European Journal of Molecular & Clinical Medicine

ISSN 2515-8260 Volume 07, Issue 11, 2020

SCREENING AND IDENTIFICATION OF POTENTIAL INHIBITORS AGAINST UDP-N ACETYL GLUCOSAMINE ENOLPYRUVYL TRANSFERASE(MURA) IN STREPTOCOCCUS PNEUMONIA: AN INSILICO APPROACH

Kutagolla Peera[1*], M. Abdul Kareem[2], Syed Rahamthulla[3] and M. Srinivasulu Reddy[1]

[1]DBT -Bioinformatics Infrastructure Facility, Department of Zoology, Sri Venkateswara University Tirupati-

[2]Dept. of Biochemistry, School of Sciences, Indira Gandhi National Open University (IGNOU), New Delh
i.

[3]Pathgene Health Care Pvt. Ltd., Tirupati-517502, Andhra Pradesh, India.

***Corresponding author** Email: drkutagollapeera2014@gmail.com

Abstract:

*Streptococcus pneumoniae are the most common cause of pneumonia and meningitis in infants and adults. Peptidoglycan layer is an essential component of cell wall composed of alternating units of N-acetylglicosamine (GlcNAc) and N-acetylmuramic acid (MurNAc). UDP-N-acetylglucosamine enolpyruvyl transferase (MurA) is a key enzyme involved in peptidoglycan synthesis. The Biosynthesis of peptidoglycan in cytoplasm is catalysed by MurA which transfer enolpyrul group from phosphophenol pyruvate (PEP) to UDP Nacetylglucosamine to form UDP -N-acetylenolpyruvate. Present study was aimed to develop novel antimicrobial agents against MurA enzyme through insilico analysis approach. The three dimensional structure of MurA enzyme was modelled computationally by using Modeller9v10. Later this enzyme model subjected to molecular dynamics simulations using NAMD 2.5 software with CHARMM27 force field tip 3p model of water. Initially energy minimization carried 5000 runs for 10 ps time and subsequent minimized model was simulated with 1,00,000 runs for 2 ns time period. The final resolved model reliability was assessed by prochek using Ramchandran plot calculations, verify 3D and WHATCHECK programs. From Zinc database 5000 similar structure compounds with fosfomycin were virtually screened against MurA by Autodock vina in PyRx virtual screening tool. The docking results reveal that the compounds Zinc50247, Zinc3020559, Zinc1884559, Zinc3154681, Zinc19286884, Zinc58219 and Zinc3978065 have -10.3, -10.1, -9.8, -9.8, - 9.2, -9.1 and -9.0 k.cal/mol binding affinity respectively, with MurA enzyme. We found that, the Amino acids Asp306, Tyr329, Gly115, Arg121, Arg322, Ser125, Leu91 and Phe125 present in the enzyme binding pocket are showing molecular interactions with ligands. MurA being a potential drug target in treating **Streptococcus pneumonia** infections, in this study we found that Zinc50247 effectively inhibits the MurA enzyme and can act as potential therapeutic agent.*

Keywords: *Streptococcus pneumonia*, MurA enzyme, Molecular modeling, Molecular dynamics and Zinc.

Introduction:

Streptococcus *pneumonia*is a gram-positive bacterium that causes pneumonia, septicemia and meningitis in children's and adults. It has several unique metabolic pathways tosurvive inside host and escaping threads. Peptidoglycan is an essential component of cell wall, conferring mechanical resistance to the high internal osmotic pressure and maintains define cell shape (Roger et.al., 1980). Peptidoglycan consists of linear glycan chains inter-linked by short peptides. The glycan chains are composed of alternating units of N-acetylglicosamine (GlcNAc) and N-acetylmuramic acid (MurNAc) residue linked by β1-4 glycosidic bonds (Vollmer et.al., 2008). Cross- linkage of the glycan chains generally occursbetween the carboxyl group of D-Alanine at position 4 and E-amino group of Lysine at position 3. This cross-linking joins the glycan chains into a macromolecular network of high tensile strength and rigidity. The Biosynthesis of peptidoglycan in cytoplasm (Bouhsset.al.,2008; Sauvageet.al.,2008)is catalysed by enzyme UDP-N-acetylglucosamineenolpyruvyl transferase (MurA) which transfersenolpyrul group from phosphophenol pyruvate (PEP) to UDP-N-acetylglucosamine to form UDP -N-acetylenolpyruvate. This is a precursor to the UDP-N-acetylmuramic acid, an essential building block for the bacterial cell wall. Skarzynskiet.al., (1996) explained the complex crystal structure of *Escherichia coli* with N-acetylglicosamine and fosfomycin.However,Eschenburg and Schonbrunet.al.,(2000) revealed unliganded*Enterobacter clocae*MurAstructure. MurA has two globular domains, connected by double stranded linker. According to *E.coli-*

MurA numbering, the first domain containing the catalytic site, Cys-115 comprises residues 22-229, and the second domain comprises residues from 1-21 and 230-419. The main chain fold of each domain is very similar, with three parallel internal helices surrounded by three helices and three four –stranded β-sheets. MurA synthesis is the only cytoplasmic phase inhibited by clinically used antibacterial agents. Fosfomycin, a naturally occurring broad spectrum antibiotic, is the best known inhibitor of MurA formation (Christensen *et.al.,* 1969; Hendlin *et.al.,* 1969). Fosfomycin has been the drug of choice for the treatment of pediatric gastrointestinal infections resulting from shiga-like toxin-producing *Escherichia coli* (STEC) in Japan.The early administration of this antibiotic is critical for the effective treatment of STEC infections (Horliet.al., 1999). Fosfomycin is awell-known agent as first line treatment formicrobial infections of the urinary tracts (Nicolle *et.al.,*2002).

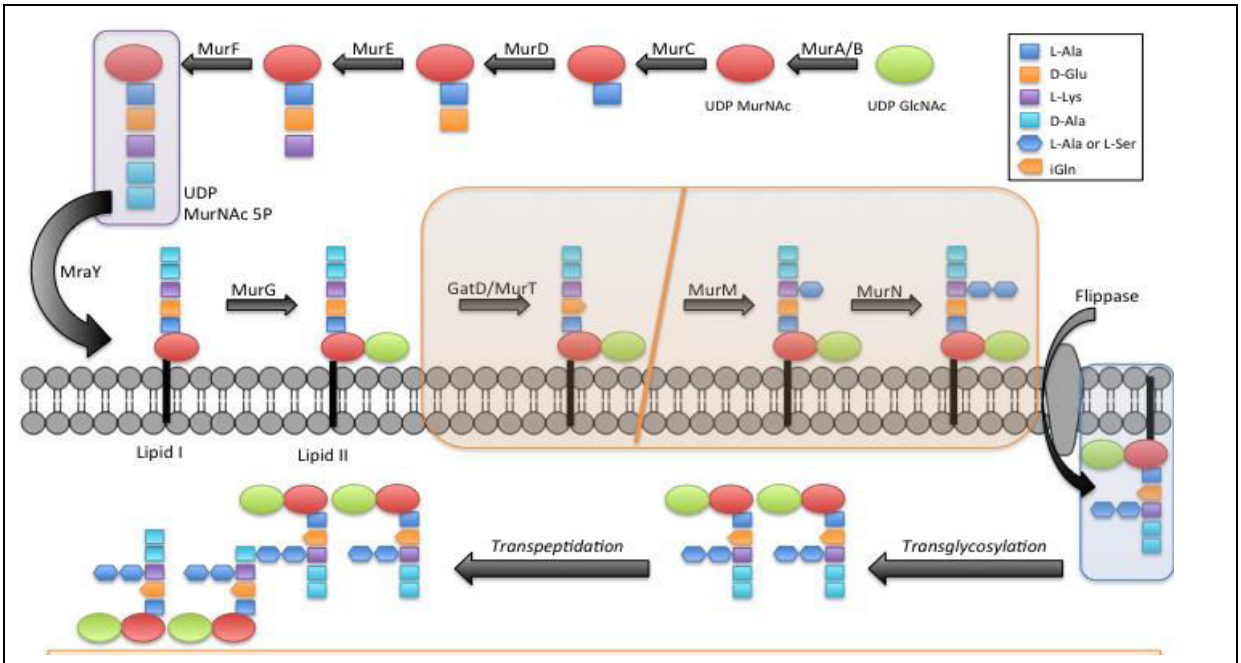


Fig-1: Biosynthesis of Peptidoglycan (Wikipedia)

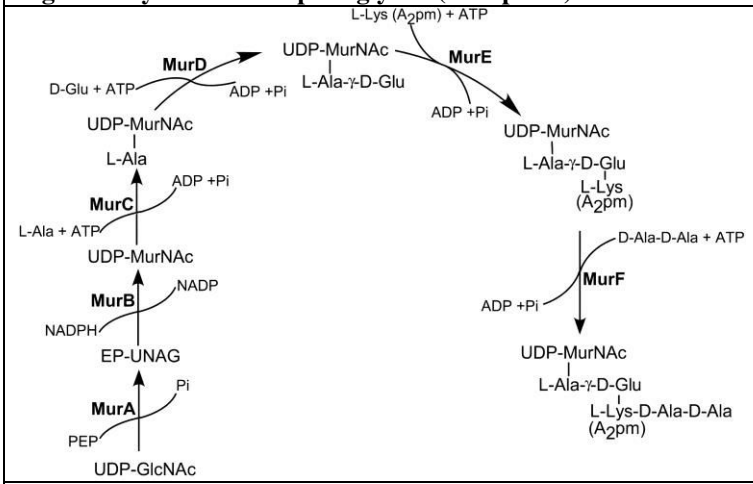


Fig-2: Mur pathwary (Wikipedia)

.

Inhibition of MurA enzyme by fosfomycin is competitive, the antibiotic acting on an analogue of PEP and forming covalent bond with Cys115 residue, as a resultMurA enzyme gets inactivated (Marqurdt*et.al.,*1994). Mutations at the active site of MurA enzyme exhibits resistance to fosfomycin while Cys residue replaced by Asp in *Mycobacterium tuberculosis* (Desmetet.al.,1999) and *Chlamyadia trachomatis* (McCoy et.al.,2003). In Streptococcus*pneumonia*, MurA is a key enzyme involved in peptidoglycan biosynthesis. Hence,it has been considered as a molecular drug target for developing novel potential antimicrobial agents.

Owing to the absence of three dimensional structures of Streptococcus *pneumonia*MurA enzyme in protein data bank (PDB) along with non-availability of homologues in humans. The present studyaimed to develop the three-dimensional structure forMurA by homology modeling followed by Molecular Dynamics Simulations. In addition to this,identification of fosfomycin analogous from Zinc database followed by Molecular interaction studies with MurAwere doneusing structure based virtual screening approach.

Materials and Methods:

3D Structure Preduction of MurA protein:

Protein sequence of UDP N-acetylglucosamineenolpyruvyltranferase (MurA)of *Streptococcus pneumoniae serotype 4 (strain ATCC BAA-334 / TIGR4)*was retrieved in FASTA format from Uniprot database. Physicochemical properties such asAliphatic index, Grand Average of Hydropathy (GRAVY), Theoretical PI value of UDP N-acetylglucosamineenolpyruvyltranferase (MurA) were calculated using PROTPARAM.

Sequence analysis:

Protein sequence of UDP N-acetylglucosamineenolpyruvyltranferase (MurA) was chosen as query sequence to search against Non-redundant protein sequences (nr) using Blastp (protein-protein BLAST).Multiple sequence alignment was performed for similar sequences using ClustalX Software(Thompson *et.al.*,1997).Percentage of identity and similarity of the query with related protein sequence were analyzed using GENEDOC Software (Nicholas *et.al*.,1997).The phylogenetic tree was constructed using TREEVIEW Software.

Secondary structure prediction:

Secondary structure elements such as alpha helix, extended sheets, beta turns and random coils were predicted by using different servers SOPMA (Deleage*et.al*.,1995), GOR4 (Garnier*et.al.,*1996), Chou &Fasman(Chou and Gerald D.Fasman*et.al.,*1974).The binding pocket sites for UDP N-acetylglucosamineenolpyruvyltranferase (MurA) were identifiedusing CastP (Computed Atlas of Surface Topography) a program for identifying and characterizing protein active sites, binding sites and functional residues located on protein surfaces and voids buried in the interior of protein by measuring concave surface regions on three-dimensional structure of protein. It can measure the area and volume of pocket (Dundal *et.al.,*2006).

3D Structure Prediction by Homology Modelling:

The protein sequence UDP N-acetylglucosamineenolpyruvyltranferase (MurA) enzyme of *Streptococcus pneumoniae*serotype 4 (strain ATCC BAA-334 / TIGR4) was obtained from uniport database.Template structure was selected on the basis of sequences identity with high score and less E-value,highest resolution and R-factor by performing the search against PDB(Protein Data Bank). The co-ordinates for the query structure were assigned from template structure by using pairwise sequence alignment using clustalX(Thompson*et.al*., 1997). The 3D Model of MurA enzyme was built by using Modeller $_{9v10}$ (Sali and Blundell,1993) against PDB.The least modeller objective (low DOPE Score) obtained was analysed through Ramachandran plot calculation to check the stereo chemical quality of protein structure using Procheck (Laskoswki*et.al*.,1993), environment profile using verify 3D(Eisenberg *et.al,*.1997) and ERRAT (Colovos*et.al*.,1993). The residue packing and atomic contact was analysed using What if (Vriend*et.a*l.,1990),Z Score of Ramachandran plot analysed using WhatCheck (Hooft*et.al.,*1996). Inorder to obtain stable conformation model, energy minimization was carried out with gromos96 force field and superimposition of model with template was performed by using SPDBV (Guex and Peitsch,1997).

Active site prediction: CastP server (Dundas j.,2006)was adopted to analyze the active sites of constructed model.This model was used for the docking analysis with selected analogue compounds.

Molecular Dynamics:

The molecular simulations for protein were carried out using NAMD-2.7 program in HP workstation Z230. The system was examined in the ensemble, while the dimension of the cell in the three direction of space fluctuates independently. The equation of the motion was integrated using multiple time steps algorithms (Izaguirre*et.al*.,1999; Jesns*et.al*.,2001). The short and long ranges forces were calculated every two and four time steps respectively, with the steps of 2.0fs. Short range interactions were smoothly truncated with an $11A^{o}$ cut-off and switching function starting at $8A^{0}$. Long range electrostatic forces were taken in account using a fast implementation of the particle mesh ewaldapproach (Darden *et.al*., 1993; Essamann., 1995). The langevin piston was employed to maintain the pressure of the cell at 1 atm and lamgeveindynamics were used to control the temperature at 300k. Chemical bonds

between hydrogen and heavy atoms were strained to their equilibrium value by means of the SHAKE/RATTLE algorithm (Rykaert*et.al*.,1997; Anderson *et.al*.,).

Ligand Preparation:

From ZINC Database(zinc.docking.org) 5000 fosfomycinanalogues were obtained. These analogues added with hydrogens and energy minimized with Uffforce field using Conjugate-gradient algorithm by Autodock and PyRx(Wolf.,2009). All ligand molecules were converted into Pdbqt format as requiredbyAutodockVina. Clog-P, solubility, molecular weight, Toxicity Risk Assessment, Overall Drug-score of compounds were predicted using Osiris property explorer.

Molecular docking studies:

Autodockvina andPyRxwere used to carry out docking studies with selected antibiotic analogous and pubchem drug molecules were docked to constructed model (Wolf., 2009). PyRx is Virtual screening software for computational Drug Discovery that can be used to screen libraries of compounds against potential drug targets. Lamarkian Genetic Algorithm (Solis *etal*.,1981) was used with the parameters; number of individual population is 150,Maximum number of energy evaluation is 25000,Top individuals to survive to next generation is 1,Gene mutation rate 0.02, Crossover rate is 0.8, Cauchy beta is 1.0 and GA window size is 10.0.The grid was set to whole protein due to the multi binding pocket at X=14.6042, Y=41.4964, Z=26.3463 and dimensions (A^0) at X=62.8291, Y=5805914, Z=66.6758 . The best docked conformation ligand molecules were identified on the basis of highest binding energy.Interactions between ligand and protein molecules were analyzed using PyMol.

Results and Discussion:

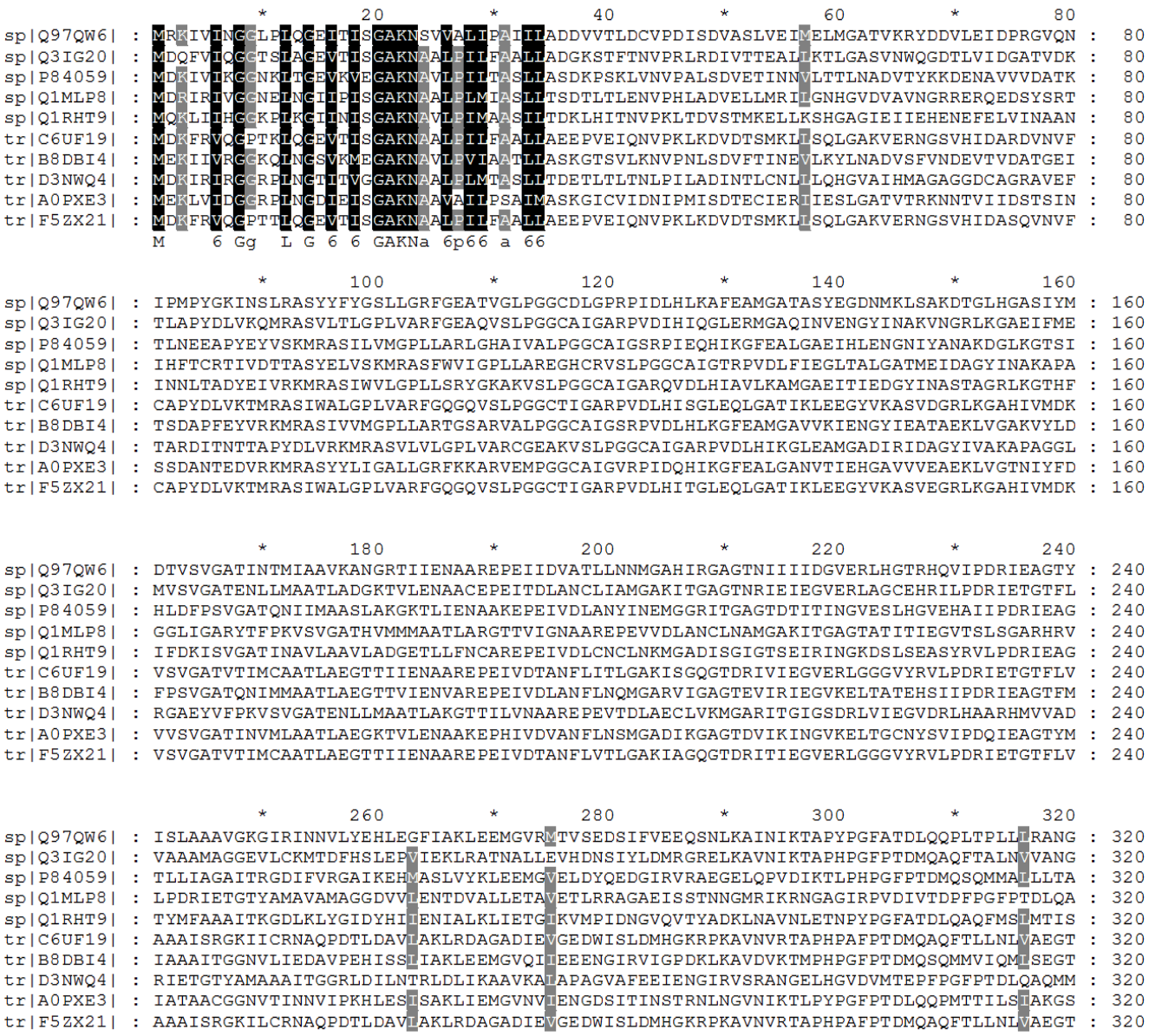
Protein sequence of MurA (Acc No:Q97QW6) of *Streptococcus pneumonia*serotype 4 strain ATCC BAA-334was retrieved in the FASTA format from SWISS PROT Database.MurA contains 419 residues with the molecular weight of 45024.8, aliphatic index 109.21, Grand average of hydropathy (GRAVY) 0.108 and Extinction coefficient, Theoretical PI value, instability index of UDP N- acetyl glucosamine 1-carboxy vinyl transferase (MurA) were found to be 19495, 5.24 and 29.39 respectively as shown in Table1.

Fasta format of MurA:

>sp|Q97QW6|MURA2_STRPN UDP-N-acetylglucosamine 1-carboxyvinyltransferase 2 OS= Streptococcus pneumoniae serotype 4 (strain ATCC BAA-334 / TIGR4) OX=170187 GN=murA2 PE=3 SV=1
MRKIVINGGLPLQGEITISGAKNSVVALIPAIILADDVVTLDCVPDISDVASLVEIMELMGATVKRYDDVLEI
DPRGVQNIPMPYGKINSLRASYYFYGSLLGRFGEATVGLPGGCDLGPRPIDLHLKAFEAMGATASYEGDN
MKLSAKDTGLHGASIYMDTVSVGATINTMIAAVKANGRTIIENAAREPEIIDVATLLNNMGAHIRGAGTNIII
IDGVERLHGTRHQVIPDRIEAGTYISLAAAVGKGIRINNVLYEHLEGFIAKLEEMGVRMTVSEDSIFVEEQSN
LKAINIKTAPYPGFATDLQQPLTPLLLRANGRGTIVDTIYEKRVNHVFELAKMDADISTTNGHILYTGGRDL
RGASVKATDLRAGAALVIAGLMAEGKTEITNIEFILRGYSDIIEKLRNLGADIRLVED

Figure 1: Pairwise alignment

ISSN 2515-8260 Volume 07, Issue 11, 2020



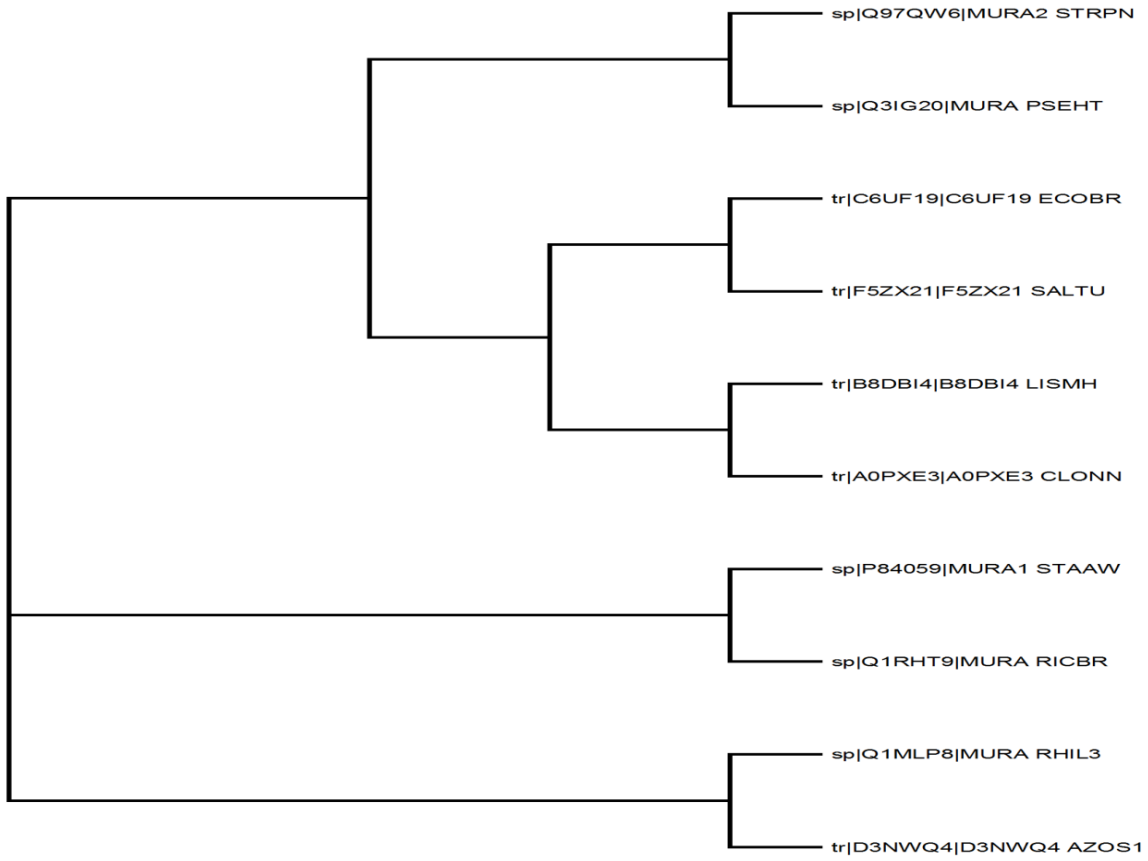
```
                    *       340         *       360         *       380         *       400
sp|Q97QW6| : RGTIVDTIYEKRVNHVFELAKMDADISTTNGHILYTGGRDLRGASVKATDLRAGAAIVIAGLMAEGKTEITNIEFILRGY : 400
sp|Q3IG20| : SATITETIFENRFMHVPELQRMGANIRLEGNTAICGDTKTLSGAQVMATDLRASASIILTGIVAQGETIVDRIYHVDRGY : 400
sp|P84059| : NGHKVVTETVFENRFMHVAEFKRMNANINVEGRSAKLEGKSQLQGAQVKATDLRAAAALIIAGLVADGKTSVTELTHLDR : 400
sp|Q1MLP8| : QFMALMTRSSGVSHVTETIFENRFMHVQELARLGARISLSGQTAKIEGVQRLRGAPVMATDLRASVSLVIAGLAAEGETT : 400
sp|Q1RHT9| : QGSSIITENIFENRFMHVPELCRMGADITVRGNQAIVQGVKGLKGAEVMASDLRASVSLILAGLSTDSETVLHRIYHLDR : 400
tr|C6UF19| : GFITETVFENRFMHVPELSRMGAHAEIESNTVICHGVEKLSGAQVMATDLRASASLVLAGCIAEGTTVVDRIYHIDRGYE : 400
tr|B8DBI4| : SIMTETVFENRFMHVEEMRRMNADMKIEGHSVIISGPAKLQGAEVAATDLRAAAALIIAGLVADGYTQVTELKYLDRGYN : 400
tr|D3NWQ4| : ALMCTAKGAAMITETIFENRFMHAPELTRMGARITVHGSSALVRGVERLTGAPVMATDLRASVSLVLAGLAAEGETTVNR : 400
tr|A0PXE3| : SIVNESIWESRFKHVDELKKMGAKISVENNIAMIEGVKSLSGAKVKATDLRAGAAMVIAGLIANGITEVTNIEHIDRGYP : 400
tr|F5ZX21| : GFITETVFENRFMHVPELSRMGARAEIESNTVICHGVETLSGAQVMATDLRASASLVLAGCIAEGTTIVDRIYHIDRGYE : 400

                    *       420         *
sp|Q97QW6| : SDIIEKLRNLGADIRLVED---------- : 419
sp|Q3IG20| : ERIEDKLSALGANIKRRSS---------- : 419
sp|P84059| : GYVDLHGKLKQLGADIERIND--------- : 421
sp|Q1MLP8| : VSRVYHLDRGFERLEEKLTRCGAIVERISE : 430
sp|Q1RHT9| : GFQNLEKKLNNCGADIKRV---------- : 419
tr|C6UF19| : RIEDKLRALGANIERVKGE---------- : 419
tr|B8DBI4| : NFHGKLQALGADVERVDDSKIDVTNLASLF : 430
tr|D3NWQ4| : VYHLDRGYERVEEKLAACGAEIERIHGGEE : 430
tr|A0PXE3| : YIEEKFNKLGAKIKRVEY---------- : 418
tr|F5ZX21| : RIEDKLRALGANIERVKGE---------- : 419
```

Table 3: sequence similarity of report file by using phylogenetic analysis

S.no	Accession number	Organism Name	Sequence similarity
1	Q97QW6	Streptococcus pneumoniae	--
2	Q3IG20	Pseudoalteromonas haloplanktis	30%
3	P84059	Staphylococcus aureus	9%
4	Q1MLP8	Rhizobium leguminosarum	13%
5	Q1RHT9	Rickettsia bellii	10%
6	C6UF19	Escherichia coli	10%
7	B8DBI4	Listeria monocytogenes	8%
8	D3NWQ4	Azospirillum sp.	11%
9	A0PXE3	Clostridium novyi	10%
10	F5ZX21	Salmonella typhimurium	10%

Figure2: Phylogenetic analysis



Secondary Structural elements such as Alpha helix, extendedstrand, Beta turns and Random coils of MurAwere indentified using varioustools as shown in Table2. These Secondary Structural analysis has shown 41.29% alpha helix with 173 residues by SOPMA, 40.10% with 168 residues by GOR4 and 69.9% with 293 by Chou &Fasman. Extended strand displayed 18.14% with 76 residues by SOPMA, 15.75% with 66 residues by GOR4 and 38.2% with 160 residues by Chou &Fasman respectively.Beta turn were predicted to be 9.3% with 138 residues by Chou &Fasman, but SOPMA and GOR4 failed to predict beta turn. Random coils were found to be 32.94% with 138 residues by SOPMA and 44.15% with 185 residues. Chou &Fasman failed to provide random coils.

MurA structure of *Streptococcus Pneumonia*:

Crystal structure of MurA of *Streptococcus pneumonia* has not been resolved yet. MurA plays key role in peptidoglycan synthesis and acts as broad spectrum drug target. In order to construct the MurA model, BlastP analysis has shown 10 similar protein structureswhich were obtained with the highest identity.3R38 was selected an reference structure to model the MurA using Modeller9v10 Co-ordinates from the reference structure to structurally conserved regions, structurally variable regions, N-termini and C-termini were assigned to the target sequence based on the satisfaction of spatial restraints. All side chains of the model protein were set by rotamers.100 models were generated and one with least DOPE score was selected for further refinement. The least DOPE score objective was furthered relined using Molecular dynamics approach and graph drawn by taking time in ps (picoseconds) on X-axis and RMSD(A^0) on Y-axis (Figure 3D).

The final refined model was further validated using standard validation tools. Ramachandran plot calculations, carried out using Procheck program revealed that 92.5% with 333 residues were plotted in with the most favoured region, 0.1% with 4 residues with in the Generously allowed region and 0.3% with 1 residue was aligned in with the disallowed region respectively (Figure 4A). The overall quality factor of 91.8% was observed by using of ERRAT environment profile (Figure 4B). Verify 3D showed that 411 residues of all the residues had an average 3D-1D score greater than 2 indicating that model was highly reliable (Figure 4C). The constructed MurA model was superimposed using SPDBV and RMSD calculated was 2.522^0 which indicate the reliability of good model (Figure 3B). Active site of the model was predicted using Cast P server (Figure 3E).

3D structure of MurA:

ISSN 2515-8260 Volume 07, Issue 11, 2020

Fig -3: Modelled 3D structure of MurA

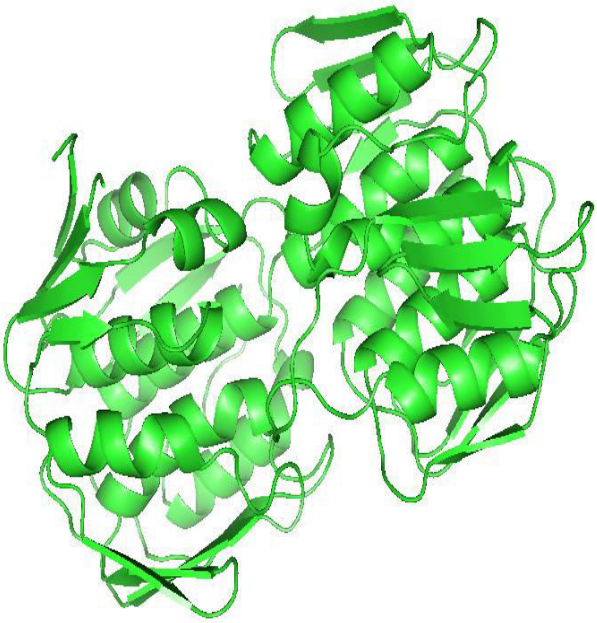


Fig -4:Superimpose of template & MurA modelled.

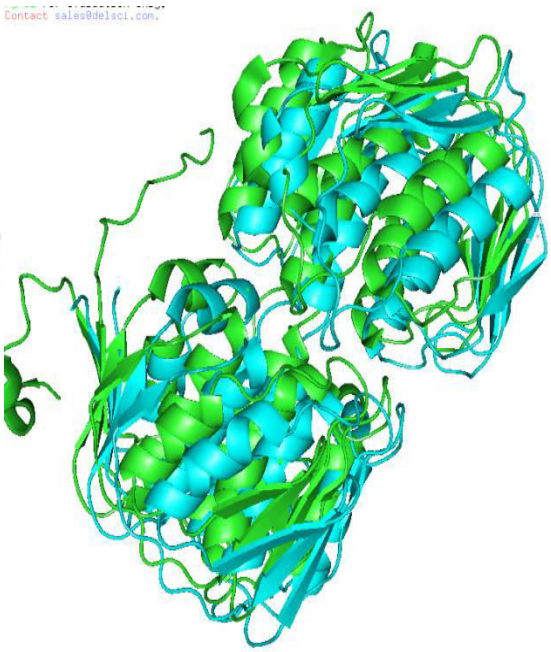


TABLE 1:
Physicochemical properties of UDP-N-acetyl glucosamine 1-carboxyvinyl transferase (MURA) were predicted by PROTPARAM.

s.no	protein secondary structure	values
1	Aliphatic index	109.21
2	Grand average of hydropathicity	0.108
3	Theoretical PI	5.24
4	Half-life	30 hours
5	Molecular Weight	45024.8
6	The Instability index	29.39
7	Extinction Coefficients	19495

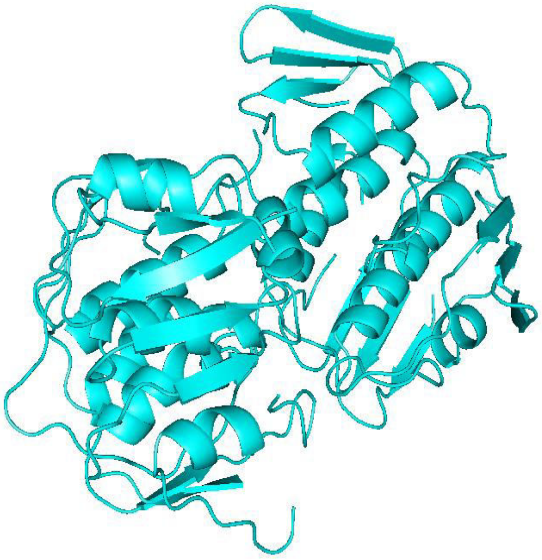
TABLE 2: Secondary structure elements were predicted by different servers:

S.no	Protein Secondary Structure	SOPMA		GOR4		CHOU & FASMAN	
		No. of Residues	% of Residues	No. of Residues	% of Residues	No. of Residues	% of Residues
1	Alpha helix	173	41.29	168	40.10	293	69.9
2	Extended strand	76	18.14	66	15.75	160	38.2
3	Beta turn	-	-	-	-	39	9.3
4	Random coil	138	32.94	185	44.15	-	-

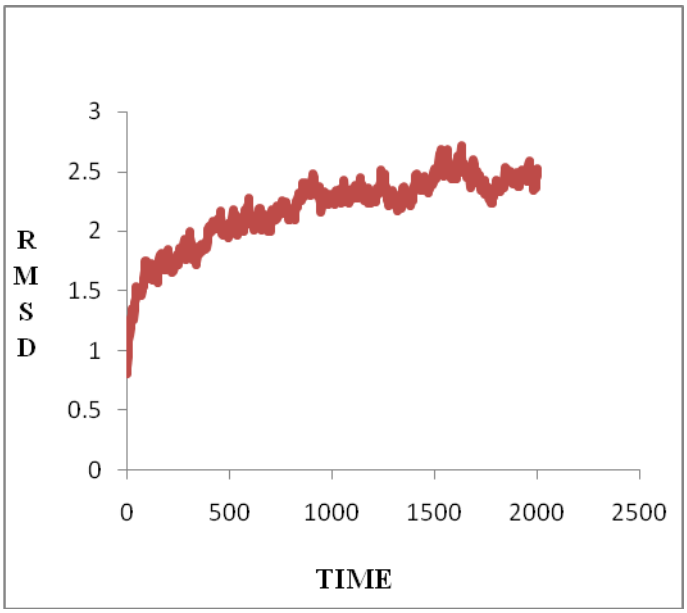
Fig- 5: 3D refined Dynamics structure of MurA.

Fig -6:RMSD Dynamics graph.

D.



E.



Structure Validation:

Fig -7:Ramachandran Plot calculations were drawn using Procheck.

1. Ramachandran Plot statistics

		No. of residues	%-tage
Most favoured regions	[A,B,L]	333	92.5%
Additional allowed regions	[a,b,l,p]	22	6.1%
Generously allowed regions	[~a,~b,~l,~p]	4	1.1%
Disallowed regions	[XX]	1	0.3%*
Non-glycine and non-proline residues		360	100.0%
End-residues (excl. Gly and Pro)		2	
Glycine residues		42	
Proline residues		15	
Total number of residues		419	

ISSN 2515-8260 Volume 07, Issue 11, 2020

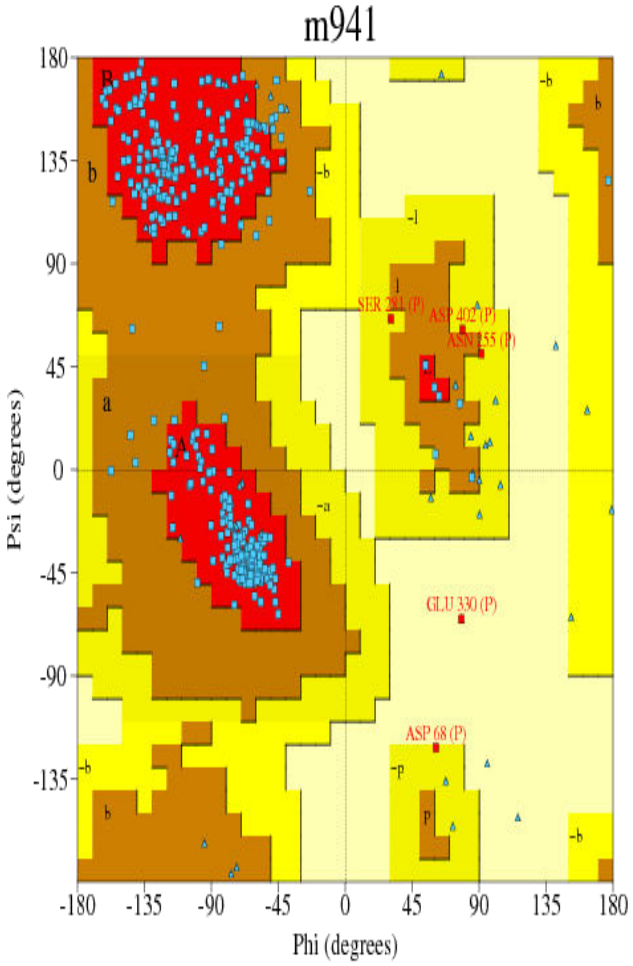


Fig -8:The over quality of MurA was observed using ERRAT environment profile.

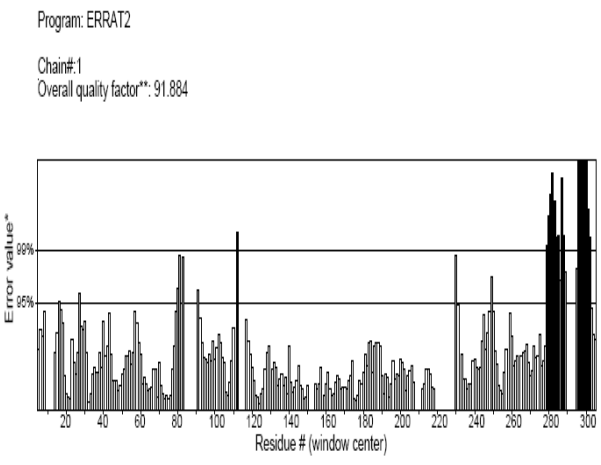


Fig -9:Verify 3D-1D graph.

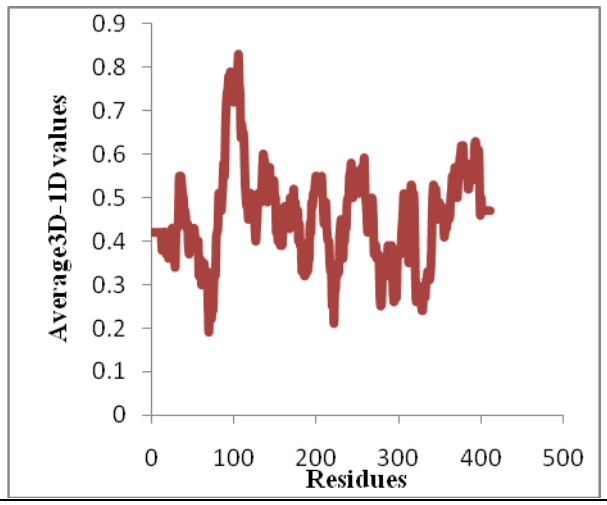
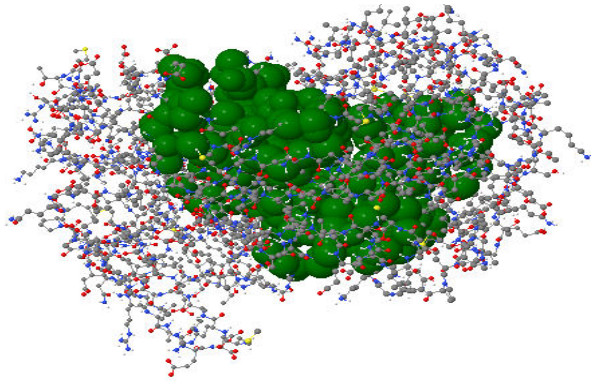
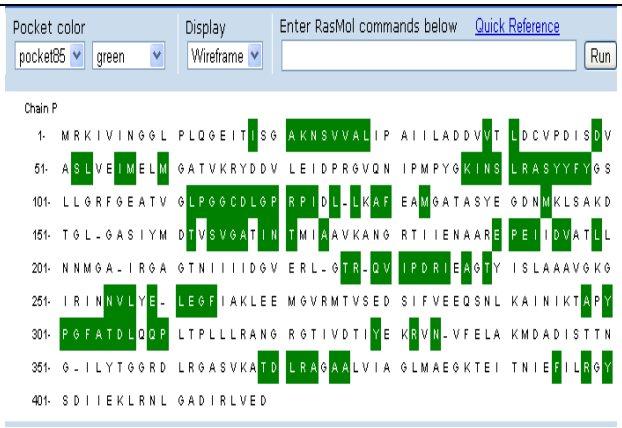
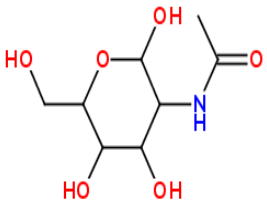


Fig -10:MurA was predicted Using Cast P Server	**Fig -11:** Amino acids present in binding pocket
C. 	

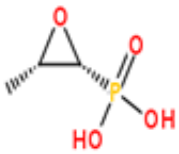
Molecular docking studies:

Molecular docking studiesperformed with substrate (GluNAC) and fosfomycin to MurA model showed that Substrate GluNAC exhibits binding energy -4.5 kcal/mol and formed three hydrogen bonding interactions with Asp89,Leu91 and Ser94 respectively (Figure 4B). Fosfomycin is broad spectrum antibiotic which showed binding affinity -4.5 kcal/mol and displayed two hydrogen bonding interactions, one with NH atom of Phe264 another one with O atom of gly302 respectively (Figure 4A).

GluNAC (Substrate)

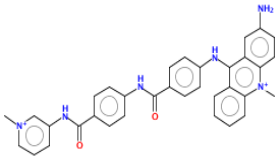


Fosfomycin(Reference drug)

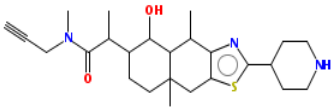


C. Selected Zinc compounds.

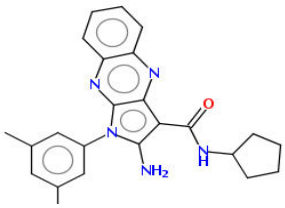
1. ZINC50247



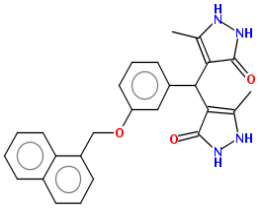
2. ZINC3820559



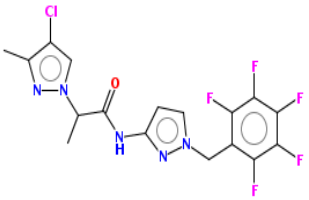
3. ZINC1884559



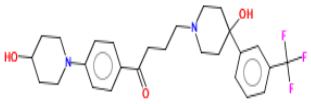
4. ZINC3154681



5. ZINC19286884



6. ZINC58219



7. ZINC3978065

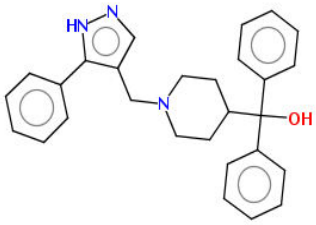


TABLE 4: Protein-ligand interaction,binding energies, bond distance and bond angle ofReference Drug Fosfomycin and Substrate GluNac with UDP-N-acetyl glucosamine 1- carboxyvinyl transferase (MURA) Zinc Compounds.

S.no	Compounds	protein-ligand interactions		Distance	Bond angle	binding energy
		protein	ligand			
1	Fosfomycin	Gly302 CA-C-O----------H9 Phe264 CA-NH----------O4P		2.2 2.5	132.7 106.1	-4.5

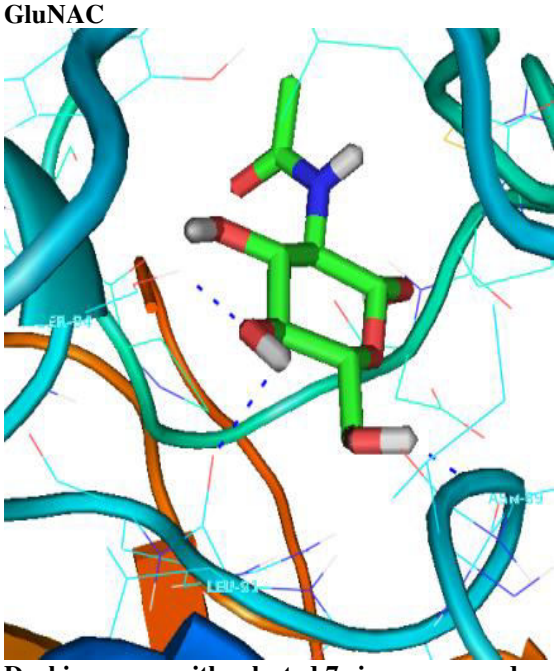
2	Substrate GluNac	Leu 91 CA-C-O----------H9O Ser 94 OG-G-H----------O14C Asn 89 CA-C-O----------H20O	2.4 2.2 2.2	141.4 132.7 110.5	-4.5

TABLE 5: Protein-ligand interaction,binding energies, bond distance and bond angle of best docked zinc compounds with (MURA) UDP-N-acetyl glucosamine 1-carboxyvinyl transferase

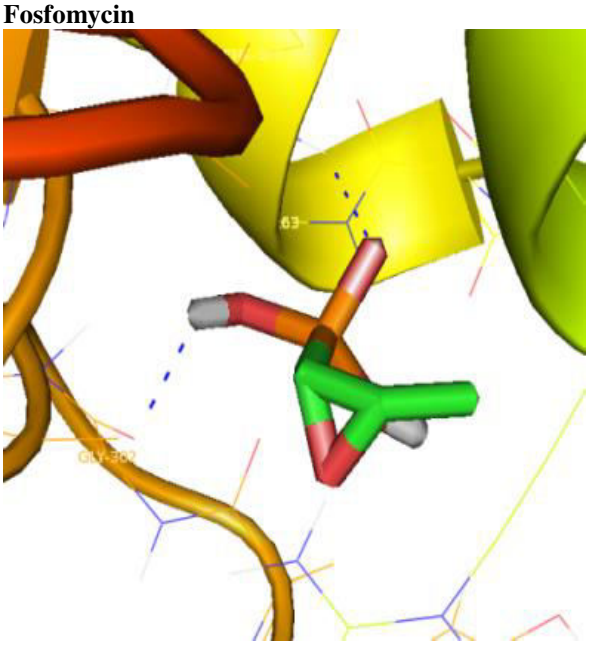
S..No	Compounds	Protein-Ligand Interactions		Distance	Bond Angle	Binding Energy
		protein	ligand			
1	ZINC50247	Asp306 CG-D1O----------H46N Asp306 CG-D2O----------H47N Tyr329 CZ-HO----------H45N		2.6 2.5 2.1	141.3 130.3 96.9	-10.3
2	ZINC3820559	Arg121 CA-N----------O29C Gly115 CO----------H32N		3.5 2.7	132.8 142.0	-10.1
3	ZINC1884559	Arg322 CG-D1O----------O30C Asn23 CZ-NH2----------HN34N		3.4 2.7	107.0 131.9	-9.8
4	ZINC3154681	Ser 125 CB-GO----------O32C		3.4	108.1	-9.8
		Leu91 C-O----------H37N		2.4	124.7	
5	ZINC19286884	Tyr329 CZ-HO----------H30N Tyr329 CZ-HO----------HN32N		2.3 2.3	115.1 97.3	-9.2
6	ZINC58219	Asn23,Ala27,Asn89,Leu91,Arg92, Tyr95,Pro113,Gly114,Gly115,Lys116,Asp117,Pro120,Arg121,Pro122,Ile123,Ser164,Val165,Gly166,Glu190,Glu192,Arg234,Asp306,Tyr329				-9.1
7	ZINC3978065	Phe125 C-O----------O24C		2.5	137.4	-9.0

Figure 4: Docking results of MurA against GluNAC, Fosfomycin, and selected ZINC compounds.

GluNAC



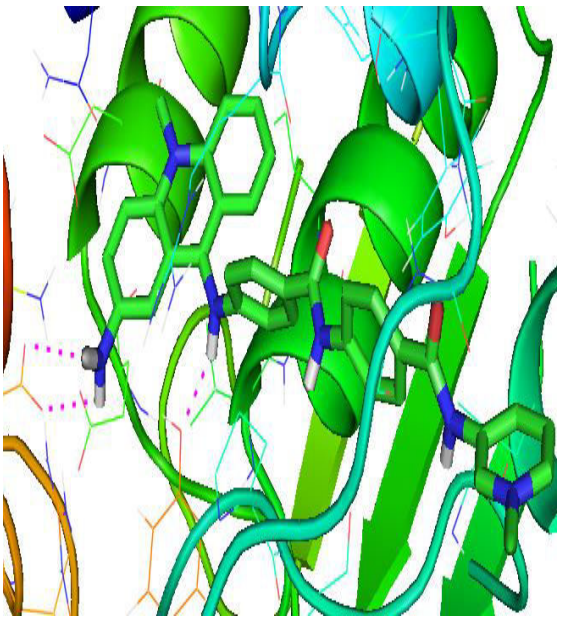
Fosfomycin



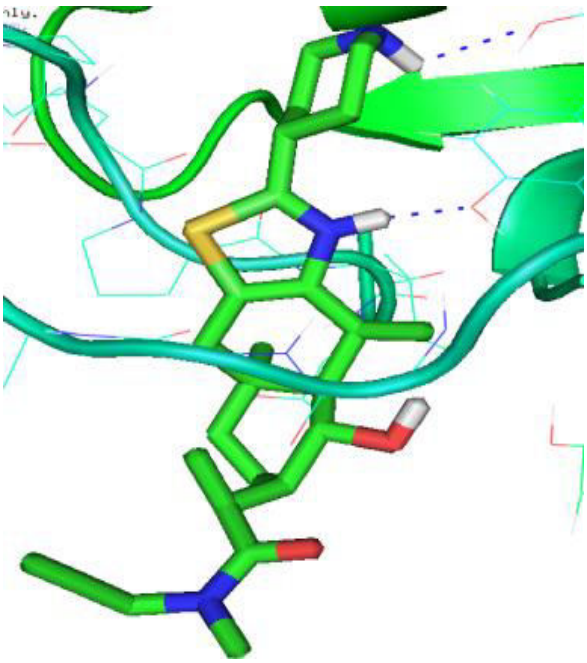
Docking poses with selected 7 zinc compounds.

ISSN 2515-8260 Volume 07, Issue 11, 2020

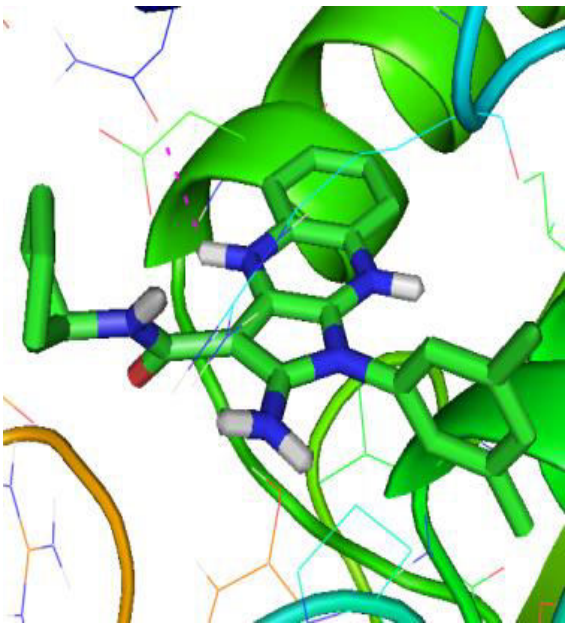
1. **ZINC50247**



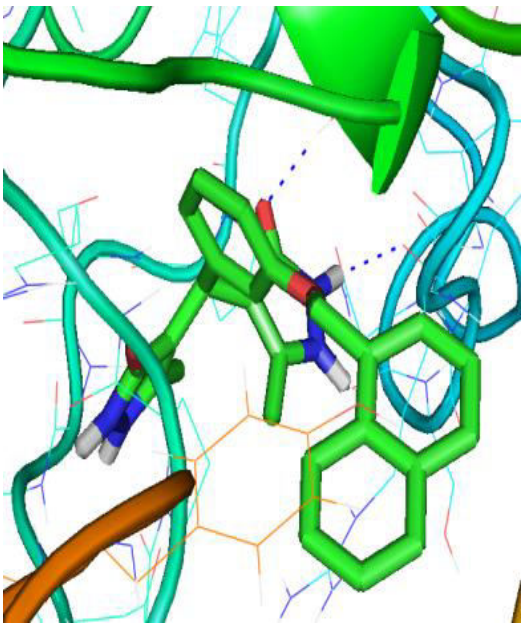
2. ZINC3820559



3. ZINC1884559

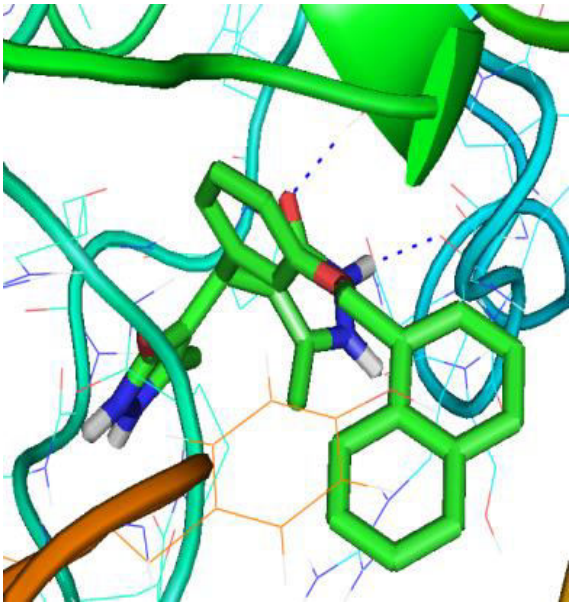


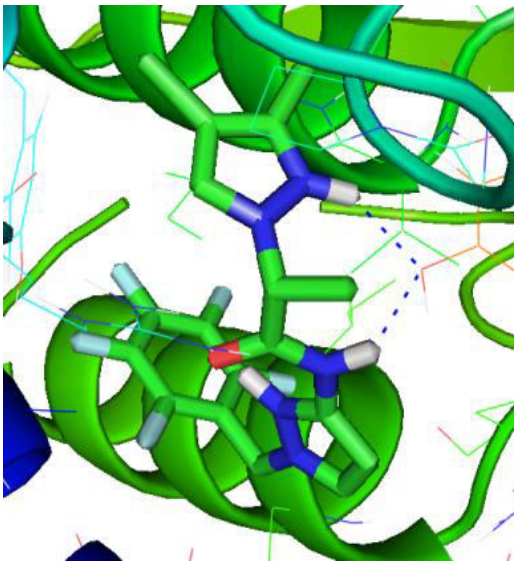
4. ZINC3154681



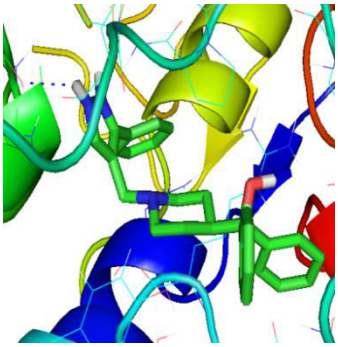
5. ZINC19286884

6. ZINC58219





7. ZINC3978065



In order to perform lead optimization, virtual screening studies were performed for 5000 compounds downloaded from ZINC Database. Seven best compounds such as ZINC50247, ZINC3820559, ZINC1884559, ZINC3154681, ZINC19286884, ZINC58219, and ZINC3978065 were obtained (Figure5) (Table5). Biological properties and Lipinski rule of best docked conformation compound were predicted using Osiris and Mol inspiration servers (Table7 and 8).

ZINC 50247 displayed three hydrogen bonding interactions, two bonds with the O atom of Asp306, one with the O atom of Thr329 and showed best binding energy -10.3 k.cal/mol. Similarly, ZINC 3820559 showed binding energy -10.1 k.cal/mol and displayed two hydrogen bonding interactions such as one with CO atom of Gly115 and one with N atom of Arg121 respectively.ZINC1884559 showed binding energy -9.8 kcal/mol and confers two hydrogen bond interactions with Asn23 and Arg121 respectively. ZINC3154681 showed similar binding energy -9.8 k.cal/mol as above and displayed two hydrogen bonding interaction with Leu91 and Ser125 respectively. ZINC19286884 showed binding energy -9.2 k.cal/mol and exhibits two hydrogen bonding interaction with the Tyr329 respectively. ZINC 58219 showed hydrophobic interaction and best binding energy -9.1 kcal/mol. ZINC 3978065 showed binding affinity -9.0 kcal/mol and displayed one hydrogen bonding interaction with Phe125 respectively.

TABLE 6: Lipinskirule of five for best docked zinc compounds.

s.no	Zinc Compound	pH range	xlogP	Apolar desolvation(kcal/mol)	Polar desolvation(kcal/mol)	H-bond donors	H-bond acceptors	Net charge	tPSA (Å²)	Molecular weigh (g/mol)	Rotatable bonds
1	ZINC50247	pH 7	2.34	-2.04	-47.39	1	6	1	51	298.37	1

2	ZINC3820559	pH 7	3.42	11.1	-44.31	5	9	1	112	472.371	7
3	ZINC1884559	pH 7	3.91	8.61	-21.76	1	5	0	72	316.316	4
4	ZINC3154681	pH 7	4.61	3.11	-16.6	1	10	0	147	407.338	7
5	ZINC19286884	pH 7	3.14	10.93	-54.17	2	7	1	64	446.575	7
6	ZINC58219	pH 7	3.66	-2.07	-9.32	1	3	0	45	301.143	1
7	ZINC3978065	pH 7	0.80	2.68	-15.17	3	6	0	104	404.503	2

TABLE 7: Molecular properties and bioactivity of best docked compounds were predicted using molinspiration : Molecular properties:

S.NO	COMPOUND ID	MI LOGP	TPSA	n-ATOMS	M.W	nON	nOHNH	nVIOLATIONS	NROTB	VOLUME
1	ZINC50247	2.637	104.016	42.0	554.654	8	5	1	6	503.989
2	ZINC3820559	2.8	65.456	31.0	443.657	5	2	0	4	430.647
3	ZINC1884559	4.97	85.839	30.0	399.498	6	3	0	3	369.48
4	ZINC3154681	3.841	106.54	33.0	440.503	7	4	0	6	395.907
5	ZINC19286884	3.079	64.75	29.0	433.768	6	1	0	5	325.612
6	ZINC58219	3.454	64.003	35.0	490.566	5	2	0	8	443.804
7	ZINC3978065	4.489	52.149	32.0	423.56	4	2	0	6	406.399
8	Fosfomycin	0.762	70.055	8.0	138.059	4	2	0	1	105.377

Table 8: Biological Properties Of Best Docked Zinc Compounds Were Predicted Using OSIRIS Server:

S.no	Compound	Mutag-enic	Tumorigenic	Irritant	Reproduction Effective	Clogp	Solubility	Mol. Wt	Drug Likeness	Drug Score
1	ZINC 50247	+	partial	-	-					
2	ZINC 3820559	-	-	-	-	1.5	-5.07	433.0	4.87	0.61
3	ZINC 1884559	partial	-	-	-	4.16	-8.57	399.0	-4.6	0.16
4	ZINC 3154681	partial	-	-	-	1.78	-6.28	440.0	3.04	0.39
5	ZINC 19286884	-	-	-	-	2.69	-4.03	433.0	-6.66	0.35
6	ZINC 58219	-	-	-	partial	3.78	-4.56	440.0	6.96	0.48
7	ZINC 3978065	-	-	-	-	1.55	-2.04	257.0	4.55	0.93

Predict Bioactivity:

s.no	compound	gpcr ligand	ion channel modulator	kinase inhibitor	nuclear receptor ligand	protease inhibitor	enzyme inhibitor
1	ZINC50247	0.07	-0.11	-0.05	-0.95	-0.04	0.21
2	ZINC3820559	0.18	-0.26	-0.27	-0.02	0.27	0.42
3	ZINC1884559	-0.33	-0.57	-0.30	-0.97	-0.51	-0.26
4	ZINC3154681	-0.25	-0.40	-0.34	-0.29	-0.34	-0.20
5	ZINC19286884	-0.27	-0.79	-0.24	-1.11	-0.59	-0.54
6	ZINC58219	0.34	0.20	0.02	0.17	0.10	0.20
7	ZINC3978065	0.11	0.02	-0.18	-0.14	-0.13	0.02
8	Fosfomycin	-2.60	-1.52	-2.51	-2.47	-1.84	-0.60

Conclusion:

After the analysis of the above data obtained through different sources, it was joined out seven novel fosfomycin structure similar compounds from Zinc data base depending on various parameters such as Lipinski rule of five, drug score, drug likeness and other adverse effects. Zinc50247 showed high binding affinity with Autodockvina binding energy of -10.3 k.cal/mol and Zinc3820559, Zinc188459, Zinc3154681, Zinc19286884, Zinc58219 and Zinc3978065 have -10.1, -9.8, -9.8, -9.2, -9.1, and -9.0k.cal/mol binding energies with active site region of MurA enzyme. All the compounds are docked with binding pocket region, forming interactions with Asp306, Tyr329, Gly115, Arg121, Arg322, Ser125, Leu91 and Phe125 residues. Hence, the lead molecule identified in the present work with be helpful for designing and synthesis of new class of antimicrobial drugs.

Acknowledgments:

Authors are thankful to the Department of Biotechnology (DBT), Ministry of Science and Technology, Government of India, New Delhifor providing necessary labfacilities through Bioinformatics Infrastructure facility (BIF) Centre, Department of Zoology, Sri Venkateswara University, Tirupati.

Reference:

1. Bouhss,A., Trunkfield,A. E., Bugg, T.D.H &Mengin-Lecreulx, D*et.al.,*(2008).The biosynthesispeptidoglycanlipid-linkedintermediates. *FEMS Microbiol Rev,*doi: 10.1111/j.1574-6976.2007.00089.
2. Brown,E.D., Marquardt,J.L., Lee,J. P., Walsh,C.T., andAnderson, K.S. *et.al.,*(1994). Detection and characterization of aphospholactoyl-enzyme adduct in the reaction catalyzedbyUDP-N-acetylglucosamineenolpyruvoyl transferase, MurZ.*Biochemistry* 33:10638–10645
3. Brown, E. D., Vivas, E. I., Walsh,C. T.&Kolter, R.,*et.al.,*(1995).MurA (MurZ),the enzyme that catalyzes the first committed step inpeptidoglycan biosynthesis, is essential in Escherichia coli. *J.Bacteriol* 177: 4194–4197.
4. Christensen,B. G., Leanza,W. J., Beattie,T.R., Patchett, A. A., Arison,B.H., Ormond, R.E., *et.al.,*(1969).Phosphonomycin:structure and synthesis. *Science* 166: 123–125.
5. De Smet,K. A., Kempsell,K. E, Gallagher,A., Duncan,K& Young, D. B*et.al.,*(1999). Alteration of a single amino acid residue reversesfosfomycin resistance of recombinant MurA fromMycobacterium tuberculosis. *Microbiology* 145: 3177–3184.
6. Eschenburg,S., and Schonbrunn,E.,*et.al.,*(2000). Comparative X-rayanalysis of the un-ligandedfosfomycin-target murA.*Proteins* 40:290–298
7. Hendlin,D., Stapley,E. O., Jackson,M., Wallick,H., Miller,A.K., Wolf, F.J., *et al.* (1969).Phosphonomycin, a new antibioticproduced by strains of streptomyces. *Science* 166:122–123.

8. Horii,T., Kimura,T., Sato,K., Shibayama,K., and Ohta,M*et.al.,*(1999). Emergence of fosfomycin-resistant isolates ofShiga-like toxin-producing *Escherichia coli* O26. *AntimicrobAgents Chemother*43:789–793
9. Kim,D. H., Lees,W. J., Kempsell,K. E., Lane,W. S., Duncan,K&Walsh,C. T(1996). Characterizationof a Cys115 to Asp substitution in theEscherichia coli cell wall biosynthetic enzyme UDP-GlcNAcenolpyruvyl transferase (MurA) that confers resistance toinactivation by theantibiotic fosfomycin. *Biochemistry* 35:4923–4928.
10. Kahan,F. M., Kahan,J. S.,Cassidy,P. J and Kropp,H. *et.al.,*(1974.)The mechanismof action of fosfomycin (phosphonomycin).*Ann. N. Y. Acad. Sci*.235:364–385.
11. Marquardt, J. L., Brown, E. D.,Walsh,C.T&Anderson,K. S., *et.al.,*(1993). Isolation and structural elucidation of a tetrahedral intermediatein the UDP-N-acetyl glucosamine enolpyruvyl transferaseenzymatic pathway.*J.AmChemSoc* 115: 10398–10399.
12. Marquardt, J. L., Brown,E. D., Lane,W. S., Haley,T. M.,Ichikawa,Y.,Wong,C. H and Walsh,C. T., *et.al.,*(1994). Kinetics, stoichiometry, and identification of the reactive thiolate in the inactivation of UDP-GlcNAcenolpyruvoyl transferaseby the antibiotic fosfomycin. *Biochemistry* 33:10646–10651.
13. McCoy, A. J &Maurelli,A.T., *et.al.,*(2005). Characterization of ChlamydiaMurC-Ddl, a fusion protein exhibiting D-alanyl-D-alanineligase activity involved in peptidoglycan synthesis andD-cycloserine sensitivity. *MolMicrobiolFEMS*57: 41–52.
14. McCoy,A. J., Sandlin,R.C &Maurelli,A.T.,*et.al.,*(2003).Invitro and invivofunctional activity of Chlamydia MurA, a UDP-N-acetylglucosamineenolpyruvyl transferase involved inpeptidoglycansynthesis and fosfomycinresistance. *J.Bacteriol* 185:1218–1228.
15. Nicolle, L.E., *et.al.,*(2002). Urinary tract infection: traditional pharmacologictherapies. *Am J Med* 113(Suppl. 1A): 35S–44S.O'Hara, K. (1993) Two different types of fosfomycin resistancein clinical isolates of *Klebsiella pneumoniae*. *FEMSMicrobiol Lett* 114:9–16
16. Nicholas,K. B., Nicholas,H.B and Deerfield,D.W., *et.al.,*(1997).GeneDoc: Analysis and visualization of genetic variation. *EMBNEW.NEWS* 4:14.
17. Rogers,H. J., Perkins,H.R., and Ward, J.B., *et.al.,*(1980).Biosynthesisof peptidoglycan.Rogers, H.J., (eds). London: Chapman &Hall,pp. *I.Microbial Cell Walls and Membranes*239–297.
18. Sauvage,E., Charlier,P., Terrak,M&Ayala,J.A.,*et.al.,*(2008). The structure of Penicillin- Binding Proteins and their role in peptidoglycan biosynthesis. *FEMS Microbiol Rev, doi*: 10.1111/j.1574-6976.2008.00105.
19. Schonbrunn,E., Svergun,D. I., Amrhein,N&Koch,M. H., (1998). Studies on the conformational changes in the bacterial cell wallbiosynthetic enzyme UDP-N-acetylglucosamineenolpyruvyltransferase (MurA).*Eur J.Biochem* 253: 406–412.
20. Schonbrunn,E., Sack,S., Eschenburg,S., Perrakis,A., Krekel,F.,Amrhein,N&Mandelkow, E., *et.al.,*(1996).Crystal structure of UDPN-acetylglucosamine enolpyruvyltransferase, the target of theantibiotic fosfomycin.*Structure* 4: 1065–1075.
21. Skarzynski,T., Mistry,A., Wonacott,A., Hutchinson,S.E.,Kelly,V. A., and Duncan,K., *et.al.,*(1996). Structure of UDP-N acetylglucosamine enolpyruvyl transferase, an enzymeessentialfor the synthesis of bacterial peptidoglycan, complexedwith substrate UDP-N-acetylglucosamine and thedrugfosfomycin. *Structure* 4**:** 1465–1474
22. Sali,A., Blundell,T.L.,*et.al.,*(1993).Comparative protein modeling by satisfaction of spatial restraints.*J.MolBiol* 234:779–815
23. Thompson,J.D, *et.al.,*(1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 1997;25:4876-4882.
24. Vollmer,W., Blanot,D&DePedro,M.A.,*et.al.,*(2008).Peptidoglycanstructure and architecture.*FEMS Microbiol Rev, doi*: 10.1111/j.1574-6976.2007.00094.
25. Vriend,G., *et.al.,*(1990). WHAT IF: a molecular modeling and drug design program. *J.Mol Graph* 8:52–56.
26. Wanke,C&Amrhein,N., *et.al.,*(1993). Evidence that the reaction of theUDP-N-acetylglucosamine 1-carboxyvinyltransferaseproceeds through the O-phosphothioketal of pyruvic acidbound to Cys115 of the enzyme. *EurJ.Biochem* 218:861–870.