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Anti-inflammatory and Cytotoxic Neoflavonoids and Benzofurans from *Pterocarpus santalinus*

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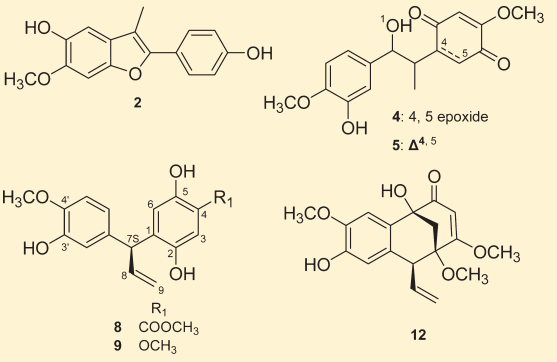
Supporting Information

ABSTRACT: Five new benzofurans, pterolinuses A–E (**1**–**5**), six new neoflavonoids, pterolinuses F–J (**8**–**13**), and five known compounds (**6**, **7**, **14**–**16**) were isolated from an extract of *Pterocarpus santalinus* heartwood. All new structures were elucidated by spectroscopic methods, and configurations were confirmed by CD spectral data and optical rotation values. The isolates were evaluated for anti-inflammatory and cytotoxic activities. Six compounds (**1**, **2**, **4**, **6**, **7**, and **15**) showed significant inhibition in at least one anti-inflammatory assay. Compound **2** showed the best selective effect against superoxide anion generation in human neutrophils with, an IC_{50} value of 0.19 μg/mL, and was 6.2-fold more potent than the positive control LY294002. Compound **14** showed the highest cytotoxicity against Ca9-22 cancer cells, with an IC_{50} value of 0.46 μg/mL.



Pterocarpus santalinus L. (Fabaceae), also named "red sanders" or "red sandalwood", is a rare, commercial tree in the Legume family. This species is distributed exclusively in well-defined forest tracts of Andhra Pradesh in Southern India. It is valuable in the international market and is most notably exported from India to Japan and other countries.[1] The fragrant red heartwood is valued for making furniture and also as a source of coloring and dyeing materials. In Buddhism, it represents holiness and is said to prevent evil; thus, it is used for carved statues and as a component of incense. In addition, *P. santalinus* has been used as a folk remedy for treatment of inflammation, such as in chronic bronchitis and chronic cystitis, fever, headaches, mental aberrations, ulcers, cancer, etc.[2,3] In previous phytochemical investigations, six sesquiterpenes,[4] one isoflavone,[5] two lignans,[2] and two aurone glycosides[6] were isolated from this species. In preliminary studies, we found that a MeOH extract of the heartwood exhibited potent cytotoxicity (IC_{50} < 20 μg/mL) against six cancer cell lines (HepG2, Hep3B, Ca9-22, A549, MCF7, MDA-MB-231). Subsequently, we isolated seven benzofurans (**1**–**7**) and nine neoflavonoids (**8**–**16**) from the extract using bioactivity-guided fractionation. Compounds **1**–**5** and **8**–**13** are new, and their structures were determined by spectroscopic methods. Cytotoxic and anti-inflammatory activities of the isolates were also investigated in this study.

RESULTS AND DISCUSSION

The CH_2Cl_2-soluble portion of a MeOH extract of *P. santalinus* heartwood was subjected to column chromatography (CC) on silica gel, C18 gel, Sephadex LH-20, and preparative TLC to yield five new benzofurans (**1**–**5**), six new neoflavonoids (**8**–**13**), and five known compounds (**6**, **7**, **14**–**16**). The known compounds were identified as dehydromelanoxin (**6**), melanoxin (**7**),[7] melanoxoin (**14**), *S*-3′-hydroxy-4,4′-dimethoxydalbergione (**15**), and melannein (**16**)[8] by comparison of the NMR and MS data with those in published literature.

Compounds **1** and **6**, isolated separately, both had the same molecular weight. The ion at *m*/*z* 300.0999 $[M]^+$ in HREIMS indicated a molecular formula of $C_{17}H_{16}O_5$ and 10 degrees of unsaturation. Compounds **1** and **6** had similar ^1H and ^{13}C NMR spectra. The IR spectra of **1** and **6** were also similar, with absorptions for OH (3443 cm^{-1}) and aromatic (1514 and

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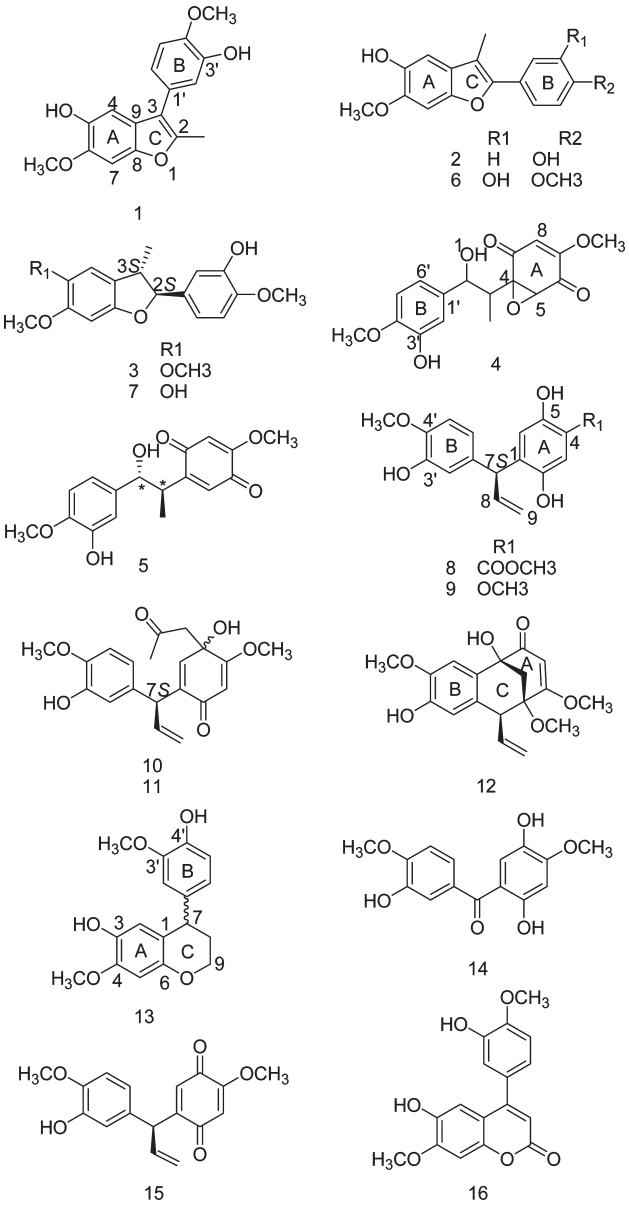

1512 cm^{-1}, respectively) groups. However, the UV spectra of **1** and **6** were significantly different. As indicated in the literature,[9] 3-methyl- and 2-methyl-benzofurans show different λ_{max} absorptions, at ca. 321 and 235 nm, respectively. The UV spectrum of **1** showed a maximum absorption at 242 nm, whereas that of **6** showed a λ_{max} at 324 nm, which were consistent with the reported data. The methyl groups of **1** and **6** showed significantly different ^{13}C NMR chemical shifts (δ_C 13.6 in **1** and δ_C 9.6 in **6**).[9] Therefore, the B rings in **1** and **6** are attached at C-3 and C-2, respectively, of the arylbenzofuran skeleton. On the basis of the ^1H and ^{13}C NMR spectra, compound **1** has a 14-carbon skeleton along with one methyl (δ_H 2.45/δ_C 13.6) and two methoxy groups (δ_H 3.91/δ_C 57.4, and δ_H 3.90/δ_C 57.0). Five olefinic sp^2 methine carbons (δ_C 96.5, 105.2, 113.5, 116.9, and 121.4) and nine quaternary carbons [six oxygenated sp^2 carbons (δ_C 145.1, 147.3, 148.1, 148.4, 149.4, 150.9)] were observed according to HSQC and DEPT spectra. In the ^1H NMR spectrum, an ABX system (δ_H 6.99, J = 2.0 Hz; 7.07, J = 8.0 Hz; 6.94, J = 8.0, 2.0 Hz) and two singlet protons (δ_H 6.97 and 7.12) were found. The latter were identified on the basis of the HMQC spectra and a previous reference[9] as corresponding to C-4 (δ_H 6.97, δ_C 105.2) and C-7 (δ_H 7.12, δ_C 96.5). Moreover, these two aromatic protons showed HMBC correlations with C-5, C-6, C-9 and C-5, C-6, C-8, C-9, respectively. The HMBC spectrum also showed correlations of the OCH_3 protons at δ_H 3.91 and 3.90 with C-6 and C-4′, which was confirmed by NOESY corrections with H-7 and H-5′, respectively. Two OH protons (δ_H 7.30, 7.75) showed HMBC correlations with C-4, C-5, C-6 and C-2′, C-3′, which indicated that they were attached at C-5 and C-3′. Consequently, compound **1** has an isoparvifuran skeleton,[9] and it was named pterolinus A.

The molecular formula ($C_{16}H_{14}O_4$) of **2** was identified from the HRESIMS ion at m/z 271.0972 $[M + H]^+$, indicating 10 degrees of unsaturation. IR absorptions at 3317(OH) and 1510 (aromatic) cm^{-1} and UV absorptions at 321 and 284 nm were characteristic of a 2-methylbenzofuran.[9,10] A 14-carbon skeleton was also proposed on the basis of the ^1H and ^{13}C NMR spectra. In contrast with **1**, the proton and carbon chemical shifts for the methyl group were observed at δ_H 2.36 s and δ_C 9.6. One OCH_3 group (δ_H 3.92), two OH groups (δ_H 7.23 br/8.60 br), and an AA′BB′ (δ_H 6.96, d, J = 8.0 Hz/δ_H 7.63, d, J = 8.0 Hz) system appeared in the ^1H NMR spectrum. The OCH_3 was connected to C-6 on the basis of a HMBC correlation and a NOE correlation with H-7. Hence, the two OH groups were assigned at C-5 and C-4′. Compound **2** was identified as indicated and has been named pterolinus B.

Compound **3** showed an $[M + H]^+$ ion at m/z 317.1387 in the HRESIMS (molecular formula $C_{18}H_{20}O_5$), and nine degrees of unsaturation were calculated. The IR spectrum showed absorptions at 3408 (OH) and 1495, 1592 (aromatic) cm^{-1}, and UV maximum absorption were found at 207, 232, and 298 nm,[7] indicating a 2,3-dihydrobenzofuran skeleton. On the basis of 1D NMR, one OH, one CH_3, and three OCH_3 groups were found. Aromatic singlet protons, at δ_H 6.83 (H-4) and 6.51 (H-7), showed NOE correlations with δ_H 3.78 (OCH_3)/1.34 (CH_3) and δ_H 3.74 (OCH_3), respectively. The third OCH_3 was located at C-4′ on the basis of an HMBC correlation with C-4′ and an NOE correlation with H-5′. The OH group connected at C-3′ according to the HMBC correlations with C-2′ (δ_C 114.4) and C-3′ (δ_C 148.2). The relative orientations of H-2 and H-3 in substituted 2,3-dihydrobenzofurans can be judged easily by ^1H NMR chemical shifts, with the *trans* form at ca. δ_H 5.11 and 3.38 vs the *cis* form at ca. δ_H 5.73 and 3.62.[10] The chemical shifts of H-2 and H-3 in **3** were δ_H 5.02 and 3.29, which are consistent with a *trans* orientation. This assignment was confirmed by the absence of a NOE correlation. In addition, a negative Cotton effect at 319 nm and a positive Cotton effect at 255 nm in the CD spectrum, as well as a specific optical rotation value of −27.8, were similar to those of a known compound ((2*S*,3*S*)-3-methyl-2-phenyl-2,3-dihydrobenzb[*b*]furan, a negative Cotton effect at 280 nm and a positive Cotton effect at 220 nm, $[\alpha]_D$ −4.8).[10] The data suggested that the absolute configurations were 2*S* and 3*S*. Thus, compound **3** was identified as shown and was named pterolinus C.

Compound **4** showed an $[M + Na]^+$ ion at m/z 357.0952 ($C_{17}H_{18}O_7Na$), corresponding to nine degrees of unsaturation. The IR spectrum showed OH, C═O, and aromatic absorptions, and a UV maximum absorption occurred at 230 nm. A 14-carbon skeleton was evident from 1D NMR, including seven methine carbons (four olefinic and three sp^3 carbons), seven quaternary carbons (three oxygenated carbons, an oxygenated sp^3 carbon, and two carbonyl carbons), a methyl, and two OCH_3 groups. In

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Table 1. ^{1}H NMR Data of Compounds 1−7 (400 MHz in acetone-d_6, J values in parentheses)

	1	2[a]	3	4	5	6	7
1				4.28 d (4.0)	4.25 d (4.0)		
2			5.02 d (8.0)	4.77 dd (10.0, 4.0)	4.68 d (7.6, 4.0)		4.98 d (8.2)
3			3.29 m (8.0, 6.8, 0.8)	1.81 dq (10.0, 7.2)	3.28 p (7.6)		3.27 p (8.2, 6.8)
4	6.97 s	6.96 s	6.83 d (0.8)			6.96 s	6.66 d (1.2)
5				3.93 s	6.62 s		
6							
7	7.12 s	7.13 s	6.51 s			7.13 s	6.49
8				5.91 s	5.99 s		
9							
1′							
2′	6.99 d (2.0)	7.63 d (8.0)	6.91 d (2.0)	6.88 d (2.0)	6.87 d (2.0)	7.28 d (2.4)	6.91 d (2.0)
3′		6.96 d (8.0)					
4′							
5′	7.07 d (8.0)	6.96 d (8.0)	6.93 d (8.0)	6.89 d (8.0)	6.86 d (8.0)	7.04 d (8.4)	6.49 d (8.0)
6′	6.94 dd (8.0, 2.0)	7.63 d (8.0)	6.86 dd (8.0, 2.0)	6.80 dd (8.0, 2.0)	6.76 d (8.0, 2.0)	7.21 dd (8.4, 2.4)	6.86 dd (8.0, 2.0)
5-OCH_3			3.78 s				
6-OCH_3	3.91 s	3.92 s	3.74 s			3.91 s	3.84 s
7-OCH_3				3.83 s	3.82 s		
4′-OCH_3	3.90 s		3.84 s	3.83 s	3.83 s	3.89 s	3.81 s
5-OH	7.30 s	7.23 br				7.34 s	6.95 s
3′-OH	7.75 s		7.73 s	7.54 s	7.50 s	7.86 s	7.65 s
4′-OH		8.60 br					
CH_3	2.45 s	2.36 s	1.34 d (6.8)	1.04 d (7.2)	0.97 d (7.6)	2.37 s	1.32 d (6.8)

[a] Recorded by 500 MHz NMR.

the ^{1}H NMR spectrum, an ABX system (δ_H 6.88, J = 2.0 Hz; 6.89 J = 8.0 Hz; 6.80 J = 8.0, 2.0 Hz) was found, together with an olefinic methine, an oxymethine, and two OH groups. A CH_3−CH−CH−OH fragment (1.04, J = 7.2 Hz, CH_3/1.81, J = 10.0, 7.2 Hz, H-3/4.77, J = 10.0, 4.0 Hz, H-2/4.28, J = 4.0 Hz, OH) was present. Proton signals at δ_H 6.88 and 6.80 showed a HMBC correlation with C-2 (δ_C 76.3), indicating that ring B was attached at C-2. Two unique carbons (δ_C 62.5, 65.1) of ring A were observed in the ^{13}C NMR spectrum, and a singlet proton at δ_H 3.93 showed HMBC correlations with δ_C 47.1, 65.1, 160.3, and 189.5. On the basis of this data, there was a C4−C-5 epoxide. The olefinic proton (δ_H 5.91, s) showed correlations with δ_C 65.1, 160.3, 189.5, and 193.5, providing evidence that ring A also contained two carbonyl groups. The OCH_3 groups in **4** showed HMBC correlations with C-7 and C-4′ and NOESY enhancements with H-8 and H-5′, which indicated that the OCH_3 substituents were located at C-6 and C-4′. The coupling constant (J = 7.6 Hz) of H-2 and H-3 in compound **4** indicated that the dihedral angle ϕ of these vicinal protons was near 0° or 150°. However, a weak NOE correlation was observed between H-2 and H-3, which suggested that both dihedral angles (0° or 150°) were possible. Therefore, we were not able to determine the structure of **4** by means of NMR techniques. We attempted to determine the absolute configuration in **4** by a Moshers' reaction, but were unable to distinguish the positive side and negative side after calculating $\delta_{S\text{-MTPA}} - \delta_{R\text{-MTPA}}$. Thus, the structure of **4** was identified as shown, and it was named pterolinus D.

Compound **5** had the molecular formula $C_{17}H_{18}O_6$, one oxygen atom less than **4**. The IR spectrum showed absorptions indicative of OH, C=O, and aromatic groups, and the UV had maximum absorption at 230 and 273 nm. The epoxide carbons of **4** were absent and instead were replaced by carbons resonating at δ_C 153.7 and 132.2. Thus, a 1,4-benzoquinone was present. H-2 and H-3 of compound **5** showed a large coupling constant (J = 10 Hz) and no NOE correlation, indicating that these two protons had an *anti* conformation. Because NOE correlations were found between H-2 and the two vicinal groups, C3-Me and H-5, any of four isomers were possible. Therefore, the stereochemistry was not defined. Compound **5** was named pterolinus E.

Compound **8** had a molecular formula of $C_{18}H_{18}O_6$, with nine degrees of unsaturation. On the basis of comparison with previously reported ^{13}C NMR data,[11] a neoflavonoid skeleton was identified in **8**. The ^{1}H NMR spectrum exhibited an ABX system (δ_H 6.66, J = 8.4, 2.4 Hz/6.86, J = 8.4 Hz/6.71, J = 2.4 Hz), two aromatic singlet signals (δ_H 6.73/7.30), three OH groups (δ_H 7.48, br/8.16, br/10.16, s), and two OCH_3 groups (δ_H 3.80, s/3.91, s). ^{1}H NMR, COSY, and HSQC data established the partial connectivity of the C7−C8−C9 segment and identified the carbons as an aliphatic CH, an olefinic CH, and an olefinic CH_2. Two singlet aromatic protons in the ^{1}H NMR spectrum revealed the presence of a 1,2,4,5-tetrasubstituted phenyl ring A. In the HMBC spectrum, both H-6 and H-6′ showed correlations with C-7, suggesting that the two phenyl groups were connected through C-7. In addition, one OCH_3 group (δ_H 3.91) showed an HMBC correlation with the carbonyl carbon at δ_C 171.7, which indicated the presence of a methyl ester. The OH proton at δ_H 10.16 showed HMBC correlations with C-4 (δc 111.5) and C-6 (δc 119.2), while H-3 showed correlations with C-1, C-2, CO, and C-5. These data indicated that ring A was *para*-hydroxy substituted. Furthermore, on the basis of the HMBC and NOESY spectra, the OCH_3 group (δ_H 3.80) was assigned at C-4′. The absolute configuration was

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Table 2. ^{1}H NMR Data of Compounds 8−15 (400 MHz in acetone-d_6, J values in parentheses)

	8	9	10[a]	11[a]	12[b]	13	14[3]	15	16[c]
1									
2		6.50 s				6.24 d (1.2)	6.86 s		7.49 s
3	7.30 s		5.42 s	5.42 s	5.11 s			6.00 s	
4									
5		6.57 s				6.38 s	7.69 s		7.07 s
6	6.73 s		6.47 s	6.48 s	2.36 d (11.4)			6.45 d (1.2)	
					2.71 dd (11.4, 1.2)				
7	5.05 brd (7.2)	4.74 brd (7.2)	4.68 d (6.5)	4.64 d (7.0)	3.50 brd (7.5)	3.98 brd (6.8)		4.79 dd (1.2, 7.2)	
8	6.31 ddd	6.26 ddd	6.10 ddd	6.09 ddd	6.07 ddd	2.01 m		6.19 ddd	6.44
	(17.0, 10.0, 7.2)	(17.0, 10.2, 7.0)	(17.0, 10.0, 6.5)	(17.0, 10.0, 7.0)	(17.4, 10.5, 7.5)	2.17 m		(17.2, 10.4, 7.2)	
9	4.97 dt (17.0, 1.6)	4.90 dt (17.0, 1.6)	4.92 d (17.0)	4.91 dt (10.0, 1.5)	4.85 ddd (17.4, 2.4, 1.8)	4.09 m		5.02 dt (17.2, 1.6)	
	5.18 dt (10.0, 1.6)	5.11 ddd (10.2, 2.4, 1.6)	5.10 d (OL)[d]	5.09 dt (1.5, 17.0)	5.05 ddd (10.5, 1.8, 1.2)			5.20 dt (10.4, 1.6)	
1′									
2′	6.71 d (2.4)	6.69 d (2.4)	6.67 d (2.0)	6.71 d (2.0)	6.52 d (0.6)	6.78 d (2.0)	7.77 d (2.0)	6.73 d (2.4)	7.01[e]
3′									
4′									
5′	6.86 d (8.4)	6.83 d (8.4)	6.83 d (8.4)	6.82 d (8.0)	7.23 s	6.75 d (8.0)	6.92 d (8.4)	6.87 d (8.4)	7.01[e]
6′	6.66 dd (8.4, 2.4)	6.64 dd (8.4, 2.4)	6.63 dd (8.4, 2.0)	6.64 dd (8.0, 2.0)		6.57 dd (8.0, 2.0)	7.38 d (8.4, 2.0)	6.67 dd (8.4, 2.4)	7.41 brdd
1-OCH_3					3.22 s				
2-OCH_3					3.72 s				
4-OCH_3		3.74 s	3.76 s	3.77 s		3.79 s	3.80 s	3.83 s	3.84 s
5-OCH_3									
3′-OCH_3						3.78			
4′-OCH_3	3.80 s	3.79 s	3.79 s	3.79 s	3.81 s		3.81 s	3.80 s	3.84 s
2-OH	8.16 br	7.57 br					13.08 s		
3-OH						6.92 s			11.50 br
5-OH	10.16 s	7.40 br	5.10 s	5.08 s	5.21 s		11.10 br		
3′-OH	7.48 br	6.88 br	7.44 s	7.39 s	7.59 s		11.65 br	7.58 br	
4′-OH						7.42 s			
COOCH_3	3.91 s								
CH_2			2.95 d (14.5)	2.93 d (14.5)					
			3.06 d (14.5)	3.04 d (14.5)					
COCH_3			2.14 s	2.10 s					

[a] Recorded by 500 MHz NMR in acetone-d_6. [b] Recorded by 600 MHz NMR in acetone-d_6. [c] Measured in pyridine-d_5. [d] Overlapping with 5-OH. [e] Overlapping.

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identified as 7*S* by comparing the $[\alpha]_D$ value (+20.6) with previous references (latifolin, 7*R*, $[\alpha]^{20}_D$ −26.7; 2,4-dihydroxydalbergiquinol, 7*S*, $[\alpha]^{22}_D$ +34.7).[11,12] Thus, the structure of **8** was elucidated, and the compound was named pterolinus F.

Compound **9** was obtained as a 2:5 mixture with **15**, according to ^{1}H NMR data, even when different purification methods were used. Compound **15** was isolated as a pure compound that contains a 1,4-benzoquinone ring, as elucidated in an earlier report,[11,13] while **9** contains 1,4-hydroxy-substituted phenyl rings (see OH signals in Table 2). To further suggest that **15** was the oxidative product of **9**, the latter compound showed an $[M]^+$ ion at *m*/*z* 302.1151 ($C_{17}H_{16}O_5$) in the HRGCEIMS, while the former compound showed an $[M + H]^+$ ion at *m*/*z* 300.97 ($C_{17}H_{17}O_5$) in the ESIMS, 2 amu less than **9**. In addition, excess $NaBH_4$ was used to reduce compound **15** to confirm that **9** was the hydroquinone counterpart of **15** (data are shown in Supporting Information). We postulate that **9** is oxidized gradually to **15** and that both compounds should have an *S* configuration at C-7. Compound **9** was named pterolinus G.

Compounds **10** and **11** were isolated by RI-recycle HPLC (EtOAc−hexanes, 2:1, t_R = 32.4 and 35.6 min). Both compounds had the molecular weight $C_{20}H_{23}O_6$. On the basis of similar NMR data, **10** and **11** both had a neoflavonoid C7−C8−C9 fragment and a 3-hydroxy-4-methoxyphenyl ring. However, unlike **15**, compounds **10** and **11** had a $-CH_2-CO-CH_3$ moiety (δ_H 2.95, 3.06, *J* = 14.5 Hz, δ_C 52.3/δ_C 205.6/δ_H 2.14, s, δ_C 31.9) and an OH (δ_H 5.10, s) rather than a second carbonyl group in ring A, based on 1D NMR and HMBC data. In the HMBC data of **10**, H-7 (δ_H 4.68) showed correlations with C-1 (δ_C 139.4) and C-2 (δ_C 185.8), and the proton of an OH group showed correlations with C-4, C-5, C-6, and $-\underline{C}H_2-CO-CH_3$. Therefore, the $-CH_2-CO-CH_3$ and OH substituents were both attached to the C-5 of ring A. On the other hand, the HMBC spectrum of **11** also showed correlation patterns similar to those of **10**. Both **10** and **11** showed positive $[\alpha]^{25}_D$ values ($[\alpha]^{25}_D$ 142.12/105.96) and a negative Cotton effect at ca. 340 nm and positive Cotton effect at ca. 300 nm. By comparisons with previous data (shown in Supporting Information),[13] we suggest that C-7 has an *S* configuration. However, we could not identify the absolute configuration at C-5. Thus, **10** and **11** were identified as epimers and were named pterolinus Ha and pterolinus Hb. However, these two epi-isomers could possibly be artifacts due to reaction with acetone.

The molecular formula of **12** was $C_{18}H_{20}O_6$, with nine degrees of unsaturation. On the basis of the ^{13}C NMR data, a 15-carbon skeleton and three OCH_3 groups were found. The ^{1}H NMR and COSY data revealed a −C7−C8−C9 moiety; however, the H-7 signal was shifted to δ_H 3.50 (d, *J* = 7.5 Hz), indicating that one aromatic ring was absent. Two quaternary, one methylene, and one carbonyl carbon were found in the 1D NMR spectra. From the HMBC data, H-7 showed key correlations with C-1, C-6, C-1′, and C-6′, revealing that this partial structure was linked to C-7. In addition, H-6a and H-6b showed HMBC correlation with C-1, C-2, C-4, C-5, and C-1′, and H-6b showed a long-range coupling (*J* = 1.2 Hz) with H-8 (δ_H 5.05). Moreover, H-5′ showed HMBC correlations with C-5, C-4′, and C-6′. Thus, compound **12** was tricyclic and linked between C-5 and C-6′. The relative configuration was assigned from the NOESY spectrum, in which H_2-6 showed no correlation with H-7, suggesting that these two protons are on a different side than H-7. The absolute configuration could not be identified in the current study. The three OCH_3 groups (δ_H 3.22, 3.72, 3.81) showed NOE correlations with H-6b, H-3, and H-5′, respectively, and the structure was assigned as shown. Compound **12** was named pterolinus I.

Compound **13** had the molecular formula $C_{17}H_{19}O_5$, corresponding to nine degrees of unsaturation. The ^{1}H NMR and HMQC spectra demonstrated that the terminal double bond (C-8, C-9) found in **8**−**12** was absent and instead was replaced by substituted methylene (δ_H 2.01, 2.17, m, δ_C 33.6) and oxygenated methylene (δ_H 4.09, m, δ_C 65.4) groups. On the basis of nine degrees of unsaturation, the presence of a heterocyclic ring C was postulated. HMBC correlations of H-5 (δ_H 6.38, s) with C-4 (δ_C 148.5) and C-6 (δ_C 149.7), as well as correlations of H-9 with C-6 and C-7 (δ_C 41.7), indicated that C-9 was linked to C-6 through an oxygen atom. The positions of the two OCH_3 and two OH groups at C-4, C-3′, C-3, and C-4′, respectively, were confirmed from the NOESY and HMBC data. Thus, **13** was identified and was named pterolinus J.

Compounds **1**−**8**, **10**, **11**, and **13**−**16** were screened for cytotoxicity against six human cancer cell lines, liver (HepG2, Hep3B), gingival (Ca9-22), lung (A549), and breast (MCF7, MDA-MB-231), and for anti-inflammatory activity based on effects against superoxide anion generation and elastase release by human neutrophils in response to fMLP/CB.

These compounds could be divided into those with a benzofuran skeleton and those with a neoflavonoid skeleton. Among the neoflavonoids (**8**, **10**, **11**, **13**−**16**), compound **14** showed the highest cytotoxicity against Ca9-22, with an IC_{50} value of 0.46 μg/mL, **15** showed significant cytotoxicity against Hep3B and MDA-MB-231 with IC_{50} values of 2.39 and 3.34 μg/mL, **10** exhibited cytotoxicity against HepG2 and MDA-MB-231 with IC_{50} values of 3.65 and 2.85 μg/mL, and compound **11** showed selective cytotoxicity against A549 with an IC_{50} value of 3.97 μg/mL. In contrast, compounds **13** and **16**, which contain a pyran or lactone ring C formed by cyclization of a −C7−C8−C9−O− moiety onto ring B, were inactive (IC_{50} >17.10 μg/mL). Therefore, the heterocyclic ring C abolished cytotoxic activity. Compounds **8**, **10**, **11**, **14**, and **15** have identical phenyl B rings, but differences in the A rings and linkage between the two rings. Compound **8**, with a *para*-dihydroxy-substituted phenyl ring A, exhibited only weak cytotoxicity (IC_{50} 11.10−16.10 μg/mL) compared with **10**, **11**, and **15**, which contain benzoquinone or similar ring systems containing carbonyl groups. Compound **14**, which has a carbonyl (C═O) linkage between the phenyl A and B rings rather than an allyl (CH−CH═CH_2) group, exhibited potent cytotoxcity against Ca9-22. Therefore, compound **14** could be a potential candidate for anticancer drug development.

Among the benzofurans (**1**−**7**), compound **2** showed the highest cytotoxicity against A549 and MCF7, with IC_{50} values of 2.34 and 1.74 μg/mL, while compound **6**, with a different substitution pattern on ring B, and compound **1**, with ring B attached at C-2 rather than C-3, exhibited no cytotoxicity. Compounds **3** and **7**, with a *trans*-configured saturated C2−C3 bond, were inactive (>19.42 μg/mL) or weakly active (7.70−15.42 μg/mL). Compound **4**, with an opened ring C and benzoquinone ring A, showed significant cytotoxicity against Hep3B and MCF7 (IC_{50} 2.08 and 3.31 μg/mL) and moderate cytotoxicity against HepG2 and MDA-MB-241 (IC_{50} 4.33 and 4.89 μg/mL). Compound **5**, which is structurally identical to **4**, except for lacking the epoxide group, exhibited reduced activity (IC_{50} 6.96−16.65 μg/mL).

Compounds **1**−**8**, **10**, **11**, and **13**−**16** were evaluated for inhibitory effects on superoxide anion generation and elastase

release by human neutrophils in response to fMLP/CB. The benzofurans generally showed more potent inhibition than neoflavonoids (Table 5). Compounds **1**, **2**, **4**, **6**, and **7** showed potent inhibition of superoxide anion generation with IC_{50} values of 0.33, 0.19, 0.29, 0.73, and 0.69 μg/mL, respectively. The inhibition was 1.6−6.2-fold greater than that of the positive control LY294002 (a phosphatidylinositol-3-kinase inhibitor).[14,15] In addition, compound **4** showed significant inhibition of human neutrophil elastase release with an IC_{50} value of 1.06 μg/mL, which was 1.9-fold higher than the positive control. Among the neoflavonoids, compound **15** exhibited potent inhibition in both assays with IC_{50} values of 0.47 and 1.44 μg/mL (Table 5). On the basis of the results, compounds **1**, **2**, **4**, **6**, **7**, and **15** merit consideration as leads for anti-inflammatory agents.

EXPERIMENTAL SECTION

General Experimental Procedures. CD data were measured on a JASCO J-815 instrument. Optical rotations were recorded on a JASCO P-1020 polarimeter. IR spectra were measured on a Mattson Genesis II FT-IR spectrophotometer. UV spectra were obtained on a JASCO V-530 UV/vis spectrophotometer. NMR spectra were run on a Varian Unity-plus 400 MHz FT-NMR or a Varian Mercury-plus 400 MHz FT-NMR spectrometer. The chemical shift (δ) values are in ppm (parts per million) with d_6-acetone, d_4-MeOH, or d_5-pyridine as the internal standard, and coupling constants (J) are in Hz. Low-resolution ESI-mass spectra were obtained on a VG Biotech Quattro 5022 mass spectrometer in a positive or negative mode, high-resolution MS spectra were obtained on Bruker Daltonics APEX α30e and JEOL JMS-700 mass spectrometers. A JASCO PU986 pump, a JASCO UV-1575 detector, a JASCO 887-30 mix. Module, a JAI LC-918 RI-recycle HPLC, and an Ascentis C18 column (250 mm × 10 mm, 5 μm) were employed for HPLC separations. The Biotage SP1 was used for flash liquid chromatography. Silica gel 60 (40−60 mesh, Merck), C-18 (40−63 mesh, Merck), Sephadex LH-20, Celite 545, and Diaion HP-20 were used for column chromatography. Silica gel plates (Kieselgel 60, F254, 0.20 nm, Merck) were used for TLC.

Plant Material. Heartwood (4.75 kg) of *P. santalinus* was imported from India, provided by Mr. Mike Y. C. Wei, and was identified by Prof. Dr. Sheng-Yang Wang, Department of Forestry, National Chung-Hsing University, Taichung, Taiwan. A voucher specimen (Pterocarpus 002) was deposited in the Graduate Institute of Natural Products, Kaohsiung, Taiwan.

Extraction and Isolation. The heartwood was powdered and extracted with MeOH (5 × 20 L). After removal of solvent, the crude extract (ca. 480 g) was partitioned between CH_2Cl_2 and 50% aqueous MeOH. The CH_2Cl_2 layer was concentrated in vacuo, and the CH_2Cl_2 extract (ca. 300 g) was subjected to CC on Celite 545 and eluted with *n*-hexane (8 L), CH_2Cl_2 (40 L), EtOAc (20 L), acetone (10 L), and MeOH (8 L). Six fractions (PS-C1 to PS-C6) were obtained. PS-C3 (ca. 100 g) was subjected to silica gel CC using a hexane−EtOAc−MeOH gradient solvent system (3:1:0, 1:1:0, 0:1:0, 0:40:1, 0:20:1, 0:10:1, 0:6:1), to give 11 fractions (PS-C3.1 to PS-C3.11).

Compounds **14** (3.6 g) and **16** (ca. 8 g) were obtained as precipitated solids from PS-C3.6 and PS-C3.7, and **6** (144 mg) was obtained from PS-C3.5. PS-C3.5 (31.2 g) was subjected to repeated silica gel CC with a CH_2Cl_2 and MeOH gradient solvent system, and 11 fractions (PS-C3.5.1 to PS-C3.5.11) were obtained. PS-C3.5.6 (20.70 g) was subjected to CC on Sephadex LH-20 and eluted with acetone and CH_2Cl_2 (1:1) to give 10 fractions (PS-C3.5.6.1 to PS-C3.5.6.10). PS-C3.5.6.3 (6.0 g) was repeatedly chromatographed on RP-MPLC, Sephadex LH-20, NP-RI-HPLC, silica gel, and PTLC to give **3** (9.8 mg), **10** (25 mg), **11** (15.1 mg), **12** (0.6 mg), and **15** (42 mg). PS-C3.5.6.5 and PS-C3.5.6.6 were mixed (total ca. 6.8 g) and subjected to CC with Sephadex LH-20, RP-MPLC, silica gel, and PTLC to give **4** (16.5 mg), **5** (12 mg), and **7** (1.6 g).

Table 3. ^{13}C NMR Data (δ) of Compounds 1−7 (100 MHz in acetone-d_6)

	1	2[a]	3	4	5	6	7
1							
2	150.9	151.1	94.0	76.3	78.1	151.3	93.8
3	117.8	110.0	47.2	47.1	41.2	125.4	47.1
4	105.2	104.2	111.0	65.1	153.7	104.9	111.6
5	145.1	148.5	151.8	62.5	132.2	147.9[b]	142.2
6	147.3	147.1	145.6	189.5	183.5	149.1	148.7[b]
7	96.5	95.7	96.8	160.3	160.0	96.3	96.1
8	149.4	144.4	155.1	110.5	109.2	145.0	153.8
9	122.8	124.8	123.6	193.5	188.4	111.2	124.3
1′	127.5	124.3	135.9	138.5	138.4	125.4	135.9
2′	116.9	128.6	114.4	115.0	115.0	114.4	114.4
3′	148.4	116.5	148.2	148.1	147.8[b]	148.2[b]	148.2[b]
4′	148.1	158.0	149.0	148.6	148.3[b]	148.7[b]	148.9[b]
5′	113.5	116.5	112.9	112.5	112.4	113.3	112.9
6′	121.4	128.6	118.9	119.8	119.5	119.4	118.9
5-OCH_3			57.0				
6-OCH_3	57.4	56.8	58.1			56.9	56.9
7-OCH_3				57.7[b]	57.2[b]		
4′-OCH_3	57.0		56.9	56.9[b]	56.9[b]	57.4	57.2
CH_3	13.6	9.6	19.4	14.7	17.8	10.3	19.3

[a] Recorded by 125 MHz NMR. [b] The chemical shifts may be interchanged.

PS-C3.5.6.7 (478 mg) was separated by RP-MPLC with a MeOH and H_2O gradient system and divided into five subfractions, while compound **13** (7.6 mg) was isolated by RP-HPLC (Acentis, 250 × 10 mm, 57% MeOH(aq)) from subfraction 3. PS-C3.5.6.8 (0.27 g) was separated by RP-MPLC with 60% MeOH(aq), and eight subfractions were obtained, while compounds **1** and **8** were isolated by PTLC with pure CH_2Cl_2 from subfraction 7 (70.7 mg). PS-C3.5.6.9 (533 mg) was separated by RP-MPLC with a MeOH and H_2O gradient to give nine fractions, while **2** (19.2 mg) and **9** (9.7 mg) were isolated by PTLC with pure CH_2Cl_2 from subfraction 3 (117.6 mg).

Pterolinus A (***1***): brown gum; UV λ^{MeOH}_{max} nm (log ε) 242 (4.13), 297 (3.97), 309 (3.84), 350 (3.33); IR (neat) ν_{max} 3443, 2930, 1621, 1514 cm^{-1}; ^{13}C and ^{1}H NMR data, see Table 1; HREIMS m/z 300.0999 $[M]^+$ (calcd 300.0999 for $C_{17}H_{16}O_5$).

Pterolinus B (***2***): amorphous, pale brown powder; UV λ^{MeOH}_{max} nm (log ε) 226 (4.00), 284 (4.05), 321 (4.28), 337 (4.08); IR (neat) ν_{max} 3317, 2921, 1677, 1510 cm^{-1}; ^{13}C and ^{1}H NMR data, see Table 2; ESIMS m/z 270.99 $[M + H]^+$; HRESIMS m/z 271.0972 $[M + H]^+$ (calcd 271.0970 for $C_{16}H_{15}O_4$).

Pterolinus C (***3***): amorphous, pale brown powder; $[\alpha]^{25}_D$ −27.8 (c 0.095, MeOH); UV λ^{MeOH}_{max} nm (log ε) 207 (4.44), 232 (3.93), 289 (3.72), 298 (3.69); CD ε_{315} −11.08, $\Delta\varepsilon_{258}$ 29.26 (MeOH; c = 0.3 mg/mL); IR (neat) ν_{max} 3408, 2928, 1592, 1495 cm^{-1}; ^{13}C and ^{1}H NMR data, see Table 3; ESIMS m/z 316.93 $[M + H]^+$; HRESIMS m/z 317.1387 $[M + H]^+$ (calcd 317.1389 for $C_{18}H_{21}O_5$).

Pterolinus D (***4***): amorphous, brown powder; $[\alpha]^{25}_D$ −116.0 (c 0.10, MeOH); UV λ^{MeOH}_{max} nm (log ε) 230 (4.04), 264 (3.86), 278 (3.86); CD $\Delta\varepsilon_{329}$ −93.23, $\Delta\varepsilon_{293}$ +26.64 (MeOH; c = 0.3 mg/mL); IR (neat) ν_{max} 3433, 2922, 1715, 1667, 1609, 1511 cm^{-1}; ^{13}C and ^{1}H NMR data, see Table 3; ESIMS m/z 357 $[M + Na]^+$; HRESIMS m/z 357.0952 $[M + Na]^+$ (calcd 357.0950 for $C_{17}H_{18}O_7Na$).

Pterolinus E (***5***): amorphous, brown powder; $[\alpha]^{25}_D$ −10.4 (c 0.10, MeOH); UV λ^{MeOH}_{max} nm (log ε) 230 (4.16), 273 (4.04), 313 (3.82); CD $\Delta\varepsilon_{384}$ +9.67, $\Delta\varepsilon_{313}$ −7.51 (MeOH; c = 0.3 mg/mL); IR (neat) ν_{max}

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Table 4. ^{13}C NMR Data (δ) of Compounds 8−15 (100 MHz in acetone-d_6)

	8	9	10[a]	11[a]	12[b]	13	14[c]	15	16[b]
1	148.5	123.3	139.4	139.5	82.2	118.2	112.6	152.0	112.4
2	142.0	148.7	185.8	185.9	185.0	117.2	159.8	187.6	112.2
3	115.6	102.1	101.8	101.8	101.3	141.7	101.1	109.1	114.9
4	111.5	147.6[d]	174.8	174.8	198.9	148.5	156.3	160.3	152.5
5	156.5	141.0	69.9	69.9	74.1	101.8	140.3	187.3	100.6
6	119.2	117.1	143.4	143.3	40.2	149.7	118.7	132.5	155.8
7	48.9	47.9	46.8	47.2	51.8	41.7	199.3	48.0	149.7
8	141.5	142.8	140.9	140.9	141.4	33.6		139.8	111.8
9	117.0	115.9	116.2	116.2	117.3	65.4		118.2	161.6
1′	136.7	138.2	135.3	135.5	133.7	139.0	132.1	134.3	129.2
2′	117.0	117.0	116.4	116.5	118.5	113.4	117.3	116.9	120.0
3′	147.6	147.6[d]	146.9	147.2	147.9	149.0	148.0	148.0[d]	148.3
4′	148.0	147.3[d]	147.2	146.9	148.0	146.7	151.8	148.2[d]	149.4
5′	113.0	112.8	112.2	112.2	109.1	116.2	111.1	113.1	112.2
6′	120.9	120.8	120.5	120.3	128.1	122.6	122.0	121.0	116.7
1-OCH_3					52.0				
2-OCH_3					57.9				
4-OCH_3		56.9	56.3	56.4		56.8		57.3	55.9
5-OCH_3							56.0		
3′-OCH_3						56.9			
4′-OCH_3	56.9	57.0	56.4	56.3	56.9		55.8	56.9	56.1
$COOCH_3$	53.4								
$COOCH_3$	171.7								
CH_2			52.3	52.1					
$COCH_3$			31.9	31.9					
$COCH_3$			205.6	205.7					

[a] Recorded by 125 MHz NMR in acetone-d_6. [b] Recorded by 150 MHz NMR in acetone- d_6. [c] Measured in pyridine-d_5. [d] The chemical shifts are interchangeable.

3419, 2924, 1681, 1606, 1509 cm^{-1}; ^{13}C and ^{1}H NMR data, see Table 3; ESIMS m/z 341 $[M + Na]^+$; HRESIMS m/z 341.0998 $[M + Na]^+$ (calcd 341.1001 for $C_{17}H_{18}O_6Na$).

Pterolinus F (**8**): yellow, colorless oil; $[\alpha]^{25}_D$ +20.6 (c 0.11, CH_2Cl_2); UV λ^{MeOH}_{max} nm (log ε) 220 (4.23), 253 (3.88), 285 (3.62), 341 (3.68); CD $\Delta\varepsilon_{363}$ −4.15, $\Delta\varepsilon_{307}$ −12.12, $\Delta\varepsilon_{266}$ +2.51 (EtOH; c = 0.3 mg/mL); ε_{329} −2.57, $\Delta\varepsilon_{266}$ +2.13, $\Delta\varepsilon_{255}$ −2.12 (MeOH; c = 0.5 mg/mL); IR (neat) ν_{max} 3419, 2955, 1677, 1623, 1510 cm^{-1}; ^{13}C and ^{1}H NMR data, see Table 3; ESIMS m/z 331.10 $[M + H]^+$; HRESIMS m/z 331.1185 $[M + H]^+$ (calcd 331.1182 for $C_{18}H_{19}O_6$).

Pterolinus G (**9**): brown gum; UV λ^{MeOH}_{max} nm (log ε) 261 (3.92); IR (neat) ν_{max} 3420, 2928, 1670, 1600, 1514 cm^{-1}; ^{13}C and ^{1}H NMR data, see Table 3; HREIMS m/z 300.1000 $[M]^+$ (calcd 300.0998 for $C_{17}H_{16}O_5$), 302.1151 $[M]^+$ (calcd 302.1151 for $C_{17}H_{18}O_5$).

Pterolinus Ha (**10**): brown gum; $[\alpha]^{25}_D$ +142.12 (c 0.12, CH_2Cl_2); UV λ^{MeOH}_{max} nm (log ε) 232 (4.43), 280 (4.12), 399 (3.37); CD ε_{343} −23.87, $\Delta\varepsilon_{325}$ −19.51, $\Delta\varepsilon_{295}$ +4.33 (EtOH; c = 0.3 mg/mL); IR (neat) ν_{max} 3392, 2922, 1704, 1664, 1609, 1507 cm^{-1}; ^{13}C and ^{1}H NMR data, see Table 3; ESIMS m/z 359.14 $[M + H]^+$; HRESIMS m/z 359.1494 $[M + H]^+$ (calcd 359.1495 for $C_{20}H_{23}O_6$).

Pterolinus Hb (**11**): brown gum; $[\alpha]^{25}_D$ +106.0 (c 0.094, CH_2Cl_2); UV λ^{MeOH}_{max} nm (log ε) 232 (4.66), 282 (4.36), 399 (3.50); CD ε_{349} −20.13, ε_{305} +24.37 (EtOH; c = 0.3 mg/mL); IR (neat) ν_{max} 3418, 2922, 1705, 1606, 1510 cm^{-1}; ^{13}C and ^{1}H NMR data, see Table 3; ESIMS m/z 359.14 $[M + H]^+$; HRESIMS m/z 359.1494 $[M + H]^+$ (calcd 359.1495 for $C_{20}H_{23}O_6$).

Table 5. Inhibitory Effects of Compounds 1−8, 10, 11, and 13−16 on Superoxide Anion Generation and Elastase Release by Human Neutrophils in Response to FMLP/CB

	superoxide anion	elastase release
compound	IC_{50}[a] (μg/mL) or (Inh %)[b]	IC_{50} (μg/mL) or (Inh %)
1	0.33 ± 0.38	2.54 ± 0.57
2	0.19 ± 0.03	(47.83 ± 4.63)[f]
3	2.28 ± 0.79	(9.73 ± 2.95)[d]
4	0.29 ± 0.07	1.06 ± 0.24
5	NT	2.13 ± 0.19
6	0.73 ± 0.20	3.69 ± 0.19
7	0.69 ± 0.19	(33.33 ± 7.33)[e]
8	NT	3.22 ± 1.01
10	(29.44 ± 7.71)[d]	(19.53 ± 7.60)
11	(34.15 ± 7.76)[d]	(2.84 ± 5.74)
13	2.10 ± 0.29	(16.59 ± 6.99)
14	NT	3.62 ± 0.71
15	0.47 ± 0.04	1.44 ± 0.11
16	3.18 ± 0.48	(−1.53 ± 2.90)
LY294002[c]	1.18 ± 0.24	1.99 ± 0.35

[a] Concentration necessary for 50% inhibition (IC_{50}). [b] Percentage of inhibition (Inh %) at 10 μg/mL concentration. Results are presented as mean ± SEM (n = 3−5). [c] LY294002, a phosphatidylinositol-3-kinase inhibitior, was used as a positive control for superoxide anion generation and elastase release. [d] $p < 0.05$ compared with the control value. [e] $p < 0.01$ compared with the control value. [f] $p < 0.001$ compared with the control value. "NT" means not tested.

Pterolinus I (**12**): colorless oil; $[\alpha]^{25}_D$ −30.7 (c 0.10, EtOH); UV λ^{MeOH}_{max} nm (log ε) 250 (3.86); CD ε_{315} −132.37 (EtOH; c = 0.3 mg/mL); IR (neat) ν_{max} 3405, 2925, 1713, 16467 1510 cm^{-1}; ^{13}C and ^{1}H NMR data, see Table 3; ESIMS m/z 333.02 $[M + H]^+$; HRESIMS m/z 333.1339 $[M + H]^+$ (calcd 333.1338 for $C_{18}H_{21}O_6$).

Pterolinus J (**13**): amorphous, white powder; $[\alpha]^{25}_D$ +11.6 (c 0.10, EtOH); UV λ^{MeOH}_{max} nm (log ε) 227 (4.10), 289 (3.83), 300 (3.76); CD ε_{320} −5.75, ε_{309}−6.17, $\Delta\varepsilon_{257}$ −23.11 (EtOH; c = 0.3 mg/mL); IR (neat) ν_{max} 3433, 2921, 1708, 1609, 1507 cm^{-1}; ^{13}C and ^{1}H NMR data, see Table 3; ESIMS m/z 302.98 $[M + H]^+$; HRESIMS m/z 303.1233 $[M + H]^+$ (calcd 303.1232 for $C_{17}H_{19}O_5$).

In Vitro Cytotoxicity Assay. Fractions and isolates were tested against lung (A549), gingival (Ca9-22), breast (MEA-MB-231 and MCF7), and liver (HepG2 and Hep3B) cancer cell lines using established colorimetric MTT assay protocols.[16] Doxorubicin was used as a positive control.

In Vitro Anti-inflammatory Assay. The assay procedure was performed as previously reported.[17−19]

■ ASSOCIATED CONTENT

Supporting Information. ^{1}H, ^{13}C NMR and HRMS data of all new compounds along with the stereochemical comparison of compounds **8**, **10**, **11**, and **15** are available free of charge via the Internet at http://pubs.acs.org.

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