JOURNAL OF NATURAL PRODUCTS

pubs.acs.org/jnp

Article

Antibacterial and Antifungal Polyketides from the Fungus *Aspergillus unguis* PSU-MF16

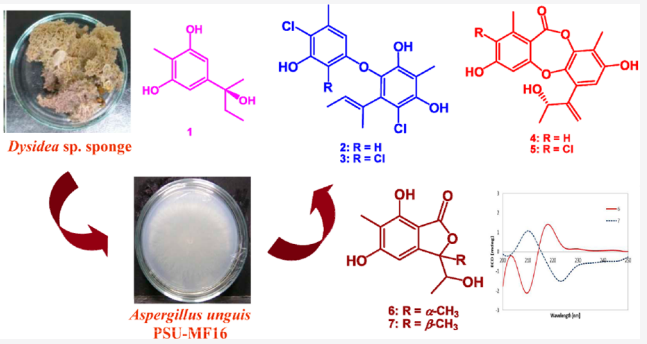
Praphatsorn Saetang, Vatcharin Rukachaisirikul,* Souwalak Phongpaichit, Sita Preedanon, Jariya Sakayaroj, Sarinya Hadsadee, and Siriporn Jungsuttiwong

Cite This: *J. Nat. Prod.* 2021, 84, 1498−1506

Read Online

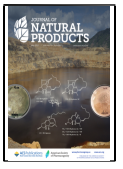
ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Seven new polyketides including a phenol (**1**), two diphenyl ethers (**2** and **3**), two depsidones (**4** and **5**), and two phthalides (**6** and **7**) were isolated from the fungus *Aspergillus unguis* PSU-MF16 along with 27 known compounds. Their structures were determined by extensive spectroscopic analysis. The absolute configurations of **1** and **4**−**7** were established using comparative analyses of calculated and experimental ECD spectra. Among the new metabolites, **2** exhibited the best antimicrobial activity against *Staphylococcus aureus*, methicillin-resistant *S. aureus*, and *Microsporum gypseum* with equal MIC values of 16 μg/mL. In addition, known emeguisin A displayed potent antimicrobial activity against *S. aureus*, methicillin-resistant *S. aureus*, and *Cryptococcus neoformans* with equal MIC values of 0.5 μg/mL, compared with the standard drugs, vancomycin and amphotericin B. The structure−activity relationship study of the isolated compounds for antimicrobial activity is discussed.



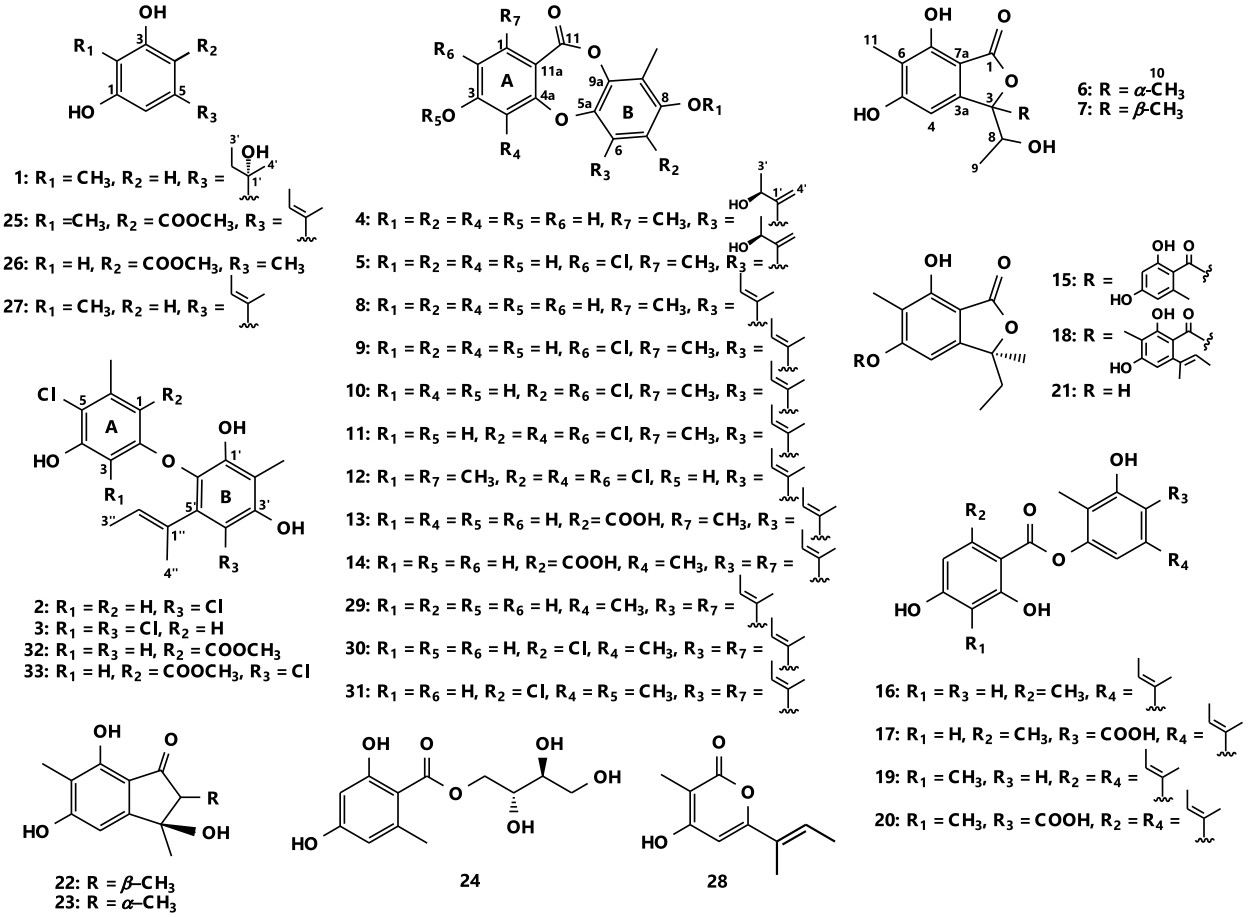
Previous investigations on secondary metabolites of the fungus *Aspergillus unguis* have provided unique types of polyketide metabolites including depsidones, depsides, diphenyl ethers, and phthalides.[1−5] The depsidone derivatives showed a wide range of biological activities such as antiaromatase,[2] antibacterial,[3] antimalarial,[3] antifungal,[5] and CFTR inhibitory[5] activities, whereas depside and diphenyl ether derivatives displayed antifungal[4] and antibacterial[5] activities, respectively. As part of our research on antimicrobial metabolites from marine-derived fungi, we chemically investigated the fungus *Aspergillus unguis* PSU-MF16 isolated from a sponge in the genus *Dysidea* collected from Koh Bulon Mai Pai, Satun Province, Thailand. The culture broth EtOAc extract displayed antimicrobial activities against *Staphylococcus aureus*, methicillin-resistant *S. aureus*, and *Cryptococcus neoformans* with MIC values of 16, 16, and 4 μg/mL, respectively. In addition, the mycelial EtOAc extract was active against *S. aureus*, methicillin-resistant *S. aureus*, *C. neoformans*, and *Candida albicans* with the respective MIC values of 8, 8, 4, and 128 μg/mL, whereas the mycelial hexane extract displayed MIC values of 4, 4, 4, and 128 μg/mL against the above human pathogens, respectively. In this study, we describe herein the isolation of one new phenol, aspergillusphenol C (**1**), and two new diphenyl ethers, aspergillusethers E (**2**) and F (**3**), together with 22 known compounds including seven depsidones, unguinol (**8**),[5,6] 2-chlorounguinol (**9**),[5,6] aspergillusidone C (**10**),[1,5] nornidulin (**11**),[5,6] nidulin (**12**),[5,6] aspergillusidone A (**13**),[1,5] and aspergisidone (**14**),[5] six depsides, asperlide (**15**),[3] aspergisides A−C (**16**−**18**),[5] and agonodepsides A (**19**)[7] and B (**20**),[7] one phthalide, (3*S*)-3-ethyl-5,7-dihydroxy-3,6-dimethylphthalide (**21**),[5,6] two indanones, asperunguisones A (**22**)[4] and B (**23**),[4] three orsellinic acid derivatives, (+)-montagnetol (**24**),[4,8] pilobolusate (**25**),[4] and methyl orsellinate (**26**),[4] two phenols, aspergillusphenol A (**27**)[2,4] and orcinol,[4] and one pyranone, 4-hydroxy-3-methyl-6-(1-methyl-1-propenyl)-2*H*-pyran-2-one (**28**),[1] from the broth extract of *A. unguis* PSU-MF16. Moreover, two new depsidones, asperunguissidones A (**4**) and B (**5**), and two new phthalides, asperunguislides A (**6**) and B (**7**), as well as five additional known compounds including three depsidones, folipastatin (**29**),[5,9] emeguisins A (**30**)[5,10] and B (**31**),[10] and two diphenyl ethers, aspergillusethers C (**32**)[4] and D (**33**),[4] were isolated from the mycelial EtOAc extract, whereas the mycelial hexane extract provided six previously isolated compounds (**8**−**10**, **29**, **30**, and **33**). The isolated compounds with sufficient quantity were evaluated for antibacterial (against *S. aureus* ATCC25923, methicillin-resistant *S. aureus*), antifungal (against *C. albicans* NCPF90028, *C. neoformans* ATCC90113, and *Microsporum*

Received: December 5, 2020
Published: April 16, 2021



https://doi.org/10.1021/acs.jnatprod.0c01308
J. Nat. Prod. 2021, 84, 1498−1506

Chart 1



gypseum, a clinical isolate) and cytotoxic (against African green-monkey fibroblasts and Vero cell lines) activities.

RESULTS AND DISCUSSION

The broth and mycelial extracts of *A. unguis* PSU-MF16 were purified using various chromatographic techniques, and the structures of the isolated compounds were elucidated on the basis of spectroscopic data including UV, IR, NMR, and MS. The relative configuration was assigned using NOEDIFF data, whereas the absolute configurations of **1** and **4**−**7** were determined by comparison of the experimental electronic circular dichroism (ECD) data with the calculated ones. For the known compounds, their structures were confirmed by comparison of the ^{1}H and ^{13}C NMR spectroscopic data as well as specific rotations with those previously reported.

Aspergillusphenol C (**1**) was obtained as a colorless gum with the molecular formula $C_{11}H_{16}O_3$ determined by the HRESIMS peak at *m*/*z* 197.1163 $[M + H]^+$. The IR spectrum showed hydroxy and double-bond absorption bands at 3385 and 1623 cm^{-1}, respectively. The UV spectrum exhibited absorption bands at 206 and 272 nm, indicating the presence of a benzene chromophore.[2] The ^{1}H and ^{13}C NMR spectroscopic data (Table 1) were similar to those of **27**[2] with the exception that proton signals for the 2-substituted-2-butenyl unit in **27** were replaced by signals for the 2-substituted-2-hydroxybutyl unit in **1**. The presence of signals for a hydroxy nonprotonated carbon (C-1′, δ_C 74.3) and a methylene carbon (C-2′, δ_C 37.7) in **1** instead of those for olefinic nonprotonated and olefinic methine carbons in **27** supported the above conclusion. The 2-substituted-2-hydroxybutyl unit was constructed on the basis of the COSY correlations of the methylene protons, H_2-2′ (δ_H 1.69), and the methyl protons, H_3-3′ (δ_H 0.75) (Figure 1), and the HMBC correlations from the methyl protons, H_3-4′ (δ_H 1.39), to C-1′ and C-2′ (Figure 1) as well as the chemical shift of C-1′ (Table 1). The HMBC correlations from H_2-2′ and H_3-4′ to C-5 (δ_C 148.4) confirmed the attachment of the 2-substituted-2-hydroxybutyl unit at this carbon. Accordingly, **1** was identified as a hydrated derivative of **27**. In order to determine the absolute configuration at C-1′, the calculated ECD spectra of both enantiomers were performed using time-dependent density functional theory (TD-DFT) calculations. The experimental ECD spectrum of **1** displayed a similar shape of curves and Cotton effects to those of the calculated ECD spectrum of the *S* isomer (Figure 2). These results established the absolute configuration of **1** to be 1′*S*.

Aspergillusether E (**2**) was obtained as a colorless gum and had the molecular formula $C_{18}H_{18}Cl_2O_4$, which was deduced from the HRESIMS peak at *m*/*z* 391.0483 $[M + Na]^+$. It exhibited UV absorption bands of a benzene chromophore at 207 and 284 nm,[2] while the IR spectrum showed absorption bands at 3447 and 1604 cm^{-1} for hydroxy and double-bond functional groups, respectively.[4] The ^{1}H NMR spectroscopic data (Table 1) were similar to those of **33**[4] except for the replacement of signals for an aromatic proton and a methoxy group of ring A in **33** with those for two *meta*-coupled aromatic protons (δ_H 6.27, H-1, and 6.22, H-3) in **2**. The replacement of signals for one methyl ester group in **33** with a

https://doi.org/10.1021/acs.jnatprod.0c01308
J. Nat. Prod. 2021, 84, 1498−1506

Table 1. ^{1}H and ^{13}C NMR Data for 1−3

	1[a]		2[a]		3[b]	
position	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1	156.7, C		110.2, CH	6.27, d (2.0)	109.0, CH	6.08, s
1-OH		7.86, brs				
2	109.0, C		158.7, C		152.8, C	
2-Me	8.5, CH_3	2.05, s				
3	156.7, C		102.5, CH	6.22, d (2.0)	107.9, C	
3-OH		7.86, brs				
4	104.8, CH	6.50, s	154.4, C		148.8, C	
4-OH				8.62, s		5.97, s
5	148.4, C		114.2, C		114.9, C	
6	104.8, CH	6.50, s	138.2, C		135.6, C	
6-Me			20.4, CH_3	2.26, s	20.2, CH_3	2.22, s
1′	74.3, C		148.6, C		146.8, C	
1′-OH				8.11, s		5.47, s
2′	37.7, CH_2	1.69, q (7.5)	112.8, C		111.2, C	
2′-Me			9.7, CH_3	2.19, s	9.3, CH_3	2.25, s
3′	8.8, CH_3	0.75, t (7.5)	149.6, C		148.1, C	
3′-OH				7.77, s		5.67, s
4′	30.6, CH_3	1.39, s	110.8, C		110.6, C	
5′			136.1, C		134.5, C	
6′			133.9, C		133.0, C	
1″			130.6, C		128.7, C	
2″			127.1, CH	5.22, qq (6.5, 1.5)	128.0, CH	5.27, q (7.0)
3″			13.7, CH_3	1.53, d (6.5)	13.7, CH_3	1.54, d (7.0)
4″			16.7, CH_3	1.68, s	16.4, CH_3	1.64, s

[a]Recorded at 500 and 125 MHz for ^{1}H and ^{13}C in acetone-d_6, respectively. [b]Recorded at 500 and 125 MHz for ^{1}H and ^{13}C in $CDCl_3$, respectively.

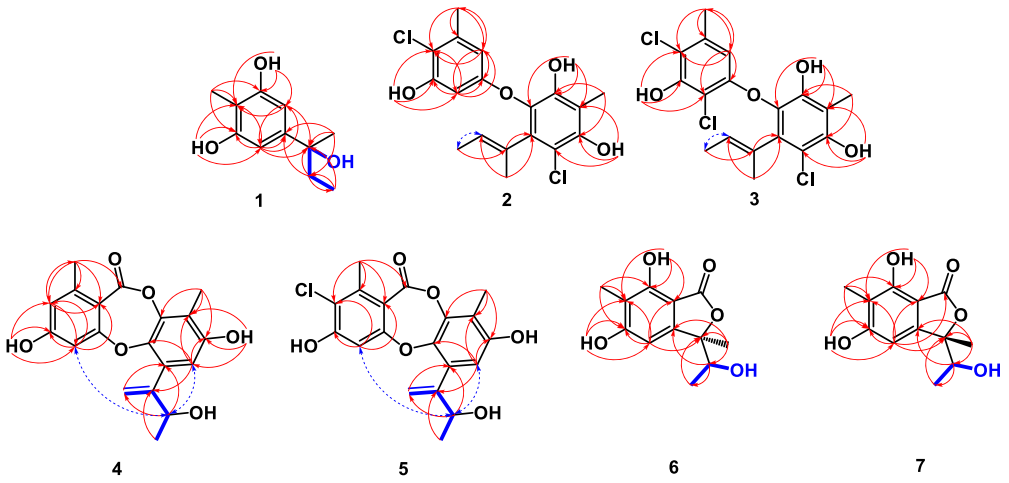


Figure 1. COSY (blue bars), key HMBC (red ↷), and NOEDIFF (blue ⇢) data of 1−7.

signal for an aromatic methine carbon in the ^{13}C NMR spectrum of 2 indicated that the methyl ester group at C-1 in 33 was replaced by an aromatic proton in 2. This assignment was confirmed by the COSY correlation of H-1 (δ_H 6.27) with H-3 (δ_H 6.22) and the HMBC correlations from H-1 with C-2 (δ_C 158.7), C-3 (δ_C 102.5), C-5 (δ_C 114.2), and 6-Me (δ_C 20.4) (Figure 1). An *E*-configuration of the 2-substituted-2-butenyl unit was assigned according to signal enhancement of H_3-3″, but not H_3-4″, upon irradiation of H-2′′ in the NOEDIFF experiment. Therefore, aspergillusether E had the structure 2.

Aspergillusether F (3) was obtained as a colorless gum with the molecular formula $C_{18}H_{17}Cl_3O_4$ determined by the HRESIMS peak at m/z 403.0253 $[M + H]^+$. The UV and IR spectra of 3 were similar to those of 2, indicating their identical chromophore and functional groups. The structural determination was accomplished by comparing its NMR spectroscopic data with those of 2. The ^{1}H NMR spectrum of 3 (Table 1) differed from that of 2 in the replacement of signals for the two *meta*-coupled aromatic protons on ring A in 2 with an aromatic proton signal of a pentasubstituted benzene resonating at δ_H 6.08 (s, 1H) in 3. This aromatic proton was assigned as H-1 based on its HMBC correlations with C-2 (δ_C 152.8), C-3 (δ_C 107.9), C-5 (δ_C 114.9), C-6 (δ_C 135.6), and 6-Me (δ_C 20.2) (Figure 1). A substituent at C-3 was assigned as a chlorine atom according to the chemical shift of C-3 and the molecular formula. Compound 3 displayed the NOEDIFF data (Figure 1) similar to those of 2, indicating the identical

https://doi.org/10.1021/acs.jnatprod.0c01308
J. Nat. Prod. 2021, 84, 1498−1506

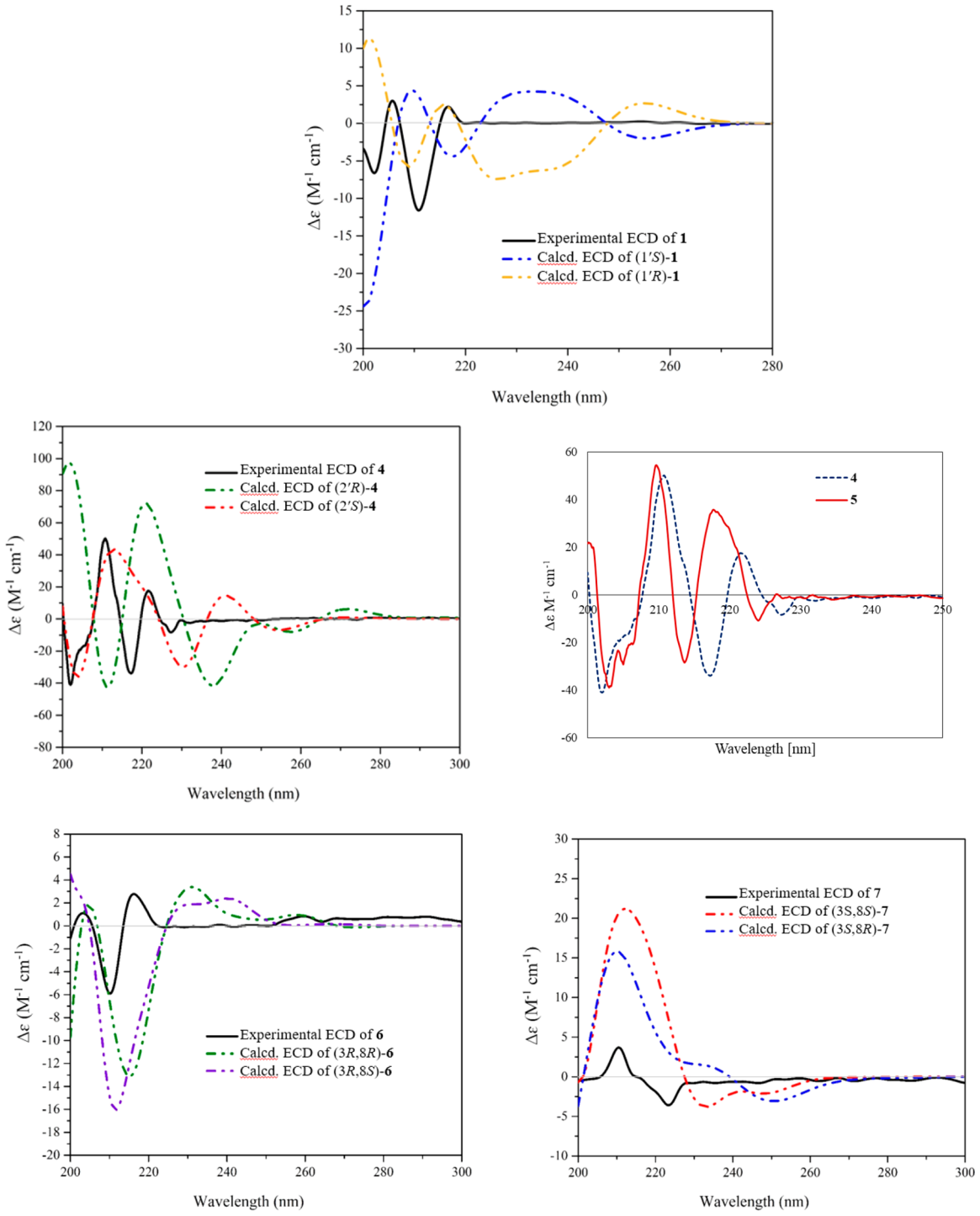


Figure 2. Experimental and calculated ECD spectra of **1**, **4**, **6**, and **7** and the experimental ECD spectrum of **5**.

configuration of the 2-substituted-2-butenyl unit. Therefore, **3** was identified as a 3-chloro derivative of **2**.

Asperunguissidone A (**4**) was isolated as a colorless gum and had the molecular formula $C_{19}H_{18}O_6$ on the basis of the HRESIMS peak at m/z 365.0998 $[M + Na]^+$. The UV absorption bands at 208 and 266 nm indicated the presence of a benzene chromophore. The IR spectrum displayed absorptions bands at 3357, 1707, and 1609 for hydroxy, ester carbonyl, and double-bond functional groups, respectively.[1] The ^1H and ^{13}C NMR (Table 2), HMBC, and COSY (Figure 1) data revealed that **4** had a similar depsidone structure to that of **8**.[5,6] The obvious difference in the ^1H NMR spectrum was the replacement of signals for the 2-substituted 2-butenyl unit in **8** with those for the 3-substituted-2-hydroxy-3-butenyl moiety in **4**. This unit was constructed on the basis of the COSY correlations of H-2′ (δ_H 4.80) with H_3-3′ (δ_H 1.18) (Figure 1) along with the HMBC correlations (Figure 1) from the geminal olefinic protons, H_{ab}-4′ (δ_H 5.63 and 5.03), and H_3-3′ to C-1′ (δ_C 152.1) and C-2′ (δ_C 70.0). The chemical shift of C-2′ indicated the attachment of a hydroxy group at this carbon. This unit was attached at C-6 (δ_C 132.7) of the depsidone skeleton because H-2′ and H_{ab}-4′

https://doi.org/10.1021/acs.jnatprod.0c01308
J. Nat. Prod. 2021, 84, 1498−1506

Table 2. ^{1}H and ^{13}C NMR Data for 4 and 5 in Acetone-d_6

	4[a]		5[b]	
position	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1	145.9, C		142.4, C	
1-Me	21.2, CH_3	2.40, s	18.5, CH_3	2.47, s
2	116.6, CH	6.63, d (2.1)	120.4, C	
3	162.6, C		158.1, C	
3-OH		9.51, s		
4	106.2, CH	6.56, d (2.1)	106.9, CH	6.83, s
4a	163.3, C		162.4, C	
5a	142.3, C		142.2, C	
6	132.6,C		132.7,C	
7	112.8, CH	6.58, s	112.9, CH	6.59, s
8	153.5, C		153.7, C	
8-OH		8.64, s		8.69, brs
9	117.0, C		117.0, C	
9-Me	9.6, CH_3	2.17, s	9.5, CH_3	2.17, s
9a	144.9, C		144.7, C	
11	164.7, C		163.1, C	
11a	113.8, C		115.6, C	
1′	152.1, C		151.9, C	
2′	70.0, CH	4.80, q (6.6)	70.0, CH	4.77, q (6.5)
3′	23.4, CH_3	1.18, d (6.6)	23.3, CH_3	1.18, d (6.5)
4′	114.0, CH_2	a: 5.63, brt (1.8)	114.3, CH_2	a: 5.63, brt (1.5)
		b: 5.03, dd (1.8, 0.9)		b: 5.03, s

[a]Recorded at 300 and 75 MHz for ^{1}H and ^{13}C, respectively. [b]Recorded at 500 and 125 MHz for ^{1}H and ^{13}C, respectively.

gave HMBC correlations with this carbon. Signal enhancement of H-4 and H-7 after irradiation of H-2′ in the NOEDIFF experiment (Figure 1) supported the assigned location. Based on the similar shape of curves and Cotton effects of the experimental ECD spectrum of **4** and the calculated ECD spectrum of the 2′-*S* isomer (Figure 2), asperunguissidone A had the depsidone structure **4** with an *S*-configuration at C-2′.

Asperunguissidone B (**5**) was isolated as a colorless gum and had the molecular formula $C_{19}H_{17}ClO_6$ on the basis of the HRESIMS peak at m/z 399.0607 $[M + Na]^+$. The UV and IR spectra were similar to those of **4**. In addition, their ^{1}H and ^{13}C NMR spectroscopic data (Table 2) were similar except that signals for two *meta*-coupled aromatic protons on ring A of **4** were replaced by a signal for an aromatic proton of a pentasubstituted benzene resonating at δ_H 6.83 (s, 1H) in **5**. This aromatic proton was assigned as H-4 on the basis of the HMBC correlations with C-2 (δ_C 120.4), C-3 (δ_C 158.1), C-4a (δ_C 162.4), and C-11a (δ_C 115.6) (Figure 1). The substituent at C-2 was assigned as a chlorine atom by comparison of the chemical shift of C-2 with that of previously reported chlorinated depsidones[6] as well as the molecular formula. The absolute configuration at C-2′ of **5** was assigned to be *S*, identical to that of **4**, on the basis of their similar experimental ECD data (Figure 2). Consequently, **5** was assigned as a 2-chloro derivative of **4**.

Asperunguislide A (**6**) was obtained as a colorless gum and had the molecular formula $C_{12}H_{14}O_5$ on the basis of the HRESIMS peak at m/z 261.0733 $[M + Na]^+$. The UV spectrum exhibited absorption bands at 216, 262, and 296 nm, indicating the presence of a conjugated carbonyl chromophore.[11] The IR spectrum showed absorption bands for hydroxy, ester carbonyl, and double-bond functional groups at 3400, 1719, and 1618 cm^{-1}, respectively.[14] The ^{1}H NMR spectroscopic data (Table 3) contained signals for two hydroxy

Table 3. ^{1}H and ^{13}C NMR Data for 6 and 7 in Acetone-d_6

	6[a]		7[b]	
position	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1	172.0, C		172.0, C	
3	90.6, C		90.8, C	
3a	152.0, C		152.6, C	
4	102.2, CH	6.63, s	101.9, CH	6.61, s
5	163.8, C		163.7, C	
5-OH		9.45, s		9.46, brs
6	111.7,C		111.7,C	
7	156.1, C		156.2, C	
7-OH		8.16, s		8.19, brs
7a	104.9, C		104.4, C	
8	72.0, CH	4.02, quint (6.0)	72.0, CH	3.90, quint (6.3)
8-OH		4.29, brd (6.0)		4.23, d (6.3)
9	17.9, CH_3	1.04, d (6.0)	18.1, CH_3	1.11, d (6.3)
10	23.2, CH_3	1.59, s	21.6, CH_3	1.57, s
11	7.9, CH_3	2.08, s	7.8, CH_3	2.08, s

[a]Recorded at 500 and 125 MHz for ^{1}H and ^{13}C, respectively. [b]Recorded at 300 and 75 MHz for ^{1}H and ^{13}C, respectively.

protons (δ_H 9.45 and 8.16 each s, 1H), one aromatic proton of a pentasubstituted benzene (δ_H 6.63, s, 1H), one 1-substituted ethanol unit [δ_H 4.02 (quint, J = 6.0 Hz, 1H), 4.29 (brd, J = 6.0 Hz, 1H), and 1.04 (d, J = 6.0 Hz, 3H)], and two methyl groups (δ_H 2.08 and 1.59, each s, 3H). The ^{13}C NMR spectrum (Table 3) displayed signals for one ester carbonyl (δ_C 172.0), six nonprotonated (δ_C 163.8, 156.1, 152.0, 111.7, 104.9, and 90.6), two methine (δ_C 102.2 and 72.0), and three methyl (δ_C 23.2, 17.9, and 7.9) carbons. These results together with the HMBC correlations (Figure 1) suggested that **6** had a similar phthalide skeleton to that of **21**[5,6] with the presence of the 1-substituted ethanol unit instead of an ethyl group in **21**. A methylene carbon resonance in the ^{13}C NMR spectrum in **21** was replaced by one oxymethine carbon resonance at δ_C 72.0 in **6**, supporting the above conclusion. The COSY correlation of the hydroxymethine proton (H-8, δ_H 4.02) with H_3-9 (δ_H 1.04) and the HMBC cross-peaks of H-8 with C-3a (δ_C 152.0) (Figure 1) confirmed the presence of the 1-substituted ethanol unit and further established the attachment of the this unit at C-3. The determination of the absolute configuration at C-8 by the modified Mosher's method was not performed because of insufficient material. In the ECD spectrum, **6** displayed a negative Cotton effect ($\Delta\varepsilon$ −2.1) at 210 nm (Figure 2) which had an opposite sign to that of **15** (211 nm, $\Delta\varepsilon$ +28.21),[3] **18** (213 nm, $\Delta\varepsilon$ +5.51),[5] and **21** (213 nm, $\Delta\varepsilon$ +5.84).[5] These results suggested an *R*-configuration at C-3. Accordingly, the absolute configuration of **6** would be either 3*R*,8*R* or 3*R*,8*S*. The ECD calculations afforded the calculated spectra that did not allow for definitive assignment of the configuration at C-8 (Figure 2). Based on these data, the absolute configuration at C-8 remained unidentified.

Asperunguislide B (**7**) was obtained as a colorless solid. It had an identical molecular formula to that of **6** based on the HRMS peak at m/z 261.0737 $[M + Na]^+$. The UV and IR absorption bands were similar to those of **6**. The ^{1}H, and ^{13}C NMR (Table 3), COSY, and HMBC (Figure 1) data of **7** indicated that **7** had an identical planar structure to **6**. The absolute configuration at C-3 was determined to be *S* on the

https://doi.org/10.1021/acs.jnatprod.0c01308
J. Nat. Prod. 2021, 84, 1498−1506

Table 4. Antibacterial, Antifungal, and Cytotoxic Activities of 2, 4, 6−21, 24−27, and 29−33

	antibacterial activity (MIC, μg/mL)		antifungal activity (MIC, μg/mL)			cytotoxicity (IC_{50}, μM)
compound	*S. aureus*	methicillin-resistant *S. aureus*	*C. albicans*	*C. neoformans*	*M. gypseum*	Vero
2	16	16	32	32	16	130
4	64	64	–[i]	–	–	ND[j]
6	–	–	–	–	200	ND
7	–	–	–	–	–	ND
8[a]	128	–	–	200	–	–
9[a]	8	8	8	32	8	86
10[a]	2	1	200	128	2	8.9
11[a]	2	2	–	8	–	110
12[a]	4	8	–	16	–	57
13[a]	–	–	–	200	–	–
14[a]	32	64	–	64	200	–
15	200	200	–	32	200	–
16[a]	8	8	200	64	128	84
17[a]	128	128	–	32	–	110
18[a]	200	200	–	200	–	45
19	2	2	–	32	32	38
20	8	16	128	32	8	–
21[a]	2	4	128	32	8	40
24[b]	–	–	–	200	–	–
25[b]	64	64	200	64	128	77
26[b]	–	–	–	200	–	–
27[b]	16	8	–	128	128	130
29[a]	2	1	–	1	–	17
30[a]	0.5	0.5	–	0.5	–	16
31	–	–	–	–	–	ND
32[b]	64	64	128	64	128	36
33[b]	64	128	16	8	–	110
control	0.5[c]	1.0[d]	0.5[e]	2.0[f]	2.0[g]	4.0[h]

[a]Values from ref 5. [b]Values from ref 4. [c]*S. aureus*: vancomycin; MIC 0.25 μg/mL for **8**−**14**, **16**−**19**, **21**, **24**−**27**, **29**, **30**, **32**, and **33**. [d]Methicillin *S. aureus*: vancomycin; MIC 0.5 μg/mL for **4**, **8**−**15**, **16**−**18**, **20**, **21**, **24**−**27**, **29**, **30**, **32**, and **33**; MIC 0.25 μg/mL for **19**. [e]*C. albicans*: amphotericin B; MIC 0.25 μg/mL for **4**, **8**−**14**, **16**−**18**, **21**, **24**−**27**, **29**, **30**, **32**, and **33**.[f]*C. neoformans*: amphotericin B; MIC 0.5 μg/mL for **4**, **15**, and **20**; MIC 0.25 μg/mL for **8**−**14**, **16**−**19**, **21**, **24**−**27**, **29**, **30**, **32**, and **33**. [g]*M. gypseum*: clotrimazole; MIC 1.0 μg/mL for **8**−**14**, **16**−**18**, **21**, **24**−**27**, **29**, **30**, **32**, and **33**; MIC 0.5 μg/mL for **4**, **15**, and **20**; MIC 0.25 μg/mL for **19**. [h]Vero: ellipticine; IC_{50} 4.6 μM for **8**−**14**, **16**−**18**, **21**, **24**−**27**, **29**, **30**, **32**, and **33**; IC_{50} 5.5 μM for **15** and **20**. [i]– = inactive at the concentration of 50 μg/mL. [j]ND = not determined.

basis of a positive Cotton effect at 210 nm in the ECD spectrum (Figure 2), the same sign as that of **15**, **18**, and **21**. Consequently, the absolute configuration of **7** would be either 3*S*,8*S* or 3*S*,8*R*. As **6** and **7** were separable by normal-phase PTLC, they were diastereomers, not a pair of enantiomers. Attempts to prepare the Mosher's esters resulted in the decomposition of material. The ECD calculations provided inadequate data for the assignment of the absolute configuration at C-8 (Figure 2). Therefore, **7** is the C-3 epimer of **6**, but the C-8 configuration remains unassigned.

The antimicrobial activities of compounds **8**−**14**, **16**−**18**, **21**, **24**−**27**, **29**, **30**, **32**, and **33** against *S. aureus* ATCC25923, methicillin-resistant *S. aureus*, *C. albicans* NCPF90028, *C. neoformans* ATCC90113, and *M. gypseum* were previously reported by our research group (Table 4).[4,5] In addition, these compounds were noncytotoxic to Vero cells (Table 4).[4,5] Accordingly, compounds **2**, **4**, **6**, **7**, **15**, **19**, **20**, and **31** with sufficient amount were evaluated for antimicrobial activity against the same pathogens (Table 4). Among the diphenyl ether derivatives (**2**, **32**, and **33**), **2**, without the methyl ester group at C-1, showed stronger activity against *S. aureus*, methicillin-resistant *S. aureus*, and *M. gypseum* than the corresponding methyl ester **33**[4] with equal MIC values of 16 μg/mL. In contrast, **2** was 2- and 4-fold less active than **33** against *C. albicans* and *C. neoformans*, respectively.[4] It is worth noting that the nonchlorinated diphenyl ether **32** was less active against *C. albicans* and *C. neoformans*, but more active against methicillin-resistant *S. aureus* and *M. gypseum* than **33**.[4] These results indicated that the methyl ester group at C-1 and the chlorine atom at C-4′ played an important role for antimicrobial activity.

For the phthalides (**6**, **7**, **15**, **18**, and **21**), compound **6**, the 8-hydroxy derivative of **21**, and **7**, the C-3 epimer of **6**, were inactive in antimicrobial assays. Compound **21** displayed interesting antibacterial (MIC values 2−4 μg/mL) and weak to moderate antifungal (MIC values 8−128 μg/mL) activities.[5] In addition, **15** and **18**,[5] the benzoate derivatives of **21**, displayed much weaker antimicrobial activity than **21**. Based on these data, a hydroxy group at C-5, but not the one at C-8, enhanced the activity.

Among the tested depside derivatives (**16**, **17**, **19**, and **20**), **19** displayed the strongest activity against *S. aureus* and methicillin-resistant *S. aureus* with equal MIC values of 2 μg/mL, whereas **20**, the carboxy derivative of **19**, was the most active against *M. gypseum*, with an MIC value of 8 μg/mL. In addition, **16** displayed much better activity against all tested pathogens, except for *C. neoformans*, than the corresponding carboxy derivative **17**.[5] However, **16** was less active against all

https://doi.org/10.1021/acs.jnatprod.0c01308
J. Nat. Prod. 2021, 84, 1498−1506

tested pathogens, except for *C. albicans*, than **19**. These results indicated that the antimicrobial activity of these depsides was controlled by the presence of a carboxyl moiety, the position of the methyl group on ring A, and the number of 2-substituted 2-butenyl units.

The tested depsidones can be divided into two types. The first type is 1-methyl-6-(2-methylbut-2-enyl)depsidone derivatives (**4** and **8**−**13**), whereas the second type is 4-methyl-1,6-di(2-methylbut-2-enyl)depsidone derivatives (**14** and **29**−**31**). All metabolites of the first depsidone type, except for nonchlorinated **4**, **8**, and **13**, displayed significant antibacterial activity against both bacterial strains with the MIC values in the range of 1−8 μg/mL.[5] These results revealed that the chlorine substituents at C-2, C-4, and C-7 dramatically enhanced antibacterial activity. Interestingly, an additional chlorine atom at C-4 in **11** and **12** remarkably increased the antifungal activity against *C. neoformans* when compared with **10**. Moreover, **4** differing from **8** in the presence of the 3-substituted-2-hydroxy-3-butenyl moiety instead of the 2-methylbut-2-enyl unit, was active against methicillin-resistant *S. aureus* and displayed 2-fold more activity than **8** against *S. aureus*. In contrast, the replacement of H-7 in **8** with a carboxyl group in **13** resulted in no antibacterial activity. For the second depsidone type, the nonchlorinated **29** was much more potent than **8** from the first type against *S. aureus*, methicillin-resistant *S. aureus*, and *C. neoformans* with the respective MIC values of 2, 1, and 1 μg/mL.[5] But **14**, the carboxyl derivative of **29**, was 16-, 64-, and 64-fold less active than **29** against the same pathogens, respectively. Again, **30**, the 7-chloro derivative of **29**, exhibited much better antibacterial and antifungal activities against the above pathogens than **29** with equal MIC values of 0.5 μg/mL.[5] Additionally, the disappearance of antimicrobial activity of **31**, the corresponding 3-methoxy derivatives of **30**, suggested the importance of a 3-hydroxy group in these activities.

Finally, the antimicrobial **2**, **15**, **19**, and **20**, which were obtained in sufficient amount, were tested for cytotoxic activity against the Vero cell line. Compounds **15** and **20** were inactive at the concentration of 50 μg/mL, whereas compounds **2** and **19** were considered to be noncytotoxic to Vero cells with IC_{50} values of >10 μM.

In summary, the fungus *A. unguis* PSU-MF16 produced seven new (**1**−**7**) and 27 known polyketides. Depsidone derivatives are obtained as major metabolites. There are only two reports on the isolation of chlorinated diphenyl ether derivatives from *A. unguis*,[1,4] while the depsidone derivatives bearing a 3-substituted-2-hydroxy-3-butenyl moiety at C-6 have never been reported. Accordingly, the diphenyl ether (**2** and **3**) and depsidone (**4** and **5**) derivatives are rare fungal metabolites. Among the tested polyketides, **10**, **11**, **19**, **29**, and **30** displayed interesting antibacterial activity against *S. aureus* and methicillin-resistant *S. aureus* with MIC values in the range 0.5−2 μg/mL. Compounds **29** and **30** were also strongly active against *C. neoformans* with respective MIC values of 1 and 0.5 μg/mL. Interestingly, among the tested compounds, **10** showed the strongest antifungal activity against *M. gypseum* with the MIC value of 2 μg/mL. In addition, antimicrobial activities of known compounds **15**, **19**, and **20** are reported for the first time.

EXPERIMENTAL SECTION

General Experimental Procedures. The specific rotations were performed with a JASCO P-2000 polarimeter. The ultraviolet absorption spectra were measured in MeOH on a Shimadzu UV-2600 UV−vis spectrophotometer. ECD spectra were recorded on a JASCO J-815 polarimeter. The infrared spectra were recorded neat using a PerkinElmer 783 FTS165 FT-IR spectrometer. The ^{1}H and ^{13}C NMR spectra were recorded on a 300 or a 500 MHz Bruker FTNMR Ultra Shield spectrometer using tetramethylsilane as an internal standard. ESI-TOF mass spectra were measured on a TOF/Q-TOF mass spectrometer. Thin-layer chromatography (TLC) and preparative TLC (PTLC) were performed on silica gel 60 GF_{254} (Merck). Column chromatography (CC) was carried out on Sephadex LH-20, silica gel (Merck) type 60 (230−400 mesh ASTM) or type 100 (70−230 mesh ASTM), or reversed-phase C_{18} silica gel.

Fungal Material. The fungus PSU-MF16 was isolated from a sponge in the genus *Dysidea* that was collected from Koh Bulon Mai Pai, Satun Province, Thailand. This fungus was deposited as BCC83151 at BIOTEC Culture Collection (BCC), National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand. The fungus PSU-MF16 was identified based on its morphological and molecular characteristics. Colonies on potato dextrose agar at 25 °C grew fast, reaching 4.2 cm in 7 days. Colony color is green. It produced a globose conidial head bearing phialides, which are the characteristics of the genus *Aspergillus*.[12] The molecular analysis of the rDNA sequence of the internal transcribed spacers (GenBank accession no. KY397987) through the BLAST search from GenBank revealed that PSU-MF16 matched with several nucleotides of *Aspergillus unguis* strains comprising *A. unguis*, KU866670, KU866641, KU866620, KU866616, and KU866612 (100% nucleotide similarity). Therefore, this fungus was then identified to be *A. unguis*.

Fermentation, Extraction, and Purification. The fermentation and extraction of the fungus *A. unguis* PSU-MF16 were carried out using the same procedure as described previously.[13] The broth EtOAc extract (1.7 g, brown gum) was subjected to CC over Sephadex LH-20 using MeOH as a mobile phase to obtain nine fractions (A1−A9). Fraction A5 (90.3 mg) was subjected to CC over silica gel using a gradient of $MeOH/CH_2Cl_2$ (1:99−100:0) as a mobile phase to yield six subfractions (A5A−A5F). Purification of subfraction A5B (38.2 mg) using PTLC with a mixture of $MeOH/CH_2Cl_2$ (1:99) (two runs) afforded **18** (7.2 mg) and **21** (18.5 mg). Purification of subfraction A5D (5.0 mg) was performed by PTLC using a mixture of acetone/hexane (3:7) (five runs) to afford **28** (2.0 mg). Subfraction A5E (8.7 mg) was purified by CC over reversed-phase C_{18} silica gel using a gradient of $MeOH/H_2O$ (3:2−5:0) to afford five subfractions (A5E1−A5E5). Subfraction A5E2 contained **1** (1.2 mg). Compound **22** (1.4 mg) was obtained from subfraction A5E4 (2.6 mg) after purification by PTLC using a mixture of acetone/hexane (1:4) (eight runs). Purification of fraction A6 (192.4 mg) using the same procedure as fraction A5 gave eight subfractions (A6A−A6H). Subfraction A6G (15.3 mg) was purified by CC over reversed-phase C_{18} silica gel with a gradient of $MeOH/H_2O$ (3:2−5:0) to afford **23** (2.3 mg). Using the same procedure as fraction A5, fraction A8 (464.1 mg) gave 10 subfractions (A8A−A8J). Purification of subfraction A8A (22.8 mg) was performed by flash column chromatography (FCC) over silica gel with a mixture of acetone/hexane (1:4) to afford eight subfractions (A8A1−A8A8). Subfraction A8A1 (13.1 mg) yielded **25** (7.4 mg) upon purification on PTLC using a mixture of acetone/hexane (1:4) (seven runs). Subfractions A8A2 and A8A8 contained **3** (1.4 mg) and **12** (3.0 mg), respectively. Compound **2** (3.5 mg) was obtained from subfraction A8A4 (4.6 mg) using PTLC with a mixture of acetone/hexane (1:4) (five runs). Using the same purification procedure as subfraction A8A, subfraction A8C (47.1 mg) yielded four subfractions (A8C1−A8C4). Subfraction A8C2 (29.6 mg) was purified by FCC over silica gel using a mixture of acetone/hexane (1:4) followed by PTLC with a mixture of EtOAc/hexane (1:4) (five runs) to give **19** (3.5 mg). Purification of subfraction A8E (90.0 mg) by dissolving with $CHCl_3$ afforded a soluble subfraction (12.6 mg), which was further purification by PTLC with a mixture of acetone/hexane (1:4) (eight runs) to give **15** (6.1 mg). Subfraction A8F (14.9 mg) was dissolved with $CHCl_3$ to give a soluble subfraction, which

https://doi.org/10.1021/acs.jnatprod.0c01308
J. Nat. Prod. 2021, 84, 1498−1506

contained **8** (8.5 mg). Subfraction A8H (27.5 mg) was purified by CC over reversed-phase C_{18} silica gel with a gradient of MeOH/H_2O (3:2) followed by PTLC with a mixture of EtOAc/hexane (1:4) (four runs) to give orcinol (5.8 mg). Subfraction A8I (91.0 mg) was fractionated using the same procedure as subfraction A6G to give eight subfractions (A8I1−A8I8). Subfraction A8I1 (11.2 mg) gave **24** (6.2 mg) upon purification on PTLC using a mixture of CH_2Cl_2/EtOAc/MeOH (8:1:1) (two runs). Subfraction A8I3 contained **14** (6.3 mg), while subfraction A8I4 (6.0 mg) was subjected to PTLC with a mixture of MeOH/CH_2Cl_2 (1:49) (three runs) to afford **27** (1.8 mg). Using the same procedure as subfraction A8I, subfraction A8I7 (18.3 mg) provided **20** (4.8 mg). Fraction A9 (561.7 mg) was purified using the same procedure as fraction A5 to give nine subfractions (A9A−A9I). Compound **10** (18.4 mg) was obtained from subfraction A9A (79.0 mg) upon purification on FCC over silica gel with a mixture of EtOAc/CH_2Cl_2 (1:1). Subfraction A9B (38.4 mg) was fractionated by FCC over silica gel with a mixture of acetone/hexane (1:4) to provide **11** (5.2 mg). Compounds **9** (19.9 mg) and **26** (5.8 mg) were obtained from subfraction A9C (176.9 mg) upon purification by CC over silica gel using the same eluting system as subfraction A9B. Purification of subfraction A9H (157.5 mg) by the same procedure as subfraction A6G yielded eight subfractions (A9H1−A9H8). Subfraction A9H2 (19.8 mg) afforded **13** (7.6 mg) upon purification by CC over reversed-phase C_{18} silica gel with a mixture of MeOH/H_2O (1:1). Compound **17** (2.6 mg) was obtained from subfraction A9H5 (61.9 mg) upon purification by CC over reversed-phase C_{18} silica gel with a mixture of MeOH/H_2O (3:2) followed by CC over reversed-phase C_{18} silica gel with a mixture of MeOH/H_2O (2:3). Subfraction A9H8 contained **16** (2.8 mg).

The mycelial EtOAc extract (7.4 g, yellow solid) was purified by CC over Sephadex LH-20 using pure MeOH as a mobile phase to give four fractions (B1−B4). Fraction B2 (162.9 mg) was purified by the same procedure as fraction A5 to give eight subfractions (B2A−B2H). Subfraction B2H (31.2 mg) was purified by CC over reversed-phase C_{18} silica gel using a gradient of MeOH/H_2O (6:4−10:0) followed by PTLC with a mixture of hexane/EtOAc/MeOH (20:9:1) as a mobile phase (nine runs) to give **6** (3.5 mg) and **7** (3.8 mg). Purification of fraction B3 (5.5 g) using the same procedure as fraction B2 yielded 12 subfractions (B3A−B3L). Compound **31** (3.2 mg) was obtained from subfraction B3A (20.3 mg) upon purification on CC over silica gel with a mixture of EtOAc/hexane (1:9) followed by PTLC using a mixture of EtOAc/hexane (1:19) (four runs). Subfraction B3D (369.8 mg) was subjected to CC over reversed-phase C_{18} silica gel using a gradient of MeOH/H_2O (7:3−10:0) followed by FCC over silica gel using a mixture of EtOAc/hexane (1:9) to yield **33** (30.9 mg). Subfraction B3E (844.1 mg) was purified by dissolving with hexane to yield a soluble subfraction (55.2 g), which was further purified using CC over reversed-phase C_{18} silica gel with a gradient of MeOH/H_2O (7:3−10:0) to afford five subfractions (B3E1A−B3E1E). Subfraction B3E1E (108.1 mg) was fractionated by FCC over silica gel with a mixture of hexane/CH_2Cl_2 (3:7) to give **30** (25.9 mg). Subfraction B3F (835.7 mg) was subjected to CC over silica gel using the same procedure as fraction B2 to yield five subfractions (B3F1−B3F5). Compound **29** (33.7 mg) was obtained from subfraction B3F4 (606.4 mg) after purification by CC over silica gel with a mixture of MeOH/CH_2Cl_2 (1:99−100:0), subsequent FCC over silica gel with a mixture of acetone/hexane (1:4), followed by CC over reversed-phase C_{18} silica gel with a gradient of MeOH/H_2O (7:3−10:0). Subfraction B3H (123.5 mg) was further purified by CC over reversed-phase C_{18} silica gel using a mixture of MeOH/H_2O (3:2) followed by PTLC with a mixture of EtOAc/hexane (1:3) to give **32** (6.5 mg). Subfraction B3J (55.2 mg) was fractionated by CC over reversed-phase C_{18} silica gel with a gradient of MeOH/H_2O (1:1−2:0) to provide six subfractions (B3J1−B3J6). Compounds **4** (2.7 mg) and **5** (1.7 mg) were obtained from subfractions B3J3 (10.1 mg) and B3J5 (5.0 mg), respectively, after purification by PTLC with a mixture of CH_2Cl_2/hexane/MeOH (25:24:1) (five runs).

The mycelial hexane extract (1.5 g, pale yellow gum) was purified by various chromatographic techniques to give **8**−**10**, **29**, **30**, and **33** (see Supporting Information).

Aspergillusphenol C (**1**): colorless gum; $[\alpha]_D^{26}$ −3.7 (*c* 1.09, MeOH)); UV (MeOH) λ_{max} nm (log ε) 206 (4.19), 272 (3.13); ECD (MeOH, 0.39 mM) λ_{max} ($\Delta\varepsilon$) 202 (−6.7), 206 (+2.8), 211 (−11.7), 217 (+2.0) nm; IR (neat) ν_{max} cm^{-1}: 3385, 1623; ^1H and ^{13}C NMR data, Table 1; HRESIMS *m*/*z* $[M + H]^+$ 197.1163 (calcd for $C_{11}H_{17}O_3$, 197.1172).

Aspergillusether E (**2**): colorless gum; UV (MeOH) λ_{max} nm (log ε) 207 (5.00), 284 (4.03); IR (neat) ν_{max} cm^{-1} 3447, 1604; ^1H and ^{13}C NMR data, Table 1; HRESIMS *m*/*z* $[M + Na]^+$ 391.0483 (calcd for $C_{18}H_{18}Cl_2O_4Na$, 391.0474).

Aspergillusether F (**3**): colorless gum; UV (MeOH) λ_{max} nm (log ε) 208 (4.59), 284 (3.58); IR (neat) ν_{max} cm^{-1} 3432, 1607; ^1H and ^{13}C NMR data, Table 1; HRESIMS *m*/*z* $[M + H]^+$ 403.0253 (calcd for $C_{18}H_{18}Cl_3O_4$, 403.0265).

Asperunguissidone A (**4**): colorless gum; $[\alpha]_D^{26}$ +67.3 (*c* 0.1, MeOH); UV (MeOH) λ_{max} nm (log ε) 208 (4.28), 266 (3.74); ECD (MeOH, 0.16 mM) λ_{max} ($\Delta\varepsilon$) 202 (−40.9), 210 (+49.4), 216 (−33.9), 221 (+16.2), 227 (−8.2) nm; IR (neat) ν_{max} cm^{-1} 3357, 1707, 1609; ^1H and ^{13}C NMR data, Table 2; HRESIMS *m*/*z* $[M + Na]^+$ 365.0998 (calcd for $C_{19}H_{18}O_6Na$, 365.0996).

Asperunguissidone B (**5**): colorless gum; $[\alpha]_D^{26}$ +39.3 (*c* 0.1, MeOH); UV (MeOH) λ_{max} nm (log ε) 208 (4.58), 266 (3.89); ECD (MeOH, 0.16 mM) λ_{max} ($\Delta\varepsilon$) 203 (−39.0), 210 (+54.4), 214 (−28.4), 218 (+34.8), 224 (−10.6) nm; IR (neat) ν_{max} cm^{-1} 3400, 1708, 1597; ^1H and ^{13}C NMR data, Table 2; HRESIMS *m*/*z* $[M + Na]^+$ 399.0607 (calcd for $C_{19}H_{17}ClO_6Na$, 399.0606).

Asperunguislide A (**6**): colorless gum; $[\alpha]_D^{26}$ +252.6 (*c* 0.05, MeOH); UV (MeOH) λ_{max} nm (log ε) 216 (4.40), 262 (4.25), 296 (3.80); ECD (MeOH, 2.0 mM) λ_{max} ($\Delta\varepsilon$) 210 (−2.1), 218 (+1.4) nm; IR (neat) ν_{max} cm^{-1} 3400, 1719, 1618; ^1H and ^{13}C NMR data, Table 3; HRESIMS *m*/*z* $[M + Na]^+$ 261.0733 (calcd for $C_{12}H_{14}O_5Na$, 261.0733).

Asperunguislide B (**7**): colorless solid; $[\alpha]_D^{26}$ +57.5 (*c* 0.29, MeOH); UV (MeOH) λ_{max} nm (log ε) 216 (4.23), 262 (4.06), 296 (3.64); ECD (MeOH, 2.0 mM) λ_{max} ($\Delta\varepsilon$) 210 (+1.1), 223 (−1.5) nm; IR (neat) ν_{max} cm^{-1} 3284, 1719, 1618; ^1H and ^{13}C NMR data, see Table 3; HRESIMS *m*/*z* $[M + Na]^+$ 261.0737 (calcd for $C_{12}H_{14}O_5Na$, 261.0733).

TD-DFT Calculations of the ECD Spectra. The optimized structure was performed by the DFT method at the B3LYP/6-311G(d,p) level of theory. The minima of structures using vibrational analysis at the B3LYP/6-311G(d,p) level of theory resulted in no imaginary frequencies. ECD spectra was carried out using the TD-DFT method at the B3LYP/6-311++G(d,p) level. The rotary strengths of 60 excited states were determined. All calculations were performed using the Gaussian09 program package. A Gaussian band shape with a bandwidth of 0.20 eV was used to simulate the ECD spectra. The ECD curves were generated by the software SpecDis 1.64 (University of Wurzburg, Wurzburg, Germany).[14]

Antimicrobial Assay. Antimicrobial activity was determined as described by the Clinical and Laboratory Standards Institute.[15] The positive controls were vancomycin for both *S. aureus* and methicillin-resistant *S. aureus*, amphotericin B for *C. albicans* and *C. neoformans*, and clotrimazole for *M. gypseum*.

Cytotoxicity Assay. Cytotoxic activity against African green monkey kidney fibroblast (Vero) cells was assessed employing the colorimetric method.[16] Ellipticine was used as a positive control.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.0c01308.

^1H and ^{13}C NMR spectra of compounds **1**−**7**; purification of compounds **8**−**30** from the culture broth and mycelial extracts (PDF)

https://doi.org/10.1021/acs.jnatprod.0c01308
J. Nat. Prod. 2021, 84, 1498−1506

AUTHOR INFORMATION

Corresponding Author

Vatcharin Rukachaisirikul − *Division of Physical Science and Center of Excellence for Innovation in Chemistry, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand;* orcid.org/0000-0003-1517-1526; Email: vatcharin.r@psu.ac.th

Authors

Praphatsorn Saetang − *Division of Physical Science and Center of Excellence for Innovation in Chemistry, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand*

Souwalak Phongpaichit − *Division of Biological Science, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand*

Sita Preedanon − *National Center for Genetic Engineering and Biotechnology (BIOTEC), Klong Luang, Pathumthani 12120, Thailand*

Jariya Sakayaroj − *School of Science, Walailak University, Thasala, Nakhonsithammarat 80161, Thailand*

Sarinya Hadsadee − *Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Ubon Ratchathani University, Ubon Ratchathani 34190, Thailand*

Siriporn Jungsuttiwong − *Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Ubon Ratchathani University, Ubon Ratchathani 34190, Thailand;* orcid.org/0000-0001-5943-6878

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jnatprod.0c01308

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

V.R. thanks the NSTDA Chair Professor grant (the fourth Grant) of the Crown Property Bureau and the National Science and Technology Development Agency. P.S. acknowledges the TRF through the Royal Golden Jubilee Ph.D. program (grant number PHD/0211/2559) for a scholarship, the Center of Excellence for Innovation in Chemistry (PERCH−CIC), Ministry of Higher Education, Science, Research and Innovation, the Graduate School and the Division of Physical Science, Faculty of Science, Prince of Songkla University, for partial support. Finally, National Center for Genetic Engineering and Biotechnology (BIOTEC) is acknowledged for the evaluation of cytotoxic activity.

REFERENCES

(1) Sureram, S.; Wiyakrutta, S.; Ngamrojanavanich, N.; Mahidol, C.; Ruchirawat, S.; Kittakoop, P. *Planta Med.* **2012**, *78*, 582−588.

(2) Sureram, S.; Kesornpun, C.; Mahidol, C.; Ruchirawat, S.; Kittakoop, P. *RSC Adv.* **2013**, *3*, 1781−1788.

(3) Klaiklay, S.; Rukachaisirikul, V.; Aungphao, W.; Phongpaichit, S.; Sakayaroj, J. *Tetrahedron Lett.* **2016**, *57*, 4348−4351.

(4) Phainuphong, P.; Rukachaisirikul, V.; Phongpaichit, S.; Preedanon, S.; Sakayaroj, J. *Tetrahedron* **2017**, *73*, 5920−5925.

(5) Phainuphong, P.; Rukachaisirikul, V.; Phongpaichit, S.; Sakayaroj, J.; Kanjanasirirat, P.; Borwornpinyo, S.; Akrimajirachoote, N.; Yimnual, C.; Muanprasat, C. *Tetrahedron* **2018**, *74*, 5691−5699.

(6) Kawahara, N.; Nakajima, S.; Satoh, Y.; Yamazaki, M.; Kawai, K.-I. *Chem. Pharm. Bull.* **1988**, *36*, 1970−1975.

(7) Cao, S.; Lee, A. S. Y.; Huang, Y.; Flotow, H.; Ng, S.; Butler, M. S.; Buss, A. D. *J. Nat. Prod.* **2002**, *65*, 1037−1038.

(8) Basset, J.-F.; Leslie, C.; Hamprecht, D.; White, A. J. P.; Barrett, A. G. M. *Tetrahedron Lett.* **2010**, *51*, 783−785.

(9) Hamano, K.; Kinoshita-Okami, M.; Hemmi, A.; Sato, A.; Hisamoto, M.; Matsuda, K.; Yoda, K.; Haruyama, H.; Hosoya, T.; Tanzawa, K. *J. Antibiot.* **1992**, *45*, 1195−1201.

(10) Kawahara, N.; Nozawa, K.; Nakajima, S.; Kawai, K.-I. *J. Chem. Soc., Perkin Trans. 1* **1988**, 2611−2614.

(11) Tayone, W. C.; Honma, M.; Kanamaru, S.; Noguchi, S.; Tanaka, K.; Nehira, T.; Hashimoto, M. *J. Nat. Prod.* **2011**, *74*, 425−429.

(12) Barnett, H. L.; Hunter, B. B. *Illustrated Genera of Imperfect Fungi*; Prentice-Hall, Inc., The American Phytopathological Society, USA, 1998.

(13) Trisuwan, K.; Khamthong, N.; Rukachaisirikul, V.; Phongpaichit, S.; Preedanon, S.; Sakayaroj, J. *J. Nat. Prod.* **2010**, *73*, 1507−1511.

(14) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A., Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, Ö.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. *Gaussin09*; Gaussian Inc.: Wallingford, 2010.

(15) Phongpaichit, S.; Rungjindamai, N.; Rukachaisirikul, V.; Sakayaroj, J. *FEMS Immunol. Med. Microbiol.* **2006**, *48*, 367−372.

(16) Hunt, L.; Jordan, M.; Jesus, M. D.; Wurm, F. M. *Biotechnol. Bioeng.* **1999**, *65*, 201−205.