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Identification of Small-Molecule Potential Inhibitor(s) for *Helicoverpa armigera* Juvenile Hormone Acid-o-Methyl Transferase (HaJHAMT) through Molecular Docking and MD Simulation Approaches †

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Abstract: Juvenile hormone compounds belong to a family of acyclic sesquiterpenoids, biosynthesized within small paired endocrine glands of corpora allata-corpora cardiaca having a neural connection with the brain. Stringent regulation of JH-levels in insect haemolymph is one of the most critical factors ensuring normal growth and development in insects. JHAMT is characterized as a key regulatory enzyme playing a pivotal role during insect metamorphosis and reproduction physiology by catalyzing the final step of SAM-dependent methylation of JH-acids into their cognate methyl esters. Recent reports on biochemical and molecular characterization of JHAMT gene from different insect species have proposed the JHAMT enzymatic activity as a potential target for the development of novel small molecule insect growth regulator(s). Therefore, in the present study, the protein sequence identity dependent homology model for JHAMT protein from a polyphagous pest, *Helicoverpa armigera* (order: Lepidoptera) has been generated and subsequently explored for virtual screening against small molecules natural product library. One such promising compouns namely, Scolimoside (HMDB05799) showing interactions with amino acid residues of enzyme's substrate-binding pocket (SBP) was further validated using molecular docking and Molecular Dynamics simulation (MD simulation). The study may pave the way forward for the design and development of novel eco-friendly small regulatory molecules as a component of integrated pest management.

Keywords: JHAMT; docking; MD Simulation; Small molecule inhibitor

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1. Introduction:

Juvenile hormone (JH) is a group of small sesqui-terpenoids molecules primarily restricted to insects. JH are known to play key roles in insect's metamorphosis and reproduction physiology, including caste differentiation in Honey bees [1]. At least eight different forms of juvenile hormones have been identified so far in insects [2]. Juvenile hormone III (JH III) is the common JH to most insects. In lepidopteran insects, five different forms of JH have been identified (JH 0, JH I, JH II, JH III, and 4-methyl JH I), whereas bis-epoxide JH III and skipped bis-epoxide (JHSB III) is commonly found in dipteran and heteropteran insect species, respectively [3]. JH's are biosynthesized within tiny paired endocrine glands, corpora allata-corpora cardiaca having a neural connection with the brain. The JH secrets into the insect hemolymph and regulates several polyphenism in insects. The biosynthetic pathway of JH in insect corpora allata glands is divided into two stages, *viz* early-stage and late-stage pathway. The steps of the early stage are called the

mevalonic pathway starts from acetyl Co-A and results in the formation of farenesyl pyrophosphate. In contrast, the steps of the late-stage pathway are rather specific to insects called as JH branch. The JH branch pathway starts from farnesyl pyrophosphate to farnesoic acid. The sequence of last two steps of JH branch are dependent on insect order. In lepidoptera, epoxidation preceeds the methylation whereas in diptera, dictyoptera, coleoptea, orthoptra and hemiptera, the methylation preceeds the epoxidation [4]. The observed variation in the sequence of the last two steps is considered due to the relative affinity of the respective enzymes towards their corresponding substrate. Due to the relative importance of JH in the insect life cycle as well as the restricted occurrence in insects, the enzymes of JH branch have been considered as a potential target for bio-rational insecticide development in pest management. The juvenile hormone acid-O-methyl transferase (JHAMT) is characterized as a key regulatory enzyme playing a pivotal role during insect metamorphosis and reproduction physiology by catalyzing the final step of SAM-dependent methylation of JH-acids into their cognate methyl esters. RNAi-mediated silencing or knockdown of JHAMT in different insects have reported the impaired larval development, including precocious metamorphosis and adults with poor reproduction ability [5–7]. *Helicoverpa armigera* L. Hubner is a polyphagous pest on several host species such as cotton, tomato, black gram, soybean, ladyfinger, etc. Apart from the chemical insecticides, Bt technology with cry toxin expressing within the plants (Bt cotton, Bt corn, etc.) has been employed in the management of this notorious pest.. However, poor refugia management in the Bt field and the un-discriminative use of insecticidal chemicals have reduced the efficacy of the pest management strategy for this pest [8]. Moreover, the residual effect of synthetic chemical insecticides also contributes to soil and environment pollution. Therefore, the identification and development of new molecules which are not only effective but also easy to degrade in the environment are considered a green approach for pest management. In this, small molecules of natural plant metabolites with intertidal properties can be viewed as potential candidates. In the present study, small molecules from Food database compounds were virtually screened and evaluated for their insecticidal properties through molecular docking and molecular dynamics (MD) simulation approaches.

2. Methods:

2.1. Homology model generation and validation:

The 284 amino acids, the full-length protein sequence of the HaJHAMT was retrieved from the NCBI database (Accession number: XP_021182380). Homology model of HaJHAMT protein was generated using I-TASSER server [9,10] with default parameters. The loops were refined and modelled by ModLoop web server [11]. The quality of the obtained protein model was assessed and optimized using various model quality assessment programs at saves server version 6.0 (https://saves.mbi.ucla.edu/). Ramachandran plot for mainchain dihedral angels was generated using procheck analysis at saves server. The model was further refined, and energy minimized using modrefiner[12] and swiss pdb-viewer version 4.1[13].

2.2. Ligand molecules preparation:

The fragment library containing 32516 natural products was retrieved from the FOODB database (https://foodb.ca/). The sdf files of farnesoic acid, juvenile hormone acid III, and juvenile hormone III were retrieved from pubchem database (https://pubchem.ncbi.nlm.nih.gov/). All these molecules were energy minimized and converted to pdbqt format using open babel software in PyRx [14].

2.3. Virtual Screening:

Virtual screening while keeping the receptor protein rigid was performed using PyRx software 0.8 [15,16] which uses autodock-vina as a search program. The files *viz*. target

protein and ligand molecules were prepared and saved in supported pdbqt format using PyRx software. Through a literature search of homologous sequences, residues spanning substrate-binding pocket were identified. Grid box parameters were selected in such a way that all active site residues lie with the box. The grid box dimensions set as X= 68, Y=62 and Z=104 with central spacing of 0.375 A^0 and grid central points x= 62.82, y= 69.997 and z= 63.521. The search exhaustiveness was set to 9. The output was ranked according to the binding free energy. The pose with the lowest binding free energy was considered the best pose.

2.4. Molecular Docking:

Flexible molecular docking was performed using the standalone autodock-vina program. The side chains of interacting conserved residues *viz* Gln 14 and Trp 114 were kept flexible to enable the docked ligand to interact freely with the receptor protein. The grid box parameters were kept the same as in virtual screening, and exhaustiveness of conformation search was also set to 9.

2.5. Molecular Dynamics Simulation:

Molecular Dynamics simulations of jhamt protein in water (jhamt apo form) and jhamt-ligand complex was run to assess the validation of docking interactions and stability of protein ligand-complex. The entire course of simulations was performed using GROMACS software's modules version 2021 (https://www.gromacs.org/) in a linux workstation. The topology of the protein and ligands molecules were written using GROMOS53a1 force field and PRODRG server[17], respectively. The system was treated as the cubic unit cell with a 1nm distance from the cell's edges and filled with water molecules using the SPC water model (Simple Point Charges) using the editconf and solvate module in GROMACS. The protein complexes were neutralized by adding 12 Na^+ ions into the system, and energy minimized using 50000 steps of the steepest descent gradient method with a total energy cutoff of 10 $KJmol^{-1}nm^{-1}$. The system then equilibrated at NVT (constant number of particle, volume, and Temperature) and then NPT (constant number of particle, Pressure, and Temperature) for 1000 ps at 300 kelvin (K). V-rescale, a modified Berendsen thermostat[18], was employed as a temperature coupler, whereas for constant Pressure, a Parrinello-Rahman barostat pressure coupler[19] was used. To constraints the covalent bond during simulation, LINCS algorithm (Linear constraints Solver) was employed [20]. A 50 ns MD simulation run was performed using leap-frog integrator[21,22] with the integration of trajectories at every two femtoseconds (fs). PME method (Particle Mesh Ewald) was used to compute long-range electrostatic interactions [23]. The trajectories of the simulation were analyzed for RMSD (Root Mean Square Deviation), RMSF (Root Mean Square Fluctuation), H-bond profile of protein-ligand docked complexes for binding stability analysis.

3. Results:

3.1. Homology Modelling and validation:

The homology model was obtained by submitting the full-length jhamt protein sequence to the I-TASSER-protein structure and function prediction server. The server outputs and ranked the predicted model according to a C-score (typical range [-5 to 2]) which actually a confidence score indicative of predicted model quality. Higher the C-score will be better model quality. Therefore, we have selected jhamt model with the highest C-Score of value -0.26. The loops were further refined by the ModLoop web server, and energy minimization was done. The quality of the geometrically optimized and energy minimized model was found to be satisfactory in the quality assessment performed using saves server programs. Ramachandran plot analysis showed more than 98 % residues in allowed regions, whereas only 1.9% residues are in the disallowed area (Figure 1a). The model also satisfies other quality check parameters like ERRAT plot (81.159 % quality

factor) (Figure 1b), VERIFY-3D score of 80.63% of the residues have averaged 3D-1D score>=0.2 (Figure 1c). The ProQ server [24] LGscore of 8.851 placed the predicted jhamt model extremely good and consistent catagory.

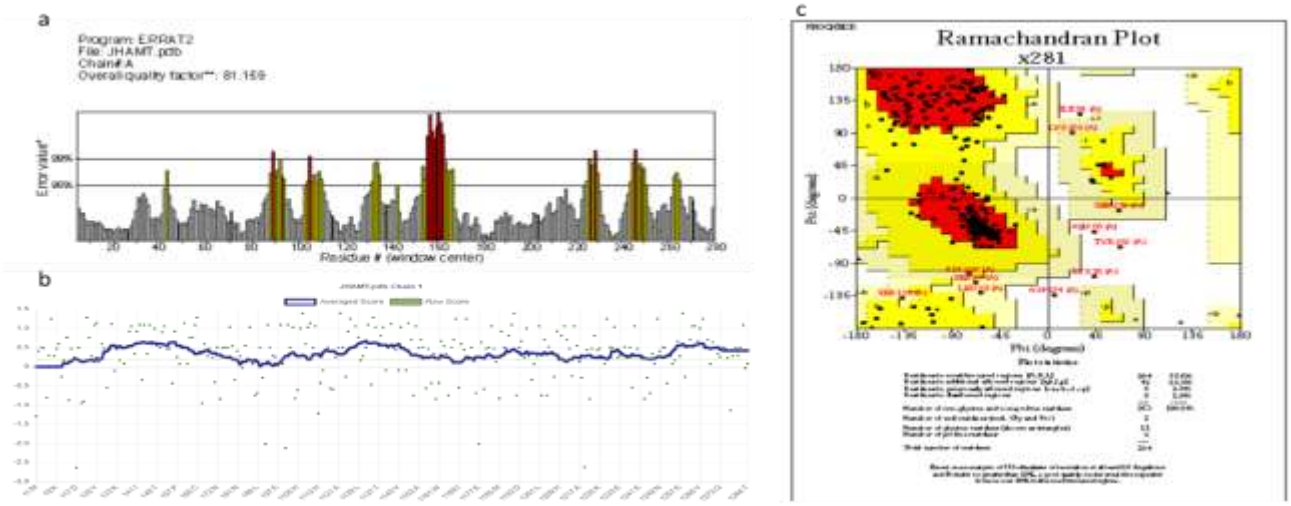

Figure 1. Assesment of quality of 3D model of jhamt protein. a) ERRAT plot b) 3D-1D score and c) Ramachandran plot.

3.2. Virtual screening:

Virtual screening was run using the PyRx version 0.8. The rigid receptor protein (jhamt) and the ligand library molecules were energy minimized and converted to pdbqt format using the open babel program. PyRx run Autodock-vina for docking pose search and ranked the best nine (exhaustiveness set to 9) conformations according to the binding energy (the pose with more negative binding energy will be ranked best). The resultant conformations for each protein-ligand pair were analyzed in PyMOL. The top hits that showed higher affinity for jhamt with binding energy ranged between -9.0 to -6.5.0 kcal mol^{-1} compared to its substrates/product *viz*. farnesoic acid (-5.9 kcal mol^{-1}) and juvenile hormone acid III (-6.2 kcal mol^{-1}) (Table1).

Table 1. Virtual screening results of top five compounds and two natural substrates using PyRx. Interaction energy is expressed as kcal per mol.

Sr. No.	Compounds	Binding Affinity (kcal mol^{-1})	Sr no.	Compounds	Binding Affinity (kcal mol^{-1})
1.	HMDB05799	-9	5.	HMDB05796	-6.9
2.	HMDB05800	-7.6	6.	Juvenile hormone acid III	-6.2
3.	HMDB05808	-7.4	7.	Farnesoic acid	-5.9

3.3. Molecular docking:

The flexible molecular docking with autodock-vina results suggest that the compound Scolimoside (compound Id: HMDB05799) interacts most stably within the active site pocket of jhamt protein. The compound Scolimoside is stabilized by making both H-bond network and non-polar π-π planer interactions involving the active site residues of Ser 10, Gln 14, Tyr 205 Ala 217, Tyr 110, Trp 114, and Phe 221 (Figure 2a, 2b, and 2c). The calculated binding energy was found to be in the order of -9.7 kcal mol^{-1}.

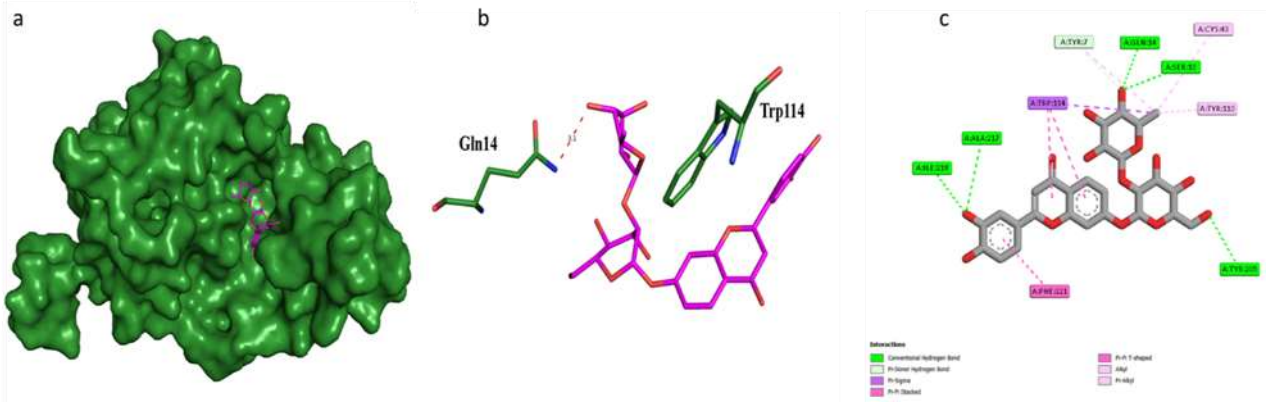

Figure 2. Molecular docking and binding interactions. a) jhamt protein with ligand (Scolimoside) docked into the substrate-binding pocket. b) H-bond interaction of amide nitrogen of Gln 14 with Scolimoside c) 2-D ligand-interaction plot showing active site residues interactions with docked Scolimoside.

3.4. MD Simulation:

The binding interactions of the jhamt-Scolimoside complex were evaluated and validated at atomic detail using MD simulation run. Therefore, a number of MD simulation parameters *viz* RMSD, RMSF and H-bond profiling were evaluated for jhamt- Scolimoside complex. RMSD analysis was done to assess the structural stability of the obtained complexe after virtual screening and also to evaluate structural agreement with the crystal structure. The average value of RMSD obtained for jhamt-Scolimoside complex was 0.55 nm which is lesser in value than the average value for jhamtin apo form (0.62 nm). The RMSD plot for the complex of the Scolimoside with jhamt protein is shown in Figure 3. All the structures under consideration showed a consistent RMSD profile throughout MD simulation till 50 ns. The apo form of jhamt protein showed larger fluctuations during the simulation run compared to the jhamt-Scolimoside complex. These variations may be attributed to the structural changes brought about by the ligand positioning in the active site of the protein. Further, the radius of gyration was used to assess the changes brought about by ligand binding to the protein. The average value of Rg for scolimoside was 1.98 nm. This value is in close agreement with the apo form, which was 2.1 nm. As shown in Figure 4, due to the compact packing of secondary components, all structures showed relatively similar and consistent Rg values during the simulation process of 50 ns. Moreover, we used the GROMACS h-bond utility to assess the distribution of hydrogen bonds between the ligand and protein during the simulation. As shown in Figure 5, a consistent number of intermolecular hydrogen bonds were established for the complex of jhamt protein with scolimoside offering the complex stability during the 50 ns simulation.

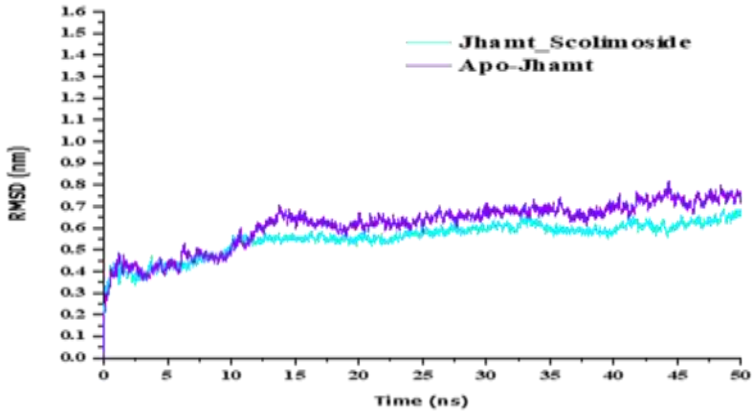

Figure 3. Root mean square deviation (RMSD) plot of jhamt apo and jhamt- Scolimoside complex calculated in MD simulation for 50 ns.

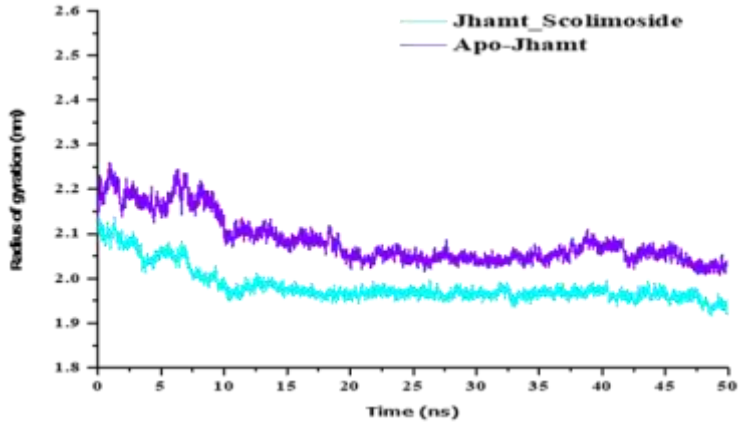

Figure 4. Radiation of gyration plot of jhamt apo and jhamt- Scolimoside complex calculated in MD simulation.at 300 K for 50 ns.

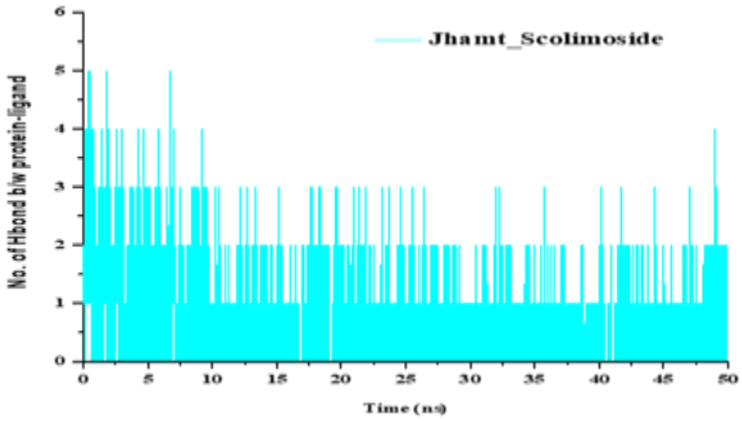

Figure 5. Inter molecular hydrogen bonds bar graphs of jhamt- Scolimoside complexe.

4. Discussion:

Juvenile hormone has been regarded as a status quo hormone in insect physiology, plays a central role in insect metamorphism and reproductive maturation [25]. Since the occurance of JH is restricted to insects and related arthropods, the enzymes of JH biosynthesis pathway have been considered as a suitable target for the bio-rational design of insecticide [2,26]. The Juvenile hormone acid-methyl transferase (JHAMT) has been characterized as a key enzyme in JH biosynthesis pathway [27–29]. Therefore thargeting the jhamt enzyme via identifying suitable, selective and eco-friendly small inhibitory from natural sources may be useful for managing the pest population below the economic threshold level. In the present study, due to the lack of x-ray crystal and NMR structure of jhamt, a homology model of jhamt protein was generated and explored for the identification of suitable inhibitory molecules. In order to get an eco-friendly and biodegradable compound as lead compounds, a fragment library of food compounds (total 32516 compounds) was selected as chemical space from FOODB database. In order to enhance the search speed and reducing the computation time for virtual screening, Jhamt protein was kept in rigid conformation. However, flexible docking was also performed with individually selected hits from virtual screening. The top five promising hits were selected based upon the binding energies (Table 1). Studies have demonstrated that the binding pocket of jhamt is mostly hydrophobic in nature and the substrates (farnesoic acid and (10R) juvenile hormone acid III) are stabilized within this hydrophobic substrate-binding pocket via H-bond and hydrophobic interactions [4]. Keeping this in view, we have performed a flexible docking of jhamt protein with the best hit compound-Scolimoside (compound ID: HMDB05799). The side chains of Gln 14 and Trp 114 were kept flexible, and site specific

docking was run using autodock-vina platform with search exhaustiveness set to 9. Although small, but comperatively higher interaction energy (-9.7 compared to 9) was observed in flexible docking. This may be attributed to additional flexibility in bond rotation and bending of side chains for selected key residues (Gln 14 and Trp 114) participating in ligand interaction and stabilization. The ligand was stabilized within the substrate-binding pocket of jhamt protein via forming H-bond network with amide nitrogen of Gln 14, similar to what was observed in the case of farnesoic acid and (10R) juvenile hormone acid III in yellow fever mosquito (*Aedes aegypti*) (Figure 2b) [4]. In addition to these H-bonds, the hydrophobic residues of the active site pocket also participate in π-π interactions with rings of ligand,contributing to overall ligand stabilization within the active site pocket (Figure 2a and 2c). To validate the stability and binding interactions of jhamt-Scolimoside complex, various parameters such as RMSD, the radius of gyration or RMSF, and h-bond profiling of the protein-ligand complex and protein in water were evaluated from the MD simulation trajectory. The structural stability of jhamt-Scolimoside complex was asses with RMSD values. The reduction in RMSD from 0.65 (apo jhamt) to 0.55 nm, suggests that the jhamt-Scolimoside complex tends to form a stable complex. However, the lower RMSD of jhamt-Scolimoside complex may be attributed to the structural changes brought about by the ligand positioning in the active site of the protein. Further, the radius of gyration was used to assess the changes brought about by ligand binding to the protein. The near values of Rg for jhamt-Scolimoside complex and jhamt in apo form may be due to the compact packing of secondary components. H-bond profiling results showed that there is a consistent number of intermolecular hydrogen bonds were established for the complex of protein with scolimoside showing the complex stability during the 50 ns simulation.

5. Conclusions:

Chemical control constitutes an integral component of an integrated pest management strategy. However, the field and laboratory evolved insecticide resistance strains against newer chemistry of chemical insecticides and reduced efficacy of Bt crops due to Bt resistance is posing a severe threat to food security. In addition, the chemical pesticide not only pollutes our environment and but also poses a serious concern for human health. Therefore, mining and developing green chemistry molecules from a natural source with drug-like properties may be an urgent need today. These natural products are biodegradable and eco-friendly in use and may perform excellent if evaluated for pest management. Therefore, in the present study, we have explored a food database of natural compounds and evaluated their efficacy for pest management or control by selectively targeting juvenile hormone acid-o- methyltransferase enzyme from the juvenile hormone biosynthesis pathway. The study will pave the way forward for developing green chemistry compounds for pest management in the future.

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References

1. Li, W.; Huang, Z.Y.; Liu, F.; Li, Z.; Yan, L.; Zhang, S.; Chen, S.; Zhong, B.; Su, S. Molecular Cloning and Characterization of Juvenile Hormone Acid Methyltransferase in the Honey Bee, Apis mellifera, and Its Differential Expression during Caste Differentiation. *PLoS One* **2013**, *8*, 68544, doi:10.1371/journal.pone.0068544.
2. Cusson, M.; Sen, S.E.; Shinoda, T. Juvenile hormone biosynthetic enzymes as targets for insecticide discovery. In *Advanced Technologies for Managing Insect Pests*; Springer Netherlands, **2013**; pp. 31–55 ISBN 9789400744974.
3. Yin, Y.; Qiu, Y.W.; Huang, J.; Tobe, S.S.; Chen, S.S.; Kai, Z.P. Enzymes in the juvenile hormone biosynthetic pathway can be potential targets for pest control. *Pest Manag. Sci.* **2020**, *76*, 1071–1077, doi:10.1002/ps.5617.
4. Defelipe, L.A.; Dolghih, E.; Roitberg, A.E.; Nouzova, M.; Mayoral, J.G.; Noriega, F.G.; Turjanski, A.G. Juvenile hormone synthesis: "esterify then epoxidize" or " epoxidize then esterify" ? Insights from the structural characterization of juvenile hormone acid methyltransferase. *Insect Biochem. Mol. Biol.* **2011**, *41*, 228–235, doi:10.1016/j.ibmb.2010.12.008.

5. Asokan, R.; Sharath Chandra, G.; Manamohan, M.; Krishna Kumar, N.K.; Sita, T. Response of various target genes to diet-delivered dsRNA mediated RNA interference in the cotton bollworm, Helicoverpa armigera. *J. Pest Sci. (2004).* **2014**, *87*, 163–172, doi:10.1007/s10340-013-0541-7.
6. Minakuchi, C.; Namiki, T.; Yoshiyama, M.; Shinoda, T. RNAi-mediated knockdown of juvenile hormone acid O-methyltransferase gene causes precocious metamorphosis in the red flour beetle Tribolium castaneum. *FEBS J.* **2008**, *275*, 2919–2931, doi:10.1111/j.1742-4658.2008.06428.x.
7. Jaiwal, A.; Natarajaswamy, K.; Rajam, M.V. RNA silencing of hormonal biosynthetic genes impairs larval growth and development in cotton bollworm, Helicoverpa armigera. *J. Biosci.* **2020**, *45*, 1–14, doi:10.1007/s12038-020-00079-6.
8. Balasubramani, G.; Raghavendra, K.P.; Das, J.; Kumar, R.; Santosh, H.B.; Amudha, J.; Kranthi, S.; Kranthi, K.R. Critical Evaluation of GM Cotton. In *Cotton Precision Breeding*; Springer International Publishing: Cham, 2021; pp. 351–410.
9. Yang, J.; Yan, R.; Roy, A.; Xu, D.; Poisson, J.; Zhang, Y. The I-TASSER suite: Protein structure and function prediction. *Nat. Methods* 2014, *12*, 7–8.
10. Yang, J.; Zhang, Y. I-TASSER server: New development for protein structure and function predictions. *Nucleic Acids Res.* **2015**, *43*, W174–W181, doi:10.1093/nar/gkv342.
11. Fiser, A.; Sali, A. ModLoop: Automated modeling of loops in protein structures. *Bioinformatics* **2003**, *19*, 2500–2501, doi:10.1093/bioinformatics/btg362.
12. Xu, D.; Zhang, Y. Improving the Physical Realism and Structural Accuracy of Protein Models by a Two-Step Atomic-Level Energy Minimization. *Biophysj* **2011**, *101*, 2525–2534, doi:10.1016/j.bpj.2011.10.024.
13. Guex, N.; Peitsch, M.C. SWISS-MODEL and the Swiss-PdbViewer: An environment for comparative protein modeling. *Electrophoresis* **1997**, *18*, 2714–2723, doi:10.1002/elps.1150181505.
14. O'Boyle, N.M.; Banck, M.; James, C.A.; Morley, C.; Vandermeersch, T.; Hutchison, G.R. Open Babel: An Open chemical toolbox. *J. Cheminform.* **2011**, *3*, 1–14, doi:10.1186/1758-2946-3-33.
15. Ahsan, T.; Sajib, A.A. Repurposing of approved drugs with potential to interact with SARS-CoV-2 receptor. *Biochem. Biophys. Reports* **2021**, *26*, doi:10.1016/j.bbrep.2021.100982.
16. Dallakyan, S.; Olson, A.J. Small-molecule library screening by docking with PyRx. *Methods Mol. Biol.* **2015**, *1263*, 243–250, doi:10.1007/978-1-4939-2269-7_19.
17. Schüttelkopf, A.W.; Van Aalten, D.M.F. PRODRG: A tool for high-throughput crystallography of protein-ligand complexes. *Acta Crystallogr. Sect. D Biol. Crystallogr.* **2004**, *60*, 1355–1363, doi:10.1107/S0907444904011679.
18. Berendsen, H.J.C.; Postma, J.P.M.; Van Gunsteren, W.F.; Dinola, A.; Haak, J.R. Molecular dynamics with coupling to an external bath. *J. Chem. Phys.* **1984**, *81*, 3684–3690, doi:10.1063/1.448118.
19. Parrinello, M.; Rahman, A. Polymorphic transitions in single crystals: A new molecular dynamics method. *J. Appl. Phys.* **1981**, *52*, 7182–7190, doi:10.1063/1.328693.
20. Hess, B.; Bekker, H.; Berendsen, H.J.C.; Fraaije, J.G.E.M. LINCS: A Linear Constraint Solver for molecular simulations. *J. Comput. Chem.* **1997**, *18*, 1463–1472, doi:10.1002/(SICI)1096-987X(199709)18:12<1463::AID-JCC4>3.0.CO;2-H.
21. Van Gunsteren, W.F.; Berendsen, H.J.C. A Leap-Frog Algorithm for Stochastic Dynamics. *Mol. Simul.* **1988**, *1*, 173–185, doi:10.1080/08927028808080941.
22. Young, P. *Physics 115/242 The leapfrog method and other "symplectic" algorithms for integrating Newton's laws of motion*;
23. Abraham, M.J.; Gready, J.E. Optimization of parameters for molecular dynamics simulation using smooth particle-mesh Ewald in GROMACS 4.5. *J. Comput. Chem.* **2011**, *32*, 2031–2040, doi:10.1002/jcc.21773.
24. Wallner, B.; Elofsson, A. Can correct protein models be identified? *Protein Sci.* **2003**, *12*, 1073–1086, doi:10.1110/ps.0236803.
25. Noriega, F.G. Juvenile Hormone Biosynthesis in Insects: What Is New, What Do We Know, and What Questions Remain? *Int. Sch. Res. Not.* **2014**, *2014*, 1–16, doi:10.1155/2014/967361.
26. Yin, Y.; Qiu, Y.W.; Huang, J.; Tobe, S.S.; Chen, S.S.; Kai, Z.P. Enzymes in the juvenile hormone biosynthetic pathway can be potential targets for pest control. *Pest Manag. Sci.* **2020**, *76*, 1071–1077, doi:10.1002/ps.5617.
27. Van Ekert, E.; Heylen, K.; Rougé, P.; Powell, C.A.; Shatters, R.G.; Smagghe, G.; Borovsky, D. Aedes aegypti juvenile hormone acid methyl transferase, the ultimate enzyme in the biosynthetic pathway of juvenile hormone III, exhibits substrate control. *J. Insect Physiol.* **2014**, *64*, 62–73, doi:10.1016/j.jinsphys.2014.03.001.
28. Sheng, Z.; Ma, L.; Cao, M.X.; Jiang, R.J.; Li, S. Juvenile hormone acid methyl transferase is a key regulatory enzyme for juvenile hormone synthesis in the eri silkworm, Samia cynthica ricini. *Arch. Insect Biochem. Physiol.* **2008**, *69*, 143–154, doi:10.1002/arch.20268.
29. Shinoda, T.; Itoyama, K. Juvenile hormone acid methyltransferase: A key regulatory enzyme for insect metamorphosis. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 11986–11991, doi:10.1073/pnas.2134232100.