# *N*-Hydroxypyridones, Phenylhydrazones, and a Quinazolinone from *Isaria farinosa*

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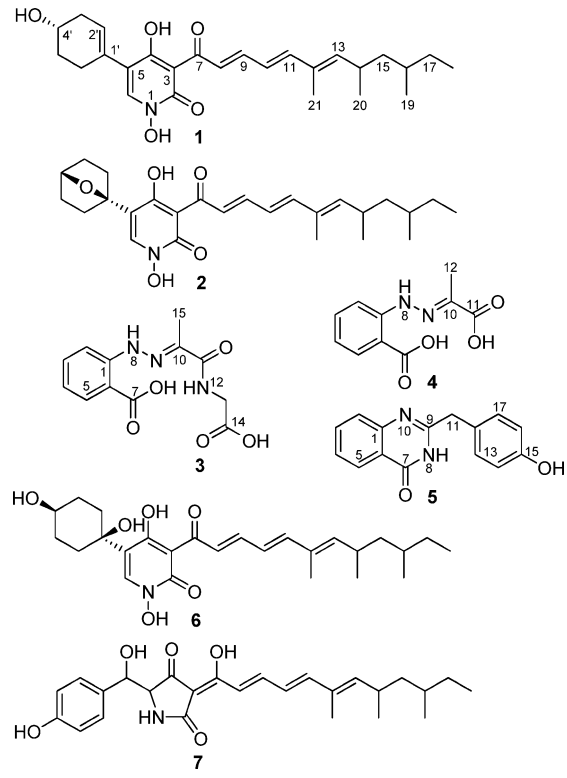
New *N*-hydroxypyridones, militarinones E (**1**) and F (**2**), phenylhydrazones, farylhydrazones A (**3**) and B (**4**), a quinazolinone, 2-(4-hydroxybenzyl)quinazolin-4(3*H*)-one (**5**), and the known militarinones A (**6**) and B (**7**) were isolated from cultures of the *Cordyceps*-colonizing fungus *Isaria farinosa*. The structures of **1**−**5** were elucidated by spectroscopic methods, and **3** was confirmed by X-ray crystallography. The absolute configuration of the C-4′ secondary alcohol in **1** was deduced via the circular dichroism data of the *in situ* formed [$Rh_2(OCOCF_3)_4$] complex. Compounds **1** and **6** showed significant cytotoxicity against A549 cells, whereas **7** was active against *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Candida albicans*.

Naturally occurring *N*-hydroxypyridone derivatives are a group of compounds with a rich diversity of structures and bioactivities. Examples from fungi include pyridoxatin, a radical scavenger from *Acremonium* sp. BX86;[1] the cordypyridones, antimalarial agents from an insect pathogenic strain of *Cordyceps nipponica*;[2] akanthomycin, an antibiotic from an entomopathogenic *Akanthomyces gracilis*;[3] TMC-69, a cytotoxic agent from *Chrysosporium* sp.;[4] fischerin, a toxin causing lethal peritonitis in mice from *Neosartorya fischeri*;[5] sambutoxin and its *N*-demethyl analogue, oxysporidinone, and its 6-*epi* and dimethyl ketal derivatives, antifungal agents from *Fusarium oxysporum*;[6] leporin A, an antiinsectan metabolite from the sclerotia of *Aspergillus leporis*;[7] and antiplasmodial agents from a plant pathogenic *Septoria pistaciarum*.[8] Despite their wide occurrence in fungi, the *N*-hydroxypyridones with a C-3-substituted enoyl moiety are relatively rare, with militarinones A (**6**) and D,[9,10] (+)-*N*-deoxymilitarinone A, and farinosones A and B[11] as reported examples. Phenylhydrazones have been used extensively for protection of the carbonyl groups in organic synthesis,[12] for derivatization, resolution, and characterization of carbonyl-containing natural products,[13] and for studying the hydrazone−enhydrazine tautomeric transformation in the synthesis of indole derivatives.[14] Although phenylhydrazones showed various bioactivities including antioxidative,[15] antiparasitic,[16] antitubercular,[17] and antiviral properties,[18] as far as we are aware, naturally occurring ones have not been reported in the literature.

In a search for new antimicrobial and cytotoxic natural products from fungi of unique niches, those that colonize *Cordyceps sinensis* (recently reclassified as *Ophiocordyceps sinensis*),[19] namely, *Cordyceps*-colonizing, were screened in our laboratory.[20] Since *C. sinensis*, endemic to alpine regions on the Tibetan plateau, is actually the combination of the fungus and the dead caterpillars of the moth *Hepialus* spp., those fungi inhabiting its fruiting body and larvae could be a valuable source of bioactive metabolites with ecological implications. *Isaria farinosa* (formerly known as *Paecilomyces farinosus*) is an entomopathogenic fungus that has been used as a biocontrol agent[21] and from which various bioactive metabolites have been reported.[22] Our initial work on a *Cordyceps*-colonizing isolate afforded two new anthranilic acid-containing cytotoxic cyclopeptides.[20e] Since the crude extract also showed antimicrobial activity and its HPLC fingerprint revealed minor components that could not be identified, the fungus was fermented in a larger scale on rice in which the cyclopeptides were isolated initially. Fractionation of an EtOAc extract afforded the new *N*-hydroxypyridones, militarinones E (**1**) and F (**2**), phenylhydrazones, farylhydrazones A (**3**) and B (**4**), a quinazolinone, 2-(4-hydroxybenzyl)quinazolin-4(3*H*)-one (**5**), and the known militarinones A (**6**) and B (**7**).[9,10] This paper describes the isolation, structure elucidation, and biological activities of these compounds.




## Results and Discussion

Militarinone E (**1**) was isolated as a yellow, amorphous solid with a molecular formula of $C_{26}H_{35}NO_5$ (10 degrees of unsaturation), determined by HREIMS (*m*/*z* 441.2519 $[M]^+$; Δ −0.4 mmu). The $^1H$ and $^{13}C$ NMR spectra of **1** showed resonances for three exchangeable protons ($\delta_H$ 4.64, 11.56, and 17.03, respectively), four methyl groups, five methylenes, three methines including one

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**Table 1.** NMR Data of **1** (acetone-$d_6$) and **2** ($CD_3OD$)

| | **1** | | | **2** | |
|---|---|---|---|---|---|
| position | $\delta_C$[a], mult. | $\delta_H$[b]($J$ in Hz) | HMBC[b] | $\delta_C$[c], mult. | $\delta_H$[d] ($J$ in Hz) |
| 2 | 157.9, qC | | | 160.5, qC | |
| 3 | 106.2, qC | | | 107.9, qC | |
| 4 | 173.6, qC | | | 175.2, qC | |
| 5 | 114.4, qC | | | 120.3, qC | |
| 6 | 136.8, CH | 7.89, s | 2, 3, 4, 5, 1′ | 139.3, CH | 7.94, s |
| 7 | 194.5, qC | | | 195.7, qC | |
| 8 | 128.1, CH | 8.05, d (15.0) | 7, 10 | 128.9, CH | 7.90, d (15.0) |
| 9 | 147.0, CH | 7.63, dd (15.0, 12.0) | 7, 10, 11 | 147.7, CH | 7.58, dd (15.0, 12.0) |
| 10 | 126.2, qC | 6.52, dd (15.0, 12.0) | 8, 9, 12 | 126.7, qC | 6.41, dd (15.0, 12.0) |
| 11 | 149.4, CH | 6.85, d (15.0) | 9, 12, 13, 21 | 149.8, CH | 6.72, d (15.0) |
| 12 | 133.8, qC | | | 134.4, qC | |
| 13 | 147.0, CH | 5.62, d (9.6) | 11, 15, 20, 21 | 147.6, CH | 5.51, d (9.0) |
| 14 | 31.5, CH | 2.72, m | 12, 13, 15, 20 | 32.3, CH | 2.61, m |
| 15a | 45.3, $CH_2$ | 1.15, m | 13, 17, 19, 20 | 46.1, $CH_2$ | 1.09, m |
| 15b | | 1.36, m | 13, 17, 19, 20 | | 1.29, m |
| 16 | 33.1, CH | 1.31, m | 14, 15, 18 | 34.0, CH | 1.24, m |
| 17a | 30.7, $CH_2$ | 1.13, m | 15, 16, 18, 19 | 31.5, $CH_2$ | 1.10, m |
| 17b | | 1.27, m | 15, 16, 18, 19 | | 1.27, m |
| 18 | 11.5, $CH_3$ | 0.84, t (9.0) | 16, 17 | 11.9, $CH_3$ | 0.81, t (9.0) |
| 19 | 19.3, $CH_3$ | 0.84, d (8.0) | 15, 16, 17 | 19.7, $CH_3$ | 0.80, d (8.0) |
| 20 | 21.5, $CH_3$ | 0.98, d (8.0) | 13, 14, 15 | 21.8, $CH_3$ | 0.93, d (8.0) |
| 21 | 12.6, $CH_3$ | 1.89, s | 11, 12, 13 | 12.9, $CH_3$ | 1.80, s |
| 1′ | 131.8, qC | | | 72.8, qC | |
| 2′a | 126.5, CH | 5.80, br s | 5, 4′, 6′ | 30.7, $CH_2$ | 1.38, br d (12.5) |
| 2′b | | | | | 2.60, m |
| 3′a | 35.6 $CH_2$ | 2.09, m | 1′, 2′, 4′ | 29.3, $CH_2$ | 1.57, br d (12.5) |
| 3′b | | 2.46, m | 1′, 5′ | | 1.91, t (12.5) |
| 4′ | 66.2, CH | 3.90, m | 6′ | 66.3, CH | 3.94, br s |
| 5′a | 32.2, $CH_2$ | 1.66, m | 1′, 3′, 4′ | 29.3, $CH_2$ | 1.57, d (12.5) |
| 5′b | | 1.91, m | 1′, 3′, 4′ | | 1.94, t (12.5) |
| 6′a | 27.7, $CH_2$ | 2.42, m | 2′, 4′ | 30.7, $CH_2$ | 1.38, br d (12.5) |
| 6′b | | 2.46, m | 2′, 4′ | | 2.60, m |
| OH-1[e] | | 11.56, s | | | 11.60, s |
| OH-4[e] | | 17.03, s | | | 17.33, s |
| OH-4′[e] | | 4.64, d (3.6) | 3′, 4′, 5′ | | |

[a] Recorded at 100 MHz. [b] Recorded at 500 MHz. [c] Recorded at 125 MHz. [d] Recorded at 500 MHz. [e] Recorded at 500 MHz in DMSO-$d_6$.

oxymethine, 12 olefinic/aromatic carbons (five of which are protonated), one carboxylic carbon ($\delta_C$ 157.9), and one α,β-unsaturated ketone carbon ($\delta_C$ 194.5). These data accounted for all the NMR resonances for **1** and are consistent with the molecular formula $C_{26}H_{35}NO_5$. Analysis of its $^1H$ and $^{13}C$ NMR spectroscopic data (Table 1) revealed structural features similar to those of militarinone A (**6**),[9] except that the C-1′ $sp^3$ quaternary carbon ($\delta_C$ 71.7) and the C-2′ methylene ($\delta_H/\delta_C$1.60; 2.42/34.7) were replaced by a C-1′/C-2′ olefin unit ($\delta_H/\delta_C$ 5.80/126.5; 131.8), which were confirmed by $^1H-^1H$ COSY NMR correlations of H-2′ with $H_2$-3′ and HMBC cross-peaks from H-2′ to C-5, C-4′, and C-6′ and from H-6 and $H_2$-3′ to C-1′. Therefore, the planar structure of **1** was determined as shown.

The relative configuration of the enoyl moiety in **1** was determined by analysis of the $^1H-^1H$ coupling constants and NOESY data and by comparison with that of the model compounds.[9,23] The C-8/C-9 and C-10/C-11 double bonds were all assigned *E*-geometry based on a large coupling constant of 15.0 Hz observed for corresponding olefinic protons, and the same assignment was made for the C-12/C-13 olefin by NOESY correlations of H-10 with $H_3$-21 and H-11 with H-13. The relative configurations of C-14 and C-16 were deduced by comparison of the $^{13}C$ NMR shift values of the C-19 and C-20 methyl groups with those of the model compounds,[9,23] and a difference of 2.2 ppm revealed their *syn*-configuration. However, this relative configuration could not be correlated to that of the distant C-4′ stereogenic center.

The absolute configuration of the C-4′ secondary alcohol was assigned via the circular dichroism data of the *in situ* formed $[Rh_2(OCOCF_3)_4]$ complex,[24] with the inherent contribution subtracted. Upon addition of $[Rh_2(OCOCF_3)_4]$ to a solution of **1** in $CH_2Cl_2$, a metal complex was generated as an auxiliary chromophore. The Rh complex of **1** showed a positive E band (at ca. 350 nm; Figure 1), correlating to the 4′*S* absolute configuration by applying the bulkiness rule.[24,25]

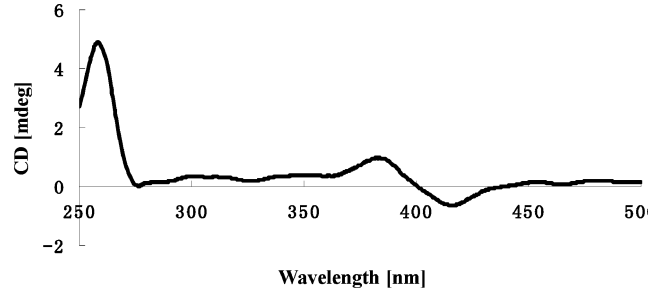



**Figure 1.** CD spectrum of the Rh complex of **1** with the inherent CD spectrum subtracted.

Militarinone F (**2**) was assigned the same molecular formula, $C_{26}H_{35}NO_5$, as **1** by HREIMS (*m/z* 441.2520 $[M]^+$; Δ −0.5 mmu). Analysis of its $^1H$ and $^{13}C$ NMR data (Table 1) revealed nearly identical structural features to those found in **6**,[9] except for different chemical shifts for C-4′ ($\delta_H/\delta_C$ 3.94/66.3 in **2**; 3.62/70.7 in **6**). In addition, the molecular weight of **2** was 18 mass units less than that of **6**, corresponding to the loss of one $H_2O$ molecule. On the basis of these data, an ether bond was proposed to link C-1′ and C-4′, and such a linkage was confirmed by an HMBC correlation from H-4′ to C-1′, leading to assignment of the gross structure of **2**. The relative configuration of **2** was deduced as shown by analogy to **1** and **6**.

Farylhydrazone A (**3**) gave a molecular ion $[M + Na]^+$ peak at *m/z* 302.0742 (Δ −0.5 mmu) by HRESIMS, corresponding to a

**Table 2.** NMR Data of **3**−**5** in DMSO-$d_6$

| | **3** | | | **4** | | **5** | | |
|---|---|---|---|---|---|---|---|---|
| position | $\delta_C$[a], mult. | $\delta_H$[b] (*J* in Hz) | HMBC[b] | $\delta_C$[a], mult. | $\delta_H$[b] (*J* in Hz) | $\delta_C$[c], mult. | $\delta_H$[b] (*J* in Hz) | HMBC[b] |
| 1 | 146.6, qC | | | 146.4, qC | | 148.9, qC | | |
| 2 | 114.2, CH | 7.98, d (8.0) | 1, 4, 6 | 114.2, CH | 7.91, d (8.0) | 126.8, CH | 7.61, d (8.0) | 3, 4, 6 |
| 3 | 134.5, CH | 7.53, t (8.0) | 1, 5 | 134.6, CH | 7.54, t (8.0) | 134.4, CH | 7.78, t (8.0) | 1, 4, 5 |
| 4 | 119.7, CH | 6.92, t (8.0) | 2, 6 | 120.2, CH | 6.95, t (8.0) | 126.0, CH | 7.47, t (8.0) | 2, 3, 6 |
| 5 | 131.7, CH | 7.91, d (8.0) | 1, 3, 7 | 131.7, CH | 7.81, d (8.0) | 125.6, CH | 8.08, d (8.0) | 1, 3, 7 |
| 6 | 113.2, qC | | | 113.2, qC | | 120.6, qC | | |
| 7 | 170.6, qC | | | 170.4, qC | | 161.9, qC | | |
| 8 | | | | | | | 12.35, s | 7 |
| 9 | | | | | | 156.5, qC | | |
| 10 | 138.3, qC | | | 136.0, qC | | | | |
| 11 | 164.9, qC | | | 166.3, qC | | 39.5, $CH_2$ | 3.81, s | 9, 13, 17 |
| 12 | | 8.51, t (6.0) | 11, 13 | | | 126.5, qC | | |
| 13 | 41.5, $CH_2$ | 3.86, d (6.0) | 11, 14 | | | 129.8, CH | 7.18, d (8.5) | 11, 15, 17 |
| 14 | 171.9, qC | | | | | 115.2, CH | 6.71, d (8.5) | 12, 15, 16 |
| 15 | 10.2, $CH_3$ | 2.04, s | 10, 11 | 11.4, $CH_3$ | 2.06, s | 156.2, qC | | |
| 16 | | | | | | 115.2, CH | 6.71, d (8.5) | 12, 14, 15 |
| 17 | | | | | | 129.8, CH | 7.18, d (8.5) | 11, 13, 15 |
| OH-7 | | 11.54, s | | | 11.65, s | | | |
| OH-15 | | | | | | | 9.41, s | |

[a] Recorded at 100 MHz. [b] Recorded at 500 MHz. [c] Recorded at 125 MHz.

molecular formula of $C_{12}H_{13}N_3O_5$ (eight degrees of unsaturation). Interpretation of its $^1H$, $^{13}C$, and HMQC NMR spectroscopic data (Table 2) revealed the presence of two exchangeable protons ($\delta_H$ 8.51 and 11.54, respectively), one methyl group, one methylene that could be heteroatom-bonded ($\delta_H/\delta_C$ 3.86/42.0), seven aromatic/olefinic carbons (four of which are protonated), and three carboxylic carbons. These data, plus the two unobserved exchangeable protons, accounted for all the $^1H$ and $^{13}C$ NMR resonances for **3**. The $^1H-^1H$ coupling patterns for the four aromatic protons, H-2, H-3, H-4, and H-5 (Table 2), suggested the presence of an *o*-substituted phenyl ring, which was confirmed by relevant $^1H-^1H$ COSY and HMBC correlations. Interpretation of the $^1H-^1H$ COSY NMR data of **3** showed the second proton spin system of N-12−C-13, and an HMBC correlation from $H_2$-13 to C-14 established the identify of the glycine (Gly) moiety. Further correlations of H-12 and $H_2$-13 with C-11, and $H_3$-15 with C-10 and C-11, established the C-10−N-12−C-14 fragment with C-15 attached to the C-10 $sp^2$ carbon. An HMBC cross-peak from H-5 to C-7 indicated that the carboxylic carbon at 170.6 ppm is attached to C-6. Considering the facts that C-10 is the only $sp^2$ carbon to be connected and two more nitrogen atoms remain unassigned, a hydrazine moiety was proposed and attached to C-1 of the aryl ring, thereby completing the structure of a phenylhydrazone. However, since the H-8 exchangeable proton was not observed, the proposed structure could not be verified by additional HMBC data. The structure of farylhydrazone A (**3**) was finally confirmed by X-ray crystallographic analysis, and a perspective ORTEP plot is shown in Figure 2.

The molecular formula of farylhydrazone B (**4**) was established as $C_{10}H_{10}N_2O_4$ (seven degrees of unsaturation) by HRESIMS (*m*/*z* 245.0530 $[M + Na]^+$; Δ −0.2 mmu). Analysis of its $^1H$ and $^{13}C$ NMR data (Table 1) revealed the same phenylhydrazone partial structure as found in **3**, except that those for the Gly moiety were absent, consistent with a 72 mass units difference. These data suggested the gross structure of **4** was as depicted.

The elemental composition of **5** was determined to be $C_{15}H_{12}N_2O_2$ (11 degrees of unsaturation) by HRESIMS (*m*/*z* 275.0789 $[M + Na]^+$; Δ +0.2 mmu). The $^1H$ and $^{13}C$ NMR data of **5** (Table 2) revealed two exchangeable protons ($\delta_H$ 9.41 and 12.35, respectively), one methylene, 13 aromatic/olefinic carbons (eight of which are protonated), and one carboxylic carbon ($\delta_C$ 161.9). Analysis of the $^1H-^1H$ coupling patterns revealed the same *o*-substituted aryl ring as found in **3** and **4**, and the remaining four aromatic protons were attributed to a *p*-substituted aryl ring with two sets of doublets (8.5 Hz each) at $\delta_H$ 6.71 and 7.18, respectively. HMBC correlation from H-5 to C-7 revealed the connection of C-6 to C-7, whereas those from $H_2$-11 to C-9, C-13, and C-17 indicated that C-11 is attached to C-12 and the remaining $sp^2$ carbon C-9 ($\delta_C$ 156.5). Considering an HMBC cross-peak of H-8 with C-7, the shift values of C-1, C-7, and C-9 ($\delta_C$ 148.9, 161.9, and 156.5, respectively), and the unsaturation requirement, a quinazolin-4(3*H*)-one moiety was deduced,[26] completing the structure of **5** as 2-(4-hydroxybenzyl)quinazolin-4(3*H*)-one.

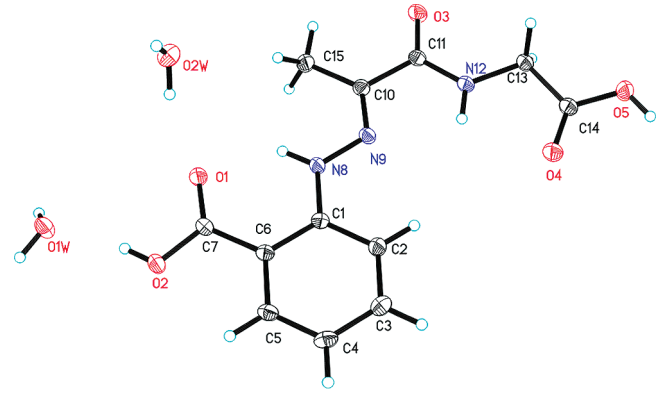



**Figure 2.** Thermal ellipsoid representation of **3**. (Note: A different numbering system is used for the structural data deposited with the CCDC.)

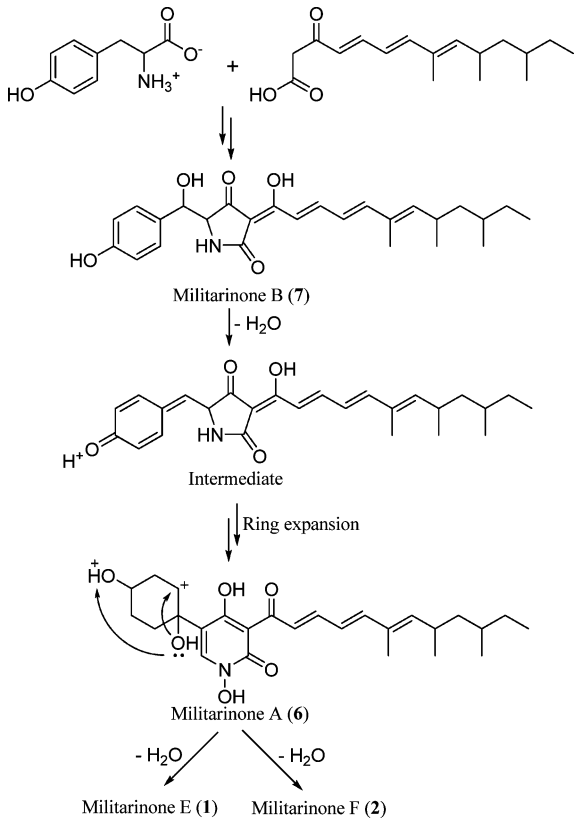
**Table 3.** Activities of Compounds **1**, **6**, and **7**

| | $IC_{50}$ (μM) | | | |
|---|---|---|---|---|
| compound | A 549 | *S. aureus* | *S. pneumoniae* | *C. albicans* |
| **1** | 1.59 | >150 | >150 | >150 |
| **6** | 1.75 | >150 | >150 | >150 |
| **7** | >120 | 2.11 ± 0.01 | 2.60 ± 0.07 | 43.4 ± 1.2 |
| cisplatin | 4.17 | | | |
| ampicillin | | 0.06 ± 0.01 | 1.08 ± 0.08 | |
| amphotericin B | | | | 0.72 ± 0.01 |

The known compounds **6** and **7** were identified as militarinones A and B, respectively, by comparison of their NMR and MS data with those reported.[9,10]

Compounds **1**−**7** were tested for antimicrobial and cytotoxic effects (Table 3). Compounds **1** and **6** displayed significant cytotoxicity against the human lung carcinoma cell line A549, with $IC_{50}$ values of 1.59 and 1.75 μM, respectively, while the positive control cisplatin showed an $IC_{50}$ value of 4.17 μM. Compound **7** was active against the Gram-positive bacteria *Staphylococcus aureus*

**Scheme 1.** Plausible Biosyntheses of Compounds **1**, **2**, **6**, and **7**[10]




(CGMCC 1.2465) and *Streptococcus pneumoniae* (CGMCC 1.1692), showing $IC_{50}$ values of 2.11 and 2.60 $\mu$M, respectively (the positive control ampicillin showed $IC_{50}$ values of 0.06 and 1.08 $\mu$M, respectively). However, **7** showed only modest activity ($IC_{50}$: 43.5 $\mu$M) against *Candida albicans* (CGMCC 2.2086). Compounds **2**−**5** did not show noticeable antimicrobial activities against the above-mentioned microorganisms ($IC_{50}$ > 150 $\mu$M) or cytotoxic effect against A549 cells ($IC_{50}$ > 120 $\mu$M).

Militarinones E (**1**) and F (**2**) are closely related to the known fungal metabolite militarinone A (**6**),[9] a relatively rare 3-enoyl-*N*-hydroxypyridone alkaloid, but differ from **6** by virtue of the presence of different substituents at C-5. Specifically, **1** and **2** could be dehydration products of **6**, occurring at C-1′/C-2′ and C-1′/C-4′, respectively. Biogenetically, this class of compounds could be derived from the condensation of an amino acid (tyrosine or possibly phenylalanine) and an activated polyketide precursors to form **7** via a tetramic acid intermediate, and subsequent rearrangement and ring expansion of **7** could afford **6** (Scheme 1). Obviously, **1** and **2** are very likely the dehydration products of **6** (Scheme 1). To our knowledge, farylhydrazones A (**3**) and B (**4**) are the first naturally occurring phenylhydrazones identified from any source; these were inactive in our bioassays. Compound **5** is a new member of the quinazolinone class of natural products known for a broad range of biological activities,[27] but only a limited number of quinazolinones have been reported from fungi. Examples include the benzomalvins and spiroquinazoline, substance P inhibitors from terrestrial fungi *Penicillium* sp.[28] and *Aspergillus flavipes*,[29] respectively; fumiquinazolines A−C, cytotoxic agents from a marine-derived *Aspergillus fumigatus*;[30] and dictyoquinazols A−C, moderate MAO inhibitors from the mushroom *Dictyophora indusiata*.[31] Although the secondary metabolites identified from this *Cordyceps*-colonizing strain of *Isaria farinosa* in the current and previous work are all nitrogen-containing, they cover broad structural classes including cyclopeptide, *N*-hydroxypyridone, arylhydrazone, and quinazolinone, implying that this class of fungi warrants further investigations.

## Experimental Section

**General Experimental Procedures.** Optical rotations were measured on a Perkin-Elmer 241 polarimeter, and UV data were recorded on a Shimadzu Biospec-1601 spectrophotometer. CD spectra were recorded on a JASCO J-815 spectropolarimeter, using MeOH as solvent. IR data were recorded using a Nicolet Magna-IR 750 spectrophotometer. $^1$H and $^{13}$C NMR data were acquired with Varian Mercury-400 and Inova-500 spectrometers using solvent signals (acetone-$d_6$: $\delta_H$ 2.05/$\delta_C$ 29.8, 206.1; $CD_3OD$; $\delta_H$ 3.35/$\delta_C$ 49.9; DMSO-$d_6$: $\delta_H$ 2.50/$\delta_C$ 39.5) as references. The HMQC and HMBC experiments were optimized for 145.0 and 8.0 Hz, respectively. ESIMS data were recorded on a Bruker Esquire 3000$^{plus}$ spectrometer, and HRESIMS data were obtained using Bruker APEX III 7.0 T and APEX II FT-ICR spectrometers, respectively. EIMS and HREIMS data were recorded on a Micromass GCT-MS spectrometer.

**Fungal Material.** The culture of *I. farinosa* was isolated by Dr. Mu Wang from a sample of *C. sinensis* (Berk.) Sacc. colleted in Linzhi, Tibet, in June 2004. The fungus was identified on the basis of sequence (Genbank accession no. AB233337.1) analysis of the ITS region of the rDNA and assigned the accession number XJC04-CT-303 in X.L.'s culture collection at the Institute of Microbiology, Chinese Academy of Sciences, Beijing. The fungal strain was cultured on slants of potato dextrose agar at 25 °C for 10 days. Agar plugs were cut into small pieces (about 0.5 × 0.5 × 0.5 cm$^3$) under aseptic conditions, 15 pieces were used to inoculate in three Erlenmeyer flasks (250 mL), each containing 50 mL of media (0.4% glucose, 1% malt extract, and 0.4% yeast extract), and the final pH of the media was adjusted to 6.5. After sterilization, three flasks of the inoculated media were incubated at 25 °C on a rotary shaker at 170 rpm for five days to prepare the seed culture. Spore inoculum was prepared by suspending the seed culture in sterile, distilled $H_2O$ to give a final spore/cell suspension of 1 × 10$^6$/mL. Fermentation was carried out in 12 Fernbach flasks (500 mL) each containing 80 g of rice. Distilled $H_2O$ (120 mL) was added to each flask, and the contents were soaked overnight before autoclaving at 15 psi for 30 min. After cooling to room temperature, each flask was inoculated with 5.0 mL of the spore inoculum and incubated at 25 °C for 40 days.

**Extraction and Isolation.** The fermented material was extracted with EtOAc (4 × 1.0 L), and the organic solvent was evaporated to dryness under vacuum to afford the crude extract (3.8 g), which was fractionated by silica gel VLC using *n*-hexanes−$CH_2Cl_2$−MeOH gradient elution. The fraction (124 mg) eluted with 100:2 $CH_2Cl_2$−MeOH was separated by Sephadex LH-20 column chromatography (CC) eluting with MeOH. The resulting subfractions were combined and further purified by RP HPLC (Agilent Zorbax SB-$C_{18}$ column; 5 $\mu$m; 9.4 × 250 mm; 77% MeOH in $H_2O$ for 2 min, followed by 77−100% for 45 min; 2 mL/min) to afford **2** (3.0 mg, $t_R$ 32.31 min), **6** (10.0 mg, $t_R$ 38.43 min), **1** (5.0 mg, $t_R$ 40.11 min), and **7** (2.5 mg, $t_R$ 43.42 min). The fraction (80 mg) eluted with 100:5 $CH_2Cl_2$−MeOH was separated by Sephadex LH-20 CC eluting with MeOH, and the resulting subfractions were purified by RP HPLC (35% MeOH in $H_2O$ for 5 min, followed by 35−72% for 45 min; 2 mL/min) to afford **5** (5.0 mg, $t_R$ 19.10 min), **3** (3.5 mg, $t_R$ 22.30 min), and **4** (3.0 mg, $t_R$ 23.82 min).

**Militarinone E (1):** yellow, amorphous powder; $[\alpha]^{25}_D$ −8.0 (*c* 0.25, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 207 (3.12), 219 (3.19), 241 (2.87) nm; CD (*c* 7.5 × 10$^{-3}$ M, $CH_2Cl_2$) $\lambda_{max}$ ($\Delta\varepsilon$) 390 (−1.1), 356 (−3.1) nm; IR (neat) $\nu_{max}$ 3386 (br), 2959, 2926, 1642, 1609, 1531, 1441, 1377, 1215, 1112 cm$^{-1}$; $^1$H, $^{13}$C NMR, and HMBC data see Table 1; Key NOESY correlations (DMSO-$d_6$, 400 MHz) H-8 ↔ H-10; H-9 ↔ H-11; H-10 ↔ H-8, $H_3$-21; H-11 ↔ H-9, H-13; H-13 ↔ H-11; $H_3$-19 ↔ $H_3$-21; $H_3$-21 ↔ H-10, $H_3$-19; HREIMS *m*/*z* 441.2519 (calcd for $C_{26}H_{35}NO_5$, 441.2515).

**Absolute Configuration of the Secondary Alcohol in 1 (refs 24, 25).** A sample of **1** (0.5 mg) was dissolved in a dry solution of [$Rh_2(OCOCF_3)_4$] complex (1.5 mg) in $CH_2Cl_2$ (200 $\mu$L) and was subjected to CD measurements at a concentration of 0.3 mg/mL The first CD spectrum was recorded immediately after mixing, and its time evolution was monitored until stationary (about 10 min after mixing). The inherent CD was subtracted. The observed sign of the E band at ca. 350 nm in the induced CD spectrum was correlated to the absolute configuration of the C-4′ secondary alcohol moiety.

**Militarinone F (2):** yellow, amorphous powder; $[\alpha]^{25}_D$ −32.5 (*c* 0.27, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 205 (3.17), 237 (3.13), 287

(3.07) nm; IR (neat) $\nu_{max}$ 3383 (br), 2959, 2928, 1642, 1607, 1452, 1376, 1229, 1112 $cm^{-1}$; $^1H$ and $^{13}C$ NMR data see Table 1; HMBC correlations ($CD_3OD$, 500 MHz) H-6 → C-2, 4, 1′; H-8 → C-7, 10; H-9 → C-7, 11; H-10 → C-8, 11, 12; H-11 → C-9, 13, 21; H-13 → C-11, 15, 20, 21; H-15a → C-13, 16, 19; H-15b → C-16, 19; H-16 → C-14, 18; H-17a → C-15, 18, 19; H-17b → C-16, 18, 19; $H_3$-18 → C-16; $H_3$-19 → C-15, 17; $H_3$-20 → C-13, 14,15; $H_3$-21 → C-11, 13; H-2′a → C-1′; H-2′b → C-3′; H-3′a → C-1′, 4′; H-3′b → C-1′; H-4′ → C-1′; H-5′a → C-1′, 4′; H-5′b → C-1′; H-6′a → C-1′; H-6′b → C-5′; NOESY correlations ($CD_3OD$, 500 MHz) H-9 ↔ H-11; H-10 ↔ $H_3$-21; H-11 ↔ H-9, H-13; H-13 ↔ H-11, $H_3$-20; H-14 ↔ $H_3$-19, $H_3$-21; H-15b ↔ $H_3$-20; $H_3$- 19 ↔ H-14; $H_3$-20 ↔ H-13, H-15b; $H_3$-21 ↔ H-10, H-14; HREIMS *m/z* 441.2520 (calcd for $C_{26}H_{35}NO$, 441.2515).

**Farylhydazone A (3):** colorless needles (MeOH−$H_2O$); mp 235−237 °C; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 205 (3.18), 232 (3.11), 285 (3.04) nm; IR (neat) $\nu_{max}$ 3392 (br), 1730, 1678, 1646, 1591, 1505, 1426, 1245, 1218, 1159 $cm^{-1}$; $^1H$, $^{13}C$ NMR, and HMBC data see Table 2; HRESIMS *m/z* 302.0742 (calcd for $C_{12}H_{13}N_3O_5Na$, 302.0747).

**X-ray Crystallographic Analysis of Faryhydrazone A (3) (ref 32).** Upon crystallization from MeOH−$H_2O$ (10:1) using the vapor diffusion method, colorless crystals were obtained for **3**. A crystal (0.23 × 0.15 × 0.15 mm) was separated from the sample and mounted on a glass fiber, and data were collected using a Rigaku RAPID IP diffractometer with graphite-monochromated Mo Kα radiation, $\lambda$ = 0.71073 Å at 173 (2) K. Crystal data: $C_{12}H_{17}N_3O_7$, $M$ = 315.29, space group tricinic, $P\bar{1}$; unit cell dimensions $a$ = 8.933(2) Å, $b$ = 9.339(2) Å, $c$ = 9.944(18) Å, $V$ = 721.5(3) $Å^3$, $Z$ = 2, $D_{calcd}$ = 1.451 $mg/m^3$, $\mu$ = 0.121 $mm^{-1}$, $F(000)$ = 332. The structure was solved by direct methods using SHELXL-97[33] and refined by using full-matrix least-squares difference Fourier techniques. All non-hydrogen atoms were refined with anisotropic displacement parameters, and all hydrogen atoms were placed in idealized positions and refined as riding atoms with the relative isotropic parameters. Absorption corrections were performed using the Siemens Area Detector Absorption Program (SADABS).[34] The 11 847 measurements yielded 3304 independent reflections after equivalent data were averaged, and Lorentz and polarization corrections were applied. The final refinement gave $R_1$ = 0.0531 and $wR_2$ = 0.1448 [$I > 2\sigma(I)$].

**Farylhydrazone B (4):** white powder; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 202 (2.72), 216 (2.81), 238 (2.79) nm; IR (neat) $\nu_{max}$ 3347 (br), 1724, 1661, 1586, 1513, 1454, 1375, 1275, 1236, 1160 $cm^{-1}$; $^1H$ and $^{13}C$ NMR data see Table 2; HRESIMS *m/z* 245.0530 (calcd for $C_{10}H_{10}N_2O_4Na$, 245.0532).

**2-(4-Hydroxybenzyl)quinazolin-4(3*H*)-one (5):** white powder; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 204 (2.78), 220 (2.86), 241 (2.83) nm; IR (neat) $\nu_{max}$ 3179 (br), 2922, 1686, 1618, 1515, 1468, 1455, 1333, 1255, 1242, 1172 $cm^{-1}$; $^1H$, $^{13}C$ NMR, and HMBC data see Table 2; HRESIMS *m/z* 275.0789 (calcd for $C_{15}H_{12}N_2O_2Na$, 275.0791).

**Militarinone A (6):** $^1H$, $^{13}C$ NMR, and the MS data were consistent with literature values.[9]

**Militarinone B (7):** $^1H$, $^{13}C$ NMR, and the MS data were consistent with literature values.[10]

**MTT Assay (ref 35).** The assay was run in triplicate. In a 96-well plate, each well was plated with $10^4$ cells. After cell attachment overnight, the medium was removed, and each well was treated with 50 μL of medium containing 0.2% DMSO, or appropriate concentrations of the test compounds, or the positive control cisplatin (10 mg/mL as stock solution of a compound in DMSO and serial dilutions; the test compounds showed good solubility in DMSO and did not precipitate when added to the cells). Cells were treated at 37 °C for 4 h in a humidified incubator at 5% $CO_2$ first and were allowed to grow for another 48 h after the medium was changed to fresh Dulbecco's modified Eagle medium. The medium was removed from the wells, and in the dark 50 μL of a solution containing 0.5 mg/mL MTT (Sigma) was dissolved in serum-free medium or phosphate-buffered saline (PBS) and then incubated at 37 °C for 3 h. Upon removal of MTT/medium, 100 μL of DMSO was added to each well and agitated at 60 rpm for 5 min to dissolve the precipitate. The assay plate was read at 540 nm using a microplate reader.

**Antimicrobial Assays.** Antibacterial and antifungal assays were conducted in triplicate following the National Center for Clinical Laboratory Standards (NCCLS) recommendations.[36,37] The bacteria, *Staphylococcus aureus* Col (CGMCC 1.2465) and *Streptococcus pneumoniae* (CGMCC 1.1692), and the yeast, *Candida albicans* (CGMCC 2.2086), were obtained from China General Microbial Culture Collection (CGMCC) and were grown on Mueller-Hinton broth (MHB) and Sabouraud dextrose broth (SDB) medium, respectively. Targeted microbes (3−4 colonies) were cultured in broth culture (the bacteria: 37 °C for 24 h; the yeast: 28 °C for 24 h), and the final suspensions of bacteria (in MHB medium) and yeast (in SDB medium) were $10^6$ and $10^5$ cells/mL, respectively. Test samples (4 mg/mL as stock solution in DMSO and serial dilutions) were transferred to 96-well clear plates in triplicate, and the suspension of the test organisms was added to each well, achieving a final volume of 200 μL (ampicillin and amphotericin B were used as the positive controls). After incubation, the absorbance at 595 nm was measured with a microplate reader (TECAN), and the inhibition was calculated and plotted versus test concentrations to afford the $IC_{50}$.

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**Supporting Information Available:** $^1H$ and $^{13}C$ NMR spectra of **1**−**5** and CD spectrum of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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