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Phenylphenalenones from the Australian Plant *Haemodorum simplex*

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Chemical investigation of the Australian plant *Haemodorum simplex* resulted in the isolation of three new phenylphenalenones, haemodorone (**10**), haemodorol (**11**), and haemodorose (**12**), together with the previously reported compounds **5**, dilatrin (**6**), and xiphidone (**8**). The first complete 2D NMR characterization for all of the compounds isolated, including several chemical shift reassignments for dilatrin (**6**), is reported. In addition this is one of the few reports to discuss the isolation of new phenylphenalenones from an Australian medicinal plant. The crude extract of both the bulbaceous and aerial components of the plant exhibited varying degrees of antibacterial, antifungal, and antiviral activity, and only the bulbs displayed potent cytotoxic activity.

Haemodorum simplex is a bulbaceous perennial herb, which grows to a height of 0.2−0.65 m, producing black/brown flowers from the months of September to December.[1] The plant is endemic to the southwest region of Western Australia, growing in the region from the north of Geraldton to the south coast, east of Albany.[2] Commonly known as *blood root*, due to its strongly red-colored bulbs, *H. simplex* belongs to the family Haemodoraceae, which is predominantly Australian.[3] The genus *Haemodorum* was first investigated in 1955 with haemocorin (**1**) being the first phenylphenalenone (perinaphthenone) pigment reported from the plant *H. corymbosum* Vahl.[3] Haemocorin (**1**) was found to display antibacterial activity against *Bacillus subtilis* and other bacteria including *Staphylococcus aureus*.[4] Over the last 50 years there have been several reports discussing the isolation of phenylphenalenones, predominately from plants such as *Lachnanthes tinctoria, Wachendorfia paniculata*, and *W. thyrsiflora* Berm., indicating that the phenylphenalenone nucleus is characteristic to the family Haemodoraceae.[5−7] Investigation of *H. distichophyllum* (Hook.), which is endemic to the southwestern region of Tasmania, afforded the dark red pigment haemodorin (**2**).[2] By the early 1970s 20 naturally occurring phenalenones had been reported from both fungi and higher plants.[8] Chemical analyses extended to other plants including the roots of *L. tinctoria* afforded compound **3**,[9] along with several naturally occurring phenolic precursors.[10] Investigation of the phenylphenalenones present in the flowers of *L. tinctoria* resulted in the isolation of **4**, which was converted into compound **5** using ethereal diazomethane.[9,11,12] It was not until the early 1990s that investigation of *Dilatris vicosa* Bergius, endemic to South Africa, resulted in the isolation of the new phenylphenalenone glycoside dilatrin (**6**), which was confirmed by 1D and 2D NMR (COSY and NOESY) experiments.[13] In determining the position of the sugar moiety in **6**, methylation was carried out, which resulted in a single monomethyl ether (**7**), suggesting that the hexose was located at C-1.[13] Hydrolysis of **7** afforded xiphidone (**8**),[6] previously isolated from the plant *Xiphidium caeruleum*.[10,13,14] A minor phenylphenalenone glycoside, lachnanthoside aglycone 1-glucoside (**9**) was also isolated from *D. vicosa*, which was methylated to afford **7**, demonstrating that the new compound was the C-1 glucoside of **9**.[13]

As part of the activities of the Marine and Terrestrial Natural Product (MATNAP) research group at RMIT University we examined a specimen of *H. simplex* from Western Australia. The crude extract of the bulbs and aerial components of the plant showed varying antibacterial, antifungal, and antiviral activities, while only the crude extract of the bulbs of the plant displayed potent cytotoxic activity. On the basis of these biological activities, subsequent chemical investigation was carried out only on the bulbs of the plant. We describe here the isolation and structure determination of three new phenylphenalenones, haemodorone (**10**), haemodorol (**11**), and haemodorose (**12**). We also report the first complete 2D NMR characterization of compound **5**, dilatrin (**6**), and xiphidone (**8**), which subsequently resulted in a number of chemical shift reassignments for dilatrin (**6**).

Results and Discussion

Once the plant was physically separated into its component bulbaceous and aerial components, 2 g of each was extracted with 3:1 CH_3OH/CH_2Cl_2. Following the results obtained from the bioassay of these extracts only the bulbaceous extract was bulk extracted and further fractioned. A larger quantity of the bulbs of the plant (see Experimental Section) was extracted with 3:1 CH_3OH/CH_2Cl_2 and then sequentially trituated (sequentially solvent partitioned) with CH_2Cl_2 followed by CH_3OH. The bulbs were further extracted with 100% CH_3OH, and this extract was combined with the CH_3OH extract generated from the first extraction step. The combined CH_3OH extract was then fractionated by C_{18} vacuum liquid chromatography (VLC), and the 40:60 and 50:50 H_2O/CH_3OH column fractions were subsequently combined. Final purification was achieved by reversed-phase HPLC to yield compounds **6**, **10**, **11**, and **12**. The CH_2Cl_2 extract of the bulbs was fractionated by silica gel VLC using a 20% stepwise solvent elution from 100% hexanes to 100% CH_2Cl_2 to 100% EtOAc and finally to 100% CH_3OH. The 100% hexanes fraction was then subjected to reversed-phase HPLC to yield compounds **5** and **8**.

Compound **5** was first isolated by Cooke in 1955 and subsequently prepared by Bazan in 1976. Only ^{1}H NMR data of **5** have been reported.[11,12] In the current study the structure of **5** was established by 1D and 2D NMR experiments, resulting in the first complete unequivocal assignment of this compound along with the first report of compound **5** occurring as a natural product. As a result of the reisolation of dilatrin (**6**), additional chemical shift assignments are reported for the sugar moiety for this compound by comparison of carbon chemical shifts to the phenylphenalenone nucleus.[10,15−17] The NMR data of **6**, which had been previously reported in either acetone-d_6 or DMSO-d_6 (the latter solvent used for the assignment of the sugar moiety protons), were compared to the literature NMR.[13] It was immediately evident that several of the carbon chemical shifts and their multiplicities were not consistent with reported data of dilatrin (**6**).[10,13] This resulted in the reassignment of a number of chemical shifts of **6**. These reassignments include the previously assigned methine proton/carbon at δ_H 7.14, δ_C 148.63 (C-2), which has now been reassigned

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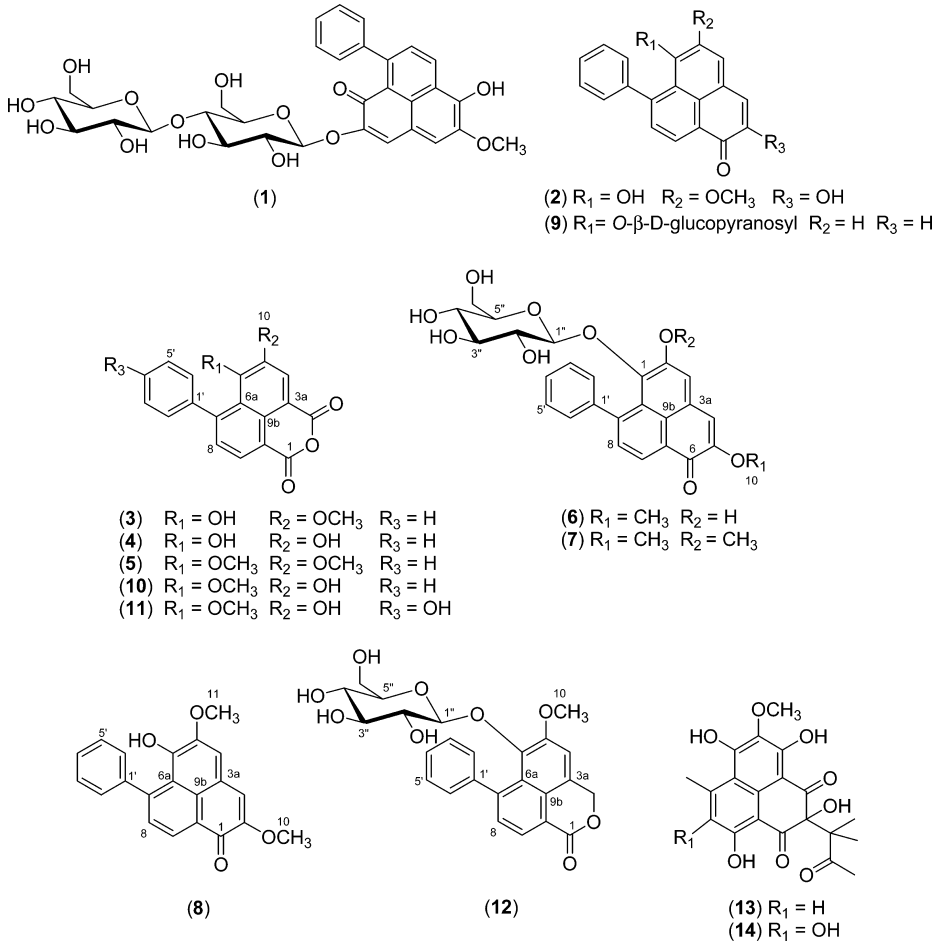
Chart 1



to a quaternary carbon bearing a hydroxy substituent at δ_C 148.1 (C-2). Position C-3 was previously assigned as a quaternary carbon (δ_C 115.27, C-3); however HSQCAD NMR experiments confirmed the $^1H-^{13}C$ connectivity and multiplicity of this carbon to be a methine (δ_H 7.62, δ_C 124.1, C-3). The carbon bearing the pendant sugar moiety was formerly assigned as δ_C 149.15 (C-1), which has now been reassigned as δ_C 140.4 (C-1), on the basis of gHMBC NMR correlations. Other chemical shift reassignments include the following: the methine previously assigned at δ_H 7.57, δ_C 121.68 (C-4) has now been reassigned to δ_H 7.23, δ_C 114.3 (C-4), the carbon at δ_C 141.88 (C-6a) is now reassigned to δ_C 130.7 (C-6a), and positions C-9a and C-9b, which were incorrectly reported as δ_C 126.64 and δ_C 129.7, respectively. These have now been reassigned on the basis of HMBC NMR experiments and comparison to carbon chemical shifts reported for the phenylphenalenone nucleus to be δ_C 127.8 and δ_C 120.7, respectively.[10,15−17] Furthermore, gHSQCAD NMR experiments confirmed direct $^1H-^{13}C$ NMR connectivity for δ_H 2.20, δ_C 73.8 (C-2″) and δ_H 2.72, δ_C 70.4 (C-4″). These chemical shifts were previously either incorrectly assigned or not assigned at all.[13] Xiphidone (**8**) had been previously reported from the roots of *L. tinctoria*[6] as well as from the neotropical plant *Xiphidium caeruleum* Aubl.[18] Once again, only the ^1H NMR of **8** has been reported.[18] Detailed spectroscopic analyses (1D and 2D NMR experiments) confirmed the structure of **8**, resulting in its first complete unequivocal assignment.

Haemodorone (**10**) was isolated as a yellow oil, for which HRESIMS established the molecular formula as $C_{19}H_{12}O_5$ (319.0610 $[M - H]^-$, calcd for $C_{19}H_{11}O_5$, 319.0685), possessing 14 degrees of unsaturation. The IR spectrum supported the presence of hydroxy (3430 cm^{-1}) and carbonyl moieties (1769 and 1725 cm^{-1}). The UV profile extracted for **10** from the HPLC contour plot displayed UV maxima (λ_{max} 218, 259, 327, 344, and 393 nm) that were similar to those previously reported for **3**, thereby suggesting a phenylphenalenone anhydride nucleus.[10] Analysis of the NMR spectra (Table 1) indicated the presence of a methoxy moiety (δ_H 3.25, s, 3H; δ_C 59.9, C-10), three aromatic methines [(δ_H 8.22, s, 1H; δ_C 124.8, C-4), (δ_H 7.53, d, J = 7.5 Hz, 1H; δ_C 130.4, C-8), and (δ_H 8.34, d, J = 7.5 Hz; δ_C 129.2, C-9)] as well as a monosubstituted phenyl moiety [(δ_H 7.44, m 2H; δ_C 127.2), (δ_H 7.37, m, C-3′, C-4′, C-5′; δ_C 128.2)]. HSQCAD NMR experiments supported the presence of one methyl and eight methine carbons (three being equivalent). Ten quaternary carbons were identified on the basis of the HMBC correlations (Table 1). A combination of the COSY and HMBC

Table 1. NMR Data (500 MHz, DMSO-d_6) for Haemodorone (**10**)

position	δ_C[a], mult	δ_H (J in Hz)	gCOSY	gHMBC
1	161.0, qC			
2				
3	160.4, qC			
3a	114.4, qC			
4	124.8, CH	8.22, s		3, 5, 6, 3a, 9b
5	149.0, qC			
6	148.6, qC			
6a	118.3, qC			
7	144.9, qC			
8	130.4, CH	7.53, d, (7.5)	9	6a, 9a, 1′
9	129.2, CH	8.34, d, (7.5)	8	1, 7, 9b
9a	125.5, qC			
9b	127.1, qC			
10	59.9, CH_3	3.25, s		6
1′	142.2, qC			
2′, 6′	127.2, CH	7.44, m		2′, 6′
3′, 4′, 5′	128.2, CH	7.37, m		7, 2′, 6′, 3′, 4′, 5′
5-OH		10.60, bs		5

[a] Carbon assignments based on HSQCAD and HMBC experiments.

Table 2. NMR Data (500 MHz, DMSO-d_6) for Haemodorol (**11**)

position	δ_C[a], mult	δ_H (J in Hz)	gCOSY	gHMBC
1	161.8, qC			
2				
3	161.4, qC			
3a	115.0, qC			
4	125.3, CH	8.20, s		3, 3a, 5, 6, 9b
5	157.0, qC			
6	149.5, qC			
6a	118.5, qC			
7	145.9, qC			
8	131.3, CH	7.51, d, (7.5)	9	1′, 6a, 9, 9a
9	130.0, CH	8.31, d, (7.5)	8	1, 7, 9b
9a	126.3, qC			
9b	127.4, qC			
10	60.8, CH_3	3.26, s		6
1′	133.2, qC			
2′, 6′	130.5, CH	7.20, d, (8.5)	3′,5′	2′, 4′, 6′, 7
4′	157.1, qC			
3′, 5′	114.8, CH	6.82, d, (8.5)	2′,6′	1′, 3′, 4′, 5′
4′-OH		9.60, bs		3′, 4′, 5′
6-OH		10.54, bs		

[a] Carbon assignments based on HSQCAD and HMBC experiments.

NMR experiments as well as comparison to the literature NMR data for the structurally related compound **3** readily confirmed the presence of a phenylphenalenone anhydride.[10] Linking of the phenylphenalenone nucleus to the phenyl ring was achieved through the observation of a HMBC correlation from the aromatic H-8 (δ_H 7.53) to the quaternary C-1′ (δ_C 142.2). The aromatic methine at H-9 (δ_H 8.34), which was coupled to H-8, showed an HMBC correlation to the ester carbon at C-1 (δ_C 161.0). In comparing the NMR data between **10** and the structurally related compound **3**, it was immediately evident that the data were not identical. In particular the upfield nature of the methoxy resonance (δ_H 3.29 in acetone-d_6) in **10**, which is shifted upfield by 0.82 ppm compared to the closely related analogue **3** (δ_H 4.11 in acetone-d_6), is attributable to the shielding effect of the aromatic ring.[10] This allowed the methoxy moiety to be positioned at position 6, adjacent to the aromatic ring.[9−11,13]

Haemodorol (**11**) was also isolated as a yellow oil, for which HRESIMS established the molecular formula as $C_{19}H_{12}O_6$ (335.0558 $[M - H]^-$, calcd for $C_{19}H_{11}O_6$, 335.0556), possessing 14 degrees of unsaturation. As in the case of compound **10**, the IR spectrum supported the presence of hydroxy (3369 cm^{-1}) and carbonyl moieties (1761 and 1729 cm^{-1}), and the UV maxima once again suggested the presence of a phenylphenalenone anhydride nucleus.[10] Analysis of the NMR spectra (Table 2) indicated that the only difference between compounds **10** and **11** was the presence of two *ortho*-substituted aromatic methines [(δ_H 7.20, d, J = 8.5 Hz; δ_C 130.5, C-2′, C-6′) and (δ_H 6.82, d, J = 8.5 Hz; δ_C 114.8, C-3′, C-5′)] in the latter, indicating that the pendant aromatic ring was 1,4-disubstituted in **11**, as supported by HMBC and COSY correlations (Table 2). HSQCAD NMR experiments indicated the presence of one *o*-methyl and seven methine carbons (two of these being equivalent), while 11 quaternary carbons were identified from the HMBC experiment (Table 2). Linking of the 1,4-disubstituted phenyl moiety to the phenylphenalenone anhydride nucleus was possible due to the observation of an HMBC correlation from the methine at H-8 (δ_H 7.51) to the quaternary carbon at C-1′ (δ_C 133.2). Once again the shielded nature of the methoxy moiety in haemodorol (**11**) at δ_H 3.26 supported its placement nearest the aromatic ring at C-6.[9−11,13]

Haemodorose (**12**) was isolated as a yellow oil, for which HRESIMS established the molecular formula as $C_{25}H_{24}O_9$ (469.1496 $[M + H]^+$, calcd for $C_{25}H_{26}O_9$, 469.1493; 491.1313 $[M + Na]^+$, calcd for $C_{25}H_{24}O_9Na$, 491.1313), possessing 14 degrees of unsaturation. The IR spectrum supported the presence of hydroxy (3421 cm^{-1}) and ester carbonyl (1711 cm^{-1}) moieties. The extracted UV

Table 3. NMR Data (500 MHz, DMSO-d_6) for Haemodorose (**12**)

position	δ_C[a], mult	δ_H (J in Hz)	gCOSY	gHMBC
1	164.6, qC			
2				
3	68.9, CH_2	5.83, bs	4	1, 3a, 4, 5, 6, 9b
3a	125.0, qC			
4	112.4, CH	7.59, s	3	3, 3a, 5, 6
5	149.9, qC			
6	139.3, qC			
6a	130.1, qC			
7	144.6, qC			
8	130.2, CH	7.33, d, (7.3)	9	7, 9a, 9b, 1′
9	125.7, CH	8.10, d, (7.3)	8	1, 7, 9a
9a	125.5, qC			
9b	119.1, qC			
10	56.8, CH_3	3.90, s		5
1′	145.4, qC			
2′, 3′, 4′, 5′, 6′	125.9, CH	7.29, m		
1″	101.4, CH	4.78, d, (7.5)	2″	6
2″	72.5, CH	1.84, ddd, (4.5, 7.5, 12.5)	1″, 2″-OH, 3″	1″, 3″
3″	76.0, CH	2.95, dd, (4.0, 8.5)	2″, 4″, 3″-OH	
4″	69.2, CH	2.76, m	3″, 5″	3″, 5″
5″	76.5, CH	2.74, m		
6″$_a$	60.6, CH_2	3.37, m		
6″$_b$	60.6, CH_2	3.20, m	6″-OH	5″
2″-OH		4.38, d, (4.5)	2″	1″, 2″, 3″
3″-OH		4.75, d, (4.5)	3″	3″
4″-OH		ND[b]		
6″-OH		4.04, t, (5.5)	6″$_b$	5″, 6″

[a] Carbon assignments based on HSQCAD and DEPT experiments. [b] ND = signal was not detected.

spectrum of **12** displayed maxima similar to the known phenylphenalenone glycoside dilatrin (**6**).[13] Analysis of the NMR spectra (Table 3) revealed chemical shifts indicative of a methoxy group (δ_H 3.90, s, 3H; δ_C 56.8, C-10), three aromatic methines [(δ_H 7.59, s, 1H; δ_C 112.4, C-4), (δ_H 7.33, d, J = 7.3 Hz, 1H; δ_C 130.2, C-8), and (δ_H 8.10, d, J = 7.3 Hz, 1H; δ_C 125.7, C-9)], as well as a monosubstituted phenyl moiety (δ_H 7.29, m 5H; δ_C 125.9, C-2′, C-3′, C-4′, C-5′, C-6′) and a deshielded oxymethylene (δ_H 5.83, bs, 2H; δ_C 68.9, C-3) together with chemical shifts consistent with the presence of a sugar moiety [(δ_H 4.78, d, J = 7.5 Hz; δ_C 101.4, C-1″), (δ_H 1.84, ddd, J = 4.5, 7.5, 12.5 Hz; δ_C 72.5, C-2″), (δ_H 2.95, dd, J = 4.0, 8.5 Hz; δ_C 76.0, C-3″), (δ_H 2.76, m; δ_C 69.2, C-4″), (δ_H 2.74, m; δ_C 76.5, C-5″), (δ_H 3.37, m and 3.20, m; δ_C 60.6, C-6″a, C-6″b), (δ_{OH} 4.38, d, J = 4.5 Hz; 2″-OH), (δ_{OH} 4.75, d, J = 4.5 Hz; 3″-OH), and (δ_{OH} 4.04, t, J = 5.5 Hz, 6″-OH)]. The doublet at δ 4.78 with a coupling constant of 7.5 Hz indicated the β-configuration of the anomeric proton, and vicinal coupling constants indicated that all protons are axial, supportive of glucose as the sugar residue. Due to the shielding effect of the phenyl ring, the sugar resonances appear at unusually high field since the sugar attached to the phenalenone ring lies within the shielding cone of the phenyl substitutent.[10,13] Similar high-field shifts in glucosides have been observed for such protons in the antibiotic moenomycin A and benzoxazinone-*N*-glucosides.[19−21] DEPT and HSQCAD NMR experiments supported the presence of one methyl, 13 methine (four of these being equivalent), and two oxymethylene carbons, with nine quaternary carbons being identified from the HMBC experiment (Table 3). The UV maxima, together with a comparison of the NMR data reported for the closely related compound dilatrin (**6**), readily established the presence of a phenylphenalenone glycoside skeleton for **12**.[13] Acid hydrolysis of haemodorose (**12**) indicated the presence of a D-β-glucopyranose moiety via TLC comparison to authentic standards in addition to its positive optical rotation.[22] A combination of HMBC and COSY correlations (Table 3) established the presence of a phenyl moiety. Linking of the phenyl group to the phenylphenalenone glycoside nucleus was achieved on the basis of a key HMBC correlation observed from H-8 (δ_H 7.33) to C-1′ (δ_C 145.4). Additional

important correlations include a COSY correlation from the aromatic methine at H-9 (δ_H 8.10) to H-8 and a HMBC correlation from H-9 (δ_H 8.10) to the ester carbon at C-1 (δ_C 164.6). The oxymethylene at H-3 (δ_H 5.83, bs) showed HMBC correlations to a quaternary carbon at C-1 (δ_C 164.6), confirming the presence of a lactone, as well as to the C-4 aromatic methine (δ_C 112.4). The deshielded nature of the methoxy moiety in **12** (δ_H 3.90) allowed for the positioning of the methoxy and sugar substituents.[13] This, together with HMBC correlations, confirmed the presence of the methoxy substituent at C-5, adjacent to the glucosidic linkage with the pendant aromatic moiety attached at C-6 (δ_C 149.9). Acid hydrolysis, optical rotation, NMR, and MS data supported the presence of a D-β-glucopyranose residue. The NMR data for the D-β-glucopyranose moiety was compared directly to the sugar residue that occurs in dilatrin (**6**) and found to be in agreement.[13]

To date, the phenylphenalenone nucleus has been isolated from the family Haemodoraceae, predominately from *Haemodorum*, *Wachendorfia*, *Anigozanthos*, *Dilatris*, *Lachnanthes*, and *Xiphidium* genera, and is regarded to be a characteristic chemotaxonomic marker.[10] The phenylphenalenones have also been reported from the families Strelitziaceae, Pontederiaceae, and Musaceae, for which the genus *Haemodorum* is restricted to the southwest region of Western Australia.[10,15−17] In terms of biological activities, phenylphenalenones have shown to function as phytoalexins (naturally occurring antimicrobials) in the banana plant species *Musa acuminata*.[23] Isolation of these compounds has also been reported from fungal species, where it was noted that they are produced through a polyketide pathway.[8] In plants, the phenylphenalenones have been shown to be biosynthesised from phenylpropanoid units derived from the shikimate pathway.[8] Extracts of the bulbs from the genus *Haemodorum* have been reportedly used by the Aborigines for a number of reasons, including medicinal uses as a purgative, applied externally for snake bites, and also as a dyeing agent for the coloring of strings, ropes, and baskets.[24−26] In a recent study of the secondary metabolites occurring in a marine-derived fungus, two novel phenalenones, sculezonone A (**13**) and sculezonone B (**14**), were reported, along with their inhibitory activity toward DNA polymerase.[27]

Compounds **5**, **6**, **8**, and **10**−**12** displayed moderate cytotoxic activities (IC_{50} of >26−39 μM when tested at 1 mg/mL). It is suggested that the principle bioactive secondary metabolite(s) are yet to be identified; however, any efforts to secure these will require a re-collection of the plant material, which is not foreseen for any time in the near future.

Experimental Section

General Experimental Procedures. For details on the general experimental procedures see Reddy et al., 2008.[28] IR spectra were recorded as KBr discs on a Perkin-Elmer Spectrum One FTIR spectrometer. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectra were acquired in DMSO-d_6 or acetone-d_6 on a 500 MHz Varian INOVA spectrometer with referencing to solvent signals (δ 2.49 and 39.5; and δ 2.05 and 30.5, respectively). 2D NMR experiments recorded included gCOSY, gHSQCAD, and gHMBC. HRESIMS was carried out on an Agilent 6200 Series TOF system (ESI operation conditions of 8 L/min N_2, 350 °C drying gas temperature, and 4000 V capillary voltage) equipped with an Agilent 1200 Series LC solvent delivery module (100% CH_3OH at a flow rate of 0.1 mL/min) in either positive or negative ionization modes. In all cases the instruments were calibrated using the Agilent Tuning Mix using purine as the reference compound and HP0921. Silica gel flash chromatography was carried out with Merck silica gel (60 mesh) using N_2 and a 20% stepwise solvent elution from 100% hexanes to 100% CH_2Cl_2 to 100% EtOAc and finally to 100% CH_3OH. C_{18} VLC was carried out using Alltech bulk high-capacity C_{18} (particle size 35−75 μm) using a 10% stepwise solvent elution from 100% H_2O to 100% CH_3OH and finally to 100% CH_2Cl_2. All analytical HPLC analyses were performed on a Dionex P680 solvent delivery system equipped with a PDA100 UV detector (operated using Chromeleon software). Reversed-phase analytical HPLC analyses were run using either a gradient method (0−2 min 10% CH_3CN/H_2O; 14−24 min 75% CH_3CN/H_2O; 26−30 min 100% CH_3CN; and 32−40 min 10% CH_3CN/H_2O) or an isocratic method (either 60% CH_3CN/H_2O or 65% CH_3CN/H_2O) on a Phenomenex Prodigy ODS (3) C_{18} 100 Å 250 × 4.6 (5 μm) and on a Phenomenex Luna ODS (3) C_{18} 100 Å 250 × 4.6 (5 μm) column at a flow rate of 1.0 mL/min. Normal-phase analytical HPLC analyses were run using a gradient method (0−20 min 65% *n*-hexane/EtOAc; 20.01−30 min 30% *n*-hexane /EtOAc; and 30.01−40 min 100% EtOAc) on a Alltech Alltima HP silica 250x 4.6 (5 μm) column at a flow rate of 1.0 mL/min. All semipreparative HPLC was carried out on a Varian Prostar 210 (solvent delivery module) equipped with a Varian Prostar 335 PDA detector using STAR LC WS version 6.0 software using either a gradient method (0−12 min 30% CH_3CN/H_2O and 13−30 min 100% CH_3CN) or an isocratic method (85% CH_3CN/H_2O) on a Phenomenex Prodigy ODS (3) 100 Å C_{18} 250 × 10 (5 μm) column at a flow rate of 3.5 mL/min.

Acid Hydrolysis of Dilatrin (6) and Haemodorose (12). Approximately 0.5 mg of compounds **6** and **12** were individually treated with 2.5 mL of 15% aqueous HCl. The reaction mixtures were heated to 50 °C and stirred for 6 h. The solutions were neutralized by dropwise addition of 5% aqueous NaOH followed by the addition of EtOAc. The aqueous partitions were individually combined and evaporated under reduced pressure and the residues spotted on aluminum-backed silica gel TLC plates (see Experimental Section) along with a standard sample of D-β-glucopyranose. The TLC plate was developed with *n*-butanol/HOAc/H_2O (3:1:1), air-dried, and then sprayed with 10% aqueous H_2SO_4, followed by heating on a hot plate. The hydrolysis products from compounds **6** and **12** each exhibited a characteristic spot (R_f = 0.24) that was identical with that observed for D-β-glucopyranose. This, together with a positive optical rotation, supported the D-β-glucopyranose moiety.

Biological Evaluation and Details of Assays. The bulbs and aerial parts of the plant were first physically separated. Crude extracts (2 g extracted with 40 mL of 3:1 CH_3OH/CH_2Cl_2) were then generated and evaluated (at 50 mg/mL) in a number of biological assays including a P388 murine leukemia cell line (antitumor assay), against *Herpes simplex* and *Polio* viruses (antiviral assays), and against a number of bacteria and fungi (antimicrobial assays) at the University of Canterbury, Christchurch, New Zealand. Potent cytotoxicity was observed for the crude extracts (IC_{50} < 4875 ng/mL), but only moderate cytotoxicity was observed for the crude extract of the aerial parts (IC_{50} 104 717 ng/mL). In addition both extracts displayed antiviral activity against the DNA *Herpes simplex* and the RNA *Polio* viruses, which showed toxicity to the BSC-1 cells (which are used to grow the viruses), making it impossible to ascertain if there was any virus inhibition. The crude extracts showed antimicrobial activity when tested as an inhibitor of bacterial and fungal pathogens. Moderate antifungal activity was detected for the bulbs and aerial components of the plant against *Trichophyton mentagrophytes*, with inhibition zones (radius of inhibition outside the 6 mm diameter application disc) of 5 and 1 mm, respectively, while antibacterial activity was detected for both the bulbs and aerial parts of the plant against *Bacillus subtilis* with inhibition zones of 3 and 3 mm, respectively. For both crude extracts no activity was observed against *Eschericha coli*, *Pseudomonas aeruginosa*, *Candida albicans*, or *Cladosporium resinae*.

Antitumor Assay (P388 murine leukemia cell line). For the antitumor assay a 2-fold dilution series of the crude extracts as well as compounds **5**, **6**, **8**, and **10**−**12** were incubated for 72 h with P388 cells. The IC_{50} for the antitumor compound adriamycin, also known as doxorubicin, is 31 ng/mL. The concentration of sample required to reduce the P388 cell growth by 50% (compared to control cells) was determined using the absorbance values obtained when the yellow dye MTT tetrazolium is reduced by healthy cells to the purple-colored MTT formazan and is expressed as an IC_{50}, in ng/mL. The P388 standard used was mitomycin C (Sigma M0503) with an IC_{50} of 183 ng/mL.

Antiviral Assays (*Herpes simplex* virus and *Polio* virus). The crude extracts were pipetted onto 6 mm diameter filter paper discs, and the solvent was evaporated. The disc was then placed directly onto BSC-1 cells (African green monkey kidney), infected with either the DNA *Herpes simplex* virus type 1 (ATCC VR-733) or the RNA *Polio* virus type 1 (ATCC VR-192), and then incubated. The assays were examined after 24 h using an inverted microscope for the size of antiviral or viral inhibition and/or cytotoxic zones and the type of cytotoxicity. The antiviral standard used was 30 μL of 0.005 mg/mL didemnum B, which displayed cytotoxicity against both viruses at this concentration.

Antimicrobial Assays. A standardized inoculum was prepared by transferring a loop of bacterial/fungal cells, from a freshly grown stock slant culture, into a 10 mL vial of sterile H_2O. This was vortexed and compared to 5% $BaCl_2$ in H_2O standard to standardize the cell density. This gave a cell density of 10^8 colony-forming units per mL. Then 10 mL of the standardized inoculum was added to 100 mL of Mueller Hinton or potato dextrose agar and mixed by swirling, giving a final cell density of 10^7 colony-forming units per mL. Then 5 mL of this was poured into sterile 85 mm Petri dishes. The suspensions were allowed to cool and solidify on a level surface to give a lawn of bacteria/fungi over the dish. The crude extract was pipetted onto 6 mm diameter filter paper discs, and their solvents were evaporated. These discs were placed onto the seeded agar dishes (with appropriate solvent and positive controls) and incubated. Active antimicrobial samples displayed a zone of inhibition outside the disc, which was measured in millimeters as the radius of inhibition for each bacteria/fungi. The six organisms were *Eschericha coli* (G −ve ATCC 25922), *Bacillus subtilis* (G +ve ATCC 19659), and *Pseudomonas aeruginosa* (G −ve ATCC27853) for bacteria and *Candida albicans* (ATCC 14053), *Trichophyton mentagrophytes* (ATCC 28185), and *Cladosporium resinae* for fungi. The standard reference compounds included 10 μg of gentamycin (Difco 6423890), which showed a 10 mm zone of inhibition against *E. coli* and *P. aeruginosa,* 30 μg of chloroamphenicol (Difco 61338910), which showed a 12 mm zone of inhibition against *Bacillus subtilis*, and 100 IU of nystatin (Difco 6753890), which showed zones of inhibition of 12, 6, and 10 mm against *Candida albicans*, *Trichophyton mentagrophytes*, and *Cladosporium resinae*, respectively.

Plant Material. The plant identified as *Haemodorum simplex* (Haemodoraceae) was collected by Mr. Allan Tinker on January 20, 2005, from the Western Flora Caravan Park, 22 km north of Eneabba (Irwin botanical province) in Western Australia (plant license for scientific or other prescribed purposes SW008335). The plant material was physically separated into its aerial and bulbaceous components, respectively. Voucher specimens designated by the codes 2005-01a (bulbaceous roots) and 2005-01b (aerial parts) are deposited at the School of Applied Sciences (Discipline of Applied Chemistry), RMIT University.

Extraction and Isolation. The bulbaceous part of the plant (22 g) was extracted with 3:1 CH_3OH/CH_2Cl_2 (400 mL) and the crude extract decanted and concentrated under reduced pressure and subsequently triturated (sequentially solvent partitioned) into CH_2Cl_2- and CH_3OH-soluble extracts, respectively. The bulbs were then re-extracted with CH_3OH (100%), and this extract was combined with the previous CH_3OH extract. The combined CH_3OH extract was fractionated using flash C_{18} VLC (10% stepwise elution from H_2O to CH_3OH and finally to CH_2Cl_2). All fractions were analyzed using a reversed-phase HPLC gradient (see Experimental Section), and on this basis, the 40:60 and 50:50 H_2O/CH_3OH column fractions were combined and subjected to semipreparative reversed-phase HPLC to yield dilatrin (**6**) (12.2 mg, 0.15%), haemodorone (**10**) (1.4 mg, 0.02%), haemodorol (**11**) (2.7 mg, 0.03%), and haemodorose (**12**) (13.1 mg, 0.16%). The CH_2Cl_2 extract was fractionated using flash silica gel column chromatography (20% stepwise elution from hexanes to 100% CH_2Cl_2 to 100% EtOAc and finally to 100% CH_3OH). The 100% hexanes silica gel column fraction was subjected to reversed-phase HPLC (85% CH_3CN/H_2O) to yield compound **5** (3.3 mg, 0.04%) and xiphidone (**8**) (2.0 mg, 0.03%).

5,6-Dimethoxy-7-phenylbenzo[*de*]isochromene-1,3-dione (5): yellow oil; UV (CH_3OH) λ (log ϵ) 210 (3.69), 245 (3.60), 315 (3.04), and 346 (2.92) nm; UV profile from HPLC (CH_3CN/H_2O) λ_{max} 216, 259, 330, 346, and 395 nm; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.39 (1H, d, J = 7.8 Hz, H-9), 8.38 (1H, s, H-4), 7.55 (1H, d, J = 7.8 Hz, H-8), 7.43 (2H, m, H-2′, H-6′), 7.35 (2H, m, H-3′, H-5′), 7.34 (1H, m, H-4′), 4.02 (3H, s, H-10), 3.28 (3H, s, H-11); ^{13}C NMR (125 MHz, d_6-DMSO) δ 160.9 (C, C-1), 160.4 (C, C-3), 150.5 (C, C-5), 150.7 (C, C-6), 145.4 (C, C-7), 142.2 (C, C-1′), 130.6 (CH, C-8), 130.3 (CH, C-9), 128.1 (CH, C-3′, C-5′), 127.3 (CH, C-2′, C-6′), 127.2 (C, C-9b), 127.1 (CH, C-4′), 125.1 (C, C-6a), 120.5 (CH, C-4), 118.3 (C, C-9a), 114.4 (C, C-3a), 60.5 (CH_3, C-11), 57.0 (CH_3, C-10); ESIMS (positive mode) m/z 335 $[M + H]^+$.

5-Hydroxy-2-methoxy-7-phenyl-6-(3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yloxy)-1*H*-phenalen-1-one (dilatrin) (6): red oil; UV (CH_3OH) λ (log ε) 214 (3.99), 280 (3.65), 375 (3.31), and 465 (3.08) nm; UV profile from HPLC (CH_3CN/H_2O) λ_{max} 214, 279, 360, 375, and 474 nm; IR (KBr) ν_{max} 3427, 1924, 2851, 1719, 1623, 1571, 1384, 1214, 1076, 1032 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.33 (1H, d, J = 7.5 Hz, H-7), 7.62 (1H, s, H-3), 7.48 (1H, d, J = 7.5 Hz, H-8), 7.30 (5H, m, H-2′, H-3′, H-4′, H-5′, H-6′), 7.23 (1H, s, H-4), 4.95 (1H, bs, 3″-OH), 4.84 (1H, d, J = 5.5 Hz, 4″-OH), 4.64 (1H, t, J = 9.0 Hz, H-1″), 4.05 (1H, bs, 2″-OH), 3.85 (3H, s, H-10), 3.46 (1H, m, H-6″a), 3.24 (1H, m, H-6″b), 3.17 (1H, d, J = 4.0 Hz, 6″-OH), 3.00 (1H, dd, J = 9.0, 9.0 Hz, H-3″), 2.80 (1H, m, H-5″), 2.72 (1H, dd, J = 5.0, 9.0 Hz, H-4″), 2.20 (1H, t, J = 9.0 Hz, H-2″); ^{1}H NMR (500 MHz, acetone-d_6) δ 8.42 (1H, d, J = 7.8 Hz, H-7), 7.58 (1H, s, H-3), 7.61 (1H, d, J = 7.8 Hz, H-8), 7.34 (5H, m, H-2′, H-3′, H-4′, H-5′, H-6′), 7.16 (1H, s, H-4), 4.50 (1H, d, J = 8.0 Hz, H-1″), 3.90 (3H, s, H-10), 3.63 (1H, m, H-6″a), 3.52 (1H, m, H-6″b), 3.33 (1H, dd, J = 8.5, 8.5 Hz, H-3″), 3.04 (1H, m, H-5″), 3.05 (1H, m, H-4″), 2.88 (1H, m, H-2″); ^{13}C NMR (125 MHz, DMSO-d_6) δ 179.2 (C, C-6), 152.5 (C, C-5), 148.1 (C, C-2), 146.7 (C, C-9), 144.4 (C, C-1′), 140.4 (C, C-1), 131.7 (CH, C-8), 130.7 (C, C-6a), 127.8 (C, C-9a), 127.2 (CH, C-7), 126.9 (CH, C-2′, C-3′, C-4′, C-5′, C-6′), 126.2 (C, C-3a), 124.1 (CH, C-3), 120.7 (C, C-9b), 114.3 (CH, C-4), 103.7 (CH, C-1″), 77.5 (CH, C-5″), 76.7 (CH, C-3″), 73.8 (CH, C-2″), 70.4 (CH, C-4″), 61.9 (CH_2, C-6″), 56.3 (CH_3, C-10); ESIMS (positive mode) m/z 481 $[M + H]^+$.

6-Hydroxy-2,5-dimethoxy-7-phenyl-1*H*-phenalen-1-one (xiphidone) (8): red oil; UV (CH_3OH) λ (log ε) 273 (3.41), 358 (3.00), and 458 (2.82) nm; UV profile from HPLC (CH_3CN/H_2O) λ_{max} 217, 251, 273, 341, 355, 373, and 462 nm; IR (KBr) ν_{max} 3422, 2924, 2854, 1730, 1630, 1594, 1385, 1359, 1211 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.10 (1H, bs, 6-OH), 8.38 (1H, d, J = 8.5 Hz, H-9), 7.56 (1H, s, H-4), 7.50 (1H, d, J = 8.5 Hz, H-8), 7.38 (2H, m, H-2′, H-6′), 7.28 (3H, d, J = 7.0 Hz, H-3′, H-4′, H-5′), 7.12 (1H, s, H-3), 3.96 (3H, s, H-11), 3.77 (3H, s, H-10); ^{13}C NMR (125 MHz, DMSO-d_6) δ 179.8 (C, C-1), 152.3 (C, C-2), 146.6 (C, C-6), 144.1 (C, C-7), 142.9 (C, C-5); 141.2 (C, C-1′), 131.2 (CH, C-8), 128.1 (CH, C-2′, C-6′), 128.0 (CH, C-3′, C-4′, C-5′), 127.6 (C, C-6a), 127.2 (CH, C-9), 126.6 (C, C-3a), 124.8 (CH, C-4), 122.9 (C, C-9b), 119.8 (C, C-9a), 111.8 (CH, C-3), 61.2 (CH_3, C-11), 55.3 (CH_3, C-10); ESIMS (positive mode) m/z 333 $[M + H]^+$; ESIMS (negative mode) m/z 331 $[M - H]^-$.

6-Hydroxy-5-methoxy-7-phenylbenzo[*de*]isochromene-1,3-dione (haemodorone) (10): yellow oil; UV (CH_3OH) λ (log ε) 205 (4.31), 255 (4.04), 345 (3.54), and 395 (3.57) nm; UV profile from HPLC (CH_3CN/H_2O) λ_{max} 218, 259, 327, 344, and 393 nm; IR (KBr) ν_{max} 3430, 2919, 1769, 1725, 1592, 1578, 1398, 1254, 1018, 1010 cm^{-1}; ^{1}H (500 MHz, DMSO-d_6) and ^{13}C (125 MHz, DMSO-d_6) NMR see Table 1; ^{1}H NMR (500 MHz, acetone-d_6) δ 8.43 (1H, d, J = 7.5 Hz, H-9), 8.33 (1H, s, H-4), 7.62 (1H, d, J = 7.5 Hz, H-8), 7.46 (5H, m, H-2′, H-3′, H-4′, H-5′, H-6′), 3.29 (3H, s, H-10);[10] ESIMS (negative mode) m/z 319 $[M - H]^-$; HRESIMS m/z 319.0610 $[M - H]^-$ (calcd for $C_{19}H_{11}O_5$, 319.0685).

6-Hydroxy-7-(4-hydroxyphenyl)-5-methoxybenzo[*de*]isochromene-1,3-dione (haemodorol) (11): yellow oil; UV (CH_3OH) λ_{max} (log ϵ) 250 (4.21), 316 (3.75), 350 (3.69) 403 (3.51) nm; UV profile from HPLC (CH_3CN/H_2O) λ_{max} 215, 259, 348, and 404 nm; IR (KBr) ν_{max} 3369, 2924, 1761, 1729, 1591, 1575, 1516, 1399, 1253, 1021 cm^{-1}; ^{1}H (500 MHz, DMSO-d_6) and ^{13}C (125 MHz, DMSO-d_6) NMR see Table 2; ESIMS (negative mode) m/z 335.1 $[M - H]^-$; HRESIMS m/z 335.0558 $[M - H]^-$ (calcd for $C_{19}H_{11}O_6$, 335.0556).

5-Methoxy-7-phenyl-6-(3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yloxy)benzo[*de*]isochromen-1(3*H*)-one (haemodorose) (12): yellow oil; $[\alpha]^{25}_D$ +13.1 (c 0.1, CH_3OH); UV (CH_3OH) λ_{max} (log ϵ) 258 (3.45), 333 (2.96), 362 (2.97), 485 (2.49) and 527 (2.19) nm; UV profile from HPLC (CH_3CN/H_2O) λ_{max} 316, 361, 323, 336, and 369 nm; IR (KBr) ν_{max} 3421, 2924, 2854, 1711, 1615, 1417, 1240, 1198, 1079 cm^{-1}; ^{1}H (500 MHz, DMSO-d_6) and ^{13}C (125 MHz, DMSO-d_6) NMR see Table 3; ESIMS (positive mode) m/z 491.4 $[M + Na]^+$; HRESIMS m/z 469.1496 $[M + H]^+$ (calcd for $C_{25}H_{25}O_9$, 469.1493) and 491.1313 $[M + Na]^+$ (calcd for $C_{25}H_{24}O_9Na$, 491.1313).

Supporting Information Available: ^{1}H, ^{13}C, COSY, HSQCAD, and HMBC NMR spectra provided for compound **10**, ^{1}H, COSY, HSQCAD, and HMBC NMR spectra provided for compound **11**, and ^{1}H, DEPT, COSY, HSQCAD, and HMBC NMR spectra provided for compound **12**. This material is available free of charge via the Internet at http://pubs.acs.org.

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References and Notes

(1) Paczkowska, G. Department of Environment and Conservation, Western Australian Herbarium. http://florabase.calm.wa.gov.au (July 24, 2008).
(2) Bick, I. R.; Blackman, A. J. *Aust. J. Chem.* **1973**, *26*, 1377–1380.
(3) Cooke, R. G.; Segal, W. *Aust. J. Chem.* **1955**, *8*, 107–113.
(4) Narasimhachari, N.; Joshi, V. B.; Krishnan, S.; Panse, M. V.; Wamburkar, M. N. *Curr. Sci.* **1968**, *37*, 288–289.
(5) Edwards, J. M. *Tetrahedron Lett.* **1972**, *17*, 1631–1634.
(6) Edwards, J. M.; Weiss, U. *Phytochemistry* **1974**, *13*, 1597–1602.
(7) Edwards, J. M. *Phytochem. Rep.* **1974**, *13*, 290–291.
(8) Thomas, R. *Pure Appl. Chem.* **1973**, *34*, 515–528.
(9) Cooke, R. G. *Phytochemistry* **1970**, *9*, 1103–1106.
(10) Opitz, S.; Holscher, D.; Oldham, N. J.; Bartram, S.; Schneider, B. *J. Nat. Prod.* **2002**, *65*, 1122–1130.
(11) Bazan, A. C.; Edwards, J. M. *Phytochemistry* **1976**, *15*, 1413–1415.
(12) Cooke, R. G.; Segal, W. *Aust. J. Chem.* **1955**, *8*, 413–421.
(13) Dora, G.; Xie, X.-Q.; Edwards, J. M. *J. Nat. Prod.* **1993**, *56*, 2029–2033.
(14) Cooke, R. G.; Dagley, I. J. *Aust. J. Chem.* **1979**, *32*, 1841–1847.
(15) Holscher, D.; Schneider, B. *J. Nat. Prod.* **2000**, *63*, 1027–1028.
(16) Otálvaro, F.; Nanclares, J.; Vásquez, L. E.; Quinones, W.; Echeverri, F.; Arango, R.; Schneider, B. *J. Nat. Prod.* **2007**, *70*, 887–890.
(17) Hölscher, D.; Reichert, M.; Görls, H.; Ohlenschlager, O.; Bringmann, G.; Schneider, B. *J. Nat. Prod.* **2006**, *69*, 1614–1617.
(18) Cremona, T. L.; Edwards, J. M. *Lloydia* **1974**, *37*, 112–113.
(19) Pauli, G. F. *J. Nat. Prod.* **2000**, *63*, 834–838.
(20) Henning, L.; Findeisen, M.; Welzel, P.; Haessner, R. *Magn. Reson. Chem.* **1998**, *36*, 615–620.
(21) Sticker, D.; Schneider, B.; Henning, L.; Knop, M.; Schulz, M. *Phtochemistry* **2001**, *58*, 812–825.
(22) Cichewicz, R. H.; Lim, K.; McKerrow, J. H.; Nair, M. G. *Tetrahedron* **2002**, *58*, 8597–8606.
(23) Borges, A. A.; Fernandez-Falcon, M. *J. Agric. Food Chem.* **2003**, *51*, 5326–5328.
(24) Li, R. W.; Myers, S. P.; Leach, D. N.; Lin, G. D.; Leach, G. *J. Ethnopharmacol.* **2003**, *85*, 25–32.
(25) Webb, L. J. *Mankind* **1969**, *7*, 137–146.
(26) Reid, E. J.; Betts, T. J. *Planta Med.* **1979**, *36*, 164–173.
(27) Perpelescu, M.; Kobayashi, J.; Furuta, M.; Ito, Y.; Izuta, S.; Takemura, M.; Suzuki, M.; Yoshida, S. *Biochemistry* **2002**, *41*, 7610–7616.
(28) Reddy, P.; Urban, S. *J. Nat. Prod.* **2008**, *71*, 1441–1446.

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