Homology modelling and evaluation of the cannabinoid receptor type 2

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Introduction

The human cannabinoid receptor type 2 (CB2) is linked to a variety of immune functional events which are found primarily in brain and tissues of immune and hematopoietic systems like spleen, tonsil, and thymus. In the absence of crystal structure of CB2 receptor, 3D homology patterns with different templates are built [1,2].

The aims of the present study are: (1) to choose within the recently published crystallographic structures templates for homology modelling of the CB2 receptor; (2) to evaluate the models with different computational tools; (3) to precise the most reliable model on the basis of the correlation between data from molecular docking and the values of the biological activity of the cannabinoid ligands.

Materials and Methods

A different software is used in the present work in order to perform computational studies. The protein sequence of the human CB2 receptor was obtained from UNIPROT (Accession number – P34972). Homology modelling studies were carried out using Swiss-Model [3]. Ligand preparation was done with Avogadro. Docking studies were performed by using GOLD 5.2 (Genetic Optimization for Ligand Docking) [4]. Molegro Molecular Viewer was used for generating figures (CLC Bio. Qiagon Inc). In order to find relationship between sets of data derived from *in vitro* assay and docking results, we tried to predict it with the help of Pearson's correlation, using GraphPad Prism 3.0.

Table 1: The values of the inhibitory constant (K_i) of the cannabinoid ligands with known affinity, used in the docking studies [5].

Ligands	CB1 Affinity (K_i)	Type
Anandamide	78 nM	Endogenous
N-Arachidonoyl dopamine	-	Endogenous
2-Arachidonoylglycerol	-	Endogenous
2-Arachidonoyl glyceryl ether	21 nM	Endogenous
Δ -9-Tetrahydrocannabinol	10 nM	Phytogenic
EGCG(Epigallocatechin Gallate)	33.6 μ M	Phytogenic
Yangonin	0.72 μ M	Phytogenic
UR-144	150 nM	Synthetic

The human CB2 receptor protein sequence was obtained from Swiss-Prot website (P34972) and aligned with the sequence of the crystal structure of the human CB1 receptor in complex with agonist AM115421U19 (PDBid:5xra.1) with transmembrane(TM) sequence identity = 46.78%, resolution = 2.8 Å using the PAM-250 matrix, which aligns the sequence based on the conservation of charged, bulky 13 aliphatic or aromatic residues (<https://swissmodel.expasy.org/>). Alignment was refined manually by taking the 7 TM regions and structurally conserved regions into consideration. The 7 TM regions were identified based on the conservation of residues in each putative TM region.

Table 2: The values of scoring functions in GOLD 5.2 for performed docking between the model of the CB2 receptor obtained by homology modelling and affinity constant K_i of the known cannabinoid ligands.

Ligands	ASP Score	ChemScore	ChemPLP	GoldScore
Δ -9-Tetrahydrocannabinol	13,99	37,30	23,33	45,24
Yangonin	14,57	30,06	18,90	40,57
UR-144	19,84	46,48	23,69	33,71
EGCG(Epigallocatechin Gallate)	17,06	30,34	0,89	37,70
Anandamide	18,03	56,40	20,03	61,24
2-Arachidonoylglycerol	21,29	54,27	18,03	53,70
N-Arachidonoyl dopamine	20,20	64,42	25,60	42,64

The binding site was defined as residues within 10 Å radius of Cys257. Docking was performed with the chosen model and 7 ligands (Table 2) [6]. There is a significant correlation between the results of the docking (ChemScore function) and the biological activity of the ligands (Table 3, Fig.1), (Pearson R = -0.945). This correlation describes the relationship between the biological activity of the compounds and the docking results.

Table 3: The values of Pearson's coefficient for the correlation between GOLD scoring functions for model of the CB2 receptor obtained by homology modelling and affinity constant K_i of the cannabinoid ligands.

ASP Score	ChemPLP	ChemScore	GoldScore
R=-0,1343 (pvalue=0,7740)	R=-0,5118 (pvalue=0,2403)	R=-0,9456 (pvalue=0,0013)	R=-0,3426 (pvalue=0,4519)

Figure 1: Pearson's correlation between the values of Affinity of cannabinoid ligands and the values of ChemScore scoring function from molecular docking.

Figure 2: Graphical representation of the docking between the cannabinoid ligand Anandamid and the Chem-Score scoring function for model of CB2 receptor obtained by homology modelling.

New model of the CB2 receptor was generated and evaluated by different approaches. Newly generated model by homology modelling could be used further for *in silico* experiments and along with the correlation found between *in vitro* and docking experiments will give possibility for faster and more correct design of selective and effective ligands for CB receptors [7-10].

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