Supplementary Information

An Assay Platform for Clinically Relevant Metallo-β-Lactamases

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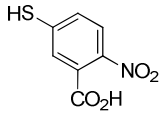
General experimental

Material and Methods

Chemicals were purchased from commonly used suppliers (Aldrich, Acros, Alfa Aesar, and TCI) and were used without further purification. (6*R*,7*R*)-4-Methoxybenzyl 3-(chloromethyl)-8-oxo-7-(2-phenylacetamido)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (PMB-protected Cep-Cl) was obtained from Activate Scientific (Prien, Germany). Solvents (including dry solvents) for chemical transformations, work-up and chromatography were purchased from Aldrich (Dorset, UK) at HPLC grade, and used without further distillation. Silica gel 60 F254 analytical thin layer chromatography (TLC) plates were from Merck (Darmstadt, Germany) and visualized under UV light, or with potassium permanganate stain. Chromatographic purifications were performed using Merck Geduran 60 silica (40-63 μm) or prepacked SNAP columns on a Biotage SP1 Purification system (Uppsala, Sweden). Deuterated solvents were obtained from Sigma and Apollo Scientific Ltd. All ^{1}H and ^{13}C NMR Spectra were recorded using a Bruker Avance 400 MHz spectrometer. All chemical shifts are given in ppm relative to the solvent peak,[1] and coupling constants (*J*) are reported in Hz to the closed 0.5. High Resolution (HR) mass spectrometry data (m/z) were obtained from a Bruker MicroTOF instrument using an ESI source and Time of Flight (TOF) analyzer. Low Resolution (LR) mass spectrometry data (m/z) were obtained from a Waters LCT Premier instrument using an ESI source and Time of Flight (TOF) analyzer. Fourier transform Infrared (FT-IR) spectra were recorded on a Bruker Tensor 27 instrument. Absorption spectra were recorded on a Varian Cary 4000 UV-Vis spectrophotometer using an 1 mL quartz cuvette. Fluorescence spectra were recorded on a Varian Cary Eclipse fluorescence spectrophotometer using an 1 mL quartz cuvette. Optical rotations were recorded on a Perkin Elmer 241 Polarimeter. Melting points were obtained from a Stuart SMP-40 automatic melting point apparatus. HPLC analysis was run on a Waters Acquity UPLC equipped with a Phenomanex Luna 5 μM C18 column (75 x 4.60 mm) using a gradient of 100% solvent A → 100% solvent B (solvent A: 10% MeCN in H_2O containing 0.05% formic acid; solvent B: 100% MeCN containing 0.1% formic acid), flow rate = 0.6 ml/min and UV detection at 254 nm.

Synthesis

5-mercapto-2-nitrobenzoic acid (**S1**)

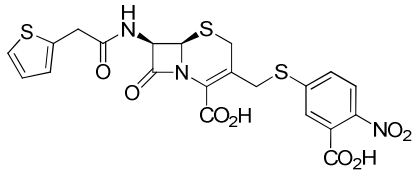


Prepared according to a modified literature reference:[2]

5,5'-Dithio-bis-(2-nitrobenzoic acid) (2.0 g, 5.05 mmol) was dissolved in aq. Tris base (0.5 M, 100 mL) and the pH was adjusted to pH 8 by addition of 2 M HCl. Dithiothreitol (1.1 g, 7.1 mmol) was added in portions (solution turned orange/red). The reaction was stirred at r.t. until TLC analysis (CH_2Cl_2/MeOH, 9:1) showed full conversion of the starting material (~2 hours). The reaction mixture was extracted with EtOAc (3 x 50 mL) before being acidified to pH 2 with 2 M HCl. The acidified H_2O layer was subsequently extracted with EtOAc (3 x 50 mL), dried over Na_2SO_4, filtered and

concentrated under reduced pressure. The crude product was purified by column chromatography (CH_2Cl_2/MeOH/AcOH, 8:2:0.05) to yield the title compound as orange solid (1.1 g, 55%). R_F = 0.2 (CH_2Cl_2/MeOH/AcOH, 8:2:0.05). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.86 (d, *J* = 8.5 Hz, 1H), 7.62 (d, *J* = 1.5 Hz, 1H), 7.48 (dd, *J* = 8.5, 2.0 Hz, 1H), 6.37 (br. s., 1H), 3.85 (s, 1H) ppm. LRMS calc. for $C_7H_4NO_4S$; M-H = 197.99, mass found; 197.9.

(6*R*,7*R*)-3-(((3-carboxy-4-nitrophenyl)thio)methyl)-8-oxo-7-(2-(thiophen-2-yl)acetamido)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (**S2**, CENTA)

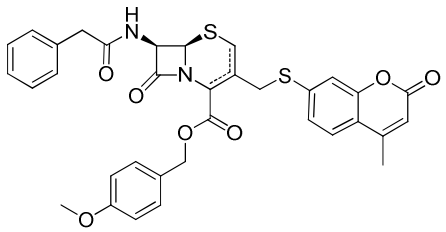


Prepared according to a modified literature reference:2

To a solution containing cephalothin sodium salt (500 mg, 1,2 mmol) in MilliQ H_2O (10 mL) was added 5-mercapto-2-nitrobenzoic acid (**S1**, 239 mg, 1.2 mmol). The pH was adjusted to pH 7.0 by the drop wise addition of 1 M NaOH and the solution was stirred at 65 °C for 6 hours (pH was monitored and kept at pH 7 throughout the course of the reaction). After cooling to r.t. the reaction mixture was extracted with EtOAc (2 x 10 mL) to remove unreacted material. The mixture was acidified to pH 4 using 1 M HCl and extracted with EtOAc (3 x 10 mL). The organic fraction was concentrated *in vacuo* and subsequently re-dissolved in sat. $NaHCO_3$ (15 mL). The H_2O layer was slowly acidified to pH 2-3 by addition of 1 M HCl (a fine suspension is formed). The acidified H_2O layer was extracted with EtOAc (3 x 15 mL), where after the combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to yield the title compound as a light brown solid (220 mg, 34%). ^{1}H NMR (400 MHz, CD_3OD) δ = 7.89 (d, *J* = 8.5 Hz, 1H), 7.75 (d, *J* = 2.0 Hz, 1H), 7.68 (dd, *J* = 8.5, 2.0 Hz, 1H), 7.29 (dd, *J* = 5.0, 1.5 Hz, 1H), 6.94-7.00 (m, 2H), 5.67 (d, *J* = 5.0 Hz, 1H), 5.09 (d, *J* = 5.0 Hz, 1H), 4.25-4.39 (m (app. AB-system), 2H), 3.82 (d, *J* = 3.0 Hz, 2H), 3.57-3.78 (m (app AB-system), 2H) ppm. LRMS calc. for $C_{21}H_{16}N_3O_8S_3$; M+H = 534.01, mass not found; fragmented mass of M+Na = 359.0 found.

(6*R*,7*R*)-4-methoxybenzyl 8-oxo-3-(((4-methyl-2-oxo-2*H*-chromen-7-yl)thio)methyl)-7-(2-phenylacetamido)-5-thia-1-azabicyclo[4.2.0]oct-**3-ene**-2-carboxylate, (**1**-Δ_2) and

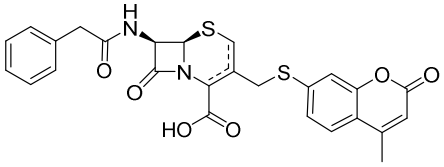
(6*R*,7*R*)-4-methoxybenzyl 8-oxo-3-(((4-methyl-2-oxo-2*H*-chromen-7-yl)thio)methyl)-7-(2-phenylacetamido)-5-thia-1-azabicyclo[4.2.0]oct-**2-ene**-2-carboxylate (**1**-Δ_3)



To a solution of chloro-cephalosporin-PMB ester (121.5 mg, 0.25 mmol) in dry DMF (2 mL) was added 7-thia-4-methyl coumarin (48.0 mg, 0.25 mmol) and DiPEA (86 µL, 0.50 mmol). The reaction was placed under an N_2-atmosphere and stirred at r.t. for 18 hours. The reaction was quenched by the addition of H_2O (5 mL) and 1M HCl (1 mL) after which extraction with CH_2Cl_2 (3 x 10 mL) was performed. The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (cHex/EtOAc, 1:1) to give the title compound **1** as slightly yellowish solid (120 mg, 75%). R_F = 0.35 (cHex/EtOAc, 1:1). ^{1}H NMR (400 MHz, $CDCl_3$) (*Mix of isomers*) δ = 7.45 (d, *J* = 8.0 Hz, 1H), 7.24 - 7.40 (m, approx. 12H ($CDCl_3$ overlap)), 7.20 (d, *J* = 1.5 Hz, 1H), 7.13 - 7.18 (m, 2H), 7.10 (d, *J* = 1.5 Hz, 1H), 6.87 (t, *J* = 8.0 Hz, 4H), 6.23 - 6.29 (m, 3H), 6.15 (d, *J* = 9.0 Hz, 1H), 6.07 (s, 1H), 5.75 (dd, *J* = 9.0, 5.0 Hz, 1H), 5.64 (dd, *J* = 8.5, 4.0 Hz, 1H), 5.20 - 5.23 (m, 2H), 5.15 (dd, *J* = 6.0, 4.5 Hz, 4H), 4.87 (d, *J* = 5.0 Hz, 1H), 4.15 (d, *J* = 13.5 Hz, 1H), 3.99 (d, *J* = 13.5 Hz, 1H), 3.80 (d, *J* = 7.0 Hz, 6H), 3.70 (d, *J* = 6.0 Hz, 2H), 3.64 - 3.68 (m, 4H), 3.62 (t, *J* = 3.5 Hz, 1H), 3.57 (s, 1H), 3.45 (s, 1H), 3.41 (s, 1H), 2.41 (d, *J* = 14.5 Hz, 7H) ppm.

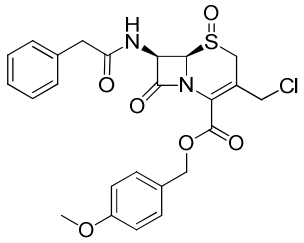
(6*R*,7*R*)-8-oxo-3-(((4-methyl-2-oxo-2*H*-chromen-7-yl)thio)methyl)-7-(2-phenylacetamido)-5-thia-1-azabicyclo[4.2.0]oct-**3-ene**-2-carboxylic acid (**FC1**-Δ_2), and

(6*R*,7*R*)-8-oxo-3-(((4-methyl-2-oxo-2*H*-chromen-7-yl)thio)methyl)-7-(2-phenylacetamido)-5-thia-1-azabicyclo[4.2.0]oct-**2-ene**-2-carboxylic acid (**FC1**-Δ_3)



Compound **1** (50 mg, 78 µmol, mixture of Δ_2-Δ_3 isomers) was dissolved in a mixture of trifluoroacetic acid (TFA)/anisole (5:1, 3 mL) while being cooled on an ice-bath. The reaction was stirred at 0 °C for 1 hour with interval monitoring of the conversion by TLC analysis (CH_2Cl_2/MeOH 9:1 + AcOH). After completion of the reaction, cold Et_2O (5 mL) was added upon which a precipitate was formed. The solid was filtered-off and washed with cold Et_2O (2 x 5 mL). The product was dried under high vacuum and obtained as an slightly yellowish solid (23 mg, 56%). R_F = 0.05 (CH_2Cl_2/MeOH/AcOH, 8:2:0.05). ^{1}H NMR (400 MHz, $CDCl_3$) (*Mix of isomers*) δ = 7.49 (d, *J* = 8.0 Hz, 2H), 7.19 - 7.40 (m, approx. 9H ($CDCl_3$ overlap)), 7.15 (d, *J* = 1.0 Hz, 1H), 6.47 (d, *J* = 8.5 Hz, 1H), 6.38 (d, *J* = 8.5 Hz, 1H), 6.27 (s, 2H), 6.17 (s, 1H), 5.76 (dd, *J* = 8.5, 5.0 Hz, 1H), 5.66 (dd, *J* = 8.0, 3.5 Hz, 1H), 5.27 (d, *J* = 4.0 Hz, 1H), 5.22 (s, 1H), 4.93 (d, *J* = 4.5 Hz, 1H), 4.20 - 4.27 (m, 1H), 4.09 - 4.15 (m, 1H), 3.86 (s, 2H), 3.66 - 3.71 (m, 5H), 3.64 (br. s., 2H), 3.48 (d, *J* = 2.5 Hz, 1H), 3.44 (s, 1H), 2.42 (d, *J* = 5.0 Hz, 6H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) (*Mix of isomers*) δ = 170.9, 170.8, 168.6, 164.7, 163.7, 163.0, 159.6 (2C), 153.3, 153.2, 153.0, 153.0, 141.5, 141.4, 135.8, 135.7, 129.5, 129.1 (2C), 129.0 (2C), 128.2 (2C), 128.2 (2C), 126.5, 126.4, 125.7, 125.5, 124.1, 123.4, 119.3, 119.1, 117.6, 117.3, 115.1, 114.2, 113.6, 113.5, 60.5, 59.0, 57.8, 52.7, 50.0, 41.6, 41.5, 36.5 (2C), 34.7, 27.2, 18.0 ppm. LRMS calcd. for $C_{26}H_{21}N_2O_6S_2$, M-H = 521.08, mass found; M-H = 521.0.

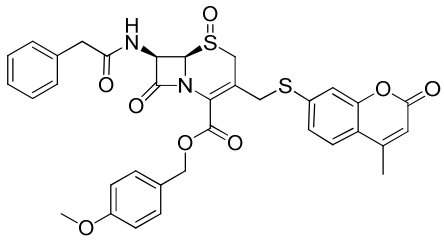
(6*R*,7*R*)-4-methoxybenzyl 3-(chloromethyl)-8-oxo-7-(2-phenylacetamido)-5-thia-1-azabicyclo-[4.2.0]oct-2-ene-2-carboxylate 5-oxide (**2**)



Prepared according to literature procedure:[3]

A solution of chloro-cephalosporin-PMB ester (234 mg, 0.5 mmol) in dry CH_2Cl_2 (7 mL) was cooled to 0 °C prior to the addition of *meta*-chloroperbenzoic acid (*m*CPBA, 86 mg, 0.5 mmol). The reaction was stirred at 0 °C for 30 min. (during this time a white precipitate was formed) and subsequently another 4 hours at r.t. (full conversion according to TLC analysis). The white solid was filtered-off and washed with cold CH_2Cl_2 (1 x 10 mL). The product was dried under high vacuum and used without further purification (200 mg, 80%, white solid). R_F = 0.25 (CH_2Cl_2/EtOAc 4:1). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.50 (d, *J* = 8.5 Hz, 1H), 7.38 (d, *J* = 8.5 Hz, 2H), 7.28 - 7.32 (m, 4H), 7.21 - 7.26 (m, 1H), 6.94 (d, *J* = 8.5 Hz, 2H), 5.85 (dd, *J* = 8.0, 5.0 Hz, 1H), 5.19 - 5.31 (m, app. q, 2H), 4.92 (d, *J* = 4.0 Hz, 1H), 4.68 (d, *J* = 11.5 Hz, 1H), 4.51 (d, *J* = 11.5 Hz, 1H), 3.93 (d, *J* = 18.5 Hz, 1H), 3.75 (s, 3H), 3.71 (d, *J* = 6.0 Hz, 1H), 3.67 (s, 1H), 3.52 - 3.57 (m, 1H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 171.1, 164.5, 160.5, 159.4, 135.8, 130.4 (2C), 129.1 (2C), 128.3 (2C), 126.8, 126.6 (2C), 124.9, 120.3, 113.8, 67.6, 66.6, 58.3, 55.1, 46.0, 44.1, 41.4 ppm. LRMS calcd. for $C_{24}H_{22}ClN_2O_6S$, M-H = 501.09, mass found; M-H = 501.1.

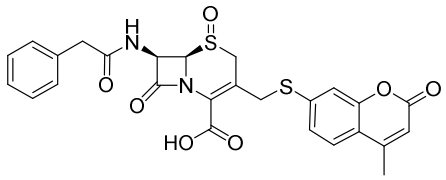
(6*R*,7*R*)-4-methoxybenzyl 3-(((4-methyl-2-oxo-2*H*-chromen-7-yl)thio)methyl)-8-oxo-7-(2-phenylacetamido)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 5-oxide (**3**)



To a solution of compound **2** (100 mg, 0.2 mmol) in dry DMF (3 mL) was added 7-thia-4-methyl coumarin (38.4 mg, 0.2 mmol) and N,N'-diisopropylethylamine (DiPEA, 80 µL, 0.45 mmol). The reaction was stirred at r.t. for 2 hours. The reaction was quenched by the addition of H_2O (5 mL) and 1M HCl (1 mL) after which extraction with CH_2Cl_2 (3 x 10 mL) was performed. The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (CH_2Cl_2/MeOH, 9:1) to give the title compound **3** as off-white solid (64 mg, 49%). R_F = 0.50 (CH_2Cl_2/MeOH, 9:1). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.43 (d, *J* = 8.5 Hz, 1H), 7.59 (d, *J* = 8.5 Hz, 1H), 7.26 - 7.33 (m, 7H), 7.19 - 7.25 (m, 2H), 6.87 (d, *J* = 8.5 Hz, 2H), 6.35 (d, *J* = 1.0

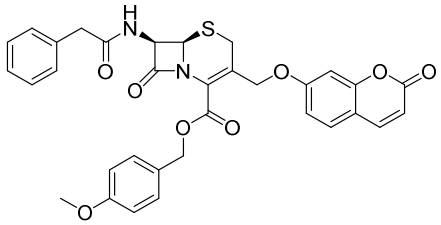
Hz, 1H), 5.79 - 5.76 (m, 1H) 5.09 - 5.18 (m, app. d, 2H), 4.85 (d, *J* = 3.5 Hz, 1H), 4.49 (d, *J* = 13.5 Hz, 1H), 3.91 - 3.99 (m, , app. dd, 2H), 3.76 (d, *J* = 18.0 Hz, 1H) 3.72 (s, 3H) 3.64 - 3.69 (m, 1H) (part of AB-system), 3.50 - 3.56 (m, 1H) (part of AB-system), 2.37 (s, 3H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 171.0, 164.2, 160.8, 159.5, 159.3, 153.1, 152.9, 140.0, 135.8, 130.2 (2C), 129.1 (2C), 128.3 (2C), 126.7, 126.5, 125.6, 124.9, 124.0, 121.1, 117.9, 116.2, 113.9, 113.7 (2C), 67.3, 66.5, 58.1, 55.1, 46.8, 41.4, 35.3, 18.0 ppm. LRMS calcd. for $C_{34}H_{29}N_2O_8S_2$, M-H = 657.14, mass found; M-H = 657.1.

(6*R*,7*R*)-3-(((4-methyl-2-oxo-2H-chromen-7-yl)thio)methyl)-8-oxo-7-(2-phenylacetamido)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid 5-oxide (**FC2**)



Compound **3** (32 mg, 48.6 µmol) was dissolved in a mixture of TFA (1.25 mL) and anisole (0.25 mL). The reaction progress was monitored by TLC analysis and was determined to be complete after 1 hour stirring at r.t. Cold Et_2O (5 mL) was added and the product precipitated out of solution. The product was filtered-off and washed with cold Et_2O (2 x 5 mL) after which the product was dried *in vacuo* overnight yielding the title compound **3** as a cream white solid (23 mg, 88%). R_F = 0.20 (CH_2Cl_2/MeOH/AcOH, 9:1:0.05). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.40 (d, *J* = 8.0 Hz, 1H), 7.66 (d, *J* = 8.5 Hz, 1H), 7.34 (s, 1H), 7.26 - 7.32 (m, 4H), 7.20 - 7.25 (m, 1H), 6.36 (s, 1H), 5.74 (dd, *J* = 8.0, 4.5 Hz, 1H), 4.84 (d, *J* = 4.0 Hz, 1H), 4.54 (d, *J* = 13.5 Hz, 1H), 3.99 (d, *J* = 13.5 Hz, 1H), 3.85 - 3.94 (m, app. d, 1H), 3.65 - 3.76 (m, 2H) (overlap of two doublets), 3.52 (d, *J* = 14.0 Hz, 1H), 2.41 (s, 3H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 171.0, 164.0, 162.3, 159.6, 153.2, 153.0, 140.6, 135.8, 129.1 (2C), 128.3 (2C), 126.5, 125.7, 125.2, 124.7, 120.2, 117.8, 115.8, 113.8, 66.3, 58.1, 46.7, 41.5, 35.2, 18.0 ppm. LRMS calcd. for $C_{26}H_{21}N_2O_7S_2$, M-H = 537.08, mass found; M-H = 537.0.

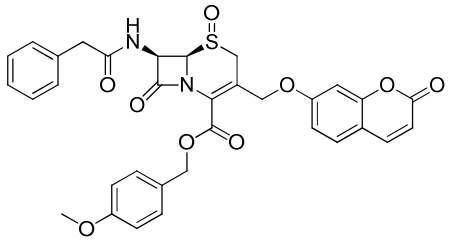
(6*R*,7*R*)-4-methoxybenzyl 8-oxo-3-(((2-oxo-2*H*-chromen-7-yl)oxy)methyl)-7-(2-phenylacetamido)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (**4**)



To a suspension of Cl-Cep-OPMB ester (680 mg, 1.4 mmol) in acetone (10 mL) was added NaI (2.10 g, 14.0 mmol, 10 eq.). The reaction was stirred at r.t. for 2 hours after which the solvent was removed *in vacuo*. The crude mixture was partitioned between H_2O (10 mL) and EtOAc (10 mL) and the layers separated. The H_2O-layer was extracted with EtOAc (2 x 15 mL) after which the combined organic layers were washed with 5% $Na_2S_2O_3$ aq. solution (2 x 20 mL) and brine (20 mL). The organic layer

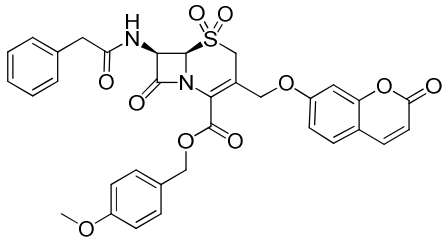
was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product dissolved in MeCN (15 mL) and 7-hydroxy-coumarin (454 mg, 2.8 mmol, 2 eq.) and K_2CO_3 (4.2 mmol, 4 eq.) were added. The conversion of the reaction was monitored by TLC analysis (cHex/EtOAc 1:1) and completion was reached after 4 hours. The solvent was evaporated *in vacuo* and the resulting crude mixture was partitioned between H_2O (10 mL) and EtOAc (10 mL). After separation of the layers, the H_2O-layer was extracted with EtOAc (2 x 15 mL), after which the combined organic layers were washed with 5% $Na_2S_2O_3$ aq. solution (2 x 20 mL) and brine (20 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography (cHex/EtOAc 1:1) to yield compound **4** as a light brown solid (392 mg, 46%). R_F = 0.30 (cHex/EtOAc 1:1). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 9.20 (d, *J* = 8.0 Hz, 1H), 7.98 (d, *J* = 9.5 Hz, 1H), 7.59 (d, *J* = 8.5 Hz, 1H), 7.17 - 7.33 (m, 8H), 6.92 (d, *J* = 1.5 Hz, 2H), 6.87 (dd, *J* = 8.5, 2.5 Hz, 1H), 6.79 (d, *J* = 8.5 Hz, 2H), 6.30 (d, *J* = 9.5 Hz, 1H), 5.44 (dd, *J* = 7.5, 4.0 Hz, 1H), 5.11 - 5.19 (m, 2H), 5.01 - 5.10 (m, app. d, 2H), 4.64 - 4.76 (m, app. q, 2H), 3.69 (s, 3H), 3.47 - 3.57 (m, 2H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 170.9, 167.0, 163.8, 160.9, 160.3, 159.3, 155.2, 144.3, 135.7, 130.0 (2C), 129.5, 129.1 (2C), 128.2 (2C), 126.9, 126.5, 122.4, 118.8, 113.7 (2C), 112.9, 112.7 (2C), 101.4, 69.8, 67.2, 60.8, 55.0, 52.9, 49.7, 41.6 ppm. LRMS calcd. for $C_{33}H_{27}N_2O_8S$, M-H = 611.15, mass found; M-H = 611.0.

(6*R*,7*R*)-4-methoxybenzyl 8-oxo-3-(((2-oxo-2*H*-chromen-7-yl)oxy)methyl)-7-(2-phenylacetamido)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 5-oxide (**5**)



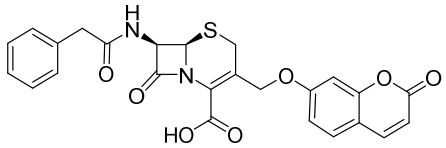
To a suspension of compound **4** (122 mg, 0.2 mmol) in dry CH_2Cl_2 (5 mL) cooled to 0 °C was added *m*CPBA (45 mg, 0.2 mmol, 1 eq.). The reaction was stirred at 0 °C for 30 min. followed by an additional hour at r.t. (a white precipitate was formed). The crude product was dry-loaded onto silica and purified by column chromatography (CH_2Cl_2/EtOAc, 8:2) to yield compound **5** as cream white solid (60 mg, 48%) R_F = 0.80 (CH_2Cl_2/MeOH, 9:1). ^{1}H NMR (400 MHz $CDCl_3$) δ = 7.64 (d, *J* = 9.5 Hz, 1H), 7.27 - 7.40 (m, 8H), 6.91 (d, *J* = 8.5 Hz, 2H), 6.74 - 6.78 (m, 2H), 6.67 (d, *J* = 10.0 Hz, 1 H), 6.30 (d, *J* = 9.6 Hz, 1 H), 6.10 (dd, *J* = 10.0, 5.0 Hz, 1H), 5.22 - 5.35 (m, app. mix of d and q, 3H), 4.80 (d, *J* = 13.5 Hz, 1H), 4.45 (dd, *J* = 5.0, 1.5 Hz, 1H), 3.99 (d, *J* = 19.0 Hz, 1H), 3.81 (s, 3H) 3.65 (m, app d, *J* = 5.5 Hz, 2H), 3.28 (d, *J* = 18.5 Hz, 1H) ppm. ^{13}C-NMR: (101 MHz, DMSO-d_6) δ = 171.1, 164.5, 160.7 (2C), 160.2, 159.4, 155.2, 144.3, 135.8, 130.4 (2C), 129.5, 129.1 (2C), 128.3 (2C), 126.8, 126.6, 125.0, 119.6, 113.7 (2C), 112.8, 101.6, 67.5, 67.3, 66.5, 58.4, 55.1, 45.4, 41.4 ppm. LRMS calcd. for $C_{33}H_{27}N_2O_9S$, M-H = 627.14, mass found; M-H = 627.0.

(6*R*,7*R*)-4-methoxybenzyl 8-oxo-3-(((2-oxo-2*H*-chromen-7-yl)oxy)methyl)-7-(2-phenylacetamido)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 5,5-dioxide (**6**)



To a suspension of compound **5** (100 mg, 0.16 mmol) in dry CH_2Cl_2 (10 mL) cooled to 0 °C was added *m*CPBA (74 mg, 0.35 mmol, 2 eq.). The reaction was stirred at 0 °C for 30 min. (formation of sulfoxide observed) after which the reaction was warmed to r.t. and stirred overnight. Upon completion of the reaction, the crude product was dry-loaded on silica and purified by column chromatography (CH_2Cl_2/MeOH 9:1) to yield the desired product as a white solid (35 mg, 34%). R_F = 0.85 (CH_2Cl_2/MeOH 9:1). ^{1}H NMR (400 MHz, DMSO-d_6) (*small amount of mCBA present*) δ = 8.93 (d, *J* = 8.6 Hz, 1H), 8.01 (d, *J* = 9.5 Hz, 1H), 7.87 - 7.93 (m, 1H), 7.63 (d, *J* = 8.5 Hz, 1H), 7.18 - 7.34 (m, 7H), 6.94 - 6.88 (m, 2H, 2 signals overlapping), 6.81 - 6.86 (m, app. d, 2H), 6.33 (d, *J* = 9.5 Hz, 1H), 6.01 (dd, *J* = 8.5, 5.0 Hz, 1H), 5.43 (d, *J* = 4.5 Hz, 1H), 5.22 (s, 2H), 4.89 - 4.84 (m, app. d, 2H), 4.46 (d, *J* = 18.5 Hz, 1H)(part of AB-system), 4.23 (d, *J* = 18.5 Hz, 1H)(part of AB-system), 3.70 (s, 3H), 3.60 (d, *J* = 5.0 Hz, 2H) ppm. ^{13}C-NMR: (101 MHz, DMSO-d_6) δ = 170.9, 164.5, 160.6 (2C), 160.2, 159.4, 155.2, 144.3, 135.6, 130.4 (2C), 129.5, 129.2 (2C), 128.2 (2C), 126.6, 126.5, 124.6, 124.0, 113.7 (2C), 112.9, 112.8, 101.6, 67.8, 66.8, 66.2, 58.4, 55.1, 50.9, 41.2 ppm. LRMS calcd. for $C_{33}H_{27}N_2O_{10}S$, M-H = 643.14, mass found; M-H = 643.0.

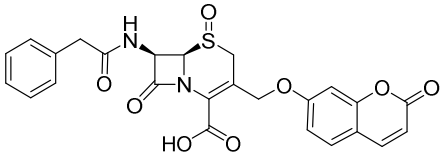
(6*R*,7*R*)-8-oxo-3-(((2-oxo-2H-chromen-7-yl)oxy)methyl)-7-(2-phenylacetamido)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (**FC3**)



Compound **4** (50 mg, 81.6 µmol) was cooled to 0 °C prior to the addition of TFA/anisole (5:1, 3 mL). The resulting reaction mixture was stirred at 0 °C for 30 min. with constant monitoring of the conversion by TLC analysis (CH_2Cl_2/EtOAC, 8:2). Upon completion of the reaction cold Et_2O (5 mL) was added which resulted in the formation of a precipitate. The solid material was filtered-off and washed with Et_2O (2 x 5 mL) and subsequently dried under high vacuum to yield the desired product as an off-white solid (15 mg, 37%). R_F = 0.25 (CH_2Cl_2/MeOH +AcOH, 9:1). ^{1}H NMR (400 MHz, DMSO-d_6) (*compound not pure due to instability*) δ = 9.18 (d, *J* = 8.0 Hz, 1H), 7.98 (d, *J* = 9.5 Hz, 1H), 7.63 (d, *J* = 9.0 Hz, 1H), 7.16 - 7.34 (m, 7H), 7.02 (s, 1H), 6.97 (dd, *J* = 8.5, 2.0 Hz, 1H) 6.85 (s, 1H), 6.29 (d, *J* = 9.5 Hz, 1H), 5.43 (dd, *J* = 8.0, 4.0 Hz, 1H), 5.17 (d, *J* = 4.0 Hz, 1H), 4.97 (s, 1H), 4.72 - 4.83 (m, 2H), 3.64 - 3.79 (m, 1H), 3.46 - 3.57 (m, 2H, overlap with H_2O in DMSO-d_6) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 170.9, 168.6, 163.7, 161.1, 160.3, 155.3, 144.3, 135.8, 129.6, 129.1 (2C), 128.2 (2C), 126.5, 121.5,

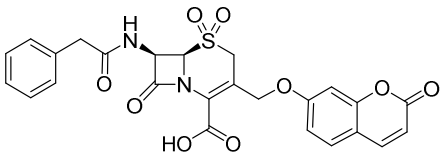
119.5, 112.9, 112.7 (2C), 101.6, 69.9, 60.6, 52.9, 49.7, 41.6 ppm. LRMS calcd. for $C_{25}H_{19}N_2O_7S$, M-H = 491.09, mass found; M-H = 491.0 and M-CO_2 = 447.0. Retention time = 7.24 min. (purity = ~80%).

(6*R*,7*R*)-8-oxo-3-(((2-oxo-2*H*-chromen-7-yl)oxy)methyl)-7-(2-phenylacetamido)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid 5-oxide (**FC4**)



Compound **5** (50 mg, 81.6 µmol) was cooled to 0 °C prior to the addition of TFA/anisole (5:1, 3 mL). The resulting reaction mixture was stirred at 0 °C for 30 min. with constant monitoring of the conversion by TLC analysis (CH_2Cl_2/EtOAC, 8:2). Upon completion of the reaction cold Et_2O (5 mL) was added which resulted in the formation of a precipitate. The solid material was filtered-off and washed with Et_2O (2 x 5 mL) and subsequently dried under high vacuum to yield the desired product as an off-white solid (15 mg, 37%). R_F = 0.10 (CH_2Cl_2/MeOH +AcOH, 9:1). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.45 (d, *J* = 8.5 Hz, 1H), 8.00 (d, *J* = 9.5 Hz, 1H), 7.65 (d, *J* = 8.5 Hz, 1H), 7.27 - 7.32 (m, 4H), 7.24 (td, *J* = 8.5, 4.0 Hz, 1H), 6.93 - 7.01 (m, 1H), 6.31 (d, *J* = 9.5 Hz, 1H), 5.81 (dd, *J* = 8.0, 4.5 Hz, 1H), 5.14 (d, *J* = 12.5 Hz, 1H), 4.87 - 4.93 (m, 1H), 3.98 (d, *J* = 18.0 Hz, 1H), 3.70 (d, *J* = 14.0 Hz, 1H) (part of AB-system), 3.62 (d, *J* = 18.5 Hz, 1H), 3.54 (d, *J* = 14.0 Hz, 1H) (part of AB-system) ppm. ^{13}C-NMR: (101 MHz, DMSO-d_6) δ = 171.1, 164.2, 162.2, 161.1, 155.3, 144.3, 135.8, 129.6, 129.1 (2C), 128.3 (2C), 126.6, 112.8 (2C), 101.6, 67.5, 66.3, 58.3, 45.3, 41.5 ppm. LRMS calcd. for $C_{25}H_{19}N_2O_8S$, M-H = 507.09, mass found; M-H = 507.0. Retention time = 7.15 min. (purity = 99%).

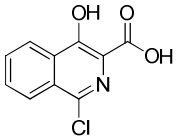
(6*R*,7*R*)-8-oxo-3-(((2-oxo-2H-chromen-7-yl)oxy)methyl)-7-(2-phenylacetamido)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid 5,5-dioxide (**FC5**)



Compound **6** (20 mg, 0.03 mmol) was cooled to 0 °C prior to the addition of TFA/anisole (5:1, 1.2 mL). The reaction was stirred at 0 °C for 30 min followed by 30 min at r.t. with constant monitoring of the conversion by TLC analysis (CH_2Cl_2/MeOH, 9:1). Upon completion of the reaction cold Et_2O (5 mL) was added which resulted in the formation of a precipitate. The solid material was filtered-off and washed with Et_2O (2 x 3 mL) and subsequently dried under high vacuum to yield the desired product as an off-white solid (10 mg, 63%). R_F = 0.10 (CH_2Cl_2/MeOH +AcOH, 9:1). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.92 (d, *J* = 9.0 Hz, 1H), 8.01 (d, *J* = 9.5 Hz, 1H), 7.66 (d, *J* = 8.5 Hz, 1H), 7.19 - 7.31 (m, 5H) 7.04 (d, *J* = 2.5 Hz, 1H), 6.99 (dd, *J* = 8.5, 2.5 Hz, 1H), 6.32 (d, *J* = 9.5 Hz, 1H), 5.97 (dd, *J* = 8.5, 4.5 Hz, 1H), 5.42 (d, *J* = 4.5 Hz, 1H), 4.90 - 5.00 (m, app. q., 2H), 4.41 (d, *J* = 18.0 Hz, 1H), 4.19 (d, *J* = 18.0 Hz, 1H), 3.54 - 3.65 (m, app. q., 2H) ppm. ^{1}H NMR (101 MHz, DMSO-d_6) δ = 170.9, 164.3, 162.1,

160.8, 160.2, 155.2, 144.3, 135.6, 129.6, 129.2 (2C), 128.2 (2C), 126.5, 125.2, 123.8, 112.9, 112.9, 101.7, 66.8, 66.3, 58.3, 50.8, 41.2 ppm. LRMS calcd. for $C_{25}H_{19}N_2O_9S$, M-H = 523.08, mass found; M-H = 523.0. Retention time = 8.89 min. (purity = 95%).

1-Chloro-4-hydroxyisoquinoline-3-carboxylic acid (**S3**)[4]

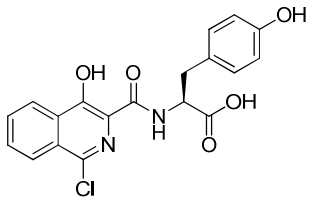


Commercially available methyl 1-chloro-4-hydroxyisoquinoline-3-carboxylate (5.0 g, 21.0 mmol) was dissolved in a mixture of H_2O and THF (1:1, 50 mL) and treated with lithium hydroxide ($LiOH \cdot H_2O$, 4.42 g, 0.21 mol, 10 eq). The reaction was stirred at room temperature for 24h. The THF was evaporated and resulting water solution extracted with EtOAc (2 x 20 mL). The aqueous phase was acidified with conc. HCl (pH = 1) and subsequently extracted with EtOAc (3 x 20 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and evaporated *in vacuo* to yield title compound as a white solid (4.47 g, 95%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.31-8.36 (m, 1H, ArH), 8.21-8.25 (m, 1H, ArH), 7.96-8.01 (m, 2H, ArH) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 171.5, 156.0, 138.8, 132.1, 131.8, 129.1, 128.8, 125.9, 123.2, 119.5 ppm. Mp = 202-205°C. HRMS (ESI-TOF) calcd. for $C_{10}H_6ClNO_3$ [$M+H^+$]: 221.9963, found: 221.9958. FT-IR ν_{max} (neat): 2966, 1656, 1312, 1233, 768 cm^{-1}.

General procedure for synthesis of amino acids conjugates of 1-chloro-4-hydroxyisoquinolines:

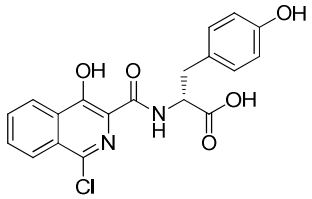
1-Chloro-4-hydroxyisoquinoline-3-carboxylic acid (300 mg, 1.35 mmol), amino acid methyl ester (1.2 eq), benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP, 1.2 eq) and triethylamine (Et_3N, 1.5 mmol) were dissolved in anhydrous DMF (5 mL) and subsequently stirred at room temperature for 24h. Upon completion of the reaction the DMF was evaporated *in vacuo* and resulting residue was suspended in CH_2Cl_2 (20 mL) and washed with H_2O (2 x 10 mL). The organic phase was dried over $MgSO_4$, filtered and subjected to column chromatography (Biotage SNAP KP-SIL^{TM} 25 g cartridge, eluent system: cHex/EtOAc, ratio for elution of each methyl ester is given along with characterization of final product). The obtained product was dissolved in a mixture of THF/H_2O (1:1, 10 mL) and subsequently treated with $LiOH \cdot H_2O$ (5 eq). The reaction was stirred at room temperature for 12h. The THF was evaporated *in vacuo* and the remaining aqueous solution was neutralized with conc. HCl. If precipitate was formed it was filtered-off and dried *in vacuo* to yield the desired product. In case that no precipitate was formed, the aqueous solution was extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over $MgSO_4$, filtered and evaporated *in vacuo* to yield desired product.

(*S*)-2-(1-Chloro-4-hydroxyisoquinoline-3-carboxamido)-3-(4-hydroxyphenyl)propanoic acid (***S*-7a**)



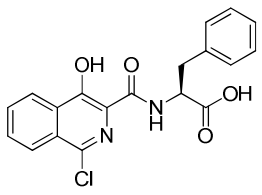
The title compound was obtained as a light grey solid (182 mg, 35%). Methyl ester was purified in linear gradient of 7-60% EtOAc in cHex over 400 mL of total solvent volume with compound elution at 40% of EtOAc. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 13.50 (br. s., 1H, OH), 9.32 (s, 1H, OH), 8.81 (d, *J* = 8.0 Hz, 1H, NH), 8.24 - 8.40 (m, 2H), 7.93 - 8.05 (m, 2H), 7.04 (d, *J* = 8.5 Hz, 2H), 6.65 (d, 2H, *J* = 8.5 Hz) 4.71 (m, 1H) 3.16 (d, *J* = 6.5 Hz, 2H) ppm. ^{13}C NMR (176 MHz, DMSO-d_6) δ = 172.6, 168.9, 156.5, 154.8, 139.1, 132.3, 130.5, 129.1, 127.7, 127.4, 126.6, 123.5, 120.6, 115.6, 53.9, 35.6 ppm. Mp = 166-170 °C, $[\alpha]^{20}_{D}$ = -49.8 (c = 0.100 in DMSO). HRMS (ESI-TOF) calcd for $C_{19}H_{15}ClN_2NaO_5$ [M+Na$^+$]: 385.0597, found: 385.0597. FT-IR ν_{max} (neat): 3367, 1736, 1533, 1174, 767 cm^{-1}. Retention time = 7.75 min. (purity = 90%).

(*R*)-2-(1-Chloro-4-hydroxyisoquinoline-3-carboxamido)-3-(4-hydroxyphenyl)propanoic acid (***R*-7a**)



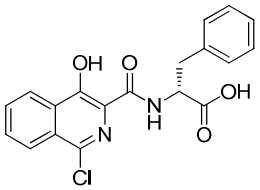
The title compound was obtained as an off-white solid (166 mg, 32%). Methyl ester was purified in linear gradient of 7-60% EtOAc in cHex over 400 mL of total solvent volume with compound elution at 40% of EtOAc. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 13.50 (br. s., 1H, OH), 9.32 (s, 1H, OH), 8.81 (d, *J* = 8.0 Hz, 1H, NH), 8.24 - 8.40 (m, 2H), 7.93 - 8.05 (m, 2H), 7.04 (d, *J* = 8.5 Hz, 2H), 6.65 (d, *J* = 8.5 Hz, 2H), 4.73 - 4.68 (m, 1H) 3.16 (d, *J* = 6.5 Hz, 2H) ppm. ^{13}C NMR (176 MHz, DMSO-d_6) δ = 172.6, 168.9, 156.5, 154.8, 139.1, 132.3, 130.5, 129.1, 127.7, 127.4, 126.6, 123.5, 120.6, 115.6, 53.9, 35.6 ppm. Mp = 173-175 °C, $[\alpha]^{20}_{D}$ = 48.0 (c = 0.179 in DMSO). HRMS (ESI-TOF) calcd for $C_{19}H_{15}ClN_2NaO_5$ [M+Na$^+$]: 385.0597, found: 385.0596. FT-IR ν_{max} (neat): 3382, 3289, 1718, 1614, 1320 cm^{-1}. Retention time = 7.74 min. (purity = 99%).

(*S*)-2-(1-Chloro-4-hydroxyisoquinoline-3-carboxamido)-3-phenylpropanoic acid (***S*-7b**)



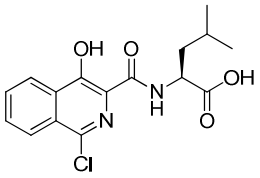
The title compound was obtained as a white solid (219 mg, 44%). Methyl ester was purified in linear gradient of 7-60% EtOAc in cHex over 400 mL of total solvent volume with compound elution at 30% of EtOAc. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.99 (d, *J* = 8.5 Hz, 1H, NH), 8.40 - 8.31 (m, 1H), 8.32 (m, 1H), 8.03 (m, 2H), 7.36 - 7.26 (m, 4H), 7.25 (m, 1H), 4.86 (dt, *J* = 8.5, 7.0 Hz, 1H), 3.35 (d, *J* = 7.0 Hz, 2H) ppm, ^{13}C NMR (101 MHz, DMSO-d_6) δ = 172.5, 168.8, 154.8, 139.0, 137.8, 132.3, 132.3, 129.9, 129.6, 129.0, 128.8, 127.1, 126.6, 123.4, 120.7, 53.6, 36.4 ppm. Mp = 180-183 °C, $[\alpha]^{20}_{D}$ = -31.1 (c = 0.100 in DMSO). HRMS (ESI-TOF) calcd for $C_{19}H_{15}ClNaN_2O_4$ [M+H$^+$]: 393.0613, found: 393.0610, FT-IR ν_{max} (neat): 3448, 3360, 2931, 1736, 1570, 1259, 773 cm^{-1}. Retention time = 9.41 min. (purity = 99%).

(*R*)-2-(1-Chloro-4-hydroxyisoquinoline-3-carboxamido)-3-phenylpropanoic acid (***R*-7b**)



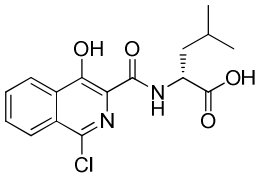
The title compound was obtained as a white solid (205 mg, 41%). Methyl ester was purified in linear gradient of 7-60% EtOAc in cHex over 400 mL of total solvent volume with compound elution at 30% of EtOAc. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.99 (d, *J* = 8.5 Hz, 1H, NH), 8.40 - 8.31 (m, 1H), 8.32 (m, 1H), 8.03 (m, 2H), 7.36 - 7.26 (m, 4H), 7.25 (m, 1H, PhH), 4.86 (dt, *J* = 8.5, 7.0 Hz, 1H), 3.35 (d, *J* = 7.0 Hz, 2H) ppm, ^{13}C NMR (101 MHz, DMSO-d_6) δ = 172.5, 168.8, 154.8, 139.0, 137.8, 132.3, 132.3, 129.9, 129.6, 129.0, 128.8, 127.1, 126.6, 123.4, 120.7, 53.6, 36.4 ppm. Mp = 168-171 °C, $[\alpha]^{20}_{D}$ = +29.7 (c = 0.100 in DMSO). HRMS (ESI-TOF) calcd for $C_{19}H_{15}ClNaN_2O_4$ [M+H$^+$]: 393.0613, found: 393.0612, FT-IR ν_{max} (neat): 3449, 3359, 2931, 1736, 1574, 1260, 770 cm^{-1}. Retention time = 9.40 min. (purity = 99%).

(*S*)-2-(1-Chloro-4-hydroxyisoquinoline-3-carboxamido)-4-methylpentanoic acid (***S*-7c**)



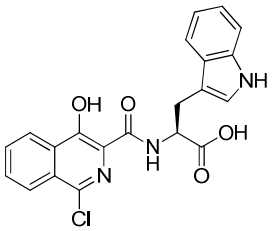
The title compound was obtained as an off-white solid (160 mg, 35%). Methyl ester was purified in linear gradient of 2-20% EtOAc in cHex over 200 mL of total solvent volume with compound elution at 9% of EtOAc. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 13.67 (br. s., 1H, OH), 9.03 (d, *J* = 8.5 Hz, 1H, NH), 8.29 - 8.35 (m, 1H), 8.24 - 8.29 (m, 1H), 7.91 - 8.06 (m, 2H), 4.44 - 4.66 (m, 1H), 1.96 (m, 1H), 1.54 - 1.77 (m, 2H), 0.91 (d, *J* = 6.0 Hz, 3H), 0.92 (d, *J* = 6.0 Hz, 3H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 174.0, 169.7, 155.3, 139.4, 132.6, 132.6, 130.4, 129.4, 126.9, 123.8, 121.2, 51.1, 39.6 (overlapped with DMSO signal), 25.4, 23.8, 22.1 ppm. Mp = 158-160 °C, $[\alpha]^{20}_{D}$ = 13.4 (c = 0.104 in DMSO). HRMS (ESI-TOF) calcd for $C_{16}H_{17}ClN_2NaO_4$ [M+Na$^+$]: 359.0769, found: 359.0763. FT-IR ν_{max} (neat): 3380, 2966, 1656, 1312, 1233, 768 cm^{-1}. Retention time = 9.70 min. (purity = 90%).

(*R*)-2-(1-Chloro-4-hydroxyisoquinoline-3-carboxamido)-4-methylpentanoic acid (***R*-7c**)



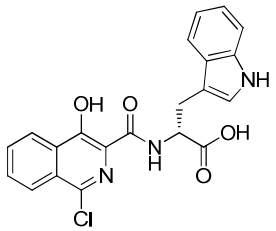
The title compound was obtained as an off-white solid (146 mg, 32%). Methyl ester was purified in linear gradient of 2-20% EtOAc in cHex over 200 mL of total solvent volume with compound elution at 9% of EtOAc. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 13.67 (br. s., 1H, OH), 9.03 (d, *J* = 8.5 Hz, 1H, NH), 8.29 - 8.35 (m, 1H), 8.24 - 8.29 (m, 1H), 7.91 - 8.06 (m, 2H), 4.44 - 4.66 (m, 1H), 1.96 (m, 1H), 1.54 - 1.77 (m, 2H), 0.91 (d, *J* = 6.0 Hz, 3H), 0.92 (d, *J* = 6.0 Hz, 3H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 174.0, 169.7, 155.3, 139.4, 132.6, 132.6, 130.4, 129.4, 126.9, 123.8, 121.2, 51.1, 39.6 (overlapped with DMSO signal), 25.4, 23.8, 22.1 ppm. Mp = 157-159 °C, $[\alpha]^{20}_{D}$ = -12.6 (c = 0.095 in DMSO). HRMS (ESI-TOF) calcd for $C_{16}H_{17}ClN_2NaO_4$ [M+Na$^+$]: 359.0769, found: 359.0765. FT-IR ν_{max} (neat): 3379, 2968, 1656, 1312, 1233, 768 cm^{-1}. Retention time = 9.65 min. (purity = 99%).

(*S*)-2-(1-Chloro-4-hydroxyisoquinoline-3-carboxamido)-3-(1H-indol-3-yl)propanoic acid (***S*-7d**)



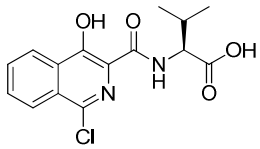
The title compound was obtained as an off-white solid (232 mg, 42%). Methyl ester was purified in linear gradient of 7-60% EtOAc in cHex over 400 mL of total solvent volume with compound elution at 35% of EtOAc. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 13.50 (s, 1H, OH), 10.93 (s, 1H, NH indole), 8.80 (d, *J* = 8.1 Hz, 1H, NH), 8.30 - 8.38 (m, 1H), 8.24 - 8.30 (m, 1H), 7.93 - 8.08 (m, 2H), 7.58 (d, *J* = 8.0 Hz, 1H), 7.33 (d, *J* = 8.0 Hz, 1H), 7.19 (d, *J* = 2.0 Hz, 1H), 7.05 (t, *J* = 7.0 Hz, 1H), 6.95 (t, *J* = 7.5 Hz, 1H), 4.83 (td, *J* = 7.5, 5.0 Hz, 1H), 3.46 (overlapping with water signal, determined by HSQC, 2H) ppm. ^{13}C NMR (176 MHz, DMSO-d_6) δ = 172.9, 168.7, 154.7, 139.0, 136.6, 132.3, 132.3, 129.9, 129.0, 127.7, 126.6, 124.2, 123.5, 121.5, 120.8, 119.0, 118.7, 111.9, 109.8, 53.3, 26.9 ppm. Mp = 205-208 °C, $[\alpha]^{20}_{D}$ = -21.7 (c = 0.133 in DMSO). HRMS (ESI-TOF) calcd for $C_{21}H_{16}ClN_3NaO_4$ [M+Na$^+$]: 432.0722, found: 432.0737. FT-IR ν_{max} (neat): 3365, 1718, 1633, 1529, 1320, 768 cm^{-1}. Retention time = 8.81 min. (purity = 99%).

(*R*)-2-(1-Chloro-4-hydroxyisoquinoline-3-carboxamido)-3-(1H-indol-3-yl)propanoic acid (***R*-7d**)



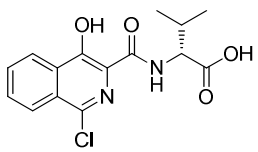
The title compound was obtained as a white solid (249 mg, 45%). Methyl ester was purified in linear gradient of 7-60% EtOAc in cHex over 400 mL of total solvent volume with compound elution at 35% of EtOAc. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 13.50 (s, 1H, OH), 10.93 (s, 1H, NH indole), 8.80 (d, *J* = 8.0 Hz, 1H, NH), 8.30 - 8.38 (m, 1H), 8.24 - 8.30 (m, 1H), 7.93 - 8.08 (m, 2H), 7.58 (d, *J* = 8.0 Hz, 1H), 7.33 (d, *J* = 8.0 Hz, 1H), 7.19 (d, *J* = 2.0 Hz, 1H), 7.05 (t, *J* = 7.0 Hz, 1H), 6.95 (t, *J* = 7.5 Hz, 1H), 4.83 (td, *J* = 7.5, 5.0 Hz, 1H), 3.46 (overlapped with water signal, determined by HSQC, 2H) ppm. ^{13}C NMR (176 MHz, DMSO-d_6) δ = 172.9, 168.7, 154.7, 139.0, 136.6, 132.3, 132.3, 129.9, 129.0, 127.7, 126.6, 124.2, 123.5, 121.5, 120.8, 119.0, 118.7, 111.9, 109.8, 53.3, 26.9 ppm. Mp = 212-214 °C, $[\alpha]^{20}_{D}$ = 24.9 (c = 0.088 in DMSO). HRMS (ESI-TOF) calcd for $C_{21}H_{16}ClN_3NaO_4$ [M+Na$^+$]: 432.0722, found: 432.0729. FT-IR ν_{max} (neat): 3362, 1634, 1529, 1319, 770 cm^{-1}. Retention time = 8.81 min. (purity = 99%).

(*S*)-2-(1-Chloro-4-hydroxyisoquinoline-3-carboxamido)-3-methylbutanoic acid (***S*-7e**)



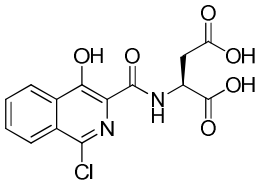
The title compound was obtained as a white solid (148 mg, 34%). Methyl ester was purified in linear gradient of 2-20% EtOAc in cHex over 200 mL of total solvent volume with compound elution at 30% of EtOAc. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.55 (d, *J* = 8.5 Hz, 1H, NH), 8.40 (m, 1H), 8.35 (m, 1H), 8.06 (m, 2H), 4.49 (dd, *J* = 8.5, 5.5 Hz, 1H), 2.38 (sptd, *J* = 7.0 × 4, 5.5 Hz, 1H), 1.04 (d, *J* = 7.0 Hz, 3H), 1.03 (d, *J* = 7.0 Hz, 3H) ppm, ^{13}C NMR (101 MHz, DMSO-d_6) δ = 172.6, 168.7, 154.8, 139.2, 132.4, 132.3, 130.0, 129.1, 126.6, 123.5, 120.6, 57.6, 30.6, 19.5, 18.6 ppm. Mp = 165-168°C, $[\alpha]^{20}_{D}$ = +16.7 (c = 0.100 in DMSO). HRMS (ESI-TOF) calcd for $C_{15}H_{15}ClNaN_2O_4$ [M+H$^+$]: 345.0613, found: 345.0611, FT-IR ν_{max} (neat): 3447, 3373, 2965, 1759, 1572, 1259, 776 cm^{-1}. Retention time = 9.25 min. (purity = 99%).

(*R*)-2-(1-Chloro-4-hydroxyisoquinoline-3-carboxamido)-3-methylbutanoic acid (***R*-7e**)



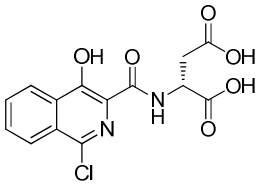
The title compound was obtained as a white solid (165 mg, 38%). Methyl ester was purified in linear gradient of 2-20% EtOAc in cHex over 200 mL of total solvent volume with compound elution at 30% of EtOAc. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.55 (d, *J* = 8.5 Hz, 1H, NH), 8.40 (m, 1H), 8.35 (m, 1H), 8.06 (m, 2H), 4.49 (dd, *J* = 8.5, 5.5 Hz, 1H), 2.38 (sptd, *J* = 7.0 × 4, 5.5 Hz, 1H), 1.04 (d, *J* = 7.0 Hz, 3H), 1.03 (d, *J* = 7.0 Hz, 3H) ppm, ^{13}C NMR (101 MHz, DMSO-d_6) δ = 172.6, 168.7, 154.8, 139.2, 132.4, 132.3, 130.0, 129.1, 126.6, 123.5, 120.6, 57.6, 30.6, 19.5, 18.6 ppm. Mp = 172-175°C, $[\alpha]^{20}_{D}$ = -18.1 (c = 0.100 in DMSO). HRMS (ESI-TOF) calcd for $C_{15}H_{15}ClNaN_2O_4$ [M+H$^+$]: 345.0613, found: 345.0614, FT-IR ν_{max} (neat): 3447, 3373, 2965, 1759, 1572, 1259, 776 cm^{-1}. Retention time = 9.25 min. (purity = 99%).

(*S*)-2-(1-Chloro-4-hydroxyisoquinoline-3-carboxamido)succinic acid (***S*-7f**)



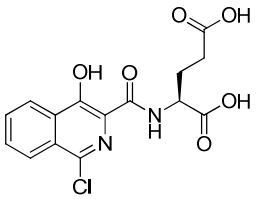
The title compound was obtained as a pink solid (186 mg, 41%). Methyl ester was purified in linear gradient of 7-40% EtOAc in cHex over 250 mL of total solvent volume with compound elution at 30% of EtOAc. ^{1}H NMR (400 MHz, DMSO-d_6) δ =13.56 (br. s., 1H, OH), 9.16 (d, *J* = 8.5 Hz, 1H, NH), 8.22 - 8.43 (m, 2H), 7.95 - 8.13 (m, 2H), 4.78 - 5.05 (m, 1H), 2.97 (d, *J* = 6.0 Hz, 2H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 172.6, 172.2, 168.7, 154.9, 139.1, 132.3, 132.3, 130.0, 129.1, 126.6, 123.5, 120.8, 48.8, 36.0 ppm. Mp = 240-243 °C, $[\alpha]^{20}_{D}$ = -14.2 (c = 0.098 in DMSO). HRMS (ESI-TOF) calcd for $C_{14}H_{11}ClN_2NaO_6$ [M+Na$^+$]: 337.0233, found 337.0247. FT-IR ν_{max} (neat): 3413, 2927, 1707, 1528, 1212, 767 cm^{-1}. Retention time = 7.05 min. (purity = 90%).

(*R*)-2-(1-Chloro-4-hydroxyisoquinoline-3-carboxamido)succinic acid (***R*-7f**)



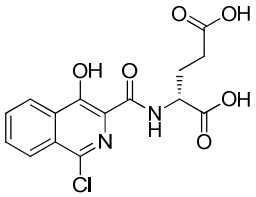
The title compound was obtained as a pink solid (177 mg, 39%). Methyl ester was purified in linear gradient of 7-40% EtOAc in cHex over 250 mL of total solvent volume with compound elution at 30% of EtOAc. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 13.56 (br. s., 1H, OH), 9.16 (d, *J* = 8.5 Hz, 1H, NH), 8.22 - 8.43 (m, 2H), 7.95 - 8.13 (m, 2H), 4.78 - 5.05 (m, 1H), 2.97 (d, *J* = 6.0 Hz, 2H), ^{13}C NMR (101 MHz, DMSO-d_6) δ = 172.6, 172.2, 168.7, 154.9, 139.1, 132.3, 132.3, 130.0, 129.1, 126.6, 123.5, 120.8, 48.8, 36.0 ppm. Mp = 239-241 °C, $[\alpha]^{20}_{D}$ = 13.8 (c = 0.102 in DMSO). HRMS (ESI-TOF) calcd for $C_{14}H_{11}ClN_2NaO_6$ [M+Na$^+$]: 337.0233, found: 337.0248, FT-IR ν_{max} (neat): 3357, 2926, 1704, 1526, 1194, 765 cm^{-1}. Retention time = 7.04 min. (purity = 90%).

(*S*)-2-(1-Chloro-4-hydroxyisoquinoline-3-carboxamido)pentanedioic acid (***S*-7g**)



The title compound was obtained as a cream white solid (133 mg, 28%). Methyl ester was purified in linear gradient of 7-40% EtOAc in cHex over 250 mL of total solvent volume with compound elution at 35% of EtOAc. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 13.63 (br. s., 1H, OH), 9.14 (d, *J* = 8.0 Hz, 1H, NH), 8.31 - 8.36 (m, 1H), 8.25 - 8.31 (m, 1H), 7.94 - 8.03 (m, 2H), 4.54 - 4.65 (m, 1H), 2.37 (t, *J* = 7.5 Hz, 2H), 2.07 - 2.31 (m, 2H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 174.2, 172.8, 169.3, 154.9, 139.1, 132.0, 132.0, 130.0, 129.0, 126.4, 123.3, 120.2, 51.8, 30.7, 26.1 ppm. Mp = 128-130 °C, $[\alpha]^{20}_{D}$ = -6.4 (c = 0.125 in DMSO). HRMS (ESI-TOF) calcd for $C_{15}H_{13}ClN_2NaO_6$ [M+Na$^+$]: 375.0354, found: 375.0352, FT-IR ν_{max} (neat): 2923, 2853, 1709, 1528, 1319, 1214, 766 cm^{-1}. Retention time = 6.95 min. (purity = 99%).

(*R*)-2-(1-Chloro-4-hydroxyisoquinoline-3-carboxamido)pentanedioic acid (***R*-7g**)



The title compound was obtained as a cream white solid (166 mg, 35%). Methyl ester was purified in linear gradient of 7-40% EtOAc in cHex over 250 mL of total solvent volume with compound elution at 35% of EtOAc. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 13.63 (br. s., 1H, OH), 9.14 (d, *J* = 8.0 Hz, 1H, NH), 8.31 - 8.36 (m, 1H), 8.25 - 8.31 (m, 1H), 7.94 - 8.03 (m, 2H), 4.54 - 4.65 (m, 1H), 2.37 (t, *J* = 7.5 Hz, 2H), 2.07 - 2.31 (m, 2H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 174.2, 172.8, 169.3, 154.9, 139.1, 132.0, 132.0, 130.0, 129.0, 126.4, 123.3, 120.2, 51.8, 30.7, 26.1 ppm. Mp = 135-138 °C, $[\alpha]^{20}_{D}$ = 6.2 (c = 0.127 in DMSO). HRMS (ESI-TOF) calcd for $C_{15}H_{13}ClN_2NaO_6$ [M+Na$^+$]: 375.0354, found: 375.0342. FT-IR ν_{max} (neat): 2923, 2853, 1709, 1527, 1319, 1214, 766 cm^{-1}. Retention time = 7.01 min. (purity = 90%).

Protein production

The pET9a-Bc II plasmid was a kind gift from Prof. dr. Moreno Galleni (Lab. Biological Macromolecules Centre for Protein Engineering, Dept. of Life Sciences, University of Liege). The pOPIN-F NDM-1 plasmid was previously described by Green et. al.[5] Plasmids expressing the VIM-2, SPM-1 and IMP-1 genes were constructed using primers (see Table below)) to amplify the relevant open reading frames (encoding the mature MBL polypeptide after removal of the *N*-terminal periplasmic export sequence). The resulting PCR products were inserted into the KpnI-HindIII sites of the pOPINF expression vector by Infusion PCR cloning according to Berrow et al.[6]

Primers:

Gene	Fwd primer	Rev primer
VIM-2 (aa 27 – 266)	aagttctgtttcagggcccgGTGGATTCGT CGGGCGAATATC	atggtctagaaagctttaTTCGACAACAGAA CGATTGGTGTG
SPM-1 (aa 29 – 276)	aagttctgtttcagggcccgAAAAGCTCTG ATCATGTTGACCTG	atggtctagaaagctttaCAGGCGCATTTCG CCAACAG

Bc II production

Bc II protein was produced in *E. coli* BL21 (DE3)pLysS cells at 37 °C using 2TY medium supplemented with 50 µg/ml ampicillin and 50 µg/ml choramphenicol. The cells were grown until an OD_{600} 0.6 - 0.7 was reached and then induced with 0.5 mM IPTG. Following it was grown at the same temperature for another 4 h. Cells were harvested by centrifugation (10 min, 10 g) and were resuspended in 50 mL lysis buffer (50 mM Tris pH 7.5, 500 mM NaCl) supplemented with DNAse, Lysosyme and EDTA-free protease-inhibitor cocktail and lysed (1 tablet / 15 g of cells). Cellular debris was removed by centrifugation and the supernatant was dialysed against 20 mM MES pH 6.35 supplemented with 0.2 mM $ZnCl_2$ (Buffer A) and loaded onto an SP-Sepharose column (1.5 × 12 cm with a 25 mL bed volume), which was also pre-equilibrated with Buffer A. Bound proteins were eluted with 0−1 M NaCl gradient in Buffer A. The purity of the fractions was determined using SDS-PAGE gels and those fractions containing pure Bc II were concentrated by centrifugal ultrafiltration. The concentration of the purified protein was determined by means of NanoDrop measurements and the consecutive concentrations used in the assay were calculated based on the original concentration.

NDM-1/VIM-2/SPM-1 production

NDM-1/VIM-2/SPM-1 proteins were produced in *E. coli* BL21 (DE3)pLysS cells at 37 °C using 2TY medium supplemented with 50 µg/ml ampicillin and 50 µg/ml chloramphenicol. Cells were grown until OD_{600} reached 0.6 - 0.7. At this point the temperature was lowered to 20 °C (for NDM-1) or 30 °C (for VIM-2 and SPM-1),expression was induced with IPTG (0.5 mM final concentration) and the cells were further incubated for 20 h (in the case of NDM-1), 8h (SPM-1) or 4h (VIM-2). Cells were harvested by centrifugation (10 min, 10 g) and were resuspended in 50 mL lysis buffer (50 mM Tris pH 7.5, 500 mM NaCl, 0.2% Tween 20, 5 mM imidazole) supplemented with DNAse, Lysosyme and EDTA-free protease-inhibitor cocktail and lysed. Cellular debris was removed by centrifugation and the supernatant was loaded onto a 5 ml HisTrap HP column followed by extensive washing with 50

mM Tris pH 7.5, 500 mM NaCl with 20 mM imidazole increasing to 500 mM imidazole. Fractions containing the purified enzyme were concentrated by centrifugal ultrafiltration and the protein was injected onto a Superdex S200 column (300 mL) and eluted with 20 mM Tris pH 7.5, 200 mM NaCl. Fractions containing the pure enzyme were incubated overnight at 4 °C with 6-His-tagged 3C protease (1:100 w/w) to remove the *N*-terminal His-tag. In order to remove the 3C protease together with any uncleaved protein the digestion mixture was passed over a second HisTrap HP column pre-equilibrated in 50 mM Tris pH 7.5 500 mM NaCl 20 mM imidazole. The active and pure enzyme fractions (i.e. untagged protein passing through the column), as identified by SDS-PAGE and a nitrocefin-based activity measurement,[7] were pooled and concentrated by centrifugal ultrafiltration. The concentrations of the purified proteins were determined by means of NanoDrop measurements and the consecutive concentrations used in the assay were calculated based on the original concentrations.

IMP-1 production

IMP-1 protein was expressed in *E. coli* BL21 (DE3)pLysS cells at 37 °C using 2TY medium supplemented with 50 µg/ml ampicillin and 50 µg/ml choramphenicol. The cells were grown until an OD_{600} 0.6 - 0.7 was reached, induced with 0.5 mM IPTG and grown at the same temperature for a further 4 h. Cells were harvested by centrifugation (10 min, 10 g) and were resuspended in 50 mL lysis buffer (50 mM Tris pH 7.0, 500 mM NaCl) supplemented with DNAse, Lysosyme and EDTA-free protease-inhibitor cocktail and lysed. Cellular debris was removed by centrifugation and the supernatant dialysed against 50 mM Tris pH 7.00 supplemented with 0.1 mM $ZnCl_2$ (Buffer A) and loaded onto an SP-Sepharose column (1.5 × 12 cm with a 25 mL bed volume), pre-equilibrated with Buffer A. Bound proteins were eluted with a 0−1 M NaCl gradient in Buffer A. Fractions containing pure and active enzyme, as identified by SDS-PAGE and a nitrocefin-based activity measurement, were pooled and concentrated and the protein injected onto a Superdex S75 column (300 mL) pre-equilibrated in, and eluted using, 50 mM Tris pH 7.5 150 mM NaCl 100 µM $ZnCl_2$. The purity of the resulting fractions was determined using SDS-PAGE gels and those fractions containing pure IMP-1 were concentrated by centrifugal ultrafiltration. The concentration of the purified protein was determined by means of NanoDrop measurements and the consecutive concentrations used in the assay were calculated based on the original concentration.

Screening assay

Prerequisites for substrates suitable for MBL inhibitor screening include rapid substrate hydrolysis (i.e. high turn-over k_{cat}) by MBLs; high binding constant (K_M), since the occupancy of the enzyme by substrate must be low to minimize any substrate inhibition effects; and finally, broad applicability to a set of different MBLs (i.e. kinetic parameters that are broadly consistent between the different enzyme targets). Since the K_M value embodies the substrate concentration at which half of the enzyme is saturated, the substrate concentration in an inhibition assay should be below K_M. In order to be able to measure slow-binding inhibitors, the total amount of converted substrate should be minimal to rule out lower conversion rates as a result of declining substrate concentrations. These two requirements are met in a high K_M value. Taking into account the aforementioned requirements, the ideal substrate should thus have a high K_M and a high k_{cat}, and is specific for set of different MBLs.

The experiments were performed by using a NovaStar microplate reader (using path length correction) and were performed at r.t. (24-25 °C). All enzymes and substrates were dissolved in the assay buffer: 50 mM HEPES-NaOH buffer (pH 7.2) supplemented with 1 μg / mL BSA (to minimize the denaturation of the enzyme), 1 μM $ZnSO_4$ and 0.01% Triton X-100.

Hydrolysis of nitrocefin, CENTA, imipenem and Fluo-cep **FC4**, was monitored by following the variation in absorbance at 492, 405 and 300 nm or fluorescence at excitation 380 nm and emission at 460 nm, respectively. In all cases 96 well flat bottom plates: μClear half area black plate for fluorescence (675096) and UV-STAR Microplate (655801) or Micro assay Plate (655095) for absorbance from Greiner Bio-One were used.

The kinetic values reported in this study are the means from at least three independent measurements. At least six different concentrations of the substrate or inhibitor were used to determinate the kinetic parameters (K_M, k_{cat} and IC_{50}). Determination of the steady state kinetic parameters for the hydrolysis of different substrates (K_M and k_{cat}) was performed by fitting the initial velocity data to the Michaelis-Menten equation using the software package Graph Prism 5.01. The IC_{50} values were determined from the plot of activity (steady state rate) versus inhibitor concentration using the same software.

The enzyme concentration used for the determination of the kinetic parameters (K_M and k_{cat}) is presented in Table 1. For IC_{50} determination we used the same fixed enzyme concentration as presented in Table 1 and the substrate concentration is approximately equal with the K_M value.

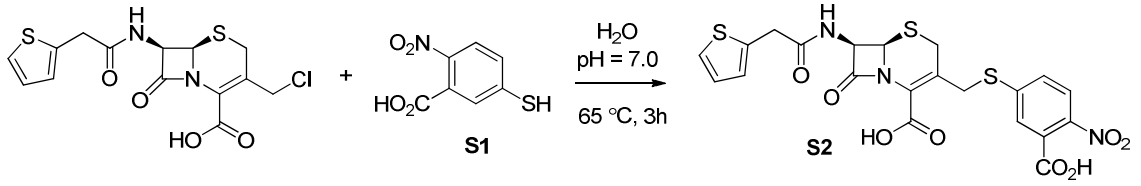
IC_{50} values (concentration required to affect 50% inhibition of enzyme activity) were determined by preincubation of the appropriate amount of enzyme with the desired compound in the assay buffer for 10 min at r.t. prior to the initiation of the assay by the addition of the substrate.[8] The compounds for inhibition study were prepared in 1 to 100 mM DMSO stock solutions. Additional tests verified that the low concentration of DMSO (0.5%) present in the reaction mixture had no inhibition effects.

***In silico* studies**

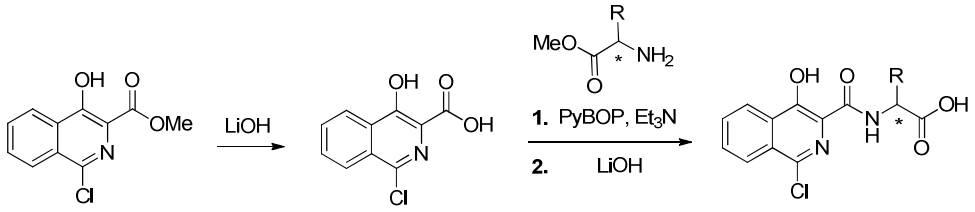
Energy minimised structures of nitrocefin, imipenem, CENTA and the fluorogenic compound **FC4**, constructed in maestro,[9] were imported into the SPROUT[10] programme and docked into the di-zinc containing active site of the crystal structures of NDM-1, IMP-1 and VIM-2 (PDB ID: 3Q6X, 1JJT and 1KO3, respectively). The resulting docking 'poses' were subsequently scored (see Table SI_1). For comparison, docking was also performed, repeating the initial docking procedure, using AutoDock 4,[11] which utilises a different docking algorithm compared to the SPROUT software. The resulting docking 'poses' were scored, both by AutoDock and the SPROUT scoring function. The predicted binding models should be regarded as speculative (see main text).

The predicted enzyme-substrate complex structures show some similarities in terms of orientation of the substrates within the binding cavity (Fig. SI_5A. Both Nitrocefin and CENTA are predicted to bind in almost identical orientations having the C-4 carboxylic acid of the cephalosporin-derived thiazine ring positioned between the two zinc atoms of IMP-1 (Fig SI_5B). Differences are found in the binding of the C-3' nitrophenyl ring systems (see Fig. SI_6 and SI_7). In case of Nitrocefin, the *ortho*-nitro groups is positioned to form a hydrogen bond with K161 (2.8 Å, Nε) which does not occur in case of CENTA. However, the carboxylic acid on the thiophenol ring of CENTA is able to form a hydrogen bond with indole nitrogen of W28. Both Imipenem and **FC4** have dissimilar zinc-binding orientations when compared to Nitrocefin and CENTA. In case of Imipenem the β-lactam carbonyl is positioned between the two zinc atoms (Fig. SI_8), whereas for **FC4** coordination to the di-zinc atoms is predicted to occur via the C-4 carboxylic acid of the cephalosporin-derived thiazine ring and the β-lactam carbonyl (Fig. SI_9). Both Imipenem and **FC4** are predicted to coordinate to K161 through their C-3/C-4 carboxylic acids located on the thiazolidine and thiazine rings, respectively. This mode of binding, unlike having the C-4 carboxylate positioned between the two zincs, is the predicted mode of productive substrate binding to MBLs.[12]

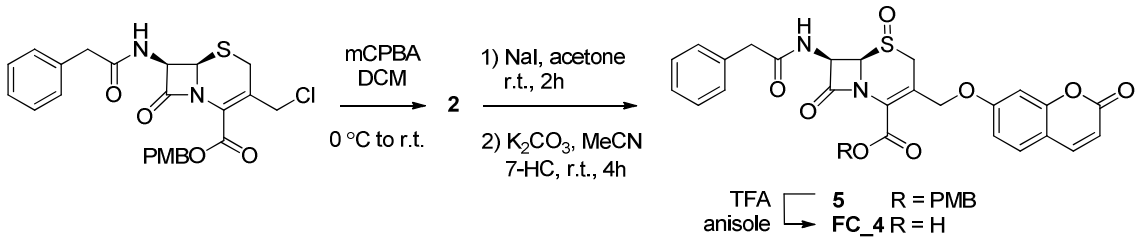
Supplementary Schemes, Figures, Tables and Graphs



Scheme SI_1. Synthesis of chromogenic substrate CENTA.



Scheme SI_2. Synthesis of functionalized chloroisoquinolinols.



Scheme SI_3. Alternative synthesis of **FC4**. To avoid Δ_2-Δ_3-isomerisation we investigated an one pot, two step procedure, using sulfoxide **2** as starting material, in order to obtain PMB-protected substrate **5**. This method, however, gave only very low yields (<10%) of the desired product **5** in several attempts using either K_2CO_3 or $CsCO_3$ as a base. Subsequent acid-mediated deprotection gave fluorogenic substrate **FC4** (70%).

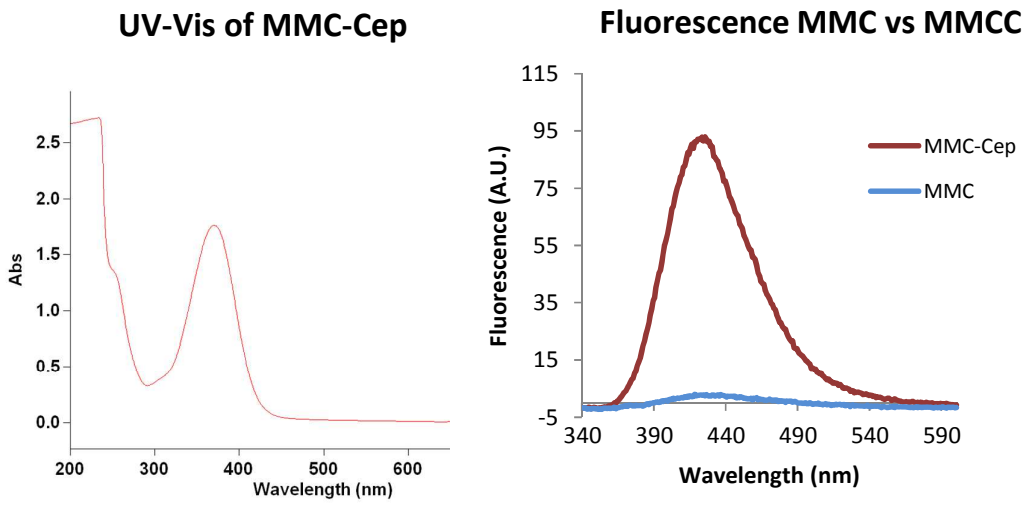


Figure SI_1. Spectroscopic data on MMC-Cep; Left) UV-Vis spectrum; Right) Fluorescence spectra of both MMC-Cep (**FC2**) and free MMC (7-mercapto-4-methyl coumarin).

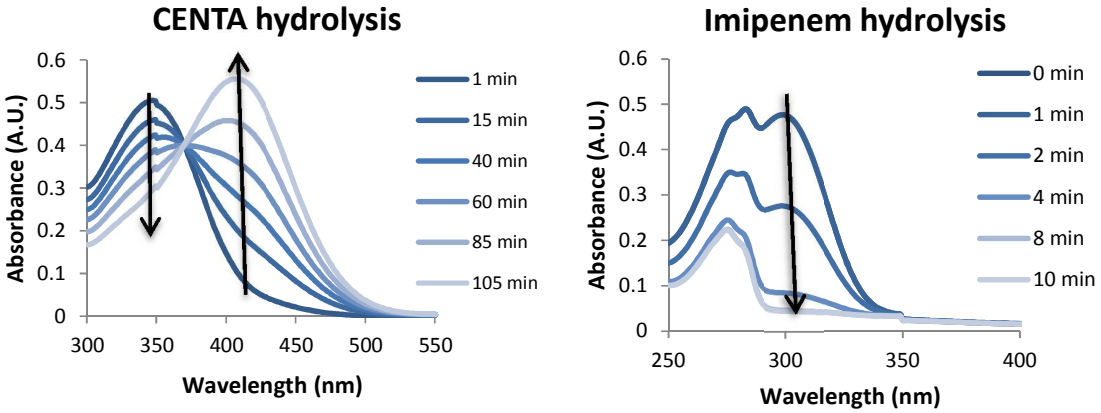


Figure SI_2. Absorption spectra over time for the hydrolysis of CENTA (left) and Imipenem (right). Substrate hydrolysis (50 µM) by NDM-1 in 50 mM HEPES (pH = 7.4). CENTA showed a distinctive increase in absorbance at 420 nm over a course of 105 minutes, while Imipenem gives a clear decrease in absorbance at 305 nm within 10 minutes.

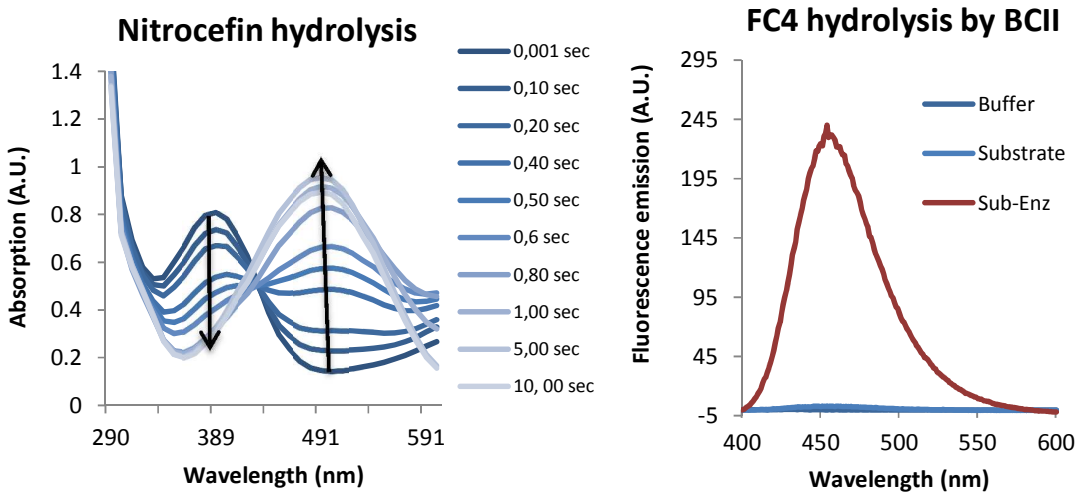


Figure SI_3. Absorption spectra over time for the hydrolysis of Nitrocefin (left) and the fluorescence spectra of the hydrolysis product of **FC4** (i.e. umbelliferone) (right). Substrate hydrolysis (50 µM) by NDM-1 in 50 mM HEPES (pH = 7.4). Nitrocefin showed a distinctive decrease in absorbance at 492 nm over a course of 10 seconds, while **FC4** showed a direct, clear increase of fluorescence at 460 nm upon addition of BcII in 50 mM HEPES (pH = 7.4).

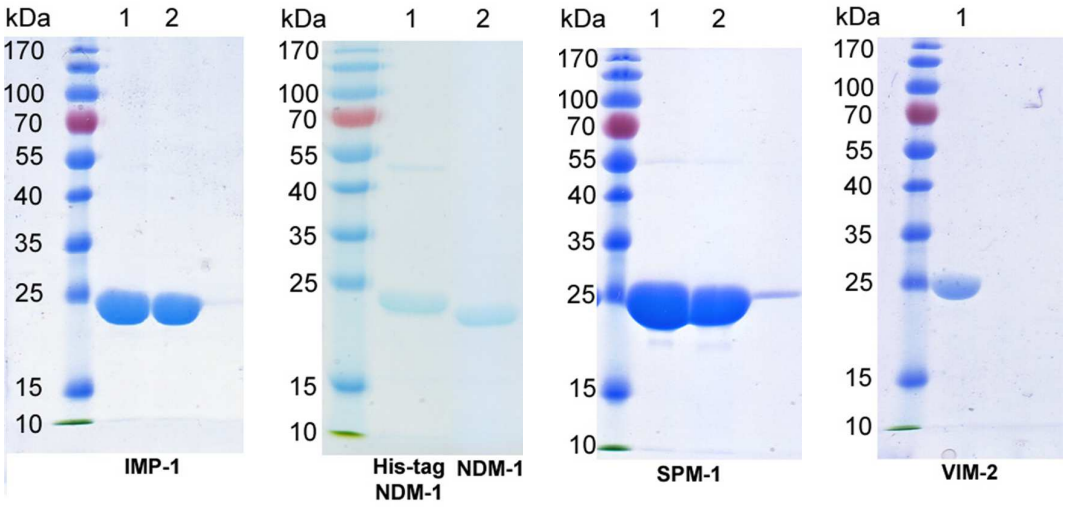


Figure SI_4. Polyacrylamide gels (12,5%); A) SDS-PAGE gel of purified IMP-1 (lanes 1 and 2); B) SDS-PAGE gel of purified NDM-1 (lane 1 His-tagged NDM-1, lane 2 NDM-1 after His-tag cleavage); C) SDS-PAGE gel of purified SPM-1 (lanes 1-3); D) SDS-PAGE gel of purified VIM-2.

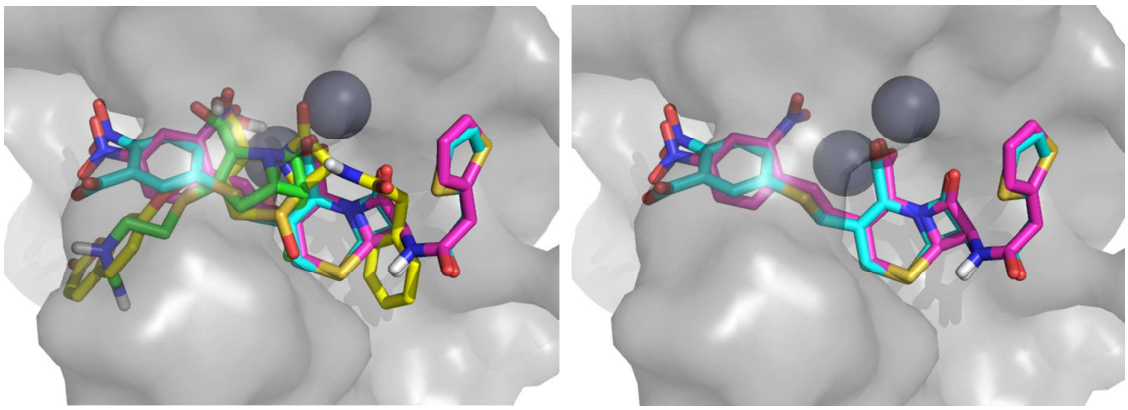


Figure SI_5. A) Overlay of Nitrocefin (pink), Imipenem (green), CENTA (blue) and **FC4** (yellow) structures, modelled in the active site of IMP-1 (PDB-ID: 1JJT); B) Overlay of Nitrocefin (pink) and CENTA (blue).

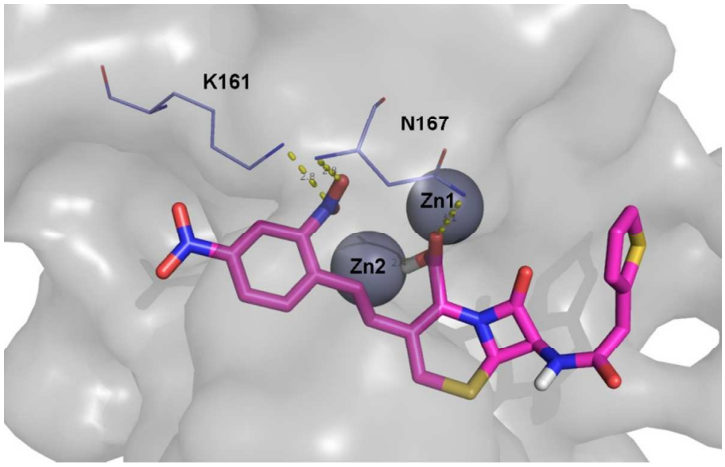


Figure SI_6. 3D spatial binding representation of nitrocefin modelled in IMP-1 (PDB-ID: 1JJT).

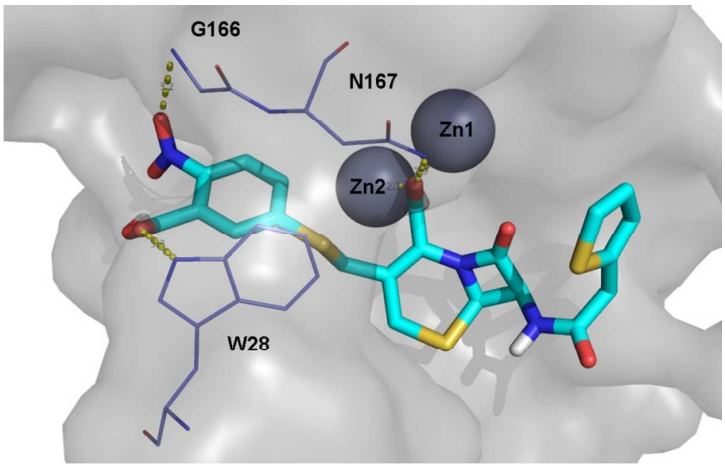


Figure SI_7. 3D spatial binding representation of CENTA modelled in IMP-1 (PDB-ID 1JJT).

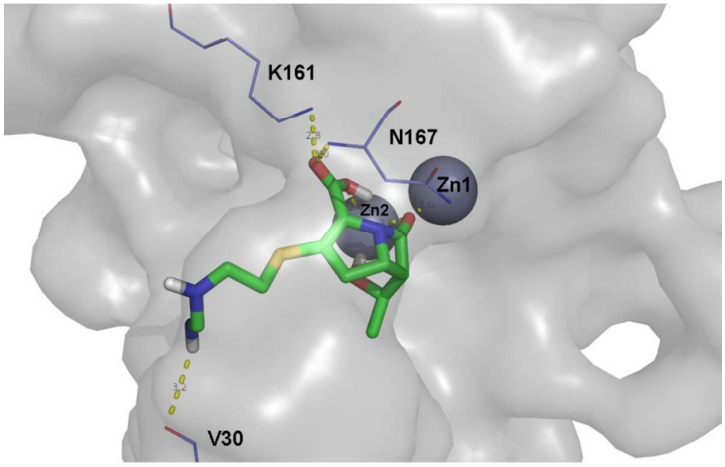


Figure SI_8. 3D spatial binding representation of Imipenem modelled in IMP-1 (PDB-ID: 1JJT).

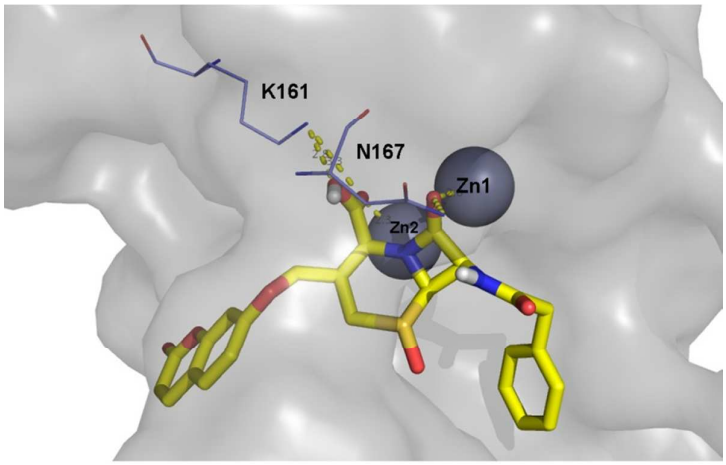


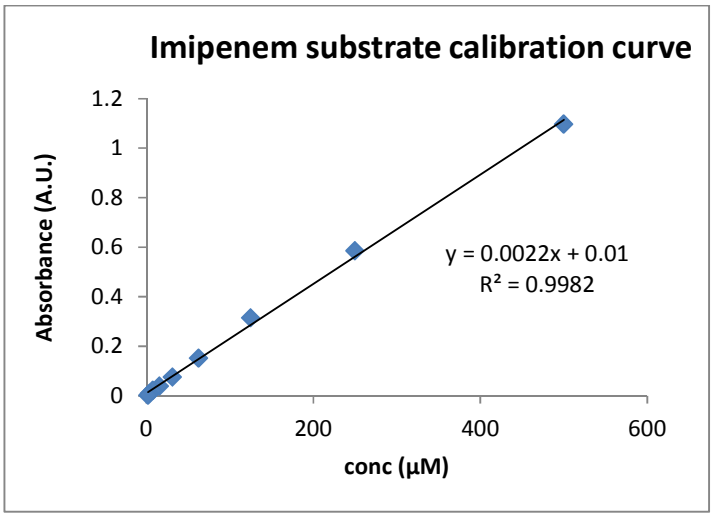
Figure SI_9. 3D spatial binding representation of **FC4** modelled in IMP-1 (PDB-ID: 1JJT).

Table SI_1. Docking scores for different substrates.

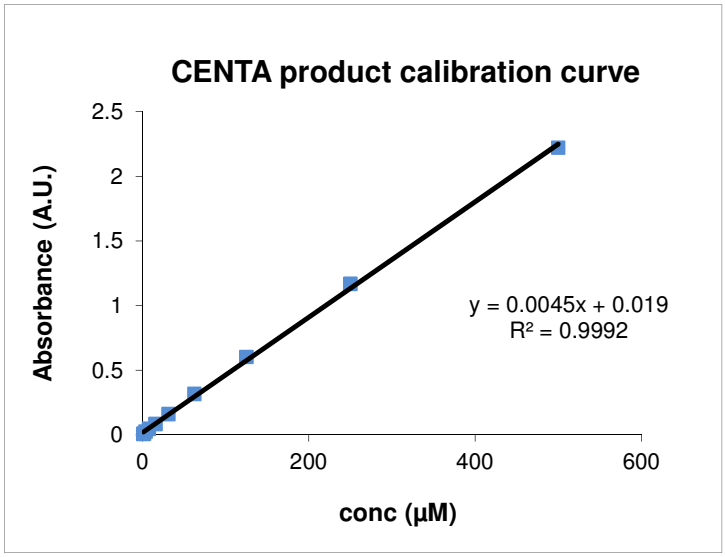
Enzyme	Substrate	Normalized AutoDock Score	Normalized SPROUT Score[a]	K_M-values (μM)
IMP-1	CENTA	1.9	1.20	17.1
	Imipenem	1.25	1.23	42.7
	Nitrocefin	1.83	1.30	55.7
	FC4	1.92	1.35	15.2
NDM-1	Imipenem	1.03	0.95	111.2
	CENTA	1.18	1.01	34.6
	Nitrocefin	1.12	1.15	8.8
	FC4	1.33	1.34	4.0
VIM-2	Imipenem	1.23	0.76	37.8
	CENTA	1.15	0.81	26.1
	Nitrocefin	1.00	1.00	7.2
	FC4	1.33	1.14	6.3

[a] Note, higher normalised score equates to tighter predicted binding. Scores are ranked based on the normalized SPROUT scores going from the lowest score to the highest score.

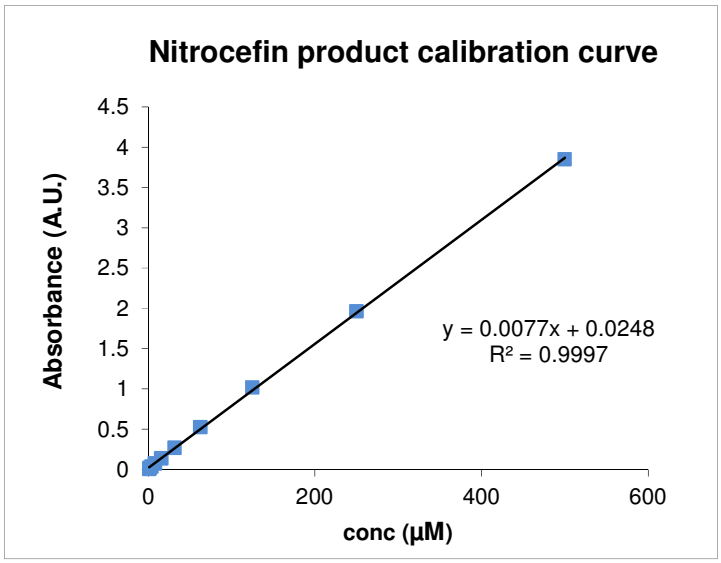
The *in silico* SPROUT docking scores generally show a better correlation with the *in vitro* obtained kinetic parameters (specifically K_M values) compared to the docking scores obtained by Autodock 4. For all three enzymes the highest ranking ligand in terms of SPROUT and Autodock score is **FC4** which was also measured to have the highest binding affinity for all three enzymes. The broad correlation found, may reflect, in part, different Zn-binding binding modes as well as variation in the orientation of the side-chains of imipenem, nitrocefin, CENTA and **FC4** (see Figures SI_5-9).



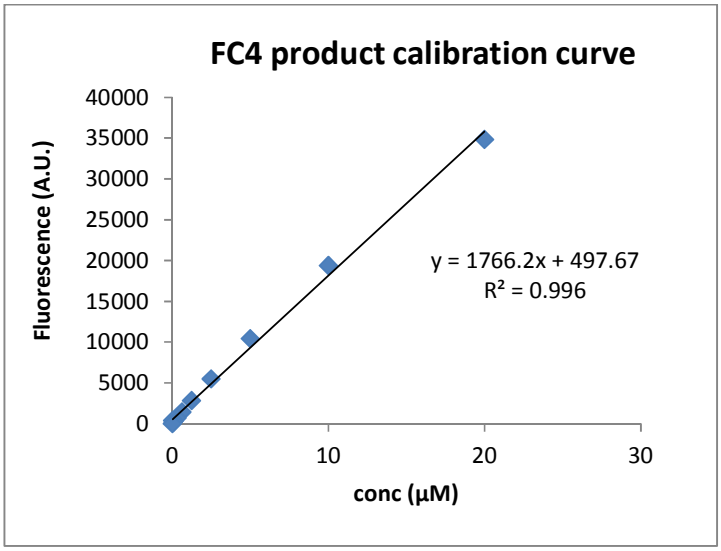
Graph SI_1. Calibration curve for the hydrolysis product of Imipenem (1.95 to 500 µM), Greiner UV Star 96 well plate.



Graph SI_2. Calibration curve for the hydrolysis product of CENTA (1.95 to 500 µM) using Greiner UV Star 96 well plate.



Graph SI_3. Nitrocefin calibration curve 0.98 to 500 µM, normal 96 well plate.



Graph SI_4. Calibration curve for the hydrolysis product of **FC4**.

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