Supporting Information

Azolylthioacetamide: a Highly Promising Scaffold for the Development of Metallo-β-lactamase Inhibitors

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Synthetic procedures

2-(2-carbamothioylhydrazinecarbonyl)benzoic acid (4)

A solution of thiosemicarbazide (9.00 g, 98.90 mmol) and phthalic anhydride (14.68 g, 99.07 mmol) in acetonitrile (350 mL) was heated under reflux for 2 h. A precipitate was formed on cooling to room temperature, then it was filtered off and washed with anhydrous ethanol. The product was dried under vacuum to obtain compound **4.** White powder, yield 88%, ^{1}H NMR (400 MHz, DMSO): δ 13.75 (s, 1H), 10.35 (s, 1H), 9.43 (s, 1H), 8.27 (d, J = 38.1 Hz, 2H), 7.93 (d, J = 7.6 Hz, 1H), 7.70 – 7.64 (m, 1H), 7.62 – 7.56 (m, 1H), 7.43 (d, J = 6.1 Hz, 1H).

4-(2-carbamothioylhydrazinyl)-4-oxobutanoic acid (5)

A solution of thiosemicarbazide (7.00 g, 76.92 mmol) and succinic anhydride (7.70 g, 77.00 mmol) in acetonitrile (300 mL) was heated under reflux for 2 h. A precipitate was formed on cooling to room temperature, then it was filtered off and washed with anhydrous ethanol. The product was dried under vacuum to obtain compound **5**. White powder, yield 85%, ^{1}H NMR (400 MHz, DMSO): δ 12.25 (s, 1H), 9.84 (s, 1H), 9.23 (s, 1H), 7.92 (s, 1H), 7.26 (s, 1H), 2.47 (t, J = 7.6 Hz, 2H), 2.33 (t, J = 6.0 Hz, 2H).

5-(2-carbamothioylhydrazinyl)-5-oxopentanoic acid (6)

A solution of thiosemicarbazide (6.00 g, 65.93 mmol) and glutaric anhydride

(7.53 g, 66.05 mmol) in acetonitrile (250 mL) was heated under reflux for 2 h. A precipitate was formed on cooling to room temperature, then it was filtered off and washed with anhydrous ethanol. The product was dried under vacuum to obtain compound **6.** White powder, yield 59%, ^{1}H NMR (400 MHz, DMSO): δ 12.08 (s, 1H), 9.69 (s, 1H), 9.15 (s, 1H), 7.85 (s, 1H), 7.44 (s, 1H), 2.22 (t, J = 6.4 Hz, 2H). 2.14 (t, J = 7.0 Hz, 2H).1.77 – 1.64 (m, 2H).

2-(5-mercapto-4H-1,2,4-triazol-3-yl)benzoic acid (7)

Compound **4** (9.50 g, 39.75 mmol) was heated under reflux in 10% NaOH solution (300 mL) overnight, after cooling to room temperature, the reaction mixture was acidified with dilute HCl and concentrated. The product was precipitated out on cooling, and dried under vacuum to obtain compound **7**. White powder, yield 56%, ^{1}H NMR (400 MHz, DMSO): δ 13.59 (s, 1H), 13.15 (s, 1H), 7.89 (d, J = 6.6 Hz, 1H), 7.66 (s, 2H), 7.60 (s, 1H).

3-(5-mercapto-4H-1,2,4-triazol-3-yl)propanoic acid (8)

Compound **5** (9.00 g, 47.12 mmol) was heated under reflux in 20% NaOH solution (120 mL) overnight, after cooling to room temperature, the reaction mixture was acidified with dilute HCl and concentrated. The product was precipitated out on cooling, and dried under vacuum to obtain compound **8**. White powder, yield 30%, ^{1}H NMR (400 MHz, DMSO): δ 13.22 (s, 1H), 12.32 (s, 1H), 2.73 (t, J = 6.6 Hz, 2H), 2.62 (t, J = 6.7 Hz, 2H).

4-(5-mercapto-4H-1,2,4-triazol-3-yl)butanoic acid (9)

Compound **6** (7.70 g, 37.56 mmol) was heated under reflux in 20% NaOH solution (70 mL) overnight, after cooling to room temperature, the reaction mixture was acidified with dilute HCl and concentrated. The product was precipitated out on cooling, and dried under vacuum to obtain compound **9.** White powder, yield 66%, ^{1}H NMR (400 MHz, DMSO): δ 13.12 (s, 1H), 12.05 (s, 1H), 2.44 (t, J = 7.0 Hz, 2H), 2.15 (t, J = 6.7 Hz, 2H), 1.80 – 1.62 (m, 2H).

General procedure A for N-substituted-2-chloroacetamides

To a stirred solution of appropriate amine (20 mmol, 1 equiv) in acetone (50 mL) was added K_2CO_3 (24 mmol, 1.2 equiv), and the solution was stirred in an ice-water bath for 5 min. After chloroacetyl chloride (24 mmol, 1.2 equiv) was added dropwise at 0-5°C, the reaction mixture was heated under reflux for 6 h. Then the reaction mixture was cooled to room temperature and slowly poured into ice water (200 mL), white powder was formed, and the resulting precipitate was separated by filtration and washed successively with water. The product was dried under vacuum to obtain *N-s*ubstituted-2-chloroacetamides.

2-chloro-N-phenylacetamide (a). White powder, yield 71%, ^{1}H NMR (400 MHz, DMSO): δ 10.32 (s, 1H), 7.58 (d, J = 7.4 Hz, 2H), 7.32 (t, J = 7.5 Hz, 2H), 7.08 (s, 1H), 4.24 (s, 2H).

N-(1H-benzo[d]imidazol-2-yl)-2-chloroacetamide (b). Pale yellow powder, yield 63%, ^{1}H NMR (400 MHz, DMSO): δ 12.09 (s, 1H), 7.44 (dd, J = 5.3, 3.0 Hz, 2H), 7.11 (dd, J = 5.3, 2.9 Hz, 2H), 4.38 (s, 2H).

2-chloro-N-(4-chlorophenyl)acetamide (d). White powder, yield 80%, ^{1}H NMR (400 MHz, DMSO): δ 10.44 (s, 1H), 7.62 (d, J = 8.5 Hz, 2H), 7.39 (d, J = 8.5 Hz, 2H), 4.26 (s, 2H).

N-benzyl-2-chloroacetamide (f). White powder, yield 38%, ^{1}H NMR (400 MHz, DMSO): δ 8.75 (s, 1H), 7.37 – 7.30 (m, 2H), 7.26 (d, J = 7.2 Hz, 3H), 4.30 (d, J = 5.7 Hz, 2H), 4.12 (s, 2H).

2-chloro-N-(2-chlorophenyl)acetamide (g). White powder, yield 69%, ^{1}H NMR (400 MHz, DMSO): δ 9.88 (s, 1H), 7.75 (d, J = 7.9 Hz, 1H), 7.51 (d, J = 7.9 Hz, 1H), 7.35 (t, J = 7.6 Hz, 1H), 7.22 (t, J = 6.9 Hz, 1H), 4.39 (s, 2H).

2-chloro-N-(3-chlorophenyl)acetamide (h). White powder, yield 77%, ^{1}H NMR (400 MHz, DMSO): δ 10.50 (s, 1H), 7.80 (s, 1H), 7.45 (d, J = 8.0 Hz, 1H), 7.35 (t, J = 8.0 Hz, 1H), 7.14 (d, J = 7.8 Hz, 1H), 4.27 (s, 2H).

General procedure B for N-substituted-2-chloroacetamides

To a stirred solution of appropriate amine (20 mmol, 1 equiv) in dichloromethane (50 mL) was added triethylamine (24 mmol, 1.2 equiv), and the solution was stirred in an ice-water bath for 5 min. After chloroacetyl chloride (24 mmol, 1.2 equiv) was added dropwise at 0-5 °C, the reaction mixture was stirred at room temperature for 5 h. Then the reaction was evaporated under reduced pressure and washed with water, a

precipitate was obtained after filtered. Recrystallisation from acetone gave the *N-s*ubstituted-2-chloroacetamides.

N-(benzo[d]thiazol-2-yl)-2-chloroacetamide (c). Red brown powder, yield 60%, ^{1}H NMR (400 MHz, DMSO): δ 12.74 (s, 1H), 7.99 (s, 1H), 7.76 (s, 1H), 7.44 (d, J = 4.4 Hz, 1H), 7.31 (d, J = 5.4 Hz, 1H), 4.47 (s, 2H).

2-chloro-N-(4-nitrophenyl)acetamide (e). Yellow powder, yield 51%, ^{1}H NMR (400 MHz, DMSO): δ 10.90 (s, 1H), 8.24 (d, J = 8.5 Hz, 2H), 7.83 (d, J = 8.5 Hz, 2H), 4.34 (s, 2H).

2-chloro-N-(2-nitrophenyl)acetamide (i). Yellow powder, yield 12%, ^{1}H NMR (400 MHz, DMSO): δ 10.71 (s, 1H), 8.02 (d, J = 8.2 Hz, 1H), 7.82 (d, J = 8.0 Hz, 1H), 7.73 (t, J = 7.6 Hz, 1H), 7.40 (t, J = 7.7 Hz, 1H), 4.38 (s, 2H).

2-chloro-N-(3-nitrophenyl)acetamide (j). Yellow power, yield 43%, ^{1}H NMR (400 MHz, DMSO): δ 10.78 (s, 1H), 8.55 (s, 1H), 7.86 (d, J = 7.7 Hz, 2H), 7.54 (t, J = 7.8 Hz, 1H), 4.29 (s, 2H).

General procedure for azolylthioacetamides (1a-j)

To a solution of compound **7** (2 mmol, 1 equiv) in acetone (40 mL) was added K_2CO_3 (2 mmol, 1 equiv), and the reaction mixture was stirred for 5 min. After N-substituted-2-chloroacetamide (**a-j**) (2 mmol, 1 equiv) dissolved in acetone (5 mL) was added dropwise, the reaction mixture was heated under reflux for 6 h. Then the reaction mixture was cooled to room temperature and the precipitate was filtered and dried under vacuum to obtain compounds **1a-j**.

2-(5-((2-oxo-2-(phenylamino)ethyl)thio)-4H-1,2,4-triazol-3-yl)benzoic acid (1a). White powder, yield 91%, ^{1}H NMR (400 MHz, DMSO): δ 10.43 (s, 1H), 8.16 (d, J = 6.8 Hz, 1H), 7.95 (d, J = 6.4 Hz, 1H), 7.60 (d, J = 7.8 Hz, 2H), 7.39 (s, 2H), 7.30 (t, J = 7.6 Hz, 2H), 7.04 (t, J = 7.1 Hz, 1H), 4.06 (s, 2H). ^{13}C NMR (100 MHz, DMSO): δ 171.2, 167.2, 158.4, 157.4, 139.5, 139.4, 132.4, 129.3, 129.2, 129.1, 128.6, 124.8, 123.8, 119.6, 36.9. HRMS(ESI$^-$) *m/z*: 353.0841 (Calcd for [M-H$^+$]$^-$: 353.0714 *m/z*).

2-(5-((2-((1H-benzo[d]imidazol-2-yl)amino)-2-oxoethyl)thio)-4H-1,2,4-triazol-3-yl)benzoic acid (1b). White powder, yield 83%, ^{1}H NMR (400 MHz, DMSO): δ 11.92 (s, 1H), 8.17 (s, 1H), 7.95 (s, 1H), 7.40 (d, J = 25.9 Hz, 4H), 7.08 (s, 2H), 4.16 (s, 2H). ^{13}C NMR (100 MHz, DMSO): δ 171.5, 167.9, 166.8, 157.9, 157.5, 141.8, 139.4, 134.8, 132.3, 132.0, 129.3, 129.1, 128.6, 127.3, 125.5, 125.0, 124.8, 36.3. HRMS(ESI$^-$) *m/z*: 393.1026 (Calcd for [M-H$^+$]$^-$: 393.0775 *m/z*).

2-(5-((2-(benzo[d]thiazol-2-ylamino)-2-oxoethyl)thio)-4H-1,2,4-triazol-3-yl)benzoic acid (1c). White powder, yield 58%, ^{1}H NMR (400 MHz, DMSO): δ 12.80 (s, 1H), 8.19 (s, 1H), 7.98 (s, 2H), 7.77 (d, J = 6.6 Hz, 1H), 7.35 (dd, J = 35.3, 19.4 Hz, 4H), 4.21 (s, 2H). ^{13}C NMR (100 MHz, DMSO): δ 171.1, 170.1, 158.6, 157.3, 149.6, 139.2, 132.7, 132.3, 129.3, 129.2, 128.7, 126.1, 124.7, 123.1, 123.0, 121.9, 120.5, 37.0. HRMS(ESI$^-$) *m/z*: 410.0495 (Calcd for [M-H$^+$]$^-$: 410.0387 *m/z*).

2-(5-((2-((4-chlorophenyl)amino)-2-oxoethyl)thio)-4H-1,2,4-triazol-3-yl)benzoic acid (1d). White powder, yield 35%, ^{1}H NMR (400 MHz, DMSO): δ 10.66 (s, 1H), 7.76 (s, 1H), 7.65 (d, J = 8.0 Hz, 4H), 7.59 (s, 1H), 7.36 (d, J = 6.5 Hz, 2H), 4.14

(s, 2H). ^{13}C NMR (100 MHz, DMSO): δ 171.6, 167.4, 166.3, 158.3, 157.2, 139.4, 138.5, 132.3, 129.5, 129.1, 128.7, 128.2, 127.4, 127.3, 124.6, 121.1, 36.8. HRMS(ESI$^-$) *m/z*: 387.0491 (Calcd for [M-H$^+$]$^-$: 387.0324 *m/z*).

2-(5-((2-((4-nitrophenyl)amino)-2-oxoethyl)thio)-4H-1,2,4-triazol-3-yl)benzoic acid (1e). Yellow powder, yield 54%, ^{1}H NMR (400 MHz, DMSO): δ 11.16 (s, 1H), 8.22 (d, *J* = 8.3 Hz, 2H), 8.13 (s, 1H), 7.94 (s, 1H), 7.88 (d, *J* = 8.3 Hz, 2H), 7.38 (s, 2H), 4.14 (s, 2H). ^{13}C NMR (100 MHz, DMSO): δ 170.8, 168.4, 158.2, 157.4, 145.7, 142.7, 139.2, 136.7, 132.6, 129.4, 129.2, 128.5, 125.5, 124.7, 119.3, 37.0. HRMS(ESI$^-$) *m/z*: 398.0653 (Calcd for [M-H$^+$]$^-$: 398.0565 *m/z*).

2-(5-((2-(benzylamino)-2-oxoethyl)thio)-4H-1,2,4-triazol-3-yl)benzoic acid (1f). White powder, yield 53%, ^{1}H NMR (400 MHz, DMSO): δ 8.76 (s, 1H), 7.80 (d, *J* = 6.7 Hz, 2H), 7.66 – 7.42 (m, 2H), 7.23 (s, 5H), 4.30 (d, *J* = 5.6 Hz, 2H), 3.95 (s, 2H). ^{13}C NMR (100 MHz, DMSO): δ 172.5, 170.8, 142.3, 137.1, 133.7, 132.8, 132.6, 131.5, 131.4, 130.5, 130.3, 129.9, 45.7, 38.5. HRMS(ESI$^-$) *m/z*: 367.0881 (Calcd for [M-H$^+$]$^-$: 367.0870 *m/z*).

2-(5-((2-((2-chlorophenyl)amino)-2-oxoethyl)thio)-4H-1,2,4-triazol-3-yl)benzoic acid (1g). White powder, yield 65%, ^{1}H NMR (400 MHz, DMSO): δ 10.00 (s, 1H), 8.19 (s, 1H), 7.94 (d, *J* = 18.0 Hz, 2H), 7.52 – 7.06 (m, 5H), 4.12 (s, 2H). ^{13}C NMR (100 MHz, DMSO): δ 173.9, 170.8, 160.9, 160.5, 141.9, 138.0, 135.3, 132.7, 132.1, 131.9, 131.5, 130.7, 129.1, 128.2, 127.7, 127.6, 39.0. HRMS(ESI$^-$) *m/z*: 387.0446 (Calcd for [M-H$^+$]$^-$: 387.0324 *m/z*).

2-(5-((2-((3-chlorophenyl)amino)-2-oxoethyl)thio)-4H-1,2,4-triazol-3-yl)benz

oic acid (1h). White powder, yield 93%, ^{1}H NMR (400 MHz, DMSO): δ 10.79 (s, 1H), 8.14 (d, J = 4.0 Hz, 1H), 7.89 (d, J = 37.3 Hz, 2H), 7.48 (d, J = 7.9 Hz, 1H), 7.39 – 7.32 (m, 3H), 7.11 (d, J = 7.6 Hz, 1H), 4.08 (s, 2H). ^{13}C NMR (100 MHz, DMSO): δ 174.1, 170.6, 160.9, 160.3, 143.7, 142.3, 136.3, 135.0, 133.7, 132.0, 131.8, 131.3, 127.6, 126.3, 121.8, 120.8, 39.6. HRMS(ESI$^-$) *m/z*: 387.0448 (Calcd for [M-H$^+$]$^-$: 387.0324 *m/z*).

2-(5-((2-((2-nitrophenyl)amino)-2-oxoethyl)thio)-4H-1,2,4-triazol-3-yl)benzoi c acid (1i). Yellow powder, yield 41%, ^{1}H NMR (400 MHz, DMSO): δ 10.91 (s, 1H), 8.15 (d, J = 7.3 Hz, 1H), 8.04 – 7.82 (m, 4H), 7.72 (t, J = 7.6 Hz, 1H), 7.43 – 7.31 (m, 2H), 4.09 (s, 2H). ^{13}C NMR (100 MHz, DMSO): δ 174.0, 170.6, 160.7, 160.2, 144.4, 142.0, 137.6, 135.2, 134.8, 132.2, 132.0, 131.4, 130.1, 128.3, 127.8, 127.4, 39.1. HRMS(ESI$^-$) *m/z*: 398.0690 (Calcd for [M-H$^+$]$^-$: 398.0565 *m/z*).

2-(5-((2-((3-nitrophenyl)amino)-2-oxoethyl)thio)-4H-1,2,4-triazol-3-yl)benzoi c acid (1j). Yellow powder, yield 56%, ^{1}H NMR (400 MHz, DMSO): δ 11.12 (s, 1H), 8.69 (s, 1H), 8.14 (s, 1H), 8.02 – 7.83 (m, 3H), 7.61 (t, J = 7.8 Hz, 1H), 7.39 (s, 2H), 4.14 (s, 2H). ^{13}C NMR (100 MHz, DMSO): δ 174.1, 171.0, 161.0, 160.1, 151.1, 143.4, 142.2, 135.1, 133.4, 132.1, 131.8, 131.3, 128.4, 127.4, 121.1, 116.4, 39.6. HRMS(ESI$^-$) *m/z*: 398.0694 (Calcd for [M-H$^+$]$^-$: 398.0565 *m/z*).

General procedure for azolylthioacetamides (2a-e)

To a solution of compound **8** (2 mmol, 1 equiv) in ethanol (40 mL) was added NaOH (2 mmol, 1 equiv), and the reaction mixture was stirred for 5 min. After

N-substituted-2-chloroacetamide **(a-e**) (2 mmol, 1 equiv) dissolved in ethanol (5 mL) was added dropwise, the reaction mixture was heated under reflux for 6 h. Then the reaction mixture was cooled to room temperature and the precipitate was filtered and dried under vacuum to obtain compounds **2a-e**.

3-(5-((2-oxo-2-(phenylamino)ethyl)thio)-4H-1,2,4-triazol-3-yl)propanoic acid (2a). White powder, yield 49%, ^{1}H NMR (400 MHz, DMSO): δ 10.67 (s, 1H), 7.72 (s, 2H), 7.40 (s, 2H), 7.15 (s, 1H), 4.13 (s, 2H), 2.93 (s, 2H), 2.62 (s, 2H). ^{13}C NMR (100 MHz, DMSO): δ 173.8, 168.5, 158.1, 157.1, 146.9, 121.6, 114.6, 114.1, 35.9, 31.7, 22.0. HRMS(ESI^{-}) *m/z*: 305.0914 (Calcd for [M-H^{+}]$^{-}$: 305.0714 *m/z*).

3-(5-((2-((1H-benzo[d]imidazol-2-yl)amino)-2-oxoethyl)thio)-4H-1,2,4-triazol -3-yl)propanoic acid (2b). White powder, yield 78%, ^{1}H NMR (400 MHz, DMSO): δ 12.09 (s, 1H), 7.74 – 7.26 (m, 2H), 7.24 – 6.93 (m, 2H), 4.12 (s, 2H), 2.85 (t, J = 6.8 Hz, 2H), 2.65 (t, *J* = 6.8 Hz, 2H). ^{13}C NMR (100 MHz, DMSO): δ 174.9, 166.7, 158.3, 156.8, 138.0, 128.6, 126.9, 120.7, 36.2, 33.3, 22.5. HRMS(ESI^{-}) *m/z*: 345.0992 (Calcd for [M-H^{+}]$^{-}$: 345.0775 *m/z*).

3-(5-((2-(benzo[d]thiazol-2-ylamino)-2-oxoethyl)thio)-4H-1,2,4-triazol-3-yl)pr opanoic acid (2c). White powder, yield 47%, ^{1}H NMR (400 MHz, DMSO): δ 12.47 (s, 1H), 7.97 (d, *J* = 7.6 Hz, 1H), 7.75 (d, *J* = 7.9 Hz, 1H), 7.44 (t, *J* = 7.3 Hz, 1H), 7.30 (t, *J* = 7.4 Hz, 1H), 4.18 (s, 2H), 2.85 (t, *J* = 6.8 Hz, 2H), 2.65 (t, *J* = 6.8 Hz, 2H). ^{13}C NMR (100 MHz, DMSO): δ 177.2, 171.3, 161.3, 159.9, 151.8, 134.7, 129.3, 126.8, 124.9, 123.8, 38.3, 35.5, 25.3. HRMS(ESI^{-}) *m/z*: 362.0495 (Calcd for [M-H^{+}]$^{-}$: 362.0387 *m/z*).

3-(5-((2-((4-chlorophenyl)amino)-2-oxoethyl)thio)-4H-1,2,4-triazol-3-yl)prop anoic acid (2d). White powder, yield 45%, ^{1}H NMR (400 MHz, DMSO): δ 10.76 (s, 1H), 7.65 (d, J = 7.9 Hz, 2H), 7.34 (d, J = 8.0 Hz, 2H), 4.03 (s, 2H), 2.95 – 2.79 (m, 2H), 2.55 – 2.48 (m, 2H). ^{13}C NMR (100 MHz, DMSO): δ 175.3, 167.2, 158.8, 157.2, 138.4, 129.1, 127.4, 121.1, 36.6, 33.8, 23.0. HRMS(ESI^{-}) *m/z*: 339.0481 (Calcd for [M-H^{+}]$^{-}$: 339.0324 *m/z*).

3-(5-((2-((4-nitrophenyl)amino)-2-oxoethyl)thio)-4H-1,2,4-triazol-3-yl)propa noic acid (2e). Yellow powder, yield 65%, ^{1}H NMR (400 MHz, DMSO): δ 11.04 (s, 1H), 8.11 (d, J = 8.6 Hz, 2H), 7.73 (d, J = 8.6 Hz, 2H), 3.97 (s, 2H), 2.78 – 2.67 (m, 2H), 2.39 – 2.33 (m, 2H). ^{13}C NMR (100 MHz, DMSO): δ 174.9, 167.6, 158.4, 156.7, 145.2, 142.2, 125.0, 118.8, 36.3, 33.3, 22.5. HRMS(ESI^{-}) *m/z*: 350.0651 (Calcd for [M-H^{+}]$^{-}$: 350.0565 *m/z*).

General procedure for azolylthioacetamides (3a-e)

To a solution of compound **9** (2 mmol, 1 equiv) in ethanol (40 mL) was added NaOH (2 mmol, 1 equiv), and the reaction mixture was stirred for 5 min. After N-substituted-2-chloroacetamide **(a-e**) (2 mmol, 1 equiv) dissolved in ethanol (5 mL) was added dropwise, the reaction mixture was heated under reflux for 6 h. Then the reaction mixture was cooled to room temperature and the precipitate was filtered and dried under vacuum to obtain compounds **3a-e.**

4-(5-((2-oxo-2-(phenylamino)ethyl)thio)-4H-1,2,4-triazol-3-yl)butanoic acid (3a). White powder, yield 23%, ^{1}H NMR (400 MHz, DMSO): δ 10.53 (s, 1H), 7.60 (d,

J = 7.4 Hz, 2H), 7.28 (t, *J* = 7.1 Hz, 2H), 7.04 (d, *J* = 6.7 Hz, 1H), 4.03 (s, 2H), 2.65 (t, *J* = 6.4 Hz, 2H), 2.19 – 2.13 (m, 2H), 1.89 – 1.75 (m, 2H). ^{13}C NMR (100 MHz, DMSO): *δ* 176.2, 167.0, 158.9, 157.3, 139.5, 129.2, 123.8, 119.6, 36.7, 35.5, 26.1, 24.1. HRMS(ESI$^-$) *m/z*: 319.0992 (Calcd for [M-H$^+$]$^-$: 319.0870 *m/z*).

4-(5-((2-((1H-benzo[d]imidazol-2-yl)amino)-2-oxoethyl)thio)-4H-1,2,4-triazol-3-yl)butanoic acid (3b). White powder, yield 60%, ^{1}H NMR (400 MHz, DMSO): *δ* 12.06 (s, 1H), 7.42 (s, 2H), 7.08 (s, 2H), 4.12 (s, 2H), 2.66 (t, *J* = 6.8 Hz, 2H), 2.56 (t, *J* = 6.8 Hz, 2H), 1.89 – 1.80 (m, 2H). ^{13}C NMR (100 MHz, DMSO): δ 174.5, 168.6, 158.5, 157.2, 146.9, 136.7, 121.6, 114.6, 35.9, 33.3, 25.6, 23.1. HRMS(ESI$^-$) *m/z*: 359.1061 (Calcd for [M-H$^+$]$^-$: 359.0932 *m/z*).

4-(5-((2-(benzo[d]thiazol-2-ylamino)-2-oxoethyl)thio)-4H-1,2,4-triazol-3-yl)butanoic acid (3c). Pale yellow powder, yield 20%, ^{1}H NMR (400 MHz, DMSO): *δ* 12.50 (s, 1H), 7.97 (d, *J* = 7.8 Hz, 1H), 7.75 (d, *J* = 8.0 Hz, 1H), 7.43 (t, *J* = 7.5 Hz, 1H), 7.30 (t, *J* = 7.4 Hz, 1H), 4.19 (s, 2H), 2.67 (t, *J* = 7.3 Hz, 2H), 2.25 (t, *J* = 7.1 Hz, 2H), 1.89 – 1.79 (m, 2H). ^{13}C NMR (100 MHz, DMSO): *δ* 174.4, 168.4, 158.2, 149.0, 131.9, 126.6, 124.1, 122.2, 121.1, 35.4, 33.3, 25.6, 23.1. HRMS(ESI$^-$) *m/z*: 376.0595 (Calcd for [M-H$^+$]$^-$: 376.0544 *m/z*).

4-(5-((2-((4-chlorophenyl)amino)-2-oxoethyl)thio)-4H-1,2,4-triazol-3-yl)butanoic acid (3d). White powder, yield 79%, ^{1}H NMR (400 MHz, DMSO): *δ* 10.68 (s, 1H), 7.62 (d, *J* = 8.5 Hz, 2H), 7.35 (d, *J* = 8.4 Hz, 2H), 4.02 (s, 2H), 2.66 (t, *J* = 6.9 Hz, 2H), 2.11 (t, *J* = 6.7 Hz, 2H), 1.86 – 1.76 (m, 2H). ^{13}C NMR (100 MHz, DMSO): *δ* 167.2, 158.7, 157.2, 138.4, 129.1, 127.3, 121.1, 36.7, 35.5, 26.1, 24.6. HRMS(ESI$^-$)

m/z: 353.0627 (Calcd for $[M\text{-}H^+]^-$: 353.0481 *m/z*).

4-(5-((2-((4-nitrophenyl)amino)-2-oxoethyl)thio)-4H-1,2,4-triazol-3-yl)butanoic acid (3e). Yellow powder, yield 17%, ^{1}H NMR (400 MHz, DMSO): δ 11.55 (s, 1H), 8.20 (s, 2H), 7.90 (s, 2H), 4.09 (s, 2H), 2.64 (s, 2H), 1.99 (s, 2H), 1.80 (s, 2H). ^{13}C NMR (100 MHz, DMSO): δ 177.2, 168.3, 159.2, 157.1, 145.8, 142.6, 125.4, 119.3, 37.2, 36.8, 26.5, 24.8. HRMS(ESI^-) *m/z*: 364.0875 (Calcd for $[M\text{-}H^+]^-$: 364.0721 *m/z*).

Over-expression and purification of MβLs

CcrA: CcrA was overexpressed and purified as previously described.[1] BL21(DE3) *E. coli* cells were transformed with plasmid pMSZ02-CcrA and a culture of these cells in LB medium was used for protein overexpression. Protein concentration was determined using Beer's law and an extinction coefficient of 39,000 $M^{-1}cm^{-1}$ at 280 nm.

NDM-1: The overexpression plasmid, pET26b-NDM-1, was used for expression of NDM-1 as previously described.[2] The over-expression plasmid, pET26b-NDM-1, was used to transform BL21(DE3) *E. coli* cells. The crude protein NDM-1 was further purified by run through a G75 column. Protein purity was ascertained by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis and protein concentration was determined using Beer's law and an extinction coefficient of 27,960 $M^{-1}cm^{-1}$ at 280 nm.

ImiS: ImiS was overexpressed and purified as previously described.[3] BL21(DE3) *E. coli* cells were transformed with a plasmid containing the gene for ImiS,

pET-26b-ImiS and a culture of these cells in LB medium was used for overexpression of protein. The concentration of ImiS 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.[4] The gene that encodes L1 was ligated into pET26b and *E. coli* DH5R cells were transformed with the resulting plasmid pET26b(+)L1. Presence of the insert was confirmed with DNA sequivuencing. BL21(DE3) *E. coli* cells were then transformed with the pET26b(+)L1 plasmid and the resulting cells were used for overexpression of protein. L1 was quantitated by monitoring the absorbance at 280 nm and using an extinction coefficient of 54,614 $M^{-1}cm^{-1}$.

Inhibition studies

The inhibition studies were conducted at 25℃ using imipenem as substrate of ImiS and cefazolin V as substrate of CcrA, NDM-1 and L1. Compounds **1a-j**, **2a-d** and **3b** were dissolved in a small volume of DMSO and diluted with 50 mM Tris, pH 7.0. The final DMSO concentration in the reaction mixture was less than 0.5%, which did not alter the enzyme activity. The substrate concentrations were varied between 24 and 140 μM, inhibitor concentrations were varied between 0 and 10 μM, and the enzyme concentrations were varied between 3 and 60 nM. The enzyme and inhibitor were pre-incubated for 30 min before starting the kinetic experiments.

Lineweaver-Burk Plot Kinetic Analysis and determination of K_i values

The mode of inhibition was confirmed by generating Lineweaver–Burk plots,[4] and K_i values for the inhibitors were determined by fitting initial velocity versus substrate concentration at each inhibitor concentration to $v_i = V_{max}[S]/[S] + K_m(1 + [I]/K_i)$ using SigmaPlot v. 12.0, where vi is initial velocity, V_{max} is maximum velocity, [S] is initial substrate concentration, K_m is the Michaelis constant, [I] is inhibitor concentration, and K_i is inhibition constant. The reported K_i values represent the averages of all calculations with each inhibitor ± standard deviations from multiple kinetic trials.[4]

Determination of MIC values

Single colonies of *E. coli* BL21(DE3) containing plasmids pMSZ02-CcrA, pET26b-NDM-1, pET26b-ImiS and pET26b-L1 and two bacteria *P. aeruginosa* and *K. pneumoniae* on LB agar plates were transferred to 5 mL of Mueller-Hinton (MH) liquid medium and were grown at 37℃ overnight. The bacterial cells were collected by centrifugation (4000 rpm for 10 min). The supernatant was discarded, and the remaining cells were re-suspended in MH medium and diluted to an $O.D_{600}$ of 0.5. MIC values were determined by using the Clinical and Laboratory Standards Institute (CLSI) macrodilution (tube) broth method.[5] Antibiotics were dissolved in ddH_2O to prepare 1280 μg/mL stock solutions and the compounds **1a-j** were dissolved in DMSO respectively to prepare 320 μg/mL stock solutions. MH solution (0.9 mL) and 0.1 mL inhibitor stock solution were added to a series of sterile test tubes, with an additional 0.5 mL MH solution and 0.1 mL inhibitor stock solution added to the first tube. Antibiotic stock solution (400 μL of 1280 μg/mL) was added to the first test

tube, and a series of twofold dilutions were prepared by transferring 1 mL to successive tubes. Cultures (5 mL) of four bacterial strains: *E. coli* BL21(DE3) containing plasmids pMSZ02-CcrA, pET26b-NDM-1, pET26b-ImiS and pET26b-L1 and two bacteria *P. aeruginosa* and *K. pneumoniae* were grown to an OD_{600} of 0.5. The concentration of bacterial strains was diluted to 10^6 colony forming units (CFU) per mL with MH medium, and 1 mL bacterial solution was added to each tube containing different concentration of antibiotic. The final concentration of bacterial strains was $5x10^5$ CFU per mL. These cultures were incubated at 37℃ for 12 h. Each measurement was performed in duplicate.

Docking analysis procedure

AutoDock 4.2[6] was used to dock inhibitors **1a**, **1c**, and **1d** into the active site of CphA (PDB code 2QDS[7]) as a representative of ImiS, for which no crystal structure has been reported to date and from which it only deviates by 4% in amino acid sequence. The carboxyl and triazole groups were deprotonated, resulting in the compounds to carry an overall charge of -2e. A charge of +1.4e was assigned to the Zn(II) in the Zn2 binding site, while +0.2e was added to each of its ligands.[8] Three scenarios for the protonation states of His118 and His196 in the empty Zn1 binding site were tested: (1) both histidines protonated, which was the protonation state determined by AutoDockTools and used in our previous study,[9] (2) both histidines neutral, His118 Nε-protonated and His196 Nδ-protonated, (3) both histidines neutral and Nε-protonated. Overall, results for these three scenarios do not differ a lot in

terms of lowest binding energies (~ -12 kcal/mol mean binding energy for top ranked cluster) and diversity of conformations. We judged that the results for scenario (3) were in best agreement with the experiments (see main text) and previously published computational studies.[10,11] The grid and docking parameter files were prepared using Zn(II) van der Waals parameters r_0 = 1.95 Å and ε = 0.25 kcal/mol.[12] CphA was treated as a rigid receptor, while ligands were treated as flexible. The grid box was centered on the active-site Zn(II), with dimensions of 70 x 70 x 70 Å 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 number of energy evaluations and generations were set at 2,500,000 and 27,000, respectively. All other parameters were kept at default values 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 populated with at least 10 conformations were closely examined. Based on visual examination, the second-highest ranked cluster was chosen for **1a** and **1c** and the highest ranked cluster for **1d**. To get more similar conformations, those within 1.5 Å RMSD of the highest ranked conformation in those clusters were selected. This resulted in 14, 13, and 10 conformations with average binding energies of -11.3, -11.8, and -11.7 kcal/mol for **1a**, **1c**, and **1d**, respectively. The conformations shown in Figure 3 are the highest ranked (lowest energy) conformations of those clusters.

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