Zinc-Binding Groups Modulate Selective Inhibition of MMPs

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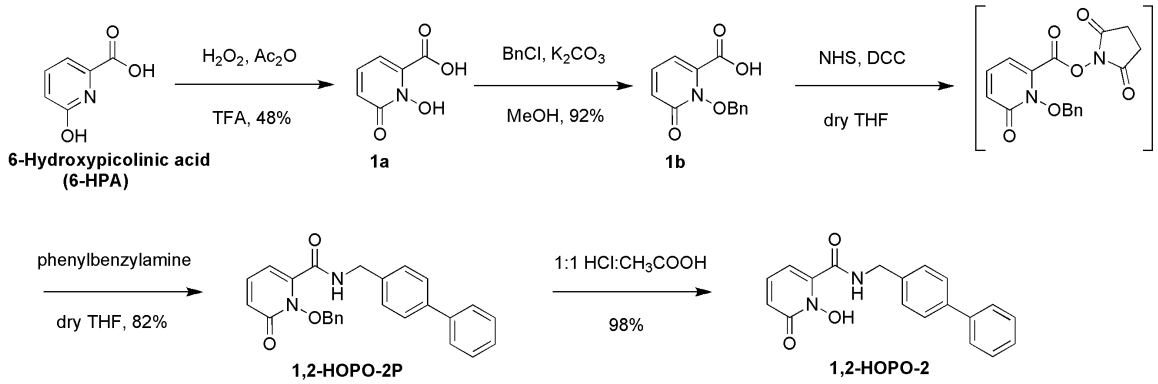
SUPPORTING INFORMATION

1,2-HOPO-2 (Compound 1)



(1a): Syntheses of **1a** and **1b** were based on literature procedures (1). 80 mL of acetic anhydride and 20 mL of 30% hydrogen peroxide were combined and stirred under N_2 for 4 h in an ice-bath to form a peracetic acid solution. 120 mL of trifluoroacetic acid (TFA) and 80 mL of glacial acetic acid were added to 20 g (144 mmol) of 6-hydroxypicolinic acid (6-HPA) in a separate flask; the solution turned a dark brown. The peracetic acid solution was added drop wise to the dark brown solution and the mixture was stirred under N_2 for 1 h. A suspension formed that fully dissolved after 1 h to a clear orange solution that was heated to reflux at 80°C for 10 h under N_2 giving an off-white precipitate. The reaction was cooled to room temperature and then cooled to 4°C for 5-6 h to allow more precipitate to crash out. The precipitate was vacuum filtered, washed with cold MeOH, and dried in a vacuum oven overnight. The dried precipitate was dissolved in 40 mL of 10% KOH and refluxed at 80°C under N_2 for 6 h to yield an off-white precipitate in a clear orange solution. Concentrated HCl was added to the orange solution, which was cooled to 4°C overnight to generate more precipitate. The combined precipitates were vacuum filtered, washed with water, and dried in a vacuum oven to yield 10.8 g (70 mmol) of an off-white solid. Yield = 48%. ^{1}H NMR (400 MHz,

DMSO-d_6): δ 6.64 (dd, J = 6.8, 1.2 Hz, 1H), 6.72 (dd, J = 9.2, 1.2 Hz, 1H), 7.43 (dd, J = 9.0, 7.2 Hz, 1H). ESI-MS(-): *m/z* 153.93 $[M-H]^-$.

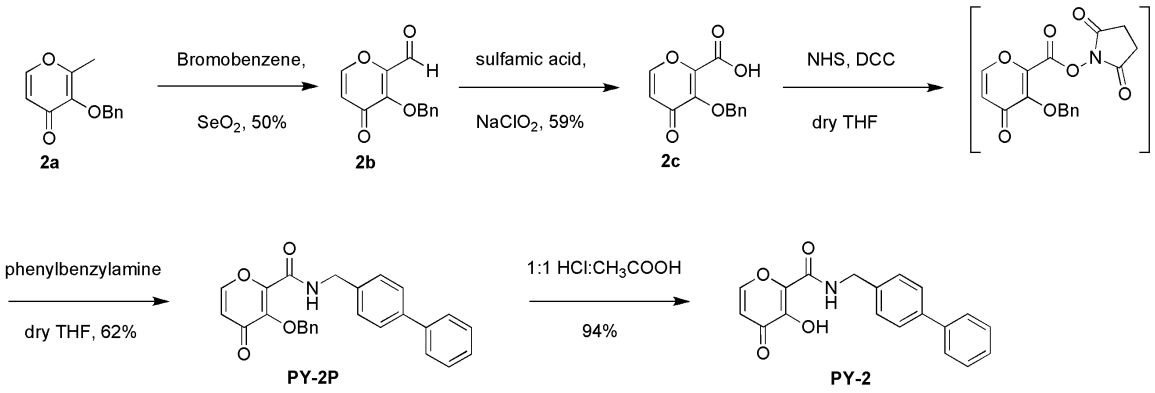
(1b): 7.2 g (46.4 mmol) of compound **1a**, 10.6 mL (11.7 g, 92.8 mmol) of benzyl chloride, and 12.8 g (92.8 mmol) of K_2CO_3 were mixed in 115 mL of MeOH. The mixture was heated to reflux for 16 h at 75°C under N_2, vacuum filtered, and the filtrate was evaporated to dryness. The lightly brown and gummy residue was dissolved in water (some heating required). The solution was acidified to pH 2 with 6N HCl, which resulted in a white precipitate. The precipitate was vacuum filtered, washed with cold water, and dried in a vacuum oven to yield 10.5 g (42.8 mmol) of an off-white solid. Yield = 92%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.27 (s, 2H), 6.56 (dd, J = 7, 1.2 Hz, 1H), 6.74 (dd, J = 9.4, 1.8 Hz, 1H), 7.40-7.51 (m, 6H).

(1,2-HOPO-2P): 10.5 g (42.8 mmol) of compound **1b** was dissolved in 150 mL of dry THF. 4.9 g (42.8 mmol) of N-hydroxysuccinimide (NHS) was added to the flask and the mixture was stirred for 30 min under N_2 to a clear yellow solution. 8.8 g (42.8 mmol) of N,N'-dicyclohexylcarbodiimide (DCC) was added to the solution and stirred for 3 h under N_2 to yield the activated ester shown in the scheme above. The solution was vacuum filtered to remove the precipitated dicyclohexylurea DCU. 7.8 g (42.8 mmol) of 4-phenylbenzylamine was added to the clear amber filtrate and the mixture was stirred for 40 h at room temperature under N_2. The solution was evaporated to an off-white residue, dissolved in a minimal amount of CH_2Cl_2, extracted (3×) with saturated $NaHCO_3$, and the organic layer was dried over anhydrous $MgSO_4$. $MgSO_4$ was filtered off and the filtrate

was evaporated to an off-white residue. The residue was sonicated in minimal amount of MeOH to remove any remaining DCU. The solution was vacuum filtered and the precipitate was dried in a vacuum oven overnight to yield 14.4 g (35 mmol) of a white solid. Yield = 82%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.48 (d, J = 6 Hz, 2H), 5.24 (s, 2H), 6.38 (dd, J = 6.4, 1.6 Hz, 1H), 6.68 (dd, J = 9.4, 1.4 Hz, 1H), 7.36-7.62 (m, 15H), 9.46 (t, J = 5.8 Hz, 1H, NH). APCI-MS(+): *m/z* 410.85 $[M+H]^+$.

(1,2-HOPO-2): To 6.2 g (15.1 mmol) of **1,2-HOPO-2P** was added 180 mL of a 1:1 solution of concentrated HCl and glacial acetic acid. The solution was stirred at room temperature for 5 d to obtain a white milky solution. The solution was evaporated to dryness to yield a white residue which was further co-evaporated with methanol (3×) and the solid was dried in a vacuum oven overnight to yield 4.8 g (14.8 mmol) of a white solid. Yield = 98%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.47 (d, J = 6 Hz, 2H), 6.35 (dd, J = 6.8, 1.6 Hz, 1H), 6.59 (dd, J = 9, 1.4 Hz, 1H), 7.32-7.65 (m, 10H), 9.35 (t, J = 5.8 Hz, 1H, NH). APCI-MS(-): *m/z* 319.11 $[M-H]^-$. HR-EI-MS Calculated for $C_{19}H_{16}N_2O_3$: 320.1155. Expected: 320.1158. Anal. Calcd (Found) for $C_{19}H_{16}N_2O_3$: C 71.24% (70.95), H 5.03% (5.15), N 8.74% (8.70).

PY-2 (Compound 2)



(2b): Synthesis of benzyl-protected maltol (**2a**) was based on literature procedures (see reference XX). 4.39 mL (5 g, 23 mmol) of benzyl maltol and 62 mL of fresh bromobenzene were heated to 105°C and stirred for 5 min. To the solution was added 7.65 g (69 mmol) of selenium dioxide and the mixture was refluxed at 150°C for 40 h under N_2. The solution turned dark brown with a black residue on the bottom. The solution was vacuum filtered over a layer of sand to remove the selenium dioxide. The filtrate was diluted with diethyl ether, filted to remove insoluble impurities, and then evaporated to dryness to yield 2.4 g (10.4 mmol) of a brown oil. Yield = 45%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.34 (s, 2H), 6.61 (d, J = 4.8 Hz, 1H), 7.32-7.39 (m, 5H), 8.23 (d, J = 5.6 Hz, 1H), 9.82 (s, 1H).

(2c): To a flask containing 2.4 g (10.4 mmol) of compound **2a** was added 50 mL of a 1:1 solution of acetone and water. 1.4 g (14.6 mmol) of sulfamic acid and 988 mg (10.9 mmol) of 80% sodium chlorite were added to the clear orange solution which turned yellow. The solution was stirred at room temperature for 6 h, evaporated to dryness, and dried in a vacuum oven to yield 1.5 g (6.1 mmol) of an off-white solid. Yield = 59%. ^{1}H

NMR (400 MHz, DMSO-d_6): δ 5.10 (s, 2H), 6.54 (d, J = 6 Hz, 1H), 7.31-7.43 (m, 5H), 8.20 (d, J = 6 Hz, 1H).

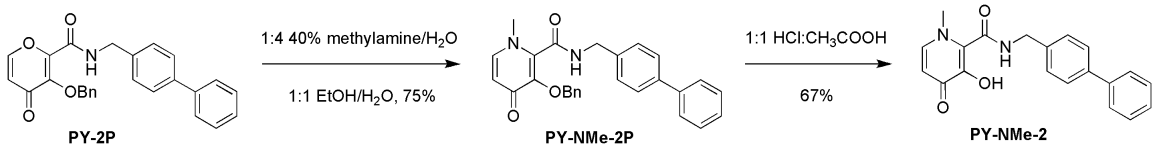
(PY-2P): 500 mg (2 mmol) of compound **2c** was dissolved in 30 mL of dry THF to give clear yellow solution. 4-Phenylbenzylamine was coupled to **2c** in an identical fashion to that reported above for compound **1b**. 520 mg (1.3 mmol) of a white solid was obtained. Yield = 62%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.45 (d, J = 6 Hz, 2H), 5.16 (s, 2H), 6.54 (d, J = 5.6 Hz, 1H), 7.29-7.64 (m, 14H), 8.22 (d, J = 5.6 Hz, 1H), 9.19 (t, J = 2.8 Hz, 1H, NH). ESI-MS(+): *m/z* 410.95 $[M+H]^+$. HR-EI-MS Calculated for $C_{26}H_{21}NO_4$: 411.1472. Expected 411.1465.

(PY-2): Deprotection of **PY-2P** was identical to that of **1,2-HOPO-2P** described above. 500 mg (1.3 mmol) of **PY-2P** was treated with acid to yield 365 mg (1.1 mmol) of a white solid. Yield = 94%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.53 (d, J = 7.6 Hz, 2H), 6.48 (d, J = 7.2 Hz, 1H), 7.39-7.65 (m, 9H), 8.18 (d, J = 7.2 Hz, 1H), 9.36 (s, 1H, NH). APCI-MS(-): *m/z* 320.06 $[M-H]^-$. HR-EI-MS Calculated for $C_{19}H_{15}NO_4$: 321.0993. Expected 321.0996. Anal. Calcd (Found) for $C_{19}H_{15}NO_4$: C 71.02% (71.15), H 4.71% (5.04), N 4.36% (4.43).

AM-2 (Compound 3)

The synthesis of this compound has been previously published (3). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.30 (s, 3H), 4.52 (d, J = 6.4 Hz, 2H), 6.32 (s, 1H), 7.32-7.64 (m, 9H), 9.34 (t, J = 6Hz, 1H, NH). ESI-MS(+): *m/z* 335.95 $[M+H]^+$. Anal. Calcd (Found) for $C_{20}H_{17}NO_4$: C 71.63% (71.44), H 5.11% (5.42), N 4.18% (4.04).

PY-NMe-2 (Compound 4)

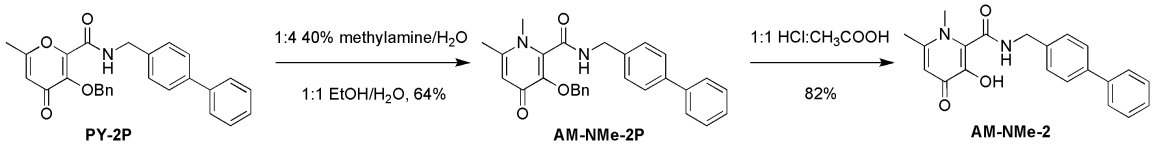


(PY-NMe-2P): Synthesis of this compound was based on a procedure by Harris (2)**.** To 100 mg (0.24 mmol) of **PY-2P** was added 5 mL of a 1:1 solution of EtOH and H_2O to produce a white suspension. The suspension was heated to 80°C. To the suspension was added 5 mL of a 1:4 solution of 40% methylamine and H_2O in a drop wise fashion. The solution turned yellow after 15 min. The reaction was stirred for 17 h and a cream colored precipitate settled on the bottom. The reaction was cooled to room temperature, the precipitate was vacuum filtered and then dried in a vacuum oven to yield 75 mg (0.18 mmol) of a white solid. Yield = 75%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.56 (s, 3H), 4.48 (d, J = 10 Hz, 2H), 5.08 (s, 2H), 6.26 (d, J = 7.2 Hz, 1H), 7.48-7.60 (m, 14H), 7.67 (d, J = 7.2 Hz, 1H), 9.44 (t, J = 6.2 Hz, 1H, NH). ESI-MS(+): *m/z* 425.02 $[M+H]^+$.

(PY-NMe-2): Deprotection of **PY-NMe-2P** was similar to that of **1,2-HOPO-2P** described above. 50 mg (0.12 mmol) of **PY-NMe-2P** was deprotected to yield 26 mg (0.08 mmol) of a white solid. Yield = 67%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.81 (s,

3H), 4.54 (d, J = 6 Hz, 2H), 6.84 (d, J = 6 Hz, 1H), 7.34-7.67 (m, 9H), 8.00 (d, J = 7.2 Hz, 1H), 9.50 (t, J = 5.8 Hz, 1H, NH). APCI-MS(-): *m/z* 333.08 $[M-H]^-$. Anal. Calcd (Found) for $C_{20}H_{18}N_2O_3 \cdot 0.4H_2O$: C 70.33% (70.20), H 5.55% (5.79), N 8.20% (8.44).

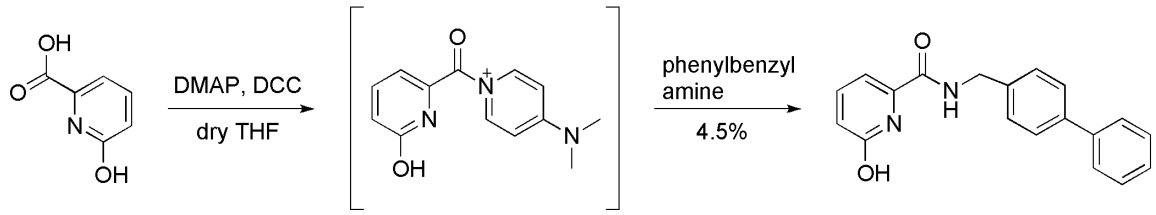
AM-NMe-2 (Compound 5)



(AM-NMe-2P): Synthesis of **AM-NMe-2P** was identical to that described above for **PY-NMe-2P**. 100 mg (0.24 mmol) of **AM-2P** was used to yield 67 mg (0.15 mmol) of a yellow solid of **AM-NMe-2P**. Yield = 64%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.31 (s, 3H), 3.45 (s, 3H), 4.48 (d, J = 7.2 Hz, 2H), 5.05 (s, 2H), 6.24 (s, 1H), 7.31-7.65 (m, 14H), 9.43 (t, J = 6.8 Hz, 1H, NH). ESI-MS(+): *m/z* 439.06 $[M+H]^+$.

(AM-NMe-2): Deprotection of **AM-NMe-2P** was similar to that of **1,2-HOPO-2P** described above 50 mg (0.11 mmol) of **AM-NMe-2P** was treated with acid to yield 32 mg (0.09 mmol) of a white solid of **AM-NMe-2**. Yield = 82%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.50 (s, 3H), 3.70 (s, 3H), 4.56 (d, J = 6 Hz, 2H), 6.93 (s, 1H), 7.33-7.68 (m, 9H), 9.54 (t, J = 5.8 Hz, 1H, NH). APCI-MS(-): *m/z* 347.05 $[M-H]^-$. HR-EI-MS Calculated for $C_{21}H_{20}N_2O_3$: 348.1470. Expected 348.1468. Anal. Calcd (Found) for $C_{21}H_{20}N_2O_3 \cdot 0.5H_2O$: C 70.57% (70.40), H 5.92% (6.00), N 7.84% (8.21).

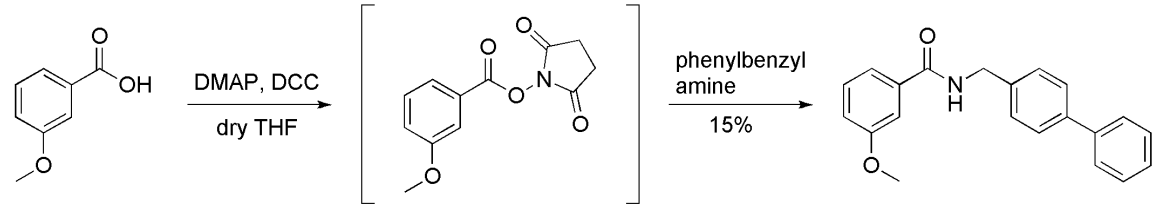
PICO-2 (Compound 6)



6-HPA **PICO-2**

(PICO-2): 2 g (14.4 mmol) of 6-HPA was dissolved in 100 mL of dry THF. To the solution was added 0.53 g (4.3 mmol) of dimethylaminopyridine (DMAP) and 2.97 g (14.4 mmol) of DCC and the mixture was stirred at room temperature for 3 h. The solution was vacuum filtered to remove DCU as a white precipitate. 2.6 g (14.4 mmol) of 4-phenylbenzylamine was added to the filtrate and the solution was stirred overnight. The solution was vacuum filtered and the filtrate was evaporated to dryness. The residue was dissolved in 300 mL of CH_2Cl_2 and 10 mL of MeOH which was then sonicated, heated, and concentrated to 70 mL. The suspension was vacuum filtered and the precipitate was dried in a vacuum oven to yield 199 mg (0.66 mmol) of a white solid. Yield = 4.5%. ^{1}H NMR (500MHz, $CDCl_3$): δ 4.67 (d, J = 6 Hz, 2H), 6.63 (d, J = 10 Hz, 1H), 7.04 (d, J = 6.5 Hz, 1H), 7.36 (t, 1H), 7.44 (dt, 4H), 7.52 (dd, 1H), 7.58 (dd, 4H), 7.64 (t br, 1H). IR (KBr pellet): ν 1302, 1537, 1647, 3060, 3304, 3435 cm^{-1}. ESI-MS(+): m/z 304.91 $[M+H]^+$. Anal. Calcd (Found) for $C_{19}H_{16}N_2O_2$: C 74.98% (74.59), H 5.30% (5.50), N 9.20% (9.46).

m-ANISIC-2 (Compound 7)



***m*-Anisic Acid** ***m*-ANISIC-2**

(*m*-ANISIC-2): 303 mg (2 mmol) of *m*-anisic acid was dissolved in 100 mL of dry THF. 230 mg (2 mmol) of NHS was added to the flask and the mixture was stirred for 30 min under N_2. 413 mg (2 mmol) of DCC was added to the solution and the mixture was stirred for 3 h to yield the activated ester. The solution was vacuum filtered to remove DCU as white precipitate. 367 mg (2 mmol) of 4-phenylbenzylamine (367 mg, 2 mmol) was added to the filtrate and the mixture was stirred for 2 d at room temperature. The solution was evaporated to dryness, redissolved in 10 ml of CH_2Cl_2, and filtered. The filtrate was loaded onto a flash silica column and eluted with 0-5% methanol in CH_2Cl_2 to isolate the product as a white precipitate. The precipitate was dissolved in 10 ml of MeOH and sonicated for 15 min. The solution was then cooled to 0°C to recrystallize the product giving 95 mg of a white solid. Yield = 15%. ^{1}H NMR (500MHz, $CDCl_3$): δ 3.86 (s, 3H), 4.69 (d, J = 5.5 Hz, 2H), 6.41 (s, 1H), 7.04 (dd, J = 2.25, 8.25 Hz, 1H), 7.27-7.47 (m, 9H, 1NH), 7.58 (dd, J = 8.0, 15.5 Hz, 2H). IR (KBr pellet): ν 1247, 1308, 1532, 1637, 2920, 3313, 3451 cm^{-1}. ESI-MS(+): m/z 318.05 $[M+H]^+$. Anal. Calcd (Found) for $C_{21}H_{19}NO_2$: C 79.47% (79.22), H 6.03% (6.40), N 4.41% (4.39).

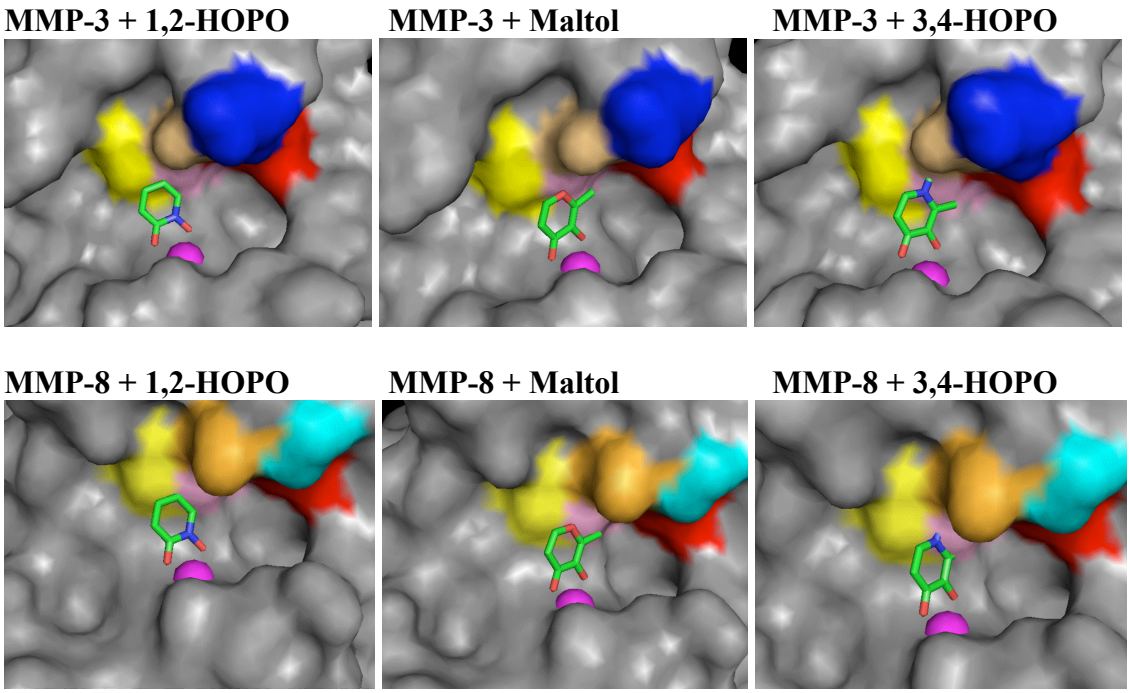


Figure S1. MMP active sites (gray surface) with different ZBGs. ZBGs are colored by atom: carbon (green), oxygen (red), nitrogen (blue). The zinc(II) ion is shown as a magenta sphere. MMP-3 is shown in the top row and MMP-8 is shown in the bottom row with 1,2-HOPO (left, ZBG in **1**), maltol (middle, ZBG in **2**), and 3,4-HOPO (right, ZBG in **4**). Active site residues in MMP-3 are colored as follows: Asn162 (blue), Val163 (beige), Leu164 (red), Ala165 (pink), His166 (yellow). Active site residues in MMP-8 are colored as follows: Gly158 (cyan), Ile159 (orange), Leu160 (red), Ala161 (pink), His162 (yellow).