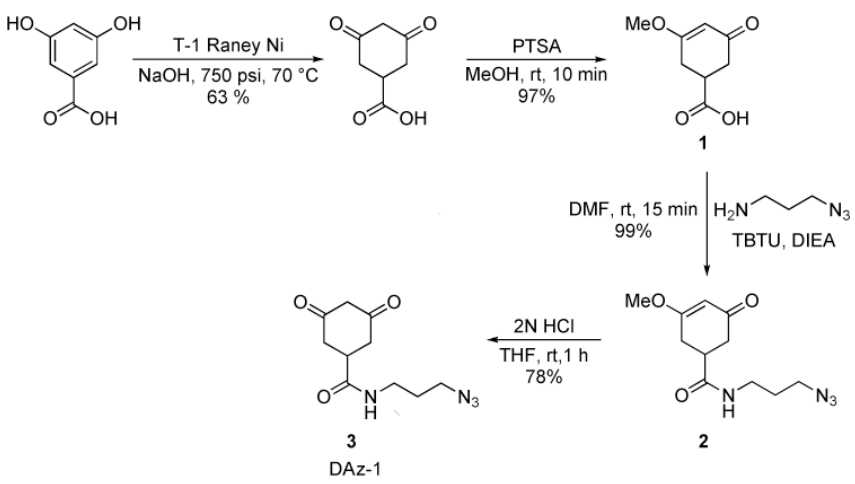
Supporting Information

Chemical Synthesis of DAz-1.

DAz-1 was synthesized as shown in Supporting Information Figure 1. Commercially available 3,5-dihydroxybenzoic acid was reduced to afford 3,5-diketohexahydrobenzoic acid in 63% yield after silica gel flash chromatography as previously described.[1] 3,5-dihydroxybenzoic acid was protected to yield compound (**1**), which was coupled to 3-aminopropylazide[2] to give (**2**) in 99% yield after flash column chromatography. Compound (**2**) was deprotected with hydrochloric acid to give (**3)** 'DAz-1' in 78% yield after reverse phase HPLC purification.



Supporting Information Figure S1. Synthesis of DAz-1, a sulfenic acid-specific chemical probe.

Chemical Methods

Unless otherwise noted, all reactions were performed under an argon atmosphere in oven-dried glassware. Methylene chloride and triethyl amine were distilled over calcium hydride prior to use. Additional reagents and solvents were purchased from Sigma or other commercial sources and were used without further purification. Thin layer chromatography (TLC) was

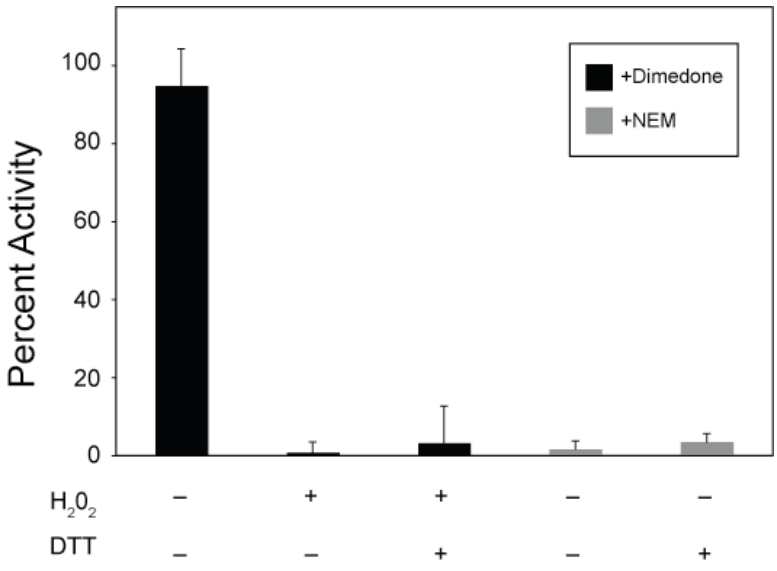
carried out using Analtech Uniplate silica gel plates. TLC plates were visualized using a combination of UV, *p*-anisaldehyde, ceric ammonium molybdate, ninhydrin, and potassium permanganate staining. Flash chromatography was performed using silica gel (32-63 μM, 60 Å pore size) from Sorbent Technologies Incorporated. NMR spectra were obtained on a Varian Inova 400 (400 MHz for ^{1}H; 100 MHz for ^{13}C), or a Varian Mercury 300 (300 MHz for ^{1}H; 75 MHz for ^{13}C NMR) spectrometer. ^{1}H and ^{13}C NMR chemical shifts are reported in parts per million (ppm) relative to TMS, with the residual solvent peak used as an internal reference. Low-resolution electrospray ionization (ESI) mass spectra were obtained with Water-Micromass LCT at the University of Michigan Mass Spectrometry Laboratory. Reversed phase HPLC purification was performed on a Beckman Coulter System Gold 126p equipped with System Gold 166p detector (λ= 220) using a C18 (21.2×150 mm) Beckman Coulter Ultraprep column.

3-Methoxy-5-oxocyclohex-3-enecarboxylic acid (1). To an oven-dried round bottom flask purged with argon was added to 3,5-diketohexahydrobenzoic acid (0.209 g, 1.34 mmol), PTSA (0.0130g, 0.0669 mmol), and 10 mL of methanol. The solution was stirred at RT for 10 min. The reaction mixture was concentrated *in vacuo* and purified by silica gel chromatography, eluting with 1:1 ethyl acetate: methanol to provide the title compound (0.221 g, 1.30 mmol) in 97% yield as a white solid. R_f: 0.55 (1:1 ethyl acetate: methanol). ^{1}H NMR (CD_3OD, 400 MHz): δ 5.36 (s, 1H), 3.72 (s, 3H), 3.04-2.94 (m, 1H), 2.70-2.66 (m, 2H), 2.54 (d, J = 10.0, 2H). ^{13}C NMR (CD_3OD, 100 MHz): δ 201.19, 180.63, 129.93, 102.55, 56.93, 40.62, 40.15, 32.64. ESI-LRMS calcd. for $C_8H_{10}NaO_4$ ($M+Na^+$) 193.0, found 193.0.

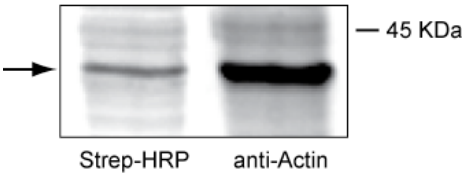
***N*-(3-Azidopropyl)-3-methoxy-5-oxocyclohex-3-enecarboxamide (2).** To a solution of (**1**)

(0.0290 g, 0.171 mmol) in 2 mL of DMF was added TBTU (0.0660 g, 0.205 mmol) followed by diisopropylethylamine (0.0360 mL, 0.205 mmol). To the stirring solution was added a solution of 3-aminopropylazide (0.0190 g, 0.188 mmol) in 1 mL of DMF. The reaction mixture was stirred at RT for 15 min. The solution was concentrated *in vacuo* and purified by silica gel chromatography, eluting with ethyl acetate to provide the title compound (0.425 g, 0.168 mmol) in 99% yield. R_f: 0.38 (ethyl acetate). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.13 (s, 1H), 5.37 (s, 1H), 3.71 (s, 3H), 3.36 (q, J = 6.3 Hz, 4H), 2.89-2.79 (m, 2H), 2.62-2.45 (m, 2H), 1.86-1.75 (m, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 197.27, 177.30, 172.20, 101.80, 56.03, 49.43, 40.72, 39.83, 37.43, 31.43, 28.60. ESI-LRMS calcd. for $C_{11}H_{16}N_4NaO_3$ ($M+Na^+$) 275.1, found 275.2.

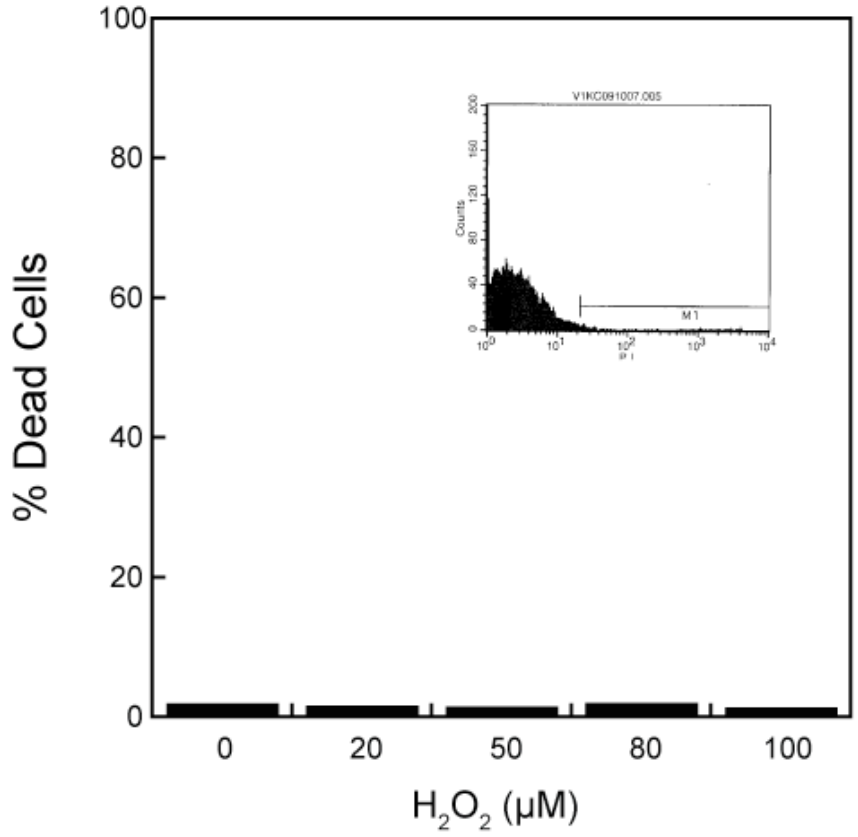
***N*-(3-Azidopropyl)-3,5-dioxocyclohexanecarboxamide (3).** To a solution of (**2**) (0.0360 g, 0.143 mmol) in 5 mL of THF was added 2 mL of 2N HCl. The solution was stirred at RT for 1 h. To the solution was added 0.3 g of silica gel and concentrated *in vacuo*. The residue powder was subjected to flash chromatography, eluting with 3:1 – 1:1 ethyl acetate:methanol to provide the title compound (0.0270 g, 0.112 mmol) in 78% yield as a white solid. The compound was further purified by C18 reversed phase HPLC (10 m, 21.2×150 mm, Beckman coulter) with a gradient of 0% to 30 % B in 30 min (buffer A: 0.1% TFA in water; buffer B: 0.1% TFA in acetonitrile) at a flow rate of 15 mL/min. R_f: 0.14 (3:1 ethyl acetate: methanol). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.99 (t, J = 5.1 Hz, 1H), 5.19 (s, 1H), 3.34 (t, J = 6.6 Hz, 2H), 3.11 (q, J = 6.3 Hz, 2H), 2.91-2.80 (m, 1H), 2.43 (dd, J = 16.8 Hz, 11.1 Hz, 2H), 2.29 (dd, J = 16.8 Hz, 4.5 Hz, 2H), 1.69-1.60 (m, 2H). ^{13}C NMR (D_2O, 100 MHz): δ 191.11, 175.07, 48.55, 39.52, 36.64, 34.02, 27.44. ESI-LRMS calcd. for $C_{10}H_{14}N_4NaO_3$ ($M+Na^+$) 261.1, found 261.0.



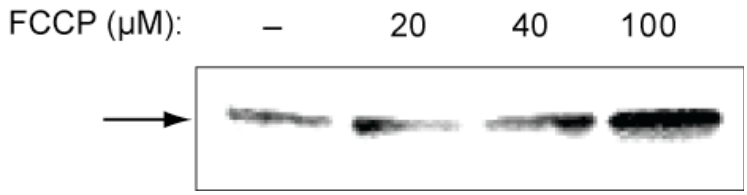
Supporting Information Figure S2. Covalent papain adducts are not reduced by DTT. Black bars: Active papain was not inhibited by 10 mM dimedone alone. Papain was inactivated by the addition of stoichiometric H_2O_2. When oxidized papain was reacted with dimedone, protease activity was not recovered by DTT treatment. Light grey bars: Active papain was inhibited by treatment with the thiol-reactive probe, NEM. The activity of NEM-modified papain was not restored by DTT.



Supporting Information Figure S3. β-actin is labeled *in vivo* by DAz-1. β-actin antibody probe shows a DAz-1 labeled biotinlyated band at ~41 kDa in the Strep-HRP blot to be actin (arrow). A streptavidin-HRP blot (left lane) from live cell labeling of Jurkats with DAz-1 (10 mM) was reprobed with anti-β-actin antibody (right lane). In brief, the blot was incubated in 15% H_2O_2 for 1 h at rt to deactivate the Strep-HRP reagent, blocked in 5% non fat dry milk, then reprobed with anti-β actin for 1 h at rt (1:10,000). After secondary antibody incubation for 1 h (rabbit anti-mouse HRP, 1:40:000), the blot was washed and developed with chemiluminescence reagents.



Supporting Information Figure S4. Jurkat cells remain viable after H_2O_2 challenge. Jurkat cell viability after 10 min H_2O_2 challenge as assessed by propidium iodide (PI) and flow cytometry. Cells were incubated in 2% FBS RPMI with H_2O_2 for 10 min at rt, washed with PBS, incubated in 250 μL PI (3 μM) for 15 min at rt then analyzed for uptake of the fluorescent PI compound. Cells showed no difference in viability compared to the control (no uptake of PI, and hence fluorescent signal is equivalent to control). Inset shows typical histogram obtained for Jurkat cells on viability analysis.



Supporting Information Figure S5. Peroxiredoxin thiols are oxidized to sulfinic and sulfonic acids after FCCP challenge. Western blot obtained from FCCP challenge of live Jurkat cells was probed with anti-peroxiredoxin-SO3 antibody (1:2000) for 1 h at rt, incubated with secondary (1:200,000) for 1 h, then developed with chemiluminescence reagents. Blot shows increased oxidation of peroxiredoxin thiols with increased concentration of FCCP oxidant.

References

1. E. E. van Tamelen and G. T. Hildahl, *J. Org. Chem.*, 1956, 78, 4405-4412.

2. N. S. Hatzakis, H. Engelkamp, K. Velonia, J. Hofkens, P. C. Christianen, A. Svendsen, S. A. Patkar, J. Vind, J. C. Maan, A. E. Rowan and R. J. Nolte, *Chem. Commun. (Camb)*, 2006, 2012-2014.