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Claurailas A−D, Cytotoxic Carbazole Alkaloids from the Roots of *Clausena harmandiana*

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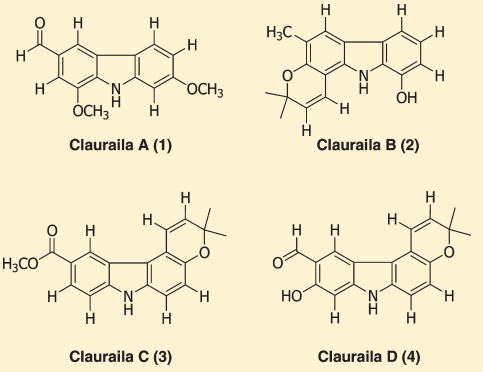
Supporting Information

ABSTRACT: Four new carbazole alkaloids, claurailas A−D (**1**−**4**), as well as 12 known carbazoles and three known coumarins were isolated from the roots of *Clausena harmandiana*. Heptaphylline (**6**) and 7-methoxyheptaphylline (**7**) showed strong cytotoxicity against NCI-H187 and KB cell lines with IC_{50} values ranging from 1.3 to 2.7 μM. Compound **7** showed no cytotoxicity against Vero cells.



Clausena harmandiana (Rutaceae), known in Thai as "Song Fa", is a medicinal plant that has shown some therapeutic activities against stomachache, headache, and sickness and as a health-promoting herb.[1] In the northeast of Thailand, the young leaves of this plant are used as a traditional vegetable and, in addition, as fodder for cattle and buffalo. Previously, the isolation of carbazole alkaloids and coumarins from the roots of this plant has been reported.[1] Biological activities of the chemical constituents from *C. harmandiana* have been evaluated as being antiplasmodial, antimalarial, anti-TB, and cytotoxic.[1,2] There are many reports on the biological activities of carbazoles as topoisomerase II inhibitors,[3] for antiplatelet aggregating and vasorelaxing activities,[4] as antitumor agents,[5] and as having cytotoxicity against leukemia HL-60 cell lines.[6] It has been reported that carbazoles exhibit antimicrobial,[7] anti-inflammatory,[8] anti HIV-1,[9] and antitrichomonal activity.[10] Coumarins from the *Clausena* genus exhibit antibacterial, antitumor promoting, antimycobacterial,[11] and antidiabetic[12] effects and showed an inhibitory effect on iNOS protein expression.[8]

In continuing our research on this plant, four new and 12 known carbazole alkaloids, as well as three known coumarins, have been isolated and identified by spectroscopic methods including 1D and 2D NMR (COSY, HMQC, and HMBC). All compounds were evaluated for their cytotoxicity against NCI-H187 (human small cell lung cancer), KB (oral human epidermal carcinoma), and Vero cell lines (African green monkey kidney, normal cells).

RESULTS AND DISCUSSION

Four new carbazole alkaloids, claurailas A−D (**1**−**4**), were isolated from the roots of *C. harmandiana* together with 12 known carbazoles, girinimbine (**5**),[13] heptaphylline (**6**),[2] 7-methoxyheptaphylline (**7**),[14] clausine E (**9**),[3] 3-formyl-1-hydroxy-7-methoxycarbazole (**10**),[15] *O*-demethylmurrayanine (**11**),[16] 7-hydroxyheptaphylline (**12**),[17] murrayanine (**15**),[18] 7-methoxymurrayanine (**16**),[4] clausine O (**17**),[4] lansine (**18**),[19] and clausine K (**19**),[19] and three known coumarins, xanthoxyletin (**8**), dentatin (**13**), and nordentatin (**14**).[11] All isolated compounds were tested for their cytotoxicity against NCI-H187, KB, and Vero cells lines, and their activities are shown in Table 3. The structures of the known compounds were characterized by spectroscopic methods, including IR, MS, and 1D and 2D NMR, and by comparison with literature values.

Claurailа A (**1**) was obtained as a yellow solid, mp 175−177 °C. It was assigned the molecular formula $C_{15}H_{14}NO_3$ as determined from its pseudomolecular ion peak at m/z 256.0969 $[M + H]^+$ in the HRESIMS. The IR spectrum showed an absorption band at 1714 cm^{-1}, while the ^{13}C NMR spectrum showed a signal at δ 191.9 indicating the presence of a carbonyl group. The ^1H NMR spectrum showed a singlet at δ 10.03, which was assigned to the formyl proton. The HMBC spectrum showed correlations between the formyl proton and C-2 (δ 102.9) and C-3 (δ 130.3). The ^1H NMR spectrum showed singlets at δ 7.41 and 8.04, which were assigned to H-2 and H-4, respectively. In addition, the HMQC spectrum showed correlations of H-2 and H-4 with carbons at δ 102.9 and 119.4, respectively. The HMBC spectrum showed correlation of these two protons with the formyl carbon (δ 191.9) and C-1a (δ 133.9), indicating the structure of a 1,3-disubstituted carbazole. The ^1H NMR and

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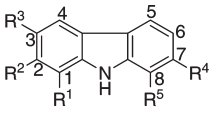
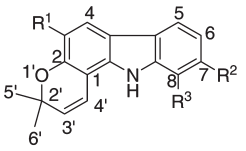
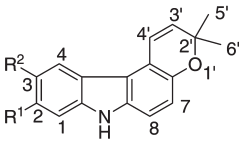


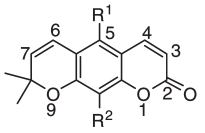
1 $R^1 = R^4 = OCH_3$, $R^2 = R^5 = H$, $R^3 = CHO$
6 R^1 = prenyl, $R^2 = OH$, $R^3 = CHO$, $R^4 = R^5 = H$
7 R^1 = prenyl, $R^2 = OH$, $R^3 = CHO$, $R^4 = OCH_3$, $R^5 = H$
9 $R^1 = OH$, $R^2 = R^4 = R^5 = H$, $R^3 = COOCH_3$
10 $R^1 = OH$, $R^2 = R^5 = H$, $R^3 = CHO$, $R^4 = OCH_3$
11 $R^1 = OH$, $R^2 = R^4 = R^5 = H$, $R^3 = CHO$
12 R^1 = prenyl, $R^2 = R^4 = OH$, $R^3 = CHO$, $R^5 = H$
15 $R^1 = OCH_3$, $R^2 = R^4 = R^5 = H$, $R^3 = CHO$
17 $R^1 = R^5 = H$, $R^2 = R^4 = OH$, $R^3 = CHO$
18 $R^1 = R^5 = H$, $R^2 = R^4 = OCH_3$, $R^3 = CHO$
19 $R^1 = R^5 = H$, $R^2 = R^4 = OCH_3$, $R^3 = COOH$



2 $R^1 = CH_3$, $R^2 = H$, $R^3 = OH$
5 $R^1 = CH_3$, $R^2 = R^3 = H$
16 $R^1 = CHO$, $R^2 = OCH_3$, $R^3 = H$



3 $R^1 = H$, $R^2 = COOCH_3$
4 $R^1 = OH$, $R^2 = CHO$



8 $R^1 = OCH_3$, $R^2 = H$
13 $R^1 = OCH_3$, R^2 = 1,1-dimethyl-2-propenyl
14 $R^1 = OH$, R^2 = 1,1-dimethyl-2-propenyl

Figure 1. Chemical structures of all compounds.

COSY spectra showed an AB system at δ 7.96 (1H, d, J = 8.6 Hz) and 6.93 (1H, d, J = 8.6 Hz), which were assigned to H-5 and H-6, respectively. The singlet at δ 6.97 was assigned to H-8, which correlated to carbons at C-6 (δ 109.6), C-5a (δ 117.4), and C-7 (δ 159.6) in the HMBC experiment. The correlation between methoxy protons (δ 3.91) and C-7 (δ 159.6) in the HMBC spectrum indicated that the methoxy group is attached to C-7 of the ring. Therefore, clauraila A was deduced to be **1**. Detailed assignments of protons and carbons of **1** are shown in Table 1.

Clauraila B (**2**) was obtained as a dark brown solid, mp 278−280 °C. It was assigned the molecular formula $C_{18}H_{18}NO_2$ as determined from its pseudomolecular ion peak at m/z 280.1339 $[M + H]^+$ in the HRESIMS. The IR spectrum showed an OH stretching band at 3434 cm^{-1}. The ^1H NMR spectrum showed a singlet at δ 7.62, which was assigned to H-4, and correlations of this signal with C-1a (δ 134.9), C-2 (δ 149.9), C-5a (δ 125.9), and the C-3 methyl group (δ 16.1) were seen in the HMBC spectrum. The C-3 methyl group (δ 2.33) displayed correlations to C-2 (δ 149.9), C-3 (δ 118.7), and C-4 (δ 121.3) in the HMBC spectrum. The ^1H NMR spectrum showed signals at δ 6.64 (1H, d, J = 9.7 Hz) and 5.68 (1H, d, J = 9.7 Hz), which were assigned to H-4′ and H-3′, respectively, and confirmed by the COSY spectrum. In the HMBC spectrum, correlations of H-4′ with C-1a (δ 134.9), C-1 (δ 104.7), C-2 (δ 149.9), and C-2′ (δ 75.9) were observed. The C-5′ and C-6′ methyl groups resonated as a singlet at δ 1.48 and displayed correlations to C-2′ (δ 75.9) and C-3′ (δ 129.3). The protons at δ 7.52 (1H, d, J = 7.8 Hz), 7.00 (1H, t, J = 7.8 Hz), and 6.74 (1H, d, J = 7.8 Hz) were assigned as H-5, H-6, and H-7, respectively. Correlations of H-7 with C-8a (δ 128.7) and C-5 (δ 112.3) were observed in the HMBC spectrum. The ^{13}C NMR spectrum showed the

Table 1. ^1H (400 MHz, $CDCl_3$) ^{13}C (100 MHz), and HMBC NMR Data of Claurailas A (1) and B (2)

	1			**2**		
no.	δ_H (J in Hz)	δ_C	HMBC[a]	δ_H (J in Hz)	δ_C	HMBC[a]
1		145.8			104.7	
1a		133.9			134.9	
2	7.41, s	102.9	C-1, 1a, 4, CHO		149.9	
3		130.3			118.7	
4	8.04, s	119.4	C-1a, 2, 5a, CHO	7.62, s	121.3	C-1a, 2, 5a, CH_3 on C-3
4a		123.9			117.1	
5	7.96, d (8.6)	121.4	C-7, 8a	7.52, d (7.8)	112.3	C-7, 8a
5a		117.4			125.9	
6	6.93, d (8.6)	109.6	C-5a	7.00, t (7.8)	119.7	C-5a, 8
7		159.6		6.74, d (7.8)	109.5	C-5, 8a
8	6.97, s	95.3	C-5a, 6, 7		140.9	
8a		140.8			128.7	
9	8.52, NH, s			8.12, NH, s		
2′					75.9	
3′				5.68, d (9.7)	129.3	C-1, 2′
4′				6.64, d (9.7)	117.3	C-1a, 1, 2, 2′
5′ and 6′				1.48, s	27.6	C-2′, 3′
CHO	10.03, s	191.9	C-2, 3			
CH_3				2.33, s	16.1	C-2, 3, 4
1-OCH_3	4.06, s	55.8	C-1			
7-OCH_3	3.91, s	55.7	C-7			

[a] HMBC correlations, optimized for 8 Hz, are from proton(s) stated to the indicated carbon.

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Table 2. ^{1}H (400 MHz, $CDCl_3$) ^{13}C (100 MHz), and HMBC NMR Data of Claurailas C (3) and D (4)

	3			4		
no.	δ_H (J in Hz)	δ_C	HMBC[a]	δ_H (J in Hz)	δ_C	HMBC[a]
1	7.36, d (8.6)	110.1	C-3, 4a	6.80, s	96.7	C-2, 3, 4a
1a		143.2			146.4	
2	8.09, d (8.6)	127.0	C-1a, 4		160.7	
3		122.9			115.5	
4	8.85, s	124.9	C-1a, 2, 5a, C=O	8.15, s	129.6	C-1a, 2, 5a, CHO
4a		121.0			117.8	
5		115.9			115.4	
5a		118.7			118.7	
6		147.2			147.7	
7	6.97, d (8.6)	116.2	C-5, 8a	6.89, d (8.5)	115.4	C-5, 6, 8a
8	7.19, d (8.6)	110.6	C-6, 5a	7.11, d (8.5)	110.4	C-6, 5a
8a		135.0			135.0	
9	8.23, NH, s			8.12, NH, s		
2′		75.4			75.4	
3′	5.87, d (9.9)	131.9	C-5, 2′	5.88, d (10.0)	132.4	C-5, 2′
4′	7.31, d (9.9)	119.8	C-6, 5, 5a	7.14, d (10.0)	119.4	C-6, 2′
5′ and 6′	1.50, s	27.2	C-3′, 2′	1.50, s	27.3	C-3′, 2′
C=O		167.9		9.91, s	195.1	C-2, 3
CH_3 (ester)	3.97, s	51.9	C=O			
OH				11.43, s		

[a] HMBC correlations, optimized for 8 Hz, are from proton(s) stated to the indicated carbon.

oxygen-bearing C-8 at δ 140.9 as correlating with H-6 in the HMBC experiment. From these data, clauraila B was characterized as **2**. Detailed assignments of protons and carbons of **2** are shown in Table 1.

Clauraila C (**3**) was obtained as a yellow solid, mp 208−210 °C. It was assigned the molecular formula $C_{19}H_{18}NO_3$ as determined from its pseudomolecular ion peak at m/z 308.1280 $[M + H]^+$ in the HRESIMS. The IR spectrum showed an absorption band at 1697 cm^{-1}, while the ^{13}C NMR spectrum showed a signal at δ 167.9, indicating the presence of an ester group. The singlet at δ 8.85 was assigned as H-4, which correlated with the carbonyl carbon (δ 167.9), C-1a (δ 143.2), C-5a (δ 118.7), and C-2 (δ 127.0) in the HMBC spectrum. The correlation between methoxy protons and the carbonyl carbon in the HMBC experiment indicated that the methyl ester group is attached to C-3. The ^{1}H NMR and COSY spectra displayed *ortho*-coupled protons at δ 7.36 (1H, d, J = 8.6 Hz) and 8.09 (1H, d, J = 8.6 Hz) assigned as H-1 and H-2, respectively. The HMQC spectrum showed correlations of H-1 and H-2 to carbons at δ 110.1 and 127.0, respectively. Two doublets at δ 7.31 (1H, d, J = 9.9 Hz) and 5.87 (1H, d, J = 9.9 Hz) were assigned to the *cis* olefinic protons, H-4′ and H-3′, respectively. The HMBC spectrum displayed correlations of H-4′ with C-6 (δ 147.2), C-5a (δ 118.7), and C-5 (δ 115.9), while H-3′ correlated with C-5 (δ 115.9) and C-2′ (δ 75.4). The doublets at δ 7.19 (1H, d, J = 8.6 Hz) and 6.97 (1H, d, J = 8.6 Hz) were assigned as H-8 and H-7, respectively. The correlations of H-7 with C-8a (δ 135.0) and C-5 (δ 115.9) and of H-8 with C-6 (δ 147.2) and C-5a (δ 118.7) were seen in the HMBC experiment. From the above spectroscopic evidence, clauraila C was determined to be **3**. Detailed assignments of protons and carbons of **3** are shown in Table 2.

Clauraila D (**4**) was obtained as a yellow solid, mp 227−229 °C. It was assigned the molecular formula $C_{18}H_{16}NO_3$ as determined from its pseudomolecular ion peak at m/z 294.1125 $[M + H]^+$ in the HRESIMS. The IR spectrum displayed an OH stretching band

Table 3. Cytotoxicity of Compounds[a]

	cytotoxicity (IC_{50}, μM)		
compound	NCI-H187[b]	KB[c]	Vero cells[d]
1	13.5	inactive[e]	inactive[e]
2	inactive[e]	inactive[e]	inactive[e]
3	inactive[e]	inactive[e]	inactive[e]
4	7.54	30.55	29.39
5	22.37	7.02	inactive[e]
6	1.32	1.61	92.47
7	1.68	2.75	inactive[e]
8	inactive[e]	133.12	inactive[e]
9	94.59	107.56	152.05
10	10.36	10.69	179.53
11	19.22	5.49	103.5
12	14.59	27.9	41.24
13	45.41	113.98	inactive[e]
14	11.81	20.62	56.35
15	11.45	72.23	inactive[e]
16	inactive[e]	inactive[e]	inactive[e]
17	74.73	59.55	64.61
18	102.01	inactive[e]	inactive[e]
19	138.83	inactive[e]	177.50
ellipticine	2.76	1.79	3.82

[a] Data shown are from triplicate experiments. [b] Human small cell lung cancer. [c] Human epidermoid carcinoma. [d] African green monkey kidney. [e] Inactive at >200 μM.

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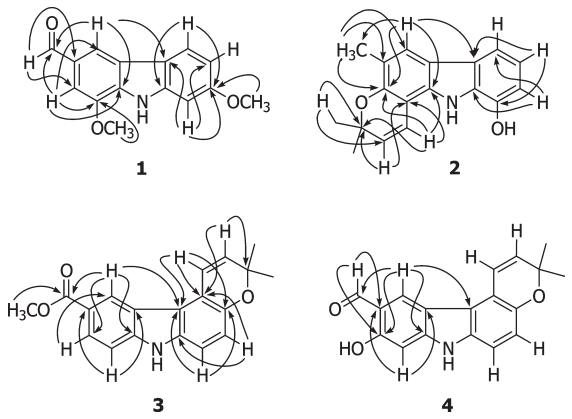

Figure 2. Key HMBC correlations for claurailas A−D (**1**−**4**).

at 3413 cm^{-1} and a carbonyl group at 1623 cm^{-1}. The ^1H NMR spectrum showed a low-field chemical shift of the hydroxy proton at δ 11.43, indicating intramolecular hydrogen bonding. The formyl proton showed a singlet at δ 9.91 and correlated with the carbonyl carbon (δ 195.1) in the HMQC spectrum. The HMBC experiment revealed correlations of the formyl proton to C-3 (δ 115.5) and C-2 (δ 160.7). The pyranocarbazole structure of this compound displayed similar proton and carbon signals to compound **3**. Two doublets (J = 10.0 Hz) at δ 7.14 and 5.88 were suggested to be H-4′ and H-3′, respectively, and two doublets (J = 8.5 Hz) at δ 6.89 and 7.11 were assigned to H-7 and H-8, respectively. The ^{13}C NMR signals at δ 147.7 and 135.0 were assigned as C-6 and C-8a, respectively. Therefore, clauraila D was characterized as **4**. Detailed assignments of protons and carbons of **4** are shown in Table 2.

Biological Activities (refs 20, 21**).** Ellipticine was used as a reference standard for cytotoxicity testing. Almost all obtained compounds showed weaker cytotoxicity than the ellipticine standard except compounds **6** and **7**. Compound **1** showed cytotoxicity against the NCI-H187 cell line with an IC_{50} value of 13.5 μM, which is about a 7.5-fold higher toxicity than **18** (IC_{50} = 102.01 μM) (Table 3). The results suggest that the C-1 methoxy group is required for activity to the NCI-H187 cell line. In addition, **1** exhibited no cytotoxicity against normal cells (Vero cells). Compound **15** showed cytotoxicity against the NCI-H187 cell line with an IC_{50} value of 11.45 μM. It should be noted that compounds **1**, **10**, and **15** showed weak and no cytotoxicity against normal cells. Compounds **10** and **11** showed cytotoxicity against the NCI-H187 cell line with IC_{50} values of 10.36 and 19.22 μM, respectively. The results demonstrate that the C-1 methoxy or hydroxy group and the C-3 formyl group may play an important role in cytotoxicity against this cell line. In addition, **10** and **11** showed cytotoxicity against the KB cell line with IC_{50} values of 10.69 and 5.49 μM, respectively, while **1** and **15** showed a weak effect and were inactive against this cell line. The results suggest that the C-1 hydroxy group is required for activity in the KB cell line. On the other hand, ester **9** showed weak cytotoxicity against all cell lines. The results indicate that the formyl group is essential for cytotoxicity. Interestingly, **6** and **7** showed strong cytotoxicity against the NCI-H187 and KB cell lines with IC_{50} values ranging from 1.3 to 2.75 μM. On the other hand, **12** showed about 8- to 17-fold weaker cytotoxicity than **6** and **7**. The results suggest that prenyl, hydroxy, and formyl groups at C-1, C-2, and C-3, respectively, are essential for cytotoxicity, but the C-7 hydroxy group led to a dramatic loss of the activity. It should be noted that carbazoles **1**, **10**, and **15** showed moderate cytotoxicity against the NCI-H187 cell line and exhibited no and weak cytotoxicity to normal cells. In addition, carbazole **5** demonstrated stronger cytotoxicity against KB cells than Vero cells.

Among the coumarins **8**, **13**, and **14**, only **14** exhibited cytotoxicity against the NCI-H187, KB, and Vero cells, with IC_{50} values of 11.81, 20.62, and 56.35 μM, respectively. It is suggested that the C-5 hydroxy group and the C-10 1,1-dimethylallyl group are important for the activity.

EXPERIMENTAL SECTION

General Experimental Procedures. NMR spectra were recorded on a Varian Mercury Plus spectrometer operating at 400 MHz (^1H) and at 100 MHz (^{13}C). IR spectra were recorded as KBr disks using a Perkin-Elmer Spectrum One FT-IR spectrophotometer. Mass spectra were determined on a Micromass Q-TOF 2 hybrid quadrupole time-of-flight (Q-TOF) mass spectrometer with a Z-spray ES source (Micromass, Manchester, UK). Melting points were determined on a Sanyo Gallenkamp melting point apparatus and were uncorrected. Thin-layer chromatography (TLC) was carried out on Merck silica gel 60 F_{254} TLC aluminum sheets. Column chromatography was performed with silica gel 0.063−0.200 mm or less than 0.063 mm. Preparative layer chromatography (PLC) was carried out on glass supported silica gel plates using silica gel 60 PF_{254} for preparative layer chromatography. All solvents were routinely distilled prior to use.

Plant Material. The roots of *C. harmandiana* were collected in April 2008 from Khon Kaen Province. The plant was identified by Dr. Pranom Chantaranothai, Faculty of Science, Khon Kaen University. A botanically identified voucher specimen (KK9807179) was deposited at the herbarium of The Forest Herbarium.

Extraction and Isolation. Air-dried and finely powdered roots (3.5 kg) of *C. harmandiana* were sequentially extracted at room temperature for three days with hexanes (2 × 5 L), EtOAc (2 × 5 L), and MeOH (2 × 5 L). The extracts were evaporated in vacuo to obtain three dry extracts, crude hexanes (60 g), crude EtOAc (110 g), and crude MeOH (405 g). The crude EtOAc extract (110 g) was subjected to column chromatography on silica gel 60 and subsequently eluted with a gradient of three solvents (hexanes, EtOAc, and MeOH) by gradually increasing the polarity of the elution solvents system. The eluents were collected and monitored by TLC, producing nine groups of eluting fractions, which were designated F1 to F9. Purification of F2 was performed on a silica gel column and elution with a gradient system of hexanes−CH_2Cl_2 to afford **5** (15 mg), **6** (8.0 g), **7** (12.2 g), and **8** (1.3 g). Fraction F3 was subjected to silica gel column chromatography using gradient elution of hexanes−EtOAc mixtures to furnish **9** (15 mg), **10** (10 mg), and **11** (12 mg). Fraction F4 was further purified by silica gel column chromatography and eluted with hexanes−EtOAc mixtures of increasing polarity, yielding **12** (40 mg) and **13** (50 mg). Purification of F5 was performed by a silica gel column chromatography and eluted with a gradient system of hexanes−CH_2Cl_2, affording five subfractions (F5.1−F5.5). Subfraction F5.3 was purified by preparative TLC using hexanes−CH_2Cl_2 (2:1) as developing solvent to give **1** (11 mg). Subfraction F5.4 was subjected to silica gel column chromatography using elution of hexanes−CH_2Cl_2 mixtures to yield **2** (10 mg) and **14** (30 mg). Fraction F6 was purified by preparative TLC using hexanes−CH_2Cl_2 (1:1) to yield **4** (13 mg) and **3** (10 mg). Fraction F7 was purified by silica gel column chromatography and eluted with hexanes−EtOAc to provide **15** (17 mg) and **16** (15 mg). Purification of F8 was carried out on silica gel column chromatography and eluted with hexanes−EtOAc to furnish **17** (13 mg). Rechromatography of F9 was performed on silica gel column

chromatography eluted with hexanes and a gradient of EtOAc to provide **18** (20 mg) and **19** (1.2 g).

Clauraila A (***1***):. yellow solid; UV ($CHCl_3$) λ_{max} (log ε) 245 (6.03), 342 (5.47) nm; IR (KBr) ν_{max} 2924, 1714, 1641, 1020 cm^{-1}; ^1H, ^{13}C, and 2D NMR, see Table 1; HRESIMS m/z 256.0969 $[M + H]^+$ (calcd for $C_{15}H_{14}NO_3$, 256.0974).

Clauraila B (***2***):. dark brown solid; UV ($CHCl_3$) λ_{max} (log ε) 245 (6.03), 342 (5.47) nm; IR (KBr) ν_{max} 3434, 1632, 1020 cm^{-1}; ^1H, ^{13}C, and 2D NMR, see Table 1; HRESIMS m/z 280.1339 $[M + H]^+$ (calcd for $C_{18}H_{18}NO_2$, 280.1334).

Clauraila C (***3***):. yellow solid; UV (CH_3OH) λ_{max} (log ε), 227 (6.32), 292 (6.41) nm; IR (KBr) ν_{max} 1697, 1615, 1294 cm^{-1}; ^1H, ^{13}C, and 2D NMR, see Table 2; HRESIMS m/z 308.1280 $[M + H]^+$ (calcd for $C_{19}H_{18}NO_3$, 308.1287).

Clauraila D (***4***):. yellow solid; UV (CH_3OH) λ_{max} (log ε) 305 (6.66), 326 (6.56) nm; IR (KBr) ν_{max} 3413, 1623, 1286 cm^{-1}; ^1H, ^{13}C, and 2D NMR, see Table 2; HRESIMS m/z 294.1125 $[M + H]^+$ (calcd for $C_{18}H_{16}NO_3$, 294.1131).

Cytotoxicity Assay. The cytotoxicity assay against the human epidermoid carcinoma (KB) and human small cell lung cancer (NCI-H187) cell lines was performed employing the resazurin microplate assay (REMA),[20] while cytotoxicity assay against Vero cells (African green monkey kidney) was performed by the green fluorescent protein (GFP)-based assay.[21] Ellipticine was included as a reference substance.

■ ASSOCIATED CONTENT

Supporting Information. ^1H and ^{13}C NMR spectra for **1**−**4** are provided free of charge via the Internet at http://pubs.acs.org.

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