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Two new 2-arylbenzofurans from *Sesbania grandiflora* L. and their cytotoxicity towards cancer cell

Tjitjik Srie Tjahjandarie, Mulyadi Tanjung, Ratih Dewi Saputri, Devina Oktari Rahayu, Alfiah Nur Irza Gunawan and Muhammad Fajar Aldin

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ABSTRACT

Two new 2-arylbenzofurans, sesbagrandiflorain D (**1**) and E (**2**) along with two known 2-arylbenzofurans, spinosan A (**3**) and spinosan B (**4**) were isolated from the stem bark of *Sesbania grandiflora* L. The structure of two new compounds established by HRESIMS, 1D NMR (^1H , ^{13}C) and 2D NMR (HMOC, HMBC) spectra. Compounds (**1-4**) assayed for their cytotoxicity towards three human cancer cells (MCF-7, HeLa, and WiDr). Compound **1** showed very high activity against MCF-7 and WiDr with an IC_{50} value of 0.06 and 0.60 $\mu\text{g}/\text{mL}$, respectively.

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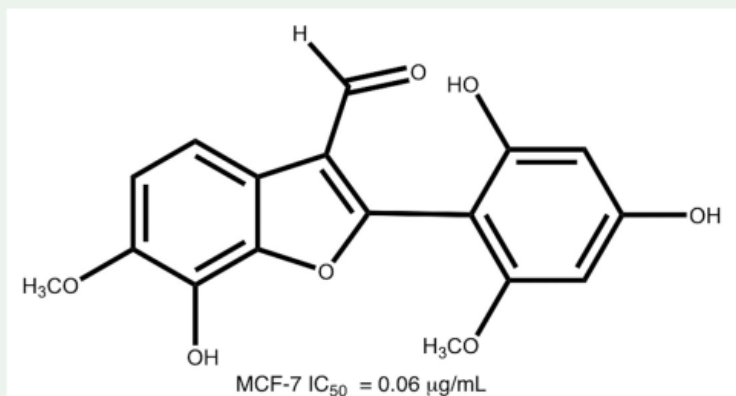
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1. Introduction

Sesbania grandiflora L. locally known as 'Turi Putih' is one species of the Fabaceae family. The flowers of this plant used as a vegetable in Indonesia. Traditionally, the decoction of roots and leaves of this plant used as a cold, fever, diarrhoea, and cancer medicine (Wagh et al. 2009). Based on literature studies, secondary metabolites of *Sesbania* include flavonoids (Messens et al. 1989), isoflavonoids, and terpenoids (Hasan

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et al. 2012). Recently studies on *S. grandiflora* L, resulted 2-arylbenzofuran derivatives, Sesbagrandidiflorains A-C which were found for the first time in genus *Sesbania*. (Noviany et al. 2018, 2020). The biological activities of *Sesbania* has been reported, include anticancer and antimicrobial. However, the information about the anticancer activity of 2-arylbenzofuran derivatives from *Sesbania* is very limited. A recent study by Noviany et al. (2020), established 2-arylbenzofuran derivatives, sesbagrandidiflorains A-C from the stem bark of *S. grandiflora* which showed moderate activity against MCF-7, HEPG2, and HeLa (Noviany et al. 2020), thus revealing the potential of the 2-arylbenzofurans as potential metabolites from the genus *Sesbania*. However, the information about the anticancer activity of 2-arylbenzofuran derivatives from *Sesbania* are scanty. On the basis of such information, the study intended to investigate the potential of more 2-arylbenzofuran derivatives from the stem bark of the *Sesbania grandiflora* against human cancer cell lines. Nowadays, the discovery of lead compound for development of natural-based medicines is very attractive to be studied. Based on the description above, the purpose of this research was to isolate 2-arylbenzofuran derivatives from the stem bark of *Sesbania grandiflora* and investigate the cytotoxicity towards human cancer cell. Two new compounds, sesbagrandidiflorain D (**1**) and E (**2**) along with two known 2-arylbenzofurans have been isolated from the stem bark of this plant. The cytotoxic activities of 2-arylbenzofurans (**1-4**) against MCF-7, HeLa, and WiDr were also reported.

2. Result and discussion

Sesbagrandidiflorain D (**1**) isolated as a yellowish oil showed a positive ion peak $[M + H]^+$ at m/z 331.0817 on HRESIMS spectra corresponding to a molecular composition of $C_{17}H_{15}O_7$. The UV spectra exhibited four maximum absorptions at λ_{max} 227, 269, 282, 337, and 398 characteristics for a 2-arylbenzofuran moiety (Belofsky et al. 2006). The IR spectrum displayed an absorption band for hydroxyl (3350 cm^{-1}), an aldehyde (1663 cm^{-1}), an aromatic ring ($1611\text{-}1502\text{ cm}^{-1}$), respectively (Wang et al. 2009). Two ortho-coupled signals at δ_H 7.11 ($J=8.4\text{ Hz}$, H-4), and δ_H 6.84 (H-5) typical for 1,2,3,4-tetrasubstituted benzene. Two meta-coupled signals at δ_H 6.69 ($J=2.0\text{ Hz}$, H-5'), and δ_H 6.34 (H-3') typical for a 1,2,4,6-tetrasubstituted benzene. The ^1H NMR spectrum also exhibited signals of an aldehyde group at δ_H 9.80 (1H, s, 3-CHO), a hydroxyl group at δ_H 10.17 (1H, s, 4'-OH), two methoxyl groups at δ_H 3.83 (3H, s, 2'-OCH₃), and δ_H 3.72 (3H, s, 6-OCH₃). The ^{13}C NMR spectrum of **1** exhibited 17 carbons separately, including two methoxyl carbons, five methine carbons, and ten quaternary carbons. The position of the aldehyde, methoxyl, and hydroxyl were determined based on HMQC and HMBC spectra. The HMBC spectrum showed long range correlation of an aromatic proton at δ_H 7.11 (H-4) to two oxyaryl carbons at δ_C 150.6 (C-8), and 147.5 (C-6). The location of the methoxyl group was identified based on the HMBC correlation between methoxyl proton signal at δ_H 3.72 with carbon at δ_C 147.5 identified as C-6 which in turn showed HMBC correlation to an aromatic proton at δ_H 6.84 (H-5). One meta coupled signal at δ_H 6.69 showed HMBC correlation to a quaternary carbon at δ_C 107.7, methine carbon at 98.9 and an oxyaryl carbon at 157.4, whereas the other meta coupled proton at δ_H 6.34 showed correlation with carbon at δ_C 107.7,

Table 1. Cytotoxic activities of 2-arylbenzofurans (**1–4**).

Compound	IC ₅₀ (μg/mL)		
	MCF-7	WiDr	HeLa
1	0.06 ± 0.01	0.60 ± 0.12	6.31 ± 0.30
2	2.98 ± 0.15	2.35 ± 0.20	>100
3	>100	>100	38.46 ± 1.69
4	4.08 ± 0.35	44.98 ± 0.85	24.26 ± 0.75
Doxorubicin	0.09 ± 0.01	0.08 ± 0.01	0.08 ± 0.02

a methine at 88.5 and an oxyaryl at 162.0, which confirmed a 1,2,4,6-tetrasubstituted benzene in the B-ring. A signal of methoxyl at δ_{H} 3.83 (2'-OCH₃) correlated to an oxyaryl carbon at δ_{C} 162.0 (C-2'), and confirmed a methoxyl group at C-2'. A signal of hydroxyl at δ_{H} 10.17 (4'-OH) correlated to an oxyaryl carbon at δ_{C} 152.8 (C-4'), and a methine carbon at 98.9 (C-3') were evident of the assigned position for the OH group at C-4'. Furthermore, an aldehyde group at δ_{H} 9.81 correlated to two quaternary carbons δ_{C} 119.1 (C-3), and 114.1 (C-9) confirmed its position to be at C-3 in the 2-arylbenzofuran moiety. The structure of compound **1** identified as 2-(2,4-dihydroxy-6-methoxyphenyl)-7-hydroxy-6-methoxybenzofuran-3-carbaldehyde with trivially named sesbagrandiflorain D.

Sesbagrandiflorain E (**2**), isolated as a yellow solid, exhibited a UV spectrum (λ_{max} 212, 269, 298, and 348), and the IR spectrum (3225, 1660, and 1521-1468 cm⁻¹) very similar with **1**. The molecular composition represented as C₁₇H₁₄O₆ with an ion peak [M + H]⁺ at m/z 315.0865 by HRESIMS spectrum. The 1D NMR (¹H, ¹³C) and 2D NMR (HMOC, HMBC) spectra of **2** exhibited very similar profile to those of compound **1**. The principal difference was that the ¹H NMR of **2** showed an ABX system in the A ring at δ_{H} 7.54 (1H, *d*, *J* = 8.4 Hz, H-4), δ_{H} 6.71 (1H, *d*, *J* = 2.2 Hz, H-7), δ_{H} 6.65 (1H, *dd*, *J* = 8.4; 2.2 Hz, H-5), and a methoxyl signal at δ_{H} 3.87 (3H, *s*, 6-OCH₃). The HMBC spectra confirmed the ABX spin system through the observed long range correlation of signal at δ_{H} 7.54 (H-4) and two oxyaryl carbons at δ_{C} 162.8 (C-8), and 160.0 (C-6). Therefore, the structure of compound **2** identified as 2-(2,4-dihydroxy-6-methoxyphenyl)-6-methoxybenzofuran-3-carbaldehyde with trivially named sesbagrandiflorain E.

Two known 2-arylbenzofurans, spinosan A (**3**), and B (**4**) compared by the 1D and 2D NMR spectra, showing the same profiles to previously published by Belofsky et al. 2006.

The cytotoxic activities of 2-arylbenzofurans (**1–4**) were investigated against MCF-7, HeLa, and WiDr (Table 1). Sesbagrandiflorain D (**1**) showed high activity against MCF-7, WiDr cells, and moderate activity against HeLa cell. Furthermore, the activity of compound **1** against MCF-7 is better than the Doxorubicin (positive control). Sesbagrandiflorain E (**2**) showed moderate activity against MCF-7, WiDr cells, and it was inactive against HeLa cells. Compound **3** was inactive towards those three human cancer cells. Compound **4** showed moderate activity against MCF-7 and HeLa, and it was inactive towards WiDr cells. Based on those data, it showed that compounds **1–2** are more active towards MCF-7, and WiDr cells compared to compounds **3–4** (Table 1). The difference between compound **1–2** with the compound **3–4** is the presence of OH group at C-6' (B ring). This hydroxyl group at compound **1–2** could exert a profound effect on increasing anticancer activity against MCF-7, and WiDr cells. Based on the

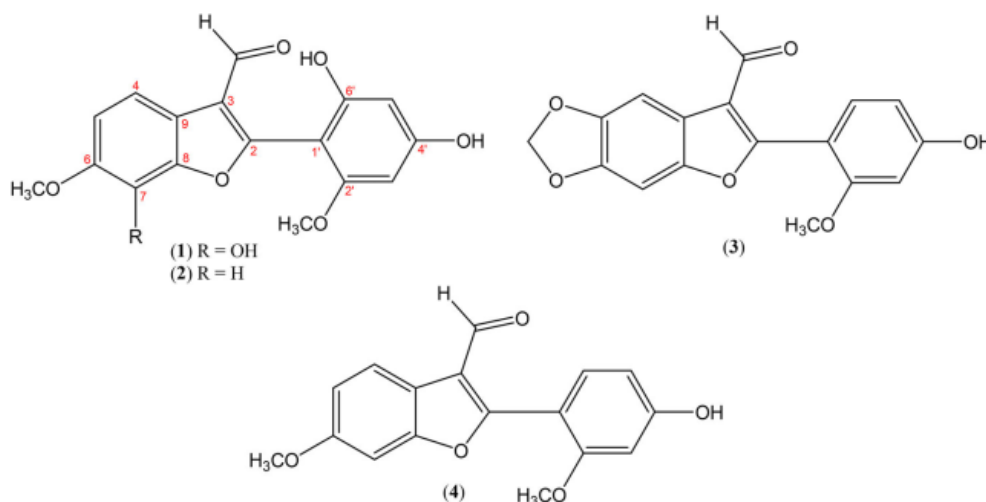


Figure 1. 2-Arylbenzofurans from *S. grandiflora* L.

structure of compound **1** and **2** (Figure 1), it suggested that the presence of OH at C-7 of compound **1** which is not present in compound **2**, appeared critical in their cytotoxic effects towards MCF-7 and WiDr cells. In summary, all of the 2-arylbenzofurans (**1-4**) were inactive against the HeLa cell.

3. Experimental

3.1. Plant material

The fresh stem barks of *S. grandiflora* L. were collected from Siwalan Panji Village, Buduran Districts, Sidoarjo, East Java, Indonesia in Dec 2018, and identified by Dr.M. Affandi, a botanist from Biology Department, Universitas Airlangga, Surabaya, East Java, Indonesia. The specimen of *S. grandiflora* L. (SG 20181203) was deposited at Herbarium Biology, Universitas Airlangga, Surabaya.

3.2. Extraction and isolation

The powdered dry stem barks (2.5 kg) of *S. grandiflora* was extracted by maceration with MeOH (2 × 15 L) at room temperature for three days, and after evaporation of the solvent under low pressure yielded MeOH extract (245 g). The MeOH extract was added water (9:1 v/v) and then partitioned with *n*-hexane (65 g) and EtOAc (17 g), successively. The *n*-hexane extract (50 g) was separated on silica gel and eluted by *n*-hexane-EtOAc (from 9:1 to 7:3) to yield compound **1** (18 mg). The separation of the EtOAc extract (16 g) with column chromatography over Si gel, and eluted with *n*-hexane-EtOAc (from 9:1 to 1:1) yielded four significant fractions, A-D. Fraction C (2.8 g) subjected to Sephadex LH-20, eluted with MeOH, gave three subfractions C₁-C₃. Subfraction C₁ was purified on CPC using *n*-hexane-EtOAc (from 9:1 to 4:1) gave compound **4** (40 mg) and further purification of subfraction C₂ by same methods using *n*-hexane-diisopropyl ether (from 1:1 to 1:4) to yield compound **2** (5 mg), and **3** (21 mg).

3.4. Spectral data

Sesbagrandiflorain D (**1**): yellowish oil, ²⁴UV/Vis (MeOH) λ_{\max} (nm) (log ϵ): 227 (4.28), 269 (3.99), 282 (4.00), 337 (3.99), and 398 (2.42) nm. IR (KBr) ν (cm^{-1}): 3350, 2980, ²⁴2928, 2860, 1663, 1611, 1502, and 1170. ¹H-NMR (400 MHz, acetone- d_6) δ_{H} ppm: 7.11 (1H, *d*, $J=8.4$ Hz, H-4), 6.84 (1H, *d*, $J=8.4$ Hz, H-5), 6.35 (1H, *d*, $J=2.0$ Hz, H-3'), 6.69 (1H, *d*, $J=2.0$ Hz, H-5'), 10.17⁴ (1H, *s*, 4'-OH), 3.72 (3H, *s*, 6-OCH₃), 3.83 (3H, *s*, 2'-OCH₃), and 9.80 (1H, *s*, 3-CHO). ¹³C-NMR (100 MHz, acetone- d_6) δ_{C} ppm: 164.4 (C-2), 119.1 (C-3), 123.1 (C-4), 112.4 (C-5), 147.5 (C-6), 139.6 (C-7), 150.6 (C-8), 114.1 (C-9), 107.7 (C-1'), 162.0 (C-2'), 98.9 (C-3'), 152.8 (C-4'), 88.5 (C-5'), 157.4 (C-6'), 61.5 (6-OCH₃), 56.1 (2'-OCH₃), and 191.1 (3-CHO). HRESIMS: m/z $[M+H]^+$ calcd. for C₁₇H₁₄O₇ 331.0817, found 331.0818.

Sesbagrandiflorain B (**2**): yellow solid, m.p. 176–178 °C, UV/Vis (MeOH) λ_{\max} (log ϵ): 212 (4.19), 269 (4.09), 298 (3.69), and 348 nm (3.90). IR (KBr) ν cm^{-1} : 3225, 1660, 1590, 1468 and 1033. ¹H-NMR (400 MHz, acetone- d_6) δ_{H} ppm: 7.54 (1H, *d*, $J=8.4$ Hz, H-4), 6.65 (1H, *dd*, $J=8.4$; 2.2 Hz, H-5), 6.71 (1H, *d*, $J=2.2$ Hz, H-7), 6.33 (1H, *d*, $J=2.0$ Hz, H-3'), 6.66 (1H, *d*, $J=2.0$ Hz, H-5'), 10.19 (1H, *s*, 4'-OH), 3.87 (3H, *s*, 6-OCH₃), 3.82 (3H, *s*, 2'-OCH₃), and 9.81 (1H, *s*, 3-CHO). ¹³C-NMR (100 MHz, acetone- d_6) δ_{C} ppm: 164.4 (C-2), 119.2 (C-3), 133.7 (C-4), 108.9 (C-5), 160.0 (C-6), 100.5 (C-7), 162.8 (C-8), 109.4 (C-9), 107.7 (C-1'), 161.9 (C-2'), 98.8 (C-3'), 152.6 (C-4'), 88.5 (C-5'), 157.3 (C-6'), 56.0 (6-OCH₃), 56.1 (2'-OCH₃), and 191.0 (3-CHO). HRESIMS: m/z $[M+H]^+$ calcd. for C₁₇H₁₄O₆ 315.0865, found 315.0869.

3.5. Cytotoxic assay

¹³The cytotoxicity of 2-arylbenzofurans (**1–4**) was evaluated by the MTT methods against human cervical cancer (HeLa), human breast cancer (MCF-7), and human colon carcinoma (WiDr). Doxorubicin was used as control positive (Saputri et al. 2018, 2019; Noviany et al. 2020).

4. Conclusions

⁹In this study, four 2-arylbenzofurans have been isolated from the stem bark of *S. grandiflora* L. Two of them are new compounds, named sesbagrandiflorain D (**1**) and E (**2**). The Cytotoxic activity of compounds (**1–4**) were investigated against MCF-7, HeLa, and WiDr. Both of the new compounds (**1–2**) exhibited higher activity compared to compounds (**3–4**). Furthermore, sesbagrandiflorain D (**1**) showed high activity against MCF-7 and WiDr cells. The activity of compound **1** against MCF-7 is better than the Doxorubicin (positive control).

Supplementary material

¹HRESIMS, 1D, and 2D NMR spectra reported in the supplementary materials as Figure S1–S12 and related to the following articles are available online.

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Disclosure statement

The authors declare no conflict of interest.

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