Structures, Biogenesis, and Biological Activities of Pyrano[4,3-*c*]isochromen-4-one Derivatives from the Fungus *Phellinus igniarius*

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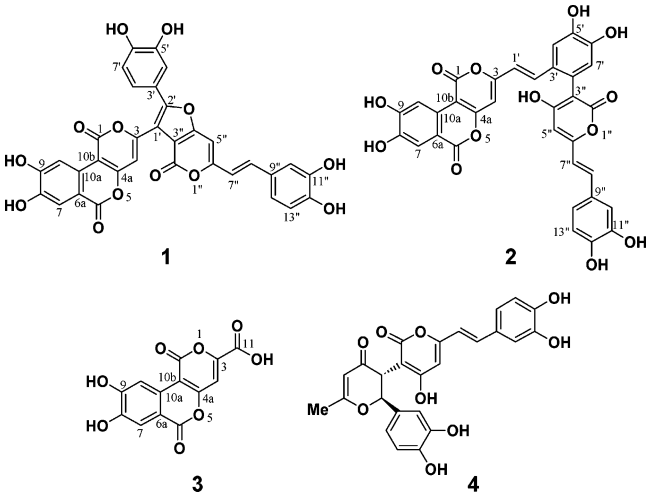
Further chemical investigation of the Chinese medicinal fungus *Phellinus igniarius* has resulted in the isolation and structural elucidation of three pyrano[4,3-*c*]isochromen-4-one derivatives, phelligridins H (**1**), I (**2**), and J (**3**), together with the known compounds davallialactone (**4**), scopolin, nebularine, uridine, trehalose, glucitol, and ethyl glucoside. The structures of **1**−**3** were elucidated by spectroscopic methods. Phelligridins H (**1**) and I (**2**) possess unprecedented carbon skeletons, and phelligridin J (**3**) is an oxidative derivative of the co-occurring phelligridin A. Compounds **1** and **2** inhibited protein tyrosine phosphatase 1B (PTP1B) and rat liver microsomal lipid peroxidation, while compound **3** exhibited cytotoxic activity against several human cancer cell lines.

Fungi are a rich source of secondary metabolites that possess a variety of structures and significant bioactivities.[1] *Phellinus igniarius* (DC. ex Fr.) Quél, a fungus belonging to the family Polyporaceae, grows on the stems of various common trees. Its fruit body is used to treat wounds, abdominalgia, and bloody gonorrhea, in traditional Chinese medicine.[2] As part of our program to systematically assess the chemical and biological diversity of several traditional Chinese medicines, more than 20 metabolites have been characterized from an ethanolic extract of the fruit body of *P. igniarius*.[3] Further investigation of this fungus has resulted in the isolation and structural elucidation of three additional pyrano-[4,3-*c*]isochromen-4-one derivatives, designated as phelligridins H−J (**1**−**3**), together with the known compounds davallialactone (**4**),[4] scopolin,[5] nebularine,[6] uridine, glucitol,[7] trehalose, and ethyl glucoside. Phelligridins H (**1**) and I (**2**) possess unprecedented carbon skeletons, and phelligridin J (**3**) is an oxidative derivative of phelligridin A. The isolation of davallialactone (**4**) from this fungus supported a previous scheme for the biogenesis of phelligridin F and inoscavin A.[3e] In this paper, we report the isolation, structure elucidation, and biological activities of **1**−**3**, together with a summary of the postulated biogenesis of the phelligridin fungal metabolites, as well as related natural products.

Compound **1** was obtained as an orange, amorphous powder and showed IR absorption bands for hydroxyl (3288 cm^{-1}) and conjugated carbonyl (1718 and 1689 cm^{-1}) groups and aromatic rings (1593, 1554, and 1522 cm^{-1}). The presence of aromatic ring and conjugated carbonyl functional groups in **1** was also indicated by the UV absorption maxima at 204 (4.75), 262 (4.51), and 406 (4.75) nm. The negative-mode ESIMS of **1** exhibited a quasi-molecular ion peak at *m*/*z* 621.2 $[M - H]^-$, and the positive-mode high-resolution FTMALDI/DHBMS at *m*/*z* 623.0820 was consistent with the molecular formula $C_{33}H_{18}O_{13}$ (calcd 623.0808 for $C_{33}H_{19}O_{13}$), requiring 25 sites of unsaturation. The ^1H NMR spectrum of **1** indicated the presence of two 1,3,4-trisubstituted phenyl moieties at δ 6.83 (d, $J = 7.5$ Hz, H-7′), 7.07 (dd, $J = 7.5$, 1.5 Hz, H-8′), and 7.08 (d, $J = 1.5$ Hz, H-4′) and at δ 6.77 (d, $J = 8.0$ Hz, H-13″), 6.98 (dd, $J = 8.0$, 1.5 Hz, H-14″), and 7.06 (d, $J = 1.5$ Hz, H-10″),



and a 1,2-disubstituted *trans*-double bond at δ 6.81 and 7.24 (d each, $J = 16.0$ Hz, H-7″ and 8″), as well as four uncoupled aromatic and/or olefinic protons at δ 7.14, 7.25, 7.58, and 8.38 (s each, H-5″, H-4, H-7, and H-10) and six broad exchangeable phenolic hydroxyl protons at δ 9.12, 9.34, 9.49, 9.60, 10.21, and 10.83 (brs each, OH-11″, OH-5′, OH-6′, OH-12″, OH-8, and OH-9). The ^{13}C NMR and DEPT spectra of **1** displayed 33 carbon signals consisting of 12 methines and 21 quaternary carbons, of which all signals appeared at a relatively low magnetic field (δ >95 ppm) and 14 quaternary carbons were assigned as oxygenated sp^2 hybrid carbons on the basis of their chemical shift values (δ >145 ppm) (Table 1). These spectroscopic data implied a highly oxygenated and highly unsaturated structure for **1** that was finally established by a comprehensive analysis of the 2D NMR spectroscopic parameters.

The $^1H-^1H$ COSY spectroscopic data confirmed the presence in the molecule of **1** of two 1,3,4-trisubstituted phenyl and 1,2-disubstituted *trans* double-bond moieties. The HSQC experiment enabled the unambiguous assignment of the protons and their corresponding carbons (Table 1). In the HMBC spectrum of **1**, a series of correlations from H-4 to C-3 (δ 151.7), C-4a (δ 160.2), and C-10b (δ 100.5), from H-7 to C-6 (δ 158.9), C-8 (δ 147.4), C-9 (δ 153.8), and C-10a (δ 126.8), and from H-10 to C-8, C-9, C-6a (δ 112.2), and C-10b (Figure 1), in combination with an uncorrelated quaternary carbon at δ 159.8 (C-1), as well as a careful

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Table 1. NMR Data for Compounds **1** and **2**[a]

	1		2	
no.	δ_H	δ_C	δ_H	δ_C
1		159.8 s		159.4 s
3		151.7 s		158.6 s
4	7.25 s	103.7 d	6.74 s	99.3 d
4a		160.2 s		160.8 s
6		158.9 s		158.7 s
6a		112.2 s		111.5 s
7	7.58 s	114.8 d	7.50 s	114.5 d
8		147.4 s		146.9 s
9		153.8 s		153.6 s
10	8.38 s	111.0 d	8.26 s	110.6 d
10a		126.8 s		127.1 s
10b		100.5 s		98.8 s
11				
1′		108.8 s	6.73 d (16.0)	115.8 d
2′		155.1 s	7.08 d (16.0)	134.0 d
3′		119.1 s		126.0 s
4′	7.08 d (1.5)	114.7 d	7.20 s	112.0 d
5′		145.5 s		145.4 s
6′		147.9 s		147.4 s
7′	6.83 d (7.5)	116.2 d	6.60 s	118.7 d
8′	7.07 dd (7.5, 1.5)	120.0 d		126.0 s
2″		157.4 s		162.7 s
3″		107.8 s		101.5 s
4″		161.0 s		166.0 s
5″	7.14 s	95.4 d	6.34 s	100.4 d
6″		158.2 s		158.3 s
7″	6.81 d (16.0)	116.4 d	6.80 d (16.0)	116.4 d
8″	7.24 d (16.0)	134.7 d	7.20 d (16.0)	134.8 d
9″		127.2 s		127.0 s
10″	7.06 d (1.5)	114.2 d	7.08 d (1.5)	114.4 d
11″		145.7 s		145.6 s
12″		147.5 s		147.4 s
13″	6.77 d (8.0)	116.1 d	6.78 d (8.0)	115.8 d
14″	6.98 dd (8.0 1.5)	120.8 d	7.01 dd (8.0, 1.5)	120.4 d

[a] NMR data were recorded in DMSO-d_6 at 500 MHz for proton and at 125 MHz for carbon. Proton coupling constants (J) in Hz are given in parentheses. The assignments were based on DEPT, ^{1}H−^{1}H COSY, HMQC, and HMBC experiments.

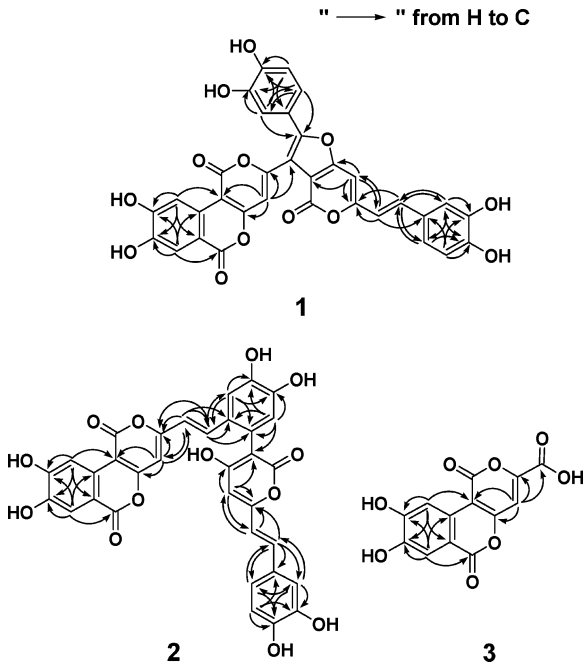

Figure 1. Key HMBC correlations of compounds **1**−**3**.

comparison of the chemical shift values of these protons and carbons with those of phelligridins A, C−E, and G,[3a,e,g] demonstrated that **1** contains a 8,9-dihydroxypyrano[4,3-*c*]isochromen-4-on-3-yl moiety, identical to that of phelligridins A, C−E, and G. Moreover, HMBC correlations from H-4′ to C-6′ (δ 147.9) and C-8′ (δ 120.0), from H-7′ to C-3′ (δ 119.1) and C-5′ (δ 145.5), and from H-8′ to C-4′ (δ 114.7) and C-6′, together with the chemical shift values of these protons and carbons, revealed the presence of a 3,4-dihydroxyphenyl group. In addition, a hispidin-3-yl unit was distinguished unequivocally by the HMBC correlations from H-5″ to C-3″ (δ 107.8), C-4″ (δ 161.0), C-6″ (δ 158.2), and C-7″ (δ 116.4), from H-7″ to C-5″ (δ 95.4), C-6″, and C-9″ (δ 127.2), from H-8″ to C-6″, C-10″ (δ 114.2), and C-14″ (δ 120.8), from H-10″ to C-8″ (δ 134.7), C-12″ (δ 147.5), and C-14″, from H-13″ to C-9″ and C-11″ (δ 145.7), and from H-14″ to C-8″, C-10″, and C-12″, together with an uncorrelated quaternary carbon at δ 157.4 (C-2″), as well as a careful comparison of chemical shift values of the above protons and carbons with those of hispidin[8] and the hispidin moiety in phelligridins B−E[3e] and phelligridimer A.[3h] The two remaining carbons at δ 108.8 (C-1′) and 155.1 (C-2′) were connected to one another to form a tetrasubstituted double-bond unit on the basis of their chemical shift values and quaternary nature. Moreover, in the HMBC spectrum, C-1′ and C-2′ were correlated with H-4, and H-8′ and H-4′ (Figure 1), respectively. This indicated that the 8,9-dihydroxypyrano[4,3-*c*]isochromen-4-on-3-yl and 3,4-dihydroxyphenyl units were substituted at C-1′ and C-2′ of the double bond, respectively, to form a structural moiety similar to phelligridin D.[3e] To satisfy the 25 sites of unsaturation of the molecular formula ($C_{33}H_{18}O_{13}$), and the chemical shift values of the terminal quaternary carbons (C-1′, C-2′, and C-3″) of the above moieties, as well as six phenolic hydroxyl groups indicated by the ^{1}H NMR spectrum, C-1′ must be connected directly with C-3″, while C-2′ must be bridged by an oxygen atom to C-4″ to form a furan ring. Consequently, the structure of **1** was determined as 8,9-dihydroxy-3-{2-(3,4-dihydroxyphenyl)-6-(5,6-dihydroxystyryl)furo-[3,2-*c*]pyran-4-oxo-3-yl}pyrano[4,3-*c*]isochromen-4-one and has been designated as phelligridin H.

Compound **2**, an orange, amorphous powder, showed IR and UV absorption bands similar to those of **1**, indicating a structural similarity between these two compounds. The negative-mode ESIMS of **2** exhibited a quasi-molecular ion peak at *m*/*z* 623.5 [M − H]$^-$, and the positive-mode high-resolution FTMALDI/DHBMS at *m*/*z* 625.0977 [M + H]$^+$ indicated a molecular formula of $C_{33}H_{20}O_{13}$ (calcd 625.0998 for $C_{33}H_{21}O_{13}$), requiring 24 sites of unsaturation. The ^{1}H NMR spectrum of **2** showed two pairs of doublets due to two 1,2-disubstituted *trans* double bonds at δ 6.73 and 7.08 (d each, J = 16.0 Hz, H-1′ and H-2′) and at δ 6.80 and 7.20 (d each, J = 16.0 Hz, H-7″ and H-8″), and an ABX coupled system attributed to a 3,4-trisubstituted phenyl unit at δ 6.78 (d, J = 8.0 Hz, H-13″), 7.01 (dd, J = 8.0, 1.5 Hz, H-14″), and 7.08 (d, J = 1.5 Hz, H-10″). These observations were confirmed by the ^{1}H−^{1}H COSY spectrum of **2**. In addition, the ^{1}H NMR spectrum showed six singlets attributed to aromatic and/or olefinic protons at δ 6.34, 6.60, 6.74, 7.20, 7.50, and 8.26 (s each, H-5″, H-7′, H-4, H-4′, H-7, and H-10) and seven broad exchangeable singlets due to phenolic hydroxyl protons at δ 9.08, 9.15, 9.48, 9.59, 10.08, 10.71, and 11.42 (brs each, OH-5′, OH-11″, OH-6′, OH-12″, OH-8, OH-9, and OH-4″). The ^{13}C NMR and DEPT spectra of **2** displayed 33 sp^2 hybrid carbon signals consisting of 13 methines and 20 quaternary carbons (13 oxygen-bearing carbons, δ >145 ppm) (Table 1). The above spectroscopic data were used to confirm that **2** possesses a structure similar to **1**.

The final structure of **2** was established on the basis of HSQC and HMBC experiments. The proton and carbon signals of the methines in the NMR spectra of **2** were assigned unambiguously by the HSQC experiment of **2** (Table 1). In the HMBC spectrum, long-range correlations from the protons to the carbons of **2** (Figure 1) revealed unequivocally the presence of 8,9-dihydroxypyrano-[4,3-*c*]isochromene-4-on-3-yl, hispidin-3-yl, and 2-substituted 4,5-dihydroxyphenyl units in this molecule. In addition, HMBC correlations from H-4 to C-1′, from H-1′ to C-3, C-4, C-2′, and

C-3′, and from H-2′ to C-3, C-1′, C-3′, C-4′, and C-8′ indicated that the 8,9-dihydroxypyrano[4,3-*c*]isochromen-4-on-3-yl and 2-substituted 4,5-dihydroxyphenyl units are located at C-1′ and C-2′ of the remaining *trans* double bond, respectively. By considering the molecular composition of **2**, the hispidin-3-yl moiety must be a substituent of the 2-substituted 4,5-dihydroxyphenyl unit, which was proved by a HMBC correlation from H-7′ to C-3″. Therefore, the structure of **2** (phelligridin I) was determined as 8,9-dihydroxy-3-{6,7-dihydroxy-4-(hispidin-3-yl)styryl}pyrano[4,3-*c*]isochromen-4-one.

Compound **3**, a yellow, amorphous powder, showed IR absorption bands for hydroxyl (3380 cm^{-1}), conjugated carbonyl (1751 and 1701 cm^{-1}), and aromatic ring (1618, 1598, and 1531 cm^{-1}) functional groups. The negative-mode ESIMS of **3** exhibited a quasi-molecular ion peak at *m*/*z* 289 $[M - H]^-$, and the negative-mode HRESIMS at *m*/*z* 289.0005 indicated the molecular formula, $C_{13}H_6O_8$ (calcd 288.9984 for $C_{13}H_5O_8$), with 11 degrees of unsaturation. The ^{1}H NMR spectroscopic data of **3** were similar to those of the co-occurring phelligridin A,[3a] except that the methyl singlet was absent in the ^{1}H NMR spectrum of **3**, and H-4 of **3** was deshielded by $\Delta\delta_H$ 0.30 ppm compared to analogous data for phelligridin A. Further comparison of ^{13}C NMR data of these two compounds indicated that a resonance assignable to an oxygenated sp^2 quaternary carbon of **3** at δ_C 157.6 ppm (C-11) replaced the resonance of the methyl carbon of phelligridin A, while C-3 of **3** was shielded by $\Delta\delta_C$ 2.9 ppm compared to that of phelligridin A. These data in combination with the molecular composition suggested that **3** is a derivative of phelligridin A in which the methyl group is oxidized as a carboxyl group. This was confirmed by the HMBC spectrum, showing a long-range correlation from H-4 to C-11 (Figure 1). Therefore, the structure of **3** (phelligridin J) was assigned as 3-carboxyl-8,9-dihydroxypyrano[4,3-*c*]isochromen-4-one.

As new members of the phelligridin family, phelligridins H (**1**) and I (**2**) have unique carbon skeletons. For the related co-occurring compounds phelligridins A−G, inoscavin A, and phelligridimer A, we have postulated a biogenetic pathway involving the fungal metabolite precursor 4-hydroxy-6-methyl-2-pyrone that couples with activated 3,4-dihydroxybenzoyl-SCoA or the co-occurring 3,4-dihydroxybenzaldehyde and/or 4-hydroxybenzaldehyde.[3e,g,h] On the basis of such a speculation, both **1** and **2** may be formed from oxidative coupling between one molecule of hispidin and a molecule of activated 3,4-dihydroxybenzoyl-SCoA and further coupled with one more hispidin precursor in a different manner involving the co-occurring phelligridin D (Scheme S1, Supporting Information). Alternatively, they may be formed by oxidative coupling of two molecules of hispidin, followed by coupling with a molecule of activated 3,4-dihydroxybenzoyl-SCoA involving the fungal metabolites hypholomine B[9] and 3,14′-bihispidinyl[10] (Scheme S2, Supporting Information), respectively. Phelligridin J (**3**) may come from the oxidation of phelligridin A (Scheme S1, Supporting Information). In addition, the isolation of davallialactone (**4**) in *P. igniarius* supports a previous postulation of the biogenesis of the co-occurring phelligridin F and inoscavin A.[3e] Although a biogenetic pathway through a plausible epoxide precursor was proposed for davallialactone,[4] the co-occurrence of these three metabolites suggests that they may most likely be biosynthesized from oxidative coupling between the co-occurring fungal metabolites hispidin and hispilone, followed by intramolecular cyclization through three tautomers (Scheme S3, Supporting Information).

Phelligridin J (**3**) showed cytotoxic activities against several human cancer cell lines (Table 2), but phelligridins H (**1**) and I (**2**) and davallialactone (**4**) were inactive against the cell lines tested (IC_{50} >10 μM). Compounds **1**−**4** exhibited antioxidant activity inhibiting rat liver microsomal lipid peroxidation with IC_{50} values of 4.8, 3.7, 6.5, and 8.2 μM, respectively. Both **1** and **2** inhibited protein tyrosine phosphatase 1B (PTP1B), with IC_{50} values of 3.1 and 3.0 μM, respectively, but phelligridin J (**3**) was inactive (IC_{50} >10 μM).

Table 2. Cytotoxic Activities of Phelligridins H−J (**1**−**3**) and Davallialactone (**4**) against Four Human Cancer Cell Lines ($IC_{50}/\mu M$)[a]

compound	A2708[b]	A549[c]	Bel-7402[d]	HCT-8[e]
3	7.2	4.2	9.2	8.4
topotecan[f]	1.2	3.1	1.4	1.6

[a] Compounds **1**, **2**, and **4** were inactive against all cell lines tested (IC_{50} >10 μg/mL). [b]Human ovary cancer cell line. [c]Human lung cancer cell line. [d]Human hepatoma cell line. [e]Human colon cancer cell line. [f]Positive control.

Experimental Section

General Experimental Procedures. Melting points were determined on an XT-4 micro melting point apparatus. UV spectra were measured with a Shimadzu UV-260 spectrometer. IR spectra were recorded as KBr disks on a Nicolet Impact 400 FT-IR spectrophotometer. NMR spectra were recorded on a Varian INOVA 500 MHz spectrometer at 500.1 MHz for ^{1}H and 125.8 MHz for ^{13}C in DMSO-d_6 with TMS as internal standard. ESIMS, HRMALDIFTMS, and HRESIMS data were measured with Micromass Autospec-Ultima ETOF and IonSpec 4.7 Tesla FTMS spectrometers. Column chromatography was performed using silica gel (160−200 mesh) and Sephadex LH-20. TLC was carried out with glass precoated silica gel GF_{254} plates. Spots were visualized under UV light and by spraying with 7% H_2SO_4 in 95% EtOH followed by heating. All solvents used were either spectral grade or distilled prior to use.

Fungal Material. *Phellinus igniarius* (DC. ex Fr.) Quél was collected in the Dandong district of Liaoning Province, People's Republic of China in September 2000. The fungus identification was verified by Professor Shufang Wang (Department of Medicinal Plants, Institute of Materia Medica, Beijing, 100050, People's Republic of China). A voucher specimen (No. 200136) was deposited at the Herbarium of the Department of Medicinal Plants, Institute of Materia Medica, Beijing, People's Republic of China.

Extraction and Isolation. The air-dried and powdered fruit body of *P. igniarius* (5 kg) was extracted with 95% EtOH at room temperature for 3 × 48 h. After the solvent was removed under reduced pressure at <40 °C, a dark brown residue (193 g) was obtained. The residue was suspended in water and then partitioned successively with EtOAc and *n*-BuOH. The EtOAc fraction (95 g) was separated by column chromatography on silica gel eluting with a gradient with increasing proportions of acetone (0−50%) in $CHCl_3$, followed by a gradient elution with MeOH (20−100%) in $CHCl_3$, to give 24 fractions (a_1−a_{24}) on the basis of TLC analysis. Fraction a_1 was chromatographed over silica gel, eluting with a gradient of acetone (0−100%) in petroleum ether (60−90 °C), to give isoergosterone (23 mg) and octadecyl ferulate (54 mg). The antioxidant fractions a_{16} (5.5 g) and a_{18} (9.0 g)[11] were further separated individually over Sephadex LH-20 using $CHCl_3$−MeOH (3:1) for a_{16} and $CHCl_3$−MeOH (2:1) for a_{18} as mobile phases, to afford **1** (35 mg) from a_{16} and **2** (53 mg) and **4** (30 mg) from a_{18}. Fractionation of the *n*-BuOH-soluble portion (60 g) via medium-pressure liquid chromatography over reversed-phase silica gel (C_{18}), eluting with a gradient of MeOH (0−100%) in acidic water containing 0.1% AcOH, gave four subfractions, nB_1−nB_4. Subfraction nB_2 was separated by reversed-phase flash column chromatography, eluting with 20% MeOH in H_2O, to give partially purified fractions nB_{2-1}−nB_{2-3}. These were separately chromatographed using Sephadex LH-20, with $CHCl_3$−MeOH (2:1) as mobile phase, to yield scopolin (28 mg) from nB_{2-1}, nebularine (17 mg) from nB_{2-2}, and **3** (15 mg) from nB_{2-3}, respectively. The H_2O phase was subjected to column chromatography over Sephadex LH-20 and eluted with 0%, 30%, 50%, and 80% MeOH in H_2O, sequentially, to yield the fractions W_1−W_4 after removal of solvent under reduced pressure. Fractions W_1 and W_2 were separately chromatographed over Sephadex LH-20, using 50% MeOH in H_2O as mobile phase, to yield glucitol (1.12 g) and trehalose (5.5 g), respectively. Fraction W_3 was separated by column chromatography over silica gel by using $CHCl_3$−MeOH (10:1) to yield uridine (31 mg) and ethyl glucoside (22 mg).

Phelligridin H (1): orange, amorphous powder, mp >300 °C; UV (MeOH) λ_{max} (log ϵ) 204 (4.75), 262 (4.51), 406 (4.75) nm; IR (KBr) ν_{max} 3288, 1718, 1689, 1593, 1554, 1522, 1294, 1261, 1201, 1016, 972, 897, 808, 781 cm^{-1}; ^1H and ^{13}C NMR data, Table 1; (−)-ESIMS *m*/*z* 621.2 $[M - H]^-$; HRMALDIFTMS *m*/*z* 623.0820 ($[M + H]^+$, calcd for $C_{33}H_{19}O_{13}$ 623.0808).

Phelligridin I (2): orange, amorphous powder, mp >300 °C; UV (MeOH) λ_{max} (log ϵ) 221 (4.28), 254 (3.64), 387 (3.52) nm; IR (KBr) ν_{max} 3406, 1691, 1610, 1545, 1516, 1292, 1147, 1016, 962 cm^{-1}; ^1H and ^{13}C NMR data, Table 1; (−)-ESIMS *m*/*z* 623.5 $[M - H]^-$; HRMALDIFTMS *m*/*z* 625.0977 ($[M + H]^+$, calcd for $C_{33}H_{21}O_{13}$ 625.0998).

Phelligridin J (3): yellow, amorphous powder, mp >300 °C; UV (MeOH) λ_{max} (log ϵ) 202 (4.13), 213 (4.11), 233 (4.04, sh), 260 (4.06), 278 (4.00, sh), 344 (3.96), 356 (3.95) nm; IR (KBr) ν_{max} 3388, 3078, 1751, 1701, 1618, 1589, 1531, 1408, 1371, 1354, 1317, 1209, 1026 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 6.90 (1H, s, H-4), 7.51 (1H, s, H-7), 8.38 (1H, s, H-10); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 159.9 (C-1), 159.5 (C-3), 100.3 (C-4), 160.2 (C-4a), 158.3 (C-6), 110.9 (C-6a), 113.7 (C-7), 146.9 (C-8), 153.6 (C-9), 110.2 (C-10), 126.2 (C-10a), 99.9 (C-10b), 157.6 (C-11); (−)-ESIMS *m*/*z* 289 $[M - H]^-$; (−)-HRESIMS *m*/*z* 289.0005 ($[M - H]^-$, calcd for $C_{13}H_5O_8$ 288.9984).

Cells, Culture Conditions, and Cell Proliferation Assay. See refs 3e, 12, and 13.

PTP1B Inhibition Assay. The recombinant GST-hPTP1B (gluthathione *S*-transferase-human protein tyrosine phosphatase 1B) bacteria pellets were purified by a GST bead column. The dephosphorylation of *para*-nitrophenyl phosphate (*p*-NPP) was catalyzed to *para*-nitrophenol by PTP1B. Enzyme activity involving an end-point assay, which intensifies the yellow color, was measured at a wavelength of 405 nm.[14] All compounds were dissolved in 100% dimethyl sulfoxide (DMSO), and reactions, including controls, were performed at a final concentration of 10% DMSO. Selected compounds were first evaluated for their ability to inhibit the PTPase reaction at a 10 μM concentration at 30 °C for 10 min, in a reaction system with 3 mM *p*-NPP in HEPES assay buffer (pH 7.0). The reaction was initiated by addition of the enzyme and quenched by addition of 1 M NaOH. The amount of *p*-nitrophenol produced was determined at 405 nm using a microplate spectrophotometer (uQuant, Bio-tek). IC_{50} values were evaluated using a sigmoidal dose−response (variable slope) curve-fitting program of GraphPad Prism 4.0 software.

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Supporting Information Available: Biogenetic schemes S1−S3 for phelligridins H (**1**) and J (**2**) and for davallialactone (**4**). MS, HRMS, IR, UV, 1D and 2D NMR spectra of phelligridins H−J (**1**−**3**). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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