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Molecular docking of triterpenoids from Neem with the ecdysone receptor of lepidopteran pests

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An *in silico* docking study was performed to evaluate the interaction of various triterpenoids present in neem with the ecdysone receptor of two economically important lepidopteran pests viz., *Helicoverpa armigera* (HaEcR) and *Plutella xylostella* (PxEcR). Twenty triterpenoids were selected for the study, and their docking scores with HaEcR and PxEcR were calculated using the program AutoDock Vina. A commercially available DAH insecticide, tebufenozide, was used as a reference ligand. Out of the twenty triterpenoids used for the study, six and nine triterpenoids recorded binding energy lower than the reference ligand, tebufenozide, when docked with HaEcR and PxEcR, respectively. Four triterpenoids, viz., isomeldenin, azdiradione, 6-deacetylnimbinene, and nimocinol, docked effectively with the ecdysone receptor of both insect pests. In addition, nimbinene and 6-deacetylnimbin also docked effectively with HaEcR and epoxyazadiradione and nimbocinol with PxEcR. Most of the lead compounds were able to form hydrogen bonds with the ecdysone receptor molecule. We found two key amino acid residues, Asn of HaEcR and Ser of PxEcR, at the 504th position, based on their ability to form hydrogen bonds with many lead triterpenoids tested. Other residues, such as Trp 526 in HaEcR and Lys 372 and Phe 520 in PxEcR, were involved in hydrophobic and π-π stacking interactions with many lead triterpenoids, suggesting these residues as an important point of interaction between receptor and ligand molecules. Triterpenoids such as tirucallol, 3-tigloylazadirachtol, and azadirone, although recorded binding energy lower than tebufenozide when docked with PxEcR, failed the prerequisite conditions laid down by Tice rule for a successful pesticide. The lower binding energy of the lead compounds suggests their stable interaction with the receptor molecule and their possible use as an ecdysone agonist or antagonist for effective insect control.

Keywords: DAH, Docking, Ecdysone receptor, Moulting, Triterpenoids

IPC code; Int. cl. (2021.01)-A01N 27/00, A01N 65/00, A01N 65/00, A01N 65/26, A01P

Introduction

Helicoverpa armigera (Hub.) (Lepidoptera: Noctuidae), commonly known as cotton bollworm, is a major pest of cotton in India, significantly affecting its productivity. Damage caused by this pest is estimated to be greater than US$2 billion annually in Asia, Africa, Europe, and Australia, where it is most prevalent[1]. *Plutella xylostella*, (L.) (Lepidoptera: Plutellidae), commonly known as diamondback moth, is another economically important pest of cruciferous crops. The annual worldwide cost associated with diamondback moth management is estimated to be between US$4 billion-US$5 billion[2]. These lepidopteran pests have shown an incidence of resistance to almost all synthetic insecticides used against them for their control. This warrants an alternative to synthetic insecticides to control these agricultural pests.

Diacylhydrazine (DAH), viz. tebufenozide (TF), methoxyfenozide (MF), and halofenozide (HF), are a class of non-steroidal compounds that emerged as a possible tool for insecticide resistance management due to their discerning insect toxicity[3]. These compounds are known to exert their insecticidal effect by mimicking the action of an important insect hormone, 'ecdysone,' thereby interfering with a vital process in insect development, i.e., metamorphosis. The action of 20–hydroxyecdysone (20E), an active metabolite of ecdysone, is mediated by its binding to the ecdysone receptor complex. The ecdysone receptor complex is a heterodimer of two proteins, the ecdysone receptor (EcR) and ultraspiracle (USP). Although ecdysone can bind to EcR on its own, the binding is significantly enhanced by the presence

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of USP. The binding of ecdysone to its receptor induces moulting and, thereby, metamorphosis in insects, and thus its release is finely regulated to coincide with the insect's major morphological transition period. DAHs, like 20E, are known to bind to the EcRs, activating them permanently (ecdysone agonist)[4]. This induces a premature and unsuccessful moult in several insect orders (coleoptera, diptera, and lepidoptera), interfering with their normal cuticle formation and ultimately leading to death[5].

Due to their specificity to a few insect orders and the hormone ecdysone being found exclusively in invertebrates, these diacylhydrazines are reported to have no negative impact on most non-target and beneficial organisms, including mammals[3]. However, recently, some studies reported DAH to interact with some crustacean ecdysone receptors, thus interfering with their physiology. Moderate toxicity risks to *Daphnia magna* and mysid shrimp by DAH pesticides, such as tebufenozide, methoxyfenozide and halofenozide have been reported[6]. Agonist and antagonist effects of these DAH insecticides were also reported for the ecdysone receptor of Cherry Shrimp (*Neocaridina davidi*)[7].

Natural compounds originating from plants that are less persistent in the environment and are safe to natural enemies of insect pests and humans are a better alternative to synthetic insecticides. The Neem tree, *Azadirachta indica,* A. Juss (Meliaceae) is one of the richest sources of biologically active compounds. All parts of this plant contain enormous amounts of active compounds, the highest being in neem seed kernels. Triterpenoids, phenolic compounds, carotenoids, steroids, ketones etc., are some major active compounds in neem seed kernels[8,9]. These compounds from neem are known to produce insecticidal, antifeedant, adult sterility and growth regulator effects in insects belonging to orders diptera, hymenoptera, coleoptera, lepidoptera, orthoptera, and hemiptera[10,11].

The present study was undertaken to evaluate the effectiveness of triterpenoids, one of the active compounds present in neem plants, to dock with the ecdysone receptors of two economically important pests, i.e., *H. armigera* and *P. xylostella*, and to further compare it with widely used and commercially successful DAH insecticide, tebufenozide (TF).

Materials and Methods

Retrieval of ecdysone receptor of *Helicoverpa armigera*

The molecular database RCBS (Repository for Biological Molecular Information) (PDB; https://www.rcsb.org/) was used to retrieve the 3D coordinate of the crystal structure of the ecdysone receptor of *H. armigera*. The crystal structure (PDB code: 3IXP) obtained is bound to the ligand BYI08346.

Retrieval and homology modelling of ecdysone receptor of *Plutella xylostella*

Since the crystal structure of *Plutella xylostella's* ecdysone receptor is not available in the PDB database, homology modelling was performed. NCBI database was used to retrieve *P. xylostella's* ecdysone receptor sequence (accession no. NP_001296080.1). The protein has 545 amino acid residues. A fully automated protein structure homology-modelling server, SWISS-MODEL[12] was used to predict the protein structure. It is widely used for the prediction of the structure and/or function of protein sequences. The generated structures were validated using a series of protein validation tools such as, Verify 3D[13], ProQ[14], ProSA[15,16], PROCHECK[17]and QMEAN[18].

Ligand retrieval

Based on the information available in the literature[19-22], 20 triterpenoids from *A. indica* were selected for docking studies. The available 3D structures of these triterpenoids were downloaded in SDF file format from the PubChem repositories (https://pubchem. ncbi.nlm.nih.gov/). Only one compound for which 3D structure was unavailable in PubChem; its 2D structure was downloaded and converted to 3D using OpenBabel[23] software for docking.

Receptor and ligand preparation

Preparation of the retrieved 3D crystal structure of *H. armigera* ecdysone receptor (HaEcR) for docking was performed using UCSF Chimera 1.15[24]. The associated ligand, other hetero atoms, and water molecules were removed from the receptor, and the final target protein was prepared by adding hydrogen atoms and Gasteiger charges. This charged protein receptor was saved as a PDB file to perform docking work. The modelled ecdysone receptor of *P. xylostella* (PxEcR) was also prepared using UCSF Chimera 1.15. Hydrogen atoms and Gasteiger charges were added to prepare this receptor for the docking studies. The prepared receptor was saved as a PDB file, which was used for docking work. All ligands selected for the docking studies were similarly prepared using UCSF Chimera 1.15 by adding hydrogen atoms and Gasteiger charges and were saved as a PDB file for later docking work.

Docking of the ligand molecules to a receptor protein

Molecular docking was performed by using Chimera 1.15 integrated AutoDock Vina program. Grid box values on HaEcR were set to encompass the site where the ligand BYI08346 (a non-steroidal EcR activator) was previously bound to the protein molecule (x, y, z coordinates as 10, 60 & 10 respectively and grid size: 40 x 40 x 40). Grid box values for PxEcR were selected based on structural comparison with HaEcR (x, y, z coordinates as 30, 01 & -5 respectively and grid size: 40 x 40 x 40). After docking, a PDBQT file was generated, which was converted to a PDB file using OpenBabel software. The docked poses were visualised using LigPlot program version 2.2.4[25] and PyMOL[26]. To view interactions other than hydrogen bond and hydrophobic interactions, protein–ligand interaction profiler (PLIP[27]) server was used.

Validation of Docking protocol

The co-crystalised ligand BYI08346 was extracted from the crystal structure of the receptor and re-docked to the prepared HaEcR used in the present docking studies. The binding interaction of the re-docked ligand with the receptor was compared with the co-crystalised structure to validate the docking protocol.

Prediction of drug-likeness property/tice rule prediction

The insecticide potency of all compounds was predicted using Tice rule[28] by using the online cheminformatics software Molinspiration[29]. According to Tice rule, a potential insecticidal compound should have: (a) molecular weight ≤ 500 g/mol, (b) number of hydrogen-bond donors ≤ 3, (c) number of hydrogen-bond acceptors ≤ 12, (d) partition coefficient (log P) ≤ 5, and (e) no. of rotatable bonds ≤ 12.

Results

Modelling of PxEcR

The homology modelling using a fully automated SWISS-MODEL server constructed eight different models. Based on the GMQE (Global Model Quality Estimation) and QMEAN (Qualitative Model Energy ANalysis) score, model 8 was selected for further quality assessment (Table 1). GMQE scoring function is used to estimate the absolute quality of the constructed protein with the scoring range 0 to 1, where close to 1 is considered as more reliable model. The 'QMEAN Z-score' calculated estimates the degree of nativeness of the structural features present in the model. It also gives an insight into its quality compared to experimental protein models available in the server. Model 8 also showed a maximum sequence identity of 87.25% with Chain A of *Heliothis virescens* EcR (PDB code: 2r40) and the least RMSD (Root Mean Square Deviation) with the template (0.048) (Table 1).

The quality of model 8 was further analysed to ensure the reliability and suitability of the model for performing molecular docking studies. Based on the PROCHECK analysis, it was observed that there is 11 alpha helix in the protein, a single beta-sheet and

Table 1 — Template description of the eight protein model constructed by SWISS-MODEL and its validation score obtained from various tools.

Model	PDB Id of the template	Seq. identity	GMQE	QMean	RMSD with the template	QMEAN DisCo	ProQ Predicted LG score (MaxSub)	ProSA	Verify 3D
Model 1	4nqa	40.42	0.52	-6.83	0.043	0.57	10.493 (-0.559)	-7.22	Fail
Model 2	4nqa	40.42	0.5	-7.38	0.038	0.57	10.896 (-0.621)	-7.33	Fail
Model 3	3e00	26.80	0.42	-10.23	2.394	0.51	9.109 (-0.576)	-5.21	Fail
Model 4	1z5x	59.18	0.33	-3.20	0.064	0.77	8.359 (-0.375)	-7.69	Pass
Model 5	1r1k	86.56	0.36	-2.98	0.066	0.79	8.662 (-0.307)	-8.07	Fail
Model 6	2r40	87.25	0.36	-0.96	0.048	0.82	8.492 (-0.355)	-8.09	Pass
Model 7	1r1k	86.56	0.41	-2.98	0.066	0.79	8.662 (-0.307)	-8.07	Fail
Model 8	2r40	87.25	0.41	-0.96	0.048	0.82	8.492 (-0.355)	-8.09	Pass

13 beta turns. This 12 helical structure is a unique characteristic feature of EcR reported from many other insect groups (Fig. 1a). The amino acid sequence between H3 and H4 ranging from 352 to 371, which is conserved in the superfamily of nuclear receptors is also observed in our protein. Ramachandran plot analysis obtained from PROCHECK shows that (Fig. 1b), the model selected for the docking study has 91.9% of amino acid residues in the most favourable regions, making it a good quality model. The other observations include amino acids in additionally allowed regions (7.2%), generously allowed regions (0.5%) and disallowed regions (0.5%). The amino acid in the disallowed region was found to be Ser331, which is distantly located from the binding site.

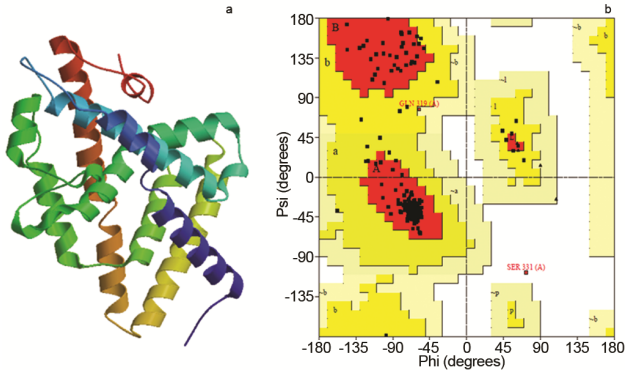

Fig. 1 — a) Homology modelled structure of the ecdysone receptor of *P. xylostella* (model 8) using Swiss Model Software; and b) Ramachandran Plot for the modeled structure of *P. xylostella* ecdysone receptor (model 8).

The Z score of model 8 based on ProSA analysis was found to be -8.09, which indicates a highly reliable structure (Table 1, Fig. 2a). This analysis helps us to evaluate the quality of the protein considering all the proteins available in PDB whose structures are experimentally derived. The Z score calculated in this analysis denotes the deviation of the total energy of the structures with respect to an energy distribution from random protein conformations derived from two different sources, i.e. X-ray and NMR. The QMEANDisCo score of model 8 was found to be 0.82, with a Z score of <1. The constructed model falls within the dark zone, which denotes the reliability of this model (Fig. 2b). The LG score of this model based on ProQ analysis was found to be 8.492 and MaxSub of -0.355. The LG score of > 4 indicates that model 8 is extremely good (Table 1).

Validation of docking protocol

The extracted co-crystalised ligand (BYI08346) was re-docked with prepared HaEcR to validate the docking procedure used in the present study. The extracted ligand bound to the same site as the native co-crystalised ligand with a very low binding energy of -12.1 kcal/mol. An RMSD of 0.001 Å was obtained on superimposing the re-docked complex with the

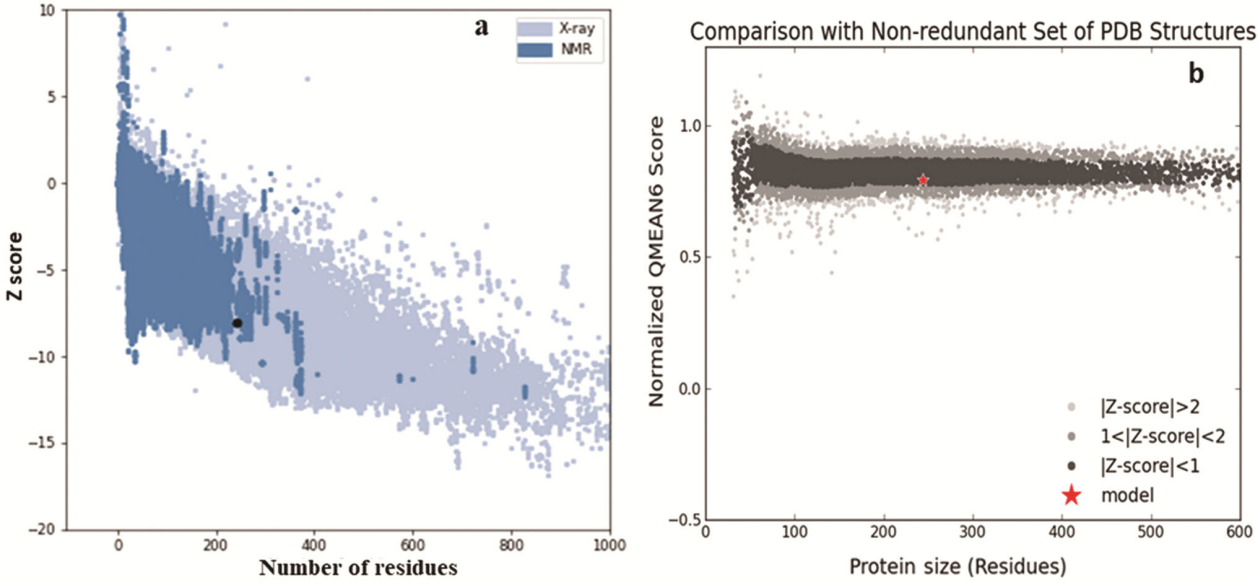

Fig. 2 — a) ProSA analysis of model 8. ProSA-web Zscore of all protein chains in PDB determined by X- ray crystallography (light blue) or NMR-spectroscopy (dark blue) with respect to their length. The z-score (-8.09) of model 8 can be seen as a large black dot; and b) Quality assessment of model 8 by QMEAN Z-score. Good models are generally located in the dark zone. (red mark shows the positioning of model 8).

native co-crystallised protein structure obtained from PDB (Fig. 3a). Superimposing re-docked ligand-HaEcR complex onto the native co-crystallised ligand-protein complex using LigPlot shows 16 out of 17 interacting amino acids of EcR to be common for both the ligands (Fig. 3b). These results suggest the reliability of the docking protocol employed in the present docking studies.

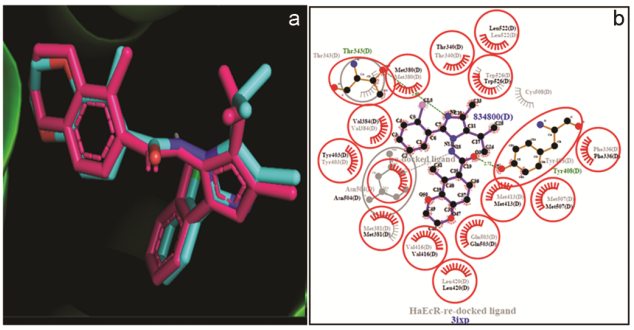

Fig. 3 — Validation of docking protocol, a) Re-docked BYI08346 (blue) in complex with HaEcR superimposed onto native co-crystallised ligand-protein complex (pink) using PyMOL (RMSD 0.001 Å); and b) Superimposed re-docked ligand-HaEcR complex onto native co-crystallised ligand-protein complex using LigPlot. (superimposed amino acids encircled in red).

Docking of Triterpenoids with HaEcR and PxEcR

The binding potential of selected triterpenoids to the ecdysone receptors of *H. armigera* (HaEcR) and *P. xylostella* (PxEcR) was evaluated based on their binding energy score calculated using the AutoDock Vina program. Docking with AutoDock Vina generates a number of poses for each receptor-ligand complex. The best pose with the highest docking score (lowest binding energy) and lowest RMSD values (usually 0) was considered for each ligand.

The binding energy scores of various ligands with HaEcR ranged from -8.7 kcal/mol to -7.3 kcal/mol. Six triterpenoids, viz. isomeldenin, 6-deactylnimbinene, nimbinene, azdiradione, nimocinol, and 6-deacetyl nimbin, scored binding energies lower than the reference ligand, tebufenozide (-8.2 kcal/mol), while one compound, viz. isosalanninolide recorded binding energy comparable to tebufenozide. Isomeldenin and 6-deactylnimbinene recorded the lowest binding energy of -8.7 kcal/mol, followed by nimbinene and azdiradione with -8.6 kcal/mol. Nimocinol and 6-deacetylnimbin binding energy scores were -8.4 kcal/mol and -8.3 kcal/mol, respectively (Table 2). All these lead compounds, except azadiradione, also established hydrogen bonds with the receptor protein. The amino

Table 2 — List of ligands with their binding energy, inhibitory constant, number of hydrogen bonds and amino acids involved in hydrogen bonds when docked with HaEcR

S. No.	Pub chem Id	Name of the Ligand	Binding Energy (kcal/mol)	Inhibition constant (Ki) (μM)	No. of Hydrogen bond	Amino acid residue forming H bond
1	CID_91773	Tebufenozide	-8.2	0.96	0	-
2	CID_76316558	Isomeldenin	-8.7	0.41	2	Ser 505, Ser 426
3	CID_102285347	6-deacetylnimbinene	-8.7	0.41	3	Pro 450, Asn 504, Arg347
4	CID_12308714	Azadiradione	-8.6	0.49	0	-
5	CID_44715635	Nimbinene	-8.6	0.49	1	Asn 504
6	CID_178770	Nimocinol	-8.4	0.68	2	Ser 505, Ser 426
7	CID_10505484	6-deacetyl nimbin	-8.3	0.81	3	Arg 498, Arg 449, Arg 347
8	CID_76316561	Isosalanninolide	-8.2	0.96	5	Asn 504, Ser 376, Ser 377, Ala374, Lys373
9	CID_76327467	Epoxyazadiradione	-8.0	1.35	3	Ser 377, Glu 378, Ala 374
10	CID_12004512	Gedunin	-8.0	1.35	1	Ser 426
11	CID_13875741	Nimbocinol	-8.1	1.14	0	-
12	CID_101919043	3-Tigloylazadirachtol	-8.1	1.14	6	Ala 390, Asp 389, Arg 387, Arg 386 (2), Asn 434
13	CID_14458886	3-deacetyl salanin	-8	1.35	2	Arg 347, Ser 377
14	CID_14194023	Nimbanal	-7.8	1.89	1	Arg 347
15	CID_6437066	Salannin	-7.8	1.89	1	Ser 377
16	CID_10906239	Azadirone	-7.7	2.24	1	Ser 426
17	CID_101257	Tirucallol	-7.7	2.24	0	-
18	CID_14194026	Salannol acetate	-7.5	3.13	1	Ser 377
19	CID_108058	Nimbin	-7.5	3.13	0	-
20	CID_101355584	Meliacinanhydride	-7.4	3.71	5	Ser 426, Arg 347 (3), Lys 430
21	CID_101355583	Zafaral	-7.3	4.39	1	Ser 426

acid residues of the protein receptor, viz. Asn 504, Ser 376, Ser 377, Ala 374, and Lys 373 were involved in hydrogen bond formation with isosalanninolide. Isomeldenin and nimocinol formed two hydrogen bonds involving Ser 505 and Ser 426 residues (Fig. 4, Table 2). 6-deactylnimbinene and 6-deacetylnimbin established three hydrogen bonds, i.e. Pro 450, Asn 504, Arg 347 and Arg 498, Arg 449, Arg 347, respectively, with Arg 347, being common. Nimbinene formed just one hydrogen bond involving the residue Asn 504 (Fig. 4, Table 2). Several hydrophobic interactions between ligands and amino acid residues of HaEcR were also observed, further contributing to the stability of the docked ligand-receptor complex. Trp 526 of HaEcR was one common residue that made hydrophobic interaction with the reference ligand, tebufenozide, as well as with five of the top scoring ligands viz., 6-deacetylnimbinene, azadiradione, nimbinene, 6-deacetylnimbin, and isosalanninolide (Fig. 4). This aromatic amino acid also contributed to π-π stacking interaction with 6-deacetylnimbinene and nimbinene as viewed using the PLIP server. Another residue, Asn 504 contributed to hydrophobic interaction with tebufenozide while it was involved in forming hydrogen bond with 6-deacetylnimbinene, nimbinene, and isosalanninolide.

Like HaEcR, the ecdysone receptor complex of *P. xylostella* (PxEcR) also recorded low binding energy scores with most of the neem triterpenoids, indicating a high affinity of the docked ligands for the protein complex. Nine out of twenty compounds recorded binding energies lower than the reference ligand, tebufenozide (-6.9 kcal/mol), when docked with the ecdysone receptor of *P. xylostella* (Table 3). The lead compound, tirucallol recorded binding energy of -11.4 kcal/mol and formed two hydrogen bonds with the amino acid residues of the protein receptor, viz., Glu 370 and Arg 382. 3-Tigloylazadirachtol (binding energy, -7.6 kcal/mol) established three hydrogen bonds with PxEcR amino acid residues, viz., Ser 504, Asp 526 and Arg 497 while nimbocinol with binding energy of -7.2 kcal/mol established two hydrogen bonds, viz., Glu 311 and Gln 308. Epoxyazadiradione and isomeldenin, both with binding energies of -7.4 kcal/mol and 6-deacetylnimbinene, with -7.2 kcal/mol, made one hydrogen bond each with the receptor protein

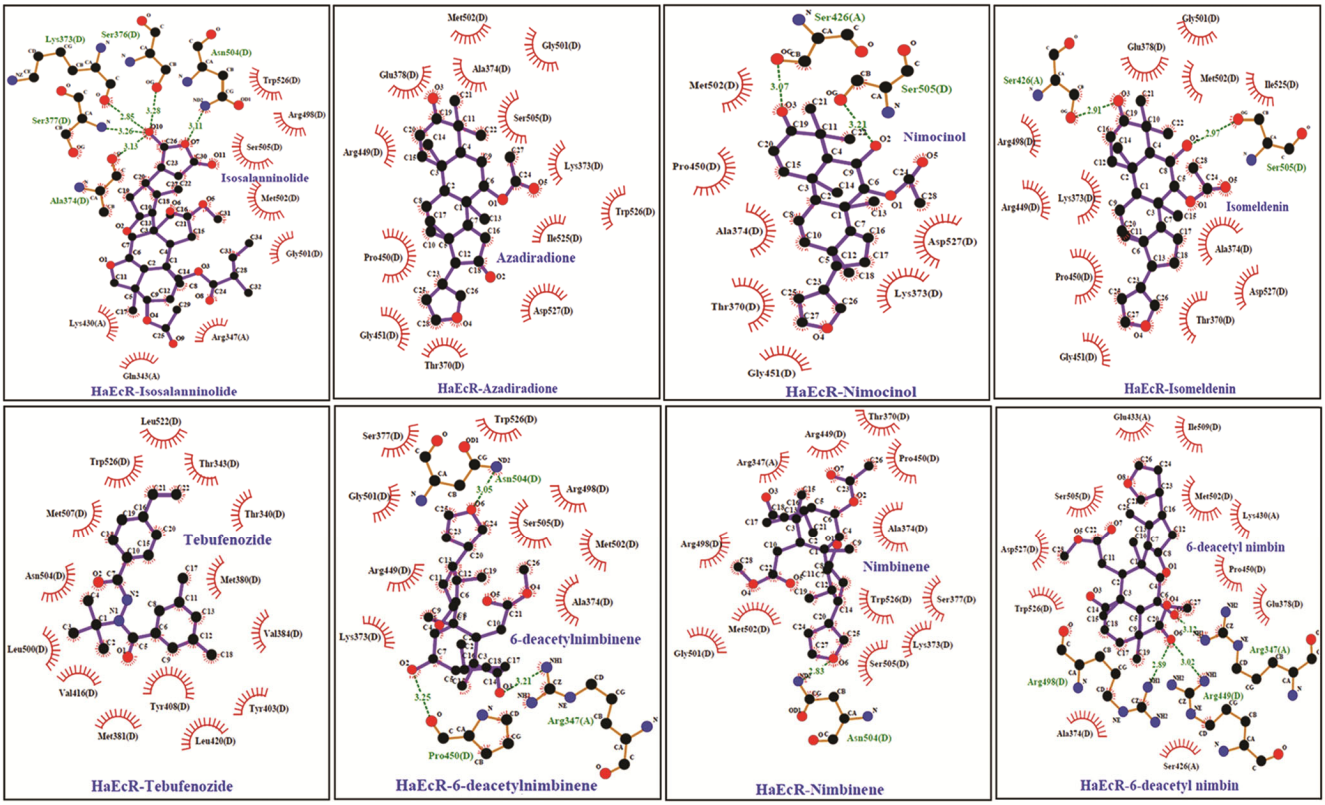

Fig. 4 — Two-dimensional plot of docked poses showing interactions of neem triterpenoids with the ecdysone receptor of *Helicoverpa armigera* (HaEcR). Dotted green lines denote hydrogen bond, and hydrophobic interactions between protein and ligand are shown by red spoked arc.

Table 3 — List of ligands with their binding energy, inhibitory constant, number of hydrogen bonds and amino acids involved in hydrogen bonds when docked with PxEcR

S. No.	Pub chem. Id	Name of the Ligand	Binding Energy (kcal/mol)	Inhibition constant (Ki) (μM)	No. of Hydrogen bond	Amino acid residue forming H bond
1	CID_91773	Tebufenozide	-6.9	8.59	0	-
2	CID_101257	Tirucallol	-11.4	4.2 nM	2	Glu 307, Arg 382
3	CID_101919043	3-Tigloylazadirachtol	-7.6	2.63	3	Ser 504, Asp 526, Arg497
4	CID_10906239	Azadirone	-7.5	3.12	0	-
5	CID_12308714	Azadiradione	-7.4	3.69	0	-
6	CID_76327467	Epoxyazadiradione	-7.4	3.69	1	Thr 369
7	CID_76316558	Isomeldenin	-7.4	3.69	1	Ser 504
8	CID_13875741	Nimbocinol	-7.2	5.17	2	Glu 311, Gln 308
9	CID_102285347	6-deacetylnimbinene	-7.2	5.17	1	Glu 523
10	CID_178770	Nimocinol	-7.0	7.26	0	-
11	CID_12004512	Gedunin	-6.8	10.17	0	-
12	CID_44715635	Nimbinene	-6.8	10.17	0	-
13	CID_101355584	Meliacinanhydride	-6.8	10.17	1	Glu 329
14	CID_101355583	Zafaral	-6.8	10.17	1	Arg 448
15	CID_14458886	3-deacetylsalanin	-6.7	12.05	0	-
16	CID_76316561	Isosalanninolide	-6.7	12.05	1	Gln 347
17	CID_10505484	6-deacetylnimbin	-6.5	16.9	0	-
18	CID_6437066	Salannin	-6.5	16.9	0	-
19	CID_108058	Nimbin	-6.4	20.01	0	-
20	CID_14194023	Nimbanal	-6.3	23.69	0	-
21	CID_14194026	Salannol acetate	-6.2	28.06	0	-

(Fig. 5, Table 3). Azadirone (-7.5 kcal/mol), azadiradione (-7.4 kcal/mol), and nimocinol (-7 kcal/mol) recorded lower binding energy than the reference ligand, tebufenozide but established no hydrogen bonds with PxEcR (Table 3). A number of amino acid residues, like, Gln 347, Gln 367, Ile 368, Ile 524, Leu 371, Lys 372, Phe 359, Phe 520, and Val 350 were found to contribute to hydrophobic interactions with tebufenozide (Fig. 5). Many of these residues of PxEcR were also found to make the same interactions with various lead triterpenoids in our docking studies. Lys 372 was involved in hydrophobic interaction with all lead triterpenoids except nimbocinol and tirucallol (Fig. 5). Of all the top triterpenoids, nimbinene made a single π-π stacking interaction with the Phe 520 residue of PxEcR.

The inhibition constant (K*i*) for each ligand was calculated from the binding energy (ΔG) using the formula: K*i* = exp(ΔG/RT), where R is the universal gas constant (1.985×10^{-3} kcal mol^{-1} K^{-1}), and T is the temperature (298.15 K)[30]. All lead triterpenoids bound to HaEcR recorded K*i* less than 1 μM (Table 2). K*i* value for tirucallol bound to PxEcR was in the nanomolar range (4.2 nM), while for other lead compounds, it was less than 8 μM (Table 3).

Prediction of insecticide potency (Tice rule)

The insecticide-likeness of the potential compounds was predicted using Tice rule. Three lead compounds, viz. tirucallol, 3-tigloylazadirachtol, and azadirone, despite docking strongly with the ecdysone receptor of *P. xylostella* could not pass all criteria put down by the Tice rule. 3-tigloylazadirachtol violated the property of molecular weight, while both tirucallol and azadirone recorded LogP values higher than the desirable limits. Other lead compounds such as azdiradione, epoxyazadiradione, nimbocinol, 6-deacetylnimbinene, isomeldenin, nimocinol, nimbinene, and 6-deacetyl nimbin recorded no Tice rule violation (Table 4).

Discussion

Bioinformatic techniques allow us to generate and evaluate various molecular conformations between ligands and proteins from their available crystal structures. This technique could be successfully employed to screen a huge number of natural or synthetic compounds for their ability to interact with target receptors or proteins and to propose a probable activity. A number of such *in silico* docking of phytochemicals with some important insect proteins

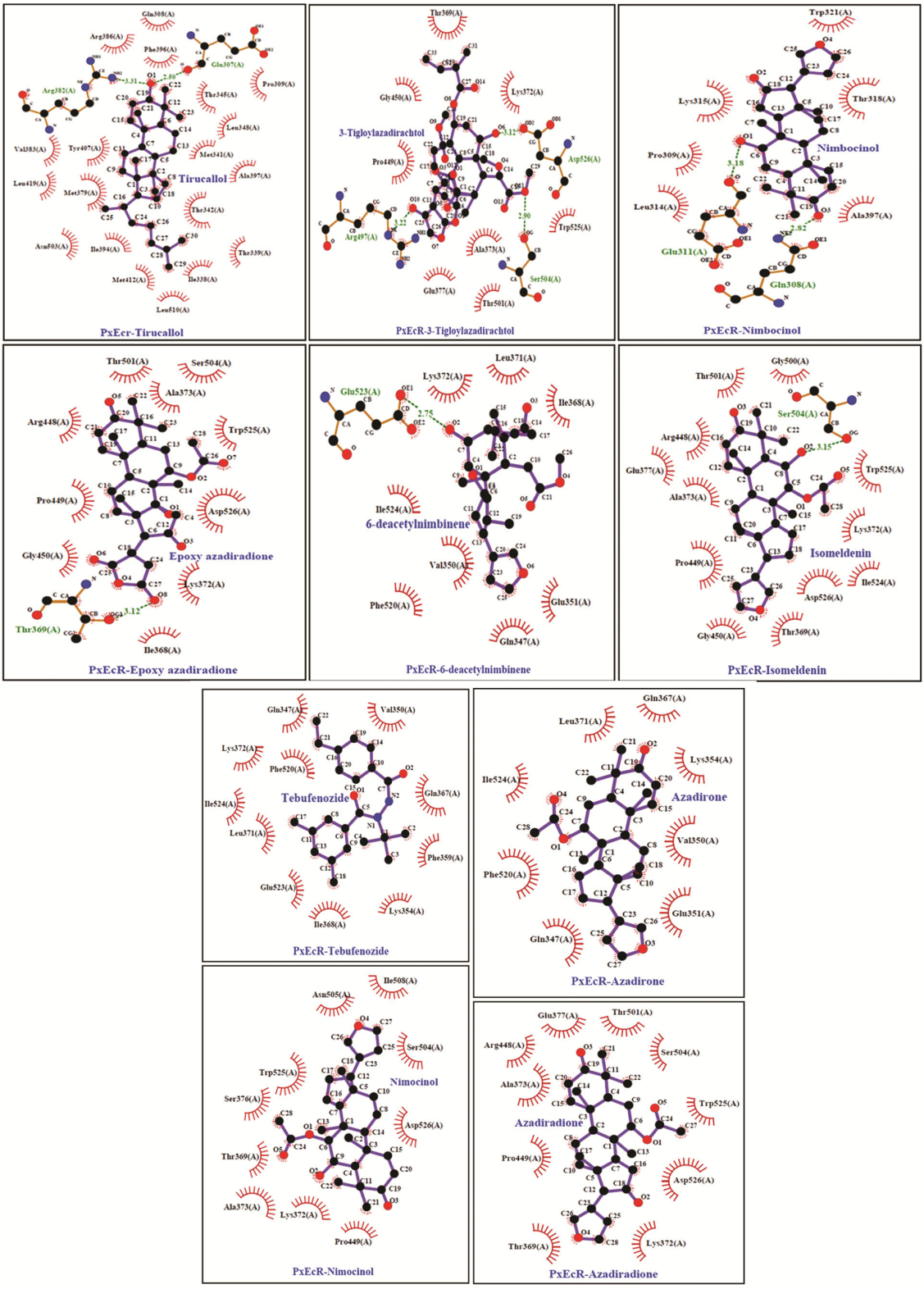

Fig. 5 — Two-dimensional plot of docked poses showing interactions of neem triterpenoids with the ecdysone receptor of *Plutella xylostella* (PxEcR). Dotted green lines denote hydrogen bond, and hydrophobic interactions between protein and ligand are shown by red spoked arc.

Table 4 — Tice rule properties of the selected compounds

S. No.	Compounds	Molecular weight (kDa/g/mol)	Hydrogen Bond Donar (OH + NH)	Hydrogen Bond Acceptor (O + N)	No. of Rotatable Bonds	Log P	No. of Violations
1	Tebufenozide	352.48	1	4	5	4.08	0
2	Nimbocinol	408.54	1	4	1	3.52	0
3	Isomeldenin	454.61	1	5	3	4.44	0
4	Zafaral	484.63	0	6	6	4.83	0
5	Gedunin	482.57	0	7	3	4.34	0
6	6-deacetyl nimbin	498.57	1	8	6	2.85	0
7	Nimbinene	482.57	0	7	6	3.89	0
8	Azadiradione	450.57	0	5	3	4.23	0
9	6-deacetylnimbinene	440.541	1	6	4	3.19	0
10	Epoxyazadiradione	498.57	1	8	3	3.01	0
11	Nimocinol	452.59	1	5	3	4.41	0
12	Salannol acetate	598.73	0	9	10	5.30	2
13	Nimbanal	510.8	0	8	7	3.51	1
14	3-deacetyl salannin	554.68	1	8	7	4.69	1
15	Nimbin	540.61	0	9	8	3.55	1
16	Meliacinanhydride	570.63	1	10	6	2.77	1
17	Isosalanninolide	628.72	1	11	9	4.75	2
18	3- Tigloylazadirachtol	662.68	3	14	8	1.9	2
19	Tirucallol	426.73	1	1	4	8.48	1
20	Azadirone	436.59	0	4	3	5.33	1
21	Salannin	596.72	0	9	9	5.40	2

have been performed before[31,32,33]. Tebufenozide and other diacylhydrazine (DAH) are ecdysone agonist compounds that mimic the action of the ecdysone hormone. Binding these compounds to insect ecdysone receptor leads to the cessation of feeding and force insects to moult prematurely, ultimately causing insect death. Ecdysone agonists are also reported to result in increased egg mortality[34] and reduced rate of reproduction[35]. Significantly high docking scores and low inhibitory constant (K*i*) of lead triterpenoids with HaEcR and PxEcR in the present study suggests that these compounds may activate the ecdysone receptor complex to bring about similar effects. Low binding energy has been reported for a few non-azadirachtin limonoids compared to the reference ligand, tebufenozide, when docked with the ecdysone receptor of *H. armigera*[36]. The potential insecticidal activity of limonoids present in calamondin was evaluated by calculating their binding affinities to EcR of *Heliothis virescens* using molecular docking studies[37]. Phenylethanoid glycoside derivatives from the plant genus, *Calceolaria*, when docked with *Heliothis virescens* ecdysone receptor, also recorded low binding energy[31]. Another *in silico* study reported a high binding affinity of triterpenoids from the plant *Centella asiatica* against RdRp enzyme. The arrest of the RdRp enzyme may stop the SARS-CoV-2 virus from replicating[38].

In addition to acting as ecdysone agonists, the binding of these compounds to the ecdysone receptor may block the binding site of the receptor or bring about changes in the overall structure of the receptor. This might inhibit or hinder the binding of ecdysone hormone to its receptor, interfering with the insect's ecdysis. Various lab studies have confirmed moult-related deformities due to neem constituents on many economically important pests viz., *Spodoptera frugiperda*, *Pectinophora gossypiella, Heliothis virescens* and *H. zea*[39], and *Spodoptera littoralis*[40]. Significant high mortality in *P. xylostella* larvae during moulting due to neem extract has also been reported[41]. Two triterpenoids viz., cucurbitacins B and D, isolated from seeds of *Iberis umbellata* (Cruciferae), produced an antagonist effect at the ecdysteroid receptor of *Drosophila melanogaster*[42]. In the same study, another triterpenoid (Hexanorcucurbitacin D*)* from the crucifer plant acted as a weak agonist rather than an antagonist. In a protein-inhibitor complex, K*i* denotes the dissociation constant (Kd) of the complex. The value of K*i* thus represents the concentration of inhibitor required to produce half maximum inhibition. The calculated K*i* for all leading triterpenoids in the present study was in the µM or nM range, which shows that these compounds can bring about the desired effect at very low concentrations.

Various types of interactions, viz., hydrogen bond[43], hydrophobic interactions[44], π-π stacking[45] etc., have been reported to contribute to the stability of the ligand-protein complexes. The amino acid residue Asn 504 of HaEcR was involved in hydrogen bond formation with three lead triterpenoids viz., 6-deacetylnimbinene, nimbinene, and isosalanninolide. The serine residue present at 504 position of PxEcR was involved in hydrogen bond formation with two lead triterpenoids viz., 3-tigloylazadirachtol and isomeldenin, suggesting it as a major point of interaction between triterpenoids and receptor protein. Amino acid residue Trp 526 in HaEcR and Lys 372 and Phe 520 in PxEcR was found to be another important residue contributing to hydrophobic interactions and π-π stacking interactions, thus increasing the stability of the docked complexes.

Tirucallol, 3-tigloylazadirachtol and azadirone despite being the top scorers with PxEcR violated a few Tice rule properties. The compound's LogP value indicates the drug's permeability to reach the target tissue in the body. Failing this important criterion makes these compounds unfit as a potential pesticide candidate. Four triterpenoids viz., azadiradione, isomeldenin, 6-deacetylnimbinene, and nimocinol docked well with ecdysone receptors of both *H. armigera* and *P. xylostella.* In addition to that, nimbinene, and 6-deacetyl nimbin with HaEcR and epoxyazadiradione and nimbocinol with PxEcR also showed promising result. However, *in vitro/in vivo* analysis is required to confirm and establish the potentiality of these compounds.

Conclusion

Ecdysone agonists and antagonists are being successfully used in integrated pest management (IPM) programmes as a sustainable approach to pest management. The low binding energy scores of the lead triterpenoids with the ecdysone receptors of *Helicoverpa armigera* and *Plutella xylostella* indicate that these compounds can be a potent candidate for insecticide formulation to be used as ecdysone agonists or antagonists for the control of these two economically important pests. The natural plant based origin of these compounds further make them a better alternative than commercially available insecticides like tebufenozide. Such virtual screening and identification of promising compounds from the plethora of plant-based compounds would be the first step for further *in vitro/in vivo* pharmacological analysis.

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Conflict of interest

All authors declare that they have no conflicts of interest.

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