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

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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 -Nacetylenolpyruvate. 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 PvRx 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 pneumoniais 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 interlinked by short peptides. The glycan chains are composed of alternating units of N-acetylglicosamine (GlcNAc) and N-acetylmuramic acid (MurNAc) residue linked by β 1-4 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 Schonbrunet.al., (2000) revealed unliganded*Enterobacter* and clocaeMurAstructure. MurA has two globular domains, connected by double stranded linker. According to E.coli-

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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).



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 threedimensional 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.al.*, 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 $11A^{\circ}$ cut-off and switching function starting at $8A^{\circ}$. 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 langeveindynamics 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

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			*	20	*	40	*	60	*	80		
sp10970W61	:	MRKIVINCG	LPIOGE	TTISGAKI	NSVVALIPAITI	ADDVVTLD	CVPDISDVAS	LVEIMELMGA	TVKRYDDVLE	IDPRGVON	:	80
sp103TG201		MDOFVTOCC	TSTACE	VTISCARI		ADGKSTETI	VVPRT.RDTVT	TEALTKTLGA	SVNWOGDTLV	TDGATVDK		80
splP840591	-	MDKTVTKCC	NKTTCE	WKVEGAKI	NAVI.PTT TASTI	ASDKPSKL	VNVPALSOVE	TTNNVT.TT.N	ADVTYKKDEN	AVVVDATK	1	80
spl01MLP8	1	MDRTRTVGG	NETNOT	TPTSCAK		TSDTLTLE	VPHTADVET.	LMR TT GNHGV	DVAVNGRBER	OEDSYSET	1	80
spl018HT91	-	MOKLTTHGG	KPLKGI	TNTSGAK	NAVT.PTMAASTI	TOKTHTTN	VPKLTDVSTM	KELLKSHGAG	TETTEHENEF	ELVINAAN	-	80
5p/@10019/	1	MDKERWOOD	TRIOCE	VTSCAR		AFFRUETO	VUDKIKDUDT	SWKT SOLCA	KUEDNCQUUT	DAPDUNUE	1	80
tr PSDPT4	1	MENTINDCC	KOINCS	WEME CAR		ACCTOUT	KNUDNT GDUE	TNEVIEVIN	IN DUGEUNDEU	TUNNE	1	80
tr D2NWO4	1	MENITVRGG	DDING					TCNLTLOUCU	ADVSEVINDEV	CACRAVEE	1	00
tribsnwQ41	1	MDAIRIRGG	RPENGI	TTTGGAK		TDETLITI	NLFILADINT	CIEDITORO	AIHMAGAGGD	CAGRAVEF	1	00
tr AUPXE3	-	MEKLVIDGG	RPINGI	TEISGARI	NAAVALIPSAIN	ASKGICVI	DNIPMISDTE	CIERTIESLG	ATVTRKNNTV	TIDSTSIN	-	80
tr F52X21	-	MDRERACE	TTEOGE		NAALELLEBAALI	AFFDAFTÖ	NABETEDADI.	SMKL	KVERNGSVHI	DASQVNVF	1	80
		M 6 Gg	L G	6 6 GAKI	Na 6p66 a 60	D						
			*	100	*	120	*	140	*	160		
sp Q97QW6	-	IPMPYGKIN	SLRAS	YFYGSLL	GRFGEATVGLPO	GCDLGPRP:	IDLHLKAFEA	MGATASYEGE	NMKLSAKDTG	LHGASIYM	1	160
sp Q3IG20	1	TLAPYDLVK	QMRAS	/LTLGPLV/	ARFGEAQVSLP	GCAIGARP	VDIHIQGLER	MGAQINVENG	YINAKVNGRL	KGAEIFME	1	160
sp P84059	:	TLNEEAPYE	YVSKMF	RASILVMG	PLLARLGHAIVA	ALPGGCAIG	SRPIEQHIKG	FEALGAEIHI	ENGNIYANAK	DGLKGTSI	:	160
sp Q1MLP8	:	IHFTCRTIV	DTTASY	CELVSKMR2	ASFWVIGPLLA	REGHCRVSL	PGGCAIGTRP	VDLFIEGLTA	LGATMEIDAG	YINAKAPA	:	160
sp Q1RHT9	:	INNLTADYE	IVRKME	RASIWVLG	PLLSRYGKAKVS	SLPGGCAIG	ARQVDLHIAV	LKAMGAEITI	EDGYINASTA	GRLKGTHF	:	160
tr C6UF19	:	CAPYDLVKT	MRASIV	ALGPLVA	RFGQGQVSLPG	GCTIGARPVI	DLHISGLEQL	GATIKLEEGY	VKASVDGRLK	GAHIVMDK	:	160
tr B8DBI4	:	TSDAPFEYV	RKMRAS	SIVVMGPL	LARTGSARVALI	PGGCAIGSRI	PVDLHLKGFE	AMGAVVKIEN	GYIEATAEKL	VGAKVYLD	:	160
tr D3NWQ4	:	TARDITNTT	APYDL	RKMRASVI	LVLGPLVARCGE	EAKVSLPGG	CAIGARPVDL	HIKGLEAMGA	DIRIDAGYIV	AKAPAGGL	:	160
tr A0PXE3	:	SSDANTEDV	RKMRAS	SYYLIGAL	LGRFKKARVEMI	GGCAIGVR	PIDQHIKGFE	ALGANVTIEH	GAVVVEAEKL	VGTNIYFD	:	160
tr F5ZX21	:	CAPYDLVKT	MRASIV	VALGPLVA	RFGQGQVSLPG	GCTIGARPVI	DLHITGLEQL	GATIKLEEGY	VKASVEGRLK	GAHIVMDK	:	160
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			*	180	*	200	*	220	*	240		
sp.1.0970W61		DTUSUCATT	* N ጥ M T Δ Z	180	* TTENAARDETT	200	* MGAHIRGAGT	220	*	240		240
sp Q97QW6	:	DTVSVGATI	* NTMIAZ	180 AVKANGRTI	* IIENAAREPEII	200 IDVATLLNN	* MGAHIRGAGT	220 NIIIIDGVER	* LHGTRHQVIP	240 DRIEAGTY	:	240
sp Q97QW6  sp Q3IG20  sp P84059		DTVSVGATI MVSVGATEN	* NTMIAA LLMAAJ	180 AVKANGRTI ILADGKTVI MAASLAKGI	* IIENAAREPEII LENAACEPEITI	200 IDVATLLNN DLANCLIAM	* MGAHIRGAGT SAKITGAGTN	220 NIIIIDGVER RIEIEGVERI	* LHGTRHQVIP AGCEHRILPD	240 DRIEAGTY RIETGTFL	:	240 240 240
sp Q97QW6  sp Q3IG20  sp P84059		DTVSVGATI MVSVGATEN HLDFPSVGA	* NTMIAZ LLMAAJ TQNIIN	180 AVKANGRT TLADGKTVI MAASLAKGI	* IIENAAREPEII LENAACEPEITI KTLIENAAKEPH	200 IDVATLLNM DLANCLIAM EIVDLANYII	* MGAHIRGAGT SAKITGAGTN NEMGGRITGA	220 NIIIIDGVER RIEIEGVERI GTDTITINGV	* LHGTRHQVIP AGCEHRILPD ESLHGVEHAI	240 DRIEAGTY RIETGTFL IPDRIEAG	: : :	240 240 240
sp Q97QW6  sp Q3IG20  sp P84059  sp Q1MLP8		DTVSVGATI MVSVGATEN HLDFPSVGA GGLIGARYT	* NTMIAA LLMAAJ TQNIIN FPKVSV	180 AVKANGRT PLADGKTVI MAASLAKGI MGATHVMMI	* IIENAAREPEII LENAACEPEITI KTLIENAAKEPF MAATLARGTTVI	200 IDVATLLNM DLANCLIAM IVDLANYII IGNAAREPEY	* MGAHIRGAGT SAKITGAGTN NEMGGRITGA VVDLANCLNA	220 NIIIIDGVER RIEIEGVERI GTDTITINGV MGAKITGAGT	* LHGTRHQVIP AGCEHRILPD ESLHGVEHAI ATITIEGVTS	240 DRIEAGTY RIETGTFL IPDRIEAG LSGARHRV	: : : :	240 240 240 240
sp Q97QW6  sp Q3IG20  sp P84059  sp Q1MLP8  sp Q1RHT9		DTVSVGATI MVSVGATEN HLDFPSVGA GGLIGARYT IFDKISVGA	* LLMAAJ TQNIIN FPKVSV TINAVI	180 AVKANGRT TLADGKTVI MAASLAKGI GATHVMMI LAAVLADGI	* IIENAAREPEII LENAACEPEITI KTLIENAAKEP MAATLARGTTVI ETLLFNCAREPI	200 IDVATLLNNI DLANCLIAM( EIVDLANYII IGNAAREPEV EIVDLANCA	* MGAHIRGAGT SAKITGAGTN NEMGGRITGA VVDLANCLNA NKMGADISGI	220 NIIIIDGVER RIEIEGVERI GTDTITINGV MGAKITGAGT GTSEIRINGK	* LHGTRHQVIP AGCEHRILPD ESLHGVEHAI ATITIEGVTS DSLSEASYRV	240 DRIEAGTY RIETGTFL IPDRIEAG LSGARHRV LPDRIEAG	: : :	240 240 240 240 240
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<pre>sp Q97QW6  sp Q3IG20  sp P84059  sp Q1MLP8  sp Q1RHT9  tr C6UF19  tr B8DBI4  tr B8DBI4  tr A0PXE3  tr F5ZX21 </pre>		DTVSVGATI MVSVGATEN HLDFPSVGA GGLIGARYT IFDKISVGA VSVGATVTI FPSVGATQN RGAEYVFPK VVSVGATIN VSVGATVTI	* LLMAAT TQNIIN FPKVSV TINAVI MCAATI IMMAAT VSVGAT VSVGAT MCAATI	180 AVKANGRT: TLADGKTVI MAASLAKGI VGATHVMMI LAAVLADGI LAEGTTIII TLAEGTTVI TENLLMAA TLAEGKTVI LAEGTTIII	* IIENAAREPEII KTLIENAAKEPI MAATLARGTTVI ETLLFNCAREPEIVDI IENVAREPEIVDI IENVAREPEIVNI LENAAKEPHIVI ENAAREPEIVDI	200 IDVATLLNNI DLANCLIAM( EIVDLANYII IGNAAREPE FANFLITLGJ DLANFLNTLGJ DVANFLNSM( FANFLVTLGJ	* MGAHIRGAGT SAKITGAGTN NEMGGRITGA VVDLANCLNA NKMGADISGI AKISGQGTDR SARVIGAGTE LAECLVKMGA GADIKGAGTD	220 NIIIIDGVER RIEIEGVERI GTDTITINGV GTSEIRINGK IVIEGVERLG VIRIEGVKEI RITGIGSDRI VIKINGVKEI ITIEGVERLG	* LHGTRHQVIP AGCEHRILPD ESLHGVEHAI DSLSEASYRV GGVYRVLPDR TATEHSIIPD VIEGVDRLHA TGCNYSVIPD GGVYRVLPDR	240 DRIEAGTY RIETGTFL IPDRIEAG LSGARHRV LPDRIEAG RIEAGTFLV RIEAGTFM ARHMVVAD QIEAGTYM IETGTFLV		240 240 240 240 240 240 240 240 240 240
<pre>sp Q97QW6  sp Q3IG20  sp P84059  sp Q1MLP8  sp Q1RHT9  tr C6UF19  tr B8DBI4  tr D3NWQ4  tr A0PXE3  tr F5ZX21 </pre>		DTVSVGATI MVSVGATEN HLDFPSVGA GGLIGARYT IFDKISVGA VSVGATVTI FPSVGATQN RGAEYVFPK VVSVGATIN VSVGATVTI	* LLMAAJ TQNIIN FPKVSV TINAVI MCAATI IMMAAJ VSVGAJ VSVGAJ VMLAAJ	180 AVKANGRT: TLADGKTVI MAASLAKGI VGATHVMMI LAAVLADGI LAEGTTII TLAEGTTVI TLAEGTTVI LAEGTTIII	* IIENAAREPEII LENAACEPEITI KTLIENAAKEPI MAATLARGTTVI ETLLFNCAREPEIVDI IENVAREPEIVDI IENVAREPEIVDI LENAAKEPHIVI ENAAREPEIVDI	200 IDVATLLNNI DLANCLIAM( EIVDLANYII IGNAAREPEY EIVDLCNCLI TANFLITLG2 DLANFLNQM( AAREPEVTDI DVANFLNSM( FANFLVTLG2	* MGAHIRGAGT SAKITGAGTN NEMGGRITGA VVDLANCLNA NKMGADISGI AKISGQGTDR SARVIGAGTE LAECLVKMGA GADIKGAGTDR	220 NIIIIDGVER RIEIEGVERI GTDTITINGV MGAKITGAGT GTSEIRINGK IVIEGVERLG VIRIEGVKEI RITGIGSDRI VIKINGVKEI ITIEGVERLG	* LHGTRHQVIP AGCEHRILPD ESLHGVEHAI DSLSEASYRV GGVYRVLPDR TATEHSIIPD VIEGVDRLHA TGCNYSVIPD GGVYRVLPDR	240 DRIEAGTY RIETGTFL IPDRIEAG LSGARHRV IPDRIEAG IETGTFLV RIEAGTFM ARHMVVAD QIEAGTYM IETGTFLV		240 240 240 240 240 240 240 240 240 240
<pre>sp Q97QW6  sp Q3IG20  sp P84059  sp Q1MLP8  sp Q1RHT9  tr C6UF19  tr B8DBI4  tr D3NWQ4  tr A0PXE3  tr F5ZX21 </pre>		DTVSVGATI MVSVGATEN HLDFPSVGA GGLIGARYT IFDKISVGA VSVGATVTI FPSVGATQN RGAEYVFPK VVSVGATIN VSVGATVTI	* LLMAAT TQNIIN FPKVSV TINAVI MCAATI IMMAAT VSVGAT VSVGAT VMLAAT	180 AVKANGRT: FLADGKTVI (AASLAKG) GATHVMM LAAVLADG LAEGTTII FENLLMAA FLAEGTTVI LAEGTTII	* IIENAAREPEII LENAACEPEITI KTLIENAAKEPE MAATLARGTTVI ETLLFNCAREPEIVDI IENVAREPEIVDI IENVAREPEIVDI LAKGTTILVNI LENAAKEPHIVDI	200 IDVATLLNNI DLANCLIAM( EIVDLANYII IGNAAREPEY EIVDLCNCLI FANFLITLG/ DLANFLNQM( DAREPEVTD) DVANFLNSM( FANFLVTLG/	* MGAHIRGAGT SAKITGAGTN NEMGGRITGA VVDLANCLNA NKMGADISGI AKISGQGTDR SARVIGAGTE LAECLVKMGA GADIKGAGTDR	220 NIIIIDGVER RIEIEGVERI GTDTITINGV MGAKITGAGT GTSEIRINGK IVIEGVERIG VIRIEGVERIG VIRIEGVERIG ITIEGVERIG	* LHGTRHQVIP AGCEHRILPD ESLHGVEHAI ATITIEGVTS DSLSEASYRV GGVYRVLPDR TATEHSIIPD VIEGVDRLHA TGCNYSVIPD GGVYRVLPDR	240 DRIEAGTY RIETGTFL IPDRIEAG LSGARHRV LPDRIEAG IETGTFLV RIEAGTFM ARHMVVAD QIEAGTYM IETGTFLV		240 240 240 240 240 240 240 240 240 240
<pre>sp Q97QW6  sp Q3IG20  sp P84059  sp Q1MLP8  sp Q1RHT9  tr C6UF19  tr B8DBI4  tr D3NWQ4  tr A0PXE3  tr F5ZX21 </pre>		DTVSVGATI MVSVGATEN HLDFPSVGA GGLIGARYT IFDKISVGA VSVGATVTI FPSVGATQN RGAEYVFPK VVSVGATIN VSVGATVTI	* NTMIAA TQNIIN FPKVSV TINAVI MCAATI IMMAA VSVGA WLAATI MCAATI	180 AVKANGRT TLADGKTVI MAASLAKGI JAAVLADGI LAEGTTVI TLAEGTTVI TLAEGTTVI LAEGKTVI LAEGKTVI LAEGTTII	* IIENAAREPEII KTLIENAAKEPE MAATLARGTTVI ETLLFNCAREPE ENAAREPEIVD IENVAREPEIVD LAKGTTILVNZ LENAAREPEIVD ENAAREPEIVD	200 IDVATLLNNI DLANCLIAM( SIVDLANYII IGNAAREPEY SIVDLCNCLI PANFLITLG/ DLANFLNQM( AAREPEVTD) DVANFLNSM( PANFLVTLG/ 280	* MGAHIRGAGT SAKITGAGTN NEMGGRITGA VVDLANCLNA NKMGADISGI AKISGQGTDR GARVIGAGTE LAECLVKMGA GADIKGAGTD AKIAGQGTDR	220 NIIIIDGVER RIEIEGVERI GTDTITINGV MGAKITGAGT GTSEIRINGK IVIEGVERIG VIRIEGVERIG VIRIEGVERIG ITIEGVERIG 300	* LHGTRHQVIP AGCEHRILPD ESLHGVEHAI ATITIEGVTS DSLSEASYRV GGVYRVLPDR TATEHSIIPD VIEGVDRLHA TGCNYSVIPD GGVYRVLPDR	240 DRIEAGTY IPDRIEAG LSGARHRV LPDRIEAG IETGTFLV RIEAGTFM ARHMVVAD QIEAGTYM IETGTFLV		240 240 240 240 240 240 240 240 240
<pre>sp Q97QW6  sp Q3IG20  sp P84059  sp Q1MLP8  sp Q1RHT9  tr C6UF19  tr B8DBI4  tr D3NWQ4  tr A0PXE3  tr F5ZX21 </pre>		DTVSVGATI MVSVGATEN HLDFPSVGA GGLIGARYT IFDKISVGA VSVGATVTI FPSVGATQN RGAEYVFPK VVSVGATIN VSVGATVTI ISLAAAVGK	* NTMIAA TQNIIN FPKVSU MCAATI IMMAAJ VSVGAJ VMLAAJ MCAATI * GIRINN	180 AVKANGRT ILADGKTVI MAASLAKGI JAAVLADGI LAEGTTVI TLAEGTTVI TAEGKTVI JAEGTTII JAEGKTVI JAEGTTII 260 IVLYEHLE(	* IIENAAREPEII KTLIENAAKEPI MAATLARGTTVI ETLLFNCAREPI ENAAREPEIVO IENVAREPEIVO ILAKGTTILVNA LENAAKEPHIVO ENAAREPEIVO * SFIAKLEEMGVI	200 IDVATLINM DLANCLIAM SIVDLANCII IGNAAREPEV SIVDLCNCLI TANFLITLG DLANFLNDM AAREPEVTD DVANFLNSM TANFLVTLG 280 280	* MGAHIRGAGTN NEMGGRITGA VVDLANCLNA NKMGADISGI AKISGQGTDR GARVIGAGTE LAECLVKMGA GADIKGAGTD AKIAGQGTDR * FVEEQSNLKA	220 NIIIIDGVER RIEIEGVERI GTDTITINGV MGAKITGAGT GTSEIRINGK IVIEGVERIG VIRIEGVERIG VIRIEGVERIG ITIEGVERIG 300 INIKTAPYPG	* LHGTRHQVIP AGCEHRILPD ESLHGVEHAI ATITIEGVTS DSLSEASYRV GGVYRVLPDR TATEHSIIPD VIEGVDRLHA TGCNYSVIPD GGVYRVLPDR * FATDLQQPLT	240 DRIEAGTY RIETGTFL IPDRIEAG LSGARHRV LPDRIEAG IETGTFLV RIEAGTFM ARHMVVAD QIEAGTYM IETGTFLV 320 PLLERANG		240 240 240 240 240 240 240 240 240 240
<pre>sp Q97QW6  sp Q3IG20  sp P84059  sp Q1MLP8  sp Q1RHT9  tr C6UF19  tr B8DBI4  tr D3NWQ4  tr A0PXE3  tr F5ZX21  sp Q97QW6  sp Q3IG20 </pre>		DTVSVGATI MVSVGATEN HLDFPSVGA GGLIGARYT IFDKISVGA VSVGATVTI FPSVGATQN RGAEYVFPK VVSVGATIN VSVGATVTI ISLAAAVGK VAAAMAGGE	* NTMIAA TQNIIN FPKVSV TINAVI MCAATI VSVGAJ VSVGAJ VMLAAJ MCAATI * GIRINN VLCKMJ	180 AVKANGRT PLADGKTVI MAASLAKGI JAAVLADG JAAVLADG JAAVLADG LAEGTTII PLAEGTTVI PENLLMAA PLAEGKTVI JAEGTTII 260 AVLYEHLEG DFHSLEE	* IIENAAREPEII LENAACEPEITI KTLIENAAKEPH MAATLARGTTVI ETLLFNCAREPEIVI IENVAREPEIVI ILAKGTTILVN2 LENAAKEPHIVI ENAAREPEIVD * GFIAKLEEMGVE	200 IDVATLINNI DIANCLIAMU EIVDLANYII IGNAAREPEV DIANFLITLG DIANFLITLG DIANFLITLG DIANFLITLG DIANFLITLG DIANFLVTLG 280 280 280 EVISEDSII	* MGAHIRGAGT SAKITGAGTN NEMGGRITGA VVDLANCLNA NKMGADISGI AKISGQGTDR GARVIGAGTE LAECLVKMGA GADIKGAGTD AKIAGQGTDR * FVEEQSNLKA LDMRGRELKA	220 NIIIIDGVER RIEIEGVERI GTDTITINGV MGAKITGAGT GTSEIRINGK IVIEGVERIG VIRIEGVERIG VIRIEGVERIG ITIEGVERIG 300 INIKTAPYPG VNIKTAPPPG	* LHGTRHQVIP AGCEHRILPD ESLHGVEHAI ATITIEGVTS DSLSEASYRV GGVYRVLPDR TATEHSIIPD VIEGVDRLHA TGCNYSVIPD GGVYRVLPDR * FATDLQQPLT FFTDMQAQFT	240 DRIEAGTY RIETGTFL IPDRIEAG LSGARHRV LPDRIEAG IETGTFLV RIEAGTFM ARHMVVAD QIEAGTYM IETGTFLV 320 PLLI RANG ALNVVANG		240 240 240 240 240 240 240 240 240 240
<pre>sp Q97QW6  sp Q3IG20  sp P84059  sp Q1MLP8  sp Q1RHT9  tr C6UF19  tr B8DBI4  tr D3NWQ4  tr A0PXE3  tr F5ZX21  sp Q97QW6  sp Q3IG20  sp P84059 </pre>		DTVSVGATI MVSVGATEN HLDFPSVGA GGLIGARYT IFDKISVGA VSVGATVTI FPSVGATQN RGAEYVFPK VVSVGATVTI ISLAAAVGK VAAAMAGGE TLLIAGAIT	* NTMIAA ILMAAJ TQNIIN FPKVSV TINAVI MCAATI VSVGAJ VSVGAJ VMLAAJ MCAATI * GIRINN VLCKMJ RGDIFV	180 AVKANGRT: PLADGKTVI MAASLAKGI JAGATHVMMI JAEGTTII PENLLMAA: PENLLMAA: PLAEGKTVI JAEGTTIII 260 IVLYEHLEQ PDFHSLEP VRGAIKEH	* IIENAAREPEII KTLIENAAKEPH MAATLARGTTVI ETLLFNCAREPEIVI IENVAREPEIVI ILAKGTTILVN2 LENAAKEPHIVI ENAAREPEIVD * SFIAKLEEMGVH VIEKLRATNALI	200 IDVATLLNNI DLANCLIAM( EIVDLANYII IGNAAREPEV EIVDLCNCLI TANFLITLG DLANFLNQM( AARE PEVTDD DVANFLNSM( FANFLVTLG 280 XVSEDSI) LEVHDNSIYJ	* MGAHIRGAGT SAKITGAGTN NEMGGRITGA VVDLANCLNA NKMGADISGI AKISQQGTDR GARVIGAGTE LAECLVKMGA GADIKGAGTD AKIAGQGTDR * FVEEQSNLKA LDMRGRELKA GIRVRAEGEL	220 NIIIIDGVER RIEIEGVERI GTDTITINGV IVIEGVERIG VIRIEGVERIG VIRIEGVERIG ITIEGVERIG 300 INIKTAPYPG VNIKTAPHPG QPVDIKTLPH	* LHGTRHQVIP AGCEHRILPD ESLHGVEHAI ATITIEGVTS DSLSEASYRV GGVYRVLPDR TATEHSIIPD VIEGVDRLHA TGCNYSVIPD GGVYRVLPDR * FATDLQQPLT FPTDMQAQFT PGFPTDMQSQ	240 DRIEAGTY RIETGTFL IPDRIEAG LSGARHRV LPDRIEAG IETGTFLV RIEAGTFM ARHMVVAD QIEAGTYM IETGTFLV 320 PLLIRANG ALNVVANG MMAILLTA		240 240 240 240 240 240 240 240 240 240
<pre>sp Q97QW6  sp Q3IG20  sp P84059  sp Q1MLP8  sp Q1RHT9  tr C6UF19  tr B8DBI4  tr D3NWQ4  tr A0PXE3  tr F5ZX21  sp Q97QW6  sp Q3IG20  sp P84059  sp Q1MLP8 </pre>		DTVSVGATI MVSVGATEN HLDFPSVGA GGLIGARYT IFDKISVGA VSVGATVTI FPSVGATQN RGAEYVFPK VVSVGATIN VSVGATVTI ISLAAAVGK VAAAMAGGE TLLIAGAIT LPDRIETGT	* NTMIAA ILMAAJ TQNIIN FPKVSV TINAVI MCAATI IMMAAJ VSVGAJ VSVGAJ VMLAAJ MCAATI * GIRINN VLCKMJ RGDIFV YAMAVA	180 AVKANGRT: LADGKTVI MAASLAKGJ JAAVLADGJ JAAVLADGJ LAEGTTII LAEGTTII LAEGTTII 260 IVLYEHLEQ TOFHSLEP /RGAIKEH AMAGGDVV	* IIENAAREPEII KTLIENAAKEPI MAATLARGTTVI ETLLFNCAREPEIVDI IENVAREPEIVDI IENVAREPEIVDI LENAAKEPHIVI ENAAREPEIVDI * SFIAKLEEMGVH VIEKLRATNALI ASLVYKLEEMG	200 IDVATLLNNI DLANCLIAM( EIVDLANYII IGNAAREPE IVDLCNCLI FANFLITLG DLANFLNTLG DVANFLNSM( TANFLVTLG 280 RMTVSEDSII EVHDNSIXI SVELDYQED( AVETLRRAG	* MGAHIRGAGT SAKITGAGTN NEMGGRITGA VVDLANCLNA NKMGADISGI AKISGQGTDR GARVIGAGTE LAECLVKMGA GADIKGAGTD AKIAGQGTDR * FVEEQSNLKA LDMRGRELKA GIRVRAEGEL AEISSTNNGM	220 NIIIIDGVER RIEIEGVERI GTDTITINGV MGAKITGAGT GTSEIRINGK IVIEGVERIG VIRIEGVERIG VIRIEGVERIG 300 INIKTAPYPG VNIKTAPHPG QPVDIKTIPH RIKRNGAGIR	* LHGTRHQVIP AGCEHRILPD ESLHGVEHAI ATITIEGVTS DSLSEASYRV GGVYRVLPDR TATEHSIIPD VIEGVDRLHA TGCNYSVIPD GGVYRVLPDR * FATDLQQPLT FPTDMQAQFT PGFPTDMQSQ PVDIVTDPFP	240 DRIEAGTY RIETGTFL IPDRIEAG LSGARHRV LPDRIEAG IETGTFLV RIEAGTFM ARHMVVAD QIEAGTYM IETGTFLV 320 PLLIRANG ALNVVANG MMALLITA GFPTDLQA		240 240 240 240 240 240 240 240 240 240
<pre>sp Q97QW6  sp Q3IG20  sp P84059  sp Q1MLP8  sp Q1RHT9  tr C6UF19  tr B8DBI4  tr A0PXE3  tr F5ZX21  sp Q97QW6  sp Q3IG20  sp P84059  sp Q1MLP8  sp Q1RHT9 </pre>		DTVSVGATI MVSVGATEN HLDFPSVGA GGLIGARYT IFDKISVGA VSVGATVTI FPSVGATQN RGAEYVFPK VVSVGATIN VSVGATVTI ISLAAAVGK VAAMAGGE TLLIAGAIT LPDRIETGT TYMFAAAIT	* NTMIAA TQNIIN FPKVSU TINAVI MCAATI IMMAAJ VSUGAJ VMLAAJ MCAATI * GIRINN VLCKMI RGDIFU YAMAV KGDLKI	180 AVKANGRT TLADGKTVI MAASLAKGI (GATHVMM LAAVLADGI LAEGTTII) TLAEGTTVI LAEGTTII 260 IVLYEHLEC TDFHSLEP IRGAIKEH MAGGDVV JYGIDYHI	* IIENAAREPEII LENAACEPEITI KTLIENAAKEPF MAATLARGTTVI ETLLFNCAREPEIVI IENVAREPEIVI IENVAREPEIVI LENAAKEPHIVI SFIAKLEEMGVI VIEKLRATNALI ASLVYKLEEMG IENTDVALLET ENIALKLIET	200 IDVATLINNI DLANCLIAM IGNAAREPE' SIVDLCNCLI IANFLITLG/ DLANFLNQM AAREPEVTDD OVANFLNSM (TANFLVTLG/ 280 RMTVSEDSI) LEVHDNSIY] SVELDYQED AVETLRRAG	* MGAHIRGAGT SAKITGAGTN NEMGGRITGA VVDLANCLNA NKMGADISGI AKISGQGTDR GARVIGAGTD AKIAGQGTDR * FVEEQSNLKA GIRVRAEGEL AEISSTNNGM GVQVTYADKL	220 NIIIIDGVER RIEIEGVERI GTDTITINGV MGAKITGAGT GTSEIRINGK IVIEGVERIG VIRIEGVERIG VIRIEGVERIG 300 INIKTAPYPG QPVDIKTLPH RIKRNGAGIF NAVNLETNPY	* LHGTRHQVIP AGCEHRILPD ESLHGVEHAI ATITIEGVTS DSLSEASYRV GGVYRVLPDR TATEHSIIPD VIEGVDRLHA TGCNYSVIPD GGVYRVLPDR * FATDLQQPLT FFFTDMQAQFT PGFFTDMQAQF PVDIVTDFFP PGFATDLQAQ	240 DRIEAGTY RIETGTFL LSGARHRV LPDRIEAG IETGTFLV RIEAGTFM ARHMVVAD QIEAGTYM IETGTFLV 320 PLL RANG ALNVVANG MMAILLTA GGFPTDLQA FMS MTIS		2400 2400 2400 2400 2400 2400 2400 2400
<pre>sp Q97QW6  sp Q3IG20  sp P84059  sp Q1MLP8  sp Q1RHT9  tr C6UF19  tr B8DBI4  tr D3NWQ4  tr A0PXE3  tr F5ZX21  sp Q97QW6  sp Q3IG20  sp P84059  sp Q1MLP8  sp Q1RHT9  tr C6UF19 </pre>		DTVSVGATI MVSVGATEN HLDFPSVGA GGLIGARYT IFDKISVGA VSVGATVTI FPSVGATQN RGAEYVFPK VVSVGATIN VSVGATVTI ISLAAAVGK VAAAMAGGE TLLIAGAIT LPDRIETGT TYMFAAAIT AAAISRGKI	* NTMIAA TQNIIN FPKVSV TINAVI MCAATI IMMAAJ VSVGAJ VSVGAJ VMLAAJ MCAATI * GIRINN VLCKMJ RGDIFV YAMAVZ KGDLKI ICRNAQ	180 AVKANGRT TLADGKTVI MAASLAKGI JAASLAKGI JAAVLADGI JAEGTTVI TLAEGTTVI TLAEGTTVI AEGTTVI 260 IVLYEHLEK TDFHSLEP VRGAIKEH MAGGDVV LYGIDYHI 20DTLDAV	* IIENAAREPEII LENAACEPEITI KTLIENAAKEPH MAATLARGTTU ETLLFNCAREPE ENAAREPEIVD IENVAREPEIVD LENAAREPEIVD * SFIAKLEEMGVH VIEKLRATNALI VASLVYKLEEMG IENTDVALLET IENIALKIETG AKLRDAGADI	200 IDVATLINNI DIANCLIAM IVDLANCIIAM IVDLANCIIAM IGNAAREPEY SIVDLCNCLI TANFLITLG DIANFLITLG DIANFLITLG OVANFLNSM TANFLVTLG 280 RMTVSEDSI EVHDNSIYI SVELDYQED IKVMPIDN SIKVMPIDN SVELDYZEJ	* MGAHIRGAGT SAKITGAGTN NEMGGRITGA VVDLANCLNA NKMGADISGI AKISGQGTDR GARVIGAGTE LAECLVKMGA GADIKGAGTD AKIAGQGTDR * FVEEQSNLKA LDMRGRELKA GIRVRAEGEL AEISSTNNGM GVQVTYADKL DMHGKRPKAV	220 NIIIIDGVER RIEIEGVERI GTDTITINGV MGAKITGAGT GTSEIRINGK IVIEGVERIG VIRIEGVERIG VIRIEGVERIG 300 INIKTAPHPG QPVDIKTLPH RIKRNGAGIF NAVNLETNPY	* LHGTRHQVIP AGCEHRILPD ESLHGVEHAI ATITIEGVTS DSLSEASYRV GGVYRVLPDR TATEHSIIPD VIEGVDRLHA TGCNYSVIPD GGVYRVLPDR * FATDLQQPLT FPTDMQAQFT PGFPTDMQSQ PVDIVTDPFP PGFATDLQQQFTL	240 DRIEAGTY RIETGTFL IPDRIEAG LSGARHRV LPDRIEAG IETGTFLV RIEAGTFM ARHMVVAD QIEAGTYM IETGTFLV 320 PLLIRANG ALNVVANG GFPLLQA FMSIMTIS LNLVAEGT		240 240 240 240 240 240 240 240 240 240
<pre>sp Q97QW6  sp Q3IG20  sp P84059  sp Q1MLP8  sp Q1RHT9  tr C6UF19  tr B8DBI4  tr D3NWQ4  tr A0PXE3  tr F5ZX21  sp Q97QW6  sp Q3IG20  sp P84059  sp Q1MLP8  sp Q1RHT9  tr C6UF19  tr B8DBI4 </pre>		DTVSVGATI MVSVGATEN HLDFPSVGA GGLIGARYT IFDKISVGA VSVGATVTI FPSVGATQN RGAEYVFPK VVSVGATIN VSVGATVTI ISLAAAVGK VAAAMAGGE TLLIAGAIT LPDRIETGT TYMFAAAT AAAISRGKI IAAAITGGN	* NTMIAZ LLMAAJ TQNIIN FPKVSVI MCAATI IMMAAJ VSVGAJ VSVGAJ VMLAAJ MCAATI * GIRINN VLCKMJ RGDIFV YAMAVZ KGDLKI ICRNAQ VLIEDZ	180 AVKANGRT ILADGKTVI MAASLAKGI JAAVLADG JAEGTTIII TLAEGTTVI TENLLMAA TAEGTTIII 260 IVLYEHLEO TDFHSLEP IRGAIKEH MAGGDVVI JYGDYLDAV AVFEHISS	* IIENAAREPEII LENAACEPEITI KTLIENAAKEPH WAATLARGTTU ETLLFNCAREPE ENAAREPEIVD IENVAREPEIVD ILAKGTTILVN2 LENAAKEPHIVI ENAAREPEIVD * SFIAKLEEMGVI VIEKLRATNALI VASLVYKLEEMG ENTALKLETA IAKLRDAGADIH IAKLEEMGVOI	200 IDVATLINNI DLANCLIAM SIVDLANCII SIVDLANCII IGNAAREPEV SIVDLCNCLI TANFLITLG DLANFLNDM AAREPEVTD DVANFLNSM TANFLVTLG SVELDYQED SVELDYQED SIKVMPIDN SVELDYQED SIKVMPIDN SVELDYQED SIKVMPIDN SIKVMPIDN SVELDYQED SIKVMPIDN SIKVMPIDN	* MGAHIRGAGT SAKITGAGTN NEMGGRITGA VVDLANCLNA NKMGADISGI AKISGQGTDR GARVIGAGTE LAECLVKMGA GARVIGAGTD * FVEEQSNLKA SIRVRAEGEL ASIRVRAEGEL ASISYTNAGK GVQVTYADGL DMHGKRPKAV VIGPDKLKAV	220 NIIIIDGVER RIEIEGVERI GTDTITINGV MGAKITGAGT GTSEIRINGK IVIEGVERIG VIRIEGVERIG VIRIEGVERIG VIKINGVKEI ITIEGVERIG 300 INIKTAPHPG QPVDIKTLPH RIKRNGAGIF NAVNLETNPA DVKTAPHPGF	* LHGTRHQVIP AGCEHRILPD ESLHGVEHAI ATITIEGVTS DSLSEASYRV GGVYRVLPDR TATEHSIIPD VIEGVDRLHA TGCNYSVIPD GGVYRVLPDR * FATDLQQPLT FFPTDMQAQFT PGFPTDMQAQFT PFDMQAQFTL PTDMQAQFTL	240 DRIEAGTY RIETGTFL IPDRIEAG LSGARHRV LPDRIEAG IETGTFLV RIEAGTFM ARHMVVAD QIEAGTYM IETGTFLV 320 PLLIRANG ALNVVANG MMALLLTA GFPTDLQA FMSLMTIS LNLVAEGT IQMISEGT		240 240 240 240 240 240 240 240 240 240
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<pre>sp Q97QW6  sp Q3IG20  sp P84059  sp Q1MLP8  sp Q1RHT9  tr C6UF19  tr B8DBI4  tr J3NWQ4  tr A0PXE3  tr F5ZX21  sp Q97QW6  sp Q3IG20  sp P84059  sp Q1RHT9  tr C6UF19  tr C6UF19  tr B8DBI4  tr J3NWQ4  tr A0PXE3 </pre>		DTVSVGATI MVSVGATEN HLDFPSVGA GGLIGARYT IFDKISVGA VSVGATVTI FPSVGATQN RGAEYVFPK VVSVGATVTI ISLAAAVGK VAAAMAGGE TLLIAGAIT LPDRIETGT TYMFAAAIT AAAISRGKI IAAAITGGN RIETGTYAM	* NTMIAA LLMAAJ TQNIIN FPKVSV TINAVI MCAATI VSVGAJ VSVGAJ VMLAAJ MCAATI * GIRINN VLCKMJ RGDIFV YAMAVA KGDLKI ICRNAÇ VLICKAZ VLIEDA AAAITC	180 AVKANGRT PLADGKTVI MAASLAKGI JAAVLADGI JAEGTTII PLAEGTTV PENLLMAA PLAEGTTVI 260 VLYEHLEG PDFHSLEP VRGAIKEH AMAGDVV LYGIDYHI PDTLDAV SGRLDILW UTEKHLES	* IIENAAREPEII LENAACEPEITI KTLIENAAKEPH MAATLARGTTVI ETLLFNCAREPEIVI IENVAREPEIVI ILAKGTTILVN2 LENAAKEPHIVI ENAAREPEIVD SFIAKLEEMGVI VIEKLRATNALI VASLVYKLEEMG IENIALKLIETO IAKLRDAGADIH IAKLEEMGVQ IRLDLIKAAVK2 ISAKLIEMGVW	200 IDVATLINNI DIANCLIAM( EIVDLANYII IGNAAREPE DIANFLITIGJ DIANFLNQM( AAREPEVTD) DVANFLNSM( FANFLVTLGJ EVHDNSIY] SVELDYQED( AVETLRRAGJ EIKVMPIDM( EVGEDWISL] I EEENGIY] ALAPAGVAFJ	* MGAHIRGAGT SAKITGAGTN NEMGGRITGA VVDLANCLNA NKMGADISGI AKISQQGTDR GARVIGAGTE LAECLVKMGA GADIKGAGTD AKIAGQGTDR * FVEEQSNLKA GIRVRAEGEL AEISSTNNGM GVQVTYADKL DMHGKRPKAV EEIENGIRVS INSTRNLNGV	220 NIIIIDGVER RIEIEGVERI GTDTITINGV IVIEGVERIG VIRIEGVERIG VIRIEGVERIG VIRIEGVERIG NIKTAPYPG VNIKTAPHPG RANGELHGVI NIKTLPYPGF	* LHGTRHQVIP AGCEHRILPD ESLHGVEHAI ATITIEGVTS DSLSEASYRV GGVYRVLPDR TATEHSIIPD VIEGVDRLHA TGCNYSVIPD GGVYRVLPDR * FATDLQQPLT FPTDMQAQFT PGFPTDMQAQFT PTDMQAQFT PTDMQAQFT PTDMQSQMV WMTEPFPGFP	240 DRIEAGTY RIETGTFL IPDRIEAG LSGARHRV LPDRIEAG IETGTFLV RIEAGTFM ARHMVVAD QIEAGTYM IETGTFLV 320 PLLI RANG ALNVVANG MMAILLTA GFPTDLQA FMSIMTIS LNLVAEGT TDLQAQMM ILS JAKGS		240 240 240 240 240 240 240 240 240 320 320 320 320 320 320 320 320 320 32
<pre>sp Q97QW6  sp Q3IG20  sp P84059  sp Q1MLP8  sp Q1RHT9  tr C6UF19  tr B8DBI4  tr A0PXE3  tr F5ZX21  sp Q97QW6  sp Q3IG20  sp P84059  sp Q1MLP8  sp Q1RHT9  tr C6UF19  tr B8DBI4  tr A0PXE3  tr F5ZX21</pre>		DTVSVGATI MVSVGATEN HLDFPSVGA GGLIGARYT IFDKISVGA VSVGATVTI FPSVGATQN RGAEYVFPK VVSVGATIN VSVGATVTI ISLAAAVGK VAAAMAGGE TLLIAGAIT LPDRIETGT TYMFAAAIT AAAISRGKI IAAAITGGN RIETGTYAM IATAACGGN	* NTMIAA LLMAAJ TQNIIN FPKVSU TINAVI MCAATI IMMAAJ VSUGAJ VSUGAJ VMLAAJ MCAATI * GIRINN VLCKMI RGDIFU YAMAVZ KGDLKI ICRNAQ VLIEDA AAAITO VTINNU LCRNA	180 AVKANGRT: TLADGKTVI MAASLAKGJ (GATHVMM LAAVLADGJ LAEGTTII) TLAEGTTVI LAEGTTVI LAEGTTII) 260 WLYEHLEK DFHSLEP MAGGDVV YGGIYHI QPDTLDAV AVPEHISS GGRLDILM /IPKHLES	* IIENAAREPEII LENAACEPEITI KTLIENAAKEPF MAATLARGTTU ETLLFNCAREPEIVD IENVAREPEIVD IENVAREPEIVD LENAAREPEIVD * SFIAKLEEMGVI ASLVYKLEEMG IENTDVALLET IENTALKLIET IAKLRDAGADIF IAKLEEMGVQ SAKLIEMGVN	200 IDVATLINNI DLANCLIAM IGNAAREPE' SIVDLCNCLI IGNAFLITLG/ DLANFLNQM AREPEVTDD DVANFLNSM FANFLVTLG/ VELDYQED VELDYQED VELDYQED SVELDYQED SVELDYQED I IEEENGIR I IEEENGIR ALAPAGVAFI VIENGDSII	* MGAHIRGAGT SAKITGAGTN NEMGGRITGA VVDLANCLNA NKMGADISGI AKISGQGTDR GARVIGAGTE AKIAGQGTDR * FVEEQSNLKA GIRVRAEGEL AKIASQGTDR GVQVTYADKL DMHGKRPKAV VIGPDKLKAV EEIENGIRVS DMHGKRPKAV	220 NIIIIDGVER RIEIEGVERI GTDTITINGV MGAKITGAGT GTSEIRINGK IVIEGVERIG VIRIEGVERIG VIRIEGVERIG 300 INIKTAPYPG QPVDIKTLPH RIKRNGAGIR NAVNLETNPY NVRTAPHPAF DVKTMPHPGF RANGELHGVU NIKTLPYPGF NVRTAPHPAF	* LHGTRHQVIP AGCEHRILPD ESLHGVEHAI ATITIEGVTS DSLSEASYRV GGVYRVLPDR TATEHSIIPD VIEGVDRLHA TGCNYSVIPD GGVYRVLPDR * FFATDLQQPLT FFTDMQAQFTL PTDMQAQFTL PTDMQAQFTL PTDMQAQFTL PTDLQQPMTT PTDLQQPTT	240 DRIEAGTY RIETGTFL LSGARHRV LPDRIEAG IETGTFLV RIEAGTFM ARHMVVAD QIEAGTYM IETGTFLV 320 PLLIRANG MMAILLTA GFPTDLQA FMSIMTIS LNLVAEGT IQMISEGT TDLQAQMM ILSTAKGS LNLVAEGT		240 240 240 240 240 240 240 240 240 320 320 320 320 320 320 320 320 320 32

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			*	340	*		360	1	*	380	*	400		
sp Q97QW6	:	RGTIVDT	IYEKRVI	HVFELAKM	CADISTING	GHI	LYTGGRDLF	GASVKAT	DLRAGAA	VIAGLMA	EGKTEITNI	EFILRGY	:	400
sp Q3IG20	:	SATITET	IFENREN	HVPELQRM	GANIRLEGN	TA	AICGETKTLS	GAQVMAT	DLRASAS	LILTGIVA	QGETIVERI	THVDRGY	:	400
sp P84059	:	NGHKVVT	ETVFENI	REMHVAEEKI	RMNANINVE	EGF	RSAKLEGKSQ	LQGAQVK	ATDLRAA	AALILAGI	VADGKTSVI	TELTHLDR	:	400
sp Q1MLP8	:	QFMALMT	RSSGVSI	IVTETIFENI	RFMHVQELZ	ARI	GARISLSG	TAKIEGV	QRLRGAP	MATCLRA	SVSLVIAGI	LAAEGETT	2	400
sp Q1RHT9	:	QGSSIIT	ENIFEN	REMHVPELCI	RMGADITVE	RGN	<b>IQAIVQGVK</b>	LKGAEVM	ASDLRAS	VSLILAGI	STDSETVLE	RIYHLDR	2	400
tr C6UF19	:	GFITETV	FENRFM	HVPELSRMG	AHAEIESNI	rvı	CHGVEKLSO	AQVMATD	LRASASL	VLAGCIAE	GTTVVDRI	HIDRGYE	:	400
tr B8DBI4	:	SIMTETV	FENRFM	IVEEMRRMN	ADMKIEGHS	IVE	ISGPAKLQG	AEVAATD	LRAAAAL	LAGLVAL	GYTQVTELF	KYLDRGYN	-	400
tr D3NWQ4	:	ALMCTAK	GAAMITH	ETIFENRFM	HAPELTRMO	GAR	RITVHGSSAL	VRGVERL	TGAPVMA	TELRASVS	LVLAGLAAE	GETTVNR	:	400
tr AOFXE3	:	SIVNESI	WESRFK	HVDELKKMG	AKISVENNI	IAM	AIEGVKSLSG	AKVKATD	LRAGAAM	IAGLIAN	GITEVTNIE	EHIDRGYP		400
tr F5ZX21	:	GFITETV	FENRFM	HVPELSRMG	ARAEIESNI	IVI	CHGVETLS	AQVMATD	LRASASL	VLAGCIAE	GTTIVDRIY	HIDRGYE	2	400
			*	420	*									
sp Q97QW6	:	SDIIEKL	RNLGAD:	IRLVED		:	419							
sp Q3IG20	:	ERIEDKL	SALGAN	IKRRSS		:	419							
sp P84059	:	GYVDLHG:	KLKQLGA	ADIERIND		-	421							
sp Q1MLP8	:	VSRVYHL	DRGFERI	LEEKLTRCG	AIVERISE	:	430							
sp Q1RHT9	:	GFQNLEK	KLNNCG	ADIKRV		:	419							
tr C6UF19	:	RIEDKLR	ALGANI	ERVKGE		:	419							
tr B8DBI4	:	NFHGKLQ	ALGADVE	ERVEDSKID	VTNLASLF	-	430							
tr D3NWQ4	:	VYHLDRG	YERVEER	KLAACGAEI	ERIHGGEE	:	430							

tr|AOFXE3| : VIELKKIKALGANIERVKGE------ : 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 structures which 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 2.522⁰ 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:** 

## 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	SOPMA		GOR4		CHOU & FA	SMAN	
	Structure	No. of	% of	No. of	% of	No. of	% of	
	~	Residues	Residues	Residues	Residues	Residues	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.

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## **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,1]	,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 resid	ues 360	100.0%
End-residues (excl. Gly and Pro)	2	
Glycine residues	42	
Proline residues	15	
Total number of residues	419	

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Fig-8:The over quality of MurA was observed using<br/>ERRAT environment profile.Fig-9:Verify3D-1D graph.





#### 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







## 7. ZINC3978065



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

S.no	Compounds	protein-ligand interacti	ons		Bond	binding	
		protein	ligand	Distance	angle	energy	
1	Fosfomycin	Gly302 CA-C-O	Н9	2.2	132.7	-4.5	
		Phe264 CA-NH	O4P	2.5	106.1		

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2	Substrate GluNac	Leu 91 CA-C-OH9C	)	2.4	141.4	-4.5
		Ser 94 OG-G-HO14	С	2.2	132.7	
		Asn 89 CA-C-OH20	0	2.2	110.5	

**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

SNo	Compounds	Protein-Ligand Inter	ractions	Distance	Bond	Binding
		protein	ligand		Angle	Energy
1	ZINC50247	Asp306 CG-D1O	H46N	2.6	141.3	-10.3
		Asp306 CG-D2O	H47N	2.5	130.3	
		Tyr329 CZ-HO	H45N	2.1	96.9	
2	ZINC3820559	Arg121 CA-N	O29C	3.5	132.8	-10.1
		Gly115 CO	H32N	2.7	142.0	
3	ZINC1884559	Arg322 CG-D10	O30C	3.4	107.0	-9.8
		Asn23 CZ-NH2	HN34N	2.7	131.9	
4	ZINC3154681	Ser 125 CB-GO	032C	3.4	108.1	-9.8
		Leu91 C-O	H37N	2.4	124.7	
5	ZINC19286884	Tyr329 CZ-HO	H30N	2.3	115.1	-9.2
		Tyr329 CZ-HO	HN32N	2.3	97.3	
6	ZINC58219	Asn23,Ala27,Asn89,I	Leu91,Arg92,			-9.1
		Tyr95,Pro113,Gly114	,Gly115,Lys116,Asp117,Pr			
		o120,Arg121,Pro122,	Ile123,Ser164,Val165,Gly1			
		66,Glu190,Glu192,Ar	g234,Asp306,Tyr329			
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.





Docking poses with selected 7 zinc compounds.



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1. ZINC50247

2. ZINC3820559





3. ZINC1884559



5. ZINC19286884

4. ZINC3154681



6. ZINC58219

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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.
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s.n 0	Zinc Compound	pH range	xlog P	Apolar desolva tion(kc al/mol)	Polar desolvati on(kcal/ mol)	H- bond dono rs	H- bond accep tors	Net char ge	tPS A (Ų)	Molecular weigh (g/mol)	Rotata ble bonds
1	ZINC50247	pH 7	2.34	-2.04	-47.39	1	6	1	51	298.37	1

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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.N	COMPOUND	MI	TPSA	n-	M.W	nO	nOHNH	nVIOLATIO	NROTB	VOLUME
0	ID	LOGP		ATO		Ν		NS		
				MS						
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.n	Compound	Mutag-	Tumori	Irrita	Reprod	Clog	Solubilit	Mol.	Drug	Dru
0		enic	genic	nt	uction	р	У	Wt	Likene	g
					Effecti				SS	Scor
					ve					e
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

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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.

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