Published in final edited form as:

J Biomol Struct Dyn. 2018 November ; 36(15): 3938–3957. doi:10.1080/07391102.2017.1404931.

Pharmacophore-based virtual screening of catechol-o-methyltransferase (COMT) inhibitors to combat Alzheimer's disease

Chirag N. Patel^a, John J. George^b, Krunal M. Modi^c, Moksha B. Narechania^d, Daxesh P. Patel^e, Frank J. Gonzalez^e, and Himanshu A. Pandya^{a,*}

^aDepartment of Botany, Bioinformatics and Climate Change Impacts Management, University School of Sciences, Gujarat University, Ahmedabad 380 009, Gujarat, India

^bDepartment of Bioinformatics, Christ College, Rajkot 360 005, Gujarat, India

^cJ. Heyrovský Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, Dolejškova 2155/3, 182 23, Prague 8, Czech Republic

^dHuman Genetics Division, Department of Zoology, BMTC and HG, University School of Sciences (USSC), Gujarat University, Ahmedabad 380009, Gujarat, India

^eLaboratory of Metabolism, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Rockville, MD, USA

Abstract

Alzheimer's disease (AD) is one of the most significant neurodegenerative disorders and its symptoms mostly appear in aged people. Catechol-o-methyltransferase (COMT) is one of the known target enzymes responsible for AD. With the use of 23 known inhibitors of COMT, a query has been generated and validated by screening against the database of 1500 decoys to obtain the GH score and enrichment value. The crucial features of the known inhibitors were evaluated by the online ZINC Pharmer to identify new leads from a ZINC database. Five hundred hits were retrieved from ZINC Pharmer and by ADMET (absorption, distribution, metabolism, excretion, and toxicity) filtering by using FAF-Drug-3 and 36 molecules were considered for molecular docking. From the COMT inhibitors, opicapone, fenoldopam, and quercetin were selected, while ZINC63625100_413 ZINC39411941_412, ZINC63234426_254, ZINC63637968_451, and ZINC64019452_303 were chosen for the molecular dynamics simulation analysis having high binding affinity and structural recognition. This study identified the potential COMT inhibitors through pharmacophore-based inhibitor screening leading to a more complete understanding of molecular-level interactions.

*Corresponding author. hapandya@gujaratuniversity.ac.in.

Disclosure statement

The authors declare no conflict of interest.

Supplemental data

The supplemental data for this article is available online at <https://doi.org/10.1080/07391102.2017.1404931>.

Keywords

Alzheimer's disease (AD); catechol-o-methyltransferase (COMT); pharmacophore; ZINC pharmer; ADMET; molecular dynamics

Introduction

Dementia has spread universally and has become a major social problem which even leads to monetary burdens on the sufferer and those around him/her. Dementia can be divided into respective forms which introduces Alzheimer's disease (AD), vascular dementia (VaD), and dementia with Lewy bodies (Muller et al., 2007) _ENREF_1. Alzheimer's disease (AD) is a neurological disorder that impacts with the brain's neurons and nerve transmission, resulting in loss of memory, decay in language behavioral changes and problems with visual spatial search etc (Wu et al., 2012). The AD is pathologically characterized by continuous intra-cerebral accumulation of beta amyloid (Ab) peptides (Murphy & LeVine III, 2010) and tau protein (microtubule associated protein) in some parts of the brain (Serrano-Pozo, Frosch, Masliah, & Hyman, 2011). Catechol O-methyltransferase (COMT) is a magnesium-dependent entresol enzyme that activates the removal of a methyl group from the common methyl donor S-adenosyl-L methionine (AdoMet or SAM) to the substrate through a dihydroxybenzene (catechol) motif, resulting in the development of mono-O-methylated products and S-adenosylhomocysteine (AdoHcy or SAH) (Tsao, Diatchenko, & Dokholyan, 2011). COMT can be found on the edge or the surroundings of the central nervous system and is mainly expressed in liver, kidney, and intestines (Guldborg & Marsden, 1975; Kurogi et al., 2012). Different types of COMT inhibitors are used for treatment of various neurological disorders on the basis of their half-life *in vivo*. For example, pyrogallol and gallic acid are first generation COMT inhibitors, which possess low *in vivo* activity, whereas entacapone, tolcapone, and nebicapone are second generation COMT inhibitors, that are more potent but have hepatotoxic activity (Goncalves, Alves, Soaresda-Silva, & Falcão, 2012). The metabolism of catecholamine neurotransmitters and estrogen is affected by COMT, and could impact the AD pathophysiology. The COMT rs4680 gene polymorphism has been explored as a susceptibility gene for the AD. By *in vitro* analysis, COMT inhibitors, blocks beta-amyloid fibrils through a replication process, thus showing the utility of COMT for AD (Serretti & Olgiati, 2012).

Molecular modeling analysis has played a role in the development of drugs for the treatment of AIDS, cancer, Alzheimers, and various other diseases (Liu, Ji, Dong, & Sun, 2009; Jatana, Sharma, & Latha, 2013). Pharmacophore modeling is used for the classification and to discover the key features of the molecules. It allows designing of the new molecules which will be helpful for the rational drug design (Khedkar, Malde, Coutinho, & Srivastava, 2007; Sliwoski, Kothiwale, Meiler, & Lowe, 2014). Toxicity prediction has also been done on the basis of the Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) criteria which show the physic-chemical properties and biological activity of the selected drugs. To discover and optimize the novel molecules, with binding affinity to a target, the Molecular Docking (Wang et al., 2009) method has been used. Molecular Dynamics (MD) simulations have been carried out to study the conformational changes (Karplus &

McCammon, 2002), structural stability, hydration, and dynamics of peptide fibrils (Das, Kang, Temple, & Belfort, 2014; Lemkul & Bevan, 2013).

Materials and methods

By using the pharmacophore modeling, molecular docking, and molecular dynamics simulations, this study was carried out to identify new leads for the treatment of AD in relation to COMT inhibition. The merged feature pharmacophore model was generated using the merged feature pharmacophore generation protocol in Zinc Pharmer as well as LigandScout 3.12 version (Wolber, Dornhofer, & Langer, 2006; Wolber & Langer, 2005). Molecular docking and Molecular dynamics were executed by the YASARA (Yet Another Scientific Artificial Reality Application) commercial package (Krieger & Vriend, 2015) to get the insights into their structural recognition.

Pharmacophore modeling

Ligand preparation—A set of ligands was collected from the literature to establish a merged feature pharmacophore (Goncalves et al., 2012; Haasio, 2010). All the ligands were constructed using Marvin Suite applications (Marvin Sketch). The most imperative stride in pharmacophore modeling is the choice of suitable inhibitors that constitute the training set. The most active molecules reflected better affinity with COMT inhibitor but inactive molecules were short of affinity and their binding mode cannot be affectively determined using molecular docking approach. In addition, we are not certainly sure the less to less binding affinity. To overcome this problem only 23 best scoring adme (absorption, distribution, metabolism, excretion) COMT inhibitors with good activity and better affinity to receptor were selected for building pharmacophore query (Kiss & Soares-da-Silva, 2014) with diverse scaffolds (Figure 1) as the training set and prepared by energy minimization using MMFF94 force field.

Pharmacophore generation—Pharmacophore model generation was performed on the set of 23 best scoring adme molecules. The ligands were subjected to alignment perspective in LigandScout for the alignment purpose. This alignment is used to obtain the merged feature pharmacophore. The multi conformers were generated by applying default FAST settings and similarity was measured using Pharmacophore RDF–Code Similarity algorithm. Based on this, the pharmacophore is generated to predict the different pharmacophore features with scoring function – Pharmacophore fit and atom overlap and number of omitted structures for merged pharmacophore was set to 4. Default values were used for all other parameters. By this approach, ten pharmacophore hypotheses were successfully generated. Subsequently, poor models were rejected and the remaining pharmacophore models with the highest score was further assessed for pharmacophore validation (Chen et al., 2009).

Pharmacophore validation—In general, pharmacophore models are used as 3D queries to search databases to discover novel and potent lead molecules. A validation of the generated pharmacophore model should be performed to determine whether the model is able to accurately differentiate between best scoring and low scoring adme molecules. The Güner-Henry (GH) scoring method was used to validate the pharmacophore hypotheses

(Guner, 2002). This method quantifies the merit of the generated model by retrieving the best scoring adme molecules from a database containing known best scoring and low scoring adme molecules. The validation for the selected model was performed by Decoys Set Method (Keasar & Levitt, 2003; Zhou & Wishart, 2013). In which 23 best scoring adme molecules were used to search against the database of 1500 decoys generated by the online DUD-E server (Waldner, Fuchs, Schauperl, Kramer, & Liedl, 2016; Xia, Tilahun, Reid, Zhang, & Wang, 2015). The GH score method was successfully applied to quantify the selectivity of the pharmacophore model and to discover the activities from a decoy database. The GH score was calculated using the formula given below.

$$\begin{aligned}\%A &= \frac{Ha}{A} \times 100 \\ \%Y &= \frac{Ha}{Ht} \times 100 \\ E &= \frac{Ha/Ht}{A/D} \\ \text{False Negative} &= A - Ha \\ \text{False Positive} &= Ht - Ha \\ GH &= \left(\frac{Ha(3A + Ht)}{4HtA} \right) \left(1 - \frac{Ht-Ha}{D-A} \right)\end{aligned}$$

where D is the total number of molecules in the database, A is the total number of actives in the database, Ht is the total hits obtained by the pharmacophore-based database search, and Ha is the true positives or the number of actives present in the hit list. % Y is the recall or the percent yield of active molecules from the database hits and % A is the precision or the percent ratio of active molecules identified from the database search. Active (known inhibitor) from the decoy collection at the 5% or 10% of ranked database. We had revised this error in the manuscript. The higher the E value, the better is the model in retrieving the actives from a library of molecules.

Database searching and ADMET-Tox filtration—Virtual screening of a chemical database often leads to the discovery of a novel and potential lead molecules for further development. The obtained pharmacophore model was used as the 3D query for screening the ZINC purchasable database (Irwin, Sterling, Mysinger, Bolstad, & Coleman, 2012). All screening experiments were performed using the online Zinc Pharmer database searching utility. Molecules that possessed all the desired pharmacophore features were considered to be the hit molecules. To obtain new drug-like leads, generated hits were subjected to Lipinski's Rule of Five (Lipinski, 2004, 2016) and ADME-Tox filtration. The ADME-Tox filtration was carried out using the FAF-Drug3 server (Lagorce, Sperandio, Galons, Miteva, & Villoutreix, 2008; Ritchie, Ertl, & Lewis, 2011). The molecules that successfully passed these initial tests were selected for the subsequent molecular docking analysis.

Molecular docking

Docking studies are a necessary step to select potential hits in virtual screening. To investigate the detailed interactions between the virtual hits and COMT, the YASARA software program was used to perform molecular docking studies. The 3D structure determined by X-ray diffraction of human COMT (PDB ID: 3a7e) protein was retrieved

from Protein Data Bank (PDB). The structure has a depth of resolution 2.8 Å and contains 216 amino acids, ligand 3, 5-dinitrocatechol (DNC), cofactor S-adenosyl L- methionine (SAM), and magnesium ion (Mg^{+2}). Removal of water molecules, energy minimization (Kumar et al., 2015; Modi et al., 2016) were performed with the help of the standard steepest decent conjugate method in YASARA (Parmar, Patel, Highland, Pandya, & George, 2016). The 36 ligands from ZINC database (Irwin et al., 2012) were docked in the place of DNC, located at the binding site of COMT. Interaction profile for all the generated hits was also studied. From the interaction profile the ligands (hits) having high binding energy were further considered for the molecular dynamics approach (Borad et al., 2016). The binding free energy G_{bind} was estimated according to Equation (1):

$$\Delta G = \Delta G_{vdW} + \Delta G_{Hbond} + \Delta G_{elec} + \Delta G_{tor} + \Delta G_{desolv} \quad (1)$$

where, G_{vdW} = van der Waals term for docking energy; G_{Hbond} = H bonding term for docking energy; G_{elec} = electrostatic term for docking energy; G_{tor} = torsional free energy term for ligand when the ligand transits from unbounded to bounded state; G_{desolv} = desolvation term for docking energy.

Molecular dynamics

A molecular dynamics simulation study was undertaken by using YASARA (Krieger & Vriend, 2015) with AMBER03 force field (Parmar et al., 2016) for 8 docked complexes. The 3a7e protein was cleaned, hydrogens were added; water molecules were removed. Steepest descent minimization was used and simulation continued by 1 ns. The following molecular dynamics simulation steps were performed: temperature of 298 K, pressure at 1 bar, coulomb electrostatics at a cut off of 7.86, 0.9% NaCl, solvent density 0.997, pH 7.0, 1-fs time steps, periodic boundaries, all atoms mobile (Parmar et al., 2016). The average structures were determined from the simulations and were used to calculate the RMSDs and RMSFs for the respective model. Furthermore, conformation of the receptor-ligand was identified through the superposition of the simulated complex (Böckmann & Grubmüller, 2002).

Results and discussion

Pharmacophore modeling

Development of merged feature pharmacophore model of COMT inhibitors—

Pharmacophore modeling is a powerful tool in virtual screening, which helps to identify novel molecules. In the present study, a ligand-based pharmacophore method was performed using COMT inhibitors, in which a; training set of 23 known COMT inhibitors were taken for the development of a merged feature pharmacophore. Ten merged feature pharmacophore hypothesis were developed from 219 compounds. Six chemical features (2-Hydrogen Bond Donor (HBD), 2-Hydrogen Bond Acceptor (HBA), 1- Aromatic Ring (AR) and 1-Hydrophobic (HYP)) were identified in 14 ligands and their pharmacophore score ranged from 55.64 to 64.91 (Table 1).

Validation of pharmacophore modeling—On the basis of these scores, the best model of the pharmacophore hypothesis was determined. The Güner-Henry (GH) scoring method was used to identify the best pharmacophore model. A decoy database was generated and it was used for the pharmacophore identification and validation; which included 23 independent, best scoring adme molecules COMT inhibitors and 1500 low scoring adme molecules from the DUD-E Server. The six pharmacophore features map very well for 14 independent COMT inhibitors, successfully. Figure 2 shows the shared pharmacophore mapped for all molecules, while the highest mapped pharmacophore features were identified in the opicapone compound 3D as well as 2D format. On the basis of these results the best model shows a strong capability with a GH scores of 0.927 (a significant model based score) and enrichment factor 63.5 which was considered further for database searching (Table 2). Among 1500 low scoring adme molecules, 219 ligands were found in the category of active feature mapping pharmacophore.

Database searching and ADME-Tox filtration—The best pharmacophore model was selected for the identification of novel molecules from the ZINC database by using the Zinc Pharmer. In Zinc Pharmer, features with exclusion sphere from the developed merged feature pharmacophore model were submitted to retrieve novel hits. In a preclinical trials of drug discovery process, Lipinski's Rule of five and ADMET properties should be followed by the molecules. To achieve these, 500 novel hits were retrieved from Zinc Pharmer and for those molecules parameters (Molecular weight, Partition coefficient, Distribution-coefficient, Aqueous solubility, Topological polar surface area, Rotatable bonds, Rigid bonds, Hydrogen bond donor and Hydrogen bond acceptor) were set to filter hits from Lipinski's Rule of five. After that, FAF-Drug3 was used for the filtration of these molecules for ADMET properties. Using FAF-Drug3 server for ADME-Tox filtration, 36 hits were retrieved from the generated 500 hits of Zinc Pharmer only and the remaining 464 hits were rejected from the study. Hence, 23 best scoring and 36 best scoring out of 219 adme molecules were studied further for the molecular docking analysis. Table 3 depicts the predicted ADMET properties of best docked molecules.

Molecular docking analysis of known inhibitors and identified hit molecules

Docking validation—Molecular docking studies show the conformational flexibility of a protein's binding site (Krieger, Darden, Nabuurs, Finkelstein, & Vriend, 2004; Mabbitt et al., 2016). COMT (PDB ID: 3a7e) was used as a docking target. The validation of the docking procedure was performed by re-docking co-crystallized DNC into the active site of COMT using YASARA software (Parmar, Highland, Desai, Patel, & George, 2015). In this analysis it was found that the re-docked DNC (Figures 3 and 4) reproduced the binding pose with a binding energy of 6.343 kcal/mol. The RMSD of the co-crystallized and experimental poses was analyzed, and the RMSD value was 0.45 Å. These results show that the docking simulations reproduced the crystal complexes very well. The alignment of the co-crystallized ligand (green) and re-docked ligand (pink) is shown in Figures 3 and 4. The YASARA program was suitable to study the binding pose of the novel hits further.

Results from docking analysis—From the interaction profile various interactions including traditional hydrogen bonding interactions, hydrophobic interaction, Van der Waals

interactions, and pi-pi interactions between selected inhibitors and retrieved Hit molecules with COMT enzyme were examined. According to the docking results, the interface points of COMT included interactions, with the responsible key residues, from Trp 38, Met 40, Lys 46, Asp 141, His 142, Trp 143, Lys 144, Asp 169, Asn 170, Pro 174, Leu 198 and Glu 199 amino acids, MG 215 and SAM 216 were found. The calculated free energy of binding 3a7e with opicapone, fenoldopam, and quercetin, were found to be the best three docked molecules with the binding energy of 7.634, 7.631, and 7.561 Kcal/mol, respectively (Figures 5–7 and Table 4). Thus, these three molecules among 23 best scoring adme molecules have the potential to prolong the interaction with a catechol structure.

In all the 36-docked models generated, the catechol moiety is bound into the catalytic pocket of COMT, with the two catechol hydroxyl oxygens forming a bidentate chelate with the Mg^{+2} . Furthermore, the two hydroxyls are consistently found at hydrogen-bonding distances from the carboxylate of Glu 199 and the e-NH₂ of Lys 144 (average donor acceptor distances of 2.79 and 3.16 Å, respectively). These ligands were docked to a wide portion of COMT surface, without constraining them to form direct interactions with the catalytic site or the Mg^{+2} . The results support earlier suggestions that the substrate (or inhibitor) recognition is primarily dependent on the presence of two adjacent hydroxyl groups bound to an aromatic ring and indicate that the docking algorithm is able to capture it.

From the Zinc Pharmer, 36 ligands were retrieved in which ZINC63625100_413, ZINC39411941_412, ZINC63234426_254, ZINC63637968_451, and ZINC64019452_303 (Figure S1) were found with the best docked binding energy of 8.278, 8.100, 7.951, 7.946, and 7.888 Kcal/mol respectively (Figures 8–12 and Table 5). The contacting receptor residues were found as Trp 38, Met 40, Asp 141, Trp 143, Lys 144, Asp 145, Asn 170, Cys 173, Pro 174, Leu 198, Glu 199, Arg 201, Mg 215, Sam 216 (Table 6).

Molecular dynamics simulations

Molecular dynamics simulation was performed to allow the protein structure to relax to its equilibrium conformation and structural integrity. The effects that molecular dynamics simulation has on molecular docking was investigated by using the structures of COMT obtained directly from the Protein Data Bank and the equilibrium structure derived from molecular dynamics simulation.

In this section, an analysis of the conformations is presented, along with the bonding and flexibility of proteins during the simulations. The simulation of eight molecules (three best scoring adme molecules-opicapone, fenoldopam, and quercetin; five ZINC molecules – ZINC63625100_413, ZINC39411941_412, ZINC63234426_254, ZINC63637968_451, and ZINC64019452_303) for 1 ns was performed to study the conformational stability of the model. The data collected throughout the trajectory was used to investigate the stability of the secondary structure of the complex by plotting root mean square deviation (RMSD) and root mean square fluctuations (RMSF).

Figure 13 shows the energy value and RMSD of bound and unbound ligands in different time intervals (picoseconds) for whole proteins, while same time intervals have been considered for the RMSD and RMSF of pocket residues. All complexes possess their

distribution between -123000 and -124000 kcal/mol during 1 ns time interval. While higher fluctuations identified in the RMSD calculations are within the range of $0.7\text{--}1$ Å. Among them opicapone showed a stable conformation toward 3a7e protein at 0.795 Å. We then analyzed the RMSD and RMSF values of pocket residues of all simulated receptor-protein complexes, and $0\text{--}1.5$ Å structural variations were identified in residues RMSD while major fluctuations were measured for the residues RMSF values which ranges from 0.5 to 2 Å. During this event various interactions have been identified and observed including Conventional Hydrogen Bonds, Carbon Hydrogen Bonds, Salt Bridge, Alkyl, Pi-Alkyl, van der Waals, Unfavorable Hydrogen Bonds, Unfavorable Negative–Negative, Pi–Sigma, Pi–Pi T-shaped interactions including respective amino acids at different distances Figure 14 and Table S1. The conformational changes were evaluated by the superposition of proteins by considering Pre-MD as well as Post-MD files (Figures 15 and 16, Table 5) which demonstrates less than 2 Å changes found in the conformation of all receptor-ligand complexes (Nitta, Okada, & Hirokawa, 2008; Parmar et al., 2015).

We have performed molecular dynamics simulation of opicapone with selected protein for 10 ns time interval based on the energy value obtained from previously carried out md simulation to decipher the structural and conformational change which ranged at 1.967 Å. Figures S2–S4 shows the analyzed results of md simulations.

The molecules derived from this study show promising results and a few of them were previously reported as a potent lead to treat Alzheimer's disease (AD), Parkinson's disease (PD), and other diseases. Opicapone is a short lived but very long acting novel and potent third-generation COMT inhibitor expected to release into the market (Rocha et al., 2013). Fenoldopam is a synthetic benzazepine derivative drug, which acts as a selective D1 receptor partial agonist. Quercetin is a powerful flavonoid commonly found in fruits and vegetables, especially onions, citrus, apples, dark berries, grapes, olive oil, green tea, and red wine. It has a wide range of health benefits, including its ability to reduce inflammation, eliminate pain, protect against cardiovascular diseases, lower blood pressure, boost the immune system, manage diabetes, prevent certain cancers, and reduce irritation of the skin (Bondonno et al., 2016; Lu et al., 2015).

Conclusion

In this study, insights into the interaction of COMT with its inhibitors were elucidated through the use of molecular modeling, pharmacophore, docking and ADMET predictions, which can be important initial steps toward the development of novel pharmaceuticals to fight against the AD. The selectivity of COMT known inhibitors with respect to the developed pharmacophore-based sequential virtual screening protocol from the ZINC database, shed some light on the effects of the related biological activity within the series of anti-Alzheimer molecules.

With the assistance of a well-defined structure and annotations, the functional and binding sites were predicted, which will further the understanding of the biological roles of COMT. The binding energies of the receptor-ligand interactions also confirm that the ligands will fit into the active pockets of receptor tightly.

The structure of, opicapone, fenoldopam, and quercetin known COMT best scoring adme molecules, and ZINC63625100_413, ZINC39411941_412, ZINC63234426_254, ZINC63637968_451, and ZINC64019452_303 ZINC molecules from the molecular dynamics analysis were obtained after 1 ns of simulation. This study indicates that hydrophobic properties with aromatic sub-structural arrangement flanked by H bonds amino acids with key residues, Met 40, Asp 141, Asp 169, and Asn 170, can be used as filters to recognize potent COMT inhibitors repertoire. Further development of these molecules may lead to the generation of novel, highly potent COMT inhibitors with improved inhibitory activity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The authors gratefully acknowledge the Department of Botany, Bioinformatics and Climate Change Impacts Management, Gujarat University for providing an opportunity to access the bioinformatics research facilities. Mr Chirag N. Patel acknowledges financial assistance from University Grants Commission (UGC), Government of India as Rajiv Gandhi National Fellowship. Ms. Moksha Narechania acknowledges financial assistance from University Grants Commission (UGC), Government of India as Maulana Azad National Fellowship. The authors also acknowledge Dr Sivakumar Prasanth Kumar for their valuable suggestion and discussions during analysis of data. The authors sincerely acknowledge Dr Frank J. Gonzalez, Dr Bharti Dave, and Dr Usha Chaturvedi for English language assessment. The authors also acknowledge Dr Linz-Buoy George for technical evaluation of whole work.

Funding

This work was supported by University Grants Commission [grant number F1-17.1/2013-14/RGNF-2013-14-SC-GUJ-55791], [grant number F1-17.1/2015-16/MANF-2015-17-GUJ-63295].

Abbreviations:

AD	Alzheimer's disease
PD	Parkinson's disease
COMT	Catechol-O-Methyltransferase
ADMET	Absorption, Distribution, Metabolism, Excretion, and Toxicity
MD	Molecular Dynamics
MMFF94	Merck Molecular Force Field94
RDF	Radial Distribution Function
GH	Güner-Henry
DUDE	A Database of Useful (Docking) Decoys-Enhanced
YASARA	Yet Another Scientific Artificial Reality Application
PDB	Protein Data Bank

DNC	3, 5-dinitrocatechol
SAM	S-Adenosyl L- Methionine
Mg⁺²	Magnesium ion
RMSD	Root-mean-square-deviation
RMSF	Root-mean-square Fluctuations
HBD	Hydrogen Bond Donor
HBA	Hydrogen Bond Acceptor
AR	Aromatic Ring
HYP	Hydrophobic
FAF	Drug3-Free ADME-Tox Filtering Tool
ZINC63625100_413	((2R)-N-[3-[2-(2H-benzimidazol-2-yl) ethyl]phenyl]-2H-indole-2-carboxamide)
ZINC39411941_412	(N-[3-[(2R)-5-benzoyl-2H-benzimidazol-2-yl]phenyl]cyclopropanecarboxamide)
ZINC63234426_254	((4S)-4-(2H-benzimidazol-2-yl)-1-(1-naphthyl)pyrrolidin-2-one)
ZINC63637968_451	(N-[2-(2H-benzimidazol-2-yl)phenyl]-4-benzylbenzamide)
ZINC64019452_303	(2-(2H-benzimidazol-2-yl)-N-phenethyl-benzamide)

References

- Böckmann RA, & Grubmüller H (2002). Nanoseconds molecular dynamics simulation of primary mechanical energy transfer steps in F1-ATP synthase. *Nature Structural and Molecular Biology*, 9, 198–202. doi:10.1038/nsb760
- Bondonno NP, Bondonno CP, Rich L, Mas E, Shinde S, Ward NC, ... Croft KD (2016). Acute effects of quercetin-3-O-glucoside on endothelial function and blood pressure: A randomized dose-response study. *The American Journal of Clinical Nutrition*, 104, 97–103. doi:10.3945/ajcn.116.131268 [PubMed: 27169837]
- Borad MA, Bhoi MN, Rathwa SK, Vasava MS, Patel HD, Patel CN, ... George JJ (2016). Microwave-assisted ZrSiO₂ catalysed synthesis, characterization and computational study of novel spiro [Indole-Thiazolidines] derivatives as anti-tubercular agents. *Interdisciplinary Sciences: Computational Life Sciences*, 1–8. doi:10.1007/s12539-016-0195-2
- Chen Z, Li H-L, Zhang Q-J, Bao X-G, Yu K-Q, Luo X-M, ... Jiang H-L (2009). Pharmacophore-based virtual screening versus docking-based virtual screening: A benchmark comparison against eight targets. *Acta Pharmacologica Sinica*, 30, 1694–1708. doi:10.1038/aps.2009.159 [PubMed: 19935678]
- Das P, Kang S-G, Temple S, & Belfort G (2014). Interaction of amyloid inhibitor proteins with amyloid beta peptides: Insight from molecular dynamics simulations. *PLoS ONE*, 9, e113041. doi: 10.1371/journal.pone.0113041 [PubMed: 25422897]

- Goncalves D, Alves G, Soares-da-Silva P, & Falcão A (2012). Bioanalytical chromatographic methods for the determination of catechol-O-methyltransferase inhibitors in rodents and human samples: A review. *Analytica Chimica Acta*, 710, 17–32. doi:10.1016/j.aca.2011.10.026 [PubMed: 22123108]
- Guldborg HC, & Marsden CA (1975). Catechol-O-methyl transferase: Pharmacological aspects and physiological role. *Pharmacological Reviews*, 27, 135–206. [PubMed: 1103160]
- Guner O (2002). History and evolution of the pharmacophore concept in computer-aided drug design. *Current Topics in Medicinal Chemistry*, 2, 1321–1332. doi:10.2174/1568026023392940 [PubMed: 12470283]
- Haasio K (2010). Toxicology and safety of COMT inhibitors. *International Review of Neurobiology*, 95, 163–189. doi:10.1016/B978-0-12-381326-8.00007-7 [PubMed: 21095462]
- Irwin JJ, Sterling T, Mysinger MM, Bolstad ES, & Coleman RG (2012). ZINC: A free tool to discover chemistry for biology. *Journal of Chemical Information and Modeling*, 52, 1757–1768. doi:10.1021/ci3001277 [PubMed: 22587354]
- Jatana N, Sharma A, & Latha N (2013). Pharmacophore modeling and virtual screening studies to design potential COMT inhibitors as new leads. *Journal of Molecular Graphics and Modelling*, 39, 145–164. doi:10.1016/j.jmgs.2012.10.010 [PubMed: 23280413]
- Karplus M, & McCammon JA (2002). Molecular dynamics simulations of biomolecules. *Nature Structural and Molecular Biology*, 9, 646–652. doi:10.1038/nsb0902-646
- Kearse C, & Levitt M (2003). A novel approach to decoy set generation: Designing a physical energy function having local minima with native structure characteristics. *Journal of Molecular Biology*, 329, 159–174. doi:10.1016/S00222836(03)00323-1 [PubMed: 12742025]
- Khedkar SA, Malde AK, Coutinho EC, & Srivastava S (2007). Pharmacophore modeling in drug discovery and development: An overview. *Medicinal Chemistry*, 3, 187–197. doi:10.2174/157340607780059521 [PubMed: 17348856]
- Kiss L. s. E., & Soares-da-Silva P (2014). Medicinal chemistry of catechol O-methyltransferase (COMT) inhibitors and their therapeutic utility. *Journal of Medicinal Chemistry*, 57, 8692–8717. doi:10.1021/jm500572b [PubMed: 25080080]
- Krieger E, Darden T, Nabuurs SB, Finkelstein A, & Vriend G (2004). Making optimal use of empirical energy functions: Force-field parameterization in crystal space. *Proteins: Structure, Function, and Bioinformatics*, 57, 678–683. doi:10.1002/prot.20251
- Krieger E, & Vriend G (2015). New ways to boost molecular dynamics simulations. *Journal of Computational Chemistry*, 36, 996–1007. doi:10.1002/jcc.23899 [PubMed: 25824339]
- Kumar R, Son M, Bavi R, Lee Y, Park C, Arulalapperumal V, ... Kim Y-S (2015). Novel chemical scaffolds of the tumor marker AKR1B10 inhibitors discovered by 3D QSAR pharmacophore modeling. *Acta Pharmacologica Sinica*, 36, 998–1012. doi:10.1038/aps.2015.17 [PubMed: 26051108]
- Kurogi K, Alazizi A, Liu M-Y, Sakakibara Y, Suiko M, Sugahara T, & Liu M-C (2012). Concerted actions of the catechol O-methyltransferase and the cytosolic sulfotransferase SULT1A3 in the metabolism of catecholic drugs. *Biochemical Pharmacology*, 84, 1186–1195. doi:10.1016/j.bcp.2012.08.009 [PubMed: 22917559]
- Lagorce D, Sperandio O, Galons H, Miteva MA, & Villoutreix BO (2008). FAF-drugs2: Free ADME/tox filtering tool to assist drug discovery and chemical biology projects. *BMC Bioinformatics*, 9, 396. doi:10.1186/14712105-9-396 [PubMed: 18816385]
- Lemkul JA, & Bevan DR (2013). Aggregation of Alzheimer's amyloid β -peptide in biological membranes: A molecular dynamics study. *Biochemistry*, 52, 4971–4980. [PubMed: 23855340]
- Lipinski CA (2004). Lead-and drug-like molecules: The rule-of-five revolution. *Drug Discovery Today: Technologies*, 1, 337–341. doi:10.1021/bi400562x [PubMed: 24981612]
- Lipinski CA (2016). Rule of five in 2015 and beyond: Target and ligand structural limitations, ligand chemistry structure and drug discovery project decisions. *Advanced Drug Delivery Reviews*, 101, 34–41. doi:10.1016/j.addr.2016.04.029 [PubMed: 27154268]
- Liu F-F, Ji L, Dong X-Y, & Sun Y (2009). Molecular insight into the inhibition effect of trehalose on the nucleation and elongation of amyloid β -peptide oligomers. *The Journal of Physical Chemistry B*, 113, 11320–11329. doi:10.1021/jp905580j [PubMed: 19719268]

- Lu T-M, Chiu H-F, Shen Y-C, Chung C-C, Venkatakrishnan K, & Wang C-K (2015). Hypocholesterolemic efficacy of quercetin rich onion juice in healthy mild hypercholesterolemic adults: A pilot study. *Plant Foods for Human Nutrition*, 70, 395–400. doi:10.1007/s11130-0150507-4 [PubMed: 26385226]
- Mabbitt PD, Correy GJ, Meirelles T, Fraser NJ, Coote ML, & Jackson CJ (2016). Conformational disorganization within the active site of a recently evolved organophosphate hydrolase limits its catalytic efficiency. *Biochemistry*, 55, 1408–1417. doi:10.1021/acs.biochem.5b01322 [PubMed: 26881849]
- Modi K, Panchal U, Dey S, Patel C, Kongor A, Pandya HA, & Jain V (2016). Thiactalix [4] arene-tetra-(quinoline-8-sulfonate): A Sensitive and selective fluorescent sensor for Co (II). *Journal of Fluorescence*, 26, 1729–1736. doi:10.1007/s10895-016-1864-6 [PubMed: 27392975]
- Muller M, Tang M-X, Schupf N, Manly JJ, Mayeux R, & Luchsinger JA (2007). Metabolic syndrome and dementia risk in a multiethnic elderly cohort. *Dementia and Geriatric Cognitive Disorders*, 24, 185–192. doi:10.1159/000105927 [PubMed: 17641531]
- Murphy MP, & LeVine H, III (2010). Alzheimer's disease and the amyloid- β peptide. *Journal of Alzheimer's Disease*, 19, 311–323. doi:10.3233/JAD-2010-1221
- Nitta R, Okada Y, & Hirokawa N (2008). Structural model for strain-dependent microtubule activation of Mg-ADP release from kinesin. *Nature Structural & Molecular Biology*, 15, 1067–1075. doi:10.1038/nsmb.1487
- Parmar R, Highland H, Desai K, Patel C, & George L-B (2015). Pesticide target protein and phytochemical interactions: A computational study mitigating mosquito-vectors.
- Parmar F, Patel C, Highland H, Pandya H, & George L-B (2016). Antiproliferative efficacy of kaempferol on cultured daudi cells: An *in silico* and *in vitro* study. *Advances in Biology*, 2016. doi:10.1155/2016/9521756
- Ritchie TJ, Ertl P, & Lewis R (2011). The graphical representation of ADME-related molecule properties for medicinal chemists. *Drug Discovery Today*, 16, 65–72. doi:10.1016/j.drudis.2010.11.002 [PubMed: 21074634]
- Rocha JF, Almeida L, Falcão A, Palma PN, Loureiro AI, Pinto R, ... Soares-da-Silva P (2013). Opicapone: A short lived and very long acting novel catechol-O-methyltransferase inhibitor following multiple dose administration in healthy subjects. *British Journal of Clinical Pharmacology*, 76, 763–775. doi:10.1111/bcp.12081 [PubMed: 23336248]
- Serrano-Pozo A, Frosch MP, Masliah E, & Hyman BT (2011). Neuropathological alterations in Alzheimer disease. *Cold Spring Harbor perspectives in medicine*, 1, a006189. doi:10.1101/cshperspect.a006189 [PubMed: 22229116]
- Serretti A, & Olgiati P (2012). Catechol-O-methyltransferase and Alzheimer's disease: A review of biological and genetic findings. *CNS and Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS and Neurological Disorders)*, 11, 299–305. doi:10.2174/187152712800672472
- Sliwoski G, Kothiwale S, Meiler J, & Lowe EW (2014). Computational methods in drug discovery. *Pharmacological Reviews*, 66, 334–395. doi:10.1124/pr.112.007336 [PubMed: 24381236]
- Tsao D, Diatchenko L, & Dokholyan NV (2011). Structural mechanism of S-adenosyl methionine binding to catechol O-methyltransferase. *PLoS ONE*, 6, e24287. doi:10.1371/journal.pone.0024287 [PubMed: 21904625]
- Waldner BJ, Fuchs JE, Schauerl M, Kramer C, & Liedl KR (2016). Protease inhibitors in view of peptide substrate databases. *Journal of Chemical Information and Modeling*, 56, 1228–1235. doi:10.1021/acs.jcim.6b00064 [PubMed: 27247997]
- Wang H-W, Noland C, Siridechadilok B, Taylor DW, Ma E, Felderer K, ... Nogales E (2009). Structural insights into RNA processing by the human RISC-loading complex. *Nature Structural & Molecular Biology*, 16, 1148–1153. doi:10.1038/nsmb.1673
- Wolber G, Dornhofer AA, & Langer T (2006). Efficient overlay of small organic molecules using 3D pharmacophores. *Journal of Computer-Aided Molecular Design*, 20, 773–788. doi:10.1007/s10822-006-9078-7 [PubMed: 17051340]
- Wolber G, & Langer T (2005). LigandScout: 3-D pharmacophores derived from protein-bound ligands and their use as virtual screening filters. *Journal of Chemical Information and Modeling*, 45, 160–169. doi:10.1021/ci049885e [PubMed: 15667141]

- Wu L, Rosa-Neto P, Hsiung G-YR, Sadovnick AD, Masellis M, Black SE, ... Gauthier S (2012). Early-onset familial Alzheimer's disease (EOFAD). *The Canadian Journal of Neurological Sciences*, 39, 436–445. doi:10.1017/S0317167100013949 [PubMed: 22728850]
- Xia J, Tilahun EL, Reid T-E, Zhang L, & Wang XS (2015). Benchmarking methods and data sets for ligand enrichment assessment in virtual screening. *Methods*, 71, 146–157. doi:10.1016/j.ymeth.2014.11.015 [PubMed: 25481478]
- Zhou J, & Wishart DS (2013). An improved method to detect correct protein folds using partial clustering. *BMC Bioinformatics*, 14, 11. doi:10.1186/1471-2105-14-11 [PubMed: 23323835]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 1.
2D structures of selected molecules from COMT inhibitors.

Figure 2.

(A) Structural alignment of all selected ligands, (B) Mapped pharmacophore features of all ligands, (C) Pharmacophore model of Opicapone generated by LigandScout (hydrogen bond Donor: green sphere, hydrogen bonds Acceptor: Red sphere and ionizable area: Blue asterisk and Aromatic rings), (D) 2D representation of pharmacophore features.

Figure 3.
Alignment of the co-crystallized ligand and re-docked ligand with the 3A7E protein.

Figure 4.

(A) and (B) Binding modes of DNC with the crystal structure of human COMT complexed with SAM and 3,5-dinitrocatechol, Tranferase protein (PDB code: 3a7e), (C) receptor-ligand interactions, (D) 2D representation of 3,5-dinitrocatechol.

Figure 5.

(A) and (B) Binding modes of Opicapone with the crystal structure of human COMT complexed with SAM and 3,5-dinitrocatecho, Transferase protein (PDB code: 3a7e), (C) receptor-ligand interactions, (D) 2D representation of Opicapone.

Figure 6.

(A) and (B) Binding modes of Fenoldopam with the crystal structure of human COMT complexed with SAM and 3,5dinitrocatecho, Tranferase protein (PDB code: 3a7e), (C) receptor-ligand interactions, (D) 2D representation of Fenoldopam.

Figure 7.

(A) and (B) Binding modes of Quercetin with the crystal structure of human COMT complexed with SAM and 3,5-dinitrocatecho, Tranferase protein (PDB code: 3a7e), (C) receptor-ligand interactions, (D) 2D representation of Quercetin.

Figure 8.

(A) and (B) Binding modes of ZINC63625100_413 with the crystal structure of human COMT complexed with SAM and 3,5-dinitrocatecho, Tranferase protein (PDB code: 3a7e), (C) receptor-ligand interactions, (D) 2D representation of ZINC63625100_413.

Figure 9.

(A) and (B) Binding modes of ZINC39411941_412 with the crystal structure of human COMT complexed with SAM and 3,5-dinitrocatecho, Tranferase protein (PDB code: 3a7e), (C) receptor-ligand interactions, (D) 2D representation of ZINC39411941_412.

Figure 10.

(A) and (B) Binding modes of ZINC63234426_254 with the crystal structure of human COMT complexed with SAM and 3,5-dinitrocatecho, Tranferase protein (PDB code: 3a7e), (C) receptor-ligand interactions, (D) 2D representation of ZINC63234426_254.

Figure 11.

(A) and (B) Binding modes of ZINC63637968_451 with the crystal structure of human COMT complexed with SAM and 3,5-dinitrocatecho, Tranferase protein (PDB code: 3a7e), (C) receptor-ligand interactions, (D) 2D representation of ZINC63637968_451.

Figure 12.

(A) and (B) Binding modes of ZINC64019452_303 with the crystal structure of human COMT complexed with SAM and 3,5-dinitrocatecho, Tranferase protein (PDB code: 3a7e), (C) receptor-ligand interactions, (D) 2D representation of ZINC64019452_303.

Figure 13. Molecular Dynamics analysis: (A) Time vs. Energy, (B) Time vs. Energy, (C) Time vs. pocket Residues, and (D) Time vs. Pocket RMSF Graph plots.

Figure 14.

Schematic representation of the interactions between 3a7e with all simulated molecules by Discovery studio visualizer: (A) Opicapone, (B) Fenoldopam, (C) Quercetin, (D) ZINC63625100_413 ((2R)-N-[3-[2-(2H-benzimidazol-2-yl)ethyl]phenyl]-2H-indole-2-carboxamide), (E) ZINC39411941_412 (N-[3-[(2R)-5-benzoyl-2H-benzimidazol-2-yl]phenyl]cyclopropanecarboxamide), (F) ZINC63234426_254 ((4S)-4-(2H-benzimidazol-2-yl)-1-(1-naphthyl) pyrrolidin-2-one), (G) ZINC63637968_451 (N-[2-(2H-benzimidazol-2-yl)phenyl]-4-benzylbenzamide) and (H) ZINC64019452_303 (2-(2H-benzimidazol-2-yl)-N-phenethyl-benzamide).

Figure 15. Superposition of the simulated complexes (Opicapone, Fenoldopam, Quercetin and ZINC63625100_413) to decipher the changes occurs in the conformation

Figure 16. Superposition of the simulated complexes (ZINC39411941_412, ZINC63234426_254, ZINC63637968_451 and ZINC64019452_303) to decipher the changes occurs in the conformation.

Table 1.

Mapped pharmacophore with distinctive features and confirmations.

Sr. No.	Name	Feature pattern mapped	Pharmacophore-fit	Cluster ID	Confirmation
1	Alpha-methyl-L-Dopa	6	64.82	11	8
2	Apomorphine	6	64.94	11	1
3	Benserazide	6	62.23	1	25
4	Caffeic Acid	6	64.82	2	2
5	Carbidopa	6	64.82	11	6
6	Catechol	6	64.60	9	1
7	DNC	5	58.99	9	1
8	Dobutamine	6	64.82	3	25
9	Dopacetamide	6	64.82	9	8
10	Dopamine Lutine	5	58.99	4	1
11	Entacapone	5	58.99	10	19
12	Fenoldopam	5	58.97	5	2
13	Galllic Acid	5	58.99	8	1
14	GPA1714	5	58.99	9	11
15	Isoprenaline	6	64.82	7	15
16	Nebicapone	5	58.99	6	25
17	Nitecapone	5	55.64	9	14
18	Opicapone	5	58.99	10	8
19	Protocatechuic Acid	6	64.91	8	1
20	Pyrogallol	6	62.19	9	1
21	Quercetin	6	64.82	11	4
22	Rimiterol	6	64.82	7	10
23	U-0521	6	64.91	9	6

Table 2.

Statistics of the selected pharmacophore model validated by validation set.

Particulars	Values
Total number of molecules in validation set (D)	1523
Total number of actives (A)	23
Total number of inactives ^a	1500
Total Hits (Ht)	24
Active Hits (Ha)	23
True positives	23
True negatives	0
False positives	1
False negatives	1499
Sensitivity	1
Specificity	1
Accuracy	1
Enrichment factor (E)	63.46
GH score	0.967

^a Inactives contained 36 Zinc randomized inactives.

Table 3.

Determined ADMET properties.

Sr. No.	Ligands	MW ^a	logP ^b	logD ^c	logSw ^d	tPSA ^e	RotatableB ^f	Rigid B ^g	Flexibility	HBD ^h	HBA ⁱ	HBDHBA
1	Fenoldopam	305.76	2.39	0.76	-3.42	77.3	1	18	0.05	4	4	8
2	Opicapone	413.17	3.42	1.93	-4.7	150.33	3	19	0.14	2	10	12
3	Quercetin	302.24	1.54	1.01	-2.99	131.03	1	18	0.05	5	7	12
4	ZINC64019452	303	3.15	4.77	-3.91	53.82	5	24	0.17	1	4	5
5	ZINC63234426	254	2.52	3.84	-3.61	45.03	2	27	0.07	0	4	4
6	ZINC63637968	451	4.67	6.86	-5.3	53.82	5	30	0.14	1	4	5
7	ZINC39411941	412	2.61	4.89	-3.8	74.13	5	28	0.15	1	5	6
8	ZINC63625100	413	3	4.77	-4.03	66.18	5	28	0.15	1	5	6

^a Molecular weight

^b Partition coefficient

^c Distribution-coefficient

^d Aqueous solubility

^e Topological polar surface area

^f Rotatable bonds

^g Rigid bonds

^h Hydrogen bond donor

ⁱ Hydrogen bond acceptor.

Table 4. Binding energy, hydrogen bonds, and contacting receptor residues of selected COMT ligands.

Sr. No	Ligand	Binding energy (kcal/mol)	No. of hydrogen bonds	Contacting receptor residues
1	Opicapone	7.634	5	Trp 38, Met 40, Asp 141, Trp 143, Lys 144, Asp 169, Asn 170, Pro 174, Glu 199, Arg 201, Mg 215, Sam 216
2	Fenoldopam	7.631	3	Trp 38, Met 40, Asp 141, Lys 144, Asn 170, Pro 174, Leu 198, Glu 199, Mg 215
3	Quercetin	7.561	2	Trp 38, Met 40, Asp 141, Trp 143, Lys 144, Asp 169, Asn 170, Pro 174, Glu 199, Mg 215
4	Apomorphine	7.338	3	Trp 38, Met 40, Asp 141, Trp A 143, Lys A 144, Asp A 169, Asn A 170, Pro A 174, Leu A 198, Glu A 199, Arg A 201, Mg A 215, Sam A 216
5	Nebicapone	7.312	4	Trp 38, Met 40, Asn 41, Trp 143, Lys 144, Asp 145, Asn 170, Pro 174, Leu 198, Glu 199, Mg 215, Sam 216
6	Dobutamine	7.084	3	Trp 38, Met 40, Lys 46, Asp 141, Trp 143, Lys 144, Asp 145, Asp 169, Asn 170, Pro 174, Leu 198, Glu 199, Mg 215, Sam 216
7	Nitecapone	7.004	5	Trp 38, Met 40, Asp 141, Trp 143, Lys 144, Asp 169, Asn 170, Pro 174, Leu 198, Glu 199, Mg 215, Sam 216
8	Rimiterol	6.756	5	Trp 38, Met 40, Asn 41, Asp 141, Trp 143, Lys 144, Asp 145, Asn 170, Pro 174, Leu 198, Glu 199, Mg 215, Sam 216
9	Entacapone	6.591	3	Trp 38, Met 40, Lys 46, Asp 141, His 142, Trp 143, Lys 144, Asn 170, Pro 174, Leu 198, Glu 199, Arg 201, Mg 215, Sam 216
10	DNC	6.343	2	Trp 38, Met 40, Lys 46, Asp 141, His 142, Trp 143, Lys 144, Asp 169, Asn 170, Pro 174, Leu 198, Glu 199, Mg 215, Sam 216
11	Benserazide	6.227	5	Trp 38, Met 40, Asn 41, Asp 141, Trp 143, Lys 144, Asp 145, Asp 169, Asn 170, Pro 174, Leu 198, Glu 199, Mg 215, Sam 216
12	Isoprenaline	6.126	2	Trp 38, Met 40, Asn 41, Asp 141, Trp 143, Lys 144, Asp 145, Asp 169, Asn 170, Pro 174, Leu 198, Glu 199, Mg 215, Sam 216
13	U-0521	6.118	2	Trp 38, Met 40, Asp 141, Trp 143, Lys 144, Asp 169, Asn 170, Pro 174, Leu 198, Glu 199, Arg 201, Mg 215, Sam 216
14	GPA1714	6.092	0	Trp 38, Met 40, Lys 46, Asp 141, Trp 143, Lys 144, Asp 169, Asn 170, Pro 174, Leu 198, Glu 199, Arg 201, Mg 215, Sam 216
15	Caffeic Acid	6.078	2	Trp 38, Met 40, Asp 141, Trp 143, Lys 144, Asp 169, Asn 170, Pro 174, Leu 198, Glu 199, Arg 201, Mg 215, Sam 216
16	Dopamine Lutine	6.064	3	Trp 38, Met 40, Asp 141, Trp 143, Lys 144, Asp 169, Asn 170, Pro 174, Leu 198, Glu 199, Mg 215, Sam 216
17	Carbidopa	5.819	4	Trp 38, Met 40, Asp 141, Trp 143, Lys 144, Asp 145, Asp 169, Asn 170, Pro 174, Glu 199, Mg 215, Sam 216
18	Galllic Acid	5.706	2	Trp 38, Met 40, Lys 46, Asp 141, Trp 143, Lys 144, Asp 169, Asn 170, Pro 174, Leu 198, Glu 199, Mg 215, Sam 216
19	Dopacetamide	5.665	0	Trp 38, Met 40, Lys 46, Asp 141, Trp 143, Lys 144, Asp 169, Asn 170, Pro 174, Leu 198, Glu 199, Mg 215, Sam 216
20	Alpha-methyl-L-Dopa	5.637	3	Trp 38, Met 40, Asp 141, Trp 143, Lys 144, Asn 170, Pro 174, Leu 198, Glu 199, Mg 215, Sam 216
21	Protocatechuic Acid	5.589	5	Trp 38, Met 40, Asn 41, Asp 141, Trp 143, Lys 144, Asp 169, Asn 170, Pro 174, Leu 198, Glu 199, Mg 215, Sam 216
22	Pyrogallol	5.475	4	Trp 38, Met 40, Asn 41, Asp 141, Trp 143, Lys 144, Asp 169, Asn 170, Pro 174, Leu 198, Glu 199, Mg 215, Sam 216

Sr. No	Ligand	Binding energy (kcal/mol)	No. of hydrogen bonds	Contacting receptor residues
23	Catechol	5.317	3	Trp 38, Met 40, Asp 141, Trp 143, Lys 144, Asp 169, Asn 170, Pro 174, Leu 198, Glu 199, Mg 215, Sam 216

Note: The arrangement layout was kept based on best binding energy obtained by every molecules.

Table 5. Binding energy, hydrogen bonds, and contacting receptor residues of selected ZINC molecules.

Sr. No	Ligand	Binding energy (kcal/mol)	Contacting receptor residues
1	ZINC63625100_413 ((2R)-N-[3-[2-(2Hbenzimidazol-2-yl)ethyl]phenyl]-2H-indole-2-carboxamide)	8.278	Trp 38, Met 40, Asp 141, Trp 143, Lys 144, Asp 145, Asn 170, Pro 174, Leu 198, Glu 199, Arg 201
2	ZINC39411941_412 (N-[3-[(2R)-5-benzoyl-2Hbenzimidazol-2-yl] phenyl]cyclopropanecarboxamide)	8.100	Trp 38, Met 40, Trp 143, Lys 144, Asp 145, Asn 170, Pro 174, Leu 198, Glu 199, Arg 201, Mg 215, Sam 216
3	ZINC63234426_254 ((4S)-4-(2H-benzimidazol-2-yl)-1-(1-naphthyl) pyrrolidin-2-one)	7.951	Trp 38, Met 40, Trp 143, Lys 144, Asp 145, Asn 170, Pro 174, Leu 198, Glu 199, Sam 216
4	ZINC63637968_451 (N-[2-(2H-benzimidazol-2-yl) phenyl]-4-benzylbenzamide)	7.946	Trp 38, Met 40, Trp 143, Lys 144, Asp 145, Pro 174, Leu 198, Arg 201
5	ZINC64019452_303 (2-(2H-benzimidazol-2-yl)-Nphenethyl-benzamide)	7.888	Trp 38, Met 40, Trp 143, Lys 144, Asp 145, Asn 170, Pro 174, Leu 198, Glu 199, Sam 216

Note: The arrangement layout was kept based on best binding energy obtained by every molecules.

Table 6.

Superposition of the all simulated complexes.

Sr. No.	MD simulated complexes	RMSD (Å)
1	3a7e - Opicapone	1.6709
2	3a7e - Fenoldopam	1.5904
3	3a7e - Quercetin	1.8154
4	3a7e - ZINC63625100_413	1.6777
5	3a7e - ZINC39411941_412	1.7290
6	3a7e - ZINC63234426_254	1.6381
7	3a7e - ZINC63637968_451	1.7128
8	3a7e - ZINC64019452_303	1.7631