Identification of Novel LRRK2 Inhibitors using Pharmacophore Modelling and Docking

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BE21B004

Introduction

Parkinson's disease is one of the most common neurodegenerative disorders in the world [1]. It primarily affects motor function, leading to slow movement, tremors, rigidity, and imbalance. It can also lead to non-motor symptoms like cognitive impairment, dementia, and sleep disorders. There is no cure for Parkinson's and current treatments only offer symptomatic relief [2].

Figure 1: Symptoms of Parkinson's Disease
(Source: Stanford Medicine)

Studies have shown that there is a correlation between certain genes and the occurrence of Parkinson's. Of these genes, *lrrk2* (leucine-rich repeat kinase 2) has been hypothesized to be behind a significant fraction of Parkinson's cases [3]. The protein encoded by this gene, LRRK2, is a 286 kDa protein with multiple domains. Its most important domains include a kinase domain and a GTPase domain.

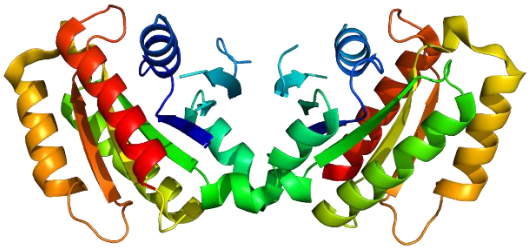

Figure 2: LRRK2, the target protein
(Source: Wikimedia Commons)

While no studies have confirmed the complete function of this protein, it has been shown to be involved in neuron plasticity, vesicle trafficking, mitochondrial activity, autophagy, and apoptosis [4]. Hyperactivation of LRRK2 may be involved in the onset and progression of Parkinson's disease. Abnormal activity in its kinase domain, due to hyperactivation, can lead to the accumulation of α-synuclein, which is a hallmark of Parkinson's [5]. Thus, targeting LRRK2 is a good strategy for the treatment of this disease.

Using previously identified inhibitors of this protein, we perform pharmacophore modelling to identify novel LRRK2 inhibitors in a small molecule database. Using these, and the existing inhibitors, we perform a docking study to determine the efficacy of their LRRK2 inhibition. Through this, we hope to discover new small molecules for the treatment of Parkinson's disease.

Methodology

Identifying LRRK2 inhibitors from literature

13 small molecule drugs that have been shown to inhibit LRRK2 were found from existing literature [6, 7]. These drugs target the kinase domain of LRRK2. Their details are summarised below:

Table 1: Small molecule LRRK2 inhibitors

Sl.	Drug Name	ZINC ID	SMILES	Ref.
1	MLi-2	ZINC220966210	C[C@H]1CN(c2cc(-c3n[nH]c4ccc(OC5(C)CC5)cc34)ncn2)C[C@@H](C)O1	[6]
2	PF-06447475	ZINC210747484	N#Cc1cccc(-c2c[nH]c3ncnc(N4CCOCC4)c23)c1	[6]
3	PF-06685360	Not available	Cn4cc(c2c[nH]c3ncnc(N1CCOCC1)c23)cc4C#N	[6]

4	GNE-0877	ZINC103260600	CNc1nc(Nc2cn(C(C)(C)C#N)nc2C)ncc1C(F)(F)F	[6]
5	GNE-7915	ZINC95578650	CCNc1nc(Nc2cc(F)c(C(=O)N3CCOCC3)cc2OC)ncc1C(F)(F)F	[6]
6	GSK2578215A	ZINC68267183	O=C(Nc1cccnc1)c1cc(-c2ccnc(F)c2)ccc1OCc1ccccc1	[6]
7	HG-10-102-1	Not available	CNc3nc(Nc2ccc(C(=O)N1CCOCC1)cc2OC)ncc3Cl	[6]
8	LRRK2-IN-1	ZINC84605049	COc1cc(C(=O)N2CCC(N3CCN(C)CC3)CC2)ccc1Nc1ncc2c(n1)N(C)c1ccccc1C(=O)N2C	[6]
9	Staurosporine	ZINC3814434	CN[C@@H]1C[C@H]2O[C@@](C)([C@@H]1OC)n1c3ccccc3c3c4c(c5c6ccccc6n2c5c31)C(=O)NC4	[7]
10	JH-II-127	ZINC253387881	CNc1[nH]c(Nc2ccc(C(=O)N3CCOCC3)cc2OC)nc2ncc(Cl)c1-2	[7]
11	TAE684	ZINC55760827	COc1cc(N2CCC(N3CCN(C)CC3)CC2)ccc1Nc1ncc(Cl)c(Nc2ccccc2S(=O)(=O)C(C)C)n1	[7]
12	GNE-9605	ZINC103260612	CNc1nc(Nc2cnn([C@H]3CCN(C4COC4)C[C@@H]3F)c2Cl)ncc1C(F)(F)F	[7]
13	Ponatinib	ZINC36701290	Cc1ccc(C(=O)Nc2ccc(CN3CCN(C)CC3)c(C(F)(F)F)c2)cc1C#Cc1cnc2cccnn12	[7]

Obtaining 3D structures of LRRK2 inhibitors

The structures of these molecules was downloaded in .sdf format from the ZINC15 database [8] and converted to .mol2 format using OpenBabel [9]. For those molecules whose structures were unavailable in ZINC, OpenBabel was used to convert their SMILES to a .mol2 file with 3D coordinates.

Pharmacophore modelling

Based on structural similarity, molecules #5, #6, and #7 were chosen for pharmacophore modelling. All three molecules contain at least 2 benzene rings. They also contain nitrogen heterocycles, ether linkages, ketone group and secondary amine groups.

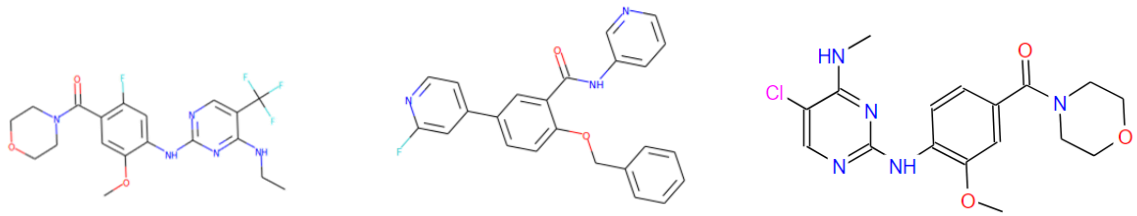

Figure 3: The structures of the 3 molecules chosen for pharmacophore modelling

Their .mol2 files were loaded in the BIOVIA Discovery Studio Visualizer. After setting rotatable bonds, the 3 molecules were aligned. The pharmacophore thus produced was saved as a .mol2 file.

Discovering novel LRRK2 inhibitors based on pharmacophore

Using the pharmacophore, the ZINCPharmer server [10] was used to identify molecules in the ZINC15 database that contain the same pharmacophore. The features selected for the query included 2 hydrophobic rings, 2 hydrogen bond acceptors, and 2 hydrogen bond donors. The query was submitted and hits from the ZINC15 database were obtained.

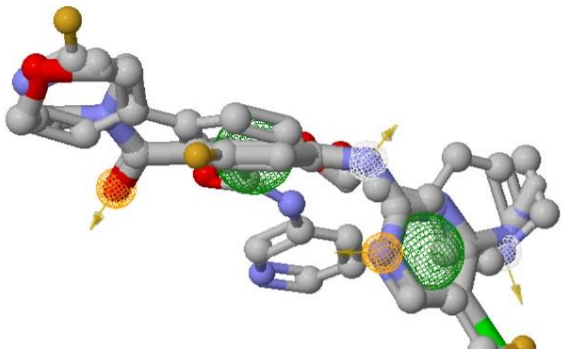

Figure 4: The pharmacophore submitted as query to ZINCPharmer

Computing ADME properties of discovered molecules

The SMILES representations of the candidate molecules from ZINCPharmer were obtained from the ZINC15 database. These were given as input to the pkCSM server [11] to compute their ADME properties.

Since LRRK2 inhibitors necessarily have to cross the blood-brain barrier in order to perform their function effectively, BBB permeability was chosen as the criteria for selecting drugs. Those with higher permeability were given higher priority for further study. 20 candidates were chosen for further analysis.

Docking

The 3D structures, in .mol2 format, of the 20 identified candidates were obtained from the ZINC15 database. The structure of the target protein, LRRK2, was obtained from the Protein Data Bank (PDB ID – 2ZEJ).

The target structure and ligand structures were prepared in .pdbqt format using AutoDock Tools [12]. After creating a configuration file describing the receptor and grid box for docking, a batch file was used to dock the original 13 ligands and the new 20 ligands with the protein. This was carried out using AutoDock Vina [13].

Table 2: Docking parameters

Parameter	Value
x_center	36
y_center	44
z_center	38
x_size	25
y_size	25
z_size	25
exhaustiveness	5

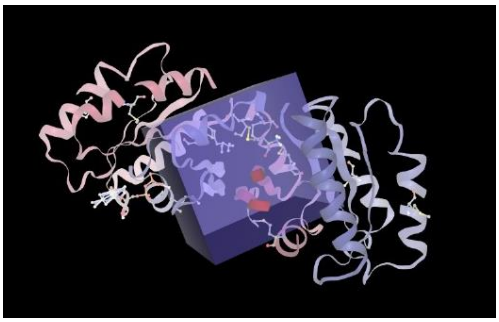

Figure 5: The grid box used for docking with AutoDock Vina

The most negative binding affinity of each ligand was used to determine the effectiveness of the LRRK2 inhibitors. Visualisations were performed using PyMOL and LigPlus.

Results

Using the BIOVIA Discovery Studio Visualizer, a pharmacophore was created for 3 LRRK2 inhibitors identified in literature.

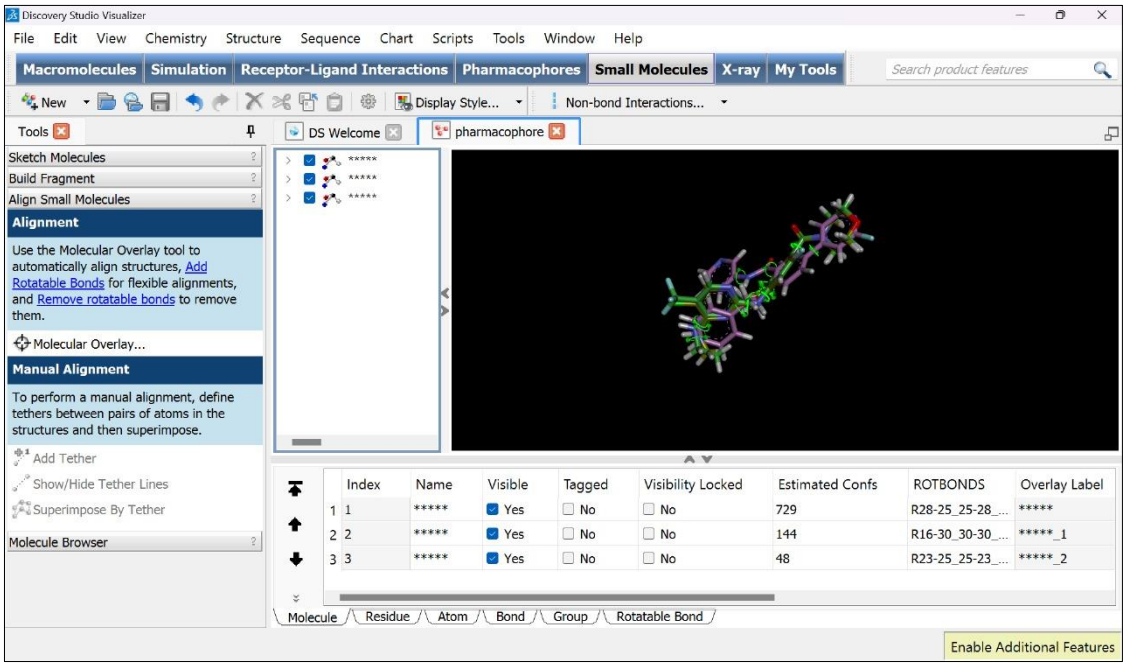

Figure 6: Pharmacophore modelling in the BIOVIA Discovery Studio Visualizer

When queried using the ZINCPharmer server, this pharmacophore yielded 30 hits in the ZINC15 database.

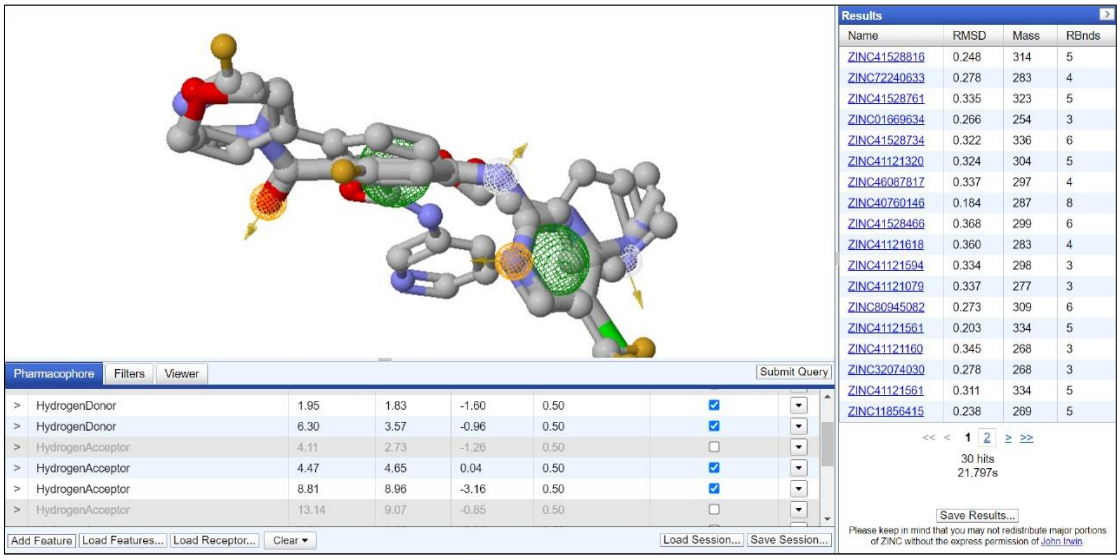

Figure 7: Hits from the ZINCPharmer server

These identified molecules were fed into the pkCSM server to compute their ADME properties. BBB permeability was chosen as the criterion to select drug candidates. The selected drugs are highlighted in green.

Table 3: Candidate LRRK2 inhibitors

Sl.	ZINC ID	SMILES	BBB perm.
1	ZINC31808880	CC(=O)c1ccc(Nc2ncnc3[nH]ncc23)cc1	−1.331
2	ZINC01669634	O=C([O-])c1ccc(Nc2ncnc3[nH]cnc23)cc1	−1.198
3	ZINC06422975	NC(=O)c1ccc(Nc2ncnc3[nH]cnc23)cc1	−1.292
4	ZINC80764347	Cc1nc(Nc2ccc3[nH]ncc3c2)c2cc[nH]c2n1	−1.577
5	ZINC41121160	CNC(=O)c1ccc(Nc2ncnc3[nH]ncc23)cc1	−1.32
6	ZINC72240636	CC(=O)c1ccc(Nc2nc(N)nc3[nH]cnc23)cc1	−1.596
7	ZINC32074030	CC(=O)Nc1ccc(Nc2ncnc3[nH]cnc23)cc1	−1.31
8	ZINC11856415	COC(=O)c1ccc(Nc2ncnc3[nH]ncc23)cc1	−1.46
9	ZINC41121079	c1cnn(-c2ccc(Nc3ncnc4[nH]ncc34)cc2)c1	−1.422
10	ZINC80945079	CC(=O)Nc1ccc(Nc2nc(C)nc3[nH]ccc23)cc1	−0.994
11	ZINC41121289	CNC(=O)c1ccc(Nc2ncnc3[nH]c(C)nc23)cc1	−1.354
12	ZINC41121618	CC(=O)c1ccc(Nc2ncnc3c2NC(=O)CN3)cc1	−1.14
13	ZINC72240633	CC(=O)Nc1ccc(Nc2nc(N)nc3[nH]cnc23)cc1	−1.604
14	ZINC40760146	CN(C)Nc1ncnc(Nc2ccc(C(=O)[O-])cc2)c1N	−0.773
15	ZINC41121076	Cc1ccn(-c2ccc(Nc3ncnc4[nH]ncc34)cc2)n1	−1.465
16	ZINC46087817	CNC(=O)c1ccc(Nc2ncnc3c2C[C@@H](C)CN3)cc1	−0.784
17	ZINC41121594	CNC(=O)c1ccc(Nc2ncnc3c2NC(=O)CN3)cc1	−1.129
18	ZINC41528466	CSc1n[nH]c2ncnc(Nc3ccc(C(C)=O)cc3)c12	−1.842
19	ZINC46087772	CNS(=O)(=O)c1ccc(Nc2ncnc3[nH]ncc23)cc1	−1.587
20	ZINC41121320	Cc1nc2c(Nc3ccc(S(N)(=O)=O)cc3)ncnc2[nH]1	−1.988
21	ZINC80764149	Cc1nc(Nc2ccc(N3CCCC3=O)cc2)c2cc[nH]c2n1	−1.264
22	ZINC80945082	Cc1nc(Nc2ccc(NC(=O)C(C)C)cc2)c2cc[nH]c2n1	−1.338
23	ZINC41528816	CNC(=O)c1ccc(Nc2ncnc3[nH]nc(SC)c23)cc1	−1.446
24	ZINC41528819	CSc1n[nH]c2ncnc(Nc3ccc(NC(C)=O)cc3)c12	−1.871
25	ZINC41528819	CSc1n[nH]c2ncnc(Nc3ccc(NC(C)=O)cc3)c12	−1.871
26	ZINC57279110	CNS(=O)(=O)c1ccc(Nc2ncnc3[nH]c(C)nc23)cc1	−1.621
27	ZINC41528761	CSc1n[nH]c2ncnc(Nc3ccc(-n4cccn4)cc3)c12	−1.963
28	ZINC41121561	CNS(=O)(=O)c1ccc(Nc2ncnc3c2NC(=O)CN3)cc1	−1.396
29	ZINC41121561	CNS(=O)(=O)c1ccc(Nc2ncnc3c2NC(=O)CN3)cc1	−1.396
30	ZINC41528734	CSc1n[nH]c2ncnc(Nc3ccc(S(N)(=O)=O)cc3)c12	−2.105

The prepared .pdbqt files of the ligands were docked with the target protein. The results of the docking were interpreted on the basis of the most negative binding affinity of the ligand.

Table 4: Docking results

Molecule	Name / ZINC ID	Binding affinity (kcal/mol)
Original 1	MLi-2	−7.1
Original 2	PF-06447475	−6
Original 3	PF-06685360	−5.5
Original 4	GNE-0877	−7
Original 5	GNE-7915	−5.6
Original 6	GSK2578215A	−7.4
Original 7	HG-10-102-1	−6.6
Original 8	LRRK2-IN-1	−7
Original 9	Staurosporine	−6.8
Original 10	JH-II-127	−7.4
Original 11	TAE684	−5.9
Original 12	GNE-9605	−7.7
Original 13	Ponatinib	−8.7
Candidate 1	ZINC31808880	−6.9
Candidate 2	ZINC01669634	−6.5
Candidate 3	ZINC06422975	−6.6
Candidate 4	ZINC80764347	−6.9
Candidate 5	ZINC41121160	−6.9
Candidate 7	ZINC32074030	−6.7
Candidate 8	ZINC11856415	−6.3
Candidate 9	ZINC41121079	−6.8
Candidate 10	ZINC80945079	−6.7
Candidate 11	ZINC41121289	−6.9
Candidate 12	ZINC41121618	−7
Candidate 14	ZINC40760146	−6.4
Candidate 15	ZINC41121076	−7.3
Candidate 16	ZINC46087817	−7.2
Candidate 17	ZINC41121594	−7.1
Candidate 21	ZINC80764149	−7.7
Candidate 22	ZINC80945082	−7.3
Candidate 23	ZINC41528816	−6.6
Candidate 28	ZINC41121561	−7.3
Candidate 29	ZINC41121561	−7.3

Discussion

- Based on the docking, we can see that the ligand with the highest affinity for the protein is Original 13 – ponatinib, which has a binding affinity of −8.7 kcal/mol. This forms multiple interactions with the target protein.

- The ligand with the next highest affinity is Original 12 – GNE-9605, which has a binding affinity of −7.7 kcal/mol. This is 1 kcal/mol higher than ponatinib.
- Candidate 21 – ZINC80764149, also has the same binding affinity of −7.7 kcal/mol. This makes it the best among the ligands identified by ZINCPharmer. It has a different binding site than that of ponatinib and forms fewer interactions.
- On average, the binding affinity of the original 13 molecules is −6.8 kcal/mol, and that of the newly identified 20 molecules is −6.9 kcal/mol. Thus, the novel inhibitors seem to have higher affinity, on average.
- The molecules used to prepare the pharmacophore for ZINCPharmer – Original 5, 6, and 7 – have an average binding affinity of −6.5 kcal/mol. Most of the hits returned by ZINCPharmer have a more negative binding energy than this, indicating that we have been successful in finding novel LRRK2 inhibitors using the pharmacophore.

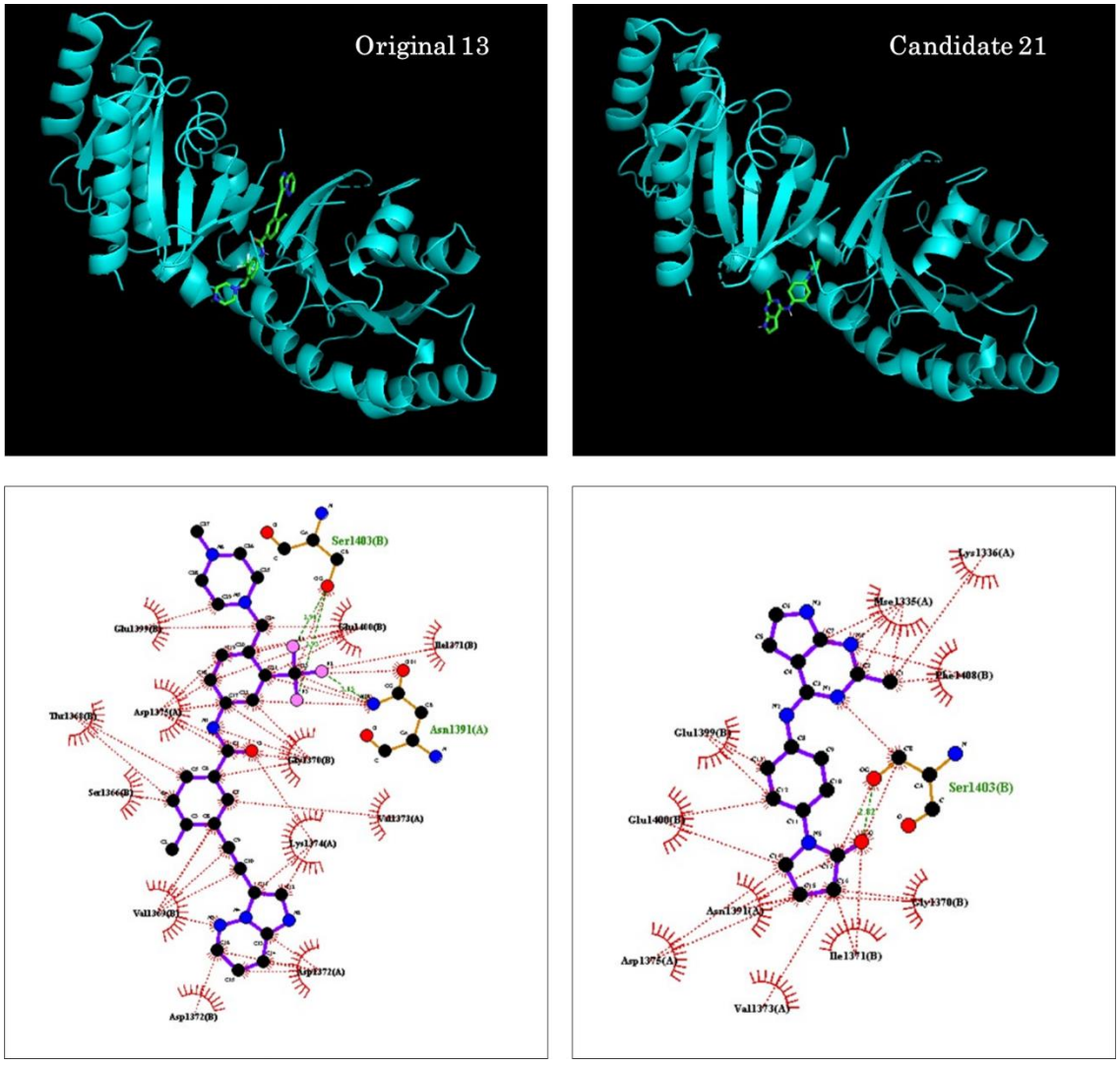

Figure 8: Visualising the protein-ligand interactions for the top 2 ligands

Conclusion

Using existing LRRK2 inhibitors, pharmacophore modelling was performed to identify novel drugs in the ZINC15 database, which yielded 30 hits. The top 20 drugs were selected on the basis of their ability to penetrate the blood-brain barrier. These were then docked with the target protein. The results were compared to the existing inhibitors.

One of the newly identified ligands, ZINC80764149 (Candidate #21), shows good promise for future *in vitro* and *in vivo* studies for the treatment of Parkinson's disease.

Thus, using pharmacophore modelling and molecular docking, we have successfully identified novel LRRK2 inhibitors that have, on average, better binding affinity than inhibitors already studied in literature.

References

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[13] AutoDock Vina. https://vina.scripps.edu/

Supplementary Files

All the data generated during this project can be found at this link:

https://drive.google.com/drive/folders/1kGjkisKaSpSa2f1iFpyrKdQ5i6IJ1SRw?usp=sharing