

DESIGN AND EVALUATION OF MOLECULES FOR PARKINSON'S DISEASE

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CERTIFICATE

This is to certify that the project entitled “Design and evaluation of molecules for Parkinson’s Disease” which is being submitted by **Sohail Sankhyan** in partial fulfillment for the award of degree of B.Tech in Biotechnology from Jaypee University of Information Technology is the record of candidate’s own work carried out under my supervision. This work has not been submitted partially or wholly to any other University or Institute for the award of this or any degree or diploma.

Dr. Udayabanu

Designation:

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Sohail Sankhyan

Summary

Cannabis basically is helpful in decreasing the progression of the Parkinson's Disease as it overcomes all the disadvantages of deficiency of dopamine and side effects of the levodopa consumption such as dyskinesia. In case of levodopa consumption or in take there is no particular starting time for the action of the drug . We basically have unpredictable function of the levodopa consumption and its conversion in to dopamine inside the brain. For example: If a Parkinson's patient takes a small scoop of levodopa in the morning then it is not known when that dose will start its action in the body, thus he/she can have its action after 2 to 3 hrs and it may last long for 15mins to 2 hrs. Thus Cannabis here is helpful in a way that its mechanism of action is instant and it lasts long. Cannabis basically has THC tetrahydrocannabinol which binds to the CB2 receptor in the brain more specifically magneta 2ki9 receptor which then targets the dopamine D2R receptor for the production of dopamine neurotransmitter in the brain. Thus combined dose of the levodopa and cannabis together will have a prolonged effect and longer action time reducing the dose per day and also improve the quality life of the patient every day. The idea was to superimpose the two drugs on the basis of chemical structural superimposition using the bioinformatics tool MAESTRO. The superimposition of chemical structure were done using insilico biotechnology but the super imposition resulted with skipping of the atoms due to presence of higher number of the atoms. MAESTRO is the latest bioinformatics tool released in January 2016.

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CHAPTER 1

Introduction

Parkinson's disease is a chronic, progressive disorder of the nervous system that causes the malfunction and death of neurons in the brain. Parkinson's disease (PD) typically starts with a minor tremor and develops gradually, eventually causing stiffness throughout the body and slowing movement. An accelerating ache of the afraid arrangement apparent by tremor, able-bodied rigidity, and slow movement, chiefly affecting middle-aged and aged people. It is associated with decline of the basal ganglia of the academician and absence of the neurotransmitter dopamine. Some neurons aftermath dopamine, a actinic that sends letters to the allotment of the academician that controls movement, and as these neurons die from Parkinson's disease, the bulk of dopamine decreases and movement is affected. After diagnosis, treatments can advice abate symptoms, but there is no cure. Acknowledgment to assertive toxins has acquired Parkinson's affects in attenuate affairs. Other still-unidentified ecology factors may as well could cause PD. Parkinson's disease has no cure but its progression can be slowed down with the help of chemical L-DOPA(**Levodopa**).

Stages of Parkinson's disease

Stage One

During this primary stage, the person has a gentle symptoms that typically don't interfere with daily activities. Tremor and alternative movement symptoms occur on one facet of the body solely. Friends and family might notice changes in walking ,posture and facial expressions.

Stage Two

Parkinson's, the symptoms begin getting worse. Tremor, rigidity and different movement symptoms have an effect on each side of the body. Walking issues and poor posture might become apparent during this stage, the person is still able to live alone, however finishing day-after-day tasks becomes harder and may take longer.

Stage Three

Stage 3 is taken into account mid-stage within the progression of the sickness. Loss of balance and slowness of movements are hallmarks of this phase. Falls are more common although the person remains totally free, symptoms considerably impair activities of daily living like dressing and meal intake.

Stage Four

During this stage of Parkinson's, symptoms are severe and extremely limiting.

It's possible to stand without help, however movement might need a walker. The person requires help with activities of daily living and is unable to live alone.

Levodopa

It is a chemical that's made and used as a part of the conventional biology of humans, some animals and plants. Some animals and humans make it via biogenesis from the amino acid L-tyrosine. L-DOPA is the precursor to the neurotransmitters dopamine. L-DOPA crosses the protecting blood-brain barrier, whereas dopamine itself cannot.

Thus, L-DOPA is used to extend the concentrations within the treatment of Parkinson's and dopamine-responsive dystonia. This treatment was made sensible and established clinically by Saint George Cotzias and his coworkers, for which they won the 1969 Lasker Prize. Once L-DOPA has entered the central nervous system, it is converted into Dopamine by the catalyst aromatic L-amino acid enzyme, also well-known as amino alkanolic acid enzyme.

Medical cannabis or medical marijuana can refer to the utilization of cannabis and its cannabinoids to treat sickness or improve symptoms. Evidence suggests that cannabis doubtless slow the progression of Parkinson's by providing neuron protective effects.

The cannabinoids found in cannabis are in a position to suppress excited toxicity, glial activation and aerophilic injury that cause the degeneration of the dopamine-releasing neurons. In addition, they improve the function of cell's mitochondria and activation of cellular rubble clearance, further encouraging nerve cell health.

Researchers have found evidence that one specific cannabinoid found in cannabis named tetrahydrocannabinol (THC), helps within the treatment of Parkinson's disease by aiding in the interference of harm caused by free radicals and activating a receptor that encourages the formation of latest mitochondria. Cannabis can facilitate patients with Parkinson's disease to manage their symptoms. One study observed that when smoking cannabis, Parkinson's disease patients saw vital enhancements in reducing motor disability and impairments, tremors, rigidity, bradykinesia, sleep and pain.

The disease will be either primary or secondary. Primary brain disorder has no noted cause, though some atypical cases have a genetic origin. Secondary degenerative disorder is as a result of the noted causes like toxins. Several risks associated protecting factors are investigated. The clearest proof is for an enhanced risk in folks exposed to some pesticides and a reduced risk in tobacco smokers.

The motor symptoms of the disease result from the death of cells within the substantia nigra, an area of the neural structure(The Mid Brain).This leads to not enough Dopamine in these areas. The rationale for this necro biosis area unit poorly understood however involves the build-up of proteins into Lewy bodies within the neurons, wherever the Lewy bodies area unit set is partially associated with the expression and degree of the symptoms. Designation of typical cases is especially supported symptoms, with tests like neuroimaging being employed for confirmation.

Treatments, usually the antiparkinson medications Levodopa and Dopastat agonists, improve the first symptoms of the disease because the disease progresses and neurons are lost, these medications become ineffective whereas at identical time turn out a complication marked by involuntary twisting movements.

Diet and a few varieties of rehabilitation have shown some effectiveness at rising symptoms. Surgery to position deep brain stimulation are used for cutting back motor symptoms in severe cases wherever medicine are ineffective.

Analysis directions involve investigations into new animal models of the disease and of the potential utility of sequence medical care, stem cell transplants, and neuroprotective agents. Medications to treat non-movement-related symptoms of Parkinson's disease, like sleep disturbances and emotional issues, also exist.

CHAPTER 2

Literature Review

Parkinson's Disease happens once the brain slowly stops generating a neurochemical known as Dopamine, that commonly assists within the transmission of signals to the section of the brain that controls movement. The disease affects these nerve cells and, with light production of Dopamine, the patient has reduced ability to manage his or her movements, body, and emotions, although the disease itself isn't fatal, complications arising from it are terribly dangerous. Since the disease affects the body increasingly, it becomes worse over time across many years. Most patients' symptoms take many years to become noticeable.

The reason for Parkinson's remains unknown, it's tied to the untimely death of specialised cells within the brain that manufacture dopamine and ends up in an absence of coordination, poor motor perform, and issue walking.

The primary symptoms of Parkinson's disease include:

1. Tremors
2. Rigidity
3. Bradykinesia (very slow physical movements)
4. Impaired balance coordination
5. Sleep disturbance
6. Neuropathic pain

Tremors are the initial and yet most typical symptom of the Parkinson's disease. However, not everybody affected by the disease can exhibit it. Tremor typically begins in one in all the limbs. Eventually, the disease can begin targeting muscles found throughout the body. This could lead to numerous issues like difficulties with swallowing food or constipation.

When the Parkinson's disease has totally developed, a patient might have a set or blank expression, difficulties in speech, and different issues. Some patients additionally experience a reduction in their psychological feature and skills.

Parkinson's symptoms commonly begin to be seen once patients are between fifty and sixty years old. However, at times, the symptoms begin appearing earlier.

After beginning Parkinson's disease treatment using medications, patients might begin experiencing notable changes (improvement) of their symptoms. With time, though, the advantages of medicines commonly reduce or abate reliable, even though the symptoms will still be fairly well managed.

Parkinson's is basically a condition of deficient dopamine within the brain, the foremost direct answer is to supplement the Dopamine. The sole downside is that Dopamine, by itself, isn't able to cross the barrier. However, its precursor, levodopa, will enter the brain simply and be reborn to Dopamine. Since the Seventies, the foremost effective treatment of Parkinson's sickness symptoms has been a combination of Brocadopa and carbidopa (carbidopa-levodopa). The carbidopa works to stop Brocadopa from untimely alteration to Dopamine before reaching wherever it's required the foremost, particularly the brain.

Marijuana As A Treatment Option :

In a recent study done by the National Institutes of Health, results instructed the effectiveness of the drug. According to the National Biotechnology information (NCBI) study in the National Institutes of Health, twenty two patients had favorable results in clinical trials both in 2011 and 2012. Users' total scores on the Motor Examination portion of the Unified Parkinson disease Rating Scale improved considerably from the mean score of thirty three. at baseline to twenty three once cannabis consumption was done .

Analysis of specific motor symptoms had vital improvement in tremors moreover also in sleep and pain scores when smoking cannabis for thirty minutes. The conclusion recommended that cannabis has a therapeutic result on Parkinson's but for that a large-scale study should be conducted for more measures for the success of the treatment.

Marijuana comes from the plant *Cannabis sativa*, that contains over 60 totally different compounds spoken as cannabinod. All of these have a major “psychoactive” part — Delta-9-tetrahydrocannabinol (THC) —that causes alterations in perception, mood and behavior. Marijuana has been used for several years across the world as a healthful herb for a large array of diseases. Throughout previous few decades there has been exaggerated interest on the employment of the herb in treating varied diseases, together with Parkinson's disease.

Our body already have cannabinoid receptors CB1 and CB2 to which the Tetrahydrocannabinol (THC) binds present in marijuana. Cannabinoids influence varied body processes like pain and inflammation. Therefore, if somebody consumes marijuana, it will assist those natural chemicals to operate with more efficiency. Specifically, cannabinoids can relieve symptoms, like dystonia and neurological disease, that are prevailing in some patients affected by the Parkinson's disease. Cannabis has been found to treat not solely the shaking ,rigidity but also the depression that accompany Parkinson's. Psychoactive drug (THC) and Cannabidiol (CBD), the first cannabinoids (active therapeutic chemicals) found in cannabis, area unit best-known to manage spasms, scale back inflammation, and stop any medicine harm.

Cannabis is helpful in Parkinson's as it is:

- >Neuroprotective
- >An antioxidant
- >Anti-Inflammatory

Parkinson's disease at present has no effective standard treatment. In fact, the first treatment involves dopamine increasing medication that additionally increase a toxin substance referred as 6-hydroxy-dopamine, and it thus will truly accelerate the progression of the disease.

This is often the reason why natural alternatives that are safe, effective, and protected by scientific evidence and are thus required nowadays. Thankfully presymptomatic analysis on cannabidiol has already discovered some promising results, as well as 2 studies in animal models of Parkinson's disease (PD) assessing its neuroprotective properties.

Cannabidiol protects against the neurotoxin referred to as MPP(+), that is believed to be accountable for the harm to the dopamine-producing cells in the substantia nigra of the Parkinson's patient, by preventing neuron death and causing neuritogenesis (a neuro-regenerative method for repairing broken neurons).

This mechanism was independent of the neural protein (NGF) pathway, although it involves ngf receptors. Cannabidiol was additionally found to extend the expression of nerve

fibre and synaptic proteins. The study resulted that CBD's neuroprotective properties may benefit the Parkinson's patients.

The data available for this research is limited work done is not to the mark for the declaration of the specified results for the use of cannabis in the treatment.

Chapter 3

Hypothesis

The basic idea is to superimpose the two chemical structures i.e levodopa and THC (Tetrahydrocannabinol) for the production of dopamine for a longer period of time, this will change the quality of life of the patient throughout the day. Levodopa is used due to the reason that dopamine itself cannot cross the blood brain barrier. Thus levodopa is a precursor of dopamine which crosses the blood brain barrier and enters the brain and then gets converted to dopamine. Basically the use of cannabis is because it has advantages which are very helpful for the Parkinson's patient. Dopamine itself has a depressant activity. Low levels of dopamine in brain has a depressant activity. Cannabis overcomes this as it targets the D2R receptor in the brain for the production of the dopamine thus having an anti-depressant activity. This basically will have a fast and enhanced effect for a longer period of time, thus will change the quality of life of the patient with day.

Cannabis is beneficial in way that it overcomes the antidepressant activity of the dopamine, this helps in reduction of the strong hallucinations caused by levodopa side effects. The effect starts as soon as it crosses the blood brain barrier, took either by smoking or ingesting. The effect last long for a minimum of 5 hours for people who often intake it and 12 hours for the beginners. Thus a Parkinson's patient can work easily and properly for a longer period of time. This reduces the intake of levodopa as production of dopamine is for a longer period of time.

Insilico (Biotech) Drug Design –

1. THC (Tetrahydrocannabinol) and Levodopa structure superimposition.
2. Active Site determination through previous studies done on cannabis.
3. Virtual Screening of the model structure.

>Final evaluation of the molecule on dopamine homeostasis.

SUPERIMPOSITION OF CHEMICAL STRUCTURES

Structural superimposition is done with the help of a tool of bio informatics known as MAESTRO.

Maestro: A powerful, all-purpose molecular modeling environment

Maestro is the unified interface for all Schrödinger software system with impressive rendering capabilities, and a powerful choice of analysis . It is easy to use design and mix to create new structures. Maestro is a flexible modeling atmosphere for all researchers.

Maestro is the linchpin of Schrödinger's process technology way more than simply a visualisation program, it conjointly helps researchers organize and analyze information. Maestro's intuitive interface makes setting up calculations simple and easy. Computed results are mechanically returned and incorporated into projects for any study. Maestro's large array of visualisation choices makes it attainable to reap insight into molecular properties additionally as careful building block interactions. Maestro is a powerful and versatile molecular modelling environment, and therefore the portal to the most advanced science in computational chemistry.

Features

- Model generation
- Flexible visualization
- 3D realism
- 2D ligand interaction diagrams
- Quantitative structural analysis
- Molecular properties
- Easy to use

Model generation-

Maestro supports several common file formats for structural input. Additionally, maestro provides an intuitive, full-featured building tool for constructing molecular models of any sort.

Flexible visualization-

Maestro provides several viewing choices to accommodate the various desires of various applications. From small molecules to the giant biomolecular complexes, maestro brings clarity to a good range of modeled systems with full-featured 3D image and increased 2d ligand interaction diagrams.

3D realism-

Maestro's superior rendering and stereographic capabilities enable researchers to look at advanced molecular systems as 3 dimensional objects with unrivaled realism.

2D ligand interaction diagrams-

Binding pocket characteristics and protein-ligand interactions are easily identified and communicated with automatically-generated 2D ligand interaction diagrams. These 2D representations of the binding pocket use distinctive colors and shapes to convey binding pocket shape, electrostatics, protein-ligand interactions, and more.

Quantitative structural analysis-

Maestro includes versatile measurement tools that give the user the ability to precisely quantify a molecule's structural features. Superimposition tools make possible detailed comparisons between structures.

Molecular properties-

Computed properties such as vibrational modes, molecular orbitals, or electron density are easily visualized in Maestro. The unique Sitemap feature locates areas within a protein that correspond to hydrophobic or hydrophilic regions.

PYMOL:

PyMOL is an open-source, user-sponsored, molecular visual image system.

PyMOL can produce high- quality 3D images of small molecules and biological macromolecules, like proteins. consistent with the first author, nearly 1 / 4 of

all published pictures of 3D macromolecule structures within the scientific literature were made using PyMOL.

Leader in 3D Molecular Visualization-

PyMOL can produce pictures of unmatched visualisation quality, whereas providing the flexibleness to Java-based development and measurability. Competitively priced and accessible for tutorial, non-profit, government, and business application, PyMOL leads the approach in molecular visualisation.

PyMOL interprets over 30 different file formats and its own format is supported on Windows, Mac, Linux, and iPad.

Create 3D Images

Complex visualization tasks that previously required expert knowledge now only require a few mouse clicks. PyMOL's straightforward graphical user interface allows first-time and expert users alike to create stunning 3D visualizations from their favorite file formats. Users can **quickly and easily create movies** that fly through a molecular landscape, elegantly depict protein structure morphing, and animate ensembles and trajectories.

Protocol – (For superimposition using MAESTRO)

1. The initial step basically involved the preparation of the ligand (inhibitor) using the extension Lig prep in maestro.
2. The second step involved preparation of the protein using protein preparation wizard in the maestro.
3. After the second step the two of the structures were taken with the format “sdf“ from drug bank and are placed in maestro which then generates the active sites.

4. After the generation of the active sites the process was then ran for the docking using Jaugar in maestro.
5. SP(Standard) Docking scores were then calculated.

Protocol (For checking the structural similarity) Using PyMoL:

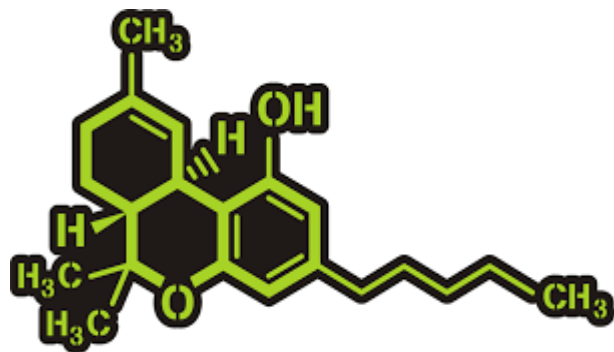
1. The initial step involved opening of the structure of THC in the pymol. THC with its receptor structure was opened in pymol.
2. The second step involved opening of the levodopa structure in pymol. Levodopa with receptor 2ki9 is opened in structural form in pymol.
3. Pymol supports “PDB” format.
4. Command was placed in the command window for superimposition.
5. Align Structure 1, Structure 2.
6. Results were recorded accordingly.

CHAPTER 4

RESULTS

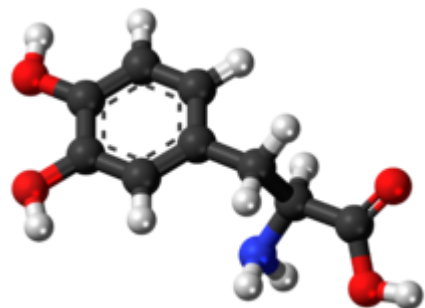
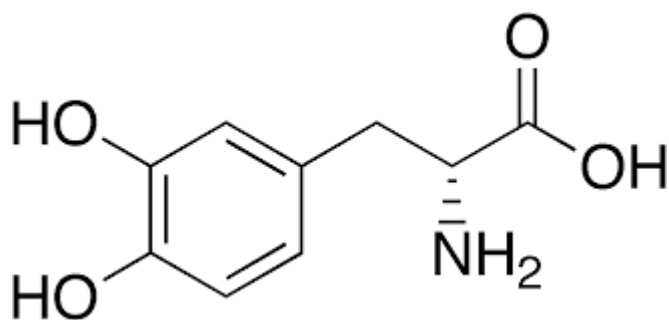
Chemical Structures involved:

Tetrahydrocannabinol (THC)



- Chemical structure and the 3D structure of THC .
- Molecular weight-314.45g/mol

Levodopa- L-DOPA.



- Chemical structure and 3D structure of Levodopa.
- Molecular weight-197.1879g/mol.

>On structural alignment using the PyMoL the alignment resulted-

- ⊙ Executive alignment= 10 atoms aligned.
- ⊙ Executive RMS= 3.013(10 to 10 atoms)
- ⊙ RMS tells the distance between the atoms.
- ⊙ More the RMS poor attachment between the atoms.

MAESTRO - Results

[running at reduced cpu priority]

Date: Friday, May 20 2016, at 14:48:07 India Standard Time

Glide version 70014

mmshare version 33014

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After readscreen, (nx, ny, nz) = (23 , 23 , 23).

Receptor setup: (nsites, nx, ny, nz, bsize)=(125 , 23 ,

Grid setup finished

%IMPACT-W: Build Primary: *Too many atoms or bonds for structure in: "*

glide-dock_SP_11.maegz"

%IMPACT-W: CREATE: *struc has too many atoms, bonds, or bonds per atom.*

Will skip atom typing, docking. Other tasks may fail.

%IMPACT-W: Ligand skipped, too many atoms, tot bonds, or bonds per

atom

%IMPACT-W: **GLIDE SKIPPING LIGAND: STRUCTURE HAS TOO MANY ATOMS OR BONDS.**

Buried polar penalty 0.000

Coulomb vdW cutoff 0.000

Total elapsed time = 8 second.

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