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Isolation and Characterization of New *p*-Terphenyls with Antifungal, Antibacterial, and Antioxidant Activities from Halophilic Actinomycete *Nocardiopsis gilva* YIM 90087

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Supporting Information

ABSTRACT: A new *p*-terphenyl **1** and a novel *p*-terphenyl derivative **3** bearing a benzothiazole moiety were isolated from halophilic actinomycete *Nocardiopsis gilva* YIM 90087, along with known *p*-terphenyl **2**, antibiotic novobiocin **4**, cyclodipeptides **5**−**13**, and aromatic acids **14** and **15**. Their structures were elucidated on the basis of the interpretation of spectral data and by comparison of the corresponding data with those reported previously. The *p*-terphenyl **1** showed antifungal activity against the three pathogenic fungi, including *Fusarium avenaceum*, *Fusarium graminearum*, and *Fusarium culmorum*, that caused Fusarium head blight with minimal inhibitory concentrations (MICs) of 8, 16, and 128 μg/mL, respectively. Compound **1** showed antifungal activity against *Candida albicans* with a MIC of 32 μg/mL and antibacterial activity against *Bacillus subtilis* with a MIC of 64 μg/mL. Novobiocin **4** showed antifungal activity against *Pyricularia oryzae* with a MIC of 16 μg/mL and antibacterial activity against *B. subtilis* with a MIC of 16 μg/mL and *Staphylococcus aureus* with a MIC of 64 μg/mL. The 1,1-diphenyl-2-picryl-hydrazyl assay suggested that **1**, **3**, and **4** exhibited 54.9% (2 mg/mL), 14.3% (4 mg/mL), and 47.7% (2 mg/mL) free radical scavenging activity, respectively. The positively charged 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid radical ($ABTS^{+\bullet}$) scavenging assay indicated that **1**, **3**, **4**, and **8** exhibited 68.6% (1 mg/mL), 28.4% (2 mg/mL), 78.2% (0.5 mg/mL), and 54.6% (2 mg/mL) $ABTS^{+\bullet}$ scavenging capacity, respectively. The superoxide anion radical scavenging assay suggested that **4** exhibited 77.9% superoxide anion radical scavenging capacity at 2 mg/mL. *N. gilva* YIM 90087 is a new resource for novobiocin **4**.

KEYWORDS: *halophilic actinomycete, Nocardiopsis gilva, p-terphenyl, benzothiazole, antifungal, antibacterial, antioxidant*

INTRODUCTION

Fusarium head blight (FHB) is a major fungal disease caused by an infection with a fungus of the genus *Fusarium*. FHB affects maize, wheat, and small-grain cereals, including barley, oats, rye, and triticale, in all cereal-growing areas of the world, which results in substantial reductions in crop yield and quality (aborted or shriveled seed and reduced seed size) and economically devastating losses around the world. Additionally, the contamination of mycotoxins produced by these pathogenic fungi is harmful to human beings and animals.[1] Three species, *Fusarium graminearum*, *Fusarium culmorum*, and *Fusarium avenaceum*, are known to be most frequently involved in FHB. Their geographical distribution is influenced by climatic conditions, especially temperature and humidity, and the species composition may vary from year to year and from region to region.[2] However, *F. avenaceum* has been isolated from and identified in diseased crops over a range of climatic zones. Usually, it is predominant in colder areas such as Central and Northern Europe and North America.[3] *F. avenaceum* is also a member of the group of root rot and seedling disease complexes of wheat.[4] The demand for potent natural chemicals to inhibit the infection of *Fusarium* species is growing.

In the course of our program initiated for the discovery of new actinomycetes, more than 100 halophilic actinomycete strains were isolated from various regions of China.[5] Little is known about the secondary metabolites of the halophilic actinomycetes.[6,7] However, our preliminary biological activity screening experiments suggested that the fermentation broth of halophilic actinomycete *Nocardiopsis gilva* YIM 90087 (YIM 90087^T = KCTC 19006^T = CCTCC $AA2040012^T$ = DSM 44841^T)[5] exhibited antifungal and antibacterial activities. Herein, we report the isolation and structural characterization of the secondary metabolites, including one new *p*-terphenyl **1**, one novel *p*-terphenyl derivative **3** bearing a benzo[*d*]thiazole moiety, and 13 known compounds of *N. gilva* YIM 90087 (Figure 1). The antifungal activity against *F. avenaceum*, *F. graminearum*, *F. culmorum*, *Trichophyton verrucosum*, *Aspergillus niger*, *Candida albicans*, and *Pyricularia oryzae* and the antibacterial activity against *Staphylococcus aureus*, *Escherichia*

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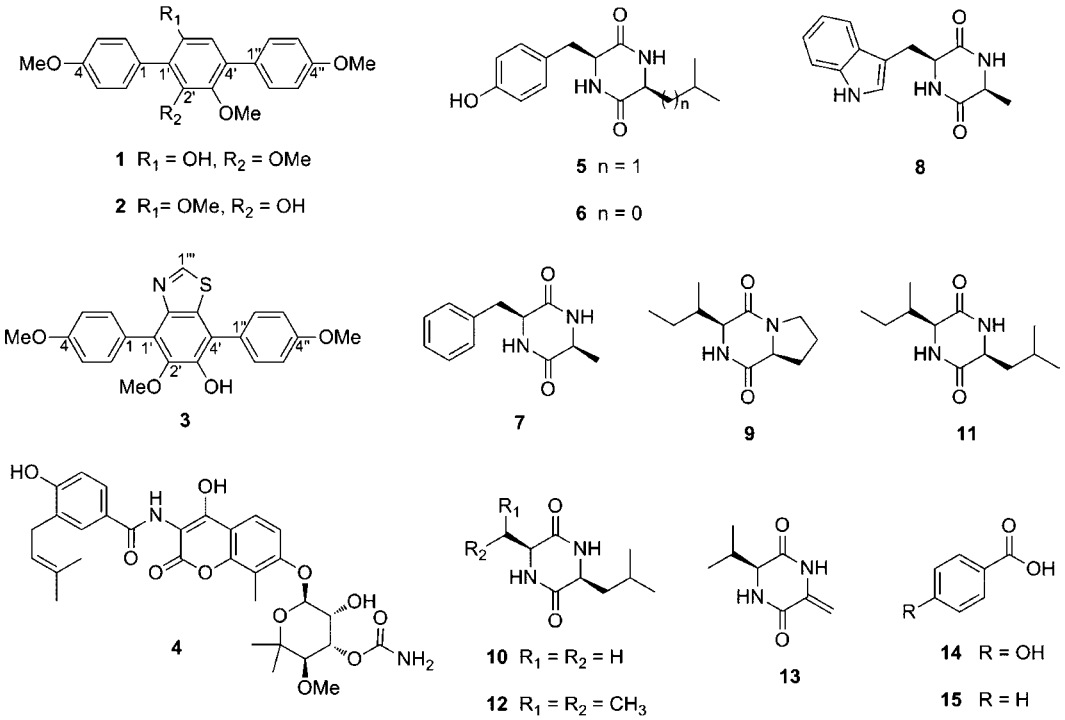

Figure 1. Chemical structures of secondary metabolites **1**−**15** of halophilic actinomycete *N. gilva* YIM 90087.

coli, and *Bacillus subtilis* of these secondary metabolites were reported. The antioxidant activity of the isolated compounds was evaluated by using the DPPH (1,1-diphenyl-2-picryl-hydrazyl) free radical assay, the ABTS (2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) cation radical assay, and the superoxide anion radical assay.

MATERIALS AND METHODS

General Experimental Procedures. Melting points were determined on an X-6 apparatus and were uncorrected. Optical rotations were measured with a Perkin-Elmer 341 automatic polarimeter. UV spectra were recorded in MeOH (1 mg/50 mL) on a Perkin-Elmer Lambda 35 spectrometer. IR spectra were recorded on a Perkin-Elmer Spectrum One spectrometer using a KBr disc with ν_{max} values given in inverse centimeters. NMR spectra were recorded on a Bruker Avance 600 spectrometer at room temperature (^{1}H, 600 MHz; ^{13}C, 150 MHz). The chemical shifts (δ) are reported in parts per million using tetramethylsilane as an internal standard, and the coupling constants (J) are given in hertz. HREIMS and EIMS data were obtained from a VG Auto Spect-3000 mass spectrometer. HRESIMS and ESIMS data were obtained on a Bruker Bio TOF IIIQ (quadrupole time-of-flight) mass spectrometer. Column chromatography (CC) was performed on self-packed open columns with silica gel [Qingdao Haiyang Chemical Co., Ltd. (QHCC), Qingdao, China] or Sephadex LH-20 (H&E Co., Ltd., Beijing, China). Thin layer chromatography (TLC) analyses were conducted on plates precoated with 10−40 μm of silica gel GF_{254} from QHCC, detected under a UV lamp at 254 or 365 nm, and visualized by spraying an 8% phosphomolybdic acid/EtOH (w/v) or 5% vanillin/H_2SO_4 (w/v) solution followed by heating or visualized with iodine (I_2). The semipreparative high-performance liquid chromatograph was equipped with a Perkin-Elmer series 200 pump, a Perkin-Elmer series UV−vis detector, a 200 μL manual injector, and a 250 mm × 10 mm (inside diameter), 5 μm, Kromasil C_{18} column. Fractions from all columns were collected by an autocollecting apparatus and combined according to TLC analyses. All other solvents were commercially purchased and distilled under normal atmospheric pressure prior to use.

Bacterial Strain and Materials. *N. gilva* YIM 90087 was isolated from saline soil samples by using modified International Streptomyces Project (ISP) 5 medium [consisting of 1 g/L L-asparagine, 10 g/L glycerol, 1 g/L K_2HPO_4, 1 mL of a trace salt solution (consisting of 1 g/L $FeSO_4·7H_2O$, 1 g/L $MnCl_2·4H_2O$, and 1 g/L $ZnSO_4·7H_2O$), 20 g/L agar, and distilled water (pH 7.2)] supplemented with 15% NaCl (w/v).[7] The stock culture of *N. gilva* YIM 90087 was grown on the slant of trypticase soy agar with 5% NaCl and maintained at 37 °C.

Fermentation of *N. gilva* YIM 90087. A two-stage fermentation procedure was employed to grow the *N. gilva* YIM 90087 strain. The seed medium [consisting of 15 g/L tryptone, 5 g/L soya peptone, 30 g/L NaCl, and distilled water (pH 7.2) and autoclaved at 121 °C for 30 min; 100 mL in a 500 mL flask] was inoculated with the strain, and the flasks were incubated on a rotary shaker at 120 rpm and 28 °C for 5 days. The seed culture (40 mL) was transferred into the fermentation medium [consisting of 10 g/L glycerol, 2 g/L peptone, 3 g/L beef extract, 3 g/L yeast extract, 1 g/L KH_2PO_4, 1 g/L K_2HPO_4, 0.3 g/L $MgSO_4·7H_2O$, 30 g/L NaCl, and distilled water (pH 7.2) and autoclaved at 121 °C for 30 min; 400 mL in a 2 L flask], and the flasks were incubated on a rotary shaker at 120 rpm and 28 °C for 15 days. A total of 80 L of fermentation culture was obtained via the two-stage fermentation procedure.

Extraction and Isolation of Secondary Metabolites from *N. gilva* YIM 90087. The fermentation cultures were centrifuged at 4500 rpm and 4 °C for 15 min to pellet the mycelia. The broth supernatant was collected and filtered to afford supernatant that was extracted three times with an equal volume of EtOAc at room temperature. The resulting organic layers were combined and dried over anhydrous Na_2SO_4. EtOAc was removed under vacuum to give the crude EtOAc extract. The mycelia were extracted three times with 4 L of acetone. The organic extracts were combined, and acetone was removed under vacuum to give the crude acetone extract. On the basis of TLC analyses, the EtOAc extract and the acetone extract were combined to offer 35 g of crude extract that was subjected to a silica gel CC eluted with a $CHCl_3$/MeOH mixture (1:0, 50:1, 20:1, 10:1, 5:1, 2:1, 1:1, and 0:1, each 4 L) to afford 20 fractions (A−T).

Fraction G (1.5 g) was separated via silica gel CC by using a petroleum ether/acetone mixture (10:1) as the eluent to afford compound **1** (250−450 mL, 12 mg).

Fraction H (1.0 g) was separated via silica gel CC eluted with a petroleum ether/acetone mixture (10:1, 5:1, 2:1, and 0:1, each 300 mL) to give four subfractions (H1−H4). Subfraction H1 (0.3 g) was

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chromatographed on silica gel eluted with a petroleum ether/$CHCl_3$ mixture (1:1, 300 mL) and then a $CHCl_3$/MeOH mixture (50:1, 300 mL) to give three subfractions (H1A–H1C). Subfraction H1C (80 mg) was separated by semipreparative reversed-phase high-performance liquid chromatography (HPLC), using a chromatograph equipped with a DAD detector, 30 to 100% MeOH in H_2O over 50 min, a flow rate of 3.0 mL/min, and a detector set at 254 nm, to afford subfractions H1C1–H1C8. Subfraction H1C8 (6 mg) was purified by semipreparative normal HPLC using a chromatograph equipped with a UV–vis detector. The mobile phase was *n*-hexane and 2-propanol (2:1); the flow rate was 3.0 mL/min, and the UV detector was set at 254 nm. The peak at 18 min was collected to give compound **3** (3.8 mg).

Compound **9** (4 mg) was crystallized from the MeOH solution of fraction J (25 mg).

Fraction K (1.4 g) was separated via silica gel CC eluted with a $CHCl_3$/MeOH mixture (50:1, 20:1, 10:1, 5:1, 2:1, and 0:1, each 300 mL) to yield eight subfractions (K1–K8). Subfraction K5 (140 mg) was subsequently separated via reversed-phase C_{18} CC by using a MeOH/H_2O mixture (4:6, 6:4, 8:2, and 0:1, each 150 mL) as the eluent to afford 13 subfractions (K5A–K5M). Subfraction K5B was separated by semipreparative reversed-phase HPLC (25% MeOH in H_2O, flow rate of 3.0 mL/min, and UV detector set at 230 nm) to give compounds **7** (10 mg; t_R = 14 min) and **10** (8.3 mg; t_R = 20 min). Subfraction K5H was separated using the same procedure to afford compound **11** (0.9 mg; t_R = 13 min). Subfraction K5C (3 mg) was purified by semipreparative reversed-phase HPLC on a chromatograph equipped with a UV–vis detector. The mobile phase was a MeOH/H_2O mixture (25:75); the flow rate was 3.0 mL/min, and the UV detector was set at 230 nm. The peak at 14 min was collected to give compound **13** (1.9 mg). Compound **12** (8 mg) was crystallized from the MeOH solution of K5F (20 mg).

Fraction P (3.0 g) was separated by reversed-phase C_{18} CC using a MeOH/H_2O mixture (5:5, 7:3, 9:1, and 0:1, each 800 mL) as the eluent to afford five subfractions (P1–P5). Subfraction P1 (80 mg) was subjected to CC over Sephadex LH-20 by using a $CHCl_3$/MeOH mixture (1:1) as the eluent to afford three subfractions (P1A–P1C). Subfraction P1B (20 mg) was separated by semipreparative reversed-phase HPLC on a chromatograph equipped with a DAD detector. The mobile phase was a MeOH/H_2O mixture (5 to 10% over 45 min); the flow rate was 3.0 mL/min, and the UV detector was set at 254 nm. The peak at 22 min was collected to afford compound **2** (3.4 mg). Subfraction P4 (0.2 g) was separated via silica gel CC by using a $CHCl_3$/MeOH mixture (10:1) as the eluent to afford five subfractions (P4A–P4E). Subfraction P4C (20 mg) was purified by semipreparative normal-phase HPLC by using an *n*-hexane/2-propanol mixture (2:1) as the eluent. The flow rate was 3.0 mL/min, and the UV detector was set at 254 nm. The peak at 15 min was collected to yield compound **4** (10.5 mg).

Fraction R (1.6 g) was chromatographed via silica gel CC using a $CHCl_3$/MeOH mixture (15:1, 10:1, 5:1, 2:1, and 0:1, each 400 mL) to give six subfractions (R1–R6). Compound **5** (0.9 mg) was crystallized from the MeOH solution of subfraction R3 (100 mg). The remaining fraction of R3 was further purified by CC over Sephadex LH-20 using a $CHCl_3$/MeOH mixture (1:1) as the eluent to give three subfractions (R3A–R3C). Subfraction R3C was separated by semipreparative reversed-phase HPLC on a chromatograph equipped with a UV–vis detector. The mobile phase was a MeOH/H_2O mixture (30 to 60% MeOH in H_2O over 50 min); the flow rate was 3.0 mL/min, and the UV detector was set at 254 nm. The peaks at 8, 10, 18, 20, 24, 30.8, and 31.5 min were collected to afford R3C1–R3C5, **14** (1.6 mg), and **15** (2.0 mg), respectively. Subfraction R3C5 (15 mg) was purified by semipreparative normal-phase HPLC on a chromatograph equipped with a UV–vis detector. The mobile phase was an *n*-hexane/2-propanol mixture (1:1); the flow rate was 3.0 mL/min, and the UV detector was set at 254 nm. The peaks at 14 and 18 min were collected to yield compounds **6** (1.6 mg) and **8** (0.3 mg), respectively.

*6′-Hydroxy-4,2′,3′,4″-tetramethoxy-p-terphenyl (**1**).* Colorless needles; UV ($CHCl_3$) λ_{max} (log ε) 239 (2.76), 270 (4.28) nm; IR (KBr) ν_{max} 3510, 3451,2939, 2836, 1608, 1519, 1483, 1397, 1352, 1294, 1246, 1178, 1102, 1024, 828, 832 cm^{-1}; ^1H NMR and ^{13}C NMR data in Table 1; HREIMS *m*/*z* 366.1474 (calcd for $C_{22}H_{22}O_5$, 366.1467, error of 1.9 ppm).

Table 1. NMR Spectroscopic Data of Compounds 1 and 3 in $CDCl_3$

	1		3	
position	δ_H, mult. (*J*)	δ_C, type	δ_H, mult. (*J*)	δ_C, type
1		124.31, C		126.62, C
2, 6	7.35, d (8.5)	131.58, CH	7.68, d (8.6)	131.64, CH
3, 5	7.06, d (8.5)	114.74, CH	7.06, d (8.6)	113.85, CH
4		159.52, C		159.41, C
1′		121.41, C		126.81, C
2′		151.45, C		144.53, C
3′		144.31, C		144.68, C
4′		135.48, C		119.03, C
5′	6.75, s	111.42, CH		131.81, C
6′		149.13, C		145.51, C
1″		130.35, C		128.72, C
2″, 6″	7.53, d (8.6)	130.22, CH	7.63, d (8.6)	130.33, CH
3″, 5″	6.97, d (8.6)	113.64, CH	7.07, d (8.6)	114.19, C
4″		158.98, C		159.38, C
4-OMe	3.88, s	55.27, CH_3	3.89, s	55.29, CH_3
2′-OMe	3.67, s	60.86, CH_3	3.53, s	61.04, CH_3
3′-OMe	3.59, s	60.67, CH_3	–	–
4″-OMe	3.86, s	55.32, CH_3	3.89, s	55.32, CH_3
6′-OH	4.86, s		–	–
3′-OH	–	–	6.24, s	
1‴	–	–	8.78, s	131.81, CH

*4,7-Bis(4-methoxyphenyl)-6-hydroxy-5-methoxybenzo[d]thiazole (**3**).* White powder; UV (MeOH) λ_{max} (log ε) 256 (4.62), 280 (4.52) nm; IR (KBr) ν_{max} 3435, 2923, 2852, 1632, 1406, 1244, 1100, 720, 609 cm^{-1}; ^1H NMR and ^{13}C NMR data in Table 1; HRESIMS (positive ion mode) *m*/*z* 394.1113 (calcd for $C_{22}H_{20}NO_4S$, 394.1108, error of −1.3 ppm).

Antifungal and Antibacterial Activity Assays. Antifungal and antibacterial assays were performed in 96-well sterilized microplates using a microdilution method described previously.[8,9] Briefly, 4-day-old spores from *F. avenaceum*, *F. graminearum*, *F. culmorum*, *T. verrucosum*, *A. niger*, *C. albicans*, and *P. oryzae* were transferred to sterile PDB broth medium, and the test concentration was 1×10^3 spores/mL. The 18-hour-old bacterial cultures from *S. aureus*, *E. coli*, and *B. subtilis* were added to sterile LB broth medium to reach 1×10^5 colony-forming units/mL. The test compounds were dissolved in DMSO, and their final concentrations ranged from 512 to 0.5 μg/mL by using a 2-fold serial dilution method. The final concentration of DMSO did not exceed 5%. The wells containing test strains and diluted compounds were incubated at 28 °C (4 days) for fungi and 37 °C (24 h) for bacteria. The wells containing a culture suspension and DMSO were run as negative controls. Nystatin (Taicheng Pharmaceutical Co., Ltd., Guangdong, China) and kanamycin (Yunke Biotechnology, Kunming, China) were introduced in the experiments as positive controls for antifungal and antibacterial assays, respectively. All experiments were repeated three times. The growth of test strains was observed with a CX21BIM-set5 microscope (Olympus Corp., Tokyo, Japan). MICs were determined as the lowest concentrations that produce complete growth inhibition of the tested microorganisms.

Antioxidant Activity Assay. Three methods, DPPH free radical scavenging assay, ABTS cation radical scavenging assay, and superoxide anion radical scavenging assay, were used to test the antioxidant activities of the isolated compounds.

DPPH Free Radical Scavenging Assay. The DPPH free radical scavenging assay was performed according to the previously reported method.[10−12] Briefly, the DPPH stock solution was prepared by

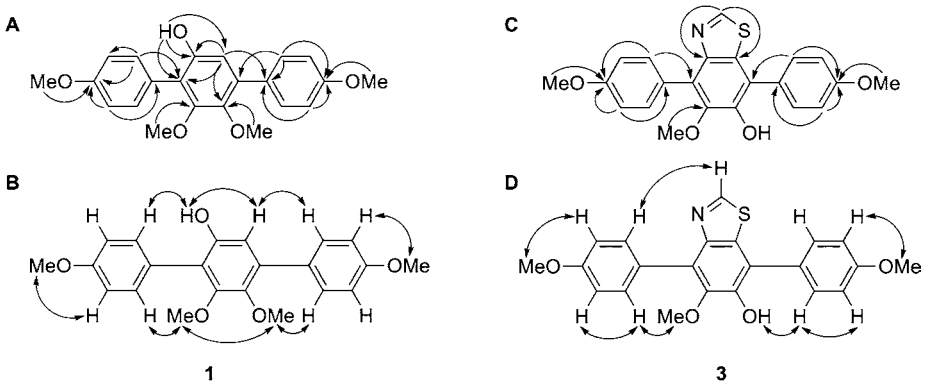

Figure 2. Key two-dimensional NMR correlations of new *p*-terphenyl **1** and its benzo[*d*]thiazole derivative **3**. (A and B) HMBC and NOESY correlations of **1**, respectively. (C and D) HMBC and NOESY correlations of **3**, respectively.

dissolving 59.3 mg of DPPH (Sigma-Aldrich, Beijing, China) in 100 mL of EtOH and stored at −20 °C in the dark. To 290 μL of a fresh DPPH work solution obtained by diluting the stock solution 1:20 in EtOH was added 10 μL of a sample solution, EtOH (negative control), or vitamin C (positive control) (Tianjin Bodi Chem. Inc., Ltd., Tianjin, China). The reaction mixture was kept for 30 min in the dark. The absorbance of the mixture was measured at 517 nm on a Varioskan Flash Reader (ThermoFisher Scientific Inc., Waltham, MA). All experiments were conducted in triplicate. The DPPH free radical scavenging activity (percent) was determined by comparison of the absorbance of the sample with that of the negative control and was calculated as $[1 - (A_e - A_s)/A_c] \times 100\%$, where A_e is the A_{517} in the presence of the sample, A_s is the A_{517} of the sample, and A_c is the A_{517} of the negative control solution).

ABTS Cation Radical Scavenging Assay. The $ABTS^{+\bullet}$ scavenging assay was performed according to the previously reported method with minor modification.[13] The $ABTS^{+\bullet}$ solution was prepared by incubating the aqueous mixture of ABTS (7 mM, final concentraction) (Sigma-Aldrich) and potassium persulfate (2.45 mM, final concentraction) (Aladdin Reagent, Shanghai, China) for 16 h in the dark at room temperature. The $ABTS^{+\bullet}$ working solution was obtained freshly by diluting 350 μL of the $ABTS^{+\bullet}$ solution to 25 mL with aqueous 80% EtOH. The sample solution or corresponding solvent (as negative control) was added to 250 μL of the $ABTS^{+\bullet}$ working solution in a 96-well plate. The mixture was shaken and incubated in the dark at room temperature for 5 min. The absorbance was measured at 734 nm on a Varioskan Flash Reader. The $ABTS^{+\bullet}$ scavenging capacity (percent) was calculated as $[1 - (A_e - A_s)/A_c] \times 100\%$, where A_e is the A_{734} in the presence of the sample, A_s is the A_{734} of the sample, and A_c is the A_{734} of the negative control solution. Vitamin C was used as a positive control. All analyses were performed in triplicate.

Superoxide Anion Radical Scavenging Assay. According to the method established previously,[14,15] superoxide anions were generated by a nonenzymatic NADH-PMS system and measured by the reduction of NBT with some modification. To each well of a 96-well plate was added 200 μL of the reaction mixture containing 150 μM NBT (Aladdin Reagent) and 234 μM NADH (Sigma-Aldrich) in 16 mM Tris-HCl buffer (pH 8.0). The sample (10 μL) was added to the reaction mixture described above, followed by the addition of 100 μL of a PMS solution [30 μM in 16 mM Tris-HCl buffer (pH 8.0)] to trigger the reaction. The 96-well plate containing the reaction mixture was shaken and incubated at room temperature for 5 min. The absorbance was measured at 560 nm on a Varioskan Flash reader. The superoxide anion scavenging capacity (percent) was calculated as $[1 - (A_e - A_s)/A_c] \times 100\%$, where A_e is the A_{560} in the presence of the sample, A_s is the A_{560} of the sample, and A_c is the A_{560} of the negative control solution. Vitamin C was used as a positive control. All analyses were performed in triplicate.

RESULTS AND DISCUSSION

Structure Elucidation of Compounds 1−15 from *N. gilva* YIM 90087. Compound **1** was isolated as colorless needles. The molecular formula of **1** was determined to be $C_{22}H_{22}O_5$ by its HREIMS data, which is consistent with the ^1H NMR and ^{13}C NMR data. The IR spectrum of compound **1** revealed the presence of a hydroxyl group (3510 cm^{-1}) and aromatic rings (1608 and 1519 cm^{-1}). The ^1H NMR data (Table 1) of **1** indicated the presence of two characteristic 1,4-disubstituted phenyls and four methoxy groups. One proton signal at δ_H 4.86 was assigned to a hydroxy group because no HSQC correlation was observed, and the assignment is consistent with its exchangeable property with D_2O. The ^1H NMR signal at δ_H 6.75 was assigned to a downfield -CH group because of its HSQC correlation with the carbon signal at δ_C 111.42. In addition to the carbon signals of the groups mentioned above, five additional quaternary carbon signals were observed in the ^{13}C NMR spectrum of compound **1** (Table 1). The presence of two 1,4-disubstituted phenyls was confirmed by the H-2/C-3, C-4 and H-3/C-1, C-4 HMBC correlations, and the H-2″/C-1″, C-4″ and H-3″/C-1″, C-4″ HMBC correlations (Figure 2A). Another substituted phenyl was constructed by the H-2/C-1′; H-2″/C-4; H-5/C-1, C-3, C-6, C-1″; and OH-6/C-1, C-5, C-6 HMBC correlations (Figure 2A). The four methoxy groups were located at C-4, C-2′, C-3′, and C-4″ by their corresponding HMBC correlations (Figure 2A). The assignment of the locations of all substituted groups was consistent with the key OMe-4/H-3, H-5; H-2/OMe-2′; H-6/OH-6′; H-5′/OH-6′, H-6″; H-3″/OMe-4″; and H-2″/OMe-3′ NOESY correlations (Figure 2B). Thus, the structure of compound **1** was determined to be 6′-hydroxy-4,2′,3′,4″-tetramethoxy-*p*-terphenyl. It is an isomer of the known compound **2**, 2′-hydroxy-4,6′,3′,4″-tetramethoxy-*p*-terphenyl, a methylated product of a toxic metabolite terphyllin from *Aspergillus candidus*.[16] It should be noted that in this study compound **2** was isolated as a natural product for the first time from the fermentation of *N. gilva* YIM 90087.

Compound **3** was isolated as a white powder. Its molecular formula, $C_{22}H_{19}NO_4S$, was provided by the $[M + H]^+$ ion in the HRESIMS spectrum of **3**. The IR spectrum displayed absorption bands for hydroxyl group (3435 cm^{-1}), C−N (1632 cm^{-1}), and C−S (1100 cm^{-1}) bonds. The NMR data of **3** (Table 1) were similar to those of compounds **1** and **2**, except that one methoxy group was absent in **3**. Careful comparison of the NMR data of **3** with those of **1** and **2** disclosed that the downfield proton at δ_H 8.78 should be connected to the carbon

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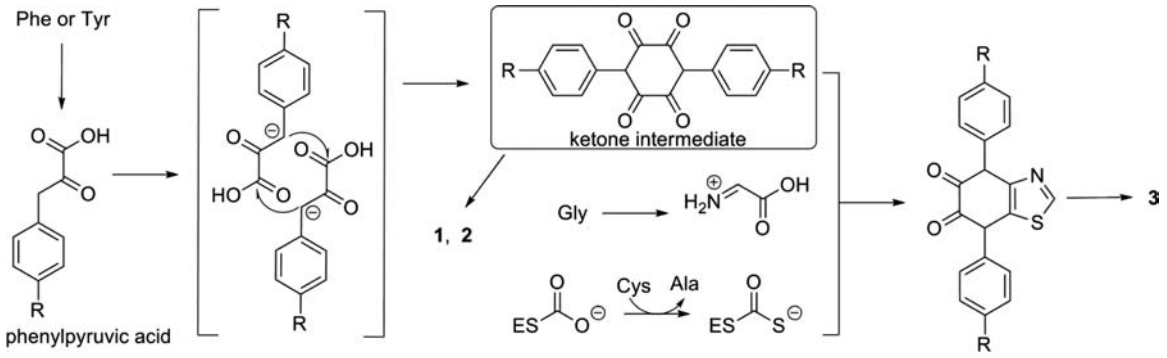

Figure 3. Proposed biosynthetic pathway of *p*-terphenyls and derivatives.

at δ_C 131.81 by its HSQC correlation, which suggests that the carbon should be connected with one heteroatom such as N or S. The H-2/C-4 and H-3/C-1, C-4; H-2″/C-4″ and H-3″/C-1″, C-4″ HMBC correlations indicated the presence of two 1,4-disubstituted phenyls, respectively (Figure 2C). A novel benzo[*d*]thiazole moiety was concluded from the H-2/C-1′; H-2″/C-4′; H-1‴/C-5′, C-6′ HMBC correlations and the molecular formula requirement of 14 degrees of unsaturation. The methoxy groups were located at C-4, C-2′, and C-4″ by their corresponding HMBC correlations (Figure 2C). The assignment of the locations of all substituted groups was consistent with the key OMe-4/H-3, H-5; H-2/OMe-2′; H-6/H-1‴; H-3″/OMe-4″; H-2″/OH-3′ NOESY correlations (Figure 2D). Thus, the structure of compound **3** was determined to be 4,7-bis(4-methoxyphenyl)-6-hydroxy-5-methoxybenzo[*d*]thiazole.

The other secondary metabolites were identified as novobiocin **4**,[17] cyclo(L-Tyr-L-Leu) **5**,[18] cyclo(L-Tyr-L-Val) **6**,[19] cyclo(L-Phe-L-Ala) **7**,[20] cyclo(L-Trp-L-Ala) **8**,[21] cyclo(L-Ile-L-Pro) **9**,[22] cyclo(L-Ala-L-Leu) **10**,[22] cyclo(L-Ile-L-Leu) **11**,[19] cyclo(L-Val-L-Leu) **12**,[22] cyclo(L-Val-Dha) (**13**),[23] *p*-hydroxybenzoic acid **14**,[24] and benzoic acid **15**[25] by data analyses and comparison of their data with those reported in the literature.

The strains of the genus *Nocardiopsis* are rich in secondary metabolite diversity. Previous chemical investigations led to the isolation of many kinds of secondary metabolites such as macrolide polyketides,[26] glycosylated macrolactones,[27] polycyclic naphthospironones,[28] apoptolidin,[29] pyranonaphthoquinone,[30,31] γ-pyrones,[32] griseusins,[33] indolocarbazole alkaloids,[34] peptides,[35−37] thiopeptide,[38] and chloroaromatic compounds related to the Bergman cyclization products of enediyne precursors.[39] Many of them display interesting bioactivities such as FKBP12 binding activity,[26] apoptosis,[29] cytotoxicity,[31,33,37] macrophage activating properties,[34] and an inhibitory effect on TNF-α-induced NFκB activation.[39] However, little is known about the secondary metabolites of halophilic actinomycetes.[6,7] The chemical investigations of the secondary metabolites of *N. gilva* YIM 90087 led to the isolation of a new *p*-terphenyl **1**, a new *p*-terphenyl derivative **3** bearing a benzothiazole moiety, and 13 known compounds, including *p*-terphenyl **2**, novobiocin **4**, nine cyclodipeptides (**5**−**13**), and two aromatic acids (**14** and **15**).

p-Terphenyls are aromatic hydrocarbons consisting of three phenyl rings in which the two terminal phenyls are para-substituted to the central phenyl.[40] Most of them show immunosuppressive, antioxidant, neuroprotective, cytotoxic, antithrombotic, and anticoagulant activities.[40] The biosynthesis of *p*-terphenyls was proposed to be initiated by the Claisen-like condensation between two molecules of phenylpyruvic acid from phenylalanine or tyrosine to generate the key ketone intermediate. Subsequent sequential oxidation and methylation led to the formation of *p*-terphenyls **1** and **2** (Figure 3).[40] For the novel *p*-terphenyl derivative **3**, the ketone intermediate was proposed to sequester the dehydroglycine from glycine and the active sulfur from cysteine, just as with the biosynthesis of thiamine,[41,42] to form the novel benzo[*d*]thiazole moiety. Subsequent sequential oxidation and methylation led to the formation of the novel *p*-terphenyl derivative **3** (Figure 3).

Biological Activity Assay of Compounds 1−15 from *N. gilva* YIM 90087. Seven pathogenic fungi (*F. avenaceum*, *F. graminearum*, *F. culmorum*, *T. verrucosum*, *A. niger*, *C. albicans*, and *P. oryzae*) and three pathogenic bacteria (*S. aureus*, *E. coli*, and *B. subtilis*) were selected for antifungal and antibacterial assays, respectively. The new *p*-terphenyl **1** showed antifungal activity against the three pathogenic fungi, including *F. avenaceum*, *F. graminearum*, and *F. culmorum*, that caused FHB with MICs of 8, 16, and 128 μg/mL, respectively. Compound **1** showed antifungal activity against *C. albicans* with a MIC of 32 μg/mL and antibacterial activity against *B. subtilis* with a MIC of 64 μg/mL. The known antibiotic novobiocin **4** showed antifungal activity against *P. oryzae* with a MIC of 16 μg/mL and antibacterial activity against *B. subtilis* with a MIC of 16 μg/mL and *S. aureus* with a MIC of 64 μg/mL. As a positive control, nystatin had antifungal activity against *F. avenaceum*, *F. graminearum*, *F. culmorum*, *T. verrucosum*, *A. niger*, *C. albicans*, and *P. oryzae* with MICs of 1, 4, 1, 4, 8, 4, and 8 μg/mL, respectively; kanamycin showed antibacterial acitivity against *S. aureus*, *E. coli*, and *B. subtilis* with MICs of 4, 1, and 1 μg/mL, respectively.

The DPPH assay suggested that the new *p*-terphenyl **1** showed 54.9 ± 2.0% free radical scavenging capacity at 2 mg/mL; the known novobiocin **4** showed 47.7 ± 0.9% free radical scavenging capacity at 2 mg/mL, whereas the novel benzothiazole **3** showed 14.3 ± 1.5% free radical scavenging capacity at 4 mg/mL. The positive control vitamin C showed 43.9 ± 0.8% free radical scavenging capacity at 0.8 mg/mL. The $ABTS^{+\bullet}$ scavenging assay indicated that compounds **1**, **3**, **4**, and **8** showed 68.6 ± 1.0, 28.4 ± 2.7, 78.2 ± 3.7, and 54.6 ± 0.6% cation radical scavenging capacity at 1, 2, 0.5, and 2 mg/mL, respectively. Meanwhile, the positive control vitamin C had 79.1 ± 4.3% cation radical scavenging capacity at 0.16 mg/mL. The superoxide anion radical scavenging assay suggested that **4** showed 77.9 ± 0.4% superoxide anion radical scavenging capacity at 2 mg/mL. The positive control vitamin C had 75.5 ± 1.3% superoxide anion radical scavenging capacity at 5 mg/mL.

The results of biological activity assays of compounds **1** and **3** indicated that *p*-terphenyls are new scaffolds for potent antifungal, antibacterial, and antioxidant agents.

Novobiocin **4**, an aminocoumarin antibiotic isolated from *Streptomyces* strains, is an inhibitor of bacterial gyrase.[43] Recently, novobiocin **4** was found to bind weakly to the newly discovered 90 kDa heat shock protein's (Hsp90) C-terminal ATP binding site and induce degradation of Hsp90 client proteins.[44] However, chemical modification of **4** led to an increase of 1000-fold in activity in antiproliferative assays,[44] which suggested that **4** is a potential scaffold for innovative drug discovery. Our chemical investigations of the halophilic *N. gilva* YIM 90087 strain suggested that *N. gilva* YIM 90087 is a new resource for novobiocin **4**. Furthermore, **4** exhibited antifungal activity, antibacterial activity, 47.7% free radical scavenging capacity, 78.2% $ABTS^{+\bullet}$ scavenging capacity, and 77.9% superoxide anion radical scavenging capacity, which suggested possible uses of the known antibiotics.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

HRMS and one- and two-dimensional NMR spectral diagrams for compounds **1** and **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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■ ABBREVIATIONS

FHB, Fusarium head blight; MIC, minimal inhibitory concentration; DPPH, 1,1-diphenyl-2-picryl-hydrazyl; ABTS, 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid; PMS, phenazine methosulfate; NBT, nitroblue tetrazolium chloride; NADH, nicotinamide adenine dinucleotide (reduced form); HSQC, heteronuclear single-quantum coherence; HMBC, heteronuclear multiple-bond correlation; NOESY, nuclear Overhauser enhancement spectroscopy

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