Supplementary data

Nitro-based selective inhibitors against matrix metalloproteinase-7 over matrix metalloproteinase-1

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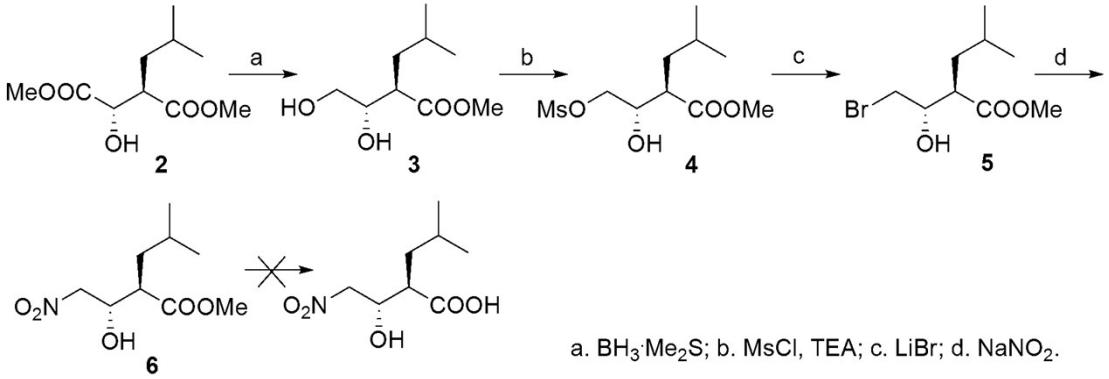
S1 Synthesis

1.1. Materials and methods

Unless otherwise noted, all chemicals were commercially available and used as received without further purification. Flash chromatography was performed with 100-200 mesh silica gel (Qingdao, China) and thin-layer chromatography (TLC) was carried out on silica coated glass sheets (Qingdao silica gel 60 F-254). Melting points were taken on a Thomas-Hoover capillary melting point apparatus and uncorrected. 1H nuclear magnetic resonance (NMR) and 13C NMR spectra were recorded with a Bruker AV 300 (300 MHz) instrument using tetramethylsilane as the internal standard. IR spectra were recorded on a Perkin-Elmer 1300 FT-IR spectrometer. High-resolution mass spectra were taken on a Shimadzu GC-MS-QP 2010. Elemental analyses were performed at Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, China.



Scheme S1. Synthesis route of (2*R*,3*S*)-2-*iso*-butyl-3-hydroxy-4-nitro butanoic acid

1.2 (2*R*,3*S*)-2-*iso*-Butyl-3-hydroxy succinic acid dimethyl ester (2)

Prepared starting from (*S*)-malic acid according to the literature.[1]

1.3 (2*R*,3*S*)-2-*iso*-Butyl-3,4-dihydroxy butanoic acid methyl ester (3)

To a solution of THF (10 mL) containing compound **2** (1.0 g, 4.58 mmol) was added dropwise 2.0 M

$BH_3 \cdot Me_2S$ in THF (2.34 mL, 4.58 mmol) at 0 °C. After stirring for 1 hour, $NaBH_4$ (14.4 mg, 0.38 mmol) was added. The reaction mixture were further kept stirring for 5 hours at room temperature and then quenched by addition of 14 mL methanol. After removal of the organic solvents under reduced pressure, the residue was separated by flash column chromatography using the mixture of n-hexane and ethyl acetate as eluent. Oil (650 mg). Yield: 74.6 %. $[\alpha]_D^{21}$ = -13.2° (*c* = 1.52，$CHCl_3$). IR (film): 3398, 3184, 1732, 1466, 1369, 1253, 1199, 1168, 1095, 1033, 979 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz), δ: 3.83-3.76 (m, 1H), 3.68 (s, 3H), 3.65 (dd, 1H, *J* = 7.6, 12.4 Hz), 3.54 (dd, 1H, *J* = 6.6, 12.4 Hz), 2.98 (br, 1H), 2.69-2.62 (m, 1H), 1.68-1.36 (m, 3H), 0.94 (d, *J* = 6.4 Hz, 3H), 0.91 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 175.95, 73.24, 64.81, 51.75, 46.12, 38.21, 26.18, 23.13, 21.70. HRMS (calcd. as $C_9H_{18}O_4$): 190.1197 (190.1205).

1.4 (2*R*,3*S*)-4-Bromo-2-*iso*-butyl-3-hydroxy butanoic acid methyl ester (5)

To a solution of dried CH_2Cl_2 (20 mL) containing compound **3** (300 g, 1.58 mmol) was added dropwise methanesulfonyl chloride (216.8 mg, 1.89 mmol) and triethyl amine (191.5 mg, 1.89 mmol) simultaneously at 0 °C. After further stirring for 2 hour, the reaction mixture was washed by 1.0 M HCl one time followed by washing by brine (10 mL × 3). After dried over anhydrous Na_2SO_4, organic solvents were removed under reduced pressure. A colorless oil (480 mg) was obtained as crude product (compound **4**), which was used for subsequent bromination without further purification.

The crude compound **4** (480 mg) was dissolved in dried THF (20 mL) and then added LiBr (776.8 mg, 8.94 mmol) under Ar atmosphere. After refluxing overnight and then cooled to room temperature, the solvent was removed under reduced pressure. The residue was dissolved in 50 mL EtOAc. The organic layer was washed by brine (20 mL × 3) and dried over anhydrous Na_2SO_4. Condensation under reduced pressure provided the residue, which was separated by flash column chromatography using the mixture of n-hexane and ethyl acetate as eluent. Compound **5** was obtained as colorless oil (310 mg). Yield: 68.4 %. $[\alpha]_D^{21}$ = -10.0° (*c* = 1.50, $CHCl_3$); IR (film): 3495, 2353, 1732, 1438, 1365, 1269, 1172, 1037, 918, 887 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 3.88 (m, 1H), 3.74 (s, 3H), 3.49 (m, 2H), 2.98 (d, *J* = 5.4 Hz, 1H), 2.89-2.82 (m 1H), 1.68-1.39 (m, 3H), 0.93 (d, *J* = 6.4 Hz, 3H), 0.90 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 175.35, 72.04, 51.99, 46.83, 38.39, 36.85, 26.07, 23.03, 21.85. HRMS (calcd. as $C_9H_{17}O_3Br$): 252.0367(252.0361).

1.5 (2*R*,3*S*)-2-*iso*-Butyl-3-hydroxy-4-nitro butanoic acid methyl ester (6)

Compound **5** (270 mg, 1.07 mmol), sodium nitrate (127.3 mg, 1.85 mmol), and phloroglucinol (144 mg, 1.14 mmol) were added to 11 mL dried DMF. The mixture were refluxed for overnight and then cooled to room temperature. After addition of 70 mL Et_2O and 20 mL water, the layered organic phase was washed by brine (20 mL × 3) and dried over anhydrous Na_2SO_4. Condensation under reduced pressure provided the residue, which was separated by flash column chromatography using the mixture of n-hexane and ethyl acetate as eluent. Compound **6** was obtained as colorless oil (60 mg). Yield: 25.7 %. $[\alpha]_D^{20}$ = -18.2° (*c* = 0.8, $CHCl_3$); IR (film): 3495, 2357, 1728, 1558, 1438, 1373, 1257, 1199, 1172, 1091 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 4.51-4.43 (m, 3H), 3.75 (s, 3H), 3.28 (d, J = 6.6 Hz, 1H), 2.69-2.63 (m, 1H), 1.77-1.53 (m, 2H), 1.47-1.38 (m, 1H), 0.92 (d, *J* = 6.4 Hz, 3H), 0.89 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 174.58, 79.03, 69.45, 52.18, 46.16, 37.86, 25.98, 22.82, 21.85. HRMS (calcd. as $C_9H_{17}NO_5$): 219.1111 (219.1107).

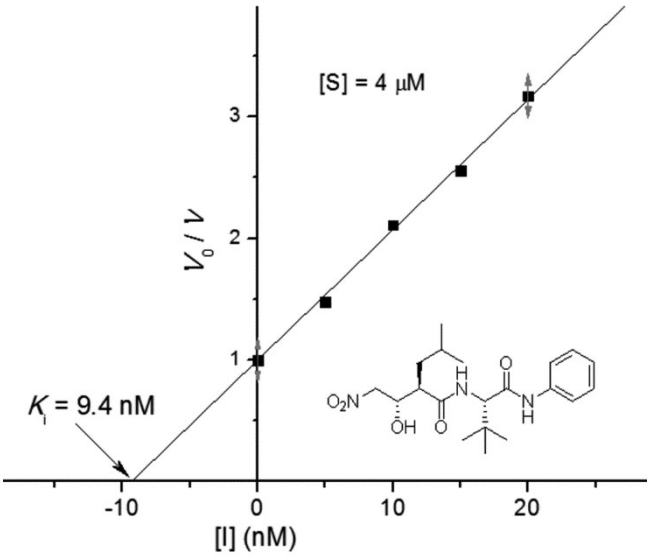
1.6 Hydrolysis of compound 6

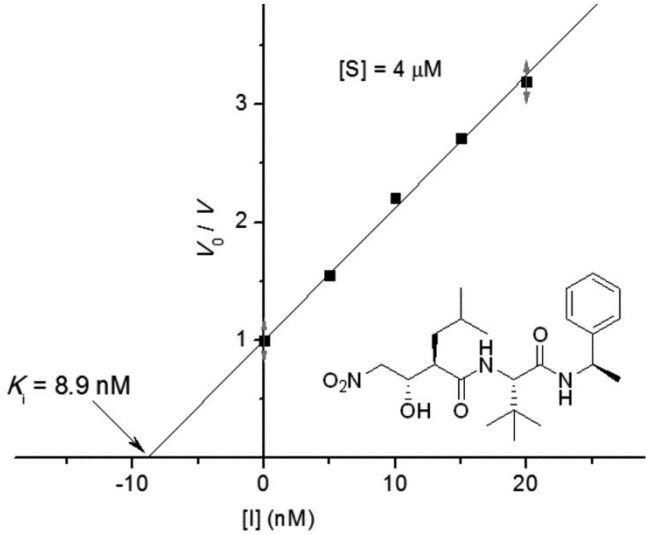
Failed either catalyzed by LiI in neutral condition[2] or enzymatic hydrolysis.[3]

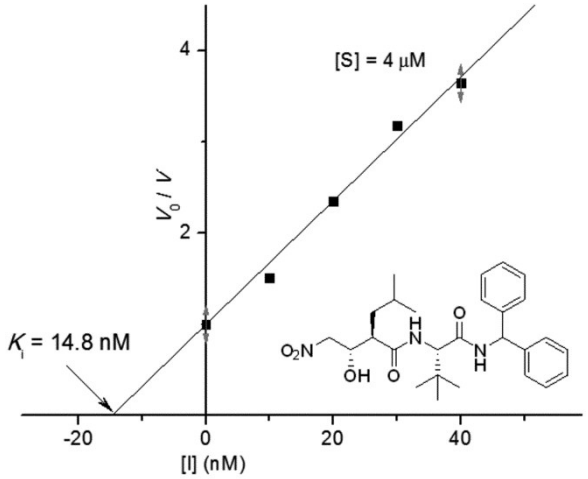
S2 MMP Inhibition Kinetics

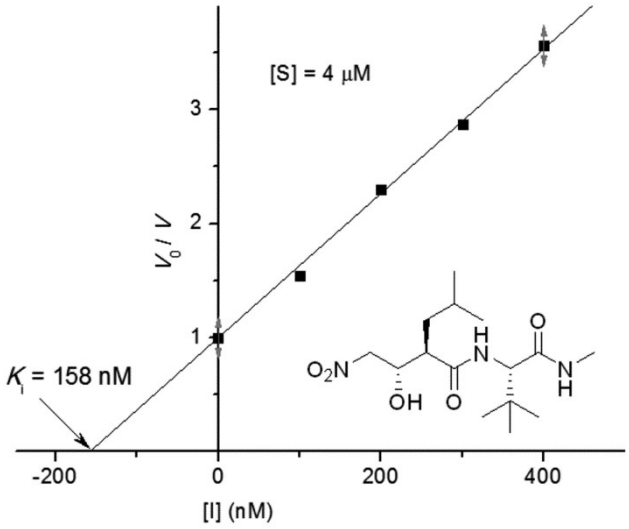
2.1 MMP-7 inhibition

Fig. S1 Inhibition kinetics of the other nitro-based compounds against MMP-7



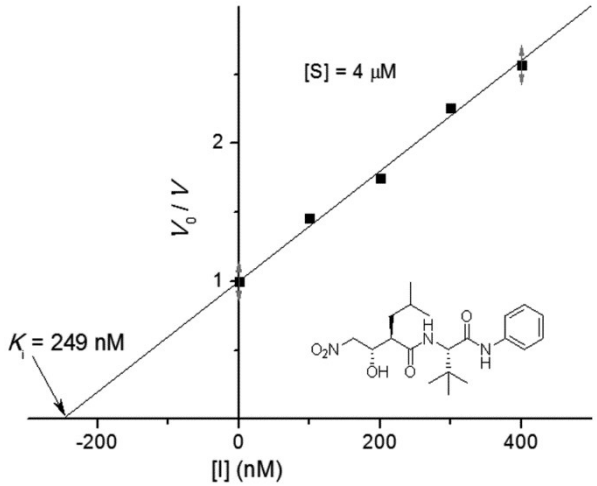


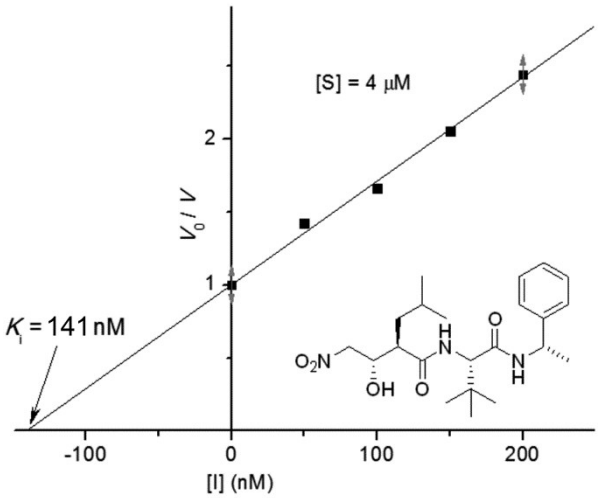


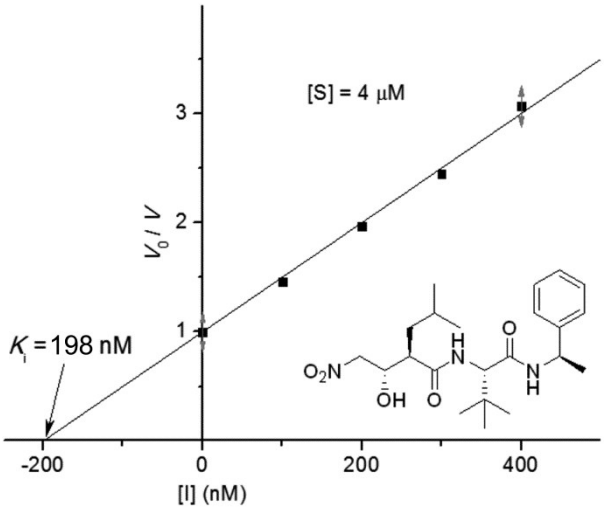


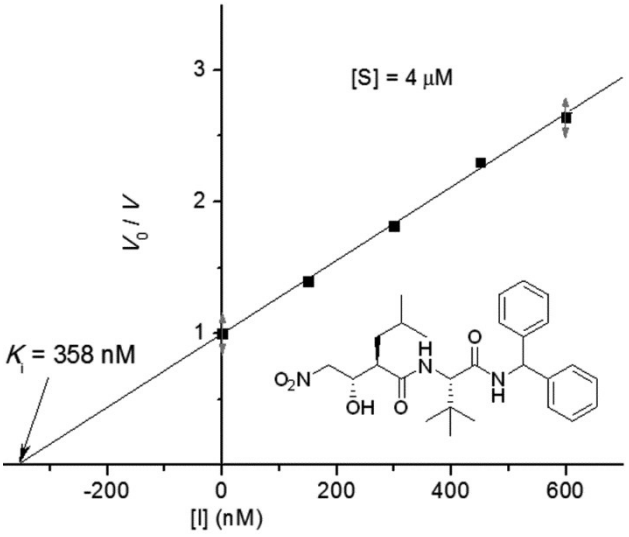
2.2 MMP-1 inhibition

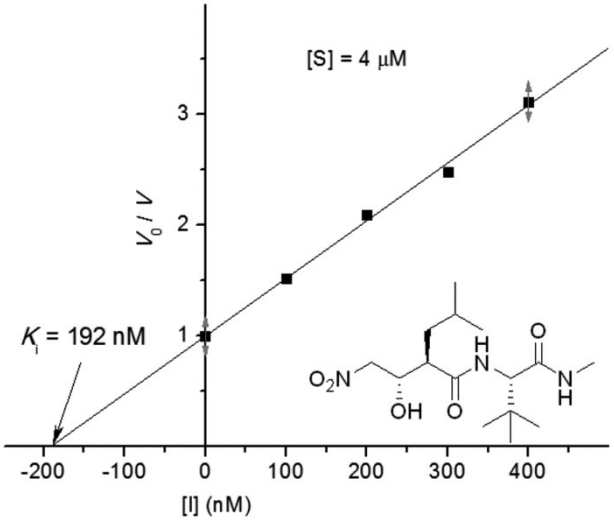
Fig. S2 Inhibition kinetics of compounds **1** against MMP-1





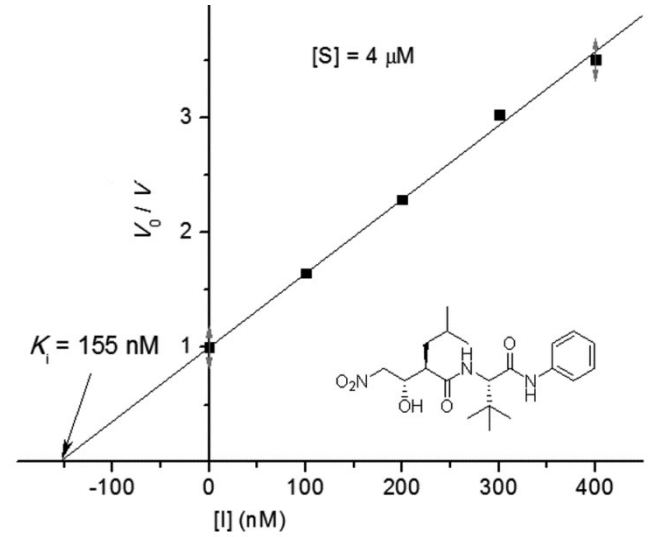

[S] = 4 μM
V_0 / V
K_i = 198 nM
[I] (nM)

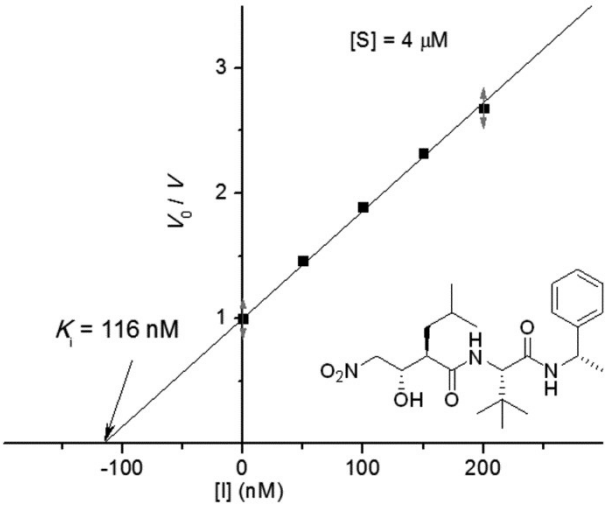

[S] = 4 μM
V_0 / V
K_i = 358 nM
[I] (nM)

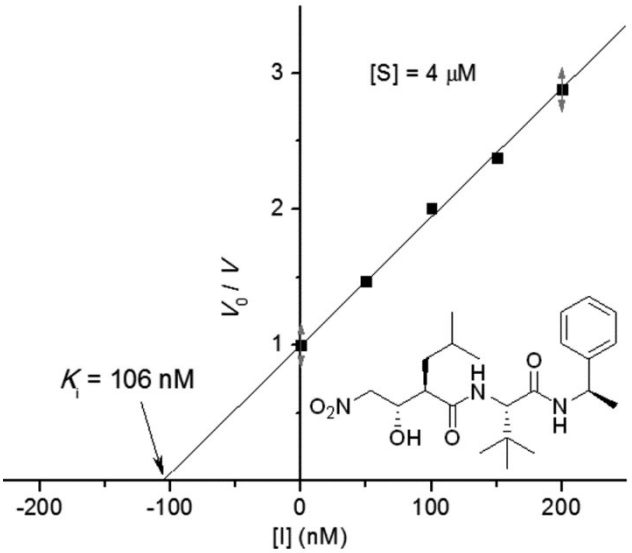

[S] = 4 μM
V_0 / V
K_i = 192 nM
[I] (nM)

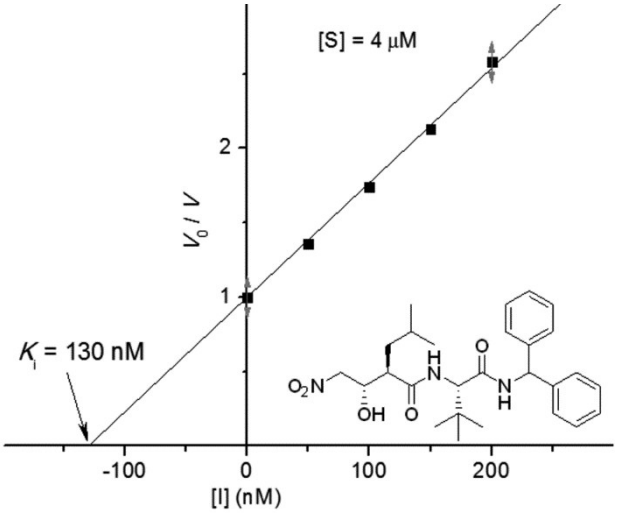
2.3 MMP-2 inhibition

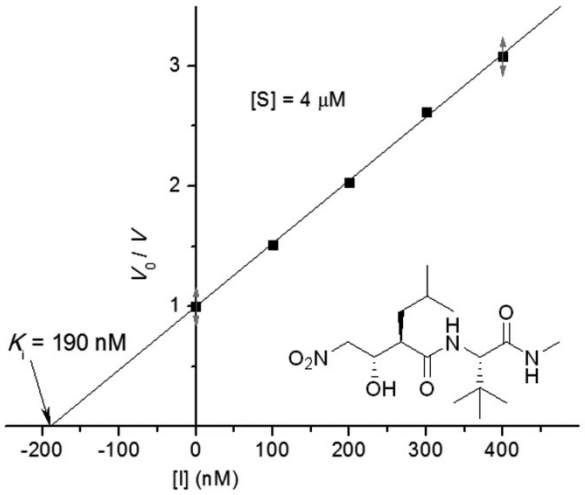
Fig. S3 Inhibition kinetics of compounds **1** against MMP-2











S3 Auto Dock studies

3.1 The binding mode of compounds 1 with MMP-1 predicated by Auto Dock shown as follows.

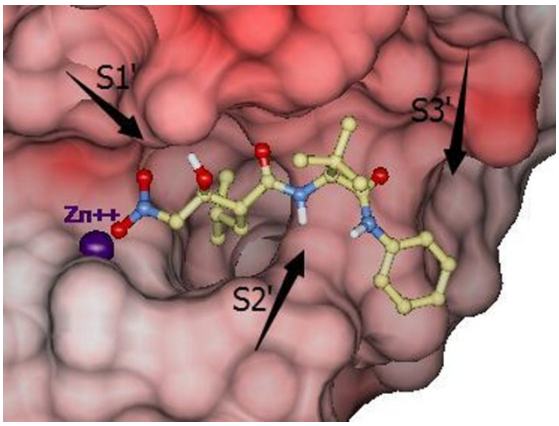


Fig. S4 Docking of compound **1a** onto the active site of MMP-1

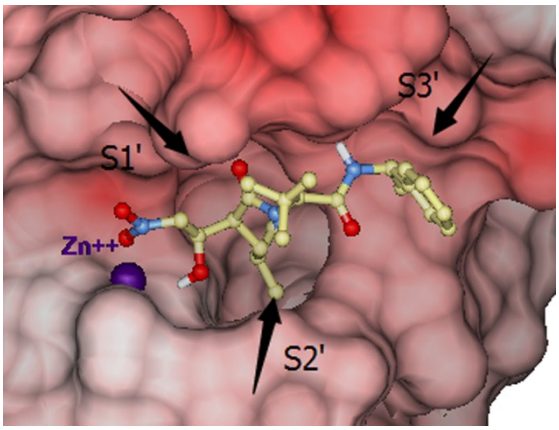


Fig. S5 Docking of compound **1c** onto the active site of MMP-1

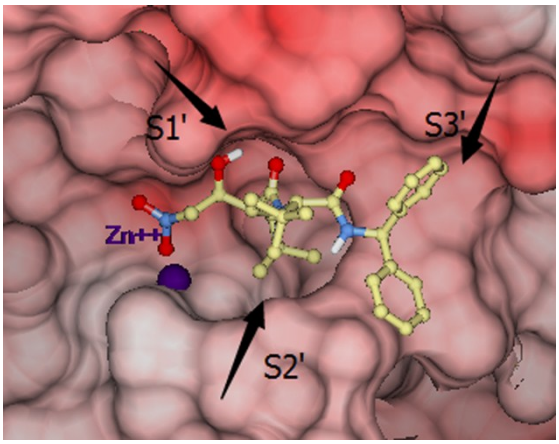


Fig. S6 Docking of compound **1d** onto the active site of MMP-1

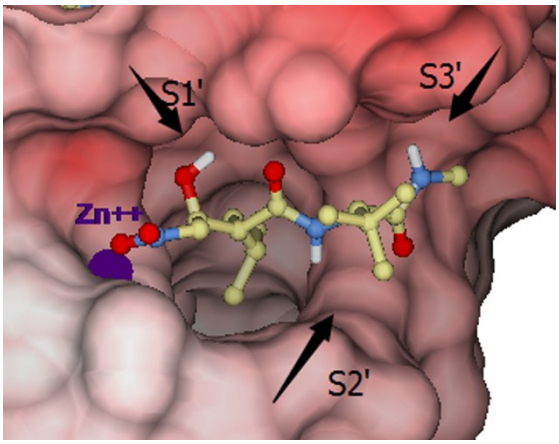


Fig. S7 Docking of compound **1e** onto the active site of MMP-1

3.2 The binding mode of compounds 1 with MMP-7 predicated by Auto Dock shown as follows.

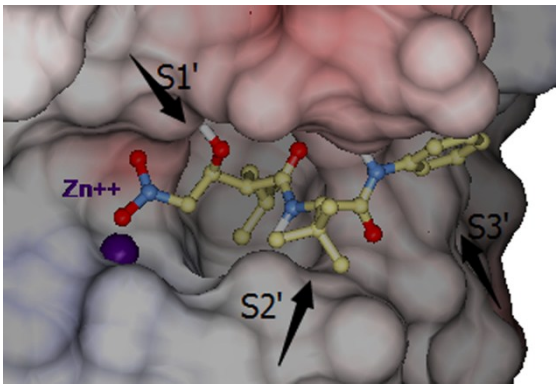


Fig. S8 Docking of compound **1a** onto the active site of MMP-7

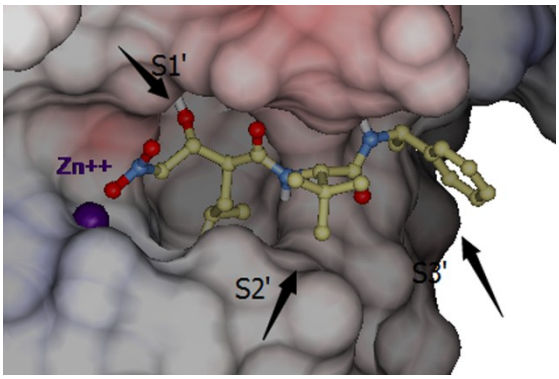


Fig. S9 Docking of compound **1c** onto the active site of MMP-7

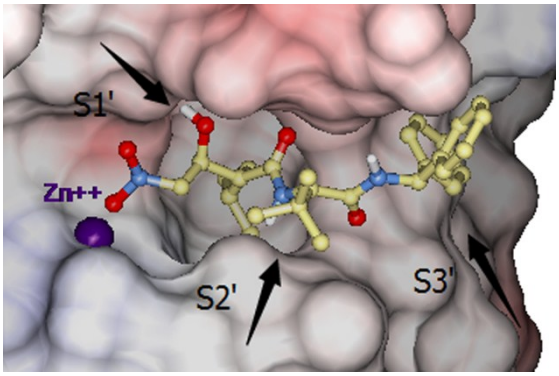


Fig. S10 Docking of compound **1d** onto the active site of MMP-7

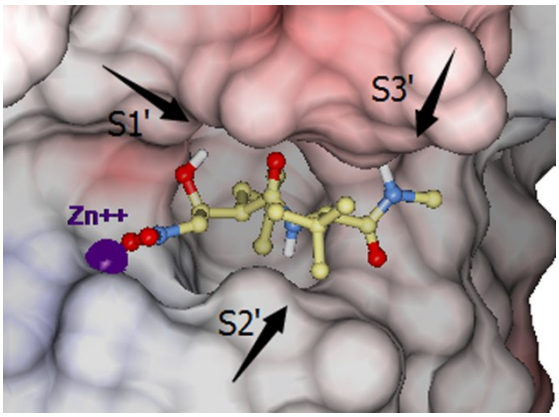


Fig. S11 Docking of compound **1e** onto the active site of MMP-7

References:

1. D．E．Levy, F．Lapierre, W. Liang, W. Ye, C. W. Lange, X. Li, D. Grobelny, M. Casabonne, D. Tyrrell, K. Holme, A. Nadzan and R. E. Galardy, *J. Med. Chem.*, 1998, **41**, 199.
2. S.-H. Wang, S.-F. Wang, W. Xuan, Z.-H. Zeng, J.-Y. Jin, J. Ma and G. R. Tian, *Bioorg. Med. Chem.,* 2008, **16**, 3596.
3. J.-Y. Jin, G. R. Tian and D. H. Kim, *Bioorg. Med. Chem.*, 2003, **11**, 4377.