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Pharmacological target based novel molecules design and validation for Parkinson's using molecular docking studies

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ABSTRACT

Parkinson's results from the degeneration of dopamine-producing nerve cells in the brain, specifically in the substantia nigra and the locus coeruleus. It is characterized by muscle rigidity, tremor, a slowing of physical movement (bradykinesia) and, in extreme cases, a loss of physical movement (akinesia). Drug designing, one of the hottest topics have found its new pathway to create a history in the field of medical science. The lead compound analysis starts with CADD, assisting to identify and to optimize the right compound. The technique helps in generating a suitable compound specific to the disease; thereby an effective treatment is achieved. Molecular modeling method has been used for modeling a new molecule for Parkinson's using Carbidopa , a drug that's already designed. This drug is drawn using hyperchem, and its R group is modified by replacing different functional groups like CL, F, CF_2OH, CCL_2OH, NH_2, CF_3, CH_2CH_3OH, and I its place and docked by using gold software. The molecules designed as such are optimized using different algorithms and their affinity is checked with protein. The binding free energy of the protein is calculated by performing docking process. The molecule with minimum binding energy will have the maximum binding affinity. The binding free energy is calculated by the formula Z = Sum of the energy of optimized ligand devoid of solvation parameters and the energy of the protein - ligand optimization. The binding free energy of the designed molecules is obtained by eliminating the energy of the main molecule i.e. Carbidopa. From the results obtained it's clear that ligand 10 & 5 (-5.63 & -2.10.) for Parkinson's have the maximum binding affinity. So these molecules are determined as the best lead molecules targeting computationally.

Keywords: Parkinson's **,** Dopamine , CADD , Carbidopa , Hyperchem

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INTRODUCTION

Parkinson's disease belongs to a group of conditions called movement disorders. It is stimulation of the motor cortex by the basal ganglia, normally caused by the insufficient formation and action of dopamine, which is produced in the dopaminergic neurons of the Parkinson's disease (also known as Parkinson disease or PD) is a degenerative disorder of the central nervous system that often impairs the sufferer's motor skills and speech[1]. Parkinson's disease is a chronic, progressive neurodegenerative movement disorder. Tremors, rigidity, slow movement (bradykinesia), poor balance, and difficulty walking (called parkinson's gait) are characteristic primary symptoms of Parkinson's disease.It is characterized by muscle rigidity, tremor, a slowing of physical movement (bradykinesia) and, in extreme cases, a loss of physical movement (akinesia). The primary symptoms are the results of decreased brain.

Secondary symptoms may include high level cognitive dysfunction and subtle language problems[2]. PD is both chronic and progressive. Parkinson's results from the degeneration of dopamine-producing nerve cells in the brain, specifically in the substantia nigra and the locus coeruleus. Dopamine is a neurotransmitter that stimulates motor neurons, those nerve cells that control the muscles. When dopamine production is depleted, the motor system nerves are unable to control movement and coordination. Parkinson's disease patients have lost 80% or more of their dopamine-producing cells by the time symptoms appear[3].

Motor symptoms

The cardinal symptoms are:

- Tremor: normally 4-7 Hz tremor, maximal when the limb is at rest, and decreased with voluntary movement. It is typically unilateral at onset. This is the most apparent and well-known symptom, though an estimated 30% of patients have little perceptible tremor; these are classified as akinetic-rigid[4].
- Rigidity: stiffness; increased muscle tone. In combination with a resting tremor, this produces a ratchety, "cogwheel" rigidity when the limb is passively moved[5].
- Bradykinesia/akinesia: respectively, slowness or absence of movement. Rapid, repetitive movements produce a dysrhythmic and decremental loss of amplitude. Also "dysdiadokinesia", which is the loss of ability to perform rapid alternating movements
- Postural instability: failure of postural reflexes, which leads to impaired balance and falls[6].

Other motor symptoms include:

Gait and posture disturbances:

- Shuffling: gait is characterized by short steps, with feet barely leaving the ground, producing an audible shuffling noise. Small obstacles tend to trip the patient
- Decreased arm swing: a form of bradykinesia
- Turning "en bloc": rather than the usual twisting of the neck and trunk and pivoting on the toes, PD patients keep their neck and trunk rigid, requiring multiple small steps to accomplish a turn[7].
- Stooped, forward-flexed posture. In severe forms, the head and upper shoulders may be bent at a right angle relative to the trunk (camptocormia).
- Festination: a combination of stooped posture, imbalance, and short steps. It leads to a gait that gets progressively faster and faster, often ending in a fall[8].
- Gait freezing: "freezing" is another word for akinesia, the inability to move. Gait freezing is characterized by inability to move the feet, especially in tight, cluttered spaces or when initiating gait[9].
- Dystonia (in about 20% of cases): abnormal, sustained, painful twisting muscle contractions, usually affecting the foot and ankle, characterized by toe flexion and foot inversion, interfering with gait. However, dystonia can be quite generalized, involving a majority of skeletal muscles; such episodes are acutely painful and completely disabling[10].

Speech and swallowing disturbances

- Hypophonia: soft speech. Speech quality tends to be soft, hoarse, and monotonous. Some people with Parkinson's disease claim that their tongue is "heavy".
- Festinating speech: excessively rapid, soft, poorly-intelligible speech.
- Drooling: most likely caused by a weak, infrequent swallow and stooped posture[11].
- Non-motor causes of speech/language disturbance in both expressive and receptive language: these include decreased verbal fluency and cognitive disturbance especially related to comprehension of emotional content of speech and of facial expression
- Dysphagia: impaired ability to swallow. Can lead to aspiration, pneumonia.

Other motor symptoms:

- fatigue (up to 50% of cases);
- masked faces (a mask-like face also known as hypomimia), with infrequent blinking;
- difficulty rolling in bed or rising from a seated position;
- micrographia (small, cramped handwriting);
- impaired fine motor dexterity and motor coordination;
- impaired gross motor coordination;

- Poverty of movement: overall loss of accessory movements, such as decreased arm swing when walking, as well as spontaneous movement[12].

Non-motor symptoms

Mood disturbances

- Estimated prevalence rates of depression vary widely according to the population sampled and methodology used. Reviews of depression estimate its occurrence in anywhere from 20-80% of cases. Estimates from community samples tend to find lower rates than from specialist centres. Most studies use self-report questionnaires such as the Beck Depression Inventory, which may overinflate scores due to physical symptoms. Studies using diagnostic interviews by trained psychiatrists also report lower rates of depression.
- More generally, there is an increased risk for any individual with depression to go on to develop Parkinson's disease at a later date.
- 70% of individuals with Parkinson's disease diagnosed with pre-existing depression go on to develop anxiety. 90% of Parkinson's disease patients with pre-existing anxiety subsequently develop depression; apathy or abulia[13].

Cognitive disturbances

- slowed reaction time; both voluntary and involuntary motor responses are significantly slowed.
- executive dysfunction, characterized by difficulties in: differential allocation of attention, impulse control, set shifting, prioritizing, evaluating the salience of ambient data, interpreting social cues, and subjective time awareness. This complex is present to some degree in most Parkinson's patients;
- dementia: a later development in approximately 20-40% of all patients, typically starting with slowing of thought and progressing to difficulties with abstract thought, memory, and behavioral regulation. Hallucinations, delusions and paranoia may develop.
- short term memory loss; procedural memory is more impaired than declarative memory. Prompting elicits improved recall.
- medication effects: some of the above cognitive disturbances are improved by dopaminergic medications, while others are actually worsened.

Sleep disturbances

- Excessive daytime somnolence
- Initial, intermediate, and terminal insomnia
- Disturbances in REM sleep: disturbingly vivid dreams, and REM Sleep Disorder, characterized by acting out of dream content - can occur years prior to diagnosis

Sensation disturbances

- impaired visual contrast sensitivity, spatial reasoning, colour discrimination, convergence insufficiency (characterized by double vision) and oculomotor control
- dizziness and fainting; usually attributable orthostatic hypotension, a failure of the autonomous nervous system to adjust blood pressure in response to changes in body position
- impaired proprioception (the awareness of bodily position in three-dimensional space)
- reduction or loss of sense of smell (microsmia or anosmia) - can occur years prior to diagnosis,
- pain: neuropathic, muscle, joints, and tendons, attributable to tension, dystonia, rigidity, joint stiffness, and injuries associated with attempts at accommodation

Autonomic disturbances

- oily skin and seborrheic dermatitis
- urinary incontinence, typically in later disease progression
- nocturia (getting up in the night to pass urine) - up to 60% of cases
- constipation and gastric dysmotility that is severe enough to endanger comfort and even health
- altered sexual function: characterized by profound impairment of sexual arousal, behavior, orgasm, and drive is found in mid and late Parkinson disease. Current data addresses male sexual function almost exclusively[14].
- weight loss, which is significant over a period of ten years - 8% of body weight lost compared with 1% in a control group.

Treatment

Parkinson's disease is a chronic disorder that requires broad-based management including patient and family education, support group services, general wellness maintenance, exercise, and nutrition. At present, there is no cure for PD, but medications or surgery can provide relief from the symptoms. Recently, Botox injections are being investigated as a non-FDA approved possible experimental treatment. The most widely used form of treatment is L-dopa in various forms. L-dopa is transformed into dopamine in the dopaminergic neurons by L-aromatic amino acid decarboxylase (often known by its former name dopa-decarboxylase). However, only 1-5% of L-DOPA enters the dopaminergic neurons. The remaining L-DOPA is often metabolised to dopamine elsewhere, causing a wide variety of side effects. Due to feedback inhibition, L-dopa results in a reduction in the endogenous formation of L-dopa, and so eventually becomes counterproductive.Carbidopa and benserazide are dopa decarboxylase inhibitors. They help to prevent the metabolism of L-dopa before it reaches the dopaminergic neurons and are generally given as combination preparations of carbidopa/levodopa (co-careldopa) (e.g. Sinemet, Parcopa) and benserazide/levodopa (co-beneldopa) (e.g. Madopar). There are also controlled release versions of Sinemet and Madopar that spread out the effect of the L-dopa. Duodopa is a combination of levodopa and carbidopa, dispersed as a viscous gel. Using a patient-operated portable pump, the drug is continuously delivered via a tube directly into the upper small intestine, where it is rapidly absorbed. Tolcapone inhibits the COMT enzyme, thereby prolonging the effects of L-dopa. The dopamine-agonists bromocriptine, pergolide, pramipexole, ropinirole , cabergoline, apomorphine, and lisuride, are moderately effective. These have their own side effects including those listed above in addition to somnolence, hallucinations and /or insomnia. Selegiline and rasagiline reduce the symptoms by inhibiting monoamine oxidase-B (MAO-B), which inhibits the breakdown of dopamine secreted by the dopaminergic neurons [15,16,17].

Surgical interventions
Speech therapies
Physical exercise
Methods undergoing evaluation
Gene therapy, Neuroprotective treatments, Neural transplantation, Nutrients.
Plan of Work

- Energy Calculations of Ligand in Air by Single Point, Geometry Optimisation, Molecular Dynamics, Monte Carlo
- Energy Calculations of Ligand with different replaced groups
- Energy Calculations of Ligands (Solvent Intra)
- Energy Calculations of Ligands (Protein Intra)
- Docking
- Free Energy Calculations for more effective drug
- Protein Analysis by different Databases

MATERIALS AND METHODS

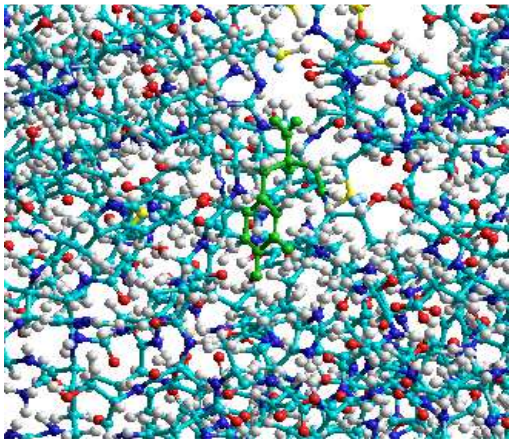
Open Eye scientific software: OpenEye Scientific Software develops large-scale modeling applications and toolkits. Primarily geared towards drug discovery and design, areas of application include structure generation, docking, shape comparison, electrostatics, chemical informatics and visualization. The software is designed for scientific rigor, as well as speed, scalability and platform independence. Ligand –solvent interactions (Inter-solvent) (-solventPB).For optimization of small molecules in solution, the electrostatic part of molecule-solvent interactions will be calculated using Poisson-Boltzmann model of Open Eye scientific software.

Gold Genetic Optimisation for Ligand Docking:GOLD is a program for calculating the docking modes of small molecules into protein binding sites. The product of collaboration between the University of Sheffield, GlaxoSmithKline plc and CCDC, GOLD is very highly regarded within the molecular modeling community for its accuracy and reliability Ligand –protein interactions (Inter-protein) (Docking) For Docking of small molecules into the protein active site, the VDW, Hydrogen bonds and hydrophobic energies of ligand-protein interactions will be calculated using GA of Gold software[18].

Hyperchem: HyperChem is a versatile molecular modeler and editor and a powerful computational package. It offers many types of molecular and quantum mechanics calculations. For optimization of small molecules in

solution and protein complex the intra molecular energies of ligand-solvent and ligand protein will be calculated using molecular mechanics calculations of Hyperchem software[19].

Structure of Aromatic-L-Amino-Acid Decarboxylase
Complexed With (-)-Carbidopa 2.3a° Resolution



Computer Aided Drug Design Approaches

Computational assessment of the binding affinity of enzyme inhibitors prior to synthesis is an important component of computer-aided drug design paradigms. In this study, the molecular mechanics (MM) method is used for the estimation of relative binding affinities of inhibitors to an enzyme. Qualitative predictions of relative binding affinities of Beta Secretase inhibitors using MM method are discussed[20]. The results indicate that the MM based method is useful in the qualitative estimation of relative binding affinities of enzyme inhibitors prior to synthesis.CADD approach has contributed to the successful discovery of numerous novel enzyme inhibitors including inhibitors of thymidylate synthase (Appelt et al., 1991 and Reddy et al., 1993), HIV-1 Protease (Reddy and Appelt, 2001) and purine nucleoside phosphorylase (Montgomery et al., 1993) inhibitors. In each case CADD was used to predict the binding affinity of an inhibitor designed from a lead compound prior to synthesis. Earlier, Jorgensen et al. (2000), reported results using the free energy perturbation calculations in an iterative structure-based design program to accurately predict relative binding affinities of COX-1, COX-2 and SRC SH2 domain and linear interaction energy results for thrombin and HIV –RT. This work focuses on lead inhibitor optimization strategies using molecular mechanics method by predicting relative binding affinities of galantamine inhibitors[21].

RESULTS

QSAR Properties

Molecule number	Surface areas (Approx) in $(A^o)^2$	Surface area (grid) In $(A^o)^2$	Volume In $(A^o)^3$	Hydration Energy (Kcal/mol)	Log P	Refractivity	Polarizability	Mass (amu)
Ligand 1 (Standard)	323.32	394.27	637.82	-24.67	1.49	58.54	22.47	226.23
Ligand 2	316.34	374.95	594.16	-26.04	-1.13	54.27	20.15	233.63
Ligand 3	309.00	388.51	614.29	-25.79	-0.78	57.10	20.55	230.20
Ligand 4	297.99	409.05	661.53	-24.50	-1.14	63.65	22.93	278.21
Ligand 5	316.48	419.63	696.61	-23.13	-0.77	73.32	26.97	311.12
Ligand 6	279.93	391.29	621.14	-28.25	-1.53	60.14	21.99	227.22
Ligand 7	315.81	402.56	659.20	-20.97	-0.68	62.24	22.20	280.20
Ligand 8	295.61	389.28	613.80	-28.83	-1.19	58.48	21.27	228.20
Ligand 9	338.30	407.53	659.44	-25.06	-1.00	69.99	25.67	338.10
Ligand 10	323.65	408.68	667.57	-23.02	-0.84	66.06	24.31	240.26

List of Inhibitors Developed

Sl.no.	Ligand	Carbidopa inhibitor with substituent
1.	Ligand -1	R1(CH3) (STD)
2	Ligand-2	R2(CL)
3	Ligand -3	R3(F)
4	Ligand -4	R4(CF2OH)
5	Ligand -5	R5(CCL2OH)
6	Ligand -6	R6(NH2)
7	Ligand -7	R7(CF3)
8	Ligand -8	R8(CH2CH3)
9	Ligand -9	R9(OH)
10	Ligand-10	R10(I)

Table 1:- Solvent (Intra)

MOLECULE	INTRA ENERGY(x_2)
R1(CH3) (STD)	-12.07
R2(CL)	-1.01
R3(F)	-23.01
R4(CF2OH)	-23.48
R5(CCL2OH)	-18.42
R6(NH2)	-22.27
R7(CF3)	-17.69
R8(CH2CH3)	-17.01
R9(OH)	-17.41
R10(I)	-19.49

Table 2:- Energy of Ligand In Air (X_1)

MOLECULE	Energy in Air (x_1)
R1(CH3) (STD)	7.66
R2(CL)	1.00
R3(F)	17.99
R4(CF2OH)	18.22
R5(CCL2OH)	11.82
R6(NH2)	19.19
R7(CF3)	12.44
R8(CH2CH3)	12.29
R9(OH)	13.49
R10(I)	13.49

Table 3:- Protein (Intra)

MOLECULE	INTRA ENERGY(y_1)
R1(CH3) (STD)	15.51
R2(CL)	13.62
R3(F)	75.76
R4(CF2OH)	15.50
R5(CCL2OH)	09.34
R6(NH2)	15.68
R7(CF3)	17.13
R8(CH2CH3)	17.01
R9(OH)	13.59
R10(I)	07.14

Table 4:- Docking (Inter)

MOLECULE	DOCKING(y_2)
R1(CH3) (STD)	-50.34
R2(CL)	-42.90
R3(F)	-45.53
R4(CF2OH)	-46.74
R5(CCL2OH)	-48.46
R6(NH2)	-45.74
R7(CF3)	-47.60
R8(CH2CH3)	-46.23
R9(OH)	-46.36
R10(I)	-49.19

Table 5:-

MOLECULE	SOLVENT($X=x_1+x_2$)	PROTEIN($Y=y_1+y_2$)
R1(CH3) (STD)	-4.41	-34.83
R2(CL)	-0.01	-29.28
R3(F)	-5.02	30.23
R4(CF2OH)	-5.26	-31.24
R5(CCL2OH)	-6.60	-39.12
R6(NH2)	-3.08	-30.06
R7(CF3)	-5.25	-30.47
R8(CH2CH3)	-4.72	-29.22
R9(OH)	-3.92	-32.77
R10(I)	-6.0	-42.05

Table 6:-Binding Free Energy Changes

S.NO	MOLECULES	Z-VALUES(Y-X)	E BIND(Z_2-Z_1)
1	R1(CH3) (STD)	-30.42 (Z_1)	0.00
2	R2(CL)	-29.27 (Z_2)	1.15
3	R3(F)	35.25 (Z_3)	65.67
4	R4(CF2OH)	-25.98 (Z_4)	4.44
5	R5(CCL2OH)	-32.52 (Z_5)	-2.10
6	R6(NH2)	-26.98 (Z_6)	3.44
7	R7(CF3)	-25.22 (Z_7)	5.20
8	R8(CH2CH3)	-24.50 (Z_8)	5.92
9	R9(OH)	-28.85 (Z_9)	1.57
10	R10(I)	-36.05 (Z_{10})	-5.63

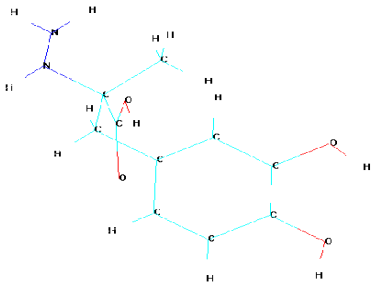
Docking(y_2) :

Fitness (y_2) = S (hb – ext) + 1.3750 × S (vdw - ext) + S (hb - int) + 1.0000 × S (vdw - int)

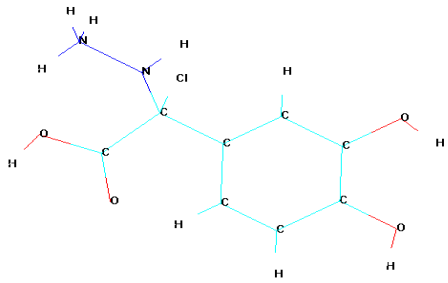
(Solvent) $X = x_1 + x_2$ (Protein) $Y = y_1 + y_2$

Binding free enrgy $Z = Y - X$

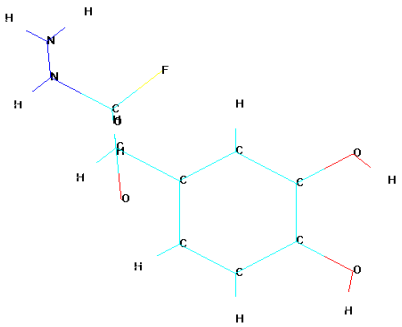
E bind $= Z_2 - Z_1$



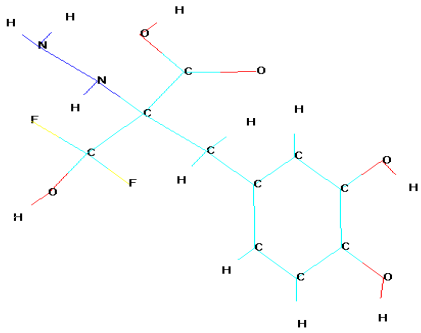
Standard Ligand 1($R=CH_3$)



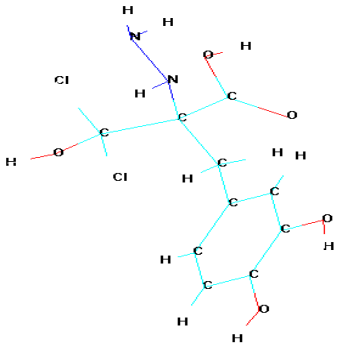
Ligand-2(R=Cl)



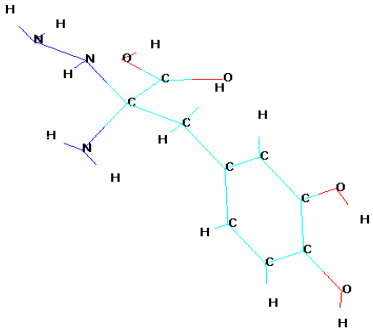
Ligand-3 (R=F)



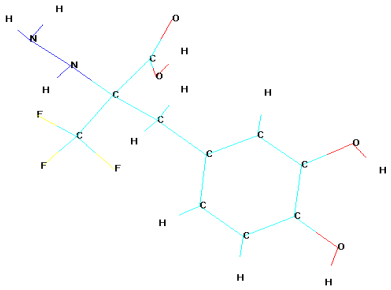
Ligand-4 (R= CF_20H)



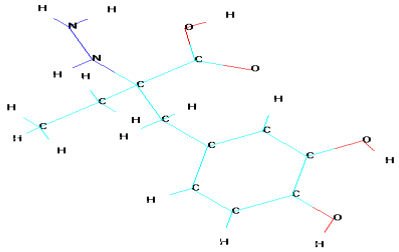
Ligand–5(R=CCL_2OH)



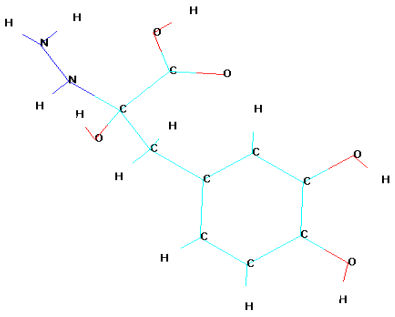
Ligand-6(R=NH_2)



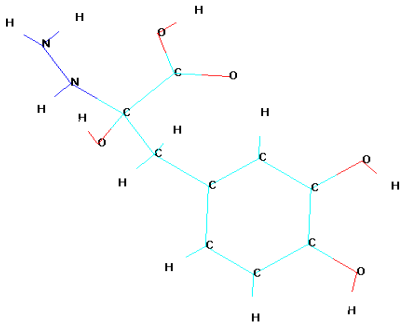
Ligand-7(R=CF_3)



Ligand-8(R=CH_2CH_3)



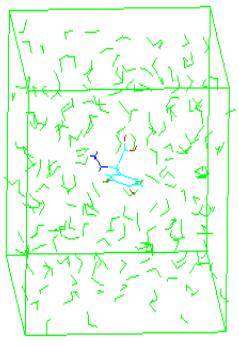
Ligand-9(R=OH)



Ligand-10(R=I)

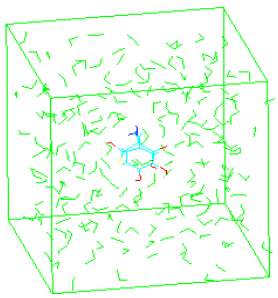
Solvent interactions:

Standard Ligand 1(R=CH_3)

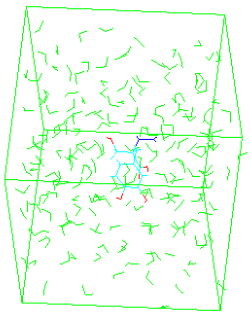


Ligand-2(R=CL)

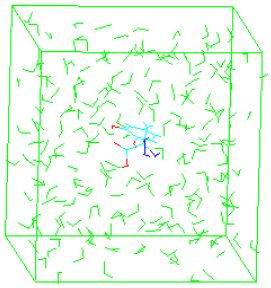
Ligand-3(R=F)



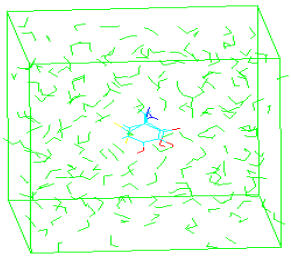
Ligand-4(R=CF_2OH)



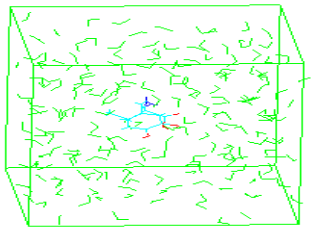
Ligand–5(R=CCL_2OH)



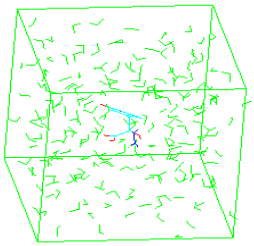
Ligand-6(R=NH_2)



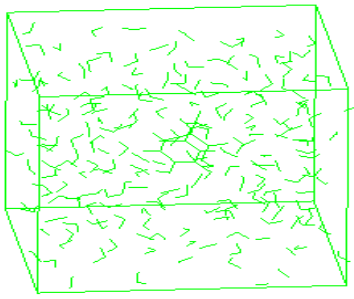
Ligand-7(R=CF_3)



Ligand-8(R=CH_2CH_3)

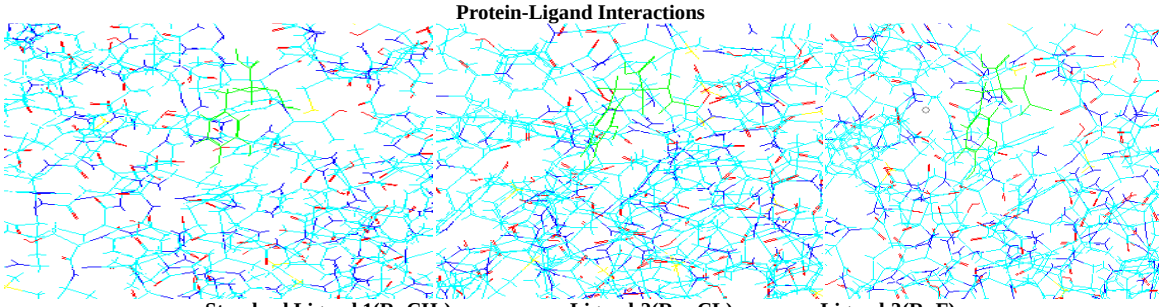


Ligand-9(R=OH)

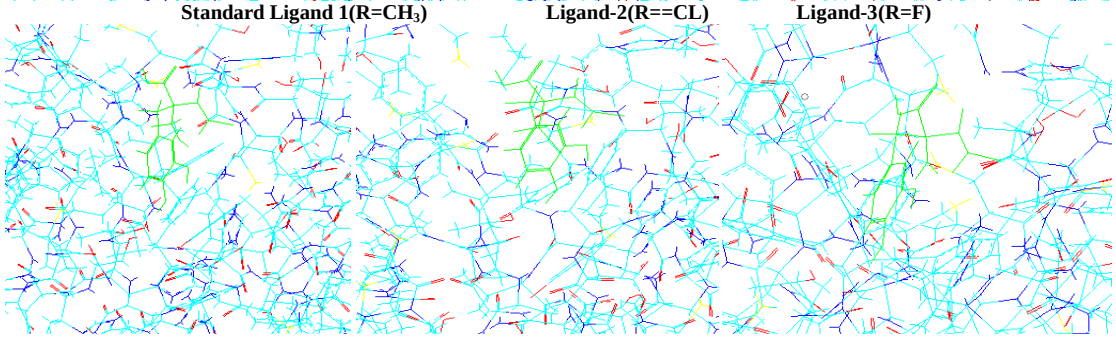


Ligand-10(R=I)

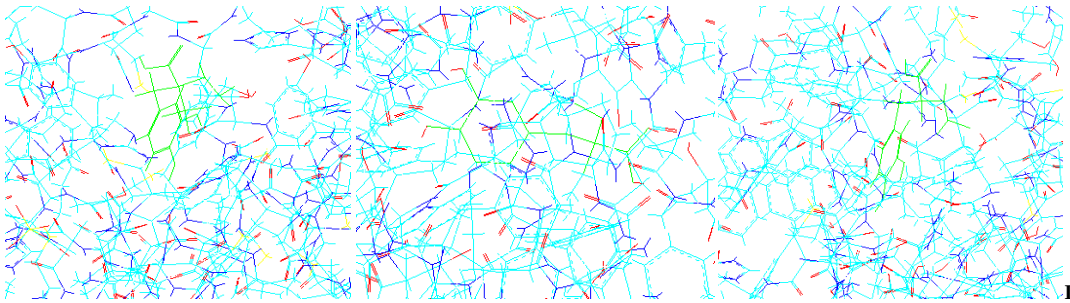
Protein-Ligand Interactions



Standard Ligand 1(R=CH_3) **Ligand-2(R==CL)** **Ligand-3(R=F)**



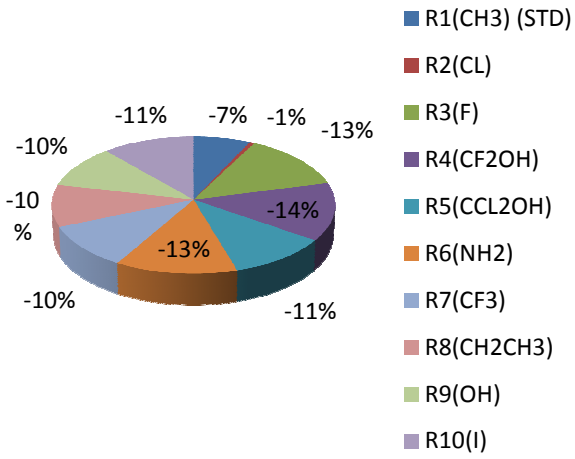
Ligand-4(R=CF_2OH) **Ligand–5(R=CCL_2OH)** **Ligand-6 (R=NH_2)**

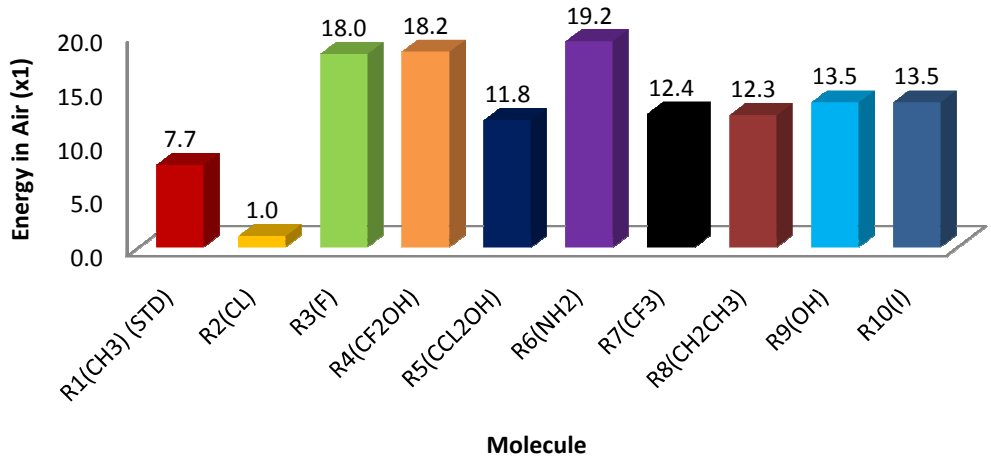


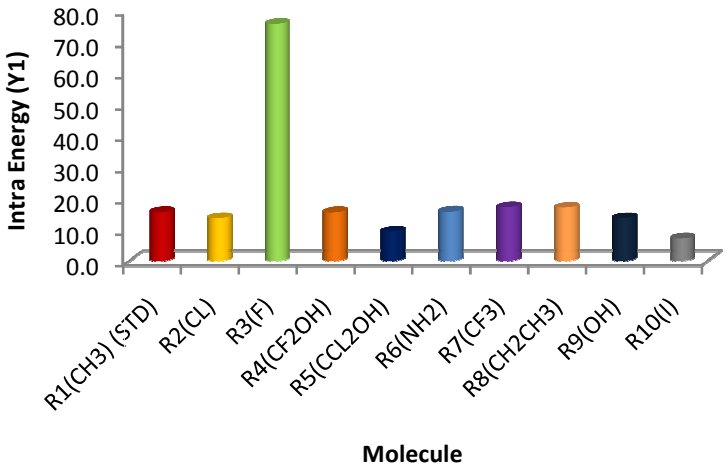
7(R=CF_3) **Ligand-8 (R=OH)** **Ligand-9(R=I)** **Ligand-**

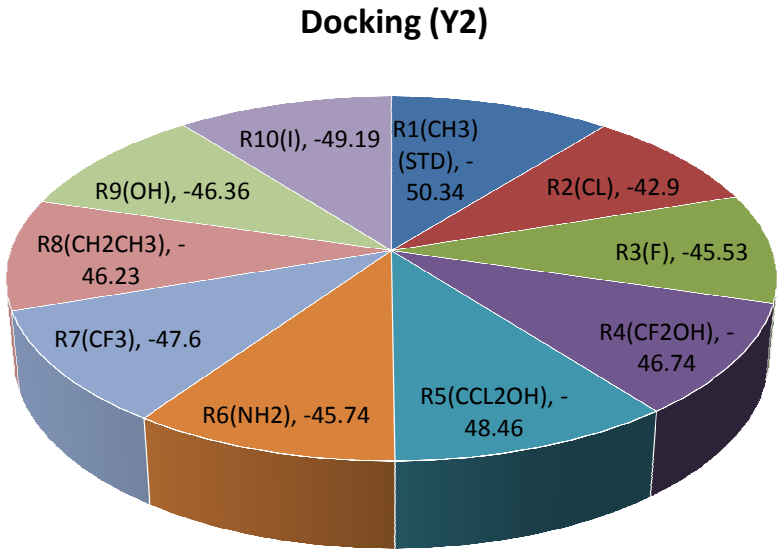
Statistical Interpretation:

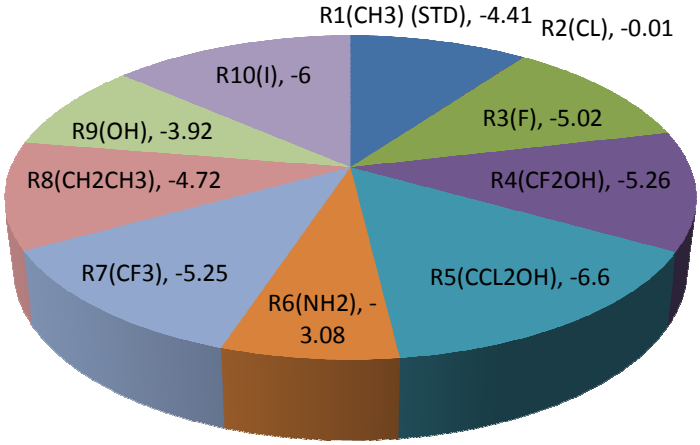
Table 1 Solvent (Intra)



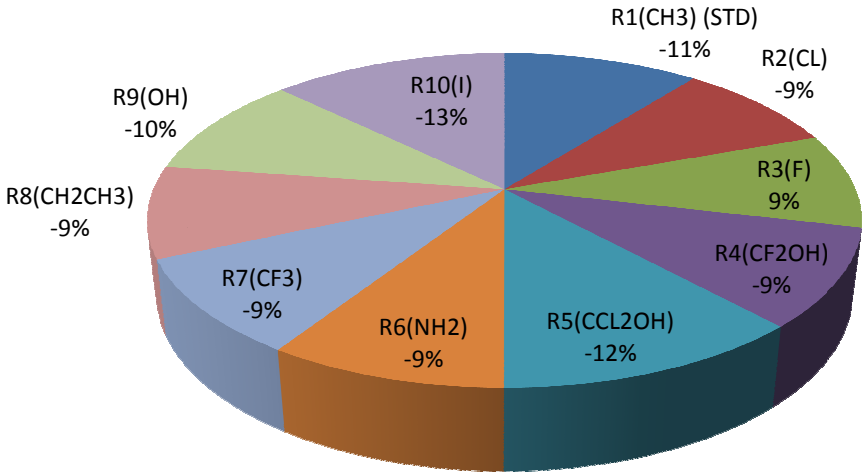



80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Intra Energy (Y1)
R1(CH3) (STD)
R2(CL)
R3(F)
R4(CF2OH)
R5(CCL2OH)
R6(NH2)
R7(CF3)
R8(CH2CH3)
R9(OH)
R10(I)
Molecule


Docking (Y2)
R1(CH3) (STD), -50.34
R2(CL), -42.9
R3(F), -45.53
R4(CF2OH), -46.74
R5(CCL2OH), -48.46
R6(NH2), -45.74
R7(CF3), -47.6
R8(CH2CH3), -46.23
R9(OH), -46.36
R10(I), -49.19



SOLVENT(X=x1+x2)



PROTEIN(Y=y1+y2)

DISCUSSION

Drug designing, one of the hottest topics have found its new pathway to create a history in the field of medical science. The lead compound analysis starts with CADD, assisting to identify and to optimize the right compound. The technique helps in generating a suitable compound specific to the disease ; thereby an effective treatment is achieved. Molecular modeling method has been used for modeling a new molecule for **Parkinson's** using **Carbidopa** , a drug that's already designed. This drug is drawn using hyperchem, and its R group is modified by replacing different functional groups like CL ,F, CF_2OH, CCL_2OH, NH_2, CF_3, CH_2CH_3, OH, I in its place and docked by using gold software. The molecules designed as such are optimized using different algorithms and their affinity is checked with protein. The binding free energy of the protein is calculated by performing docking process. **The molecule with minimum binding energy will have the maximum binding affinity.** The binding free energy is calculated by the formula Z = Sum of the energy of optimized ligand devoid of solvation parameters and the energy of the protein - ligand optimization. The binding free energy of the designed molecules is obtained by eliminating the energy of the main molecule i.e.**Carbidopa** .From the results obtained it's clear that ligand 10 & 5 for **Parkinson's** have the maximum binding affinity. So these molecules are determined as the best lead molecules targeting computationally. We can findout the drug binding affinity by using fitness of the drug , which can bind to target protein during the docking process and second way is using Gibbs free energy calculations.According to

this more negative value, we can consider as more effective drug. Here the following replacement groups for **Parkinson's** such as **I & CCL_2OH** found to be -5.63 & -2.10. So we can predict the above mentioned replaced groups found to be more effective.

CONCLUSION

A comparison of the calculated binding free energies for structurally similar inhibitors to CARBIDOPA molecular mechanics methods gave suitable analogues. These results clearly indicate that before synthesis and biochemical testing of new analogs, one can use molecular mechanics based methods for qualitative assessment of relative binding affinities for speeding up drug discovery process by eliminating less potent compounds from synthesis. The inhibitor 10 & 5 with the substituents I & CCL2OH identified as the most suitable analogues of CARBIDOPA in the present study, and need to be further evaluated in laboratory.

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