APPLICATIONS OF
MODELLING AND SIMULATION
http://arqiipubl.com/ams eISSN 2600-8084 VOL 6, 2022, 122-133

Ligand-Based Pharmacophore Modelling Targeting α-Synuclein Misfolding for an Effective Treatment of Parkinson's Disease

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Submitted 30 September 2022, Revised 3 November 2022, Accepted 16 November 2022, Available online 4 December 2022.

Abstract: Parkinson's disease (PD) is a progressive neurodegenerative disease prominently observed in elderly population. Aggregation and misfolding of α-synuclein protein is strongly implicated as an underlying pathogenesis of the disease. PD is characterised by high amount of intracellular α-synuclein as the main constituents in Lewy bodies. However, PD drugs in clinical conditions elicit unpleasant side effects and develop resistance after long time application, making a search for better candidates imperative. This study is aimed at identifying potent inhibitors of α-synuclein from interbioscreen (IBS) database using ligand-based pharmacophore modelling, Glide standard precision docking, molecular dynamics, and pk-CSM pharmacokinetics parameters. Among the ten models generated, only one of the pharmacophore models was validated with Guner-Henry's goodness of hits (GH) scoring method and enrichment factor (EF) of 0.87 and 23.43 respectively, making it ideal for database screening. The pharmacophore model with features, HHHHHHDDDDA identified 100 hits from "877,337" IBS database natural compounds. The top hits, STOCK2S-85121, STOCK3S-13122, STOCK2S-57139, STOCK7S-07150, and STOCK4S-24924 demonstrated better docking scores of -4.789, -4.451, -4.413, -4.365 and -4.227 kcal/mol respectively, while the standard, Levodopa has docking score of -3.556 kcal/mol. The retrieved compounds have similar amino acid interactions with the standard compound (levodopa). The ligands are predicted to have good Blood Brain Barrier permeation, central nervous system penetrations and absorption, distribution, metabolism, elimination, and toxicity (ADMET) properties. Molecular dynamics for 50 ns further suggested that the highest docked compound (STOCK2S-85121) was stable into the binding pocket and displayed strong hydrogen bond interactions. Therefore, the compound is recommended for further evaluations as potential anti-PD agents.

Keywords: *In silico* ADMET; Ligand-based drug design; Natural products; Parkinson's disease; Pharmacophore model; α-synuclein inhibitors.

1. BACKGROUND

Brain is one of the vital organs in human system. Its functions deteriorate over time due to inevitable process of aging and accumulation of oxidative biomolecules, leading to neurodegenerative disease. Parkinson's disease (PD) is a chronic progressive neurodegenerative disease prominently observed in elderly population with characteristic movement disorders [1]. Globally, one out of every 100 people are affected by PD and yet limited effective curative options are available [2-4]. Specific course of PD is not fully understood and one of the most affected areas in the brain by PD is the substantia nigra, resulting to substantial loss of dopaminergic neurons and a concomitant decrease in dopamine levels within the striatum. Consequently, most prominent PD symptoms are manifested as impaired motor activity including muscle rigidity, and resting tremor [5]. The disease is characterized by intracellular inclusions known as Lewy bodies (LBs) and the main constituent of LBs is α-synuclein [6]. Aggregation of α-synuclein is one of the underlying molecular pathogeneses of PD. This is demonstrated when soluble α-synuclein monomers form oligomers to aggregated small protofibrils, and progressively to insoluble amyloid fibrils. These amyloid fibrils are neurotoxic, causing dopamine cells death. Therefore, this strongly supports the notion that α-synuclein is an essential target in the molecular pathogenesis of PD [2]. The α-synuclein protein is abundant in the brain, having short amino acid (AA) of 140 residues, and high concentration at presynaptic terminals as both soluble and membrane-associated fractions of the brain. The protein is estimated to account for as much as 1% of the total protein as a soluble cytosolic brain

fractions [7]. It is divided into three segments: (a) N-terminal consisting of 1-60 AA residues mainly involved in membrane binding, (b) hydrophobic domain AA residues 61-95, also known as non-amyloid-beta component (NAC) responsible for aggregation, and (c) C-terminal domain with an AA residue 96-140, which is essential for protein-small molecules and protein-protein binding. Therefore, it is important to target both the binding site (C-terminal) and the aggregation site (NAC region) or hydrophobic domain to ensure effective ligand-receptor binding and inhibition of the protein [2, 7]. Although, several functions for α-synuclein have been postulated, including the regulation of neurotransmitter release, synaptic vesicle recycling and synthesis, and physiological regulation of specific enzymes and transporters. However, its high negative pathological implication in fibrillation and oligomerization, constitutes a vital target for effective management of PD [8].

The predominant clinical drugs currently used to manage PD are dopamine agonist precursors such as levodopa, monoamine oxidase inhibitors (selegiline), and catechol-o-methyl transferase (entacapone). These drugs have symptomatic efficacy and provoke severe and unwanted side effects, especially upon a long-term application. Some of the common drugs used to manage PD are indicated in Figure 1. Therefore, it is pertinent to search for alternative inhibitors that could be developed as curative and preventive agents [9, 10]. Focusing on NAC and C-terminal domains as a strategy for effective binding and inhibition of α-synuclein will undoubtedly provide an alternative route to develop new agents that could be potential anti-PD agents [11]. Thus, the discovery of therapeutic inhibitors against α-synuclein progressive aggregation remains imperative [12].

Although, many researchers reported inhibitors of α-synuclein fibrillation, the physicochemical, pharmacokinetics and toxicological properties of the compounds are poor to aid their development as potential anti-PD drugs [11]. Finding therapeutic and successful candidate drug compounds through experimental studies are expensive and time-consuming. Moreover, most of the reported inhibitors have inactive structural features that further limit their therapeutic effects [13]. Recently, utilization of computer-aided drug design (CADD) with virtual screening techniques provides powerful tools for developing potential compounds, reducing the cost up to 50%, and elimination of candidate compounds with poor pharmacokinetic properties such as absorption, distribution, metabolism, elimination, and toxicity (ADMET) before their applications. Therefore, application of CADD and the breakdown of all binding affinity into its interaction components are necessary to assist in pharmacophore modelling and virtual screening of small molecules aimed at identification for effective therapeutic drugs against PD [14].

The CADD techniques are broadly classified into ligand-based drug design (LBDD) and structure-based drug design (SBDD) [15]. In SBDD, it is essential to have a high-resolution crystal structure of the target macromolecule in complex with a bound ligand. The target protein should have a signal in a solution-state nuclear magnetic resonance interacting within a specified region, while structural information on the entire region of the target protein must be provided through X-ray crystallography [6, 16]. The α-synuclein lacks some of the basic information such as macromolecule in complex with a bound ligand, as stipulated above. Therefore, LBDD approach was employed in this study, using a diverse set of active ligands to generate pharmacophore model for accelerated screening of large compound databases [17]. To ease selection of chemical entities as potential therapeutic compounds, diverse parameters are employed to evaluate large number of ligands. A large number of molecular structures are evaluated according to diverse parameters to aid selection of the chemicals for synthesis, testing, and promote them with the goal to identify those with better chance to become potential therapeutics [17]. Therefore, breakdown of all binding affinity into its interaction components are necessary to assist in pharmacophore modelling and virtual screening of small molecules aimed at identification of effective therapeutic drugs for PD treatment [14].

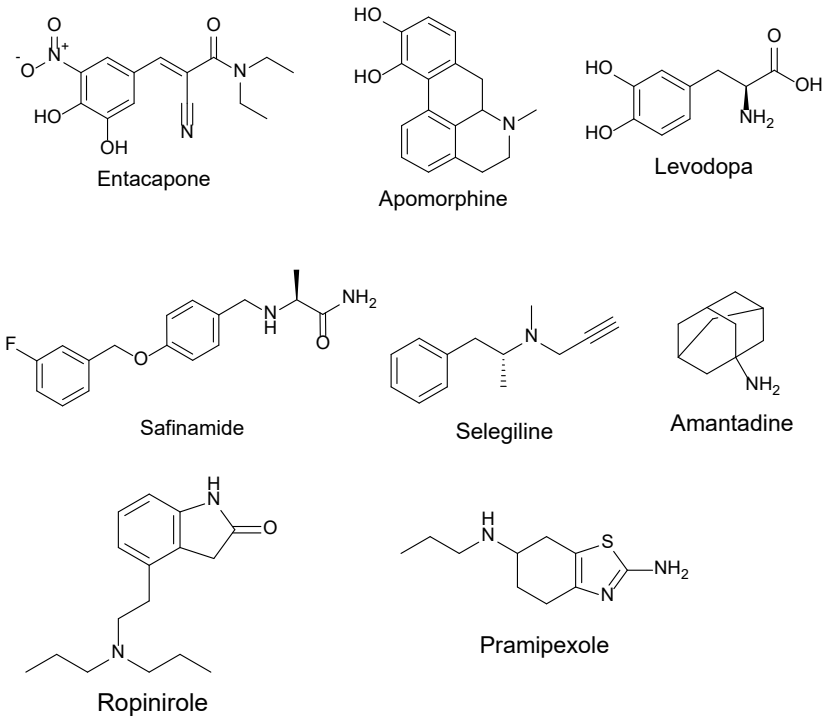

Figure 1. Some of the drugs use to manage Parkinson's disease symptoms

This research is aimed to develop ligand-based pharmacophore model having therapeutic inhibition against α-synuclein fibrillation that could selectively pick novel compounds from interbioscreen (IBS) database containing natural compounds (877,337), for developing anti-PD drugs with good physicochemical properties through ligand-based pharmacophore modelling, Glide standard precision and pk-CSM pharmacokinetics parameters.

2. EXPERIMENTAL DATA

All computational analyses were carried out on Dell Desk Top running on Windows 10 in high performance computer with Intel® Core Quad (TM) i7 processor, running on 8.00 GB and 64 bit operating system.

2.1 Generation of Ligand-based Pharmacophore

Available inhibitors of α-synuclein protein aggregation were collated from different literature [2, 13, 18-20], to generate ligand-based pharmacophore model using pharmacophore RDF code similarity embedded in LigandScout 4.4 software version, available from Intel Ligand, GmbH, Vienna, Austria (http://www.inteligand.com/ligandscout) [21]. Known inhibitors of the α-synuclein (Figure 2) were employed as training sets having minimum inhibitory concentrations (IC_{50}) between 0.2-4.8 μM ($\leq 0.2 > 5\mu M$). The structural formula and the smiles of the ligands were generated using ChemDraw Ultra 8.0 and reconverted into standard data-file (sdf) using molecular Operating Environments obtained from Chemical Computing Groups Inc. Pharmacophore features of the model were then generated by aligning most rigid molecules having same cluster-ID and highest cluster scores. Subsequently, an independent model was created comprising chemical features present in the training set only by applying OMEGA conformation modelling method, multiple acceptable comformers embedded in the LigandScout [21, 22]. Finally, an independent pharmacophore models were created. The pharmacophore model included only the chemical features present in all the training set compounds. Each model will be validated before chosen the best one.

2.2 Pharmacophore and Model Validation

Using successive Guner-Henry scoring functions, the selected pharmacophore model was validated. A robust model should display an optimum specificity and sensitivity for accuracy of the validation protocol. To detect active compounds, high SE is seen that can discriminate it from inactive ligands (decoys). The SP indicated error reduction to which distinguishes between active and inactive ligands. Here to discriminate between active and decoy compounds, the model generated consist of 16 actives and 328 decoy compounds. The decoy sets were generated from directorate of decoy finder (http://decoys.docking.org) [23]. Using the LigandScout internal generator database, the compounds were converted to ligand database format (.ldb) from sdf format. To efficiently validate the pharmacophore model generated, parameters such as enrichement factor (EF) and Guner-Henry's goodness of hits (GH) scores were determined for accuracy of screened hits. In addition, area under the curve (AUC 100%) was determined. Moreover, external validation filters, especially, decoy and receiver operating characteristics (ROC) were used as validation protocol. The validation parameters were expressed as follows:

$$GH = \frac{H_a(3A + H_t)}{4H_tA} + 1 - \frac{H_t - H_a}{D - A} \tag{1}$$

$$\%Y = \frac{H_a}{H_t} \times 100 \tag{2}$$

$$EF = \frac{H_a}{H_t} \div \frac{A}{D} \tag{3}$$

$$\%A = \frac{H_a}{A} \times 100 \tag{4}$$

where Y is percentage yield, A total actives, D total decoys (inactives), H_t is total hits and H_a is true positive respectively. It is important to note that the physicochemical properties of a decoy sets such as molecular weight (MW), hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), and number of rotatable bonds must be similar to the reported ligands [24-26].

2.3 Virtual Database Screening

Identification of potential hits that could selectively inhibit α-synuclein protein is crucial, here pharmacophore virtual screening was utilised [27]. The 3D query of the validated model was transferred to the screening perspective emdeded in the ligandScout to search for potential lead molecules from IBS database containing "877,337" natural compounds. The default parameters of the LigandScout were used for the screening to check for scoring functions, retrieval mode which indicates "pharmacophore fit" [21, 28].

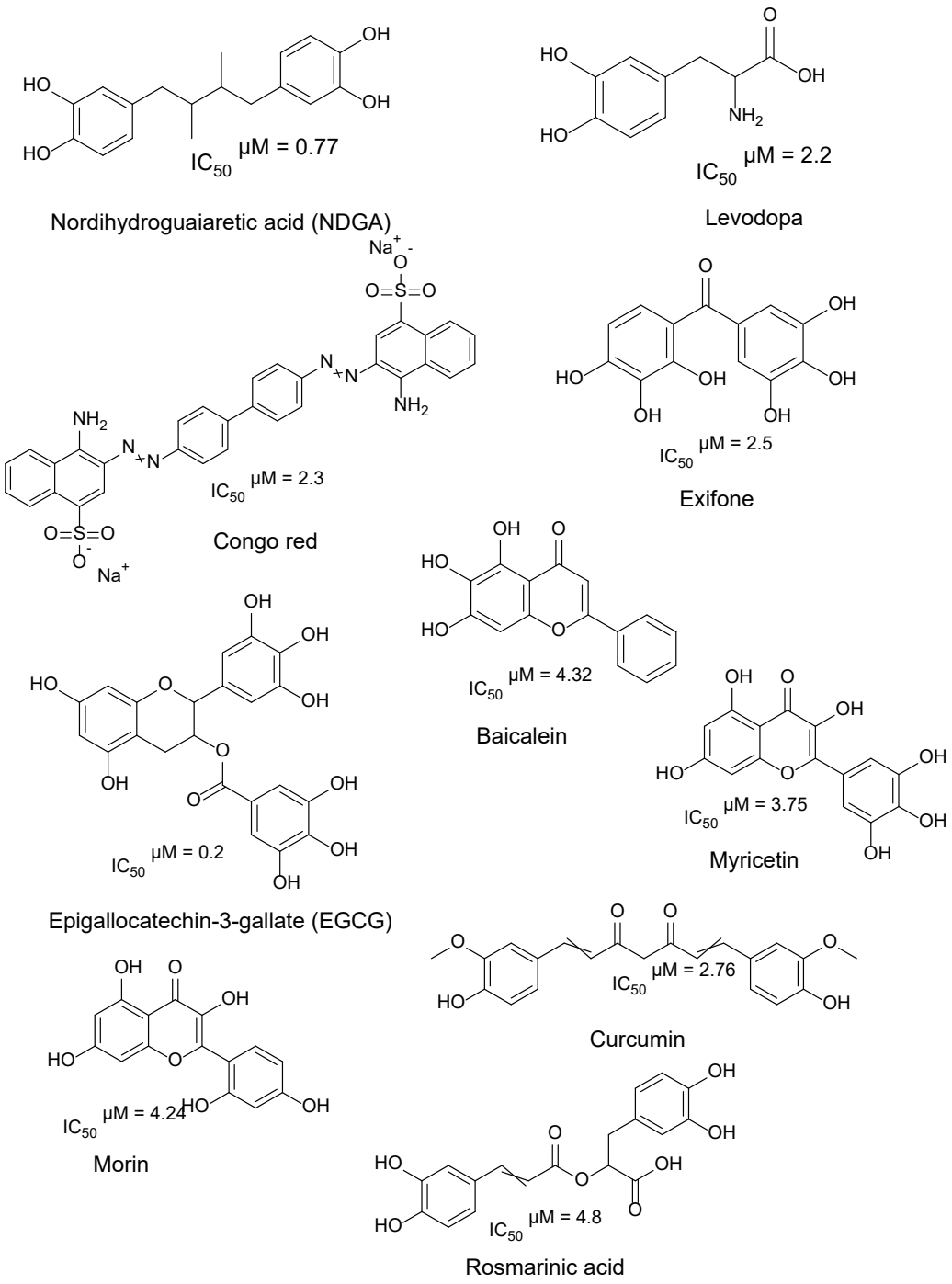

Figure 2. Molecular structures of 10 ligands used as training sets and their inhibitory concentrations

2.4 *In Silico* BBB Permeation and ADMET Studies of Clustering Candidates

It is essential to ensure that drugs reach the target sites adequately to elicit its pharmacological response. The predicted parameters were used to quantify ADMET properties of the potential hit compounds and such parameters include solubility (Logs), lipophilicity (LogP), molecular weight (MW), number of hydrogen bond acceptors (HBA), number of hydrogen bond donors (HBD), topological polar surface area (TPSA), and number of rotatable bonds indicating flexibility. Moreover, the Blood Brain Barrier (BBB) permeation together with ADMET studies of the hits were computed using *in silico* pkCSM-pharmacokinetics server [29]. The smiles string of the ligands was uploaded into the software and ADMET prediction parameters was run to obtain pharmacokinetics parameters such as *in vivo* absorption (water solubility in buffers, *in vivo* Cacao2 cell permeability for human colorectal cancer), distribution, BBB permeability and CNS penetration, metabolism including metabolic parameters using *in vivo* Cytochrome P450 substrate were all considered. Total clearance and toxicity studies were computed for the compounds intended for further development [29].

2.5 Protein Preparation

The structure of α-synuclein protein was obtained from RCSB protein data bank DOI: 10.2210/pdb1XQ8/pdb (1XQ8). The protein structure was prepared for docking using Maestro interface in Schrodinger software. Protein preparation Wizard was

applied aimed at checking the structures of the protein to correct assignment of protein, peptide bond orders, charges, add missing hydrogen atoms, ionization states, and optimize the structures of the protein [30]. OPLs2005 force field with implicit solvation was used for energy minimization of the protein, until an average root means square deviation (RMSD) of heavy atoms reached 0.3 Å. All hetero atoms of the PDB structure were removed, hydrogen atoms were added. Grid centre and dimensions of binding sites were identified [31-34].

2.6 Ligands Preparation

The 100 hit compounds obtained from the virtual screening, as well as the reference compound (Levodopa), and other known inhibitors of α-synuclein were visualized in Schrodinger software using maestro interface. The structures of the ligands were checked to correct the bond orders, add missing hydrogen atoms, and finally optimize the structures of the ligands.

2.7 Molecular Docking

Molecular docking panel was utilised in Maestro interface. The optimized and minimized α-synuclein structure was used in molecular docking study. Initially, the protein structure was loaded, followed by locating the active sites [34]. After identifying the active site, the ligands were loaded; and their energies were minimized using OPL2005 force field. Finally, the ligands were docked using Glide standard precision into the identified active site of the target protein and a maximum of 20 poses per ligand were retained. Ligands were ranked based on their docking scores, binding energies, and selectivity to α-synuclein. Those that display poses with the most negative binding energies (favourable binding) against α-synuclein were identified as the best poses. Levodopa was used as a standard for evaluating the affinity and selectivity of the hits.

2.8 Molecular Dynamics Simulations (MDS) Study

The top ranked ligand-receptor complexes having pass the ADMET prediction profiles was subjected to molecular dynamics simulation using the academic version of Desmond 2020-3 (Maestro-Desmond Interoperability Tools, Schrodinger, New York, NY, 2021) for 50 ns. The simulated complex's binding free energy was calculated using 50 ns using MD trajectories [35-37]. Several trajectories were produced within 50 ns and analysed using simulation interaction diagram tool kit [37, 38].

3. RESULTS

3.1 Ligand-based Pharmacophore Modelling

In this study, the pharmacophore model represents steric and electronic features of chemical compounds that ensure exact interactions with α-synuclein protein. The ligand-based pharmacophore (LBP) model was generated based on the pharmacophoric features present in the training set compounds selected from literature [2, 12, 19]. LigandScout inserted common pharmacophore properties for best-aligned solutions. Ten pharmacophore hypotheses were built with a tolerance scale of 1.0 and ranked following the Pharmacophore-Fit score [39]. The generated LBP (Figure 3A) model comprised of eleven pharmacophoric features (Figure 3B), six hydrogen bond acceptor (HBA), four hydrogen bond donor (HBD), and one aromatic group (AR).

3.2 Pharmacophore Model Validation Using GH Scoring

Following the generation of LBP, the model retrieved 50% of active compounds, while none of the inactive (decoys) was retrieved. The validation results (Table 1) suggest that the pharmacophore model recognized seven hits and all are true positives while predicting none from the decoy sets as false positive compounds. The statistical parameters calculated include total hits, H_t, false negatives (FN), false positive (FP), the goodness of hit score (GH), and enrichment factor (E-value) using the decoy set [40].

The generated model in this research has GH score of 0.87 and EF of 23.43, indicating a good model generated. The efficiency of the pharmacophore model was evaluated by Receiver Operating Characteristic (ROC) curve (Figure 4), which compares sensitivity versus selectivity, indicating good sensitivity to the actives from a large set of decoys against the target.

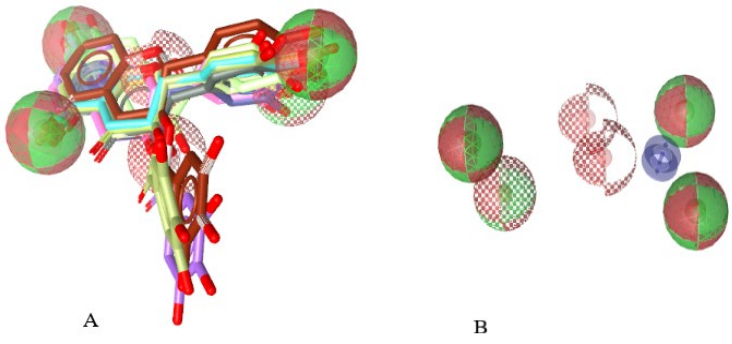

Figure 3. Indicating model with the aligned ligands (A) and features (B) of the generated ligand-based pharmacophore model for α-synuclein protein, consisting of six HBA (red), four HBD (green), and one AR (blue)

Table 1. Validation parameters of the shared feature pharmacophore model

S/N	Parameters	Value
1	Total molecules in database, D	342
2	Total number of actives in database, A	14
3	Total Hits, H_t	7
4	True Positive, TP	7
5	%Yield of activities, $(TP/H_t) \times 100$	100
6	True negatives, TN	0
7	False-negative, $FN = A - TP$	7
8	False-positive, $FP = H_t - TP$	0
9	Sensitivity, $SE = TP/A$	0.5
10	Specificity, $SP = TN/(D - A)$	0
11	Enrichment factor, $EF = (TP \times D)/(H_t \times A)$	23.43
12	Accuracy $= (TP + TN)/(TP + FP + TN + FN)$	0.5
13	Guner-Henry's goodness of hit scores, GH	0.87

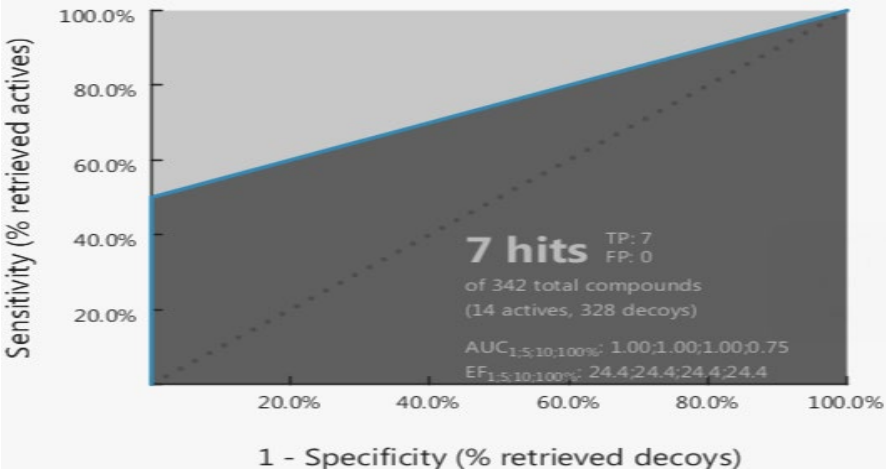

Figure 4. The ROC curve of the validated pharmacophore model

3.3 Pharmacophore-based Virtual Screening

Using pattern recognition, the 3D query of the pharmacophore model should match with conformation of a given molecule in the database. The pharmacophore model with features HHHHHHDDDDA was used as a query to screen chemical libraries of IBS database. The virtual screening resulted in screening a total of 100 hits matching the model.

3.4 *In Silico* BBB Permeation and ADMET Profiles of the Selected Hits

3.4.1 Assessments of Pharmacokinetics using ADMET

It is essential to ensure that drugs reach the target sites adequately, after absorption and then distributed through the body to elicit its pharmacological response [41]. According to the ADMET prediction results, the pharmacokinetic properties of the top five shortlisted compounds namely STOCK2S-85121, STOCK3S-13122, STOCK2S-57139, STOCK7S-07150, and STOCK4S-24924 were in desirable and acceptable range for drug-likeness (Table 2). The compounds demonstrated sufficient physicochemical properties for drug like molecules and fall within Lipinski's rule of 5. The selected hits have MW between 45-450, HBA < 10, LogP -1.2 to +1.7, TSPA 18-175 and rotatable bond between 0-9. Although, most of the hits have HBD >5, STOCK7S-07150 and STOCK4S-24924 have HBD < 5 which is quite like Levodopa. This indicates that the selected hits have potential physicochemical properties that could be considered for further evaluation as drugs [42].

In addition to chemical properties of selected compounds (Table 2), the pharmacokinetics, pharmacodynamics, and toxicological properties of the top 5 ranked ligands are presented in Table 3. This will help provide sufficient evidence on how the compound s could pass the BBB for as potential drugs for PD treatment.

Table 2. Structure and chemical properties of best five screened compounds

Descriptor	Compound name and values					
	STOCK2S-85121	STOCK3S-13122	STOCK2S-57139	STOCK7S-07150	STOCK4S-24924	Levodopa
Molecular weight[a]	372.337	414.418	334.292	383.436	45.041	197.19
LogP[b]	0.4995	1.6698	-1.1799	0.9764	0.0762	0.0522
#Rotatable bonds[c]	6	9	5	5	0	3
#Acceptors[d]	8	8	8	9	2	4
#Donors[e]	6	6	6	4	1	4
Surface area[f]	152.644	171.739	133.614	145.465	18.383	80.410
Lipinki's Rule of 5	0	0	0	0	0	0

[a]Molecular weight (acceptable range < 500), [b]Lipophilicity acceptable range 1.35-1.80), [c]Number of rotatable bonds (acceptable range ≤ 10), [d]Hydrogen bond acceptors (acceptable range ≤ 10), [e]Hydrogen bond donors (acceptable range < 5), [f]Surface area (acceptable range > 60 < 90 Å passive diffusion & 140 Å active diffusion.

Table 3. ADME properties of best five compounds from virtual screening

Compound	QPlogS[a]	IA[b]	Log VDss[c]	QPlogKp[d]	LogBB[e]	LogPS[f]
STOCK2S-85121	-2.887	54.951	-0.037	-2.736	-1.376	-3.75
STOCK3S-13122	-3.184	62.405	0.108	-2.735	-1.526	-3.54
STOCK2S-57139	-2.67	45.532	-0.426	-2.735	-1.895	-5.241
STOCK7S-07150	-2.675	68.497	0.06	-2.746	-1.615	-3.361
STOCK4S-24924	0.826	97.546	-0.229	-3.221	-0.331	-2.627

[a]Predicted aqueous solubility S in mol 1^{-1} (acceptable range -6.5 to 0.5), [b]Predicted Human intestinal absorption (acceptable range > 30%), [c]Predicted steady state volume of drug distribution (acceptable range -0.15 to 0.45), [d]Predicted skin permeability (acceptable range < -2.5), [e]Predicted blood-brain barrier permeability (acceptable range -1.0 to 0.3), [f]Predicted central nervous system permeability (acceptable range -3.0 to -2.0).

3.5 Molecular Docking Studies

The binding interactions of the selected hits against human α-synuclein protein were demonstrated, and the x-ray structure of the protein has co-ordinates of 228.13, 2.81 and -18.89 Å, within the x, y, and z and a length of 20 Å of the receptor grid box surface using ligand-receptor docking simulation. From the docking results (Table 4), the top hit compounds interact strongly with amino acid residues via hydrogen bonding compared to the standard compound, and this could be due to conformational flexibility of the compounds and the amino acid residues responsible for self-aggregation of the protein [43]. Finally, the docking analyses of this study suggested that the top best five compounds with better docking scores could be selected for further evaluations.

The binding interactions (Figure 5) between the selected compounds and amino acid residues further illustrated their inhibitory potentials via occupying similar sub-active cavities of the receptor. The AA residues between 64 and 100 are crucial for binding to α-synuclein protein [43].

Table 4. Amino acid interactions and binding energy of the best five compounds obtained from molecular docking

S/N	Ligand	Docking score	Binding energy (kcal/mol)	RMSD (Å)	Amino acid interactions
1	STOCK2S-85121	-4.789	-33.586	1.079	GLY101, LYS102, GLU104, GLU105
2	STOCK3S-13122	-4.451	-33.138	0.726	LYS102, ASN103, GLU104
3	STOCK2S-57139	-4.413	-28.249	2.129	LYS96, LYS97, LYS102
4	STOCK7S-07150	-4.365	-36.57	2.453	LYS97, LEU100
5	STOCK4S-24924	-4.277	-30.003	1.252	GLY101, LYS102, GLU104, GLU105
6	Levodopa	-3.556	-27.446	0.627	LYS96, LYS97 ASP98

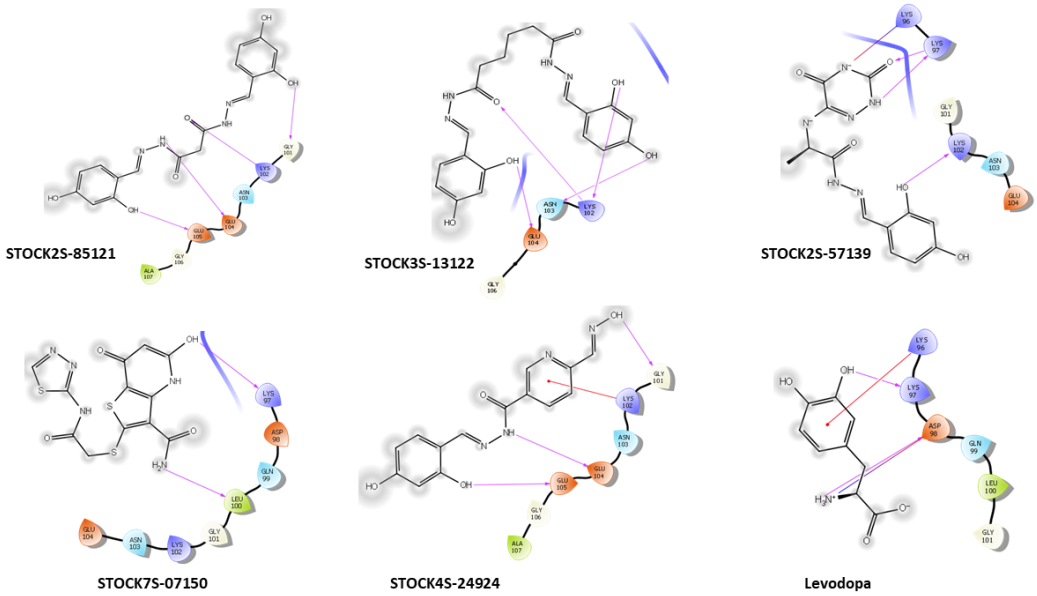

Figure 5. Binding poses of the best five hits showing bonding and nonbonding interactions of the selected ligands with amino acid residues. Hydrogen bonding (magenta arrow), π - cation (red line), salt bridge (blue line) and π - π stacking (green line)

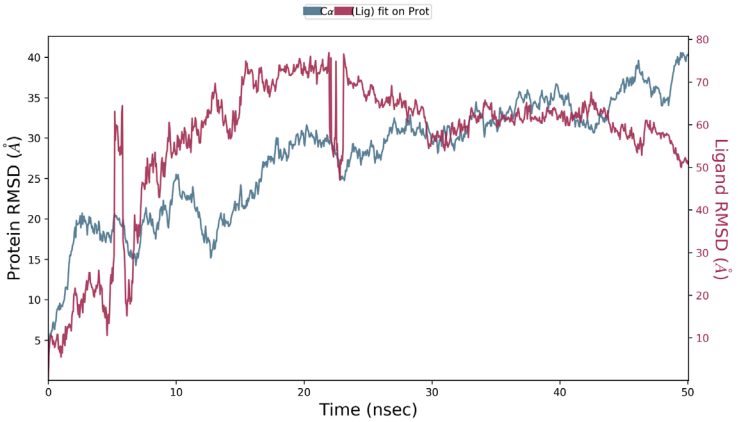

Figure 6. Time-dependent protein-ligand root mean square deviation (RMSD) plots (Angstrom)

3.6 Molecular Docking Studies

The thermodynamic stability of the selected ligand-receptor complex was studied using MDS. Although MD run of ≤ 10 ns is sufficient for premilitary study of protein-ligand complex in silico [44]. However, here 50 ns MDS was run to observe an independent biophysical and structural probe of the complex. The 2D graph of RMSD, RMSF and hydrogen bonds the top docked compound were generated and studied for understanding stability of the system complex in a native motion (Figures 6 and 7). The RMSD of the ligand (STOCK2S-85121), indicated initial fluctuation at around 2.5 Å, this demonstrates stability of the ligand having small RMSF. Furthermore, the ligand-protein complex showed favourable contacts of the ligands with Glu104, Glu101, and Lys102, where all amino acid residues indicate H-bond interactions which contributed to the binding affinity of the ligand (Figure 8).

4. DISCUSSION

The pharmacophore model was optimized by changing the size and number of features. Consequently, one of the pharmacophore hypotheses gave the best pharmacophoric features with reasonable specificity and selectivity as the LBP model. EF reflects sensitivity and specificity of a model generated and specifies concentration of the active compounds in the generated model relative to the decoy sets uses. GH score and EF are the most critical parameters for validation, the higher the EF, the higher the acceptability and validity of a model. Generally, EF value from 20 is considered good for validation. The GH scores ranges from 0 to 1, indicating null hypothesis and ideal model respectively. Moreover, a model with GH score from 0.6 and above is considered reliable for screening large set of databases. Many studies have reported a good model with GH score from 0.67 [45, 46]. Importantly, the model generated in this study has GH score of 0.87 and EF of 23.43, indicating validity and robustness of the model for screening.

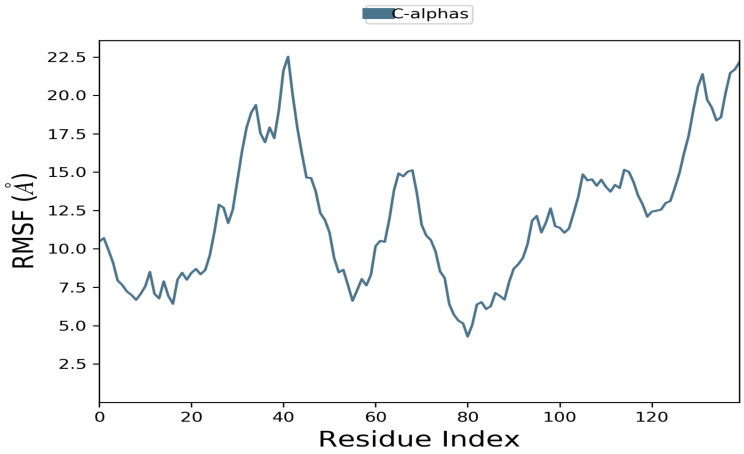

Figure 7. Time-dependent protein root mean square fluctuation (RMSF) plots (Angstrom)

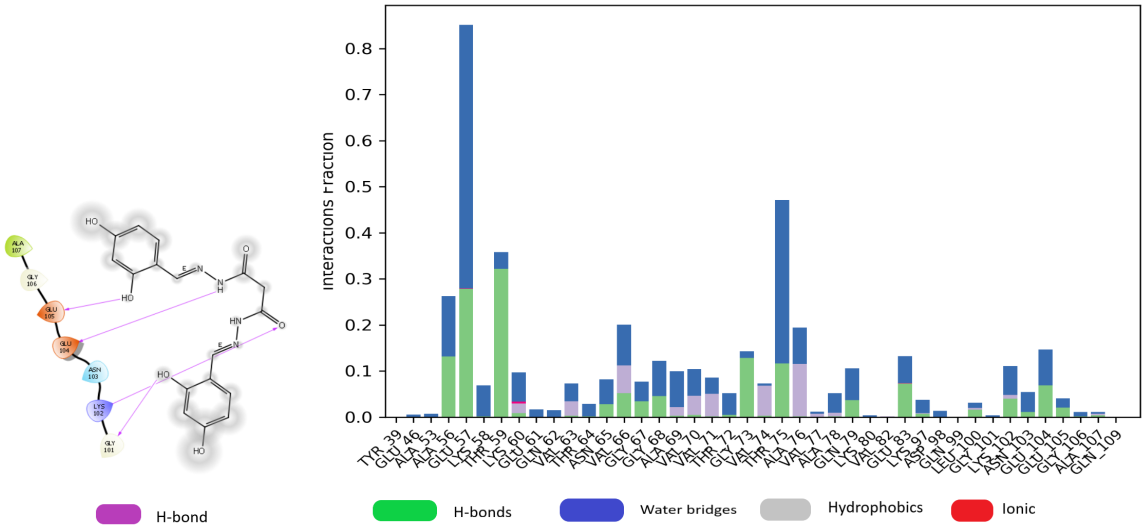

Figure 8. Simulation interactions diagram, (2D binding interaction of STOCK2S-85121 along with bar diagram indicating the fold of interaction, fraction, and contacts)

Compounds intended to be applied as potential anti-PD agents must be able to cross the BBB in the brain. Therefore, BBB permeability of drugs is a complex process that involves passive transcellular diffusion of molecules and contributions of active transport across the brain [47]. Compounds with MW > 500 Da, TSPA between <60-90 Å and above 150 Å are said to cross the BBB through passive and active diffusion respectively [47, 48]. The selected hits have their MW and TPSA falls within that range, comparable to the standard Levodopa. Moreover, most of the predicted values of the hit compounds were found to be within the acceptable range, indicating that the selected compounds could possess favourable pharmacokinetic properties [49]. Among the top hits, STOCK3S-13122 showed the highest volume of distribution (VDs) comparable to Levodopa followed by STOCK7S-07150. The VDs reveals the theoretical dose needed to distribute any drug in plasma and tissue. Moreover, STOCK4S-24924 indicated a better and favourable ability to cross the BBB and central nervous system (CNS) compared to other compounds, ultimately, revealing lipophilic superiority of the ligands compared to STOCK4S-24924, STOCK2S-85121, and STOCK7S-07150 respectively. Therefore, the compounds have potential to transit across the BBB in order to elicit their pharmacological effect. Additionally, the presence of tertiary nitrogen in the desired compound is a predictor indicating CNS penetration and a higher degree of permeability to BBB [49]. The compounds were predicted to have good total drug clearance, suggesting their inability to cause renal system toxicity.

Molecular docking study plays a vital key role in a selection of compounds *in silico*. It identifies correct conformation of ligands in relation to binding site at the protein and prediction of affinity between ligands and protein [41]. The α-synuclein protein is a small protein having 140 amino acid residues with no crystal structure [3]. Therefore, dopamine was used as a positive control while some decoy sets were used as negative control to validate the docking scores. The positive control compound has high docking score of -4.786 kcal/mol, while some decoys set (negative control) display docking scores of less than one. The docking score suggested that compounds such as STOCK2S-85121, STOCK3S-13122, and STOCK2S-57139, have produced good conformation of binding to the protein compare with the positive control. Other hit compounds also produce good docking scores against the receptor, thereby validating the docking protocols as good [50, 51]. Interestingly,

other known inhibitors (Thiazole-5-carboxamide, Epigallo catechin gallate) of α-synuclein are known to have activity against the protein and used to compare the docking score obtained from the hits and their binding poses. Currently, Levodopa is used to manage PD, thiazol-5-carboxamide is in phase II clinical trial while epicatechin produced the highest docking scores among known inhibitors of α-synuclein [52, 53].

From the binding poses (Figure 5), all ligands have strong H-bonding with Lys102 except for STOCK7S-07150 that interacted with Lys 97and Leu 100. Two of the selected ligands (STOCK2S-57139 and STOCK4S-24924) have pie cation and salt bridge bonding with Lys 102 and Lys 96, respectively, while other ligands expressed H-bonding only. The reference compound levodopa display interaction with AA Lys 96, and Lys 97 like STOCK2S-57139 and STOCK7S-07150. On the other hand, thiazole-5-carboxamide and STOCK2S-85121 had good hydrogen bonding interaction with Gly 101. Other selected ligands display interactions with other amino acid residues such as Gln 99, Asn 103, Glu104, Glu105 through strong H-bonding and Lys102 mainly through pie cation interaction, and this is a strong indication of electrostatic interactions for better inhibitory potentials against the receptor in references to the standards used [52]. Therefore, the top five highlighted ligands could serve as potent inhibitors of α-synuclein protein for further studies. Typically, it was observed that N- and C-terminals fluctuates more than the NAC-region, where secondary elements like alpha-helices and beta strands are more rigid. Thus, the NAC region fluctuates less due to its important for aggregation of the protein and subsequent formation of β-sheet structures essential for formation of fibrils [2, 7]. The essential H-bond interaction with STOCK2S-85121 support the potential inhibition of the compound against α-synuclein aggregation process.

5. CONCLUSION

In summary this study, succesfully built a pharmacophore model that consist of eleven features, including six HBA, four HBD, and one AR, with significant statistical parameters. The model generated was validated using GH scoring functions, this is indicated by the ability of the model to pick 100 hit compounds after performing 3D query to screen more than eight hundred thousand molecules, indicating powerful ability of the model to discriinate active ligands from decoy sets. We collected 100 similar analogs that matched the model from virtual screening using IBS database,. Top docked compounds demonstrated good docking scores, potential BBB permeability and ADMET profiles. Based on lipophilic properties, ability to cross the BBB and CNS, produce good conformation of binding to the protein, and displaying acceptable ADMET profiles, compounds STOCK2S-85121 could be the final compounds to be evaluated for further studies. MDS study of the compound suggested that comples was stable for 50 ns. The compound showed crucial H-bond interaction with Glu104, Lys102 residues at the C-termnal chain. Thus, it can be concluded that this molecule could be potential inhibitors of α-synuclein aggregation. This research shade more light on a better understanding of potential active inhibitors of α-synuclein aggregation and develop druggable compounds that could be translated upon further evaluations for treatment of synucleinopathies, especially Parkison's disease.

REFERENCES

[1] A. Kouli, K. M. Torsney and W. L. Kuan, Parkinson's Disease: Etiology, Neuropathology, and Pathogenesis. In: Stoker TB, Greenland JC, editors. *Parkinson's Disease: Pathogenesis and Clinical Aspects*. Brisbane (AU): Codon Publications, 2018.

[2] H. Liu, L. Chen, F. Zhou, Y. -X. Zhang, J. Xu, M. Xu, et al. Anti-oligomerization sheet molecules: Design, synthesis and evaluation of inhibitory activities against α-synuclein aggregation, *Bioorganic & Medicinal Chemistry*, 27(14), 2019, 3089-3096.

[3] H. Liu, L. Chen, F. Zhou, Y. -X. Zhang, J. Xu, M. Xu, et al. Anti-oligomerization sheet molecules: Design, synthesis and evaluation of inhibitory activities against α-synuclein aggregation, *Bioorganic & Medicinal Chemistry*, 27(14), 2019, 3089-3096.

[4] E. Mariani, M. C. Polidori, A. Cherubini and P. Mecocci, Oxidative stress in brain aging, neurodegenerative and vascular diseases: an overview, *Journal of Chromatography B, Analytical Technologies in the Bimedical and Life Sciences*, 827(1), 2015, 65-75.

[5] M. T. Ardah, K. E. Paleologou, G. Lv, S. B. Abul Khair, A. S. Kazim, S. T. Minhas, et al., Structure activity relationship of phenolic acid inhibitors of α-synuclein fibril formation and toxicity, *Frontiers in Aging Neuroscience*, 6, 2014, 197.

[6] F. E. Herrera, A. Chesi, K. E. Paleologou, A. Schmid, A. Munoz, M. Vendruscolo, et al., Inhibition of α-synuclein fibrillization by dopamine is mediated by interactions with five C-terminal residues and with E83 in the NAC region, *PLoS One*, 3(10), 2008, e3394.

[7] L. Breydo, J. W. Wu and V. N. Uversky, α-Synuclein misfolding and parkinson's disease, *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, 1822(2), 2012, 261-285.

[8] L. Yu, J. Cui, P. K. Padakanti, L. Engel, D. P. Bagchi, P. T. Kotzbauer, et al., Synthesis and in vitro evaluation of α-synuclein ligands, *Bioorganic & Medicinal Chemistry*, 20(15), 2012, 4625-4634.

[9] N. Török, Z. Majláth, L. Szalárdy and L. Vécsei, Investigational α-synuclein aggregation inhibitors: hope for Parkinson's disease, *Expert Opinion on Investigational Drugs*, 25(11), 2016, 1281-1294.

[10] I. Zahoor, A. Shafi and E. Haq, Pharmacological treatment of Parkinson's disease, *Exon Publications*, 2018, 129-44.

[11] S. S. Jafaripour, S. Gharaghani, E. Nazarshodeh, S. Haider and A. A. Saboury, In silico drug repositioning of FDA-approved drugs to predict new inhibitors for alpha-synuclein aggregation, *Computational Biology and Chemistry*, 88, 2020, 107308.

[12] R. Kumar, R. Bavi, M. G. Jo, V. Arulalapperumal, A. Baek and S. Rampogu, et al., New compounds identified through in silico approaches reduce the α-synuclein expression by inhibiting prolyl oligopeptidase in vitro, *Scientific Reports*, 7(1), 2017, 1-14.
[13] J. H. Lu, M. T. Ardah, S. S. K. Durairajan, L. F. Liu, L. X. Xie, W. F. D. Fong, et al., Baicalein inhibits formation of α-synuclein oligomers within living cells and prevents Aβ peptide fibrillation and oligomerisation, *Chembiochem*, 12(4), 2011, 615-624.
[14] K. K. Sahu, M. T. Woodside and J. A. Tuszynski, α-synuclein dimer structures found from computational simulations, *Biochimie*, 116, 2015, 133-140.
[15] S. Kamzolova, V. Sivozhelezov, A. Sorokin, T. Dzhelyadin, N. Ivanova and R. Polozov, RNA polymerase-promoter recognition. Specific features of electrostatic potential of "early" T4 phage DNA promoters, *Journal of Biomolecular Structure and Dynamics*, 18(3), 2000, 325-334.
[16] C. Charlier, G. Bouvignies, P. Pelupessy, A. Walrant, R. Marquant, M. Kozlov, et al., Structure and dynamics of an intrinsically disordered protein region that partially folds upon binding by chemical-exchange NMR, *Journal of the American Chemical Society*, 139(35), 2017, 12219-12227.
[17] V. Prachayasittikul, A. Worachartcheewan, W. Shoombuatong, N. Songtawee, S. Simeon, V. Prachayasittikul, et al., Computer-aided drug design of bioactive natural products, *Current Topics in Medicinal Chemistry*, 15(18), 2015, 1780-1800.
[18] M. Masuda, N. Suzuki, S. Taniguchi, T. Oikawa, T. Nonaka, T. Iwatsubo, et al., Small molecule inhibitors of α-synuclein filament assembly, *Biochemistry*, 45(19), 2006, 6085-6094.
[19] M. G. Tigan, A. Ghahghaei and M. Lagzian, In-vitro and in-silico investigation of protective mechanisms of crocin against E46K α-synuclein amyloid formation, *Molecular Biology Reports*, 46(4), 2019, 4279-4292.
[20] Y. Zhao, F. Ye, J. Xu, Q. Liao, L. Chen, W. Zhang, et al., Design, synthesis and evaluation of novel bivalent β-carboline derivatives as multifunctional agents for the treatment of Alzheimer's disease, *Bioorganic & Medicinal Chemistry*, 26(13), 2018, 3812-3824.
[21] G. Wolber and T. Langer, LigandScout: 3-D pharmacophores derived from protein-bound ligands and their use as virtual screening filters, *Journal of Chemical Information and Modeling*, 45(1), 2005, 160-169.
[22] G. Marcou and A. Varnek, Relational chemical databases: creation, management, and usage, *Tutorials in Chemoinformatics*, 2017, 37-66.
[23] C. M. Adrià, S. Garcia-Vallvé and G. Pujadas, DecoyFinder, a tool for finding decoy molecules, *Journal of Cheminformatics*, 4(1), 2012.
[24] L. Wang, X. Pang, Y. Li, Z. Zhang and W. Tan, RADER: a RApid DEcoy Retriever to facilitate decoy based assessment of virtual screening, *Bioinformatics*, 33(8), 2017, 1235-1237.
[25] R. Pascual, C. Almansa, C. Plata-Salamán and J. M. Vela, A new pharmacophore model for the design of sigma-1 ligands validated on a large experimental dataset, *Frontiers in Pharmacology*, 10(519), 2019.
[26] I. Wallach and R. Lilien, Virtual decoy sets for molecular docking benchmarks, *Journal of Chemical Information and Modeling*, 51(2), 2011, 196-202.
[27] M. M. Niu, J. Y. Qin, C. P. Tian, X. H. Yan, F. G. Dong, Z. Q. Cheng, et al., Tubulin inhibitors: pharmacophore modeling, virtual screening and molecular docking, *Acta Pharmacologica Sinica*, 35(7), 2014, 967-979.
[28] V. Temml, U. Garscha, E. Romp, G. Schubert, J. Gerstmeier, Z. Kutil, et al., Discovery of the first dual inhibitor of the 5-lipoxygenase-activating protein and soluble epoxide hydrolase using pharmacophore-based virtual screening, *Scientific Reports*, 7(1), 2017, 1-8.
[29] D. E. Pires, T. L. Blundell and D. B. Ascher, pkCSM: predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures, *Journal of Medicinal Chemistry*, 58(9), 2015, 4066-4072.
[30] T. S. Ulmer, A. Bax, N. B. Cole and R. L. Nussbaum, Structure and dynamics of micelle-bound human α-synuclein, *Journal of Biological Chemistry*, 280(10), 2005, 9595-9603.
[31] R. L. Jayaraj and N. Elangovan, In silico identification of potent inhibitors of alpha-synuclein aggregation and its in vivo evaluation using MPTP induced Parkinson mice model, *Biomedicine & Aging Pathology*, 4(2), 2014, 147-152.
[32] R. L. Jayaraj, V. Ranjani, K. Manigandan and N. Elangovan, In silico docking studies to identify potent inhibitors of alpha-synuclein aggregation in Parkinson disease, *Asian Journal of Pharmaceutical and Clinical Research*, 6(4), 2013, 127-131.
[33] K. Jenkins, T. Mateeva, I. Szabó, A. Melnik, P. Picotti, A. Csikász-Nagy, et al., Combining data integration and molecular dynamics for target identification in α-Synuclein-aggregating neurodegenerative diseases: Structural insights on Synaptojanin-1 (Synj1), *Computational and Structural Biotechnology Journal*, 18, 2020, 1032-1042.
[34] T. Mohankumar, V. Chandramohan, H. S. Lalithamba, R. L. Jayaraj, P. Kumaradhas, M. Sivanandam, et al., Design and molecular dynamic investigations of 7, 8-dihydroxyflavone derivatives as potential neuroprotective agents against alpha-synuclein, *Scientific Reports*, 10(1), 2020, 1-10.
[35] I. Ahmad, H. Jadhav, Y. Shinde, V. Jagtap, R. Girase and H. Patel, Optimizing Bedaquiline for cardiotoxicity by structure based virtual screening, DFT analysis and molecular dynamic simulation studies to identify selective MDR-TB inhibitors, *In Silico Pharmacology*, 9(1), 2021, 23.
[36] W. L. Jorgensen, D. S. Maxwell, J. Tirado-Rives, Development and testing of the OPLS all-atom force field on conformational energetics and properties of organic liquids, *Journal of the American Chemical Society*, 118(45), 1996, 11225-11236.
[37] H. M. Patel, I. Ahmad, R. Pawara, M. Shaikh and S. Surana, In silico search of triple mutant T790M/C797S allosteric inhibitors to conquer acquired resistance problem in non-small cell lung cancer (NSCLC): a combined approach of

structure-based virtual screening and molecular dynamics simulation, *Journal of Biomolecular Structure & Dynamics*, 39(4), 2021, 1491-1505.

[38] G. Kalibaeva, M. Ferrario and G. Ciccotti, Constant pressure-constant temperature molecular dynamics: a correct constrained NPT ensemble using the molecular virial, *Molecular Physics*, 101(6), 2003, 765-778.

[39] M. C. Gidaro, S. Alcaro, D. Secci, D. Rivanera, A. Mollica, M. Agamennone, et al., Identification of new anti-Candida compounds by ligand-based pharmacophore virtual screening, *Journal of Enzyme Inhibition and Medicinal Chemistry*, 31(6), 2016, 1703-1706.

[40] A. Vuorinen, R. Engeli, A. Meyer, F. Bachmann, U. J. Griesser, D. Schuster, et al., Ligand-based pharmacophore modeling and virtual screening for the discovery of novel 17β-hydroxysteroid dehydrogenase 2 inhibitors, *Journal of Medicinal Chemistry*, 57(14), 2014, 5995-6007.

[41] T. Sindhu, T. Venkatesan, G. R. Gracy, S. K. Jalali and A. Rai, Exploring the resistance-developing mutations on Ryanodine receptor in diamondback moth and binding mechanism of its activators using computational study, *Biochemical Engineering Journal*, 121, 2017, 59-72.

[42] D. E. Pires, T. L. Blundell and D. B. Ascher, pkCSM: predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures, *Journal of Medicinal Chemistry*, 58(9), 2015, 4066-4072.

[43] O. M. El-Agnaf, K. E. Paleologou, B. Greer, A. M. Abogrein, J. E. King, S. A. Salem, et al., A strategy for designing inhibitors of α-synuclein aggregation and toxicity as a novel treatment for Parkinson's disease and related disorders, *The FASEB Journal*, 18(11), 2004, 1315-1317.

[44] N. D. Gajjar, T. M. Dhameliya and G. B. Shah, In search of RdRp and Mpro inhibitors against SARS CoV-2: Molecular docking, molecular dynamic simulations and ADMET analysis, *Journal of Molecular Structure*, 1239, 2021, 130488.

[45] S. John, S. Thangapandian, S. Sakkiah and K. W. Lee, Potent BACE-1 inhibitor design using pharmacophore modeling, in silico screening and molecular docking studies, *BMC Bioinformatics*, 12 (Suppl 1), 2011, S28.

[46] R. Kumar, R. Bavi, M. G. Jo, V. Arulalapperumal, A. Baek, S. Rampogu, et al., New compounds identified through in silico approaches reduce the α-synuclein expression by inhibiting prolyl oligopeptidase in vitro, *Scientific Reports*, 7(1), 2017, 10827.

[47] C. Suenderhauf, F. Hammann and J. Huwyler, Computational prediction of blood-brain barrier permeability using decision tree induction, *Molecules*, 17(9), 2012, 10429-10445.

[48] U. Norinder and M. Haeberlein, Computational approaches to the prediction of the blood–brain distribution, *Advanced Drug Delivery Reviews*, 54(3), 2002, 291-313.

[49] L. Ioakimidis, L. Thoukydidis, A. Mirza, S. Naeem and J. Reynisson, Benchmarking the reliability of QikProp. correlation between experimental and predicted values, *QSAR & Combinatorial Science*, 27(4), 2008, 445-456.

[50] A. Castro-Alvarez, A. M. Costa and J. Vilarrasa, The performance of several docking programs at reproducing protein-macrolide-like crystal structures, *Molecules*, 22(1), 2017.

[51] D. Ramirez and J. Caballero, Is it reliable to take the molecular docking top scoring position as the best solution without considering available structural data?, *Molecules*, 23(5), 2018.

[52] M. Deffains, M. H. Canron, M. Teil, Q. Li, B. Dehay, E. Bezard, et al., L-DOPA regulates α-synuclein accumulation in experimental parkinsonism, *Neuropathology and Applied Neurobiology*, 47(4), 2021, 532-543.

[53] J. Joubert, Bicyclic bis-heteroaryl derivatives as inhibitors of the α-synuclein protein: a patent evaluation of WO2018138088A1, *Expert Opinion on Therapeutic Patents*, 28(12), 2018, 939-945.