Supporting Information

Meta-substituted benzenesulfonamide: a potent scaffold for the development of metallo-β-lactamase ImiS inhibitors

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^{1}H and ^{13}C NMR and MS characterization

***N*-[4-(aminosulfonyl) phenyl]benzamide (1a)**

White solid, yield 74%. ^{1}H NMR (400 MHz, DMSO) δ 10.56 (s, 1H), 7.97 (t, 4H), 7.82 (d, *J* = 8.8 Hz, 2H), 7.63 (t, *J* = 7.3 Hz, 1H), 7.56 (t, *J* = 7.4 Hz, 2H), 7.28 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 120.40, 120.40, 127.02, 127.02, 129.02, 129.02, 130.26, 130.26, 133.62, 137.26, 139.36, 142.43, 165.31. HRMS (ESI) m/z: 299.0459 (Calcd. for $[M+Na^{+}]^{+}$ 299.0461m/z).

***N*-[4-(aminosulfonyl) phenyl]-3-bromobenzamide (1b)**

Pale yellow solid, yield 62%. ^{1}H NMR (400 MHz, DMSO) δ 10.66 (s, 1H), 8.19 (s, 1H), 8.01 (s, 1H), 7.98 (d, J = 8.8 Hz, 2H), 7.86 (d, J = 8.4 Hz, 3H), 7.55 (t, J = 7.9 Hz, 1H), 7.32 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 120.45, 120.45, 122.21, 127.04, 127.04, 127.49, 130.84, 131.18, 135.09, 137.09, 139.45, 142.33, 164.85. HRMS (ESI) m/z: 378.9551 (Calcd for [M+Na+]+ 378.9546 m/z).

***N*-[4-(aminosulfonyl) phenyl]-3-nitrobenzamide (1c)**

White solid, yield 59%. ^{1}H NMR (400 MHz, DMSO) δ 10.88 (s, 1H), 8.81 (s, 1H), 8.46 (d, *J* = 8.2 Hz, 1H), 8.43 (d, *J* = 7.8 Hz, 1H), 7.96 (d, *J* = 8.7 Hz, 2H), 7.86 (d, *J* = 8.4 Hz, 1H), 7.84 (d, 2H), 7.31 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 120.62, 120.62, 123.03, 126.95, 127.08, 127.08, 130.76, 134.80, 136.30, 136.69, 142.12, 148.23, 164.23. HRMS (ESI) m/z: 360.0059 (Calcd for $[M+K^{+}]^{+}$ 360.0051m/z).

***N*-[4-(Aminosulfonyl) phenyl]-4-(trifluoromethyl)benzamide (1d)**

White solid, yield 68%. ^{1}H NMR (400 MHz, DMSO) δ 10.79 (s, 1H), 8.16 (d, *J* = 8.1 Hz, 2H), 7.96 (d, *J* = 5.6 Hz, 2H), 7.94 (d, *J* = 4.8 Hz, 2H), 7.84 (d, *J* = 8.8 Hz, 2H), 7.32 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 120.50, 120.50, 123.00, 125.94, 125.94, 125.97, 125.97, 127.07, 127.07, 129.22, 132.28, 138.17, 139.57, 142.23, 165.34. HRMS (ESI) m/z: 367.0333 (Calcd for $[M+Na^{+}]^{+}$367.0035m/z).

***N*-[4-(Aminosulfonyl) phenyl]-4-chlorobenzamide (1e)**

Light pink solid, yield 73%. ^{1}H NMR (400 MHz, DMSO) δ 10.62 (s, 1H), 8.00 (d, *J* = 8.6 Hz, 2H), 7.94 (d, *J* = 8.8 Hz, 2H), 7.83 (d, *J* = 8.8 Hz, 2H), 7.65 (d, *J* = 8.6 Hz, 2H), 7.29 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 120.40, 120.40, 127.02, 127.02, 129.02, 129.02, 130.26, 130.26, 133.62, 137.26, 139.36, 143.32, 165.31. HRMS (ESI) m/z: 348.9816 (Calcd for $[M+K^{+}]^{+}$ 348.9816m/z).

***N*-[4-(Aminosulfonyl) phenyl]-4-(1, 1-dimethylethyl)benzamide (1f)**

Light pink solid, yield 61%. ^{1}H NMR (400 MHz, DMSO) δ 10.50 (s, 1H), 7.96 (d, *J* = 8.7 Hz, 2H), 7.92 (d, *J* = 8.3 Hz, 2H), 7.84 (d, *J* = 8.6 Hz, 2H), 7.60 (d, *J* = 8.2 Hz, 2H), 7.29 (s, 2H), 1.35 (s, 9H). ^{13}C NMR (101 MHz, DMSO) δ 31.38, 35.20, 120.50, 120.19, 120.19, 125.72, 125.72, 1265.99, 126.99, 128.15, 128.15, 132.26, 139.70, 142.72, 155.30, 166.36. HRMS (ESI) m/z: 371.0830 (Calcd for $[M+K^{+}]^{+}$ 371.0826m/z).

4-(acetyloxy)-*N*-[4-(Aminosulfonyl) phenyl]benzamide (1g)

White solid, yield 69%. 1H NMR (400 MHz, DMSO) δ 10.60 (s, 1H), 8.02 (d, J = 8.7 Hz, 2H), 7.94 (d, J = 8.8 Hz, 2H), 7.83 (d, J = 8.8 Hz, 2H), 7.34 (d, J = 8.7 Hz, 2H), 7.30 (s, 2H), 2.32 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 21.33, 120.34, 120.34, 122.42, 122.42 127.07, 127.07, 129.87, 129.87, 132.50, 139.22, 142.63, 153.66, 165.74, 169.47. HRMS (ESI) m/z: 357.0519 (Calcd for [M+Na+]+ 357.0516m/z).

***N*-[4-(Aminosulfonyl) phenyl]benzenepropanamide (1h)**

White solid, yield 68%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.26 (s, 1H), 7.74 (s, 4H), 7.29 (d, *J* = 13.1 Hz, 2H), 7.26 (d, *J* = 6.1 Hz,2H), 7.25 (s, 2H), 7.19 (t, *J* = 6.9 Hz, 1H), 2.93 (t, *J* = 7.6 Hz, 2H), 2.67 (t, *J* = 7.7 Hz, 2H). ^{13}C NMR (101 MHz, DMSO) δ 30.63, 39.51, 118.53, 118.53, 125.98, 126.66, 126.66, 128.22, 128.22, 128.32, 128.32, 138.15, 141.00, 142.07, 170.98. HRMS (ESI) m/z: 343.0513 (Calcd for $[M+K^{+}]^{+}$ 343.0513m/z).

***N*-[4-(aminosulfonyl) phenyl]-4-hydroxybenzamide (1i)**

White solid, yield 78%. ^{1}H NMR (400 MHz, DMSO) δ 10.35 (s, 1H), 10.24 (s, 1H), 7.97 (d, *J* = 8.7 Hz, 2H), 7.93 (d, *J* = 8.5 Hz, 2H), 7.84 (d, *J* = 8.6 Hz, 2H), 7.31 (s, 2H), 6.94 (d, *J* = 8.5 Hz, 2H). ^{13}C NMR (101 MHz, DMSO) δ 115.48, 115.48, 120.12, 120.12, 125.35, 126.94, 126.94, 130.40, 130.40, 138.76, 142.96, 161.35, 165.92. HRMS (ESI) m/z: 315.0405 (Calcd for $[M+Na^{+}]^{+}$ 315.0410m/z).

***N*-[4-(Aminosulfonyl) phenyl]-β-phenyl-benzenepropanamide (1j)**

White solid, yield 79%. ^{1}H NMR (400 MHz, DMSO) δ 10.33 (s, 1H), 7.70 (d, *J* = 8.9 Hz, 2H), 7.66 (d, *J* = 8.9 Hz, 2H), 7.39 – 7.26 (m, 8H), 7.23 (s, 2H), 7.17 (s, 2H), 4.58 (t, *J* = 7.9 Hz, 1H), 3.14 (d, *J* =

8.0 Hz, 2H). ^{13}C NMR (101 MHz, DMSO) δ 42.16, 47.10, 118.98, 118.98, 126.76, 126.76, 127.15, 127.15, 128.01, 128.01, 128.01, 128.01, 128.04, 128.04, 128.04, 128.04, 138.67, 142.42, 144.57, 144.57, 170.29. HRMS (ESI) m/z: 403.1091 (Calcd for $[M+Na^+]^+$ 403.1087m/z).

***N*-[3-(aminosulfonyl) phenyl]benzamide (2a)**

White solid, yield 76%. ^{1}H NMR (400 MHz, DMSO) δ 10.55 (s, 1H), 8.39 (s, 1H), 7.99 (d, *J* = 7.2 Hz, 2H), 7.97 (d, *J* = 3.3 Hz, 1H), 7.63 (t, *J* = 7.2 Hz, 1H), 7.60 – 7.48 (m, 4H), 7.39 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 166.27, 145.01, 140.02, 134.93, 132.34, 129.83, 128.94, 128.94, 128.21, 128.21, 123.63, 121.21, 117.77. HRMS (ESI) m/z: 299.0465 (Calcd for $[M+Na^+]^+$ 299.0461m/z).

***N*-[3-(aminosulfonyl) phenyl]-3-bromobenzamide (2b)**

White solid, yield 68%. ^{1}H NMR (400 MHz, DMSO) δ 10.66 (s, 1H), 8.36 (s, 1H), 8.19 (s, 1H), 7.98 (d, *J* = 7.4 Hz, 2H), 7.82 (d, *J* = 7.8 Hz, 1H), 7.59 (d, 1H), 7.57 (t, 1H), 7.53 (t, *J* = 7.9 Hz, 1H), 7.44 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 164.70, 145.06, 139.76, 137.07, 135.08, 131.23, 130.80, 129.92, 127.48, 123.72, 121.50, 117.87. HRMS (ESI) m/z: 378.9554 (Calcd for $[M+Na^+]^+$ 378.9546m/z).

***N*-[3-(aminosulfonyl) phenyl]-3-nitrobenzamide (2c)**

White solid, yield 74%. ^{1}H NMR (400 MHz, DMSO) δ 10.87 (s, 1H), 8.84 (s, 1H), 8.58 – 8.42 (m, 2H), 8.36 (s, 1H), 8.02 (d, *J* = 6.0 Hz, 1H), 7.87 (t, *J* = 7.9 Hz, 1H), 7.71 (s, 1H), 7.60 (d, *J* = 6.6 Hz, 2H), 7.42 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 164.08, 148.26, 145.11, 139.56, 136.28, 134.79, 130.79, 129.99, 126.96, 123.84, 122.97, 121.69, 117.99. HRMS (ESI) m/z: 344.0317 (Calcd for $[M+Na^+]^+$ 344.0312m/z).

***N*-[3-(Aminosulfonyl) phenyl]-4-(trifluoromethyl)benzamide (2d)**

White solid, yield 69%. ^{1}H NMR (400 MHz, DMSO) δ 10.71 (s, 1H), 8.37 (s, 1H), 8.19 (d, *J* = 8.1 Hz, 2H), 7.98 (d, *J* = 7.5 Hz, 1H), 7.94 (d, *J* = 8.3 Hz, 2H), 7.61 (d, *J* = 7.8 Hz, 1H), 7.60 (d, 1H), 7.58 (t, 1H), 7.36 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 165.11, 145.09, 139.68, 138.74, 131.92, 129.93, 129.93, 129.17, 129.17, 125.97, 125.94, 123.74, 123.02, 121.58, 117.89. HRMS (ESI) m/z: 367.0333 (Calcd for $[M+Na^+]^+$ 367.0335m/z).

***N*-[3-(Aminosulfonyl) phenyl]-4-chlorobenzamide (2e)**

White solid, yield 76%. ^{1}H NMR (400 MHz, DMSO) δ 10.61 (s, 1H), 8.36 (s, 1H), 8.02 (d, *J* = 8.4 Hz, 2H), 7.96 (d, *J* = 6.4 Hz, 1H), 7.65 (d, *J* = 8.4 Hz, 2H), 7.58 (d, *J* = 6.3 Hz, 1H), 7.56 (t, 1H), 7.41 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ 165.16, 145.04, 139.83, 137.22, 133.61, 130.18, 130.18, 129.86, 129.02, 129.02, 123.71, 121.39, 117.86. HRMS (ESI) m/z: 333.0071 (Calcd for $[M+Na^{+}]^{+}$ 333.0076m/z).

***N*-[3-(Aminosulfonyl) phenyl]-4-(1, 1-dimethylethyl)benzamide (2f)**

Light pink solid, yield 60%. ^{1}H NMR (400 MHz, DMSO) δ 10.48 (s, 1H), 8.38 (s, 1H), 7.97 (t, 1H), 7.92 (d, *J* = 8.5 Hz, 2H), 7.58 (d, *J* = 2.0 Hz, 2H), 7.56 (d, 2H), 7.39 (s, 2H), 1.33 (s, 9H). ^{13}C NMR (101 MHz, DMSO) δ 166.18, 155.22, 145.02, 140.13, 132.23, 129.77, 128.10, 128.10, 125.71, 125.71, 123.57, 121.10, 117.73, 35.19, 31.39. HRMS (ESI) m/z: 355.1089 (Calcd for $[M+Na^{+}]^{+}$ 355.1087m/z).

4-(acetyloxy)-*N*-[3-(Aminosulfonyl) phenyl]benzamide (2g)

Light pink solid, yield 70%. ^{1}H NMR (400 MHz, DMSO) δ 10.57 (s, 1H), 8.38 (s, 1H), 8.03 (d, *J* = 8.7 Hz, 2H), 7.97 (t, 1H), 7.57 (d, *J* = 0.9 Hz, 2H), 7.40 (s, 2H), 7.34 (d, *J* = 8.7 Hz, 2H). ^{13}C NMR (101 MHz, DMSO) δ 169.50, 165.51 , 153.59 , 145.02 , 139.98 , 132.47 , 129.86 ,129.79 , 129.79 ,123.59 , 122.44 , 122.44 ,121.25 , 117.72 , 21.38. HRMS (ESI) m/z: 357.0526 (Calcd for $[M+Na^{+}]^{+}$ 357.0516m/z).

***N*-[3-(Aminosulfonyl) phenyl]benzenepropanamide (2h)**

White solid, yield 73%. ^{1}H NMR (400 MHz, DMSO) δ 10.26 (s, 1H), 8.18 (s, 1H), 7.74 (s, 1H), 7.51 (d, *J* = 4.3 Hz, 2H), 7.40 (s, 2H), 7.29 (d, *J* = 7.5 Hz, 2H), 7.28 (d, *J* = 7.2 Hz, 2H), 7.20 (t, *J* = 6.7 Hz, 1H), 2.94 (t, *J* = 7.4 Hz, 2H), 2.67 (t, *J* = 7.5 Hz, 2H). ^{13}C NMR (101 MHz, DMSO) δ 171.24, 145.02, 141.50, 139.96, 129.89, 128.80, 128.80, 128.70, 128.70, 126.45, 122.24, 120.56, 116.42, 38.86, 31.14. HRMS (ESI) m/z: 343.0518 (Calcd for $[M+K^{+}]^{+}$ 343.0513m/z).

***N*-[2-(Aminosulfonyl) phenyl]-4-chlorobenzamide (3e)**

White solid, yield 71%. ^{1}H NMR (400 MHz, DMSO) δ 10.37 (s, 1H), 8.43 (d, *J* = 8.2 Hz, 1H), 7.93 (d, *J* = 8.4 Hz, 2H), 7.91 (d, 1H), 7.76 (s, 2H), 7.70 (d, *J* = 8.5 Hz, 2H), 7.65 (d, 1H), 7.36 (t, *J* = 7.5 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ 164.18, 137.54, 135.43, 133.58, 133.48, 129.59, 129.59, 129.44, 129.44, 128.56, 124.82, 123.16. HRMS (ESI) m/z: 348.9817 (Calcd for $[M+Na^{+}]^{+}$ 348.9817m/z).

***N*-[2-(Aminosulfonyl) phenyl]-4-(1,1-dimethylethyl)benzamide (3f)**

White solid, yield 65%. ^{1}H NMR (400 MHz, DMSO) δ 10.38 (s, 1H), 8.51 (d, *J* = 8.3 Hz, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 7.86 (d, *J* = 8.3 Hz, 2H), 7.79 (s, 2H), 7.66 (d, *J* = 7.4 Hz, 1H), 7.63 (d, *J* = 8.4 Hz, 2H), 7.33 (t, *J* = 7.7 Hz, 1H), 1.34 (s, 9H). ^{13}C NMR (101 MHz, DMSO) δ 165.00, 155.73, 135.92, 133.60, 131.97, 131.87, 128.60, 127.56, 127.56, 126.20, 126.20, 124.25, 122.38, 35.23, 31.34. HRMS (ESI) m/z: 355.1086 (Calcd for $[M+Na^{+}]^{+}$ 355.1087m/z).

***N*-(6-(diethylamino)-9-(2-((4-sulfamoylphenoxy)carbonyl)phenyl)-3H-xanthen-3-ylidene)-*N*-ethylethanaminium (RS)**

Dark purple crystals, yield 45%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 (d, J = 7.7 Hz, 1H), 7.92 (d, J = 8.5 Hz, 2H), 7.87 (d, J = 7.4 Hz, 1H), 7.80 (t, J = 7.5 Hz, 1H), 7.37 (d, J = 7.4 Hz, 1H), 7.11 (d, J = 9.5 Hz, 2H), 6.88 (d, J = 8.5 Hz, 4H), 6.75 (d, J = 1.7 Hz, 2H), 6.23 (s, 2H), , 3.59 (q, J = 7.0 Hz, 8H), 1.28 (t, J = 7.0 Hz, 12H). ^{13}C NMR (101 MHz, DMSO) δ 167.67, 157.13, 154.14, 152.16, 152.16, 148.98, 148.98, 141.45, 140.62, 134.36, 129.26, 129.11, 128.69, 128.69, 126.56, 126.56, 125.42, 125.42, 124.24, 123.58, 108.84, 105.76, 97.79. HRMS (ESI) m/z: 598.2369 (Calcd for $[M]^{+}$ 598.2370m/z).

Over-expression and purification of MβLs

NDM-1: 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 of 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 an 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: 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 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 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 an YM-10 membrane. The crude protein ImiS was run through a G75 column and eluted with 30 mM Tris, pH 7.0, 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 37,250 $M^{-1}cm^{-1}$ at 280 nm.

L1: 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 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 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, 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 8.5, 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.

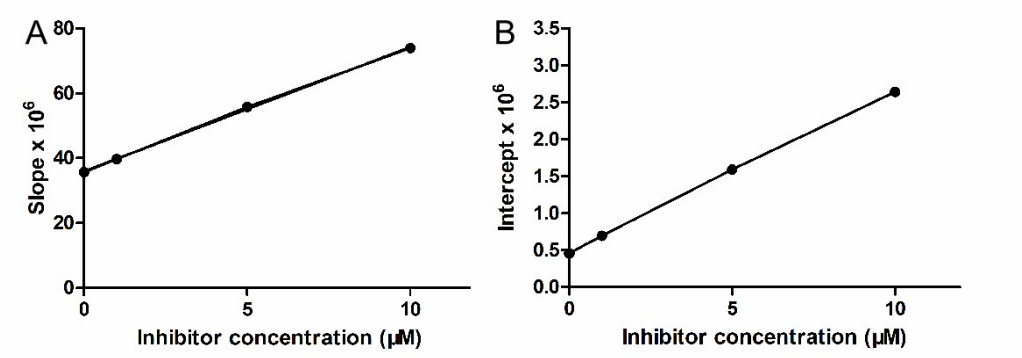

Fig. S1 Slope (A) and intercept (B) replots from the data of Fig. 5A, respectively.

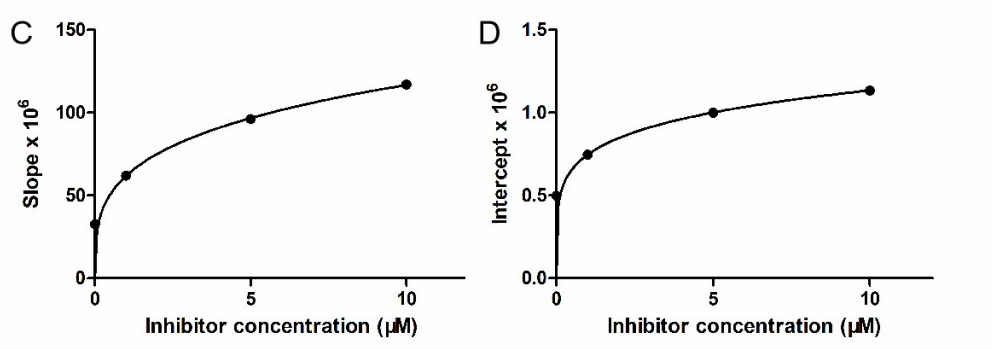

Fig. S2 Slope (C) and intercept (D) replots from the data of Fig. 5B, respectively.

Docking studies

Docking studies of the compounds **2a** into the active site of CphA (PDB: 2QDS, which was used as a closely related relative of ImiS) were conducted by AutoDock 4.2[4]. Active-site Zn(II) were assigned a charge of +1.4 e and +0.2 e was added to the Zn(II)-coordinating ligands[5]. The grid and docking parameter files were prepared using Zn(II) van der Waals parameters = 0.25 kcal/mol and r0= 1.95 Å[6]. Enzyme was treated as a rigid receptor, while ligands were treated as flexible. The grid box was centered between the active-site Zn (II) ions in the CphA, 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 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 lowest-binding energy clusters and most populated clusters or clusters that contained comparable conformations with other predominant clusters were further studied.

Cytotoxicity evaluation

A cytotoxicity assay was performed to evaluate the toxicity of **1e**, **2e**, **2g** and **3f** to mouse fibro-blast cells (L929) as previously reported[7]. The cells were seeded into 96-well plates at cell density of 1.0 × 104 cells/well in 100 μL of culture medium and maintained for 24 h. Then solutions of inhibitors **1e**, **2e**, **2g** and **3f** with different concentrations (6.25, 12.5, 25, 50, 100, 200

μM) were added to 96-well plates 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 without inhibitor. Six wells containing only the complete medium were used as the blank control. Following that, the medium was removed and 20 μL MTT were added to each well. After incubation for 4 h, 150 μL of DMSO were added to each well and the 96-well plates 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. The results are shown in Fig. 9.

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