Int J Pharm Bio Sci 2012 Oct; 3(4): (B) 193 - 203

Research Article **Bioinformatics**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

IN-SILICO INTERACTION STUDIES ON INHIBITORY ACTION OF TETRANORTRITERPENOIDS ON ACTIN

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ABSTRACT

In-silico interaction studies on forty two tetranortriterpenoids, which include four classes of compounds azadiratchins, salannins, nimbins and intact limonoids, with actin have been carried out using Autodock Vina and Surflex Dock. The docking scores and predicted hydrogen bonds along with spatial confirmation of the molecules indicate that actin could be a possible target for insect antifeedant studies, and a good correlation has been obtained between the percentage feeding index (PFI) and the binding energy of these molecules. The enhancement of the activity in the photo products and its reduction in microwave products observed in *in-vivo* studies are well brought out by this study. The study reveals Arg 183 in actin to be the most favoured residue for binding in most compounds whereas Tyr 69 is favoured additionally for salannin and nimbin type of compounds. In the case of limonoids Gln 59 seems to have hydrogen bonding interactions with most of the compounds. The present study reveals that the fit for PFI vs. binding energy is better for individual classes of compounds and can be attributed to the binding of ligand with different residues. This comprehensive in-silico analysis of interaction between actin as a receptor and tetranortriterpenoids may help in the understanding of the mode of action of bioinsecticides, and designing better lead molecules.

KEY WORDS: **tetranortriterpenoids, actin, docking, hydrogen bonding interactions**

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INTRODUCTION

Among the plant-derived chemicals, terpenoids exhibit potent activity as insect antifeedants [1] and the noxious nature of these molecules makes them important as antifeedant agents. They are the largest group of plant chemicals with a common origin via mevalonate biosynthetic pathway[2]. Of the various biosynthetic reactions involved in secondary metabolism, the terpenoid pathway is perhaps the best suited to generate the incredible structural diversity and complexity [2]. The insecticidal activity, which is a function of antifeedancy, is widely spread among the compounds isolated from various species belonging to the family of Rutaceae and more than 300 limonoids and 100 quassinoids have been isolated till date [3]. Many tetranortriterpenoids isolated from Rutaceae show antifeedant, repellent and molt inhibitory effects and hence are considered as bioinsecticidal, and are being looked upon as alternatives to synthetic insecticides[4]. Among the four major classes of tetranortriterpenoids viz., azadiratchins, nimbins, salannins and intact limonoids, Azadirachtin-A has been widely used as a insect control agent in the last two decades and has been shown to arrest the G2/M phase of cell division [5]. Another study has indicated that the azadirachtin has pronounced effect in actin polymerization [6]. Though the insecticidal activity of these compounds has been well established the receptor molecules are not yet identified. Hence it is necessary to look for alternate methods to study such interactions. A recent i*n-silico* study on a synthetic pyrethroid used in agriculture shows that it interacts with actin from and brings out changes in the ATP binding pocket of actin in mammals which is responsible for its low toxicity [7]. Another docking study of the modelled actin of Drosophila explains the mechanism of Azadirachtin. [8]. Though the Azadirachtins are the highly active compounds, they are present only 3% in the neem kernels and also have a low shelf life which minimizes its use in the field. Hence, it is of interest to study the binding of other tetranortriterpenoids with actin to understand the mode of binding. In the present study we have carried out the docking of forty two tetranortriterpenoids with actin from *Drosophila* using two docking programs, Autodock Vina, an automated program released by Scripps Research Institute[9] and Surflex Dock incorporated in SYBYL-X.

MATERIALS AND METHODS

1. Dataset

The structures of the tetranortriterpenoids from Rutales and their insect antifeedant activities were obtained [10] and classified into four different classes viz., azadirachtins, nimbins, salannins and intact limonoids based on their geometry. These include compounds exhibiting antifeedant activities at different levels. Among these (comma,) azadirachtins show the highest activity and the other compounds have activity less than the azadirachtins. Energy minimized structures were used in all calculations for uniformity as the crystal structures of only a few compounds are known. The structure of each compound was drawn in 2D and was converted to 3D using Hyperchem 7[11], and pre-minimized by Polak–Ribiere geometry optimization using MM+. These structures were then used as the starting point for re-minimization by Polak–Ribiere optimization using AM1 semi-empirical quantum mechanical method. Energy minimization was performed until the absolute value of the largest partial derivative of energy with respect to the coordinates was below 0.01 kcal mol^{-1} A^{-1}

2. Molecular docking:

2.1 Actin receptor:

The percentage feeding index (PFI) is a function of insecticidal activity and has been experimentally determined using the third instar larvae of *Spodoptera litura[10];*however the structure of actin from this species has not yet been reported. Recently the three dimensional structure of the actin from *Drosophila melanogaster* has been reported (GI: 114794360),(PDB id:3EKS)[12]) and the

same was used in the present study as the actins are known to be conserved in insects[13].To recheck this similarity the sequences of actin from *Caenorhabditis elegans* (GI:14278147) , *Spodoptera littoralis* (GI: 600075) and *Drosophila melanogaster* (GI:114794360) were retrieved from NCBI protein sequence database and aligned using BLOSUM62 cost matrix on a GENEIOUS PRO[14] platform with a gap open penalty of 12 and gap extension penalty of 3.This resulted in a pair wise identity of 97.6% showing good homology in the sequences(Fig 1).

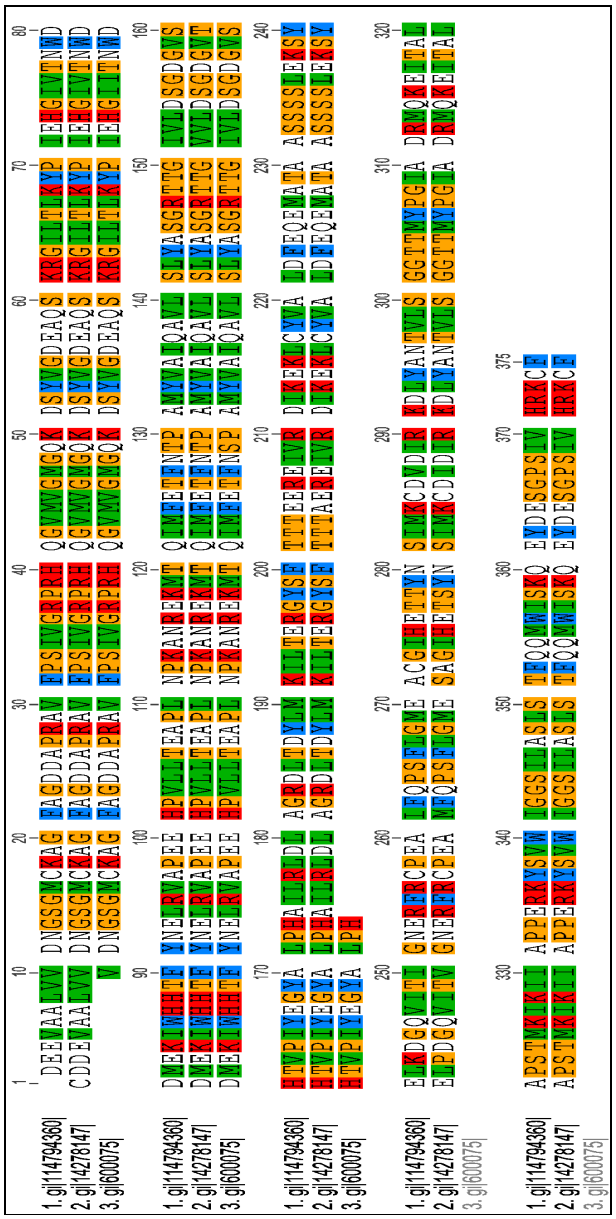

Figure 1
Sequence alignment showing the homology in actin from Drosophila melanogaster(row 1), Caenarohabditis elegans (row 2) and Spodoptera littoralis (row 3).

2.2 Docking of Actin from Drosophila:

Autodock was used to prepare the receptor and the ligands for docking studies[15]. The partial charges on atoms were assigned after adding the polar hydrogens. A grid spacing of 0.375A was used for docking using Autodock Vina and the results were analysed with LIGPLOT [16; 17], a program to generate schematic diagrams of protein–ligand interactions. Initial docking studies were carried out with whole modelled actin receptor molecules in the grid identified 2 major binding pockets and the same was also verified using Pocket-Finder[18]. The two results combined with earlier reported mechanism of ATP hydrolysis based on water diffusion[19] and actin depolymerisation was used to select the active site. The hydrogen bonds as well as the hydrophobic interactions in the binding pocket were analysed by LIGPLOT. Alternately docking was also performed using Surflex Dock[20] integrated in SYBYL-X program which is based on an empirical scoring function and Protomol[21], a computational representation of the specified binding site used to guide docking process. In the present study Protomol was specified using the receptor residues which had earlier been found to interact with the ligand in Autodock and also the binding cavity generated automatically by Surflex Dock. Interestingly both the sites were identical and have been used in this study. Surflex Dock scores are expressed in $-\log_{10}(K_d)$ units to represent binding affinities.

RESULTS

Autodock Vina allowed flexible docking of ligands into the active site of the receptor and gave a number of confirmations of the ligand from which the most probable binding mode can be identified. When the docking scores were close to each other the rmsd was used to select the best conformation. Table I lists the binding energy along with the PFI value for each compound and a linear fit could be seen between the free binding energy and the PFI of the molecules; however the correlation is found to be better for the individual classes of compounds (Fig 2).

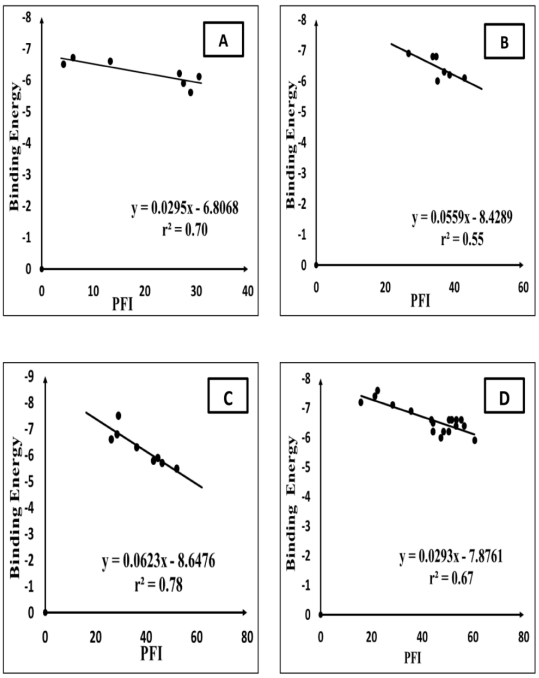

Figure 2

Correlation between PFI (obs.) and binding energy. azadiratchins B. Nimbins C. salannins D. intact limonoids

It can be seen that the slopes of A & D are similar; but are slightly different from B & C showing the effect of geometry on the activity. In A & D Gln 59 of actin participate in hydrogen bonding with a few azadirachtins and intact limonoids molecules (Comma) whereas in C & D Tyr 69 is the interacting residue. The small change in the linear fits for individual compounds may also be attributed to the differences in the mode of binding. Hydrogen bonding and hydrophobic interactions play important roles in the receptor-ligand interaction. The LIGPLOT analysis shows that azadirachtins, the most active molecules, have a larger number of hydrogen bonding interactions than the other classes of compounds. Similar results have been obtained from Surflex Docking using SYBYL-X. The high affinity of azadiratchins for actin can be visualised from the large Surflex scores ranging from -120 to -150 for azadirachtins compared to -90 or less for other classes of tetranortriterpenoids (Fig 3).

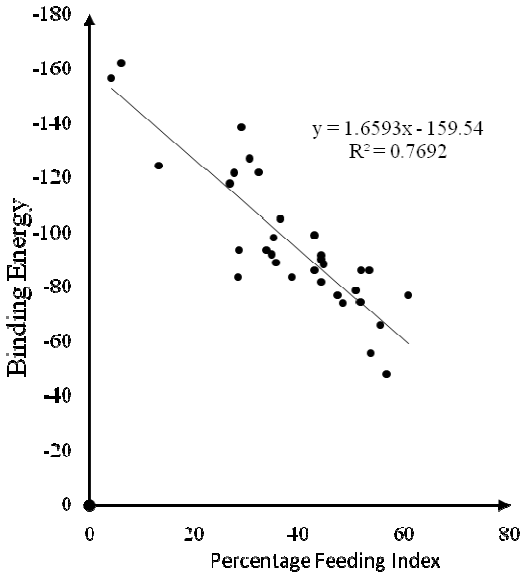

Figure 3
A plot of PFI(obs) vs the docking score using Surflex Dock. A few compounds showing large deviations are excluded from the calculation.

A schematic representation of the interacting residues for the different classes of compounds under study obtained from LIGPLOT is given in Fig 4. It can be seen from Fig 4 and Table I that Arg 183 is the most favoured residue for binding of most of the compounds whereas Tyr 69 is favoured additionally for salannin and nimbin type of compounds. Gln 59 seems to have hydrogen bonding interaction with most of the limonoids.

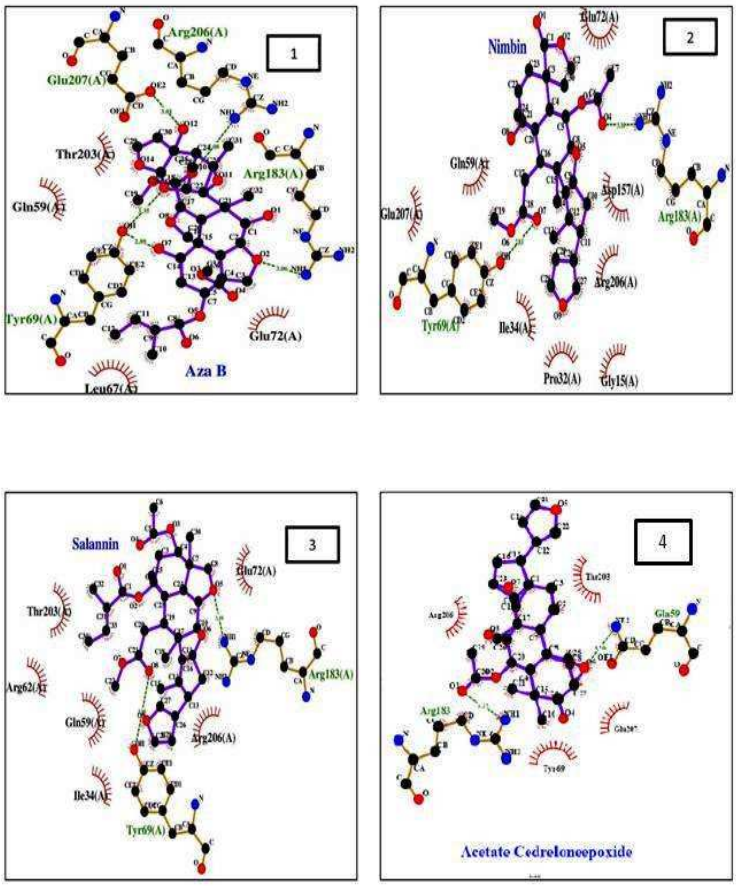

Figure 4

The interaction of amino acid residues in actin with 1. Azadiratchins 2.nimbins 3.salannins 4. Intact limonoids. The hydrophobic interactions are shown by red arcs and the hydrogen bond interactions by green dotted lines.

Table I

PFI(obs), binding energy and the amino acid residues participating in the hydrogen bonding interaction and hydrophobic interactions obtained using LIGPLOT. rmsd represents root mean squares deviation; ub and lb refer to upper and lower limits respectively.

S.no	Name of the Ligand	PFI	Binding Affinity (KCal\ mole)	Rmsd (ub)	Rmsd (lb)	Hydrogen Bonding Interactions	Hydrophobic interactions
	Azadiratchins						
1	Acetoxy-azadirachtol	4.2	-6.5	0.0	0.0	LYS50(2),SER52, ASN92(2)	ARG95,TYR91,HIS88, VAL54,HIS87
2	Dihydroxy-azdirachtol	6.1	-6.7	0.0	0.0	ASP184(4), ASP179	HIS 73,MET 269,LEU267,TYR188, ARG183,ASP187
3	Tigloyl-melicarpin	13.3	-6.6	8.7	5.6	ARG183(2),ASP 187(2)	TYR 69, ARG206, ASP184,LEU267, TYR188
4	Azadirachtin B	26.7	-6.2	7.4	3.9	TYR 69(2),	GLU72,LEU67,GLN59

						GLU207 , ARG183 ,ARG 206	,THR203
5	Azadirachtin A	27.5	-5.9	15.7	12.0	GLU207,ARG206, TYR69	ARG183,ASP157,GLN 59,LEU67, ARG62,THR203
6	Azadirachtin D	28.9	-5.6	4.5	2.3	LYS243,SER60	ARG62,ILE208,LEU24 2,TYR240, GLU204,GLU207,GLN 59
7	Azadirachtin H	30.5	-6.1	15.3	12.0	ARG-183	GLU72,LEU67,TYR69 ,GLY15, GLU207,THR203,AR G206
	Nimbins						
8	Nimbinolide	26.7	-6.9	0.0	0.0	ARG183,TYR69	ARG62,THR203, GLN59,ILE34, PRO32, ASP157, ARG206,GLU72
9	Nimbin	33.7	-6.8	0.0	0.0	ARG183,TYR69	ARG206,PRO32, GLY15,GLN59, GLU207,GLU72, ASP157,ILE34
10	Desacetyl-nimbin	34.7	-6.8	0.0	0.0	ARG-183,TYR-69	GLU72,PRO32, GLN59,ASP157, ILE34,ARG62, ARG206,
11	Desacetyl-nimbin lactone	35.1	-6.0	29.7	26.2	ARG-183,TYR-69	GLU72,ASP157, ARG62,PRO32, ILE34,ARG206, GLN59,THR203
12	Nimbolide	37	-6.3	2.4	1.8	ARG-183, TYR-69	LEU67,GLN59,ILE34, PRO32,ASP157, ARG206,GLU72
13	6-homo-desacetyl nimbin	38.6	-6.2	33.4	29.0	ARG-183, TYR-69	ARG206,ARG62,ASP 157,ILE34, GLN59,PRO32
14	Nimbinlactone	42.9	-6.1	34.3	30.0	ARG-183, TYR-69	PRO32,THR203, ARG206,GLU207, ASP157,ILE34,GLY15 ,GLN59
	Salannins						
15	Salanninpp 29	25.1	-7.5	0.0	0.0	TYR-69,TYR-69	ARG62,GLU207, ARG206,LEU67, GLN59, ILE34, ARG183,GLU72, THR203
16	Desacetyl-salannolide	28.44	-6.8	0.0	0.0	TYR-69	GLU72,ARG183,ILE3 4,ASP157, GLY15,PRO32,GLN59 ,ARG206,THR203,GL U207
17	Salannolide	26.3	-6.6	0.0	0.0	ARG-62,ARG-210,ASP211	LYS243,ILE208, GLU204,GLN59, GLU207
18	Salannin	36.3	-6.3	0.0	0.0	ARG-183,TYR-69	GLU72,THR203, ARG62,GLN59, ILE34,ARG206
19	Desacetyl-salanin	44.67	-5.9	14.9	11.6	ARG-183,TYR-69	ARG62,THR203, ARG206,ILE34, GLN59,GLU207,GLU7

							2
20	Oxosalannin	42.9	-5.8	9.9	5.7	ARG-183,TYR-69	ARG62,THR203, ARG206,ILE34, GLN59, GLU207, GLU72
21	Salannin mw	46.3	-5.7	11.2	6.0	ARG-183,TYR-69	ARG62,THR203, ARG206,ILE34, GLN59,GLY15, GLU207,PRO32, ASP157
22	Salannol	52.1	-5.5	15.9	11.8	TYR-69 ,GLN59	ILE34,GLU207, ARG206,ASP157, ARG183,LEU67, ARG62,THR203
	Intact limonoids						
23	Nimonol pp2	15.8	-7.2	0.0	0.0	ARG-183,TYR69, GLN59	ARG62,LEU67,THR66 ,ARG206,ASP187, THR203
24	Nimonol pp1	21.3	-7.4	0.0	0.0	NIL	ARG206,ARG183, ASP157,THR186, GLY15, MET16, PRO32,ARG210, GLU207, GLN59, ILE34,TYR69,
25	Cedrelone-ppOH	22.4	-7.6	0.0	0.0	TYR69,ASP157,	PRO32,ARG210, GLU207,GLN59, ARG206,ARG183
26	Isomeldeninpp	28.3	-7.1	0.0	0.0	GLN-59,TYR240, ARG62, SER60	GLU207,ILE208, GLU204,ARG210
27	Azadiradione	35.5	-6.9	2.2	1.6	NIL	GLU207,ASP187,ARG 206,TYR69, ARG183,ASP157
28	Cedrelone-epoxide	43.5	-6.6	3.6	2.4	GLN59	GLU207,ARG206, ARG183,TYR69, LEU65,THR203,
29	Cedrelone22-epoxide	44.2	-6.5	7.5	5.0	TYR-69	LEU67,ARG62, THR203,ARG206, GLN59,ASP157, GLU207,ARG210
30	Nimonolmw	44.2	-6.2	15.0	11.4	ARG62	GLU207,GLN59, ARG183,TYR69, ARG206,THR203, LEU67
31	Oxonimonol	44.2	-6.2	14.6	11.1	NIL	GLU207,GLN59, ARG183,TYR69, ARG206,THR203, ILE34,LEU67, ARG62,GLN59
32	Nimonol	47.3	-6.0	32.7	29.2	NIL	GLU207,ARG183, TYR69,ARG206, THR203,LEU67, ARG62,GLN59
33	Azadirone	48.3	-6.2	35.2	30.1	TYR69,GLN59	ARG183,LEU67, THR66,LEU65, ARG62,THR203
34	Epoxy-azadiradione	50.4	-6.2	35.9	32.5	ARG-183	ARG206,GLU72, TYR69
35	Dihydro-cedrelone	50.5	-6.6	28.7	26.7	ARG-183	ASP157,GLY15, PRO32,

							GLU59,GLU207, TYR69,ILE34,ARG206
36	Cedrelone	51.5	-6.6	4.4	2.5	ARG183	ASP157,GLY15, PRO32,GLU59, GLU207,TYR69,ILE34 ,ARG206
37	7-desacetyl-15methoxy - azadiradione	51.6	-6.6	31.5	28.3	GLN59	ARG62,THR203, PRO32,GLY15, SER33,ILE34,ASP157 ,SER14,ARG183, TYR69,ARG206, GLU207
38	Acetate-cedrolone epoxide	53.2	-6.4	42.2	37.5	ARG183,GLN59	THR203,GLU207,AR G206,TYR69
39	Cedrelone-butyro lactone	53.4	-6.6	40.4	38.7	GLN-59	LEU67,THR203, ARG206,ILE34, ARG183,ASP157, TYR69,GLU207, MET16,ARG210, PRO32
40	Desepoxy-cedrelone	55.3	-6.6	9.3	4.2	NIL	THR203,ARG206, TYR69,ARG183, ASP157,GLY15, PRO32
41	Metether Dihydro-cedrelone	56.5	-6.4	2.0	1.5	NIL	ASP157,ILE34,GLN59 ,TYR69,PRO32, GLY15,SER14, GLU207,ARG183, ARG206
42	Isomeldenin	60.6	-5.9	14.7	11.4	NIL	ILE34,TYR69,ARG183 ,ARG206,GLN59, GLU207,PRO32, MET16,ARG210, ASP157,GLY15, THR186,

The higher activity of the photo products of salannin and the intact limonoids seen in *in-vivo* studies is corroborated by the maximum binding energy. These results may be taken into account in modifying the functional groups while designing better compounds.

CONCLUSION

The *in silico* interaction studies on the inhibitory action of tetranortriterpenoids on actin using Autodock Vina and Surflex Dock confirm the earlier studies on azadirachtins [6; 8] that actin may be one of the possible receptors for tetranortriterpenoids and hence for determining the insecticidal activity. A linear relationship of the free binding energy with the PFI values of these molecules is seen through these studies. The hydrogen bond interactions calculated for these compounds with actin show Arg 183 to be the most favoured residue for binding with most of the compounds whereas Tyr 69 is additionally favoured for nimbin and salannin type of compounds. There is a good correlation of the PFI values with the binding energy for all classes of compounds and the equations obtained can be used to design better molecules with enhanced activity. These studies also confirm the enhanced activity of the photo products. As the azadirachtins, the most potent insect antifeedants, are less stable in fields the photo products of intact limonoids and salannin can be used with high stability. One can also try with this method to modify the functional groups of these compounds to obtain an active compound with better activity.

ACKNOWLEDGEMENTS

The authors dedicate this paper to their Beloved Founder Chancellor, Bhagwan Sri Sathya Sai Baba, who offered them free and value-based education in the University. Thanks are due to Prof. Mukesh Doble, Department of Biotechnology, IIT, Madras for the support in using the SYBYL software and the teams of Autodock and LIGPLOT for their free software tools.

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