



Review Article

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Exploring the Evolutionary Patterns of Fish and Rat Melatonin Receptor Responses to Pineal Peptides: A Bioinformatics Analysis

Ayushi Singh[#], Umang Verma[#] and Somenath Ghosh^{*}

Dr Harisingh Gour Vishwavidyalaya (A Central University), Sagar, Madhya Pradesh 470003, India

***Corresponding author:** Dr. Somenath Ghosh, Assistant Professor, Department of Zoology, Dr. Harisingh Gour Vishwavidyalaya (A Central University), Sagar 470003, Madhya Pradesh, India.

To Cite This article: Ayushi Singh[#], Umang Verma[#] and Somenath Ghosh^{*}, Exploring the Evolutionary Patterns of Fish and Rat Melatonin Receptor Responses to Pineal Peptides: A Bioinformatics Analysis. *Am J Biomed Sci & Res.* 2026 32(3) AJBSR.MS.ID.004169,

DOI: [10.34297/AJBSR.2026.32.004169](https://doi.org/10.34297/AJBSR.2026.32.004169)

Received: 📅 October 09, 2026 Published: 📅 October 13, 2026

Abstract

Melatonin Receptor Proteins (MRPs) are G-protein-coupled receptors that regulate reproduction, immune function, and bone metabolism across vertebrates, including fish. Using an integrated bioinformatics pipeline, we modelled, docked, and simulated the rat melatonin receptors MT1A and MT1B and the fish (*Labeo rohita*) receptors Mel1a, Mel1b, and Mel1c in complex with the synthetic pineal tetrapeptide epitalon. Subcellular localisation predictions placed all five receptors predominantly at the inner plasma and cytoplasmic membranes. Ramachandran plot analysis confirmed high-quality homology models: 96.0% of residues in rat MT1A and 94.1% in fish Mel1b occupied the most favoured regions, with 0.0% and 0.4% of residues, respectively, in disallowed regions. Molecular docking with AutoDock Vina yielded binding energies of -5.6 and -7.2 kcal/mol for rat MT1A and MT1B, and -7.4, -6.3, and -7.2 kcal/mol for fish Mel1a, Mel1b, and Mel1c, respectively. Epitalon engaged the receptor active sites through hydrogen bonding, involving residues such as TYR142, ARG140, ILE141, and SER143 in rat MT1A; PHE192, THR191, and GLN194 in rat MT1B; and ASN189, GLY131, GLY135, ASN282, TRP278, THR205, and GLN208 in fish Mel1a. A 10 ns molecular dynamics simulation of each receptor-epitalon complex showed that four of the five receptors remained conformationally stable, whereas fish Mel1c displayed markedly higher RMSD, RMSF, and radius-of-gyration values, indicating greater structural flexibility and reduced complex stability. Together, these findings characterise the structural basis of epitalon binding to melatonin receptors and provide a foundation for designing receptor-targeted therapies for reproductive disorders in fish and for metabolic and circadian disorders in mammals.

Keywords: Fish, Melatonin receptor, Molecular docking, Molecular dynamics, Rat, Epitalon

Introduction

The pineal gland comprises pinealocytes, interstitial cells, perivascular phagocytes, pineal neurons, and peptidergic neuron-like cells, of which the pinealocytes secrete melatonin (N-acetyl-5-methoxytryptamine), first isolated from bovine pineal gland by *Lerner, et al.* (1958) [2]. Melatonin synthesis occurs predominantly during

the dark hours and is regulated by norepinephrine, folate, and vitamin B6; synthesis increases when norepinephrine levels rise or β 1-adrenergic receptors are activated [3], using tryptophan as the precursor amino acid [33,34]. Beyond regulating the day-night circadian rhythm and seasonal biorhythms [9], melatonin acts as

a potent antioxidant and anti-ageing agent and can reduce chemotoxicity [21]. It also influences the neuroendocrine control of sexual maturation: the pattern of melatonin secretion under the light-dark cycle, and in some species the photoperiod, determines the timing of puberty [25].

Melatonin acts on the brain, retina, cardiovascular system, liver, intestines, kidneys, and immune cells through the G-protein-coupled receptors MT1 and MT2 [7]. While mammals, including rats, express only MT1 and MT2, fish and amphibians possess an additional subtype, Mel1c (MTNR1C) [11,17]. MT1 receptors primarily mediate melatonin’s effects on the suprachiasmatic nucleus, with MT2 receptors playing a secondary role [28]. Although predominantly located on the plasma membrane, these receptors are also found in mitochondria [4], consistent with a broader physiological role in redox balance, circadian regulation, and ageing [31]. Once activated, MT1 and MT2 trigger multiple signal transduction pathways that produce tissue- and context-specific cellular responses [30], a functional complexity compounded by the range of proteins and ligands with which these receptors interact.

Comparing melatonin’s pharmacological effects and evolutionary history across species such as rats and fish clarifies these mechanisms further. In fish embryos, for example, melatonin causes pigment granules to aggregate toward the cell centre, producing a paler phenotype that aids camouflage under low-light conditions

[16]. Beyond melatonin itself, the pineal gland secretes several peptides that regulate additional physiological functions [8], most notably the synthetic tetrapeptide epitalon (Ala-Glu-Asp-Gly) and the synthetic tripeptide pinealon (Glu-Asp-Arg).

In the present study, pineal peptide sequences were retrieved from peptide repositories, and comparative structural modelling and molecular dynamics (MD) simulation were used to characterise receptor–peptide complexes [19,29]. Docking simulations were used to predict receptor–peptide interactions [5,6] and indicated that pineal peptides engage the extracellular loops of melatonin receptors, with binding affinities that vary by species. This in-silico study identifies both shared and distinct structural features of pineal peptide–receptor interactions in fish and rats, providing a foundation for future therapeutic and experimental work on metabolic and circadian disorders.

Methodology

Identification and Protein Sequence Retrieval

UniProt (<https://www.uniprot.org/>) is an open-access resource that provides programmatic access to curated protein knowledge [1] (Ahmad et al., 2025). Figure 1 summarises the overall workflow of the study, and Table 1 lists the bioinformatics tools and databases used.

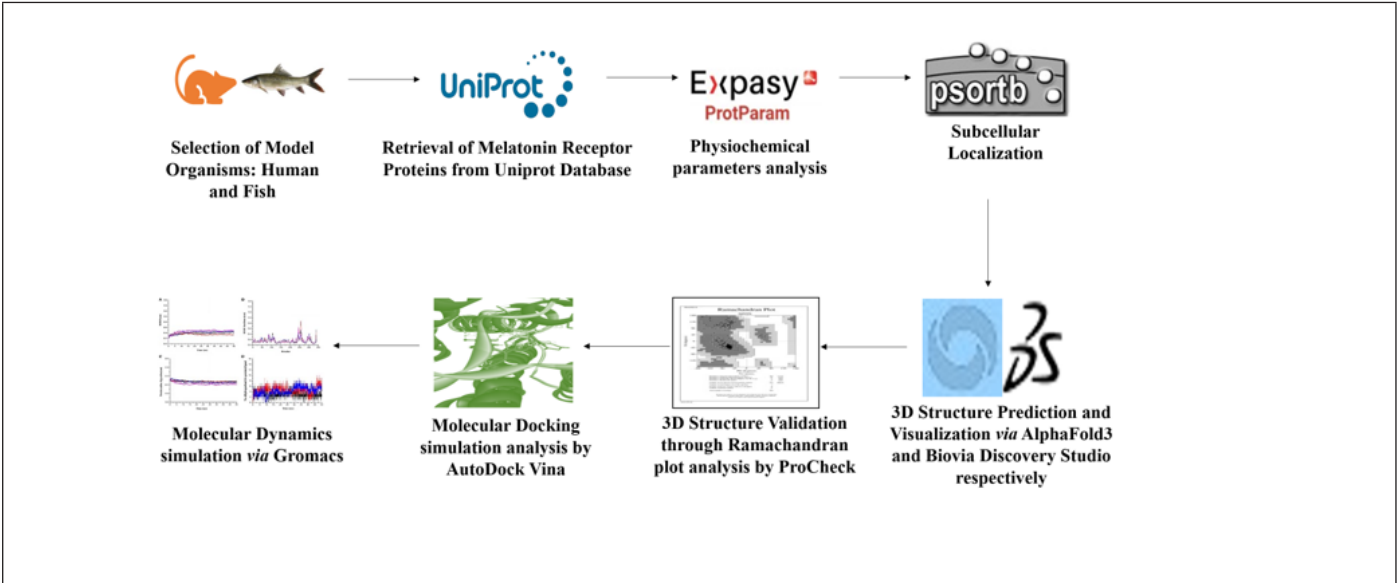


Figure 1: Overall methodological workflow of the study.

Table 1: Software and databases used in the study.

S. No.	Software/Database	Link
1	UniProt	https://www.uniprot.org/
2	ProtParam	https://web.expasy.org/protparam/
3	PSORTb	http://www.psort.org/psortb

4	AlphaFold 3	https://alphafoldserver.com/
5	BIOVIA Discovery Studio (BDS)	https://discover.3ds.com/discovery-studio-visualizer-download
6	PROCHECK	https://www.ebi.ac.uk/thorntonsrv/software/PROCHECK/
7	PubChem	https://pubchem.ncbi.nlm.nih.gov/
8	AutoDock Vina	http://autodock.scripps.edu/resources/raccoon
9	GROMACS	https://visualdynamics.fiocruz.br/login

Proteomic Analysis

The physicochemical properties of all melatonin receptor proteins — theoretical pI, molecular weight, number of negatively and positively charged residues, grand average of hydropathy (GRAVY), instability index, and aliphatic index — were computed using the ProtParam server (<https://web.expasy.org/protparam/>).

Subcellular Localization of Proteins

Subcellular localisation was predicted for each receptor using PSORTb (<http://www.psort.org/psortb>; [32] Yu, *et al.*, 2010), which classifies proteins as extracellular, outer membrane, periplasmic, inner membrane, or cytoplasmic — information relevant to understanding receptor function and drug design.

Structure Prediction and Visualization

Three-dimensional structures of the melatonin receptors in fish and rats were predicted from their amino acid sequences using AlphaFold 3 (<https://alphafoldserver.com/>; [12] Krokidis, *et al.*, 2025), a template-based (homology) modelling approach, and visualised using BIOVIA Discovery Studio (BDS) Visualizer (<https://discover.3ds.com/discovery-studio-visualizer-download>; [26] Umi Baroroh, *et al.*, 2023).

Structure Validation

Model quality was assessed by Ramachandran plot analysis in PROCHECK (<https://www.ebi.ac.uk/thorntonsrv/software/PROCHECK/>; [13] Laskowski, *et al.*, 2013), examining the proportion of residues in favoured and allowed regions, the distribution of glycine and proline residues, and the phi/psi dihedral angles and backbone conformation of each model.

Molecular Docking Simulation Analysis

Epitalon was retrieved from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>) in SDF format [20] and converted to PDB format using PyMOL. Docking of epitalon against each melatonin receptor was performed using AutoDock Vina.

(<http://autodock.scripps.edu/resources/raccoon>; [6] Eberhardt, *et al.*, 2021).

Molecular Dynamics Simulation

For each receptor, the docking pose with the lowest binding energy was selected for further analysis. Following system neutralisation, a 10 ns molecular dynamics simulation with periodic boundary conditions (500,000 steps) was performed for each receptor–ligand complex using GROMACS (<https://visualdynamics.fiocruz.br/login>). Trajectories were analysed for root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF), and radius of gyration (ROG) [27] to characterise the kinetic and thermodynamic stability of each complex.

Results and Discussion

Retrieved Protein Sequence Analysis

Melatonin receptor sequences for rat MT1A and MT1B and for *Labeo rohita* Mel1a, Mel1b, and Mel1c were retrieved from UniProt in FASTA format (Table 2) and used as input for structure prediction, docking, and molecular dynamics simulation. Both species possess MT1 and MT2 orthologues, but fish additionally express Mel1c, whose signalling pathway may be more complex than in mammals [22]; differences in the melatonin-synthesising enzyme arylalkylamine N-acetyltransferase (AANAT) further reflect this divergence between fish and rats [10]. These sequences also carry substantial information on the genetic composition of each organism [23].

Table 2: Accession ID, sequence length, and receptor type of the melatonin receptor proteins used in this study.

S. No.	Accession ID	Sequence length	Receptor type	Organism
1	A6JPS4	353 aa	MT1A	<i>Rattus norvegicus</i>
2	P49287	364 aa	MT1B	<i>Rattus norvegicus</i>
3	A0A6P8M1G9	344 aa	Mel1a	<i>Labeo rohita</i>
4	A0A6P8MAC0	297 aa	Mel1b	<i>Labeo rohita</i>
5	A0A498NF27	442 aa	Mel1c	<i>Labeo rohita</i>

Analysis of Physicochemical Characteristics

Physicochemical analysis of the five receptors (Table 3) showed molecular weights ranging from 33,695.81 Da (Mel1b) to 49,064.78 Da (Mel1c), with theoretical pI values above 7 for all receptors. Instability indices ranged from 32.42 to 44.03 — lower values denoting greater stability — while aliphatic indices, which correlate positively with thermal stability, ranged from 114.41 to 118.92.

GRAVY values ranged from 0.56 (rat MT1A) to 0.674 (Mel1b); lower GRAVY values indicate more favourable interaction with the aqueous environment (Wang, *et al.*, 2021). Molecular weight and other physicochemical properties are relevant to drug-target selection, as proteins near the optimal molecular weight for drug development (~110 kDa) are generally easier to purify and remain functionally stable across a wider range of conditions (Naz, *et al.*, 2015).

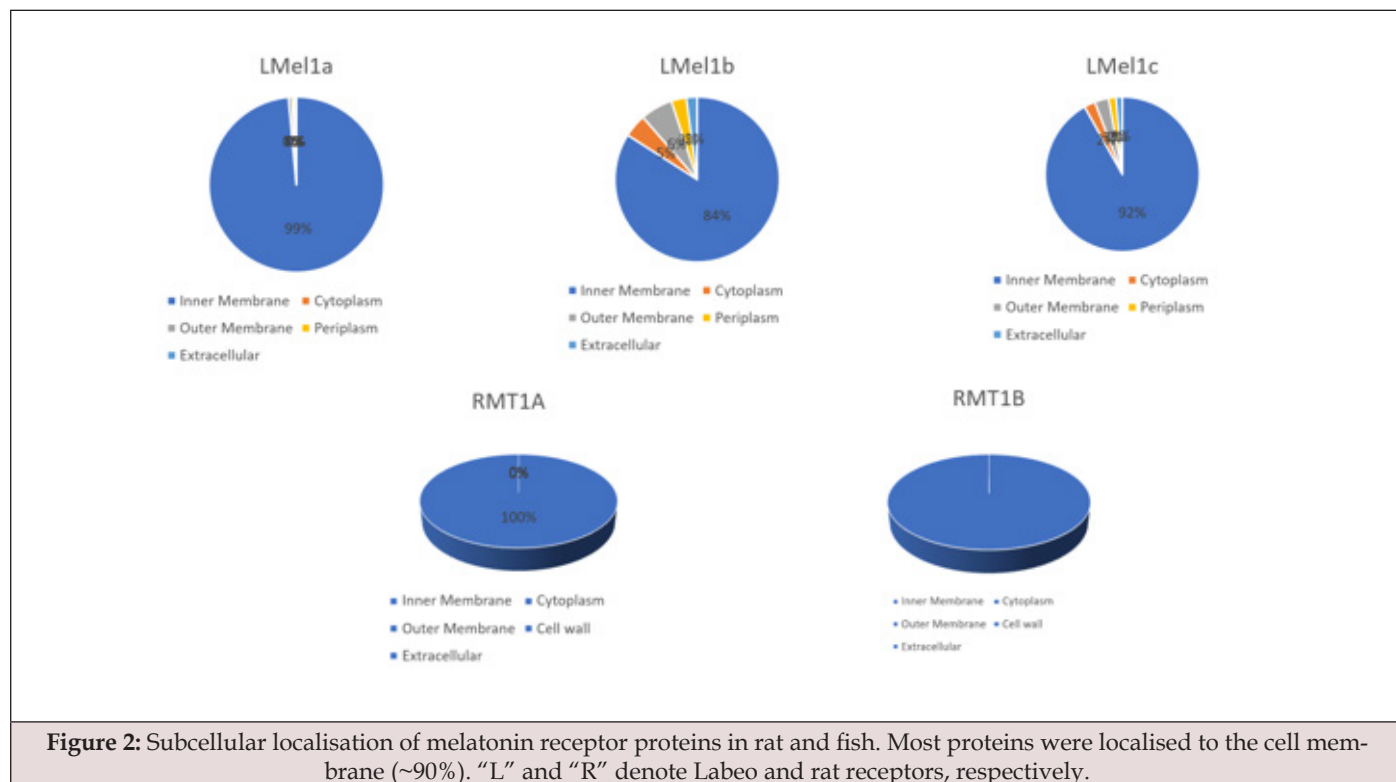
Table 3: Physicochemical properties of the melatonin receptors in rat and fish.

Receptor	No. of AA	Theoretical pI	MW (Da)	Negative residues	Positive residues	Instability index	Aliphatic index	GRAVY
LMel1a	344	9.42	38991.24	17	32	32.42	118.92	0.583
LMel1b	297	9.39	33695.81	14	24	37.44	114.41	0.674
LMel1c	442	9.14	49064.78	20	33	35.94	115.97	0.653
RMT1A	353	9.41	39790.19	16	31	39.78	118.73	0.56
RMT1B	364	8.78	40194.45	19	26	44.03	115.8	0.61

Localization of Melatonin Receptor Proteins

Subcellular localisation predictions (Figure 2) placed most melatonin receptor proteins at the cell membrane (~90%), with the remainder distributed across the cytoplasm, periplasm, outer

membrane, and extracellular compartments. Because subcellular localisation is central to understanding protein activity, trafficking, and drug accessibility [15,24], the predominance of membrane localisation supports the pharmacological relevance of these receptors as therapeutic targets.



Homology Modelling and Structure Prediction

Structural models of all five receptors displayed the canonical seven-transmembrane-helix architecture characteristic of the GPCR family (Figure 3), predicted using AlphaFold 3 [25] and visualised in BIOVIA Discovery Studio. This template-based approach successfully generated reliable models even where no closely related reference structure was available. Surface analysis revealed a comparatively low-hydrophobicity binding pocket in the rat re-

ceptors that favours ligand binding, with the MT1 and MT2 loop regions contributing to ligand selectivity and receptor activation [18]. Fish receptor surfaces were structurally more diverse, likely reflecting variation in light intensity and physiological demands; the larger, more exposed binding site of Mel1c, for example, may allow it to accommodate a broader range of ligands [16]. These structural differences inform both GPCR-targeted drug design for bone and psychiatric disorders and our understanding of the evolutionary divergence of melatonin receptors between fish and rats.

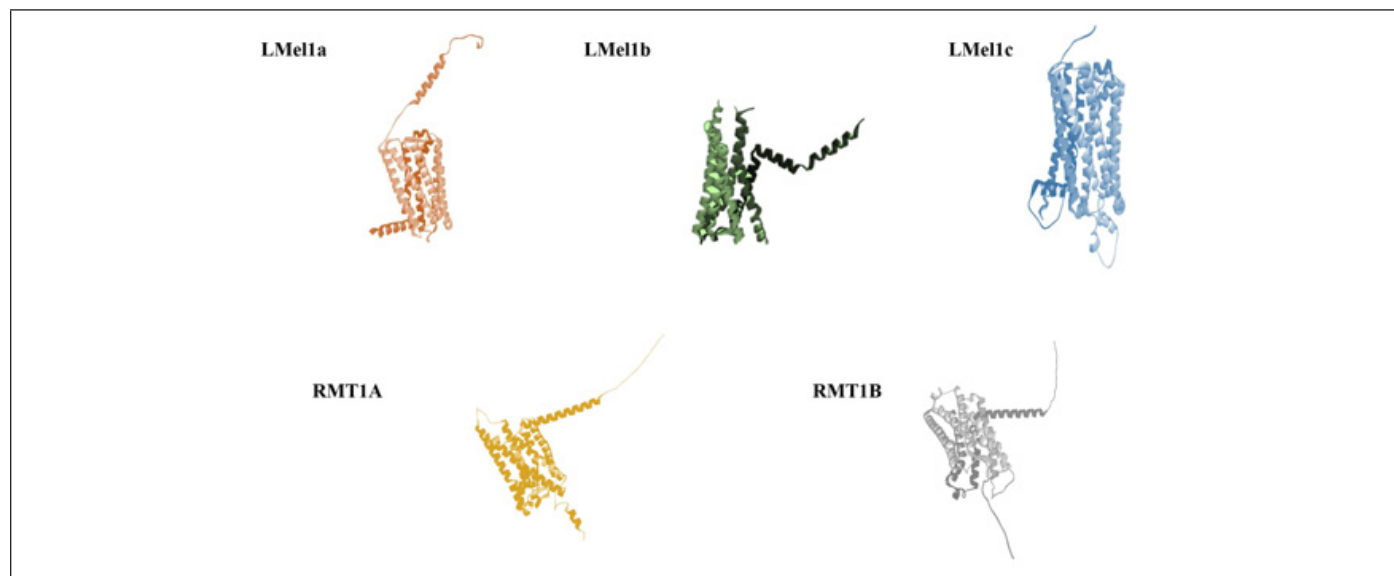


Figure 3: Overall three-dimensional structures of the melatonin receptor proteins in rat and fish. “L” and “R” denote Labeo and rat receptors, respectively.

Structure Validation of Melatonin Receptors

Model quality was assessed by Ramachandran Plot analysis prior to docking, with residues in the most favoured regions shown in red and those in disallowed regions in white; models with more than 90% of residues in favoured regions were considered suitable for docking.

Ramachandran plot analysis for structure validation in rats:

For the rat receptors (Figure 4a), 96.0% and 92.3% of residues occupied the most favoured regions of MT1A and MT1B, respectively, with 0.0% of residues in disallowed regions for both. Most dihedral angles clustered within the stereochemically stable alpha-helical regions typical of GPCRs, and both models were considered suitable for molecular docking.

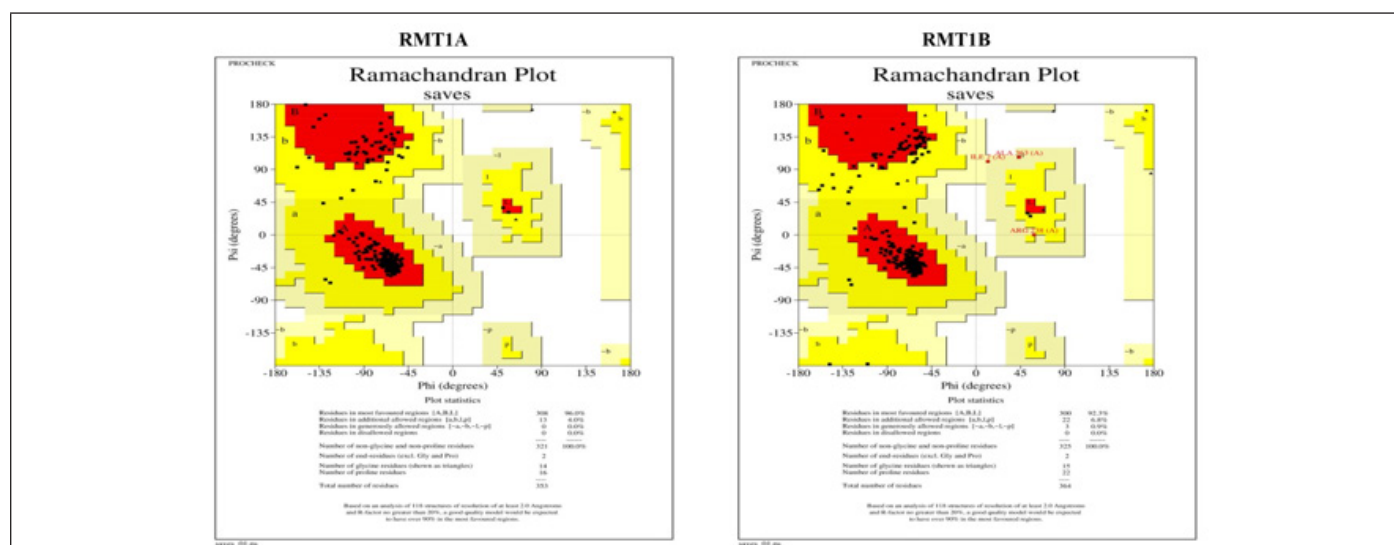


Figure 4(a): Ramachandran Plot analysis for structure validation of the rat RMT1A and RMT1B receptor proteins.

Ramachandran plot analysis for structure validation in fish:

For the fish receptors (Figure 4b), 92.5%, 94.1%, and 94.0%

of residues fell within the most favoured regions of Mel1a, Mel1b, and Mel1c, respectively, with 0.0%, 0.4%, and 0.0% of residues in disallowed regions. All three models met the quality threshold for docking studies.

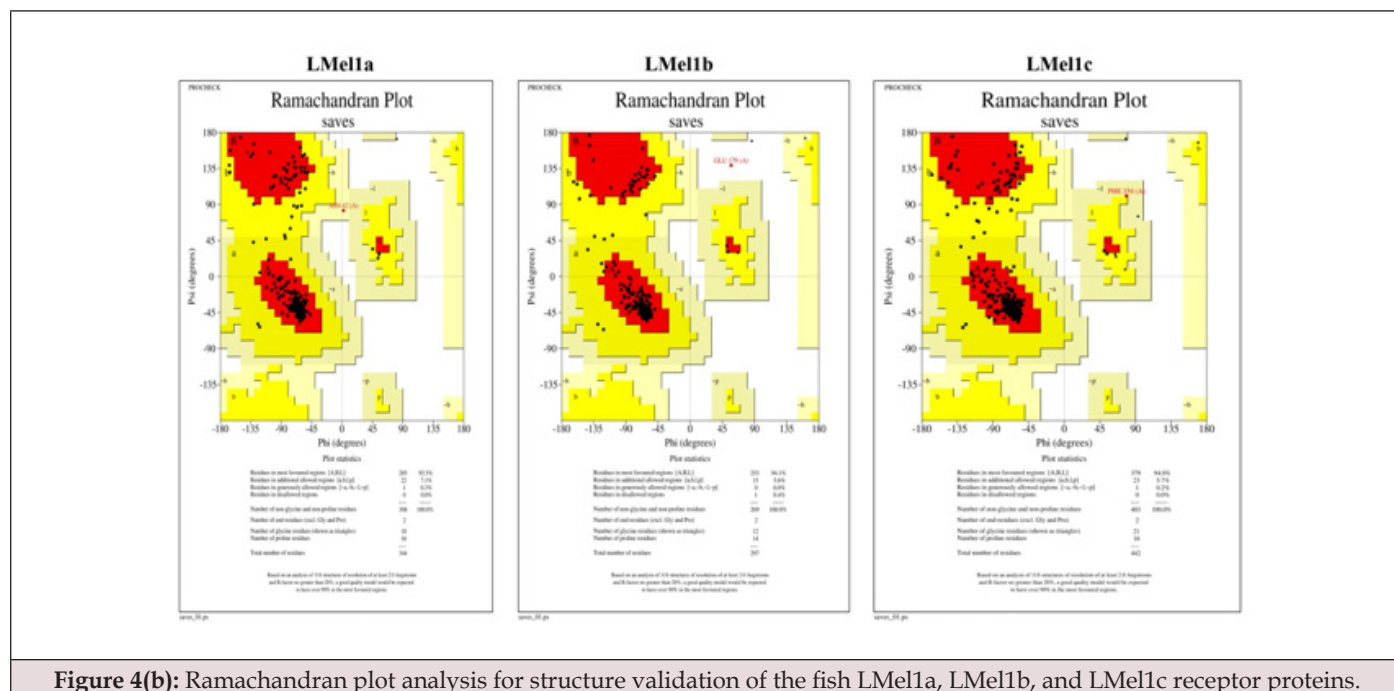


Figure 4(b): Ramachandran plot analysis for structure validation of the fish LMel1a, LMel1b, and LMel1c receptor proteins.

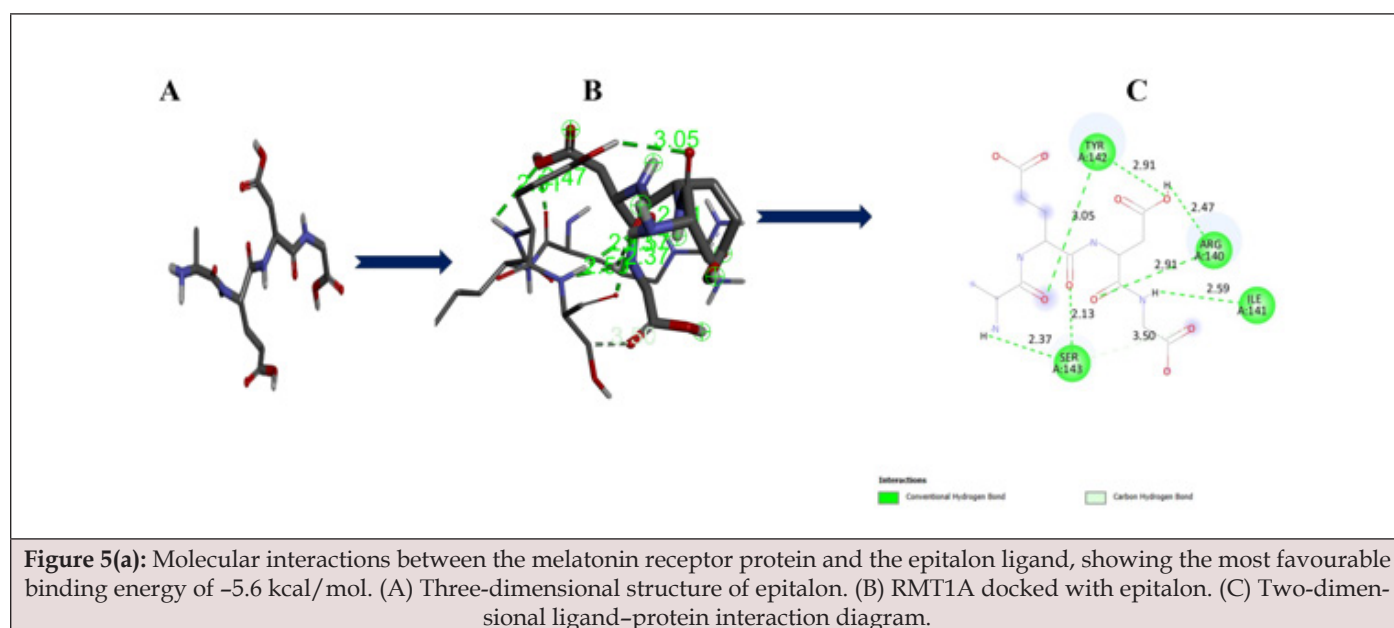
Molecular Docking Simulation and Intermolecular Interaction Analysis

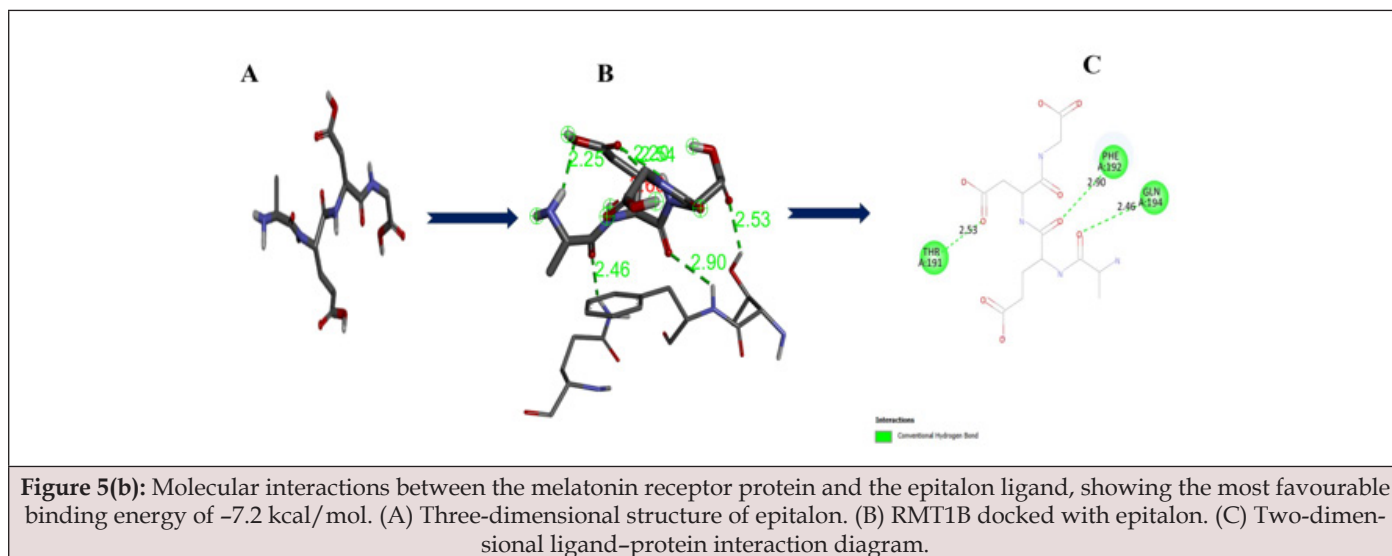
Molecular docking is a widely used approach for virtual screening of candidate ligands. Docking of epitalon against the rat receptors MT1A and MT1B and the fish receptors Mel1a, Mel1b, and Mel1c using AutoDock Vina yielded binding energies of -5.6 and -7.2 kcal/mol for MT1A and MT1B, and -7.4, -6.3, and -7.2 kcal/mol for Mel1a, Mel1b, and Mel1c, respectively, indicating favourable

binding across both species.

Evaluation of ligand–protein binding affinities and residue interactions in rats:

Molecular interactions between epitalon and the rat melatonin receptors were analysed using BIOVIA Discovery Studio Visualizer. Epitalon formed four hydrogen bonds with the active-site residues TYR142, ARG140, ILE141, and SER143 in MT1A, and with PHE192, THR191, and GLN194 in MT1B (Figure 5a, b).

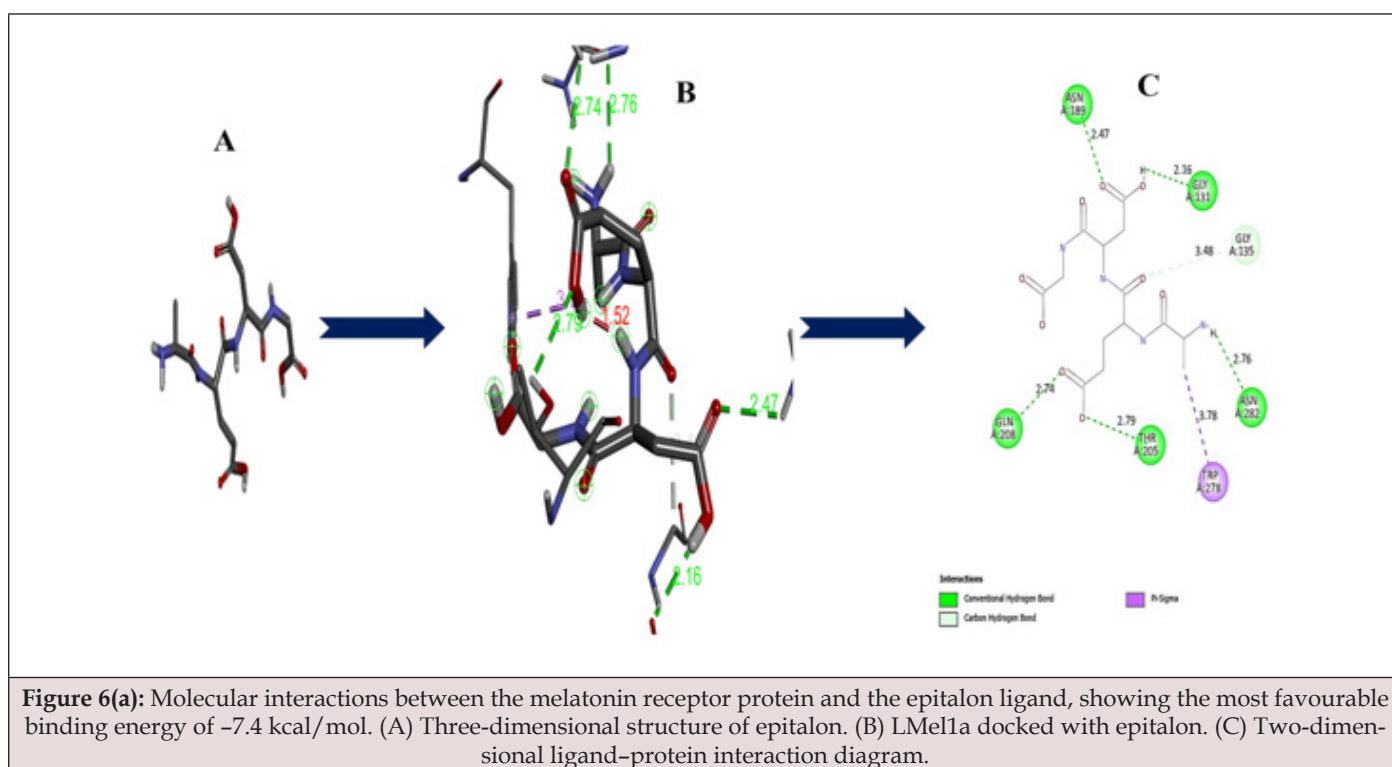




Evaluation of ligand-protein binding affinities and residue interactions in fish:

In the fish receptors, epitalon engaged ASN189, GLY131,

GLY135, ASN282, TRP278, THR205, and GLN208 in Mel1a; GLU259, PRO7, SER9, LEU8, and ASN253 in Mel1b; and VAL196, PHE183, ASN257, and TYR191, among more than twenty residues, in Mel1c (Figure 6a-c).



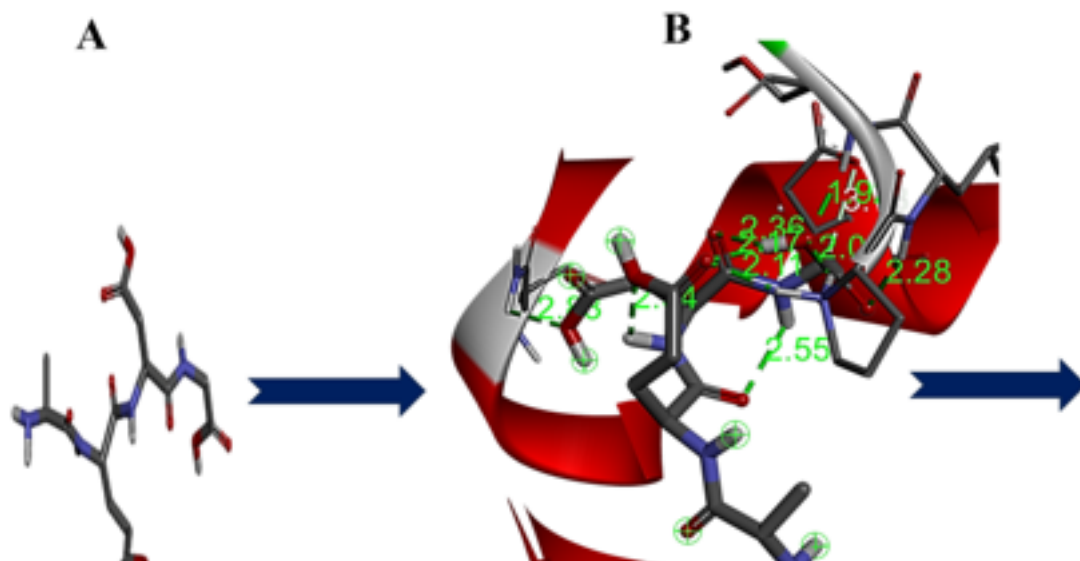


Figure 6(b): Molecular interactions between the melatonin receptor protein and the epitalon ligand, showing the most favourable binding energy of -6.3 kcal/mol. (A) Three-dimensional structure of epitalon. (B) LMel1b docked with epitalon. (C) Two-dimensional ligand-protein interaction diagram.

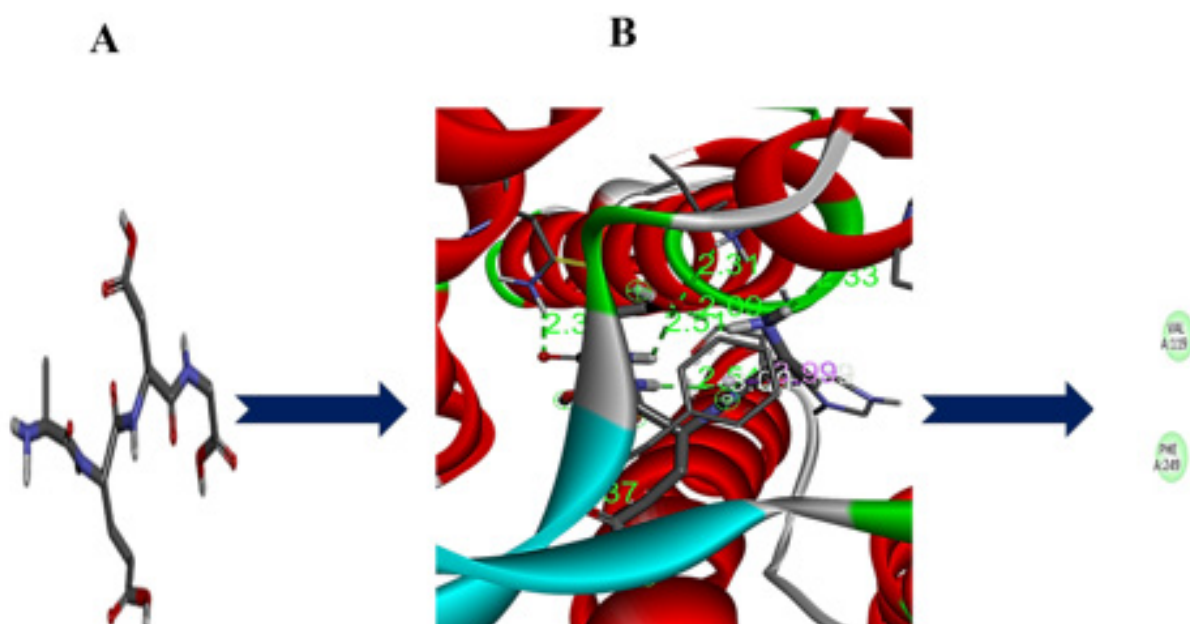


Figure 6(c): Molecular interactions between the melatonin receptor protein and the epitalon ligand, showing the most favourable binding energy of -7.2 kcal/mol. (A) Three-dimensional structure of epitalon. (B) LMel1c docked with epitalon. (C) Two-dimensional ligand-protein interaction diagram.

Molecular Dynamics Simulation Analysis

Molecular dynamics (MD) simulation is a key tool for understanding the structural and functional stability of proteins. A 10 ns MD simulation of each receptor-epitalon complex was performed, sufficient to capture side-chain reorganisation at the binding interface, and trajectories were analysed for RMSD, RMSF, and radius of

gyration (ROG).

RMSD values (Figure 7a) were 0.34 nm and 0.24 nm for rat MT1A and MT1B, respectively, and 0.3, 0.4, and 1.3 nm for fish Mel1a, Mel1b, and Mel1c, respectively. The markedly higher RMSD of Mel1c relative to the other four receptors indicates that its complex with epitalon is comparatively less stable.

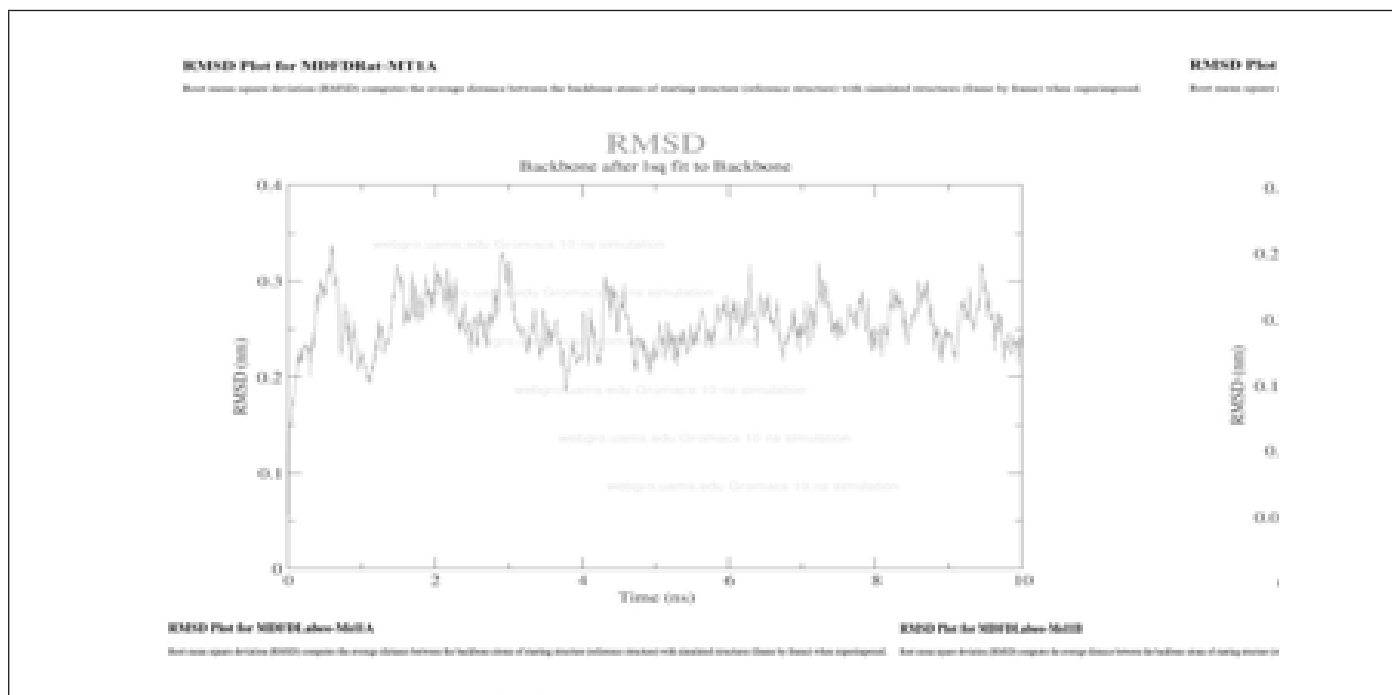


Figure 7(a): RMSD of the melatonin receptor-epitope complexes in rat and fish over the course of the 10 ns simulation.

RMSF values (Figure 7b), which reflect local residue flexibility, were higher in MT1A than in MT1B (maxima of ~0.73 nm and ~0.35 nm, respectively), with low fluctuation in the conserved transmembrane helices and higher fluctuation in the extracellular loops involved in ligand binding. Mel1a and Mel1b showed com-

parable, moderate flexibility (maxima of ~0.75 nm and ~0.65 nm, respectively), whereas Mel1c showed markedly higher flexibility (~1.9 nm), consistent with its lower conformational stability among the five receptors.

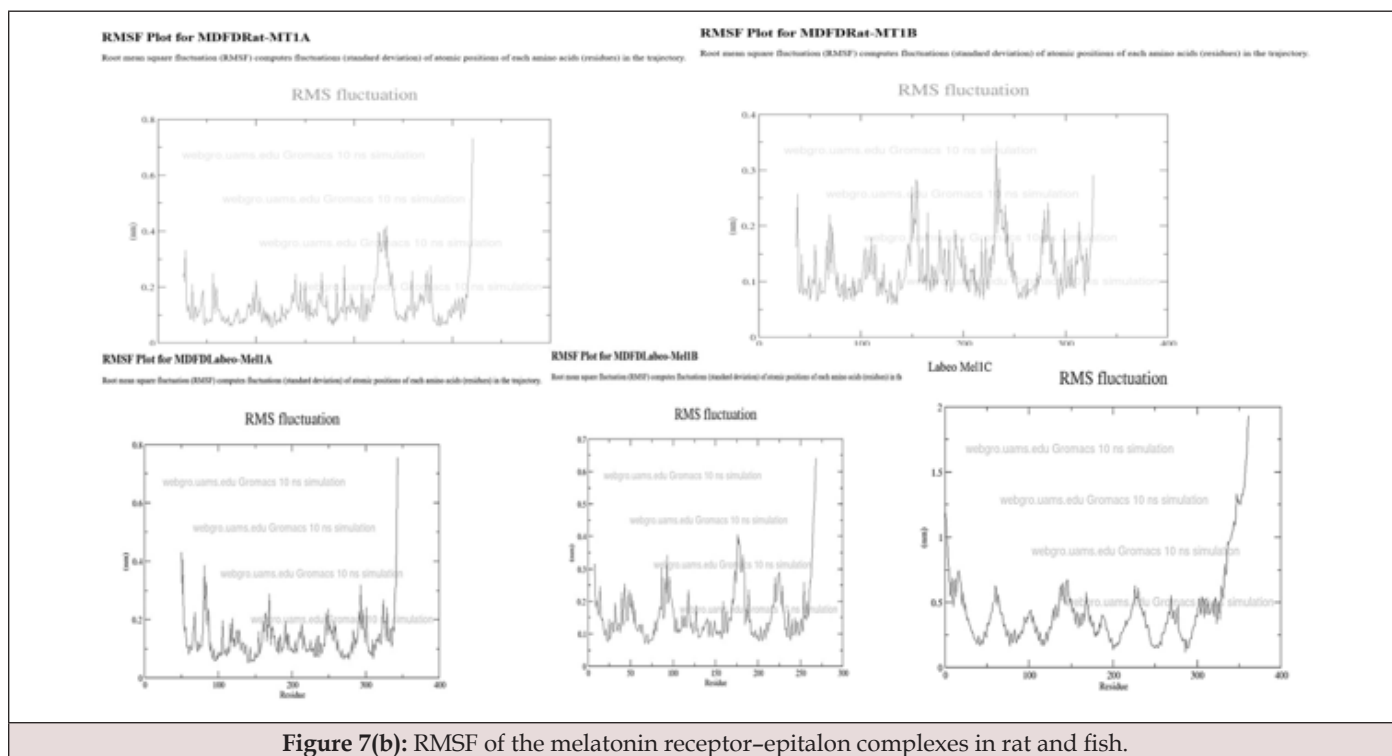


Figure 7(b): RMSF of the melatonin receptor-epitope complexes in rat and fish.

Radius of gyration (Figure 7c) indicated that rat MT1A remained highly compact and rigid throughout the simulation (~2.24 nm), while MT1B showed moderate fluctuation (~2.10 nm). Among the fish receptors, Mel1a was the most compact (~2.15 nm), fol-

lowed by Mel1b (~2.0 nm), whereas Mel1c showed the largest and most variable radius of gyration (~3.08 nm), further supporting its reduced structural stability relative to the other four receptors.

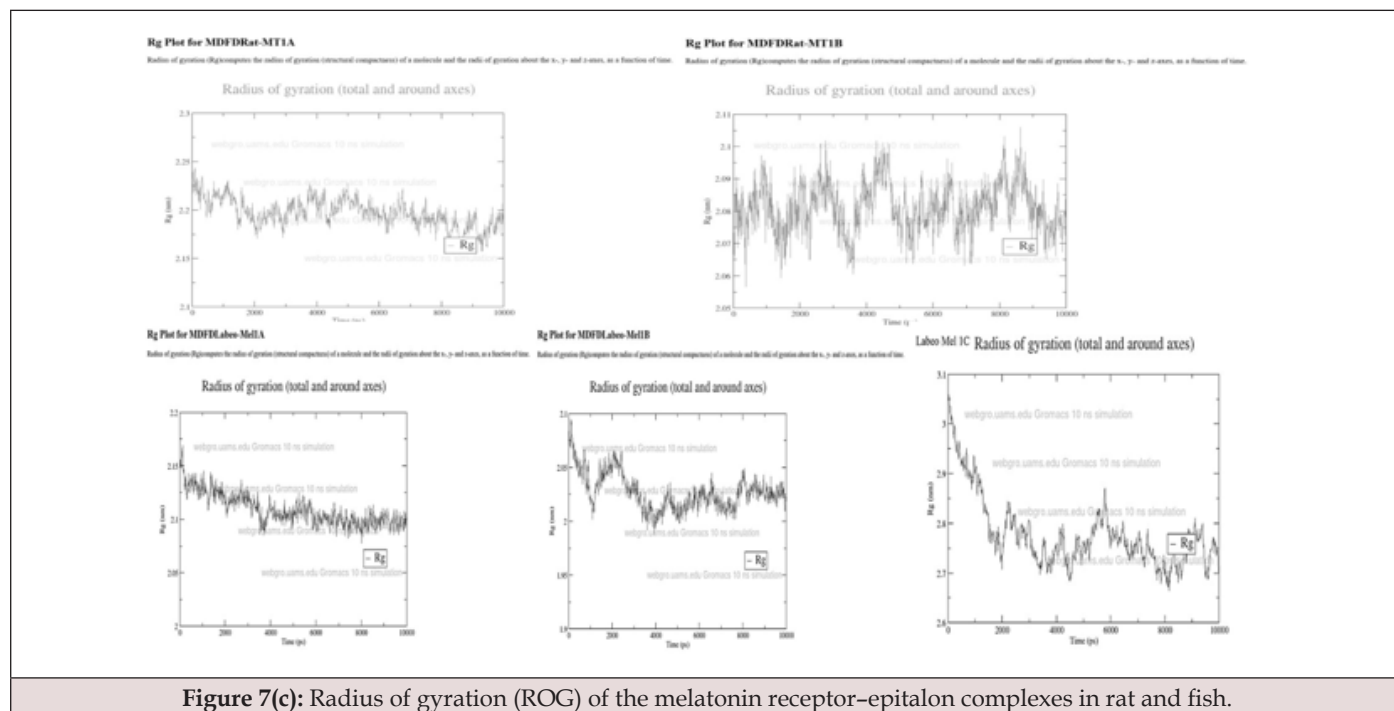


Figure 7(c): Radius of gyration (ROG) of the melatonin receptor-epitagon complexes in rat and fish.

Conclusion

This study integrates homology modelling, molecular docking, and molecular dynamics simulation to characterise the structural basis of epitagon binding to melatonin receptors in rats and fish. Four of the five receptors examined, rat MT1A and MT1B and fish Mel1a and Mel1b formed stable complexes with epitagon, whereas fish Mel1c showed comparatively lower stability, highlighting species- and subtype-specific differences in receptor-ligand dynamics. These findings clarify the structure-function relationship of melatonin receptors across vertebrate lineages and provide a structural rationale for the design of epitagon-based or receptor-targeted therapies for reproductive disorders in fish and for metabolic and circadian disorders in mammals. Extending this comparative framework to additional vertebrate classes, together with in vitro validation of the predicted binding interactions, will be an important next step in confirming the therapeutic potential identified here.

Credit Authorship Contribution Statement

Ayushi Singh: Writing, original draft, Investigation, Formal analysis, Validation, Methodology, Investigation. Umang Verma: Writing, review & editing. Somenath Ghosh: Supervision, Software, Methodology, Funding acquisition, Data curation, Conceptualization.

Declaration of Competing Interests

The authors declare no conflict of interest.

Acknowledgement

Somenath Ghosh acknowledges the Department of Zoology, School of Biological Sciences. The authors thank Dr Harisingh Gour Vishwavidyalaya (A Central University), Sagar, Madhya Pradesh, 470003, for providing research facilities.

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