Fissistiganoids A and B: two new flavonoids from the *Fissistigma tungfangense*

Wan-Lin Zhonga,b, Xue-Ming Zhoua,b, Ji-Ling Yia,b, Xin-Ming Songa,b, Bin Zhanga,b, Jing-Yu Yanga,b and Guang-Ying Chena,b

*a Key Laboratory of Tropical Medicinal Resource Chemistry of Ministry of Education, College of Chemistry and Chemical Engineering, Hainan Normal University, Haikou, People’s Republic of China; b Key Laboratory of Tropical Medicinal Plant Chemistry of Hainan Province, College of Chemistry and Chemical Engineering, Hainan Normal University, Haikou, People’s Republic of China*

**ABSTRACT**

Two new flavanoids fissistiganoids A and B (1 and 2), together with two known pterocarpans derivatives (3 and 4), were isolated from the stems of *Fissistigma tungfangense*. The structures of these compounds were elucidated using comprehensive spectroscopic methods. The absolute configurations of fissistiganoids A and B (1 and 2) were determined by comparing their ECD spectra with quantum-mechanics ECD calculations. The inhibitory activities of all compounds against three cancer cell lines HeLa, MCF-7 and A549 were evaluated. Compounds 1–4 showed moderate inhibitory effects on HeLa, MCF-7 and A549 cells with IC₅₀ values ranging from 12.5 to 42.3 μM.

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1. Introduction

The genus *Fissistigma* (Annonaceae) consists of approximately 80 species, which are mainly distributed in Australia and Asia. *Fissistigma tungfangense* Tsiang et P. T. Li is a middle-sized vine, mainly distributed in southern China (Kuo et al. 2002). The dried stems of several *Fissistigma* species (Annonaceae) as traditional medicine are widely used as traditional medicine for the treatment of various diseases, such as dampness arthralgia, lumbago, stomachache, traumatic injury (SFDA 1997; Pham and Nguyen-Ngoc 2020). Alkaloids, fluorenones, sesquiterpenoids and cyclopentenones were reported as the constituents of this plant (Fu et al. 2011 and Chia et al. 2000; Ge et al. 2011).
In our search for new natural anti-tumor products from the stems of *F. tungfangense*, we found that the ethanol extract of *F. tungfangense* has cytotoxic activity and two new flavonoids fissistiganoids A and B (1 and 2), together with two known pterocarpans derivatives (3 and 4) were isolated from the stems of *F. tungfangense*. The structures of these compounds were elucidated using comprehensive spectroscopic methods. The absolute configurations of fissistiganoids A and B (1 and 2) were determined by comparing their ECD spectra with quantum-mechanics ECD calculations. The inhibitory activities of all compounds against three cancer cell lines HeLa, MCF-7 and A549 were evaluated.

### 2. Results and discussion

Compound 1 was obtained as white amorphous powder, with the molecular formula C_{15}H_{12}O_{4} from HRESIMS data combined with ¹H and ¹³C NMR spectroscopic data. The ¹H NMR data revealed two olefinic protons δ_H 6.50 (1H, dd, 10.0, 1.6 Hz) and δ_H 5.71 (1H, dd, 10.0, 3.6 Hz), six aromatic protons δ_H 7.09 (1H, td, 8.0, 2.0 Hz), 6.97 (1H, dd, 8.0, 2.0 Hz), 6.92 (1H, d, 8.4 Hz), 6.81 (1H, td, 8.0, 1.2 Hz), 6.70 (1H, d, 8.0 Hz) and 6.58 (1H, d, 8.4 Hz), one oxygenated methine proton δ_H 6.21 (1H, dd, 3.6, 1.6 Hz). The ¹³C NMR data showed 15 resonances, including fourteen olefinic carbons and one oxygenated carbon. These data indicated that compound 1 was a flavonoid. The ¹H-¹H COSY correlation of H-2/H-3, H-3/H-4, H-5/H-6, H-6/H-7 and H-7/H-8 combined with the HMBC correlations from H-2 and H-4 to C-8a, H-4 to C-5, H-6 to C-4a, H-6 to C-8a, indicated the presence of fragments from O-1 to C-8a. The ¹H-¹H COSY correlation of H-5'/H-6' combined with the HMBC correlations from H-6 to C-2'/4', H-5' to C-1'/3', indicated the presence of fragments from C-1' to C-6'. The HMBC correlations from H-2 to C-2'/6' indicated that C-2 linked with C-1'. The absolute configuration of 1 (The 1D structure of compound 1 has been reported by Zhou et al. 2020) was confirmed by comparing experimental and calculated ECD spectra for the (2R)-1 and (2S)-1 using TDDFT (see supporting information). The theoretical spectrum of (2R)-1 showed an excellent fit with the experimental plot recorded in MeOH. Thus, the absolute configuration of 1 was established as 2R (Figure 1) and named fissistiganoid A.

Compound 2 was also obtained as white amorphous powder, with the molecular formula C_{17}H_{16}O_{4} from HRESIMS data combined with ¹H and ¹³C NMR spectroscopic data. The first preliminary investigation of its ¹H and ¹³C NMR showed that 2 was closely related to 1, except for the presence of two methoxy group signals (δ_H 3.98/3.87, s and δ_C 61.6/56.4) in 2. The location of the two methoxy groups at C-2' and C-4' were confirmed by the HMBC correlations from H-(2'-OCH₃) to C-2', from H-(4-OCH₃)
 Detailed analysis of 2D NMR (HSQC, $^1$H-$^1$H COSY and HMBC) spectra confirmed that the other part of the molecule were the same as those of 1. The ECD spectra of 1 and 2 showed the similar spectral feature in the 200–400 nm range, so they must have the same stereochemistry. Thus, the absolute stereochemistry of 2 was determined as $2\text{R}$ and named fissistiganoid B.

The structures of known compounds, 4-methoxymedicarpin (3) (Miller et al. 1988), (6aR,12aR)-(-)-4-Methoxymaackiain (4) (Posri et al. 2019) were identified by comparison of their spectroscopic data with those in the literature.

All compounds were tested for cytotoxic activities against HeLa, MCF-7 and A549 cells (see supporting information Table S2). Compounds 1–4 showed moderate inhibitory effects on HeLa, MCF-7 and A549 cells with IC$_{50}$ values ranging from 12.5 to 42.3 µM.

3. Experimental

3.1. General

Optical rotations were measured on a JASCO P-1020 digital polarimeter. IR spectra were recorded on a Nicolet 6700 spectrophotometer. CD spectra were recorded on a Bruker AV spectrometer (400 MHz for $^1$H and 100 MHz for $^{13}$C). TMS was used as an internal standard. HRESIMS spectra were measured on a Q-TOF Ultima Global GAA076 LC mass spectrometer. Semi-preparative HPLC was performed on an Agilent 1260 LC series with a DAD detector using an Agilent Eclipse XDB-C18 column (9.4 × 250 mm, 5 µm). Silica gel (Qing Dao Hai Yang Chemical Group Co.; 200–300 mesh), octadecylsilyl silica gel (YMC; 12 nm–50 µm) were used for column chromatography (CC). Precoated silica gel plates (Yan Tai Zi Fu Chemical Group Co.; G60, F-254) were used for thin-layer chromatography (TLC).

3.2. Plant material

The stems of F. tungfangense were collected from Changjiang County, Hainan Province, China in June 2015, and identified by Prof. Qiong-Xin Zhong, College of Life Science, Hainan Normal University. A voucher specimen (No. GFM20150618) was deposited at the Key Laboratory of Tropical Medicinal Plant Chemistry of Ministry of Education, Hainan Normal University.

3.3. Extraction and isolation

The air-dried and powdered stems (10 kg) of F. tungfangense were extracted with 80% EtOH (3 × 20 L, 7 days each) at room temperature. After concentration under reduced pressure, the water-soluble residue was partitioned successively with petroleum ether and EtOAc. The EtOAc extract (120 g) was separated using a silica gel column chromatography (CC) (petroleum ether, EtOAc, MeOH v/v, gradient) to generate seven fractions (Frs. 1–9). Frs. 3 (20 g) was applied to silica gel CC eluted with EtOAc-MeOH (from 40: 1 to 1: 1) to afford three subfractions (3a–3c). Subfraction 3a was further
separated by semi-preparative HPLC (CH$_3$CN/H$_2$O, 45: 55 v/v) to obtain 1 (8 mg) and 2 (3 mg). Subfractions 3 b was further purified by using octadecylsilyl silica gel (40% MeOH/H$_2$O) to obtain 3 (15 mg) and 4 (23 mg), respectively.

3.3.1. Fissistiganoid A (1)
White amorphous powder; colorless gum; CD (c 2 x 10$^{-4}$ mol/L, MeOH) $\lambda_{\max}$ (\Delta$\varepsilon$) 292 (0.9), 244 (0.9), 225 (0.9) and 203 (6.4); UV (MeOH) $\lambda_{\max}$ (log$e$) 206 (4.2), 271 (1.1) and 311 (0.3) nm; IR (KBr) $\nu_{\max}$ 3437, 1611 and 1462 cm$^{-1}$; $^1$H and $^{13}$C NMR: see supporting information; HRESIMS m/z 255.0658 [M - H]$^-$ (calcd for C$_{15}$H$_{11}$O$_4$, 255.0663).

3.3.2. Fissistiganoid B (2)
White amorphous powder; colorless gum; CD (c 2 x 10$^{-4}$ mol/L, MeOH) $\lambda_{\max}$ (\Delta$\varepsilon$) 296 (1.3), 249 (1.3), 229 (1.3) and 211 (3.7); UV (MeOH) $\lambda_{\max}$ (log$e$) 205 (3.8), 271 (0.9) and 310 (0.2) nm; IR (KBr) $\nu_{\max}$ 3431, 1608 and 1442 cm$^{-1}$; $^1$H and $^{13}$C NMR: see supporting information; HRESIMS m/z 283.0972 [M–H]$^-$ (calcd for C$_{17}$H$_{15}$O$_4$, 283.0976).

3.4. Biological assays
Cytotoxic activity was evaluated by the MTT method as described previously (Scudiero et al. 1988). HeLa, MCF-7 and A549 were provided by College of Pharmacy, Hebei University and maintained in DMEM medium (Gibco) containing 5% fetal bovine serum (Gibco) at 37 $^\circ$C in air with 5% CO$_2$. Compounds 1–4 showed moderate inhibitory effects on HeLa, MCF-7, and A549 cells.

4. Conclusion and prospect
Flavonoids, a class of important secondary metabolites, are widely distributed in the plant kingdom. Based on the oxidation and saturation status of the C ring, flavonoids are classified into different subgroups, mainly including flavones, flavonols, flavanones, flavanols, isoflavones, aurones, anthocyanins, and proanthocyanidins. In addition, flavonoids possess rich biological activities for instance hepatoprotection, anti-mutagenesis, anti-inflammation, anti-oxidation, anti-cancer, anti-bacterial and anti-viral (Kumar and Pandey 2013; Deng and Lu 2017). Based on previously reported, chalcone synthase acts in the first step of the flavonoid biosynthetic pathway. It catalyzes the iterative condensation and subsequent intramolecular cyclisation of one p-coumaroyl-CoA with three acetate residues from malonyl-CoA molecules to form chalcone. In the second step, chalcone isomerase catalyzes the stereospecific isomerisation of chalcone into flavanone. Thereafter, flavone synthase introduces a double bond between the C-2 and C-3 positions of flavanone, converting flavanone into flavone (Ferrer et al. 1999; Jez et al. 2000).

In this study, two new flavanoids fissistiganoids A and B (1 and 2), together with two known pterocarpsans derivatives (3 and 4), were isolated from the stems of F. tungfangense. The absolute configurations of fissistiganoids A and B (1 and 2) were determined by comparing their ECD spectra with quantum-mechanics ECD
calculations. Compounds 1–4 showed moderate inhibitory effects on HeLa, MCF-7 and A549 cells with IC50 values ranging from 12.5 to 42.3 μM.

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