Available online at www.jcpronline.in

Journal of Current Pharma Research 5 (4), 2015, 1632-1639.

Journal of Current Pharma Research
www.jcpronline.in

Original Research Article

Role of Aromatic Amino Acids in Stabilizing Organophosphate and Human Acetylcholinesterase Complex.

Anuj Ranjan*, Ashwani Kumar, Khushbu Gulati, Shalini Thakur, Tanu Jindal

Amity Institute of Environmental Toxicology, Safety and Management; Amity University, Noida - 201303, Uttar Pradesh, India.

Received 20 May 2015; received in revised form 18 August 2015; accepted 19 August 2015
Available online 04 October 2015

Abstract

Organophosphate (OP) poisoning induces inhibition of acetylcholinesterase (AChE) thereby accumulation of a neurotransmitter acetylcholine in synaptic gap. Simulated binding of OPs with human AChE was performed to study mechanistic insight of binding and inhibition. High precision flexible ligand docking of seventy-five OPs with human AChE enzyme (HuAChE) individually reveals binding energies of OPs; lowest in Pyraclofos followed by Phenthoate acid, Prothiophos, Methyl isofenphos, Sulprophosoxon and Propaphos. Aromatic amino acids such as Trp86, Phe295, Arg296, Tyr337, Phe338, Tyr341 etc were noticed in Pi interactions due to partial negative charges on surface of aromatic group. Pi-cation interactions were most favored by OPs and were contributed by Trp86 due to presence of anionic indole ring. Although Phe295, Phe298 and Tyr341 were also involved in Pi -sigma or Pi - Pi interactions, Trp86 played a key role in amino acid stabilizing OP-HuAChE complex by Pi-cation interaction. Study suggested that amino acid Trp86 has provided a most potent Pi-cation binding site. Trp86 along with other aromatic residues played crucial role in stabilizing organophosphate-human acetylcholinesterase complex thereby inhibition of target enzyme.

Keywords: Human Acetylcholinesterase (HuAChE); Ops, docking, amino acids, aromatic.

1. Introduction

Organophosphates (OP) are esters or thiols derived from phosphoric, phosphonic, phosphinic or phosphor- amidic acid of broad class insecticides extensively used to enhance agricultural produce and household pest control. A total of about 890 active ingredients are registered as pesticides in USA and currently marketed in some 20,700 pesticide products. World pesticide expenditures totaled more than $39 billion in 2007[1]. Some OPs have medical importance, e.g. diisopropyl phosphorofluoridate (DFP), tetraethyl pyrophosphate (TEPP), and octomethyl pyrophosphotetramide (OMPA) for the treatment of myasthenia gravis and an organophosphate esteret ecothiopate, still being used to treat glaucoma [2, 3, 4].

Corresponding author
E-mail address: aranjan@amity.edu
(Anuj Ranjan)

They are also used as plasticizers, stabilizers in lubricating and hydraulic oils, flame retardants, and gasoline additives [5].

General population is inevitably exposed to OP pesticides since residues of many of them are environmentally persistent. Every year thousands of individuals suffer OP poisoning from dietary, household, accidental or occupational exposure [6]. The extensive application or mishandling of these compounds result in deleterious health hazards including numerous documented cases of human fatalities and also associated with serious human toxicity, accounting for more than 80% of pesticide-related hospitalizations [7]. The use of OP have increased considerably due to their low persistence in the mammalian system compared to organochlorine pesticides [8] but indiscriminate and excessive application has lead to the common occurrence of residues in

food crops, natural water systems, soil and atmosphere, which are considered as the major sources of exposure[9,10,11].

In technical words organophosphates refer to a group of insecticides and nerve agents that inhibit AChE [12]. AChE is a serine hydrolase enzyme mainly available at neuromuscular junctions and cholinergic brain synapses. Its main biological role in body is to terminate cholinergic impulse transmission by hydrolyzing the neurotransmitter acetylcholine to acetate and choline [13]. Because of the ubiquity of cholinergic neurotransmission in the animals, AChE is the target of numerous pesticides, including organophosphate and carbamate insecticides. AChE inhibition leads to accumulation of acetylcholine at the synapses causing cholinergic hyper stimulation and neurotoxicity followed by loss of metabolic balance which may lead to death in absence of any effective treatment [14]. AChE is very catalytic in nature [15] and being coded by AChE gene on chromosome 7 at 7q22 [16]. This polypeptide has 614 amino acid length of sequence which carries signal peptide of first thirty-one amino acids. The OPs exert their main toxicological effects through non-reversible phosphorylation of esterases in the central nervous system. The acute toxic effects are related to irreversible inactivation of AChE. OPs are substrate analogues to acetylcholine, and like natural substrate enter the active site covalently binding to serine –OH group. During acetylation, OP is split and the enzyme is phosphorylated, Phosphate radicals of OP bind covalently to the active sites of the cholinesterase, transforming them into enzymatically inert proteins [7, 17]. Dephosphorylation of AChE is very slow (on the order of days), and phosphorylated enzyme cannot hydrolyze the neurotransmitter which leads to accumulation and continuous transmission of signals [18].

HuAChE has an ellipsoidal shape with dimensions ~ 45 Å by 60 Å by 65 Å. The enzyme is monomer and has 12 stranded central mixed β sheets surrounded by 14 α helices. The most remarkable feature of the structure is a deep and narrow gorge ~ 20 Å long penetrating halfway into the enzyme and widens out close to its base [19] which is lined with aromatic amino acids that compose various subsites: the hydrophobic patch containing the choline binding site and the hydrophobic site for the alkoxy leaving group of the substrate, the peripheral site, and the acyl pocket. The narrowest part of the gorge, the bottleneck, is about halfway down the gorge and is comprised of the aromatic amino acids Tyr121, Phe330, and Phe331 in *Torpedo californica* AChE [20]. Sussman's structure of huAChE of 2.8 Å resolution on X ray diffraction [20] provided valuable insights on many fronts, not the least of which is the cation-ð interaction. The active site lies at the bottom of a deep, narrow gorge, a substantial portion of which is lined by 14 conserved aromatic residues. At the active site, the quad of ACh is in contact with the side chain of the highly conserved Trp-84. AChE enzyme and its inhibitors is target to many X-ray crystallographic and molecular modeling studies. The X-ray crystal structures of AChE from different species have been documented in the protein data bank e.g. 1EVE [24]. A number of ligands have been co-crystallized with the enzyme. However, X-ray crystallographic structure of human AChE with OPs has not been documented so far, so further details of binding mechanism between the two are very limited. This research has focused on role of active site aromatic amino acids in inhibition and binding pattern of huAChE with OPs. The study included random selection of seventy five OPs commonly marketed in India, considering exposure to the population is random. Model of HuAChE (PDB ID- 1B41) was used as reference model to execute OP-HuAChE interaction study.

2. Materials and Methods

2.1 Tools used for editing molecules, molecular dynamics and docking

Discovery studio visualizer 3.1 was used to analyze ligand-HuAChE complexes and also to edit enzyme molecules prior to docking. Argus lab 4.0.1 was used to run docking program which allows flexible as well as rigid docking. Marvin sketch was used to generate lower energy conformers and Chimera was used for molecular dynamic simulation and minimization program.

2.2 Preparation of Protein model

3D model of HuAChE based on the crystal structure of human AChE enzyme was retrieved from Protein Data Bank, PDB ID 1B41. Enzyme was complexed with a snake venom toxin fasciculin-Ⅱ and three other ligands alpha-l-fucose, n-acetyl-d-glucosamine and 2 - (acetylamino) – 2 – deoxy – a - d-glucopyranose. In order to recover the structure of the enzyme from the above complex, ligands were removed using a molecular editor (Discovery studio visualizer) and hydrogen atoms were added to the enzyme. Model was optimized by energy minimization. Hundred steps of each minimization steepest decent was carried out followed by conjugate gradient.

2.3 Active site analysis

By considering the experimental facts that the active site of AChE includes Ser203, Glu334, and His447, it was found that this site was composed of 29 residues (Kryger et al. 2000 and Zheng et al. 2009). Figure 1 represents the binding site (Highlighted residues).

These residues are: Gln71-Tyr-Val-Asp-Thr-Leu76, Gly82-Thr-Glu84, Trp86- Asn-Pro88, Leu130, Tyr133, Glu202-Ser-Ala204, Trp286, Phe295, Phe297, Glu334, Tyr337-Phe338, Tyr341, Trp439, His447-Gly-Tyr449, and Ile451.

2.4 Ligand preparation

Seventy-five OP molecules were retrieved from pubchem library. Using Marvin sketch tool, lowest energy conformers of each was generated and saved as .mol2 format making them compatible for Argus lab program.

2.5 Docking studies

Docking studies were performed by Argus lab 4.0.1 software which is a freeware provided by Planaria Software LLC. Grid docking was allowed at 0.4 Å resolutions and 150 maximum numbers of poses with high precision flexible ligand dock. Binding site of AChE was composed of 29 amino acids on X=23.634, Y=26.89, Z=24.627 Å coordinates.

3. Results and Discussion

OP molecules were simulated individually for interacting *in–silico* with HuAChE enzyme on a defined binding site of ~27Å. Figure 1 represents model of HuAChE and binding site.

3.1 Dock score

Best dock score that is lowest binding energy were exhibited by Pyraclofos (-12.7036 Kcal/mol) followed by Phenthoate acid (-12.5184 Kcal/mol), prothiophos (-12.4092 Kcal/mol), Methylisofenphos (-11.927 Kcal/mol), Sulprophosoxon (-11.707 Kcal/mol) and Propaphos. table 1 includes list of OPs used to perform docking. Pyroclofos which exhibited best dock score binds on AChE's binding site by one Hydrogen bond contributed by Hydrogen on Phenyl group of Tyr124 to O4. Interestingly three interactions were stabilizing the complex; PI-PI: Benzene ring of Pyraclofos and imidazole ring of His447, PI-cation: Tyr334- central phosphorous of Pyraclofos and PI-sigma was observed in Trp236-H37 of Pyraclofos.

3.2 Amino acids contributing OP-HuAChE binding

Analyzing all individual OP-AChE complexes, study infers that OP prefers anionic binding site composed of two pockets connected by a narrow gorge. Amino acids on anionic pockets are preferably Phe295, Arg296, Tyr337, Phe338, Tyr341, His447 etc. Amino acid Tyr124 and Tyr337 played an important role in contributing H-bonds with most of the OP ligands, cases of such bonding were observed with Ser125 and Gly121 also. Tyr124 and ser125 were not included on binding site still they were showing good frequency while interacting with ligands. Anionic surface of indole ring of Trp86 helped in contributing maximum Pi-cation interactions although, several Pi interaction were noticed with Phe295,Tyr337 and Tyr341. Figure 1 explains the contribution of binding site amino acids in OP-HuAChE binding.

3.3 Pi interaction and role of aromatic amino acids

Aromatic amino acids which have negative charge clouds over the rings are helpful in stabilizing OP-HuAChE complexes. Trp86 provided maximum of both Pi-Pi and Pi-cation interaction that is six and fifty one respectively in number. Maximum number of Pi-sigma that is seven was provided by Tyr341. This amino acid was also noticed in backing Pi-cation followed by Phe295 after Trp86. Amino acids Gly122, Ser125, Gly121, Ser203, Tyr124 etc played an important role in binding of OPs with

AChE by means of hydrogen bonds, but aromatic amino acid such as Trp86, Tyr337, Phe338, Tyr341 etc also played excellent role in stabilizing OP-AChE complexes by contributing Pi-Pi, Pi-cation, Pi-sigma interactions. Role of Trp86 was surprisingly active in providing electrostatic potential between indole ring of tryptohan and central cation of the OPs there by forming Pi-cation interaction.

A similar theoretical model of Ho¨ltje and Kier also suggested that an aromatic ring could play the role of the anion at the binding site [21]. Studies on the esterase by Cohen also downplayed the anionic nature of the binding site, recognizing that it must be somewhat hydrophobic and suggesting the term "trimethyl" subsite. [22, 23] In 1990, studies on cyclophane binding of quats led us to propose that cation-*ð* interactions would be important in binding ACh.

Conclusion

Analyzing all individual OP-AChE complexes, it seems convincing to say that, OP prefer anionic binding site composed of two pockets connected by a narrow gorge on HuAChE. Anionic pocket is lined by aromatic amino acids on anionic pockets are preferably Phe295, Arg296, Tyr337, Phe338, Tyr341, His447 etc and Trp86 on the neck of the gorge. These amino acids are actively stabilizing Pi interactions. Anionic surface of aromatic ring of these amino acids help in contributing Pi interaction. Trp86 was noticed as key involvement in Pi-cation interaction since sixty eight percent of OPs were stabilized by Pi -cation interaction. On an average sixty eight percent of the OPs are found to have Pi-cation interaction led by Trp86. The flat face of aromatic rings has partial negative charge owing to the Pi electrons. Positive charge deficit of central atom of OP and partial negative charge on rings of aromatic amino acids are helpful in creating electrostatic potential by stabilizing Pi -cation interaction

References

1. US EPA Pesticide Industry Sales and Usage: 2006 and 2007 Market Estimates, updated Oct 2012.
2. Grob, D and Harvey, A.M. Bull Johns Hopkins Hospital. 84 (1949) 533-567.
3. Rider, J.A., Schulman,S., Richtern,R.B., Moeller, H.D. and DuBois, K.P., The J Amer Med Asso. 145 (1951) 967-972.
4. Comroe,J.H., Todd, J., Gammon,G.D, Leopold, I.H., Koelle,G.B. and Bodansky,O. Amer J Med sci. 212(1946) 641-651.
5. De-Bleeker, J.L., DeReuck, J.L. and Willems, J.L. Neurological aspects of organophosphate poisoning. Clin Neur and Neurosurg. 94 (1992) 93-103.
6. Nigg, H.N., Beier, R.C., Catrer, O., Chaisson, C., Franklin, C., Lavy, T., Lewis, R.G.,Lombardo, P., McCarthy, J.F., Maddy, K.T., Moses, M., Norris, D., Peck, C., Skinner, K. and Tardiff, R.G. The Effects of Pesticides on Human Health. S. R. Baker and C. F. Wilkinson, Edison,18: PP 35–130.
7. Haddad, L. and Winchester, J Clin Manag Poison Overdose, Philedelphia, WB Saunders.(1983) 575-586.
8. Kamath, V. and Rajini, P.S. Altered glucose homeostasis and oxidative impairment in pancreas of rats subjected to dimethoate intoxication. Toxicology231(2007**)**137-146.
9. Caldasa, E.D., De Souzab, M.V. and Jardim, A.N.O. Dietary risk assessment of organophosphorus and dithiocarbamate pesticides in a total diet study. Food Addit Contam.28(2011)71–79.
10. Ahmadi, F., Assadi, Y., Milani Hosseini, S.M.R. and Rezaee, M. Determination of organophosphorus pesticides in water samples by single drop microextraction and gas chromatography-flame photometric detector. J Chrom1101 (2006),307-12.
11. Schipper, P.N., Vissers, M.J. and Van-Der L.A.M. Pesticides in groundwater and drinking water wells: overview of the situation in the Netherlands. Water Sci Tech 8 (2008)1277-1286.
12. Bajgar, J., Kassa J., Pohanka, M., Karasova, J.Z,, Novotny, L., Fusek, F.

and Blah. V. Inhibition of blood and tissue cholinesterases by soman in guinea pigs in vivo. J Appl Med. 9 (2011) 35-41.

13. Gabriel Z. and Soreq, H. Termination and beyond: acetylcholinesterase as a modulator of synaptic transmission. Cell and Tiss Res.326 (2006)655-669.

14. Worek, F., Aurbek, N. and Thiermann, H. Reactivation of organophosphate-inhibited human AChE by combinations of obidoxime and HI-6 in vitro. J Appl Toxicol. 27(2007)582-588.

15. Quinn, D.M. Acetylcholinesterase: enzyme structure, reaction dynamics, and virtual transition states. Chem. Rev.87(1987) 955-979.

16. Getman, D.K., Eubanks, J.H., Camp, S., Evans, G.A. and Taylor, P. The human gene encoding acetylcholinesterase is located on the long arm of chromosome 7. Amer J Hum Gen. 51(1992)170–177.

17. Namba, T., Nolte, C.T., Jackrel, J. and Grob, D. Poisoning due to organophosphate insecticides.Acute and chronic manifestations. Amer J Med.50 (1971) 475–492.

18. Boublik, Y., Saint-Aguet, P., Lougarre, A., Arnaud, M., Villatte, F., Estrada-Mondaca, S. and Fournier, D. Acetylcholinesterase engineering for detection of insecticide residues. Prot Engin Des and Selec. 15(2002), 43-50.

19. Manavalan, P., Taylor, P. and Johnson Jr. W.C. Circular dichroism studies of acetylcholine sterase conformation. Comparison of the 11 S and 5.6 S species and the differences induced by inhibitory ligands.BBA-Prot Str and Mol Enzymol. 829,3 (1985) 365-370.

20. Sussman, J.L., Harel,M., Frolow,F., Oefner, C., Goldman,A., Toker,L. and Silman, I. Atomic structure of acetylcholinesterase from Torpedo californica: a prototypic acetylcholine-binding protein. Science.253 (1991**)** 872–879.

21. Ho¨ltje, H.D. and Kier, L.B. Nature of anionic or α-site of cholinesterase. J Pharm Sci. 64 (1975) 418-420.

22. Cohen S.G., Elkind, J.L., Chishti, S.B., Giner, J.L., Reese, H. and Cohen J.B. Effects of volume and surface property in hydrolysis by acetylcholinesterase. The trimethyl site. J Med Chem. 27(1984)643-1647.

23. Cohen, S.G., Chishti, S.B., Elkind, J.L., and Reese. H. Effects of charge, volume, and surface on binding of inhibitor and substrate moieties to acetylcholinesterase. J Med Chem. 28(1985**)**1309-1313.

24. Kryger, G., Silman, I. and Sussman, J.L. Structure of acetylcholinesterase complexed with E2020 (Aricept): Implications for the design of new anti-Alzheimer drugs. Structure. 7(1999**)** 297–307.

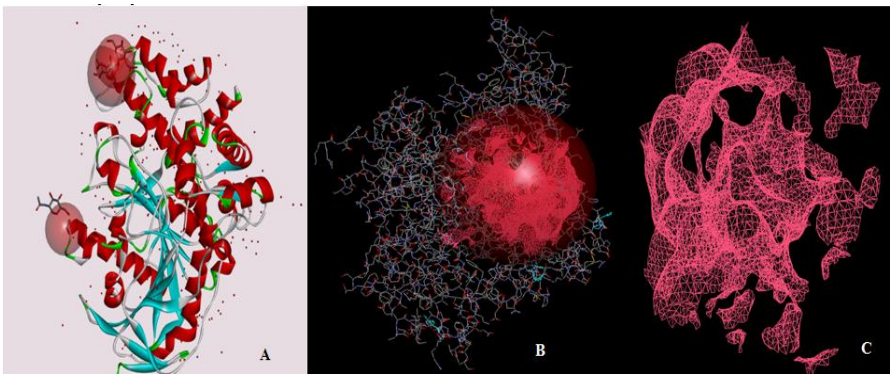

Figure 1. Model of HuAChE (PDB ID-1B41) (A) crystal structure in native state (B) amino acids on binding site highlighted within red sphere (C) wireframe depiction of binding site.

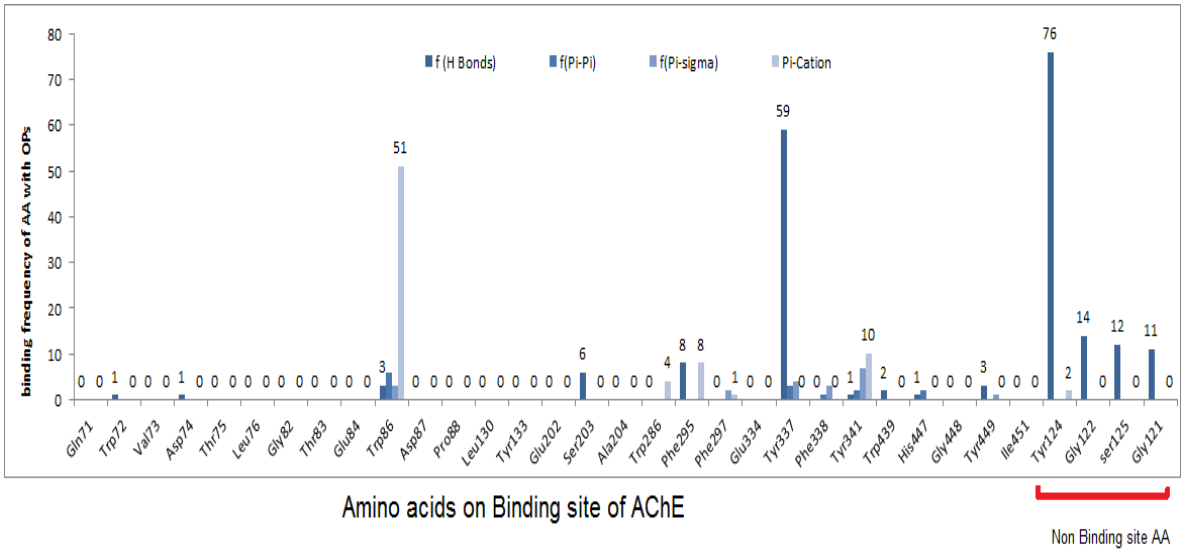

Figure 2. Bars showing contribution of active site amino acids in binding of OP ligands with HuAChE enzyme model. Amino acid Tyr124, Gly122, Ser125 and Gly121were not defined on binding site but they were found actively participating in H-bond network.

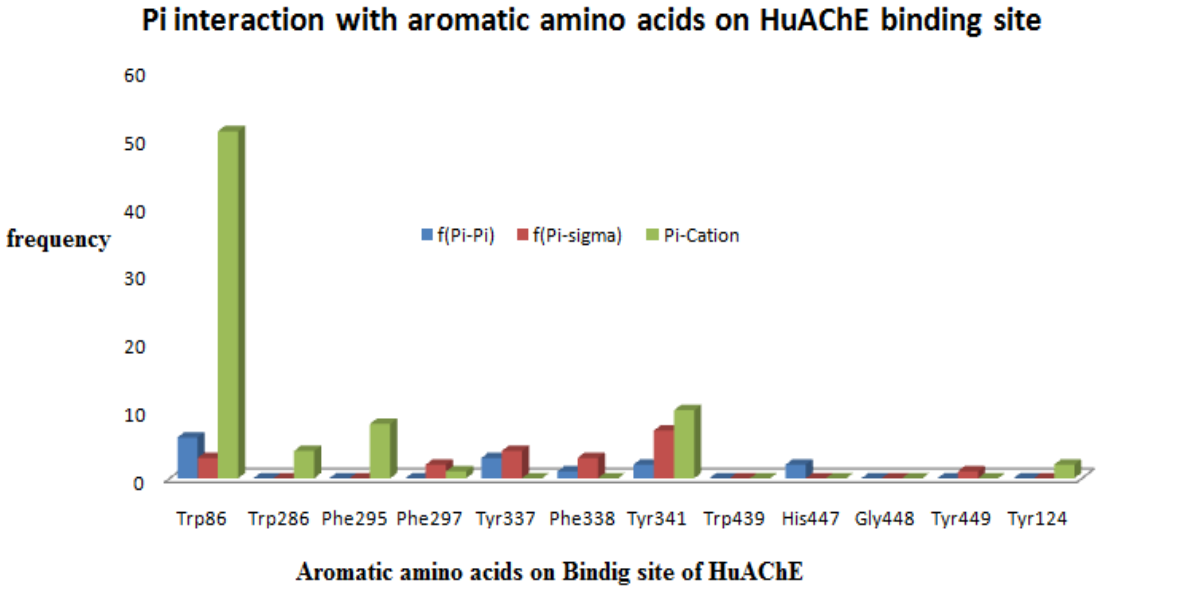

Figure 3. Bars showing contribution of aromatic amino acids on binding of HuAChE model with OP ligands. Amino acid Trp86, Trp286, Phe295,Tyr337,Phe338 and Tyr341 were actively involved in binding with OPs by Pi-cation, Pi-sigma or Pi-Pi interactions. Trp86 shows highest binding frequency by forming Pi-cation interaction with OPs.

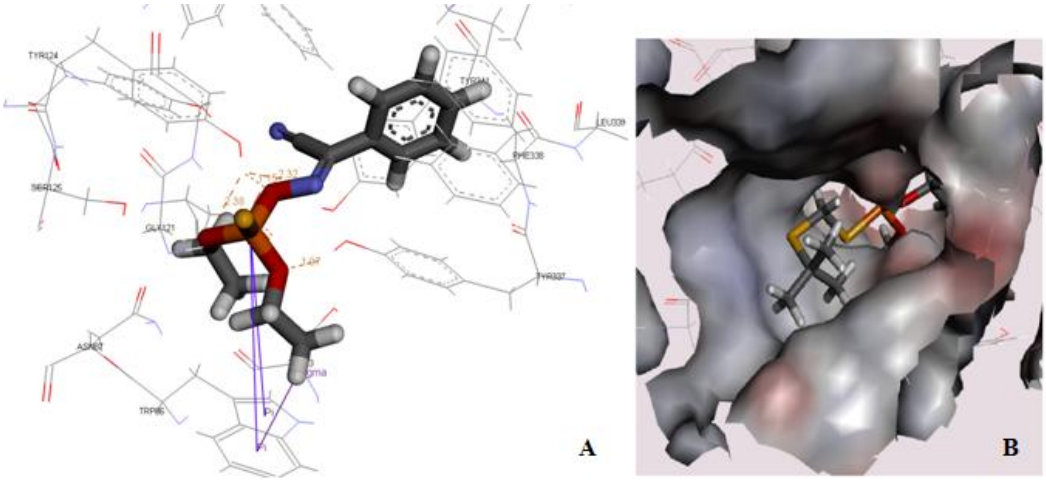

Figure 4. Trp-86 intaeracting with terbufos through Pi cation interaction. (A) Pi-cation bond hilighted in blue line (B) solid suface view of terbufos embded in binding pocket of HuAChE, benzene ring is on the other side of binding pocket

Table 1: Dock scores (in Kcal/mol), represent the lowest overall binding energy of the organophosphate ligands with human acetylcholinesterase enzyme model (PDB ID-1B41). Dock score of a ligand is determined by selecting best binding pose with minimum energy.

Organophosphates	Dock score (kcal/mol)	Organophosphates	Dock score (kcal/mol)
Pyraclofos	-12.7036	Phosmet	-9.62957
Phenthoate acid	-12.5184	Quinalphos	-9.5814
Prothiophos	-12.4092	Prothoate	-9.56214
Methyl isofenphos	-11.927	Pp-211 (pirimiphosethy)	-9.55637
Sulprophosoxon	-11.707	salithion	-9.556
Propaphos	-11.6749	Phoratoxonsulfone	-9.44407
Sulprofos	-11.5227	Malathion	-9.38016
Phenthoate	-11.4252	Quinalphos-methyl	-9.32877
Profenofos	-11.3512	Terbufossulfone	-9.77642
4-tert-butyl-2-chlorophenol	-11.2563	Acephate	-9.27297
Phoximo,o-diisopropyl phosphate	-11.0853	Thiometon	-9.27282
Phosmethylan	-10.9647	Phosmetoxon	-9.24824
Phoxim	-10.9621	4-aminofenitrothion	-9.11045
Propaphossulfoxide	-10.9449	S-[(tert-butylsulfinyl)methyl] o,o-diethyl phosphorothioate	-9.09675
Bromophos	-10.9221	Pirimiphos methyl	-8.94031
Temefos	-10.8382	Trans-dioxathion	-8.7646
Stirofox	-10.7713	Trans-phosphamidon	-8.73127
Plondrel	-10.7631	Propetamphos	-8.71535
Propaphossulfone	-10.7499	Terbufossulfoxide	-8.67139

Terbufos	-10.7473	Vamidothion	-8.66077
Methylephoxim	-10.7415	Sulfotepp	-8.63165
Phoxim ethyl phosphonate	-10.7029	(2,2,2-trichloro-1-hydroxyethyl)phosphonic acid	-8.52373
Phosalone	-10.6713	Methylsystoxsulfone	-8.52084
Phenthoateoxon	-10.5674	Phosphamidon	-8.46453
Tetrachlorvinphos	-10.5499	DSStox	-8.43271
Pyridafenthion	-10.4504	Cebetox	-8.27635
S-fonofos	-10.4272	Dichlorovos	-8.27097
R-fonofos	-10.3989	Oxydeprofos	-8.04162
Terbufos	-10.3944	Trichlorfon ethyl	-8.01142
Chloropyrifos	-10.3438	Tetraethyl pyrophosphate	-7.94173
Phorate	-10.2948	Phoratoxonsulfoxide	-7.86383
Pyridafenthion	-10.255	Phosphamidon amide	-7.84851
Phoratesulfone	-10.2515	Trichlorfon	-7.77697
2,5-dichloro-4-bromophenol	-10.2421	Phoratesulfoxide	-7.60604
Triazophos	-9.94821	Trans-mevinphos	-7.43863
Ronoxon	-9.93202	Trans-methacrifos	-7.43165
Phoratoxon	-9.83439	Thionazin	-7.3713
Diazinon	-9.6993		

Source of Support: Nil.

Conflict of Interest: None declared

****