Contents lists available at UGC-CARE

International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

journal home page : http://ijpsdr.com/index.php/ijpsdr

Research Article

Identification of Novel Inhibitors for TG2 (Tissue Transglutaminase) and DJ-1 (Protein Deglycase) against Parkinson's Diseases by Pharmacophore-based Approaches

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ARTICLE INFO

Article history:
Received: 24 May, 2020
Revised: 17 July, 2020
Accepted: 18 August, 2020
Published: 30 September, 2020

Keywords:
ADMET, DJ-1,
Multiple docking,
NPACT database,
Parkinson's diseases,
Pharmacophore modeling, TG2.

DOI:
10.25004/IJPSDR.2020.120512

ABSTRACT

Parkinson's disease (PD) is one of the most common neurodegenerative diseases affecting the central nervous system (CNS), characterized by a multitude of motor and non-motor clinical symptoms. The hallmark of PD motor manifestation includes progressive tremor, rigidity, bradykinesia, and postural instability. There are many proteins involved in the progression of this disease, including TG2 (tissue transglutaminase) and DJ-1 (protein deglycase) protein. The present is focused on finding the novel inhibitor-based from phytochemicals category to inhibit the activity of TG2 and DJ-1 protein. The cheminformatics pipeline used include adsorbtion, distribution, metabolism, excretion, toxicity (ADMET) analysis, pharmacophore modeling, and molecular docking. Six best hit molecules were mapped with the e-pharmacophore features of TG2 and DJ-1 protein. These pharmacophores were further analyzed by molecular docking, protein-ligand interactions, and *in silico* ADMET studies. The molecular docking analysis revealed that hydroxywogonin and 2',3',5,7-tetrahydroxy flavones had good binding energy and satisfied the Lipinski rule of five, and had no toxicity.

Introduction

Parkinson's disease (PD), also known as shaking palsy, is a common neurodegenerative disorder clinically characterized by stilly shacking, bradykinesia, rigidity muscles, abnormal posture, and pace first systematically described by an English doctor named James Parkinson.[1] Then, neuropathological hallmarks are characterized by a progressive loss of dopaminergic neurons in the substantianigra pars compacta (SNpc), leads to the characteristic motor features of tremor, rigidity, and bradykinesia, while more-widespread neuronal changes lead to complex and variable nonmotor symptoms.[2] Traditionally, PD has been considered a sporadic neurodegenerative disorder. However, increasing evidence of family aggregation genes involved in PD.[3] There are many proteins involved in the progression of this disease. Among them are DJ-1and TG2.

DJ-1 belongs to the functionally diverse and large family DJ-1/PfpI present in almost all organisms. It is the most studied protein as it has a role in neurological disorders, especially in Parkinson's and cancer; DJ-1 is a dimeric protein composed of 189 amino acids.[4] DJ-1 has been involved in different cellular processes, such as, homeostatic control of reactive oxygen species (ROS), transcription regulation, protein folding, modulation of glucose levels, fertility, and cellular transformation, a

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

role in neuronal protection against oxidative stress seems the most widely accepted.[5]

Apart from DJ-1, the recent data indicated the possible involvement of TG2 in PD progression by catalyzing the formation of protein aggregates.[6] TG2 belongs to the transglutaminase family, which catalyzes transamidating acyltransferases and (Ca^{2+})-dependent protein.[7] It also carried out several important cellular functions, including apoptosis, extracellular matrix formation, cell signaling, etc. The protein is chiefly located in the cytoplasm but also present on the surface in various cell types and in the extracellular matrix, endoplasmic reticulum, and Golgi apparatus.[8] Overexpressed TG2 also cancer and PD activity, so it is also considered the therapeutic target for PD (Rudlong *et al.*).[9]

In silico approaches, such as, e-pharmacophore modeling, followed by multiple docking, would help define TG2 and DJ-1 inhibitors.[10] Two e-pharmacophore models defined increases the diversity of the compound and are used for pharmacophore.[11] The ability of defined e-pharmacophore to retrieve actives was evaluated or validated using multiple docking strategies rigid receptor docking (RRD), and mm-GBSA calculations were followed to propose antagonists against DJ-1 with a wide variety of scoring functions that defines better active affinities, orientations, and free energies of the docked complexes and finally the cocrystal-DJ-1 and TG2 complex was subjected, such as, docking and virtual screening and QikProp, that have also made important strides in advancing drug discovery, which would be useful for treating TG2and DJ-1 mediated Parkinson's disease by designing novel and potent inhibitor.[11,12]

Methodology

Preparation of Target Proteins

In the current study, the workflow is meant for designing and prioritization of potential inhibitors targeted against DJ-1 and TG2 essential structural features of active site regions was elucidated with co-crystal structure; ande-pharmacophore models were constructed using Sun Microsystems (Maestro 9.8, Schrodinger, New York, USA) workstation running on CentOS 6. Available co-crystal structures DJ-1 (3cz9-P4C) and TG2 (4pyg-GTP) were downloaded from the protein data bank (PDB). (http://www.rcsb.org) of leucine-rich repeat kinase 2 (LRRK2) (Laskowski).[13] Among the two structures, the lowest resolution < 1.15 Å of 3CZ9 and 2.8 Å 4PYG structure was considered to propose antagonists through e-pharmacophore-based modeling, multiple docking, and Qikprop properties.[14]

Protein Structure Preparation and Active Site Analysis

The protein preparation 3CZ9 and 4PYG was carried out by the protein preparation wizard. It finished with a partially optimized protein-ligand complex to which hydrogens have been added, subjected to adjustment of protonation states for ionizable residues, modification of tautomeric forms, and repositioning of reorientable hydrogens.[15] Active site was predefined for further study as the residues contribute to the functionality of the protein. The co-crystal ligand interactions with GTP-TGM2 and P4C-DJ-1 were analyzed using PDBsum. Protein structure minimization was carried out using the OPLS_2003 force field by converging the heavy atoms to an root-mean-square deviation of atomic positions (RMSD) of 0.3 Å.[16]

Preparation of Ligands

The structure data format (SDF) structure of selected 18 natural compounds was retrieved from the naturally occurring plant-based anti-cancer compound-activity-target (NPACT) database (Table 1). The structures of the ligands were sketched using Marvin's sketch.[17] Each structure was then executed for energy minimization in the same workstation. These obtained conformations were saved in the PDB file and further used as starting conformations to perform docking analysis.[18]

Generation of E-Pharmacophore Model

The structure-based pharmacophore model has also become increasingly important in computational drug design. The pharmacophore model is used to identify the key features vital to inhibition activity for drug molecules. The co-crystal ligand was docked in the generated grid using Glide XP docking. Glide XP energies were added together for all the atoms, which comprises the pharmacophoric sites. A fitness score is a measure of how well the ligand fits into the receptor with reference to the ligand. Pharmacophoric sites with fitness scores less than -0.5 were rejected.[19] Receptor based e-pharmacophore model of TG2 and DJ-1 was generated from 3CZZ9 and 4PYG docked complex using the protein-ligand coordinates.[20]

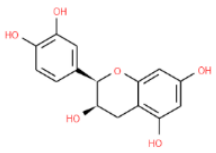
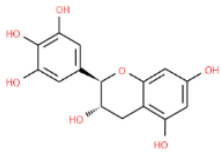
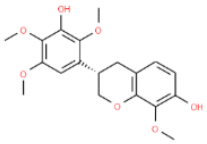
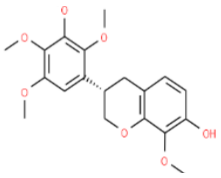
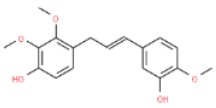
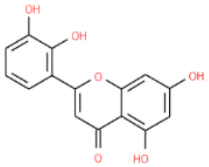
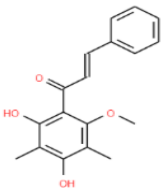
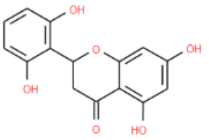
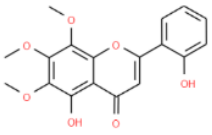
Pharmacophore Model Evaluation

Two e-pharmacophore models of LRRK2 were generated from GTP and P4C ligands. The two e-pharmacophore hypotheses were considered for validating. The co-crystal ligands were considered actives, which were then combined to decoys set with 1,000 drug-like compounds retrieved from Schrodinger[21] to form an internal library of 1,015 compounds. The e-pharmacophore was taken as a query to screen the internal library to generate a library of TG2 and DJ-1 activators and was docked.

E-Pharmacophore-based Database Screening

E-pharmacophores that are validated were considered for shape screening. Structure-based similarity screening was performed using the PHASE v5.0 module of Schrodinger for

Table 1: List of TG2 and DJ-1: their best phytochemical compound, Pubchem ID SMILES, IUPAC name, 2-D structure, and molecular weight

Pubchem ID	*Compound*	*Molecular weight (g/mol)*	*IUPAC name*	*SMILES*	*2-D structure*
72276	Epicatechin	290.27	2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol	O1[C@@H]([C@H](O)Cc2c1cc(O)cc2O)c1cc(O)c(O)cc1	
65084	Gallocatechin	306.27	2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol	O1[C@@H]([C@@H](O)Cc2c1cc(O)cc2O)c1cc(O)c(O)c(O)c1	
471695	3',7-dihydroxy-2',4',5',8-tetramethoxyisoflavan	362.4	(3-hydroxy-2,4,5-trimethoxyphenyl)-8-methoxy-3,4-dihydro-2H-chromen-7-ol	O1C[C@@H](Cc2c1c(OC)c(O)cc2)c1c(OC)c(O)c(OC)c(OC)c1	
471694	7-hydroxy-2',3',4',5',8-pentamethoxyisoflavan	376.4	8-methoxy-3-(2,3,4,5-tetramethoxyphenyl)-3,4-dihydro-2H-chromen-7-ol	O1C[C@@H](Cc2c1c(OC)c(O)cc2)c1c(OC)c(OC)c(OC)c(OC)c1	
44254875	Candenatenin A	316.3	3-(3-hydroxy-4-methoxyphenyl)prop-2-enyl]-2,3-dimethoxyphenol	O(c1c(C/C=C/c2cc(O)c(OC)cc2)ccc(O)c1OC)C	
5321864	2',3',5,7-tetrahydroxy flavone	286.24	2-(2,3-dihydroxyphenyl)-5,7-dihydroxychromen-4-one	o1c2c(c(=O)cc1c1c(O)c(O)ccc1)c(O)cc(O)c2	
10424762	2',4'-dihydroxy-6'-methoxy-3',5'-dimethylchalcone	298.3	(E)-1-(2,4-dihydroxy-6-methoxy-3,5-dimethylphenyl)-3-phenylprop-2-en-1-one	O(c1c(c(O)c(c(O)c1C(=O)/C=C/c1ccccc1)C)C)C	
42607849	2',5,6',7-tetrahydroxy flavanone	286.24	2-(2,6-dihydroxyphenyl)-5,7-dihydroxy-2,3-dihydrochromen-4-one	O1C(CC(=O)c2c1cc(O)cc2O)c1c(O)cccc1O	
159029	Tenaxin I	344.3	5-hydroxy-2-(2-hydroxyphenyl)-6,7,8-trimethoxychromen-4-one	o1c2c(c(O)c(OC)c(OC)c2OC)c(=O)cc1c1c(O)cccc1	

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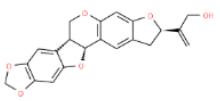
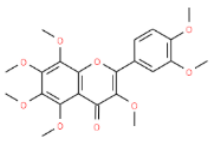
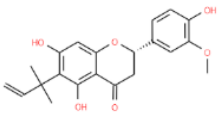
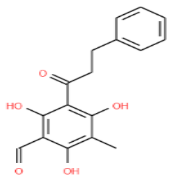
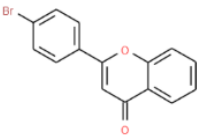
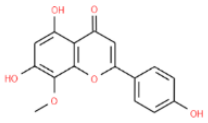
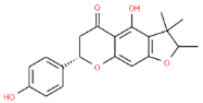
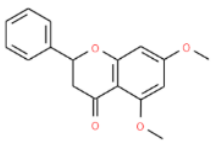
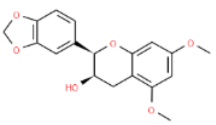
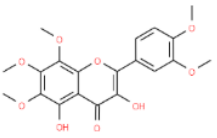
46939793	2'R,4'-hydroxyemoroidocarpan	366.4	2-[(1*R*,6*R*,13*R*)-7,11,17,19,23-pentaoxahexacyclo tricosa-21-hexaen-6-yl] prop-2-en-1-ol	O1[C@@H]2[C@H](c3c1cc1OCOc1c3)COc1c2cc2C[C@@H](Oc2c1)C(=C)CO	
150893	3,3',4',5,6,7, 8-heptamethoxyflavone	432.4	2-(3,4-dimethoxyphenyl)-3,5,6,7,8-pentamethoxychromen-4-one	o1c2c(c(OC)c(OC)c(OC)c2OC)c(=O)c(OC)c1c1cc(OC)c(OC)cc1	
10761665	3'-O-methyl-6-(1,1-dimethylallyl)eriodictyol	370.4	(2*S*)-5,7,4'-trihydroxy-3'-methoxy-6-(1,1-dimethylallyl)flavanone	O1[C@@H](CC(=O)c2c(O)c(C(C)(C)C=C)c(O)cc12)c1cc(OC)c(O)cc1	
11033908	3'-formyl-2',4',6'-trihydroxy-5'-methyldihydrochalcone	300.3	2,4,6-trihydroxy-3-methyl-5-(3-phenylpropanoyl) benzaldehyde	Oc1c(C(=O)CCc2ccccc2)c(O)c(c(O)c1C)C=O	
1686	4'-bromoflavone	301.13	2-(4-bromophenyl) chromen-4-one	Brc1ccc(c2oc3c(c(=O)c2)cccc3)cc1	
5322078	4'-hydroxy wogonin	300.26	5,7-dihydroxy-2-(4-hydroxyphenyl)-8-methoxychromen-4-one	o1c2c(c(=O)cc1c1ccc(O)cc1)c(O)cc(O)c2OC	
10664858	5,4'-dihydroxy-4",4"-dimethyl-5"-methyl-5"-H-dihydrofuruno[2",3":6,7] flavonone	340.4	(7S)-4-hydroxy-7-(4-hydroxyphenyl)-2,3,3-trimethyl-6,7-dihydro-2H-furo[3,2-g]chromen-5-one	O1[C@@H](C(c2c1cc1O[C@@H](CC(=O)c1c2O)c1ccc(O)cc1)(C)C)C	
378567	5,7-dimethoxy-3',4'-methylenedioxyflavanone	284.31	5,7-dimethoxy-2-phenyl-2,3-dihydrochromen-4-one	O1[C@H](CC(=O)c2c1cc(OC)cc2OC)c1ccccc1	
10336729	5,7-dimethyl-3',4'-methylene(-)-epicatechin	330.3	(2R,3R)-2-(1,3-benzodioxol-5-yl)-5,7-dimethoxy-3,4-dihydro-2H-chromen-3-ol	O1[C@@H]([C@H](O)Cc2c1cc(OC)cc2OC)c1cc2OCOc2cc1	
10453852	5-desmethylnobiletin	404.4	2-(3,4-dimethoxyphenyl)-3,5-dihydroxy-6,7,8-trimethoxychromen-4-one	o1c2c(c(O)c(OC)c(OC)c2OC)c(=O)c(O)c1c1cc(OC)c(OC)cc1	

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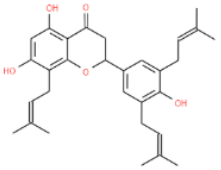
42607927	5-hydroxysophoranone	476.6	5,7-dihydroxy-2-[4-hydroxy-3,5-bis(3-methylbut-2-enyl)phenyl]-8-(3-methylbut-2-enyl)-2,3-dihydrochromen-4-one	O1[C@H](CC(=O)c2c1c(CC=C(C)C)c(O)cc2O)c1cc(c(O)c(c1)CC=C(C)C)CC=C(C)C	

the multiple e-pharmacophore models generated with the selected features.[22] Multiple conformers were generated for all the tautomer's matched from the screening. In order to reduce the false positives, inactive compounds were rejected. To refine the screening process, receptor-based excluded volumes were also integrated into shape screening.[15] All the hits obtained from the shape-based similarity search based on the e-pharmacophore model and co-crystal ligand were exported as a library of TG2 and DJ-1.

Molecular Docking

Multiple docking strategy protocols were employed to predict the scoring and active site interactions between GTP-4PYG and P4C-3CZ9. The prepared in-house library was then docked into the TG2 and DJ-1 active site. Energy minimization was done at neutral pH 7 ± 2 units. Ligands with a reactive functional group, high ionization energy/tautomer states were removed from the generated conformations.[15] Ligands that are not obeying Lipinski's rule of five were discarded from the multiple conformations generated. A receptor grid of 10 × 10 × 10 Å was generated around the active site residues of GTP-4PYG and P4C-3CZ9 crystal structure, using Glide v.5.9 (Grid-based Ligand Docking with Energetics). Intermediate charge sets of LRRK2 and lead complexes calculated earlier in the Q-Site refinement were re-docked through Glide extra precision mode to get the empirical atomic charges and further carried out for binding free energy (ΔG) calculations.[23]

Free Energy Calculations

Using molecular mechanics/generalized Born surface area (MM/GBSA), the binding free energy (ΔG) of TG2 and DJ-1 complexes was calculated by the Prime approach. For each TG2 and DJ-1 complexes, ΔG was calculated by using the equation as follows:

ΔGbinding = ΔGcomplex - (ΔG TG2 and DJ-1 + ΔGlead)

Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) Studies of Compounds

ADME study is an essential and primary step of pharmacological drug screening. It includes properties of structural analogs; it predicts both physically significant descriptors and pharmaceutically relevant properties. It consists of principle descriptors and physicochemical properties with a detailed analysis of the log P (octanol/water), log S, molecular weight, etc. It also calculates the analogs depending upon Lipinski's rule of five,[24] which is an important step for rational drug design. The properties were predicted using the SwissADME tool.[25]

Results

Cheminformatics Pipeline

TG2 and DJ-1 protein was found to localize throughout the nigrostriatal dopaminergic pathway, with the highest levels detected in the striatum but also at lower levels in the SNpc. TG2 and DJ-1 protein was found to localize throughout the cytoplasm of neuronal perikarya and dendritic processes, where it is associated with vesicular and membranous structures, the microtubule network, mitochondria, and other membrane-bound organelles. The membrane localization of TGM2 and DJ-1 was resistant to solubilization by non-ionic detergent, indicating that TGM2 and DJ-1 associate with lipid rafts, which play important roles in signal transduction, membrane trafficking, and cytoskeletal organization.[26] The expression of TGM2 and DJ-1 mRNA and protein has also been examined in neurologically normal human brains. Both mRNA and protein expression were found in brain regions of direct relevance to the pathogenesis of PD, including the cerebral cortex, caudate-putamen, and SNpc. The overview flow chart of the cheminformatics pipeline for the present study is shown in Fig. 1.

Receptor Preparation and Active Site Analysis

The structure of TG2 (PDB ID: 4PYG) was retrieved from PDB. It was determined by X-ray crystallography with a bond length of 0.019 Å and bond angles of 1.966°. The structure also contains 29,223 reflections used and protein of 16,257, respectively. DJ-1 (3cz9) is composed of 189 amino acids, mainly located in mitochondria, plasma membrane, and nucleus (Fig. 1).

The active site defines the favorable surface, which was desirable for ligand binding towards receptor; hence, the active site was analyzed. The active site was defined with the residues present around the 4 Å region surrounding the ligand co-crystallized in PDB structures. The predefined active site was cross-checked with PDBsum. The binding site region of human TG2 contains 13 amino acid residues. Lys 173, Phe 174, Arg 478, Arg 476, Val 479, Gly 480, Gln 481, Ser 482, Met 483, Arg 580, Leu 582, Tyr 583, and Glu 585 were selected from PDBsum as binding site cavity in the current study, while in case of DJ-1 protein only one amino acid is present in the active site (Fig. 2).

E-Pharmacophore Generation

Available co-crystal structures for TGM2 and DJ-1 in the PDB, energy-optimized structure-based pharmacophore method was practiced in the present study. E-pharmacophores were developed for 4PYG and 3CZ9 co-crystal structures of TG2 and DJ-1 by Glide XP docking. As a pharmacophoric site based on the structural and interactional energy-information between the TG2, DJ-1, and co-crystal ligand, one e-pharmacophores were developed from 4PYG and 3CZ9, such that all the Glide XP energetic terms were mapped on to the atoms.[27] E-pharmacophores were written with the selected features, such that it could effectively map all the pharmacophoric features which were responsible for TG2 and DJ-1 bioactivity. The generated e-pharmacophore was validated using enrichment studies and was used for screening the small-molecule databases. Each e-pharmacophores differ from another in screening performance based on the pharmacophoric features. Multiple e-pharmacophores improved overall small molecule databases screening efficiency. The derived e-pharmacophore model has six features (AAADDNN) and three hydrogen bond acceptors (A), two hydrogen bond donors (D), and two negatively ionizable groups (N); four features (AAAAD), such as, four hydrogen acceptors (A) and one hydrogen donor (D). The pharmacophoric sites having a fitness score more than -0.5 were written as pharmacophore hypothesis, as it measures how well the pharmacophore site points are aligned with those of the co-crystal ligand.[28] The generated e-pharmacophore model and their pharmacophoric sites were shown in Fig. 3.

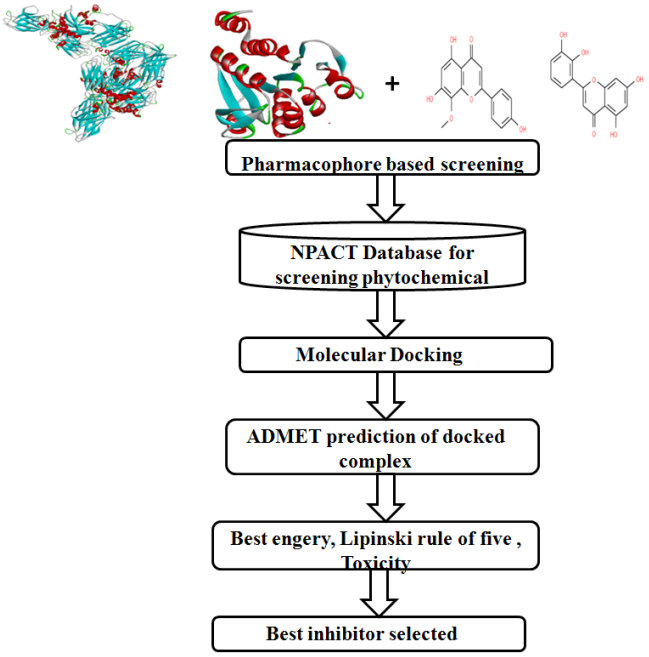


Fig. 1: Detailed workflow of present study; cheminformatics part of pipeline indicates pharmacophore modeling, virtual screening, molecular docking, and *in silico* ADMET analysis

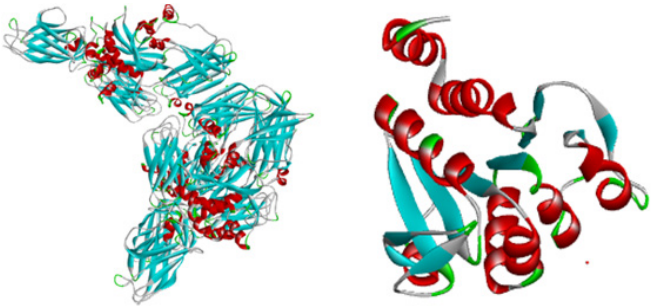


Fig. 2: 3-D structure protein TG2 (4PYG) and DJ-1 (3cz9)

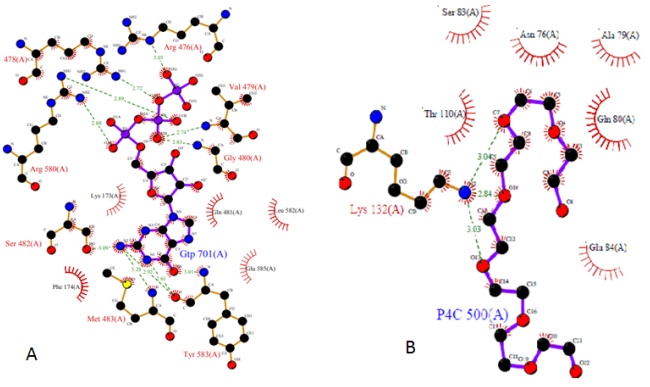


Fig. 3: Active site of both proteins: **A)** TG2 protein; **B)** DJ-1

Multiple Docking

In the present study, a genetic algorithm is used to predict protein-ligand interaction. This docking procedure predicts the site of a ligand when it is bound to its protein. Docking algorithms predict all possible structures by means of scoring each structure. The selected ligand molecules were docked into the active site of TG2 and DJ-1; based on the binding conformation, Schrodinger generated binding energies for all molecules. Table 2 shows TG2 and DJ-1. The best top two small binding energies, protein-ligand interaction residues, angles, the distance between hydrogen bonds, and the number of hydrogen bonds. Docking of human TG2 and DJ-1, with TG2 and DJ-1 ligand library. The RRD method was streamlined through HTVS-, SP-, and XP-docking methods to find potential lead molecules. Out of 18 ligands docked in the HTVS method, nine top-ranked ligands were re-docked using the SP method. Similarly, five top-ranked leads obtained through the SP method were re-docked using the XP docking method. The two docked complexes were rescored for Prime/MM-GBSA. The result was recorded in the form of binding energies, protein-ligand interaction residues, angles, the distance

Table 2: Multiple docking study of TG2 and DJ-1 protein

Compound	*Volume*	*Glide energy*	*Binding interaction*
Hydroxywogonin	1,025.295	-27.176	-2.496
2',3',5,7-tetrahydroxy flavone	958.783	-27.029	-2.382

Table 3: Druglikeness property of top two protein-ligand complex

Compound	*Mol. wt.*	*Rotor*	*SASA*	*FOSA*	*WPSA*	*PISA*	*Volume*	*Donor*	*Acceptor*
Hydroxywogonin	316.394	5	575.837	410.719	0	0	102.095	4	10.2
2',3',5,7-tetrahydroxy flavone	302.367	5	541.641	541.641	0	0	958.783	5	10.2

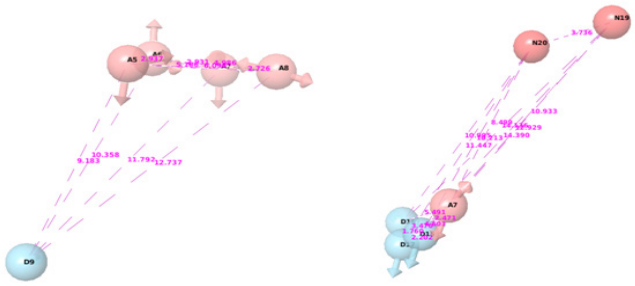


Fig. 4: Generated pharmacophore model with six features and five features for selected proteins

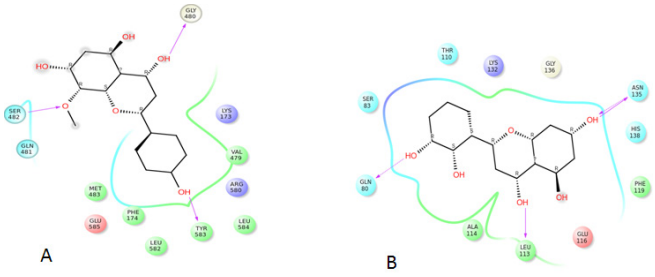


Fig. 5: Top two ligand molecular poses of docking protein-ligand interaction analysis of TG2 and DJ-1 protein complex: **A)** Hydroxywogonin; **B)** 2',3',5,7-tetrahydroxy flavones

between hydrogen bonds, and the number of hydrogen bonds.

The rescoring was performed as it was proved by various research groups that Prime/MM-GBSA rescoring of docking complex (DG) showed better correlation to their experimental binding affinity compared to XP Gscore.[29] The top 2 ligands better than the co-crystal ligands were subjected to re-dock with TG2 and DJ-1 using mmGBSA for evaluating relative active interactions and the strength of each potential lead with 4PYG and 3CZ9 by accurate charge calculation through hybrid quantum mechanics and molecular mechanics method. The compounds were ranked based on MMGBSA and DG scores. The different docking strategies of RRD and MM-GBSA rescoring further affirmed the co-crystal ligand's ability as a potent TG2 and DJ-1 inhibitor. The GTP and P4C site geometry of a protein complex depends heavily upon conformational changes induced by the bound ligand. Further, the active site residues of human TG2 and DJ-1 with ligand were compared with the best resolute reference co-crystal structure, 4PYG, and 3CZ9 (Fig. 4).

Multiple docking strategies revealed that hydroxywogonin has active free energy and docking scores than the existing inhibitors, co-crystal ligand. The good binding affinity of hydroxywogonin is due to hydrogen bonding, hydrophobic interactions, hydrophilic interactions, electrostatic interactions, and steric interactions with Glide score of -6.396 kcal/mol; ΔG value as -59.28 kcal/mol in RRD. Hydroxywogonin bound to the TG2 and 3CZ9 with seven hydrogen bond interactions, in which six hydrogen bonds were observed with backbone residues of Phe 174, Val 479, Tyr 483, Leu 582, Tyr 583, and Leu 584. Hydroxywogonin docking interactions well collaborated with the co-crystal structure of 4PYG and 3CZ9 (Fig. 5). Molecular interactions of TGM2 and DJ-1-hydroxywogonin docking complex showed six hydrogen bond interactions, revealing high stability.

Chemical Analysis of Drug-likeness

All 20 inhibitors were performed Lipinski "rule of five" and "drug-likeness" by Quickpro tool. The compounds showed log P ≤ 5, relative molecular mass ≤ 500, range of HBA (hydrogen bond acceptors) ≤ 10, and range of hydrogen bond donors (HBD) ≤ 5, considering the best ligand molecules were used as drug leads for biological activity (Table 2). Lipinski's rule of five could be a rule of thumb designed to evaluate the drug-likeness or decide whether a substance through a particular pharmacologic or biological action may create a credible, verbally energetic compound in humans. The results showed that two molecules satisfied the rule of five and drug-likeness (Table 3).

Prediction of Physicochemical Descriptors and ADMET Parameters

The present study analyzed physicochemical descriptors and ADMET parameters by SwissADME analysis to find the solubility and permeability of the 18 ligand molecules to use them for experimental assays and reach their site of action in an accurate drug ability. The fifteen ligands' molecular complexity could be measured by the number of rings and aromatic rings, the fraction of carbons that were sp3 hybridized (Fsp3), or the number of stereocenter and ADMET properties, which were all computed by SwissADMET. The TG2 and DJ-1 protein top compounds showed the best result. The *in silico* ligand toxicity and biological property predictions are faster and more reliable approaches to take before further exploring experimental authentications, such as, *in vitro* and *in vivo* tests. Therefore, these inhibitors are most appropriate for additional drug discovery approaches to drug discovery.

Discussion

The present study screened for novel small inhibitors that can specifically inhibit TG2 and DJ-1 interaction and downstream signalling. We identified the key amino acid residues involved in the interactions for selected proteins. We screened the lead compound hits for proteins from the NPACT using structure-based pharmacophore modeling, virtual screening, and molecular docking along

with *in silico* ADMET analysis. The identified novel small inhibitors can potentially be utilized for anti-inflammatory agents to treat relevant disorders.

The pharmacophore features are the key elements to screen for the best, potent small molecules binding to target proteins from publicly available databases. Pharmacophore-based approaches were widely used in virtual screening, *de novo* design, and other applications, such as, lead optimization and multitarget drug design. For TG2 and DJ-1, six pharmacophore features were selected with the default ligand. Hydroxywogonin molecule HN group interacts with one hydrogen bond with binding energy -27.176 and amino acid GLY480, SER 482, TYR 583. 2',3',5,7-tetrahydroxy flavones interact with three amino acid residues GLN 180, ASN 130, and LEU 113 with binding energy -27.029. The *in silico* ADMET results revealed that all the top two TG2 and DJ-1 inhibitors are virtually safe and active. These novel inhibitors are worthy of further assessment for safety and efficacy *in vitro* and *in vivo*.

Conclusion

In the present work, the pharmacophore model was to recognize vitally assorted lead hits for TG2 and DJ-1. The recognized hit compounds were utilized to create novel, strong inhibitors for the targets and further assessed by docking and *in silico* ADMET studies. Two compounds satisfied all the criteria and serve as novel, structurally diverse inhibitors for protein.

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HOW TO CITE THIS ARTICLE: Vaidya GS, Naik VU, Parvatikar P. Identification of novel inhibitors for TG2 (tissue transglutaminase) and DJ-1 (protein deglycase) against Parkinson's Diseases by Pharmacophore-based approaches. Int. J. Pharm. Sci. Drug Res. 2020;12(5):509-516. **DOI:** 10.25004/IJPSDR.2020.120512