Supporting Information for

A small molecule antagonist of ghrelin *O*-acyltransferase (GOAT)

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A. General Methods and Materials

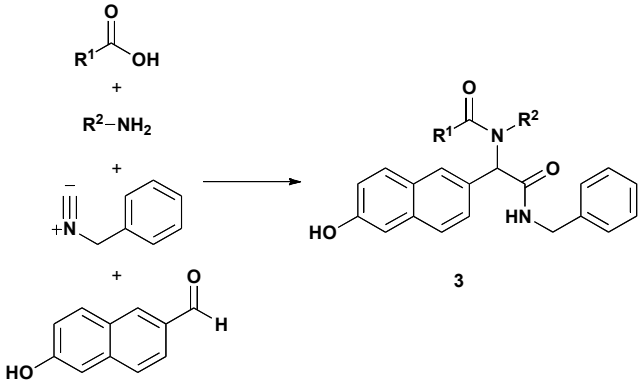
General chemistry methods. Reactions were carried out under a nitrogen atmosphere with dry, freshly distilled solvents under anhydrous conditions, unless otherwise noted. Yields refer to chromatographically and spectroscopically homogenous materials, unless otherwise stated. Reactions were monitored 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.

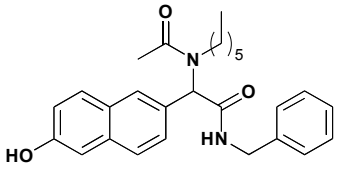
Materials. Reacti-Bind™ High Binding Capacity Streptavidin-coated plates (96-well, black) were purchased from Thermo Scientific. Octynoyl-CoA and HRP-N_3 were prepared as previously described.[1] THPTA was received from the laboratory of Professor M. G. Finn (The Scripps Research Institute). Enhanced Chemifluorescent HRP Substrate kit (amplex red) was purchased from Thermo Scientific.

B. Synthesis of Compounds 3a and 3b



*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***. ^{1}H NMR (400 MHz, $CDCl_3$, 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); ^{13}C NMR (100 MHz, $CDCl_3$, 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, 127.3, 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* calcd for $C_{27}H_{33}N_2O_3$ $[M+H]^+$ 433.2413, found 433.5546.

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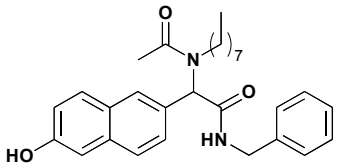
TIFF (Uncompressed) decompressor
are needed to see this picture.

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



*Compound **3b***. ^{1}H NMR (400 MHz, $CDCl_3$, 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); ^{13}C NMR (100 MHz, $CDCl_3$, 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, 127.3, 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* calcd for $C_{29}H_{37}N_2O_3$ $[M+H]^+$ 461.2726, found 461.4077.

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Figure S2 HPLC analysis (254 nm) for compound **3b** (both enantiomers).

C. Other Ugi Library Members

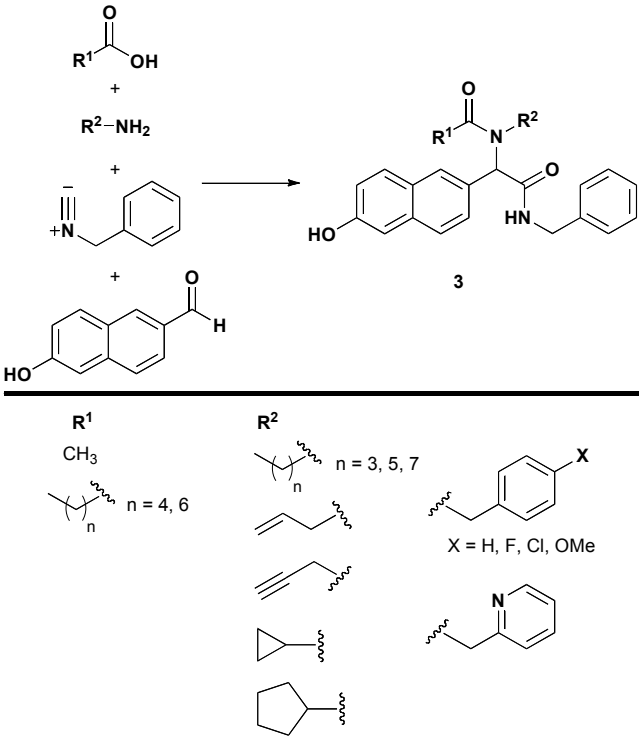

Figure S3 Structures of other library members examined in cat-ELCCA for GOAT. All compounds were tested as racemates.

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-N_3 (1.0 μM), $CuSO_4$ (100 μM), THPTA (500 μM; 5.0 equiv with respect to $CuSO_4$)[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.[5,6] 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_2O_2 solution (QuantaRed Enhanced Chemifluorescent HRP Substrate kit) was added to each well, and the fluorescence (λ_{ex} = 560 nm, λ_{em} = 585 nm) was measured over time (~10 min) on a SpectraMax M2^e 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**.*

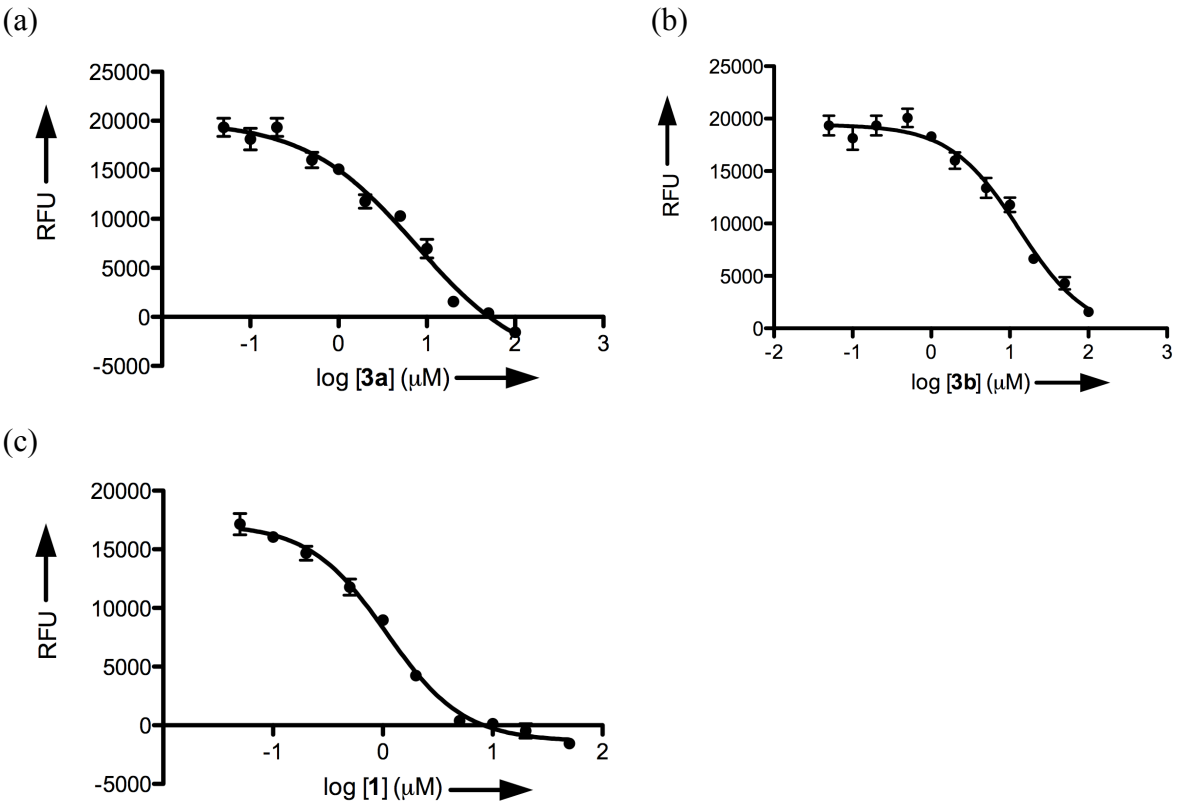

Figure S4 Inhibition curves for compounds **3a** (a), **3b** (b) and **1** (c). Data are represented as normalized with respect to the negative control. Negative fluorescence values were obtained after normalization.

E. References

1. A. L. Garner and K. D. Janda, *Angew. Chem. Int. Ed.*, 2010, **49**, 9630-9634.
2. J. Yang, T.-J. Zhao, J. L. Goldstein and M. S. Brown, *Proc. Natl. Acad. Sci., U. S. A.*, 2008, **105**, 10750-10755.
3. Y. Xu, J. Shi, N. Yamamoto, J. A. Moss, P. K. Vogt and K. D. Janda, *Bioorg. Med. Chem.*, 2006, **14**, 2660-2673.
4. Y. Xu, H. Lu, J. P. Kennedy, X. Yan, L. A. McAllister, N. Yamamoto, J. A. Moss, G. E. Boldt, S. Jiang and K. D. Janda, *J. Comb. Chem.*, 2006, **8**, 531-539.
5. V. Hong, S. I. Presolski, C. Ma and M. G. Finn, *Angew. Chem. Int. Ed.*, 2009, **48**, 9879-9883.
6. V. Hong, N. F. Steinmetz, M. Manchester and M. G. Finn, *Bioconj. Chem.* 2010, **21**, 1912-1916.