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In Silico Characterization of Human Serotonin Receptor 1A Protein

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Abstract

Prediction of structure and function of protein is a great challenge in the era of proteomics, particularly for those proteins which are wholly or partially unstructured such as Serotonin receptor 1A protein. It is an auto receptor that binds with serotonin (neurotransmitter) and induces downstream cellular signalling. It has been well studied for its role in various physiological functions but information about its structural and conformational aspects is not known, which is necessary to understand the structural and functional interactions. Therefore, in present study we studied structural characteristic features of 5-HTR 1A protein by using various bioinformatics tools. Swiss-Model server was used to construct the three-dimensional Model for 5-HTR 1A protein that revealed its seven-transmembrane helical structure. Quality of predicted model was validated by Ramachandran plot analysis. It showed that 95.3% of residues present in favoured region, that predicting best fit model. This receptor is consisting of 48.1% hydrophobic, 7.35% acidic, 11.85% basic and 32.7% neutral residues. 3D structure and Stereo packing of predicted model evaluated by VADAR (Volume Area Dihedral Angle Reporter) server. It showed that hydrophobic residues covered more assessable surface area as compared to hydrophilic residues. Fractional volume analysis showed efficient packing of protein in predicted model of 5-HTR 1A. Subcellular localization analysis predicted that it localized in plasma membrane. Furthermore, Protein-protein interaction showed that 5-HTR 1A interacts mainly with Pro-opiomelanocortin (POMC) protein, which involved in feeding behaviour. These results from computational analysis will further contribute to understand the drug related characteristic features of serotonin receptor 1A.

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Introduction

Serotonin receptor 1A (5-HTR 1A) is a subtype of serotonin receptor family. It is an auto receptor that binds with serotonin (neurotransmitter) and induce downstream cellular signalling. It regulates neurotransmission and control activities of other serotonergic receptors (Gilliam *et al.*, 1989; Celada *et al.*, 2004). It is one of the most important serotonin receptor which contribute in various physiological functions such as aggression (De Boer and Koolhaas, 2005), sociability (Thompson *et al.*, 2007), impulsivity (Winstanley *et al.*, 2005) food intake (Ebenezer *et al.*, 2007), sleep (Ansseau *et al.*, 2004), depression (Meyer *et al.*, 2006), behaviour (Müller *et al.*, 2007) and sex. Role of this receptor in cellular interactions involving various activities highlights that this receptor can be a potential therapeutic candidate (Fernández-Guasti *et al.*, 1997). But studies related to its structural and functional interaction with other compounds or respective cellular interactions are not available till date.

There is only one study available in which limited structure and function relationship of this receptor has been studied. Generally experimental investigations face major challenge of unviability of functional assay in molecular biology (Nelson, 1991). Nowadays, owing to the development of computational simulation technology, with help of various bioinformatic tools, it is possible to unfold the information about structural features of proteins. Understanding the structure of 5-HTR 1A at protein level is necessary, as this receptor can be targeted in various diseases and drug interaction studies, based upon structural information. In present study, we aimed to determine the structural model of 5-HTR 1A, validate the model and predict the structural characteristic features by using various bioinformatics tools. The results of this study will help to understand potential drug related interactions of 5-HTR 1A in various physiological functions.

Material and methods

Human 5-HTR 1A protein sequence retrieved from uniport data base (Uniprot ID: P08908) (www.uniprot.org) and used for the prediction of structural features and characteristics.

Prediction of three-dimensional structure

Swiss-Model Server was used to predict the three-dimensional structure of 5-HTR 1A protein model by employing protein sequence. The same server was used to predict QMEAN score (Biasini *et al.*, 2014).

Qualitative analysis of predicted model

Predicted model of serotonin 1A receptor protein was evaluated for its quality and reliability. Ramachandran plot version 2.0 (Ramachandran *et al.,* 1963) was employed to calculate the conformational statistics of backbone by evaluating the phi and psi (Ψ) torsion angles. Energy plots and Z-score calculated by using QMEAN and ProSA to assess the absolute quality of predicted protein model. Volume Area Dihedral Angle Reporter (VADAR) server was used to find the quality profile index of hydrophobic and hydrophilic residues and their respective fractions of accessible surface area (Willard *et al.,* 2003).

Intrinsic disordered regions (IDRs) identification

Presence of intrinsic disordered regions in protein structure play an important role in regulation of protein activity. The estimation of IDRs of the 5-HTR 1A were made by means of DisEMBL (Linding *et al.,* 2003) and results also validated by three other softwares such as MetaPrDOS (Ishida and Kinoshita, 2008), PSIPRED (Buchan *et al.*, 2013) and IUPred (Deng *et al.*, 2012). Position of each amino acid measured as disordered score from 0 to 1 in predicted protein model. Cut-off value was set at 0.5 and amino acid position scored above this value was categorized as disordered region.

Analysis of hydrophobic character

To find out the hydrophobic characteristics of Serotonin receptor 1A protein "PEPTIDE.2" program (www.peptide2.com) was used. Hydrophobic characteristics imparted by small molecules are very important structural features which contribute in various protein-protein and protein-drug interactions. Therefore, hydrophobic residues and transmembrane regions of Serotonin receptor 1A protein predicted by employing TM finder program (Deber *et al.,* 2001).

According to the criteria, if mean values of hydrophobicity and helicity of contributing amino acids are more than threshold limit, it will be categorized as transmembrane.

Analysis of Subcellular localization

Subcellular localization analysis was performed to understand the protein suitability and its function as vaccine/target. CELLO2GO (Yu *et al.,* 2013) program was applied to identify the subcellular localization of 5-HTR 1A protein. Other programs including SCL-Epred (Mooney *et al.,* 2011), TOPCONS (Bernsel *et al.,* 2009), R3P-Loc (Wan *et al.*, 2014) and TMHMM (Krogh *et al.,* 2001) were also used to validate the predicted results.

Analysis of protein-protein interactions

An electronic database for interaction analysis of known and predicted proteins is known as Search Tool for Retrieval of Interacting Genes and proteins (STRING). It has been used to find protein-protein interactions of 5-HTR 1A with other proteins (Szklarczyk *et al.*, 2011).

Results

The human Serotonin receptor 1A protein sequence used in this study is given below:

>P08908_*Homo sapiens*

mdvlspgqgnnttsppapfetggnttgisdvtvsyqvitslllgtlifcavlg nacvvaaialerslqnvanyligslavtdlmvsvlvlpmaalyqvlnkwt lgqvtcdlfialdvlcctssilhlcaialdrywaitdpidyvnkrtprraaali sltwligflisippmlgwrtpedrsdpdactiskdhgytiystfgafyiplll mlvlygrifraarfrirktvkkvektgadtrhgaspapqpkksvngesgs rnwrlgveskaggalcangavrqgddgaalevievhrvgnskehlplps eagptpcapasferknernaeakrkmalarerktvktlgiimgtfilcwl pffivalvlpfcesschmptllgaiinwlgysnsllnpviyayfnkdfqnaf kkiikckfcrq. Sequence length is consisting of 422 amino acids. (www.ncbi.nlm.nih.gov/gene0).

Three-dimensional structural analysis of 5-HTR 1A

Three-dimensional structural analysis of 5-HTR 1A protein was carried out by using the "Swiss-Model server". Results showed six best models. Among these six models, the best matched model showed validation with highest score for amino acid sequence identity 41.60% with the sequence of human 5-HTR 1A was Chimera protein of human 5-HTR 1B and *E. coli* soluble cytochrome b562 (PDB ID:4iar.1. A) (Fig. 1).

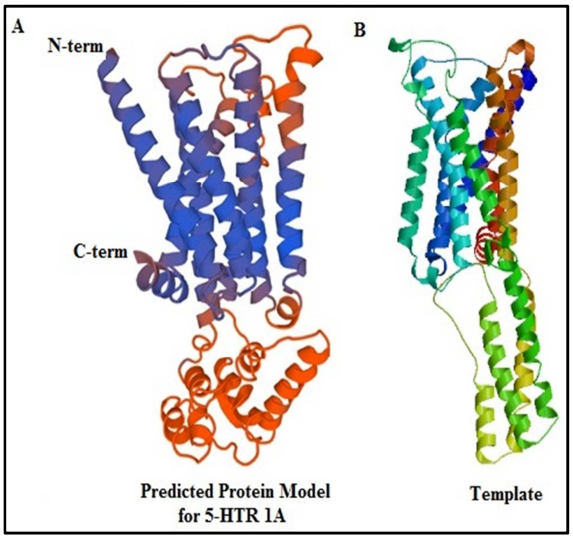


Fig. 1. Predicted three-dimensional structure of human 5-HTR 1A using Swiss model server (A). Crystal structure of template protein 4iar.1. A (Chimera protein of human 5-HTR 1B and *E. coli*, soluble cytochrome b562). N-terminus of model represented by blue colour and C-terminus presented in red colour (B).

The predicted model of 5-HTR 1A protein was analysed through QMEAN. QMEAN score assessed the global and per-residue stereo chemical qualities of model (Table 1). Generally, reliability of model based upon in the range of 0 to 1. The QMEAN4 and QMEAN6 score of the predicted model was close to the value of 0 and it shows the good quality of model. Further confirmation was carried out for Psi/Phi angels by using Ramachandran plot (Fig. 2).

Table 1. QMEAN global scores for 5-HTR 1A model obtained from Swiss modelling.

S.No	Scoring function term	Z-Score
1.	QMEAN4 score	-5.67
2.	QMEAN6 score	-6.50
3.	All-atom pairwise energy	-0.98
4.	C_beta interaction energy	-4.77
5.	Solvation energy	1.59
6.	Torsion angle energy	-5.34
7.	SS Agreement	-4.56
8.	ACC Agreement	-1.42

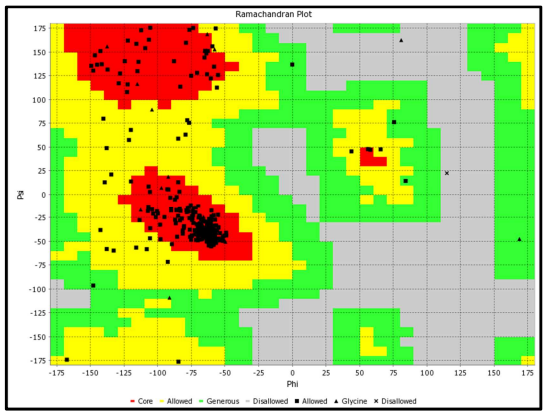


Fig. 2. Ramachandran plot of human 5-HTR 1A protein.

Ramachandran plot analysis revealed that most of the residues were present in the core and allowed regions. It is predicted that 95.3% residues present in favoured region, 3.4% residues in allowed region whereas 1.3% was present in outlier region.

This information supports the good quality of model. VADAR (Volume Area Dihedral Angle Reporter) server was used to predict the accessible surface areas by each amino acid present in side chains and core structure of predicted model of human 5-HTR 1A protein (Fig. 3).

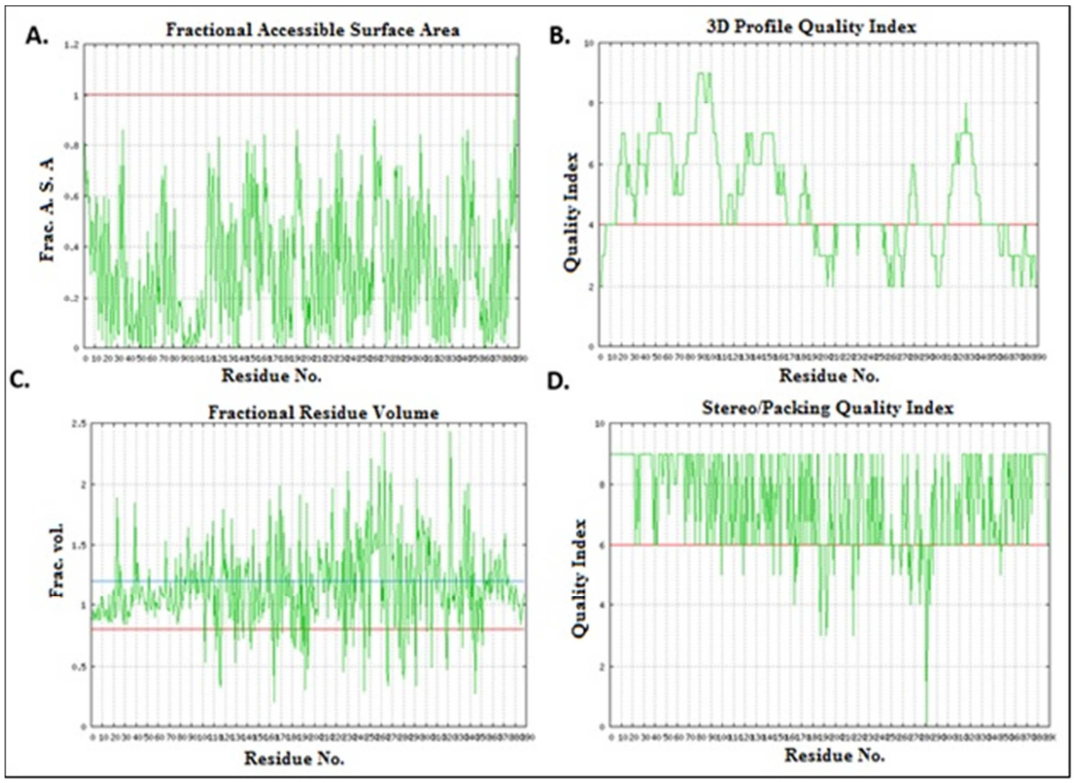


Fig. 3. The fractional A. S.A (A). Quality index of 3D profile (B). Fractional residue volume (C) and packing quality index (D) of 5-HTR 1A protein by using VADAR server.

ASA represents the area accessible to the water molecules in the protein structure. It is calculated in square angstroms and value ranging from 0 to 1. Results revealed that hydrophobic residues occupied a large fraction of ASA whereas; hydrophilic residues covered only a small area and revealed tightly packed stereo conformation. Predicted model of human 5-HTR 1A protein has ASA scores less than 0.8, indicating compact folding. This conformation offers less accessibility to water molecules for residues. Quantitative analysis indicated that all residues have fractional volume close to the 1.0 ± 0.1, which showed efficient packing of protein in predicted model of 5-HTR 1A. The quality of the model with respect to the stereo packing and 3D profile was also assessed.

Prediction of Intrinsic disorder regions

Purpose of identification for intrinsic disorder regions (IDRs) is to understand the mechanism of folding in secondary and tertiary structure which ultimately

contributes to determine protein characteristics and precise function. In this study we studied the IDRs in predicted protein model of 5-HTR 1A by using DisEMBL and results were verified by three other software tools such as MetaPrDOS, IUPred and PSIPRED as well. Those residues which score above than 0.5, based upon real score of disordered propensities produced by MetaPrDOS were considered as disordered (Fig. 4). Results showed that the number of residues which scores below the grey line is more as compared to the disordered residues that revealed more degree of flexibility in 5-HTR 1A predicted protein model (Fig. 5A). Coloured residues represent the disordered regions and black showed ordered regions in 5-HTR 1A predicted protein model (Fig. 5B).

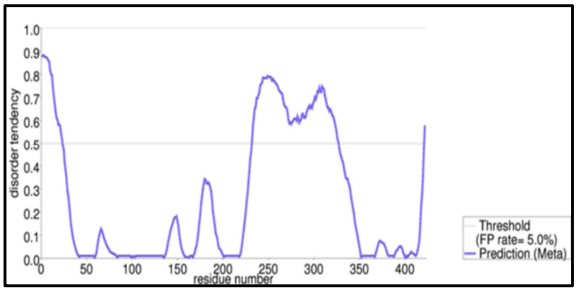


Fig. 4. Intrinsic disorder regions using MetaPrDOS server. Grey line represents disordered tendency, threshold was set at 0.5.

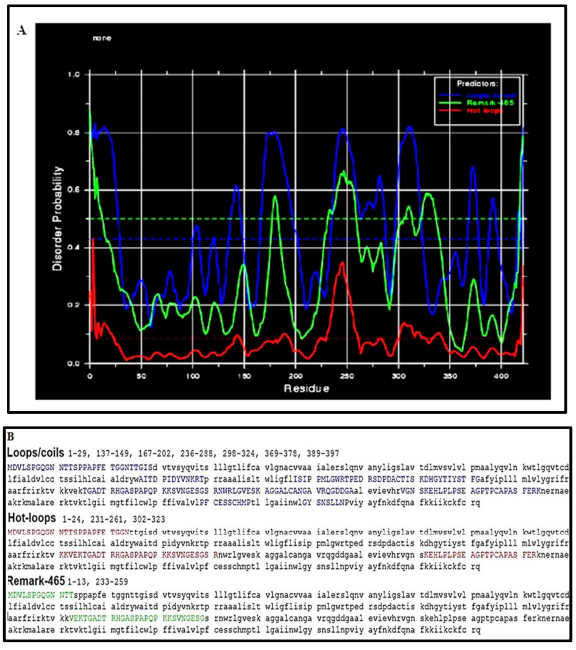


Fig. 5. Intrinsic disordered regions by using DisEMBL server (A). Coloured residues are disordered residues and black are ordered residues (B).

Prediction of Hydrophobic characteristics

Hydrophobic characteristics are important to understand the protein-protein or/and protein-drug interactions. We evaluated the hydrophobic regions of 5-HTR 1A protein with the help of TM-Finder and PEPTIDE 2. Results showed that predicted 5-HTR 1A protein model was consisted of seven helical structures. Residues which contribute in transmembrane regions are 48.1% hydrophobic, 7.35% acidic, 11.85% basic and 32.7% neutral residues.

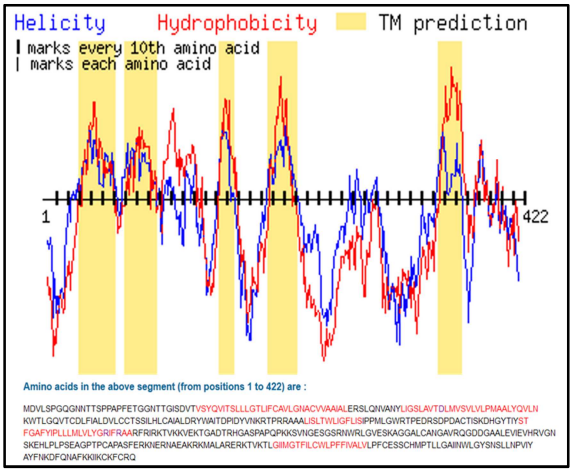


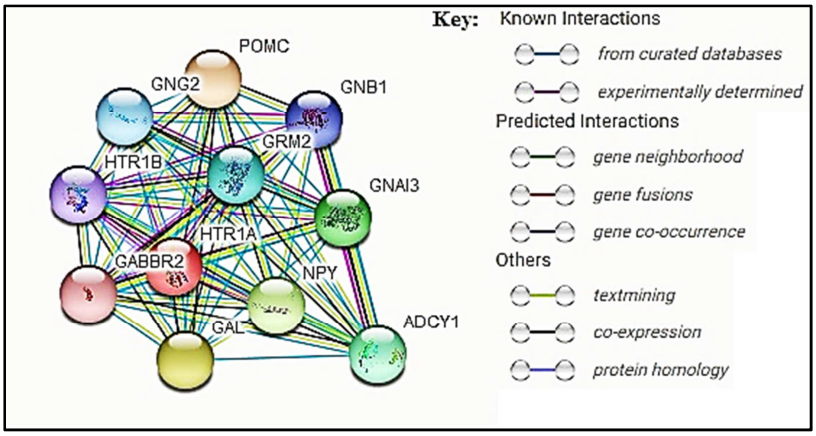
Fig. 6. Hydrophobicity prediction of 5-HTR 1A protein by using PEPTIDE 2 and TM-Finder. Amino acids which participate in transmembrane segment represented by red colour and charged amino acids are represented by purple colour.

Predicted Protein-protein interactions

Protein-protein interactions of 5-HTR 1A protein model studied by using STRING platform. Results predicted that 5-HTR 1A protein interacts with many proteins such as POMC, GNG2, GNB1, GRM2, HTR1B, GABBR2, GAL, NPY, ADCY1 and GNAI3 proteins but interaction score was highest with Pro-opiomelanocortin (POMC) (Fig. 7).

Analysis of Subcellular localization

Subcellular localization analysis of 5-HTR 1A protein was performed by CELLO2GO, SCL-Epred, R3P-Loc, TOPCONS and TMHMM. Results revealed that this protein localized in extracellular space, cytoplasm, endoplasmic reticulum, lysosomes, mitochondria, chloroplast, peroxisomes, vacuole and nuclear membrane. Highest localization probability is in plasma membrane with 91.3% probability (Fig. 8).



Predicted Functional Partners:

POMC	Proopiomelanocortin; ACTH stimulates the adrenal glands to release cortisol (267 aa)	0.969
GAL	galanin/GMAP prepropeptide; Endocrine hormone of the central and peripheral nervous systems that binds and activates the ...	0.964
NPY	Neuropeptide Y; NPY is implicated in the control of feeding and in secretion of gonadotrophin-release hormone (97 aa)	0.948
GNAI3	Guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 3; Heterotrimeric guanine nucleotide-bindin...	0.947
ADCY1	Adenylate cyclase 1 (brain); Catalyzes the formation of the signaling molecule cAMP in response to G-protein signaling. Media...	0.946
GRM2	Glutamate receptor, metabotropic 2; G-protein coupled receptor for glutamate. Ligand binding causes a conformation change ...	0.945
GNG2	Guanine nucleotide binding protein (G protein), gamma 2; Guanine nucleotide-binding proteins (G proteins) are involved as a m...	0.940
GNB1	Guanine nucleotide binding protein (G protein), beta polypeptide 1; Guanine nucleotide-binding proteins (G proteins) are involv...	0.940
HTR1B	5-hydroxytryptamine (serotonin) receptor 1B, G protein-coupled; G-protein coupled receptor for 5-hydroxytryptamine (serotonin...	0.939
GABBR2	Gamma-aminobutyric acid (GABA) B receptor, 2; Component of a heterodimeric G-protein coupled receptor for GABA, formed ...	0.938

Fig. 7. Protein-protein interaction analysis for 5-HTR 1A protein by using STRING server.

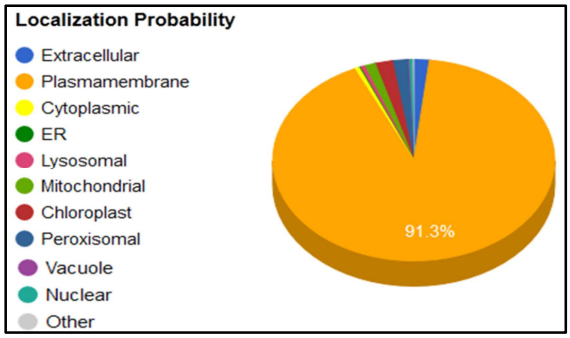


Fig. 8. Subcellular localization prediction of 5-HTR 1A protein by using CELLO2GO server.

Discussion

Among all macromolecules protein structure expresses more complex form of compounds present in the nature. Generally large number of atomic composition, variability, respective topology and surface features make structural description of protein a challenging task. Along with that quantitative assessment is also a difficult task, as it needs precise experimental determination. In this scenario Protein Data Bank (PDB) is providing the large number of related or similar protein structures. It helps to evaluate the protein structure based upon predictability with similar protein sequences in data base (Willard *et al.*, 2003).

Among all known serotonin receptors, only 5-HTR 1A is most studied receptor with multifocal aspects in biology (Alfredo, 2014). Despite of the fact that continues efforts are going on to resolve the structural mysteries of serotonin 1A receptor, but still experimental analysis is facing some limitations. In this study by applying computational homology modelling, we constructed protein model for human serotonin receptor 1A protein and evaluated its quality. Predicted model was consisted of seven transmembrane alpha helixes containing more hydrophobic and less hydrophilic residues. Based on this characteristic feature Serotonin receptor 1A protein is categorized into G protein coupled receptor family (Celada *et al.*, 2004).

It involves in inhibition of neurotransmission by coupling with Gi protein and regulates activities of other serotonergic receptors as well (Gilliam *et al.*, 1989; Celada *et al.*, 2004). Predicted protein-protein interactions revealed that serotonin 1A receptor interacts more with proopiomelanocortin (POMC) protein. Previous study reports that serotonin interacts with postsynaptic serotonin receptor 1A and release peptides which further interacts with

proopiomelanocortin protein during food intake regulation process, which shows that this receptor is involve in feeding behaviour (Collin *et al.*, 2002). Furthermore, regarding cellular localization of this receptor, computational method was employed. Results revealed that probability of localization of serotonin receptor 1A is highest in plasma membrane. This localization is also confirmed by secondary structure analysis, which exhibit transmembrane alpha helical regions. Literature also indicated that 5-HT1A receptors mostly located on the dendrites, cell body, axons, both post-synaptically and pre-synaptically in synapses or nerve terminals (Hjorth *et al.*, 2000). Previous *in vitro* study results also described that experimental analysis on mutant N1E-115 cells indicated that this receptor is present mostly in the plasma membrane whereas intracellular compartments also showed its minor distribution (Gorinski *et al.*, 2012). In conclusion computational analysis revealed that serotonin receptor 1A protein is a seven-transmembrane receptor. The results of bioinformatic analysis might be useful as the basis to study structural and functional interactions of 5-HT1A receptor.

Acknowledgements

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Conflict of interests

There is no competing interest among the authors of this study.

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