, OH-5), 7.96 (1H, s, H-2), 6.57 (1H, d, $J = 2.1$ Hz, H-6), 6.75 (1H, d, $J = 2.1$ Hz, H-8), 6.73 (1H, s, H-3'), 6.99 (1H, s, H-6'), 3.74 (3H, s, OMe-2'), 3.80 (3H, s, OMe-5'), 2.60 (2H, q, $J = 7.4$ Hz, $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH CO-7,4}'$), 1.76 (2H, dq, $J = 14.9, 7.3$ Hz, $\text{CH}_3\text{CH}_2\text{CH CH}_2\text{CO-7,4}'$), 1.47 (2H, dq, $J = 14.5, 7.3$ Hz, $\text{CH}_3\text{CH CH}_2\text{CH}_2\text{CO-7,4}'$), 0.98 (3H, t, $J = 7.4$ Hz, $\text{CH CH}_2\text{CH}_2\text{CH}_2\text{CO-7,4}'$) ppm; ^{13}C NMR (100 MHz, CDCl_3): 181.3 (C-4), 171.7/172.2 ($\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{cmb.b.lineCO-7,4}'$), 162.7 (C-5), 157.3 (C-7), 156.4 (C-8a), 156.1 (C-2), 151.9 (C-2'), 145.4 (C-5'), 141.1 (C-4'), 121.1 (C-3), 117.1 (C-1'), 116.6 (C-3'), 109.9 (C-6'), 107.9 (C-4a), 105.9 (C-6), 101.4 (C-8), 57.1 (OMe-2'), 56.9 (OMe-5'), 34.2/34.5 ($\text{CH}_3\text{CH}_2\text{CH}_2\text{CH CO-7,4}'$), 27.2/27.5 ($\text{CH}_3\text{CH}_2\text{CH CH}_2\text{CO-7,4}'$), 22.6 ($\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CO-7,4}'$), 13.8 ($\text{CH CH}_2\text{CH}_2\text{CH}_2\text{CO-7,4}'$) ppm; HRESI-MS m/z 499.1971 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{27}\text{H}_{31}\text{O}_9$, 499.1968).

5-Hydroxy-7,2',4',5'-tetramethoxyisoflavone (**5**) Yellow powder; mp 164–165 °C; FT-IR (neat) ν_{max} cm^{-1} : 2942, 2839, 1656, 1610, 1514, 1499, 1294, 1207, 1155, 1034, 819; ^1H NMR (400 MHz, CDCl_3): δ 7.97 (1H, s, H-2), 12.86 (1H, s, OH-5), 6.35 (1H, d, $J = 2.3$ Hz, H-6), 6.96 (1H, d, $J = 2.2$ Hz, H-8), 6.61 (1H, s, H-3'), 6.88 (1H, s, H-6'), 3.77 (3H, s, OMe-7), 3.91 (3H, s, OMe-2'), 3.84 (3H, s, OMe-4'), 3.85 (3H, s, OMe-5') ppm; ^{13}C NMR (100 MHz, CDCl_3): 180.8 (C-4), 165.4 (C-7), 162.6 (C-5), 157.9 (C-8a), 154.9 (C-2), 152 (C-2'), 150.1 (C-4'), 143.1 (C-5'), 120.6 (C-3), 115.1 (C-1'), 110.7 (C-6'), 106.3 (C-4a), 98.1 (C-6), 98.1 (C-3'), 92.4 (C-8), 56.8 (OMe-2'), 56.4 (OMe-4), 56.2 (OMe-5), 55.8 (OMe-7) ppm; HRESI-MS m/z 359.1129 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{19}\text{H}_{19}\text{O}_7$, 359.1131).

5,7,2',4',5'-Pentamethoxyisoflavone (**6**) black needles; mp 188–189 °C; FT-IR (neat) ν_{max} cm^{-1} : 2942, 2845, 1615, 1457, 1282, 1210, 1150; ^1H NMR (400 MHz, CDCl_3): δ 7.97 (1H, s, H-2), 6.35 (1H, d, $J = 2.3$ Hz, H-6), 6.96 (1H, d, $J = 2.2$ Hz, H-8), 6.61 (1H, s, H-3'), 6.88 (1H, s, H-6'), 3.77 (3H, s, OMe-7), 3.91 (3H, s, OMe-2'), 3.84 (3H, s, OMe-4'), 3.85 (3H, s, OMe-5'), 3.85 (3H, s, OMe-5') ppm; ^{13}C NMR (100 MHz, CDCl_3): 175.1 (C-4), 163.5 (C-7), 161.1 (C-5), 159.7 (C-8a), 152.1 (C-2), 151.6 (C-2'), 149.3 (C-4'), 142.6 (C-5'), 122.2 (C-3), 115.5 (C-1'), 112.2 (C-6'), 109.8 (C-4a), 97.9 (C-3'), 95.9 (C-6), 92.4 (C-8), 56.7 (OMe-2'), 56.3 (OMe-4'), 56.1 (OMe-5'), 55.9 (OMe-5), 55.5 (OMe-7) ppm; HRESI-MS m/z 373.1284 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{20}\text{H}_{21}\text{O}_7$, 373.1287).

1-(5,7-dihydroxy-4-oxo-4H-1-benzopyran-3-yl)-4-hydroxy-3-nitro-2,5-quinone (**7**) brown solid; mp 190–191 °C; FT-IR (neat) ν_{max} cm^{-1} : 3377, 2924, 2855, 1620, 1495, 1325, 1285, 1233, 1161, 1047; ^1H NMR (400 MHz, DMSO-d_6): δ 12.57 (1H, s, OH-5), 8.30 (1H, s, H-2), 6.21 (1H, s, H-6), 6.37 (1H, s, H-8), 6.75 (1H, s, H-6') ppm; ^{13}C NMR (100 MHz, CDCl_3): 185.4 (C-2'), 179.1 (C-4), 171.5 (C-5'), 166.1 (C-7), 163.4 (C-4'), 161.9 (C-5), 157.4 (C-8a), 157.2 (C-2), 140.7 (C-1'), 134.4 (C-3'), 131.5 (C-6'), 116.8 (C-3), 103.8 (C-4a), 99.9 (C-6), 94.4 (C-8) ppm; HRESI-MS m/z 346.0193 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{15}\text{H}_8\text{NO}_9$, 346.0194).

5,7,4'-Trihydroxy-2',5'-dimethoxyisoflavanone (**8**) brown solid; mp 176–177 °C; FT-IR (neat) ν_{max} cm^{-1} : 3343, 2939, 2843, 1629, 1512, 1455, 1380, 1267, 1196, 1165, 1094, 1035; ^1H NMR (400 MHz, $\text{CDCl}_3 + \text{CD}_3\text{OD}$): δ 12.27 (1H, s, OH-5), 6.60 (1H, s, H-3'), 6.63 (1H, s, H-6'), 5.99 (1H, d, $J = 2.2$ Hz, H-8), 5.94 (1H, d, $J = 2.2$ Hz, H-6), 4.53 (1H, t, $J = 11.4$ Hz, H-2), 4.40 (1H, dd, $J =$



11.0, 5.6 Hz, H-2), 4.29 (1H, dd, $J = 11.9, 5.6$ Hz, H-3), 3.82 (3H, s, OMe-2'), 3.73 (3H, s, OMe-5') ppm; ^{13}C NMR (100 MHz, CDCl_3): 197.3 (C-4), 166.3 (C-7), 164.1 (C-5), 163.4 (C-8a), 152.2 (C-2'), 146.4 (C-4'), 141.0 (C-5'), 113.8 (C-1'), 113.1 (C-6'), 102.7 (C-4a), 100.4 (C-3'), 96.4 (C-6), 95.3 (C-8), 70.3 (C-2), 56.7 (OMe-2), 56.0 (OMe-5'), 46.9 (C-3) ppm; HRESI-MS m/z 334.1034 $[\text{M} + 2\text{H}]^+$ (calcd for $\text{C}_{17}\text{H}_{18}\text{O}_7$, 334.1042).

5-Hydroxy-7,2',4',5'-tetramethoxyisoflavanone (9) white powder; mp 97–99 °C; FT-IR (neat) ν_{max} cm^{-1} : 2995, 2938, 2836, 1640, 1575, 1511, 1437, 1316, 1271, 1205, 1156, 1033; ^1H NMR (400 MHz, CDCl_3): δ 12.24 (1H, s, OH-5), 6.66 (1H, s, H-6'), 6.57 (1H, s, H-3'), 6.07 (1H, d, $J = 2.2$ Hz, H-8), 6.00 (1H, d, $J = 2.2$ Hz, H-6), 4.53 (1H, t, $J = 12.0$ Hz, H-2), 4.43 (1H, dd, $J = 10.9, 5.7$ Hz, H-2), 4.28 (1H, dd, $J = 11.9, 5.6$ Hz, H-3), 3.88 (3H, s, OMe-2'), 3.82 (3H, s, OMe-5'), 3.80 (3H, s, OMe-4'), 3.77 (3H, s, OMe-7) ppm; ^{13}C NMR (100 MHz, CDCl_3): 197.3 (C-4), 167.7 (C-7), 164.6 (C-5), 163.3 (C-8a), 151.9 (C-2'), 149.7 (C-4'), 143.3 (C-5'), 114.3 (C-1'), 113.9 (C-6'), 103.4 (C-4a), 98.2 (C-3'), 95 (C-8), 94 (C-6), 70.4 (C-2), 55.7 (OMe-7), 56.7 (OMe-2'), 56.5 (OMe-4'), 56.2 (OMe-5'), 47.3 (C-3) ppm; HRESI-MS m/z 361.1288 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{19}\text{H}_{21}\text{O}_7$, 361.1287).

5,7,2',4',5'-Pentamethoxyisoflavanone (10) white powder; mp 121–123 °C; FT-IR (neat) ν_{max} cm^{-1} : 2940, 2843, 1675, 1606, 1514, 1460, 1213, 1160, 1118, 1032; ^1H NMR (400 MHz, CDCl_3): δ 6.65 (1H, s, H-6'), 6.54 (1H, s, H-3'), 6.09 (1H, s, H-6), 6.09 (1H, s, H-8), 4.52 (1H, t, $J = 12.0$ Hz, H-2), 4.44 (1H, dd, $J = 10.8, 5.3$ Hz, H-2), 4.24 (1H, dd, $J = 11.2, 5.3$ Hz, H-3), 3.87 (3H, s, OMe-5), 3.77 (3H, s, OMe-7), 3.87 (3H, s, OMe-2'), 3.77 (3H, s, OMe-4'), 3.83 (3H, s, OMe-5') ppm; ^{13}C NMR (100 MHz, CDCl_3): 190.1 (C-4), 165.7 (C-7), 165.3 (C-5), 162.7 (C-8a), 152.1 (C-2'), 149.2 (C-4'), 143.1 (C-5'), 115.6 (C-1'), 114.3 (C-6'), 106.8 (C-4a), 98.1 (C-3'), 93.4 (C-8), 93.1 (C-6), 70.8 (C-2), 56.8 (OMe-2'), 56.7 (OMe-4'), 56.2 (OMe-5), 55.7 (OMe-7), 53.2 (OMe-5'), 48.0 (C-3) ppm; HRESI-MS m/z 375.1444 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{20}\text{H}_{23}\text{O}_7$, 375.1444).

5,7,2',4',5'-Pentamethoxyisoflavan (11) brown liquid; FT-IR (neat) ν_{max} cm^{-1} : 2937, 2843, 1603, 1509, 1457, 1316, 1206, 1117, 1037; ^1H NMR (400 MHz, CDCl_3): δ 6.71 (1H, s, H-6'), 6.55 (1H, s, H-3'), 6.08 (1H, s, H-6), 6.08 (1H, s, H-8), 4.26 (1H, ddd, $J = 10.2, 3.4, 2.0$ Hz, H-2), 3.98 (1H, t, $J = 10.0$ Hz, H-2), 3.89 (3H, s, OMe-5), 3.81 (3H, s, OMe-2'), 3.80 (3H, s, OMe-5'), 3.79 (3H, s, OMe-4'), 3.72 (3H, s, OMe-7), 3.54 (1H, m, Hz, H-3), 2.87 (1H, dd, $J = 5.5, 1.8$ Hz, H-4), 2.69 (1H, dd, $J = 16.4, 10.8$ Hz, H-4) ppm; ^{13}C NMR (100 MHz, CDCl_3): δ 159.4 (C-7), 158.7 (C-5), 155.8 (C-8a), 151.8 (C-2'), 148.4 (C-4'), 143.2 (C-5'), 121.3 (C-1'), 112.1 (C-6'), 103.9 (C-4a), 97.9 (C-3'), 93.3 (C-8), 91.3 (C-6), 70 (C-2), 56.9 (OMe-2'), 56.5 (OMe-4'), 56.3 (OMe-5), 55.5 (OMe-7), 55.4 (OMe-5'), 31.5 (C-3), 25.2 (C-4) ppm; HRESI-MS m/z 361.1650 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{20}\text{H}_{24}\text{O}_6\text{Na}$, 361.1651).

5,7,4'-Trihydroxy-2',5'-dimethoxyisoflavan hydrazone (12) light brown solid; mp 245–246 °C; FT-IR (neat) ν_{max} cm^{-1} : 3363, 2940, 2841, 1630, 1600, 1513, 1460, 1236, 1194, 1038; ^1H NMR (400 MHz, $\text{CDCl}_3 + \text{CD}_3\text{OD}$): δ 4.13 (2H, br s, H-2), 4.39 (1H, s, H-3), 5.85 (1H, d, $J = 2.2$ Hz, H-6), 5.96 (1H, d, $J = 2.2$ Hz, H-8), 6.39 (1H, s, H-3'), 6.46 (1H, s, H-6'), 3.71 (3H, s, OMe-2'), 3.48 (3H, s, OMe-5') ppm; ^{13}C NMR (100 MHz, CDCl_3): δ 160.6 (C-4), 159.9 (C-7), 152.3 (C-8a), 158.1 (C-5), 151.3 (C-2'), 146.7 (C-4'), 141.2 (C-

5'), 113.7 (C-1'), 112.2 (C-6'), 99.8 (C-4a), 99.2 (C-3'), 97.0 (C-6), 95.4 (C-8), 69.6 (C-2), 56.6 (OMe-2'), 55.9 (OMe-5'), 32.2 (C-3) ppm; HRESI-MS m/z 347.1235 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{17}\text{H}_{19}\text{N}_2\text{O}_6$, 347.1243).

5,7,4'-Trihydroxy-2',5'-dimethoxyisoflavan oxime (13) brown solid; mp 172–174 °C; FT-IR (neat) ν_{max} cm^{-1} : 3353, 2939, 2843, 1629, 1512, 1455, 11380, 1288, 1165, 1158, 1093, 1035, 826; ^1H NMR (400 MHz, $\text{CDCl}_3 + \text{CD}_3\text{OD}$): δ 6.50 (1H, s, H-6'), 6.32 (1H, s, H-3'), 6.02 (1H, s, H-8), 4.81 (1H, br s, H-3), 4.25 (1H, d, $J = 11.0$, Hz, H-2), 4.13 (1H, dd, $J = 11.0, 4.0$ Hz, H-2), 5.89 (1H, d, s, H-6), 3.74 (3H, s, OMe-2'), 3.50 (3H, s, OMe-5') ppm; ^{13}C NMR (100 MHz, CDCl_3): δ 160.2 (C-4), 159.1 (C-7), 158.2 (C-5), 155.1 (C-8a), 151.5 (C-2'), 145.7 (C-4'), 140.5 (C-5'), 116.4 (C-1'), 112.3 (C-6'), 100.0 (C-4a), 98.3 (C-3'), 96.8 (C-6), 95.4 (C-8), 69.5 (C-2), 56.7 (OMe-2'), 56.0 (OMe-5'), 32.1 (C-3) ppm; HRESI-MS m/z 349.1142 $[\text{M} + 2\text{H}]^+$ (calcd for $\text{C}_{17}\text{H}_{19}\text{NO}_7$, 349.1151).

5-Hydroxy-7,2',4',5'-tetramethoxyisoflavanone hydrazone (14) dark brown solid; mp 103–105 °C; FT-IR (neat) ν_{max} cm^{-1} : 2994, 2937, 2842, 1632, 1588, 1512, 1461, 1314, 1204, 1154, 1034; ^1H NMR (400 MHz, CDCl_3): δ 6.57 (1H, s, H-6'), 6.56 (1H, s, H-3'), 6.26 (1H, d, $J = 2.2$ Hz, H-8), 6.03 (1H, d, $J = 2.4$ Hz, H-6), 4.56 (1H, br s, H-3), 4.29 (2H, br s, H-2), 3.89 (3H, s, OMe-2'), 3.88 (3H, s, OMe-5') 3.78 (3H, s, OMe-4'), 3.61 (3H, s, OMe-7) ppm; ^{13}C NMR (100 MHz, CDCl_3): δ 163.1 (C-4), 160.8 (C-7), 158.2 (C-5), 152.3 (C-8a), 151.1 (C-2'), 149.8 (C-4'), 143.5 (C-5'), 114.7 (C-1'), 112.6 (C-6'), 100.1 (C-4a), 97.4 (C-3'), 95.9 (C-8), 94.3 (C-6), 69.8 (C-2), 56.8 (OMe-2'), 56.4 (OMe-4'), 56.3 (OMe-5'), 55.5 (OMe-7), 32.6 (C-3) ppm; HRESI-MS m/z 375.1555 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{19}\text{H}_{23}\text{N}_2\text{O}_6$, 375.1556).

5-Hydroxy-7,2',4',5'-tetramethoxyisoflavanone oxime (15) light yellow solid; mp 213–214 °C; FT-IR (neat) ν_{max} cm^{-1} : 3408, 2943, 2847, 2353, 1632, 1576, 1515, 1456, 1203, 1155, 1089, 1034; ^1H NMR (400 MHz, CDCl_3): δ 6.57 (1H, s, H-6'), 6.42 (1H, s, H-3'), 6.29 (1H, d, $J = 2.4$ Hz, H-8), 6.05 (1H, d, $J = 2.4$ Hz, H-6), 4.92 (1H, m, H-3), 4.43 (1H, dd, $J = 11.2, 1.4$ Hz, H-2), 4.22 (1H, dd, $J = 11.2, 3.4$ Hz, H-2), 3.88 (3H, s, OMe-2'), 3.87 (3H, s, OMe-5'), 3.79 (3H, s, OMe-4'), 3.59 (3H, s, OMe-7) ppm; ^{13}C NMR (100 MHz, CDCl_3): δ 163.4 (C-4), 159.7 (C-7), 158.6 (C-5), 156.5 (C-8a), 151.0 (C-2'), 149.1 (C-4'), 142.8 (C-5'), 116.7 (C-1'), 112.5 (C-6'), 98.3 (C-4a), 97.7 (C-3'), 95.7 (C-8), 94.4 (C-6), 69.5 (C-2), 56.7 (OMe-2'), 56.4 (OMe-4'), 56.1 (OMe-5'), 55.4 (OMe-7), 32.2 (C-3) ppm. HRESI-MS m/z 376.1396 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{19}\text{H}_{22}\text{NO}_7$, 376.1396).

5,7,2',4',5'-Pentamethoxyisoflavan oxime (16) dark brown solid; mp 194–196 °C; FT-IR (neat) ν_{max} cm^{-1} : 3180, 2939, 2844, 1609, 1511, 1460, 1310, 1210, 1157, 1114, 1031, 931; ^1H NMR (400 MHz, CDCl_3): δ 6.55 (1H, s, H-3'), 6.55 (1H, s, H-6'), 6.22 (1H, d, $J = 2.2$ Hz, H-8), 6.20 (1H, d, $J = 2.1$ Hz, H-6), 4.94 (1H, m, H-3), 4.41 (1H, dd, $J = 11.4, 1.4$ Hz, H-2), 4.31 (1H, dd, $J = 11.4, 3.7$ Hz, H-2), 4.15 (3H, s, OMe-5'), 3.87 (3H, s, OMe-5), 3.86 (3H, s, OMe-4'), 3.85 (3H, s, OMe-7), 3.59 (3H, s, OMe-2'), ppm; ^{13}C NMR (100 MHz, CDCl_3): δ 167.4 (C-4), 162.1 (C-7), 161.1 (C-5), 153.0 (C-8a), 151.6 (C-2'), 150.3 (C-4'), 143.0 (C-5'), 113.5 (C-1'), 112.9 (C-6'), 98.0 (C-4a), 95.9 (C-3'), 95.3 (C-8), 93.8 (C-6), 69.5 (C-2), 57.5 (OMe-2), 57.4 (OMe-4'), 56.67 (OMe-5'), 56.2 (OMe-7), 56.2 (OMe-5), 33.1 (C-3) ppm; HRESI-MS m/z 390.1554 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{20}\text{H}_{24}\text{NO}_7$, 390.1553).



Cytotoxicity assay

Cytotoxic activity assay. The cytotoxicity evaluation was examined using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. In brief, all cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, penicillin (100 U mL⁻¹) and streptomycin (100 µg mL⁻¹) (Gibco-BRL, USA) and incubated at 37 °C in 5% CO₂ humidified atmosphere. For preliminary testing, cells were exposed to the selected compounds at a concentration of 100 µg mL⁻¹ for 24, 48 and 72 hours. The compounds that caused less than 50% cell viability were further evaluated for their half maximal inhibitory concentration (IC₅₀) values. Control groups were treated with a mixture of solvent (1 : 1 DMSO/EtOH). After incubation, the medium was replaced with 110 µL of fresh medium containing MTT (0.5 mg mL⁻¹ in PBS) (Sigma Chemical Co., St Louis, MO, USA) and incubated for 2 h. The formazan formed after conversion of MTT was dissolved in DMSO. The absorbance of formazan was measured with a microplate reader (Bio-Rad Laboratories, USA) at the wavelength of 550 nm using 655 nm as a reference wavelength. Each assay was replicated four times. The percentage of viable cells which corresponds to the production of formazan was calculated as previously described.²⁵

$$\text{Cell viability (\%)} = \frac{[\text{sample}(A_{550} - A_{655})/\text{control}(A_{550} - A_{655})]}{100} \times 100$$

4. Conclusions

The major constituent from the stems of *D. stipulacea* was found as a isoflavonoid, olibergin A. Due to the cytotoxicity of olibergin A showed weak activity against HCT-116, HT-29, MCF-7 and vero cell lines, then this compound was used as the starting material for structural modification and led to sixteen derivatives. All derivatives were evaluated for the activity using MTT assay and the results displayed isoflavone **5** was the most active compound (IC₅₀ ≅ 10–19 µM). It should be note that isoflavanone **10** and oxime **13** exhibited strong cytotoxicity against HCT-116 and HT-29 cell lines, respectively, and showed weak activity toward normal cells. In addition, oxime **15** displayed cytotoxicity against all cell lines with IC₅₀ values around 26–38 µM. These information may be useful to develop the structure and cytotoxicity of isoflavonoid derivatives.

Conflicts of interest

The authors declare no conflict of interest.

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