MODELING COMPUTATIONAL STUDIES OF MODIFIED DRUG MOLECULES BINDING TO THE LRRK2 PROTEIN IN THE TREATMENT OF PARKINSON'S DISEASE

Hetvi Shah, Jerry A. Darsey*

Center for Molecular Design and Development,
University of Arkansas at Little Rock,
Little Rock, Arkansas USA 72204
*e-mail: jadarsey@ualr.edu

Abstract

Parkinson's disease is a neurodegenerative and progressive disease of the central nervous system. It affects more than 10 million patients worldwide and the symptoms allow for little to no control for movement. These symptoms appear because the chemical messenger dopamine is being made in small quantities from impaired cells. However, the disease often forms when there is a mutation in the LRRK2 gene, as the functions of the protein become abnormal. The IC50 value is essential information about molecules because it measures their effectiveness. The goal of this research was to design molecules with a lower IC50 value. This was first done by modeling molecules on the molecular modeling program, Gaussian 09. Modifications were made to molecules that were said to bind to the LRRK2 protein. Modifications ranged from adding a single atom or replacing atoms with groups. After running these molecules on the program, the total energy was found. Using the equation found from the correlation between 1/IC50 and the total energy, the IC50 value was predicted for each of the modified molecules. Many of the modified molecules portrayed a positive percent difference between the original IC50 value and the new one. This saves both time and money because the molecules with lower IC50 values can be made, preserving the resources. After creating the molecule with a low IC50 value, further experimental procedures can be taken; this is a large step in assisting researchers to reach a potential treatment because it is more efficient.

Keywords: *LRRK2 proteins, Parkinson, Computational Modeling, IC50, Density Functional Theory, DFT*

[Engineering of Biomaterials 145 (2018) 2-7]

Introduction

Parkinson's disease is a progressive, neurodegenerative disease that affects the central nervous system. A patient experiencing this disease suffers from a lack of movement and muscle control. More than 10 million people worldwide are affected by this and the United States receives about 60,000 cases per year [1]. Likewise, Parkinson's disease stands as the top 14th cause of death in the United States. Doctors are able to diagnose patients with Parkinson's if they are experiencing symptoms, such as bradykinesia, tremor, and rigidity. Although symptoms can be controlled using medication and surgical therapy, there is no standard treatment for Parkinson's disease [2].

The neurotransmitter, or chemical messenger, dopamine is responsible for controlling movement and emotions, including pleasure and pain. Those who are diagnosed with Parkinson's have impaired cells making dopamine. As this disease progresses, cells that are producing dopamine die and the brain eventually reaches a state in which no dopamine is being produced in a significant amount. This is the leading cause of abnormal movement as a symptom of Parkinson's disease [2].

The precise cause of Parkinson's is unknown, however research shows that it can be caused by genetic and environmental factors. It is known that the disease results from a loss of cells in the brain (substantia nigra). This area of the brain produces the neurotransmitter dopamine, which will transmit signals that allow for movement. A loss of this, as stated above, leads a patient to have no control over their movement. Some researchers have found that Parkinson's disease develops from a genetic mutation that is passed from generation to generation. It has been increasingly evident that the mutation occurs in the Leucine-Rich Repeat Kinase 2 (LRRK2) gene, causing it to produce an abnormal protein [3,4].

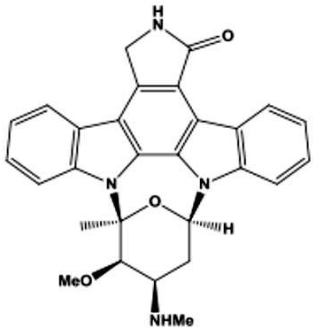
The LRRK2 gene is located on chromosome 12 and it gives proper instructions to make the LRRK2 protein as a kinase. It is made as a kinase to maintain a cell's ability to survive. However, mutations in this gene can make it hyperactive, causing cell death. A study illustrates that the LRRK2 gene is negatively affecting a set of proteins that originally function to traffic cargo. Thus, a mutation in this gene is shown to cause traffic jams in the cell [4].

Many organizations are working towards this specific issue from using drug therapy to using technology. They are focusing on the LRRK2 gene because it is responsible for a defect. The Michael J. Fox Foundation (MJFF) is taking a novel and therapeutic approach to develop a drug for the gene. They are studying the mutation and its surroundings and plan to take a further step by determining drugs that could bind to the active site of the protein [5][6]. Finding a balance that will lower the kinase activity allows the pharmaceutical industry to believe that this gene is drugable. The Dawson Duo, in a recent study, showed that regulating a low dose of anisomycin will help tremendously by blocking protein production. They further established that removing the phosphate group that attaches to the ribosomal s15 could prevent degeneration and brain cell death.

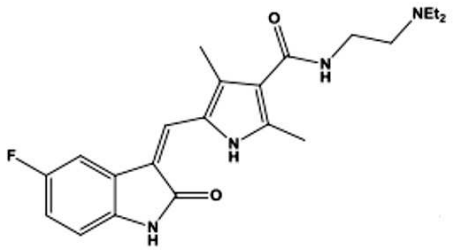
Several studies have shown that having a specific molecule bind to the LRRK2 gene may result in further progress towards creating a drug to bind to the protein in the treatment of Parkinson's disease. The plan of this research is to design new molecules that could potentially bind to the LRRK2 protein and to see how well this can be done by looking at the IC50 values [4]. The IC50 value is a measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function or the concentration of a potential drug molecule with 50% inhibition; this allows the data to be quantified. The values will be found by using a correlation that was found in a previous study [4]. The new molecules designed can be more efficient by saving both time and money.

Original Molecules

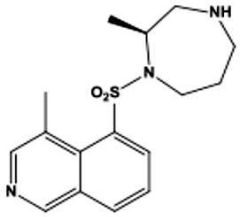
A) Staurosporine



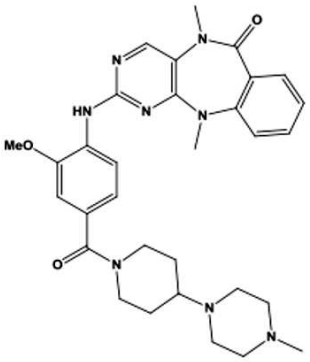
B) Sunitinib



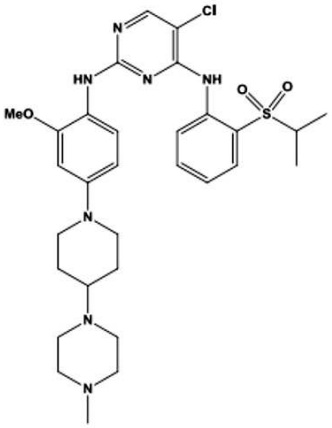
C) H1152



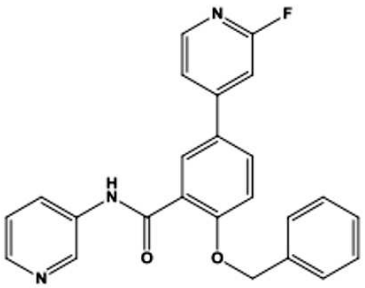
D) LRRK2-IN-1



E) TAE684



F) GSK2578215A



G) HG-10-102-01

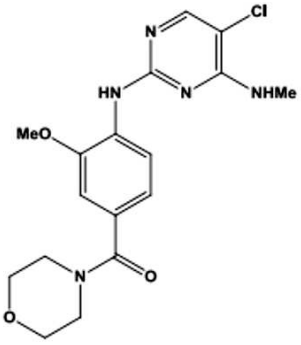


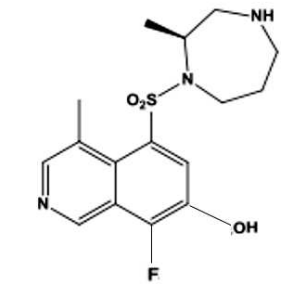
FIG. 1. These are all of the molecules that are modified and said to bind to the LRRK2 protein.

TABLE 1. The table displays all of the modifications made to specific molecules, as well as the IC50 values, the total energy, and the percent difference between the old IC50 value and the new one. The new IC50 values were found by using the strong correlation found in previous research. The top eight compounds that indicated a significant percent difference is highlighted by *.

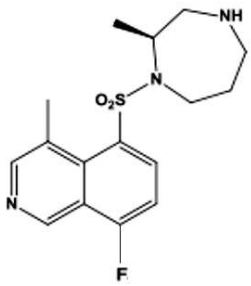
	Total Energy	1/IC50	IC50	Original IC50	Percent Difference
Staurosporine(1) - changed C to N	-1556.16	0.0338	29.57	40	29.98
Staurosporine(2) * - added =O	-1599.44	0.0380	26.30	40	41.32
Sunitinib(1) * - added a carboxyl	-1512.00	0.0295	33.86	79	79.99
Sunitinib(2) * - changed F to Cl	-1683.86	0.0462	21.64	79	113.99
H1152(1) * - added F	-1433.63	0.0219	45.61	244	137.01
H1152(2) * - added OH	-1409.61	0.0196	51.03	244	130.81
H1152(3) * - added F and OH	-1508.82	0.0292	34.22	244	150.80
LRRK2-IN-1(1) - added NH	-1923.65	0.0695	14.39	13	-10.16
LRRK2-IN-1(2) - added a carboxyl	-2056.80	0.0824	12.14	13	6.88
LRRK2-IN-1(3) - added OH^-	-1943.49	0.0714	14.00	13	-7.44
LRRK2-IN-1(4) - added F	-1967.48	0.0737	13.56	13	-4.23
LRRK2-IN-1(5) * - added Cl	-2327.88	0.1087	9.20	13	34.25
LRRK2-IN-1(6) * - added a carboxyl and Cl	-2516.36	0.1270	7.87	13	49.12
TAE684(1) - added methyl	-2674.10	0.1423	7.03	7.8	10.43
TAE684(2) - changed F to Cl	-2274.42	0.1035	9.66	7.8	-21.30
TAE684(3) - added a carboxyl	-2823.26	0.1568	6.38	7.8	20.06
TAE684(4) - added a carboxyl and methyl	-2862.57	0.1606	6.23	7.8	22.44
TAE684(5) - added OH	-2709.96	0.1458	6.86	7.8	12.84
TAE684(6) - added NH	-2690.11	0.1439	6.95	7.8	11.51
TAE684(7) - added OH, NH, carboxyl, and methyl	-2993.06	0.1733	5.77	7.8	29.90
GSK2578215A(1) - added a carboxyl	-1528.00	0.0311	32.17	10.9	-98.77
GSK2578215A(2) - added O_2S	-1848.64	0.0622	16.08	10.9	-38.38
GSK2578215A(3) - added NH	-1394.84	0.0182	55.06	10.9	-133.90
GSK2578215A(4) - added OH	-1414.69	0.0201	49.78	10.9	-128.15
GSK2578215A(5) - changed F to Cl	-1699.86	0.0478	20.94	10.9	-63.05
HG-10-102-01(1) - changed C to NH	-1635.02	0.0415	24.11	20	-18.65
HG-10-102-01(2) - added methyl	-1658.35	0.0437	22.86	20	-13.37
HG-10-102-01(3) - added carboxyl	-1807.54	0.0582	17.18	20	15.18
HG-10-102-01(4) - added OH	-1694.22	0.0472	21.18	20	-5.73
HG-10-102-01(5) - added NH	-1674.37	0.0453	22.08	20	-9.88

Top Modified Molecules

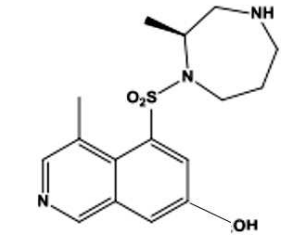
A) H1152(3)



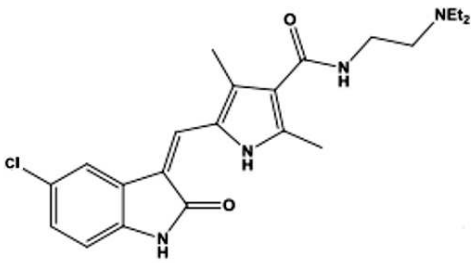
B) H1152(1)



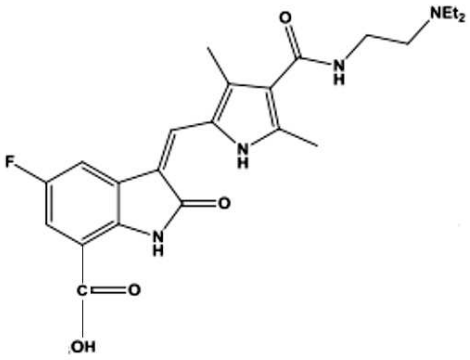
C) H1152(2)



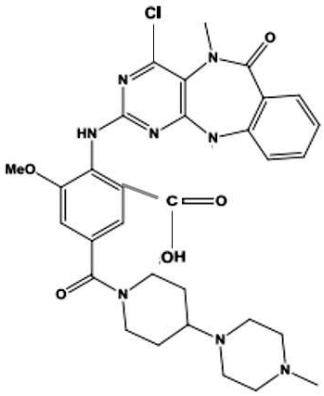
D) Sunitinib(2)



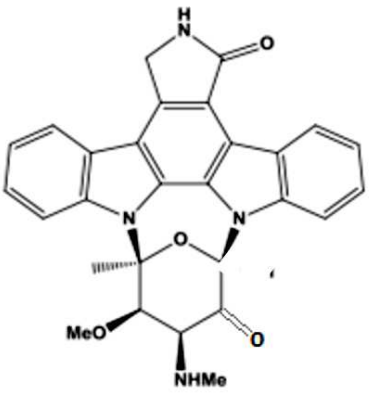
E) Sunitinib(1)



F) LRRK2-IN-1(6)



G) Staurosporine(2)



H) LRRK2-IN-1(5)

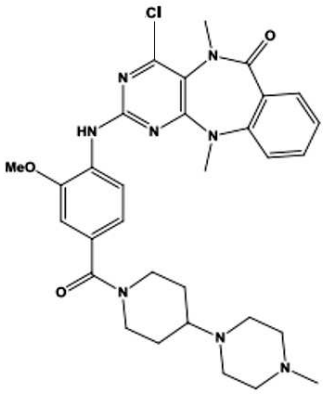


FIG. 2. These are the top eight modified molecules with the greatest percent difference between the original IC50 value and the new IC50 value (ordered with decreasing percent difference).

Materials and Methods

Designing and Modeling New Molecules

A strong correlation has been found in a previous study between the 1/IC50 of molecules that are said to bind to the LRRK2 protein and the total energy of those molecules [7]. This research aims to use this correlation to design new molecules with significantly improved IC50 values. The molecules that were used were modified in this research. Modifications consisted of adding a certain group or element, such as adding a methyl, carboxyl acid, OH, fluorine, chlorine, etc. In order to modify a compound, the original compound was opened in the Gaussian 09 program and then the corresponding group or element that needed to be added was performed. The proper bonds were made as well. After modifying the compound, the Gaussian 09 program was used under the DFT and 6-38G settings; the total energy of the modified compound was recorded. A total of 30 new molecules were designed.

Analysis - Determining the New IC50 Value

Using the total energy of each molecule in the previous step, a table was made. The table listed the name of the original compound, the total energy, the original IC50, the modified IC50 (which was calculated using the equation from the graph "1/IC50 vs. Total Energy"), and the percent difference. The equation that was used was y = -10304x - 1207.7, where y represents the total energy and x represents the 1/IC50 value. FIG. 1 represents the top eight compounds that illustrated the highest percent difference, which significantly lowered the IC50 value of the molecule before it was modified.

Results

In order to determine the IC50 value of the modified molecules, a correlation that was previously found (between 1/IC50 and total energy) was used [7]. The equation given from the line of best fit allowed the total energy to be plugged in and the IC50 value to be solved. The molecules that were modified were chosen because research has shown that they will bind to the active site of the LRRK2 protein [8]. The total energies of the new and modified molecules were found using the computation molecular modeling program, Gaussian 09. Each molecule was modeled and ran under the density functional theory (DFT) method of this program. This method was used because it examines the electronic structure of the molecule, allowing the total energy to be utilized. This research plans to take a further step in targeting the goal of binding a molecule/drug to the active site of the LRRK2 protein; thus, the strength of a binding affinity is essential information. This is because the binding affinity is the capability of ligands to form bonds with a reception. Because the total energy is an interactive force of attraction that can affect the strength, it can be related to the IC50 value. Additionally, the IC50 value is necessary information because it will tell the effectiveness of a substance; predicting the IC50 value before making a molecule is more efficient because only the better ones can be made, saving both time and money.

TABLE 1 summarizes the results of the modifications to the original structures, which include the percent difference is the difference between the original IC50 value and the new IC50 value. Most of the molecules showed a positive percent difference, whereas some had a negative percent difference. A positive percent difference means that the new IC50 value was better and a negative percent difference means that the new IC50 value was worse. The top 4 compounds had a percent difference in the range of 113-150% and they include the following: Sunitinib (Mod. 2), H1152 (Mod. 1), H1152 (Mod. 2), and H1152 (Mod. 3). This illustrates a significant change in the molecules IC50 value, displayed in FIG. 2 and TABLE 1. As a result, these molecules could be made with the modification because the IC50 value was significantly better. For example, after adding a fluorine atom and a hydroxyl group to the H1152 molecule, the percent difference was 150.80% because the IC50 value went from 244 to 34.22.

Some of the molecules that were modeled had a positive percent difference that was very low. This was because the original IC50 value was already significantly low. For example, when adding a hydroxyl group, a NH, a carboxyl, and a methyl to the TAE684 molecule, the IC50 value went from 7.8 to 5.77. This is important because a low original IC50 value decreased to an even lower number. Despite this, some modifications to molecules did not result in a positive percent difference at all, such as GSK2578215A(1) and parts of HG-10-102-01(5). If we are able to find molecules with more efficient mechanisms for treatment of the negative symptoms of Parkinson's disease, life for those afflicted with this disease could be vastly improved [10].

Discussion

A mutation in the LRRK2 protein causes a change in function, resulting in Parkinson's disease [9]. This research designed new molecules and found the new IC50 value that it had by using a correlation that was found in previous research between the total energy and the 1/IC50 value. Finding the new IC50 value as was done in this research can save time and money because resources don't have to be spent for every molecule made; rather only the ones with positive percent differences can be made to be tested.

In computational molecular modeling, finding a reference when creating new molecules is necessary and helpful. Consequently, one of the biggest applications of this project is the fact that it can be used as a reference for future procedures. This research designs new molecules that can bind to the active site of the LRRK2 protein. After running the modified molecules on the Gaussian 09 program, the total energy can be found. The total energy that was found gives the y-value of the equation that is used. After plugging in the y-value, the x-value can be found by solving for it. Because the definition of IC50 states the concentration of a potential drug molecule with 50% inhibition, the lower the value, the stronger the interaction. For the graph with the outcome of a strong correlation, the x-axis is "1/IC50." This means that the highest value of 1/IC50 produces the lower value of IC50. Therefore, the graph can be used to estimate an approximate IC50 value, increasing efficiency and saving time. If the IC50 value is good, then the experimental procedures can be taken in the future.

Each molecule that was modified had a different effect on the IC50 value. It is plausible that the sole reason for this depended on the effects of the new atoms or groups that were added or replaced. FIG. 1 illustrates the original molecules and FIG. 2 illustrates the top eight modified molecules when regarding the percent difference. Examples of modifications included adding fluorine or chlorine atoms or adding carboxyl or hydroxyl groups. Furthermore, the placement (ortho, meta or para) of each of these atoms and groups could create a larger effect. However, this is countered with the fact that modifications to some molecules always lead to a positive percent difference, such as shown in FIG. 2 with the H1152 molecule.

Acknowledgements

The authors would like to thank the organizers of the 4th International Conference on Biomedical Polymers & Polymeric Biomaterials ISBPPB 2018 for the invitation to present at this conference and to publish in their society's journal. Also thanks to the Center for Molecular Design and Development for sponsoring this trip.

References

[1] R. R. Neubig, International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification. XXXVIII. Update on terms and symbols in quantitative pharmacology. Pharmacol Rev 55 (4) (2003) 597-606.

[2] What Is Parkinson's? (2018, March 13). Retrieved April 15, 2018, from http://www.parkinson.org/understanding-parkinsons/what-is-parkinsons

What is Parkinson's Disease. (2016). Retrieved December 11, 2016, from http://www.pdf.org/about_pd

[3] Renter, E. (2012, October 30). Monsanto's Roundup, Glyphosate Linked To Parkinson's And Similar Diseases. Retrieved November 12, 2016, from http://naturalsociety.com/monsantos-roundup--glyphosate-parkinsons-neurodegenerative/

[4] Reina N. Fuji, Michael Flagella, Miriam Baca, Marco A. S. Baptista, Jens Brodbeck, Bryan K. Chan, Brian K. Fiske: Effect of selective LRRK2 kinase inhibition on nonhuman primate lung Lee H, Effect of selective LRRK2 kinase inhibition on nonhuman primate lung. Science Translational Medicine 04 Feb 2015: Vol. 7, Issue 273, pp. 273ra15.

[5] The Parkinson Study Group PRECEPT Investigators. Mixed lineage kinase inhibitor CEP-1347 fails to delay disability in early Parkinson disease. Neurology 69 (2007) 1480-1490.

[6] The Science of Parkinson's. (2012). Retrieved December 11, 2016, from http://tanz.med.utoronto.ca/page/science-parkinsons.

[7] Shah H., Darsey J.: Computational Modeling Studies of the LRRK2 Protein in the Mechanism of Parkinson's Disease. Curr. Trends Biomedical Eng & Biosci. 7(1) (2017) 1-6.

[8] Gilligan P.J.: Inhibitors of Leucine-Rich Repeat Kinase 2 (LRRK2): Progress and Promise for the Treatment of Parkinson's Disease. Current Topics in Medicinal Chemistry 15 (10) (2015) 927-938.

[9] LRRK2 gene - Genetics Home Reference. (2017). Retrieved January 23, 2017, from https://ghr.nlm.nih.gov/gene/LRRK2#conditions

[10] Truong DD., Bhidayasiri R., Wolters E.: Management of non--motor symptoms in advanced Parkinson disease. J Neurol Sci. 266 (2008) 216-228.