Published in final edited form as:

J Nat Prod. 2017 August 25; 80(8): 2215–2223. doi:10.1021/acs.jnatprod.7b00066.

Antifungal and Cytotoxic β -Resorcylic Acid Lactones from *Paecilomyces* sp. SC0924

Liangxiong Xu[†], Ping Wu[†], Jinghua Xue[†], Istvan Molnar[‡], and Xiaoyi Wei^{†,*}

[†]Key Laboratory of Plant Resources Conservation and Sustainable Utilization/Guangdong Provincial Key Laboratory of Applied Botany, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, P. R. China

[‡]Natural Products Center, School of Natural Resources and the Environment, University of Arizona, Tucson, AZ 85706, United States of America

Abstract

Eight new β -resorcylic acid lactones (RALs), including the hypothemycin-type compounds paecilomycins N–P (**1–3**) and the radicol-type metabolites dechloropochonin I (**4**), monocillins VI (**5**) and VII (**6**), 4'-hydroxymonocillin IV (**7**), and 4'-methoxymonocillin IV (**8**), along with nine known RALs (**9–17**), were isolated from the cultures of *Paecilomyces* sp. SC0924. Compounds **1** and **2** feature a novel 6/11/5 ring system and **3** is the first 5'-keto RAL. The structures of **1–8** were elucidated on the basis of extensive spectroscopic analysis, X-ray diffraction analysis, and theoretical calculations of ECD spectra. Compounds **3**, **5**, and **6** exhibit cytotoxicity against MCF-7, A549, and HeLa cells, and compounds **5** and **7** display antifungal activity against *Peronophythora litchii*.

Graphical abstract

β -Resorcylic acid lactones (RALs) are benzomacrolides produced by a variety of fungal strains via polyketide biosynthesis.^{1–3} They have diverse biological activities^{1,3} including antitumor^{4–6} and antimalarial activities,^{7–9} and they inhibit ATPases and kinases.^{10–12} Most RALs possess a 14-membered lactone ring (RAL₁₄), though 12- and 10-membered RALs (RAL₁₂ and RAL₁₀) have also been reported.² Several acyl resorcylic acid congeners of RAL₁₄, such as aigialomycins F and G,⁸ paecilomycins C and D,⁹ and aigialospirol¹³ are

*Corresponding Author: Tel (X. Wei): +86-20-3725-2538. Fax: +86-20-3725-2537. wxy@scbg.ac.cn.

Notes

The authors declare no competing financial interest.

Supporting Information. Computational details of **1–3**, **5** and **6**, 1D and 2D NMR spectra and HR-ESIMS of **7–8**, and ECD spectra of **7** and **8**. These materials are available free of charge via the Internet at <http://pubs.acs.org>.

found to contain benzopyran or benzofuran ring systems. In some macrocyclic RALs, including pochonins G, H, J,¹⁴ radicicol D,¹⁵ paecilomycins J–M,^{16,17} greensporones F and G, and dechlorogreensporone F,⁶ the 14-membered macrolactone is further bridged by an oxygen-containing five- or six-membered ring. Among the RALs so far reported, the dibenzomacrolide pochonin I is unique in featuring a carbon-carbon bridge between C-3' and C-8'.¹⁴ RAL₁₄ and their congeners and derivatives can also be classified according to the absolute configuration of C-10'. Thus, hypothemycin-type RAL₁₄ feature a 10'*S*, while radicicol-type RAL₁₄ display a 10'*R* configuration. The polyketide backbones of these two types of RAL₁₄ have been elucidated to be biosynthesized by different polyketide synthase enzyme systems.^{18–21} Previous studies showed that each RAL₁₄-producing strain harbors only one RAL₁₄ biosynthetic gene cluster^{18–21} and produces either a hypothemycin-type or a radicicol-type RAL₁₄ skeleton but not both.³

Extracts of *Paecilomyces* sp. SC0924, a hypocrealean fungal strain isolated from soil, were found to be active against the economically important Oomycete phytopathogen *Peronophythora litchii*.⁹ Eleven hypothemycin-type RALs, including six new ones (paecilomycins A–F), were isolated from *Paecilomyces* sp. SC0924 cultivated on wheat grains.⁹ In a subsequent study, we found that solid-state fermentation of this fungus on rice grains was even more effective to yield a diverse array of RALs. Thus, the metabolite profile of strain SC0924 was re-investigated using this fermentation method, leading to the isolation of seven new hypothemycin-type RAL₁₄ named paecilomycins G–M.^{16,17,22} In continuing our study on the fermentation products of strain SC0924, eight new (**1–8**) and nine known (**9–17**) hypothemycin- and radicicol-type RALs were obtained from rice-grown cultures. Amongst these, paecilomycins N (**1**) and O (**2**) feature unprecedented 6/11/5 ring systems, while paecilomycin P (**3**) is the first RAL containing a C-5' ketone. The isolated metabolites were evaluated for antifungal activity against *P. litchii* and *Fusarium verticillioides*, and for cytotoxicity against MCF-7, A549, and HeLa tumor cell lines. Herein, we report the isolation, structural elucidation, and biological activities of these new RALs.

RESULTS AND DISCUSSION

Paecilomyces sp. SC0924 was fermented and the resulting cultures were extracted as described previously.²² The CHCl₃-soluble extract was separated by silica gel, polyamide, ODS, and Sephadex LH-20 column chromatography (CC), followed by preparative HPLC to furnish the new compounds paecilomycins N–P (**1–3**), dechloropochonin I (**4**), monocillins VI (**5**) and VII (**6**), 4'-methoxymonocillin IV (**7**), and 4'-hydroxymonocillin IV (**8**), and the known compounds 2' α -hydroxymonocillin II (**9**),²³ monocillins I–IV (**10–13**),²⁴ radicicol (**14**),²⁵ monorden D (**15**),²⁶ lasicicol (**16**),²⁷ and hypothemycin (**17**).²⁸ The known compounds were characterized by comparing their spectroscopic data with the published values. Compound **9**, previously obtained from monocillin II by semi-synthesis, was isolated as a natural product for the first time.

Paecilomycin N (**1**), obtained as a yellow powder, has a molecular formula of C₁₉H₂₀O₇ as determined by (-)-HRESIMS data in experimental section. The ¹H and ¹³C NMR data (Table 1), with the aid of the HSQC data, displayed signals for two aromatic methines [δ_{H} 6.71 and 6.94 (each 1H, d, J = 2.5 Hz); δ_{C} 101.1 and 106.1], a chelated phenolic hydroxy group [δ_{H} 12.28 (1H, br s)], an aromatic methoxy group [δ_{H} 3.69 (3H, s); δ_{C} 55.6], an ester carbonyl (δ_{C} 171.2), and four sp² carbons (δ_{C} 104.3, 143.3, 165.6, and 166.3), which are characteristic of a 4-*O*-methyl β -resorcylic acid moiety.⁷⁻⁹ The remaining proton and carbon resonances were ascribed to a conjugated keto carbonyl [δ_{C} 202.4 (C-6')], a tetrasubstituted olefin [δ_{C} 142.8 (C-4') and 151.8 (C-5')], four saturated methines with three being oxygenated [δ_{H} 5.04 (H-10'), 4.50 (H-1'), and 2.78 (H-2'); δ_{C} 74.5 (C-10'), 61.4 (C-1'), and 59.6 (C-2')], three methylenes, and a secondary methyl group [δ_{H} 1.39 (H₃-11'); δ_{C} 20.6 (C-11')]. Analysis of the ¹H-¹H COSY data revealed the structural fragments from C-1' to C-3' and from C-7' to C-11' (Figure 1). In the HMBC data, key correlations (Figure 1) were observed from H-1' to C-1, C-5, and C-6, from H₂-3' to C-4', C-5', and C-8', from H₂-7' to C-6', and from H-10' to C-7, indicating the carbon-carbon connections of C-1' with C-6, C-3' with C-4', C-4' with C-8', and C-6' with C-7', and the ester linkage binding at C-10' to construct a unique 11-membered benzolactone ring fused with a cyclopentenone ring. The upfield ¹³C NMR shifts of C-1' and C-2' and the downfield shift of C-5', in combination with the molecular formula, showed the presence of 1',2'-epoxy and 5'-OH groups to complete the gross structure of **1** as shown in Figure 1.

The ¹H NMR data gave no coupling constant ($J_{1',2'} \approx 0$ Hz) between H-1' and H-2', revealing a *trans* configuration of the 1',2'-epoxide ring as in hypothemycin and its analogues.^{7,28} In the NOESY data, NOE interactions were observed between H-1'/H-3' β , H-1'/H-9' β , H-1'/H₃-11', H-3' β /H-9' β , H-2'/H-8', and H-8'/H-10' (Figure 2). These

findings in association with the theoretical lowest-energy conformation (Figure 2) indicated that H-1' and CH₃-11' are in a β -orientation while H-2' and H-8' are α -oriented, assigning the relative configuration of **1** as shown.

Paecilomycin O (**2**), a yellow amorphous solid, was determined to have the same molecular formula as **1** on the basis of its HRESIMS data. The ¹H and ¹³C NMR data of **2** (Table 1) were closely similar to those of **1** except for small differences in chemical shifts of the protons and carbons in the macrolide ring. Analysis of the ¹H-¹H COSY, HSQC, and HMBC data of **2** indicated a planar structure identical to that of **1**. However, in the NOESY spectrum of **2** (Figure 2), the key correlations of H-3' β /H-9' β , H-2'/H-8', and H-8'/H-10' observed in **1** were absent. Instead, strong NOE interactions of H-3' β /H-8' and H-10'/H-7' α were observed, suggesting a β -orientation of H-8' in **2**. Thus, **2** was established as the 8'-epimer of **1**.

As efforts failed to obtain single crystals of either **1** or **2**, assignments of the absolute configurations of **1** and **2** were made by quantum chemical calculations of the ECD spectra using previously described procedures.^{17,29} As can be seen in Figure 3, the B3LYP/TZVP calculated ECD spectra for 1'R,2'R,8'S,10'S-**1** and 1'R,2'R,8'R,10'S-**2** in MeOH solution are highly consistent with the experimental data of **1** and **2**, respectively. The significant difference in the curves around 250 nm between the measured spectra of **1** and **2** was well simulated (Figure 3). The CAM-B3LYP/TZVP calculations gave the same results (Figure S8, Supporting Information). Therefore, the absolute configurations of **1** and **2** were assigned as depicted.

Comparisons with the RALs already obtained from this fungus^{9,16,22} indicates that **1** and **2** are closely related to hypothemycin (**17**),²⁸ one of the main metabolites of this fungal strain,³⁰ in both their basic structures and configurations except for the connection between C-4' and C-8'. Based on this finding, we conclude that **1** and **2** are likely derived from **17**. During biosynthesis, **17** would be dehydrated to generate the direct precursor 5',7'-dien-6'-one **17a** which would undergo a Nazarov-type cyclization to yield **1** and **2** via intermediate **17b** (Scheme 1).

Paecilomycin P (**3**) was obtained as a yellowish solid. Its molecular formula, C₁₈H₂₀O₅, was determined from its HRESIMS data. Analysis of the ¹H and ¹³C NMR data (Table 1), by the aid of the HSQC data, showed **3** to be an RAL₁₄ containing two *trans* 1,2-disubstituted olefins [$\delta_{\text{H}}/\delta_{\text{C}}$: 6.84 (1H, d, J = 16.0 Hz)/129.9, 6.01 (1H, dt, J = 16.0, 5.0 Hz)/130.3, 5.95 (1H, ddd, J = 15.0, 9.6, 5.0 Hz)/130.0, 5.62 (1H, ddd J = 15.0, 9.6, 5.0 Hz)/126.9] and a ketone carbonyl (δ_{C} 210.0) in the macrolide ring, suggesting a structure similar to that of 7'-dehydrozearalenone.²² However, the presence of two isolated spin systems, H-1'/H-2'/H₂-3'/H₂-4' and H₂-6'/H-7'/H-8'/H₂-9'/H-10'/H₃-11', in the ¹H-¹H COSY data and long-range H-C correlations from H-3', H-4' and H-6' to C-5' in the HMBC data located the ketone carbonyl at C-5' instead of C-6' in 7'-dehydrozearalenone. The *S* configuration of the only chiral center C-10' was suggested by the presence of the *trans* C-1'/C-2' double bond, a structural feature only found in some hypothemycin-type RALs,³ and supported by ECD/TDDFT calculations which provided a simulated ECD curve consistent with the measured spectrum (Figure 4).

To the best of our knowledge, paecilomycin P (**3**) is the first RAL with a keto group at C-5'. It is likely derived from aigialomycin D, a known RAL₁₄ obtained from this strain in our previous study,⁹ by dehydration to generate a 5'-en-5'-ol intermediate, followed by the enol-keto isomerization of the latter.

Compound **4**, obtained as colorless needles, has a molecular formula of C₁₈H₁₆O₅ on the basis of its HRESIMS data. Its ¹H and ¹³C NMR data (Tables 2 and 3) were similar to those of pochonin I,¹⁴ except for the absence of the chlorinated aromatic carbon. Instead, the ¹H NMR data displayed an extra aromatic proton at δ 6.30 (d, J = 2.3 Hz), which was *meta* coupled with H-3 [δ 6.34 (d, J = 2.3 Hz)]. This finding together with the molecular formula suggested a dechlorinated derivative of pochonin I. The structure was supported by the ¹H-¹H COSY, HSQC, and HMBC data and confirmed by the X-ray diffraction analysis using Cu $K\alpha$ radiation (Figure 5).

Monocillin VI (**5**) was isolated as a white powder. It has a molecular formula of C₁₈H₂₀O₅ according to its HRESIMS data. Analysis of its ¹H, ¹³C (Tables 2 and 3), and 2D NMR data constructed a structure closely similar to monocillin II (**11**), except for the *cis*-configured C-3'/C-4' double bond as evidenced by the proton coupling constant between H-3' and H-4' (J = 11.5 Hz). The *R* configuration of the only chiral center C-10' in **5** was assignable when considering its structural similarity to monocillin II. However, the assignment was required to be substantiated by further evidence since recent studies demonstrated that some monocillin-like RAL₁₄ have the 10' *S* configuration.^{6, 31, 32} Consequently, theoretical computations of ECD spectrum were carried out for (10' *R*)-**5**. As can be seen in Figure 6, the calculated ECD spectrum is in an excellent fit with the experimental spectrum. Therefore, the structure of **5** was elucidated as shown.

Monocillin VII (**6**) gave a molecular formula of C₁₈H₂₀O₆ as determined from its HRESIMS data. The ¹H and ¹³C NMR data (Tables 2 and 3), in combination of the HSQC data, revealed resonances indicating an RAL₁₄ with a ketone carbonyl (δ_C 205.1), a *cis*-configured ($J_{6'/7'} = 10.5$ Hz) 1,2-disubstituted double bond [δ_H/δ_C 5.78/124.6 (CH-6') and 5.63/129.8 (CH-7')], and two oxymethines [δ_H/δ_C 4.38/74.3 (CH-8') and 4.33/65.0 (CH-4')] in the macrocyclic lactone ring. The presence of an isolated AB system in the downfield region [δ_H 5.22 and 3.79 (each 1H, d, J = 18.3 Hz, H₂-1')] and a spin system successively from H₂-3' to H₃-11' in the ¹H-¹H COSY spectrum (Figure 7) assigned the ketone carbonyl to C-2', two oxymethines to C-4' and C-8', and the double bond between C-6'/C-7'. The presence of long-range correlations of H-4'/C-8' and H-8'/C-4' in HMBC spectrum (Figure 7), together with the unsaturation requirement, indicated C-4' being connected with C-8' via an oxygen bridge to form a pyran ring.

In the NOESY spectrum, key mutual NOE correlations between H-3' β /H-4', H-9' β /H-4', H-9' α /H-7', and H₃-11'/H-8' (Figure 7) suggested H-8' and CH₃-11' were on the same side of the macrocyclic ring and α -oriented, while H-4' was on the opposite side of the ring and in the β -orientation. Compound **6** is a radicicol-type RAL₁₄ as suggested by the presence of a C-2 ketone carbonyl and thus should have the 4' *R*, 8' *S*, 10' *R* configuration. To verify the absolute configuration, structure **6** was subjected to ECD/TDDFT calculations and

afforded a simulated ECD spectrum matching well with the experimental spectrum (Figure 8). Consequently, the complete structure of **6** was established as shown.

Compound **7**, a white powder, has a molecular formula of C₁₉H₂₄O₆ according to HRESIMS analysis. The ¹H and ¹³C NMR data (Tables 2 and 3), in combination with the HSQC data, suggested an RAL₁₄ sharing similarity to the co-occurring known compound monocillin IV (**13**), except for replacement of one of the methylenes by an oxygenated methine (δ_{H} 3.78; δ_{C} 77.2) and the presence of a methoxy group (δ_{H} 3.36; δ_{C} 56.3). Analysis of ¹H-¹H COSY and HMBC data established the planar structure as 4'-methoxymonocillin IV. The structure was confirmed and its absolute configuration was unambiguously assigned to be 4'*R*,10'*R* by X-ray crystallography using Cu K α radiation (Figure 9).

Compound **8** was determined to have the molecular formula C₁₈H₂₂O₆ from (+)-HRESIMS analysis. This compound is a 4'-*O*-desmethyl derivative of **7** as evidenced by the HRESIMS data as well as the ¹H and ¹³C NMR data (Tables 2 and 3), which were almost superimposable on those of **7** except for the absence of signals for the methoxy group and the upfield shift of C-4' (δ_{C} 77.2 in **7** vs. δ_{C} 68.7 in **8**). The absolute configuration of **8** was assigned to be the same as that of **7** on the basis of the similarity of its ECD spectrum with that of **7** (Figure S64, Supporting Information).

Compounds **5–8** are proposed to be derived from monocillin II (**11**) in a plausible biosynthetic pathway as shown in Scheme 2. The initial reaction would be the hydration of **11** to generate **8** which, as a key precursor, undergoes *cis*-dehydration, *O*-methylation, or oxidation to yield **5**, **7**, or the 7',8'-epoxide intermediate **8a**, respectively. The epoxide **8a** then affords **6** by intramolecular S_N2 substitution followed by dehydration via pochonin J.¹⁴

The isolation of radicicol-type compounds **4–16** from the cultures of *Paecilomyces* sp. SC0924 was quite unexpected, as the RAL congeners obtained in our previous work from this fungal strain were all hypothemycin-type,^{9,16,17,22} and no RAL-producing fungi had been reported to be capable to biosynthesize both hypothemycin- and radicicol-type metabolites.³

The isolated compounds, except **9**, **15**, and **16**, were evaluated for their cytotoxicity against A549, HeLa, and MCF-7 tumor cells. As shown in Table 4, all tested RAL₁₄ with no further bridging within the macrocycle, except **7** and **8**, exhibited cytotoxicity (IC₅₀ < 10 μ M) against at least one of the three tested tumor cell lines, with the best activity shown by radicicol (**14**, IC₅₀ = 0.1–0.4 μ M) followed by hypothemycin (**17**, IC₅₀ = 1.0–3.3 μ M). Among these compounds, all the radicicol-type RAL₁₄ with a 3'-en-2'-one functionality (**5**, **10–12**, **14**) exhibited strong cytotoxicity (mostly IC₅₀ < 5.0 μ M), while those with a saturated 2'-keto function displayed decreased activity (**7**, **8**, and **13** vs. **11**). The activity profiles of these compounds were in agreement with the previously proposed structure-activity relationships (SARs).²³ Noteworthy is that RAL₁₄ **3**, with a 5'-keto function, demonstrated good activity (IC₅₀ = 3.6–6.9 μ M) against the three test cell lines, and a change of the configuration of the 3'-en-2'-one moiety from *trans* to *cis* had no significant effect on cytotoxicity (**11** vs. **5**). Regarding the RALs featuring bridged macrolactones, it is

remarkable that **1** and **2** with the 6/11/5 and **4** with the 6/10/6 ring system were almost inactive, while **6** with the oxygen-bridged 6/12/6 ring system was active ($IC_{50} = 3.9\text{--}4.9\text{ }\mu\text{M}$) against all tested tumor cell lines.

These RALs were also evaluated for their antifungal activity against the phytopathogenic fungi *P. litchii* and *F. verticillioides* using spore germination tests. New compounds **5–7** exhibited antifungal activity against *P. litchii* with IC_{50} values of 9.2, 41.0, and 19.3 μM , respectively, but none of the new compounds were active against *F. verticillioides* (Table 4). Analysis of the antifungal activity data revealed SARs similar to those for cytotoxicity, except that the cytotoxic **3** showed almost no antifungal activity, while the non-cytotoxic **7** displayed potent activity against *P. litchii*.

EXPERIMENTAL SECTION

General Experimental Procedures

Optical rotations were obtained on a Perkin-Elmer 343 spectropolarimeter. UV measurements were performed with a Perkin Elmer Lambda 650 UV/vis spectrometer. CD data were collected by a Jasco J-810 CD spectrometer (Jasco Inc., Japan). ^1H NMR, ^{13}C NMR, and 2D NMR spectra were recorded on a Bruker Avance DRX 500 or Bruker DRX-400 instrument with TMS as a reference. ESIMS data were obtained on an MDS SCIEX API2000 LC/MS/MS instrument. HRESIMS data were collected on Bruker Bio TOF IIIQ mass spectrometer. Preparative HPLC was performed with a Shimadzu LC-6AD pump and a Shimadzu RID-10A refractive index detector using an YMC-pack ODS-A column (5 μm , 250 mm $\times$ 20 mm). For column chromatography (CC), silica gel 60 (100–200 mesh, Qingdao Marine Chemical Ltd., Qingdao, China), polyamide (60–100 mesh, Taizhou Luqiao Si-jia Biochemical Plastic Company, Zhejiang, China) and Sephadex LH-20 (GE Healthcare, Uppsala, Sweden) were used. Cytotoxic assays were performed with a Genois microplate reader (Tecan Group, Männedorf, Zürich, Switzerland). Spore germination percentages were determined using a B203LED optical microscope (Chongqing Optec instrument Co. Ltd., Chongqing, China).

Producing Fungus and Fermentation

The producing fungus, *Paecilomyces* sp. SC0924, was isolated from a soil sample collected in the Dinghu Mountain Biosphere Reserve, Guangdong, China, in March 2003. It was authenticated on the basis of its morphological characteristics and ITS DNA sequence data (GenBank: KR011745.1) by Prof. Tai-hui Li, Guangdong Institute of Microbiology, Guangzhou, China. Fermentation of the fungus was performed as previously described.²² Briefly, the fungal strain was grown in rice broth (2 % rice powder in H_2O) for 5 days in the dark at 28 °C, 120 rpm. The culture was transferred into 115 $\times$ 5 L Erlenmeyer flasks each containing 500 mL of water and 500 g of rice. Fermentation was conducted under stationary condition for 20 days in the dark at 28 °C.

Extraction and Isolation

The mycelial culture (115 L) was extracted with 95 % EtOH. The resultant extract, after excluding EtOH, was partitioned with CHCl_3 . The CHCl_3 -soluble extract (975.50 g) was

chromatographed on silica gel eluted with CHCl_3 -MeOH mixture of increasing polarity (100:0–80:20) to obtain six fractions (A–F). Fraction C was subjected to silica gel CC using petroleum ether-acetone (90:10–50:50) to afford seven fractions (C1–C7). Fraction C3 was further separated by polyamide CC eluted with aqueous EtOH (30–90 %) to obtain six subfractions (C3-1–C3-6). Fraction C3-3 in MeOH afforded colorless needles which were collected by filtration to obtain **11** (250 mg). The filtrate was further separated by Sephadex LH-20 CC eluted with MeOH to afford **7** (120 mg) and **8** (70 mg). Fraction C3-5 was separated by Sephadex LH-20 CC eluted with MeOH to obtain **4** (45 mg). Fraction C3-6 was separated by ODS CC using aqueous MeOH (60–80 %) followed by HPLC using 75 % MeOH to afford **5** (22 mg), **13** (6 mg), **15** (28 mg), and **16** (3 mg). Fr. C4 was separated by polyamide CC eluted with aqueous EtOH (30–90 %) to obtain six sub-fractions (C4-1–C4-6). Fraction C4-3 was separated by silica gel CC, eluted with petroleum ether-acetone (90:10–50:50), followed by HPLC using 55 % MeOH to obtain **1** (62 mg), **2** (167 mg), **10** (83 mg), **12** (280 mg), and **6** (18 mg). Fraction C4-4 was dissolved in MeOH to yield colorless needles **14** (450 mg), and the mother liquor was purified by HPLC using 65 % MeOH to afford **9** (19 mg). Fraction C4-5, when dissolved in MeOH, yielded the crystal **17** (20 g). Fraction D was subjected to silica gel CC using petroleum ether-acetone (90:10–50:50) to afford nine fractions (Frs. D1–D9). Fraction D5 was further separated by Sephadex LH-20 CC using MeOH to obtain **3** (42 mg).

Paecilomycin N (1): yellow amorphous solid, $[\alpha]_D^{20} -103.6$ (c 0.25, MeOH); UV (MeOH) λ_{max} (log ϵ) 215 (4.4), 262 (4.3), 302 (3.8) nm; CD (MeOH) ϵ 227 (+10.1), 269 (–24.5), 325 (+1.9); ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) see Table 1; (+)-ESIMS m/z 383 $[\text{M} + \text{Na}]^+$; (–)-ESIMS m/z 359 $[\text{M} - \text{H}]^-$, 395 $[\text{M} + \text{Cl}]^-$; (–)-HRESIMS m/z 359.1131 $[\text{M} - \text{H}]^-$ (calcd for $\text{C}_{19}\text{H}_{19}\text{O}_7$, 359.1131).

Paecilomycin O (2): yellow amorphous solid, $[\alpha]_D^{20} +17.6$ (c 0.25, MeOH); UV (MeOH) λ_{max} (log ϵ) 215 (4.5), 263 (4.4), 302 (3.9) nm; CD (MeOH) ϵ 227 (+13.3), 255 (+3.9), 270 (–9.8), 294 (+0.8), 316 (–0.8); ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) see Table 1; (+)-ESIMS m/z 383 $[\text{M} + \text{Na}]^+$; (–)-ESIMS m/z 395 $[\text{M} + \text{Cl}]^-$; (–)-HRESIMS m/z 359.1116 $[\text{M} - \text{H}]^-$ (calcd for $\text{C}_{19}\text{H}_{19}\text{O}_7$, 359.1131).

Paecilomycin P (3): yellow amorphous solid; $[\alpha]_D^{20} +57.6$ (c 0.25, MeOH); UV in MeOH: 234 (4.3), 271 (3.9), 309 (3.6); CD (MeOH) ϵ 239 (+8.3), 255 (+3.9), 267 (–0.7), 296 (+1.1); ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) see Table 1; (–)-ESIMS m/z 315 $[\text{M} - \text{H}]^-$, 631 $[\text{2M} - \text{H}]^-$; (–)-HRESIMS m/z 315.1230 $[\text{M} - \text{H}]^-$ (calcd for $\text{C}_{18}\text{H}_{19}\text{O}_5$, 315.1232).

Dechlorpochonin I (4): colorless needles (MeOH); $[\alpha]_D^{20} +109.2$ (c 0.25, MeOH); UV in MeOH: 208 (4.5), 267 (4.0), 305 (3.8); ^1H NMR (600 MHz) and ^{13}C NMR (150 MHz) see Tables 2 and 3; (+)-ESIMS m/z 335 $[\text{M} + \text{Na}]^+$; (–)-ESIMS m/z 311 $[\text{M} - \text{H}]^-$; (+)-HRESIMS m/z 313.1073 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{18}\text{H}_{17}\text{O}_5$, 313.1071).

X-ray Crystallographic Analysis of Dechlorpochonin I (4)

$\text{C}_{18}\text{H}_{16}\text{O}_5$, $M = 312.31$, colorless needles, $0.50 \times 0.10 \times 0.05 \text{ mm}^3$, orthorhombic, space group: $P2_1 2_1 2_1$, $a = 5.08803(6) \text{ \AA}$, $b = 11.94598(15) \text{ \AA}$, $c = 23.4043(3) \text{ \AA}$, $\alpha = \gamma = 90^\circ$, β

$= 90^\circ$, $V = 90 \text{ \AA}^3$, $Z = 4$, $D_c = 1.458 \text{ g/cm}^3$, $F_{000} = 656$, Cu K α radiation, $\lambda = 1.54184 \text{ \AA}$, $T = 293(2) \text{ K}$, theta range for data collection: 1.89° to 71.55° ; 2782 reflections collected, 2626 unique ($R_{\text{int}} = 0.0456$), completeness to theta = 71.98° (99.53%). The structure was refined by full-matrix least-squares on F^2 . Final R indices $I > 2\sigma(I)$: 0.0342 , $wR_2 = 0.0888$ for 211 parameters, GOF = 1.056. Absolute structure parameter: Flack = $-0.12(18)$. Data were collected on Agilent Xcalibur Nova single crystal diffractometer using Cu-K α radiation. Crystallographic data for the structure of **4** have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 1486556).

Monocillin VI (5): white powder; $[\alpha]_D^{20} -173.75$ (c 0.25, MeOH); UV (MeOH) λ_{max} (log ϵ) 217 (4.3), 263 (3.9), 305 (3.6) nm; ^1H NMR (500 MHz) and ^{13}C NMR (150 MHz) see Tables 2 and 3; (+)-ESIMS m/z 317 $[\text{M} + \text{H}]^+$, 339 $[\text{M} + \text{Na}]^+$, 655 $[\text{2M} + \text{Na}]^+$; (–)-ESIMS m/z 315 $[\text{M} - \text{H}]^-$, 351 $[\text{M} + \text{Cl}]^-$, 631 $[\text{2M} - \text{H}]^-$. (+)-HRESIMS m/z 317.1382 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{18}\text{H}_{21}\text{O}_5$, 317.1384).

Monocillin VII (6): brown powder, $[\alpha]_D^{20} -31.2$ (c 0.25, MeOH); UV (MeOH) λ_{max} (log ϵ) 216 (4.4), 262 (4.0), 304 (3.8) nm; ^1H NMR (500 MHz) and ^{13}C NMR (150 MHz) see Tables 2 and 3; (+)-ESIMS m/z 333 $[\text{M} + \text{H}]^+$, 355 $[\text{M} + \text{Na}]^+$, 687 $[\text{2M} + \text{Na}]^+$; (–)-ESIMS 331 $[\text{M} - \text{H}]^-$, 663 $[\text{2M} - \text{H}]^-$. (+)-HRESIMS m/z 355.1155 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{18}\text{H}_{20}\text{O}_6\text{Na}$, 355.1152).

4'-Methoxymonocillin IV (7): colorless needles, $[\alpha]_D^{20} +25.6$ (c 0.25, MeOH); UV (MeOH) λ_{max} (log ϵ) 217 (4.3), 265 (4.0), 305 (3.7) nm; ^1H NMR (500 MHz) and ^{13}C NMR (150 MHz) see Tables 2 and 3; (+)-ESIMS m/z 349 $[\text{M} + \text{H}]^+$, 371 $[\text{M} + \text{Na}]^+$, 387 $[\text{M} + \text{K}]^+$, 719 $[\text{2M} + \text{Na}]^+$; (–)-ESIMS 347 $[\text{M} - \text{H}]^-$, 383 $[\text{M} + \text{Cl}]^-$, 695 $[\text{2M} - \text{H}]^-$. (+)-HRESIMS m/z 371.1466 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{19}\text{H}_{24}\text{O}_6\text{Na}$, 371.1465).

X-ray Crystallographic Analysis of 4'-Methoxymonocillin IV (7)

$\text{C}_{19}\text{H}_{24}\text{O}_6$, $M = 328.38$, colorless needles, $0.50 \times 0.10 \times 0.05 \text{ mm}^3$, monoclinic, space group: $P 1 2_1 1$, $a = 9.25830(10) \text{ \AA}$, $b = 8.03346(8) \text{ \AA}$, $c = 12.00210(14) \text{ \AA}$, $\alpha = \gamma = 90^\circ$, $\beta = 103.3025(11)^\circ$, $V = 868.719(17) \text{ \AA}^3$, $Z = 2$, $D_c = 1.332 \text{ g/cm}^3$, $F_{000} = 372$, Cu K α radiation, $\lambda = 1.54184 \text{ \AA}$, $T = 293(2) \text{ K}$, theta range for data collection: 3.78° to 71.73° ; 3382 reflections collected, 3320 unique ($R_{\text{int}} = 0.0414$), completeness to theta = 71.98° (99.08%). The structure was refined by full-matrix least-squares on F^2 . Final R indices $I > 2\sigma(I)$: 0.0283 , $wR_2 = 0.0719$ for 230 parameters, GOF = 1.046. Absolute structure parameter: Flack = $0.04(12)$. Data were collected on Agilent Xcalibur Nova single crystal diffractometer using Cu-K α radiation. Crystallographic data for the structure of **7** have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 1062538).

4'-Hydroxymonocillin IV (8): colorless needles, $[\alpha]_D^{20} +58.4$ (c 0.25, MeOH); UV (MeOH) λ_{max} (log ϵ) 217 (4.3), 265 (4.0), 306 (3.7) nm; ^1H NMR (500 MHz) and ^{13}C NMR (150 MHz) see Tables 2 and 3; (+)-ESIMS m/z 357 $[\text{M} + \text{Na}]^+$; (–)-ESIMS 333 $[\text{M} - \text{H}]^-$, 667 $[\text{2M} - \text{H}]^-$. (+)-HRESIMS m/z 357.1312 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{18}\text{H}_{22}\text{O}_6\text{Na}$, 357.1309).

2'-Hydroxymonocillin II (9): colorless needles, $[\alpha]_D^{20} +73.2$ (c 0.25, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 220 (4.4), 264 (4.1), 304 (3.7) nm; ^1H NMR (500 MHz) and ^{13}C NMR (150 MHz) see Supporting Information; (+)-ESIMS m/z 319 $[\text{M} + \text{H}]^+$, 341 $[\text{M} + \text{Na}]^+$, 659 $[2\text{M} + \text{Na}]^+$; (–)- ESIMS 317 $[\text{M} - \text{H}]^-$, 353 $[\text{M} + \text{Cl}]^-$, 635 $[2\text{M} - \text{H}]^-$. (+)-HRESIMS m/z : 341.1356 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{19}\text{H}_{24}\text{O}_6\text{Na}$, 341.1359).

Cytotoxicity assay

Cytotoxic activities were determined using the MTT colorimetric method as previously described.³³ Compounds in DMSO were serially diluted with culture medium. The final concentrations of each of the compounds were 50, 10, 2, 0.4, 0.08, and 0.016 μM , and the experiments were performed in quadruplicate for each concentration. Adriamycin (ADM) was used as a positive control. The inhibitory rate of cell growth was calculated according to the following formula: Inhibition rate (%) = $\{1 - (\text{OD}_{\text{treated}} - \text{OD}_{\text{control}})/(\text{OD}_{\text{control}} - \text{OD}_{\text{blank}})\} \times 100\%$. IC_{50} values were determined by nonlinear regression analysis of logistic dose–response curves (SPSS 16.0 statistic software).

Antifungal activity assay

Antifungal activities were determined by a conidial germination assay using 48-well culture plates.³⁴ Conidia of *P. litchii* and *F. verticillioides* were collected from 7- and 3-day-old cultures of fungi growing on PDA, respectively. Conidia were diluted with half strength potato dextrose broth (PDB:water = 1:1), counted and immediately used in the bioassay. Test samples were dissolved and diluted with DMSO to yield a two-fold dilution series, and 10 μL of the sample solution was added to each well containing 490 μL arthroconidium suspensions to obtain the final concentration from 0.625 to 80 $\mu\text{g/mL}$. After 8 h incubation at 30°C on a rotary shaker at 150 rpm, both germinated and non-germinated conidia were counted under an optical microscope to calculate the spore germination percentages. A spore was considered to be germinated when the germ tube length was 1.5 times the spore diameter. Three duplicates were performed for each concentration and more than 200 conidia in each duplicate were counted. Thiram (tetramethylthiuram disulfide) was used as the positive control, and DMSO was used as the negative control. Inhibition rates and IC_{50} values were calculated by nonlinear regression analysis of logistic dose–response curves (SPSS 16.0 statistic software).

Computational Methods

Molecular Merck force field (MMFF) and *ab initio* calculations were performed with the Spartan' 14 (Wavefunction Inc., Irvine, CA, USA) and the Gaussian09³⁵ program packages, respectively, using default grids and convergence criteria. Low-energy conformers within a 10 kcal/mol energy window, generated by MMFF conformational searches, were subjected to geometry optimization using the density functional theory (DFT) method at the B3LYP/6-31G(d,p) (for **1** and **2**) or B3LYP/6-31G (d) (for **3**, **5**, **6**) level. Frequency calculations were run at the same level to verify that each optimized conformer was a true minimum and to estimate their relative thermal free energies (G) at 298.15K. Energies of the low-energy conformers in MeOH were calculated at the B3LYP/6-311+G (2d,p) (for **1** and **2**) or B3LYP/def2-TZVP (for **3**, **5**, **6**) level. Solvent effects were taken into account by using polarizable

continuum model (PCM). The time-dependent density functional theory (TDDFT) calculations were performed using the hybrid B3LYP,³⁶ CAM-B3LYP,³⁷ PBE1PBE,³⁸ ω B97X,³⁹ and/or TPSSH⁴⁰ functionals, and Ahlrichs' basis set TZVP (triple zeta valence plus polarization).⁴¹ The number of excited states per each molecule was 30–36. The ECD spectra were generated by the program SpecDis⁴² using Gaussian band shapes from dipole-length dipolar and rotational strengths. Equilibrium population of each conformer at 298.15K was calculated from its relative free energies using Boltzmann statistics. The calculated spectra of compounds were generated from the low-energy conformers according to the Boltzmann weighting of each conformer in MeOH solution.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Prof. Tai-Hui Li, Guangdong Institute of Microbiology, for morphological authentication of the producing fungus, Ms. Aijun Sun, South China Sea Institute of Oceanology, Chinese Academy of Sciences, for HR-ESIMS measurements, Ms Yun-Yun Chen, School of Pharmaceutical Science, Sun Yat-Sen University, for X-ray diffraction analysis, Prof. Liangcheng Du from Chemistry Department, Nebraska University-Lincoln, NE, U.S.A and Prof. Ru-Qian Pan from South China Agricultural University for the test fungi. This work was supported by grants from the National Science Foundation of China (Nos. 81172942 to X. W., 30901856 to L. X.), the U.S. National Institutes of Health (NIGMS R01GM114418-01A1 to I. M.), and the Guangzhou Science Technology and Innovation Commission (No. 201604020048 to L. X.). I. M. has a disclosed financial interest in TEVA Pharmaceuticals Hungary, which is unrelated to the subject of the research presented here. All the other authors declare no competing financial interests.

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Figure 1.
¹H-¹H COSY (bold lines) and key HMBC (arrows) correlations of **1**.

Figure 2.
Key NOESY correlations (dashed arrows) and the theoretical lowest-energy conformations of **1** and **2**.

Figure 3.

Comparison between B3LYP/TZVP calculated and experimental ECD spectra of **1** and **2** in MeOH. Vertical bars represent rotational strengths R of the global energy minima). Parameters for calculated ECD spectra: $\sigma = 0.29$ eV (for both), shift = -6 (for **1**)/ -4 (for **2**) nm, and scaling factor = 0.85 (for **1**)/ 0.53 (for **2**).

Figure 4. Comparison between the ω B97X/TZVP calculated and the measured ECD spectra of **3** in MeOH. Parameters for calculated ECD spectrum: $\sigma = 0.35$ eV, shift = +20 nm, and scaling factor = 0.24.

Figure 5.
X-ray structure of **4**.

Figure 6.

Comparison between the PBE1PBE/TZVP calculated and the measured ECD spectra of **5** in MeOH. Vertical bars represent rotational strengths R of the lowest-energy conformer. Parameters for calculated ECD spectrum: $\sigma = 0.27$ eV, shift = +8 nm, and scaling factor = 0.27.

Figure 7. ¹H-¹H COSY (bold lines), key HMBC (arrows), and key ROESY (dashed arrows in 3D structure) correlations of **6**. The 3D-conformer represents the global energy minimum afforded by the theoretical conformational analysis.

Figure 8.

Comparison between the TPSSh/TZVP calculated and experimental ECD spectra of **6** in MeOH. Vertical bars represent rotational strengths R of the lowest-energy conformer. Parameters for calculated ECD spectrum: $\sigma = 0.33$ eV, shift = -14 nm, and scaling factor = 0.40.

Figure 9.
X-ray structure of **7**.

Scheme 1.
Plausible Biogenetic Pathway for 1 and 2

Scheme 2.
Proposed Biosynthetic Pathway of 5–8

Table 1

¹H (400 MHz) and ¹³C (100 MHz) NMR Data (δ in ppm) of Compounds 1–3

position	¹ a			² a			³ b		
	δ_{C} , type	δ_{H} , mult., (<i>J</i> in Hz)	δ_{C} , type	δ_{H} , mult., (<i>J</i> in Hz)	δ_{C} , type	δ_{H} , mult., (<i>J</i> in Hz)	δ_{C} , type	δ_{H} , mult., (<i>J</i> in Hz)	
1	104.3, C		104.5, C		103.7, C				
2	166.3, C		165.6, C		164.5, C				
3	101.1, CH	6.71 d (2.5)	100.8, CH	6.56 d (2.5)	102.2, CH	6.25 d (2.4)			
4	165.6, C		165.3, C		161.0, C	6.41 d (2.4)			
5	106.1, CH	6.94 d (2.5)	105.8, CH	6.87 d (2.5)	107.8, CH				
6	143.3, C		143.6, C		142.6, C				
7	171.2, C		170.8, C		170.9, C				
1'	61.4, CH	4.50 br s	60.9, CH	4.38 d (2.0)	129.9, CH	6.84 d (16.0)			
2'	59.6, CH	2.78 br d (10.1)	57.2, CH	3.79 br d (10.3)	130.3, CH	6.01 dt (16.0, 5.0)			
3'	29.0, CH ₂	α 3.98 d (14.8) β 2.42 dd (14.8, 10.1)	31.2, CH ₂	α 3.52 br d (13.2) β 2.15 dd (13.2, 10.3)	25.4, CH ₂	α 2.69 m β 2.41 m			
4'	142.8, C		143.1, C		39.0, CH ₂	α 3.25 m β 2.46 m			
5'	151.8, C		153.3, C		210.0, C				
6'	202.4, C		201.3, C		47.7, CH ₂	α 3.33 dd (11.8, 10.0) β 3.04 dd (12.0, 5.0)			
7'	42.5, CH ₂	α 2.86 dd (18.6, 6.5) β 2.22 d (18.6)	37.4, CH ₂	β 2.56 dd (18.9, 6.3) α 2.40 d (18.9)	126.9, CH	5.62 ddd (15.0, 9.6, 5.0)			
8'	33.6, CH	2.68 m	35.7, CH	2.83 m	130.0, CH	5.95 ddd (15.0, 9.6, 5.0)			
9'	40.3, CH ₂	β 2.18 dd (14.8, 13.2) α 1.47 dd (14.8, 5.8)	36.4, CH ₂	β 2.38 m α 1.73 dd (15.7, 4.6)	37.1, CH ₂	α 2.59 ddd (13.8, 9.6, 3.7) β 2.34 m			
10'	74.5, CH	5.04 m	69.5, CH	5.03 dq (11.8, 6.1)	70.9, CH	5.52 m			
11'	20.6, CH ₃	1.39 d (6.1)	21.4, CH ₃	1.32 d (6.1)	18.4, CH ₃	1.41 d (6.6)			
4-OCH ₃	55.6, CH ₃	3.69 s	55.5, CH ₃	3.63 s					
2-OH		12.28 br s		11.91 br s					

^a C₅D₅N used as solvent.

^b CDCl₃ used as solvent.

Table 2

¹H NMR (500 MHz) Data [δ_{H} in ppm, mult., (J in Hz)] of Compounds 4–8

position	δ^a	δ^b	δ^a	δ^b
3	6.34 d (2.3)	6.30 d (2.4)	6.83 d (2.4)	6.32 d (2.5)
5	6.30 d (2.3)	6.03 d (2.4)	6.67 d (2.4)	6.18 d (2.5)
1'	α 4.56 d (16.8) β 3.78 d (16.8)	α 4.52 d (18.0) β 3.56 d (18.0)	α 3.79 d (18.3) β 5.22 d (18.3)	α 4.22 d (17.4) β 3.40 d (17.4)
3'		6.28 d (11.5)	α 2.54 t (12.4) β 2.82 dd (12.4, 3.0)	α 2.76 dd (19.4, 3.5) β 2.50 dd (19.4, 6.6)
4'	7.32 dd (6.5, 2.4)	6.08 td (11.5, 6.5)	4.33 m	3.78 dddd (9.5, 5.8, 3.5, 2.1)
5'	7.37 td (6.5, 2.4)	α 3.46 dt (11.5, 10.5) β 2.28 td (11.5, 5.5)	α 1.91 m β 1.91 m	α 1.89 ddt (14.0, 6.6, 2.5) β 1.52 dddd (14.0, 11.8, 9.5, 2.5)
6'	7.38 td (6.5, 2.3)	α 2.37 m β 1.97 m	5.78 m	α 2.05 m β 2.32 m
7'	7.24 dd (6.5, 2.3)	5.33 m	5.63 br d (10.5)	5.30 m
8'		5.24 dt (15.5, 7.4)	4.38 br d (11.3)	5.50 ddd (15.0, 9.2, 5.0)
9'	α 2.54 dd (14.5, 2.4) β 3.64 dd (14.5, 4.4)	α 2.56 ddd (14.5, 8.0, 3.1) β 2.16 ddd (14.5, 5.6, 4.3)	α 1.36 d (15.7) β 2.28 dt (15.7, 10.8)	α 2.20 dt (15.0, 4.8) β 2.64 ddd (15.0, 9.2, 3.8)
10'	5.71 qdd (6.5, 4.4, 2.4)	5.36 m	5.22 m	5.31 m
11'	1.12 d (6.5)	1.32 d (6.6)	1.24 d (6.1)	1.36 d (6.6)
4'-OCH ₃				3.36 s
2-OH	11.66 br s	12.15 br s	12.04 br s	11.96 br s

^a CDCl₃ used as solvent.

^b C₅D₅N used as solvent.

Table 3

¹³C NMR (125 MHz) Data (δ_C in ppm, type) of Compounds 3–7

position	4 ^a	5 ^a	6 ^b	7 ^a	8 ^a
1	104.7, C	105.0, C	107.3, C	105.5, C	105.5, C
2	166.1, C	166.0, C	163.8, C	166.0, C	166.2, C
3	102.9, CH	103.1, CH	102.8, CH	103.1, CH	102.9, CH
4	161.2, C	161.2, C	165.1, C	161.2, C	161.0, C
5	113.5, CH	113.3, CH	113.9, CH	112.6, CH	113.0, CH
6	139.8, C	138.6, C	139.6, C	138.6, C	139.4, C
7	169.1, C	170.7, C	171.8, C	170.3, C	170.4, C
1'	51.4, CH ₂	52.5, CH ₂	48.2, CH ₂	50.7, CH ₂	51.2, CH ₂
2'	205.3, C	199.7, C	205.1, C	209.2, C	206.4, C
3'	143.4, C	127.4, CH	50.2, CH ₂	47.7, CH ₂	47.2, CH ₂
4'	123.9, CH	148.2, CH	65.0, CH	68.7, CH	77.2, CH
5'	127.0, CH	27.2, CH ₂	31.2, CH ₂	33.4, CH ₂	29.6, CH ₂
6'	129.3, CH	31.2, CH ₂	124.6, CH	29.8, CH ₂	29.6, CH ₂
7'	132.7, CH	131.4, CH	129.8, CH	134.7, CH	134.6, CH
8'	131.1, C	124.5, CH	74.3, CH	124.7, CH	124.8, CH
9'	37.3, CH ₂	37.0, CH ₂	37.8, CH ₂	37.1, CH ₂	37.4, CH ₂
10'	72.0, CH	71.9, CH	74.1, CH	72.7, CH	73.0, CH
11'	18.4, CH ₃	18.0, CH ₃	21.7, CH ₃	18.3, CH ₃	18.1, CH ₃
4'-OCH ₃					56.3, CH ₃

^a CDCl₃ used as solvent.

^b C₅D₅N used as solvent.

Table 4

Cytotoxicity and Antifungal Activity of Compounds 1–8, 10–14, and 17

compound	cytotoxic activity (IC ₅₀ , μM)			antifungal activity (IC ₅₀ , μM)		
	MCF-7	A549	HeLa	<i>P. litchii</i>	<i>F. verticillioides</i>	
3	6.9 ± 0.41 ^a	3.9 ± 0.27	3.6 ± 0.24	> 100	> 100	
5	1.7 ± 0.08	4.1 ± 0.21	3.6 ± 0.03	9.2 ± 0.3	> 100	
6	4.9 ± 0.54	4.1 ± 0.65	3.9 ± 0.26	41.0 ± 5.7	> 100	
7	> 10.0	> 10.0	> 10.0	19.3 ± 1.7	> 100	
10	8.1 ± 0.42	2.6 ± 0.27	2.7 ± 0.39	7.9 ± 1.5	> 100	
11	4.1 ± 0.17	1.7 ± 0.1	2.7 ± 0.35	9.8 ± 0.9	> 100	
12	> 10.0	2.1 ± 0.33	3.4 ± 0.38	12.0 ± 0.6	> 100	
13	3.6 ± 0.5	> 10.0	> 10.0	33.6 ± 1.6	> 100	
14	0.4 ± 0.04	0.1 ± 0.01	0.1 ± 0.0	1.4 ± 0.5	8.0 ± 2.5	
17	3.3 ± 1.08	1.0 ± 0.04	1.0 ± 0.11	1.9 ± 0.3	1.1 ± 0.3	
ADM	0.2 ± 0.03	0.1 ± 0.02	0.3 ± 0.02	NT	NT	
Thiram	NT	NT	NT	4.6 ± 1.7	1.7 ± 0.8	

^a Values represent means ± SD based on three individual experiments.

^b NT: not tested.

Compounds **1**, **2**, **4**, and **8**, being neither cytotoxic (IC₅₀ > 10.0 μM) nor antifungal (IC₅₀ > 100 μM), are not listed. Positive controls: ADM, adriamycin; Thiram, tetramethylthiuram disulfide.