Supporting Information

Asterogynins: Secondary Metabolites from a Costa Rican Endophytic Fungus

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Materials and Methods

General experimental procedures. All NMR experiments were carried out on a Varian INOVA 600 MHz spectrometer. asterogynins A (1) and B (2) were purified from active fractions on an Agilent 1100 series HPLC (Agilent Technologies) using a preparative Phenomenex C18 column (Luna, 25 cm x 21.2 mm, 5 μm particle size). [α]₀ measurements were obtained using a Jasco P-2000 digital polarimeter with a sodium lamp.

Culturing and extraction. Agar plugs of CR1488E were initially grown at 25°C on yeast malt agar plates supplemented with 30 μg/ml streptomycin and 12 μg/ml chlortetracycline. After one week, agar plugs of this plate were placed in 150 ml of rich seed media in 1 L flasks. They were grown at 25 °C and 150 rpm for 6 days. 450 ml of 0.66% (w/v) malt extract and 10 g HP-20 resin were then added to each flask, and the fungi were cultured under the same conditions for 21 days. The fungal cultures were then held at 25 °C without shaking for 5 days. Extraction of the mycelium was accomplished by three rounds of sonication in ethanol. Rich seed media: 5 g peptone, 10 g dextrose, 3 g yeast extract, 10 g malt extract per 1 L water (pH 6.2).

Separation. Extract CR1488E was suspended in aqueous MeOH (MeOH-H2O, 9:1, 100 mL) and extracted with hexane (3 × 100 mL portions). The aqueous layer was then diluted to 70% MeOH with H2O and extracted with CH2Cl2 (3 × 100 mL portions). The CH2Cl2 extract (326.5 mg) was active with an EC₅₀ value of 23.92±15.44 μg/ml (EC₅₀ ± 95% C.I), while the hexane and the aqueous MeOH extracts were much less active. The CH2Cl2 extract was chromatographed on a C18 HPLC column (Phenomenex, Luna, 250 × 21.2 mm, 5 μ) using MeOH-H2O (50%-50%-100%, 0-10-30 min, 10 mL/min) to yield four major compounds, 1 (tᵣ 10.2 min, 8 mg), 2 (tᵣ 13.8 min, overlapped with other compounds), 3 (tᵣ 20 min, 6 mg), and 4 (tᵣ 23 min, 11 mg). Compound 2 (tᵣ 23.8 min, 0.3 mg) was further purified using a phenyl-hexyl HPLC column (Phenomenex, Luna, 250 × 10 mm, 5 μ; 60% MeOH/H2O; 2 mL/min).
**Sequencing and species identification.** For identification by internal transcribed spacer (ITS) sequencing, CR1488E cultured in potato dextrose broth for 5 days. The mycelium was then retrieved by filtration and ground to a fine powder in liquid N2. Genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega), and large subunit rDNA was amplified by PCR using primers LR5 (5’-TCCTGAGGGAAACTTCG-3’) and LROR (5’-ACCCGCTGAACCTAAGC-3’). PCR products were transformed into *E. coli* TOP10 cells using a TOPO TA Cloning Kit (Invitrogen), according to manufacturer’s protocols. Transformed plasmids were isolated and sequenced at Genewiz (http://www.genewiz.com/). The following consensus sequence were used in a BLAST search against deposited sequences:

**1488E_rDNA-LROR_A02.ab1**
TACCTCNNAACGGCGAGNGANGCGGTAATAGCTCAAATTTGAAATCTGGGCTTCGG
TCCGAGTTGTAATTTTGAGAAGATCAGTTCCGTTGGTGTGGCCTCAGTCTAAGTTTTGG
AACAGAGCTAGTCATAGAGGTGAGAATCCCGTATGTGAATGATCTGCTTCCGCTATGT
GAAGCTCTTTCCACGAGTCGATTTGGTGTGGGAAATGCAGCCTCAAAATATTGGGTGATAT
TTTATCTAAGCTAAATTTTACGTCAGCGGAGGCTACAGATATCACGAGGATGATAGTG
TCCGTGTCCAGCCGAGCATCGTGTTGGTTGGTGTGGTGAATAGCCCTGGGGAATGT
GTGCTCCTCAGGGAGTTTTATAGCCCTCAGTTGCAATGCACGCTACTGAGCCAGAGG
ACCAGCGCTCCCGCTAGGTAGTGGCCGTAATGATTTGCAAACGGAGCCGCTTTGATCT
GGTGGAGAGTCGACGCGCTCGGATGCATTACGTGCAATTTGGGTAANNNNNTGCTGA
AAATCGTGAACAGGAAAGCATCCAAATTTGACGATCGATTTGGCAGTCGAACCCGCTG
AGCCTCAAACGAGATTTCATCGTCTCGTCAACCTAGCCATCAGGCAATTTGGAATCG
GGTCCCAACAGCTATGCTCTTACTCAGATCCTTCCGCAAAGACATCGACGACGGCTG
AAATGCAACCTTTAAGGGCTTCATCTCCGTTACGTTAAATACGCATATTGGGTGTTAA
ACCCAAATTACTGCAGATAGTTGACTTTGTCGTTTGCAGTGATAGGCGCTG
TACAAATGCTTACGGCAGATCTAGTACGCGGATATGCTGCTTGGACGAGCTCTGCT
ATTGCAGAGGCTATAAACACTCTCCCAAGGAGAGGAGGACATCCCACAAGGCCTTTATC
CAACCCAAACCAGATGCTGCGCTTAAGGAGGAAACCCAGGTGACGTCGAGAACCC
AGATGATCAGGCTAAGCTGATTGCAAAACGCTTCCTTTTACAAATTTTCAG
TACTGTATTAACCTCTTTTACAAAGGAGTTTATCTTCTCAGTACATCTCTTGTGCCGCT
ATCGGTCTCTGGCCATATTTGACGCTTTAGGAAATATACCaACCCCACATTGTGACTGC
ATTGCCCAAAACACTCGACTGCGAAAGAGCCTCAGTACATAGGCAAGGCAACCGTAC
ACATACGGGAGTTTCTCACCCTCTATGAGCTGTCTGTTCCCAAGGAAACTTAGACCGAGGC
ACACCCGAAGCATTTCTCACAATTTACACACTCGACGCAAGCGCATTTCCAATTTTG
**Hsp90 protein expression and purification.** A codon-optimized PfHsp86 gene (DNA 2.0) was inserted into pET101D. The codon-optimized PfHsp86-pET101D plasmid was transformed into BL21(DE3)Star *E. coli*. Cells were grown in Terrific Broth containing Ampicillin (100 µg/ml). Cells were grown to 0.6 OD$_{600}$ at 30 °C, 250 rpm. To induce protein expression, 0.2 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) was added. The cells were then grown 9 hours at 20°C. Cells were pelleted by centrifugation and frozen before processing. Cells were resuspended in ice-cold lysis buffer (50 mM HEPES pH 7.5, 500 mM NaCl, 40 mM imidazole, 0.1% Triton X-100, 5 mM beta-mercaptoethanol added fresh) supplemented with a protease inhibitor cocktail for his-tagged proteins (Sigma). Cells were lysed by sonication and the addition of lysozyme. The lysate was centrifuged (20000g, 20 minutes), and the resulting clarified supernatant was incubated with a Nickel-NTA agarose resin (Invitrogen) at 4°C. The resin was washed with lysis buffer, and protein was eluted with the same buffer supplemented with an additional 360 mM imidazole. The buffer was changed to a freezing solution by dialysis (50 mM HEPES pH 7.5, 150 mM NaCl, 0.1% Triton X-100, 10% glycerol) and the protein was then aliquoted and flash-frozen in liquid nitrogen and stored at -80°C.

**Hsp90 fluorescence polarization assay.** The fluorescence polarization assay developed for human Hsp90 was adapted to PfHsp86. 4 nM fluorescein isothiocyanate (FITC) linked to geldanamycin at the C17 position served as the ligand to be displaced. Protein was used at a concentration where 55% of the geldanamycin-FITC in solution was bound. Compounds were dissolved in DMSO, which had no effect on geldanamycin-FITC displacement. Geldanamycin-FITC displacement was measured in an Envision2 plate reader (Perkin Elmer). Unlabeled geldanamycin was used as a positive control; DMSO, as a negative control. The data were normalized to samples with protein, fluorescent tracer,
and no test compound (i.e., fully bound background signal). Data was analyzed with Prism 5.0 software. Initial fractionation was done without replicates to conserve samples. Purified compounds and controls were tested in triplicate, and are shown as the mean ± SEM.

| Sample                    | EC50 ± 95% C.I. | Units  |
|---------------------------|-----------------|--------|
| 1488E-DCM                 | 23.9 ± 15.4     | ug/ml  |
| 1488E-DCM-D (viridiol, 3) | 5.5 ± 1.2       | ug/ml  |
| Geldanamycin              | 8.3 ± 1.4       | nM     |
| DMSO                      | n/a             | v/v%   |

1 Kim, J.; Felts, S.; Llauger, L.; He, H.; Huezo, H.; Rosen, N.; Chiosis, G. *J. Biomol. Screening* **2004**, *9*, 375-381.
Asterogynin A (1) – $^1$H NMR spectrum in CD$_3$OD

Asterogynin A (1) – $^{13}$C NMR spectrum in CD$_3$OD
Asterogynin A (1) – gHSQC spectrum in CD$_3$OD
Asterogynin A (1) – gHMBC spectrum in CD$_3$OD

Asterogynin A (1) – $^1$H NMR spectrum in Pyridine-$d_5$
Asterogynin A (1) – gHSQC spectrum in Pyridine-$d_5$

Asterogynin A (1) – gHMBC spectrum in Pyridine-$d_5$
Asterogynin A (1) – ROESY spectrum in Pyridine-$d_5$

Asterogynin B (2) – $^1$H NMR spectrum in CD$_3$OD
Asterogynin B (2) – gHSQC spectrum in CD$_3$OD

Asterogynin B (2) – gHMBC spectrum in CD$_3$OD
Selected ion chromatogram and HRMS of asterogynin A (1)
Selected ion chromatogram and HRMS of asterogynin B (2)