Herbicidin Congeners, Undecose Nucleosides from an Organic Extract of Streptomyces sp. L-9-10

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Supporting Information

ABSTRACT: Four new undecose nucleosides (herbicidin congeners), three known herbicidins, and 9-((β-D-arabinofuranosyl)hypoxanthine (Ara-H) were isolated from the organic extract of a fermentation culture of Streptomyces sp. L-9-10 using proton NMR-guided fractionation. Their structures were elucidated on the basis of comprehensive 1D and 2D NMR and mass spectrometry analyses. These structures included 2′-O-demethylherbicidin F (1), 9′-deoxy-8′,8′′-dihydroxyherbicidin B (2), 9′-deoxy-8′-oxoherbicidin B (2a), and the 8′-epimer of herbicidin B (3). This is the first detailed assignment of proton and carbon chemical shifts for herbicidins A, B, and F. The isolated compounds were evaluated for cancer chemopreventive potential based on inhibition of tumor necrosis factor alpha (TNF-α)-induced nuclear factor-kappa B (NF-κB) activity.

Actinomycetes represent a ubiquitous, dominant group of Gram-positive bacteria. In the past few decades studies of the bioactive metabolites isolated from Actinomycetes have demonstrated that these compounds are valuable leads for the development of new pharmaceutical agents.12,13 Herbicidins, adenine nucleoside antibiotics belonging to a chemical class of undecose (C11)-based nucleosides, have rarely been reported from natural sources. Since the original report of the herbicidin chemical scaffold from Streptomyces saganonensis in 1976, only 11 congeners have been discovered. These include herbicidins A–C (4–6), E (7), F (8), and G and H3–7 S12245,8 antibiotic BE-65932,9 aureonucleomycin (9),10 and the synthetic 6′-epi-herbicidin B.11 Some of these naturally occurring nucleoside derivatives (4, 5, and 9) showed herbicidal and antialgal activities and significantly inhibited the growth of Xanthomonas oryzae, which causes rice leaf blight; they were also selectively toxic toward dicotyledons.10,11 In addition, the herbicidins have attracted considerable attention as synthetic targets due to their complex chemical structures. This unique nucleoside motif consists of a heterocyclic adenine base, an unusual undecose sugar moiety (consisting of a tricyclic furano-pyano-pyran ring system), an internal hemiketal that links C-3′ and C-7′, and four substituents at the C-7′, C-8′, C-9′, and C-10′ positions on the pyran ring that are fixed in axial positions.12–18 The first total synthesis of herbicidin B (5) was accomplished in 1999 by Matsuda et al.,11 and the total syntheses of herbicidin C and aureonucleomycin were achieved in 2012 by Trauner et al.19 As part of our continuing search for novel bioactive molecules from terrestrial bacteria of the Actinomycetaceae family,20,21 we undertook an investigation of the organic extract of a fermentation culture of Streptomyces sp. L-9-10, a strain isolated from the lichen Platismatia glauca. The butanol-soluble extract of the culture inhibited hyphae formation in our hyphae formation inhibition (HFI) assay, which is a surrogate for kinase inhibition. During the search for active kinase inhibitors, a series of herbicidins were also isolated via an NMR-guided fractionation process. New herbicidin congeners, 2′-O-demethylherbicidin F (1), 9′-deoxy-8′,8′′-dihydroxyherbicidin B (2) and its dehydrated product (2a), and the 8′-epimer of herbicidin B (3), were isolated from the broth and characterized spectroscopically. The known compounds herbicidins A (4), B (5), and F (8), along with the known 9′-(β-D-arabinofuranosyl)hypoxanthine (Ara-H), were also isolated. We report herein the isolation, structure elucidation, and biological activities of herbicidins from Streptomyces sp. L-9-10.

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RESULTS AND DISCUSSION

The molecular formula of compound 1 was determined as C_{22}H_{32}N,O_{10} (12 degrees of unsaturation) by HRESIMS. Analysis of its 1H and 13C NMR spectra (Tables 1 and 2) and HSQC experiments revealed the presence of three methyl groups (including a methyl ester), one methylene, eight oxygenated methines, one sp<sup>3</sup> oxygenated quaternary carbon (likely a hemiketal), six olefinic carbons, and two carbons for carboxylic acids or derivatives.

The 1H NMR data (Table 1) recorded in methanol-d<sub>4</sub> showed two singlet peaks at δ<sub>H</sub> 7.98 (1H, s, H-8) and 8.23 (1H, s, H-2), which in conjunction with the five nitrogen atoms deduced by HRESIMS suggested the presence of an adenine unit (fragment A) in 1 (Figure 1). This conclusion was further supported by the HMBC correlations that were observed from H-8 to C-4/C-5 and from H-2 to C-4/C-6. A second subunit (fragment B) was assembled based on analysis of the COSY spectrum, which connected an olefinic proton that resonated at δ<sub>H</sub> 6.68 (1H, q, J = 7.2 Hz, H-3′) with a methyl doublet at δ<sub>H</sub> 1.89 (3H, d, J = 7.2 Hz, H-4′). The HMBC correlations observed from H-3′ to C-4/C-1′ and from H-2/C-5′ to C-2′/C-3′/C-1′ in the HMBC experiments suggested a 2-methyl-2-butenolic (tiglic) group was present in 1. A methyl ester was deduced from the 13C NMR resonances at δ<sub>C</sub> 169.8 (C-11a) and 51.3 (11′-OCH<sub>3</sub>) and the HMBC correlation from 11′-OCH<sub>3</sub> (δ<sub>C</sub> 3.62) to C-11′. Connections of the tiglic and methoxycarboxyl groups were established through analysis of the HMBC experiments. The HMBC correlations from H-8′ to C-7′ (the quaternary hemiketal carbon) and C-1′ indicated the tiglic group was attached at the C-8′ position (Figure 1). This carbon (C-8′) could then be connected to the carbonyl C-11′ through a series of HMBC correlations: H-9′ to C-8′, C-7′, and C-11′; H-10′ to C-9′ and C-11′. Fragment C was deduced as follows. The COSY data contained cross-peaks consistent with the connections of H-1′-H-2′ and H-3′-H-6′. This information, in combination with the HMBC correlations from H-1′ to C-2′, C-3′, and C-4′ and from H-2′ to C-3′ and C-4′ suggested the presence of fragment C (Figure 1). These three fragments were then assembled into a larger structure that fulfilled most of the structural requirements. The observed HMBC cross-peaks of H-1′ to C-4/C-8 suggested fragments A and C were connected at C-1′ of the adenine residue through an N=C glycosidic bond. The HMBC correlations from both H-5′ and H-6′ to C-7′ revealed a direct C-6′/C-7′ linkage, and the HMBC correlation from H-5′ to C-4′ demonstrated the presence of a furan ring, while the HMBC correlation from H-10′ to C-6′ established the linkage of C-6′ and C-10′ via an ether bond to give a pyran ring (Figure 1). The above assignments accounted for 11 out of the 12 degrees of unsaturation. Therefore, one more ring was required to complete a planar structure. In principle, three possible cyclic hemiketal structures could be generated: C2′−O−C7′, C3′−O−C7′, and C9′−O−C7′. After

Table 1. 1H NMR Data (400 MHz, in CD3OD) of 1–5 and 8, δ in ppm and J in Hz

| no. | 1      | 2<sup>a</sup> | 3      | 4      | 5      | 8      |
|-----|--------|--------------|--------|--------|--------|--------|
| 2   | 8.23, s| 8.15, s      | 8.19, s| 8.24, s| 8.23, s| 8.23, s|
| 8   | 7.98, s| 8.60, s      | 8.59, s| 8.17, s| 8.01, s| 8.70, s|
| 1′  | 6.01, d| 6.01, d      | 6.07, br| 6.13, br| 6.08, d| 6.19, d|
| 2′  | 4.41, br| 4.01, br| 3.79, br| 4.23, br| 4.08, d| 4.01, br|
| 3′  | 4.38, d| 4.31, br s  | 4.38, br s| 4.44, br d| 4.52, br d| 4.47, br d|
| 4′  | 4.52, m| 4.35, m      | 4.41, m | 4.48, m | 4.43, m| 4.45, m|
| 5′  | 2.27, m| 1.98, m      | 2.17, m | 2.28, dd| 2.28, m| 2.26, m|
| 6′  | 4.52, t| 4.35, m      | 4.34, m | 3.90, dd| 4.55, dd| 4.69, t|
| 8′  | 5.01, d| 3.60, d     | 3.60, d| 3.60, d| 5.09, d| 3.74, d|
| 9′  | 4.34, dd| 2.32, dd| 2.32, dd| 2.60, d| 4.35, dd| 4.36, dd|
| 10′ | 4.47, br| 4.40, br d| 4.98, d| 4.56, br s| 4.49, br s| 4.39, br s|
| 3′  | 6.68, q| 6.80, q     | 6.80, q| 1.98, q| 1.91, q| 1.91, q|
| 4′  | 1.89, d| 1.87, s      | 4.43, m| 1.89, s| 3.37, s| 3.34, s|
| 5′  | 1.87, s| 3.37, s      | 3.43, s| 3.49, s| 3.42, s| 3.48, s|
| 2′−OCH<sub>3</sub> | 3.62, s | 3.58, s | 3.59, s | 3.74, s | 3.65, s | 3.74<sup>a</sup> |
| 7′α−OH | 5.72, s | 5.97, s |
| 8′β−OH | 5.92, s |

<sup>a</sup>Measured in DMSO-d<sub>4</sub>. <sup>b</sup>−<sup>c</sup>Signals with the same letter were partly overlapped.
carefully checking the literature and comparing our NMR spectroscopic data to those reported for herbicidin F (8), we concluded that 1 had a C3′−O−C7′ linkage due to the similarity of the NMR data. The only difference between the two was the replacement of the 2′-OCH3 in 8 with 2′-OH in 1.

The physicochemical analyses and the key NOESY correlations from H-8 to H-2′/H-3′, from H-3′ to H-6′/11′-OCH3, and from H-1′ to H-3′/H-4′ (Figure 2) of 1 supported the same relative configuration compared to those of herbicidins B (5) and G. On the basis of these data, the structure of 1 was determined to represent a new compound and was named 2′-O-demethylherbicidin F.

Compound 2 was isolated as a white solid. Its molecular formula was determined as C18H23N5O9 on the basis of the HRESIMS data. Comparison of the 1H and 13C NMR data of 2 and 1 (Tables 1 and 2) indicated that they were very closely related analogues, differing only in the presence of an additional methoxy (δH 3.37; δC 57.9, 2′-OCH3), an oxygenated quaternary carbon (δC 92.6, C-8′), and a methylene [δH 2.32 (dd, J = 8.0, 13.6 Hz) and 2.19 (br d, J = 13.6 Hz)] in 2, instead of the tiglyl group at C-8′ and an oxygenated methine group at C-9′ found in 1. These conclusions were supported by the HMBC correlations from H-9′ to C-11′/C-8′/C-7′, of H-10′ to C-9′/C-6′, of 7′-OH (δH 5.72) to C-8′/C-6′, and of two OH protons (δH 5.92 and 5.97) to C-9′.

Figure 1. Selected HMBC and COSY correlations of 1.

Figure 2. Selected NOESY correlations of 1.

Table 2. 13C NMR Data (100 MHz, in CD3OD) of 1–5 and 8, δ in ppm

| no. | 1 | 2a | 2a′ | 3 | 4 | 5 | 8 |
|-----|---|----|-----|---|---|---|---|
| 2   | 152.7, CH | 153.2, CH | 153.5, CH | 152.8, CH | 152.8, CH | 152.6, CH | 152.7, CH |
| 4   | 149.2, C | 149.5, C | 149.7, C | 149.1, C | 149.1, C | 148.9, C | 149.1, C |
| 5   | 118.3, C | 118.8, C | 118.5, C | 118.0, C | 118.1, C | 118.0, C | 118.0, C |
| 6   | 156.0, C | 156.5, C | 156.5, C | 155.9, C | 156.0, C | 155.8, C | 156.0, C |
| 8   | 139.2, CH | 140.7, CH | 138.9, CH | 139.0, CH | 139.5, CH | 141.0, CH | 139.5, CH |
| 1′  | 89.6, CH | 87.2, CH | 86.8, CH | 88.0, CH | 87.1, CH | 87.7, CH | 87.3, CH |
| 2′  | 81.0, CH | 90.8, CH | 89.7, CH | 89.5, CH | 90.3, CH | 90.8, CH | 90.3, CH |
| 3′  | 76.4, CH | 73.0, CH | 72.7, CH | 72.5, CH | 73.1, CH | 72.4, CH | 73.2, CH |
| 4′  | 77.5, CH | 77.8, CH | 77.5, CH | 78.4, CH | 77.7, CH | 78.3, CH | 77.5, CH |
| 5′  | 25.3, CH2 | 26.4, CH2 | 25.5, CH2 | 25.0, CH2 | 25.1, CH2 | 25.0, CH2 | 25.2, CH2 |
| 6′  | 65.2, CH | 66.3, CH | 57.9, CH | 69.6, CH | 65.1, CH | 64.1, CH | 65.2, CH |
| 7′  | 91.8, C | 94.5, C | 94.1, C | 94.2, C | 92.0, C | 93.3, C | 91.9, C |
| 8′  | 70.5, CH | 92.6, C | 200.5, C | 68.4, CH | 70.5, CH | 69.9, CH | 70.5, CH |
| 9′  | 69.0, CH | 38.4, CH2 | 40.6, CH2 | 70.7, CH | 69.2, CH | 73.0, CH | 69.1, CH |
| 10′ | 76.9, CH | 71.0, CH | 74.6, CH | 79.0, CH | 76.9, CH | 76.6, CH | 76.9, CH |
| 11′ | 169.8, C | 172.3, C | 170.9, C | 169.9, C | 170.0, C | 170.4, C | 169.8, C |
| 1′r | 165.8, C | | | 164.7, C | | | 165.7, C |
| 2′r | 129.6, C | | | 131.1, C | | | 127.0, C |
| 3′r | 140.4, CH | | | 144.3, CH | | | 140.3, CH |
| 4′r | 13.6, CH3 | | | 13.7, CH3 | | | 13.7, CH3 |
| 5′r | 10.9, CH3 | | | 54.9, CH2 | | | 10.9, CH3 |

Table notes:
- Measured in DMSO- d6.

Herbicidins B (5) and G. On the basis of these data, the structure of 1 was determined to represent a new compound and was named 2′-O-demethylherbicidin F.

Compound 2 was isolated as a white solid. Its molecular formula was determined as C18H23N5O9 on the basis of the HRESIMS data. Comparison of the 1H and 13C NMR data of 2 and 1 (Tables 1 and 2) indicated that they were very closely related analogues, differing only in the presence of an additional methoxy (δH 3.37; δC 57.9, 2′-OCH3), an oxygenated quaternary carbon (δC 92.6, C-8′), and a methylene [δH 2.32 (dd, J = 8.0, 13.6 Hz) and 2.19 (br d, J = 13.6 Hz)] in 2, instead of the tiglyl group at C-8′ and an oxygenated methine group at C-9′ found in 1. These conclusions were supported by the HMBC correlations from H-9′ to C-11′/C-8′/C-7′, of H-10′ to C-9′/C-6′, of 7′-OH (δH 5.72) to C-8′/C-6′, and of two OH protons (δH 5.92 and 5.97) to C-9′. 

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Figure 3. Key HMBC and NOESY correlations of 2 and 2a.

Figure 4. Key NOESY correlations of 3.

Figure 5. CD and UV spectra of 3 and 5 in MeOH.
hydroxyl substitutions or oxygenation at nine of the 11
undecene backbone carbons. Their complex structures have
made their structure elucidation and data assignment
challenging, especially before the advent of 2D NMR
techniques. Our work represents the first complete NMR
assignment of the herbicidin family of natural products and the
first isolation of a naturally occurring herbicidin with an 8′α-
OH (3).

Compounds 2 and 2a, with partial structural similarity to
spectinomycin and adenylenated spectinomycin,22 are of special
interest, as they are the first herbicidin analogues without a
hydroxyl substituent at the C-9′ position. The discovery of 2 led
us to speculate the herbicidins may be formed in a biogenic
pathway originally from adenosine and a deoxyhexose, possibly
derived from glucose, as its nitrogen and carbon sources.

Compounds 1−5 and 8 were evaluated for their inhibitory
activities against Streptomyces 8SE in the HFI assay, according
to an established protocol.21 However, this series of nucleoside
antibiotics were inactive in the HFI assay. On the other hand,
when evaluated in a mammalian cell-based assay designed to
monitor TNF-α-induced NF-κB activity, 1−5 and 8 were found
to mediate inhibitory responses with IC50 values in a range of
about 0.5−4.9 μM (Table 3). With the exception of 5, when
growth inhibitory activity was not observed with the hormon-
responsive breast cancer cell line MCF-7 (data not shown).
Thus, the effect of these herbicidins on NF-κB appears
somewhat unique and worthy of further investigation.

In summary, a 1H NMR-guided fractionation of the culture
extract of terrestrial Streptomyces sp. L-9-10 led to the isolation
and elucidation of three new herbicidin congeners, one epimer
of known herbicin B, and three known analogues as well as 9-(β-D-
arabinofuranosyl)hypoxanthine. Moreover, this work
represents the first report of the complete NMR assignments
for the herbicidins and the first report on the isolation of an
8′α-OH herbicidin from nature. Biological testing showed that
the herbicidins exhibited modest inhibition against TNF-α-
induced NF-κB activity. Further work will be performed to
design and develop novel herbicidin analogues with potential
improved efficacy and selectivity.

### EXPERIMENTAL SECTION

#### General Experimental Procedures.

Specific rotations were recorded on an Autopol IV automatic polarimeter. UV spectra were measured on a Shimadzu PharmaSpec-1700 UV−visible spectrophotometer. CD spectra were measured on a JASCO J-810 spectropolarimeter. IR spectra were measured on a Bruker Tensor-27 spectrometer. 1D and 2D NMR spectra were recorded on Bruker AVANCE (400 MHz) and INOVA Unity (500 MHz) Varian spectrometers. Mass spectra and high-resolution MS spectra were obtained with a BioTOF II ESI mass spectrometer. Semi preparative HPLC was conducted on a Beckman Coulter Gold-168 system equipped with a photodiode array detector using an Alltech reversed-
phase Econosil C18 column (10 μm, 10 × 250 mm) with a flow rate of
2.0 mL/min. Column chromatography was carried out on Merck silica
gel 60 (70−230 mesh) and preparative TLC plates (0.2 × 20 × 20 cm).
Precoated plates of silica gel 60 F254 were used for analytical
purposes.

#### Microbial Material and Sample Collection.

The organism was isolated from the surface of the lichen Platismatia glauca growing on a Douglas fir in the Arboretum at the University of British Columbia Malcolm Knapp Research Forest, Maple Ridge, British Columbia, in February 2003. The strain was isolated using ISP#4 (International Streptomyces Project 4, inorganic salts starch agar) media plates supplemented with 50 μg/mL cycloheximide and 20 μg/mL nalidixic acid. The Streptomyces sp. L-9-10 is maintained in the laboratory collection at the University of British Columbia under the accession number DCA 2501. The strain shared >99% identity (853 base pairs of 863) with the partial 16S rRNA gene of an unpublished strain, Streptomyces sp. MJM9209 (accession GU296743, positions 24 to 858). Its sequence has been deposited in GenBank with the accession number KJ123644.

#### Culturing and Harvesting Streptomyces sp. L-9-10.

The same method22 as previously described was used to culture and harvest Streptomyces sp. L-9-10. For detailed protocols, see the Supporting Information.

#### Extraction and Isolation.

The fermentation broth (100 L) was centrifuged, and the supernatant was partitioned successively with CHCl3−MeOH (40:1) to obtain fractions E1−E10. Fraction E3 (231 g) was subjected to silica gel CC (500 g, 400−600 mesh) and eluted with a gradient of CHCl3−MeOH (0:1−1:1) to afford 100 fractions, E1−E10. Fraction E10 (2.5 g), with a 17 mm clear zone of inhibition in the HFI assay, was separated using Sephadex LH-20 (280 g, MeOH) to afford six fractions, Eα−Eγ. Fraction Eα (1.2 g) was further purified by silica gel CC eluting with hexane−acetone (2:1) to obtain fractions Eα1−Eα6. Fraction Eα4 was further separated with silica gel CC eluting with CHCl3−MeOH (40:1) to obtain fractions Eα4a−Eα4d. Subfraction Dα4 (40 mg) was separated by preparative TLC (2.0 mm, 20 × 20 cm) using CHCl3−EtOH (5:1, Rf 0.6) to yield 8 (7.0 mg). Then 1H NMR-

#### Table 3. Inhibitory Effect of Compounds 1−5 and 8 against TNF-α-Induced NF-κB Activity

| Compound | NF-κB % inhibitiona | NF-κB % survivalb | NF-κB IC50 (μM) | HEK293 cell line cytotoxicity IC50 (μM) |
|----------|---------------------|-------------------|-----------------|-------------------------------------|
| 1        | 92.4 ± 8.3          | 42.3 ± 3.9        | 4.9 ± 1.1       | 2.2 ± 0.7                           |
| 2        | 96.2 ± 4.9          | 45.1 ± 5.7        | 2.4 ± 0.9       | 13.7 ± 3.3                          |
| 3        | 92.2 ± 2.8          | 53.0 ± 3.9        | 3.2 ± 0.6       | 43.7 ± 3.9                          |
| 4        | 80.9 ± 5.2          | 45.8 ± 2.9        | 1.8 ± 0.3       | 2.7 ± 1.3                           |
| 5a       | 94.2 ± 1.6          | 95.6 ± 6.6        | 2.9 ± 0.8       | >50                                 |
| 8        | 83.4 ± 3.3          | 59.8 ± 8.0        | 0.5 ± 0.1       | 2.3 ± 0.8                           |
| TPCKb    | 3.8 ± 0.9           | 11.1 ± 1.6        |                 |                                     |
| BAY-11c  | 2.0 ± 0.4           | 3.8 ± 1.5         |                 |                                     |

a% inhibition of NF-κB at 50 μM. b% survival at a concentration of 50 μM. cPositive control for NF-κB.

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**Author contributions.** A.M. performed the experiments and analyzed the data. T.A. and P.C.G. designed the experiments and analyzed the data. C.A. performed the natural products isolation and analyzed the data. L.W. supervised the experiments and analyzed the data. All authors contributed to the writing of the manuscript.}

**Conflicts of interest.** The authors declare that they have no conflicts of interest.

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Aromatic fractionation was used for fractions E4 and E5, which exhibited characteristic proton signals at 8.00 and 4.00 ppm in their 1H NMR spectra. Fraction E4 (3.6 g) was separated by Sephadex LH-20 (280 g, MeOH) to obtain seven subfractions (E4a−E4g). Fraction E4e (1.1 g) was further subjected to silica gel CC eluting with hexane−acetone (1:2) to yield the main portion, then by a preparative TLC (2.0 mm, 20 × 20 cm) purification using CHCl3−EtOH (5:1, R0.4) to give 4 (13.5 mg). Fraction E5 (5.8 g) was subjected to silica gel CC eluting with EtOAc−EtOH (20:1−5:1) to obtain 12 subfractions (E5a−E5l). Fraction E5e was separated by Sephadex LH-20 (70 g, MeOH) to give four fractions (e1−e4). Fraction E5d was applied to silica gel CC eluting with hexane−acetone (2:3) to obtain 1 (23 mg). Fraction E5d (0.8 g) was separated with Sephadex LH-20 (70 g, MeOH) to obtain four subfractions (E5i−E5l), and fraction E5i was repeatedly separated by reversed-phase HPLC eluting with MeOH−H2O (55:45) to afford 2 (2.8 mg, mp 128.8−129.8 min), 3 (11.4 mg, mp 198 min), and 5 (27.8 mg, mp 243.2 min). Fraction E6 (2.2 g) was subjected to Sephadex LH-20 (280 g, MeOH) to yield four subfractions (E6a−E6d). Fraction E6C was applied to silica gel and eluted with CHCl3−MeOH (5:2) to afford crude Ara-H, which was purified by recrystallization from MeOH.

2’-O-Demethylherbicidin F (1): white, amorphous solid; [α]232 D 78.1 (c 0.032, MeOH); UV (MeOH) λmax (log ε) 212 (4.27), 259 (4.03) nm; IR (KBr) 3396, 3193, 2951, 2921, 1729, 1638, 1597, 1249, 1057, 955 cm−1; 1H and 13C NMR (CD3OD, 400/100 MHz) see Tables 1 and 2; HRESIMS m/z 522.1821 [M + H]+ (calcd for C22H28N5O10, 522.1836). 2’-Deoxy-8’-8’-dihydroxyherbicidin B (2): white solid; [α]232 D 48.0 (c 0.050, MeOH); UV (MeOH) λmax (log ε) 210 (4.13), 259 (4.00) nm; IR (KBr) 3332, 3212, 2849, 1732, 1642, 1597, 1574, 1299, 1197, 1019, 948 cm−1; 1H and 13C NMR (DMSO-d6, 400/100 MHz) see Tables 1 and 2; HRESIMS m/z 454.1560 [M + H]+ (calcd for C18H24N5O9, 454.1574).

Herbicidin A (4): white solid; [α]232 D 58.3 (c 0.075, MeOH); lit. [α]25 D 61.7 (c 1.0, MeOH); 1H and 13C NMR (CD2OD, 400/100 MHz) see Tables 1 and 2.

Herbicidin B (5): white, amorphous powder; [α]232 D 55.0 (c 0.050, MeOH); lit. [α]25 D 63.0 (c 1.0, MeOH); CD (1.0, MeOH) λmax (Ar) 260 (−0.5), 218 (−0.5) nm; 1H and 13C NMR (CD2OD, 400/100 MHz) see Tables 1 and 2.

Tumor Necrosis Factor (TNF-α)-Induced Nuclear Factor-Kappa B (NF-κB) Assay. We employed human embryonic kidney cells 293 Panomic for monitoring changes occurring along the NF-κB pathway.23 Stable constructed cells were seeded into 96-well plates at 20 × 103 cells per well. Cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) (Invitrogen Co.), supplemented with 10% FBS, 100 units/mL penicillin, 100 μg/mL streptomycin, and 2 mM L-glutamine. After 48 h incubation, the medium was replaced and the cells were treated with various concentrations of test substances. TNF-α (human, recombinant, E. coli, Calbiochem) was used as an activator at a concentration of 2 ng/mL (0.14 nm). The plate was incubated for 6 h. Spent medium was discarded, and the cells were washed once with PBS. Cells were lysed using 50 μL (for 96-well plate) of reporter lysis buffer from Promega, by incubating for 5 min on a shaker, and stored at −80 °C. The luciferase assay was performed using the Luc assay system from Promega. The gene product, luciferase enzyme, reacts with luciferase substrate, emitting light, which was detected using a luminometer (LUMiStar Galaxy BMG). Data for NF-κB inhibition are expressed as IC50 values (i.e., concentration required to inhibit TNF-induced NF-κB activity by 50%). As a positive control, two known NF-κB inhibitors were used: TPCK and BAY-11.

The inhibition of hyphae-formation in Streptomyces SS5 was performed on pure compounds as described previously.20

**ASSOCIATED CONTENT**

**Supporting Information**

Culturing and harvesting *Streptomyces* sp. L-9-10. The NMR spectra comparison for 2 and 2a. 1H and 13C NMR, DEPT, COSY, HSQC, HMBC, and NOESY data of compounds 1−5, 8, and Ara-H, as well as experimental data for the known compounds 4, 5, and 8. This material is available free of charge via the Internet at http://pubs.acs.org.

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**Notes**

The authors declare no competing financial interest.

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