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In *silico* design, synthesis and evaluation of MurD and MurE ligase inhibitors as antibacterial agents

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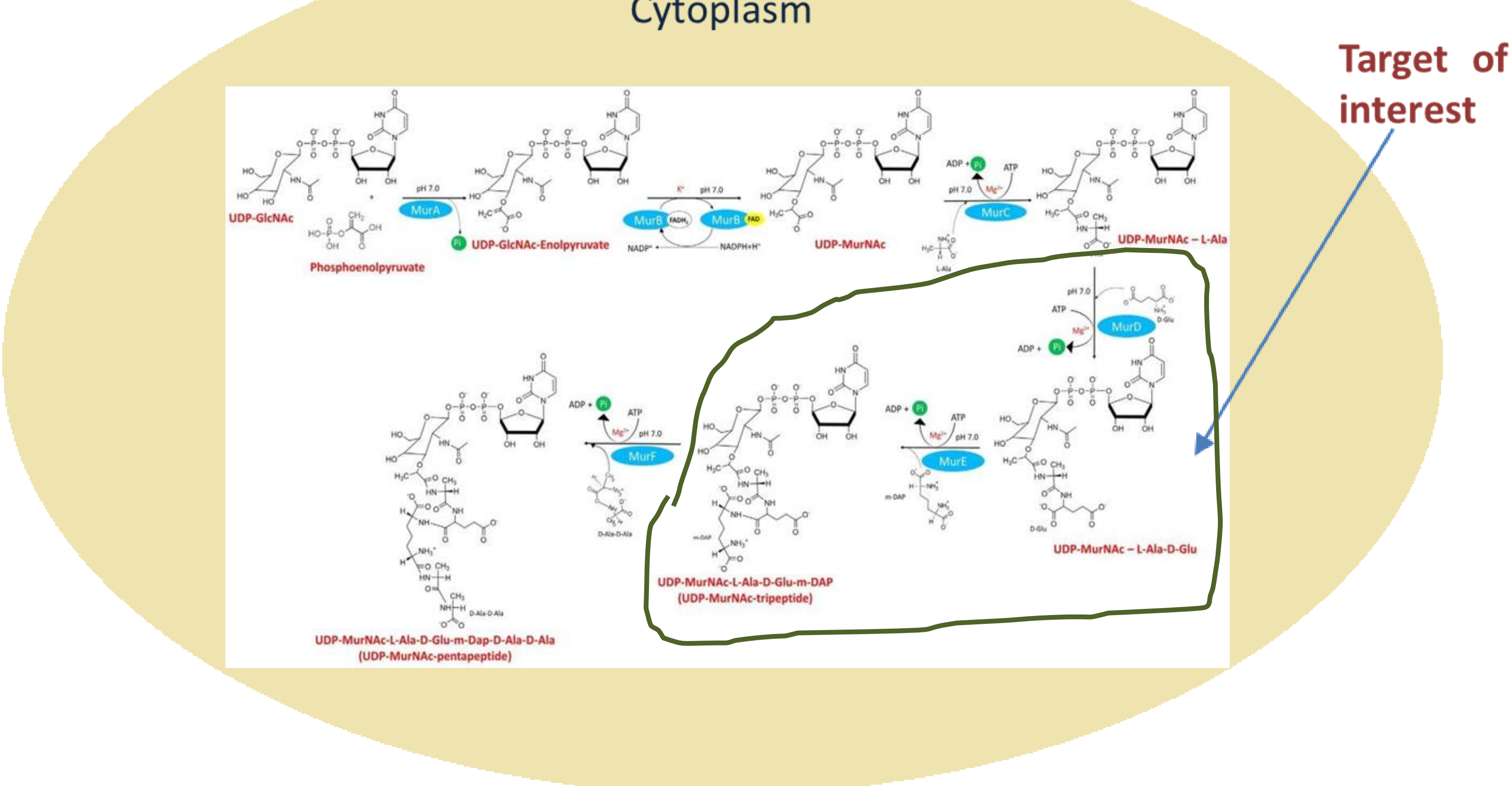
Dual Inhibition of MurD and MurE: A strategy for Anti-resistant Antibiotics Development

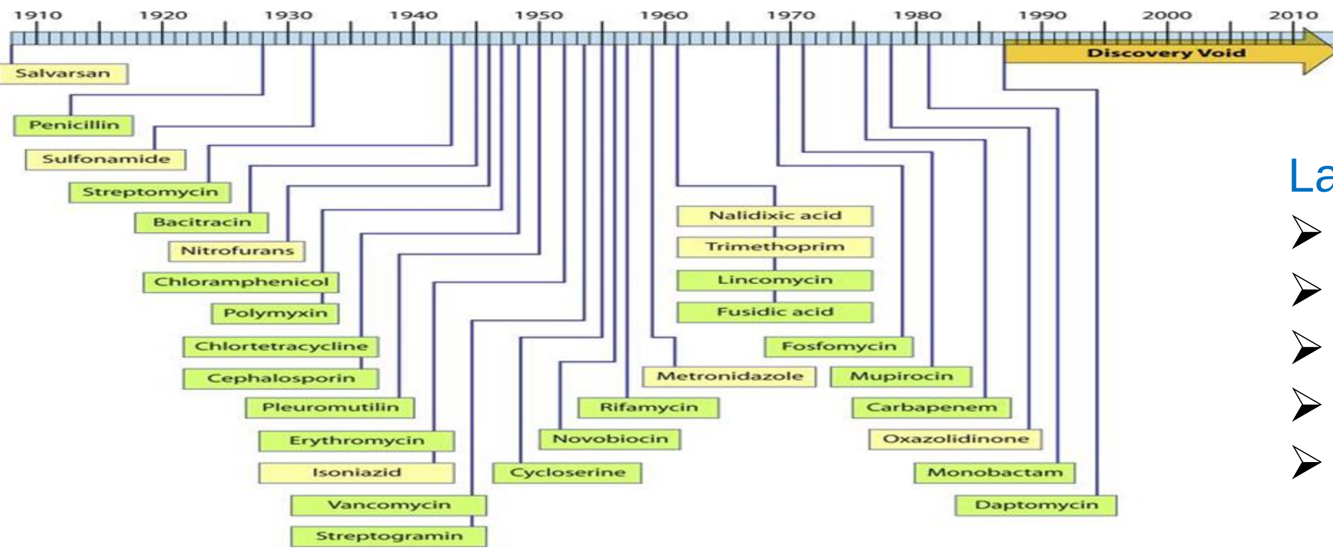
Graphical Abstract



Abstract:

Bacterial resistance is one of the biggest threat to health community, especially hospital acquired MRSA. There are various mechanisms are involved in bacterial resistance out of which, the penetration of cell wall and the mutation of target receptor are the most important. From the beginning, the later stage of Peptidoglycan synthesis has been targeted which occurs outside the cytoplasm. The early stage of peptidoglycan synthesis has never been exploited. Inside the cytoplasm a group of enzyme known as Mur enzymes having similar mechanism of action using ATP, act consecutively and the active residues for all the enzymes are conserved. These make them ideal for multi-target. The MurD involve in adding the D-glu amino acid whereas MurE involve for L-Lys/m-DAP amino acid addition. The MurE act as a gatekeeper for gram-positive and gram-negative bacteria. The product of the previous enzyme act as a substrate for the next one. By designing similar chemical nature to the MurD product, will be having the dual affinity. But the major drawback of these inhibitors are penetration. The IC_{50} values and the MIC values have not correlated for most of the inhibitors. The current work is focused on this problem and we have designed some novel scaffold using various drug designing tools to get the desired hits. The MIC values and time kill studies of the synthesized compound has been carried out against MRSA (ATCC-43300). All the MICs were within µg/ml and better time–kill studies shown against the standard drug ciprofloxacin. These hits can optimized further to get the desired lead.
Keywords: MRSA; MurD; MurE.

Introduction



Last resort antibiotics:

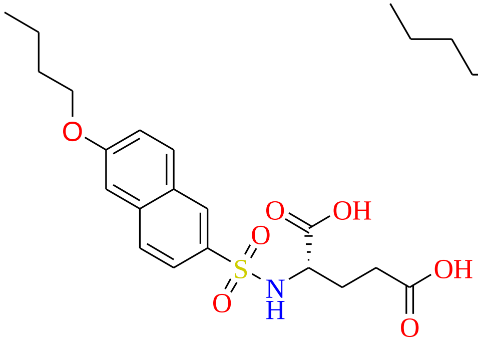
- Vancomycin
- Linezolid
- Colistin
- Daptomycin
- 3rd and 4th generation cephalosporins

Antibiotics approved by the U.S. Food and Drug Administration (FDA) Jan- 2010 to Dec 2017 were

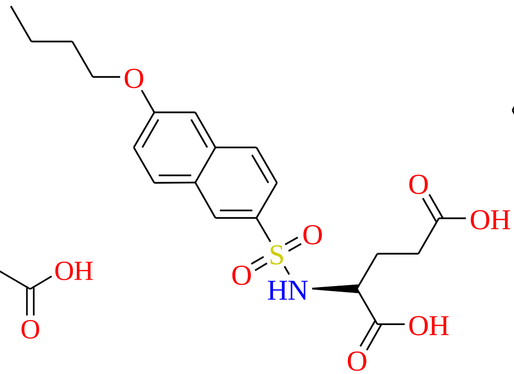
- Ceftaroline
- Fidaxomicin
- Bedaquiline
- Dalbavancin
- Tedizolid
- Ceftolozane–tazobactam
- Ceftazidime–avibactam
- Meropenam
- Delafloxacin

?

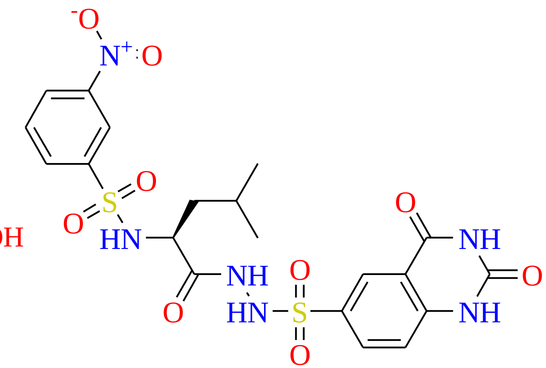
Literature survey (MurD)



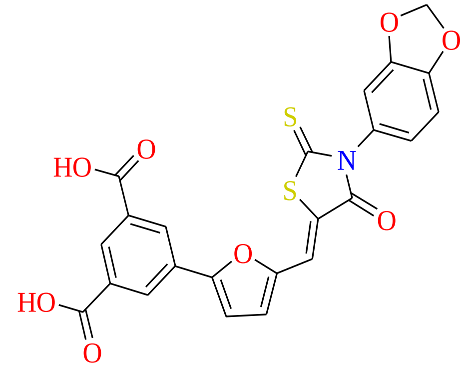
E.coli MurD IC_{50} 280 µM



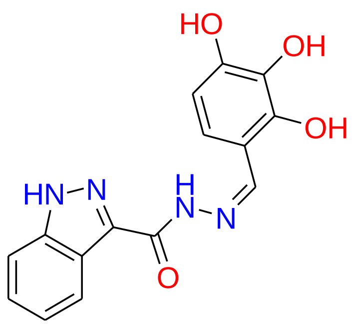
E.coli MurD IC_{50} 280 µM



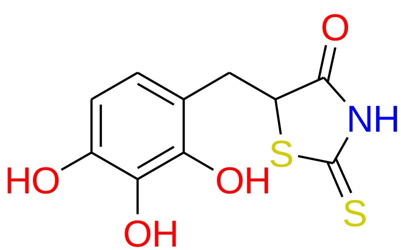
E. coli MurD 56% residual activity at 500 µM



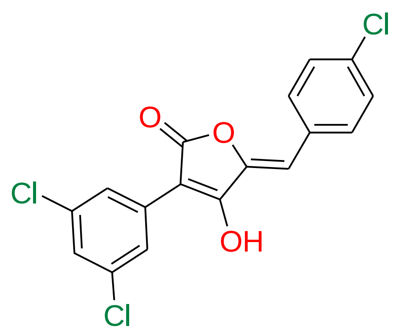
E. coli MurD IC_{50} 270 µM; *E. coli* MurE IC_{50} 32 µM



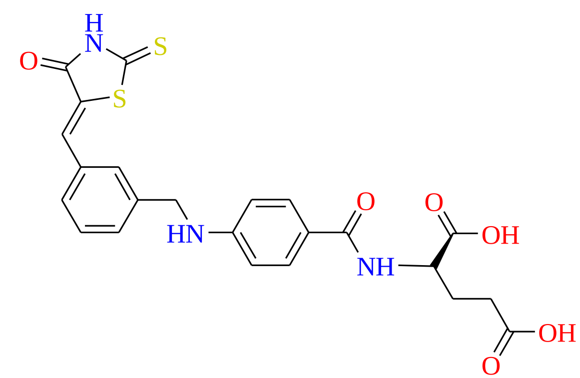
E.coli MurD IC_{50} 230 µM MIC-128 µg/ml



E. coli MurD IC_{50} 2 µM; *S.aureus* MurE IC_{50} 6 µM
MIC- >128 µg/ml for *E.coli* and *S.aureus*

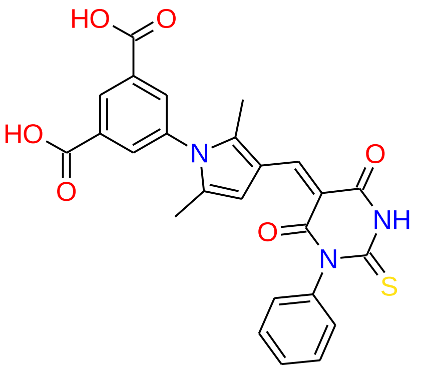


S.aureus MurD IC_{50} 13 µM; *S.aureus* MurE IC_{50} 13 µM; MIC- S.aureus 1-2 µg/ml

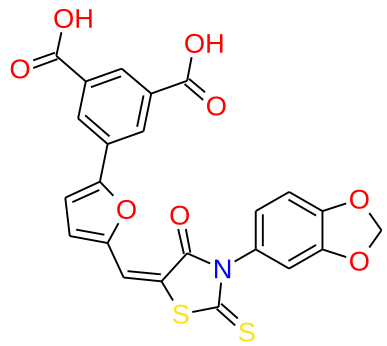


S. aureus MurD (IC_{50} **6.4** µM)and MurE (IC_{50} **17** µM) and *E. coli* MurD (IC_{50} **8.2**µM) and MurE (IC_{50} **180** µM) MIC- S.aureus (MRSA) 8 µg/ml

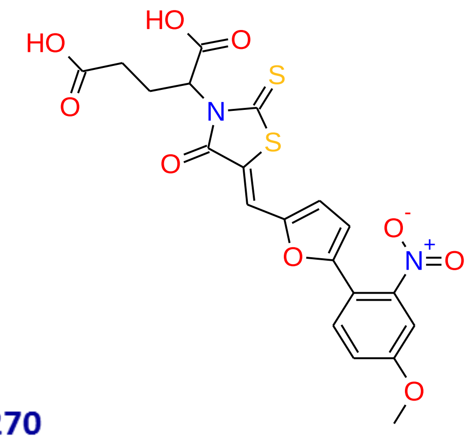
Literature survey (MurE)



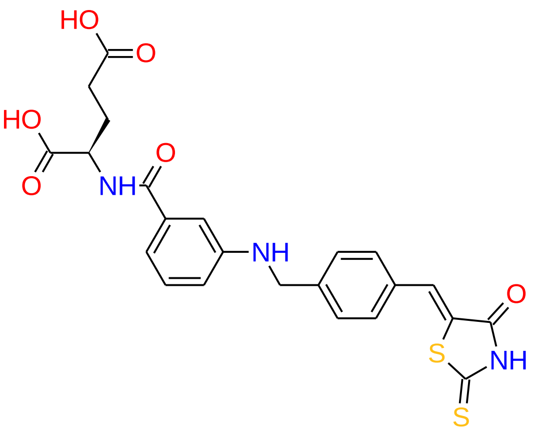
E. coli MurD (IC_{50} **690** μM) and MurE (IC_{50} **89** μM)



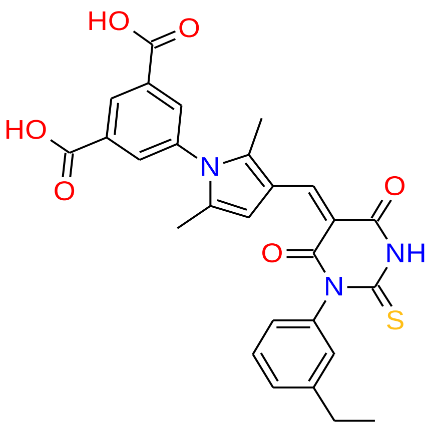
E. coli MurD (IC_{50} **270** μM) and MurE (IC_{50} **32** μM)



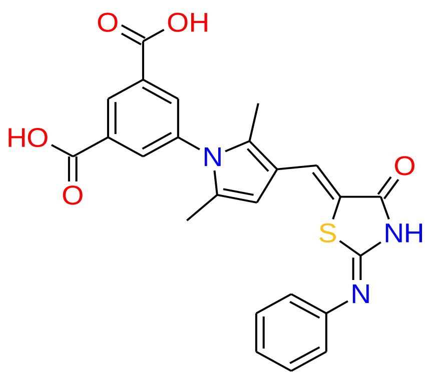
E.coli MurE(IC_{50} **61** μM)



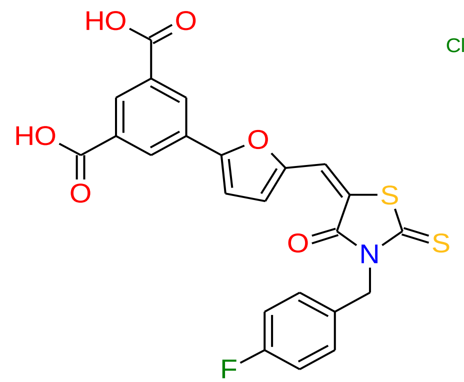
S. aureus MurD (IC_{50} **6.4** μM)and MurE (IC_{50} **17** μM) and *E. coli* MurD (IC_{50} **8.2**μM) and MurE (IC_{50} **180** μM)



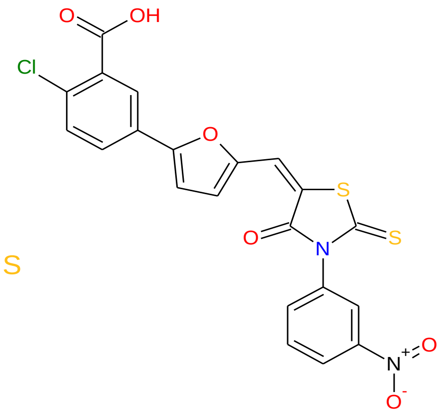
E.coli MurE(IC_{50} **330** μM)



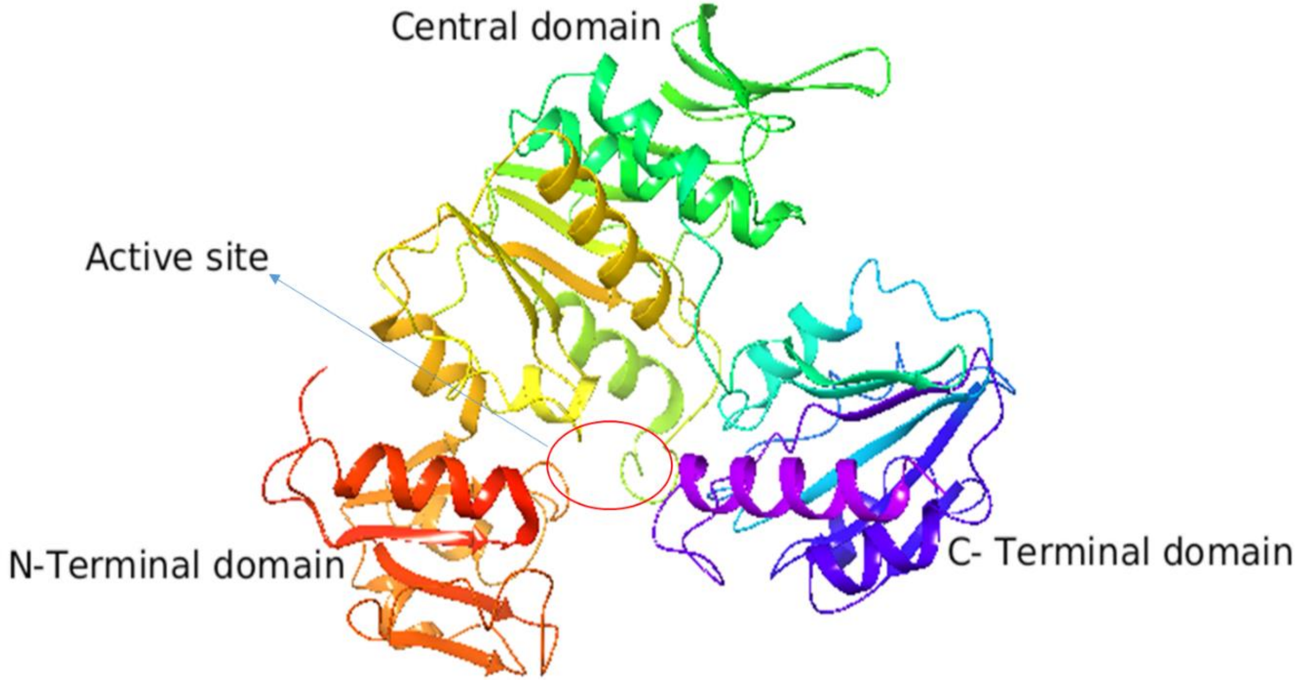
E. coli MurD (IC_{50} **206** μM) and MurE (IC_{50} **494** μM)



E.coli MurE(IC_{50} **330** μM)



E. coli MurD (IC_{50} **148** μM) and MurE (IC_{50} **16** μM)

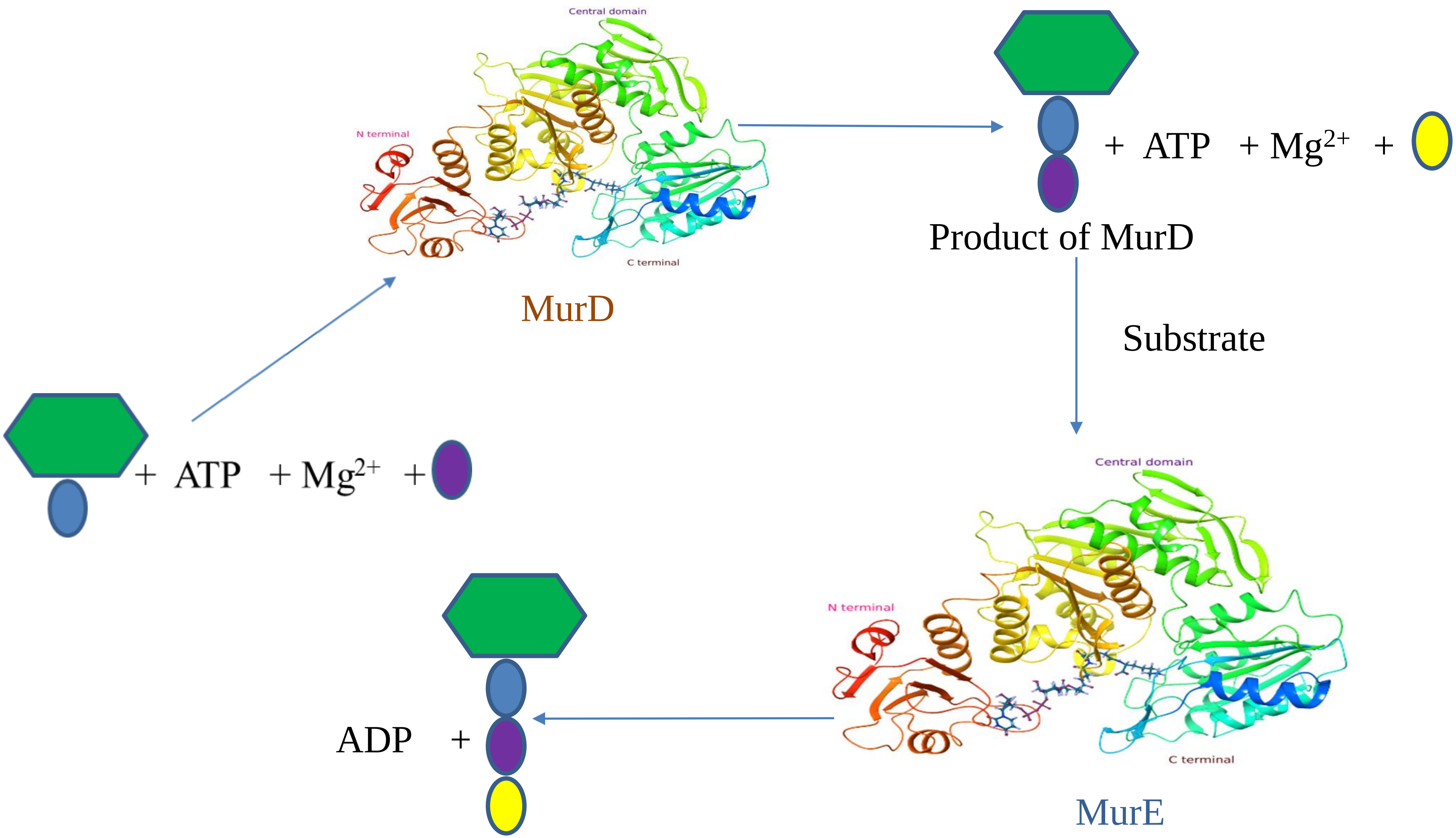


Domain representation in the 3D-crystal structure of MurD and MurE Ligase enzyme

N-Terminal Domain: 1-99
UMA

Central Domain: 100-304
ATP

C-Terminal Domain: 305-449
Incoming amino-acid


Central domain
N terminal
C terminal
MurD
+ ATP + Mg2+ +
Product of MurD
+ ATP + Mg2+ +
Substrate
Central domain
N terminal
C terminal
MurE
ADP +

Why MurD and MurE ?

- Both MurD & MurE enzymes are present only in bacteria with high specificity towards their amino acid and also no structural homology with mammalian enzymes.
- All Mur ligases presumably act through an analogous sequential enzymatic mechanism, as corroborated by structural, biochemical and computational studies.
- The binding site residues of both the enzymes are conserved for different bacterial species.
- As both MurD & MurE are consecutive enzymes with similar catalytic mechanism and binds to the substrate having same structural features.
- This leads our attention to design a ligand which will act as analog of MurD product and MurE substrate.
- As MurE plays a decisive role in cell wall synthesis which differentiate gram positive and gram negative bacteria. So, inhibitors of this enzyme will inhibit cell wall synthesis for both gram positive and gram negative bacteria .

Aim

- To design molecules having similar nature to the product of the MurD enzyme which then act as a substrate for MurE, which posses the dual affinity.

- The closed form of the enzyme structure, which is bound with the product has been used for the HTVS and designing.

Homology modeling of *S. aureus* MurD enzyme using uniprot Fasta sequence (accession code: Q6GHQ2)

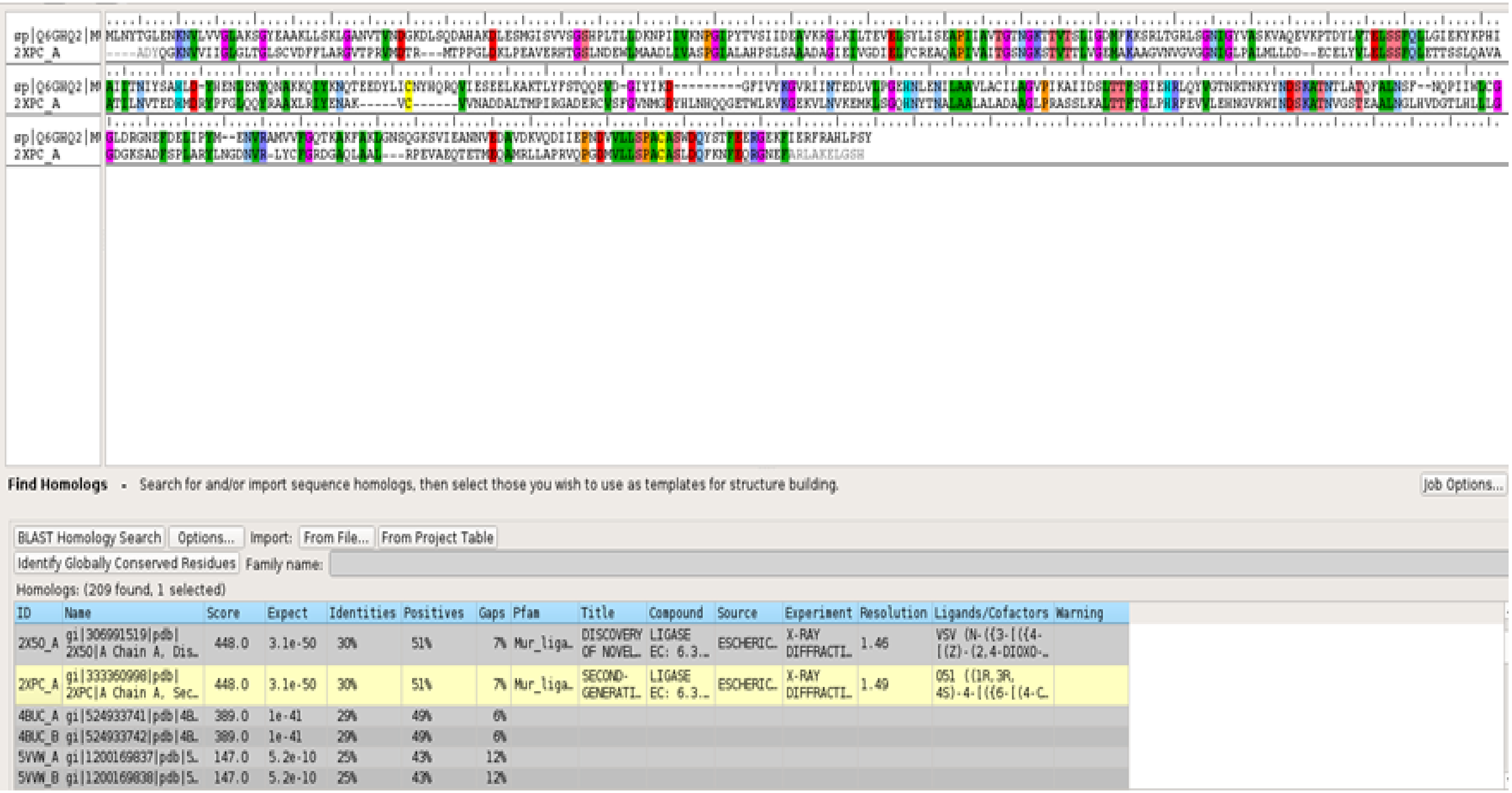


Fig 1: Alignment of template and query sequence and the alignment score of 2XPC.

Homology modeling (MurD from *S.aureus*)

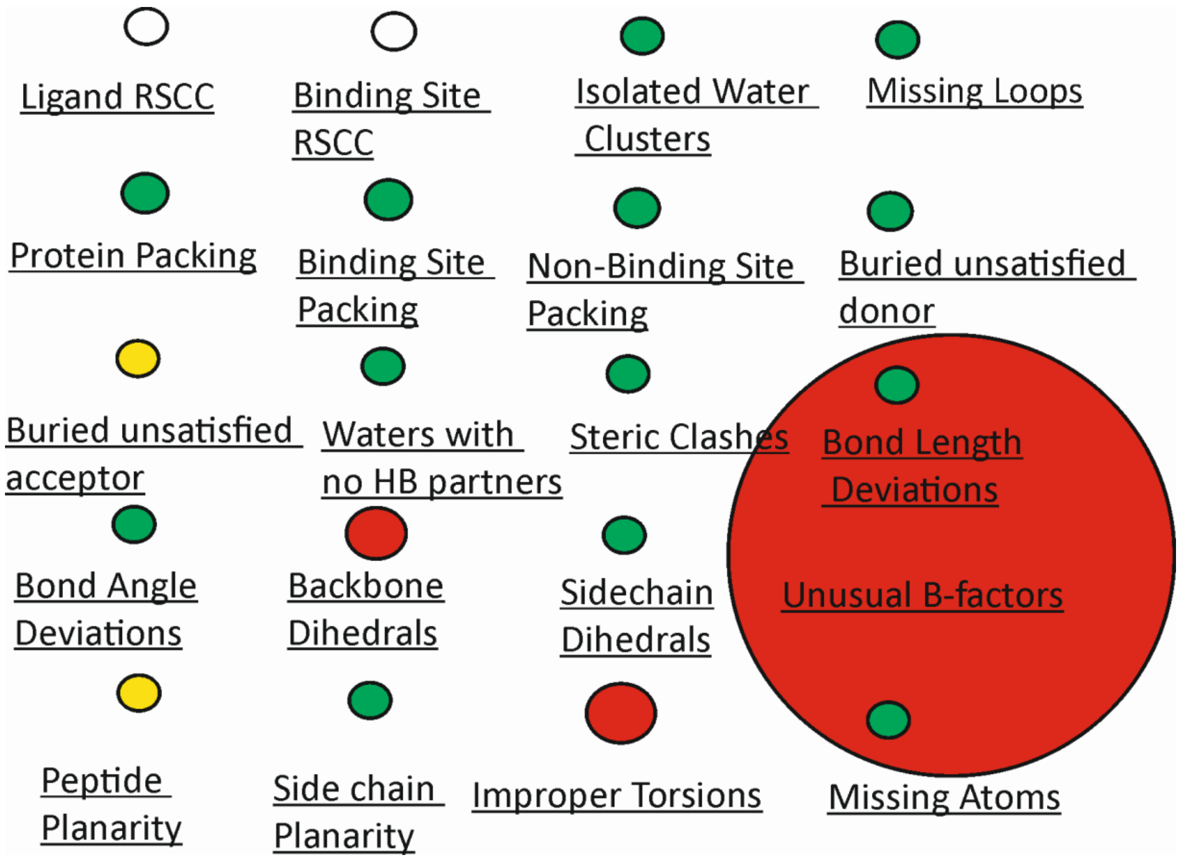
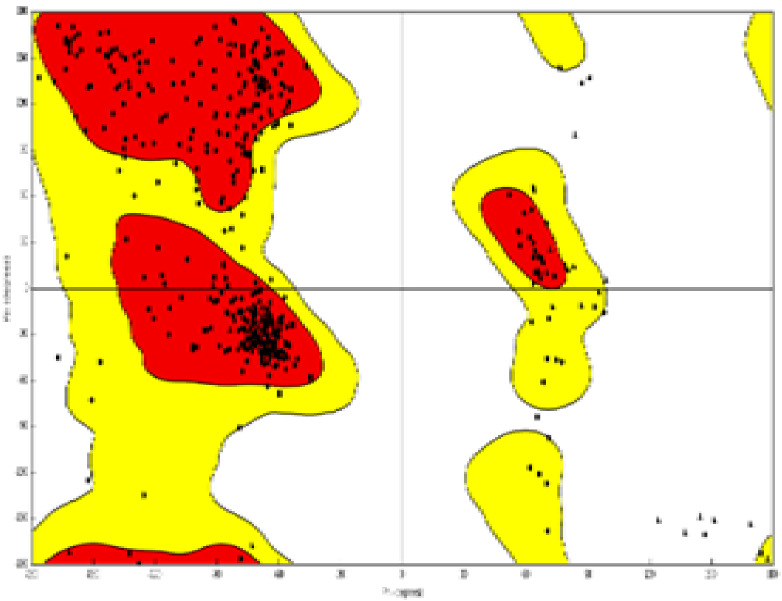


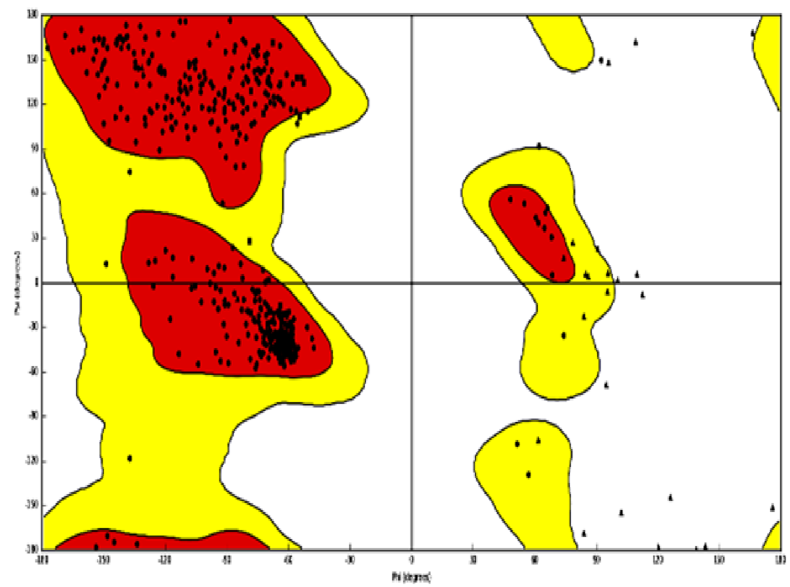
Fig 2: Protein reliability report of the homology modeled *S. aureus* MurD enzyme.

Protein Backbone



Ramachandran plot of MurD enzyme (Uniprot accession code: Q6GHQ2) homology model from *S.aureus*

Ramachandran plot of MurE enzyme X-ray crystal structure (PDB ID- 4C13) from *S.aureus.*



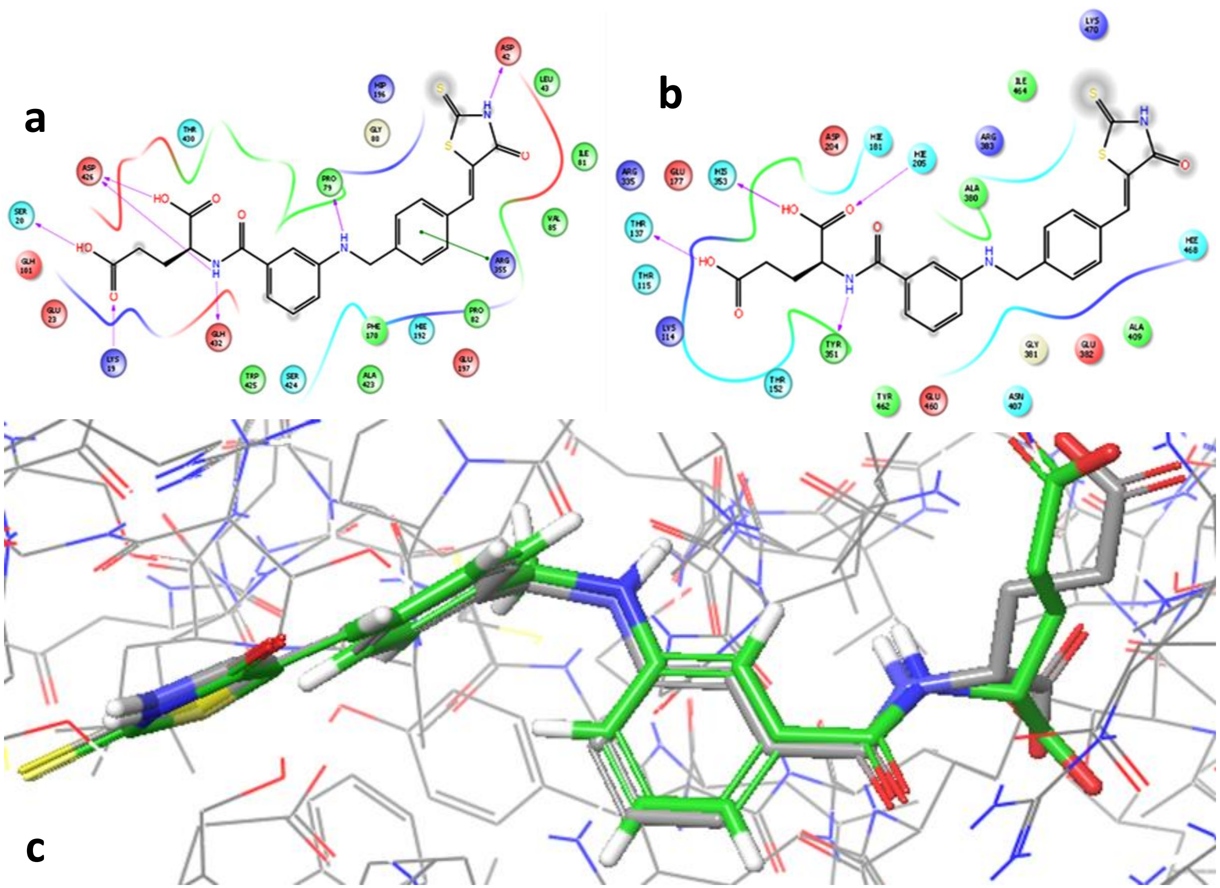


Fig 3: Pictorial representation of docking pose of co-crystal molecule in the active site of **a**) MurD enzyme model **b**) MurE enzyme (4C13) **c**) Overlay of the docking conformation with the co-crystal of ligand (RMSD 0.790).

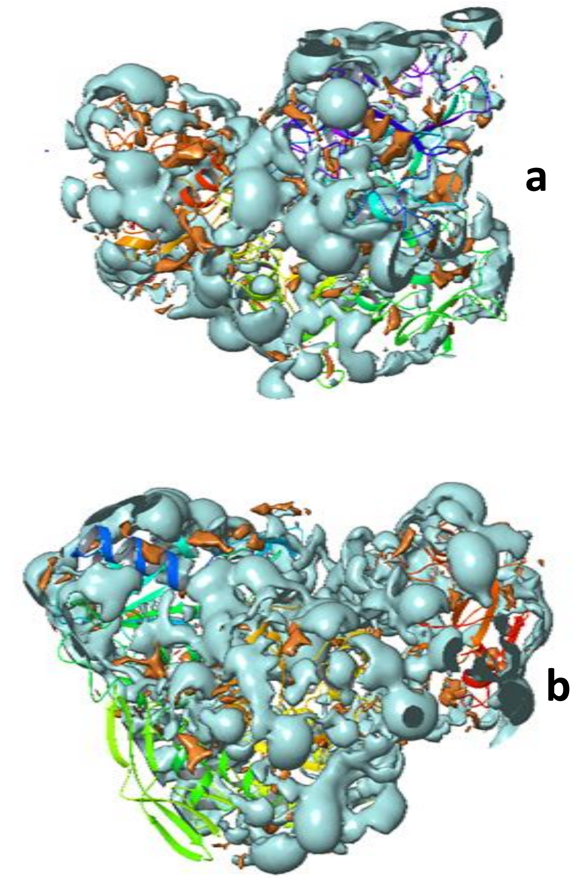


Fig 4: Hydrophillic **(Blue)** and Hdrophobic **(Brown)** surface mapping of the protien **a)** homology model of MurD and **b)** crystalographic structure of MurE (Pdb Id-4C13) *S. aureus*.

S. aureus MurD ligase active site (Modeled protein)

ASINEX inspired by Nature

Enamine

specs

TimTec

Molecular Docking (HTVS, SP and XP) and Binding free energy calculation studies

Molecular Docking (XP) and Binding free energy calculation studies

Optimization of ligands

ADMET prediction

HTVS 4.8 million → **Virtual hits 3820** → **SP-docking 350** → **XP-docking MM/GBSA 70** → **Top 10 virtual hits**

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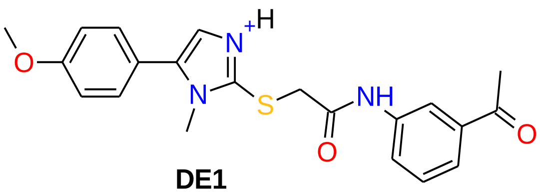
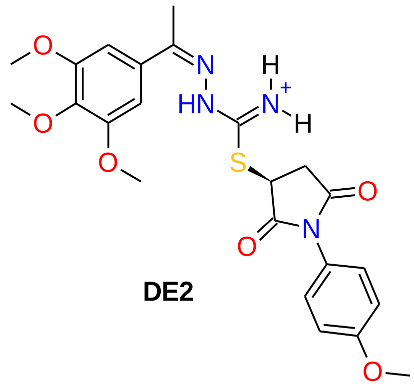
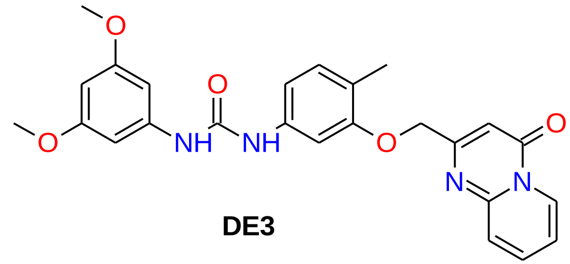
Selected Top 10 HTVS



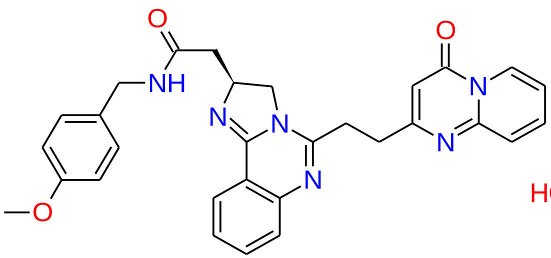
DE1



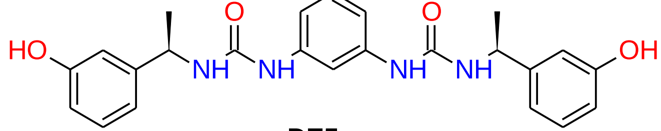
DE2



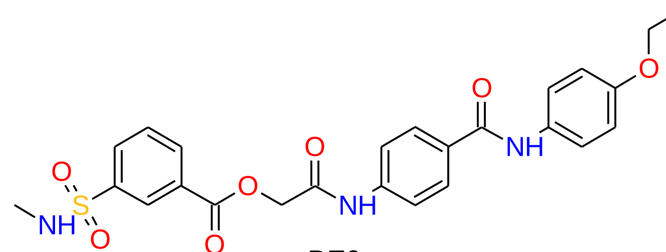
DE3



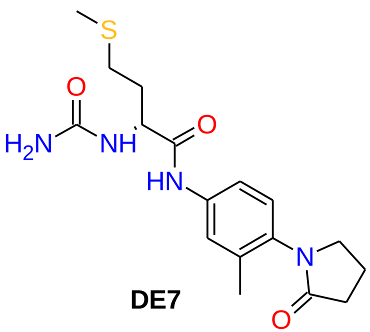
DE4



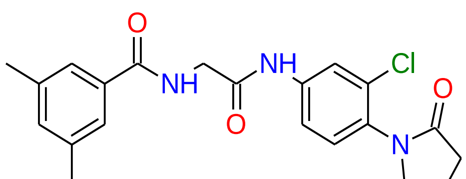
DE5



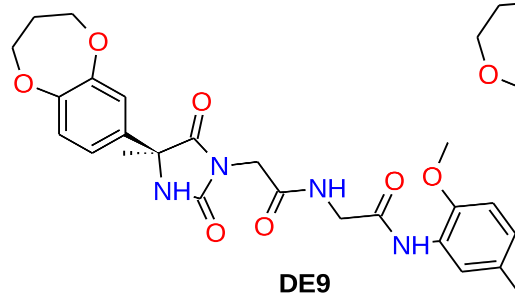
DE6



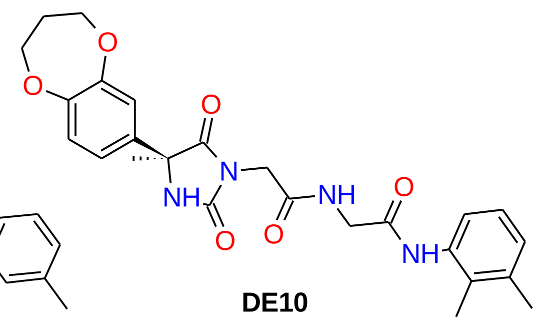
DE7



DE8



DE9



DE10

Docking result of *in silico* HTVS hits (DE1-DE10)

Table 1: Docking result of the virtual hits in MurD homology (Uniprot accession code: Q6GHQ2) model active site of *S .aureus*.

Comp.	Library	docking score	[a]glide ecoul	[b]glide evdw	[c]glide emodel	[d]glide energy	[e]XP Lipophilic EvdW
DE1	Life chemicals F223-0271	-6.2	-9.2	-47.2	-65.2	-48.3	-3.2
DE2	Timtec ST003236	-5.4	-7.2	-38.6	-62.4	-50.6	-3.8
DE3	Chem Div G756-0425	-5.1	-12.6	-36.5	-61.2	-50.3	-3.5
DE4	Chemdiv K279-1370	-5.0	-10.5	-32.0	-60.3	-48.4	-4.8
DE5	Enamine T6806127	-5.5	-11.8	-40.1	-55.6	-55.3	-4.2
DE6	Enamine T5346963	-9.7	-14.6	-42.8	-85.1	-59.2	-3.3
DE7	Enamine T6299159	-9.9	-19.4	-50.2	-102.5	-68.2	-4.9
DE8	Enamine T6067464	-9.5	-17.7	-58.3	-93.7	-65.6	-4.3
DE9	Enamine T6520315	-9.5	-17.5	-45.6	-88.3	-68.5	-3.8
DE10	Enamine T6004991	-9.4	-16.8	-42.3	-93.5	-65.8	-4.1

[a]glide Coulomb energy; [b]glide van der Waals energy; [c]glide model energy; [d]glide energy; [e]glide lipophilic contact plus phobic attractive term in the glide score.

Table 2: Docking result of the virtual hits in the catalytic pocket of *S. aureus* MurE (PDB ID- 4C13) enzyme.

Comp.	Libery	docking score	[a]glide ecoul	[b]glide evdw	[c]glide emodel	[d]glide energy	[e]XP Lipophilic EvdW
DE1	Life chemicals F223-0271	-6.3	-16.20	-36.2	-49.2	-50.0	-3.4
DE2	Timtec ST003236	-6.2	-15.10	-48.5	-43.2	-49.3	-3.1
DE3	Chem Div G756-0425	-7.0	-12.60	-60.0	-81.2	-63.7	-4.1
DE4	Chemdiv K279-1370	-7.2	-9.30	-59.3	-83.2	-62.5	-4.7
DE5	Enamine T6806127	-7.3	-9.80	-65.6	-89.0	-60.5	-4.5
DE6	Enamine T5346963	-5.9	-14.20	-39.2	-40.1	-54.3	-3.3
DE7	Enamine T6299159	-4.1	-11.40	-55.8	-46.3	-38.6	-2.5
DE8	Enamine T6067464	-4.8	-7.70	-42.8	-36.5	-37.2	-2.6
DE9	Enamine T6520315	-5.9	-17.20	-51.8	-53.8	-46.7	-2.7
DE10	Enamine T6004991	-5.7	-10.30	-53.5	-52.1	-48.4	-3.2

Binding free energy calculation of *in silico* HTVS hits (DE1-DE10)

Table 3: Binding free energy calculation of HTVS hits by MM-GBSA approach against *S. aureus* MurD modeled protein.

Comp.	Library	[a]ΔG Coul	[b]ΔGLipo	[c]ΔG Solv GB	[d]ΔG vdW	[e]ΔG
DE1	Life chemicals F223-0271	48.2	-19.2	-3.8	-50.0	-50.0
DE2	Timtec ST003236	35.3	-9.1	-2.8	-52.1	-58.6
DE3	Chem Div G756-0425	12.1	-11.8	-2.3	-49.5	-55.7
DE4	Chemdiv K279-1370	-5.2	-105	9.2	-53.2	-59.3
DE5	Enamine T6806127	-9.1	-17.1	-11.3	-63.8	-67.3
DE6	Enamine T5346963	-3.8	-13.1	-15.7	-66.6	-60.4
DE7	Enamine T6299159	61.1	-6.7	21.0	-69.1	-55.5
DE8	Enamine T6067464	23.6	-5.6	16.8	-68.3	-50.6
DE9	Enamine T6520315	-5.8	-5.5	-12.6	-55.2	-60.6
DE10	Enamine T6004991	-5.2	-7.1	-12.4	-60.3	-61.8

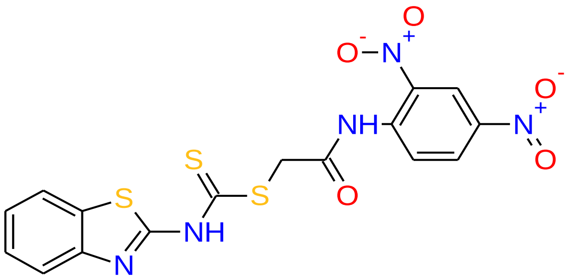
[a]Coulomb energy, [b]Lipophilic energy, [c]electrostatic solvation energy, [d]van der Waal energy, [e]Free energy binding.

Table 4: Free energy calculation by MMGBSA method for MurE (PDB ID-4C13) of *S.aureus* in the active site (kcal/mol)

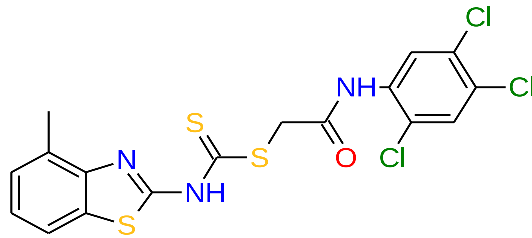
Comp.	Libery	[a]ΔG Coul	[b]ΔG Lipo	[c]ΔG Solv GB	[d]ΔG vdW	[e]ΔG
DE1	Life chemicals F223-0271	-15.5	-25.3	-8.5	-70.8	-73.3
DE2	Timtec ST003236	-7.6	-14.6	-12.4	-73.5	-67.8
DE3	Chem Div G756-0425	-5.4	-13.5	-16.5	-63.5	-64.3
DE4	Chemdiv K279-1370	-4.4	-21.2	-16.2	-73.8	-70.0
DE5	Enamine T6806127	-14.5	-18.7	-18.9	-83.3	-75.7
DE6	Enamine T5346963	-5.2	-11.6	-14.3	-53.8	-60.6
DE7	Enamine T6299159	10.5	-13.6	-12.1	-63.2	-59.4
DE8	Enamine T6067464	-3.6	-7.3	-17.1	-64.6	-56.8
DE9	Enamine T6520315	21.8	-9.3	-13.6	-42.4	-43.7
DE10	Enamine T6004991	19.7	-10.4	-14.1	-47.4	-49.3

[a]Coulomb energy, [b]Lipophilic energy, [c]electrostatic solvation energy, [d]van der Waal energy, [e]Free energy binding.

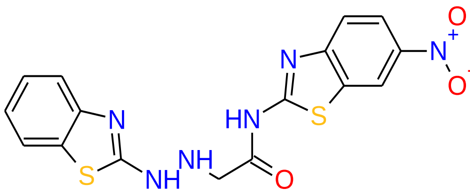
Structure of designed molecules which has been synthesized



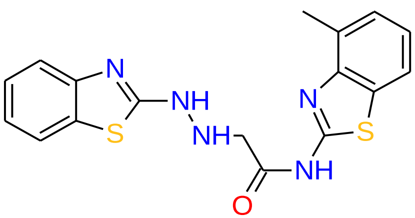
A1



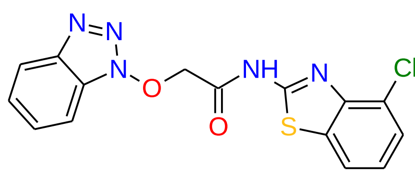
A8



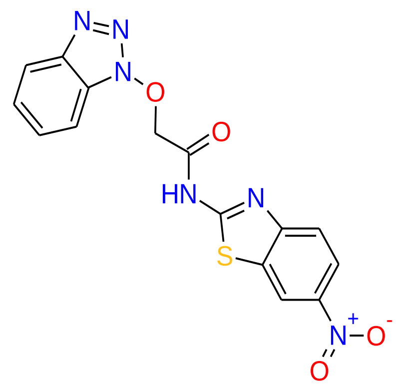
B10



B11



C2



C3

Docking result of designed molecules

Table 5: Docking result of synthesized molecules in the catalytic pocket of *S. aureus* MurD (kcal/mol)

Title	docking score	[a]glide ecoul	[b]glide evdw	[c]glide emodel	[d]XP HBond	[e]XP Lipophili cEvdW	[f]glide energy
A1	-4.5	-7.0	-49.4	-75.4	-0.4	-3.5	-56.4
A2	-6.3	-7.9	-45.0	-75.1	-1.2	-4.4	-52.9
A3	-5.4	-7.9	-54.6	-92.6	-0.7	-4.4	-62.5
A4	-4.6	-8.5	-55.8	-87.0	-1.0	-3.3	-64.3
A5	-5.0	-8.2	-53.0	-81.5	-0.3	-4.3	-61.2
A6	-5.4	-10.8	-51.9	-86.9	-0.3	-4.4	-62.7
A7	-4.8	-5.0	-49.5	-75.4	-0.1	-4.6	-54.6
A8	-6.1	-5.8	-46.9	-71.5	-0.9	-4.7	-52.6
B1	-5.5	-11.1	-38.2	-63.9	-1.9	-2.6	-49.4
B2	-4.7	-14.8	-32.2	-60.1	-1.9	-1.8	-47.0
B3	-6.3	-12.4	-39.0	-68.6	-1.9	-3.1	-51.4
B4	-5.8	-11.0	-40.7	-65.8	-2.1	-2.9	-51.7
B5	-6.3	-14.9	-39.3	-68.3	-2.2	-2.9	-54.3
B6	-5.4	-10.1	-35.2	-59.2	-1.9	-2.4	-45.3
B7	-5.3	-10.8	-39.8	-69.6	-1.9	-2.4	-50.6
B8	-5.2	-6.5	-48.7	-77.5	-0.9	-4.0	-55.2
B9	-5.2	-12.1	-40.9	-70.1	-1.2	-3.0	-53.0
B10	-5.7	-15.5	-35.6	-75.8	-1.8	-2.7	-51.1
B11	-5.2	-10.3	-43.7	-68.4	-1.2	-3.3	-54.1
C1	-4.6	-7.6	-35.8	-55.5	-0.6	-3.3	-43.4
C2	-4.7	-9.7	-40.0	-59.4	-0.5	-3.5	-49.7
C3	-4.1	-5.9	-43.2	-65.6	-0.7	-3.4	-49.1
C4	-4.8	-5.4	-38.8	-58.9	-1.1	-3.5	-44.2
C5	-4.7	-2.6	-38.5	-54.3	-0.8	-3.4	-41.1

Table 6: Docking result of synthesized molecules in the catalytic pocket of *S. aureus* MurE (PDB ID-4C13) (kcal/mol)

Title	docking score	[a]glide ecoul	[b]glide evdw	[c]glide emodel	[d]XP HBond	[e]XP Lipophilic EvdW	[f]glide energy
A1	-4.5	-4.2	-45.2	-67.5	-0.4	-4.0	-49.4
A2	-3.7	-5.1	-36.1	-57.2	-0.6	-2.9	-41.2
A3	-3.6	-5.5	-44.6	-70.3	0	-3.5	-50.1
A4	-4.1	-4.1	-47.2	-66.7	-0.5	-3.6	-51.4
A5	-4.7	-5.0	-44.4	-65.6	-0.5	-4.1	-49.4
A6	-4.0	-5.2	-41.3	-65.6	-0.5	-3.3	-46.5
A7	-3.4	-4.0	-41.8	-66.2	-0.7	-3.7	-45.8
A8	-4.1	-3.2	-49.2	-71.3	-0.7	-4.1	-52.4
B1	-6.5	-9.7	-40.6	-63.3	-2.0	-3.6	-50.4
B2	-7.7	-14.3	-41.5	-75.7	-1.7	-3.5	-55.9
B3	-7.4	-16.5	-34.6	-70.6	-2.9	-1.8	-51.1
B4	-8.0	-13.3	-39.1	-81.9	-1.9	-3.5	-52.4
B5	-6.1	-13.4	-42.1	-70.9	-1.8	-3.2	-55.6
B6	-4.1	-6.8	-31.8	-49.0	-0.7	-2.4	-38.6
B7	-5.1	-5.6	-47.8	-59.1	-0.1	-4.3	-53.4
B8	-5.0	-9.4	-38.7	-61.7	-2.1	-3.1	-48.1
B9	-5.0	-6.5	-39.4	-61.1	-1.3	-2.8	-45.9
B10	-5.3	-7.6	-32.8	-64.4	-2.0	-2.5	-40.5
B11	-6.0	-9.2	-42.3	-65.5	-2.6	-3.5	-51.5
C1	-3.3	-5.7	-30.7	-49.0	-0.7	-2.1	-36.4
C2	-4.0	-4.5	-35.4	-56.0	-0.3	-2.4	-40.0
C3	-4.2	-8.1	-39.7	-61.8	-0.4	-2.4	-47.8
C4	-3.8	-4.9	-38.8	-59.3	-0.9	-3.4	-43.7
C5	-4.6	-4.8	-31.6	-49.8	-0.8	-2.1	-36.4

[a]glide Coulomb energy; [b]glide van der Waals energy; [c]glide model energy; [d]extra-precision hydrogen bond [e]glide lipophilic contact plus phobic attractive term in the glide score; [f]glide energy.

Table 7: Binding free energy (MM-GBSA) calculation of designed molecules and *S. aureus* MurD complexes (kcal/mol)

Title	[a]ΔG Bind Coulomb	[b]ΔG Bind Lipo	[c]ΔG Bind Solv GB	[d]ΔG Bind vdW	[e]ΔG Bind
A1	-42.4	-37.0	26.1	-109.2	-123.9
A2	-36.4	-40.0	38.3	-88.0	-90.7
A3	-51.9	-25.8	42.2	-79.1	-81.4
A4	-26.5	-36.6	12.9	-100.5	-108.5
A5	-39.7	-22.9	21.9	-48.8	-81.8
A6	-35.3	-33.1	19.6	-85.2	-111.5
A7	-73.0	-32.9	53.9	-78.0	-85.9
A8	-126.2	-45.1	106.7	-88.6	-115.6
B1	-39.3	-19.1	3.0	-26.0	-85.4
B2	-16.4	-29.9	19.7	-87.3	-87.1
B3	30.9	-23.7	-23.5	-55.3	-71.1
B4	33.2	-23.6	-28.5	-49.4	-63.1
B5	-29.3	-30.6	48.8	-87.3	-58.9
B6	-4.0	-23.2	-5.2	-39.9	-59.6
B7	-70.7	-33.9	29.2	-69.6	-107.4
B8	-21.2	-25.7	32.0	-61.9	-70.6
B9	-4.3	-15.4	3.6	-52.3	-57.5
B10	-44.6	-11.8	24.0	-41.7	-65.8
B11	-13.6	-22.7	42.0	-93.4	-59.1
C1	-69.1	-28.0	57.2	-59.3	-86.1
C2	-75.7	-31.6	44.2	-87.1	-111.4
C3	-8.4	-21.7	6.2	-68.6	-85.0
C4	-5.9	-16.9	-17.4	-52.3	-83.1
C5	-60.7	-30.1	51.2	-71.5	-85.2

Table 8: Binding free energy (MM-GBSA) calculation of designed molecules and *S. aureus* MurE (pdb.C13)

Title	[a]ΔG Bind Coulomb	[b]ΔG Bind Lipo	[c]ΔG Bind Solv GB	[d]ΔG Bind vdW	[e]ΔG Bind
A1	88.7	-9.6	-54.1	-55.6	-52.1
A2	-24.9	-3.3	33.4	-18.2	-41.7
A3	21.2	-14.8	-9.4	-49.6	-62.7
A4	69.1	-15.6	-18.2	-50.5	-42.6
A5	-24.3	-20.5	15.6	-42.3	-73.3
A6	77.1	-18.4	-30.6	-37.9	-44.7
A7	-29.9	-19.8	31.8	-66.3	-69.4
A8	-118.1	-5.5	79.3	0.2	-45.2
B1	-14.6	-1.0	13.5	-10.8	-46.2
B2	68.0	1.1	-38.4	-38.5	-31.9
B3	26.7	-2.1	-2.0	-25.5	-25.3
B4	15.2	2.1	-24.9	-14.6	-37.2
B5	7.9	-0.1	18.6	-13.9	-8.2
B6	-44.3	-5.4	35.6	-8.5	-26.3
B7	-40.9	-4.7	59.9	-17.7	11.4
B8	-73.4	-1.8	37.8	-1.9	-57.7
B9	-45.7	0.2	45.3	-17.7	-42.7
B10	-7.9	2.7	-11.5	-4.0	-54.0
B11	-45.0	2.6	29.7	-4.5	-50.5
C1	-19.0	-5.7	45.7	-17.5	-13.6
C2	7.5	-5.3	36.0	-43.3	-23.2
C3	-5.6	-4.1	57.8	-47.8	8.4
C4	-47.9	-5.5	37.9	-13.2	-38.9
C5	-36.1	2.4	41.7	-9.5	-15.9

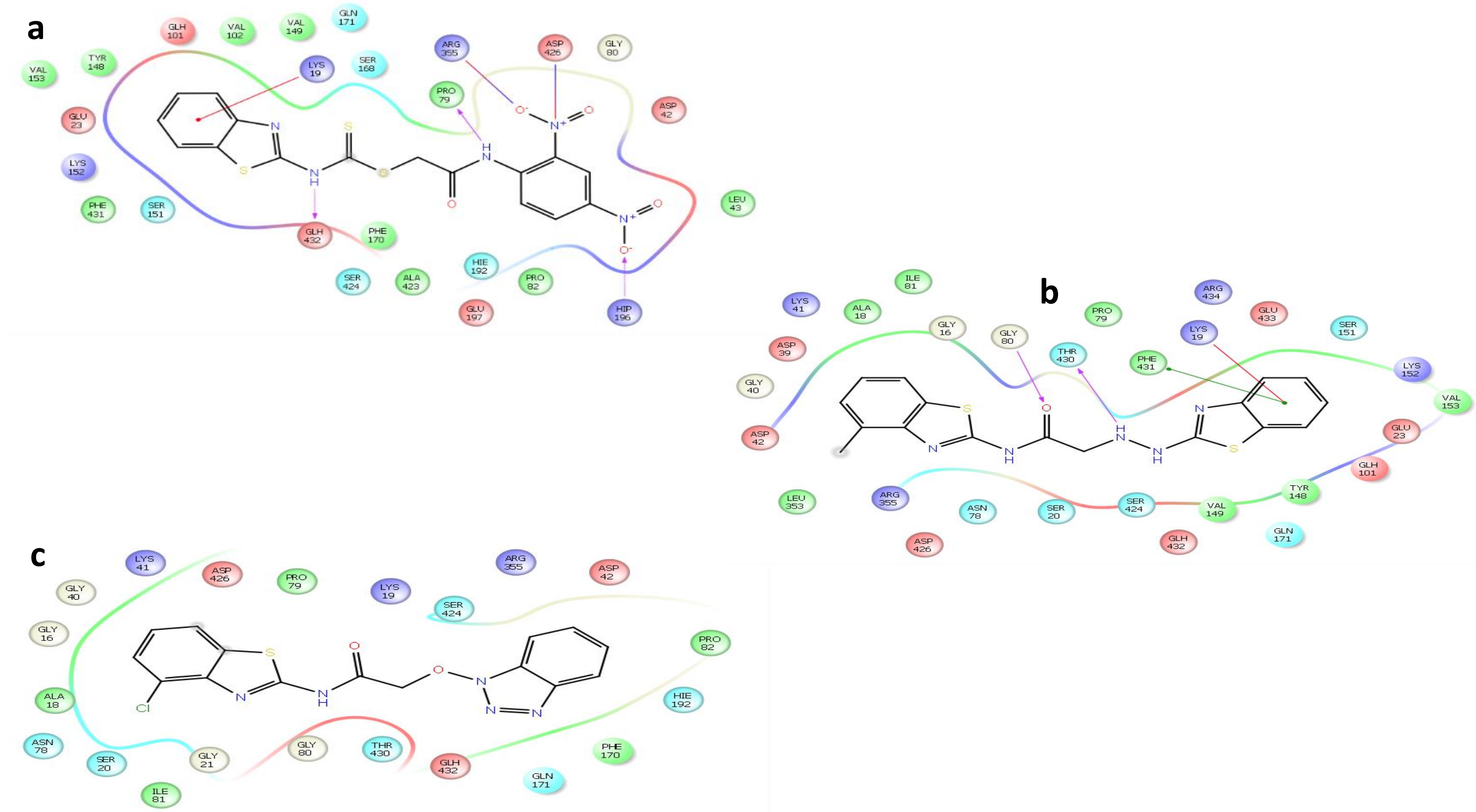


Figure 5: 2D-Pictorial representation of docking interaction in the binding pocket of MurD *S. aureus* homology model (2XPC) of compound **(a) A1**, **(b) B11**, **(c) C2.**

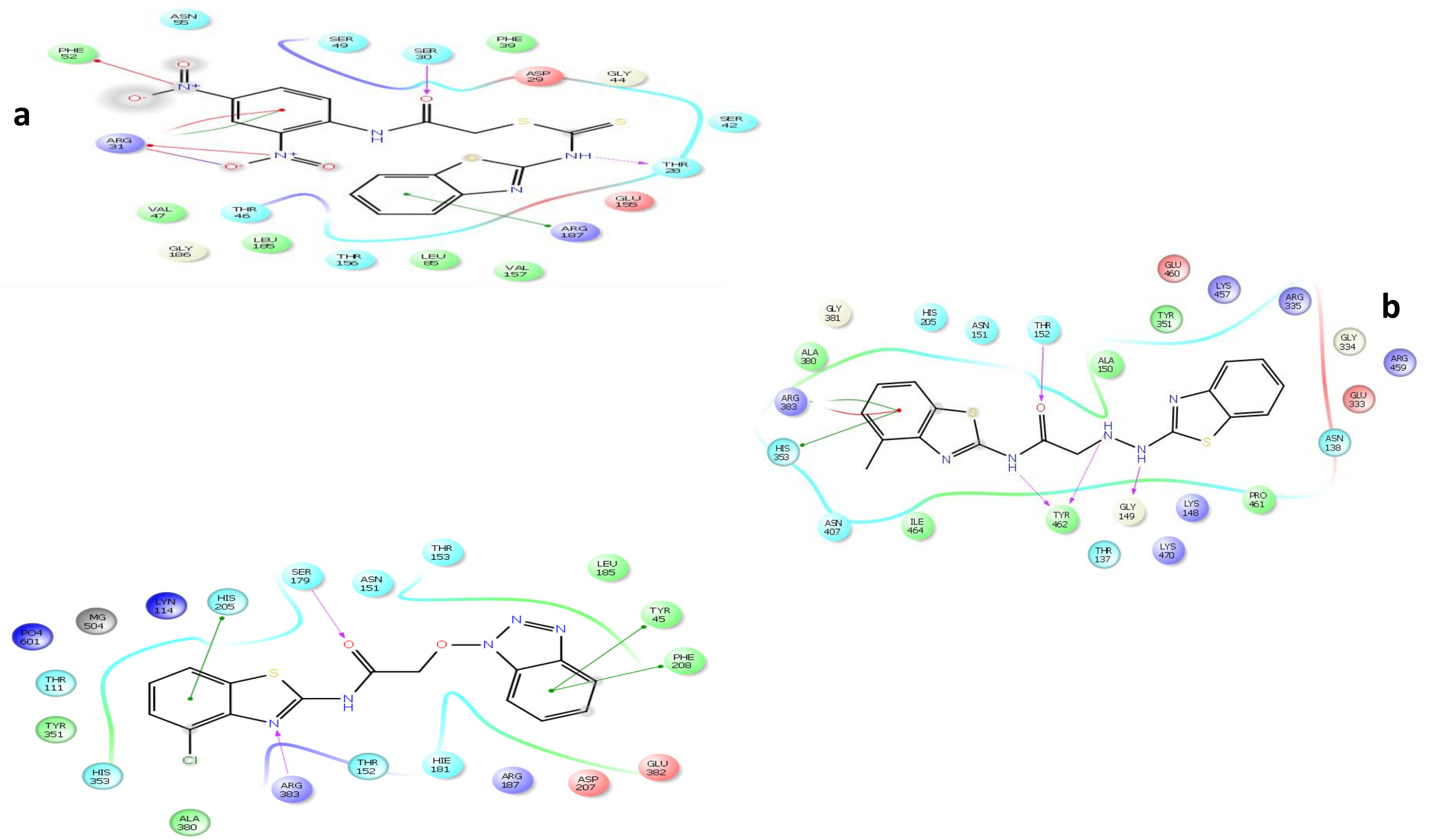
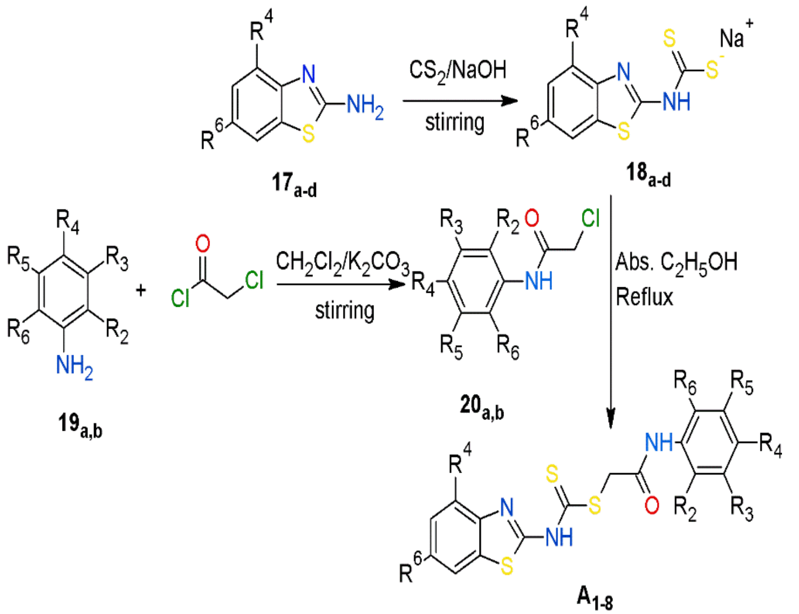


Figure 6: 2D-Pictorial representation of docking interaction in the binding pocket of *S. aureus* MurE (4C13) of compound **A1 (a)**, **(b) B11**, **(c) C2**.

Table 9: Predicted ADMET profile of synthesized compounds.

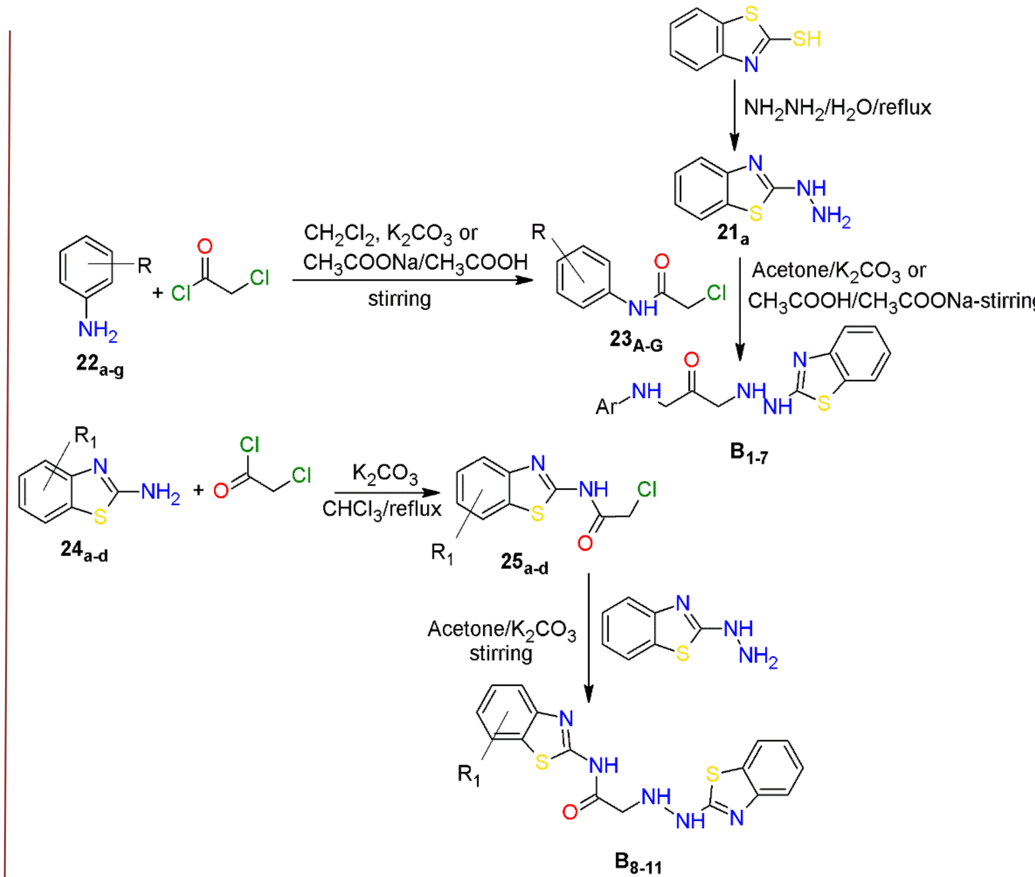
Title	CNS	SASA	Donor HB	Acceptor HB	QP log Po/w	QP PCaco	PSA	Rule Of Three	Rule Of Five
A1	-2	699.2	2	8	2.4	33.7	154.6	0	0
A2	2	684.4	2	5	5.5	3186.7	50.8	1	1
A3	-2	728.6	2	9	1.5	4.2	196.8	1	1
A4	-2	745.0	2	8.7	2.5	23.2	164.5	0	1
A5	0	767.9	2	6.7	5.3	1295.7	77.9	1	2
A6	-2	692.4	2	7	3.9	145.9	116.1	1	1
A7	-2	687.8	2	8	2.4	36.2	152.7	0	0
A8	2	709.1	2	5	5.7	3348.4	49.5	1	1
B1	-2	606.5	2	6	1.4	17.9	126.2	1	0
B2	-2	626.8	3	7	-0.2	3.9	131.2	1	0
B3	-2	656.5	3	6.7	-0.1	2.5	149.8	1	0
B4	-2	642.4	3	8	-1.0	0.7	171.0	1	0
B5	-2	637.6	2	6	0.9	7.2	125.4	1	0
B6	1	563.6	2	5	2.1	157.6	81.2	0	0
B7	-2	651.0	2	6	2.0	28.4	120.3	0	0
B8	1	716.3	1	6.7	3.5	224.5	83.8	0	0
B9	1	627.5	2	6.5	2.3	145.0	89.3	0	0
B10	-2	655.7	2	7.5	1.6	16.2	133.7	1	0
B11	1	624.3	2	6.5	2.5	157.8	86.3	0	0
C1	0	594.6	1	6	3.3	1051.2	75.4	0	0
C2	-1	602.7	1	7.5	2.3	549.4	90.0	0	0
C3	-2	620.2	1	8.5	1.1	56.1	136.3	0	0
C4	-2	666.2	1	8.2	2.3	464.9	98.9	0	0
C5	-2	581.4	1	7.5	1.8	483.0	90.8	0	0
Recommended limits	-2 to +2	300-1000	0.0-6.0	2.0-20.0	-2 to 6.5	<25 poor > 500 great nm/sec	7-200	Max 4	Max 3

Synthesis Scheme (Scheme 1& 2)



Scheme 1

Fig 7: Schematic representation of synthesis **Scheme 1**.



Scheme 2

Fig 8: Schematic representation of synthesis **Scheme 2**.

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Synthesis scheme 3

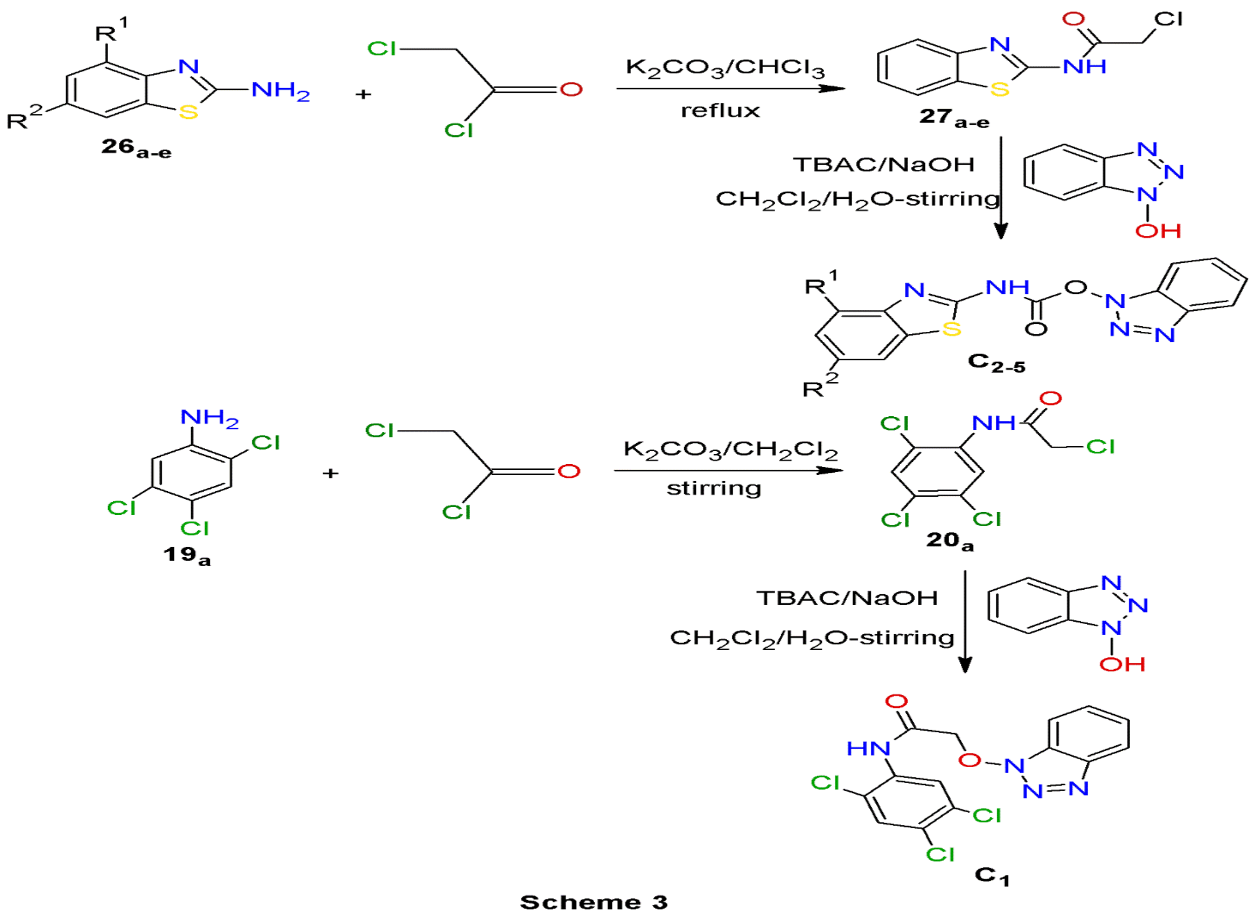

Fig 9: Schematic representation of synthesis **Scheme 3**.

Spectral data of the synthesized compound **A1**

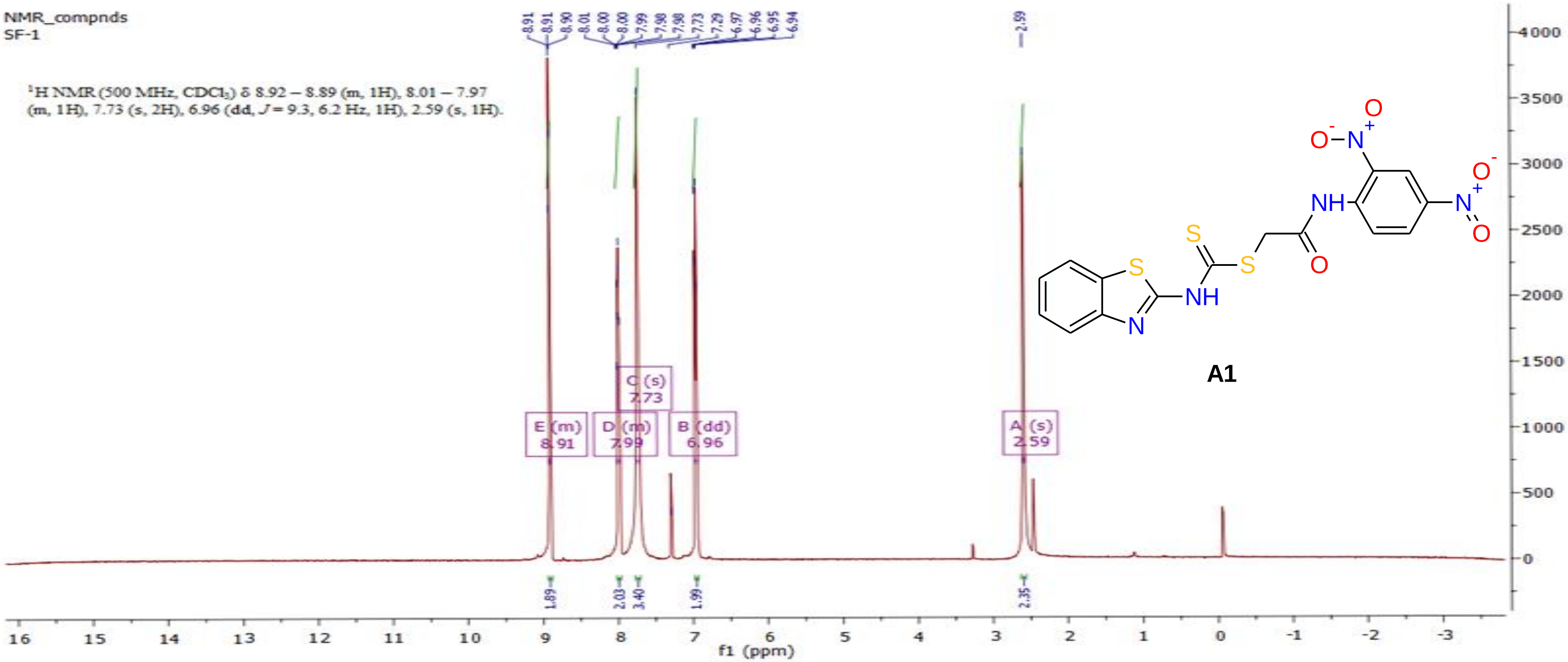

Fig 10: H[1] NMR spectrum of the synthesized compound **A1**.

Spectral data of synthesized compound **B11**

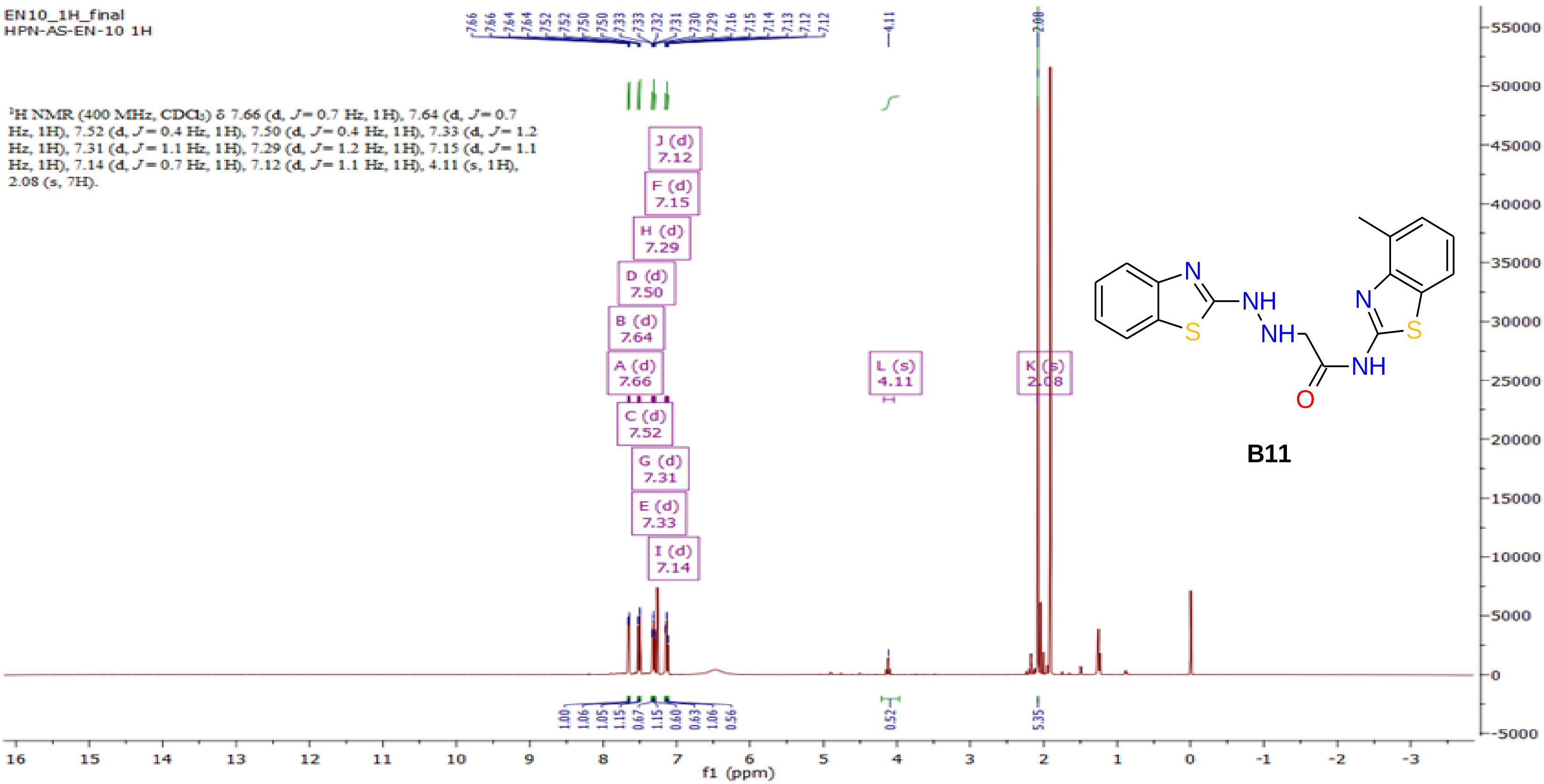

Fig 11. H[1] NMR of synthesized compound **B11**.

Spectral data of synthesized compound B11

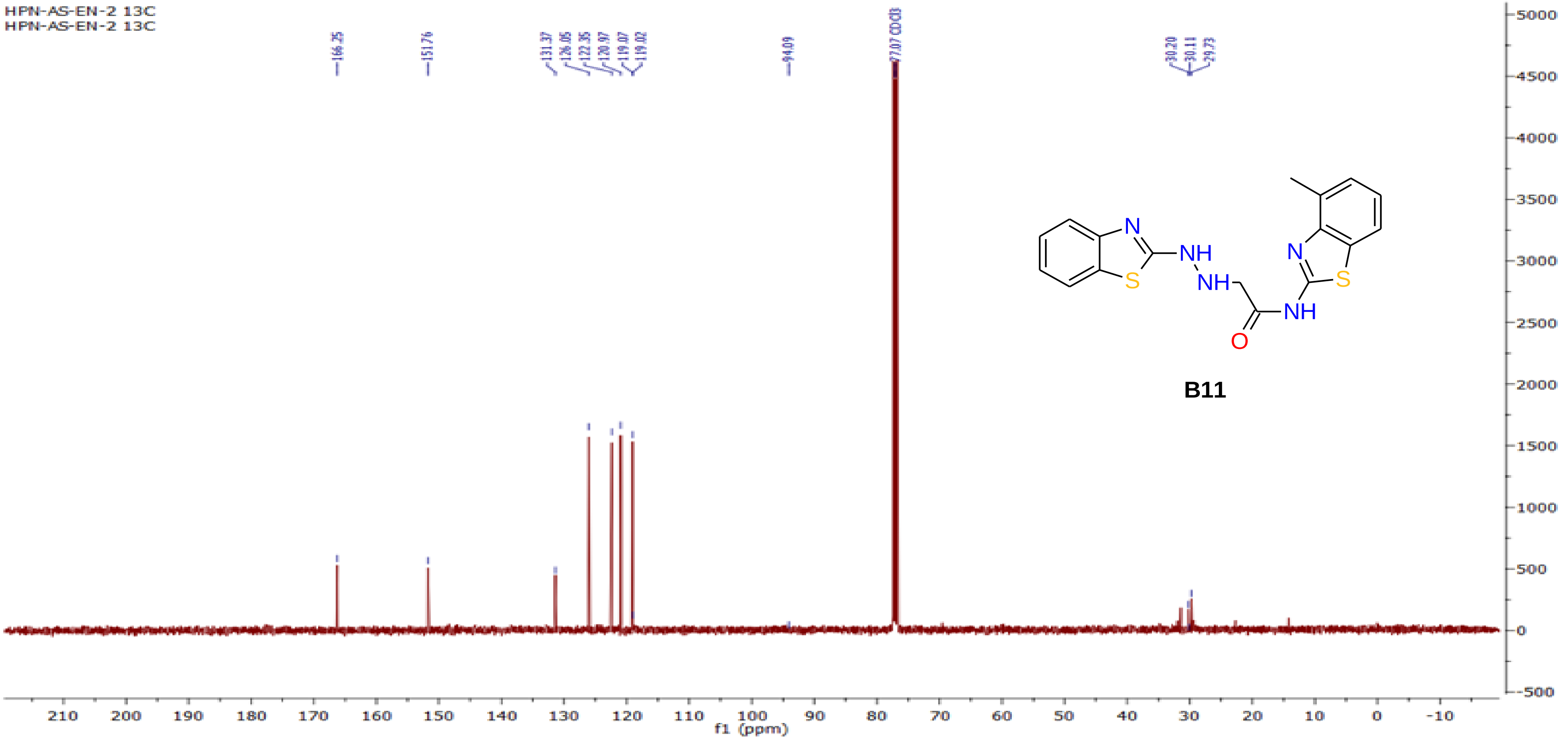

Fig 12: C^{13} NMR of the synthesized compound **B11**.

Molecular dynamics study of literature molecule

Fig 13: The ligand-receptor complex during MD simulation different plots represent a) RMSD plot of C-α and backbone of the enzyme b) RMSF of all the MurE enzyme residues c) interaction of residues and types of interaction with time fraction for whole simulation time d) interaction profile of the ligand with different residues of MurE enzyme (**PDB ID 4C13**).

Molecular dynamics study of literature molecule

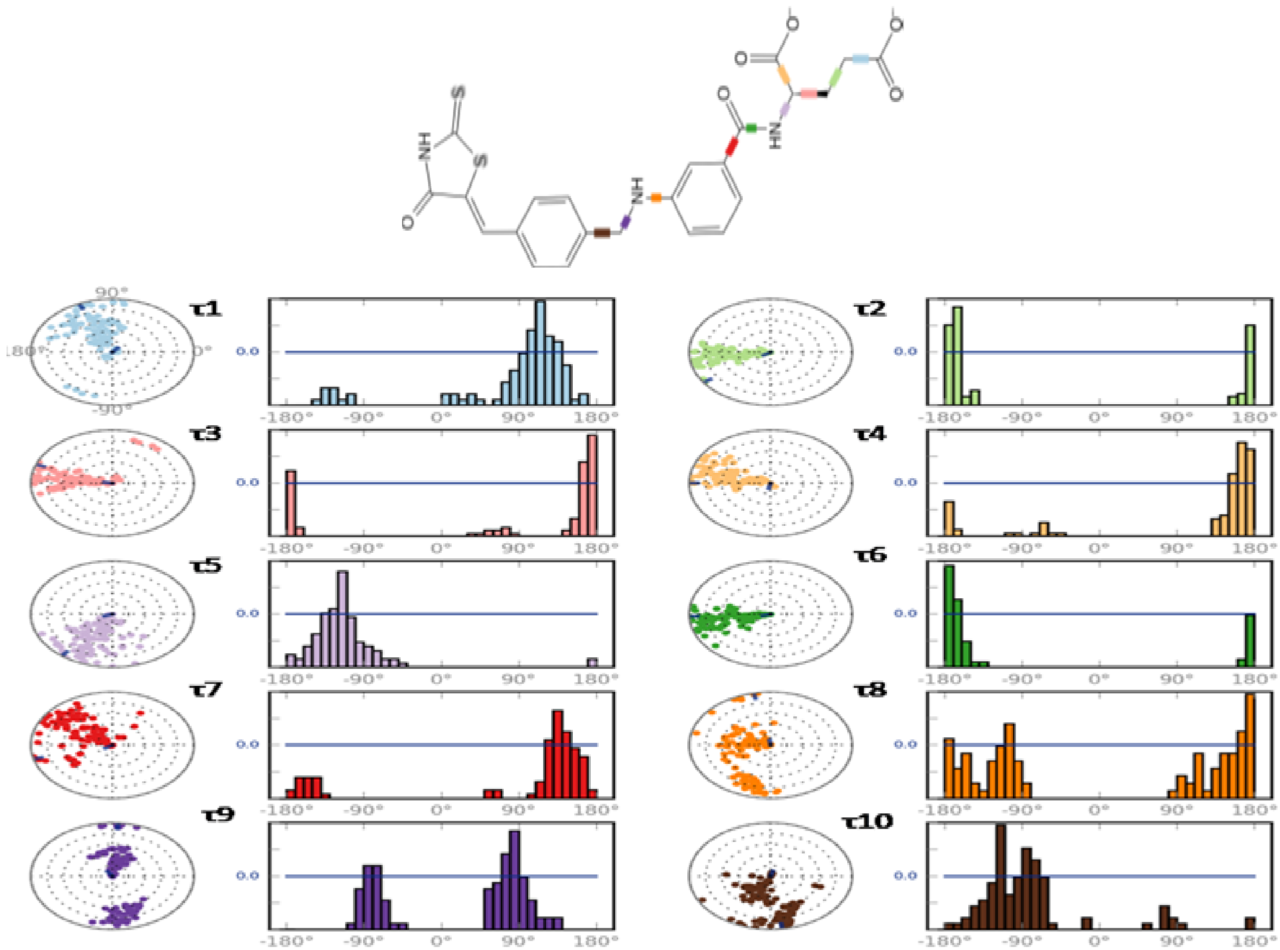

Fig 14: Representation of all the rotatable bonds torsion angle which represent the conformational change of the ligand during the whole MD simulation.

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RESEARCH ARTICLE

An explorative study on *Staphylococcus aureus* MurE inhibitor: induced fit docking, binding free energy calculation, and molecular dynamics

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ABSTRACT

Staphylococcus aureus MurE enzyme catalyzes the addition of L-lysine as third residue of the peptidoglycan peptide moiety. Due to the high substrate specificity and its ubiquitous nature among bacteria, MurE enzyme is considered as one of the potential target for the development of new therapeutic agents. In the present work, induced fit docking (IFD), binding free energy calculation, and molecular dynamics (MD) simulation were carried out to elucidate the inhibition potential of 2-thioxothiazolidin-4-one based inhibitor 1 against *S. aureus* MurE enzyme. The inhibitor 1 formed majority of hydrogen bonds with the central domain residues Asn151, Thr152, Ser180, Arg187, and Lys219. Binding free-

ARTICLE HISTORY
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KEYWORDS
MurE inhibitor; induced fit docking; binding free energy; molecular dynamics

Table 10: MIC$_{50}$ values of all compound **A1** and **C2**.

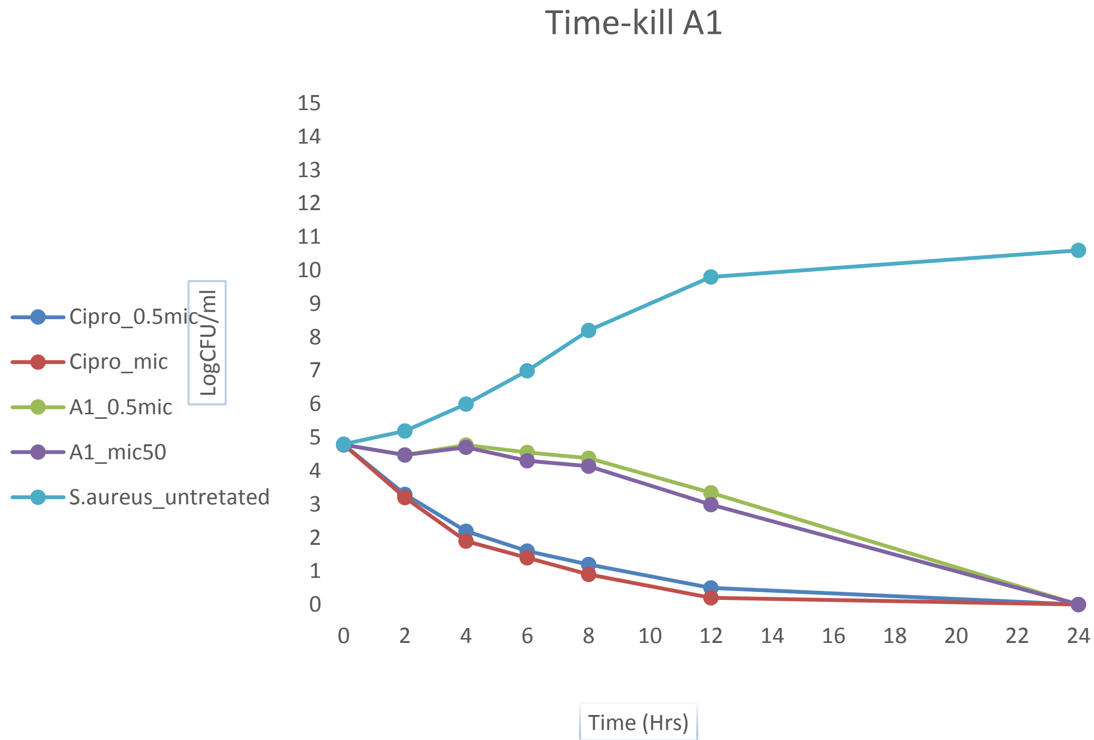
Title	[a]SA-5021	[b]SA-5022	[c]SA-43300	[d]KP-2706	[e]PA-2036	[f]EC-2567
A1	0.64	28.28	5.56	17.04	1.4	1.7
B11	72.37	2.07	12.98	36.41	1.41	1.72
C2	**69.41**	**8.27**	**1.41**	**>250**	**1.45**	**1.70**
Ciprofloxacin	10.34	6.37	16.49	1.39	6.87	1.55

(a) *S. aureus* (NCIM-5021) (b) *S. aureus* (NCIM-5022) (c) *S. aureus* (ATCC-43300) (d) *K. Pneumonia* (NCIM-2706) (e) *P. aureginosa* (NCIM-2036) (f) *E. coli* (NCIM-2567). **Concentration range has been used 250-1.95 µg/ml and as per CLSI guidelines.

Table 11. MBC values of compounds **A1** and **C2** 3 strains of *S. aureus* (µg/ml)

Title	MRSA (ATCC 43300)			*S. aureus* (NCIM 5021)			*S. aureus* (NCIM 5022)		
	MIC	MBC	MBC/ MIC*	MIC	MBC	MBC/ MIC*	MIC	MBC	MBC/ MIC*
A1	5.56	62.5	11.24	0.64	~~-do-~~	-	28.28	72.5	2.56
C2	1.41	10	7.09	69.41	15	0.21	8.27	12.5	1.51

***Values ≤4 are bactericidal and ≥32 are having the chances of developing tolerance**



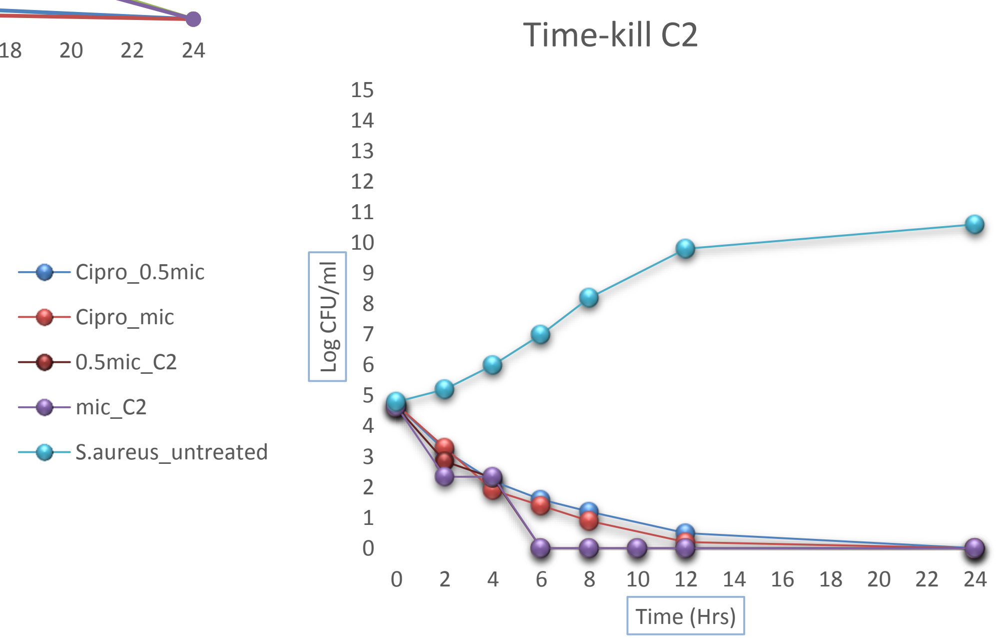


Fig 15: Graphical representation of Time kill studies of synthesized compound **A1** and **C2** against MRSA strain (ATCC-43300) using ciprofloxacin as a standard.

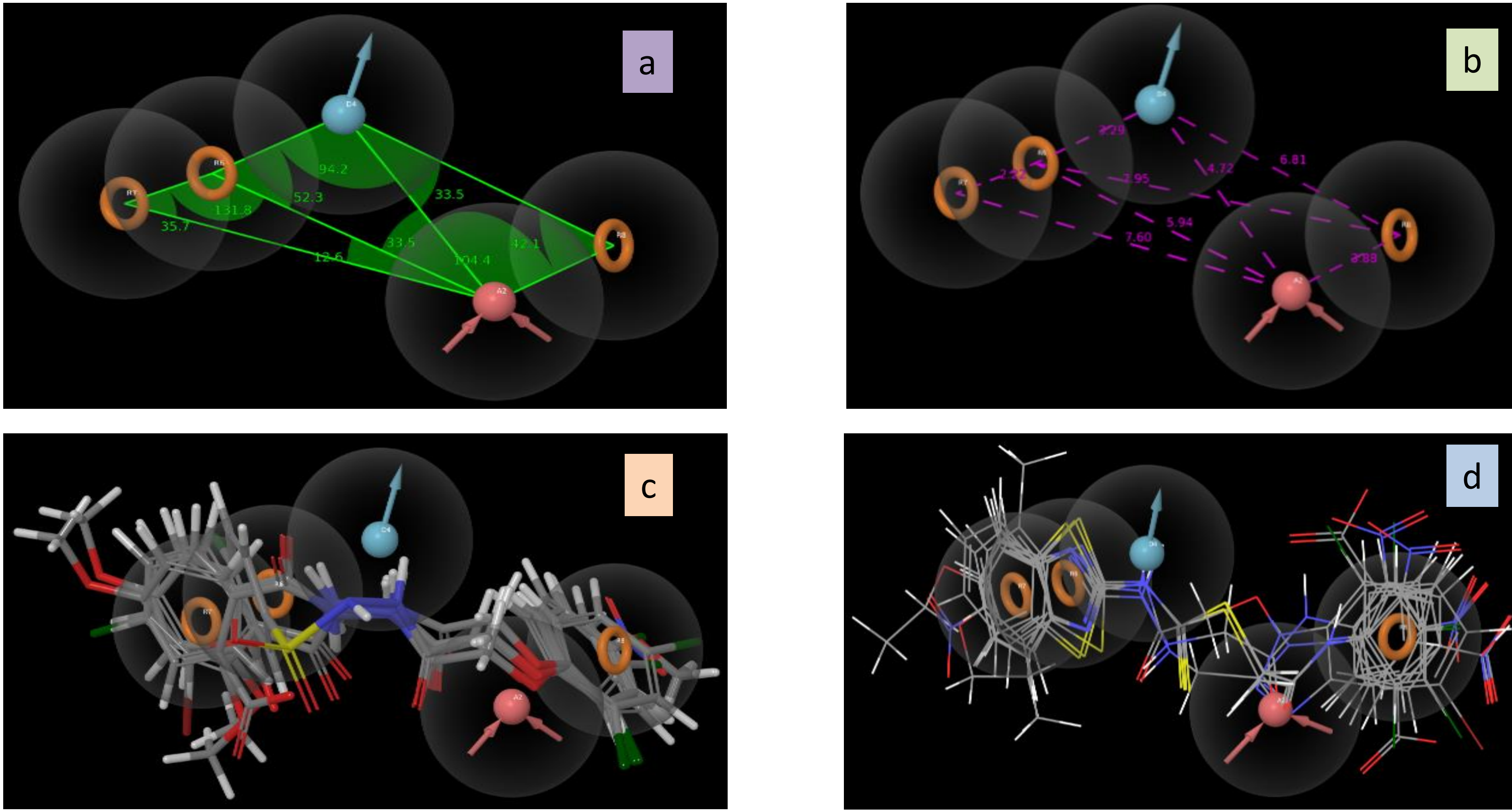
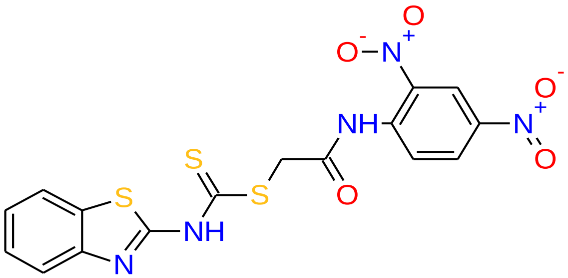


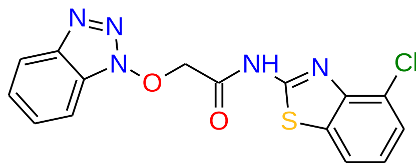
Fig 16: Represents pharmacophore model ADRRR_1 inter-site (a) distances in Å unit and (b) angles between the pharmacophoric points. Hydrogen bond acceptor (A): Pink sphere with arrow; Aromatic ring (R): yellow open circle; Hydrogen bond donor (D): blue sphere with arrow. (c) alignment of active compounds on the generated pharmacophore model (d) alignment of inactive compounds on the generated pharmacophore model.

Conclusion



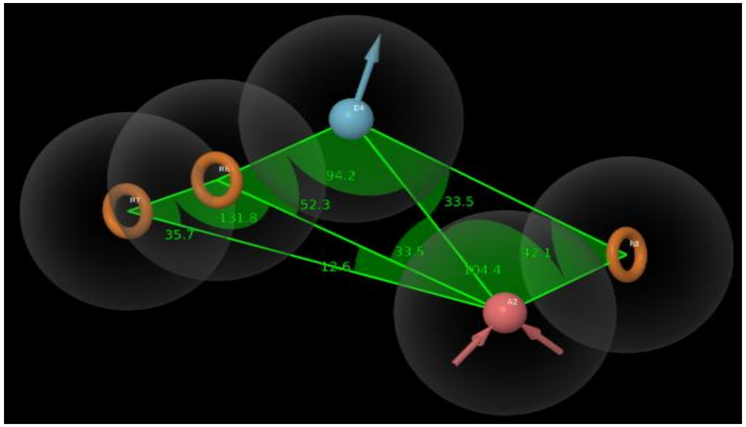
A1

MIC **5.56** and MBC **62.5** µg/ml (ATCC-43300)MRSA

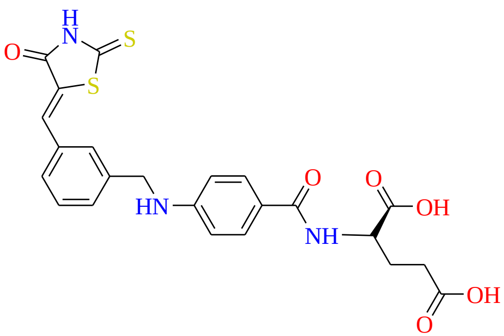


C2

MIC **1.41** and MBC **10** µg/ml (ATCC-43300) MRSA



Literature Molecule



S. aureus MurD (IC$_{50}$ **6.4** µM)and MurE (IC$_{50}$ **17** µM) and *E. coli* MurD (IC$_{50}$ **8.2**µM) and MurE (IC$_{50}$ **180** µM) MIC- S.aureus (MRSA) 8 µg/ml

The finding from our studies, the literature molecule MIC is higher than compound A1 and C2 which attributes the penetration through the cell membrane. The penetration problem may be the presence of ionized glutamic acid which is hindering the penetration for the literature molecule. Compound A1 showed MIC$_{50}$ 5 fold more than compound C2 and also the MBC is higher , which can be used for lead optimization . This two compound can further used for in-vitro studies for understanding the mechanism of action which will in turn help to develop the lead. All the previous attempt has failed because of the imbalance in the MIC and IC$_{50}$, which we think our studies can be one of the best way to develop novel antibiotic by targeting novel enzymes.

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