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Cytotoxic and Antimycobacterial Prenylated Flavonoids from the Roots of *Eriosema chinense*

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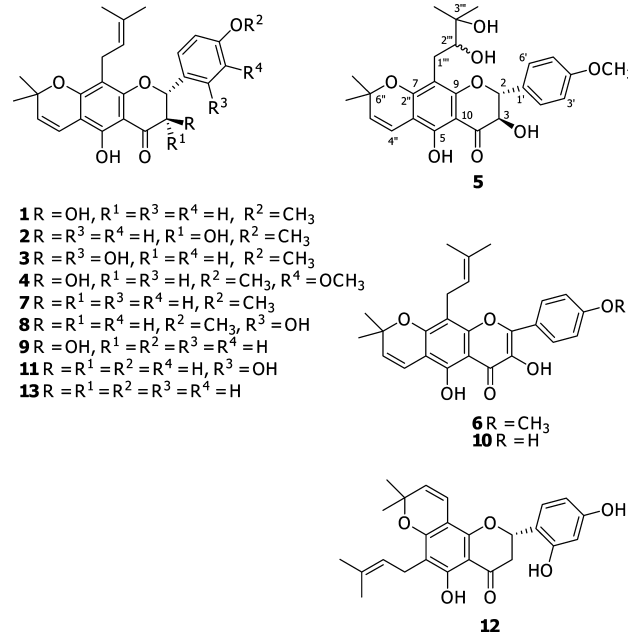
Eight new prenylated flavonoids, khonklonginols A−H (**1**−**8**), together with six known compounds including five flavonoids, lupinifolinol (**9**), dehydrolupinifolinol (**10**), flemichin D (**11**), eriosemaone A (**12**), and lupinifolin (**13**), and one lignan, yangambin (**14**), have been isolated from hexane and dichloromethane extracts of the roots of *Eriosema chinense*. The structures of **1**−**8** were elucidated by spectroscopic methods. The compounds were evaluated for cytotoxic activity against the small-cell lung (NCI-H187) and oral epidermal carcinoma (KB) human cell lines as well as for antimycobacterial activity against *Mycobacterium tuberculosis* H37Ra.

Eriosema chinense Vogel (Leguminosae-Papilionoideae) is a small plant that is the only member of its genus found in Thailand.[1] There have been no previous reports on the biological activity or phytochemical investigation of this species. Preliminary cytotoxic activity assays indicated the hexane extract of the root to be active, showing IC_{50} values of ca. 10 μg/mL using two cancer cell lines and also showing anti-TB activity against *Mycobacterium tuberculosis* H37Ra with a MIC value of 50 μg/mL. Chromatographic separation of the hexane and dichloromethane extracts led to the isolation of compounds **1**−**8** in addition to six known compounds, comprising lupinifolinol (**9**),[2,3] dehydrolupinifolinol (**10**),[4] flemichin D (**11**),[5] eriosemaone A (**12**),[5] lupinifolin (**13**),[2,6] and yangambin (**14**).[7] To our knowledge **10**, although previously obtained as a synthetic product from **9**,[4] has been isolated from a natural source for the first time in this study. We report herein the isolation and structural identification of eight new prenylated flavonoids, khonklonginols A−H, in addition to the biological activity of some of the isolates.

Results and Discussion

Compound **1** was isolated as a yellow liquid with a molecular formula of $C_{26}H_{28}O_6$, as determined from the HREIMS (found M^+ at *m*/*z* 436.1873). The FT-IR spectrum showed absorption bands for hydroxy (ν_{max} 3467 cm^{-1}) and conjugated carbonyl (ν_{max} 1626 cm^{-1}) functional groups. The ^1H and ^{13}C NMR spectra showed characteristic sets of signals at δ_H 4.98 (1H, d, J = 12.0 Hz, H-2) and 4.42 (1H, d, J = 12.0 Hz, H-3) and at δ_C 82.9 (CH, C-2) and 72.6 (CH, C-3) of a 3-hydroxyflavanone skeleton.[8] A low-field singlet at δ_H 11.41 indicated a C-5 OH group hydrogen-bonded to a carbonyl carbon at C-4. Aromatic proton signals at δ_H 7.46 (2H, d, J = 8.8 Hz, H-2′, H-6′) and 6.96 (2H, d, J = 8.8 Hz, H-3′, H-5′) could be assigned as 1,4-disubstituted aromatic ring B protons, as evident from the HMBC correlations from H-2′ and H-6′ to C-2. The ^3J correlations of H-2′, H-3′, and a singlet at δ_H 3.83 to C-4′ (δ_C 160.3, qC) indicated the attachment of a OCH_3 group at C-4′. The ^1H NMR signals at δ_H 5.51 (d, J = 10.0 Hz, H-5″), 6.62 (d, J = 10.0 Hz, H-4″), 1.44 (s), and 1.43 (s) showed ^1J correlations with the ^{13}C NMR signals at δ_C 126.2, 115.4, and 28.4 (2×), respectively, and were assigned to a dimethylchromene group. In turn, the ^1H NMR signals at δ_H 3.16 (2H, d, J = 6.8 Hz, H-1‴), 5.11 (dt, J = 6.4, 1.6 Hz, H-2‴), and two singlets at δ_H 1.63 and 1.59 and ^{13}C NMR signals at δ_C 21.4, 122.3, 131.3, 25.7, and 17.8 were assigned to a dimethylallyl group. The key HMBC correlations between H-4″/C-5 (δ_C 156.1) required the placement of a chromene ring at C-6 and C-7, and the correlations of H-1‴ with C-8 (δ_C 109.3, qC) and C-9 (δ_C 159.5, qC) indicated a 3‴,3‴-dimethylallyl group at C-8. Compound **1** was identified as 3,5-dihydroxy-4′-methoxy-6″,6″-dimethylpyrano(2″,3″:7,6)-8-(3‴,3‴-dimethylallyl)flavanone. This compound was given the trivial name khonklonginol A. The absolute configurations at C-2 and C-3 were proposed as 2*R*,3*R*, based on the large $J_{2,3} = J_{3,2}$ values of 12.0 Hz, indicating H-2 and H-3 to be *trans*, and from the circular dichroism spectrum, which showed a positive n → π^* Cotton effect at 362 nm.[9] The data obtained were consistent with those reported for lupinifolinol,[2,3] which was also isolated in this study, as well as for jayacanol, previously isolated from *Lonchocarpus oaxacensis*.[10] Full assignments of ^1H and ^{13}C NMR chemical shifts are as shown in Tables 1 and 2.



Compound **2** was assigned a molecular formula of $C_{26}H_{28}O_6$ from the HRMS. The ^1H and ^{13}C NMR spectra showed a pattern of signals similar to those of compound **1** (Tables 1 and 2), except for the presence of two less shielded doublet signals at δ_H 5.64 and 4.71, both mutually coupled with a vicinal coupling constant of 5.1 Hz and assignable to H-2 and H-3 of a 3-hydroxyflavanone skeleton, respectively.[8] The smaller $J_{2,3}$ value indicated compound **2** to possess a different configuration at C-3 from compound **1**,

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Table 1. ^{1}H NMR Spectroscopic Data of **1**−**5** (in $CDCl_3$, δ ppm, mult. J in Hz)[a]

position	1	2	3	4	5
2	4.98, d (12.0)	5.64, d (5.1)	5.28, d (12.0)	4.97, d (11.9)	5.01, d (11.9) 4.50, d (11.9)
3	4.42, d (12.0)	4.71, d (5.1)	4.50, d (12.0)	4.49, d (11.9)	4.50, d (11.9) 4.49, d (11.9)
2′	7.46, d (8.8)	7.29, brd (8.9)		7.07, d (2.0)	7.43, d (8.7)
3′	6.96, d (8.8)	6.82, d (8.7)	6.54, d (2.5)		6.96, d (8.8) 6.94, d (8.8)
5′	6.96, d (8.8)	6.82, d (8.7)	6.58, d (8.7)	6.91, d (8.8)	6.96, d (8.8) 6.94, d (8.8)
6′	7.46, d (8.8)	7.29, d (8.9)	7.42, brd (8.6)	7.08, dd (8.8, 2.0)	7.43, d (8.7)
4″	6.62, d (10.0)	6.58, d (9.9)	6.62, d (10.1)	6.62, d (10.0)	6.64, d (10.0) 6.63, d (10.0)
5″	5.51, d (10.0)	5.48, d (9.8)	5.53, d (10.0)	5.51, d (10.0)	5.52 d (10.2)
CH_3-6″	1.44, s 1.43, s	1.43, s 1.41, s	1.44, s	1.44, s 1.43, s	1.47, 1.45, 1.44, 1.43, s
1‴	3.16, d (6.8)	3.21, d (7.1)	3.21, brt (8.0)	3.16, brd (6.9)	3.16, brd (6.9)
2‴	5.11, dt (6.4, 1.6)	5.10, t (7.3)	5.10, brt (7.4)	5.13, brt (6.8)	5.13, brt (6.1)
CH_3-3‴	1.63, s 1.59, s	1.69, s 1.62, s	1.66, s 1.65, s	1.62, s 1.59, s	1.17, 1.16, 1.14, 1.135, s
OCH_3-4′	3.83, s	3.76, s	3.79, s	3.90, s[a]	3.82, s
OCH_3-3′				3.91, s[a]	
O*H*-5	11.41, s	11.39, s	11.29, s[b]	11.41, s	11.40, s

[a] Assignments may be reversed. [b] O*H*-2′ in **3** was detected as a singlet at δ_H 6.82.

Table 2. ^{13}C NMR Spectroscopic Data of **1**−**5** (in $CDCl_3$, δ ppm, mult.)

position	1	2	3	4	5
2	82.9, CH	80.0, CH	79.0, CH	83.1, CH	83.1, CH 83.0, CH
3	72.6, CH	71.5, CH	73.1, CH	72.6, CH	72.4, CH
4	196.4, qC	194.6, qC	195.3, qC	196.1, qC	196.4, qC 196.3, qC
5	156.1, qC	156.1, qC	156.1, qC	156.0, qC	156.5, qC
6	103.2, qC	102.9, qC	103.5, qC[a]	103.2, qC	103.4, qC 103.3, qC
7	160.7, qC	160.6, qC	160.9, qC	160.7, qC	160.48, qC 160.45, qC
8	109.3, qC	109.1, qC	109.6, qC	109.3, qC	106.5, qC
9	159.5, qC	158.2, qC	159.0, qC	159.3, qC	159.8, qC
10	100.4, qC	100.9, qC	100.2, qC	100.3, qC	100.5, qC 100.4, qC
1′	128.8, qC[a]	126.8, qC	116.3, qC	129.0, qC	128.1, qC
2′	128.8, CH[a]	128.7, CH	155.3, qC	110.1, CH	128.6, CH
3′	114.0, CH	113.8, CH	103.5, CH[a]	149.1, qC	114.2, 114.1, CH
4′	160.3, qC	159.6, qC	161.2, qC	149.7, qC	160.3, qC
5′	114.0, CH	113.8, CH	107.3, CH	111.0, CH	114.2, 114.1, CH
6′	128.8, CH[a]	128.7, CH	127.9, CH	120.2, CH	128.6, CH
4″	115.4, CH	115.4, CH	115.3, CH	115.4, CH	115.4, CH
5″	126.2, CH	126.1, CH	126.5, CH	126.3, CH	126.2, CH
6″	78.5, qC	78.4, qC	78.7, qC	78.5, qC	79.2, qC
CH_3-6″	28.4, (2×) CH_3	28.4, 28.5, CH_3	28.4, (2×) CH_3	28.3, (2×) CH_3	28.6, (2×), 28.5, 28.4, CH_3
1‴	21.4, CH_2	21.3, CH_2	21.3, CH_2	21.3, CH_2	25.2, 25.1, CH_2
2‴	122.3, CH	122.2, CH	122.0, CH	122.2, CH	79.1, 78.9, CH
3‴	131.3, qC	131.3, qC	131.7, qC	131.3, qC	72.8, qC
CH_3-3‴	25.7, 17.8, CH_3	25.5, 17.9, CH_3	25.8, 17.9, CH_3	25.7, 17.8, CH_3	25.97, 25.91, 23.38, 23.37, CH_3
OCH_3-4′	55.3, CH_3	55.2, CH_3	55.3, CH_3	55.94, CH_3[b]	55.3, CH_3
OCH_3-3′				55.91, CH_3[b]	

[a] Overlapped signals. [b] Assignments may be reversed.

impling H-2 and H-3 to be *cis*. The CD spectrum showed a positive Cotton effect at 356 nm.[9] The absolute configurations at C-2 and C-3 of **2**, a C-3-epimer of **1**, could be proposed as 2*R*,3*S*, and **2** was given the trivial name khonklonginol B.

Compound **3** was obtained as a pale yellow liquid. The HRMS revealed a molecular formula of $C_{26}H_{28}O_7$. The ^{1}H and ^{13}C NMR signals were similar to those of compound **1** (Tables 1and 2), with differences detected among the aromatic proton signals. Instead of a 1,4-disubstituted pattern as observed in **1** and **2**, the aromatic ring B was deduced as being trisubstituted. Assignments of the signals at δ_H 7.42 (brd, J = 8.6 H) for H-6′, δ_H 6.58 (d, J = 8.7 Hz) for H-5′, and δ_H 6.54 (d, J = 2.5 Hz) for H-3′ were based on the long-range ^{1}H−^{13}C correlations between H-2 (δ_H 5.28)/C-1′ (δ_C 116.3, qC), C-2′ (δ_C 155.3, qC), C-6′ (δ_C 127.9, CH), as well as H-3′/C-1′, C-2′, C-4′ (δ_C 161.2, qC), C-5′ (δ_C 107.3, CH). The ^3J correlations of OCH_3 (δ_H 3.79), H-6′, and H-5′ with C-4′ required placement of the OCH_3 group at C-4′, thus implying the presence of an OH group at C-2′. Compound **3** (khonklonginol C) was established as 3,5,2′-trihydroxy-4′-methoxy-6″,6″-dimethylpyrano(2″,3″:7,6)-8-(3‴,3‴-dimethylallyl)flavanone.

Table 3. ^{1}H and ^{13}C NMR Spectroscopic Data of **6**−**8** (in $CDCl_3$, δ ppm, mult. J in Hz)

	6		**7**		**8**	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2		145.4, qC	5.33, dd (12.8, 2.7)	78.6, CH	5.54, dd (13.0, 3.0)	77.7, CH
3		135.5, qC	3.03, dd (17.1, 12.8) 2.78, dd (17.1, 2.7)	43.3, CH_2	3.09, dd (17.4, 13.1) 2.86, dd (17.3, 3.1)	41.9, CH_2
4		175.5, qC		196.4, qC		196.4, qC
5		153.0, qC		156.6, qC		156.8, qC
6		104.9, qC		102.7, qC		103.3, qC
7		156.9, qC		159.3, qC		159.8, qC
8		107.7, qC		108.6, qC		108.8, qC
9		153.6, qC		159.8, qC[a]		158.6, qC
10		103.5, qC		102.8, qC		102.7, qC
1′		123.7, qC		130.9, qC		116.6, qC
2′	8.15, d (8.8)	129.3, CH	7.35, d (8.4)	127.5, CH		155.4, qC
3′	7.01, d (8.8)	114.1, CH	6.92, d (8.5)	114.1, CH	6.46, d (2.2)	102.9, CH
4′		161.0, qC		159.8, qC[a]		161.2, qC
5′	7.01, d (8.8)	114.1, CH	6.92, d (8.5)	114.1, CH	6.49, dd (8.5, 2.2)	106.4, CH
6′	8.15, d (8.8)	129.3, CH	7.35, d (8.4)	127.5, CH	7.16, d (8.4)	127.9, CH
4″	6.72, d (9.9)	115.7, CH	6.61, d (10.0)	115.7, CH	6.62, d (9.9)	115.6, CH
5″	5.62, d (10.1)	128.1, CH	5.48, d (10.0)	125.9, CH	5.50, d (9.9)	126.3, CH
6″		77.8, qC		78.1, qC		78.3, qC
CH_3-6″	1.45, s	28.3, CH_3	1.43, 1.41, s	28.4, 28.3, CH_3	1.42, 1.43, s	28.4, 28.3, CH_3
1‴	3.49, d (7.0)	21.5, CH_2	3.19, t (7.2)	21.5, CH_2	3.20, t (7.0)	21.4, CH_2
2‴	5.21, dt (7.0, 6.1)	122.2, CH	5.15, t (7.2)	122.6, CH	5.09, t (7.2)	122.3, CH
3‴		131.8, qC		131.0, qC		131.8, qC
CH_3-3‴	1.67, 1.82, s	25.7, 18.0, CH_3	1.63, s (2 ×)	25.8, 17.8, CH_3	1.65, s (2 ×)	25.7, 17.8, CH_3
O*H*-5	11.93, s		12.24, s		12.23, s	
OCH_3-4′	3.87, s	55.3, CH_3	3.82, s	55.3, CH_3	3.77, s	41.9, CH_3
OH-	6.63, brs (O*H*-3)				6.26, brs (O*H*-2′)	

[a] Overlapped signals.

Compound **4** was isolated as a yellow liquid and assigned a molecular formula of $C_{27}H_{30}O_7$ based on the $[M + 1]^+$ ion at m/z 467.2063 in the HRESIMS. The ^{1}H NMR spectrum indicated **4** to have a core skeleton similar to those of **1** and **3**. The aromatic ring B protons revealed a trisubstituted pattern as in compound **3**, but with some differences. The partially overlapped doublet of doublets signal at δ_H 7.08 (J = 8.8, 2.0 Hz), a doublet signal at δ_H 7.07 (J = 2.0 Hz), and a doublet at δ_H 6.91 (J = 8.8 Hz) were assigned to H-6′, H-2′, and H-5′, respectively, due to the ^3J correlations in the HMBC spectrum between H-2 (δ_H 4.97)/C-2′ (δ_C 110.1, CH), C-6′ (δ_C 120.2, CH), and C-1′ (δ_C 129.0, qC), and of H-2′, H-5′, and H-6′ with C-1′. HMBC correlations also led to the assignment of signals at δ_H 3.91 and 3.90 for O*CH*$_3$-3′ and O*CH*$_3$-4′, respectively. Compound **4** (khonklonginol D) was proposed as 3,5-dihydroxy-3′,4′-dimethoxy-6″,6″-dimethylpyrano(2″, 3″: 7,6)-8-(3‴,3‴-dimethylallyl)flavanone.

Compound **5** was obtained as a yellow liquid and assigned a molecular formula of $C_{26}H_{30}O_8$ from its HRESIMS, with the $[M + Na]^+$ ion at m/z 493.1830. The ^{1}H and ^{13}C NMR spectra of **5** were similar to those of **1** (Tables 1and 2) except for the absence of signals for a dimethylallyl group. Two sets of partially overlapped signals for an oxymethine proton at δ_H 3.45 and 3.42, as well as for benzylic protons at δ_H 2.76, 2.75, 2.53, and 2.51, indicated the presence of two forms of a vicinal diol at C-2‴ and C-3‴, as reported for 2‴,3‴-dihydroxylupinifolin.[11] Compound **5** (khonklonginol E) was proposed as 3,5-dihydroxy-4′-methoxy-6″,6″-dimethylpyrano(2″,3″:7,6)-8-(3‴,3‴-dimethyl-2‴,3‴-dihydroxypropyl)flavanone.

Compound **6** was obtained as a pale yellow solid with a molecular formula of $C_{26}H_{26}O_6$. The ^{1}H NMR spectrum exhibited signals for chromene, dimethylallyl, and chelated OH groups (Table 3). Two pairs of doublet signals at δ_H 8.15 and 7.01 (both corresponding to 2H, J = 8.8 Hz) of the 1,4-disubstituted aromatic ring were also observed. The OCH_3 group that resonated at δ_H 3.87 was assigned at C-4′, as indicated from the HMBC correlations between H-2′, H-6′, and O*CH*$_3$/C-4′. The molecular weight was determined as 2 amu lower than that of compound **1**. This information, along with the absence of ^{1}H NMR doublet signals for the H-2 and H-3 protons at approximately δ_H 4.98 and 4.42, as observed in **1**−**4**, and the presence of two quaternary carbon signals at δ_C 145.4 and 135.5, in addition to the HMBC correlations of a hydroxy proton signal at δ_H 6.63/C-2 (δ_C 145.4) and C-4, implied the presence of a double bond at C-2 and a hydroxy group at C-3. Compound **6** (khonklonginol F) could thus be established as a flavonol and was elucidated as 3,5-dihydroxy-4′-methoxy-6″,6″-dimethylpyrano(2″,3″:7,6)-8-(3‴,3‴-dimethylallyl)flavone.

Compound **7** was obtained as a pale yellow, amorphous solid, and its mass spectrum exhibited a $[M + 1]^+$ ion at m/z 421.2012, corresponding to a molecular formula of $C_{26}H_{28}O_5$. The ^{1}H NMR spectrum (Table 3) also showed the presence of a chelated hydroxyl proton, a 1,4-disubstituted aromatic ring, a dimethyl chromene, and a dimethylallyl group. The location of each functional group was confirmed by the use of 2D NMR spectroscopic techniques, suggesting that these groups are present at similar positions to **1** and **2**. Two missing doublets at ca. δ_H 4.98 and 4.42 were replaced by resonances for an ABX system at δ_H 5.33 (1H, dd, J = 12.8, 2.7 Hz), 3.03 (1H, dd, J = 12.8, 17.1 Hz), and 2.78 (1H, dd, J = 17.1, 2.7 Hz) of a flavanone. The configuration at C-2 was assigned as *S* based on a vicinal coupling constant of 12.8 Hz, in comparison to those of previously reported flavanones.[12] Compound **7** (khonklonginol G) was thus identified as 5-hydroxy-4′-methoxy-6″,6″-dimethylpyrano(2″,3″:7,6)-8-(3‴,3‴-dimethylallyl)flavanone.

Compound **8** was obtained as a yellow liquid. The molecular formula of $C_{26}H_{28}O_6$ was based on its HRESIMS, with the $[M + 1]^+$ ion at m/z 437.1951. The ^{1}H and ^{13}C NMR data of compound **8** were very similar to those of **7** (Table 3), except for aromatic proton signals at δ_H 6.46 (d, J = 2.2 Hz), 6.49 (dd, J = 8.5, 2.2 Hz), and 7.16 (d, J = 8.4 Hz), implying a trisubstituted aromatic ring. The key HMBC correlations between H-2 and δ_C 116.6 (qC, C-1′), 155.4 (qC, C-2′), and 127.9 (CH, C-6′), and H-6′ (δ_H 7.16)/C-2′, C-4′ (δ_C 161.2, qC) as well as O*CH*$_3$/C-4′ required the placement of the OCH_3 and OH groups at C-4′ and C-2′, respectively. Compound **8** (khonklonginol H) was concluded as being 5,2′-dihydroxy-4′-methoxy-6″,6″-dimethylpyrano(2″,3″:7,6)-8-(3‴,3‴-dimethylallyl)flavanone.

Table 4. Biological Activity of Some Isolates from *E. chinense*

compound	anti-TB[a]	KB[b]	NCI H187[b]	Vero cell[b]
1	25	3.1	3.0	7.9
2	50	3.8	4.3	6.9
6	100	6.7	2.4	7.0
8	25	5.4	3.3	6.4
9	25	1.73	3.5	nd[c]
10	12.5	5.8	3.9	11.1
11	12.5	3.3	2.1	nd[c]
12	12.5	5.8	6.0	nd[c]
13	12.5	2.4	6.5	nd
hexane extract	50	12.0	9.9	5.8
ellipticine[d]		0.37	0.44	
doxorubicin[d]		0.12	0.042	
isoniazid[d]	0.023−0.046			
streptomycin[d]	0.156−0.313			

[a] MIC in μg/mL. [b] IC_{50} in μg/mL. [c] nd = not determined. [d] Positive control substance.

Cytotoxicity against small-cell lung (NCI-H187) and oral epidermal carcinoma (KB) human cell lines and antimycobacterial activity against *Mycobacterium tuberculosis* H37Ra of the isolates were evaluated, with the results are presented in Table 4. Among the three cytotoxic 3-hydroxylflavanones (**1**, **2**, **9**), the inhibitory activity against the NCI-H187 cell line was found to be comparable, and **9** was the most active against the KB cell line. Compound **11**, with a linear pyrano ring, was found to be more active than **12**, a regioisomer of **11** with an angular pyrano ring, against both cell lines. Compounds **10**−**13** were the most active antimycobacterial substances, and all exhibited a MIC value of 12.5 μg/mL. Compounds **1**, **8**, and **9** were less potent, with a MIC value of 25 μg/mL.

Experimental Section

General Experimental Procedures. Melting points were measured using an Electrothermal melting point apparatus and are uncorrected. Optical rotations were recorded on a JASCO DIP 1020 polarimeter and CD spectra obtained on a JASCO J-810 spectropolarimeter. The IR spectra were run on a Perkin-Elmer 1760x FT-IR spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AVANCE 400 MHz spectrometer. Chemical shifts are referenced to the residual solvent signals ($CDCl_3$: δ_H 7.24 and δ_C 77.0 ppm). HRESIMS and HREIMS were recorded on a Bruker Daltonics microTOF instrument and a Finnigan MAT 8200 mass spectrometer, respectively.

Plant Material. The roots of *Eriosema chinense* (Leguminosae-Papilionoideae), known in Thailand as "Toon Khonklong" and "Haeo Praduu", were collected from Kangsrikotr subdistrict, Siridhorn district, Ubonrachathani Province, Thailand, in June 2003. The plant was identified by Assoc. Prof. Dr. Wongsatit Chuakul, Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University, Thailand, and also by comparison with the herbarium collections (SN 218063 BK 60048 of Bangkok Herbarium, Sirindhorn Museum, Department of Agriculture, Ministry of Agriculture and Cooperatives). A voucher specimen (SSECH/2003) is maintained at the Chemistry Department, Ramkhamhaeng University.

Extraction and Isolation. The dried roots (6.3 kg) were extracted successively with hexane, CH_2Cl_2, and MeOH using a Soxhlet extractor to obtain hexane (116.0 g), CH_2Cl_2 (53.5 g), and MeOH (490.3 g) extracts, respectively. The hexane extract (115 g) was fractionated using silica gel column chromatography with gradients of hexane−CH_2Cl_2 and CH_2Cl_2−MeOH to give six major fractions. Fraction 2 (6.02 g) was subjected to silica gel column chromatography (hexane−CH_2Cl_2, 50:50, to CH_2Cl_2−MeOH, 50:50) to obtain 11 subfractions (2.1−2.11). Subfraction 2.5 after reversed-phase column chromatography (C_{18}, H_2O−MeOH, 15:85) yielded **1** (175 mg). Subfraction 2.8 (523 mg) after reversed-phase column chromatography (C_{18}, H_2O−MeOH, 30:70) gave **6** (18 mg). Fraction 3 (6.5 g) was column chromatographed (silica gel, hexane−CH_2Cl_2, 30:70, to CH_2Cl_2−MeOH, 50:50) to give nine subfractions (3.1−3.9), of which subfraction 3.5 (134 mg) after purification using reversed-phase column chromatography (C_{18}, H_2O−MeOH, 30:70) gave **2** (24 mg). Subfraction 3.6 after purification using silica gel column chromatography (hexane−EtOAc, 92:8 to 0:100) yielded compounds **8** (21 mg) and **13** (83 mg). Fraction 4 (3.8 g) was fractionated using silica gel column chromatography (hexane−CH_2Cl_2, 45:55, to CH_2Cl_2−MeOH, 50:50) to give six subfractions (4.1−4.6). Subfraction 4.2 (673.5 mg) yielded compounds **3** (4.5 mg), **4** (8.7 mg), and **6** (18 mg), after reversed-phase column chromatography (C_{18}, H_2O−MeOH, 20:80).

The CH_2Cl_2 extract (53.5 g) was fractionated using silica gel column chromatography with gradient elution (hexane−CH_2Cl_2, 80:20, to CH_2Cl_2−MeOH, 50:50) to obtain seven fractions. Fraction 3 was column chromatographed (silica gel, hexane−CH_2Cl_2, 50:50, to CH_2Cl_2−MeOH, 95:5) to afford six subfractions (3.1−3.6). Subfraction 3.4 gave an additional quantity of **1** (77 mg) and **7** (9 mg) after column chromatography (silica gel, hexane−CH_2Cl_2, 60:40, to CH_2Cl_2−MeOH, 50:50). Fraction 5 was purified using column chromatography (hexane−CH_2Cl_2, 99:1, to CH_2Cl_2−MeOH, 50:50) to obtain **9** (309 mg). Fraction 6 (3.3 g) was further separated using silica gel column chromatography (hexane−CH_2Cl_2, 75:25, to CH_2Cl_2−MeOH, 50:50) to yield seven subfractions (6.1−6.7). Subfraction 6.2 after column chromatography using reversed-phase silica gel (C_{18}, H_2O−MeOH, 30:70 to 0:100) yielded **10** (7 mg). Subfraction 6.3 (1.2 mg) after column chromatography (silica gel, CH_2Cl_2−MeOH, 99.5:0.5 to 50:50) gave five subfractions (6.3.1−6.3.5). Purification of subfraction 6.3.2 by reversed-phase column chromatography (C_{18}, H_2O−MeOH 40:60 to 0:100) gave **15** (72 mg) and **9** (258 mg). Fraction 6.4 was purified using reversed-phase column chromatography (C_{18}, H_2O−MeOH, 40:60 to 0:100) to obtain **14** (6 mg) and **5** (24 mg). Subfraction 6.5 (457 mg) after silica gel column chromatography (CH_2Cl_2−MeOH, 99:1 to 50:50) gave six subfractions (6.5.5−6.5.6), and subfraction 6.5.4 gave **11** (26 mg), **12** (17 mg), **9** (210 mg), and an additional quantity of **1** (102 mg) after reversed-phase column chromatography (2×, C_{18}, H_2O−MeOH, 30:70 to 0:100, then H_2O−MeOH, 40:60 to 0:100).

Khonklonginol A [1; 3,5-Dihydroxy-4′-methoxy-6″,6″-dimethylpyrano(2″,3″:7,6)-8-(3‴,3‴-dimethylallyl)flavanone]: yellow liquid; $[\alpha]_D$ +18.9 (*c* 0.5, $CHCl_3$); CD nm (*c* 0.12, EtOH, 23 °C, rel [θ]) 359 (+6.68), 321 (−0.13), 301.5 (−19.58), 280.5 (−1.02), 274.5 (+70.15), 267.5 (+56.54); IR (KBr) ν_{max} 3467, 2975, 2926, 1626, 1516, 1463, 1379, 1289, 1251, 1191, 1126, 1026, 943, 907, 830, 737, 641, 575 cm^{-1}; ^{1}H and ^{13}C NMR data (in $CDCl_3$), see Tables 1 and 2; HREIMS $[M]^+$ *m*/*z* 436.1873 (calcd for $C_{26}H_{28}O_6$, 436.1886).

Khonklonginol B [2; 3,5-Dihydroxy-4′-methoxy-6″,6″-dimethylpyrano(2″,3″:7,6)-8-(3‴,3‴-dimethylallyl)flavanone]: yellow liquid; $[\alpha]_D$ −191.2 (*c* 0.29, $CHCl_3$); CD nm (*c* 0.25, EtOH, 23 °C, rel [θ]) 355 (+15.12), 338 (−0.17), 298.5 (−14.34); IR (KBr) ν_{max} 3436, 2975, 2926, 1645, 1622, 1583, 1515, 1463, 1380, 1298, 1252, 1192, 1135, 1120, 1102, 1030, 956, 890, 829, 771, 737, 637, 577, 524 cm^{-1}; ^{1}H and ^{13}C NMR data (in $CDCl_3$), see Tables 1 and 2; HRESIMS $[M + 1]^+$ *m*/*z* 437.1954 (calcd for $C_{26}H_{29}O_6$ 437.1956).

Khonklonginol C [3; 3,5,2′-Trihydroxy-4′-methoxy-6″,6″-dimethylpyrano(2″,3″:7,6)-8-(3‴,3‴-dimethylallyl)flavanone]: yellow liquid; $[\alpha]_D$ −66.5 (*c* 0.40, $CHCl_3$); CD nm (*c* 0.25, EtOH, 23 °C, rel [θ]) 365.5 (+13.62), 328 (+11.45), 306.5 (−24.37), 243.5 (+22.14); IR (KBr) ν_{max} 3391, 2973, 2925, 2855, 1622, 1520, 1462, 1379, 1287, 1239, 1192, 1165, 1124, 1024, 843, 765, 734, 634 cm^{-1}; ^{1}H and ^{13}C NMR data (in $CDCl_3$), see Tables 1 and 2; HRESIMS $[M + 1]^+$ *m*/*z* 453.1898 (calcd for $C_{26}H_{29}O_7$, 453.1905).

Khonklonginol D [4; 3,5-dihydroxy-3′,4′-dimethoxy-6″,6″-dimethylpyrano(2″,3″:7,6)-8-(3‴,3‴-dimethylallyl)flavanone]: yellow liquid; $[\alpha]_D$ −39.1 (*c* 0.98, $CHCl_3$); IR (KBr) ν_{max} 3430, 2926, 1645, 1627, 1518, 1463, 1379, 1291, 1264, 1190, 1164, 1125, 1026, 896, 851, 807, 765, 736, 608 cm^{-1}; ^{1}H and ^{13}C NMR data (in $CDCl_3$), see Tables 1 and 2; HRESIMS $[M + 1]^+$ *m*/*z* 467.2063 (calcd for $C_{27}H_{31}O_7$, 467.2061).

Khonklonginol E [5; 3,5-Dihydroxy-4′-methoxy-6″,6″-dimethylpyrano(2″,3″: 7,6)-8-(3‴,3‴-dimethyl-2‴,3‴-dihydroxypropyl)flavanone]: yellow liquid; $[\alpha]_D$ −1.9 (*c* 0.18, $CHCl_3$); IR (KBr) ν_{max} 3401, 2975, 2932, 1645, 1633, 1588, 1516, 1463, 1381, 1288, 1251, 1192, 1127, 1030, 879, 831, 776, 736, 575, 520 cm^{-1}; ^{1}H and ^{13}C NMR data (in $CDCl_3$), see Tables 1 and 2; HRESIMS $[M + Na]^+$ *m*/*z* 493.1830 (calcd for $C_{26}H_{30}O_8Na$, 493.1833).

Khonklonginol F [6; 3,5-Dihydroxy-4′-methoxy-6″,6′-dimethylpyrano(2″,3″:7,6)-8-(3‴,3‴-dimethylallyl)flavone]: yellow solid; $[\alpha]_D$ −19.0 (*c* 0.16, $CHCl_3$); IR (KBr) ν_{max} 3316, 2924, 2852, 1736, 1651, 1620, 1596, 1552, 1483, 1428, 1359, 1303, 1256, 1187, 1159, 1125, 1091, 1052, 1035, 999, 899, 878, 834, 803, 772, 648, 585, 508

cm^{-1}; ^{1}H and ^{13}C NMR data (in $CDCl_3$), see Table 3; HRESIMS [M + 1]$^+$ *m/z* 435.1800 (calcd for $C_{26}H_{27}O_6$, 435.1800).

Khonklonginol G [7; 5-Hydroxy-4′-methoxy-6″,6″-dimethylpyrano(2″,3″:7,6)-8-(3‴,3‴-dimethylallyl)flavanone]: yellow, amorphous solid; $[\alpha]_D$ −13.2 (*c* 0.39, $CHCl_3$); ν_{max} 2918, 2850, 1740, 1644, 1628, 1584, 1516, 1447, 1379, 1298, 1242, 1196, 1161, 1122, 1089, 1033, 900, 831, 740, 620 cm^{-1}; ^{1}H and ^{13}C NMR data (in $CDCl_3$), see Table 3; HRESIMS [M + 1]$^+$ *m/z* 421.2012 (calcd for $C_{26}H_{29}O_5$, 421.2010).

Khonklonginol H [8; 5,2′-Dihydroxy-4′-methoxy-6″,6″-dimethylpyrano(2″,3″:7,6)-8-(3‴,3‴-dimethylallyl)flavanone]: yellow liquid; $[\alpha]_D$ −29.6 (*c* 0.28, $CHCl_3$); IR (KBr) ν_{max} 3363, 2973, 2924, 1624, 1520, 1446, 1380, 1296, 1239, 1197, 1163, 1120, 1036, 945, 896, 835, 742, 680, 618 cm^{-1}; ^{1}H and ^{13}C NMR data (in $CDCl_3$), see Table 3; HRESIMS [M + 1]$^+$ *m/z* 437.1951 (calcd for $C_{26}H_{29}O_6$, 437.1959).

Dehydrolupinifolinol [10; 3,5,4′-Trihydroxy-6″,6″-dimethylpyrano(2″,3″:7,6)-8-(3‴,3‴-dimethylallyl)flavone]: ^{1}H NMR (in $CDCl_3$) δ_H 8.05 (2H, d, *J* = 8.9 Hz, H-2′, H-6′), 6.90 (2H, d, *J* = 8.9 Hz, H-3′, H-5′), 6.68 (1H, d, *J* = 10 Hz, H-4″), 5.59 (1H, d, *J* = 10 Hz, H-5″), 5.17 (1H, dd, *J* = 7.1, 5.8 Hz, H-2‴), 3.45 (2H, brd, *J* = 7.1 Hz, H-1‴), 1.77 (3H, s, C*H*$_3$-3‴), 1.63 (3H, s, C*H*$_3$-3‴), 1.41 (6H, s, C*H*$_3$-6″); ^{13}C NMR (in $CDCl_3$) δ_C 175.5 (C, C-4), 157.6 (C, C-4′), 157.0 (C, C-7), 153.6 (C, C-9), 153.0 (C, C-5), 145.4 (C, C-2), 135.4 (C, C-3), 131.8 (C, C-3‴), 129.5 (CH, C-2′, C-6′), 128.2 (CH, C-5″), 123.6 (C, C-1′), 122.2 (CH, C-2‴), 115.7 (CH, C-4″), 115.6 (CH, C-3′, C-5′), 107.7 (C, C-8), 104.9 (C, C-6), 103.6 (C, C-10), 77.9 (C, C-6″), 28.3 (2×, CH_3, CH_3-6″), 25.7 (CH_3, CH_3-3‴) 21.5 (CH_2, C-1‴), 18.1 (CH_3, CH_3-3‴).

Bioassays. The cytotoxicity assay was performed using the colorimetric method of Skehan and co-workers.[13] The antimycobacterial activity assay was performed against *Mycobacterium tuberculosis* H37Ra using the microplate Alamar Blue assay.[14]

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of compounds **1**−**8** (Figures S1−S16) and HMBC correlations of **1**−**8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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