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Research Article

Phytoconstituent Isolation and Cytotoxicity Evaluation of the Egyptian *Cassia occidentalis* L. Possessing Selective Activity against Lung Carcinoma

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Ethyl acetate fraction column chromatographic analysis was used to isolate eleven compounds (numerically tagged 1–10) from *Cassia occidentalis* L. in this study. Two unique metabolites, including a neolignan compound designated as occidentalignan I (9) and a flavonoidal glycoside, chrysin-7-O-α-L-rhamnopyranosyl (10), were identified, while silybin A (8) was the first flavonolignan to be isolated from the Fabaceae family. Four compounds, including β-sitosterol-3-*O*-β-D-glucopyranoside (1), stigmasterol-3-*O*-β-D-glucopyranoside (2), betulinic acid (3), and vanillic acid (4) were isolated from *C. occidentalis* for the first time. In addition, four known compounds, cinnamic acid (5), p-hydroxybenzoic acid (6), β- resorcylic acid (7), and citric acid (11), were also detected. The *in-vitro* cytotoxicity assessment of the methanolic extract of *C. occidentalis* on seven cancer cell lines, including A-549, Colo-205, Huh-7, HCT-116, PANC-1, SKOV-3, and BNL, demonstrated its selective potent cytotoxicity on lung cancer cells without affecting normal BNL cells. In contrast, the methanolic extract showed moderate activity on Colo-205 and Huh-7 and nearly no activity on HCT-116, PANC-1, and SKOV-3 cell lines. These results suggest that the methanolic extract of *C. occidentalis* is an excellent candidate with potential antiproliferative activity against lung cancer; however, further studies are necessary to clarify its mechanism of action.

1. Introduction

Cancer treatment remains a significant healthcare challenge that constantly necessitates the discovery of new therapeutic candidates. Numerous synthetic cancer medications are currently available; however, the majority exhibit fatal drawbacks to normal healthy cells, such as cell toxicity, genotoxicity, carcinogenicity, and teratogenic effects [1]. Thus, despite their high efficiency in targeted cancer cell treatments, the adverse effects of these available agents limit their use as synthetic chemotherapeutics. Consequently, there is an urgency to discover new sources for safe anticancer agents with selective activity on cancer cells and without harmful effects on normal cells. Nature is a rich source of highly effective therapeutic agents with the capacity to treat several deadly ailments at very low toxicity margins.

C. occidentalis L. is a pharmacologically significant herb and a candidate with crucial anticarcinogenic properties. The herb belongs to the family Fabaceae (Leguminosae), and its different tissues have been used in folk medicine. *C. occidentalis* is native to the western hemisphere, principally South and Central America, and was introduced to India and China and then to African countries, such as Egypt and Libya [2, 3]. The extract of the whole plant has been used ethnomedicinally to cure eye inflammation, while it is extensively used in Jamaica to treat various diseases, such as eczema, constipation, diarrhea, venereal diseases, fever, dysentery, and cancer [4]. Infusions of *C. occidentalis* roots have been used for stomach pain, whereas uses in veterinary

medicine as antidotes for animal treatments have been reported. In addition, boiled *C. occidentalis* roots are used to treat constipation, whooping cough, and lactogogue [5], while its decoction mixed with black pepper is used in the treatment of filarial disease [6]. In Brazil, the roots are also used as a tonic, antidiuretic, and febrifuge and in the treatment of liver diseases, anemia, fever, and tuberculosis [7].

C. occidentalis L. leaves have been used topically as a paste for the treatment of wounds, cutaneous diseases, sores, bone fractures, ringworms, fever, and throat infections, while their extracts have also been shown to pharmacologically possess antibacterial, antimalarial, antimutagenic [8–10], immunosuppressive [11], anticarcinogenic, and hepatoprotective activities [10, 12, 13]. Other uses, such as anti-inflammatory, antidermatophyte, antibacterial, antiplasmodial, antifertility, antimalarial, and antidiabetic activities, as well as its capacity to repair, protect, and normalize liver functions have been reported [10, 14].

Previous studies on *Cassia* species reported the isolation and identification of a wide range of secondary metabolites with numerous biological potentials. Consequently, this study explored *C. occidentalis* with an aim to elucidate its phytochemical and biological properties and to extensively demonstrate evidence of its usage in various pharmaceutical products.

2. Methods and Materials

2.1. Ethical Statement. All experiments and procedures were performed by following the relevant guidelines and regulations of the Faculty of Pharmacy, Assiut University, Assiut, Egypt.

2.2. General Experimental Procedures. Infrared (IR) spectra were recorded on a Shimadzu Infrared-400 spectrometer (Kyoto, Japan) and ultraviolet (UV) spectra were obtained on a Shimadzu 1601 UV/VIS spectrophotometer. Nuclear magnetic resonance spectroscopy (NMR) spectra were obtained on a Bruker Avance III 400 spectrometer using precoated silica gel 60 F_{254} TLC (0.25 mm, Merck, Germany) and RP-18 F_{254} plates (0.25 mm, Merck, Germany). In electron-ionization mass spectrometry (EIMS), measurements were obtained on a Bruker, Mass DIP meth-mass spectrometer.

2.3. Plant Material. The whole aerial flowering parts of *C. occidentalis* were collected between April and July 2014, from plants cultivated at the Experimental Station, Faculty of Pharmacy, Assiut University, Assiut, Egypt. The plant was identified by Dr. A. A. Fayed, Prof. of Plant Taxonomy, Faculty of Science, Assiut University, Assiut, Egypt. A voucher sample was kept in the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Assiut University, Assiut, Egypt.

2.4. Extraction and Fractionation. The air-dried powdered samples from whole aerial flowering parts (3 kg) of *C. occidentalis* L. were extracted by maceration in 70% methanol (10 L × 4). The alcoholic extract was concentrated, and 350 g of the solvent-free residue (11.7%) was mixed with 500 mL of distilled water and then was subjected to successive solvent fractionation with *n*-hexane, chloroform, ethyl acetate, and *n*-butanol till complete exhaustion, followed by extract storage in a vacuum desiccator until use. After monitoring all fractions with TLC using different solvent systems and spraying with different reagents, the ethyl acetate fraction was selected for column chromatographic isolation of compounds as it contained several different compounds of important chemical classes with vital biological functions, such as flavonoids and phenolics.

2.5. Phytochemical Studies

2.5.1. Column Chromatographic Separations. The ethyl acetate fraction (40 g) was subjected to vacuum liquid chromatography with chloroform-methanol gradient elution. Five subfractions labeled as COEt-I-COEt-V.Subfraction COEt-II (7.5 g) were rechromatographed on silica gel column chromatography (280 g) and eluted with a chloroform-methanol gradient. Fractions of 50 mL each were collected and monitored by thin-layer chromatography (TLC). Similar fractions were collected and grouped. Fractions eluted with chloroform/methanol in the ratios of 9 : 1, 8 : 2, and 7 : 3 generated 50, 20, and 25 mg of compounds **(1)**, **(2)**, and **(5)**, respectively. Subfraction COEt-III (8.5 g) was rechromatographed on silica gel column chromatography (320 g) and eluted with a chloroform/methanol gradient. Fractions of 50 mL each were collected and grouped. Fractions eluted with chloroform/methanol in the ratios of 9 : 1, 8 : 2, and 6 : 4 yielded 18 g, 22 mg, and 18 mg of compounds **(6)**, **(7)**, and **(4)**, respectively. Subfraction COEt-IV (9.5 g) was rechromatographed on silica gel column chromatography (350 g) and then eluted with a chloroform/methanol gradient in the ratios of 8 : 2, 7 : 3, 6 : 4, and 1 : 1 to generate compounds **(3)**, **(9)**, and **(8)** with 15, 25, and 30 mg, respectively. Subfraction COEt-V (9 g) was rechromatographed on silica gel column chromatography (300 g) and eluted with a chloroform/methanol gradient in the ratios of 7 : 3, 6 : 4, and 1 : 1 to yield compounds **(10)** and **(11)** with mass of 18 and 25 mg, respectively.

2.5.2. Acid Hydrolysis. A solution of the isolated glycoside (5 mg in 10 mL MeOH) was treated with 1.5 mL of 3% H_2SO_4 and heated at 100°C for 1 h. The aglycone was extracted with ethyl acetate, concentrated under reduced pressure, and identified by co-TLC with an authentic sample by using a suitable system. The sugars in the aqueous layer were identified by paper chromatography with authentic standards using system n-butanol-acetic acid-water (4 : 1 : 2 drops) v/v and sprayed with aniline hydrogen phthalate [15].

2.6. Pharmacological Studies

2.6.1. In Vitro Assay

(1) Cell Culture. The lung cancer (A-549), colorectal cancer (Colo-205), liver cancer (Huh-7), colon cancer (HCT-116), pancreatic cancer (PANC-1), and ovarian cancer (SKOV-3) cell lines, as well as normal hepatocyte cell line (BNL) were obtained from Nawah Scientific Inc. (Mokatam, Cairo, Egypt). Cell lines were maintained in a Roswell Park Memorial Institute (RPMI) medium supplemented with 100 mg/mL of streptomycin, 100 units/mL of penicillin, and 10% of heat-inactivated fetal bovine serum and then incubated at 37°C in a humidified 5% (v/v) CO_2 atmosphere [16].

(2) Cytotoxicity Assay. Cell viability was assessed using a sulforhodamine B (SRB) assay. About 100 L of cell suspension (5×10^3 cells) was transferred to a 96-well plate and incubated in a complete medium for 24 h. The cells were then treated with 100 L of medium containing samples at 10 and 100 g/mL concentrations. After 72 h of exposure, the cells were fixed by replacing the medium with 150 L of 10% trichloroacetic acid and incubating at 4°C for 1 h. Subsequently, the trichloroacetic acid solution was removed and the cells were washed five times with distilled water. Approximately, 70 L of SRB solution (0.4% w/v) was added and the mixture was incubated in the dark at an ambient temperature for 10 min. The plates were then washed three times with 1% acetic acid and allowed to air-dry overnight. After drying, 150 L of 10 mM tris (hydroxymethyl) aminomethane (TRIS) was added to dissolve the protein-bound SRB stain, and then the absorbance was measured at 540 nm using a BMG LABTECH-FLUOstar Omega microplate reader (Ortenberg, Germany) [16].

2.7. Statistical Analysis. Data are expressed as mean ± SD for all parameters. Graph Pad Prism software package was used for multiple comparisons of data, and a one-way analysis of variance (ANOVA) test was used to infer statistical significance at $P < xx$.

3. Results and Discussion

3.1. Phytochemical Studies

3.1.1. Screening and Isolation of Compounds. Silica gel column chromatographic analysis of the ethyl acetate fraction obtained from the whole aerial flowering parts of *C. occidentalis* identified eleven compounds labeled **1**–**11** (Figure 1). The structures of these compounds were identified and confirmed by one-dimensional (1D) and 2D NMR analysis and mass measurements and by comparing results with the reported data that were previously published.

Four known compounds (**1**–**4**) identified as *β*-sitosterol-3-*O*-*β*-D-glucopyranoside (**1**), stigmasterol-3-*O*-*β*-D-glucopyranoside (**2**), betulinic acid (**3**), and vanillic acid (**4**) were for the first time in *Cassia occidentalis* L, while in addition, three known compounds, cinnamic acid (**5**), *p*-hydroxybenzoic acid (**6**), and *β*- resorcylic acid **(7),** were also identified. Silybin A (**8**) is the first flavonolignan to be identified in the family Fabaceae and could serve as a taxonomic marker of *C. occidentalis*. All physical and spectral data of these compounds were consistent with those reported in the previous data (8).

Compound (1) was isolated as a granular powder (50 mg, MeOH), and electron-ionization mass spectrometry (EI-MS), *m*/*z* (rel.int%), of 413 $[M\text{-sugar}]^+$ (10%), 394 (20%), 399 (10%), 264 (20%), 83 (60%), and 57 (100%) was detected (Figure S1). On the basis of the EI-MS, ^{1}H-, and ^{13}C-NMR (Figures S2 and S3) and by comparison with the literature [17, 18], compound (1) was identified as *β*-sitosterol-3-*O*-*β*-D-glucopyranoside (**1**). To our knowledge, this study represents the first report of its isolation from *C. occidentalis* L.

Compound (2) was isolated as a white granular powder (20 mg, MeOH), and electron-ionization mass spectrometry (EI-MS), *m*/*z* (rel.int%), of 411 $[M\text{-sugar}]^+$(5%), 394 (5%), 300 (30%), 255 (20%), 83 (30%), and 70 (100%) was observed (Figure S4). On the basis of EI-MS, ^{1}H-, and ^{13}C-NMR (Figures S5 and S6) and by comparison with the literature [18, 19], compound (2) was identified as stigmasterol-3-*O*-*β*-D-glucopyranoside (**2**). To our knowledge, this represents its first isolation from *C. occidentalis*.

Compound (3) was isolated as a white needle-shaped substance (15 mg, MeOH) with a melting point (m.p) of 295–298°C. EI-MS, *m*/*z* (rel.int%), of 456 $[M]^+$ (60%), 411 $[M\text{-}COOH]^+$ (5%), 438 $[M\text{-}H_2O]^+$ (5%), 248 (10%), 207 (22%), and 189 (20%) was detected (Figure S7). The ^{1}H-NMR spectrum analysis of compound (3) (Table S1 and Figure S8) showed that it exhibited a triterpenoid skeleton that revealed the presence of six methyl group signals, and five of them at δ_H 0.65, 0.76, 0.89, 0.91, and 0.93 parts per million (ppm) were assigned to Me-25, Me-23, Me-24, Me-27, and Me-26, respectively. In addition, a vinylic methyl at δ_H 1.64 ppm assigned to Me-30, a methine proton at δ_H 2.97 ppm (1H, m) attributed to H-19, and one oxymethine proton at δ_H 3.33 ppm (overlapped with solvent signal) assigned to H-3 were detected, which were confirmed by IR band at ν 3400 cm^{-1} [20]. Two olefinic protons at δ_H 4.56 and 4.69 ppm were attributed to H-29a and H29b, respectively. The mass spectrum (Figure S7) showed a molecular ion peak at *m*/*z* 456, which corresponded to a molecular formula $C_{30}H_{48}O_3$, with characteristic mass fragments at *m*/*z* 411, 219, 248, 218, and 207, typical of betulinic acid [21]. The ^{13}C-NMR spectrum of compound (3) (Table S1; Figures S9 and S10) confirmed its triterpenoid nature and revealed signals for thirty carbons. The compound exhibited a carbonyl carboxylic acid moiety at δ_C 177.77 ppm assigned to C-28, in addition to two olefinic carbons at δ_C 110.11 and 150.78 ppm assigned to C-29 and C-20, respectively, and one oxymethine at δ_C 78.70, suggesting a lupane skeleton. Previous studies suggested that the compound was betulinic acid [21]; thus, it was designated as betulinic acid (**3**). To our knowledge, this represents its first isolation from *C. occidentalis*.

Compound (4) was isolated as a white amorphous powder (18 mg, MeOH). EI-MS, *m*/*z* (rel.int%), of 168 $[M]^+$ (10%), 153 $[M\text{-}CH_3]^+$ (15%), 137 $[M\text{-}OCH_3]^+$ (60%), 129

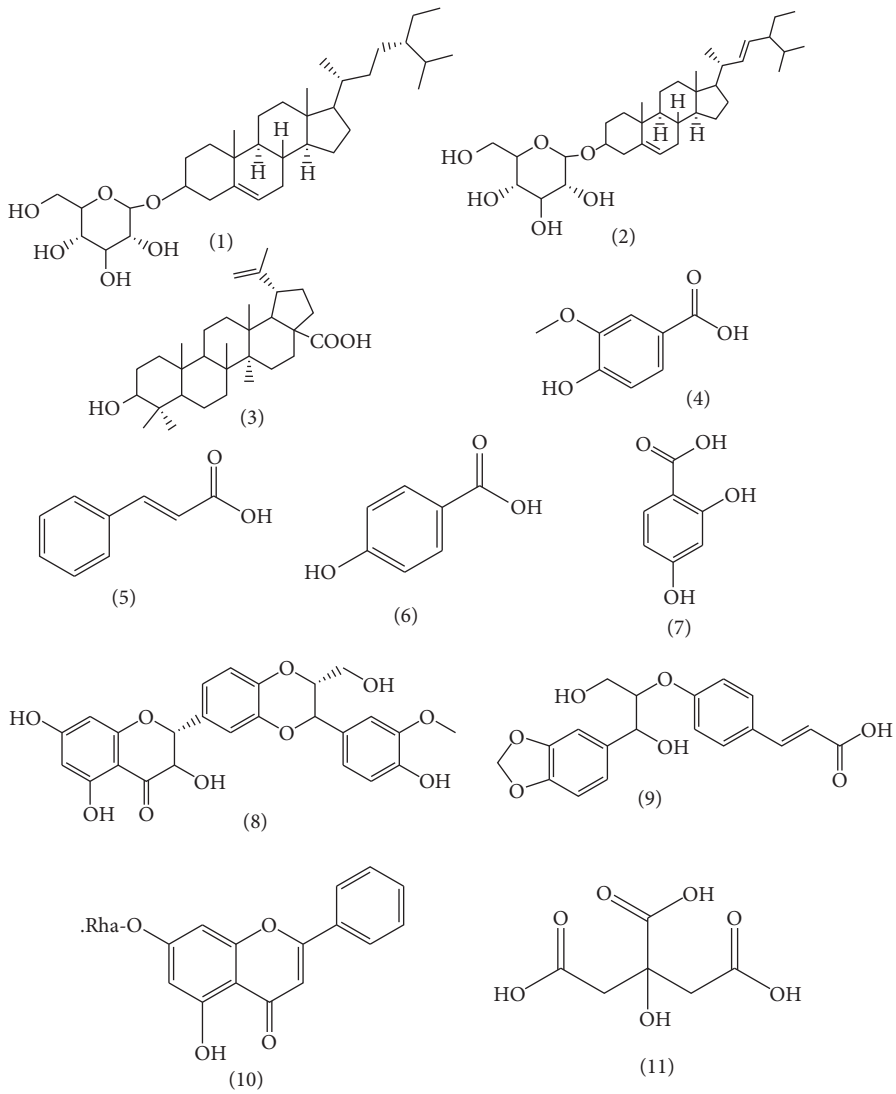

Figure 1: Structures of compounds isolated from the whole aerial flowering parts of *C. occidentalis* L.

(90%), 111 (50%), and 87 (100%) was observed (Figure S11). Analysis of ^{1}H- and ^{13}C-NMR spectra data of compound (4) (Table S2; Figures S12 and S13) showed that it exhibited three signals in the aromatic region at δ_H 7.45 ppm (1H, s), 6.84 ppm (1H, d, J = 8.8 Hz), and 7.44 ppm (1H, d, J = 8.8 Hz), indicating the presence of a trisubstituted benzene ring. A singlet corresponding to three protons at δ_H 3.80 ppm suggested the presence of a methoxy group, which was confirmed by δ_C 55.97 ppm and another singlet at δ_H 9.01 ppm for the aromatic hydroxyl group. The ^{13}C-NMR spectrum revealed seven aromatic carbon signals, with downfield one at δ_C 167.80 ppm being attributed to a carboxy carbonyl group, while the other six signals were similar to the previously reported compounds [22, 23]. The mass with the molecular ion peak at m/z 168 and ^{13}C-NMR spectral data of compound (4) suggested a molecular formula of $C_8H_8O_4$, thus the compound was considered to be 3-methoxy, 4-hydroxy benzoic acid (vanillic acid) (**4**), which to our knowledge represents its first isolation from *C. occidentalis*.

Compound (5) was isolated as white crystals (25 mg, MeOH) with an m.p of 133135°C. EI-MS, m/z (rel.int%), of 148 $[M]^+$ (77%), 147 $[M\text{-}1]^+$ (100%), 131 $[M\text{-}OH]^+$ (27%), 103 $[M\text{-}45]^+$ (69%), 91 (40%), and 77 (65%) was detected (Figure S14). Compound (5) exhibited a ^{1}H-NMR spectrum (Table S3 and Figure S15) for a monosubstituted phenyl ring at δ_H 7.68 ppm (2H, d, J = 8.0 Hz) corresponding to H-2 and H-6, in addition to AB system with resonance at δ_H 7.60 and 6.54 ppm that had a large coupling constant, indicating a transgeometry. The ^{13}C- and ^{13}C-DEPT (distortionless enhancement by polarization transfer) NMR spectra of compound (5) (Table S3; Figures S16, and S17) displayed seven carbon signals, with one of them being attributed to acid carbonyl at δ_C 168.05 ppm, which predicted a cinnamic acid structure. The mass spectrum (Figure S7) exhibited a molecular ion peak at m/z 148 that corresponded to $C_9H_8O_2$, thus validating the predicted structure. The resulting spectral data were consistent with that of cinnamic acid in a previous study [24], and it was therefore isolated as

cinnamic acid (**5**), which to our knowledge represents its first isolation from the genus *Cassia*.

Compound (6) was isolated as a white needle-shaped compound (18 mg, MeOH) with m.p of 215–217°C. EI-MS, *m*/*z* (rel.int%), of 138 $[M]^+$ (70%), 121 $[M\text{-}OH]^+$ (100%), 93 $[M\text{-}COOH]^+$ (32%), and 65 (40%) was obtained (Figure S18). The ^{1}H-NMR spectrum analysis of the compound (Table S4 and S19) showed that it exhibited two doublet signals at δ_H 7.80 ppm (2H, d, J = 8.4 Hz) and 6.83 ppm (2H, d, J = 8.4 Hz), which were equivalent to protons attributed to a paradisubstituted benzene ring, in addition to the two downfield singlet signals at δ_H 12.28 and 10.32 ppm that corresponded to carboxylic OH and 4-OH groups. The ^{13}C- and DEPT ^{13}C-NMR (Figures S20 and S21) spectra revealed five signals, which could be attributed to seven carbons including a signal at δ_C 167.64 ppm that corresponded to carboxy carbonyl. The DEPT experiment revealed three quaternary carbons, which were attributed to C-1 and C-4 at the δ_C 121.84 and 162.06 ppm and a carboxylic one at δ_C 167.64. The mass spectrum (Figure S18) generated a molecular ion peak at *m*/*z* 138, which predicted a molecular formula of $C_7H_6O_3$. The obtained data were consistent with those of *p*-hydroxybenzoic acid [25], thus the compound was identified as *p*-hydroxybenzoic acid (**6**), which to our knowledge represents its first isolation from the genus *Cassia*.

Compound (7) was isolated as a white needle-shaped compound (22 mg, MeOH) with m.p of 208–211°C. EI-MS, *m*/*z* (rel.int%), of 154 $[M]^+$ (30%), 136 $[M\text{-}H_2O]^+$ (100%), 108 $[M\text{-}COOH]^+$ (46%), and 80 (60%) was obtained (Figure S22). Its ^{1}H-NMR spectrum (Table S5 and Figure S23) exhibited three aromatic proton signals, which were characteristic to the trisubstituted benzene ring at δ_H 7.18 ppm (1H, br.s), 6.78 ppm (1H, d, J = 8.8 Hz), and 6.96 ppm (1H, d, J = 8.8 Hz), which were assigned to H-3, H-5, and H-6, respectively. An additional downfield singlet at δ_H 9.30 ppm for phenolic hydroxyl was also detected. The ^{13}C-NMR spectrum of compound (7) (Table S5 and Figure S24) showed seven carbon signals and included a signal at δ_C 172.14 ppm, which was assigned to the carboxy carbonyl group. DEPT experiment (Figure S25) revealed four quaternary carbons attributed to C-1, C-2, and C-4 at δ_C 113.08, 149.83, and 154.57 ppm, respectively, in addition to a carboxylic group at δ_C 172.14. The mass spectrum (Figure S22) revealed a molecular ion peak at *m*/*z* 154 that predicted a molecular formula of $C_7H_6O_4$. The obtained data suggested that the compound was 2,4-dihydroxy benzoic acid, and previous spectral data report [23] confirmed its identity as 2,4-dihydroxy benzoic acid (β-resorcylic acid) (**7**), which to our knowledge represents its first isolation from the genus *Cassia*.

Compound (8) was isolated as a yellowish-white granular powder (30 mg, MeOH). The UV spectral analysis of the compound λ_{max} (MeOH) showed absorption at 217, 290, and 330 nm. The IR (KBr) spectrum showed the following absorption bands at ν cm^{-1} 3431 (phenolic OH), 1704 (C=O), 1552, 1440, 1250, 1237, 1080, 992, and 680 (aromatic system). EI-MS, m/*z* (rel.int%), of 482 $[M]^+$ (21%), 465 $[M\text{-}OH]^+$ (8%), 195 (3%), 109 (16%), 180 [M-cinnamoyl]$^+$ (30%), 153 (60%), 137 (90%), 133 (10%), and 124 (100%) (Figure S26) was obtained. The ^{1}H-NMR spectrum evaluation of the compound (Table S6 and Figure S27) displayed typical features of 5,7-dihydroxy-substituted flavonol skeleton [15] with signals at δ_H 5.88 ppm (1H, br.s, H-6), 5.92 ppm (1H, br.s, H-8), 5.08 ppm (1H, d, J = 7.2 Hz), and 4.62 ppm (1H, m), which were characteristic to the H-2 and H-3 of the dihydro-flavonol skeleton [26]. In addition, the six protons in the aromatic region could be attributed to the two sets of 1, 3, and 4-trisubstituted aromatic rings, with one belonging to the B-ring of the dihydro-flavonol group at δ_H 7.09 ppm (1H, br.s.), 6.97 ppm (1H, d, J = 8.0 Hz), 6.99 ppm, and (1H, d, J = 8.0 Hz) for H-2′, 5′, and 6′, respectively, while the other belonging to the cinnamic alcohol groups at δ_H 7.01 (1H,br.s.), 6.81 (1H,d, J = 8.0 Hz), and 6.87 (1H,d, J = 8.0 Hz) for H-2″, H-5″, and H-6″, respectively. Four protons at δ_H 4.90 ppm (1H, d, J = 8.0 Hz, H-7″), 4.17 ppm (1H, m, H-8″), 3.55 ppm (1H, m, H-9a″), and 3.40 ppm (1H, m, H-9b″) could be assigned to a propanol moiety connected to a dioxane ring [26]. The ^{1}H-NMR spectra also exhibited a three-proton singlet at δ_H 3.78 ppm that was assigned to the aromatic methoxy group and three hydroxyls attached to the aromatic skeleton at δ_H 11.89, 10.24, and 9.15 ppm (each as one proton singlet), which suggested the 5-OH, 7-OH, and 4″-OH groups, respectively, and were confirmed by the IR absorption band at 3431 cm^{-1}. In addition, two proton signals were attributed to olefinic hydroxyls at δ_H 5.81 ppm (1H, d, J = 6.0 Hz) and 4.91 ppm (1H, d, J = 7.6 Hz) for 3-OH and 9″-OH, respectively. The mass spectrum (Figure S26) showed a molecular ion peak at *m*/*z* 482 and the ^{13}C-NMR spectrum (Table S6 and Figure S28) revealed 25 carbon signals, suggesting a molecular formula of $C_{25}H_{22}O_{10}$. Carbon signals at δ_C 83.02 and 71.80 ppm corresponded to C-2 and C-3, in addition to the downfield one at δ_C 198.20 ppm, which was assigned to the C-4, thereby confirming a dihydro-flavonol skeleton with hydroxylated C-5 and C-7 [27]. The remaining ^{13}C-NMR signals were consistent with those previously reported for a flavonolignan skeleton [26, 28]. The DEPT experiment (Table S6 and Figure S29) detected 1, 1, 12, and 11 signals for methoxy, CH_2, CH, and quaternary carbons, respectively. The obtained data suggested a dihydro-flavonol skeleton and a lignan moiety, and their connection was verified by comparing the obtained spectral data with those in the literature [28]. Complete assignment of the ^{1}H- and ^{13}C-NMR signals was achieved by analyzing their ^{1}H^{1}H correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), and heteronuclear multiple bond correlation (HMBC) spectra (Figures S30–S32). The stereochemistry of compound (8) was based on the carbon chemical shifts, which shared a close resemblance with those previously reported for silybin A (**8**) (2R,3R)-3,5,7-trihydroxy-2-[(2S,3S)-3-(4-hydroxy-3-methoxyphenyl)-2-hydroxymethyl-2,3-dihydro-benzol [1, 4] dioxin-6-yl]-chroman-4-one [28]. On the basis of previously reported spectral data and by comparison with the available literature [26, 28], compound (8) was verified to be silybin A (**8**). To our knowledge, this represents its first isolation from

Table 1: ^{1}H-NMR, ^{13}C-NMR, DEPT, and HMBC (400 and 100 MHz, DMSO-d_6) data of compound(**9**).

Carbon no.	^{1}H-NMR (m, in Hz)	^{13}C-NMR	DEPT	HMBC
1	—	131.30	C	—
2	7.17 (1H, br.s)	104.40	CH	3, 4, 6
3	—	148.40	C	—
4	—	148.20	C	—
5	6.91 (1H, *d*, *J* = 7.6 Hz)	109.0	CH	1, 3, 4
6	6.98 (1H, *d*, *J* = 7.6 Hz)	123.0	CH	1, 2, 4
7	3.52 (1H, m)	70.10	CH	-—
8	3.41 (1H, m)	73.20	CH	—
9	9_a 4.20 (1H, m) 9_b 4.46 (1H, m)	62.50	CH_2	—
1′	—	148.40	C	—
2′,6′	6.94 (2H, *d*, *J* = 8.1 Hz)	126.10	CH	1′, 3′, 4′, 5′
4′	—	131.30	C	—
3′,5′	6.87 (2H, *d*, *J* = 8.1 Hz)	138.20	CH	1′, 4′, 7′, 2′, 6′
7′	7.21 (1H, *d*, *J* = 14.8 Hz)	142.40	CH	3′, 5′, 9′
8′	6.67 (1H, *d*, *J* = 14.8 Hz)	121.20	CH	3′, 5′, 9′
9′	—	164.90	C	—
9′-OH	13.13 (1H, br.s.)	—	—	—
O-CH_2-O	6.02 (s)	101.70	CH_2	3, 4

Table 2: UV spectral data of compound **(10)** in methanol and with different ionizing and complexing reagents.

Band	λ_{max} (nm)										
	MeOH	+NaOMe		+$AlCl_3$		+$AlCl_3$/HCl		+NaOAc		+NaOAc/H_3BO_3	
	λ_{max}	λ_{max}	Δ λ_{max}	λ_{max}	Δ λ_{max}	λ_{max}	Δ λ_{max}	λ_{max}	Δ λ_{max}	λ_{max}	Δ λ_{max}
II	283	290	+7	295	+12	293	+8	285	+3	290	+7
I	335	350	+15	386	+56	370	+45	345	+15	344	+9

the genus *Cassia* and family Fabaceae; thus, it might serve as a taxonomic marker of *C. occidentalis* as flavonolignans have not been reported in the family.

Compound 9 was obtained as a yellow granular powder (25 mg, MeOH). EI-MS, *m*/*z* (rel.int%), of 358 $[M]^+$ (27%), 357 $[M\text{-}H]^+$ (89%), 341 $[M\text{-}OH]^+$ (2%), 327 $[M\text{-}CH_2OH]^+$ (3%), 313 $[M\text{-}COOH]^+$ (2%), 300 (36%), 287 [M-propenoic acid]$^+$ (3%), 195 (5%), 163 (13%), and 71 [propenoic acid] (45%) was obtained (Figure S33). The molecular formula was established to be $C_{19}H_{18}O_7$, which implied 11 degrees of unsaturation. The IR (KBr) spectrum showed the following absorption bands at ν cm^{-1} 3400 (OH), 2830, 1705 (C=O), 1601, 1580, 1509, 1280, 750 (C=C aromatic ring), 1038, and 930 (methylenedioxy). The ^{1}H-NMR spectrum (Table 1 and Figure S34) first revealed the protons in the aromatic region at δ_H 6.98–7.17 ppm that displayed seven signals, which were attributed to two metadisubstituted and paradisubstituted phenyl rings, then followed by two transolefinic protons at δ_H 6.67 and 7.21 (d, *J* = 14.8 Hz) [29] and one methylenedioxy group at δ_H 6.02 ppm [29]. In addition, four signals at δ_H 3.52–3.41 ppm could be attributed to the protons attached to carbons with adjacent oxygen, leading to the assignment of an oxyneolignan structure with propenyl moiety. Based on signal characteristics (HSQC, COSY, and HMBC), protons at δ_H 7.17 ppm (1H, s), 6.91 ppm (1H, d, *J* = 7.6 Hz), and 6.98 ppm (1H, d, *J* = 7.6 Hz) could be assigned to H-2, H-5, and H-6 (ring A), while protons at δ_H 6.94 ppm (2H, d, *J* = 8.1 Hz) and 6.87 ppm (2H, d, *J* = 8.1 Hz) were assigned to the *para*disubstituted phenyl ring (ring B). Besides, the ^{13}C-NMR indicated the presence of 19 carbons, while DEPT ^{13}C-NMR data (Table 1; Figures S35 and S36), confirmed the presence of two phenylpropanoid units [30]. The DEPT experiment revealed the presence of an alcoholic methylene signal at δ_C 62.50 ppm and a signal at δ_C 101.70 ppm, confirming the methylenedioxy group [29]. In addition, six quaternary carbons at δ_C 131.30, 148.40, and 148.20 ppm were assigned to C-1, C-3, and C-4 of ring A, while the signals at δ_C 148.40 and 131.30 ppm were assigned to C-1′ and C-4′ of ring B, in addition to carboxylic carbonyl at δ_C 164.90 ppm, which confirmed the *trans*substituted neolignan. The NMR results and the detection of significant mass peaks at *m*/*z* 71 and 287 confirmed the presence of propenoic (acrylic) acid moiety [30]. Complete assignment of the ^{1}H- and ^{13}C-NMR signals was confirmed from their ^{1}H-^{1}H COSY, HSQC, and HMBC (Figures S37–S39) spectra analysis. The spectral data and their comparison with similar compounds in the literature [29–31] could assign compound **9** as (E)-3-[4-(1-(benzol) [d] [1, 3] dioxol-6-yl)-1, 3-dihydroxy propan-2) phenyl] propanoic acid, which can loosely be called occidentalignan I (**9**). This represents a new compound and the first report of its isolation from *C. occidentalis* [32].

Table 3: ^{1}H-NMR, ^{13}C-NMR, and DEPT (400 and 100 MHz, DMSO-d_6) data of compound **(10)**.

Carbon no.	^{1}H-NMR (m, in Hz)	^{13}C-NMR	DEPT
2	—	164.92	C
3	6.93 (1H, s)	105.61	CH
4	—	182.31	C
5	—	161.91	C
6	6.20 (1H, *d*, $J = 1.6$ Hz)	99.50	CH
7	—	163.65	C
8	6.51 (1H, *d*, $J = 1.6$ Hz)	94.60	CH
9	—	157.92	C
10	—	104.41	C
1′	—	131.15	C
2′, 6′	8.04 (2H, *d*, $J = 8.0$ Hz)	126.84	CH
3′, 5′	7.59 (3H, *dd*, $J = 8.0$, 1.8 Hz)	129.60	CH
4′		132.48	CH
1″	4.81 (1H, s)	101.91	CH
2″	3.08–3.63 (remaining sugar protons, m)	72.16	CH
3″		72.85	CH
4″		73.97	CH
5″		68.16	CH
6″	1.10 (3H, *d*, $J = 6.40$ Hz)	18.44	CH
5-OH	12.80 (1H, s)	—	—

Compound 10 was obtained as a yellow amorphous powder (18 mg, MeOH). EI-MS (Figure S40) showed a molecular ion peak at m/z 254 [M-sugar]$^+$ and a calculated molecular formula of $C_{15}H_{10}O_4$ (chrysin). The UV spectral data in MeOH (Table 2) showed two absorption bands at λ_{max} 283 nm (band II) and 335 nm (band I), which were the characteristic of the flavone skeleton [15], with 5-OH (bathochromic replaced in band I with $AlCl_3$) and the absence of dihydroxyl substitution at C-4` or C-7 position (no shift with NaOMe (band I) or with NaOAc (band II)). The ^{1}H-, ^{13}C-, and DEPT ^{13}C-NMR spectra of compound **10** (Table 3; Figures S41–S43) exhibited a characteristic pattern of flavone skeleton [15], similar to those of chrysin [33]. In addition, it displayed anomeric sugar proton at δ_H 4.81 ppm (1H, s) with a δ_C at 101.91 as well as an upfield three-proton signal at δ_H 1.10 ppm (3H, d, $J = 6.40$ Hz) attributed to the methyl group of rhamnose moiety, which was confirmed by CH_3 signal at δ_C 18.44 ppm. The appearance of carbon signal at δ_C 68.16 ppm was attributed to C-5, indicating α-L-rhamnose [27]. Glycosylation of chrysin was determined to occur at the C-7 (no NaOAC bathochromic shift in band II), which was confirmed by the upfield shift of C-7 by 1.33 ppm in the ^{13}C-NMR spectrum in comparison to chrysin. The ^{13}C-NMR data displayed 15 carbon signals of a flavone skeleton [27] at δ_C 72.16–68.16, which could be assigned to the sugar moiety. The DEPT experiment revealed seven quaternary carbon and 13 CH signals. Acid hydrolysis of compound **(10)** as described in the methods resulted in the identification of a hydrolysate. The aglycone was confirmed to be chrysin by direct comparison with an authentic sample, and the sugar fraction was identified as L-rhamnose using paper chromatography. From the spectral data and their comparison with similar compounds in the literature [34], compound (10) was identified as chrysin-7-*O*-α-L-rhamnopyranosyl (**10**), which to our knowledge represents a new compound and the first report of its isolation from a plant-based natural source.

Compound (11) was isolated from ethyl acetate fraction as a colorless needle-shaped substance (25 mg, MeOH) with an m.p of 152-153°C. EI-MS, m/z (rel.int%), of 192 [M]$^+$ (5%), 147 [M-COOH]$^+$ (5%), 102 [M-2COOH]$^+$ (25%), 57 [M-3COOH]$^+$ (32%), and 45 (100%) was observed (Figure S44). The ^{1}H-NMR spectrum of compound (11) (Table S7 and Figure S45) exhibited two sets of enantiotropic pairs of hydrogen with an identical chemical shift at δ_H 2.60 and 2.72 ppm, each for two protons with a coupling constant of 15.6 Hz. The ^{13}C-NMR spectrum (Table S7 and Figure S46) displayed four carbon signals attributed to six carbons, with two downfield signals being assigned to three carboxylic groups at δ_C 175.03 and 171.77 ppm (for two chemically equivalent groups), in addition to an oxygenated carbon at δ_C 72.91 and two chemically equivalent ethylenic carbons at δ_C 43.20. Mass spectrum (Figure S44) showed a molecular ion peak at m/z 192 with a calculated molecular formula of $C_6H_8O_7$ and characteristic mass peaks at m/z 147, 102, and 57 for successive loss of three carboxylic groups, which was confirmed from the ^{13}C-NMR data. The obtained spectral data were inconsistent with those previously reported for citric acid [35]. Based on the comparison between previously identified and published spectral data [35], compound (11) was identified as citric acid (**11**), representing its isolation from the genus *Cassia*.

3.2. Pharmacological Studies

3.2.1. In Vitro Cytotoxicity assay. The total methanolic extract of the whole aerial flowering parts of *C. occidentalis* was screened for its cytotoxic activity (Table 4). As reported, the cytotoxicity of *C. occidentalis* is due to anthraquinones (major active constituents in the plant) which are found in high concentrations. Since this class of compounds (anthraquinones) is present in higher concentrations in the total extract than other fractions, it was chosen in our study to confirm the plant's activity [36] against seven cancer cell lines, including lung cancer (A-549), colorectal carcinoma (Colo-205), hepatocellular carcinoma (Huh-7), colon cancer (HCT-116), pancreatic cancer (PANC-1), ovarian cancer (SKOV-3), and mouse normal liver cells BNL, using SRB screening assay at sample concentrations of 10 and 100 g/mL. The cytotoxic activity revealed that the extract exhibited selective potent cytotoxicity on lung cancer cells without affecting BNL normal cells, while the extract showed moderate activity on Colo-205 and Huh-7 and nearly no activity on HCT-116, PANC-1, and SKOV-3 cell lines.

The optical microscope-stained images (Figure 2) recorded the SRB cytotoxicity assay results for the total methanolic extract of the whole aerial flowering parts of *C. occidentalis* at the two tested concentration points against

Table 4: Rapid SRB cytotoxicity screening results of total methanolic extract from the whole aerial flowering parts of *C. occidentalis* on several cell lines.

Conc. of the tested sample (μg/mL)	Cell viability (%)						
	Cancer cell lines						Normal cell line
	A-549	Colo-205	Huh-7	HCT-116	PANC-1	SKOV-3	BNL
10	98.5338	100.512	96.0551	98.9648	100.038	102.567	99.0442
100	65.9283	83.916	93.2841	98.6645	93.1391	100.09	96.3608

±SD ($n = 3$).

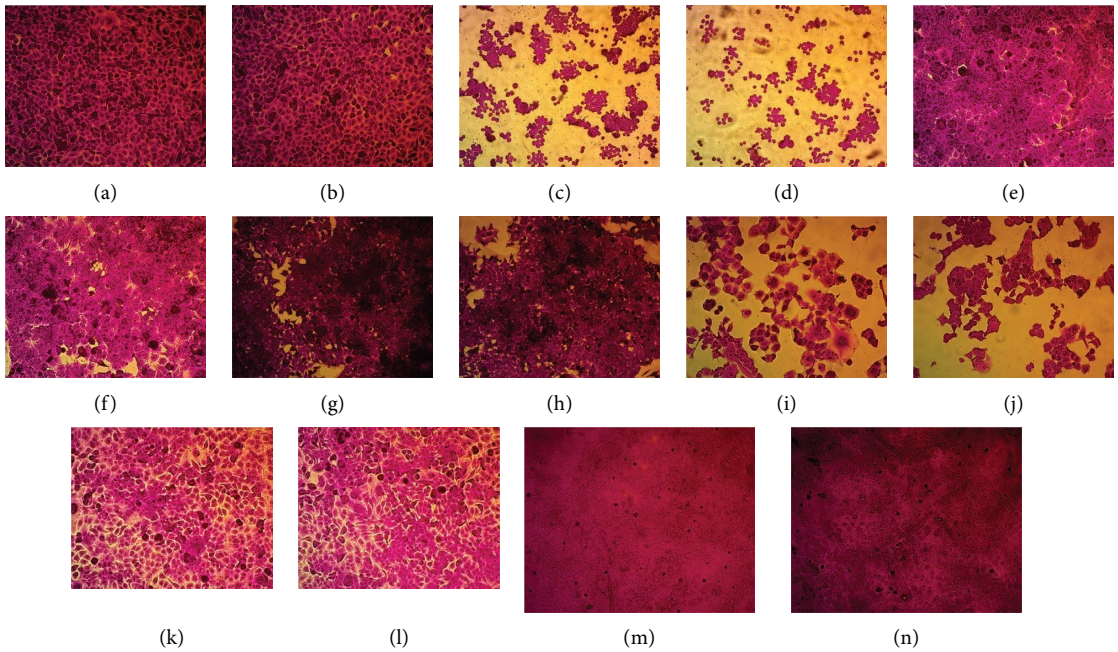

Figure 2: The optical microscope stained images of cytotoxicity assays of the seven cell lines: (a) A-549 (10 μg/mL), (b) A-549 (100 μg/mL), (c) colo-205 (10 μg/mL), (d) colo-205 (100 μg/mL), (e) huh-7 (10 μg/mL), (f) huh-7 (100 μg/mL), (g) HCT-116 (10 μg/mL), (h) HCT-116 (100 μg/mL), (i) PANC-1 (10 μg/mL), (j) PANC-1 (100 μg/mL), (k) SKOV-3 (10 μg/mL), (l) SKOV-3 (100 μg/mL), (m) BNL (10 μg/mL), and (n) BNL (100 μg/mL), magnification power: 200×.

the seven cell lines. As a result, clear significant morphological changes in lung cancer cells were detected at both extract concentrations, while moderate changes were observed at higher (100 g/mL) concentrations on Colo-205 and Huh-7. In contrast, no changes were detected on HCT-116, PANC-1, and SKOV-3. These results confirmed the cytotoxic activity of the total methanolic extract of *C. occidentalis* on the A-549 cell line.

To investigate the safety of *C. occidentalis* methanolic extract on normal cells and its selective cytotoxicity on cancer cells, the cytotoxic activity was tested against mouse normal liver cells BNL. The results showed the percentage cell viability of 96.3608 for the methanolic extract at a concentration of 100 μg/mL against the BNL normal cell line. In contrast, it demonstrated a potent cytotoxic effect with a percentage cell viability of 65.9283 on the A-549 cell line with the same concentration, which suggested its safety on normal cell lines and selective cytotoxicity on lung cancer cell lines.

4. Conclusion

In summary, this study used ethyl acetate fraction column chromatography to isolate eleven compounds of different classes from the whole aerial flowering parts of *C. occidentalis*. Two new metabolites, including a neolignan, designated as occidentalignan I (**9**) and a flavonoidal glycoside, named chrysin-7-*O*-α-L-rhamnopyranosyl (**10**), were identified based on spectroscopic evidence. In addition, a flavonolignan compound, silybin A (**8**), was isolated for the first time from the family Fabaceae, while four compounds, including β-sitosterol-3-*O*-β-D-glucopyranoside (**1**), stigmasterol-3-*O*-β-D-glucopyranoside (**2**), betulinic acid (**3**), and vanillic acid (**4**), as well as four known compounds, cinnamic acid (**5**), *p*-hydroxybenzoic acid (**6**), β-resorcylic acid (**7**), and citric acid (**11**), were isolated for the first time in *C. occidentalis* L. *In vitro* cytotoxicity assessment of the methanolic extract on seven different cancer cell lines, A-549, Colo-205, Huh-7, HCT-116, PANC-1, SKOV-3, and

BNL, showed a selective potent cytotoxicity of *C. occidentalis* extract on lung cancer cells without affecting BNL normal cells. The extract also showed moderate activity on Colo-205 and Huh-7, and nearly no activity on HCT-116, PANC-1, and SKOV-3. These results indicate that the *Cassia occidentalis* methanolic extract is potentially a good candidate for the treatment of lung cancer cell lines; however, further studies are required to clarify its underlying mechanism of action.

Abbreviations

A-549: Lung cancer
br.s: Broad singlet
BNL: Normal hepatocyte cell line
COEt: *Cassia occidentalis* ethyl acetate extract
Colo-205: Colorectal cancer
DEPT: Distortions enhancement by polarization transfer
EI-MS: Electron ionization-mass spectrometry
HMBC: Heteronuclear multiple bond correlation
HSQC: Heteronuclear single-quantum correlation
Huh-7: Liver cancer
HCT-116: Colon cancer
PANC-1: Pancreatic cancer
SKOV-3: Ovarian cancer
TLC: Thin-layer chromatography.

Data Availability

All data generated or analyzed during this study are included in this published article and its supplementary materials.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

All authors conceptualized the study, curated the data, performed the formal analysis, acquired the funding, investigated the data, developed the methodology, administered the project, collected the resources, developed the software, visualized, validated, and supervised the study, wrote of the original draft, and reviewed and edited the data.

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Supplementary Materials

Supplementary Figure (S1): EI-MS spectrum of compound (1). Supplementary Figure (S2): 1H-NMR spectrum of compound (1) (DMSO-d6-400 MHz). Supplementary Figure (S3): 13C-NMR spectrum of compound (1) (DMSO-d6-100 MHz). Supplementary Figure (S4): EI-MS spectrum of compound (2). Supplementary Figure (S5): 1H-NMR spectrum of compound (2) (DMSO-d6-400 MHz). Supplementary Figure (S6): 13C-NMR spectrum of compound (2) (DMSO-d6-100 MHz). Supplementary Figure (S7): EI-MS spectrum of compound (3). Supplementary Figure (S8): 1H-NMR spectrum of compound (3) (DMSO-d6-400 MHz). Supplementary Figure (S9): 13C-NMR spectrum of compound (3) (DMSO-d6- 100 MHz). Supplementary Figure (S10): Expansion of 13C-NMR spectrum of compound (3) (DMSO-d6- 100 MHz). Supplementary Figure (S11): EI-MS spectrum of compound (4). Supplementary Figure (S12): 1H-NMR spectrum of compound (4) (DMSO-d6-400 MHz). Supplementary Figure (S13): 13C-NMR spectrum of compound (4) (DMSO-d6-100 MHz). Supplementary Figure (S14): EI-MS spectrum of compound (5). Supplementary Figure (S15): H-NMR spectrum of compound (5) (DMSO-d6-400 MHz). Supplementary Figure (S16): 13C-NMR spectrum of compound (5) (DMSO-d6-100 MHz). Supplementary Figure (S17): DEPT 13C-NMR spectrum of compound (5) (DMSO-d6-100 MHz). Supplementary Figure (S18): EI-MS spectrum of compound (6). Supplementary Figure (S19): 1H-NMR spectrum of compound (6) (DMSO-d6-400 MHz). Supplementary Figure (S20): 13C-NMR spectrum of compound (6) (DMSO-d6-100 MHz). Supplementary Figure (S21): DEPT 13C-NMR spectrum of compound (6) (DMSO-d6- 100 MHz). Supplementary Figure (S22): EI-MS spectrum of compound (7). Supplementary Figure (S23): 1H-NMR spectrum of compound (7) (DMSO-d6-400 MHz). Supplementary Figure (S24): 13C-NMR spectrum of compound (7) (DMSO-d6-100 MHz). Supplementary Figure (S25): DEPT 13C-NMR spectrum of compound (7) (DMSO-d6-100 MHz). Supplementary Figure (S26): EI-MS spectrum of compound (8). Supplementary Figure (S27): 1H-NMR spectrum of compound (8) (DMSO-d6-400 MHz). Supplementary Figure (S28): 13C-NMR spectrum of compound (8) (DMSO-d6-100 MHz). Supplementary Figure (S29): DEPT 13C-NMR spectrum of compound (8) (DMSO-d6-100 MHz). Supplementary Figure (S30): 1H-1H COSY spectrum of compound (8) (DMSO-d6). Supplementary Figure (S31): HSQC spectrum of compound (8) (DMSO-d6). Supplementary Figure (S32): HMBC spectrum of compound (8) (DMSO-d6). Supplementary Figure (S33): EI-MS spectrum of compound (9). Supplementary Figure (S34): 1H-NMR spectrum of compound (9) (DMSO-d6-400 MHz). Supplementary Figure (S35): 13C-NMR spectrum of compound (9) (DMSO-d6-100 MHz). Supplementary Figure (S36): DEPT 13C-NMR spectrum of compound (9) (DMSO-d6-100 MHz). Supplementary Figure (S37): 1H-1H COSY spectrum of compound (9) (DMSO-d6). Supplementary Figure (S38): HSQC spectrum of compound (9) (DMSO-d6). Supplementary Figure (S39): Extended HSQC spectrum of compound (9) (DMSO-d6). Supplementary Figure (S40): EI-MS spectrum of compound (10). Supplementary Figure (S41): 1H-NMR spectrum of compound (10) (DMSO-d6-400 MHz). Supplementary Figure (S42): DEPT 13C-NMR spectrum of compound (10) (DMSO-d6- 100 MHz). Supplementary Figure (S43): DEPT 13C-NMR spectrum of

compound (10) (DMSO-d6-100 MHz). Supplementary Figure (S44): EI-MS spectrum of compound (11). Supplementary Figure (S45): 1H-NMR spectrum of compound (11) (DMSO-d6-400 MHz). Supplementary Figure (S46): 13C-NMR spectrum of compound (11) (DMSO-d6-100 MHz). Supplementary Table (S1): 1H-, 13C-, and 13C-NMR (400 and 100 MHz, DMSO-d6) data of compound (3). Supplementary Table (S2): 1H- and 13C-NMR (400 and 100 MHz, DMSO-d6) data of compound (4). Supplementary Table (S3): 1H-, 13C-, and DEPT 13C-NMR (400 and 100 MHz, DMSO-d6) data of compound (5). Supplementary Table (S4): 1H-, 13C-, and DEPT 13C-NMR (400 and 100 MHz, DMSO-d6) data of compound (6). Supplementary Table (S5): 1H-, 13C-, and DEPT 13C-NMR (400 and 100 MHz, DMSO-d6) data of compound (7). Supplementary Table (S6): 1H-, 13C-, and DEPT 13C-NMR (400 and 100 MHz, DMSO-d6) data of compound (8). Supplementary Table (S7): 1H- and 13C-NMR (400 and 100 MHz, DMSO-d6) data of compound (11). (*Supplementary Materials*)

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