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Regulation of Metabolic Syndromes by means of controlling diseased Ache and Bche with Multitarget Inhibitors through *in silico* techniques

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ABSTRACT

Alzheimer's and D2M are the metabolic syndromes that are interlinked due to interaction of regulatory proteins or enzymes such as AchE and BchE. The function of AchE and BchE relies on specific regulation on its expression and localization in biological systems has been proved experimentally, and the interaction study and docking with virtual ligands has been conducted in the present work. Prediction of the binding of a ligand to a target protein is important in computational drug design and discovery. Alzheimer's and D2M are the metabolic syndromes that are interlinked due to interaction of regulatory proteins such as AchE and BchE. Docking has been made for the energy minimized ligands with the AchE (2X8B.pdb) and BchE (2XMB.pdb) proteins using docking softwares such as AutoDock, Hex and iGemDock. Docking results predicted that Huperzine and Galanthamine have better drug activity with AchE protein. Docking results also predicted that Dibucaine and propionyl thiocholine have better drug activity with BchE.

Key words: Alzheimer's Disease, Diabetes Mellitus Type 2, Docking.
List of abbreviations. AD - Alzheimer's Disease, D2M - Diabetes Mellitus Type 2, AchE - Acetylcholinestarase, BchE - Butyrylcholinesterase

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INTRODUCTION

1.1 Enzymes involved in Alzheimer's Disease [AD] and Diabetes Mellitus Type 2 [D2M]

Based on previous reports, two enzymes AchE and BchE may be involved in relation with Alzheimer's and D2M.

1.1.1 Acetylcholinestarase (AchE)

Acetylcholinesterase (EC 3.1.1.7) belongs to family of α/β hydrolases which catalyses the hydrolysis of neurotransmitter acetylcholine (Ach) at the cholinergic synapse [1]. AchE is inhibited by excess of substrate and selectively by 1,5-bis(4-allyl dimethyl amminopropyl)pentan-3-ondibromide(BW284C51) [2]. The function of AchE depends on precise regulation on its expression and localization. Alternative splicing in the 3' region of the primary transcript generates the sub-units of AchE which contains same catalytic domain but distinct C-terminal peptides determine the post-translational maturation and oligomeric assembly [3].

1.1.2 Butyrylcholinesterase (BchE)

Butyrylcholinesterase (E.C.3.1.1.8) is a pseudocholinesterase and non-specific cholinesterase that have no specific physiological function. It hydrolysis acetylcholine as well as many other esters. BchE can be inhibited by 1,5-bis(4-allyl dimethyl amminopropyl)pentan-3-ondibromide (BW284C51) and N N'-di-isopropyl phosphorodiamidic anhydride. BchE is responsible for the hydrolysis of succinylcholine drug used in surgery as a short acting blocker of acetylcholine receptor. In case of AD the level of BchE raises with decrease progression 10-15% of ChE-positives cells human amygdale and hippocampus are regulated by BchE [4].

AD is because of accumulation of α-amyloid in brain leading to nerve cell death. In AD patients BchE levels increases aggravating the toxicity of β-amyloid peptide. BchE co-localize within the brain in amyloid plaques to form insoluble β-amyloid fibrils [5].

1. 2 Aging diseases

The role and activity of AchE and BchE in aging diseases like Alzheimer's disease and D2M is predicted in the present *in silico* analysis.

1. 2. 1 Alzheimer's Disease

The Alzheimer brain was first described by Dr. Alzheimer in 1906. Dr.Alzheimer described about senile plaques and neurofibrillary tangles (NFT's), later proteins that form plaques and NFT's were described which has proven paramount to understanding of the disease pathogenesis. AD therapeutic development are decreasing synthesis of Aβ and tau, preventing misfolding and aggregation of tau protein and neutralizing or removing the toxic aggregate or misfolds from proteins [6].

AD associated with loss of memory is characterised by low concentration of acetylcholine in the hippocampus and cortex part of the brain. Low amounts of acetylcholine in the hippocampus and cortex are considered as one of the cause for AD [7].

1.2.2 Diabetes Mellitus Type 2

D2M causes abnormal carbohydrate, lipid and protein metabolism associated with insulin resistance and impaired insulin secretion [8]. Changes in the activity of AchE and BchE shows effect on type 1 and type 2 diabetes. BchE may play a role in altering lipoprotein metabolism in hypertriglyceridaemia associated with insulin insensitivity or insulin deficiency [9]. D2M is a risk factor for AD because phosphorylation of tau protein at some of the AD abnormal hyper phosphorylation sites increased D2M and O-Glc-Nacylation levels of global proteins and tau proteins decreased D2M[10]. Glucose intolerance and diabetes are abnormal elevations of blood glucose which increases risk for micro vascular and macro vascular disease [11].

1.3 Protein DataBank (PDB)

Brookhaven National Laboratories (BNL) established the protein databank in 1971 as an archive for biological macromolecular crystal structure [12]. The data included in archive are as follows, atomic co-ordinates, NMR experimental data and crystallographic structure factors. Each of the depositions in PDB includes names of molecules primary, secondary structure information, sequence solution bibliographic citations and ligand and biological assembly information [13]. The processing of deposited data in PDB is done using software tools like Autodep Input tool (ADIT) and the Macromolecular Exchange and Input Tool (MAXIT) [14].

1.4 Docking

Prediction of the binding of a ligand to a target protein is a first step in computational drug design and discovery. The computational scheme for predicting ligand binding occurrence, affinity and orientation is referred to as "molecular docking" [15]. Ligands are small molecules which bind at active site of a protein. The interaction between the ligand and the protein can be found by solving the combined Schrodinger equation of both ligand and protein system and by applying quantum mechanics [16]. Docking starts with an efficient search algorithm which places the ligand in active site of a protein in numerous different positions, orientation and conformations. Then these are evaluated by a scoring function to differentiate between good (near native) and bad (decoy) [17].

1.4.1 iGemDock

iGemDock combines two methods such as structure based virtual screening and post-screening analysis, where false positives are reduced from large compound database. This is the main step in finding the lead compound for which iGemDock a graphical-automatic drug discovery system was developed. GemDock generate can be visualized using molecular visualization tool and can be analysed by post-analysis tools. The post analysis tools works by using K-means and hierarchal clustering methods [18].

First in GEMDOCK interactive interfaces will be provided for preparing the binding site of target proteins and the screening compound library. Compounds from library are then docked into binding site by in-house docking tool GEMDOCK. Then protein-compound interaction profiles of electrostatic (E), hydrogen bonding (H) and vanderwaals interactions are generated in iGemDock. Then the profiles are analyzed by post-analysis tools and finally iGemDock ranks and visualizes the compound based on pharmacological interactions and energy based scoring function [19].

1.4.2 AutoDock

The interaction of small molecules with macromolecular targets can be done using the program AutoDock. Here AutoDock uses Monte Carlo simulated annealing technique for configurational exploration with a rapid energy evaluation using grid based molecular affinity potentials [20]. AutoDock calculations are performed in steps they are preparation of co-ordinate files using AutoDock tool, Pre calculations of atomic affinities using AutoGrid, Docking of ligand using AutoDock and Analysis of results using AutoDock tools [20].

1.4.3 Hex

Hex is an interactive molecular graphics program for calculating and displaying feasible docking modes of pairs of proteins and DNA molecules. Assuming the ligand is rigid and it can superpose pairs of molecules using the knowledge of 3D shapes. Hex can calculate protein ligand docking. Few of the docking program in Hex are built in graphics to view the result and even Hex uses Spherical Polar Fourier (SPF) correlations to accumulate the calculations [21]. The protein structure for Hex should be uploaded in PDB format. After completion of docking, a ranked list of predicted complexes can be downloaded [22].

MATERIALS AND METHODS

The following softwares have been used in the present experimentation. AchE (2X8B.pdb) and BchE (2XMB.pdb) has been retrieved from Protein DataBank and has docked with virtually screened molecules that act as ligands. 2X8B is a X-ray structured molecule and 2XMB is a BchE synthetic molecule. In the present experimentation, only the protein structures have been taken, removing other unwanted molecules and has submitted as receptors.

2.1 Pathwaylinker

PathwayLinker identifies and visualizes the first neighbor interactor network of the queried proteins submitted as protein names, analyzes the signaling pathway memberships of the proteins in this subnet, and provides links to further online resources. Biomedical research often focuses on altering the functions of selected proteins to signaling pathways through protein-protein and/or genetic interactions.

2.2 iGemdock v2.0

It is a Generic Evolutionary Method for molecular DOCKing GEMDOCK is a program for computing a ligand conformation and orientation relative to the active site of target protein.

2.3 Autodock v4.2

AutoDock is a suite of automated docking tools. It is designed to predict how small molecules, such as substrates or drug candidates, bind to a receptor of known 3D structure. AutoDock consist of two generations of software: AutoDock 4 and AutoDock Vina. AutoDock has applications in X-ray crystallography, structure-based drug design, lead optimisation, virtual screening (HTS), combinatorial library design, protein-protein docking and chemical mechanism studies.

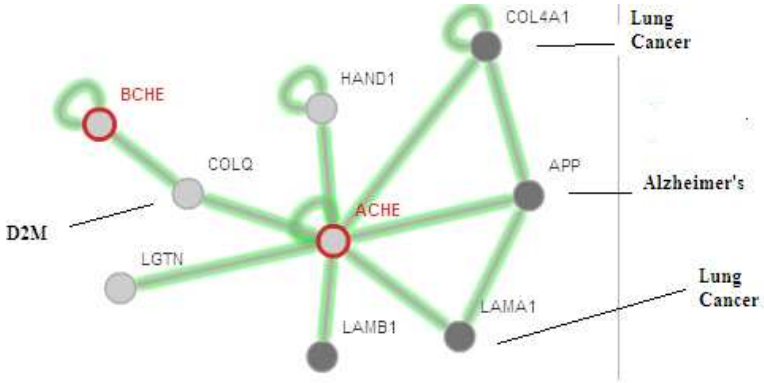
2.4 Hex v6.0

Hex is an interactive protein docking and molecular superposition program. Researchers can upload either protein or DNA structures in PDB format, and Hex can also read small-molecule SDF files.

RESULTS AND DISCUSSION

The interactions between the proteins is provided in Figure 1. These interactions has shown the links to Alzheimer, D2M and Cancer. The present work has been constructed to dock the ligands with Alzheimer and D2M.

Figure 1: Protein interactions in AchE and BchE using SignalLink



Ligands that bind with AchE and BchE protein were selected and these proteins were docked with ligands using some of the docking software like AutoDock, Hex and iGemDock. Docking was performed for AchE protein with its Ligands using AutoDock. Ten docking conformations were obtained for each ligand and among those ten conformations the one with minimum energy is considered as better one. Huperzine with energy of -9.11 showed good activity (Table 1, Figure 2).

Figure 2: Docking Results for AchE Protein with Huperzine using AutoDock

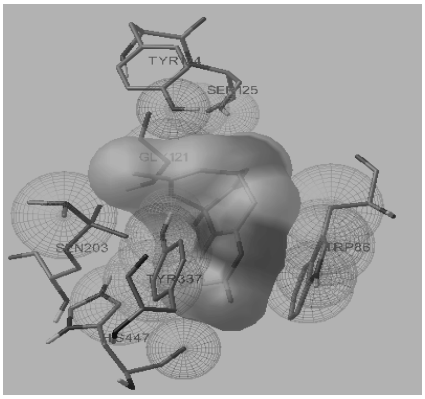


Table 1: Docking Results for AchE Protein using AutoDock

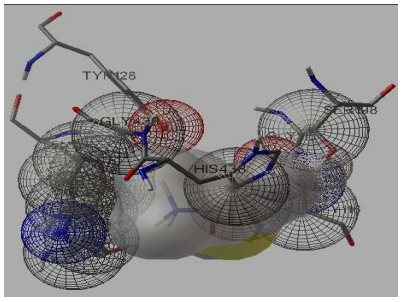
Properties	**Donepenzil**	**Galanthamine**	**HI_6**	**Rivastigmine**	**Huperzine**	**Ortho-7**	**Ambenonium**	**AchE inhibitor substrate**	**Pyridostigmin E**
Binding-energy	24.97	28.19	**-5.53**	**-6.25**	**-9.26**	9.56	352.46	**-4.08**	**-5.11**
Active site	TYR124 SER125 LEU130 ASN87 ASP74 GLY120 SEN203 TYR337 GLU202 HIS447 GLY448 TYR449 TYR133	TYR337 SEN203 ASP74 TYR124 SER125 GLY121 GLY120 TYR133	SEN203 TYR337 GLY121 TYR86 ASN87 ASP74 SER125 GLY126	GLN71 SER125 PRO88 ASN87 GLY121 TRP86 SEN203 TYR337 TYP133 GLU202 HIS447 GLY448	SEN203 TYR337 HIS447 TRP86 GLY121 SER125 TYR124	TYR124 SER125 GLY126 ASP74 TYR337 SEN203 GLY448 HIS447 GLY202 TRP86	GLY122 GLY121 SER125 ASP74 ASN87 THR83 TYR133 TYR337 VAL132 ILE451 GLU202 SEN203 ALA204	GLY126 GLY121 SEN203 HIS447 TYR337 TRP86 LEU130 TYR133 GLY120	TYR124 ASP74 SER125 GLY126 GLY121 LEU130 TRP86 GLY120 TYR133

Docking for BchE with its nine ligands were done using AutoDock software and its results are shown in tabular form in table 2. Propionyl thiocholine showed better docking results compared to other ligands with energy of -5.37 (Figure 3).

Table 2: Docking Results for BchE and its Ligands using AutoDock

Properties	Chlorpyrifos-oxon	1-anilino-8-naphthalene sulfonic acid	Dibucaine	Procainamide	Benzoylcholine	Propionylthiocholine	Acetyl Thio Choline	Dialkyl Phenyl Phosph Ates	Tetra ethyl ammonium
Binding- energy	13.64	**-4.54**	534.48	66.93	-0.15	**-5.37**	-2.75	-3.44	-3.96
Active site of protein	TYR128 SER198 HIS117 HIS438 GLY115 TYR114	TRP82 LEU125 TYR128 GLU197 TYR114 GLY115 HIS438 GLY439	TYR440 ILE442 TYR128 GLY439 MET437 HIS438 GLU197 PHE195 GLY116 PHE195 GLY196 TRP112 ILE113	HIS438 TRP231 PHE329 LEU236 LEU125 GLY115 TRP82 SER198 PHE398	TRP82 LEU125 TYR128 GLY115 HIS488 TYR114 SER198 GLY116 HIS117	TYR128 GLY439 HIS438 SER198 LEU125 GLY116 TRP82	LEU125 GLY439 HIS438 GLY115 GLY116 TRP82	PHE329 TRP82 SER198 HIS438 GLY115 TYR128 GLY128 HIS117	TYR128 GLU197 HIS438 GLY115 GLY116 TRP82

Figure 3:Docking Results for BchE and its Ligand (Propylthiocholine) using AutoDock

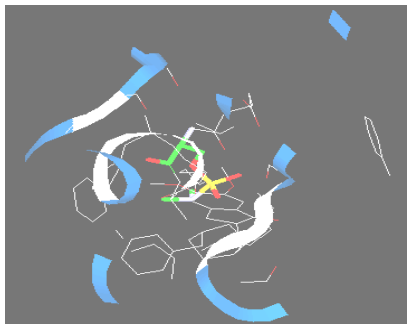


The AchE protein was docked with nine different ligands using iGemDock and its results are shown in the table 3. Donepenzil has shown best docking result with AchE with energy value -92.38 (Figure 4).

Table 3: Docking Results for AchE and its Ligand using iGemDock

Compound	Energy
Ambenoniun	-64.1845
AchE inhibitor Substrate	-52.3985
Donepezil	**-92.3878**
Galanthamine	-89.9345
HI-6	-83.656
Huperzine	-68.1222
Ortho-7	**-73.3213**
Pyridostigmine	-66.1134
Rivastigmine	-68.5023

Figure 4: Docking Results for Donepezil with AchE using iGemDock

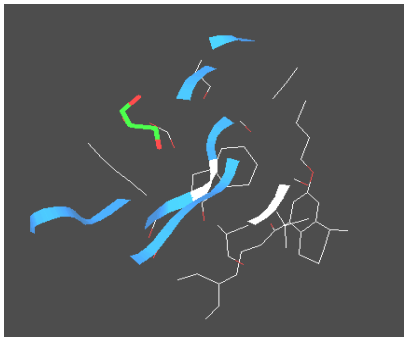


iGemDock is an tool used for docking using this tool BchE protein was docked with its ligands and results were retrieved which are shown in the table4. Out of 9 ligands, Dibucine showed better docking results with BchE with binding energy value of -65.3 (Figure 5).

Table 4: BchE and its Ligands Docking Results using iGemDock

Compound	Energy
Acetylthiocholine	-35.022
1-anilino-8-naphthalenesulfonicacid	**-59.871**
Benzoylcholine	-49.1163
Chlorpyrifos	-54.4679
Dialkylphenylphosphates	-50.2987
Dibucaine	**-65.3374**
Procainamide	-48.351
Tetraethylammonium	-28.2795
Propionylthiocholine	-41.4

Figure 5: Docking Results for Dibucaine with BchE using iGemDock

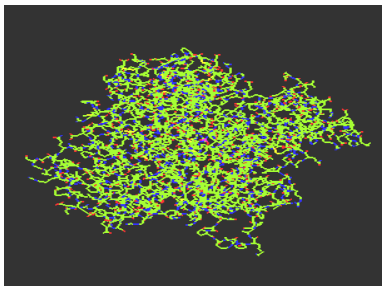


AchE protein was docked with different ligands using hex docking tool and energies and R-value for each ligand were retrieved.Galanthamine showed best docking results with AchE and its energy value is -4101 (Table 5, Figure 6).

Table 5: Docking results for AchE and its Ligand using Hex

Ligand	Etotal	R-Value
Donepezil	-287.92	16.8
Galanthamine	-41015608	13.6
HI-6	-229.82	19.2
Rivastigmine	-222.32	16.8
Huperzine	-17716850	19.2
Ortho-7	-292.80	16.8
HLo-6	-283	16.8
Pyridostigmine	-3638.00	12.8
Ambenoniun	-309.39	21.6
AchE inhibitor substrate	-768.00	16.8

Figure 6: Docking Results for Galanthamine with AchE using Hex

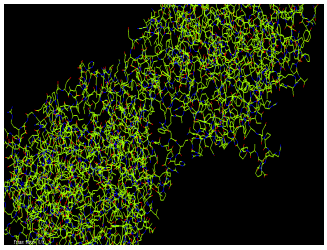


Docking was performed for BchE with its ligands using the tool Hex, an docking tool and the results were retrieved. Dialkyl phenyl phospate showed good docking results with BchE protein with an energy value of -641.00 (Table 6, Figure 7).

Table 6: Docking Results for BchE and its Ligands using Hex

Ligands	Etotal	R-value
Chlorpyrifos-oxon	-185.00	24.0
1-analino-8-naphtalene sulfonic acid	-206.43	24.8
Dibucaine	-273.00	28.0
Procainamide	-206.54	26.4
Benzoylcholine	-200.84	26.4
Propionyl thiocholine	-400.00	29.6
Acetylthiocholine	-139.89	24.0
Di alkyl phenyl phosphates	-641.00	29.6
Tetra ethyl ammonium	-119.30	26.4

Figure 7: Docking Results for Acetylcholine with BchE using Hex



Ligands which bind to AchE and BchE were searched and retrieved from different articles and to this ligands 2D model was built in Hyperchem and energy minimization was done using Steepest Descent algorithm, has also been build by Zaheer et al., 2010 on thiophene-2,3-dihydro-1,5-benzothiazepine against BChE [23]. Docking was performed for the energy minimized ligands with the AchE and BchE using docking software AutoDock, Hex and iGemDock.

Huperzine was observed as ligand with good docking activity with AchE protein using AutoDock [24, 25]. 1-anilino-8-naphthalene sulfonic acid was observed as ligand with good docking activity with BchE protein using Autodock. HLo-6 was observed as ligand with good docking activity with AchE protein using iGemDock. Dibucaine was observed as ligand with good docking activity with BchE protein using iGemDock.

Galanthamine was observed as ligand with good docking activity with AchE protein using Hex. Propionyl thiocholine was observed as ligand with good docking activity with BchE protein using Hex. Docking results provided the information that Huperzine and Galanthamine has better drug activity with AchE protein. Jian et al, 2009 designed and synthesised highly potent anti-acetylcholinesterase activity huperzine A derivatives [26]. Docking results provided the information that Dibucaine and propionyl thiocholine has better drug activity with BchE.

CONCLUSION

AchE and BchE are interlinked with various aging diseases like Alzheimer, Parkinson's, D2M etc, that are proposed to be as metabolic and genetic syndromes. Computational analysis provides an information regarding the drugs that act against these diseases. The screening and predicting data provides good results in control of these diseases.

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