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Spoxazomicin D and Oxachelin C, Potent Neuroprotective Carboxamides from the Appalachian Coal Fire-Associated Isolate *Streptomyces* sp. RM-14-6

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Abstract

The isolation and structure elucidation of six new bacterial metabolites [spoxazomicin D (**2**), oxachelins B and C (**4**, **5**), and carboxamides **6–8**] and 11 previously reported bacterial metabolites (**1**, **3**, **9–12a**, and **14–18**) from *Streptomyces* sp. RM-14-6 is reported. Structures were elucidated on the basis of comprehensive 1D and 2D NMR and mass spectrometry data analysis, along with direct comparison to synthetic standards for **2**, **11**, and **12a,b**. Complete 2D NMR assignments for the known metabolites lenoremycin (**9**) and lenoremycin sodium salt (**10**) were also provided for

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Supporting Information

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Synthesis of compounds **2**, **11**, and **12a,b**, biological activity data, workup isolation scheme, chemical structures of compounds **14–18**, HPLC-MS/UV, HRESIMS, and NMR (1D and 2D) spectra of compounds **1–12b** and **14–18** (PDF)

the first time. Comparative analysis also provided the basis for structural revision of several previously reported putative aziridine-containing compounds [exemplified by madurastatins A1, B1, C1 (also known as MBJ-0034), and MBJ-0035] as phenol-dihydrooxazoles. Bioactivity analysis [including antibacterial, antifungal, cancer cell line cytotoxicity, unfolded protein response (UPR) modulation, and EtOH damage neuroprotection] revealed **2** and **5** as potent neuroprotectives and lenoremycin (**9**) and its sodium salt (**10**) as potent UPR modulators, highlighting new functions for phenol-oxazolines/salicylates and polyether pharmacophores.

Graphical abstract

Roughly 0.3%¹ of reported microbial metabolites are classified as phenol-oxazolines or salicylate-containing natural products, where salicylate C7 amino acid or peptide modification is a common feature (e.g., spoxazomicins,^{2,3} oxachelin,^{4,5} amyachelin,⁶ madurastatins,^{7,8} acinetobactin,⁹ asterobactins,¹⁰ carboxymycobactins,^{11–13} exochelins,¹⁴ and nocardimicins^{15,16}). While most commonly reported as metal chelators/siderophores,^{4,6,10} representative members have also been noted to display cancer cell line cytotoxicity and antimicrobial and antitrypanosomal activities.^{2–4,7,17} As part of an effort to explore the microbial diversity and corresponding metabolic potential of actinomycetes associated with thermal vents emanating from underground coal mine fires in Appalachia,^{18–24} herein we report the discovery of three new members of this family [spoxazomicin D (**2**) and oxachelins **4** and **5**] from the Ruth Mullins coal fire-affiliated isolate *Streptomyces* sp. RM-14-6.²¹ Of this set of new microbial products, **2** and **5** notably displayed activity in an EtOH damage neuroprotection assay using rat hippocampal-derived primary cell cultures.^{23,25,26} As neurodegeneration and gliotoxicity are hallmarks of protracted EtOH dependence,^{27,28} this work exposes a new, clinically relevant activity of the phenol-oxazoline/salicylate pharmacophore and establishes a corresponding preliminary structure–activity relationship of spoxazomicins C and D (**1** and **2**) and oxachelins (**3–5**). Furthermore, a comparison of spectral data of the phenoloxazoline moiety of compounds **1–3** with that of several aziridine-containing peptides, including madurastatins A1 (**19**),⁷ B1 (**20**),⁷ C1 (**21**; also known as MBJ-0034),^{8,29} and MBJ-0035 (**22**)²⁹ (Figure 4A), provides the basis for the revision of structures of madurastatins A1, B1, C1, and MBJ-0035 as dihydrooxazole-based metabolites (Figure 4B). In addition, assessment of the cumulative set of metabolites isolated from *Streptomyces* sp. RM-14-6 in an unfolded protein response (UPR) assay³⁰ revealed previously reported polyether lenoremycin **9** and its sodium salt **10** as potent modulators of protein folding capacity.

RESULTS AND DISCUSSION

Metabolic profiling using AntiBase as the primary reference¹ implicated *Streptomyces* sp. RM-14-6 as capable of unique metabolic potential. While *Streptomyces* sp. RM-14-6 was previously noted as a producer of isoptercarpolone (**17**),²¹ further resolution of *Streptomyces* sp. RM-14-6 fermentation organic extracts using progressive chromatography led to the isolation and characterization of six new bacterial metabolites [spoxazomicin D (**2**; 1.2 mg), oxachelin B (**4**; 8.8 mg), oxachelin C (**5**; 6.2 mg), 4-(methylamino) benzamide (**6**; 2.7 mg), α -hydroxybenzamide (**7**; 1.8 mg), and pyrrol-2-carboxamide (**8**; 2.4 mg), the latter three of which have been previously reported as synthetic products^{31–33}] (Figures 1–3 and S4). The known compounds spoxazomicin C (**1**; 41.4 mg), oxachelin (**3**; 1.03 g), lenoremycin (**9**; 8.3 mg), lenoremycin sodium salt (**10**; 6.2 mg), *N*-salicyloyl-2-aminopropane-1,3-diol (**11**; 17.1 mg),³⁴ (*R*)-(-)-*N*-salicyloyl-2-aminopropan-1-ol (**12a**; 1.8 mg; the absolute configuration for which is reported herein for the first time based on comparison to synthetic standards **12a,b**, see Supporting Information for details),³⁴ *N*-acetyltyramine (**14**; 2.3 mg),³⁵ cyclo(*D*-*cis*-Hyp-L-Leu) (**15**; 12.0 mg),³⁶ 2-phenacetamide (**16**; 14.3 mg), isoptercarpolone (**17**; 7.1 mg), and oxachelin-Fe-complex (**18**; 1.21 g) were also isolated and identified (Figures 1 and S4–S6). The full 1D and 2D NMR assignments for **9** and **10** (Supporting Information, Table S2 and Figure S9) are reported herein for the first time.

Structure Elucidation

The physicochemical properties of compounds **1–10** are summarized in the Experimental Section. Compound **1** was obtained as a white powder by sequential chromatographic techniques (Figure S4). HRESIMS and 1D/2D ¹H and ¹³C NMR data (Tables 1 and S3) were consistent with spoxazomicin C,^{2,3} an antitrypanosomal alkaloid from *Streptosporangium oxazolinicum* K07-0460 structurally confirmed by X-ray crystallography (also previously reported with incorrect stereochemistry as nocazoline A).³⁷ Compound **2** was obtained as a white solid and displayed similar UV and physicochemical properties to **1**. The molecular formula of **2** was deduced as C₁₀H₁₁NO₃ from HRESIMS and ¹H and ¹³C NMR data. Comparison of the **1** and **2** spectral data (Tables 1 and S3) revealed the absence of the **1** 12-CH₂ (δ_C 64.8) and the presence of a carboxamide signature carbonyl carbon (δ_C 173.3) and two amide proton signals (δ 6.35 and 5.61), which was further confirmed by 2D NMR (Figures 2 and 3). Structure **2** was further validated via direct comparison to a synthetic standard (see Supporting Information for details). As a new naturally occurring dihydrooxazole-carboxamide closely related to spoxazomicin C (**1**), **2** was subsequently named spoxazomicin D. The related methyl-2-(2'-hydroxyphenyl)-2-oxazoline-4-carboxylate (**13**; previously reported as a metabolite of *Actinomadura* sp. MJ502-77F8)³⁸ also displayed similar spectral characteristics to **1** and **2** (Tables 1 and S2), where the notably distinct optical rotation ($[\alpha]^{25}_D = -50.1$) may implicate distinct **13** 10-CH stereochemistry.

It is important to note that the spectral features of the dihydrooxazole core of **1**, **2**, and **13** share striking similarities with those reported for the putative aziridine moiety within madurastatins A1 (**19**),⁷ B1 (**20**),⁷ C1 (**21**; also known as MBJ-0034),^{8,29} and MBJ-0035 (**22**)²⁹ (Figure 4A). While key 2D NMR experiments including HMBC and COSY cannot

differentiate between **1** and **1a** (Figure 2), the distinct ^{13}C NMR chemical shift of $\text{CH}_2\text{-9}$ (δ_{C} 70.2) implicates the presence of a $\text{CH}_2\text{-O}$ (**1**) rather than a $\text{CH}_2\text{-N}$ (**1a**) connection. Consistent with this, a survey of the spectral features for other reported aziridine-containing natural products¹ reveals the ^{13}C chemical shift of the aziridine ring carbons to be in the range δ_{C} 33–44 ppm across well-established comparators (including azinomycins,³⁹ mitomycins,^{40,41} albomitomycins/isomitomycins,⁴¹ mitiomycins,⁴² ficellomycin,⁴³ FR-66979,⁴⁴ and other closely related structures⁴⁵). In contrast, the ^{13}C NMR chemical shift of the putative aziridine carbons of **19–22** are ~ 70 ppm. Comparison of ^1H and ^{13}C NMR shifts of isolated dihydrooxazoles **1** (a structure previously confirmed by X-ray crystallography)² and **2** (a structure confirmed herein via chemical synthesis) to that corresponding to the NMR simulation⁴⁶ for **1a** and the putative phenol-aziridine moiety in **19–21** (Tables 1 and S2) thereby provides a strong basis for revision of madurastatins A1, B1, C1, and MBJ-0035 as the corresponding dihydrooxazoles **23–26**, respectively (Figure 4A,B).

Compound **4** was obtained as a white solid (8.8 mg, Figure S4) using various chromatographic techniques and displayed UV–vis (Figure S48) and physicochemical properties common to oxachelin (**3**). The molecular formula of **4** was established as $\text{C}_{27}\text{H}_{39}\text{N}_7\text{O}_{12}$ by HRESIMS and ^1H and ^{13}C NMR data. Compared to **3**, the $m/z = 18$ of **4** implicated the addition of H_2O . The ^1H and ^{13}C NMR spectra of **3** and **4** (Table 2) in $\text{DMSO}-d_6$ revealed both to share a common peptide chain and salicylic acid core (Table 2) where the oxazole of **3** is replaced by serine in **4**. Consistent with this, the 19-NH/20-OH proton signals of the serine moiety in **4** were observed at δ 8.90 (d, $J = 7.0$ Hz) and 5.10 (brs), respectively. Cumulative COSY, TOCSY, HMBC, and NOESY spectroscopic data (Table 2, Figures 2, 3, and S8) set the relative stereochemistry of where the proposed absolute stereochemistry is based on the established X-ray structure of the structurally related amyachelin⁶ and the corresponding stereochemical relationship to synthetic **2**. As an unprecedented oxachelin analogue, **4** was subsequently named oxachelin B.

Compound **5** was also obtained as a white solid (6.2 mg, Figure S4) and displayed oxachelin-like UV–vis (Figure S48) and physicochemical properties. While the determined molecular weights of **4** and **5** were identical ($\text{C}_{27}\text{H}_{39}\text{N}_7\text{O}_{12}$), they displayed distinct chromatographic properties (Figure S48). Key ^1H and ^{13}C NMR distinctions of **5** centered around shift changes corresponding to the serine backbone (C-18, CH-19, and $\text{CH}_2\text{-20}$), the lack of a signature corresponding to the **4** 20-OH, and the presence of resonances corresponding to a free NH_2 (δ 8.41), implicating a salicylicoyl ester. This was further confirmed by a full suite of 2D NMR experiments (Table 2, Figures 2, 3, and S8), where the observed $\text{CH}_2\text{-20}$ to C-21 (δ_{C} 165.6) $^3J_{\text{HMB}}$ cross-peak served as key evidence of a C-21 ester reminiscent of a salicylicoyl ester in the closely related madurastatin A3 from *Actinomadura madurae* IFM 0745.⁷ As a new oxachelin analogue, **5** was thereby designated as oxachelin C.

Compounds **6**, **7**, and **8** were obtained as colorless solids using a series of chromatographic techniques (Figure S4), and their molecular formulas were established as $\text{C}_8\text{H}_{10}\text{N}_2\text{O}$, $\text{C}_7\text{H}_8\text{NO}_2$, and $\text{C}_5\text{H}_6\text{N}_2\text{O}$, respectively, by HRESIMS and ^1H and ^{13}C NMR analysis. On

the basis of the full 1D and 2D NMR spectroscopic data (Figures 1, 2, and S8) along with HRMS analysis, the structures of compounds **6–8** were confirmed as 4-(methylamino)benzamide, 2-hydroxybenzamide, and pyrrole-2-carboxamide, respectively. Compounds **6–8** are three carboxamides reported herein for the first time as new bacterial metabolites, where 4-(methylamino)benzamide (**6**) was previously reported as a substructure of the anticoccidial antibiotic cytosaminomycin B from *Streptomyces amakusaensis* KO-8119.^{47,48}

Biological Activity

To interrogate potential variant bioactivity, all isolated metabolites were tested in a broad array of assays including an EtOH damage neuroprotection assay,^{23,25,26} an unfolded protein response assay,³⁰ and standard antibacterial, antifungal, and cancer cell line cytotoxicity assays. For the former, compounds **1–5** were assessed for their ability to protect against EtOH (100 mM)-induced cytotoxicity of rat organotypic hippocampal slice cultures over 48 h. Cytotoxicity was determined by uptake of propidium iodide, a highly polar nucleic acid intercalating agent that labels compromised cells. Mean increases of approximately 170% of ethanol-naïve control levels were observed with each replication. Coexposure of compounds **1, 3, and 4** in this model did not significantly attenuate EtOH-induced cytotoxicity at 48 h. In contrast, compound **2** was found to attenuate EtOH-induced increases in propidium iodide at 48 h. Post hoc analyses revealed that this reversal of propidium iodide uptake by compound **2** was observed at 0.01 μM (Figure 5). Compound **2** did not reveal any significant effects in EtOH-naïve medium. Additionally, compound **5** was also found to attenuate EtOH-induced increases in propidium iodide at 48 h. Post hoc analyses demonstrated this reduction in propidium iodide uptake was observed with the coexposure of 0.01 and 0.10 μM compound **5** (Figure 5). In EtOH-naïve medium, coexposure to compound **5** did reveal significant increases in propidium iodide uptake at 0.01 and 1.0 μM . Cumulatively, this analysis revealed **2** and **5** as potent neuroprotectants against EtOH-induced damage, highlighting a new potential application of the phenol-oxazole/salicylic acid scaffolds.

Unfolded protein response is a stress response specific to the endoplasmic reticulum (ER), the cell-based assay for which employed asialoglycoprotein receptor 1 (ASGR) and *Cypridina* luciferase (Cluc) fusion protein (ASGR-Cluc) to enable the identification of compounds that enhance protein folding capacity as monitored by ASGR-Cluc secretion levels.³⁰ Evaluation of the cumulative set of metabolites isolated from *Streptomyces* sp. RM-14-6 in this assay revealed the polyether antibiotics lenoremycin (**9**; also known as Ro 21-6150⁴⁹) and lenoremycin sodium salt (**10**) as potent modulators of protein folding capacity (EC_{50} = 38 and 109 nM, respectively) (Figures S1 and S2 and Table S1). This study notably highlights a putative new function of polyether antibiotics.

With the exception of **9** and **10**, none of the isolated compounds displayed antimicrobial activity (up to 60 μM) or cancer cell line cytotoxicity (up to 20 μM) (Table S4 and Figure S10). Consistent with prior reports,^{49,50} both **9** and **10** displayed antibacterial and antifungal activity (MICs 0.1–3 μM , Figure S10 and Table S3), where the corresponding cytotoxicity of **9** (A549 IC_{50} = 1.2 μM ; Figure S10) and **10** (A549 IC_{50} = 1.9 μM ; Figure S10) may restrict their utility as potential probes/leads.

Discussion

Including spoxazomicin D (**2**) and oxachelins B and C (**4** and **5**) reported herein for the first time, phenoxazoles/salicylic acid core structures make up 97 of the 16 765 cataloged naturally occurring bacterial metabolites,^{1,51} where they have been reported to contribute to cancer cell line cytotoxicity and antibacterial, antifungal, free radical scavenger, metal chelating, and neuroprotective activities.^{2–4,7,8,10,13–17,29,52–70} Likewise, including the new bacterial carboxamides **6** and **7** reported herein, over 60 benzamide derivatives have been reported from bacteria, many of which also reportedly display interesting biological and pharmaceutical properties.⁷¹ Importantly, the current study is the first to assess spoxazomicins or oxachelins in the context of neuroprotection against EtOH-induced damage, where corresponding bioactivity comparisons among the analogues tested highlight the importance of the spoxazomicin D (**2**) carboxamide and oxachelin C (**5**) salicylate as critical to this newly discovered function. While the molecular mechanism and/or specific target of spoxazomicin D (**2**) and oxachelin C within this context remains to be determined, it is important to note that structurally related thiazoline-based pulicatinins were recently identified as modulators of Ca²⁺ influx in a mouse dorsal root ganglion neuronal assay and as high-affinity ligands for the human serotonin 5-HT_{2B} receptor.⁷² The fermentation-based and synthetic strategies put forth set the stage for future spoxazomicin D (**2**) and oxachelin C target identification and mechanistic studies and potentially probe pulicatin functional relationships. Notably, this work also presents key revisions of the previously reported structures for madurastatins A1, B1, C1, and MBJ-0035 and may serve as a framework to facilitate future structure clarification among oxazolines and aziridines. Finally, the discovery of representative polyether antibiotics as potent inhibitors of UPR presents a new potential pharmacophore/probe of potential relevance to novel strategies to target human pathologies including diabetes, neurodegenerative diseases, and cancer.^{73,74}

EXPERIMENTAL SECTION

General Experimental Procedures

Optical rotation was recorded on a Jasco DIP-370 digital polarimeter. UV spectra were recorded on an Ultrospec 8000 spectrometer (GE, Pittsburgh, PA, USA). NMR spectra were measured using Varian (Palo Alto, CA, USA) Vnmr 500 (¹H, 500 MHz; ¹³C, 125.7 MHz) and Vnmr 400 (¹H, 399.8 MHz; ¹³C, 100.5 MHz) spectrometers where δ -values were referenced to respective solvent signals [CDCl₃, δ_H 7.24 ppm, δ_C 77.23 ppm; CD₃OD, δ_H 3.31 ppm, δ_C 49.15 ppm; DMSO-*d*₆, δ_H 2.50 ppm, δ_C 39.51 ppm]. High-resolution electrospray ionization (HRESI) mass spectra were recorded on an AB SCIEX Triple TOF 5600 system. ESI mass spectra were recorded on a Finnigan LCQ ion trap mass spectrometer. HPLC-MS analyses were accomplished using a Waters (Milford, MA, USA) 2695 LC module (Waters Symmetry Anal. C₁₈, 4.6 × 250 mm, 5 μ m; solvent A: H₂O/0.1% formic acid, solvent B: CH₃CN/0.1% formic acid; flow rate: 0.5 mL min⁻¹; 0–4 min, 10% B; 4–22 min, 10–100% B; 22–27 min, 100% B; 27–29 min, 100–10% B; 29–35 min, 10% B). Semipreparative HPLC was accomplished using a Phenomenex (Torrance, CA, USA) C₁₈ column (10 × 250 mm, 5 μ m) on a Varian ProStar model 210 equipped with a photodiode array detector and a gradient elution profile (solvent A: 0.05% TFA/H₂O, solvent B: CH₃CN; flow rate: 5.0 mL min⁻¹; 0–2 min, 25% B; 2–15 min, 25–100% B; 15–

17 min, 100% B; 17–18 min, 100–25% B; 18–19 min, 25% B). All solvents used were of ACS grade and purchased from Pharmco-AAPER (Brookfield, CT, USA). R_f values were measured on Polygram SIL G/UV254 (Macherey-Nagel & Co., Dueren, Germany). C_{18} -functionalized silica gel (40–63 μ m) was purchased from Material Harvest Ltd. (Cambridge, UK). Amberlite XAD16N resin (20–60 mesh) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Size exclusion chromatography was performed on Sephadex LH-20 (25–100 μ m; GE Healthcare, Piscataway, NJ, USA). *Staphylococcus aureus*, *Salmonella enterica*, and *Saccharomyces cerevisiae* strains and A549 cells were obtained from ATCC (Manassas, VA, USA); *Micrococcus luteus* and *Escherichia coli* were obtained from NRRL (Peoria, IL, USA). All other reagents used were reagent grade and purchased from Sigma-Aldrich.

Isolation of *Streptomyces* sp. RM-14-6 and Its Taxonomy

See ref 21 for details about the isolation and taxonomy of *Streptomyces* sp. RM-14-6.

Culture Media, Fermentation, Extraction, Isolation, and Purification

The terrestrial *Streptomyces* sp. RM-14-6²¹ was cultivated on M₂-agar plates [glucose (4.0 g), malt extract (10.0 g), yeast extract (4.0 g), and agar (15.0 g) dissolved in 1 L of H₂O (pH 7.2) and sterilized by autoclaving for 33 min at 121 °C] at 28 °C for 3 days. To prepare the seed culture, small pieces of agar with the fully grown strain were used to inoculate three 250 mL baffled flasks, each containing 50 mL of A-medium [glucose (10.0 g), yeast extract (5.0 g), soluble starch (20.0 g), peptone (5.0 g), NaCl (4.0 g), K₂HPO₄ (0.5 g), MgSO₄·7H₂O (0.5 g), and calcium carbonate (2 g) dissolved in 1 L of H₂O (pH 7.0) and sterilized by autoclaving for 33 min at 121 °C], and the culture was grown at 28 °C with shaking (210 rpm) for 3 days. An aliquot of seed culture (3 mL) was used to inoculate 80 250 mL baffled flasks each containing 100 mL of A-medium. Fermentation was continued at 28 °C with shaking (210 rpm) for 7 days. The obtained yellowish-green culture broth was centrifuged and filtered over Celite. The supernatant was extracted by mixing with XAD-16 (4%) resin overnight, followed by filtration. The resin was washed with water (3 × 500 mL) followed by extraction with MeOH (3 × 600 mL). The methanolic extract was subsequently evaporated in vacuo to afford 11.8 g of yellowish-green, oily crude extract. The biomass (mycelium) was extracted with MeOH (3 × 700 mL) followed by acetone (1 × 300 mL) and then evaporated in vacuo to yield 16.9 g of a yellowish-green crude extract. Both extracts revealed an identical set of metabolites based on HPLC and TLC analyses and were therefore combined for a cumulative 28.8 g of crude extract.

As highlighted in Figure S4, the obtained crude extract (28.8 g) was subjected to silica gel column chromatography (column 5 × 45 cm, 250 g) and fractionated with a gradient of CH₂Cl₂/0–100% CH₃OH, affording 10 fractions, FI–X [1.0 L 0% MeOH → fraction FI (0.92 g); 0.5 L 5% MeOH → fraction FII; 0.5 L 7% MeOH → fraction FIII; 0.5 L 10% MeOH → fraction FIV; 0.5 L 15% MeOH → fraction FV; 0.5 L 20% MeOH → fraction FVI; 0.5 L 30% MeOH → fraction FVII; 0.5 L 50% MeOH → fraction FVIII; 0.5 L 20% MeOH → fraction FIX (1.17 g); and 0.5 L 100% MeOH → fraction FX (6.01 g)]. Fractions FII–FIV were combined based on the HPLC and TLC similarity, affording 0.65 g. Similarly, fractions FV–FVIII were combined to afford 6.2 g. Fraction FI (0.92) was identified as fats based on TLC and corresponding indicators. Further fractionation of the

combined fractions FII–FIV using preparative TLC (CH₂Cl₂–CH₃OH, 95:5) gave six subfractions, FIIA–F. Purification of subfraction FIIB using Sephadex LH-20 (CH₂Cl₂–CH₃OH, 6:4; column 2.5 × 50 cm) and semipreparative HPLC afforded spoxazomicin C (**1**; 35.8 mg) as white powder. Semipreparative HPLC purification of subfraction FIIC afforded spoxazomicin C (**1**; white powder, 5.6 mg), spoxazomicin D (**2**; white solid, 1.2 mg), *o*-hydroxybenzamide (**7**; colorless, amorphous solid, 1.8 mg), (*R*)-(–)-*N*-salicyloyl-2-aminopropan-1-ol (**12a**; colorless, amorphous solid, 1.8 mg), 2-phenacetamide (**16**; colorless, amorphous solid, 14.3 mg), and isopterocarpolone (**17**; colorless oil, 7.1 mg) in pure form. Similarly, semipreparative HPLC purification of subfraction FIID gave 4-(methylamino)benzamide (**6**; colorless solid, 2.7 mg), pyrrol-2-carboxamide (**8**; colorless solid, 2.4 mg), and *N*-acetyltyramine (**14**; white solid, 2.3 mg). In the same manner, further purification of subfraction FIIE using semipreparative HPLC and Sephadex LH-20 (CH₂Cl₂–CH₃OH, 6:4; column 1 × 40 cm) afforded *N*-salicyloyl-2-aminopropane-1,3-diol (**11**; colorless, amorphous solid, 17.1 mg) and cyclo(D-*cis*-Hyp-L-Leu) (**15**; colorless, amorphous solid, 12.0 mg).

The major fractions FV–FVIII (6.2 g) were combined based on their similarity in TLC and HPLC analyses, dissolved in 50% MeOH–H₂O (100 mL), and fractionated with a gradient of H₂O/0–100% CH₃CN, using RP-18 column chromatography (column 3 × 40 cm, 80 g) to provide seven subfractions [0.5 L 0% CH₃CN and 0.5 L 10% CH₃CN → subfraction FV-A; 0.5 L 20% CH₃CN → subfraction FV-B; 0.5 L 30% CH₃CN → subfraction FV-C; 0.5 L 40% CH₃CN → subfraction FV-D; 0.5 L 50% CH₃CN → subfraction FV-E; 0.5 L 60% CH₃CN, 0.5 L 80% CH₃CN → subfraction FV-F; 0.8 L 100% CH₃CN → subfraction FV-G]. Subfraction FV-C was identified as oxachelin-Fe-complex (**18**; orange powder, 1.21 g, Supporting Information, Figures S6 and S38) on the basis of HPLC/UV/MS analysis. Semipreparative HPLC of subfraction FV-D afforded oxachelin (**3**; white powder, 1.03 g) as the major metabolite from the strain crude extract. Further purification of subfraction FV-E using semipreparative HPLC gave oxachelins B (**4**; 8.8 mg) and C (**5**; 6.2 mg) as white solids. Finally, subfraction FV-G was subsequently purified by preparative TLC (CH₂Cl₂–MeOH, 9:1) and Sephadex LH-20 (CH₂Cl₂–MeOH, 6:4; column 1 × 40 cm), to afford the polyether antitumor antibiotics lenoremycin (**9**; 8.3 mg) along with its sodium salt (**10**; 6.2 mg) as white, amorphous solids. Remaining fractions and subfractions of this strain have been excluded, as they mainly identified as fats, sugars, and/or media components based on HPLC analysis, TLC, and corresponding indicators (Supporting Information, Figure S4).

Spoxazomicin C (1): white powder; *R*_f 0.44 (CH₂Cl₂–MeOH, 95:5); UV/vis (MeOH) $\lambda_{\max}$ (log ϵ) 243 (4.00), 250 (4.02), 259 (3.70) sh, 306 (3.73) nm; ¹H NMR (CD₃OD, 400 MHz; CDCl₃, 500 MHz; and DMSO-*d*₆, 400 MHz), see Supporting Information, Table S3; ¹³C NMR (CD₃OD, CDCl₃, DMSO-*d*₆, 100 MHz), see Table 1; (+)-APCI-MS *m/z* 194 [M + H]⁺; (+)-HRESIMS *m/z* 194.0809 [M + H]⁺ (calcd for C₁₀H₁₂NO₃, 194.0812).

Spoxazomicin D (2): white solid; *R*_f 0.33 (CH₂Cl₂–MeOH, 95:5); pale yellow with anisaldehyde/H₂SO₄ spraying reagent; [α]_D²⁵ +62 (*c* 1.0, MeOH–DMSO, 85:15); UV/vis (MeOH) $\lambda_{\max}$ (log ϵ) 244 (4.02), 251 (4.03), 261 (3.74) sh, 307 (3.71) nm; ¹H NMR (CDCl₃, 400 MHz), see Supporting Information, Table S3; ¹³C NMR (CDCl₃, 100 MHz), see Table 1; (+)-APCI-MS *m/z* 207 [M + H]⁺; (–)-APCI-MS *m/z* 205 [M – H][–]; (+)-ESIMS

m/z 207 $[M + H]^+$, 162 $[(M - CONH_2) + H]^+$; (+)-HRESIMS m/z 207.0744 $[M + H]^+$ (calcd for $C_{10}H_{11}N_2O_3$, 207.0764), 229.0567 $[M + Na]^+$ (calcd for $C_{10}H_{10}N_2O_3Na$, 229.0583); (–)-HRESIMS m/z 205.0620 $[M - H]^-$ (calcd for $C_{10}H_9N_2O_3$, 205.0619).

Oxachelin (3): white powder; R_f 0.22 (CH_2Cl_2 –MeOH, 8:2); pale yellow with anisaldehyde/ H_2SO_4 spraying reagent; UV/vis (MeOH) λ_{max} (log ϵ) 245 (3.65) sh, 253 (3.75), 262 (3.44) sh, 308 (3.37) nm; 1H NMR (DMSO- d_6 , 500 MHz) and ^{13}C NMR (DMSO- d_6 , 100 MHz), see Table 2; (+)-APCI-MS m/z 636 $[M + H]^+$; (–)-APCI-MS m/z 634 $[M - H]^-$; (+)-HRESIMS m/z 636.2569 $[M + H]^+$ (calcd for $C_{27}H_{38}N_7O_{11}$, 636.2623). For the 1H , 1H -COSY, HMBC, TOCSY, and NOESY correlations, see Supporting Information, Figure S7.

Oxachelin B (4): white solid; R_f 0.30 (CH_2Cl_2 –MeOH, 8:2); pale yellow with anisaldehyde/ H_2SO_4 spraying reagent; UV/vis (MeOH) λ_{max} (log ϵ) 243 (4.16), 261 (3.40) sh, 303 (3.77) nm; 1H NMR (DMSO- d_6 , 500 MHz) and ^{13}C NMR (DMSO- d_6 , 100 MHz), see Table 2; (+)-APCI-MS m/z 654 $[M + H]^+$; (–)-APCI-MS m/z 652 $[M - H]^-$; (+)-HRESIMS m/z 676.2590 $[M + Na]^+$ (calcd for $C_{27}H_{39}N_7O_{12}Na$, 676.2549), 654.2770 $[M + H]^+$ (calcd for $C_{27}H_{40}N_7O_{12}$, 654.2729).

Oxachelin C (5): white solid; R_f 0.27 (CH_2Cl_2 –MeOH, 8:2); pale yellow with anisaldehyde/ H_2SO_4 spraying reagent; UV/vis (MeOH) λ_{max} (log ϵ) 242 (3.92), 310 (3.48) nm; 1H NMR (DMSO- d_6 , 500 MHz) and ^{13}C NMR (DMSO- d_6 , 100 MHz), see Table 2; (+)-APCI-MS m/z 654 $[M + H]^+$; (–)-APCI-MS m/z 652 $[M - H]^-$; (+)-HRESIMS m/z 676.2596 $[M + Na]^+$ (calcd for $C_{27}H_{39}N_7O_{12}Na$, 676.2549), 654.2768 $[M + H]^+$ (calcd for $C_{27}H_{40}N_7O_{12}$, 654.2729).

4-(Methylamino)benzamide (6): colorless solid; R_f 0.41 (CH_2Cl_2 –MeOH, 95:5); pale yellow with anisaldehyde/ H_2SO_4 spraying reagent; UV/vis (MeOH) λ_{max} (log ϵ) 211 (3.21), 310 (3.56) nm; 1H NMR (DMSO- d_6 , 500 MHz) δ 7.65 (d, J = 8.5 Hz, 2H, 2-H/6-H), 7.53 (brs, 1H, 7-NH_a), 6.83 (brs, 1H, 7-NH_b), 6.50 (d, J = 8.5 Hz, 2H, 3-H/5-H), 6.16 (brs, 1H, 4-NH), 2.70 (brs, 3H, CH₃-8); 1H NMR ($CDCl_3$, 400 MHz) δ 7.64 (d, J = 8.4 Hz, 2H, 2-H/6-H), 6.55 (d, J = 8.8 Hz, 2H, 3-H/5-H), 5.70 (brs, 2H, 7-NH₂), 2.86 (s, 3H, CH₃-8); 1H NMR (CD_3OD , 500 MHz) δ 7.68 (dd, J = 7.2, 1.6 Hz, 2H, 2-H/6-H), 2.81 (s, 3H, CH₃-8); ^{13}C NMR (DMSO- d_6 , 100 MHz), δ 168.0 (Cq-7), 152.2 (Cq-4), 129.1 (CH-2/6), 110.3 (CH-3/5), 29.3 (CH₃-8); (+)-HRESIMS m/z 173.0672 $[M + Na]^+$ (calcd for $C_8H_{10}N_2ONa$, 173.0684), 151.0851 $[M + H]^+$ (calcd for $C_8H_{11}N_2O$, 151.0865).

2-Hydroxybenzamide (7): colorless solid; R_f 0.26 (CH_2Cl_2 –MeOH, 95:5); pale yellow with anisaldehyde/ H_2SO_4 spraying reagent; UV/vis (MeOH) λ_{max} (log ϵ) 211 (3.09), 241 (3.41), 305 (3.12) nm; 1H NMR (DMSO- d_6 , 400 MHz) δ 12.99 (brs, 1H, 2-OH), 8.42 (brs, 1H, 7-NH_a), 7.86 (brs, 1H, 7-NH_b), 7.83 (d, J = 7.7 Hz, 1H, 6-H), 7.37 (t, J = 8.4 Hz, 1H, 4-H), 6.92 (d, J = 8.0 Hz, 1H, 3-H), 6.84 (t, J = 7.6 Hz, 1H, 5-H); 1H NMR ($CDCl_3$, 400 MHz) δ 12.13 (brs, 1H, 2-OH), 7.41 (t, J = 7.6 Hz, 1H, 4-H), 7.36 (dd, J = 8.0, 1.6 Hz, 1H, 6-H), 6.98 (d, J = 8.0 Hz, 1H, 3-H), 6.85 (t, J = 6.8 Hz, 1H, 5-H), 5.90 (brs, 2H, NH₂); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.7 (Cq-7), 162.3 (Cq-2), 135.2 (CH-4), 126.6 (CH-6), 119.0 (CH-3), 119.0 (CH-5), 113.2 (Cq-1); (+)-ESIMS m/z 138 $[M + H]^+$; (+)-HRESIMS m/z

138.00552 $[M + H]^+$ (calcd for $C_7H_8NO_2$, 138.0555); (–)-HRESIMS m/z 136.0399 $[M - H]^-$ (calcd for $C_7H_6NO_2$, 136.0404).

Pyrrole-2-carboxamide (8): colorless, amorphous solid; R_f 0.29 (CH_2Cl_2 –MeOH, 95:5); yellow and changed later to orange with anisaldehyde/ H_2SO_4 spraying reagent; UV/vis (MeOH) λ_{max} (log ϵ) 203 (3.28), 251 (3.74), 306 (3.31) nm; 1H NMR (DMSO- d_6 , 400 MHz) δ 11.37 (brs, 1H, NH-1), 7.44 (brs, 1H, NH), 6.88 (brs, 1H, NH), 6.83 (m, 1H, 3-H), 6.75 (m, 1H, 5-H), 6.05 (m, 1H, 4-H); ^{13}C NMR (DMSO- d_6 , 100 MHz) δ 162.3 (2-CO), 126.3 (Cq-2), 121.3 (CH-5), 110.6 (CH-3), 108.5 (CH-4); (+)-ESIMS m/z 111 $[M + H]^+$; (+)-HRESIMS m/z 111.0553 $[M + H]^+$ (calcd for $C_5H_7N_2O$, 111.0553).

Lenoremycin (9): colorless, amorphous solid; R_f 0.42 (CH_2Cl_2 –MeOH, 95:5); yellowish-green then turned orange and finally to reddish-brown with anisaldehyde/ H_2SO_4 spraying reagent; 1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Supporting Information, Table S2; (+)-ESIMS m/z 873 $[M + Na]^+$; (–)-ESIMS m/z 849 $[M - H]^-$; (+)-HRESIMS m/z 873.5335 $[M + H]^+$ (calcd for $C_{47}H_{78}O_{13}Na$, 873.5335); (–)-HRESIMS m/z 849.5370 $[M - H]^-$ (calcd for $C_{47}H_{77}O_{13}$, 849.5370). For the 1H , 1H -COSY, HMBC, TOCSY, and NOESY correlations of compound **9**, see Supporting Information, Figure S9.

Lenoremycin sodium salt (10): colorless, amorphous solid; R_f 0.33 (CH_2Cl_2 –MeOH, 95:5); yellowish-green then turned orange and finally to reddish-brown with anisaldehyde/ H_2SO_4 spraying reagent; 1H NMR (CD_3OD , 500 MHz), ^{13}C NMR ($CDCl_3$, 100 MHz), and ^{13}C NMR (CD_3OD , 100 MHz), see Supporting Information, Table S2; (+)-ESIMS m/z 868 $[M + NH_4]^+$; 873 $[M + Na]^+$; 889 $[M + K]^+$; (–)-ESIMS m/z 849 $[M - H]^-$; (+)-HRESIMS m/z 873.5330 $[M + Na]^+$ (calcd for $C_{47}H_{78}O_{13}Na$, 873.5335); (–)-HRESIMS m/z 849.5370 $[M - H]^-$ (calcd for $C_{47}H_{77}O_{13}$, 849.5370). Note: this compound in solution turned to its free acid form, and it shows the same mass and molecular formula as lenoremycin (**9**). For the 1H , 1H -COSY, HMBC, TOCSY, and NOESY correlations of compound **10**, see Supporting Information, Figure S9.

Synthesis of Spoxazomicin D (**2**), *N*-Salicyloyl-2-aminopropane-1,3-diol (**11**), (*R*)-(–)-*N*-Salicyloyl-2-aminopropan-1-ol (**12a**), and (*S*)-(+)-*N*-Salicyloyl-2-aminopropan-1-ol (**12b**)

See Supporting Information.

Antibacterial, Antifungal, and Cancer Cell Line Viability Assays

Antibacterial (*Staphylococcus aureus* ATCC 6538, *Micrococcus luteus* NRRL B-287, *Escherichia coli* NRRL B-3708, and *Salmonella enterica* ATCC 10708), antifungal (*Saccharomyces cerevisiae* ATCC 204508), and cell line cytotoxicity (non-small-cell lung A549, Figure S10; INS-1E cell line, Figure S2) assays were accomplished in triplicate following our previously reported protocols.^{24,30,75} Antibacterial/antifungal MIC values were obtained after 16–48 h of incubation (Table S4). Kanamycin and ampicillin (*S. aureus*, *M. luteus*, *S. enterica*, and *E. coli*), amphotericin B (*S. cerevisiae*), and actinomycin D (non-small-cell lung A549) were used as positive controls (Table S4 and Figure S10).

EtOH Damage Neuroprotection Assay

Assays to assess modulation of EtOH damage were accomplished following previously reported protocols (see Supporting Information).^{23,25,26} Briefly, hippocampal slices from 8-day-old Sprague–Dawley rats were transferred to plates containing 1 mL of culture medium containing 3.74 μ M propidium iodide (control) in the absence or presence of 100 mM EtOH and varied test agent concentrations. After 48 h of incubation, all cultures were removed from incubators and imaged to assess the intensity of propidium iodide uptake as a measure of cellular integrity.

Unfolded Protein Response Assay

Assays to assess modulation of UPR were accomplished following a previously reported protocol (see Supporting Information).³⁰ Briefly, this assay employs ASGR-Cluc cells (HEK293 cell line) that produce an asialoglycoprotein receptor 1 (ASGR1)–*Cypridina noctiluca* luciferase (Cluc) fusion protein as a specific and quantitative reporter of ER folding capacity. Cells were incubated in the presence of vehicle (DMSO, negative control), forskolin or tunicamycin (positive controls), or test agents for 48 h. After the incubation period, 2 μ L of Cluc reagent (Cypridina Luciferase assay kit; BioLux, Vancouver, BC, Canada) was added, and the amount of secreted ASGR-Cluc determined by luminescence. In a similar manner, the impact of test agents on insulin secretion was determined employing INS-1E β cells and the HTRF insulin assay kit (Cisbio Assay, Bedford, MA, USA) followed by FRET imaging.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Laatsch, H. AntiBase 2014, The Natural Compound Identifier. Wiley-VCH; Weinheim: 2014.
2. Inahashi Y, Iwatsuki M, Ishiyama A, Namatame M, Nishihara-Tsukashima A, Matsumoto A, Hirose T, Sunazuka T, Yamada H, Otoguro K, Takahashi Y, Omura S, Shiomi KJ. Antibiot. 2011; 64:303–307.
3. Inahashi Y, Matsumoto A, Omura S, Takahashi Y. J Antibiot. 2011; 64:297–302. [PubMed: 21386852]
4. Sontag B, Gerlitz M, Paululat T, Rasser HF, Grun-Wollny I, Hansske FG. J Antibiot. 2006; 59:659–663. [PubMed: 17191682]
5. Grün-wollny, I., Paululat, T., Hansske, FG., Sontag, B. Novel Drug: Oxachelin and its Derivatives. WO. 2005/051411. 2005.
6. Seyedsayamdost MR, Traxler MF, Zheng SL, Kolter R, Clardy J. J Am Chem Soc. 2011; 133:11434–11437. [PubMed: 21699219]

7. Harada K, Tomita K, Fujii K, Masuda K, Mikami Y, Yazawa K, Komaki H. *J Antibiot.* 2004; 57:125–135. [PubMed: 15112961]
8. Mazzei E, Iorio M, Maffioli SI, Sosio M, Donadio SJ. *Antibiot.* 2012; 65:267–269.
9. Yamamoto S, Okujo N, Sakakibara Y. *Arch Microbiol.* 1994; 162:249–254. [PubMed: 7802543]
10. Nemoto A, Hoshino Y, Yazawa K, Ando A, Mikami Y, Komaki H, Tanaka Y, Grafe U. *J Antibiot.* 2002; 55:593–597. [PubMed: 12195966]
11. Ratledge C, Ewing M. *Microbiology.* 1996; 142:2207–2212. [PubMed: 8800816]
12. Lane SJ, Marshall PS, Upton RJ, Ratledge C, Ewing M. *Tetrahedron Lett.* 1996; 37:1–1.
13. Lane SJ, Marshall PS, Upton RJ, Ratledge C, Ewing M. *Tetrahedron Lett.* 1995; 36:4129–4132.
14. Gobin J, Moore CH, Reeve JR Jr, Wong DK, Gibson BW, Horwitz MA. *Proc Natl Acad Sci U S A.* 1995; 92:5189–5193. [PubMed: 7761471]
15. Ikeda Y, Nonaka H, Furumai T, Onaka H, Igarashi YJ. *Nat Prod.* 2005; 68:1061–1065.
16. Ikeda Y, Furumai T, Igarashi Y. *J Antibiot.* 2005; 58:566–572. [PubMed: 16320761]
17. Tsukamoto M, Murooka K, Nakajima S, Abe S, Suzuki H, Hirano K, Kondo H, Kojiri K, Suda H. *J Antibiot.* 1997; 50:815–821. [PubMed: 9402985]
18. Wang X, Shaaban KA, Elshahawi SI, Ponomareva LV, Sunkara M, Zhang Y, Copley GC, Hower JC, Morris AJ, Kharel MK, Thorson JS. *J Nat Prod.* 2013; 76:1441–1447. [PubMed: 23944931]
19. Shaaban KA, Wang X, Elshahawi SI, Ponomareva LV, Sunkara M, Copley GC, Hower JC, Morris AJ, Kharel MK, Thorson JS. *J Nat Prod.* 2013; 76:1619–1626. [PubMed: 23947794]
20. Wang X, Elshahawi SI, Shaaban KA, Fang L, Ponomareva LV, Zhang Y, Copley GC, Hower JC, Zhan CG, Kharel MK, Thorson JS. *Org Lett.* 2014; 16:456–459. [PubMed: 24341358]
21. Shaaban KA, Singh S, Elshahawi SI, Wang X, Ponomareva LV, Sunkara M, Copley GC, Hower JC, Morris AJ, Kharel MK, Thorson JS. *Nat Prod Res.* 2014; 28:337–339. [PubMed: 24237421]
22. Wang X, Shaaban KA, Elshahawi SI, Ponomareva LV, Sunkara M, Copley GC, Hower JC, Morris AJ, Kharel MK, Thorson JS. *J Antibiot.* 2014; 67:571–575. [PubMed: 24713874]
23. Wang X, Reynolds AR, Elshahawi SI, Shaaban KA, Ponomareva LV, Saunders MA, Elgumati IS, Zhang Y, Copley GC, Hower JC, Sunkara M, Morris AJ, Kharel MK, Van Lanen SG, Prendergast MA, Thorson JS. *Org Lett.* 2015; 17:2796–2799. [PubMed: 25961722]
24. Shaaban KA, Singh S, Elshahawi SI, Wang X, Ponomareva LV, Sunkara M, Copley GC, Hower JC, Morris AJ, Kharel MK, Thorson JS. *J Antibiot.* 2014; 67:223–230. [PubMed: 24252813]
25. Prendergast MA, Harris BR, Mullholland PJ, Blanchard JA 2nd, Gibson DA, Holley RC, Littleton JM. *Neuroscience.* 2004; 124:869–877. [PubMed: 15026127]
26. Zimmer J, Kristensen BW, Jakobsen B, Noraberg J. *Amino Acids.* 2000; 19:7–21. [PubMed: 11026469]
27. Crews FT, Nixon K. *Alcohol Alcohol.* 2009; 44:115–127. [PubMed: 18940959]
28. Collins MA, Neafsey EJ. *Neurotoxic Res.* 2012; 21:70–78.
29. Kawahara T, Itoh M, Izumikawa M, Sakata N, Tsuchida T, Shin-Ya K. *J Antibiot.* 2014; 67:577–580. [PubMed: 24619229]
30. Fu S, Yalcin A, Lee GY, Li P, Fan J, Arruda AP, Pers BM, Yilmaz M, Eguchi K, Hotamisligil GS. *Sci Transl Med.* 2015; 7:292ra98.
31. Selva M, Tundo P, Foccardi T. *J Org Chem.* 2005; 70:2476–2485. [PubMed: 15787533]
32. Dündar Y, Özatik Y, Özatik O, Ergin V, Onkol T, Menevse A, Erol K, Sahin MF. *Med Chem.* 2012; 8:481–90. [PubMed: 22530891]
33. Kanchanadevi A, Ramesh R, Semeril D. *Inorg Chem Commun.* 2015; 56:116–119.
34. Shamim Hossain M, Aslam Hossain M, Mukhlesur Rahman M, Mojib Mondol MA, Bhuiyan MSA, Gray AI, Flores ME, Rashid MA. *Phytochemistry.* 2004; 65:2147–2151. [PubMed: 15279987]
35. Ding L, Qin S, Li F, Chi X, Laatsch H. *Curr Microbiol.* 2008; 56:229–235. [PubMed: 18214603]
36. Shigemori H, Tenma M, Shimazaki K, Kobayashi J. *J Nat Prod.* 1998; 61:696–698. [PubMed: 9599283]
37. Fu P, Liu P, Qu H, Wang Y, Chen D, Wang H, Li J, Zhu W. *J Nat Prod.* 2011; 74:2219–2223. [PubMed: 21958359]

38. Sasaki T, Otani T, Yoshida K, Unemi N, Hamada M, Takeuchi T. *J Antibiot.* 1997; 50:881–883. [PubMed: 9402996]
39. Yokoi K, Nagaoka K, Nakashima T. *Chem Pharm Bull.* 1986; 34:4554–4561. [PubMed: 3829181]
40. Urakawa C, Tsuchiya H, Nakano K, Nakamura NJ. *Antibiot.* 1981; 34:1152–1156.
41. Kono M, Saitoh Y, Kasai M, Shirahata K. *J Antibiot.* 1995; 48:179–181. [PubMed: 7706131]
42. Kono M, Kasai M, Shirahata K, Hirayama N. *J Antibiot.* 1991; 44:309–312. [PubMed: 2026557]
43. Kuo MS, Yurek DA, Mizesak SA. *J Antibiot.* 1989; 42:357–360. [PubMed: 2708128]
44. Terano H, Takase S, Hosoda J, Kohsaka M. *J Antibiot.* 1989; 42:145–148. [PubMed: 2921221]
45. Moran-Ramallal R, Liz R, Gotor V. *Org Lett.* 2007; 9:521–524. [PubMed: 17249802]
46. ACD/NMR Predictor. Advanced Chemistry Development, Inc; Toronto, Ontario (Canada): 2002.
47. Shiomi K, Haneda K, Tomoda H, Iwai Y, Omura SJ. *Antibiot.* 1994; 47:782–786.
48. Haneda K, Shinose M, Seino A, Tabata N, Tomoda H, Iwai Y, Omura S. *J Antibiot.* 1994; 47:774–781. [PubMed: 8071122]
49. Liu CM, Evans R Jr, Fern L, Hermann T, Jenkins EJ. *Antibiot.* 1976; 29:21–28.
50. Kevin DA, Meujo DAF, Hamann MT. *Expert Opin Drug Discovery.* 2009; 4:109–146.
51. Elshahawi SI, Shaaban KA, Kharel MK, Thorson JS. *Chem Soc Rev.* 2015; 44:7591–7697. [PubMed: 25735878]
52. Merkal RS, Mccullough WG. *Curr Microbiol.* 1982; 7:333–335.
53. Mccullough WG, Merkal RS. *Curr Microbiol.* 1982; 7:337–341.
54. Cavanaugh PF Jr, Porter CW, Tukalo D, Frankfurt OS, Pavelic ZP, Bergeron RJ. *Cancer Res.* 1985; 45:4754–4759. [PubMed: 4027962]
55. Bergeron RJ, Dionis JB, Elliott GT, Kline SJ. *J Biol Chem.* 1985; 260:7936–7944. [PubMed: 4008483]
56. Okujo N, Saito M, Yamamoto S, Yoshida T, Miyoshi S, Shinoda S. *BioMetals.* 1994; 7:109–116. [PubMed: 8148612]
57. Okujo N, Akiyama T, Miyoshi S, Shinoda S, Yamamoto S. *Microbiol Immunol.* 1996; 40(8):595–598. [PubMed: 8887355]
58. Murakami Y, Kato S, Nakajima M, Matsuoka M, Kawai H, Shin-Ya K, Seto H. *J Antibiot.* 1996; 49:839–845. [PubMed: 8931715]
59. Ratledge C, Snow GA. *Biochem J.* 1974; 139:407–413. [PubMed: 4614794]
60. Suenaga K, Kokubo S, Shinohara C, Tsuji T, Uemura D. *Tetrahedron Lett.* 1999; 40:1945–1948.
61. Kokubo S, Suenaga K, Shinohara C, Tsuji T, Uemura D. *Tetrahedron.* 2000; 56:6435–6440.
62. Beiderbeck H, Taraz K, Budzikiewicz H, Walsby AEZ. *Naturforsch, C: J Biosci.* 2000; 55:681–687.
63. Ito Y, Ishida K, Okada S, Murakami M. *Tetrahedron.* 2004; 60:9075–9080.
64. Itou Y, Okada S, Murakami M. *Tetrahedron.* 2001; 57:9093–9099.
65. Tsuda M, Yamakawa M, Oka S, Tanaka Y, Hoshino Y, Mikami Y, Sato A, Fujiwara H, Ohizumi Y, Kobayashi J. *J Nat Prod.* 2005; 68:462–464. [PubMed: 15787462]
66. Hoshino Y, Mukai A, Yazawa K, Uno J, Ando A, Mikami Y, Fukai T, Ishikawa J, Yamaguchi K. *J Antibiot.* 2004; 57:803–807. [PubMed: 15745115]
67. Hoshino Y, Mukai A, Yazawa K, Uno J, Ishikawa J, Ando A, Fukai T, Mikami Y. *J Antibiot.* 2004; 57:797–802. [PubMed: 15745114]
68. Mukai A, Fukai T, Matsumoto Y, Ishikawa J, Hoshino Y, Yazawa K, Harada K, Mikami Y. *J Antibiot.* 2006; 59:366–369. [PubMed: 16915823]
69. Schneider K, Rose I, Vikineswary S, Jones AL, Goodfellow M, Nicholson G, Beil W, Sussmuth RD, Fiedler HP. *J Nat Prod.* 2007; 70:932–935. [PubMed: 17536856]
70. Tsunakawa, M., Chang, L., Mamber, SW., Bursuker, I., Huggill, R. *Antitumor Antibiotic BMS-199687.* US. 5,811,440. 1998.
71. Shaaban KA, Shepherd MD, Ahmed TA, Nybo SE, Leggas M, Rohr J. *J Antibiot.* 2012; 65:615–622. [PubMed: 23047245]

72. Lin Z, Antemano RR, Hughen RW, Tianero MD, Peraud O, Haygood MG, Concepcion GP, Olivera BM, Light A, Schmidt EW. *J Nat Prod.* 2010; 73:1922–1926. [PubMed: 21028889]
73. Rivas A, Vidal RL, Hetz C. *Expert Opin Ther Targets.* 2015; 19:1203–1218. [PubMed: 26166159]
74. Wang M, Kaufman RJ. *Nature.* 2016; 529:326–335. [PubMed: 26791723]
75. Shaaban KA, Elshahawi SI, Wang X, Horn J, Kharel MK, Leggas M, Thorson JS. *J Nat Prod.* 2015; 78:1723–1729. [PubMed: 26091285]

Figure 1.
Chemical structures of compounds **1–13**.

Figure 2.
 ^1H , ^1H -COSY and selected HMBC correlations in compounds **1** (putative structure **1a**), **2**, and **4-6**.

Figure 3.
Key NOESY correlations in compounds **1**, **2**, **4**, and **5**.

Figure 4.
(A) Previously reported chemical structures of madurastatins A1, B1, C1, and MBJ-0035 (19–22). (B) Revised chemical structures of madurastatins A1, B1, C1, and MBJ-0035 (23–26).

Figure 5.

EtOH damage neuroprotection assay (propidium iodide uptake in rat-derived organotypic hippocampal slice primary cell cultures). (A) DMSO control; (B) 48 h exposure to 100 mM EtOH; (C) 48 h exposure to 100 mM EtOH and 10 nM **2**; (D) 48 h exposure to 100 mM EtOH and 10 nM **5**. (E) Dose–response with 48 h exposure to EtOH (100 mM) in the absence or presence of **2** on propidium iodide uptake. (F) Dose–response with 48 h exposure to EtOH (100 mM) in the absence or presence of **5** on propidium iodide uptake. * $p < 0.001$ vs control; ** $p < 0.001$ vs EtOH.

¹³C NMR Spectroscopic Data of Compounds 1 and 2 in Comparison with the Reported Data of Spoxazomicin C (1), Methyl-2-(2-hydroxyphenyl)-2-oxazoline-4-carboxylate (13), and the Aziridine Moiety of Madurastatin C1 (21)

position	1 (literature) ²		21 (aziridine moiety) ⁸		1		1a ⁴⁶		2		13	
	δ_C , ^{a,b} type	δ_C , ^{a,c} type	δ_C , ^{a,c} type	δ_C , ^{a,c} type	δ_C , ^{c,d} type	δ_C , ^{c,e} type	δ_C , type	δ_C , ^{c,d} type	δ_C , ^{c,d} type	δ_C , ^{c,d} type	δ_C , ^{c,d} type	δ_C , ^{c,d} type
1	111.9, C	109.7, C	112.0, C	110.6, C	110.2, C	110.2, C	120.2, C	110.1, C	110.0, C	110.0, C	110.0, C	110.0, C
2	161.0, C	159.5, C	161.2, C	160.0, C	159.2, C	159.2, C	159.1, C	159.9, C	159.9, C	159.9, C	159.9, C	159.9, C
3	117.5, CH	116.2, CH	117.6, CH	117.0, CH	116.4, CH	116.4, CH	117.5, CH	117.2, CH	116.9, CH	116.9, CH	116.9, CH	116.9, CH
4	134.4, CH	133.7, CH	134.6, CH	133.8, CH	133.6, CH	133.6, CH	131.1, CH	134.6, CH	133.9, CH	133.9, CH	133.9, CH	133.9, CH
5	119.7, CH	118.7, CH	119.9, CH	119.0, CH	118.8, CH	118.8, CH	121.1, CH	119.5, CH	118.7, CH	118.7, CH	118.7, CH	118.7, CH
6	129.2, CH	128.0, CH	129.3, CH	128.4, CH	127.7, CH	127.7, CH	128.6, CH	128.8, CH	128.3, CH	128.3, CH	128.3, CH	128.3, CH
7	167.4, C	167.0, C	167.6, C	167.1, C	165.1, C	165.1, C	172.5, C	168.3, C	167.5, C	167.5, C	167.5, C	167.5, C
9	70.1, CH ₂	69.3, CH ₂	70.2, CH ₂	68.7, CH ₂	68.9, CH ₂	68.9, CH ₂	29.9, CH ₂	69.8, CH ₂	68.8, CH ₂	68.8, CH ₂	68.8, CH ₂	68.8, CH ₂
10	68.5, CH	67.9, CH	68.7, CH	67.1, CH	66.7, CH	66.7, CH	34.7, CH	68.0, CH	67.1, CH	67.1, CH	67.1, CH	67.1, CH
12	64.6, CH ₂	169.2, C	64.8, CH ₂	64.1, CH ₂	62.6, CH ₂	62.6, CH ₂	68.3, CH ₂	173.3, C	170.9, C	170.9, C	170.9, C	170.9, C
12-OCH ₃									52.7, CH ₃	52.7, CH ₃	52.7, CH ₃	52.7, CH ₃

^aCD₃OD.

^b₇₅ MHz.

^c₁₀₀ MHz.

^dCDCl₃.

^eDMSO-*d*₆: NMR simulation for **1a**. See Supporting Information for the NMR spectra. Assignments supported by HSQC and HMBC experiments.

Table 2
 ^{13}C (100 MHz) and ^1H (500 MHz) NMR Spectroscopic Data of Compounds 3–5 in DMSO- d_6

position	oxachelin (3) ^d			oxachelin B (4) ^d			oxachelin C (5) ^d		
	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)	
<i>N</i> -Hydroxy-D-ornithine									
1	164.9, C		164.9, C		164.9, C		164.9, C		
2	49.5, CH	4.30, m	49.4, CH	4.32, m	49.4, CH	4.31, m	49.4, CH	4.31, m	
2-NH		8.12, d (8.0)				8.17, d (8.5)		8.17, d (8.5)	
3	27.7, CH ₂	1.88, m	27.6, CH ₂	1.87, m	27.6, CH ₂	1.89, m	27.7, CH ₂	1.89, m	
		1.63, m		1.65, m		1.65, m		1.65, m	
4	20.3, CH ₂	1.86, m	20.3, CH ₂	1.87, m	20.3, CH ₂	1.89, m	20.3, CH ₂	1.89, m	
5	51.3, CH ₂	3.45, m	51.2, CH ₂	3.45, brn	51.3, CH ₂	3.46, m	51.3, CH ₂	3.46, m	
5- <i>N</i> -OH		9.70, brs		9.62, brs		9.60, brs		9.60, brs	
<i>β</i> -Alanine									
6	170.0, C		170.1, C		170.0, C		170.0, C		
7	35.3, CH ₂	2.25, t (7.0)	35.2, CH ₂	2.27, t (6.5)	35.1, CH ₂	2.28, t (7.0)	35.1, CH ₂	2.28, t (7.0)	
8	35.4, CH ₂	3.22, m	35.4, CH ₂	3.25, m	35.3, CH ₂	3.23, m	35.3, CH ₂	3.23, m	
8-NH		7.92, brn		7.85, brn		7.85, brn		7.85, brn	
<i>N</i> -Hydroxy- <i>N</i> -formyl-D-ornithine									
9	171.2, C		171.1, C		171.2, C		171.2, C		
10	52.2 [52.4], ^b CH	4.20, m	52.2 [52.3], ^b CH	4.19, brn	52.1 [52.4], ^b CH	4.21, brn	52.1 [52.4], ^b CH	4.21, brn	
10-NH		8.08, brn		7.97, brs		8.23, d (10.0)		8.23, d (10.0)	
11	28.8 [29.3], ^b CH ₂	1.68–1.40, m	28.6 [29.1], ^b CH ₂	1.65, m	28.8 [29.3], ^b CH ₂	1.63, m	28.8 [29.3], ^b CH ₂	1.63, m	
				1.50, m		1.50, m		1.50, m	
12	22.6 [23.1], ^b CH ₂	1.68–1.40, m	22.6 [23.1], ^b CH ₂	1.50, m	22.6 [23.1], ^b CH ₂	1.56, m	22.6 [23.1], ^b CH ₂	1.56, m	
13	45.5 [48.8], ^b CH ₂	3.35, m	45.5 [48.8], ^b CH ₂	3.30, m	45.6 [48.8], ^b CH ₂	3.38, m	45.6 [48.8], ^b CH ₂	3.38, m	
13- <i>N</i> -OH		9.70, brs		9.62, brs		9.60, brs		9.60, brs	
14	157.2 [161.8], ^b CH	7.86 [8.23], ^b brs	157.0 [161.7], ^b CH	7.81 [8.22], ^b brs	157.1 [161.7], ^b CH	7.89 [8.21], ^b brs	157.1 [161.7], ^b CH	7.89 [8.21], ^b brs	
<i>L</i> -Serine									

position	oxachelin (3) ^d		oxachelin B (4) ^d		oxachelin C (5) ^d	
	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)
15	169.5, C		169.8, C		169.1, C	
16	55.3, CH	4.38, m	55.5, CH	4.28, brd (5.0)	55.1, CH	4.48, q (6.0)
16-NH		8.27, brn		8.19, d (8.5)		8.80, d (8.0)
17	61.8, CH ₂	3.63, brd (5.5)	61.6, CH ₂	3.63, m	61.2, CH ₂	3.54, m
17-OH				4.94, brs		5.02, brs
L-Serine						
18	169.9, C		170.1, C		165.6, C	
19	67.3, CH	5.07, dd (10.0, 7.5)	55.6, CH	4.58, brd (5.5)	51.3, CH	4.38, brn
19-NH/NH ₂				8.90, d (7.0)		8.41, brs
20	69.6, CH ₂	4.64, dd (10.0, 9.0)	61.6, CH ₂	3.75, m	63.3, CH ₂	4.62, m
20-OH		4.50, dd (8.5, 8.0)		5.10, brs		
2-Hydroxybenzoic acid						
21	166.1, C		167.0, C		167.7, C	
22	109.9, C		116.7, C		112.6, C	
23	159.1, C		158.2, C		160.1, C	
23-OH		11.78, brs		11.81, brs		10.29, brs
24	116.6, CH	7.01, d (8.5)	117.1, CH	6.93, d (8.5)	117.3, CH	7.00, dd (8.5, 1.0)
25	134.2, CH	7.47, ddd (8.5, 7.5, 1.5)	133.5, CH	7.39, t (7.5)	136.0, CH	7.55, ddd (8.5, 7.5, 2.0)
26	119.2, CH	6.96, ddd (8.0, 8.0, 1.5)	119.0, CH	6.92, t (8.0)	119.3, CH	6.96, ddd (8.0, 8.0, 1.0)
27	128.1, CH	7.64, dd (8.0, 1.5)	129.5, CH	7.94, d (7.5)	131.0, CH	7.95, dd (8.0, 1.5)

^aSee Supporting Information for the NMR spectra.^bDuplicate signals due to rotamers. Assignments supported by HSQC and HMBC experiments.