Tannic Acid Potentiates Carbapenem Activity in Class A and B Carbapenemase-Producing Enterobacteriaceae

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Running Title: Tannic acid, a potential carbapenemase inhibitor.

1.1. Molecular modelling

1.1.1. System preparation

The crystal structures (PDB ID: 3QX6, 3RXW, 5ACU and 5FAS) were retrieved from the RSCB Protein Data Bank (https://www.rcsb.org/pdb/). The missing residues were added using a graphical user interface of Chimera, a molecular modelling tool [22]. A ligand interaction map was generated using the web version of PoseView [23].

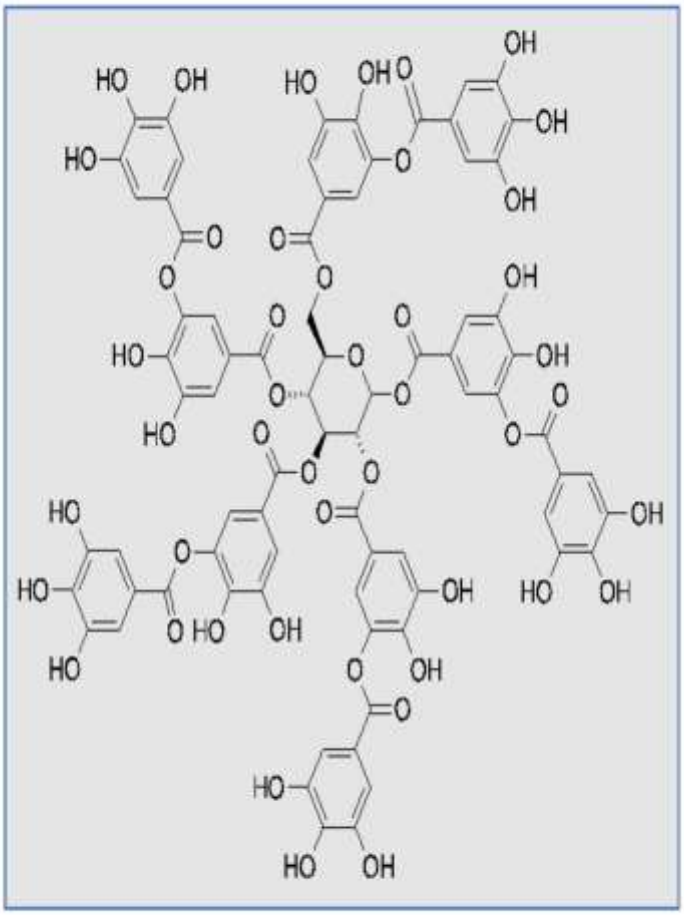
1.1.2. Molecular Docking

Docking calculations were obtained using AutoDock Vina software [24]. Geister partial chargers were assigned and the AutoDock atom types were defined using the AutoDock graphical user interface supplied by MGL tools [25]. The docked conformations were generated using the Lamarckian genetic algorithm (LGA), which is considered to be one of the best docking methods available [26]. The reports for each calculation were in (Kcal/mol). This technique has been validated in previous studies [27].

1.1.3. Molecular dynamics simulation

Due to the lack of parameters needed for the ligand in the Cornell et al. force field [28], the missing parameters were created. Optimization of the ligands were first performed at the HF/6-31G* level with the Gaussian 03 package [29]. The restrained electrostatic potential (RESP) procedure [30] was used to calculate the partial atomic charges. GAFF [31] force field parameters and RESP partial charges were assigned using the ANTECHAMBER module in the Amber14 package. Hydrogen atoms of the proteins were added using the Leap module in Amber12. The standard AMBER force field for bioorganic systems (ff03) was used to define the enzyme parameters. Counter ions were added to neutralize the charge enzyme. The system was enveloped in a box of equilibrated TIP3P water molecules with 8 Å distance around the enzyme. Cubic periodic boundary conditions were imposed and the long-range electrostatic interactions were treated with the particle-mesh Ewald method [32] implemented in Amber12 with a non-bonding cut-off distance of 10 Å.

Initial energy minimization, with a restraint potential of 2 kcal/mol Å^2 applied to the solute, was carried out using the steepest descent method in Amber12 for 1000 iterations followed by conjugate gradient protocol for 2000 steps. The entire system was then freely minimized for 1000 iterations. Harmonic restraints with force constants 5-kcal/mol Å^2 were applied to all solute atoms during the heating phase. A canonical ensemble (NVT) MD was carried out for 50 ps, during which the system was gradually annealed from 0 to 300 K using a Langevin thermostat with a coupling coefficient of 1/ps. Subsequently, the system was equilibrated at 300 K with a 2 fs time step for 100 ps whilst maintaining the force constants on the restrained solute. The SHAKE algorithm [33] was employed on all atoms covalently bonded to a hydrogen atom during equilibration and production runs. With no restraints imposed, a production run was performed for 2 ns in an isothermal isobaric (NPT) ensemble using a Berendsen barostat [34] with a target pressure of 1 bar and a pressure coupling constant of 2 ps. The coordinate file was saved every 1 ps and the trajectory was analyzed every 1 ps using the Ptraj module implemented in Amber14.



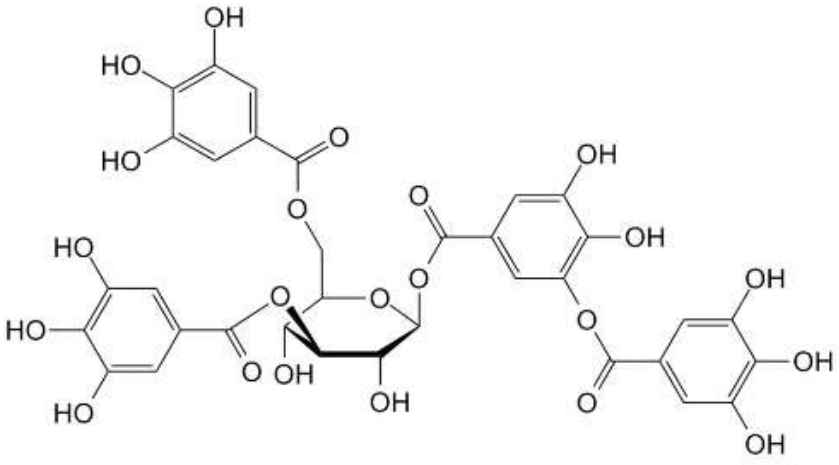


Fig. S1: Chemical structure of tannic acid

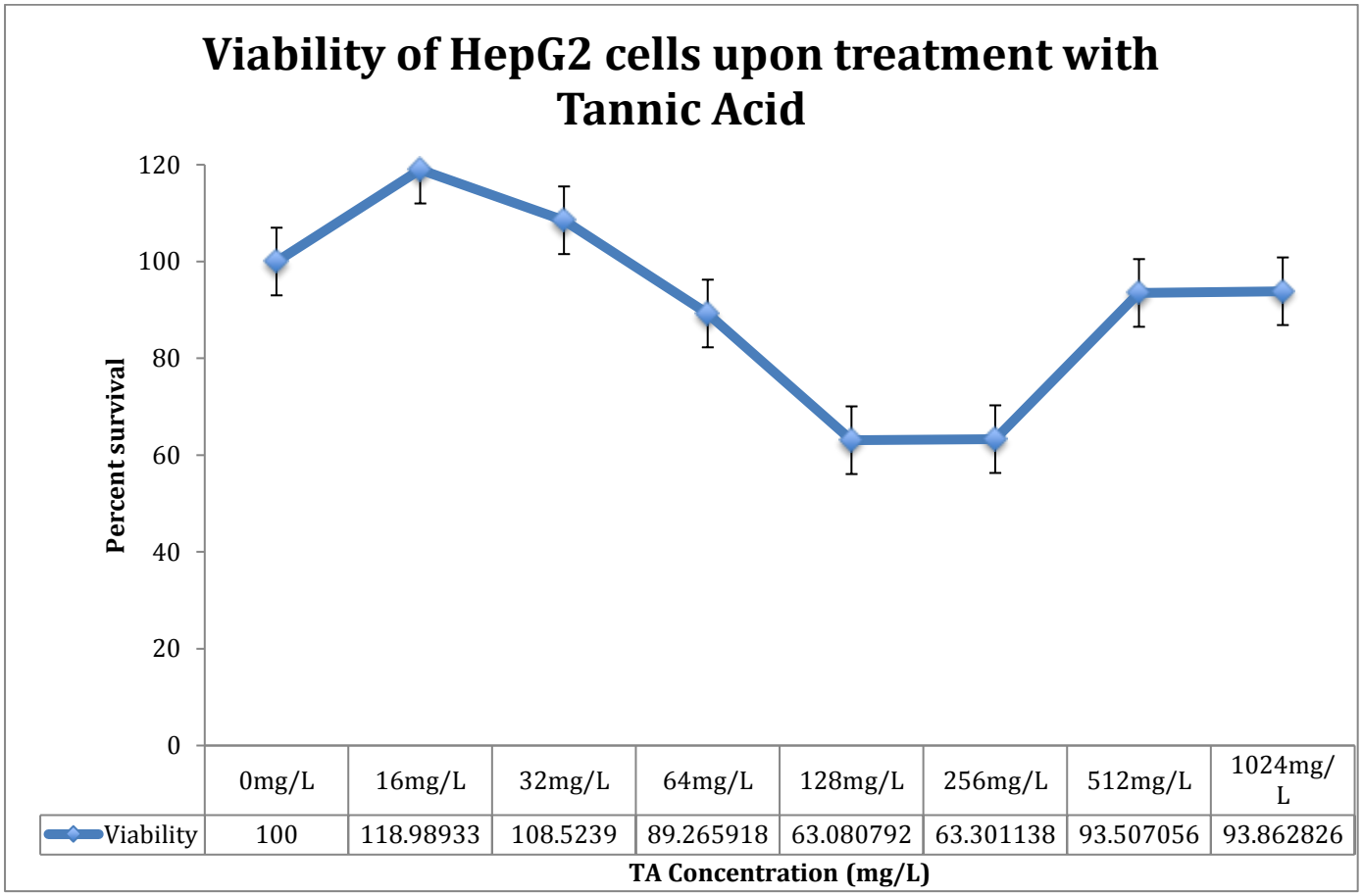


Fig S2: A figure showing HepG2 cell viability following treatment with TA for 24 hours. TA regression analysis yielded a biphasic curve with no significant reduction in cell viability at 512 and 1024mg/L.

Table S1: ***P-values of the MIC fold changes of the various carbapenemases upon inhibition by tannic acid***

ENZYMES	Antibiotics	Tannic acid [MIC mg/L]			
		64	128	256	512
CLASS B					
NDM-1/4/5	MEM	*	**	***	***
	IMP	*	**	***	***
VIM-1/19	MEM	*	**	***	***
	IMP	*	**	***	***
IMP-1	MEM	ns	*	*	**
	IMP	ns	*	*	**
IMP-8	MEM	ns	ns	*	*
	IMP	ns	ns	*	*
CLASS A					
KPC-2	MEM	ns	ns	ns	ns
	IMP	ns	*	*	*
GES-5	MEM	ns	ns	ns	*
	IMP	ns	ns	ns	*
SME 1/2	MEM	ns	**	**	**
	IMP	ns	ns	ns	ns
IMI-1	MEM	ns	*	*	*
	IMP	ns	ns	ns	ns
CLASS D					
OXA-48	MEM	ns	ns	ns	ns
	IMP	ns	ns	ns	ns
OXA-181	MEM	ns	ns	ns	ns
	IMP	ns	ns	ns	ns
OXA-232	MEM	ns	ns	ns	ns
	IMP	ns	ns	ns	ns

ns --> not significant as fold change was < 4. P-value < 0.0001 ---- ***, < 0.001 --- ** < 0.01 --- *

Table S2: ***P-values of the MIC fold changes of NDM-1-producing isolates upon addition of tannic acid***

NDM-1-positive Organism	Antibiotics	Tannic acid [MIC mg/L]			
		64	128	256	512
Klebsiella species (pneumoniae/oxytoca)	MEM	*	**	***	***
	IMP	*	**	***	***
Serratia marcescens	MEM	*	*	**	**
	IMP	*	*	**	**
Enterobacter species	MEM	*	**	***	***
	IMP	*	**	***	***
Escherichia coli	MEM	*	**	***	***
	IMP	*	**	***	***
Citrobacter freundii	MEM	*	**	***	***
	IMP	*	**	***	***

ns --> not significant. P value < 0.0001 ---- ***, < 0.001 --- **, < 0.01 --- *

Table S3: ***MIC ranges of the inhibitors alone and their sub-MICs used for modulating meropenem and imipenem activity against the clinical South African and reference CREs***

INHIBITORS	MIC [mg/L]	
	Range	Modulator
Tannic acid (TA)	>512	64 - 512
Thioridazine (TZ)	>512 -128	32
Chlorpromazine (CPZ)	>512 - 32	16
Reserpine (RSP)	≥512	256
Verapamil (VRP)	≥512	256
CCCP	64 - 16	10

Table S4: ***MIC ranges of the inhibitors and their sub-MICs used for modulating meropenem and imipenem activity against E. coli ATCC 25922***

INHIBITORS	MIC [mg/L]	
	Range	Modulator
Tannic acid (TA)	>512	64 - 512
Thioridazine (TZ)	64	32
Chlorpromazine (CPZ)	32	16
Reserpine (RSP)	≥512	256
Verapamil (VRP)	>512	256
CCCP	16	10

Table S5: ***Minimum inhibitory concentrations of meropenem and imipenem in the presence and absence of carbonyl cyanide m-chlorophenylhydrazine (CCCP), thioridazine (TZ), chlorpromazine (CPZ), verapamil (VRP) and reserpine (RSP) on the clinical South African strains.***

Isolates	MIC [mg/L]					
	Antibiotics alone	+ CCCP [10 mg/L]	+ TZ [32 mg/L]	+ CPZ [16 mg/L]	+ VRP [256 mg/L]	+ RES [256 mg/L]
Reference strain						
E. coli ATCC 25922	MEM- 0.0075 [1]	0.0075 [1]	0.0075 [1]	0.0075 [1]	0.0075 [1]	0.0075 [1]
	IMP- 0.06 [1]	0.06 [1]	0.06 [1]	0.06 [1]	0.06 [1]	0.06 [1]
Klebsiella pneumoniae						
C(UNN_S3)	MEM- 256	128 (2)	256 (1)	256 (1)	256 (1)	256 (1)
	IMP- 128	64 (2)	128 (1)	128 (1)	128 (1)	128 (1)
D(UNN_S4)	MEM- 512	128 (4)	128 (4)	256 (2)	512 (1)	512 (1)
	IMP- 128	64 (2)	64 (2)	128 (1)	128 (1)	128 (1)
I(UNN_S9)	MEM- 512	512 (1)	256 (2)	256 (2)	512 (1)	512 (1)
	IMP- 256	256 (1)	128 (2)	256 (1)	256 (1)	256 (1)
J(UNN_S10)	MEM- 128	128 (1)	128 (1)	128 (1)	128 (1)	128 (1)
	IMP- 256	256 (1)	256 (1)	256 (1)	256 (1)	256 (1)

3_S2	MEM- 128	128 (1)	128 (1)	128 (1)	128 (1)	128 (1)
	IMP- 128	128 (1)	128 (1)	128 (1)	128 (1)	128 (1)
12_S5	MEM- 64	64 (1)	64 (1)	64 (1)	64 (1)	64 (1)
	IMP- 128	128 (1)	128 (1)	128 (1)	128 (1)	128 (1)
13_S6	MEM- 128	128 (1)	128 (1)	128 (1)	128 (1)	128 (1)
	IMP- 64	64 (1)	64 (1)	64 (1)	64 (1)	64 (1)
15_S8	MEM- 16	16 (1)	16 (1)	16 (1)	16 (1)	16 (1)
	IMP- 128	128 (1)	128 (1)	128 (1)	128 (1)	128 (1)
18_S10	MEM- 512	256 (2)	128 (4)	256 (2)	512 (1)	512 (1)
	IMP- 512	256 (2)	128 (4)	256 (2)	512 (1)	512 (1)
20_S11	MEM- 256	128 (2)	128 (2)	256 (1)	256 (1)	256 (1)
	IMP- 128	64 (2)	64 (2)	128 (1)	128 (1)	128 (1)
21_S12	MEM- 512	256 (2)	512 (1)	512 (1)	512 (1)	512 (1)
	IMP- 128	128 (1)	128 (1)	128 (1)	128 (1)	128 (1)
29_S13	MEM- 512	256 (1)	128 (4)	128 (4)	512 (1)	512 (1)
	IMP- 128	128 (1)	64 (2)	64 (2)	128 (1)	128 (1)
30_S14	MEM- 256	256 (1)	128 (2)	256 (1)	256 (1)	256 (1)
	IMP- 128	128 (1)	128 (1)	128 (1)	128 (1)	128 (1)
32_S15	MEM- 128	128 (1)	128 (1)	128 (1)	128 (1)	128 (1)
	IMP- 32	32 (1)	32 (1)	32 (1)	32 (1)	32 (1)
34_S15	MEM-512	512 (1)	512 (1)	512 (1)	512 (1)	512 (1)
	IMP-512	512 (1)	512 (1)	512 (1)	512 (1)	512 (1)
35_S17	MEM-512	256 (2)	512 (1)	512 (1)	512 (1)	512 (1)
	IMP-512	512 (1)	512 (1)	512 (1)	512 (1)	512 (1)
36_S18	MEM-128	128 (1)	128 (1)	128 (1)	128 (1)	128 (1)
	IMP-128	128 (1)	128 (1)	128 (1)	128 (1)	128 (1)
38_S19	MEM-16	16 (1)	16 (1)	16 (1)	16 (1)	16 (1)
	IMP-32	32 (1)	32 (1)	32 (1)	32 (1)	32 (1)
52_S26	MEM-512	512 (1)	512 (1)	512 (1)	512 (1)	512 (1)
	IMP-256	256 (1)	256 (1)	256 (1)	256 (1)	256 (1)
53_S27	MEM-256	128 (2)	256 (1)	256 (1)	256 (1)	256 (1)

	IMP-128	64 (2)	128 (1)	128 (1)	128 (1)	128 (1)
Serratia marcescens						
B (UNN38 _S2)	MEM->512	64 (**>8**)	64 (4)	64 (>4)	512 (1)	512 (1)
	IMP-512	256 (2)	64 (4)	64 (4)	512 (1)	512 (1)
E (UNN41 _S5)	MEM-128	64 (2)	64 (2)	64 (2)	128 (1)	128 (1)
	IMP-128	128 (1)	64 (2)	64 (2)	128 (1)	128 (1)
G (UNN43 _S7)	MEM-16	16 (1)	16 (1)	16 (1)	16 (1)	16 (1)
	IMP-512	256 (2)	256 (2)	256 (2)	512 (1)	512 (1)
K (UNN47 _S11)	MEM-128	64 (2)	64 (2)	128 (1)	128 (1)	128 (1)
	IMP-256	256 (1)	128 (2)	256 (1)	256 (1)	256 (1)
L (UNN38 _S12)	MEM-128	64 (2)	128 (1)	128 (1)	128 (1)	128 (1)
	IMP-128	128 (1)	128 (1)	128 (1)	128 (1)	128 (1)
7_S3	MEM-64	64 (2)	64 (1)	64 (1)	64 (1)	64 (1)
	IMP-32	32 (1)	32 (1)	32 (1)	32 (1)	32 (1)
45_S21	MEM-32	32 (1)	32 (1)	32 (1)	32 (1)	32 (1)
	IMP-32	8 (4)	32 (1)	32 (1)	32 (1)	32 (1)
56_S29	MEM-512	512 (1)	256 (2)	256 (2)	512 (1)	512 (1)
	IMP-256	128 (2)	128 (2)	128 (2)	256 (1)	256 (1)
59_S30	MEM-512	256 (2)	128 (4)	256 (2)	512 (1)	512 (1)
	IMP-256	128 (2)	128 (2)	128 (2)	256 (1)	256 (1)
67_S33	MEM-256	128 (2)	128 (2)	128 (2)	256 (1)	256 (1)
	IMP-512	256 (2)	256 (2)	256 (2)	512 (1)	512 (1)
68_S34	MEM-64	64 (1)	64 (1)	64 (1)	64 (1)	64 (1)
	IMP-32	32 (1)	32 (1)	32 (1)	32 (1)	32 (1)
71_S36	MEM->512	512(>1)	>512 (1)	>512 (1)	>512 (1)	>512 (1)
	IMP-128	64 (2)	128 (1)	128 (1)	128 (1)	128 (1)
***Enterobacter cloacae* (unless otherwise stated in the footnote)**						
A (UNN37 _S1)	MEM-64	64 (1)	64 (1)	64 (1)	64 (1)	64 (1)
	IMP-32	32 (1)	32 (1)	32 (1)	32 (1)	32 (1)
F (UNN42 _S6)	MEM-512	512 (1)	256 (2)	256 (2)	512 (1)	512 (1)
	IMP-128	128 (1)	64 (2)	128 (1)	128 (1)	128 (1)

H (UNN44 _S8)	MEM->512	512 (>1)	>512 (1)	>512 (1)	>512 (1)	>512 (1)
	IMP-128	64 (2)	128 (1)	128 (1)	128 (1)	128 (1)
1_S1	MEM-2	2 (1)	2 (1)	2 (1)	2 (1)	2 (1)
	IMP-0.5	0.5 (1)	0.5 (1)	0.5 (1)	0.5 (1)	0.5 (1)
16_S9	MEM-256	256 (1)	64 (4)	128 (2)	256 (1)	256 (1)
	IMP-128	128 (1)	64 (2)	64 (2)	128 (1)	128 (1)
43_S20	MEM->512	512 (>1)	>512 (1)	>512 (1)	>512 (1)	>512 (1)
	IMP-64	64 (1)	64 (1)	64 (1)	64 (1)	64 (1)
49_S24	MEM-512	256 (2)	512 (1)	512 (1)	512 (1)	512 (1)
	IMP-128	128 (1)	128 (1)	128 (1)	128 (1)	128 (1)
51_S25	MEM->512	512 (>1)	>512 (1)	>512 (1)	>512 (1)	>512 (1)
	IMP-128	64 (2)	128 (1)	128 (1)	128 (1)	128 (1)
55_S28	MEM-512	256 (2)	256 (2)	256 (2)	512 (1)	512 (1)
	IMP-256	128 (2)	128 (2)	128 (2)	256 (1)	256 (1)
63_S31	MEM-512	256 (2)	512 (1)	512 (1)	512 (1)	512 (1)
	IMP-64	64 (1)	64 (1)	64 (1)	64 (1)	64 (1)
65_S32	MEM-512	512 (1)	256 (2)	512 (1)	512 (1)	512 (1)
	IMP-256	128 (2)	128 (2)	256 (1)	256 (1)	256 (1)
Escherichia coli						
10_S4	MEM->512	512 (>1)	>512 (1)	>512 (1)	>512 (1)	>512 (1)
	IMP-512	512 (1)	256 (2)	512 (1)	512 (1)	512 (1)
Citrobacter freundii						
48_S23	MEM-512	256 (2)	256 (2)	512 (1)	512 (1)	512 (1)
	IMP-128	64 (2)	64 (2)	128 (1)	128 (1)	128 (1)
***Klebsiella* oxytoca**						
69_S35	MEM-512	512 (1)	512 (1)	512 (1)	512 (1)	512 (1)
	IMP-128	128 (1)	128 (1)	128 (1)	128 (1)	128 (1)

<u>Table S6:</u> Minimum inhibitory concentrations of meropenem and imipenem in the presence and absence of carbonyl cyanide m-chlorophenylhydrazine (CCCP), thioridazine (TZ), chlorpromazine (CPZ), verapamil (VRP) and reserpine (RSP) on the reference strains.

Isolates		MIC [mg/L]				
	Antibiotics alone	CCCP [10 mg/L]	TZ [32 mg/L]	CPZ [16 mg/L]	VER [256 mg/L]	RES [256 mg/L]
Reference strains						
E. coli ATCC 25922	MEM- 0.0075 [1]	0.0075 [1]	0.0075 [1]	0.0075 [1]	0.0075 [1]	0.0075 [1]
	IMP- 0.06 [1]	0.06 [1]	0.06 [1]	0.06 [1]	0.06 [1]	0.06 [1]
CLASS A						
KPC-2						
E. coli KPC-2	MEM- 16	16 [1]	16 [1]	16 [1]	16 [1]	16 [1]
	IMP- 64	64 [1]	64 [1]	64 [1]	64 [1]	64 [1]
E. cloacae KPC-2	MEM- 32	32 [1]	32 [1]	32 [1]	32 [1]	32 [1]
	IMP- 128	128 [1]	64 [2]	128 [1]	128 [1]	128 [1]
C. freundii KPC-2	MEM- 16	16 [1]	16 [1]	16 [1]	16 [1]]	16 [1]
	IMP- 64	64 [1]	32 [2]	32 [2]	64 [1]	64 [1]
GES-5						
E. cloacae GES-5	MEM- 64	64 [1]	64 [1]	64 [1]	64 [1]	64 [1]
	IMP- 128	128 [1]	128 [1]	128 [1]	128 [1]	128 [1]
IMI-1						
E. asburiae IMI-1	MEM- 256	256 [1]	128 [2]	256 [1]	256 [1]	256 [1]
	IMP- >512	512 [>1]	256 [>2]	>512 [1]	>512 [1]	>512 [1]
SME-1						
S. marcescens SME-1	MEM- 512	256 [2]	64 [8]	128 [4]	256 [2]	256 [2]
	IMP- >512	512 [>1]	128 [>4]	256 [>2]	256 [>2]	512 [>1]
SME-2						
S. marcescens SME-2	MEM- 512	256 [2]	64 [8]	256 [2]	256 [2]	256 [2]

	IMP->512	256 [>2]	128 [>4]	256 [>2]	256 [>2]	512 [>1]
CLASS D						
OXA-48/181						
E. coli OXA-48	MEM- 1	1 [1]	0.5 [2]	1 [1]	1 [1]	1 [1]
	IMP- 2	2 [1]	2 [1]	2 [1]	2 [1]	2 [1]
K. pneumoniae OXA-48	MEM- 8	8 [1]	8 [1]	8 [1]	8 [1]	8 [1]
	IMP- 8	8 [1]	8 [1]	8 [1]	8 [1]	8 [1]
K pneumoniae OXA-181	MEM- 1	1 [1]	1 [1]	1 [1]	1 [1]	1 [1]
	IMP- 4	4 [1]	1 [1]	1 [1]	1 [1]	4 [1]
P. rettgeri OXA-181	MEM- 0.5	0.5 [1]	0.5 [1]	0.5 [1]	0.5 [1]	0.5 [1]
	IMP- 1	1 [1]	1 [1]	1 [1]	1 [1]	1 [1]
CLASS B						
NDM-1/NDM-4						
E. coli NDM-1	MEM- 64	64 [1]	32 [2]	64 [1]	64 [1]	64 [1]
	IMP- 128	128 [1]	128 [1]	128 [1]	128 [1]	128 [1]
E. cloacae NDM-1	MEM- 16	16 [1]	16 [1]	16 [1]	16 [1]	16 [1]
	IMP- 32	32 [1]	32 [1]	32 [1]	32 [1]	32 [1]
C. freundii NDM-1	MEM- 16	16 [1]	16 [1]	16 [1]	16 [1]	16 [1]
	IMP- 8	8 [1]	8 [1]	8 [1]	8 [1]	8 [1]
E. coli NDM-4	MEM- 256	128 [2]	128 [2]	128 [2]	256 [1]	256 [1]
	IMP- 128	128 [1]	64 [2]	128 [1]	128 [1]	128 [1]
VIM-1/19						
E. coli VIM-1	MEM- 64	32 [2]	16 [4]	32 [2]	32 [2]	64 [1]
	IMP-128	32 [4]	16 [8]	32 [4]	64 [2]	128 [1]
E. cloacae VIM-1	MEM- 8	8 [1]	4 [2]	4 [2]	8 [1]	8 [1]
	IMP- 16	16 [1]	8 [2]	16 [1]	16 [1]	16 [1]
IMP-1/8						
E. cloacae IMP-1	MEM- 64	64 [1]	16 [4]	32 [2]	32 [2]	32 [2]
	IMP- 128	128 [1]	32 [4]	128 [1]	64 [2]	64 [2]
E. coli IMP-1	MEM- 16	16 [1]	16 [1]	16 [1]	16 [1]	16 [1]
	IMP- 32	32 [1]	16 [2]	32 [1]	32 [1]	32 [1]

S. marcescens IMP-1	MEM- 128	64 [2]	32 [4]	32 [4]	128 [1]	128 [1]
	IMP- 128	64 [2]	32 [4]	64 [2]	64 [2]	128 [1]
E. coli IMP-8	MEM- 16	16 [1]	16 [1]	16 [1]	16 [1]	16 [1]
	IMP- 32	32 [1]	32 [1]	32 [1]	32 [1]	32 [1]
E. cloacae IMP-8	MEM- 8	4 [2]	8 [1]	8 [1]	8 [1]	8 [1]
	IMP- 16	8 [2]	16 [1]	16 [1]	16 [1]	16 [1]

Table S1: Docking score of TA with the target enzymes

Systems/Enzymes	PDB code	Docking score
NDM-1	3QX6	-13.6
VIM-2	5ACU	-14.1
KPC-2	3RXW	-10
OXA-48	5FAS	-9.3