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HOMOLOGY MODELLING OF NEUROMEDIN-U TYPE-2 RECEPTOR AND MOLECULAR DOCKING STUDIES OF NMUR2 SPECIFIC AGONIST ICARIIN

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ABSTRACT: Neuromedin-U type 2 (NMUR2) receptor is a centrally located receptor, specifically in the hypothalamus region of the brain. The NMUR2 receptor is proven to trigger signal responses against food intake when bound to a neuropeptide, Neuromedin-U, thereby reducing body weight in mice and rats. The findings from these experiments also indicated that discovery of an agonist specific to NMUR2 could be a potential drug against obesity. Icariin, a flavonol glycoside was screened as an agonist selective to NMUR2 with similar mode of action as that of NMU. Computer Aided Drug Discovery (CADD) is an *in-silico* approach for identification of new drugs. The objective of this study was to build a 3-D model of NMUR2 using homology modelling method and to perform docking studies of icariin onto the modeled NMUR2. The receptor was modelled using MODELLER (9.22) software. The resultant model was a typical G- protein coupled receptor with seven transmembrane α- helices connected by loops and a small stretch of anti-parallel β-sheet. Then the model was validated by plotting Ramachandran plot using RAMPAGE server that showed 92.7% residues in most favored region, which indicated fairly good quality. The ligand icariin was then docked onto the model using AutoDock Vina software. Docking results showed that icariin was well bound onto the receptor by establishing hydrogen bonds with six residues. Therefore, it could positively perform agonist action by initiating responses against food intake. Hence, it was concluded that icariin can be developed as a potential anti-obesity drug in near future.

Keywords: Homology modelling, molecular docking, computer-aided drug discovery, NMUR2, icariin, anti-obesity drug.

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1.INTRODUCTION

Obesity is a lifestyle disease which is now a global epidemic, with about 42.4% prevalence in 2017-2018, according to the National Centre for Health Statistics. It affects individuals of all age groups and is associated with multiple risk factors including diabetes, cardiovascular diseases, hypertension and cancer [1]. It could be defined as "a state of imbalance in the body's energy homeostasis" i.e., imbalance between energy intake and energy expenditure, which ultimately results in an obese phenotype. In last few years, lot of research has been carried out and they suggest that, a neuropeptide, neuromedin U (NMU) is involved in regulating energy homeostasis [2][3]. Two G-protein coupled receptors NMUR1 and NMUR2 have been identified as receptors for neuromedin-U [4][5]. Of the two, NMUR1 is expressed in peripheral tissues like stomach, pancreas, liver and intestine, while NMUR2 is expressed mainly in central nervous system, especially in paraventricular nucleus of hypothalamus [6]. Andrea Peier and co-workers pointed out through their research [7] that weight-reducing potential of intracerebroventricularly (ICV) administered NMU is mediated by NMUR2. Therefore, identification of compounds that could act as agonists to NMUR2 could lead to discovery of potential anti-obesity drugs. On screening of several extracts of natural products, a flavonoid, icariin extracted from *Herba epidemii* was identified to be acting as a strong agonist selective to NMUR2 [8].Computer-aided drug discovery (CADD) is the latest trend in the field of drug discovery. It is fast, feasible and saves significant amount of expenses in the initial stages of drug discovery. Any *in-silico*drug discovery begins with modelling of the protein receptors. Homology modelling is a computational method for predicting secondary structures of proteins. It uses template-based modelling strategy to build 3-dimensional model of a protein with a known homologous structure as a reference. The modelled protein receptors should be validated for its secondary structures. Ramachandran plot is used to validate the predicted protein based on ψ and φ rotational angles. The validated protein receptors are ready for molecular docking studies. Docking is another important computational method employed in drug discovery process. It is used to study the interaction between a drug-like molecule and protein of interest. In this paper, we report the initial steps in the course of CADD, modelling of neuromedin-U type 2 receptor using homology modelling method and molecular docking of probable drug-like molecule icariin onto the receptor.

2. MATERIALS AND METHODS

2.1 Homology Modelling of NMUR2

The receptor was modelled using MODELLER (version 9.22), (University of California, USA) [9]. Firstly, the FASTA file containing 415 amino acid sequence of NMUR2 (Accession number: NP_0.64552.3) was retrieved from NCBI database. Then the sequence was fed as an input in PIR format for the MODELLER to perform homology search. A suitable template with good crystallographic resolution and sequence identity was identified and the template sequence was aligned with that of the target sequence. Based on this alignment, the software was commanded to

build three models. The model with the least DOPE score was considered as the best possible model for NMUR2. The final model was visualized using PyMol (version 0.99 rc6) (DeLano Scientific LLC, California, USA)

2.2 Energy Minimization and Structure Verification

The modelled receptor protein was submitted to ModRefiner serverfor energy minimization. ModRefiner removes side-chain clashes and other atomic restrains, thereby reducing the overall energy of the molecule [10]. The energy minimized model was validated by plotting Ramachandran plot using RAMPAGE server (University of Cambridge, UK) [11].

2.3 Binding Site Prediction

Binding site prediction was done using PockDrug server. The server first identifies binding sites on the protein surface using fpocket server and then predicts druggability of each pocket based on factors like number of binding site residues, hydrophobicity and pocket size [12].

2.4 Docking Icariin to the Receptor

For docking, AutoDock Vina (version 4.2) (The Scripps Research Institute, California, USA) [13] was used. The 3-dimensional structure of icariin was retrieved from PubChem in SDF format. The SDF structure was then converted into PDB format using PyMol. Ligand preparation and protein preparation was done using AutoDock tools. For the receptor, Kollman charges and polar hydrogens were added. Ligand and protein were saved as PDBQT file, readable by AutoDock Vina. The coordinates for the grid box were set based on the pocket position identified by PockDrug server and other parameters were kept default. The software was the run to dock icariin within the specified grid box on the receptor. The software was commanded to produce nine conformations until the best conformation is obtained. A blind docking was also performed in order to verify the predicted binding site.

3. RESULTS AND DISCUSSION

3.1 Homology Modelling

The homology search resulted in 142 hits of homologous templates. Among them, five templates (PDB_IDs 6B73 A and B chain, 4BUO A chain, 4BV0 A chain, 5DHG A chain) were chosen based on the sequence identity and E-value for the next step. In spite of highest sequence identity of 6B73 chain A and B, 4BUO, A chain of Neurotensin type 2 receptor of *Rattus norvegicus*, was selected as the template due to its better crystallographic resolution of 2.8 Å. The software produced three models of the target protein with A chain of neurotensin type 2 receptor as a template (Fig. 1). Model qseq1. B99990001 with the least DOPE score of -46276.33203 was chosen as the best possible model of neuromedin U type 2 receptor. The model was a typical GPCR protein with seven transmembrane α-helices, connected by intracellular and extracellular loops. The structure also contained a small stretch of anti-parallel β-sheet of about 10 amino acids between 4^{th} and 5^{th} helix.

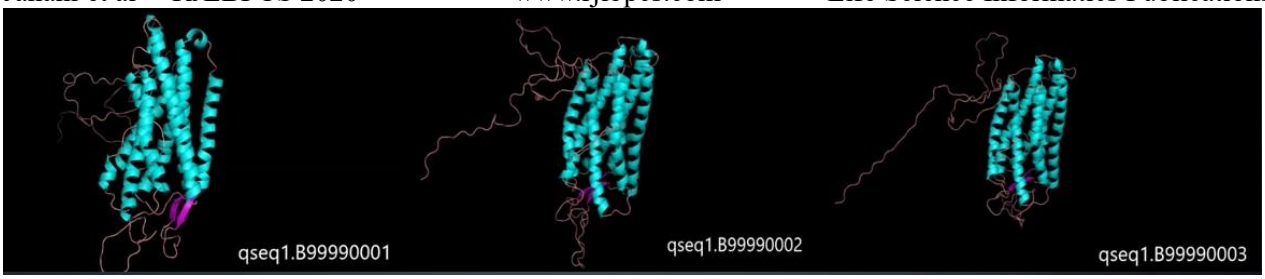

Fig. 1: Three homology models predicted by MODELLER.

3.2 Energy Minimization and Quality Verification

A much stable model was obtained after energy minimization step. The tension between the α-helices was relaxed and the structure was stabilized by minimizing the overall energy. Ramachandran plot for this structure showed 92.7% (381/415) in the most favored region, 5.6% (23/415) in generously allowed region and only 1.7% (7/415) amino acids were the outliers. Since >90% of the residues fell in the allowed region, the predicted model was concluded to be of fairly good quality.

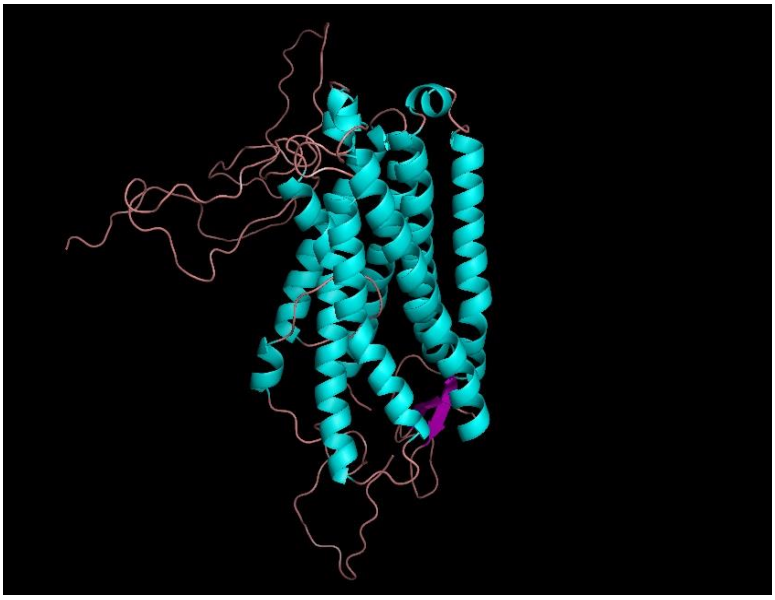

Fig. 2: Receptor after energy minimization.

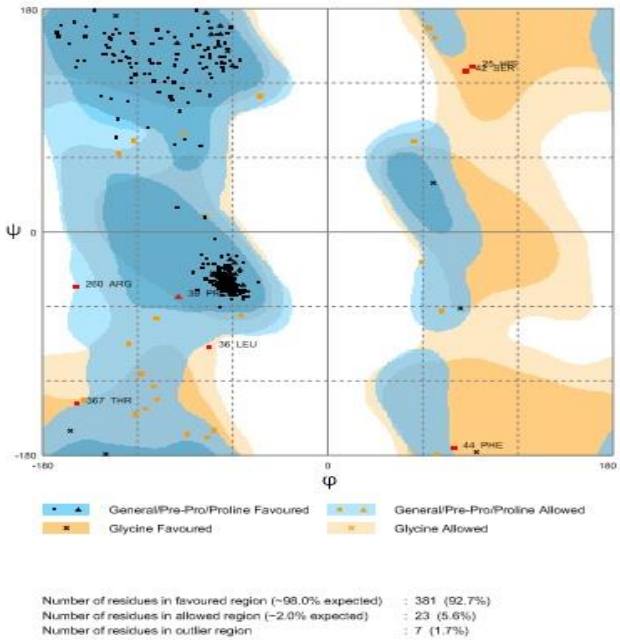

Fig 3: Ramachandran plot for NMUR2 plotted by RAMPAGE.

3.3 Binding Site Prediction

The PockDrug server identified seven binding pockets having >14 residues and ten small pockets having fewer than 14 residues. Pocket with 24 binding site residues and highest druggability probability of 0.98 was selected for docking.

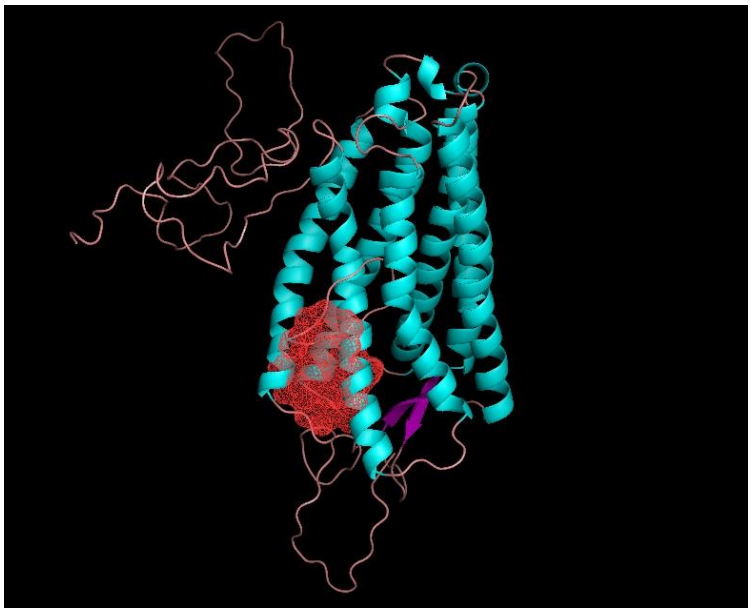

Fig. 4: Binding pocket predicted by PockDrug server.

3.4 Docking Results

AutoDock Vina produced nine different docking conformations along with their binding energies and root mean square deviation values. The binding energies ranged from -7.7kcal/mol to as low as -9.6kcal/mol. The first conformation with the least binding energy of -9.6kcal/mol and 0.0 RMSD value was selected as the best possible conformation. The binding energy of the best conformer from

blind docking was -7.7kcal/mol which was higher than the energy of the predicted binding site. This further establishes the druggability of the predicted binding site. Analysis of the protein-ligand complex showed that icariin was well bound to the receptor by forming hydrogen bonds with six amino acids- MET1, GLU102, GLU105, LYS112, ARG288, TRP297.

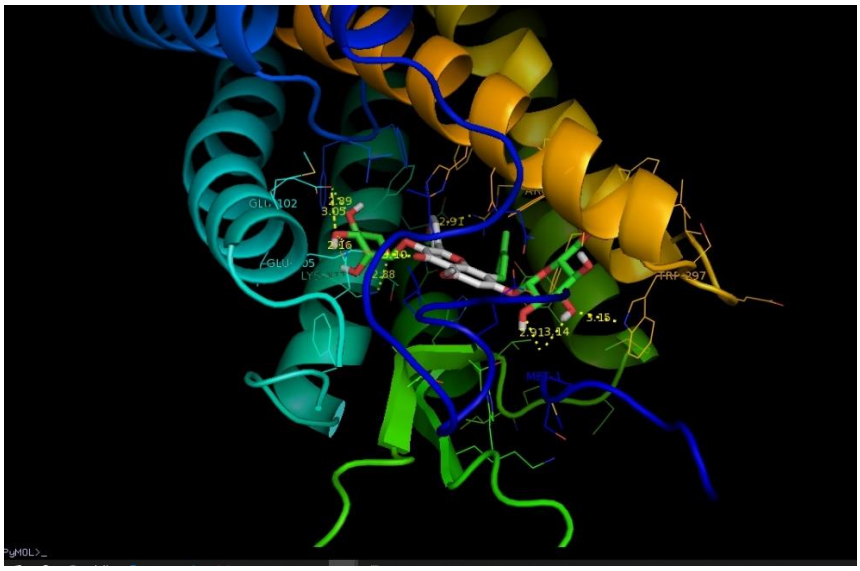

Fig. 5: Residues participating in hydrogen bonding with icariin.
Bond lengths measured are represented as yellow dots.

G-protein coupled receptors represent the largest family of membrane proteins [14]. They are of utmost importance due to their versatile physiological functions. Therefore, they are pharmacologically most sought-after drug targets. They represent about 17% of total drug targets for approved drugs [15]. There are still numerous orphan GPCRs whose endogenous ligands have not been identified and their physiological functions are yet to be explored. In the year 2000, an orphan GPCR, TGR1 was deorphanized when it was identified as a receptor specific to a neuropeptide, neuromedin- U [4]. The receptor then came to be known as neuromedin- U type 2 receptor (NMUR2). Since then, extensive research is being carried out on the structure and function of NMUR2, especially their role in suppressing food intake [2] [3] [4] [16] [17] [18] [19]. These studies, revealed that NMUR2 could be a drug target for anti-obesity drugs. Homology modelling is the most reliable method for protein structure prediction. Numerous drug targets have been modelled using homology modelling [20]. Hence, in our study, we used homology modelling method to predict the structure of our target protein NMUR2, with neurotensin type 2 receptor of rat as the template. The resultant model justified all the molecular signatures of a GPCR structure, *viz*., 7 transmembrane α-helices in the transmembrane region, N- terminus and 3 extracellular loops (ECL1-ECL3) in the extracellular region; and C-terminus and 3 intracellular loops (ICL1-ICL3) in the intracellular region [21].Quality assessment of the model plotting Ramachandran plot showed >90% residues in the most favored region. Although, there were 7 residues in the outlier region. This could be due to low sequence identity of the template (34%). Ideally, high quality models are

produced from a template of more than 60% sequence identity, which probably explains the large stretches of loops. Interestingly, all 7 outlier residues were located on loop region. They were ignored as they did not interfere in the predicted catalytic site of the protein. Icariin was identified as an agonist for NMUR2 [8]. The function of an agonist is to mimic the natural ligand and bind the receptor and produce a similar response as that of the natural ligand. For the agonist to elicit a biological response, it must first form a stable complex with the receptor. Hydrophobic interactions are the most common type of interaction between a ligand and its receptor. These interactions also largely contribute towards the stability of the complex. Followed by hydrophobic bonds, it its hydrogen bonds that are generally established [22]. Here, in this study, icariin forms hydrogen bonds with six binding site residues (MET1, GLU102, GLU105, LYS112, ARG288, TRP297, most of them were N-H--O bonds. The ligand is seen to be tightly bound and enclosed within the receptor with a stable binding energy of – 9.6kcal/mol. Hydrogen bonds contribute a lot more in molecular recognition and specificity more rather than to stability [22].

4. CONCLUSION

Supported by *in vivo* evidences of effects of icariin in rat models, this study was an *in-silico* approach to understand the structure of human NMUR2 receptor and icariin's ability to form a stable interaction with the receptor, so as to produce agonist effects. The docking results were satisfactory in explaining how icariin could be a potential candidate for anti-obesity drugs. Icariin is not only specific, but also selective to NMUR2 over NMUR1 indicating its effectiveness against obesity. These data could be further exploited in the anti-obesity drug discovery procedure.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this research are available within the article.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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