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Serpulanines A to C, N-Oxidized Tyrosine Derivatives Isolated from the Sri Lankan Fungus *Serpula* sp.: Structure Elucidation, Synthesis, and Histone Deacetylase Inhibition

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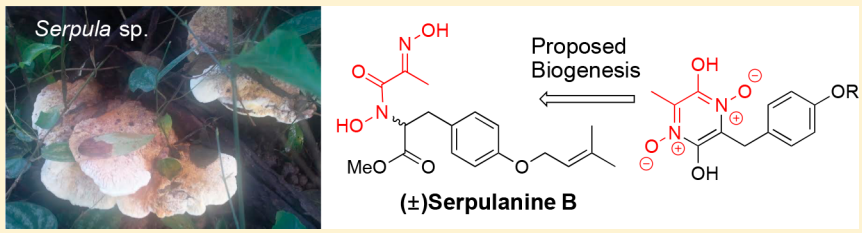
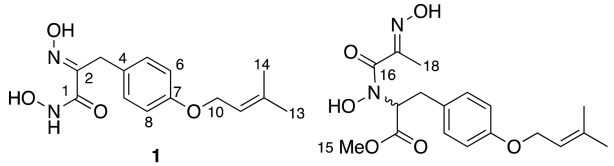
Supporting Information



ABSTRACT: Serpulanines A (**1**), B (**2**), and C (**3**) have been isolated from extracts of the rare Sri Lankan macrofungus *Serpula* sp. The structures of **1**, **2**, and **3** were elucidated by a combination of spectroscopic and single-crystal X-ray diffraction analyses. Serpulanines A (**1**) and B (**2**) both contain the rare (*E*)-2-hydroxyimino hydroxamic acid functional group array. A proposed biogenesis for serpulanine B (**2**) suggests that its (*E*)-2-hydroxyimino hydroxamic acid moiety arises from a diketopiperazine precursor. Synthetic serpulanine A (**1**) inhibited class I/II histone deacetylases in murine metastatic lung carcinoma cells with an IC_{50} of 7 μM.

Sri Lanka has a high rate of endemic speciation in both plants and microbes,[1] which offers great potential for the discovery of novel secondary metabolites with medicinal or agricultural utility.[2−4] As part of an ongoing program aimed at exploring the secondary metabolites of Sri Lankan fungi,[5−7] and particularly macrofungi,[8,9] we have investigated the rare macrofungus *Serpula* sp. collected from a wooded area in the Monaragala District. Chemical investigation of the dried fruiting bodies of *Serpula* sp. has resulted in the discovery of serpulanines A (**1**), B (**2**), and C (**3**). Serpulanines A (**1**) and B (**2**) contain the rare (*E*)-2-hydroxyimino hydroxamic acid functional group array. Details of the isolation and structure elucidation of **1**, **2**, and **3**, along with the synthesis of serpulanine A (**1**) and its inhibition of class I/II histone deacetylases (HDAC) in murine metastatic lung carcinoma cells, are presented herein.



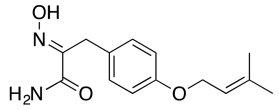


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Table 1. NMR Data (600 MHz, DMSO-d_6) for Serpulanines A (1), B (2), and C (3)

	serpulanine A (1)			serpulanine B (2)		serpulanine C (3)		
position	δ_C, type	δ_H (J in Hz)	δ_N[a]	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_N[a]
1	161.3 br, C			169.4, C		165.1, C		
2	151.1 br, C			59.8/64.6 br, C	5.14/5.18 br	152.1, C		
3	28.4 br, CH_2	3.72, s		31.9/32.8 br, CH_2	2.97 dd (14.0, 10.7) 3.09 bm	27.7, CH_{2s}	3.69 s	
4	128.1 br, C			128.7/128.9 br, C		128.7, C		
5/9	129.8, CH	7.08 d (8.6)		130.2/130.5 br, CH	7.12 bm	129.7, CH	7.08 d (8.7)	
6/8	114.4, CH	6.80 d (8.6)		114.2/114.5 br, CH	6.81 d (8.5)	114.3, CH	6.79 d (8.7)	
	156.9, C			157.1, C		156.7, C		
10	64.2, CH_2	4.45 bd (6.7)		64.2, CH_2	4.46 d (6.6)	64.1, CH_2	4.44 bd (6.7)	
11	120.1, CH	5.39 tm (6.7)		120.1, CH	5.38 m	120.1, CH	5.39 tm (6.7)	
12	136.8, C			136.8, C		136.8, C		
13	25.4, CH_3	1.72 bs		25.4, CH_3	1.72 bs	25.4, CH_3	1.71 bs	
14	18.0, CH_3	1.67 bs		18.0, CH_3	1.68 bs	18.0, CH_3	1.67 bs	
15				52.3, CH_3	3.66 s			
16				163.6/166.8 br, C				
17				149.9/151.1 br, C				
18				12.0/11.0 br, CH_3	1.71/1.29 bs			
1-NH[a]		10.71 bs	−217.8				7.28 bs/7.19 bs	−284.1
1-NH-OH		8.91 bs						
2-N-OH		11.68 bs			9.89/10.07 bs[b]		11.75 s	no[c]
17-N-OH			no		11.19/11.50 bs[b]			

[a]The ^{15}N assignments were not calibrated with an external standard. The values have an accuracy of about 1 ppm in reference to CH_3NO_2 (0 ppm) and are assigned on the basis of ^{15}NgHSQC and ^{15}NlrHMQC correlations. [b]Assignments maybe interchanged. [c]no, not observed.

RESULTS AND DISCUSSION

Air-dried fruiting bodies of *Serpula* sp. were extracted with MeOH and CH_2Cl_2. The combined organic extracts were fractionated by solvent partitioning, Sephadex LH 20 chromatography, and HPLC to give serpulanines A (**1**), B (**2**), and C (**3**). Serpulanine A (**1**) gave an $[M + Na]^+$ ion in the HRESIMS at m/z 301.1169, appropriate for a molecular formula of $C_{14}H_{18}N_2O_4$ requiring 7 sites of unsaturation. The ^{1}H, ^{13}C, gCOSY60, gHSQC, and gHMBC NMR data (Table 1, Supporting Information) obtained for **1** identified an oxygen-bearing isoprene fragment and a *para*-disubstituted benzene ring, as illustrated in Figure 1, which accounted for 5 of the sites of unsaturation. Correlations in the gHMBC between the isoprene methylene doublet resonating at δ 4.45 (H_2-10) and a downfield aromatic carbon resonance at δ 156.9 (C-7) showed that the isoprene fragment was attached to the phenyl ring via an ether linkage between C-10 and C-7. Additional gHMBC correlations observed between a two-proton singlet at δ 3.72 (H_2-3) and aromatic carbon resonances at δ 128.1 (C-4) and 129.8 (C-5/C-9) and between a two-proton aromatic doublet at δ 7.08 (H-5/H-9) and a carbon resonance at δ 28.4 (C-3) defined the second substituent of the *para*-disubstituted benzene as a methylene carbon at C-4.

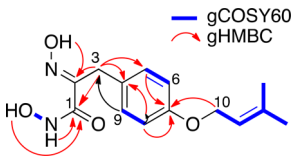

Figure 1. Selected gCOSY60 and gHMBC correlations used to assign the structure of serpulanine A (**1**).

The methylene resonance at δ 3.72 (H_2-3) also showed gHMBC correlations to carbon resonances at δ 161.3 (C-1) and 151.1 (C-2). A broad proton singlet at δ 10.71 (1-NH), which was shown in the g^{15}NHSQC experiment to correlate to a nitrogen at δ −217.8, also correlated in the gHMBC to the carbon at δ 161.3 (C-1), while a proton resonance at δ 11.68 (2-N-OH), which did not correlate to either a carbon or nitrogen atom in the gHSQC experiments, showed a gHMBC correlation to the carbon resonance at δ 151.1 (C-2). The structure proposed for serpulanine A (**1**) that contains both oxime and hydroxamic acid functionalities is consistent with the NMR data discussed above, and it satisfies the unsaturation number requirements of the molecular formula. Additional support for the 2-hydoxyimino hydroxamic acid substructure in **1** came from the observation that a broad proton singlet at δ 8.91 (1-NH-OH), which did not correlate to carbon or nitrogen atoms in gHSQC experiments, showed a weak correlation to the resonance at δ 10.71 (1-NH) in the gCOSY60 spectrum and a weak gHMBC correlation to the resonance at δ 161.3 assigned to C-1. The ^{13}C NMR resonances at δ 161.3, 151.1, 28.4, and 128.1, assigned to C-1, C-2, C-3, and C-4, respectively, were all broadened or slightly split into two unequal peaks, presumably due to some slow dynamic process such as hindered rotation and/or tautomerization in the oxime/hydroxamic acid functional group array.

Serpulanine A (**1**) gave crystals from MeOH that were suitable for single-crystal X-ray diffraction analysis. The resultant structure obtained for serpulanine A (Figure 2) showed that the compound crystallizes with two molecules of **1** and two molecules of water in the asymmetric unit, and it confirmed the constitution that was proposed from analysis of the NMR and MS data and assigned the oxime configuration in the crystals as *E*. The 2-hydroxyimino hydroxamate substructure in serpulanine A (**1**) is related to the 2-hydroxyimino amide substructures in the bastadin and related families of sponge metabolites.[10] It has been shown that the oxime functionality in the bastadins interconverts between the *E* and *Z* configurations.[11] An *E* to *Z* oxime

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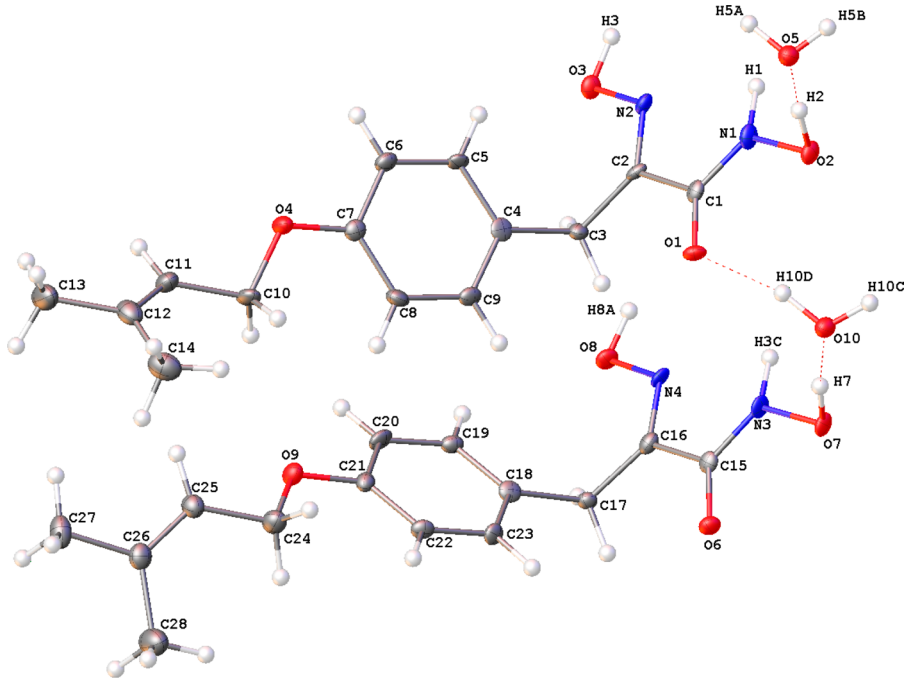

Figure 2. ORTEP-style diagram generated by single-crystal X-ray diffraction analysis of serpulanine A (**1**).

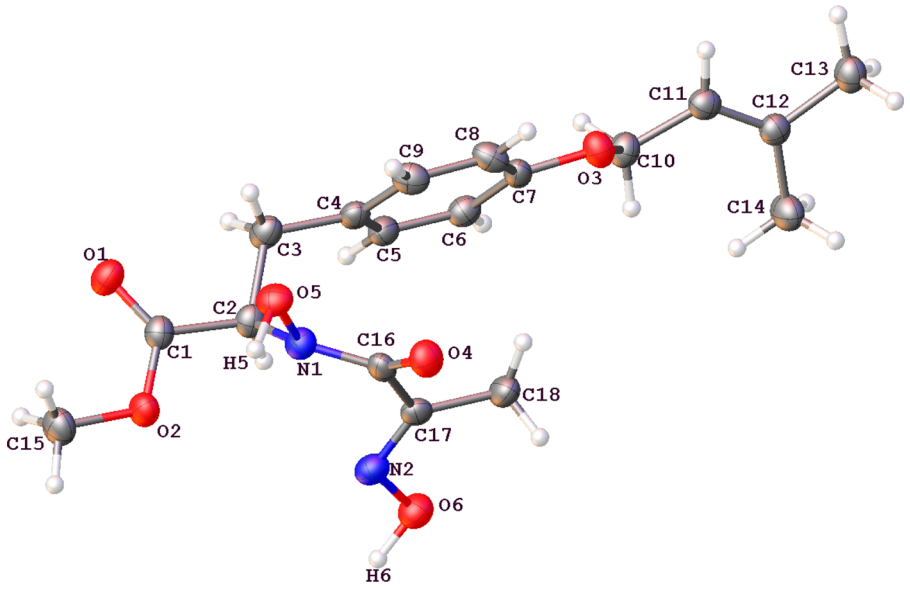

Figure 3. ORTEP-style diagram generated by single-crystal X-ray diffraction analysis of serpulanine B (**2**).

equilibrium in serpulanine A (**1**) might account for the doubling of signals that were observed in its solution NMR data.

Serpulanine B (**2**) gave an $[M + Na]^+$ ion in the HRESIMS at m/z 387.1545, appropriate for a molecular formula of $C_{18}H_{24}N_2O_6$, requiring 8 sites of unsaturation. The 1D and 2D NMR data (Table 1, Supporting Information) obtained for **2** indicated that serpulanine B (**2**) contained the identical C-3 to C-14 *para*-disubstituted benzene fragment found in serpulanine A (**1**). In parallel with serpulanine A (**1**), the ^{13}C NMR resonances assigned to the C-3, C-4, C-5/C-9, and C-6/C-8 resonances in serpulanine B (**2**) were all broadened or doubled. gCOSY and gHMBC correlations showed that serpulanine B (**2**) had a heteroatom-substituted methine with broad and doubled resonances at C-2 [$^1H/^{13}C$: δ 5.14, 5.18/59.8, 64.6 (CH-2)] and a methyl ester at C-1 [^{13}C: δ 169.4 (C-1); $^1H/^{13}C$: δ 3.66 s/52.3 (Me-15)]. The significant broadening and doubling of the remaining unassigned $^1H/^{13}C$ resonances meant that it was not possible to unambiguously assign a complete structure to serpulanine B (**2**) via analysis of the NMR and HRESIMS data alone.

Serpulanine B (**2**) was initially isolated as a viscous oil. Co-crystallization of **2** with L-(−)-malic acid gave colorless needles that were suitable for single-crystal X-ray diffraction analysis. The X-ray analysis of the crystals of serpulanine B generated the ORTEP-style diagram shown in Figure 3 that identified the complete constitution of the natural product and assigned the oxime configuration in the crystals as *E*. There were two molecules of serpulanine B (**2**) in the unit cell, which were enantiomers, demonstrating that the natural product was racemic. Once again, an *E* to *Z* oxime equilibrium in solution

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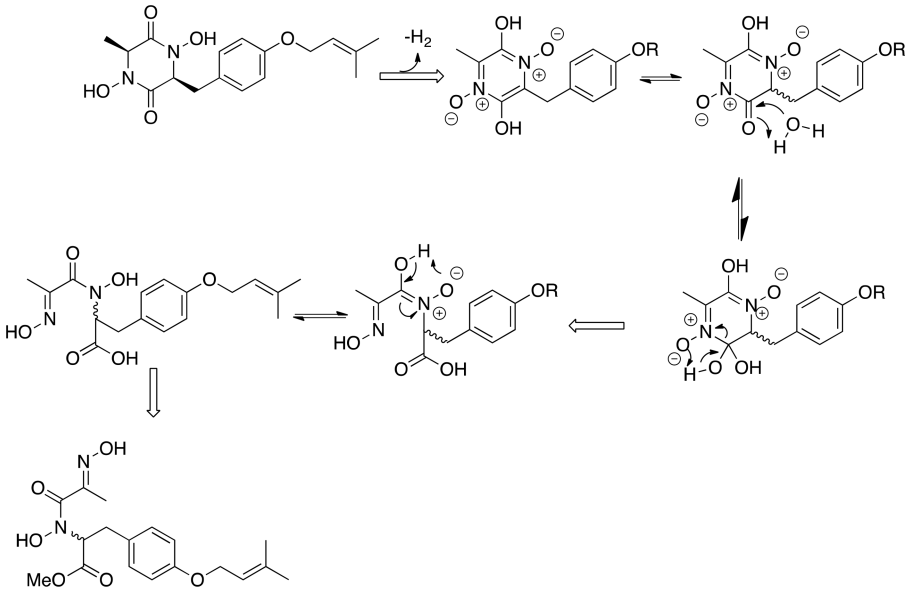
Scheme 1. Proposed Biogenesis of Serpulanine B (2)



might account for the complexity of the NMR data recorded for serpulanine B (**2**).

Serpulanine C (**3**) gave an $[M + Na]^+$ ion in the HRESIMS at m/z 285.1218 appropriate for a molecular formula of $C_{14}H_{18}N_2O_3$, which differs from that of **1** by the loss of one O atom but still requires 7 sites of unsaturation. The 1D and 2D NMR data (Table 1, Supporting Information) obtained for **3** were markedly similar to the spectra observed for **1**. Significant differences in the NMR data obtained for **1** and **3** were only found in the resonances assigned to the exchangeable protons (δ 8.91, 10.71, 11.68 in **1**; δ 7.19, 7.28, 11.75 in **3**) and the resonances assigned to both C-1 (δ 161.3 in **1**; δ 165.1 in **3**) and the C-1 nitrogen substituent (δ −217.8 in **1**; −284.1 in **3**). A proton resonance at δ 11.75 (2-N-OH) in the ^{1}H NMR spectrum of **3** that did not correlate to a carbon or nitrogen atom in the gHSQC experiments showed a gHMBC correlation to a carbon resonance at δ 152.1 assigned to C-2. The chemical shift of C-2 (δ 152.1) in **3** was nearly identical to the chemical shift of C-2 (δ 151.1) in **1**, leading to the identification of an oxime functionality at C-2 in serpulanine C (**3**). The remaining unassigned carbon, oxygen, nitrogen, and proton atoms were assigned to a primary amide since the two broad singlets at δ 7.19 and 7.28 correlated to the nitrogen at δ −284.1 in the g^{15}NHSQC and to C-1 (δ 165.1) and C-2 (δ 152.1) in the gHMBC experiment. Therefore, the complete constitution of serpulanine C was assigned as **3**, and we have assumed that the oxime configuration is *E* as in **1** and **2**.

The serpulanines have a mixed isoprenoid/amino acid biogenesis with a single isoprene unit being added at some stage to a tyrosine phenol as an alkyl ether. Serpulanine C (**3**) requires conversion of the tyrosine carboxylic acid to a primary amide and the oxidation of the tyrosine α-amino group to an oxime, presumably by a P450 enzyme, as has been well documented in the biosynthesis of glucosinolates in plants.[12,13] Further N-hydroxylation of the primary amide in **3** would lead to the hydroxamic acid functionality in serpulanine A (**1**). A recent study has shown that the terminal hydroxamate in the microbial metabolite trichostatin A (**4**)[14] arises from enzymatic transfer of an intact hydroxylamine from a side chain hydroxamic acid in an oxidized glutamine (GluHx) to a terminal carboxylic acid precursor of **4**. Although this could also be a possible origin for the terminal hydroxamate functionality in serpulanine A (**1**), the co-occurrence of serpulanine C (**3**) argues for a two-step pathway to **1** involving primary amide formation followed by N-hydroxylation.

Baran et al. have reported the synthesis and structural reassignment of sarcodonin (**5**), the only other natural product that contains the 2-hydroxyimino hydroxamate functional group array found in serpulanine B (**2**). They proposed that the oxime and hydroxamate functionalities in the natural product **5** arise from an Ile-Ile diketopiperazine precursor.[15] Scheme 1 shows a putative biogenesis of serpulanine B (**2**), modeled on Baran's proposal, that proceeds through an N,N′-oxidized Ala-Tyr diketopiperazine. This pathway can epimerize the tyrosine α carbon stereogenic center as it proceeds through a planar aromatic intermediate, which is consistent with the fact that the natural product **2** is racemic.

Serpulanine A (**1**) shows a structural resemblance to the hydroxamic acid-containing natural product trichostatin A (**4**) isolated from the actinomycete *Streptomyces hygroscopious*.[16] Trichostatin A is a potent inhibitor of mammalian HDACs both *in vitro* and *in vivo*, and it also shows potent *in vitro* antiproliferative activity against a variety of human cancer cells lines. It has not been effective in anticancer clinical trials, a failure attributed to poor pharmacokinetics. Crystal structures of trichostatin (**4**) bound to a histone-deacetylase-like protein showed that it acts like a substrate mimic, blocking access of acetylated lysine residues on the histones to the enzyme active site. The hydoxamate functionality in **4** binds to an essential zinc atom in the active site, and the benzene ring occupies a lipophilic binding pocket.

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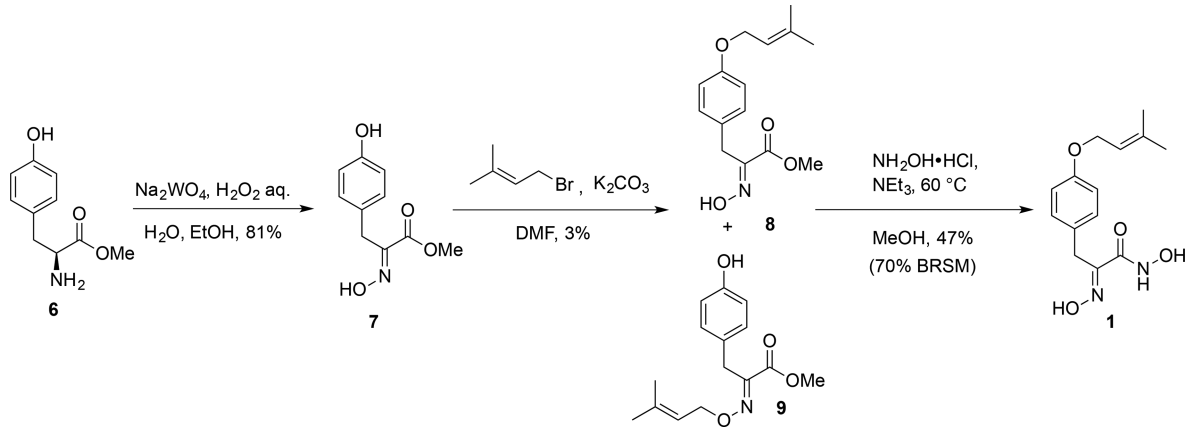
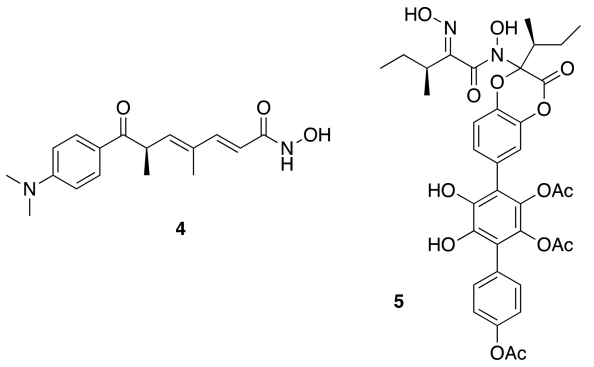
Scheme 2. Synthesis of Serpulanine A (1)





The ongoing interest in HDAC inhibitors as potential therapeutic agents,[17] combined with the linear structure of serpulanine A (**1**), which contains a terminal hydroxamic acid functionality and a *para*-disubstituted benzene ring similar to the structural elements in trichostatin A (**4**), prompted us to test **1** for its ability to inhibit HDACs. Since the natural product was only available in very small amounts, a synthesis of serpulanine A (**1**) was carried out in order to provide the material required for preliminary biological evaluation.

Our synthetic route to serpulanine A (**1**) is shown in Scheme 2. Treatment of L-tyrosine methyl ester (**6**) with a stoichiometric amount of sodium tungstate afforded the oxime **7** in 82% yield.[18] Alkylation of the phenol **7** with dimethylallyl bromide gave the oxime-alkylated product **9** as the major product and the desired phenol-alkylated product **8** in low yield. Extensive efforts to find a protecting group strategy that would have allowed only the desired selective alkylation at the phenol all proved futile. Therefore, since the starting material **6** was cheap, the chromatographic separation of **8** and **9** was routine, and only tens of milligrams of **1** was needed for the preliminary biological evaluation, we accepted the low yield of **8** and proceeded with the synthesis. Treatment of the methyl ester **8** with excess hydroxylamine and triethylamine in MeOH at 60 °C gave serpulanine A (**1**) in 47% yield. Synthetic serpulanine A (**1**) was identical with the natural product by NMR, MS, and TLC comparison (Supporting Information).

Serpulanine A (**1**) was evaluated for HDAC inhibition compared with a trichostatin A (**4**) control using a commercial luminescent cell-based assay that measures relative activity of total HDAC class I and II enzymes. Synthetic serpulanine A (**1**) was found to inhibit total class I/II HDAC enzymatic activity in murine metastatic lung carcinoma cells[19] in a dose-dependent manner with an IC_{50} of 7 μM (Supporting Information). The small amount of natural serpulanine B (**2**) that was available was not sufficient to allow testing it in the cell-based assay for total class I/II HDAC inhibition. Therefore, **2** was tested for *in vitro* inhibition of pure HDAC6 and HDAC9 enzymes at a single concentration of 330 μM. Even at this high concentration, serpulanine B (**2**) inhibited HDAC6 enzymatic activity by only 69%, and it showed no inhibition of HDAC9 enzymatic activity. This limited bioactivity evaluation of serpulanine B (**2**) indicates that it is a significantly less potent HDAC inhibitor than serpulanine A (**1**).

CONCLUSION

Serpulanines A (**1**) and B (**2**) isolated from the Sri Lankan macrofungus *Serpula* sp. both contain the rare 2-hydroxyimino hydroxamic acid functional group array. The only previously known natural product that has this functional group array is the fungal metabolite sarcodonin (**5**), although 2-hydroxyimino amides are common substructures in the bastadins and related compounds isolated from marine sponges.[10,11] Serpulanine A (**1**) is an O-prenylated N-oxidized tyrosine amide derivative, while serpulanine B (**2**) is a prenylated and N-oxidized Ala-Tyr dipeptide. A proposed biogenesis for serpulanine B illustrated in Scheme 1 proceeds through an Ala,Tyr diketopiperazine intermediate, paralleling the proposed biogenesis of sarcodonin (**5**),[15] resulting in the observed racemization of the natural product **2**. Serpulanine A (**1**) represents a new natural product HDAC inhibitory scaffold that is synthetically accessible and active in murine metastatic lung carcinoma cells with an IC_{50} of 7 μM.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured using a Jasco P-1010 polarimeter with sodium light (589 nm). UV spectra were recorded with a Waters 996 photodiode array detector. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV-600 spectrometer with a 5 mm CPTCI cryoprobe. ^{1}H chemical shifts are referenced to the residual DMSO-d_6 (δ 2.49 ppm), and ^{13}C chemical shifts are referenced to the DMSO-d_6 solvent peak (δ 39.5 ppm). Low- and high-resolution ESI-QIT-MS were recorded on a Bruker-Hewlett-Packard 1100 Esquire−LC system mass spectrometer. Merck type 5554 silica gel plates and Whatman MKC18F plates were used for analytical thin-layer chromatography (TLC). Reversed-phase HPLC purifications were performed on a Waters 600E system controller liquid chromatograph attached to a Waters 996 photodiode array detector. All solvents used for HPLC were Fisher HPLC grade.

Fungal Material. The rare fungus *Serpula* sp. was collected from a wooded area in Bibile (geographical coordinates 7°10′0″ N, 81°13′0″

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E) in the Monaragala District of Sri Lanka in December 2012. A voucher specimen (UOC:BIB:MB4) has been deposited in the Department of Plant Sciences, University of Colombo.

The fungus was identified based on macro- and micromorphological characteristics: Macromorphological characteristics recorded are effused reflexed, semicircular, up to 12.0 cm wide and 11.0 cm from base to margin; upper surface, off-white, smooth, fine hair present; lower surface, yellow, maze-like, 1−2 pores/mm; context, white, 0.2−2.0 cm. Micromorphological characteristics recorded are hyphal system monomitic, hyphae hyaline, septate and rarely clamped; spore, spherical to ovoid, pale yellow, 2.8−3.8 × 3.7−5.7 μm. These descriptions matched the morphological description of *Serpula himantoides* described in *The Complete Encyclopedia of Mushrooms* (Gerrit J. Keizer, Rebo International, The Netherlands, 1998) except for the fact that *S. himantoides* grows spreading on the substrate, whereas this specimen is bracket-like (effuse reflexed). When an Internet-based search was conducted (http://www.thebeamguy.com/rotfungi.php; http://www.gobice.com/?action=details&gid=2171) for *Serpula*, it was found that spore characteristics of this specimen and *Serpula* sp. (Pers.) Gray matched. Hence the specimen was inferred to be a *Serpula* sp.

Extraction of *Serpula* sp. and Isolation of Serpulanines A (2), B (2), and C (3). The fruiting bodies of *Serpula* sp. (30 g) were air-dried in the shade for 2 weeks, powdered, and extracted sequentially with 250 mL each of MeOH, 1:1 MeOH/CH_2Cl_2, and CH_2Cl_2. The three organic extracts were combined and concentrated under reduced pressure to give 6 g of crude extract.

The crude extract was suspended in 4:1 MeOH/H_2O (500 mL) and defatted by extraction with hexanes (2 × 250 mL). The polarity of the aqueous layer was adjusted to 3:2 MeOH/H_2O and extracted with $CHCl_3$ (2 × 250 mL). The $CHCl_3$ extract was concentrated under reduced pressure to yield a brown, amorphous solid (2 g). The $CHCl_3$-soluble material was chromatographed on a reversed-phase Si gel cartridge (step gradient: 1:1 MeOH/H_2O to MeOH, 10 g Sep pak). The resulting fractions were combined according to TLC profiles. Si gel column chromatography (2.5 × 22 cm; 270−400 mesh) of one of the major combined fractions (300 mg) with gradient elution (5:1 hexane/EtOAc to EtOAc), followed by size-exclusion chromatography on Sephadex LH-20 (1 × 45 cm) with MeOH as eluent, resulted, after recrystallization from MeOH, in the isolation of serpulanine A (**1**) (5.0 mg) as colorless plate crystals. Fractionation of LH-20 fractions eluting just after serpulanine A (**1**) by C_{18} reversed-phase HPLC using an InertSustain, 5 μm, 25 × 1 cm column for 38 min under isocratic conditions with 75:25 H_2O/MeCN as eluent followed by a linear gradient to 55:45 MeCN/H_2O over an additional 52 min gave pure samples of **1** (retention time = 82 min, 2.2 mg) along with serpulanine B (**2**) (retention time = 97 min, 2.8 mg) as a viscous oil and serpulanine C (**3**) (retention time = 88 min, 0.6 mg) as an amorphous solid.

Crystallization of Serpulanine B (2) in the Presence of L-(−)-Malic Acid. To serpulanine B (**2**) (1.87 mg, 5.1 μmole) was added L-(−)-malic acid (0.69 mg, 5.1 μmol) dissolved in 200 μL of MeOH. This solution was left to evaporate slowly at 3 °C. The resulting colorless needle crystals were subjected to single-crystal X-ray diffraction analysis.

Serpulanine A (1): colorless plate crystals; mp 129−133 °C; UV (1:1 H_2O/MeCN) λ_{max} 203, 226 (sh), 271 (sh) nm; ^1H and ^{13}C NMR, see Table 1; positive ion HRESIMS $[M + Na]^+$ m/z 301.1169 (calcd for $C_{14}H_{18}N_2O_4Na$, 301.1164).

Serpulanine B (2): racemic colorless needle crystals; mp 76−78 °C; UV (3:1 H_2O/MeCN) λ_{max} 206, 228, 271 nm; ^1H and ^{13}C NMR, see Table 1; positive ion HRESIMS $[M + Na]^+$ m/z 387.1545 (calcd for $C_{18}H_{24}N_2O_6Na$, 387.1532).

Serpulanine C (3): colorless, amorphous solid; UV (1:1 H_2O/MeCN) λ_{max} 201, 224 (sh), 276 (sh) nm; ^1H and ^{13}C NMR, see Table 1; positive ion HRESIMS $[M + Na]^+$ m/z 285.1218 (calcd for $C_{14}H_{18}N_2O_3Na$, 285.1215).

Preparation of (E)-Oxime 7. L-Tyrosine methyl ester **6** (2.00 g, 10.2 mmol) was added to EtOH (30 mL) at 0 °C to form a suspension. $Na_2WO_4·2H_2O$ (169 mg, 0.51 mmol), hydrogen peroxide solution (30% w/w in water 10 mL), and H_2O (20 mL) were added in sequence to form a light yellow suspension. After stirring at 0 °C for 45 min and at RT for 30 h, this reaction suspension was extracted with EtOAc (50 × 3 mL). The EtOAc extracts were combined, washed with aqueous $Na_2S_2O_3·5H_2O$ (10% w/w, 60 × 3 mL) and brine (60 mL), dried with $MgSO_4$, filtered, and then concentrated *in vacuo*. Product **7** (1.73 g, 81%) was a yellow solid, which was pure enough to be used without any further purification. Following a similar procedure, the same product was obtained in 82% yield using 1 equiv of $Na_2WO_4·2H_2O$ in 4.5 h at RT.

7: ^1H NMR (300 MHz, acetone-d_6) δ 3.12 (br s, 1 H), 3.76 (s, 3 H), 3.88 (s, 2 H), 6.77 (d, J = 8.5 Hz, 2 H), 7.15 (d, J = 8.5 Hz, 2 H); ^{13}C NMR (75 MHz, acetone-d_6) δ 52.4, 116.0, 128.1, 130.9, 151.9, 156.8, 165.2, 206.4; HRESIMS $[M + Na]^+$ calcd for $C_{10}H_{11}NO_4Na$ 232.0586, found 232.0583.

Preparation of Methyl Esters 8 and 9. Oxime **7** (2.35 g, 11.2 mmol) and K_2CO_3 (1.86 g, 13.5 mmol) were added to dry dimethylformamide (40 mL) to provide a white suspension. Prenyl bromide (1.37 mL, 11.2 mmol) was added via syringe to give a brown suspension. After stirring at room temperature for 4 h, the reaction was quenched by adding H_2O (60 mL). The aqueous layer was acidified (pH < 1) by adding concentrated HCl; then EtOAc (60 × 4 mL) was used for extraction. The combined EtOAc layers were washed with H_2O (100 mL) and brine (100 mL), dried over $MgSO_4$, filtered, and evaporated *in vacuo* to give the crude. The crude was purified by Si gel chromatography (hexane/EtOAc, 4:1) to give **8** (88 mg, 3%) as a yellow oil and **9** (1.64 g, 53%) as a yellow oil,

8: ^1H NMR (400 MHz, $CDCl_3$) δ 1.73 (s, 3 H), 1.78 (s, 3 H), 3.83 (s, 3 H), 3.92 (s, 2 H), 4.47 (d, J = 6.5 Hz, 2 H), 5.48 (t, J = 6.1 Hz, 1 H), 6.82 (d, J = 8.5 Hz, 2 H), 7.22 (d, J = 8.5 Hz, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.3, 26.0, 29.8, 52.9, 64.9, 114.8, 119.8, 127.6, 130.3, 138.3, 152.1, 157.8, 164.0; HRESIMS $[M + Na]^+$ calcd for $C_{15}H_{19}NO_4Na$ 300.1212, found 300.1204.

9: ^1H NMR (300 MHz, $CDCl_3$) δ 1.71 (s, 3 H), 1.78 (s, 3 H), 3.81 (s, 3 H), 3.85 (s, 2 H), 4.79 (d, J = 7.1 Hz, 2 H), 5.03 (s, 1 H), 5.45 (t, J = 7.1 Hz, 1 H), 6.71 (d, J = 8.5 Hz, 2 H), 7.13 (d, J = 8.5 Hz, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.4, 25.9, 30.5, 52.9, 72.6, 115.4, 119.1, 128.2, 130.5, 139.5, 150.7, 154.4, 164.2; HRESIMS $[M + Na]^+$ calcd for $C_{15}H_{19}NO_4Na$ 300.1212, found 300.1215.

Preparation of Serpulanine A (1). Methyl ester **8** (38.0 mg, 0.14 mmol) was dissolved in MeOH (5 mL) to form a colorless solution; then $NH_2OH·HCl$ (95.2 mg, 1.4 mmol) and NEt_3 (0.38 mL, 2.7 mmol) were added. After stirring at 60 °C for 2 days, the reaction was worked up by adding H_2O (10 mL). The aqueous layer was acidified (pH < 1) with concentrated HCl, and then EtOAc (10 × 3 mL) was used for extraction. The combined EtOAc layers were washed with brine (20 mL), dried over $MgSO_4$, filtered, and evaporated *in vacuo*. The crude EtOAC-soluble material was purified by Si gel chromatography (1:1 hexane/EtOAc) to recover the starting material **8** (12.5 mg) and crude product **1** (18 mg, 47%, 70% BRSM) as a white solid. The synthetic serpulanine A (**1**) was further purified using reversed-phase HPLC (eluting with 2:3 MeCN/H_2O, retention time = 25.0 min).

^1H NMR (400 MHz, MeOH-d_4) δ 1.73 (s, 3 H), 1.77 (s, 3 H), 3.84 (s, 2 H), 4.48 (d, J = 6.5 Hz, 2 H), 5.43 (t, J = 6.5 Hz, 1 H), 6.78 (d, J = 8.5 Hz, 2 H), 7.16 (d, J = 8.5 Hz, 2 H); ^{13}C NMR (100 MHz, MeOH-d_4) δ 18.2, 25.8, 29.4, 65.8, 115.6, 121.4, 129.7, 131.1, 138.4, 153.2, 158.8, 164.2; HRESIMS $[M + Na]^+$ calcd for $C_{14}H_{18}N_2O_4Na$ 301.1164, found 301.1161. See Supporting Information for ^1H NMR spectrum comparison with the natural product in DMSO-d_6.

Cell-Based HDAC Inhibition Assays. Serpulanine A (**1**) was analyzed for its effect on histone deacetylase activity in the A9 murine metastatic lung carcinoma cell line[19] using the HDAC-GloTM I/II assay and screening system (Promega) as instructed by the manufacturer. The linear range of A9 cells was established in a black-walled, clear-bottomed 96-well plate (PerkinElmer). Cells were diluted to 105 cells/mL and serial diluted by 2-fold, to a final concentration of 98 cells/mL. All dilutions were plated in triplicate in a volume of 100 μL per well. Cells were cultured at 37 °C for 24 h before addition of HDAC class I/II reagent. Luminescence was read after 30 min of incubation with HDAC class I/II reagent. After determination of an optimal cell density of 30 000 cells/well, cells were plated in a 96-well plate and left for 24 h at 37 °C. Medium was used as a blank control, and a positive control provided in the HDAC assay kit was included consisting of HeLa cells.

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The next day, medium was removed from the wells and new media containing the treatments—vehicle (negative control), trichostatin A (TSA) (**4**) (positive control), or a range of dilutions of serpulanine A (**1**) (29 to 0.9 μM)—were added in triplicate and incubated for 30 min. HDAC class I/II reagent was then added and incubated for 30 min before luminescence was measured using an Infinite M200 (Tecan) with the i-control software (Tecan).

Individual HDAC Enzyme Inhibition Assays. The activity of serpulanine B (**2**) was assessed with purified human HDAC 6 and 9 enzymes using HDAC fluorogenic assay kits (BPS Biosciences). All assays were completed in black-sided clear-bottom 96-well plates (PerkinElmer), and all treatments were plated in triplicate. The assays were measured using a Synergy HI hybrid reader (BioTek) and Gen5 software (BioTek); excitation was set to 360 nm, and detection was measured at 450 nm with a gain of 100. Following optimization, each HDAC was run following the Promega protocol in black-sided clear-bottomed 96-well plates in triplicates with the same treatments listed above (PerkinElmer). Luminescence was read 30 min after HDAC-GloI/II reagent was added using the Synergy HI hybrid reader (BioTek) and Gen5 software (Bio-Tek). For all assays, vehicle (1% DMSO) was used as a negative control and TSA (25 nM) was used as a positive control, and all assays contained multiple blank controls. To calculate percent activity, the average of blank wells was subtracted from all treatment groups. The relative mean of activity of the HDAC being measured was determined, and all wells that received treatment were divided by this average, to give a percentage of activity. All individualized enzyme assays were analyzed for significance using an unpaired *t* test that compared the different treatment concentrations of the compounds directly to untreated HDACs.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.7b00680.

1D and 2D NMR spectra for **1**, **2**, and **3**; experimental details for the X-ray diffraction analyses of **1** and **2** (PDF)

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Notes

The authors declare no competing financial interest.

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