Analysis of potentiality of Cyclotide, A Major Compound from *Clitoria Ternatea* as Anti-Parkinsonism Drug: A Pilot *In Silico* Study

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ABSTRACT

Parkinson's illness (PD) is a main source of mental handicap and passing around the world. Despite the fact that there are many advances in drug improvement against PD, an intense low measurement drug with less secondary effects is still in its nursery. This is a trailblazer in silico endeavor to test the counter PD activities of cyclotide to go about as clever medication.In this review, utilizing Auto dock devices 4.2, cyclotide was anticipated for its inhibitory activities with Alpha-Synuclein (AS) Apo site, Dopamine D3 Receptor (D3R), Glycogen Synthase Kinase-3 Beta (GSK3β), Mono Oxidase B (MAO-B), Parkin and Tyrosine 3-Hydroxylase (TH) with levodopa standard. The dependability of the 3D anticipated model of these proteins was broke down utilizing RAMPAGE. Further, the blood-cerebrum boundary (BBB) crossing capacity of the normal mixtures were examined utilizing cbligand. The In silico ADME (Absorption, Distribution, Metabolism, Excretion) properties of cyclotide was contrasted and that of levodopa utilizing molinspiration and admetSAR @ LMMD programming. The expectations were that cyclotide, being blood-mind hindrance positive (BBB+) with less secondary effects could be a strong enemy of PD drug. Cyclotide was anticipated to be great inhibitors of AS, MAO-B and Parkin. The review uncovered that cyclotide could be an intense enemy of PD drug, being BBB+. Cyclotide was furthermore anticipated as a decent inhibitor of AS, MAO-B and Parkin than levodopa standard.

KEY WORDS: AutoDock 4.2, Esculin, Hinokitol, Rampage, Admet SAR, Molinspiration
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INTRODUCTION

Among the whole total populace, PD, is the second most normal neurodegenerative infection influencing maturing populaces with a commonness of 0.3% [1]. PD, is one of the conspicuous, moderate development problem portrayed by postural flimsiness, inflexibility, resting quake and bradykinesia [1], coming about basically from neurolysis influencing dopaminergic neurones in the substantia nigra standards compacta (SNpc). Moreover, assuming the job of a few synapses in PD, a

few neuro-structures, for example, front olfactory designs, amygdala, dorsal engine core of the vagus, caudal raphe cores, locus coeruleus, autonomic sensory system, hippocampus, and cerebral cortex has been connected to PD. Furthermore, non-engine side effects, including uneasiness and gloom, olfactory and memory hindrances, rest anomalies, as well as gastrointestinal aggravation are additionally pervasive in PD patients [2].

PD, different framework decay and dementia show articulation of alpha synuclien (AS) with conspicuous lewy bodies [3]. AS, which are the histological signs of PD is the significant constituent of proteinaceous amyloidogenic incorporations known as lewy bodies, among PD patients. The 14 kDa polypeptide AS, alongside the passing of dopaminergic neurones inside the SN of the cerebrum, makes the sickness communicated conspicuously. In an in vitro disconnected framework, AS collects as opposed to developing into mature fibrils as 5-hydroxytryptamine receptors (5-HT) ties and settles the totals [2]. GSK3β, one of the GSK3 isomers, an engaging helpful objective for neurodegenerative problems assumes a significant part in neuronal apoptosis. Additionally, its hindrance diminishes articulation of AS, which make this kinase substantially more alluring for neurobiologists [4].

Catechol-O-methyl transferase (COMT) and monoamine oxidase (MAO) inhibitors will delay the half-existence of levodopa and dopamine [5-7]. An adjusted MAOB action has been seen in PD patient's platelets, which was embroiled in the PD neuropathology for its part in using the neurotoxin 1methyl-4phenyl-1,2,3,6tetra-hydro pyridine, and framing H2O2

In the prefrontal cortex, restraint of D3R upgrades dopamine and acetylcholine discharge. Furthermore, D3R, adjusts the prefrontal cortex cholinergic levels [9]. Decarboxylation of L-3,4-dihydroxy-phenylalanine (L-DOPA) and L-5-hydroxytryptophan by DOPA decarboxylase (DDC) is liable for the blend of the key synapses dopamine and serotonin individually. DDC is one of the significant markers of PD and hypertension [10]. Most Parkin knockout mouse models neglected to create the pathophysiology of PD and show the specific dopamine (DA) neurone misfortune. This new comprehension of the component and the significance of mitochondrial quality control may likewise reach out to other normal neurodegenerative illnesses related with mitochondrial brokenness, for example, Alzheimer's infection and Amyotrophic horizontal sclerosis [11]. It has been recorded that TH phosphorylation during maturing diminishes in the striatum, which was the sign of striatal DA misfortune in human. Along these lines, the striatal TH guideline during maturing by>70% loss of striatal DA will bring about the presence of crazy engine side effects related with PD [12]. during dopamine digestion [8].

As of late, bioactive mixtures, have been disconnected from different plant materials, with potential antineuralgic impacts. These bioactive mixtures basically incorporate plant optional metabolites like saponins, steroids, flavanones, quinones, glycosides, etc. Current engineered drug treatment attempts to further develop memory, learning capacity, and furthermore attempting to quiet the patients, however every one of the medications utilized for this design are having their profile of genuine antagonistic impacts. At this point, research must be finished integrating different frameworks of medication, particularly the Ayurvedic framework to recognize the most important pathogenesis additionally to get a decent fix or preventive medication for PD. In the Ayurvedic arrangement of medication 'Medhya medications' — a gathering of home grown prescriptions — are known for their activities on the sensory system [13]. These 'Medhya medications' referenced in Ayurvedic texts are said to further develop mental capacities like learning and memory improvement, upper impact, additionally successful in forestalling temperament fluctuating issues [14]. Clitoriaternatea L. is one of the recorded 'Medhya medications' [15, 16]; In this paper, as a trailblazer in silico study, the bio animation of plant auxiliary

metabolites, cyclotide was analyzed by foreseeing their neuroprotective activity by repressing AS, D3R, GSK3β, MAO-B, Parkin and TH receptors engaged with PD neuropathology keeping levodopa as standard.

MATERIALS AND METHODS

Softwares

The receptor model was worked by utilizing Auto Dock instruments 1.5.6 and MGL devices 1.5.6 bundles (The Scripps Research Institute, Molecular Graphics Laboratory, and 10550 North Torrey Pines Road, CA, 92037). Cbligand instruments were utilized to check for in silico BBB crossing capacity of the ligands. Molinspiration devices were utilized for examining ADME and atomic physio-synthetic properties of levodopa and cyclotide. Harmfulness forecast was finished by admetSAR @ LMMD. The unwavering quality of the 3D anticipated models of the protein and receptor models were examined utilizing RAMPAGE.

Molecular docking studies

The 3-layered construction of Levadopa (PubChem ID: 6047),Cyclotide (PubChem ID: 161211025) were recovered from PubChem and enhanced for docking utilizing Discovery studio. The protein Crystallographic designs of receptors Alpha-Synuclein Apo site (3Q25), Dopamine D3 Receptor (3BPL), Glycogen Synthase Kinase-3 Beta (1H8F), Mono Oxidase B (1OJA), Parkin (5C9V) and Tyrosine 3-Hydroxylase (1TOH) were recovered from www. rcsb. Organization was ready for docking by erasing all heteroatoms, ligands and water particles and enhanced by minimization of energy by utilizing Discovery studio. The got structures were sav ed and utilized for the docking studies. The 3D construction of proteins was produced after enhancement in Discovery studio. Auto Dock devices consequently process gasteiger charges and decide rotatable obligations of the ligand to have the option to produce different conformers for the docking [17]. Receptor matrices were created utilizing 40 × 40 × 40 network focuses in xyz with a lattice dividing of 1.000 Å. Framework boxes were created utilizing autogrid4. The lamarckian hereditary calculation was utilized for all sub-atomic docking recreations. The collaborations of the protein-ligand buildings were likewise recovered through Auto Dock apparatuses 1.5.6. Dockings were completed with following boundaries for the quantity of runs: 50, populace size: 150, the quantity of assessments: 2,500,000 and number of ages: 27,000 quantities of ages [18, 19].

Ramachandran plot Rampage Analysis

Ramachandran plot was utilized to really take a look at the dependability of the anticipated 3D model. The pre-arranged designs of the multitude of six receptors were checked for the primary unwavering quality through frenzy investigation [20].

ADME studies

An in silico investigation of the integrated mixtures was performed for the forecast of ADME properties. Absolute polar surface region (TPSA), miLog P, the quantity of rotatable bonds, the quantity of a hydrogen giver and acceptor molecules were determined upon the premise of Lipinski's standard of five [21]. BBB forecast was finished by using an online program (www. cbligand. organization/BBB). The mixtures with positive qualities can cross promptly in the BBB, while compounds with negative qualities are ineffectively appropriated across the cerebrum.

Toxicity estimation

It was seen that an excessive number of mixtures were ended in clinical advancement in light of inadmissible pharmacokinetics or in silico ADME studies. It is currently conceivable to anticipate the action and harmfulness dangers of mixtures through dependable Bioinformatics apparatuses, e. g., admetSAR @ LMMD. In the current review, we have determined harmfulness risk boundaries like mutagenicity, tumorigenicity, and biodegradability of chosen drugs; cyclotide with the standard medication levodopa.

RESULTS AND DISCUSSION

AutoDock

AutoDock is performed by assessing energies for both the bound and unbound states. It additionally consolidates another charge-based desolvation technique that utilizes a common arrangement of molecule types and

charges. The limiting energy of the medications; in particular cyclotide was contrasted and levodopa, which showed the limiting energies fluctuating from-5.3 to-9.1 Kcal/Mol. The generally speaking inhibitory activity of cyclotide with the chose receptors in table 1 must be valued as to standard levodopa. Alpha synuclien Apo site has an intense planned site of inhibitory activity for cyclotide (- 6.9 Kcal/Mol), when contrasted and standard levodopa (- 7.3 Kcal/Mol). The GSK3β showed just 5.6 Kcal/Mol for Cyclotide and a lot lesser than levodopa, which was-5.8 Kcal/Mol. MOA-B is another receptor, which was profoundly repressed by Cyclotide with a prognostic positive energy of-7.2 Kcal/Mol. Parkin uncovered its greatest inhibitory activity with cyclotide, being-5.1Kcal/Mol. The TH showed a really impressive measure of restraint with the cyclotide as-7.1Kcal/Mol. The conceivable dynamic site amino acids, which are connecting with the locales, are demonstrated in fig. 1-6.

Table 1: Auto Dock Vina 4.2 results showing binding energy/affinity energy (Kcal/Mol) of receptors with the ligands

Receptors (PDB ID)	**Ligand (PUBCHEM ID)**	
	Levodopa (6047)	**Cyclotide (3611)**
α-Synuclein Apo site (3Q25)	-7.3	-6.9
Dopamine D3 Receptor (3BPL)	-5.8	-8.3
Glycogen Synthase Kinase-3β (1H8F)	-5.8	-5.6
Mono Oxidase B (1OJA)	-7.7	-7.2
Parkin (5C9V)	-5.3	-5.1
Tyrosine 3-Hydroxylase (1TOH)	-6.9	-7.1

Fig. 1: The fig.showing active site interactions between Alpha-synuclein Apo site and ligands. A. Between alpha-synuclein Apo site and levodopa B. between alpha-synuclein Apo site and cyclotide.

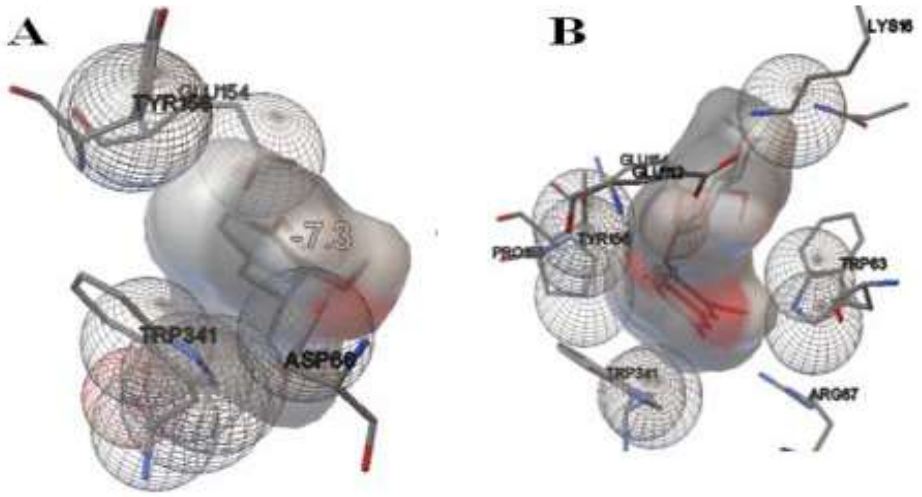


Fig. 2: The fig. showing active site interactions between dopamine D3 Receptors and ligands. A. Between dopamine D3 Receptors and levodopa B. Between dopamine D3 Receptors and cyclotide.

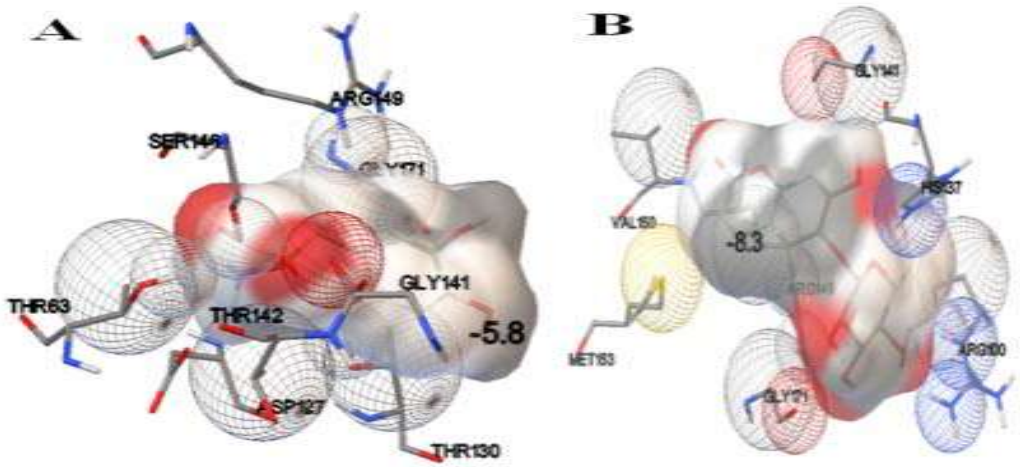


Fig. 3: The fig. showing active site interactions between glycogen synthase kinase-3-beta and ligands. A. between glycogen synthase kinase-3-beta and levodopa B. between glycogen synthase kinase-3-beta and cyclotide.

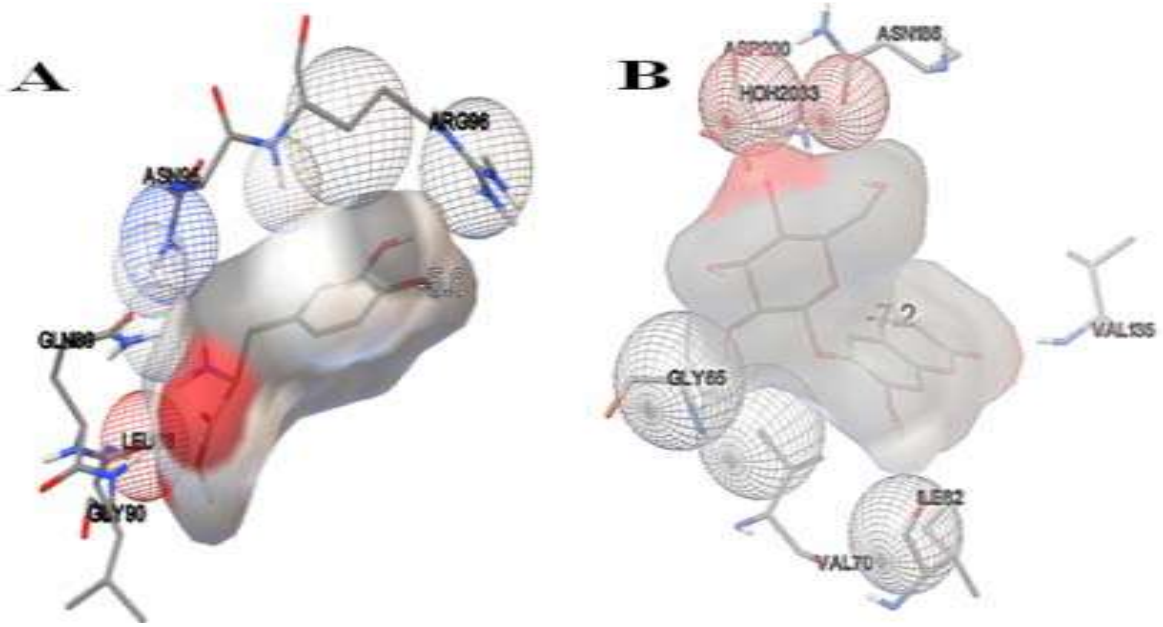


Fig. 4: The fig. showing active site interactions between mono oxidase B and ligands. A. between mono oxidase B and levodopa B. Between mono oxidase B and hinokitol

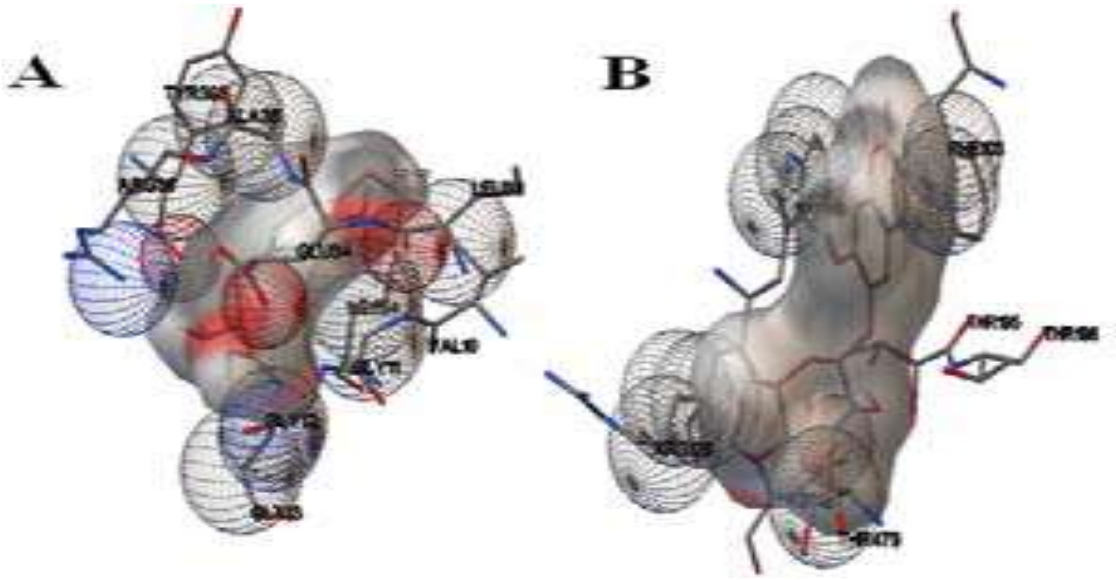


Fig. 5: The fig. showing active site interactions between Parkin and ligands. A. Between parkin and levodopa B. Between parkin and cyclotide.

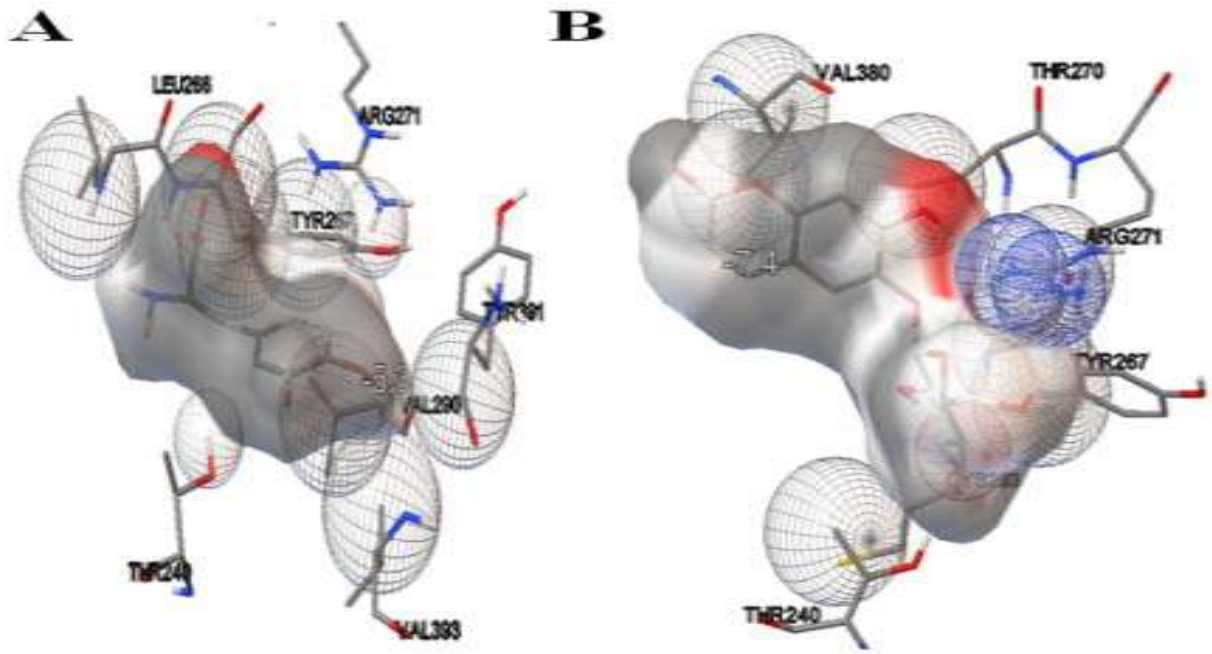
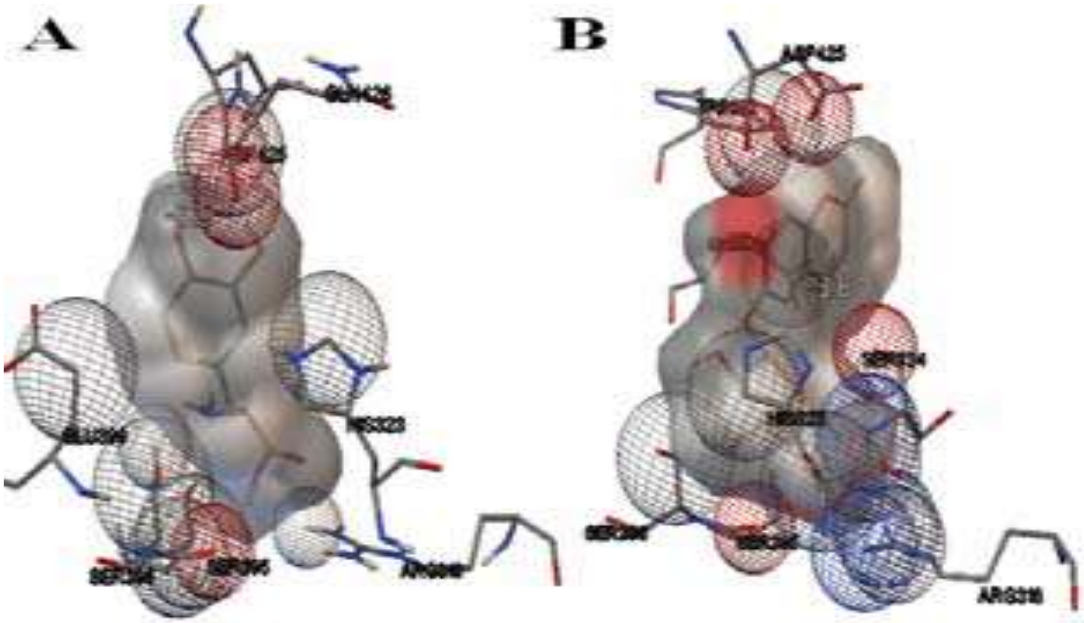


Fig. 6: The fig. showing active site interactions between tyrosine 3-hydroxylase and ligands. A. between tyrosine 3-hydroxylase and levodopa B.between tyrosine 3-hydroxylase and cyclotide.



Rampage analysis

The level of buildups in the leaned toward area for every one of the chose six receptors changed from 91.1-98.0. The equivalent for MOA-B was viewed as 98%. The receptors like D3R and Parkin offered 96% of the inclined toward district amino corrosive arrangement. Around 95% for synuclien were evaluated for the good buildups, which could be surveyed under the dynamic locales on the receptors. In the interim, it was 91% for GSK3beta and 90% in the MOA-B (table 2, fig. 7-12).

Table 2: The rampage stereochemical quality of receptors by analyzing residue geometry and overall structure geometry

Receptor(PDB ID)	Expected percentage(%)of residues in		Actual percentage (%) of residues in		
	Favored region	Allowed region	Favored region	Allowed region	Outlier region
α-Synuclein Apo site (3Q25)	98.00	2.00	95.40	3.70	0.9
Dopamine D3 Receptor (3BPL)	98.00	2.00	96.2	3.8	0.0
Glycogen Synthase Kinase-3 β(1H8F)	98.00	2.00	91.1	6.6	2.3
Mono Oxidase B (1OJA)	98.00	2.00	98.0	2.0	0.0
Parkin (5C9V)	98.00	2.00	96.3	3.7	0.0
Tyrosine 3-Hydroxylase (1TOH)	98.00	2.00	96.9	2.8	0.3

Fig. 7: Ramachandran plot analysis of alpha-synuclein Apo site

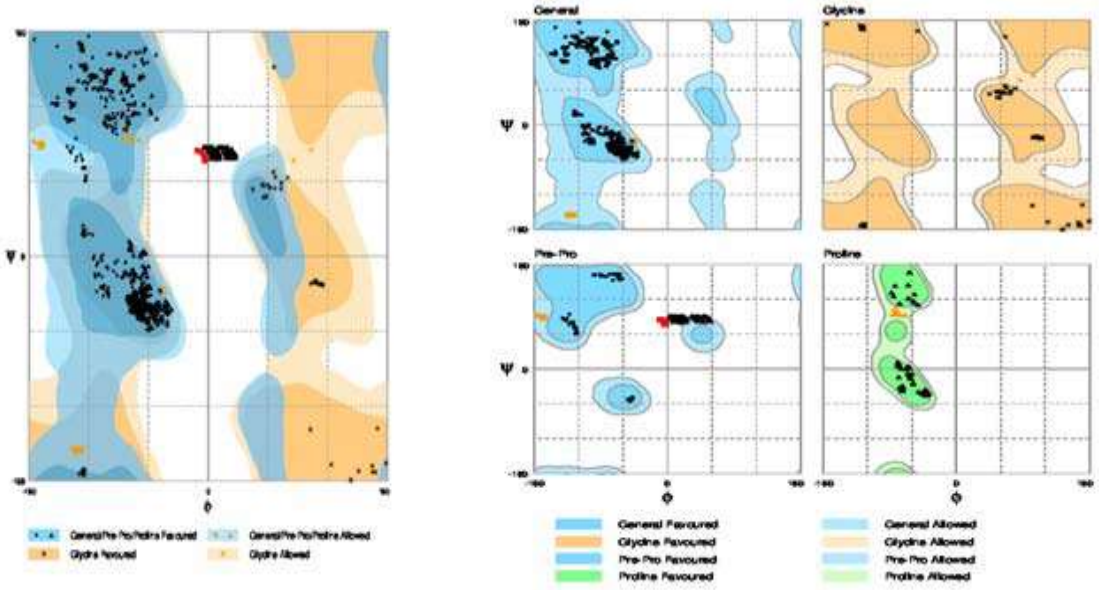


Fig. 8: Ramachandran plot analysis of human dopamine 3 receptors

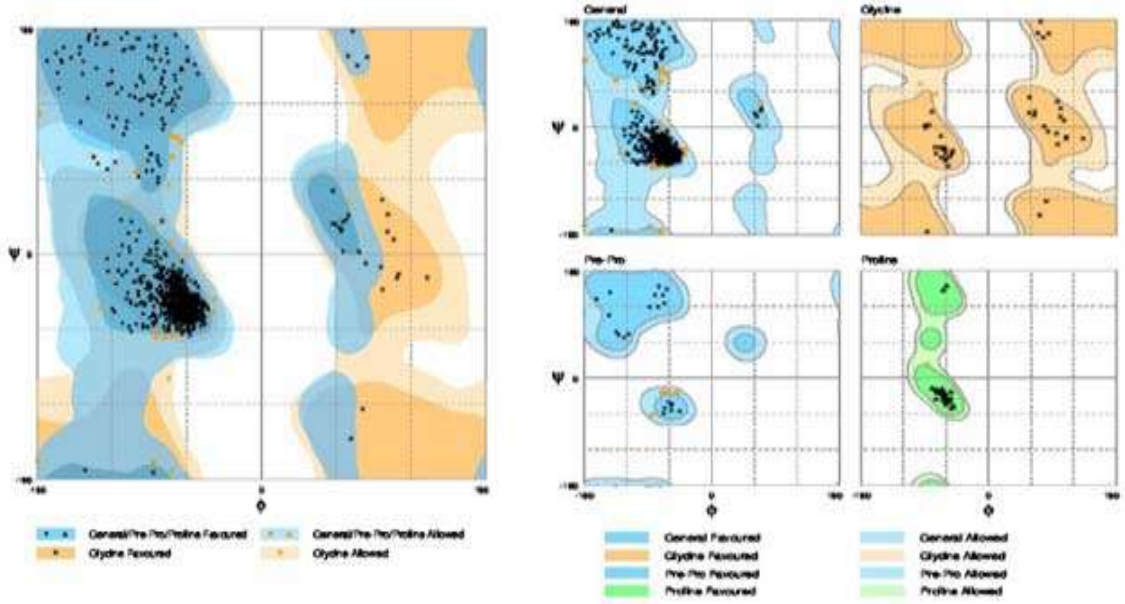


Fig. 9: Ramachandran plot analysis of human glycogen kinase 3 β

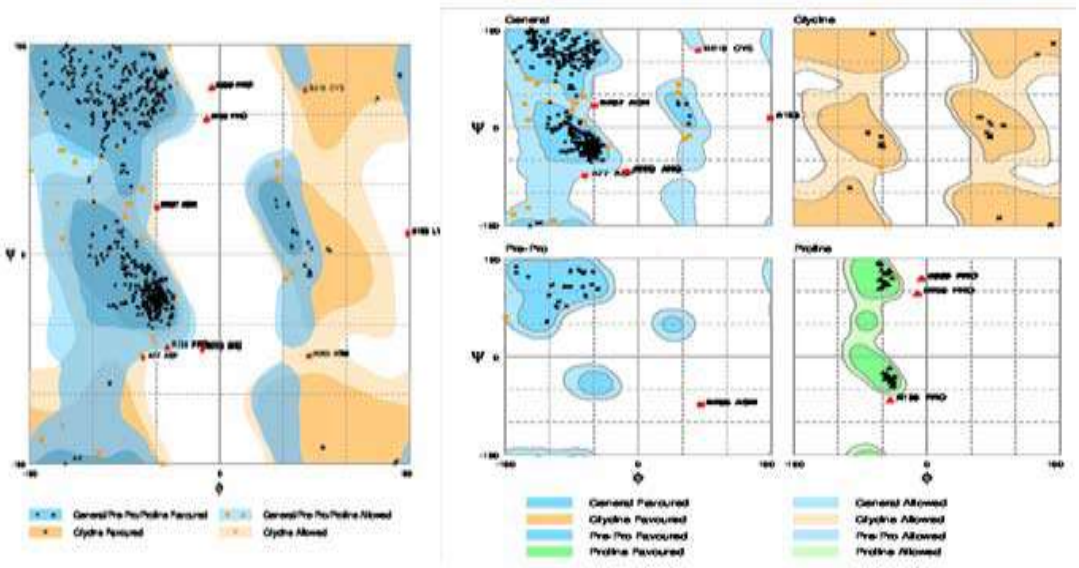


Fig. 10: Ramachandran plot Analysis of human Mono Oxidase B

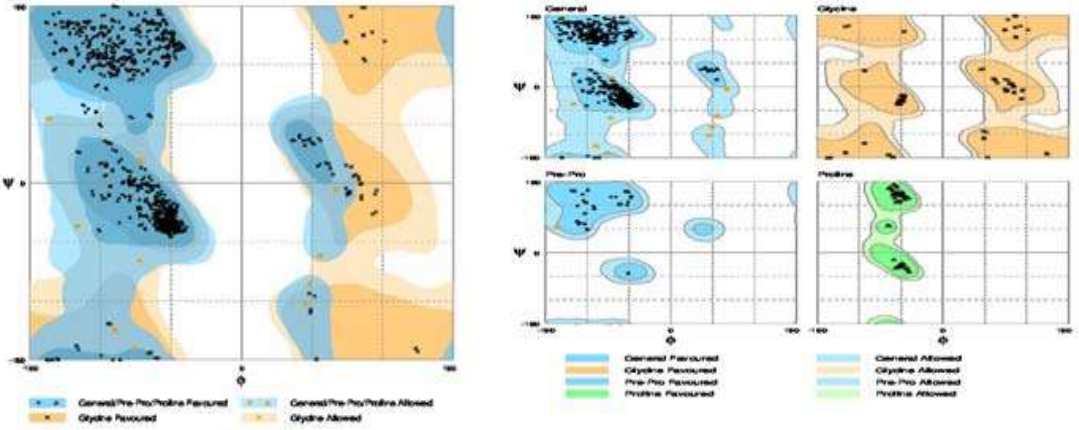


Fig. 11: Ramachandran plot Analysis of parkin

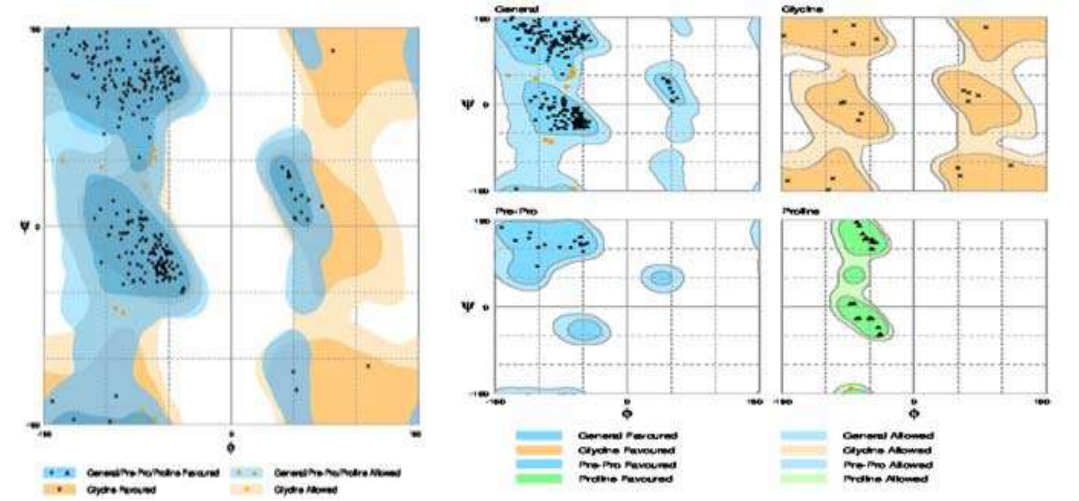
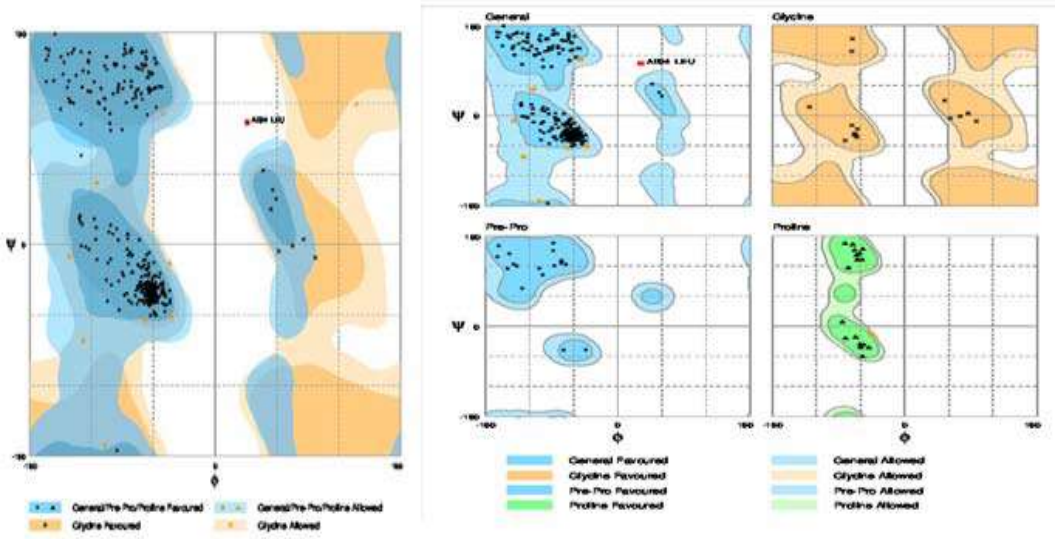


Fig. 12: Ramachandran plot analysis of human tyrosine 3-hydroxylase



Blood-brain barrier permeability

As indicated by the program www. cbligand. Organization/BBB, the blood-cerebrum obstruction porousness of the chose two medications alongside standard levodopa was surveyed. This shows that the medication esculin couldn't cross the BBB (fig. 13-14). Concerning cyclotide and levodopa, both were BBB porous with a higher penetrability rate with levodopa (penetrability score: +0.681).

Fig. 13: Blood brain barrier permeability scores for levodopa. A. Pub Chem 2D structureof levodopa. B. Graph showing BBB permeability score of levodopa

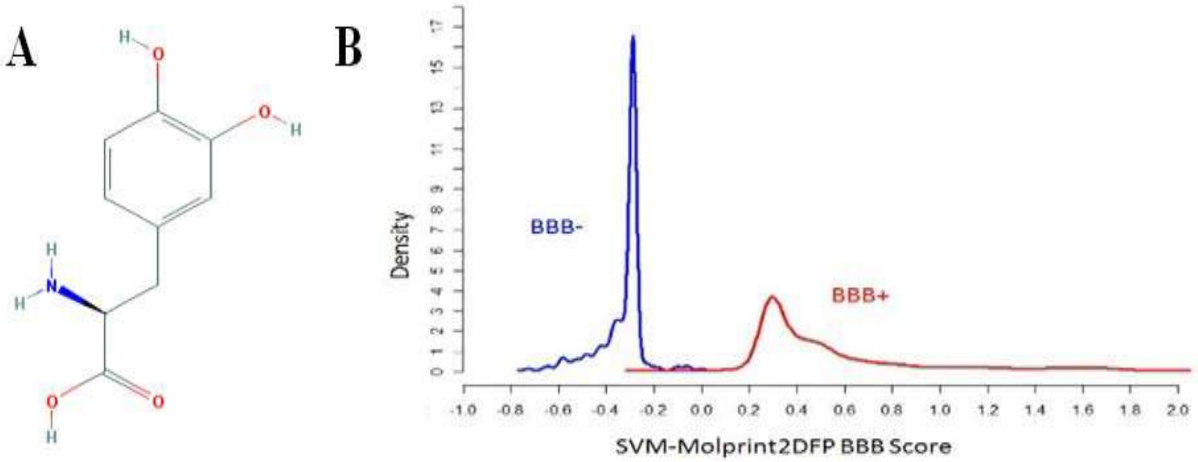
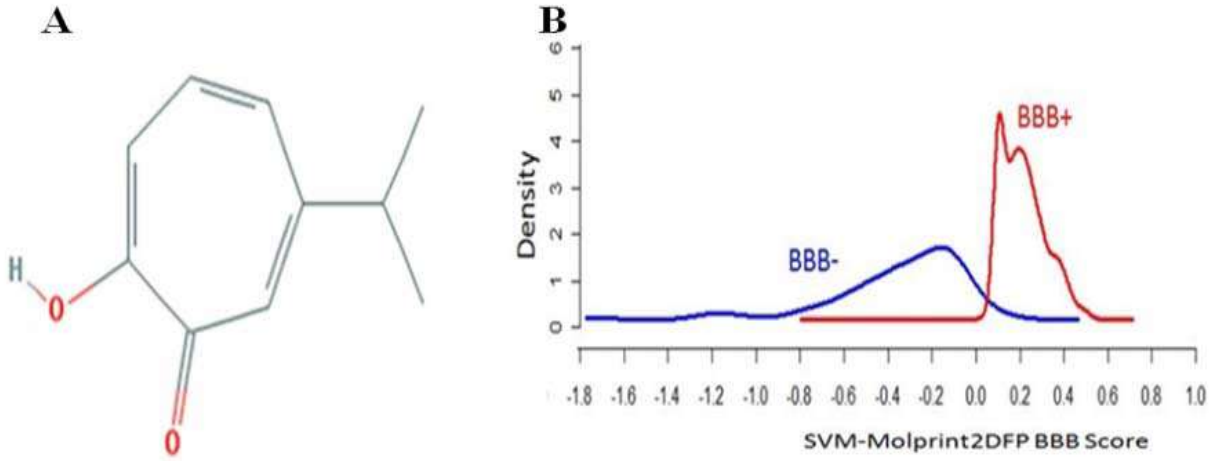


Fig. 14: Blood-brain barrier permeability scores for Cyclotide A. Pub Chem 2D structure of cyclotide B. graph showing BBB permeability score of cyclotide.



Molecular physio-chemical properties

The *in silico* studies suggested that the Total Polar Surface Area (TPSA) for cyclotide was 37.30 .While TPSA for levodopa was 103. 78. The mi log P for cyclotide was+2.08. While the same for levodopa was-2.20 (table 3).

Table 3: Calculation of molecular physiochemical properties

Compound	Number of atoms	Molecular weight	TPSA	miLogP	N Rtb	nON	nOHNH	Rule of Violation	BBB Permeability scores (+/-)
Levodopa	14	197.19	103.78	-2.20	3	5	5	0	+0.681
Cyclotide	12	164.20	37.30	+2.08	1	2	1	0	+0.317

Bioactivity studies

The bioactivity concentrates on uncovered the conceivable bioactivity scores of the medications to go about as an intense G-protein coupled receptor (GPCR) ligand, particle channel modulator, kinase modulator, atomic receptor ligand, protease inhibitor as well as a compound inhibitor (table 4). The review uncovered the bioactivity scores of cyclotide as a solid GPCR ligand with-1.19 score. cyclotide can be additionally treated as a potential atomic receptor ligand (bioactivity score:- 1.02) and a protease inhibitor (bioactivity score:- 1.27).

Table 4: Bioactivity predictions using molinspiration tools

Bioactive properties	Bioactivity scores	
	Levodopa	Cyclotide

GPCR Ligand	-0.04	-1.19
Ion Channel Modulator	+0.39	-0.59
Kinase Modulator	-0.60	-0.94
Nuclear Receptor Ligand	-0.17	-1.02
Protease Inhibitor	-0.01	-1.27
Enzyme Inhibitor	+0.29	-0.22

ADMET studies

The ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) review, showed the chose medications can without much of a stretch go through human gastrointestinal assimilation and are non-inhibitors of P-glycoproteins (able 5). They even go about as non-inhibitor of renal natural cations. With respect to dissemination and digestion, contrasted with standard levodopa, the properties, for example, CYP450 2C9 substrate, CYP450 2D6 substrate, CYP450 3A4 substrate CYP450 1A2 inhibitor CYP450 2C9 inhibitor CYP450 2D6 inhibitor CYP450 2C19 inhibitor CYP450 3A4 inhibitor and CYP inhibitory wantonness were by and large same for cyclotide and standard Levodopa. Whenever the discharge and poisonousness instrument of the medications are concerned, every one of the three medications are non-inhibitor of human etheragogo related quality restraint and are non-cancer-causing agents.The relapse profile assessed the rodent intense poisonousness (LD50, Mol/kg) for cyclotide as 2.7972 than levodopa being 2.3137 (LD50, Mol/kg). The fish poisonousness (pLC50, mg/l) was 1.0043 and-1.4952 for levodopa, and cyclotide separately.

Table 5: ADMET profile of the selected drugs

ADMET properties	Levodopa	Cyclotide
Absorption		
Human Intestinal Absorbtion	+	+
CaCo-2 Permeability	-	+
P-glycoprotein Substrate	Non-Substrate	Non substrate
P-glycoprotein Inhibitor	Non inhibitor	Non inhibitor
Renal Organic Cation Inhibitor	Non inhibitor	Non inhibitor
Distribution, Metabolism		
CYP450 2C9 Substrate	Non-Substrate	Non Substrate
CYP450 2D6 Substrate	Non-Substrate	Non Substrate
CYP450 3A4 Substrate	Substrate	Non Substrate
CYP450 1A2 Inhibitor	Inhibitor	Inhibitor
CYP450 2C9 Inhibitor	Inhibitor	Non inhibitor
CYP450 2D6 Inhibitor	Inhibitor	Non inhibitor
CYP450 2C19 Inhibitor	Inhibitor	Non inhibitor
CYP450 3A4 Inhibitor	Inhibitor	Non inhibitor
CYP Inhibitory Promiscuity	Low CYP Inhibitory Promiscuity	Low CYP Inhibitory Promiscuity
Excretion, Toxicity		
Human Etheragogo Related Gene Inhibition	Non-Inhibitor	Non-Inhibitor

AMES Toxicity	AMES Toxic	Non-AMES Toxic
Carcinogens	Non-Carcinogen	Non-Carcinogen
Fish Toxicity	High	High
Tetrahymena Pyriformis Toxicity	High	High
Honey Bee Toxicity	Low	High
Biodegradation	Readily Biodegradable	Readily Biodegradable
Acute Oral Toxicity	III	III
ADMET predicted profile regression		
Aqueous solubility (LogS)	-2.1345	-4.1536
Caco2 Permeability (LogPapp, cm/s)	-0.6730	0.8364
Rat Acute Toxicity (LD_{50}, mol/kg)	2.3137	2.7972
Fish Toxicity(pLC50, mg/l)	1.0043	-1.4952
Tetrahymena Pyriformis Toxicity (pIGC50, ug/l)	0.2883	0.6404

Auto Dock utilizes a semiempirical free energy force field to anticipate restricting free energies of little atoms to macromolecular targets [22]. The power field depends on a far reaching thermodynamic model that permits fuse of intramolecular energies into the anticipated free energy of restricting. The technique has been adjusted for a bunch of 188 different protein-ligand buildings of known construction and restricting energy, showing a standard blunder of around 2-3 kcal/Mol in the expectation of restricting free energy in cross-approval studies. The docked complex was pictured in AutoDock Tools [23] showing how the ligand associates with the proteins. Toward the finish of each docking run, AutoDock yields an outcome which is the most reduced energy compliance of the ligand it found during that run. This conformity is a mix of interpretation, quaternion and twist points and is described by intermolecular energy, inner energy and the torsional energy [24]. The initial two of these consolidated structures give the 'docking energy' while the first and third give 'restricting energy.' AutoDock additionally stalls the all-out energy into a vdW energy and an electrostatic energy for every molecule. The 'best' docking result can be viewed as the adaptation with the most minimal (docked) energy. The relative examination of the cyclotide with levodopa assisted with finding the connecting amino acids in the dynamic locales of the multitude of chosen proteins, which are basically adding to the neurodegenerative problem's starting pathways.

The quick hydrolysis of acetylcholine (AChE) into acetyl-coA and choline happens at the end of the nerve drive in cholinergic neural connections, which is intervened by AChE. Also, there is great proof to give interrelationship among TH and cerebrum cholinergic framework, with the diminished degrees of AChE and the general shortages to the cholinergic framework [25]. TheAS development plays a stripping job in the movement of neurodegeneration. Additionally, in mouse hippocampal neurones, AS has been displayed to confine vesicle dissemination away from neurotransmitters, reliable with vesicle grouping hindrance intervened by AS. In rodent hippocampal neurons, AS-instigated surrenders in vesicle reclustering following endocytosis were clarified [26]. Thusly, the hindrance of Tyrosine 156 of the Apo-site of AS, in this study clears another way through which the medication of determination, cyclotide could go about as like of standard levodopa drug.

The digestion of significant monoamine synapses, for example, 5-HT, norepinephrine and dopamine in the CNS is controlled by a flavoenzyme MAO. It exists in two isoforms in particular MAO-An and MAO-B. Since days of yore, a large number of the human MAO-An inhibitors are utilized as against tension as well as upper specialists. While, human MAO-B inhibitors are utilized alone or in mix as against Alzheimer's and hostile to Parkinsonian drugs [27]. The prior reports [28] prompts the improvement of quinazolinone subordinates as anticancer leads by in silico plan, in the comparable way. In this review, Valine 106 on account of MOA-B showed better cooperation.

Ubiquitin frequently consolidates with Parkin as both activator and substrate for alteration of Parkin movement. Phosphorylation of Ser 56 is the justification behind both initiation and hindrance [11]. This restraint of Parkin can be one more huge answer for decreasing the movement of neurodegeneration. In this review, docking concentrates on uncovered that the amino corrosive Thr 240 could be an imminent site of hindrance by cyclotide, in examination with levodopa.

The atomic physiochemical properties examination of the medications uncovered that cyclotide was BBB+ showing a positive incentive for its BBB penetrability, guaranteeing it as a certifiable medication for neurodegenerative illnesses. In addition, the miLogP worth of cyclotide was positive, which further affirms its capability to go about as an enemy of neurodegenerative medication. Moreover, cyclotide can go about as certain GPCR ligand, atomic receptor as well as protease inhibitor ligands. Subsequently, this guarantees that cyclotide could be a superior medication of decision for anticipation of movement of neurodegenerative infections like PD. The ADMET concentrates additionally support that cyclotide is a decent decision to be focused on as an enemy of Parkinsonian drug. Notwithstanding, further, sub-atomic unique recreation studies, in vitro too as in vivo ordered trials could get out the specific capacity free from cyclotide against PD.

The review anticipated the capability of cyclotide as a strong enemy of neurodegenerative medication which could target sicknesses, particularly PD, being great inhibitors of AS, MAO-B and Parkin. The medication was anticipated to be BBB positive as they could be BBB porous. The ADMET concentrates on additionally uncovered its capacity to go about as a high-conceived drug with negligible poisonousness. This in-silico investigation could be taken for additional trial and error in-vitro and in vivo models to reconfirm its expected component of activity, to use the medication as an honorable medication for neurodegenerative problems.

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