Electronic Supplementary Material (ESI) for ChemComm.

Exploring Hydrogen Peroxide Responsive Thiazolidinone-Based Prodrugs

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Supplementary Information

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General: All chemicals were purchased from commercial suppliers (Sigma–Aldrich, Acros Organics, TCI America) and were used without further purification. Chromatography was performed using a CombiFlashRf 200 automated system from TeledyneISCO (Lincoln, NE USA). NMR spectra were recorded on a Varian FT 400 NMR instrument. Mass spectrometry was performed at the Molecular Mass Spectrometry Facility (MMSF) in the Department of Chemistry & Biochemistry at the University of California, San Diego.

Abbreviations

DCC: *N,N′*-Dicyclohexylcarbodiimide

DMAP: 4-Dimethylaminopyridine

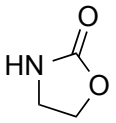
HOBT: N-Hydroxybenzotriazole

HATU: 2-(1H-7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate

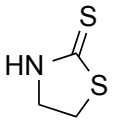
EDC: 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide

Synthesis

Synthesis of compounds **B**, **D**, **1** and **4**-**8** had been previously reported.[1-7]

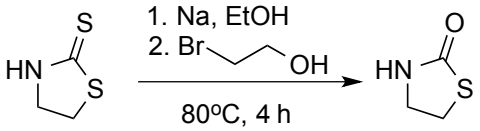


A



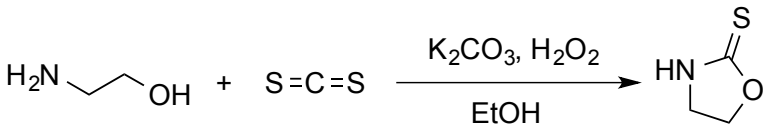
D

Commercially available **Oxazolidin-2-one (A)** and **thiazolidine-2-thione (D)**.



B

Synthesis of Thiazolidin-2-one (B). To a solution of anhydrous EtOH (10 mL) in a dry vessel was added sodium (0.25 g, 11 mmol). The mixture was kept under N_2 atmosphere and stirred at RT for ~30 mins. To this was added thiazolidine-2-thione **(D)** (1.2 g, 10 mmol) and 2-bromoethanol (1.3 g, 10 mmol). The mixture was heated to reflux for 4 h, allowed to cool to room temperature, then filtered to remove insoluble white solids, which were rinsed with anhydrous EtOH (3x10 mL). The filtrate was concentrated, then purified via silica gel chromatography eluting with hexanes and EtOAc to afford **B** in 56% yield (0.64 g, 6.2 mmol). ^{1}H NMR (400 MHz, Acetone-d_6) δ 6.91 (br s, 1H), 3.62 (t, J = 7.2 Hz, 2H), 3.44(t, J = 7.2 Hz, 2H). ESI-MS(+): *m/z* 104.0 $[M+H]^+$, 126.0 $[M+Na]^+$.

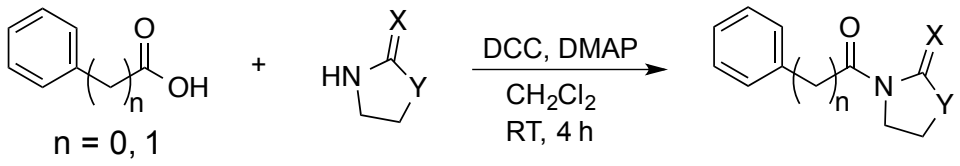


C

Synthesis of Oxazolidine-2-thione (C). To a solution of 2-aminoethanol (6.1 g, 100 mmol) in EtOH (250 mL) was added K_2CO_3 (6.9 g, 50 mmol) and carbon disulfide (15.2 g, 200 mmol). The mixture was heated to 40 °C, and H_2O_2 (30% w/w) (15.3 mL, 150 mmol) was added over 1 h. The reaction was cooled to room temperature and stirred for an additional 4 h then sat. NH_4Cl (aq, 10 mL) was added, and the solution was extracted with EtOAc (3x150 mL). The organic phases were combined, dried with $MgSO_4$, concentrated, and purified via silica gel chromatography, eluting hexanes and EtOAc to afford **C** in 78% yield (4.0 g, 39 mmol). ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.63 (br s, 1H), 4.66 (t, J = 8.7 Hz, 2H), 3.81 (t, J = 8.7 Hz, 2H). ESI-MS(+): *m/z* 104.0 $[M+H]^+$, 126.0 $[M+Na]^+$.

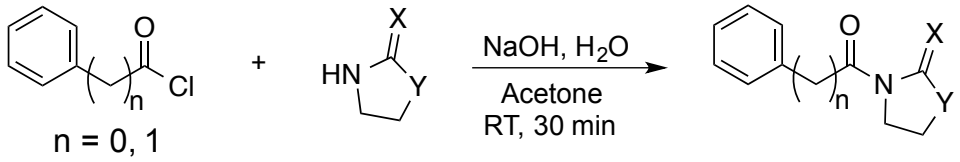
General Procedure for Synthesis of Model Compounds **1**-**8**:

Protocol for the amide coupling (Method i):

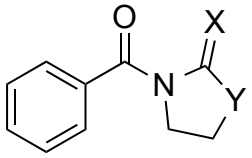


To a solution of carboxylic acid (1 mmol) in anhydrous CH_2Cl_2 (10 mL), was added DCC (1.1 mmol) and DMAP (1.1 mmol). The mixture was stirred at room temperature for 20 min followed by the addition of the corresponding amine (**A**-**D)** (1 mmol) and stirred for an additional 4 h. The resulting solution was concentrated, then purified via silica gel chromatography eluting with a gradient of 0-30% EtOAc in hexanes.

Protocol for Schotten-Baumann reaction (Method ii)



To a solution of **A**-**D** (12 mmol) in H_2O (5 mL) was added NaOH (15 mmol), followed by acetone (45 mL), then the corresponding acyl chloride (15 mmol). The mixture was stirred for 30 min at room temperature. Acetone was removed from solution under reduced pressure and the remaining aqueous solution was further diluted with H_2O (20 mL) then extracted with EtOAc (3x20 mL). The organic phases were combined and dried with $MgSO_4$, concentrated, then purified via silica gel chromatography eluting with a gradient of 0-30% EtOAc in hexanes.



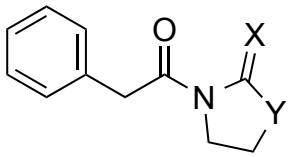
1: X=Y=O
2: X=O, Y=S
3: X=S, Y=O
4: X=Y=S

3-Benzoyloxazolidin-2-one (1). Yield via Method i: 72% (0.14 g, 0.72 mmol). ^{1}H NMR (400 MHz, Acetone-d_6) δ 7.64-7.66 (m, 2H), 7.52-7.56 (m, 1H) 7.40-7.45 (m, 2H) 4.55 (t, J = 7.7 Hz, 2H) 4.18 (t, J = 7.7 Hz, 2H). ESI-MS(+): *m/z* 192.2 $[M+H]^+$, 214.1 $[M+Na]^+$.

3-Benzoylthiazolidin-2-one (2). Yield via Method i: 50% (0.10 g, 0.50 mmol. ^{1}H NMR (400 MHz, Acetone-d_6) δ 7.62-7.65 (m, 2H), 7.52-7.56 (m, 1H), 7.41-7.45 (m, 2H), 4.25 (t, J = 7.0 Hz, 2H), 3.55 (t, J = 7.0 Hz, 2H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 172.4, 169.5, 134.8, 132.0, 129.1, 128.0, 48.7, 25.9. ESI-MS(+): *m/z* 230.0 $[M+Na]^+$.

Phenyl(2-thioxooxazolidin-3-yl)methanone (3). Yield via Method i: 72% (0.15 g, 0.72 mmol). ^{1}H NMR (400 MHz, Acetone-d_6) δ 7.71-7.73 (m, 2H), 7.54-7.58 (m, 1H), 7.42-7.46 (m, 2H), 4.77 (t, J = 7.0 Hz, 2H), 4.36 (t, J = 7.0 Hz, 2H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 187.7, 171.1, 134.5, 132.3, 129.5, 128.1, 68.2, 48.4. ESI-MS(+): *m/z* 230.0 $[M+Na]^+$.

Phenyl(2-thioxothiazolidin-3-yl)methanone (4). Yield via Method i: 86% (0.19 g, 0.86 mmol). ^{1}H-NMR (400 MHz, Acetone-d_6) δ 7.73-7.76 (m, 2H), 7.55-7.59 (m, 1H), 7.43-7.47 (m, 2H), 4.58 (t, J = 7.2 Hz, 2H), 3.68 (t, J = 7.2 Hz, 2H). ESI-MS(+): *m/z* 224.1 $[M+H]^+$, 246.0 $[M+Na]^+$.



5: X=Y=O
6: X=O, Y=S
7: X=S, Y=O
8: X=Y=S

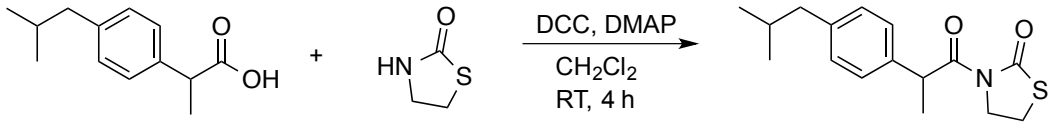
3-(2-Phenylacetyl)oxazolidin-2-one (5). Yield via Method ii: 82% (2.5 g, 12.3 mmol). ^{1}H NMR (400 MHz, Acetone-d_6) δ 7.24-7.31 (m, 5H), 4.47 (t, J = 7.8 Hz, 2H) 4.34 (s, 2H), 4.03 (t, J = 7.8 Hz, 2H). ESI-MS(+): *m/z* 206.0 $[M+H]^+$, 226.0 $[M+Na]^+$.

3-(2-Phenylacetyl)thiazolidin-2-one (6). Yield via Method i: 41% (0.09 g, 0.41 mmol). ^{1}H-NMR (400 MHz, Acetone-d_6) δ 7.24-7.32 (m, 5H), 4.17-4.20 (m, 4H), 3.42 (t, J = 7.4 Hz, 2H). ESI-MS(+): *m/z* 222.1 $[M+H]^+$, 244.0 $[M+Na]^+$.

2-Phenyl-1-(2-thioxooxazolidin-3-yl)ethan-1-one (7). Yield via Method i: 63% (0.14 g, 0.63 mmol). ^{1}H NMR (400 MHz, Acetone-d_6) δ 7.25-7.32 (m, 5H), 4.70 (s, 2H), 4.64 (t, J = 7.6 Hz, 2H), 4.29 (t, J = 7.6 Hz, 2H). ESI-MS(+): *m/z* 244.0 $[M+Na]^+$.

2-Phenyl-1-(2-thioxothiazolidin-3-yl)ethan-1-one (8). Yield via Method i: 73% (0.17 g, 0.73 mmol). ^{1}H NMR (400 MHz, Acetone-d_6) δ 7.25-7.33 (m, 5H), 4.63 (s, 2H), 4.61 (t, J = 7.6 Hz, 2H), 3.44 (t, J = 7.6 Hz, 2H). ESI-MS(+): *m/z* 260.0 $[M+Na]^+$.

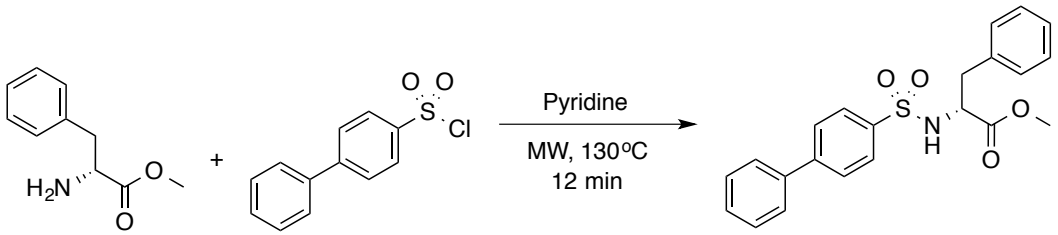
Synthesis of proIBU



proIBU

3-(2-(4-Isobutylphenyl)propanoyl)thiazolidin-2-one (proIBU). Yield via Method i: 70% (0.2 g, 0.7 mmol). ^{1}H NMR (400 MHz, Acetone-d_6) δ 7.19 (d, J = 8.1 Hz, 2H), 7.10 (d, J = 8.1 Hz, 2H), 4.93 (q, J = 7.0 Hz, 1H), 4.15-4.20 (m, 2H), 3.28-3.37 (m, 2H), 2.44 (d, J = 7.2 Hz, 2H), 1.84 (sep J = 6.7 Hz, 1H), 1.30 (d, J = 7.0 Hz, 3H), 0.88 (d, J = 6.7 Hz, 6H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 174.0, 172.2, 140.1, 138.5, 129.1, 127.8, 47.8, 44.7, 43.7, 30.1, 24.6, 21.8, 19.21. ESI-MS(+): *m/z* 292.2 $[M+H]^+$, 314.2 $[M+Na]^+$. HRMS calcd for $C_{16}H_{21}NO_2SNa$: 314.1185; Found: 314.1192.

Synthesis of proMMPi



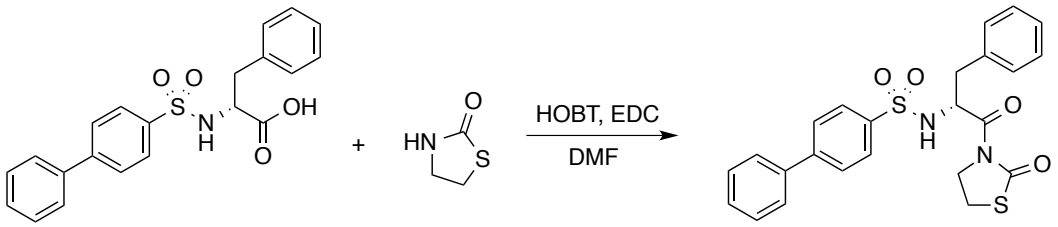
9

Methyl([1,1'-biphenyl]-4-ylsulfonyl)-*D*-phenylalaninate (9). A solution of D-phenylalanine methyl ester hydrochloride (0.5 g, 2.3 mmol) and [1,1'-biphenyl]-4-sulfonyl chloride (1.76 g, 6.9 mmol) in pyridine (11 g, 139 mmol) was irradiated in a microwave reactor (CEM Discover) for 12 min (130 °C, 250 max psi). The resulting solution was concentrated in vacuo. The crude oil was purified via silica gel chromatography eluting a gradient of 0-50% EtOAc in hexanes. Yield = 0.85 g (93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, J = 8 Hz, 2H), 7.63 (d, J = 8 Hz, 2H), 7.59 (d, J = 8.0 Hz, 2H), 7.49-7.09 (m, 8H), 5.45 (d, J = 9.2 Hz, 1H), 4.25 (dd, J = 8 Hz, 6 Hz, 1H), 3.49 (s, 3H), 3.06-3.02 (m, 2H).

9 **10**

([1,1'-biphenyl]-4-ylsulfonyl)-*D*-phenylalanine (10). To a solution of **9** (0.85 g, 2.16 mmol) in THF (30 mL) was added 2M NaOH (25 mL), and the mixture heated to 55 °C for 4 h. THF was removed in vacuo. The aqueous layer was acidified to pH 2-3 using 6M HCl, which caused the formation of an off-white precipitate. The precipitate was

filtered to afford the carboxylic acid product. Yield = 0.81 g (99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, J = 8.4 Hz, 2H), 7.58-7.40 (m, 7H), 7.21-7.08 (m, 5H), 5.28 (d, J = 9.2 Hz, 1H), 4.24 (m, 1H), 3.13 (dd, J = 13.6 Hz, 4.8 Hz, 1H), 2.97 (dd, J = 14 Hz, 7.2 Hz, 1H). ESI-MS(-): *m/z* 380.26 $[M-H]^-$



10 **B** **proMMPi**

(*R*)-*N*-(1-oxo-1-(2-oxothiazolidin-3-yl)-3-phenylpropan-2-yl)-[1,1'-biphenyl]-4-sulfonamide (proMMPi). To a solution of **10** (0.25 g, 0.65 mmol) in DMF (10 mL) was added HOBT (0.15 g, 0.98 mmol) and EDC (0.18 g, 0.98 mmol), and the reaction was stirred under N_2 atmosphere at room temperature for 15 min. To this was then added **B** (0.34 g, 3.3 mmol) and the mixture was allowed to stir overnight. Solvent was removed via rotary evaporation, and the resulting crude oil was purified via silica gel chromatography eluting 0-80% EtOAc in hexanes. Yield = 0.06 g (19%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, J = 8.4 Hz, 2H), 7.59-7.42 (m, 7H), 7.18-7.12 (m, 5H), 5.50 (d, J = 10.4 Hz, 1H), 5.37 (m, 1H), 3.95 (m, 2H), 3.15 (t, J = 7.2 Hz ,2H), 3.07 (dd, J = 13.6 Hz, 4.8 Hz, 1H), 2.77 (dd, J = 8.8 Hz, 4.8 Hz, 1H). ESI-MS(+): *m/z* 467.09 $[M+H]^+$, 489.19 $[M+Na]^+$. HRMS calcd for $C_{24}H_{22}N_2O_4S_2Na$: 489.0913; Found: 489.0906.

HPLC Analysis

Analytical HPLC was performed on a HP Series 1050 System equipped with an Agilent Poroshell 120 reverse-phase column (EC-C18, 4.6x100mm, 2.7µm). Separation was achieved with a flow rate of 1 mL min^{-1} and the following mobile phase: 2.5% ACN + 0.1% formic acid in H_2O (A) and 0.1% formic acid in ACN (B). Starting with 95% A and 5% B, a linear gradient was run for 15 min to a final solvent mixture of 5% A and 95% B, which was held for 5 min before ramping back down to 95% A and 5% B over the course of 2 min, with constant holding at this level for 4 additional min. Injections consisted of 100 µL.

Activation of Compounds by ROS

Activation of compounds **1**, **3**-**5, 7**-**8** and proIBU was performed at a concentration of 1 mM in a 40% DMSO/60% Buffer (100 mM Tris-Cl, pH 7.4) solution by adding H_2O_2 (20 equiv, 20 mM) and incubating at 37 °C. For compounds **2** and **6,** activation was performed at a concentration of 1 mM in a 5% DMSO/95% Buffer (100 mM Tris-Cl, pH 7.4) solution by adding H_2O_2 (20 equiv, 20 mM) and incubating at 37 °C. ProMMPi activation was determined in a 100 µM solution in 40% DMSO/60% Buffer (100 mM Tris-Cl, pH 7.4) by adding H_2O_2 (20 equiv, 2 mM) or NaClO (20 equiv, 2 mM) and incubating at 37 °C. HPLC traces were obtained at different time points. Conversion to corresponding acid was determined by integration of area under the curve.

Stability of Model Compounds

Aqueous stability of compounds was determined by making a 1 mM stock solution in 40% DMSO/60% Buffer (100 mM Tris-Cl, pH 7.4) and incubating at 37 °C for 24 h. Stability in the presence of nucleophiles was determined by making a 1 mM stock solution in 40% DMSO/60% Buffer (100 mM Tris-Cl, pH 7.4) and adding the corresponding nucleophile (20 equiv, 20 mM) and incubating at 37 °C for 24 h.

Enzyme Inhibition Assays

MMP Inhibition Assays

Inhibition values of proMMPi were determined using a commercially available fluorescent-based assay kit. MMP-2 and OmniMMP fluorogenic subsbtrate were purchased from Enzo Life Sciences (Farmingdale, NY). MMP activity was measured in 96-well plates using a Bio-Tek Synergy HT fluorescent plate reader. ProMMPi was dissolved in DMSO to a concentration of 100 mM and further diluted with Tris-Cl buffer (100 mM, pH 7.4) to a concentration of 1 mM (40% DMSO/ 60% Buffer). To the sample was added H_2O_2 (50 equiv, 50 mM), followed by incubation at 37 °C until full deprotection was observed via analytical HPLC. The treated compound was then added to appropriate wells near its IC_{50} value (350 nM). Each well contained 20 µL of MMP-2 (1.16 U), 60 µL MMP assay buffer (50 mM HEPES, 10mM $CaCl_2$, 0.10% Brij-35, pH 7.5), and the H_2O_2-treated MMPi (10 µL). After a 30 min incubation at 37 °C, a reaction was initiated with the addition of 10 µL (40 µM) of the fluorescent substrate (Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2) where Mca = (7-methoxycoumarin-4-yl)-acetyl and Dpa = *N*-3-(2,4-dinitrophenyl)-L-α-β-diaminopropionyl)) and activity was monitored every 30 sec for 30 min with excitation and emission wavelengths at 320 nm and 400

nm, respectively. Enzymatic activity and thus inhibition was calculated with respect to the control experiment (no inhibitor present). Measurements were performed in triplicate with three independent experiments.

COX-1 Inhibition Assays

Inhibition values of proIBU was determined using a commercially available fluorescent-based assay kit *Cayman Chemical – Cox Fluorescent Inhibitor Screening Assay Kit Item No. 700100*. COX-1 activity was measured in 96-well plates using a Bio-Tek Synergy HT fluorescent plate reader. ProIBU was dissolved in DMSO to a concentration of 100 mM and further diluted in Tris-Cl buffer (100 mM, pH 8.0) to a concentration of 1 mM (40% DMSO/60% Buffer). To the sample was added H_2O_2 (50 equiv), and incubation at 37 °C was allowed until full deprotection was observed via analytical HPLC. The treated compound was then added to appropriate wells near its IC_{50} value (2μM). Protocol was carried out as instructed by supplier.

Crystallographic Data

A concentrated solution of either compound **2** or **3** was in prepared in a 1:1 (by volume) mixture of CH_2Cl_2 and EtOAc. The solution was transferred to a vial, layered with hexanes, and sealed. The solution was allowed to stand at room temperature for several days, yielding X-ray quality crystals of the desired compound.

A single crystal of **2** or **3** taken from a mixture of CH_2Cl_2:EtOAc:hexanes was mounted on nylon loops with paratone oil and placed under a nitrogen cold stream. Data was collected on a Bruker Apex diffractometer using Mo Kα (λ= 0.71073 Å)

radiation controlled using the APEX 2010 software package. The data was collected up to 0.83 Å. A multi-scan method utilizing equivalents was employed to correct for absorption. All data collections were solved and refined using the SHELXTL software suite. Details for these structures can be obtained from the Cambridge Crystallographic Data Centre (CCDC) under deposition numbers 1038268-1038269.

Cytotoxicity Assay

NIH 3T3 cell line was kindly donated by Dr. Richard Klemke (Department of Pathology and Moores Cancer Center, UCSD) and DMEM medium supplemented with 10% fetal bovine serum (FBS, Gibco, Grand Island, NY, USA) at 37 °C in an incubator with 5% CO_2. The CellTiter 96 AQ_{ueous} One Solution Cell Proliferation assay (MTS) kit was purchased from Promega (Madison, WI, USA). Cytotoxicity of thiazolidinone was measured using the MTS assay according to the manufacturer's protocol. To start the assay, NIH3T3 cells were counted with a hemocytometer and diluted with fresh medium to the proper concentration, such that 5000 cells per well were seeded in a 96 well plate. The NIH 3T3 cells were then incubated at 37 °C with 5% CO_2 for 16 h. Following this 16 h incubation, the cells were treated with various concentrations of thiazolidinone (ranging from 0.25 μM to 170 μM) for 60 h. Each concentration was conducted in triplicate in three independent experiments. After the 60 h incubation, 20 μL of CellTiter 96 AQ_{ueous} One Solution was added to each well and the cells were incubated at 37 °C for 2 h. Following this 2 h incubation, the absorbance at 490 nm was recorded using a BioTek Synergy HT microplate reader.

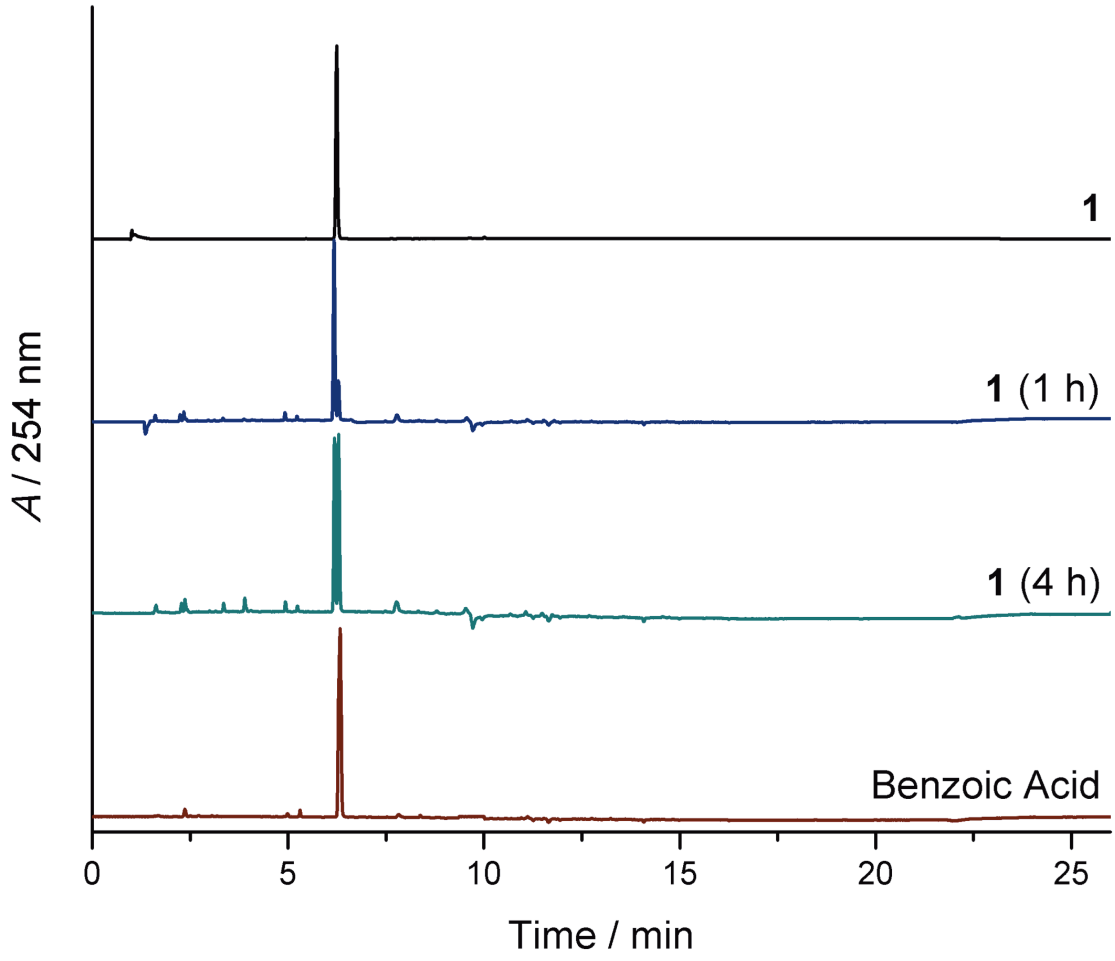


Figure S1.

HPLC trace of an authentic sample of **1** (RT = 6.1 min, black), **1** (1 mM, 40% DMSO/ 60% 100 mM Tris-Cl, pH 7.4 buffer) in the presence of H_2O_2 (20 mM, 37 °C), at 1 h (blue), and at 4 h (green). The expected product, benzoic acid, is also shown for comparison (RT = 6.2 min, brown).

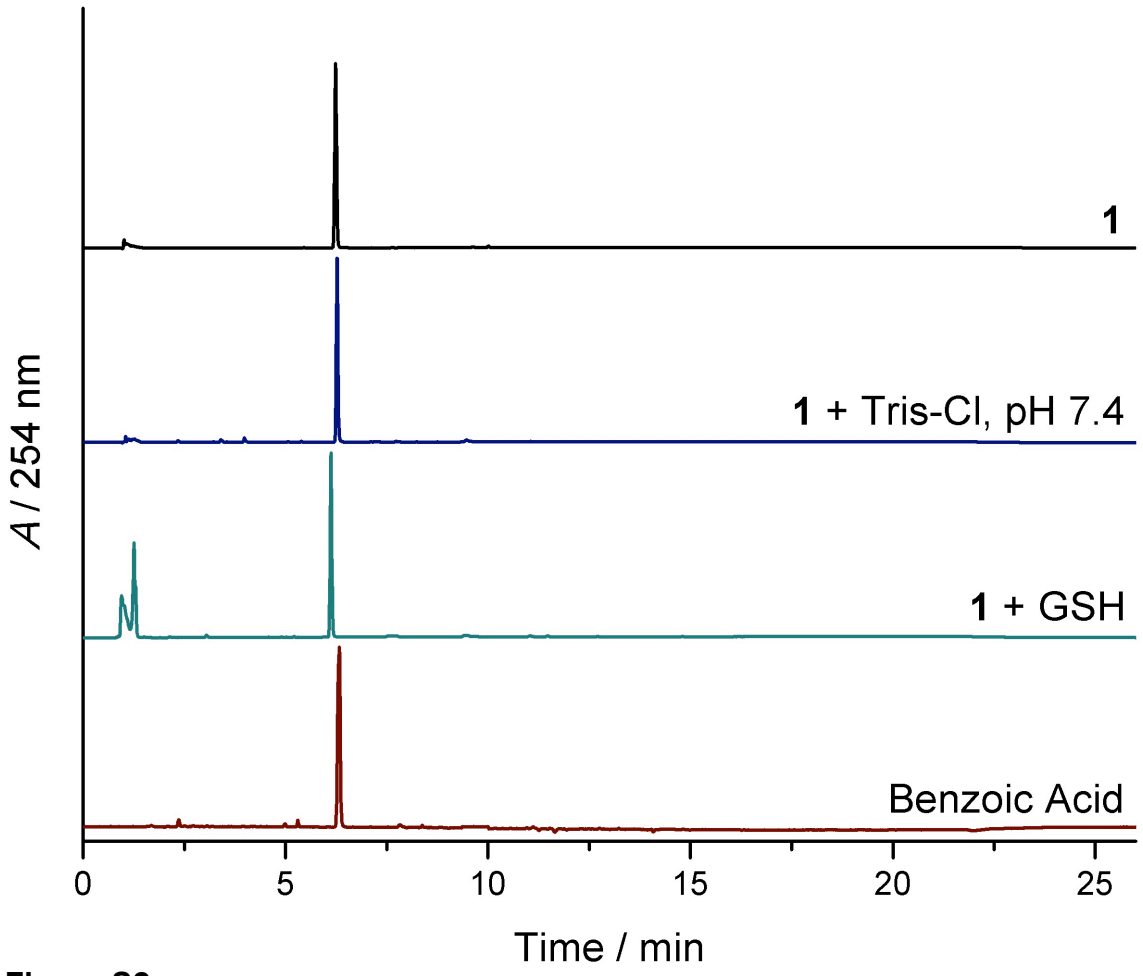


Figure S2.

HPLC trace of an authentic sample of **1** (RT = 6.1 min, black), **1** (1 mM, 40% DMSO/ 60% 100 mM Tris-Cl, pH 7.4 buffer) after 24 h (37 °C) incubation (blue), and **1** under the same conditions, with the addition of glutathione (20 mM, green). The expected product, benzoic acid, is also shown for comparison (RT = 6.2 min, brown).

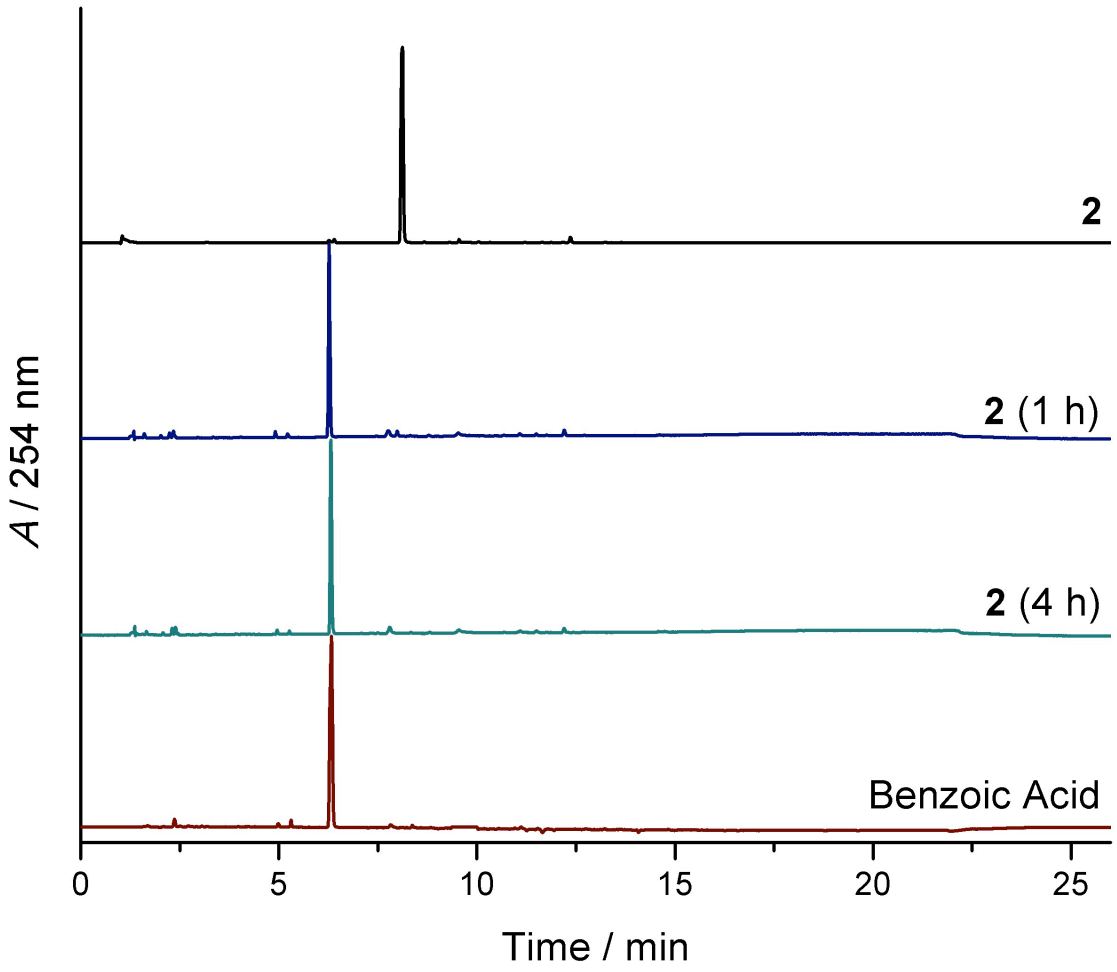


Figure S3

HPLC trace of an authentic sample of **2** (RT = 8.1 min, black), **2** (1 mM, 5% DMSO/ 95% 100 mM Tris-Cl, pH 7.4 buffer) in the presence of H_2O_2 (20 mM, 37 °C) at 1 h (blue) and at 4 h (green). The expected product, benzoic acid, is also shown for comparison (RT = 6.2 min, brown).

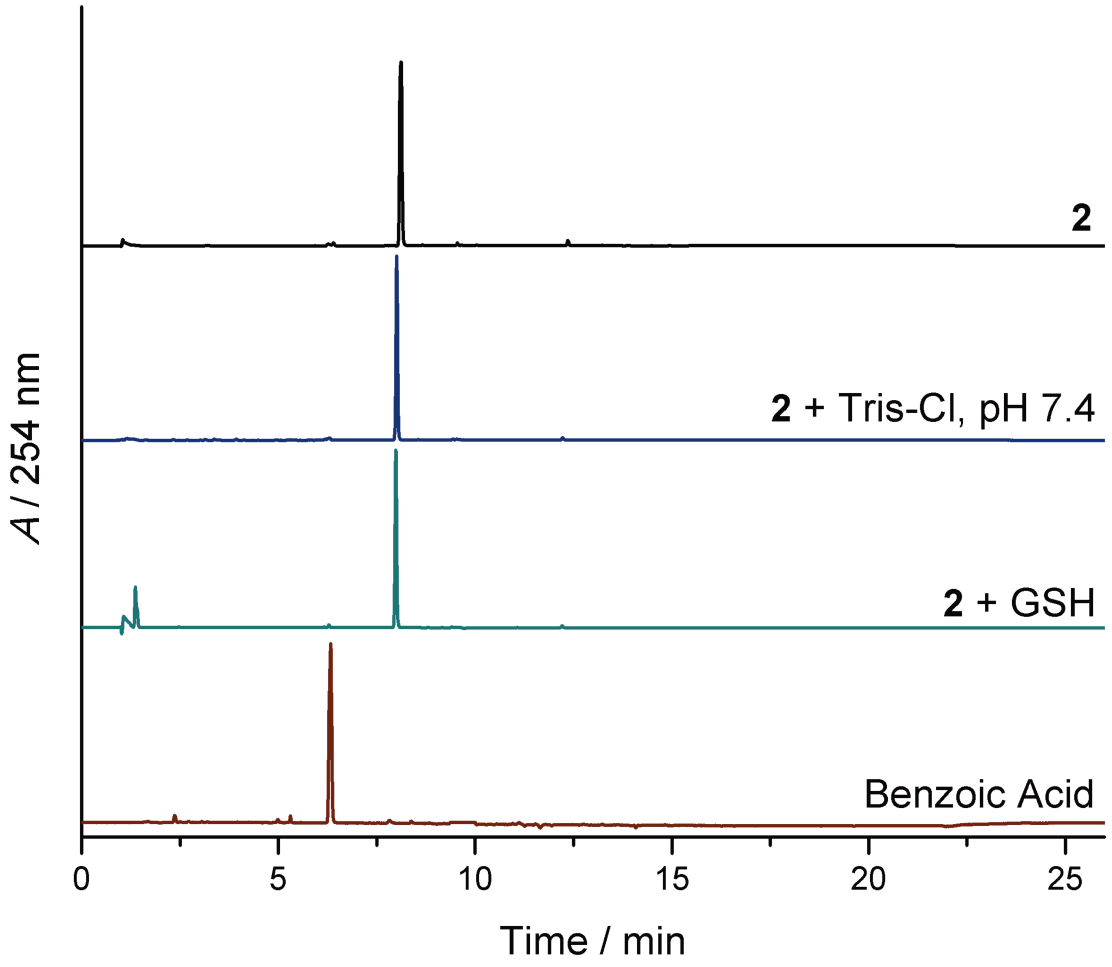


Figure S4

HPLC trace of an authentic sample of **2** (RT = 8.1 min, black), **2** (1 mM, 40% DMSO/ 60% 100 mM Tris-Cl, pH 7.4 buffer) after 24 h (37 °C) incubation (blue), and **2** under the same conditions, with the addition of glutathione (20 mM, green). The expected product, benzoic acid, is also shown for comparison (RT = 6.2 min, brown).

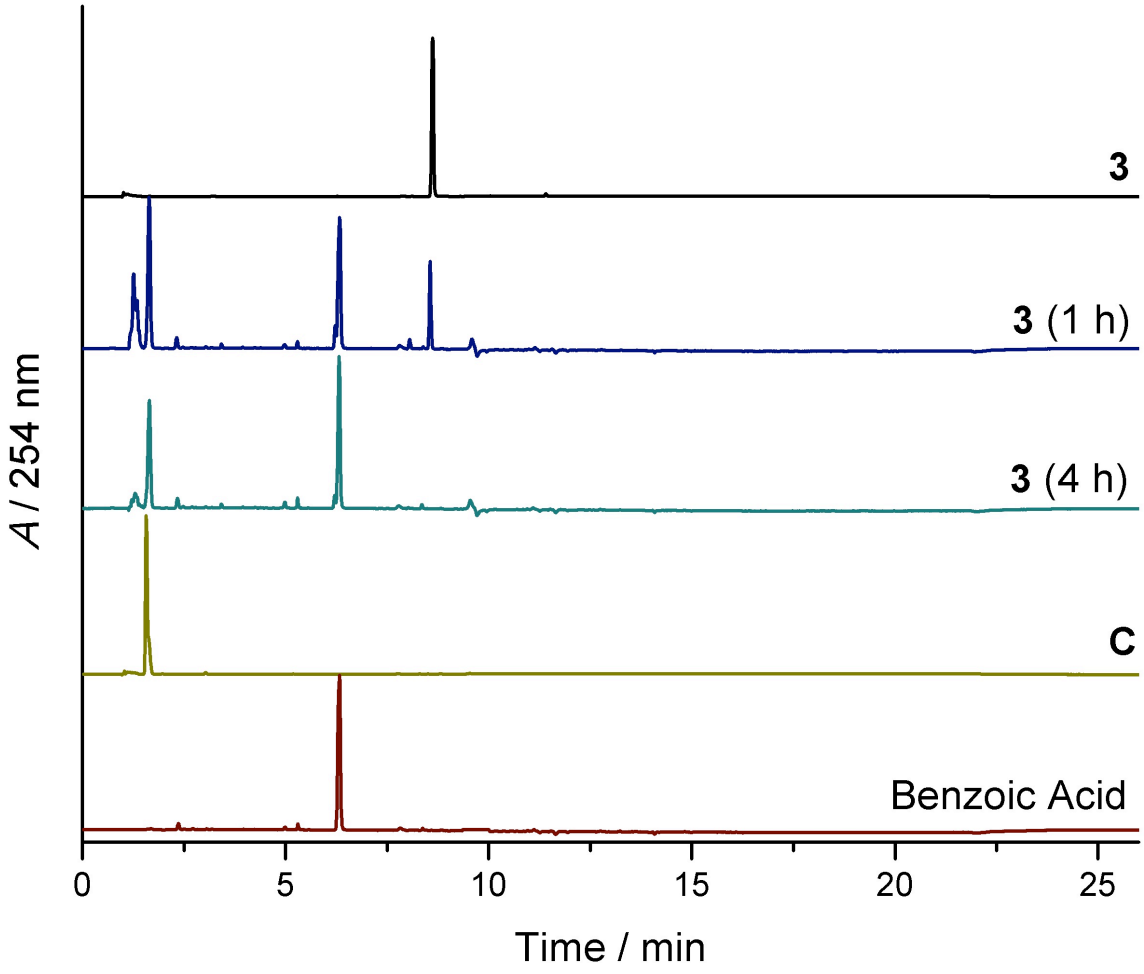


Figure S5

HPLC trace of an authentic sample of **3** (RT = 8.4 min, black), **3** (1 mM, 40% DMSO/ 60% 100 mM Tris-Cl, pH 7.4 buffer) in the presence of H_2O_2 (20 mM, 37 °C) at 1 h (blue) and at 4 h (green). The expected products, **C** (RT = 1.6 min, yellow) and benzoic acid are also shown for comparison (RT = 6.2 min, brown).

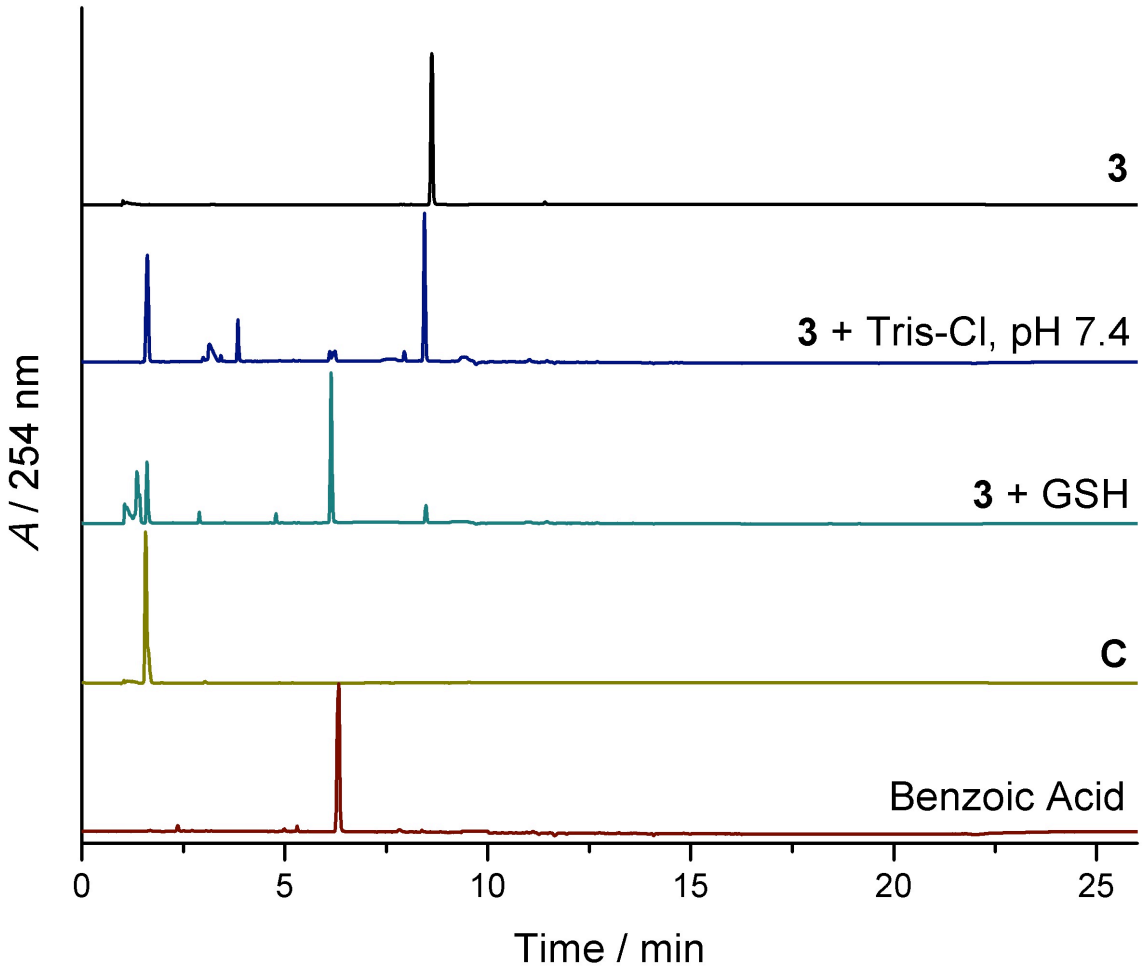


Figure S6

HPLC trace of an authentic sample of **3** (RT = 8.4 min, black), **3** (1 mM, 40% DMSO/ 60% 100 mM Tris-Cl, pH 7.4 buffer) after 24 h (37 °C) incubation (blue) and **3** under the same conditions, with the addition of glutathione (20 mM, green). The expected products, **C** (RT = 1.6 min, yellow) and benzoic acid are also shown for comparison (RT = 6.2 min, brown).

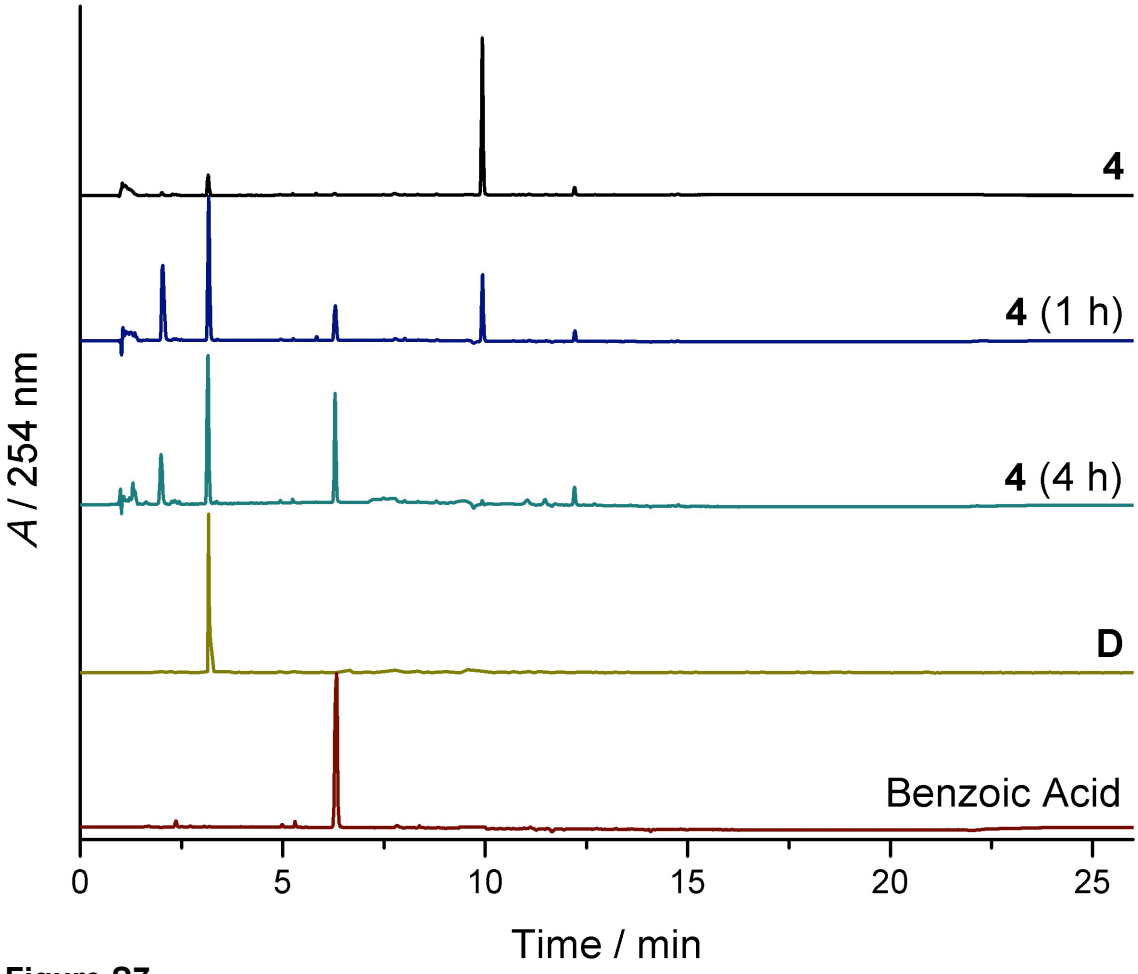


Figure S7

HPLC trace of an authentic sample of **4** (RT = 9.9 min, black), **4** (1 mM, 40% DMSO/ 60% 100 mM Tris-Cl, pH 7.4 buffer) in the presence of H_2O_2 (20 mM, 37 °C) at 1 h (blue) and at 4 h (green). The expected products, **D** (RT = 3.1 min, yellow) and benzoic acid are also shown for comparison (RT = 6.2 min, brown).

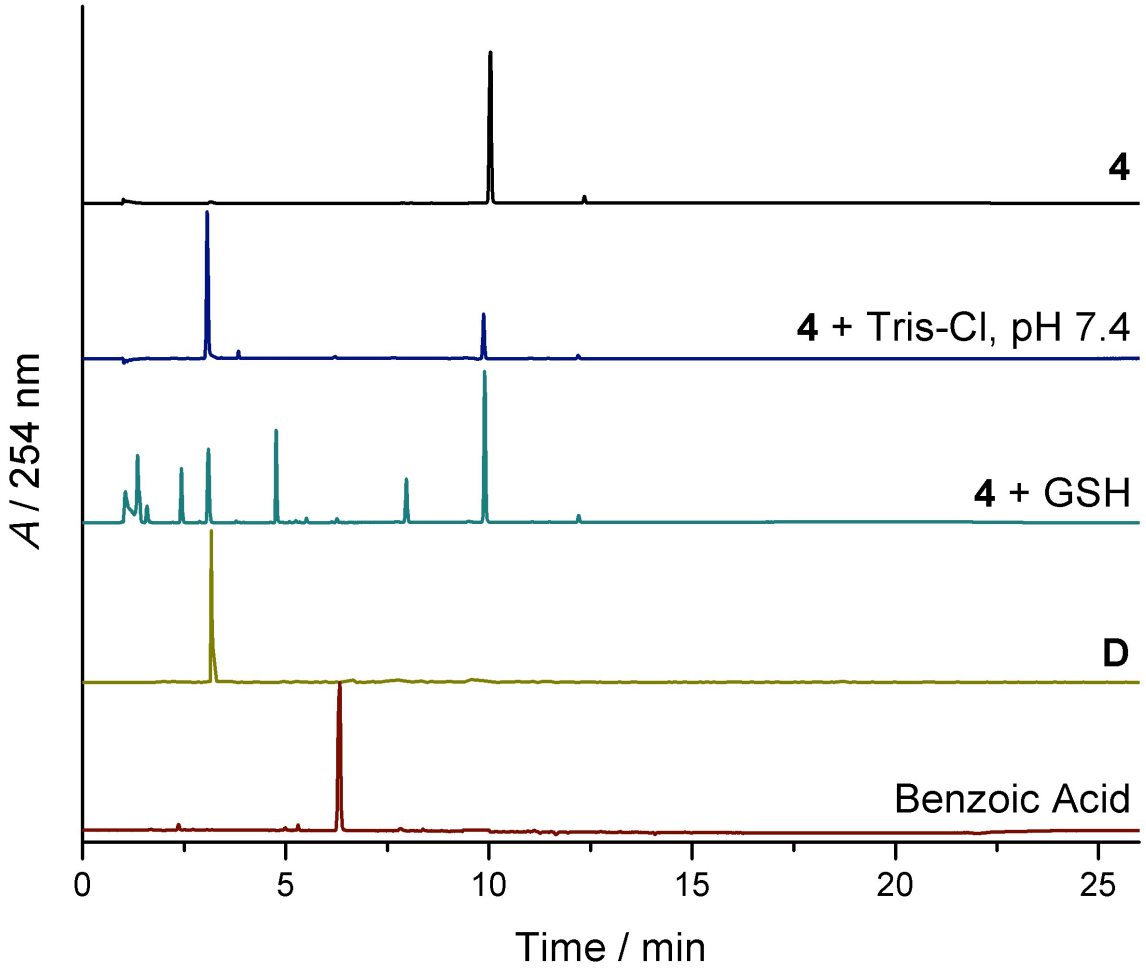


Figure S8

HPLC trace of an authentic sample of **4** (RT = 9.9 min, black), **4** (1 mM, 40% DMSO/ 60% 100 mM Tris-Cl, pH 7.4 buffer) after 24 h (37 °C) incubation (blue), and **4** under the same conditions, with the addition of glutathione (20 mM, green)The expected products, **D** (RT = 3.1 min, yellow) and benzoic acid are also shown for comparison (RT = 6.2 min, brown).

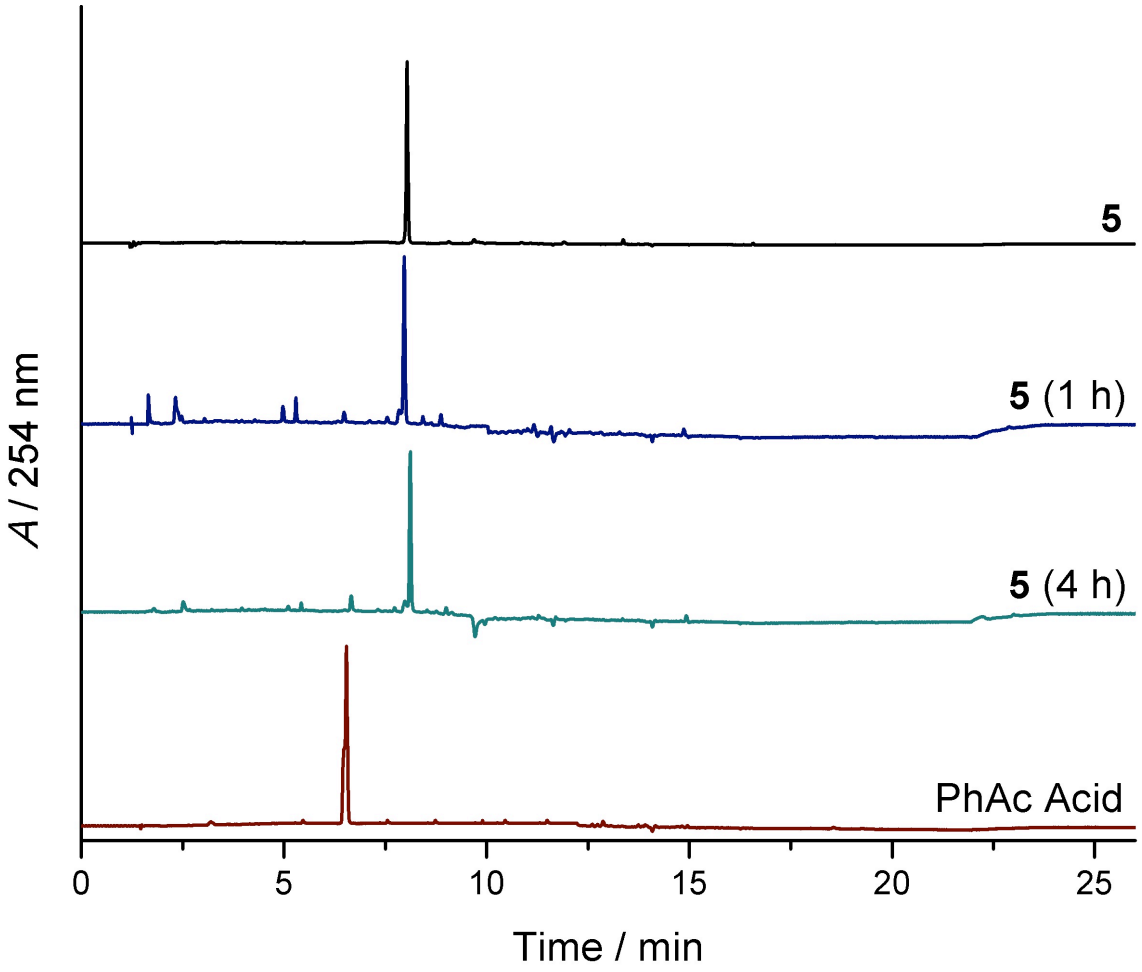


Figure S9

HPLC trace of an authentic sample of **5** (RT = 7.9 min, black), **5** (1 mM, 40% DMSO/ 60% 100 mM Tris-Cl, pH 7.4 buffer) in the presence of H_2O_2 (20 mM, 37 °C) at 1 h (blue) and at 4 h (green). The expected product, phenylacetic acid is also shown for comparison (RT = 6.4 min, brown).

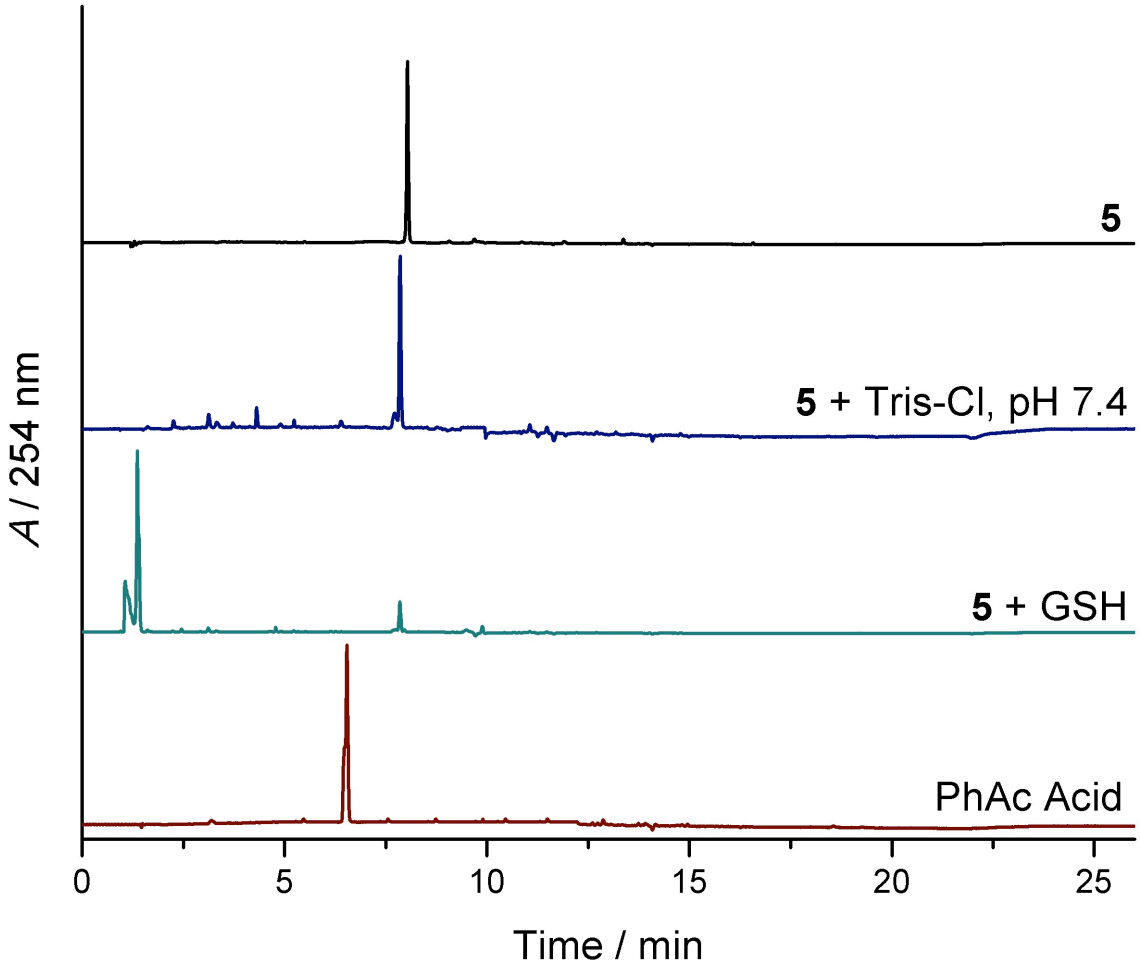


Figure S10

HPLC trace of an authentic sample of **5** (RT = 7.9 min, black), **5** (1 mM, 40% DMSO/ 60% 100 mM Tris-Cl, pH 7.4 buffer) after 24 h (37 °C) incubation (blue), and **5** under the same conditions, with the addition of glutathione (20 mM, green). The expected product, phenylacetic acid is also shown for comparison (RT = 6.4 min, brown).

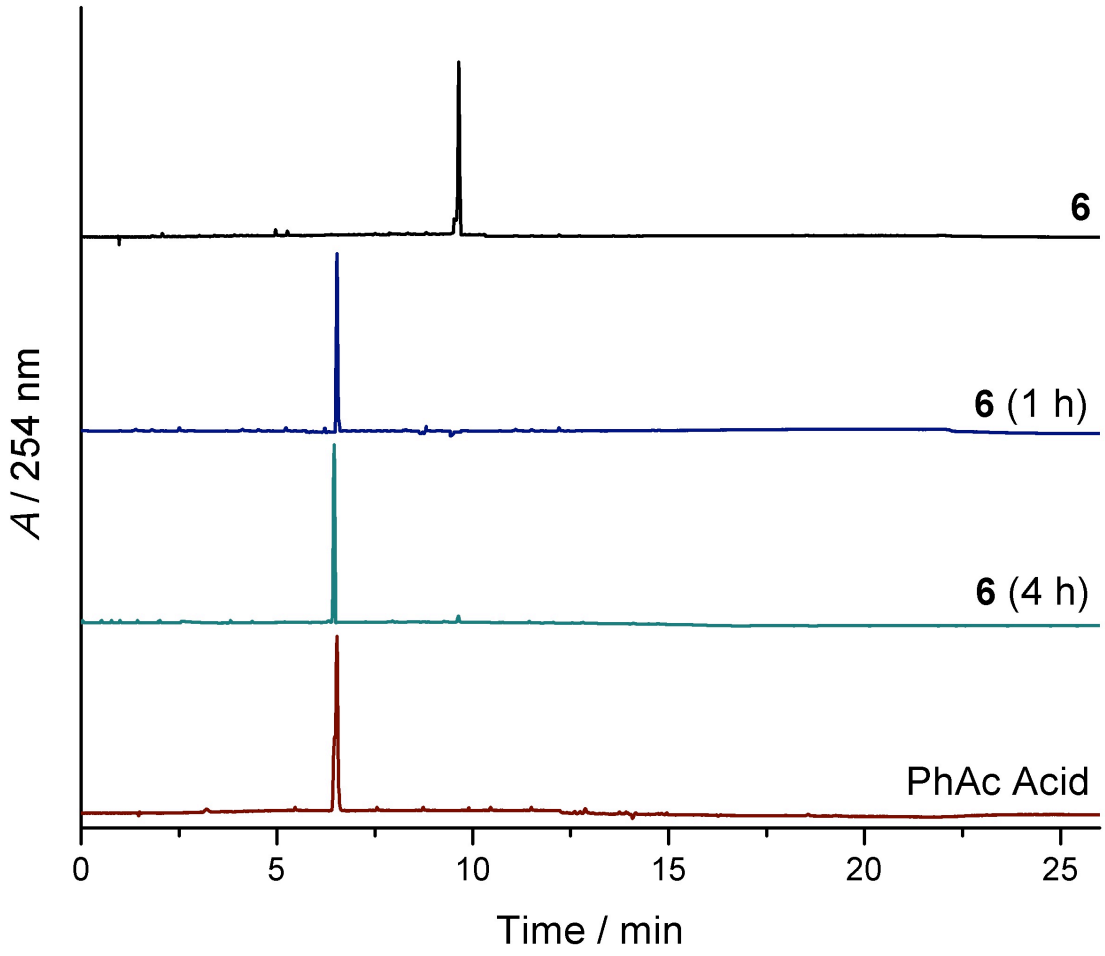


Figure S11

HPLC trace of an authentic sample of **6** (RT = 9.6 min, black), **6** (1 mM, 5% DMSO/ 95% 100 mM Tris-Cl, pH 7.4 buffer) in the presence of H_2O_2 (20 mM, 37 °C) at 1 h (blue) and at 4 h (green). The expected product, phenylacetic acid is also shown for comparison (RT = 6.4 min, brown).

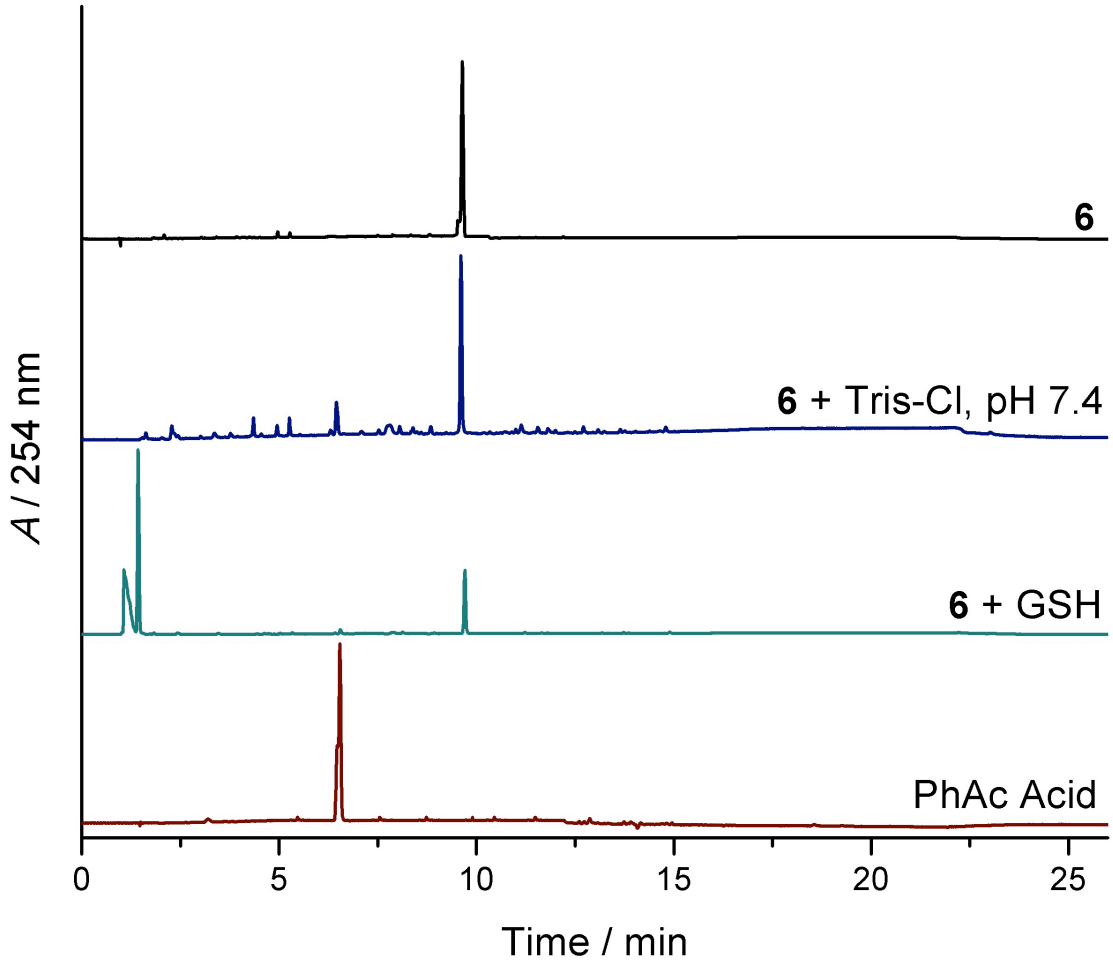


Figure S12

HPLC trace of an authentic sample of **6** (RT = 9.6 min, black), **6** (1 mM, 40% DMSO/ 60% 100 mM Tris-Cl, pH 7.4 buffer) after 24 h (37 °C) incubation (blue), and **6** under the same conditions, with the addition of glutathione (20 mM, green). The expected product, phenylacetic acid is also shown for comparison (RT = 6.4 min, brown).

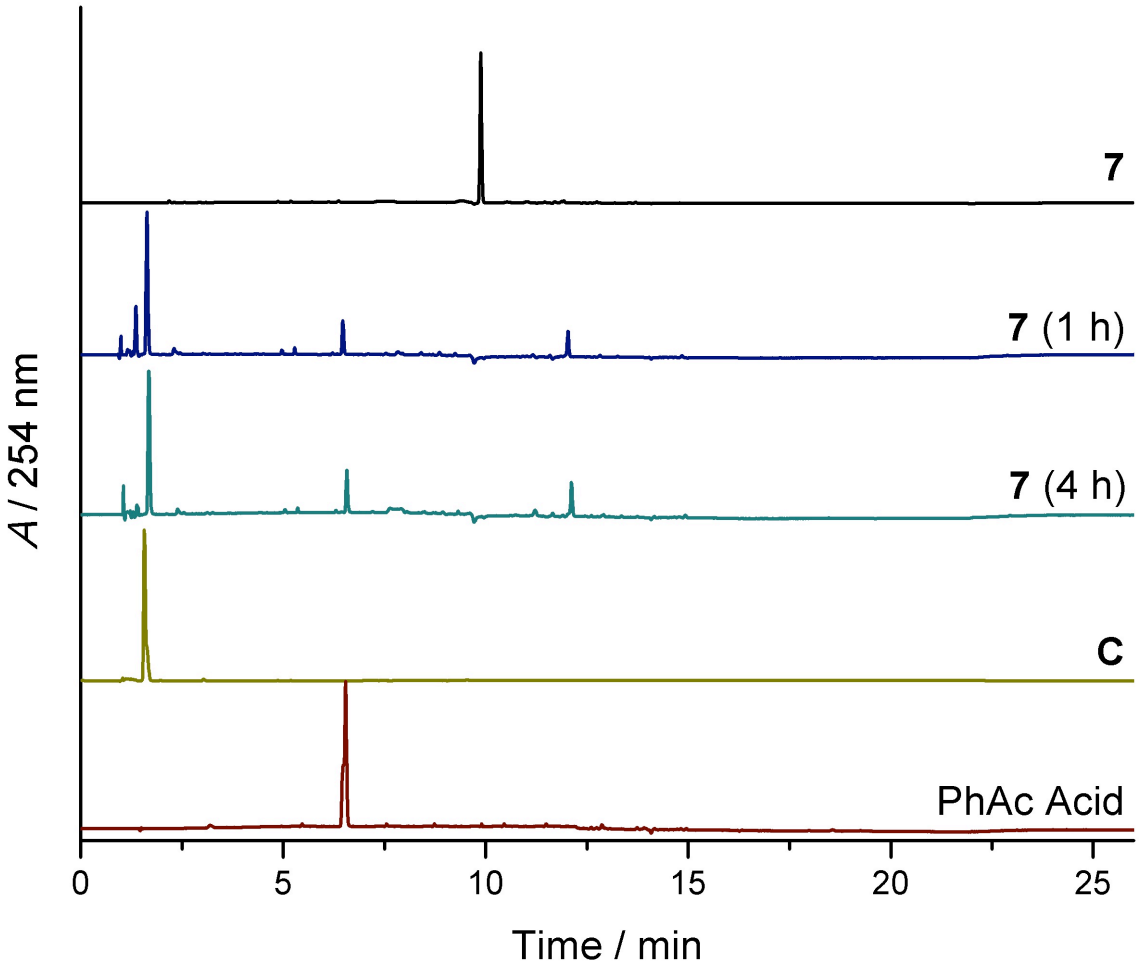


Figure S13

HPLC trace of an authentic sample of **7** (RT = 10.0 min, black), **7** (1 mM, 40% DMSO/ 60% 100 mM Tris-Cl, pH 7.4 buffer) in the presence of H_2O_2 (20 mM, 37 °C) at 1 h (blue) and at 4 h (green). The expected products, **C** (RT = 1.6 min, yellow) and phenylacetic acid are also shown for comparison (RT = 6.4 min, brown).

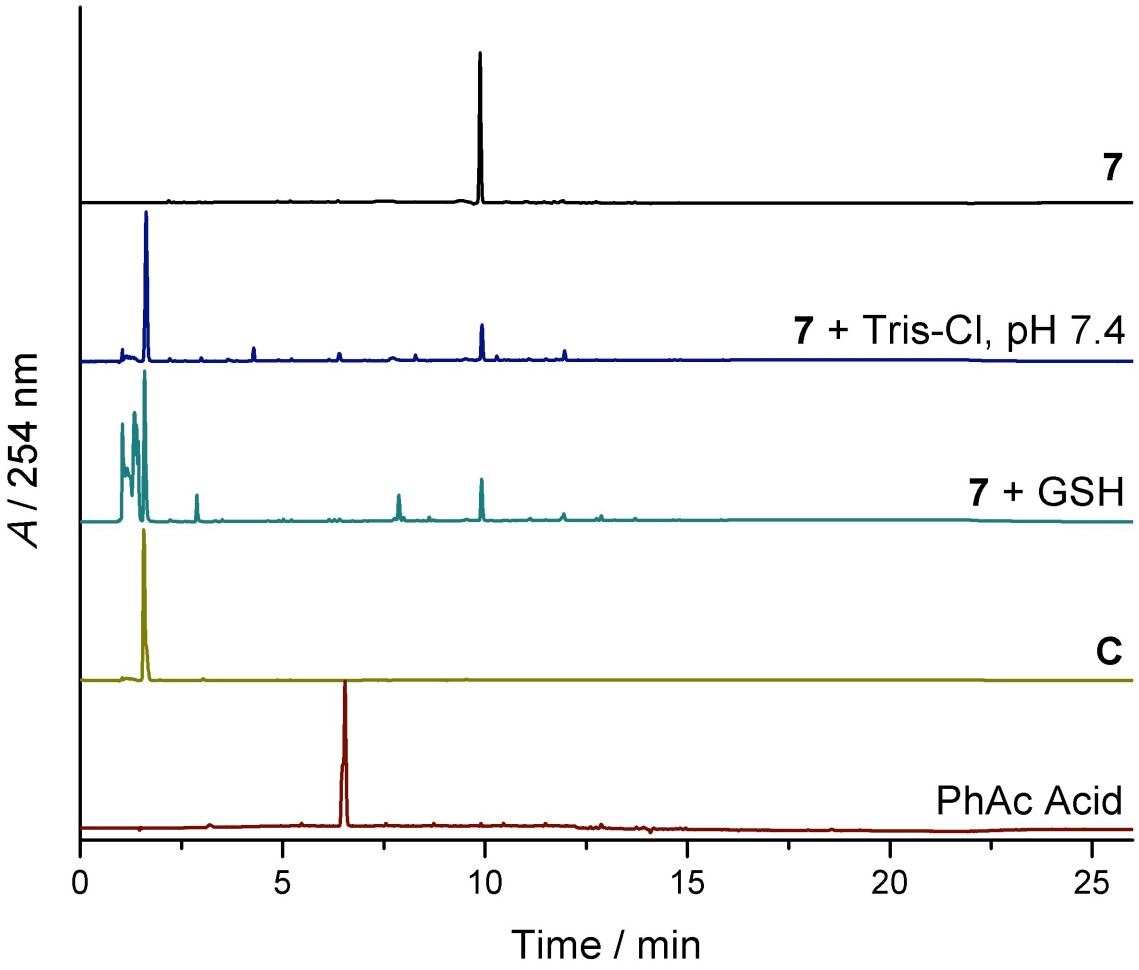


Figure S14

HPLC trace of an authentic sample of **7** (RT = 10.0 min, black), **7** (1 mM, 40% DMSO/ 60% 100 mM Tris-Cl, pH 7.4 buffer) after 24 h (37 °C) incubation (blue), and **7** under the same conditions, with the addition of glutathione (20 mM, green). The expected products, **C** (RT = 1.6 min, yellow) and phenylacetic acid are also shown for comparison (RT = 6.4 min, brown).

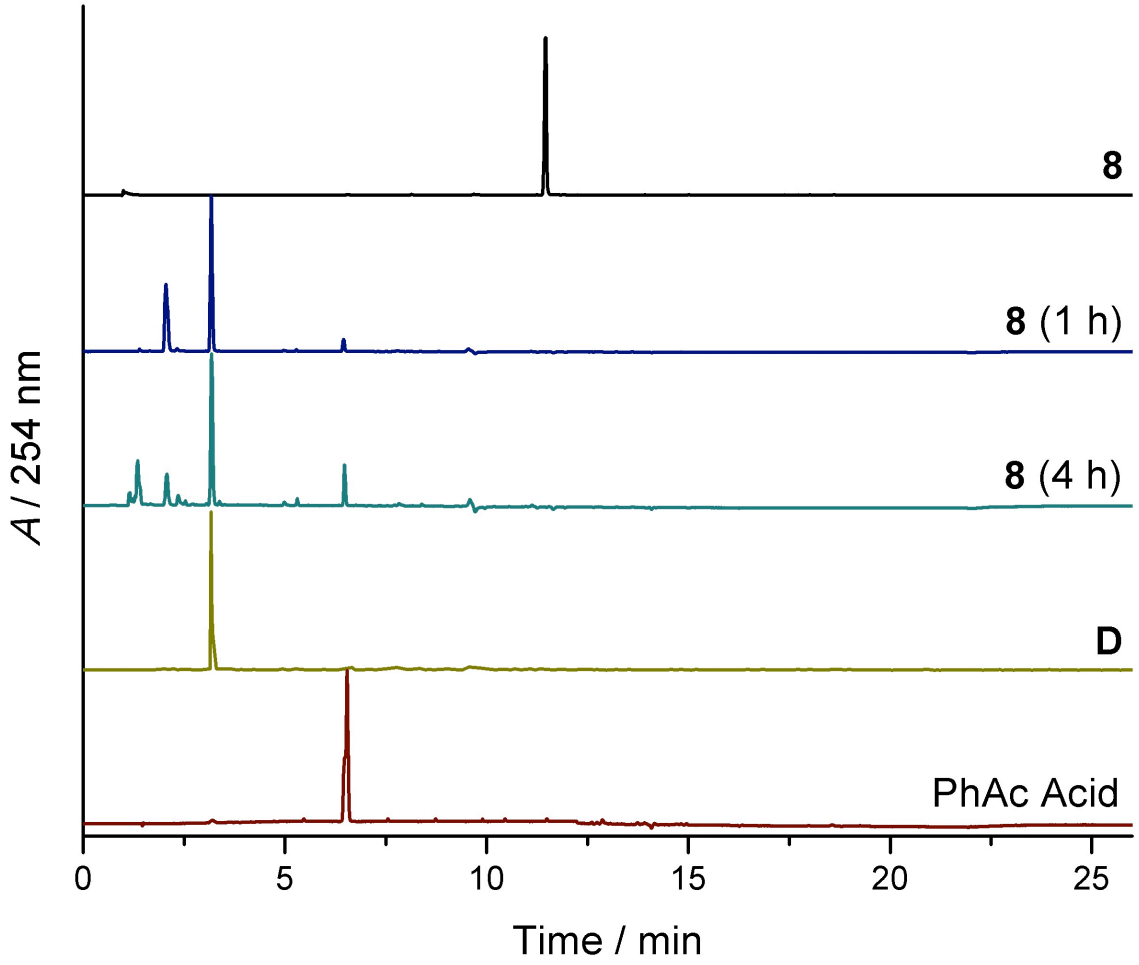


Figure S15

HPLC trace of an authentic sample of **8** (RT = 11.5 min, black), **8** (1 mM, 40% DMSO/ 60% 100 mM Tris-Cl, pH 7.4 buffer) in the presence of H_2O_2 (20 mM, 37 °C) at 1 h (blue) and at 4 h (green). The expected products, **D** (RT = 3.1 min, yellow) and phenylacetic acid are also shown for comparison (RT = 6.4 min, brown).

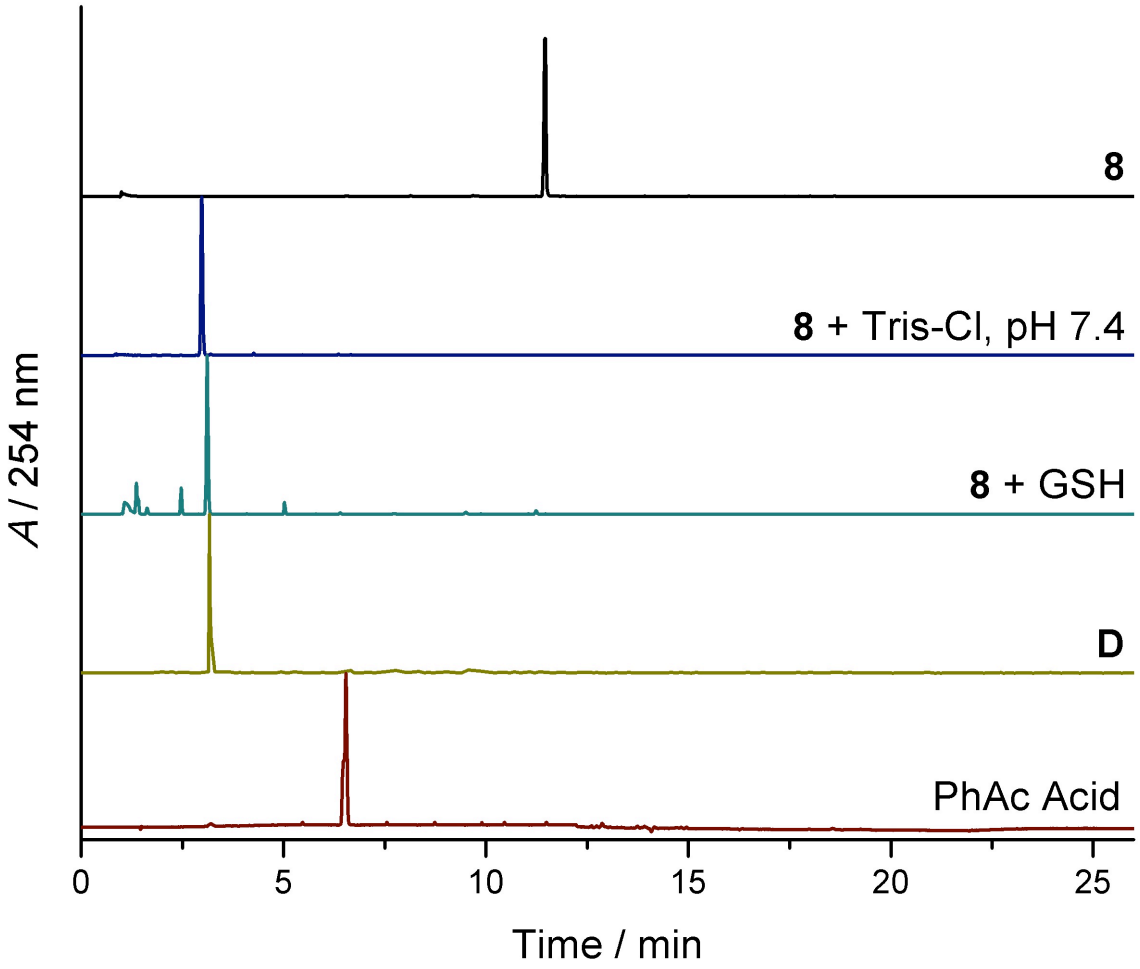


Figure S16

HPLC trace of an authentic sample of **8** (RT = 11.5 min, black), **8** (1 mM, 40% DMSO/ 60% 100 mM Tris-Cl, pH 7.4 buffer) after 24 h (37 °C) incubation (blue), and **8** under the same conditions, with the addition of glutathione (20 mM, green). The expected products, **D** (RT = 3.1 min, yellow) and phenylacetic acid are also shown for comparison (RT = 6.4 min, brown).

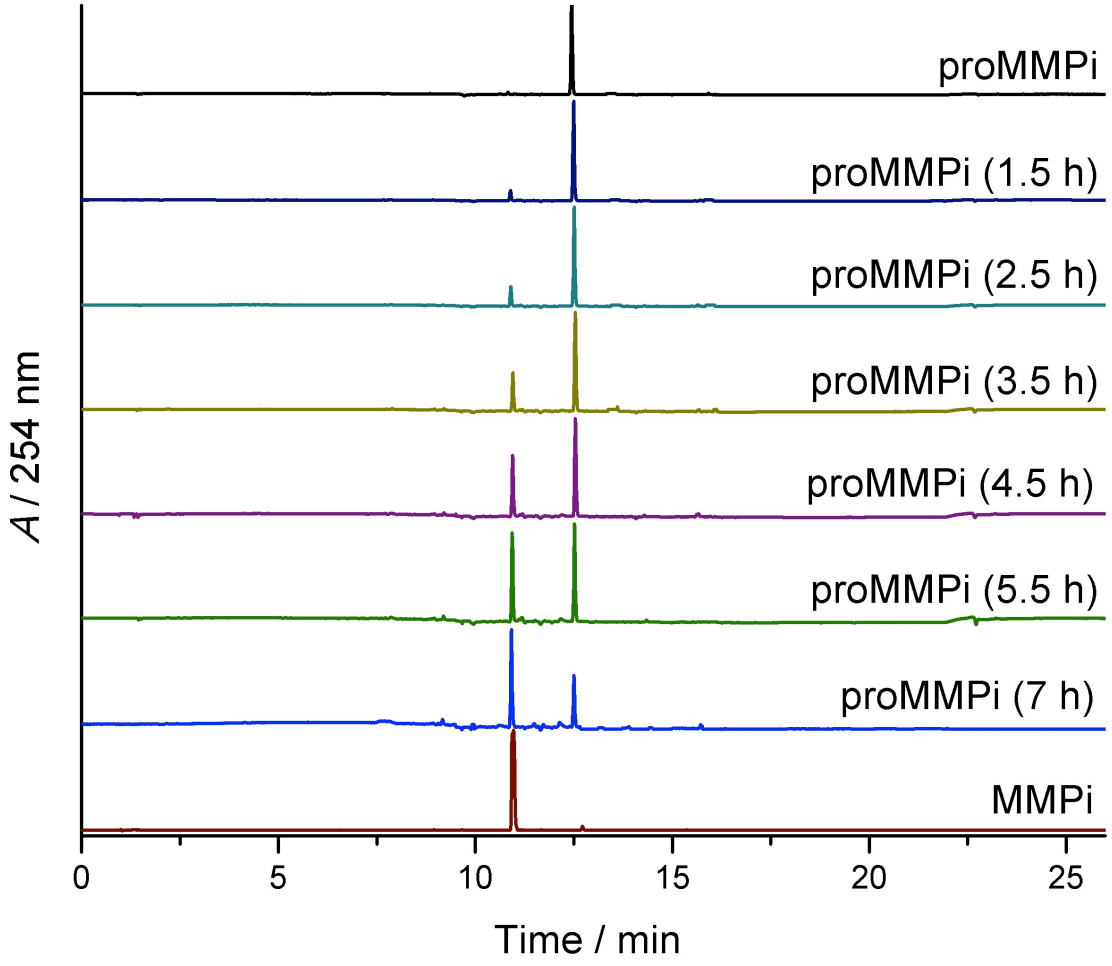


Figure S17

HPLC trace of an authentic sample of proMMPi (RT = 12.6 min, black), proMMPi (100 μM, 40% DMSO/ 60% 100 mM Tris-Cl, pH 7.4 buffer) in the presence of H_2O_2 (2 mM, 37 °C) at several time points. The expected MMPi product is also shown for comparison (RT = 11.0 min, brown).

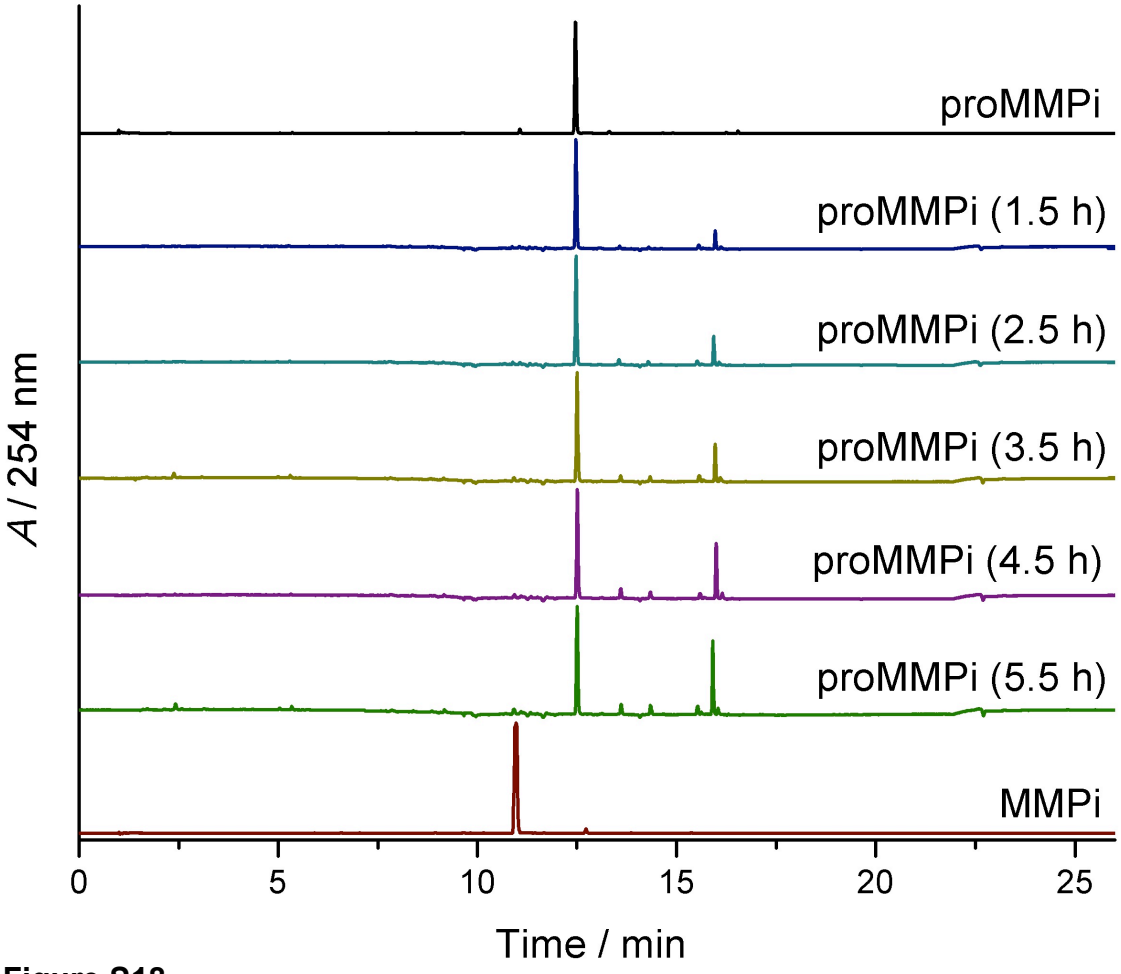


Figure S18

HPLC trace of an authentic sample of proMMPi (RT = 12.6 min, black), proMMPi (100 μM, 40% DMSO/ 60% 100 mM Tris-Cl, pH 7.4 buffer) in the presence of NaClO (2 mM, 37 °C) at several time points. The expected MMPi product is also shown for comparison (RT = 11.0 min, brown).

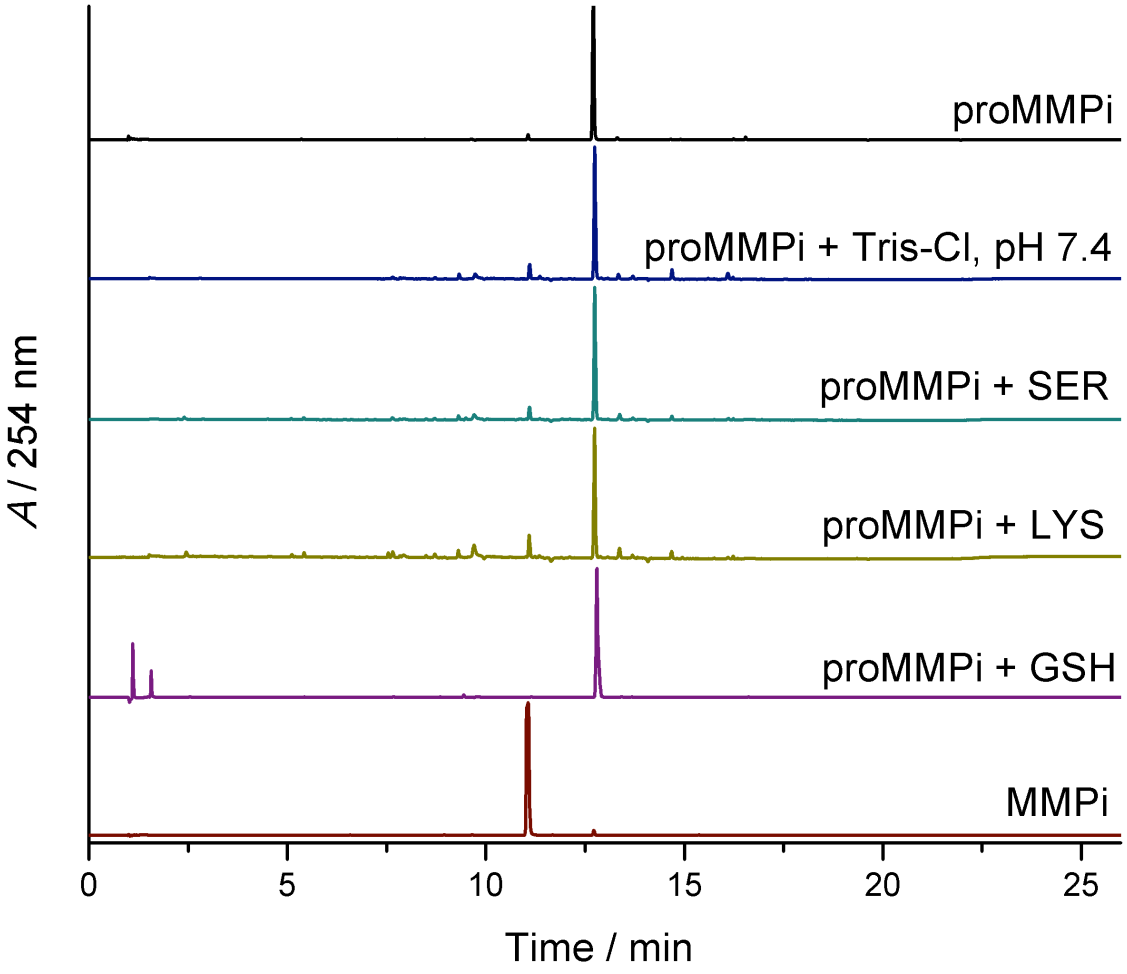


Figure S19.

HPLC trace of an authentic sample of proMMPi (RT = 12.6 min, black), proMMPi (1 mM, 40% DMSO/ 60% 100 mM Tris-Cl, pH 7.4 buffer) after 24 h (37 °C) incubation (blue), proMMPi under the same conditions, with the addition serine (20 mM, teal), lysine (20 mM, yellow), and glutathione (20 mM, purple). The expected MMPi product is also shown for comparison (RT = 11.0 min, brown).

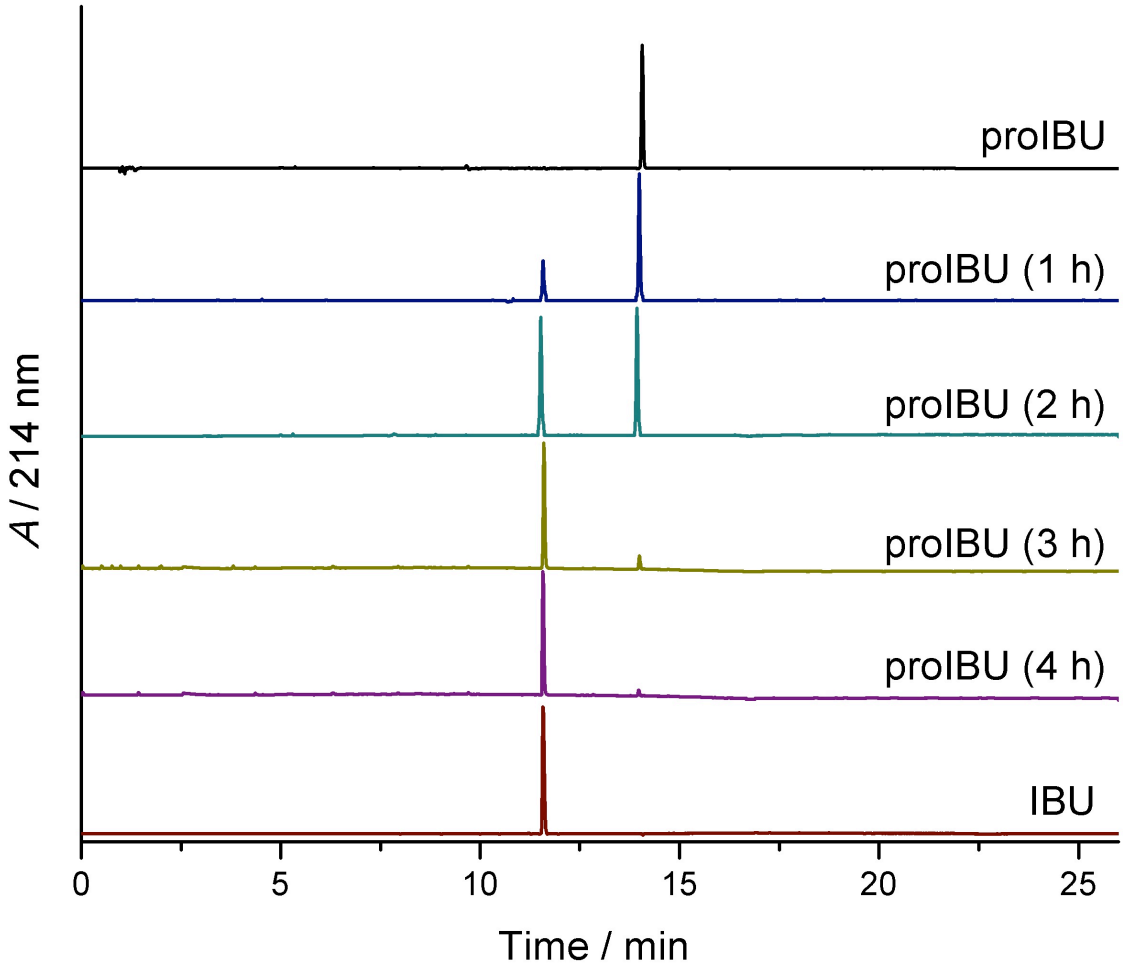


Figure S20

HPLC trace of an authentic sample of proIBU (RT = 14.1 min, black), proIBU (1 mM, 40% DMSO/ 60% 100 mM Tris-Cl, pH 7.4 buffer) in the presence of H_2O_2 (20 mM, 37 °C) at several time points. The expected IBU product is also shown for comparison (RT = 11.6 min, brown).

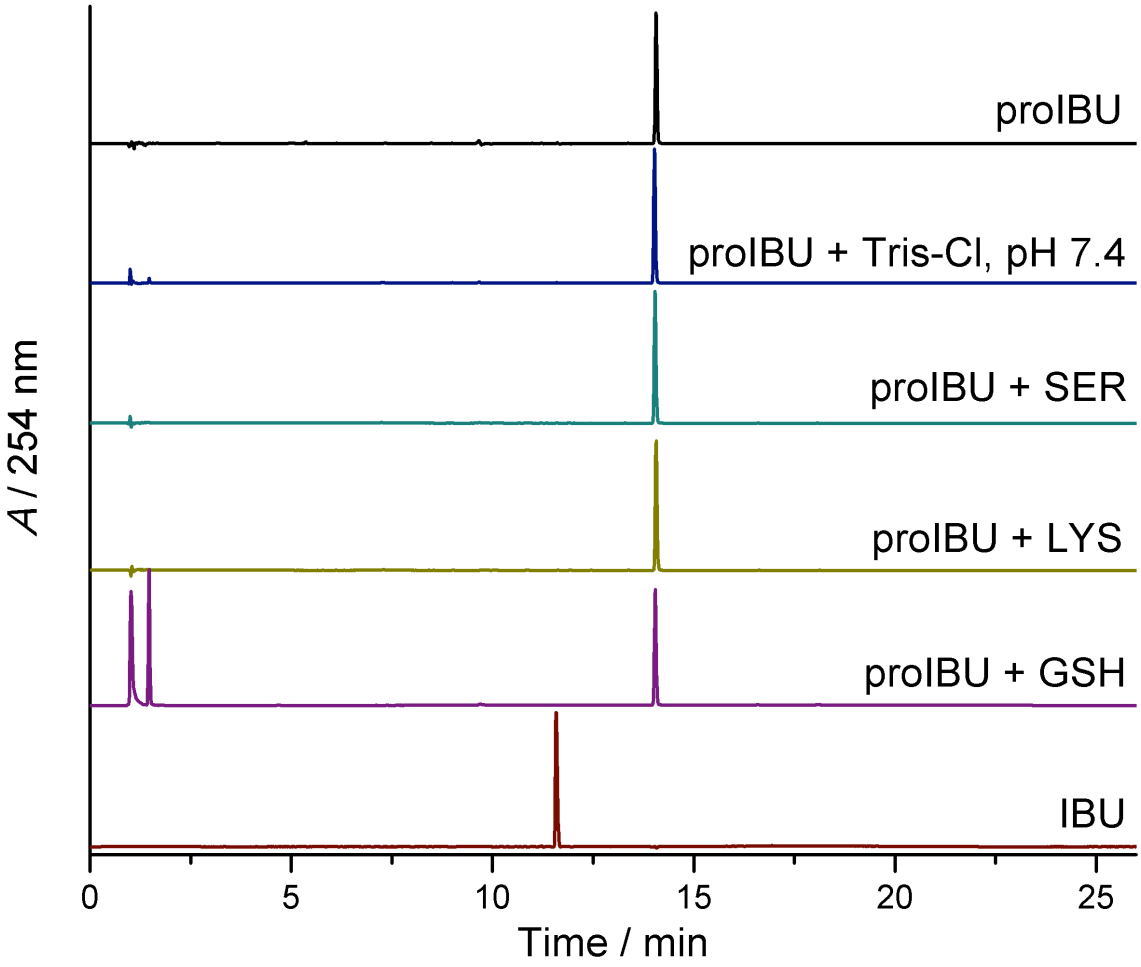


Figure S21

HPLC trace of an authentic sample of proIBU (RT = 14.1 min, black), proIBU (1 mM, 40% DMSO/ 60% 100 mM Tris-Cl, pH 7.4 buffer) after 24 h (37 °C) incubation (blue), proIBU under the same conditions, with the addition serine (20 mM, teal), lysine (20 mM, yellow), and glutathione (20 mM, purple). The expected IBU product is also shown for comparison (RT = 11.6 min, brown).

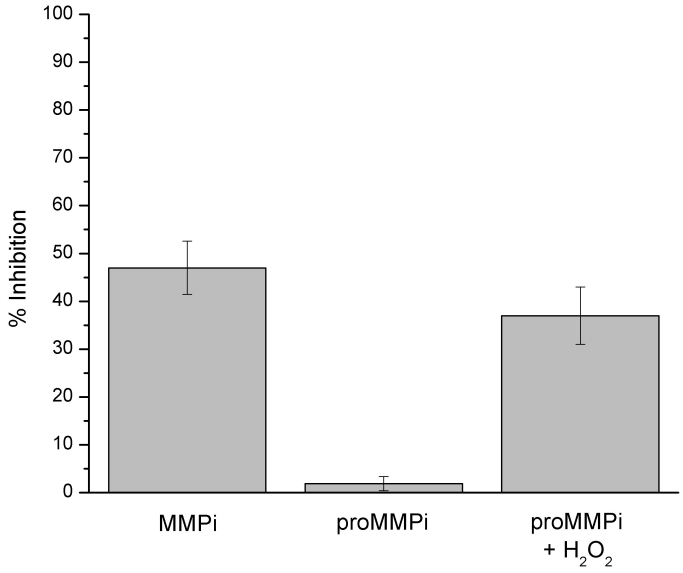


Figure S22

Inhibition assay results for proMMPi against MMP-2 in the absence and presence of H_2O_2. Inhibition for MMPi was measured 47±6%, proMMP 3±2%, and proMMPi + H_2O_2 at 37±6%.

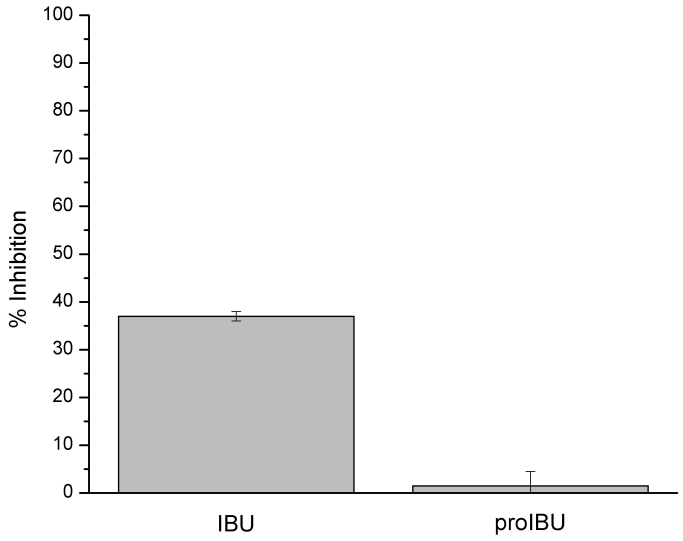


Figure S23

Inhibition assay results for proIBU against COX-1 in the absence and presence of H_2O_2. Inhibition for **IBU** was measured 37±2% and **proIBU** 2±2.

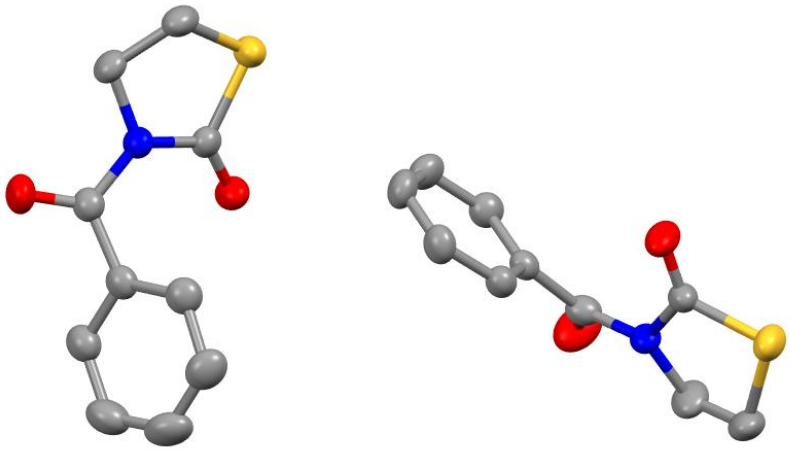


Figure S24

Crystal structure of compound **2,** showing the asymmetric unit. Thermal ellipsoids are shown at 50% probability. Hydrogen atoms are omitted for clarity. Color scheme: Carbon (grey), nitrogen (blue), oxygen (red), and sulfur (yellow).

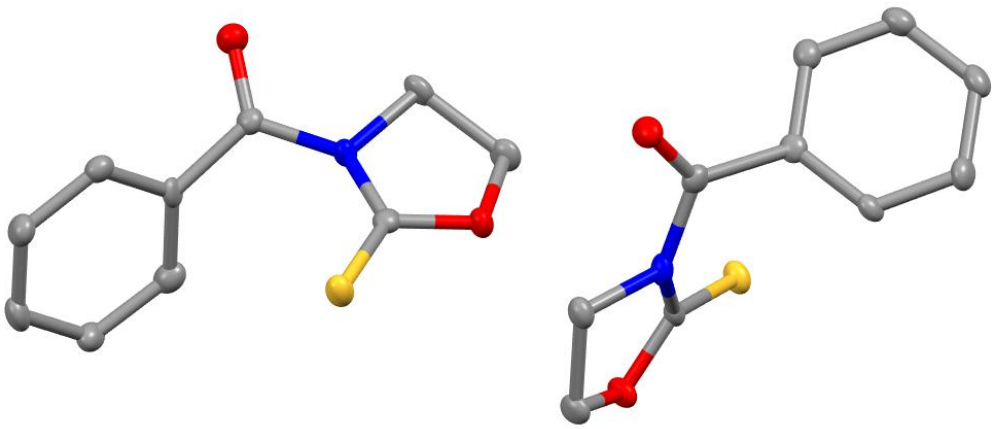


Figure S25

Crystal structure of compound **3,** showing the asymmetric unit. Thermal ellipsoids are shown at 50% probability. Hydrogen atoms are omitted for clarity. Color scheme: Carbon (grey), nitrogen (blue), oxygen (red), and sulfur (yellow).

Table S1. Crystal data and structure refinement for compounds **2** and **3**

Compound	**2**	**3**
Empirical Formula	$C_{10}H_9NO_2S$	$C_{10}H_9NO_2S$
Formula Weight	207.24	207.24
Collection *T* (K)	250(2)	100(2)
λ (Å)	0.71073	0.71073
Crystal system	Monoclinic	Orthorhombic
Space group	$P2_1/n$	$Pca2_1$
a (Å)	11.222(2)	13.7485(7)
b (Å)	12.458(3)	5.4360(3)
c (Å)	13.769(3)	25.063(1)
α (deg)	90	90
β (deg)	91.055(11)	95.1570(10)
γ (deg)	90	90
V (Å^3)	1924.6(7)	1873.1(2)
Z	8	8
D_{calcd} (g cm^{-3})	1.430	1.470
μ (mm^{-1})	0.306	0.315
min/max *T*	0.8713/0.9692	0.8713/0.9692
hkl ranges	-13<h<13	-16<=h<=16
	-14<k<14	-6<=k<=6
	-16<l<16	-30<=l<=28
Total reflections	12019	11582
Independent reflections	3538	3362
R (int)	0.0439	0.0381
Parameters/restraints	254/0	253/1
R_1 (all data)	0.0796	0.0498
R_1 [$I > 2\sigma(I)$]	0.0505	0.0463
wR_2 (all data)	0.1295	0.1152
wR_2 [$I > 2\sigma(I)$]	0.1101	0.1121
Max/Min residual (e$^-$/Å^3)	0.284/-0.281	0.284/-0.281
G.O.F.	1.153	1.073

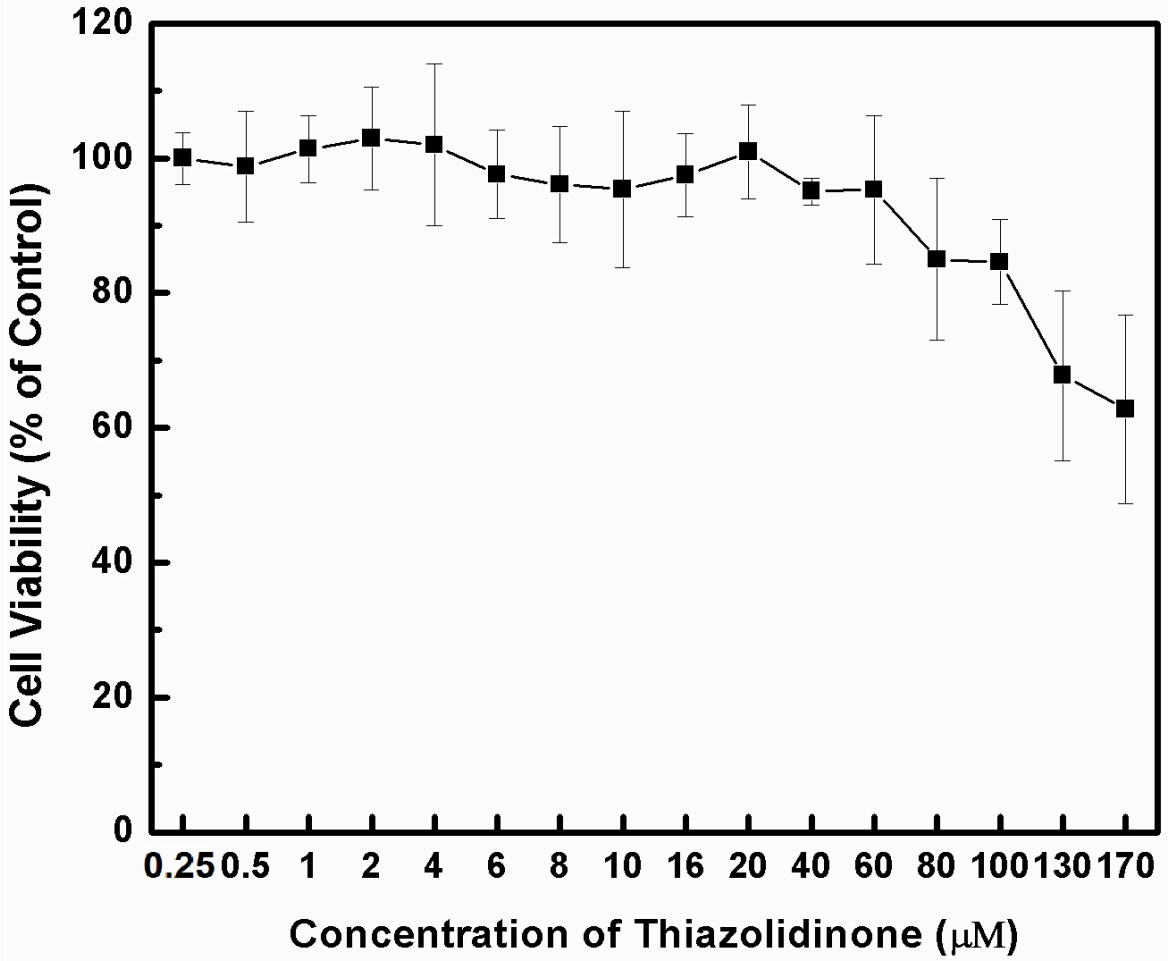


Figure S26

Cytotoxicity assay for thiazolidinone promoiety **B**. Each data point is the result of three independent experiments (error bars shown).

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