In Silico Evaluation of 18 Kda Translocator Protein Specific Ligands

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Abstract

In-silico technique that successfully integrates the prediction of physicochemical and pharmacokinetic characteristics, molecular docking, QSAR, and ADMET studies were used in an effort to rationalize the search for novel potential therapeutic drugs on the TSPO receptor (PDBID 4UC1). A library of 51 compounds which are TSPO specific was designed based on a lead pharmacophore model. It was found that virtually all of the compounds meet the criteria for physicochemical principles, which is a crucial characteristic for drug-likeness. Almost all of the compounds had favourable docking, and other scores and drug-likeness characteristics. Among the 51 ligand derivatives, it was found that the docking score values range from -13.3299 to -36.2151.In case of binding affinity, values obtained are very good and range from -28 KJ/mole to -61 KJ/mole.

Finally, it was found that N-(2-((dimethylamino)methyl)phenyl)-N-methyl-2-(2-oxo-5-phenylbenzo[d]oxazol-3(2H)-yl)acetamide&N-benzyl-N-methyl-2-(2-oxo-5-phenylbenzo[d]oxazol-3(2H)-yl)acetamide exhibit the best outcomes out of all the analysed ligands on the basis of their docking score, binding affinity and ligand efficiency and may be utilized as a therapeutic in the field of medicine. In this analysis, various kinds of bonds were identified to be present, as given below: hydrophilic interaction, conventional hydrogen

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bonds, hydrophobic interactions: ð-ð stacked and ð-ð T-shaped, Other interactions, Vander Waals forces and halogen. These compounds show great promise as TSPO-targeted options, according to our analysis.

***Keywords**: -Translocator Protein (TSPO), Molecular docking, ADMET &QSAR.*

1. Introduction

To develop a drug that cures a disease in less time and at a cheaper cost is the main aim of most of the drug researchers. Drug design incorporates all the hard work, procedures and strategies followed by the researchers in order to achieve the same [1-3]. Formulating a drug can be achieved via various methods, but the best one would be that which requires lesser time and money investment. A drug prepared by the classical methods requires a large amount of time, money, and it is quite interesting to know that this expenditure can be reduced by switching towards the CADD method [4-6]. Furthermore, since biological structural knowledge and computing power have improved, it is now possible to apply computational tools adequately during different stages of the drug discovery and development process [7-9].

The computer-aided drug design (CADD) is a boon to researchers in the field of pharmacology [10]. The application of various computational techniques and programs in the preparation of a drug for a specific target (receptor)molecule saves the drug from the hardships faced by researchers during the execution of classical methods of drug development (without CADD involvement) [11-13]. This could be understood like this, as in CADD methods one can pick-out virtually several lead molecules which are stronger than the other available molecules before going for the actual selection. The complicated interactions between the drug and the receptor molecule can be imitated virtually for easy understanding [14,15].

The CADD can be implemented in the two kinds of techniques viz., structure-based drug design (SBDD) and Ligand-based drug design (LBDD) based on the structure of the protein and the biological and physicochemical characteristics of the bound ligand [16,17]. One can separate a few most potent ligands from a large number of available ones by scoring them and then by applying molecular docking, very easily observe and understand the binding of the probably the strongest ligand to the target molecule, and that too in the probably the most correct orientation. This is done while going with the structure-based drug design method by the application of tools like virtual screening, molecular docking and scoring functions [18].

Apart from that, the quantitative structure-activity Relationship (QSAR), ligand-based pharmacophore modelling and scaffold hopping etc. can be utilized to achieve the expected target-ligand interaction and hence, the preparation of a drug for the required disease in the case of ligand- based drug design. CADD, after including the concepts like machine learning (ML), deep learning (DL) and artificial

intelligence (AI), in the preparation of a drug for a specific disease, has become more convenient in terms of selection, optimization and interaction of the correct drug with the most suitable receptor molecule. If we talk about these concepts, machine learning it enables us to convert this complicated process into a simulated pattern in order to develop an understanding of the vital information of both the interacting partners, i.e., the drug and the receptor molecule. While the deep learning provides access to pick up the molecules with the help of specific forms and in speedily spotting of illness and, hence, its treatment. Apart from that, this high-dimension data integration of AI and ML and its potent capabilities have advanced. Predicting the results of clinical trials using AI/ML integrated Models has the potential to minimize the cost of those trials while increasing the likelihood of success [19, 20].

Translocator Protein (TSPO) Protein Data Bank (RCSB PDB): The protein with PDBID 4UC1 was downloaded from RCSB Protein Data Bank. Translocator protein (TSPO) has a very strong biological importance (18 kDa), such as trans membrane transporter binding, +ve regulation of necrotic cell death and a component of the mitochondrial outer membrane, etc [20].

2. Methodology

2.1. Preparation of ligand

TSPO, a Translocator protein present in the outer mitochondrial membrane, binds the drug molecule. We started with three pharmacophore structures, as seen in the first, second, and third pharmacophore structures of TSPO PET tracers represented below. By making different replacements on different orientations, we were able to produce 51 derivatives. We had drawn the structures and obtained their IUPAC names, physical and chemical properties, with the help of software, viz. ChemDraw Professional 16.O by PerkinElmer [Figure 1].

Select the desired ligand derivative from the table (prepared before). Copy and paste on Chem3D, then perform energy minimization by clicking MM2, or go to calculation, then go to MMFF94. Then perform minimization, then click on run. This gives the value of dipole /dipole and total energy in K Cal/mole after the calculation ended. Save the file in mol.2 format.

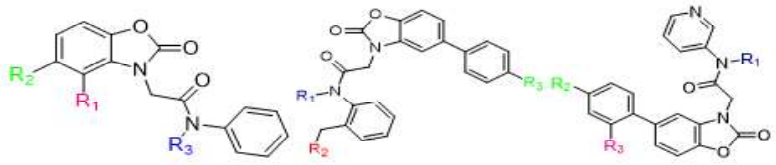


Figure 1: Ligands structures on the basis of different permutations & orientation

In the series, docking of fifty-one molecules has been performed with TSPO protein PDB-ID (4UC1) whose nucleus was "benzoxazolone", The benzoxazolone nucleus is an excellent scaffold for drug design because of its distinct physicochemical profile, bioisosteric preference over pharmacokinetically weaker moieties, weakly acidic behaviour, presence of both lipophilic and hydrophilic fragments on a single framework, and a wider range of chemical properties. These are organic substances that have a benzene ring attached to an oxazole ring, an aliphatic ring with five members that consists of three carbon atoms, one oxygen atom, and one nitrogen atom, and which also bears a ketone group. The different structures were prepared by substituent addition like +I (-O-, -COO-, $-CR_3$, $-CHR_2$, $-CH_3$, etc.), -I (X = Halogens like Cl, Br, I, etc.), +M, -M, groups and alteration in the position of already attached functional group. The process of docking done by LEADT software, by analysing the magnitude of HYDE scoring assessment, one can predict the best orientation of the ligand possible for protein interaction.

2.2. Preparation of receptor molecule

Downloaded a protein from the PDB bank with ID 4UC1. Open the Lead IT software, then molecule, then prepare receptor, then open the downloaded (previously) protein as shown in Figure 2, i.e., 4UC1, then click on the arrow. Select all the three chains A, B and C. Then go for automatic detection (here amino acid residue radius was taken 6.4 Å. Selected all the pockets of the protein, viz. Pocket A, B, C, D and E. Clicking on the next arrow followed by the run button we get a prepared protein molecule ready to dock with the prepared ligand molecule.

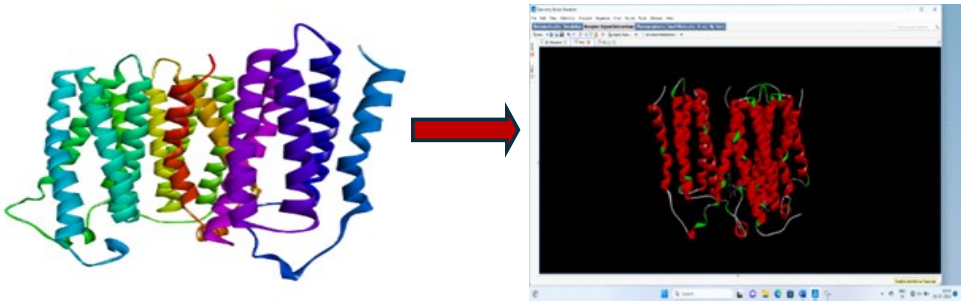


Figure 2: Preparation of receptor molecule

Click on docking, then select molecular Flex docking, then a dialogue box appears which asks for a certain ligand, here we have to load the ligand molecule (prepared in mol.2 Format). Click on OK now, select the top 10 poses on the top left side of the same dialogue box. Now, click on apply and dock. This gives a tabular data of the top 10 poses with essential details, viz., docking score, ligand

efficiency, binding energy, lipophilicity, etc. Among these top ten poses, we have to select the best pose depending on the high value of docking score, binding energy and ligand efficiency. The selected pose is saved as a file in .sdf format. The whole top ten poses are saved as a project file in .fxx format. The overall details of the selected pose are saved in a tabular form for each of the 51 ligands in a Word file.

2.3. ADME

The analysis of physicochemical properties, lipophilicity, solubility, Pharmacokinetics, drug likeness and various inhibitor interactions or impacts were done as ADME. Now, with the help of an online software, SwissADME, the further analysis of physicochemical properties, lipophilicity, solubility, pharmacokinetics, drug likeness and various inhibitor interactions or impacts were done as ADME. SwissADME software was employed to evaluate ADMET properties. The molecular drawing interface was opened via the "Draw Molecule" option on the SwissADME homepage, and the prepared structure was submitted for analysis. Structures could also be generated from SMILES (Simplified Molecular Input Line Entry Specification), a feature available in the free version of ChemSketch, though it is not commonly used by general users. Alternatively, molecules were manually drawn in ChemSketch. The ADMET parameters assessed included molecular weight, log P (partition coefficient), log S (solubility), Lipinski's rule of five, hydrogen bond donors and acceptors, and blood–brain barrier permeability.

2.4. QSAR

In QSAR analysis, the StarDrop software was utilized following a structured workflow. The dataset was first formatted and imported into StarDrop, after which appropriate molecular descriptors were selected based on relevant physicochemical properties. Lipinski's rule of five was then applied to evaluate drug-likeness and identify core features. Graphical visualization enabled the recognition of patterns and correlations, and subsequent comparisons were performed to reveal relationships and support predictive model development.

3. Result and Discussion

3.1. Docking studies of selected compound

The analysis of the above results for the interaction of ligands of series of 51 ligand derivatives with the TSPO protein molecule (PDBID 4UC1) based on docking score and binding affinity we have found that's 12 ligands showing most favourable and showing their best result with more negative docking score and better hyde score therefore based on below mentioned docking result the ligands were renamed as M-01 to M-12 as shown in Table 1.

Table 1: Docking results of PDE10A derivatives with PDBID:4UC1.

Compound	Compound Renaming	HS (KJ/mol)	DS	LE	MATCH	LIPO	AMBIG	CLASH	ROT	Match
14	M-01	-61	-13.3299	0.47	-8.9103	-21.0299	-6.2468	11.857	5.6	21
13	M-02	-57	-23.0932	0.49	-17.1245	-21.7442	-7.2136	11.9891	5.6	31
8	M-03	-52	-28.1393	0.43	-18.9571	-20.0739	-5.2762	5.168	5.6	27
36	M-04	-52	-22.4213	0.44	-17.2551	-17.5509	-6.2106	7.5953	5.6	29
4	M-05	-47	-29.8175	0.42	-19.2018	-20.7734	-6.2482	8.206	2.8	27
22	M-06	-44	-30.3797	0.36	-23.4527	-18.5417	-5.5936	7.6084	4.2	32
39	M-07	-44	-25.8191	0.33	-15.882	-19.8348	-7.8244	3.9221	8.4	25
34	M-08	-42	-27.0615	0.35	-12.6165	-19.9541	-11.0055	6.9147	4.2	18
19	M-09	-41	-28.8392	0.36	-15.1196	-18.0513	-6.9889	3.1206	2.8	22
11	M-10	-40	-21.8808	0.38	-13.7676	-19.6775	-5.7383	9.1026	2.8	27
1	M-11	-39	-24.2147	0.44	-20.1109	-12.5837	-4.2669	4.5467	2.8	23
31	M-12	-33	-23.0645	0.26	-9.9862	-20.9671	-7.0477	5.3365	4.2	24

Docking Score (DS), hyde score (HS), ligand efficiency (LE), lipophilicity contact area (LIPO), ambiguous score (AMBIG); clash penalty score (CLASH).

The docking study of all designed TSPO derivatives against the protein (PDB ID: 4UC1) shows that most of the compounds bind well inside the active site of the target enzyme. The docking score (DS) is an important parameter to judge binding strength; more negative values indicate stronger and more stable binding. In case of binding affinity, Values obtained are very good and range from -28 KJ/mole to -61 KJ/mole. The most common value is -37 KJ/mole and is shown by nine molecules. Among all the derivatives, compound M-01 showed the best binding affinity with the lowest docking score (-61 kJ/mol). This means M-01 fits very well inside the binding pocket and forms strong interactions with the amino acid residues of the protein. The next best compounds were M-02 (-57 kJ/mol) and M-03/M-08 (around -52 kJ/mol), which also demonstrated good binding stability. The ligand efficiency (LE) values for most compounds ranged between 0.33 and 0.49, indicating that these molecules bind efficiently relative to their size. Higher LE values suggest that the compounds are using their chemical structure effectively to interact with the target site. The lipophilic contact area (LIPO) values were largely negative, which shows that hydrophobic interactions play a major role in stabilizing the ligand–protein complex. These hydrophobic contacts help the ligand stay firmly inside the active pocket of TSPO.

The ambiguous score (AMBIG) and clash score (CLASH) provide information about the quality of fitting. Moderate AMBIG values indicate that the ligand orientation is clear and stable, while acceptable CLASH values show that

there are minimal steric hindrances between ligand atoms and protein residues. This confirms that the designed molecules are properly accommodated in the binding cavity without unfavourable overlaps.

Rotatable bonds (ROT) describe molecular flexibility. Compounds having moderate flexibility were able to adjust their conformation to fit better into the binding site, which improved their docking performance. However, excessive flexibility did not always result in better binding, suggesting that an optimal balance between rigidity and flexibility is important for stable interaction.

Overall, the docking results clearly suggest that compounds M-01, M-02, and M-03 exhibit the most promising interaction profiles with the PDE10A enzyme. Their strong binding affinity, good ligand efficiency, Favorable hydrophobic contacts, and low steric clashes indicate that these molecules could serve as potential lead candidates. These findings support further optimization and experimental validation of these derivatives for the development of effective PDE10A-targeted therapeutic agents.

Two compounds, N-(2-((dimethylamino)methyl)phenyl)-N-methyl-2-(2-oxo-5-phenylbenzo[d]oxazol- 3(2H)-yl)acetamide and N-benzyl-N-methyl-2-(2-oxo-5-phenylbenzo[d]oxazol-3(2H)-yl)acetamide, Emerged as top performers among the analysed ligands, showing the best docking scores, binding affinity, And ligand efficiency. These compounds hold potential as therapeutic agents in medicine.

3.2. Computational ADME assessment and predictive outcomes

The pharmacokinetic and drug-likeness evaluation of the selected TSPO derivatives (M-01 to M-12) demonstrates that most compounds possess favourable ADME properties, as shown in Table 2.

The molecular weight of the molecules ranges approximately from 282.29 to 434.46 g/mol, which lies within the acceptable limit for oral drug candidates, suggesting good permeability and absorption potential. The hydrogen bond acceptor (HBA) values vary between 3 and 6, while hydrogen bond donor (HBD) values remain 0–1, indicating compliance with Lipinski's rule of five and supporting good membrane permeability. The molar refractivity values (80.47–123.38) and TPSA values (52.21–68.34 Å^2) suggest balanced polarity and suitable bioavailability. Log P values ranging from 2.41 to 4.92 reflect optimal lipophilicity, favouring effective interaction with biological membranes, whereas Log S values (-3.37 to -5.69) indicate moderate aqueous solubility.

Most derivatives exhibit positive BBB permeability, implying their ability to cross the blood–brain barrier, which is crucial for targeting TSPO in the central nervous system. Additionally, the majority of compounds are non-substrates for P-gp and show minimal inhibition of CYP1A2 and CYP2C19 enzymes, suggesting a

lower risk of efflux and metabolic drug–drug interactions. Overall, the ADME profile confirms that these derivatives possess suitable pharmacokinetic characteristics and promising drug-likeness for further development as TSPO-targeting therapeutic agents, as shown in **Table 3**.

Table 2: Pharmacokinetics and drug- likeness properties of selected derivatives of TSPO

Compound	MW	HBA	HBD	MREFR ACTIVITY	TPSA	LogP	LogS	BBB	P- GP	DRU GLI KEN ESS	CYP 1A2 INHI BIT ORS	CYP 2C19 INHI BIT ORS
M-01	415.48	4	0	123.38	58.69	3.87	-4.89	YES	NO	NO	YES	YES
M-02	372.42	3	0	109.18	55.45	3.97	-4.81	YES	NO	YES	YES	YES
M-03	386.44	3	0	115.52	55.45	4.29	-5.01	YES	NO	YES	YES	YES
M-04	374.39	4	0	106.99	64.68	3.94	-4.8	YES	NO	YES	YES	YES
M-05	358.39	3	0	105.91	55.45	4.03	-4.85	YES	NO	YES	YES	YES
M-06	388.42	4	0	112.4	64.68	4	-4.91	YES	NO	YES	YES	YES
M-07	434.46	5	0	112.06	64.68	4.71	-5.39	YES	NO	NO	YES	YES
M-08	388.42	4	0	112.4	64.68	4	-4.91	YES	NO	YES	YES	YES
M-09	359.38	4	0	103.7	68.34	3.3	-4.39	YES	NO	YES	YES	YES
M-10	329.35	3	0	96.89	52.21	4.36	-4.99	YES	NO	YES	YES	YES
M-11	282.29	3	0	80.47	55.45	2.41	-3.37	YES	NO	YES	YES	YES
M-12	426.39	6	0	110.91	55.45	4.92	-5.69	NO	NO	YES	YES	YES

Molecular weight (MW), Hydrogen Bond Acceptor (HBA), Hydrogen Bond Donor (HBD), Molar Refractivity (M-REFRACTIVITY), Rotatable bond (ROT), Topological Polar Surface Area (TPSA), Partition coefficient (LOGP), Solubility (LOGS), Blood Brain Barrier (BBB),P-Group Substrate; CYP1A2 INHIBITOR & CYP2C19 INHIBITOR types of cytochrome inhibitors.

Table 3: Overview of the ADME study results of 51 ligand derivatives.

S.No	ADMEReport	Range	Highestvalue
1.	Molecularweight	269.25 g/mol – 451.52 g/mol	451.52 g/mol
2.	Hydrogenbondaccepter(HBA)	2-8	8
3.	Hydrogenbonddonor(HBD)	0-1	1
4.	M-Refractivity	73.75 – 136.67	136.67
5.	TPSA	38.38 – 110.50	110.50
6.	LogP	1.89 – 5.27	5.27
7.	LogS	(-3.05) – (-6.04)	(-6.04)

3.3. Binding interaction and interpretation of selected compound -

Discovery Studio is a molecular modelling software used to analyse small molecules and macromolecular systems such as proteins. It provides a clear visualization and structural analysis of molecules. The software is developed and distributed by BIOVIA and supports academic research. It is applied in ligand library design and optimization, pharmaceutical modelling, including model building and

virtual screening, validation and rational design of macromolecules, and macromolecular engineering for scientific investigations, as shown in Figure 3.

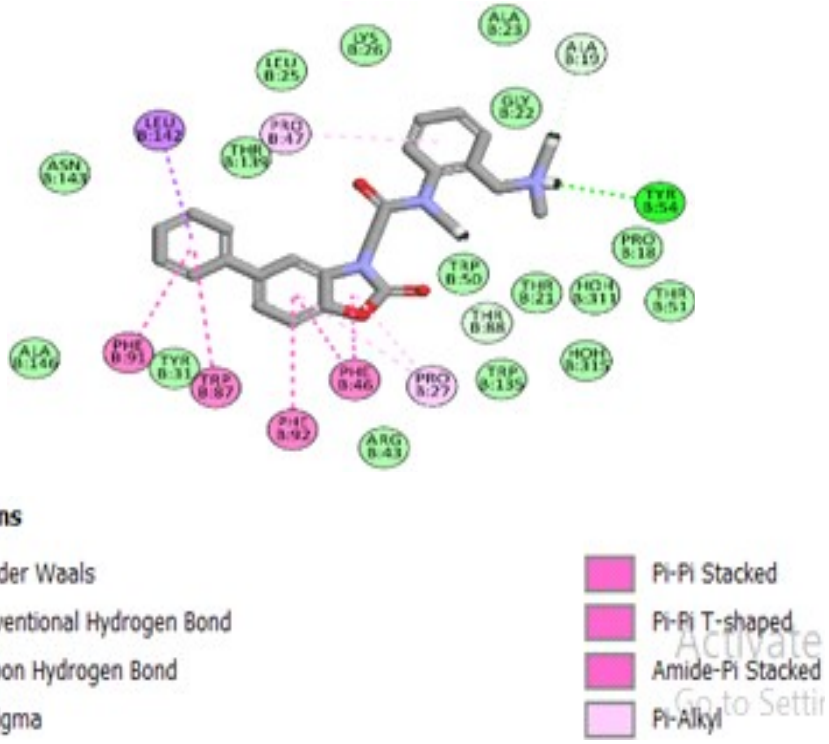


Figure 3:Binding interaction and interpretation of selected compound

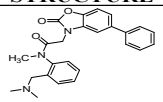
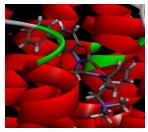
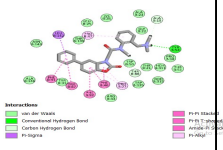
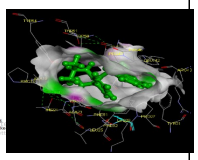
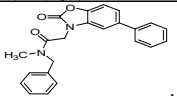
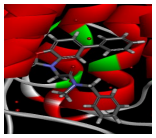
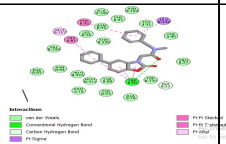
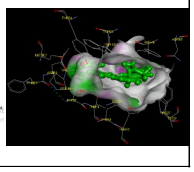
The 2-D interaction study shows how the ligands bind inside the active site of the TSPO protein and what types of interactions help in stable binding. Hydrogen bond interactions are mainly formed with ARG-43 and TRP-50 residues, which help in stabilizing the ligand in the binding pocket. Attractive charge interactions are observed with residues like PHE-46 and PHE-92, contributing to electrostatic stability. In addition, several hydrophobic and van der Waals interactions with residues such as GLY-22, ALA-23, LEU-25, TYR-31, TRP-39, TRP-44, TRP-87, THR-88, THR-139, and ASN-84 further strengthen the ligand–protein complex. The involvement of aromatic residues like TRP and PHE also enhances binding through ð–ð interactions. Overall, the results show that the ligands fit well into the TSPO binding pocket and form stable interactions, indicating their potential as effective TSPO-targeting drug candidates, as demonstrated in Tables 4 and 5.

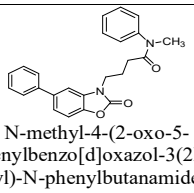
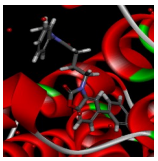
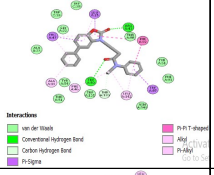
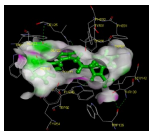
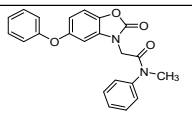
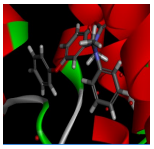
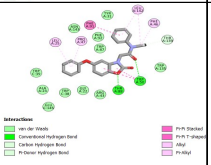
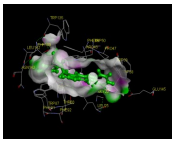
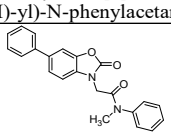
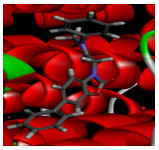
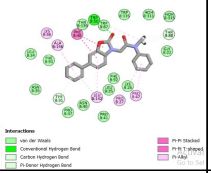
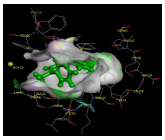
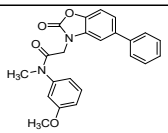
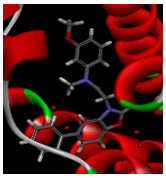
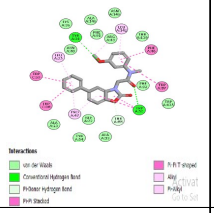
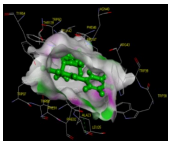
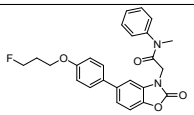
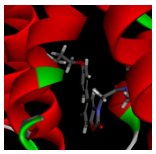
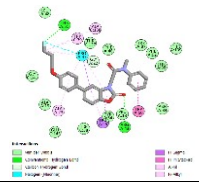
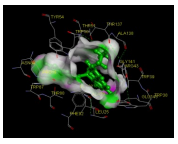
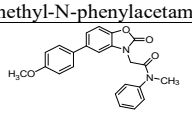
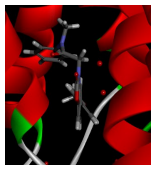
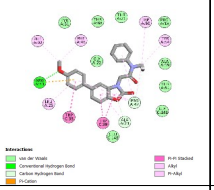
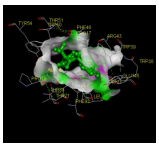
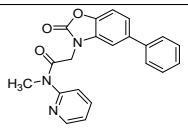
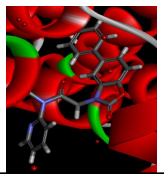
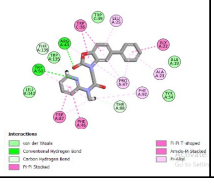
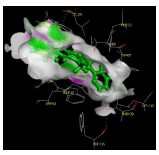
Table 4: Interaction results of TSPO derivatives with PDBID:4UC1

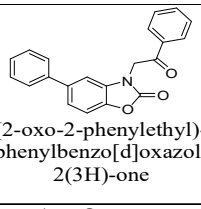
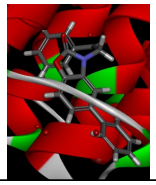
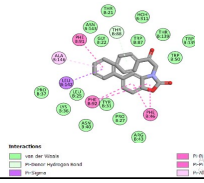
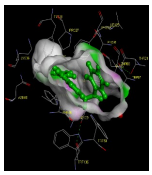
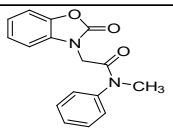
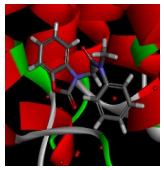
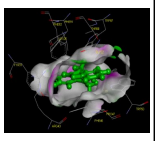
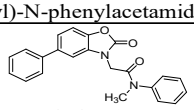
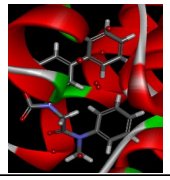
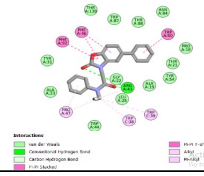
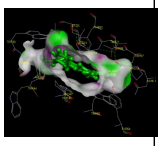
Compound	H-Bond	HBI(Pi-Pi bond)	VanderWaals	Halogen Bond
M-01	TYR-54	PHE-46 TRP-87 PHE-91 PHE-92	PRO-18 THR-21 GLY-22 ALA-23 LEU-25 LYS-26 TYR-31 ARG-43 TRP-50 THR-51 THR-135 THR-139 ASN-143 ALA-146	
M-02	TRP-50	TRP-50 TRP-87 PHE-92	PRO-18 ALA-19 PRO-27 TYR-31 LYS-37 ASN-40 PHE-46 TYR-54 PHE-83 ASN-84 THR-88 PHE-51 VAL-104 TRP-135 THR-139 ASN-143 ALA-146	
M-03	ARG-43 TRP-50	PHE-92	GLY-22 ALA-23 TYR-31 TRP-38 TRP-39 THR-51 TYR-54 THE-88 PHE-51 TRP-135 ASN-143	
M-04	TRP-50 THR-88	PHE-91	GLY-22 ALA-23 TYR-31 TRP-38 TRP-39 ARG-43 TRP-87 PHE-92 TRP-135 ASN-143 GLU-145	
M-05	TRP-50	PHE-46	GLY-22 LEU-25 LYS-26 LEU-34 ASN-35 PRO-37 ASN-40 PRO-41 TRP-87 PHE-91 PHE-92 TRP-135 TRP-139	
M-06	TYR-31 TRP-50	TRP-38 TRP-39 PHE-46 TRP-50 TRP-87	ALA-19 GLY-22 ALA-23 LYS-36 ASN-40 ARG-43 TYR-54 PHE-91 PHE-92 TRP-135 THR-139 ASN-143	
M-07	ARG-43 THR-51	TRP-50	PRO-18 ALA-19 TRP-38 PHE-46 LEU-38 TYR-54 ASN-84 TRP-87 THR-88 THE-92 THR-137 THR-139 GLY-141 GLU-135	PRO-47
M-08	ARG-43	TRP-38 TRP-39	PRO-18 ALA-19 THR-21 GLY-22 TYR-31 THR-51 THR-88 GLY-141 GLU-145	
M-09	ARG-43 TRP-50	GLY-22 TRP-38 PHE-46 TRP-87	ALA-19 TRP-39 TYR-54 TRP-135 LEU-142	
M-10		PHE-46 PHE-91 PHE-92	THR-21 GLY-22 LEU-25 PRO-27 TYR-31 LYS-36 PRO-37 ASN-40 ARG-43 TRP-50 TRP-87 TRP-135 THR-139 ASN-143	
M-11	ARG-43TRP-50	PHE-46 PHE-92	GLY-22 TYR-31 ASN-40 TRP-87 THR-88 PHE-91 TRP-135 THR-139 ASN-143	
M-12	TRP-50	TRP-38 PHE-46 PHE-92	PRO-18 ALA-19 THR-21 ALA-23 TYR-31 TYR-54 TRP-87 HOH-313	GLY-22 GLU-145

Tyrosine (TYR), Phenylalanine (PHE), Proline (PRO), Threonine (THR), Glutamine (GLY), Leucine (LEU), Lysine (LYS), Arginine (ARG),Tryptophan (TRP), Asparagine (ASN), Alanine (ALA), Valine (VAL), Glutamine (GLU).

Table 5: 2D and 3D interaction images of selected derivatives with TSPO protein before and after binding

Sr. No.	MOLECULE STRUCTURE	3D (BEFORE BINDING)	2D INTERACTION	3D (AFTER BINDING)
M-01	 N-(2-((dimethylamino)methyl)phenyl)-N-methyl-2-(2-oxo-5-phenylbenzo[d]oxazol-3(2H)-yl)acetamide			
M-02	 N-benzyl-N-methyl-2-(2-oxo-5-phenylbenzo[d]oxazol-3(2H)-yl)acetamide			

M-03	 N-methyl-4-(2-oxo-5-phenylbenzo[d]oxazol-3(2H)-yl)-N-phenylbutanamide			
M-04	 N-methyl-2-(2-oxo-5-phenoxybenzo[d]oxazol-3(2H)-yl)-N-phenylacetamide			
M-05	 N-methyl-2-(2-oxo-6-phenylbenzo[d]oxazol-3(2H)-yl)-N-phenylacetamide			
M-06	 N-(3-methoxyphenyl)-N-methyl-2-(2-oxo-5-phenylbenzo[d]oxazol-3(2H)-yl)acetamide			
M-07	 2-(5-(4-(3-fluoropropoxy)phenyl)-2-oxobenzo[d]oxazol-3(2H)-yl)-N-methyl-N-phenylacetamide			
M-08	 2-(5-(4-methoxyphenyl)-2-oxobenzo[d]oxazol-3(2H)-yl)-N-methyl-N-phenylacetamide			
M-09	 N-methyl-2-(2-oxo-5-phenylbenzo[d]oxazol-3(2H)-yl)-N-(pyridin-2-yl)acetamide			

M-10	 3-(2-oxo-2-phenylethyl)-5-phenylbenzo[d]oxazol-2(3H)-one			
M-11	 N-methyl-2-(2-oxobenzo[d]oxazol-3(2H)-yl)-N-phenylacetamide			
M-12	 N-methyl-2-(2-oxo-5-phenylbenzo[d]oxazol-3(2H)-yl)-N-phenylacetamide			

3.4. QSAR

While working with StarDrop software, I analysed graphical plots that compared the magnitude of various molecular descriptors between the reference compound and the isolated ligand. The values shown in the QSAR graphs represent physicochemical or biological descriptors that influence a molecule's drug likeness and binding behaviour.

MW vs BBB

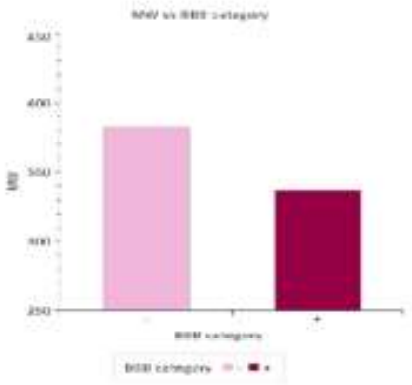


Figure 4. Molecular Weight Vs Blood Brain Barrier

The numeric values on the y axis indicate the count or magnitude of molecules in each category. A higher pink bar means more compounds possess the favourable property (e.g., better solubility or lower molecular weight), which is desirable for drug design as shown in Figure 4.

Overall QSAR Analysis and Key Observations -

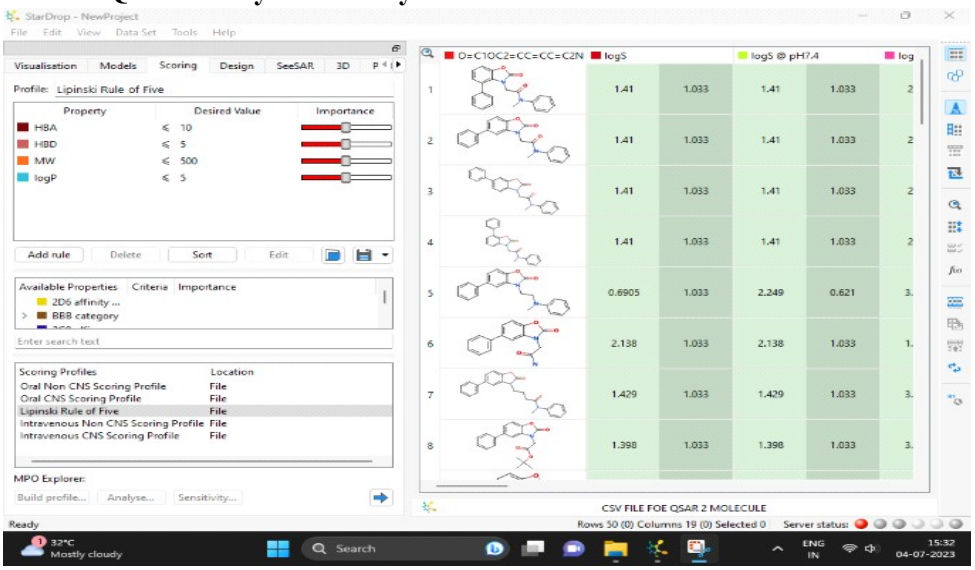


Figure 5. Important properties to study through QSAR based upon Lipinski rule of five.

These values are analyzed to ensure the ligands obey Lipinski's rule of five (d"/ 5 H bond donors, d"/ 10 H bond acceptors, MW/ </ 500, log/ P/ </ 5), which predicts oral bioavailability and informs modifications to improve docking scores and binding affinity with the TSPO protein. The distribution indicates which value ranges are most common, guiding optimization toward those efficient ranges as shown in Figure 5.

4. Conclusion and Future Aspects

In this work, the ultimate goal was to rationalize the search for novel potential therapeutic initially, the TSPO PET tracers having the N-(2-((dimethylamino)methyl)phenyl)-N-methyl-2-(2-oxo-5-phenylbenzo[d]oxazol-3(2H)-yl)acetamide&N-benzyl-N-methyl-2-(2-oxo-5-phenylbenzo[d]oxazol-3(2H)-yl)acetamide core which by various substitution(s) provided various ligand molecules which were then analysed for their physicochemical and ADME analysis. Secondly, upon docking with the TSPO Protein (PDBID 4UC1) it was found that molecule

no. 13 (M-01) and molecule no. 14 (M-02), come out to be the best out of all the ligands on the Basis of their docking score, binding affinity and ligand efficiency. The work explores the principles of drug design and discovery, highlighting the importance of computational software and methodologies in streamlining the process. Researchers can design and develop eco-friendly drugs with reduced toxicity and environmental impact. The concept of bioisosterism, a lead modification technique, is particularly effective in minimizing toxicity and altering the activity of lead compounds, while also optimizing their pharmacokinetics.

The integration of computational methods, such as docking and QSAR analysis, has revolutionized the drug discovery process, reducing the need for resource-intensive laboratory experimentation. LEADIT software, utilized in this study, has facilitated the novel synthesis of ligands with potential therapeutic applications. By applying computational chemistry metrics, such as the HYDE scoring function and physicochemical properties, researchers can identify lead compounds with improved reduced toxicity.

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