

## Sesbagrandidflorain F, a New 2-Arylbenzofuran from the Stem Bark of *Sesbania grandiflora* L.

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**Abstract** – Sesbagrandidflorain F (**1**), a novel 2-arylbenzofuran, and two more 2-arylbenzofurans (**2-3**), were isolated from the stem bark of *Sesbania grandiflora* L. Based on information HRESIMS data, 1D, and 2D NMR spectra, the structure of **1** was fully assigned. Compounds **1-3** were tested for cytotoxicity in MCF-7 and HeLa cells. Compounds **1** and **3** showed moderate activity against MCF-7 cells with an IC<sub>50</sub> value of 2.68 and 4.08 µg/mL, respectively. Conversely, all of the isolates were inactive towards HeLa cells.

**Keywords** – Sesbagrandidflorain F, 2-Arylbenzofuran, *Sesbania grandiflora*, Cytotoxic

### Introduction

The plant *Sesbania grandiflora* L. (Fabaceae), sometimes known as ‘Turi,’ is endemic to Indonesia. The leaves and roots of this plant are used to treat fevers, colds, and diarrhea. In addition, the blossoms are also used as a vegetable. Secondary metabolites discovered in the *Sesbania* genus include flavonoids, isoflavonoids, and 2-arylbenzofurans. Studies have demonstrated that *Sesbania grandiflora* has anticancer and antimicrobe activities.<sup>1-4</sup> However, data of the anticancer effectiveness of 2-arylbenzofurans from *Sesbania* is limited.<sup>1-3</sup> Sesbagrandidflorain D, a 2-arylbenzofuran derivative found in *Sesbania grandiflora* L., was firmly against MCF-7 and WiDr cells.<sup>3</sup> The purpose of this study was to discover the cytotoxicity of 2-arylbenzofurans from *S. grandiflora* L. In addition, we reported the isolation of sesbagrandidflorain F (**1**), a new 2-arylbenzofuran derivative, as well as two known 2-arylbenzofurans, spinosans A (**2**) and B (**3**), from *S. grandiflora* L. stem barks (Fig. 1). Compounds **1-3** were cytotoxic to breast cancer cells (MCF-7) and human cervical cells (HeLa).

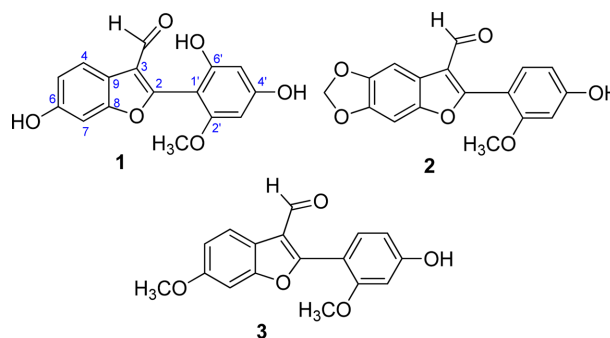


Fig. 1. 2-Arylbenzofurans (**1-3**) from *S. grandiflora* stem barks.

### Experimental

**Modify the formation procedures as the following –** The UV-VIS spectrophotometer (UV-1800-Shimadzu) was used to determine each component's maximum absorption ( $\lambda_{\text{max}}$ ). The functional groups of compounds **1-3** were recorded using an FTIR spectrophotometer recorded the (IR Tracer-100- Shimadzu). The NMR spectra of compounds in acetone-*d*<sub>6</sub> were examined using a JEOL FTNMR ECA 400 spectrometer. A Waters LCT PremierTM XE mass spectrometer was used to examine the chemical formula of isolates. Column chromatography was performed using Si gel G<sub>60</sub> and Sephadex LH-20. The use of a UV lamp and cerium sulfate reagent to

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visualize chemicals on TLC.

**Plant materials** – Dr. M. Affandi, a botanist from the Biology Department, Universitas Airlangga, identified fresh stem barks of *Sesbania grandiflora* L. collected in Kemiri Village, Districts Sidoarjo, East Java, Indonesia, in December 2018, and a specimen (SG 20181203) was deposited.

**Extraction and isolation** – The dry stem bark powder from *S. grandiflora* (3.0 kg) was extracted in 90% methanol ( $2 \times 30$  L) for three days at room temperature, giving methanol extract (350 g) following vaporization under low pressure. The methanol extract was 9:1 watered down before being partitioned with n-hexane (80 g) and EtOAc (20 g) in that order. By separating EtOAc extract (19 g) on a silica gel column chromatography and eluting it with a mobile phase of increasing polarity (hexane, hexane-EtOAc, EtOAc), five fractions (A-E) were produced. Fraction D (3.0 g) was chromatographed on a Sephadex LH-20 column and eluted with methanol, yielding three subfractions D<sub>1</sub>-D<sub>3</sub>. Compounds **2** (25 mg) and **3** (25 mg) were purified on silica gel radial chromatography using n-hexane-EtOAc (from 9:1 to 4:1 v/v). Purification of subfraction D<sub>3</sub> using the same techniques and n-hexane-diisopropyl ether (from 1:1 to 1:4), and 100% diisopropyl ether to obtain compound **1** (13 mg).

**Sesbagrandidflorain F (1)** – Yellow solid, UV (MeOH)  $\lambda_{\max}$  nm (log  $\epsilon$ ): 268 (4.11), 296 (3.70), and 346 (3.91) nm. IR (KBr)  $\nu_{\max}$   $\text{cm}^{-1}$ : 3373, 1663, 1602, and 1516. Table 1 shows the NMR spectral data of **1**. HRESIMS:  $m/z$

$[\text{M}+\text{H}]^+$  calculated for  $\text{C}_{16}\text{H}_{12}\text{O}_6$  300.0643, found 300.0641.

**Spinosan A (2)** – Yellow solid, UV (MeOH)  $\lambda_{\max}$  nm (log  $\epsilon$ ): 250 (4.07), 295 (3.71), and 345 (3.85) nm. The NMR spectra of **2** have similarities to data from the literature.<sup>5</sup>

**Spinosan B (3)** – Yellow solid, UV (MeOH)  $\lambda_{\max}$  nm (log  $\epsilon$ ): 246 (4.27), 280 (3.79), and 342 (3.95) nm. The NMR spectra of **3** were compared and found to be almost equivalent to the data in the literature.<sup>5</sup>

**Cytotoxic activity** – The MTT assay was used to assess the cytotoxic activity of **1-3** against human cervical cells (HeLa) and human breast cells (MCF-7) following the previous experiment.<sup>6-8</sup> For 48 hours, HeLa and MCF-7 cells were grown in RPMI-1640 media containing 10% FBS at 37 °C with 5%  $\text{CO}_2$  flow. Compounds **1-3** were introduced to HeLa and MCF-7 cells in 96-well plates and incubated for 24 hours at 37 °C with 5%  $\text{CO}_2$ . The microplate reader spectrometer measured the active compound's capacity to kill cancer cells at  $\lambda$  590 nm. As a positive control for the cytotoxic assay, doxorubicin was used.<sup>9-10</sup>

## Result and Discussion

Compound **1** (sesbagrandidflorain F) was produced as a yellow solid and exhibited the chemical formula  $\text{C}_{16}\text{H}_{12}\text{O}_6$  by high-resolution ESIMS at  $[\text{M}+\text{H}]^+$  ion  $m/z$  300.0641 (calcd 300.0643). The UV spectra of **1** revealed the maximum absorption at  $\lambda_{\max}$  (log  $\epsilon$ ): 268 (4.11), 296

**Table 1.**  $^1\text{H}$  (400 MHz),  $^{13}\text{C}$  NMR data (100 MHz) of **1** in acetone- $d_6$

No.C	$\delta_{\text{H}}$ (mult, $J$ in Hz)	$\delta_{\text{C}}$	HMBC
2	-	164.6	-
3	-	118.7	-
4	7.52 ( <i>d</i> , 8.6)	133.5	C-6; C-8
5	6.65 ( <i>dd</i> , 8.4; 2.2)	109.1	C-7; C-9
6	-	157.8	-
7	6.66 ( <i>d</i> , 2.2)	104.1	C-8; C-9
8	-	162.4	-
9	-	108.2	-
1'	-	107.8	-
2'	-	161.8	-
3'	6.33 ( <i>d</i> , 2.0)	98.8	C-1', C-2'; C-4'; C-5'
4'	-	152.7	-
5'	6.71 ( <i>d</i> , 2.0)	88.4	C-1', C-3'; C-6'
6'	-	157.3	-
4'-OH	10.26 ( <i>s</i> )	-	C-3'; C-4'
2'-OCH <sub>3</sub>	3.82 ( <i>s</i> )	56.0	C-2'
CHO	9.96 ( <i>s</i> )	191.3	C-3; C-9

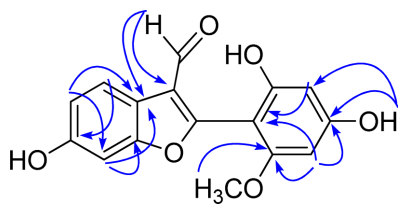


Fig. 2. HMBC of sesbagrandidflorain F (1).

(3.70), and 346 (3.91) nm characteristics for a 2-arylbenzofuran skeleton.<sup>5</sup> According to the IR spectra, the functional group of sesbagrandidflorain F comprises hydroxyl groups ( $3373\text{ cm}^{-1}$ ), aldehyde ( $1663\text{ cm}^{-1}$ ), and aromatic C=C ( $1516$  and  $1602\text{ cm}^{-1}$ ).<sup>1</sup> The protons of two aromatic units, hydroxy, methoxy, and aldehyde, are found in the  $^1\text{H}$  NMR data (Table 1). In-ring A, there is an ABX system at  $\delta_{\text{H}}$  7.52 (1H, *d*,  $J=8.4$  Hz, H-4),  $\delta_{\text{H}}$  6.66 (1H, *d*,  $J=2.2$  Hz, H-7),  $\delta_{\text{H}}$  6.65 (1H, *dd*,  $J=8.4$ ;  $2.2$  Hz, H-5), and two *meta*-coupled ( $J=2.0$  Hz) protons in the ring C at  $\delta_{\text{H}}$  6.71 (H-5'), and  $\delta_{\text{H}}$  6.33 (H-3'). In addition, the protons of a hydroxy at  $\delta_{\text{H}}$  10.26 (1H, *s*, 4'-OH), a methoxy at  $\delta_{\text{H}}$  3.82 (3H, *s*, 2'-OCH<sub>3</sub>), and an aldehyde at  $\delta_{\text{H}}$  9.96 (1H, *s*, 3-CHO) were exposed by sesbagrandidflorain F. The  $^{13}\text{C}$  NMR of sesbagrandidflorain F exhibits signals for sixteen carbons, including a methoxy carbon, five methine carbons, and ten quaternary carbons (see Table 1). The HMBC and HMQC spectra confirmed the positioning of hydroxy, methoxy, and aldehyde groups in the structure of 2-arylbenzofuran. An ABX system proton at  $\delta_{\text{H}}$  7.52 (H-4) was found to be connected to two oxyaryl carbons at  $\delta_{\text{C}}$  162.4 (C-8) and  $\delta_{\text{C}}$  157.8 (C-6) in the HMBC spectrum (Fig. 2). A signal at  $\delta_{\text{H}}$  6.65 (H-5) revealed long-range correlations with a methine carbon [ $\delta_{\text{C}}$  104.1 (C-7)], and a quaternary carbon [ $\delta_{\text{C}}$  108.2 (C-9)], as well as a proton at  $\delta_{\text{H}}$  6.66 (H-7) correlated to C-8, and C-9 in the ring A. In the ring C, a *meta* coupled proton at  $\delta_{\text{H}}$  6.63 (H-3') showed HMBC correlation to a methine carbon at  $\delta_{\text{C}}$  88.4 (C-5'), a quaternary carbon at  $\delta_{\text{C}}$  107.7 (C-1'), and two oxyaryl carbons at  $\delta_{\text{C}}$  161.8 (C-2') and  $\delta_{\text{C}}$  152.7 (C-4'). In contrast, the other *meta*-coupled proton at  $\delta_{\text{H}}$  6.71 (H-5') showed a correlation with C-1', a methine at  $\delta_{\text{C}}$  98.8 (C-3'), and an oxyaryl at  $\delta_{\text{C}}$  157.3 (C-6'). The designated position for the hydroxy group at C-4' was clear by a hydroxy proton at  $\delta_{\text{H}}$  10.26 (4'-OH) connected to C-3' and C-4'. A methoxy signal at  $\delta_{\text{H}}$  3.82 (2'-OCH<sub>3</sub>) was also found to be connected to C-2'. In addition, an aldehyde group at  $\delta_{\text{H}}$  9.96 (3-CHO) was discovered to be linked to two quaternary carbons in the 2-arylbenzofuran skeleton,  $\delta_{\text{C}}$  119.1 (C-3), and 114.1 (C-9). Based on NMR data, the structure of compound **1** was determined to be 2-(2,4-

Table 2. Cytotoxicity data of compounds **1-3**

Compounds	IC <sub>50</sub> (μg/mL)	
	MCF-7	HeLa
Sesbagrandidflorain F ( <b>1</b> )	2.68 ± 0.83	> 100
Spinosan A ( <b>2</b> )	> 100	90.05 ± 1.20
Spinosan B ( <b>3</b> )	4.08 ± 1.03	44.98 ± 0.85
Doxorubicin	5.67 ± 0.74	4.60 ± 0.23

dihydroxy-6-methoxyphenyl)-6-hydroxy-benzofuran-3-carbaldehyde, which was given as the name sesbagrandidflorain F.

Compounds **1-3** were tested for cytotoxicity towards MCF-7 and HeLa cells (Table 2) using the MTT assay.<sup>6-10</sup> Compounds **1** and **3** had moderate activity against MCF-7 cells, with IC<sub>50</sub> values of 2.68 and 4.08 μg/mL, respectively. In addition, none of the isolates had any effect on HeLa cells. The only variations between compounds **1-3** are the hydroxy, methoxy, and methylenedioxy groups in the 2-arylbenzofuran structure. Compound **1** is distinguished from compound **2-3** by an -OH group at C-6' in the ring B, which increases cytotoxic action.

In conclusion, a new 2-arylbenzofuran, sesbagrandidflorain F (**1**), and spinosans A (**2**) and B (**3**) were isolated from the stem bark of *S. grandiflora*. Compounds **1** and **3** exhibited moderate activity against MCF-7 cells with an IC<sub>50</sub> value of 2.68 and 4.08 μg/mL. However, all of the 2-arylbenzofurans were inactive against HeLa cells lines.

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