WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 6, 2058-2067. Research Article ISSN 2277– 7105

MOLECULAR DOCKING STUDIES OF SOME NOVEL BENZOFLAVANONE AND BENZOFLAVONE DERIVATIVES ON ENTEROTOXIN A OF STAPHYLOCOCCUS AUREUS

Manju Kumari* and Birendra Kumar

Department of Chemistry, Gaya College, Gaya, 823001(Bihar), India.

Article Received on
19 April 2016,

Revised on 09 May 2016,
Accepted on 29 May 2016

DOI: 10.20959/wjpr20166-6441

***Corresponding Author**
Manju Kumari
Department of Chemistry,
Gaya College, Gaya,
823001(Bihar), India.

ABSTRACT

In this work, we collected the three dimensional structure of Enterotoxin A from Staphylococcus aureus which plays an important role in staphylococcus pathway. The protein structure was collected from PDB data bank. From the 3D structures of the proteins, the targeted derivatives were designed. Docking studies was performed with designed compounds. The compounds docked to the protein by hydrogen boding interactions and these interactions play an important role in the binding studies. Docking results showed the best compounds among the derivatives.

KEYWORDS: Antibacterial activity, docking studies, enterotoxin A.

INTRODUCTION

Staphylococcal Enterotoxins (SEs) are a family of structurally related basic secretory proteins that are important virulence factors for the pathogen.[1] By mediating massive cellular proliferation and cytokine secretion at extremely low concentrations, these SAgs can cause systemic pathology in the host, ranging from nausea and fever up to toxic shock and death.[2] SEs can bind relatively non-polymorphic regions outside the peptide binding groove of MHC class II molecules on Antigen Presenting Cells (APCs) as well as conserved Vβ regions of TCR molecules leading various groups to hypothesize that these SAgs may act as a binding "bridge" between MHC class II and TCR, resulting in downstream signaling events and immune activation.[3-5] One of the most potent SEs is Staphylococcal Enterotoxin A (SEA), with an exceedingly low half-maximum stimulating dose of 0.1 pg/mL. SEA is somewhat atypical in that it has two binding sites for two corresponding sites on MHC class II, a high

affinity Zinc coordinating site on the β chain of MHC class II and a second weaker (>1 μM affinity) binding site on the α chain that has been shown to play an important role in the complete functional activity of SEA.[6] Studies have suggested a cooperative model where the binding of one SEA to MHC class II favors the binding of the second SEA molecule and MHC class II - (SEA)2 trimers have been isolated in solution.[7] These results have led to the speculation that SEA could crosslink multiple MHC class II molecules on the surface of APCs.[8-12] Indeed, when MHC class II expressing cell lines were treated with SEA, but not with mutants missing either binding site or toxins with one MHC class II binding site, downstream signaling inflammatory cytokine gene upregulation[13] and homotypic aggregation was observed, even in the absence of T cells. These results hint a role for a multivalent binding mode between SEA and MHC class II, and indeed, many subsequent studies on superantigens have assumed this multivalency of SEA as part of its functionality.[14-16] However, the actual membrane reorganization of MHC class II on the surface of a cell in response to SEA treatment has not been directly probed, and as such, remains unknown.

Methodology

The series were docked to **Enterotoxin A** of Staphylococcus aureus 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

Active site of **Enterotoxin A** 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 as partial flexibility of protein and full flexibility of ligand. The compounds are docked to the active site of the **Enterotoxin A**. 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), number of operations (10,000), number of 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 A** obtained is shown in Figure 1.

Fig 1: structure of Enterotoxin A

Active site Identification of **Enterotoxin A**

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

Fig 2: active site of Enterotoxin A

Docking of inhibitors with the active site

Docking of the inhibitors with **Enterotoxin A** 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 cocrystalized 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 were added. Docking of best inhibitor with the active site of protein showed the activity of the molecule on protein function.

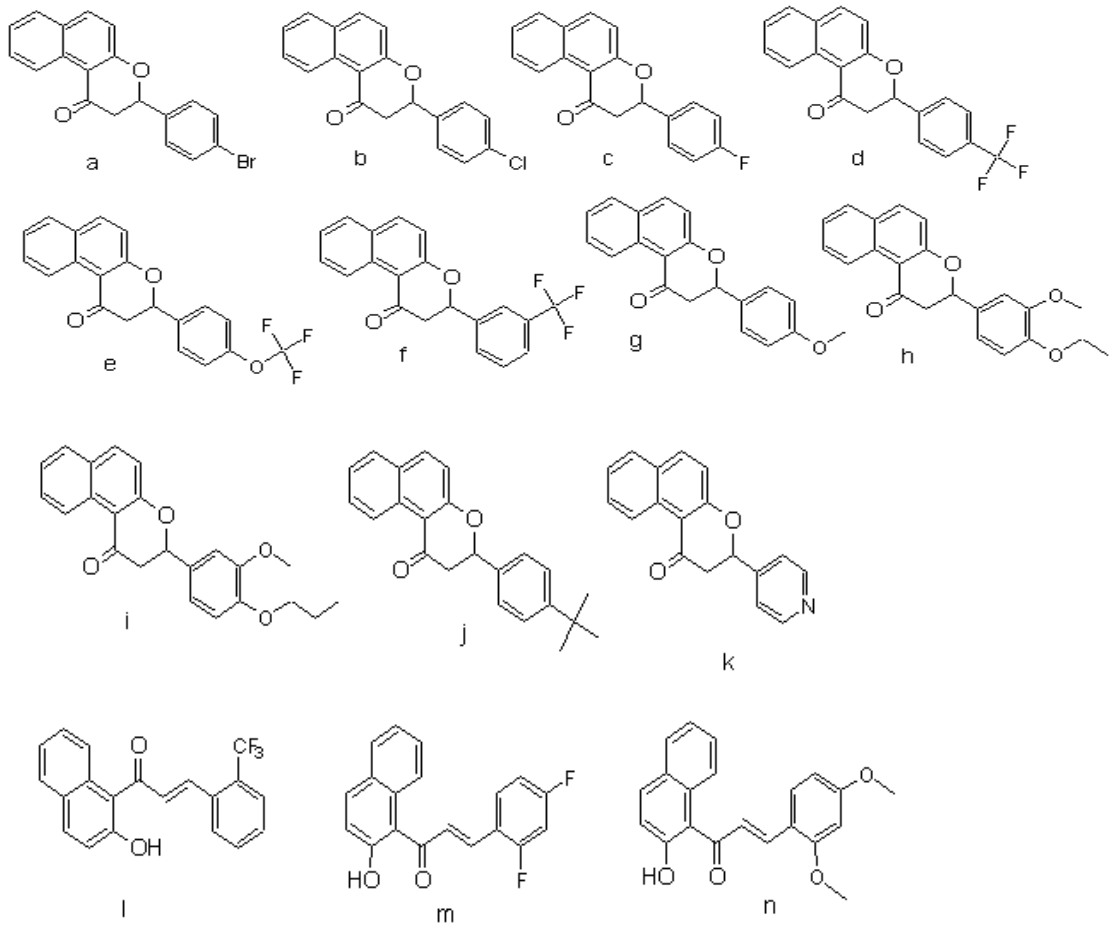
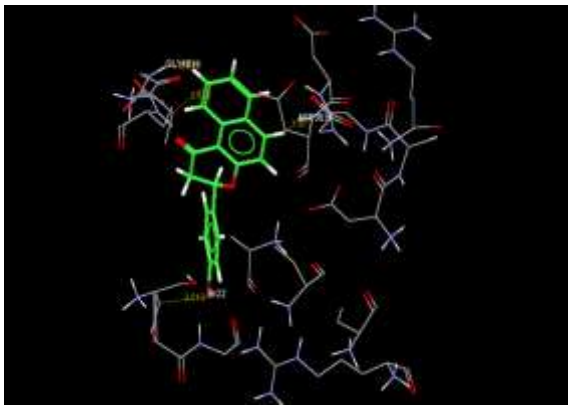
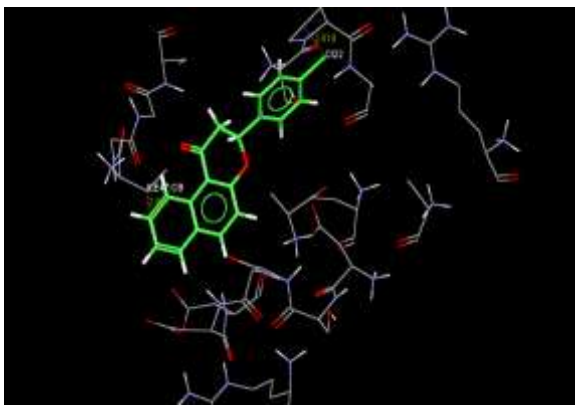


Fig 3: structures of compounds

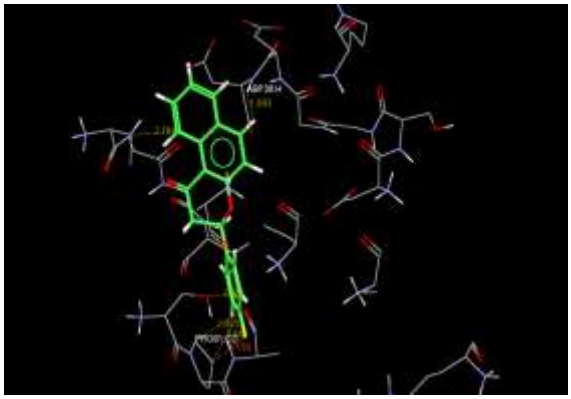
In the binding pocket, common H-bonding interactions were formed between all docked compounds and GLY27, GLY48, ASP30 ILE50, THR80. In order to explain the binding of these compounds, the H-bonding interactions with the other surrounding residues in the hydrophobic binding pocket were also investigated. In Figure 4, strong H-bonding interactions between the methoxyl oxygen (O) of compounds with hydrogen atom of protein.



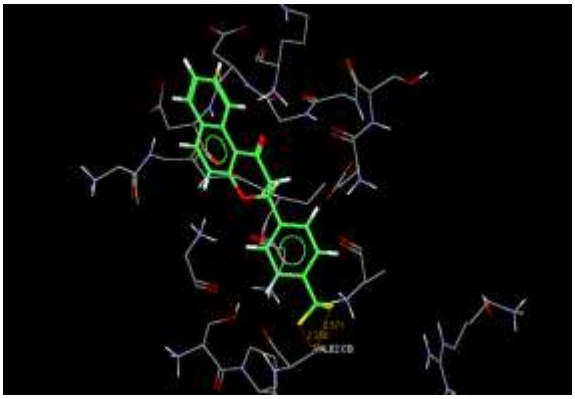
Compound a



Compound b

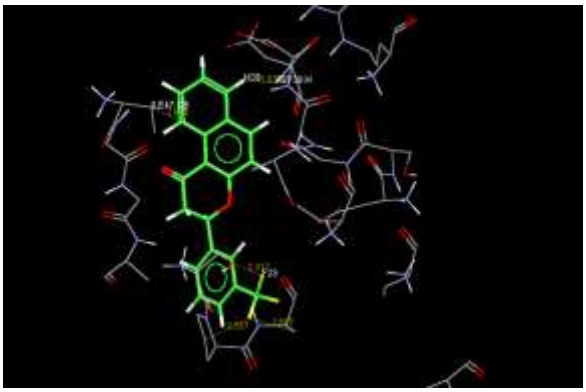


Compound c

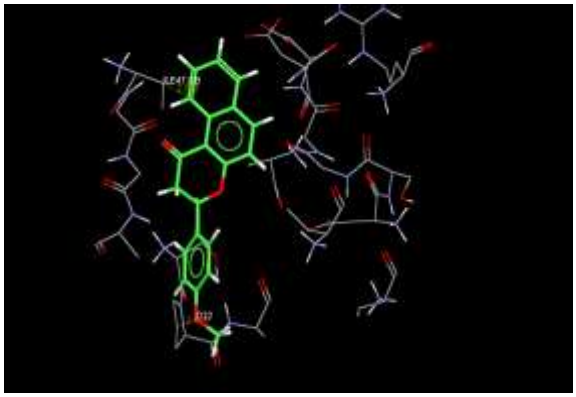


Compound d

Compound e

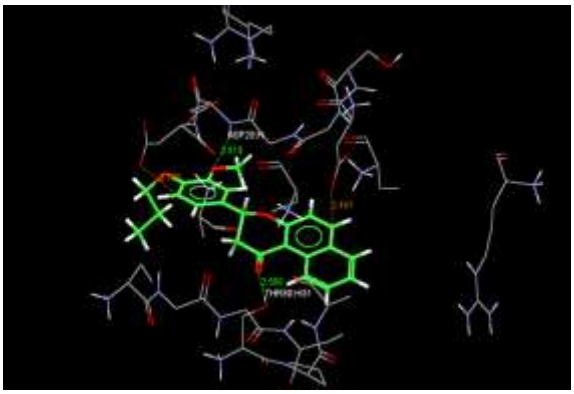


Compound f

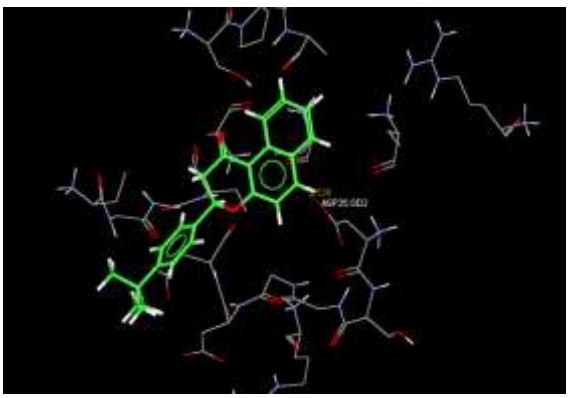


Compound g

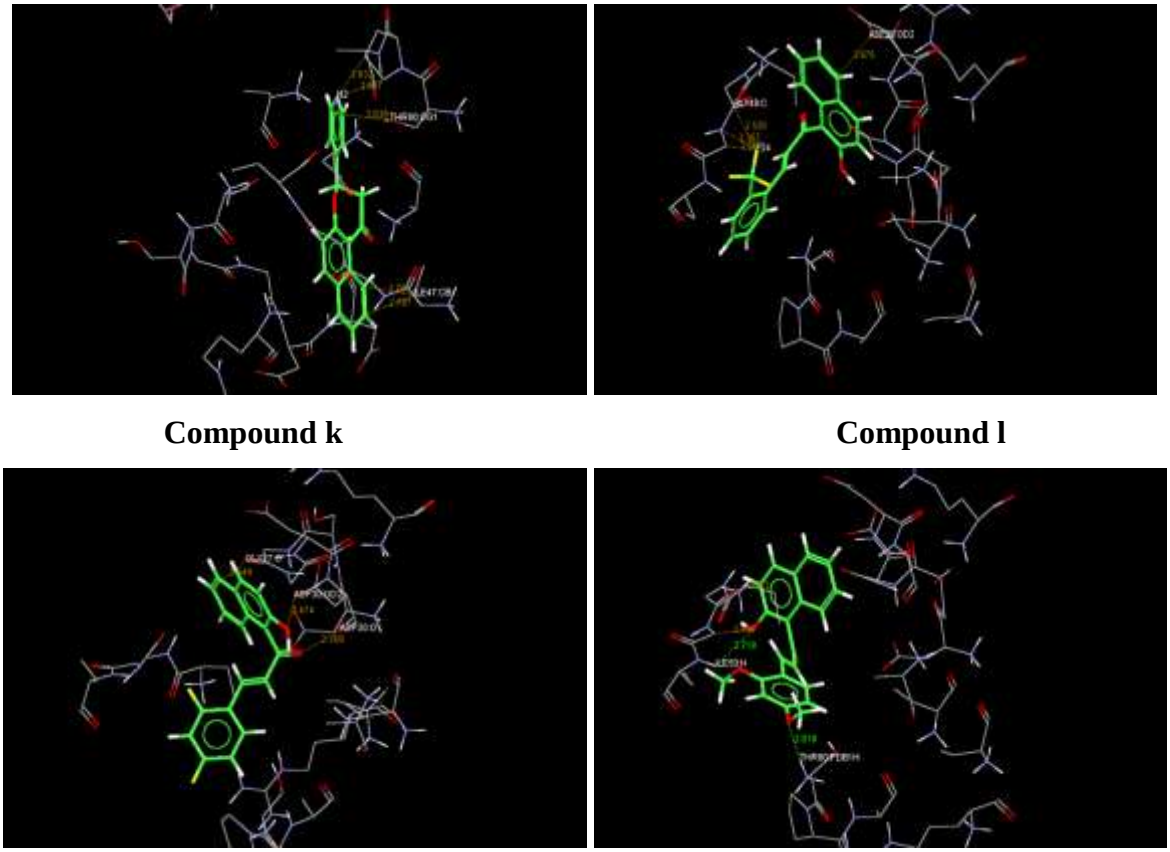
Compound h



Compound i



Compound j



Compound k **Compound l**

Compound m **Compound n**

Fig 4: docking studies of compounds

Table 1: docking results

Fitness	S(hb_ext)	S(vdw_ext)	S(hb_int)	S(int)	Ligand name
31.94	0.00	23.06	0.00	0.23	compound a
31.73	0.00	22.87	0.00	0.28	compound b
31.22	0.00	22.61	0.00	0.13	compound c
29.71	0.00	21.81	0.00	-0.29	compound d
33.18	6.00	21.58	0.00	-2.49	compound e
34.05	0.00	24.87	0.00	-0.15	compound f
32.53	0.00	24.57	0.00	-1.26	compound g
32.57	2.00	24.70	0.00	-3.39	compound h
32.51	1.54	26.70	0.00	-5.74	compound i
25.90	0.00	25.19	0.00	-8.75	compound j
31.94	0.00	23.12	0.00	0.14	compound k
24.18	0.00	24.10	0.00	-8.96	compound l
27.52	0.70	25.92	0.00	-8.82	compound m
27.88	6.00	24.05	0.00	-11.20	compound n

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.

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 a research on 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 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 f is one of the good inhibitory compounds of

enterotoxin of staphylococcus. The application of computational sciences to pharmaceutical research is a discipline, which is phenomenal.

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