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Virtual high-throughput screening of insect acetylcholinesterase inhibitors

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Abstract
Acetylcholinesterase (Ache, EC 3.1.1.7) is a substrate specific enzyme that catalyzes the hydrolysis of the neurotransmitter acetylcholine (Ach) in the nerve synapses. Hydrolysis of the neurotransmitter leads to decrease in the concentration of the acetylcholine. An optimum level of Ach should be maintained in the brain for its proper function. An increased and decreased level of Ach hampers the functioning of brain. Thus Ache performs a critical function in the nervous system of all organisms including insects. Any impairment of Ache action leads to imbalance in the nervous system and will be fatal for organism concerned. Therefore, Ache seems to be an attractive target for identifying receptor specific insecticides. The present study is focused on finding the more efficient inhibitors for Ache through virtual high-throughput screening methods.

Keywords: acetylcholinesterase, acetylcholine, molecular docking, ADME-T prediction

Introduction

Insects are most diverse and abundant of all terrestrial animals. Insects dominated the earth by successfully adapting to wide range of ecosystem. As a part of the ecosystem, they compete for food with other biota and in turn they cause various infections to vegetation and make it livestock and humans. At the same time they may cause direct injury to the plants by feeding on leaves or burrowing in stems, fruits or roots leading to loss in crop yield. There are hundreds of insect/pest species of this type which in both larval and adult stage harm the crops. They can also cause indirect damage in which insect itself does little or no harm but transmits bacterial, viral or fungal infection into a crop. These insect pests have several enzymes and each of these enzymes plays a key role in their life processes at one or the other stage for eg. Acetylcholineesterase, aconitase, GABA, Acetyl COA carboxylase, mitochondrial ATP synthase etc. All of these enzymes are critical for the normal metabolism of these insects. If the normal functioning of these enzymes is disturbed, their normal life cycle may also be affected thereby causing death of the insect. In the present study we focused only on enzyme acetylcholinesterase (Ache). Ache is one of the most essential enzyme in the family of Serine hydrolases. It is a substrate specific enzyme that catalyzes the hydrolysis of the neurotransmitter acetylcholine (Ach) in the nerve synapses. Thus, acetylcholine is the ultimate substrate of Ache. An optimum level of Ach should be maintained in the brain for its proper function. An increased and decreased level of Ach disturbs the functioning of brain. Thus Ache performs a important function in the nervous system of all organisms including insects and alterations created in its action can lead to disturbance in the nervous system and will ultimately effect the life cycle of organism concerned. Therefore, some insecticides are so designed that they inhibit the activity of Ache leading to increased level of Ach in brain. Two groups of insecticides that are actively used to inhibit the activity of Ache are organophosphates and carbamates. Both these inhibit Ache but have different mechanism of action. The inhibition of Ache by organophosphorus ester takes place *via* a chemical reaction in which the serine hydroxyl moiety present in the active site of enzyme is phosphorylated in a manner analogus to the acetylation of Ache. In contrast to the acetylated enzyme, which rapidly break down to give acetic acid and regenerated enzyme, the phosphprylated enzyme is highly stable, and in some cases, depending on the group attached to the phosphorus atom it is irreversibly inhibited. Similarly carbamates inhibit the Ache by carbamylation of the serine hydroxyl and thus resulting in the inhibition of the enzyme. Thus, the phosphorylated and carbamylated enzyme is no longer capable of hydrolyzing Ach and results in the buildup of Ach at a nerve synapse or neuromuscular junction leading to the abnormal functioning of the nervous system of the insects. The following work is aimed to find out the lead molecules which can act as inhibitor for the Ache of the insects as well as are non-toxic for the non targeted organisms especially human beings.

Methodology

1. Retrieval of Structure of the receptor

The 3D structure of the acetylcholinesterase of *Drosophila melanogaster* was retrieved from protein data bank (PDB) (PDB ID -1dx4).

2. Preparation of library of small molecules (ligands)

A combinatorial library of 6199 molecules was prepared using ZINC database. Library was prepared from clean leads of the ZINC database, by filtering the database on the basis of properties like molecular weight, xlogP, apolar desolvation, polar desolvation, of the known inhibitors of the Ache. Minimun and maximum values that were used to filter the database are shown below:

Table 1: criteria that was applied on the properties to filter the ligands from ZINC database

PROPERTY	MINIMUM VALUE	MAXIMUM VALUE
X Log P	-2.97	6.13
Apolar desolvation (kcal/mol)	-3.08	22.7
Polar desolvation (kcal /mol)	-44.64	-7.27
H-bond donor	0	2
H-bond acceptor	2	7
Net charge	-1	0
Molecular weight(g/ mol)	141.32	466.479
Rotatable bond	3	14
Polar surface area(a^{o2})	18	74

3. Preparation of the receptor: program chimera UCSF was used to prepare the receptor (1dx4) as input for DOCK calculations. Ligands, solvent molecules that were already present with the enzyme were deleted and along with this hydrogen atoms and partial charges were also added, making the receptor ready for sphere generation.

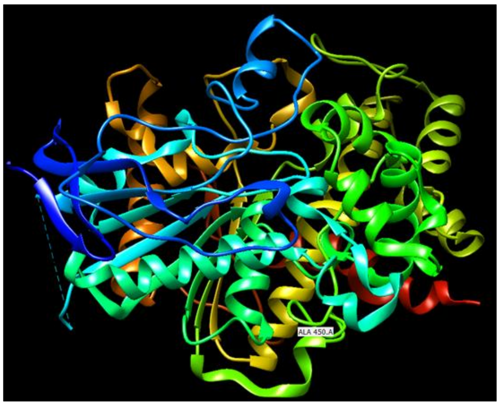

Fig 1: Ache (1dx4) after addition of hydrogen and partial charges

4. Sphere generation

the molecular surface of the target was generated using write DMS tool of the chimera UCSF. The spheres from molecular surface were generated with the help of sphgen program, an accessory of DOCK. Out of total 111 clusters generated, cluster 4 containing 35 spheres was selected as docking site as the said cluster contains the atoms that constitute the active site of the enzyme.

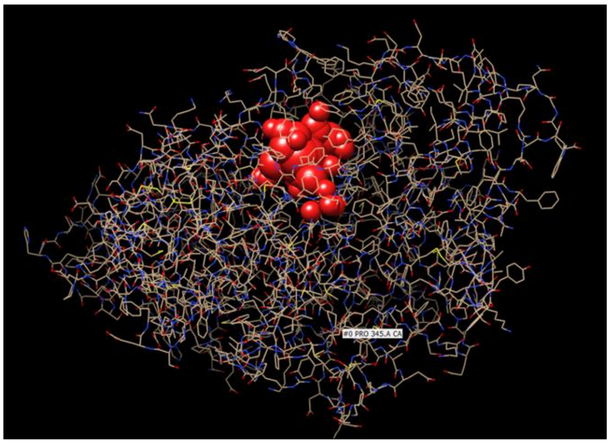

Fig 2: position of the cluster 4(active site) in the enzyme 1dx4

5. Grid generation

A box with dimensions of 31 A^o ×31 A^o ×31A^o was created around the active site of the enzyme for restricting the docking area.

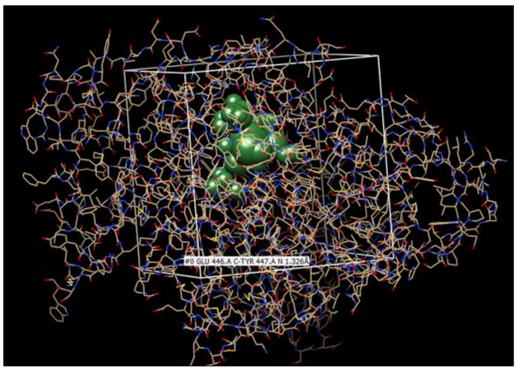

Fig 3: box with dimensions of 31 A^o ×31 A^o ×31A^o containing the cluster 4

The scoring grids were stored in the contact, energy and bump files ending with *.cnt, *.nrg and *.bmp extensions respectively. When docking, each scoring function is applied independent of the others and the results are written to separate output files.

6. Molecular Docking

the program dock6 which is distributed with DOCK was utilized for carrying out the molecular docking. Input parameters thatwere used for dock6 program are shown below

ligand_atom_file	**/home/neha/Downloads/ligands/ligands.mol2**
limit_max_ligands	no
skip_molecule	no
read_mol_solvation	no
calculate_rmsd	yes
use_rmsd_reference_mol	no
use_database_filter	no
orient_ligand	yes
automated_matching	yes
receptor_site_file	/home/neha/Downloads/dock6/bin/selectedsphere.sph
max_orientations	100
critical_points	no
chemical_matching	no
use_ligand_spheres	no
use_internal_energy	yes
internal_energy_rep_exp	12
flexible_ligand	no
bump_filter	yes
bump_grid_prefix	grid
max_bumps_anchor	2
max_bumps_growth	2
score_molecules	yes
contact_score_primary	no
contact_score_secondary	no
grid_score_primary	yes
grid_score_secondary	no
grid_score_rep_rad_scale	1
grid_score_vdw_scale	1
grid_score_es_scale	1
grid_score_grid_prefix	grid
multigrid_score_secondary	no
dock3.5_score_secondary	no
continuous_score_secondary	no
descriptor_score_secondary	no
gbsa_zou_score_secondary	no
gbsa_hawkins_score_secondary	no
SASA_descriptor_score_secondary	no
amber_score_secondary	no
minimize_ligand	yes
simplex_max_iterations	1000
simplex_tors_premin_iterations	0
simplex_max_cycles	1
simplex_score_converge	0.1
simplex_cycle_converge	1.0
simplex_trans_step	1.0
simplex_rot_step	0.1
simplex_tors_step	10.0
simplex_random_seed	0
simplex_restraint_min	no
atom_model	all
vdw_defn_file	/home/neha/Downloads/dock6/parameters/vdw_AMBER_parm99.defn
flex_defn_file	/home/neha/Downloads/dock6/parameters/flex.defn
flex_drive_file	/home/neha/Downloads/dock6/parameters/flex_drive.tbl
ligand_outfile_prefix	rigid
write_orientations	no
num_scored_conformers	1
rank_ligands	yes

7. Adme-Toxicity Prediction
program T.E.S.T was used to check the developmental toxicity of the best docked ligands.

Results and discussion
Out of 6199 molecules that were docked against the receptor, top 500 molecules with the lowest grid score were chosen to be the best docked ligands. The grid score of these 500 compounds ranges from -26.058649 to -46.723248.These selected ligands were further analysed for ADME-TOXICITY. Out of the 500 molecules that were tested for developmental toxicity only the molecules enlisted in table 1 were found to be developmental non- toxicant. And grid score of these non-toxicant ligands falls within the range of the grid score for the known inhibitors of the Ache (as shown in table 2), so these compounds may serve as potential lead molecules for further investigation.

Table2: developmental non-toxicant ligands with their ZINC IDs and grid score which is sum of grid van der walls (grid_vdw) and grid electrostatic (grid_es) score.

S.NO	ZINC ID	GRID_vdw	GRID es	GRId SCORE
1.	ZINC06205620	-34.569965	-6.360187	-40.930153
2.	ZINC00477670	-32.559513	-3.456164	-36.015678
3.	ZINC0301649	-29.604439	-5.370246	-34.974686
4.	ZINC03093776	-31.312859	-3.533874	-34.846733
5.	ZINC03884546	-30.692122	-2.786835	-33.478958
6.	ZINC98607992	-30.011175	-3.246643	-33.257816
7.	ZINC08729968	-29.394236	-3.745631	-33.140068
8.	ZINC43827756	-27.183678	-5.882127	-33.065804
9.	ZINC00333995	-29.977016	-3.065800	-33.042816
10.	ZINC00166278	-29.809349	-3.218256	-33.027607
11.	ZINC12336452	-27.277872	-5.729635	-33.007507
12.	ZINC12336325	-29.737612	-3.160244	-32.897854
13.	ZINC03052393	-23.513195	-9.315744	-32.828941
14.	ZINC91626008	-28.735294	-3.745988	-32.481281
15.	ZINC43827581	-30.615202	-1.811385	-32.426586
16.	ZINC32911298	-26.913084	-5.451757	-32.364811
17.	ZINC08670302	-29.421093	-2.921671	-32.342762
18.	ZINC03164230	-27.805542	-4.423042	-32.228584
19.	ZINC36007031	-24.206549	-8.018499	-32.225048
20.	ZINC00169827	-31.565145	-0.551237	-32.116383
21.	ZINC00078287	-26.009140	-5.549062	-31.558201
22.	ZINC4382817	-25.630413	-5.908417	-31.538830
23.	ZINC34450518	-26.947472	-4.343295	-31.290766
24.	ZINC43827910	-27.801559	-3.358407	-31.159967
25.	ZINC18179951	-29.486115	-1.673462	-31.159576
26.	ZINC02567128	-26.041773	-4.989551	-31.031322
27.	ZINC01686242	-27.423388	-3.588266	-31.011654
28.	ZINC00169808	-29.214743	-1.593172	-30.807915
29.	ZINC00170272	-27.672369	-3.058184	-30.730553
30.	ZINC18249651	-26.957809	-3.616042	-30.513853
31.	ZINC0383519	-28.189976	-2.350101	-30.540077
32.	ZINC72457156	-26.351589	-4.125213	-30.476803
33.	ZINC20246730	-24.875189	-5.533664	-30.408845
34.	ZINC14008096	-27.627542	-2.662513	-30.290054
35.	ZINC15919789	-25.705900	-4.449705	-30.155605
36.	ZINC00270582	-27.38722	-2.716162	-30.054884
37.	ZINC01095288	-27.997849	-2.032580	-30.030430
38.	ZINC03885162	-25.787922	-4.221018	-30.008940
39.	ZINC20246724	-2.655993	-6.346838	-30.002831
40.	ZINC34541063	-27.016802	-2.897913	-29.914715
41.	ZINC06293463	-24.902861	-4.983112	-29.885973
42.	ZINC03884306	-25.466469	-4.358174	-29.824642
43.	ZINC00169484	-23.123234	-6.660034	-29.783268
44.	ZINC08729737	-24.825073	-4.896463	-29.721537
45.	ZINC00167611	-26.523102	-3.114714	-29.637815
46.	ZINC01394846	-26.164135	-3.300260	-29.464394
47.	ZINC00169383	-24.160492	-5.156468	-29.316959
48.	ZINC12336455	-25.251989	-3.959017	-29.211006
49.	ZINC00169340	-23.077948	-6.090942	-29.168890
50.	ZINC00167418	-24.718792	-4.303121	-29.021912
51.	ZINC00110481	-24.66486	-4.866215	-28.932701
52.	ZINC00053159	-23.679676	-5.118351	-28.798027
53.	ZINC08729980	-24.949219	-3.834661	-28.783880
54.	ZINC00153480	-2.755901	-5.002867	-28.758768
55.	ZINC03276907	-26.038979	-2.685035	-28.724014

56.	ZINC03883332	-23.700750	-5.011236	-28.711987
57.	ZINC00169269	-27.750309	-0.958693	-28.709002
58.	ZINC12336461	-26.412075	-2.261765	-28.673840
59.	ZINC95529861	-24.126379	-4.294539	-28.420918
60.	ZINC63110855	-25.993254	-2.408064	-28.401318
61.	ZINC01406459	-27.289051	-1.101953	-28.391005
62.	ZINC0650167513	-24.171265	-4.151603	-28.322868
63.	ZINC03228258	-24.571165	-3.677851	-28.249016
64.	ZINC00158918	-26.426979	-1.770891	-28.197870
65.	ZINC08730207	-28.044395	-0.151646	-28.196041
66.	ZINC00168151	-24.940968	-3.159984	-28.100952
67.	ZINC00170275	-25.927147	-2.121030	-28.048176
68.	ZINC02163750	-23.999456	-4.028463	-28.027920
69.	ZINC63110854	-25.179968	-2.803339	-27.983307
70.	ZINC12336564	-22.955420	-4.906759	-27.862179
71.	ZINC20246733	-24.376238	-3.478530	-27.854767
72.	ZINC06183801	-24.669735	-3.183522	-27.85326
73.	ZINC03885559	-23.899265	-3.932860	-27.832125
74.	ZINC12336462	-25.560513	-2.108775	-27.669287
75.	ZINC02384436	-28.603092	0.953713	-27.649380
76.	ZINC00378381	-24.401205	-3.184525	-27.585730
77.	ZINC989602534	-21.194975	-6.375389	-27.570364
78.	ZINC12336514	-26.757618	-0.777774	-27.535393
79.	ZINC08781870	-27.043352	-0.405603	-27.468954
80.	ZINC03883550	-23.019615	-4.078003	-27.097618
81.	ZINC00170209	-23.472584	-3.579739	-27.052322
82.	ZINC66323419	-24.000708	-3.025113	-27.025822
83.	ZINC39018450	-23.023013	-3.657316	-26.680389
84.	ZINC91426509	-22.683739	-3.921895	-26.665633
85.	ZINC05439951	-24.384628	-2.251575	-26.63204
86.	ZINC00153453	-23.668455	-2.958514	-26.626968
87.	ZINC00167745	-22.440626	-3.868490	-26.309120
88.	ZINC08730087	-24.795477	-1.442480	-26.237957
89.	ZINC08729791	-26.92664	0.736156	-26.190487
90	ZINC40456635	-25.826941	-0.357485	-26.184425
91.	ZINC03263976	-25.866034	0.206168	-26.072201

Table 3: Grid score of the known inhibitors of the acetylcholinesterase

S. NO	ZINC ID	GRID_vdw	GRID_es	GRID SCORE
1.	ZINC02034740	-30.335100	-15.263934	-45.599033
2.	ZINC01590886	-30.036537	-1.579863	-44.616402
3.	ZINC02039915	-23.6310069	-17.125387	-40.756454
4.	ZINC00900715	-26.850388	-13.881322	-40.731709
5.	ZINC02040888	-31.279955	-9.230075	-40.510029
6.	ZINC2031565	-27.155378	-12.786576	-39.941956
7.	ZINC01530799	-38.124130	-1.457214	-39.581345
8.	ZINC01587404	-24.810219	-14.544174	-39.354393
9.	ZINC02035982	-26.592115	-12.370466	-38.962582
10.	ZINC02014295	-25.315798	-13.181273	-38.497070
11.	ZINC13827879	-24.935621	-13.446167	-38.381796
12.	ZINC13531994	-31.320381	-6.765086	-38.085468
13.	ZINC02040123	-32.906582	-5.097175	-38.003757
14.	ZINC00900772	-33.141869	-4.480683	-37.622551
15.	ZINC02034488	-21.055012	-16.465862	-37.520874
16.	ZINC02041343	-25.123188	-11.225976	-36.349163
17.	ZINC05734608	-27.246298	-8.577759	-35.824059
18.	ZINC03874471	-29.101372	-6.246050	-35.347424
19.	ZINC02036016	-30.111372	-5.071684	-35.183056
20.	ZINC00001934	-31.973572	-2.791286	-34.764858
21.	ZINC01849749	-24.660295	-9.293062	-33.953358
22.	ZINC00896311	-31.284468	-2.301396	-33.585865
23.	ZINC31881392	-29.292040	-4.1522929	-33.444969
24.	ZINC01590889	-29.685209	-3.654871	-33.3400880
25.	ZINC02011298	-29.564171	-3.768016	-33.332188
26.	ZINC03874232	-14.658882	-18.138012	-32.796894
27.	ZINC02545289	-26.980169	-5796215	-32.776382
28.	ZINC01849744	-28.260662	-40458489	-32.719151
29.	ZINC01693865	-27.438858	-4.828415	-32.267273

30.	ZINC00056963	-28.033615	-4.150985	-32.184601
31.	ZINC02036014	-28.289717	-3.784123	-32.073841
32.	ZINC05922461	-24.390873	-7.669128	-32.060001
33.	ZINC02003718	-23.571722	-8.129255	-31.700977
34.	ZINC05860119	-23.331947	-8.170019	-31.501966
35.	ZINC91693008	-28.790195	-2.627421	-31.417616
36.	ZINC02038416	-25.463457	-5.887455	-31.417616
37.	ZINC015321114	-25.372267	-5.936839	-31.309165
38.	ZINC01590885	-27.778057	-3.452426	-31.230484
39.	ZINC03874261	-25.376625	-5.834987	-31.211613
40.	ZINC02019782	-26.722643	-4.377883	-31.100527
41.	ZINC01530958	-24.757486	-6.294577	-31.052063
42.	ZINC00001443	-22.861067	-8.105390	-30.966457
43.	ZINC35328278	-30.400133	-0.170072	-30.570206
44.	ZINC004001576	-26.796009	-3.640451	-30.436460
45.	ZINC02015426	-27.145056	-3.083387	-30.228443
46.	ZINC02019828	-25.851368	-3.993394	-29.844761
47.	ZINC02040889	-26.107519	-3.84028	-29.791546
48.	ZINC00001090	-24.769737	-4.929896	-29.699635
49.	ZINC02018990	-22.607317	-7.021122	-29.628439
50.	ZINC00608250	-27.548439	-1.470949	-29.019388
51.	ZINC03977733	-21.661783	-7.141456	-28.803240
52.	ZINC00002006	-21.661783	-7.141456	-28.803240
53.	ZINC01667585	-24.595045	-3.810468	-28.405514
54.	ZINC00900708	-19.677322	-8.650154	-28.327477
55.	ZINC020388307	-26.384859	-1.832522	-28.217381
56.	ZINC00001434	-20.601980	-6.988829	-27.590809
57.	ZINC00135179	-24.305195	-3.265555	-27.570749
58.	ZINC02038975	-25.988283	-0.784930	-26.773212
59.	ZINC01853866	-23.481167	-1.774195	-25.255362
60.	ZINC01648341	-19.215219	-4.547895	-23.763115
61.	ZINC02009586	-21.467396	-1.710228	-23.117624
62.	ZINC00900687	-22.615202	-0.25631	-22.871513
63.	ZINC00001309	-21.047499	-1.222937	-22.270435
64.	ZINC02006793	-17.938263	-3.604666	-21.542929
65.	ZINC02014879	-18.765406	0.481818	-18.283587
66.	ZINC00900717	-11.248289	-6.476459	-17.724747

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