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Article

Proversilins A−E, Drimane-Type Sesquiterpenoids from the Endophytic *Aspergillus versicolor*

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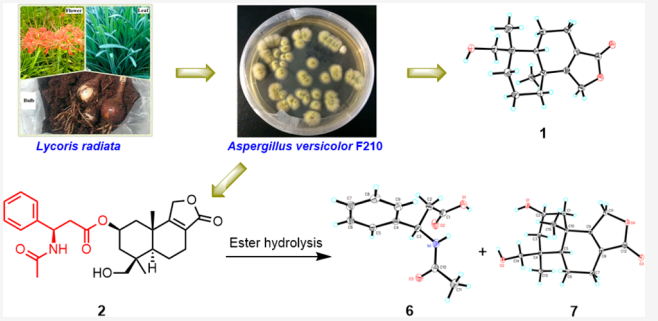
ABSTRACT: Five new drimane-type sesquiterpenoids, named proversilins A−E (**1**−**5**), were isolated from the endophytic fungus *Aspergillus versicolor* F210 isolated from the bulbs of *Lycoris radiata*. Their structures and absolute configurations were characterized by extensive spectroscopic analysis, including 1D and 2D NMR and HRESIMS data, comparison of experimental and calculated electronic circular dichroism data, and X-ray crystallography. Proversilins B−E (**2**−**5**) represent the first examples of natural products featuring an *N*-acetyl-β-phenylalanine moiety. Compounds **3** and **5** inhibited the growth of HL-60 cells with IC_{50} values of 7.3 and 9.9 μM, respectively.



Sesquiterpenoids, which are derived from three five-carbon isoprene units,[1] have attracted much attention from the scientific community because of their diverse chemical skeletons, as exemplified by chain, monocyclic, dicyclic, tricyclic, tetracyclic, and polymer architectures,[2] and their broad spectrum of bioactivities, such as antimalarial,[3] cytotoxic,[4] antibacterial,[5] and antiviral activities.[6] As a subclass, drimane-type sesquiterpenoids, which are widespread metabolites isolated from plant and microorganisms, are derived from (or related to) the sesquiterpenoid alcohol drimenol.[7] They have antifungal,[8] cytotoxic,[9] antifeedant, and insecticidal activities[10] and plant-growth regulatory[11] and neurological effects[12] and are a research target in the fields of agriculture, medical and health care, and chemistry and chemical engineering.[13]

In recent years, our research group has studied the chemical diversity of metabolites of *Aspergillus* fungi, which have become a research hotspot in natural products chemistry because of their biosynthetic capacities to produce structurally diverse and/or bioactive metabolites, such as alkaloids,[14,15] terpenoids,[16] meroterpenoids,[17] and cytochalasins.[18] As part of our ongoing commitment to discover additional structurally intriguing, and potentially biologically active, metabolites from *Aspergillus versicolor* F210, a fungus isolated from the bulbs of *Lycoris radiata*[19] was chemically investigated, resulting in five new drimane-type sesquiterpenoids, named proversilins A−E (**1**−**5**), being isolated. They were identified by interpretation of spectroscopic data. Proversilins B−E (**2**−**5**) represent the first examples of natural products featuring an *N*-acetyl-β-phenylalanine moiety. Herein, we describe the isolation, structure elucidation, and bioactivity evaluation of these compounds.

RESULTS AND DISCUSSION

Proversilin A (**1**), isolated as colorless needle crystals, was shown to have a molecular formula of $C_{15}H_{22}O_3$ by HRESIMS analysis, indicating five indices of hydrogen deficiency. The ^{1}H NMR spectrum showed two singlet methyl groups (δ_H 1.05 and 1.16) and four oxygenated protons [δ_H 4.73 (1H, dd, J = 16.9, 2.8 Hz), 4.65 (1H, dd, J = 16.9, 2.8 Hz), 3.75 (1H, d, J = 10.9 Hz), and 3.57 (1H, d, J = 10.9 Hz)]. Its ^{13}C and DEPT NMR spectra displayed 15 carbon resonances including two methyl groups, seven methylene carbons (two oxygenated), one methine carbon, and five non-proton-bearing carbons (one carbonyl and two olefinic). Analysis of the 1D NMR and ^{1}H−^{1}H COSY and HMBC spectra demonstrated that **1** was a normal drimane-type sesquiterpenoid (Figure 1).[7]

The key NOE correlations of Me-13 (δ_H 1.05)/H-5 (δ_H 1.37) and H_2-14 (δ_H 3.57 and 3.75)/Me-15 (δ_H 1.16) indicated that Me-13 and H-5 were cofacial and Me-15 was on the opposite face (Figure 2). Therefore, the relative configuration of **1** was determined. A recrystallization experiment furnished a suitable crystal of **1**, and a subsequent single-crystal X-ray diffraction analysis (Figure 3) was performed using Cu Kα radiation, which confirmed the absolute configuration of **1** to be 4*S*,5*R*,10*S* with a Flack parameter of −0.01(3). Accordingly, compound **1** was defined and named proversilin A.

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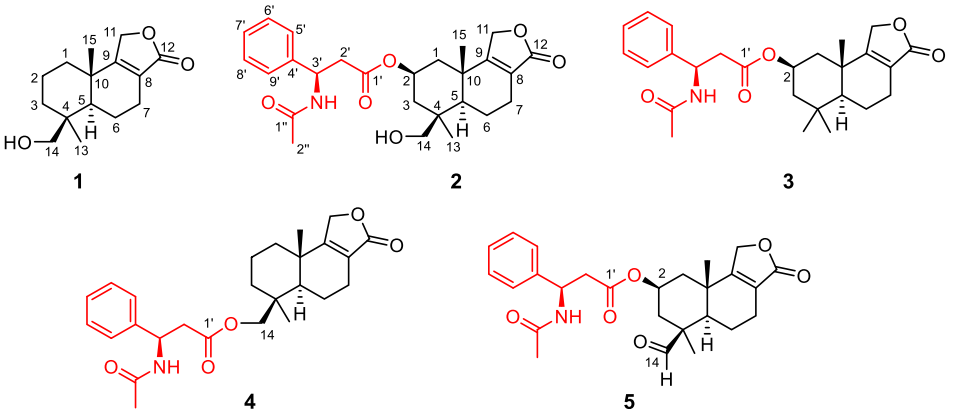
Chart 1



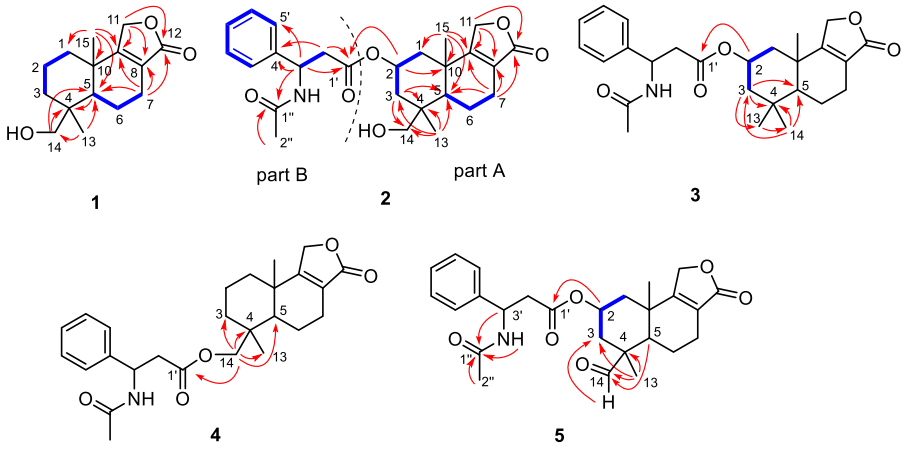


Figure 1. Key $^{1}H-^{1}H$ COSY correlations (blue bonds) and key HMBC correlations (red arrows) of proversilins A–E (**1**–**5**).

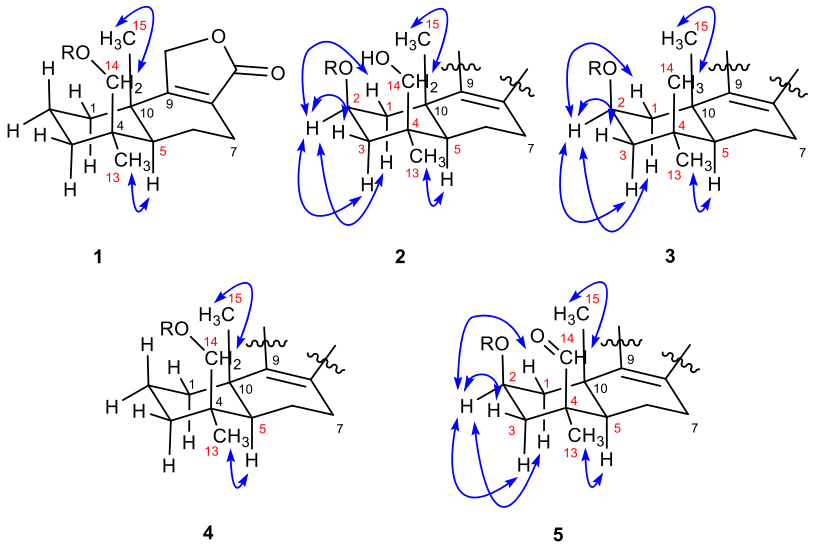


Figure 2. Key NOESY correlations (blue arrows) of proversilins A–E (**1**–**5**).

Proversilin B (**2**) was isolated as a white amorphous powder, and its molecular formula was determined to be $C_{26}H_{33}NO_6$, based on the ^{13}C NMR data and HRESIMS analysis, suggesting 11 indices of hydrogen deficiency. The IR spectrum showed the presence of hydroxy (3421 cm^{-1}), ester carbonyl (1736 cm^{-1}), and aromatic ring (1661, 1543, and 1445 cm^{-1}) groups. The ^{1}H NMR spectrum showed three singlet methyl groups at δ_H 1.07 (Me-13), 1.28 (Me-15), 1.96 (Me-2′), five aromatic protons at δ_H 7.24–7.37, and signals attributable to methylene and methine groups. Moreover, 26 carbon resonances were revealed in the ^{13}C and DEPT NMR spectra and were assigned to three methyl groups, seven methylene carbons (two oxygenated), eight methine carbons (five olefinic and one oxygenated), and eight non-proton-bearing carbons

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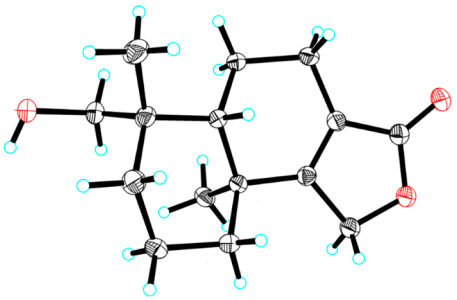

Figure 3. ORTEP drawing of compound **1**.

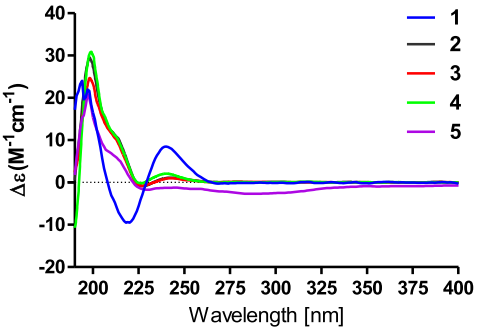

Figure 4. Experimental ECD spectra of compounds **1**−**5**.

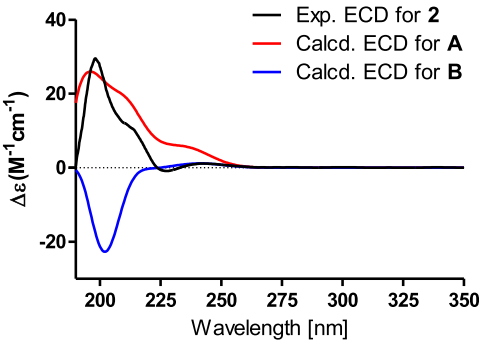

Figure 5. Comparison of experimental ECD spectrum of proversilin B (**2**) and calculated ECD curves of **A** (2*S*,4*S*,5*R*,10*S*,3′*R*) and **B** (2*S*,4*S*,5*R*,10*S*,3′*S*).

(three carbonyl and three olefinic). Additionally, the presence of an amino group (−NH−) was assigned on the basis of the HRESIMS data.

By interpretation of the ^{1}H− ^{1}H COSY and HMBC spectra, the planar structure of **2** was elucidated. A sesquiterpene lactone (part A) was established on the basis of two spin-coupling systems of H_2-1/H-2/H_2-3 and H-5/H_2-6/H_2-7 and key HMBC correlations from Me-13 to C-3, C-4, C-5, and C-14, from Me-15 to C-1, C-5, C-9, and C-10, from H_2-7 to C-5, C-8, C-9, and C-12, and from H_2-11 to C-8, C-9, and C-12 (Figure 1). The core structure of part A was similar to compound **1**. A monosubstituted benzene group was characterized by the spin-coupling system of H-5′/H-6′/H-7′/H-8′/H-9′. The ^{1}H−^{1}H COSY spectrum showed a cross-peak of H_2-2′/H-3′. The HMBC spectrum displayed correlations from H_2-2′ to C-1′ and C-4′, from H-3′ to C-1′, C-4′, C-5′, and C-1″, and from Me-2″ to C-1″. Comprehensive analyses of the above information suggested a substructure of *N*-acetyl-*β*-phenylalanine (part B). The linkage of parts A and B was established by an ester bond on the basis of the key HMBC correlation from H-2 to C-1′ (Figure 1) and the downfield chemical shift of CH-2 (δ_H 5.15; δ_C 71.5). Thus, the planar structure of **2** was confirmed.

The relative configuration of part A was revealed by the NOESY spectrum. The correlation between Me-15 and H_2-14 indicated that they were on the same face and were both assigned as *β*-orientations. Meanwhile, H-5 was determined to be *α*-oriented by a key NOESY correlation of Me-13/H-5. Additionally, the NOESY cross-peaks of H-2/H_{ax}-1, H-2/H_{eq}-1, H-2/H_{ax}-3, and H-2/H_{eq}-3 indicated that H-2 was *α*-oriented by analyzing the preponderant chair conformation of a cyclohexane (Figure 2). The absolute configuration (2*S*,4*S*,5*R*,10*S*) of part A was deduced based on the shared biogenesis of drimane-type sesquiterpenoids showing the absolute configuration of 5*R*,10*S*.[7]

Quantum chemical ECD calculation is one of the most powerful and reliable methods to address the configurational problems of natural products when useful crystals are hard to obtain.[20] To define the absolute configurations of C-3′, the DFT-calculated ECD spectra of the lowest-energy conformers of a pair of epimers [**A** (2*S*,4*S*,5*R*,10*S*,3′*R*); **B** (2*S*,4*S*,5*R*,10*S*,3′*S*)] were performed at the B3LYP/6-311++G(2d,p) level. The results implied that the absolute configuration of **2** was 2*S*,4*S*,5*R*,10*S*,3′*R* (Figure 5). To confirm the above conclusion, the ester hydrolysis reaction for **2** was carried out,[21] which furnished two hydrolysates, (3*R*)-*N*-acetyl-*β*-phenylalanine (**6**) and a sesquiterpenoid (**7**, named proversilin F), and their planar and absolute structures were confirmed by the single-crystal X-ray diffraction analyses (Figure 6). Accordingly, the structure of **2** (2*S*,4*S*,5*R*,10*S*,3′*R*) was defined.

The HRESIMS data of proversilin C (**3**) pinpointed that its molecular formula was $C_{26}H_{33}NO_5$, indicating an oxygen atom less than that of **2**. The 1D and 2D NMR data of **3** showed the coexistence of a drimane-type sesquiterpenoid and an *N*-acetyl-phenylalanine substructure, whose framework was similar to that of **2**. Evident differences were the presence of a methyl group and the absence of a hydroxymethyl group in **3**, which were supported by a singlet methyl group (δ_H 1.09) in the ^{1}H NMR spectrum and the observable HMBC correlations from Me-14 (δ_H 1.09) to C-3 (δ_C 44.5), C-4 (δ_C 33.4), and C-5 (δ_C 51.3), from H_2-3 (δ_H 1.55 and 1.78) to C-4, C-5, and C-13 (δ_C 33.9), and from Me-13 (δ_H 0.99) to C-14 (Figure 1). H-5 (δ_H 1.41) and Me-15 (δ_H 1.29, δ_C 23.0) were determined to have the opposite orientations based upon the NOESY correlations of Me-13/H-5 and Me-14/Me-15 (Figure 2). The H-2 was unambiguously confirmed to be equatorial (*α*-orientation) via the strong NOESY correlations of H-2 with H_{ax}-1, H_{eq}-1, H_{ax}-3, and H_{eq}-3 (Figure 2), similar to those of **2**. The similar ECD curves of **2** and **3** suggested the absolute configuration of **3** to be 2*R*,5*S*,10*S*,3′*R*.

The molecular formula of proversilin D (**4**) was determined to be $C_{26}H_{33}NO_5$ from HRESIMS data. The ^{1}H and ^{13}C NMR spectra showed that **4** was also a drimane-type sesquiterpenoid derivative, bearing an *N*-acetyl-*β*-phenylalanine moiety. Comparing the 1D NMR data of **4** with those of **2**, the presence of a methylene group and the absence of a methine group in **4** was the major difference, which was supported by the ^{1}H− ^{1}H COSY correlations of H_2-1/H_2-2/H_2-3. Analysis of the HMBC spectrum revealed that an *N*-acetyl-*β*-phenyl-alanine (part B) group was linked to C-14 by an ester bond on the basis of the HMBC correlations from H_2-14 to C-3, C-4,

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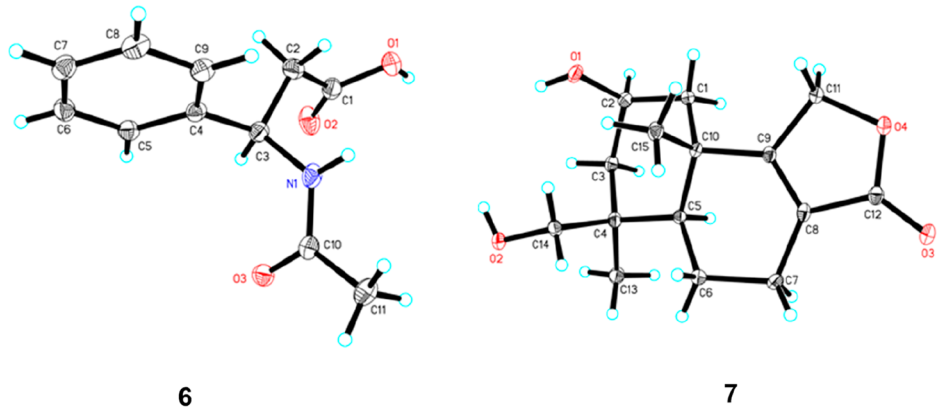

Figure 6. ORTEP drawings of compounds **6** and **7** (ester hydrolysates of **2**).

Table 1. ^{1}H NMR Data of Compounds 1−5 (δ in ppm, J in Hz)

no.	1[a]	2[b]	3[b]	4[b]	5[a]
1	1.37, m	1.74, m	1.70, m	1.44, m	1.56, m
	1.68, m	1.87, m	1.84, m	1.73, m	1.86, m
2	1.56, m	5.15, p (3.9)	5.13, m	1.53, m	5.10, m
	2.00, m			1.68, m	
3	1.06, m	1.34, dd (15.3, 4.0)	1.55, dd (15.0, 4.2)	1.10, m	1.32, dd (15.4, 4.1)
	1.87, m	2.13, m	1.78, ddd (15.0, 3.5, 1.9)	1.68, m	2.52, m
5	1.37, m	1.53, dd (12.7, 1.5)	1.41, dd (12.5, 1.9)	1.42, m	1.58, m
6	1.56, m	1.71, m	1.65, m	1.57, m	1.97, m
	2.00, m	2.06, m	1.96, m	1.99, m	2.21, m
7	2.14, m	2.09, m	2.11, m	2.09, m	2.13, m
	2.38, m	2.31, m	2.35, dd (17.6, 6.2)	2.31, m	2.44, m
11	4.65, dd (16.9, 2.8)	4.75, m	4.73, ddd (17.3, 3.6, 1.6)	4.75, ddd (17.4, 3.5, 1.6)	4.58, m
	4.73, dd (16.9, 2.8)	4.83, m	4.82, m	4.87, m	4.63, m
13	1.05, s	1.07, s	0.99, s	0.99, s	1.06, s
14	3.57, d (10.9); 3.75, d (10.9)	3.61, d (10.9); 3.91, d (10.9)	1.09, s	4.00, d (11.1); 4.24, d (11.1)	9.64, s
15	1.16, s	1.28, s	1.29, s	1.15, s	1.08, s
2′		2.79, dd (15.1, 6.8); 2.85, dd (15.1, 8.3)	2.77, dd (15.1, 7.8); 2.86, dd (15.1, 7.8)	2.85, m; 2.90, m	2.69, d (1.7); 2.71, d (3.5)
3′		5.35, dd (8.3, 6.8)	5.36, t (7.8)	5.36, t (7.5)	5.36, m
5′		7.34, m	7.33, m	7.34, m	7.26, m
6′		7.35, m	7.35, m	7.33, m	7.27, m
7′		7.28, m	7.28, m	7.25, m	7.19, m
8′		7.35, m	7.35, m	7.33, m	7.27, m
9′		7.34, m	7.33, m	7.34, m	7.26, m
2″		1.96, s	1.95, s	1.94, s	1.96, s
NH					6.64, d (7.9)

[a]Recorded at 400 MHz in $CDCl_3$. [b]Recorded at 400 MHz in CD_3OD.

C-5, C-13, and a carbonyl carbon (C-1′). Thus, the gross structure of **4** was determined. Together with the NOESY cross-peaks of H_2-14/Me-15 and H-5/Me-13, the ECD curve of **4** (Figure 4) was similar to that of **2**, showing that the absolute configuration of **4** was 4*S*,5*R*,10*S*,3′*R*.

Proversilin E (**5**) had a molecular formula of $C_{26}H_{31}NO_6$, as designated by the HRESIMS and ^{13}C NMR data. The structure with an *N*-acetyl-*β*-phenylalanine moiety linked to a drimane-type sesquiterpenoid at C-2 by an ester bond was confirmed by analyzing the 1D and 2D NMR data. This compound possesses a formyl group at C-14, which was supported by the HSQC cross-peak (δ_H 9.64 and δ_C 203.5) and the HMBC correlations from H-14 to C-3 (δ_C 37.0), from H-5 (δ_H 1.58) to C-14, and from Me-13 (δ_H 1.06) to C-3, C-4 (δ_C 47.2) and C-14 (Figure 1). Therefore, the planar structure of **5** was determined. Similar NOESY correlations (Figure 2) of H-2/H_{ax}-1, H-2/H_{eq}-1, H-2/H_{ax}-3, H-2/H_{eq}-3, H-5/H-14, and Me-13/Me-15 (δ_H 1.08) as well as the ECD curve (Figure 4) signified the absolute configuration of **5** to be 2*S*,4*S*,5*R*,10*S*,3′*R*.

Compounds **1**−**5** were evaluated for *in vitro* cytotoxicity against human tumor HL-60, SMMC-7721, A-549, MCF-7, and SW-480 cell lines and the normal colonic epithelial cells NCM460. As shown in Table 3, compounds **3** and **5** showed moderate cytotoxic activity with IC_{50} values ranging from 7.3 to 28.4 μM.[15,22]

EXPERIMENTAL SECTION

General Experimental Procedures. An X-5 microscopic melting point apparatus (Beijing Tech) was used, and the reported melting

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Table 2. ^{13}C NMR Data of Compounds 1−5 (δ in ppm)

no.	1[a]	2[b]	3[b]	4[b]	5[a]
1	36.2, CH_2	39.2, CH_2	39.1, CH_2	36.9, CH_2	38.6, CH_2
2	18.1, CH_2	71.5, CH	71.8, CH	19.1, CH_2	68.5, CH_2
3	35.5, CH_2	38.9, CH_2	44.5, CH_2	37.4, CH_2	37.0, CH_2
4	38.5, C	39.0, C	33.4, C	38.0, C	47.2, C
5	51.8, CH	51.7, CH	51.3, CH	52.9, CH	51.0, CH
6	18.4, CH_2	19.0, CH_2	18.8, CH_2	19.5, CH_2	17.7, CH_2
7	21.8, CH_2	22.6, CH_2	22.2, CH_2	22.7, CH_2	21.6, CH_2
8	123.5, C	123.5, C	123.5, C	124.0, C	123.6, C
9	170.3, C	172.9, C	173.2, C	173.2, C	168.0, C
10	36.4, C	37.1, C	37.3, C	37.7, C	36.0, C
11	68.2, CH_2	70.1, CH_2	70.1, CH_2	70.1, CH_2	68.2, CH_2
12	174.5, C	176.9, C	177.0, C	177.0, C	173.7, C
13	26.9, CH_3	27.8, CH_3	33.9, CH_3	27.8, CH_3	24.5, CH_3
14	65.3, CH_2	66.4, CH_2	23.5, CH_3	68.5, CH_2	203.5, CH
15	21.3, CH_3	23.6, CH_3	23.0, CH_3	21.2, CH_3	22.0, CH_3
1′		171.3, C	171.3, C	172.3, C	169.9, C
2′		42.7, CH_2	42.5, CH_2	41.8, CH_2	41.1, CH_2
3′		51.8, CH	51.7, CH	51.7, CH	50.0, CH
4′		142.7, C	142.6, C	142.5, C	140.9, C
5′		127.7, CH	127.8, CH	127.7, CH	126.2, CH
6′		129.7, CH	129.7, CH	129.7, CH	128.7, CH
7′		128.6, CH	128.7, CH	128.7, CH	127.6, CH
8′		129.7, CH	129.7, CH	129.7, CH	128.7, CH
9′		127.7, CH	127.8, CH	127.7, CH	126.2, CH
1″		172.4, C	172.3, C	172.2, C	169.9, C
2″		22.6, CH_3	22.7, CH_3	22.6, CH_3	23.2, CH_3

[a]Recorded at 100 MHz in $CDCl_3$. [b]Recorded at 100 MHz in CD_3OD.

points were uncorrected. Optical rotations were recorded by an AUTOPOL IV-T automatic polarimeter (Rudolph Research Analytical, Hackettstown, NJ, USA) in MeOH. A PerkinElmer Lambda 35 UV spectrophotometer (PerkinElmer, Inc., Fremont, CA, USA) was used to measure UV spectra. ECD curves and FT-IR spectra were collected by a JASCO-810 ECD spectrometer (JASCO Co., Ltd., Tokyo, Japan) and a Bruker Vertex 70 instrument (Bruker, Karlsruhe, Germany), respectively. NMR spectra were acquired on Bruker AM-400 and -600 spectrometers (Bruker, Karlsruhe, Germany). The ^1H and ^{13}C NMR chemical shifts were referenced to the solvent or solvent impurity peaks for CD_3OD (δ_H 3.31 and δ_C 49.0) and $CDCl_3$ (δ_H 7.26 and δ_C 77.0). HRESIMS data of all compounds were obtained on a Thermo Fisher LTQ XL spectrometer (Thermo Fisher, Palo Alto, CA, USA). Column chromatography (CC) was performed with silica gel (80−120, 100−200, and 200−300 mesh; Qingdao Marine Chemical Inc., China), ODS (50 μm, YMC, Tokyo, Japan), and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Sweden). Semipreparative HPLC was measured on an Agilent 1260 with dual pumps and a DAD detector by an RP-C_{18} column (5 μm, 10 × 250 mm, Welch Ultimate XB-C_{18}). Thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} (Yantai Chemical Industry Research Institute, Yantai, China).

Fungal Material. The strain *Aspergillus versicolor* F210 was isolated from the bulbs of *Lycoris radiata*, collected in Yichang City of Hubei Province and identified by Prof. Jianping Wang of Huazhong University of Science and Technology. The sequence data for this strain have been submitted to DDBJ/EMBL/GenBank under accession No. MG821480. A voucher sample (no. TJ-LHQ-AVF210) has been deposited in the culture collection of Tongji Medical College, Huazhong University of Science and Technology, China.

Fermentation and Extraction. The strain was cultured on potato dextrose agar (PDA) at 28 °C for 7 days and then inoculated into 400 sterilized 1 L Erlenmeyer flasks, each containing 250 g of rice and 250 mL of H_2O. After incubating for 30 days at 28 °C, each flask was soaked in 300 mL of 95% EtOH for 24 h. Then, the cultures were poured into buckets (100 L), and the solvent was decanted and removed under reduced pressure. The cultures were soaked with the recycled EtOH seven times (24 h each time) until the solvent extract was almost colorless at room temperature. The dry extract was suspended in water (2 L) and extracted with EtOAc (1:1, v/v) three times.

Purification. The dry EtOAc extract (520 g) was subjected to a silica gel CC eluted with petroleum ether (PE)−EtOAc (25:1−0:1, v/v) progressively to obtain seven major fractions (Fr.1−Fr.7). Fr.5 (PE−EtOAc, 3:1, v/v, 42 g) was subjected to RP-C_{18} silica gel CC eluted with MeOH−H_2O (20:80−100:0, v/v, gradient system) to obtain seven subfractions (Fr.5.1−Fr.5.7). The second subfraction (Fr.5.2, 6.3 g, 20−40% MeOH−H_2O, v/v) was subsequently fractionated by a Sephadex LH-20 column (MeOH) to get four parts (Fr.5.2.1−Fr.5.2.4). Fr.5.2.1 (1.2 g) was fractionated by a silica gel CC with CH_2Cl_2−MeOH to afford compound **2** (23 mg). Fr.5.2.2 (220 mg) was further purified by semipreparative HPLC (MeOH−H_2O, 35:65, v/v, 2 mL/min) to acquire compounds **3** (3.8 mg, t_R = 13.3 min), **4** (6.6 mg, t_R = 22.1 min), and **5** (2.2 mg, t_R = 18.6 min). The subfraction Fr.5.3 (11.2 g, 40% MeOH−H_2O, v/v) was subjected to Sephadex LH-20 (MeOH) to get three parts (Fr.5.3.1−Fr.5.3.3). Fr.5.3.2 (2.6 g) was applied to a silica gel column eluted with CH_2Cl_2−MeOH (50:0−10:1, v/v) to produce six parts (Fr.5.3.2.1−Fr.5.3.2.6). The fourth part (Fr.5.3.2.4, 134 mg) was separated by semipreparative HPLC (MeOH−H_2O, 45:55, v/v, 2.0 mL/min) to give compound **1** (28 mg, t_R = 17.4 min).

Proversilin A (1): $C_{15}H_{22}O_3$; colorless needle crystals; mp 185−186 °C; $[\alpha]^{25}_D$ +23.0 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 218 (4.16) nm; IR (KBr) ν_{max} 3434, 2962, 2930, 1759, 1741, 1669, 1443, 1383, 1187, 1019 cm^{-1}; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 199 (+18.3), 220 (−9.5), 240 (+8.5) nm; ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) data, see Tables 1 and 2; HRESIMS $[M + Na]^+$ *m*/*z* 273.1465 (calcd for $C_{15}H_{22}NaO_3^+$, 273.1461).

Proversilin B (2): $C_{26}H_{33}NO_6$; white amorphous powder; $[\alpha]^{25}_D$ +73.8 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.30) nm; IR (KBr) ν_{max} 3421, 2954, 2929, 1736, 1661, 1543, 1445, 1376, 1296, 1028 cm^{-1}; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 198 (+29.5) and 243 (+1.3) nm; ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) data, see Tables 1 and 2; HRESIMS $[M + H]^+$ *m*/*z* 456.2362 (calcd for $C_{26}H_{34}NO_6^+$, 456.2381).

Table 3. Cytotoxicity of Compounds 1−5 against Human Tumor Cell Lines[a]

compound	HL-60	SMMC-7721	A-549	MCF-7	SW-480	NCM460
1	>40	>40	>40	>40	>40	>40
2	>40	>40	>40	>40	>40	>40
3	7.3 ± 1.2	12.6 ± 0.9	15.0 ± 0.8	11.8 ± 0.5	12.4 ± 0.4	>40
4	>40	>40	>40	>40	>40	>40
5	9.9 ± 1.4	19.4 ± 0.7	28.4 ± 1.2	18.3 ± 1.2	16.4 ± 1.0	>40
cis-platin[b]	1.9 ± 0.8	10.8 ± 0.6	14.0 ± 0.7	18.5 ± 0.4	10.1 ± 1.0	6.3 ± 0.6
paclitaxel[b]	<0.008	<0.008	<0.008	<0.008	<0.008	<0.008

[a]Results are expressed as IC_{50} values in μM. [b]*cis*-Platin and paclitaxel were used as positive controls.

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*Proversilin C (**3**):* $C_{26}H_{33}NO_5$; white amorphous powder; $[\alpha]^{25}_D$ +59.0 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 207 (4.28) nm; IR (KBr) ν_{max} 3422, 2928, 1738, 1660, 1542, 1443, 1380, 1165, 1025 cm^{-1}; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 198 (+24.7) and 243 (+1.0) nm; ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) data, see Tables 1 and 2; HRESIMS $[M + H]^+$ *m*/*z* 440.2440 (calcd. for $C_{26}H_{34}NO_5^+$, 440.2431).

*Proversilin D (**4**):* $C_{26}H_{33}NO_5$; white amorphous powder; $[\alpha]^{25}_D$ +68.0 (*c* 0.4, MeOH); UV (MeOH) λ_{max} (log ε) 206 (4.29) nm; IR (KBr) ν_{max} 3417, 2928, 1740, 1662, 1544, 1449, 1378, 1164, 1027 cm^{-1}; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 199 (+30.8) and 240 (+2.1) nm; ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) data, see Tables 1 and 2; HRESIMS $[M + H]^+$ *m*/*z* 440.2421 (calcd for $C_{26}H_{34}NO_5^+$, 440.2431).

*Proversilin E (**5**):* $C_{26}H_{31}NO_6$; white amorphous powder; $[\alpha]^{25}_D$ +44.4 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 206 (4.29) nm; IR (KBr) ν_{max} 3419, 2925, 1733, 1668, 1541, 1445, 1381, 1167, 1027 cm^{-1}; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 197 (+20.9) and 284 (−2.9) nm; ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) data, see Tables 1 and 2; HRESIMS $[M + Na]^+$ *m*/*z* 476.2071 (calcd for $C_{26}H_{31}NNaO_6^+$, 476.2044).

Ester Hydrolysis Reaction. Aqueous NaOH (1 M, 1.5 equiv) was combined with tetrahydrofuran (THF, $V_{THF} = 2.5V_{NaOH}$) and cooled to 0 °C. Substrate **2** (13.6 mg) was dissolved in the solution and then warmed to rt and stirred overnight. Afterward, aqueous HCl (2 M) was added to the solution to acidify to pH 1 and then evaporated under reduced pressure. The products were dissolved in MeOH−H_2O (1:1, 2 mL) and isolated by semipreparative HPLC (MeCN−H_2O, 20:80 to 40:60, v/v, *t* = 50 min, 2 mL/min) to acquire compounds **6** (3.8 mg, t_R = 14.6 min) and **7** (6.2 mg, t_R = 33.7 min).

*(R)-N-acetyl-β-phenylalanine (**6**):* $C_{11}H_{13}NO_3$, colorless block crystals; mp 195−196 °C; $[\alpha]^{25}_D$ +75.0 (*c* 0.2, MeOH); ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 213 (+8.40) nm; ^1H NMR (600 MHz) and ^{13}C NMR (150 MHz) data, see Table S1; HRESIMS $[M + H]^+$ *m*/*z* 208.0979 (calcd for $C_{11}H_{14}NO_3^+$, 208.0968).

*Proversilin F (**7**):* $C_{15}H_{22}O_4$, colorless needle crystals; mp 189−190 °C; $[\alpha]^{25}_D$ +124.3 (*c* 0.2, MeOH); ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) data, see Table S1; HRESIMS $[M + H]^+$ *m*/*z* 267.1582 (calcd for $C_{15}H_{23}O_4^+$, 267.1591).

X-ray Crystal Structure Analysis. The crystals of compounds **1**, **6**, and **7** were obtained in MeOH by the solvent vapor diffusion method. A suitable needle crystal of **1** was analyzed on a Rigaku XtaLAB PRO MM007HF. ShelXL was used to analyze the structure solution program. Refinement was measured by a least-squares procedure. The crystals of **6** and **7** were collected on a Rigaku Oxford Diffraction Supernova Dual Source. The data were collected and processed using CrysAlisPro. The structures were solved by direct methods using Olex2 software, and the non-hydrogen atoms were located from the trial structure and then refined anisotropically with SHELXL-2018 using a full-matrix least-squares procedure. The crystallographic data for **1** (deposition no. CCDC 1908632), **6** (deposition no. CCDC 2004222), and **7** (deposition no. CCDC 2004221) have been deposited in the Cambridge Crystallographic Data Centre.

*Crystal data for proversilin A (**1**):* $C_{15}H_{22}O_3$, M = 250.32, *a* = 9.77217(5) Å, *b* = 9.81597(5) Å, *c* = 13.76057(8) Å, α = 90°, β = 90°, γ = 90°, *V* = 1319.959(12) $Å^3$, *T* = 100.01(10) K, space group $P2_12_12_1$, *Z* = 4, μ(Cu Kα) = 0.689 mm^{-1}, density (calcd) is 1.260 g/cm^3, 28 879 reflections collected, 2658 independent reflections (R_{int} = 0.0190) were used in all calculations. The final R_1 was 0.0267 and wR_2 was 0.0684 ($I > 2\sigma(I)$). Flack parameter was −0.01(3).

*Crystal data for (R)-N-acetyl-β-phenylalanine (**6**):* $C_{11}H_{13}NO_3$, M = 207.22, *a* = 5.42071(10) Å, *b* = 12.6801(3) Å, *c* = 14.9194(3) Å, α = 90°, β = 90°, γ = 90°, *V* = 1025.49(4) $Å^3$, *T* = 100.00(10) K, space group $P2_12_12_1$, *Z* = 4, μ (Cu Kα) = 0.812 mm^{-1}, density (calcd) is 1.342 g/cm^3, 12 007 reflections were collected, 2064 independent reflections (R_{int} = 0.0396, R_{sigma} = 0.0226) were used in all calculations. The final R_1 was 0.0448 and wR_2 was 0.1128 ($I > 2\sigma(I)$). Flack parameter was 0.07(9) and Hooft parameter was 0.10(9).

*Crystal data for proversilin F (**7**):* $C_{15}H_{22}O_4$, M = 266.32, *a* = 6.34756(13) Å, *b* = 7.9249(2) Å, *c* = 13.5609(3) Å, α = 90°, β = 93.6003(19)°, γ = 90°, *V* = 680.82(3) $Å^3$, *T* = 100.00(10) K, space group $P2_1$, *Z* = 2, μ(Cu Kα) = 0.758 mm^{-1}, density (calcd) is 1.299 g/cm^3, 7784 reflections were collected, 2533 independent reflections (R_{int} = 0.0475, R_{sigma} = 0.0300) were used in all calculations. The final R_1 was 0.0384 and wR_2 was 0.1027 ($I > 2\sigma(I)$). Flack parameter was 0.06(13) and Hooft parameter was 0.06(7).

ECD Calculations for Proversilin B (2). The conformation optimization, ECD spectrum calculation, and final ECD combination were performed as previously described.[23]

Cytotoxicity Assay. The cytotoxic activity of compounds **1**−**5** was evaluated against HL-60 (acute leukemia), SMMC-7721 (liver cancer), A-549 (lung cancer), MCF-7 (breast cancer), SW-480 (colon cancer), and normal colonic epithelial cells, NCM460. The cell survival assay was performed using the MTT method. Each tumor cell line was exposed for 48 h to the tested compounds at concentrations ranging from 0.0625 to 40 μM, with *cis*-platin and paclitaxel as positive controls. The absorbance at 570 nm was measured and data are expressed as averages of three replicates. The IC_{50} values were calculated by using a standard dose−response curve fitting with Prism (version 5.0, GraphPad Software, La Jolla, CA, USA).[15,22]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.0c00298.

1D and 2D NMR, HRESIMS, IR, and UV spectra of compounds **1**−**5** and 1D NMR and HRESIMS spectra of **6** and **7** (PDF)

X-ray crystallographic data of **1** (CIF)

X-ray crystallographic data of **6** (CIF)

X-ray crystallographic data of **7** (CIF)

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Notes

The authors declare no competing financial interest.

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