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ORIGINAL ARTICLE

# Saccharosporones A, B and C, cytotoxic antimalarial angucyclinones from *Saccharopolyspora* sp. BCC 21906

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**Three new angucyclinones, saccharosporones A, B and C, together with (+)-ochromycinone, (+)-rubiginone $B_2$, tetrangulol methyl ether and fujianmycin A, were obtained from fermentation of the terrestrial actinomycete of the genus *Saccharopolyspora* BCC 21906 isolated from a soil collected in Chanthaburi Province, Thailand. Structures of the new compounds and their relative configurations were assigned by NMR spectral data interpretation. Saccharosporones A and B exhibited antimalarial activity against *Plasmodium falciparum* K1 with $IC_{50}$ values of 4.1 and 3.9 μM. Both metabolites also possessed cytotoxic activities against cancer cell lines (KB, MCF-7 and NCI-H187) and nonmalignant Vero cell, while saccharosporone C only showed cytotoxic activity against NCI-H187.**
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**Keywords:** actinomycete; angucyclinone; antimalarial activity; cancer cell cytotoxicity; natural product; *Saccharopolyspora* sp.

## INTRODUCTION

As angucycline metabolites are a large class of antibiotics with a broad spectrum of biological activities and interesting chemical structures, they have attracted much attention.[1,2] In general, a compound from this family is characterized by an angular tetracyclic framework that possesses a benz[*a*]anthracene ring system. In the course of our screening program of rare actinomycetes in Thailand for novel bioactive secondary metabolites, fermentation of *Saccharopolyspora* sp. BIOTEC Culture Collection (BCC) 21906 led to the isolation of three new angucyclines saccharosporones A (**1**), B (**2**) and C (**3**), (Figure 1) and several known metabolites, including ochromycinone (**4**),[3,4] (+)-rubiginone $B_2$ (**5**),[5] tetrangulol methyl ether (**6**)[6,7] and fujianmycin A.[8] In this paper, we report the isolation, structure elucidation and biological activities of these new angucyclinone polyketides.

The culture of *Saccharopolyspora* sp. BCC 21906 was extracted exhaustively with ethyl acetate. The crude substance was evaporated to dryness and fractionated by partition on the HP-20SS resin, subsequently eluted with acetone/water mixtures to obtain 20, 30, 40, 50, 60, 70, 80, 90 and 100% acetone fractions. Further purification of 70% and 80% acetone–water fractions by reversed-phase $C_{18}$ HPLC furnished compounds **1**–**3** as minor constituents.

## RESULTS AND DISCUSSION

Saccharosporone A (**1**) was obtained as a yellowish powder and its molecular formula was established as $C_{19}H_{20}O_5$ by high-resolution HRESITOFMS (*m/z* 327.1231, $[M-H]^-$) and its NMR data. The LRESIMS spectra showed ions corresponding to $[M+Na]^+$ and $[M+H]^+$ at *m/z* 351 and 329, respectively. Analysis of the IR spectroscopic data showed an absorption band at 3474 $cm^{-1}$, indicating hydroxyl functionality, and a signal at 1632 $cm^{-1}$, which suggested the presence of a chelated quinone carbonyl group.[9] The violet color reaction with the addition of sodium hydroxide indicated a peri-hydroxy quinone functional group.

The $^1H$ NMR spectrum of **1**, measured in acetone-$d_6$ (Table 1), illustrated three olefinic/aromatic proton signals at $\delta$ 7.26, 7.58 and 7.71, three hydroxy proton signals at $\delta$ 3.65, 4.24 and 12.14, in addition to one methyl, three methine and four methylene proton signals. The analysis of $^1H$–$^1H$ COSY NMR spectrum showed three coupled spin systems. The first spin system consisted of three doublets of doublets at $\delta$ 7.26 7.58 and 7.71, which can be attributed to 1,2,3-trisubstituted aromatic ring system. The second spin system involved a hydroxy ($\delta$ 4.24)-bearing methine ($\delta$ 4.04) adjacent to a methylene group ($\delta$ 2.88–2.90). The final spin system had a methine ($\delta$ 2.77) connected to two correlated methylenes ($\delta$ 1.06, 1.70 and 1.92) attached to a methine at $\delta$ 2.29 that also coupled to a methyl group ($\delta$ 0.89) and another methylene ($\delta$ 1.38 and 2.06).

The $^{13}C$ NMR/DEPT spectrum of **1** showed 19 carbon resonances of a methyl group ($\delta$ 22.4), 2 carbonyl carbons ($\delta$ 183.5 and 190.8), 3 non-oxygenated olefinic/aromatic methine carbons ($\delta$ 118.4, 123.2 and 136.2), 5 olefinic/aromatic quaternary carbons, including 1 bearing oxygen, 1 oxygenated quaternary carbon, 3 methines and

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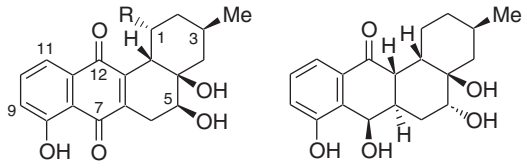
4 methylene carbons. The major portion of the angucyclinone backbone could be assembled through the interpretation of COSY and HMBC correlations (Figure 2). The cross-peaks between H-9 ($\delta$ 7.26) and H-10 ($\delta$ 7.71) to C-8 ($\delta$ 161.1) suggested the presence of an oxygenated carbon in the aromatic ring. The $^1$H NMR signal at $\delta$ 12.14 could be assigned to a chelated peri-hydroxy group attached to C-8 according to its HMBC correlations to the carbons at C-7a ($\delta$ 114.9), C-8 and C-9 ($\delta$ 123.2). The aromatic methine at H-11 ($\delta$ 7.58) correlated to carbonyl at C-12 ($\delta$ 183.5) indicated the carbonyl position adjacent to the trisubstituted aromatic ring. Both methine signal at H-12b ($\delta$ 2.77) and methylene protons at $H_2$-6 ($\delta$ 2.88–2.90) showed HMBC correlations to the same carbons at C-4a ($\delta$ 69.1), C-5 ($\delta$ 72.7), C-6a ($\delta$ 141.1) and C-12a ($\delta$ 147.4), while H-12b correlated to a carbonyl carbon at C-12 and H-6 to a carbonyl at C-7 ($\delta$ 190.8), confirming the second spin system next to the quinone ring. The HMBC correlations from methine proton at H-5 ($\delta$ 4.04) to carbons at C-4a, C-6a and C-12b ($\delta$ 42.3) and COSY correlations between $\delta$ 4.04 and $\delta$ 4.24 established a secondary alcohol at C-5, whereas HMBC correlations from hydroxyl proton at $\delta$ 3.65 to C-5 and C-12b supported the position of a tertiary alcohol at C-4a. Analysis of the COSY allowed the assignment of C-12b/C-1/C-2/C-3/C-4 connectivities, which made up a major portion of the third spin system. In addition, methylene signals at $H_2$-4 ($\delta$ 1.38, 2.06) showed correlations to carbons at C-4a, C-5 and C-12b, while the other methylene at $\delta$ 1.92 correlated to C-4a, C-12a and C-12b, establishing the core structure of the third spin system. Unlike the other angucyclinone polyketides, saccharosporone A lacked a carbonyl group at C-1 but instead contained a secondary alcohol at C-5 position.




**1** R = H
**2** R = OH

**3**

**Figure 1** The structures of saccharosporones A (**1**), B (**2**) and C (**3**) from *Saccharopolyspora* sp. BCC 21906.

An attempt to determine the absolute configuration of **1** was carried out using Mosher's method under several conditions but none of the derivatization reactions was successful, possibly because of the steric hindrance of secondary alcohol in the axial orientation. The relative stereochemistry of molecule **1** was determined by the interpretation of the NOESY NMR spectroscopic data. The strong NOESY correlations from methylene signal at $H_e$-4 ($\delta$ 2.06) to methine protons at H-3 ($\delta$ 2.29) and H-5 ($\delta$ 4.04) suggested that these protons were on the same side of the ring. In addition, the important correlations between a methine proton H-12b ($\delta$ 2.77) and two methylene signals at $H_a$-2 ($\delta$ 1.06) and $H_a$-4 ($\delta$ 1.38), along with the fact that no correlation between H-5 and H-12b has been observed, confirmed that H-5 and H-12b were on the opposite side of the ring. The *cis* relative configuration of two hydroxyl groups at

**Table 1 $^1$H and $^{13}$C NMR spectroscopic data (500 MHz, acetone-$d_6$) for saccharosporones A (1), B (2) and C (3)**

| | *Saccharosporone A (**1**)* | | *Saccharosporone B (**2**)* | | *Saccharosporone C (**3**)* | |
|---|---|---|---|---|---|---|
| *Position* | $\delta_C$, *type* | $\delta_H$(*J in Hz*) | $\delta_C$, *type* | $\delta_H$ (*J in Hz*) | $\delta_C$, *type* | $\delta_H$(*J in Hz*) |
| 1 | 28.5, $CH_2$ | 1.92, m | 83.5, CH | 4.25, d (5.3) | 22.6, $CH_2$ | 1.81, qd (12.7, 3.3)<br>1.32, dq (12.7, 3.5) |
| 2 | 34.6, $CH_2$ | 1.70, m 1.06, m | 39.5, $CH_2$ | 1.84, m 1.17, m | 34.9, $CH_2$ | 1.62–1.64, m 0.88–0.90, m |
| 3 | 29.5, CH | 2.29, m | 25.9, CH | 2.12–2.14, m | 29.8, CH | 2.33–2.35, m |
| 3-Me | 22.4, $CH_3$ | 0.89, d (6.6) | 20.5, $CH_3$ | 1.02, d (6.4) | 22.7, $CH_3$ | 0.83, d (6.6) |
| 4 | 49.8, $CH_2$ | 2.06, m 1.38, t (12.9) | 45.4, $CH_2$ | 2.09-2.11, m 1.64, t (12.5) | 50.5, $CH_2$ | 2.00–2.02, m 1.19, t (12.8) |
| 4a | 69.1, C | | 73.7, C | | 71.4, C | |
| 4a-OH | | 3.65, s | | 4.49, s | | 3.23, s |
| 5 | 72.7, CH | 4.04, m | 76.7, CH | 4.09, m | 75.5, CH | 3.85, m |
| 5-OH | | 4.24, br d (3.1) | | | | |
| 6 | 30.7, $CH_2$ | 2.88–2.90, m | 31.7, $CH_2$ | 2.83, dd (19.1, 1.8)<br>2.65, dd (19.1, 3.3) | 34.5, $CH_2$ | 2.60, td (13.2, 2.7)<br>1.63, dt (13.3, 2.7) |
| 6a | 141.1, C | | 143.7, C | | 32.4, CH | 2.44, tt (12.5, 2.7) |
| 7 | 190.8, C | | 190.7, C | | 62.8, CH | 5.07, br m |
| 7-OH | | | | | | 3.99, br m |
| 7a | 114.9, C | | 115.4, C | | 132.2, C | |
| 8 | 161.1, C | | 161.2, C | | 154.5, C | |
| 8-OH | | 12.14, s | | 12.11, s | | |
| 9 | 123.2, CH | 7.26, dd (8.4, 1.0) | 123.6, CH | 7.28, dd (8.4, 1.0) | 119.7, CH | 7.11, dd (8.0, 1.0) |
| 10 | 136.2, CH | 7.71, dd (8.4, 7.5) | 136.3, CH | 7.73, dd (8.4, 7.5) | 128.4, CH | 7.24, t (7.8) |
| 11 | 118.4, CH | 7.58, dd (7.5, 1.0) | 118.5, CH | 7.59, dd (7.5, 1.0) | 117.7, CH | 7.46, d (7.7) |
| 11a | 132.8, C | | 132.5, C | | 133.7, C | |
| 12 | 183.5, C | | 182.7, C | | 200.3, C | |
| 12a | 147.4, C | | 145.8, C | | 43.1, CH | 3.37, dd (12.5, 4.5) |
| 12b | 42.3, CH | 2.77, t (8.8) | 46.8, CH | 3.04, d (22.4) | 43.9, CH | 2.30, m |

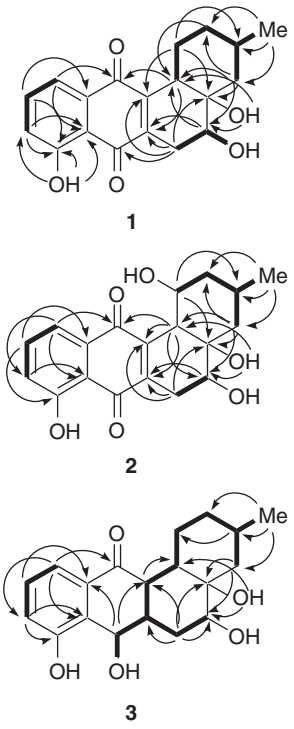



**Figure 2** Selected COSY (bold lines) and HMBC (arrows) correlations observed for **1**, **2** and **3**.

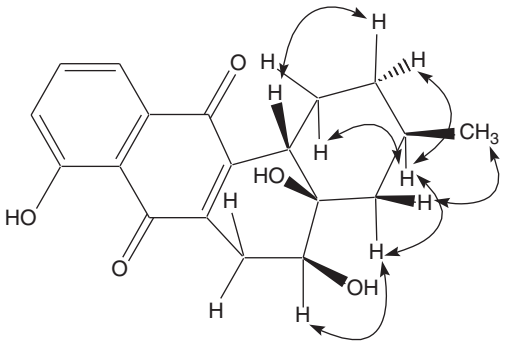



**Figure 3** Assigned relative configuration of **1** from selected NOESY NMR correlations.

C-4a and C-5 could be proposed on the basis of the analysis by NOESY and 1D NOE difference spectrum. Irradiation of the methylene proton at $H_2$-1 ($\delta$ 1.92) showed correlation to $H_2$-6 ($\delta$ 2.88–2.90), which supported this arrangement (Figure 3).

Saccharosporone B (**2**) was obtained as a yellowish powder and its structure was also elucidated on the basis of the interpretation of 2D NMR data. In comparison to **1**, both $^1$H and $^{13}$C NMR chemical shifts were quite comparable (Table 1), except the major difference at C-1 ($\delta$ 83.5) attributed to oxygenated methine ($\delta$ 4.25) instead of a methylene group. The correlation patterns of the COSY and HMBC NMR spectral data were revealed to be very similar to **1** (Figure 2). The molecular formula of **2** was established as $C_{19}H_{20}O_6$ by HRESITOFMS and only differed from **1** by having one additional hydroxy group instead. This conclusion was supported by its $^{13}$C NMR chemical shift in the downfield region at C-1. Therefore, saccharosporone B (**2**) could be established as a 1-hydroxy analogue of **1**. The relative configuration of **2** was also derived based on the interpretation of NOESY spectrum, and the same correlation pattern has been observed in comparison to **1**. The methine proton at H-12b ($\delta$ 3.04) showed correlations to the methylene signals at $H_a$-2 ($\delta$ 1.17) and $H_a$-4 ($\delta$ 1.64), while the oxygenated methine at H-5 ($\delta$ 4.09) correlated to the methylene proton $H_e$-4 ($\delta$ 2.09–2.11) and a methine signal at H-3 ($\delta$ 2.12–2.14). Accordingly, the configuration of two hydroxy groups at C-4a ($\delta$ 73.7) and C-5 ($\delta$ 76.7) could be proposed to stay on the same ring side as similar to **1**. The presence of a cross-peak between H-1 ($\delta$ 4.25) and H-12b, whereas no correlation between H-1 and $H_a$-3 has been recorded, confirming that the additional secondary alcohol at C-1 was on the opposite side of the ring when compared with the other two hydroxy groups at C-4a and C-5. Both **1** and **2** possessed similar relative configurations as well as their optical rotations that exhibited the same negative sign. The color reaction with sodium hydroxide from yellow to violet also supported the presence of a peri-hydroxy quinone functional group.

Compound **3**, saccharosporone C, was also obtained as a yellowish powder and its structure was found to be closely related to compound **1**. The $^1$H and $^{13}$C NMR spectra of **3** is mostly similar to **1** with the same 1,2,3-trisubstituted aromatic ring. The major difference is the change in the quinone ring, as only one carbonyl carbon remained at C-12 ($\delta$ 200.3), whereas C-7 ($\delta$ 62.8) has been altered to an oxygenated methine signal. Furthermore, two olefinic quaternary carbons, which formed the double bond between C-6a and C-12a, have been replaced with two aliphatic methine carbons. A molecular formula of $C_{19}H_{24}O_5$ of **3** was established by HRESITOFMS ($m/z$ 333.1697, $[M+H]^+$). Analysis of the COSY data allowed the assignment of the C-12a/C-6a/C-7/hydroxyl group ($\delta$ 3.99) connectivities, and HMBC correlations from H-7 ($\delta$ 5.07) to C-11a ($\delta$ 133.7) and C-12a ($\delta$ 43.1) further confirmed that a carbonyl at C-7 in **1** and **2** has been replaced by a methine carbon attached to a hydroxy group, while the double bond between C-6a and C-12a became saturated. The relative configuration of **3** was proposed on the basis of NOESY spectrum and by analysis of vicinal coupling constants. The configurations at C-3, C-4a and C-12b were established to be similar to those of compound **1**. Important correlation between H-6a ($\delta$ 2.44) and $H_a$-1 ($\delta$ 1.81) confirmed the assignment of configurations at C-4a and C-12b. The position of a hydroxyl group at C-5 was opposite to **1** and **2** as suggested by the lack of NOESY correlations from H-5 to H-3 and H-6a. The *trans* configuration at C-6a and C-12a was supported by NOESY correlations from a methine signal at H-6a to $H_a$-1, $H_e$-6 ($\delta$ 1.63) and H-7, along with correlations from H-12a ($\delta$ 3.37) to $H_a$-6 ($\delta$ 2.60) and H-12b ($\delta$ 2.30). Furthermore, the large coupling constant between H-6a and H-12a ($J_{\text{H-6a/H-12a}} = 12.5$ Hz) represented a diaxial relationship of these protons. The relative configuration of the hydroxy at C-7 was confirmed by NOESY correlations from H-7 to $H_e$-6 ($\delta$ 1.63) and H-6a and from the lack of correlation between H-7 and H-12a or $H_a$-6.

Isolated pure metabolites were screened for antimalarial, antibacterial, antifungal and cytotoxic activities. Compounds **1**, **2**, **5** and **6** exhibited antimalarial activities against *Plasmodium falciparum* K1 with respective $IC_{50}$ values of 4.1, 3.9, 6.0 and 4.6 μM, whereas **1**, **2**, **4** and **6** showed growth inhibition against *Mycobacterium tuberculosis* with $IC_{50}$ values of 76.2, 72.7, 40.8 and 19.7 μM, respectively. None of them exhibited activities against *Bacillus cereus*, while only **2** displayed antifungal activity against *Candida albicans* with an $IC_{50}$ value of 46.2 μM. New angucyclinones **1** and **2** showed cytotoxic activities against all three cancer cell lines (KB, MCF-7

**Table 2 Cytotoxic activities of compounds 1–6 against Vero cell and various cancer cell lines**

| | *Vero cells* | *KB* | *MCF-7* | *NCI-H187* |
|---|---|---|---|---|
| *Compound* | | $IC_{50}$ (μM) | | |
| **1** | 9.1 | 9.1 | 3.4 | 7.7 |
| **2** | 12.3 | 4.9 | 3.6 | 4.5 |
| **3** | Inactive | Inactive | Inactive | 41.5 |
| **4** | Inactive | Inactive | Inactive | 30.3 |
| **5** | Inactive | 49.8 | 51.3 | 14.7 |
| **6** | 25.7 | 26.5 | 16.9 | 5.5 |

and NCI-H187) while **3** only exhibited weak cytotoxicity against NCI-H187 (Table 2).

## EXPERIMENTAL PROCEDURES

### General

Optical rotations were measured on a JASCO P-1030 digital polarimeter. UV spectra were obtained on a SPEKOL 1200 spectrophotometer (Analytikjena, Jena, Germany). FT-IR spectra were taken on a Bruker Alpha-E spectrometer (Bruker Optik, GmbH, Ettlingen, Germany). $^{1}H$, $^{13}C$, DEPT, COSY, HMQC, HMBC and NOESY NMR spectra were recorded on a Bruker AV500D spectrometer (Bruker BioSpin AG Fällanden, Switzerland). ESI-TOF mass spectrometer measurements were obtained on a Micromass LCT (Micromass UK Limited, Manchester, UK) and a Bruker micrOTOF mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany).

### Biological material

The actinomycete *Saccharopolyspora* sp. (BCC 21906) was isolated from a soil collected at Evergreen forest, Khao Kitchakut National Park, Chanthaburi Province, Thailand. Analysis of the nucleotide sequence of the 16S rRNA gene of *Saccharopolyspora* sp. BCC 21906 exhibited a sequence similarity to the genes of *S. antimicrobica* I05-00074(T) (98.62%) and *S. hirsuta* subsp. kobensis JCM 9109(T) (98.14%). The collection and taxonomic identification were performed by Mr Chanwit Suriyachadkun (BIOTEC). A specimen has been deposited at BIOTEC Culture Collection and designated as BCC 21906.

### Culture conditions

*Saccharopolyspora* sp. BCC 21906 was maintained on the International Streptomyces Project medium 2 at 28 °C for 20–30 days. Later, the agar was cut into pieces ($1 \times 1\,cm^2$) and inoculated into $3 \times 250$ ml Erlenmeyer flasks each containing 25 ml of Bio 19.1 medium (glucose 20 g, peptone 5 g, yeast extract 2 g, meat extract 5 g, NaCl 0.5 g, $CaCO_3$ 3 g and vitamin B complex solution 1 ml, per 1 liter of distilled water). After the incubation on a rotary shaker (250 r.p.m.) at 28 °C for 7 days, each primary culture was transferred into a 1000-ml Erlenmeyer flask containing 250 ml of the same liquid medium and incubated at 28 °C for 7 days on a rotary shaker (250 r.p.m.). Each 25 ml portion of the secondary cultures was transferred into $20 \times 1$ liter Erlenmeyer flasks each containing 225 ml of production medium LS2 (mannitol 20 g, soy meal 20 g and trace element mix 0.25 ml, per 1 liter of distilled water). The mixture of trace elements consisted of (w/v): $CaCl_2$, 0.4%; $ZnSO_4$, 0.2%; $Na_2B_4O_7$, 0.01%; $FeSO_4$, 0.5%; KI, 0.005%; $CoCl_2$, 0.05%; $CuSO_4$, 0.02%; $MnCl_2$, 0.2%; $Na_2MoO_4$, 0.005%; (v/v) $H_2SO_4$ (95–97% p.a.), 0.1%. A total of 5 l ($20 \times 250$ ml) of BCC 21906 culture was incubated on rotary shakers (250 r.p.m.) for 7 days at 28 °C.

### Extraction and isolation

The organic constituents from a 5 l culture of *Saccharopolyspora* sp. strain BCC 21906 were extracted with ethyl acetate. The crude organic layer was concentrated *in vacuo* to yield a dark substance (3.7 g), which was partitioned by Diaion HP20SS (Supelco Bellefonte, PA, USA) column chromatography ($3.5 \times 25\,cm^2$, acetone:water) to yield nine fractions (20, 30, 40, 50, 60, 70, 80, 90 and 100% acetone mixtures). The fractions eluted with 70% and 80% acetone–water (160 mg) were combined and subjected to further purification by gradient preparative HPLC using a reversed-phase column (Phenomenex Luna 10u C18(2) 100A (Phenomenex Torrance, CA, USA), $21.2 \times 250\,mm^2$, 10 μm; 10 ml $min^{-1}$, 45% MeCN/$H_2O$ over 10 min, 45–50% MeCN/$H_2O$ over 10 min, 50% MeCN/$H_2O$ over 10 min, 50–100% MeCN/$H_2O$ over 20 min, 100% MeCN over 20 min) to afford compounds **1** (9.7 mg), **2** (1.9 mg), **3** (2.4 mg) and **5** (12.9 mg). The 90% (183.2 mg) and 100% (57.7 mg) acetone–water fractions were also subjected to the purification by gradient HPLC (Phenomenex Luna $C_{18}$ preparative, 10 ml $min^{-1}$, 50% MeCN/$H_2O$ over 10 min, 50–60% MeCN/$H_2O$ over 10 min, 60% MeCN/$H_2O$ over 20 min, 60–100% MeCN/$H_2O$ over 10 min, 100% MeCN over 20 min) to yield compounds **4** (35.1 mg), **5** (1.6 mg) and **6** (3.7 mg).

### Saccharosporone A (1)

Yellow amorphous solid; $[\alpha]_D$ −103.2 (*c* 0.3, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 215 nm (4.05), 245 nm (3.87), 273 nm (3.91), 421 nm (3.52); IR $\nu_{max}$ (ATR) 3474, 2947, 1632, 1611, 1455, 1284, 1237, 1065 $cm^{-1}$; for NMR data, see Table 1; ESIMS $[M+Na]^+$ *m/z* 351; ESIMS $[M+H]^+$ *m/z* 329; HRESITOFMS $[M-H]^-$ *m/z* 327.1231 (calcd for $C_{19}H_{19}O_5$, 327.1232).

### Saccharosporone B (2)

Yellow amorphous solid; $[\alpha]_D$ −87.3 (*c* 0.03, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 215 nm (4.03), 245 nm (3.83), 273 nm (3.82), 421 nm (3.49); IR $\nu_{max}$ (ATR) 3470, 2925, 1635, 1612, 1457, 1288, 1267, 1243, 1038 $cm^{-1}$; for NMR data, see Table 1; ESIMS $[M-H_2O+Na]^+$ *m/z* 349; HRESITOFMS $[M-H_2O+Na]^+$ *m/z* 349.1047 (calcd for $C_{19}H_{18}O_5Na$, 349.1052).

### Saccharosporone C (3)

Yellow amorphous solid; $[\alpha]_D$ −20.1 (*c* 0.07, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 222 nm (4.10), 259 nm (3.93), 313 nm (3.48); IR $\nu_{max}$ (ATR) 3402, 2952, 2923, 1703, 1665, 1588, 1291, 1038 $cm^{-1}$; for NMR data, see Table 1; ESIMS $[M+Na]^+$ *m/z* 355; HRESITOFMS $[M+H]^+$ *m/z* 333.1697 (calcd for $C_{19}H_{25}O_5$, 333.1702).

### Biological assays

Antimalarial activity against *P. falciparum* K1 was evaluated by the microculture radioisotope technique.[10] The $IC_{50}$ value of a standard antimalarial agent, dihydroartemisinin, was 0.001 μM. Antimicrobial assay against *B. cereus* was performed using the resazurin microplate technique.[11] Vancomycin, used as a standard antibacterial drug, showed an MIC value of 0.69 μM. Growth inhibition against *M. tuberculosis* ($H_{37}Ra$ strain) was determined by a green fluorescent protein microplate assay.[12] The MIC value of a standard drug, isoniazid, was 0.69 μM. A green fluorescent protein-based assay[13] was used to determine cytotoxicity against Vero cell (African green monkey kidney fibroblasts), while antifungal activity against *C. albicans* and cytotoxic tests against cancer cell lines, including KB (human epidermoid carcinoma), MCF-7 (human breast cancer) and NCI-H187 (human small cell lung cancer), were performed using the resazurin microplate assay.[14] The standard antifungal agent, amphotericin B, exhibited antifungal activity with an $IC_{50}$ value of 0.13 μM, while ellipticine used as standard control for cyctotoxic assay against vero cell lines, displayed an $IC_{50}$ value of 5.07 μM. Doxorubicin was used as positive controls for cytotoxic tests against KB, MCF-7 and NCI-H187 with respective $IC_{50}$ values of 0.65, 16.3 and 0.1 μM. MIC and $IC_{50}$ values $>50\,\mu g\,ml^{-1}$ were reported as inactive.

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