Supporting Information

Rhodanine as a potent scaffold for the development of broad-spectrum metallo-β-lactamases inhibitors

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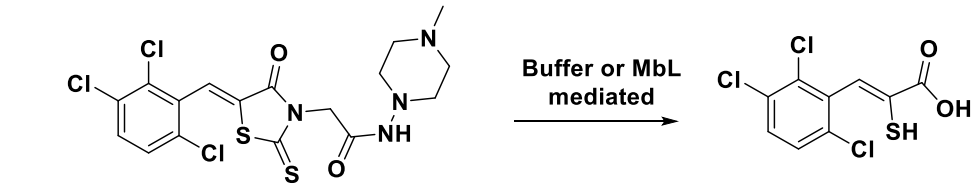

Trichlorobenzylidene substituted rhodanine **Thioenolate**

Scheme S1. Rhodanine hydrolysis by MβL VIM-2 to yield a thioenolate.[1]

General methods

The reagents were purchased from Aladdin (Shanghai) Trading Co., Ltd, and 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 F_{254} plates with visualization by ultraviolet radiation. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 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), and multiplet (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. Single crystal X-ray diffraction data were collected on a Bruker SMART APEX II CCD X-ray crystallography instrument. 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 of compounds

General procedure 1 for synthesis of N-substituted rhodanines

To a solution of the corresponding amine (100 mmol) in water (200 mL) was added NaOH (8.0 g, 200 mmol) and CS_2 (6.03 mL, 100 mmol), stirring for 16 h at room temperature. Then, a solution of sodium chloroacetate (9.45 g, 100 mmol) was added, the mixture was stirred for 3 h at room temperature before acidifying with HCl (6 M, 90 mL). The resulting solution was refluxed for 16 h, and then cooled to room temperature. The crude product was extracted with ethyl acetate, and the combined organic layer was dried on $MgSO_4$, filtered, and the solvent was removed *in vacuo* to given the raw product. The raw product was recrystallized with EtOH to offer compounds **1** or **2**.

2-(4-oxo-2-thioxothiazolidin-3-yl)acetic acid (1). Pale yellow solid (15.6 g, 82%), ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.56 (s, 2H), 4.41 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 203.3, 174.2, 167.8, 45.3, 36.5. HRMS(ESI) *m/z*: 189.9631 (Calcd for $C_5H_4NO_3S_2$ $[M-H]^-$ 189.9638).

4-(4-oxo-2-thioxothiazolidin-3-yl)benzoic acid (2). Yellow solid (18.7 g, 74%), ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.08 (d, J = 8.4 Hz, 2H), 7.42 (d, J = 8.4 Hz, 2H), 4.39 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 203.9, 174.4, 167.1, 139.8, 132.0, 130.7, 129.6, 37.8. HRMS(ESI) *m/z*: 251.9797 (Calcd for $C_{10}H_6NO_3S_2$ [M-H]$^-$ 251.9789).

General procedure 2 for rhodanines (1a-m)

To a solution of the aldehydes (1 mmol) in glacial acetic acid (5 mL) and sodium acetate (660 mg, 4 mmol) was added **1** (380 mg, 2 mmol). The mixture was refluxed for 3 h at 120 ℃, cooled to room temperature, and aqueous HCl (6 M, 5 mL) was added, and refluxed for a further 1 h. The reaction mixture was cooled to room temperature again. The precipitate was collected *via* vacuum filtration, washed with water and hot EtOH, and gave the pure products **1a-m**.

(Z)-2-(5-benzylidene-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (1a). Yellow solid (153 mg, 55%), m.p: 245-246 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.89 (s, 1H), 7.67 (m, 2H), 7.56 (m, 3H), 4.74 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 198.5, 172.5, 171.6, 139.2, 138.0, 136.5, 136.0, 134.8, 127.0, 50.2. HRMS(ESI) *m/z*: 277.9938 (Calcd for $C_{12}H_8NO_3S_2$ [M-H]$^-$ 277.9946).

(Z)-2-(5-(furan-2-ylmethylene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (1b). Yellow solid (187 mg, 69%), m.p: 209-210 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.47 (s, 1H), 8.19 (s, 1H), 7.76 (s, 1H), 7.29 (s, 1H), 6.82 (s, 1H), 4.72 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 194.5, 167.8, 166.5, 149.9, 149.4, 121.6, 120.2, 118.9, 114.6, 45.4. HRMS(ESI) *m/z*: 267.9745 (Calcd for $C_{10}H_6NO_4S_2$ [M-H]$^-$ 267.9738).

(Z)-2-(4-oxo-5-(thiophen-2-ylmethylene)-2-thioxothiazolidin-3-yl)acetic acid (1c). Yellow solid (201 mg, 71%), m.p: 241-242 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.48 (s, 1H), 8.21 (s, 1H), 8.18 (s, 0H), 7.84 (s, 1H), 7.37 (s, 1H), 4.76 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 192.6, 167.7, 166.5, 137.7, 136.9, 135. 0, 130.0, 127.5, 119.4 45.6. HRMS(ESI) *m/z*: 283.9527 (Calcd for $C_{10}H_6NO_3S_3$ [M-H]$^-$ 283.9515).

(Z)-2-(4-oxo-5-(pyridin-3-ylmethylene)-2-thioxothiazolidin-3-yl)acetic acid (1d). Yellow solid (165 mg, 59%), m.p: 262-263 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.54 (s, 1H),8.93 (s, 1H), 8.70 (s, 1H), 8.06 (d, 1H), 7.97 (s, 1H), 7.61 (s, 1H), 4.76 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 193.3, 167.7, 166.6, 152.5, 151.6, 137.1, 131.11, 129.4, 124.9 124.5, 45.5. HRMS(ESI) *m/z*: 278.9918 (Calcd for $C_{11}H_7N_2O_3S_2$ [M-H]$^-$ 278.9903).

(Z)-2-(5-(naphthalen-1-ylmethylene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (1e). Yellow solid (208 mg, 63%), m.p: 251-252 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.56 (s, 1H), 8.56 – 8.47 (m, 1H), 8.19 (d, J = 7.4 Hz, 1H), 8.12 (d, J = 8.0 Hz, 1H), 8.05 (m, 1H), 7.78 – 7.62 (m, 4H), 4.79 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 194.2, 167.8, 166.2, 133.7, 132.1, 131.5, 131.1, 130.3, 129.4, 128.2, 127.7, 127.4, 126.2, 125.5, 123.8, 45.5. HRMS(ESI) *m/z*: 328.0118. (Calcd for $C_{16}H_{10}NO_3S_2$ [M-H]$^-$ 328.0107).

(Z)-2-(5-(naphthalen-2-ylmethylene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (1f). Yellow solid (198 mg, 60%), m.p: 238-239 ℃. H NMR (400 MHz, DMSO-d_6) δ 13.54 (s, 1H), 8.29 (s, 1H), 8.06 (t, J = 8.5 Hz, 2H), 7.97 (d, J = 5.2 Hz, 2H), 7.73 – 7.57 (m, 3H), 4.77 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 193.7, 167.8, 166.8, 134.4, 134.0, 133.2, 132.6, 130.8, 129.6, 129.5, 129.0, 128.2, 127.7, 126.7, 122.3, 45.5. HRMS(ESI) *m/z*: 328.0121. (Calcd for $C_{16}H_{10}NO_3S_2$ [M-H]$^-$ 328.0107).

(Z)-2-(5-(4-methoxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (1g). Yellow solid (189 mg, 61%), m.p: 254-255 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.46 (s, 1H), 7.87 (s, 1H), 7.67 (d, J = 8.8 Hz, 2H), 7.14 (d, J = 8.8 Hz, 2H), 4.74 (s, 2H), 3.85 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 193.5, 167.8, 166.9, 162.2, 134.6, 133.61, 125.8, 118.9, 115.7, 56.1, 45.4. HRMS(ESI) *m/z*: 308.0069. (Calcd for $C_{16}H_{10}NO_3S_2$ [M-H]$^-$ 308.0056).

(Z)-2-(5-(4-chlorobenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (1h). Yellow solid (201 mg, 64%), m.p: 258-259 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.24 (s, 1H), 7.92 (s, 1H), 7.67 (d, J = 8.5 Hz, 2H), 7.60 (d, J = 8.6 Hz, 2H), 4.75 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 193.4, 167.7, 166.7, 136.4, 132.9, 132.1, 130.1, 122.9, 45.5. HRMS(ESI) *m/z*: 311.9553. (Calcd for $C_{12}H_7ClNO_3S_2$ [M-H]$^-$ 311.9561).

(Z)-2-(5-(2,4-dichlorobenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (1i). Yellow solid (212 mg, 61%), m.p: 243-244 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.57 (s, 1H), 7.90 (s, 1H), 7.89 (s, 1H), 7.64 (s, 2H), 4.76 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 193.3, 167.7, 166.5, 136.7, 136.3, 131.2, 130.6, 130.2, 129.1, 127.8, 126.5, 45.6. HRMS(ESI) *m/z*: 345.9180. (Calcd for $C_{12}H_6Cl_2NO_3S_2$ [M-H]$^-$ 345.9166).

(Z)-2-(5-(4-fluorobenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (1j). Yellow solid (201 mg, 68%), m.p: 264-265 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.90 (s, 1H), 7.74 (s, 2H), 7.40 (s, 2H), 4.74 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 193.6, 167.8, 162.5, 133.9, 133.8, 133.3, 129.9, 121.9, 117.3, 117.1, 45.5. HRMS(ESI) *m/z*: 295.9867. (Calcd for $C_{12}H_7FNO_3S_2$ [M-H]$^-$ 295.9851).

(Z)-2-(4-oxo-2-thioxo-5-(3-(trifluoromethyl)benzylidene)thiazolidin-3-yl)acetic acid (1k). Yellow solid (235 mg, 67%), m.p: 200-201 ℃. ^{1}H NMR (400MHz, DMSO-d_6) δ 13.56 (s, 1H), 8.11 (s, 1H), 8.04 (s, 1H), 7.98 – 7.88 (m, 2H), 7.86 – 7.78 (m, 1H), 4.77 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 193.3, 167.2, 166.6, 134.3, 133.7, 132.6, 131.2, 128.3, 128.3, 127.6, 125.5, 124.5, 45.5. HRMS(ESI) *m/z*: 345.9813. (Calcd for $C_{13}H_7F_3NO_3S_2$ [M-H]$^-$ 345.9825).

(Z)-2-(5-(4-nitrobenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (1l). Saffron yellow solid (178 mg, 55%), m.p: 267-268 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.59 (s, 1H), 8.37 (d, J = 8.8 Hz, 2H), 8.02 (s, 1H), 7.94 (d, J = 8.8 Hz, 2H), 4.77 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 198.0, 172.4, 171.4, 153.0, 144.1, 136.8, 136.3, 131.3, 129.7, 50.3. HRMS(ESI) *m/z*: 322.9812. (Calcd for $C_{12}H_7N_2O_5S_2$ [M-H]$^-$ 322.9796).

(Z)-2-(5-(2,4-dinitrobenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (1m). Yellow solid (134 mg, 36%), m.p: 204-205 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.55 (s, 1H), 8.87 (d, J = 2.3 Hz, 1H), 8.66 (dd, J = 8.5, 2.3 Hz, 1H), 8.16 (s, 1H), 8.06 (d, J = 8.5 Hz, 1H), 4.77 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 193.4, 167.7, 165.9, 148.2, 134.9, 131.64, 129.3, 129.3, 129.2, 129.2, 121.3, 45.6. HRMS(ESI) *m/z*: 367.9653. (Calcd for $C_{12}H_6N_3O_7S_2$ [M-H]$^-$ 367.9647).

General procedure 3 for rhodanines (2a-m). To a solution of aldehyde (1 mmol) and compound **2** (250 mg, 1 mmol) in acetic acid (4 mL) was added ammonium acetate (308 mg, 4 mmol). The mixture was stirred at 120 ℃ until the aldehyde reacted completely, about 4 h. After cooling, the precipitate was collected by filtration and washed with water. The solid was recrystallized with ethanol and filtrated to afford the desired compound **2a-2m**.

(Z)-4-(5-benzylidene-4-oxo-2-thioxothiazolidin-3-yl)benzoic acid (2a) Yellow solid (223 mg, 66%), m.p: >298 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.26 (s, 1H), 8.11 (s, 2H), 7.87 (s, 1H), 7.70 (s, 2H), 7.59 (s, 5H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 194.6, 167.2, 139.4, 133.4, 133.3, 132.3, 131.5, 131.1, 130.7 130.1, 129.7, 123.8. HRMS(ESI) *m/z*: 340.0116. (Calcd for $C_{17}H_{10}NO_3S_2$ [M-H]$^-$ 340.0102).

(Z)-4-(5-(furan-2-ylmethylene)-4-oxo-2-thioxothiazolidin-3-yl)benzoic acid (2b) Yellow solid (208 mg, 63%), m.p: >295 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.25 (s, 1H), 8.19 (s, 1H), 8.13 – 8.07 (m, 2H), 7.70 (s, 1H), 7.58 – 7.50 (m, 2H), 7.26 (s, 1H), 6.82 (s, 1H). ^{13}C NMR (101MHz, DMSO-d_6) δ 195.1, 167.0, 166.9, 150.1, 149.2, 139.5, 132.2, 130.2, 129.7, 121.1, 120.4, 119.3, 114.6. HRMS(ESI) *m/z*: 329.9982. (Calcd for $C_{15}H_8NO_4S_2$ [M-H]$^-$ 329.9895).

(Z)-4-(4-oxo-5-(thiophen-2-ylmethylene)-2-thioxothiazolidin-3-yl)benzoic acid (2c). Yellow solid (251 mg, 72%), m.p: >298 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.23 (s, 1H), 8.18–8.06 (m, 4H), 7.80 (s, 1H), 7.61–7.53 (m, 2H), 7.36 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 193.1, 167.1, 166.9, 139.5, 137.9, 136.5, 135.4, 132.2, 131.8, 131.4, 130.7, 129.8, 126.5, 120.9. HRMS(ESI) *m/z*: 345.9678. (Calcd for $C_{15}H_8NO_3S_3$ [M-H]$^-$ 345.9666).

(Z)-4-(4-oxo-5-(pyridin-3-ylmethylene)-2-thioxothiazolidin-3-yl) benzoic acid (2d). Yellow solid (211 mg, 62%), m.p: >300 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.27 (s, 1H), 8.94 (s, 1H), 8.70–8.65 (m, 1H), 8.16–8.09 (m, 2H), 8.06–8.00 (m, 1H), 7.90 (s, 1H), 7.64–7.54 (m, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 193.8, 167.1, 167.0, 152.4, 151.4, 139.3, 136.9, 132.3, 130.7, 130.3, 129.7, 129.6, 129.6, 126.0, 124.9. HRMS(ESI) *m/z*: 341.0073. (Calcd for $C_{16}H_9N_2O_3S_2$ [M-H]$^-$ 341.0055).

(Z)-4-(5-(naphthalen-1-ylmethylene)-4-oxo-2-thioxothiazolidin-3-yl)benzoic acid (2e). Yellow solid (198 mg, 56%), m.p: >298 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.26 (s, 1H), 8.50 (s, 1H), 8.20–8.08 (m, 4H), 8.06–7.99 (m, 1H), 7.76–7.71 (m, 1H), 7.71–7.62 (m, 3H), 7.62–7.56 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 194.7, 167.1, 166.7, 139.4, 133.8, 132.3, 131.9, 131.5, 130.8, 130.5, 129.9, 129.7, 129.4, 128.1, 127.5, 127.4, 127.1, 126.3, 123.9. HRMS(ESI) *m/z*: 390.0266. (Calcd for $C_{21}H_{12}NO_3S_2$ [M-H]$^-$390.0259).

(Z)-4-(5-(naphthalen-2-ylmethylene)-4-oxo-2-thioxothiazolidin-3-yl)benzoic acid (2f). Yellow solid (251 mg, 64%), m.p: >298 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.29 (s, 1H), 8.32 (s, 1H), 8.16 – 8.01 (m, 4H), 7.98 (s, 2H), 7.80 – 7.72 (m, 1H), 7.70 – 7.53 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 194.2, 167.3, 167.1, 133.9, 133.3, 132.3, 132.2, 131.0, 130.7, 129.7, 129.4, 128.9, 128.2, 127.8, 126.8, 123.9. HRMS(ESI) *m/z*: 390.0273. (Calcd for $C_{21}H_{13}NO_3S_2$ [M-H]$^-$ 390.0259).

(Z)-4-(5-(4-methoxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)benzoic acid (2g). Yellow solid (265 mg, 71%), m.p: >295 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.11 (d, *J* = 8.3 Hz, 2H), 7.83 (s, 1H), 7.68 (d, *J* = 8.7 Hz, 2H), 7.58 (d, *J* = 8.3 Hz, 2H), 7.16 (d, *J* = 8.7 Hz, 2H), 3.87 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 194.0, 167.3, 162.1, 139.6, 133.5, 130.7, 129.7, 126.0, 120.4, 115.7, 56.1. HRMS(ESI) *m/z*: 370.0207. (Calcd for $C_{18}H_{12}NO_4S_2$ [M-H]$^-$ 370.0213).

(Z)-4-(5-(4-chlorobenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)benzoic acid (2h). Yellow solid (157 mg, 42%), m.p: >295 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.29 (s, 1H), 8.13 (d, *J* = 8.4 Hz, 2H), 7.87 (s,

1H), 7.73 (d, *J* = 8.6 Hz, 2H), 7.65 (d, *J* = 8.6 Hz, 2H), 7.58 (d, *J* = 8.4 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 193.9, 167.2, 167.1, 139.4, 136.1, 132.71, 132.3, 131.8, 130.7, 130.1, 129.7, 124.6. HRMS(ESI) *m/z*: 373.9730. (Calcd for $C_{17}H_9ClNO_3S_2$ [M-H]$^-$ 373.9712).

(Z)-4-(5-(2,4-dichlorobenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)benzoic acid (2i). Saffron yellow solid (252 mg, 62%), m.p: >297 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.31 (s, 1H), 8.12 (d, *J* = 8.5 Hz, 2H), 7.89 (d, *J* = 1.0 Hz, 1H), 7.86 (s, 1H), 7.66 (d, *J* = 0.8 Hz, 2H), 7.59 (d, *J* = 8.5 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 193.8, 167.0, 166.9, 139.2, 136.5, 136.2, 132.4, 131.0, 130.7, 130.3, 129.6, 129.1, 128.0, 126.5. HRMS(ESI) *m/z*: 407.9335. (Calcd for $C_{17}H_8Cl_2NO_3S_2$ [M-H]$^-$ 407.9323).

(Z)-4-(5-(4-fluorobenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)benzoic acid (2j). Yellow solid (195 mg, 54%), m.p: >298 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.26 (s, 1H), 8.15–8.05 (m, 2H), 7.87 (s, 1H), 7.76 (s, 2H), 7.63 – 7.53 (m, 2H), 7.46–7.33 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 194.1, 167.2, 167.1, 139.4, 133.7, 133.6, 132.2, 130.7, 130.2, 129.7, 123.4, 117.4, 117.1. HRMS(ESI) *m/z*: 358.0029. (Calcd for $C_{17}H_9FNO_3S_2$ [M-H]$^-$ 358.0013).

(Z)-4-(4-oxo-2-thioxo-5-(3-(trifluoromethyl) benzylidene) thiazolidin-3-yl)benzoic acid (2k). Yellow solid (234 mg, 57%), m.p: 276-277 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ13.27 (s, 1H), 8.16–8.09 (m, 2H), 8.08 (s, 1H), 7.97 (s, 1H), 7.95–7.91 (m, 1H), 7.89–7.85 (m, 1H), 7.84–7.77 (m, 1H), 7.63–7.51 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 193.8, 167.1, 139.3, 134.6, 133.6, 132.3, 131.3, 131.2, 130.7, 129.6, 128.1, 127.5, 126.0, 125.6. HRMS(ESI) *m/z*: 407.9988. (Calcd for $C_{18}H_9F_3NO_3S_2$ [M-H]$^-$ 407.9976).

(Z)-4-(5-(4-nitrobenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)benzoic acid (2l). Brown solid (178 mg, 46%), m.p: >297 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.28 (s, 1H), 8.40 (d, *J* = 8.9 Hz, 2H), 8.16 – 8.10 (m, 2H), 7.97 (s, 2H), 7.95 (s, 1H), 7.62 – 7.58 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 193.8, 167.0, 148.1, 139.6, 139.2, 132.4, 131.9, 130.8, 130.2, 129.6, 128.2, 124.9. HRMS(ESI) *m/z*: 384.9962. (Calcd for $C_{17}H_9N_2O_5S_2$ [M-H]$^-$ 384.9953).

(Z)-4-(5-(2,4-dinitrobenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)benzoic acid (2m). Yellow solid (261 mg, 61%), m.p: 284-285 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.28 (s, 1H), 8.89 (d, *J*=2.1 Hz, 1H), 8.70 (dd, *J*=8.5, 2.2 Hz, 1H), 8.13 (d, *J*=8.3 Hz, 2H), 8.10–8.04 (m, 2H), 7.62 (d, *J* = 8.3 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 193.9, 167.0, 166.4, 148.5, 148.1, 139.1, 135.0, 132.4, 131.5, 131.2, 130.8, 129.6, 129.3, 127.4, 121.4 HRMS(ESI) *m/z*: 429.9802. (Calcd for $C_{17}H_8N_3O_7S_2$ [M-H]$^-$ 429.9804).

Synthesis of (Z)-2-mercapto-3-(4-methoxyphenyl)acrylic acid (3a). Compound **2g** (186 mg, 0.5 mmol) was dissolved in 1 M NaOH (4 mL), upon which a color change to deep red was observed. The clear reaction mixture was warmed to 60 °C and stirred for 45 min (the reaction colour changed from deep red to yellow), consumption of starting material was observed. The reaction mixture was cooled to room temperature and subsequently carefully acidified with 1 M HCl (5 mL). A faint yellow precipitate was formed and collected, after removal of the liquid phase, the white solid was re-suspended in H_2O (2 mL) and collected. This process was repeated twice. The obtained solid was dried under high vacuum overnight. The product was obtained as faint yellow solid (53 mg, 25%), m.p: 163 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.72 (s, 1H), 7.68 (s, 1H), 7.66 (s, 1H), 7.07 (s, 1H), 7.05 (s, 1H), 3.81 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.0, 160.1, 133.9, 132.0, 127.8, 121.5, 114.7, 55.8. HRMS(ESI) *m/z*:209.0280. (Calcd for $C_{10}H_9O_3S$ [M-H$^+$]$^-$209.0275).

The small molecule X-ray structure of rhodanine derivatives

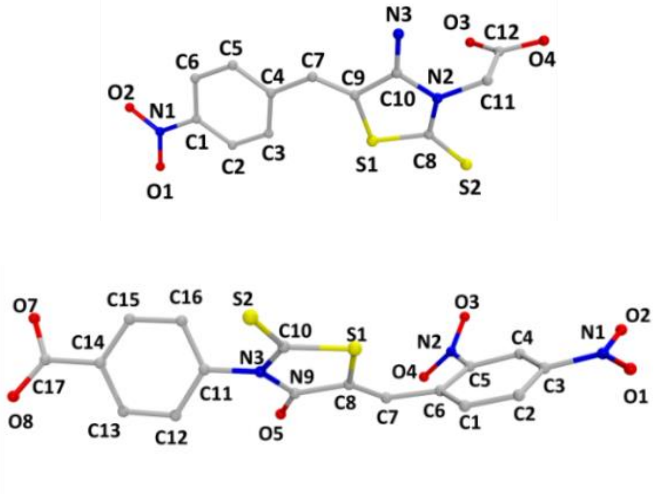

Figure S1. Crystal structures of rhodanine **1l** (top) and **2m** (bottom). The molecules are shown as ball and sticks models (C, gray; N, blue; O, red; S, yellow).

Purity analysis of compound 2l by HPLC

The purity analysis was done by SHIMADZU (LC-2010C HT) analytical reverse phase HPLC using water/acetonitrile (5-90%) as mobile phase for 30 min. A C18 column (10 mm diameter, 250 mm length) and UV detector (at 375 nm wavelength) were used. The result is shown in Figure. S2.

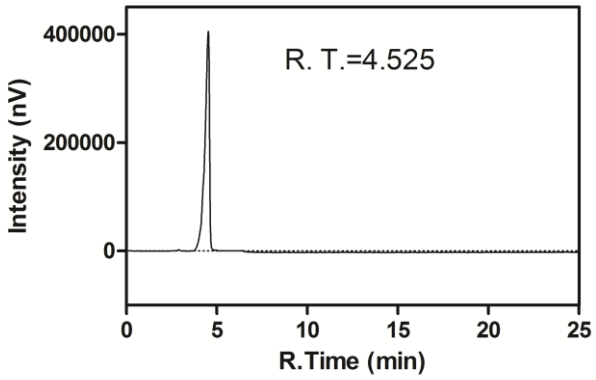

Figure S2. Purity analysis of compound **2l** by HPLC.

Over-expression and purification of MβLs

NDM-1: The overexpression plasmid, pET26b-NDM-1, was used for expression of NDM-1 as previously described.[2] *E. coli* BL21(DE3) cells were transformed with the over-expression plasmid, pET26b-NDM-1. A 10 mL overnight culture of these cells in lysogeny broth (LB) was used to inoculate 4 × 1 L of LB medium containing 25 μg/mL kanamycin. The cells were allowed to grow at 37 °C with shaking

until they 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. Cells were collected by centrifugation (30 min at 8,275 x g) and resuspended in 25 mL of 30 mM Tris, pH 8.0, containing 500 mM NaCl. The cells were lysed by ultrasonication, and cell debris was separated by centrifugation (30 min at 32,583 x 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 x 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 an YM-10 membrane. The crude protein NDM-1 was run through a G75 column and eluted with 30 mM Tris, pH 7.5, containing 200 mM NaCl. Protein purity was ascertained by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) and protein concentration was determined using Beer's law and an extinction coefficient of 27,960 $M^{-1}cm^{-1}$ at 280 nm.

VIM-2: VIM-2 was overexpressed and purified as previously described.[3] *E. coli* BL21(DE3) cells were transformed with plasmid pET24a-VIM-2. A 10 mL overnight culture of these cells in lysogeny broth (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 they 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 overnight. Cells were removed by centrifugation (30 min at 8,275 x g) and the supernatant was concentrated using an Amicon ultrafiltration cell equipped with a YM-10 membrane. The concentrated protein was dialyzed *versus* 30 mM Tris, pH 7.6, containing 100 μM $ZnCl_2$ for 4 h, three times at 4 °C, centrifuged (25 min at 32,583 x 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 7.6, containing 100 μM $ZnCl_2$ at 2 mL/min. Fractions (2 mL) containing VIM-2 were pooled and concentrated with an Amicon ultrafiltration cell equipped with an YM-10 membrane. The crude protein VIM-2 was run through a G75 column and eluted with 30 mM Tris, pH 7.6, 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 39,000 $M^{-1}cm^{-1}$ at 280 nm.

ImiS: ImiS was overexpressed and purified as previously described.[4] *E. coli* BL21(DE3) cells were transformed with a plasmid containing the gene for ImiS, pET-26b-ImiS. A 10 mL overnight culture of these cells in lysogeny broth (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 they 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. Cells were collected by centrifugation (30 min at 8,275 x 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 x g). The cleared supernatant was dialyzed *versus* 30 mM Tris, pH 7.0, containing 100 μM $ZnCl_2$ for 36 h at 4 °C, centrifuged (25 min at 32,583 x 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 an 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: L1 was overexpressed and purified as previously described.[5] The gene that encodes L1 was ligated

into pET26b and *E. coli* BL21(DE3) cells were transformed with the resulting plasmid pET26b(+)-L1. A 10 mL overnight culture of these cells in lysogeny broth (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 they 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 x g) and resuspended in 25 mL of 30 mM Tris, pH 8.5, containing 500 mM NaCl. The cells were lysed byultrasonication, and the cell debris was separated by centrifugation (30 min at 32,583 x g). The cleared supernatant was dialyzed versus 30 mM Tris, pH 8.5, containing 100 μM $ZnCl_2$ for 36 h at 4 °C, centrifuged (25 min at 32,583 x 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 ultrafiltration cell equipped with an 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.

Stability assays of rhodanine in aqueous solution at different pH

The hydrolysis of rhodanine **2g** (50 μM) in MES (pH 5.5, 6.0, and 6.5), Tris (pH 7.0, 7.5, 8.0, and 8.5), and Tris (pH 7.0) containing L1 (10 μM) were assayed on an Agilent UV8453 spectrometer at 25 °C through monitoring the absorbance change at 401 nm for 24 h. The UV-Vis spectra were recorded once every 4 h, and the results are shown in Figure. S3. It is clearly observed that the rhodanine was not hydrolyzed in the buffer with a pH ranging 6.0-7.0 (Figure S3B-D), but it was hydrolyzed in the buffer with pH 5.5, 7.5, 8.0 and 8.5 (Figure S3A and 3E-G), and also the hydrolysis of rhodanine accelerated with the increase of pH from 7.5 to 8.5. Further assays of **2g** in Tris (pH 7.0) containing L1 (10 μM) showed that the rhodanine was not hydrolyzed (Figure S3H).

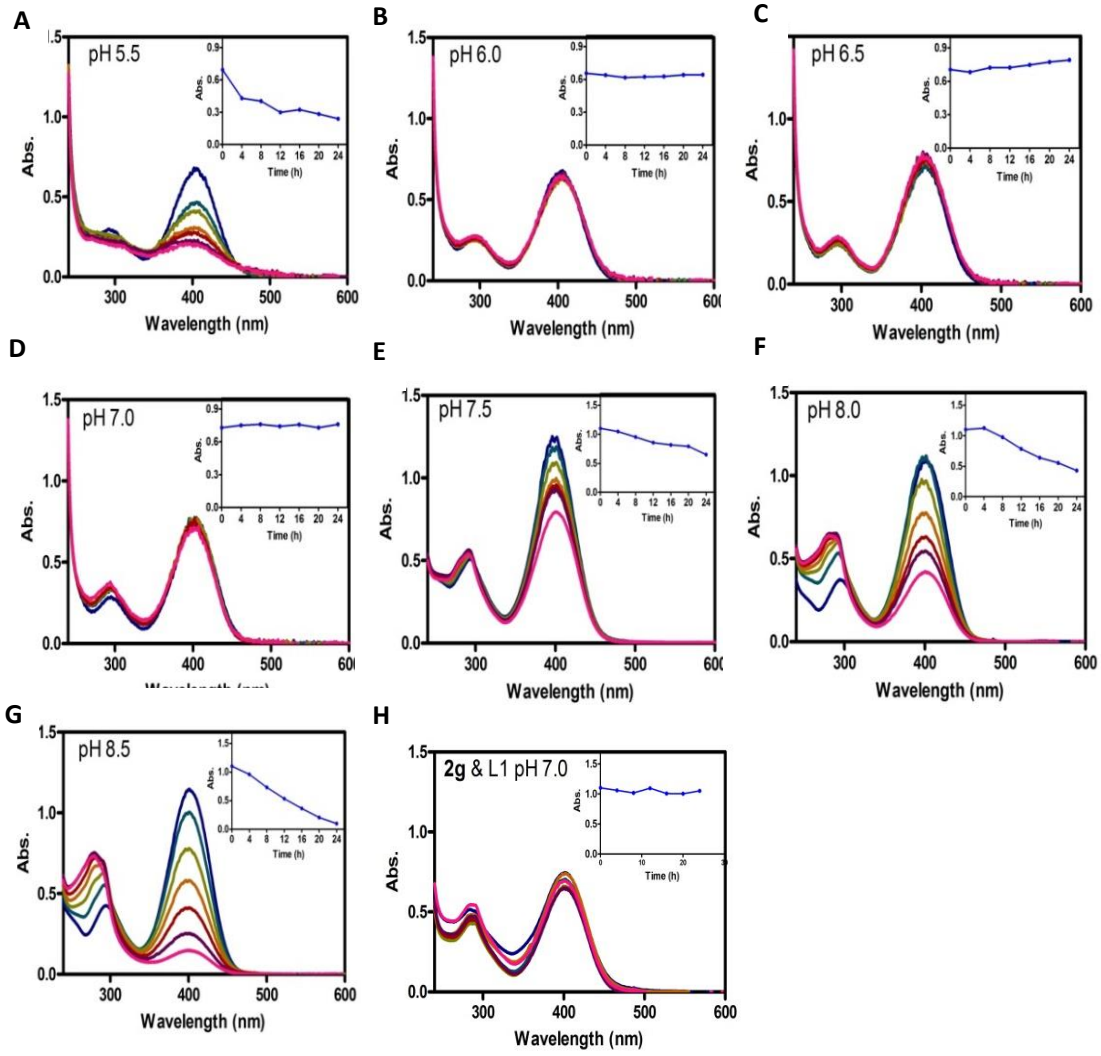

Figure S3. UV-Vis spectra monitoring rhodanine **2g** (50 μM) hydrolysis in buffers with different pH over 24 hours (blue, initial spectrum; pink, final spectrum). MES, pH 5.5 (A), 6.0 (B), and 6.5 (C); Tris, pH 7.0 (D), 7.5 (E), 8.0 (F), and 8.5(G), and Tris containing L1, pH 7.0 (H). Insets show absorbance at 401 nm over time.

Inhibition kinetic studies

The inhibitor concentrations causing 50% decrease of enzyme activity (IC_{50}) were determined. Enzymes were expressed and purified as described in the Supporting Information. The rhodanine derivatives **1a-1m**, **2a-2m** and the thioenolate **3a** were dissolved in a small volume of DMSO and diluted with ddH_2O, and the lyophilized MβLs and substrates were dissolved in 50 mM Tris (pH 7.0). The final concentrations of DMSO in inhibition experiments were below 0.5%, and control experiments verified that 0.5% DMSO had no inhibitory activity against the tested MβLs. The IC_{50} values were determined in triplicate against these four enzymes, where the inhibitor concentrations were varied between 0 and 50 μM. The final concentrations of NDM-1, VIM-2, ImiS, and L1 were 13, 100, 50 and 58 nM, respectively. 50 μM cefazolin was used as substrate of NDM-1, VIM-2 and L1, and 60 μM imipenem was used as substrate of ImiS, and the hydrolysis of substrate was monitored at 262 and 300 nm, respectively, on an Agilent UV8453 spectrometer at 25 °C. The tested MβLs and inhibitors were premixed at room temperature in buffer (50 mM Tris-HCl, pH 7.0), and then substrate in the same buffer was added to the mixture, the initial rates of antibiotic hydrolysis were recorded at each inhibitor concentration. All experimental hydrolytic rates were determined in triplicate. The percent inhibition was calculated by equation $\%I = 1-(V_i/V_0)$ and IC_{50} values were calculated by plotting the average percentage inhibition against inhibitor concentration and sigmoidal fitting of the data.

Antibacterial activity assays

The minimum inhibitory concentration (MIC) of compound alone and antibiotics in the presence and absence of enzyme inhibitors were determined using the broth micro-dilution method.[6] Single colonies of *E. coli* DH10B containing plasmid, pBCSK-NDM-1, pBCSK-VIM-2, pBCSK-ImiS or pBCSK-L1 in lysogeny broth agar plates were transferred into 5 mL of Mueller-Hinton (MH) liquid medium. Strains grown in MH medium to OD_{600} = 0.45 were used as inocula after 84-fold dilution to 1×10^5 CFU/mL in MH medium. Cefazolin was dissolved in MH medium to prepare 4096, 2048, 1024, 512, 256, 128, 64 and 32 (for *E.coli* producing VIM-2) and 64, 32, 16, 8, 4, 2, 1 and 0.5 μg/mL (for *E. coli* expressing NDM-1 and L1), and imipenem was dissolved in MH medium to prepare 64, 32, 16, 8, 4, 2, 1 and 0.5 μg/mL (for *E. coli* expressing ImiS) stock solutions, respectively. Compounds **1a-1m**, **2a-2m** and **3a** as inhibitors were dissolved in DMSO and MH media to prepare 128 μg/mL stock solutions. The prepared solutions with different antibiotic concentrations (50 μL) were diluted to 100 μL with 50 μL inhibitor solution, then 100 μL inoculum was added sequentially into the prepared solutions to yield a quarter of the initial concentrations.

Furthermore, **2l** and **2m** were dissolved in DMSO and MH medium to prepare 1024, 512, 256, 128, and 64 μg/mL stock solutions, 50 μL inhibitor solution was diluted to 200 μL with 50 μL substrate solution and 100 μL inoculum to yield final concentrations of 256, 128, 64, 32, and 16 μg/mL. The mixtures were incubated at 37 °C for 16 h. The MIC results were taken as the lowest concentration that completely inhibited visible growth. Each measurement was performed in duplicate.

pH Monitoring of culture medium

Monitoring of pH values of culture medium during *E. coli* reproduction showed that the pH value of culture media decreased from 7.1 to 6.4 in 4 h, then stabilized at about 6.4 in 20 h, and finally increased to 7.4 after 24 h (Figure S4). It means that the rhodanine was not hydrolyzed during the process of MIC test.

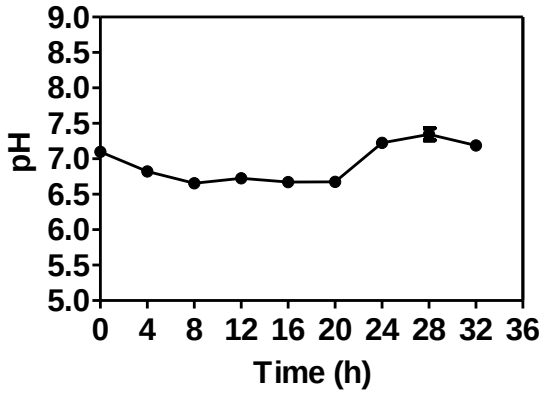

Figure S4. Monitored pH values of culture medium during *E. coli* reproduction.

Cytotoxicity evaluation

A cytotoxicity assay was performed to evaluate the toxicity of **1l**, **1m**, **2l**, **2m** and **3a** to mouse fibroblast cells (L-929). 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 the inhibitors with working concentrations (12.5, 25, 50, 100, 200 and 400 μM) were added to the 96-well plates and incubated for another 48 h. Three wells containing only cells were suspended in a mixture solution of 98 μL complete medium and 1 μL DMSO as control for cell viability without inhibitor. Three wells containing only the complete medium were used as blank control. Following that, the medium was removed, and 100 μL of fresh culture medium and 10 μL CC-K8 were added to each well. After incubation for 4 h, the trays were then vigorously shaken to solubilize the formed product and the absorbance at a wavelength of 490 nm was read on a microplate reader and analyzed. All experiments were conducted in triplicate and data are expressed as mean ± standard deviation. The results are shown in Figure S5.

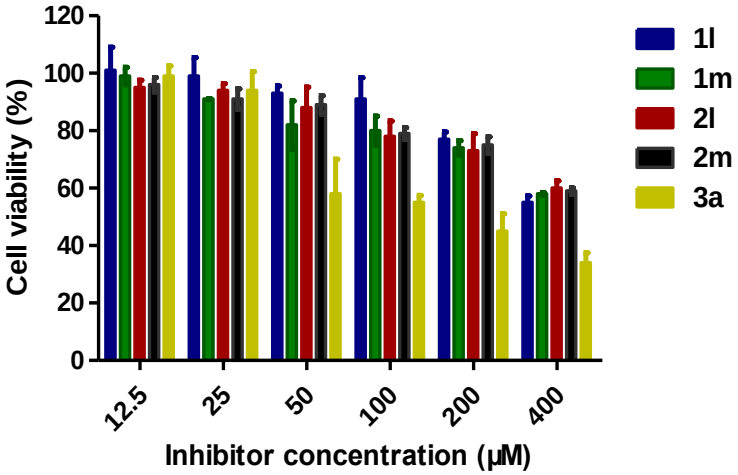

Figure S5. Percent cell viability (relative to without compound) of L-929 mouse fibroblastic cells in the presence of **1l**, **1m**, **2l**, **2m** or **3a** at concentrations ranging from 12.5 to 400 μM.

Docking studies

Docking studies of compound **2l** into the active site of NDM-1 (PDB: 4EYL[7]), VIM-2 (PDB: 4NQ2[8]), CphA (PDB: 2QDS,[9] which was used as a closely related relative of ImiS) and L1 (PDB: 2AIO[10]) were performed by AutoDock 4.2.[11] The carboxyl groups were deprotonated, resulting in the overall charge of -1e for all 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.[12] 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.[13] Enzymes were treated as a rigid receptor, while ligands were treated as flexible. The grid box was centered between the two active-site Zn(II) ions in the binuclear enzymes and Zn2 CphA, with dimensions of 60 x 60 x 60 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 parameters 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 conformations shown in Figure 3 are the lowest-energy conformations of those clusters, and views of **2l** in complex with the four enzymes represented as a surface are shown in Figure S6.

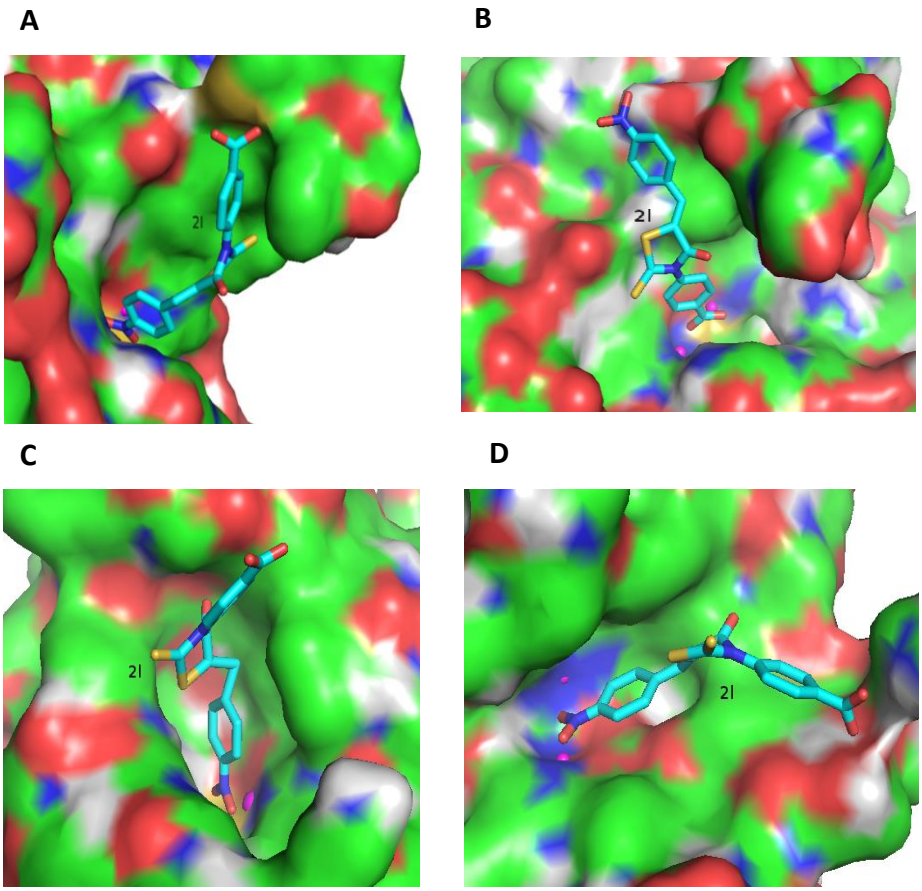

Figure **S6**. Graphical representations of **2l** with the enzymes (A, NDM-1; B, VIM-2; C, CphA; D, L1) represented as a surface (C, green; N, blue; O, red; S, yellow; H, white). Zn(II) ions are shown as a magenta spheres. The ligands are shown as ball and sticks (C, cyan; N, blue; O, red; S, yellow). The pictures were generated with PyMOL software.

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