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Supplementary Material

Mercaptoacetate thioesters and their hydrolysate mercaptoacetic acid jointly inhibit Metallo-β-Lactamase L1

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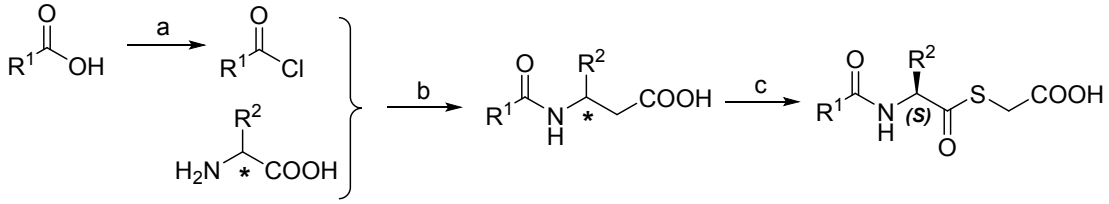
General methods

The amines, chloroacetyl chloride and mercaptoacetic acid were purchased from Sigma-Aldrich (Shanghai) Trading Co. Ltd. All other starting materials were purchased from commercial sources and purified using standard methods. Analytical Thin Layer Chromatography (TLC) was carried out on silica gel GF254 plates with visualization by ultraviolet radiation. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III 400 MHz NMR spectrometer. Chemical shifts are given in parts per million (ppm) on the delta scale. The peak patterns are reported as singlet (s), doublet (d), triplet (t), quartet (q), doublet doublet (dd), and mul-tiplet (m). The spectra were recorded with TMS as internal standard. Coupling constants (*J*) were reported in Hertz (Hz). Mass spectra were obtained on a micro TOF-Q (BRUKER) mass spectrometer. Activity evaluation of inhibitors was performed on an Agilent 8453 UV-Vis spectrometer.

Synthetic procedures, and ^{1}H and ^{13}C NMR and MS characterization

N-substituted mercaptoacetate thioesters **1**-**10** were synthesized by a synthetic route shown in Scheme S1.



Scheme S1. Synthetic route of mercaptoacetate thioesters. Reagents and conditions: (a) $SOCl_2$, DMF, 80°C, 3 h; (b) NaOH, diethyl ether, HCl; (c) mercaptoacetic acid, ethyl chloroformate, Et_3N, AcOEt/DMF

2-(((4-Nitrobenzoyl)tryptophyl)thio)acetic acid (1)

Substituted benzoic acid (0.80 g, 4 mmol) was added to a mixture of 6 mL ethyl acetate and 1 mL DMF containing ethyl chloroformate (0.4 mL, 4 mmol) and triethylamine (1.12 mL, 8 mmol) with stirring at -5°C. After 15 min, mercaptoacetic acid (0.28 mL, 4 mmol) and triethylamine (0.56 mL, 4 mmol) were added. The solution was stirred at room temperature for 1 h and then at 50°C for 3 h. The reaction mixture was acidified with 2 M HCl, and the resulting organic layer was isolated and dried over anhydrous sodium sulfate. The crude product was further purified via silica gel chromatography to give 0.85 mg compound **1** as yellow solid. Yield 52 %, ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.84 (s, 1H), 9.54 (s, 1H), 8.34 (d, *J* = 7.8 Hz, 2H), 8.08 (d, *J* = 8.0 Hz, 2H), 7.58 (d, *J* = 7.6 Hz, 1H), 7.32 (d, *J* = 7.3 Hz, 1H), 7.19 (s, 1H), 7.03 (dd, *J* = 13.2, 6.4 Hz, 2H), 4.87-4.75 (m, 1H), 3.71 (s, 2H), 3.22-3.14 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 200.20, 169.74, 165.99, 149.77, 139.34, 136.22, 129.40, 126.91, 123.39, 121.36, 118.94, 118.24, 111.67, 109.26, 60.80, 31.41, 26.97. HRMS(ESI) *m/z*: 426.0754 (Calcd for $[M-H]^-$ 426.0765 *m/z*). Compounds **2-10** were obtained by similar procedure of compound **1**.

2-(((3-Nitrobenzoyl)tryptophyl)thio)acetic acid (2)

White powder, yield 57 %, ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.84 (s, 1H), 9.59 (d, *J* = 5.4 Hz, 1H), 8.72 (s, 1H), 8.28 (dd, *J* = 8.2, 10.4 Hz, 2H), 7.80 (t, *J* =6.5 Hz, 1H), 7.59 (t, *J* = 7.5 Hz, 1H), 7.31 (d, *J* = 7.8 Hz, 1H), 7.20 (s, 1H), 6.98-6.84 (m, 2H), 4.92-4.85 (m, 1H), 3.71 (s, 2H), 3.24-3.02 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 200.11, 170.03,

165.22, 148.24, 136.35, 135.25, 134.43, 130.61, 127.38, 126.83, 124.15, 122.57, 121.53, 119.03, 118.34, 111.98, 110.00, 61.14, 31.65, 27.11. HRMS(ESI) *m/z*: 426.0758 (Calcd for $[M-H]^-$ 426.0765*m/z*).

2-(((4-Fluorobenzoyl)tryptophyl)thio)acetic acid (3)

White solid, ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.83 (s, 1H), 9.40 (d, *J* = 7.6 Hz, 1H), 8.03 (d, *J* = 7.4 Hz, 2H), 7.95 (m, 2H), 7.57 (t, *J* = 7.6 Hz, 1H), 7.31 (s, 1H), 7.18 (t, *J* = 7.6 Hz, 1H), 7.06 (t, *J* = 7.3 Hz, 1H), 4.88-4.82 (m, 1H), 3.70 (s, 2H), 3.19-3.03 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 200.52, 170.21, 166.22, 137.68, 136.54, 131.97, 128.69, 127.58, 125.85, 124.15, 121.68, 118.99, 118.24, 111.98, 109.85, 61.04, 31.53, 27.16. HRMS(ESI) *m/z*: 399.0840 (Calcd for $[M-H]^-$ 399.0820 *m/z*).

2-(((4-Chlorobenzoyl)tryptophyl)thio)acetic acid (4)

White solid, ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.82 (s, 1H), 9.22 (d, *J* = 7.6, 1H), 7.86 (d, *J* = 7.8, Hz, 2H), 7.57 (d, *J* = 8.2, 2H), 7.32 (d, *J* = 6.8, 1H), 7.17 (s, 1H), 7.01 (d, *J* = 7.9, 2H), 4.85-4.76 (m, 1H), 3.69 (s, 2H), 3.20-3.02 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 200.55, 170.45, 165.99, 137.01, 136.31, 132.69, 129.85, 127.35, 124.12, 121.56, 118.92, 118.25, 112.00, 110.05, 60.89, 31.54, 27.11. HRMS(ESI) *m/z*: 415.0522 (Calcd for $[M-H]^-$ 415.0525 *m/z*)

2-(((4-Bromobenzoyl)tryptophyl)thio)acetic acid (5)

White solid, ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.87 (s, 1H), 8.79 (d, *J* = 7.8 Hz, 1H), 7.77 (d, *J* = 8.2 Hz, 2H), 7.67 (d, *J* = 8.3 Hz, 2H), 7.59 (d, *J* = 7.8 Hz, 1H), 7.32 (d, *J* = 8.0 Hz, 1H), 7.19 (d, *J* = 2.4 Hz, 1H), 7.02 (dt, *J* = 30.2, 7.3 Hz, 2H), 4.65-4.51 (m, 1H), 3.73 (s, 2H), 3.24-3.01 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.69, 165.92, 136.62, 133.40, 131.71, 129.99, 127.70, 125.50, 124.02, 121.42, 118.86, 118.58, 111.91, 110.82, 54.21, 30.54, 27.06. HRMS(ESI) *m/z*: 459.0058 (Calcd for $[M-H]^-$ 459.0043 *m/z*).

2-(((3-Bromobenzoyl)tryptophyl)thio)acetic acid (6)

White solid, ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.84 (s, 1H), 9.28 (d, *J* = 7.8 Hz, 1H), 8.04 (s, 1H), 7.79 (dd, *J* = 26.8, 7.7 Hz, 2H), 7.57 (d, *J* = 7.8 Hz, 1H), 7.46 (t, *J* = 7.8 Hz, 1H), 7.31 (d, *J* = 7.9 Hz, 1H), 7.19 (s, 1H), 7.03 (dt, *J* = 30.1, 7.1 Hz, 2H), 4.87-4.69 (m, 1H), 3.71 (s, 2H), 3.33-3.28 (m, 1H), 3.20-3.12 (m, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 200.58, 170.12, 165.87, 136.53, 136.10, 134.97, 131.18, 130.43, 127.30, 127.15, 124.13, 122.12, 121.58, 118.99, 118.34, 111.91, 110.02, 60.92, 31.63, 27.05. HRMS(ESI) *m/z*: 459.0058 (Calcd for $[M-H]^-$ 459.0040 *m/z*).

2-(((4-(Trifluoromethyl)benzoyl)tryptophyl)thio)acetic acid (7)

White solid, ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.83 (s, 1H), 9.40 (d, *J* = 7.8 Hz, 1H), 8.03 (d, *J* = 7.9 Hz, 2H), 7.90 (d, *J* = 8.2 Hz, 2H), 7.57 (d, *J* = 7.4 Hz, 1H), 7.31 (d, *J* = 7.6 Hz 1H), 7.18 (s, 1H), 7.06 (dt, *J* = 29.8, 7.4 Hz, 2H), 4.88-4.75 (m, 1H), 3.70 (s, 2H), 3.19-3.14 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 200.52, 170.21, 166.22, 137.68, 136.54, 131.97, 128.69, 127.58, 125.85, 124.15, 121.68, 118.99, 118.24, 111.98, 109.85, 61.04, 31.53, 27.16. HRMS(ESI) *m/z*: 449.0845 (Calcd for $[M-H]^-$ 449.0818 *m/z*).

2-(((4-Nitrobenzoyl)methionyl)thio)acetic acid (8)

White solid, ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.04 (d, *J* = 8.0 Hz, 1H), 8.32 (d, *J* = 12.0 Hz, 2H), 8.12 (d, *J* = 8.0 Hz, 2H), 4.55-4.42 (m, 1H), 3.71 (s, 2H), 2.56-2.24 (m, 2H), 2.06 (s, 5H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 200.60, 169.10, 165.61, 149.34, 139.34, 129.55, 124.13 , 59.18, 58.98, 31.56, 30.73, 30.07. HRMS(ESI) *m/z*: 371.0358 (Calcd for $[M-H]^-$ 371.0377 m/z).

2-(((4-Chlorobenzoyl)methionyl)thio)acetic acid (9)

White solid, ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.16 (d, *J* = 4.2 Hz, 1H), 7.93 (d, *J* = 7.6 Hz, 2H), 7.60 (d, *J* = 7.4 Hz, 2H), 4.76-4.59 (m, 1H), 3.65 (s, 2H), 2.58-2.38 (m, 2H), 2.04 (s, 5H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 200.54, 170.13, 166.62, 137.21, 132.71, 129.98, 129.01, 59.02, 31.49, 30.74, 30.11, 14.97. HRMS(ESI) *m/z*: 360.0118 (Calcd for $[M-H]^-$ 360.0137 *m/z*).

2-(((3-Bromobenzoyl)methionyl)thio)acetic acid (10)

White solid, ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.76 (s, 1H), 8.78 (d, *J* = 7.7 Hz, 1H), 8.10 (d, *J* = 12.0 Hz, 2H), 7.91-7.89 (m, 2H), 7.78 (d, *J* = 9.8 Hz, 2H), 7.59-7.45 (m, 2H), 4.57-4.46 (m, 1H), 3.66 (s, 2H), 2.59 – 2.46 (m, 2H), 2.14 (s, 5H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 200.92, 173.78, 166.11, 136.48, 131.23, 131.06, 127.29, 122.09, 59.02, 52.18, 36.29, 30.56, 15.16. HRMS(ESI) *m/z*: 403.9642 (Calcd for $[M-H]^-$ 403.9631 *m/z*).

Over-expression and purification of MβLs

NDM-1 (B1): NDM-1 was overexpressed and purified as previously described[1]. *E. coli* BL21(DE3) cells were first transformed with the over-expression plasmid pET26b-NDM-1 and the cells were plated on LB-agar plates containing 25 μg/mL kanamycin. A single colony was used to inoculate 50 mL of LB containing 25 μg/mL of kanamycin. After the preculture grew overnight at 37 °C, 10 mL overnight culture of these cells in LB was used to inoculate 4 × 1 L of LB containing 25 μg/mL kanamycin. The cells were allowed to grow at 37 °C with shaking until the cells reached an optical density at 600 nm of 0.6-0.8. Protein production was induced with 1 mM IPTG, and the cells were shaken at 25 °C for 3 h. The cells were collected by centrifugation (30 min at 8,275 × g) and resuspended in 25 mL of 30 mM Tris, pH 8.0, containing 500 mM NaCl. The cells were lysed by ultrasonication, and the cell debris was separated by centrifugation (30 min at 32,583 × g). The cleared supernatant was dialyzed versus 30 mM Tris, pH 8.0, containing 100 μM $ZnCl_2$ for 36 h at 4 °C, centrifuged (25 min at 32,583 × g) to remove insoluble matter, and loaded onto an equilibrated Q-Sepharose column. Bound proteins were eluted with a 0-500 mM NaCl gradient in 30 mM Tris, pH 8.0, containing 100 μM $ZnCl_2$ at 2 mL/min. Fractions (2 mL) containing NDM-1 were pooled and concentrated with an Amicon ultrafiltration cell equipped with a YM-10 membrane. The crude protein NDM-1 was run through a G75 column and eluted with 30 mM Tris, pH 8.0, containing 200 mM NaCl. Protein purity was ascertained by SDS PAGE and protein concentration was determined using Beer's law and an extinction coefficient of 27,960 $M^{-1}cm^{-1}$ at 280 nm.

ImiS (B2): ImiS was overexpressed and purified as previously described[2]. *E. coli* BL21(DE3) cells were first transformed with the over-expression plasmid pET-26b-ImiS and the cells were plated on LB-agar plates containing 25 μg/mL kanamycin. A single colony was used to inoculate 50 mL of LB containing 25 μg/mL kanamycin. After the preculture grew overnight at 37 °C, 10 mL overnight culture of these cells in LB was used to inoculate 4 × 1 L of LB containing 25 μg/mL kanamycin. The cells were allowed to grow at 37 °C with shaking until the cells reached an optical density at 600 nm of 0.6-0.8. Protein production was induced with 1 mM IPTG, and the cells were shaken at 25 °C for 3 h. The cells were collected by centrifugation (30 min at 8,275 × g) and resuspended in 25 mL of 30 mM Tris, pH 7.0, containing 500 mM NaCl. The cells were lysed by ultrasonication, and the cell debris was separated by centrifugation (30 min at 32,583 × g). The cleared supernatant was dialyzed versus 30 mM Tris, pH 7.0, containing 100 μM $ZnCl_2$ for 36 h at 4 °C, cen-trifuged (25 min at 32,583 × g) to remove insoluble matter, and loaded onto an equilibrated SP-Sepharose column. Bound proteins were eluted with a 0-500 mM NaCl gradient in 30 mM Tris, pH 7, containing 100 μM $ZnCl_2$, at 2 mL/min. Fractions (2 mL) containing ImiS were pooled and concentrated with an Amicon ultrafiltration cell equipped with a YM-10 membrane. The crude protein ImiS was run through a G75 column and eluted with 30 mM Tris, pH 7.0,

containing 200 mM NaCl. Protein purity was ascertained by SDS PAGE and protein concentration was determined using Beer's law and an extinction coefficient of 37,250 $M^{-1}cm^{-1}$ at 280 nm.

L1 (B3): L1 was overexpressed and purified as previously described[3]. *E. coli* BL21(DE3) cells were first transformed with the over-expression plasmid pET26b(+)-L1 and the cells were plated on LB-agar plates containing 25 µg/mL kanamycin. A single colony was used to inoculate 50 mL of LB containing 25 µg/mL kanamycin. After the preculture grew overnight at 37 °C, 10 mL overnight culture of these cells in LB was used to inoculate 4 × 1 L of LB containing 25 µg/mL kanamycin. The cells were allowed to grow at 37 °C with shaking until the cells reached an optical density at 600 nm of 0.6-0.8. Protein production was induced with 1 mM IPTG, and the cells were shaken at 37 °C for 3 h. The cells were collected by centrifugation (30 min at 8,275 × g) and resuspended in 25 mL of 30 mM Tris, pH 8.5, containing 500 mM NaCl. The cells were lysed by ultrasonication, and the cell debris was separated by centrifugation (30 min at 32,583 × g). The cleared supernatant was dialyzed versus 30 mM Tris, pH 8.5, containing 100 µM $ZnCl_2$ for 36 h at 4 °C, cen-trifuged (25 min at 32,583 × g) to remove insoluble matter, and loaded onto an equilibrated Q-Sepharose column. Bound proteins were eluted with a 0-500 mM NaCl gradient in 30 mM Tris, pH 8.5, containing 100 µM $ZnCl_2$ at 2 mL/min. Fractions (2 mL) containing L1 were pooled and concentrated with an Amicon ultra-filtration cell equipped with a YM-10 membrane. The crude protein L1 was run through a G75 column and eluted with 30 mM Tris, pH 8.5, containing 200 mM NaCl. Protein purity was ascertained by SDS PAGE and protein concentration was determined using Beer's law and an extinction coefficient of 54,614 $M^{-1}cm^{-1}$ at 280 nm.

Percent inhibition of MβLs

The percent inhibition of MβLs from the subclasses B1 (NDM-1), B2 (ImiS) and B3 (L1) by the mercaptoacetate thioesters at 20 µM were determined, and the results are shown in Fig S1.

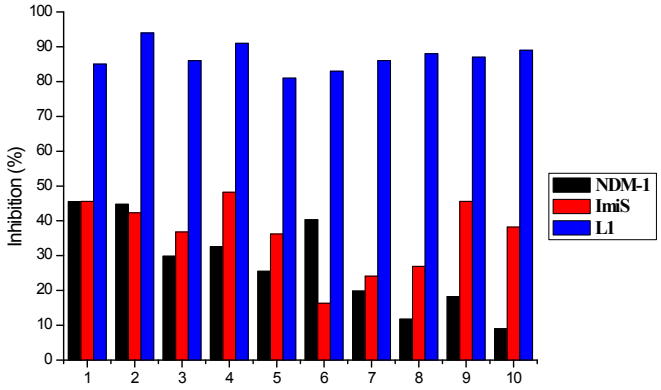


Fig. S1. Percent inhibition of mercaptoacetate thioesters (20 µM) against MβLs. Cefazolin was used as substrate for B1 and B3 subclasses enzymes, and imipenem as substrate for B2 subclass enzyme.

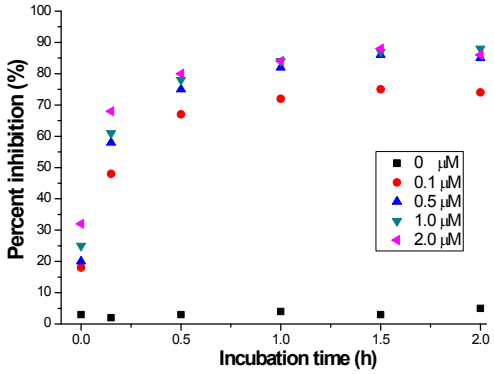


Fig. S2. Time-dependent and concentration-dependent inhibition of L1 by thioester **9**.

IC_{50} determination

The inhibitor concentration causing 50% decrease of enzyme activity (IC_{50}) were determinated at 25 ℃ using imipenem as substrate of ImiS and cefazolin as substrate of NDM-1 and L1. Compounds **1**-**10** were dissolved in a small volume of DMSO and then diluted with 30 mM Tris, pH 7.0 for NDM-1, ImiS and L1. The final concentrations of DMSO in inhibition experiments were below 0.1%; control experiments verified that the 0.1% DMSO had no inhibitory activity against the MβLs tested. The inhibitor concentrations were varied between 0.01 and 2 μM, and substrate concentrations were 50 μM. The enzyme and inhibitor were pre-incubated for 30 min before starting the kinetic experiments.

Isothermal titration calorimetry (ITC) experiments

Isothermal titration calorimetry (ITC) experiments were performed on a Malvern MicroCal iTC 200 instrument at 25°C[4]. All solution used in this experiment was degassed prior to titration. The concentration of NDM-1 enzyme in 30 mM Tris-HCl, pH 7.5, was 50 μM. The protein solution was placed in a 210 μL sample cell at 25℃. Inhibitor was dissolved in the same buffer at concentrations of 500 μM and loaded in the syringe. Titration was performed by titrating over 20 injections using 38 μL of inhibitor at 120 s intervals. In the top panel each peak represents a single injection of the inhibitor into protein solution. The bottom panel shows an integrated plot of the amount of heat liberated per injection as a function of the molar ratio of the inhibitor to protein. By integrating the area under the peaks in the isotherm, the heat of reaction per injection was determined in one site binding mode. Several binding properties from the experiment were obtained, such as enthalpy change (ΔH), Entropy (ΔS), the dissociation constant (K_d), binding stoichiometry (N).

Stability assays of thioester

As a representatives of thioesters (in this manuscript), the stabilities of compound **9** in the same buffer (Tris) at pH 6.0, 7.0 and 8.5 were assayed by monitoring their absorbance change over 24 hours. Their UV-Vis absorbance spectra (λ= 245 nm) were recorded every 2 hours (Fig. S2A-C) and with L1 enzyme concentration increasing, pH 7.0 (Fig. S2D).

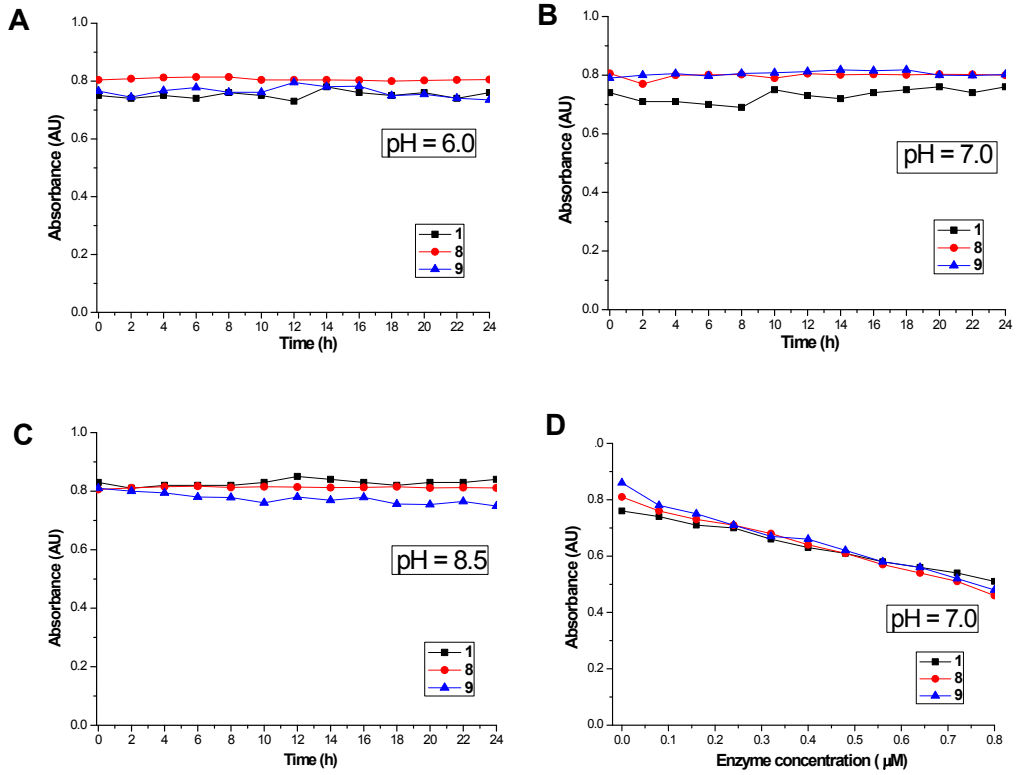


Fig. S3. UV-Vis absorbance change (λ= 245 nm) of thioesters **1**, **8** and **9** after incubation of 24 hours in Tris buffer with a pH 6.0 (A), 7.0 (B), 8.5 (C) and with L1 enzyme concentration increasing in Tris buffer pH=7.0 (D).

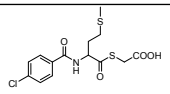
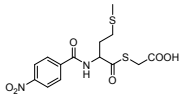
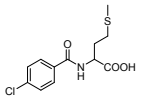
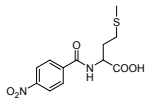
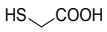
Monitoring of thioester hydrolysis by HPLC

As a representatives of thioesters**,** the hydrolysis of compound **9** by enzyme L1 were assayed by HPLC under the conditions of IC_{50} assay. HPLC analyses were carried out using the Analytical column 150 mm × 4.6 mm, Sepax (C18, 5 µm), at a flow rate of 1.0 mL/min. Solvent A: 100% H_2O. Solvent B: 100% CH_3CN. The following gradients were used: t = 2 min (5% B), t = 15 min (15% B), t = 30 min (95% B). The eluted peaks were detected using a UV detector at 245 nm.

STD NMR experiments

The NMR measurements were performed on a Bruker Avance III 400 MHz spectrometer (5 mm BBI probe and *z*-axis gradient) at 298 K. We prepared 100 mM stock solutions in DMSO-d_6 of compound **9**. All samples were measured under conditions of 5% DMSO-d_6, D_2O, 150 mM NaCl at pH 7.5 after 1 hour of pre-incubation. ^{1}H STD-NMR experiments were performed under saturation at 300 MHz (*on*-resonance spectra), 2000 MHz (*off*-resonance spectra) and a total saturation time of 1.5 s (relaxation delay of 2.5 s)[5]. STD experiment of a sample of compound **9** (2, 8 and 10 mM) and L1 (50 µM) led to the spectra Figure 4A (Reference spectrum for the mixture L1 and **9**) and 4B (Corresponding STD-NMR spectrum with different concentration of **9**).

Table S1. Inhibitory activities of thioester **8** and **9** and its two hydrolytic components against MβL L1

Components	IC_{50}/μM
	0.97±0.09
	0.17±0.03
	NI
	NI
	0.32±0.03

NI: No inhibition

MIC determination

A single colony of *E. coli* BL21(DE3) containing plasmids pET26b-L1 on LB agar plates was transferred to 5 mL of Mueller-Hinton (MH) liquid medium and grown at 37 °C overnight. The bacterial cells were collected by centrifugation (4,000 rpm for 10 min). After discarding the supernatant, the pelleted cells were re-suspended in MH medium and diluted to an OD_{600} of 0.1. MIC values were determined by using the Clinical and Laboratory Standards Institute (CLSI) macrodilution (tube) broth method. The MIC was interpreted as the lowest concentration of the drug that completely inhibited the visible growth of bacteria after incubating plates for at least 16 hrs at 37 °C. Each inhibitor was tested in triplicate in at least two independent experiments and the highest MIC value was reported.

Docking studies

Docking studies of the compounds **4** and **9** into the active site of L1 (PDB code 2AIO) were performed by AutoDock 4.2. The carboxyl groups were deprotonated, resulting in the overall charge of -1e for the compounds. A charge of +1.4e was assigned to the two Zn(II) in the active site, while +0.2e was added to each of its ligands.8 The grid and docking parameter files were prepared using Zn(II) van der Waals parameters = 0.25 kcal/mol and r_0 = 1.95 Å.9 9 L1 was treated as a rigid receptor, while ligands as flexible. The grid box was centered between the two active-site Zn(II) ions, with dimensions of 70 x 70 x 70 grid points with grid points spaced at 0.375 Å. The mutation rate and crossover rates were set at 0.02 and 0.8, respectively, while the maximum energy evaluations and generations' numbers were set at 2,500,000 and 27,000, respectively. Default values were kept for all other parameter and no constraints were used. Fifty conformations were generated according to the Lamarckian genetic algorithm and grouped into clusters based on a root mean square deviation (RMSD) tolerance of 2.0 Å. The lowest-energy (highest ranked) clusters were closely examined. Based on visual examination, the highest ranked cluster was chosen for **4** and **9**. The several higher-energy clusters showed very similar conformations as the lowest-energy clusters. This resulted in 11 and 19 conformations with average binding energies of -12.1 and -10.8 kcal/mol for **4** and **9**, respectively. The conformations are shown in Fig. S3 are the highest ranked (lowest energy) conformations.

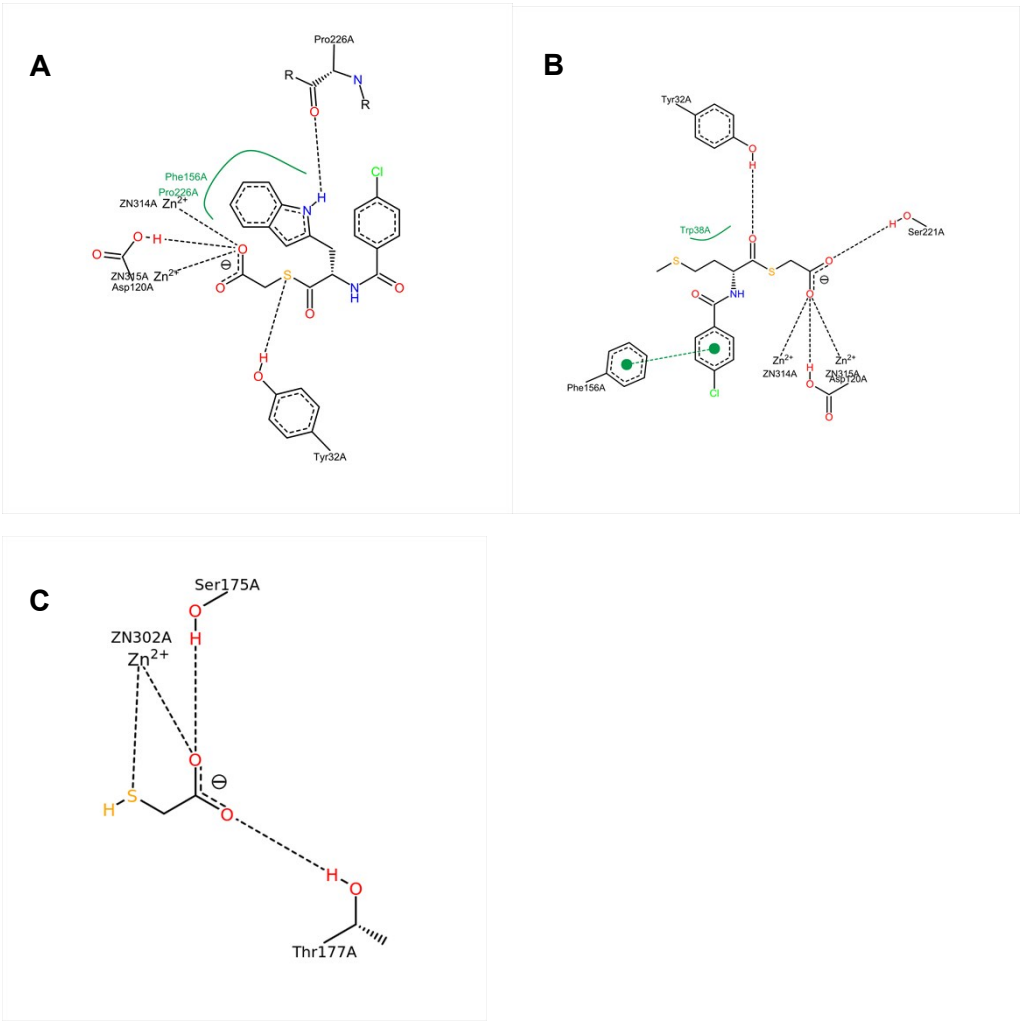


Fig. S4. Lowest-energy conformations of compound **4** and **9** docked into the active site of enzymes L1. Picture **A**-**B** are key interaction of **4** and **9**, with Zn(II) and residues of MβL L1, respectively, with dashed lines (A-B), the binding mode of mercaptoacetate targeting to the subclass B3 metallo-β-lactamase SMB-1(PDB: 3VQZ) (C). All 2D images were generated with free PoseView webservice.

Cytotoxicity assay

A cytotoxicity assay was performed to evaluate the toxicity of inhibitors **8** and **9** to mouse fibro-blast cells (L929). The cells were seeded into 96-well plates at cell density of 1.0×10^4 cells/well in 100 µL of culture medium and maintained for 24 h. Then solutions of inhibitors **8** and **9** with different concentrations were added to 96-well plates, respectively, and incubated for another 48 h. Six wells containing only cells suspended in a mixture of 99 µL of complete medium and 1 µL of DMSO were used as the control for investigating cell-viability. Six wells containing only the complete medium were used as the blank control. Following that, the medium was removed. Finally, 100 µL of fresh culture medium and 10 µL of Cell Counting Kit solution (purchased from 7Sea) were added to each well. After incubation for 4 h, the 96-well plates were then vigorously shaken to solubilize the formazan product and the absorbance at a wavelength of 450 nm was read on a Microplate Reader and analyzed. All experiments were conducted in triplicate. The results are shown in Fig. S4.

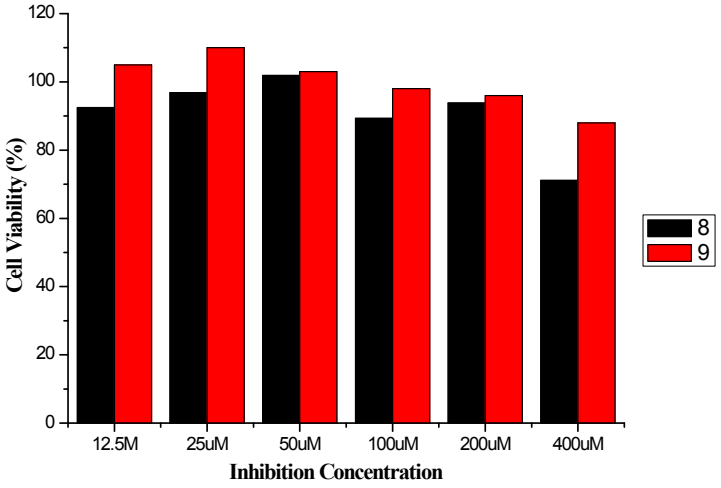


Fig. S5. Percent cell viability (relative to without compound) of L-929 mouse fibroblastic cells in the presence of **8** and **9** at concentrations of 12.5, 25, 50, 100, 200 and 400 μM.

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