Bioactive Constituents of Safflower plant as potential inhibitors of mutant Leucine-rich repeat kinase 2 (LRRK2): Docking, Pharmacokinetics properties and Molecular Dynamics studies

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Research Article

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Abstract

Background

Parkinson's disease has posed a global health threat with no disease-modifying treatment and prolonged use of available drugs such as Levodopa (L-dopa) causes debilitating side effects. This study focused on adopting *in-silico* approach to investigate the drug likeliness and pharmacokinetics of some Safflower plant phytochemicals as potential therapeutics. A manually curated library of 120 phytochemicals (from different parts of Safflower plant) was first screened virtually for adherence to the Lipinski's rule of 5 using the SwissADME server. Binding interactions were studied using Discovery Studio Visualizer. Site-specific docking was performed using AutoDock Vina and the docking parameters were set based on the score of Standard drug. The docking scores were validated by Mcule server. ADMET (Adsorption, distribution, metabolism, excretion and toxicity) properties were predicted using swissadme while carcinogenicity and toxicity were predicted using CarcinoPred-EL and PkCSM tools respectively. MD (Molecular dynamics) simulations were performed in GROMACS for 200ns to estimate the stability of the receptor and the protein – ligand complexes.

Results

Eight compounds; Serotobenine, Kaempferol, Nb-PCoumaroyltryptamine, N-Coumaroyl Serotonin, N-Feruloylserotonin, Scutellarein, Acacetin and Trans-Chalcone were identified as potential inhibitors of LRRK2 with no or mild toxicity. Molecular dynamics show more stability in molecular recognition to C1B (N-Coumaroyl-Serotonin) compound than C4B (Serotobenine).

Conclusion

Some of the compounds identified in the Safflower have the potential to be drug candidates for the treatment of Parkinson's disease caused by mutant LRRK2 (Leucine-rich repeat kinase 2), owing to their recorded low binding energy and stability in the target.

Background

Parkinson's Disease is a debilitating neurodegenerative disorder which mainly affects dopamine-producing (dopaminergic) neurons in a specific area of the brain called substantia nigra (Shivaniet et al. 2019). It results in the loss of motor movement and motor sense to perform daily tasks. Reports have shown that more than 6 million individuals have Parkinson's disease worldwide and the number will be doubled by 2050 (Fothergill-Misbah et al. 2020). Furthermore, a disease-modifying treatment for PD (Parkinson's disease) to halt or reverse the disease progression remains an unmet medical need. Only symptomatic therapies with minimum satisfactory effects are known and there is no treatment known to decelerate the disease progression (Rai et al. 2021).

Biochemical, pathological, and genetic studies have identified 18 types of PD caused by various genes including LRRK2, α-Syn, parkin, tau, GBA (Glucocerebrosidase), PARK7 (Parkinson Associated Deglycase 7), SNCA (Synuclein Alpha) and UCHL1 (Ubiquitin Carboxy – Terminal Hydrolase L1), by promoting cognitive impairment (Gourmaud et al. 2020). Parkinson's disease (PD) is either familial or sporadic resulting mostly from a complex interaction of environmental and genetic factors. Mutations in the LRRK2, PARK7, PINK1 (PTEN – induced kinase 1), PRKN, or SNCA gene causes familial cases of Parkinson disease. Also, mutations in some of these genes may play a role in cases that appear to be sporadic (not inherited) (Quadri et al. 2018). Among the listed genes implicated in the pathology of PD, Leucine-rich repeat kinase 2 (LRRK2) is of interest because of its abnormal activity in elevating PD among carriers of mutant LRRK2, particularly those with G2019S type of mutation.

LRRK2 is a multifunctional protein containing 2527 amino acids with a molecular weight of 286 kDa. The protein consists of different domains namely; Ras of complex (ROC) domain with GTPase activity, MAPK (mitogen-activated protein kinase) domain and C-terminal of ROC (COR) surrounding several putative protein-protein scaffolding domains (Ding and Ren. 2020). The effect of mutant LRRK2 in Parkinson's disease (PD) have features consistent with idiopathic PD (DeRight 2022). Even though age 50 is usually the onset, early onset at 20 and late onset at 90 have been observed (Saunders-Pullman et al. 2019). The LRRK2 gene is active in the brain and other tissues throughout the body providing instructions for synthesis of dardarin. Leucine-rich region is a segment of this protein and its name results from the large amount of leucine it contains which is a protein building block (amino acid) (Kilarski et al. 2012). Studies have indicated that dardarin has an enzyme function known as kinase activity, which moves phosphate group from the energy molecule ATP (Adenosine triphosphate) to amino acids (phosphorylation) in certain proteins. This protein has a second enzyme function referred to as a GTPase activity, whose activity resides in ROC domain (Guo et al. 2007).

Safflower plant has been previously described as an important medicinal plant due to the presence of commercially important secondary metabolites (Golkar et al. 2019). Specifically, the presence of phenolic compounds is a consequence of its known antioxidant activities. Parkinson's diseases which has been described to be associated with accumulation of oxidative stress-induced neuronal damage resulting from increased release of reactive oxygen species (ROS) at the cellular levels, currently has no drug with total curative potency. However, the symptoms are alleviated using dopaminergic prodrugs and agonists (Park and Ellis 2020). Due to the presence of antioxidants in safflower, it could serve as a potential inhibitor of mutant LRRK2 which elevates PD. Therefore, this research aims to investigate potency of bioactive constituents of safflower as potential inhibitors of mutant LRRK2.

Methods

Ligand selection and preparation

The chemical structures of one hundred and twenty (120) phytochemicals were obtained from the PubChem database of compounds (Kim et al. 2016). Open babel was used to concatenate the downloaded compounds and converted to PDB followed by conversion to PDBQT format.

Target selection and preparation

The structure of mutant LRRK2 G2019S known to be one of the causes of Parkinson's disease-pathogenesis was downloaded from the Protein Data Bank (PDB) with PDB ID 7LI3. The protein was prepared using MGTools by removing bound complex molecules, non-essential water molecules, non – polar hydrogens and all heteroatoms. Polar hydrogens and gasteiger charges were added. The co-crystallized unique ligands were extracted from the active site to reveal the coordinate of the grid around the binding pocket.

Grid box dimensions for virtual screening

The grid resolution was centered at 200.666 x 178.348 x 270.880 along x, y, and z-axes, respectively, for a grid size of 40 x 40 x 40 Å to define the binding site for site -1 while 220.958 x 212.312 x 229.094 along x, y, and z-axes, respectively, for a grid size of 40 x 40 x 40 Å to define the binding site for site -2.

Molecular docking using Autodock Vina

The method of Arannilewa *et al.* (Arannilewa et al. 2018) was adopted for molecular docking of the selected compounds. Molecular anchor (docking) analysis was done by autodock vina on the prepared receptor, standard and test ligands. In this study, we employed the exhaustive search anchoring function. The grid resolution was centered to define the binding site after energy minimization and the binding affinity generated was extracted.

Validation of docking results and generation of some physicochemical properties

Docking results were validated through a structure based virtual screening carried out using the Mcule pipeline custom flows. The workflow was set so as to allow zero violation of Lipinski's rule of five. This limitation set is important in determining the potentiality of the molecules having oral drug likeness as molecules of moderate lipophilicity are major characteristics of most orally administered drugs (Karavasili et al. 2020). The results of the docking validation were generated alongside some physicochemical properties of interest.

Visualization and 2D structure generation

Discovery Studio was employed to visualize the receptor-ligand interactions with the lowest energy model (the hit compounds). The benchmark docking score was set to the binding score of the standard drug (levodopa). The amino acids interacting with the ligand, hydrogen bonds, and specific atoms were documented. Among other hydrophobic interactions, non-covalent interaction, including the occurrences of hydrogen, Vanderwaal, π–π, π-sigma bond, and π-sulfur were observed.

Drug likeliness, Pharmacokinetics and Admet Predictions

The drug likeliness and pharmacokinetics parameter of the hit compounds (with binding score of ≤ -7) was checked by subjecting the compounds to absorption, distribution, metabolism and excretion (ADME) predictions with SwissADME (Bakchi et al. 2022). While CarcinoPred-EL (Zhang 2017), a web-based tool that predicts carcinogenicity with ensemble learning methods was employed in predicting carcinogenicity of the hit compounds. The Oral rat acute toxicity LD_{50} was determined using PkCSM, a toxicity prediction which uses graph-based signatures. This web tool also predicted the Maximum tolerated dose in humans (Pires et al. 2015).

Molecular dynamics simulation

Using four steps, MD simulations were performed in GROMACS v2021.4 (Abraham et al. 2015). First, in 5000 steps (until reaching an energy < 10 kJ/mol/nm), energy minimization was carried out using the steepest descent algorithm to eliminate bad contacts followed by an NVT equilibrium phase at 310 K for 2 ns to equilibrate the system temperature. In next step, an NPT equilibrium phase at 1 bar for 4 ns was done to equilibrate the system pressure. The Berendsen thermostat and the Parrinello-Rahman barostat (Parrinello and Rahman 1981) were used in the equilibrium phases, respectively. Finally, with integration steps of 2 fs, under constant pressure and temperature using leap-frog integration algorithm, a production simulation for 200 ns was obtained (Van Gunsteren and Berendsen 1988). The LINCS algorithm was used to constrain the interactions during equilibrium so as to generate the trajectories, while the long-range ionic interactions were constrained using the Particle-Mesh Ewald algorithm.

Molecular Data Analysis. Using geometrical and structural properties, the trajectories were analyzed so as to determine the influence of ligand protein dynamics (Michaud-Agrawal et al. 2011).

Results

Tables 1–4 and Figs. 1–4 contain the summary of the results of this study. It presents important properties of phytochemical compounds from safflower plants that have the potential of attenuating Parkinson's disease.

Table 1
Site specific docking scores of the top ten compounds using Autodock Vina and validated with Mcule

Molecule	ADV score (active Site 1)	ADV score (active Site 2)	Mucle docking score (active site 1)	Mcule docking score (active site 2)
Reference drug (Levodopa)	-7	-7	-6.2	-6.4
Serotobenine	-8.8	-8.7	-8.4	-8.7
N-Coumaroyl Serotonin	-8.2	-7.8	-7.2	-8
Quercetin	-8.2	-8	-7.6	-8.1
Kaempferol	-7.7	-8	-7.4	-7.8
N-Feruloylserotonin	-7.6	-7.9	-7.1	-8.3
Nb-PCoumaroyltryptamine	-7.2	-7.9	-7.3	-7.6
Scutellarein	-7.7	-8.2	-7.1	-8
Acacetin	-7.2	-7.9	-6.8	-7.6
Trans-Chalcone	-6.9	-7.4	-6.7	-7.1
4-Hydroxybenzhydrazide	-6.7	-6.1	-7	-6.1

[a]ADV = autodock vina

Table 2
Physicochemical properties of hit compounds

Compound	TMW(g/mol)	RB	HA	HD	TPSA	Logp
Serotobenine	350.367	2	4	3	83.58	2.25
N-Coumaroyl Serotonin	322.357	6	3	4	85.35	2.57
Quercetin	302.235	1	7	5	131.36	1.23
Kaempferol	286.235	1	6	3	111.13	1.58
N-Feruloylserotonin	352.383	7	4	4	94.58	2.58
Nb-P-Coumaroyltryptamine	306.357	6	2	3	65.12	2.82
Scutellarein	286.235	1	6	4	111.13	1.81
Acacetin	284.262	2	5	2	79.9	2.52
Trans-Chalcone	208.254	3	1	0	17.07	3.3
4-Hydroxybenzhydrazide	152.151	2	3	3	75.35	0.09

[a]TPSA = Topological Polar Surface Area; [b]TMW = Total Molecular Weight; [c]HA = Hydrogen acceptor; [d]HD = Hydrogen donor; [e]RB = Rotatable bonds

Table 3
Drug likeliness and pharmacokinetics of the hit compounds

Compound	Solubility class	GI absorption	BBB permeant	P-gp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
Serotobenine	Soluble	High	No	Yes	Yes	Yes	No	Yes	Yes
N-Coumaroyl Serotonin	Moderately soluble	High	No	No	Yes	No	No	Yes	No
Quercetin	Soluble	High	No	No	Yes	No	No	Yes	Yes
Kaempferol	Soluble	High	No	No	Yes	No	No	Yes	Yes
N-Feruloylserotonin	Moderately soluble	High	No	Yes	Yes	No	No	Yes	No
Nb-PCoumaroyltryptamine	Soluble	High	Yes	Yes	Yes	Yes	No	Yes	No
Scutellarein	Soluble	High	No	No	Yes	No	No	Yes	Yes
Acacetin	Moderately soluble	High	No	No	Yes	No	Yes	Yes	Yes
Trans-Chalcone	Soluble	High	Yes	No	No	Yes	No	No	No
4-Hydroxybenzhydrazide	Very soluble	High	No	No	No	No	No	No	No

[a] GI = gastrointestinal, [b] BBB = blood brain barrier, [c] P-gp = permeability glycoprotein, [d] CYP = Cytochrome.

Table 4
Toxicity of hit compounds

Compound	Ames toxicity	Oral rat acute toxicity LD50 (mol/kg)	Max. tolerated dose human (log mg/kg/day)	Carcinogenicity
Serotobenine	No	2.244	0.145	NO
N-Coumaroyl Serotonin	No	2.151	0.276	NO
Quercetin	Yes	2.625	0.512	NO
Kaempferol	No	2.524	0.543	NO
N-Feruloylserotonin	No	1.908	0.637	NO
Nb-PCoumaroyltryptamine	No	2.274	0.000	NO
Scutellarein	No	2.622	0.542	NO
Acacetin	No	2.37	0.028	NO
Trans-Chalcone	No	1.854	0.738	NO
4-Hydroxybenzhydrazide	Yes	2.76	1.254	NO

Discussion

The mutant LRRK2 G2019S has two active sites. The active site − 1 is where the unique ligand GDP (Guanosine diphosphate) is located while Active site − 2 is the ATP bond location. (Myasnikov et al. 2021). In this study, levodopa (Hornykiewicz 2010) was adopted as the reference drug on which selection of the lead compounds were based on. However, our interest is to select compounds as potent as levodopa but without side effects as the reference drug. The reference drug gave a binding score of -7 for both actives sites but − 6.2 and − 6.4 were got as binding score for active site 1 (GDP location) and active site 2 (ATP location) in a validation docking using Mcule webserver. The docking score threshold was set based on these values. Even though the docking score values of 4-Hydroxybenzhydrazide do not correspond with the threshold values set for docking score of active sites 1 and 2, the score obtained for active site 1 is close to the threshold score while the validated score is same as the threshold cut off. Table 1 shows the docking scores of the first 10 phytochemical compounds of safflower against the active sites of the LRRK2 ligand. Out of these top 10 compounds, only the first nine molecules have a docking score, less or equal to the threshold docking score. The phytocompound with the lowest score, Serotobenine was reported as a novel phenolic amide from safflower seeds in 1985 which was isolated together with the already known compounds; *N*-ferloyltyptamine and *N-(P*-coumaroyl)tryptamine (Sato et al. 1985). Also, by construction of an optically active dihydrobenzofuran through rhodium mediated intramolecular C-H insertion, (-) – serotobenine has been synthetically generated (Koizumi et al. 2008). Other methods have been employed in an attempt to carry out a total synthesis of serotobenine (Hu et al. 2013). Though no significant activity has been recorded for this compound, it's derivatives alongside Decursivine which is structurally related to it have been shown to have anti- plasmodial activity (Qin et al. 2011). Quercetin has been reported to have various biological activity which includes but not limited to anti-inflammatory, antibacterial, anticancer and antioxidant activity, however it has mutagenic effects which corresponds to our toxicity predictions in this study (Batiha et al. 2020). The only structural difference between Kaempferol and Quercetin is the presence of an additional hydroxyl group in its benzene ring (Liu et al., 2008). Like quercetin it has numerous reported biological activities and their similarity in activity can be attributed to their structural resemblance, thus a proof of therapeutic dependence on molecular structure (Ren et al. 2019). Nb-P-Coumaroyltryptamine isolated from *Phragmites australis* (cav.) Trin. Ex steud has shown to have cytotoxic activity and even though it has been reported to inhibit the production of proinflammatory cytokins, it exhibited an activity lower than N(P-Coumaroyl)serotonin (Chen et al. 2021). More so, the antioxidant activity of N-Coumaroyl Serotonin has been reported alongside N-Feruloylserotonin of which both were isolated from safflower seeds (Lee et al. 2008). Scutellarin is another constituent of safflower that has been described as possessing multiple pharmacological activities including but not limited to myocardial protection, anticoagulation, antiplatelet, vascular relaxation, anti-inflammation and anti-oxidant. It has also been described to be effective towards diabetic complications, stroke and myocardial infarction treatment. Reports of its recent medicinal modification, formulation with improved safety, efficacy and bioavailability supports the appreciable admet and pharmacokinetics properties reported by our study on this compound and may thus be repurposed alongside its bioactive derivatives towards PD treatment (Wang and Ma 2018). Anticancer and anti-inflammatory activity have been reported for acacetin (Sun et al. 2018) while anti-inflammatory and alpha – amylase activity has also been reported for Transchalcone (Staurengo-Ferrari et al. 2018). Since oxidative stress has been reported to cause cognitive impairment in PD, the selected molecules that have previously shown to possess oxidative potency may likely be efficient in PD treatment therapy.

Table 2 presents some physicochemical properties of the lead compounds. These properties determine the pharmacodynamic process and pharmacokinetics of drugs and thus are given significant consideration in design, discovery and development of drugs (Jia et al. 2020). Molecular weight determines the drug-likeness of any molecule under consideration especially for oral drugs. While Lipinski's rule assumes a molecular weight of ≤ 500 as an optimal weight of good oral drugs, lead-likeness prediction assumes a molecular weight in the range 250 ≤ MW ≤ 350 (Protti et al. 2021). The number of hydrogen acceptors and donors present in a compound has been described to affect its drug-likeness. Hydrogen bonding is characterized by the donor and acceptor group which creates the needed hydrophobic interactions that is essential for chemotherapeutic effects. It has been established decades ago that the action of local anesthetics is characterized by a formation of a hydrogen – bonded complex between the acceptor group and the drug (Sax and Pletcher 1969). Also, inhibitors and non-inhibitors of organic anion transporters (OAT) have number of hydrogen -bond donors and acceptors, topological surface area, molecular weight and number of rotatable bonds attributed to their inhibitory or non-inhibitory effect. OAT family is reported to mediate between clinically important drugs and the body disposition (Duan et al. 2012). Partition coefficient is as important as other physicochemical properties, thus an indispensable property in drug design and development. It measures the lipophilicity of a molecule and describes the extent to which a drug would remain either in the lipid membrane or in an aqueous medium. Swiss adme uses five (5) predictive models, however the consensus estimation is chosen for accuracy (Mannhold et al. 2009).

Among other hydrophobic interactions, non-covalent interactions, including the occurrences of hydrogen, Vanderwaals, π–π, π-sigma bond, conventional hydrogen bond, π – π T – shaped, π – alkyl and π – stacked interactions were observed. However, for serotonin and N-Feruloylserotonin, Vanderwaals, conventional hydrogen bond, π – π T – shaped, π – alkyl and π – stacked interactions were observed after visualization with discovery studio (Fig. 1). The conventional hydrogen bond, having a relative strongest interaction is responsible for the high stability observed in (N-Coumaroyl-Serotonin) – 7li3 complex during molecular dynamics study.

Examining the pharmacokinetics profile of a potential drug is one of the major routes to drug discovery and development. Carrying out all required processes in the wet laboratory is not only time consuming but also yield an extreme cost. Therefore, pharmacokinetics prediction is a route to salvaging time, resources and cost in finding potential lead molecule as it filters possible lead compounds at a minimal cost (Bandyopadhyay et al. 2021). Table 3 shows the drug likeliness and pharmacokinetics of the hit compounds. Swiss ADME uses three models in solubility prediction. However, we have presented the results from ALI model only, since it demonstrates a stronger linear relation between predicted and experimental values (R^2 = 0.81). Most predicted molecules are soluble in this model, though N-Coumaroyl Serotonin and N-Feruloylserotonin are moderately soluble. Even though solubility is a key property in design of drugs, insoluble potent drug candidates can be converted to the salt of the parent compound to enhance its solubility. Some drugs such as diclofenac are marketed in this form (Al Ragib et al. 2018). Gastrointestinal absorption and brain accessibility prediction is one of the preliminary tests to discover the fate of a drug candidate within the biological system of an organism. The model employs the computation of polarity of small molecules and their lipophilicity. This physicochemical property determines the extent of a drug's bioavailability. The bioavailability of a drug entails a systemic distribution of its molecules which also determines its therapeutic efficiency, metabolism and excretion (Maderuelo et al. 2019). Poor permeability of potential drugs has been revealed to be the cause of the prevailing limited therapeutic treatment for Parkinson's diseases, thereby necessitating our study to also consider potential drug candidates with positive test result for blood brain barrier (BBB) permeability (Shaltiel-Karyo et al. 2013). Though, only Nb-PCoumaroyltryptamine and Trans-Chalcone show positive result for this test, other lead compounds can still be subjected to *in vivo* testing for further verification. It has been reported in previous research that water-soluble molecules can be transformed into lipid soluble molecules so as to enhance blood brain barrier permeability owing to the observation that lipid solubility plays significant roles in passive diffusion into BBB. This transformation introduces lipid groups at the polar ends of the drug candidate (Bellettato and Scarpa 2018). An alternative method reported to solve the challenge posed by this, is the use of intranasal drug delivery method which entails a direct transport into the cerebrospinal fluid; a process that delivers molecules of interest direct to the central nervous system in minutes time interval with a bypass of the BBB (Mathison et al. 1998). Other reported alternative methods include use of transport/carrier systems, inhibition of efflux transports that impede drug delivery, trojan horse approach, chimeric peptides, monoclonal antibody fusion proteins, pro-drug bioconversion strategy, nanoparticles-based technologies, gene therapy and intracerebral gene therapy (Bellettato and Scarpa 2018). All the selected lead molecules in this study show high GI (Gastrointestinal) absorption prediction. The P-gp (permeability glycoprotein) otherwise known as multi-drug resistance protein is a constituent of the cell membrane that exudes foreign substances out of the cell. The ability of a drug to act as either P-gp-substate or non-P-gp substrate determines both the total clearance time and bioavailability. Pumping of drugs back to lumen by this protein reduces absorption. In summary, drugs that inhibit P-glycoprotein would increase absorption while drugs that induce it leads to reduction in absorption.

CYPP450 (Cytochrome P450) enzymes are very essential in metabolism of many drugs. Although there exist about 50 cytochrome enzymes that are involved in drug metabolism, ninety percent of drugs are metabolized by only six of these enzymes. In this research, we present the possible inhibitors of CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4 of which CYP3A4 and CYP2D6 have been described as the most important. Drug candidates that inhibit all of these enzymes may not be subjected to metabolism and thus would increase the chance of the

drug's toxicity (Das et al. 2009). In our study, each of the selected drug candidate is a non-inhibitor to at least one of these enzymes, making them possible of being metabolized and excreted.

The toxicity predictions of the hit compounds (Table 4) show that, Quercetin and 4-Hydroxybenzhydrazide are not suitable as drugs as they are predicted to be ames mutagenic. A potential drug candidate with no tolerable toxicity profile would not be included in the next stage of a drug discovery process, although there should a consideration of concentration/dosage dependence of toxicity. The Oral rat acute toxicity (LD50) estimates the amount of a drug candidate that would lead to 50% death of the test animal group. The prediction reveals the relative toxicity profile of the selected compounds. Ames mutagenicity involves the use of bacteria to investigate mutagenic potential of a given molecule in the DNA (deoxyribonucleic acid) while the toxicity threshold dose in humans is determined by maximum tolerated dose in human. CarcinoPred EL was used to predict the carcinogenicity of the selected compounds. Quercetin in previous research has been demonstrated to be toxic and precisely has exhibited carcinogenicity in the kidney of male rats, leading to benign tumors of the renal tubular epithelium, however, further research has demonstrated that synergistic therapy can attenuate its possible toxicity by reducing the dosage in take (Zou et al. 2021), a proof why the phytocompound does not pose much risk to the consumption of fruits that contains it, since there is a synergistic action with other phytochemicals. Also, this compound has been evaluated previously for its ability to attenuate cognitive impairment with 6-hydroxydopamine induced PD in rats and the result proved that quercetin enhances spatial memory (Sriraksa et al. 2012) and has been considered as PD supplemental therapy (Tamtaji et al. 2020).

LRRK2 being a 286-kDa multidomain protein makes it more computationally expensive to carryout out a simulation using the entire protein. In order to minimize time and computational cost, Kinase (KIN), ROC, and COR domains which contain the mutation sites (Myasnikov et al. 2021) were used for a complete model simulation, though RMSD (Root Mean Square Deviation) was obtained using the full protein structure (7LI3) (Fig. 2). The RMSD values for the COR domain of 7LI3 complex and apo were within 2.5 to 5.6 Amstrong. The RMSD of the COR domain of 7LI3 is stable for the apoprotein after about 15ns, for C4B, stability is attained after about 80ns. C1B showed a stark increase in its RMSD after about 80ns and remains stable throughout the rest of the duration. This is suggested to be as a result of change in conformation (Bandyopadhyay et al. 2021); however, it maintains an RMSD similar to the apoprotein indicating that the C1B complex results in a similar conformation as the native conformation (Swargiary et al. 2022). The RMSD values for the ROC domain for 7LI3 complex and apo were within 1 to 5 Amstrong. It is stable for the apoprotein, C1B and C4B after about 5ns. C1B showed a stark increase in its RMSD after about 124 and 175ns. This is perhaps due to a conformational change; however, it maintained an RMSD similar to the apoprotein indicating that the C1B complex results in a similar conformation as the native conformation. C1B remains stable at around 3 Armstrong throughout the rest of the duration, maintaining the lowest deviation from its native conformation (Lower than the apo state). The RMSD values for the KIN domain for 7LI3 complex and apo were within 1 to 4 Amstrong. It is stable for the apoprotein, C1B and C4B after about 2ns. C1B remains stable at around 2.5 Armstrong throughout the rest of the duration, maintaining the lowest deviation from its native conformation (Lower than the apo state).

For the 200ns simulation, RMSF (Root mean square fluctuation) (Fig. 3) was carried out to detect the local changes along 7LI3 amino acid residues (Ishola et al. 2021). It was observed that the alpha helices and bet strands of the apo structure and docked systems oscillated within 5 to 30 Amstrong with the most fluctuation observed in C4B. The loop regions of all the simulations showed large fluctuations up to 40, 29 and 35 Armstrong for C4B C1B and the apo state respectively for the COR domain. For the ROC domain, loop regions of all the simulations showed large fluctuations up to 45, 27 and 40 Armstrong for C4B, C1B, and apo state respectively while large fluctuations were observed up to 45, 27 and 47 Armstrong for C4B, C1B, and apo state respectively in the KIN domain.

In MD simulation, the gyration radius is an index to monitor the structural formation process. A relative steady value of Rg (Radius of Gyration) (Fig. 4) indicates that the complex is stable (Morgan et al. 2017).

The results of our study reveal that C1B and C4B complex with 7LI3 were found to be stable in the binding sites with insignificant structural movements and lesser conformational changes to the overall structure hence, presenting them as potential inhibitors against LRRK2 (Bandyopadhyay et al. 2021).

Conclusion

Parkinson's disease is one of the neurodegenerative diseases that attracts a universal interest as only symptomatic therapies are available with unsatisfactory therapeutic effects. There have been series of research to discover a better therapy to decelerate the disease's progression. In order to contribute towards ongoing researches in this area, our study has used an *in-silico* approach; docking, ADMET and molecular dynamics evaluation to investigate the potencies of constituents of safflower plant in inhibiting mutant LRRK 2, a cause of familial cases of Parkinson's diseases. This research has proven that safflower plants contain phytocompounds with LRRK2 inhibitory potency. It identified Serotobenine, Kaempferol, Nb-PCoumaroyltryptamine, N-Coumaroyl Serotonin, N-Feruloylserotonin, Scutellarein, Acacetin and Trans-Chalcone as potential drug candidates for treatment of PD. However, Nb-PCoumaroyltryptamine and Trans-Chalcone appears more

promising since they have been predicted to be permeable to the blood brain barrier membrane, a factor which also limits therapeutic efficiency in treatment of this disease. The result of the molecular dynamics study supports the significant stability of Serotobenine and N-Coumaroyl Serotonin at the active sites of the receptor protein through conventional hydrogen bonding and other hydrophobic interactions. This research also validates previous findings on quercetin, acacetin and kaempferol neuroprotection potency.

Abbreviations

ADMET – Absorption, Distribution, Metabolism, Excretion and Toxicity

COR – C – Terminal of ROC

GBA – Glucocerebrosidase

KIN - Kinase

LRRK2 - Leucine-Rich Repeat Kinase 2

MAPK – Mitogen Activated Protein Kinase

MD -Molecular dynamics

PARK – Parkinson Associated Deglycase 7

PD – Parkinson's disease

PINK 1 - PTEN – induced kinase 1

RMSD – Root Mean Square Deviation

RMSF – Root mean square fluctuation

RGYR – Radius of Gyration

ROC – Ras – of- Complex

SNCA – Synuclein Alpha

UCHL1 – Ubiquitin Carboxy – Terminal Hydrolase L1

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

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Figures

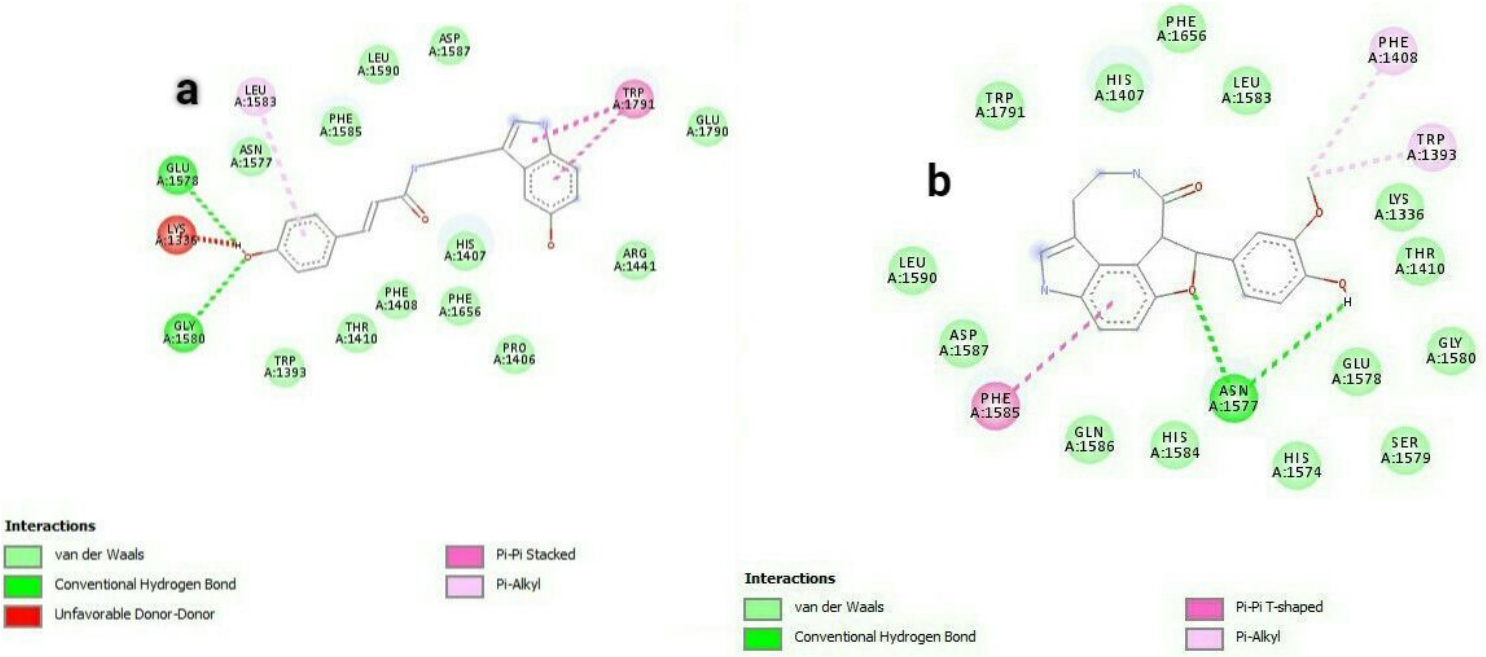

Figure 1

2D representation of the interactions between ligands and the receptor (a) N-Coumaroyl-Serotonin and 7li3 (b) Serotobenine

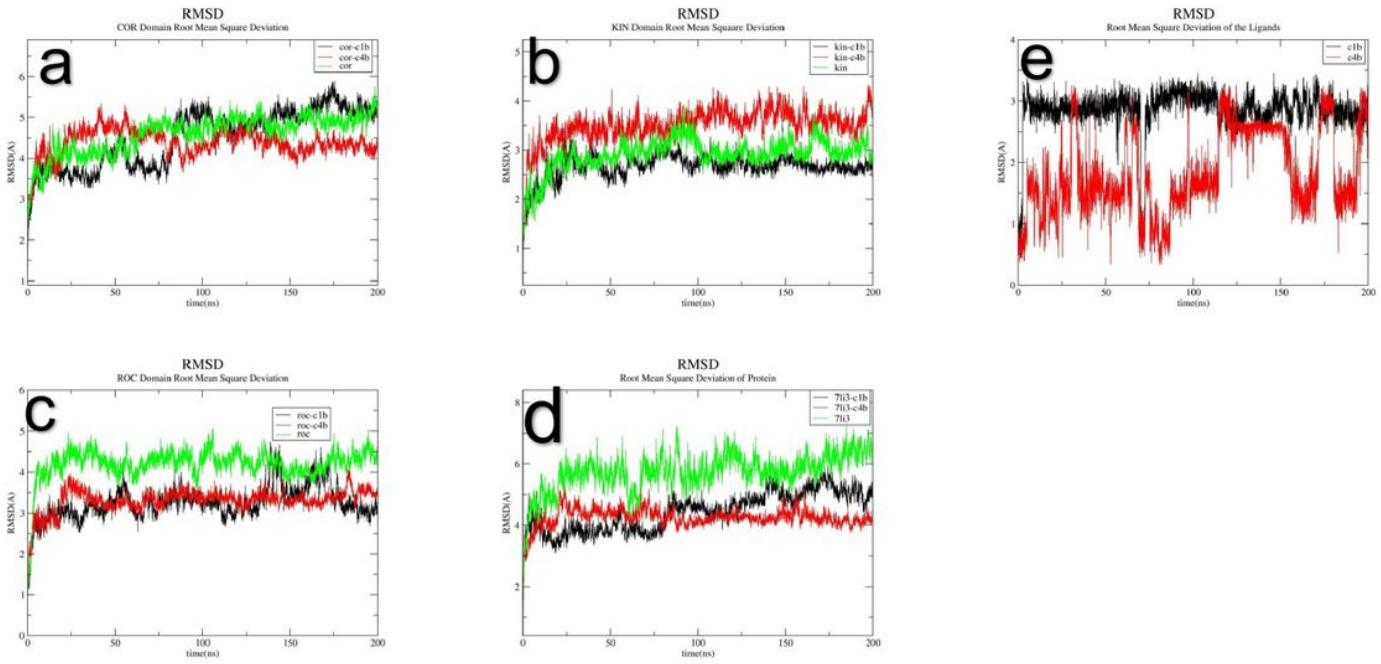

Figure 2

(a) RMSD of COR (apo) and COR – ligands (complexed) (b) RMSD of KIN (apo) and KIN – ligands (complexed) (c) RMSD of ROC (apo) and ROC – ligand (complexed) (d) RMSD of protein target (apo, PDB ID: 7li3) and protein – ligand (e) RMSD of ligands (N-Coumaroyl-Serotonin and Serotobenine)

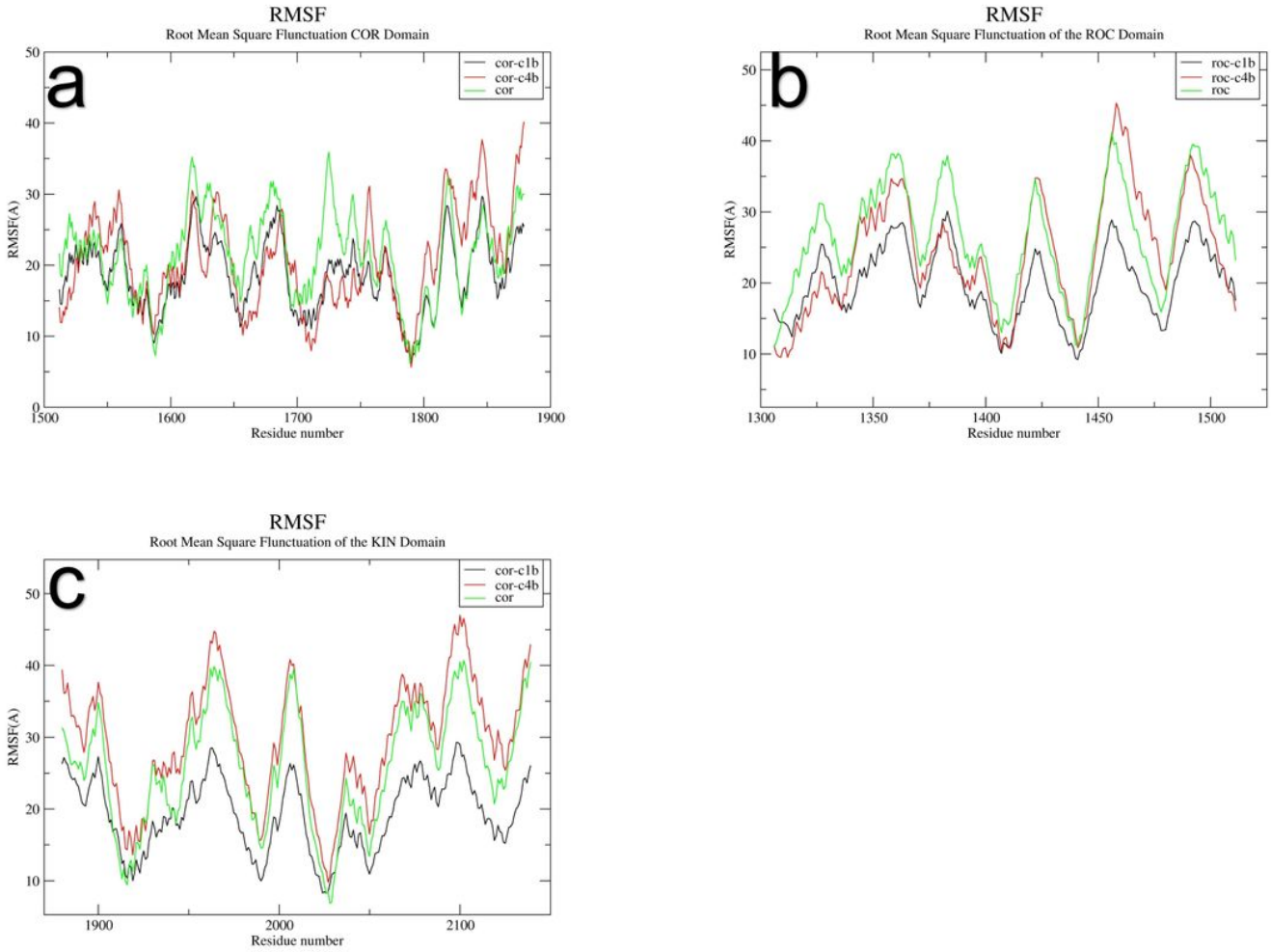

Figure 3

(a) RMSF of COR (apo) and COR – ligands (complexed) (b) RMSF of ROC (apo) and ROC – ligands (complexed) (c) RMSF of KIN (apo) and KIN – ligand (complexed)

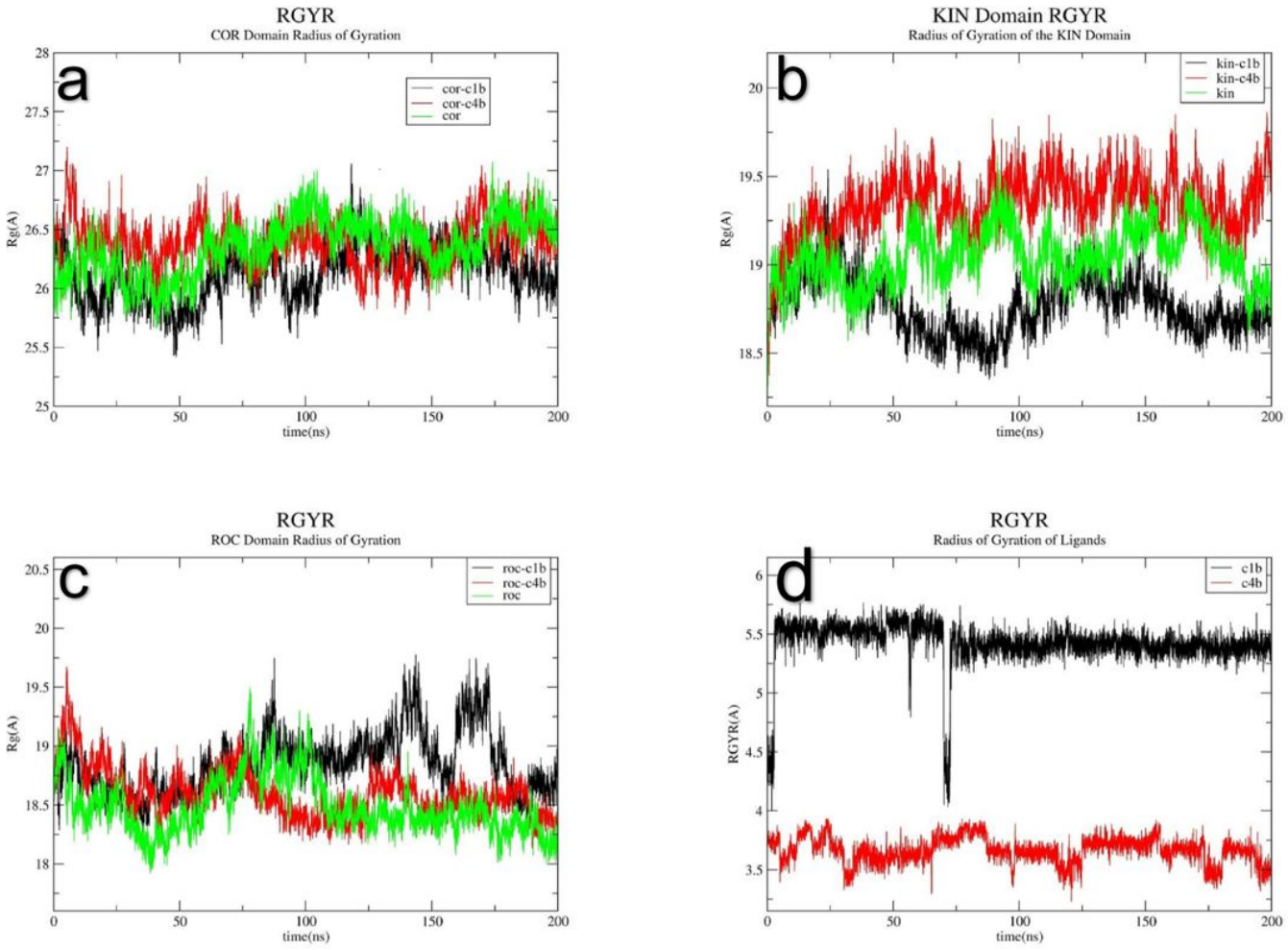

Figure 4

(a) RGYR of COR (apo) and COR – ligands (complexed) (b) RGYR of KIN (apo) and KIN – ligands (complexed) (c) RGYR of ROC (apo) and ROC – ligand (complexed) (d) RGYR of ligands (N-Coumaroyl-Serotonin and Serotobenine).