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Supporting Information

69451 Weinheim, Germany

Redox-Based Probes for Protein Tyrosine Phosphatases**

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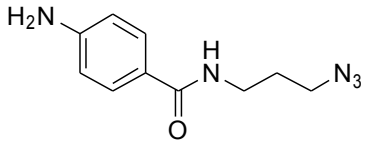
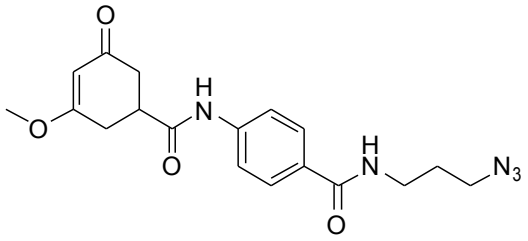
Materials. Reagents and solvents were purchased from Sigma or other commercial sources and were used without further purification. DAz-1 was synthesized as previously described.[1] YopH was expressed and purified as previously reported.[2] Recombinant PTP1b protein was purchased from Enzo Life Sciences.

Chemical Methods. All reactions were performed under an argon atmosphere in oven-dried glassware. Analytical thin layer chromatography (TLC) was carried out using Analtech Uniplate silica gel plates and visualized using a combination of UV, potassium permanganate, and ninhydrin staining. Flash chromatography was performed using silica gel (32–63 μM, 60Å pore size) from Sorbent Technologies Incorporated. NMR spectra were obtained on a Varian Inova 400 (400 MHz for ^{1}H; 100 MHz for ^{13}C). ^{1}H and ^{13}C NMR chemical shifts are reported in parts per million (ppm) referenced to the residual solvent peak. High-resolution electrospray ionization (ESI) mass spectra were obtained with a Micromass AutoSpec Ultima Magnetic sector mass spectrometer at the University of Michigan Mass Spectrometry Laboratory. Low-resolution ESI mass spectra were obtained with a Micromass LCT Time-of-Flight mass spectrometer. Microwave reactions were performed in a Biotage Initiator Microwave Synthesizer.



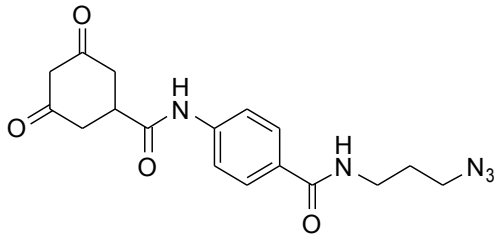
Amino-N-(3-azidopropyl)benzamide (9): A 2-5 mL process vial flushed with argon was charged with a solution of 4-aminobenzoic acid (100 mg, 0.73 mmol), EDC (280 mg, 1.46 mmol) and DMAP (179 mg, 1.46 mmol) in dry DMF (5 mL). Subsequently 3-azidopropylamine (146 mg, 1.46 mmol) and TEA (0.204 mL, 1.46 mmol) were added to the solution. The vial was sealed, placed into the cavity of

the microwave reactor and irradiated at 120 °C for 0.5 h. The DMF was removed *in vacuo* and the resulting oil was extracted with DCM/H_2O (3 x 15 mL). The organic phases were combined, dried over Na_2SO_4, filtered, and concentrated. Flash column chromatography was used for purification (EtOAc/Hexanes 6:4) to yield an oil, 4-amino-N-(3-azidopropyl)benzamide (**9**) (80 mg, 50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (d, J = 8.5 Hz, 2H), 6.80 (s, 1H), 6.56 (d, J = 8 Hz, 2H), 4.14 (s, 2H), 3.431 – 3.383 (m, 2H), 3.30 (t, J = 6.7 Hz, 2H), 1.824 – 1.757 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.7, 149.9, 128.6, 123.0, 49.3, 37.4, 28.8. ESIHRMS calcd. for $C_{10}H_{13}N_5O$ (M + Na) 242.11, found 242.1024.

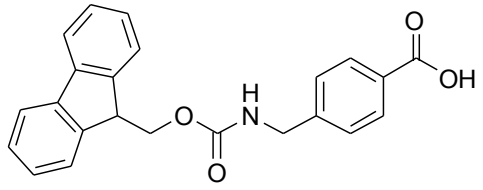


N-(3-azidopropyl)-4-(3-methoxy-5-oxocyclohex-3-enecarboxamido)benzamide (10): A 2-5 mL process vial flushed with argon was charged with 4-amino-N-(3-azidopropyl)benzamide (**9**) (363 mg, 1.66 mmol) and TEA (0.28 mL, 1.99 mmol)in dry DMF (2.5 mL). To this was added a solution of 3-methoxy-5-oxocyclohex-3-enecarboxylic acid (339 mg, 1.99 mmol), EDC (382 mg, 1.99 mmol), and DMAP (243 mg, 1.99 mmol) in dry DMF (2.5 mL). The vial was sealed, placed into the cavity of the microwave reactor and irradiated at 120 °C for 0.5 h. The DMF was removed *in vacuo* and the resulting oil was extracted with DCM/H_2O (3 x 15 mL). The organic phases were combined, dried over Na_2SO_4, filtered, and concentrated. Purification was completed with flash column chromatography (EtOAc/Hexanes 1:1 to EtOAc) to yield N-(3-azidopropyl)-4-(3-methoxy-5-oxocyclohex-3-enecarboxamido)benzamide (**10**) (129 mg, 21%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.95 (s, 1H), 7.68 (d, J = 8.4 Hz, 2H), 7.62 (d, J = 8.3 Hz, 2H), 6.73 (s, 1H), 5.37 (s, 1H), 3.70 (s, 3H), 3.55 – 3.49 (m, 2H), 3.42 (t, J = 6.5 Hz, 2H), 3.14 – 3.10 (m, 1H), 2.95 – 2.88 (m, 2H), 2.69 – 2.52 (m, 2H),

1.92 – 1.86 (m, 2H). ^{13}C NMR (100 MHz, CD_3OD) δ 198.8, 178.9, 168.2, 141.5, 129.4, 127.7, 118.9, 100.8, 55.5, 48.0, 40.8, 38.7, 37.0, 36.9, 30.9, 28.4. ESIHRMS calcd. for $C_{18}H_{21}N_5O_4$ (M + Na) 394.16, found 394.1495.

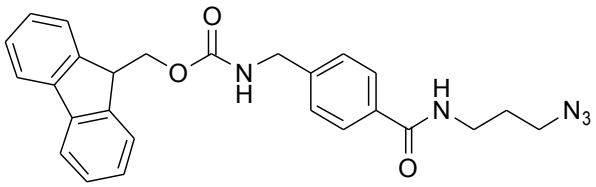


N-(3-azidopropyl)-4-(3,5-dioxocyclohex-3-enecarboxamido)benzamide (3): N-(3-azidopropyl)-4-(3-methoxy-5-oxocyclohex-3-enecarboxamido)benzamide (**10**) (129 mg, .348 mmol) was added to a solution of ACN/H_2O (1:1 v/v, 10 mL) with 10 mol% CAN (19 mg, .0348 mmol) and refluxed at 95 °C for 3 h. The reaction was cooled and concentrated. Flash column chromatography was used for purification (EtOAc to EtOAc/MeOH 9:1) to yield a yellow solid (**3**) (123 mg, 99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.22 (s, 1H), 8.41 (t, J = 5.4 Hz, 1H), 7.81 (d, J = 8.7 Hz, 2H), 7.66 (d, J = 8.7 Hz, 2H), 5.24 (s, 1H), 3.40 (t, J = 6.7 Hz, 2H), 3.32 – 3.28 (m, 2H), 3.19 – 3.10 (m, 1H), 2.57 – 2.52 (m, 2H), 2.50 – 2.42 (m, 2H), 1.80 – 1.73 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.9, 166.1, 141.9, 129.4, 128.4, 118.7, 103.7, 48.9, 39.9, 28.8. ESIHRMS calcd. for $C_{17}H_{19}N_5O_4$ (M + Na) 380.14, found 380.1325.



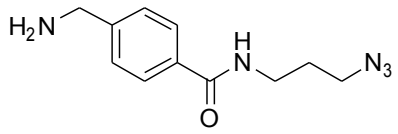
4-(((((9H-fluoren-9-yl)methoxy)carbonyl)amino)methyl)benzoic acid (11): To a solution of $NaHCO_3$ (10% w/v, 10 mL) was added 4-(aminomethyl)benzoic acid (300 mg, 1.99 mmol). FMOC-OSU (805 mg, 2.98 mmol) was solubilized in THF (5 mL) and added drop wise to the reaction with

vigorous stirring. The mixture was stirred at RT overnight. 1 N HCl was added to acidify the solution. The aqueous phase was then extracted with EtOAc (3 x 15 mL), the organic phases were combined, dried over Na_2SO_4, filtered, and concentrated. Purification was carried out by flash column chromatography (EtOAc/Hexanes 6:4 to EtOAc) giving the final product (**11**) (342 mg, 46%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.95 – 7.91 (m, 2H), 7.89 (d, J = 6.9 Hz, 2H), 7.70 (d, J = 7.4 Hz, 2H), 7.42 (t, J = 7.4 Hz, 2H), 7.34 (d, J = 3.5 Hz, 2H) 7.32 (d, J = 4.8 Hz, 2H), 4.39 (d, J = 6.7 Hz, 2H), 4.24 (t, J = 7.7 Hz, 2H), 2.59 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.2, 167.6, 156.8, 145.3, 144.2, 141.2, 129.7, 128.0, 127.4, 125.5, 120.5, 65.7, 47.2, 43.9, 25.6. ESIHRMS calcd. for $C_{23}H_{19}NO_4$ (M + Na) 396.1312, found 396.1212.

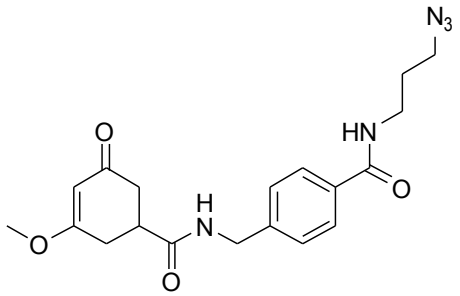


(9H-fluoren-9-yl)methyl 4-((3-azidopropyl)carbamoyl)benzylcarbamate (12): To an oven-dried flask, flushed with argon, was added a solution of 4-(((((9H-fluoren-9-yl)methoxy)carbonyl)amino)methyl)benzoic acid (**11**) (318 mg, .853 mmol) in anhydrous DMF (5 mL). TFA-Pfp was added (0.18 mL, 1.02 mmol) as well as TEA (0.143 mL, 1.02 mmol). The mixture was stirred at RT for 2 h before adding 3-azidopropylamine (103 mg, 1.02 mmol) and TEA (0.14 mL, 1.02 mmol). The reaction mixture was then left stirring overnight at RT. DMF was removed *in vacuo* and the resulting oil was extracted with DCM/H_2O (3 x 15 mL). The organic phases were combined, dried with Na_2SO_4, filtered, and concentrated. Purification was carried out by flash column chromatography (EtOAc/Hexanes 2:8 to 6:4) yielding the product (**12**) (243 mg, 63%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48 (t, J = 5.4 Hz, 1H), 7.89 (d, J = 7.4 Hz, 2H), 7.79 (d, J = 8.1 Hz, 2H), 7.70 (d, J = 7.4 Hz, 2H), 7.42 (t, J = 7.4 Hz, 2H), 7.33 (t, J = 7.4 Hz, 2H), 7.29 (d, J = 8.1 Hz, 2H), 4.39 – 4.35 (m, 2H), 4.24 (t, J =6.1 Hz, 2H), 3.41 (t, J = 6.7 Hz, 2H), 3.33 – 3.30 (m, 2H), 1.82 – 1.74 (m, 2H). ^{13}C

NMR (100 MHz, DMSO-d_6) δ 166.5, 156.8, 144.2, 143.3, 141.1, 133.4, 128.0, 127.6, 127.4, 127.1, 125.5, 120.5, 65.7, 48.9, 47.2, 37.04, 28.8. ESIHRMS calcd. for $C_{26}H_{25}N_5O_3$ (M + Na) 478.20, found 478.1855.

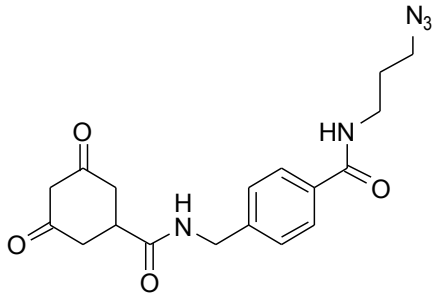


4-(aminomethyl)-N-(3-azidopropyl)benzamide (13): In a round bottom flask, (9H-fluoren-9-yl)methyl 4-((3-azidopropyl)carbamoyl)benzylcarbamate (**12**) (119 mg, 0.261 mmol) was added to a solution of ethanolamine in DCM (1:1 v/v, 5 mL) and stirred for 3 h. The reaction mixture was washed with saturated $NaHCO_3$ and extracted with DCM (3 x 15 mL). The organic phases were combined, dried over Na_2SO_4, filtered, and concentrated. Flash column chromatography was used for purification (EtOAc/Hexanes 8:2 to EtOAc/MeOH 9:1) to yield 4-(aminomethyl)-N-(3-azidopropyl)benzamide (**13**) (40 mg, 66%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, J = 8.0 Hz, 2H), 7.37 (d, J = 8.0 Hz, 2H), 6.50 (s, 1H), 3.92 (s, 2H), 3.56 – 3.52 (m, 2H), 3.46 – 3.42 (m, 2H), 2.12 (s, 2H), 1.92 – 1.87 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.4, 146.4, 132.9, 128.0, 126.9, 49.5, 45.8, 37.7, 28.7. ESIHRMS calcd. for $C_{26}H_{25}N_5O_3$ (M + H) 234.13, found 234.1355.



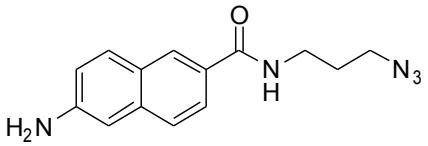
N-(3-azidopropyl)-4-((3-methoxy-5-oxocyclohex-3-enecarboxamido)methyl)benzamide (14): In an oven-dried round bottom flask, EDC (50 mg, .258 mmol) and DMAP (32 mg, .258 mmol) were added to a solution of 3-methoxy-5-oxocyclohex-3-enecarboxylic acid (44 mg, .2575 mmol) in anhydrous

DMF (2.5 mL) under argon**.** To this was added 4-(aminomethyl)-N-(3-azidopropyl)benzamide (**13**) (40 mg, 0.172 mmol) in anhydrous DMF (2.5 mL) and TEA (0.04 mL, 0.258 mmol) and the reaction was stirred overnight at 45 °C. The DMF was removed *in vacuo* and the resulting oil was extracted using DCM/H_2O (3 x 15 mL). The organic phases were combined, dried with Na_2SO_4, filtered, and concentrated. Flash column chromatography was used to purify the product (EtOAc/Hexanes 3:7 to EtOAc/MeOH 9:1) yielding N-(3-azidopropyl)-4-((3-methoxy-5-oxocyclohex-3-enecarboxamido)methyl)benzamide (**14**) (34 mg, 52%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (d, J = 8.3 Hz, 2H), 7.25 (d, J = 7.3 Hz, 2H), 5.35 (s, 1H), 4.46 (m, 2H), 3.70 (s, 3H), 3.56 – 3.51 (m, 2H), 3.44 (t, J = 6.5 Hz, 2H), 2.95 – 2.82 (m, 1H), 2.79 – 2.72 (m, 2H), 2.48 – 2.41 (m, 2H), 1.92 – 1.87 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.6, 177.5, 172.6, 167.8, 141.8, 133.4, 127.2, 101.4, 56.0, 49.3, 42.8, 40.3, 37.6, 31.4, 29.6, 28.65. ESIHRMS calcd. for $C_{19}H_{23}N_5O_4$ (M + Na) 408.18, found 408.1648.



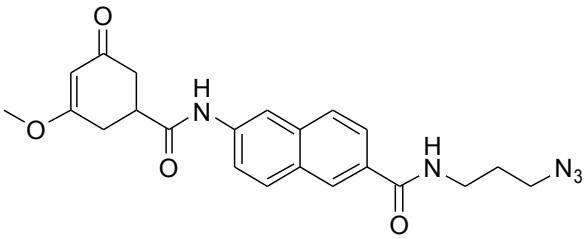
N-(3-azidopropyl)-4-((3,5-dioxocyclohex-3-enecarboxamido)methyl)benzamide (4): In a round bottom flask, N-(3-azidopropyl)-4-((3-methoxy-5-oxocyclohex-3-enecarboxamido)methyl)benzamide (**14**) (100 mg, 0.260 mmol) was added to a solution of ACN/H_2O (1:1 v/v, 5 mL) with 10 mol% CAN (14 mg, 0.026 mmol) and refluxed at 95 °C for 3 h. Solvent was removed *in vacuo.* Flash column chromatography was used to purify the product (EtOAc to EtOAc/MeOH 9:1) yielding (**4**) (89 mg, 92%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.53 (t, J = 5.7 Hz, 1H), 8.48 (t, J = 5.5 Hz, 1H), 7.79 (d, J = 8.2 Hz, 2H), 7.30 (d, J = 8.1 Hz, 2H), 5.20 (s, 1H), 4.32 (d, J = 5.8 Hz, 2H), 3.40 (t, J = 6.7 Hz, 2H), 3.34 – 3.29 (m, 2H), 3.01 – 2.97 (m, 1H), 2.46 (d, J = 10.9 Hz, 2H), 2.38 – 2.32 (m, 2H), 1.82 – 1.72

(m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.7, 166.5, 143.0, 133.4, 127.6, 127.1, 103.7, 48.9, 42.2, 37.0, 28.8. ESIHRMS calcd. for $C_{18}H_{21}N_5O_4$ (M + Na) 394.16, found 394.1491.



6-amino-N-(3-azidopropyl)-2-naphthamide (15):

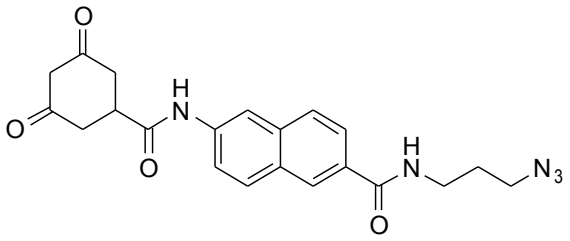
A 2-5 mL process vial flushed with argon was charged with a solution of 6-amino-2-naphthoic acid (50 mg, 0.27 mmol), EDC (58 mg, 0.30 mmol) and DMAP (52 mg, 0.30 mmol) in dry DMF (3 mL). Subsequently 3-azidopropylamine (54 mg, 0.54 mmol) and TEA (0.080 mL, 0.54 mmol) were also added to the solution. The vial was sealed, placed into the cavity of the microwave reactor and irradiated at 120 °C for 0.5 h. The DMF was removed *in vacuo* and the resulting oil was extracted with DCM/H_2O (3 x 15 mL). Flash column chromatography was used for purification (EtOAc/Hexanes 1:1) to yield an orange solid (**15)** (35 mg, 48%). ^{1}H NMR (400MHz, CD_3OD): δ 8.16 (s, 1H), 7.71 (d, *J*=8.8, 1H), 7.68 (d, *J*=9.2, 1H), 7.75 (d, *J*=8.8, 1H), 7.03 (d, *J*=8.4, 1H), 6.96 (d, *J*= 1.6, 1H), 3.47 (t, *J*=6.8, 2H), 3.40 (t, *J*= 6.8, 2H), 1.88 (q, *J*=6.4, 2H) ^{13}C NMR (100MHz, CD_3OD): δ 169.36, 147.78, 137.02, 129.75, 127.42, 126.85, 126.29, 125.31, 123.58, 118.9, 106.93, 48.84, 36.99, 28.50. ESIHRMS calcd. for $C_{11}H_{17}N_3O_2$ (M + Na) 292.1174, found 292.1175.



N-(3-azidopropyl)-6-(3-methoxy-5-oxocyclohex-3-enecarboxamido)-2-naphthamide (16):

A 2-5 mL process vial flushed with argon was charged with a solution of 3-methoxy-5-oxocyclohex-3-

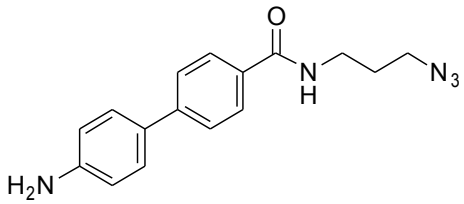
enecarboxylic acid (22 mg, 0.13 mmol), EDC (27 mg, 0.14 mmol) and DMAP (25 mg, 0.14 mmol) in dry DMF (3 mL). 6-amino-N-(3-azidopropyl)-2-naphthamide (**15**) (35 mg, 0.13 mmol) and TEA (0.02 mL, 0.13 mmol) in dry DMF (2 mL) were then added to the solution. The vial was sealed, placed into the cavity of the microwave reactor and irradiated at 120 °C for 0.5 h. The DMF was removed *in vacuo* and the resulting oil was extracted with DCM/H_2O (3 x 15 mL). The organic phases were combined, dried over Na_2SO_4, filtered, and concentrated. Flash column chromatography was used for purification (EtOAc/Hexanes 1:1 to EtOAc) to yield an orange solid (**16**) (33 mg, 62%). ^{1}H NMR (400MHz, DMSO-d_6): δ 8.60 (t, *J*= 4.8, 1H), 8.33 (d, *J*=9.2, 2H), 8.16 (d, *J*=6.8, 1H), 7.93 (d, *J*=8.8, 1H), 7.84 (s, 1H), 7.60 (d, *J*= 7.2, 1H), 6.90 (d, *J*=6.4, 1H), 5.35 (s, 1H), 3.68 (s, 3H), 3.41 (t, *J*=6.8, 2H), 3.18 (t, *J*= 2.4, 2H), 3.13 (s, 2H), 3.12-2.56 (m, 3H), 1.78 (q, *J*=6.8, 2H). ^{13}C NMR (100MHz, DMSO-d_6): δ 196.64, 176.97, 171.89, 167.43, 166.87, 138.42, 135.13, 130.94, 130.00, 129.18, 127.74, 127.56, 125.10, 120.97, 115.31, 56.51, 49.00, 40.58, 37.15, 31.28, 28.88. ESIHRMS calcd. for $C_{22}H_{23}N_5O_4$ (M + Na) 444.1458, found 444.1649.



N-(3-azidopropyl)-6-(3,5-dioxocyclohex-3-enecarboxamido)-2-naphthamide (5):

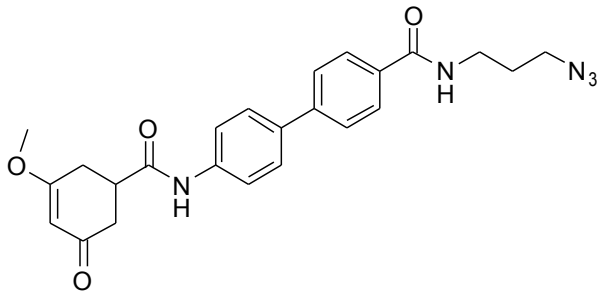
In a round bottom flask, (**16**) (33 mg, 0.08 mmol) was added to a solution of ACN/H_2O (1:1 v/v, 5 mL) with 10% CAN (4.4 mg, 0.008 mmol) and refluxed at 95 °C for 3 h. The reaction was cooled and concentrated. Flash column chromatography was used for purification (EtOAc to EtOAc/MeOH 9:1) to yield an orange solid (**5**) (30 mg, 92%). ^{1}H NMR (400MHz, DMSO-d_6): δ 8.29 (s, 2H), 7.92 (d, *J*=8.8, 1H), 7.84 (s,*2* H),7.63 (d, *J*=8.8, 1H), 5.10 (s, 1H), 3.49 (t, *J*=4.4, 2H), 3.43 (t, *J*= 6.8, 2H), 2.75-2.53 (m, 4H), 1.9 (q, *J*=7.2, 2H), 1.56 (t, *J*= 7.2, 1H). ESIHRMS calcd. for C21H21N5O4 (M + Na)

430.1491, found 430.1489.



4'-amino-N-(3-azidopropyl)-[1,1'-biphenyl]-4-carboxamide (17):

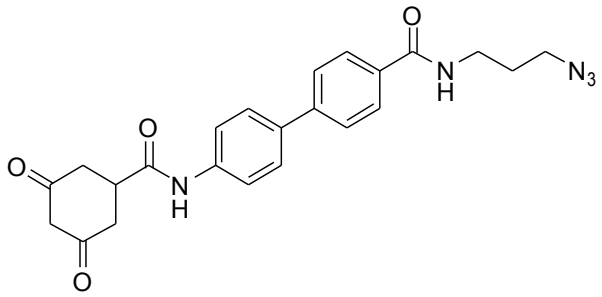
A 2-5 mL process vial flushed with argon was charged with a solution of 4'-amino-[1,1'-biphenyl]-4-carboxylic acid (125 mg, 0.587 mmol), EDC (122 mg, 0.646 mmol) and DMAP (110 mg, 0.30 mmol) in dry DMF (3 mL). Subsequently 3-azidopropylamine (88 mg, 0.88 mmol) and TEA (0.13 mL, 0.88 mmol) were also added to the solution. The vial was sealed, placed into the cavity of the microwave reactor and irradiated at 120 °C for 0.5 h. The DMF was removed *in vacuo* and the resulting oil was extracted with DCM and 10% (v/v) sodium bicarbonate (3 x 15 mL). Flash column chromatography was used for purification (EtOAc/Hexanes 4:6) to yield a white solid (**17**) (39 mg, 23%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.87 – 7.79 (m, 2H), 7.73 (dd, *J* = 5.8, 3.3 Hz, 1H), 7.67 – 7.60 (m, 2H), 7.45 (d, *J* = 8.5 Hz, 2H), 6.80 (d, *J* = 8.5 Hz, 2H), 3.48 (t, *J* = 6.8 Hz, 2H), 3.43 (t, *J* = 6.7 Hz, 2H), 1.90 (dd, *J* = 13.6, 6.9 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.42, 146.56, 144.27, 131.80, 129.96, 128.08, 127.29, 126.22, 115.32, 49.59, 37.75, , 28.81. ESILRMS calcd. for $C_{16}H_{17}N_5O$ (M + H) 296, found 296.



N-(3-azidopropyl)-4'-(3-methoxy-5-oxocyclohex-3-enecarboxamido)-[1,1'-biphenyl]-4-

carboxamide (18):

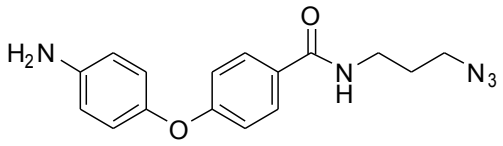
A 2-5 mL process vial flushed with argon was charged with a solution of 3-methoxy-5-oxocyclohex-3-enecarboxylic acid (23.8 mg, 0.14 mmol), EDC (26.8 mg, 0.14 mmol) and DMAP (25 mg, 0.14 mmol) in dry DMF (3 mL). 4'-amino-N-(3-azidopropyl)-[1,1'-biphenyl]-4-carboxamide (**17**) (39 mg, 0.13 mmol) and TEA (0.02 mL, 0.13 mmol) in dry DMF (2 mL) were also added to the solution. The vial was sealed, placed into the cavity of the microwave reactor and irradiated at 120 °C for 0.5 h. The DMF was removed *in vacuo* and the resulting oil was extracted with DCM/H_2O (3 x 15 mL). The organic phases were combined, dried over Na_2SO_4, filtered, and concentrated. Flash column chromatography was used for purification (EtOAc/Hexanes 6:4 to EtOAc) to yield a brown solid (**18**) (12 mg, 21%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, *J* = 8.4 Hz, 2H), 7.61 (d, *J* = 8.4 Hz,6H), 7.58 (s, 1H), 7.55 (d, *J* = 8.2 Hz, 1H), 6.40 (s, 1H), 5.42 (s, 1H), 3.71 (d, *J* = 12.8 Hz, 3H), 3.65 – 3.56 (m, 2H), 3.56 – 3.41 (m, 2H), 3.08 – 2.85 (m, 2H), 2.77 – 2.50 (m, 3H), 1.98 – 1.79 (m, 2H). ESIHRMS calcd. for $C_{24}H_{25}N_5O_4$ (M + Na) 470.1804, found 470.1790.



N-(3-azidopropyl)-4'-(3,5-dioxocyclohex-3-enecarboxamido)-[1,1'-biphenyl]-4-carboxamide (6):

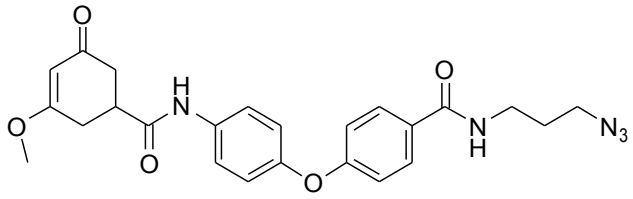
In a round bottom flask, (**18**) (12 mg, 0.027 mmol) was added to a solution of ACN/H_2O (1:1 v/v, 5 mL) with 10% CAN (1.6 mg, 0.003 mmol) and refluxed at 95 °C for 3 h. The reaction was cooled and concentrated. Flash column chromatography was used for purification (EtOAc to EtOAc/MeOH 9:1) to yield a yellow solid (**6**) (10 mg, 85%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.58 (s, 1H), 7.89 (d, *J* = 8.6 Hz, 2H), 7.69 (dt, *J* = 17.3, 8.8 Hz, 6H), 5.39 (s, 1H), 3.49 (d, *J* = 5.6 Hz, 2H), 3.43 (t, *J* = 6.7 Hz, 2H),

2.66 (ddd, *J* = 40.4, 25.6, 22.0 Hz, 3H), 2.40 – 1.97 (m, 2H), 1.95 – 1.85 (m, 2H). ESILRMS calcd. for C23H23N5O4 (M + H) 434, found 434.



4-(4-aminophenoxy)-N-(3-azidopropyl)benzamide (19):

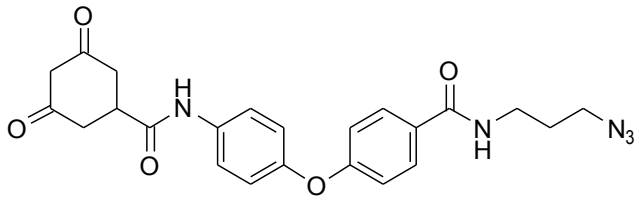
A 2-5 mL process vial flushed with argon was charged with a solution of 4-(4-aminophenoxy) benzoic acid (73.5 mg, 0.32 mmol), EDC (67 mg, 0.35 mmol) and DMAP (60.4 mg, 0.35 mmol) in dry DMF (3 mL). Subsequently 3-azidopropylamine (64 mg, 0.64 mmol) and TEA (0.09 mL, 0.64 mmol) were also added to the solution. The vial was sealed, placed into the cavity of the microwave reactor and irradiated at 120 °C for 0.5 h. The DMF was removed *in vacuo* and the resulting oil was extracted with DCM/H_2O (3 x 15 mL). Flash column chromatography was used for purification (EtOAc/Hexanes 4.5:5.5) to yield a brown solid (**19**) (31 mg, 31%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, *J* = 7.8 Hz, 2H), 6.89 – 6.74 (m, 4H), 6.66 – 6.56 (m, 2H), 3.21 – 3.15 (m, 2H), 3.13 – 3.06 (m, 2H), 1.79 (dt, *J* = 12.8, 6.3 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 128.60, 121.59,116.43,116.24, 49.61, 37.76, 28.78. ESILRMS calcd. for $C_{16}H_{17}N_5O_2$ (M + H) 312, found 312.



N-(3-azidopropyl)-4-(4-(3-methoxy-5-oxocyclohex-3-enecarboxamido)phenoxy)benzamide (20):

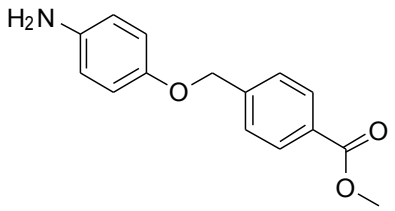
A 2-5 mL process vial flushed with argon was charged with a solution of 3-methoxy-5-oxocyclohex-3-enecarboxylic acid (19 mg, 0.11 mmol), EDC (21 mg, 0.11 mmol) and DMAP (19 mg, 0.11 mmol) in dry DMF (3 mL). Subsequently 4-(4-aminophenoxy)-N-(3-azidopropyl)benzamide (**19**) (31 mg, 0.1

mmol) and TEA (0.01 mL, 0.1 mmol) in dry DMF (2 mL) were also added to the solution. The vial was sealed, placed into the cavity of the microwave reactor and irradiated at 120 °C for 0.5 h. The DMF was removed *in vacuo* and the resulting oil was extracted with DCM/H_2O (3 x 15 mL). The organic phases were combined, dried over Na_2SO_4, filtered, and concentrated. Flash column chromatography was used for purification (EtOAc/Hexanes 1:1 to 8:2) to yield a yellow solid (**20**) (10 mg, 22%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (s, 1H), 7.68 (t, *J* = 10.9 Hz, 2H), 6.90 (dd, *J* = 19.4, 8.4 Hz, 4H), 6.72 – 6.57 (m, 2H), 6.33 (s, 1H), 5.39 (s, 1H), 3.70 (t, *J* = 12.3 Hz, 3H), 3.52 (dt, *J* = 18.2, 9.0 Hz, 2H), 3.45-3.42 (m, 2H), 3.04 (s, 1H), 2.79 – 2.45 (m, 2H), 1.93 – 1.85 (m, 2H), 1.85 – 1.74 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 128.75, 121.76, 120.50, 117.40, 109.99, 101.84, 56.08, 49.57, 49.28, 41.63, 39.62, 37.85, 35.84, 31.34, 28.74. ESILRMS calcd. for C24H25N5O5 (M + H) 464, found 464.



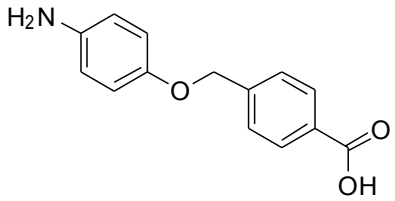
N-(3-azidopropyl)-4-(4-(3,5-dioxocyclohex-3-enecarboxamido)phenoxy)benzamide (7):

N-(3-azidopropyl)-4-(4-(3-methoxy-5-oxocyclohex-3-enecarboxamido)phenoxy)benzamide (**20**) (10 mg, 0.022 mmol) was added to a solution of ACN/H_2O (1:1 v/v, 10 mL) with 10 mol% CAN (1.2 mg, 0.002 mmol) and refluxed at 95 °C for 3 h. The reaction was cooled and concentrated. Flash column chromatography was used for purification (EtOAc to EtOAc/MeOH 9:1) to yield a white solid (**7**) (8 mg, 81%) ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.21 (d, *J* = 6.9 Hz, 2H), 7.51 – 7.42 (m, 2H), 7.12 (dt, *J* = 10.8, 5.4 Hz, 2H), 6.96 (d, *J* = 7.3 Hz, 2H), 5.25 (s, 1H), 3.46 (s, 2H), 3.33 (s, 2H), 3.00 – 2.82 (m, 3H), 1.83 – 1.77 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 128.51, 125.90, 60.20, 49.02, 46.15, 40.50, 40.29, 40.08, 21.19, 14.51, 9.04. ESILRMS calcd. for $C_{23}H_{23}N_5O_5$ (M + H) 450, found 450.



Methyl 4-((4-aminophenoxy)methyl)benzoate (21):

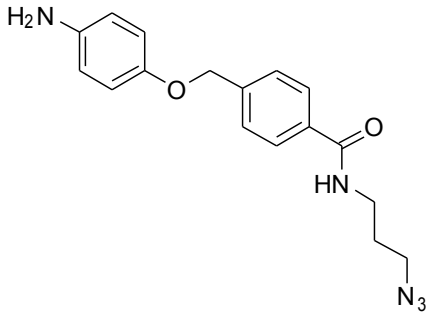
In an oven-dried round bottom flask flushed with argon NaH (60% suspension 60 mg, 1.5mM) was added to 4-aminophenol (163.5 mg, 1.5 mmol) in dry DMF (5 mL) and stirred for 1 h at 0 °C. Methyl 4-(bromomethyl)benzoate (230 mg, 0.13 mmol) in dry DMF (5 mL) was slowly added to the solution. The reaction stirred for 2 h. The DMF was removed *in vacuo* and the resulting oil was extracted with DCM/10% sodium bicarbonate (3 x 15 mL). The organic phases were combined, dried over Na_2SO_4, filtered, and concentrated. Flash column chromatography was used for purification (EtOAc/Hexanes 1:1 to EtOAc) to yield a white solid (**21**) (265 mg, 62%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (t, J = 14.1 Hz, 2H), 7.47 (t, J = 10.1 Hz, 2H), 6.84 – 6.77 (m, 2H), 6.67 – 6.59 (m, 2H), 5.07 (d, J = 19.9 Hz, 2H), 3.91 (s, 3H), 3.25 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.86, 151.58, 142.78, 140.45, 129.77, 129.46, 126.94, 116.32, 116.04, 70.10, 52.08. ESILRMS calcd. for $C_{15}H_{15}NO_3$ (M + H) 258, found 258.



4-((4-aminophenoxy)methyl)benzoic acid (22):

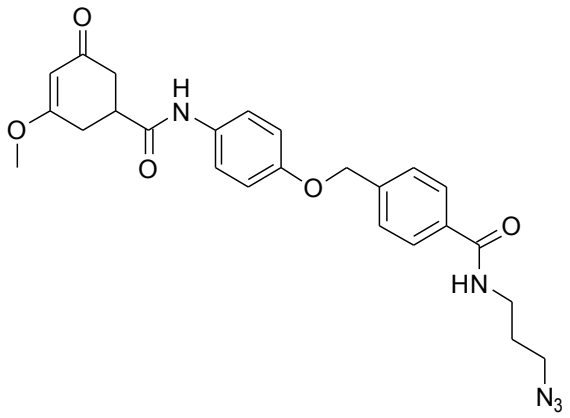
In a round bottom flask at 0 °C, potassium *tert*-butoxide (1.06 g, 11 mM) was stirred in 50 mL of ether for 15 min. H_2O (0.05 mL, 2.76 mmol) was then added to the slurry. After 5 min methyl 4-((4-aminophenoxy)methyl)benzoate (**21**) (265 mg, 0.92 mmol) was added and the reaction was stirred for

48 h. The ether was removed *in vacuo* and flash column chromatography was used for purification (EtOAc/MeOH 9:1) to yield a yellow solid (**22**) (192 mg, 86%). ^{1}H NMR (400 MHz, CD_3OD) 7.97 (t, J = 10.0 Hz, 2H), 7.46 (d, J = 8.4 Hz, 2H), 6.87 – 6.73 (m, 2H), 6.71 (dd, J = 6.6, 2.3 Hz, 2H). ^{13}C NMR (100 MHz, CD_3OD) δ 152.10, 141.74, 140.05, 129.22, 126.53, 116.83, 115.57, 110.12, 69.79. ESILRMS calcd. for $C_{13}H_{13}NO_3$ (M + H) 244, found 244.



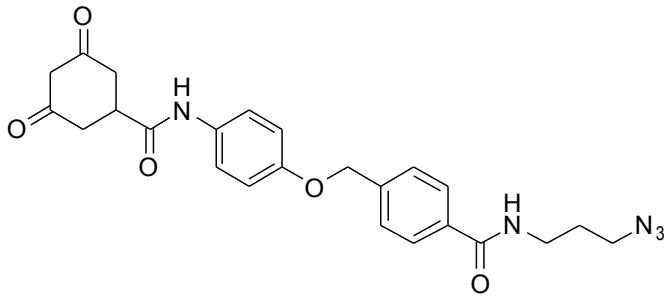
4-((4-aminophenoxy)methyl)-N-(3-azidopropyl)benzamide (23):

A 2-5 mL process vial flushed with argon was charged with a solution of 4-((4-aminophenoxy)methyl)benzoic acid (**22**) (100 mg, 0.41 mmol), EDC (86 mg, 0.45 mmol) and DMAP (60.4 mg, 0.45 mmol) in dry DMF (3 mL). Subsequently 3-azidopropylamine (45 mg, 0.45 mmol) and TEA (0.06 mL, 0.45 mmol) were also added to the solution. The vial was sealed, placed into the cavity of the microwave reactor and irradiated at 120 °C for 0.5 h. The DMF was removed *in vacuo* and the resulting oil was extracted with DCM/10% sodium bicarbonate (3 x 15 mL). The organic phases were combined, dried over Na_2SO_4, and concentrated. Flash column chromatography was used for purification (EtOAc/Hexanes 7:3) to yield a brown solid (**23**) (34 mg, 48**%**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 – 8.13 (m, 2H), 6.81 – 6.72 (m, 2H), 6.66 – 6.58 (m, 2H), 6.54 (dd, J = 5.4, 1.5 Hz, 2H), 5.00 (s, 2H), 3.75 (dd, J = 9.3, 4.5 Hz, 2H), 3.28 (dd, J = 7.0, 4.4, 1.7 Hz, 2H), 1.84 – 1.74 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.22, 140.77, 140.05, 127.25, 126.99, 116.31, 116.07, 70.11, 49.26, 35.85, 34.91, 29.67, 28.67, 14.88. ESILRMS calcd. for $C_{17}H_{19}N_5O_2$ (M + H) 326, found 326.



N-(3-azidopropyl)-4-((4-(3-methoxy-5-oxocyclohex-3-enecarboxamido)phenoxy)methyl)benzamide (24):

A 2-5 mL process vial flushed with argon was charged with a solution of 3-methoxy-5-oxocyclohex-3-enecarboxylic acid (17 mg, 0.10 mmol), EDC (19 mg, 0.1 mmol) and DMAP (17 mg, 0.1 mmol) in dry DMF (3 mL). Subsequently 4-((4-aminophenoxy)methyl)-N-(3-azidopropyl)benzamide (**23**) (34mg, 0.09 mmol) and TEA (0.01 mL, 0.1 mmol) in dry DMF (2 mL) were also added to the solution. The vial was sealed, placed into the cavity of the microwave reactor and irradiated at 120 °C for 0.5 h. The DMF was removed *in vacuo* and the resulting oil was extracted with DCM/H_2O (3 x 15 mL). The organic phases were combined, dried over Na_2SO_4, filtered, and concentrated. Flash column chromatography was used for purification (EtOAc/Hexanes 7:3 to EtOAc) to yield a yellow solid (**24**) (12.8 mg, 30%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 – 7.67 (m, 2H), 7.44 (dd, *J* = 14.9, 8.5 Hz, 2H), 6.98 – 6.90 (m, 2H), 6.85 (d, *J* = 2.2 Hz, 1H), 5.29 – 5.25 (m, 1H), 5.05 (s, 2H), 3.85 (s, 1H), 3.50 (d, *J* = 6.0 Hz, 2H), 3.39 (t, *J* = 6.5 Hz, 2H), 2.73 – 2.44 (m, 3H), 1.89 – 1.82 (m, 2H). ESILRMS calcd. for $C_{25}H_{27}N_5O_5$ (M + Na) 500.1, found 500.2.



N-(3-azidopropyl)-4-((4-(3,5-dioxocyclohexanecarboxamido)phenoxy)methyl)benzamide (8): N-(3-azidopropyl)-4-((4-(3-methoxy-5-oxocyclohex-3-enecarboxamido)phenoxy)methyl)benzamide (**24**) (15 mg, 0.03 mmol) was added to a solution of ACN/H_2O (1:1 v/v, 10 mL) with 10% CAN (1.6 mg, 0.003 mmol) and refluxed at 95 °C for 3 h. The reaction was cooled and concentrated. Flash column chromatography was used for purification (EtOAc to EtOAc/MeOH 9:1) to yield a white solid (**8**) (11.8 mg, 85%) ^{1}H NMR (400 MHz, CD_3OD) δ 7.81 (s, 2H), 7.61 (s, 2H), 7.03 (s, 4H), 5.12 (s, 2H), 3.65 (s, 2H), 3.41 (s, 2H), 2.88 (s, 2H), 2.75 – 2.55 (m, 2H), 1.87 (s, 2H). ESILRMS calcd. for $C_{24}H_{25}N_5O_5$ (M + H) 464, found 464.

General procedure for PTP activity assay. Steady-state phosphatase assays were performed by following the enzymatic turnover of 4-methylumbelliferyl phosphate (4-MUP) by the protein tyrosine phosphatase YopH or PTP1b.[3] Briefly, to 30 μL of YopH or PTP1b (35 nM) in buffer (32 mM HEPES pH 7.2, 5 mM NaCl, 2.5 mM EDTA, 0.83% glycerol, 0.002% Brij-35) was added 5 μL of the azido-probe or DMSO control and incubated for 15 min. The assay was initiated by addition of 20 μL of 4-MUP (500 μM). The fluorescence owing to dephosphorylation of 4-MUP was measured over 15 min at 25 °C using a SpectraMax M5 plate reader (Costar 94-well plate, λ_{ex} = 358 nm, 449 nm emission filter). To evaluate compounds for aggregation-based inhibition, experiments were performed as described above except that 0.02% Triton-X100 was included in the assay buffer.

LC/MS analysis of dimedone-tagged YopH. YopH (3 μM) was incubated with dimedone (10 mM)

and H_2O_2 (30 μM) or DMSO alone in buffer (32 mM HEPES pH 7.2, 5 mM NaCl, 2.5 mM EDTA, 0.83% glycerol, 0.002% Brij-35, 0.02% Triton X-100) with rocking for 6 h at RT. The resulting samples were concentrated and exchanged into 0.1% formic acid using Amicon Ultra Centrifugal Filters (Amicon Ultra, 0.5 mL, 10k MWCO). The concentrated samples were then subjected MS analysis. An Agilent Eclipse XDB-C8 2.1mm x 15mm trap with mobile phases A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile) was used to trap, desalt and elute proteins by a linear gradient 5-90% of mobile phase B over 7 minutes at a flow rate of 200 uL/min. The desalted proteins were eluted directly on to an electrospray linear ion trap mass spectrometer (LTQ XL, Thermo Scientific) to measure protein mass.

Determination of the dissociation constant for inhibitor binding (K_i). In the absence of oxidant, dimedone-based probes function as reversible inhibitors. The compound-dependence of PTP inhibition was fit to a simple model of competitive inhibition (eq 1) to yield apparent inhibitor binding constants (K_i):

$$\frac{v}{[\mathrm{YopH}]} = \frac{k_{cat}[\mathrm{S}]}{(K_m + [\mathrm{S}])(1 + [\mathrm{I}]/K_i)} \qquad (1)$$

where [I] is the inhibitor concentration, [S] is the substrate (4-MUP) in excess, and K_i is the apparent inhibitor binding constant.

Detecting reversible PTP oxidation with azido-probes. YopH sulfenic acid was generated by oxidizing protein (30 μM) with 100 eq H_2O_2 for 1 h at RT in buffer (32 mM HEPES pH 7.2, 5 mM NaCl, 2.5 mM EDTA, 0.83% glycerol, 0.002% Brij-35, 0.02% Triton-X100). Following oxidation,

catalase (100 units) was added for 15 min at RT to remove excess H_2O_2. Sulfenic acid modification of YopH was monitored by incubating protein (3 μM) with azido-probes **2**-**8** (0.5 mM) or DMSO (5% v/v) for 15 min at RT. In some reactions, YopH (30 μM) was pretreated with dimedone (50 mM) or TCEP (0.9 mM) for 0.5 h at RT and then incubated with the azido-probe. In subsequent steps, azide-tagged YopH was conjugated to phosphine-activated biotin (p-biotin; 200 μM) via the Staudinger ligation for 2 h at RT.[4] To detect PTP1B oxidation, the phosphatase was treated with H_2O_2 and probed with **2**, **5**, **6** or DMSO (5% v/v) in buffer (50 mM HEPES pH 7.2, 1 mM EDTA, 0.05% NP-40), as described above.

Probing sulfenic acid modification of GAPDH. Oxidized GAPDH (15 μM) in buffer (32 mM HEPES pH 7.2, 5 mM NaCl, 2.5 mM EDTA, 0.83% glycerol, 0.002% Brij-35, 0.02% Triton-X100) was incubated with DAz-1 (0.5 mM), compound **5** or **6** (0.5 mM) or DMSO (5% v/v) for 0.5 h at 37 °C. Staudinger ligation was carried out by incubation of purified protein with p-biotin (200 μM) for 2 h at 37 °C.

Western blot. Biotinylated proteins were separated by SDS−PAGE using Criterion XT 4−20% Bis-Tris gels (BioRad) and transferred to a polyvinylidene difluoride (PVDF) membrane (BioRad). After transfer, the PVDF membrane was blocked with 3% BSA in phosphate-buffered saline Tween-20 (PBST) for 1 h at RT. The membrane was washed with PBST (2 × 10 min) and then incubated with HRP-streptavidin (1:5,000 to 1:50,000; Pierce). PVDF membrane was washed with PBST (2 × 5 min, 1 × 10 min) and then developed with ECL Plus chemiluminescence (GE Healthcare). GAPDH was probed with anti-GAPDH (1:1,000; Santa-Cruz) and rabbit anti-mouse-HRP (1:35,000; Invitrogen). The quality of protein transfer and loading was ascertained by staining the PVDF membrane with Ponceau S.

Computational method for Autodock calculations: Coordinates for compounds were built with ideal geometry in InsightII (Accelrys, Inc.), including coordinates for the Cβ and Sγ positions of the cysteine adduct. The Cβ position was then overlapped on CYS403 in PDB entry 3blt. Autodock[5] was used to perform a simulated annealing conformation search, keeping the Cβ atom at the crystallographic position and searching through rotational and torsional degrees of freedom. Initial simulations revealed that the cyclic diketone formed close contacts with amino acids surrounding the active site, giving highly unfavorable interaction energies for all conformations. We then modeled induced fit by calculating a smoothed energy function. Smoothing is performed by calculating the energy potential, then scanning through the potential with a moving window, taking the minimum energy within the window at each point. This has the effect of widening the favorable basins in the potential. The default width for this smoothing window in AutoDock is 0.5 Å. In the current study, we increased this to 1.5 Å for atoms in the cyclic diketone, and kept the default values for the variable portions of the compounds.

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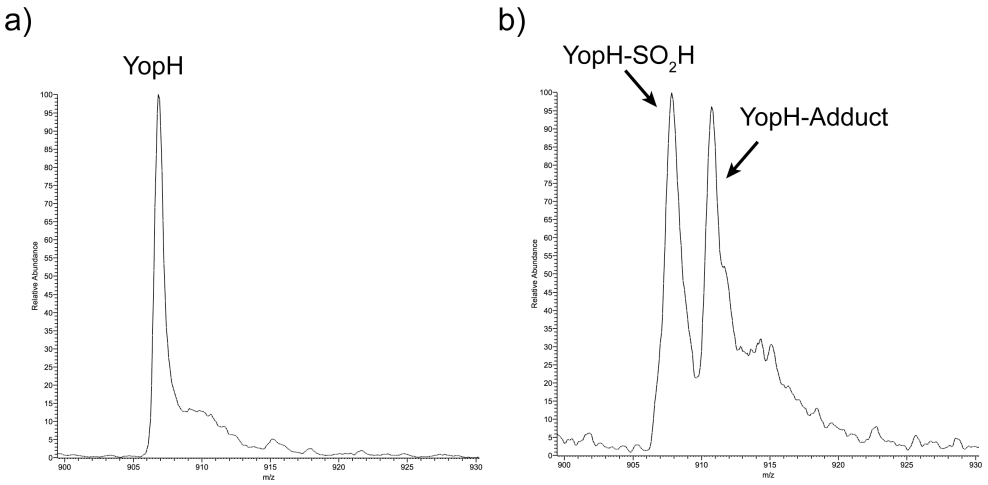

Figure S1. YopH forms a covalent adduct with dimedone with 1:1 stoichiometry. a) ESI mass spectra of active, unmodified YopH (33514.8 Da expected; 33514.6 Da observed). b) ESI mass spectrum of H_2O_2-treated YopH incubated with dimedone (33653.6 Da expected; 33653.2 Da observed). The signal at ~908 *m/z* corresponds to sulfinic acid-modified YopH (33546.8 expected; 33549.7 Da observed).

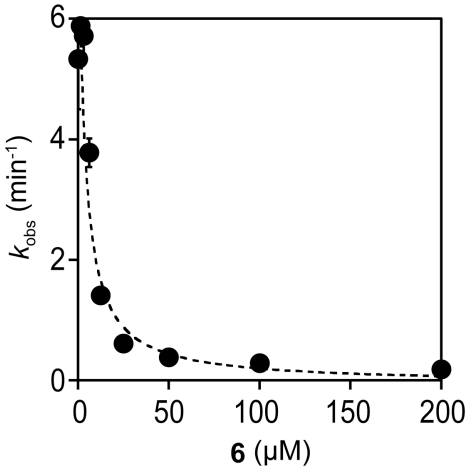

Figure S2. Plot of averaged initial rate versus compound **6** used to determine the averaged K_i for the inhibitor (n=3, error bars show the standard deviation). Dashed line represents fit to a simple model for competitive inhibition $R^2 \geq 98\%$.

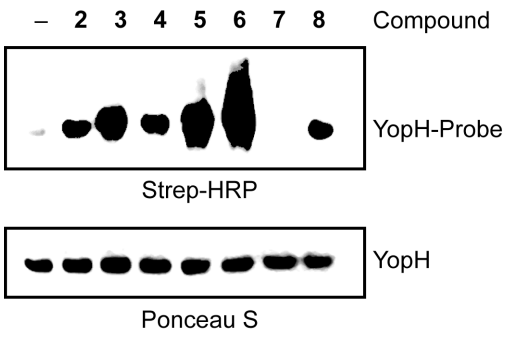

Figure S3. Detecting sulfenic acid modification of YopH. The phosphatase was oxidized with H_2O_2, incubated with compounds **2**-**8** or DMSO alone (–) and analyzed by streptavidin-HRP western blot as described above. Figure S3 represents a longer exposure of the autoradiographic film from Figure 4a in the main text.

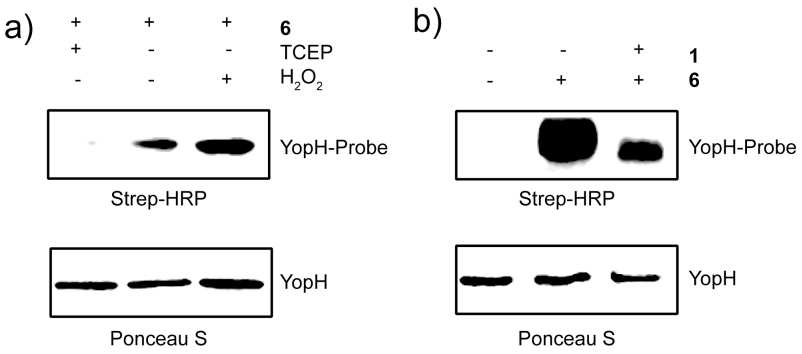

Figure S4. Compound **6** selectively modifies sulfenic acid-modified YopH. a) YopH labeling by compound **6** requires oxidation by H_2O_2. YopH was treated with the reducing agent TCEP, buffer alone, or H_2O_2 and then incubated with compound **6**. Following Staudinger ligation, covalent modification of YopH by **6** was determined by streptavidin-HRP western blot. b) Dimedone pre-treatment blocks YopH modification by compound **6**. YopH was treated with DMSO alone, compound **6**, or pre-treated with dimedone **1** and then incubated with **6**, as described above. Following Staudinger ligation, covalent modification of YopH by **6** was determined by streptavidin-HRP western blot.

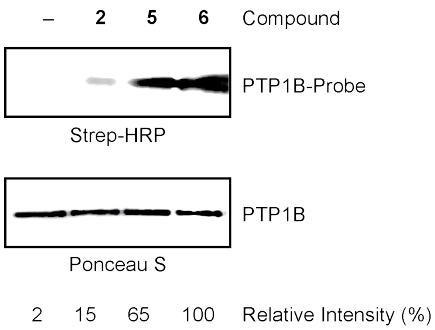

Figure S5. Analysis of RBP selectivity for oxidized PTP1B. a) RBP **5** and **6** detect sulfenic acid in oxidized PTP1B with increased sensitivity over the parent compound DAz-1 **2**. PTP1B was oxidized with H_2O_2 and incubated with compounds **2**, **5**, **6** or DMSO (–) alone. Following p-biotin conjugation, reactions were analyzed by streptavidin-HRP western blot (top). Equal loading was verified by Ponceau S staining (bottom).