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Eremophilane Sesquiterpenes and Diphenyl Thioethers from the Soil Fungus *Penicillium copticola* PSU-RSPG138

Charuwan Daengrot,[†] Vatcharin Rukachaisirikul,*[,†] Chittreeya Tansakul,[†] Tienthong Thongpanchang,[‡] Souwalak Phongpaichit,[§] Kawitsara Bowornwiriyapan,[§] and Jariya Sakayaroj[⊥]

[†]Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand

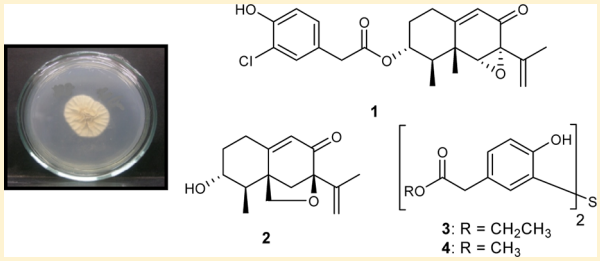
[‡]Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Mahidol University, Rama 6 Road, Bangkok 10400, Thailand

[§]Natural Products Research Center of Excellence and Department of Microbiology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand

[⊥]National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand Science Park, Khlong Luang, Pathum Thani 12120, Thailand

Supporting Information

ABSTRACT: Four new compounds including two eremophilane sesquiterpenes, penicilleremophilanes A (**1**) and B (**2**), as well as two sulfur-containing biphenols, penicillithiophenols A (**3**) and B (**4**), were isolated from the soil fungus *Penicillium copticola* PSU-RSPG138 together with 16 known compounds. Their structures were elucidated by spectroscopic methods. Known sporogen AO-1 exhibited significant antimalarial activity against *Plasmodium falciparum* with an IC_{50} value of 1.53 μM and cytotoxic activity to noncancerous (Vero) cell lines with an IC_{50} value of 4.23 μM. Although compound **1** was approximately half as active against *P. falciparum* with the IC_{50} value of 3.45 μM, it showed much weaker cytotoxic activity.



Fungi in the genus *Penicillium* are an important source of natural products with unique structures and interesting biological activities, for example, anti-H1N1 indole-diterpenoids,[1] cytotoxic sporogen AO-1 and dihydrosporogen AO-1,[2] cytotoxic sumalarins A-C and dehydrocurvularin,[3] cytotoxic expansols C and E,[4] and antibacterial penicillanthranin A.[5] As part of our ongoing search for bioactive metabolites from soil fungi, *Penicillium copticola* PSU-RSPG138 was isolated from soil collected from the Ratchaprapa Dam in Suratthani Province, Thailand. Both the broth and mycelial ethyl acetate extracts from *P. copticola* PSU-RSPG138 exhibited antimalarial (*Plasmodium falciparum*, K1 strain) and cytotoxic (Vero-African green monkey kidney fibroblast cell lines and KB-oral cavity cancer cells) activities with IC_{50} values of 0.30, 4.29, and 0.85 μg/mL for the broth extract and 2.11, 10.00, and 2.02 μg/mL for the mycelial extract, respectively. A chemical investigation of the broth extract led to the identification of four novel compounds, two eremophilane sesquiterpenes, penicilleremophilanes A (**1**) and B (**2**), and two sulfur-containing biphenols, penicillithiophenols A (**3**) and B (**4**). The following known compounds were also identified: 3-chloro-4-hydroxyphenylacetic acid (**5**),[6] (*E*)-but-2-enedioic acid monomethyl ester (**6**),[7] (3*R*,4*R*,5*R*,6*R*,7*R*)-sporogen AO-1 (**7**),[8] (3*R*,4*R*,5*R*,6*R*,7*S*,8*S*)-dihydrosporogen AO-1 (**8**),[9] a 8-epimer of **8** (**9**),[10] (3*R*,4*R*,5*R*,6*R*,7*R*)-phomenone (**10**),[11] (3*R*,4*R*,5*R*,6*R*,7*R*)-3-acetyl-13-deoxyphomenone (**11**),[12] (3*R*,4*R*,5*R*,7*S*)-petasol (**12**),[9] (3*R*,4*R*,5*R*,7*S*)-7-hydroxypetasol (**13**),[13] 7-hydroxy-4a,5-dimethyl-3-prop-1-en-2-yl-3,4,5,6,7,8-hexahydronaphthalen-2-one (**14**),[14] (3*R*,4*R*,5*S*,7*S*)-JBIR-27 (**15**),[15] (3*R*,4*R*,5*R*)-6-dehydropetasol (**16**),[13] 3α,13-dihydroxyeremophila-6,9,11-triene-8-one (**17**)[16] and (3*R*,4*R*,5*R*,7*S*,11*S*)-11,12-epoxypetasol (**18**).[13] Two additional known eremophilanes, (4*S*,5*R*,7*S*)-neopetasane (**19**)[17] and (3*R*,4*R*,5*R*)-isopetasol (**20**),[18] were obtained from the mycelial extract along with 3-chloro-4-hydroxyphenylacetic acid (**5**), (3*R*,4*R*,5*R*,6*R*,7*R*)-sporogen AO-1 (**7**), (3*R*,4*R*,5*R*,6*R*,7*S*,8*S*)-dihydrosporogen AO-1 (**8**), (3*R*,4*R*,5*R*,7*S*)-petasol (**12**), and (3*R*,4*R*,5*R*)-6-dehydropetasol (**16**). 7-Hydroxy-4a,5-dimethyl-3-prop-1-en-2-yl-3,4,5,6,7,8-hexahydronaphthalen-2-one (**14**) and 3α,13-dihydroxyeremophila-6,9,11-triene-8-one (**17**) which were previously obtained by chemical synthesis, were isolated as natural products for the first time. The isolated compounds were evaluated for antimycobacterial (*Mycobacterium tuberculosis*, H_{37}Ra strain), antimalarial (*P. falciparum*, K1 strain), and cytotoxic (Vero, KB and MCF-7-human breast cancer cells) activities.

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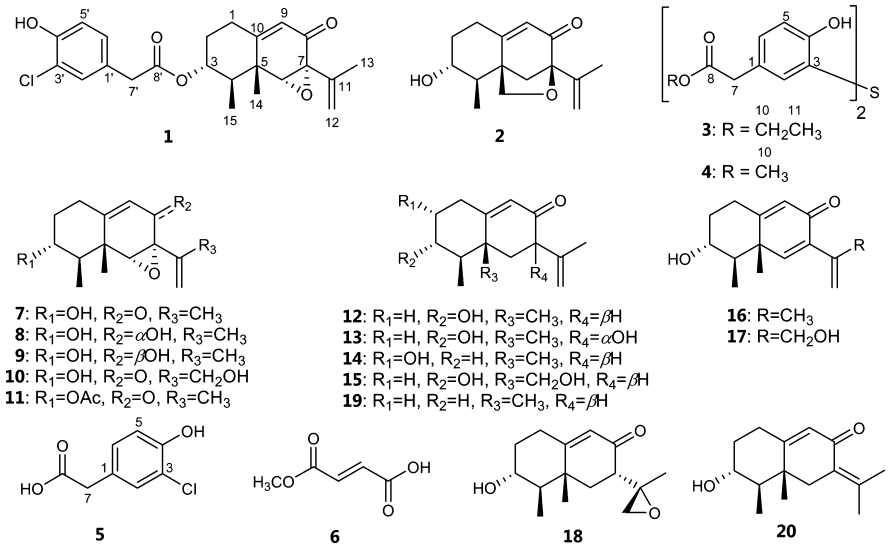

Figure 1. Structures of compounds **1**–**20** isolated from *Penicillium copticola* PSU-RSPG138.

RESULTS AND DISCUSSION

All compounds (**1**–**20**) (Figure 1) were obtained using chromatographic purification. Their structures were determined by spectroscopic analysis. The relative configuration was assigned according to the NOEDIFF data. The absolute configuration of the isolated compounds was established by comparison of their optical rotations with those of known or structurally related compounds (Table S1). For the new eremophilanes (**1** and **2**), the absolute configuration was established according to the assigned absolute configuration of the cometabolite **7** which was obtained as the major component in both the broth and mycelia extracts (Table S2). The relative configuration of **7** was determined to be identical to that of sporogen AO-1 according to the NOEDIFF data. The absolute configuration at C-3 in **7** was established to be *R* by Mosher's method[19] (Figure 2) and THENA-ester[20]

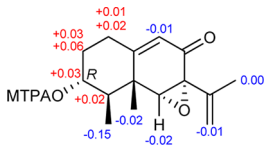

Figure 2. $\Delta\delta$ (= $\delta_S - \delta_R$) values for (*S*)- and (*R*)-MTPA-esters of compound **7**.

(Figure 3). Subsequently, the absolute configurations for the remaining chiral carbons, C-4, C-5, C-6, and C-7, were all *R*, identical to those of sporogen AO-1. These assignments were supported by their similar optical rotations, $[\alpha]_D^{25}$ of **7** +179.5 (*c* 1.0, $CHCl_3$) and $[\alpha]_D$ of sporogen AO-1 +214 (*c* 1.0, $CHCl_3$). On the basis of absolute configuration of **7**, the absolute configurations for the chiral centers C-3, C-4, and C-5 of the new and known isolated compounds could be assigned to be identical to those of **7** on the basis of biogenetic considerations. The absolute configuration of **17** was then assigned for the first time as 3*R*, 4*R*, and 5*R*. The absolute configurations at C-3 and C-8 of **8** were confirmed by THENA-ester (Figure 3) to be *R* and *S* configurations, respectively, identical to those previously reported. Furthermore, the NMR spectroscopic data of **9** and **14** are reported for the first time.

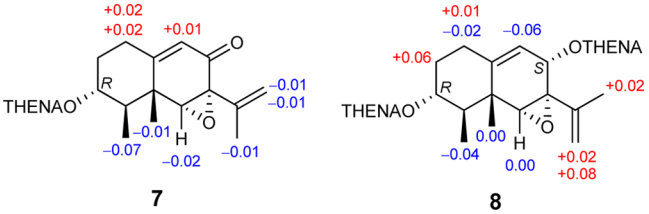

Figure 3. $\Delta\delta$ (= $\delta_S - \delta_R$) values for (*S*)- and (*R*)-THENA-esters of compounds **7** and **8**.

Penicilleremophilane A (**1**) was obtained as a colorless gum with the molecular formula $C_{23}H_{25}ClO_5$ according to the HRESIMS peak at *m*/*z* 439.1283 $[M + Na]^+$. The UV spectrum showed absorption bands at 231 and 281 nm for α,β-unsaturation carbonyl and aromatic functional groups.[8] The IR spectrum showed absorption bands at 3405, 1731, and 1673 cm^{-1} for hydroxy, ester carbonyl, and α,β-unsaturated carbonyl groups, respectively. The ^1H NMR spectroscopic data (Table 1) contained signals for three aromatic protons of a 1,2,4-trisubstituted benzene [δ_H 7.26 (brd, *J* = 2.1 Hz), 7.09 (dd, *J* = 2.1 and 8.4 Hz), 6.97 (d, *J* = 8.4 Hz), each 1H], one olefinic proton of a trisubstituted alkene (δ_H 5.76, d, *J* = 2.1 Hz), two geminal olefinic protons (δ_H 5.11, brs), one hydroxy proton (δ_H 5.54, s), two oxymethine protons (δ_H 4.86, dt, *J* = 4.5 and 11.1 Hz and 3.30, s, each 1H), two equivalent methylene protons (δ_H 3.54, s, 2H), two sets of nonequivalent methylene protons [δ_H 2.54 (ddt, *J* = 2.1, 5.1, and 14.7 Hz, 1H)/2.35 (ddd, *J* = 3.0, 4.2, and 14.7 Hz, 1H) and 2.14 (m, 1H)/1.43 (dtd, *J* = 4.2, 11.1, and 14.7 Hz, 1H)], one methine proton (δ_H 2.06, qd, *J* = 6.6 and 11.1 Hz), and three methyl groups [δ_H 1.86 (s), 1.24 (s) and 1.02 (d, *J* = 6.6 Hz), each 3H]. The ^{13}C NMR spectrum (Table 1) displayed signals for two carbonyl carbons (δ_C 192.4 for an α,β-unsaturated ketone carbonyl carbon and δ_C170.9 for an ester carbonyl carbon), seven quaternary, seven methine, four methylene and three methyl carbons. The ^{13}C chemical shifts of the oxymethine carbon at δ_C 68.1 and the quaternary oxycarbon at δ_C 63.5 indicated the presence of an epoxide.[8] The ^1H–^1H COSY and HMBC correlations (Figure 4) showed that **1** possessed the same eremophilane core structure as **7**.[8] However, a significant difference was that H-3 (δ_H 4.86) in **1** resonated at much lower field than that in **7** (δ_H 3.63). The

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Table 1. ^{1}H and ^{13}C NMR Data for Penicilleremophilanes A (1) and B (2) in $CDCl_3$

	1		2	
position	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1α	30.4, CH_2	2.35, ddd (3.0, 4.2, 14.7)	29.1, CH_2	2.72, dtd (2.1, 5.1, 16.8)
1β		2.54, ddt (2.1, 5.1, 14.7)		2.57, dddd (1.2, 5.1, 11.4, 16.8)
2α	31.3, CH_2	1.43, dtd (4.2, 11.1, 14.7)	32.5, CH_2	1.68, m
2β		2.14, m		2.12, m
3	73.6, CH	4.86, dt (4.5, 11.1)	72.5, CH	3.52, dt (4.8, 10.2)
4	41.4, CH	2.06, qd (6.6, 11.1)	40.7, CH	1.92, qd (6.9, 10.2)
5	41.1, C		51.5, C	
6α	68.1, CH	3.30, s	47.3, CH_2	2.22, d (12.3)
6β				2.18, d (12.3)
7	63.5, C		88.3, C	
8	192.4, C		195.4, C	
9	121.5, CH	5.76, d (2.1)	124.2, CH	5.90, dd (1.2, 2.1)
10	161.6, C		165.9, C	
11	138.9, C		142.4, C	
12a	114.5, CH_2	5.11, brs	112.2, CH_2	5.18, q (1.5)
12b		5.11, brs		5.08, quin (1.5)
13	19.7, CH_3	1.86, s	20.1, CH_3	1.85, t (1.5)
14a	18.6, CH_3	1.24, s	69.8, CH_2	4.12, d (7.5)
14b				3.42, d (7.5)
15	11.1, CH_3	1.02, d (6.6)	11.4, CH_3	1.18, d (6.9)
1′	127.0, C			
2′	129.6, CH	7.26, brd (2.1)		
3′	119.9, C			
4′	150.6, C			
4′−OH		5.54, s		
5′	116.3, CH	6.97, d (8.4)		
6′	129.3, CH	7.09, dd (2.1, 8.4)		
7′	40.5, CH_2	3.54, s		
8′	170.9, C			

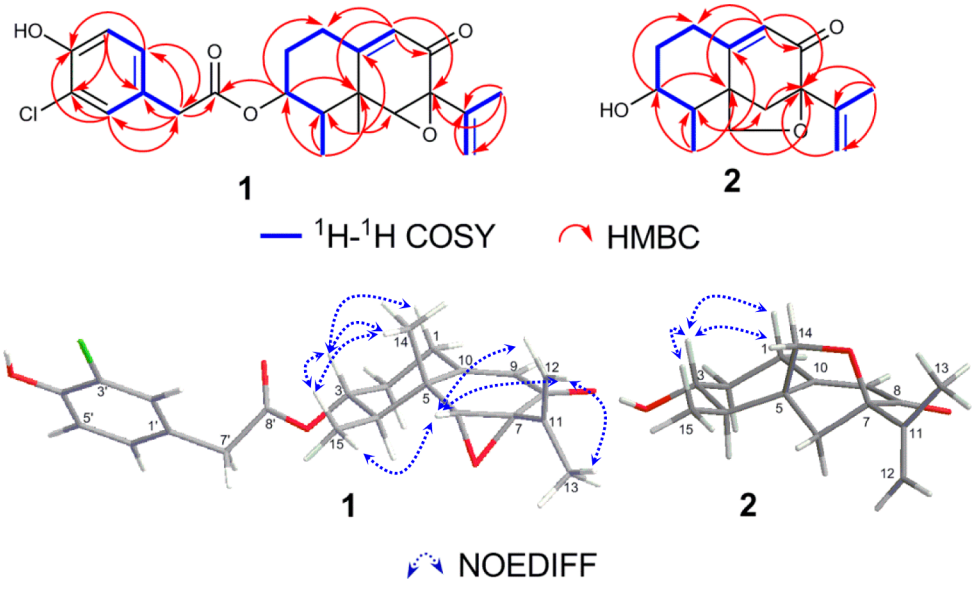

Figure 4. Key $^{1}H-^{1}H$ COSY and HMBC correlations and NOEDIFF data of compounds 1 and 2.

aromatic protons resonating at δ_H 7.26, 6.97, and 7.09 were attributed to H-2′, H-5′ and H-6′, respectively, according to their multiplicities and coupling constants. Both H-2′ and H-6′ gave the HMBC cross peaks with C-7′ (δ_C 40.5), which displayed the HMQC correlation with the methylene protons at δ_H 3.54 (H_2-7′). These results together with the HMBC correlations from H_2-7′ to the ester carbonyl carbon (C-8′, δ_C 170.9) established the attachment of a CH_2COO- unit at C-1′. This assignment was confirmed by the HMBC correlations from H_2-7′ to C-1′ (δ_C 127.0), C-2′ (δ_C 129.6) and C-6′ (δ_C 129.3). The chemical shifts of C-3′ (δ_C 119.9) and C-4′ (δ_C 150.6) together with the molecular formula indicated the attachment of a chlorine atom and a hydroxy group at C-3′ and C-4′, respectively. Thus, a 3′-chloro-4′-hydroxyphenylacetoxyl

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Table 2. ^{1}H and ^{13}C NMR Data for Penicillithiophenols A (3) and B (4) in $CDCl_3$

	3			4		
position	δ_C, type	δ_H (J in Hz)	HMBC	δ_C, type	δ_H, (J in Hz)	HMBC
1	127.2, C			127.1, C		
2	129.7, CH	7.26, d (2.1)	C-3, C-4, C-6, C-7	129.7, CH	7.26, d (2.1)	C-3, C-4, C-6, C-7
3	119.8, C			119.9, C		
4	150.5, C			150.5, C		
4-OH		5.63, s	C-3, C-4, C-5		5.57, brs	C-3, C-4, C-5
5	116.3, CH	6.94, d (8.4)	C-1, C-3, C-4, C-7	116.3, CH	6.96, d (8.4)	C-1, C-3, C-4
6	129.3, CH	7.08, dd (2.1, 8.4)	C-2, C-4, C-7	129.3, CH	7.08, dd (2.1, 8.4)	C-2, C-4, C-7
7	40.2, CH_2	3.52, brs	C-1, C-2, C-6, C-8	39.9, CH_2	3.54, s	C-1, C-2, C-6, C-8
8	171.5, C			171.8, C		
10	61.0, CH_2	4.15, q (7.2)	C-8, C-11	52.1, CH_3	3.70, s	C-8
11	14.1, CH_3	1.26, t (7.2)	C-10			

unit was established. This unit was attached at C-3 (δ_C 73.6) of the eremophilane unit on the basis of the HMBC correlation from H-3 to C-8′. The relative configuration of the eremophilane moiety in **1** was determined by the coupling constants observed in the ^{1}H NMR spectrum and the NOEDIFF experiments (Figure 4). H-3 was coupled with H_α-2 (δ_H 1.43) and H-4 (δ_H 2.06) with equal large coupling constants of 11.1 Hz, indicating their axial orientation. Irradiation of H-3 affected signal intensity of H_3-14 (δ_H 1.24) and H_3-15 (δ_H 1.02), while signal intensity of H-3, H-6 (δ_H 3.30) and H_3-14 was enhanced upon irradiation of H_3-15. In addition, signal enhancement of H-6 and H_3-13 (δ_H 1.86) was observed after irradiation of H_2-12 (δ_H 5.11). These results indicated *cis*-relationships of H-3, H-6, H_3-14, H_3-15 and the vinyl group and their location at β positions, identical to those of **7**. Compound **1** gave a similar optical rotation, $[\alpha]_D^{25}$ +195.3 (*c* 1.0, $CHCl_3$), to that of the cometabolite **7**.[8] Thus, the absolute configurations at C-3, C-4, C-5, C-6, and C-7 in **1** were proposed to be all *R*, identical to those of **7**. Therefore, **1** was assigned as a new ester derivative which would be derived from the esterification reaction between the acid **5** and the alcohol **7**.

Penicilleremophilane B (**2**) was obtained as a colorless gum. The molecular formula was $C_{15}H_{20}O_3$, corresponding to six degrees of unsaturation, on the basis of the HRESIMS peak at m/z 271.1315 $[M + Na]^+$. The UV spectrum showed an absorption band at 240 nm for an α,β-unsaturation carbonyl functionality. The IR spectrum showed absorption bands at 3424 and 1669 cm^{-1} for hydroxy and α,β-unsaturated carbonyl groups, respectively. The ^{1}H NMR spectroscopic data (Table 1) contained signals for one olefinic proton of a trisubstituted alkene (δ_H 5.90, dd, J = 1.2 and 2.1 Hz), two nonequivalent geminal-olefinic protons (δ_H 5.18, q and 5.08, quin, each J = 1.5 Hz, 1H), two nonequivalent oxymethylene protons (δ_H 4.12 and 3.42, each d, J = 7.5 Hz, 1H), one oxymethine proton (δ_H 3.52, dt, J = 4.8 and 10.2 Hz), three sets of nonequivalent methylene protons [δ_H 2.72 (dtd, J = 2.1, 5.1, and 16.8 Hz, 1H)/2.57 (dddd, J = 1.2, 5.1, 11.4, and 16.8 Hz, 1H), 2.22/2.18 (each d, J = 12.3 Hz, 1H) and 2.12/1.68 (each m, 1H)], one methine proton (δ_H 1.92, qd, J = 6.9 and 10.2 Hz) and two methyl groups [δ_H 1.85 (t, J = 1.5 Hz) and 1.18 (d, J = 6.9 Hz), each 3H]. These data were similar to those of **15**.[15] The obvious difference was the replacement of the methine carbon (C-7, δ_C 55.3) in **15** with an oxyquaternary carbon (δ_C 88.3) in **2**. The assignment of the oxyquaternary carbon as C-7 was confirmed by the HMBC correlations from this carbon to $H_{\alpha\beta}$-6 (δ_H 2.22 and 2.18), H-9 (δ_H 5.90), H_{ab}-12 (δ_H 5.18 and 5.08), and H_3-13 (δ_H 1.85). An ether linkage between C-7 and C-14 was established according to the HMBC correlations from H_{ab}-14 (δ_H 4.12 and 3.42) to C-7. The relative configuration of **2** was determined by the NOEDIFF experiments (Figure 4). Irradiation of H-3 (δ_H 3.52) affected signal intensity of H_{ab}-14 and H_3-15 (δ_H 1.18), whereas irradiation of H_3-13 and H_{ab}-12 did not affect signal intensity of H_{ab}-14. These data indicated that H-3, H_{ab}-14, and H_3-15 had close proximity and had a trans-relationship to the vinyl group. The absolute configurations at C-3, C-4, C-5, and C-7 were proposed to be *R*, *R*, *S*, and *R*, respectively, on the basis of the absolute configuration of **7** and **15**. Therefore, **2** was identified as a new naturally occurring eremophilane sesquiterpene.

Penicillithiophenol A (**3**) was obtained as a colorless gum with the molecular formula $C_{20}H_{22}O_6S$ determined from the HRESIMS peak at m/z 431.1035 $[M + Na]^+$. It exhibited UV absorption bands at 220 and 282 nm, whereas the IR spectrum showed characteristic absorption bands of hydroxy and ester carbonyl groups at 3414 and 1729 cm^{-1}, respectively. The ^{1}H and ^{13}C NMR spectroscopic data (Table 2) as well as the HMBC and ^{1}H−^{1}H COSY correlations were similar to those of the 3-chloro-4-hydroxyphenylethanoyl unit of **1**. However, **3** had additional proton signals of an ethoxy group at δ_H 4.15 (q, J = 7.2 Hz, 2H) and 1.26 (t, J = 7.2 Hz, 3H). The HMBC correlation of the methylene protons of the ethoxy group with C-8 (δ_C 171.5) attached the ethoxy group at C-8. These data together with the molecular formula indicated that the chlorine atom in the 3-chloro-4-hydroxyphenylethanoyl unit was replaced by a sulfur atom which joined two identical phenyl units with a sulfide linkage. Consequently, **3** was identified as a new sulfur-containing biphenol.

Penicillithiophenol B (**4**) was obtained as a colorless gum and had the molecular formula $C_{18}H_{18}O_6S$ as determined by the HRESIMS peak at m/z 385.0736 $[M + Na]^+$. The ^{1}H and ^{13}C NMR data (Table 2) were almost identical to those of **3** with the replacement of signals for the ethoxy group in **3** with a singlet signal of a methoxy group (δ_H 3.70, δ_C 53.1) in **4**. This conclusion was confirmed by the HMBC correlation of the methoxy protons with C-8 (δ_C 171.7). Consequently, **4** was identified as a methyl ester derivative of **3**.

Compound **9** was obtained as a colorless gum with the molecular formula $C_{15}H_{22}O_3$ as deduced by the HRESIMS peak at m/z 273.1466 $[M + Na]^+$. The UV, IR, ^{1}H, and ^{13}C NMR data were almost identical to those of **8**.[9] However, in the ^{1}H NMR spectrum of **9**, the oxymethine proton (H-8, δ_H 4.46) appeared as a doublet with a coupling constant of 5.1 Hz instead of a broad singlet at δ_H 4.51 in **8**. Irradiation of H-8 in

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Table 3. Antimycobacterial, Antimalarial, and Cytotoxic Activities for Compounds 1, 7−8, 10−12, and 15−16

	antimycobacterial (MIC, μg/mL)	antimalarial (IC_{50}, μM)	cytotoxic (IC_{50}, μM)		
compd	*M. tuberculosis* H_{37}Ra strain	*P. falciparum*	KB	MCF-7	Vero
1	50.00	3.45	56.95	39.55	21.08
7	IN	1.53[a]	17.84[b]	IN	4.23[c]
8	IN	IN	IN	IN	24.05
10	IN	5.41	31.82	IN	6.05
11	IN	4.55	35.72	IN	7.51
12	IN	39.32	186.01	IN	44.50
15	IN	IN	IN	IN	56.25
16	IN	15.71	IN	IN	34.61
rifampicin	0.025				
streptomycin	0.625				
isoniazid	0.094				
ofloxacin	0.391				
ethambutol	0.983				
mefloquine		0.03			
dihydroartemisinin		1.26[d]			
ellipticine			1.98		3.80
doxorubicin			0.91	11.52	
tamoxifen				18.71	

IN = inactive. [a]0.38 μg/mL. [b]4.43 μg/mL. [c]1.10 μg/mL. [d]nM.

the NOEDIFF experiment enhanced signal intensity of H-9 (δ_H 5.43, dd, J = 1.8 and 5.1 Hz) and H_3-13 (δ_H 1.92, brs), but not H_3-14 (δ_H 1.16, s), indicating the α-orientation of H-8. Therefore, **9** was identified as a C-8 epimer of dihydrosporogen AO-1 (**8**). According to the absolute configuration of the cometabolite **8**, the absolute configuration at C-8 of **9** was assigned as *R* configuration.

Compound **14** was obtained as a colorless gum with the molecular formula $C_{15}H_{22}O_2$, as determined by the HRESIMS peak at m/z 235.1696 $[M + H]^+$. The UV and IR data were almost identical to those of **12**.[9] The ^{1}H NMR spectrum was similar to that of **12** except that the oxymethine proton in **14** resonated at much lower field (δ_H 4.22, qn, J = 3.3 Hz in **14** and δ_H 3.62, dt, J = 4.5 and 10.8 Hz, H-3 in **12**). It was attributed to H-2 on the basis of the ^{1}H−^{1}H COSY correlations between $H_{\alpha\beta}$-1 (δ_H 2.61, ddd, J = 2.1, 3.3, and 15.3 Hz and 2.38, brd, J = 15.3 Hz) and H_2-3 (δ_H 1.70, m) and the HMBC correlations from $H_{\alpha\beta}$-1, H_2-3 and H-4 (δ_H 2.00, m) to C-2 (δ_C 67.2). The small coupling constant of 3.3 Hz between H-2 and $H_{\alpha\beta}$-1 as well as H_2-3 established the location of H-2 at an equatorial position which was identical to that of 2-hydroxyaristolone.[21] This assignment was confirmed by signal enhancement of $H_{\alpha\beta}$-1 and H_2-3 upon irradiation of H-2 in the NOEDIFF experiment. In addition, the relative configuration at C-4, C-5, and C-7 in **14** was identical of that in **12** on the basis of the NOEDIFF data. Because they were cometabolites, **14** would have 4*R*, 5*R* and 7*S* configurations, identical to those of **12**. Accordingly, the absolute configuration at C-2 in **14** was purposed to be *S*.

Compounds **1**−**5** and **7**−**16**, which were obtained in sufficient amounts, were tested for antimycobacterial (*Mycobacterium tuberculosis*, H_{37}Ra strain), antimalarial (*P. falciparum*, K1 strain), and cytotoxic (KB, MCF-7 and Vero cells) activities (Table 3). Compounds **2**−**5**, **9**, and **13**−**14** were noncytotoxic against all tested cell lines and showed no other activities. Only **1** exhibited mild antimycobacterial activity and weak cytotoxic activity against MCF-7 cell lines. For antimalarial activity, **1**, **7**, **10**, and **11** displayed significant activity with the IC_{50} values in the range of 1.53−5.41 μM. For cytotoxic activity toward KB cell lines, **1**, **7** and **10**−**12** showed weak activity. However, **7**, **10**, and **11** exhibited much stronger cytotoxic activity to Vero cells than **1**, **8**, **12**, **15**, and **16**. Interestingly, **1** was approximately half as active as **7** against *P. falciparum*, but it showed much weaker cytotoxic activity to Vero cell lines. These data indicated that 3-OH, epoxide, vinylic methyl, and ketone functionalities would be important for antimalarial and cytotoxic (against KB and Vero cell lines) activities. From these results, we conclude that the biological activities of both broth and mycelial extracts might be governed by **7**, which is the major component in both extracts.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were recorded on a JASCO P-1020 polarimeter. The ultraviolet (UV) absorption spectra were measured in MeOH on a PerkinElmer Lambda 45 spectrophotometer. The infrared (IR) spectra were recorded neat using a PerkinElmer 783 FTS165 FT-IR spectrometer. Mass spectra were obtained from a MAT 95 XL mass spectrometer (Thermo Finnigan), Bruker MicrOTOF mass spectrometer or a liquid chromatograph−mass spectrometer (2090, LCT, Waters, Micromass). ^{1}H and ^{13}C NMR spectra were recorded on a 300 or 500 MHz Bruker FTNMR Ultra Shield spectrometer. Chemical shifts are expressed in δ (parts per million, ppm), referring to the tetramethylsilane peak. Thin-layer chromatography (TLC) and preparative TLC (PTLC) were performed on silica gel 60 GF254 (Merck). Column chromatography (CC) was carried out on Sephadex LH-20 with MeOH, silica gel (Merck) type 60 (230−400 mesh ASTM) or type 100 (70−230 mesh ASTM), or on reversed phase C_{18} silica gel.

Fungal Material. The soil fungus PSU-RSPG138 was isolated from soil samples collected from the Plant Genetic Conservation Project under the Royal Initiation of Her Royal Highness Princess Maha Chakri Sirindhorn at Ratchaprapa Dam in Suratthani province, Thailand. This fungus was deposited as BCC56847 at the BIOTEC Culture Collection, National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand. The fungus PSU-RSPG138 was identified based on its morphological characteristics and the ITS rDNA sequence analysis. Colony of the fungus PSU-RSPG138 on potato dextrose agar (PDA) was corrugated and white to gray surface. It produced brush-like conidial bearing structures which are typical characteristics of the genus *Penicillum*. Its ITS sequence (GenBank

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accession no. KC478549) matched with a closely related sequence of *Penicillium copticola* JN617685 with 99.5% nucleotide sequence identity. Thus, the fungus PSU-RSPG138 could be identified as *Penicillium copticola*.

Fermentation, Extraction, and Purification. The soil fungus *P. copticola* PSU-RSPG138 was grown on potato dextrose agar at 25 °C for 5 days. Five pieces (0.5 × 0.5 cm^2) of mycelial agar plugs were inoculated into 500 mL Erlenmeyer flasks containing 300 mL of potato dextrose broth at room temperature for 3 weeks. The flask culture (16.5 L) was filtered to separate into the filtrate and wet mycelia. The filtrate was extracted twice with ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure to afford a dark brown gum (7.89 g). The mycelial cakes were extracted with MeOH (500 mL). The MeOH layer was concentrated under reduced pressure. To the MeOH extract was added H_2O (150 mL), and the mixture was washed with hexane (500 mL). The aqueous residue was extracted three times with an equal amount of EtOAc. The EtOAc layer was dried over anhydrous Na_2SO_4 and then evaporated to dryness to obtain a brown gum (470 mg).

The broth extract was separated by CC over Sephadex LH-20 using MeOH to afford six fractions (A-F). Fraction B (358.2 mg) was purified by CC over silica gel using a gradient of acetone-EtOAc-CH_2Cl_2 (2:3:95 to 1:0:0) to provide seven fractions (B1−B7). Fraction B3 (68.3 mg) was purified by CC over silica gel using a gradient of acetone-CH_2Cl_2 (2:23 to 1:1) to afford **12** (26.9 mg). Fraction B4 (30.6 mg) was purified by CC over silica gel using a gradient of acetone-CH_2Cl_2 (2:23 to 1:1) followed by PTLC using acetone-CH_2Cl_2 (1:19) as a mobile phase (three runs) to provide **16** (8.1 mg). Fraction B6 (68.8 mg) was further separated by CC over reversed phase C_{18} silica gel using MeOH-H_2O (3:2) to give **8** (39.3 mg). Fraction C (5.94 g) was purified using the same procedure as fraction B3 to afford eight fractions (C1−C8). Fraction C1 (48.8 mg) was rechromatographed on CC over silica gel using a gradient of EtOAc-CH_2Cl_2 (3:97 to 1:1) followed by PTLC using CH_2Cl_2 as a mobile phase (two runs) to provide **11** (5.2 mg). Fraction C2 (3.67 g) was purified using the same procedure as fraction B3 to afford **7** (2.25 g) and **12** (1.38 g). Fraction C3 (807.3 mg) was separated by CC over reversed phase C_{18} silica gel using MeOH-H_2O (7:3) to afford **7** (12.5 mg), **13** (25.4 mg), and **16** (2.9 mg). Fraction C4 (273.9 mg) was subjected to CC over reversed phase C_{18} silica gel using MeOH-H_2O (3:2) to give six subfractions. The first subfraction (100.8 mg) was subjected to CC over silica gel using MeOH−CH_2Cl_2 (1:49) to give **13** (6.4 mg). The second subfraction (34.6 mg) was rechromatographed on CC over reversed phase C_{18} silica gel using MeOH-H_2O (3:2) followed by PTLC using a mixture of acetone-EtOAc-CH_2Cl_2 in a ratio of 2:3:45 as a mobile phase (two runs) to provide **18** (1.1 mg). The third subfraction (12.9 mg) was purified by PTLC using acetone-CH_2Cl_2 (1:19) to afford **2** (5.0 mg). The fifth subfraction (10.8 mg) was purified by PTLC using acetone-CH_2Cl_2 (1:19) (three runs) followed by PTLC using acetone-CH_2Cl_2 (2:23) (five runs) to provide **14** (3.5 mg). Fraction C5 (28.5 mg) was subjected to CC over reversed phase C_{18} silica gel using MeOH-H_2O (3:2) followed by PTLC using EtOAc-CH_2Cl_2 (3:17) as a mobile phase (three runs) to give **9** (5.6 mg). Fraction C6 (124.3 mg) was purified by CC over silica gel using MeOH−CH_2Cl_2 (2:49) to provide **8** (50.5 mg). Fraction C7 (329.5 mg) was purified by CC over silica gel using MeOH−CH_2Cl_2 (1:19) to afford five subfractions. Compound **8** (241.6 mg) was obtained from the second subfraction. The fourth subfraction (11.5 mg) was purified by PTLC using EtOAc-CH_2Cl_2 (3:7) as a mobile phase (five runs) to afford **10** (4.7 mg). Fraction C8 (274.8 mg) was purified by CC over silica gel using a gradient of MeOH−CH_2Cl_2 (1:19 to 1:0) to provide three subfractions. The second subfraction (28.5 mg) was further purified by CC over silica gel using a gradient of acetone-petroleum ether (1:3 to 1:0) followed by PTLC using EtOAc-CH_2Cl_2 (3:7) as a mobile phase (five runs) to afford **17** (1.8 mg). The last subfraction (139.3 mg) was purified using the same procedure as faction B3 to give **15** (29.8 mg). Fraction D (1.23 g) was purified by CC over silica gel using a gradient of acetone-CH_2Cl_2 (1:4 to 1:0) to afford seven subfractions (D1-D7). Fraction D2 (45.6 mg) was subjected to CC over silica gel using a gradient of EtOAc-petroleum ether (1:9 to 1:1) to provide three subfractions. Compound **3** (15.2 mg) was obtained from the first subfraction. The second subfraction (16.1 mg) was purified by PTLC using acetone-petroleum ether (1:4) as a mobile phase (two runs) to afford **4** (8.7 mg). Fraction D4 (103.3 mg) was purified by CC over silica gel using a gradient of EtOAc-CH_2Cl_2 (2:23 to 1:1) to afford **1** (4.7 mg) and **7** (73.2 mg). Fraction D6 (163.0 mg) was purified by CC over silica gel using a gradient of MeOH−CH_2Cl_2 (1:49 to 1:1) to afford **5** (49.7 mg). Fraction E (398.6 mg) was separated by dissolving with $CHCl_3$ to give a $CHCl_3$ soluble fraction (EB) and a $CHCl_3$ insoluble fraction (EA). Fraction EA (235.5 mg) was purified by CC over silica gel using a gradient of MeOH−CH_2Cl_2 (3:17 to 1:1) to afford **4** (10.2 mg) and **5** (71.8 mg). Fraction EB (154.1 mg) was purified by CC over silica gel using a gradient of acetone-CH_2Cl_2 (3:17 to 1:1) to afford **6** (1.3 mg). The mycelial ethyl acetate extract (473.8 mg) was purified by CC over silica gel using a gradient of EtOAc-CH_2Cl_2 (3:97 to 1:0) to provide five fractions (CE1-CE5). Fraction CE2 (22.0 mg) was then purified by PTLC using EtOAc-petroleum ether (1:19) as a mobile phase (four runs) to afford **19** (1.2 mg). Fraction CE3 (132.9 mg) was purified using the same procedure as fraction B3 to afford **7** (99.0 mg), **12** (33.2 mg), and **16** (30.0 mg). Fraction CE4 (18.3 mg) was purified using the same procedure as fraction B4 to provide **20** (1.4 mg). Fraction CE5 (197.3 mg) was purified using the same procedure as the mycelial extract to give **5** (25.0 mg) and **8** (32.0 mg).

*Penicilleremophilane A (**1**).* Colorless gum; $[\alpha]_D^{27}$ +195.3° (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} (logε): 231(4.53), 281(3.72) nm; IR (neat)ν_{max} 3405, 2982, 1731, 1673 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z*: $[M + Na]^+$ calcd for $C_{23}H_{25}ClNaO_5$, 439.1288; found, 439.1283.

*Penicilleremophilane B (**2**).* Colorless gum; $[\alpha]_D^{27}$ +139.6° (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (logε): 240 (3.99) nm; IR (neat)ν_{max} 3424, 2971, 1669 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z*: $[M + Na]^+$ calcd for $C_{15}H_{20}O_3Na$, 271.1310; found, 271.1315.

*Penicillithiophenol A (**3**).* Colorless gum; UV (MeOH) λ_{max} (logε) 220(4.03), 282(3.50) nm; IR (neat)ν_{max} 3414, 2983, 1729, 1714 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRESIMS *m/z*: $[M + Na]^+$ calcd for $C_{20}H_{22}O_6SNa$, 431.1035; found, 431.1035.

*Penicillithiophenol B (**4**).* Colorless gum; UV (MeOH) λ_{max} (logε) 221(4.20), 281(3.71) nm; IR (neat)ν_{max} 3418, 2953, 1736, 1721 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRESIMS *m/z*: $[M + Na]^+$ calcd for $C_{18}H_{18}O_6SNa$, 385.0722; found, 385.0736.

*(3R,4R,5R,6R,7S,8R)-Dihydrosporogen AO-1 (**9**).* Colorless gum; $[\alpha]_D^{25}$ −35.0° (*c* 0.1, MeOH); IR (neat)ν_{max} 3397, 2975 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ_H 5.43 (dd, *J* = 1.8 and 5.1 Hz, 1H, H-9), 5.18 (quin, *J* = 1.5 Hz, 1H, H_a-12), 5.11 (brs, 1H, H_b-12), 4.46 (brd, *J* = 5.1 Hz, 1H, H-8), 3.54 (dt, *J* = 4.5 and 11.1 Hz, 1H, H-3), 3.30 (brs, 1H, H-6), 2.32 (ddt, *J* = 1.8, 4.5, and 14.4 Hz, 1H, H_β-1), 2.19 (ddd, *J* = 2.7, 4.2, and 14.4 Hz, 1H, H_α-1), 2.07 (m, 1H, H_β-2), 1.92 (brs, 3H, H_3-13), 1.60 (m, 1H, H-4), 1.32 (m, 1H, H_α-2), 1.16 (s, 3H, H_3-14), 1.16 (d, *J* = 6.6 Hz, 3H, H_3-15); ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C 142.6 (C, C-11), 141.9 (C, C-10), 118.3 (CH, C-9), 116.6 (CH_2, C-12), 72.1 (CH, C-3), 65.2 (CH, C-6), 65.2 (C, C-7), 64.6 (CH, C-8), 44.4 (CH, C-4), 39.6 (C, C-5), 36.3 (CH_2, C-2), 30.9 (CH_2, C-1), 19.2 (CH_3, C-13), 18.8 (CH_3, C-14), 11.0 (CH_3, C-15); HRESIMS *m/z*: $[M + Na]^+$ calcd for $C_{15}H_{22}O_3Na$, 273.1467; found, 273.1466.

*7-Hydroxy-4a,5-dimethyl-3-prop-1-en-2-yl-3,4,5,6,7,8-hexahydronaphthalen-2-one (**14**).* Colorless gum; $[\alpha]_D^{27}$ +55.1° (*c* 0.8, $CHCl_3$); UV (MeOH) λ_{max} (logε) 240 (3.58) nm; IR (neat)ν_{max} 3415, 2962, 1671 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ_H 5.83 (d, *J* = 2.1 Hz, 1H, H-9), 4.99 (quin, *J* = 1.5 Hz, 1H, H_a-12), 4.83 (brs, 1H, H_b-12), 4.22 (qn, *J* = 3.3 Hz, 1H, H-2), 3.15 (dd, *J* = 6.0 and 13.2 Hz, 1H, H-7), 2.61 (ddd, *J* = 2.1, 3.3, and 15.3 Hz, 1H, H_β-1), 2.38 (brd, *J* = 15.3 Hz, 1H, H_α-1), 2.00 (m, 1H, H-4), 1.98 (m, 2H, H_2-6), 1.75 (brs, 3H, H_3-13), 1.70 (m, 2H, H_2-3), 1.17 (s, 3H, H_3-14), 0.93 (d, *J* = 6.9 Hz, 3H, H_3-15); ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C 198.5 (C, C-8), 166.4 (C, C-10), 144.0 (C, C-11), 127.5 (CH, C-9), 114.5 (CH_2, C-12), 67.2 (CH, C-2), 51.4 (CH, C-7), 41.7 (CH_2, C-6), 40.4 (CH_2, C-1), 39.7 (C, C-5), 37.6 (CH_2, C-3), 36.7 (CH, C-4), 20.4 (CH_3, C-13),

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15.8 (CH_3, C-14), 15.0 (CH_3, C-15); HRESIMS m/z: $[M + H]^+$ calcd for $C_{15}H_{23}O_2$, 235.1698; found, 235.1696.

Preparation of the (*R*)- and (*S*)-MTPA Ester Derivative of 7..[19,23] Pyridine (100 μL) and (+)-(*R*)-MTPACl (40 μL) were added to a CH_2Cl_2 solution (300 μL) of **7** (5.8 mg). The reaction mixture was stirred at room temperature for 2 days. After removal of the solvent, the mixture was purified by PTLC using EtOAc-petroleum ether (1:4) (two runs) to afford the (*S*)-MTPA ester (11.3 mg, 97.4% yield). Compound **7** (2.5 mg) was treated in a similar way with (−)-(*S*)-MTPACl, and after purification by PTLC, (*R*)-MTPA ester (4.7 mg, 93.3% yield) was obtained.

Preparation of the (*R*)- and (*S*)-THENA Ester Derivative of 7 and 8.[20] The solution of acid chloride (*S*)-THENA-Cl, generated from (*S*)-THENA (21.3 mg, 0.11 mmol), in CH_2Cl_2 (200 μL), was added to the solution of **7** (2.6 mg, 0.010 mmol), pyridine (100 μL, 7.2 mmol), and a catalytic amount of dimethylaminopyridine in CH_2Cl_2 (300 μL). The reaction mixture was stirred at room temperature overnight. The reaction mixture was then quenched with saturated $NaHCO_3$ aqueous solution, and the crude mixture was extracted with CH_2Cl_2 (three times). The organic layer was dried over anhydrous $MgSO_4$ and evaporated to dryness. The crude product was purified by CC (silica gel; hexane-EtOAc 7:3 as an eluent) to give compound (*S*)-THENA-**7** (4.4 mg, 84% yield) as a white solid. Compound **7** (2.4 mg, 0.010 mmol) was treated in a similar way with (*R*)-THENA (23.6 mg, 0.12 mmol), and after purification by CC, (*R*)-THENA-**7** (3.9 mg, 95% yield) was obtained. Compound **8** was reacted with the same method as compound **7**, which **8** (2.2 mg, 0.009 mmol) was treated with (*S*)-THENA (42.6 mg, 0.22 mmol) to yield (*S*)-THENA-**8** (5.1 mg, 98% yield), and **8** (2.6 mg, 0.010 mmol) was treated with (*R*)-THENA (47.3 mg, 0.25 mmol) to give (*R*)-THENA-**8** (6.1 mg, 98% yield).

Antimycobacterial Assay. Antimycobacterial activity was determined against *gfp* recombinant *Mycobacterium tuberculosis* H37Ra (H37Ra *gfp*) using a green fluorescent protein (GFP)-based fluorescent detection method.[24] Briefly, 5 μL of each test compound serially diluted in 5% DMSO was mixed with 45 μL of H37Ra *gfp* suspended in Middlebrook 7H9 medium (approximately 2×10^4 cfu/well). The assay was performed in duplicate in 384-well plate format. Plates were incubated at 37 °C for 7 days, and the fluorescence signals were measured using SpectraMax M5 microplate reader (Molecular devices, U.S.A.) in the bottom-reading mode at the excitation and emission wavelengths of 485 and 535 nm. Fluorescence signals at day zero were used as background. The percentage of growth inhibition was calculated from the mean of fluorescence unit of the treated (FU_T) and untreated cells (FU_C) as the following equation: % inhibition = $[1 - (FU_T/FU_C)] \times 100$. The lowest concentration that inhibited cell growth by 90% was recorded as the minimum inhibitory concentration (MIC). Standard drugs rifampin, streptomycin, isoniazid, ofloxacin, and ethambutol were used as positive controls and gave MICs of 0.025, 0.625, 0.094, 0.391, and 0.983 μg/mL, respectively. DMSO (0.5%) was used as a negative control.

Antimalarial Assay. The activity was evaluated against the parasite *Plasmodium falciparum* K1(multidrug resistant stain) using the microculture radioisotope technique based on the method described by Desjardins et al.[25] Briefly, 25 μL of test solution in RPMI-1640 was mixed in microplate wells with 200 μL of a 1.5% erythrocyte suspension with 1% parasitaemia in the early ring stage and incubated in a CO_2 incubator (5% O_2, 5% CO_2, and 90%N_2) at 37 °C for 24 h. Subsequently, 25 μL of ^{3}H-hypoxanthine in culture medium (0.5 μCi) was added into each well. The plates were further incubated for an additional 24 h. Levels of labeled hypoxanthine incorporation indicating parasite growth were determined by the TopCount microplate scintillation counter (Packard, U.S.A.). The percentage of parasite growth was calculated from the signal count per minute of treated (CPM_T) and untreated conditions (CPM_U) by the following formula: % parasite growth = $CPM_T/CPM_U \times 100$. Inhibition concentration recorded as IC_{50} value was the concentration which indicates 50% reduction in parasite growth. Dihydroartemisinin and mefloquine were used as the standard antimalarial drugs with IC_{50} values of 1.26 nM and 0.03 μM, respectively.

Cytotoxicity Assays. The cytotoxicity assay against African green monkey kidney cell lines (Vero, ATCC CCL-81) was performed in triplicate employing the method described by Hunt and co-workers.[26] The GFP-expressing Vero cell line was generated in-house by stably transfecting the Vero cells with pEGFP-N1 plasmid (Clontech) and maintained in minimal essential medium supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 1.5 g/L sodium bicarbonate, and 0.8 mg/mL geneticin at 37 °C in an incubator with 5% CO_2. Vero cell suspension (45 μL, 3.3×10^4 cells/mL) was added into each well of 384-well plates containing 5 μL of test compounds. Plates were incubated in a CO_2 incubator at 37 °C for 4 days. Fluorescence signals were measured using SpectraMaxM5 microplate reader (Molecular Devices, U.S.A.), and the percentages of cytotoxicity were calculated in the same manner as for the anti-*Mycobacterial* assay. IC_{50} values were derived from dose−response curves using six concentrations of 2-fold serially diluted test compounds by the SOFTMax Pro software. Ellipticine was used as a positive control and gave the IC_{50} value of 3.80 μM.

The antiproliferative activities against human oral epidermoid carcinoma (KB) cell lines (ATCC CCL-17) and human breast adenocarcinoma MCF-7) cell lines (ATCC HTB-22) were evaluated using the resazurin microplate assay.[27] Briefly, 5 μL of each test compound diluted in 5%DMSO was mixed with 45 μL of KB suspension (2.2×10^4 cells/mL) and MCF-7 (3.3×10^4 cells/mL) in a 384-well plate and incubated at 37 °C in 5% CO_2 incubator 3 days. After incubation, cell growth was determined by adding 12.5 μL of 62.5 μg/mL resazurin solution and incubated further for 4 h. Fluorescence signals were measured using SpectraMax M5 microplate reader (Molecular devices, U.S.A.) in the bottom-reading mode at the excitation and emission wavelengths of 530 and 590 nm. The percentage of growth inhibition was calculated from the mean of fluorescence unit of the treated (FU_T) and untreated cells (FU_C) as the following equation: % inhibition = $[1 - (FU_T/FU_C)] \times 100$. The IC_{50} value was obtained from the dose response curve using the SOFTMax Pro software. Ellipticine and doxorubicin were used as positive controls for KB cells and gave IC_{50} values of 1.98 and 0.91 μM, respectively, whereas tamoxifen and doxorubicin were positive controls for MCF-7 cells with the IC_{50} values of 18.71 and 11.52 μM, respectively. DMSO (0.5%) was used as a negative control.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H and ^{13}C NMR spectra for penicilleremophilanes A (**1**) and B (**2**), penicillithiophenols A (**3**) and B (**4**), 8-epimer of **8** (**9**) and 7-hydroxy-4a,5-dimethyl-3-prop-1-en-2-yl-3,4,5,6,7,8-hexahydronaphthalen-2-one (**14**), optical rotations of known compounds, and yields of the isolated compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: vatcharin.r@psu.ac.th. Tel.: +66 74 288 435. Fax: +66 74 558 841.

Notes

The authors declare no competing financial interest.

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