Research Article

Discovery of Novel Monoamine Oxidase-B Inhibitors by Molecular Docking Approach for Alzheimer's and Parkinson's Disease Treatment.

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ABSTRACT

The main aim of the present study was aimed to screen the herbal lead such as arecoline, apigenin, chlorogenic acid, curcumin, kaempferol, luteolin, quercetin along with standard drug rasagiline and selegiline against the target enzyme monoamine oxidase -B by in-silico virtual screening techniques. Results obtained from the study shown that all the selected lead has shown good binding affinity towards target enzyme in which curcumin, luteolin, apigenin, quercetina and chlorogenic acid exhibit significant binding toward target enzyme similar to that of the standard drug. Hence it was concluded that lead from traditional medicine with biologically significant properties and structural diversity, have often served as valuable drug candidate for the treatment of neuro degenerative disorders like alzheimer's disease, parkinson's diseases by replacing the synthetic drug with known side effects.

Keywords: Monoamine oxidase, In-silico, Herbal lead, alzheimer's disease, parkinson's diseases.

INTRODUCTION

Enzyme hyperactivity in central nervous system promotes certain degenerative disorders like Alzheimer's and Parkinson's disease. Increased level of the enzyme acetylcholinesterase (AchE) leads to depletion in the level of acetylcholine a vital neurotransmitters required for the process of memory and learning may leads to Alzheimer's disease (AD). Similarly increased level of monoamine oxidase (MAO) enzyme involved in degradation of norepinephrine, serotonin and dopamine an neurotransmitters which is required for proper muscle coordination, mood, behavior, learning and adaptation.

Monoamine oxidase (MAO) is one of the main enzymes which catabolize the catecholamine and serotonin in the brain and in the periphery.

MAO is widely distributed in the central nervous system, and is present in 2 main isoforms, A and B.

In the brain, the A isoform appears to be present mainly in catecholaminergic neurons, whereas the B form is primarily present in ganglia and in serotonergic neurons[1].

Both monoamine oxidase (MAO) A and MAO B in the brain have been implicated in the etiology of Alzheimer's disease. MAO B is elevated in plaque-associated ganglia in Alzheimer brain.

Elevations in MAO A in Alzheimer neurons have been linked to increase in neurotoxic metabolites and neuron loss.

MAO B activity and mRNA have been reported to be elevated in several brain areas including hippocampus and cerebral cortex as well as in platelets of Alzheimer patients. MAO A activity and mRNA has been reported to be elevated in several brain areas of Alzheimer patients including frontal lobe of the neocortex, parietal cortex, occipital cortex, temporal cortex and frontal cortex[2].

There is also some data suggesting that and MAO inhibitor can improve cognitive function in Alzheimer patients and that neurotoxic catecholamine metabolites produced by MAO activity are present intraneuronally at high levels in Alzheimer brain[3].

There is also reason to believe that the changes in brain MAO in alzheimer's may be an insignificant by-product of changes that are more intimately related to the etiology of the disease. Cholinergic function declines in alzheimer's patients and this is thought to be critical to the memory dysfunction that accompanies this disease. Selective disruption of hippocampal cholinergic neurons in rats leads to widespread increases in brain MAO B suggesting that MAO B elevations may be secondary to cholinergic disruption.

Neuronal decay can lead to gliosis, and ganglia appear to have a higher concentration of MAO B than neurons[4].

So elevated MAO B in alzheimer's may reflect the increased numbers or activation of ganglia that have been observed in several cortical areas in Alzheimer patients.

Similarly, glucocorticoids elevate brain MAO A and B in elderly rats increase MAO B in cultured astrocytes.

Glucocorticoids increase has been linked to neuronal death and cognitive decline, and glucocorticoids appear to be elevated in Alzheimer patients and the MAO increase in Alzheimer's may be secondary to glucocorticoid elevation.

Another possibility is that MAO activity appears to be enhanced by aluminum ions and aluminum may be elevated in alzheimer brains. MAO B elevations may simply be a consequence of amyloid beta peptide elevations in plaques, as exposure of cultured ganglia to amyloid beta 25–35 peptide increases their MAO B. It was found that three is threefold increase in monoamine oxidase B activity in particular to temporal, parietal and frontal cortices of Alzheimer disease cases[5].

L-Deprenyl has been used for the treatment of depression and Parkinson's disease. L-Deprenyl, a levorotatory acetylenic derivative of phenethylamine, is an irreversible monoamine oxidase inhibitor (MAOI) that selectively inhibits MAOB at low doses.

Double-blind, placebo-controlled trials with small patient samples suggest that subchronic treatment with 10 mg/day L-deprenyl improves aspects of cognition, behavior, and performance[6].

The predictive mechanism underlying neuronal degeneration induced by MAO enzyme activity in AD is that degradation of dopamine by hyperactive MAO enzyme liberates hydrogen peroxide (H2O2) and other toxic aldehyde metabolites of DA (Dihydro phenyl acetic acid). Further H2O2 may undergo Fenton reaction and in turn liberates more hydroxyl free radical. Hydroxyl radical damage the neuronal membrane which has high amount of poly unsaturated fatty acid and also damage the nucleic acid in the DNA of the mitochondria.

Increased level of H2O2 promotes condition called cell acidosis by increasing the influx of calcium which decreases the cell pH which leads to neuronal damage and degeneration. This degeneration of neurons may leads to the condition of neuro inflammation which grabs the attention of microglia. Activation of this immune cell release cytokines such as interleukin and tumor necrosis factor. The brain has high lipid content and poor antioxidant effect in addition to high metabolic rate and abundant supply of the necessary transition metals makes the brain an ideal target for the free radical attack. Free radicals produced during oxidative stress are speculated to be pathologically important in Alzheimer's disease (AD) and other neurodegenerative diseases[7].

The leads of Central Nervous System (CNS) active medicinal plants, that have emerged besides *Rawolfia serpentina, Mucuna pruriens* for Parkinson's disease, *Ocimum santum* as an antistress agent, *Withania somnifera* as anxiolytic, *Centella asiatica* and *Bacopa monneria* for learning and memory disorders. *Bacopa monneria*, *Ginkgo biloba* and *Ipomoea Aquatica* for Alzheimer's disease. The study related to Alzheimer's disease (A.D) is focused towards the traditionally used rejuvenating and neurotonic agents[8]. The recent trends in the pharmacological studies are based on the biochemical and molecular mechanism that leads to the development of CNS active principles from the herbal drugs.

MATERIALS AND METHODS

Software's Required

Various computational tools and software's are used to analyze the target protein human monoamine oxidase B (PDB Code: 2V5Z) and to study the binding energy properties with Arecoline, Apigenin, Chlorogenic acid, Curcumin, Kaempferol, Luteolin, Quercetin along with Rasagiline and Selegiline a MAO-B inhibitor as a standard. Monoamine oxidase B enzyme with pdb code 2V5Z sequence was obtained from protein data bank (www.pdb.org/pdb/). To get insight the intermolecular interactions, the molecular docking studies were done for the above mentioned phytoconstituents along with MAO-B inhibitor as a standard at the active site 3D space of enzyme of interest MAO-B using online DOCKING SERVER web tool module.

Ligand Preparation

The ligands such as Arecoline, Apigenin, Chlorogenic acid, Curcumin, Kaempferol, Luteolin, Quercetin along with standard drug Rasagiline and Selegiline were built using Chemsketch and optimized using Docking server online web tool as shown in Figure 1 and 2 for docking studies by using Geometry optimization method MMFF94 and charge calculation was carried out based on Gasteiger method at PH 7 as shown in Table 1.

Protein preparation

The target protein human monoamine oxidase B was retrieved from protein Data Bank (www.rcsb.org) and crystallographic water molecules and bound inhibitor safinamide and two coumarin derivatives were removed from the protein. The chemistry of the protein was corrected for missing hydrogen followed by correcting the disorders of crystallographic structure by filling the valence atoms using alternate conformations and valence monitor options. As shown in Figure 3.

Active Site Prediction

Active site of enzyme was obtained by LIGSITE web server by using the automatic identification of pockets on protein surface given 3D coordinates of protein.

The potential ligand binding sites in MAO-B target protein is identified using grid space of 1 and probe of radius 5.0 angstrom[9]. Ligand site prediction was performed by using online tool GHECOM and the respective pockets calculations[10,11]. As shown in Figure 4.

Docking Methodology

Docking calculations were carried out using Docking Server[12,13] Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined. Docking calculations were carried out based on the binding free energy on the following compounds like Arecoline, Apigenin, Chlorogenic acid, Curcumin, Kaempferol, Luteolin, Quercetin along with standard drug Rasagiline and

Selegiline with respect to their binding affinity towards the target protein MAO-B (PDB Code: 2V5Z).

Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of Auto Dock tools. Affinity (grid) maps of Å grid points and 0.375 Å spacing were generated using the Autogrid program. Auto Dock parameter set and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis and Wets local search method[14]. Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 2 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied[15].

RESULTS

Interaction of lead with the protein target plays a significant role in structural based drug designing. In the present study, protein monoamine oxidase B (MAO-B) was docked with phytochemical leads derived from the indigenous plant of Indian system of traditional medicine. The different score such as binding free energy, inhibition constant, intermolecular energy and electrostatic energy values represented in Table 2.

The results showed that all the selected compounds showed binding energy ranging between -7.12 kcal/mol to -4.13 kcal/mol when compared with that of the standard Rasagiline (-6.32 kcal/mol) Selegiline (-5.62 kcal/mol). Electrostatic energy (-0.82 kcal/mol to -0.07 kcal/mol) of the ligands also coincide with the binding energy. All the phytochemical lead compounds contributed MAO-B enzyme inhibitory activity because of its structural parameters.

The docking calculations of all seven compounds at the active sites of MAO-B revealed that the compounds bound to the active site of enzyme with lower docking (D energy) when compared with standard drug Rasagiline and Selegiline.

Compound Luteolin exhibited quite tight binding against MAO-B enzyme with binding energy -7.12 Kcal/mol and ranks first in the compound series. The second best score was ranked by compound Apigenin with binding energy -6.97 Kcal/mol followed by this MAO-B Inhibitor Rasagiline with binding energy -6.32 Kcal/mol.

Inhibition constant is directly proportional to binding energy. Inhibition constant ranges from (939.67 µM to 5.99 µM). Thus from the report it was clear that all the phytoconstituents having promising MAO-B inhibition activity when compared to standard rasagiline and selegiline with inhibition constant 23.23 and 76.30 µM. Intermolecular energy of all seven compounds ranging between -7.49 to -4.87 kcal/mol which was lesser when compared to the standard rasagiline (-7.11 Kcal/mol) and selegiline (-6.84 Kcal/mol). Intermolecular energy is also directly proportional to binding energy. We found a decrease in intermolecular energy of all the selected compounds coincide with the binding energy.

Binding of lead with the sequential amino acid residue on the target enzyme site plays a significant role in prediction the inhibition potential of the drug candidate, as a justification of this statement the results obtained from the study reveals that lead such as Curcumin, Quercetin, Chlorogenic acid, Kampherol, Arecoline and Apigenin has interacted with amino acids at enzyme binding site which is closely resemble to that of the standard drug rasagiline and selegiline as represented in Table 3.

In respect of scoring the lead based on the total interaction surface on the active site of the target enzyme.

Out of all compounds screened for MAO-B inhibition potential the lead Curcumin ranks first with the largest interaction surface of about 1223.91 followed by this Chlorogenic acid ranks second with 1006.37 and Quercetin ranks third with interaction of about 934.67.

DISCUSSION AND CONCLUSION

Now a days computer aided drug discovery attains greater importance mainly because of the reliability in the results and also paves a new way for the research focus towards the alternative animal models.

The ultimate scope of the docking study is to predict the binding nature of the ligand molecule with the target may be an enzyme.

Efficacy of the ligand predicated by its nature of interaction with the specific functional amino acid residue on the active site of the receptor.

Interaction attained with the help of hydrogen bonding and hydrophobic bonding which are key players in stabilizing energetically-favored ligands, in an open conformational environment of protein structures.

Pharmacophore modeling and structural activity relation techniques aids the researcher in new drug discovery process of inventing novel therapeutic agents[16].

In conclusion, the results obtained from the current investigation clearly demonstrated the in silico molecular docking studies of selected phytoconstituents along with standard drug selected phytoconstituents against MAO-B enzyme.

These results clearly indicates that the leads especially Curcumin, Quercetin, Chlorogenic acid, Kampherol, Luteolin and Apigenin shown similar binding sites and interactions with MAO-A enzyme compared to the standard drug rasagiline and selegiline.

This in silico docking screening is actually an added advantage to screen the potential lead against MAO-B inhibition activity. Now a day's phytoconstituents from the natural derivatives may serve as therapeutic leads in the development of clinically effective MAO-B inhibitor.

Further investigations on the above compounds on preclinical and clinical studies are necessary to develop potential drug candidate for the treatment of neuro degenerative disorders like AD and PD.

Table 1: Ligand Properties

Compounds	molar weight g/mol	Molecular Formula	H Bond Donor	H Bond Acceptor	Rotatable bonds	Log P	pKa
Arecoline	155.19	$C_8H_{13}NO_2$	0	3	2	0.17	6.84
Apigenin	270.24	$C_{15}H_{10}O_5$	3	1	1	1.22	8.23
Chlorogenic acid	354.30	$C_{16}H_{18}O_9$	6	9	5	0.37	3.9
Curcumin	368.38	$C_{21}H_{20}O_6$	2	6	8	2.85	7.8
Kaempferol	286.23	$C_{21}H_{10}O_6$	4	6	1	1.9	6.44
Luteolin	286.24	$C_{15}H_{10}O_6$	4	6	1	01.5	6.63
Quercetin	302.23	$C_{15}H_{10}O_7$	5	7	1	1.5	7.15
Rasagiline	171.23	$C_{12}H_{13}N$	1	1	2	3.12	6.95
Selegiline	187.28	$C_{13}H_{17}N$	0	1	4	2.68	7.53

Table 2: Summary of the Molecular Docking Studies of Compounds against MAO-B Enzyme

Compounds	Binding Free Energy Kcal/mol	Inhibition Constant Ki µM	Electrostatic Energy Kcal/mol	Intermolecular Energy Kcal/mol
Arecoline	-5.18	160.64	-0.70	-4.87
Apigenin	-6.97	7.81	-0.07	-7.49
Chlorogenic acid	-4.13	939.67	-0.09	-6.20
Curcumin	-6.31	23.58	-0.09	- 9.04
Kaempferol	-5.99	40.58	-7.07	-7.16
Luteolin	-7.12	5.99	-0.07	-7.43
Quercetin	-4.51	494.85	-0.10	-5.24
Rasagiline	-6.32	23.23	-0.49	-7.11
Selegiline	-5.62	76.30	-0.82	-6.84

Table 3: Interaction of Lead Compounds with Active Site Amino Acid Residue of MAO-B Enzyme.

Compounds	Target binding Amino acid residue
Arecoline	34 GLU,35 ALA,36 ARG, 264 ILE, 393 TYR
Apigenin	42 ARG, 60 TYR, 426 THR, 436 MET
Chlorogenic acid	13 GLY,15 SER.42 ARG,59 SER, 397 CYS, 398 TYR, 425 GLY, 435 TYR, 436 MET
Curcumin	34 GLU, 42 ARG,264 ILE, 393 TYR, 397 CYS, 426 THR
Kaempferol	42 ARG, 60 TYR, 393 TYR,397 CYS, 426 THR, 435 TYR, 436 MET
Luteolin	42 ARG, 60 TYR, 397 CYS, 426 THR, 435 TYR, 436 MET
Quercetin	14 ILE,42 ARG,397 CYS,398 TYR, 426 THR, 435 TYR, 436 MET
Rasagiline	34 GLU,35 ALA,36 ARG,42 ARG,264 ILE,393 TYR,397 CYS, 398 TYR, 426 THR,434 GLY,436 MET
Selegiline	34 GLU,35 ALA,36 ARG,42 ARG,264 ILE,393 TYR,397 CYS, 398 TYR, 426 THR,436 MET

Figure 1 Showing 2D structure of lead. 1. Arecoline 2. Apigenin 3. Chlorogenic acid 4. Curcumin 5. Kaempferol 6. Luteolin 7. Quercetin 8. Rasagiline and 9. Selegiline

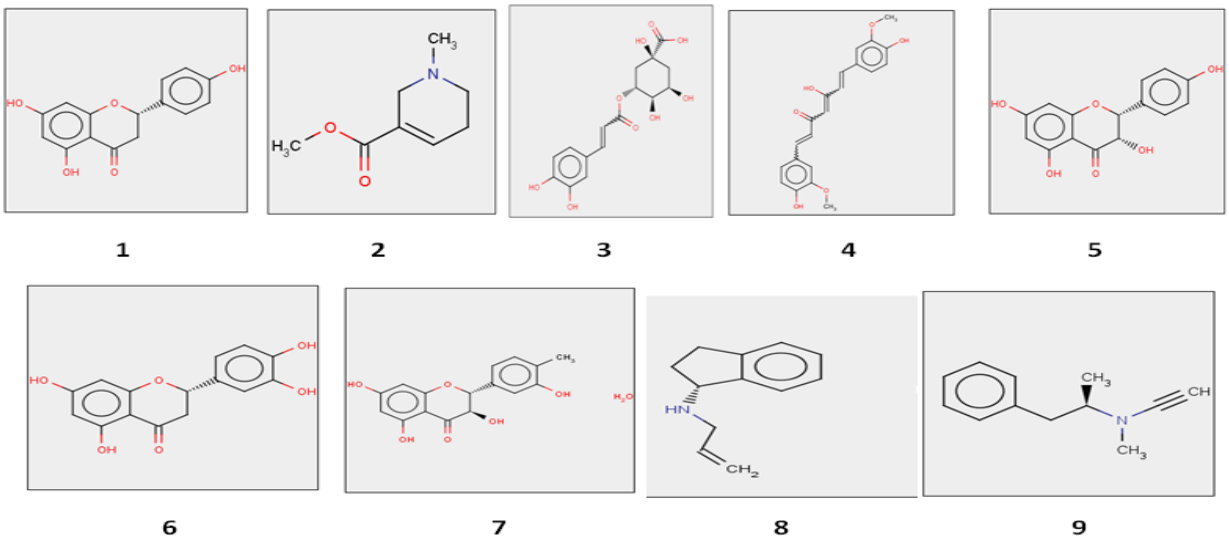

Figure 1: 2D Structure of Lead

Figure 2 Showing 3D structure of lead. 1. Arecoline 2. Apigenin 3. Chlorogenic acid 4. Curcumin 5. Kaempferol 6. Luteolin 7. Quercetin 8. Rasagiline and 9. Selegiline

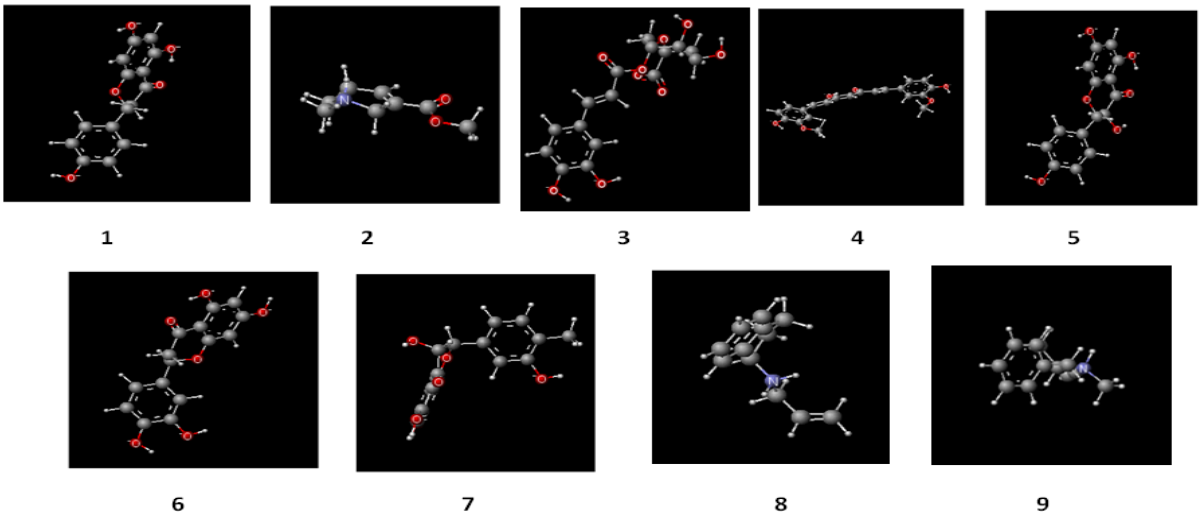

Figure 2: 3D Structure of Lead

Figure 3: Target protein Human Monoamine oxidase B. PDB 2V5Z

Figure 4 Showing the possible ligand binding pockets on the surface of target enzyme MAO-B. Pockets calculated by GHECOM.

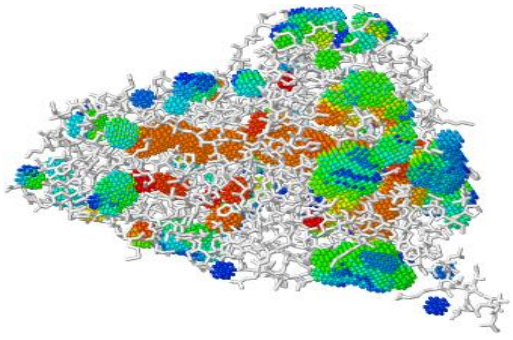

Figure 4: Ligand binding pockets on the surface of target enzyme MAO-B

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