Supporting Information for

A small molecule antagonist of ghrelin O-acyltransferase (GOAT)
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A. General Methods and Materials

General chemistry methods. Reactions were carried out under a nitrogen atmosphere with dry, freshly distilled solvents under anhydrous conditions, unless otherwise noted. Yields refer to chromatographically and spectroscopically homogenous materials, unless otherwise stated. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25-mm EMD silica gel plates (60F-254) using UV-light (254 nm). Flash chromatography separations were performed on Silicycle silica gel (40-63 mesh). Purity analyses were performed using HPLC (254 nm). NMR spectra were recorded on Bruker 400 MHz spectrometer instruments 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.

General molecular biology methods. GOAT membranes were isolated as previously described.1, 2

Data analysis. All data was analyzed using GraphPad Prism version 5.0a for Mac OS X (GraphPad Software, www.graphpad.com). Data are represented as normalized with respect to the negative control.

Materials. Reacti-Bind™ High Binding Capacity Streptavidin-coated plates (96-well, black) were purchased from Thermo Scientific. Octynoyl-CoA and HRP-N₃ were prepared as previously described.1 THPTA was received from the laboratory of Professor M. G. Finn (The Scripps Research Institute). Enhanced Chemifluorescent HRP Substrate kit (amplex red) was purchased from Thermo Scientific.

B. Synthesis of Compounds 3a and 3b

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\begin{align*}
\text{O} & \quad \text{R}^1\text{OH} \\
\text{R}^2\text{NH}_2 & \quad \text{N} \\
\text{H} & \quad \text{N} \\
\text{+} & \quad \text{O} \\
\text{HO} & \quad \text{HO} \\
\text{R}^1 & \quad \text{O} \\
\text{N} & \quad \text{R}^2
\end{align*}
\]

General synthetic procedure for compounds 3a and 3b.3, 4 A stirring solution of 6-hydroxy-2-naphthaldehyde (1.0 equiv) in MeOH at 25 °C was treated with carboxylic acid (2.0 equiv), amine (2.0 equiv) and benzylisonitrile (2.0 equiv). The solution was heated to reflux, and stirred for 24 h. The solution was then cooled to 25 °C and concentrated in vacuo. The crude residue
was purified via flash column chromatography (10–50% EtOAc in hexanes) to afford the purified product. Compound 3a was obtained in 71% yield and compound 3b was obtained in 77% yield.

**Compound 3a.** ¹H NMR (400 MHz, CDCl₃, 25 °C): δ = 0.66 (t, J = 7.2 Hz, 3H), 0.90–1.10 (m, 2H), 1.34–1.56 (m, 6H), 2.41 (s, 3H), 3.30–3.37 (m, 2H), 4.63 (s, 2H), 6.00 (s, 1H), 6.94–7.34 (m, 11H); ¹³C NMR (100 MHz, CDCl₃, 25 °C): δ = 13.4, 19.9, 28.0, 29.2, 31.9, 43.6, 47.2, 65.2, 109.1, 117.4, 126.9, 127.1, 127.3, 127.5, 127.6, 128.3, 128.4, 128.5, 129.0, 129.8, 129.9, 130.7, 138.0, 156.4, 167.8, 169.9; HRMS (ESI-TOF) m/z calc'd for C₂₇H₃₃N₂O₃ [M+H]⁺ 433.2413, found 433.5546.

**Figure S1** HPLC analysis (254 nm) for compound 3a (both enantiomers).

**Compound 3b.** ¹H NMR (400 MHz, CDCl₃, 25 °C): δ = 0.68 (t, J = 7.2 Hz, 3H), 0.90–1.12 (m, 2H), 1.34–1.61 (m, 10H), 2.40 (s, 3H), 3.31–3.35 (m, 2H), 4.62 (s, 2H), 5.98 (s, 1H), 6.91–7.36 (m, 11H); ¹³C NMR (100 MHz, CDCl₃, 25 °C): δ = 13.4, 19.9, 21.2, 23.5, 28.0, 29.2, 31.9, 43.6, 47.2, 65.2, 109.1, 117.4, 126.9, 127.1, 127.3, 127.5, 127.6, 128.3, 128.4, 128.5, 129.0, 129.8, 129.9, 130.7, 138.0, 156.4, 167.8, 169.9; HRMS (ESI-TOF) m/z calc'd for C₂₉H₃₇N₂O₃ [M+H]⁺ 461.2726, found 461.4077.

**Figure S2** HPLC analysis (254 nm) for compound 3b (both enantiomers).
C. Other Ugi Library Members

![Chemical structure](image)

**Figure S3** Structures of other library members examined in cat-ELCCA for GOAT. All compounds were tested as racemates.

D. Assay Protocols and Supplementary Material

*Plate loading.* A 10 µg/mL solution of biotinylated ghrelin(1–5)pentapeptide was prepared and used for immobilization in Reacti-Bind™ High Binding Capacity Streptavidin-coated plates (96-well, black). Wells were first washed with 25 mM TBST (3 × 200 µL), and then 100 µL of ghrelin(1–5)pentapeptide stock solution was added. The plate was covered and allowed to shake for 2 h at 25 °C. The ghrelin(1–5)pentapeptide solution was removed, and the wells were again washed with TBST (3 × 200 µL) followed by HEPES buffer (50 mM, pH 7.0) (3 × 200 µL).

*General assay procedure.* Membrane-bound GOAT (~50 µg), compound (varying concentrations, 5% total DMSO) and HEPES buffer (50 mM, pH 7.0) were added to wells containing immobilized ghrelin(1–5)pentapeptide, and the plate was incubated at 37 °C for 5 min. Palmitoyl-CoA (50 µM) and n-octynoyl-CoA (1.0 µM) were then added to the mixture to initiate the enzyme-catalyzed reaction (total volume = 100 µL), and the plate was incubated at 37 °C for another 5 min. Negative controls did not contain either membrane-bound GOAT or n-octynoyl-CoA. Positive controls contained no compound or DMSO in place of compound. The GOAT reaction mixture was then removed, and the wells were washed with TBST (3 × 200 µL) followed by PBS (pH 7.4) (3 × 200 µL). Fresh PBS (pH 7.4), HRP-N₃ (1.0 µM), CuSO₄ (100 µM), THPTA (500 µM; 5.0 equiv with respect to CuSO₄)⁵,⁶ and sodium ascorbate (5 mM) were then added (total volume = 100 µL), and the plate was covered and allowed to shake at 25 °C for
2 h. The click reaction mixture was then removed, and the wells were washed again with TBST (3 × 200 µL) and PBS (pH 7.4) (3 × 200 µL). For detection, 100 µL of amplex red/H$_2$O$_2$ solution (QuantaRed Enhanced Chemifluorescent HRP Substrate kit) was added to each well, and the fluorescence ($\lambda_{ex} = 560$ nm, $\lambda_{em} = 585$ nm) was measured over time (~10 min) on a SpectraMax M2e Microplate Reader (Molecular Devices). All reactions were performed in triplicate.

**Click Chemistry Notes:**

1. A 40 µM stock solution of HRP-N$_3$ was prepared in pH 9.4 carbonate-bicarbonate buffer.
2. A 20 mM stock solution of CuSO$_4$ was prepared in water and used in the assay.
3. A 50 mM stock solution of THPTA was prepared in water and used in the assay.
4. A 100 mM stock solution of sodium ascorbate was prepared in water and used in the assay. This stock solution was prepared fresh for each experiment.

**Inhibition curves for compounds 3a, 3b and 1.**

![Inhibition curves](image)

**Figure S4** Inhibition curves for compounds 3a (a), 3b (b) and 1 (c). Data are represented as normalized with respect to the negative control. Negative fluorescence values were obtained after normalization.
E. References