Supporting Information

An inexpensive and straightforward method for the selective isolation of histidine-derived natural products using nickel (II) phosphate

Muhammad Saalim,[†] Lu Ya,[†] Kenneth J. Woycechowsky,[†‡] and Benjamin R. Clark[†,*]

[†]School of Pharmaceutical Science and Technology, Tianjin University, 92 Weijin Road, Tianjin 300092, People's Republic of China

[‡] Current Address: School of Molecular Sciences, Arizona State University, Tempe, AZ, USA

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Isolation of gartryprostatin C and meleagrin using conventional methods

Gyroprostatin C and meleagrin were isolated from two different fungi using conventional methods i.e., large-scale culture in steamed rice followed by solvent extraction, fractionation, and purification with preparative HPLC. Meleagrin was isolated from a strain of *Penicillium chrysogenum* WX-6 collected from Caldara di Manziana Nature Reserve, Rome, Italy (GeneBank accession number: PP087909) whereas gartryprostatin C was isolated from a strain of *Aspergillus sclerotiorum* L3, a lab strain found originally as contamination in our lab (GeneBank accession number: PP087906). Structures were confirmed by matching spectroscopic (NMR and HRMS) data with the literature.[1-3]

Large-scale culture and extraction of *Aspergillus sclerotiorum* L3

Aspergillus sclerotiorum L3 was cultured on steamed (autoclaved) rice medium by inoculating steamed rice in 1 L conical flask using fungal discs of fungi cultured on PDA plates. After 14 days, the biomass was extracted with methanol and methanol crude extract was dispersed in water and extracted with ethyl acetate using separating funnel.

Isolation of gartryprostatin C from crude extract

The ethyl acetate crude extract from large scale culture of *Aspergillus sclerotiorum* L3 (300 mg) was subjected to fractionation with Sephadex LH-20 column using methanol with a flow rate of 0.8 ml/min. Gartryprostatin C was obtained in fraction 8 (5.4 mg). The fraction was then purified by preparative HPLC using isocratic conditions i.e., 50% ACN, flowrate = 8 ml/min to obtain 2.5 mg of gartryprostatin C at 26 mins.

Data for gartryprostatin C: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 12.25 (s), 11.85 (s), 8.14 (s), 7.81 (s), 6.68 (s), 6.08 (dd, J = 17.7 Hz, 10.9 Hz), 5.07 (d, J = 10.52 Hz), 5.02 (d, J = 17.83 Hz), 4.01 (s), 1.41 (s); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 162.9, 158.8, 145.7, 136.3, 134.3, 131.7, 124.1, 111.8, 103.4, 44.7, 37.4, 27.9; HRESIMS m/z 259.1197 $[M - H]^-$ (calculated for $C_{13}H_{15}N_4O_2$, 259.1200).

Large-scale culture and extraction of *Penicillium chrysogenum* WX-6

Penicillium chrysogenum WX-6 was cultured on steamed (autoclaved) rice medium with pH adjusted to 3 with citric acid prior to autoclaving. After two weeks the biomass was extracted with methanol. The methanol crude extract was dispersed in water and extracted with ethyl acetate using separating funnel.

Isolation of meleagrin from crude extract

The ethyl acetate extract (8 g) was subjected to fractionation with HP-20 resin by first washing with water followed by gradient elution with methanol. The 40% methanol fraction (569 mg) was first separated by preparative HPLC using gradient conditions i.e., 18-48% ACN to give various flow fractions with a flow rate of 8 ml/min. Flow fraction P22 (94 mg) was further separated using isocratic conditions (20% ACN) to give meleagrin at 27 mins (10 mg).

Data for meleagrin: ^{1}H-NMR (400 MHz, methanol-d_4) δ 8.29 (s), 7.79 (s), 7.59 (d, J = 7.8 Hz), 7.27 (t, J = 7.6 Hz), 7.06 (t, J = 7.6 Hz) 6.99 (d, J = 8.0 Hz), 6.10 (brs), 5.38 (s), 5.05 (m), 3.74 (s), 1.31 (s), 1.29 (s); ^{13}C-NMR (100 MHz, methanol-d_4) δ 167.0, 160.8, 148.1, 144.3, 141.8, 138.0, 133.0, 129.4, 127.4, 126.0, 125.9, 124.5, 112.9, 113.7, 109.1, 108.0, 103.2, 35.6, 54.0, 43.5, 24.3; HRESIMS m/z 434.1823 $[M + H]^+$ (calculated for $C_{23}H_{24}N_5O_4$, 434.1833).

^{1}H-NMR of Meleagrin and Gartryprostatin C isolated using $Ni_3(PO_4)_2$

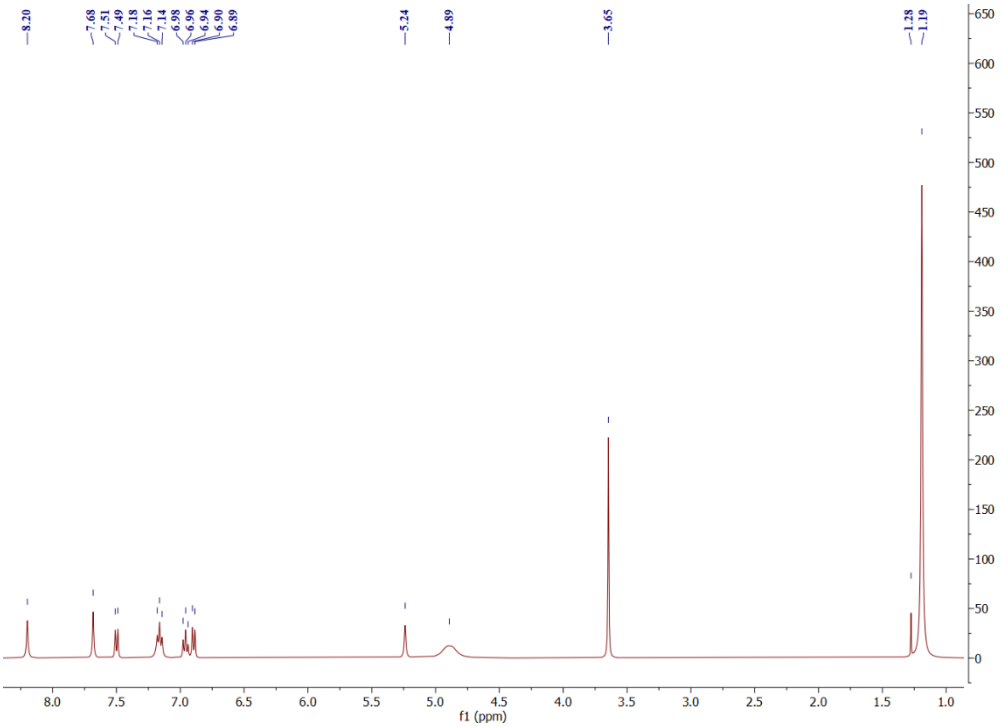

Figure S1. ^{1}H-NMR (400 MHz, d_4-MeOH) of meleagrin isolated using $Ni_3(PO_4)_2$

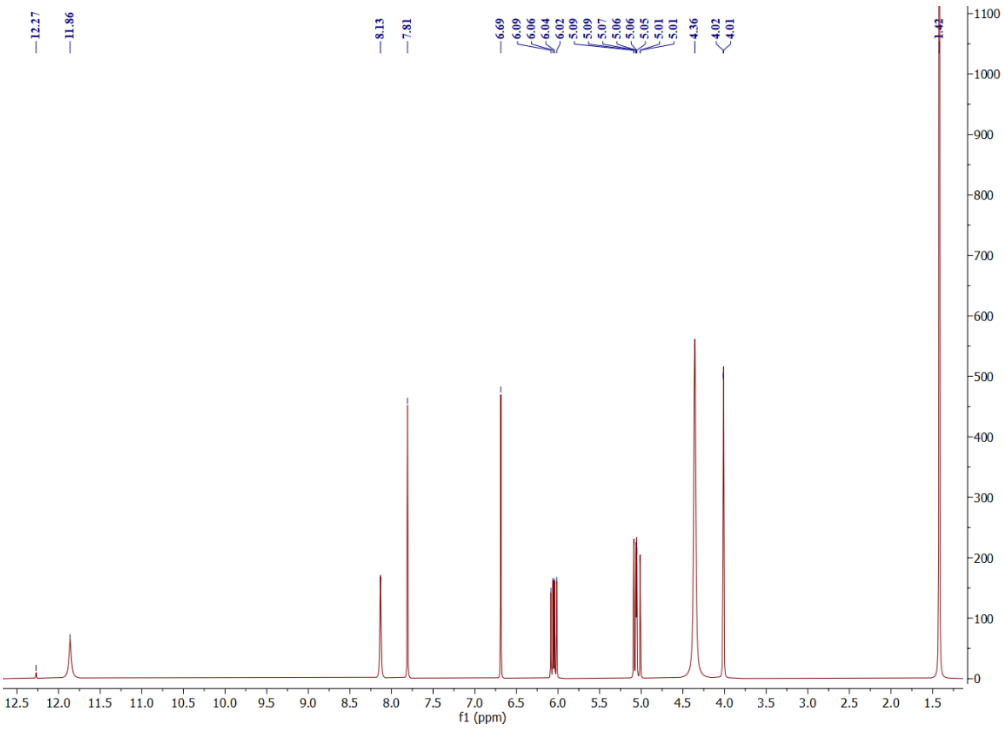

Figure S2. ^{1}H-NMR (400 MHz, d_6-DMSO) of gartryprostatin C isolated using $Ni_3(PO_4)_2$.

Estimation of Ni^{2+} concentration

Ni^{2+} concentrations were estimated using a colorimetric method. A standard curve (Figure S1) was constructed using serial twofold dilutions of a soluble nickel (II) salt ($NiSO_4.6H_2O$), measuring absorbance at 750 nm, where other components are unlikely to absorb. The elution fraction from meleagrin had an absorbance of 0.298, corresponding to a concentration of 17.0 mM.

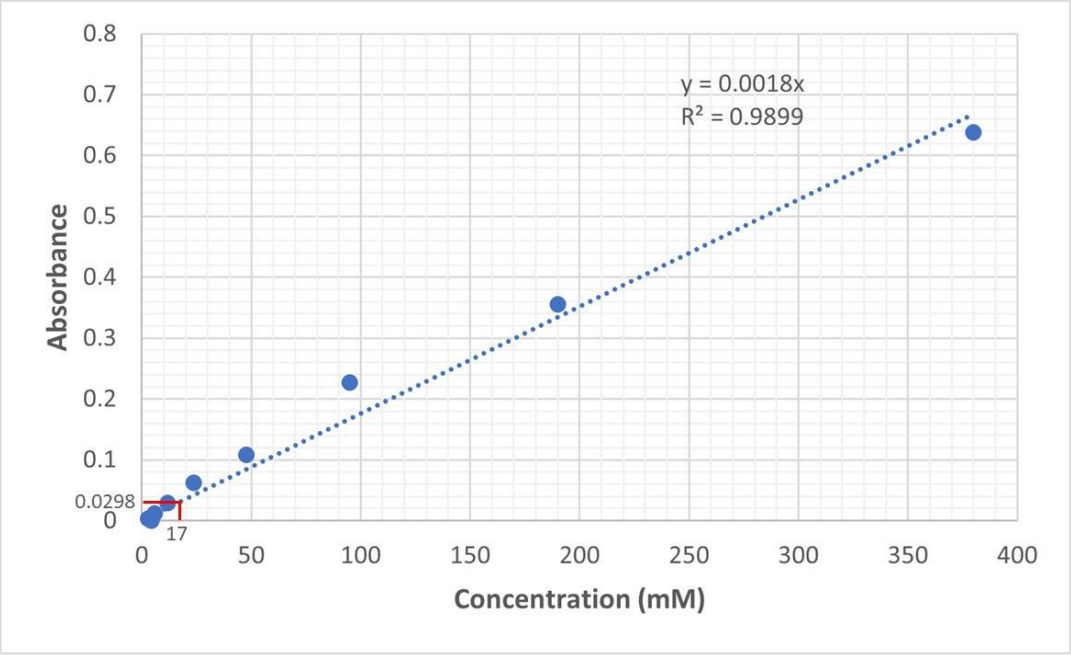

Figure S3. Calibration curve for Ni^{2+} (Absorbance at 750 nm). Nickel concentration in the meleagrin eluent is marked in red on the curve.

REFERENCES

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