REPORT

Computational drug designing of newly synthesized triazoles against potential targets of methicillin resistant *Staphylococcus aureus*

Umair Ilyas[1,3], Reem Altaf[1], Syed Aun Muhammad[*2], Muhammad Imran Qadir[2], Humaira Nadeem[3] and Safia Ahmed[*1]

[1]Department of Microbiology, Quaid-I-Azam University Islamabad, Pakistan
[2]Institute of Molecular Biology and Biotechnology, Bahauddin Zakariya University Multan, Pakistan
[3]Riphah Institute of Pharmaceutical Sciences (RIPS) Islamabad, Pakistan

Abstract: Methicillin resistant *Staphylococcus aureus* (MRSA) is resistant to known antibiotics and has become a great challenge for healthcare professionals, therefore new molecules are needed to manage this situation. In this study, new lead molecules 4-Amino-5-(2-Hydroxyphenyl)-1,2,4-Triazol-3-Thione (U1) and4-(2-hydroxybenzalidine) amine-5-(2-hydroxy) phenyl-1,2,4-triazole-3-thiol(U1A Schiff base) were synthesized by fusion method that showed promising antibacterial activity (U1A: 26mm and U1: 14mm) against MRSA.FT-IR and NMR were used for structural characterization of these derivatives and their toxicity properties were assessed by Lipinski's rule of 5. New potential drug targets of this bacterium were also identified by comparative and subtraction genomics techniques. In particular, octanoyl-[GcvH]: protein N-octanoyl transferase and phosphor mevalonate kinase were used as potential targets in AutoDock Vina studies. This study can provide a framework to find potential drug targets for other pathogenic microorganisms that can successfully be docked with compound U1 and U1A.

Keywords: MRSA, triazoles derivatives, targets identification, drug designing.

INTRODUCTION

MRSA is a lethal infectious agent that has become a major concern in health care setting because of its emerging new strains causing infections in healthy people as well. The bacteria not only possess the ability of causing diverse infections in both hospital and community acquired settings but also have intrinsic virulence activity and capacity to adapt to diverse environmental conditions (Franklin 2003) and involved in infectious diseases of skin, blood stream, respiratory tract and soft tissues causing minor to life threatening infections (DeLeo and Chambers 2009). Although continuous work on development of novel antibiotics has increased their availability yet the mortality rate of MRSA remains to be approximately 20-40% (Mylotte *et al*., 1987) and has now become a leading cause of bacterial infection in developed countries worldwide (DeLeo and Chambers 2009). This ongoing emergence and development of resistance since past decade has increased the necessity for the development of new antibiotics, other compounds and strategies to overcome the growing concern of mortality and morbidity. Synthesizing new derivatives of known ligands is one of the ways of developing some promising compounds for treating MRSA infections.

For many years, triazoles have been considered as important pharmacophore for the development of novel ligands and therefore they have been successfully being used against microbes. Due to these effective biological activities, 4-amino-1,2,4-triazoles with their derivatives have been recognized as valuable drug candidates and gained significant attention in pharmaceutical industries. These derivatives have been reported to have antibacterial (Alagarsamy *et al*., 2006), anti-fungal (Ragenovic *et al*., 2001), anti-tumor (Bekircan and Nurhan 2005), anti-migraine (Williamson *et al*., 2001) and anti-oxidant (Ilango and Valentina 2010).

Another interesting aspect of development of novel drug candidates is with the help of *in silico* approaches. This has become possible after the development and completion of host and pathogen genomic sequences which allowed the identification and location of target sites in pathogen making drug discovery process easier. With the availability of genomic sequences of pathogens beneficial information has been provided to design drug and vaccine targets. These target sites identified in bacterium must be essential for its growth and survival and must also be conserved in different bacterial genome while at the same time absent in host to avoid any undesirable effects (Ko *et al*., 2006). For the development of effective antimicrobials and vaccines the genomic approaches are being widely used among which *in silico* comparative, subtractive and functional approaches are presently common (Reddy *et al*., 2007). *In silico* virtual

**Corresponding author:* e-mail: aunmuhammad78@yahoo.com

screening involves docking of the target enzymes or proteins with drug-like molecules and then ranking them according to calculated binding affinity in order to determine the conformation and orientation of these ligands (Breda *et al*., 2008). This framework can be successfully used in drug discovery and development (Reddy *et al*., 2007).

The present study was designed to determine the anti-MRSA activity of new triazoles derivatives against prioritized protein targets of *Staphylococcus aureus*.

MATERIALS AND METHODS

Synthesis of ligands

Ligands U1 and U1A were synthesized as given in reaction scheme:

Synthesis of 1a

The reactants (30 ml of hydrazine hydrate and 15ml of carbon disulfide) were reacted about 4 hours using reflux-condenser in the presence of 150 ml of ethyl alcohol. The precipitates of thiocarbo-hydrazide were collected on cooling.

Synthesis of 2a(ligand-U1)

Similar quantities of reactants (0.1 moles of thiocarbohydrazide and salicylic acid) were mixed with continuous stirring in oil bath for 2 hours at 160°C. At the end of the reaction, the mixture was re-crystallized using 70% solvent (ethanol).

Synthesis of 2b(ligand-U1A)

1 gm of the ligand-U1 (synthesized in previous step) was dissolved in ethyl alcohol using water bath and benzaldehyde (1gm) was added into it (for about 4 hours). The ligand U1-A was filtered and re-crystallized using similar solvent. Thin layer chromatography was used to assess the reaction mixture.

Structural characterization by FT-IR and NMR

FT-IR (Bruker Germany Alpha model) spectra of U1 and U1A were observed in the 3600-650cm^{-1} region while ^{1}H-NMRwas analyzed using Bruker 300 MHz spectrometer (15 mg of samples dissolved in 0.5 ml D_2O).

Activity against MRSA

The activity against methicillin resistant *Staphylococcus aureus* (MRSA) was determined by agar well-diffusion method according to Wiegand *et al*., (2008). MIC was determined according to CLSI protocol (Cockerill *et al*., 2012).

Drug target prioritization

A computational based cellular analysis to identify alternative candidate drug targets for MRSA was studied. The entire genomic sequence of MRSA (2829 mbp size) was obtained from Comprehensive Microbial Resource (CMR) database.

Screening for essentiality

The complete nucleotide and amino acid sequences of MRSA genome were subjected to BLASTn and BLASTp analysis respectively using Database of Essential Genes (DEGs) to screen out the essential genes of this organism as the target for new antimicrobial drugs must be essential for the growth, replication, or survival of the bacterium (Kim *et al*., 2004). For the selection of essential genes, the sequences of more than 30% similarity with an E-value of 0.0001 were selected (Barh *et al*., 2011; Ren and Yan 2004).

Sequence analysis against human genomes

To find human-homolog and non-human homolog proteins sequences, the selected essential proteins were searched against human reference genome on NCBI using BLASTp option. The sequences showed similarity score value ≥100 and E-value database (NCBI≤0.0001 were considered as human homologs and discarded while the essential non-human homologs were retained.

Sequence analysis with against human gut flora

The selected non-human homolog protein sequences of MRSA were mapped with the protein sequences of the gut flora. The entire genomic sequence of MRSA was searched against gut flora using BLASTp option at the Human Micro biome Project database server (http://www.hmpdacc-resources.org)with an E=0.01.

Pathways analysis and subcellular localization Prediction

The role of non-human and non-homolog to gut flora proteins in biological pathways were studied using the KEGG database and subsequently analyzed for biological distribution of these proteins in cellular compartments using the CELLO v.2.5 Sub cellular Localization Predictor tool (Lu *et al*., 2004).

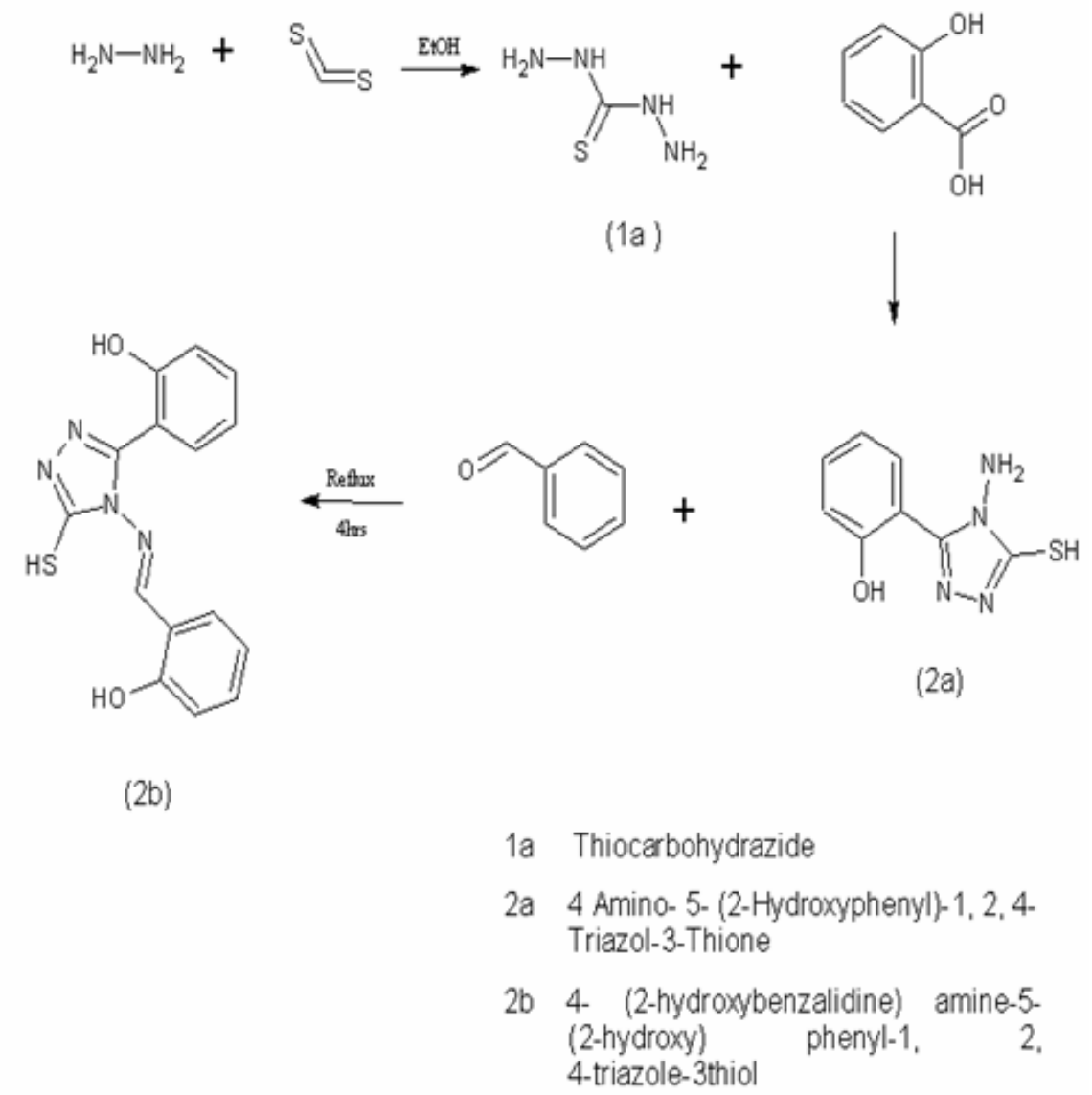
Computational drug designing

Preparation of ligands files

Chem Bio Draw Ultra software was used to draw the chemical structures of U1 and U1A molecules to produce the MOL format of these compounds. Prior to use PyRx software, PDBQT files of these derivatives were generated after uploading the MOL format onto software. The atoms of the molecule were labeled by Discovery Studio 3.0 visualizer.

Analysis of pharmacokinetics properties

To analyze the drug likeness and pharmacokinetics properties of U1 and U1A compounds, Chem informatics Mol inspiration server (http://www.molinspiration.com) was used. According to it, for the ligand molecules to have good absorption and permeation, it must contain H-bond donors ≤5 (OH and NH groups), H-bond acceptors ≤ 10 (N and O atoms), molecuar weight less than 500 dalton and log *P* coefficient (Clog P) less than 5.



Reaction Scheme: Synthesis of 4-Amino-5-(2-Hydroxyphenyl)-1, 2, 4-Triazol-3-Thione [Compound U1 (2a)] and its Schiff base (2b).

Target proteins

The 3-D structures of phosphor mevalonate kinase and octanoyl-[GcvH]: protein N-octanoyl transferase were built on The Protein Model Portal based on the templates PDB: 3K17 and PDB: 2P5I respectively. These templates were retrieved from the Protein Data Bank (PDB).

Analysis of binding sites

The binding sites of phosphor mevalonate kinase and octanoyl-[GcvH]: protein N-octanoyl transferase was studied by Auto Dock-Vina based on the scoring algorithms. The active site means posing of the coordinates of the ligands in the target protein pockets. The Ramachandran-plot was applied to assess the quality of these protein models that also the shows the graphical representation of the local backbone conformation of each residue of these drug targets.

Molecular docking

Molecular docking was performed to find structural interaction of the phosphomevalonate kinase and octanoyl-[GcvH]: protein N-octanoyl transferase with U1 and U1A derivatives. Docking was executed by PyRx virtual software. The energies during interaction of these structures were calculated at each step of the simulation (by software) that is a free-energy based expression involving dispersion/repulsion energy, directional hydrogen bonding.

RESULTS

Design and synthesis of triazoles

Sequential reaction scheme was designed to synthesize the biological active anti-MRSA triazoles (U1 and U1A) having melting point of 120^0C and 170^0C respectively. These ligands were soluble in organic solvent (70%

ethanol) and R_f value 0.16 (U1) and 0.12 (U1A) respectively was observed in methanol-chloroform solvent (table 1).

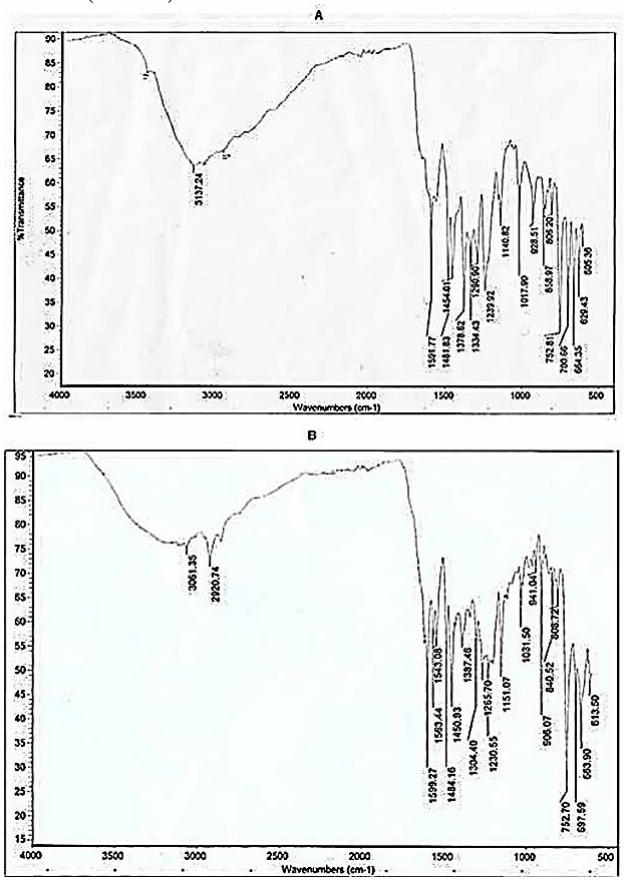


Fig. 1: FTIR analysis (A) U1 Compound (B) U1A derivative

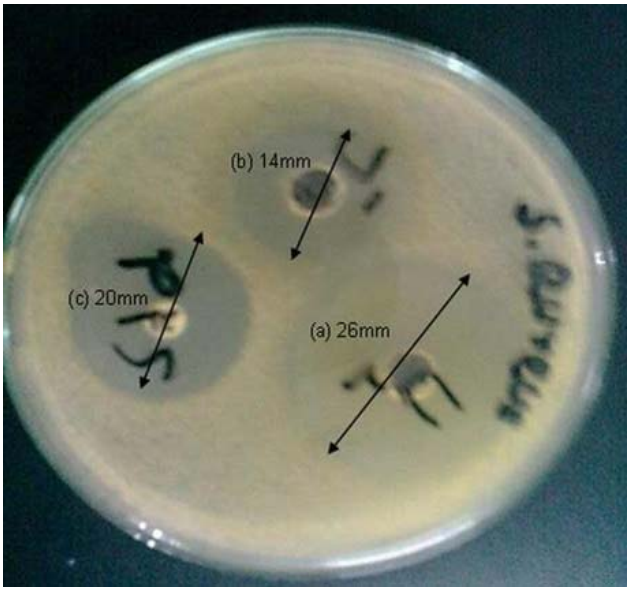


Fig. 2: Antibacterial activity of synthesized compounds (in terms of zone of inhibition) against methicillin resistant *Staphylococcus aureus* (MRSA) (a) compound U1A (b) compound U1 (c) Ofloxacin as standard

FT-IR analysis

The FT-IR spectral data of U1 and U1A revealed the peak values of functional groups, for O-H in case of U1 appeared at 3137 cm^{-1} while for U1A: 3061-3100cm^{-1}. For C=N, U1 and U1A were observed at 1591 and 1599cm^{-1}respectively. N-H and C=O spectral peaks do not appear that validates the formation of these ligands by cyclo-condensation (fig. 1).

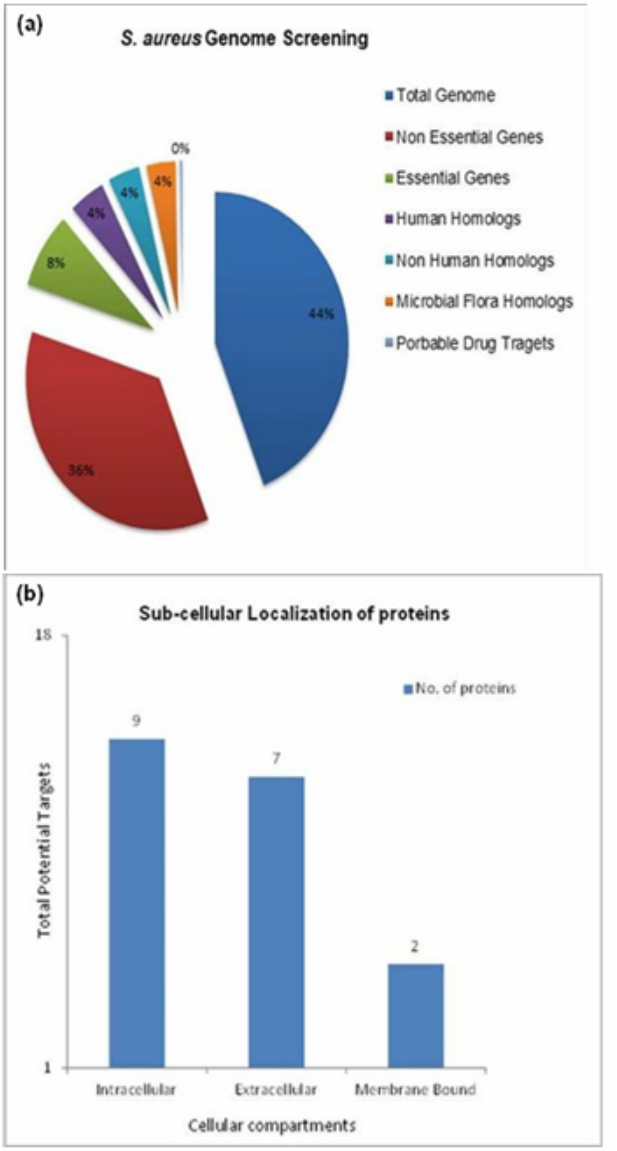


Fig. 3: (a) Screening of genes in methicillin resistant *Staphylococcus aureus* (b) Distribution of potential drug targets in cellular components

NMR analysis

The ^{1}H-NMR spectral signals showed that the derivatives belongs to aliphatic and aromatic groups: for U1-ligand [H NMR (DMSO; 300MHZ; 5 ppm): 7.25-7.37 (m; 4H;Ar-H), 5.37 (s; 2H; NH_2), 10.3 (bS; 1H; 0H), 13.9 (S; 1H; SH)] and for U1A [7.28-7.43 (m; 8H;Ar-H), 9.1 (S;

1H; CH), 10.5 (bS; 2H; OH), 13.8 9S; 1H; SH)]. The proton-signal for C-H of the triazole ring was detected as a singlet between 5.32-5.87 ppm, the proton-signal for the SH-proton on this ring was examined at 11.79-12.68 ppm.

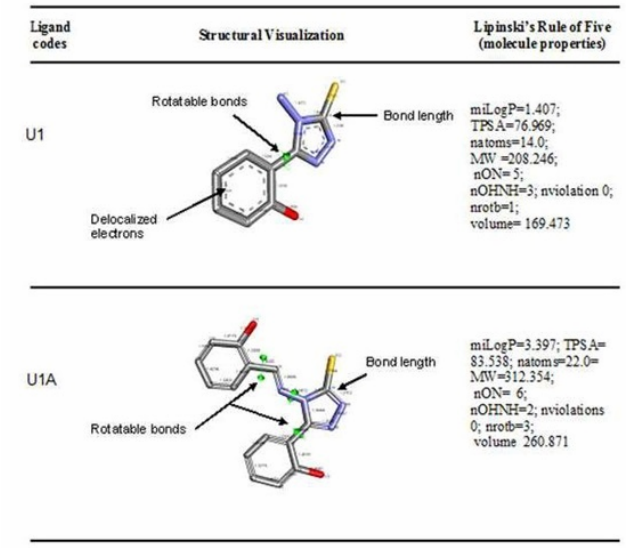


Fig. 4: Pharmacokinetics properties of U1 and U1A molecules using Lipinski's rule of 5

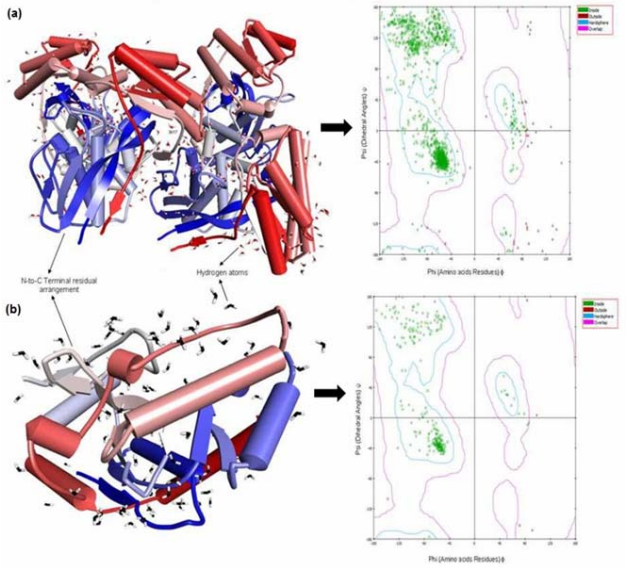


Fig. 5: Qualitative 3D Model of Protein Targets from N-to-C terminal (a) Phospho mevalonate kinase with Ramachandran plots (b) Octanoyl-[GcvH]: protein N-octanoyl transferase with Ramachandran plots

Anti-MRSA activity

The activity of U1A derivative against MRSA by agar well-diffusion method exhibited significant zone of inhibition (26 mm) as compared to U1 (14mm). Ofloxacin was used as standard (20 mm) (fig. 2). The tube dilution method for MIC determination against MRSA revealed the minimum inhibitory concentration of U1 and U1A were 64µg and 32µg respectively (table 1).

Drug targets prioritization

For drug target prioritization, the entire genomic sequence of MRSA (2829 mbp) was passed through the BLASTx screening analysis using Database of Essential Genes (DEG) resulting 523 essential genes of this bacterium. Protein sequences that were homologous to human genes or human gut flora were excluded. Analysis with human genome using NCBI server resulted 245-essential and non-human homolog genes while the comparative genomic analysis with micro biota at the Human Micro biome Project (HMP) database server exposed 18 non-human and non-gut flora homologs genes as probable drug targets in MRSA (fig. 3a).

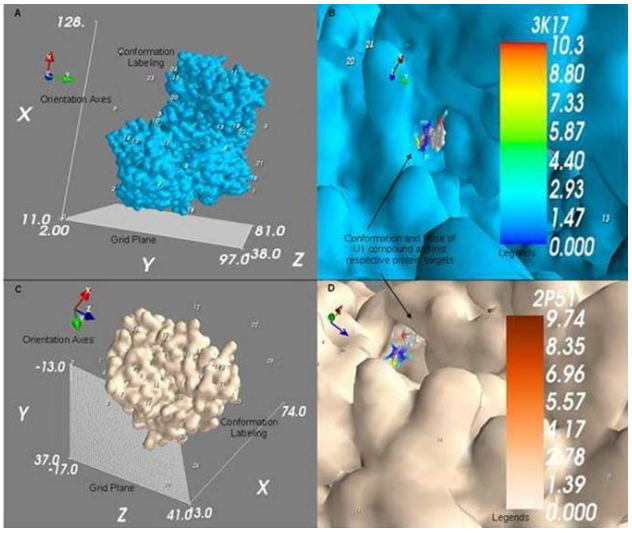


Fig. 6: Docking steps of U1 (A) The Auto Grid dimensions between U1 derivative and 3K17 target protein atoms (A, HD, OA, and N) are: Grid Center X: 68.5151, Y: 55.4765, Z: 23.1276 with total number of points X:Y: Z: 50 and Spacing (Angstrom): 0.3750 (B) Confirmation and pose of U1 with 3K17 protein target (C) The Auto Grid dimensions between U1 derivative and 2P51 target protein atoms are: Grid Center X: 44.0954, Y: 10.8384, Z: 12.9584 with total number of points X:Y: Z: 25 and Spacing (Angstrom): 0.3750 (D) Pose of the U1 compound in 2P51 target enzyme on the basis of RMSD calculation

The sub cellular localization of these 18 probable targets showed: 9 proteins were cytoplasmic, 2 were membrane bound and 7 were extra cellular proteins (fig. 3b). Studying the cell-insights in more detail by considering signaling pathways provided the comprehensive information about potential targets. The selected probable targets (table 2) were mapped in their metabolic pathways using KEGG database which showed 3 important pathways involved in *Staphylococcus aureus*. In pathway analysis, it was found that phosphor mevalonate kinase and Octanoyl-[GcvH]: protein N-octanoyl transferase in MRSA as a potential target involved in mevalonate pathway and lipoate biosynthetic process respectively.

Table 1: Physical and activity data of compound U1 [*4 Amino-5-(2-Hydroxyphenyl)-1, 2, 4-Triazol-3-Thione)]* and U1A [4-(2-hydroxybenzalidine) amine-5-(2-hydroxy) phenyl-1, 2, 4-triazole-3-thiol].

Compound	U1	U1A
Molecular Formula	$C_8H_8N_4OS$	$C_{15}H_{12}N_4O_2S$
Color	Yellow	Yellow
Solubility Water Ethanol	 Not soluble Soluble on heating	 Not soluble Soluble on heating
Melting Point	120 °C	170 °C
Form	Crystalline	Crystalline
R_f -value	0.16	0.20
MIC (µg) (MRSA)	64	32

Table 2: Subcellular localization of potential protein targets of methicillin resistant *Staphylococcus aureus.*

Cellular Compartments	Total number of genes/proteins	Accession Number	Protein Name
Intracellular	9	SaurJH1_0542	Septum formation initiator
		SaurJH1_0627	Lipoate-protein ligase A protein
		SaurJH1_0630	Phospho mevalonate kinase
		SaurJH1_0655	*Staphylococcal* accessory regulator
		SaurJH1_0818	Conserved hypothetical protein
		SaurJH1_1172	Protein of unknown function DUF1447
		SaurJH1_1571	Ferredoxin
		SaurJH1_1876	Conserved hypothetical protein
		SaurJH1_1935	Helix-turn-helix domain protein
Extracellular	7	SaurJH1_0811	Conserved hypothetical protein
		SaurJH1_0857	CsbD family protein
		SaurJH1_1209	Ribosomal protein L32
		SaurJH1_1264	Cell division protein FtsL
		SaurJH1_1568	Peptidoglycan-binding LysM
		SaurJH1_1622	Lipoprotein, putative
		SaurJH1_2533	Prevent-host-death family protein
Membrane Bound	2	SaurJH1_0572	Preprotein translocase
		SaurJH1_2369	Conserved hypothetical Protein

Table 3: Energy and RMSD values obtained during docking application of U & U1A as ligand molecules with Phospho mevalonate kinase (PDB: 3K17) and Octanoyl-[GcvH]: N-octanoyl transferase (PDB: 2P51) as target proteins.

Target-Ligand	Binding Affinity (Kcal/mol)	RMSD/UB	RMSD/LB
3K17_U1	-8.3	0	0
3K17_U1	-8.1	3.278	2.789
3K17_U1	-8.1	2.961	2.149
3K17_U1	-7.9	2.44	2.219
3K17_U1A	-12.1	0	0
3K17_U1A	-11.8	4.415	2.294
3K17_U1A	-11.6	2.305	1.641
3K17_U1A	-11.4	2.498	1.757
2P51_U1	-8.8	0	0
2P51_U1	-8.8	1.518	1.145
2P51_U1	-8.8	2.74	1.754
2P51_U1	-8.8	9.756	9.284
2P51_U1A	-13.2	0	0
2P51_U1A	-12.7	3.191	2.334
2P51_U1A	-12.5	7.917	6.922
2P51_U1A	-12.4	8.543	7.862

Study of drug likeness properties

The drug likeness properties (pharmacokinetics) of U1 and U1A had passed the Lipinski's Rule of 5. For U1: partition-coefficient log p: 1.407, molecular weight: 208.40gm, number of violations: 0.00 and for U1A: log p-value: 3.397, molecular weight: 312.35gm, number of violations 0.00 (fig. 4).

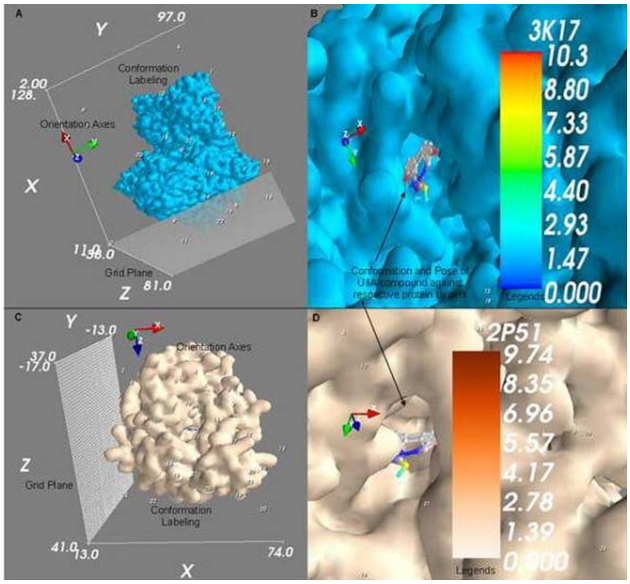


Fig. 7: Docking steps of U1A (A) The Auto Grid dimensions between U1A derivative and 3K17 target protein atoms (A, HD, OA, and N) are: Grid Center X: 68.5151, Y: 55.4765, Z: 23.1276 with total number of points X:Y: Z: 25 and Spacing (Angstrom): 0.3750 (B) Confirmation and pose of U1A with 3K17 protein target (C) The Auto Grid dimensions between U1A derivative and 2P51 target protein atoms are: Grid Center X: 44.0954, Y: 10.8384, Z: 12.9584 with total number of points X:Y: Z: 25 and Spacing (Angstrom): 0.3750 (D) Pose of the U1A compound in 2P51 target enzyme on the basis of RMSD calculation

Protein models analysis

The protein models of phosphor mevalonate kinase and octanoyl-[GcvH]: protein N-octanoyl transferase were obtained from the Protein Model Portal based on the templates PDB: 3K17 and PDB: 2P5I (fig. 5a) respectively. Ramachandran plots were built using Discovery Studio 3.0 to evaluate the quality of these models. These plots indicate the presence of amino acid in the "allowed region" or "disallowed region". The protein model was considered as "good" as ≥90% amino acid residues were present in the "allowed" region and <2% in the "disallowed region" of the plot. The graphical representation of the protein model is shown in fig. 5b.

Drug-targets interaction

It was observed that ligand atoms involved in docking with the target enzymes (fig. 6 and 7). The Auto Grid points indicated the most stable and favorable pose of U1 and U1A derivative to these targets. PyRx software was used and during calculation it was observed that the weight averaged grids perform best. The U1 and U1A derivatives exhibited the optimum interaction with target proteins based on the RMSD-algorithms. Beside RMSD calculations, software also measured the binding free energies of this interaction (table 3). During docking, the H-bond between ligands (U1 and U1A) and drug targets are important, as involve in the binding affinity. The calculated final docked energies for U1 are: -8.3 Kcal/mol and -8.8 Kcal/mol and for U1A are: 12.1 Kcal/mol and -13.2 Kcal/mol against phosphor mevalonate kinase and octanoyl-[GcvH]: protein N-octanoyl transferase respectively. The results clearly revealed that U1 and U1A molecules accurately interacted with phosphor mevalonate kinase and octanoyl-[GcvH]: protein N-octanoyl transferase protein targets.

DISCUSSION

In this study, triazoles derivatives were synthesized against the potential drug targets of MRSA, as this bacterium is resistant to broad spectrum antibiotics including penicillin and cephalosporins (Dancer 2001; Kisgen and Whitney2008). MRSA is a main cause of nosocomial and community-acquired infections throughout the world and a major cause of morbidity and mortality (Klein *et al.,* 2007). Koçyiğit-Kaymakçıoğlu *et al*. (2010) synthesized triazoles derivative and produced schiff bases in sequential steps. Therefore, the development of novel anti-bacterial agents has become essential to overcome the complications encountered due to bacterial resistance against these infectious microorganisms.

The FT-IR spectral data identified the functional groups of triazoles derivatives. Murti *et al*. (2011) determined the FT-IR spectra of triazole ring that revealed the absorption bands of C=N and C-N at 1562-1598cm^{-1} and 1313-1365cm^{-1} respectively. The H-signal for C-H stretching for the triazole was observed as a singlet between 5.32-5.87 ppm, while the H-signal for the S-H proton on this ring was analyzed at 11.79-12.68 ppm (Prakash *et al*., 2004; Sztanke *et al*., 2006; Liu *et al*., 2008).

Idhayadhulla *et al.* (2011) designed and synthesized a series of triazole derivatives including 5,5'-(3,5-dimethyl-1H-pyrrole-2,4-diyl)bis(4-amino-4H-1,2,4-triazole-3-thiol), 5,5'-(3,5-dimethyl-1H-pyrrole-2,4-diyl)bis(4H-1,2,4-triazole-3-thiol), 5,5'-(3,5-dimethyl-1Hpyrrole-2,4-diyl)bis(1,3,4-thiadiazol-2-amine) and determined antibacterial activity of these compounds against *Escherichia coli* (MTCC-739), *Pseudomonas aeruginosa* (MTCC-2435), *Streptococcus epidermidis*, *Klebsiella pneumonia*, and methicillin resistant *Staphylococcus aureus* (MTCC- 96) by disc diffusion assay. Each compound was tested at a concentration of 100µg/mL in DMSO and ciprofloxacin was used as standard and these

compounds showed no activity against *Escherichia coli* and *Staphylococcus aureus* as compared to our compounds.

Targeting an essential gene is indispensable for microbial cell existence may give an active way to cure infectious diseases. Therefore, these essential translated products of microbial cellular system are confident novel targets for antibiotic molecules (Barh *et al*., 2011; Ren and Yan 2004). One approach is to synthesize new antibacterial compounds are to find out new drug targets and this scheme is being used by the available genomic database sources (McDevitt and Rosenberg 2001). As, genome sequence databases are easily available for several bacterial species, therefore such sort of study has been described for a number of pathogenic bacterial species like *Helicobacter pylori* (Dutta *et al*., 2006), *Pseudomonas aeruginosa* (Perumal *et al*., 2007) and even *Mycobacterium tuberculosis* (Singh *et al*., 2006). The subcellular localization revealed that some of which could be a potential vaccine targets (Lu *et al*., 2004). Materi and Wishart (2007) analyzed the cellular systems by investigating the integrated pathways and other biological routes to estimate the importance of each macromolecule that may biologically occur to compensate for the lack of that protein molecule. Due to importance of systems biology analysis, broad-spectrum procedures have been established and used for the modeling simulation and investigation of these systems. The similar pathway analysis in *Neisseria* species was studied by Sarangi *et al*. in 2009. During this pathway, phosphor mevalonate kinase is the critical enzyme of mevalonate pathway required for isopentenyl diphosphate synthesis, crucial and unique to bacterial cell survival as it is necessary for the integrity of bacterial cellular membrane. Targeting this enzyme will block and inhibit the synthesis of mevalonate pathway and ultimately bacterial cell membrane (Miziorko2011). Similarly, octanoyl-[GcvH]: protein N-octanoyl transferase is required for lipoic acid synthesis that is involved in the formation of lipoylated proteins, responsible for oxidative defense, bacterial sporulation, and gene expression. Microbes use several adaptations to uptake lipoic acid that affects their pathogenicity and virulence. If lipoic acid formation is inhibited defense mechanisms of the bacterial specie will be collapsed and eventually there will be reduced chances of survival of bacterial pathogens (Christensen *et al*., 2011).

The drug likeness and pharmacokinetic properties of the ligands (Zaid *et al*., 2010) revealed that both U1 & U1A had complied the Lipinski's Rule (RO5). The computational docking showed that all lowest energy complexes of phosphor mevalonate kinase and octanoyl-[GcvH]: protein N-octanoyl transferase were stabilized by intermolecular H-bonds and stacking interactions. These molecules (ligand and targets) have rotatable bonds that showed the flexibility of these compounds.

CONCLUSIONS

These triazole derivatives have been effectively used against potential target proteins of MRSA. The *in-silico* approaches and simulation screening techniques have been proved as a worthy procedure for drug designing and development.

REFERENCES

Franklin DL (2003). Antimicrobial resistance: the example of *Staphylococcus aureus. J. Clin. Invest,* **111**: 1265-1273.

DeLeo FR and Chambers HF (2009). Reemergence of antibiotic-resistant *Staphylococcus aureus* in the genomics era. *J. Clin. Invest*, **119**: 2464-2474.

Mylotte JM, McDermott C and Spooner JA (1987). Prospective study of 114 consecutive episodes of *Staphylococcus aureus* bacteremia. *Rev. Infect. Dis*., **9**: 891-907.

Alagarsamy V, Giridhar R, Yadav MR, Revathi R, Rukmani K and Clercq DE (2006). Anti-HIV, Antibacterial, Antifungal activities of some novel 1, 4-disubstituted-1, 2, 4-triazolo [4, 3-a] quinazolin-5(4H)-ones. *Ind. J. Pharm. Sci*., **68**: 532-535.

Ragenovic1 KC, Vesna D, Vlado K, Dora GM and Aleksandra B (2001). Synthesis, Antibacterial and Antifungal Activity of 4-Substituted-5-Aryl-1, 2, 4-Triazoles. *Molecules*, **6**: 815-824.

Bekircan O and Nurhan G (2005). Synthesis of some 3, 5-diphenyl-4H-1, 2, 4-triazoe derivatives as antitumor agents. *Ind. J. Chem*., **44B**: 2107.

Williamson DJ, Hill RG, Shepheard SL and Hargreaves RJ (2001). The anti-migraine 5-HT(1B/1D) agonist rizatriptan inhibits neurogenic dural vasodilation in anaesthetized guinea-pigs. *Br. J. Pharmacol*., **133**: 1029-1034.

Ilango K and Valentina P (2010). Synthesis and biological activities of novel 1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazoles. *Der. Pharma. Chemica*., **2**: 16-22.

Ko, Soo K, Lee JY, Song JH, Baek JY and Oh WS *et al*. (2006). Screening of Essential Genes in *Staphylococcus aureus* N315 Using Comparative Genomics and Allelic Replacement Mutagenesis. *Microbiol. Biotechnol*., **16**: 623-632.

Reddy AS, Pati SP, Kumar PP, Pradeep HN and Sastry GN (2007). Virtual Screening in Drug Discovery A Computational Perspective. *Curr. Protein Pept. Sci*., **8**: 329-351.

Breda A, Basso LA, Santos DS and Azevedo WF (2008). Virtual Screening of Drugs: Score Functions, Docking, and Drug Design. *Curr. Computer-Aided Drug Design*, **4**: 265-272.

Wiegand I, Hilpert K and Hancock REW (2008). Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nat. Prot*., **3**: 2.

Cockerill FR, Wikler MA, Alder J, Dudley MN and Eliopoulos GM *et al*. (2012). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard Ninth Edition. CLSI, **32**: 2 M07-A9.

Kim YC, Kim CS, Cho BH and Anderson AJ (2004). Major Fe-super oxide dimutase (FeSOD) activity in *Pseudomonas putida* is essential for survival under conditions of oxidative stress during microbial challenge and nutrient limitation. *J. Microbiol. Biotechnol.*, **14**: 859-862.

Barh D, Tiwari S, Jain N, Ali A and Santos AR *et al*. (2011). *In Silico,* Subtractive Genomics for Target Identification in Human Bacterial Pathogens. *Drug Dev. Res*., **72**:1-16.

Ren Z and Yan L (2004). DEG 5.0, a database of essential genes in both prokaryotes and eukaryotes. *Nucleic Acids Res*., **32**: D271.

http://www.hmpdacc-resources.org/hmp_catalog/. Accessed 23 September 2013.

Lu Z, Szafron D, Greiner R, Lu P and Wishart DS (2004). Predicting Subcellular Localization of Proteins using Machine- Learned Classifiers. *Bioinformatics*, **20**: 547-556.

http://www.molinspiration.com/. Accessed 23 September 2013.

Dancer SJ (2001). The problem with cephalosporins. *J. Antimicrob. Chemother*., **48**: 463-478.

Kisgen J and Whitney D (2008). Ceftobiprole, a Broad-Spectrum Cephalosporin with Activity against Methicillin-Resistant *Staphylococcus aureus* (MRSA). *P.T*., **33**: 631-634.

Klein E, Smith DL and Laxminarayan R (2007). Hospitalizations and deaths caused by methicillin-resistant *Staphylococcus aureus*, United States, 1999-2005. *Emerg. Infect. Dis*., **13**: 1840-1846.

Koçyiğit-Kaymakçıoğlu B, Çalışır MM, Özbek B and Ötük G (2010). Synthesis and antimicrobial activities of schiff bases derived from 4-amino-5-(1-phenylethyl)-2,4-dihydro-3H-1,2,4-triazole 3-thione. *E. J. Chem*., **7**: 458-464.

Murti Y, Agnihotri R and Pathak D (2011). Synthesis, Characterization and Pharmacological screening of Some Substituted 1,2,3-& 1,2,4-Triazoles. *Amer. J. Chem*., **1**: 42-46.

Prakash OP, Bhardwaj V, Kumar R and Aneja KR (2004). Organoiodine (III) mediated synthesis of 3-aryl/hetryl-5,7-dimethyl-1,2,4-triazolo[4,3-a] pyrimidines as antibacterial agents. *Eur. J. Med. Chem*., **39**: 1073-1077.

Sztanke R, Pasternak K and Jozwiak K (2006). Synthesis of imidazoline and imidazo[2,1c][1,2,4]triazole aryl derivatives containing the methylthio group as possible antibacterial agents. *Biorg. Med. Chem*., **14**: 3635-3642.

Liu P, Zhu S, Xie P, Xie W, Jiang Y and Zhang D (2008). Synthesis and SAR studies of biaryloxy-substituted triazoles as antifungal agents. *Biorg. Med. Chem. Lett*., **18**: 3261-65.

Idhayadhulla A, Kumar RS, Nasser AJA, Selvin J and Manilal A (2011). *Arabian J. Chem*., **12**: 0-25.

Mc Devitt D and Rosenberg M (2001). Exploiting genomics to discover new antibiotics. *Trends Microbiol.*, **9**: 611-617.

Dutta A, Singh SK, Ghosh P, Mukherjee R and Mitter S *et al*. (2006). *In silico* identification of potential therapeutic targets in the human pathogen *Helicobacter pylori*. *In Silico. Biol*., **6**: 43-47.

Perumal D *et al*. (2007). Differential genome analyses of metabolic enzymes in *Pseudomonas aeruginosa* for drug target identification. *In Silico Biol*., **7**: 453-465.

Singh NK, Selvam SM and Chakravarthy P (2006). T-iDT: tool for identification of drug target in bacteria and validation by *Mycobacterium tuberculosis*. *In Silico Biol*., **6**: 485-493.

Materi W and Wishart DS (2007). Computational systems biology in drug discovery and development: Methods and applications. *Drug Discov. Today*., **12**: 295-303.

Sarangi AN, Aggarwal R, Rahman Q and Trivedi N (2009). Subtractive Genomics Approach for *in Silico* Identification and Characterization of Novel Drug Targets in *Neisseria Meningitides* Serogroup. *B. J. Comput. Sci. Syst. Biol*., **2**: 255-258.

Miziorko HM (2011). Enzymes of the Mevalonate Pathway of Isoprenoid Biosynthesis. *Arch. Biochem. Biophys*., **505**: 131-143.

Christensen QH, Martin N, Mansilla MC, de Mendoza D and Cronan JE (2011). A novel amidotransferase required for lipoic acid cofactor assembly in *Bacillus subtilis*. *Mol. Microbiol*., **80**: 350-363.

Zaid H, Raiyn J, Nasser A, Saad B and Rayan A (2010). Physicochemical Properties of Natural Based Products versus Synthetic Chemicals. *The Open Nutraceut. J.*, **3**: 194-202.