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# Identification of Inhibitors Targeting the Mutated Lrrk2 Proteins by Structure Based Pharmacophore Modeling

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**ABSTRACT**
*Mutations in LRRK2 gene are associated with Parkinson disease and over expression of this gene result in reduction of dopamine gaining its attention as a promising therapeutic target. Parkinson disease is a second most neurodegenerative disorder of central nervous system. Keeping in view the importance of LRRK2 we attempt to uncover novel inhibitor of LRRK2 through pharmacophore modeling, which is key step in drug designing. In the present study 6 mutated proteins were taken, individual pharmacophore and their shared feature pharmacophore were generated. Shared feature pharmacophore of LRRK2 mutated proteins contain two aromatic rings and two HBD. Virtual screening was done in order to get hit compounds against the shared feature pharmacophore. Total of 19 hit compounds were obtained by virtual screening, out of nineteen only four compounds were selected which were following the Lipinski rule of five and lies within toxicity class of four and five. These four compounds were docked with the mutated proteins of LRRK2 and only three compounds shows best docking results. It is concluded that three compounds could be effective enough in the treatment of LRRK2 mutations in PD and could be used as an inhibitor to control over-expression of LRRK2 gene.*
***Keywords:*** *LRRK2, Pharmacophore, Parkinson, Virtual Screening, Molecular Docking*

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## INTRODUCTION

Parkinson disease PD is a frequent neurodegenerative disorder of the Central Nervous System (CNs). Those individuals that have age limit of 65 years the probability of PD is 2% whereas having age limit 85 are more likely to have this disease i.e.5% [7].It is estimated that with increase in the age chances of neurodegenerative disorder are becoming a trend [6,10,18]. PD is slowly progressive disorder accompanied by the rigidity of the muscles and shaking of hands. Other symptoms includes dementia, depression, Brady-kinesia ,autonomal dysfunction as a result of depletion of the dopamine [18, 25] . A complex disorder that is characterized by multiple factors having unknown etiology [3, 6]. Epidemiology shows that smoking and the consumption of caffeinated coffee results in lowering the risk of PD. On the other hand risk of developing the disease increases in those having pesticide exposure [24].

LRRK2 gene is located on chromosome 12p11.2-q13.1 and it produces a protein known as dardarin [19]. Dardarin is most conserved large 286-kDa protein having multiple independent domains belonging to ROCD protein family [15].

LRRK2 is present in various regions involving those of nuclear region which is affected in Parkinson disease. Level of both LRRK2 protein and the messenger RNA are high in most of the regions including cortical region and cerebellum [11, 12]. In adult's neurogenesis level of LRRK2 protein is found to be high and also associated with the reduction in neuritic length. LRRK2 mutants are also responsible for the degeneration of dopaminergic neurons whereas overexpression of this protein results in the loss of dopamine cells. Important fact is that knowledge about the LRRK2's function is essential in order to get its role in signaling pathways e.g. alternations in case of MAP signaling pathways has been associated with Parkinson disease [4, 5].

PD associated mutation in LRRK2 includes p.G2019S, p.X1699C, p.12020T and p.N1437H. Only six out of 20 mutations in LRRK2 has been demonstrated to be pathogenic [14,15,21]. LRRK2 mutation especially

G2019s any account for 1 to 2% of familial and 3 to 6% of sporadic case of Parkinson disease and cause worse neuro-degeneratin [28, 31].

The concept of pharmacophore was introduced in 19th century by Paul Ehrlich. Paul Ehrlich suggested that biological activity of a molecule depends on the specific group within a molecule. According to international union of Pure and Applied Chemistry pharmacophore can be defined as "the ensemble of steric and electronic features that is necessary to ensure the optimal supra-molecular interactions with a specific biological target structure and to trigger (or to block) its biological response" [21]. Pharmacophore modeling is a key element in the drug designing. It represents the 3D arrangement of chemical and structural features of a drug and helps in the screening of potential inhibitor [17]. The pharmacophore modeling applied in drug discovery, lead identification, structure activity relationship, chemical functionalities and to identify the compounds that are responsible for the modulation of enzyme activity to block the desired biological effect [12, 13].

Structure base pharmacophore modeling is an efficient method when there is little information on ligand that acts as an inducer and proved to trigger the activity of specific therapeutic target. We can directly work on receptor site and get more information from the receptor site i.e. 3D structure of protein [9, 11, 23, 30]. Pharmacophore validation is performed to check whether the generated model is good enough to predict the active compounds. In the same way virtual screening is a process used to detect potential leads with different scaffolds and high inhibitory activity [1, 2, 26, 29].

Up till now there is very limited work on structural based pharmacophore modeling against mutations in the LRRK2 gene. Keeping in consideration we attempt to design potent novel inhibitors by adopting a structure based approach .The purpose of our research work is to quantify the effect of structure based pharmacophore modeling in finding novel drugs for the inhibition of LRRK2 mutations. Virtual screening and molecular docking also have been performed in order to achieve our task.

## MATERIAL AND METHODS

To generate the structure based pharmacophore against the mutation in LRRK2 protein we have adopted the following methodology.

### Gene Selection

Through literature we have selected the genes that are involved in Parkinson disease (PD). LRRK2, SNCA, PRKN are highly mutated genes that are involved in this disease. For confirmation we have checked the involvement of these genes through GeneCards database (http://www.genecards.org/ ). On the basis of highest score we have selected LRRK2 gene.

### Screening and Selection of LRRK2 Proteins

The highly mutated proteins coded by LRRK2 gene were selected from Protein Data Bank (RCSB-PDB) (https://www.rcsb.org/ ). We have applied the following filters for the selection of proteins i.e. organism selected as Homo-sapiens, taxonomy selected as eukaryota, and resolution greater than 2.0Å. There were total of 27 proteins of Homo-sapiens coded by LRRK2 gene and among them 17 were mutated. After applying the above mention filters we get 6 proteins id's named as 5OOT, 5OP4, 5OPS, 5OQ7, 5OQ8 and 4PY1. The structures of all these mutated proteins were downloaded in .pdb format.

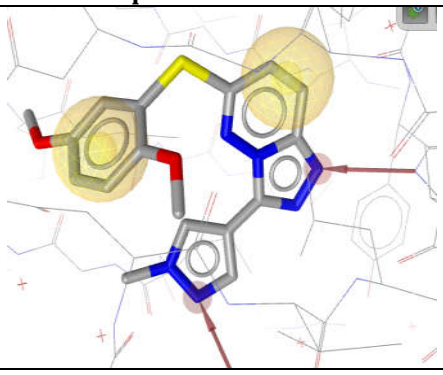
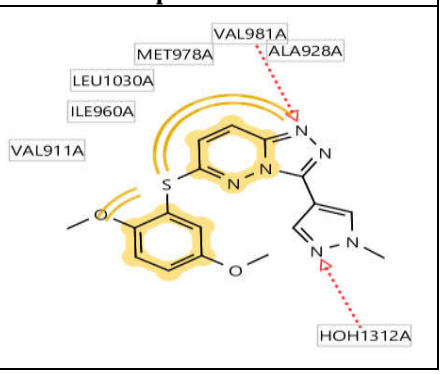
### Identification of Target Binding Sites

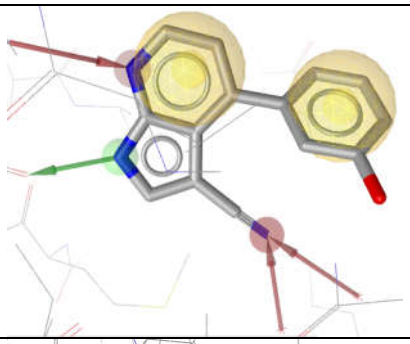
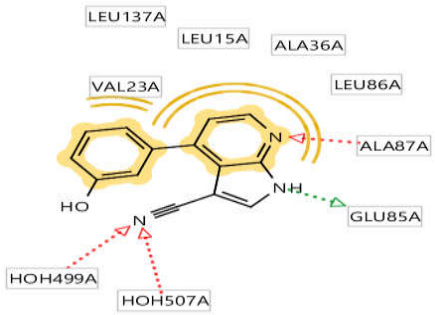
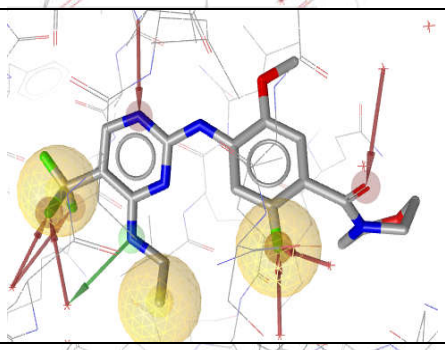
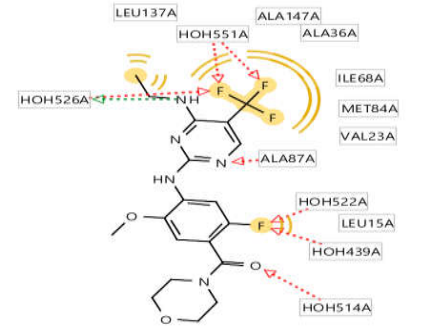
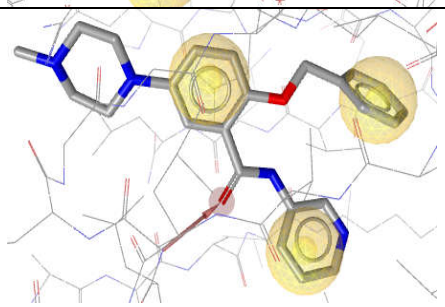
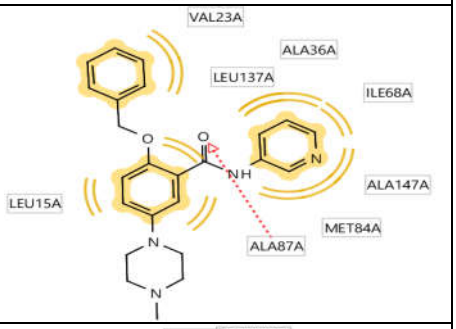
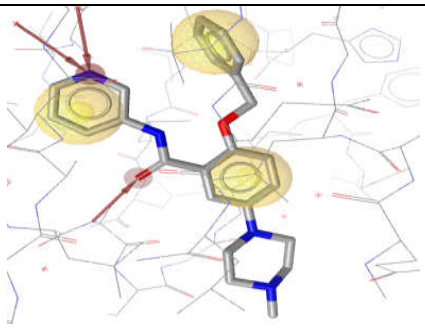
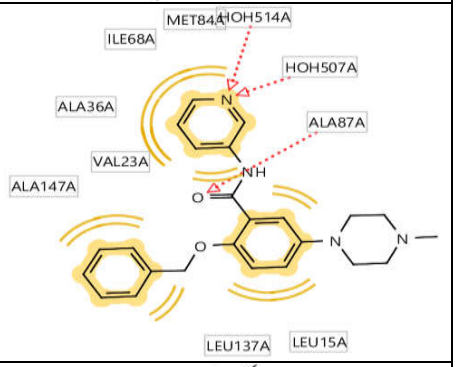
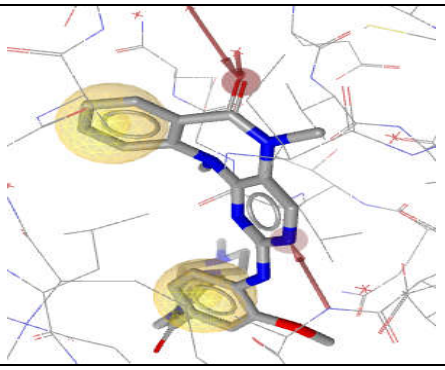
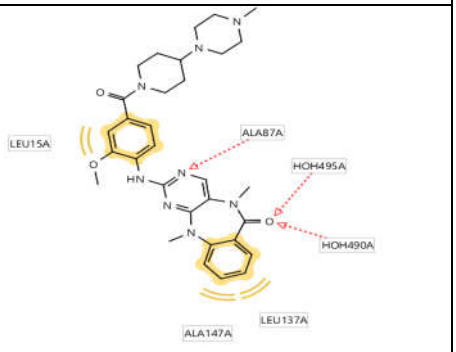
In structure based pharmacophore modeling we first identify the target binding sites. Binding sites of the target is the active site (pocket) where ligand molecule attaches. Once the target site has been identified it would be effective enough for designing the lead compound against the targets. 5OOT is the member of the transferase family in complex with 2-[(2-methoxy-4-{[4-(4-methylpiperazin-1-yl)piperidin-1-yl]carbonyl}phenyl)amino]-5,11-dimethyl-5,11-dihydro-6H-pyrimido[4,5-b][1,4]benzodiazepin-6-one ligand with molecular formula C31 H38 N8 O3. 5OP4 is the 3D structure of LRRK2 also belong to transferase family with ligand binding domain in complex with [4-[[4-(ethylamino)-5-(trifluoromethyl) pyrimidin-2-yl] amino]-2-fluoranyl-5-methoxy-phenyl]-morpholin-4-yl-methanone ligand with molecular formula C19 H21 F4 N5 O3. 5OPS is another transferase with ligand 4-(3-hydroxyphenyl)-1~ {H}-pyrrolo [2, 3-b] pyridine-3-carbonitrile having molecular formula C14 H9 N3 O. Another mutated protein 5OQ7 with 5-(4-methylpiperazin-1-yl)-2-phenylmethoxy-~{N}-pyridin-3-yl-benzamide(C24 H26 N4 O2) ligand. 5-(4-methylpiperazin-1-yl)-2-phenylmethoxy-~ {N}-pyridin-3-yl-benzamide (C24 H26 N4 O2) as a ligand molecule is attached with 5OQ8 protein. 4PY1 receptor with ligand binding domain in complex with 6-[(2,5 di-methoxy-phenyl)sulfanyl]-3-(1-methyl-1H-pyrazol-4-yl)[1,2,4]triazolo[4,3-b]pyridazine (C17 H16 N6 O2 S) The structures of all these proteins were opened into the structure-based Pharmacophore program of Ligand Scout [28]. The protein structural accuracy was confirmed through alignment prospective.

**Structure-Based Pharmacophore Generation:**
After confirming the structural accuracy we have generated the individual Pharmacophore model from receptor binding site.
**Shared-Feature Pharmacophore Generation:**
By importing the individual Pharmacophore in the alignment tool of the Ligand Scout we have generated their shared-feature Pharmacophore. As a result we get functional group in the shared feature Pharmacophore that are involved in the bioactivity of toward targeted proteins.
**Virtual Screening of Hit Compounds against Shared Feature Pharmacophore:**
We have selected the shared feature Pharmacophore in the alignment tab of Ligand Scout we set it as a reference. Virtual screening was performed in order to find the potent inhibitor to stabilize the expression of LRRK2. Virtual screening was performed through the library obtained from zinc database in order to get the hit compounds against the shared feature Pharmacophore.
**Selection of Compounds on basis of Lipinski Rule of Five:**
All the hit compounds were checked whether they are following Lipinski Rule of Five which illustrates that the compounds should have H-Bond donor less than 5, H-Bond acceptor less than 10, molecular weight should be less than 500 and logP less than 5. Toxicity class of the selected compounds was checked by using Protox Server (http://tox.charite.de/protox_II/ ). Only those compounds were selected which were following the Lipinski Rule of five and having toxicity class 4 and above.
**Docking of Hit Compounds with LRRK2 Proteins**
Docking of the selected compounds was performed through PatchDock server (https://bioinfo3d.cs.tau.ac.il/PatchDock/ ). Only those compounds were docked with the mutated proteins of LRRK2 which were following the Lipinski Rule of Five. Docking have been performed in order to identify the preferred orientations and also to find the possible interactions. manually before its entry into the computer.

**RESULT**
6 mutated proteins of LRRK2 along with their ligands were selected. The protein 5OQ8 consist of total of 261 amino acids residues and comprised of chain A, number of mutations were found to be 12 i.e. F20Y, S150G, A87C, M84L, Y149F, L86Y, I68V, S91E, L59N, S19A, A147S, H134E. 5OPS mutated protein having 10 no of mutations: S150G, A87C, M84L, Y149F, L86Y, I68V, S91E, L59N, A147S and H134E, chain A with 258 amino acids residues. 5OOT accompanied by 10 mutations: S150G, A87C, M84L, Y149F, L86Y, I68V, S91E, L59N, A147S, H134E, and only chain A whereas amino acids residues were 260 in numbers. Mutated protein 5OP4 contains 259 amino acids residues with chain A and mutations were found to be S150G, A87C, M84L, Y149F, L86Y, I68V, S91E, L59N, A147S, H134E. 4OQ7 is another mutated protein of LRRK2 with chains A and B consisting of 515 amino acids residues. The numbers of mutations were found in 5OP7 are 8: A87C, M84L, I68V, L86Y, S91E, L59N, A147S, H134E. 4PY1 protein contains about 290 amino acids with 6 numbers of mutations i.e. A105Q, A278C, S152A, A108K, A107E and A72C in chain A. All the selected proteins were shown in Fig 1.
Pharmacophore is considered to be an essential feature for drug designing. The pharmacophore of selected proteins i.e. 4PY1, 5OPS, 5OQ4, 5OQ7, 5OQ8 and 5OOT are generated in structure base pharmacophore tab of ligand scout. All the pharmacophores contain hydrogen bond donor (HBD), Hydrogen bond acceptor (HBA) and Aromatic Ring that are represented by green arrows, red arrows and yellow sphere respectively. The pharmacophores of representative 4PY1, 5OPS, 5OQ4, 5OQ7, 5OQ8 and 5OOT protein ligands are represented in table 1.

**Table.1: Pharmacophore model of selected Proteins**

| S. No. | Proteins | Pharmacophore | 2D Pharmacophore |
|---|---|---|---|
| 1. | 4PY1 |  |  |

| | | | |
|---|---|---|---|
| **2.** | **5OPS** |  |  |
| **3.** | **5OP4** |  |  |
| **4.** | **5OQ7** |  |  |
| **5.** | **5OQ8** |  |  |
| **6.** | **5OOT** |  |  |

Shared feature pharmacophore of the selected mutated proteins were generated which consist of two aromatic rings along with two hydrogen bond acceptor.

After performing screening nineteen hit compounds were obtained against share feature pharmacophore with maximum pharmacophore fitness score of 45.98. we have selected only those 4 compounds that are following the Lipinski rules of five and their toxicity class is 4 or above. Selected 4 compounds following Lipinski rules of fives are shown in table 2.

**Table 2: Selected Hit Compounds Fulfilling Lipinski Rule of Five**

| S.No | Hit Compounds | xLogP | H-bond Donor | H-Bond Acceptor | Molecular Weight |
|---|---|---|---|---|---|
| 1. | ZINC03901268 | 2.67 | 1 | 5 | 292.36 |
| 2. | ZINC00666987 | 3.9 | 0 | 9 | 420.425 |
| 3. | ZINC00633982 | 2.86 | 2 | 7 | 464.378 |
| 4. | ZINC00633999 | 2.68 | 2 | 7 | 484.376 |

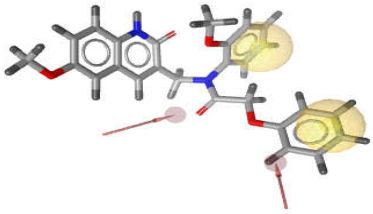
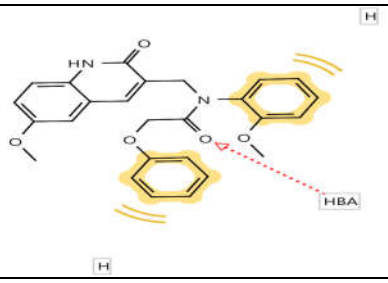
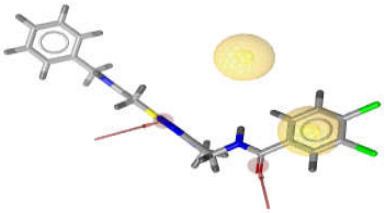
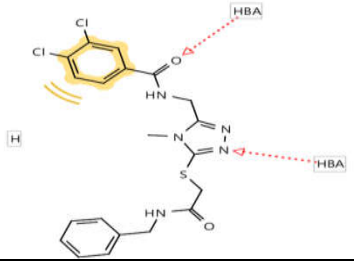
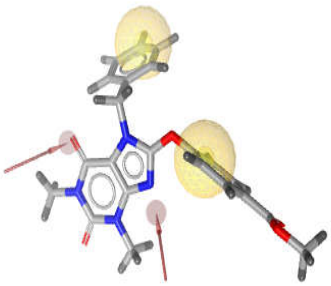
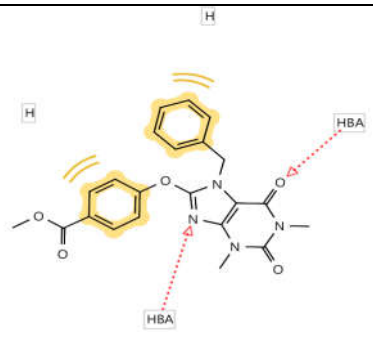
The predicted toxicity class of selected 4 hit compounds range from 5 which is best and maximum predicted LD50 value is 3500mg/kg with scoring shown in table 3.

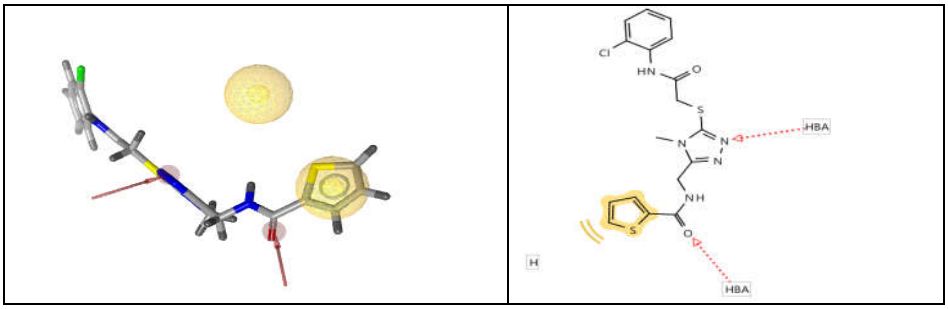
**Table 3: Hit Compounds with their Scoring, Toxicity class and predicted LD50**

| S.No | Hit Compounds | Scoring | Toxicity Class | Predicted LD50 |
|---|---|---|---|---|
| 1. | ZINC03901268 | 36.63 | 5 | 3500mg/kg |
| 2. | ZINC00666987 | 45.98 | 4 | 1500 mg/kg |
| 3. | ZINC00633982 | 38.00 | 4 | 1000 mg/kg |
| 4. | ZINC00633999 | 37.66 | 4 | 500 mg/kg |

Chemical structure of hit compounds following Lipinski rules of five are shown in Fig 2.
Four pharmacophore models with hit compounds having highest pharmacophore fit score and following Lipinski rule of five are shown in table 4.

**Table 4: Pharmacophore models of compounds with highest fitness scores.**

| | |
|---|---|
| a) | |
|  |  |
| b) | |
|  |  |
| c) | |
|  |  |
| d) | |




Selected compounds were docked with the total selected protein individually through patch dock. 24 docking results were obtained then these docking results were visualized and analyzed through discovery studio. 2D interaction, bumps, Vander wall, pi donor, hydrogen bond, pi alkyl and pi sulphur bond were analyzed. Among 24 results 5 best were selected which shows high interaction and no bump. Out of 4 selected solutions 3 solutions show best interactions with the LRRK2 proteins. Results were shown in fig 3 and 4.

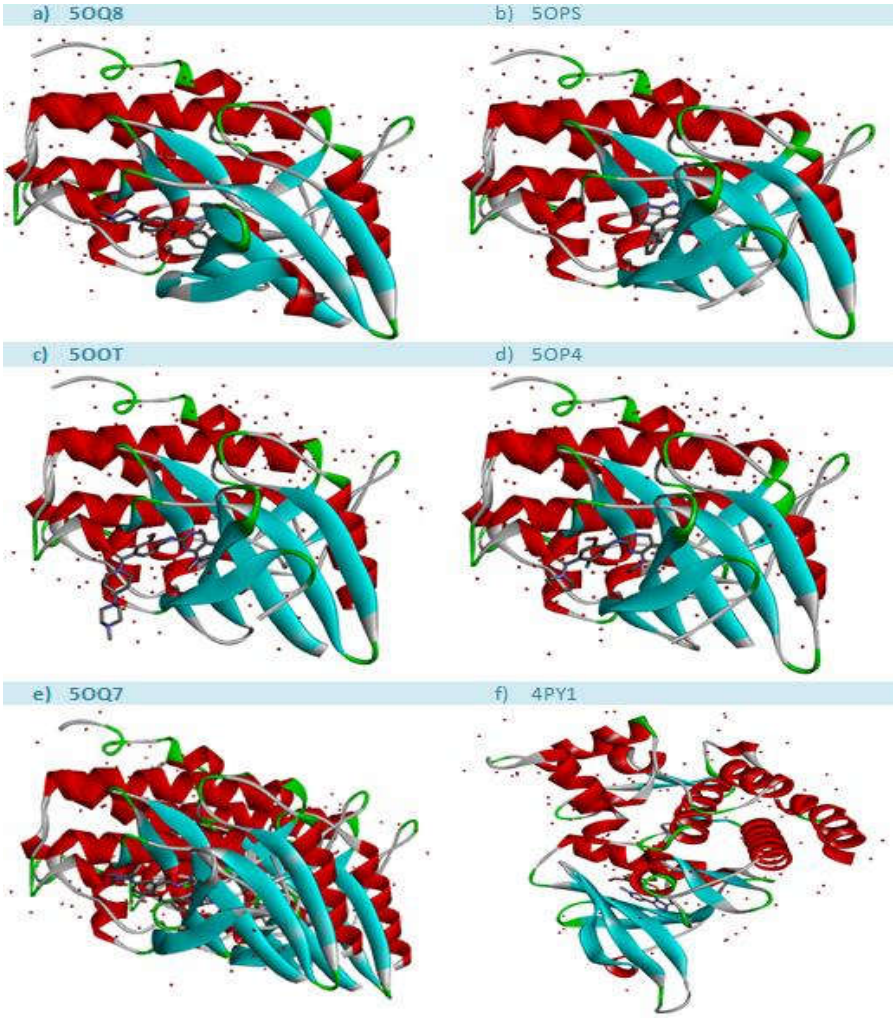



**Figure 1:** Targeted proteins structure along with their ligands. a) 5OQ8 receptor with arylbenzamide ligand. b) 5OPS receptor with pyrrolopyridine ligand. c) 5OOT receptor with aminopyrimido-benzodiazepinone ligand. d) 5OP4 receptor with aminopyrimidine ligand. e) 50Q7 receptor with arylbenzamide ligand. f) 4PY1 receptor ligand complex.

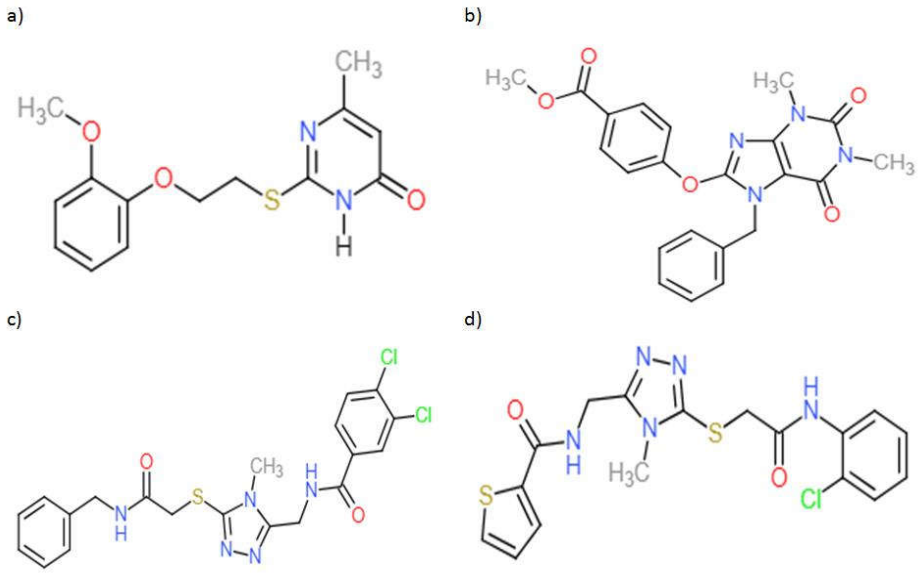



**Figure 2:** Chemical Structures of Hit Compounds

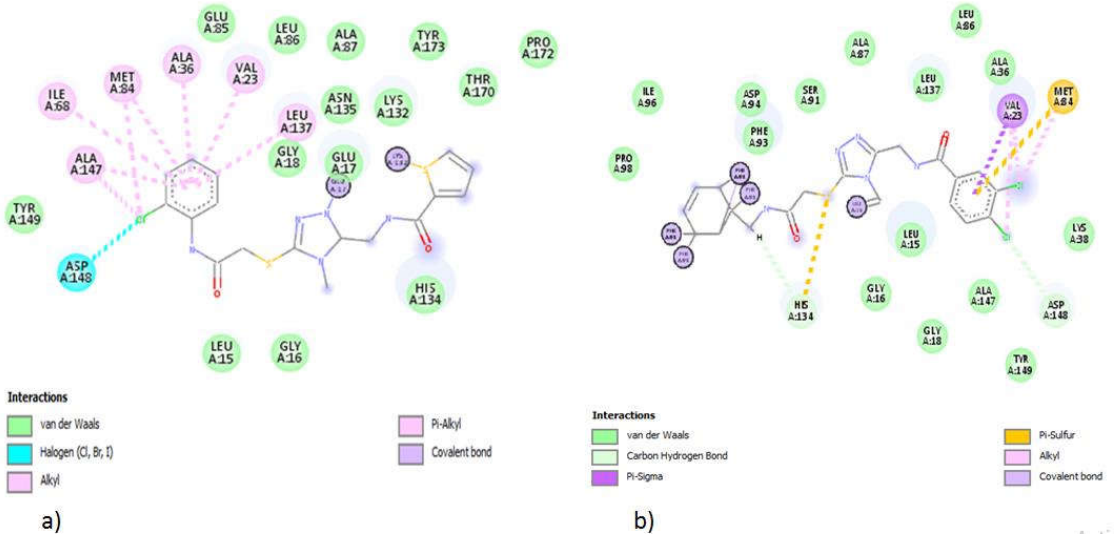



**Figure 3:** Ideal docking results of compounds with proteins. a) Docking of ZINC00633999 compound with 5OQ8 b) Docking of ZINC00633982 compound with 5OQ8 receptor.

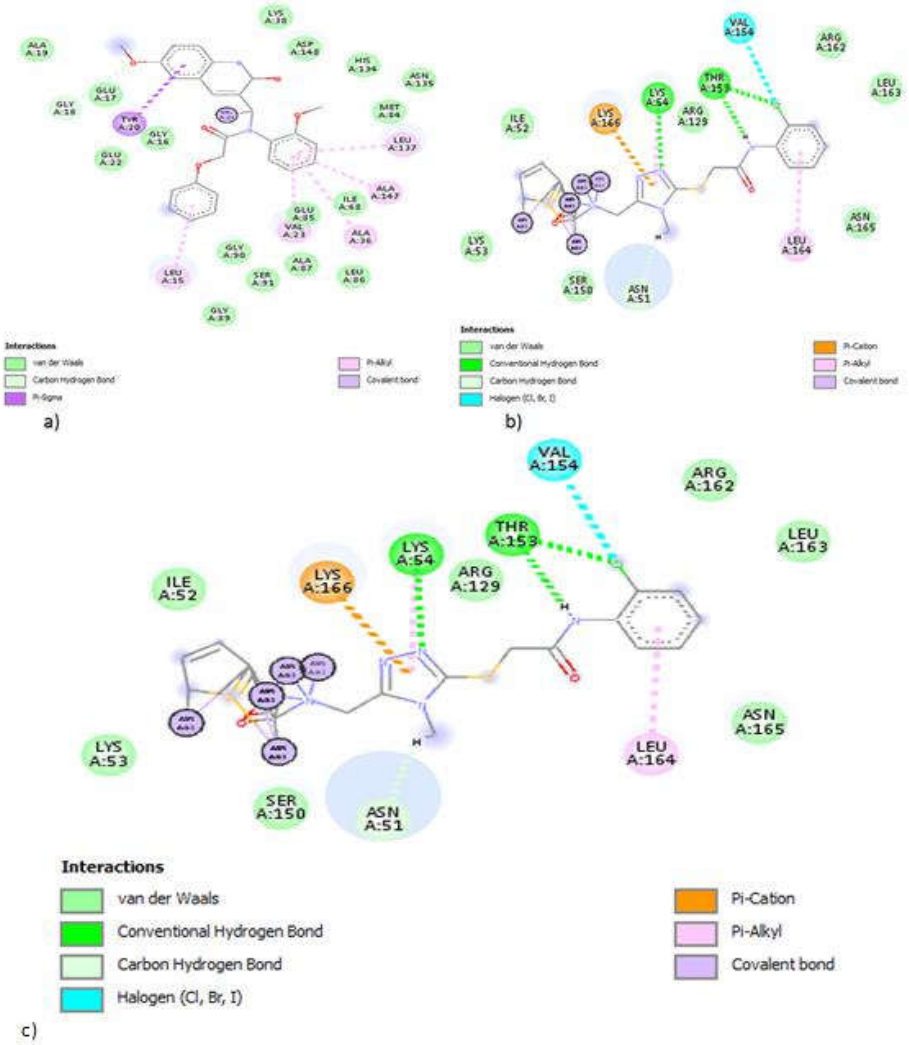



**Figure 4:** Docking results of compounds with receptor proteins. a) Docking of ZINC03901268 compound with 5OOT b) Docking of ZINC00633999 compound with 5OP4 c) Docking of ZINC00633999 compound with 5OPS receptor.

## DISCUSSION

Pharmacophore modeling is a vast and successful field of computer aided drug designing. It is basically applied for rational drug design. Structure based pharmacophore modeling involves the virtual screening for the lead identification and could be used as alternative approach to experimental high through-put screening that work efficiently for the discovery and development of drugs with structural diversity over ligand based pharmacophore search. This involves docking of receptor molecule with lead compounds based on pharmacophore fit scores. This technique is more effective when there is absence of the knowledge about the ligand molecules. It is inventive process of designing new medication based on the knowledge of biological target [2, 22, 26, 27]

Virtual screening is used for the identification of hit compounds that are the lead compounds in pharmacophore modeling and would be effective enough and their biological activity could be analyzed experimentally [28].

In our research work we have generated the shared feature pharmacophore and identified lead compounds through virtual screening against shared feature pharmacophore that would be effective against LRRK2 mutations in Parkinson disease. Molecular docking technique is utilized for the further validation of the three compounds i.e. ZINC00633982, ZINC00633999 and ZINC03901268 .These virtual hit compounds might prove promising lead compounds to be tested as potential LRRK2 inhibitors.

## CONCLUSION

Our study was intended to find novel potent inhibitor for the inhibition of LRRK2 mutations through pharmacophore modeling approach. These pharmacophore models should be effective enough to uncover

the anti-neurodegenerative compounds for Parkinson disease. From the present research work it is concluded that the selected inhibitors could be used for drug designing and would aid in designing new compounds against LRRK2 proteins. However, our work required experimental validation for further efficacy and adequacy.

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**CONFLICT OF INTEREST**
Authors declare no conflict of interest.

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