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Research Article

Studies on 3D Structure Prediction and Binding Modes of Kisspeptin Receptor 1 Complexed with Kisspeptin 1 using Molecular Docking Approach

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ABSTRACT

Kisspeptin (Kiss), a neuropeptide belongs to the RF-amide peptide family which activates the kisspeptin receptor at puberty. Kisspeptin is recognized as an upstream regulator of reproductive events in teleost. The popular food and game fish golden mahseer (Tor putitora) is an important aquaculture species in recent years due to high market demand. kisspeptin1 and its receptor modulation is a significantly important event during early development and gonadal sex maturation. The present study was aimed to characterize the kisspeptin1 and its receptor from human, mouse, fish (Tor putitora) and also to identify the binding mode of kisspeptin1 and its receptor. The tertiary structure of Kiss1r was developed using the Swiss model server. The best model was selected based upon the Ramachandran plot and Errat server. Molecular docking method was applied to identify the binding modes between Kiss1r and Kiss1 using ZDOCK server. The physicochemical properties revealed that the kisspeptin and its receptor is basic in nature, unstable, lower extinction coefficient in case of kiss1 but higher in case of kiss1r. kisspeptin1 is hydrophilic in nature whereas kisspeptin1r is hydrophobic in nature. The functional properties showed that kisspeptin1 had no transmembrane helix whereas kisspeptin1 receptor was composed of seven transmembrane helices. The secondary structure revealed the dominance of random coils followed by the alpha helix, extended strands and beta turns in kisspeptin1 whereas, alpha helix dominated followed by the random coils, extended strands and beta turns for the kisspeptin1r. The docking study revealed the highest binding energy between Kiss1r and Kiss1.

Key words: *Kisspeptin, Molecular docking, Ramachandran plot and Errat server*

INTRODUCTION

Puberty is a highly complex biological process which involves rapid linear growth, sexual development and adrenal maturation and further characterized by the onset of spermatogenesis in male and oogenesis in female[10]. GnRH of hypothalamus stimulates the synthesis and release of the gonadotropins, FSH and LH from the anterior pituitary.

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These hormones act on the gonad to induce oogenesis and spermatogenesis by stimulating the production of sex steroids[15]. Kisspeptin (KISS) is a neuropeptide belonging to the RF-amide peptide family. It is largely expressed in the hypothalamic region of the brain[7]. KISS1 was originally identified as a human metastasis suppressor gene that has the ability to suppress melanoma and breast cancer metastasis. The mutations in KISS1R were found to be associated with the idiopathic hypothalamic hypogonadism (IHH) syndrome in human, which impairs puberty[2,11]. This syndrome is characterized by the delayed or absence of pubertal development and a gonadotropin secretion deficiency. Thus, it was demonstrated that the ligand kisspeptin (KISS) and its receptor (KISSR) regulate the segregation of the gonadotropin-releasing hormone (GnRH), and in consequence this system is one of the principal regulators of the early development and gonadal sex differentiation[9], puberty onset[16,14] and seasonal reproduction in teleosts[8,5,1]. Therefore, kisspeptin is recognized as an upstream regulator of reproductive events in teleosts[5,1]. Most mammals possess only one kisspeptin gene (Kiss1)[3]. But multiple forms of kisspeptin and their receptor genes have been reported in several non-mammalian vertebrates[7].

Golden mahseer, *Tor putitora* (Hamilton, 1822; family - Cyprinidae) is a popular food and game fish of the Himalayan region of northern India[12]. This species is also found in several south and south-east Asian countries such as Indonesia, Malaysia, Java, Nepal, Pakistan, Sri Lanka, Afghanistan, Bangladesh, Bhutan, Myanmar, Thailand and Iran[6], and is being targeted for aquaculture in recent years, due to its high market demand. The KISS1 and their receptors are characterized and their expression profiles were studied from golden mahseer[13].

However, to our knowledge, there is no published report so far on structural features of KISS1 and their receptors using computational modeling approaches. Hence, in the present study, we try to understand the physicochemical properties of KISS1 protein and its receptor from human, rat and golden mahseer by comparative study. We also predicted the 3D structure of KISS1 of golden mahseer and molecular docking between KISS1 and their receptors.

MATERIAL AND METHODS

Sequence retrieval

Kisspeptin1 (KISS1) and its receptor (KISS1r) of *Homo sapiens* (Human), *Rattus norvegicus* (Rat), *Tor putitora* (Golden mahseer) were retrieved in FASTA format from NCBI protein database and were used for further analysis. The NCBI ID is shown in Table1.

Primary structure analysis

The physicochemical parameters were computed using the Expasy's ProtParam tool **(**http://web.expasy.org/protparam/).

Transmembrane region analysis

The identification of transmembrane regions of a protein was determined by applying a hidden Markov model using the TMHMM Server v. 2.0 (trans-membrane Hidden Markov Model) servers (http://www.cbs.dtu.dk/services/TMHMM/).

Secondary structure analysis

SOPMA server (https://npsa prabi.ibcp.fr/NPSA/npsa_sopma.html) was used to predict the secondary structure of the protein in the form of α-helical, β-strand and coiled regions in percentages.

Tertiary structure prediction

The modeling of the 3D structure of KISS1 (AJT39600.1) and KISS1r (AKI84606.1) amino acid sequence was predicted by SWISS-MODEL. The predicted model was validated by SAVES including PROCHECK and ERRAT server. Python molecular viewer (PyMol) was used to visualize the tertiary structure of the protein.

Molecular docking

KISS1 was downloaded from NCBI database in fasta format. The docking was done by ZDOCK 3.0.2 Server.

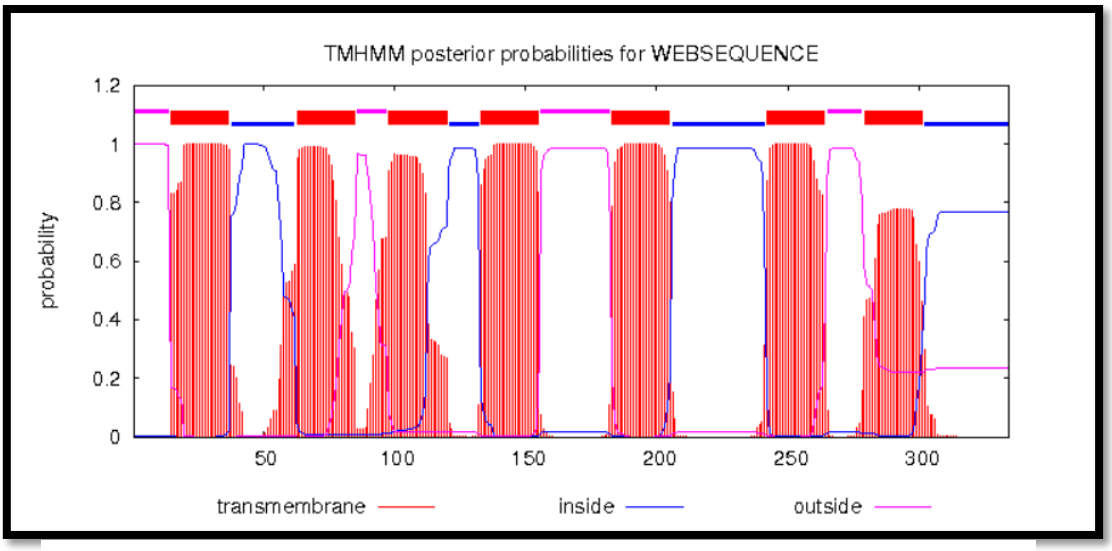

Fig. 1: Transmembrane Topology prediction of KISS1r using TMHMM Server

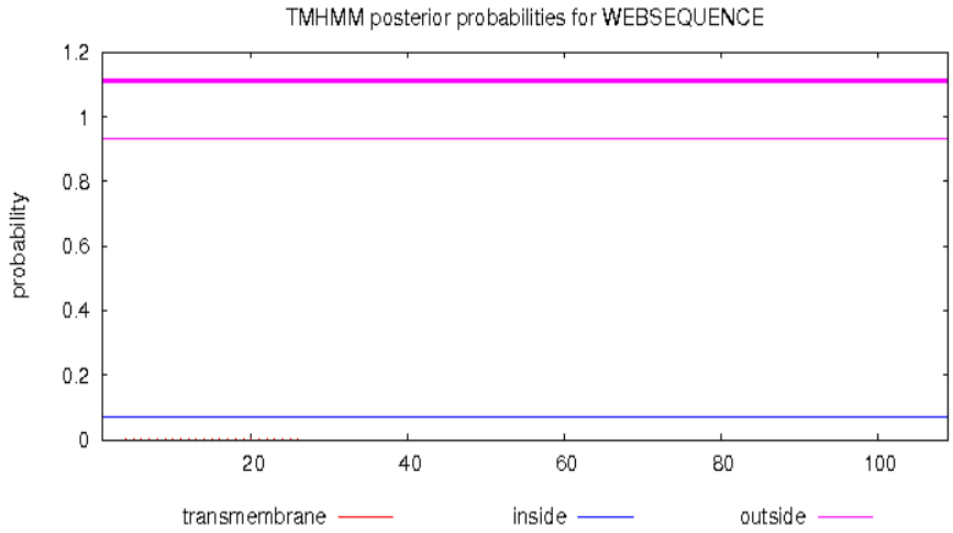

Fig. 2: Transmembrane Topology prediction of KISS1 using TMHMM Server

Table 1: Kiss1 and Kiss1r retrieved from NCBI database

ACCESSION NUMBER	SEQUENCE DESCEIPTION	ORGANISM
NP_002247.3	Metastasis-suppressor KiSS-1 preproprotein	*Homo sapiens*
NP_859043.1	Metastasis-suppressor KiSS-1 precursor	*Rattus norvegicus*
AJT39600.1	Kisspeptin 1	*Tor putitora*
NP_115940.2	KISS-1 receptor	*Homo sapiens*
Q924U1.2	KISS-1 receptor	*Rattus norvegicus*
AKI84606.1	KISS-1 receptor	*Tor putitora*

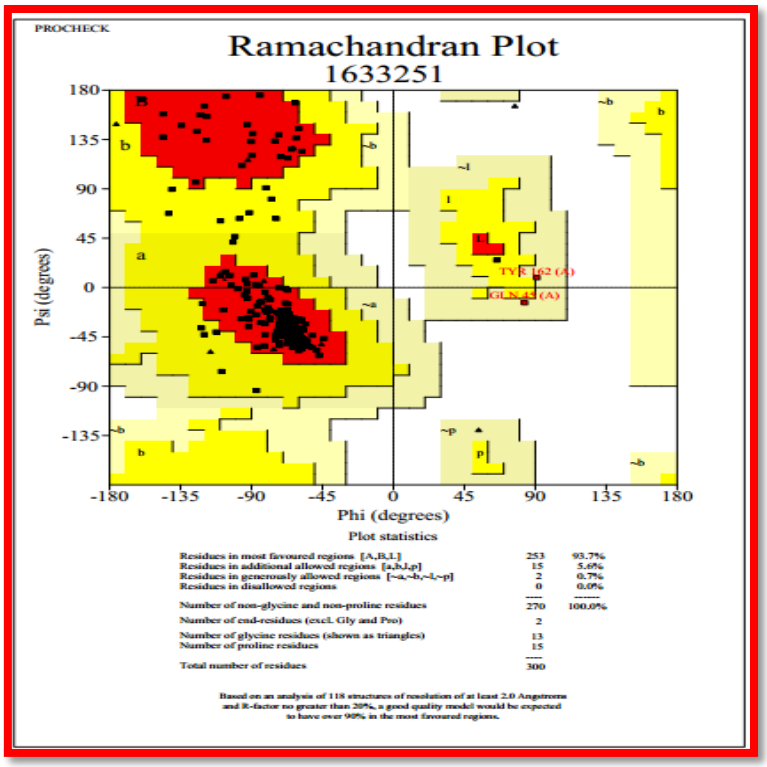

Fig. 3: Ramachandran plot of the Predicted Tertiary Structure of KISS1r of *Tor putitora* by Saves Server (Procheck)

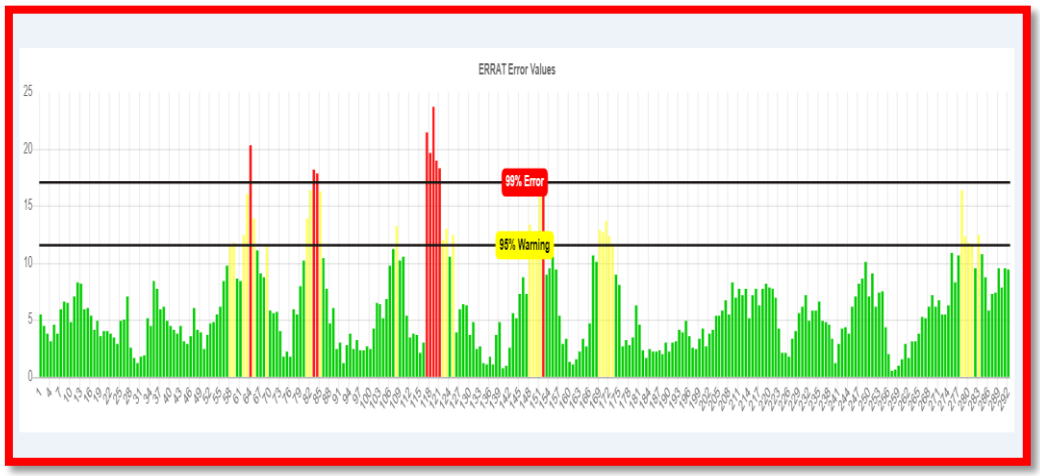

Fig. 4: Errat plot for *Tor putitora* KISS1r model. Red bars show misfolded region located distantly from the active site, Yellow bars demonstrate the error region between 95% and 99%, green bars indicate the region having less error rate for protein folding

RESULTS AND DISCUSSIONS

Physicochemical characteristics:

The primary structure analysis was done and different parameters computed using the ExPasy Protparam tool. The amino acid composition of KISS1 and KISS1r (*Tor putitora*) is tabulated in Table 2. The physicochemical parameters of KISS1 and KISS1r is tabulated in Table 3. The Molecular mass of protein was estimated by sum of the average isotopic mass of amino acids in the target protein and the average isotopic mass of one water molecule. The value of molecular weight ranged from 12534.01 to 42889.14 Dalton (Table 3). In the present study, the value of isoelectric point ranged from 6.57 to 10.85 indicating basic nature except for AJT39600.1 (Table 3). The isoelectric point is useful for developing a buffer system for the proteins which are essential to preserve the

proteins for studies. In the present study, EC values ranged from 12615 to 68255 M^{-1} cm^{-1} indicating a lower concentration of Cys, Trp, and Tyr in case of kiss1 and higher concentration of Cys, Trp, and Tyr in case of kiss1r (Table 3). Amino acids with aromatic side chains (i.e., tyrosine phenylalanine, and tryptophan) exhibit strong UV-light absorption. It helps in the quantitative study of protein-protein and protein-ligand interactions in solution. In the present study, the instability index ranged from 32.01 to 60.57 indicating that kisspeptin1and its receptor is unstable except for AKI84606.1 (Table 3). The instability index provides an estimate of the stability of the protein in a test tube. In general, a protein whose instability index is lesser than 40 are predicted as stable and a value above 40 indicates that the protein may be unstable. In the present study, the value of the aliphatic index ranged from 65.32 to 99.67 indicating stable nature for a wide range of temperature (Table 3). The Aliphatic Index (AI) evaluates the relative volume of the protein occupied by the aliphatic side chains and helps to study the thermal properties of the protein. The aliphatic side chains include Ala, Val, Leu, and Ile. In the present study, the value of GRAVY ranged from -0.489 to 0.358 indicating that kisspeptin1 is hydrophilic in nature whereas kisspeptin1r is hydrophobic in nature.

The physicochemical properties of KISS1 and KISS1r from human, rat and fish species revealed that the majority of the properties are the same. It is concluded that the physiological function of KISS1 and KISS1r might be same in human, rat, and fish.

Transmembrane analysis:

Analysis of the transmembrane region of the kisspeptin1 and their receptor using the TMHMM server showed that kisspeptin1 had not transmembrane helix whereas, the kisspeptin1 receptor was composed of seven transmembrane helices. The sequence position of transmembrane helices is provided in Table 4, and the posterior probability of the transmembrane is shown in Fig 1 and 2.

Secondary structure analysis:

SOPMA server was used to derive quantitative values for the number of alpha-helices, beta sheets and coils present within the amino acid stretch of the protein. The predicted secondary structure of Kiss1 and Kiss1r in the present study revealed that random coils dominated the secondary structure followed by the alpha helix, extended strands and beta turns except for AKI84606.1. While other features of secondary structure such as 310 helix, Pi helix, Ambiguous states, Bend region, and Beta Bridge were not found in any sequences (Table 4).

Homology modeling:

Neuropeptide Y receptor type 1, T4 Lysozyme, Neuropeptide Y receptor type 1 was used as a template for the tertiary structure prediction as it had 24.12% amino acid sequence identity with *Tor putitora* kiss1r protein. The predicted structure is shown in fig 3. The analysis of the tertiary structure showed the GMQE value 0.63 and QMEAN value -4.96 between the query sequence and the template. The analysis of the Ramachandran plot revealed that of the amino acid residues 93.7% were found in most favored region, 5.6% amino acid residues in additional allowed region, 0.7% in generously allowed region and 0.0% amino acid residues in the disallowed region (Fig 4), indicating the high crystallographic quality of the predicted tertiary structure. ERRAT is a protein structure verification algorithm used for evaluating the crystallographic structure building and refining. This plot is extremely useful for making the decision on the reliability of the model. The normally accepted value of ERRAT overall quality factor is >50 for a high-quality model. The ERRAT value for the Swiss model was 86.6712% in the present study. It further confirms the reliability of the models developed.

Molecular docking: ZDOCK Server is a user friendly, fast and effective web interface that produces models of protein–protein complexes and symmetric multimers. The main activity of ZDOCK is to search all possible binding modes in the translational and rotational space

between the two proteins and evaluates each pose using an energy-based scoring function. In this study, the grid dimension was used in the docking was 140x140x140, the spacing between grid cells was by default 1.2 angstrom and receptor was fixed during docking. The orientation of a ligand with respect to fixed coordinate system is -3.141593, 1.599406, and 1.426070. The orientation of a receptor with respect to fixed coordinate system is 2.380392, 2.500101, -0.120466.

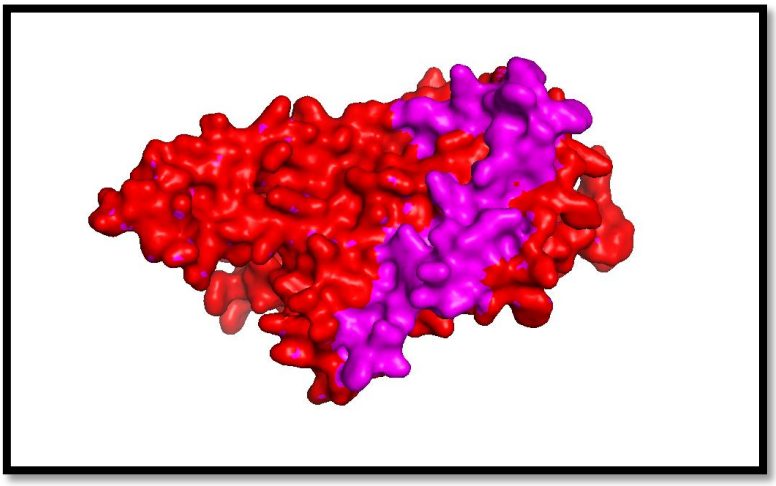

Fig. 5: Surface representation of the protein-protein interactions of the compound viewed n PyMol (KISS1R – red in color, KISS1 – magenta in color)

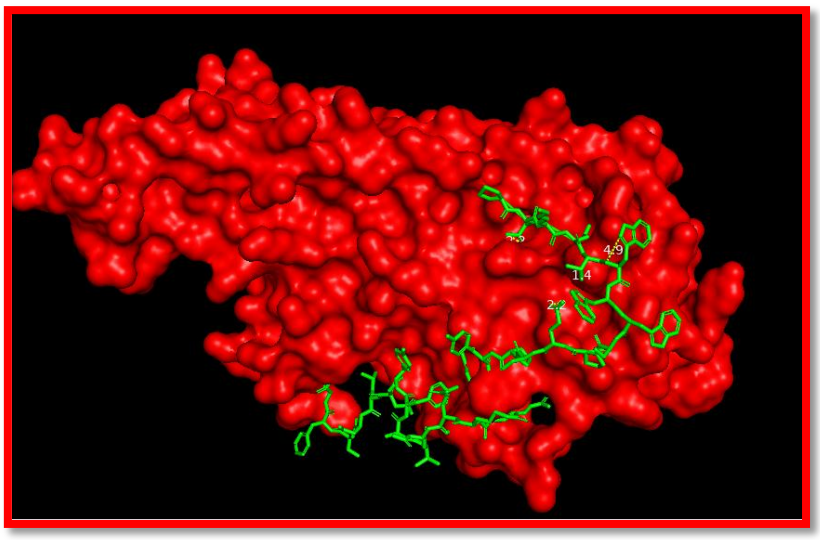

Fig. 6: Hydrogen bond representation of the protein-protein interactions of the compound viewed n PyMol (KISS1R – red in color, KISS1 – green in color)

Table 2: Amino acid composition of considered Kiss1 and Kiss1r (in percentage) computed using ExPasy tool

Accession Number	Ala	Arg	Asn	Asp	Cys	Gln	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val
NP_002247.3	8.7	8.0	4.3	0.7	1.4	6.5	5.8	10.9	2.2	0.7	12.3	2.9	0.7	2.9	10.9	11.6	3.6	1.4	0.7	3.6
NP_859043.1	11.5	10.8	3.8	0.8	2.3	6.2	4.6	8.5	0.8	1.5	9.2	3.1	2.3	1.5	11.5	7.7	3.1	2.3	2.3	6.2
AJT39600.1	3.7	8.3	4.6	5.5	0.0	3.7	6.4	7.3	0.9	2.9	11.0	3.7	2.8	1.8	7.3	9.2	9.2	2.8	6.4	2.8
NP_115940.2	16.6	7.8	2.8	2.0	3.8	2.3	1.3	6.3	2.5	1.5	13.8	1.3	1.8	3.8	9.0	6.8	3.5	2.0	3.3	8.5
Q924U1.2	14.6	7.1	2.8	2.0	3.8	3.3	1.3	5.6	3.8	1.8	13.1	1.0	1.8	4.0	8.6	7.1	4.8	2.3	3.0	8.3
AKI84606.1	6.3	5.1	3.3	2.7	3.0	3.0	3.0	4.2	2.4	9.3	8.7	4.8	3.3	8.4	4.8	7.5	6.0	1.8	5.1	7.5

Table 3: Physicochemical parameter of considered kiss1 and kiss1r are computed using ExPasy Protparam tool

Accession Number	No of amino acid	M. wt.	PI	(-) R	(+) R	EC	II	AI	GRAVY
NP_002247.3	138	14704.57	10.17	9	15	12615	60.57	70.07	-0.567
NP_859043.1	130	14188.34	10.85	7	18	21095	56.14	71.38	-0.489
AJT39600.1	109	12534.01	6.57	13	13	26930	55.51	65.32	-0.816
NP_115940.2	398	42586.04	9.93	13	36	64245	48.25	99.67	0.383
Q924U1.2	396	42889.14	9.70	13	32	68255	43.29	96.92	0.318
AKI84606.1	334	38483.43	9.35	19	33	58955	32.29	98.05	0.358

Legends: M. wt., PI, (-) R, (+) R, EC, II, AI and GRAVY denotes Molecular weight, Isoelectric point, Positive R group, Negative R group, Extinction coefficient, Instability index, Aliphatic index and The grand average hydropathy

Table 4: Transmembrane region identified by TMHMM Server

Accession Number	TM1	TM2	TM3	TM4	TM5	TM6	TM7
NP_002247.3	No Transmembrane Helix						
NP_859043.1	No Transmembrane Helix						
AJT39600.1	No Transmembrane Helix						
NP_115940.2	44-66	79-101	121-138	158-180	204-226	264-286	306-328
Q924U1.2	44-66	79-101	121-138	158-180	204-226	261-283	306-330
AKI84606.1	15-37	63-85	98-120	133-155	183-205	244-264	279-301

Table 5: Secondary structure prediction by SOPMA server

Accession Number	NP_002247.3	NP_859043.1	AJT39600.1	NP_115940.2	Q924U1.2	AKI84606.1
Random coil	72.46%	66.92%	54.13%	42.21%	42.68%	34.13%
Alpha helix	17.39%	26.15%	19.27%	39.20%	40.91%	47.31%
Extended strand	8.70%	6.92%	22.02%	15.33%	14.14%	15.87%
Beta turn	1.45%	0.00%	4.59%	3.27%	2.27%	2.69%
310 helix	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Pi helix	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Beta bridge	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Bend region	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Ambiguous state	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Other states	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%

CONCLUSION

Kisspeptin and its receptor showed a pivotal role in early development, gonadal sex differentiation, puberty onset and seasonal reproduction. The present comparative study showed that the physicochemical properties of kisspeptin and its receptor in human, rat and fishes are similar with minor exception; this may be due to slight variation in amino acid sequences. Hence, the current study reveals various aspects of kisspeptin and its receptor in various species including docking result of

kisspeptin and its receptor of *Tor putitora* which can help to extend the knowledge in other areas.

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