Phelligridins C−F: Cytotoxic Pyrano[4,3-*c*][2]benzopyran-1,6-dione and Furo[3,2-*c*]pyran-4-one Derivatives from the Fungus *Phellinus igniarius*

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Three unique pyrano[4,3-*c*][2]benzopyran-1,6-dione derivatives and a new furo[3,2-*c*]pyran-4-one, named phelligridins C−F (**2**−**5**), together with hispolon (**8**), (*E*)-4-(3,4-dihydroxyphenyl)but-3-en-2-one (**9**), 4-hydroxybenzaldehyde, protocatechualdehyde, syringic acid, protocatechuic acid, caffeic acid, isoergosterone, and octadecyl ferulate were isolated and identified from the ethanolic extract of *Phellinus igniarius*. Their structures were determined by spectroscopic methods including IR, MS, and 1D and 2D NMR experiments. The structures of the new compounds were characterized as 3-(4-hydroxystyryl)-8,9-dihydroxypyrano[4,3-*c*]isochromene-4-one (**2**), 3-(3,4-hydroxystyryl)-8,9-dihydroxypyrano[4,3-*c*]isochromene-4-one (**3**), 8,9-dihydroxy-3-{5′,6′-dihydroxy-5″-methyl-3″-oxo-spiro[fural-2″(3″H),1′-indene]-2′-yl}-1*H*,6*H*-pyrano[4,3-*c*][2]benzopyran-1,6-dione (**4**), and (3*Z*)-3-(3,4-dihydroxybenzylidene)-6-(3,4-dihydroxystyryl)-2,3-dihydro-2-methoxy-2-(2-oxo-propyl)furo[3,2-*c*]pyran-4-one (**5**), respectively. Some compounds including **2** and **3** showed in vitro selective cytotoxicity against a human lung cancer cell line (A549) and a liver cancer cell line (Bel7402). Possible biogenetic sequences to the formation of **1**−**9** are postulated.

Fungi produce an astonishing variety of structurally unusual secondary metabolites and seem to be a rich source of novel structures. *Phellinus igniarius* (DC. Ex Fr.) Quél, a fungus belonging to the Polyporaceae family, preferably hosts on the stems of aspen, robur, and birch. Its fruit body is used to treat fester, abdominalgia, and bloody gonorrhea in traditional Chinese medicine.[1] Previous chemical investigations indicated that polysaccharides,[2] phenolic pigment,[3] and peroxidases[4,5] were metabolites of this fungus, but few structures were definitely assigned. In our investigation of the chemical constituents of the fruit body of *P. igniarius*, seven flavonoids, phelligrins A and B, naringenin, sakuranetin, aromadendrin, folerogenin, and eriodictyol, and two coumarins, coumarin and scopoletin, have been isolated and identified.[6,7] In the continuation of our investigation on the same material, four structurally unique pyrano[4,3-*c*][2]-benzopyran-1,6-dione derivatives (**1**−**4**), two unusual furo[3,2-*c*]pyran- 4-one derivatives (**5**, **6**), and a hispidin derivative (**7**) were obtained and characterized together with hispolon (**8**),[8] (*E*)-4-(3,4-dihydroxyphenyl)but-3-en-2-one (**9**),[9] 4-hydroxybenzaldehyde, protocatechualdehyde,[10] syringic acid,[11] protocatechuic acid,[10,12] caffeic acid,[10,13] isoergosterone,[14,15] and octadecyl ferulate.[16] In a short letter,[17] we have described the isolation and structural elucidation of **1** and **7**, named phelligridins A and B, respectively. Compound **6** (inoscavin A) is a free radical scavenger and was isolated from the fungus *Inonotus xeranticus*.[18] We report herein the isolation and structural elucidation of **2**−**5**, as well as in vitro cytotoxic activities of compounds **1**−**3** and **5**−**9**, in addition to the postulations regarding the biogenetic formation of **1**−**9**.

Results and Discussion

The EtOH extract of the fruit body of the fungus was evaporated to dryness and then suspended in water and partitioned with EtOAc and *n*-butanol. The EtOAc-soluble extract was subjected to column chromatography over silica gel eluting with a gradient of increasing MeOH (0−100%) in $CHCl_3$. The subsequent fractions were further purified by a variety of chromatographic techniques to yield the new compounds **2**−**5** and other known compounds. Compounds **2**−**5** gave amorphous powders in a variety of solvents (MeOH, Me_2CO, EtOAc, H_2O, and mixtures of MeOH/H_2O, Me_2CO/H_2O, MeOH/EtOAc, etc). The normal 1D NMR spectra of **2**−**5** showed limited proton spin couplings along with an indication of more than 10 sp^2 quaternary carbons in each of the structures due to the highly oxygenated and conjugated properties. In the structural elucidation HMQC and HMBC experiments played powerful roles.

Compound **2** was obtained as a yellow amorphous powder (MeOH), mp 272−276 °C. The IR spectrum of **2** showed absorption bands for a hydroxyl group (3288 and 3091 cm^{-1}), a conjugated carbonyl group (1668 cm^{-1}), and aromatic rings (1604, 1549, and 1514 cm^{-1}). The ESI mass spectrum exhibited a quasi-molecular ion at *m*/*z* 365 [M + H] $^+$, and the HRFABMS at *m*/*z* 365.0698 [M + H]$^+$ established the molecular formula as $C_{20}H_{12}O_7$. The ^{1}H NMR spectrum of **2** showed signals attributable to the AA′BB′ spin coupling system of a *para*-disubstituted phenyl moiety at δ 6.83 (2H, d, J = 8.5 Hz, H-5′ and H-7′), 7.54 (2H, d, J = 8.5 Hz, H-4′ and H-8′), a *trans* disubstituted double bond at δ 6.90 (1H, d, J = 15.5 Hz, H-1′), 7.38 (1H, d, J = 15.5 Hz, H-2′), three exchangeable phenolic hydroxyl protons at δ 10.78, 10.10, and 9.98 (each 1H), and three singlets at δ 8.35 (1H, s, H-10), 7.53 (1H, s, H-7), and 6.70 (1H, s, H-4). The ^{13}C NMR and DEPT spectra of **2** showed 20 sp^2 carbon signals including nine methines and 11 quaternary carbons (seven of which are oxygenated, δ > 145 ppm) (see Table 1). The protonated carbons and their corresponding protons were unambiguously assigned by the HMQC experiment of **2** (see Table 1), and the connectivities of carbons in **2** were established by the HMBC experiment (see Figure 1). In the HMBC spectrum, the correlations from H-1′ to C-3′, H-2′ to C-4′ and C-8′, both H-4′ and H-8′ to C-6′, and both H-5′ and H-7′ to C-3′ revealed the presence of the *para*-hydroxystyryl moiety. In addition, the three bond correlations from H-4 to C-10b, H-7 to C-6, C-9, and C-10a, and H-10 to C-6a, C-8, and C-10b showed that **2** possesses a structural moiety of 8,9-dihydroxy-1*H*,6*H*-

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Table 1. ^{1}H and ^{13}C NMR Data for Compounds **2**−**5**[a]

no.	**2** δ_H	**2** δ_C	**3** δ_H	**3** δ_C	**4** δ_H	**4** δ_C	**5** δ_H	**5** δ_C
1		159.5 s		159.4 s		158.9 s		
2								114.0 s
3		158.4 s		158.4 s		153.2 s		127.2 s
3a								102.8 s
4	6.70 s	99.0 d	6.66 s	98.9 d	6.17 s	95.9 d		157.7 s
4a		160.7 s		160.7 s		160.0 s		
6		158.7 s		158.6 s		158.2 s		161.7 s
6a		111.4 s		111.4 s		111.6 s		
7	7.53 s	114.4 d	7.51s	114.4 d	7.51 s	114.4 d	6.61 s	94.7 d
7a								169.1 s
8		146.7 s		146.8 s		147.1 s		
9		153.7 s		153.5 s		153.5 s		
10	8.35 s	110.4 d	8.33 s	110.4 d	8.30 s	110.6 d		
10a		127.0 s		127.0 s		126.7 s		
10b		98.8 s		98.7 s		99.3 s		
1′	6.90 d (15.5)	115.6 d	6.77 d (15.9)	115.4 d		94.6 s	6.76 d (15.5)	116.0 d
2′	7.38 d (15.5)	135.5 d	7.27 d (15.9)	135.8 d		131.4 s	7.27 d (15.5)	136.0 d
3′		126.2 s		126.7 s	7.86 s	141.4 d		126.7 s
3′a						132.2 s		
4′	7.54 d (8.5)	129.6 d	7.07 d (1.5)	114.1 d	6.98 s	111.4 d	7.06 s	114.2 s
5′	6.83 d (8.5)	115.9 d		145.5 s		147.0 s		145.9 s
6′		159.3 s		147.7 s		146.8 s		147.8 s
7′	6.83 d (8.5)	115.9 d	6.76 d (7.7)	115.8 d	6.62 s	109.5 d	6.77 d (8.0)	115.8 d
7′a						133.8 s		
8′	7.54 d (8.5)	129.6 d	6.99 dd (7.7, 1.5)	120.8 d			6.98 dd (8.0, 2.0)	120.9 d
1″							7.24 s	124.2 d
2″								126.2 s
3″						198.4 s	7.06 s	116.3 d
4″					6.08 s	105.0 d		145.6 s
5″						192.3 s		145.3 s
6″					2.49 s	16.7 q	6.74 d (8.0)	115.8 d
7″							6.89 dd (8.5, 2.0)	121.4 d
1‴							3.23 s	47.7 t
2‴								202.8 s
3‴							2.01 s	31.0 q
OCH_3							3.27 s	50.3 q

[a] NMR data were measured in DMSO 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.

pyrano[4,3-*c*][2]benzopyran-1,6-dione, which is similar to phelligridin A (**1**) except for the *para*-hydroxystyryl moiety replacing the methyl group in **1**. Three bond correlations from H-1′ to C-4, H-2′ to C-3, and H-4 to C-1′ confirmed that the *para*-hydroxystyryl moiety was located at C-3. Consequently, the structure of **2** was unambiguously established as 3-(4-hydroxystyryl)-8,9-dihydroxypyrano[4,3-*c*]isochromene-4-one and named phelligridin C.

Compound **3**, obtained as yellow powder, mp > 300 °C, showed IR spectral features similar to those of **2**. The EIMS gave a molecular ion at *m*/*z* 380 [M]$^+$. The molecular formula of **3** was determined as $C_{20}H_{12}O_8$ by the HREIMS at *m*/*z* 380.0542, which was one more oxygen atom than that of **2**. This suggested that compound **3** is an analogue of **2** with one more hydroxyl group. The ^{1}H NMR spectroscopic data of **3** were similar to those of **2** except that the AA′BB′ spin system of the *para*-hydroxystyryl moiety of **2** was replaced by an AMX system at δ 6.76 (d, $J = 7.7$ Hz), 6.99 (dd, $J = 7.7$ and 1.5 Hz), and 7.07 (d, $J = 1.5$ Hz), suggesting the presence of a 5′,6′-dihydroxystyryl in **3** instead of the 6′-hydroxystyryl in **2**. This suggestion was further confirmed by the ^{13}C NMR spectral data of **3** (see Table 1), as well as by the HMQC and HMBC experiments (see Figure 2). Therefore, the structure of **3** was assigned as 3-(3,4-hydroxystyryl)-8,9-dihydroxypyrano[4,3-*c*]isochromene-4-one and designated phelligridin D.

Compound **4** was obtained as an orange powder, mp 178−182 °C, $[\alpha]^{18}_D$ 0° (*c* 0.16, MeOH/DMSO, 1:1). The IR spectrum showed absorption bands for hydroxyl groups (3454 cm^{-1}), conjugated carbonyl groups (1684 cm^{-1}), and aromatic rings (1587, 1544, and 1520 cm^{-1}). The FABMS exhibited a quasi-molecular ion at *m*/*z* 475 [M + H]$^+$, and

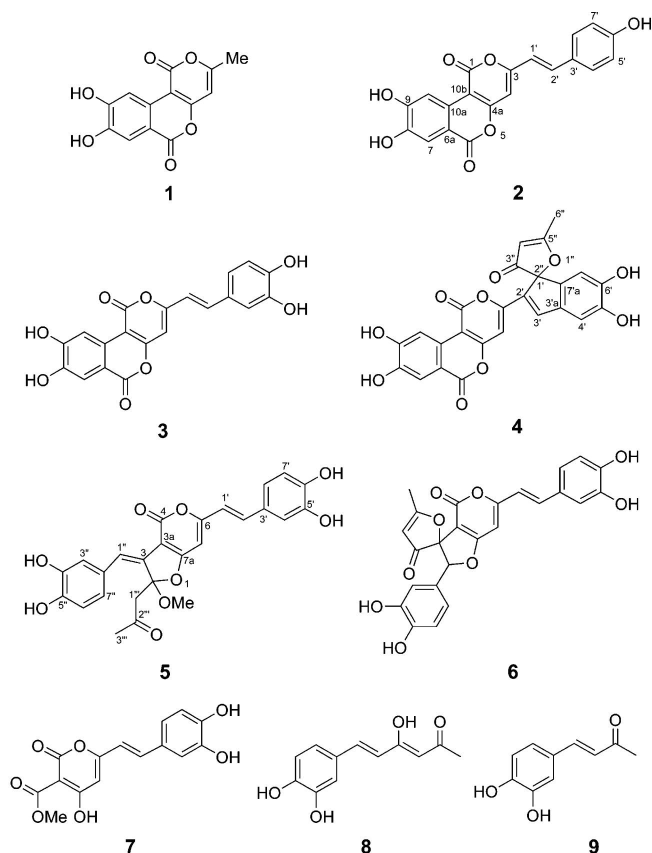


Figure 1. Structures of compounds **1**−**9**.

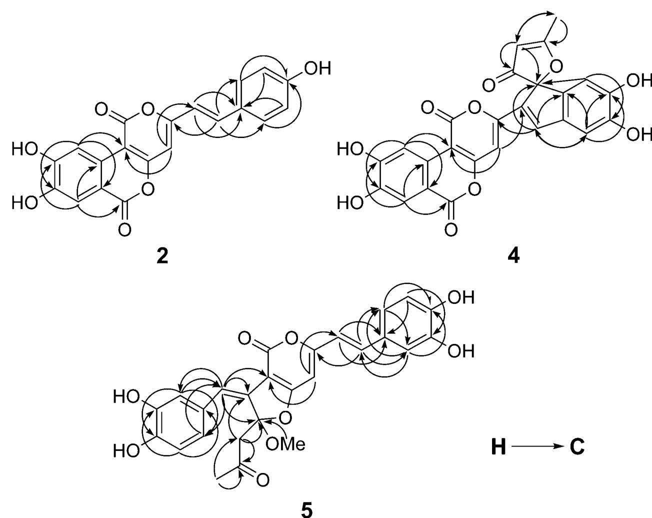


Figure 2. Key HMBC correlations of compounds **2**, **4**, and **5**.

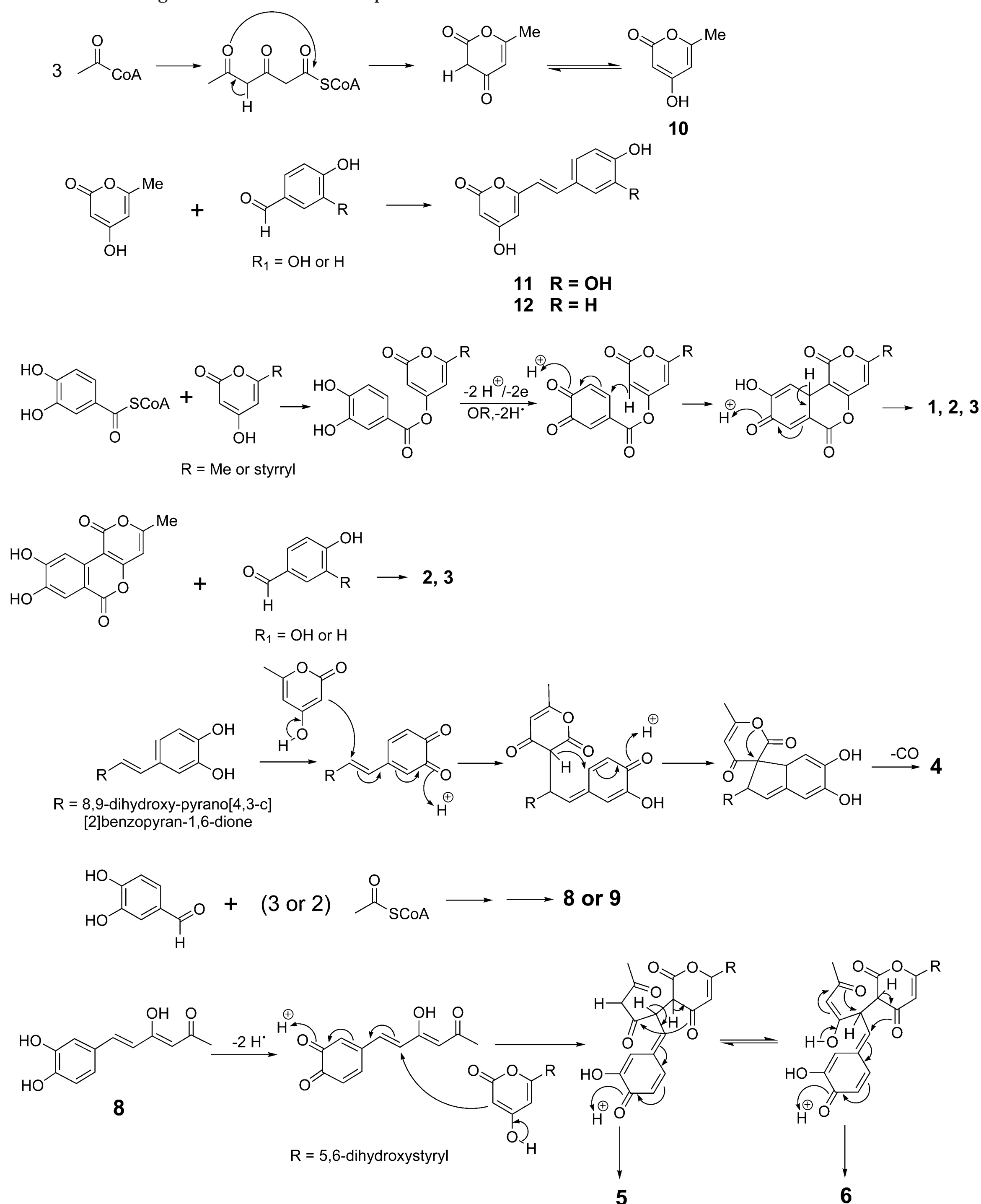
the HRFABMS at *m*/*z* 475.0661 [M + H]$^+$ established the molecular formula as $C_{25}H_{14}O_{10}$. The ^{1}H NMR spectrum of **4** showed seven singlets at δ 6.08 (1H, s, H-4″), 6.17 (1H, s, H-4), 6.62 (1H, s, H-7′), 6.98 (1H, s, H-4′), 7.51 (1H, s, H-7), 7.86 (1H, s, H-3′), and 8.30 (1H, s, H-10) in the aromatic region and one methyl proton singlet at δ 2.49 (3H, s, CH_3) in the higher field region. The ^{13}C NMR spectrum of **4** displayed 25 carbon signals, and the DEPT spectrum revealed that these comprised one methyl, seven sp^2 methines, and 17 quaternary carbons (10 oxygenated sp^2 carbons, δ > 145 ppm) (see Table 1). The signals of the methine carbons and their corresponding protons in the NMR spectra were unambiguously assigned by an HMQC experiment. In the HMBC spectrum (see Figure 2), three bond correlations from H-4 to C-10b, H-7 to C-6, C-9, and C-10a, and H-10 to C-8, C-6a, and C-10b, in combination with the chemical shift values of these carbons, demonstrated that **4** contains the structural unit 8,9-dihydroxy-1*H*,6*H*-pyrano[4,3-*c*][2]benzopyran-1,6-dione, which is identical to that of **2** (or **3**). A 2′,7′a-disubstituted 5′,6′-dihydroxystyryl moiety located at C-3 of the above structural moiety was revealed by correlations from H-4 to C-2′, H-3′ to C-3, C-4′, and C-7′a, H-4′ to C-3′, C-7′a, and C-6′, and H-7′ to C-3′a and C-5′. In addition, the correlations from the methyl protons (H-6″) to C-5″ and C-4″ and H-4″ to C-2″, C-3″, and the methyl carbon unequivocally established the presence of a 2″,2″-disubstituted 5″-methyl-3″-(2″*H*)-furanone moiety in **4**. The chemical shift values of the carbons of this moiety are in good agreement with the corresponding carbons of the 5-methyl-3(2*H*)-furanone moiety of inoscavin A (**6**).[18] Furthermore, long-range correlations from both H-3′ and H-7′ to C-2″ in combination with the chemical shift values of C-1′ (δ 94.6, s) and C-7′a (δ 133.8, s) indicated that C-2″ of the 2″,2″-disubstituted 5″-methyl-3″(2″*H*)-furanone moiety was connected to both C-2′ and C-7′a to form a spiroindene structural moiety. Therefore, the structure of **4** was unequivocally determined as 8,9-dihydroxy-3-{5′,6′-dihydroxy-5″-methyl-3″-oxo-spiro-[furan-2″(3″*H*),1′-indene]-2′-yl}-1*H*,6*H*-pyrano[4,3-*c*][2]-benzopyran-1,6-dione and named phelligridin E. Compound **4** was optically inactive and hence a racemate. This indicates that the biosynthetic formation of the spiroindene moiety of **4** is nonstereoselective.

Compound **5** was obtained as an orange powder, mp 215−218 °C, $[\alpha]^{18}_D$ −3.23 (*c* 0.31, MeOH/DMSO, 1:1). Its IR spectrum showed absorption bands for hydroxyls (3329 cm^{-1}), conjugated carbonyl groups (1687 cm^{-1}), and aromatic rings (1601, 1539, and 1444 cm^{-1}). The FAB mass spectrum showed a quasi-molecular ion at *m*/*z* 479 [M + H]$^+$, and the HRFABMS at *m*/*z* 479.1358 [M + H]$^+$ established the molecular formula $C_{26}H_{22}O_9$. The ^{1}H NMR and ^{1}H−^{1}H COSY spectra of **5** showed two ABX spin systems assignable to two 1,3,4-trisubstituted benzene rings at δ 6.98 (1H, dd, *J* = 8.0, 2.0 Hz, H-8′), 6.77 (1H, d, *J* = 8.0 Hz, H-7′), and 7.06 (1H, s, H-4′), and 6.89 (1H, dd, *J* = 8.5, 2.0 Hz, H-7″), 6.74 (1H, d, *J* = 8.5 Hz, H-6″), and 7.06 (1H, s, H-3″), an AM spin system originating from a *trans*-1,2-disubstituted double bond unit at δ 6.76 (1H, d, *J* = 15.5 Hz, H-1′) and 7.27 (1H, d, *J* = 15.5 Hz, H-2′), and a singlet of a conjugated olefinic methine proton at δ 7.24 (1H, s, H-1″), together with three singlets at δ 3.27 (3H, s, OCH_3), 3.23 (2H, s, H-1‴), and 2.01 (3H, s, H-3‴) attributable to an O-methyl, an sp^3 methylene, and a methyl, respectively. The ^{13}C NMR and DEPT spectra of **5** proved the existence of the protonated carbons corresponding to the above protons, while showing 13 quaternary carbons including a ketone (δ 202.8) and seven oxygenated sp^2 carbons (δ > 145) (see Table 1). The structure of **5** was established on the basis of the HMQC and HMBC experiments. The connections of C−H and C−C in **5** were unambiguously established by the HMQC and HMBC spectra (see Figure 1). The presence of the hispidin moiety was revealed by the long-range correlations from H-1′ to C-7 and C-3′, H-2′ to C-6, C-4′, and C-8′, H-4′ to C-2′, C-6′, and C-8′, H-7′ to C-3′ and C-5′, and H-7 to C-7a, C-3a, and C-1′ in combination with the comparison of chemical shift values of corresponding carbons in **6** and **7**. The presence of the 3,4-dihydroxybenzylidene moiety in the structure of **5** was demonstrated by the three bond correlations from H-1″ to C-3″ and C-7″, H-3″ to C-1″ and C-5″, and H-6″ to C-2″ and C-4″. The 2-oxopropyl unit was indicated by correlations from H-1‴ to C-2‴ and C-3‴ and from H-3‴ to C-1‴ and C-2‴. The above three moieties and the O-methyl group were connected through the remaining two quaternary carbons C-2 and C-3 on the basis of long-range correlations from both H-1″ and H-1‴ to C-2 and C-3, H-1″ to C-3a, and the O-methyl protons to C-2, as depicted in Figure 2. The chemical shift of C-2 at δ 114.0 would suggest the possibility of an sp^2 carbon, whereas the attachment of the O-methyl at C-2 establishes that C-2 is an sp^3 acetal carbon. To satisfy the molecular formula $C_{26}H_{22}O_9$ and the 16 degrees of unsaturation, C-2 and C-7a must be connected through an oxygen atom to form a furo[3,2-*c*]pyran-4-one moiety. Consequently, the structure of **5** was elucidated as (3*Z*)-3-(3,4-dihydroxybenzylidene)-6-(3,4-dihydroxystyryl)-2,3-dihydro-2-methoxy-2-(2-oxo-propyl)furo[3,2-*c*]-pyran-4-one and named phelligridin F. The geometry of the double bond between C-3 and C-1″ was determined to be *cis* by the NOE difference experiment showing strong negative enhancements of H-3″ and H-7″ by irradiation of H-1‴. The absolute stereochemistry at C-2 has not been determined yet.

Although compounds **1**−**9** were not isolated by following a specific bioassay-guided separation protocol, follow-up biological screening of **1**−**9** indicated in vitro cytotoxicity against several human cancer cell lines (see Table 2). Of the nine compounds, phelligridins C (**2**) and D (**3**) showed selectivity against A549 and Bel7402 with IC_{50} values of 0.012, 0.016, 0.010, and 0.008 μM, respectively, and hispolon (**8**) was more sensitive to MCF-7 and Bel7402 with IC_{50} values of 0.025 and 0.038 μM, respectively.

Compounds **1**−**5** possess unique carbon skeletons, although some structurally related compounds were reported from the mushrooms *Inonotus xeranticus*,[18] *Hypericum chinense*,[19] and *Dorstenia brasiliensis*.[20,21] The widespread

Scheme 1. Postulated Biogenetic Formation of Compounds **1**−**11**



fungal metabolite hispidin (**11**) was previously reported from the fungus *Phellinus igniarius*.[3] The investigation of the biosynthetic pathway of hispidin in *Polyporus hispidus* by Towers et al. revealed that it is biosynthesized from phenylalanine through a cinnamyl derivative (*p*-coumaryl-SCoA or caffeoyl-ScoA) that is combined with either acetate or malonate through the polyketide pathway.[22,23] The co-occurrence of hispidine, phelligridin B (**7**), and hispolon (**8**) in this report suggests that this pathway may also exist in *P. igniarius* and that hispidine may well be the precursor of compounds **2**−**6**. However, the co-occurrence of compounds **1**−**7** with 3,4-dihydroxybenzoic acid, 3,4-dihydroxybenzyaldehyde, and 4-hydroxybenzaldehyde in *P. igniarius* prompted us to postulate another biosynthetic pathway mediated by 4-hydroxy-6-methyl-2-pyrone (**10**) for compounds **1**−**7**. Compound **10** may be formed by three molecules of the basic biosynthetic unit acetyl-SCoA and

Table 2. Cytotoxicity of Compounds **1**−**9**

compd	IC$_{50}$ value (μM)					
	A549	BGC823	MCF-7	Bel7402	Ketr3	HCT-8
1	>0.192	0.181	0.109	0.110	>0.192	>0.192
2	0.012	>0.137	0.072	0.010	0.094	0.126
3	0.016	>0.131	0.037	0.008	0.090	0.099
4	0.079	0.096	0.070	0.055	>0.105	>0.105
5	0.084	0.092	0.085	0.046	>0.104	>0.104
6	>0.108	>0.108	>0.108	0.088	>0.108	>0.108
7	>0.164	0.146	0.143	0.050	0.144	0.139
8	0.183	0.205	0.025	0.038	0.206	0.199
9	>0.280	0.243	0.141	0.153	0.245	0.227
toptecan (control)	0.0032	0.0043	0.0018	0.0012	0.0049	0.0015

then may couple with one molecule of activated 3,4-dihydroxybenzoyl-SCoA or 3,4-dihydroxybenzyaldehyde and/or 4-hydroxybenzaldehyde to give **1** or **11** and/or bisnoryangonin (**12**), respectively. Further coupling of **1** with 4-hydroxybenzaldehyde (or 3,4-dihydroxybenzyaldehyde) would generate **2** (or **3**), which may also be produced by coupling of **12** (or **11**) with 3,4-dihydroxybenzoyl-SCoA. Compound **4** might be formed from the coupling of **3** with another molecule of 4-hydroxy-6-methyl-2-pyrone. 3,4-Dihydroxybenzyaldehyde couples with three (or two) molecules of the basic biosynthetic unit acetyl-SCoA, followed by a decarboxylation, to give **8** (or **9**). Compounds **5** and **6** might originate from the coupling between **8** and **11**. In addition, compound **7** might be generated from the coupling of two molecules of acetyl-SCoA, one molecule of manonyl-SCoA, and one molecule of 3,4-dihydroxybenzyaldehyde. According to the dimerization procedure of **11** proposed by Steglich and co-workers,[24,25] the postulated biogenetic formation of **1**−**11** is depicted in Scheme 1.

The 4-hydroxy-6-methyl-2-pyrone[26] and many related metabolites have been obtained from different fungi;[27−29] however, their biogenetic importance has not been realized yet.[30] The above coupling process could be catalyzed by phenol peroxidases. Although peroxidases have been detected and reported from this fungus,[4,5] the biosynthesis of the above phenol metabolites and the enzymes involved in the biosynthetic process remains unknown.

Experimental Section

General Experimental Procedures. Melting points (uncorrected) were determined on an XT-4 micro melting point apparatus. Optical rotations were measured on a P-E 241 MC automatic polarimeter. UV spectra were measured with a Shimadaz 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 500.103 MHz for ^{1}H and 125.762 MHz for ^{13}C in DMSO-d_6 with TMS as internal standard. EIMS, HREIMS, FABMS, HRFABMS, ESIMS, and HRESIMS data were measured with Micromass Autospec-Ultima ETOF and VGZAB-2F spectrometers. Column chromatography was performed on silica gel (160−200 mesh) and Sephadex LH-20. HPLC separation was performed on a chromatograph consisting of a Waters 600 controller, a Waters 600 pump, and a Waters 2487 dual λ absorbance detector with an Alltima 250 cm × 2.2 cm i.d. preparative column packed with C_{18} (10 μm). TLC was carried out with glass precoated silica gel GF254 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 spectral grade or were distilled prior to use.

Fungal Material. *P. igniarius* (DC. Ex Fr.) Quél was collected in the Dandong District of Liaoning Province, China, in September 2000. The fungus identification was verified by Prof. Shufang Wang (Department of Medicinal Plants, Institute of Materia Medica, Beijing, 100050, 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* (DC. Ex Fr.) Quél (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 with EtOAc and *n*-BuOH successively. The EtOAc fraction (95 g) was separated by column chromatography on silica gel eluting with a gradient of increasing acetone (0−50%) in $CHCl_3$ followed by a gradient elution with MeOH (20−100%) and $CHCl_3$ to give 24 fractions (a_1−a_{24}) on the basis of TLC analyses. Fraction a_1 was chromatographed over silica gel eluting with a gradient of increasing acetone (0−100%) in petroleum ether (60−90 °C) to give isoergosterone (23 mg) and octadecyl ferulate (54 mg). Fraction a_2 was chromatographed over Sephadex LH-20 eluting with petroleum ether/$CHCl_3$/MeOH (5:5:1), followed by purification by preparative HPLC eluting with MeOH/H_2O/HOAc (6:4:0.01) to yield hispolon (112 mg) and syringic acid (30 mg). Fraction a_3 was subjected to chromatography over Sephadex LH-20 with petroleum ether (60−90 °C)/$CHCl_3$/MeOH (5:5:1), and subsequent subfractions were purified by preparative HPLC using MeOH/H_2O/HOAc (7:3:0.01) to (*E*)-4-(3,4-dihydroxyphenyl)but-3-en-2-one (312 mg), 4-hydroxybenzaldehyde (33 mg), and protocatechualdehyde (301 mg). Fraction a_5 was chromatographed over Sephadex LH-20 eluting with petroleum ether (60−90 °C)/$CHCl_3$/MeOH (5:5:1), and then purified by preparative HPLC eluting with MeOH/H_2O/HOAc (5:5:0.01) to yield phelligridin C (**2**, 66 mg), inoscavin A (**6**, 85 mg), protocatechuic acid (40 mg), and caffeic acid (65 mg). Fraction a_6 was separated by chromatography over Sephadex LH-20 with $CHCl_3$/MeOH (3:1) to give four subfraction. The later three subfractions were purified by preparative HPLC using MeOH/H_2O/HOAc (6:4:0.01) as the mobile phase to yield phelligridin D (**3**, 17 mg), phelligridin E (**4**, 15 mg), and phelligridin F (**5**, 22 mg).

Phelligridin C (2): yellow powder, mp 272−275 °C (MeOH); UV (MeOH) λ_{max} (logϵ) 212.4 (2.60), 250.2 (3.59), 406 (3.54) nm; IR (KBr) ν_{max} 3288, 3091, 1668, 1604, 1549, 1514, 1406, 1279, 1171, 1138, 1018, 970, 897, 823 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) data, see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz) data, see Table 1; ESIMS *m*/*z* 365.1 [M + H]$^+$; HRFABMS *m*/*z* 365.0698 [M + H]$^+$ (calcd for $C_{20}H_{13}O_7$ 365.0661).

Phelligridin D (3): yellow powder (MeOH), mp > 300 °C; UV (MeOH) λ_{max} (log ϵ) 214.4 (4.75), 253.8 (4.75), 403.0 (4.65) nm; IR (KBr) ν_{max} 3475, 3089, 1666, 1604, 1552, 1516, 1408, 1286, 1128, 1016 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) data, see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz) data, see Table 1; EIMS *m*/*z* 380 [M]$^+$; HREIMS *m*/*z* 380.0542 [M]$^+$ (calcd for $C_{20}H_{12}O_8$ 380.0532).

Phelligridin E (4): orange powder (MeOH), mp 178−181 °C; $[\alpha]^{18}_D$ 0° (*c* 0.16, MeOH/DMSO, 1:1); UV (MeOH) λ_{max} (log ϵ) 211.8 (4.33), 267.2 (3.62), 441.0 (4.22) nm; IR (KBr) ν_{max} 3454, 1684, 1587, 1544, 1520, 1329, 1201, 1118, 1032, 803 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) data, see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz) data, see Table 1; FABMS *m*/*z* 474.8 [M + H]$^+$; HRFABMS *m*/*z* 475.0661 [M + H]$^+$ (calcd for $C_{25}H_{15}O_{10}$ 475.0665).

Phelligridin F (5): orange powder (MeOH), mp 215−217 °C; $[\alpha]^{18}_D$ −3.23 (*c* 0.31, MeOH/DMSO, 1:1); UV (MeOH) λ_{max} (log ϵ) 210.4 (4.00), 269.4 (4.00), 445.0 (3.91) nm; IR (KBr) ν_{max} 3329, 1687, 1601, 1539, 1444, 1361, 1290, 1194, 1111, 958, 901, 810 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) data, see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz) data, see Table 1; FABMS *m*/*z* 479.1 [M + H]$^+$; HRFABMS *m*/*z* 479.1358 [M + H]$^+$ (calcd for $C_{26}H_{23}O_9$ 479.1342).

Cells and Culture Conditions. Human lung adenocarcinoma (A549), human stomach cancer (BGC-823), human breast cancer (MCF-7), human hepatoma (Bel7402), human kidney cancer (Ketr3), and human colon cancer (HCT-8) cell lines were obtained from ATCC. Cells were maintained in RRMI1640 supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin, and 100 μg/mL streptomycin. Cultures were incubated at 37 °C in a humidified 5% CO_2 atmosphere.

Cell Proliferation Assay. A549, BGC823, MCF-7, Bel7402, Ketr3, and HCT-8 cells were seeded in 96-well microtiter plates at 1200 cells/well. After 24 h, the compounds were added to the cells. After 96 h of drug treatment, cell viability was determined by measuring the metabolic conversion of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) into purple formazan crystals by active cells.[31,32] MTT assay results were read using an MK 3 wellscan (Labsystem Drogon) plate reader at 570 nm. All compounds were tested in five concentrations and were dissolved in 100% DMSO with a final DMSO concentration of 0.1% in each well. Each concentration of the compounds was tested in three parallel wells. IC_{50} values were calculated using Microsoft Excel software.

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Supporting Information Available: MS and 1D and 2D NMR spectra of compounds **1**−**5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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