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COMPUTATIONAL DRUG DESIGNING AGAINST *SALMONELLA TYPHI*: A CAUSATIVE AGENT OF TYPHOID

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ABSTRACT

Typhoid fever, caused by *Salmonella typhi*, is characterized by gastrointestinal illness and fatal bloody diarrhea. The emerging epidemic cases and resistance of this bacterium to broad spectrum antibiotics demands the development of new anti-typhoid drugs. In this study, oxadiazole has been synthesized with significant anti-Salmonella activity. This compound was structurally characterized through FTIR and NMR. The computational docking studies of oxadiazole with essential target protein (UDP-N-acetylmuramoyl-L-alanine:D-glutamate ligase) of *Salmonella typhi* showed stable binding affinity with minimum binding energy of -9.9 Kcal/mol. This computational study will add to our understanding of the drug designing and development, and to target these pathogenic bacteria to treat typhoid infections.

Keywords: Oxadiazole, *Salmonella typhi*, typhoid, computational analysis

INTRODUCTION

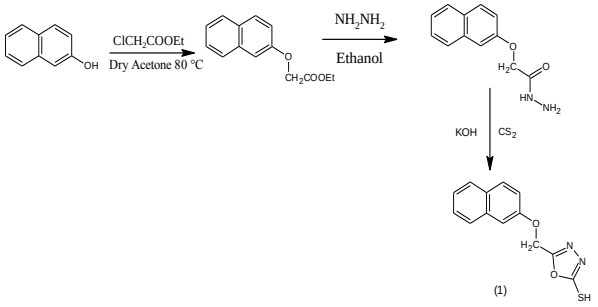
Typhoid is life threating bacterial infection transmitted by the ingestion of unhygienic food or feces contaminated water [1]. This infection is more commonly found in low-income countries, where unsanitary conditions are more likely to occur, and can affect as many as 21.5 million persons each year and an estimated 16–33 million cases has been reported annually. Its incidence is highest in children and young adults between 5 and 19 years old [2]. These epidemic cases and resistance of *Salmonella typhi* to ciprofloxacin, ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, streptomycin and other multidrug resistant typhoid (MDR typhoid) bacterial strains is now alarming problem, especially in the Indian subcontinent and Southeast Asia [3].
Therefore, there is urgent need of establishing new anti-typhoid drugs development strategies to cope this situation. In this regard, computational approaches have the edges of high speed, economical and, even more importantly, enable researchers to raise questions that would otherwise be difficult to address experimentally. These *in silico* studies are widely used and have become vital component of drug discovery and development programmes [4].
Here, we have proposed new oxadiazole ligand molecule against *Salmonella typhi* that showed optimum binding affinity with bacterial target enzyme. This compound inhibits the cell wall synthesis by interfering the action of UDP-N-acetylmuramoyl-L-alanine:D-glutamate ligase enzyme that cross-links peptidoglycan chains to form rigid cell envelop [5]. Therefore, this compound can get a pharmaceutical application on future directions in anti-typhoid drug development.

MATERIALS AND METHODS

Synthesis of Lead compound: Ethyl-2-(naphthalene-2-yloxy) acetate was synthesized from 2-naphthol and ethyl chloro-acetate in the presence of potassium carbonate following the reported procedure [6]. The ester was condensed with hydrazine hydrate in ethanol to the corresponding 2-(naphthalene-2-yloxy) acetic acid hydrazide. The

hydrazide was used for the synthesis of target molecule (1). A mixture of 2-(naphthalene-2-yloxy) acetic acid hydrazide (0.01 mol), carbon disulphide (0.04mol) and potassium hydroxide (0.04mol) in 25 ml absolute ethanol was refluxed for 6-8 h, until the evolution of hydrogen sulphide ceased. The reaction mixture was cooled and acidified with 4N HCl to pH of 2-3. The solid separated was filtered, washed with water and recrystallized from 70 % ethanol.

Structural Characterization: Structural characterization of oxadiazole was carried out by FTIR (Bruker Germany Alpha model) and NMR spectra were recorded on Bruker 300 MHz spectrometer equipped with 5 mm of probehead for ^{1}H analysis.



Drug-Targets Interaction Analysis

Accession of ligand and target protein: The chemical structure of oxadiazole was prepared by ChemBioDraw and MOL SDF format were converted to PDBQT file using PyRx tool to generate atomic coordinates while 3D structure of UDP-N-acetylmuramoyl-L-alanine:D-glutamate ligase enzyme was accessed from Protein Data Bank (PDB:1E0D).

Molecular docking analysis: The active sites of target protein were analyzed using the Drug Discovery Studio version 3.0. A computational ligand-target docking approach was used to determine structural complexes of the target enzyme with heterocyclic oxadiazole. Docking was carried out by PyRx, AutoDock Vina option based on scoring functions. At each step of the simulation, the binding energies of ligand and enzyme were evaluated.

RESULT AND DISCUSSION

Structural Characterization and *Spectral Analysis:* In this study, we synthesized oxadiazole heterocyclic compounds as anti-typhoid agent which reveals significant binding affinity to Salmonella UDP-N-acetylmuramoyl-L-alanine:D-glutamate ligase enzyme. Among the heterocyclic compounds, 1,3,4-oxadiazole-2-thiol is a highly privileged structure. Its derivatives exhibit wide range of biological activities including antibacterial, anti-tubercular, antifungal, analgesic, anti-inflammatory, vasodilatory, cytotoxic, ulcerogenic and anticancer activities [7-8]. Following data of oxadiazole compound (1) was obtained: *5-(2-naphthyloxy)methyl-1,3,4-oxadiazole-2-thiol (1):* Yield 78%, white solid, m.p. 225°C, R_f 0.61 (ethyl acetate: pet. ether 4:1); IR (KBr) cm^{-1}: 2903 (C-H), 1546 (C=C), 1508 (C=N); ^{1}H-NMR (DMSO, ppm); δ = 13.6 (s, 1H, SH), 7.15-6.75 (m, 7H, naphthyl protons), 4.98 (s, 2H, OCH_2), MS(m/z) 258 (M^+), Elemental analysis: $C_{13}H_{10}O_2N_2S$; Calculated % C, 60.46; N, 10.85; H, 3.87, Found % C, 60.12; N, 10.07; H, 3.8.

Drug-Target Interaction

Active Binding Site and Docking Analysis: The active binding sites of UDP-N-acetylmuramoyl-L-alanine:D-glutamate ligase enzyme using virtual tools revealed that the probable amino acid residues around ligand are: THR, MET, GLU, and HIS. The binding residues that define the coordinates of the ligand [9] on to the surface of target protein are analyzed confirming that these ligand molecules can enter the substrate-binding region of the protein active sites. Before docking study, the hydrophobicity maps were constructed to analyze the progress of hydrophobicity clusters on the membrane surface of UDP-N-acetylmuramoyl-L-alanine:D-glutamate ligase along the molecular dynamics run and the features of interface between the membrane and membrane-binding molecule (Figure 1) by PLATINUM server [10]. The docking of target with synthesized compound (1) using AutoDock procedure showed the computationally predicted lowest energy complex of target enzyme are stabilized by intermolecular hydrogen bonds and stacking interactions. The AutoGrid Model presented the most energetically favorable binding mode. This inhibitor is docked into the generated combined grids and the RMSD from native pose and the binding energy are evaluated and it is observed that the weight averaged grids performs best. During these interaction procedures, the hydrogen bond between the ligand and target are most important, as it can decide the binding strength and the conformation of ligand (Figure 2).

The calculated final docked energies of this antibacterial compound are -9.9 Kcal/mol (Table 1). Docking results clearly demonstrated that oxadiazole accurately interact with ligase protein target.

CONCLUSION

No doubt the availability of computational and *in silico* approaches is the milestones in drug discovery

progression. UDP-N-acetylmuramoyl-L-alanine:D-glutamate ligase enzyme is an important enzyme involved in the synthesis of bacterial cell envelop and so interference in biosynthesis of cell wall will lead to the death of *Salmonella typhi*. The synthesized compound oxadiazole has been virtually screened and selected as anti-typhoid ligand due to its binding affinity with this target. This newly synthesized organic molecule can be clinically investigated for establishing safe doses.

Table 1. Energy and RMSD values obtained during docking analysis of oxadiazole as ligand molecule and UDP-N-acetylmuramoyl-L-alanine:D-glutamate ligase (PDB:1E0D) as target protein

COMPLEX	BINDING AFFINITY	RMSD/UB*	RMSD/LB*
1e0d_oxadiazole	-9.9	0	0
1e0d_oxadiazole	-9.9	21.59	19.049
1e0d_oxadiazole	-9.6	20.194	17.623
1e0d_oxadiazole	-9.5	21.515	19.92
1e0d_oxadiazole	-9.3	21.057	19.524
1e0d_oxadiazole	-9.3	21.901	19.397
1e0d_oxadiazole	-9.2	21.752	19.163
1e0d_oxadiazole	-8.9	2.274	1.867
1e0d_oxadiazole	-8.8	20.688	18.68

*RMSD/UB: Root Mean Square Deviation/Upper Bond; *RMSD/LB: Root Mean Square Deviation/Lower Bond

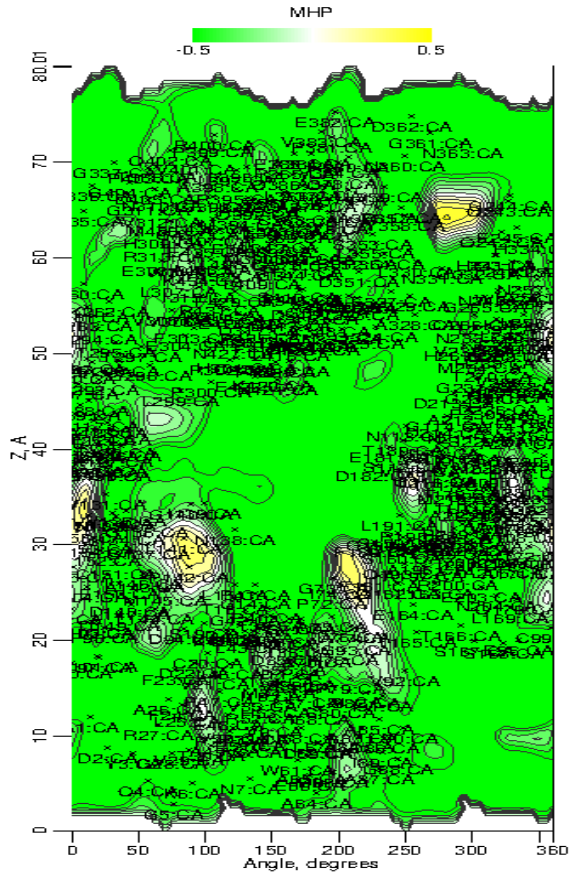


Figure 1. Hydrophobicity map for upper layer of UDP-N-acetylmuramoyl-L-alanine:D-glutamate ligase target protein membrane, position of the protein is projected along the Z-axis

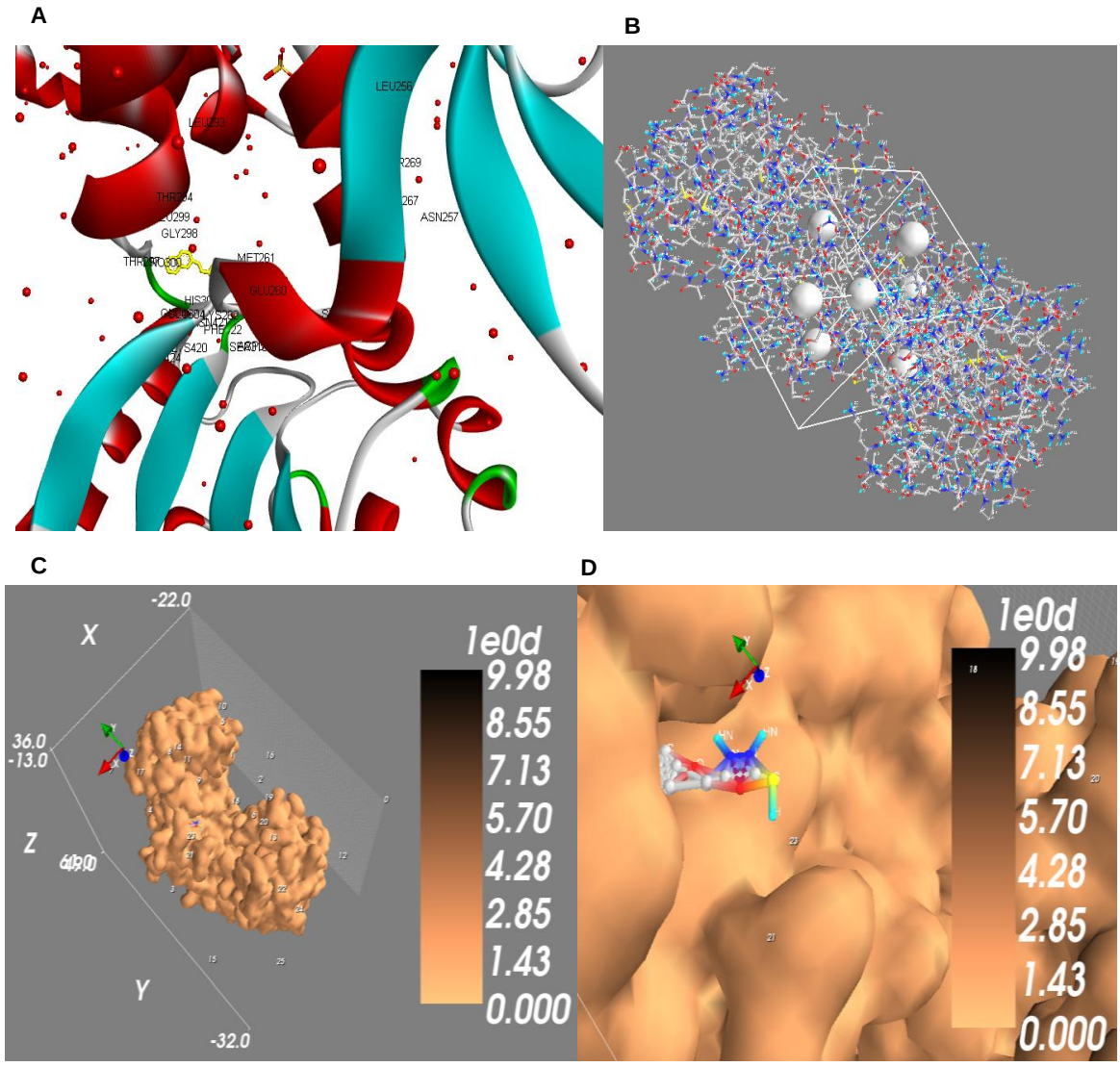


Figure 2: Various views of docking interaction steps of oxadiazole ligand molecule (A) Binding site atoms of ligase amino acids residues THR, MET, GLU, and HIS around ligand (B) The AutoGrid dimensions between ligand and ligase (PDB:1E0D) target protein atoms (A, HD, OA, and N) are: Grid Center X=6.6296, Y=9.2803, Z=24.1419 with dimension (Angstrom) X:Y: Z: 25.0000 (C and D) Confirmation and pose of ligand molecule with protein target

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