Published in final edited form as:

Phytochem Lett. 2020 February ; 35: 211–215. doi:10.1016/j.phytol.2019.12.008.

Structure Characterization and Biological Activity of 2-Arylbenzofurans from an Indonesian Plant, *Sesbania grandiflora* (L.) Pers

Noviany Noviany^{a,*}, Arash Samadi^{b,c}, Nita Yuliyana^a, Sutopo Hadi^a, Muhammad Aziz^d, Neny Purwitasari^e, Suriyati Mohamad^f, Nur Najihah Ismail^f, Kevin P. Gable^c, Taifo Mahmud^{b,c}

^aDepartment of Chemistry, Faculty of Mathematics and Natural Sciences, University of Lampung, Bandar Lampung 35145, Lampung, Indonesia

^bDepartment of Pharmaceutical Sciences, Oregon State University, Corvallis, Oregon 97331-3507, USA

^cDepartment of Chemistry, Oregon State University, Corvallis, Oregon 97331-3507, USA

^dInstitute of Innovative Research, Tokyo Institute of Technology, Ookayama, Meguro-ku, Tokyo 152-8550, Japan

^eDepartment of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, University of Airlangga, Surabaya 60286, Indonesia

^fSchool of Biological Sciences, Universiti Sains Malaysia, Minden 11800, Penang, Malaysia

Abstract

A new 2-arylbenzofuran, sesbgrandiflorain C (**1**), together with four known compounds, 2-(3,4-dihydroxy-2-methoxyphenyl)-4-hydroxy-6-methoxybenzofuran-3-carbaldehyde (**2**), 2-(4-hydroxy-2-methoxyphenyl)-5,6-dimethoxybenzofuran-3-carboxaldehyde (**3**), sesbgrandiflorain A (**4**) and sesbgrandiflorain B (**5**), have been isolated from the stem bark of an Indonesian plant, *Sesbania grandiflora* (L.) Pers. The chemical structure of compound **1** was elucidated by UV, IR, MS, and NMR spectroscopic techniques. The proton and carbon NMR resonances of **1** were also compared with the predicted chemical shifts obtained from DFT quantum mechanical calculations with Gaussian. None of the compounds showed antibacterial activity against *Bacillus subtilis*, *Escherichia coli*, *Mycobacterium smegmatis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* in an agar diffusion assay. However, sesbgrandiflorains A (**4**) and B (**5**) exhibited moderate activity against *Mycobacterium tuberculosis* H37Rv. In addition, compounds **1** – **5** have moderate cytotoxicity against HeLa, HepG2, and MCF-7 cancer cell lines.

*Corresponding author at: Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Lampung, Bandar Lampung 35145, Lampung, Indonesia, (N. Noviany). noviany@fmipa.unila.ac.id.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflict of Interest

The authors declare that they have no conflict of interest.

Graphical abstract

Keywords

herbal medicine; natural product; 2-arylbenzofuran; sesbagrandidflorain; *Sesbania grandiflora*

1. Introduction

The Fabaceae is the third largest and one of the most economically important families of flowering plants. Many fabaceous plants, particularly those from the Papilionoideae subfamily, are frequently used as traditional medicines to treat illnesses such as diabetes, cough, urinary problems, eye diseases, lung diseases, toothache, fever, dysentery and other infections (Neto et al., 2008; Roosita et al., 2008; Vitor et al., 2004; Watjen et al., 2007). Members of this family of plants have been known to produce alkaloids, non-proteinogenic amino acids, anthraquinones, coumarins, cyanogenic glycosides, flavonoids, isoflavonoids, phenylpropanoids, and terpenoids (Wink and Mohamed, 2003; Kobayashi et al., 1996, 1997; Kitagawa et al., 1996). Among them, isoflavonoids are mainly identified in plants from the Papilionoideae subfamily (Kırmızıbekmez et al., 2015). They demonstrate a wide-range of bioactivities, such as anti-microbial, anti-insecticidal, and allelopathic activities (Dixon and Sumner, 2003).

Among members of the Papilionoideae subfamily is *Sesbania grandiflora*, a flowering plant that is native to tropical Asia including Indonesia. Different parts of this plant have been used as herbal medicines to treat anemia, bronchitis, fever, and tumors (Wagh et al., 2009; Laladhas et al., 2010; Powell et al., 1984). Earlier studies on the leaves, seeds, and roots of *S. grandiflora* showed the presence of various secondary metabolites, e.g., α -5-methyl-5-pentacosanol, galactomannan, and flavonoids, some of which exhibited antituberculosis activity against *Mycobacterium tuberculosis* H37Rv (Tiwari and Bajpai, 1964; Pollard et al., 2011; Hasan et al., 2012; Noviany et al., 2012). Recently, we reported the isolation of two phenolic compounds, sesbagrandidflorains A (**4**) and B (**5**), from the stem bark of *S. grandiflora* (Noviany et al., 2018). However, their biological properties were unknown. Here, we report the isolation and structural characterization of other bioactive constituents in the EtOAc extract of the stem bark of *S. grandiflora* including a new 2-arylbenzofuran, sesbagrandidflorain C (**1**) (Fig. 1), and two known compounds, 2-(3,4-dihydroxy-2-methoxyphenyl)-4-hydroxy-6-methoxybenzofuran-3-carbaldehyde (**2**) and 2-(4-hydroxy-2-methoxyphenyl)-5,6-dimethoxybenzofuran-3-carboxaldehyde (**3**) (Fig. 1S, supplementary data). Compounds **4** and **5** were also isolated from this extract as major metabolites.

Furthermore, we determined the antibacterial activity and the cytotoxicity of compounds **1** – **5**, as well as the antituberculosis activity of compounds **4** and **5**.

2. Results and discussion

The stem bark of *S. grandiflora*, collected from Sumberdadi Village, Pringsewu, Bandar Lampung, Indonesia, was macerated sequentially using *n*-hexane, EtOAc, and 90% aqueous MeOH at room temperature. Repeated silica gel column chromatography of the EtOAc extract afforded a single spot on TLC, which gave high quality 1D and 2D NMR spectra (Fig. 2S – 20S, supplementary data). Analysis of the NMR data showed two sets of resonances similar to those reported previously for sesbagrandiflorain A with equal intensities, suggesting a possibility of a dimer compound. However, high-resolution (+)-ESI-MS analysis of the compound showed signals corresponding to sesbagrandiflorain “monomers”, indicating that the sample contained two equal amounts of closely related sesbagrandiflorain analogs. Further purification using high-performance liquid chromatography (HPLC) provided a new 2-arylbenzofuran, sesbagrandiflorain C (**1**), and a previously reported related compound **2**, which was isolated from the leaves of *Andira inermis* (Kraft et al., 2001).

Sesbagrandiflorain C (**1**) was isolated as a yellowish solid. Its molecular formula was established to be $C_{17}H_{14}O_6$ (m/z 315.08765 $[M+H]^+$) by HR-TOF-MS analysis. The 1H NMR spectrum of **1** is similar to that of sesbagrandiflorain A (**4**) including a unique aldehyde proton at 10.05 ppm (Table 1). However, compound **1** lacks the two aromatic proton resonances with J values of ~2 Hz (typical for meta aromatic protons) found in **4**. Instead, compound **1** has two additional aromatic proton resonances (δ_H 6.87; 7.10 ppm) with J values of 8.0 Hz, indicating different positions of aromatic substitutions in **1**. The ^{13}C NMR spectrum of **1** exhibited the existence of two methyl carbons, five sp^2 aromatic ring carbons, nine oxygenated/non-oxygenated quaternary carbons and a carbonyl carbon (δ_C 186.8) (Table 1). Detailed analysis of 2D NMR data [1H - 1H COSY, HSQC, HMBC and NOESY (Fig. 2) and (Figs. 8S – 11S, supplementary data)] for **1** revealed that the A and C rings of **1** are identical to those in **4**, whereas the B ring is different. While no HMBC correlations were observed to connect the C-2 and C-3 fragments in the C-ring of **1**, direct comparisons of the NMR data with those for **2** – **5** revealed that **1** also has a benzofuran-3-carbaldehyde skeleton. HMBC correlations between the OCH_3 protons (δ_H 3.73) and C-2' (δ_C 147.1) as well as between H-6' (δ_H 7.10) and C-2' (δ_C 147.1) indicate that the OCH_3 group is located at C-2' (Fig. 2). All together the data support the chemical structure of **1** to be 2-(3,4-dihydroxy-2-methoxyphenyl)-6-methoxybenzofuran-3-carbaldehyde (Fig. 1).

To confirm the 1H and ^{13}C NMR assignments of compound **1**, we performed computational NMR chemical shifts prediction using DFT quantum mechanical calculations [Willoughby et al., 2014]. Conformational analyses of **1** were performed in Gaussian 16 using DFT/B3LYP functional with the 6-31+G(d,p) basis set for geometry optimization and frequency calculations for the candidate structure. All conformers with a relative energy difference <5 kcal/mol from the most stable conformer (Fig. 2d) were kept and carried forward for NMR analysis. NMR shielding tensors were computed with the GIAO (gauge-independent (or including) atomic orbitals) method in Gaussian 16 using the DFT/MPW1PW91 functional

with the 6–311+G(2d,p) basis set, with acetone as the solvent, and the obtained tensor values were then converted to the predicted chemical shifts using the equation described in (Willoughby et al., 2014) (Table 1S, supplementary data). The mean absolute error (MAE) between the computed and the experimental data sets was 0.0825, which is within the “correct fit” cut off of 0.10 ppm [Willoughby et al., 2014].

The 2-arylbenzofuran-3-carbaldehyde **2** was isolated as a yellowish solid and the molecular formula was established to be $C_{17}H_{14}O_7$ (m/z 331.08261 $[M+H]^+$), suggesting the presence of an additional hydroxyl substitution in **2**. This is consistent with the 1H NMR spectrum of **2**, in which only four aromatic protons are present as opposed to five protons in **1** (Table 1, Fig. 12S, supplementary data). The ^{13}C NMR spectrum of **2** showed resonances of two methyl carbons, four sp^2 aromatic ring carbons, ten oxygenated/non-oxygenated quaternary carbons and a carbonyl (δ_C 190.3 ppm) (Table 1, Fig. 13S, supplementary data). Further detailed assessment of the 2D NMR data [HSQC and HMBC (Figs. 14S and 15S, supplementary data)] for **2** as well comparisons of its 1H and ^{13}C NMR data with those reported in the literature (Kraft et al., 2001) revealed the chemical structure of **2** as depicted in Fig. 1S.

From another fraction of the EtOAc extract, we also isolated a known 2-arylbenzofuran compound, 2-(4-hydroxy-2-methoxyphenyl)-5,6-dimethoxybenzofuran-3-carboxaldehyde (**3**) (Fig. 1S, supplementary data), using a combination of SiO_2 column chromatography and reversed-phase HPLC. This compound was previously isolated from the roots of *Ononis vaginalis* plants (Abdel-Kader, 2001). Compound **3** was acquired as a colorless solid. HR-TOF-MS analysis of **3** revealed a molecular formula of $C_{18}H_{16}O_6$ (m/z 329.10330 $[M+H]^+$), one carbon atom more than **1** or **2**. 1H and ^{13}C NMR spectra of **3** showed the existence of an additional methoxy group in **3**. The complete chemical structure of **3** was determined based on its 1D and 2D NMR (HSQC, HMBC, and NOESY) data (Figs. 16S – 20S, supplementary data) as well comparisons of its 1H and ^{13}C NMR data with those reported in the literature (Abdel-Kader, 2001).

In addition, we have isolated the previously reported major metabolites sesbagrandiflorins A (**4**) and B (**5**) (Fig. 1S, supplementary data) (Noviany et al., 2018) from a different fraction of the EtOAc extract. Together, compounds **1** – **5** were tested for their microbial property against *Staphylococcus aureus*, *Bacillus subtilis*, *Mycobacterium smegmatis*, *Pseudomonas aeruginosa*, and *Escherichia coli* using an agar diffusion assay. However, none of them showed any activity in this assay. The relatively abundant compounds **4** and **5** were then evaluated for their antituberculosis activity against *Mycobacterium tuberculosis* H37Rv by tetrazolium micro-plate assay (TEMA) (Hasan et al., 2012). The results showed that compounds **4** and **5** have moderate activity against *M. tuberculosis* with the minimum inhibition concentration (MIC) values of 200 and 12.5 $\mu g/mL$, respectively. The greater activity of compound **5** compared to compound **4** suggests that a free hydroxy group at C-6 is important for their activity. However, a more detailed structure-activity relationship study is required to provide better understanding of their anti-TB activity. Additionally, compounds **1** – **5** were tested for their cytotoxicity against cancer cell lines HeLa (cervical adenocarcinoma), HepG2 (liver carcinoma), and MCF-7 (mammary adenocarcinoma). The

results showed that **1** – **5** have moderate cytotoxicity against two or more of the tested cancer cell lines (Table 2).

3. Experimental

3.1. General experimental procedures

TLC was done on silica gel 60 GF₂₅₄ plates (Merck; 0.25 mm) and sprayed with the staining reagent Ce(SO₄)₂. Column chromatography (CC) was performed using silica gel (Kieselgel 60, 70–230 mesh ASTM; Merck). Preparative TLC was conducted on square glass plates (Kieselgel F₂₅₄; Merck). HPLC was carried out using a Shimadzu dual LC-20AD solvent delivery system with a Shimadzu SPD-M20A UV/vis photodiode array detector. ¹H and ¹³C NMR spectra were measured in acetone-d₆ (TMS as an internal standard), on an Agilent 500 MHz spectrophotometer (Agilent Technologies) or Bruker Avance III 700 MHz spectrometer equipped with a 5 mm ¹³C NMR cryogenic probe or a Bruker 500 MHz spectrometer. HRESI-MS was carried out in positive ion mode on a 6230 TOF mass spectrometer (Agilent Technologies). IR spectra were produced using a Nicolet IR100 FT-IR spectrophotometer (Thermo Fisher Scientific). UV spectra were produced using an Eppendorf BioSpectrometer® kinetic instrument.

3.2. Bacteria strains, cell lines and biochemicals

Middlebrook 7H9 broth and the albumin-dextrose-catalase (ADC) and oleic acid-albumin-dextrose-catalase (OADC) growth supplements were purchased from Difco. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl-tetrazolium bromide] for biochemistry was purchased from Sigma-Aldrich. Tween-80 was purchased from Merck. Isoniazid was purchased from Duchefa Biochemie. *Mycobacterium tuberculosis* H37Rv ATCC 27294 was purchased from the American Type Culture Collection.

3.3. Plant material

Samples of *S. grandiflora* stem bark were collected on 27 November 2016 from Sumberdadi Village, Pringsewu, Lampung Province, Indonesia. The plant specimen (NV5/NRGD/2016) was identified at the Herbarium Bogoriense, LIPI Bogor, Indonesia.

3.4. Extraction and isolation

The extraction and purification of the active metabolites were done according to the methods described in our previous paper (Noviany et al., 2018). Briefly, the air-dried plant material (3.0 kg) was macerated consecutively with *n*-hexane, EtOAc, and 90% aqueous MeOH. The EtOAc extract (40 g) was fractionated using SiO₂ vacuum liquid chromatography (VLC) (35–70 Mesh), eluted with *n*-hexane–EtOAc gradient from 100% *n*-hexane to 100% EtOAc, to give fractions E1–E5. Fraction E4 (9.5 g) was then fractionated using VLC (silica gel, 100% *n*-hexane to 100% EtOAc) to yield sub-fractions E4.1–E4.9. Fraction E4.6 was subsequently subjected to VLC and SiO₂ column chromatography (70–230 mesh), eluted with *n*-hexane–acetone (23:2 v/v), to afford compound **4** (230 mg). Fraction E4.7 (1.0 g) was also subjected to SiO₂ column chromatography using *n*-hexane–EtOAc (19:1–1:1 v/v) as the mobile phase to yield fractions E4.7.1–E4.7.6. Fractions E4.7.3 and E4.7.4 were further chromatographed individually using SiO₂ column eluted with *n*-hexane–acetone

(19:1–1:1 v/v) to give a mixture of compounds **1** and **2**. The mixture was then separated by reverse-phase HPLC (YMC ODS, 250 × 10 mm, gradient 5–100% MeOH in H₂O for 70 min, 3 mL/min) to yield compounds **1** (2.0 mg) and **2** (5.5 mg). Fractions E4.7.5 was also chromatographed using SiO₂ column eluted with *n*-hexane–acetone (19:1–1:1 v/v) to give subfractions, from which compound **5** was obtained as yellow crystals (after the fractions were kept at room temperature for several days). Fraction E4.8 (1.4 g) was subjected to SiO₂ column chromatography and eluted with *n*-hexane–EtOAc (19:1–1:1 v/v) as the mobile phase to give 32 sub-fractions (E4.8.1– E4.8.32). Sub-fractions E4.8.19 – E4.8.21 were pooled and further purified by reverse-phase HPLC (YMC ODS, 250 × 10 mm, isocratic 60% MeOH in H₂O for 75 min; 3 mL/min) to afford compound **3** (4.5 mg).

3.4.1. Sesbagrandidflorain C (1)—Compound **1** was obtained as yellowish solid; IR (KBr) $\nu_{\max}$ cm^{−1} 3397, 2927, 1654, 1498, 1193, 1142; UV (MeOH) $\lambda_{\max}$ (nm) (ε) 206 (9370), 240 (4970), 341 (2720); ESI-TOF-MS m/z 315.08765 [M+H]⁺, calculated for C₁₇H₁₅O₆ for 315.08631; ¹H and ¹³C NMR spectral data (acetone-*d*₆): see Table 1.

3.4.2. 2-(3,4-Dihydroxy-2-methoxyphenyl)-4-hydroxy-6-methoxybenzofuran-3-carbaldehyde (2)—Compound **2** was obtained as yellowish solid; IR (KBr) $\nu_{\max}$ cm^{−1} 3397, 3218, 2943, 1601, 1499, 1192, 1145; UV (MeOH) $\lambda_{\max}$ (nm) (ε) 216 (7190), 266 (3320), 359 (1610); ESI-TOF-MS m/z 331.08261 [M+H]⁺, calculated for C₁₇H₁₅O₇ for 331.08233; ¹H and ¹³C NMR spectral data (acetone-*d*₆): see Table 1.

3.4.3. 2-(4-Hydroxy-2-methoxyphenyl)-5,6-dimethoxybenzofuran-3-carboxaldehyde (3)—Compound **3** was obtained as colorless solid; IR (KBr) $\nu_{\max}$ cm^{−1} 3489, 2922, 1684, 1204; UV (MeOH) $\lambda_{\max}$ (nm) (ε) 211 (7470), 248 (4120), 288 (2080), 350 (2470). ESI-TOF-MS m/z 329.10330 [M+H]⁺, calculated for C₁₈H₁₇O₆ for 329.10196; ¹H and ¹³C NMR spectral data (acetone-*d*₆): see Table 1.

3.5. Computational calculation

Conformational analysis of sesbagrandidflorain C (**1**) was performed in Gaussian 16 using DFT/B3LYP functional with the 6–31+G(d,p) basis set for geometry optimization and frequency calculations for the candidate structure. All conformers with energy difference <5 kcal/mol from the most stable conformer were kept and carried forward for NMR analysis. NMR shielding tensors were computed with the GIAO (gauge-independent (or including) atomic orbitals) method in Gaussian using the DFT/MPW1PW91 functional with the 6–311+G(2d,p) basis set, with acetone as the solvent. The obtained tensor values were then converted to predicted chemical shifts using the equation described in Willoughby et al. (2014) with appropriate scaling and referencing factors (slope and intercept, respectively) obtained from <http://cheshirenmr.info/ScalingFactors.htm>. Assessment of goodness of fit was done by calculating the mean absolute error (MAE) between the computed and the experimental data sets. MAEs of 0.10 ppm are considered to be ‘correct’ fits and those of 0.20 ppm are considered to be ‘incorrect’ structure matches (Willoughby et al., 2014).

3.6. Antibacterial activity assay

Agar disc diffusion assay was used to evaluate the antibacterial activity of the EtOAc extract. Five different bacteria, *S. aureus*, *B. subtilis*, *P. aeruginosa*, *M. smegmatis*, and *E. coli* were used. The EtOAc extract was dissolved in MeOH to a concentration of 10 mg/mL. The positive control for this experiment was either ampicillin (for *S. aureus*, *B. subtilis*, *P. aeruginosa*, and *E. coli*) or apramycin (for *M. smegmatis*). Both the extract and the positive control (10 µL each) were loaded onto sterile diffusion discs and left to dry for 20 min. For *S. aureus*, *B. subtilis*, *P. aeruginosa*, and *M. smegmatis*, the agar plates were prepared by adding a layer of bacterial infused YMG soft agar to an YMG plate and left to solidify. The bacterial infused YMG soft agar was prepared by growing each of the bacteria in separate 15 mL falcon tubes with liquid YMG medium for two days and mixed it with warm YMG agar. The paper discs, impregnated with the extract or the positive control, were placed onto each plates using antiseptic techniques. For *E. coli*, all procedures mentioned above were done using Luria-Bertani (LB) medium instead of YMG. In addition, the *E. coli* plates and liquid cultures were incubated at 37 °C. After 24 hours of incubation, the plates were stained with MTT (1 mg/mL in de-ionized water) to enhance the contrast of the inhibition zones to the bacterial growth.

3.7. Antituberculosis assay

The antituberculosis activity was performed by a colorimetric tetrazolium micro-plate assay (TEMA) with minor modifications as described in our previous paper (Hasan et al., 2012).

3.8. Cytotoxicity assay

Cytotoxicity assay was performed based on a method described by O'Brien et al., 2000. HepG2, MCF-7, and HeLa cells were maintained in culture in Eagle's Minimum Essential Medium (EMEM, ATCC, cat # 30-2003) supplemented with 10% fetal bovine serum (FBS, Heat Inactivated, Gibco, cat # 10082-147) at 37 °C and 5% CO₂. Cells were dispensed into black, clear bottom, 384-well plates 24 h prior to compound treatment. One column in each plate did not receive cells to serve as a low-signal controls. Compounds were dissolved in DMSO and added with the D300 digital dispenser (HP) as a 12-point, half-log titration series in triplicate. Sixteen wells in each plate were left untreated to serve as high-signal controls. DMSO was normalized to 0.5% in every well. After 48 h at 37 °C and 5% CO₂, a solution of resazurin (Acros, cat # 189900050) in PBS was added to every well to a final concentration of 44 µM. After 4–6 h at 37 °C and 5% CO₂, fluorescence was measured with the microplate reader (Synergy 4, Biotek).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank the Directorate of Research and Community Services, The Ministry of Research, Technology and Higher Education, Republic of Indonesia for providing funds through PSN Grant (No.393/UN26.21/PN/2018) and WCP Program-Scheme B 2018 (No.123.44/D2.3/KP/2018). Cytotoxicity assay was performed in the Oregon State University College of Pharmacy High Throughput Screening Services Facility. We acknowledge the support of the

Oregon State University NMR Facility funded in part by the National Institutes of Health, HEI Grant 1S10OD018518, and by the M. J. Murdock Charitable Trust grant #2014162.

Abbreviations:

ESI-MS	electrospray ionization time-of-flight mass spectrometry
EtOAc	ethyl acetate
HMBC	heteronuclear multiple bond correlation
HMQC	heteronuclear multiple quantum coherence
HR-TOF-MS	high resolution time-of-flight mass spectrometry
HSQC	heteronuclear single quantum coherence
IR	infrared
<i>M. tuberculosis</i>	<i>Mycobacterium tuberculosis</i>
NMR	nuclear magnetic resonance
NOESY	nuclear overhauser effect spectroscopy
RP HPLC	reverse phase high performance liquid chromatography
TLC	thin layer chromatography
UV	ultraviolet

References

- Abdel-Kader MS, 2001 Phenolic constituents of *Ononis vaginalis* roots. *Planta Med.* 67, 388–390. [PubMed: 11458469]
- Dixon RA, Sumner LW., 2003 Legume natural products: understanding and manipulating complex pathways for human and animal health. *Plant Physiol.* 131, 878–885. [PubMed: 12644640]
- Hasan N, Osman H, Mohamad S, Chong WK, Awang K, Zahariluddin ASM, 2012 The chemical components of *Sesbania grandiflora* roots and their antituberculosis activity. *Pharmaceuticals* 5, 882–889. [PubMed: 24280680]
- Kırmızıbekmez H, Uysal GB, Masullo M, Demirci F, Bağcı Y, Kan Y, Puiacente S, 2015 Prenylated polyphenolic compounds from *Glycyrrhiza iconica* and their antimicrobial and antioxidant activities. *Fitoterapia* 103, 289–293. [PubMed: 25963162]
- Kitagawa I, Simanjuntak P, Mahmud T, Kobayashi M, Fujii S, Uji T, Shibuya H, 1996 Indonesian medicinal plants. XIII. Chemical structures of caesaldekars c, d, and e, three additional cassane-type furanoditerpenes from the roots of *Caesalpinia major* (Fabaceae). Several interesting reaction products of caesaldekars provided by N-bromosuccinimide treatment. *Chem. Pharm. Bull.* 44, 1157–1161.
- Kobayashi M, Mahmud T, Yoshioka N, Shibuya H, Kitagawa I, 1997 Indonesian medicinal plants. XXI. Inhibitors of Na⁺/H⁺ exchanger from the bark of *Erythrina variegata* and the roots of *Maclura cochinchinensis*. *Chem. Pharm. Bull.* 45, 1615–1619. [PubMed: 9353889]
- Kobayashi M, Mahmud T, Yoshioka N, Hori K, Shibuya H, Kitagawa I, 1996 Indonesian medicinal plants. XVIII. Kompasol A, a new stilbeno-phenylpropanoid from the bark of *Koompassia malaccensis* (Fabaceae). *Chem. Pharm. Bull.* 44, 2249–2253.

- Kraft C, Jenett-Siems K, Siems K, Solis PN, Gupta MP, Bienzle U, Eich E, 2001 Andinermals A-C, antiplasmodial constituents from *Andira inermis*. *Phytochemistry* 58, 769–774. [PubMed: 11672743]
- Laladhas KP, Cheriyan VT, Puliappadamba VT, Bava SV, Unnithan RG, Vijayammal PL, Anto RJ, 2010 A novel protein fraction from *Sesbania grandiflora* shows potential anticancer and chemopreventive efficacy, *in vitro* and *in vivo*. *J. Cell. Mol. Med* 14, 636–646. [PubMed: 19183244]
- Neto MM, Neto MA, Filho RB, Lima MAS, Silveira ER, 2008 Flavanoids and alkaloids from leaves of *Bauhinia unguilata* L. *Biochem. Syst. Ecol* 36, 227–229.
- Noviany, Osman H, Chong WK, Awang K, Manshoor N, 2012 Isolation and characterisation of 1,1'-binaphthalene-2,2'-diol, a new biaryl natural product from *Sesbania grandiflora* root. *J. Basic & Appl. Sci* 8, 253–256.
- Noviany N, Nurhidayat A, Hadi S, Suhartati T, Aziz M, Purwitasari N, Subasman I, 2018 Sesbigrandiflorain A and B: isolation of two new 2-arylbenzofurans from the stem bark of *Sesbania grandiflora*. *Nat. Prod. Res* 32, 2558–2564. [PubMed: 29338338]
- O'Brien J, Wilson I, Orton T, Pognan F, 2000 Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. *Eur. J. Biochem* 267, 5421–5426. [PubMed: 10951200]
- Pollard MA, Fischer P, Windhab EJ, 2011 Characterization of galactomannans derived from legume endosperms of genus *Sesbania* (Fabaceae). *Carbohydr. Polym* 84, 550–559.
- Powell RG, Smith CRJ, Weisleder D, 1984 Sesbanimide A and related tumor inhibitors from *Sesbania drummondii*: Structure and chemistry. *Phytochemistry* 23, 2789–2796.
- Roosita K, Kusharto CM, Sekiyama M, Fachrurrozi Y, Ohtsuka R, 2008 Medicinal plants used by the villagers of a Sundanese community. *J. Ethnopharmacol* 115, 72–81. [PubMed: 17961941]
- Tiwari RD, Bajpai RK, 1964 Chemical examination of *Sesbania grandiflora* leaves. II. Isolation and study of a saponin. *Proceedings of the National Academy of Sciences, India Section A: Phys. Sci* 34, 239–244.
- Vitor RF, Mota-Filipe H, Teixeira G, Borges C, Rodrigues AI, Teixeira A, Paulo A, 2004 Flavonoids of an extract of *Pterospartum tridentatum* showing endothelial protection against oxidative injury. *J. Ethnopharmacol* 93, 363–370. [PubMed: 15234778]
- Wagh VD, Wagh KV, Tandale YN, Salve SA, 2009 Phytochemical, pharmacological and phytopharmaceutics aspects of *Sesbania grandiflora* (Hadga): a review. *J. Pharm. Res* 2, 889–892.
- Watjen W, Kulawik A, Suckow-Schnitker AK, Chovolou Y, Rohrig R, Ruhl S, Kampkotter A, Addae-Kyereme J, Wright CW, Passreiter CM, 2007 Pterocarpans phaseollin and neurautenol isolated from *Erythrina addisoniae* induce apoptotic cell death accompanied by inhibition of ERK phosphorylation. *Toxicology* 242, 71–79. [PubMed: 17964704]
- Willoughby PH, Jansma MJ, Hoye TR, 2014 A guide to small-molecule structure assignment through computation of (1H and 13C) NMR chemical shifts. *Nat. Protoc* 9, 643–660. [PubMed: 24556787]
- Wink M, Mohamed GIA, 2003 Evolution of chemical defense traits in the Leguminosae: mapping of distribution patterns of secondary metabolites on a molecular phylogeny inferred from nucleotide sequences of the *rbcl* gene. *Biochem. Syst. Ecol* 31, 897–917.

Highlights

- A new 2-arylbenzofuran and four known compounds have been isolated from the stem bark of *Sesbania grandiflora*
- The chemical structures and phytopharmacological properties of the compounds have been evaluated
- The finding expands the chemical diversity of the natural constituents of *Papilionoideae*

Sesbagrandidiflorain C (1)

Fig. 1.
Chemical structure of compound 1.

Fig. 2. 2D NMR and conformational analyses of compound **1**. (a) ^1H - ^1H COSY correlations; (b) HMBC correlations; (c) NOESY correlations; (d) the lowest energy conformation of compound **1** obtained from quantum mechanical calculations with Gaussian 16.

Table 1.¹H and ¹³C NMR data for compounds **1** – **3**.

Position	1		2		3	
	δ_C^a	δ_H^c	δ_C^a	δ_H^c	δ_C^b	δ_H^c
2	162.9	-	163.8	-	162.5	-
3	117.1	-	118.4	-	117.1	-
4	122.0	8.04 (d, $J = 8.5$)	156.6	-	103.3	7.65 (s)
5	113.2	7.03 (dd, $J = 8.5, 2.0$)	98.1	6.37 (d, $J = 2.0$)	148.0	-
6	159.0	-	161.3	-	149.0	-
7	95.7	7.23 (d, $J = 2.0$)	87.7	6.73 (d, $J = 2.0$)	95.4	7.25 (s)
8	155.5	-	152.1	-	149.1	-
9	118.3	-	106.9	-	117.3	-
1'	113.6	-	113.2	-	108.9	-
2'	147.1	-	146.9	-	159.0	-
3'	138.8	-	138.8	-	99.7	6.78 (d, $J = 2.0$)
4'	149.5	-	149.9	-	161.8	-
5'	111.6	6.87 (d, $J = 8.0$)	111.7	6.88 (d, $J = 7.0$)	108.0	6.68 (dd, $J = 8.5, 2.0$)
6'	122.1	7.10 (d, $J = 8.0$)	122.2	7.13 (d, $J = 7.0$)	132.5	7.50 (d, $J = 8.5$)
MeO-C(6)	55.2	3.91 (s)	55.3	3.86 (s)	55.2	3.87 (s)
MeO-C(2')	60.4	3.73 (s)	60.6	3.76 (s)	61.4	3.90 (s)
MeO-C(5)	-	-	-	-	55.6	3.91 (s)
HO-4	-	-	-	10.18 (s)	-	-
CHO	186.8	10.05 (s)	190.3	9.83 (s)	186.9	10.03 (s)

^a measured at 125 MHz;^b measured at 175 MHz;^c measured at 500 MHz

Table 2.

Cytotoxicity of compounds **1** – **5** on a number of cancer cell lines.

Compound	CC ₅₀ (μM)		
	HeLa	HepG2	MCF-7
1	>100	8.25	35.1
2	31.5	25.4	10.2
3	31.5	>100	9.0
4	31.3	30.8	0.65
5	30.5	31.3	21.3