BIOINFORMATION
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Volume 7(5)

Molecular interaction of fenvalarate with actin

Prashantha Karunakar[1], Venkatappa Krishnamurthy [1], Chamarahalli Ramakrishnaiyer Girija[2]*, Venkatarangaiah Krishna[3], Dindare Eswarappa Vasundhara[4], Noor Shahina Begum[4], Akheel Ahmed Syed[5]

[1]Department of Biotechnology, PES Institute of Technology, BSK III Stg, Bangalore - 560085, India; [2]Department of Chemistry, SSMRV College, Jayanagar, Bangalore - 560041, India; [3]Department of Biotechnology and Bioinformatics, Kuvempu University, Shankarghatta - 577451, India; [4]Department of Chemistry, Bangalore University, Central College Campus, Bangalore-560001, India; [5]Department of Chemistry, University of Mysore, Manasagangothri, Mysore 570006, India. Chamarahalli Ramakrishnaiyer Girija – Email: girija.shivakumar@rediffmail.com; Phone: +91 98864 19952; * Corresponding author

Received September 28, 2011; Accepted October 2, 2011; Published October 31, 2011

Abstract:
The structure of α-Cyano-3-phenoxybenzyl-2-(4-chlorophenyl)-3-methylbutyrate (Fenvalarate) has been established by X-ray crystallography to understand the structure-activity relationship, which is of paramount importance in the toxicological studies of the compound. Fenvalarate is stabilized by intermolecular C-H...O, C-H...Cl, C-H...π and C-H...N interactions which are responsible for the stability of the compound and its interaction with the Actin. The crystallographic coordinates of the compound was extrapolated to docking studies to elucidate the action of fenvalarate against neural cytoskeletal protein of insect and mammalian β-actin. A strong affinity was observed in binding of fenvalarate with insect β-actin (-7.71kcal/mol, Ki = 2.23μM) indicating it as a potent insecticide and moderate toxicity towards mammalian β-actin (-7.07kcal/mol, Ki=6.54μM).

Keywords: Synthetic pyrethroids, intermolecular interactions, β-actin, docking

Background:
Fenvalarate, a synthetic pyrethroid is used in agriculture and other domestic applications due to its high insecticidal activity, low mammalian and phytotoxicity **[1, 2].** It has easy biodegradability compared to organo-chlorides and organo-phosphates **[3].** Its stability in sunlight allows its application against a wide range of pests. Encouraged by these wide varieties of applications the study of intermolecular interactions and its correlation to biological activity was undertaken.

Actin is the most abundant intracellular cytoskeletal protein in a eukaryotic cell. In neuronal cells, actin cytoskeleton is involved in important cellular events like cell migration, intracellular transport, cellular secretions, neuronal signaling, organization of endomembranes, cell division (cytokinesis) etc **[4].**

The insect brain tissue undergoes transition phase from a larva to an adult during which complex cellular events lead to the cellular reorganization and differentiation within the brain tissue. This transition phase is a highly potential target and can be exploited for pest control **[5].** Thus, disruption of actin filaments could lead to drastic effects on the cell morphology and functioning.

Methodology:
X-ray Analysis
Fenvalarate was obtained from Rallis India Ltd., Bangalore. Single crystals were grown from methanol at room temperature by slow evaporation process. The X-ray diffraction data were collected on a Bruker Smart CCD Area Detector System, at IISc, Bangalore, using MoKa (0.71073 Å) radiation. Intensity data were collected up to a θ_{max} of 25.00° for the compound in the ω–ф scan mode. The data were reduced using SAINTPLUS **[6]** and an empirical absorption correction was applied using the package SADABS **[7].** A total of 15499 reflections were collected, resulting in 3878 independent reflections of which the number of reflections satisfying I > 2 σ (I) criteria were 2416 and were

treated as observed. The structure was solved by direct methods and difference Fourier synthesis using SHELXS97 **[8]**. The positions of all non-hydrogen atoms were included in the full-matrix least-square refinement using SHELXL97 **[8].** The hydrogen atoms were fixed geometrically and allowed to ride on their parent C atoms and refined isotropically. Molecular diagrams were generated using ORTEP **[9].** The chlorine, one of the oxygen and two butyrate carbons were disordered during refinement. By using the split atom model, proper site occupancy factors and displacement parameters for the Cl atoms (Cl1A and Cl1B) the model converged to an acceptable R factor of 0.0725.

Multiple Sequence Alignment

ClustalW **[10]** was used to align the amino acid sequences of actin from different organisms, actin sequence of *Bos taurus* (gi|157878210|pdb|1HLU|A) and *Homo sapiens* (gi|4501885|ref|NP_001092.1) representing mammals and *Drosophila melanogaster* (gi|114794361|pdb|2HF4|A) and *Helicoverpa armigera* (gi|1296534|emb|CAA66219.1) from insects were compared.

Molecular docking study

Molecular docking simulation of Fenvalarate to β-actin of mammal and insect were performed in order to gain functional and structural insight into the mechanism of inhibition. AutoDock 4.0 suite was used as molecular-docking tool **[11].**

Fenvalarate and ATP Structure

Topology file and other force field parameters were generated for Fenvalarate and ATP using the PRODRG program **[12].** Flexible torsions of Fenvalarate and ATP were defined using AUTOTORS.

Actin Structure

The coordinates of crystal structure of monomeric actin in its ATP-Bound State of *Drosophila melanogaster* (PDB ID: 2HF4) and Structure of Bovine Beta-Actin-Profilin Complex with Actin Bound ATP Phosphates Solvent Accessible of *Bos taurus* (PDB ID: 1HLU) were obtained from the Protein Data Bank. The structures were edited by deleting Calcium, ATP and water molecules from 2HF4 and Calcium, ATP and P-Chain from 1HLU.

Fenvalarate – Actin Interaction

From the ADT package hydrogen atoms were added, Non-polar hydrogens and lone pairs were merged and each atom within the macromolecule was assigned a Gasteiger partial charge. A grid box of 40×40×40 points, with a spacing of 0.375 Å was positioned at the active-site residues where ATP was bound to protein using AUTOGRID. The Lamarckian genetic algorithm (LGA) **[13]** was employed with the settings of population size of 150 individuals, maximum number of generations and energy evaluations of 27,000 and 2.5 million respectively. From the estimated free energy of ligand binding (ΔG), the inhibition constant (Ki) for each ligand was calculated. Only the best pose (the one with the lowest binding energy) was considered for each ligand. To evaluate the accuracy of AutoDock 4.0 as an appropriate docking tool for the present purpose, the co-crystallized ligand (ATP for 2HF4 and 1HLU respectively) were re-docked within the inhibitor binding cavity of β-actin as reference. The best poses of docked ATP and fenvalarate were within reasonable proximity (root mean square deviation, RMSD ≤2 Å) of the original poses in the crystal structures of β-actin and the poses were obtained.

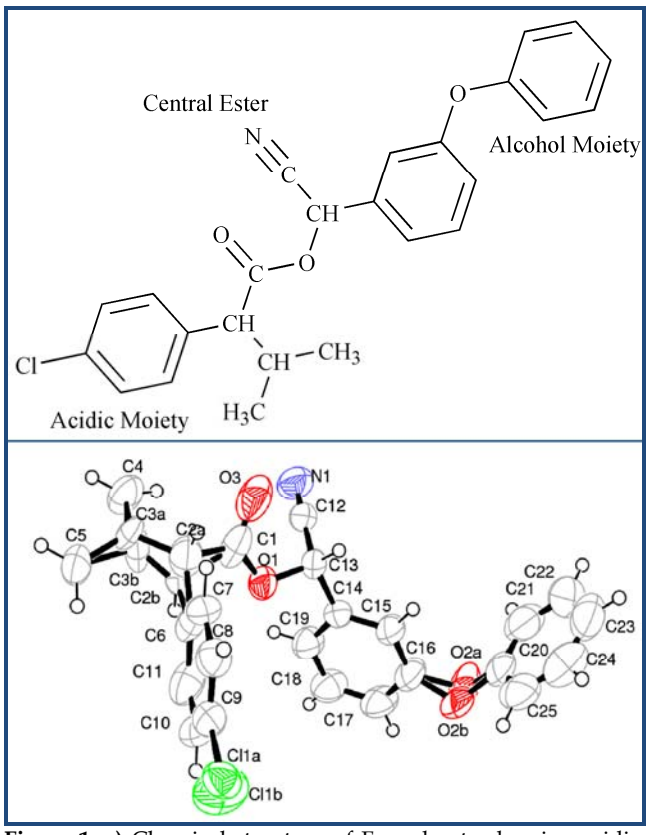

Figure 1: a) Chemical structure of Fenvalarate showing acidic, ester and alcohol moieties. **b)** Chemical structure of Fenvalarate showing ORTEP plot of the molecule drawn with 50% ellipsoidal probability

Discussion:

The molecular structure and the atomic numbering of compound are shown in **Figure 1a** and **Figure 1b,** respectively. The dihedral angle between the 4-chlorophenyl ring and the methyl butyrate chain is 78.48°, which indicate the non-planarity of the compound. This allows the bond to rotate and have six degrees of freedom and the phenoxy and benzyl rings are 80.77^0 apart from each other providing two degrees of freedom which in turn has a flexible interaction with β-actin.

The molecular packing reveals a two dimensional sheet like structure formed by a combination of C-H...O, C-H...Cl **[14, 15]** and C-H...N interactions. These non-covalent interactions not only structurally stabilize the compound but also allow predicting the probable hydrogen bond formation between the Fenvalarate and the active site residues of protein **[16]**.

Comparison of amino acid sequences shows that actin is highly conserved among insects and mammalian species. The mammalian actins share 97% sequences similarity with that of insects, but differ in ten positions which are marked in black box in the **Figure 2**. Although 97% similarity is conserved with Actin of insect and mammal, change in amino acid at MET152, THR159, SER270 and HIS72, SER159, LEU152 which are in the

ATP binding pocket of interaction of Fenvalarate to mammalian (1HLU) Actin and insect (2HF4) Actin, respectively caused the significant steric hindrance in the binding pocket resulting in difference in binding energy.

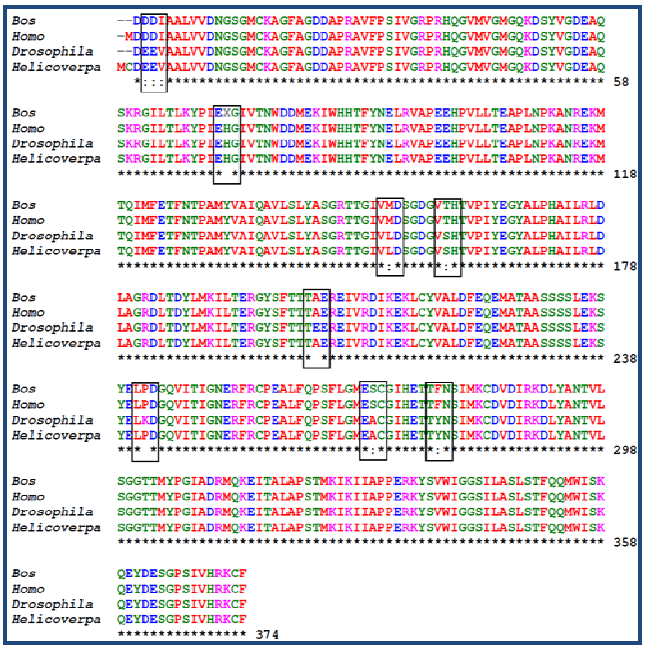

Figure 2: Sequence alignment of β-actin of mammalian and insect organisms with the bottom line showing identical (*), conserved (:) and dissimilar (with space). Residues are labeled according to bottom line.

Based on the binding energy and maximum hydrogen bond formation the chemical screening was done. The affinity of fenvalarate to the insect as well as mammalian Actin was compared against the standard ATP. Compound with Actin having lesser binding energy when compared to ATP are considered as toxic to insect/mammal and those with higher energy are considered safe.

It is observed that the oxygen of alcohol moiety of fenvalarate hydrogen bonding with the nitrogen of Gly302 of Drosophila Actin with bond distance of 2.187 and also hydrogen bond formation with the central ester oxygen of fenvalarate to zeta position hydrogen of Lys336 with bond distance of 1.939 **(Figure 3a)** is more strong compared to one hydrogen bond in the central ester oxygen of fenvalarate with the NH of Ser14 of Bovine actin with bond distance of 2.017 **(Figure 3b).** Residues interacting within 1Å of van der Waals radii are showed in **Table 1 (see supplementary material)**.

The interaction of Fenvalarate with active site of Drosophila actin shows the binding affinity (-7.71kcal/mol) and IC_{50} (2.23μM) as compared to ATP binding energy (-6.25kcal/mol) and IC_{50} (26.39 μM). This shows that Fenvalarate has high affinity and toxic effect against insect actin compared to ATP binding. The structural changes in the active site of Fenvalarate with Bovine actin is responsible for the high binding affinity (-7.07kcal/mol) and IC_{50} (6.54μM) as compared to ATP binding energy (-6.52kcal/mol), and IC_{50} (16.51 μM) signifies that Fenvalarate has moderate toxicity to mammals as the IC_{50} value is not significantly of much difference from ATP. The observation reinforces the fact that Fenvalarate toxicity is specific to insect population and fairly safe to human exposure.

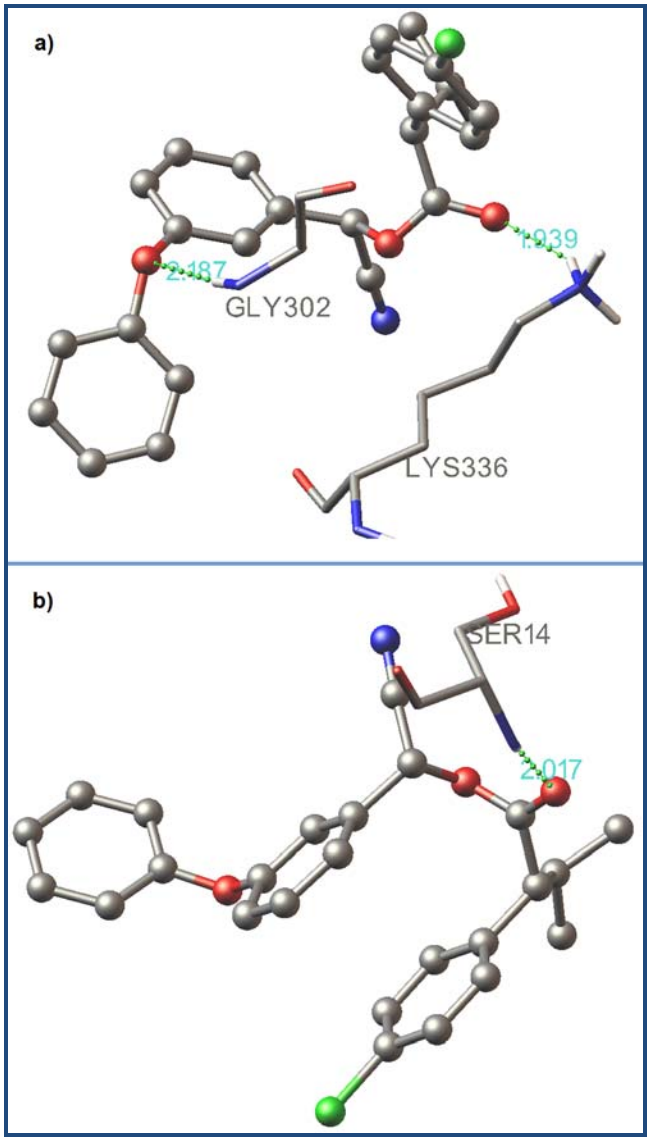

Figure 3: Interaction of Fenvalarate (Ball and stick model) at ATP binding pocket, atom coloring to both ligand and protein. Green dotted lines represent the Hydrogen bond **a)** With Drosophila Actin; **b)** With Bovine actin.

Conclusion:

Although Fenvalarate is used worldwide, in the present study its topological analysis of weak and strong non-covalent interactions using crystallographic method is performed and further extrapolated to molecular docking analysis to know the structure-activity relationship. From the analysis it was elucidated, why the biological activity of Fenvalarate has toxic effect against insect and moderate toxic effect against mammals. As the change in amino acids at the ATP binding pocket in both insect and mammalian β-actin, the steric hindrance was caused and sufficiently altered the shape of binding cavity resulting in change in binding affinity. These structural investigations and

binding interaction studies of Fenvalarate should be further explored to develop a novel insecticidal agent.

Acknowledgement:
PK and CRG thank Chemistry Research Center, SSMRV Degree College, Bangalore and RSST for providing research facility. PK thanks PES Institute of Technology, Bangalore and Kuvempu University, Shankarghatta for their kind suggestions. The authors also thank the DST, India for data collection on the CCD facility set up under the IRPHA DST program. DEV thanks the UGC-FIP for Teacher fellowship.

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Edited by P Kangueane
Citation: Karunakar *et al.* Bioinformation 7(5): 234-238(2011)

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Supplementary material:

Table 1: Molecular interactions of ATP and Fenvalarate into insect and mammalian β Actin

	Drosophila (Insect) PDB: 2HF4						Bovine(Mammal) PDB: 1HLU					
Compound	**Binding Energy (Kcal/mol)[a]**	**Ki[b]**	**H - bond donor**	**H - bond acceptor**	**Length of H-bond (Å)**	**Residues involved in van der Waals interaction (Scaling Factor= 1.00 Å)**	**Binding Energy (Kcal/mol)[a]**	**Ki[b]**	**H- bond donor**	**H - bond acceptor**	**Length of H-bond (Å)**	**Residues involved in van der Waals interaction (Scaling Factor= 1.00 Å)**
ATP	-6.25	26.39	ATP:H8L ATP:H8M	ASP157:OD2 ASP157:OD2	1.849 1.878	GLY 15 MET 16 LYS 18 ASP 157 GLY 182 LYS 213 GLU 214 GLY 302 THR 303 TYR 306 LYS 336	-6.52	16.51	ATP:H8L ATP:H8M	ASP157:OD1 GLU214:OE2	1.964 1.727	GLY 13 MET 16 LYS 18 GLY 156 ASP 157 LYS 213 GLU 214 GLY 301 GLY 302 MET 305 TYR 306 LYS 336
Fenvalarate	-7.71	2.23	GLY302:HN LYS336:HZ2	FEN:OAV FEN:OAM	2.187 1.939	MET 16 LYS 18 VAL 30 ASP 154 GLY 156 ASP 157 GLU 214 GLY 301 GLY 302 TYR 306 LYS 336 VAL 339	-7.07	6.54	SER14:HN	FEN:OAM	2.017	GLY 13 SER 14 GLY 15 MET 16 LYS 18 ASP 154 GLY 156 ASP 157 GLY 158 GLY 182

[a]Binding energy ΔG Calculated from (Kcal/mol) [b] Calculated IC_{50} in micro molar from AutoDock4