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HOMOLOGY MODELING OF MTNR1B AND *INSILICO* STRUCTURE ACTIVITY RELATIONSHIP STUDY OF MELATONIN ANALOGS FOR THERAPEUTIC APPLICATION IN INSOMNIA AND INSOMNIA RELATED DIABETES.

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ABSTRACT

Melatonin is a circulating hormone that is primarily released from the pineal gland and performs a circadian rhythm in human and plays a vital role in insomnia and diabetes. In order to search promising MTNR1B homolog inhibitors, an *in-silico* study is carried out. Since there is no reported MTNR1B crystal structural data, three dimensional structure of MTNR1B was modeled based on crystal structure of the Mos1 mariner (PDB: 3HOT_A) and validated using PROCHECK, Ramchandran plot and energy optimization techniques. Further implementing the *Insilico* molecular modeling and interaction study approaches were performed with 6 different inhibitors including melatonin and reference molecule as a Ramelteon with 5 different cavities detected by MVD where Cavity 2 resulted to a least and favorable energy among all. Melatonin with acetyl side chain extended with butyryl which followed Lipinski rule of five, and a combinatorial library of 177 analogs are designed. After several docking study and Lipinski screening, successively 3 melatonin analogs are designed and screened, which may have a better therapeutic application to the melatonin.

KEYWORDS: MTNR1A, MTNR1B, Structure Activity Relationship, MVD, melatonin analogs.

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INTRODUCTION

Melatonin is a circulating hormone that is primarily released from the pineal gland and also known as a regulator of seasonal and circadian rhythms; its levels are high during the night and low during the day [1][2]. An internal biological clock regulates the timing for sleep in humans. The activity of this clock makes us sleepy at night and awake during the day. Our clock cycles with an approximately 24-hour period and is called a circadian clock. In humans, this clock is located in the Suprachiasmatic nucleus (SCN) of the hypothalamus in the brain [3]. Melatonin is an example of chemical output form and input to the SCN. Melatonin promotes sleep by binding to and regulating the G protein–coupled receptors MT1 and MT2 in the SCN. Through projections to the dorsomedial hypothalamus (DMH) and downstream nuclei, the SCN promotes sleep at night and wakefulness during the day. The recently developed drug ramelteon (or Rozerem) is a sleep-inducing synthetic melatonin receptor agonist and hebce considered as a reference drug in the study [4]. A melatonin receptor is a G protein-coupled receptor (GPCR) which binds melatonin. Three types of melatonin receptor have been cloned. The MT1 (or Mel1A or MTNR1A) and MT2 (or Mel1B or MTNR1B) receptor subtypes are present in humans and other mammals, while an additional melatonin receptor subtype MT3 (or Mel1C or MTNR1C) has been identified in amphibians and birds [5-7]. The physiological actions of melatonin are mediated by two G-protein coupled membrane receptors, MT_1 and MT_2, and the MT_3 binding site, which belongs to the family of the Quinone reductases. The majorities of the high-affinity MT1 and MT2 receptors is expressed in the SCN and have distinct functional roles in sleep regulation. Activation of the MT_1 receptor suppresses neuronal firing rate in the SCN, while MT_2 acts mainly by inducing circadian rhythm phase shifts. Both MT_1 and MT_2 receptors are also expressed in peripheral organs and cells and contribute to other physiological functions [8]. In diabetic patient, sugary food raises the blood sugar level. The pancreas does not make adequate insulin to keep blood glucose levels regular, often because the body does not. This prevents the amino acid tryptophan from entering the brain and hence, tryptophan will not be converted into the hormone serotonin (which promotes relaxation). In darkness, serotonin will not get converted into melatonin in the pineal gland. As less melatonin is there it may lead to Insomnia [9, 10]. Our body stores ready-to-use energy as glycogen in the liver. Sufficient glycogen storage is necessary for restful sleep. When your liver runs out of glycogen at night, your brain starts to trigger stress hormones such as cortisol and adrenalin to convert protein muscle into glucose. This excess of cortisol promotes anxiety and makes it difficult to relax, and fall asleep [9, 10]. Melatonin induces the tyrosine phosphorylation of IRS-1(Insulin receptor substrate 1), which plays an important role in the stimulation of muscle glucose uptake. So if melatonin is less, Glucose uptake will be less and resulting to Hyperglycemia. If above situation occurs frequently then it may cause B-cell dysfunction in pancreatic cell which may happen to Diabetes. [11]

MTNR1B was selected as the target for the further studies based on following reference study:-

A common variant in MTNR1B was associated with fasting glucose, HbA1C and HOMA-B but not with sleep status in Chinese Hans from Shanghai, strengthening the role of MTNR1B rs10830963 in fasting glycemia and impaired beta-cell function [12]. Also a common variant in MTNR1B, encoding melatonin receptor 1B, is associated with type 2 diabetes and fasting plasma glucose [13, 16]. So based on above references it was confirmed that MTNR1B is a common factor in Insomnia and Diabetes. Melatonin promotes sleep by binding to and regulating the G protein–coupled receptors MT1 and MT2. Through projections to the dorsomedial hypothalamus (DMH) and

downstream nuclei, the SCN promotes sleep at night and wakefulness during the day. The available drug Melatonin and Ramelteon (or Rozerem) is a sleep-inducing synthetic melatonin receptor agonist [4, 17].The reference structures were taken from published data of Melatonin and Ramelteon[18].

Methodology

Target Selection.

There are two subtypes of the Melatonin receptor in humans, melatonin receptor 1 (MT1) and melatonin receptor 2 (MT2) [19-21]. MTNR1B was selected as the target for the further studies based on the reference study [22, 23].

Modeling of MTNR1B.

The modeling of MTNR1B was done using Automated Modeling servers as well as through homology modeling using MODELLER tool [19-21] [24 - 26]. Automated modeling servers such as: GOR, [27-29] SOPMA [30], BHAGEERATH [31 - 34], (PS) 2 : Protein Structure Prediction Server [35], CPH Model 3.0 Server [36] and ESyPred3D Web Server 1.0. [37]. MTNR1B was selected as the target for the further studies based on the reference study [19 -21]. 3HOT_A with 44% in PSI-BLAST was selected as a template for homology modeling of MTNR1B using Modeller9v8 tool [38] [39, 40] [19-21] resulted with a dope score of -33254.34375. The 3-D structure of MTNR1B obtained by homology modeling was evaluated and validated using PROCHECK [41, 42] comprising 90.9% amino acid residues in favored region of Ramachandran plot [43]. Energy minimization for the predicted model was also performed using SPDBV [44-47], YASARA [48] and MVD [49] side chain minimization. Further predicted structure of MTNR1B was simulated using CHARMM algorithm [50], refined and flexible docking was performed with 6 different inhibitors with 5 different cavities detected by MVD [49] and the interaction studies were carried out for all the 6 inhibitors. Melatonin [1] and Ramelteon [4] were considered for further studies.

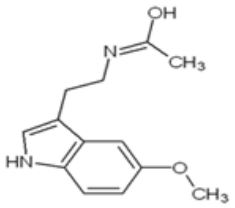

Figure 1a. Melatonin.

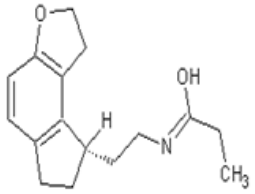

Figure 1b. Ramelteon

A molecular interaction study has been carried using basic scaffold of Melatonin and Ramelteon with MTNR1B resulted a good binding affinity, an extension of aliphatic chain has been carried on both the lead molecules and again the second iteration of docking is carried out. For Ramelteon, at 3, N (7) position, which showed interactions with the receptor (MTNR1B) protein, the side chain extension was carried out using aliphatic chain -CH_3 along with -NH_2 and -OH groups. Literature reveals presence of halogen at 6th position and further exploration acetyl side chain to butyl was carried out on melatonin [51]. After several docking studies, Cavity 2 showed best result with the least energy compared to other cavities detected by MVD. Henceforth it can be concluded that Cavity 2 of MTNR1B can be indigenous active site, And in case of Melatonin structural analogues, Melatonin with acetyl side chain extended with butyryl which followed Lipinski rule of five[52], showed consistent least energy result for all the five cavities. So, combinatorial library consisting of 177 structural analogues of Melatonin with acetyl side chain extended with butyryl was generated based on its nucleophilic interaction at 3 different positions with the receptor protein and their

docking was carried out on cavity number 2 of MTNR1B modeled structure. A Lipinski filter was performed for the structures having the best i.e. least energy at each 3 positions where substitutions were done. Out of 177 analogues, 3 have successfully followed Lipinski rule of five. While comparing the docking energies, these analogues showed least docking energy compared to Melatonin as well as Melatonin with acetyl side chain and extended with butyryl. Melatonin drug showed -102.075 as the docking energy, while analogs of melatonin acetyl side chain extended with butyryl also followed Lipinski rule of five, showed docking energy of -177.3 , -161.6 and -165.3kj/mol.

RESULT

Secondary Structure of MTNR1B: The secondary and tertiary structure of MTNR1B is carried out and the result is on following table: 1, 2 and graph 1.

Table 1
Secondary structure prediction result.

Algorithm	Alpha Helix	Extended Strand	Beta turn	Random coil
GOR	73	102	00	187
SOPMA	143	66	16	137

Table 2
Tertiary Structure Prediction result.

Evaluation on Ramachandran Plot				
Algorithm	Most Favored region	Additional allowed regions	Generously allowed region	Disallowed regions
BHAGEERATH	89.3	7.2	1.9	1.6
(PS)2: Protein Structure Prediction Server	85.0	11.0	2.8	1.3
CPH Model 3.0 Server	85.3	11.0	2.7	1.0
ESyPred3D Web Server 1.0	89.0	7.3	2.3	1.3
Modeler	90.9	7.8	0.3	0.9

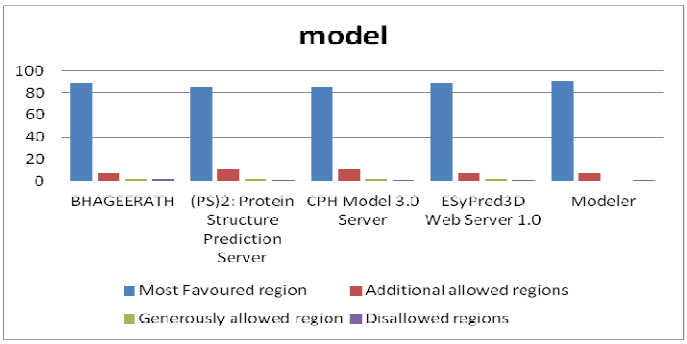

Figure 2
Comparative model result of 3D structure.

The most favoring results are from the Modeler using the homology modeling concept, with a 90.9% of allowed regions, optimizing and simulation of structure resulted to better orientation of the structure. The optimization is carried out using Gromos = -9711.929 KJ/mol. Yasara = 26286.5 KJ/mol and MVD = 13557.8 KJ/mol with a side chain optimization (fig 1, 2).

Submitting the structure to identify the active site resulted in 5 optimum hydrophobic voids (fig 2) that can be a possible place to interact the ligands and proved to be a better therapeutic space. Selected six indigenous inhibitor of MTNR1B were interacted with all the 5 cavities of MTNR1B results are described in (table 2).

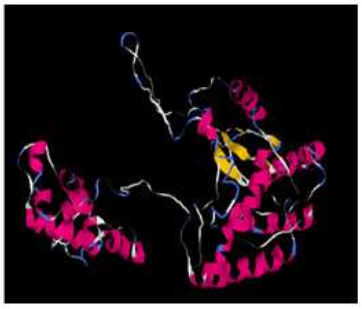

Figure 3a
Otimized 3D structure of MTNR1B.

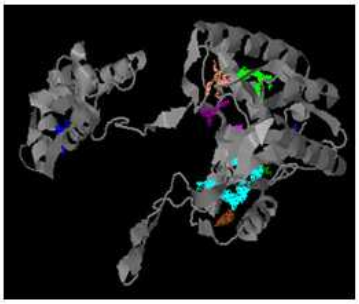

Figure 3b.
Predicted active site using Q-Site finder.

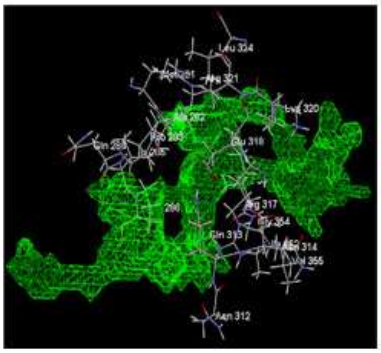

Figure 3c.
Docked complex of MTNR1B

Table 2
molecular interaction data of MTNR1B and its six inhibitors.

Drug	Cavity and its Volume in Amstrong				
	Cavity 1 (454.656) A	Cavity 2 (183.296) A	Cavity 3 (142.336) A	Cavity 4 (124.928) A	Cavity 5 (102.4) A
2-Iodomelatonin	-105.327	-109.518	-89.2221	-107.98	-103.385
6-Chloromelatonin	-99.4306	-99.8873	-92.525	-97.586	-99.7621
Agomelatine	-85.3883	-91.0485	-80.6741	-78.0029	-82.3026
Melatonin	-103.445	-102.075	-85.1302	-99.5092	-93.851
Ramelteon	-117.389	-122.006	-106.656	-117.961	-122.366
Tasimelteon	-115.932	-108.198	-98.2549	-115.701	-108.318

Performing the molecular interaction mapping of melatonin –MTNR1B complex, active site-2 resulted to a most prominent as compare to other 4 cavities (table 3), (fig 4).

Table 3
Molecular interaction mapping of Melatonin to all 5 cavity of MTNR1B.

Sr no	Drug	Cavity number	Energy	Amstrong distance	No of Amino acids	Amino acids
1	Melatonin	1	-95.1383	10	2	Arg317, Gln313
2	Melatonin	2	-102.075	13	4	Gly288, His156, Thr157, Ser180.
3	Melatonin	3	-85.1302	10	2	Ala83, Glu110
4	Melatonin	4	-96.7846	15	1	Glu287
5	Melatonin	5	-92.7815	17	2	Pro283, Glu318

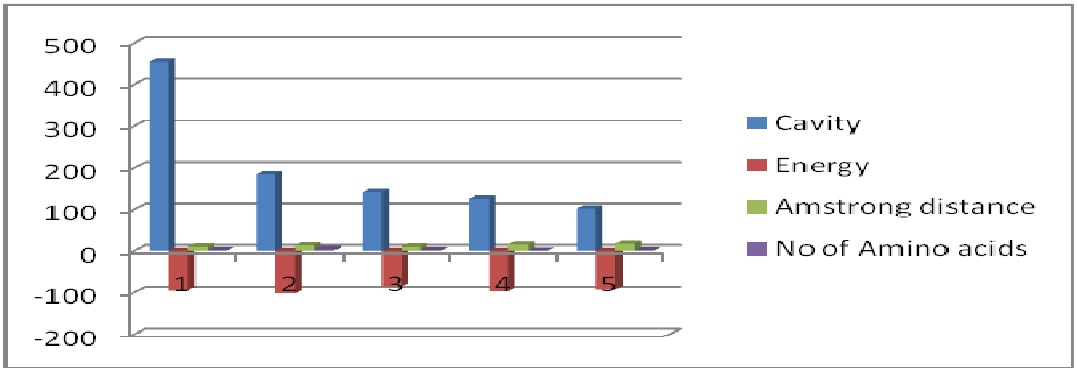

Figure 4
Molecular interaction mapping of Melatonin to all 5 cavity of MTNR1B.

SAR results of Melatonin and Ramelteon

The outcomes of mapping subjected to SAR of melatonin and ramelteon, and subjected to docking anaysis, again the cavity 2 scored the most stable complexes in MTNR1B with respect to melatonin and ramelteon (fig 5-5a.), (fig 6-6a), (Table 4a, 4b).

Figure 5
Melatonin.

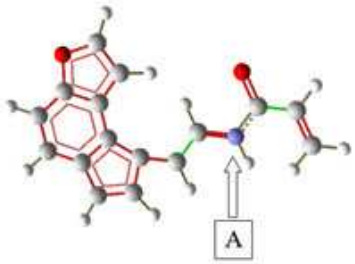

Figure 6
Ramelteon

In case of Ramelteon, different structural analogues showed best energies and interactions results for 5 different cavities. For Cavity 1 & 3, Ramelteon-N-C_9 showed best energy results, for Cavity 2 & 5, Ramelteon-N-C_7 showed best results and for Cavity 4, Ramelteon-N-C_6 showed best result. But for Melatonin, Melatonin with the extension of acetyl side chain to butyryl showed the consistent best result for all the 5 different cavities. For this reason, Melatonin with acetyl side chain extended with butyryl was selected for the further studies. From the interaction study of Melatonin most of the interactions were founded at position A and B and Ramelteon, most of the interactions were at nitrogen that is position “A”. So, at position “A” in (Fig 59), the side chain extension was done. But no specific research paper denoting SAR of Ramelteon was found. So randomly the combinatorial library was prepared.

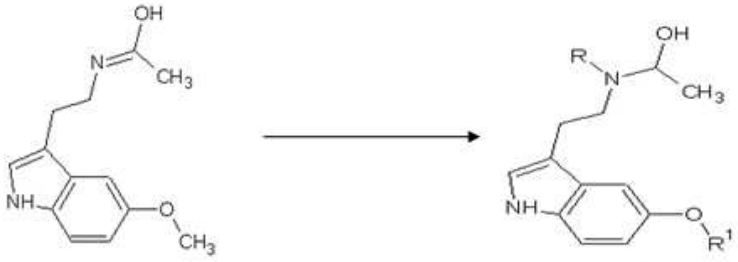

Figure 5.
Melatonin.

Figure 5a.
Melatonin

1) R = Br, $R^1 = C_3H_5O$
2) R = Cl, R^1 = C3H7CO
3) R = F
4) R = I
5) R^1 = C3H5O
6) R = C3H7CO

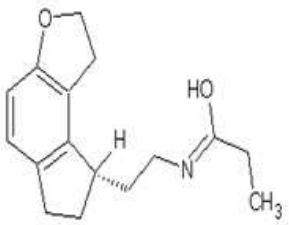

Figure 6
Ramelteon.

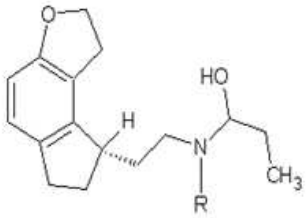

Fig 6a
Ramelteon.

1) R = $-CH_3$. 2) R = $-C_2H_5$ 3) R = $-C_3H_7$.
4) R = $-C_4H_9$. 5) R = $-C_5H_1$ 6) R = $-C_6H_{13}$.
7) R = $-C_7H_{15}$. 8) R = $-C_8H_{17}$ 9) R = $-C_9H_{19}$.
10) R = $-C_{10}H_{21}$. 11) R = $-NH_2$. 12) R = -OH.
13) R = $-C_6H_7N$. 14) R = $-C_5H_5N$.

Table 4a
Melatonin and its analogues MVD result energy comparison

Cavity	Melatonin Energy	Best Inhibitor	Energy
Cavity 1	-95.1383	Melatonin-butyryl	-116.457
Cavity2	-102.075	Melatonin-butyryl	*-125.951*
Cavity 3	-85.1302	Melatonin-butyryl	-102.003
Cavity 4	-99.5092	Melatonin-butyryl	-118.904
Cavity 5	-91.6057	Melatonin-butyryl	-108.846

Table 4b.
Ramelteon and its analogues MVD result energy comparison.

Cavity	Ramelteon Energy	Best Inhibitor	Energy
Cavity 1	-112.267	Ramelteon-N-C_9	-137.797
Cavity2	-122.006	Ramelteon-N-C_7	*-163.712*
Cavity 3	-103.447	Ramelteon-N-C_9	-152.112
Cavity 4	-117.961	Ramelteon-N-C_6	-145.304
Cavity 5	-110.489	Ramelteon-N-C_7	-123.257

Table 4c.
Active atoms from ligand interacting with receptor protein.

Atom position in ligand.	Receptor (MTNR1B) interacting amino acid.	Interaction type.
N_{14}	Thr292	Hydrogen bond acceptor
O_{15}	Ser180	Hydrogen bond acceptor
O_{19}	Thr157	Hydrogen bond acceptor
O_{19},C_{18},O_{20}	His160	Negative Ionizable Area
C_2, C3, C_{12}, C_{11}, C_6, and C_{10}.	Val291,Thr157	Hydrophobic Interactions

Combinatorial library of Melatonin_butyryl.

As shown in (Table 4c), first 3 atoms of ligand were Hydrogen accepter therefore electron donor that is nucleophilic. So other 14 functional groups having similar nucleophilic properties were added. Functional groups added at positions “R”,”R^1” and”R^2”.

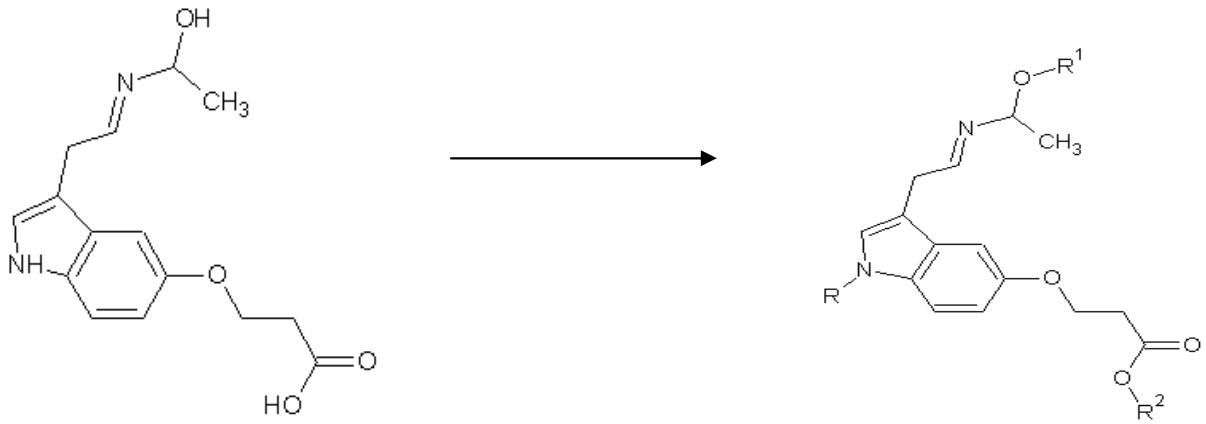

Fig 5a. $C_{15}H_{18}N_2O_4$. Fig 5b. $C_{15}H_{18}N_2O_4$-R, R^1, R^2

Fig 5a. $C_{15}H_{18}N_2O_4$.

Fig 5b. $C_{15}H_{18}N_2O_4$-R, R^1, R^2

1) –Br	2) –Cl	3) –F	4) –OH
5) –I	6) $-N_3$	7) –CN	8) $-NH_2$
9) –S	10) $-C_2H_4O_2^-$	11) $-C_3H_6O_2^-$	12) $-C_4H_8O_2^-$
13) $-C_5H_{10}O_2^-$	14) $-C_6H_{12}O_2^-$	15) $-C_7H_{14}O_2^-$	16) $C_8H_{16}O_2^-$
17) $C_9H_{18}O_2^-$	18) $-C_{10}H_{20}O_2^-$	19) $-C_{11}H_{22}O_2^-$	20) $-C_2H_4O_2$
21) $-C_3H_6O_2$	22) $-C_4H_8O_2$	23) $-C_5H_{10}O_2$	24) $-C_6H_{12}O_2$
25) $-C_7H_{14}O_2$	26) $-C_8H_{16}O_2$	27) $-C_9H_{18}O_2$	28) $-C_{10}H_{20}O_2$
29) $-C_{11}H_{22}O_2$	30) $-CH_3O^-$	31) $-C_2H_5O^-$	32) $-C_3H_7O^-$
33) $-C_4H_9O^-$	34) $-C_5H_{11}O^-$	35) $-C_6H_{13}O^-$	36) $-C_7H_{15}O^-$
37) $-C_8H_{17}O^-$	38) $-C_9H_{19}O^-$	39) $-C_{10}H_{21}O^-$	40) $-CH_4O$
41) $-C_2H_6O$	42) $-C_3H_8O$	43) $-C_4H_{10}O$	44) $-C_5H_{12}O$
45) $-C_6H_{14}O$	46) $-C_7H_{16}O$	47) $-C_8H_{18}O$	48) $-C_9H_{20}O$
49) $-C_{10}H_{22}O$	50) $-CH_3S^-$	51) $-C_2H_5S^-$	52) $-C_3H_7S^-$
53) $-C_4H_9S^-$	54) $-C_5H_{11}S^-$	55) $-C_6H_{13}S^-$	56) $-C_7H_{15}S^-$
57) $-C_8H_{17}S^-$	58) $-C_9H_{19}S^-$	59) $-C_{10}H_{21}S^-$	

After final and third iteration of docking on MTNR1B with 177 melatonin analogs the three analogs have reported a consistent efficient binding score, stable complex and also followed Lipinski rule with respect to their specific substitution position to R, R1 and R2 (table 5).

Table 5
Optimum 3 Melatonin analogs

Inhibitors	Binding Energy	Lipinski filter				
		Molecular Weight <= 500 Daltons	Hydrogen Bond Donor <= 5	Hydrogen Bond Acceptor <= 10	Log P <= 5	Molar Refractivity 40 to 130
$C_8H_{16}O_2$ (Position R at 14N).	-177.3	388.00	1	5	2.764	109.752
C_4CO_2 (Position R^1 at Position 15O).	-161.6	349.00	2	6	3.292	98.505
$C_{10}S$ (Position R^2 at Position 19O.	-165.3	415.00	2	3	5.258	130.125

CONCLUSION

Modeling of MTNR1B has revealed its more significant structural properties, by identifying the cavity area and better interaction with melatonin analogs. Whereas the Structure Activity Relationship of Melatonin has significantly helped in enhancing the binding efficiency of the Melatonin analogs, by screening of unwanted chemical structure that may land to erroneous result. As the complete work was done under the review of Insilico methodology to gain a more approximate result, one has to validate the results with wet lab scenario. Several docking experiments were performed into the active site of receptor reveals that, cavity 2 may be an indigenous site for melatonin analogs. The proposed methodology denotes that one can increase the binding efficiency by implementing the *Insilico* molecular modeling and interaction study, whereas the screening of analogues by considering the Lipinski rule of five reduces the work of scientific community for further toxic interferences. It still needs lots of wet lab experiments for verification. So based on this *Insilco* study we can say that we have enhanced the activity of original Melatonin drug. As we are enhancing the activity of Melatonin, we can say that we are improving the sleep quality in diabetics which would have a similar beneficial effect as the most commonly used anti-diabetes drugs.

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