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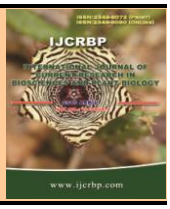

Original Research Article

Molecular Docking of *Annona muricata* L. Compounds Targeting against Mosquito Acetylcholine Esterase

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Abstract

Molecular docking is a commonly used method in structure based rational inhibitors and drug design. It is used for assessing the complex formation of small ligand molecules with proteins to predict the potency of the bonding forces and finding lead compounds. A series of nine compounds from *Annona muricata* L. were investigated as potential acetylcholine esterase inhibitors. The enzyme was modeled using SWISS-MODEL and the compounds were tested for their ability to inhibit acetylcholine esterase (AChE) of mosquito *Anopheles gambiae*. The mode of binding in the active site of AChE was investigated by molecular docking with AutoDock 4.2. Only five of the selected compounds (Syepharine, Murisolin, Corrossolone, Coreximine and Coclaurine) expressed significant AChE inhibitory activity.

Keywords

Acetylcholine esterase

Annona muricata

Anopheles gambiae

Docking

Syepharine

Introduction

Mosquitoes transmit more diseases than any other group of arthropods and affect public throughout the world. *Anopheles gambiae* and *Anopheles stephensi* are the major vectors that transmit malarial parasites among human beings. Mosquito control is essential to prevent the proliferation of mosquito borne diseases and improve quality of environmental and human health (Anupam et al., 2012). One of the most effective and safer approach is to explore botanical bio-pesticides that can paralyze the insects by inhibiting acetylcholine esterase enzyme. Anti-cholinesterase pesticides are developed for controlling wide spectrum of insects including mosquitoes and agricultural pests. Chemical pesticides react with a serine residue at the catalytic site, thereby disabling the function of AChE (Dengfeng et al., 2013). In both mammals and in insects, Acetylcholinesterase (AChE) belonging to serine hydrolase enzyme regulates acetylcholine action. This enzyme is being used as a target for pesticides (Mirjana et al., 2013). AChE has a deep and narrow active site, the bottom and the opening regions of which are known as catalytic and peripheral sites respectively (Tougu et al., 2001). Since serine residue is present in mammalian AChEs, the application of such chemical pesticides is severely limited by their toxicity to mammals (Zaidi and

Soltani, 2013). Even at low concentrations, pests are more sensitive to the chemicals than humans (Weill et al., 2004). The use of anti-cholinesterase pesticides has also been limited by resistance problems caused by mosquitoes possessing AChE mutants such as the G119S mutant that is insusceptible to current pesticides (Alout et al., 2012). Control of mosquito-borne diseases through the use of effective and safer pesticides requires thorough *in silico* investigation for conserved target sites that are particularly present in mosquito AChEs. Amino acid sequences in this region can be used as specific target sites for designing new pesticides that will not lead to mammalian toxicity and reduce the pesticidal resistance problems (Rozengart et al., 2006).. Though three-dimensional (3D) model of *Anopheles gambiae* AChE (*Ag*AChE) has been reported in the previous studies, conserved and mosquito-specific region of *Ag*AChE has to be modulated using molecular modeling software. This *in silico* investigation needs sequence analysis of *Ag*AChEs from GenBank and 3D model of *Ag*AChE generated by homology modeling (Pang, 2006).

Annona muricata L. is an undersized, deciduous, and roundish canopy-like tree. Height of this fruit bearing tree is measured to be in the range between 5 and 8 m. Traditionally, the leaves are used for headaches, insomnia, cystitis, liver problems, diabetes, and hypertension. The leaves were also found to have anti-inflammatory, pesticides, antispasmodic and antidysenteric (Di Stasi et al., 2002, Orlando et al., 2010). The phytochemicals isolated and characterized from leaves of the plant include annonacin, annocatalin, annomonicin, annomuricin, annomuricatin, corossolone, epomuricenin, gigantetrocin, javoricin, muricine, muricinine, muricapentocin, muricoreacin, montanacin, montecristin, muracin, muricatalin, muricin, murisolin, robustocin, and solamin (Zeng et al., 1996; Benkert et al., 2011). In the present study, nine compounds of the *Annona muricata* leaves were subjected to docking studies against the AChE of *Anopheles gambiae*.

Materials and methods

Homology model

The homology model of the *Anopheles gambiae* acetylcholine esterase (*Ag*AChE) was modelled using SWISS-MODEL program. Swiss model is a web based automatic protein structure modelling software based on target-template alignment. The crystal structure of mouse AChEs (1J07) was assigned as the template structure for the target sequence. The 1J07 was crystallized with an inhibitor 3,8-Diamino-5,10'-(Trimethylammonium) Decyl-6-Phenyl Phenanthridinium. The homology model was generated using default settings without any manual adjustments. The target protein sequence of the *Ag*AChE was obtained from NCBI (Genbank accession number: BN000066). The alignment between the target and template sequence were generated by using the T-COFFEE web server. The aligned file was saved in FASTA format and used for the construction of the homology model. The final model was then built by manually docking acetylcholine into the active site of the homology model (Mariani et al., 2011; Robert et al., 2011).

Ligand and protein preparation

The phytoconstituents such as Annomuricatin B, Annomuricin A, Annomuricin B, Annonacin, Coclaurine, Coreximine, Corrossolone, Murisolin, and Syepharine are present in *Annona muricata* leaves. All these plant derived ligands were downloaded from chemical databases PUBCHEM compound and chemspider. The downloaded ligands were converted to PDB format using suitable software. The homology modeled receptor was used for docking. AutoDock uses an adapted AMBER force field and so the atoms of the protein and the ligands have to be set up in accordance with this. The missing hydrogen atoms were added for the ligand and protein. A torsion search was made to the ligand and the default number of torsion is set for each ligand. Then the ligand was saved as PDBQT file format for further analysis. Similarly for proteins the heteroatom was removed and additional chains were deleted to get a monomer (Morris et al., 2009).

Docking procedure

Docking calculations were performed using AutoDock software (version 4.2). Desired compounds were docked into the active site of target enzyme *Ag*AChE. In order to assign the perfect grid of each ligand, grid box values were obtained from trial and error and previous studies. The implementing Lamarckian Genetic Algorithm (LGA), considered as one of the best docking methods available in AutoDock, was adopted to perform the molecular docking studies. The parameters for LGA were defined as follows: a

maximum number of 250,000 energy evaluations; a maximum number of generations of 27,000; and mutation and crossover rates of 0.02 and 0.8, respectively. Both Autogrid and AutoDock computations were performed on Cygwin and ten independent docking runs were performed for each compound. The docked conformations of each ligand were ranked into clusters based on the binding energy and the top ranked conformations were visually analyzed. Hydrogen bonding and hydrophobic interactions between docked potent agents and macromolecule were analyzed using AutoDock (version1.50) Tools (Dhananjayan et al., 2014; Andrew et al., 2011). The proper coordinates were set so that the grid can be specific to the binding site of the enzyme. The grids file was generated and saved in GPF format with default setting (Pang et al., 2003).

Results and discussion

Homology modeling

There was no crystal structure of *Anopheles gambiae* AChE on protein data bank (PDB). This necessitated the modeling of protein crystal structure in order to identify the compounds that can bind and inhibit *Ag*AChE. In PDB, AChE crystal structure of other species was available and was used as template structure for homology modeling of *Ag*AChE (Fig. 1). SWISS MODEL PROGRAM tool (http:// swissmodel.expassy.org//SWISS-MODEL.html) was used for construction of homology model. Swiss model is automatic protein prediction software. Swiss model allows the user to find out the template structure and use the template structure for the prediction of the protein AChE. The AChE protein structure of *Anopheles gambiae* was predicted based on the target template alignment. The sequence of AChE was obtained from NCBI (GenBank access number BN0000661). The template structure was identified by the program *insert*. The PDB ID of the protein was 1507 and it belongs to the *Mus musculus* species. The sequence identity between *Ag*AChE and *Mus musculus* AChE was 50.19%. The sequence similarity was 0.45. Since sequence identity of 30% and more than 30% is the acceptable range, the similarity obtained in the present investigation cab ne used to generate homology model (Sibhghatulla et al., 2014). The essential amino acids for ligand interaction and grids calculated for Lamarckian algorithm are given in Fig.2 and Fig. 3 respectively.

Fig. 1: Image of the Homology Modeled Receptor (*Ag*AChE).

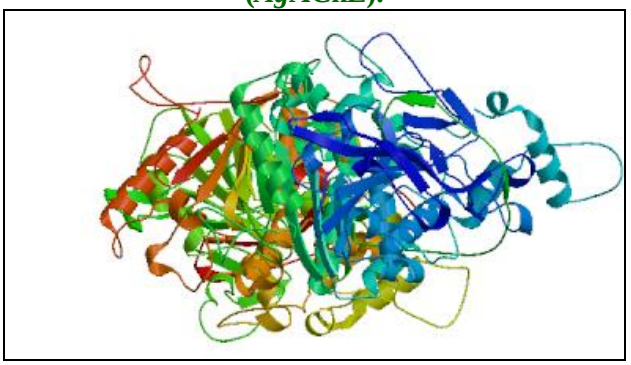


Fig. 2: Essential amino acids for ligand interaction.

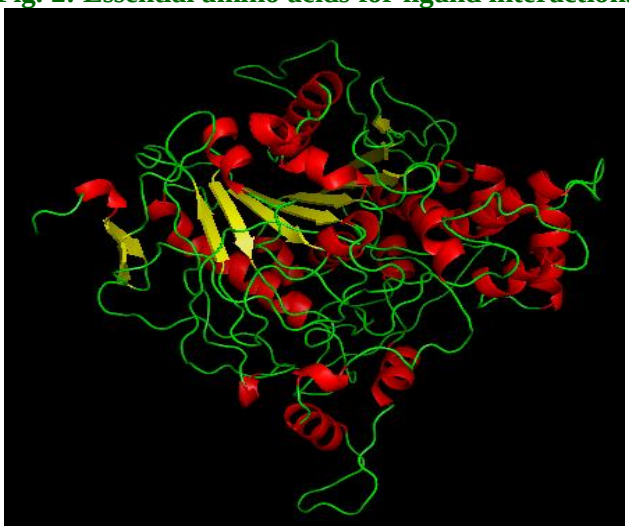
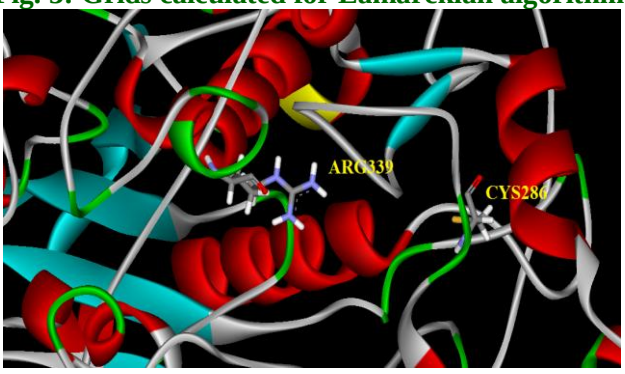


Fig. 3: Grids calculated for Lamarckian algorithm.



Docking studies

AutoDock 4.2 uses a semi-empirical free energy force field to evaluate conformations during docking simulations. The H bond and covalent interactions in amino acids are essential for the reported ligands to inhibit *Ag*AChE. Interaction of hydrogen bond with Arginine 339 of the *Ag*AChE causes reversible

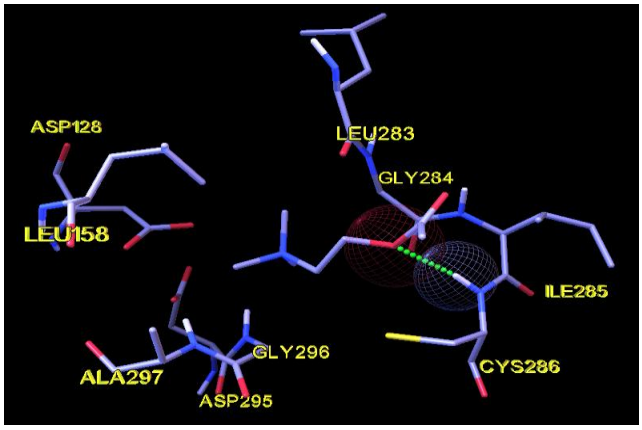
inhibition of the AChE enzyme. Instead, the covalent bond interaction with Serine 286 plays important role for irreversible inhibition of the enzyme (Bulbuli et al., 2013). A reactive group is considered to form a covalent bond in docking experiments. The compounds chosen for the study do not have any reactive group. Hence, H Bond interaction of the compounds with the amino acids Arginine 337 and Serine 286 was extensively investigated. Acetylcholine was used as standard for docking experiment and it forms H Bond interaction with Serine 286 (Fig. 4).

Table 1: Docking Scores and Hydrogen bond interaction

S. No.	Ligand	Docking score Kcal/mol	Amino acids involved in H-Bond interaction		
1	Acetylcholine	-4.67	C286	-	-
2	Annomuricatin B	-	-	-	-
3	Annomuricin A	-	-	-	
4	Annomuricin B	-	-	-	-
5	Annonacin	-	-	-	-
6	Coclaurine	-6.88	-	R339	E336
7	Coreximine	-6.61	C286	-	I285
8	Corrossolone	-2.67	-	R339	-
9	Murisolin	-2.31	-	R339	K340
10	Synepharine	-11.78	-	R339	E287/R290

The compounds Annomuricatin A, B, Annomuricin B, and Annonacin did not bind with the enzyme target site. Hence, these four compounds might be inactive against *Ag*AChE enzyme. The calculations reveal that these compounds form H-Bond interaction with Arginine 339 and glutamate 336. Hydrogen atom of the hydroxyl group or H-Bond donor might form hydrogen bond interaction with oxygen atom of the above mentioned amino acids.

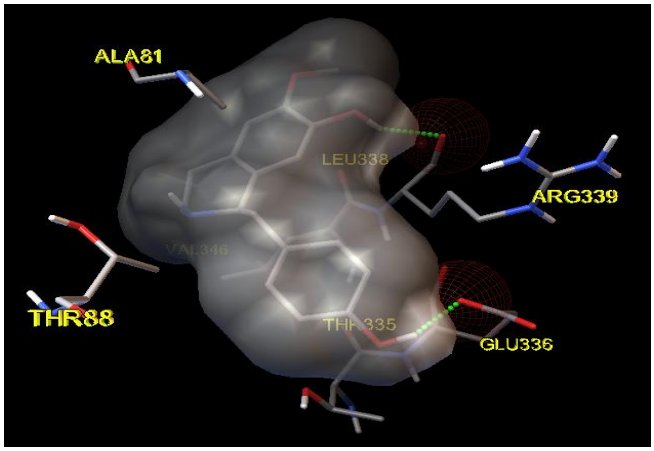
Fig. 4: Acetylcholine, H-Bond interaction with close contact amino acids



Coclaurine

The Coclaurine was found to generate one hydrogen bond interaction with Arginine 339 of the enzyme. The hydroxyl group was involved in H-bond formation. The oxygen atom of the hydroxyl group functioned as a donor and formed hydrogen bond with the hydrogen atom of amino group of Arginine 339 (Fig. 5).

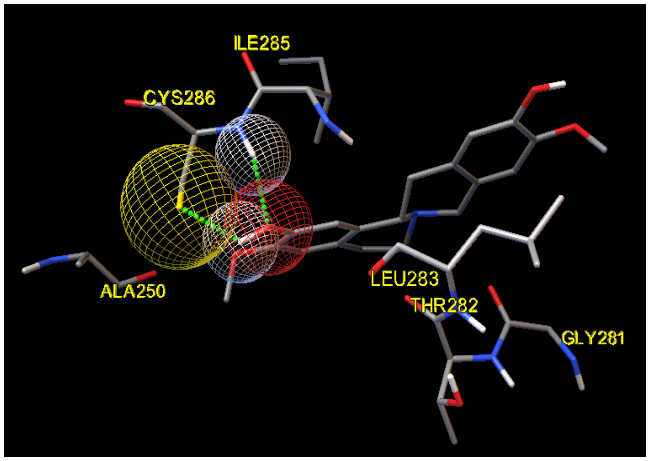
Fig. 5: Coclaurine, H-Bond interaction with close contact amino acids.



Coreximine

Coreximine was noticed to exert two hydrogen bonds with Cystene 286. The H-bonds were formed with the hydroxyl group of the ligand compound. The hydrogen atom was the H bond donor group and further the H-bond interaction was seen in sulphur atom of Cysteine 286. The oxygen atom of the hydroxyl group acted as H-bond donor group and formed H bond interaction with hydrogen atom of the amine group (Fig. 6).

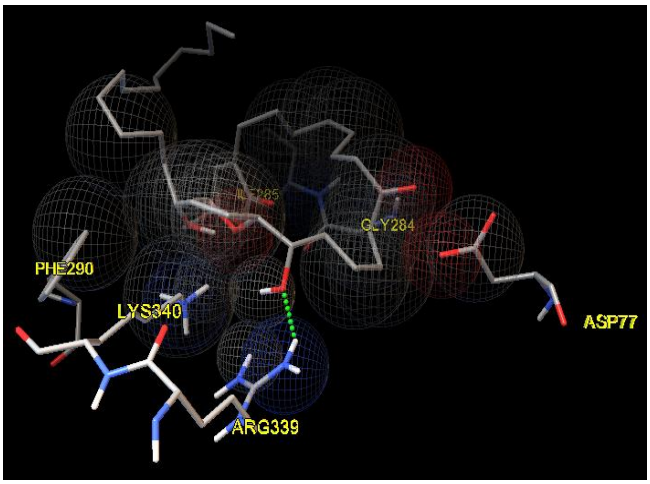
Fig. 6: Coreximine, H-Bond interaction with close contact amino acids.



Corrossolone

One hydrogen bond of Corrossolone was found to involve in the interaction with Arginine 339. The hydroxyl group was engaged in H-bond formation. The oxygen atom of the hydroxyl group performed as the hydrogen bond donor to interact with the hydrogen atom of amino group of Arginine 339 (Fig. 7).

Fig. 7: Corrossolone, H-Bond interaction with close contact amino acids.



Murisolin

The Murisolin was found to have one hydrogen bond interaction with Arginine 339. The hydroxyl group was involved in H bond formation. The oxygen atom of the hydroxyl group functions as a donor and forms hydrogen bond with the hydrogen atom of amino group of Arg 339 (Fig. 8).

Syepharine

The Syepharine was found to show one hydrogen bond interaction with Arginine 339. The hydroxyl group was involved in H bond formation. The oxygen atom of the hydroxyl group functioned as a donor which in turn formed hydrogen bond with the hydrogen atom of amino group of Arg 339 (Fig. 9).

Fig. 8: Murisolin, H-Bond interaction with close contact amino acids.

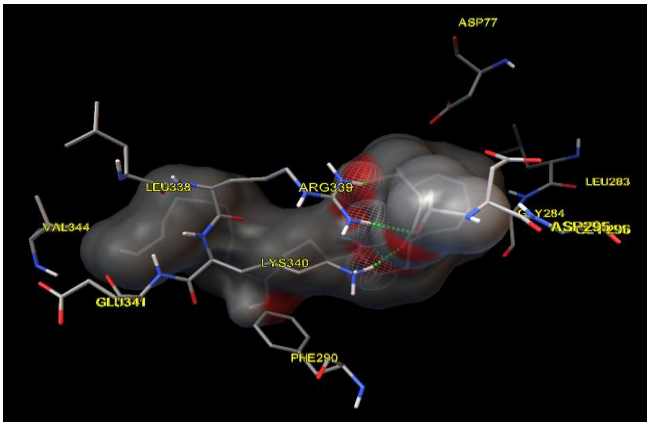
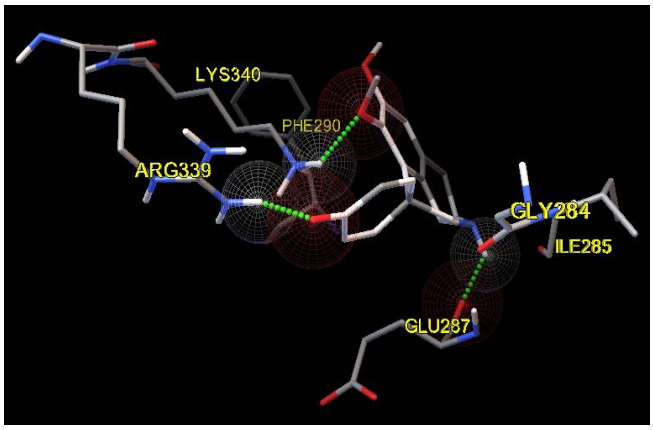


Fig. 9: Syepharine, H-Bond interaction with close contact amino acids.



The findings from the *in silico* docking suggest that Sypharine, Murisolin, Corrossolone, Coreximine and Coclaurine were found to inhibit the action of AChE of *Anopheles gambiae* by interacting with the active site aminoacids. Neurotransmitters normally control the endocrine gland and motor neurons activity in the insects (Bulbuli et al., 2013). The reported compounds might block the enzyme thereby paralyzing the mosquitoes. Hence, *Annona muricata* compounds might be useful to develop effective mosquitocidal pesticides in near future.

Conclusion

Computational designing and docking studies of *Annona muricata* compounds exhibited better binding affinity and inhibitory action up on the Acetylcholine esterase enzyme of *Anopheles gambiae*. The binding energy evaluation revealed the importance of hydroxyl groups at various positions of selected phyto-compounds. These ligands can be further evaluated using wet lab experiments for development of anti-choline esterase pesticides.

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