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Abstract

A new 2-arylbenzofuran, spathobenzofuran (1), together with ten known compounds including a 2-arylbenzofuran, three pterocarpans and six isoflavones were isolated from the acetone crude extract of the stems of Spatholobus parviflorus. All compounds were characterised by spectroscopic methods. Compound 4 was active (MIC 8 μg/mL) against Gram-negative Pseudomonas aeruginosa TISTR 781 while compound 2 had modest activity against Gram-positive Staphylococcus aureus TISTR 1466 with a MIC value of 16 μg/mL. All isolated compounds showed no cytotoxicity against Vero and KB cells.

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ABSTRACT

A new 2-arylbenzofuran, spathobenzofuran (**1**), together with ten known compounds including a 2-arylbenzofuran, three pterocarpans and six isoflavones were isolated from the acetone crude extract of the stems of *Spatholobus parviflorus*. All compounds were characterised by spectroscopic methods. Compound **4** was active (MIC 8 µg/mL) against Gram-negative *Pseudomonas aeruginosa* TISTR 781 while compound **2** had modest activity against Gram-positive *Staphylococcus aureus* TISTR 1466 with a MIC value of 16 µg/mL. All isolated compounds showed no cytotoxicity against Vero and KB cells.

Keywords: *Spatholobus parviflorus*; arylbenzofuran; antibacterial activity; cytotoxicity

1. Introduction

Spatholobus parviflorus (Fabaceae) is a woody climbing plant known as "Thao Pun Sai" and "Kwang Phu" in Thai. Four species from this genus have been found in Thailand (Smitinand 2014). A water decoction of the stems and leaves (Mahidol University 1996) of *S. parviflorus* is used in traditional medicine by tribal communities in the North of Thailand to treat indigestion, gastritis, menstrual pain, and other diseases. Plants of the genus *Spatholobus* are a major source of flavonoids (Yoon et al. 2004; Tang et al. 2012; Wang et al. 2017). Recently, Sichaem and co-workers reported the isolation of flavonoids and phenolic derivatives and their cholinesterase activity from the extracts of the stems of *S. parviflorus* (Sichaem et al. 2018). From our ongoing search for bioactive compounds from Thai medicinal plants, we report here the isolation, structural characterisation, and bioactivities of a new 2-arylbenzofuran (**1**) as well as ten known compounds (**2**-**11**) from the stem extracts of this plant. The isolation of 2-arylbenzofurans and compounds **3**, **5**, **9**, and **11** from plants of this genus are reported here for the first time.

2. Results and discussion

The dried stems of *S. parviflorus* were extracted with acetone and the crude extract was separated by column chromatography which led to the isolation of a new 2-

arylbenzofuran, spathobenzofuran (**1**), and ten known compounds; vignafuran (**2**) (Csékei et al. 2008), lyratin B (**3**) (Zhang et al. 2010), (6a*R*, 11a*R*)-medicarpin (**4**) (Yoon et al. 2016), phaseollidin (**5**) (Dagne et al. 1993), daidzein (**6**) (Takeya and Itokawa 1982), formononetine (**7**) (Granados-Covarrubias and Maldonado 2009), genistein (**8**) (Huang et al. 2013), 3´-methoxydaidzein (**9**) (Goto et al. 2009), 8-*O*-methylretusin (**10**) (Mizuno et al. 1990) and 7,3´-dihydroxy-8,4´-dimethoxyisoflavone (**11**) (Puebla et al. 2010) as shown in Figure 1. The structures of all isolated compounds were elucidated by spectroscopic methods, especially 1D and 2D NMR spectroscopy, mass spectrometry and physical data.

Compound **1** was obtained as a yellow gum with a molecular formula of $C_{17}H_{14}O_6$ from the HRESI-MS ion peak at *m/z* 315.0867 $[M+H]^+$ (calculated for $C_{17}H_{15}O_6$, 315.0869). The UV spectrum showed maximum absorption bands at λ_{max} 247, 289 and 341 nm which corresponded to a 2-arylbenzofuran derivative (Abdel-Kader 2001; Belofsky et al. 2006; Wu et al. 2018). The IR spectrum show stretching bands for a hydroxy group and a carboxaldehyde group at 3362 and 1700 cm^{-1}, respectively. The ^1H NMR spectrum of compound **1** (Table S1) showed resonances for a carboxaldehyde group [δ_H 9.93 (*s*, 1H)], two isolated aromatic protons [δ_H 7.60, 7.00 (each *s*, 1H)], a 1,2,4-trisubstituted benzene [δ_H 7.42 (*d*, *J* = 8.4 Hz, 1H), 6.61 (*d*, *J* = 2.2 Hz, 1H), 6.55 (*dd*, *J* = 8.4 and 2.2 Hz, 1H)] and two methoxy groups [δ_H 3.94, 3.83 (each *s*, 3H)]. The ^{13}C NMR spectrum (Table S1) indicated resonances for 17 carbons, including a carboxaldehyde group (δ_c 189.7), two methoxy groups (δ_c 56.9 and 56.1) and 14 aromatic carbons (9 ArC and 5 ArCH) which contributed further proof that compound **1** contained a core 2-arylbenzofuran structure. From 2D NMR spectral data, the low field singlet aromatic proton resonance at δ_H 7.60 was assigned to H-4 (δ_c 104.1) on the basis of its HMQC spectrum and its HMBC correlations with C-3 (δ_c 118.6), C-6 (δ_c 150.7) and C-8 (δ_c 147.3). The carboxaldehyde group (δ_H 9.93) was located at C-3 of the furan ring from the HMBC correlations of the aldehyde proton with C-3 and C-9 (δ_c 117.7). The other singlet aromatic proton, resonating at δ_H 7.00 was attached to C-7 (δ_c 98.9) on the basis of the HMQC spectrum and the HMBC correlations of H-7 with C-5 (δ_c 147.9), C-6 and C-9. The substituents at C-5 and C-6 were identified as a methoxy group (δ_H 3.94) from the HMBC spectrum and a hydroxy group based on the downfield ^{13}C NMR chemical shift of C-6 (δ_c 150.7), respectively. While the C-8 position (δ_c 147.3) was connected to the oxygen atom that

formed part of the furan ring. The 1,2,4-trisubstituted benzene ring substitutent was linked to C-2 (δ_c 163.1) of the furan ring on the basis of the HMBC correlations of H-6´ (δ_H 7.42) with C-2, C-2´ (δ_c 160.3) and C-4´ (δ_c 164.7). Two aromatic protons resonating at δ_H 6.61 and 6.55 were then attributed to H-3´ and H-5´, respectively, according to their splitting patterns and coupling constants. The substituents at C-2´ and C-4´ were identified as a methoxy group (δ_H 3.83), on the basis of the HMBC spectrum, and a hydroxy group, based on its ^{13}C NMR chemical shift (δ_c 164.7), respectively. In support of these assignments for the positions of these methoxy groups, NOESY NMR experiments were performed. These showed cross peaks between the methoxy group at δ_H 3.94 and δ_H 7.60 (H-4), and the methoxy group at δ_H 3.83 and δ_H 6.61 (H-3´). Therefore, the structure of compound **1** was elucidated as 6-hydroxy-2-(4´-hydroxy-2´-methoxyphenyl)-5-methoxy-3-benzofuran-carboxaldehyde.

All compounds, except for **3**, **5**, and **9**, were evaluated for their antibacterial activities against Gram-positive bacteria (*Bacillus cereus*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*) and Gram-negative bacteria (*Escherichia coli*, *Salmonella typhymurium*, *Pseudomonas aeruginosa*, and *Serratia marcescens*). Compound **4** presented the best activity against *P. aeruginosa* with a MIC value of 8 µg/mL while its activity against *B. cereus* was moderate with a MIC value of 32 µg/mL. Compound **2** showed antibacterial activity against *S. aureus* with a MIC value of 16 µg/mL, while its activity against *E. coli* and *S. typhymurium*m was modest with MIC values of 32 µg/mL. The other compounds exhibited weak antibacterial activities with MIC values ranging between 64 and 128 µg/mL (Table S2). All isolated compounds were evaluated for their cytotoxicities against human mouth epidermal carcinoma cells (KB) and Vero cells. None of the tested compounds showed cytotoxicity in these assays.

3. Experimental

For the details of the isolation of all compounds see the Supplementary material.

Spathobenzofuran (**1**): yellow gum; UV (MeOH) λ_{max} (log ε): 247 (3.5), 289 (3.3), 341 (3.3) nm; IR (neat) ν: 3362, 2851, 2836, 1700 and 1620 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD): δ_H 9.93 (*s*, 1H, H-10), 7.60 (*s*, 1H, H-4), 7.42 (*d*, J = 8.4 Hz, 1H, H-6´), 7.00 (*s*, 1H, H-7), 6.61 (*d*, J = 2.2 Hz, 1H, H-3´), 6.55 (*dd*, J = 8.4 and 2.2 Hz, 1H, H-5´), 3.94 (*s*, 3H, 5-OCH_3), 3.83 (*s*, 3H, 6´-OCH_3); ^{13}C NMR (125 MHz, CD_3OD): δ_C 189.7 (C-10),

164.7 (C-4´), 163.1 (C-2), 160.3 (C-2´), 150.7 (C-6), 147.9 (C-5), 147.3 (C-8), 118.6 (C-3), 117.7 (C-9), 110.3 (C-1´), 133.7 (C-6´), 109.1 (C-5´), 104.1 (C-4), 100.5 (C-3´), 98.9 (C-7), 56.9 (5-OCH_3), 56.1 (2´-OCH_3) (HRESI-MS *m/z* $[M+H]^+$ 315.0867 (calcd for $C_{17}H_{15}O_6$, 315.0869)).

4. Conclusions

A phytochemical investigation of the stems extracts of *S. parviflorus* led to the isolation and identification of a new arylbenzofuran (**1**) and ten known compounds (**2**-**11**). The arylbenzofurans **1** and **2**, and compounds **3**, **5**, **9**, and **11** were found for the first time from plants in this genus, while the other compounds (**4**, **6**, **7**, **8** and **10**) were found in a very recent study of this plant (Sichaem et al. 2018). In addition, the arylbenzofuran (**2**) was moderately active against a Gram-positive strain (*S. aureus*) while pterocarpan (**4**) showed slightly better activity against a Gram-negative strain (*P. aeruginosa*). These compounds showed no cytotoxicity to KB and Vero cells.

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Disclosure statement

The authors declare no conflict of interest

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