

## Antimalarial and Cytotoxic Depsidones from the Fungus *Chaetomium brasiliense*

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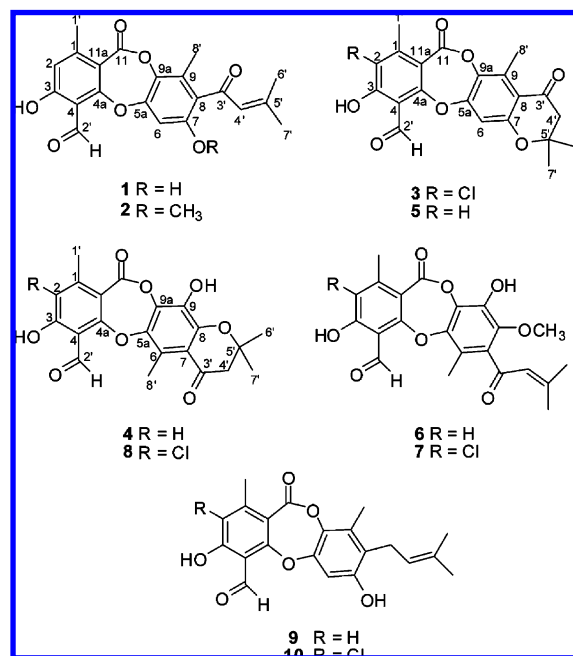
Four new depsidones, mollicellins K–N (**1–4**), and six known depsidones, mollicellins B (**5**), C (**6**), E (**7**), F (**8**), H (**9**), and J (**10**), along with two known sterols were isolated from the fungus *Chaetomium brasiliense*. Their structures were elucidated on the basis of 1D and 2D NMR spectroscopic data and chemical transformation. Among these isolates, **1–3**, **5–7**, and **10** exhibited antimalarial activity against *Plasmodium falciparum*. Only **1** exhibited antimycobacterial activity against *Mycobacterium tuberculosis* and antifungal activity against *Candida albicans* using in vitro assays. In addition, **1–10** showed cytotoxicity against the KB, BC1, NCI-H187, and five cholangiocarcinoma cell lines.

The fungus *Chaetomium brasiliense* is one of ca. 22 *Chaetomium* species that have been found in Thailand.<sup>1,2</sup> Previous investigations on secondary metabolites from *Chaetomium* species resulted in the isolation of compounds such as benzoquinone derivatives,<sup>3</sup> tetra-*S*-methyl derivatives,<sup>4</sup> azaphilones,<sup>5–7</sup> bis-azaphilones,<sup>7</sup> indol-3-yl-[13]cytochalasans, and chaetoglobosin analogues.<sup>8–14</sup> In addition, anthraquinone-chromanone,<sup>13</sup> orsellinic acid, and globosumones<sup>15</sup> have also been reported. *Chaetomium brasiliense*<sup>16</sup> has been reported to produce chaetochalasin A<sup>17</sup> and four depsidones, mollicellins D, H, I, and J.<sup>18</sup> As part of our work on bioactive constituents from *Chaetomium* species, we noted that hexane and EtOAc extracts of *C. brasiliense*, a strain isolated from Thai soil, showed in vitro antimalarial activity against *Plasmodium falciparum* (IC<sub>50</sub> 3.0 and 2.9 μg/mL, respectively), and the EtOAc extract also showed antimycobacterial activity against *Mycobacterium tuberculosis* (MIC 50 μg/mL). We report herein the isolation, structural characterization, and bioactivity of four new depsidones (**1–4**), six known depsidones (**5–10**), and two known sterols from *C. brasiliense*.

### Results and Discussion

Depsidones **1–10** and two sterols were isolated as solids from hexane, EtOAc, and MeOH extracts of dried mycelial mat of *C. brasiliense* using a combination of silica gel column chromatography (CC) and preparative TLC. Structures of the known compounds were identified by physical and spectroscopic data measurements (IR, <sup>1</sup>H and <sup>13</sup>C NMR, 2D NMR, and MS) and by comparing the data obtained with published values, as 24(*R*)-5α,8α-epidioxyergosta-6,22-diene-3β-ol,<sup>17</sup> ergosterol,<sup>19</sup> and mollicellins B, C, E, F (**5–8**),<sup>20</sup> H and J (**9** and **10**).<sup>18</sup> The details of physical properties and spectroscopic data of mollicellins B, C, E, and F (**5–8**)<sup>20</sup> are also presented, since they have not yet been reported.

Compound **1** was obtained as a white solid, and its molecular formula, C<sub>21</sub>H<sub>18</sub>O<sub>7</sub>, was deduced from the HRESITOFMS (observed *m/z* 383.1161 [M + H]<sup>+</sup>), indicating 13 degrees of unsaturation. The IR spectrum showed the presence of OH (3407 cm<sup>-1</sup>), carbonyl ester (1731 cm<sup>-1</sup>), aromatic aldehyde (1656 cm<sup>-1</sup>), α,β-unsaturated ketone (1638 cm<sup>-1</sup>), and aromatic (1594 cm<sup>-1</sup>) groups. The <sup>13</sup>C NMR and DEPT spectra (Table 1) indicated 21 signals attributable to 13 sp<sup>2</sup> quaternary (including two carbonyl groups), four sp<sup>2</sup>



methine (including an aldehyde group), and four methyl carbons. The <sup>13</sup>C NMR data together with the degrees of unsaturation revealed that **1** contained two aromatic rings in the molecule. The IR absorption band at 1731 cm<sup>-1</sup> and the <sup>13</sup>C NMR resonance signal at δ 163.7 suggested the presence of a conjugated carbonyl ester group.<sup>18,21</sup> The <sup>1</sup>H NMR data showed two singlet signals of aromatic protons at δ 6.72 (H-2) and 6.69 (H-6), as well as two aromatic methyl substituents at δ 2.51 (H<sub>3</sub>-1') and 2.53 (H<sub>3</sub>-8'). The low-field singlet signal (δ 12.06) was assigned as a chelated OH involving the carbonyl of an aldehyde group (δ 10.53) at the *ortho* position. The <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data of **1** were comparable to an analogue, mollicellin H (**9**),<sup>18</sup> except for the prenyl group at C-8, which was replaced by a 1-oxo-3-methylbut-2-enyl moiety. This unit was deduced from the <sup>1</sup>H and <sup>13</sup>C NMR signals at δ<sub>H</sub> 6.31 (s, H-4'), 2.23 (s, Me-6'), and 2.01 (s, Me-7') and δ<sub>C</sub> 196.0 (C-3'), 126.0 (C-4'), 158.3 (H-5'), 21.5 (Me-6'), and 28.1 (Me-7'), and it was located at C-8 on the basis of the HMBC correlations of H-8' to C-8, C-9, C-9a, and C-3', and H-6 to C-5a, C-7, C-8, C-9a, and C-3'. The HMBC spectrum of **1** also showed correlations of H-1' to C-1, C-2, C-11, C-4a, and C-11a; H-2 to C-3, C-4, C-11a, C-1', C-4a, and C-11; the OH proton at C-3 to

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**Table 1.**  $^1\text{H}$  NMR Data ( $\delta$ , ppm) of Compounds **1–8** in  $\text{CDCl}_3$ 

position	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>
2	6.72 (s)	6.71 (s)		6.72 (s)	6.71 (s)	6.71 (s)		
6	6.69 (s)	6.57 (s)	6.66 (s)		6.66 (s)			
1'	2.51 (s)	2.52 (s)	2.60 (s)	2.53 (s)	2.52 (s)	2.51 (s)	2.57 (s)	2.53 (s)
2'	10.53 (s)	10.60 (s)	10.54 (s)	10.85 (s)	10.53 (s)	10.83 (s)	10.81 (s)	10.78 (s)
4'	6.31 (s)	6.19 (s)	2.68 (s)	2.74 (s)	2.67 (s)	6.25 (s)	6.24 (s)	2.68 (s)
6'	2.23 (s)	2.20 (s)	1.41 (s)	1.47 (s)	1.41 (s)	2.23 (s)	2.23 (s)	1.40 (s)
7'	2.01 (s)	1.93 (s)	1.41 (s)	1.47 (s)	1.41 (s)	1.96 (s)	1.96 (s)	1.40 (s)
8'	2.53 (s)	2.23 (s)	2.67 (s)	2.59 (s)	2.66 (s)	2.17 (s)	2.16 (s)	2.52 (s)
OH-3	12.06 (s)	12.01 (s)	12.68 (s)	12.19 (s)	12.03 (s)	12.17 (s)	12.75 (s)	12.77 (s)
OH-7	10.88 (s)							
OMe-7		3.76 (s)						
OMe-8						3.73 (s)	3.72 (s)	
OH-9				5.67 (s)		5.84 (s)		5.64 (s)

**Table 2.**  $^{13}\text{C}$  NMR Data ( $\delta$ , ppm) of Compounds **1–8** in  $\text{CDCl}_3$ 

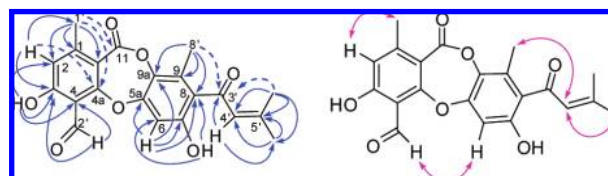
position	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>
1	153.4	154.3	150.3	153.2	153.6	153.1	149.8	149.8
2	117.8	118.2	121.1	117.9	117.9	117.7	121.3	121.1
3	165.3	165.7	161.0	165.3	165.2	165.2	161.2	161.0
4	110.7	111.2	110.6	111.0	110.7	110.9	110.8	110.9
4a	161.6	162.3	161.2	163.7	161.5	161.4	160.9	161.3
5a	153.5	151.0	154.7	137.5	154.7	140.2	140.0	137.3
6	106.8	101.6	107.5	122.6	107.5	134.0	134.2	122.6
7	158.3	153.8	158.6	115.8	158.6	117.5	117.4	115.9
8	122.3	129.4	117.2	146.2	117.0	141.2	141.2	146.2
9	131.1	131.8	134.4	142.0	134.3	139.2	139.3	142.0
9a	135.8	137.1	136.5	135.4	136.7	138.7	138.8	135.4
11	163.7	165.2	161.3	161.5	163.5	164.5	162.2	161.4
11a	112.5	113.3	114.0	112.6	112.5	112.7	114.2	114.1
1'	22.8	22.8	19.7	22.1	22.2	22.1	19.5	19.5
2'	192.6	193.3	192.5	195.4	192.6	193.4	193.3	195.1
3'	196.0	194.6	192.5	192.0	192.5	195.4	195.2	191.9
4'	126.0	126.0	50.1	50.4	50.1	125.2	125.1	50.4
5'	158.6	158.1	79.5	80.9	79.3	159.1	159.3	81.1
6'	21.5	21.6	26.3	26.4	14.2	21.1	21.2	26.4
7'	28.1	28.5	26.3	26.4	14.2	28.0	28.0	26.4
8'	16.3	13.5	14.2	13.1	26.3	12.1	12.1	13.0
OMe-7		56.8						
OMe-863.1						63.1	63.1	

C-2, C-3, and C-4; H-6 to C-5a, C-7, C-8, and C-9a; the aldehyde proton (H-2') to C-3 and C-4; the OH proton at C-7 to C-6, C-7, and C-8; H-4' to C-3', C-6', and C-7'; H-6' to C-4', C-5', C-7', and C-3'; and H-7' to C-4', C-5', and C-6' to confirm the connectivity in the molecule (Figure 1). Surprisingly, the  $^3J$  correlation of the aldehyde proton (H-2') to C-4a was not observed in the HMBC experiment. Thus, the chemical shift of C-4a was then identified by comparison with those reported for the analogues mollicellins I and J<sup>18</sup> and also from the  $^4J$  correlations of H-2 and H-1' in the HMBC spectrum. In addition, the NOESY spectrum of **1** demonstrated correlations between aldehyde proton H-2' and H-6, between H-1' and H-2, and from H-4' and H-8' to H-7' (Figure 1). Chemical transformation of **1** by cyclization with MeOH in the presence of *p*-toluenesulfonic acid yielded the product that was identical (mp, IR, NMR, and behavior on TLC) to natural mollicellin B (**5**) (Tables 1 and 2). On the basis of the above data, the structure of **1** was defined as a new depsidone and has been named mollicellin K.

Compound **2** was obtained as a white solid, and its molecular formula,  $\text{C}_{22}\text{H}_{20}\text{O}_7$ , was deduced from HRESITOFMS (observed  $m/z$  397.1286  $[\text{M} + \text{H}]^+$ ), indicating 13 degrees of unsaturation. The IR spectrum showed the presence of OH ( $3439\text{ cm}^{-1}$ ), carbonyl ester ( $1729\text{ cm}^{-1}$ ), aromatic aldehyde ( $1677\text{ cm}^{-1}$ ),  $\alpha,\beta$ -unsaturated ketone ( $1644\text{ cm}^{-1}$ ), and aromatic ( $1608\text{ cm}^{-1}$ ) groups. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of **2** were similar to those of **1**, except for the OH group at C-7, which was substituted by an  $\text{OCH}_3$  group ( $\delta_{\text{H}}$  3.76,  $\delta_{\text{C}}$  56.8). The complete assignments of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR signals of **2** were established from the DEPT, COSY, HSQC, HMBC, and NOESY data (Tables 1 and 2). The NOESY spectrum of **2** showed correlations between an aldehyde proton (H-2')

H-6, H-6, and the  $\text{OCH}_3$  protons at C-7 to support the structure of **2**. Thus, **2** was defined as a new depsidone and has been named mollicellin L.

Compound **3** was obtained as a white solid,  $\text{C}_{21}\text{H}_{17}\text{ClO}_7$ , as deduced from HRESITOFMS (observed  $m/z$  439.0561  $[\text{M} + \text{Na}]^+$  and its  $^{37}\text{Cl}$  isotope  $m/z$  441.0601  $[\text{M} + 2 + \text{Na}]^+$ ), implying 13 degrees of unsaturation. The IR spectrum of **3** showed characteristics of OH ( $3454\text{ cm}^{-1}$ ), ester carbonyl ( $1736\text{ cm}^{-1}$ ), aromatic aldehyde ( $1688\text{ cm}^{-1}$ ), conjugated ketone ( $1652\text{ cm}^{-1}$ ), and aromatic ( $1600\text{ cm}^{-1}$ ) groups. The  $^{13}\text{C}$  NMR and DEPT spectra revealed 21 signals attributable to 13  $\text{sp}^2$  quaternary (including two carbonyl groups), one  $\text{sp}^3$  quaternary, two  $\text{sp}^2$  methine (including an aldehyde group), one  $\text{sp}^3$  methylene, and four methyl carbons. From these data, **3** contained two aromatic rings and a conjugated carbonyl ester as described in **1**. The  $^1\text{H}$  NMR spectrum of **3** (Table 1) showed only one aromatic singlet signal at  $\delta$  6.66 (H-6) and two aromatic methyl substituents at  $\delta$  2.60 (H-1') and 2.68 (H-8'), which were different from **1**. The 1-oxo-3-methylbut-2-enyl moiety at C-8

**Figure 1.** Key HMBC  $^2J$  and  $^3J$  correlations (H $\rightarrow$ C) and  $^4J$  correlations (H $\rightarrow$ C, dashed arrows) and NOESY correlations of mollicellin K (**1**).

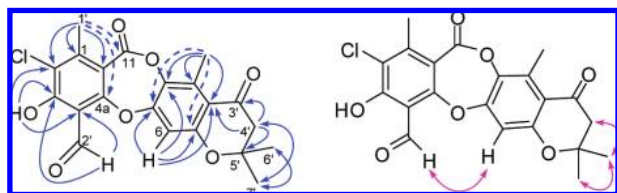
**Table 3.** Biological Activities of the Isolated Compounds

compound	antimalarial	anti-TB	antifungal	cytotoxicity (IC <sub>50</sub> , μg/mL)		
	(IC <sub>50</sub> , μg/mL)	(MIC, μg/mL)	(IC <sub>50</sub> , μg/mL)	KB <sup>a</sup>	BC1 <sup>b</sup>	NCI-H187 <sup>c</sup>
<b>1</b>	1.2	12.5	1.2	1.9	6.8	0.35
<b>2</b>	3.4	inactive	inactive	33.9	nd	9.5
<b>3</b>	2.9	inactive	inactive	Inactive	inactive	0.68
<b>4</b>	inactive	inactive	inactive	25.9	nd	13.5
<b>5</b>	4.7	inactive	inactive	37.1	nd	14.7
<b>6</b>	9.1	inactive	49.9	46.3	inactive	3.1
<b>7</b>	3.2	inactive	39.7	nd	nd	1.0
<b>8</b>	inactive	inactive	nd	37.9	nd	13.1
<b>9</b>	nd	inactive	nd	16.6	nd	3.9
<b>10</b>	4.9	inactive	nd	29.1	nd	23.3
artemisinin	0.001					
isoniazid		0.05				
kanamycin sulfate		2.5				
amphotericin B			0.034			
ellipticine				0.36	0.26	0.32

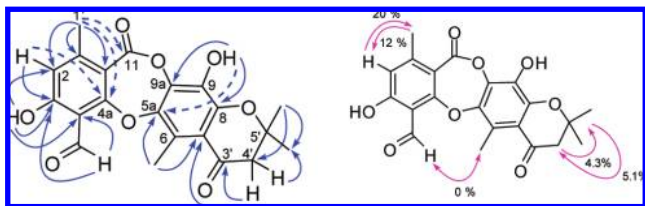
<sup>a</sup> Human epidermoid carcinoma of the mouth. <sup>b</sup> Human breast cancer. <sup>c</sup> Human small cell lung cancer. nd = not determined. Inactive at >50 μg/mL.

in **1** was replaced by a dihydropyrone ring fused to an aromatic ring, as shown by the signals of two geminal methyl groups both at  $\delta$  1.41 (H-6' and H-7') and one methylene group at  $\delta$  2.67 (s, H-4'). The low-field singlet signal at  $\delta$  12.68 was assigned as an OH chelated to an aldehyde carbonyl group ( $\delta$  10.54) at the *ortho* position as in **1**. The structure of **3** was constructed by a combination of 2D NMR analyses. The HMBC spectrum demonstrated correlations of H-1' to C-1, C-2, C-11, C-4a, and C-11a; the OH proton at C-3 to C-2, C-3, and C-4; H-6 to C-5a, C-7, C-8, and C-9a; the aldehyde proton (H-2') to C-3 and C-4; H-4' to C-8, C-3', C-5', C-6', and C-7'; H-6' to C-4', C-5', and C-7'; H-7' to C-4', C-5', and C-6'; and H-8' to C-7, C-5a, C-8, C-9, C-3', and C-9a (Figure 2). In addition, the Cl-C(2) was determined by comparing its <sup>13</sup>C NMR spectrum with those reported for mollicellins J,<sup>18</sup> K (**1**), and B (**5**), as well as the HMBC correlation of H-1' to C-2 ( $\delta_C$  121.1). The NOESY spectrum of **3** also supported the structure via the correlations between H-2' and H-6 and between H-4' and H-6' and H-7' (Figure 3) On the basis of the above data, the structure of **3** was defined as a new depsidone, and it has been named mollicellin M.

Compound **4** was obtained as a white solid, and its molecular formula, C<sub>21</sub>H<sub>18</sub>O<sub>8</sub>, was deduced from HRESITOFMS (observed *m/z* 421.0897 [M + Na]<sup>+</sup>), implying 13 degrees of unsaturation. The IR spectrum of **4** indicated OH (3355 cm<sup>-1</sup>), ester carbonyl (1738 cm<sup>-1</sup>), conjugated aldehyde (1688 cm<sup>-1</sup>), conjugated ketone



**Figure 2.** Key HMBC <sup>2</sup>J and <sup>3</sup>J correlations (H→C) and <sup>4</sup>J correlations (H→C, dashed arrows) and NOESY correlations of mollicellin M (**3**).



**Figure 3.** Key HMBC <sup>2</sup>J and <sup>3</sup>J correlations (H→C) and <sup>4</sup>J correlations (H→C, dashed arrows) and NOE-difference data of mollicellin N (**4**).

**Table 4.** Biological Activities of the Isolated Compounds against Cholangiocarcinoma

compound	cytotoxicity (IC <sub>50</sub> , μg/mL)				
	KKU-100 <sup>a</sup>	KKU-M139 <sup>b</sup>	KKU-M156 <sup>c</sup>	KKU-M213 <sup>d</sup>	KKU-M214 <sup>e</sup>
<b>1</b>	4.46 ± 0.08	13.94 ± 2.87	6.65 ± 1.15	nd	4.92 ± 0.26
<b>3</b>	6.28 ± 0.94	8.55 ± 3.20	6.04 ± 0.15	13.19 ± 1.17	4.51 ± 0.18
<b>4</b>	6.46 ± 1.24	4.34 ± 0.08	2.90 ± 0.07	3.00 ± 0.36	5.05 ± 0.50
<b>5</b>	4.63 ± 0.01	11.66 ± 2.70	15.66 ± 0.88	6.94 ± 1.02	4.50 ± 0.39
<b>6</b>	5.12 ± 0.17	2.51 ± 0.36	4.18 ± 0.11	3.2 ± 0.52	3.35 ± 0.04
<b>7</b>	4.83 ± 0.05	5.03 ± 0.16	5.22 ± 0.08	4.44 ± 0.05	7.81 ± 1.13
<b>8</b>	5.21 ± 0.04	5.21 ± 0.04	5.36 ± 0.15	4.98 ± 0.05	4.40 ± 0.02
ellipticine	7.11 ± 0.09	1.21 ± 0.03	2.02 ± 0.11	0.30 ± 0.001	0.21 ± 0.04

<sup>a</sup> Poorly differentiated adenocarcinoma. <sup>b</sup> Squamous carcinoma. <sup>c</sup> Moderately differentiated adenocarcinoma. <sup>d</sup> Adenosquamous carcinoma. <sup>e</sup> Moderately differentiated denocarcinoma. nd = not determined.

(1644 cm<sup>-1</sup>), and aromatic (1574 cm<sup>-1</sup>) groups. The <sup>13</sup>C NMR and DEPT spectra revealed 21 signals attributable to 13 sp<sup>2</sup> quaternary (including two carbonyl groups), one sp<sup>3</sup> quaternary, two sp<sup>2</sup> methine (including an aldehyde group), one sp<sup>3</sup> methylene, and four methyl carbons. The <sup>1</sup>H and <sup>13</sup>C NMR spectra of **4** (Tables 1 and 2) showed splitting patterns similar to those of **3** with four singlet methyl ( $\delta_H$  2.53, 2.59, 1.47, and 1.47), one methylene ( $\delta_H$  2.74), and two methine groups ( $\delta_H$  6.72 and 10.85). However, groups substituted on the aromatic ring and chromone units of **4** were located at different positions than in **3**. The HMBC spectrum exhibited correlations of H-1' to C-1, C-2, C-4a, C-11, and C-11a; H-2 to C-1, C-3, C-4, C-4a, and C-11a; the OH group at C-3 to C-2, C-3, and C-4; the OH group at C-9 to C-8, C-5a, and C-9a; aldehyde proton H-2' to C-3 and C-4; H-4' to C-7, C-3', C-5', C-6', and C-7'; H-6' to C-4', C-5', and C-7'; and H-7' to C-4', C-5', and C-6', confirming the structure of **4** (Figure 3). The NOESY spectrum of **4** showed correlations between H-2 and H-1' and between H-4' and H-6' and H-7'. Unfortunately, the correlation between aldehyde proton H-2' and C<sub>3</sub>-8' was not observed and the NOE-difference data showed the same correlations of protons as in the NOESY experiment (Figure 3). This suggested that the distance between the aldehyde proton (H-2') and C<sub>3</sub>-8' is more than 4.2 Å.<sup>22</sup> On the basis of the above evidence, compound **4** was determined to be a new depsidone, and it was named mollicellin N.

The isolated compounds were tested for their bioactivities at the Bioassay Research Facility of the National Center for Genetic Engineering and Biotechnology (BIOTEC), NSTDA, Thailand, and the results are shown in Table 3. Cytotoxicity tests against cholangiocarcinoma were performed at the Liver Fluke and Cholangiocarcinoma Research Center, Faculty of Medicine, Khon Kaen University, Thailand, and the results are given in Table 4.

Mollicellins K–M (**1–3**), B (**5**), C (**6**), E (**7**), and J (**10**) showed antimalarial activity against *Plasmodium falciparum* with IC<sub>50</sub> ranging from 1.2–9.1 μg/mL. Only **1** showed moderate activity



against *Mycobacterium tuberculosis* (MIC 12.5  $\mu\text{g/mL}$ ) and potent activity against *Candida albicans* (1.2  $\mu\text{g/mL}$ ), as well as cytotoxicity against KB cells (1.9  $\mu\text{g/mL}$ ). However, compounds **1**, **3**, **6**, **7**, and **9** exhibited significant cytotoxicity against NCI-H187 cell lines with  $\text{IC}_{50}$  values of 0.35, 0.68, 3.1, 1.0, and 3.9  $\mu\text{g/mL}$ , respectively (Table 3). In addition, compounds **1** and **3–8** exhibited significant cytotoxicity against five cholangiocarcinoma cell lines (KKU-100, KKU-M139, KKU-M156, KKU-M213, and KKU-M214) with  $\text{IC}_{50}$  values ranging from 2.5 to 15.7  $\mu\text{g/mL}$ . It should be noted that all compounds exhibited  $\text{IC}_{50}$  values against KKU-100 ranging from 4.5 to 6.5  $\mu\text{g/mL}$  and were more cytotoxic than the control drug ellipticine (Table 4).

The antimalarial and antimycobacterial activities of the isolated compounds corresponded to the preliminary screening tests for the crude extracts. Among the seven depsidones that exhibited antimalarial activity, mollicellin K (**1**) was more active than its crude extracts. In addition, mollicellin K (**1**) was the only one that exhibited antimycobacterial activity and was also more potent than crude extracts. Moreover, of the compounds that were tested for cytotoxicity against several cancer cell lines, most of them showed significant cytotoxicity, especially against cholangiocarcinoma cells.

## Experimental Section

**General Experimental Procedures.** Melting points were determined using a Gallenkamp melting point apparatus and were uncorrected. UV spectra were measured on an Agilent 8453 UV-visible spectrophotometer. IR spectra were taken on a Perkin-Elmer Spectrum One spectrophotometer. NMR spectra were recorded in  $\text{CDCl}_3$  on a Varian Mercury Plus 400 spectrometer, using residual  $\text{CHCl}_3$  as an internal standard. HRESITOFMS were recorded on a Micromass Q-TOF-2 spectrometer. Column chromatography and preparative TLC were carried out on silica gel 60 (230–400 mesh) and  $\text{PF}_{254}$ , respectively.

**Fungal Material.** The fungus *C. brasiliense* was collected from Doi Inthanon, Jomtong District, Chiangmai Province, Thailand, in June 2006 and was identified by K.S. A voucher specimen (no. Chbr01) was deposited at the Department of Plant Pest Management, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. The fungus was cultured in conical flasks (1 L, 65 flasks) with potato dextrose broth (PDB) (200 mL/flask) and incubated in standing condition at 25–28 °C for 4 weeks. The culture broth was filtered to give a wet mycelial mat and then air-dried at room temperature.

**Extraction and Isolation.** The air-dried mycelial mat (300 g) was ground and extracted successively at room temperature with hexane (700 mL  $\times$  3), EtOAc (700 mL  $\times$  3), and MeOH (700 mL  $\times$  3) to give crude hexane (6.8 g), EtOAc (17.8 g), and MeOH (20.6 g) extracts.  $\text{CH}_2\text{Cl}_2$  (35 mL)–hexane (300 mL) was added to the hexane extract to give a solid (95 mg), which was recrystallized from EtOAc–hexane to give mollicellin B (**5**) (34 mg). The filtrate was evaporated to yield a residue, which was subjected to silica gel flash column chromatography (FCC), eluted with a gradient system of hexane–EtOAc to give six fractions,  $F_1$ – $F_6$ . The solid in fraction  $F_2$  was recrystallized from EtOAc–hexane to give 24(*R*)-5 $\alpha$ ,8 $\alpha$ -epidioxyergosta-6,22-diene-3 $\beta$ -ol (74 mg). The filtrate was evaporated to yield a residue, which was further subjected to silica gel FCC eluted with a gradient system of hexane–EtOAc to give ergosterol (104 mg). Fraction  $F_3$  was purified by preparative TLC using 20% EtOAc–hexane to give mollicellin E (**7**) (24 mg). Fraction  $F_4$  was rechromatographed by FCC, eluted with 20% EtOAc–hexane, to afford additional mollicellin B (**5**) (26.3 mg) and mollicellin K (**1**) (43 mg). Fraction  $F_5$  was purified by silica gel FCC, eluted with a gradient of hexane–EtOAc to give four subfractions,  $F_{5.1}$ – $F_{5.4}$ . Subfraction  $F_{5.2}$  was subjected to silica gel FCC, eluted with a gradient of hexane–EtOAc, to give mollicellin L (**2**) (18 mg). Fraction  $F_6$  was purified by silica gel FCC, eluted with a gradient of hexane–EtOAc, to give subfractions  $F_{6.1}$ – $F_{6.4}$ . Subfraction  $F_{6.1}$  was rechromatographed by FCC, eluted with 20% EtOAc–hexane, to yield additional amounts of mollicellin B (**5**) (26.3 mg) and mollicellin K (**1**) (45 mg). Fraction  $F_{6.3}$  was rechromatographed by FCC, eluted with 40% EtOAc–hexane to give additional mollicellin E (**7**) (31.1 mg).

The EtOAc extract (17.8 g) was initially subjected to silica gel FCC, eluted with the same gradient system as the hexane extract above to give 10 fractions,  $F1/2_1$ – $F1/2_{10}$ . Fraction  $F1/2_1$  was subjected to silica gel FCC, eluted with a gradient of hexane–EtOAc, to give mollicellin

J (**10**) (54 mg), an additional amount of mollicellin E (**7**) (15.3 g), and mollicellin N (**4**) (6 mg). Fraction  $F1/2_4$  was separated by FCC, eluted with a gradient of hexane–EtOAc, to yield additional mollicellin K (**1**) (67 mg) and mollicellin B (**5**) (30 mg). Fraction  $F1/2_5$  was subjected to FCC, eluted with a gradient of hexane–EtOAc, to give mollicellin C (**6**) (13.2 mg), mollicellin B (**5**) (10 mg), mollicellin M (**3**) (7.8 mg), and mollicellin N (**4**) (16 mg). Fraction  $F1/2_6$  was separated by silica gel FCC, eluted with a gradient of hexane–EtOAc, to give three subfractions,  $F1/2_{6.1}$ – $F1/2_{6.3}$ . Subfraction  $F1/2_{6.1}$  was rechromatographed by FCC, eluted with 40% EtOAc–hexane, to afford additional mollicellin L (**2**) (6.3 mg). Subfraction  $F1/2_{6.2}$  was rechromatographed by FCC, eluted with 50% EtOAc–hexane, to yield mollicellin H (**9**) (14 mg). Fraction  $F1/2_7$  was further purified by silica gel FCC, eluted with a gradient of hexane–EtOAc, to give three subfractions,  $F1/2_{7.1}$ – $F1/2_{7.3}$ . Subfraction  $F1/2_{7.1}$  was further purified by preparative TLC using 20% EtOAc–hexane as eluent to yield additional mollicellin B (**5**) (15 mg). Subfraction  $F1/2_{7.2}$  was rechromatographed by FCC, eluted with 50% EtOAc–hexane, to give mollicellin F (**8**) (21 mg).

The MeOH extract (20.6 g) was subjected to silica gel FCC, eluted with a gradient of hexane–EtOAc and EtOAc–MeOH to yield fractions  $F'''_1$ – $F'''_4$ . Fraction  $F'''_2$  yielded additional mollicellin C (**6**) (7 mg). Fraction  $F'''_3$  afforded additional mollicellin E (**7**) (11.4 mg).

**Mollicellin K (1):** white solid; mp 178–181 °C; UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 203 (4.47), 264 (4.52) nm; IR (KBr)  $\nu_{\text{max}}$  3407, 2977, 2360, 1731, 1656, 1638, 1594, 1573  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 1 and 2; HRESITOFMS  $m/z$  383.1161 [ $\text{M} + \text{H}$ ] $^+$  (calcd for  $\text{C}_{21}\text{H}_{18}\text{O}_7 + \text{H}$ , 383.1131).

**Mollicellin L (2):** white solid; mp 215–219 °C; UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 203 (4.27), 264 (4.47) nm; IR (KBr)  $\nu_{\text{max}}$  3439, 3028, 2983, 2931, 1729, 1677, 1644, 1608, 1568  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 1 and 2; HRESITOFMS  $m/z$  397.1286 [ $\text{M} + \text{H}$ ] $^+$  (calcd for  $\text{C}_{22}\text{H}_{20}\text{O}_7 + \text{H}$ , 397.1287).

**Mollicellin M (3):** white solid; mp 247–250 °C; UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 201 (4.01), 261 (3.95) nm; IR (KBr)  $\nu_{\text{max}}$  3454, 2979, 2917, 1736, 1688, 1652, 1600, 1562  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 1 and 2; HRESITOFMS  $m/z$  439.0561 [ $\text{M} + \text{Na}$ ] $^+$  (calcd for  $\text{C}_{21}\text{H}_{17}\text{ClO}_7 + \text{Na}$ , 439.0561).

**Mollicellin N (4):** white solid; 251–253 °C; UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 201 (3.67), 267 (3.89) nm; IR (KBr)  $\nu_{\text{max}}$  3355, 2979, 2932, 1738, 1688, 1644, 1574  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 1 and 2; HRESITOFMS  $m/z$  421.0897 [ $\text{M} + \text{Na}$ ] $^+$  (calcd for  $\text{C}_{21}\text{H}_{18}\text{O}_8 + \text{Na}$ , 421.0899).

**Mollicellin B (5):** white solid; mp 203–205 °C; UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 201 (4.05), 261 (3.95) nm; IR (KBr)  $\nu_{\text{max}}$  3461, 3089, 2979, 2929, 2854, 1739, 1686, 1651, 1602, 1574  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 1 and 2; HRESITOFMS  $m/z$  405.0953 [ $\text{M} + \text{Na}$ ] $^+$  (calcd for  $\text{C}_{21}\text{H}_{18}\text{O}_7 + \text{Na}$ , 405.0950).

**Mollicellin C (6):** white solid; mp 200–202 °C; UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 206 (4.21), 267 (3.97) nm; IR (KBr)  $\nu_{\text{max}}$  3389, 2928, 2358, 1734, 1645, 1559  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 1 and 2; ESITOFMS  $m/z$  435.0648 [ $\text{M} + \text{Na}$ ] $^+$  (calcd for  $\text{C}_{22}\text{H}_{20}\text{O}_8 + \text{Na}$ , 435.1055).

**Mollicellin E (7):** white solid; mp 169–170 °C; UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 205 (4.18), 267 (3.80) nm; IR (KBr)  $\nu_{\text{max}}$  3382, 2921, 2850, 1741, 1651, 1624, 1566  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 1 and 2; ESITOFMS  $m/z$  469.0427 [ $\text{M} + \text{Na}$ ] $^+$  (calcd for  $\text{C}_{22}\text{H}_{19}\text{ClO}_8 + \text{Na}$ , 469.0665).

**Mollicellin F (8):** white solid; 256–257 °C; UV (MeOH)  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 201 (3.71), 267 (3.86), 224 (4.05) nm; IR (KBr)  $\nu_{\text{max}}$  3400, 2980, 2924, 1741, 1688, 1644, 1565  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 1 and 2; ESITOFMS  $m/z$  455.0318 [ $\text{M} + \text{Na}$ ] $^+$  (calcd for  $\text{C}_{21}\text{H}_{17}\text{ClO}_8 + \text{Na}$ , 455.0406).

**Cyclization of 1.** To a solution of **1** (32.1 mg) in MeOH (5 mL) was added *p*-toluenesulfonic acid (9.4 mg), and the solution was stirred at 50 °C for 4 h. Cooled water was added to the reaction mixture, and it was extracted with EtOAc (10 mL  $\times$  3). The organic layer was combined, washed with water and brine, and dried over anhydrous  $\text{Na}_2\text{SO}_4$ . The filtrate was evaporated to dryness, and the residue was separated by preparative TLC (10% EtOAc–hexane) to give **5** (29.2 mg, 91%); mp 202–204 °C; IR and NMR spectra were identical to those of mollicellin B (**5**) (Tables 1 and 2).

**Antimalarial Assay.** Antimalarial activity was evaluated against the parasite *Plasmodium falciparum* (K1, multidrug-resistant strain), using the method of Trager and Jensen.<sup>23</sup> Quantitative assessment of activity in vitro was determined by means of the microculture radioisotope

technique based upon the method described by Desjardins et al.<sup>24</sup> The inhibitory concentration (IC<sub>50</sub>) represents the concentration that causes 50% reduction in parasite growth as indicated by the in vitro uptake of [<sup>3</sup>H]-hypoxanthine by *P. falciparum*. The standard compound was artemisinin (Table 3).

**Antimycobacterial Assay.** Antimycobacterial activity was assessed against *Mycobacterium tuberculosis* H37Ra using the microplate Alamar Blue assay (MABA).<sup>25</sup> The standard drugs isoniazid and kanamycin sulfate were used as the reference compounds (Table 3).

**Antifungal Assay.** An antifungal assay was performed against clinical isolated *Candida albicans* using a method modified from the soluble formazan assay described by Scudiero and co-workers.<sup>26</sup> The number of living cells was determined by measuring the absorbance of XTT formazan at 450 nm. The reference substance was amphotericin B (Table 3).

**Cytotoxicity Assay.** Cytotoxic assays against human epidermoid carcinoma (KB), human breast cancer (BC1), human small cell lung cancer (NCI-H187), and cholangiocarcinoma cell lines were performed employing the colorimetric method as described by Skehan and co-workers.<sup>27</sup> The reference substance was ellipticine (Tables 3 and 4).

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**Supporting Information Available:** <sup>1</sup>H NMR, <sup>13</sup>C NMR, and NOESY spectra for mollicellins K–N (1–4) and B (5). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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