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MOLECULAR MODELING STUDY OF VARIOUS SYNTHETIC JHAS CONTAINING VARIED FUNCTIONALITY

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ABSTRACT

Virtual screening emerged as an important tool in our quest to access novel pesticides like compounds. The capability to propose feasible ways of binding a putative ligand to a known receptor site is crucial to the structure – based drug design. Molecular docking is one of the most important methods in computer assisted screening of compounds. A docking approach is to "dock" ligand and receptor molecules together in many different ways and then score each orientation by applying different evaluation function. AutoDock 4.2 is an unbiased type docking program in which a ligand finds an optimal position inside the binding cavity of the receptor. In this paper, we reported the protein-ligand interactions using a standard protocol of docking. We have carried out the docking study of a number of synthesized Juvenile Hormone Analogues with receptor binding proteins in order to design targeted JHAs with improved biological activities by understanding their interaction behavior. Among the synthesized derivatives of sulfonamide; 10^{th} analog showed the comparable behavior in terms of their Binding Energy (B.E.) and Inhibitory constant (Ki) with natural JH III and commercial in use IGRs like Pyriproxyfen and Fenoxycarb.

Keywords: ***AutoDock 4.2 software, Juvenile Hormone Analogues, Juvenile hormone binding proteins, Galleria mellonella, Insect Growth Regulators***

I. INTRODUCTION

The study of insect juvenile hormone has assumed great importance because of the possibility of its use as insecticides and pesticides. The use of insect growth regulators such as juvenile hormone and ecdysone has captured worldwide attention in recent past. Insects have a tendency to multiply rapidly; therefore, controlling the vast population of insect is a major problem. The regular and continues use of classical insecticides has made a drastic impact on environment as well as on warm blooded animals. A number of new methods and tools have been proposed in direction of extraction and development of natural and synthetic compounds which should be capable of interfering with growth, development and metamorphosis processes of diverse insect species [1]. These compounds have been called as an insect growth regulators (IGR) or third generation insecticides [2]. Juvenile Hormone (JH) is the main hormone able to regulate all aspects of insect life. They are involved in majority of physiological processes in both developing and mature insects [3]. Juvenile hormone (JH) regulates insect development by bounding to a specific glycoprotein, Juvenile Hormone Binding Protein (JHBP), present in the hemolymph which transport the juvenile hormone (JH) from the site of synthesis to target tissues and serve as JH reservoir. High-affinity juvenile hormone (JH) binding proteins are crucial for proper

insect development, acting as transporters, protectors and reservoirs of the highly hydrophobic and chemically labile JH [4-6]. JH has profound influence on the insect life cycle by hindering larva metamorphosis and by regulating embryogenesis, stimulating reproductive maturation, and controlling the metabolism and migratory behavior of adults. The hemolymph juvenile hormone binding proteins (JHBPs) bind 99% of JH in the hemolymph despite their low abundance, it shows high affinity towards JH molecules (Kd below 10^{-6} M) [7-9]. JHBPs have been isolated from several insect species like *Bombyx mori, Heliothis virescens, Manduca sexta* and *Galleria mellonella*. Among the JHBPs, the protein from *Galleria mellonella* is the object of the present study. *Galleria mellonella*, commonly known as greater wax moth, perceived as the worst beehive pest in the world. Devastation of honeycombs results from the nutritional habits of the larvae, which ingest honey, pollen and wax. *Galleria mellonella* JHBP is a glycoprotein of molecular mass 25880 Da having 245 amino acid residues. Juvenile hormone analogues may act at the membrane level by interacting with various proteins present in the hemolymph or at genetic level by associating with transcription of mRNA [10-15]. They may keep the insects in an immature and potentially injurious stage longer than normal and ultimately affect insect population. Therefore, interest has been generated to design new compounds with different structural features and to study their effect on insect hormonal activity. This will prove very useful in evolving an alternative method in order to avoid the use of toxic chemicals. It has been found experimentally that JHBP of *G. mellonella* undergoes a profound conformational change upon JH binding and this finally provides a foundation for JH docking into JHBP. This leads to a rational approach for designing a large number of molecules that might be used to control insect development [16-18].

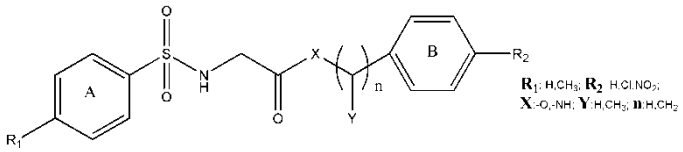

Fig.1 Structure of N-[2-oxo-3-oxa-4-methyl-4- (p-chlorophenyl)butanyl] benzene sulfonamide and analogs (1-10)

In this paper we have reported the docking study of synthesized JHAs containing sulphonamide feature in the main chain with JHBP of *Galleria mellonella*. We have carried out comparative free binding energy analysis of all the synthesized compounds with natural occurring JH III and synthetic IGRs.

II. MATERIAL AND METHOD

2.1 Docking Protocol

The 3D structures of the ligands are constructed using pymol software tool (www.pymol.com) and optimized with AMBER force field of the AutoDock 4.2. The PDB file of the selected structure of *G. mellonella* binding protein (2RCK) is downloaded from the protein data bank. Polar hydrogens are added to the macromolecule (2RCK) using the ADDSOL utility of AutoDock 4.2 (The Scripps Research Institute La Jolla, CA 92037-1000, U.S.A.) and saved in a similar manner as *protein.pdbqt* format. Default values of atomic solvation parameters are used throughout the calculations. Amino acid residues at binding site are selected [9]. Grid box is generated for the calculation of docking interaction energy followed by the generation of grid parameter file *pro.gpf* of protein using AutoGrid Tool of the software. Lamarckian Genetic Algorithm (LGA) protocol is used for protein fixed: ligand flexible model. Lamarckian Genetic Algorithm (LGA) protocol incorporates a local minimization for a given fraction of the population. The LGA mixes a global search for ligand conformation and orientation along with an adaptive local search to perform energy minimization. The scoring function is a sum of Vander

Waals, H-bonding and distance dependent dielectric electrostatics as well as conformational torsional restriction entropy and empirical solvation energetic in terms of ligand-protein complex Docking calculations begins with an initial population of 150 randomly placed conformation of ligands. Therefore, in totality hundred search attempts (ga_run parameter) are performed for each ligand. The maximum number of energy evaluations and generations before the termination of LGA run are 2.5x 10^6 and 2.7 x 10^4 respectively. For the local search, the pseudo-Solis and Wets algorithm are applied. Other docking parameters are set to default values. Final docking orientations lying within 2 Å of the root-mean square deviation (rmsd) tolerance of each other are represented as most favorable conformation with low free energy of binding (ΔG_b). The ligands are ranked according to their binding free energy (B.E.) in kcal/mol and inhibition constant (Ki) in µM at 298.15K. As a results of docking simulation , AutoDock computes intermolecular energy, internal energy and torsional energy as an output; the first two forms the 'docking energy', while the first and the third combine together to give 'binding energy'. [*All the Calculations of Autogrid and Autodock are performed on Linux operating system with system Properties (Intel(R) Pentium(R) D CPU 2.80GHz, 4.0 GB of RAM)]*.

2.2 Virtual screening of JHBP – JHAs complex

We have synthesized Juvenile Hormone mimics with varied functionalities in order to develop better agonist of JH activity in comparison to synthetic IGR like fenoxycarb, S-21149, Compound1 and pyriproxyfen . Docking study of synthesized Juvenile hormone mimics, JH-III and synthetic IGRs with the JHBP of the *Galleria mellonella* is carried out to learn the preferred conformation and configuration of the ligand inside the binding pocket [19-24] (fig 2(a,b)).

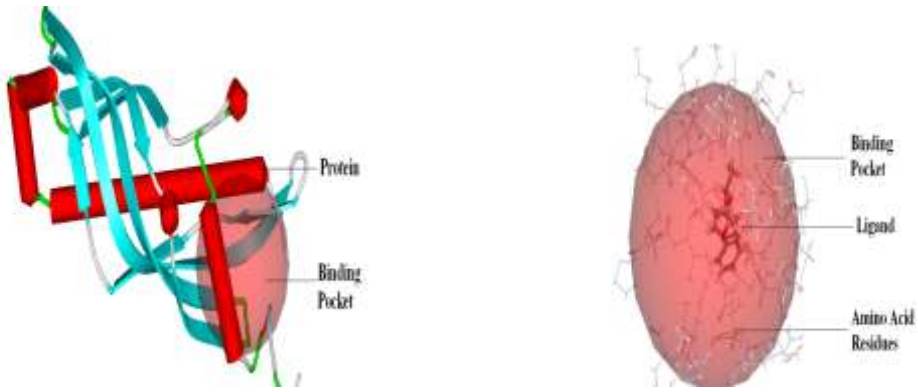

Fig: 2 (A) Complete Structure of The Binding Protein (PDB 2RCK); (B) Ligand Inside Binding Pocket of Protein

Interaction energy of ligands complexed to amino acid residue of binding pocket of the receptor protein is calculated using AUTODOCK 4.2. Docking process in a complex network of interaction is a difficult task. It requires the generation of a large number of configurations of the ligands inside the binding pocket where each being used as an initial docking configuration. Further it identifies the correct pose of the ligand inside the binding pocket and calculates the affinity between the ligand and receptor. The amino acid residues lining inside the pocket region exhibit strong interactions with the synthesized Juvenile Hormone mimics along with JH-III and synthetic IGRs [25-28] . Overall trend for the Binding Energy (BE) Profile is as under:

Pyriproxyfen< Compound 1 < 10 < 4< 8 < 6 < 7 < 9 < 1 <3 < 5 < 2 < S-21149 < Fenoxycarb < Nat. JH III

Among sulfonamide series analog 4, 8 and 10th gave the lowest binding energy profile. All synthetic sulfonamide showed B.E. behavior better then Fenoxycarb, S-21149 and Nat JH III. But higher in comparison to Compound 1 and Pyriproxyfen (Fig. 3).

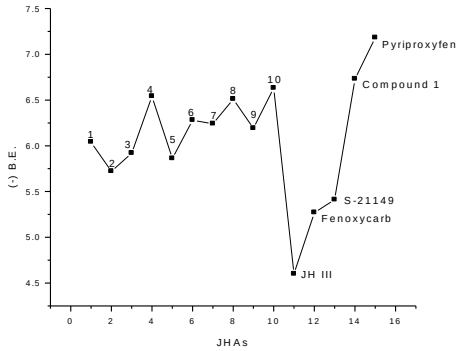

Fig 3: Binding Energy Profile of All The Analogues (1-10) Along With Natural JH III and Synthetic Jhas Fenoxycarb, S-21149, Compound1 and Pyriproxyfen

Protein-ligand binding does not depend only on shape complementary but also on the physiochemical properties. The balance of the Vander Waals, electrostatics, hydrogen bonding and hydrophobic interactions must result in energetically favored binding. Binding is a score of contributing and opposing terms. Therefore, the binding energy is a result of enthalpy-entropy compensation. There are different kinds of interactions operational inside the binding site of the protein depending upon the chemical nature of the ligand molecule. The electrostatic interaction is the main contributor of the interaction energy governing the strength of bonds, the strength of non-bonded interactions and molecular reactivity. In case of the protein-ligand interaction at the active site, ligand experiences a unique environment in terms of electrostatic, steric and hydrophobic properties. Variations in these properties near the active site of the proteins can contribute towards its selectivity and specificity.

In the present study interaction of synthesized JH mimics (1-10), nat JH-III and synthetic IGRs with receptor binding protein at active site show the hydrogen, vander waal and electrostatic interactions. Synthesized JHAs shows main interactions with the binding pocket having amino acid moieties- Thr 22, Tyr 128,130, Lys 218, Ala 21, 220, Ile 18, Cys10 which explain the hydrophobic, acidic and basic nature of the binding pocket. Based upon binding energy profile by docking study we can conclude that sulfonamides along with substituted chloro and phenoxy moiety at the terminal position will be effective IGR In comparison to commercial IGRs. Further replacement of benzene ring by toluene increases the binding energy profile.

III. CONCLUSIONS

In this research paper we have reported the docking study of some of the synthesized JHAs (1-10) having sulphonamide group along with oxa and aza features. In order to design and develop more effective JHAs and to speed up the process ; we have carried out docking study of the synthesized compounds with its receptor site and also compared the study with naturally occurring JH III and commercial in use IGRs (Fenoxycarb, S-21149, Pyriproxyfen and Compound 1). We have studied the receptor –ligand interaction on crystallized JHBP of *G. mellonella.* The crystal structure of JHBPs of *G. mellonella* is established and reported in literature [9]. In order to screen, check and compare the activity of these synthesized JHAs, we have performed docking study using

AutoDock 4.2 software modules and studied receptor-ligand interactions using binding free energy estimation. The docking study of synthesized juvenile hormones and their analogues with the receptor sites in *Galleria mellonella* JHBP suggests that juvenile hormone analogues having minimum score, will fit better inside the binding pocket of the receptor proteins. Aza features corresponds to the more negative score as compared to the oxa feature, therefore, oxa series of compounds believed to be more biological active as compared to aza series. The binding free energy of 10^{th} analog is comparable with natural occurring JH III and Pyriproxyfen. Therefore, 10^{th} analogues containing aza features are suggested to be more effective IGR. In addition to the oxa and aza feature, we have added sulphonamide functionality in the side chain and it has been observed that binding affinity get elevated with the addition of this functionality. We are also in the progress of evaluating this synthetic compound on biological models as well which will be reported later. Besides, further investigation regarding the effect on non-target organism is extremely important and imperative in the near future.

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