Studies on the Reactions of Biapenem with VIM metallo β-lactamases and the serine β-lactamase KPC-2

Supporting Information

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Position	^{13}C (ppm)	^{1}H (ppm)
1	43.97	2.56
2	57.33	3.68
3		
4		
5	72.8	4.35
6	55.92	2.6
7	180.18	
8	68.11	4.03
9	20.75	1.19
10	172.45	
11	13.49	1.01
12,14	53.66	4.57,5.04
13		
15,16	142.84	8.93

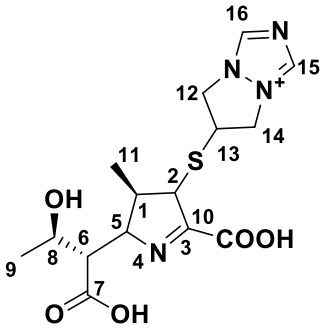

Table S1*. Chemical shift assignments for the major biapenem-derived hydrolysis product.* The chemical shifts and the C2 coupling constants (J 3.6, 1.3 Hz) of this hydrolysis product suggest that it is likely the (2*S*)-imine based on previous studies [8,9].

	VIM-2: biapenem
PDB accession	6Y6J
Data collection	
Facility/Beamline	Diamond I04-1
Space group	*I*222
Molecules/ASU	1
Cell dimensions	
a, b, c (Å)	64.324, 74.02, 78.354
α, β, γ (°)	90.0, 90.0, 90.0
Wavelength (λ)	0.9159
Resolution (Å)	27.61-1.33 (1.36-1.33)
R_{pim}	0.030 (0.205)
CC 1/2	0.998 (0.872)
I / σI	14.6 (3.0)
Completeness (%)	99.56 (98.96)
Multiplicity	13.3 (12.6)
Refinement	
Resolution (Å)	27.61-1.5
No. reflections	30179
R_{work} / R_{free}	0.1287 / 0.1573
No. non-H atoms	2048
Protein	1792
Solvent	200
Zn	3
Ligands	56
Avg. B-factors (Å^2)	
Protein	17.40
Solvent	31.03
Ligands	16.49
R.m.s. deviations	
Bond lengths (Å)	0.009
Bond angles (°)	1.15
Ramachandran (%)	
Outliers	0.00
Favoured	98.25

Table S2. Data collection and refinement statistics. High resolution statistics (1.36-1.33 Å) are in parentheses. Due to poor completeness, the resolution for data processing was cut of to 1.5 Å, with a high resolution shell of 1.556-1.5 Å.

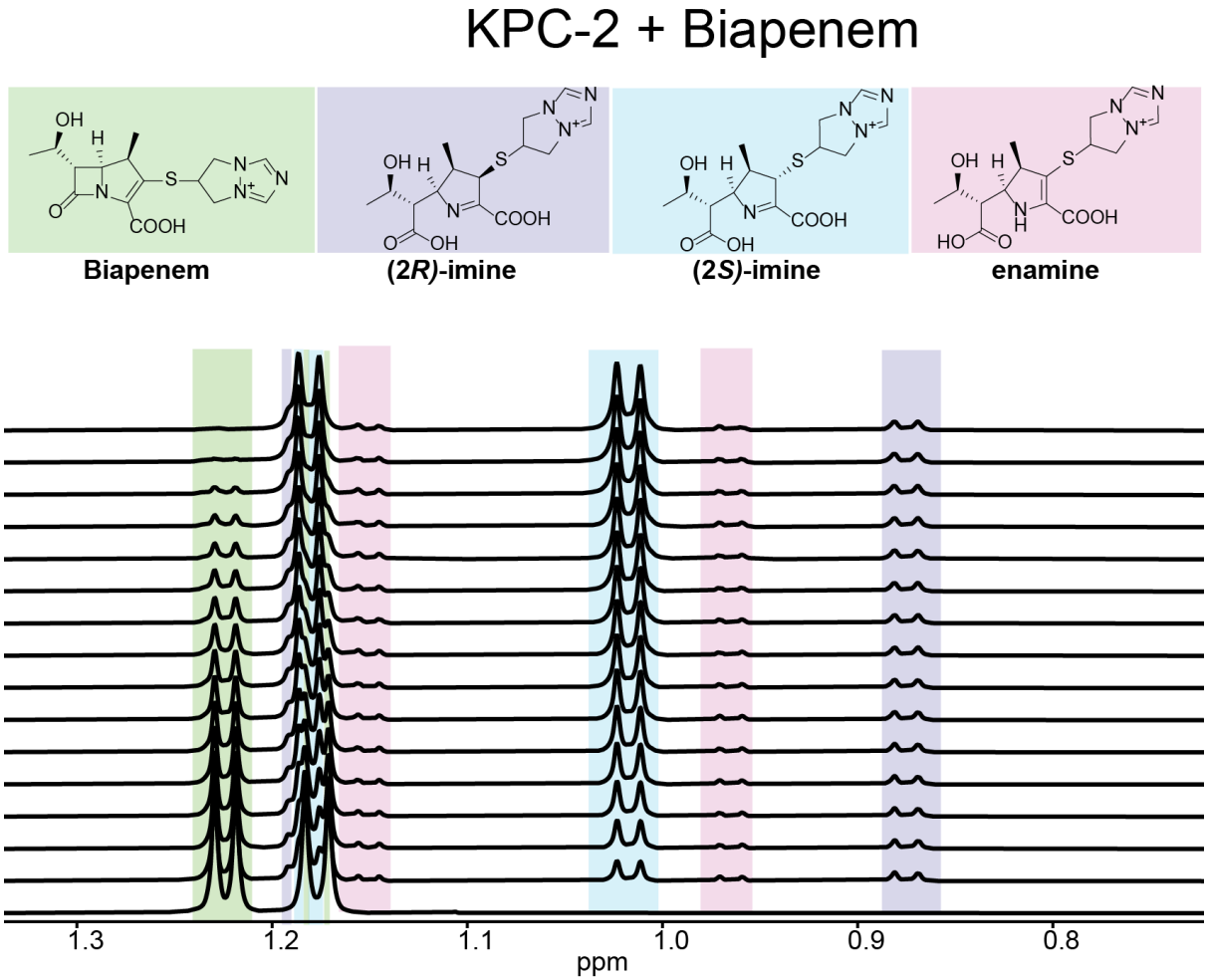

***Figure* S1. 1H NMR time course analysis of methyl group resonances of species formed on reaction of biapenem with KPC-2.** Biapenem (5 mM, green) was treated with KPC-2 (280 nM) in 50 mM sodium phosphate at pH 7.5, 10% (v/v) D_2O. Evidence was accrued for formation of (2*R*)-imine, (2*S*)-imine and enamine products (blue, purple, and pink, respectively). 15 spectra were acquired (16 scans, every 2.5 minutes) over 37.5 minutes (stacked from bottom / first spectrum to top). The bottom spectrum is of biapenem (5 mM) without added enzyme.

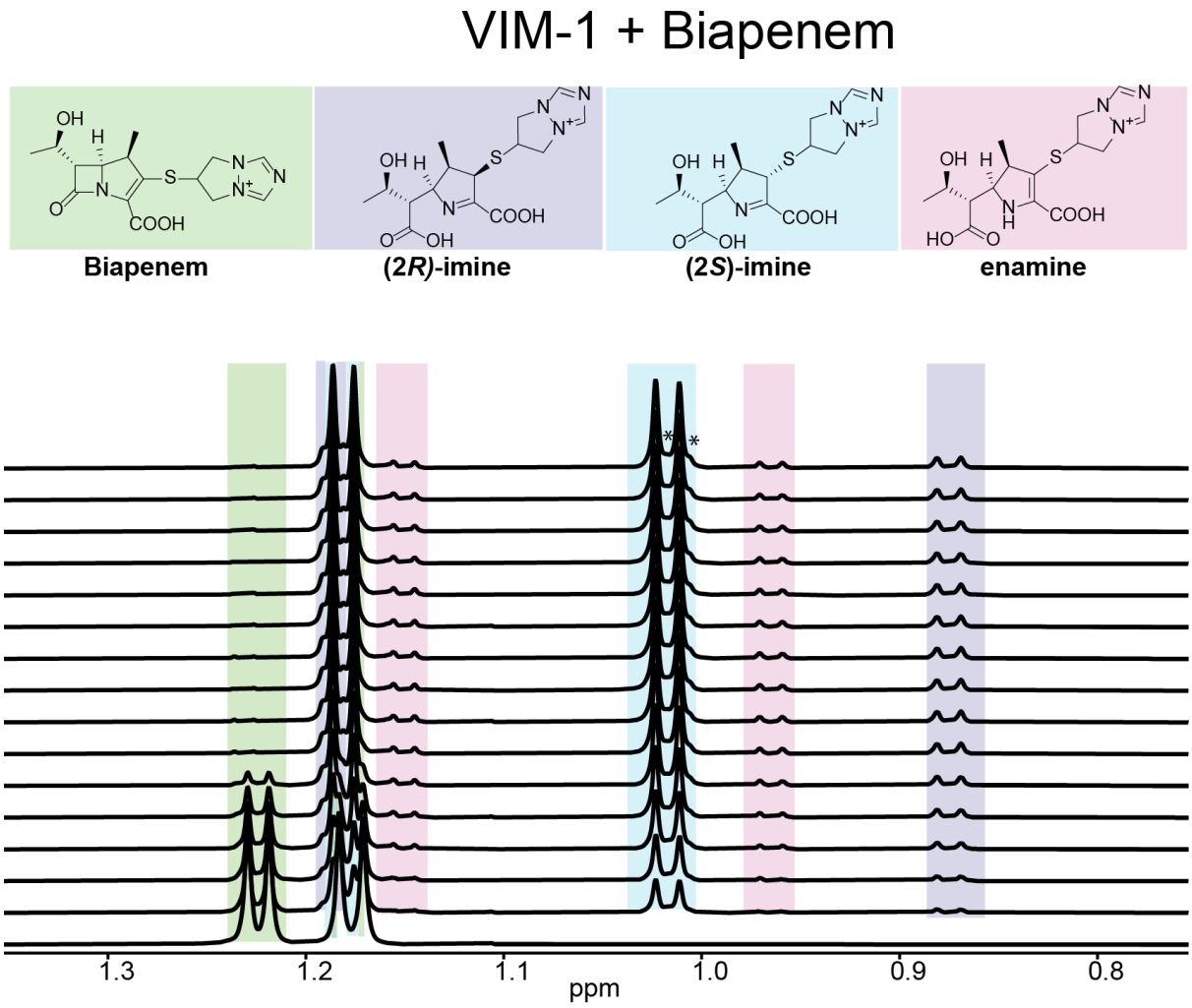

Figure S2. **^{1}H NMR time course analysis of methyl group resonances of species formed on reaction of biapenem with VIM-1**. Biapenem (5 mM, green) was treated with VIM-1 (280 nM) in 50 mM sodium phosphate at pH 7.5, 10% (v/v) D_2O. Unassigned product peaks are labelled with asterisks. Evidence was accrued for formation of (2*R*)-imine, (2*S*)-imine and enamine products (blue, purple, and pink, respectively). 15 spectra were acquired (16 scans, every 2.5 minutes) over 37.5 minutes (stacked from bottom / first spectrum to top). The bottom spectrum is biapenem (5 mM) without added enzyme.

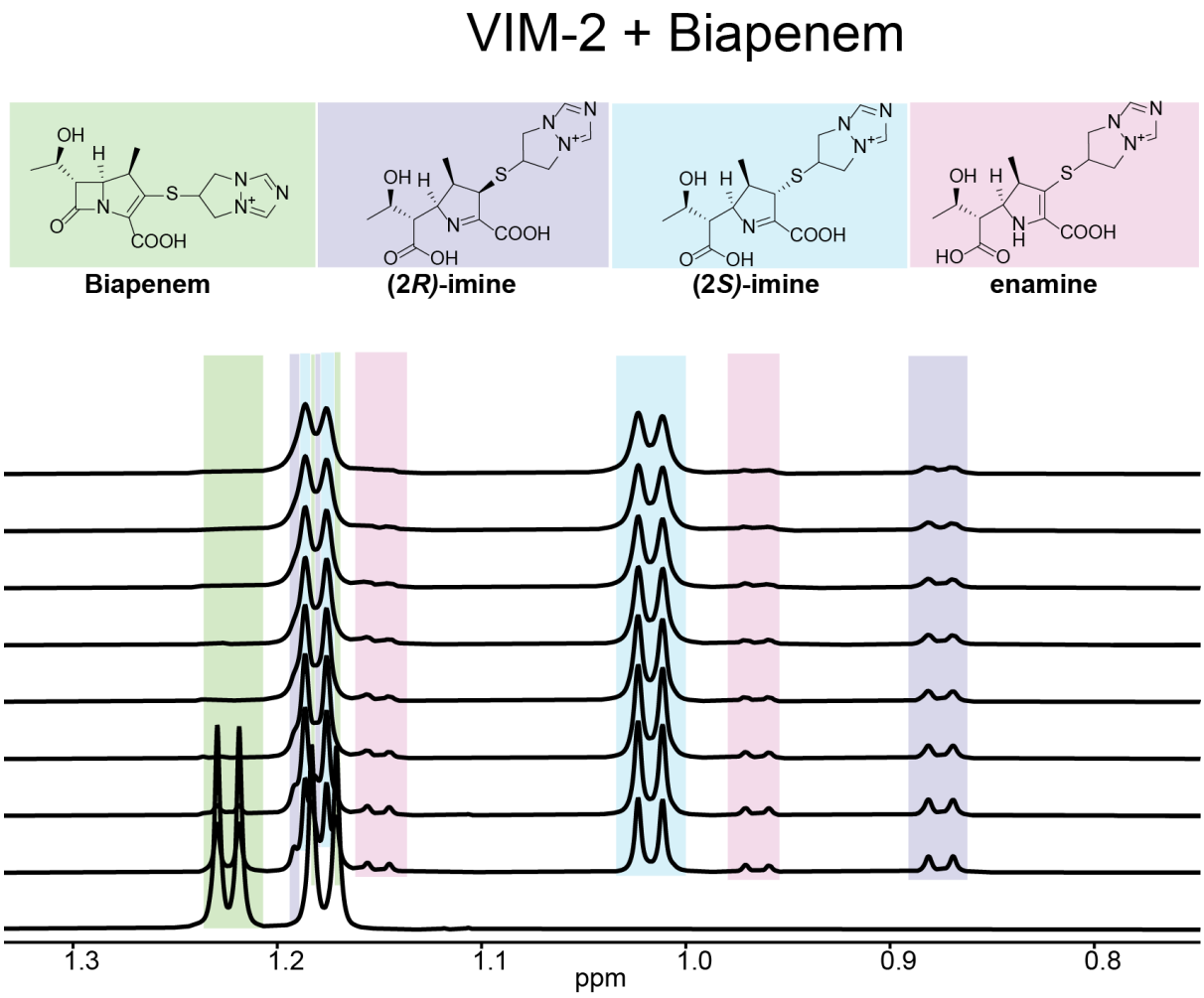

***Figure S3*. ^{1}H NMR time course analysis of methyl group resonances of products formed on reaction of biapenem with VIM-2**. Biapenem (5 mM, green) was treated with VIM-2 (280 nM) in 50 mM sodium phosphate at pH 7.5, 10% (v/v) D_2O. Evidence was accrued for formation of (2*R*)-imine and (2*S*)-imine and enamine products (blue, purple, and pink, respectively). 8 spectra were acquired (16 scans, every 2.5 minutes) over 20 minutes (stacked from bottom / first spectrum to top). The bottom spectrum is biapenem (5 mM) without added enzyme.

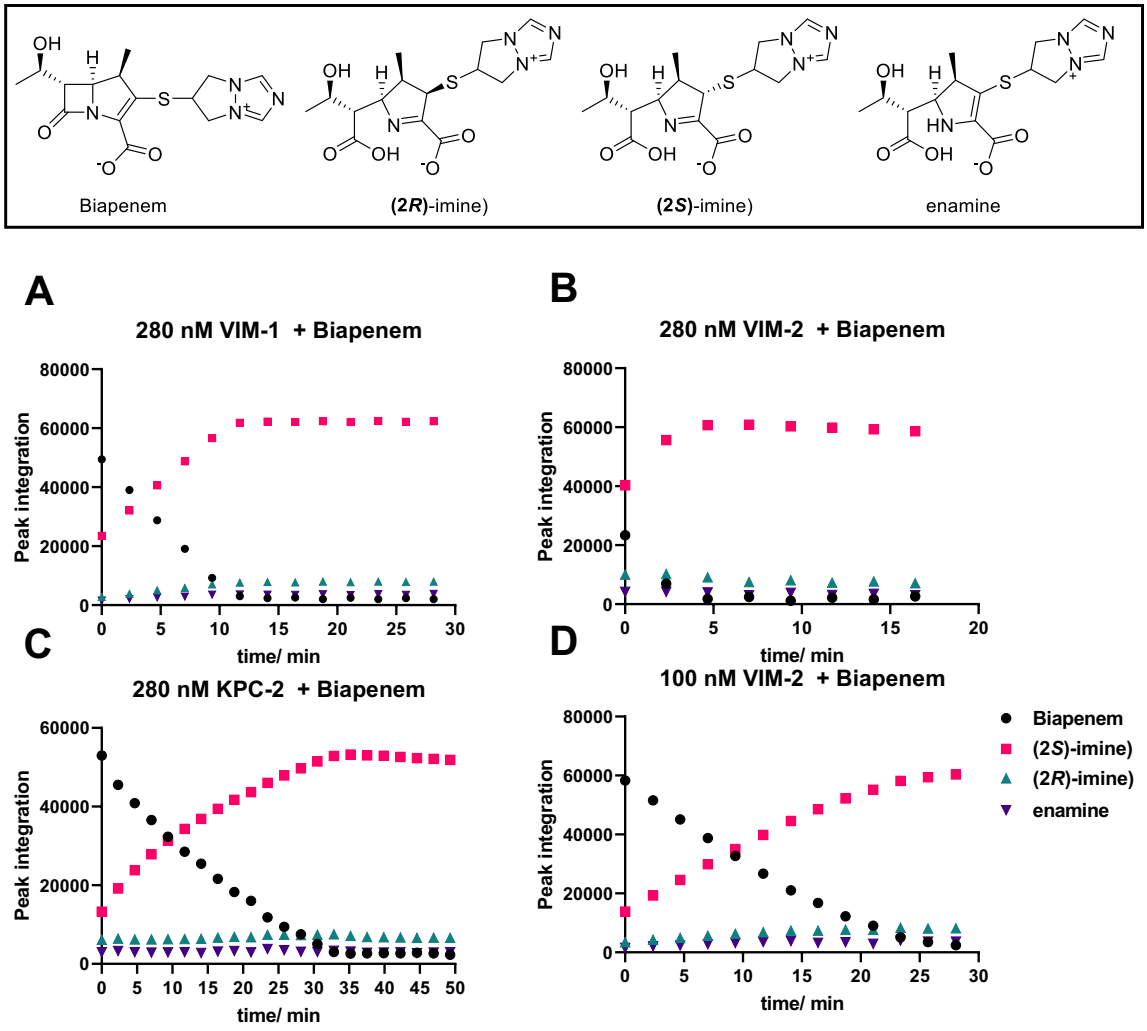

Figure S4 **1H-NMR time course analyses for reaction of biapenem with VIM-1 and VIM-2 (MBLs) and KPC-2 (an SBL).** Methyl group resonance peak integrations from time course data were analysed to monitor reaction of biapenem to give (2*R*)-imine, (2*S*)-imine and enamine (blue, pink, and purple, respectively) products using a Bruker AVIIIHD 600 MHz machine. Biapenem (5 mM) was treated with **(A)** VIM-1 (280 nM), **(B)** VIM-2 (280 nM), **(C)** KPC-2 (280 nM) and **(D)** VIM-2 (100 nM) in 50 mM sodium phosphate at pH 7.5, 10% (v/v) D_2O. Integration of methyl resonance of biapenem was normalised ((integration of biapenem) / (integration of biapenem + (2*R*)-imine + (2*S*)-imine + enamine) *100 was used to calculate the percentage of biapenem turnover.

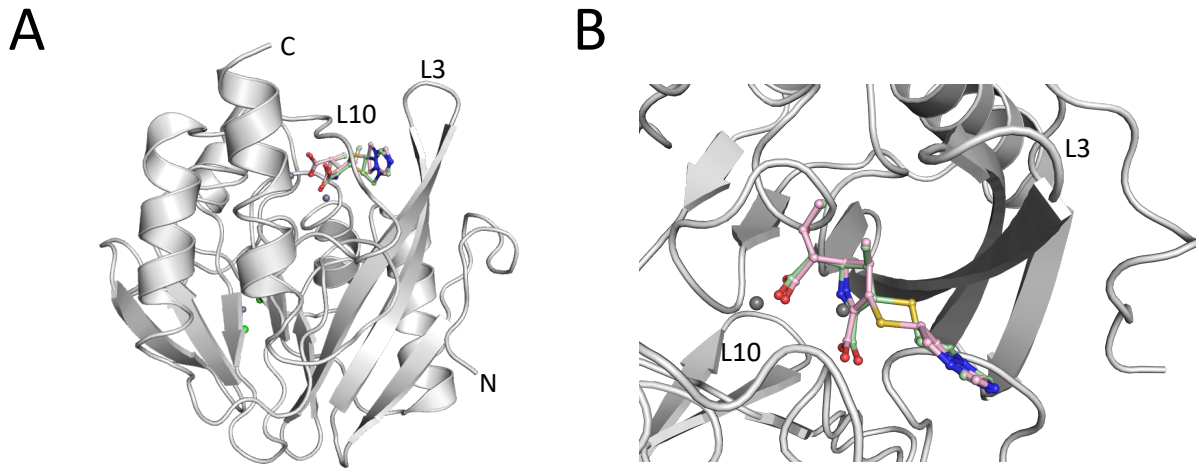

Figure S5. View of the overall fold of the VIM-2: biapenem derived complex. A) The structure was processed in the space group *I*222 (PDB 6Y6J). Zinc ions are shown as grey spheres and chloride ions are in green. B) Active site view of VIM-2 complexed with biapenem derived products. Relevant loops for interaction are labelled. The biapenem derived enamine ligand is in pale pink, and the biapenem derived imine ligand is in pale green (Figure S6).

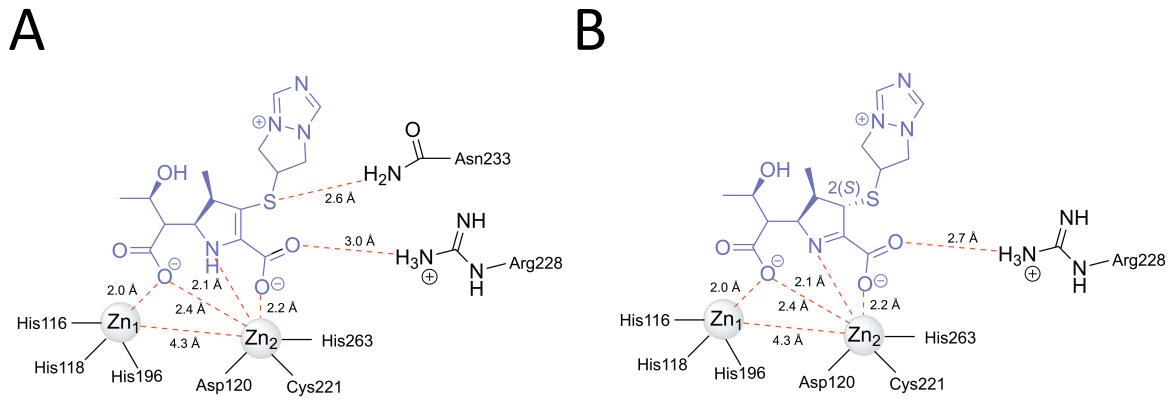

Figure S6. (A) Interactions between the biapenem derived enamine ligand and the VIM-2 active site. (B) Interactions between the biapenem derived (2S)-imine derived ligand and the VIM-2 active site**.**

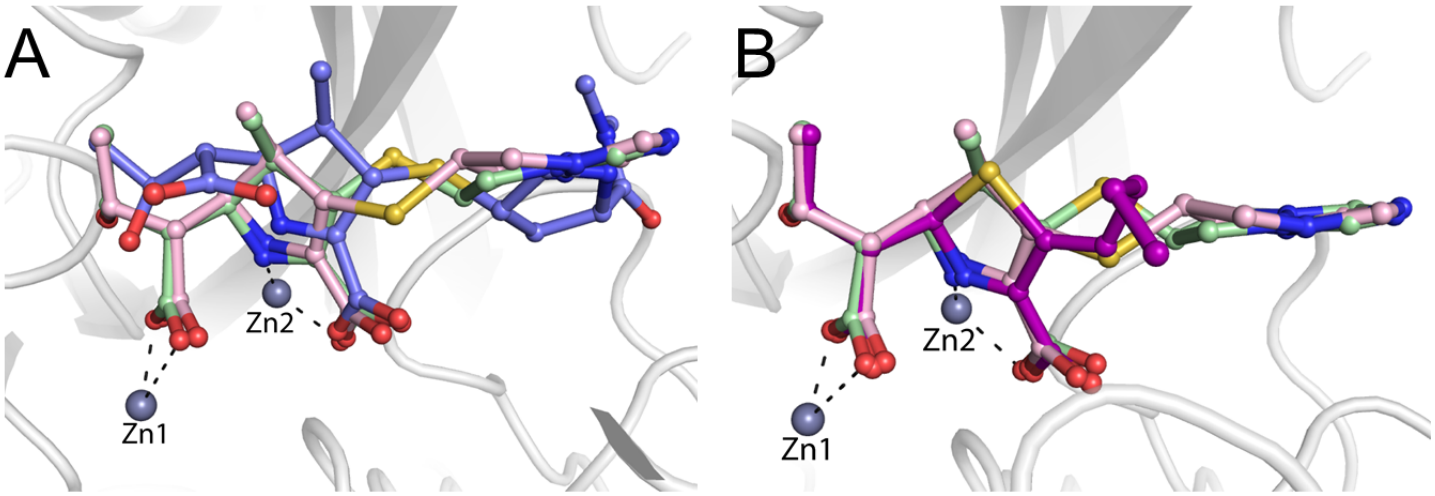

Figure S7. Comparisons of conformations of carbapenem and penem derived ligands with VIM-1 and VIM-2. (**A**) View of VIM-1 complexed with (i) the meropenem derived (2*S*)-imine ligand in slate blue (PDB 5N5I [19]); (ii) the VIM-2 biapenem derived (2*S*)-imine in green; and (iii) the VIM-2 biapenem derived enamine in pink. (PDBs: 6Y6J). (**B**) View of VIM-2 complexed with the (i) faropenem derived product (purple) (PDBs: 7A5Z [20]); (ii) the biapenem derived (2*S*)-imine in green and (iii) the biapenem derived enamine in pink. (PDBs: 6Y6J). Note that the binding modes of the faropenem derived imine (*Z*)-alkene (dark purple) and biapenem (2*S*)-imine derived ligands are similar.

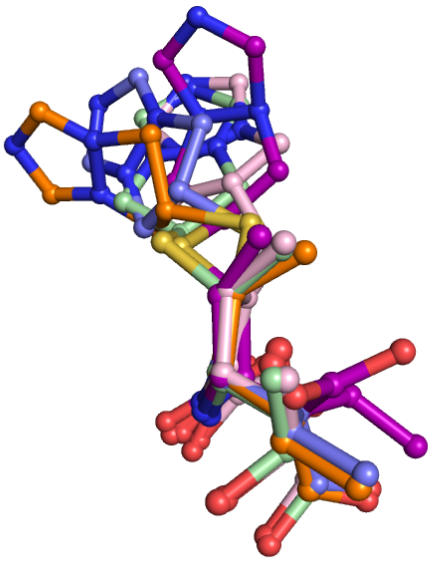

Figure S8. Overlay of the conformations of biapenem derived enamine and imine ligands with β-lactamases and penicillin binding proteins. Colour code: VIM-2 (class B1 MBL) complexed with the biapenem derived enamine - light pink; VIM-2 complexed with the biapenem derived (2*S*)-imine - light green (PDB 6Y6J); PBP2X complexed with the biapenem derived enamine - slate blue (PDB 2ZC3), PBP1A complexed with the biapenem substrate - orange) (PDB 2ZC5) [24], and CphA (class B2 MBL) complexed with the biapenem derived enamine - purple (PDB 1X8I)[23].