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Cytotoxic eremophilane sesquiterpenoids from the saprobic fungus *Berkleasmium nigroapicale* BCC 8220

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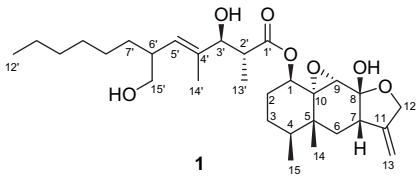
ABSTRACT

Berkleasmins A–E, five new eremophilane sesquiterpenoids were isolated from the saprobic fungus *Berkleasmium nigroapicale* BCC 8220. The structures of the new compounds were elucidated by analyses of NMR spectroscopic and mass spectrometry data in combination with chemical means. Berkleasmins A and C exhibited cytotoxic activity against cancer cell-lines (NCI-H187, MCF-7, and KB) as well as non-malignant Vero cells with IC_{50} values of 1.1–7.5 μg/mL, and these compounds also showed antimalarial activity with respective IC_{50} of 3.1 and 2.8 μg/mL.

1. Introduction

As part of our research program on the utilization of fungal sources in Thailand, we have investigated secondary metabolites of a saprobic fungus *Berkleasmium nigroapicale* BCC 8220 as an extract of this fungus showed antituberculosis activity with an MIC of 25 μg/mL and exhibited a unique ^{1}H NMR spectroscopic profile. The genus *Berkleasmium*, comprising 34 described species, has been relatively rarely distributed. To our knowledge, there has been no reported isolation of new compounds from this genus. BCC 8220 is a type strain of *B. nigroapicale*, which was isolated from a wild ginger in Chiang Mai Province, Thailand, and described in 2001.[1] Scale-up fermentation of BCC 8220 and chemical studies led to the isolation of five new elemophilane sesquiterpenoids, berkleasmins A–E (**1**–**5**). Described herein are the detailed isolation, structure elucidation, and biological activities of these compounds.



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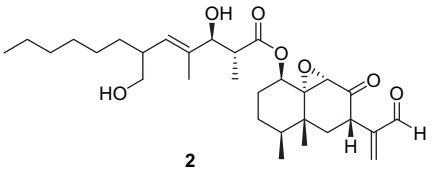

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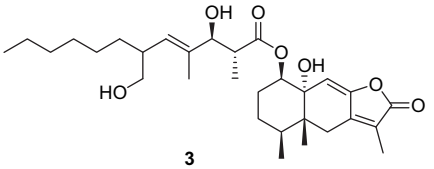

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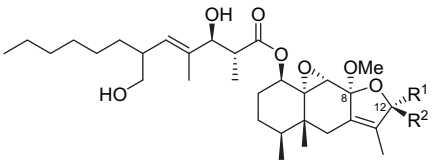

4 R^1 = H, R^2 = OMe
5 R^1 = OMe, R^2 = H

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2. Results and discussion

Berkleasmin A (**1**) was isolated as the most abundant sesquiterpene constituent of the mycelial extract. The molecular formula of **1** was determined by HRMS (ESI-TOF) as $C_{30}H_{48}O_7$. The IR spectrum exhibited absorption bands at ν_{max} 3424 (broad) and 1730 cm^{-1}, which suggested the presence of hydroxy groups and an ester. Inspection of ^1H and ^{13}C NMR, DEPT135, and HMQC data revealed that **1** contained a carbonyl carbon (δ_C 175.3), two olefinic quaternary carbons, an olefinic methine, an olefinic methylene, a quaternary acetal carbon (δ_C 102.4), an oxyquaternary carbon, three oxymethines, two oxymethylenes, an aliphatic quaternary carbon, four methines, eight methylenes, and five methyl groups (Table 1). COSY and HMBC data (Fig. 1) demonstrated that **1** is composed of a tricyclic sesquiterpene core attached with a long-chain acid through an ester linkage. The decalin unit of the sesquiterpene was deduced by HMBC correlations from the methyl protons (H_3-14, s) to quaternary carbons at δ_C 36.1 (C-5) and 63.0 (C-10), a methine carbon (δ_C 38.6, C-4), and a methylene carbon (δ_C 36.9, C-6). A hydroxyl proton at δ_H 5.16 (br s) showed HMBC correlations to a δ_C 102.4 quaternary carbon (C-8) and methines at δ_C 44.6 (C-7) and 62.4 (C-9), which indicated a hemiacetal. The tetrahydrofuran form was evident from a key HMBC correlation from one of the diastereotopic oxymethylene protons at δ_H 4.45 (H-12) to C-8. An exomethylene group was located as C-11/C-13, which was addressed by HMBC correlations from H-7 and H_2-12 to an olefinic methylene (δ_C 104.3, C-13) and a quaternary carbon (δ_C 151.2, C-11). The rest of protons and carbons were assignable to a long-chain ester moiety (Fig. 1). COSY correlations indicated the connection of C-2′ with C-3′ and C-13′, as well as the linkage from C-5′ to C-12′ and a substitution of a hydroxymethyl (C-15′) at C-6′. These two units were connected via a trisubstituted olefin (C-4′/C-5′), which was also flanked by a methyl group (C-14′). The *E*-configuration of the trisubstituted olefin was assigned by NOESY correlations from H-3′ to H-5′, and from H-6′ to H_3-14′. The ester linkage to C-1 of the sesquiterpene was indicated by HMBC correlations from H-1 (δ_H 4.51), H-2′, H-3′, and H_3-13′ to a carbonyl carbon at δ_C 175.3 (C-1′).

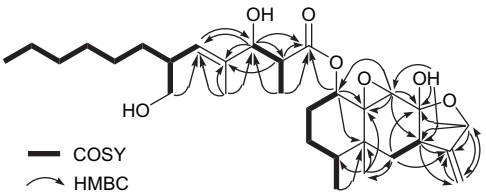


Figure 1. COSY and key HMBC correlations for **1**.

The relative configuration of the eremophilane core was deduced on the basis of NOESY data. One of the diastereotopic methylene protons, H_α-6 (δ_H 1.36), was coupled to H-7 with J=13.2 Hz, but these protons lacked a NOESY correlation. The other methylene proton (H_β-6, δ_H 1.52) showed an intense NOESY cross-peak with H-7, hence, there was an antiperiplanar relation between H_α-6 and H-7. A NOESY correlation between H_α-6 and H-4 indicated that these protons are coplanar (α-face). Proton H_β-6 exhibited NOESY cross-peaks with H-7 and H_3-14 (β-face). In addition, one of

Table 1
^{13}C (125 MHz) and ^1H (500 MHz) NMR data for berkleasmins A–C (**1**–**3**) in $CDCl_3$

Position	**1**		**2**		**3**	
	δ_C, mult.	δ_H, mult. (J in Hz)	δ_C, mult.	δ_H, mult. (J in Hz)	δ_C, mult.	δ_H, mult. (J in Hz)
1	74.6, CH	4.51, m	74.3, CH	4.52, t (2.6)	75.1, CH	5.03, m
2	28.5, CH_2	1.88, m; 1.77, m	29.1, CH_2	1.94, m; 1.91, m	25.8, CH_2	2.18, m; 1.72, m
3	25.7, CH_2	1.70, m; 1.47, m	25.5, CH_2	1.75, m; 1.52, m	25.6, CH_2	1.53, dq (3.8, 12.8); 1.40, m
4	38.6, CH	1.62, m	39.3, CH	1.75, m	33.8, CH	2.19, m
5	36.1, C		36.8, C		43.2, C	
6	36.9, CH_2	α 1.36, t (13.2) β 1.52, dd (13.2, 6.7)	37.1, CH_2	α 1.80, t (12.8) β 1.67, dd (12.8, 6.5)	32.7, CH_2	α 2.73, br d (16.3) β 2.47, d (16.7)
7	44.6, CH	2.60, m	42.9, CH	3.41, dd (12.6, 6.5)	148.0, C	
8	102.4, C		204.0, C		151.8, C	
9	62.4, CH	3.34, s	62.3, CH	3.45, s	110.5, CH	5.67, s
10	63.0, C		67.0, C		73.5, C	
11	151.2, C		149.5, C		123.2, C	
12	69.9, CH_2	4.57, br d (12.8); 4.45, m	192.5, CH	9.49, s	171.0, C	
13	104.3, CH_2	4.94, m; 4.89, m	136.1, CH_2	6.33, s; 6.18, s	8.6, CH_3	1.92 (d, 1.4)
14	15.5, CH_3	1.18, s	15.8, CH_3	1.26, s	15.5,[b] CH_3	1.02, s
15	15.2,[a] CH_3	0.91, d (6.7)	15.4, CH_3	0.92, d (6.1)	15.4,[b] CH_3	0.91, d (6.9)
1′	175.3, C		174.7, C		175.5, C	
2′	42.6, CH	2.74, dq (7.0, 7.3)	43.4, CH	2.66, dq (9.2, 7.2)	43.5, CH	2.61, dq (9.1, 7.2)
3′	78.8, CH	4.10, br d (7.0)	80.4, CH	4.12, d (9.2)	80.3, CH	4.07, d (9.1)
4′	137.1, C		137.2, C		137.2, C	
5′	130.2, CH	5.25, d (10.1)	130.2, CH	5.17, br d (9.8)	131.9, CH	5.07, d (9.8)
6′	40.8, CH	2.58, m	40.9, CH	2.56, m	40.8, CH	2.55, m
7′	31.4, CH_2	1.28, m; 1.15, m	31.5, CH_2	1.39, m; 1.16, m	31.4, CH_2	1.31, m; 1.12, m
8′	27.3, CH_2	1.28–1.20,[c] m	27.3, CH_2	1.30–1.16,[d] m	27.2, CH_2	1.28–1.20,[e] m
9′	29.4, CH_2	1.28–1.20,[c] m	29.4, CH_2	1.30–1.16,[d] m	29.3, CH_2	1.28–1.20,[e] m
10′	31.8, CH_2	1.28–1.20,[c] m	31.8, CH_2	1.30–1.16,[d] m	31.8, CH_2	1.28–1.20,[e] m
11′	22.6, CH_2	1.28–1.20,[c] m	22.6, CH_2	1.30–1.16,[d] m	22.6, CH_2	1.28–1.20,[e] m
12′	14.1, CH_3	0.88, t (7.2)	14.1, CH_3	0.88, t (7.1)	14.1, CH_3	0.87, t (7.1)
13′	15.1,[a] CH_3	1.17, d (7.2)	14.3, CH_3	1.05, d (7.2)	14.3, CH_3	1.01, d (7.2)
14′	12.5, CH_3	1.63, br s	11.3, CH_3	1.65, d (0.9)	11.4, CH_3	1.62, d (0.9)
15′	66.6, CH_2	3.63, m 3.35, m	66.6, CH_2	3.59, dd (10.8, 5.1) 3.37, dd (10.8, 8.6)	66.6, CH_2	3.59, dd (10.5, 4.6) 3.33, dd (10.5, 9.2)

[a] The assignment of carbons can be interchanged.
[b] The assignment of carbons can be interchanged.
[c] The proton signals are overlapped in the same index.
[d] The proton signals are overlapped in the same index.
[e] The proton signals are overlapped in the same index.

the exomethylene protons at δ_H 4.89 (H-13) displayed intense cross-peaks to H_β-6 and H-7, whereas it showed relatively much weaker correlation to H_α-6. These data strongly suggested a cis ring junction and a β-face orientation of the hemiacetal OH. The configuration of the epoxide and the stereochemistry of the ester unit could not be established from the available NMR spectroscopic data of **1**.

To simplify the spectroscopic analysis, we examined cleavage of the ester linkage. Base promoted hydrolysis was unsuccessful giving a complex mixture of products; however, $LiBH_4$ reduction of **1** worked fruitfully giving a mixture of products (**6**–**8**) corresponding to the sesquiterpene core and a triol fragment (**9**) of the long-chain ester. The sesquiterpene fragments were separated and purified by Si gel and ODS (HPLC) column chromatography. The gross structures of **6**–**9** and relative configurations of **6**–**8** were unambiguously determined by HRMS and NMR (1D and 2D) spectroscopic analysis. Compounds **6** and **7** were shown to be C-8 epimers. Apparently, compound **8** was produced by reductive cleavage of the C-9–O bond. The *J*-values and NOESY correlations

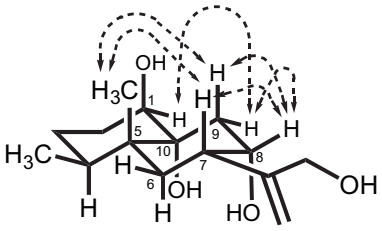


Figure 2. Selected NOESY correlations for **8**.

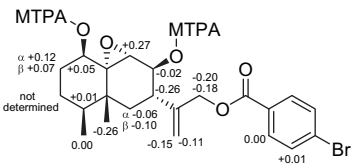


Figure 3. Δδ–Values ($\delta_S-\delta_R$) of bis-(*S*)- and bis-(*R*)-MTPA esters **12a** and **12b**.

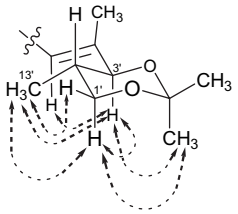


Figure 4. Selected NOESY correlations for **13**.

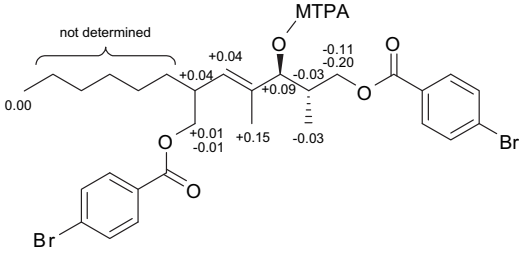
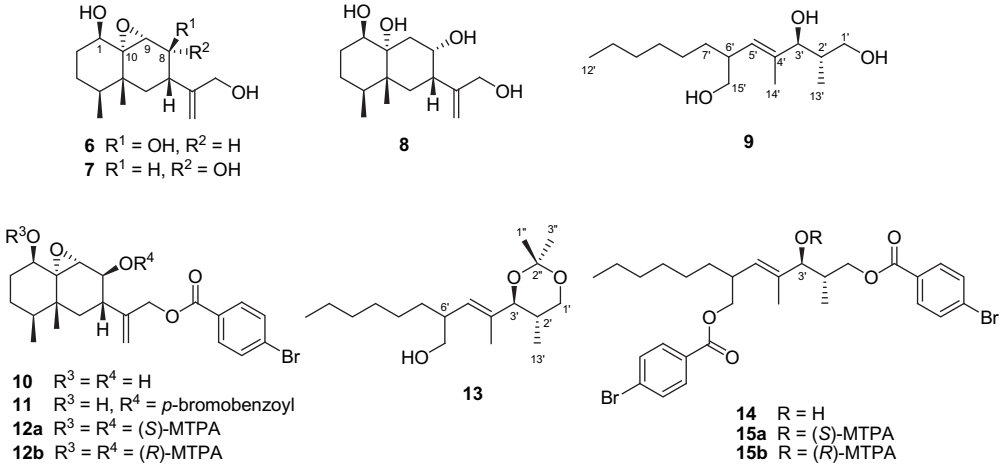


Figure 5. Δδ–Values ($\delta_S-\delta_R$) of the (*S*)- and (*R*)-MTPA esters **15a** and **15b**.

of **8** clearly indicated a *trans*-decalin unit adopting a chair–chair conformation (Fig. 2). These results indicated that the epoxide oxygen of **1** should be placed on the α-face. The absolute configuration of the eremophilane was addressed by application of modified Mosher method.[2,3] Sesquiterpene fragment **6** was reacted with *p*-bromobenzoyl chloride in pyridine to afford mono- and bis-*p*-bromobenzoate derivatives **10** and **11**, respectively. Although the (*R*)-MTPA ester of **11** was obtained by reaction with (+)-(*S*)-MTPACl in pyridine, similar reaction using (−)-(*R*)-MTPACl did not proceed under the same conditions. Repeat of the preparation of the (*S*)-MTPA ester derivative using DMAP gave a complex mixture of products. In the presence of DMAP, the reaction gave a complex mixture of products. However, synthesis of the Mosher esters of diol **10** was successful. Thus, bis-(*S*)- and bis-(*R*)-MTPA esters **12a** and **12b**, respectively, were prepared by reactions of **10** with (*R*)- and (*S*)-MTPACl in pyridine. The Δδ-values (Fig. 3) were consistent with the 1*R*,8*S* configuration and clearly ruled out an *ent*-elemophilane (2*S*,8*R*). The stereochemistry of the long-chain ester moiety of **1** was also addressed by a combination of chemical means. Compound **9** was converted into an acetonide derivative **13**. The *J*-values and NOESY data for **13** (Fig. 4) indicated that it adopted a chair conformation with axial orientation of H-2′ and H-3′ (J=10.3 Hz), hence, berkleasmin A (**1**) should possess either 2′*R*,3′*S* or 2′*S*,3′*R* configuration. The absolute configuration of C-3′ was addressed by application of modified Mosher method. Triol **9** was treated with *p*-bromobenzoyl chloride (2.1 equiv) in pyridine to afford a bis-*p*-bromobenzoate derivative **14**, which was further converted into (*S*)- and (*R*)-MTPA esters **15a** and **15b**, respectively. The Δδ-values unambiguously indicated the 3′*S* configuration (Fig. 5). Taking together, the 2′*R*,3′*S* configuration of **1** was established. The absolute configuration of C-6′ remains unassigned.



6 R^1 = OH, R^2 = H
7 R^1 = H, R^2 = OH

8

9

10 $R^3 = R^4$ = H
11 R^3 = H, R^4 = *p*-bromobenzoyl
12a $R^3 = R^4$ = (*S*)-MTPA
12b $R^3 = R^4$ = (*R*)-MTPA

13

14 R = H
15a R = (*S*)-MTPA
15b R = (*R*)-MTPA

Berkleasmin B (**2**) possessed the molecular formula of $C_{30}H_{46}O_7$ (HRESIMS), containing two less hydrogen atoms than **1**. The ^1H and ^{13}C NMR spectroscopic data, in particular the resonances of the long-chain ester moiety, were similar to those of **1**. Significant differences were the absence of a hemiacetal quaternary carbon (C-8 of **1**) and an oxymethylene group (CH_2-12 of **1**), instead of the presence of a ketone (δ_C 204.2) and a formyl group (δ_H 9.49, s; δ_C 192.5). In addition, exomethylene protons (H_2-13; δ_H 4.89, 4.94 in **1**) and carbon (C-13, δ_C 104.3 in **1**) were shifted downfield in **2** (δ_H 6.33, 6.18; δ_C 136.1). The replacement of the hemiacetal (C-8) with a ketone was confirmed by the HMBC correlations from H_β-6, H-7, and H-9 to the δ_C 204.2 quaternary carbon. The formyl group was assigned to the C-12 position, since HMBC correlations were observed from the formyl proton (H-12) to C-7 and C-11, and from one of the exomethylene protons at δ_H 6.33 to the formyl carbon (C-12), C-7 and C-11. The methine proton H-7 was coupled to H_α-6 with J=12.6 Hz, which indicated an antiperiplanar relation similar to **1**. An intense NOESY cross-peak between H_α-6 and H-13 (δ_H 6.33) further confirmed the β-orientation of H-7. Therefore, berkleasmin B (**2**) is the analogue of **1**, wherein the hydroxymethyl group (CH_2-12) is replaced by a formyl group.

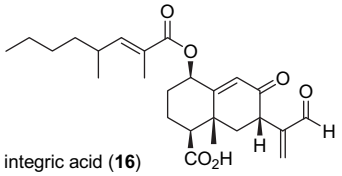
The molecular formula of berkleasmin C (**3**) was established by HRESIMS as $C_{30}H_{46}O_7$. The IR spectrum showed characteristic absorption bands at ν_{max} 1776 and 1666 cm^{-1}, in addition to those of hydroxyl groups (3450 cm^{-1}, broad) and an ester (1736 cm^{-1}, shoulder). Detailed analysis of 2D NMR spectroscopic data revealed that the long-chain ester moiety of **3** was identical to that of **1** and **2**. When compared to **1**, berkleasmin C (**3**) lacked the epoxide functionality (C-9/C-10), replaced by an olefinic methine (δ_H 5.67, s; δ_C 110.5) and a tertiary alcohol (δ_C 73.5, C-10). The exomethylene double bond (C-11/C-13) in **1** was shifted to *endo* as a tetrasubstituted olefin at δ_C 148.0 (C-7)/123.2 (C-11). The allylic methyl protons (H_3-13, δ_H 1.92) exhibited long range coupling (J=1.4 Hz) with H_α-6. The C-12 position was oxygenated to a lactone carbonyl (δ_C 171.0). The proposed sesquiterpene core structure was confirmed by key HMBC correlations: from H-9 to C-1, C-5, C-7, C-8, and C-10, from H_α-6 to C-5, C-7, C-8, C-10, and C-11, and from H_3-13 to C-7, C-11, and C-12. The coupling constants of an axial proton H_β-3 (δ_H 1.53, dq, J=3.8, 12.8 Hz) strongly suggested that ring-A adopts a chair conformation placing 10-OH and CH_3-14 in axial position.

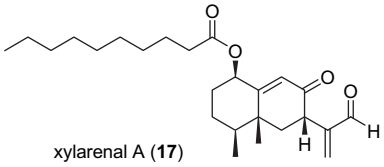
The molecular formula of berkleasmin D (**4**) was determined to be $C_{32}H_{52}O_8$ by HRESIMS. The ^1H and ^{13}C NMR and HMQC spectroscopic data indicated that two extra carbons are those of two methoxy groups resonated at δ_H 3.39 (3H, s; δ_C 54.3) and 3.31 (3H, s; δ_C 50.1). Since the NMR spectroscopic data for the long-chain ester moiety were identical to those of **1**–**3**, the methoxy groups should be involved in the sesquiterpene moiety. HMBC correlations from methoxyl protons at δ_H 3.39 and 3.31, respectively, to a methine at δ_C 110.2 and a quaternary carbon at δ_C 106.8 revealed the presence of two acetals. The sesquiterpene core contained a tetrasubstituted olefin (δ_C 133.4 and 132.9) and an allylic methyl group (δ_H 1.67, 3H, s; δ_C 9.4). The unusual bridged bis-acetal structure **4** was confirmed on the basis of the HMBC correlations: from H_2-6 to C-8 (δ_C 106.8), C-7, and C-11, from H-9 to C-7 and C-8, and from H_3-13 to C-7, C-11, and C-12. A NOESY cross-peak between H-12 and 8-OCH_3 indicated their coplanar relation. An intense NOESY correlation between H_β-6 and H_3-13 and a relatively much weaker cross-peak between H_α-6 and H_3-13 strongly suggested that the five-membered ring is flipped to the β-face, hence, α-orientation of 8-OCH_3. This assignment was supported by conformational analysis of the two possible isomers, 8α-OCH_3/12β-OCH_3 and 8β-OCH_3/12α-OCH_3, using a MM2 program (ChemDraw) and also examined with a molecular model. The former configuration preferred a conformation, which is consistent with the NOESY data.

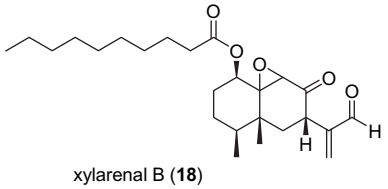
Berkleasmin E (**5**) possessed the same molecular formula as **4** ($C_{32}H_{52}O_8$), and the NMR spectroscopic data were similar to those of **4**. Significant chemical shift differences were observed at the C-12 acetal methine (**5**, δ_H 5.29, δ_C 109.6; **4**, δ_H 5.71, δ_C 110.2) and 12-OCH_3 (**5**, δ_H 3.51, δ_C 55.8; **4**, δ_H 3.39, δ_C 54.3). Detailed analysis of the 2D NMR data for **5** led to the elucidation of the same gross structure as **4**. Similar to **4**, compound **5** also exhibited an intense NOESY correlation from H_3-13 to H_β-6, but very weakly to H_α-6. On the other hand, NOESY correlation between H-12 and 8-OCH_3 was not observed, therefore, the configuration of berkleasmin E (**5**) was assigned as 8α-OCH_3/12α-OCH_3.

Minor derivatives, berkleasmins D (**4**) and E (**5**), were probably produced during methanolic mycelial extraction or silica gel column chromatography ($MeOH/CH_2Cl_2$), through double bond migration of berkleasmin B (**2**) from *exo* (C-11/C-13) to *endo* (C-7/C-11). It should be noted that compounds **4** and **5** were obtained in low quantity from a fraction mainly composed of **2**. The second batch of large-scale culture did not provide with **4** and **5**, while compounds **1**–**3** were isolated in good yields. Structurally similar bridged bis-acetals were reported as constituents of aerial part of *Senecio flavus*,[4] and an acid treatment product of integric acid is reported to possess a bis-hemiacetal structure.[5]

Eremophilane-type sesquiterpenes, including those with similar skeletons as berkleasmins A–C, widely exist as constituents of various plants, while there have been several reports as fungal secondary metabolites mostly from family Xylariaceae. A characteristic of the fungal metabolites is that they form long-chain acid esters at 1β-position. Integric acid (**16**)[6] and related compounds, 07H239A,[7] Sch 420789,[8] and eremoxylarins A and B,[9] possess the sesquiterpene core similar to berkleasmin B (**2**); however, these compounds possess a C-15 carboxylic acid functionality. Xylarenals A (**17**) and B (**18**) are most structurally close to berkleasmin B.[10] The stereochemistry of the epoxide moiety of **18** was not presented in the original report.[10]







Compounds **1**–**3** were subjected to our bioassay protocols inclusive of cytotoxicity against three cancer cell-lines (NCI-H187, MCF-7, and KB) and noncancerous Vero cells, and antimalarial (*Plasmodium falciparum* K1) and antituberculosis (*Mycobacterium tuberculosis* H37Ra) activities (Table 2). These compounds exhibited moderate cytotoxicity and antimalarial activity. The weak antituberculosis activities of the major eremophilane constituents (**1**–**3**) were consistent with that of a screening sample (MIC 25 μg/mL).

Table 2
Biological activities of compounds **1**–**3**

Compound	Cytotoxicity (IC_{50}, μg/mL)[a]				Antimalaria[b] (IC_{50}, μg/mL)	Antituberculosis[c] (MIC, μg/mL)
	NCI-H187	MCF-7	KB	Vero		
Berkleasmin A (**1**)	1.8	1.1	1.6	5.2	3.1	25
Berkleasmin B (**2**)	38	15	32	37	>10	25
Berkleasmin C (**3**)	4.1	2.4	2.9	7.5	2.8	12.5

[a] The IC_{50} values of a standard compound, doxorubicin, against NCI-H187, MCF-7, and KB cells were 0.061, 0.82, and 0.15 μg/mL, respectively. Ellipticine was used as a standard compound for the cytotoxicity assay against Vero cells (IC_{50} 0.43 μg/mL).
[b] Antimalarial activity against *P. falciparum* K1. Standard antimalarial drug, dihydroartemisinin, showed an IC_{50} value of 0.0012 μg/mL.
[c] Antituberculosis activity against *M. tuberculosis* H37Ra. Standard anti-TB drug, isoniazid, showed MIC values of 0.023–0.046 μg/mL.

A broad range of biological activities are reported for the integric acid and related compounds such as HIV-1 integrase inhibitory,[5,6] phospholipase D inhibitory,[8] calcineurin inhibitory,[9] and cytotoxic[7] activities, all with moderate potency. Xylarenals are reported to be selective ligands for the NPY Y5 receptor.[10] It should be noted that berkleasmin B (**2**), which possesses integric acid—like sesquiterpene skeleton, showed lower biological activities than other derivatives (**1** and **3**) in our assays. Therefore, all berkleasmins deserve further biological evaluation.

3. Experimental

3.1. General procedures

Melting points were measured with an Electrothermal IA9100 digital melting point apparatus. Optical rotations were measured with a JASCO P-1030 polarimeter. UV spectra were recorded on a GBC Cintra 404 spectrophotometer. FTIR spectra were taken on a Bruker VECTOR 22 spectrometer. NMR spectra were recorded on Bruker DRX400 and AV500D spectrometers. ESI-TOF mass spectra were measured with Micromass LCT and Bruker micrOTOF mass spectrometers.

3.2. Fungal material

B. nigroapicale (Dematiaceae) was isolated on dead pseudostems of *Amomum siamense* (Zingiberaceae) at Doi Suthep-Pui National Park, Chiang Mai province, on October 15, 2000, as a part of the survey of saprobic fungi in northern Thailand by one of the authors (S.L.). The living culture was deposited at the BIOTEC Culture Collection as BCC 8220 on February 21, 2001. It is the type strain of *B. nigroapicale*, whose taxonomy is detailed in the original report by Bussaban et al.[1]

3.3. Fermentation and isolation

The fungus *B. nigroapicale* BCC 8220 was maintained on potato dextrose agar (PDA) at 25 °C. The agar plugs (1×1 cm) were further cut into small pieces and inoculated into 3×1 L Erlenmeyer flasks each containing 250 mL of Difco™ potato dextrose broth (PDB; composition, potato starch 4.0 g/L, dextrose 20.0 g/L). After incubation at 25 °C for 7 days on a rotary shaker (200 rpm), the primary culture (700 mL) was transferred into a 10 L stirred-tank bioreactor containing 6.3 L of half-concentration PDB (composition, potato starch 2.0 g/L, dextrose 10.0 g/L). The fermentation was performed at 25 °C for 13 days under agitation of 350 rpm and aeration rate of 0.3 vvm. The culture was filtered and the residue (wet mycelium) was macerated in MeOH (800 mL, 2 days) and filtered. To the filtrate was added with H_2O (200 mL) and partitioned with hexane (1 L). The aqueous MeOH phase was partially evaporated. The residual aqueous solution was extracted with EtOAc (1.5 L), concentrated under reduced pressure to leave a brown gum (2.51 g). This extract was fractionated by column chromatography (CC) on Si gel (5.5×20 cm, step gradient elution with 0–20% MeOH/CH_2Cl_2) to obtain five fractions. Fraction 3 (367 mg) was further separated by CC on Si gel (3.0×17 cm, step gradient elution with 0–15% MeOH/CH_2Cl_2) to furnish subfractions 3-1 to 3-5. Subfraction 3-1 (120 mg) was subjected to preparative HPLC using a reversed-phase column (Prep Nova-Pak™ HR C_{18}, 6 μm, 25×100 mm; MeCN/H_2O=60:40; flow rate 8 mL/min) to furnish **2** (9 mg), **4** (18 mg), and **5** (5 mg). Subfraction 3-2 (121 mg) was also purified by preparative HPLC (MeCN/H_2O=60:40) to obtain **3** (27 mg). Fraction 4 (1.28 g) was fractionated by CC on Si gel (MeOH/CH_2Cl_2), and a major fraction (645 mg) was further purified by preparative HPLC (MeCN/H_2O=60:40) to furnish **1** (547 mg). Another fermentation (7 L) provided compounds **1** (1.71 g), **2** (69 mg), and **3** (24 mg); however, **4** and **5** were not detected in the extract.

*3.3.1. Berkleasmin A (**1**).* Colorless amorphous solid; $[\alpha]_D^{26}$ +22 (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 269 (3.41) nm; IR (KBr) ν_{max} 3424, 2929, 1730, 1460, 1172, 1039, 756 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see Table 1; HRMS (ESI-TOF) *m*/*z* 543.3301 $[M+Na]^+$ (calcd for $C_{30}H_{48}O_7Na$, 543.3292).

*3.3.2. Berkleasmin B (**2**).* Colorless amorphous solid; $[\alpha]_D^{26}$ +6 (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 270 (3.67) nm; IR (KBr) ν_{max} 3444, 2928, 1736, 1460, 1384, 1170 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see Table 1; HRMS (ESI-TOF) *m*/*z* 541.3155 $[M+Na]^+$ (calcd for $C_{30}H_{46}O_7Na$, 541.3136).

*3.3.3. Berkleasmin C (**3**).* Colorless amorphous solid; $[\alpha]_D^{26}$ +8 (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 275 (4.19) nm; IR (KBr) ν_{max} 3450, 2928, 1776, 1736 sh, 1666, 1378, 1167, 1053, 1007 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see Table 1; HRMS (ESI-TOF) *m*/*z* 541.3148 $[M+Na]^+$ (calcd for $C_{30}H_{46}O_7Na$, 541.3136).

*3.3.4. Berkleasmin D (**4**).* Colorless amorphous solid; $[\alpha]_D^{27}$ −4 (*c* 0.15, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 271 (3.72) nm; IR (KBr) ν_{max} 3442, 2928, 1736, 1460, 1165, 1111, 1040, 986, 756 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.71 (1H, br s, H-12), 5.13 (1H, br d, *J*=10.0 Hz, H-5′), 4.39 (1H, br t, *J*=2.7 Hz, H-1), 4.09 (1H, d, *J*=9.3 Hz, H-3′), 3.58 (1H, s, H-9), 3.57 (1H, dd, *J*=10.5, 5.1 Hz, Ha-15′), 3.39 (3H, s, 12-OCH_3), 3.37 (1H, dd, *J*=10.5, 7.7 Hz, Hb-15′), 3.31 (3H, s, 8-OCH_3), 2.58 (1H, dq, *J*=9.3, 7.2 Hz, H-2′), 2.55 (1H, m, H-6′), 2.02 (1H, d, *J*=12.4 Hz, H_β-6), 1.96 (1H, m, Ha-2), 1.92 (1H, br d, *J*=12.4 Hz, H_α-6), 1.88 (1H, m, H-4), 1.81 (1H, m, Hb-2), 1.68 (1H, m, Ha-3), 1.67 (3H, s, H_3-13), 1.63 (3H, d, *J*=1.1 Hz, H_3-14′), 1.49 (1H, m, Hb-3), 1.39 (1H, m, Ha-7′), 1.28–1.20 (8H, m, H_2-8′, H_2-9′, H_2-10′, and H_2-11′), 1.15 (1H, m, Hb-7′), 1.05 (3H, s, H_3-14), 0.99 (3H, d, *J*=7.2 Hz, H_3-13′), 0.91 (3H, d, *J*=6.8 Hz, H_3-15), 0.88 (3H, t, *J*=7.1 Hz, H_3-12′); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.1 (C, C-1′), 137.6 (C, C-4′), 133.4 (C, C-7), 132.9 (C, C-11), 131.5 (CH, C-5′), 110.2 (C, C-12), 106.8 (C, C-8), 80.4 (CH, C-3′), 74.9 (CH, C-1), 66.6 (CH_2, C-15′), 64.1 (C, C-10), 63.8 (CH, C-9), 54.3 (CH_3, 12-OCH_3), 50.1 (CH_3, 8-OCH_3), 43.7 (CH, C-2′), 40.9 (C, C-5), 40.9 (CH, C-6′), 38.7 (CH, C-4), 31.8 (CH_2, C-10′), 31.5 (CH_2, C-7′), 30.8 (CH_2, C-6), 29.4 (CH_2, C-9′), 28.3 (CH_2, C-2), 27.2 (CH_2, C-8′), 26.3 (CH_2, C-3), 22.6 (CH_2, C-11′), 15.3 (CH_3, C-14), 14.9 (CH_3, C-15), 14.2 (CH_3, C-13′), 14.1 (CH_3, C-12′), 11.2 (CH_3, C-14′), 9.4 (CH_3, C-13); HRMS (ESI-TOF) *m*/*z* 587.3569 $[M+Na]^+$ (calcd for $C_{32}H_{52}O_8Na$, 587.3560).

*3.3.5. Berkleasmin E (**5**).* Colorless amorphous solid; $[\alpha]_D^{24}$ +29 (*c* 0.09, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 276 (3.06) nm; IR (KBr) ν_{max} 3444, 2926, 1773, 1736, 1460, 1168, 1099, 756 cm^{-1}; ^1H NMR

(500 MHz, $CDCl_3$) δ 5.29 (1H, br s, H-12), 5.18 (1H, br d, J=9.8 Hz, H-5′), 4.38 (1H, m, H-1), 4.13 (1H, d, J=9.0 Hz, H-3′), 3.59 (1H, dd, J=10.5, 5.1 Hz, Ha-15′), 3.51 (3H, s, 12-OCH_3), 3.50 (1H, s, H-9), 3.39 (1H, dd, J=10.5, 8.4 Hz, Hb-15′), 3.38 (3H, s, 8-OCH_3), 2.62 (1H, dq, J=9.0, 7.2 Hz, H-2′), 2.56 (1H, m, H-6′), 2.07 (1H, d, J=12.5 Hz, H$_\beta$-6), 1.95 (1H, m, Ha-2), 1.94 (1H, br d, J=12.5 Hz, H$_\alpha$-6), 1.88 (1H, m, H-4), 1.80 (1H, m, Hb-2), 1.70 (3H, s, H_3-13), 1.66 (3H, d, J=1.2 Hz, H_3-14′), 1.63 (1H, m, Ha-3), 1.49 (1H, m, Hb-3), 1.40 (1H, m, Ha-7′), 1.30–1.20 (8H, m, H_2-8′, H_2-9′, H_2-10′, and H_2-11′), 1.16 (1H, m, Hb-7′), 1.05 (3H, d, J=7.2 Hz, H_3-13′), 0.96 (3H, s, H_3-14), 0.92 (3H, d, J=6.8 Hz, H_3-15), 0.88 (3H, t, J=7.0 Hz, H_3-12′); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.7 (C, C-1′), 137.1 (C, C-4′), 133.1 (C, C-7), 131.8 (C, C-11), 131.5 (CH, C-5′), 109.6 (C, C-12), 105.3 (C, C-8), 80.0 (CH, C-3′), 75.1 (CH, C-1), 66.6 (CH_2, C-15′), 64.2 (C, C-10), 63.7 (CH, C-9), 55.8 (CH_3, 12-OCH_3), 50.4 (CH_3, 8-OCH_3), 43.6 (CH, C-2′), 41.0 (C, C-5), 40.9 (CH, C-6′), 38.7 (CH, C-4), 31.8 (CH_2, C-10′), 31.5 (CH_2, C-7′), 31.0 (CH_2, C-6), 29.4 (CH_2, C-9′), 28.4 (CH_2, C-2), 27.3 (CH_2, C-8′), 26.1 (CH_2, C-3), 22.6 (CH_2, C-11′), 15.3 (CH_3, C-14), 14.9 (CH_3, C-15), 14.4 (CH_3, C-13′), 14.1 (CH_3, C-12′), 11.5 (CH_3, C-14′), 10.0 (CH_3, C-13); HRMS (ESI-TOF) m/z 587.3558 $[M+Na]^+$ (calcd for $C_{32}H_{52}O_8Na$, 587.3560).

3.4. $LiBH_4$ reduction of 1

To a solution of **1** (100 mg) in THF (1.5 mL) at 0 °C was added $LiBH_4$ (30 mg). The cooling bath was removed and the mixture was stirred at room temperature for 7 days. The reaction was terminated by addition of H_2O, and the mixture was extracted with EtOAc. The organic layer was concentrated under reduced pressure to leave a colorless gum (91 mg), which was separated by CC on Si gel ($MeOH/CH_2Cl_2$) to obtain **9** (39 mg) and a mixture (23.3 mg) of **6**, **7**, and **8**. The mixture was subjected to preparative HPLC (LiChroCART® 250-10, 10 μm, 1.0×25.0 cm; $MeOH/H_2O$=30:70; flow rate 4 mL/min) to furnish pure compounds; **6** (5.9 mg, t_R 32 min), **7** (2.5 mg, t_R 18 min), and **8** (5.6 mg, t_R 27 min).

*3.4.1. Compound **6**.* Colorless solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.14 (1H, br s, Ha-13), 5.02 (1H, s, Hb-13), 4.18 (1H, d, J=12.1 Hz, Ha-12), 4.11 (1H, d, J=12.1 Hz, Hb-12), 3.83 (1H, d, J=8.7 Hz, H-8), 3.31 (1H, br s, H-1), 3.08 (1H, s, H-9), 2.22 (1H, ddd, J=13.2, 8.7, 3.3 Hz, H-7), 1.86 (1H, m, H$_\alpha$-2), 1.78 (1H, m, H$_\beta$-2), 1.77 (1H, m, H$_\beta$-3), 1.62 (1H, m, H-4), 1.47 (1H, t, J=13.2 Hz, H$_\alpha$-6), 1.39 (1H, m, H$_\alpha$-3), 1.25 (3H, s, H_3-14), 1.23 (1H, dd, J=13.2, 3.3 Hz, H$_\beta$-6), 0.83 (3H, d, J=6.8 Hz, H_3-15); ^{13}C NMR (125 MHz, $CDCl_3$) δ 151.3 (C, C-11), 113.2 (CH_2, C-13), 73.6 (CH, C-1), 70.8 (CH, C-8), 67.1 (CH_2, C-12), 65.3 (C, C-10), 63.4 (CH, C-9), 41.7 (CH, C-7), 39.0 (CH, C-4), 36.0 (CH_2, C-6), 35.9 (C, C-5), 30.8 (CH_2, C-2), 25.5 (CH_2, C-3), 16.7 (CH_3, C-14), 14.9 (CH_3, C-15); HRMS (ESI-TOF) m/z 291.1565 $[M+Na]^+$ (calcd for $C_{15}H_{24}O_4Na$, 291.1567).

*3.4.2. Compound **7**.* Colorless solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.21 (1H, s, Ha-13), 4.95 (1H, s, Hb-13), 4.17 (1H, d, J=12.6 Hz, Ha-12), 4.11 (1H, d, J=12.6 Hz, Hb-12), 4.25 (1H, br s, H-8), 3.37 (1H, d, J=4.4 Hz, H-9), 3.31 (1H, m, H-1), 2.55 (1H, ddd, J=13.0, 8.74.7, 2.8 Hz, H-7), 2.28 (1H, br s, 8-OH), 1.91 (1H, m, H$_\alpha$-2), 1.77 (1H, m, H$_\beta$-2), 1.76 (1H, m, H$_\beta$-3), 1.66 (1H, m, H-4), 1.55 (1H, t, J=13.0 Hz, H$_\alpha$-6), 1.53 (1H, br s, 1-OH), 1.42 (1H, m, H$_\alpha$-3), 1.24 (1H, dd, J=13.0, 2.8 Hz, H$_\beta$-6), 1.18 (3H, s, H_3-14), 0.87 (3H, d, J=6.7 Hz, H_3-15); ^{13}C NMR (125 MHz, $CDCl_3$) δ 148.5 (C, C-11), 113.7 (CH_2, C-13), 73.9 (CH, C-1), 68.1 (C, C-10), 66.0 (CH_2, C-12), 64.7 (CH, C-8), 62.8 (CH, C-9), 39.5 (CH, C-4), 38.9 (CH, C-7), 36.2 (C, C-5), 33.1 (CH_2, C-6), 31.4 (CH_2, C-2), 25.3 (CH_2, C-3), 16.3 (CH_3, C-14), 14.8 (CH_3, C-15); HRMS (ESI-TOF) m/z 291.1568 $[M+Na]^+$ (calcd for $C_{15}H_{24}O_4Na$, 291.1567).

*3.4.3. Compound **8**.* Colorless solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.27 (1H, s, Ha-13), 5.12 (1H, s, Hb-13), 4.18 (1H, d, J=12.7 Hz, Ha-12), 4.17 (1H, m, H-8), 4.11 (1H, d, J=12.7 Hz, Hb-12), 3.60 (1H, br s, H-1), 2.66 (1H, dt, J=13.2, 2.8 Hz, H-7), 2.36 (1H, dd, J=14.8, 3.4 Hz, H$_\beta$-9), 2.16 (1H, ddt, J=5.3, 3.5, 14.0 Hz, H$_\alpha$-2), 2.02 (1H, m, H-4), 1.91 (1H, t, J=13.2 Hz, H$_\alpha$-6), 1.73 (1H, dd, J=14.8, 2.6 Hz, H$_\alpha$-9), 1.56 (1H, m, H$_\beta$-3), 1.51 (1H, m, H$_\beta$-2), 1.30 (1H, m, H$_\alpha$-3), 1.24 (1H, dd, J=13.2, 3.7 Hz, H$_\beta$-6), 1.06 (3H, s, H_3-14), 0.78 (3H, d, J=7.0 Hz, H_3-15); ^{13}C NMR (125 MHz, $CDCl_3$) δ 149.7 (C, C-11), 115.0 (CH_2, C-13), 75.2 (CH, C-1), 74.6 (C, C-10), 69.7 (CH, C-8), 65.1 (CH_2, C-12), 41.8 (CH, C-7), 40.0 (C, C-5), 35.5 (CH_2, C-9), 34.4 (CH, C-4), 32.6 (CH_2, C-6), 28.7 (CH_2, C-2), 25.3 (CH_2, C-3), 15.7 (CH_3, C-14), 15.0 (CH_3, C-15); HRMS (ESI-TOF) m/z 293.1728 $[M+Na]^+$ (calcd for $C_{15}H_{26}O_4Na$, 293.1723).

*3.4.4. Compound **9**.* Colorless gum; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.08 (1H, br d, J=9.9 Hz, H-5′), 3.88 (1H, d, J=9.3 Hz, H-3′), 3.72 (1H, dd, J=10.8, 3.6 Hz, Ha-1′), 3.65 (1H, dd, J=10.8, 8.0 Hz, Hb-1′), 3.60 (1H, dd, J=10.6, 4.4 Hz, Ha-15′), 3.32 (1H, dd, J=10.6, 9.5 Hz, Hb-15′), 2.53 (1H, m, H-6′), 1.93 (1H, m, H-2′), 1.66 (3H, d, J=1.0 Hz, H-14′), 1.31 (1H, m, Ha-7′), 1.30–1.20 (8H, m, H-8′, H-9′, H-10′, and H-11′), 1.11 (1H, m, Hb-7′), 0.88 (3H, t, J=7.0 Hz, H-12′), 0.71 (3H, d, J=7.0 Hz, H-13′); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.8 (C, C-4′), 131.3 (CH, C-5′), 84.8 (CH, C-3′), 68.1 (CH_2, C-1′), 66.7 (CH_2, C-15′), 40.7 (CH, C-6′), 37.0 (CH, C-2′), 31.8 (CH_2, C-10′), 31.4 (CH_2, C-7′), 29.3 (CH_2, C-9′), 27.2 (CH_2, C-8′), 22.6 (CH_2, C-11′), 14.1 (CH_3, C-12′), 13.6 (CH_3, C-13′), 11.3 (CH_3, C-14′); HRMS (ESI-TOF) m/z 281.2091 $[M+Na]^+$ (calcd for $C_{15}H_{30}O_3Na$, 281.2093).

3.5. Synthesis of a *p*-bromobenzoate derivatives 10 and 11 and application of the modified Mosher method

A mixture of compound **6** (4.9 mg) and *p*-bromobenzoyl chloride (10.8 mg, 2.2 equiv) in pyridine (0.2 mL) was stirred for 17 h. The mixture was diluted with EtOAc and washed with H_2O and 1 M $NaHCO_3$, and the organic layer was concentrated in vacuo. The residue was separated by CC on Si gel ($MeOH/CH_2Cl_2$) to furnish mono- and bis-*p*-bromobenzoate derivatives **10** (1.1 mg) and **11** (1.1 mg), respectively. Compound **10** (0.4 mg) was treated with (−)-(*R*)-MTPACl (5 mg) in pyridine (0.2 mL) at room temperature for 16 h. The mixture was diluted with EtOAc and washed with H_2O and 1 M $NaHCO_3$, and the organic layer was concentrated in vacuo to afford a bis-(*S*)-MTPA ester derivative **12a**. Similarly, bis-(*R*)-MTPA ester derivative **12b** was prepared from **10** and (+)-(*S*)-MTPACl. Assignments of protons of the Mosher ester derivatives **12a** and **12b** were established on the basis of COSY and NOESY data.

*3.5.1. Compound **10**.* Colorless solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.92 (2H, d, J=8.5 Hz, *p*-bromobenzoyl), 7.60 (2H, d, J=8.5 Hz, *p*-bromobenzoyl), 5.24 (1H, s, Ha-13), 5.12 (1H, s, Hb-13), 4.81 (2H, s, H_2-12), 4.00 (1H, br d, J=8.9 Hz, H-8), 3.30 (1H, br s, H-1), 3.09 (1H, s, H-9), 2.28 (1H, ddd, J=13.3, 8.9, 3.3 Hz, H-7), 1.84 (1H, m, H$_\alpha$-2), 1.77 (1H, m, H$_\beta$-2), 1.76 (1H, m, H$_\beta$-3), 1.62 (1H, m, H-4), 1.43 (1H, t, J=13.3 Hz, H$_\alpha$-6), 1.39 (1H, m, H$_\alpha$-3), 1.28 (1H, dd, J=13.3, 3.3 Hz, H$_\beta$-6), 1.25 (3H, s, H_3-14), 0.82 (3H, d, J=6.8 Hz, H_3-15); HRMS (ESI-TOF) m/z 473.0938 and 475.0959 $[M+Na]^+$ (calcd for $C_{22}H_{27}O_5{}^{79}BrNa$ and $C_{22}H_{27}O_5{}^{81}BrNa$, 473.0934 and 475.0917, respectively).

*3.5.2. Compound **11**.* Colorless solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 (4H, d, J=8.5 Hz, *p*-bromobenzoyl), 7.55 (4H, d, J=8.5 Hz, *p*-bromobenzoyl), 5.32 (1H, d, J=9.6 Hz, H-8), 5.23 (1H, s, Ha-13), 5.15 (1H, s, Hb-13), 4.78 (2H, s, H_2-12), 3.29 (1H, br s, H-1), 3.13 (1H, s, H-9), 2.71 (1H, ddd, J=13.2, 9.6, 3.4 Hz, H-7), 1.90 (1H, m, H$_\alpha$-2), 1.80–1.75 (2H, m, H$_\beta$-2 and H$_\beta$-3), 1.64 (1H, m, H-4), 1.54 (1H, t, J=13.2 Hz, H$_\alpha$-6), 1.43 (1H, m, H$_\alpha$-3), 1.42 (1H, dd, J=13.2, 3.4 Hz, H$_\beta$-6), 1.32 (3H, s, H_3-14), 0.86 (3H, d, J=6.7 Hz, H_3-15); HRMS

(ESI-TOF) *m*/*z* 633.0476, 635.0463, and 637.0454 $[M+H]^+$ (calcd for $C_{29}H_{31}O_6{}^{79}Br_2$, $C_{29}H_{31}O_6{}^{79}Br^{81}Br$, and $C_{29}H_{31}O_6{}^{81}Br_2$, 633.0482, 635.0464, and 637.0450, respectively).

3.5.3. Bis-(S)-MTPA ester ***12a***. Colorless gum; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.861 (2H, d, *J*=8.5 Hz, *p*-bromobenzoyl), 7.582 (2H, d, *J*=8.5 Hz, *p*-bromobenzoyl), 7.55–7.23 (10H, m, phenyl×2), 5.424 (1H, d, *J*=9.9 Hz, H-8), 5.160 (1H, s, Ha-13), 4.978 (1H, s, Hb-13), 4.570 (1H, m, H-1), 4.532 (2H, s, H_2-12), 3.590 (3H, br s, –OCH_3), 3.562 (3H, br s, -OCH_3), 3.368 (1H, s, H-9), 2.168 (1H, ddd, *J*=13.3, 9.9, 3.4 Hz, H-7), 1.997 (1H, m, H_α-2), 1.849 (1H, m, H_β-2), 1.580 (1H, m, H-4), 1.570 (1H, m, H_β-3), 1.439 (1H, m, H_α-3), 1.351 (1H, t, *J*=13.3 Hz, H_α-6), 1.200 (1H, dd, *J*=13.3, 3.4 Hz, H_β-6), 0.697 (3H, d, *J*=6.0 Hz, H_3-15), 0.446 (3H, s, H_3-14); MS (ESI-TOF) *m*/*z* 905.2 and 907.2 $[M+Na]^+$.

3.5.4. Bis-(R)-MTPA ester ***12b***. Colorless gum; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.863 (2H, d, *J*=8.5 Hz, *p*-bromobenzoyl), 7.571 (2H, d, *J*=8.5 Hz, *p*-bromobenzoyl), 7.52–7.25 (10H, m, phenyl×2), 5.440 (1H, d, *J*=9.8 Hz, H-8), 5.270 (1H, s, Ha-13), 5.127 (1H, s, Hb-13), 4.517 (1H, br t, *J*=2.7 Hz, H-1), 4.730 (1H, d, *J*=13.5 Hz, Ha-12), 4.707 (1H, d, *J*=13.5 Hz, Hb-12), 3.508 (3H, br s, –OCH_3), 3.341 (3H, br s, –OCH_3), 3.103 (1H, s, H-9), 2.430 (1H, ddd, *J*=13.2, 9.8, 3.3 Hz, H-7), 1.879 (1H, m, H_α-2), 1.780 (1H, m, H_β-2), 1.569 (1H, m, H-4), 1.408 (1H, t, *J*=13.2 Hz, H_α-6), 1.299 (1H, dd, *J*=13.2, 3.3 Hz, H_β-6), 1.29–1.20 (2H, overlapped in lipid resonances, H_α-3 and H_β-3), 0.708 (3H, s, H_3-14), 0.697 (3H, d, *J*=6.0 Hz, H_3-15); MS (ESI-TOF) *m*/*z* 905.2 and 907.2 $[M+Na]^+$.

3.6. Synthesis of the acetonide derivative 13

To a suspension of **9** (5.0 mg) in 2,2-dimethoxypropane (0.5 mL) was added *p*-TsOH·H_2O (ca. 0.5 mg), and the mixture was stirred at room temperature for 4 h. The mixture was diluted with EtOAc and washed with 1 M $NaHCO_3$. The organic layer was concentrated under reduced pressure to obtain compound **13** (5.0 mg).

3.6.1. Compound ***13***. Colorless gum; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.15 (1H, dq, *J*=9.8, 1.3 Hz, H-5′), 3.86 (1H, d, *J*=10.3 Hz, H-3′), 3.76 (1H, dd, *J*=11.6, 5.0 Hz, H_{eq}-1′), 3.56 (1H, t, *J*=11.6 Hz, H_{ax}-1′), 3.55 (1H, m, Ha-15), 3.43 (1H, dd, *J*=10.6, 7.9 Hz, Hb-15′), 2.56 (1H, m, H-6′), 1.89 (1H, m, H-2′), 1.68 (3H, d, *J*=1.3 Hz, H_3-14′), 1.47 (3H, s, H_3-1″), 1.42 (1H, m, Ha-7′), 1.41 (3H, s, H_3-3″), 1.30–1.20 (8H, m, H_2-8′, H_2-9′, H_2-10′, and H_2-11′), 1.17 (1H, m, Hb-7′), 0.87 (3H, t, *J*=7.0 Hz, H_3-12′), 0.66 (3H, d, *J*=6.8 Hz, H_3-13′); ^{13}C NMR (125 MHz, $CDCl_3$) δ 136.6 (C, C-4′), 131.4 (CH, C-5′), 82.8 (CH, C-3′), 66.5 (CH_2, C-15′), 66.0 (CH_2, C-1′), 98.2 (C, C-2″), 40.8 (CH, C-6′), 31.8 (CH_2, C-10′), 31.6 (CH_2, C-7′), 30.8 (CH, C-2′), 29.9 (CH_3, C-3″), 29.3 (CH_2, C-9′), 27.2 (CH_2, C-8′), 22.6 (CH_2, C-11′), 19.2 (CH_3, C-1″), 14.0 (CH_3, C-12′), 12.6 (CH_3, C-13′), 11.6 (CH_3, C-14′); HRMS (ESI-TOF) *m*/*z* 321.2413 $[M+Na]^+$ (calcd for $C_{18}H_{34}O_3Na$, 321.2400).

3.7. Synthesis of a bis-*p*-bromobenzoate derivative 14 and application of the modified Mosher method

A mixture of compound **9** (10.3 mg) and *p*-bromobenzoyl chloride (22 mg, 2.1 equiv) in pyridine (0.2 mL) was stirred at room temperature for 18 h. The mixture was diluted with EtOAc and washed with H_2O and 1 M $NaHCO_3$, and the organic layer was concentrated in vacuo. The residue was subjected to CC on Si gel ($MeOH/CH_2Cl_2$) to furnish a bis-*p*-bromobenzoate derivative **14** (14.2 mg) and a mono-*p*-bromobenzoate derivative (2.3 mg, 15′-OCOAr). Mosher esters **15a** and **15b** were prepared using the same procedure as described above. The assignments of protons were supported by COSY data.

3.7.1. Compound ***14***. Colorless gum; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (2H, d, *J*=8.5 Hz, *p*-bromobenzoyl), 7.86 (2H, d, *J*=8.5 Hz, *p*-bromobenzoyl), 7.58 (2H, d, *J*=8.5 Hz, *p*-bromobenzoyl), 7.55 (2H, d, *J*=8.5 Hz, *p*-bromobenzoyl), 5.21 (1H, br d, *J*=9.8 Hz, H-5′), 4.44 (2H, d, *J*=4.8 Hz, H_2-1′), 4.20 (2H, d, *J*=6.7 Hz, H_2-15′), 3.85 (1H, d, *J*=9.1 Hz, H-3′), 2.82 (1H, m, H-6′), 2.10 (1H, m, H-2′), 1.90 (1H, br s, 3′-O*H*), 1.67 (3H, s, H_3-14′), 1.53 (1H, m, Ha-7′), 1.35–1.20 (9H, m, Hb-7′, H_2-8′, H_2-9′, H_2-10′, and H_2-11′), 0.91 (3H, d, *J*=7.0 Hz, H_3-13′), 0.86 (3H, t, *J*=6.9 Hz, H_3-12′); HRMS (ESI-TOF) *m*/*z* 645.0840, 647.0808, and 649.0806 $[M+Na]^+$ (calcd for $C_{29}H_{36}O_5{}^{79}Br_2Na$, $C_{29}H_{36}O_5{}^{79}Br^{81}BrNa$, and $C_{29}H_{36}O_5{}^{81}Br_2Na$, 645.0827, 647.0809, and 649.0796, respectively).

3.7.2. (S)-MTPA ester ***15a***. Colorless gum; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.890 (2H, d, *J*=8.5 Hz, *p*-bromobenzoyl), 7.835 (2H, d, *J*=8.5 Hz, *p*-bromobenzoyl), 7.609 (2H, d, *J*=8.5 Hz, *p*-bromobenzoyl), 7.500 (2H, d, *J*=8.5 Hz, *p*-bromobenzoyl), 7.403 (2H, d, *J*=7.4 Hz, phenyl), 7.338 (1H, t, *J*=7.4 Hz, phenyl), 7.274 (2H, t, *J*=7.4 Hz, phenyl), 5.503 (1H, br d, *J*=9.7 Hz, H-5′), 5.404 (1H, d, *J*=9.7 Hz, H-3′), 4.305 (1H, dd, *J*=11.1, 2.7 Hz, Ha-1′), 4.239 (1H, dd, *J*=10.8, 6.3 Hz, Ha-15′), 4.166 (1H, dd, *J*=10.8, 7.3 Hz, Hb-15′), 3.909 (1H, dd, *J*=11.1, 5.3 Hz, Hb-1′), 3.403 (3H, s, –OCH_3), 2.832 (1H, m, H-6′), 2.306 (1H, m, H-2′), 1.699 (3H, s, H_3-14′), 1.5 (overlapped with H_2O signal, Ha-7′), 1.35–1.20 (9H, m, Hb-7′, H_2-8′, H_2-9′, H_2-10′, and H_2-11′), 0.946 (3H, d, *J*=7.0 Hz, H_3-13′), 0.863 (3H, t, *J*=6.6 Hz, H_3-12′); HRMS (ESI-TOF) *m*/*z* 861.1216, 863.1216, and 865.1200 $[M+Na]^+$ (calcd for $C_{39}H_{43}O_7{}^{79}Br_2F_3Na$, $C_{39}H_{43}O_7{}^{79}Br^{81}BrF_3Na$, and $C_{39}H_{43}O_7{}^{81}Br_2F_3Na$, 861.1220, 863.1204, and 865.1194, respectively).

3.7.3. (R)-MTPA ester ***15b***. Colorless gum; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.882 (2H, d, *J*=8.5 Hz, *p*-bromobenzoyl), 7.860 (2H, d, *J*=8.5 Hz, *p*-bromobenzoyl), 7.603 (2H, d, *J*=8.5 Hz, *p*-bromobenzoyl), 7.524 (2H, d, *J*=8.5 Hz, *p*-bromobenzoyl), 7.409 (2H, d, *J*=7.5 Hz, phenyl), 7.358 (1H, t, *J*=7.5 Hz, phenyl), 7.302 (2H, t, *J*=7.5 Hz, phenyl), 5.460 (1H, br d, *J*=9.6 Hz, H-5′), 5.310 (1H, d, *J*=9.7 Hz, H-3′), 4.410 (1H, dd, *J*=11.1, 2.9 Hz, Ha-1′), 4.253 (1H, dd, *J*=10.7, 6.3 Hz, Ha-15′), 4.154 (1H, dd, *J*=10.7, 7.3 Hz, Hb-15′), 4.111 (1H, dd, *J*=11.1, 5.5 Hz, Hb-1′), 3.414 (3H, s, -OCH_3), 2.792 (1H, m, H-6′), 2.332 (1H, m, H-2′), 1.546 (3H, s, H_3-14′), 1.5 (overlapped with H_2O signal, Ha-7′), 1.35–1.20 (9H, m, Hb-7′, H_2-8′, H_2-9′, H_2-10′, and H_2-11′), 0.974 (3H, d, *J*=7.0 Hz, H_3-13′), 0.863 (3H, t, *J*=6.5 Hz, H_3-12′); HRMS (ESI-TOF) *m*/*z* 861.1227, 863.1230, and 865.1241 $[M+Na]^+$ (calcd for $C_{39}H_{43}O_7{}^{79}Br_2F_3Na$, $C_{39}H_{43}O_7{}^{79}Br^{81}BrF_3Na$, and $C_{39}H_{43}O_7{}^{81}Br_2F_3Na$, 861.1220, 863.1204, and 865.1194, respectively).

3.8. Biological assays

Assay for activity against *P. falciparum* (K1, multi-drug resistant strain) was performed using the microculture radioisotope technique described by Desjardins et al.[11] Growth inhibitory activity against *M. tuberculosis* H37Ra and cytotoxicity to Vero cells (African green monkey kidney fibroblasts) were performed using the green fluorescent protein microplate assay (GFPMA).[12] Cytotoxicity against KB cells (human oral epidermoid carcinoma), MCF-7 cells (human breast cancer), and NCI-H187 cells (human small-cell lung cancer) were evaluated using the resazurin microplate assay.[13]

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