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Research Article

IN-SILICO MODELING AND DOCKING STUDIES OF $AA_{2B}R$ WITH CATECHIN TO EXPLORE THE ANTI DIABETIC ACTIVITY

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ABSTRACT

The AA_{2B} receptor ($AA_{2B}R$) acts as a target for large number of therapeutic applications due to it's wide spread occurrence in diverse tissues. This receptor is involved in the control of mast cell degranulation, interleukin-8 synthesis and cell growth. To date, there is no available PDB structure for $AA_{2B}R$. Hence it is essential to model the structure of $AA_{2B}R$. $AA_{2B}R$ is responsible for inducing insulin resistant via cytokine activation. Therefore, seeking for the highly selective $AA_{2B}R$ antagonists has been one of great interest. In this study herbal catechin, established for its anti-diabetic activity *in-vivo*, was docked with the $AA_{2B}R$ by Autodock 4.00. The results confirm the potential antagonistic effect of catechin against $AA_{2B}R$. This in-turn substantiates the hypoglycemic effect of catechin. Therefore, targeting $AA_{2B}R$ could be a better approach for discovering novel drugs for type-II diabetes.

Keywords: Adenosine A_{2B} receptor, Type 2 diabetes, Catechin, Insulin resistance.

INTRODUCTION

Diabetes mellitus, simply referred to as **diabetes**, is a group of metabolic diseases characterized by an increased amount of serum glucose, either because the patients' body does not produce enough insulin, or because cells do not respond to the insulin that is produced. Elevated levels of glucose in blood generate the classical symptoms such as polyuria, polydipsia and polyphagia[1]. World Health Organization (WHO) alarming the world in the number of cases with diabetes exponentially increases each year. As many as 1.5 million patients have type 1 diabetes in the United States; with some additional 10,000 to 12,000 new cases diagnosed each year[2]. Type I diabetes occurs when the pancreatic β cells fail to produce enough insulin in response to glucose[3]. Research for a decade proposes that the pathogenesis of non-insulin-dependent diabetes mellitus (NIDDM) remains obscure[4]. It affects nearly 2-5% of the world's population. Type II diabetes occurs as a set of disorders collectively called as "**Ominous Octet"**[5]**.** Insulin resistance in muscle, liver and adipocytes is a core defect in type II diabetes[4, 6-8] which keeps the body from properly handling sugar. Karen Vaughan a Registered Herbalist (AHG) reported that it is found to be that in case of insulin resistant there is a massive reduction in the number of receptors found on the surface of the cell and results in free floating of insulin and glucose in the blood. Hence therapies are needed for diabetes. The broad scope of anti-diabetic therapy is to restrict blood glucose control, by controlling fasting glucose levels and by controlling elevations in postprandial blood glucose[9] in which it adapts some definite mechanism.

Diabetes plays a major role in adenosine accumulation and signaling[10]. Adenosine is an anti-inflammatory and immunosuppressive molecule released from cells into the extracellular space at sites of inflammation and tissue injury and binds to its specific receptors, which are the adenosine A_1, A_{2A}, A_{2B}, and A_3 receptors[11-12]. The $AA_{2B}R$ are widely expressed in tissues and cells and has the lowest affinity for adenosine[12]. The activation of $AA_{2B}R$ results in IL-6 production (proinflammaory mediators) in macrophages and endothelial cells[13]**.** Circulating Interleukin (IL) 6 are responsible for insulin resistant[14-16]impaired glucose tolerance[17]by inhibiting IR signal transduction as well as glycogen synthesis[18]. According to Robert, (2011)[13] blocking of $AA_{2B}R$ could treat insulin resistance. Therefore targeting $AA_{2B}R$ provides new strategy in developing drugs for type II diabetes.

Traditional medicine plays an important role in the health care of human population where 80% of the world population depends on herbal medication[19]. Several studies proved for herbal plants in diabetic treatment. Hence the present study was aimed in establishing antagonistic effect of herbal catechin on modeled $AA_{2B}R$. Our previous investigations proved that catechin isolated from the methanol extract of *Cassia fistula* stem bark possesses anti-diabetic activity. We observed that there was a significant reduction in the blood glucose level after the oral administration of catechin for 6 weeks in streptozotocin –induced diabetic Wistar rats. But there was not an increase in the serum C-peptide and insulin levels. Furthermore, the histological studies showed that there was no regeneration of β cells in pancreas[20]. Hence in the present study, we propose that the strategy for the anti-diabetic activity of catechin may be due to the binding of catechin to the active site of $AA_{2B}R$ and block this protein which might prevent insulin resistant of the cell and tissues.

MATERIALS AND METHODS

Homology model development

Homology modeling is a theoretical method that is used to predict the structure of a sequence with an accuracy that is comparable to the best results achieved experimentally. The modeled protein quality is extremely dependent on the identity between the target and template proteins[21]. The sequence of human $AA_{2B}R$ (AA2BR_HUMAN) was collected from the Swiss-Prot Protein Database (accession number: **P29275**). A similarity search for $AA_{2B}R$ protein in the Protein Data Bank (http://www.rcsb.org) was performed using the BLAST server. The protein similarity search identified a very similar protein structure belonging to the Adenosine receptor A_{2A} protein of human (PDB ID: 2YDO), which has sequence identity with AA2BR_HUMAN, so this structure was used as a template to generate the model. The model was generated using Modeller9.9[22] and visualization of the 3-D structure in PYMOL was done[23].

Model evaluation and validation

The required number of models would be generated after performing homology modeling with Modeller 9.9. Dope scores of the generated models could be calculated using the command model-single.py. The 3-D structure of $AA_{2B}R$ was validated with the programs PROCHECK[24] and WHATIF. These programs generated Ramachandran plots of the amino acid residues in the allowed region.

The root mean square deviation (RMSD) between the main chain atom of the model and the template was calculated by superimposing the structure of the template on the predicted

structure of $AA_{2B}R$ in order to assess the reliability of the model using PyMol[25].

Active site analysis

Binding sites of the target protein were predicted using Q-site finder. (Active site prediction tool). The residues in receptor sites are summarized as residue name and corresponding residue numbers in the 3-D protein structure[26].

Chemoinformatics

Chemoinformatics is one of the emerging and applied branches of Drug Discovery by which one can easily solve the chemical problem with the help of computational approach Docking operations. Chemsketch is one of the important tools applicable for the lead designing using SMILES (Simplified Molecular Input Line Entry Specification)[27]. In this current study, the structure of catechin has been obtained from ChemSketch using SMILES.

Docking operations

The molecular docking operations in our studies were performed by Auto Dock 4.00 package[28-29] to investigate the interactions between modeled $AA_{2B}R$ and catechin isolated from *Cassia fistula*. During the whole docking process, drug molecules were flexible, while the protein molecule kept rigid. Auto Dock can generate a diversified set of conformations by making random changes of the coordinates of drug molecules. When a new conformation of drug molecules was generated, the search for the favorable bindings was conducted within a specified 3D docking box using genetic algorithm[30]. This approach can seek to optimize the purely spatial contacts as well as electrostatic interactions

RESULTS AND DISCUSSION

Sequence alignment between target and template

Homology modeling produced high quality structural models when the target and template are closely related. Protein structure modeling was undertaken in this present work. The main criteria in homology modeling were template selection and sequence alignment between the target and the template. According to literature[31-33] this approach would give reasonable results based on the assumption that the tertiary structures of two proteins will be similar if their sequences are similar. In this respect, in the present study, the BLAST searching result showed that, the sequence identity between the target $AA_{2B}R$ and the template human $AA_{2A}R$ is 59% which allowed for rather straightforward sequence alignment (Fig 1).

```
Target      MLLETQDALYVALELVIAALSVAGNVLVCAAVGTANTLQTPTNYFLVSLAAADVAVGLFA 60
Template    -MPIMGSSVYITVELAIAVLAILGNVLVCWAVWLNSNLQNVTNYFVVSAAAADILVGVLA 59
             :    .::*:::**.**.*:: ****** **   ..**. ****:** ****: **::*

Target      IPFAITISLGFCTDFYGCLFLACFVLVLTQSSIFSLLAVAVDRYLAICVPLRYKSLVTGT 120
Template    IPFAIAISTGFCAACHGCLFIACFVLVLTASSIFSLLAIAIDRYIAIRIPLRYNGLVTGT 119
            *****:** ***:  :****:******** ********:*:***:** :****:.*****

Target      RARGVIAVLWVLAFGIGLTPFLGWNSKDSATNNCTEPWDGTTNESCC---LVKCLFENVV 177
Template    RAKGIIAICWVLSFAIGLTPMLGWN-------NCGQPKEGKAHSQGCGEGQVACLFEDVV 172
            **:*:**:  ***:*.*****:****        ** :* :*.::.. *    * ****:**

Target      PMSYMVYFNFFGCVLPPLLIMLVIYIKIFLVACRQLQRTELM----DHSRTTLQREIHAA 233
Template    PMNYMVYFNFFACVLVPLLLMLGVYLRIFLAARRQLKQMESQPLPGERARSTLQKEVHAA 232
            **.********.*** ***:** :*::***.* ***:: *       :::*:***:*:***

Target      KSLAMIVGIFALCWLPVHAVNCVTLFQPAQGKNKPKWAMNMAILLSHANSVVNPIVYAYR 293
Template    KSLAIIVGLFALCWLPLHIINCFTFFCPDCS-HAPLWLMYLAIVLSHTNSVVNPFIYAYR 291
            ****:***:********:* :**.*:* *  . : * * * :**:***:******::****

Target      NRDFRYTFHKIISRYLLCQADVKSGNGQAGVQPALGVGL 332
Template    IREFRQTFRKIIRSHVLRQQEPFKAAAAENLYFQ----- 325
             *:** **:***  ::* * :  .. .  .:
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Fig. 1: It shows the alignment between target ($AA_{2B}R$) and template sequence ($AA_{2A}R$) obtained from ClustalW. The asterisk showed the identity of amino acids present in two protein sequences.

Homology modeling of $AA_{2B}R$ protein and its evaluation

A required number of models were generated after performing homology modeling with Modeller 9.9. Dope scores of the generated models were calculated using the command model-single.py. Based on the literature[26,34] the predicted model with a minimum dope score should considered as a best model. With respect to this the model TvLDH.B99990005.pdb, of the present study having minimum dope score of -42521.93359 was considered as the best model of protein $AA_{2B}R$.(Table-1)

The best ranked model was then subjected for evaluation to check the quality. The modeled $AA_{2B}R$ protein was used for further optimization and validation. Ribbon diagram of the modeled AA_{2B} receptor of human in Fig 2.

Generally, if the RMSD value between target and template is lower, then it indicates that the target and template are stereo chemically similar[21, 35-36]. As the calculated RMSD value of the present study is 0.102 Å, (Fig 3) which goes parallel to the literature, we concluded that the target $AA_{2B}R$ and the template human $AA_{2A}R$ (2YDO) are stereo chemically similar.

Geometric validation of the modeled 3D structure of $AA_{2B}R$ was performed using PROCHECK by calculating the Ramachandran plot (Fig 4). Based on the theory originally proposed by G. N. Ramachandran (1963)[37] the plot represents the distribution of the phi and psi angles for the amino acid residues. For the present study the percentage of phi and psi angles that occur in the most favoured region was 94.3 %, 4.4% in the allowed region, 1.3% in the generously allowed region, whereas this percentage was 0.0% for the residues located in the disallowed regions. These results revealed that the majority of the amino acids are in a phi-psi distribution, and the model is reliable and of good quality which is parallel to the report proposed by Dhanachandra singh et al (2007)[35].So, the quality of Ramachandran plots is acceptable and the stereo chemical quality of the model was found to be satisfactory.

Table 1: Shows dope score of successfully produced models

S. No	File Name	Dope Score
1	TvLDH.B99990001.pdb	42354.69531
2	TvLDH.B99990002.pdb	-41558.51563
3	TvLDH.B99990003.pdb	-41250.07031
4	TvLDH.B99990004.pdb	-41370.28906
5	TvLDH.B99990005.pdb	-42521.93359

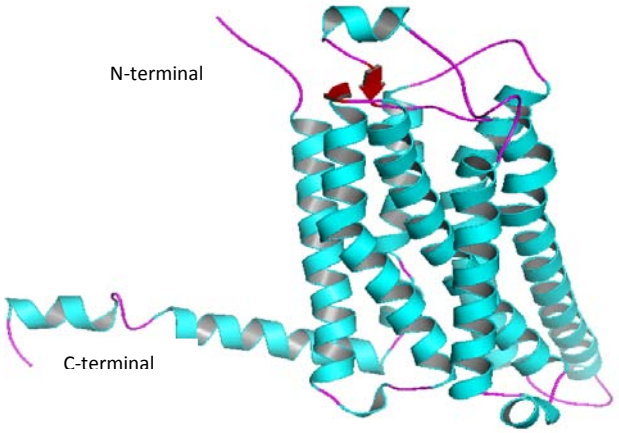

Fig. 2: Ribbon diagram of the modeled $AA_{2B}R$ of human showing α-helices (cyan color), β-strands(red color) and loops (magenta color).

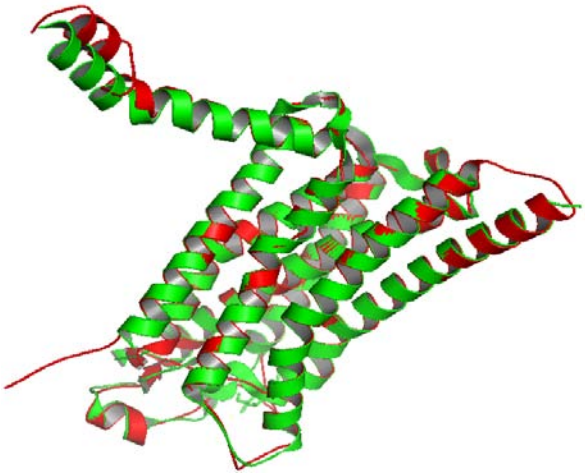

Fig. 3: Superimposition of modeled $AA_{2B}R$ protein (target) and template ($AA_{2A}R$) using Pymol. In this picture red color represents target and green represents template.

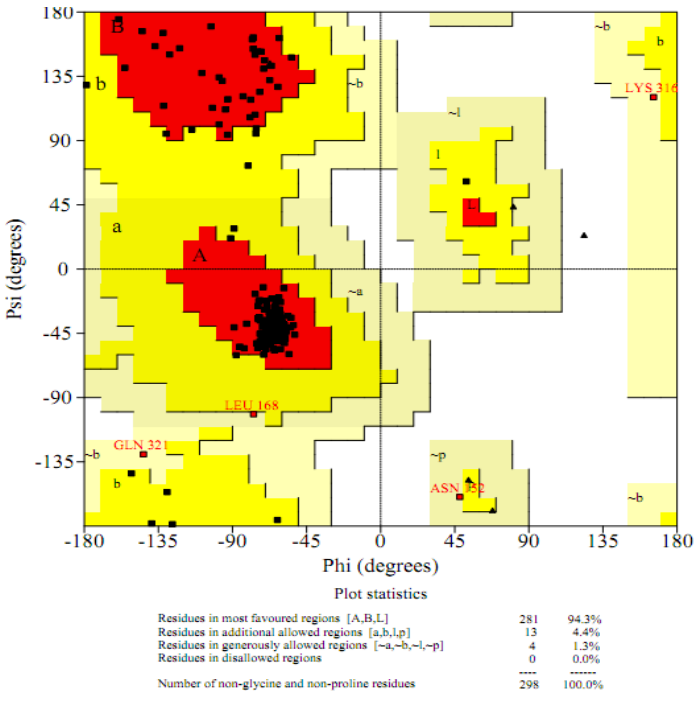

Fig. 4: Ramachandran plot of $AA_{2B}R$ protein obtained from PROCHECK

Active site analysis

Binding sites of the target protein were predicted using Q-site finder (Active site prediction tool) (fig: 5).The residues in receptor sites were summarized as residue name and corresponding residue numbers in the 3-D protein structure. The residues were identified on the protein receptor sites as ASP 7, LEU 9, TYR 10, LEU 13, GLU 14, LYS 269, TRP 270, ASN 273, MET 274, LEU 277, LEU 278 with hydrophobic nature on the surface of the protein.

Molecular docking results

Further docking analyses were carried out using Autodock4.4. The 3D structure of catechin generated by ChemSketch was docked with modeled $AA_{2B}R$. The ligand showed best interaction with the active sites of $AA_{2B}R$ (ASN 273 MET 274) which was predicted by Q-site finder (Fig 6). The interaction of ligand with receptor on the basis of docking several energies, i.e. docking energy, inter molecular energy, torsional energy, internal energy, were given in Table 2 and the RMSD value was 18.676 **Å**. Literature states that the lowest docking energy always correlated with highest binding affinity in protein–drug interactions[34,38]. As the docking energy of the catechin was -6.54 kcal/mol which seems to be low, so we confirm that the interaction between $AA_{2B}R$ and catechin of the present study is satisfied.

The interaction of catechin with $AA_{2B}R$ result in the inhibition of the receptor (Fig 7). The report of Robert et al (2011), states that the activation of $AA_{2B}R$ in mice elevates fasting blood glucose levels and reduces whole body glucose disposal, due to elevated $AA_{2B}R$ mRNA expression and elevated $A_{2B}R$ mediated cytokine production. This infers that the inhibition of the receptor would result in the reduction of blood glucose level and increases the body glucose disposal.Our precious study on the effect of catechin on STZ-induced diabetic Wistar rats[20] states that the oral administration of catechin reduces blood glucose level and increases body glucose disposal. Hence, we derived the strategy for the good docking interaction between modeled protein $AA_{2B}R$ and catechin of the present study must have been resulted in the inhibition of the receptor and not in the activation. Further *in-vivo* investigations for the lower expression of $AA_{2B}R$ mRNA and lower production of cytokine after the catechin administration is under process to substantiate the present *in- silico* study.

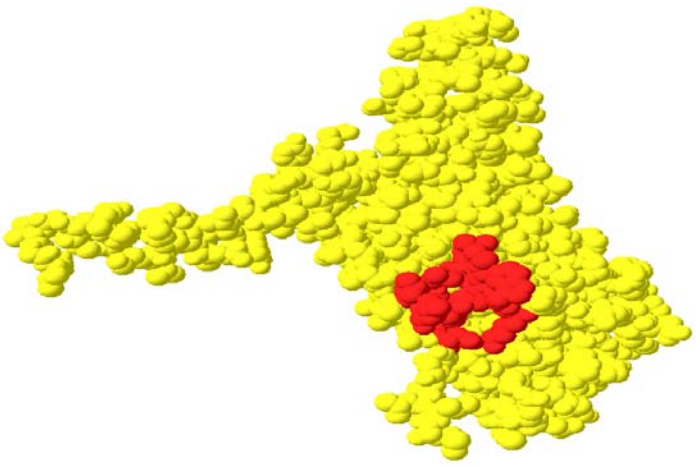

Fig. 5: The active site region of modeled protein $AA_{2B}R$ (red color).

Table 2: shows the calculated interaction energy of (kcal mol–1) of catechin with $AA_{2B}R$

S. No.	Interaction energy	Energy values (kcal/mol)
1	Binding energy	-6.54
2	Intermolecular Energy	-7.17
3	Torsional Energy	+1.49
4	Internal Energy	-0.86

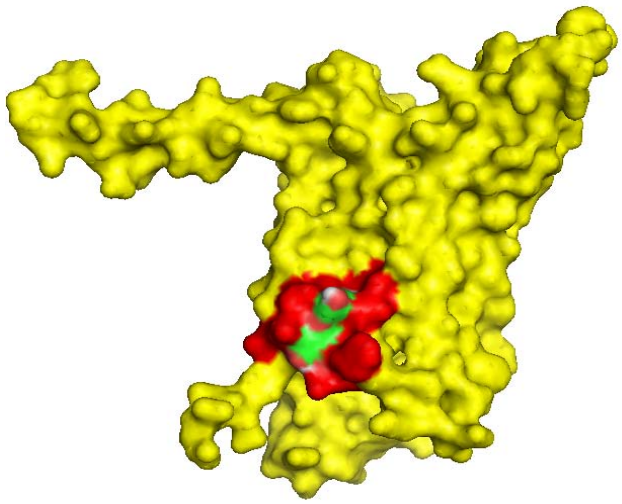

Fig. 6: The docked protein-ligand complexes with hydrogen interactions in binding pocket. Catechin (green color) was complexed with the active site (red color) of modeled protein $AA_{2B}R$ (yellow color)

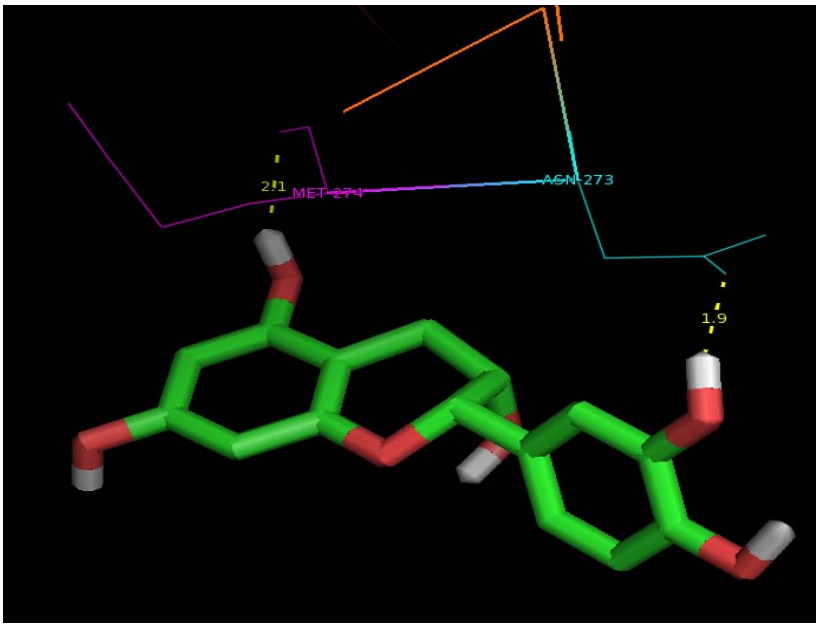

Fig. 7: Hydrogen bond interaction between modeled $AA_{2B}R$ with catechin. The amino acids MET274 (magenta color) ASN273 (cyan color) showed interaction with the ligand with bond length 2.1 Å and 1.9 Å respectively

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