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# Bioactive Carbazole Alkaloids from *Clausena wallichii* Roots

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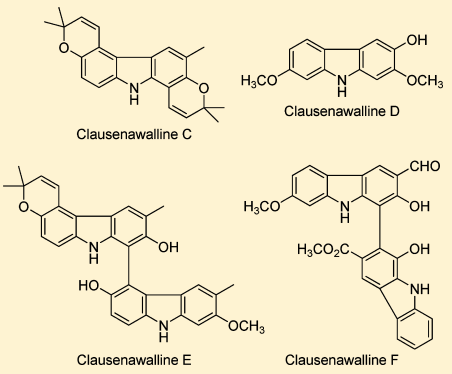
Supporting Information

**ABSTRACT:** Four new carbazole alkaloids, clausenawallines C–F (**1**–**4**), along with 18 known compounds (**5**–**22**) were isolated from the roots of *Clausena wallichii*. Compounds **3**, **9**, and **22** exhibited significant antibacterial activity against methicillin-resistant *Staphylococcus aureus* SK1 (MRSA SK1) and *Staph. aureus* TISTR 1466 with MIC values in the range 4–16 μg/mL, whereas compound **4** showed the highest cytotoxicity against oral cavity cancer (KB) and small-cell lung cancer (NCI-H187) with $IC_{50}$ values of 10.2 and 4.5 μM, respectively.




Rutaceae plants are rich sources of coumarins, amides, and carbazole alkaloids.[1−4] The *Clausena* genus of the Rutaceae family is known to produce carbazole alkaloids that possess a number of interesting pharmacological activities including antimalarial, antibacterial, anti-TB, anti-HIV reverse transcriptase-inhibitory, and cytotoxic activities.[5−7] In our continuation of the study of biologically active natural products from *Clausena* plants,[8−11] we report here the isolation and structure elucidation of four new (**1**–**4**) and 18 known carbazole alkaloids (**5**–**22**) from the roots of *C. wallichii* Oliv. The antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus* TISTR 1466 and methicillin-resistant *Staph. aureus* SK1) and Gram-negative bacteria (*Escherichia coli* TISTR 780 and *Salmonella typhimurium* TISTR 292), as well as the cytotoxicity against three human cancer cell lines, oral cancer (KB), breast cancer (MCF-7), and small-cell lung cancer (NCI-H187), are also reported.

## RESULTS AND DISCUSSION

The acetone extract of the roots of *C. wallichii* was subjected to column chromatography over Si gel to give four new carbazole alkaloids (**1**–**4**) together with 18 known compounds, **5**–**22** (Figure 1).

Clausenawalline C (**1**) was obtained as a yellow solid, mp 239–240 °C. The molecular formula, $C_{23}H_{24}NO_2$, was assigned from the ESI-TOF-MS ion peak at *m*/*z* 346.1790 $[M + H]^+$ (calcd 346.1807). The UV spectrum showed absorption maxima bands at $\lambda_{max}$ 206, 235, 295, 310, 337, 349, 390, and 399 nm. The IR spectrum indicated the presence of an NH stretching band at 3299 $cm^{-1}$,[12] which was further supported by the $^1H$ NMR N*H* resonance at δ 7.71 (br s) in the spectrum (Table 1). A methyl singlet at δ 2.32 was assigned to the aryl methyl group located at C-3 of the carbazole skeleton based on its $^2J$ and $^3J$ HMBC correlations (Table 1) to C-2 (δ 149.5), C-3 (δ 119.3), and C-4 (δ 123.2). The $^1H$ NMR spectrum also displayed a set of *ortho*-coupled aromatic signals at δ 6.82 (1H, d, *J* = 8.8 Hz, H-7) and 7.12 (1H, d, *J* = 8.8 Hz, H-8), together with an aromatic singlet at δ 7.71 (1H, s, H-4). Two 2*H*-pyran moieties were also evident. One [δ 6.59 (1H, d, *J* = 9.6 Hz, H-1′), 5.68 (1H, d, *J* = 9.6 Hz, H-2′), and 1.47 (6H, s, H-4′ and H-5′)] was located at C-1/C-2 on the basis of the $^2J$ and $^3J$ correlations between H-1′ (δ 6.59) and C-2 (δ 149.5), C-9a (δ 136.0), C-1 (δ 104.3), and C-3′ (δ 75.8) in the HMBC spectrum. The other [δ 7.20 (1H, d, *J* = 9.6 Hz, H-1″), 5.79 (1H, d, *J* = 9.6 Hz, H-2″), and 1.47 (6H, s, H-4″and H-5″)] was located at C-5/C-6 on the basis of the HMBC correlations between H-1″ (δ 7.20) and C-6 (δ 146.6), C-4b (δ 119.3), C-5 (δ 114.9), and C-3″ (δ 75.0). Detailed assignments of the protons and carbons as well as HMBC correlations are shown in Table 1, facilitating assignment of structure **1** to clausenawalline C.

Clausenawalline D (**2**) was obtained as a brown solid, mp 247–249 °C. Its molecular formula was determined as $C_{14}H_{14}NO_3$ ($[M + H]^+$ *m*/*z* 244.0968, calcd 244.0974) by ESI-TOF-MS. The UV and IR spectra were similar to that of **1**, indicating compound **2** had the same carbazole alkaloid skeleton. Analysis of its NMR spectra, including COSY, HMQC, and HMBC data, allowed unambiguous assignment of all proton and carbon signals. The $^1H$ NMR data (Table 1)

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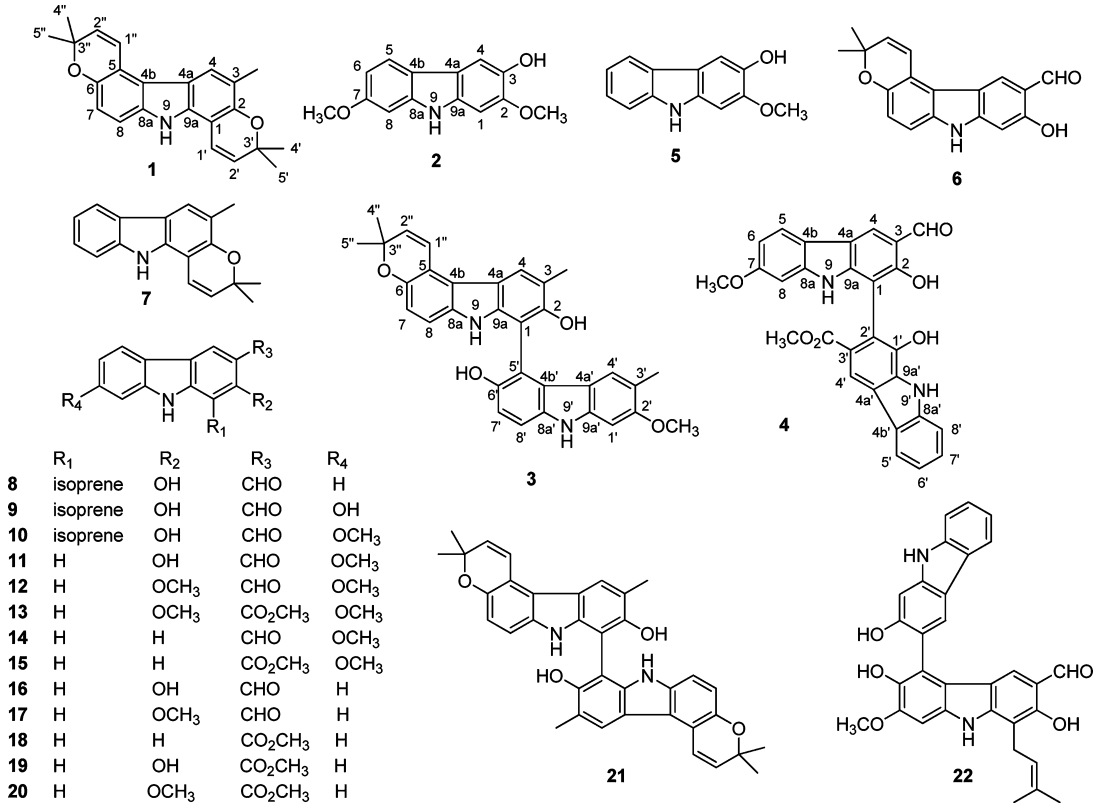


**Figure 1.** Carbazole alkaloids from *C. wallichii* roots.

**Table 1. NMR Spectroscopic Data (400 MHz) for Clausenawallines C (1) and D (2)**

| | 1[a] | | | 2[b] | | |
|---|---|---|---|---|---|---|
| position | $\delta_H$ ($J$ in Hz) | $\delta_C$ | HMBC | $\delta_H$ ($J$ in Hz) | $\delta_C$ | HMBC |
| 1 | | 104.3 | | 7.03, s | 95.2 | 2, 3, 4a |
| 2 | | 149.5 | | | 147.5 | |
| 3 | | 119.3 | | | 142.0 | |
| 4 | 7.71, s | 123.2 | 2, 4b, 9a, 3-$CH_3$ | 7.43, s | 105.2 | 2, 3, 4b, 9a |
| 4a | | 110.1 | | | 118.2 | |
| 4b | | 119.3 | | | 117.0 | |
| 5 | | 114.9 | | 7.78, d (8.4) | 120.6 | 4a, 7, 8a |
| 6 | | 146.6 | | 6.71, dd (8.4, 2.0) | 108.2 | 4b, 7, 8 |
| 7 | 6.82, d (8.8) | 113.7 | 5, 6, 8a | | 158.9 | |
| 8 | 7.12, d (8.8) | 110.1 | 4b, 6 | 6.94, d (2.0) | 95.4 | 4b, 6, 7 |
| 8a | | 134.7 | | | 142.2 | |
| 9a | | 136.0 | | | 135.0 | |
| 1′ | 6.59, d (9.6) | 117.1 | 1, 2, 9a, 3″ | | | |
| 2′ | 5.68, d (9.6) | 120.2 | 1, 3″, 4″, 5″ | | | |
| 3′ | | 75.8 | | | | |
| 4′ | 1.47, s | 27.5 | 2″, 3″, 5″ | | | |
| 5′ | 1.47, s | 27.5 | 2″, 3″, 4″ | | | |
| 1″ | 7.20, d (9.6) | 120.2 | 4b, 5, 6, 3′ | | | |
| 2″ | 5.79, d (9.6) | 123.2 | 5, 3′, 4′, 5′ | | | |
| 3″ | | 75.0 | | | | |
| 4″ | 1.47, s | 27.2 | 2′, 3′, 5′ | | | |
| 5″ | 1.47, s | 27.2 | 2′, 3′, 4′ | | | |
| 2-$OCH_3$ | | | | 3.90, s | 56.5 | 2 |
| 3-$CH_3$ | 2.32, s | 16.1 | 2, 3, 4 | | | |
| 3-OH | | | | 7.05, br s | | 3 |
| 7-$OCH_3$ | | | | 3.82, s | 55.6 | 7 |
| 9-NH | 7.71, br s | | | 9.85, br s | | |

[a]Recorded in $CDCl_3$. [b]Recorded in acetone-$d_6$.


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Table 2. NMR Spectroscopic Data for Clausenawallines E (3)[a] and F (4)[b]

| | 3 | | | 4 | | |
|---|---|---|---|---|---|---|
| position | $\delta_H$ ($J$ in Hz) | $\delta_C$ | HMBC | $\delta_H$ ($J$ in Hz) | $\delta_C$ | HMBC |
| 1 | | 101.0 | | | 96.1 | |
| 2 | | 150.9 | | | 159.3 | |
| 3 | | 119.2 | | | 106.5 | |
| 4 | 8.05, s | 128.8 | 2, 3, 4b, 9a | 8.33, s | 126.7 | 2, 3, 4b, 9a |
| 4a | | 116.8 | | | 116.4 | |
| 4b | | 119.5 | | | 118.3 | |
| 5 | | 114.8 | | 7.99, d (8.8) | 121.3 | 4b, 7, 8a |
| 6 | | 146.6 | | 6.85, dd (8.8, 2.4) | 109.4 | 4b, 8 |
| 7 | 6.76, d (8.5) | 114.0 | 5, 6, 8a | | 160.0 | |
| 8 | 6.91, d (8.5) | 110.4 | 4b, 6, 8a | 6.90, d (2.4) | 96.5 | 4b, 6, 8a |
| 8a | | 134.7 | | | 143.5 | |
| 9a | | 138.8 | | | 147.2 | |
| 1′ | 6.77, s | 91.9 | 2, 3, 4a, 8a | | 160.0 | |
| 2′ | | 157.8 | | | 115.6 | |
| 3′ | | 117.4 | | | 121.1 | |
| 4′ | 6.57, s | 122.5 | 2′, 3′, 4b′, 8a′, 3′-$CH_3$ | 8.50, s | 117.0 | 2′, 4b′, 9a′, 3′-$\underline{C}O_2CH_3$ |
| 4a′ | | 115.1 | | | 123.8 | |
| 4b′ | | 122.3 | | | 124.1 | |
| 5′ | | 108.2 | | 8.25, d (8.0) | 121.3 | 7′, 8a′ |
| 6′ | | 148.0 | | 7.29, dd (8.0, 7.6) | 120.6 | 4b′, 8′ |
| 7′ | 7.15, d (8.5) | 113.2 | 5′, 6′, 8a′ | 7.48, dd (8.0, 7.6) | 127.6 | 5′, 8a′ |
| 8′ | 7.41, d (8.5) | 112.3 | 4b′, 6′ | 7.65, d (8.0) | 112.4 | 4b′, 6′ |
| 8a′ | | 134.4 | | | 141.5 | |
| 9a′ | | 140.3 | | | 142.3 | |
| 1″ | 7.30, d (9.5) | 120.2 | 4b, 6, 3″ | | | |
| 2″ | 5.84, d (9.5) | 131.2 | 5, 3″, 4″, 5″ | | | |
| 3″ | | 75.1 | | | | |
| 4″ | 1.49, s | 27.3 | 3″, 5″ | | | |
| 5″ | 1.49, s | 27.2 | 3″, 4″ | | | |
| 2-OH | 4.95, br s | | 3 | | | |
| 3-$CH_3$ | 2.50, s | 16.5 | 2, 3, 4 | | | |
| 3-CHO | | | | 10.01, s | 196.7 | 2′, 3′, 4′ |
| 7-OMe | | | | 3.80, s | 55.8 | 7′ |
| 9-NH | 7.45, br s | | 4a, 4b, 8a, 9a | 10.23, br s[c] | | |
| 2′-$OCH_3$ | 3.82, s | 55.4 | 2′ | | | |
| 3′-$CH_3$ | 1.93, s | 16.6 | 2′, 3′, 4′ | | | |
| 3′-$\underline{C}O_2CH_3$ | | | | | 172.4 | |
| 3′-$CO_2\underline{CH_3}$ | | | | 3.51, s | 51.6 | 3′-$\underline{C}O_2CH_3$ |
| 6′-OH | 5.25, br s | | | | | |
| 9′-NH | 7.94, br s | | 4a′, 4b′, 8a′, 9a′ | 10.74, br s[c] | | |

[a]Recorded in $CDCl_3$ at 500 MHz. [b]Recorded in acetone-$d_6$ at 400 MHz. [c]The chemical shift may be interchangeable.

showed a broad N*H* singlet at $\delta$ 9.85, an ABX aromatic system [$\delta$ 7.78 (1H, d, $J$ = 8.4 Hz, H-5), 6.94 (1H, d, $J$ = 2.0 Hz, H-8), and 6.71 (1H, dd, $J$ = 8.4 and 2.0 Hz, H-6)], and two *para*-aromatic protons [$\delta$ 7.43 (1H, s, H-4) and 7.03 (1H, s, H-1)]. On the basis of HMBC correlations (Table 1), two methoxy groups were observed at $\delta$ 3.90 and 3.82 (both s, 3H) and located at C-2 and C-7, respectively. The C-3 resonance at $\delta$ 142.0 was assigned to a nonprotonated aromatic carbon carrying a hydroxy group. Detailed assignments of the protons and carbons as well as HMBC correlations are shown in Table 1, facilitating assignment of structure **2** to clausenawalline D.

Clausenawalline E (**3**) was obtained as a yellow solid, mp 196−197 °C. The ESI-TOF-MS gave a pseudomolecular ion peak at $m/z$ 505.2121 $[M + H]^+$ (calcd 505.2127), consistent with the molecular formula $C_{32}H_{29}N_2O_4$. The IR and UV spectra were similar to those of clausenawalline C. The NMR data (Table 2) indicated an unsymmetrical carbazole-type heterodimeric structure for clausenawalline E. One carbazole unit was similar to that of glycoborinine[12] except that in **3** the H-1 signal was absent in the $^1$H NMR spectrum, indicating substitution at this position. The second unit was identified as 6-hydroxy-2-methoxy-3-methylcarbazole, which showed $^1$H NMR signals for two *ortho*-coupled aromatic protons at $\delta$ 7.41 (1H, d, $J$ = 8.5 Hz, H-8′) and 7.15 (1H, d, $J$ = 8.5 Hz, H-7′), two aromatic singlets at $\delta$ 6.77 (1H, s, H-1′) and 6.57 (1H, s, H-4′), a N*H* at $\delta$ 7.94 (1H, br s), a methoxy group at $\delta$ 3.82 (3H, s, 2′-$OCH_3$), and an aryl methyl group at $\delta$ 1.93 (3H, s, 3′-$CH_3$). The HMBC correlations (Table 2) as well as an MS fragment ion at $m/z$ 227.0933 $[M - C_{18}H_{16}NO_2 + H]^+$ gave further support for this structural unit. The carbazole units were connected by a carbon−carbon linkage between C-1 and C-5′ because of the lack of signals for H-1 in the glycoborinine unit and for H-5′ of the 6-hydroxy-2-methoxy-3-methylcarbazole moiety. The detailed assignments of the protons, carbons, and


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**Table 3. Biological Activity of Isolated Compounds from *C. wallichii* Roots**

| | antibacterial activity (MIC, μg/mL) | | | | cytotocixity ($IC_{50}$, μM) | | |
|---|---|---|---|---|---|---|---|
| compound | MRSA SK1 | *Staph. aureus* | *E. coli* | *S. typhimurium* | KB[a] | MCF-7[b] | NCI-H187[c] |
| **1** | inactive | inactive | 128 | 128 | inactive | inactive | inactive |
| **2** | inactive | inactive | 128 | 128 | | | |
| **3** | 8 | 8 | 128 | 128 | | | |
| **4** | 64 | 64 | 128 | inactive | 10.2 | 62.3 | 4.5 |
| **5** | inactive | inactive | 128 | 128 | 213.0 | 31.4 | 27.4 |
| **6** | 128 | inactive | 64 | 128 | 293.1 | 66.1 | 60.8 |
| **7** | inactive | inactive | 128 | inactive | 263.1 | 28.8 | 58.9 |
| | inactive | inactive | 128 | 128 | 94.2 | 171.0 | 20.2 |
| **9** | 4 | 8 | 64 | 64 | 107.1 | inactive | 19.6 |
| **10** | inactive | inactive | 128 | 128 | inactive | 53.9 | 42.3 |
| **11** | 128 | inactive | inactive | 128 | 181.4 | 69.1 | 45.9 |
| **12** | inactive | inactive | 128 | inactive | inactive | inactive | inactive |
| **13** | inactive | inactive | 128 | 128 | inactive | inactive | inactive |
| **14** | 128 | Inactive | 128 | inactive | inactive | inactive | inactive |
| **15** | inactive | inactive | inactive | 128 | inactive | inactive | inactive |
| **16** | 64 | inactive | 128 | 128 | inactive | inactive | inactive |
| **17** | 128 | inactive | inactive | 128 | 103.1 | 111.0 | 18.2 |
| **18** | inactive | inactive | 128 | 128 | 59.7 | 169.8 | 95.8 |
| **19** | 64 | 64 | 128 | 32 | inactive | 204.8 | 134.2 |
| **20** | inactive | inactive | 128 | 128 | inactive | inactive | inactive |
| **21** | 128 | 64 | inactive | 128 | | | |
| **22** | 16 | 8 | 128 | 128 | | | |
| vancomycin | 1 | 0.25 | | | | | |
| gentamycin | | | 0.25 | 0.125 | | | |
| doxorubicin | | | | | 1.7 | 16.8 | 0.1 |
| ellipticine | | | | | 5.2 | | 3.1 |

[a]KB = oral cavity cancer. [b]MCF-7 = breast cancer. [c]NCI-H187 = small-cell lung cancer.

HMBC correlations are summarized in Table 2. Thus, the structure of **3** was identified as clausenawalline E.

Clausenawalline F (**4**) was obtained as a brown solid, mp 236−237 °C, which showed a pseudomolecular ion $[M + H]^+$ at *m/z* 481.1394 (calcd 481.1400) from the ESI-TOF-MS, corresponding to a molecular formula of $C_{28}H_{21}N_2O_6$. The NMR data (Table 2) showed the combination of two unsymmetrical carbazole units including a 2-hydroxy-7-methoxy-9*H*-carbazole-3-carbaldehyde and a methyl 1-hydroxy-9*H*-carbazole-3-carboxylate moiety (Table 2). The $^1$H NMR data of the 2-hydroxy-7-methoxy-9*H*-carbazole-3-carbaldehyde moiety displayed signals for an N*H* at δ 10.23 (br s), a formyl proton at δ 10.01 (1H, s, 3-CHO), a methoxy group at δ 3.80 (3H, s, 7-$OCH_3$), H-4 at δ 8.33 (1H, s, H-4), and an ABX aromatic proton system at δ 7.99 (1H, d, *J* = 8.8 Hz, H-5), 6.90 (1H, d, *J* = 2.4 Hz, H-8), and 6.85 (1H, dd, *J* = 8.8 and 2.4 Hz, H-6). The $^1$H NMR spectrum of the methyl 1-hydroxy-9*H*-carbazole-3-carboxylate subunit showed the protons of a 1,2-disubstituted aromatic ring at δ 8.25 (1H, d, *J* = 8.0 Hz, H-5′), 7.65 (1H, d, *J* = 8.0 Hz, H-8′), 7.48 (1H, dd, *J* = 8.0 and 7.6 Hz, H-7′), and 7.29 (1H, dd, *J* = 8.0 and 7.6 Hz, H-6′), a singlet aromatic proton at δ 8.50 (1H, H-4′), and a methyl ester signal at δ 3.51 (3H, s, 3′-$CO_2CH_3$). These carbazole moieties were linked between C-1 and C-2′ due to the lack of proton signals of H-1 and H-2′ as well as the HMBC correlation of H-4′ (δ 8.50) with δ 115.6 (C-2′). The detailed assignments of the protons, carbons, and HMBC correlations are summarized in Table 2. Thus, the structure of **4** was identified as clausenawalline F.

The remaining 18 known compounds were characterized as 3-hydroxy-2-methoxy-9*H*-carbazole (**5**),[13] clauraila D (**6**),[14] girinimbine (**7**),[15] heptaphylline (**8**),[16] 2,7-dihydroxy-3-formyl-1-(3′-methyl-2′-butenyl)carbazole (**9**),[17] 7-methoxyheptaphylline (**10**),[18] 2-hydroxy-3-formyl-7-methoxycarbazole (**11**),[18] 3-formyl-2,7-dimethoxycarbazole (**12**),[19] clauszoline C (**13**),[20] clauszoline K (**14**),[20] clausine C (**15**),[21] mukonal (**16**),[22] 2-methoxy-3-formylcarbazole (**17**),[23] methyl carbazole-3-carboxylate (**18**),[2] mukonidine (**19**),[24] clausine L (**20**),[25] clausenawalline A (**21**),[11] and clausenawalline B (**22**).[11]

All compounds were evaluated for their antibacterial activity against Gram-positive bacteria (MRSA SK1 and *Staph. aureus* TISTR 1466) and Gram-negative bacteria (*E. coli* TISTR 780 and *S. typhimurium* TISTR 292) as summarized in Table 3. All carbazole alkaloids showed weak (MIC 64−128 μg/mL) or no antibacterial activity against *E. coli* TISTR 780 and *S. typhimurium* TISTR 292, except for compound **19**, which showed moderate activity against *S. typhimurium* TISTR 292 with an MIC value of 32 μg/mL. Compounds **9** and **3** exhibited good antibacterial activity against MRSA SK1 (4 μg/mL) and *Staph. aureus* TISTR 1466 (8 μg/mL), respectively. Compound **22** showed good antibacterial activity against *Staph. aureus* (MIC 8 μg/mL) and weaker activity against MRSA SK1 (MIC 16 μg/mL). The other compounds had either weak (MIC 64−128 μg/mL) or no antibacterial activity against both *Staph. aureus* and MRSA SK1.

All isolates, except compounds **2** and **3**, were evaluated for their cytotoxicity against KB (oral cancer), MCF-7 (breast cancer), and NCI-H187 (small-cell lung cancer) cell lines (Table 3). Compounds **1**, **12**−**16**, and **20** had no cytotoxicity against all three cancer cell lines. Compounds **4**−**11**, **18**, and **19** showed cytotoxicity against the NCI-H187 cell line with $IC_{50}$ values in the range 4.5−134.2 μM, and compound **4** showed


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the highest cytotoxicity against the NCI-H187 cell line ($IC_{50}$ 4.5 $\mu$M). Also, compound **4** exhibited the highest cytotoxicity against the KB cell line ($IC_{50}$ 10.2 $\mu$M), whereas the other compounds were weakly active ($IC_{50}$ 59.7–293.1 $\mu$M) or had no activity. With respect to cytotoxicity against the MCF-7 cell line, compounds **4**–**8**, **10**, **11**, and **17**–**19** exhibited weak activity (28.8–204.8 $\mu$M).

## EXPERIMENTAL SECTION

**General Experimental Procedures.** Melting points were measured with a Buchi melting point B-540 apparatus. The UV spectra were recorded with a PerkinElmer UV–vis spectrophotometer. The IR spectra were recorded with a Perkin-Elmer FTS FT-IR spectrophotometer. The NMR spectra were recorded using 400 MHz Bruker and 500 MHz Varian UNITY INOVA spectrometers. Chemical shifts are recorded in parts per million ($\delta$) in $CDCl_3$ and acetone-$d_6$ with TMS as an internal reference. The ESI-TOF-MS data were measured on a MicroTOF, Bruker Daltonics mass spectrometer. Quick column chromatography (QCC) and CC were carried out on Si gel 60 H (Merck, 5–40 $\mu$m) and Si gel 100 (Merck, 63–200 $\mu$m), respectively. Precoated plates of Si gel 60 $F_{254}$ were used for analytical purposes.

**Plant Material.** The *C. wallichii* roots were collected in Phrae Province of Thailand, in June 2010. The plant was identified by Dr. Monthon Norsangsri, and a voucher specimen (No. QBG 4533) was deposited at the Herbarium of Queen Sirikit Botanic Garden, Mae Rim, Chiang Mai, Thailand.

**Extraction and Isolation.** Air-dried *C. wallichii* roots (1.02 kg) were extracted with acetone over a period of 3 days at room temperature and evaporated under reduced pressure to provide the crude acetone extract (18.68 g). This extract was subjected to QCC over Si gel and subsequently eluted with increasing polarity of the elution solvent system of hexanes and EtOAc (100% hexanes to 100% EtOAc) to afford five fractions (A–E). Fraction B (1.83 g) was further isolated by QCC with 5% EtOAc–hexanes to give four subfractions (B1–B4). Subfraction B2 (281.4 g) was separated by repeated CC using 30% $CH_2Cl_2$–hexanes to yield compound **7** (17.1 mg), whereas compounds **6** (17.0 mg), **8** (3.8 mg), **16** (2.1 mg), and **18** (2.1 mg) were derived from subfraction B4 (176.8 mg) by repeated CC with 50% $CH_2Cl_2$–hexanes. Fraction C (2.01 g) was further separated by QCC with 10% EtOAc–hexanes to 20% EtOAc–hexanes to afford five subfractions (C1–C5). Subfraction C2 (285.4 mg) was subjected to CC with 20% EtOAc–hexanes to give seven fractions (C2a–C2g). Fractions C2b (30.2 mg) and C2d (34.3 mg) were repeatedly purified by CC using 20% $CH_2Cl_2$–hexanes, yielding compounds **1** (5.8 mg) and **10** (16.6 mg), respectively, while compound **20** (2.8 mg) was isolated from fraction C2f (27.6 mg) by CC with 45% $CH_2Cl_2$–hexanes. Fraction C4 (181.9 mg) was subjected to repeated CC using 20% EtOAc–hexanes to give five subfractions (C4a–C4d). Subfractions C4b (55.4 mg) and C4d (24.6 mg) were further purified by CC with 80% $CH_2Cl_2$–hexanes to give compounds **17** (3.7 mg) and **15** (6.5 mg), respectively. Fraction D (3.21 g) was subjected to QCC and eluted with increasing polarity of the elution solvent system of hexanes and EtOAc (20% EtOAc–hexanes to 100% EtOAc) to provide seven subfractions (D1–D7). Subfraction D2 (118.9 mg) was further isolated by CC with 30% EtOAc–hexanes to yield compound **11** (12.8 mg). Fractions D3 (1.18 g) and D5 (862.0 mg) were subjected to Sephadex LH-20 using MeOH to afford four (D3a–D3d) and five (D5a–D5e) subfractions, respectively. Subfraction D3b (751.0 mg) was separated by CC with 2% EtOAc–$CH_2Cl_2$ to yield compound **9** (3.0 mg) and six subfractions (D3b1–D3b6). Subfractions D3b2 (25.1 mg) and D3b4 (30.5 mg) were further separated by CC with 25% EtOAc–hexanes to give compounds **5** (9.5 mg) and **14** (2.1 mg), respectively, while compound **19** (3.0 mg) was derived from subfraction D3d (114.2 mg) by repeated CC with 20% EtOAc–hexanes. Fraction D5b (259.1 mg) was further isolated by CC with 5% EtOAc–$CH_2Cl_2$ to afford compounds **2** (2.1 mg), **12** (19.0 mg), and **13** (8.5 mg). Finally, compounds **3** (1.7 mg) and **4** (6.2 mg) were isolated from fraction D5d (51.6 mg) by CC using 5% EtOAc–$CH_2Cl_2$. The isolation of compounds **21** and **22** as well as their cytotoxicity has been described by Maneerat et al.[11]

*Clausenawalline C (**1**):* yellow solid (acetone); mp 239–240 °C; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 206 (4.13), 235 (4.34), 295 (3.92), 310 (3.96), 337 (3.78), 349 (3.76), 390 (3.36), 399 (3.23) nm; IR (neat) $\nu_{max}$ 3299, 2970, 1639, 1659 $cm^{-1}$; $^1H$ and $^{13}C$ NMR (400 MHz, $CDCl_3$), see Table 1; ESI-TOF-MS $m/z$ 346.1790 $[M + H]^+$ (calcd for $C_{23}H_{24}NO_2$, 346.1807).

*Clausenawalline D (**2**):* brown solid (acetone); mp 247–249 °C; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 234 (4.69), 265 (4.30), 312 (4.24), 326 (4.04) nm; IR (neat) $\nu_{max}$ 3508, 3395, 1618, 1580 $cm^{-1}$; $^1H$ and $^{13}C$ NMR (400 MHz, acetone-$d_6$), see Table 1; ESI-TOF-MS $m/z$ 244.0968 $[M + H]^+$ (calcd for $C_{14}H_{14}NO_3$, 244.0974).

*Clausenawalline E (**3**):* yellow solid (acetone); mp 196–197 °C; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 230 (4.73), 266 (4.50), 303 (4.35), 311 (4.38), 336 (3.74) nm; IR (neat) $\nu_{max}$ 3849, 3396, 2923, 1620 $cm^{-1}$; $^1H$ and $^{13}C$ NMR (500 MHz, $CDCl_3$), see Table 2; ESI-TOF-MS $m/z$ 505.2121 $[M + H]^+$ (calcd for $C_{32}H_{29}N_2O_4$, 505.2127).

*Clausenawalline F (**4**):* brown solid (acetone); mp 236–237 °C; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 222 (4.43), 233 (4.44), 277 (4.22), 300 (4.31), 340 (3.77) nm; IR (neat) $\nu_{max}$ 3346, 1704, 1614, 1564 $cm^{-1}$; $^1H$ and $^{13}C$ NMR (400 MHz, acetone-$d_6$), see Table 2; ESI-TOF-MS $m/z$ 481.1394 $[M + H]^+$ (calcd for $C_{28}H_{21}N_2O_6$, 481.1400).

**Antibacterial Assay.** *E. coli* TISTR 780, *S. typhimurium* TISTR 292, and *Staph. aureus* TISTR 1466 were derived from the Microbiological Resources Center of the Thailand Institute of Scientific and Technological Research, whereas MRSA SK1 was derived from the Department of Microbiology, Faculty of Science, Prince of Songkla University, Thailand. The minimum inhibitory concentrations (MICs) were determined by a 2-fold serial dilution method using Mueller Hinton broth according to the Clinical and Laboratory Standards Institute recommendations (CLSI, 2002).[26] The test substances were dissolved in DMSO. Vancomycin and gentamycin were used as standard drugs.

**Cytotoxic Assay.** The cytotoxic assays against the three cancer cell lines oral cavity cancer (KB), breast cancer (MCF-7), and small-cell lung cancer (NCI-H187) were performed using the resazurin microplate assay, which was modified for mammalian cell cytotoxicity.[27]

## ASSOCIATED CONTENT

### Supporting Information

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

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### Notes

The authors declare no competing financial interest.

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