SUPPLEMENTARY INFORMATION FOR:-

SAR studies leading to the identification of a novel series of metallo-β-lactamase inhibitors for the treatment of carbapenem-resistant Enterobacteriaceae infections that display efficacy in an animal infection model

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22 Pages; 2 Figures; 3 Tables

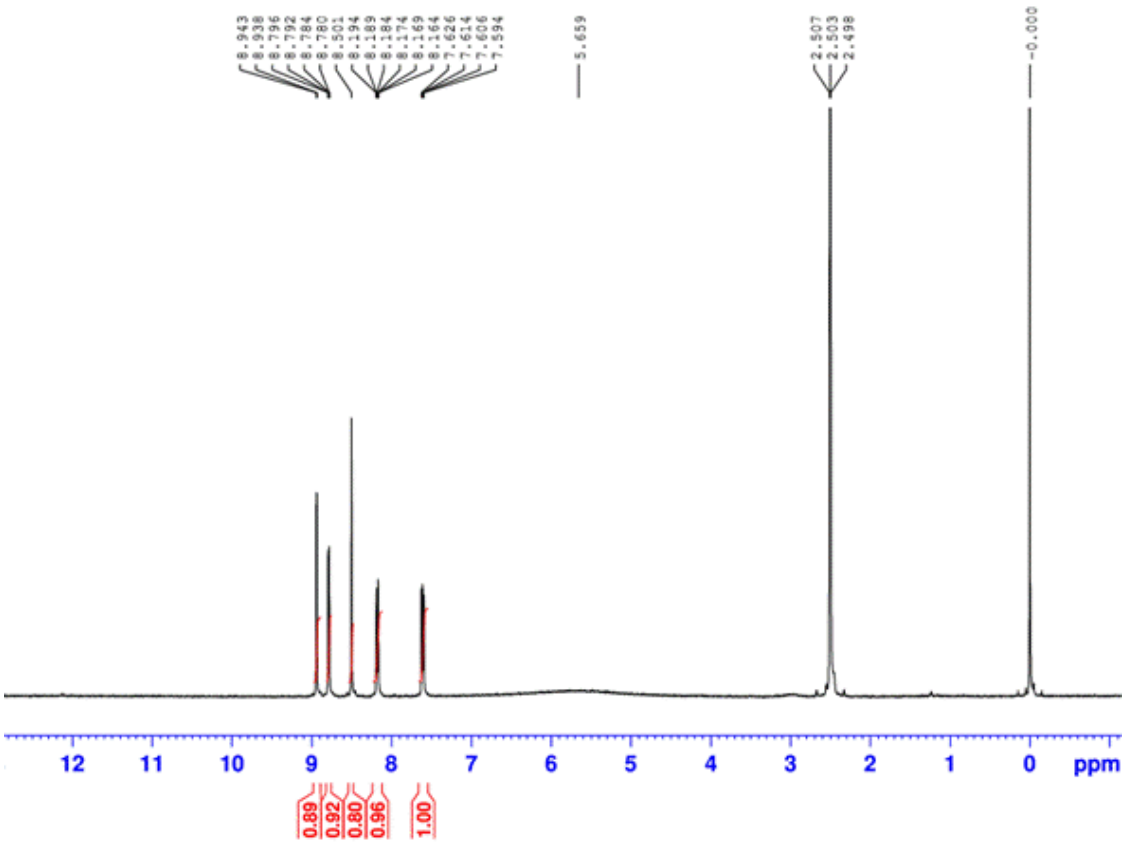


Figure S1 H[1] NMR spectrum of 5-(pyridine-3-sulfonamido)-1, 3-thiazole-4-carboxylic acid (76), (ANT431)

3-[2-(2,6-Dichlorophenyl)acetamido]pyridine-2-carboxylic acid (22)

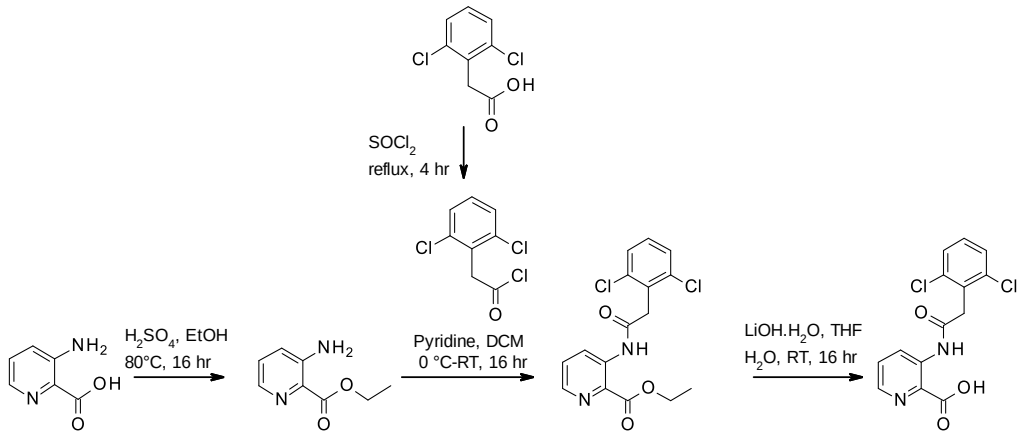


Figure S2

(a) **Ethyl 3-aminopyridine-2-carboxylate**

This preparation is based on the procedure of Carpino, L.A. *et al*., (Journal of Organic Chemistry, 2004, **69**, 54-61) but the compound is also commercially available. A solution of 3-aminopyridine-2-carboxylic acid (5 g, 36.2 mmol) in ethanol (25 mL) was treated dropwise with sulfuric acid (5.8 mL, 108.6 mmol) at 0 °C. The resulting reaction mixture was stirred at 80 °C for 16 hours then the solvent evaporated under reduced pressure. The crude material was dissolved in ethyl acetate (100 mL), washed with aqueous $NaHCO_3$ solution (60 mL), water and brine solution. The organic layer was dried over Na_2SO_4, filtered and concentrated to give crude compound which was triturated with n-pentane affording a light brown solid (2.6g, 43%).

^{1}H NMR (400 MHz, CDCl3): δ 8.10 (1H, dd), 7.20 (1H, dd), 7.00 (1H, dd), 5.80 (2H, s), 4.40 (2H, q), 1.40 (3H, t).

(b) **3-[2-(2,6-Dichlorophenyl)acetamido]pyridine-2-carboxylic acid**

A solution 2-(2,6-dichlorophenyl)acetic acid (185 mg, 0.90 mmol) in $SOCl_2$ (3 mL) was heated to reflux for 4 hours under a nitrogen atmosphere. The reaction mixture was concentrated under reduced pressure. The crude 2-(2,6-dichlorophenyl)acetyl chloride was azeotroped with toluene to remove any traces of thionyl chloride affording an oil (0.2g). This acid chloride formation is based on the procedure of Ruechardt, C. et al., (Chem. Ber., 1975, **108**, 2448-2464).

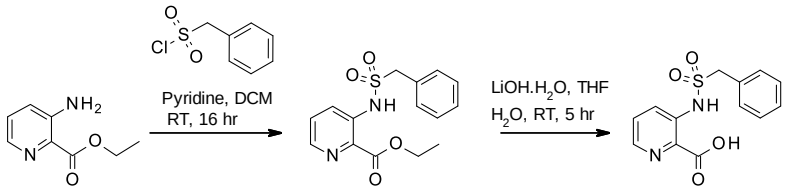
A solution ethyl 3-aminopyridine-2-carboxylate (150 mg, 0.90 mmol) in dichloromethane was treated with pyridine (0.4 mL, 4.5 mmol) a under a nitrogen atmosphere. Then 2-(2,6-dichlorophenyl)acetyl chloride (0.2g, 0.90 mmol) was added to the reaction mixture at 0 °C. The resulting reaction mixture was stirred at room temperature for 16 hours then evaporated under reduced pressure. The residue was dissolved in 10% methanol/DCM (10 mL) and washed with saturated sodium bicarbonate solution (10 mL), water (10 mL) and brine solution (6 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated to give a pale brown solid (320 mg, spectroscopic data consistent with ethyl 3-[2-(2,6-dichlorophenyl)acetamido]pyridine-2-carboxylate). This was dissolved in THF/ water (3:1, 4 mL) and treated with $LiOH.H_2O$ (56.9 mg, 1.35 mmol). The resulting mixture was stirred for 16 hours then concentrated under reduced pressure. The crude product was dissolved in water (4 mL) and acidified with 1N HCl to pH ~ 4.0. The precipitated solid was filtered and washed with diethyl ether (2 x 5 mL) and dried under high vacuum affording a white solid (50 mg, 17% over 2 steps).

^{1}H NMR (400 MHz, DMSO-d_6): δ 11.47 (br s, 1 H), 8.85 (dd, *J* = 8.4 Hz, *J* = 0.8 Hz, 1 H), 8.37 (d, *J* = 4.4 Hz, 1 H), 8.67 (dd, *J* = 8.4 Hz, *J* = 4.4 Hz, 1 H), 7.56-7.52 (m, 2 H), 7.42-7.37 (m, 1 H), 4.13 (s, 2 H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.5, 167.3, 142.2, 137.3, 135.6, 134.2, 130.8, 130.0, 129.1, 128.4, 127.9, 40.1-38.8 (obscured) ppm.

m/z: 325 $[M+H]^+$.

HRESIMS (High-resolution electrospray ionisation mass spectrometry): *m/z* 325.0146 $[M+H]^+$ (325.0147 calculated for $C_{14}H_{11}Cl_2N_2O_3$)

3-(Phenylmethanesulfonamido)pyridine-2-carboxylic acid (63)



(a) **Ethyl 3-(phenylmethanesulfonamido)pyridine-2-carboxylate**

To a stirred a solution of ethyl 3-aminopyridine-2-carboxylate (1 g, 6.0 mmol) in dichloromethane (10 mL) was added pyridine (2.4 mL, 30.0 mmol). After 10 min, benzylsulfonyl chloride (2.8 g, 15.0 mmol) was added. The mixture was stirred for 16 hours then diluted with DCM (20 mL) and washed with water (2 x 20 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated to give a solid. This was purified by column chromatography using 25% ethyl acetate in petroleum ether as the eluent affording an off-white solid (850 mg, 44%).

m/z: 321 $[M+H]^+$.

^{1}H NMR (400 MHz, CDCl3): δ 8.40 (dd, 1 H), 7.85 (dd, 1 H), 7.20-7.40 (m, 6 H), 4.40 (m, 4 H), 1.30 (t, 3H).

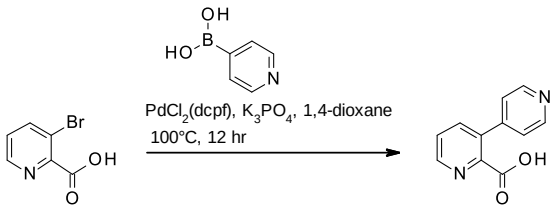
(b) **3-(Phenylmethanesulfonamido)pyridine-2-carboxylic acid**

To a stirred solution of ethyl 3-(phenylmethanesulfonamido)pyridine-2-carboxylate (850 mg, 2.65 mmol) in THF: water (1:1, 10 mL) was added $LiOH.H_2O$ (555 mg, 13.2 mmol). The resulting reaction mixture was stirred for 5 hours then the mixture was evaporated under reduced pressure and water (5 mL) was added to the residue. The aqueous solution was washed with Et_2O (2 x 5 mL) and then acidified to pH 4.0 using 1N HCl. The resulting precipitate was filtered, washed with diethyl ether (2 x 5 mL) and dried under high vacuum affording an off-white solid (420 mg, 54%).

m/z: 291.0 $[M-H]^-$

^{1}H NMR (500 MHz, DMSO-d_6): δ 11.34 (br s, 1 H), 8.33 (dd, *J* = 9.5 Hz, 1.5 Hz, 1 H), 7.89 (dd, *J* = 8.5 Hz, 1.0 Hz, 1 H), 7.59 (dd, *J* = 8.5 Hz, 4.5 Hz, 1 H), 7.32-7.27 (m, 5 H), 4.77 (s, 2 H).

[3,4'-Bipyridine]-2-carboxylic acid (12)



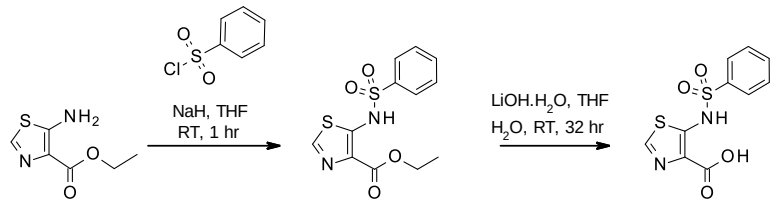
A solution of 3-bromopyridine-2-carboxylic acid (100 mg, 0.49 mmol) in 1,4-dioxane (4 mL) was purged with argon for 10 minutes. Then (pyridin-4-yl)boronic acid (73 mg, 0.59 mmol), K_3PO_4 (315 mg, 1.48 mmol) and [1,1′-bis(di-cyclohexylphosphino) ferrocene] dichloropalladium (II) (18 mg, 0.02 mmol) were added under an argon atmosphere. The resulting reaction mixture was heated to 100°C for 12 hours then allowed to cool to room

temperature, filtered through a celite pad washing with with 10% methanol in dichloromethane. The combined extracts were concentrated under reduced pressure to get a brown solid. This was purified using a mass-directed auto purification system on an Agilent 1260 infinity machine with an XSelect CHS Prep C18 column, eluting with 0.1% formic acid in water/acetonitrile (detection with a Quadruploe LC/MS) affording the title compound as an off-white solid (100 mg, 27%).

m/z: 201.0 $[M+H]^+$

^{1}H NMR (400 MHz, DMSO-d_6): δ 13.59 (br s, 1 H), 9.20 (d, *J* = 1.6 Hz, 1 H), 8.75 (d, *J* = 4.8 Hz, 2 H), 8.41 (dd, *J* = 8.0 Hz, *J* = 1.6 Hz, 1 H), 8.26 (d, *J* = 8.0 Hz, 1 H), 8.12 (d, *J* = 4.8 Hz, 2 H).

5-(Phenylsulfonamido)-1,3-thiazole-4-carboxylic acid (62)



(a) **Ethyl 5-(phenylsulfonamido)-1,3-thiazole-4-carboxylate**

To a stirred suspension of NaH (348 mg, 8.71 mmol) in THF (5 mL) was added ethyl 5-amino-1,3-thiazole-4-carboxylate (500 mg, 2.90 mmol) in THF (5 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min. Then, phenylsulfonyl chloride (615 mg, 3.48 mmol) in THF (5 mL) was added dropwise to the reaction mixture. The resulting reaction mixture was stirred for 1 hour then quenched with saturated NH_4Cl solution (10 mL) and extracted with EtOAc (2x15 mL). The combined organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated

to afford crude product. This was purified by Combi-flash column chromatography (12 g silica cartridge) using 30% EtOAc/ pet ether as an eluent to afford ethyl 5-(phenylsulfonamido)-1,3-thiazole-4-carboxylate as a yellow solid (240 mg, 26%).

^{1}H NMR (400 MHz, CDCl3): δ 10.15 (1H, bs), 8.25 (1H, s), 7.90 (2H, m), 7.60 (1H, m), 7.50 (2H, d), 4.40 (2H, q), 1.40 (3H, t).

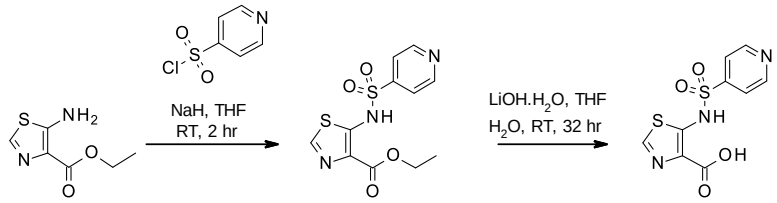
m/z: 313.1 $[M+H]^+$

(b) **5-(Phenylsulfonamido)-1,3-thiazole-4-carboxylic acid**

To a stirred solution of ethyl 5-(phenylsulfonamido)-1,3-thiazole-4-carboxylate (240 mg, 0.76 mmol) in THF (3 mL): water (1 mL) was added LiOH.H_2O (322 mg, 7.68 mmol). The resulting reaction mixture was stirred for 32 hours. then concentrated under reduced pressure. The residue was dissolved in water (4 mL) and washed with diethyl ether (2x3 mL). The aqueous phase was acidified with 1N HCl to pH ~4.0. The resulting precipitate was filtered, washed with diethyl ether (2x3 mL) and dried under high vacuum affording the title compound as a white solid (81 mg, 37%).

m/z: 285.2 $[M+H]^+$

^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.55 (s, 1 H), 7.83 (dd, *J* = 7.2 Hz, *J* = 0.8 Hz, 2 H), 7.70-7.65 (m, 1 H), 7.58 (dd, *J* = 8.0 Hz, *J* = 7.2 Hz, 2 H) .5-(Pyridine-4-sulfonamido)-1,3-thiazole-4-carboxylic acid (77)

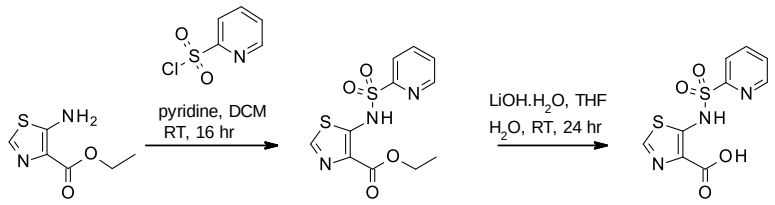


To a stirred suspension of NaH (693 mg, 29.0 mmol) in THF (5 mL) was added ethyl 5-amino-1,3-thiazole-4-carboxylate (1 g, 5.80 mmol) in THF (10 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min. Then, pyridine-4-sulfonyl chloride (2.06 g, 11.6 mmol) in THF (5mL) was added drop wise. The resulting mixture was stirred for 2 hours at room temperature then quenched with 1 N citric acid solution at 0 °C and extracted with EtOAc (2x50 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated to a solid. The crude product was purified by silica gel column chromatography eluting with 70% EtOAc/ petroleum ether an off-white solid (500 mg, 28%), LCMS analysis of which showed an approximate 2:1 mixture of ethyl 5-(pyridine-4-sulfonamido)-1,3-thiazole-4-carboxylate: ethyl 5-amino-1,3-thiazole-4-carboxylate. This material was dissolved in THF (15 mL): water (15 mL) and treated with $LiOH.H_2O$ (321 mg, 7.65 mmol). The resulting reaction mixture was stirred for 16 hours then concentrated under reduced pressure. The residue was dissolved in water and acidified to pH ~4.0 with 1N HCl. The precipitated solid was filtered and dried under high vacuum (260 mg). This was purified by preparative HPLC affording the title compound as a yellow solid (33 mg).

m/z: 285.9 $[M+H]^+$

^{1}H NMR (DMSO-d_6, 500 MHz) δ: 8.76 (dd, J = 4.5 Hz, J = 1.5 Hz, 2 H), 8.28 (s, 1 H), 7.69 (dd, J = 4.5 Hz, J = 1.5 Hz, 2 H).

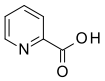
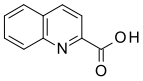
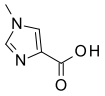
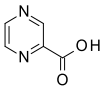
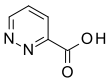
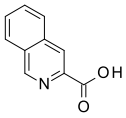
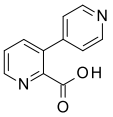
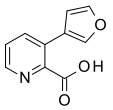
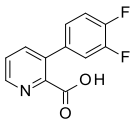
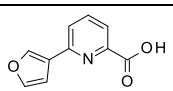
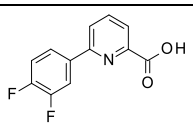
5-(Pyridine-2-sulfonamido)-1,3-thiazole-4-carboxylic acid (75)

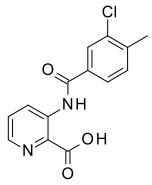
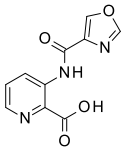
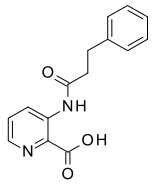
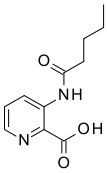
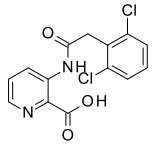
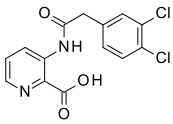
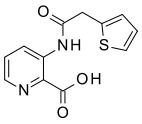
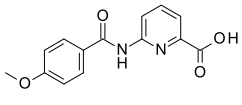
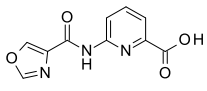
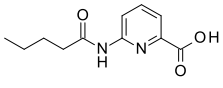


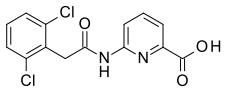
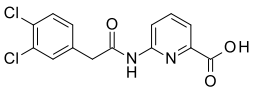
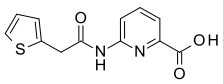
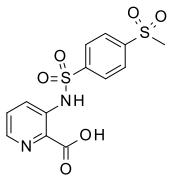
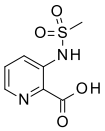
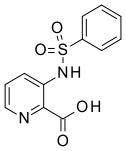
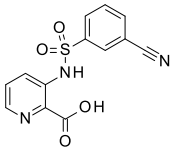
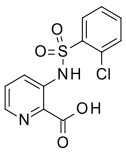
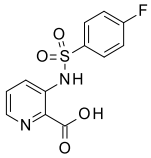
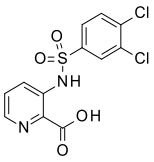
To a stirred solution of ethyl 5-amino-1,3-thiazole-4-carboxylate (150 mg, 0.87 mmol) in DCM (5 mL) under N_2 atmosphere was added pyridine (0.2 mL, 2.61 mmol). After 10 minutes, pyridine-2-sulfonyl chloride (185 mg, 1.04 mmol) was added at. The resulting reaction mixture was stirred for 16 hours then was diluted with DCM (20 mL), washed with water (15 mL) and 1N HCl solution (10 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated to obtain a brown solid (65 mg). This was dissolved in THF: H_2O (3:1 mL) and treated with $LiOH.H_2O$ (24 mg, 0.57 mmol). The resulting reaction mixture was stirred for 24 hours then concentrated under reduced pressure. The crude residue was partitioned between water (5 mL) and EtOAc (5 mL). The aqueous layer was acidified with 1N HCl solution to pH ~4. The resulting precipitate was filtered, washed with diethyl ether (2x3 mL) and dried under high vacuum affording a pale yellow solid (19 mg, 34%).

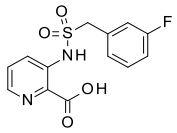
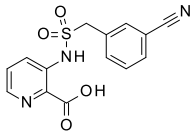
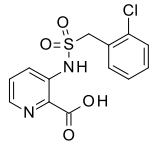
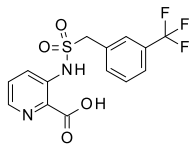
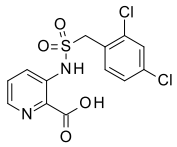
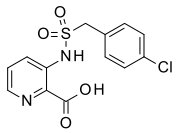
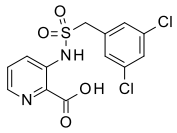
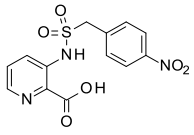
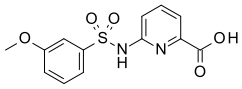
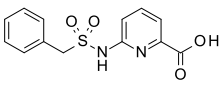
m/z: 283.9 $[M-H]^-$

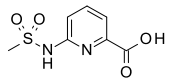
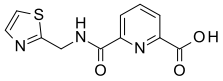
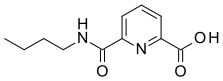
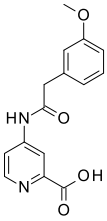
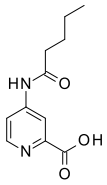
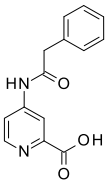
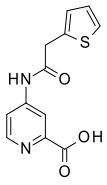
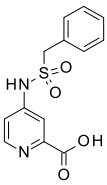
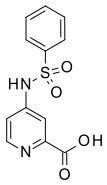
^{1}H NMR (300 MHz, DMSO-d_6): δ 8.67 (dd, *J* = 4.8 Hz, *J* = 0.9 Hz, 1 H), 8.49 (s, 1 H), 8.06 (dt, *J* = 7.8 Hz, *J* = 1.5 Hz, 1 H), 7.98 (dd, *J* = 7.8 Hz, *J* = 0.9 Hz, 1 H), 7.67-7.63 (m, 1 H).

Compound number	Structure	IC_{50} NDM-1/µM; (MIC vs NTBC121*)	IC_{50} VIM-1/µM; (MIC vs NTBC055**)	IC_{50} IMP-1/µM (MIC vs NTBC062***)
1		17.1	> 200	> 200
7		40.9	> 200	> 200
8		> 200	> 200	> 200
9		> 200	> 200	> 200
10		> 200	> 200	> 200
11		19.4	> 200	> 200
12		> 200	> 200	> 200
13		> 200	> 200	> 200
14		> 200	> 200	> 200
15		162.6	> 200	> 200
16		> 200	> 200	> 200

17		8.46	95.7	87.6
18		> 200	> 200	> 200
19		20.3	> 200	34.1
20		11.1	> 200	156.6
21		8.99	> 200	> 200
22		21.1	> 200	> 200
23		6.56	133	146
24		20.3	> 200	156
25		55.9	> 200	> 200
26		19.4	> 200	> 200

27		> 200	> 200	80
28		3.56	> 200	82.2
29		> 200	> 200	> 200
30		69.9	> 200	> 200
31		68.8	> 200	> 200
32		49.9 (16)	> 200 (4)	> 200 (2)
33		147.5	> 200	> 200
34		> 200	> 200	> 200
35		52.1	> 200	> 200
37		25.2	> 200	195.5

36		> 200	107.9	151.7
37		116.5 (32)	> 200 (2)	150.1 (1)
39		32.6 (16)	10.9 (0.5)	20.9 (1)
40		17.9 (16)	71.6 (2)	> 200 (1)
41		80.6 (16)	17.5 (1)	74.3 (1)
42		18.4	88.4	146.3
43		18.6 (8)	57.2 (1)	180 (1)
44		119.5	> 200	> 200
45		43.3	> 200	> 200
46		43.7	> 200	> 200

47		32.8	> 200	> 200
48		> 200	> 200	> 200
49		> 200	> 200	> 200
50		35.3	> 200	> 200
51		11.5	> 200	> 200
52		11.6	> 200	> 200
53		43	> 200	> 200
54		65	> 200	> 200
55		145.4	> 200	> 200

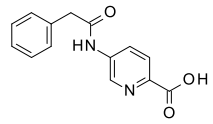
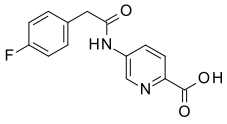
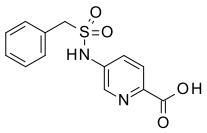
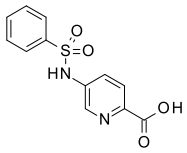
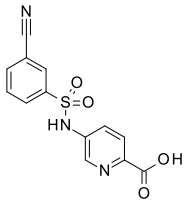
56		21.4	> 200	> 200
57		27.9	> 200	> 200
58		3.99	> 200	> 200
59		2.17	109.1	113.8
60		7.2	> 200	162.4

Table S1. Array of pyridine-2-carboxylate analogues

* NDM-1 producing strain; E. coli NTBC121; MIC meropenem 32 ug/mL (no inhibitor)

** VIM-1 producing strain; K. pneumo NTBC055; MIC meropenem 16 ug/mL (no inhibitor)

*** IMP-1 producing strain; K. pneumo NTBC062; MIC meropenem 4 ug/mL (no inhibitor)

The inhibitor was present at 100 µM concentration, the meropenem MICs are reported in ug/mL.

Enzyme tested / ANT431	IC50 (µM)	SD (µM)	N test
NDM-1	2.67	0.24	3
VIM-1	>200	ND	2
VIM-2	6.7	1.8	4
IMP-1	54	8.2	4

Table S2 Enzyme inhibition data for ANT431

X-ray crystallography

The purified VIM-2 enzyme (4.5 mg/ml) was crystallized in 24-well sitting-drop plates (Cryschem plate; Hampton Research) using 0.1 M cacodilate (pH 6.5), 5 mM DTT , 0.2 M Na-acetate, 26% PEG 8000 as the precipitant solution (drop volume, 4 µl; reservoir volume 800 µl).[1,2] Crystals of VIM-2 were subsequently soaked for 30 to 60 min with solutions of test compounds (inhibitors were resuspended in a DMSO/PEG-800 mixture, 1:9 vol/vol; final inhibitor concentration in the drop, 0.5 to 2 mM) prior to flash freezing in liquid nitrogen. Diffraction data were collected at both the European Synchrotron Radiation Facility (ESRF, Grenoble, France) and at the Diamond Light Source (Didcot, UK). Data were processed using MOSFLM[3] and scaled with SCALA[4] or AIMLESS from the CCP4 suite[5]. The structure of the VIM-2 complexes was obtained by molecular replacement using the program MOLREP[6] and PDB entry 1KO3[2] (waters omitted) as the search model. Isotropic refinement of the structures was carried out with the program REFMAC5[7] from the CCP4 suite. In between refinement cycles, the model was subjected to manual rebuilding using Coot[8]. Water molecules were added using the standard procedure within the ARP/wARP suite[9]. The stereochemical quality of the

refined model was assessed using the program PROCHECK[10] and Coot. The ligand placement was checked in the final models through the calculation of the omit maps and validated during the deposition in the protein data bank. Figures were prepared using the PyMOL software (http://www.pymol.org). Data collection, data reduction and structure refinement statistics are reported in Table SI1.

TABLE S3. Data collection, refinement and ligand validation statistics.

Parameter	VIM-2:32	VIM-2:62	VIM-2:76
X-ray source	DLS I04	DLS I04	DLS I04-1
Wavelength (Å)	0.97949	0.97949	0.91587
Data collection temp (K)	100	100	100
Space group	I222	I222	I222
Cell dimensions (Å)	a = 67.91, b = 77.90, c = 79.45	a = 67.65, b = 78.38, c = 78.82	a = 67.29, b = 77.69, c = 79.32
subunits/asymmetric unit	1	1	1
Matthews coefficient ($Å^3$ Da^{-1})	2.06	2.06	2.10
Solvent content (%)	40.30	39.96	41.44
Resolution limits (Å)[a]	55.62 – 2.00 (2.08 – 2.00)	31.23 – 1.75 (1.78 – 1.75)	51.32 – 1.80 (1.90 – 1.80)
No. of reflections measured[a]	91,927 (7,464)	140,931 (6,974)	144,351 (21,129)
No. of unique reflections[a]	14,585 (1,175)	21,376 (1,160)	19,621 (2,800)
Completeness (%)[a]	100.0 (100.0)	99.6 (99.7)	100.0 (100.0)
R_{merge} (%)[a,b]	11.7 (64.7)	13.9 (67.0)	5.8 (45.1)
Multiplicity[a]	6.3 (6.4)	6.6 (6.0)	7.4 (7.5)

$I/\sigma(I)$[a]	9.8 (2.8)	7.5 (1.8)	16.2 (3.4)
Wilson B factor (Å^2)	16.985	16.280	24.600
R_{cryst} (%)[a,b]	18.1	20.0	18.9
R_{free} (%)[a,b]	22.7	25.0	23.4
Protein atoms	1768	1761	1731
Ligand atoms	19	18	18
Water molecules	76	118	114
Avg B factor (Å^2)	25.38	23.99	33.08
RMSD bond length (Å)	0.019	0.018	0.020
RMSD bond angle (°)	1.987	2.042	1.952

[a]Data in parentheses refer to results for the highest-resolution shell.

[b]$R_{merge} = \Sigma_h\Sigma_i|I_{i,h} - \bar{I}_h|/\Sigma_h\Sigma_i I_{i,h} \times 100$. $R_{cryst}(R_{free}) = \Sigma_h||F_{h,obs}| - |F_{h,calc}|/\Sigma_h||F_{h,obs}| \times 100$.

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(2) Garcia-Saez, I., Docquier, J.D., Rossolini, G.M. & Dideberg O. (2008) The three-dimensional structure of VIM-2, a Zn-β-lactamase from Pseudomonas aeruginosa in its reduced and oxidised form. *J. Mol. Biol. 375*, *604-611*.

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(4) Evans, P.R. (2006) Scaling and assessment of data quality. *Acta Crystallogr. D Biol Crystallogr. 62, 72–82*.

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