# ACTIVE SITE STRUCTURE PREDICTION, VIRTUAL SCREENING AND MOLECULAR DOCKING FOR LIGANDS OF THE REVERBA ORPHAN NUCLEAR RECEPTOR/DNACOMPLEX:1A6Y

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***Abstract:*** **An orphan receptor is a protein that has a similar structure to other identified receptors but whose endogenous ligand has not yet been identified. If a ligand for an orphan receptor is later discovered, the receptor is referred to as an "adopted orphan". The orphan receptor REVERBA orphan nuclear receptor/DNA complex is studied. The active site is predicted for this protein so further docking studies as well as ligand that are optimally bound to the particular protein can be found through virtual screening and docking studies by using the software such as Pyrx, Autodock. Later on, the toxicity information has been denoted by the use of SwissADME software.**

***Index Terms*** **– Orphan Receptor, ReV-Erba, indole, Pyrx, Auto dock, binding affinity, virtual screening, molecular docking**

## I. INTRODUCTION

Classification: TRANSCRIPTION/DNA
Organism (s): Homo sapiens
Doi: 10.2210/pdb1A6Y/Pdb
Expression system: Escherichia coli BL21

Nuclear receptor subfamily 1 group D member 1 (NR1D1) or simply REV-ERB alpha (Rev-Erba), located on chromosome 17, is one of two Rev-Erb proteins in the nuclear receptor (NR) family of intracellular transcription factors that encodes the protein REV-ERBa in humans. NR1D1 and THRA cDNA are complementary on 269 bases as they are transcribed from the opposite strand of the human thyroid hormone receptor alpha (THRA, c-erbAα). Human brain and metabolic tissues are the most expressed sites of NR1D1 (REV-ERBα).

## II. FUNCTION

**Role of Rev-erb alpha in metabolism and molecular clock:**

The Rev-erb alpha plays an important role in stabilizing circadian oscillations. This gene expression in skeletal muscle, kidney and thymus of mice was linked to circadian oscillations and metabolism. Periodic circadian rhythms were shortened and impaired cholesterol and bile acid metabolism were observed due to the lack of this nuclear receptor [1]. This gene was also found to be involved in lipid metabolism [2]. Mice lacking Rev-erb alpha demonstrated impaired regulation of body temperature. Various polymorphic Rev-erb alpha genes in humans were associated with obesity. Upregulation of this gene in visceral adipose tissue was observed in obese women [3]. Which shows that there is a direct correlation between Rev-erb alpha and the BMI. Rev-erb alpha shows negative effect on the genes involved in muscle differentiation. Rev-erb alpha affects functioning of mitochondria by altering the AMPK (AMP activated protein kinase) pathway in skeletal muscle. Deficiency of Rev-erb alpha leads to inhibition of the liver kinases and signaling pathways in skeletal muscle.

Both Rev-erb alpha and Rev-erb beta, were known to involve in circadian rhythm generation. Lack of Rev-erb alpha and Rev-erb beta repealed the circadian gene expression and loss of rhythmicity, wheel running behavior in mouse [4]. Reverb alpha represses hydroxylase enzyme involved in the biosynthesis of dopamine in mid brain and hippocampus parts of brain. In mice lacking Rev-erb alpha, impaired memory, novelty and hyperactivity are observed due to impaired hippocampus functioning whereas aggression, anxiety and depression-like behaviors are observed due to impaired functioning of midbrain. Synthetic Rev-erb ligands altered the circadian behavior and increased mitochondrial content and exercise capacity in skeletal muscle.

**Role of Rev-erb alpha in insulin and glucagon secretion**:

Rev-erb alpha is expressed in the pancreas of rat, mouse, and in human islets. The lipogenic genes sterol regulatory element binding protein 1c (Srebp-1c) and fatty acid synthase (Fas) were expressed in smaller concentrations with the small interfering RNA (siRNA) which was used to examine the role of Rev-erb alpha in MIN6 cells and in pancreatic islets [5]. Down-regulation of Rev-erb alpha in MIN6 cells decreased the proliferation but did not affect the apoptosis which was also related to the changes in the genes regulating islet growth and development. Treatment with siRNA led to the down-regulation of Rev-erb alpha that led to the impaired glucose-stimulated insulin secretion (GSIS), but this does not lead to insulin expression or insulin protein content, but led to decreased expression of genes like Vamp3, Munc18, Snap25 and Syntaxyn1A that are important in exocytosis. Lean mice treated with a normal diet exhibited a circadian pattern in isolated islets with Rev-erb alpha expression. In obese mice circadian expression was altered from lean mice. Further studies showed that the function of this gene affects insulin secretion. Hemin which is a natural ligand of Rev-erb alpha when used in MIN6 cells along with synthetic modulators, caused rapid GSIS change and thus effects the insulin secretion in the pancreatic β-cell [6]. Rev-erb alpha plays an important role in exocytosis and

glucagon secretion. It is expressed in mouse at low glucose concentrations. Elevated glucose levels decrease Rev-erb alpha levels in mouse pancreatic α-cells. Activation of AMPK protein in αTC1-9 cells, which plays a key role in glucose-modulated glucagon secretion, by metformin affects Rev-erb alpha expression. NAMPT inhibitor incubated αTC1-9 cells led to the decreased Rev-erb alpha expression and glucagon release at low glucose concentrations. Hemin treated αTC1-9 cells increased glucagon release at both low and high glucose levels. SR8278, a Rev-erb alpha antagonist showed the opposite effect on glucagon release and regulates the activity of $Ca^{+2}$ channels [7].

## III. INTRODUCTION TO INDOLES

Synonyms: 2,3-benzopyrrole, ketole 1-benzazole.

Indole is an aromatic six-membered ring compound, fused to pyrrole. It is the main chemical constituent in amino acid tryptophan. It is present in tryptophan-containing proteins, alkaloids, and in pigments. It is also the main chemical constituent in plant hormone auxin (indole-3-acetic acid), the ant-inflammatory drug indomethacin, and the β-blocker pindolol. Indole is the most important moiety in number of biologically active heterocycles like benzofuran, benzimidazole, pyrimidines etc. Largely this is due to the varied biological activity of many natural indole derivatives which created an interest in their structure elucidation and synthesis. This has led to the preparation of many of its synthetic analogues as potential chemotherapeutics. In recent years, mechanistic studies have clarified the theoretical understanding of indole and its reactivity.

Indole has lot of importance in the synthesis of natural products and pharmaceutical products. Various methods are developed for the preparation of indoles. Newer methodologies are emerging from time to time for the synthesis of indole. Numerous methods exist for the preparation of indole (Fig.1) and its derivatives [8]. However, Fischer indole synthesis is the most widely used strategy. Acid catalysis of aryl hydrazone or thermal [3,3]-sigma tropic rearrangement of an ene-hydrazine tautomer generates the indole skeleton, via elimination of ammonia. Various protonic acid catalysts like HCl/EtOH, $H_2SO_4$, PPA, $OEt_2$/AcOH, polyphosphoric acid trimethylsilyl ester (PPSE) were reported giving rise to higher yields.

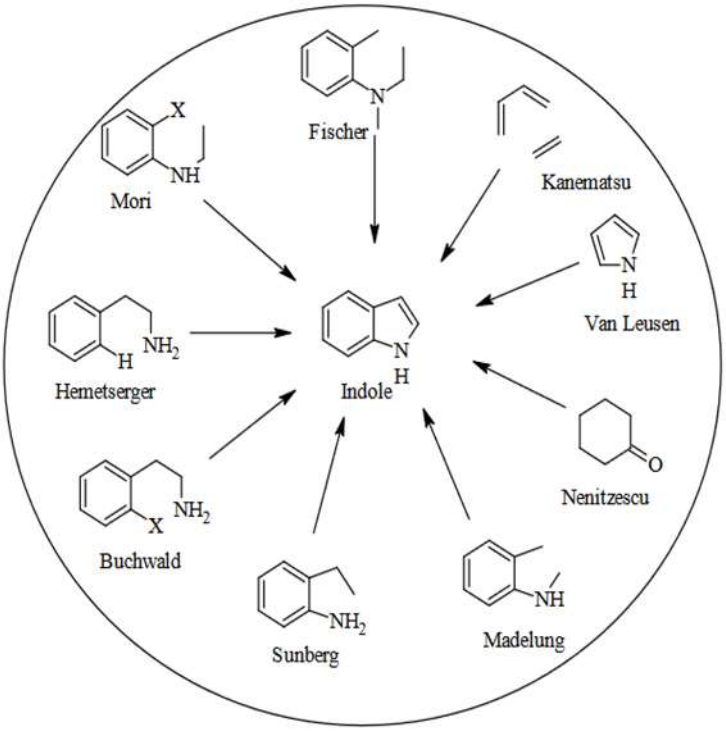



Fig. 1: Nine methods of synthesis of indoles. *(Image courtesy: Taber DF, Tirunahari PK. 2011. Indole synthesis: a review and proposed classification. Tetrahedron. 67(38):7195-7210)*

## BIOLOGICAL ACTIVITIES OF INDOLE DERIVATIVES

Istvan Borza *et al.,* reported indole-2-carboxamides as selective NMDA receptor antagonists [9] (Fig.2a). Sandra battaglia *et al.,* reported the synthesis and histamine H1–receptor antagonist activity of novel indole amide derivative (Fig.2b) [10]. Damian O. Arniaz *et al.,* synthesized and conducted the *in vitro* biological activity of indole-based Factor Xa inhibitor (Fig.2c) [11]. Mohammed A.A. Radwan *et al.,* reported the synthesis and biological activity of new 3-substituted indole derivative as a potential anti-inflammatory and analgesic agent (Fig.2d) [12]. Sunkyung Lee *et al.,* reported the synthesis of benzopyranlindoline as cardio selective anti-ischemic ATP- sensitive potassium channel (K ATP) opener (Fig.2e) [13]. Athanasia Varvaresou *et al.,* synthesized the indole- containing derivatives of 1,3,4-thiadiazole and 1,2,4- triazole and determined the activity as antimicrobial, antidepressant and anticonvulsant (Fig.2f) [14]. Luis Chacon-Garcia *et al.,* synthesized and conducted the *in vitro* cytotoxic activity of pyrrolo[2,3-e] indole derivative (Fig.2g) [15]. Wenhui Hu *et al*, reported the synthesis of 2- Sulfonyl-phenyl-3-phenyl-indole, a selective COX-2 inhibitor (Fig.2h) [16].

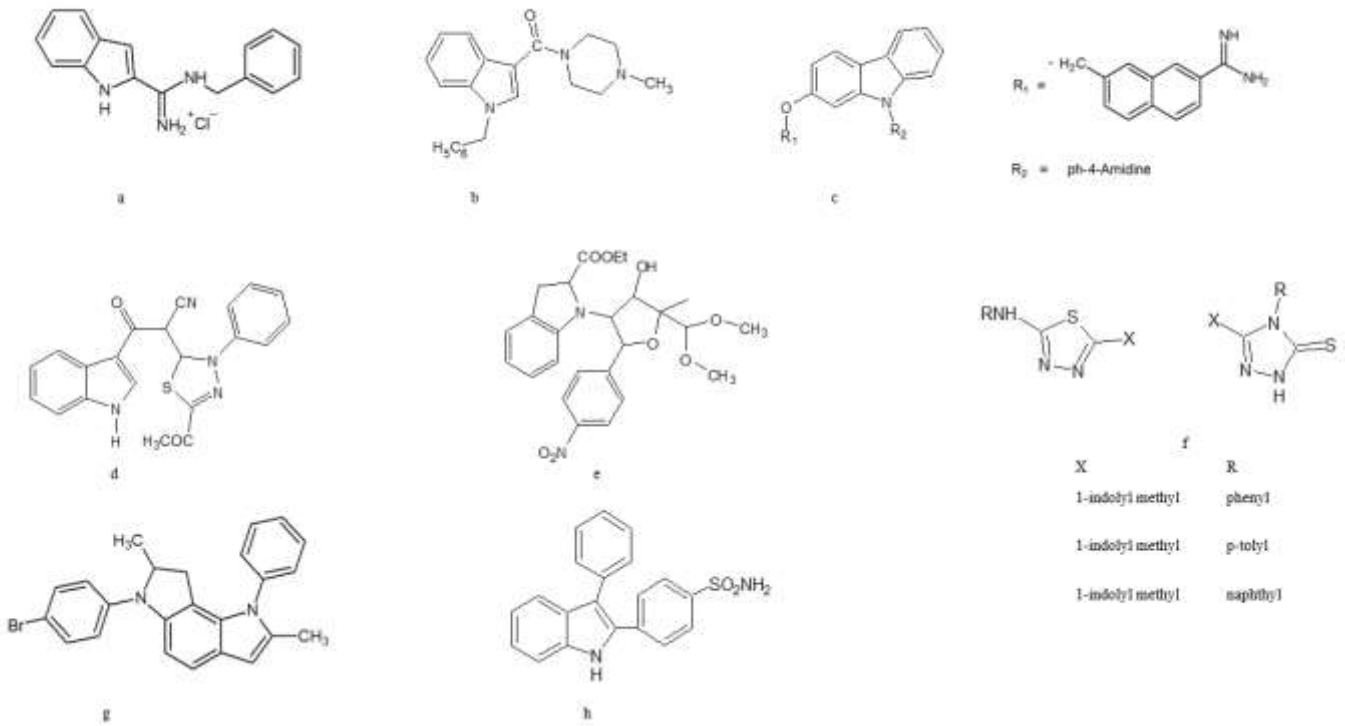



Fig. 2: Biological activities of indole derivatives

**VIRTUAL SCREENING AND MOLECULAR DOCKING STUDIES ON INDOLE DERIVATIVES**

Virtual screening of indole acyl guanidines were reported by Zou Y *et al* as potent β-secretase (BACE1) inhibitors (Fig.3a) [17]. Virtual screening of Methylene-Indole-2-Carbohydrazide Schiff 's Base derivatives were reported by Amira A Sadawe *et al* as COX Inhibitors (Fig.3b) [18]. Novel dihydrobenzo-indole-6-sulfonamide derivatives were reported by Yan Zhang *et al* (Fig.3c) as new RORγ inhibitors using virtual screening [19]. Roaiah HM *et al* reported a series of new indole derivatives 1-18 was synthesized and tested for their cytotoxic activity on a panel of 60 tumor cell lines. Binding pattern and binding affinity studies were conducted in the VEGFR-2 active site using molecular docking [20]. Molecular docking studies of indole derivatives containing cyanide group as polymerase inhibitors were reported by Thayaillany Rajandran *et al* (Fig.3d). Auto dock 4.2 is used to perform molecular docking in this study [21].

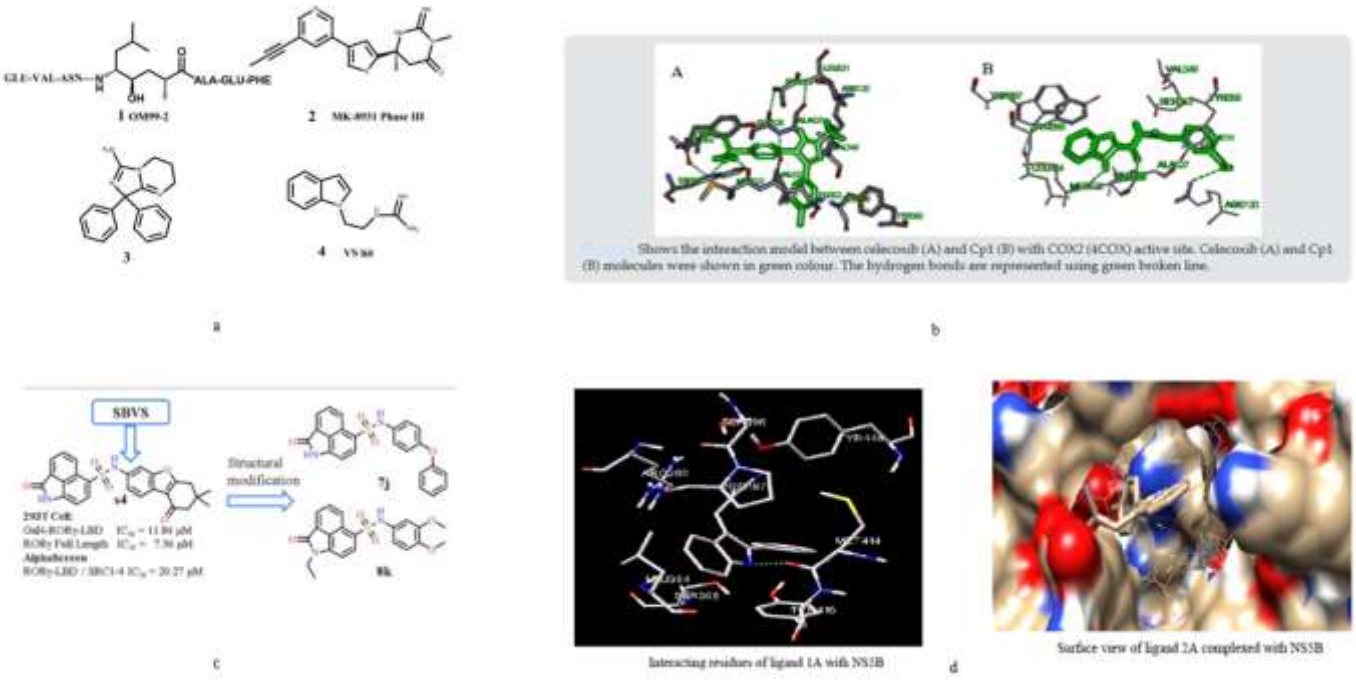



Fig. 3: Virtual screening and molecular docking studies on indole derivatives

**IV. PLAN OF WORK:**

In order to know the amino acids, present in the active site, active site prediction by using the software CASTp is done to know the location of active site. Then after the active site is predicted then virtual screening of ligands to that active site is conducted. The ligands giving optimal binding affinities are selected. The virtual screening studies are carried out using software called PyRX. Then docking studies is carried out by using the software Dock Thor. Then the drug ligand interactions are carried out by using the software BIOVIA Discovery studio. Finally, the toxicity and physicochemical parameters are known by using software SwissADME. The PDB Id of the protein is 1A6Y. This PDB Id can be taken from the protein data bank. And the protein is

downloaded from the protein data bank in PDB format. With the help of the PDB Id we can find the number of chains in the particular protein and its active site along with its amino acids present in the active site with the help of a software called as CASTp.

**EXPERIMENTAL PROCEDURES**

**Experimental Method #1: CASTp:** CASTp is an online tool that locates and measures pockets and voids on 3D protein structures. The new version of CASTp includes annotated functional information of specific residues on the protein structure.
The procedure with which we can find the active site of the protein is as follows:
1. Download the protein from the protein data bank in PDB format.
2. Open CASTp software and upload the protein in the software then run the program.
3. The protein with the active site pocket indicated in color will appear along at the side indicating its amino acids that are present in the active pocket.
4. Below there is an indication of the number of chains the protein contains.
5. PyRx: "PyRx is Virtual Screening software for Computational Drug Discovery that can be used to screen libraries of compounds against potential drug targets. PyRx enables medicinal chemists to run virtual screening from any platform and helps users in every step of this process - from data preparation to job submission and analysis of the results [22].

**Experimental Method #2: Virtual screening using pyrx**

Procedure:
1. Download PyRx software in the respective windows.
2. Select file option on the right upper corner in the software and upload the protein.
3. Convert the protein from pdb to pdbqt form by selecting the option autodock through right clicking it and further selecting the option "make macromolecule".
4. Then upon clicking on the protein we can select the amino acids that we got as a result from the CASTp software.
5. After selecting the amino acids we click upon the 'Toggle selection spheres' option above it. This will cause the software to show the active site of the protein on the protein so we could further carry out the process.
6. In order to open upload the ligand with which we are going to conduct virtual screening we click on the 'open babel option' and further click on the 'insert new item' option. Then the ligand molecules which were downloaded from PubChem are uploaded and are converted into pdbqt form.
7. Then 'Vina wizard' option is clicked and the software is run by clicking start button.
8. After running the program we are able to attain the binding affinity of the ligand to that particular active site of the protein molecule.
9. Like this several ligands were taken and the above process is repeated.

Upon carrying out the above process, the optimal binding affinities were noted when bonded with the following ligands while taking triphenyl indole as the lead molecule. Dock Thor: The implemented DockThor® program is a flexible-ligand and rigid-receptor grid-based method that employs a multiple solution genetic algorithm and the MMFF94S molecular force field scoring function [23].

**Experimental Method#3**

Procedure:
1. Dockthor website is opened as it is a free software there is easy access.
2. The option docking is selected and the protein file is uploaded into the software and send button is clicked
3. Then as there are no cofactors, we can skip this step and directly upload the ligand which is regarded as the HIT molecules.
4. Then the program is run to obtain the docking score.
5. This is then downloaded for further study into our windows.

Like this the 8 ligands with which we got the optimum binding affinities were seen for docking studies. With this software we get the accurate docking results of the ligand to the protein. Further study of the ligand and protein relationship is conducted by the use of BIOVIA Discovery studio.

**BIOVIA Discovery Studio**: Discovery Studio is a suite of software for simulating small molecule and macromolecule systems. It is developed and distributed by Dassault Systems BIOVIA.

**Experimental Method#4**

**Procedure**:
1. The software is downloaded into the system
2. As the software is opened the option 'File' is chosen and the protein is uploaded into the software.
3. Later the ligand is also uploaded into the software.
4. The information in the protein tab is then copy pasted into the best ranking ligand tab.
5. The protein was selected and then beside the tab 'Define protein' option was also selected.
6. Afterwards the ligand was selected and beside the tab 'Define ligand' option was also selected.
7. Then below there is a 'Show 2D diagram option with which the results are given.

**Experimental Method #5**

1. Later the physicochemical parameters and toxicity studies were conducted of the respective ligands by using the software's such as SwissADME, Pass Online, and Molinspiration.
2. SwissADME: This website allows you to compute physicochemical descriptors as well as to predict ADME parameters, pharmacokinetic properties, druglike nature and medicinal chemistry friendliness of one or multiple small molecules to support drug discovery.

The main article describing the web service and its underlying methodologies is SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules [24].

PASS Online: It is a free online (software) predicts over 3500 kinds of biological activity, including pharmacological effects, mechanisms of action, toxic and adverse effects, interaction with metabolic enzymes and transporters, influence on gene expression, etc. To obtain the predicted biological activity profile for your compound, only structural formula is necessary; thus, prediction is possible even for virtual structure designed in computer but not synthesized yet.

Accessing to PASS Online service requires a prior registration, which is free but one should agree with the terms & conditions for usage of this service. Prediction is based on the analysis of structure activity-relationships for more than 250,000 biologically active substances including drugs, drug-candidates, leads and toxic compounds.

## V. RESULTS AND DISCUSSION

The amino acids that are present in the active site of the protein are given as follows in Table 1:

Table 1: List of amino acids present in the active site of the protein

| S.No | Amino acid number | Amino acid |
|---|---|---|
| 1 | 106 | Aspartate |
| 2 | 119 | Glutamate |
| 3 | 120 | Glycine |
| 4 | 122 | Lysine |
| 5 | 123 | Glycine |
| 6 | 124 | Phenyl alanine |
| 7 | 126 | Arginine |
| 8 | 127 | Arginine |
| 9 | 147 | Arginine |
| 10 | 148 | Isoleucine |
| 11 | 149 | Asparagine |
| 12 | 150 | Arginine |
| 13 | 151 | Asparagine |
| 14 | 152 | Arginine |
| 15 | 157 | Arginine |

Now that we have the basic information about the protein and its biological activity, there are some possible ligands with which the retinoid acid orphan receptors beta cells can bind to. In order to check if a particular ligand can be bound to the protein, we need to download the ligands from a database such as PubChem first.

PubChem: PubChem is the world's largest database having a collection of freely accessible chemical information which is searched by chemical name, molecular formula, structure, and other identifiers. Using this data base one can find information about chemical and physical properties, biological activities, safety and toxicity, patents, literature citations and more. The ligands are downloaded in 2D form in the form of an SDF file. Then the downloaded ligands are virtually screened with the help of software called as PyRx. Upon virtual screening with the help of the software PyRX, the lead molecule was found to be triphenylindole and its derivative (Fig.4).

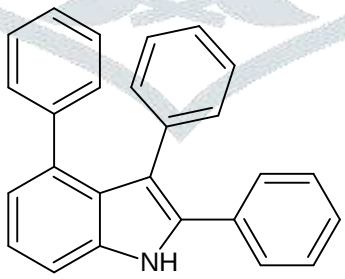



Fig. 4: Lead molecule triphenyl indole derivative

**VIRTUAL SCREENING RESULTS**

Virtual screening results determined using PyRx software are summarized in the below table 2:

Table 2: Virtual screening results

| S.NO | PUBCHEM COMPOUND ID | PyRx SCORE |
|---|---|---|
| 1. | 89657875 | -11.2 |
| 2. | 89657879 | -10.7 |
| 3 | 151325346 | -10.6 |
| 4. | 151196049 | -10.3 |
| 5. | 25194820 | -10.1 |
| 6. | 135427495 | -9.9 |
| 7. | 89657836 | -9.9 |
| 8. | 17820846 | -9.9 |
| 9. | 58341469 | -9.9 |
| 10. | 21287130 | -8.2 |
| 11. | 101478245 | -8.0 |
| 12. | 631377 | -7.5 |
| 13. | 44208480 | -7.5 |
| 14. | 101147032 | -7.5 |
| 15. | 101785377 | -7.5 |
| 16. | 101478245 | -7.5 |
| 17. | 147370154 | -7.6 |
| 18. | 144182104 | -7.5 |
| 19. | 101478241 | -7.7 |
| 20. | 141388482 | -7.4 |
| 21. | 440208480 | -7.5 |
| 22. | 132532640 | -7.3 |
| 23. | 18320586 | -7.3 |
| 24. | 102369586 | -7.3 |
| 25. | 132538907 | -7.2 |
| 26. | 423586 | -7.2 |

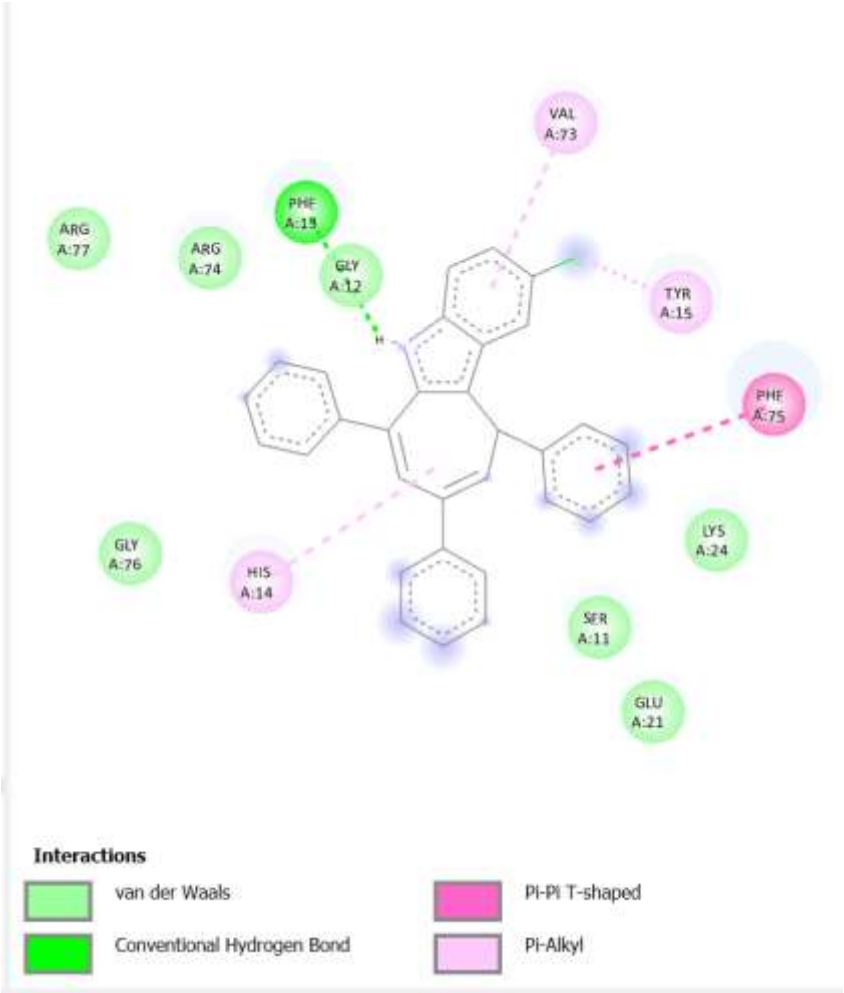
| | | |
|---|---|---|
| 27. | 101785375 | -7.1 |
| 28. | 129787113 | -7.1 |
| 29. | 101863964 | -7.1 |
| 30. | 101479252 | -7.1 |
| 31. | 151965791 | -7.1 |
| 32. | 10479252 | -7.0 |
| 33. | 101785372 | -7.0 |
| 34. | 150886969 | -7.0 |
| 35. | 86147948 | -6.8 |
| 36. | 150277976 | -6.8 |
| 37. | 896578882 | -6.8 |
| 38. | 68653683 | -6.8 |
| 39. | 101785378 | -6.7 |
| 40. | 129706222 | -6.7 |
| 41. | 23880702 | -6.6 |
| 42. | 91739124 | -6.5 |
| 43. | 89658814 | -6.5 |
| 44. | 896547878 | -6.4 |
| 45. | 14823737 | -6.1 |
| 46. | 89657877 | -5.9 |
| 47. | 394101 | -5.9 |
| 48. | 151008893 | -5.8 |
| 49. | 152690497 | -5.8 |
| 50. | 89657881 | -5.8 |

The X, Y, Z coordinate were also noted down:X = 8.3, Y= 1.16 Z=-14.6 After this virtual screening process docking studies are carried out by using the free online software “DockThor”.

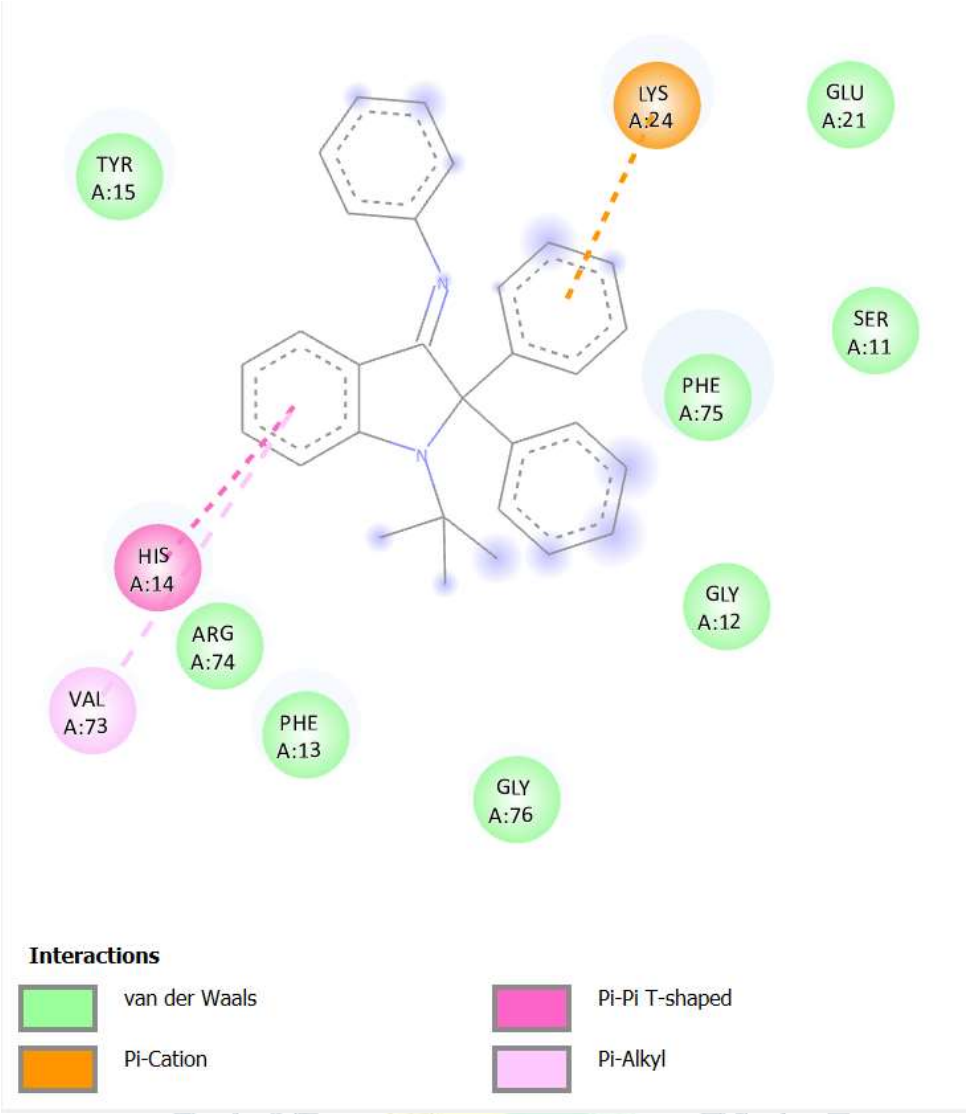
Table 3: Ligands and their scores

| S.No. | LIGAND | PUBCHEM COMPOUND ID | PYRX SCORE | DOCKTHOR SCORE |
|---|---|---|---|---|
| 1 | 2-chloro-6,8,10-triphenyl-5,10-dihydrocyclohepta[b]indole | 44208480 | -7.5 | -9.1 |
| 2 | 1-tert-butyl-N,2,2-triphenylindole-3-imine | 89657876 | -11.2 | -8.5 |
| 3 | (2R,3R)-2,3,4-triphenyl-1H-cyclopenta[b]indole-2,3-diol | 101147032 | -7.5 | -8.6 |
| 4 | 1-methoxy-6,8,10-triphenyl-5,10-dihydrocyclohepta[b]indole | 101478243 | -8 | -8.1 |
| 5 | 2,3,7-triphenyl-1H-indole | 631377 | -7.5 | -8.3 |
| 6 | N,2,2-triphenyl-1H-indole-3-imine | 135427495 | -9.9 | -7.1 |
| 7 | 2-amino-4,5,7-triphenyl-1H-indole-3,6-dicarbonitrile | 25194820 | -10.1 | -7.8 |
| 8 | 4,6,7-triphenyl-2,3-dihydro-1H-indole | 21287130 | -8.2 | -7.1 |

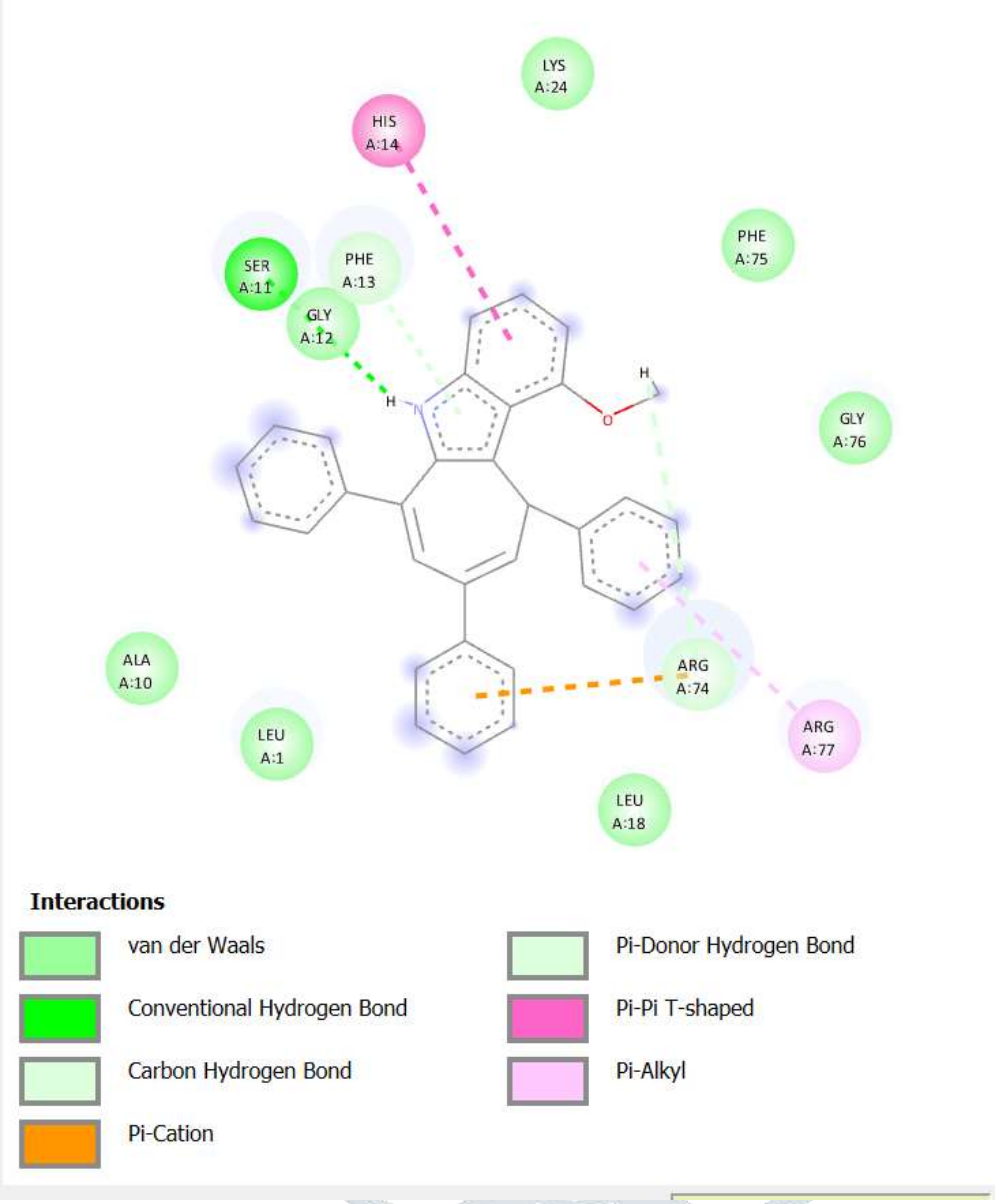
**RESUIT 1:** LIGAND:2-chloro-6,8,10-triphenyl-5,10-dihydrocyclohepta[b]indole**.** PUBCHEM ID- 44208480



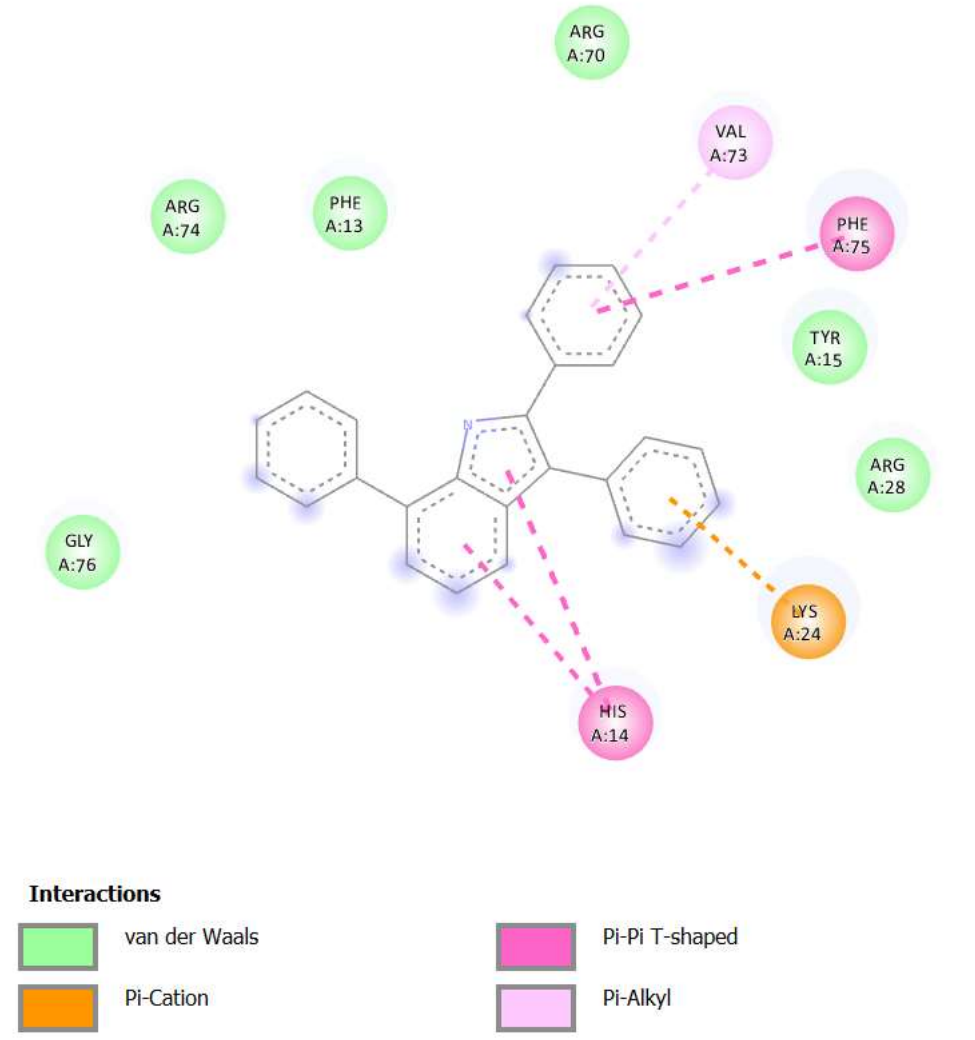
**RESULT 2:** LIGAND:1-tert-butyl-N,2,2-triphenylindole-3-imine. PUBCHEM ID-89657876



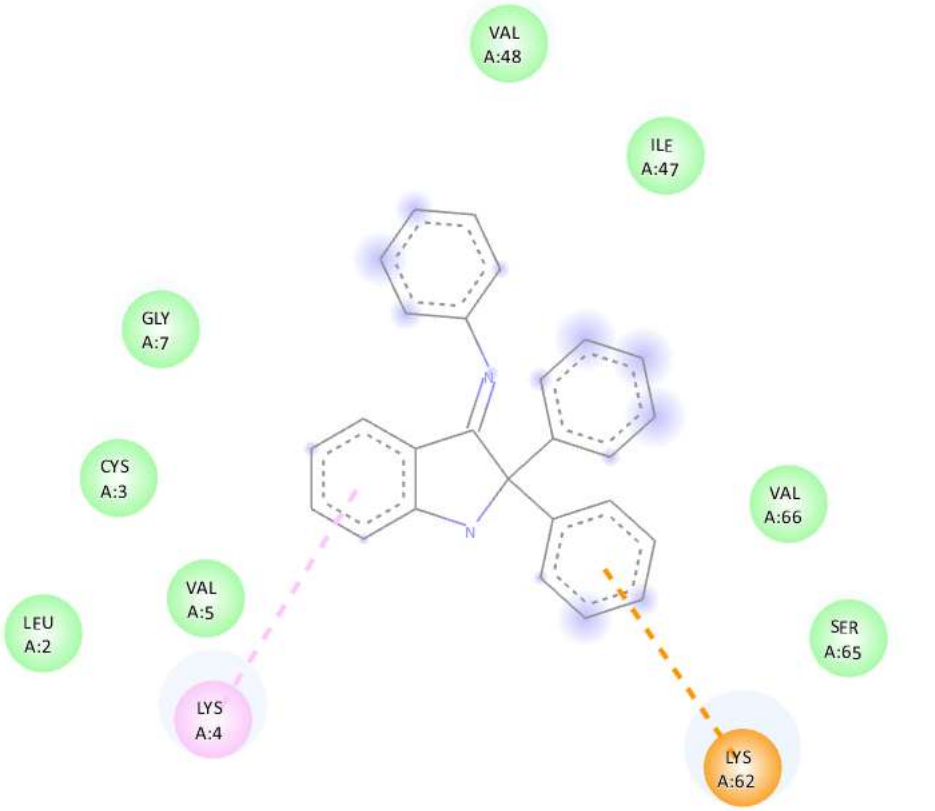
**RESULT 3:** LIGAND: 1-methoxy-6,8,10-triphenyl-5,10-dihydrocyclohepta[b]indole. PUBCHEM ID-101478243



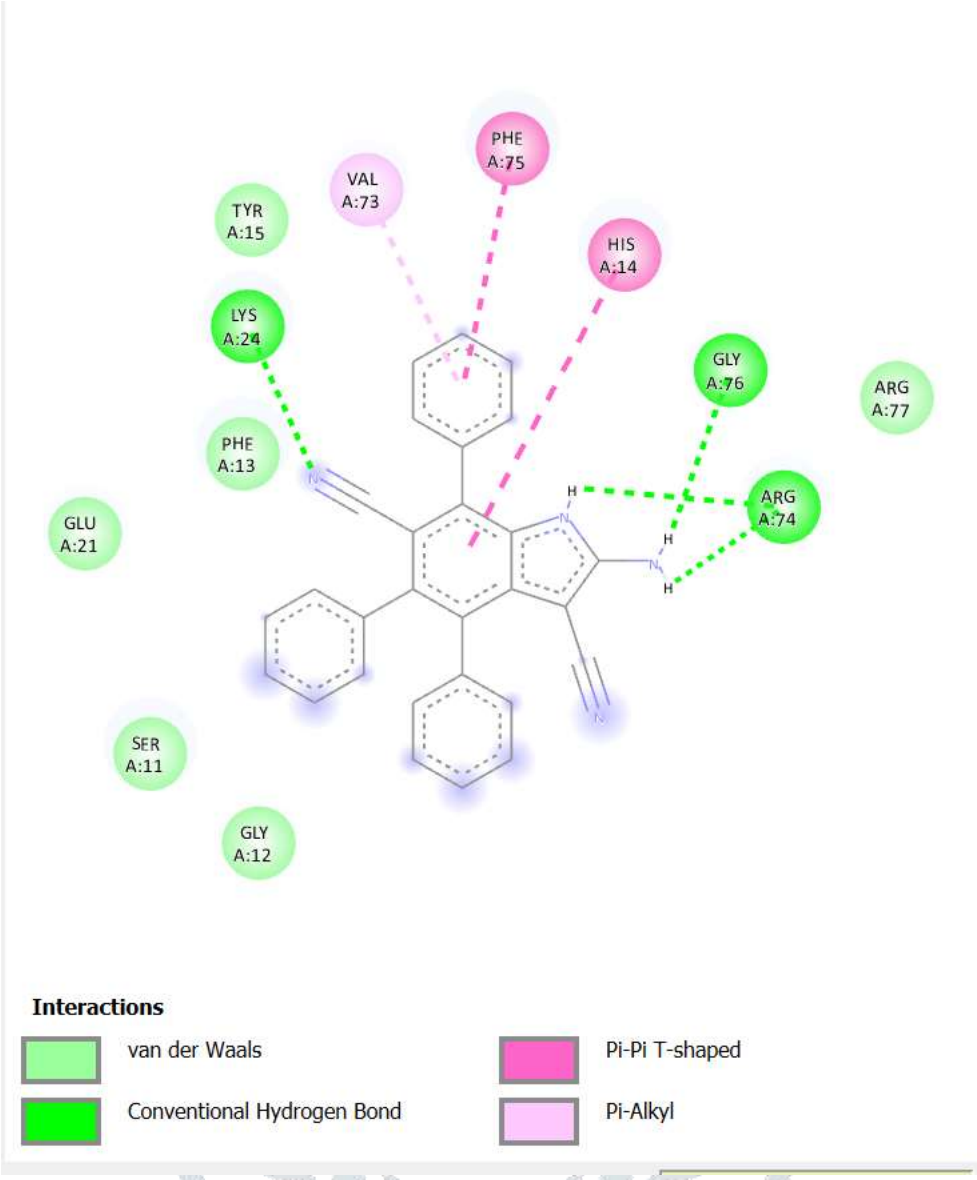
**RESULT 4:** LIGAND: 2,3,7-triphenyl-1H-indole. PUBCHEM ID-6313



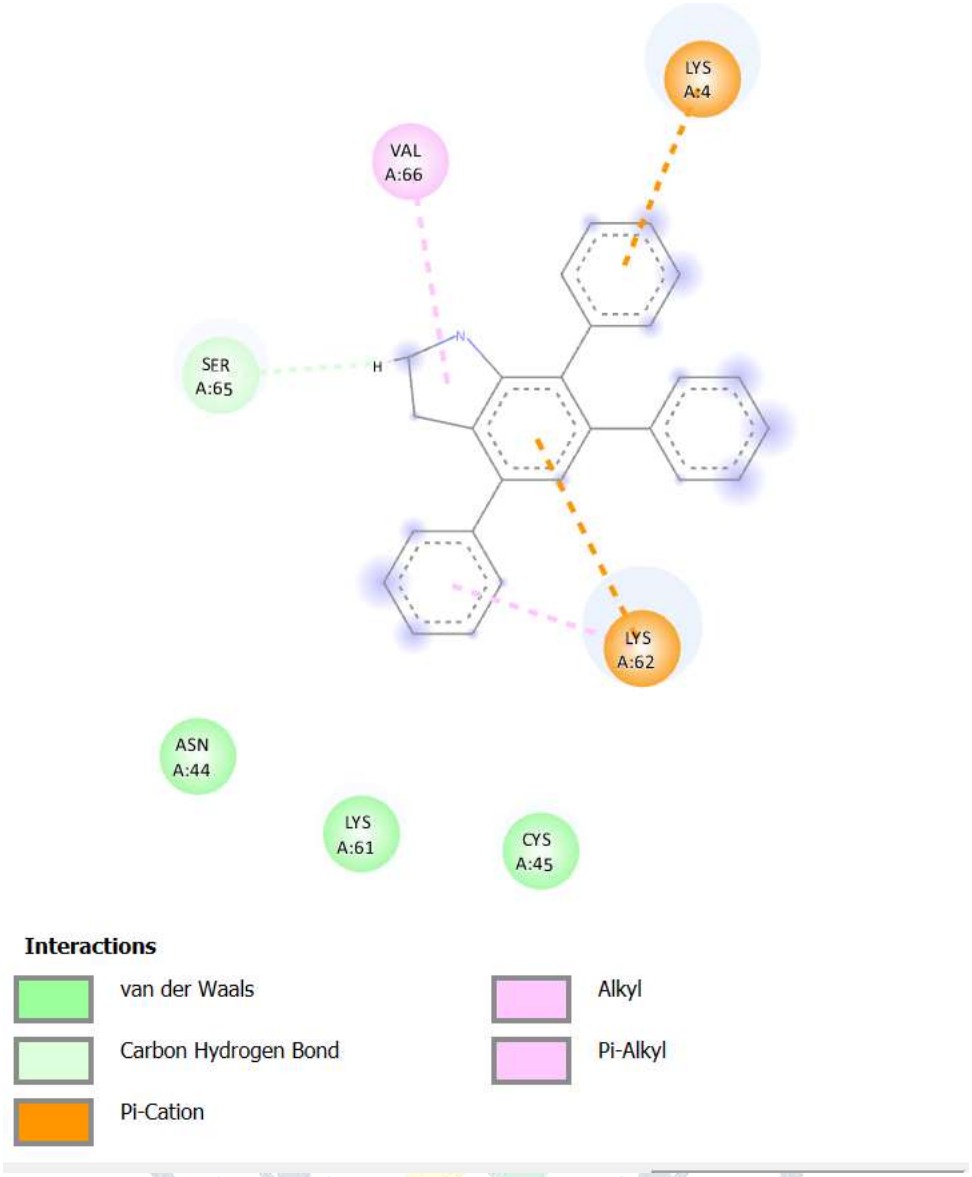
**RESULT 5:** LIGAND: N,2,2-triphenyl-1H-indole-3-imine. PUBCHEM ID-13542749



**RESULT 6:** LIGAND: 2-amino-4,5,7-triphenyl-1H-indole-3,6-dicarbonitrile. PUBCHEM ID-2519482



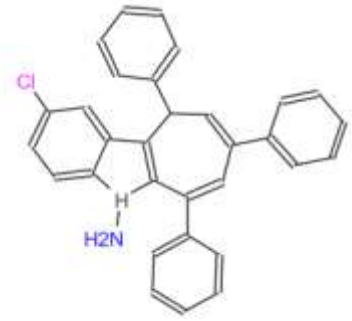
**RESULT 7: LIGAND:** 4,6,7-triphenyl-2,3-dihydro-1H-indole. PUBCHEM ID-2128713

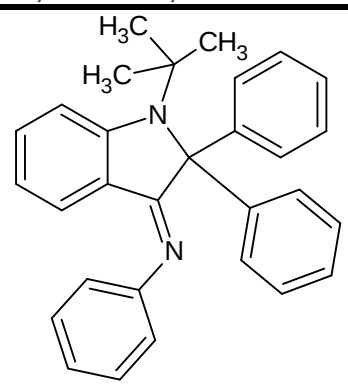
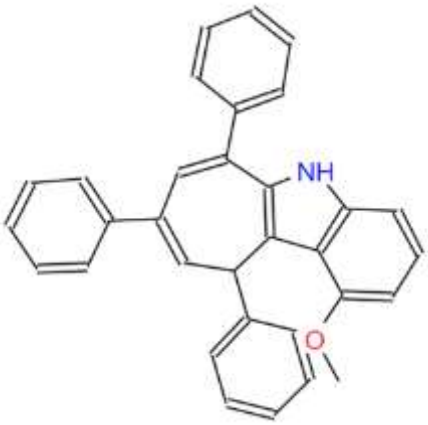
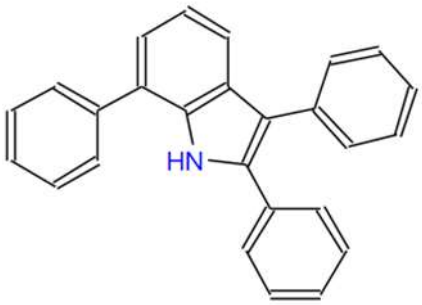


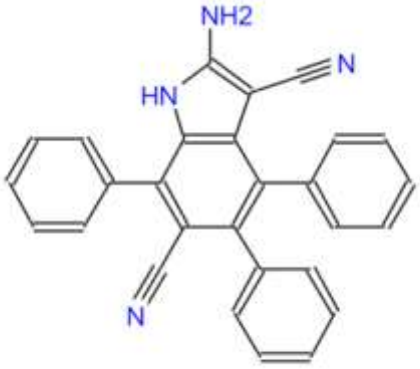
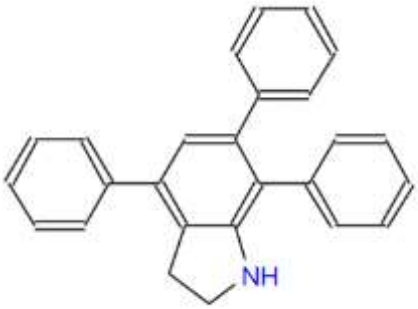

**MOLECULAR PROPERTY AND TOXICITY PREDICTION STUDIES**

Molecular property and toxicity prediction studies are done using BIOVIA DISCOVERY STUDIO and SWISS ADME software respectively. The results are summarized in the below table 4.

Table 4: Molecular properties of the lead molecules

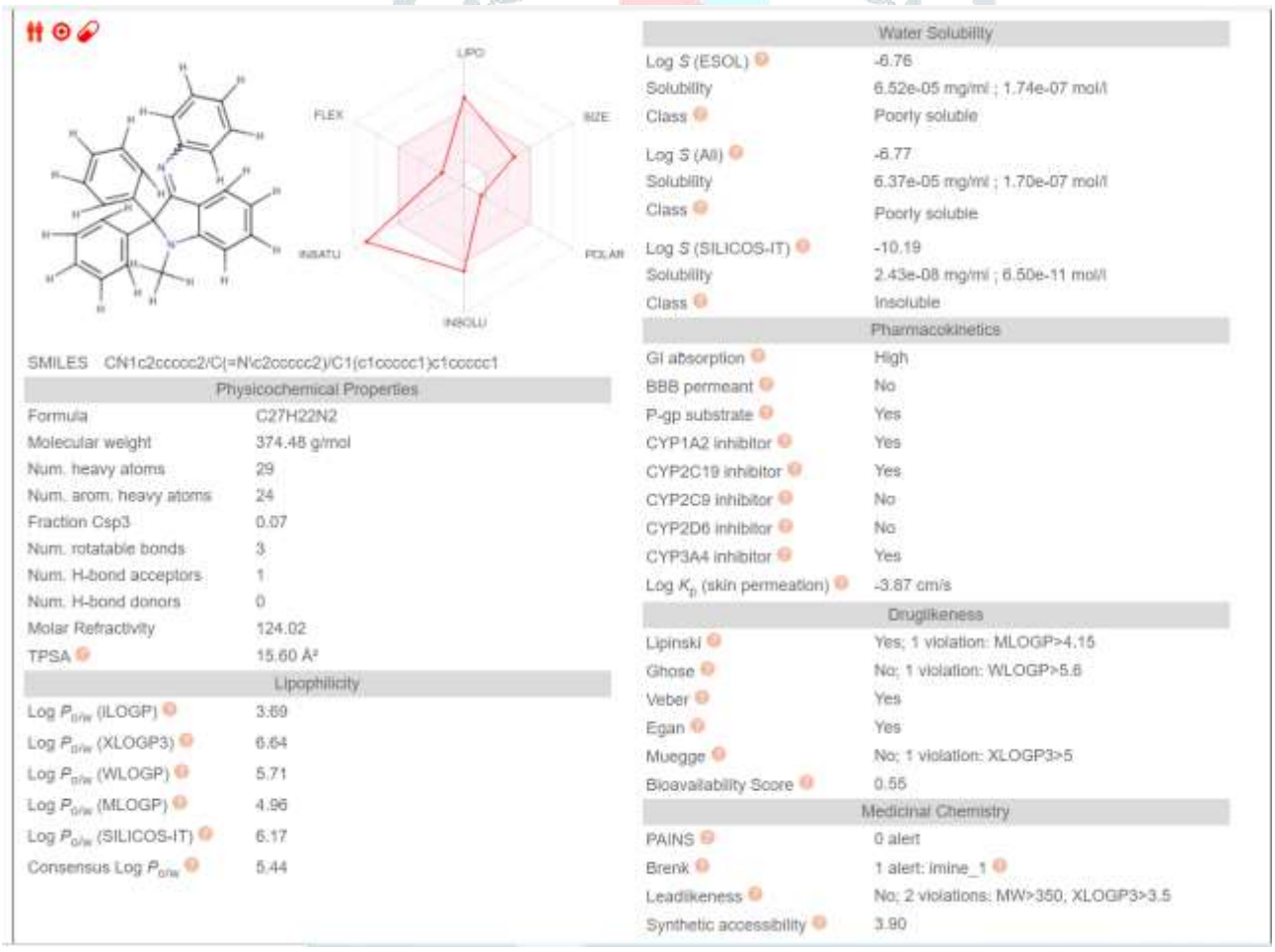
| S.No | Structure of the lead molecule | Hydrogen bonding | Hydrophobic interaction |
|---|---|---|---|
| 1 | 2-chloro-6,8,10-Triphenyl-5,10-dihydrocyclohepta[b]indole   | Convetional hydrogen bond PHE 13 | Pi-Pi T-Shaped PHE75 Pi-Alkyl VAL 73 TYR 15 HIS 14 |
| 2 | 1-tert-butyl-N,2,2-triphenylindole-3-imine | | Pi-Pi T-Shaped: HIS14 Pi -Alkyl: VAL 73 Pi -Cation: LYS 24 |

| | | | |
|---|---|---|---|
| |  | | |
| 3 | 1-methoxy-6,8,10-triphenyl-5,10-dihydrocyclohepta[b]indole  | Conventional hydrogen Bond SER11, Carbon hydrogen bond ARG74, PHE13 | Pi-cation: Pi-Donor hydrogen bond: ARG74, PHE13 Pi-Pi T-Shaped: HIS14 Pi-Alkyl: ARG77 |
| 4 | 2,3,7-triphenyl-1H-indole  | | Pi-Pi T-Shaped: HIS14, PHE75 Pi Cation: LYS24 Pi-Alkyl: VAL73 |
| 5 | N,2,2-triphenyl-1H-indole-3-imine  | | Pi -Cation: LYS62 Pi-Alkyl: LYS4 |
| 6 | 2-amino-4,5,7-triphenyl-1H-indole-3,6-dicarbonitrile | Convential hydrogen bond ARG74, GLY76, LYS24 | Pi-Pi T-shaped: HIS14, PHE75 Pi-Alkyl: VAL73 |

| | | | |
|---|---|---|---|
| |  | | |
| 7 | 4,6,7-triphenyl-2,3-dihydro-1H-indole  | Carbon hydrogen bond SER65 | Pi-Cation: LYS62 LYS4<br><br>Pi -Alkyl: VAL66 |

**TOXICITY STUDIES:**

**LIGAND 1:**2-chloro-6,8,10-triphenyl-5,10-dihydrocyclohepta[b]indole**;** PUBCHEM ID:44208480



SMILES CN1c2ccccc2/C(=N\c2ccccc2)/C1(c1ccccc1)c1ccccc1

| Physicochemical Properties | |
|---|---|
| Formula | C27H22N2 |
| Molecular weight | 374.48 g/mol |
| Num. heavy atoms | 29 |
| Num. arom. heavy atoms | 24 |
| Fraction Csp3 | 0.07 |
| Num. rotatable bonds | 3 |
| Num. H-bond acceptors | 1 |
| Num. H-bond donors | 0 |
| Molar Refractivity | 124.02 |
| TPSA | 15.60 Å² |

| Lipophilicity | |
|---|---|
| Log $P_{o/w}$ (iLOGP) | 3.69 |
| Log $P_{o/w}$ (XLOGP3) | 6.64 |
| Log $P_{o/w}$ (WLOGP) | 5.71 |
| Log $P_{o/w}$ (MLOGP) | 4.96 |
| Log $P_{o/w}$ (SILICOS-IT) | 6.17 |
| Consensus Log $P_{o/w}$ | 5.44 |

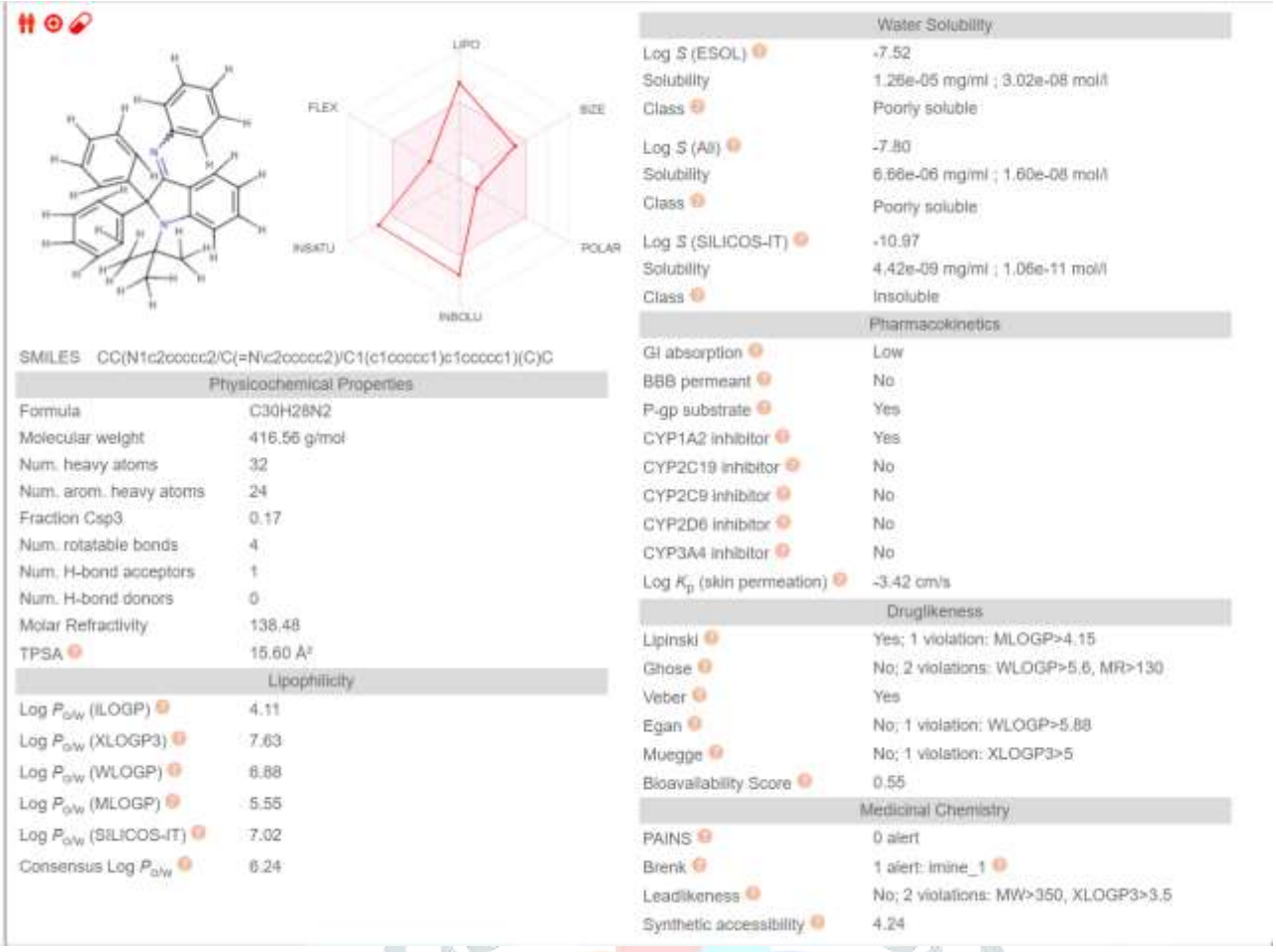
| Water Solubility | |
|---|---|
| Log *S* (ESOL) | -6.76 |
| Solubility | 6.52e-05 mg/ml ; 1.74e-07 mol/l |
| Class | Poorly soluble |
| Log *S* (Ali) | -6.77 |
| Solubility | 6.37e-05 mg/ml ; 1.70e-07 mol/l |
| Class | Poorly soluble |
| Log *S* (SILICOS-IT) | -10.19 |
| Solubility | 2.43e-08 mg/ml ; 6.50e-11 mol/l |
| Class | Insoluble |

| Pharmacokinetics | |
|---|---|
| GI absorption | High |
| BBB permeant | No |
| P-gp substrate | Yes |
| CYP1A2 inhibitor | Yes |
| CYP2C19 inhibitor | Yes |
| CYP2C9 inhibitor | No |
| CYP2D6 inhibitor | No |
| CYP3A4 inhibitor | Yes |
| Log $K_p$ (skin permeation) | -3.87 cm/s |

| Druglikeness | |
|---|---|
| Lipinski | Yes; 1 violation: MLOGP>4.15 |
| Ghose | No; 1 violation: WLOGP>5.6 |
| Veber | Yes |
| Egan | Yes |
| Muegge | No; 1 violation: XLOGP3>5 |
| Bioavailability Score | 0.55 |

| Medicinal Chemistry | |
|---|---|
| PAINS | 0 alert |
| Brenk | 1 alert: imine_1 |
| Leadlikeness | No; 2 violations: MW>350, XLOGP3>3.5 |
| Synthetic accessibility | 3.90 |

**LIGAND 2:**1-tert-butyl-N,2,2-triphenylindole-3-imine; PUBCHEM ID:89657876

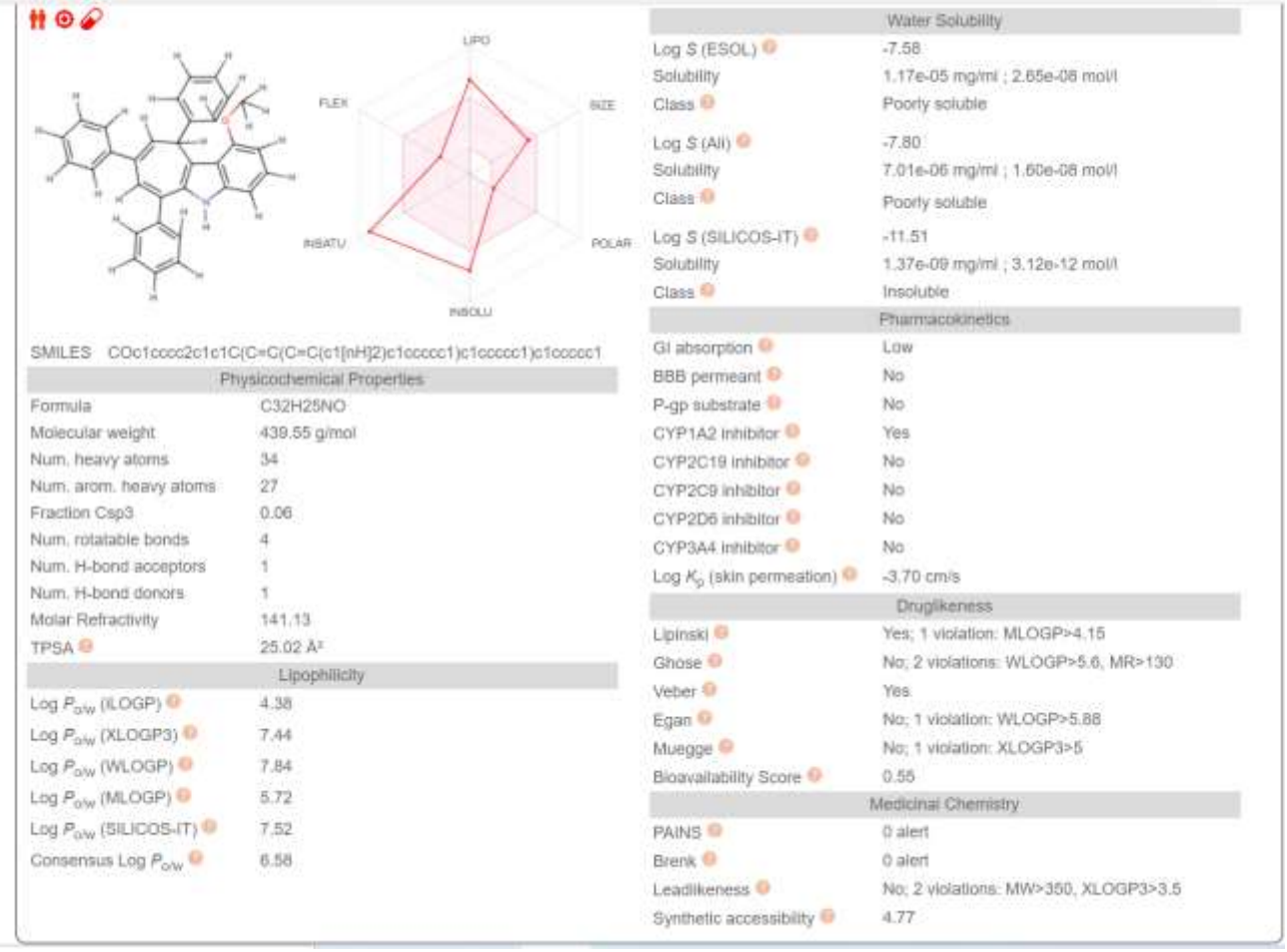



SMILES CC(N1c2ccccc2/C(=N\c2ccccc2)/C1(c1ccccc1)c1ccccc1)(C)C

| Physicochemical Properties | |
|---|---|
| Formula | C30H28N2 |
| Molecular weight | 416.56 g/mol |
| Num. heavy atoms | 32 |
| Num. arom. heavy atoms | 24 |
| Fraction Csp3 | 0.17 |
| Num. rotatable bonds | 4 |
| Num. H-bond acceptors | 1 |
| Num. H-bond donors | 0 |
| Molar Refractivity | 138.48 |
| TPSA | 15.60 Å² |
| **Lipophilicity** | |
| Log $P_{o/w}$ (iLOGP) | 4.11 |
| Log $P_{o/w}$ (XLOGP3) | 7.63 |
| Log $P_{o/w}$ (WLOGP) | 6.88 |
| Log $P_{o/w}$ (MLOGP) | 5.55 |
| Log $P_{o/w}$ (SILICOS-IT) | 7.02 |
| Consensus Log $P_{o/w}$ | 6.24 |

| Water Solubility | |
|---|---|
| Log *S* (ESOL) | -7.52 |
| Solubility | 1.28e-05 mg/ml ; 3.02e-08 mol/l |
| Class | Poorly soluble |
| Log *S* (Ali) | -7.80 |
| Solubility | 6.66e-06 mg/ml ; 1.60e-08 mol/l |
| Class | Poorly soluble |
| Log *S* (SILICOS-IT) | -10.97 |
| Solubility | 4.42e-09 mg/ml ; 1.06e-11 mol/l |
| Class | Insoluble |
| **Pharmacokinetics** | |
| GI absorption | Low |
| BBB permeant | No |
| P-gp substrate | Yes |
| CYP1A2 inhibitor | Yes |
| CYP2C19 inhibitor | No |
| CYP2C9 inhibitor | No |
| CYP2D6 inhibitor | No |
| CYP3A4 inhibitor | No |
| Log $K_p$ (skin permeation) | -3.42 cm/s |
| **Druglikeness** | |
| Lipinski | Yes; 1 violation: MLOGP>4.15 |
| Ghose | No; 2 violations: WLOGP>5.6, MR>130 |
| Veber | Yes |
| Egan | No; 1 violation: WLOGP>5.88 |
| Muegge | No; 1 violation: XLOGP3>5 |
| Bioavailability Score | 0.55 |
| **Medicinal Chemistry** | |
| PAINS | 0 alert |
| Brenk | 1 alert: imine_1 |
| Leadlikeness | No; 2 violations: MW>350, XLOGP3>3.5 |
| Synthetic accessibility | 4.24 |

**LIGAND 3:** 1-methoxy-6,8,10-triphenyl-5,10-dihydrocyclohepta[b]indole; PUBCHEM ID:1014782434

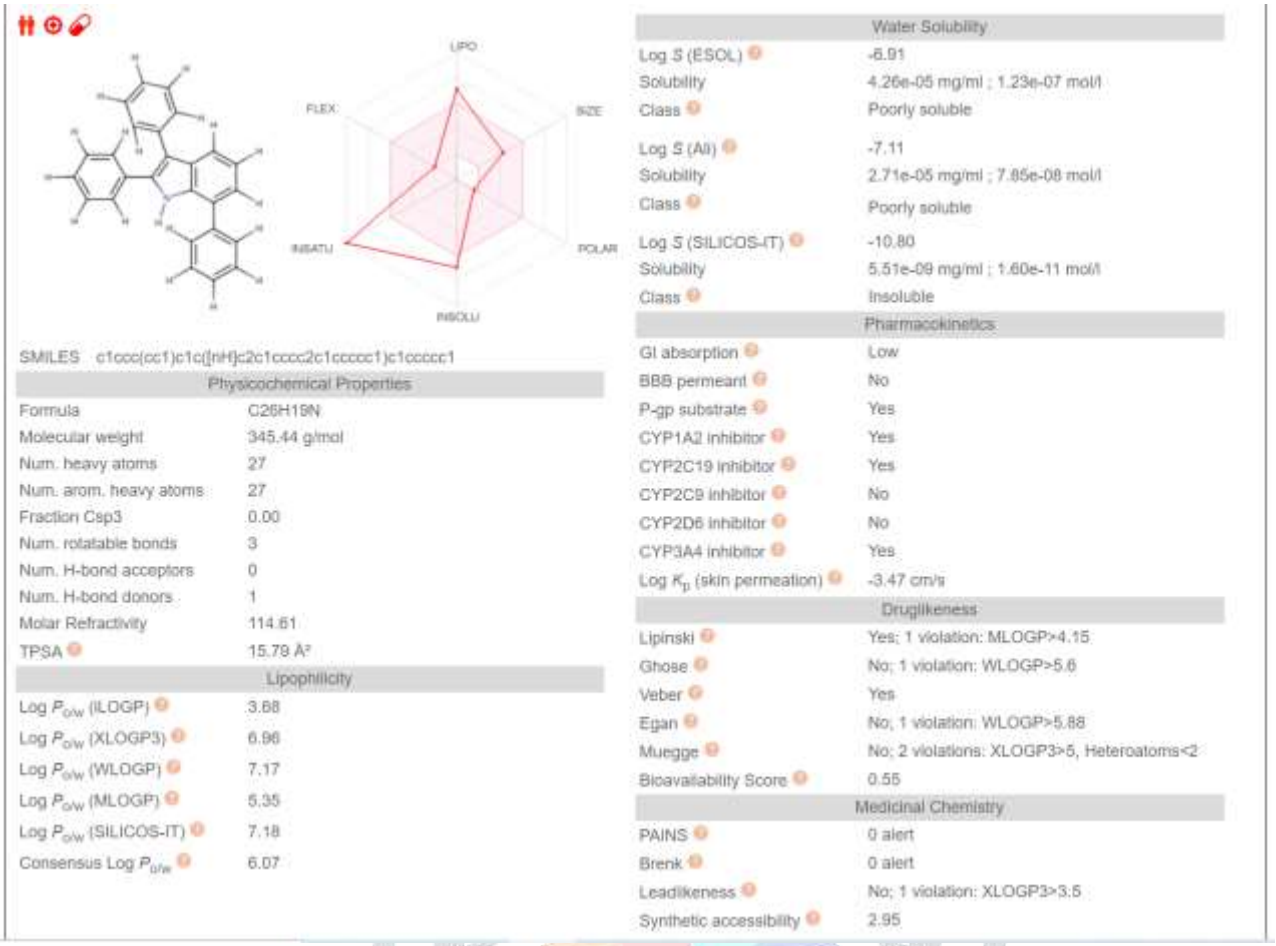



SMILES COc1cccc2c1c1C(C=C(C=C(c1[nH]2)c1ccccc1)c1ccccc1)c1ccccc1

| Physicochemical Properties | |
|---|---|
| Formula | C32H25NO |
| Molecular weight | 439.55 g/mol |
| Num. heavy atoms | 34 |
| Num. arom. heavy atoms | 27 |
| Fraction Csp3 | 0.06 |
| Num. rotatable bonds | 4 |
| Num. H-bond acceptors | 1 |
| Num. H-bond donors | 1 |
| Molar Refractivity | 141.13 |
| TPSA | 25.02 Å² |
| **Lipophilicity** | |
| Log $P_{o/w}$ (iLOGP) | 4.38 |
| Log $P_{o/w}$ (XLOGP3) | 7.44 |
| Log $P_{o/w}$ (WLOGP) | 7.84 |
| Log $P_{o/w}$ (MLOGP) | 5.72 |
| Log $P_{o/w}$ (SILICOS-IT) | 7.52 |
| Consensus Log $P_{o/w}$ | 6.58 |

| Water Solubility | |
|---|---|
| Log *S* (ESOL) | -7.58 |
| Solubility | 1.17e-05 mg/ml ; 2.65e-08 mol/l |
| Class | Poorly soluble |
| Log *S* (Ali) | -7.80 |
| Solubility | 7.01e-06 mg/ml ; 1.60e-08 mol/l |
| Class | Poorly soluble |
| Log *S* (SILICOS-IT) | -11.51 |
| Solubility | 1.37e-09 mg/ml ; 3.12e-12 mol/l |
| Class | Insoluble |
| **Pharmacokinetics** | |
| GI absorption | Low |
| BBB permeant | No |
| P-gp substrate | No |
| CYP1A2 inhibitor | Yes |
| CYP2C19 inhibitor | No |
| CYP2C9 inhibitor | No |
| CYP2D6 inhibitor | No |
| CYP3A4 inhibitor | No |
| Log $K_p$ (skin permeation) | -3.70 cm/s |
| **Druglikeness** | |
| Lipinski | Yes; 1 violation: MLOGP>4.15 |
| Ghose | No; 2 violations: WLOGP>5.6, MR>130 |
| Veber | Yes |
| Egan | No; 1 violation: WLOGP>5.88 |
| Muegge | No; 1 violation: XLOGP3>5 |
| Bioavailability Score | 0.55 |
| **Medicinal Chemistry** | |
| PAINS | 0 alert |
| Brenk | 0 alert |
| Leadlikeness | No; 2 violations: MW>350, XLOGP3>3.5 |
| Synthetic accessibility | 4.77 |

**LIGAND 4:**2,3,7-Triphenyl-1H-indole; PUBCHEM ID:631377

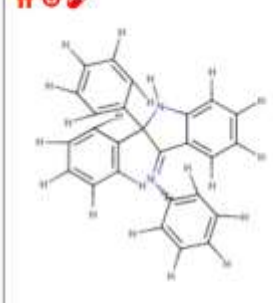



SMILES c1ccc(cc1)c1c([nH]c2c1cccc2c1ccccc1)c1ccccc1

| Physicochemical Properties | |
|---|---|
| Formula | C26H19N |
| Molecular weight | 345.44 g/mol |
| Num. heavy atoms | 27 |
| Num. arom. heavy atoms | 27 |
| Fraction Csp3 | 0.00 |
| Num. rotatable bonds | 3 |
| Num. H-bond acceptors | 0 |
| Num. H-bond donors | 1 |
| Molar Refractivity | 114.61 |
| TPSA | 15.79 Å² |
| **Lipophilicity** | |
| Log $P_{o/w}$ (ILOGP) | 3.68 |
| Log $P_{o/w}$ (XLOGP3) | 6.96 |
| Log $P_{o/w}$ (WLOGP) | 7.17 |
| Log $P_{o/w}$ (MLOGP) | 5.35 |
| Log $P_{o/w}$ (SILICOS-IT) | 7.18 |
| Consensus Log $P_{o/w}$ | 6.07 |

| Water Solubility | |
|---|---|
| Log *S* (ESOL) | -6.91 |
| Solubility | 4.26e-05 mg/ml ; 1.23e-07 mol/l |
| Class | Poorly soluble |
| Log *S* (Ali) | -7.11 |
| Solubility | 2.71e-05 mg/ml ; 7.85e-08 mol/l |
| Class | Poorly soluble |
| Log *S* (SILICOS-IT) | -10.80 |
| Solubility | 5.51e-09 mg/ml ; 1.60e-11 mol/l |
| Class | Insoluble |
| **Pharmacokinetics** | |
| GI absorption | Low |
| BBB permeant | No |
| P-gp substrate | Yes |
| CYP1A2 inhibitor | Yes |
| CYP2C19 inhibitor | Yes |
| CYP2C9 inhibitor | No |
| CYP2D6 inhibitor | No |
| CYP3A4 inhibitor | Yes |
| Log $K_p$ (skin permeation) | -3.47 cm/s |
| **Druglikeness** | |
| Lipinski | Yes; 1 violation: MLOGP>4.15 |
| Ghose | No; 1 violation: WLOGP>5.6 |
| Veber | Yes |
| Egan | No; 1 violation: WLOGP>5.88 |
| Muegge | No; 2 violations: XLOGP3>5, Heteroatoms<2 |
| Bioavailability Score | 0.55 |
| **Medicinal Chemistry** | |
| PAINS | 0 alert |
| Brenk | 0 alert |
| Leadlikeness | No; 1 violation: XLOGP3>3.5 |
| Synthetic accessibility | 2.95 |

**LIGAND 5:** N,2,2-triphenyl-1H-indole-3-imine; PUBCHEM ID:135427495

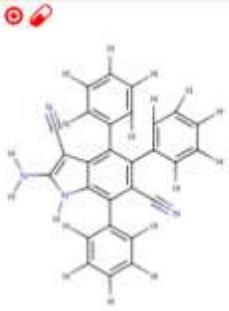



SMILES c1ccc(cc1)/N=C/1\c2ccccc2NC1(c1ccccc1)c1ccccc1

| Physicochemical Properties | |
|---|---|
| Formula | C26H20N2 |
| Molecular weight | 360.45 g/mol |
| Num. heavy atoms | 28 |
| Num. arom. heavy atoms | 24 |
| Fraction Csp3 | 0.04 |
| Num. rotatable bonds | 3 |
| Num. H-bond acceptors | 1 |
| Num. H-bond donors | 1 |
| Molar Refractivity | 119.12 |
| TPSA | 24.39 Å² |
| **Lipophilicity** | |
| Log $P_{o/w}$ (ILOGP) | 3.41 |
| Log $P_{o/w}$ (XLOGP3) | 6.50 |
| Log $P_{o/w}$ (WLOGP) | 5.50 |
| Log $P_{o/w}$ (MLOGP) | 4.76 |
| Log $P_{o/w}$ (SILICOS-IT) | 6.23 |
| Consensus Log $P_{o/w}$ | 5.28 |

| Water Solubility | |
|---|---|
| Log *S* (ESOL) | -6.61 |
| Solubility | 8.93e-05 mg/ml ; 2.48e-07 mol/l |
| Class | Poorly soluble |
| Log *S* (Ali) | -6.81 |
| Solubility | 5.60e-05 mg/ml ; 1.55e-07 mol/l |
| Class | Poorly soluble |
| Log *S* (SILICOS-IT) | -10.53 |
| Solubility | 1.08e-08 mg/ml ; 2.99e-11 mol/l |
| Class | Insoluble |
| **Pharmacokinetics** | |
| GI absorption | High |
| BBB permeant | Yes |
| P-gp substrate | Yes |
| CYP1A2 inhibitor | Yes |
| CYP2C19 inhibitor | Yes |
| CYP2C9 inhibitor | No |
| CYP2D6 inhibitor | No |
| CYP3A4 inhibitor | Yes |
| Log $K_p$ (skin permeation) | -3.88 cm/s |
| **Druglikeness** | |
| Lipinski | Yes; 1 violation: MLOGP>4.15 |
| Ghose | Yes |
| Veber | Yes |
| Egan | Yes |
| Muegge | No; 1 violation: XLOGP3>5 |
| Bioavailability Score | 0.55 |
| **Medicinal Chemistry** | |
| PAINS | 0 alert |
| Brenk | 1 alert: imine_1 |
| Leadlikeness | No; 2 violations: MW>350, XLOGP3>3.5 |
| Synthetic accessibility | 3.78 |

**LIGAND 6:** 2-amino-4,5,7-triphenyl-1H-indole-3,6-dicarbonitrile; PUBCHEM ID:25194820

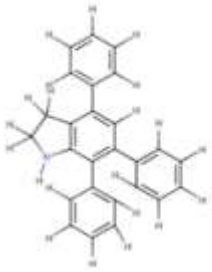



SMILES N#Cc1c(N)[nH]c2c1c(c1ccccc1)c(c1ccccc1)c(c2c1ccccc1)C#N

| Physicochemical Properties | |
|---|---|
| Formula | C28H18N4 |
| Molecular weight | 410.47 g/mol |
| Num. heavy atoms | 32 |
| Num. arom. heavy atoms | 27 |
| Fraction Csp3 | 0.00 |
| Num. rotatable bonds | 3 |
| Num. H-bond acceptors | 2 |
| Num. H-bond donors | 2 |
| Molar Refractivity | 128.44 |
| TPSA | 89.39 Å² |
| **Lipophilicity** | |
| Log $P_{o/w}$ (iLOGP) | 2.97 |
| Log $P_{o/w}$ (XLOGP3) | 6.57 |
| Log $P_{o/w}$ (WLOGP) | 6.50 |
| Log $P_{o/w}$ (MLOGP) | 3.63 |
| Log $P_{o/w}$ (SILICOS-IT) | 6.49 |
| Consensus Log $P_{o/w}$ | 5.23 |

| Water Solubility | |
|---|---|
| Log *S* (ESOL) | -6.95 |
| Solubility | 4.60e-05 mg/ml ; 1.12e-07 mol/l |
| Class | Poorly soluble |
| Log *S* (All) | -8.25 |
| Solubility | 2.33e-06 mg/ml ; 5.67e-09 mol/l |
| Class | Poorly soluble |
| Log *S* (SILICOS-IT) | -10.56 |
| Solubility | 1.13e-08 mg/ml ; 2.75e-11 mol/l |
| Class | Insoluble |
| **Pharmacokinetics** | |
| GI absorption | Low |
| BBB permeant | No |
| P-gp substrate | Yes |
| CYP1A2 inhibitor | Yes |
| CYP2C19 inhibitor | Yes |
| CYP2C9 inhibitor | No |
| CYP2D6 inhibitor | No |
| CYP3A4 inhibitor | No |
| Log $K_p$ (skin permeation) | -4.14 cm/s |
| **Druglikeness** | |
| Lipinski | Yes; 0 violation |
| Ghose | No; 1 violation: WLOGP>5.6 |
| Veber | Yes |
| Egan | No; 1 violation: WLOGP>5.88 |
| Muegge | No; 1 violation: XLOGP3>5 |
| Bioavailability Score | 0.55 |
| **Medicinal Chemistry** | |
| PAINS | 0 alert |
| Brenk | 0 alert |
| Leadlikeness | No; 2 violations: MW>350, XLOGP3>3.5 |
| Synthetic accessibility | 3.18 |

**LIGAND 7:** 4,6,7-triphenyl-2,3-dihydro-1H-indole; PUBCHEM ID:21287130




SMILES c1ccc(cc1)c1cc(c2ccccc2)c(c2c1CCN2)c1ccccc1

| Physicochemical Properties | |
|---|---|
| Formula | C26H21N |
| Molecular weight | 347.45 g/mol |
| Num. heavy atoms | 27 |
| Num. arom. heavy atoms | 24 |
| Fraction Csp3 | 0.08 |
| Num. rotatable bonds | 3 |
| Num. H-bond acceptors | 0 |
| Num. H-bond donors | 1 |
| Molar Refractivity | 117.84 |
| TPSA | 12.03 Å² |
| **Lipophilicity** | |
| Log $P_{o/w}$ (iLOGP) | 3.62 |
| Log $P_{o/w}$ (XLOGP3) | 6.82 |
| Log $P_{o/w}$ (WLOGP) | 6.08 |
| Log $P_{o/w}$ (MLOGP) | 5.49 |
| Log $P_{o/w}$ (SILICOS-IT) | 6.82 |
| Consensus Log $P_{o/w}$ | 5.77 |

| Water Solubility | |
|---|---|
| Log *S* (ESOL) | -6.75 |
| Solubility | 6.17e-05 mg/ml ; 1.78e-07 mol/l |
| Class | Poorly soluble |
| Log *S* (All) | -6.88 |
| Solubility | 4.57e-05 mg/ml ; 1.32e-07 mol/l |
| Class | Poorly soluble |
| Log *S* (SILICOS-IT) | -10.51 |
| Solubility | 1.07e-08 mg/ml ; 3.08e-11 mol/l |
| Class | Insoluble |
| **Pharmacokinetics** | |
| GI absorption | Low |
| BBB permeant | No |
| P-gp substrate | Yes |
| CYP1A2 inhibitor | Yes |
| CYP2C19 inhibitor | Yes |
| CYP2C9 inhibitor | No |
| CYP2D6 inhibitor | No |
| CYP3A4 inhibitor | Yes |
| Log $K_p$ (skin permeation) | -3.58 cm/s |
| **Druglikeness** | |
| Lipinski | Yes; 1 violation: MLOGP>4.15 |
| Ghose | No; 1 violation: WLOGP>5.6 |
| Veber | Yes |
| Egan | No; 1 violation: WLOGP>5.88 |
| Muegge | No; 2 violations: XLOGP3>5, Heteroatoms<2 |
| Bioavailability Score | 0.55 |
| **Medicinal Chemistry** | |
| PAINS | 0 alert |
| Brenk | 0 alert |
| Leadlikeness | No; 1 violation: XLOGP3>3.5 |
| Synthetic accessibility | 2.80 |

Table 5: Molecular property calculation of the title compound

| S.NO | PUBCHEM ID | MLogP | TPSA | No. of Atoms | MOL.WT | No. H | No. NH | Violation | Rotatable bonds | Volume |
|---|---|---|---|---|---|---|---|---|---|---|
| 1. | 44208480 | 6.65 | 15.79 | 33 | 443.97 | 1 | 1 | 1 | 3 | 401.60 |
| 2. | 89657876 | 5.55 | 15.60 | 32 | 416.56 | 2 | 0 | 1 | 4 | 406.16 |
| 3. | 101478243 | 5.72 | 25.02 | 34 | 439.55 | 2 | 1 | 1 | 4 | 413.61 |
| 4. | 631377 | 5.35 | 15.79 | 27 | 345.44 | 1 | 1 | 1 | 3 | 327.25 |
| 5. | 135427495 | 4.76 | 24.39 | 28 | 360.45 | 2 | 1 | 1 | 3 | 339.59 |
| 6. | 25194820 | 3.63 | 89.39 | 32 | 410.47 | 4 | 3 | 1 | 3 | 372.25 |
| 7. | 21287130 | 5.45 | 12.03 | 27 | 347.45 | 1 | 1 | 1 | 3 | 333.43 |

Rev-erba is a member of the nuclear hormone receptor superfamily whose ligand is unknown. Expression of Rev-erba is confined in the brain and metabolic tissues such as skeletal muscle, adipose tissues, and the liver. The active site of the Rev-erba receptor is predicted using CASTP and X, Y, Z dimensions for receptor grid generation have been assessed and virtual screening, molecular docking studies have been performed with the receptor grid generated. From the observation of virtual screening, molecular studies, indole containing ligands have been selected for study. From the results obtained, the following points can be discussed: Triphenyl derivatives of indole are found reported to be more potent than other substituted derivatives. The extent of binding interactions also confirmed the significance of these substitutions. Ex: The indole derivatives with PubChem ids 25194820, 135427495, 101478243, 89657876 which are triphenyl derivative of indoles (Fig.5) were found be more potent in terms of binding affinity rather than other derivatives.

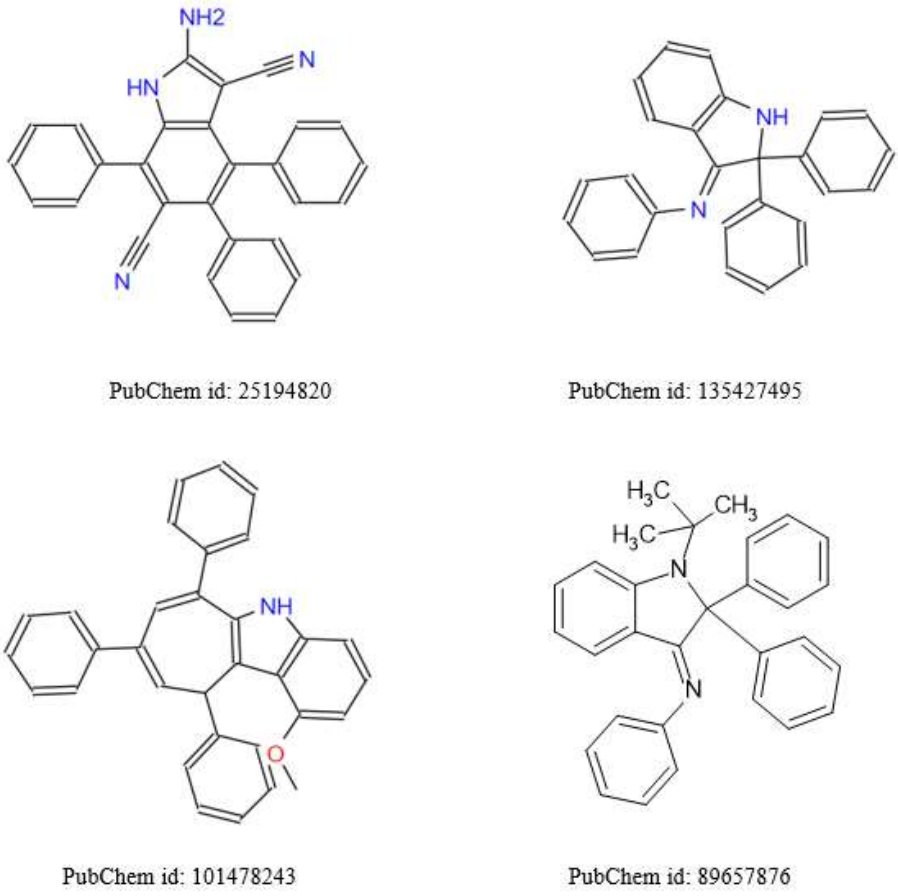



Fig.5: Triphenyl derivative of indoles

**CONCLUSION**

Through the use of the software CASTP the drugs active site was predicted and the amino acids were found out in that particular protein REVERBA ORPHAN NUCLEAR RECEPTOR. Then further ligand-based screening was done by using the software PyRx which gave us the information about the optimal binding affinity obtained by using the lead compound triphenyl indole and its derivatives. To receive more accurate binding affinities further docking studies were performed by using the software Dockthor.

Then the drug interaction of the protein with the ligand were known by using the software BIOVIA Discovery Studio. Lastly the toxicity studies are documented by using the software SwissADME.

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