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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

In silico docking studies of non-azadirachtin limonoids against ecdysone receptor of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae)

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Abstract Although specific binding of 20-hydroxyecdysone (20E) and its analogs (ecdysteroids) to the ecdysone receptor ligand-binding domain (EcR-LBD) in insects has been well documented, information on the EcR-ligand binding in *Helicoverpa armigera* is limited. Hence, an attempt has been made to screen effective natural plant-based agonists from a library of 25 non-azadirachtin neem limonoids and was compared with the commercially available insecticide, tebufenozide, through in silico approach. Results indicated that six compounds, namely nimbolide, azadirone, nimolinone, meliacinol, nimbocinol, azadiradione, efficiently docked with the active site of *H. armigera* EcR-LBD. The binding energies of top-ranked six molecules ranged from −10.54 to −12.22 kcal/mol, which was superior to the third-generation insect growth regulator (IGR), tebufenozide RH5992. Two factors are especially important in binding: (1) the residues Cys 508 and Asn 504, which are the most common in hydrogen-bonding interactions and (2) hydrophobic pocket residues—Asn 504, Met 507, Val 416, Tyr 408 and Thr 343. We also recognized one aromatic ring, 3–7 vicinal acceptors and 1–3 distal hydrophobic groups as minimum pharmacophoric feature. A significant correlation coefficient of 0.6823 was observed supporting positively the docking studies. These data could help in the application of natural compounds as alternatives to chemicals in pest management.

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Introduction

Helicoverpa armigera is one of the world's most devastating pest species feeding on more than 100 major agricultural crops (Sharma, 2001) and is found in Asia, Europe, Africa and Australia causing an estimated damage between US $ 2 and 5 billion annually (Lammers and MacLeod, 2007; Tay *et al.,* 2013). *H. armigera* has developed resistance to synthetic insecticides (Gunning and Easton, 1994; Srinivas *et al.,* 2004) due to its biological characteristics such as polyphagy, high mobility and fecundity, facultative diapause and high population build-up (Fitt, 1989; Yang *et al.,* 2013).

The ecdysone receptor complex is the key element, which enacts the ecdysteroid-induced physiological and morphological changes during insect moulting regulated by ecdysteroid hormones like 20-hydroxyecdysone (20E) and its analogs that bind to the ligand-binding domain of the ecdysone receptor (Jayachandran *et al.,* 2013). The ecdysone receptor (EcR) belongs to the nuclear hormone receptor superfamily that functions as a ligand-activated transcription factor. The basic structure of EcR consists of five modular domains referred to as A/B (transcriptional activation domain), C (DNA-binding domain; DBD), D (hinge region), E (ligand-binding domain; LBD) and F (not well-defined region) (Thummel, 1995). It is a heterodimer of two proteins, EcR-LBD (which contains the active site for ecdysteroids) and the ultraspiracle protein (USP), which is a homologue of the retinoid X receptor (RXR; Oro *et al.,* 1990).

The moulting process is initiated by a number of transcription factors in the nuclear receptor superfamily. This

results in the up-regulation of several late genes in the hormone pathway and help in mediating the moulting process (Zheng *et al.,* 2010). Since these receptors are limited to invertebrates, they have been exploited as an attractive target for insecticide development (Graham, 2002; Palli *et al.,* 2005). The ecdysteroid agonists, such as tebufenozide (RH-5992), mimic the natural function of the endogenous insect moulting hormone 20-hydroxyecdysone (20E), inducing premature lethal moulting in larval stages and aborting reproduction in adults, especially in Lepidoptera and Coleoptera (Nagata *et al.,* 2005).

Azadirachta indica A. Juss (Meliaceae), commonly known as "neem", yields more than 300 bioactive chemical compounds such as terpenoids, limonoids, flavonoids, amino acids and carbohydrates (Alland *et al.,* 2005). Limonoids from the neem tree have attracted considerable research attention in recent years owing to their wide range of bioactivities such as insect anti-feedant, anti-microbial, anti-cancer, anti-malarial, anti-inflammatory, antioxidant, anti-proliferative effects, cytotoxic and growth regulatory properties (Murugan *et al.,* 1998; Nanduri *et al.,* 2003; Setzer and Setzer, 2003; Kumar *et al.,* 2008). Apart from azadirachtin, several non-azadirachtin limonoids (NAL) inhibit feeding in some specific insect pests, yet have not been given enough importance (Koul *et al.,* 1996). These studies have provided impetus for screening other neem compounds in detail to identify potential phytochemicals that could be used in commercial formulations (Koul *et al.,* 2003). Bioinsecticides being less hazardous to the environment and human health can serve as the best alternative for pest management (Murray and Isman, 2006).

The present study is focused on the screening of potent insect growth regulators by choosing twenty-five non-azadirachtin limonoids to test its insecticidal activity through molecular docking studies in *H. armigera* ecdysone receptor (HaEcR).

Materials and methods

Retrieval of HaEcR protein structure and preparation for docking

The 3D coordinates of the crystal structure of EcR bound to bisacylhydrazine compound BYI-06830 (PDB code: 3IXP) at a resolution of 2.85 Å were retrieved from protein data bank (PDB; Berman *et al.,* 2007). Hetero atoms, ligands and water molecules were removed from the protein structure of EcR. Preparation of the target protein with AutoDock tools (ADT) involved the addition of polar hydrogens to the macromolecule, an essential step to correct the calculation of partial charge. Finally, Gasteiger charges were calculated for each atom of the macromolecule (Gasteiger *et al.,* 1990). The charged protein was converted to the "PDBQT" format and read through AutoGrid.

Ligand retrieval and preparation for docking

Twenty-five limonoid compounds from *A. indica* possessing insecticidal activity were selected for the study on the basis of literature survey (Table 1; Fig. 1). 2D structures of these compounds were downloaded in SDF format from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/; Bolton *et al.,* 2008). These 2D structures were converted to 3D structures with the help of Open Babel, using PyRx software (http://pyrx.sourceforge.net/) and were energy minimized by mmff94 force field with an energy gradient of 0.05 (Halgren, 1996; Wolf, 2009).

Setting grid map parameters

Grid map was created with a 3D lattice of regularly spaced points, surrounding (entirely or partially) and centred on the active site on chain D of HaEcR-LBD (3IXP). AutoGrid programme was used to generate the grid maps. The grid dimensions were 46 Å × 48 Å × 64 Å with points separated by 0.375 Å with grid centre 5.838 × 65.407 × 12.076 to encompass entire active site.

Running AutoDock

Molecular docking was performed in AutoDock 4.2 (Morris *et al.,* 1998; Huey *et al.,* 2007) using a Genetic Algorithm–the Least Square (GA–LS), which performs a semi-flexible docking keeping the protein itself as rigid while the ligand as flexible. The parameters were set to 10 runs, population size to 150, maximum number of energy evaluations to 25 million and 27,000 generations, mutation rate of 0.02, crossover rate of 0.80 and the rest of the parameters were set to default values. The docking results were saved as ".dlg" file, and all the docked conformation outputs were viewed using inbuilt visualization features of AutoDock. For each ligand, the best docked conformation with the least energy and high stability was selected and the complex file was exported. The analysis and visualization of the binding interaction of protein and ligands were performed using Discovery Studio Visualizer V4.0 (Accelrys Software Inc.). The docking results were clustered on the basis of root mean square deviation (RMSD) and ranked on the basis of free energy of binding.

Validation of docking

Redocking of co-crystallized ligand was performed, and the bound ligand, BYI-06830 (bisacylhydrazine compound), found in the crystal structure was extracted and

Table 1 Structure of ligands and AutoDock binding energy, no. of hydrogen bonds of top scorer non-azadirachtin limonoid compounds

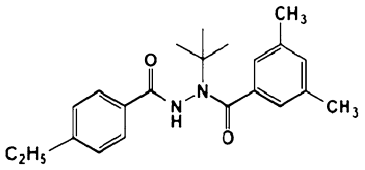
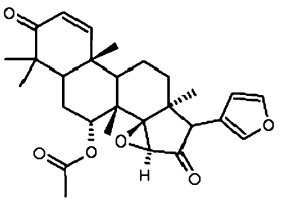
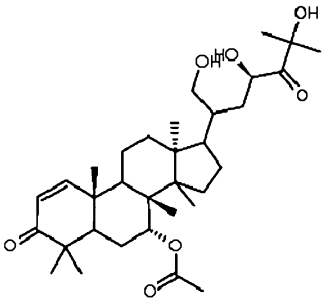
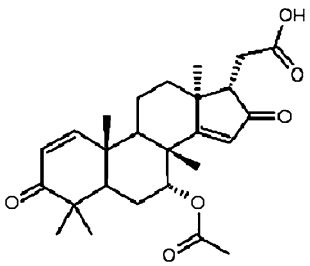
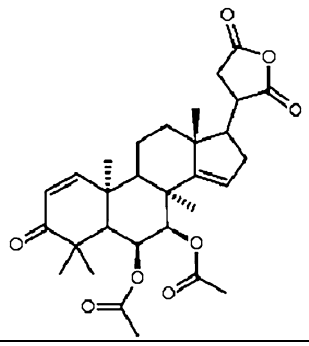
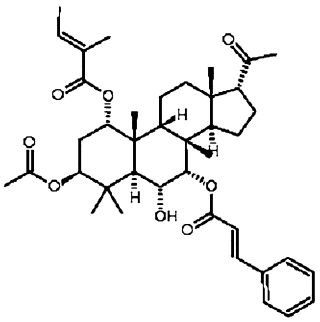
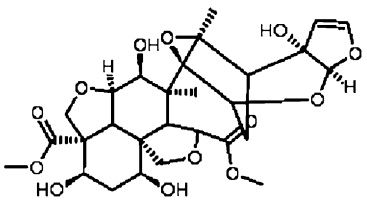
PubChem ID	Name of compound	Chemical structure	Binding energy (kcal/mol)	No. of hydrogen bonds
CID 91773	Tebufenozide		−10.46	2 (Asn 504, Tyr 408)
CID 100017	Nimbolide		−12.22	–
CID 10906239	Azadirone		−11.31	1 (Cys 508)
CID 56841069	Nimolinone		−11.29	1 (Cys 508)
CID 71717035	Meliacinol		−11.08	1 (Asn 504)

Table 1 continued

PubChem ID	Name of compound	Chemical structure	Binding energy (kcal/mol)	No. of hydrogen bonds
CID 13875741	Nimbocinol		−10.73	1(Asn 504)
CID 12308714	Azadiradione		−10.54	1(Cys 508)

docked into the corresponding binding pocket. The ability of the ligand to reproduce the orientation and the position of the ligand in the bound form were determined.

Prediction of insecticide potency (Tice rule)

Bioavailability and bioactivity of compounds are considered as important parameters for development of potential insecticides. The insecticide-likeness of the potential compounds was predicted using Tice rule. According to Tice rule, insecticidal compounds 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 (Tice, 2001). The molecular properties of investigated compounds were computed by using online Molinspiration cheminformatics software (www.molinspiration.com).

Correlation between experimental IC_{50} and docking results

The experimental inhibitory concentration (IC_{50} in μg/ml) values of gedunin (50.8), salanin (74.5), nimbolide (1,000), nimbocinol (250.8), azadiradione (249.3) and tebufenozide (20) were collected from the available literature (Rochanakij *et al.,* 1985; Carlson, 2000; Koul *et al.,* 2003, 2004) and were correlated with the docking score. The data were prepared as mean ± standard error mean (SEM), and linear regression analysis was performed considering experimental IC_{50} (μg/ml) values and binding energy (kcal/mol) as independent and dependent variables, respectively, by using SPSS ver.20 software. $P < 0.0001$ was considered to be significant.

Results and discussion

HaEcR-ligand-binding domain

From the several crystal structures determined, it was observed that the DNA-binding domain (DBD) and LBD are highly conserved in insect EcRs (Iwema *et al.,* 2009). DBD usually involves in hormone response element (HRE) recognition (Thomson *et al.,* 2009) and the LBD in receptor dimerization, ligand recognition and cofactor interactions (Nakagawa and Henrich, 2009). Apart from these, the LBD also contains the ligand-binding pocket (LBP), which binds ecdysteroids as well as certain non-steroidal EcR agonists such as the DAH-based insecticides (Tohidi-Esfahani *et al.,* 2011). It is now known that EcR and USP form a heterodimer, which functions as the natural ecdysone receptor. Although USP is an essential partner for the high affinity binding of ecdysteroids, the ligand-binding pocket resides in the EcR protein and the presence of ligand class enhances heterodimer stability for transcription (Graham *et al.,* 2007a, 2007b). HaEcR has very high identity to other lepidopteran EcRs (*Spodoptera exigua* 90 %, *Plodia interpunctella* 83 % and *Plutella xylostella* 80 %), but less with *Aedes albopictus* and *Drosophila pseudoobscura* (72 % identity; Jayachandran *et al.,* 2013). The bisacylhydrazine insecticidal compound, tebufenozide (RH5992), binds in the Lepidoptera EcR cavity that overlaps the pocket occupied by bound ecdysteroids

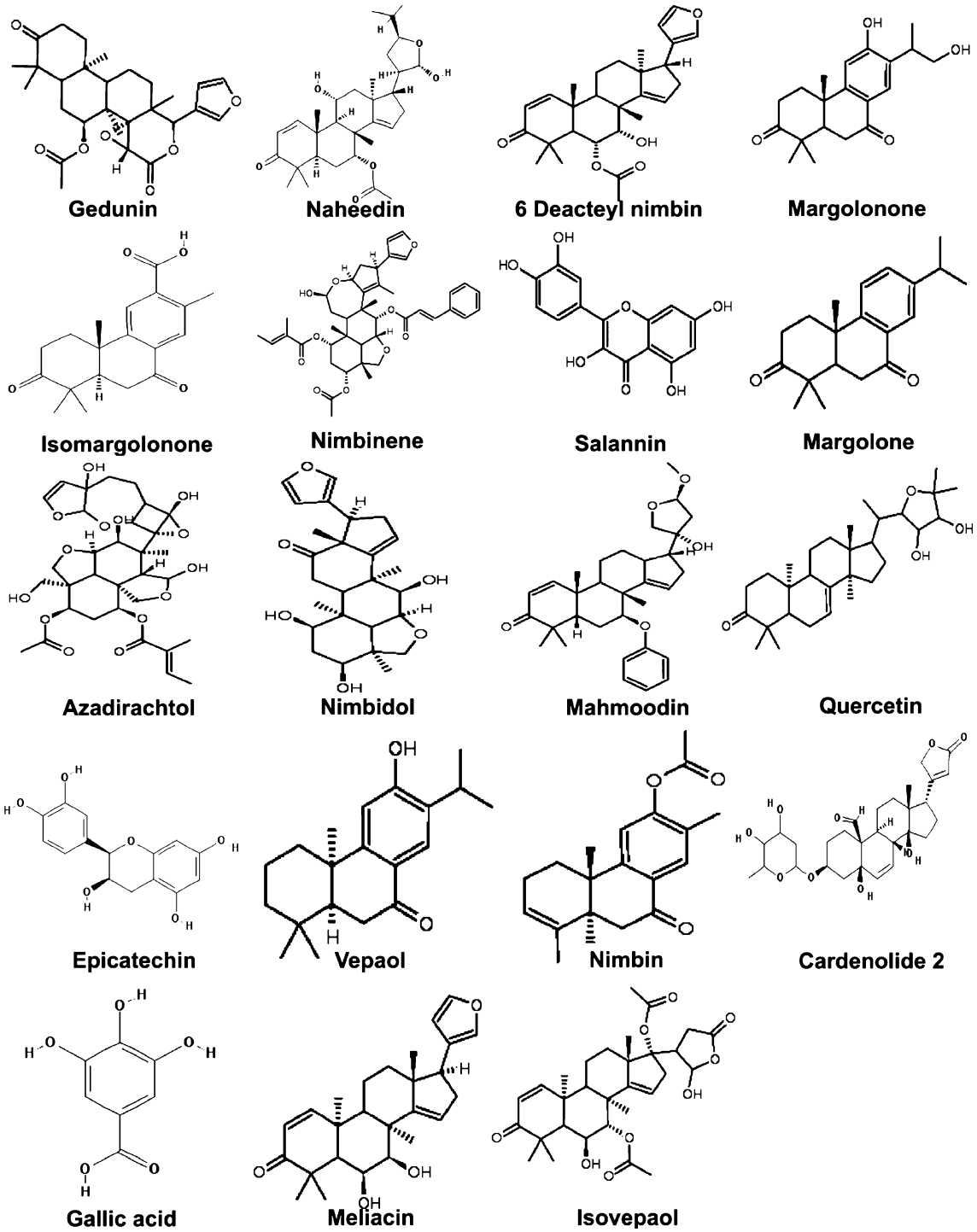


Fig. 1 Chemical structures of the non-azadirachtin limonoids selected for the study

(Tohidi-Esfahani *et al.,* 2011). The structures of the nuclear receptors constantly provided valuable information about ligand recognition and the activation mechanism of nuclear receptors. Studies building homology models based on a comparison of the EcR-LBD with known crystal structures have been employed to determine the three-dimensional (3D) structure of the EcR-LBD (Wurtz *et al.,* 2000), and docking studies have also been carried out to simulate how a candidate ligand binds to a receptor (Kasuya *et al.,* 2003).

Docking of non-azadirachtin limonoids (NAL)

Limonoids of Meliaceae have complex structure with a very high degree of oxidation and rearrangement (Roy and Saraf,

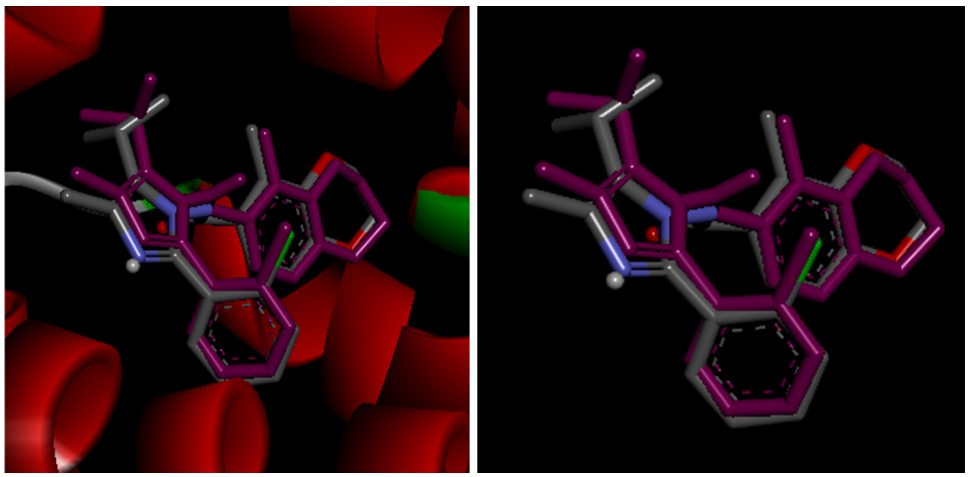


Fig. 2 Redocked conformation of crystal structure of BYI-06830 (*in purple*) with HaEcR-LBD receptor (rmsd = 1.29 Å) (Color figure online)

2006). In silico docking, experiment was performed to evaluate the binding potential of non-azadirachtin limonoids from *A. indica* to the HaEcR-LBD. We selected 25 limonoid compounds and a non-steroidal ecdysone agonist (tebufenozide), as a reference (Table 1; Fig. 1). AutoDock run resulted in the energy scores between −2.34 and −12.22 kcal/mol. Out of 25 chosen non-azadirachtin limonoids, nimbolide (−12.22 kcal/mol), azadirone (−11.31 kcal/mol), nimolinone (−11.29 kcal/mol), meliacinol (−11.08 kcal/mol), nimbocinol (–10.73 kcal/mol) and azadiradione (−10.54 kcal/mol) were able to dock (higher binding affinity) better than the reference ligand (Tebufenozide- −10.46 kcal/mol) in the active site (Table 1). The best docked ligand molecules were selected based on the binding energy and good interaction with the active site's residues. Lesser the inhibitory constant (Ki), higher the binding affinity of limonoids. Nimbolide (1.1 nM), azadirone (5.16 nM), nimolinone (5.33 nM), meliacinol (7.54 nM), nimbocinol (13.74 nM) and azadiradione (18.85 nM) came out to be the most promising hits with K in nanomolar range. All the top scorers along with their binding energies and no. of hydrogen bonds are listed in Table 1. The top score NAL ligand values include steric and H-bonding intermolecular function, stronger receptor ligand binding, lipophilic interactions, polar attractive/repulsive interactions, solvation of the protein and ligand, entropy term for the ligand and binding free energy. The order of ligands based on docking score is nimbolide > azadirone > nimolinone > meliacinol > nimbocinol > azadiradione > tebufenozide. Azadirone and azadiradione shared one hydrogen bond with Cys 508, while meliacinol and nimbocinol moiety with Asn 504 (Table 1; Fig. 3).

The docking results showed that ligands bind to the hydrophobic core of the EcR-LBD, which consist of hydrophobic amino acids (Cys, Leu, Val, Ile, Phe and Met), lipophobic amino acids (Thr and Tyr) and hydrophilic amino acids (Lys, Gly, Thr and Asp). Three polar amino acid residues (Tyr 408, Asn 504 and Cys 508) are implicated in the hydrogen-bond interaction network with the ligands. In the six top-scored NAL compounds, nine amino acids (Leu, Trp, Thr, Met, Asn, Gln, Tyr, Ser and Val) make up the binding cavity including the reference ecdysteroid agonist, tebufenozide, indicating the significant roles of these residues in binding. The six top-most scorers comprised five amino acids (Phe, Ile, Gln, Asp and Cys) in the binding pocket of the HaEcR-LBD (Fig. 3b–g), which were not present in HaEcR-LBD–tebufenozide complex (Fig. 3a).

The electrostatic bond and van der Waals interactions between the binding pocket of HaEcR-LBD and the ligands are presented in Table 1 and Fig. 3. Nimbolide seemed to be the most potent hit having −12.22 kcal/mol as binding energy. This compound was bound tightly to the binding site by electrostatic bonds (Leu 420, Tyr 403, Met 380/507, Val 416, Gln 503, Thr 343, Asn 504, Cys 508) and van der Waals interactions (Asp 419, Met 413, Tyr 408, Leu 500/511, Phe 336, Ile 339, Trp 526). One side of the binding site was completely hydrophobic with residues like Met 380, Leu 420 and Val 416 while lipophobic residues are concentrated in the same site including Gln 503 and Tyr 403/408 (Fig. 3b). Azadirone interacts with lipophobic (Thr 343 and Tyr 408) and hydrophobic (Cys 508, Leu 511/518/522, Ile 339, Met 507/381 and Phe 336) residues (Fig. 3c). Nimolinone moiety occupied a hydrophobic pocket of Cys 508, Leu 500/522, Val 416, Phe 408 and Met 507/381/413 with only one hydrogen bond between Cys 508 with cyclic carbonyl oxygen, while the remaining part is buried inside a region rich with amino acids having low hydropathy index (Asn 504 and Thr 340; Fig. 3d). The moiety of meliacinol contains hydrophobic, lipophobic, hydrophilic residues along with low hydropathy index (Fig. 3e). In all top six structures, one aromatic ring, 3–7 vicinal acceptors and 1–3 distal hydrophobic groups were observed, which could be the minimum pharmacophoric

feature. Superimposed graphical representation of the six best docked compounds with EcR-LBD active site is shown in Fig. 4.

Criteria for avoidance of local minima and false-positives

Graham et al. (2007a, b) have experimented on the recombinant EcR-LBD and found that tebufenozide, a commercially available insecticide, has differential binding affinity across the taxonomic orders and reflects the selective toxicity against lepidopteran pests. They have also been demonstrated to have no/low toxicity towards non-lepidopteran species as well as pollinators, predators and parasites (Dhadialla *et al.,* 1998; Retnakaran *et al.,* 2003). In order to avoid inaccuracy in the scoring function of docking study, non-steroidal ecdysteroid agonist—tebufenozide was docked against the protein HaEcR-LBD as a reference and the results were used as a benchmark. The rank of each non-azadirachtin limonoid compound was determined by the binding free energy of the lowest energy cluster. In all the cases, densely populated cluster coincided well with the lowest energy cluster resulted 2–3 clusters with a single conformation. The clusters possessed 99 % conformation with −8.56 kcal/mol average binding energy. The estimated free energy of binding should not be used as a sole criterion for the selection of ligand ranking. To avoid irrelevant local minima and minimize the false-positives, the following criteria's were followed: (1) The non-azadirachtin limonoid ligand was bound inside the pocket in the HaEcR-LBD receptor; (2) the non-polar/polar atoms in the ligand docked were near the non-polar/polar atoms of the receptor; and (3) hydrogen bonding and hydrophobic interactions.

Validation of docking study and confirmation of bound ligand, BYI-06830

Docking procedures aim to identify correct poses of ligands in the binding pocket of a protein and to predict the affinity between the ligand and the protein. In other words, docking describes a process by which two molecules fit together in three-dimensional space. To check the validity of docking study, redocking with bound ligand (BYI-06830) to the EcR protein was performed. The RMSD of the result was determined to be 1.29 Å, which suggests the reliability of the molecular docking procedure (Fig. 2).

Tice rule and potency of ecdysteroid agonists

No violation of Tice rule was observed in nimbolide, nimolinone, nimbocinol and azadiradione, whereas azadirone and meliacinol violated the properties of LogP and molecular weight, respectively (Table 2). Nimbolide, nimolinone, nimbocinol and azadiradione are proved to be potent ecdysteroid agonists against *H. armigera* (Praveena and Sanjayan, 2011). Based on Tice rule, it can be presumed that nimbolide may be a potent inhibitor of HaEcR-LBD receptor through its ecdysteroid agonist activity. Further, in-depth laboratory and field studies are needed to support this claim.

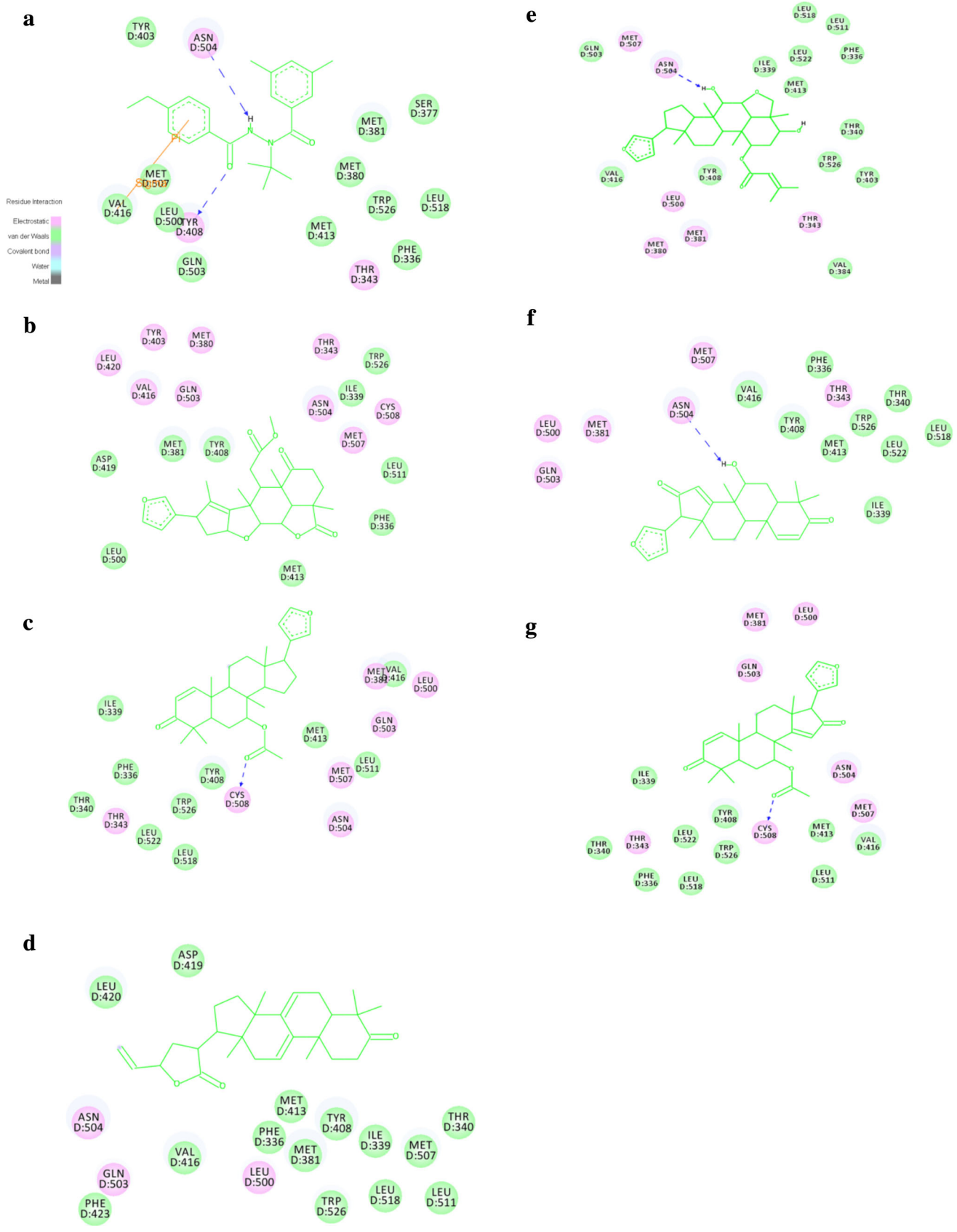
Regression analysis between experimental IC_{50} and binding energy

A linear regression analysis was performed to examine whether the docking score of non-azadirachtin limonoids (gedunin, salanin, nimbolide, nimbocinol, azadiradione and tebufenozide) can be correlated with the experimental IC_{50} values of *H. armigera*. Docking score was predicted as gedunin (−10.44), salanin (−9.25), nimbolide (−12.22), nimbocinol (−10.73), azadiradione (−10.54) and tebufenozide (−10.43). The predicted binding energies (kcal/mol-AutoDock) were plotted against available experimental IC_{50} values from the literature (Rochanakij *et al.,* 1985; Carlson, 2000; Koul *et al.,* 2003, 2004). The experimental IC_{50} values of NAL showed a linear correlation ($r = 0.8260$) with the calculated binding energy of the chosen limonoids ($y = 0.0023x - 4{,}170.5$; $F_{1,22} = 47.239$; $P < 0.0001$). The

Table 2 Tice rule properties of top-scored non-azadirachtin limonoid compounds

Non-azadirachtin limonoid	Molecular weight (kDa)	Hydrogen-bond donor (OH + NH)	Hydrogen-bond acceptor (O + N)	LogP[a]	No. of rotatable bonds	nviolations[a]
Tebufenozide	351.27	1	2	4.082	5	0
Nimbolide	466.53	0	7	1.940	4	0
Azadirone	436.59	0	4	5.329	3	1
Nimolinone	422.60	0	3	4.803	2	0
Meliacinol	510.67	2	6	5.106	4	2
Nimbocinol	408.53	1	4	3.524	1	0
Azadiradione	450.56	0	5	4.228	3	0

[a] *LogP* logarithm of the octanol/water partition coefficient, *nviolations* number of violations of the Tice rule


a
TYR D:403
ASN D:504
SER D:377
MET D:381
MET D:380
MET D:507
VAL D:416
LEU D:500
TYR D:408
TRP D:526
LEU D:518
MET D:413
PHE D:336
GLN D:503
THR D:343
Residue Interaction
Electrostatic
van der Waals
Covalent bond
Water
Metal
b
TYR D:403
MET D:380
THR D:343
TRP D:526
LEU D:420
VAL D:416
GLN D:503
ILE D:339
ASN D:504
CYS D:508
MET D:381
TYR D:408
MET D:507
ASP D:419
LEU D:511
PHE D:336
LEU D:500
MET D:413
c
MET D:381
VAL D:416
LEU D:500
ILE D:339
GLN D:503
MET D:413
PHE D:336
TYR D:408
LEU D:511
MET D:507
CYS D:508
THR D:340
TRP D:526
THR D:343
ASN D:504
LEU D:522
LEU D:518
d
ASP D:419
LEU D:420
MET D:413
ASN D:504
TYR D:408
THR D:340
PHE D:336
ILE D:339
VAL D:416
MET D:381
MET D:507
GLN D:503
LEU D:500
LEU D:518
LEU D:511
PHE D:423
TRP D:526
e
LEU D:518
LEU D:511
MET D:507
GLN D:503
PHE D:336
ASN D:504
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ILE D:339
MET D:413
THR D:340
TRP D:526
VAL D:416
TYR D:408
TYR D:403
LEU D:500
THR D:343
MET D:381
MET D:380
VAL D:384
f
MET D:507
PHE D:336
VAL D:416
THR D:343
THR D:340
ASN D:504
LEU D:500
MET D:381
TYR D:408
TRP D:526
LEU D:518
MET D:413
LEU D:522
GLN D:503
ILE D:339
g
MET D:381
LEU D:500
GLN D:503
ILE D:339
ASN D:504
TYR D:408
MET D:507
CYS D:508
MET D:413
VAL D:416
THR D:340
THR D:343
LEU D:522
TRP D:526
PHE D:336
LEU D:518
LEU D:511

◀**Fig. 3** Two-dimensional plot of docked poses and binding interactions of *Helicoverpa armigera* ecdysone receptor ligand-binding pocket (HaEcR-LBD) to the non-steroidal ecdysone agonist, tebufenozide (**a**) and non-azadirachtin limonoid compounds nimbolide (**b**), azadirone (**c**), nimolinone (**d**), meliacinol (**e**), nimbocinol (**f**) and azadiradione (**g**)

coefficient of determination ($r^2 = 0.6823$) shown a good fit with the statistical model, which is an acceptable value (SD = 0.5134) in such docking practice. These results suggest that AutoDock has performed well in predicting the binding energies and also rationalized the mechanism by which these ecdysteroid agonists work (Fig. 5).

Many studies had been focused on determining the distribution, nature, and practical use of plant-derived ecdysteroid substances that have moulting inhibitory activity against *H. armigera* (Cohen *et al.,* 1996; Dhadialla *et al.,* 1998; Murugan *et al.,* 1998; Retnakaran *et al.,* 2003). Various NAL compounds existing in neem plant either jointly or

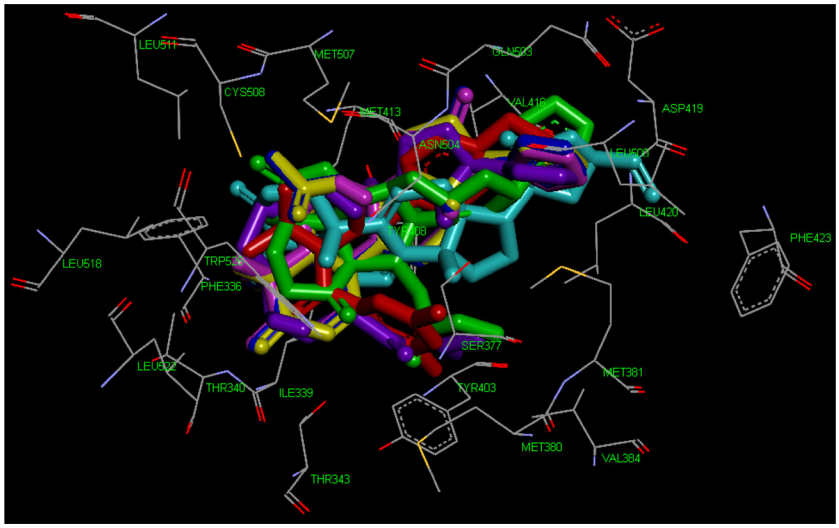

Fig. 4 Superimposed structure of docked poses of the six non-azadirachtin limonoid compounds along with ecdysone agonist, tebufenozide, at binding pocket of *Helicoverpa armigera* ecdysone receptor (HaEcR-LBD) with colour codes **a** tebufenozide in *red* (reference), **b** nimbolide in *green*, **c** azadirone in *yellow*, **d** nimolinone in *light blue*, **e** meliacinol in *purple*, **f** nimbocinol in *pink* and **g** azadiradione in *deep blue* (Color figure online)

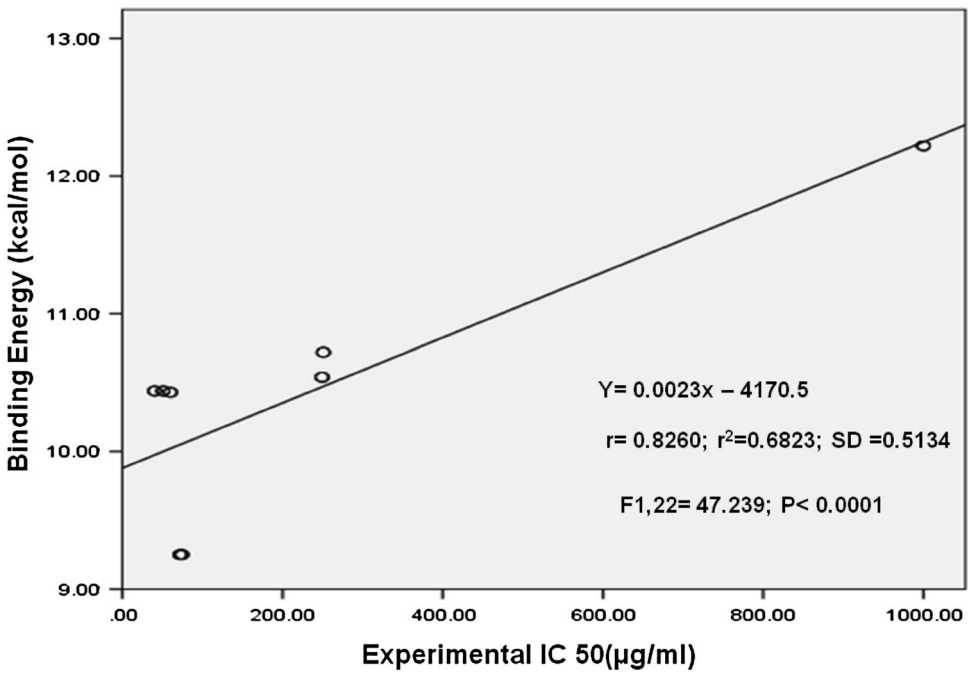

Fig. 5 Correlation between experimental IC_{50} values (µg/ml) of non-azadirachtin limonoid compounds and binding energy (kcal/mol) calculated from docking results

independently contribute to behavioural efficacy (e.g., repellence and feeding deterrence) and physiological efficacy, and/or as acute toxicity and developmental disruption against *H. armigera* (Murray and Isman, 2006; Lammers and Macleod, 2007; Jaychandrana *et al.,* 2013). Koul *et al.,* 2003 have further shown that azadirachtin being the most active compound in neem is not synergized or influenced by any other limonoid, but other non-azadirachtin limonoids show synergism in specific combinations, which may be due to their different modes of action. In this study, the chemical interaction between the selected non-azadirachtin ligands (nimbolide, azadirone, nimolinone, meliacinol, nimbocinol and azadiradione) and the target protein (HaEcR-LBD) has been found to be good and has the best interaction scores. Similar to the identified ligands of phytochemical origin, it indicated that these limonoids are safer to the environment. Since permanent activation of EcRs by certain compounds has been reported (Wing *et al.,* 1988; Dhadialla *et al.,* 1998; Nakagawa, 2005) for inhibiting metamorphosis process, we hypothesize that these non-azadirachtin neem limonoid compounds, possessing a greater binding affinity than tebufenozide could also serve as an agonist against EcR, can be used for the development of potent insecticides.

Conclusion

In conclusion, the present study reports the insecticidal effect of six plant-based non-azadirachtin limonoids on HaEcR-LBD. Docking experiment suggested that these compounds showed higher interaction energies compared to the DBH-based insecticide, tebufenozide, where Cys 508 and Asn 504 involve in hydrogen-bonding interactions. Tice rule of insecticide likeliness also indicated that lead compounds can be potent candidates as ecdysteroid agonists. Linear regression analysis in terms of predicted binding energy and its experimental activities strongly suggests that these could be applied successfully in pest management programs against *H. armigera.*

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Conflict of interest The authors declare that there is no conflict of interest.

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