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Posted Date: 18 May 2023

doi: 10.20944/preprints202305.1276.v1

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Article

New Phenolic Dimers from Plant *Paeonia suffruticosa* and Their Bioactivity

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Abstract: The *Paeonia suffruticosa*, called as 'Feng Dan', has been used for thousands of years in traditional Chinese medicine. In our chemical investigation on the root bark of the plant, five new phenolic dimers, namely paeobenzofuranone A–E (**1**–**5**), have been characterized. Their structures were determined by spectroscopic analysis including 1D and 2D NMR, HRESIMS, UV, and IR, as well as ECD calculations. Compounds **2**, **4**, and **5** showed cytotoxicity against three human cancer cell lines with IC_{50} values ranging from 6.7 to 25.1 μM. Compounds **1** and **2** showed certain inhibitory activity on NO production. To the best of our knowledge, the benzofuranone dimers and their cytotoxicity of *P. suffruticosa* are reported for the first time.

Keywords: *Paeonia suffruticosa*; benzofuranones; cytotoxicity; NO production inhibition

1. Introduction

Paeonia suffruticosa is a perennial deciduous shrub belonging to the family Paeoniaceae. The dried root bark of *P. suffruticosa* is called 'Feng Dan' or 'Mudanpi' in China, which is used as a traditional Chinese medicine to clear pathogenic heat from the blood, promot blood circulation to remove blood stasis, as recorded in Chinese Pharmacopoeia (2020 edition). Nowadays, pharmacological studies on *P. suffruticosa* have demonstrated anti-inflammatory, antioxidant, and anti-tumor activities, as well as central nervous system and cardiovascular system protective activities [1–3]. *P. suffruticosa* has been chemically investigated and is rich in phenolics, monoterpenes and glycosides, flavonoids, triterpenes, sesquiterpenes and lignans. Approximately, 190 constituents have been reported in recent ten years with anti-inflammatory and antitumor properties, representatives are paeonisides A and B, mudanpiosides C and F, and suffruticosol A [4–8].

As part of our ongoing work on bioactive natural products from plants [9–14], the study on the chemical constituents of the root bark of *P. suffruticosa* was carried out. As a result, five new phenolics were isolated, namely paeobenzofuranone A–E (**1**–**5**). Their structures (Figure 1) were determined by extensive spectroscopic methods. All compounds were evaluated for their cytotoxicity against three human cancer cell lines including breast cancer MDA-MB-231, human myeloid leukemia HL-60, and colon cancer SW480. In addition, their anti-inflammatory activity by inhibiting NO production was also evaluated. Herein, the isolation, structural elucidation, and bio-activities of the compounds from *P. suffruticosa* are reported.

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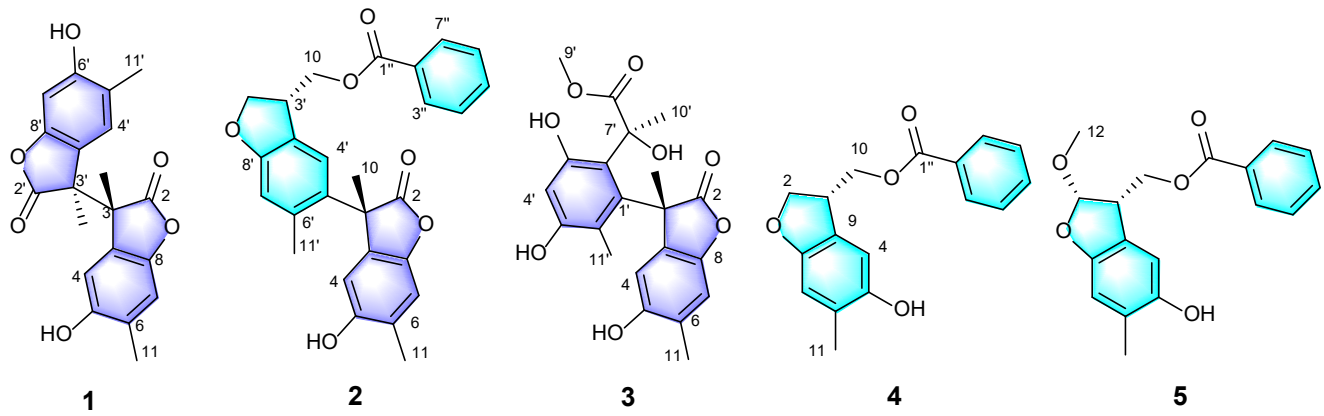

Figure 1. Structures of compounds **1**–**5**.

2. Results and Discussion

2.1. Structural Elucidation of Compounds 1–5

Compound **1** was obtained as colorless oily liquid. Its molecular formula was determined as $C_{20}H_{18}O_6$ by the HR-ESI-MS with a molecular ion peak at *m*/*z* 377.09952 ([M + Na]$^+$, calcd. 377.10011). The UV absorption peaks at λ_{max} 290 and 230 nm indicated the presence of a benzene ring and an ester carbonyl. The IR spectrum indicated that **1** possessed hydroxyl (3406 cm^{-1}) and lactone (1716 cm^{-1}) groups. The ^{1}H NMR spectroscopic data (Table 1) of **1** showed two methyl signals at δ_H 1.67 (s, 3H) and 2.15 (s, 3H), and two singlets for aromatic protons at δ_H 6.61 (1H, s, H-7) and 7.18 (1H, s, H-4). Interpretation of the ^{13}C NMR (Table 2) and DEPT spectra data displayed 10 carbon signals, which indicated six non-protonated carbons (C-2: δ_C 179.4, C-3: δ_C 52.6, C-5: δ_C 113.3, C-6: δ_C 153.7, C-8: δ_C 146.2, C-9: δ_C 127.4), two CH (C-4: δ_C 128.1, C-7: δ_C 110.3) and two CH_3 (C-10: δ_C 18.7, C-11: δ_C 16.6). Preliminary analysis of these data suggested that **1** should be a benzofuranone derivative with a structure similar to that of 4,6-dihydroxy-3,5-dimethylcoumaran-2-one [15]. The presence of a methyl at δ_C 18.7 and a carbonyl carbon at δ_C 179.4 indicated the differences. In the HMBC spectrum (Figure 2), correlations from H_3-11 (δ_H 2.11) to C-4 (δ_C 128.1), C-5 (δ_C 113.3), and C-6 (δ_C 153.7), and from H_3-10 (δ_H 1.67) to C-3 (δ_C 52.6), C-2 (δ_C 179.4), and C-9 (δ_C 127.4) enabled the assignment of the methyl and the carbonyl carbon. Further analysis of ^{13}C NMR data for a quaternary carbon at C-3 (δ_C 52.6) revealed that **1** should be a symmetric dimer with a linkage by bond of C-3/C-3'. It was also supported by the MS data analysis. The stereochemistry of C-3 and C-3' was identified as 3*R* and 3'*S* by ECD calculations (Figure 3). Finally, the structure of **1** was identified and trivially named as paeobenzofuranone A.

Table 1. ^{1}H NMR Spectroscopic Data of Compounds **1**–**5** in Methanol-d_4 (600 MHz, *J* in Hz).

Position	1	2	3	4	5
2				4.46, m	5.48, t (1.8)
3				3.81, t (7.4)	3.80, t (7.5)
4	7.18, s	6.61, s	6.96, s	6.74, s	6.72, s
7	6.61, s	6.52, s	6.33, s	4.37, dd (18.0, 9.1) 4.44, dd (10.7, 5.9)	4.46, dd (11.1, 5.5) 4.33, dd (11.1, 7.8)
10	1.67, s, 3H	1.75, s, 3H	1.72, s, 3H	4.43, m	4.48, m
11	2.15, s, 3H	2.13, s, 3H	2.02, s, 3H	2.14, s, 3H	2.15, s, 3H
12					3.48, s
2′		4.43, m; 4.06, m		8.00, d (1.5)	7.97, d (1.5)
3′		3.81, t (7.5)		7.44, m	7.45, m

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4′	7.18, s	6.63, s	6.88, s	7.58, m	7.61, m
5′	6.61, s	6.62, s		7.49, m	7.48, m
6′	1.67, s, 3H			8.01, d (1.3)	7.99, d (1.3)
7′	2.15, s, 3H	6.85, s			
9′			3.73, s		
10′		4.60, dd (18.0, 9.1); 4.44, dd (10.8, 5.9)	1.63, s, 3H		
11′		2.05, s, 3H	1.77, s, 3H		
3″		8.01, d (1.2)			
4″		7.48, t (7.8)			
5″		7.56, m			
6″		7.48, t (7.8)			
7″		8.00, d (1.4)			

Table 2. ^{13}C NMR Spectroscopic Data of Compounds **1–5** in Methanol-d_4 (150 MHz).

Position	1	2	3	4	5
2	179.4, C	179.3, C	182.7, C	73.5, CH_2	111.2, CH
3	52.6, C	52.6, C	50.2, C	42.0, CH	50.5, CH
4	128.1, CH	112.2, CH	115.6, CH	110.8, CH	112.6, CH
5	113.3, C	149.3, C	149.2, C	148.9, C	150.9, C
6	153.7, C	126.3, C	125.0, C	128.5, CH	124.4, CH
7	110.3, CH	112.4, CH	118.9, CH	110.6, CH	112.3, CH
8	146.2, C	125.9, C	144.3, C	153.4, C	153.2, C
9	127.4, C	127.4, C	126.4, C	125.6, C	129.8, C
10	18.7, CH_3	18.6, CH_3	22.3, CH_3	66.4, CH_2	66.3, CH_2
11	16.6, CH_3	16.6, CH_3	15.9, CH_3	15.6, CH_3	17.0, CH_3
12					56.2, OCH_3
1′			133.7, C		
2′	179.4, C	73.1, CH_2	153.2, C		
3′	52.6, C	43.5, CH	122.0, C		
4′	128.1, CH	111.7, CH	115.9, CH		
5′	113.3, C	126.4, C	144.6, C		
6′	153.7, C	131.4, CH	125.7, C		
7′	110.3, CH	110.3, CH	75.1, C		
8′	146.2, C	154.9, C	176.8, C		
9′	127.4, C	124.4, C	53.2, OCH_3		
10′	18.7, CH_3	66.9, CH_2	26.0, CH_3		
11′	16.6, CH_3	16.8, CH_3	10.4, CH_3		
1″		168.1, C		166.5, C	167.9, C
2″		130.7, C		129.7, C	131.2, C
3″		129.7, CH		129.2, CH	130.4, CH
4″		128.1, CH		128.2, CH	129.5, CH
5″		134.5, CH		132.9, CH	130.7, CH
6″		129.7, CH		128.2, CH	129.5, CH
7″		128.1, CH		129.1, CH	130.4, CH

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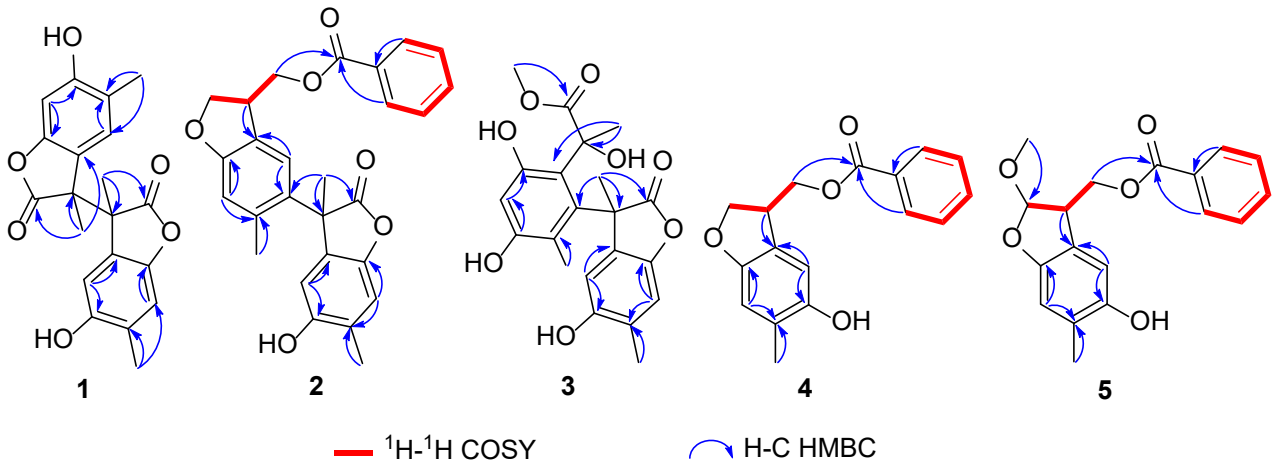

Figure 2. Selected HMBC and ^{1}H–^{1}H COSY correlations of compounds **1–5**.

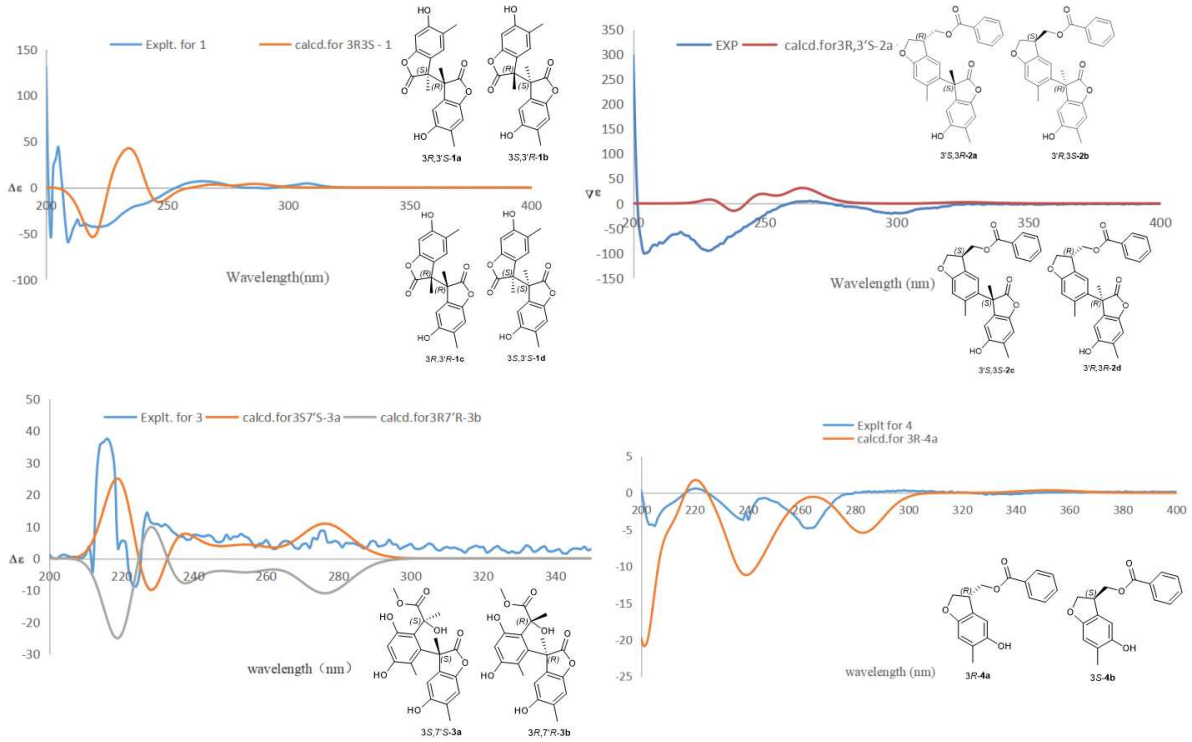

Figure 3. ECD calculations for compounds **1–4**.

Compound **2** was obtained as white powder. Its molecular formula was determined as $C_{27}H_{24}O_6$ by HR-ESI-MS (measured at *m/z* 445.16489 $[M + Na]^+$; calcd. 445.16511). The UV spectrum revealed the benzene ring and ester carbonyl by peaks at λ_{max} 295 and 230 nm. The 1D spectra data of **2** (Tables 1 and 2) are partially identical to those of **1**. Interpretation of the ^{1}H and ^{13}C spectroscopic data of **2** showed two benzofuran parts and an additional benzoyl moiety. The locations of the benzofuran parts were assigned by HMBC correlations from H-10 (δ_H 1.75) to C-3 (δ_C 52.6), C-6 (δ_C 126.3), C-2 (δ_C 179.3), as well as from H-4 (δ_H 6.61) to C-5 (δ_C 126.3) and C-3 (δ_C 52.6) (Figure 2). Furthermore, the location of the benzoyl was assigned by key HMBC peaks from H-3' (δ_H 3.81) to C-10' (δ_C 66.9), from H-2' (δ_H 4.43) to C-3' (δ_C 43.5) and C-2' (δ_C 73.1); from H-10' (δ_H 4.44) to C-3' (δ_C 43.5) and C-10' (δ_C 66.9). The stereochemistry of C-3 and C-3' was established as 3*S* and 3'*R* by ECD calculations (Figure 3). Then, the structure of **2** was established and named as paeobenzofuranone B.

Compound **3** was obtained as white powder. The IR spectrum indicated that **3** possessed hydroxyl (3394 cm^{-1}) and lactone (1712 cm^{-1}) groups. Its molecular formula was determined as

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$C_{21}H_{22}O_8$ by HR-ESI-MS data analysis (*m/z* 425.12030 ([M+Na]$^+$, calcd. 425.12124). The ^{1}H and ^{13}C NMR spectra data of **3** (Tables 1 and 2) are partially the same as those of **1**. Interpretation of the ^{1}H and ^{13}C spectroscopic data of **3** revealed one benzofuran part and one benzene ring. The benzofuran part was assigned by HMBC correlations from H-10 (δ_H 1.75) to C-3 (δ_C 50.2), C-1' (δ_C 133.7) and C-9 (δ_C 126.4), from H-11 (δ_H 2.02) to C-7 (δ_C 118.9), C-9 (δ_C 126.4), C-5 (δ_C 149.2); and from H-4' (δ_H 6.88) to C-2' (δ_C 153.2), C-5' (δ_C 144.6) (Figure 2). The ^{1}H-^{1}H COSY cross peaks from δ_H 2.02 (3H, s, H-11) to δ_H 6.33 (1H, s, H-7), and from δ_H 1.77 (3H, s, H-11') to δ_H 6.88 (1H, s, H-4') verified the location of 10-CH_3 and 11-CH_3. The HMBC correlations verified the benzofuran group attached to C-8 (δ_C 144.3). Furthermore, from HMBC correlations, the signal of another ester carbonyl group was connected to the benzene ring through the C-7' (δ_C 75.1), as evidenced from δ_H 1.63 (3H, s, H-10') to δ_C 75.1 (CH, C-7'), and from δ_H 3.73 (OCH_3, s, H-9') to δ_C 176.8 (C, C-8'). The stereochemistry of C-3 and C-7' was established as 3*S* and 7'*S* by ECD calculations (Figure 3). Eventually, the structure of **3** was elucidated as paeobenzofuranone C.

Compound **4** was obtained as white powder. The UV data absorption showed the ester carbonyl as determined by peaks with λ_{max} at 230 nm. The IR spectrum indicated that **4** possessed hydroxyl (3383 cm^{-1}) and lactone (1708 cm^{-1}) groups. Its molecular formula was determined as $C_{17}H_{16}O_4$ by HR-ESI-MS data analysis (*m/z* 285.11215 ([M + H]$^+$, calcd. 285.11268). The ^{1}H and ^{13}C spectra data of **4** (Tables 1 and 2, Supplementary data) are partially the same as those of **1**, expect for the benzoyl and hydroxymethyl groups in **4**. Interpretation of the ^{1}H and ^{13}C spectroscopic data of **4** implied one benzofuran part and one benzoyl. The locations of the benzofuran parts were assigned by correlations revealed in the HMBC experiment (Figure 2) between the 11-CH_3 (δ_H 2.14) and C-5 (δ_C 148.9), C-7 (δ_C 110.6), C-8 (δ_C 153.4), as well as from H-4 (δ_H 6.74) to C-5, C-8, C-9 (δ_C 125.6); and from H-3 (δ_H 3.81) to C-10 (δ_C 66.4), H-7 (δ_H 4.37) to C-1'' (δ_C 166.5). The ^{1}H-^{1}H COSY correlations from δ_H 3.81 (1H, s, H-3) to δ_H 4.43 (1H, s, H-10), and from δ_H 4.43 (1H, s, H-10) to δ_H 6.74 (1H, s, H-4) verified the location of benzofuran part and one benzoyl connecting by C-3 and C-10. The stereochemistry of C-3 was established as 3*R* by ECD calculations (Figure 4). Therefore, the structure of compound **4** was elucidated as paeobenzofuranone D.

Compound **5** was obtained as white powder. Its molecular formula was determined as $C_{18}H_{18}O_5$ by HR-ESI-MS data analysis (*m/z* 313.10959 [M-H]$^-$, calcd. 313.10743). The ^{1}H and ^{13}C NMR data resembled those of **4** (Tables 1 and 2), expect for the presence of an additional methoxy at C-2 in **5**, which was confirmed by the key HMBC correlation of H-12 (δ_H 3.48) with C-2 (δ_C 111.2). Comprehensive analysis of 2D NMR data indicated that other parts of **5** were the same to those of **4**. The stereochemistry of C-2 and C-3 was established as 2*S* and 3*S* by ECD calculations (Figure 4). Thus, the structure of compound **5** was established as paeobenzofuranone E.

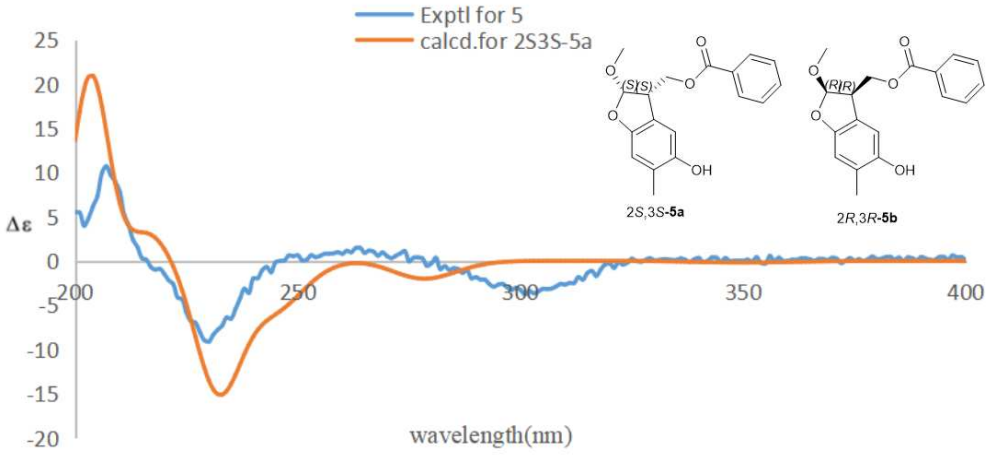

Figure 4. ECD calculations for compound **5**.

2.2. Bioactivity Analysis

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Five new compounds were tested for their inhibitory activities on nitric oxide production in the model of lipopolysaccharide-activated macrophages. As shown in Table 3, compounds **1** and **2** showed comparable inhibitory activity with the positive control at the concentration of 50 μM. In addition, all compounds were evaluated for their anti-tumor activity against HL-60, SW480, and MDA-MB-231 cell lines. As shown in Table 4, compounds **2, 4** and **5** demonstrated cytotoxicity against three human cancer cell lines. In particular, they exhibited potent cytotoxicity against HL-60 cells, with IC_{50} of 6.8, 19.1 and 11.1 μM, comparable to those of the positive control. In addition, compounds **4** and **5** showed no cytotoxicity to MDA-MB-231, indicating selectivity to the cancer cell lines.

Table 3. Inhibitory activities of compounds **1–5** on NO production.

Compound	Concentration (μM)	Inhibition Activity (100%)
L-NMMA[a]	50	52.0 ± 1.96
1	50	43.9 ± 2.07
2	50	44.6 ± 0.52
3	50	13.0 ± 1.59
4	50	33.7 ± 2.24
5	50	30.9 ± 1.56

[a]L-NMMA (NG-monomethyl-L-arginine, monoacetate salt) was use as the positive control.

Table 4. Cytotoxicity of compounds **2**, **4** and **5** (IC_{50} ± SD, μM).

Compound	HL-60	MDA-MB-231	SW480
2	6.8 ± 0.11	20.9 ± 0.46	12.6 ± 0.73
4	19.1 ± 0.32	–	8.9 ± 0.40
5	11.1 ± 1.61	–	10.7 ± 0.43
DDP[a]	23.5 ± 0.77	16.9 ± 1.19	25.1 ± 1.26

[a]DDP (Cisplatin) was used as the positive control.

3. Experimental

3.1. General Experimental Procedures

UV spectra were obtained on a UH5300 UV-VIS Double Beam Spectrophotometer. IR spectra were accessed by a Shimadzu Fourier transform infrared spectrometer with KBr pellets. HRESIMS were measured on a Thermo Scientific Q Exactive Orbitrap mass spectrometer system. The NMR spectra (^{1}H, ^{13}C, and 2D NMR) were run on a Bruker Avance III NMR instruments at 600 MHz for ^{1}H and 150 MHz for ^{13}C NMR, while tetramethylsilane (TMS) was used as an internal standard. Column chromatography (CC) was executed on silica gel (200−300 mesh, Qingdao Marine Chemical Ltd.), Sephadex LH-20 (Pharmacia Fine Chemical Co., Ltd.), and Reverse phase silica gel (20−45 μm, Fuji Silysia Chemical Ltd.). Medium pressure liquid chromatography (MPLC) was applied to Biotage SP2 equipment, and columns were packed with reverse phase silica gel (C_{18}). An Agilent 1260 series high-performance liquid chromatography (HPLC) system was used for sample analysis (ZORBAX-SB C_{18} column, 5 μm, 4.6 × 250 mm, flowing rate = 1 mL/min) and preparation (ZORBAX-SB C_{18} column, 5 μm, 9.4 × 150 mm, flowing rate = 6 mL/min). All fractions were monitored by thin-layer chromatography (TLC) over GF 254 and silica gel 60 plates. The spots were visualized by heating silica gel plates soaked with vanillin-sulfuric acid color component solvent.

3.2. Plant Material

The root barks of *P. suffruticosa* were collected in August 2021 from Tongling County, Anhui Province, People's Republic of China. It was identified by Zhenghui Li. (Associate Professor of South-Central Minzu University, Wuhan, Hubei 430074, PR China). A voucher specimen (2021123 FD) is deposited at School of Pharmaceutical Sciences, South-Central Minzu University.

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3.3. Extraction and Isolation

The root bark of *P. suffruticosa* (50 kg) were mechanically crushed and extracted with MeOH/H_2O (80:20) at 52°C for 4 times. The solvent was evaporated in vacuo to give a dark gum (9.3 kg). The latter was dissolved in a liter of water and then respectively extracted with petroleum ether (PE, 8L × 4) and dichloromethane (DCM, 8L × 4) to give PE (1.3 kg) and DCM parts (560 g). DCM part was separated by a silica gel column eluted with PE: acetone (50:1, 40:1, 30:1, 20:1, 10:1) to give eight fractions (TPG-1–8). Fraction TPG-3 (28.5 g) was subjected to ODS silica gel CC and eluted with MeOH/H_2O (20:90→90:10, v/v) to yield 10 fractions (Fr. 3-1→3-10). Fr. 3-2 (210 mg) was purified by Sephadex LH-20 (MeOH:DCM = 1:1) to afford three fractions (Fr. 3-2-1, 3-2-2, 3-2-1). Fr. 3-2-3 was purified by preparative HPLC with CH_3CN/H_2O (30:70→60:40, v/v, 30 min) to give **1** (15.6 mg, t_R = 12.5 min), **2** (9.7 mg, t_R = 14.8 min), **4** (3.3 mg, t_R = 16.9 min), **5** (4.9 mg, t_R = 17.8 min), respectively. Fr. 3-2-1 was prepared by HPLC with CH_3CN/H_2O (37:63→60:40, v/v, 30 min) to give **3** (3.2 mg) at 17.8 min.

3.3.1. Paeobenzofuranone A (1)

Colorless oil; $[\alpha]_D^{26}$ –98.6 (*c* = 0.09, MeOH); UV (MeOH) λ_{max} (log ε): 230 (3.6) nm; IR (KBr) ν_{max} 3406, 1716, 1450, 1346, 1315, 1276, 1026, 1049, and 713 cm^{-1}; HRESIMS: *m/z* 377.09952 $[M + Na]^+$, (calcd for $C_{20}H_{18}O_6Na^+$, 377.10011). The ^1H and ^{13}C NMR data were displayed on Tables 1 and 2.

3.3.2. Paeobenzofuranone B (2)

White powder; UV (MeOH) λ_{max} (log ε): 230 (3.2) nm; $[\alpha]_D^{26}$ –15.1 (*c* = 0.09, MeOH); HRESIMS: *m/z* 445.16489 $[M + H]^+$ (calcd for $C_{27}H_{24}O_6^+$ 445.16511). ^1H and ^{13}C NMR data were displayed on Tables 1 and 2.

3.3.3. Paeobenzofuranone C (3)

White powder; $[\alpha]_D^{22}$ –5.6 (*c* = 0.12, MeOH); UV (MeOH) λ_{max} (log ε): 230 (3.2) nm; IR (KBr) ν_{max}: 3394, 1712, 1450, 1346, 1315, 1276, 1176, and 1072 cm^{-1}; HRESIMS: *m/z* 425.12030 ($[M + Na]^+$ (calcd for $C_{21}H_{22}O_8Na^+$, 425.12124). ^1H and ^{13}C NMR data were displayed on Tables 1 and 2.

3.3.4. Paeobenzofuranone D (4)

White powder; $[\alpha]_D^{22}$ –3.8 (*c* = 0.11, MeOH); UV (MeOH) λ_{max} (log ε): 230(1.973) nm; IR (KBr) ν_{max}: 3383, 1708, 1450, 1342, 1315, 1276, 1176 and 1072 cm^{-1}; HRESIMS *m/z* 285.11215 ($[M + H]^+$ (calcd for $C_{17}H_{16}O_4^+$, 285.11268). ^1H and ^{13}C NMR data were displayed on Tables 1 and 2.

3.3.5. Paeobenzofuranone E (5)

White powder; $[\alpha]_D^{26}$ –13.8(c = 0.09, MeOH); UV (MeOH) λ_{max} (log ε): 230(3.136) nm; HRESIMS *m/z* 313.10959 ($[M – H]^-$ (calcd for $C_{18}H_{18}O_5^-$, 313.10743). ^1H and ^{13}C NMR data were displayed on Tables 1 and 2.

3.4. Cytotoxicity Assay

The cytotoxicity for the isolates was evaluated using the MTS assay. Briefly, 1×10^5 cells/mL from three human cancer cell lines, breast cancer MDA-MB-231, human myeloid leukemia HL-60, and colon cancer SW480 were seeded in 96-well plates. After 24 h incubation, cells were treated with test compounds or cisplatin (DDP, positive control) at a given concentrations (40、8、1.6、0.32、0.064 μM) for 48 h. MTS was then added to each well, and the plates were stored for 4 h. Absorbance was read at 490 nm. IC_{50} (50% concentration of inhibition) was calculated by Reed & Muench method [16,18].

3.5. Anti-Inflammatory Activity Assays

Mouse mononuclear macrophages RAW264.7 were seeded into 96-well plates, induced, and stimulated with 1 μg/mL LPS; at the same time, five new compounds with different concentrations to be tested were added. The drug-free group and the L-NMMA positive drug group were set approximately equal as a comparison. After the cells were cultured overnight, the medium was taken to detect the production of NO, and the absorbance was measured at 570 nm. MTS was added to the remaining medium for cell viability assays to exclude the toxic effects of compounds on cells. The assays were performed as triplicate batch experiments. NO production inhibition rate (%) = ($OD_{570\ nm}$ of non-drug treatment group - $OD_{570\ nm}$ of sample group)/$OD_{570\ nm}$ of non-drug treatment group × 100% [17,19].

3.6. ECD Calculations

The conformers of the five calculated compounds were generated by MMFF in ChemDraw. The ECD were calculated at the B3LYP/6-31+G(d,p) level in methanol with the PCM model. The calculated ECD curves and weighted ECD were all generated using SpecDis 1.71 based on the Boltzmann distribution theory, the simulated spectra of all the predominant conformers were averaged to obtain the final conformation-ally averaged data [20]. All of the Density functional theory (DFT) calculations were implemented by the Gaussian 16 software package with Gaussian 09 default keyword. For computational details of compounds **1–5**, see the Supporting Information.

4. Conclusions

In present study, the chemical investigation on *Paeonia suffruticosa* resulted in the isolation of five new benzofuran ring compounds, containing rare dimers (compounds **1–3**) and hetero-dimers (compounds **4** and **5**). Their structures were determined by extensive spectroscopic methods. These kinds of constituents were rarely reported from natural resources. This work represents the first report of five new benzofuran dimer compounds of *P. suffruticosa* and their cytotoxicity. This research broadens the horizon of the structural diversity of *P. suffruticosa*.

Supplementary Materials: The following supporting information can be downloaded at XXX. Figures S1-S35: HRESIMS, 1D & 2D NMR, ECD data of compounds **1–5**.

Author Contributions: T.F. and J.K.L. designed and guided the experiment; Q.Q.M. performed the isolation and identification of the compounds, and wrote the manuscript; S.Y.T and Y.Q.Z. contributed to the isolation of these compounds; X.R.P. reviewed the manuscript; Z.H.L. plant material and identification; T.F. and J.K.L. reviewed the manuscript. All authors have agreed to the published version of this manuscript.

Funding: This work was financially supported by the National Natural Science Foundation of China (22177138, 21961142008).

Acknowledgments: The authors thank the Bioactivity Screening Center, Kunming Institute of Botany, Chinese Academy of Sciences for screening the bioactivity of the compounds.

Conflicts of Interest: The authors declare no conflict of interest.

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