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Depsidone and phthalide derivatives from the soil-derived fungus *Aspergillus unguis* PSU-RSPG199

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ABSTRACT

Two new compounds including one phthalide (asperlide, **1**) and one depsidone (aspersidone, **2**) together with twelve known compounds were isolated from the soil-derived fungus *Aspergillus unguis* PSU-RSPG199. Known emeguisin A exhibited potent antibacterial activity against *Staphylococcus aureus* and methicillin-resistant *S. aureus* as well as strong antifungal activity against *Cryptococcus neoformans*, each with MIC values of 0.5 μg/mL. Additionally, known pilobolusate was strongly active against the human oral carcinoma (KB) cell line with an IC_{50} value of 4.5 μM. Interestingly, emeguisin A and pilobolusate were noncytotoxic against noncancerous (Vero) cell lines.

Fungi are a significant source of structurally diverse metabolites, many of which are excellent sources of pharmaceuticals such as cancer drugs.[1] The soil fungi in the genus *Aspergillus* has afforded interesting bioactive compounds such as antiviral meroesquiterpenoids,[2] cholesterol lowering lovastatins,[3] and cytotoxic phenalenone.[4] In our ongoing search for bioactive secondary metabolites from soil fungi, *Aspergillus unguis* PSU-RSPG199 was isolated from a soil sample collected from the Plant Genetic Conservation Project under the Royal Initiation of Her Royal Highness Princess Maha Chakri Sirindhorn at Ratchaprapa Dam in Surat Thani Province, Thailand. The broth extract exhibited antibacterial activity against *Staphylococcus aureus* and methicillin-resistant *S. aureus*, each with minimum inhibitory concentration (MIC) values of 32 μg/mL, while the mycelial extract displayed antibacterial activity against both bacterial stains with MIC values of 8 μg/mL. Both extracts showed weak antifungal activity against *Candida albicans*, *Cryptococcus neoformans*, and *Microsporum gypseum* with MIC values in the range of 64–200 μg/mL. Additionally, the broth extract showed antimalarial activity against *Plasmodium falciparum* (K1 strain) with an IC_{50} value of 8.46 μg/mL, while the mycelial extract was inactive. Regarding the cytotoxic activity against MCF-7 breast cancer, KB oral cavity cancer and noncancerous Vero (African green monkey kidney fibroblasts) cell lines, only the mycelial extract exhibited cytotoxicity toward KB and Vero cell lines with IC_{50} values of 36.6 and 19.15 μg/mL, respectively. Herein, we describe the isolation and characterization of secondary metabolites from the broth and mycelial extracts of the fungus PSU-RSPG199. One new phthalide, asperlide (**1**) together with four known compounds, 3-ethyl-5,7-dihydroxy-3,6-dimethylphthalide (**3**),[5] aspergillusphenol A (**4**),[6] methyl orsellinate (**5**)[7] and (+)-montagnetol (**6**),[8] were isolated from the broth extract. Moreover, one new depsidone, aspersidone (**2**), one known orsellinate, pilobolusate (**7**),[9] and seven known depsidones, 3-chlorounguinol (**8**),[5] nornidulin (**9**),[5] unguinol (**10**),[5] nidulin (**11**),[5] aspergillusidone C (**12**),[10] emeguisin A (**13**),[11] and folipastatin (**14**)[12] were obtained from the mycelial extract. Some of the isolated compounds were evaluated for antimicrobial (against *S. aureus* ATCC25923, methicillin-resistant *S. aureus*, *C. albicans* NCPF3153, *C. neoformans* ATCC90113 flucytosine-resistant, and *M. gypseum* clinical isolate), antimalarial (against *P. falciparum*), anticancer (against MCF-7 and KB cell lines), and cytotoxic (against Vero cell line) activities.

All compounds (**1**–**14**, Fig. 1) were obtained using chromatographic purification, and their structures elucidated using various spectroscopic techniques (ESI). The absolute configuration of compound **3** was assigned for the first time as 3*S* by the opposite sign of

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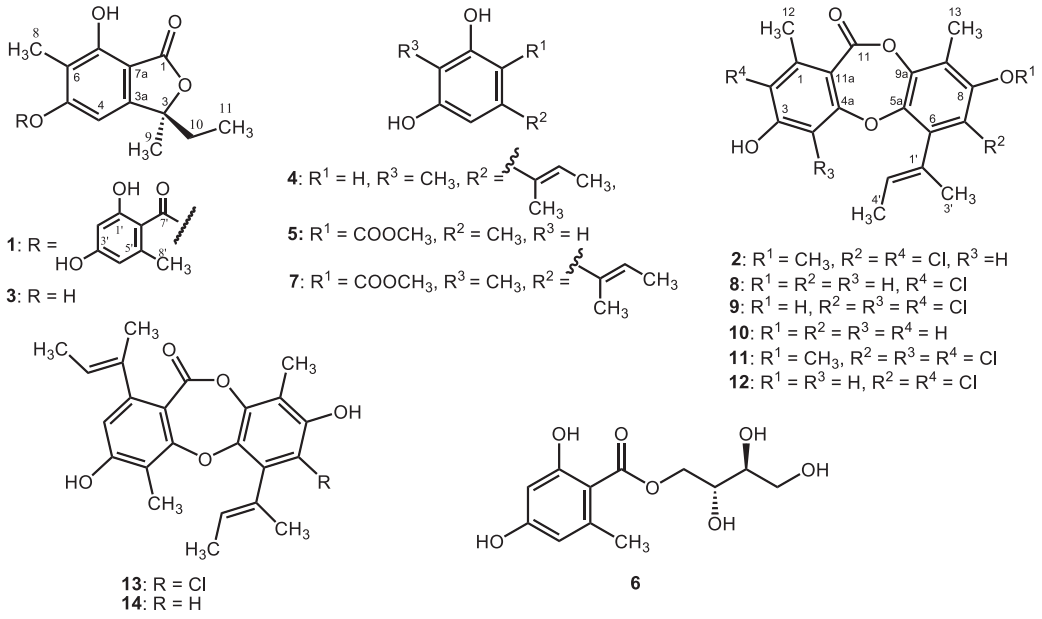

Figure 1. Structures of compounds **1**–**14** isolated from *Aspergillus unguis* PSU-RSPG199.

Table 1
NMR data of asperlide **1**

Position	δ_H,[a] *J* in Hz	δ_C,[b] type	HMBC	NOEDIFF
1		171.3, C		
3		90.2, C		
3a		151.6, C		
4	6.62, s	106.7, CH	C-3, C-5, C-6, C-7a	H_3-9, H_{ab}-10, H_3-11, H_3-8′
5		155.2, C		
6		118.4, C		
7		155.7, C		
7-OH	8.14, s		C-6, C-7, C-7a	
7a		108.9, C		
8	2.14, s	8.9, CH_3	C-5, C-6, C-7	H_3-8′
9	1.65, s	25.5, CH_3	C-3, C-3a, C-10	H-4, H_{ab}-10, H_3-11
10	a: 2.04, dq (14.4, 7.2) b: 1.91, dq (14.4, 7.2)	32.9, CH_2	C-3, C-3a, C-11	
11	0.84, t (7.2)	7.9, CH_3	C-3, C-10	H-4, H_3-9, H_{ab}-10,
1′		166.6, C		
1′-OH	11.30, s		C-1′, C-2′, C-6′	
2′	6.35, s	101.7, CH	C-1′, C-3′, C-4′, C-6′, C-7′	1′-OH
3′		161.6, C		
4′	6.35, s	112.1, CH	C-2′, C-3′, C-6′, C-7′, C-8′	H_3-8′
5′		144.4, C		
6′		104.3, C		
7′		169.5, C		
8′	2.66, s	24.7, CH_3	C-4′, C-5′, C-6′	H-4, H_3-8, H-4′

[a] Recorded at 300 MHz in $CDCl_3$.
[b] Recorded at 75 MHz in $CDCl_3$.

both the CD data ($\Delta\varepsilon$ +12.51 at 211 nm) and specific rotation ($[\alpha]_D^{25}$ −48.9, *c* 0.078, MeOH) to those of (3*R*)-pseudaboydin B ($\Delta\varepsilon$ −7.22 at 211.2 and $[\alpha]_D^{25}$ +53.6, *c* 0.078, MeOH).[13] The absolute configuration of compound **6** was established by comparison of its specific rotation with that reported in the literature.[8]

Asperlide (**1**)[14] was isolated as a colorless gum with the molecular formula $C_{20}H_{20}O_7$ as identified from the HRESIMS peak at *m*/*z* 395.1108 $[M+Na]^+$. It showed UV absorption bands of a conjugated carbonyl chromophore at 270 and 300 nm.[5] The IR spectrum showed characteristic absorption bands of hydroxy, γ-lactone carbonyl, and ester carbonyl groups at 3424, 1757, and 1726 cm^{-1}, respectively.[5,9] The ^1H NMR spectroscopic data (Table 1) were similar to those of compound **3**.[5] The differences in their ^1H NMR spectroscopic data were the presence of signals for a hydrogen bonded hydroxy proton (δ_H 11.30), two aromatic protons of a 1,2,3,5-tetrasubstituted benzene ring (δ_H 6.35, s), and one methyl group (δ_H 2.66, s). The ^{13}C NMR data contained additional signals for an ester carbonyl (δ_C 169.5) as well as four quaternary (δ_C 166.6, 161.6, 144.4 and 104.3), two methine (δ_C 112.1 and 101.7), and one methyl (δ_C 24.7) carbons. The hydrogen bonded

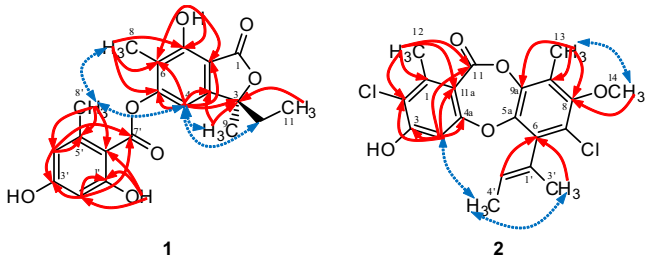

Figure 2. Selected HMBC (↷) and NOEDIFF data (⇢) for compounds **1** and **2**.