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ORIGINAL PAPER

Activation of sulfonate ester based matrix metalloproteinase proinhibitors by hydrogen peroxide

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Abstract This study details the development of matrix metalloproteinase inhibitor prodrugs (proMMPi) that are activated in the presence of reactive-oxygen species (ROS). Conventional matrix metalloproteinase inhibitors (MMPi) utilize a zinc-binding group (ZBG) that chelates to the catalytic zinc(II) ion of matrix metalloproteinases (MMPs) to inhibit their activity. To create ROS-sensitive prodrugs, sulfonate esters were used as a protecting group for the ZBG to block their metal binding ability. Surprisingly, these sulfonate esters were found to be cleaved by H_2O_2 only when the ZBG contained an *N*-oxide donor atom moiety. Sulfonate ester derivatives of full-length MMPi based on these ROS-triggerable systems were synthesized. It was found that proMMPi with sulfonate ester protecting groups showed relatively high rates of cleavage in the presence of H_2O_2 to release the active MMPi. In vitro MMP inhibition studies confirmed a significant increase in inhibitory activity of proMMPi upon addition of H_2O_2, demonstrating the use of sulfonate esters to act as cleavable triggers for ROS-activated prodrugs.

Keywords Matrix metalloproteinases · Reactive-oxygen species · Prodrugs

Introduction

Matrix metalloproteinases (MMPs) comprise a family of Zn(II)-dependent endopeptidases involved in the cleavage of extracellular proteins [1–3]. MMPs are secreted as zymogens and become activated by a variety of pathways through proteolytic cleavage of the propeptide domain by proteases, other MMPs, and reactive-oxygen species (ROS) [1, 3]. Owing to the ability of MMPs to cleave proteins in the extracellular matrix, overexpression and misregulation of MMPs has been associated with a variety of pathologic disorders, including arthritis, cancer, and cardiovascular disease [2, 4–6]. Of particular note is MMP activation associated with stroke, which is a leading cause of disablement and death [7–10]. In stroke, the inflammatory response induced by ischemia initiates the formation of ROS, which activates MMPs, and can lead to the breakdown of the blood–brain barrier, resulting in cell death and tissue damage [7–11]. The association of ROS with MMP activation after ischemia has generated interest in using MMP inhibitors (MMPi) to treat reperfusion injury associated with stroke and other cardiovascular ailments [12]. It has been shown that MMP inhibition with broad-based inhibitors upon the onset of stroke can reduce ischemic-related brain injury [9, 13, 14]. However, because MMPs have both beneficial and pathogenic roles with respect to normal physiologic function and disease progression, systemic inhibition of MMPs can lead to side effects such as musculoskeletal syndrome [1, 4, 5, 15–18]. Hence, there is an impetus to control both the spatial and temporal activity of these inhibitors [19].

To localize the activity of MMPi, a prodrug approach can be employed to develop latent forms of the inhibitor (i.e., proinhibitor) that are activated under specific, desired conditions. Prochelators activated by biological or chemical stimuli have been explored as a means to modulate metal ion chelation in various disease states [20–25]. By blocking the zinc-binding group (ZBG) of the inhibitor [which coordinates to the catalytic Zn(II) ion] with a

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stimulus-responsive protecting group, one can greatly attenuate or abolish the inhibitory activity of the MMPi. Under the appropriate biological stimulus, the proinhibitor can be activated, releasing the MMPi, thereby allowing for localized delivery and inhibition. In general, metalloenzyme proinhibitors have not been widely investigated, with only a few systems reported for MMPs [26–31]. To establish the use of ZBG protecting groups for the development of MMP proinhibitors, MMPi containing carbohydrate protecting groups that are cleaved in the presence of β-glucosidase were described [30]. Quotient IC_{50} values, which represent the ratio of the IC_{50} values of the MMPi prodrug (proMMPi) to IC_{50} values of the active inhibitor [32], were excellent at greater than 1,000 with MMP-8 [30]. Similarly, proMMPi based on concepts from prochelators and fluorescent probes sensitive to ROS [33, 34] were developed for potential use in ischemic injury [31]. Boronic ester protecting groups appended via a self-immolative linker to the ZBG of MMPi were reported by our group and showed rapid activation in the presence of H_2O_2, leading to the release of the free MMPi [31].

We sought to identify additional protecting groups to develop proMMPi that could be triggered in an ischemic setting by ROS. Fluorescent probes incorporating sulfonate ester protecting groups have been shown to be sensitive for detecting several ROS (hydrogen peroxide, superoxide anion) [35–37]. These studies prompted the use of sulfonate esters as selective protecting groups for proMMPi that could be cleaved under conditions of oxidative stress (e.g., ischemia). The work described herein demonstrates that various ZBGs could be protected with sulfonate esters, creating molecules that can be activated with H_2O_2. Four different ZBGs employing oxygen donor atoms were selected for this study. The ability of H_2O_2 to liberate the ZBGs was assessed, and suitable candidates were developed into full-length proMMPi that show increased potency against MMP-12 in the presence of H_2O_2.

Materials and methods

General

Starting materials and solvents were purchased from commercial suppliers (Sigma–Aldrich, Alfa Aesar, Fisher, and others) and used as received. $^1H/^{13}C$ NMR spectra were recorded at ambient temperature with a 400 or 500 MHz Varian Fourier transform NMR instrument or a 500 MHz JEOL instrument, located in the Department of Chemistry and Biochemistry at the University of California, San Diego. Mass spectra were obtained at the Molecular Mass Spectrometry Facility in the Department of Chemistry and Biochemistry at the University of California, San Diego. Elemental analyses was preformed by NuMega Resonance Labs, San Diego.

General procedure for the synthesis of sulfonate ester ZBGs

The ZBG compound was dissolved in pyridine on ice. To this was added the desired sulfonyl chloride. The reaction flask was removed from the ice bath and left stirring overnight under nitrogen while warming to room temperature. The pyridine was removed by rotary evaporation and the resulting oil was redissolved in dichloromethane and washed with 1 M HCl (approximately 30 mL), water, and brine. The organic layer was dried over $MgSO_4$, filtered, and then concentrated via rotary evaporation. The product was purified on a silica gel column and eluted with 1% MeOH in dichloromethane unless otherwise noted.

2-Oxopyridin-1(2*H*)-yl benzenesulfonate

2-Hydroxypyridine-1-oxide (**ZBG1**; 1.0 g, 9.1 mmol) was reacted with benzenesulfonyl chloride (1.27 mL, 10.0 mmol) in 75 mL of pyridine to afford 2-oxopyridin-1(2*H*)-yl benzenesulfonate (**PZBG-1a**) in 77% yield (1.75 g, 7.0 mmol). ^1H NMR (500 MHz, $CDCl_3$) $\delta = 8.02$ (d, $J = 8.0$ Hz, 2H), 7.75 (t, $J = 8.0$ Hz, 1H), 7.59 (m, 3H), 7.28 (dt, $J_1 = 6.9$ Hz, $J_2 = 2.3$ Hz, 1H), 6.52 (d, $J = 9.8$ Hz, 1H), 6.15 (t, $J = 7.5$ Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 157.0$, 139.7, 137.1, 136.0, 133.8, 130.0, 129.5, 123.4, 105.4. Electrospray ionization mass spectrometry (ESI-MS)(+): m/z 252.01 $[M + H]^+$, 273.95 $[M + Na]^+$. Anal. calcd for $C_{11}H_9NO_4S$: C, 52.58; H, 3.61; N, 5.57. Found: C, 52.21; H, 3.99; N, 5.44.

2-Oxopyridin-1(2*H*)-yl 4-methylbenzenesulfonate

ZBG1 (0.5 g, 4.5 mmol) was reacted with *p*-toluenesulfonyl chloride (2.57 g, 13.5 mmol) in 40 mL of pyridine to afford 2-oxopyridin-1(2*H*)-yl 4-methylbenzenesulfonate (**PZBG-1b**) in 89% yield (1.06 g, 4.0 mmol). ^1H NMR [500 MHz, dimethyl sulfoxide (DMSO)-d_6] $\delta = 7.82$ (d, $J = 8.6$ Hz, 2H), 7.75 (dd, $J_1 = 7.5$ Hz, $J_2 = 1.8$ Hz, 1H), 7.49 (d, $J = 8$ Hz, 2H), 7.41 (dt, $J_1 = 7.5$ Hz, $J_2 = 1.7$ Hz, 1H), 6.48 (dd, $J_1 = 9.2$ Hz, $J_2 = 1.8$ Hz, 1H), 6.21 (dt, $J_1 = 7.5$ Hz, $J_2 = 1.8$ Hz, 1H), 2.42 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 156.6$, 148.9, 141.2, 138.1, 131.1, 130.5, 130.0, 122.9, 106.1, 22.0. ESI-MS(+): m/z 266.10, $[M + H]^+$, 287.99 $[M + Na]^+$. Anal. calcd for $C_{12}H_{11}NO_4S$: C, 54.33; H, 4.18; N, 5.28. Found: C, 54.24; H, 4.35; N, 5.24.

2-Oxopyridin-1(2*H*)-yl 4-nitrobenzenesulfonate

ZBG1 (0.5 g, 4.5 mmol) was reacted with 4-nitrobenzenesulfonyl chloride (3.0 g, 13.5 mmol) in 40 mL of pyridine to afford 2-oxopyridin-1(2*H*)-yl 4-nitrobenzenesulfonate (**PZBG-1c**) in 92% yield (1.23 g, 4.2 mmol). ^{1}H NMR (500 MHz, $CDCl_3$) $\delta = 8.42$ (d, $J = 8.6$ Hz, 2H), 8.23 (d, $J = 9.2$ Hz, 2H), 7.65 (dd, $J_1 = 7.5$ Hz, $J_2 = 1.8$ Hz, 1H), 7.34 (dt, $J_1 = 9.2$ Hz, $J_2 = 1.7$ Hz, 1H), 6.52 (d, $J = 9.2$ Hz, 1H), 6.22 (t, $J = 7.7$ Hz, 1H). ^{13}C NMR (100 MHz, DMSO) $\delta = 156.6$, 152.3, 141.6, 139.4, 138.3, 131.8, 125.6, 122.8, 106.4. ESI-MS(+): *m/z* 297.28 $[M + H]^+$, 319.02 $[M + Na]^+$.

2-Oxopyridin-1(2*H*)-yl 2,4-dinitrobenzenesulfonate

ZBG1 (0.5 g, 4.5 mmol) was reacted with 2,4-dinitrobenzenesulfonyl chloride (1.32 g, 5.0 mmol) in 40 mL of pyridine to afford 2-oxopyridin-1(2*H*)-yl 2,4-dinitrobenzenesulfonate (**PZBG-1d**) in 31% yield (0.48 g, 1.4 mmol). ^{1}H NMR (500 MHz, $CDCl_3$) $\delta = 8.96$ (d, $J = 2.3$ Hz, 1H), 8.42 (dd, $J_1 = 9.2$ Hz, $J_2 = 2.3$ Hz, 1H), 7.68 (dd, $J_1 = 6.9$ Hz, $J_2 = 1.7$ Hz, 1H), 7.50 (dt, $J_1 = 7.5$ Hz, $J_2 = 2.3$ Hz, 1H), 7.06 (d, $J = 8.1$ Hz, 1H), 6.82 (dd, $J_1 = 9.2$ Hz, $J_2 = 1.8$ Hz, 1H), 6.35 (dt, $J_1 = 6.9$ Hz, $J_2 = 1.8$ Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 157.0$, 155.6, 140.5, 135.1, 129.6, 124.0, 122.9, 116.0, 106.6.

4-(((2-Oxopyridin-1(2*H*)-yl)oxy)sulfonyl) benzoic acid

ZBG1 (0.21 g, 1.9 mmol) was reacted with 4-(chlorosulfonyl) benzoic acid (0.62 g, 2.8 mmol) in 5 mL of pyridine. The solvent was evaporated, leaving a yellow oil. Addition of 5 mL of dichloromethane followed by the addition of 5 mL of ethyl acetate allowed for precipitation of 4-(((2-oxopyridin-1(2*H*)-yl)oxy)sulfonyl)benzoic acid (**PZBG-1e**) in 30% yield (0.17 g, 0.57 mmol). ^{1}H NMR (400 MHz, DMSO) $\delta = 8.18$ (d, $J = 8.8$ Hz, 2H), 8.08 (d, $J = 8.4$ Hz), 7.88 (dd, $J_1 = 7.6$ Hz, $J_2 = 2$ Hz), 7.45 (td, $J_1 = 8.2$ Hz, $J_2 = 2$ Hz, 1 H), 6.50 (dd, $J_1 = 9.2$ Hz, $J_2 = 1.6$ Hz, 1 H), 6.26 (td, $J_1 = 7$ Hz, $J_2 = 1.6$ Hz, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 166.5$, 156.6, 141.5, 138.2, 137.9, 137.3, 131.2, 130.4, 122.8, 106.2. ESI-MS(−): *m/z* 294.26 $[M - H]^-$. Anal. calcd. for $C_{12}H_9NO_6S$: C, 48.81; H, 3.07; N, 4.74. Found: C, 48.91; H, 3.37; N 4.84.

2-Methyl-4-oxo-4*H*-pyran-3-yl benzenesulfonate

3-Hydroxy-2-methyl-4*H*-pyran-4-one (**ZBG2**; 1.0 g, 7.9 mmol) was reacted with benzenesulfonyl chloride (3.0 mL, 23.7 mmol) in 75 mL of pyridine to afford 2-methyl-4-oxo-4*H*-pyran-3-yl benzenesulfonate (**PZBG-2a**) in 71% yield (1.50 g, 5.6 mmol). ^{1}H NMR (500 MHz, $CDCl_3$) $\delta = 8.12$ (d, $J = 8.6$ Hz, 2H), 7.69 (t, $J = 7.5$ Hz, 1H), 7.65 (d, $J = 5.8$ Hz, 1H), 7.58 (t, $J = 8.0$ Hz, 2H), 6.33 (d, $J = 5.2$ Hz, 1H), 2.46 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 172.1$, 163.1, 154.3, 138.4, 136.6, 134.7, 129.2, 129.0, 117.7, 16.3. ESI-MS(+): *m/z* 267.06 $[M + H]^+$, 289.03 $[M + Na]^+$.

2-Methyl-4-oxo-4*H*-pyran-3-yl
4-methylbenzenesulfonate

ZBG2 (0.5 g, 4.0 mmol) was reacted with *p*-toluenesulfonyl chloride (2.27 g, 11.9 mmol) in 40 mL of pyridine to afford 2-methyl-4-oxo-4*H*-pyran-3-yl 4-methylbenzenesulfonate (**PZBG-2b**) in 64% yield (0.71 g, 2.5 mmol). ^{1}H NMR (500 MHz, $CDCl_3$) $\delta = 7.99$ (d, $J = 8$ Hz, 2H), 7.64 (d, $J = 5.8$ Hz, 1H), 7.37 (d, $J = 8$ Hz, 2H), 6.34 (d, $J = 5.8$ Hz, 1H), 2.46 (s, 3H, CH_3), 2.45 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 127.2$, 163.2, 154.2, 145.8, 138.4, 133.6, 129.8, 129.0, 117.7, 22.0, 16.3. ESI-MS(+): *m/z* 281.01 $[M + H]^+$, 303.03 $[M + Na]^+$.

2-Methyl-4-oxo-4*H*-pyran-3-yl
4-nitrobenzenesulfonate

ZBG2 (0.5 g, 4.0 mmol) was reacted with 4-nitrobenzenesulfonyl chloride (0.88 g, 4.0 mmol) in 15 mL of pyridine to afford 2-methyl-4-oxo-4*H*-pyran-3-yl 4-nitrobenzenesulfonate (**PZBG-2c**) in 58% yield (0.71 g, 2.3 mmol). ^{1}H NMR (500 MHz, $CDCl_3$) $\delta = 8.42$ (d, $J = 9.2$ Hz, 2H), 8.31 (d, $J = 9.2$ Hz, 2H), 7.69 (d, $J = 5.7$ Hz, 1H), 6.34 (d, $J = 5.8$ Hz, 1H), 2.53 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 171.8$, 163.5, 154.6, 151.2, 142.3, 138.6, 130.4, 124.3, 117.6, 16.3. ESI-MS(+): *m/z* 312.06 $[M + H]^+$.

2-Methyl-4-oxo-4*H*-pyran-3-yl
2,4-dinitrobenzenesulfonate

ZBG2 (0.5 g, 4.0 mmol) was reacted with 2,4-dinitrobenzenesulfonyl chloride (1.58 g, 5.9 mmol) in 40 mL of pyridine to afford 2-methyl-4-oxo-4*H*-pyran-3-yl 2,4-dinitrobenzenesulfonate (**PZBG-2d**) in 28% yield (0.39 g, 1.1 mmol). ^{1}H NMR (500 MHz, $CDCl_3$) $\delta = 8.72$ (d, $J = 2.3$ Hz, 1H), 8.56 (dd, $J_1 = 9.2$ Hz, $J_2 = 2.3$ Hz, 1H), 8.44 (d, $J = 8.6$ Hz, 1H), 7.71 (d, $J = 5.8$ Hz, 1H), 6.31 (d, $J = 5.8$ Hz, 1H), 2.53 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 171.6$, 163.3, 154.9, 139.3, 136.4, 133.8, 126.8, 120.5, 117.5, 16.1. ESI-MS(+): *m/z* 357.03 $[M + H]^+$, 378.99 $[M + Na]^+$.

1,2-Dimethyl-4-oxo-1,4-dihydropyridin-3-yl benzenesulfonate

3-Hydroxy-1,2-dimethylpyridin-4(1*H*)-one (**ZBG-3**; 0.5 g, 3.6 mmol) was reacted with benzenesulfonyl chloride (0.51 mL, 4.0 mmol) in 40 mL of pyridine to afford 1,2-himethyl-4-oxo-1,4-dihydropyridin-3-yl benzenesulfonate (**PZBG-3a**) in 43% yield (0.43 g, 1.5 mmol). ^{1}H NMR (500 MHz, $CDCl_3$) $\delta = 8.19$ (d, $J = 6.9$ Hz, 2H), 7.66 (t, $J = 7.5$ Hz, 1H), 7.57 (t, $J = 7.5$ Hz, 2H), 7.23 (d, $J = 8$ Hz, 1H), 6.34 (d, $J = 8.1$ Hz, 1H), 3.63 (s, 3H, NCH_3), 2.49 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 171.3$, 144.8, 140.7, 139.9, 137.3, 134.3, 129.0, 128.9, 118.3, 41.8, 14.7. ESI-MS(+): *m/z* 280.09 $[M + H]^+$.

1,2-Dimethyl-4-oxo-1,4-dihydropyridin-3-yl 4-methylbenzenesulfonate

ZBG-3 (0.2 g, 1.4 mmol) was reacted with *p*-toluenesulfonyl chloride (0.82 g, 4.3 mmol) in 10 mL of pyridine to afford 1,2-dimethyl-4-oxo-1,4-dihydropyridin-3-yl 4-methylbenzenesulfonate (**PZBG-3b**) in 86% yield (0.35 g, 1.2 mmol). ^{1}H NMR (500 MHz, $CDCl_3$) $\delta = 7.99$ (d, $J = 8.4$ Hz, 2H), 7.40 (d, $J = 7.6$ Hz, 1H), 7.33 (d, $J = 8$ Hz, 2H), 6.40 (d, $J = 7.2$ Hz, 1H), 3.64 (s, 3H), 2.42 (s, 3H), 2.41 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 170.71$, 145.67, 145.54, 141.48, 134.07, 129.72, 128.96, 126.07, 117.53, 42.25, 22.00, 14.7. ESI-MS(+): *m/z* 294.05 $[M + H]^+$, 315.97 $[M + Na]^+$.

1,2-Dimethyl-4-oxo-1,4-dihydropyridin-3-yl 4-nitrobenzenesulfonate

ZBG-3 (0.2 g, 1.5 mmol) was reacted with 4-nitrobenzenesulfonyl chloride (0.488 g, 2.2 mmol) in 10 mL of pyridine to afford 1,2-dimethyl-4-oxo-1,4-dihydropyridin-3-yl 4-nitrobenzenesulfonate (**PZBG-3c**) in 50% yield (0.23 g, 0.7 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 8.41$–8.34 (m, 4H), 7.26 (d, $J = 7.6$ Hz, 1H), 6.33 (d, $J = 7.6$ Hz, 1H), 3.67 (s, 3H), 2.53 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 170.9$, 150.9, 144.9, 143.2, 140.9, 140.1, 130.4, 124.0, 118.4, 41.9, 14.6. ESI-MS(+): *m/z* 325.11 $[M + H]^+$, 346.96 $[M + Na]^+$.

1,2-Dimethyl-4-oxo-1,4-dihydropyridin-3-yl 2,4-dinitrobenzenesulfonate

ZBG-3 (0.10 g, 0.73 mmol) was reacted with 2,4-dinitrobenzenesulfonyl chloride (0.3 g, 1.1 mmol) in 10 mL of pyridine to afford 1,2-dimethyl-4-oxo-1,4-dihydropyridin-3-yl 2,4-dinitrobenzenesulfonate (**PZBG-3d**) in 23% yield (0.07 g, 0.2 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 8.83$ (d, $J = 2.8$ Hz 1H), 8.37 (dd, $J_1 = 9.6$ Hz, $J_2 = 2.8$ Hz, 1 H), 7.82 (d, $J = 7.6$ Hz, 1 H), 7.03 (d, $J = 9.2$ Hz, 1 H), 6.25 (d, $J = 7.6$ Hz, 1 H), 3.68 (s, 3H), 2.31 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 169.35$, 154.98, 144.00, 142.61, 141.42, 140.76, 138.54, 129.69, 122.24, 118.15, 116.78, 59.63, 13.29.

7-Oxocyclohepta-1,3,5-trien-1-yl benzenesulfonate

2-Hydroxycyclohepta-2,4,6-trienone (**ZBG4**; 0.2 g, 1.7 mmol) was reacted with benzenesulfonyl chloride (0.63 mL, 4.9 mmol) in 5 mL of pyridine to afford 7-oxocyclohepta-1,3,5-trien-1-yl benzenesulfonate (**PZBG-4a**) in 76% yield (0.33 g, 1.3 mmol). ^{1}H NMR (400 MHz, DMSO) $\delta = 7.95$ (d, $J = 7.6$ Hz, 2H), 7.80 (t, $J = 7.6$ Hz, 1H), 7.67 (t, $J = 8$ Hz, 2H), 7.43–7.37 (m, 2H), 7.25 (t, $J = 8.4$ Hz, 1H), 7.14–7.09 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 179.24$, 154.79, 141.19, 138.21, 136.37, 136.33, 135.57, 131.96, 130.92, 130.27, 128.76. ESI-MS(+): *m/z* 262.97 $[M + H]^+$, 279.72 $[M + NH_4]^+$, 284.99 $[M + Na]^+$.

7-Oxocyclohepta-1,3,5-trien-1-yl 4-methylbenzenesulfonate

ZBG4 (0.2 g, 1.7 mmol) was reacted with *p*-toluenesulfonyl chloride (0.41 g, 2.0 mmol) in 10 mL of pyridine to afford 7-oxocyclohepta-1,3,5-trien-1-yl 4-methylbenzenesulfonate (**PZBG-4b**) in 63% yield (0.04 g, 0.1 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 7.92$ (d, $J = 8.4$ Hz, 2H), 7.46 (d, $J = 9.2$ Hz, 1H), 7.35 (d, $J = 8.4$ Hz, 2H), 7.26–7.16 (m, 2H), 7.13–7.06 (m, 1H), 6.98 (t, $J = 10$ Hz, 1H), 2.45 (s, 3H). ^{13}C (125 MHz, $CDCl_3$) $\delta = 179.41$, 155.15, 145.50, 141.23, 136.32, 134.61, 133.41, 130.81, 130.00, 129.60, 128.59, 21.78. ESI-MS(+): *m/z* 277.21 $[M + H]^+$, 293.99 $[M + NH_4]^+$.

7-Oxocyclohepta-1,3,5-trien-1-yl 4-nitrobenzenesulfonate

ZBG4 (0.2 g, 1.7 mmol) was reacted with 4-nitrobenzenesulfonyl chloride (1.1 g, 4.9 mmol) in 5 mL of pyridine. Addition of 10 mL of water allowed for precipitation of 7-oxocyclohepta-1,3,5-trien-1-yl 4-nitrobenzenesulfonate (**PZBG-4c**) in 71% yield (0.38 g, 1.2 mmol) without the need for further purification. ^{1}H NMR (400 MHz, DMSO) $\delta = 8.45$ (d, $J = 8.8$ Hz, 2H), 8.22 (d, $J = 8.8$ Hz, 2H), 7.52 (d, $J = 9.2$ Hz, 1H), 7,46 (dd, $J_1 = 10.2$ Hz, $J_2 = 3.6$ Hz, 1 H), 7.31 (dd, $J_1 = 9.8$ Hz, $J_2 = 2$ Hz, 1 H), 7.15 (dd, $J_1 = 10.6$ Hz, $J_2 = 3.6$ Hz, 1 H). ^{13}C (100 MHz, $CDCl_3$) $\delta = 179.09$, 154.86, 151.50, 141.95, 141.27, 138.59, 136.87, 132.06, 131.48, 130.42, 125.42. ESI-MS(+): *m/z* 308.01 $[M + H]^+$, 324.73 $[M + NH_4]^+$.

7-Oxocyclohepta-1,3,5-trien-1-yl 2,4-dinitrobenzenesulfonate

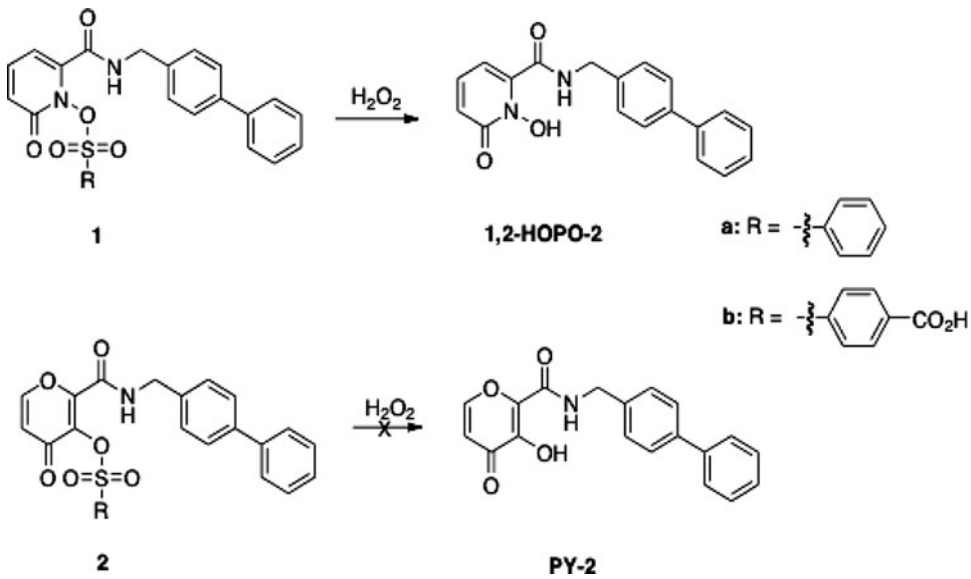
ZBG4 (0.2 g, 1.7 mmol) was reacted with 2,4-dinitrobenzenesulfonyl chloride (0.54 g, 2.0 mmol) in 10 mL of pyridine to afford 7-oxocyclohepta-1,3,5-trien-1-yl 2,4-dinitrobenzenesulfonate (**PZBG-4d**) in 6% yield (0.04 g, 0.1 mmol). ^{1}H NMR (400 MHz, DMSO) $\delta = 9.00$ (d, $J = 2.4$ Hz 1H), 8.66 (dd, $J_1 = 8.8$ Hz, $J_2 = 2.4$ Hz, 1 H), 8.38 (d, $J = 8.8$ Hz 1H), 7.66 (d, $J = 9.6$ Hz 1H), 7.50 (td, $J_1 = 8.4$ Hz, $J_2 = 3.6$ Hz, $J_3 = 1.2$ Hz, 1 H), 7.35 (td, $J_1 = 8.4$ Hz, $J_2 = 2.4$ Hz, 1 H), 7.20–7.15 (m, 2H). ^{13}C (125 MHz, DMSO) $\delta = 178.71$, 155.30, 151.20, 147.90, 140.96, 138.83, 137.10, 134.58, 133.22, 131.93, 131.55, 127.92, 121.11. ESI-MS(+): *m/z* 353.15 $[M + H]^+$, 375.11 $[M + Na]^+$.

6-(([1,1′-Biphenyl]-4-ylmethyl)carbamoyl)-2-oxopyridin-1(2*H*)-yl benzenesulfonate

1,2-HOPO-2 (Scheme 1) was prepared as previously reported [38]. In a 10-mL round-bottom flask was dissolved 0.05 g (0.16 mmol) of **1,2-HOPO-2** in 3 mL of pyridine. To this was added 60 μL (0.5 mmol) of benzenesulfonyl chloride. The reaction mixture was left stirring under nitrogen at room temperature overnight. After 16 h, the solvent was evaporated to leave an orange oil which was dissolved in dichloromethane and washed once with 1 M HCl then brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The product was purified on a silica gel column and eluted with 1% MeOH in dichloromethane to afford 6-(([1,1′-biphenyl]-4-ylmethyl)carbamoyl)-2-oxopyridin-1(2*H*)-yl benzenesulfonate (**1a**) in 67% yield (0.05 g, 0.1 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 8.01$ (d, $J = 7.6$ Hz, 2H), 7.76 (t, $J = 7.6$ Hz, 1 H), 7.61 (m, 6H), 7.47 (m, 4H), 7.38–7.31 (m, 2H), 6.62 (d, $J = 8.0$ Hz, 1H), 6.57 (d, $J = 6.4$ Hz, 1H), 4.63 (d, $J = 5.2$ Hz, 2H). ^{13}C (100 MHz, $CDCl_3$) $\delta = 159.2$, 157.0, 142.7, 141.2, 140.8, 138.9, 136.0, 134.5, 129.9, 129.5, 129.1, 128.9, 127.8, 127.7, 127.3, 125.2, 107.6, 44.5. ESI-MS(+): *m/z* 461.13 $[M + H]^+$, 483.13 $[M + Na]^+$. Anal. calcd for $C_{25}H_{20}N_2O_5S{\cdot}0.5\,H_2O$: C, 63.95; H, 4.51; N, 5.99. Found: C, 63.68; H, 5.14; N, 5.99.

4-(((6-(([1,1′-Biphenyl]-4-ylmethyl)carbamoyl)-2-oxopyridin-1(2*H*)-yl)oxy)sulfonyl) benzoic acid

1,2-HOPO-2 (0.20 g, 0.6 mmol) was dissolved in 5 mL of pyridine. To this was added 4-(chlorosulfonyl) benzoic acid (0.21 g, 1.0 mmol). The reaction was allowed to proceed overnight at room temperature. The solvent was evaporated and to the remaining oil was added 5 mL of dichloromethane followed by 5 mL of ethyl acetate, allowing for precipitation. The solid white product was filtered off and collected to afford 4-(((6-(([1,1′-biphenyl]-4-ylmethyl)carbamoyl)-2-oxopyridin-1(2*H*)-yl)oxy)sulfonyl) benzoic acid (**1b**) in 14% yield (0.04 g, 0.08 mmol). ^{1}H NMR (400 MHz, DMSO) $\delta = 9.36$ (t, $J = 5.6$ Hz, 1H, N*H*), 8.18 (d, $J = 8.8$ Hz, 2H), 8.06 (d, $J = 8.8$ Hz, 2H), 7.66–7.61 (m, 4H), 7.53 (dd, $J_1 = 7.8$ Hz, $J_2 = 2.8$ Hz, 1H), 7.45 (t, $J = 7.6$ Hz, 2H), 7.38–7.33 (m, 3H), 6.67 (dd, $J_1 = 9.2$ Hz, $J_2 = 1.6$ Hz, 1H), 6.47 (dd, $J_1 = 6.4$ Hz, $J_2 = 1.2$ Hz, 1H), 4.27 (d, $J = 5.6$ Hz, 2H). ^{13}C NMR (100 MHz, DMSO) $\delta = 166.55$, 159.45, 156.86, 143.21, 140.61, 140.54, 139.68, 138.26, 138.02, 137.61, 130.97,



Scheme 1 Activation of proinhibitor **1a** or **1b** with H_2O_2 results in generation of the inhibitor **1,2-HOPO-2**. In contrast, treatment of proinhibitor **2a** with H_2O_2 did not result in production of the inhibitor **PY-2**

130.09, 129.62, 128.73, 128.08, 127.33, 127.28, 124.19, 107.11, 43.05. ESI-MS(−): m/z 502.89 $[M - H]^-$. Anal. calcd for $C_{26}H_{20}N_2O_7S \cdot 0.25$ HCl: C, 60.80; H, 3.97; N, 5.45. Found: C, 60.92; H, 4.30; N, 5.73.

2-(([1,1′-Biphenyl]-4-ylmethyl)carbamoyl)-4-oxo-4*H*-pyran-3-yl benzenesulfonate

PY-2 (Scheme 1) was prepared as previously reported [38]. In a 50-mL round-bottom flask was dissolved 0.20 g (0.6 mmol) of **PY-2** in 15 mL of pyridine. To this was added 240 μL (1.8 mmol) of benzenesulfonyl chloride. The reaction mixture was left stirring under nitrogen at room temperature overnight. After 16 h, the solvent was evaporated to leave a red oil which was dissolved in dichloromethane and washed once with 1 M HCl then brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The product was precipitated from MeOH to afford 2-(([1,1′-biphenyl]-4-ylmethyl)carbamoyl)-4-oxo-4*H*-pyran-3-yl benzenesulfonate (**2a**) in 12% yield (0.03 g, 0.07 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 8.10$ (d, $J = 8.4$ Hz, 2H), 7.80 (d, $J = 5.6$ Hz, 1 H), 7.71 (t, $J = 7.6$ Hz, 1H), 7.61 (m, 6H), 7.47–7.36 (m, 6H), 6.47 (d, $J = 6.0$ Hz, 1H), 4.67 (d, $J = 6.0$ Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 172.7$, 157.3, 154.8, 151.3, 141.2, 140.8, 139.2, 136.0, 135.8, 135.2, 129.3, 129.2, 129.1, 128.9, 127.8, 127.7, 127.3, 118.4, 44.3. ESI-MS(+): m/z 461.98 $[M + H]^+$, 484.02 $[M + Na]^+$.

UV–vis spectroscopy

Absorption spectra of compounds were taken with a PerkinElmer Lambda 25 UV–vis spectrophotometer. To a 1.0 mL solution at 0.05 mM concentration of each compound in *N*-(2-hydroxyethyl)piperazine-*N*′-ethanesulfonic acid (HEPES) buffer (50 mM, pH 7.5) was added H_2O_2 (10 μL, 0.09 M in HEPES) and absorption spectra were monitored over time at room temperature. Hydrolytic stability was measured by monitoring each sample in HEPES buffer over a 24-h period.

Calculation of rate constant

The pseudo-first-order rate constant was calculated following a literature procedure [20]. To a 1.0 mL solution of **PZBG-1a**, **PZBG-1b**, **PZBG-1e**, **1a**, and **1b** in HEPES buffer at 50 μM was added H_2O_2 to final concentrations of 150, 250, 500, 750, and 900 μM. Spectra were monitored over 15–30 min at room temperature, with at least 50 spectra recorded at every concentration. The change in absorbance at 298 nm was monitored for **PZBG-1a** and **PZBG-1b**, whereas the change in absorbance at 288 nm was recorded for **PZBG-1e**, and at 310 nm for **1a** and **1b**. The rate constant (k_{obs}) was found from the linear slope of $\ln[(A - A_{ZBG})/(A_o - A_{ZBG})]$ versus time, where A_{ZBG} is the absorbance of a 50 μM sample of the ZBG or full-length inhibitor and A_o is the initial absorbance of **PZBG-1a**, **PZBG-1b**, **PZBG-1e**, **1a**, and **1b**. The rate of conversion was determined from the slope of the line of k_{obs} versus H_2O_2 concentration.

High-performance liquid chromatography

Analytical High-performance liquid chromatography (HPLC) was performed with a HP series 1050 system equipped with a Vydac® C_{18} reverse-phase column (218TP, 250 mm × 4.6 mm, 5 μm). Separation was achieved with a flow rate of 1 mL min^{-1} and the following solvents: solvent A was 5% MeOH and 0.1% formic acid in H_2O and solvent B was 0.1% formic acid in MeOH. Starting with 95% solvent A and 5% solvent B, we ran an isocratic gradient for 15 min to a final solvent mixture of 5% solvent A and 95% solvent B, which was held for 5 min before ramping back down to 95% solvent A and 5% solvent B in 2 min and holding for an additional 4 min. **ZBG-1a** and **PZBG-1a** were prepared in HEPES buffer (50 mM, pH 7.5) at a concentration of 1 mM and retention times were determined. To evaluate cleavage by H_2O_2, a 1 mM solution of **PZBG-1a** in HEPES buffer was reacted with a 20-fold excess of H_2O_2 before analysis under identical HPLC conditions as before.

Inhibition assays

MMP-12 (catalytic domain, human recombinant) was purchased from Enzo Life Sciences. The assays were carried out in a 96-well plate using a Bio-Tex Flx 800 plate reader. The activity of MMP-12 was evaluated after a 30-min incubation in the presence of H_2O_2 and proMMPi. The concentration of proMMPi used was selected to be close to the IC_{50} value of the parent full-length inhibitors, **1,2-HOPO-2** and **PY-2** [38]. In each well, 1 μL of proinhibitors **1a**, **1b**, and **2a** and the inhibitors **1,2-HOPO-2** and **PY-2** in DMSO (5 μM) were incubated for 30 min at 37 °C with 20 μL of MMP-12 (0.35 U mL^{-1}), 10 μL H_2O_2 (1 mM in HEPES buffer, pH 7.5), and MMP assay buffer (50 mM HEPES, 10 mM $CaCl_2$, 0.10% Brij-35, pH 7.5) for a total volume of 99 μL. A control sample containing 10 μL H_2O_2 (1 mM in HEPES buffer, pH 7.5) in MMP assay buffer was also prepared to confirm that H_2O_2 did not inhibit MMP-12. The reaction was initiated by the addition of 1 μL (400 μM) of the fluorescent substrate Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2 [where Mca is (7-methoxycoumarin-4-yl)acetyl and Dpa is *N*-3-(2,4-dinitrophenyl)-L-α,β-diaminopropionyl)] after the 30-min incubation period and kinetic activity was measured every minute for 20 min with excitation and emission

wavelengths at 335 and 405 nm, respectively. Enzyme activity with the inhibitor was calculated with respect to the control experiment—no inhibitor present. Measurements were performed in duplicate in two independent experiments.

Results and discussion

Assessment of sulfonate esters as suitable protecting groups for ZBGs

Previous studies utilizing fluorescent probes have shown sulfonate esters to be suitable protecting groups of hydroxyl groups that show a turn-on response upon exposure to ROS, including H_2O_2 and superoxide anion [35–37]. To investigate the use of sulfonate ester protecting groups for the development of ROS-activated proMMPi, a small library of compounds was synthesized. As shown in Fig. 1, sulfonate esters with different substituents were appended to **ZBG1-4** to evaluate which protected ZBGs (PZBGs) provided efficient activation in the presence of H_2O_2. These PZBGs were readily prepared by combining a ZBG with the appropriate sulfonyl chloride in pyridine. In total, 17 PZBGs (**PZBG-1a**–**PZBG-1e**, **PZBG-2a**–**PZBG-2d**, **PZBG-3a**–**PZBG-3d**, and **PZBG-4a**–**PZBG-4d**) were prepared and tested for cleavage in the presence of H_2O_2.

To evaluate cleavage of the compounds by ROS, a sample of each **PZBG** in HEPES buffer (50 mM, pH 7.5) was activated with excess H_2O_2 (0.9 mM, 18 equiv) and the change in absorbance was monitored over time via electronic spectroscopy. Surprisingly, only compounds derived from **ZBG-1** showed a change in absorbance (corresponding to the formation of the free ZBG) upon exposure to H_2O_2. The fact that only the five **PZBG-1** derivatives (out of 17 total combinations) were cleaved in the presence of H_2O_2 strongly suggests that the N–O group is essential for the observed reactivity. Figure 2 shows representative absorption spectra of **PZBG-1a** in the presence of H_2O_2. A decrease in absorbance at 298 nm over time is noted, representing the disappearance of **PZBG-1a** and a gradual increase in absorbance at 312 nm is observed, indicating the emergence of **ZBG-1**. In addition, analytical HPLC was used to confirm that **ZBG-1** was the product after reaction with H_2O_2 (Fig. 2). Upon the addition of H_2O_2 to **PZBG-1a** for 60 min, a peak with a retention time of 5.0 min was observed, which is identical to an authentic sample of **ZBG-1**. Similarly, treatment of **PZBG-1b** with H_2O_2 resulted in nearly identical spectra as found with **PZBG-1a**, whereas **PZBG-1c** showed rapid hydrolysis upon the addition of H_2O_2 (data not shown). It should be noted that the absorption spectra of **PZBG-1d** were not readily interpreted, owing to the overlapping of absorption profiles of the protecting group and the free ZBG; however, thin-layer chromatography showed the emergence of the free ZBG, demonstrating rapid hydrolytic cleavage of the protecting group (even in the absence of H_2O_2). These findings taken together prompted the synthesis of **PZBG-1e**, a more water-soluble alternative to **PZBG-1c**, with a carboxylic acid attached to the *para* position of the sulfonate ester. A substantial increase in solubility in buffered solution was noted, and the cleavage behavior was similar to that of the other compounds reported (see below), making **PZBG-1e** an attractive candidate for development into a full-length proMMPi. As mentioned above, sulfonate ester derivatives of **ZBG-2a**–**ZBG-2d**, **ZBG-3a**–**ZBG-3d**, and **ZBG-4a**–**ZBG-4d** did not show any change in absorbance over a period of 1 h with an 18 M excess of H_2O_2. These findings suggest that the N–O bond in **ZBG-1** is required for facile cleavage of the sulfonate ester group in this series of ligands, although this will require verification by additional studies. It is interesting to note that the compounds tested that did not contain the N–O moiety (those based on **PZBG-2**, **PZBG-3**, and **PZBG-4**) appeared to be stable in aqueous buffer (over at least a 1-h period).

One key factor for any prodrug approach is the stability of the protecting group in the absence of the triggering stimuli. To test the stability of the sulfonate esters in buffer, absorption spectra for **PZBG-1a**, **PZBG-1b**, and **PZBG-1e** were collected over 24 h. These stability studies showed approximately 50% cleavage of **PZBG-1a** and **PZBG-1e** in 6 h, whereas **PZBG-1b** was approximately 30% cleaved in 24 h. The rates of conversion of **PZBG-1a**, **PZBG-1b**,

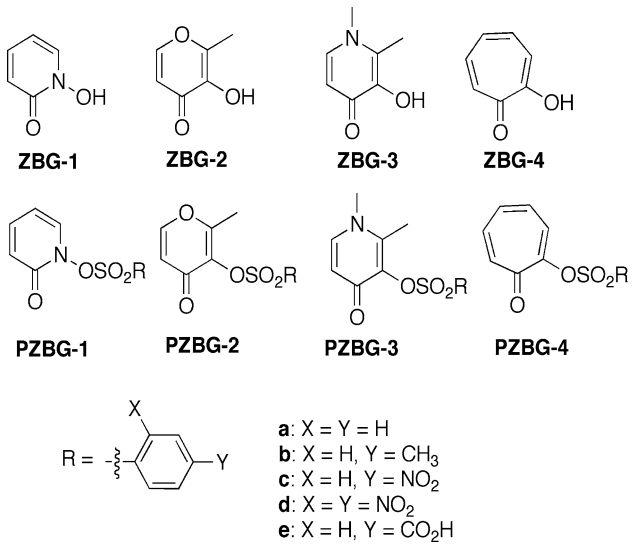


Fig. 1 Zinc-binding groups (ZBGs) and their sulfonate ester derivatives (protected ZBGs; PZBGs) examined in this study

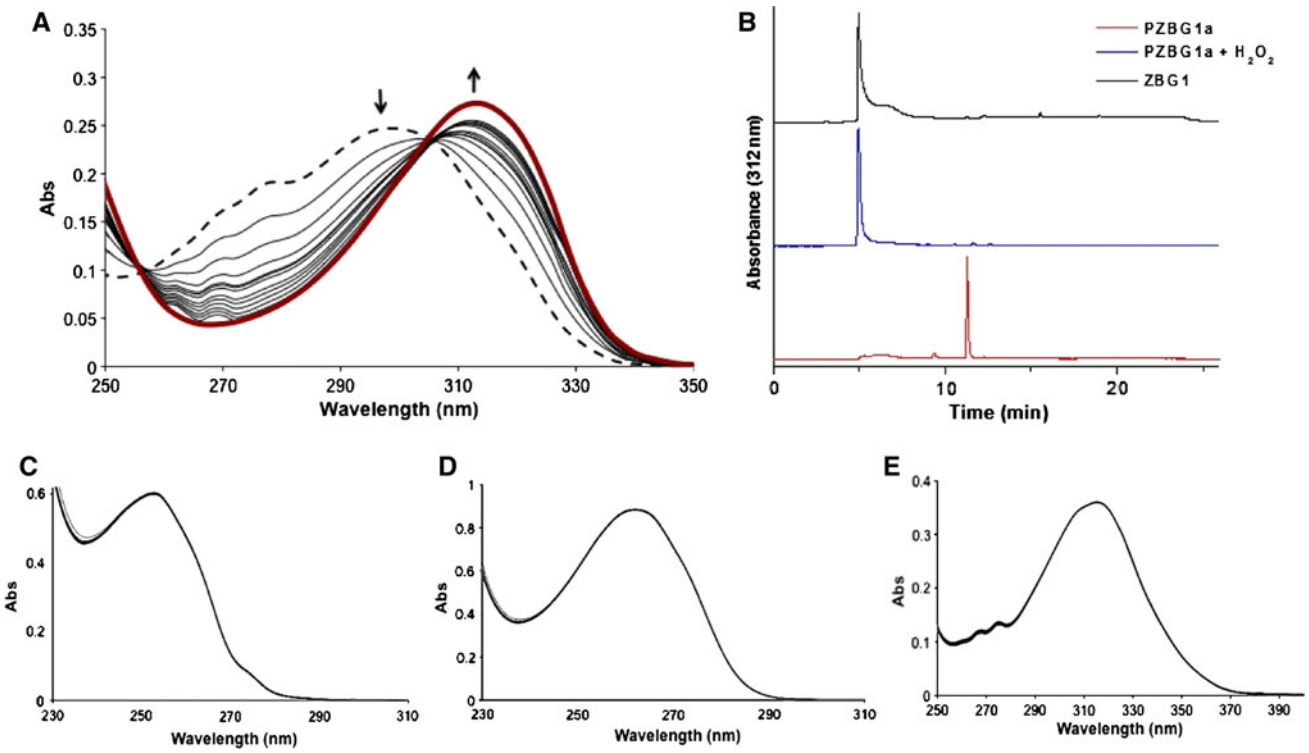


Fig. 2 Analysis of PZBGs in the presence of H_2O_2. **a** Absorption spectra of **PZBG-1a** [0.05 mM, 50 mM *N*-(2-hydroxyethyl)piperazine-*N*′-ethanesulfonic acid (HEPES) buffer, pH 7.5] in the presence of H_2O_2 (0.9 mM, 18 equiv) monitored every 5 min for 60 min. The *dashed line* represents the initial spectrum, and an authentic sample of **ZBG-1** is shown in *red*. The *arrows* indicate changes in spectra over time. **b** High performance liquid chromatography chromatograms of **PZBG-1a**, **PZBG-1a** + H_2O_2, and **ZBG-1**. The retention times are 11.5 min for **PZBG-1a** and 5.0 min for **ZBG-1**. **c–e** Absorption spectra of **PZBG-2a**, **PZBG-3a**, and **PZBG-4a**, respectively (0.05 mM, 50 mM HEPES buffer, pH 7.5), in the presence of H_2O_2 (0.9 mM, 18 equiv) monitored every 5 min for 60 min. The overlapping spectra indicate that no cleavage of the protecting group is occurring in the presence of H_2O_2

and **PZBG-1e** were determined by monitoring the change in absorbance using pseudo-first-order reaction conditions with an excess of H_2O_2 as previously reported [20]. The calculated rate constants indicate that **PZBG-1e** had the fastest rate constant at 1.3 M^{-1} s^{-1}, whereas rate constants of 0.7 and 0.3 M^{-1} s^{-1} were determined for **PZBG-1a** and **PZBG-1b**, respectively. It should be noted that the rate constants determined do take into account background hydrolysis and reactivity with H_2O_2; however, all kinetic measurements were taken over a 15–30-min period, which is before a measurable amount of hydrolysis was observed. Experiments with **PZBG-1c** and **PZBG-1d** showed the fastest cleavage kinetics upon exposure to H_2O_2, with complete dissociation achieved in less than 3 min (no rate constants determined). The rates of conversion for the **PZBG-1** compounds are consistent with the nature of the respective substituents on the leaving groups. When substituents are varied on an aromatic ring, the change in free energy of activation for a given reaction is proportional to the change in Gibbs free energy, as summarized by the Hammett equation [39]. Of the molecules for which rate constants were obtained, **PZBG-1e**, which contains an electron-withdrawing group ($–CO_2H$) in the *para* position, dissociates the fastest. **PZBG-1b**, on the other hand, with an electron-donating group ($–CH_3$) in the *para* position, had the slowest rate of the compounds tested. **PZBG-1a**, with no substituents, had a cleavage rate falling between the others, consistent with the Hammett relationship. The results with **PZBG-1c** and **PZBG-1d** are also consistent with this relationship, with the strongly electron withdrawing nitro groups producing the fastest rates, resulting in the inability to acquire precise values.

Development of full-length proMMPi

Having demonstrated the ability of sulfonate esters to act as cleavable protecting groups for **ZBG-1**, we incorporated this chelator into a full-length proMMPi. The corresponding full-length inhibitor of **ZBG-1** with a hydrophobic biphenyl backbone, **1,2-HOPO-2**, has been previously prepared and studied [38]. **1,2-HOPO-2** is an effective inhibitor of MMP-3, MMP-8, and MMP-12, with IC_{50} values under 100 nM [38]. Two full-length MMPi (**1,2-HOPO-2** and **PY-2**) were prepared by previously reported procedures and then

protected in pyridine with an excess of the appropriate sulfonyl chloride to generate proMMPi **1a**, **1b**, and **2a** (Scheme 1). The proMMPi **1a** and **1b** were evaluated for activation by H_2O_2 via electronic spectroscopy in the same manner as with the PZBG compounds. As shown in Scheme 1, the activation of **1a** and **1b** to the known MMPi **1,2-HOPO-2** is achieved upon the addition of H_2O_2. Figure 3 shows the absorption spectra of **1b**, for which a decrease at 310 nm over time is observed, indicating the disappearance of the protected MMPi, and a gradual increase in absorbance at 350 nm is observed, demonstrating the emergence of **1,2-HOPO-2**. Pseudo-first-order rate constants were determined with an excess of H_2O_2 as described earlier. Rate constants of 0.3 and 1.1 M^{-1} s^{-1} were obtained for **1a** and **1b**, respectively, which are in good agreement with the rate constants determined for the protected chelators **PZBG-1a** and **PZBG-1e**. To evaluate the stability of the sulfonate esters, absorption spectra of **1a** and **1b** were collected in buffer alone. Compounds **1a** and **1b** showed approximately 50% hydrolysis after 9 and 3 h, respectively.

To further confirm that the behavior of the protected chelators (PZBGs) was readily translated to a complete proMMPi, a proinhibitor based on **PZBG-2a** was synthesized. Compound **2a**, which contains a biphenyl backbone like **1a**, was prepared. Upon cleavage of the protecting group, **2a** should produce **PY-2**, a known inhibitor of several MMPs [38]. Treatment of **2a** with excess H_2O_2 over the course of 60 min did not result in cleavage of the sulfonate ester (Scheme 1), as evidenced by absorption spectroscopy (data not shown). This negative result is consistent with all of the findings described above, showing that the cleavage behavior of the PZBG is retained in its full-length proMMPi.

MMP inhibition studies

To monitor the ability of the protected compounds to inhibit MMP-12 in the presence of H_2O_2, a fluorescence-based assay was used [40]. Compounds **1a**, **1b**, and **2a** were tested at concentrations close to the IC_{50} values of their active parent molecules, **1,2-HOPO-2** and **PY-2**, against MMP-12. The percent inhibition of proinhibitors **1a**, **1b**, and **2a** at 50 nM was evaluated after 30 min of incubation with and without H_2O_2 (Fig. 4). Before treatment with H_2O_2, **1a** and **1b** were shown to exhibit approximately 20% and 30% inhibition of MMP-12, respectively. This significant level of inhibition is likely due to the hydrolysis of these compounds to the active MMPi during the incubation period, as described earlier for the stability studies. In addition, the inhibition assays were performed at a higher temperature than our kinetic experiments, which may result in an increase in the rate of hydrolysis and therefore lead to higher inhibition than might otherwise be expected. After treatment with 100 μM H_2O_2, the percent inhibition by proinhibitors **1a** and **1b** increased to approximately 30% and 50%, respectively, indicative of activation to **1,2-HOPO-2**. Proinhibitor **2a** was used as a negative control, and as expected, displayed no significant change in inhibitory activity upon exposure to H_2O_2.

Conclusions

In summary, we have demonstrated an alternative method to prepare proMMPi that are activated in the presence of ROS. The addition of sulfonate ester protecting groups to

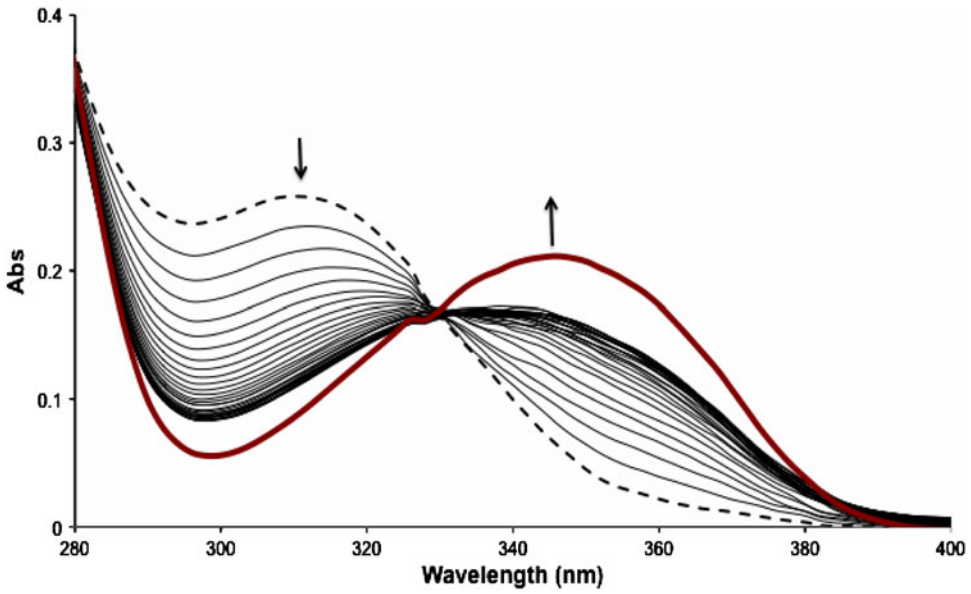


Fig. 3 Absorption spectra of **1b** (0.05 mM, 50 mM HEPES buffer, pH 7.5) in the presence of H_2O_2 (0.9 mM, 18 equiv excess) monitored every 5 min for 60 min. The *dashed line* represents the initial spectrum, and an authentic sample of **1,2-HOPO-2** is shown in *red*. The *arrows* indicate changes in spectra over time

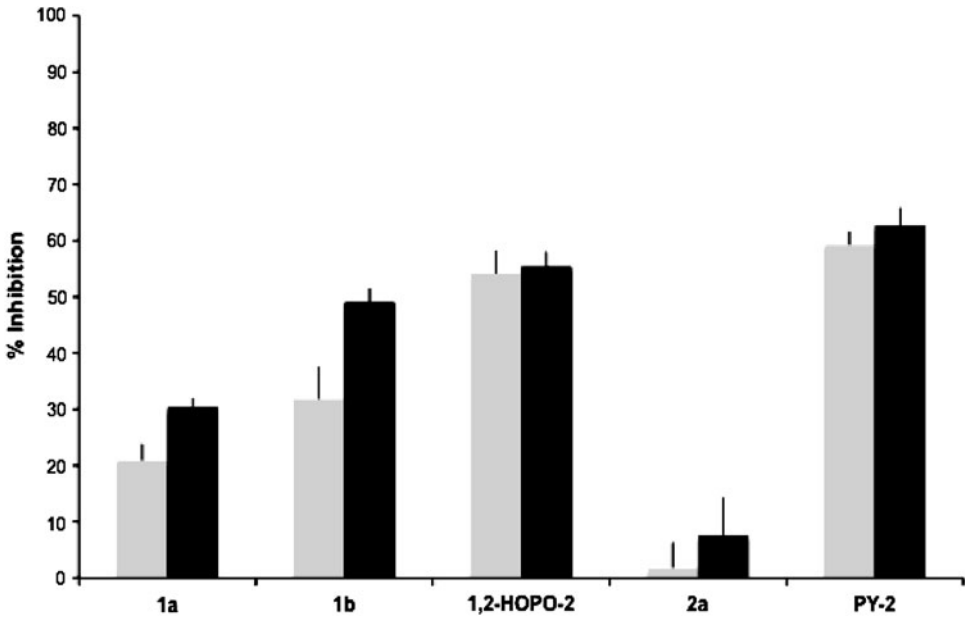


Fig. 4 Percent inhibition of MMP-12 with proinhibitors **1a**, **1b**, and **2a** tested at 50 nM in the absence (*gray bars*) and presence (*black bars*) of H_2O_2 after 30 min of activation

the ZBG blocks the chelating ability of the inhibitors, thereby attenuating their activity. The sulfonate ester proMMPi are easy to synthesize, have tunable rates of cleavage (modulated by appropriate substituents on the leaving group), and can be made reasonably water soluble by addition of substituents, such as carboxylic acids, on the aromatic ring of the protecting group. Unfortunately, the limited hydrolytic stability of the sulfonate ester protected ZBGs is a significant drawback. Nearly complete hydrolysis of the sulfonate ester proMMPi was observed after 24 h. In contrast, self-immolative boronic ester derivatized proMMPi were entirely stable under identical conditions [31]. In addition, the sulfonate esters were only cleaved from one (**ZBG-1**) of four different chelators tested, suggesting that the present strategy may not be readily applied to inhibitors with other ZBGs. Nonetheless, complete proMMPi based on these sulfonate ester protected chelators were activated after treatment with H_2O_2 at relatively fast rates, resulting in an increase in inhibition of MMP-12. It is expected that combining sulfonate esters with a self-immolative strategy may produce more stable, and more rapidly cleavable proinhibitors [31, 41, 42]. These and related experiments are under way.

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References

1. Whittaker M, Flyod CD, Brown P, Gearing AJH (1999) Chem Rev 99:2735–2776
2. Coussens L, Fingelton B, Matrisian LM (2002) Science 295:2387–2392
3. Jacobsen JA, Major Jourden JL, Miller MT, Cohen SM (2010) Biochim Biophys Acta 1803:72–94
4. Fingleton B (2008) Semin Cell Dev Biol 19:61–68
5. Renkiewicz R, Qiu L, Lesch C, Sun X, Devalaraja R, Cody T, Kaldjian E, Welgus H, Baragi V (2003) Arthritis Rheum 48:1742–1749
6. Basset P, Bellocq JP, Wolf C, Stoll I, Hutin P, Limacher JM, Podhajcer OL, Chenard MP, Rio MC, Chambon P (1990) Nature 348:699–704
7. Wang J, Tsirka SE (2005) Brain 128:1622–1633
8. Wang Q, Tang XN, Yenari MA (2007) J Neuroimmunol 184:53–68
9. Rosenberg GA, Cunningham LA, Wallace J, Alexander S, Estrada EY, Grossetete M, Razhagi A, Miller K, Gearing A (2001) Brain Res 893:104–112
10. Mun-Bryce S, Rosenberg GA (1998) J Cereb Blood Flow Metab 18:1163–1172
11. Fishman R (1975) N Engl J Med 293:706–711
12. Haorah J, Ramirez SH, Schall K, Smith D, Pandya R, Persidsky Y (2007) J Neurochem 101:566–576
13. Copin J-C, Merlani P, Sugawara T, Chan PH, Gasche Y (2008) Exp Neurol 213:196–201
14. Yang Y, Estrada EY, Thompson JF, Liu W, Rosenberg GA (2007) J Cereb Blood Flow Metab 27:697–709
15. Overall CM, Kleifeld O (2006) Br J Cancer 94:941–946
16. Nagase H, Woessner JF Jr (1999) J Biol Chem 274:21491–21494
17. Nelson AR, Fingleton B, Rothenberg ML, Matrisian LM (2000) J Clin Oncol 18:1135–1149
18. Peterson JT (2006) Cardiovasc Res 69:677–687
19. Jin R, Yang G, Li G (2010) Neurobiol Dis 38:376–385
20. Dickens MG, Franz KJ (2010) ChemBioChem 11:59–62
21. Perez LR, Franz KJ (2010) Dalton Trans (39):2177–2187
22. Schugar H, Green DE, Bowen ML, Scott LE, Storr T, Bohmerle K, Thomas F, Allen DD, Lockman PR, Merkel M, Thompson KH, Orvig C (2007) Angew Chem Int Ed 46:1716–1718

23. Storr T, Merkel M, Song-Zhao GX, Scott LE, Green DE, Bowen ML, Thompson KH, Patrick BO, Schugar H, Orvig C (2007) J Am Chem Soc 129:7453–7463
24. Wei Y, Guo M (2007) Angew Chem Int Ed 46:4722–4725
25. Wei Y, Zhang Y, Liu Z, Guo M (2010) Chem Commun 46:4472–4474
26. Failes TW, Cullinane C, Diakos N, Yamamoto N, Lyons JG, Hambley TW (2007) Chem Eur J 13:2974–2982
27. Failes TW, Hambley TW (2006) Dalton Trans (15):1895–1901
28. Failes TW, Hambley TW (2007) J Inorg Biochem 101:396–403
29. Mitchell MB, Whitcombe IWA (2000) Tetrahedron Lett 41:8829–8834
30. Major Jourden JL, Cohen SM (2010) Chem Commun 46:1241–1243
31. Major Jourden JL, Cohen SM (2010) Angew Chem Int Ed 49:6795–6797
32. Tietze LF, Feuerstein T (2003) Aust J Chem 56:841–854
33. Miller EW, Tulyathan O, Isacoff EY, Chang CJ (2007) Nat Chem Biol 3:263–267
34. Miller EW, Albers AE, Pralle A, Isacoff EY, Chang CJ (2005) J Am Chem Soc 127:16652–16659
35. Maeda H, Fukuyasu Y, Yoshida S, Fukuda M, Saeki K, Matsuno H, Yamauchi Y, Yoshida K, Hirata K, Miyamoto K (2004) Angew Chem Int Ed 43:2389–2391
36. Maeda H, Yamamoto K, Nomura Y, Kohno I, Hafsi L, Ueda N, Yoshida S, Fukuda M, Fukuyasu Y, Yamauchi Y, Itoh N (2005) J Am Chem Soc 127:68–69
37. Xu K, Tang B, Huang H, Yang G, Chen Z, Li P, An L (2005) Chem Commun (48):5974–5976
38. Agrawal A, Romero-Perez D, Jacobsen JA, Villarreal FJ, Cohen SM (2008) ChemMedChem 3:812–820
39. Hammett LP (1937) J Am Chem Soc 59:96–103
40. Knight CG, Willenbrock F, Murphy G (1992) FEBS Lett 296:263–266
41. Haba K, Papkov M, Shamis M, Lerner RA, Barbas CF III, Shabat D (2005) Angew Chem Int Ed 44:716–720
42. Weinstain R, Baran PS, Shabat D (2009) Bioconjug Chem 20:1783–1791