ANALOGS OF CARBIDOPA: *IN SILICO* DESIGN & DEVELOPMENT OF NOVEL DOPA DECARBOXYLASE INHIBITORS IN THE TREATMENT OF PARKINSON'S DISEASE

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Abstract
Parkinson's disease (PD) is the second most common neurodegenerative disorder. Pharmacotherapy with levodopa in combination with carbidopa provides symptomatic relief in patients suffering from PD. However, several studies have been conducted in an attempt to improve the therapeutic efficiency of levodopa by using drugs that preferentially inhibit extracerebral decarboxylase. Yet, none of them has shown convincingly significant results. In this study, we have used computational methods to design novel carbidopa analogues and evaluated them for interaction with the enzyme DOPA decarboxylase (DDC) through in silico analysis. Here, we report that carbidopa analogue-8 ((S)-3-(3,4-dihydroxyphenyl)-2-(hydroxyamino)-2-methylpropanoic acid) has shown higher affinity and better interaction with DDC than carbidopa. DDC protein interactions with carbidopa analogues were confirmed by binding energy given by GOLD docking software.
Keywords- Parkinson's disease, carbidopa, levodopa and DOPA decarboxylase.

INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by resting tremor, rigidity, bradykinesia and gait impairment. It is estimated that approximately 1-2% of the population over the age of 60 years are affected [1]. Pathological hallmarks for PD include degeneration of dopaminergic neurons in the substantia nigra with consequent depletion of the neurotransmitter dopamine. Dopamine depletion causes major physiological disruptions in the basal ganglia-thalamocortical motor circuit, which is responsible for controlling the motor activity [2]. At present, there is no cure for PD. However, dopamine replacement therapy with levodopa, a dopamine precursor resulted in significant symptomatic relief [3]. Levodopa is converted to dopamine by an enzyme known as DOPA decarboxylase (DDC). But the chronic use of levodopa is associated with development of motor fluctuations and dyskinesia [4]. When oral dose of levodopa is administered, about 95% of the drug is metabolized in extracerebral tissues causing a dramatic reduction in the amount of levodopa available for transport to the brain. Moreover, peripheral decarboxylation of levodopa to dopamine causes prominent nausea and vomiting [5]. To overcome these side effects, levodopa is administered with a DDC inhibitor such as carbidopa. These DDC inhibitors do not penetrate through blood-brain barrier and show their inhibitory action mainly at extracerebral tissues. Even though the combinational treatment with levodopa and carbidopa provide symptomatic relief, the pharmacological therapy can be more effective if DDC inhibitors that possess higher affinity than carbidopa are administered. In this computational study, we report few analogues of carbidopa that show higher affinity and better interaction with the enzyme DDC. These results have been confirmed by the binding energy given by GOLD docking software.

MATERIALS AND METHODS:

Ligand and protein preparation-

The enzyme DOPA decarboxylase was retrieved from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/) with PDB Id- 1JS3 [6]. Active sites of the enzyme were identified by RCSB PDB Ligand Explorer and

LigandScout. Then, active sites were verified by using PDBSum and CASTp server. CASTp (Computed Atlas of Surface Topography of proteins) is used to verify the binding sites of a protein. It includes annotated functional information of specific residues on the protein structure [7].

The analogues of DDC inhibitor carbidopa were designed by using Accelry's Symyx Draw 4.0, according to Lipinski's rule that satisfies the structure-activity relationship (SAR) properties.

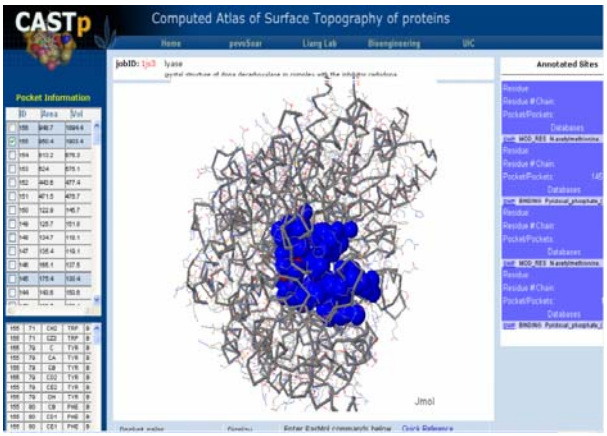


Fig 1: Active site cavity of 1JS3 from CASTp online server

Optimization of protein and ligands

Protein- The protein DDC downloaded from PDB was prepared for docking by deleting all hetero atoms, ligands and water molecules and optimized by minimization of energy by using Hyperchem 8.0. Parameters were maintained as RMS Gradient-0.01 k.Cal/Mol, CHARMm- 27 force field with Polak-Ribiere (conjugate gradient) minimizer, in In Vacco conditions [8].

Ligands- Ligands designed by using Accelry's Symyx Draw were optimized by using Hyperchem 8.0 and parameters were maintained as RMS Gradient 0.01 k.Cal/Mol, OPLS force field, in vacco media and Polak-Ribiere minimizer. Later, all the optimized ligands were saved in .MOL format.

Molecular descriptors

The molecular descriptors of each analogue were predicted by loading the carbidopa analogues into an online server, OSIRIS property explorer (http://www.organic-chemistry.org/prog/peo/) [9]. This prediction process depends on comparison between precomputed set of structural moieties whose properties are already known and the structural moieties of loaded molecules. Molecular descriptors like C logP, solubility, drug score and side effects such as mutagenicity, carcinogenicity and teratogenicity were determined. To calculate the overall drug score, OSIRIS combined logP, logS, molar mass, drug-likeness and toxicity risks into a single number to predict the molecule's over all drug potential.

Docking studies

All the 9 ligands, including carbidopa and its 8 analogues were docked with DDC enzyme in the software GOLD version 3.0. Software GOLD (Genetic Optimization for Ligand Docking) uses a genetic algorithm to explore wide range of ligand conformational flexibility and rotational flexibility of selected receptor hydrogens [10]. The docking orientations are ranked based on a molecular mechanics–like scoring function known as Goldscore. HIS-192 is set as active site residue. In the aspect of producing binding energy estimates, the Goldscore function appears to be more reliable than the Chemscore function [11]. The compound binding site was defined within a 10 Å radius around the binding sites of DDC. Standard default parameter settings were used to evaluate the protein-ligand interactions [12].

RESULTS & DISCUSSIONS

Target 1JS3 taken form PDB website is and screened for its active site cavities by using RCSB Lignad Explorer, LigandScout, PDBSum, CASTP online server. DOPA decarboxylase enzyme protein downloaded from RCSB PDB server is prepared by removing water and other hetero molecues and it was geometrically minimized with CHARMm force field and its obtained minimization values are energy: -24017.10558 K.cal/mol and RMS Gradient is 0.009974. All the ligands are optimized to its least possible energy conformation and final minimized energies are listed in Table 1.

Table 1: Carbidopa & its analogs: Structure, Nomenclature, & energy minimization values

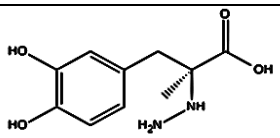
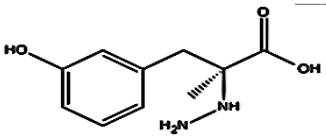
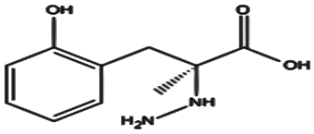
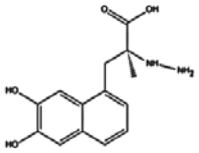
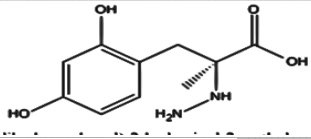
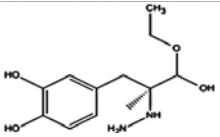
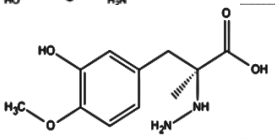
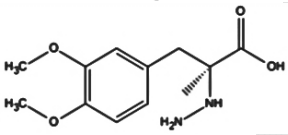
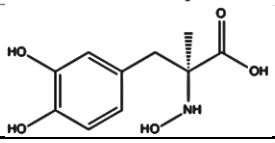
Ligand	*Structure*	*IUPAC name*	*Optimization values*	
			Energy (K.Cal/Mol)	*Gradient (K.Cal/Mol)*
Carbidopa (lead)		(S)-3-(3,4-dihydroxyphenyl)-2-hydrazinyl-2-methylpropanoic acid	0.064899	0.009561
Ligand 1		(S)-2-hydrazinyl-3-(3-hydroxyphenyl)-2-methylpropanoic acid	0.372280	0.007245
Ligand 2		(S)-2-hydrazinyl-3-(2-hydroxyphenyl)-2-methylpropanoic acid	0.650728	0.009083
Ligand 3		(S)-3-(6,7-dihydroxynaphthalen-1-yl)-2-hydrazinyl-2-methylpropanoic acid	0.451660	0.009867
Ligand 4		(S)-3-(2,4-dihydroxyphenyl)-2-hydrazinyl-2-methylpropanoic acid	0.510878	0.009773
Ligand 5		4-((2S)-3-ethoxy-2-hydrazinyl-3-hydroxy-2-methylpropyl) benzene-1,2-diol	0.289676	0.009816
Ligand 6		(S)-2-hydrazinyl-3-(3-hydroxy-4-methoxyphenyl)-2-methylpropanoic acid	0.447278	0.009794
Ligand 7		(S)-3-(3,4-dimethoxyphenyl)-2-hydrazinyl-2-methylpropanoic acid	0.840508	0.009453
Ligand 8		(S)-3-(3,4-dihydroxyphenyl)-2-(hydroxyamino)-2-methylpropanoic acid	0.35482	0.009548

Table 2: Toxicity properties of ligands: OSIRIS Property explorer

ligand	*mutagenic*	*carcinogenic*	*irritant*	*teratogenic*	*clogP*	*solubility*	*Drug score*
Ligand 1	no	no	no	no	-1.05	-1.23	0.51
Ligand 2	no	no	no	no	-0.75	-1.52	0.49
Ligand 3	no	no	no	no	0.95	-2.66	0.46
Ligand 4	no	no	no	no	-1.05	-1.23	0.49
Ligand 5	no	no	no	no	-0.73	-1.49	0.5
Ligand 6	no	no	no	no	-0.70	-1.65	0.63
Ligand 7	no	no	no	no	-0.51	-1.96	0.79
Ligand 8	Partial	no	no	no	-0.43	-1.26	0.73

Table 3: GOLD Docking: fitness scores of carbidopa & its analogs with 1JS3

ligand	*S(hb_ext)*	*S(vdw_ext)*	*S(hb_int)*	*S(vdw_int)*	*Fitness*
Carbidopa	3.20	26.16	0.00	-6.59	32.58
Ligand 1	5.30	24.34	0.00	-3.85	34.91
Ligand 2	2.22	25.36	0.00	-3.61	33.48
Ligand 3	4.35	29.79	0.00	-8.01	37.31
Ligand 4	0.69	26.89	0.00	-5.85	31.81
Ligand 5	5.15	34.17	0.00	-8.29	43.84
Ligand 6	3.01	31.66	0.00	-7.01	39.52
Ligand 7	3.86	31.38	0.00	-6.04	40.97
Ligand 8	27.69	20.83	0.00	-0.58	55.74

Protein - ligand interactions are studied by using GOLD 3.01 docking software. A scoring function to rank different binding modes; the Gold score function is a molecular mechanics–like function with four terms:

GOLD Fitness = S_{hb_ext} + 1.375(S_{vdw_ext}) + S_{hb_int} + S_{vdw_int}

where Shb_ext is the protein–ligand hydrogen-bond score and Svdw_ext is the protein-ligand van der Waals score. Shb_int is the contribution to the Fitness due to intramolecular hydrogen bonds in the ligand; this term is switched off in all calculations presented in this work: Svdw_int is the contribution due to intramolecular strain in the ligand. The GOLD Fitness score are shown in Table 3.

Figure- 3: Shows the bond distances from amino acid residues to ligand depicted by using RCSB PDB Ligand Explorer. Pink color dotted lines indicate hydrogen bonding interactions and green color dotted lines indicates the hydrophobic interactions.

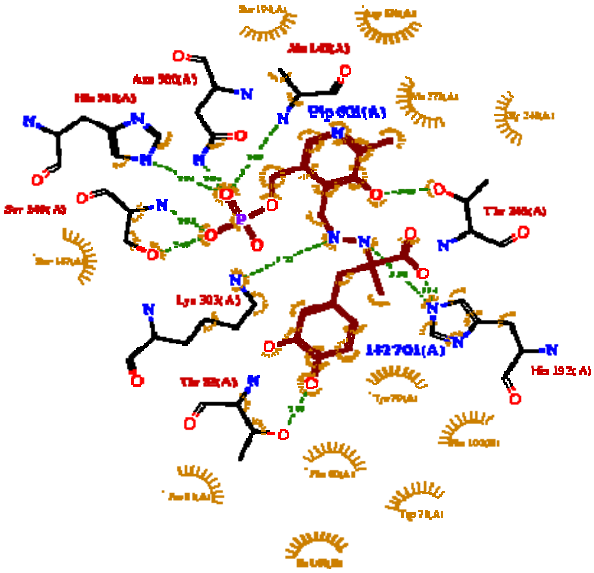


Fig 2: 1JS3 active sites residues interaction with Carbidopa from PDBSum online server

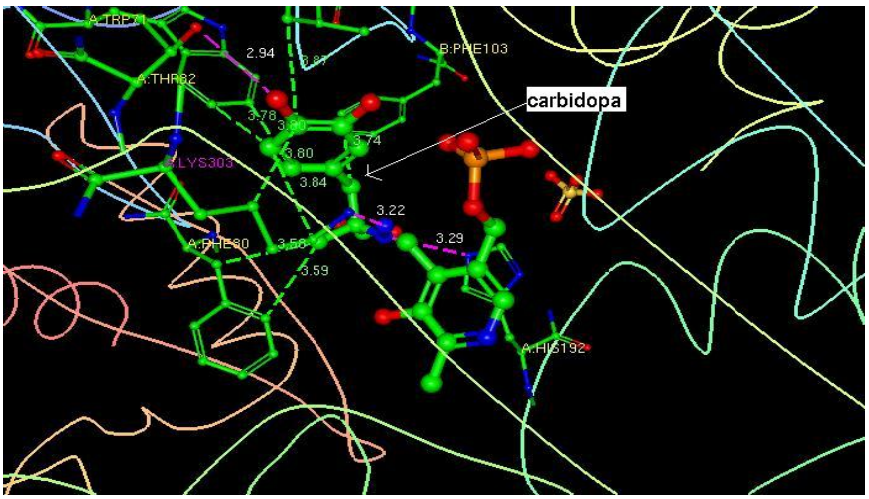


Fig 3: ligand – Protein interactions from RCSB PDB Ligand Explorer

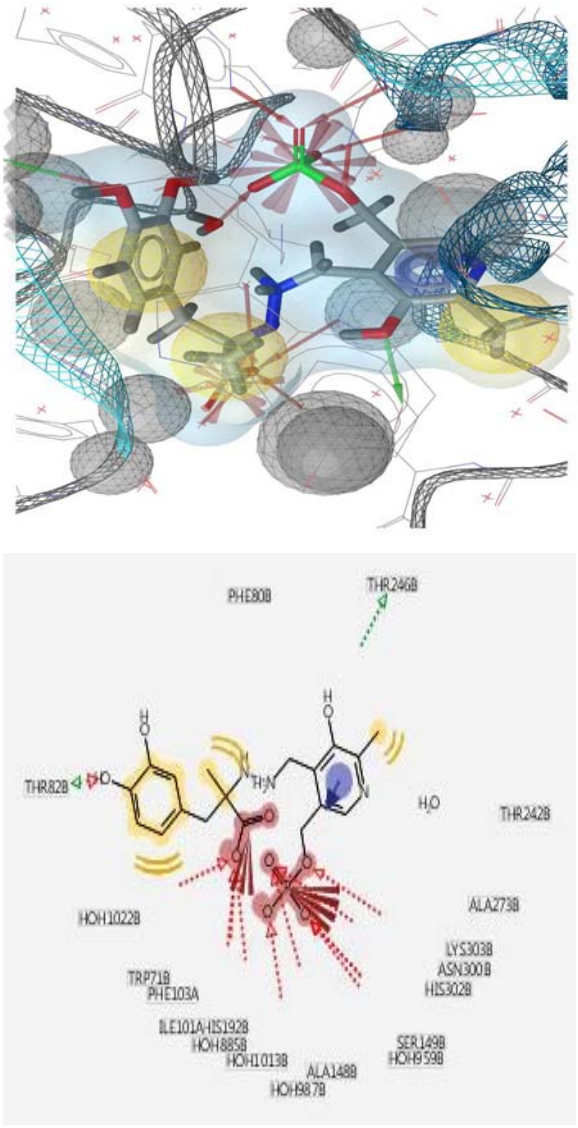


Fig 4: Protein - Ligand Interactions from LigandScout

In Fig. 4, the yellow color spheres indicate the hydrophobic interactions of ligand and the red colour dotted lines indicate the hydrogen bonds depicted by using LigandScout. Same figure shows the identification of active site amino acids with different types of interactions.

Carbidopa is the lead moiety, a potent DOPA decarboxylase inhibitor. When compared to carbidopa fitness score, ligands 8, 5, 7, 6, 3 possess higher and even considerable binding affinity towards the receptor protein, and ligands 1, 2 have partially higher fitness score than carbidopa. Ligand 4 has lesser fitness score than carbidopa, because of its hydrogen bonding interactions being lesser when compared to carbidopa.

It can assumed that ligand 8 (hydroxylamine derivative) is having much higher external hydrogen bonding energy that leads to better binding than any other ligands. Ligand 8 shows higher drug score when compared to other ligands as it shows higher H-bond & Vander Waal's interactions. Binding interactions of the ligand with various residues like LYS 303, HIS 302 can be visualized in the Fig 5.

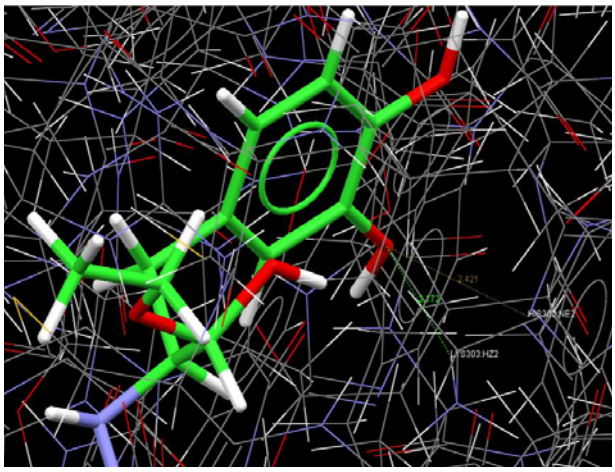


Fig 5: Binding interactions of ligand with DDC protein

CONCLUSION

The interactions between DOPA decarboxylase enzyme and the ligands were studied by using various computational methods. Based on binding energy, and hydrogen bond formed, docking results were analyzed. The results were compared to carbidopa to find out the best ligand which can inhibit the property of the DDC. Based on these observations, Ligand 8 has high values to inhibit the DDC among the ligands and Carbidopa. Thus the *in silico m*ethod adopted in the present study helped in identifying the ligands using the commercial software and online tools for the treatment of neurodegenerative Parkinson's disorder. This method reduces the time and cost in designing a drug as well as in analyzing the drug likeliness before it enters the clinical trials.

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