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# Anti-*Pseudomonas aeruginosa* xanthones from the resin and green fruits of *Cratoxylum cochinchinense*

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ABSTRACT

Cochinchinones I–L (**1**–**3** and **13**) along with 11 known xanthones (**4**–**12**, **14**, and **15**) were isolated from the resin and green fruits of *Cratoxylum cochinchinense*. In addition, four new acetylated compounds (**16**–**19**) were derivatized from 7-geranyloxy-1,3-dihydroxyxanthone (**14**) and 3-geranyloxy-1,7-dihydroxyxanthone (**15**). All compounds were characterized on the basis of spectroscopic analyses. The structures of cochinchinone I (**1**), a monoacetate (**18**) and a dibrosylate (**20**), were also confirmed by X-ray diffraction analysis. The antibacterial and antifungal activities of selected compounds were evaluated as well.



## 1. Introduction

*Cratoxylum cochinchinense* belongs to the family Guttiferae, which is distributed in several parts of Thailand.[1] This plant is a well-known tropical tree, and is commonly known in Thailand as Tui Kliang. The bark, roots, and leaves of this plant have been used in folk medicine to treat fever, coughs, diarrhea, itches, ulcers, and abdominal complaints.[2] Previous investigations revealed the major components from the *Cratoxylum* genus as xanthones and anthraquinones.[3–6] Xanthones have been reported to exhibit biological activities such as antibacterial,[3–5] antioxidant,[6,7]cytotoxic,[3–5,8,9] and antiprotozoal activities.[10] A preliminary screening of the bioactivity of the crude extracts from the resin and green fruits of *C. cochinchinense* has shown strong antibacterial activity specifically against *Pseudomonas aeruginosa*. Although *Pseudomonas sepsis* is a major cause of death in transplant recipients, while *P. aeruginosa* is one of the most common Gram-negative pathogenic bacteria causing life-threatening infections.[11–13] This result led us to investigate the bioactive compounds from this plant. The antibacterial and antifungal activities of isolated compounds were also evaluated.

## 2. Results and discussion

The crude $CH_2Cl_2$ extract of the resin of *C. cochinchinense* was subjected to chemical investigation leading to the isolation of three new xanthones, cochinchinones I–K (**1**–**3**), together with nine known xanthones identified as cochinchinone A (**4**),[6] 1,3,7-trihydroxy-2,4-diisoprenylxanthone (**5**),[14] celebixanthone methyl ether (**6**),[15] dulxis-xanthone F (**7**),[15] β-mangostin (**8**),[16] α-mangostin (**9**),[16] macluraxanthone (**10**),[17] pruniflorone G (**11**),[3] and a caged-prenylated xanthone (**12**).[6] Their structures were elucidated by NMR analysis and comparison of their spectroscopic data with those reported in the literature.

Cochinchinone I (**1**) was obtained as a yellow powder, which was further recrystallized from acetone to yield yellow single crystals. The X-ray structure (Fig. 1) suggested a xanthone skeleton with a chromene ring. A molecular formula $C_{28}H_{30}O_5$ was implied by HRMS. Its structure was supported by the $^1H$ and $^{13}C$ NMR spectral data (Table 1). The $^1H$ and $^{13}C$ NMR spectral data of **1** were

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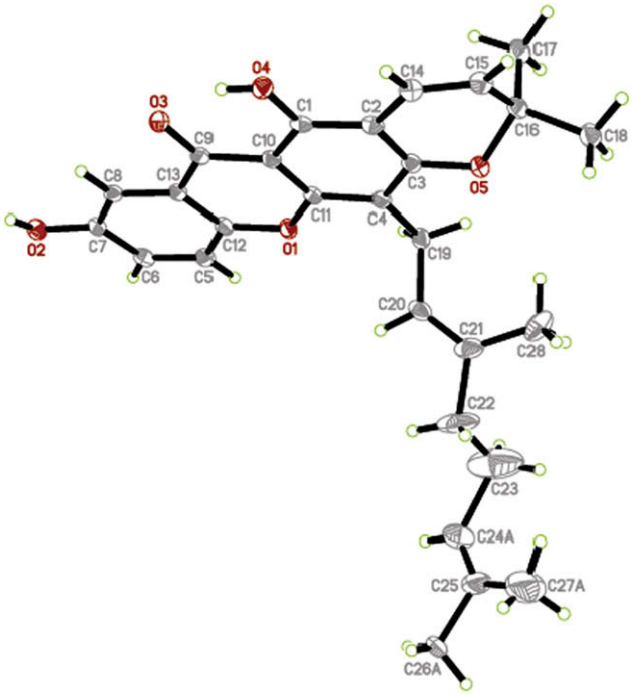



**Figure 1.** The X-ray structure of **1**.

comparable to cochinchinone A (**4**), which was previously isolated from the roots of *C. cochinchinense*.[6] The main difference was observed at C-2 and C-3, where the $^1$H NMR spectral data of **1** showed the signals of a chromene ring at $\delta$ 6.74 (d, *J* 9.9 Hz, H-1′), 5.60 (d, *J* 9.9 Hz, H-2′), and 1.48 (s, Me-4′ and Me-5′) instead of a prenyl group at C-2 and a free hydroxyl group at C-3 as in **4** (Table 1). The chromene ring was connected to a xanthone skeleton in a linear fashion whose structure was confirmed by HMBC correlations as summarized in Table 2, which agreed well with the X-ray structure (Fig. 1). From these data, the structure of cochinchinone I was assigned as **1**. In addition, the asymmetric unit of a cocrystal (Fig. 1a) consisted of a disordered mixture of cochinchinone I (**1**) (Fig. 1) and a new chromenoxanthone **1a** (Fig. 1b). The latter was different from **1** in which a geranyl side chain in **1** was replaced by a (*E*)-3″,7″,7″-trimethyloct-2″-ene side chain in **1a**. Therefore, a trace amount of chromenoxanthone **1a** in a single crystal should be an unexpected contaminated compound from cochinchinone I (**1**), which might be transformed during the resin formation.

Cochinchinone J (**2**) was obtained as a yellow viscous oil with a molecular ion peak at *m*/*z* 446.2092 $[M]^+$ in the HRMS, corresponding to a molecular formula of $C_{28}H_{30}O_5$. The UV spectrum showed absorption bands of a xanthone (243, 289, 298, 320, 351, and 391 nm),[14] while the IR spectrum exhibited the hydroxyl and conjugated carbonyl functionalities at $\nu_{max}$ 3397 and 1649 cm$^{-1}$, respectively. The $^1$H and $^{13}$C NMR data of **2** (Table 1) were similar to those of **4**,[6] except for the appearance of the signals of a chromene ring bearing a methyl group and six-carbon side chain of 4-methylpent-3-enyl group, which appeared at $\delta_H$ 5.10 (br t, *J* 7.2 Hz, H-6″), 1.89 (m, 1H-4″), 1.68 (m, 1H-4″), 2.12 (m, $H_2$-5″), 1.66 (s, Me-8″), and 1.57 (s, Me-10″) (Table 1) instead of a geranyl moiety at C-4 as in **4**. The loss of 4-methylpent-3-enyl moiety in EIMS, *m*/*z* 363 ($[M]^+ - 83$), also supported the proposed structure. Finally, the location of the angular chromene ring was confirmed by HMBC correlations (Table 2) in which the methine proton H-1″ ($\delta$ 6.88) was correlated with C-3 ($\delta$ 158.9), C-4 ($\delta$ 100.2), C-4a ($\delta$ 150.2), and C-3″ ($\delta$ 80.6). Therefore, the structure of cochinchinone J was deduced to be **2**.

Compound **3** was isolated as an inseparable mixture with compound **2**, and the mixture was thus acetylated with $Ac_2O$ in pyridine. The resulting product was further separated by CC eluting with 70% $CHCl_3$–hexane to give monoacetates **3a** (18.9 mg) and **2a**

**Table 1**
$^1$H and $^{13}$C (300 MHz) spectral data of **1**, **2**, **3a**, and **4** in $CDCl_3$

| Position | **1** | | **2** | | **3a** | | **4** | |
|---|---|---|---|---|---|---|---|---|
| | $^1$H (*J* in Hz) | $^{13}$C ($\delta$) | $^1$H (*J* in Hz) | $^{13}$C ($\delta$) | $^1$H (*J* in Hz) | $^{13}$C ($\delta$) | $^1$H (*J* in Hz) | $^{13}$C ($\delta$) |
| 1-OH | 12.97, s | 155.5 | 13.15, s | 160.2 | 13.02, s | 158.4 | 12.79, s | 158.1 |
| 2 | | 104.2 | | 111.2 | | 103.9 | | 109.1 |
| 3 | | 158.4 | | 158.9 | | 159.6 | | 161.1 |
| 4 | | 107.4 | | 100.2 | | 107.3 | | 104.9 |
| 5 | 7.30, d, 9.0 | 119.0 | 7.35, d, 8.7 | 118.9 | 7.46, d, 8.7 | 118.7 | 7.04, d, 9.0 | 118.7 |
| 6 | 7.23, dd, 2.4, 9.0 | 124.1 | 7.25, m | 123.8 | 7.39, dd, 8.7, 2.7 | 128.6 | 7.07, br d, 9.0 | 124.7 |
| 7 | | 150.5 | | 150.3 | | 146.3 | | 150.1 |
| 8 | 7.60, d, 2.4 | 108.9 | 7.60, br d, 1.8 | 109.3 | 7.92, d, 2.7 | 117.8 | 7.44, br s | 108.7 |
| 9 | | 180.9 | | 180.5 | | 180.3 | | 180.9 |
| 4a | | 154.5 | | 150.2 | | 152.4 | | 152.9 |
| 4b | | 152.4 | | 152.2 | | 153.7 | | 152.4 |
| 8a | | 120.6 | | 121.1 | | 121.2 | | 120.2 |
| 9a | | 103.3 | | 102.9 | | 102.3 | | 103.0 |
| 1′ | 6.74, d, 9.9 | 115.8 | 3.36, d, 7.2 | 21.2 | 2.75, br t, 6.9 | 16.2 | 3.32, d, 6.9 | 21.8 |
| 2′ | 5.60, d, 9.9 | 127.3 | 5.25, br t, 7.2 | 122.1 | 1.81, br t, 6.9 | 31.7 | 5.19, br t, 6.9 | 121.6 |
| 3′ | | 78.1 | | 131.5 | | 76.3 | | 134.8 |
| 4′ | 1.48, s | 28.4 | 1.68, s | 25.8 | 1.39, s | 26.9 | 1.55, s | 25.6 |
| 5′ | 1.48, s | 28.4 | 1.81, s | 17.9 | 1.39, s | 26.9 | 1.75, s | 17.9 |
| 1″ | 3.46, d, 7.2 | 21.3 | 6.88, d, 10.2 | 115.9 | 3.48, d, 7.5 | 21.5 | 3.39, d, 6.9 | 21.6 |
| 2″ | 5.22, br t, 7.2 | 122.1 | 5.54, d, 10.2 | 125.4 | 5.22, br t, 6.3 | 122.3 | 5.16, br t, 6.9 | 121.7 |
| 3″ | | 135.0 | | 80.6 | | 134.9 | | 137.9 |
| 4″ | 1.99, m[a] | 39.7 | 1.89, m,[a] 1.68, m[a] | 41.8 | 1.96, m | 39.8 | 1.99, m[a] | 39.7 |
| 5″ | 2.04, m[a] | 26.6 | 2.12, m[a] | 22.8 | 2.03, m | 26.7 | 1.97, m[a] | 26.4 |
| 6″ | 5.04, br t, 6.6 | 124.2 | 5.10, br t, 7.2 | 123.8 | 5.05, br t, 6.6 | 124.4 | 4.95, br t, 7.2 | 123.9 |
| 7″ | | 131.3 | | 131.9 | | 131.3 | | 131.8 |
| 8″ | 1.59, s | 25.6 | 1.66, s | 25.6 | 1.60, s | 25.6 | 1.67, s | 25.8 |
| 9″ | 1.86, s | 16.3 | 1.44, s | 27.1 | 1.86, s | 16.3 | 1.78, s | 16.2 |
| 10″ | 1.53, s | 17.6 | 1.57, s | 17.6 | 1.55, s | 17.7 | 1.48, s | 17.6 |
| 7-OAc | | | | | 2.34, s | 20.9/169.4 | | |

[a] Deduced from HMQC experiment.

**Table 2**
HMBC (300 MHz) spectral data of **1**, **2**, **3a**, and **4** in $CDCl_3$

| Position | 1 | 2 | 3a | 4 |
|---|---|---|---|---|
| 1-OH | C-1, C-2, C-9a | C-1, C-2, C-9a | C-1, C-2, C-9a | C-1, C-2, C-9, C-9a |
| 5 | C-7, C-9, C-4b, C8a | C-7, C8a | C-7, C-8a, C-9 | C-7, C-9, C-4b, C8a |
| 6 | C-5, C-7, C-8, C-4b | C-7, C-4b | C-5, C-7, C-4b | C-7, C-8 |
| 8 | C-6, C-7, C-9, C-4b | C-8 | C-6, C-7, C-4b, C-9 | C-6, C-7, C-9, C-4b |
| 1′ | C-1, C-2, C-3, C-4, C-3′ | C-1, C-2, C-2′, C-3′ | C-1, C-2, C-3, C-2′, C-3′ | C-1, C-2, C-3, C-2′, C-3′ C-4′, C-5′ |
| 2′ | C-2, C-3′, C-4′, C-5′ | C-2′ | C-2, C-1′, C-3′, C-4′, C-5′ | C-2, C-1′, C-5′ |
| 4′ | C-2′, C-3′, C-5′ | C-2′, C-3′, C-5′ | C-2′, C-3′ | C-2′, C-3′, C-5′ |
| 5′ | C-2′, C-3′, C-4′ | C-2′, C-3′, C-4′ | C-2′, C-3′ | C-2′, C-3′, C-4′ |
| 1″ | C-3, C-4, C-4a, C-2″, C-3″ | C-3, C-4, C-4a, C-3″ | C-3, C-4, C-4a, C-2″, C-3″ | C-3, C-4, C-4a, C-2″, C-3″, C-9″ |
| 2″ | C-4, C-1″, C-4″, C-9″ | C-4, C-3″, C-4″, C-5″, C-9″ | C-1″, C-3″, C-4″, C-9″ | C-4, C-1″, C-4″, C-9″ |
| 4″ | C-2″, C-3″, C-5″ | C-2″, C-3″ | C-2″, C-3″, C-6″ | C-2″, C-6″ |
| 5″ | C-3″, C-4″, C-6″, C-7″ | C-6″, C-7″ | C-3″, C-6″, C-7″ | C-3″, C-4″, C-6″, C-7″ |
| 6″ | C-4″, C-5″, C-8″, C-10″ | C-6″ | C-5″, C-8″, C-10″ | C-4″, C-5″, C-8″ |
| 8″ | C-6″, C-7″, C-10″ | C-6″, C-7″, C-10″ | C-6″, C-7″, C-10″ | C-6″, C-7″, C-10″ |
| 9″ | C-2″, C-3″ | C-2″, C-3″, C-4″ | C-2″,C-3″ | C-2″, C-3″, C-4″ |
| 10″ | C-6″, C-7″, C-8″ | C-6″, C-7″, C-8″ | C-6″, C-7″, C-10″ | C-6″, C-7″, C-8″ |
| 7-OAc | | | C-7 | |

(5.1 mg). The latter was confirmed as an acetylated derivative of **2** by comparison of its spectral data with those of compound **2**.

Compound **3a** is a yellow powder. The HRMS spectrum showed a molecular ion peak at $m/z$ 490.2355 $[M]^+$, corresponding to $C_{30}H_{34}O_6$. The UV spectrum showed absorption bands of a xanthone (243, 271, 303, 326, and 383 nm),[14] while the IR spectrum exhibited the hydroxyl and conjugated carbonyl functionalities at $\nu_{max}$ 3392 and 1648 $cm^{-1}$, respectively. The $^1H$ and $^{13}C$ NMR spectral data of **3a** (Table 1) were closely related to those of **1**, except for the appearance of the signals of a dimethylchromane ring and acetoxy group revealed at $\delta_H$ 2.75 (br t, $J$ 6.9 Hz, H-1′), 1.81 (br t, $J$ 6.9 Hz, H-2′), 1.39 (s, Me-4′ and Me-5′), and 2.34 (s, 7-OAc) (Table 1) instead of a chromene ring and a hydroxyl group in **1**. The chromane ring was fused to the xanthone nucleus in a linear fashion, which was confirmed by HMBC correlations (Table 2), in which the methylene protons $H_2$-1′ at $\delta_H$ 2.75 were correlated with C-1 ($\delta$ 158.4), C-2 ($\delta$ 103.9), C-3 ($\delta$ 159.6), C-2′ ($\delta$ 31.7), and C-3′ ($\delta$ 76.3), while the H-bonded hydroxyl proton 1-OH ($\delta$ 13.02) was correlated with C-1 ($\delta$ 158.4), C-2 ($\delta$ 103.9), and C-9a ($\delta$ 102.3). Therefore, the structure of **3a**, an acetylated form of **3**, was deduced. Compound **3** was named cochinchinone K.

It is interesting to note that the three new chromenoxanthones (**1** and **2**) and chromanoxanthone (**3**) could be derived from cochinchinone A (**4**). The isoprenyl or geranyl side chains of **4** were epoxidized and further cyclized via a free hydroxyl at C-3 to produce the chromanol ring[18] as shown in Scheme 1. Further dehydration led to the linear or angular chromene rings of **1** and **2**, respectively. On the other hand, the new chromanoxanthone (**3**) could be produced by cyclization via a free hydroxyl group to an isoprenyl side chain of **4** to afford the chromane ring (Scheme 1). Therefore, cochinchinone A (**4**) should be a precursor of cochinchinones I–K (**1**–**3**).

The investigation of the crude $CH_2Cl_2$ extract of the green fruits (approximate 4 weeks maturity stage) led to the isolation and identification of a new xanthone, cochinchinone L (**13**) along with two major known oxygeranyl xanthones **14**[14] and **15**.[19] Compounds **14** and **15** were structural isomers of xanthones with an oxygeranyl

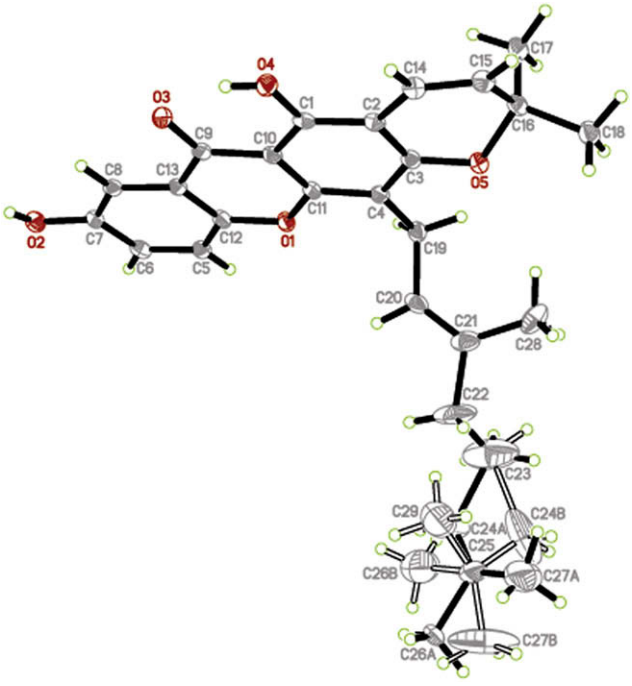



**Figure 1a.** The asymmetric unit of a cocrystal of **1** and **1a**.

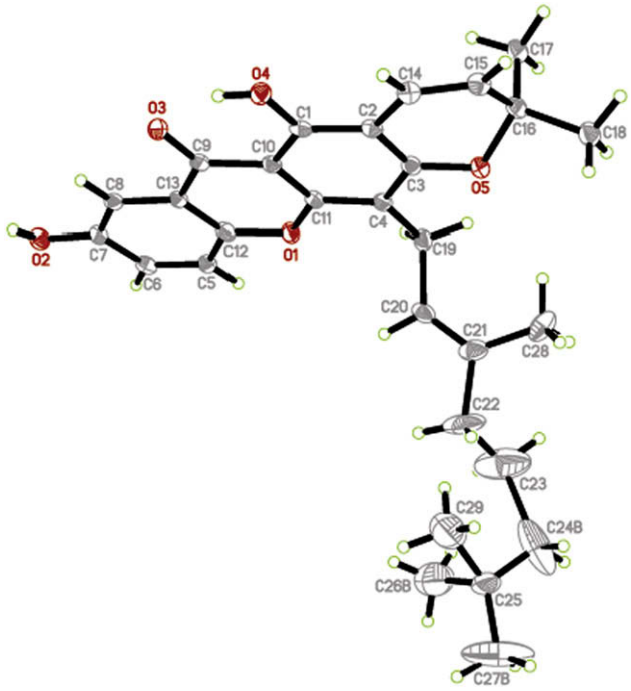
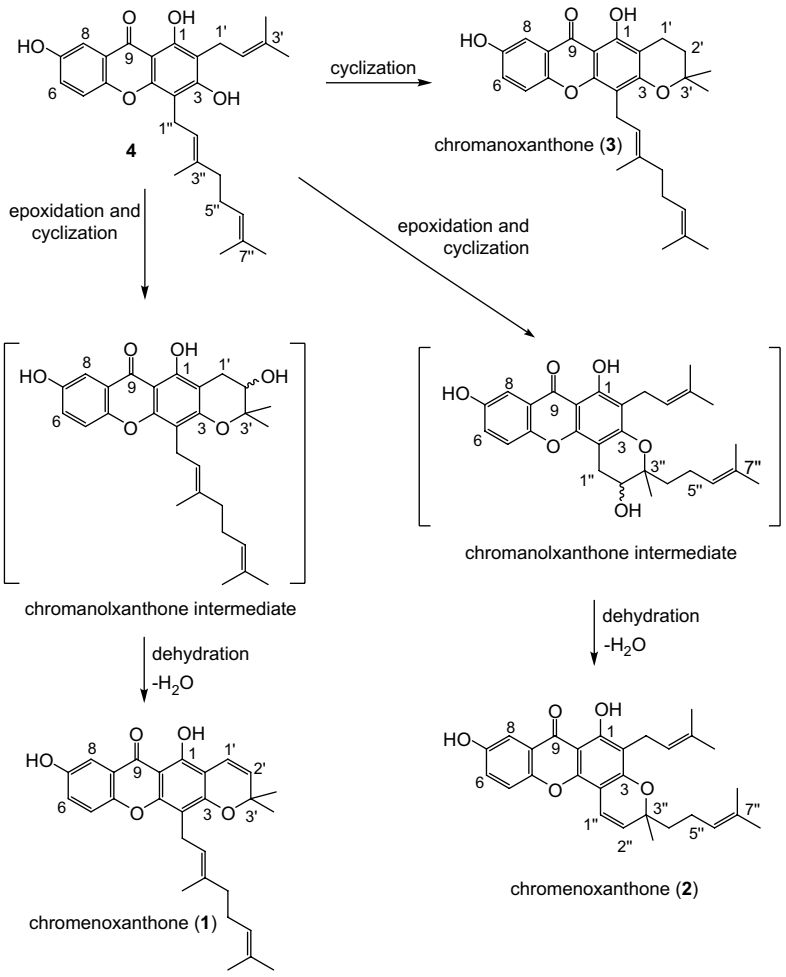



**Figure 1b.** The X-ray structure of **1a**.




**Scheme 1.** Plausible biosynthesis pathway of **1**, **2**, and **3**.

side chain. The $^{1}H$ and $^{13}C$ NMR spectral data of both **14** and **15** (Tables 3 and 4) were almost identical. However, the main difference was observed in the NOESY experiment in which $H_2$-1′ ($\delta$ 4.45) of **14** showed a cross-peak with H-8 ($\delta$ 7.40) and H-6 ($\delta$ 7.18), whereas the $H_2$-1′ ($\delta$ 4.63) of **15** showed a cross-peak with H-4 ($\delta$ 6.40) and H-2 ($\delta$ 6.34). These results implied that the oxygeranyl side chain of **14** was connected to C-7 and that of **15** to C-3. To confirm these structural assignments of **14** and **15**, compound **14** was further reacted with *p*-bromobenzenesulfonyl chloride in $CH_2Cl_2$ at room temperature to afford a crystalline dibrosylate **20**

**Table 3**
$^{1}H$ NMR (300 MHz) spectral data of **13**–**19** in $CDCl_3$

| Position | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
|---|---|---|---|---|---|---|---|
| 2 | 6.43, d, 2.1 | 6.22, d, 1.8 | 6.34, d, 2.4 | 6.42, br d, 2.1 | 7.15, d, 2.1 | 6.19, br d, 2.1 | 6.41, d, 2.1 |
| 4 | 6.57, d, 2.1 | 6.23, d, 1.8 | 6.40, d, 2.4 | 6.59, br d, 2.1 | 6.79, d, 2.1 | 6.24, br d, 2.1 | 6.46, d, 2.1 |
| 5 | 7.08, br d, 9.0 | 7.15, br d, 9.0 | 7.30, d, 9.3 | 7.21, br d, 8.1 | 7.23, d, 9.3 | 7.25, d, 9.3 | 7.25, d, 8.1 |
| 6 | 7.10, br d, 9.0 | 7.18, br d, 9.0 | 7.26, d, 9.3 | 7.20, br d, 8.1 | 7.28, dd, 9.3, 2.1 | 7.29, br d, 9.3 | 7.26, br d, 8.1 |
| 8 | 7.45, br s | 7.40, br s | 7.59, br s | 7.44, br s | 7.55, d, 2.1 | 7.76, br s | 7.78, br d, 1.5 |
| 1′ | 4.42, d, 6.3 | 4.45, d, 6.3 | 4.63, d, 6.6 | 4.50, d, 6.6 | 4.56, d, 6.3 | 4.49, d, 6.3 | 4.51, d, 6.3 |
| 2′ | 5.73, t, 6.3 | 5.39, br t, 6.3 | 5.50, br t, 6.6 | 5.40, br t, 6.6 | 5.48, br t, 6.3 | 5.37, br t, 6.3 | 5.37, br t, 6.3 |
| 4′ | 1.99, m | 2.01, m | 2.13, m | 2.03, m | 2.11, m | 2.03, m | 2.03, m |
| 5′ | 1.97, m | 1.95, m | 2.10, m | 2.02, m | 2.09, m | 1.97, m | 2.01, m |
| 6′ | 4.98, t, 6.6 | 4.98, br t, 5.7 | 5.11, br t, 5.7 | 5.00, br t, 6.3 | 5.09, br t, 6.3 | 4.99, br t, 6.9 | 4.99, br t, 6.6 |
| 8′ | 1.57, s | 1.57, s | 1.69, s | 1.57, s | 1.66, s | 1.57, s | 1.57, s |
| 9′ | 1.60, s | 1.64, s | 1.78, s | 1.68, s | 1.73, s | 1.66, s | 1.65, s |
| 10′ | 1.50, s | 1.50, s | 1.62, s | 1.51, s | 1.59, s | 1.51, s | 1.51, s |
| 1-OAc | 2.38, s | — | — | — | — | — | — |
| 1-OH | — | 12.85, s | 12.72, s | 12.71, s | — | 12.55, s | — |
| 3-OH | — | 7.86, br s | | — | — | — | — |
| 7-OH | — | — | 7.03, br s | — | — | — | — |
| 1-OAc | — | — | — | — | 2.47, s | — | 2.40, s |
| 3-OAc | — | — | — | 2.23, s | 2.27, s | — | — |
| 7-OAc | — | — | — | — | — | 2.22, s | 2.19, s |

(Fig. 3), while compound **15** was acetylated in the usual manner with $Ac_2O$ in pyridine to give a crystalline monoacetate **18** (Fig. 2). Both **20** and **18** were subjected to X-ray diffraction analysis whose results supported the structures of **14** and **15**.

Cochinchinone L (**13**) was isolated as a yellow powder, with a molecular ion peak at *m*/*z* 422.1718 $[M]^+$ in the HRMS, corresponding to a molecular formula of $C_{25}H_{26}O_6$. The $^1H$ and $^{13}C$ NMR spectral data of **13** (Tables 3 and 4) showed characteristic signals similar to those of 7-geranyloxy-1,3-dihydroxyxanthone (**14**),[14] except that the hydroxyl group at C-1 of **14** was replaced by an acetoxy group in **13**, which appeared as a proton singlet signal at $\delta$ 2.38. The higher field chemical shift of C-1 ($\delta$ 151.2) and lower field resonances of protons and carbons of C-2 ($\delta_H$ 6.43 (d, *J* 2.1 Hz, H-2); $\delta_C$ 108.3) and C-4 ($\delta_H$ 6.57 (d, *J* 2.1 Hz, H-4); $\delta_C$ 101.3) as compared to those of **14** ($\delta_H$ 6.22 (d, *J* 1.8 Hz, H-2); $\delta_C$ 98.5 and $\delta_H$ 6.23 (d, *J* 1.8 Hz, H-4); $\delta_C$ 94.4) supported the title structure (Tables 3 and 4). The $^4J$ HMBC correlations of methyl protons of the acetoxy group ($\delta$ 2.38) with C-1 ($\delta$ 151.2) supported its location at C-1, which was also confirmed by NOESY cross-peak between acetoxy moiety with H-2. Cochinchinone L (**13**), an acetylated analogue of

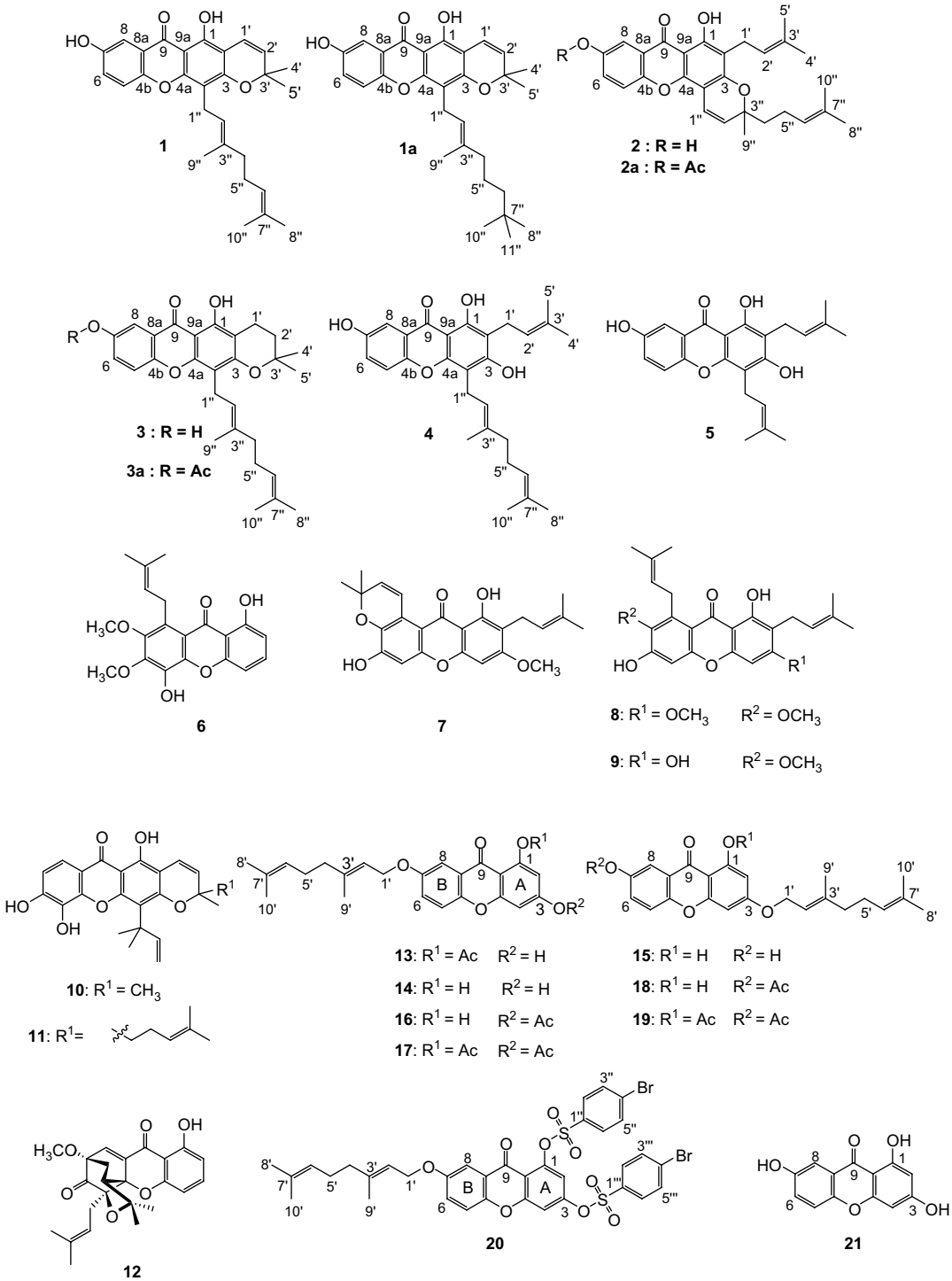


**Table 4**
$^{13}$C NMR (75 MHz) spectroscopic data of **13**–**19** in $CDCl_3$

| Position | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
|---|---|---|---|---|---|---|---|
| 1 | 151.2 | 163.8 | 163.2 | 162.8 | 150.9 | 163.3 | 158.7 |
| 2 | 108.3 | 98.5 | 97.6 | 104.0 | 108.7 | 97.8 | 99.4 |
| 3 | 162.6 | 164.0 | 166.1 | 156.8[a] | 154.5 | 166.2 | 163.6 |
| 4 | 101.3 | 94.4 | 93.2 | 100.7 | 112.4 | 93.4 | 108.1 |
| 5 | 118.8 | 118.9 | 188.9 | 119.0 | 118.9 | 118.7 | 118.6 |
| 6 | 125.1 | 125.5 | 124.2 | 126.0 | 125.2 | 128.9 | 128.3 |
| 7 | 155.4 | 155.2 | 152.5 | 155.4 | 155.5 | 146.5 | 146.7 |
| 8 | 106.4 | 105.9 | 109.0 | 106.0 | 106.6 | 117.8 | 118.4 |
| 9 | 175.7 | 180.5 | 180.5 | 181.1 | 174.7 | 179.8 | 174.1 |
| 4a | 158.8 | 157.8 | 157.8 | 156.7[a] | 157.7 | 157.5 | 151.4 |
| 4b | 150.0 | 150.7 | 150.5 | 150.8 | 149.9 | 153.3 | 152.7 |
| 8a | 122.2 | 120.3 | 120.9 | 120.7 | 122.3 | 121.1 | 123.6 |
| 9a | 107.8 | 103.3 | 103.5 | 106.6 | 112.2 | 103.4 | 108.6 |
| 1′ | 65.51 | 65.6 | 65.6 | 65.6 | 65.5 | 65.6 | 65.8 |
| 2′ | 118.8 | 118.6 | 118.3 | 118.8 | 118.8 | 118.4 | 118.0 |
| 3′ | 141.9 | 142.1 | 142.3 | 141.9 | 141.5 | 142.2 | 142.6 |
| 4′ | 39.5 | 39.5 | 39.5 | 39.5 | 39.5 | 39.5 | 39.5 |
| 5′ | 26.3 | 26.3 | 26.2 | 26.3 | 26.3 | 26.2 | 26.2 |
| 6′ | 123.8 | 123.8 | 123.6 | 123.7 | 123.8 | 123.9 | 123.6 |
| 7′ | 131.8 | 131.8 | 131.9 | 131.8 | 131.7 | 131.9 | 131.9 |
| 8′ | 25.7 | 26.3 | 25.6 | 25.6 | 25.6 | 25.6 | 25.6 |
| 9′ | 16.7 | 16.7 | 16.7 | 16.7 | 16.7 | 16.7 | 16.7 |
| 10′ | 17.7 | 17.7 | 17.7 | 17.7 | 17.6 | 17.7 | 17.7 |
| 1-OAc | 171.0 | — | — | — | 21.1 | — | 21.1 |
| | 21.3 | — | — | — | 169.2 | — | 169.5 |
| 3-OAc | — | — | — | 21.1 | 21.0 | — | — |
| | — | — | — | 168.2 | 167.8 | — | — |
| 7-OAc | — | — | — | — | — | 20.9 | 20.9 |
| | — | — | — | — | — | 169.2 | 169.2 |

[a] May be interchangeable.

**14**, was therefore assigned as 1-acetoxy-3-hydroxy-7-geranyloxyxanthone.

The isolated compounds were evaluated for their antibacterial activities against both Gram-positive bacteria: *Bacillus subtilis*, *Staphylococcus aureus*, *Enterococcus faecalis* TISTR 459, Methicillin-Resistant *S. aureus* (MRSA) ATCC 43300, Vancomycin-Resistant *E. faecalis* (VRE) ATCC 51299 and Gram-negative bacteria: *Salmonella typhi*, *Shigella sonei*, and *P. aeruginosa*. All compounds were also subjected to antifungal assay against *Candida albicans*. The results showed that most of the isolated compounds (**4**–**6** and **13**–**15**) exhibited strong antibacterial activity specifically against *P. aeruginosa* (Table 5). Interestingly, only compounds **9** and **10** exhibited strong activity against *C. albicans*. It is important to note that compound **9** also exhibited broad spectrum antimicrobial activity against all Gram-positive bacteria. Nearly all compounds, which are active against *P. aeruginosa* are the 1,3,7-trihydroxy xanthones (**4** and **5**) or 1,3,7-trioxygenated xanthones with an oxygeranyl side chain either at C-3 or C-7 and dihydroxyl groups (**14**–**15**) whose indicated structures might contribute to the strong antibacterial activity specifically against *P. aeruginosa*. However, when the free hydroxyl group of the 1,3,7-trihydroxy xanthone was cyclized onto the isoprenyl or geranyl side chain to form a chromane or chromene ring, the antibacterial activity against *P. aeruginosa* decreased drastically as shown in compounds **1**, **2**, and **3a**. In other previous reports, it was suggested that hydroxy xanthones play important roles in biological activity such as antibacterial,[3,5] α-glucosidase inhibitory,[20] anti-tumor,[21,22] and anti-inflammatory effects.[23] Because compounds **14** and **15** are the major components obtained from this plant, this prompts us to modify their structures for the structure–activity relationships (SARs). To investigate whether the free hydroxyl group was responsible for antibacterial activity, the acetylation with acetic anhydride in pyridine was therefore applied to **14** and **15**. Four acetylated geranyloxyxanthone derivatives: 3-acetoxy-7-geranyloxy-1-hydroxyxanthone (**16**) and 1,3-diacetoxy-7-geranyloxyxanthone (**17**) were obtained from **14**, whereas 7-acetoxy-3-geranyloxy-1-hydroxy-xanthone (**18**) and 1,7-diacetoxy-3-geranyloxy-xanthone (**19**) were obtained from **15**. The assignments of the $^{1}$H and $^{13}$C NMR spectral data of **16**–**19** are summarized in Tables 3 and 4. The antibacterial activity of acetylated geranyloxyxanthone derivatives **16**–**19** and dibrosylate **20** was evaluated. All of them showed strong anti-*P. aeruginosa* (Table 5). This implied that the free hydroxyl groups should not be responsible for the inhibition of *P. aeruginosa*. For further investigation of the role of an oxygeranyl side chain, a 1,3,7-trihydroxyxanthone (**21**) obtained from the dried fruits of *C. cochinchinense*[24] was tested against *P. aeruginosa*. It was inactive against Gram-negative bacteria as shown in Table 5. From these results, it can be suggested that the geranyl side chain is necessary for anti-*P. aeruginosa* activity. Moreover, mixtures of compound **4** with compound **5**, and compound **14** with compound **15** were subjected to antimicrobial assay. Interestingly, the mixture of compounds **14** and **15** significantly increased the antibacterial activity against MRSA compared with the pure forms as indicated by the lower MIC values shown in Table 5. The mixture of compounds **4** and **5**, on the other hand, did not show any significant differences for antibacterial activity compared to the pure forms as also shown in Table 5. Therefore, it may be proposed that the 1,3,7-trihydroxyxanthone with the isoprenyl or geranyl side chain at C-2 and C-4 in

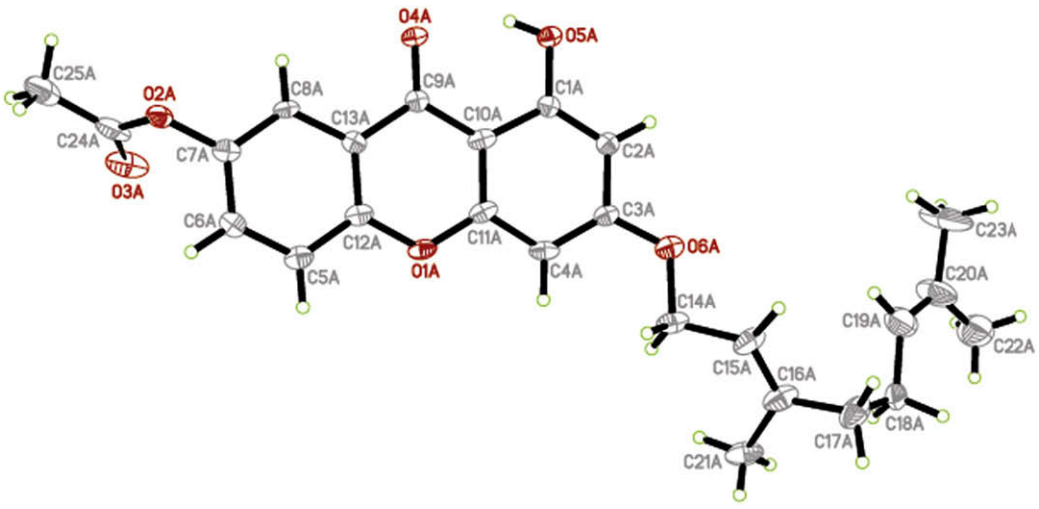



**Figure 2.** ORTEP plot of a monoacetate **18**.

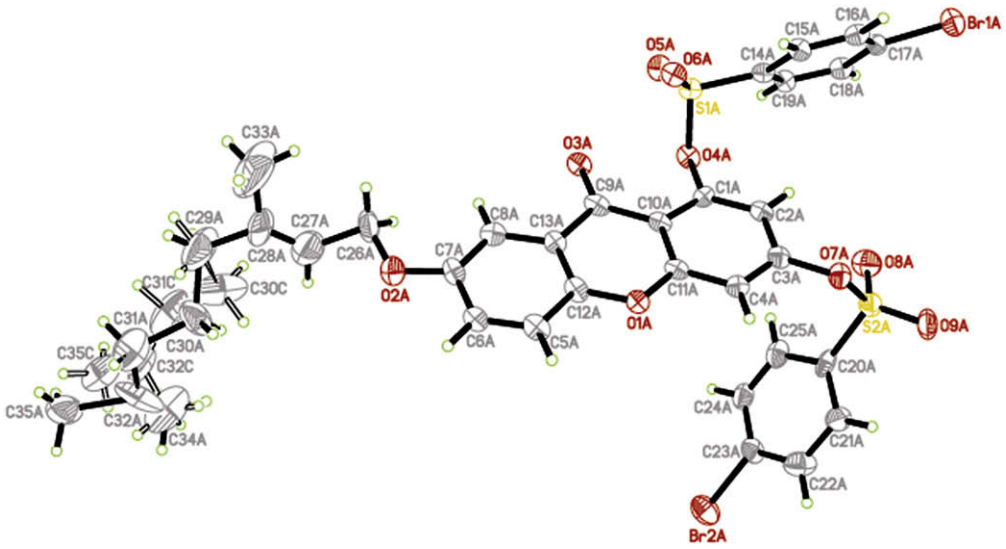



**Figure 3.** ORTEP plot of a dibrosylate **20**.

**4** and **5**, respectively, and 1,3,7-trioxygenated xanthone with the geranyl side chain at C-3 or C-7 in **13**–**19** are essential for their antibacterial activity against *P. aeruginosa*. Therefore, 1,3,7-trihydroxyxanthones (**4** and **5**) and 1,3,7-trioxygenated xanthone with geranyl side chain (**13**–**19**) should be considered as potent candidates as anti-*P. aeruginosa*.

We further studied the possible mode of action of compounds **4** and **13** against *P. aeruginosa* by observing the bacteria cell morphology through scanning electron microscopy (SEM) at 3, 6, 9, and 15 h after applying compounds **4** and **13**. From the SEM results (see Figs 4 and 5), it was clearly indicated that the cell morphology of *P. aeruginosa*, when treated with compounds **4** and **13**, started to deform at 3 h onward, and at 15 h, most cells were completely deformed whose results was correlated to their strong antibacterial activity (Table 5). Therefore, it can be suggested that compounds **4** and **13** may interact with or damage the cell wall of *P. aeruginosa* as seen by the formation of pores on the cell wall of *P. aeruginosa* (Figs 4 and 5).

## 3. Experimental

### 3.1. General experimental procedures

Melting points were determined on a Fisher–John melting point apparatus. Optical rotations were measured on a JASCO P-1020 digital polarimeter. UV and IR spectra were recorded on SPECORD S 100 (Analytikjena) and Perkin–Elmer FTS FT-IR spectrophotometer,

**Table 5**
Antimicrobial activity (MIC, μg/ml) of **1**–**21**

| No. | Antibacterial activity | | | | | | | | Antifungal activity |
|---|---|---|---|---|---|---|---|---|---|
| | Gram-positive bacteria[a] | | | | | Gram-negative bacteria [b] | | | |
| | *B. subtilis* | *S. aureus* | *E. faecalis* | MRSA | VRE | *S. typhi* | *S. sonei* | *P. aeruginosa* | *C. albicans*[c] |
| **1** | >300 | >300 | >300 | >300 | >300 | >300 | >300 | >300 | >300 |
| **2** | 300 | 300 | >300 | 300 | 300 | >300 | >300 | 300 | 75 |
| **3a** | 75 | >300 | 300 | >300 | >300 | >300 | >300 | >300 | 300 |
| **4** | 150 | 150 | 150 | 9.37 | 150 | >150 | >150 | 4.7 | 75 |
| **5** | 150 | 75 | 150 | 37.5 | 75 | >150 | >150 | 4.7 | 37.5 |
| **4**+**5** | 75 | 150 | 75 | 9.37 | 150 | >150 | >150 | 4.7 | 75 |
| **6** | >150 | >150 | >150 | 150 | 150 | >150 | >150 | 4.7 | 150 |
| **7** | 150 | 300 | 300 | 150 | 150 | >300 | >300 | 150 | 300 |
| **8** | 300 | 75 | 150 | 75 | 150 | >300 | >300 | 300 | 150 |
| **9** | 9.37 | 9.37 | 9.37 | 9.37 | 9.37 | >300 | >300 | 18.75 | 2.4 |
| **10** | 18.75 | 37.5 | 37.5 | 37.5 | 37.5 | >300 | >300 | 37.5 | 4.7 |
| **11** | >300 | 300 | >300 | 150 | 150 | >300 | >300 | >300 | 300 |
| **12** | 75 | >300 | 300 | 150 | 150 | >300 | >300 | >300 | >300 |
| **13** | 150 | >150 | 150 | 37.5 | 150 | >150 | >150 | 4.7 | 18.75 |
| **14** | 150 | >150 | 150 | 18.7 | 150 | >150 | >150 | 4.7 | 75 |
| **15** | 150 | 150 | 150 | 9.3 | 150 | >150 | >150 | 4.7 | 37.5 |
| **14**+**15** | 75 | 37.5 | 37.5 | 4.7 | 37.5 | >150 | >150 | 4.7 | 37.5 |
| **16** | 150 | >150 | 150 | 18.75 | 150 | >150 | >150 | 4.7 | 150 |
| **17** | 75 | >150 | 75 | 37.5 | 150 | >150 | >150 | 4.7 | 18.75 |
| **18** | >150 | >150 | 150 | 18.75 | 150 | >150 | >150 | 4.7 | 150 |
| **19** | >150 | >150 | >150 | 37.5 | 150 | >150 | >150 | 4.7 | 150 |
| **20** | >150 | >150 | 150 | 300 | >300 | >150 | >150 | 4.7 | >300 |
| **21** | 37.5 | 75 | >300 | 150 | 300 | >300 | >300 | 300 | 300 |

[a] *B. subtilis*, *S. aureus*, *E. faecalis* TISTR 459, Methicillin-Resistant *S. aureus* (MRSA) ATCC 43300, Vancomycin-Resistant *E. faecalis* (VRE) ATCC 51299.
[b] *S. typhi*, *S. sonei*, and *P. aeruginosa*.
[c] *C. albicans*.

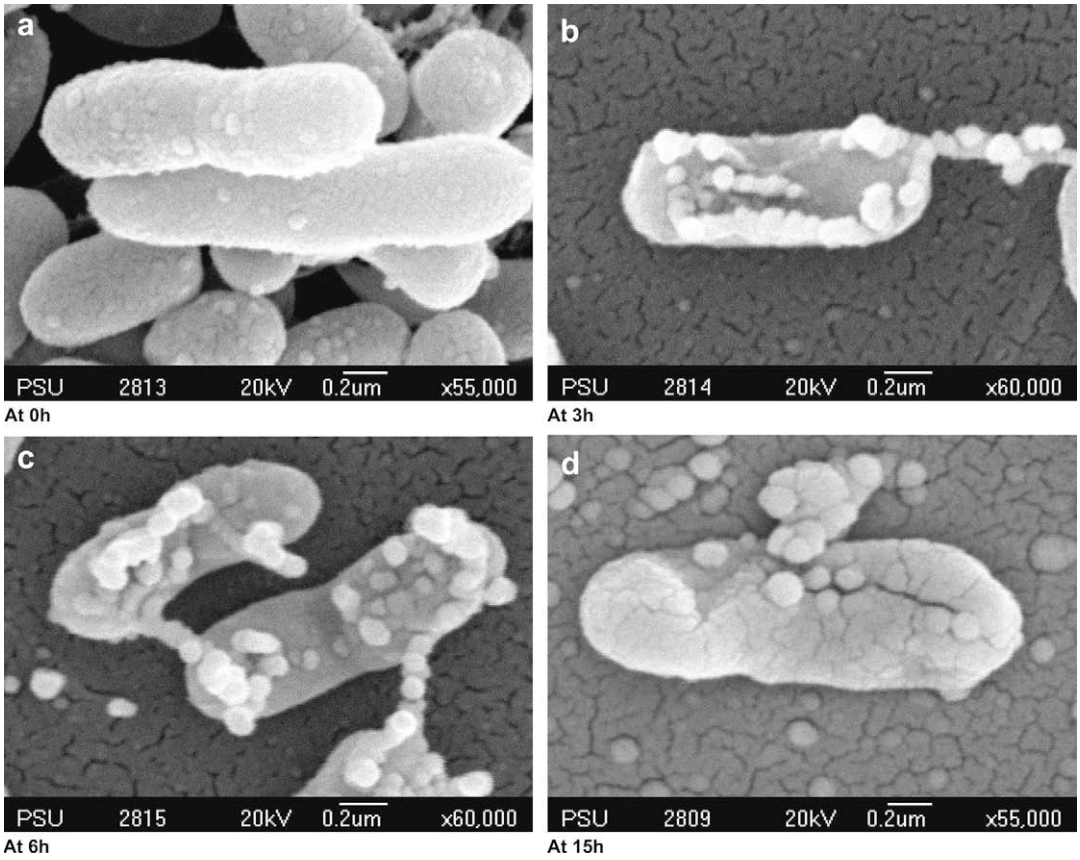



**Figure 4.** Scanning electron microscopy of cell morphology of *P. aeruginosa* treated with compound **4** at different time.

respectively. The $^{1}H$ and $^{13}C$ NMR spectra were recorded on 300 MHz Bruker FTNMR Ultra Shield™ spectrometer in $CDCl_3$ with TMS as the internal standard. Chemical shifts are reported in $\delta$ (ppm) and coupling constants ($J$) are expressed in hertz. EI and HREI mass spectra were measured on a Kratos MS 25 RFA spectrometer. Quick column chromatography (QCC) and column chromatography (CC) were carried out on silica gel 60 $F_{254}$ (Merck) and silica gel 100 (Merck), respectively. All the bacteria images were viewed with a JSM-5800LV, JEOL SEM (scanning electron microscope).

### 3.2. Plant material

The resin of *C. cochinchinense* was collected in October 2003 at Prince of Songkla University, Hat-Yai campus, whereas the green fruits of *C. cochinchinense* were collected in October 2007 at Kaun Kha Long district, Satun Province, Southern part of Thailand. Botanical identification was achieved through comparison with a voucher specimen No. SL-1 (PSU) in the herbarium of Department of Biology, Prince of Songkla University, Songkhla, Thailand.

### 3.3. Isolation and extraction

The resin of *C. cochinchinense* (87.75 g) was extracted with $CH_2Cl_2$ (2×2.0 L, for a week) at room temperature and was evaporated under reduced pressure to afford a deep green crude $CH_2Cl_2$ extract (47.04 g), which was subjected to QCC (Quick column chromatography) on silica gel (Merck 60 $F_{254}$) using hexane as a first eluent and then increasing the polarity with acetone to give 16 fractions (FR1–FR16). Fractions FR4 and FR5 were separated by CC eluting with a gradient of acetone–hexane to give eight subfractions (FR4A–FR4H) and **8** (150.2 mg) ($R_f$ (15% acetone–hexane (three runs)) 0.39). Subfraction FR4B was further purified by CC and eluted with a gradient of acetone–hexane to give **7** (31.4 mg) ($R_f$ (15% acetone–hexane) 0.31) and **12** (56.5 mg) ($R_f$ (15% acetone–hexane) 0.28). Fractions FR6 and FR7 were separated by QCC and eluted with a gradient of $CH_2Cl_2$–hexane to give six subfractions (FR6A–FR6F). Subfraction FR6B was further separated by QCC eluting with a gradient of acetone–hexane to give **6** (35.7 mg) ($R_f$ (15% acetone–hexane (three runs)) 0.41), **8** (83.2 mg) ($R_f$ (15% acetone–hexane (three runs)) 0.39), and **11** (1.8 mg) ($R_f$ (15% acetone–hexane (three runs)) 0.24). Subfraction FR6E was purified by CC on reversed-phase silica gel C-18 eluting with MeOH to give **4** (849.4 mg) ($R_f$ (MeOH) 0.54) and **5** (1.25 g) ($R_f$ (MeOH) 0.62). Fractions FR8 and FR9 were separated by QCC and eluted with a gradient of $CH_2Cl_2$–hexane to afford **5** (551.3 mg) ($R_f$ (60% $CH_2Cl_2$–hexane (three runs)) 0.05), **7** (18.2 mg) ($R_f$ (60% $CH_2Cl_2$–hexane (three runs)) 0.29), **8** (116.6 mg) ($R_f$ (60% $CH_2Cl_2$–hexane (three runs)) 0.61), and **9** (148.7 mg) ($R_f$ (60% $CH_2Cl_2$–hexane (three runs)) 0.10). Fractions FR10 and FR11 were separated by QCC and eluted with a gradient of acetone–hexane to give seven subfractions (FR10A–FR10G) and **7** (25.9 mg) ($R_f$ (15% acetone–hexane) 0.31). Subfraction FR10B was further purified by CC on silica gel C-18 and eluted with MeOH to furnish **1** (8.5 mg) ($R_f$ (MeOH) 0.36). The mixture of **1** and **1a** could not be separated due to the very low amount of **1a**. The purity of **1** is much more than 90% base on a single spot on TLC and the integral area signal in the $^{1}H$ NMR spectrum of **1**. Subfraction FR10D was separated by QCC eluting with a gradient of acetone–hexane to give six subfractions (FR10D1–FR10D6). Subfraction FR10D2 was further separated by CC and eluted with a gradient of acetone–hexane to give five subfractions (FR10D2A–FR10D2E), **2** (1.8 mg) ($R_f$ (20% acetone–hexane)

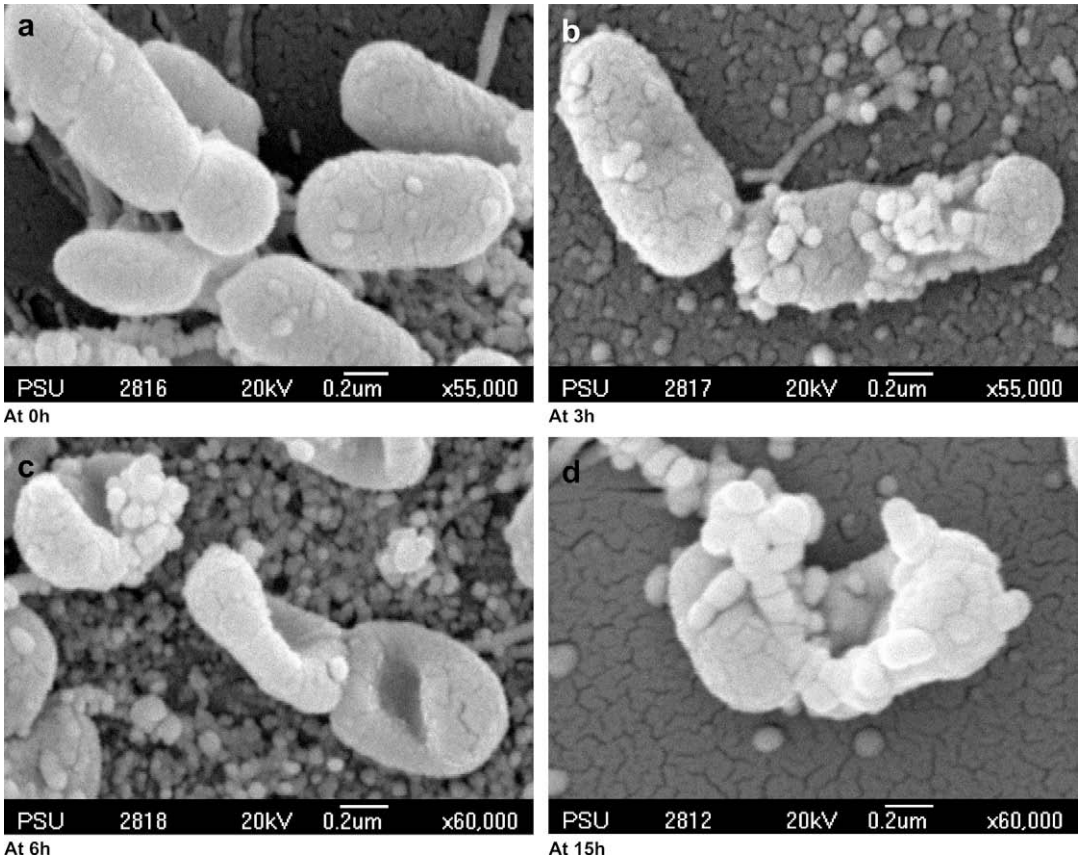



**Figure 5.** Scanning electron microscopy of cell morphology of *P. aeruginosa* treated with compound **13** at different time.

0.36), **4** (23.1 mg) ($R_f$ (20% acetone–hexane) 0.22), **5** (34.2 mg) ($R_f$ (20% acetone–hexane) 0.17), and an inseparable mixture of **2** and **3** (20.2 mg) ($R_f$ (20% acetone–hexane) 0.35). The mixture was separated by acetylation with $Ac_2O$ (0.1 ml) in pyridine (2.0 ml) and stirred overnight at room temperature to give a yellow gum, which was further purified by CC eluting with 70% $CHCl_3$–hexane to give acetylated derivatives **2a** (5.1 mg) ($R_f$ (70% $CHCl_3$–hexane) 0.68) and **3a** (18.9 mg) ($R_f$ (70% $CHCl_3$-hexane) 0.40), respectively. Subfraction FR10D2E was purified by CC and eluted with 80% $CH_2Cl_2$–hexane to give **8** (16.7 mg) ($R_f$ (80% $CH_2Cl_2$–hexane) 0.91), **10** (5.0 mg) ($R_f$ (80% $CH_2Cl_2$–hexane) 0.85), and **11** (1.5 mg) ($R_f$ (80% $CH_2Cl_2$–hexane) 0.38).

Air-dried green fruits of *C. cochinchinense* (5.5 kg) were extracted with $CH_2Cl_2$ (2×20 L, for a week) at room temperature and was evaporated under reduced pressure to afford a deep green crude $CH_2Cl_2$ extract (40.04 g), which was subjected to QCC on silica gel using hexane as a first eluent and increasing polarity with EtOAc to give nine fractions (FS1–FS9). Fraction FS5 was purified by CC eluting with pure $CHCl_3$ to give **14** (1.88 g) ($R_f$ (70% $CHCl_3$–hexane) (three runs) 0.46). Fraction FS6 was further separated by CC eluting with pure $CHCl_3$ to furnish six subfractions (FS6A–FS6F), **14** (2.10 g) ($R_f$ (70% $CHCl_3$–hexane) (three runs) 0.46) and **15** (490.2 mg) ($R_f$ (70% $CHCl_3$–hexane) (three runs) 0.39). Subfraction FS6B was further purified by CC eluting with a gradient of acetone–hexane to give **13** (53.3 mg) ($R_f$ (15% acetone–hexane (three runs)) 0.17).

### 3.3.1. *Cochinchinone I (**1**)*

Yellow needle single crystals, mp 160–162 °C; UV ($CHCl_3$) $\lambda_{max}$ (log $\varepsilon$) 261 (4.01), 297 (4.31), 342 (3.69), 393 (3.46) nm; IR (neat) $\nu_{max}$ 3406, 1650, 1612 $cm^{-1}$; HRMS *m*/*z* 446.2279 for $C_{28}H_{30}O_5$ (calcd 446.2093). EIMS *m*/*z* (rel int.): 446 $[M]^+$ (76), 431 (100), 377 (73), 363 (76), 323 (26), 307 (15), 295 (13), 137 (5), 69 (6). For $^1H$ and $^{13}C$ NMR spectroscopic data, see Table 1.

### 3.3.2. *Cochinchinone J (**2**)*

Yellow viscous oil; $[\alpha]_D^{25}$ −69.8 (*c* 0.08, $CHCl_3$); UV ($CHCl_3$) $\lambda_{max}$ (log $\varepsilon$) 243 (3.90), 289 (4.10), 298 (4.13), 320 (3.68), 351 (3.47), 391 (3.33) nm; IR (neat) $\nu_{max}$ 3397, 1649, 1613 $cm^{-1}$; HRMS *m*/*z* 446.2092 for $C_{28}H_{30}O_5$ (calcd 446.2093). EIMS *m*/*z* (rel int.): 446 $[M]^+$ (11), 363 (100), 307 (18), 69 (5). For $^1H$ and $^{13}C$ NMR spectroscopic data, see Table 1.

### 3.3.3. *Acetylated derivative of cochinchinone K (**3a**)*

Yellow powder, mp 85–87 °C; UV ($CHCl_3$) $\lambda_{max}$ (log $\varepsilon$) 243, 271, 303, 326, 383 nm; IR (neat) $\nu_{max}$ 3392, 1709, 1648, 1612 $cm^{-1}$; HRMS *m*/*z* 490.2355 for $C_{30}H_{34}O_6$ (calcd 490.2355). EIMS *m*/*z* (rel int.): 490 $[M]^+$ (53), 473 (53), 419 (44), 405 (31), 365 (100), 323 (52), 311 (41), 267 (51), 69 (24). For $^1H$ and $^{13}C$ NMR spectroscopic data, see Table 1.

### 3.3.4. *Cochinchinone L (**13**)*

Yellow powder, mp 114–116 °C; UV ($CHCl_3$) $\lambda_{max}$ (log $\varepsilon$) 248 (4.59), 273 (4.03), 305 (4.13), 354 (3.80) nm; IR (neat) $\nu_{max}$ 3237, 1774, 1728, 1628 $cm^{-1}$; HRMS *m*/*z* 422.1718 for $C_{25}H_{26}O_6$ (calcd 422.1729). EIMS *m*/*z* (rel int.): 422 $[M]^+$ (1), 286 (40), 244 (100), 187 (4), 81 (9), 69 (28). For $^1H$ and $^{13}C$ NMR spectroscopic data, see Tables 3 and 4.

## 3.4. Acetylation of 14 and 15

Compound **14** (82.5 mg) was treated with $Ac_2O$ (2.5 ml) in pyridine (2.0 ml) and stirred for 6 h at room temperature. The reaction mixture was diluted with water and extracted with $CH_2Cl_2$.

The combined organic extract was washed with 10% HCl and then washed with water again. After the organic solvent was removed, the resulting residue was dried over anhydrous $Na_2SO_4$. Chromatography over silica gel yielded a pale yellow powder of **16** (80.6 mg) ($R_f$ (5% acetone–hexane (three runs)) 0.44).

Compound **14** (200.5 mg) was treated with $Ac_2O$ (6.0 ml) in pyridine (3.0 ml) and stirred overnight at room temperature. Chromatography over silica gel yielded a pale yellow powder of **16** (10.6 mg) ($R_f$ (5% acetone–hexane (three runs)) 0.44) and **17** (177.8 mg) ($R_f$ (15% acetone–hexane (three runs)) 0.46).

Compound **15** (85.5 mg) was treated with $Ac_2O$ (2.5 ml) in pyridine (2.0 ml) and stirred for 6 h at room temperature. Chromatography over silica gel yielded a pale yellow powder of **18** (83.7 mg) ($R_f$ (5% acetone–hexane (three runs)) 0.32), which was further recrystallized from MeOH–acetone (1:99 v/v) to give yellow single crystals.

Compound **15** (190.0 mg) was treated with $Ac_2O$ (6.0 ml) in pyridine (3.0 ml) and stirred overnight at room temperature. Chromatography over silica gel yielded a pale yellow powder of **18** (8.7 mg) ($R_f$ (5% acetone–hexane (three runs)) 0.32) and **19** (170.0 mg) ($R_f$ (15% acetone–hexane (three runs)) 0.37).

*3.4.1. 3-Acetoxy-7-geranyloxy-1-hydroxyxanthone (**16**)*

Yellow powder, mp 94–95 °C; UV ($CHCl_3$) $\lambda_{max}$ (log $\varepsilon$) 239 (4.40), 262 (4.66), 289 (3.97), 379 (3.93) nm; IR (neat) $\nu_{max}$ 3429, 1768, 1649, 1612 $cm^{-1}$; HRMS *m/z* 422.1725 for $C_{25}H_{26}O_6$ (calcd 422.1729). EIMS *m/z* (rel int.): 422 $[M]^+$ (1), 286 (43), 244 (100), 187 (4), 81 (9), 69 (24). For $^1H$ and $^{13}C$ NMR spectroscopic data, see Tables 3 and 4.

*3.4.2. 1,3-Diacetoxy-7-geranyloxyxanthone (**17**)*

Yellow powder, mp 96–97 °C; UV ($CHCl_3$) $\lambda_{max}$ (log $\varepsilon$) 253 (4.59), 300 (3.45), 361 (3.86) nm; IR (neat) $\nu_{max}$ 3429, 1776, 1656, 1624 $cm^{-1}$; HRMS *m/z* 464.1838 for $C_{27}H_{28}O_7$ (calcd 464.1835). EIMS *m/z* (rel int.): 464 $[M]^+$ (2), 328 (3), 286 (58), 244 (100), 187 (5), 81 (17), 69 (36). For $^1H$ and $^{13}C$ NMR spectroscopic data, see Tables 3 and 4.

*3.4.3. 7-Acetoxy-3-geranyloxy-1-hydroxy-xanthone (**18**)*

Yellow powder, mp 104–106 °C; UV ($CHCl_3$) $\lambda_{max}$ (log $\varepsilon$) 243 (4.44), 257 (4.52), 310 (4.29), 358 (3.82) nm; IR (neat) $\nu_{max}$ 3429, 1758, 1665, 1607 $cm^{-1}$; HRMS *m/z* 422.1726 for $C_{25}H_{26}O_6$ (calcd 422.1729). EIMS *m/z* (rel int.): 422 $[M]^+$ (5), 286 (16), 244 (100), 187 (2), 81 (16), 69 (56). For $^1H$ and $^{13}C$ NMR spectroscopic data, see Tables 3 and 4.

*3.4.4. 1,7-Diacetoxy-3-geranyloxyxanthone (**19**)*

Yellow powder, mp 85–87 °C; UV ($CHCl_3$) $\lambda_{max}$ (log $\varepsilon$) 246 (4.61), 275 (4.01), 302 (4.28), 334 (3.86) nm; IR (neat) $\nu_{max}$ 3453, 1770, 1655, 1629 $cm^{-1}$; HRMS *m/z* 464.1834 for $C_{27}H_{28}O_7$ (calcd 464.1835). EIMS *m/z* (rel int.): 464 $[M]^+$ (4), 328 (4), 286 (32), 244 (100), 187 (2), 81 (24), 69(73). For $^1H$ and $^{13}C$ NMR spectroscopic data, see Tables 3 and 4.

### 3.5. Brosylation of 14

Compound **14** (40.0 mg, 105.14 mmol) was stirred overnight at room temperature with *p*-bromobenzenesulfonyl chloride (40.30 mg, 190.2 mmol) and $K_2CO_3$ (44.1 mg, 315.4 mmol) in $CH_2Cl_2$ (3 ml). After the reaction was complete, water (10 ml) was added to the reaction mixture. The resulting solution was then extracted with $CH_2Cl_2$ (10 ml, three times). The combined organic extract was dried over anhydrous sodium sulfate and evaporated under reduced pressure to give a crude extract, which was further purified by column chromatography over silica gel eluting with 5% acetone–hexane to yield the dibrosylate **20** (75.2 mg) ($R_f$ (5% acetone–hexane (three runs)) 0.20). Yellow needle-shaped single crystals of **20** were obtained after recrystallization from $CH_3OH$–$CHCl_3$ (1:4 v/v), mp 106–108 °C. $\delta_H$ (300 MHz, $CDCl_3$+$CD_3OD$) 7.82 (dd, *J* 9.0, 2.1 Hz, H-2″, H-6″), 7.68 (d, *J* 2.4 Hz, H-4), 7.65 (dd, *J* 8.7, 2.1 Hz, H-3″, H-5″, H-2‴, H-6‴), 7.63 (dd, *J* 8.7, 2.1 Hz, H-3‴, H-5‴), 7.50 (d, *J* 2.4 Hz, H-8), 7.32 (d, *J* 2.4 Hz, H-2), 7.26 (d, *J* 9.0 Hz, H-5), 7.19 (dd, *J* 9.3, 2.4 Hz, H-6), 5.44 (br t, *J* 6.6, H-2′), 5.04 (br t, *J* 6.3 Hz, H-6′), 4.56 (d, *J* 6.6 Hz, H-1′), 2.07 (m, 2H-5′), 2.05 (m, 2H-4′), 1.71 (s, 3H-9′),1.60 (s, 3H-8′), 1.54 (s, 3H-10′); $\delta_C$ (75 MHz, $CDCl_3$+$CD_3OD$) 181.6 (C-9), 157.4 (C-1), 155.9 (C-7), 151.9 (C-3), 149.7 (C-4b), 148.6 (C-4a), 142.1 (C-3′), 134.3 (C-1″), 133.4 (C-1‴), 133.1 (C-3″, C-5″), 132.5 (C-3‴, C-5‴), 131.8 (C-7′), 130.6 (C-4″), 130.2 (C-2″, C-6″), 130.1 (C-4‴), 129.8 (C-2‴, C-6‴), 124.9 (C-6), 123.7 (C-6′), 122.4 (C-8a), 118.8 (C-5), 118.6 (C-2′), 114.2 (C-9a), 112.8 (C-4), 111.1 (C-2), 106.8 (C-8), 65.6 (C-1′), 39.5 (C-4′), 26.2 (C-5′), 25.5 (C-8′), 17.5 (C-10′), 16.6 (C-9′). EIMS *m/z* (rel int.): 816 $[M-2]^+$ (1), 683 (7), 681 (14), 679 (7), 619 (5), 617 (10), 615 (5), 461 (55), 463 (56), 399 (41), 397 (41), 371 (19), 369 (19), 357 (34), 355 (34), 244 (27), 229 (84), 215 (100), 186 (16), 157 (53), 155 (53), 131 (11), 108 (13), 76 (16).

### 3.6. X-ray crystallographic studies of cocrystal of 1 and 1a, monoacetate 18, and dibrosylate 20

Crystallographic data were collected at 100.0 (1) K with the Oxford Cryosystem Cobra low-temperature attachment. The data were collected using a Bruker Apex2 CCD diffractometer with a graphite monochromated Mo Kα radiation at a detector distance of 5 cm using *APEX2*.[25] The collected data were reduced using *SAINT* program,[25] and the empirical absorption corrections were performed using *SADABS* program.[25] The structures were solved by direct methods and refined by least-squares using the *SHELXTL* software package[26] All non-hydrogen atoms were refined anisotropically, whereas all H atoms were placed in calculated positions with an O–H distance of 0.82 Å and C–H distances in the range 0.93–0.98 Å after checking their positions in the difference map. The $U_{iso}$ values were constrained to be $1.5U_{eq}$ of the carrier atoms for methyl H atoms and $1.2U_{eq}$ for hydroxyl and the other H atoms. The final refinement converged well. Materials for publication were prepared using *SHELXTL*[26] and *PLATON*.[27]

*Crystal data for* cocrystal of **1** and **1a**: $C_{28.60}H_{32.40}O_5$, $M$=465.15, 0.60×0.19×0.10 $mm^3$, monoclinic, $P2_1/c$, $a$=21.6161(6) Å, $b$=5.3826(2) Å, $c$=22.8296(8) Å, $\alpha$=90.00°, $\beta$=121.267°(2), $\gamma$=90.00°, $V$=2270.44(13) $Å^3$, $Z$=4, $D_x$=1.334 Mg $m^{-3}$, $\mu$(Mo Kα)=2.512 $mm^{-1}$, 29,338 reflection measured, 6634 unique reflections, $R$=0.0995, $R_w$=0.2794.

*Crystal data for* **18**: $C_{25}H_{26}O_6$, $M$=422.46, 0.58×0.27×0.06 $mm^3$, orthorhombic, $P2_12_12_1$, $a$=7.3280(2) Å, $b$=13.0634(5) Å, $c$=45.6190 (18) Å, $\alpha=\beta=\gamma$=90.00°, $V$=4367.0(3) $Å^3$, $Z$=4, $D_x$=1.285 Mg $m^{-3}$, $\mu$(Mo Kα)=0.091 $mm^{-1}$, 81,117 reflection measured, 4877 unique reflections, $R$=0.1258, $R_w$=0.3299.

*Crystal data for* **20**: $C_{35}H_{30}Br_2O_9S_2$, $M$=818.53, 0.60×0.18×0.03 $mm^3$, triclinic, $P-1$, $a$=9.0779(4) Å, $b$=19.3468(7) Å, $c$=20.7939(7) Å, $\alpha$=108.160(2)°, $\beta$=91.755(2)°, $\gamma$=90.046(2)°, $V$=3468.3(2) $Å^3$, $Z$=4, $D_x$=1.568 Mg $m^{-3}$, $\mu$(Mo Kα)=2.512 $mm^{-1}$, 41,231 reflection measured, 12,219 unique reflections, $R$=0.0817, $R_w$=0.2121.

The crystallographic-information files for cocrystal of **1** and **1a**, **18**, and **20** have been deposited in the Cambridge Crystallographic Data Centre as CCDC694467, CCDC689924, and CCDC689378, respectively. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/data_request/cif, or by e-mailing data_request@ccdc.cam.ac.uk, or by contacting the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

### 3.7. Antimicrobial activity

*3.7.1. Antibacterial assay*

The isolated compounds from the resin and green fruits of *C. cochinchinense* were tested against both Gram-positive and Gram-negative bacteria: *B. subtilis*, *S. aureus*, TISTR517, *E. faecalis* TISTR459, Methicillin-Resistant *S. aureus* (MRSA) ATCC43300, Vancomycin-Resistant *E. faecalis* (VRE) ATCC 51299, *Streptococcus faecalis*, *S. typhi*, *S. sonei* and *P. aeruginosa*. The microrganisms were obtained from the culture collections, Department of Industrial Biotechnology and Department of Pharmacognosy and Botany, PSU, except for the TISTR and ATCC strains, which were obtained from Microbial Research Center (MIRCEN), Bangkok, Thailand. The antibacterial assay employed was the same as described in Boonsri et al.[5] Vancomycin, which was used as a standard, showed antibacterial activity against Vancomycin-Resistant *E. faecalis* (VRE) ATCC 51299 at 75.0 μg/ml.

*3.7.2. Antifungal assay*

*C. albicans* was obtained from Department of Pharmacognosy and Botany, PSU. The antifungal assay employed was the same as described in Boonsri et al.[5] by using amphotencin B as a positive control.

*3.7.3. Antibacterial assay against P. aeruginosa and SEM analysis for treated cells*

Antibacterial activity against *P. aeruginosa* and the potential mode of action of compounds **4** and **13** were investigated by the same antibacterial assay. The cells of *P. aeruginosa* were sampled at 0, 3, 6, and 15 h for analysis by SEM.

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