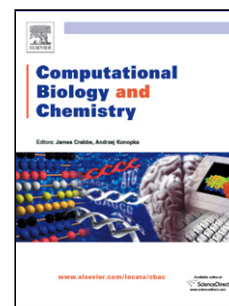# Journal Pre-proof

In silico drug repositioning of FDA-approved drugs to predict new inhibitors for alpha-synuclein aggregation

Sedighe Sadat Jafaripour (Conceptualization) (Methodology) (Software) (Data curation) (Writing - original draft), Sajjad Gharaghani (Visualization) (Investigation) (Supervision) (Writing - review and editing), Elmira Nazarshodeh (Software), Shozeb Haider (Software) (Validation) (Writing - review and editing), Ali Akbar Saboury (Visualization) (Investigation) (Supervision) (Writing - review and editing)

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In silico drug repositioning of FDA-approved drugs to predict new inhibitors for alpha-synuclein aggregation

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Graphical abstract
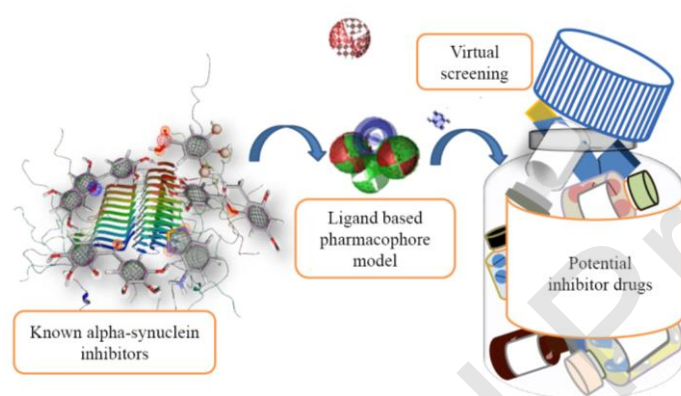
Highlights

- 3D ligand-based pharmacophore model for alpha- synuclein inhibition generated pharmacophore model consists of three HBD, two HBA and two AR.
- Several new compounds identified as potential alpha- synuclein inhibitors. The first report to identify drugs for repositioning against alpha- synuclein aggregation inhibition

One of the hallmarks of Parkinson's disease (PD), a long-term neurodegenerative syndrome, is the accumulation of alpha-synuclein (α-syn) fibrils. Despite numerous studies and efforts, inhibition of α-syn protein aggregation is still a challenge. To overcome this issue, we propose an *in silico* pharmacophore-based repositioning strategy, to find a pharmaceutical drug that, in addition to their defined role, can be used to prevent aggregation of the α-syn protein. Ligand-based pharmacophore modeling was developed and the best model was selected with validation parameters including 72% sensitivity, 98% specificity and goodness score about 0.7. The optimal model has three groups of

hydrogen bond donor (HBD), three groups of hydrogen bond acceptor (HBA), and two aromatic rings (AR). The FDA-Approved reports in the ZINC15 database were screened with the pharmacophore model taken from inhibitor compounds. The model identified 22 hits, as promising candidate drugs for Parkinson's therapy. It is noteworthy that among these, 10 drugs have been reported to inhibition of α-syn aggregation or treat/reduce Parkinson's pathogenesis. This model was used to virtual screen ZINC, NCI databases, and natural products from the pomegranate. The results of this screen were filtered for their inability to cross the blood-brain barrier, poor oral bioavailability, etc. Finally, the selected compounds of two ZINC and NCI databases were combined and structurally clustered. Remained compounds were clustered in 28 different clusters, and the 17 compounds were introduced as final candidates.

Introduction

Parkinson's disease (PD) is a neurodegenerative disease, which has a direct relationship with aging. PD can be seen in patients with delayed voluntary movement. Studies showed that neurodegenerative diseases will become the second disease-related causes of human mortality after cardiovascular disease by 2040 (Valera and Masliah, 2016). Researchers have been trying to develop drugs for complementary treatment or at least for control of this disease (Kalia et al., 2013) (Naeem et al, 2018). A characteristic of PD is the accumulation of spherical insoluble bodies called the Lewy bodies around nerve cells, which filled with the aggregation of α-Synuclein (α-syn) proteins. α-syn is an abundant presynaptic brain protein, which belongs to the family of Intrinsically disordered proteins or intrinsically disordered regions (IDPs or IDRs), (Breydo et al., 2012), that lack a rigid 3D structure under physiological conditions. These proteins adopt a variety of structural conformations upon interaction with different partners and play crucial biological roles in cellular processes (Daughdrill et al., 2005)(Uversky et al., 2008).

There is no doubt that strategy, which can inhibit or disrupt aggregation, can be effective routes for treatment or prevention of PD and thereby contributing to increased longevity of life. Thus the discovery of compounds that can serve as strong inhibitors and delay α-syn aggregation remains an important challenge. For this purpose, significant research has been done to identify α-syn fibrillation inhibitor compounds. To the best of our knowledge, more than 60 small molecule inhibitors against α-syn aggregation have been reported (Ardah et al., 2014a)(Caruana et al., 2011a)(Masuda et al., 2006b). Nevertheless, experimental studies to find successful candidate compounds are limited while conducting tests for all existing compounds is time-consuming and not cost-effective. Also, each of the reported compounds has substructures that further limit their application for therapeutic purposes.

Nowadays, virtual screening has become an essential part of the drug discovery process that endeavors to reduce time, costs, and removal of drug candidates with poor absorption, distribution, metabolism, elimination, and toxicity (*ADMET*) properties at the early stage before their synthesis. In general, this method tries to lead optimization by reducing laboratory tests. In rational drug design, the pharmacokinetic properties of compounds will be used to describe their inhibitory characteristics. The recent concept of 'polypharmacology', which proposes that drugs work mostly by acting on multiple targets, is being increasingly accepted (Macdonald et al., 2006)(Xie et al., 2012). Furthermore, polypharmacological features could help to find new uses of existing drugs via drug repositioning (Ashburn and Thor, 2004). Eventually, these approaches can help to find drugs for new targets, in addition to their well-established role.

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In this manuscript, we report a pharmacophore-based drug-repositioning method for drug discovery. To achieve this goal we built a pharmacophore model of α-syn fibrillation inhibitors. This pharmacophore model used as a template to screen various databases such as NCI (290000 molecules), ZINC (Irwin and Shoichet, 2005) (270000 compounds), natural product compounds from pomegranate, a fruit that has remarkable antioxidant effects and FDA approved drugs ("Pomegranate and its Many Functional Components as Related to Human Health : A Review," 2010).

Materials and methods

Data collection

To create a ligand-based pharmacophore model, we collected all α-syn aggregation inhibitors that are publicly available. A dataset was obtained containing 60 reported inhibitor combinations belonging to various chemical structures, 8 clusters, using the Pharmacophore RDF-Code similarity implemented in ligand Scout and the cluster distance was set to 0.4 with average calculation method. This set has diverse activities from 61 nm-< 80 μm. The structure of the molecules was taken from the PubChem. Compounds were divided into two groups: strong and weak, with an IC_{50} value of about 10 μM and those between 40-80 μM, respectively (Masuda et al., 2006a) (S1, S2 ad S3).

Pharmacophore model generation

Pharmacophore can be modeled based on two methods i.e. structure-based or ligand-based (Angelbello et al., 2016). To create a structure-based model, the three-dimensional structure of α-syn is required. The conformational space of IDPs is extremely challenging. This is because they lack a unique structure and in turn can adapt to different complexes in interaction with partners. In solution-state NMR, signals of the IDR in the interacting region becomes unrecognizable, while X-ray crystallography gives structural information on entirely ordered regions (Charlier et al., 2017). For this reason, we ignored the structure-based pharmacophore-model and explored the ligand-based method instead. Ligand-based pharmacophore model carried out using default settings of Ligand Scout 4.2 software available from InteLigand, GmbH, Vienna, Austria (http://www.inteligand.com/ligandscout)(Wolber and Langer, 2005a).

Pharmacophore model validation

The predictive performance of the models was evaluated by validation filters. The validation process usually helps to optimize or refine the model. A good model displays significant sensitivity and specificity. High sensitivity makes it possible to detect active compounds among the many inactive ones that are present in the group. The specificity reduces the error in distinguishing active

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compounds from the inactive. These two parameters are complementary and work in tandem. An ideal model has an optimal balance between these two parameters. The scoring function or the 'Fit scores' is also indicative of how functional groups of the compounds are matched with the pharmacophore model. The external validation approaches are test set/ decoy set and receiver operating characteristics (ROC) analysis is also considered as a signal-noise ratio.

In the test set method, 58 molecules with reported experimental activity values from 61 nm to >80 µm used to detect the predictive ability of the pharmacophore model (the results shown in Table 1). In the decoy set validation, a decoy database including 352 compounds generated by the virtual decoy set method (Wallach and Lilien, 2011). Physicochemical characteristics (molecular weight, hydrogen bond donor and acceptor count, number of rotatable bonds, the octanol/water partition coefficient) of the decoy were similar to the 18 reported active compounds, but spatial arrangements were different and calculated according to the Tanimoto coefficient (Wallach and Lilien, 2011).

Database screening
The model with the best scores in validation was used as a template for searching against various databases to find molecules that can potentially have an inhibitory effect. The screening was done using Ligand- Scout's default parameters: the scoring function, the retrieval mode, and the screening mode were set to "pharmacophore-fit", "Stop after first matching conformation" and "match all query features" respectively. The maximum number of omitted features was set to zero and check exclusion volume turned off. (Wolber and Langer, 2005b)(Temml et al., 2017).

Drug-likeness selection and clustering of candidates
At this stage, selected hits from NCI and ZINC (Irwin and Shoichet, 2005) databases were screened and covalent inhibitors, duplicate compounds, CNS (molecular mass ≤ 582 Da; the number of hydrogen bonding donors ≤ 3, number of hydrogen bond acceptors ≤ 5 and octanol-water partition coefficient (log P) ≤ 6) and "Pan Assay Interference Compounds" (PAINS) (false positive molecules) were identified and removed. The FAFdrug3 (Lagorce et al., 2015) online server (*http://fafdrugs3.mti.univ-paris-diderot.fr*) was engaged to check the described properties of candidate ligands.

Dataset for drug repositioning
The optimal pharmacophore model is used as a template for screening 1448 Food and Drug Administration approved (FDA-approved) drugs from the ZINC15 database (*http://ZINC15.docking.org*) (Sterling and Irwin, 2015).

Molecular docking

A molecular docking study was performed to investigate the binding affinity of the alpha-synuclein with the screened molecule. The 3D NMR structure of alpha-synuclein of Homo sapiens, 1XQ8, was taken from the protein data bank. We used to MTiAutoDock 1.0, a free online (*http://bioserv.rpbs.univ-paris-diderot.fr/services/MTiOpenScreen/*) and open-source protein-ligand docking *tool that* it is based on the AutoDock 4.2 and MGLTools. All HETATMs of the PDB structure were removed, hydrogen atoms were added and grid center and dimensions of binding sites were identified (Labbé et al., 2015).

The top 10 ranked poses are given as output and the pose with minimum energy was detected.

Results and discussion

Pharmacophore model generation

The structures of 60 α-syn aggregation inhibitors were gathered from the literature (Caruana et al., 2011b)(Ardah et al., 2014b)(Masuda et al., 2006b). Ligands set conformers were produced using the default settings of OMEGA, while the RMSD threshold was set to 0.7. Ligand Scout was used to generating conformations, which built a maximum of 500 conformers per molecule. Pharmacophore models were created with different training set groups. Ligand Scout inserted common pharmacophore properties for the best-aligned solutions. 10 pharmacophore hypotheses were built with a tolerance scale of 1.0 and ranked following the Pharmacophore-Fit score (Gidaro et al., 2016). The applied scoring function was the ''Pharmacophore-Fit score and Atom Overlap" and the chosen pharmacophore type was the "shared" pharmacophore features. The pharmacophore model was optimized by changing the size and number of features. Finally, the model with the training group including Gallic acid and Exifone (belong to two different structural clusters) was introduced as an optimal model and is illustrated in Fig. 1. Gallic acid is a known potent inhibitor of α-syn aggregation, with inhibitory activity of about 90% (Ardah et al., 2014b).
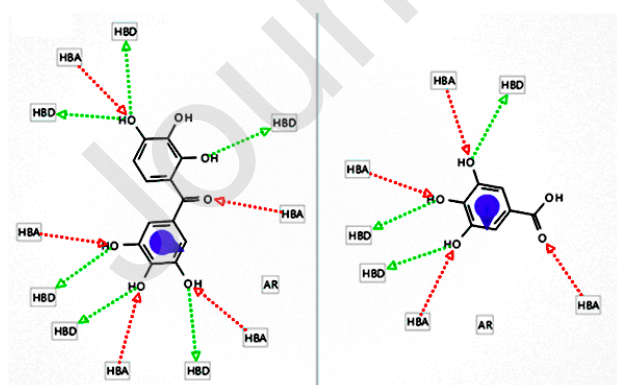

Figure 1. Chemical structures and Pharmacophore model of compounds Exifone(left) and gallic acid()
(training set).

The features and the tolerance radii (Å) of the optimum model are shown in Fig. 2 and consist of two HBA, three HBD, and two AR.
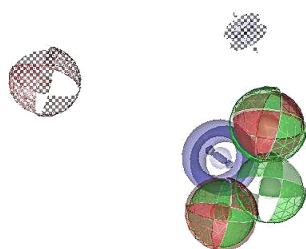

Figure2. Generated a ligand-based Pharmacophore model against alpha-synuclein aggregation that consists of three HBA (red), three HBD (green), two AR (blue) features.

Evaluating the performance of the pharmacophore model

This model has been validated by using test sets and decoy set methods, which display an extremely improved hit rate. Statistical parameters were calculated using the decoy set. These parameters include Total Hits (Ht), false negatives (FN), false positive (FP), goodness-of-hit score (GH), and enrichment factor (E-value) (Vuorinen et al., 2014). The statistical parameters obtained from the decoy test are listed in Table 1. The main parameters, which play a significant role in recognizing the ability of the pharmacophore models, are E-value and GH score.

Table 1. Statistical parameters obtained from decoy test

No	Parameters	Value
1	Total Molecules in Database (D)	370
2	Total Number of Actives in Database (A)	18
3	Total Hits (Ht)	19
4	True positives (TP)	13
5	% Yield of actives $\lceil$ = {(TP/Ht)100}	68.4%
6	True negative (TN)	346
7	False negatives (FN) = [A-TP]	5
8	False positives (FP)= [Ht-TP]	6
9	Sensitivity = TP/A	0.72
10	Specificity = TN/D-A	0.98
11	Enrichment factor ((E-value))={(TP$_x$D)/(Ht$_x$A)}	14.06
12	Accuracy = (TP+TN)/(TP+TN+FP+FN)	0.97
13	Goodness of Hit Score (GHScore): [(TP/4HtA)(3A + Ht)(1 _ (Ht _ TP)/(D _ A))]	0.68

 The model with a GH score of ~0.7 is very good (Niu et al., 2014). The efficiency of each pharmacophore model was evaluated by a ROC curve, which compares sensitivity versus the

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specificity (Fig. 3). This efficiency is based on their ability to selectively obtain various active compounds from a large list of decoys or inactive against the selected targets.
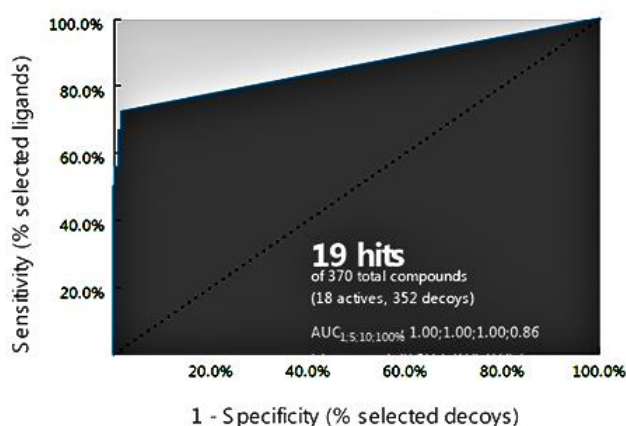

Figure 2. ROC Validation Curve for the Pharmacophore model

Pharmacophore-based drug repositioning

The ligand-based pharmacophore model identified 22 drugs that can be potential drug repositioning candidates (Table 2). The inhibitory effect of the drugs 2,3,4,15 and 20, which include Entacapone, Tolcapone, (Giovanni et al., 2010), Pyridoxine, Levodopa and Rifampicin (Török et al., 2016)(Li et al., 2004) respectively, have been previously used to study fibrillation of α-syn. These drugs are used today to control the fibrillation of α-syn and prevent the progression of Parkinson's disease. Drugs 8, 9, 10, 14, and 16 (Epinephrine, Isoproterenol, Dopamine, Dobutamine, Nordefrin) are derivatives of dopamine that have shown inhibitory effect against α-syn fibrillation (Conway et al., 2016). Interestingly, among these drug candidates, Carbidopa and Fenoldopam were suggested in previous studies to control the side effects of Parkinson's disease (Martino et al., 2016)(Twery, 1994). However, the effect of these drugs, as an inhibitor of fibrillation of α-syn has not been reported. Also, it has recently been shown that asthma drugs can be considered for regulating and influencing the SNCA gene to control Parkinson's disease (Ridler, 2017). Interestingly, drugs No. 5, 6 (Salbutamol, and Formoterol) that were identified are two asthma drugs. Of the 22 candidates identified for drug repurposing, the inhibitory activity of 10 drugs has been previously reported. Therefore, the remaining candidate compounds, which include drugs 1, 5, 6, 7, 11, 12, 13, 17, 18, 19, 21, and 22, can be potential inhibitors of α-syn and also for the treatment of Parkinson's disease.

Table 2.virtual screening of ZINC15 database with the pharmacophore model

No.	Name	Fit Score	ZINC15_id	**Energy (Kcal/mol)*** or Docking score*	Class	Water solubility (mg/L)
1						
	FENOLDOPAM	74.94	ZINC 000022116612	-8.49	Benzazepines	4000
2						
	ENTACAPONE (comtan)	66.6	ZINC 000035342787	-8.63	Cinnamic acids and derivatives	_
3						
	TOLCAPONE (tasmar)	66.6	ZINC 000035342789	-8.71	Benzene and substituted derivatives	_
4						
	PYRIDOXINE	65.67	ZINC 000000049154	-8.74	Pyridines and derivatives	2.2E+ 005
5						
	Salbutamol (ALBUTEROL)	65.39	ZINC 00000007601	-8.54	Benzene and substitued derivaties	1.41E+ 004
6						

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7	FORMOTEROL	65.08	ZINC 000000000856	-8.67	Benzene and substituted derivatives	Slightly
8	NORADRENALINE (Adrenor)	64.97	ZINC 000000057624	-8.68	Phenols	_
9	EPINEPHRINE	64.89	ZINC 000000039089	-8.68	Phenols	less than 100 (at 64° F)
10	ISOPROTERENOL	64.89	ZINC 000000056652	-8.69	Phenols	_
11	DOPAMINE	64.82	ZINC 000000033882	-8.71	Phenols	0.6
12	METHYLDOPA (RACEMIC)	64.81	ZINC 000000020255	-8.73	Phenylpropanoic acids	1E+004
	TIPIRACIL	64.8	ZINC 000100032379	-8.71	Diazines	5000 (warmed)

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13						
	CARBIDOPA	64.56	ZINC 000019168887	-8.45	Phenylpropano ic acids	3.8
14						
	DOBUTAMINE	64.56	ZINC 000000003911	-8.69	Phenols	_
15						
	LEVODOPA	64.56	ZINC 000000895199	-8.64	Carboxylic acids and derivatives	5000 (at 20 °C)
16						
	NORDEFRIN	64.56	ZINC 000000034157	-8.64	Benzene and substitued derivaties	_
17						
	L-Dops	64.51	ZINC 000002015035	-8.67	Carboxylic acids and derivatives	_
18						
	CEFDINIR	64.44	ZINC 000003927198	-8.48	Lactams	_
19						

	Name	Fit score	ZINC ID	Docking*	Class	
20	RIFAXIMIN	64.33		-8.72	Macrolactams	_
21	RIFAMPICIN	64.32	ZINC 000169621223	-8.59	Macrolactams	1400 (at 25 °C)
22	METAPROTERENOL (orciprenaline)	64	ZINC 000000002273	-8.62	Phenols	9.7
	TERBUTALINE	63.88	ZINC 000000002281	-8.73	Phenols	213000

* : binding affinity (docking) values of the hit compounds

Database screening

The results obtained from the mentioned analyses (model validation and pharmacophore-based drug repositioning) show that the validation of the model is sufficient enough for using it to screen other databases. Therefore, three databases were used for screening to identify new potential active compounds against α-syn fibrillation. Hence, the validated pharmacophore model was applied as a template to search the NCI (*https://www.cancer.gov/*), ZINC (*ZINC.docking.org/*) databases (Table 3) and natural products from the Pomegranate fruit because of its unique characteristics ("Pomegranate and its Many Functional Components as Related to Human Health : A Review," 2010) (which contains 290981, 271910, and 22 molecules, respectively). Results of screening natural products from pomegranate fruit are shown in Table 4. 11 hits were identified from the pomegranate fruit compounds. Compounds No. 7, 8, and 11, have a fit score between 67-72. This can be considered a good fit score with the optimal pharmacophore model, have low conformers (Günther et al., 2006) and are therefore expected to be plausible inhibitors.

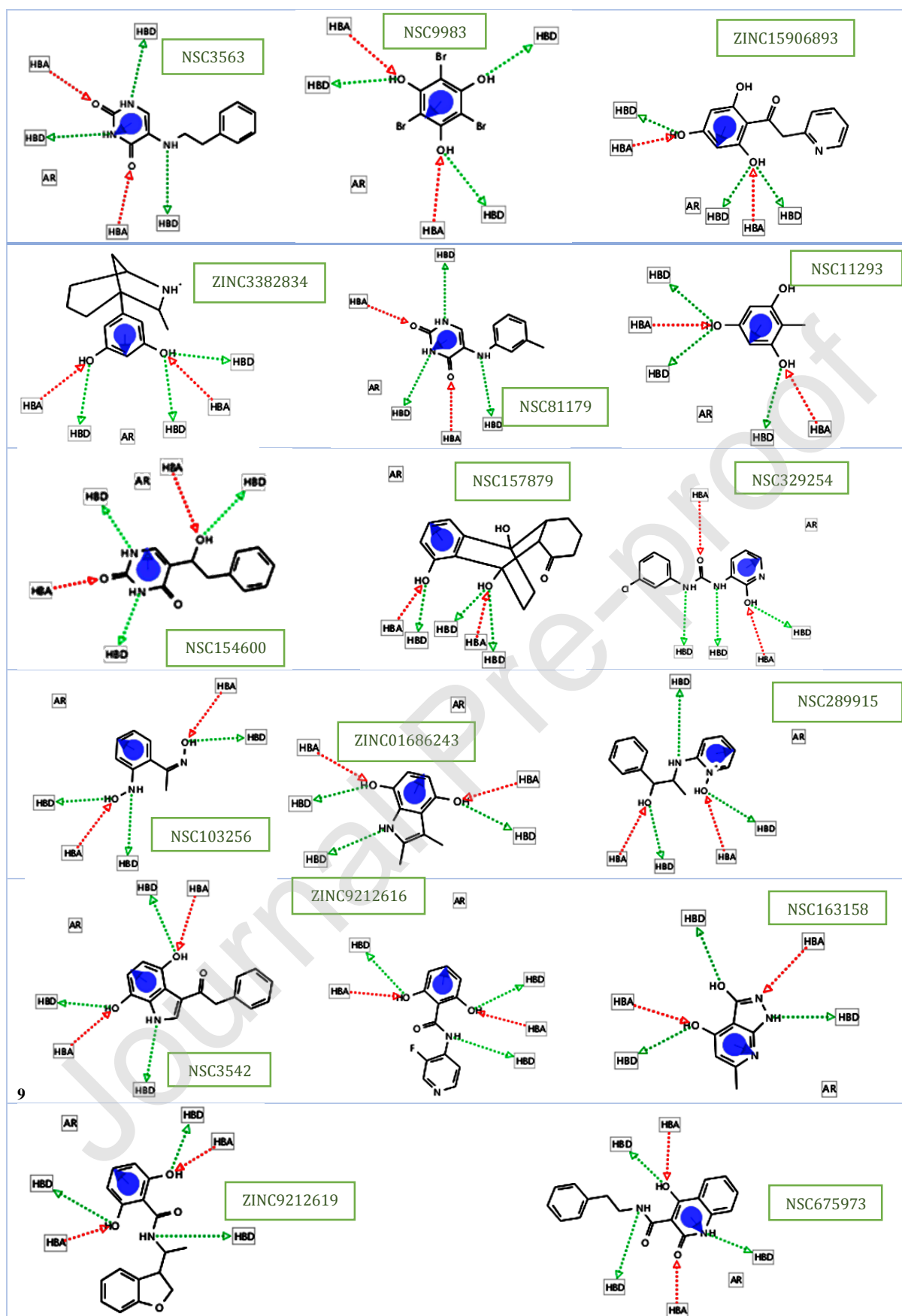*Table 3. Selected virtual hits from the ZINC and NCI subset databases*

Table 4. Screening results of natural products from pomegranate

No	compounds	**Energy (Kcal/mol)*** or Docking score*	Number of conformers	Pharmacophore Fit Score
1	Quercetin	-8.68	2	63.80
2	Epicatechin	-8.7	6	63.91
3	5-O-Caffeoylquinic acid	-8.72	100	64.51
4	Galloyl-HHDP-hexoside (Corilagin)	-8.71	89	71.60
5	Brevifolin carboxylic acid	-8.7	2	67.65
6	Valoneic acid bilactone	-8.64	10	67.62
7	Delphinidin-3-O-glucoside	-8.71	25	72.18
8	Cyanidin-3-O-glucoside	-8.68	31	63.77
9	Cyanidin-3-O-rutinoside	-8.63	98	63.76
10	Punicalagin	-8.71	15	72.23

*: binding affinity (docking) values of the hit compounds

Compound No. 10 (Punicalagin; Fig. 4) has been shown to possess strong antioxidant properties of pomegranate (Kulkarni and Aradhya, 2004)(Llorach and Tomás-barberán, 2003)(Rosenblat et al., 2013)(Ulkarni et al., 2007)(Seeram et al., 2005).
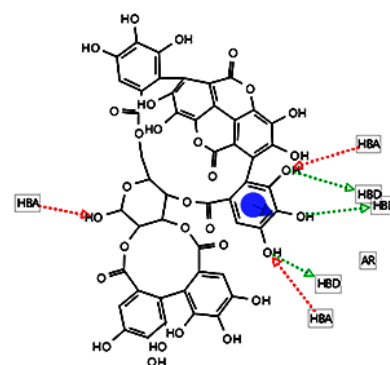
Figure4. Structure of Punigalagin fitted into the pharmacophore model

Additionally, the candidate compounds from ZINC and NCI were pre-filtered by the FAFdrug3 server (Yang et al., 2016). From the screening of ZINC and NCI databases, 44 and 53 compounds were selected respectively. Candidate compounds from these two databases were clustered into 28 different structural groups (Vuorinen et al., 2014). From each cluster, a member that has the best-fit score and compatibility with our pharmacophore model was selected. Finally, out of 28 candidate compounds, 18 compounds with up to 100 conformers were introduced as the final candidates (Günther et al., 2006). The results are shown in Table. 3. It is hoped that an experimental test will be done for these compounds in the future.

Molecular docking

To investigate the binding modes of the candidate compounds to the alpha-synuclein protein, molecular docking experiments were performed, which can evaluate the regional influence on biological activity. To find minimized affinity values, molecular docking of Exifone and Gallic acid, which was the compounds of the training set, was performed. The minimum free energy binding for these two compounds was -8.69 and -8.68 Kcal/mol, respectively. By performing molecular docking for the hit compounds of the ZINC15 and Pomegranate databases, the minimum free energy binding of these compounds was investigated (the results are shown in Tables 1 and 2). As it turns out, these minimum energies are close to the values obtained from the training set, and therefore confirm the model results.

Conclusions

In this study, we build a 3D ligand-based pharmacophore model that consists of three hydrogen bond donor (HBD), two hydrogen bond acceptor (HBA), and two aromatic rings (AR), with significant statistical parameters such as Sensitivity = 0.72, Specificity =0.98 and GH-score ~0.7, which indicates the powerful ability of the model to pick out active molecules from the database. To perform drug repositioning, this model was used as a 3D query to screen the ZINC15 database. The inhibitory effect of a significant number of candidate drugs, such as Entacapone, Tolcapone, Pyridoxine,

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Levodopa, and Rifampicin was demonstrated in previous experimental studies. We then screened NCI, ZINC databases, and natural compounds from the pomegranate. Some compounds were proposed from each of these databases that could be potential inhibitors of α-syn aggregation. Once again, the strength of the model was demonstrated by comparing the docking score of the selected ZINC and Pomegranate compounds with the training set. To best of our knowledge, this is the first report where a ligand-based pharmacophore model has been built and used to identify drugs for repositioning against α-syn aggregation inhibition. Ultimately, we believe that this research will shed new light on better understanding pharmacophore features of potentially active compounds against the α-syn aggregation. This study also certifies that there is a need to further *in vitro* and *in vivo* studies soon to develop potent drug inhibitors of the fibrillation of α-syn for the treatment of synucleinopathy disorders like Parkinson's disease.

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 Sedighe sadat jafaripour: Conceptualization, Methodology, Software, Data curation, Writing-Original draft preparation. Nazarshode: Software, Gharaghani and saboury: Visualization, Investigation, Supervision, Reviewing and Editing: Shozeb Haider: Software, Validation, Reviewing and Editing,

Conflict of interest
The authors declare that there is no conflict of interest

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