INTRODUCTION

⇒ $\alpha_2A$-adrenoceptor (ADA2A) is a membrane bound receptor which has been classified as the member of larger superfamily G-protein coupled receptors (GPCRs); also known as seven transmembrane (7-TM) domains receptor
⇒ Around 50% drugs currently available in the market exert their effects through GPCRs
⇒ Membrane proteins are difficult to crystallize as compared to soluble proteins
⇒ There is a need of 3D-structure of ADA2A to understand binding modes of various agonists and antagonists and hence homology model of ADA2A was developed
⇒ Homology model of ADA2A constructed based on crystal structure of $\beta_2$-adrenoceptor
⇒ The crystal structure of $\beta_2$-adrenoceptor (PDB ID: 2RH1) was used as template, which has good sequence identity and higher resolution (2.4 Å) and models were generated using MODELLER9v7, among them some models were selected based on molpdf and DOPE score
⇒ The built homology model was evaluated using various programs like ERRAT, PROCHECK, PROSA2003, and WHAT-IF
⇒ The built homology model can be useful for designing more potent subtype selective antagonists or/and agonists and can provide guidance for mutagenesis studies

OBJECTIVES

⇒ Development of homology model of $\alpha_2A$-adrenoceptor
⇒ Validate the built homology model using different software

METHODOLOGY

⇒ Sequence of $\alpha_2A$-adrenoceptor was retrieved from UniProtKB/Swiss-Prot
⇒ Query sequence was subjected to BLASTP against Protein Data Bank database
⇒ Four top hits were obtained viz. 2RH1, 3D4S, 2R4R and 2R4S
⇒ Between these four top hits 2RH1 was selected as template
⇒ Template sequence and query sequence was aligned using ClustalX program
⇒ 100 models were generated using MODELLER9v7 program
⇒ Based on Discrete Optimized Protein Energy (DOPE), molpdf and GA341 scores, five models were selected
⇒ Selected models were assessed using PROCHECK, ERRAT plot and Verify3D software
⇒ Among these models best model was selected based on the results of various assessment tests
⇒ The best model was further undergone for loop modeling, energy minimization and rotamer search using MODELLER9v7 and MOE2007.09 software
⇒ An optimized model was validated using various software: PROCHECK, PROSA2003, WHAT-IF and VERIFY3D

RESULTS AND DISCUSSION

⇒ BLASTP results gave different top hits as shown in Table-1. Among these 2RH1 was selected due to higher sequence identity and good crystal structure resolution

<table>
<thead>
<tr>
<th>PDB-ID</th>
<th>IDENTITY</th>
<th>SIMILARITY</th>
<th>SCORE</th>
<th>EXPECT</th>
<th>RESOLUTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>2RH1</td>
<td>31</td>
<td>46</td>
<td>179</td>
<td>1.00E-45</td>
<td>2.4 Å</td>
</tr>
<tr>
<td>3D4S</td>
<td>31</td>
<td>46</td>
<td>179</td>
<td>2.00E-45</td>
<td>2.8 Å</td>
</tr>
<tr>
<td>2R4R</td>
<td>35</td>
<td>52</td>
<td>142</td>
<td>2.00E-34</td>
<td>3.4 Å</td>
</tr>
<tr>
<td>2R4S</td>
<td>35</td>
<td>52</td>
<td>142</td>
<td>3.00E-34</td>
<td>3.4 Å</td>
</tr>
</tbody>
</table>

⇒ From PROCHECK analysis, Ramachandran plot indicated that all residues phi/psi angle distribution was within core and allowed regions and G-factor score was 0.2 which also showed reliability of developed homology model
⇒ Superimposition of template and target indicated less difference between C-alpha backbone and root mean square deviation (rmsd) was 0.1518 Å, calculated using Sybyl7.1 software
⇒ Verify3D profile for the model designated that 81.04% of residues of the model had a score over 0.2, a value used as judgment for a good model

CONCLUSIONS

⇒ The 3D-structure of $\alpha_2A$-adrenoceptor (ADA2A) obtained by homology modeling showed high structural similarity to the template 2RH1 protein
⇒ Results of structure validation using different software: PROCHECK, WHAT-IF, ERRAT plot, Verify3D and PROSA2003 showed that the built homology model have overall good structure quality
⇒ Knowledge based approach such as comparative modeling can be used as an important tool in rational drug design/docking analysis, mutagenesis studies and structure-based drug design

REFERENCES