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Molecular docking experiments of cannabinoid receptor

F. I. Sapundzhi[1*], T. A. Dzimbova[2], N. S. Pencheva[1], P. B. Milanov[1,3]

[1]*South-West University Neofit Rilski, 2790 Blagoevgrad, Bulgaria*
[2]*Institute of Molecular Biology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria,*
[3]*Institute of Mathematics and Informatics, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria*

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The cannabinoid receptor is a part of the endocannabinoid signaling system. CB1 is a therapeutic drug target, and its structure and conformational changes after ligand binding are of great interest. The present study aimed to investigate the interaction between the crystal structure of the human cannabinoid (CB1) receptor (PDB id:5TGZ) and several known cannabinoid ligands in order to determine the structure-activity relationship by using molecular docking with software GOLD 5.2. Four scoring functions provided with GOLD 5.2 were used for molecular docking between the crystal structure of CB1 receptor and the cannabinoid ligands. The obtained results could be used further for *in silico* experiments of the cannabinoid receptor-ligand interactions.

Keywords: Cannabinoid receptors, CB1, Molecular docking experiments, Ligand-receptor interactions, Structure-activity relationship

INTRODUCTION

Human cannabinoid receptor type 1 (CB1) is a part of the class G-protein coupled receptors (GPCRs) that represent the largest membrane protein family and are of great pharmacological importance. It is a therapeutically useful target involved in a different physiological process such as pain, metabolic regulation, craving, anxiety, etc. [1,2]. The drugs target cannabinoid receptors for the treatment of chemotherapy-induced nausea and vomiting, relieving neuropathic pain, etc. [3]. Nowadays, drugs targeting CB1 receptor are constantly being developed [4-6].

The agonists of the cannabinoid receptor can be divided into four structurally distinct classes of compounds. These include classical cannabinoids (like Δ-^{9}-THC), non-classical cannabinoids, represented by CP55940, aminoalkylindoles, such as WIN55212-2, and endogenous cannabinoids such as anandamide (AEA) [7]. In the present study we use known cannabinoid ligands with established binding affinity and selectivity from literature [8].

The knowledge of the 3D structure of the cannabinoid receptors could be useful in the task of understanding their function and in the design of specific ligands. Therefore, many biochemical, pharmacological, and computational studies have been carried out on cannabinoid receptors.

The crystal structure of the CB1 receptor was determined in RCSB (PDBid: 5TGZ) [9,10]. This is very helpful for the computational modeling of structure-activity relationships between the receptor and its ligands.

The present research aimed to study the interaction between the CB1 receptor (PDB id:5TGZ) [10] and several known cannabinoid ligands in order to determine the structure-activity relationship by using molecular docking with software GOLD 5.2 [11]. Four scoring functions provided in the software were used for molecular docking experiments [11-14].

MATERIALS AND METHODS

Cannabinoids used in the present work

Receptor

The crystal structure of the CB1 receptor published in RCSB Protein Data Base (PDB id: 5TGZ, www.rcsb.org) was used [9,10]. It was obtained by X-ray diffraction with 2.8 Å resolution. Length: 452 amino acids.

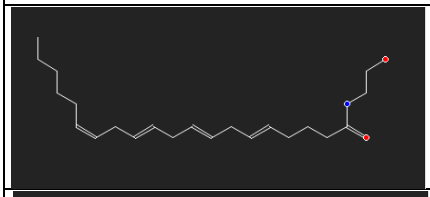
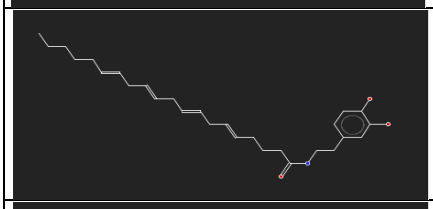
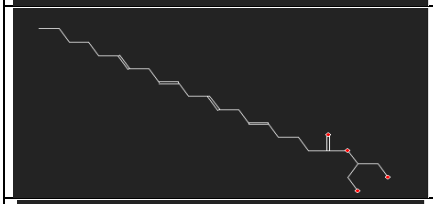
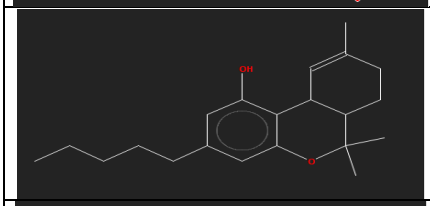
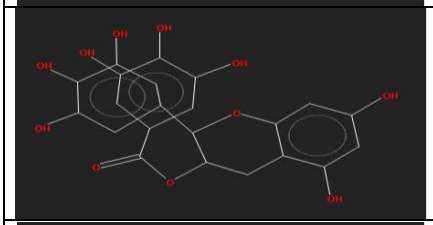
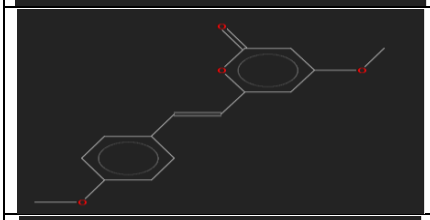
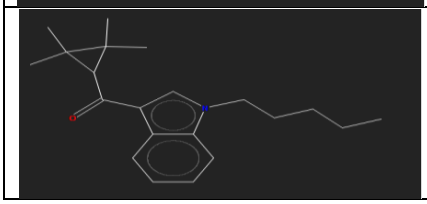
The CB1 receptor belongs to the Class A of rhodopsin class GPCRs. It has been proposed that there exists a hydrophobic binding pocket that interacts with the alkyl chain of classical and non-classical cannabinoids. One of the important points is Asp 366 residue where the polar parts of the ligands bind [15].

Computational tools

Ligand preparation was done with software Avogadro (an open-source molecular builder and visualization tool - Version 1.0.3) [16]. Image generation and interaction studies were done after docking with Molegro Molecular Viewer (MMV) [17]. A GraphPad Prism 3.0 was used for the correlations [18-25].

* To whom all correspondence should be sent.
E-mail: sapundzhi@swu.bg

Table 1. Structure, affinity and efficacy of the ligands of CB1

Structure	Ligands	Efficacy towards CB1
	Anandamide	Full agonist
	N-Arachidonoyl dopamine	Agonist
	2-Arachidonoylglycerol	Full Agonist
	Δ-^{9}-Tetrahydrocannabinol	Partial Agonist
	EGCG (Epigallocatechin gallate)	Agonist
	Yangonin	-
	UR-144	Full Agonist

Docking of the cannabinoids

Docking studies were performed by using GOLD 5.2 (Genetic Optimization for Ligand Docking) [11], run on Scientific LINUX 5.5 operating system. It uses a genetic algorithm and considers full ligand conformational flexibility and partial protein flexibility. The active center of the receptor was determined using substrate position in the crystal structure obtained from RCSB [9,10]. Four scoring functions of GOLD 5.2 (ChemScore, ChemPLP, GoldScore, and ASP) were used in order to determine the best algorithm for docking of this class of compounds [11-14]. The conformations of the compounds with the best values of the scoring functions were selected.

RESULTS AND DISCUSSION

Molecular docking was performed with the CB1 receptor (PDB id: 5TGZ) and the ligands from literature [8] (Table 1). The scoring functions embedded in GOLD 5.2 were used. The obtained results of the molecular docking are presented in the Table 2.

Table 2. CB1 receptor affinity and scoring functions values of the ligands.

Ligands	Affinity, CB_1	ASP score	Goldscore	ChemPLP	ChemScore
Anandamide	78 nM	20.28	49.82	56.92	20.43
N-Arachidonyl dopamine	-	21.62	55.64	64.8	22.76
2-Arachidonylglycerol	-	18.74	56.54	59.34	16.4
Δ-9-Tetrahydrocannabinol	10 nM	25.9	41.61	44.48	9.16
EGCG (Epigallocatechin gallate)	33.6 μM	22.27	39.30	53.88	25.82
Yangonin	0.72 μM	19.06	38.63	47.03	19.23
UR-144	150 nM	20.56	32.29	44.56	19.82

Correlations of data from molecular docking and affinity of the cannabinoid ligands were performed with GraphPad Prism 3.0 [18]. When the results were analysed we found correlation between the docking results (the values of all four scoring functions available in GOLD 5.2) and the values of affinity of cannabinoid ligands. The correlation between these data was assessed by the Pearson's correlation coefficient [18] (Table 3).The correlation coefficients for ASP and ChemScore scoring functions are with higher values, but for ASP scoring function it is positive and for ChemScore scoring function it is negative.When the correlation coefficient of Pearson is positive higher affinity corresponds to higher scoring function value, when the Pearson's correlation coefficient is negative higher affinity corresponds to lower scoring function value. Only the first correlation has biological meaning because the value of the scoring function shows how the ligand binds to the crystal structure of the CB1 receptor. As higher is that value the binding is better.

Shim *et al.* [15] proposed that there exists a hydrophobic binding pocket that interacts with the alkyl chain of the classical and non-classical cannabinoids. He showed that docking is more effective when the polar residue from the receptor sequence was chosen. In our case this residue is Asp366. All of the ligands bind near this residue mainly interacting with the nonpolar residues around but forming hidrogen bonds with their hydroxyl groups and Lys370 from the receptor sequence.

Table 3. Pearson's correlation coefficients for the crystal structure of CB1 and different scoring functions of GOLD 5.2.

Scoring functions	Values of Pearson's correlation coefficient
ASP score	Pearson R = 0,8815, P_{value}=0,0202
ChemPLP	Pearson R = -0,5056, P_{value} = 0,3063
ChemScore	Pearson R = -0,8313, P_{value} = 0,0403
GoldScore	Pearson R = -0,09325, P_{value} = 0,8605

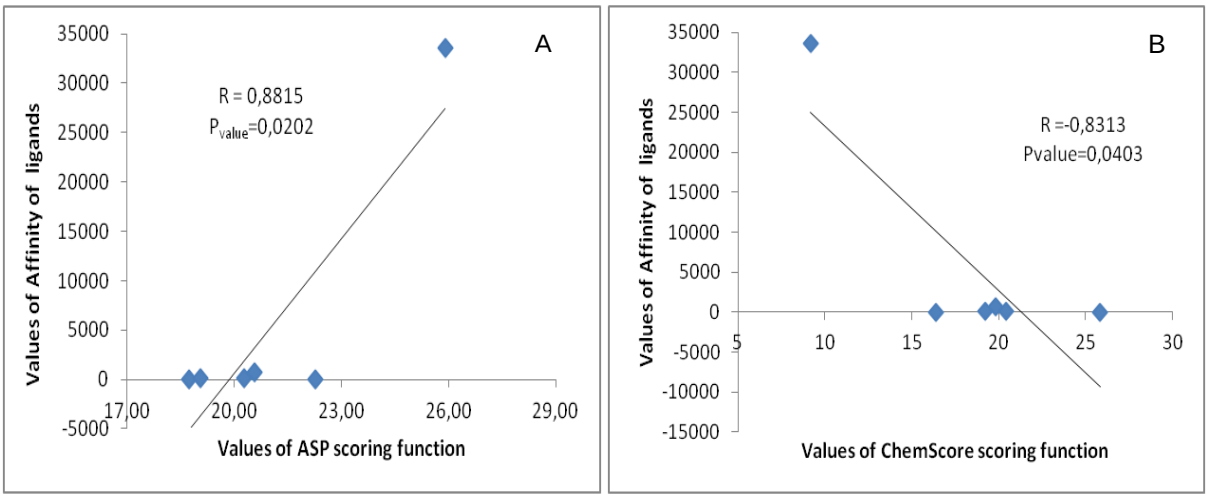


Fig. 1. Pearson's correlation between: A - the values of *affinity* of cannabinoid ligands and the values of ASP scoring function; B - the values of *affinity* of cannabinoid ligands of ChemSore scoring function.

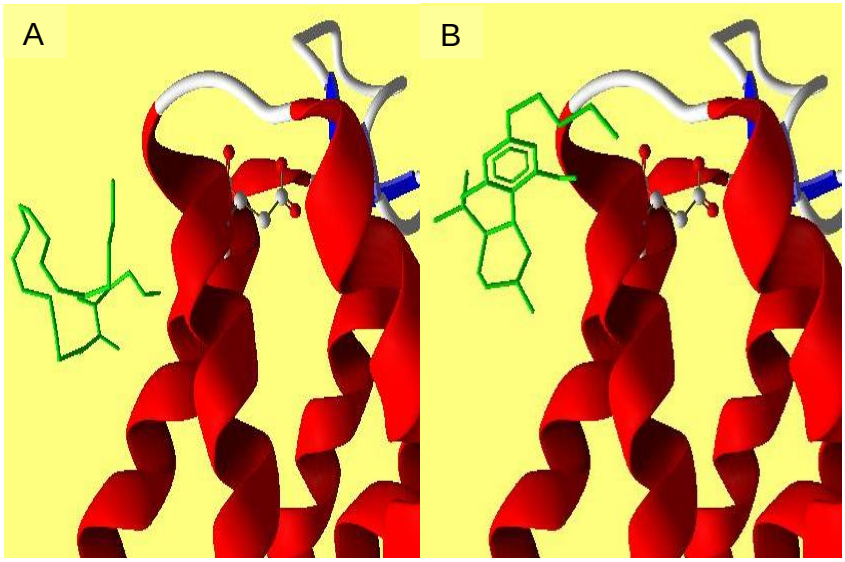


Fig. 2. Schematic diagram of the ligand-receptor complex between: A - CB1 receptor (PDBid:5tgz) and ligand anandamide; B - CB1 receptor (PDBid:5tgz) and ligand Δ-^{9}-tetrahydrocannabinol. The receptor is presented in ribbons and helixes. The ligands are presented in green. These diagrams were generated with the MMV.

Given a protein target – in our case model of the CB1 receptor, molecular docking with software GOLD 5.2 generates several probable ligand binding conformations at the active site - Asp366 around the receptor. The ASP scoring function from the program was used to rank the ligand conformations by evaluating the binding density of each of the probable complexes.

As a conclusion we found that the molecular docking between the cannabinoid ligands and the model of the CB1 with crystal structure should be performed using ASP scoring function of GOLD 5.2 as the correlations with the biological results are the best. These data indicate that the software GOLD 5.2 gives reliable results in the docking of cannabinoid ligands with the crystal structure of human cannabinoid receptor (PDBid:5tgz). For some work along these lines, see [25-31].

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