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ACTIVE SITE STRUCTURE PREDICTION, VIRTUAL SCREENING AND MOLECULAR DOCKING FOR LIGANDS OF THE ORPHAN NUCLEAR RECEPTOR RORB: 1K4W

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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". In the present work, the Orphan receptor: Nuclear receptor ROR-beta (protein) was studied. Recent studies have established roles for the RORs in physiological development and the advent of disease. Identification of ligands for the RORs, both endogenous and synthetic, has established these receptors as attractive new therapeutic targets for the treatment of ROR-related diseases. The findings of recent studies concerning the association between tumorigenesis and circadian rhythm have shown that an aberrant circadian rhythm may promote tumorigenesis and tumor progression. The present work describes a virtual screening methodology that generates a ranked list of high-binding small molecule ligands for orphan G protein-coupled receptors (oGPCRs), circumventing the active site prediction of the receptor using its three-dimensional structure determination. The active site structure is predicted for this protein and further docking studies as well as ligands that are optimally bound to the particular protein were be found through virtual screening and molecular docking studies by using the softwares such as CASTp, PyRx, dockthor.

Later on, the molecular property prediction and toxicity information of the hit molecules have been studied by the use of softwares like SwissADME.

IndexTerms **- Orphan Nuclear Receptor, RORB: 1K4W, Indole derivatives, Virtual Screening, Molecular Docking.**

I. INTRODUCTION

- Classification: HORMONE/GROWTH FACTOR
- Macromolecule: Nuclear receptor ROR-beta (protein)
- Alternative name(s):Nuclear receptor RZR-beta
- Nuclear receptor subfamily 1 group F member 2
- TARGET GENES:NR1F2, RZRB
- Expression System: *Escherichia coli* BL21(DE3)

Nuclear receptors are a super family of transcription factors and the orphan nuclear receptor family. Retinoic acid-related orphan receptor (ROR) β, as a member of the orphan nuclear receptor family, plays an important regulatory role in the maintenance of a variety of physiological and pathological processes. (1)

Function: The nuclear receptors (NRs) are a large family of ligand-regulated transcriptional factors and include the receptors for steroid hormones, thyroid hormones, lipophilic vitamins, and cholesterol metabolites. NRs are involved in a wide variety of biological processes, such as cell proliferation, differentiation, development, and homeostasis. (2). The mechanisms discussed in

this review demonstrate how aberrant RORβ-induced circadian rhythm may become a new direction for future studies on tumorigenesis and strategy design for cancer prevention. (2)

Role of ROR β in Circadian Rhythm: The retinoic acid-related orphan receptor β (RORβ) exhibits a highly restricted neuronal-specific expression pattern in brain, retina and pineal gland.(3) ROR receptors are critical regulators of cellular differentiation and the development of several tissues. In addition, RORs play an important role in the regulation of circadian rhythms. The *in vivo* activity of ROR and consequently the physiological processes controlled by RORs are regulated by endogenous ligands has yet to be determined. This overview shows that great insights have been obtained into the physiological function of RORs in several tissues; however, RORs are expressed in many other tissues in which the physiological function of RORs still needs to be uncovered.(3)So far, neither a natural RORβ target gene nor a functional ligand have been identified, and the physiological role of the receptor is not well understood.(5)The retinoid Z receptor beta (RZR beta), an orphan receptor, is a member of the retinoic acid receptor (RAR)/thyroid hormone receptor (TR) subfamily of nuclear receptors. So far, no natural RZR beta target gene has been identified and the physiological role of the receptor in transcriptional regulation remains to be elucidated. (6)

ROR β-induced circadian rhythm abnormalities and tumorigenesis

- Circadian rhythms are the daily cycles of biochemistry, behavioral and physiological changes regulated by the endogenous circadian clock, which plays an important role in the physiological function and behaviour of the body (7). A series of physiological processes including sleep, body temperature, energy metabolism, cell cycle and hormone secretion are controlled by circadian rhythms. The association between circadian rhythm abnormalities and tumorigenesis has drawn increasing attention. Circadian rhythms of mammals are mainly controlled by hypothalamic SCN and are independent of the light-sensitive system. Destruction of SCN can cause rhythm abnormalities in experimental animals and sleep disorders in patients. Circadian behaviours can be restored in SCN-ablated rodents following re-implantation of the perinatal SCN into the brain. Core clock components generally refer to the genes that are essential for the generation and regulation of circadian rhythms in individual cells and organisms, which primarily include the period and crypto chrome families.
- Circadian rhythms are regulated by RORs. Although there is little evidence supporting the regulatory effects of ROR β on clock genes, RORs possess structural homology and when compared with ROR α and ROR γ, a high expression of ROR β is intensively confined to the SCN, pineal gland, and retina, which are the major elements responsible for the regulation of circadian rhythms. The night time peak level of mRNA of RORβ2 has been detected in the pineal gland and retina whose expression shows a significant circadian rhythm.
- Moreover, ROR β−/− mice are endowed with circadian rhythm abnormalities (8). The mechanisms of how ROR β-induced circadian rhythm abnormalities promote tumorigenesis and tumour development may become a new direction for future investigations on tumour etiology.(9)
- Knowledge of the genes which are regulated by ROR β as well as the factors that regulate ROR β will be prerequisite to a sound understanding of the molecular function of this orphan nuclear receptor. (10) Dysfunction of NR signalling leads to various diseases such as cancer, diabetes, obesity, and autoimmune disorders. The NR super family is one of the primary classes of therapeutic drug targets for human disease. However, evidence has been provided indicating that in cultured cells ROR transcriptional activity can be modulated by ligands. These observations leave open the possibility that synthetic (ant) agonists might be useful in the development of new therapeutic strategies for several human diseases in which RORs are implicated (11).

III. 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 [12].

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 [13].

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 [14].

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.

Table:1 Physico-chemical Parameters used for screening compounds using PUBCHEM database

HBD	0-5
HBA	0-10
Log P	0-5
Molecular weight (M. wt)	160-500
TSPA	20-140A2
Number of rotatable bond	0-10

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.

IV. RESULTS AND DISCUSSION

The amino acids that are present in the active site of the protein are given as follows in Table 2:

Table 2: List of amino acids present in the active site of the protein

227CYS	269 ALA	304LEU	338LEU
228GLN	272TYR	306ARG	339VAL
229TYR	273VAL	307MET	342ALA
234LEU	300LEU	309ARG	343PHE
259TRP	320PHE	310ALA	419VAL
262CYS	322GLY	318VAL	420CYS
263ALA	320PHE	319LEU	423HIS
265GLN	333LEU	266ILE	273VAL
272TYR	446TYR	303VAL	330PHE

Now that we have the basic information about the protein and it's 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

VIRTUAL SCREENING RESULTS

Virtual screening results determined using PyRx software are summarized in the below table 3:

Table 3: Virtual screening results of for Antiviral activity on various Indole derivatives.

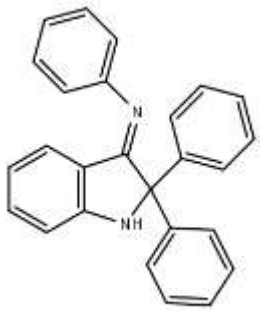
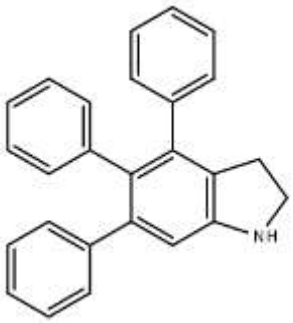
S.No	PUBCHEM COMPOUND ID	PyRX Scores
1	17820846	-10.9
2	58341469	-10.9
3	22978825	-10.7
4	89657836	-10.6
5	135427495	-10.2
6	21287130	-9.0
7	423586	-8.6
8	18320586	-8.2
9	129787113	-7.9
10	23880702	-7.7
11	23880702	-7.5
12	631377	-7.4
13	146077	-7.3
14	68653683	-7.2
15	71361834	-7.4
16	19799483	-7.0
17	101437842	-6.9
18	7021099	-6.9
19	67486	-6.8
20	4066404	-6.7
21	85622851	-6.6
22	102035092	-6.5
23	74621	-6.5
24	10932124	-6.4
25	15557208	-6.4
26	10256	-6.3
27	397	-6.3
28	102409425	-6.2
29	10615829	-6.2
30	1895688	-6.2
31	70336035	-6.2
32	78502	-6.1
33	98617	-6.1

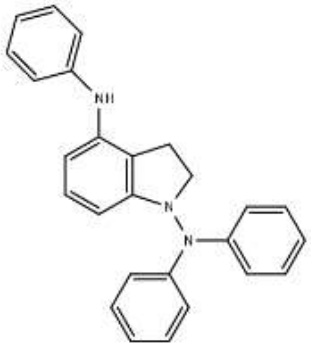
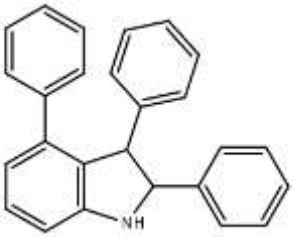
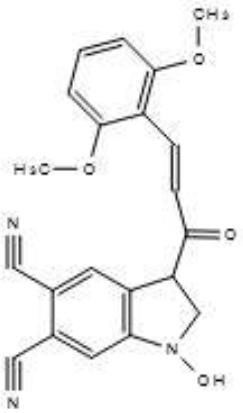
34	10615829	-6.0
35	394101	-6.2
36	162870	-5.8
37	723230	-5.8
38	11572620	-5.7
39	85146	-5.6
40	7129540	-5.5
41	11694169	-5.4
42	22036816	-5.3
43	74706	-5.3
44	76924	-5.2
45	73166	-5.2
46	31576	-5.1
47	1044	-5.0
48	76660	-5.0
49	217796439	-4.9
50	2774463	-4.9

MOLECULAR DOCKING

In the field of molecular modelling, docking is a method which predicts the preferred orientation of ligand in the binding pocket of the target protein. As the binding affinity studies between ligands and their receptors form the basis of physiological activity and pharmacological effects of chemical compounds. We carried out docking studies to investigate the correct binding pose of potent & derived (novel) compounds in the active site pocket of the G-Glycoprotein to evaluate the affinity of the title compounds towards the protein in order to assess their potency in anticancer activity.

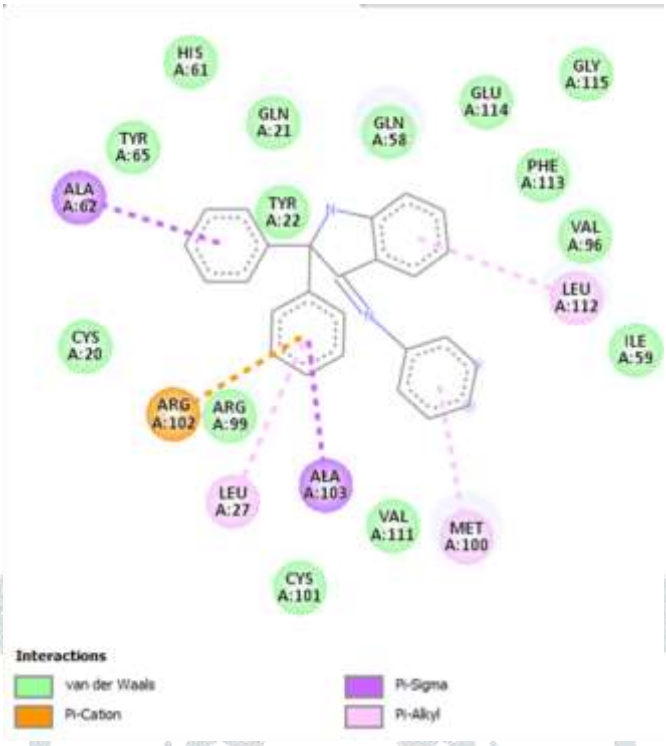
Table :4 Molecular Docking Studies of Potent Molecules (Screened by using Virtual Screening)

S.NO	STRUCTURE OF LEAD MOLECULES	Hydrogen bonding	Hydrophobic interactions
1.		_____	Pi-Sigma: Ala A:62,103 Pi-Cation: Arg A:102 Pi-Alkyl: leu A:27, Met100, Leu112
2.		Carbon HB: Leu A:112,	**Amide Pi stacked**- GLN58 **Pi-sigma** – Ala103,62 **Pi-Alkyl**: Met100, Val111, Ile59, val 96 , Arg99

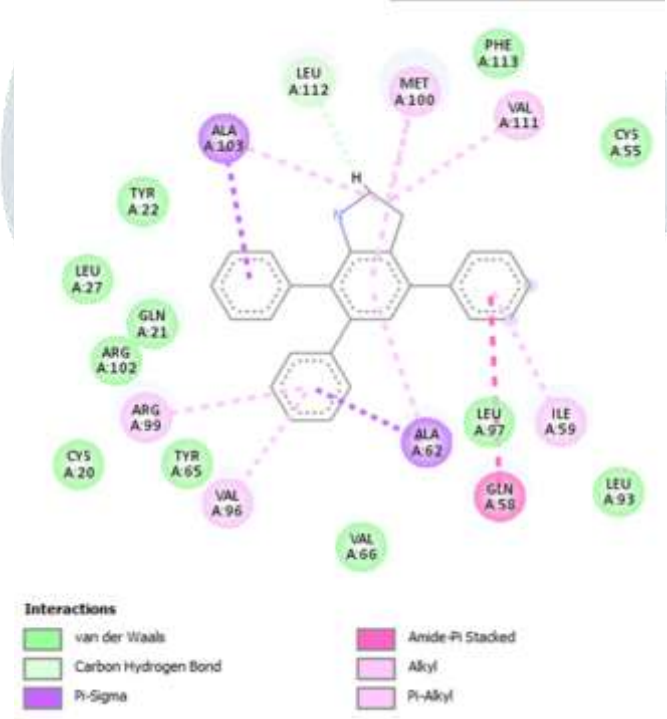
3.		**ConventionalHB**: Arg99,102, GLN21, Tyr22, Leu112 **Carbon HB**: Ile59	**Pi-Sulphur**- leuA:93
5.		**Conventional HB**: leu112	**Pi-Sulphur**-Met100 **Pi-sigma** -val96 **Pi-Alkyl**: Ala62, Ile59, Leu93
6.		_____	**Pi-sigma** –Ala62, Met100 **Pi-Alkyl**: val96, Arg99, Ala103, Leu112

Ligand- Receptor interactions of the lead molecules

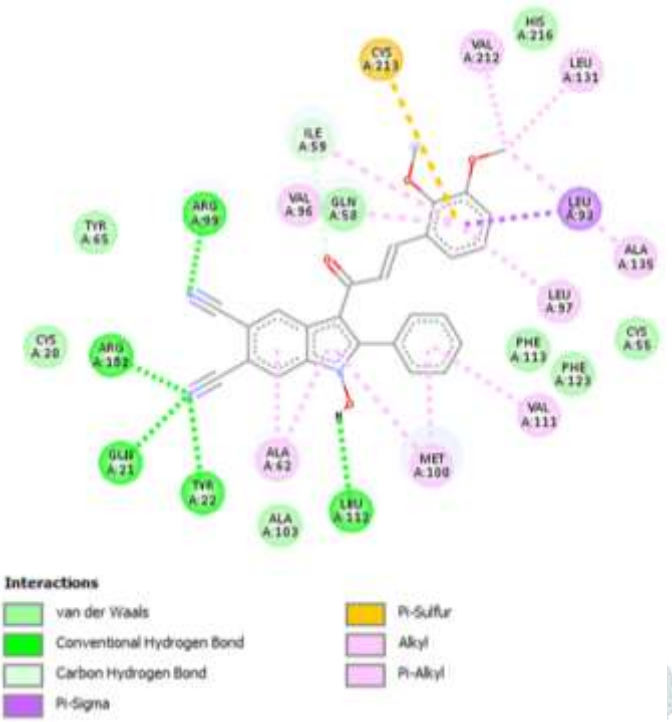
Result 1 N,2,2-Triphenylindol-3-amine



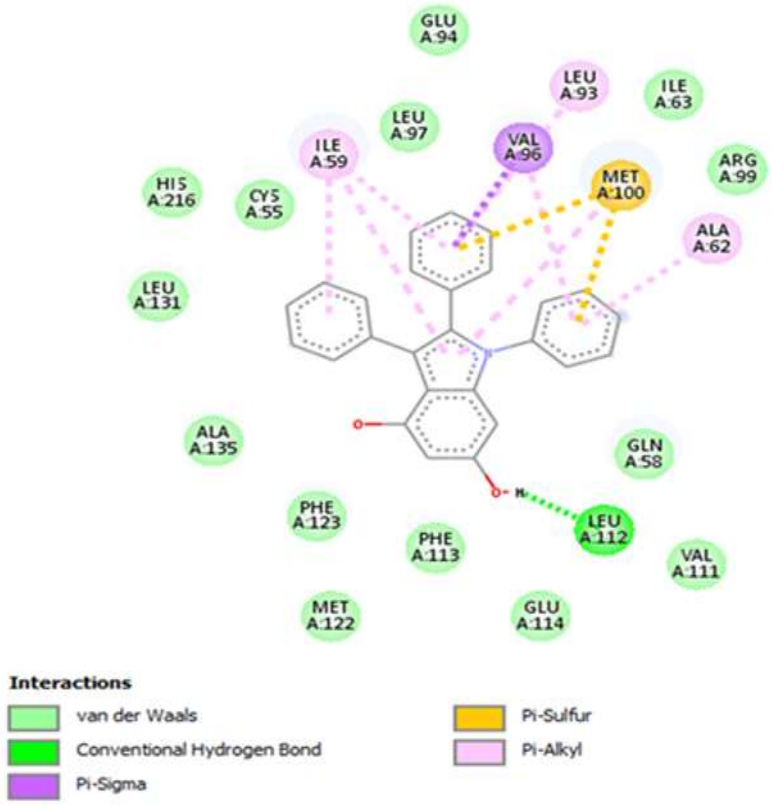
Result 2 4,6,7-Triphenyl-2,3-dihydro-1H-indole



Result 3 :4-N-Methyl-1-N,1-N,4-N-triphenylindole-1,4-diamine



Results 4 Triphenylindole



MOLECULAR PROPERTY CALCULATION AND TOXICITY PREDICTION

Bioactivity Prediction of Indole derivatives using Molinspiration software:

Table-5 The Lipinski rule of five (Lipinski rule *et al* 1997) was adopted to sort out the drug likeness of synthesized compounds. The results are presented in the following table:

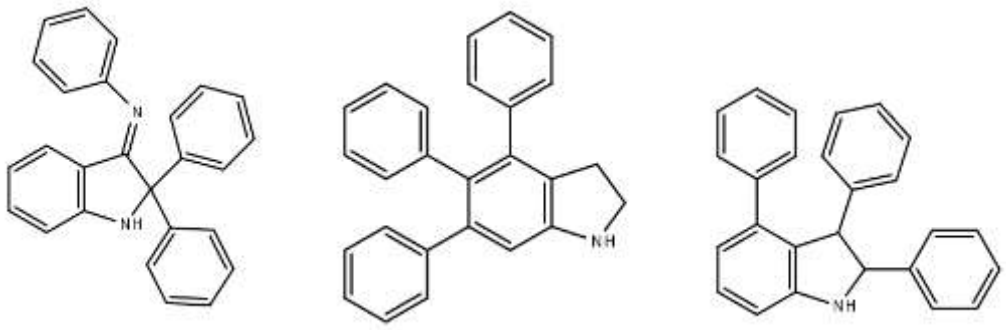
The molecular property of the newly synthesized compounds were calculated values of some basic molecular description such as logp, logs, molecular weight, Polar Surface Area, number of hydrogen bonds donor and number of hydrogen bonds acceptor in molecule membrane hydrophobicity and bioavailability were predicted.

S. No	PUBCHEM ID	mlogp	TPSA	N atoms	Mol.wt	No N	No NH	Violations	Rotatable bonds	Volume
1	135427495	4.69	35.83	29	376.45	1	2	2	3	339.59
2	21287130	5.49	12.03	27	347.46	2	2	2	3	333.43
3	423586	7.34	0	27	344.45	0	0	1	3	331.35
4	18320586	5.90	11.41	30	389.49	0	0	2	5	369.76
5	129787113	5.35	15.79	27	354.44	1	0	1	3	327.25
6	23880702	4.35	45.39	29	377.43	2	2	2	3	343.36
7	13227795	2.00	108.27	34	449.46	7	1	0	6	395.45

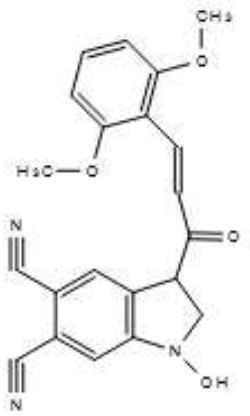
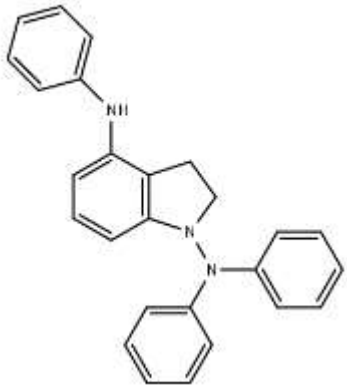
IV. Discussion

ROR beta is a member of the nuclear hormone receptor super family whose ligand is unknown. Expression of ROR beta is confined to the central nervous system and its pattern suggests that this orphan nuclear receptor is implicated in the processing of sensory information and in circadian timing. The active site the ROR beta receptor is predicted using CASTP and XYZ dimensions for receptor grid generation have been assessed and virtual screening, molecular docking studies have been performed with the receptor grid generated. From the observations of virtual screening, Molecular studies Indole containing ligands have been selected for study. From the results obtained the following points can be discussed.

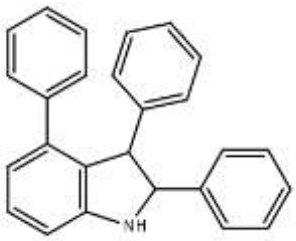
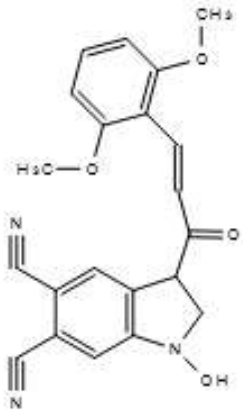
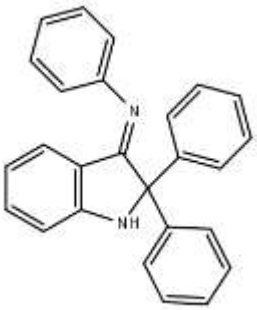
- Triphenyl derivatives of indole are found reported to be more potent than other substituted derivatives.
- The extent of binding interactions also confirmed the significance of these substitutions.
- Eg: The Indole derivatives with pubchem ids 135427495, 18320586, 23880702 which are Triphenyl derivative of indoles was found be more potent in terms of binding affinity rather than other derivatives.



- Indole –NH plays a significant role in mediating the interactions of all the molecules with various aminoacid residues like Arg99,102, GLN21, Tyr22, Leu112 of ROR beta receptor. Therefore free –N-H is essential for mediating there interactions with the protein and substitution of position-1 decreases the anticancer activity.
- Eg: The following two are molecules where the N-1 substitutions have decreased binding scores of -7.2 and 8.6 respectively which is comparatively less than the binding scores offered by other selected indole derivatives.

- Introduction of Triphenyl substitution at position-1,2,4 contributed to the anticancer activity of these derivatives by mediating hydrophobic interactions.
- Hydrophobic interactions of the molecule with Ala62, Ile59, Leu93 is attributed because of the phenyl substitution at position-1,4,5 of the indole ring.
- From the molecular property and toxicity studies of the lead molecules suggested that only three compounds 135427495, 23880702, 13227795 of lead molecules (triphenylindole) were obeying lipsinki rule and are considered to be druggable candidates

13227795 23880702 13542749

V. Conclusion

As there is no specific work reported on ROR beta orphan receptors currently, the designed molecules through our *insilico* approaches can be used for the development of new potent anti cancer agents.With the observations of the SAR studies on the tri substituted indole derivatives screened by ligand based virtual screening, the druggable lead molecules could be further developed as a potent anti cancer agents. Ligand-Binding Pocket (LBP) is strictly hydrophobic, it was predicted that hydrophobic molecules with a carboxylic head are more likely to be ligand candidates for RORβ

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VI. Acknowledgments

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