Bioactive Compounds from *Bauhinia purpurea* Possessing Antimalarial, Antimycobacterial, Antifungal, Anti-inflammatory, and Cytotoxic Activities

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Eleven new secondary metabolites (**1**−**11**), together with two known flavanones (**12** and **13**) and five known bibenzyls (**14**−**18**), were isolated from the root extract of *Bauhinia purpurea*. New compounds include eight dihydrodibenzoxepins (**1**−**8**), a dihydrobenzofuran (**9**), a novel spirochromane-2,1′-hexenedione (**10**), and a new bibenzyl (**11**). Antimycobacterial, antimalarial, antifungal, cytotoxic, and anti-inflammatory activities of the isolated compounds are reported, and biosynthetic pathways of these compounds are also discussed.

Bauhinia purpurea L. (family Leguminosae) is a shrubby tree, commonly called "Chong Kho" or "Siao Dok Daeng" (in Thai). This plant has been reported to contain cytotoxic oxepins,[1] flavone glycosides,[2] flavanones,[3] and lectins.[4] *B. purpurea* lectins are of great interest and have been practically employed as potential biological tools for binding with cellular proteins and carbohydrates.[5−7] Previously, the genus *Bauhinia* has been found to be a rich source of bioactive metabolites,[8] and we had isolated bioactive bibenzyls[8a,c] and dibenzo[*b*,*f*]oxepins[8b] from *Bauhinia* species. Our continuing search for bioactive compounds from this plant genus led to the isolation of 11 new secondary metabolites (**1**−**11**), together with two known flavanones (**12** and **13**) and five known bibenzyls (**14**−**18**), from the roots of *B. purpurea*. These new compounds include eight dihydrodibenzoxepins (**1**−**8**), a dihydrobenzofuran (**9**), a novel spirochromane-2,1′-hexenedione (**10**), and a new bibenzyl (**11**). Antimycobacterial, antimalarial, antifungal, cytotoxic, and anti-inflammatory activities of the isolated compounds are also reported here.

Results and Discussion

A crude CH_2Cl_2 extract of *B. purpurea* was purified by Sephadex LH-20 and silica gel column chromatography to yield 11 new compounds (**1**−**11**), two known flavanones (**12** and **13**), and five known bibenzyls (**14**−**18**). Spectroscopic data of the known flavanones **12** and **13** and bibenzyls **14**−**18** were identical to those published.[9,10]

The ESITOFMS established the molecular formula of bauhinoxepin C (**1**) as $C_{16}H_{16}O_4$. The ^{1}H NMR data (acetone-d_6) of bauhinoxepin C (**1**) showed two multiplets of deshielded methylene protons (H-5 and H-6), two methyl singlets (2-Me and 3-OMe), and several aromatic protons resonating at 6.28−6.99. Analyses of ^{13}C NMR, DEPT, and HMQC spectra revealed the presence of four methine, two methylene, two methyl, and eight quaternary carbons. The ^{1}H and COSY spectra suggested the presence of an aromatic ABC system. The HMBC spectrum permitted the assembly of a gross structure of dihydrodibenzoxepin, showing correlations from H-4 to C-2, C-3, and C-4a; H-5 to C-4, C-4a, and C-6a; H-6 to C-7, C-6a, and C-10a; H-8 to C-6a and C-7; H-10 to C-6a and C-10a; and 2-Me to C-1, C-2, and C-3. The 3-OMe group was located at C-3, as revealed by the NOESY correlation between 3-OMe and H-4. On the basis of the spectroscopic data, bauhinoxepin C (**1**) was identified as 5,6-dihydro-1,7-dihydroxy-3-methoxy-2-methyldibenz[*b*,*f*]oxepin. Proton and carbon resonances in **1** were assigned by analysis of 2D NMR spectra (Table 1).

Bauhinoxepin D (**2**) possessed the same molecular formula ($C_{16}H_{16}O_4$) as that of bauhinoxepin C (**1**) and also showed similar ^{1}H and ^{13}C NMR resonances (acetone-d_6) to those of **1**, suggesting that **2** was an isomer of **1**. Analysis of the HMBC and NOESY spectra revealed that the position of the methoxy group in **2** was located at C-1; a NOESY correlation between the 1-OMe and 2-Me protons was observed, but none between the methoxy protons and H-4. On the basis of these data, bauhinoxepin D (**2**) was a regioisomer of **1** and identified as 5,6-dihydro-3,7-dihydroxy-1-methoxy-2-methyldibenz[*b*,*f*]oxepin. Assignment of proton and carbon resonances in the NMR spectra of bauhinoxepin D (**2**) is shown in Table 1.

The ESITOFMS revealed the molecular formula of bauhinoxepin E (**3**) as $C_{17}H_{18}O_5$. The ^{1}H and ^{13}C NMR spectra (acetone-d_6) of bauhinoxepin E (**3**) were similar to those of **1** and **2**. The H-4 methine resonance in **3** was missing in the ^{1}H NMR spectrum; however an additional *O*-methyl singlet was observed. The HMBC correlation from 4-OH to C-4 and C-4a readily placed this hydroxy group at C-4. In addition, the NOESY correlations between 1-OMe and 3-OMe with 2-Me indicated that both methoxy groups were proximal to 2-Me. Analysis of 2D NMR data led to the assignment of proton and carbon resonances of **3** (Table 1). Bauhinoxepin E (**3**) was thus identified as 5,6-dihydro-4,7-dihydroxy-1,3-methoxy-2-methyldibenz[*b*,*f*]oxepin.

Bauhinoxepin F (**4**) had the same molecular formula, $C_{17}H_{18}O_5$, as that of bauhinoxepin E (**3**), as revealed by the ESITOFMS. The ^{1}H NMR spectrum (acetone-d_6) of **4** indicated the presence of an aromatic ABC system and two methoxy groups, similar to that of **3**. These spectroscopic data suggested that **4** was an isomer of **3**. However, NMR data acquired in acetone-d_6 exhibited several overlapping resonances, which complicated assignment of proton resonances, particularly those of the methoxy and aromatic protons. This problem was resolved by using $CDCl_3$ as an NMR solvent, which showed better resolution, enabling the structural assignment of **4**. The HMBC correlations ($CDCl_3$) from H-5 to C-4, C-4a, and C-11a; 1-OMe to C-1; 2-Me to C-1, C-2, and C-3; and 4-OMe to C-4 readily assigned the methoxy positions in **4**. Assignment of proton and carbon resonances in **4** was accomplished by analyses of 2D NMR data (Table 2). On the basis of these spectroscopic data, bauhinoxepin F (**4**) was identified as 5,6-dihydro-3,7-dihydroxy-1,4-methoxy-2-methyldibenz[*b*,*f*]oxepin.

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Chart 1

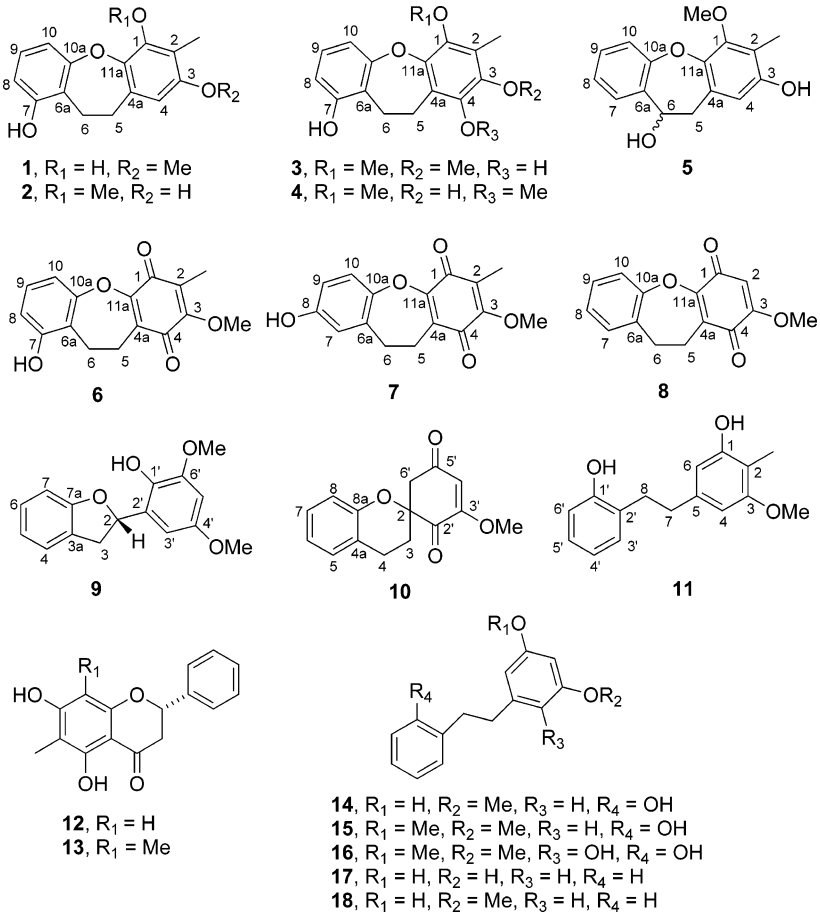

Table 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Spectroscopic Data (acetone-d_6) of Bauhinoxepins C−E (**1**−**3**)

	bauhinoxepin C (**1**)		bauhinoxepin D (**2**)		bauhinoxepin E (**3**)	
C	δ_C, mult.[a]	δ_H (mult., *J* in Hz)	δ_C, mult.[a]	δ_H (mult., *J* in Hz)	δ_C, mult.[a]	δ_H (mult., *J* in Hz)
1	146.7, s		150.7, s		142.7,[d] s	
2	110.4, s		115.9, s		121.9, s	
3	154.4, s		151.9, s		142.0,[d] s	
4	101.6, d	6.28 (s)	109.5,[b] d	6.50 (s)	143.1, s	
4a	130.2, s		133.4, s		119.6,[c] s	
5	24.4, t	3.06 (m)	28.9, t	3.03 (t, 6.2)	22.1, t	3.12 (t, 6.3)
6	29.5, t	3.04 (m)	25.7, t	2.93 (t, 6.2)	23.9, t	2.95 (t, 6.3)
6a	118.8, s		117.8, s		119.0,[c] s	
7	156.1, s		156.4, s		156.1, s	
8	110.6, d	6.65 (dd, 8.1, 1.5)	109.9,[b] d	6.58 (br d, 8.1)	110.4, d	6.61 (dd, 8.1, 0.9)
9	126.5, d	6.96 (t, 8.1)	126.4, d	6.94 (t, 8.1)	126.5, d	6.95 (t, 8.1)
10	112.3, d	6.99 (dd, 8.1, 1.5)	112.6, d	6.75 (br d, 8.1)	112.6, d	6.74 (br d, 8.1)
10a	158.4, s		158.0, s		158.3, s	
11a	139.2, s		144.2, s		147.8, s	
1-OMe			60.6, q	3.86 (s)	60.7, q	3.81 (s)
2-Me	7.9, q	2.05 (s)	8.2, q	2.06 (s)	8.6, q	2.15 (s)
3-OMe	55.1, q	3.74 (s)			59.9, q	3.65 (s)
4-OH						7.60 (br s)
7-OH						8.43 (br s)

[a] Multiplicity was determined by DEPT spectra. [b,c,d] May be interchanged in the same column.

The molecular formula, $C_{16}H_{16}O_4$, for bauhinoxepin G (**5**) was deduced from the ESITOFMS. The ^{1}H and ^{13}C NMR spectra (acetone-d_6) of **5** (Table 2) shared similarities with those of **1** and **2**. The ^{1}H NMR and COSY spectra of **5** indicated the presence of a 1,2-disubstituted aromatic ring and the replacement of one of the methylenes with an oxygenated sp^3 methine. The HMBC correlations from H-7 to C-6; H-4 to C-5; and H-5 to C-4, C-4a, and C-11a readily placed the oxygenated sp^3 methine at C-6. Other HMBC correlations also secured the structure of **5** (H-4 to C-3, C-11a, and C-5; 1-OMe to C-1; both 2-Me and 3-OH to C-2 and C-3; H-8 to C6a; and H-10 to C-6a and C-10a). The NOESY correlation between the 1-OMe protons with 2-Me, but none between 1-OMe and H-4, confirmed the position of the methoxy group at C-1. Bauhinoxepin G (**5**) was therefore identified as 5,6-dihydro-3,6-dihydroxy-1-methoxy-2-methyldibenz[*b*,*f*]oxepin. Bauhinoxepin G (**5**) is an optically active molecule, $[\alpha]^{27}_D$ −29.5 (*c* 0.09, CH_3OH); however, the absolute configuration at C-6 could not be addressed with available spectroscopic data. The amount of the isolated material also limited further studies on the C-6 stereochemistry.

Bauhinoxepin H (**6**) possessed the molecular formula $C_{16}H_{14}O_5$, as deduced by the ESITOFMS. The IR absorption bands at 1651

Table 2. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Spectroscopic Data of Bauhinoxepin F (**4**) (in $CDCl_3$ or acetone-d_6) and Bauhinoxepins G (**5**) (in acetone-d_6)

	bauhinoxepin F (**4**)				bauhinoxepin G (**5**)	
C	δ_C, mult.[a] ($CDCl_3$)	δ_C, mult.[a] (acetone-d_6)	δ_H (mult., *J* in Hz) ($CDCl_3$)	δ_H (mult., *J* in Hz) (acetone-d_6)	δ_C, mult.[a]	δ_H (mult., *J* in Hz)
1	148.3, s	148.2, s			152.0, s	
2	116.1, s	116.1, s			116.4, s	
3	142.1, s	142.9, s			150.5, s	
4	143.3, s	142.6, s			111.1, d	6.51 (s)
4a	123.7, s	124.4, s			128.4, s	
5	23.6, t	28.8, t	3.15 (m)	3.10 (m)	39.1, t	3.12 (dd, 13.5, 9.2); 3.20 (dd, 13.5, 3.9)
6	23.0, t	22.7, t	3.10 (m)	3.05 (m)	68.1, t	4.96 (m)
6a	119.4, s	119.1, s			134.0, s	
7	157.9, s	158.3, s			130.8, d	7.52 (d, 7.3)
8	111.4, d	110.8, d	6.59 (dd, 8.0, 0.9)	6.65 (dd, 8.1, 1.0)	123.4, d	7.10 (m)
9	127.0, d	126.6, d	7.04 (t, 8.0)	6.95 (t, 8.1)	128.1, d	7.21 (m)
10	113.1, d	112.4, d	6.76 (dd, 8.0, 0.9)	6.95 (t, 8.1)	120.8, d	7.21 (br d, 7.3)
10a	154.4, s	156.0, s			155.9, s	
11a	140.1, s	140.7, s			144.0, s	
1-OMe	60.5, q	59.6, q	3.80 (s)	3.73 (s)	60.6, q	3.89 (s)
2-Me	8.8, q	8.3, q	2.19 (s)	2.11 (s)	8.2, q	2.08 (s)
3-OH				7.60 (br s)		8.13 (br s)
3-OMe						
4-OMe	61.1, q	60.3, q	3.79 (s)	3.73 (s)		
6-OH						4.50 (d, 6.8)
7-OH				8.56 (br s)		

[a] Multiplicity was determined by DEPT spectra.

Table 3. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Spectroscopic Data (acetone-d_6) of Bauhinoxepins H−J (**6**−**8**)

	bauhinoxepin H (**6**)		bauhinoxepin I (**7**)		bauhinoxepin J (**8**)	
C	δ_C, mult.[a]	δ_H (mult., *J* in Hz)	δ_C, mult.[a]	δ_H (mult., *J* in Hz)	δ_C, mult.[a]	δ_H (mult., *J* in Hz)
1	182.3, s		182.4, s		181.0, s	
2	125.8, s		125.8, s		105.1, s	5.98 (s)
3	155.6, s		155.6, s		159.0, s	
4	183.6, s		183.6, s		182.1, s	
4a	124.8, s		123.2, s		123.6, s	
5	25.1, t	2.64 (t, 6.1)	25.9, t	2.71 (t, 6.0)	25.9, t	2.75 (t, 5.9)
6	20.3, t	3.09 (t, 6.1)	29.6, t	2.98 (t, 6.0)	29.4, t	3.09 (t, 5.9)
6a	120.8, s		134.6, s		133.5, s	
7	154.9, s		115.3, d	6.72 (d, 2.9)	129.6, d	7.28 (br d, 7.4)
8	112.0, d	6.73 (br d, 8.1)	154.8, s		125.6, d	7.16 (dd, 7.7, 7.4)
9	127.1, d	6.99 (t, 8.1)	113.7, d	6.68 (dd, 8.6, 2.9)	127.7, d	7.26 (dd, 7.7, 7.4)
10	111.7, d	6.63 (br d, 8.1)	121.2, d	6.97 (d, 8.6)	120.5, d	7.15 (d, 7.7)
10a	157.8, s		149.0, s		155.9, s	
11a	152.3, s		152.6, s		152.3, s	
1-OMe						
2-Me	7.8, q	1.87 (s)	7.7, q	1.89 (s)		
3-OMe	60.3, q	3.95 (s)	60.3, q	3.96 (s)	56.0, q	3.84 (s)
7-OH		8.74 (br s)				
8-OH				8.48 (br s)		

[a] Multiplicity was determined by DEPT spectra. [b,c,d] May be interchanged in the same column.

and 1613 cm^{-1}, together with the ^{13}C NMR resonances at δ_C 182.3 and 183.6, indicated the presence of ketone carbonyls in **6**. The presence of H-5 (δ_H 2.64) and H-6 (δ_H 3.09) methylenes suggested that bauhinoxepin H (**6**) was also a dihydrodibenz[*b*,*f*]-oxepin. The ^{1}H NMR spectrum of **6** showed the same ABC aromatic system as those for **1** and **2**, implying that one of the aromatic rings in the dihydrodibenzoxepin **6** was the same as that in **1** and **2**. Analysis of 2D NMR data indicated that another aromatic ring in **6** was in an oxidized 1,4-quinone form. The HMBC correlations from 2-Me to C-1, C-2, and C-3; 3-OMe to C-3; and H-5 to C-4, C-4a, and C-11a unambiguously assigned the C-1 and C-4 carbonyls (Table 2). Other key HMBC correlations were from H-5 to C-6a; H-6 to C-4a, C-6a, C-7, and C-10a; H-8 to C-6a; and H-10 to C-6a and C-10a. On the basis of these spectroscopic evidence, bauhinoxepin H (**6**) was identified as 5,6-dihydro-7-hydroxy-3-methoxy-1,4-dione-2-methyldibenz[*b*,*f*]-oxepin (Table 3).

The molecular formula of $C_{16}H_{14}O_5$ was established for bauhinoxepin I (**7**) by ESITOFMS. In general, the ^{1}H NMR spectrum of **7** was similar to that of bauhinoxepin H (**6**), except for the replacement of the ABC aromatic spin system in **6** with an ABX aromatic system in **7**. The HMBC correlations were from 2-Me to C-1, C-2, and C-3; 3-OMe to C-3 and C-4; H-5 to C-4, C-4a, C-6a, and C-11a; H-6 to C-4a, C-6a, C-7, and C-10a; H-7 to C-6, C-6a, and C-10a; and H-10 to C-6a, C-8, and C-10a. On the basis of these data, the structure of bauhinoxepin I (**7**) was identified as 5,6-dihydro-8-hydroxy-3-methoxy-1,4-dione-2-methyldibenz[*b*,*f*]-oxepin (Table 3).

Bauhinoxepin J (**8**) possessed a molecular formula of $C_{15}H_{12}O_4$, as deduced from ESITOFMS. The ^{1}H NMR and COSY spectra (acetone-d_6) showed the presence of a 1,2-substituted aromatic ring in **8** like that found in **5**. Unlike bauhinoxepins C−I (**1**−**7**), the ^{1}H and ^{13}C NMR spectra indicated the absence of the 2-Me resonances in bauhinoxepin J (**8**). An H-2 methine resonance (δ_H 5.98 (s); δ_C 105.1) was observed in the ^{1}H and ^{13}C NMR spectra of **8**. Analyses of HMBC data established the dihydrodibenz[*b*,*f*]oxepin skeleton in **8**, with the following key correlations: H-2 to C-1, C-3, and C-11a; 3-OMe to C-3; H-5 to C-4, C-4a, C-6a, and C-11a; H-6 to

Table 4. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Spectroscopic Data (acetone-d_6) of Bauhibenzofurin A (**9**), Bauhispirorin A (**10**), and Bauhinol E (**11**)

	bauhibenzofurin A (**9**)		bauhispirorin A (**10**)		bauhinol E (**11**)	
C	δ_C, mult.[a]	δ_H (mult., *J* in Hz)	δ_C, mult.[a]	δ_H (mult., *J* in Hz)	δ_C, mult.[a]	δ_H (mult., *J* in Hz)
1					155.6, s	
2	79.2, d	6.01 (dd, 9.4, 7.9)	79.1, s		109.2, s	
3	37.2, t	3.05, dd, 15.8, 7.9; 3.07, dd, 15.8, 9.5	27.9, t	2.02 (m); 2.43 (ddd, 14.1, 11.1, 5.5)	158.6, s	
3a	126.9, s					
4	124.9, d	7.18 (br d, 7.7)	21.2, t	2.78 (m); 2.77 (m)	102.4, d	6.35 (br s)
4a			121.0, s			
5	120.4, d	6.83 (dd, 7.7, 7.4)	129.2, d	7.06 (d, 7.9)	140.8, s	
6	127.8, d	7.15 (dd, 7.7, 7.4)	120.7, d	6.85 (ddd, 7.9, 7.9, 0.9)	108.0, d	6.41 (br s)
6a						
7	108.9, d	6.85 (br d, 7.4)	127.4, d	7.09 (dd, 7.9, 0.9)	36.0, t	2.77 (m)
7a	159.9, s					
8			116.3, d	6.78 (d, 7.9)	32.2, t	2.87 (m)
8a			153.3, s			
1′	136.8, s				155.0, s	
2′	128.8, s		190.4, s		128.2, s	
3′	101.5, s	6.53 (d, 3.1)	161.6, s		130.0, d	7.07 (dd, 7.4, 1.5)
4′	153.1, s		112.0, d	6.12 (s)	119.4, d	6.73 (td, 7.4, 1.1)
5′	98.5, d	6.53 (d, 3.1)	192.7, s		126.9, d	7.01 (td, 7.8, 1.6)
6′	147.8, s		48.6, t	3.05 (d, 16.3); 3.20 (d, 16.3)	114.9, d	6.83 (dd, 7.8, 0.8)
2-Me					7.42, q	2.00 (s)
3-OMe					54.9, q	3.75 (s)
3′-OMe			56.3, q	3.85 (s)		
4′-OMe	55.0, q	3.68 (s)				
6′-OMe	55.6, q	3.85 (s)				
1-OH						8.00 (br s)
1′-OH		7.33 (br s)				8.27 (br s)

[a] Multiplicity was determined by DEPT spectra. [b,c,d] May be interchanged in the same column.

Table 5. Biological Activities of Compounds from *B. purpurea*

				anti-inflammatory (IC_{50}, μM)		cytotoxicity (IC_{50}, μM)	
compound	antimycobacterial activity (MIC, μM)	antimalarial activity (IC_{50}, μM)	antifungal activity (IC_{50}, μM)	COX-1	COX-2	KB	BC
1	183.8	>73.5	79.4	ND[a]	ND	44.8	32.3
2	183.8	>73.5	>183.8	ND	ND	41.5	45.5
3	331.1	>66.2	>165.5	>33.1	>33.1	>66.2	>66.2
4	662.2	>66.2	77.8	>33.1	6.9	29.4	27.4
5	91.9	>73.5	>183.8	ND	ND	>73.5	>73.5
6	87.4	11.2	>174.8	>34.9	>34.9	15.7	11.5
7	174.8	10.5	>174.8	>34.9	10.1	38.8	41.9
8	24.4	5.8	>195.3	ND	ND	10.5	12.1
9	367.6	>73.5	130.1	ND	ND	>73.5	>73.5
10	ND	ND	ND	ND	ND	ND	ND
11	193.7	>77.5	>193.7	ND	ND	>77.5	>77.5
12	>740.7	>74.0	>185.1	ND	ND	>74.0	>74.0
13	>704.2	9.5	>176.0	ND	ND	>70.4	>70.4
14	204.6	>81.8	>204.6	>40.9	>40.9	>81.8	>81.8
15	193.7	>77.5	49.6	ND	ND	>77.5	>77.5
16	91.2	>72.9	>182.4	ND	ND	>72.9	>72.9
17	116.8	>93.4	>233.6	>46.7	>46.7	>93.4	>93.4
18	219.2	>87.7	68.4	ND	ND	51.7	72.3
isoniazid	0.29−0.66						
kanamycin sulfate	3.43−8.58						
dihydroartemisinin		$(4.2 \pm 0.7) \times 10^{-3}$ ($n = 3$)					
amphotericin B			0.04 ± 0.01 ($n = 3$)				
aspirin				11.4	19.8		
ellipticine						5.3	6.1

[a] ND = not determined.

C-4a, C-6a, C-7, and C-10a; H-7 to C-6, C-6a, and C-10a; and H-10 to C-6a and C-10a. Bauhinoxepin J (**8**) was thus identified as 5,6-dihydro-3-methoxy-1,4-dionedibenz[*b*,*f*]oxepin, and its proton and carbon resonances were assigned as indicated in Table 3.

The ESITOFMS revealed a molecular formula of $C_{16}H_{16}O_4$ for bauhibenzofurin A (**9**). The ^{1}H NMR spectrum showed the spin pattern of a 1,2-substituted aromatic ring. The HMBC correlations from H-3 to C-3a and C-7a; H-4 to C-3 and C-7a; and H-2 to C-7a established a partial structure of a 2-substituted dihydrobenzofuran in **9**. A small coupling constant (3.1 Hz) suggested *meta* coupling between H-3′ and H-5′. In the HMBC spectrum of **9**, the deshield oxygenated sp^3 methine H-2 (δ_H 6.01) correlated to C-1′, C-2′, and C-3′, and H-3′ correlated to C-2, indicating the connection of an aromatic ring to C-2 of the dihydrobenzofuran unit. The HMBC correlations from 1′-OH to C-1′ and C-6′; 6′-OMe to C-1′ and C-5′; 4′-OMe to C-4′; H-3′ to C-2, C-1′, C-2′, and C-5′; and H-5′ to C-1′, C-4′, and C-6′ readily defined the positions of 1′-OH, 4′-OMe, and 6′-OMe groups on the aromatic ring. On the basis of

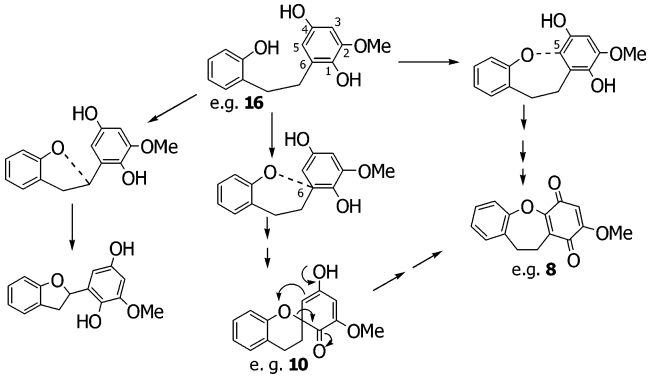

Figure 1. Proposed biosynthetic pathways for bauhispirorin A (**10**), dihydrobenzofuran (e.g., **9**), and dihydrodibenz[*b*,*f*]oxepins (e.g., **8**).

these spectroscopic data, bauhibenzofurin A (**9**) was identified as 2-(2,3-dihydrobenzofuran-2-yl)-4′,6′-dimethoxyphenol. Proton and carbon resonances of bauhibenzofurin A (**9**) were readily assigned as shown in Table 4. The coupling constants of 9.4 and 7.9 Hz suggested an axial orientation of H-2 in **9**; however, the absolute configuration of C-2 could not be established on the basis of available spectroscopic data.

Bauhispirorin A (**10**) was obtained as a viscous liquid, $[\alpha]^{27}_D$ −2.53 (*c* 0.05, MeOH). The ESITOFMS indicated a molecular formula of $C_{15}H_{14}O_4$. The IR absoption peaks at 1676 and 1650, together with the ^{13}C NMR resonances at δ 192.7 and 190.4, revealed the presence of conjugated ketone groups in **10**. The ^1H NMR and COSY data of **10** indicated the presence of a 1,2-substituted aromatic ring and also showed resonances of three methylenes (H-3, H-4, and H-6′), an sp^2 methine singlet (H-4′), and a methoxy group (3′-OMe). The HMBC data permitted the assembly of the gross structure of **10**, showing correlations from H-3 to C-2, C-4a, C-2′, and C-6′; H-4 to C-2, C-4a, and C-8a; H-5 to C-4 and C-8a; H-4′ to C-2′, C-3′, C-5′, and C-6′; H-6′ to C-2, C-3, C-2′, C-4′, and C-5′; and 3′-OMe to C-3′. However, the absolute configuration at C-2 of **10** could not be established due to the limited amounts of the material isolated. Bauhispirorin A (**10**) was therefore identified as 3′-methoxy-3,4-dihydro-2′*H*,5′*H*-spiro-[chromene-2,1′-cyclohex[3]ene]-2′,5′-dione. Complete assignment of proton and carbon resonances in **10** is shown in Table 4. Although compounds with the spirochroman-2,1′-hexenedione skeleton were previously synthesized,[11] bauhispirorin A (**10**) is the first natural product possessing the spirochroman-2,1′-hexenedione skeleton.

Previously, the biosynthesis of oxepins (i.e., bauhinoxepins A and B) was proposed to proceed via bibenzyl intermediates.[8c] Such direct oxidative coupling to form a seven-membered ring was readily established for the cularine alkaloid.[12a,b] However, Kametani and Fukumoto[12a] also speculated that the seven-membered ring in cularine might be derived from the spirodienone (structurally related to compound **10**). Interestingly, in the present study, the spirodienone (e.g., **10**), dihydrodibenz[*b*,*f*]oxepins, and bibenzyls were isolated within the same plant; therefore the possibility that the oxepins were biogenetically derived via the spirodienone **10** could not be ruled out. As shown in Figure 1, the oxidative coupling of the bibenzyl (e.g., **16**) at the *ortho* (C-6) position of the 1-OH phenol gave rise to the novel skeleton of a spirochroman-2,1′-hexenedione (e.g., **10**), while the *ortho* oxidative coupling of the 4-OH phenol resulted in the formation of dihydrodibenz[*b*,*f*]oxepins (e.g., **8**). Alternatively, dihydrodibenz[*b*,*f*]oxepins may be biosynthesized from bauhispirorin A (**10**) by the rearrangement as shown in Figure 1. The dihydrobenzofuran, such as bauhibenzofurin A (**9**), may also be derived from its bibenzyl substrate by the coupling between methylene and OH as shown in Figure 1.

Bauhinol E (**11**) exhibited the molecular formula $C_{16}H_{18}O_3$, as deduced from ESITOFMS. The ^1H NMR and COSY data revealed the presence of two aromatic rings in bauhinol E (**11**), one of which was a 1,2-disubstituted benzene. The presence of the C-7 and C-8 methylenes, together with the molecular formula obtained from the ESITOFMS, indicated that bauhinol E (**11**) was a bibenzyl.[8c] The HMBC spectrum of **11** readily established the structure of bauhinol E (**11**), showing the correlations from 1-OH to C-2; 2-Me to C-1, C-2, and C-3; 3-OMe to C-3; H-4 to C-3, C-6, and C-7; H-6 to C-1, C-2, C-4, and C-7; H-7 to C-4, C-5, C-6, and C-2′; H-8 to C-5, C-1′, C-2′, and C-3′; H-3′ to C-8, C-1′, and C-2′; and H-5′ to C-1′. On the basis of these spectroscopic data, bauhinol E (**11**) was identified as 5-[2-(2-hydroxyphenyl)ethyl]-3-methoxy-2-methylphenol (Table 4). Bauhinol E (**11**) is an oxidized form of bauhinol C, which was previously isolated from *B. saccocalyx*.[8c]

All the isolated compounds from *B. purpurea*, except compounds **10**, **12**, and **13**, exhibited antimycobacterial activity with MIC values ranging from 24.4 to 740.7 μM (Table 5). It should be noted that compound **8** exhibited relatively potent antimycobacterial activity (MIC 24.4 μM), compared to those of closely related dihydrodibenz-[*b*,*f*]oxepins **1**−**7**. Compound **8** had no 2-Me group, unlike those for oxepins **1**−**7**. Therefore, the missing 2-Me group in compound **8** might enhance the antimycobacterial activity. Among the isolated metabolites, compounds **6**, **7**, **8**, and **13** exhibited antimalarial activity (IC_{50} 5.8−11.2 μM), while compounds **1**, **4**, **9**, **15**, and **18** exhibited antifungal activity (IC_{50} 49.6−130.1 μM) (Table 5). Compounds **4** and **7** possessed potent anti-inflammatory activity, inhibiting the COX-2 enzyme with IC_{50} values of 6.9 and 10.1 μM, respectively (Table 5). Interestingly both **4** and **7** were inactive at 33.1−34.9 μM against the COX-1 enzyme, suggesting the selectivity toward COX-2 is at least 1 order of magnitude. Compounds **1**, **2**, **4**, **6**, **7**, **8**, and **18** exhibited cytotoxicity toward KB and BC cell lines with IC_{50} values ranging from 10.5 to 72.3 μM (Table 5).

Previously, dibenzo[*b*,*f*]oxepins, bauhinoxepins A and B, were isolated from *B. saccocalyx*,[8b] while a derivative, dihydrodibenz-[*b*,*f*]oxepin, was isolated from *B. variegata*.[13] This is the first report on the presence of a dihydrobenzofuran (e.g., **9**) in *Bauhinia* species. Bioactive bibenzyls are commonly found in *Bauhinia* species[8a,b] and may be precursors of oxepins, dihydrobenzofurans, and a spirochroman-2,1′-hexenedione (e.g., **10**), as shown in Figure 1. A flavanone, structurally related to flavanones **12** and **13**, was previously found in *B. variegata*.[13] Stilbenoid derivatives (e.g., **14**−**18**) are common metabolites of conifer trees; however, they are also found in the *Stemona* species.[14] Structurally diverse secondary metabolites in *B. purpurea* reported here prove that the *Bauhinia* species are a rich source of bioactive compounds.

Experimental Section

General Experimental Procedures. Melting points were measured on a digital Electrothermal 9100 melting point apparatus and are reported without correction. Optical rotations were measured on a JASCO DIP 370 polarimeter, while UV spectra were recorded on a Cary-1E UV−vis spectrophotometer. The FT-IR spectra were measured on a Perkin-Elmer GX spectrophotometer. ^1H and ^{13}C, DEPT, $^1H-^1H$ COSY, NOESY, HMQC, and HMBC experiments were carried out on a Bruker AV500 NMR spectrometer, operating at 500 MHz for hydrogen and 125 MHz for carbon. ESITOF mass spectra were obtained from a Micromass LCT mass spectrometer.

Plant Material. The roots of *B. purpurea* were collected in May 2004, from Phitsanulok Province, Thailand. A voucher specimen (W132) was deposited at the Department of Biology, Faculty of Science, Naresaun University, Phitsanulok Province, Thailand.

Extraction and Isolation. Dried roots of *B. purpurea* (5.9 kg) were macerated in CH_2Cl_2 (15 L) for 2 days. A crude CH_2Cl_2 extract (11.6 g) was chromatographed on a Sephadex LH-20 column (MeOH as eluent), and 10 fractions (A1−A10) were collected. Fraction A10 was separated on Sephadex LH-20 to yield 132 mg of bauhinoxepin C (**1**). Fraction A9 was purified by silica gel column chromatography, eluted with a gradient system of EtOAc/CH_2Cl_2 (from 1:99 to 25:75), yielding

bauhinoxepin D (**2**) (24 mg) and (−)-strobopinin (**12**) (8.7 mg). Fraction A8 was washed with CH_2Cl_2, and the residue was crystallized from CH_2Cl_2 to yield bauhinoxepin F (**4**) (243 mg). The CH_2Cl_2-soluble part of A8 was chromatographed on a silica gel column, eluted with a gradient system of EtOAc/CH_2Cl_2 (from 1:99 to 25:75), to afford bauhinoxepin E (**3**) (3.8 mg), demethoxymatteucinol **13** (24 mg), and bibenzyl **14** (28 mg). Fraction A7 was washed with CH_2Cl_2, and the residue was crystallized from CH_2Cl_2 to yield bauhinoxepin H (**6**) (181 mg), while the CH_2Cl_2-soluble part of A7 was further purified on a silica gel column, eluted with a gradient solvent system of EtOAc/CH_2Cl_2 (from 1:99 to 50:50), to give bauhibenzofurin A (**9**) (7.4 mg), bauhinol E (**11**) (5.6 mg), bibenzyl **15** (16 mg), bibenzyl **16** (24 mg), and bibenzyl **17** (6.4 mg). Fraction A6 was purified by silica gel column chromatography (a gradient solvent system of EtOAc/CH_2Cl_2, from 1:99 to 70:30) to yield bauhinoxepin G (**5**) (3.7 mg), bauhinoxepin I (**7**) (3.0 mg), bauhinoxepin J (**8**) (9.6 mg), bauhispirorin A (**10**) (2.1 mg), and bibenzyl **18** (10.5 mg).

Bauhinoxepin C (1): colorless powder; IR (thin film of $CHCl_3$) ν_{max} cm^{-1} 3435, 2925, 2853, 1611, 1587, 1504, 1461, 1426, 1279, 1234, 1131, 1090; UV (CH_3OH) λ_{max} nm 207, 222, 281; ESITOFMS *m*/*z* 295.0937 $(M + Na)^+$, calcd for $(C_{16}H_{16}O_4+Na)^+$, 295.0941; ^1H and ^{13}C NMR, see Table 1.

Bauhinoxepin D (2): colorless powder; IR (thin film of $CHCl_3$) ν_{max} cm^{-1} 3387, 2926, 2854, 1610, 1584, 1459, 1417, 1278, 1219, 1174, 1089, 1014; UV (CH_3OH) λ_{max} nm 207, 222, 281; ESITOFMS *m*/*z* 295.0951 $(M + Na)^+$, calcd for $(C_{16}H_{16}O_4+Na)^+$, 295.0941; ^1H and ^{13}C NMR, see Table 1.

Bauhinoxepin E (3): colorless powder; IR (thin film of $CHCl_3$) ν_{max} cm^{-1} 3401, 2927, 2853, 1611, 1594, 1460, 1414, 1259, 1216, 1176, 1065, 1009, 758; UV (CH_3OH) λ_{max} nm 206, 226, 279; ESITOFMS *m*/*z* 325.1052 $(M + Na)^+$, calcd for $(C_{17}H_{18}O_5+Na)^+$, 325.1046; ^1H and ^{13}C NMR, see Table 1.

Bauhinoxepin F (4): colorless powder; IR (thin film of $CHCl_3$) ν_{max} cm^{-1} 3406, 2929, 2853, 1610, 1461, 1417, 1259, 1216, 1175, 1069, 756; UV (CH_3OH) λ_{max} nm 204, 223, 280; ESITOFMS *m*/*z* 325.1051 $(M + Na)^+$, calcd for $(C_{17}H_{18}O_5+Na)^+$, 325.1046; ^1H and ^{13}C NMR, see Table 2.

Bauhinoxepin G (5): colorless powder; $[\alpha]^{27}_D$ −29.5 (*c* 0.09, CH_3OH); IR (thin film of $CHCl_3$) ν_{max} cm^{-1} 3355, 2926, 2854, 1608, 1483, 1462, 1418, 1233, 1104, 1085, 758; UV (CH_3OH) λ_{max} nm 204, 278; ESITOFMS *m*/*z* 295.0937 $(M + Na)^+$, calcd for $(C_{16}H_{16}O_4+Na)^+$, 295.0941; ^1H and ^{13}C NMR, see Table 2.

Bauhinoxepin H (6): orange needles (CH_2Cl_2); mp 170−172 °C; IR (thin film of $CHCl_3$) ν_{max} cm^{-1} 3392, 2925, 2854, 1651, 1613, 1464, 1298, 1248, 1154, 1099; UV (CH_3OH) λ_{max} nm 204, 223, 277; ESITOFMS *m*/*z* 287.0913 $(M + H)^+$, calcd for $(C_{16}H_{14}O_5+H)^+$, 287.0920; ^1H and ^{13}C NMR, see Table 3.

Bauhinoxepin I (7): orange-brown needles (CH_2Cl_2); mp 158−161 °C; IR (thin film of $CHCl_3$) ν_{max} cm^{-1} 3377, 2924, 1648, 1611, 1494, 1250, 1189, 1149; UV (CH_3OH) λ_{max} nm 203, 228, 277; ESITOFMS *m*/*z* 287.0913 $(M + H)^+$, calcd for $(C_{16}H_{14}O_5+H)^+$, 287.0919; ^1H and ^{13}C NMR, see Table 3.

Bauhinoxepin J (8): yellow liquid; IR (thin film of $CHCl_3$) ν_{max} cm^{-1} 2939, 1663, 1605, 1581, 1489, 1456, 1356, 1256, 1227, 1192, 1099, 768; UV (CH_3OH) λ_{max} nm 204, 229, 275, 317; ESITOFMS *m*/*z* 257.0824 $(M + H)^+$, calcd for $(C_{15}H_{12}O_4+H)^+$, 257.0814; ^1H and ^{13}C NMR, see Table 3.

Bauhibenzofurin A (9): colorless powder; $[\alpha]^{26}_D$ +4.2 (*c* 0.02, CH_3OH); IR (thin film of $CHCl_3$) ν_{max} cm^{-1} 3436, 2938, 2841, 1615, 1498, 1480, 1463, 1432, 1375, 1233, 1198, 1150, 1054, 752; UV (CH_3OH) λ_{max} nm 206, 227, 288; ESITOFMS *m*/*z* 273.1125 $(M + H)^+$, calcd for $(C_{16}H_{16}O_4+H)^+$, 273.1127; ^1H and ^{13}C NMR, see Table 4.

Bauhispirorin A (10): viscous liquid; $[\alpha]^{27}_D$ −2.5 (*c* 0.05, CH_3OH); IR (thin film of $CHCl_3$) ν_{max} cm^{-1} 2925, 2853, 1676, 1650, 1602, 1457, 1233, 755; UV (CH_3OH) λ_{max} nm 203, 268; ESITOFMS *m*/*z* 281.0806 $(M + Na)^+$, calcd for $(C_{15}H_{14}O_4+Na)^+$, 281.0790; ^1H and ^{13}C NMR, see Table 4.

Bauhinol E (11): colorless powder; IR (thin film of $CHCl_3$) ν_{max} cm^{-1} 3402, 2925, 2854, 1592, 1505, 1455, 1421, 1228, 1145, 1110, 753; UV (CH_3OH) λ_{max} nm 204, 225, 274; ESITOFMS *m*/*z* 259.1341 $(M + H)^+$, calcd for $(C_{16}H_{18}O_3+H)^+$, 259.1334; ^1H and ^{13}C NMR, see Table 4.

(−)-Strobopinin (12): colorless powder; $[\alpha]^{26}_D$ −78.54 (*c* 0.01, CH_3OH); ESITOFMS *m*/*z* 293.0797 $(M + Na)^+$, calcd for $(C_{16}H_{14}O_4+Na)^+$, 293.0790.

Demethoxymatteucinol (13): colorless powder; $[\alpha]^{26}_D$ −10.30 (*c* 0.01, CH_3OH); ESITOFMS *m*/*z* 285.1123 $(M + H)^+$, calcd for $(C_{17}H_{16}O_4+H)^+$, 285.1127.

Bioassays. Cytotoxicity was determined by employing the colorimetric method described by Skehan and co-workers.[15] The reference compound, ellipticine, exhibited activity toward KB and BC cell lines with respective IC_{50} values of 5.3 and 6.1 μM. Antimycobacterial activity was assessed against *Mycobacterium tuberculosis* H37Ra using the microplate Alamar Blue assay (MABA).[16] The mycobacterium *M. tuberculosis* H37Ra was cultured in Middlebrook 7H9 broth. The standard drugs, isoniazid and kanamycin sulfate, showed MIC values of 0.29−0.66 and 3.43−8.58 μM, respectively. Antimalarial activity *in vitro* was determined by means of the microculture radioisotope technique based on the method described by Desjardins et al. (1979).[17] The parasite *Plasmodium falciparum* (K1, multidrug-resistant strain) was cultured continuously according to the method of Trager and Jensen (1976).[18] An IC_{50} value of $(4.2 \pm 0.7) \times 10^{-3}$ μM ($n = 3$) was observed for the standard compound, dihydroartemisinin. Antifungal activity was assessed against *Candida albicans*, employing a colorimetric method (Hawser et al., 1998).[19] Amphotericin B and 10% DMSO were used as a positive and a negative control, respectively. In our system, the IC_{50} value of the standard drug, amphotericin B, was 0.04 ± 0.01 μM ($n = 3$). Anti-inflammatory activity was determined by measuring the inhibition of COX enzymes using the radioimmunoassay method.[20] Aspirin was used as a positive control and was almost equally effective against COX-1 and COX-2. IC_{50} values of aspirin for COX-1 and COX-2 are 11.4 and 19.8 μM, respectively.

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Supporting Information Available: ^1H and ^{13}C NMR spectra of compounds **1**−**11**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Pettit, G. R.; Numata, A.; Iwamoto, C.; Usami, Y.; Yamada, T.; Ohishi, H.; Cragg, G. M. *J. Nat. Prod.* **2006**, *69*, 323−327.
(2) Yadava, R. N.; Tripathi, P. *Fitoterapia* **2000**, *71*, 88−90.
(3) Kuo, Y. H.; Yeh, M. H.; Huang, S. L. *Phytochemistry* **1998**, *49*, 2529−2530.
(4) Wu, A. M.; Wu, J. H.; Liu, J. H.; Singh, T. *Life Sci.* **2004**, *74*, 1763−1779.
(5) Horan, N.; Yan, L.; Isobe, H.; Whitesides, G. M.; Kahne, D. *Proc. Natl. Acad. Sci.* **1999**, *96*, 11782−11786.
(6) Liang, R.; Yan, L.; Loebach, J.; Ge, M.; Uozumi, Y.; Sekanina, K.; Horan, N.; Gildersleeve, J.; Thompson, C.; Smith, A.; Biswas, K.; Still, W. C.; Kahne, D. *Science* **1996**, *274*, 1520−1522.
(7) Kuemmel, T. A.; Thiele, J.; Blaeser, A. H.; Wickenhauser, C.; Baldus, S. E.; Fischer, R. *Histochem. J.* **1997**, *29*, 695−705.
(8) (a) Kittakoop, P.; Kirtikara, K.; Tanticharoen, M.; Thebtaranonth, Y. *Phytochemistry* **2000**, *55*, 349−352. (b) Kittakoop, P.; Nopichai, S.; Thongon, N.; Charoenchai, P.; Thebtaranonth, Y. *Helv. Chim. Acta* **2004**, *87*, 175−179. (c) Apisantiyakom, S.; Kittakoop, P.; Manyum, T.; Kirtikara, K.; Bremner, J. B.; Thebtaranonth, Y. *Chem. Biodiversity* **2004**, *1*, 1694−1701. (d) Anjaneyulu, S. R.; Reddy, A. V.; Reddy, D. S. K.; Cameron, S. T.; Roe, S. P. *Tetrahedron* **1986**, *42*, 2417−2420. (e) Maillard, M. P.; Recio-Iglesias, M. C.; Saadou, M.; Stoeckli-Evans, H.; Hostettmann, K. *Helv. Chim. Acta* **1991**, *74*, 791−799.
(9) (a) Kuo, Y. C.; Yang, L. M.; Lin, L. C. *Planta Med.* **2004**, *70*, 1237−1239. (b) Hsieh, Y. L.; Fang, J. M.; Cheng, Y. S. *Phytochemistry* **1998**, *47*, 845−850. (c) Williams, C. A.; Harborne, J. B.; Newman, M.; Greenham, J.; Eagles, J. *Phytochemistry* **1997**, *46*, 1349−1353. (d) Salem, M. M.; Werbovetz, K. A. *J. Nat. Prod.* **2005**, *68*, 108−111. (e) Wu, J. H.; Wang, X. H.; Yi, Y. H.; Lee, K. H. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1813−1815.
(10) (a) Kaganda, N. G.; Adesanya, S. A. *J. Nat. Prod.* **1990**, *53*, 1345−1346. (b) Asakawa, Y.; Hashimoto, T.; Takikawa, K.; Tori, M.; Ogawa, S. *Phytochemistry* **1991**, *30*, 235−251. (c) Takasugi, M.; Kawashima, S.; Monde, K.; Katsui, N.; Masamune, T.; Shirata, A. *Phytochemistry* **1987**, *26*, 371−375. (d) Ireland, C. R.; Schwabe, W. W.; Coursey, D. G. *Phytochemistry* **1981**, *20*, 1569−1571. (e) Hashimoto, T. Jpn. Kokai Tokkyo Koho JP 49086536, 1974.
(11) (a) Choudhury, A. M. *J. Chem. Soc., Perkin Trans. 1* **1974**, 132−134. (b) Schofield, K.; Ward, R. S.; Choudhury, A. M. *J. Chem. Soc. C* **1971**, 2834−2837.

(12) (a) Kametani, T.; Fukumoto, K. *J. Chem. Soc. D, Chem. Commun.* **1971**, 352−353. (b) Blaschke, G.; Scriba, G. *Phytochemistry* **1985**, *25*, 111−113.
(13) Reddy, M. V.; Reddy, M. K.; Gunasekar, D.; Caux, C.; Bodo, B. *Phytochemistry* **2003**, *64*, 879−882.
(14) (a) Adams, M.; Pacher, T.; Greger, H.; Bauer, R. *J. Nat. Prod.* **2005**, *68*, 83−85. (b) Kostecki, K.; Engelmeier, D.; Pacher, T.; Hofer, O.; Vajrodaya, S.; Greger, H. *Phytochemistry* **2004**, *65*, 99−106. (c) Pacher, T.; Seger, C.; Engelmeier, D.; Vajrodaya, S.; Hofer, O.; Greger, H. *J. Nat. Prod.* **2002**, *65*, 820−827.
(15) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Nat. Cancer Inst.* **1990**, *82*, 1107−1112.
(16) Collins, L.; Franzblau, S. G. *Antimicrob. Agents Chemother.* **1997**, *41*, 1004−1009.
(17) Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. *Antimicrob. Agents Chemother.* **1979**, *16*, 710−718.
(18) Trager, W.; Jensen, J. B. *Science* **1976**, *193*, 673−675.
(19) Hawser, S. P.; Norris, H.; Jessup, C. J.; Ghannoum, M. A. *J. Clin. Microbiol.* **1998**, *36*, 1450−1452.
(20) Kirtikara, K.; Morham, S. G.; Raghow, R.; Laulederkind, S. J.; Kanekura, T.; Goorha, S.; Ballou, L. R. *J. Exp. Med.* **1998**, *187* (4), 517−523.

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