Electronic Supplementary Material (ESI) for Chemical Communications

Antagonism of a Zinc Metalloprotease Using a Unique Metal-Chelating Scaffold: Tropolones as Inhibitors of *P. aeruginosa* Elastase

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SUPPORTING INFORMATION

A. General Methods and Materials

General chemistry methods. Reactions were carried out under standard atmospheric conditions. Yields refer to chromatographically and spectroscopically homogenous materials, unless otherwise stated. All reactions were conducted in a CEM Discover® Microwave Reactor and were monitored by thin-layer chromatography (TLC) carried out on silica gel plates using UV-light (254 nm). THF was dried and stored over molecular sieves. Flash chromatography separations were performed using a CombiFlash® Rf automated chromatography system by Teledyne Isco. All compounds were confirmed to have ≥95% purity by HPLC (254 or 280 nm). NMR spectra were recorded on a Bruker or Varian 300 or 400 MHz spectrometers at 25 °C and calibrated using a solvent peak as an internal reference. The following abbreviations are used to indicate the multiplicities: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad.

Data measurement and analysis. All fluorescence readings were measured in black 96-well microtiter plates with clear bottoms (Corning Costar) on a SpectraMax M2e Microplate Reader (Molecular Devices). GraphPad Prism version 5.0a for Mac OS X (GraphPad Software, www.graphpad.com) was used for all IC_{50} and K_i analyses.

Materials. LasB was purchased from Elastin Products Company and used as received. The LasB pro-fluorescent substrate, Abz-Ala-Gly-Leu-Ala-p-Nitro-Benzyl-Amide (SAG-3905-PI), was purchased from Peptides International and used as received. Molecular biology grade DMF and DMSO were purchased from Sigma Aldrich and used as received.

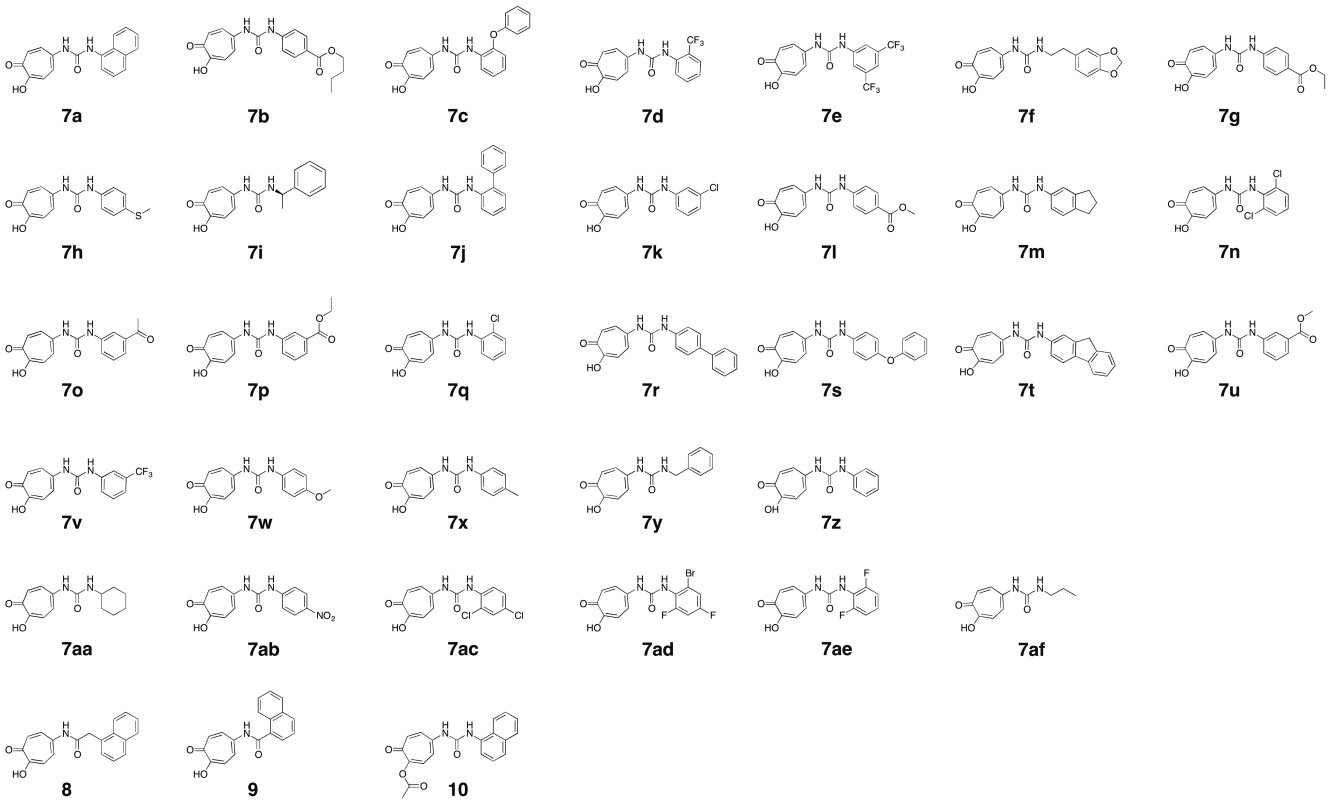


Figure S1. List of tropolone sublibrary compounds, including control compounds **8**, **9**, and **10**. Compounds are arranged based on relative potency against LasB.

B. Synthesis and Characterization for Tropolone Compounds

Acetyltropolone (4). A 25-mL round bottom flask was charged with tropolone (**3**, 300 mg, 2.45 mmol) and acetic anhydride (5 mL) and the resulting solution was stirred at 25 °C. To this mixture, 4 drops of concentrated sulfuric acid was added, and the reaction was left to stir under nitrogen for 4 h at 25 °C. The reaction was monitored by TLC, and upon disappearance of starting material, the reaction mixture was quenched with ice. Excess acetic anhydride was evaporated via high vacuum and the crude mixture was dissolved in CH_2Cl_2 and extracted 3× with water. The organic fractions were collected and purified via automatic column chromatography (0–7% MeOH in CH_2Cl_2) to yield **4** (125 mg, 31%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.31–7.23 (m, 5H), 2.26 (s, 3H). ^{13}C NMR (200 MHz, DMSO-d_6): δ 175.5, 161.5, 148.1, 139.7, 138.4, 131.0, 128.1, 124.2, 30.1. ESI-MS(-) *m/z* 163.12, found for $C_9H_7O_3$ $[M-H]^-$.

5-Aminotropolone (6). The synthesis of **6** was adapted from previous procedure.[12] A 50-mL round-bottom flask was charged with tropolone (**3**, 5.0 g, 41 mmol) and water (14.5 mL) and the resulting solution was stirred at 25 °C. In a separate beaker, acetic acid (12 mL, 210 mmol) and sodium nitrite (3.1 g, 45 mmol) were combined and quickly added to the water solution. An additional 5 mL of acetic acid was used to rinse the beaker, ensuring that all of the sodium nitrite was added. The reaction solution was then stirred at 25 °C for 3 h under nitrogen. The resulting brown solid was collected over a frit and washed with 50 ml of cool water to yield 5-nitrosotropolone (62% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.85 (d, J = 40.0 Hz, 2H), 7.58 (dd, J = 12.2, 1.5 Hz, 2H). ^{13}C NMR (200 MHz, DMSO-d_6): δ 185.7, 184.2, 152.3, 139.7, 130.3, 128.1, 124.2. ESI-MS(-) *m/z* 150.12, found for $C_7H_4NO_3$ $[M-H]^-$.

The synthesis of **6** was adapted from that described previously. In a large Parr bottle, 5-nitrosotropolone (2.2 g, 14.6 mmol) was dissolved in 100 mL of ethanol. Palladium on carbon (10%, 0.029 g, 0.028 mmol) was then added. The bottle was placed in a hydrogenator and purged three times with hydrogen gas before being set to shake for 18 h at 22 psi. The reaction was monitored by TLC, and upon disappearance of 5-nitrosotropolone, the reaction mixture was filtered over celite and the filtrate evaporated to dryness. The dark orange residue was then dissolved in 50 mL of boiling ethyl acetate, and the turbid mixture was hot filtered, reduced in volume to 35 mL, and allowed to cool. The resulting golden precipitate was filtered and collected to yield **6** (1.66 g, 83% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ δ: 7.11 (d, J = 12.1 Hz, 2H), 6.72 (d, J = 12.1 Hz, 2H), 6.27 (s, 2H). ^{13}C NMR (200 MHz, DMSO-d_6): δ 185.7, 184.2, 161.1, 139.7, 156.5, 120.1, 124.2. ESI-MS(-) *m/z* 136.11, found for $C_7H_6NO_2$ $[M-H]^-$.

General synthetic procedure for aminotropolone derivatives (7). 5-Aminotropolone (**6,** 100 mg, 0.73 mmol) and an isocyanate (1.1 mmol, 1.5 equiv) were dissolved in THF (4 mL) in a 10-mL microwave reaction tube. The reaction vial was then placed in the microwave heater and heated to 95 °C for 120 min with the following settings: maximum pressure 250 psi, 300 watts, and medium stirring. Reaction progress was monitored by TLC and upon disappearance of **6**, the mixture was transferred to a round-bottomed flask and concentrated in vacuo. Compounds (**7**) were isolated via hot filtration with boiling MeOH and recrystallization or automated flash column chromatography (0–8% MeOH in CH_2Cl_2).

Compound **7a**. 96 mg, 31% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.26 (s, 1H), 8.88 (s, 1H), 8.12 (d, J = 8.4 Hz, 1H), 7.95 (t, J = 7.5 Hz, 2H), 7.69–7.65 (m, 3H), 7.61–7.56 (m, 2H), 7.50 (d, J = 7.6 Hz, 1H), 7.27 (dd, J = 12.2, 0.9 Hz, 2H). ^{13}C NMR (200 MHz, DMSO-d_6): δ 173.9, 173.9, 153.7,

144.9, 140.6, 137.9, 136.9, 133.2, 128.3, 126.1, 126.0, 125.2, 124.4, 121.6, 119.8, 118.8, 108.1, 106.2. ESI-MS(-) *m/z* 304.94, found for $C_{18}H_{13}N_2O_3$ $[M-H]^-$.

Compound **7b**. 39 mg, 14%% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.23 (s, 1H), 9.01 (s, 1H), 7.89 (d, J = 8.6 Hz, 2H), 7.58 (dd, J = 10.6, 4.3 Hz, 4H), 7.23 (d, J = 12.2 Hz, 2H), 4.24 (t, J = 6.5 Hz, 2H), 1.67 (dd, J = 14.4, 6.6 Hz, 2H), 1.42 (dd, J = 15.0, 7.3 Hz, 2H), 0.93 (t, J = 7.4 Hz, 3H). ^{13}C NMR (200 MHz, DMSO-d_6): δ 169.7, 166.0, 153.0, 144.6, 139.9, 131.0, 128.8, 125.6, 123.7, 118.2, 61.0, 50.9, 14.9. ESI-MS(+) *m/z* 357.39, found for $C_{19}H_{21}N_2O_5$ $[M+H]^+$.

Compound **7c**. 64 mg, 24% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.47 (s, 1H), 8.56 (s, 1H), 8.25 (d, J = 8.2 Hz, 1H), 7.59 (d, J = 12.3 Hz, 2H), 7.43 (t, J = 8.0 Hz, 2H), 7.24 (d, J = 12.2 Hz, 2H), 7.18 (td, J = 7.4, 1.1 Hz, 1H), 7.15–7.10 (m, 1H), 7.07 (dd, J = 7.7, 1.0 Hz, 2H), 7.01–6.97 (m, 1H), 6.85 (dd, J = 8.1, 1.2 Hz, 1H). ^{13}C NMR (200 MHz, DMSO-d_6): δ 169.0, 156.8, 152.5, 145.4, 139.8, 131.0, 127.5, 125.3, 124.1, 123.8, 122.8, 120.0, 118.6, 118.5. ESI-MS(+) *m/z* 348.88, found for $C_{20}H_{17}N_2O_4$ $[M+H]^+$.

Compound **7d**. 41 mg, 17% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.19 (d, J = 0.5 Hz, 1H), 9.03 (s, 1H), 8.01 (s, 1H), 7.59–7.50 (m, 4H), 7.33 (d, J = 7.7 Hz, 1H), 7.23 (d, J = 10.8 Hz, 2H). ^{13}C NMR (200 MHz, DMSO-d_6): δ 174.8, 171.3, 166.5, 137.6, 134.6, 133.9, 130.1, 129.0, 128.2, 127.5, 125.7, 123.6, 120.3, 119.9. ESI-MS(+) *m/z* 324.96, found for $C_{15}H_{12}F_3N_2O_3$ $[M+H]^+$.

Compound **7e**. 72 mg, 24% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.52 (s, 1H), 9.19 (s, 1H), 8.14 (s, 2H), 7.67 (s, 1H), 7.57 (d, J = 12.1 Hz, 2H), 7.23 (d, J = 12.0 Hz, 2H). ^{13}C NMR (200 MHz, DMSO-d_6): δ 169.5, 169.9, 153.2, 140.4, 138.5, 128.4, 127.7, 127.6, 127.5, 126.9, 126.1, 125.8, 119.4, 119.2. ESI-MS(-) *m/z* 390.92, found for $C_{16}H_9F_6N_2O_3$ $[M-H]^-$.

Compound **7f**. 103 mg, 32% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.22 (s, 1H), 8.99 (s, 1H), 7.89 (s, 1H), 7.56 (dd, J = 10.7, 4.3 Hz, 4H), 7.21 (d, J = 12.1 Hz, 2H), 4.22 (t, J = 6.5 Hz, 2H), 1.66 (dt, J = 14.1, 6.9 Hz, 2H), 1.40 (dd, J = 15.0, 7.3 Hz, 2H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 174.7, 172.9, 154.9, 150.9, 148.4, 146.7, 137.8, 136.4, 131.3, 121.5, 120.7, 119.7, 110.0, 109.1, 108.0, 51.1, 49.1. ESI-MS(+) *m/z* 328.98, found for $C_{17}H_{17}N_2O_5$ $[M+H]^+$.

Compound **7g**. 81 mg, 34% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.08 (s, 1H), 8.92 (s, 1H), 8.16 (t, J = 1.9 Hz, 1H), 7.65 (d, J = 8.1 Hz, 1H), 7.58 (d, J = 12.2 Hz, 3H), 7.43 (t, J = 7.9 Hz, 1H), 7.24–7.21 (m, 2H), 4.31 (q, J = 7.1 Hz, 2H), 1.32 (t, J = 7.1 Hz, 3H). ^{13}C NMR (200 MHz, DMSO-d_6): δ 169.7, 166.0, 153.0, 144.6, 139.9, 131.0, 128.8, 125.6, 123.7, 118.2, 61.0, 14.9. ESI-MS(-) *m/z* 326.99, found for $C_{17}H_{15}N_2O_5$ $[M-H]^-$.

Compound **7h**. 76 mg, 34% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.89 (s, 1H), 8.82 (s, 1H), 7.57 (dd, J = 11.0, 1.3 Hz, 2H), 7.44–7.41 (m, 2H), 7.24–7.21 (m, 4H), 2.44 (s, 3H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 169.4, 153.2, 140.4, 137.6, 131.1, 128.4, 128.2, 125.7, 119.9, 16.5. ESI-MS(-) *m/z* 300.87, found for $C_{15}H_{13}N_2O_3S$ $[M-H]^-$.

Compound **7i**. 101 mg, 36% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.67 (s, 1H), 7.53 (d, J = 12.3 Hz, 2H), 7.34 (d, J = 4.4 Hz, 4H), 7.24 (q, J = 4.4 Hz, 1H), 7.17 (d, J = 12.3 Hz, 2H), 6.80 (d, J = 7.8 Hz, 1H), 4.80 (t, J = 7.3 Hz, 1H), 1.39 (d, J = 6.9 Hz, 3H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 169.0, 154.9, 145.5, 141.4, 129.0, 128.3, 127.4, 126.5, 126.0, 121.3, 49.5, 23.6. ESI-MS(-) *m/z* 283.05, found for $C_{16}H_{15}N_2O_3$ $[M-H]^-$.

Compound **7j**. 59 mg, 23% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.21 (s, 1H), 7.88 (d, J = 8.2 Hz, 1H), 7.78 (s, 1H), 7.54-7.49 (m, 4H), 7.42 (td, J = 8.0, 1.4 Hz, 3H), 7.34 (dd, J = 8.2, 7.3 Hz, 1H), 7.24–7.15 (m, 4H). ^{13}C NMR (200 MHz, DMSO-d_6): δ 169.4, 153.4, 140.6, 139.1, 135.9, 133.8, 131.1, 129.8, 129.5, 128.5, 128.2, 128.0, 125.8, 124.4, 123.6. ESI-MS(+) *m/z* 333.76, found for $C_{20}H_{17}N_2O_3$ $[M+H]^+$.

Compound **7k**. 65 mg, 15% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.05 (s, 1H), 9.00 (s, 1H), 7.71 (d, J = 2.1 Hz, 1H), 7.58 (d, J = 12.4 Hz, 2H), 7.31 (d, J = 6.9 Hz, 2H), 7.24 (d, J = 12.2 Hz, 2H), 7.06 (dd, J = 5.1, 3.6 Hz, 1H). ^{13}C NMR (200 MHz, DMSO-d_6): δ 174.0, 172.9, 145.5, 137.2, 136.2, 134.6, 131.2, 129.8, 125.7, 125.3, 124.9, 124.2, 122.0, 119.3. ESI-MS(+) *m/z* 291.03, found for $C_{14}H_{12}ClN_2O_3$ $[M+H]^+$.

Compound **7l**. 88 mg, 38% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.23 (s, 1H), 9.01 (s, 1H), 7.90 (d, J = 8.7 Hz, 2H), 7.60–7.56 (m, 4H), 7.23 (d, J = 12.3 Hz, 2H), 3.81 (s, 3H). ^{13}C NMR (200 MHz, DMSO-d_6): δ 169.7, 166.0, 153.0, 144.6, 139.9, 131.0, 128.8, 125.6, 123.7, 118.2, 52.8. ESI-MS(-) *m/z* 312.89, found for $C_{16}H_{13}N_2O_5$ $[M-H]^-$.

Compound **7m**. 48 mg, 22% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.86 (s, 1H), 8.66 (s, 1H), 7.60–7.56 (m, 2H), 7.37 (s, 1H), 7.24–7.21 (m, 2H), 7.16–7.11 (m, 2H), 2.81 (dt, J = 14.0, 7.2 Hz, 4H), 2.00 (quintet, J = 7.4 Hz, 2H). ^{13}C NMR (200 MHz, DMSO DMSO-d_6): δ 169.7, 166.0, 153.0, 146.0, 144.6, 143.8, 139.7, 137.6, 133.3, 129.5, 124.0, 121.4, 120.4, 120.0, 43.2, 43.1, 20.1. ESI-MS(-) *m/z* 294.92, found for $C_{17}H_{15}N_2O_3$ $[M-H]^-$.

Compound **7n**. 109 mg, 46% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.20 (s, 1H), 8.38 (s, 1H), 7.57 (dd, J = 14.3, 10.3 Hz, 4H), 7.33 (t, J = 8.1 Hz, 1H), 7.22 (d, J = 12.3 Hz, 2H). ^{13}C NMR (200 MHz, DMSO-d_6): δ 169.4, 158.2, 157.3, 156.3, 140.5, 135.9, 130.6, 128.4, 125.8, 123.5, 120.9, 120.4, 118.4. ESI-MS(-) *m/z* 322.83, found for $C_{14}H_9Cl_2N_2O_4$ $[M-H]^-$.

Compound **7o**. 64 mg, 29% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.04 (s, 1H), 8.95 (s, 1H), 8.08 (s, 1H), 7.67 (dd, J = 8.0, 1.1 Hz, 1H), 7.61–7.57 (m, 3H), 7.45 (t, J = 7.9 Hz, 1H), 7.23 (d, J = 12.2 Hz, 2H), 2.56 (s, 3H). ^{13}C NMR (200 MHz, DMSO-d_6): δ 171.2, 169.0, 154.9, 149.5, 136.5, 136.0, 133.4, 129.0, 128.3, 127.4, 126.5, 126.0, 23.6. ESI-MS(-) *m/z* 296.98, found for $C_{16}H_{13}N_2O_4$ $[M-H]^-$.

Compound **7p**. 81 mg, 34% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.08 (s, 1H), 8.92 (s, 1H), 8.16 (t, J = 1.9 Hz, 1H), 7.65 (d, J = 8.1 Hz, 1H), 7.58 (d, J = 12.2 Hz, 3H), 7.43 (t, J = 7.9 Hz, 1H), 7.24–7.21 (m, 2H), 4.31 (q, J = 7.1 Hz, 2H), 1.32 (t, J = 7.1 Hz, 3H). ^{13}C NMR (200 MHz, DMSO-d_6): δ 169.7, 166.0, 153.0, 144.6, 141.0, 139.9, 137.3, 131.0, 128.8, 125.6, 123.7, 118.2, 79.1, 31.1. ESI-MS(-) *m/z* 326.99, found for $C_{17}H_{15}N_2O_5$ $[M-H]^-$.

Compound **7q**. 89 mg, 40% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.59 (s, 1H), 8.41 (s, 1H), 8.12 (d, J = 8.4 Hz, 1H), 7.60 (d, J = 12 Hz, 2H), 7.48 (d, J = 7.9 Hz, 1H), 7.31 (t, J = 8 Hz, 1H), 7.24 (d, J = 12 Hz, 2H), 7.06 (t, J = 7.4 Hz, 1H). ^{13}C NMR (200 MHz, DMSO-d_6): δ 174.8, 171.3, 152.5, 137.6, 134.6, 132.7, 130.1, 129.0, 128.2, 127.5, 125.7, 123.6, 120.2, 119.6. ESI-MS(+) *m/z* 292.00, found for $C_{14}H_{12}ClN_2O_3$ $[M+H]^+$.

Compound **7r**. 96 mg, 40% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.93 (d, J = 8.1 Hz, 2H), 7.65–7.54 (m, 8H), 7.44 (s, 2H), 7.31 (t, J = 7.4 Hz, 1H), 7.24 (d, J = 12.3 Hz, 2H). ^{13}C NMR (200 MHz, DMSO-d_6): δ 174.8, 172.9, 152.5, 145.5, 140.6, 139.4, 138.4, 137.9, 137.0, 129.2, 128.2, 127.9, 127.6, 125.8, 121.5, 119.7 (only 16 of 20 carbons found). ESI-MS(-) *m/z* 330.94, found for $C_{20}H_{15}N_2O_3$ $[M-H]^-$.

Compound **7s**. 50 mg, 20% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.89 (s, 1H), 8.81 (s, 1H), 7.58 (d, J = 12.3 Hz, 2H), 7.47 (d, J = 8.9 Hz, 2H), 7.36 (d, J = 0.9 Hz, 2H), 7.23 (d, J = 12.3 Hz, 2H), 7.09 (t, J = 7.4 Hz, 1H), 6.97 (dd, J = 10.0, 8.5 Hz, 4H). ^{13}C NMR (200 MHz, DMSO-d_6): δ 169.4, 158.2, 153.3, 151.6, 140.5, 135.9, 130.6, 128.4, 125.8, 123.5, 120.9, 120.4, 118.4. ESI-MS(-) *m/z* 346.98, found for $C_{20}H_{15}N_2O_4$ $[M-H]^-$.

Compound **7t**. 63 mg, 25% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.92 (d, J = 15.4 Hz, 2H), 7.80 (d, J = 8.2 Hz, 3H), 7.60 (d, J = 12.3 Hz, 2H), 7.54 (d, J = 7.4 Hz, 1H), 7.41 (dd, J = 8.2, 2.0 Hz, 1H), 7.35 (t, J = 7.3 Hz, 1H), 7.27–7.22 (m, 3H), 3.90 (s, 2H). ^{13}C NMR (200 MHz, DMSO-d_6): δ 174.7, 172.7, 154.1, 153.0, 145.5, 143.4, 143.1, 141.1, 140.0, 137.9, 135.5, 129.1, 126.8, 125.8, 121.6, 120.3, 119.9, 117.3, 115.9, 105.3, 36.5. ESI-MS(-) *m/z* 342.94, found for $C_{21}H_{15}N_2O_3$ $[M-H]^-$.

Compound **7u**. 88 mg, 38% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.09 (s, 1H), 8.94 (s, 1H), 8.21 (s, 1H), 7.63-7.56 (m, 4H), 7.44 (t, J = 7.9 Hz, 1H), 7.22 (d, J = 11.7 Hz, 2H), 3.85 (s, 3H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 169.7, 166.0, 153.0, 145.8, 142.1, 138.8, 137.3, 131.0, 128.8, 125.6, 123.7, 118.2, 55.9. ESI-MS(-) *m/z* 312.93, found for $C_{16}H_{13}N_2O_5$ $[M-H]^-$.

Compound **7v**. 67 mg, 27% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.53 (s, 1H), 8.18 (s, 1H), 7.89 (d, J = 8.2 Hz, 1H), 7.71–7.58 (m, 4H), 7.31 (t, J = 7.6 Hz, 1H), 7.25–7.22 (m, 2H). ^{13}C NMR (200 MHz, acetone-d_6): δ 169.3, 133.0, 129.0, 128.7, 126.2, 126.4, 125.8, 125.6, 124.1. ESI-MS(+) *m/z* 326.01, found for $C_{15}H_{11}F_3N_2O_3$ $[M+H]^+$.

Compound **7w**. 90 mg, 21% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.84 (s, 1H), 8.62 (s, 1H), 7.58 (d, J = 12.4 Hz, 2H), 7.37-7.35 (m, 2H), 7.23 (d, J = 12.2 Hz, 2H), 6.90-6.87 (m, 2H), 3.72 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 169.4, 153.2, 140.4, 137.6, 132.2, 128.4, 127.0, 125.7, 119.9, 35.7. ESI-MS(+) *m/z* calcd for $C_{15}H_{14}N_2O_4$ 287.21 $[M+H]^+$.

Compound **7x**. 93 mg, 47% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.70 (s, 1H), 7.55 (dd, J = 12.2, 0.8 Hz, 2H), 7.19 (dd, J = 12.2, 0.8 Hz, 2H), 6.32 (t, J = 5.7 Hz, 1H), 3.04 (q, J = 6.5 Hz, 2H), 1.45 (dt, J = 14.5, 7.2 Hz, 2H), 0.87 (t, J = 7.4 Hz, 3H). ^{13}C NMR (200 MHz, DMSO-d_6): δ 173.3, 172.7, 151.5, 145.9, 139.9, 138.0, 136.1, 128.0, 127.0, 126.2, 121.6, 119.1, 26.1. ESI-MS(+) *m/z* 271.50, found for $C_{15}H_{15}N_2O_3$ $[M+H]^+$.

Compound **7y**. 30 mg, 15% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.84 (s, 1H), 7.57 (d, J = 11.9 Hz, 2H), 7.36–7.18 (m, 7H), 6.80 (t, J = 3.5 Hz, 1H), 4.30 (d, J = 5.6 Hz, 2H). ^{13}C NMR (200 MHz, DMSO-d_6): δ 173.8, 172.6, 151.3, 146.8, 143.7, 140.1, 137.9, 132.9, 128.9, 128.1, 122.0, 119.8, 44.1 (only 13 of 15 carbons found). ESI-MS(-) *m/z* 269.02, found for $C_{15}H_{13}N_2O_3$ $[M-H]^-$.

Compound **7z**. 75 mg, 40% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.85 (d, J = 40.0 Hz, 2H), 7.58 (dd, J = 12.2, 1.5 Hz, 2H), 7.45 (d, J = 8.1 Hz, 2H), 7.31–7.21 (m, 4H), 6.99 (t, J = 7.4 Hz, 1H). ^{13}C NMR (200 MHz, DMSO-d_6): δ 169.9, 157.8, 142.7, 127.3, 126.0, 120.3, 120.3, 120.2, 115.5, 113.9, 105.2. ESI-MS(+) *m/z* 257.22, found for $C_{14}H_{13}N_2O_3$ $[M+H]^+$.

Compound **7aa**. 30 mg, 15% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.56 (s, 1H), 7.52 (d, J = 12.3 Hz, 2H), 7.17 (d, J = 12.3 Hz, 2H), 6.22 (d, J = 7.8 Hz, 1H), 3.43 (dd, J = 6.8, 2.9 Hz, 1H), 1.77 (d, J = 12.1 Hz, 2H), 1.66–1.62 (m, 2H), 1.52 (d, J = 12.1 Hz, 1H), 1.30 (t, J = 11.2 Hz, 2H), 1.20–1.11 (m, 3H). ^{13}C NMR (200 MHz, DMSO-d_6): δ 168.9, 154.8, 141.7, 127.3, 126.0, 48.5, 48.5, 48.4, 33.5, 25.9, 25.0. ESI-MS(-) *m/z* 261.06, found for $C_{14}H_{17}N_2O_3$ $[M-H]^-$.

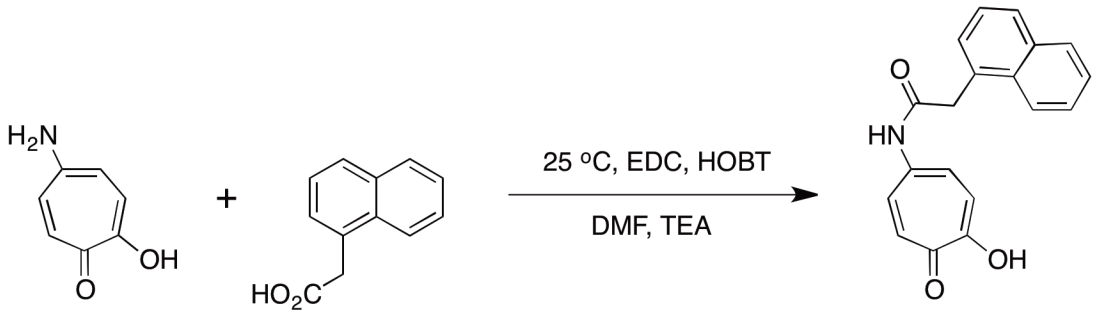
Compound **7ab**. 30 mg, 15% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.53 (s, 1H), 9.09 (s, 1H), 8.18 (d, J = 9.2 Hz, 2H), 7.68 (d, J = 9.3 Hz, 2H), 7.55 (dd, J = 12.1, 1.1 Hz, 2H), 7.23–7.20 (m, 2H). ^{13}C NMR (200 MHz, DMSO-d_6): δ 174.2, 172.9, 154.3, 145.7, 145.3, 141.7, 137.5, 132.0, 125.8, 124.1, 121.6, 119.9. ESI-MS(-) *m/z* 299.8, found for $C_{14}H_{17}N_2O_3$ $[M-H]^-$.

Compound **7ac**. 180 mg, 76% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.59 (s, 1H), 8.47 (s, 1H), 8.15 (d, J = 9.0 Hz, 1H), 7.63–7.57 (m, 3H), 7.39 (dd, J = 9.0, 2.5 Hz, 1H), 7.25–7.22 (m, 2H). ^{13}C NMR (200 MHz, DMSO-d_6): δ 169.5, 153.2, 140.4, 139.5, 134.6, 129.6, 128.4, 127.7, 127.5, 126.8, 125.8, 119.4, 119.2. ESI-MS(-) *m/z* 322.9, found for $C_{14}H_9Cl_2N_2O_3$ $[M-H]^-$.

Compound **7ad**. 71 mg, 26% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.23 (s, 1H), 8.20 (s, 1H), 7.60–7.57 (m, 3H), 7.50–7.44 (m, 1H), 7.22 (d, J = 12.2 Hz, 2H). ^{13}C NMR (200 MHz, DMSO-d_6): δ 174.9, 172.9, 166.6, 152.4, 145.5, 139.2, 137.9, 129.7, 125.8, 125.8, 124.5, 121.2, 116.5, 103.9. ESI-MS(-) *m/z* 368.71, found for $C_{14}H_8BrF_2N_2O_3$ $[M-H]^-$.

Compound **7ae**. 66 mg, 31% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.18 (s, 1H), 8.28 (s, 1H), 7.60–7.57 (m, 2H), 7.37–7.30 (m, 1H), 7.24–7.14 (m, 4H). ^{13}C NMR (200 MHz, DMSO-d_6): δ 169.4, 158.2, 157.3, 156.3, 140.5, 135.9, 134.6, 128.4, 125.8, 124.4, 120.9, 120.4, 119.6. ESI-MS(+) *m/z* 293.10, found for $C_{14}H_{11}F_2N_2O_3$ $[M+H]^+$.

Compound **7af**. 66 mg, 31% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.65 (s, 1H), 7.55 (dd, J = 12.2, 0.8 Hz, 2H), 7.19 (dd, J = 12.2, 0.8 Hz, 2H), 6.32 (t, J = 5.7 Hz, 1H), 3.04 (q, J = 6.5 Hz, 2H), 1.45 (dt, J = 14.5, 7.2 Hz, 2H), 0.87 (t, J = 7.4 Hz, 3H). ^{13}C NMR (200 MHz, DMSO-d_6): δ 169.4, 158.2, 156.3, 140.5, 135.9, 50.5, 30.2, 26.8. ESI-MS(+) *m/z* 223.70, found for $C_{11}H_{15}N_2O_3$ $[M+H]^+$.



6 **8**

IC_{50}: 180(1) μM 9.86(0.13) μM

Scheme S1. Synthesis of compound **8**. A similar procedure was used for compound **9**.

Compound **8.** A flame-dried 50-mL round-bottom flask was charged with **6** (250 mg, 1.8 mmol) and dry DMF (20 mL), and the resulting solution was stirred under nitrogen at 25 °C. To this solution, triethylamine (280 μL, 2.0 mmol, 1.1 equiv) and EDC (419 mg, 2.2 mmol, 1.2 equiv) were added and the reaction mixture was stirred under inert atmosphere for 10 min. HOBT (335 mg, 2.2 mmol, 1.2 equiv) was then added, and following 10 min of stirring at 25 °C, 2-(naphthalen-1-yl)acetic acid (407 mg, 2.2 mmol, 1.2 equiv) was introduced to the reaction mixture. The resulting solution was stirred for 18 h at 25 °C, and upon the consumption of the starting material (determined by TLC) the DMF was evaporated under vacuum and the pale yellow residue was purified via automated column chromatography to yield **8** (70 mg, 13% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.48 (s, 1H), 8.14 (d, J = 8.1 Hz, 1H), 7.97 (d, J = 8.2 Hz, 1H), 7.90–7.87 (m, 1H), 7.72 (d, J = 12.1 Hz, 2H), 7.60–7.51 (m, 4H), 7.24 (d, J = 11.7 Hz, 2H), 4.19 (s, 2H). ^{13}C NMR (200 MHz, DMSO-d_6): δ 173.9, 173.9, 153.7, 144.9, 140.6, 137.9, 136.9, 133.2, 128.3, 126.1, 126.0, 125.2, 124.4, 121.6, 119.8, 118.8, 108.1, 106.2, 43.8. ESI-MS(-) *m/z* 304.08, found for $C_{19}H_{14}NO_3$ $[M-H]^-$.

Compound **9.** Compound **9** was prepared as described for **8**, but using 1-naphthoic acid. 82 mg, 15% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.83 (d, J = 8.4 Hz, 1H), 8.27 (dd, J = 17.3, 7.7 Hz, 2H), 8.07 (d, J = 8.0 Hz, 1H), 7.70–7.63 (m, 3H), 7.25 (d, J = 12.0 Hz, 2H), 7.08 (s, 2H). ^{13}C NMR (200 MHz, DMSO-d_6): δ 169.7, 168.2, 161.2, 153.7, 137.6, 136.4, 136.1, 134.7, 133.9, 132.7, 131.1, 130.0, 126.1, 121.6, 120.5, 120.3, 119.8, 105.8. ESI-MS(-) *m/z* 289.89, found for $C_{18}H_{12}NO_3$ $[M-H]^-$.

Compound **10.** A 25-mL round-bottom flask was charged with **7a** (125 mg, 0.41 mmol) and acetic anhydride (3.0 mL, 32 mmol) at 25 °C. Sulfuric acid (22 μL, 0.41 mmol) was added to the stirring solution, and the reaction mixture was stirred at 25 °C under a nitrogen atmosphere. After 24 h, the reaction was quenched with ice and extracted 3× with CH_2Cl_2. The organic fractions were collected and purified via automated column chromatography (0–7% MeOH in CH_2Cl_2) to yield **10**. 125 mg, 31% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.57 (s, 1H), 8.99 (s, 1H), 8.06 (d, J = 6.3 Hz, 1H), 7.91 (t, J = 6.0 Hz, 2H), 7.67 (d, J = 6.0 Hz, 1H), 7.60–7.52 (m, 4H), 7.46 (t, J = 6.0 Hz, 1H), 7.30 (d, J = 9.0 Hz, 2H), 2.20 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 168.7, 153.3, 145.8, 134.4, 134.1, 129.1, 127.0, 126.7, 126.6, 126.5, 124.6, 122.1, 119.2, 21.1. ESI-MS(+) *m/z* 348.98, found for $C_{20}H_{17}N_2O_4$ $[M+H]^+$.

C. Enzymatic Assays

Fluorescence Assay for LasB Activity. This assay was adapted from that previously reported.[3]

Assay Buffers:

A: 50 mM Tris-HCl, 2.5 mM $CaCl_2$ (pH 7)

To prepare: 25 mL Tris-HCl (1 M solution), 475 mL H_2O, 139 mg $CaCl_2$

B: 50 mM Tris-HCl, 2.5 mM $CaCl_2$, 1% DMF (pH 7)

To prepare: 25 mL Tris-HCl (1 M solution), 475 mL H_2O, 139 mg $CaCl_2$, 5 mL DMF (molecular biology grade)

Assay Stock Solutions:

LasB: 0.1 mg/mL in Buffer A

LasB substrate: 5 mM in DMF (molecular biology grade)

To prepare: 3 mg (MW: 583.64 g/mol), 1 mL DMF

Inhibitor Stock Solutions:

10 mM, 5 mM, 2.5 mM, 500 μM, 250 μM, 50 μM, 25 μM in DMSO

Note: 2 μL of each stock used in the assay

Stock	Assay Final Concentration (μM)
10 mM	200
5 mM	100
2.5 mM	50
500 μM	10
250 μM	5
50 μM	1
25 μM	0.5

Assay Protocol:

1. To the wells of a 96-well microtiter plate (Corning®Costar®; black with clear bottom), add 91 μL **Buffer B**, 2 μL of each **inhibitor stock** and 2 μL **LasB** solution (2 mg/mL final) (in this order)

   Notes:

   i. Positive control: 2 μL DMSO in place of inhibitor

   ii. Negative control: 4 μL DMSO in place of LasB and inhibitor stock
2. Incubate plate at 37 °C for 30 min
3. While incubating, turn on and set up fluorescence plate reader: λ_{ex} = 340 nm, λ_{em} = 415 nm, 37°C, read on kinetic setting over 30 min
4. After 30 min incubation at 37 °C, add 5 μL **LasB substrate** solution (250 mM final) to each well
5. Transfer plate to fluorescence plate reader and measure fluorescence

   Note: For an inhibitor, *decreased* fluorescence signal will be observed in comparison to the positive control

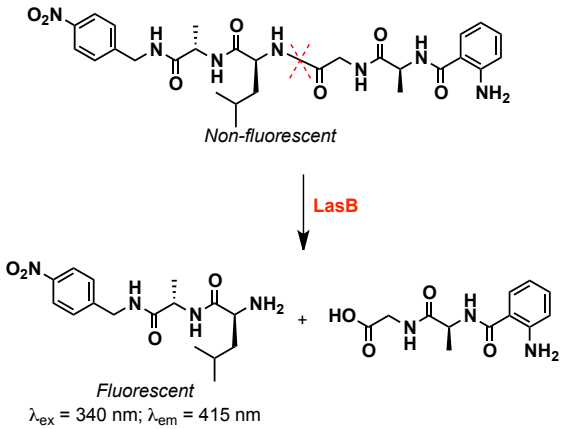


Figure S2. Fluorescence assay for LasB activity.

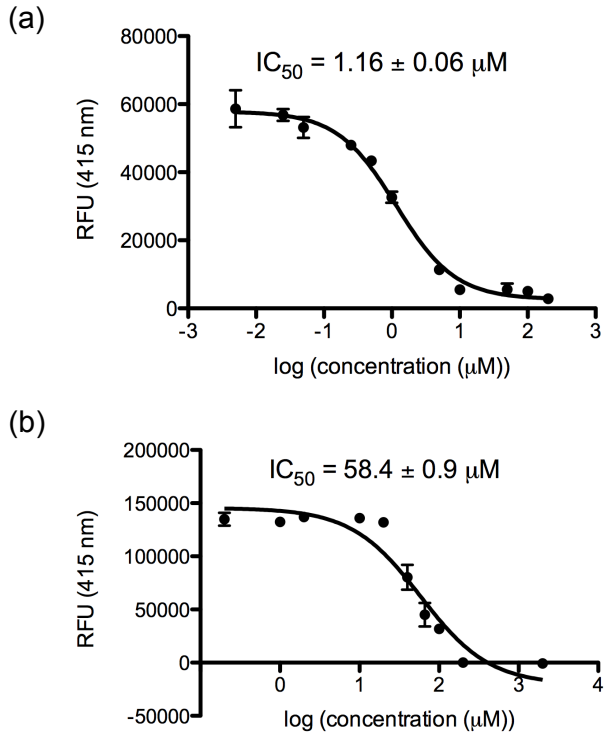


Figure S3. Inhibition curves for compounds (a) **7a** and (b) **10**. The IC_{50} values were determined from triplicate measurements. RFU = relative fluorescence units at 415 nm.

Table S1. IC_{50} values for tropolone sublibrary against LasB.

Compound	IC_{50}	Compound	IC_{50}
7a	1.16 ± 0.06 μM	**7q**	5.40 ± 0.01 μM
7b	1.28 ± 0.04 μM	**7r**	7.65 ± 0.08 μM
7c	1.73 ± 0.01 μM	**7s**	7.80 ± 0.32 μM
7d	2.51 ± 0.03 μM	**7t**	8.73 ± 0.04 μM
7e	3.09 ± 0.10 μM	**7u**	9.00 ± 0.14 μM
7f	1.64 ± 0.03 μM	**7v**	9.56 ± 0.03 μM
7g	1.90 ± 0.10 μM	**7w**	11.0 ± 0.03 μM
7h	2.30 ± 0.25 μM	**7x**	14.1 ± 0.08 μM
7i	2.40 ± 0.21 μM	**7y**	14.8 ± 0.06 μM
7j	2.60 ± 0.01 μM	**7z**	19.0 ± 0.05 μM
7k	3.15 ± 0.04 μM	**7aa**	20.4 ± 0.15 μM
7l	4.00 ± 0.08 μM	**7ab**	24.1 ± 0.08 μM
7m	4.03 ± 0.07 μM	**7ac**	31.7 ± 0.19 μM
7n	4.10 ± 0.18 μM	**7ad**	43.6 ± 0.14 μM
7o	4.60 ± 0.13 μM	**7ae**	45.3 ± 0.36 μM
7p	5.30 ± 0.08 μM	**7af**	46.7 ± 0.53 μM

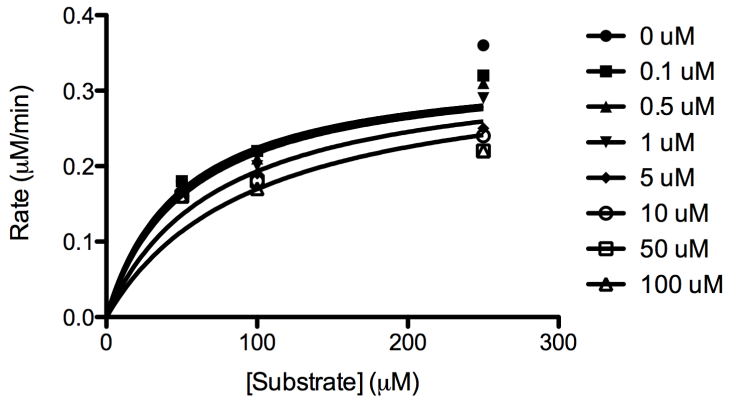


Figure S4. Competitive inhibition curve for **7a**. K_i was determined to be 336±24 nM using Michaelis-Menten kinetics.

Fluorescence Assay for MMP-2 and MMP-9. The assay was carried out in white NUNC* 96-well opaque round-bottomed plates as previously described.[4] For the assay, each well contained a total volume of 90 μL including buffer (50 mM HEPES, 10 mM $CaCl_2$, 0.05% Brij-35, pH 7.5), human recombinant MMP (ENZO Life Sciences; 1.16 U MMP-2 and 0.9 U MMP-9), and compound **7** (50 μM final concentration). After a 30 min incubation period at 37 °C, the reaction was initiated by the addition of 10 μL of the fluorogenic MMP substrate (4 μM final concentration, Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2·AcOH, ENZO Life Sciences). Fluorescence measurements were recorded using a Bio-Tek Flx 800 fluorescence plate reader every minute for 20 min at excitation and emission wavelengths of 320 and 400 nm, respectively. The rate of fluorescence increase was compared for samples and negative controls (no inhibitor, arbitrarily set as 100% activity). CGS 27023A, a broad spectrum MMP inhibitor, was used as a positive control (IC_{50} value ~20 and 8 nM of MMP-2 and MMP-9, respectively; MacPherson et al. *J. Med. Chem.*, **1997**, *40*, 2525-2532).

Mushroom Tyrosinase (TY). The assay was carried out as previously described (Liu, J.; Yi, W.; Wan, Y.; Ma, L.; Song, H. *Bioorg. Med. Chem.* **2008**, *16*, 1096-1102). The assay was performed in black 96-well clear flat-bottomed plates with each well containing a total volume of 100 μL. This included buffer (5 mM phosphate, pH 6.8), mushroom TY (30 U, Sigma-Aldrich), inhibitor (50 μM), and *L*-dopamine (0.5 mM, Sigma-Aldrich). Mushroom TY and inhibitor were preincubated in the buffer solution at room temperature for 10 min. A background absorbance reading at 475 nm was recorded using a Bio-Tek ELx 808 colorimetric plate reader. *L*-Dopamine was added to initiate the reaction, which was allowed to proceed for 10 min before a second absorbance reading at 475 nm was taken. After subtracting the background absorbance, the remaining absorbance of the negative controls (no inhibitor) was arbitrarily set as 100% activity. The ratio of absorbance between inhibitor and control wells was defined as percent TY activity.

Human Carbonic Anhydrase II (hCAII). hCAII activity was measured in 50 mM Tris (pH 8) using an esterase activity assay with 4-nitrophenyl acetate as the substrate. Conversion of 4-nitrophenyl acetate to 4-nitrophenol by hCAII is observed as an increase in absorbance at 405 nm, which was read on a BioTek ELx808 plate reader. Protein (100 nM, final concentration, expressed as previously described in Monnard F.W.; Heinisch, T.; Nogueira, E.S.; Schirmer, T; Ward, T.R. *Chem. Commun.*, **2011**, *47*, 8238-8240) was incubated with inhibitor (50 μM) for 10 min at room temperature followed by addition of substrate (in DMSO, 500 μM final concentration). The total well volume (clear, flat-bottom, 96-well plates) was 100 μL and contained 5% DMSO. Initial linear rates were compared to both negative (inhibitor-free) and positive (100% inhibition with 50 μM)[5] controls in order to determine percent inhibition. Acetazolamide, a clinically used hCAII inhibitor, was used as a positive control (IC_{50} value ~12 nM; Krishnamurthyet al. *Chem Rev*. **2008**, *108*, 946-1051).

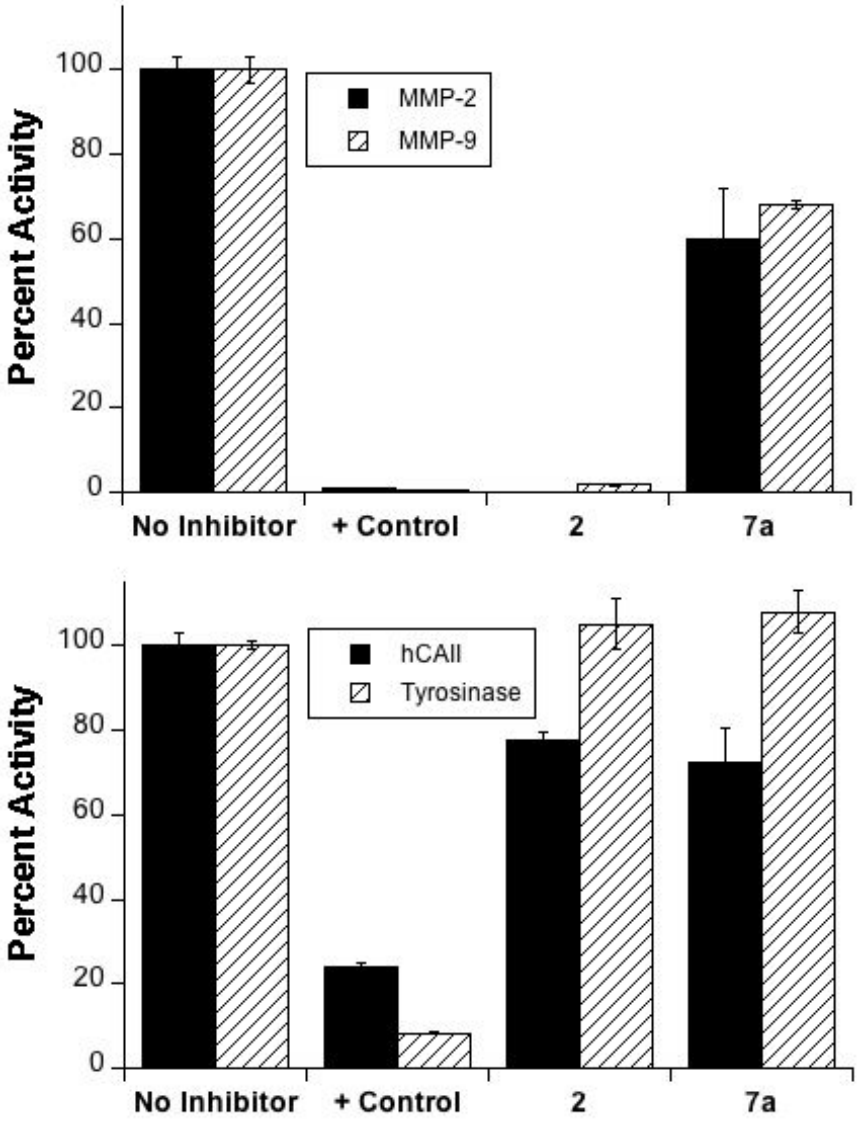


Figure S5. Metalloenzyme specificity of **2** versus **7a** (50 μM). Positive control compound for MMP-2 and MMP-9 was CGS 27023A (IC_{50} value ~20 and 8 nM of MMP-2 and MMP-9, respectively), for hCAII was acetazolamide (IC_{50} value ~12 nM), and for tyrosinase was tropolone (IC_{50} value ~400 nM, **3**).

D. Bacterial Viability and Swarming

Overnight cultures of *P. aeruginosa* strain PA14, prepared in Tryptic soy broth (TSB) at 37 °C (250 rpm), were diluted with fresh media (1:1,000) and treated with varying concentrations of **7a**. Bacterial viability was then determined by measuring the OD_{600} of the culture in the presence of compound. All concentrations were analyzed in triplicate and the average of the results with 1:1000 dilution are shown in Figure S3. Toxicity was observed at concentrations >25 μM.

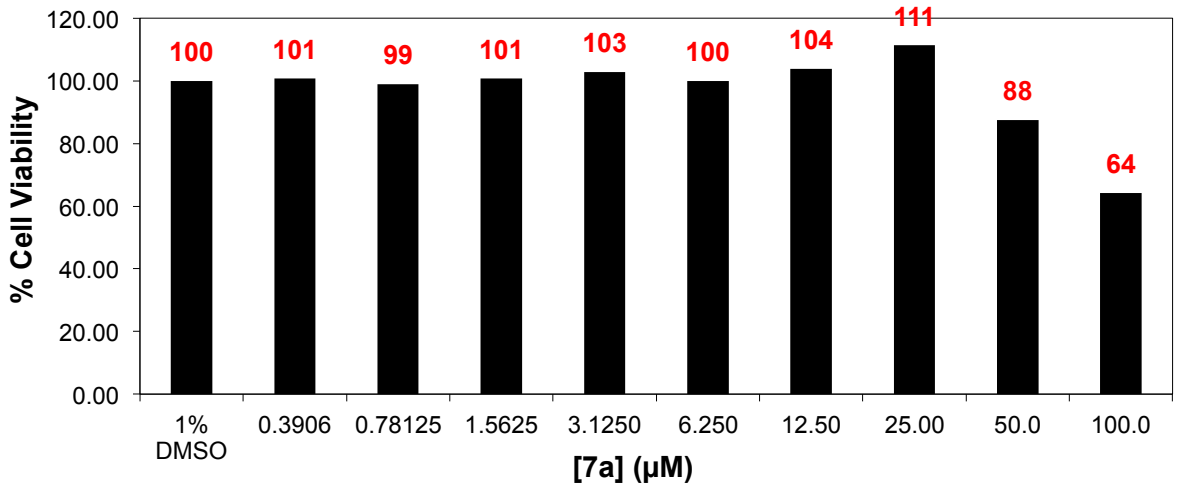


Figure S6. Viability of PA14 in the presence of **7a**.

P. aeruginosa Swarming Assay. A swarming motility assay using *P. aeruginosa* strain PA14 was executed as previously described.[6] Overnight cultures of PA14 strain, prepared in Tryptic soy broth (TSB) at 37 °C (250 rpm), were washed (3×) with phosphate-buffered saline buffer (pH 7.4). The washed culture was then diluted with the same buffer to an OD_{600} of ~3.0. Swarm agar medium was a modified M9 agar medium and contained: 20 mM NH_4Cl, 12 mM Na_2HPO_4, 22 mM KH_2PO_4, 8.6 mM NaCl, 1 mM $MgSO_4$, 1 mM $CaCl_2$•2 H_2O, 11 mM dextrose, 0.5% casamino acids (Difco) and Bacto-agar (Difco). The medium was autoclaved, and upon cooling was diluted with filter-sterilized $MgSO_4$ and $CaCl_2$•$2H_2O$. ~20 mL of swarm agar medium containing compound **7a** (25 μM) was poured into 100×25 mm Petri dishes housed in a laminar flow cabinet and dried for 1 h. 5 μL of the bacterial culture (OD_{600} of ~3.0) was spotted onto each plate followed by incubation at 30 °C for 18 h.

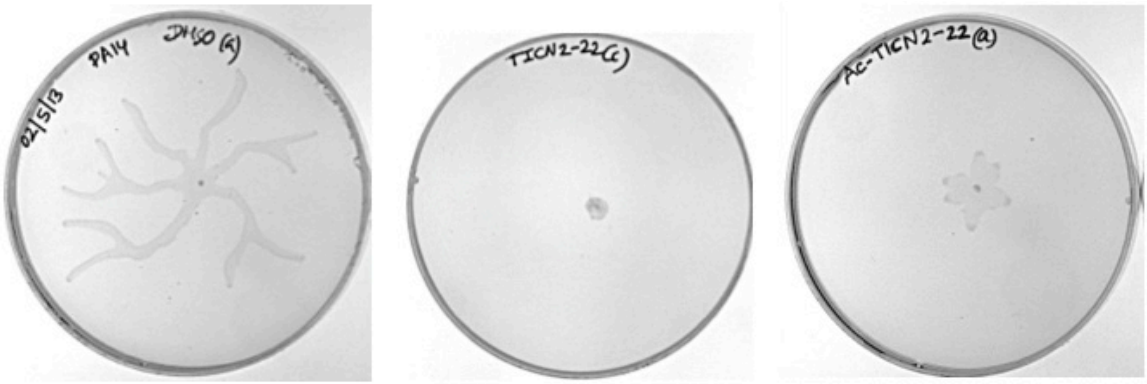


Figure S7. Swarming of *P. aeruginosa* strain PA14 in the absence (left, DMSO control) or presence of **7a** (middle, 25 μM) or **10** (right, 25 μM). Reduced swarming in the presence of **10** is attributed to some hydrolysis of **10** to **7a** (Figure S8).

Stability of Compound 10. The stability of compound **10** was assessed in Tryptic soy broth (TSB). A sample containing 50 μM compound was incubated at 37 °C for 24 h to mirror swarming conditions and analyzed using LC-MS to monitor for hydrolysis. Samples were analyzed at 6 h and 24 h (Figure S8). After 24 h, complete hydrolysis of compound **10** to **7a** was observed.

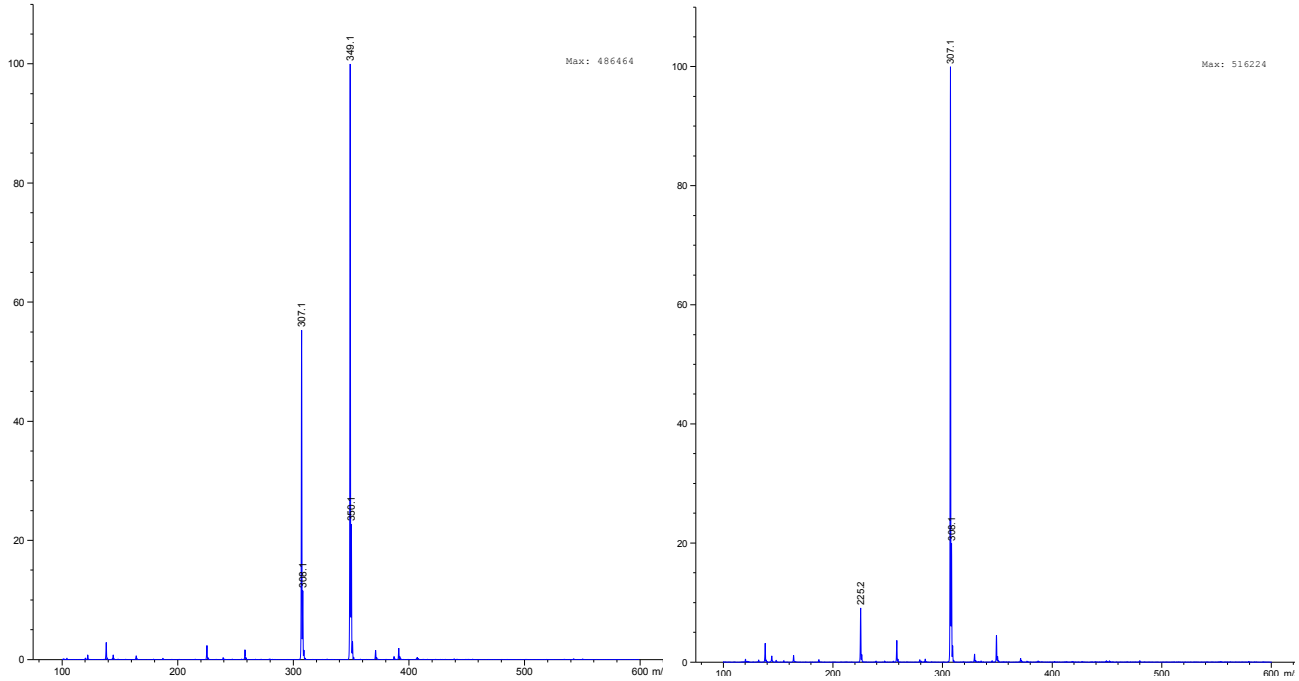


Figure S8. ESI-MS examining the stability of compound **10** in Tryptic soy broth (TSB). Samples were analyzed at 6 h (left) and 24 h (right). After 24 h, complete hydrolysis of compound **10** to **7a** was observed (right).

E. References

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