Int J Pharm Bio Sci 2014 Jan; 5(1): (B) 94 - 102

Research Article **Bioinformatics**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

INSILICO IDENTIFICATION OF SUITABLE ANTAGONISTS FOR GLYCOGEN SYNTHASE KINASE (GSK-3BETA) IN ALZHEIMER'S DISEASE

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ABSTRACT

Alzheimer's disease is also caused by the formation of β Amyloid Plaques and Neurofibrillary Tangles (NFT) due to structural changes in Tau protein. Recent studies have implicated molecular and cellular signaling cascades of Serine Threonine Kinase and Glycogen Synthase Kinase 3β (GSK-3β) in pathogenesis of AD. GSK-3β plays an important role in the formation of NFT and senile plaques in AD. In view of this, the present study focused on identification of GSK-3β inhibitors. The interaction between GSK-3β and selected analogues have studied with modern bioinformatics programs to indentify the best lead molecule, which strongly binds to the active site of the GSK-3β and inhibits its biological activity. The results observed that among 27 Indirubin analogues docked against GSK-3β, Isoindigotin &Indirubin-3-monoxime showed best affinity with GSK-3β than others, Isoindigotin exhibited best biological activity. Therefore, it is suggested that Indirubin analogues controls biochemical process involved in formation of NFT in AD.

KEYWORDS: **Alzheimer's disease, Neurofibrillary Tangles, GSK-3β and Indirubin analogues.**

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INTRODUCTION

Alzheimer's disease (AD) is a fatal brain disorder (Selkoe, D. J. 1997) and in United States alone approximately 4.5 millionAmericans are suffering from this disease which is expected to reach 16 million by 2050 (Alzheimer's Disease Fact sheet, NIH. 2005). Alzheimer's disease is a form of dementia, in which nerve cells in memory areas of brain begin to die at accelerated rate resulting in gradual deterioration of several mental functions, such as loss in Memory, Language, Orientation and Judgment. AD is characterized by the formation of senile plaques and neuro fibrillary tangles resulting in neuronal destructions. Over 400 genes have been tested for their association with late-onset sporadic AD, most with null results. In Alzheimer's disease, changes in tau protein lead to the disintegration of microtubules in brain cells hence AD is also considered as Tauopathy. It has been reported that deposition of Amyloid plaques does not correlate well with neuronal loss (Schmitz *et al*., 2004). In this model, Hyperphosporylated tau begins to pair with other threads of tau and they become tangled up together inside nerve cell bodies in masses known as neuro fibrillary tangles (Goedert *et al*., 1992). After this occurs, the microtubules disintegrate, collapsing the neuron's transport system (Iqbal *et al*., 2005), Thus resulting in malfunctions in biochemical communication between neurons and finally death of Neurons. In this connection, Tau was first discovered as a microtubule associated protein (MAP) that stimulates tubulin assembly into microtubules in the brain (Weingarten MD. *et al*., 1975). Recent reports suggest that glycogen synthase kinase-3β (GSK3β) play an important role in NFT formation, because aberrant activation of this Wnt signalling molecule has been shown to modulate tau phosphorylation (Anderton *et al*., 2001; Kaytor and Orr, 2002) and thus GSK-3β is considered as an important target enzyme in AD treatment. Drugs available today in the market for AD, target neurotransmitters in general and cholinergic inhibitors in particular and some drugs targets β-amyloid protein. But reports suggest that tau protein is also responsible for the occurrence of Alzheimer's disease by forming the neurofibrillary tangles (Experimental Alzheimer drug. Fact sheet: 2005). Hence, identification of the NFT forming protein sequence and blocking the active sites with selected ligands, has become one the best method to control the NFT formation. In view of the above earlier findings, the present study is an attempt to identify the active sites in the target molecule with suitable ligands with suitable tools.

MATERIALS AND METHODS

The protein sequence for GSK-3β (PDB ID-IJIB) was retrieved from NCBI by using URL www.ncbi.nlm.nih.gov, the 3D Structures for Tau protein 1J1B from PDB by using URL http://www.rcsb.org/PDB, Ligands (Indirubin derivatives) from the Literature (Sophie Leclerc et.al ., 2001) and their structures were taken from PubChem database.

The following tools and software were employed for determination of parameters

S.No.	Name of the parameter	Tool/Software employed
1.	Energy Minimization	Aurgus lab
2.	Active site Predication	CAST-P server
3.	Protein ligand docking	Autodock Vina (PyRx)
4.	Interaction and Visualization	Pymol
5.	Biochemical test of lead molecule	1. OSIRIS 2. PASS Prediction

RESULTS

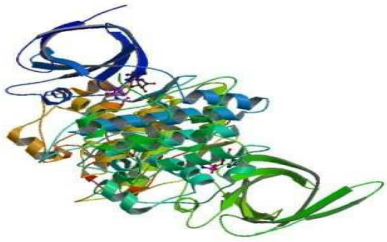
I. Structural details for the Protein 1J1B

From the Protein structural details for (GSK-3β) 1J1B, retrieved from PDB, it was observed that the Human Tau Protein Kinase-I (TPK-I), also known as Glycogen Synthase Kinase 3 Beta (GSK-3Beta) is a Serine Threonine Protein Kinase, composed of three active domains.

1. An N-terminal domain consisting of a closed beta-barrel structure.
2. A terminal domain containing a 'Kinase fold' structure.
3. A small extra-domain subsequent to the C-terminal domain.

The Catalytic site is between the two major domains and has a ATP-analogue molecule in its ATP binding Site. The adenine ring is buried in the hydrophobic pocket and interacts specifically with the main chain atoms of the hinge loop (NCBI).

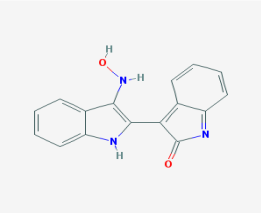
Figure 1
PDB 3D Structure for the Protein, (1J1B)

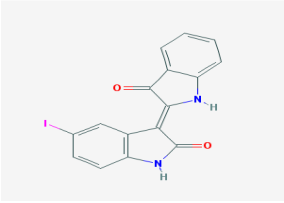


II. Ligands / Antagonists

The ligands visually Indirubins and its derivatives selected in the present study were derived from natural plant sources, and were widely used in traditional Chinese medicine for treatment of Neurodegenerative disorders. (Han, R., 1998). Indirubins constitute a group of low nanomolar lead compounds, which effectively inhibit kinase activity of the enzymes. Docking studies on the 27 selected kinase inhibitors for the enzyme, GSK-3β using Autodock Vina software showed the following structural details.

Ligands selected for docking studies


Indirubin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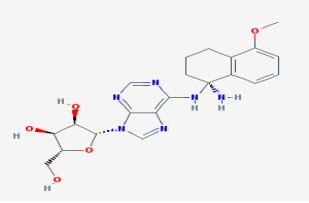
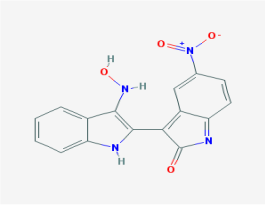

5-methyl indirubin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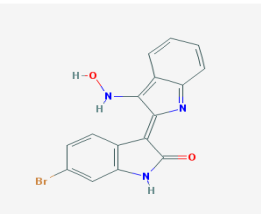
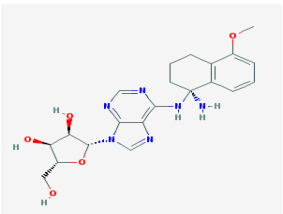
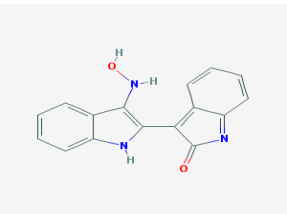
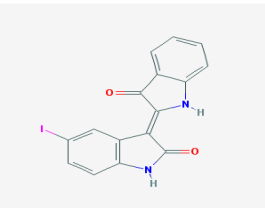
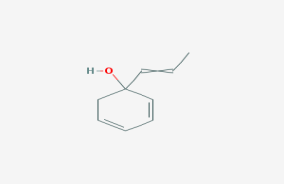

5'-bromoindirubin

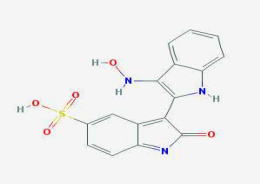

5-5'dibromoindirubin


Indirubin-3-monoxime

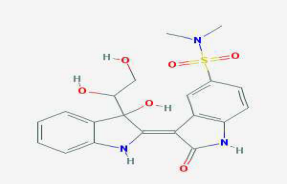

6-iodoindirubin



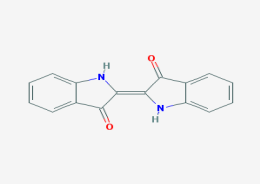
1-methyl indirubin



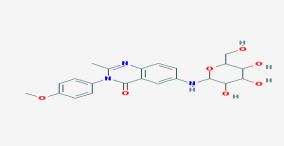
Indirubin-3-monoxime -5-sulphonic acid



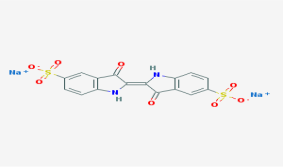
Indirubin-5-sulphonamide



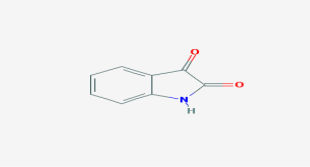
Indigo



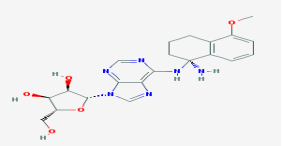
5,5',7,7'indigotetra sulfonic acid



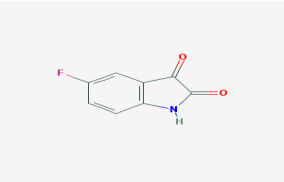
Indigocarmine



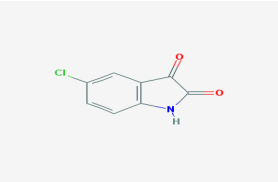
Isatin



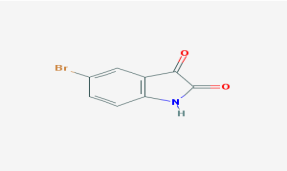
5-iodoisatin



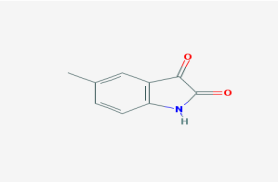
5-fluroisatin



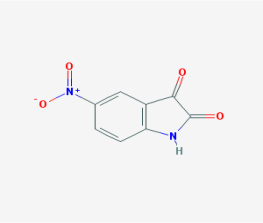
5-chloroisatin



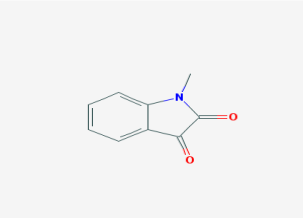
5-bromoisatin



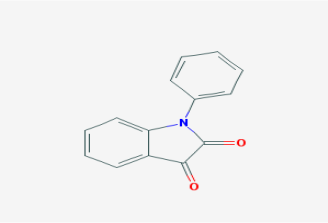
5-methylisatin



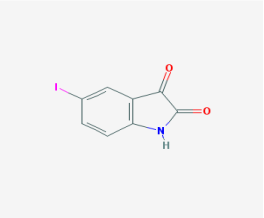
5-nitroisatin



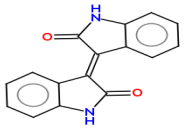
1-methylisatin



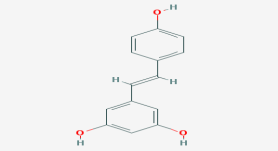
1-phenyl isatin



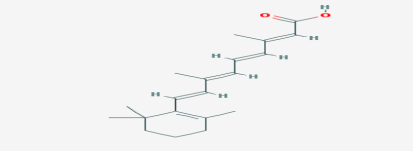
6-iodoisatin



Isoindigotin



Resveratrol



Tretinoin

III. ACTIVE SITE PREDICTION

From CAST-P results, the active sites predicted showed, that 1J1B was having the following Amino acid residues at the protein binding pocket which participate in the protein ligand interactions.

ILE-62, GLY-63, ASN-64, SER-66, GLY-65, PHE-67, GLY-68, VAL-70, ALA-83, LYS-85, VAL-87, LEU-88, GLN-89, ASP-90, LYS-1, ARG-92, PHE-93, LYS-94, ASN-95, ARG-96, GLU-97, MET-101, VAL-110, LYS-122, LYS-123, ASP-124, GLU-125, VAL-126, TYR-127, LEU-128, LEU-130, LEU-132, ASP-133, TYR-134, VAL-135, PRO-136, GLU-137, THR-138, VAL-139, THY-140, ARG-141, ALA-143, ARG-144, TYR-146, SER-147, ARG-148, LYS-150, GLN-151, THR-152, LEU-153, ARG-180, ASP-181, LYS-183, PRO-184, GLN-185, ASN-186, LEU-188, CYS-199, ASP-200, PHE-201, GLY-202, SER-203, ALA-204, LYS-205, PRO-212, ASN-213, VAL-214, ILE-217, CYS-218, SER-219, ARG-220, TYR-221, TYR-222, VAL-246, GLU-249, LEU-250.

IV. Docking Studies: (Table No-1)

The results generated by Autodock Vina revealed that, among the 27 lead molecules only Seven molecules showed best binding

interactions with 1J1B. Out of these, Isoindigotin (-10.3) and Indurubin-3-Monoxime (-9.8) were having the highest binding affinity for 1J1B.

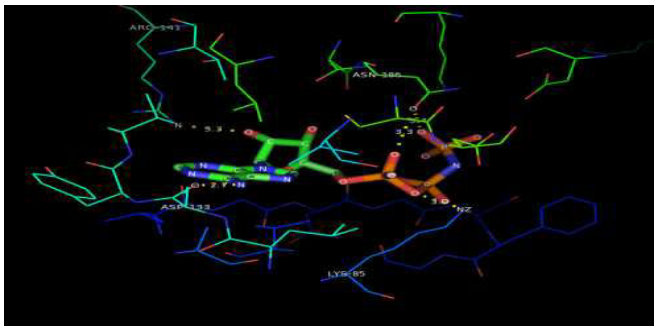
Table No 1
Showing the binding affinity between protein (1J1B) and the ligands

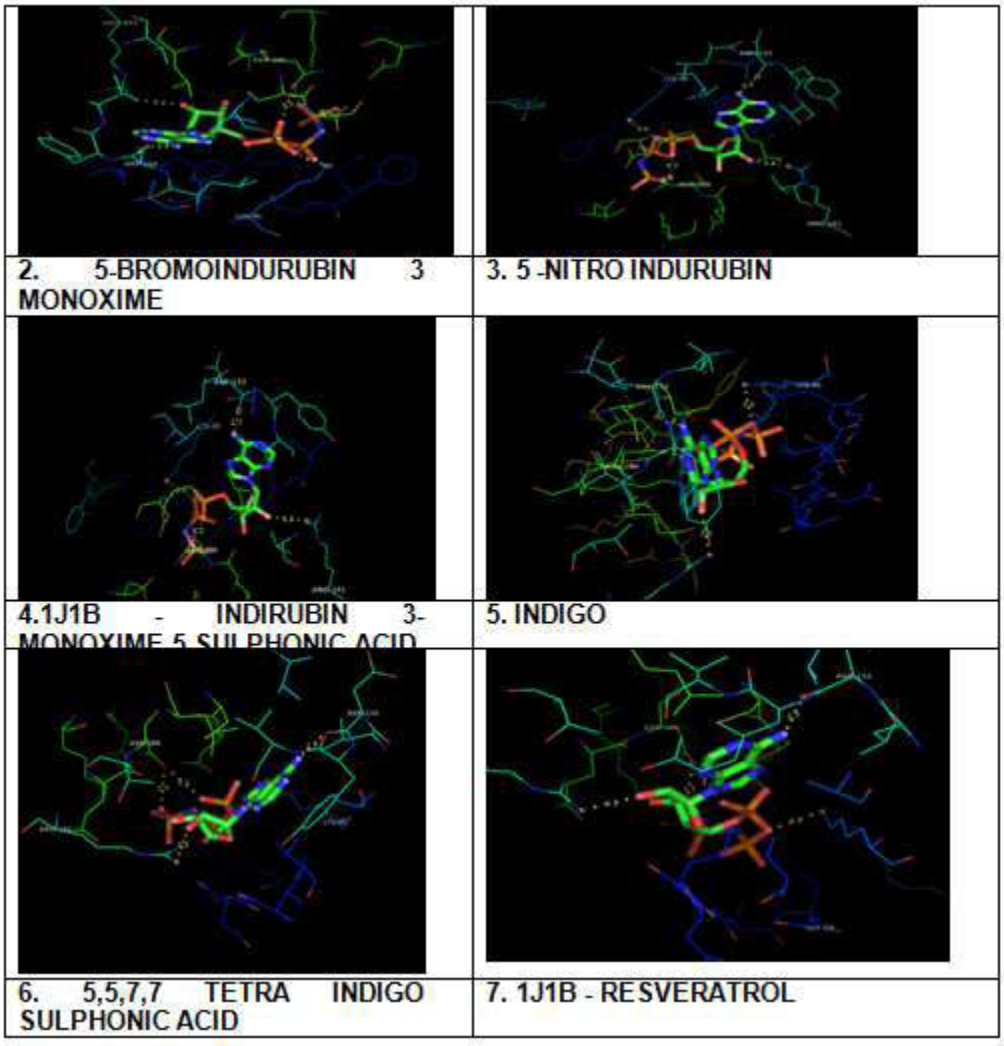
LIGAND	HYDROGEN BONDING			DISTANCE	BINDING AFFINITY
	RESIDUE	ATOM	LIGAND		
ISOINDIGOTIN	**ARG-141**	**N**	**O**	**3.3**	**-10.3**
	ASN-186	**O**	**O**	**3.3**	
	ASN-186	**O**	**O**	**3.4**	
	LYS-85	**N**	**O**	**3.0**	
	ASP-133	**O**	**N**	**2.7**	
5-BROMO INDURUBIN 3 MONOXIME	ASN-186	O	O	3.4	-9.8
	ASN-186	O	O	3.3	
	ARG-141	N	O	3.3	
	ASP-133	N	O	2.7	
	LYS-85	N	O	3.0	
5 -NITRO INDURUBIN	ASN-186	N	O	3.0	-9.6
	ASN-186	O	O	3.3	
	ASN-186	O	O	3.4	
	ASP-133	O	N	2.7	
	ARG-141	N	O	3.3	
INDURUBIN 3-MONOXIME 5 SULPHONIC ACID	ARG-141	N	O	3.3	-9.2
	LYS-85	N	O	3.0	
	ASN-186	O	O	3.3	
	ASP-133	O	N	2.7	
INDIGO	ARG-141	N	O	3.3	-9.1
	ASN-186	O	O	3.3	
	ASN-186	O	O	3.4	
	LYS-85	N	P	3.0	
	ASP-133	O	N	2.7	
5,5,7,7 TETRA INDIGO SULPHONIC ACID	ARG-141	N	O	3.3	-8.5
	ASN-186	O	O	3.3	
	ASN-186	O	O	3.4	
	LYS-85	N	O	3.0	
	ASP-133	O	N	2.7	
7.RESVERATROL	GLY-68	N	O	3.0	-7.6
	ASP-133	O	N	2.7	
	ARG-141	N	O	3.3	
	ASN-186	O	O	3.3	
	ASN-186	O	O	3.4	

V. Protein ligand Interaction and Visualization by Pymol: (Fig No.1 to 7)

From the Protein-Ligand interaction studies, it was observed that the selected lead molecules showed the best interaction with the following amino acids ARG-141, ASN-186, LYS-85, ASP-133 in the binding pocket of 1J1B.

Figure No 2
Picture showing the interaction between (1J1B) and Isoindigotin.





VI. Bioactivity analysis for ligands (Table no.2)

Result obtained from the Osiris output, revealed that Isoindigotin and Indirubin 3-Monoxime showed the best drug related properties such as Solubility, Drug Likeness, Drug Score etc. Further, we found that Isoindigotin did not show any mutagenic properties when compare to other lead molecules.

Table No 2

Data generated on biological activity properties for selected analogues by Osiris.

S.No	Ligand	C LogP	Solubility	Molecular Weight	Drug Likenes	DrugScore
1	ISOINDIGOTIN	1.24	-2.99	262	3.19	0.89
2	INDIRUBIN 3-MONOXIME	2.24	-2.62	277	2.02	0.68
3	INDIGO	2.25	-4.62	262	1.95	0.42
4	INDIRUBIN 3-MONOXIME 5-SULPHONIC ACID	-0.54	-2.53	341	2.39	0.69
5	5 NITERRO INDIRUBIN 3-OXIME	2.63	-2.74	291	2.5	0.68
6	5,5,7,7 TETRA INDIGO SULPHONIC ACID	6.87	-3.85	342	-4.89	0.07
7	RESVERATROL	3.12	-2.86	228	-3.25	0.22

The output of Pass prediction revealed that Isoindigotin showed the best biological inhibitory activities on the enzyme, Kinases based on the Structure of the lead molecule (Table No.3).

Table No 3

Data generated for selected analogue for the prediction of biological activity (Pass prediction).

S.No	Pa	Pi	Activity
1.	0.883	0.003	Kinase inhibitor
2.	0.756	0.005	Neurotrophic factor enhancer
3.	0.741	0.008	Acute neurologic disorders treatment
4.	0.681	0.003	Protein kinase inhibitor
5.	0.691	0.042	Neuroprotector
6.	0.604	0.077	Nerve growth factor agonist
7.	0.527	0.014	Neurotrophic factor
8.	0.476	0.077	Alzheimer's disease treatment

Other Biological activities shown by the Isoindigotin are as follows: CDK2/cyclin A inhibitor, CDK1/cyclin B inhibitor, Apoptosis agonist, Anticarcinogenic, Acetylcholine release stimulant, GABA receptor agonist, GABA C receptor antagonist, GABA receptor antagonist, Phosphorylase kinase inhibitor, Amyloid beta precursor protein antagonist.

DISCUSSION

From our present docking studies, it was observed that the analogues of Indirubin were showing best affinity with Glycogen Synthase Kinase - (GSK-3β), which is responsible for abnormal hyperphosphorylation of the microtubule and NFT formation in Alzheimer's disease (Kaytor and Orr, 2002). Our observation derives strong support from previous in vivo studies, which demonstrated that (Hoessel et. al., 1999, Sai Madhukar et. al., 2013) indirubins were potent inhibitors of an evolutionarily related kinase. The structure-activity relationship studies also suggested that indirubins bind to GSK-3β's ATP binding pocket in a way similar to their binding to Cyclin Dependent Kanases, both are excellent target for Indirubin and their analogues (Sophie Leclerc et al., 2000). Further from our observations on interaction studies between indirubins and GSK -3β, it was evident that the amino acids, ARG, ASN, LYS, ASP, CYS present in the binding pocket of GSK -3β form Hydrogen bonds with Indirubin. It assures that Indirubins can effectively revert the GSK -3β's function and stop the hyperphosphorylation of the microtubule and prevent NFT formation in manifestation of AD. Based on These observations, it may be inferred that good CDK inhibitors are good GSK-3β inhibitors (Sophie Leclerc et al., 2000). The three groups of proteins namely Presenilins, Amyloid β peptides, and Microtubule-binding Tau protein are implicated in the development of AD (Selkoe, D. J. 1998; Haas, C., and Mandelkow,

E., 1999). Earlier reports demonstrated that exposure of hippocampal neurons to Amyloid β peptides leads to GSK-3β stimulation and enhanced tau Phosphorylation (Takashima et al., 1998). GSK--3β inhibitor (indirubins) and their compound significantly reduced tau phosphorylation in a mouse model (Tang, W., and Eisenbrand, G. 1992). Indirubin-3-monoxime also both Indirubin and its derivatives inhibits the *in vivo* Phosphorylation of DARPP-32 by CDK5 on Thr-75, thereby mimicking one of the effects of dopamine in the striatum (Edward Rockensten et al., 2007). So GSK-3b is an Potential target enzyme for the treatment of Alzheimer's disease (AD).

CONCLUSION

Our preliminary investigation on identification of the best Ligand for GSK-3b, were Virtually screened analogues revealed that among 27 Indirubins, Isoindigotin and Indirubin-3-monoxime were showing the best affinity for GSK-3β (-10.3 and -9.8). The Osiris output also further reiterated that only these three, Isoindigotin compound possessed significant drug like activity (drug Score 3.19) than Indirubin-3-monoxime. Based on the structure of the indirubins, we predicted that GSK-3β has several properties biological activity GSK -3β such as inhibitor, Neurotrophic factor enhancer, Neurotrophic factor and Nerve growth factor agonist finally, it has been concluded Since Indirubin inhibits, GSK--3β functions, so it can act as a potential therapeutic drug target for treatment of AD (Diane P. Hanger and Wendy Nobel, 2011).

ACKNOWLEDGEMENT

We thank the DBT supported Bioinformatics Infrastructure Facility for providing the networking facilities to execute the research work.

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