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Original Article

MOLECULAR DOCKING STUDIES OF SOME NOVEL THIOPHENE CARBOHYDRAZIDE DERIVATIVES ON ENTEROTOXIN OF BACILLUS CEREUS

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ABSTRACT

Objective: In this work, we collected the three-dimensional structure of Enterotoxin from Bacillus cereus which plays an important role in the pathway.

Methods: The protein structure was collected from PDB data bank. From the 3D structures of the proteins, the targeted derivatives were designed. Docking studies were performed with designed compounds.

Results: The compounds docked to the protein by hydrogen bonding interactions and these interactions play an important role in the binding studies. Docking results showed the best compounds among the derivatives.

Conclusion: The docking results agreed well with the observed *in vitro* data, in which the anti-microbial activity of the analogs was higher than other drugs and formed hydrogen bonds.

Keywords: Antibacterial activity, Docking studies, Enterotoxin

INTRODUCTION

Bacillus cereus is a Gram-positive, rod-shaped, aerobic, motile, beta hemolytic bacterium commonly found in soil and food. Some strains are harmful to humans and cause foodborne illness, while other strains can be beneficial as probiotics for animals [1]. It is the cause of "fried rice syndrome", as the bacteria are classically contracted from fried rice dishes that have been sitting at room temperature for hours [2]. *B. cereus* bacteria are facultative anaerobes, and like other members of the genus *Bacillus*, can produce protective endospores. Its virulence factors include cereolysin and phospholipase C [3]. It was from this species that two new enzymes, named AlkC and AlkD, which are involved in DNA repair, were discovered in 2006. *B. cereus* is responsible for a minority of foodborne illnesses (2–5%), causing severe nausea, vomiting, and diarrhea. *Bacillus* foodborne illnesses occur due to the survival of the bacterial endospores when food is improperly cooked [4]. Cooking temperatures less than or equal to 100 °C (212 °F) allow some *B. cereus* spores to survive. This problem is compounded when food is then improperly refrigerated, allowing the endospores to germinate. Cooked foods not meant for either immediate consumption or rapid cooling and refrigeration should be kept at temperatures below 10 °C or above 50 °C (50 °F and 122 °F). Germination and growth generally occur between 10 °C and 50 °C, though some strains are psychrotrophic [5]. Bacterial growth results in the production of enterotoxins, one of which is highly resistant to heat and acids (pH levels between 2 and 11); ingestion leads to two types of illness, diarrheal and emetic (vomiting) syndrome [6-10]. The timing of the toxin production was previously thought to be possibly responsible for the two different courses of disease, but in fact the emetic syndrome is caused by a toxin, cereulide, found only in emetic strains and is not part of the "standard toolbox" of *B. cereus*. Cereulide is a cyclic polypeptide containing three repeats of four amino acids: D-oxy-Leu—D-Ala—L-oxy-Val—L-Val (similar to valinomycin produced by *Streptomyces griseus*) produced by nonribosomal peptide synthesis. Cereulide is believed to bind to 5-hydroxytryptamine 3 (5-HT3) serotonin receptors, activating them and leading to increased afferent vagus nerve stimulation [11]. It was shown independently by two research groups to be encoded on multiple plasmids: pCERE01 or pBCE4810 [12]. Plasmid pBCE4810 shares homology with the *Bacillus anthracis* virulence plasmid pXO1, which encodes the anthrax toxin. Periodontal isolates of *B. cereus* also possess distinct pXO1-like plasmids [13-15]. Like most of the cyclic peptides containing nonproteogenic amino acids, cereulid is resistant to heat, proteolysis, and acid conditions. *B. cereus* is also known to cause difficult-to-eradicate chronic skin infections, though less aggressive than necrotizing fasciitis. *B. cereus* can also cause keratitis.

MATERIALS AND METHODS

Methodology

The series were docked to Enterotoxin of *Bacillus cereus* was obtained from PDB database. After the unnecessary chains and hetero atoms were removed using SPDBV software, hydrogens were added to the protein and used for active site identification.

Active site identification

The active site of Enterotoxin was identified using CASTp server. A new program, CASTp, for automatically locating and measuring protein pockets and cavities, is based on precise computational geometry methods, including alpha shape and discrete flow theory. CASTp identifies and measures pockets and pocket mouth openings, as well as cavities. The program specifies the atoms lining pockets, pocket openings, and buried cavities; the volume and area of pockets and cavities; and the area and circumference of mouth openings.

Docking method

Docking was carried out using GOLD (Genetic Optimization of Ligand Docking) software which is based on genetic algorithm (GA). This method allows the partial flexibility of protein and full flexibility of ligand. The compounds are docked to the active site of the Enterotoxin. The interaction of these compounds with the active site residues are thoroughly studied using molecular mechanics calculations. The parameters used for GA were population size (100), selection pressure (1.1), the number of operations (10,000), the number of the island (1) and niche size (2). Operator parameters for crossover, mutation and migration were set to 100, 100 and 10 respectively. Default cutoff values of 3.0 A ° (dH-X) for hydrogen bonds and 6.0 A ° for vanderwaals were employed. The number of poses for each inhibitor was set 100, and early termination was allowed if the top three bound conformations of a ligand were within 1.5A ° RMSD. After docking, the individual binding poses of each ligand were observed and their interactions with the protein were studied. The best and most energetically favorable conformation of each ligand was selected.

Gold Score fitness function

Gold Score performs a force-field based scoring function and is made up of four components: 1. Protein-ligand hydrogen bond energy (external H-bond); 2. Protein-ligand vander Waals energy (external vdw); 3. Ligand internal vander Waals energy (internal vdw); 4. Ligand intramolecular hydrogen bond energy (internal-H-bond). The external vdw score is multiplied by a factor of 1.375 when the total fitness score is computed. This is an empirical correction to encourage protein-ligand hydrophobic contact. The fitness function has been optimized for the prediction of ligand binding positions.

GoldScore = S (hb_ext)+S (vdw_ext)+S (hb_int)+S (vdw_int)

Where S (hb_ext) is the protein-ligand hydrogen bond score, S (vdw_ext) is the protein-ligand van der Waals score, S (hb_int) is the score from intramolecular hydrogen bond in the ligand and S (vdw_int) is the score from intramolecular strain in the ligand.

RESULTS AND DISCUSSION

From the PDB databank, the PDB file was collected. The final stable structure of the Enterotoxin obtained is shown in fig. 1.

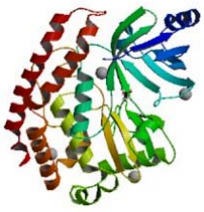


Fig. 1: Structure of enterotoxin

Active site identification of enterotoxin

After the final model was built, the possible binding sites of Enterotoxin was searched based on the structural comparison of the template and the model build and also with CASTP server and was shown in fig. 2. Infact from the final refined model of Enterotoxin domain using SPDBV program, it was found that secondary structures are highly conserved.

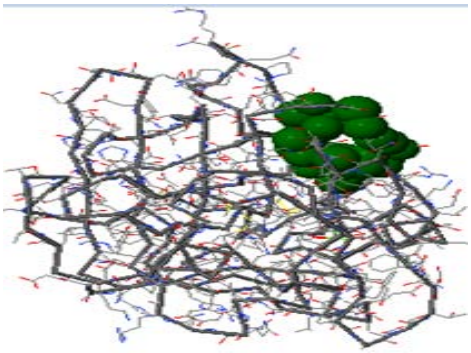


Fig. 2: Active site of enterotoxin

Docking of inhibitors with the active site

Docking of the inhibitors with enterotoxin domain was performed using GOLD 3.0.1, which is based on genetic algorithimn. This program generates an ensemble of different rigid body orientations (poses) for each compound conformer within the binding pocket and then passes each molecule against a negative image of the binding site. Poses clashing with this 'bump map' are eliminated. Poses surviving the bump test are then scored and ranked with a Gaussian shape function. We defined the binding pocket using the ligand-free protein structure and a box enclosing the binding site. This box was defined by extending the size of a cocrystallized ligand by 4A. This dimension was considered here appropriate to allow, for instance, compounds larger than the cocrystallized ones to fit into the binding site. One unique pose for each of the best-scored compounds was saved for the subsequent steps. The compounds used for docking was converted in 3D with SILVER. To this set, the substrate corresponding to the modeled protein was added. Docking of the best inhibitor with the active site of protein showed the activity of the molecule on protein function.

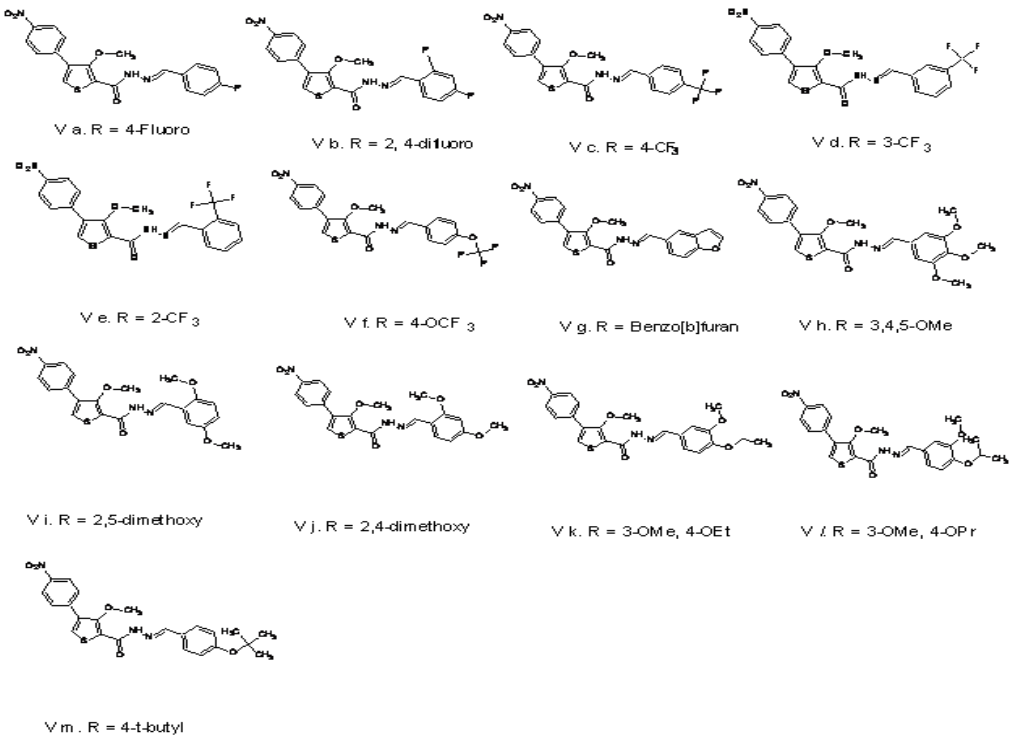
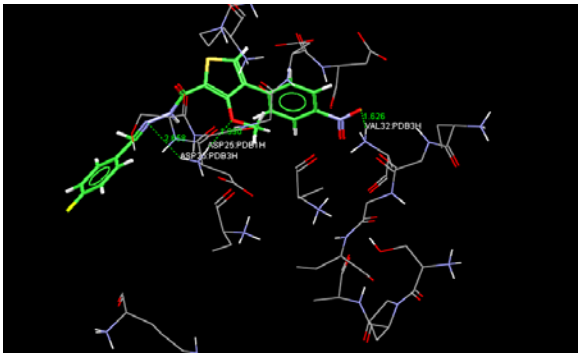
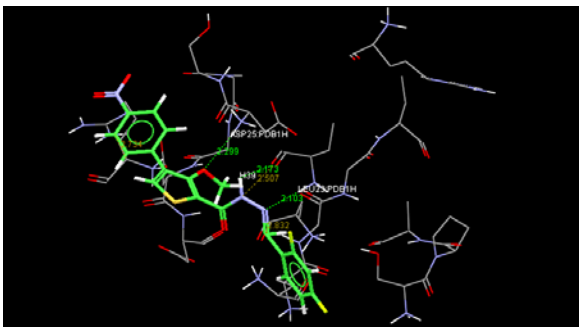


Fig. 3: Structures of compounds

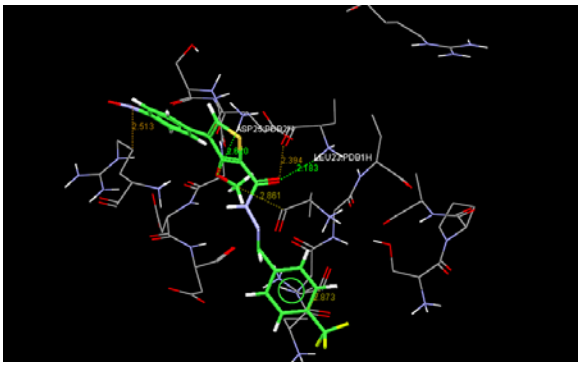
In the binding pocket, common H-bonding interactions were formed between all docked compounds and VAL32, ASP25, LEU23, ARG87. In order to explain the binding of these compounds, the H-bonding interactions with the other surrounding residues in the hydrophobic binding pocket were also investigated. In fig. 4, strong H-bonding interactions between the methoxy oxygen (O) of compounds with the hydrogen atom of protein.



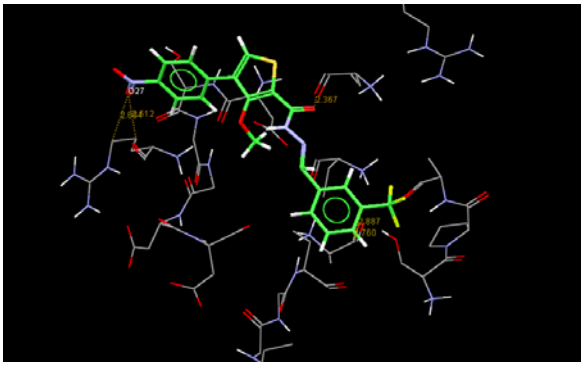
Docking studies of Va



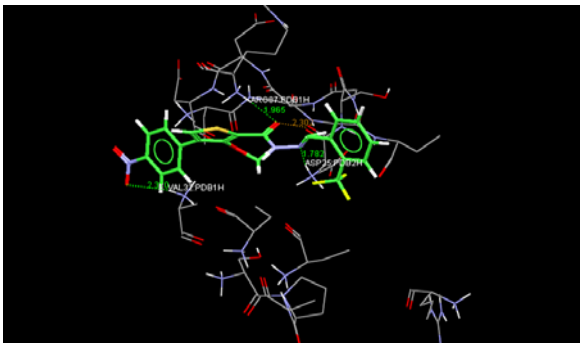
Docking studies of Vb



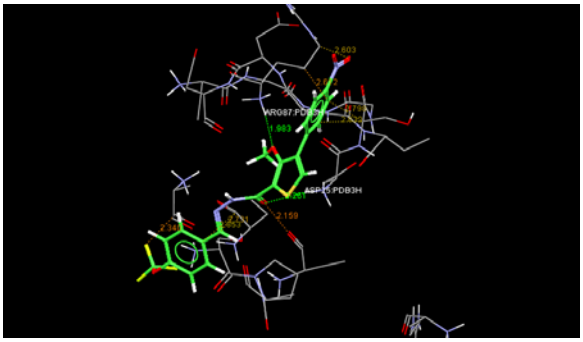
Docking studies of Vc



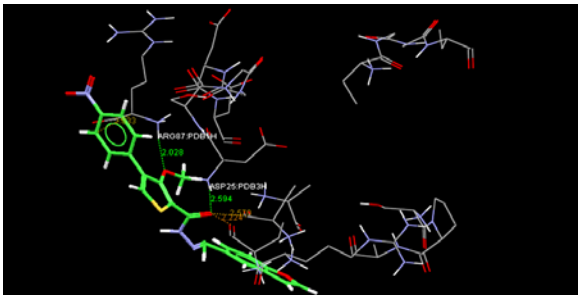
Docking studies of Vd



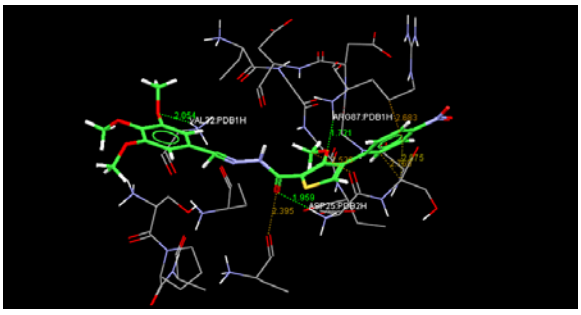
Docking studies of Ve



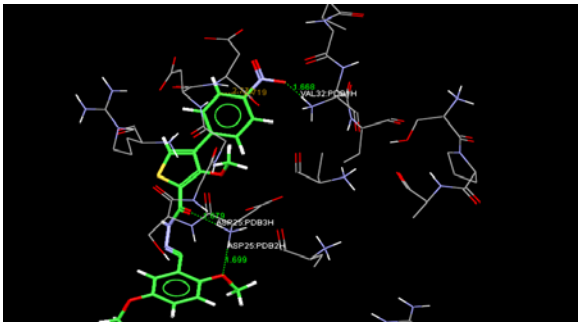
Docking studies of Vf



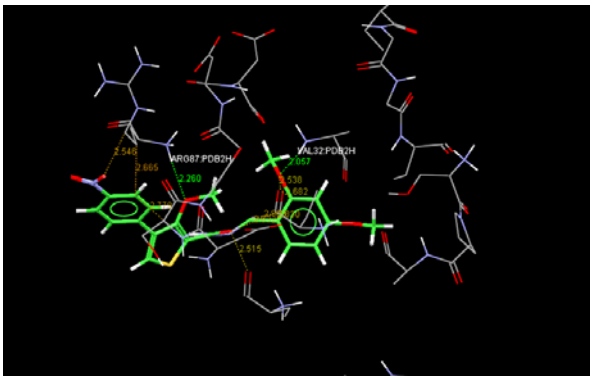
Docking studies of Vg



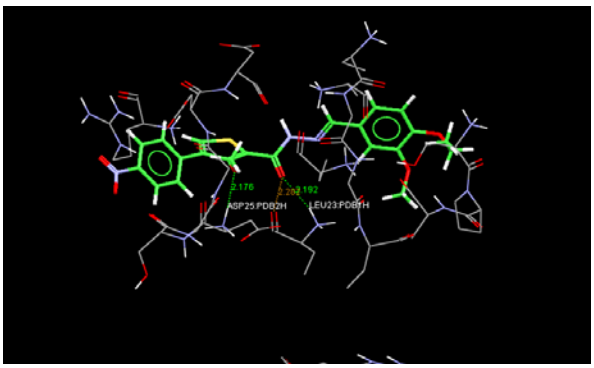
Docking studies of Vh



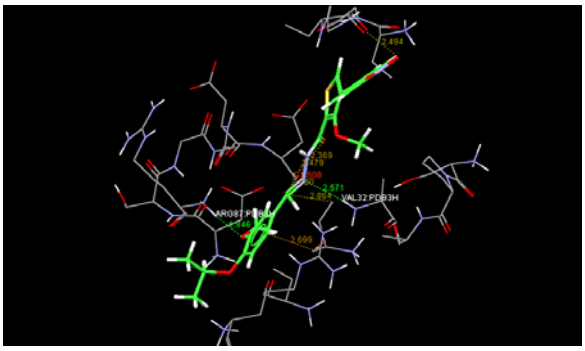
Docking studies of Vi



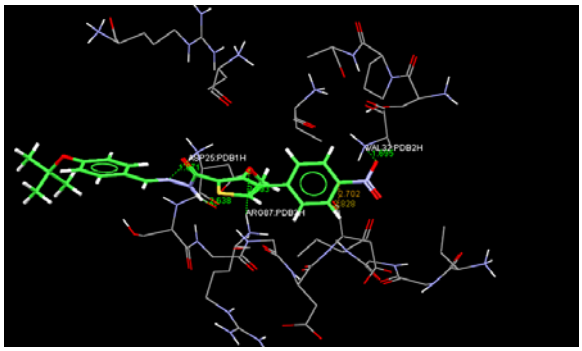
Docking studies of Vj



Docking studies of Vk



Docking studies of Vl



Docking studies of Vm

Fig. 4: Docking studies of compounds

Table 1: Docking results

Fitness	S(hb_ext)	S(vdw_ext)	S(hb_int)	S(int)	Ligand name
24.01	13.80	23.72	0.00	-22.40	Va
22.15	13.44	22.82	0.00	-22.66	Vb
21.99	10.02	26.31	0.00	-24.21	Vc
22.15	7.14	27.60	0.00	-22.93	Vd
22.01	15.43	21.77	0.00	-23.35	Ve
31.02	10.92	25.99	0.00	-15.64	Vf
25.11	10.35	26.90	0.00	-22.22	Vg
22.28	14.04	22.81	0.00	-23.12	Vh
26.43	15.47	25.65	0.00	-24.31	Vi
26.06	14.32	27.92	0.00	-26.65	Vj
29.41	11.02	26.70	0.00	-18.31	Vk
24.53	10.18	29.38	0.00	-26.05	Vl
28.25	13.96	24.28	0.00	-19.10	Vm

The synthetic drugs being pure synthetic chemicals induce cellular changes and act as foreign substances to the growth of the bacteria. The application of scientific methodology to validate the medicinal and document the toxicological properties of drugs has been stressed as an important requirement for improving the quality of medical practice. The very real potential for discovering new drugs provides additional motivation for the pharmacological evaluation of material media.

The Classical approach to the pharmacological evaluation of designed drugs has largely involved primary screening of the drugs. In as much as the objectives of primary screening usually do not extend to characterizing the complete pharmacologic profile of a drug. The chemical complexity of drugs specifically may confuse any pharmacologic extrapolation based exclusively on primary screening procedures. To make medicine to function successfully in organized health care delivery, it is important to document the pharmacology and toxicology of drugs in a comprehensive manner. The knowledge to acquire basic biologic information and in-depth pharmacologic evaluations of drugs subsequently becomes important in systemic secondary efforts to search for active fractions or individual compounds which may be used more safely and effectively as drugs for primary health care.

Some synthesized compounds are used as antimicrobial agents by the practitioners of in India, but their use is not supported by scientific study. In the search for possible cheaper antimicrobial drugs, the present study initiated research on the screening of synthesized compounds plants for antimicrobial activities. Success in these studies may lead to the development of cheaper antimicrobial drugs and hence reduce the cost of supplying health services to the majority of the population of the country.

Inhibitory activity of series to human pathogens forms the basis for their importance from the medical point of view and may be used as bactericidal agents.

CONCLUSION

The docking results agreed well with the observed *in vitro* data, in which the anti-microbial activity of the analogues was higher than other drugs and formed hydrogen bonds. The docking study revealed the binding orientation of compounds in the enterotoxin

binding pocket surrounding the active site, which resulted in inhibition of enzyme activity. From these results, we can conclude that compound Vf is one of the good inhibitory compounds of enterotoxin of Bacillus. The application of computational sciences to pharmaceutical research is a discipline, which is phenomenal.

CONFLICT OF INTERESTS

Declare none

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