

# Computer modeling of Cannabinoid receptor type 1

Fatima Sapundzhi<sup>1,\*</sup>, Tatyana Dzimbova<sup>1,2</sup>, Nevena Pencheva<sup>1</sup> and Peter Milanov<sup>1,3</sup>

<sup>1</sup>South-West University "Neofit Rilski", Bulgaria, 2700 Blagoevgrad

<sup>2</sup>Institute of Molecular Biology, Bulgarian Academy of Sciences, Bulgaria, 1113 Sofia

<sup>3</sup>Institute of Mathematics and Informatics, Bulgarian Academy of Sciences, Bulgaria, 1113 Sofia

**Abstract.** Cannabinoid receptors are important class of receptors as they are involved in various physiological processes such as appetite, pain-sensation, mood, and memory. It is important to design receptor-selective ligands in order to treat a particular disorder. The aim of the present study is to model the structure of cannabinoid receptor CB1 and to perform docking between obtained models and known ligands. Two models of CBR1 were prepared with two different methods (Modeller of Chimera and MOE). They were used for docking with GOLD 5.2. It was established a high correlation between inhibitory constant  $K_i$  of CB1 cannabinoid ligands and the ChemScore scoring function of GOLD, which concerns both models. This suggests that the models of the CB1 receptors obtained could be used for docking studies and in further investigation and design of new potential, selective and active cannabinoids with the desired effects.

## 1 Introduction

Cannabinoid receptors (CBR) are class of transmembrane receptors part of G protein-coupled receptors (GPCRs). They are located mainly in the brain and are involved in various physiological processes such as appetite, pain-sensation, mood, and memory [1, 2].

CBR can be activated by three major groups of ligands, endocannabinoids (produced by the mammalian body), phytocannabinoids (produced by the cannabis plant) and synthetic cannabinoids.

Two subtypes of CBR are currently known, cannabinoid receptor type 1 (CB1) and cannabinoid receptor type 2 (CB2) [3, 4]. The CB1 receptor is expressed mainly in the brain, but also in the lungs, liver and kidneys. The CB2 receptor is expressed in the immune system and in hematopoietic cells. Begg et al. evidence suggests that there are novel cannabinoid receptors [5], which are expressed in endothelial cells and in the CNS.

The protein sequences of CB1 and CB2 receptors are about 44% similar [6, 7]. Cannabinoids bind reversibly and stereo-selectively to the CBR. The CB1 receptors are thought to be one of the most widely expressed GPCRs in the brain. They are also found in other parts of the body. For instance, in the liver, activation of the CB1 receptor is known to increase *de novo* lipogenesis [8].

It is very important to develop subtype selective cannabinoid ligands in order to treat different diseases, including obesity. Docking studies carried out with

various groups of cannabinoids and with cannabinoid receptors, would allow to establish the structure-activity relationships, to search for potent analogues and to understand drug-receptor interactions. But the crystal structures of the CBR are still not available in the data bases.

The aim of the present study is to build a model of CB1 receptor by homology modelling in order to use it in further research.

## 2 Methods

The amino acid sequence of the cannabinoid receptor was obtained from UniProtKB (P21554) [9]. 3D structure of the CB1 receptor was generated by using two different methods.

The first method consists of several steps that are held on different software. The structure of rhodopsin (PDB id: 2Z73, crystal structure of squid rhodopsin) was used as template [10]. The alignment of the two sequences was performed in CLUSTALW and the obtained alignment file was used in Chimera for generating 3D-structure of the receptor. Five structures were generated and the structure with the best GA341 was chosen and additionally analyzed with PROCHECK [11] and MolProbity [12].

The second method uses the software MOE (Molecular Operating Environment) in direct generation of the receptor structure [13]. The software MOE is a

\* Corresponding author: [sapundzhi@swu.bg](mailto:sapundzhi@swu.bg)

chemical computing and molecular modelling tool, which is a very widely used program in scientific applications. The steps included: searching for the template in the data base (the structure with the highest per cent of identity of the amino acid residues), modeling of the 3D structure of the desired receptor and checking the obtained model.

GOLD (Genetic Optimisation for Ligand Docking) 5.2 [14] was used for the molecular docking studies of the obtained two models of CB1 receptors by homology modelling with the ligands known from the literature, presented in Table 1 [15-18].

**Table 1.** The values of the inhibitory constant (Ki) of CB1 cannabinoid receptor ligands with known affinity<sub>2</sub> used in the docking studies.

Ligands	CB1 Affinity (Ki)
Anandamide	78 nM
N-Arachidonoyl dopamine	-
2-Arachidonoylglycerol	-
2-Arachidonoyl glyceryl ether	21 nM
Δ-9-Tetrahydrocannabinol	10 nM
EGCG (Epigallocatechin Gallate)	33.6 μM
Yangonin	0.72 μM
UR-144	150 nM

GOLD 5.2 uses generic algorithm and considers full ligand conformational flexibility and partial protein flexibility. All scoring functions available in the tool are used: GoldScore, ChemScore, ChemPLP (Piecewise Linear Potential), and ASP (Astex Statistical Potential) scoring functions, [19-23]. GoldScore fitness function is a molecular mechanics-like function (Eq.1):

$$GoldScore = S_{hb\_ext} + S_{vdw\_ext} + S_{hb\_int} + S_{vdw\_int} \quad (1)$$

where  $S_{hb\_ext}$  is protein-ligand hydrogen bond energy,  $S_{vdw\_ext}$  - protein-ligand van der Waals energy,  $S_{hb\_int}$  is intramolecular hydrogen bonds in the ligand and  $S_{vdw\_int}$  is intramolecular strain in the ligand [21].

$$ChemScore = \Delta G_{binding} + E_{clash} + E_{int} + E_{cov} \quad (2)$$

ChemScore scoring function takes count of hydrophobic-hydrophobic contact area, ligand flexibility, hydrogen bonding and metal interaction (Eq.2) [21], where  $\Delta G_{binding}$  is the free energy of binding of the ligand to the protein;  $E_{clash}$  is a protein-ligand clash-energy term,  $E_{int}$  - ligand-internal-energy term.

ChemPLP is the most effective scoring function for pose prediction in cognate protein-ligand complexes, out of the four available in GOLD [20] (Eq. 3):

$$f_{CHEMPLP} = f_{PLP} - (f_{chem-hb} + f_{chem-cho} + f_{chem-met}) \quad (3)$$

where  $f_{PLP}$  is the modelling of the steric complementarity between protein and ligand,  $f_{chem-hb}$  is a distance-dependent hydrogen bonding term,  $f_{chem-cho}$

is an angle-dependent hydrogen bonding term,  $f_{chem-met}$  is a distance-dependent metal bonding term.

ASP scoring function is an atom-atom distance potential derived from a database of protein-ligand complexes and can be compared to other knowledge-based scoring potentials [22] (Eq. 4):

$$ASP \text{ Fitness} = -C_s S(map) - c_{int} E_{int} - c_{clash} E_{clash} \quad (4)$$

where  $C_s$  is a scaling factor,  $S(map)$  - total statescore - all combinations of protein and ligand atoms,  $c_{int}$  - internal energy,  $c_{clash}$ -clash coefficients,  $E_{clash}$  and  $E_{int}$  are terms like in ChemPLP function.

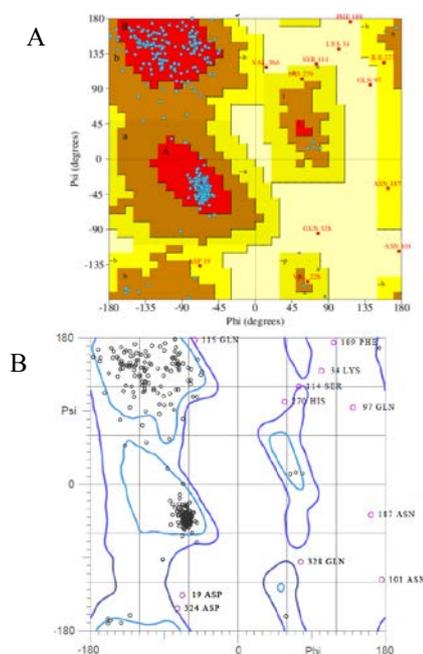
In order to find relationship between the affinity of cannabinoid ligands (Table 1) and the docking results for the obtained models of CB1 receptors by homology modelling, we tried to predict it with the help of Pearson's correlation, using GraphPad Prism 3.0 (<http://www.graphpad.com/scientific-software/prism>).

### 3 Results and discussion

By using the first method for generation of the 3D model of CB1 receptor, five models were obtained. The model with the best value of GA345 was chosen for further analysis.

Evaluations of 3D models were done for various levels of structural organization and on the base of *in vitro* studies. We generate 40 models using Modeller of Chimera. One of them with best GA345 (more than 0.9) was chosen for further evaluation.

The geometry of the obtained model, analyzed with PROCHECK and MolProbity, and the respective Ramachandran plots are presented in Figure 1.



**Fig. 1.** Ramachandran plots from PROCHECK (A) and MolProbity (B).

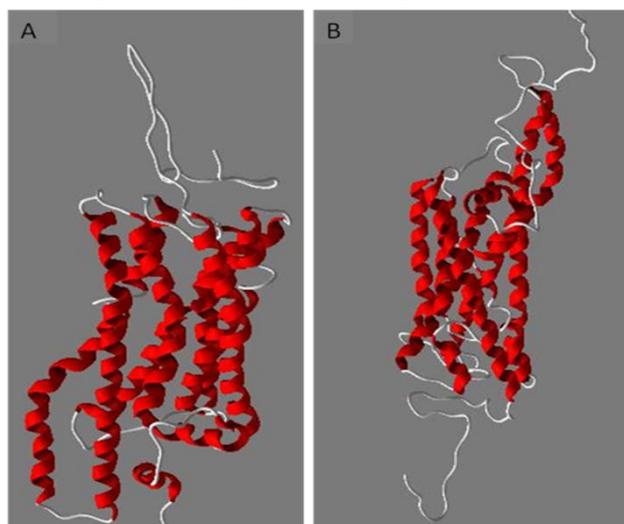
The results are good as the sum of the most favored and additional allowed regions is more than 90% - 97.3% in PROCHECK analysis [11] and 96.6% in MolProbity analysis [12].

The second model was obtained with the help of MOE software [13]. The program automatically found the structure similarity with the same template used in the first method (the crystal structure of squid rhodopsin (PDBid:2z73) [10]). The standard molecular mechanics AMBER 99 forcefield was used. The backbone fragments from a high-resolution structural database were collected and alternative side chain conformations for non-identical residues were assembled from an extensive rotamer library during data collection. During model building in software MOE [13], ten independent models were created based upon loop and side chain placements scored by a contact energy function (Figure 2).

	mol	name	RMSD to Mean	CA RMSD to Mean	Contact Energy
1		Model1 #1(1)	0.5321	0.4335	-170.5224
2		Model1 #2(1)	0.5999	0.4840	-171.1597
3		Model1 #3(1)	0.6230	0.4688	-175.4267
4		Model1 #4(1)	0.5001	0.4120	-170.9495
5		Model1 #5(1)	0.5971	0.5120	-167.9658
6		Model1 #6(1)	0.5601	0.4499	-166.6386
7		Model1 #7(1)	0.6687	0.5838	-161.2423
8		Model1 #8(1)	0.6448	0.5364	-172.5911
9		Model1 #9(1)	0.5660	0.4784	-168.1365
10		Model1 #10(1)	0.5322	0.4337	-168.3179

**Fig. 2.** Screenshot of database obtained after modelling by MOE.

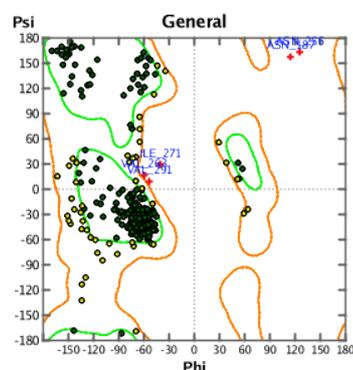
The final, refined model was loaded in MOE and Chimera after the calculation finished. Graphical representations of the obtained model of homology modelling of CB1 are presented in Figure 3.



**Fig. 3.** Graphical representation of the obtained model of homology modelling of CB1 by the software MOE (A) and the software Chimera (B).

After that, the stereochemistry of homology models for unusual or geometrically unreasonable features was examined. Our model has RMSD  $0.6230 < 3\text{\AA}$ , which means that the generated model is built correctly.

The model with the best contact energy was chosen (-175,4267) and it was validated directly in MOE. The stereochemical quality of the modeled proteins is assessed from Ramachandran validation score for favoured regions and allowed regions (Figure 4). In general, a score close to 100% implies good stereochemical quality of the models.



**Fig. 4.** Ramachandran Plot generated by MOE after optimization of the structure.

The molecular docking experiments with the obtained models of CB1 receptor by homology modelling in Chimera and in MOE and the ligands from the literature (Table 1) were carried out with software GOLD 5.2 and all four scoring functions embedded in the program: GoldScore, ChemScore, ASP and ChemPLP [19-23]. According to Shim et al. [24] there exists a hydrophobic binding pocket that interacts with the alkyl chain of the cannabinoids. The docking is effective when the polar residue from the receptor sequence was chosen - Asp366 and the investigated ligands bind near to it.

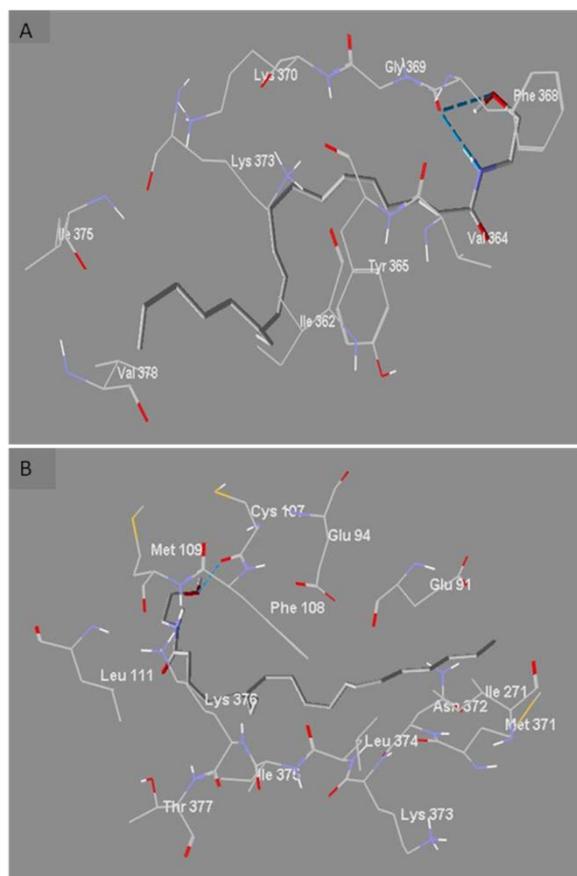
Correlations between the values of the respective GOLD functions, obtained for the models of CB1 receptor with Modeller of Chimera and MOE, and the affinity constant -  $K_i$  of the cannabinoid ligands, calculated with Pearson's coefficient, are presented in Table 2. These data show that the best correlation concerns the model of CB1 receptor obtained by MOE and the scoring function ChemScore, because the value of the Pearson's coefficient is highest and the relationship is negative. For the CB1 model, generated by Chimera and the same scoring function, the correlation coefficient was similar [25-28].

**Table 2.** The values of Pearson's coefficient for the correlation between GOLD scoring functions for models of the CB1 receptor (Chimera and MOE; homology modeling) and affinity constant  $K_i$  of the cannabinoid ligands.

GOLD Scoring Function	Pearson correlation coefficient - R	
	CB1 model with Chimera	CB1 model with MOE
ASP	0.9248	0.9594
ChemPLP	- 0.4851	- 0.0817

ChemScore	- 0.9491	- 0.9944
GoldScore	- 0.3001	- 0.0651

Thus, both models of the CB1 receptors, created by Chimera or MOE are suitable for docking studies, but using only ChemScore scoring function.



**Fig. 5.** Graphical representation of the docking between the cannabinoid ligand Anandamid and the ASP scoring function (A) and ChemScore scoring function (B).

In our previous studies it was generated a 3D model of Delta opioid receptor (DOR) by using homology modelling [27]. Newly generated model of DOR was used for *in silico* studies and it was compared with recently published data for crystal structure of DOR [28-31]. It was established that the most appropriate combination for analysis of investigated compounds and DOR is that between the model of DOR obtained by homology modelling and the ASP scoring function (available in GOLD 5.2) [31-34]. These "preferences" of the opioids to this scoring function and of the cannabinoids to the ChemScore function, found in this study, need further investigations.

In statistics the negative correlation is a relationship between two variables in which one variable increases as the other decreases, and vice versa. A negative correlation between the values for obtained models by homology modelling of the CB1 receptor (Chimera and MOE) for ChemScore scoring function and the values of affinity of the cannabinoid ligands was established. In our case, these obtained negative values

of Pearson's correlation coefficient have a biological meaning. As lower the value of the constant  $K_i$  is the biological effect is the stronger.

The presented work opens wide space for the design of novel cannabinod analogues, selective to the CB1 receptor and their assessment with the molecular docking studies. The obtained models of CB1 cannabinoid receptors allow the studying of the structure-activity relationship of synthetic, endogenous or plant-derived ligands that can act as agonists or antagonists to the CB1 receptor.

## Conclusions

In the present study there are generated two models of the CB1 receptor, obtained with the Modeller of Chimera and MOE. The models are suitable for docking studies, because it was established a high correlation between  $K_i$  of CB1 cannabinoid ligands and the ChemScore scoring function of GOLD, which concerns both models.

## Acknowledgements

This work is partially supported by the project of the Bulgarian National Science Fund, entitled: Bioinformatics research: protein folding, docking and prediction of biological activity, NSF I02/16, 12.12.14.

## References

1. A.Howlett. Prostaglandins & Other Lipid Mediators **68-69**, 619-631 (2002)
2. K. Mackie. Journal of Neuroendocrinology, **20** (1), 10-14 (2008)
3. L. Matsuda, et al. Nature **346**, 561 - 564 (1990)
4. C. Gérard, C. Mollereau, G. Vassart & Parmentier. Biochem J **279**, 129-134 (1991)
5. M. Begg, et al. Pharmacology & Therapeutics **106**, 133 - 145 (2005)
6. D. Latek, et al. J Mol Model **17**, 2353-2366 (2011)
7. S. Munro, K. Thomas, M.Abu-Shaar, Nature **365**, 61-65 (1993)
8. D. Osei-Hyiaman, et al. J Clin Invest. **115**, 1298-1305 (2005)
9. <http://www.uniprot.org/uniprot/P21554>
10. M. Murakami, T. Kouyama, Nature **453**, 363-367 (2008)
11. R. Laskowski, M. MacArthur, D. Moss, J. Thornton, J Appl Cryst **26**, 283-291 (1993)
12. V. Chen, W. Arendall, J. Headd, D. Keedy, R. Immormino, G. Kapral, L. Murray, J. Richardson, D. Richardson, Acta Crystallogr D Biol Crystallogr, **66**, 12-21 (2010)
13. <http://www.chemcomp.com/>
14. G. Jones, P. Willett, R. Glen, A. Leach, R. Taylor, J Mol Biol. **267**, 727-748 (1997)
15. A. Ligresti, R. Villano, M. Allarà, I. Ujváry, V. Di Marzo, Pharmacological Research, **66** (2), 163-169 (2012)

16. A. Makriyannis, H. Deng, "Cannabimimetic indole derivatives", granted 2001-06-07
17. J. Frost et al., *J. Med. Chem.* **53** (1), 295–315 (2010)
18. M. Aung, et al., *Drug Alcohol Depend.* **60** (2), 133–140 (2000)
19. GOLD, version 5.2 UserGuide, CCDC Software Ltd. Cambridge, UK (2010).
20. C. Baxter, C. Murray W, D. Clark, D. Westhead, M. Eldridge. *Proteins*, **33**, 367–382 (1998)
21. O. Korb, T. Stütze, T. Exner, *J. Chem. Inf. Mod.*, **49**, 84-96, (2009)
22. M. Verdonk, J. Cole, M. Hartshorn, C. Murray, R. Taylor, *Proteins*, **52**, 609-623, (2003)
23. R. Reinscheid, H. Nothacker, A. Bourson, A. Ardati, R. Henningsen, J. Bunzow, D. Grandy, H. Langen, F. Monsma, O. Civelli, *Science*, **270**, 792-794 (1995)
24. Joong-Youn Shim, A. Bertalovitz, D. Kendall. *Journal of biological chemistry*, **286** (38), 33422–33435 (2011)
25. F. Sapundzhi, T. Dzimbova, N. Pencheva, P. Milanov, *Proceedings of Biomath Communications Supplement*, 25-30 June 2017, Skukuza Camp, South Africa, **4**(1), (2017)
26. F. Sapundzhi, T. Dzimbova, N. Pencheva, P. Milanov, *Proceedings of Seventh International Conference of FMNS 2017*, 14-18 June 2017, Blagoevgrad, Bulgaria, **1**, (2017)
27. T. Dzimbova, F. Sapundzhi, N. Pencheva, P. Milanov, *Journal of Peptide Science*, **18** (S1) S84, P072, (2012)
28. F. Sapundzhi, T. Dzimbova, N. Pencheva, P. Milanov, *Journal of Peptide Science*, **20** (S1), S294, (2014)
29. T. Dzimbova, F. Sapundzhi, N. Pencheva, P. Milanov, *Int. J. Bioautomation*, **17**, 5-16 (2013)
30. F. Sapundzhi, T. Dzimbova, N. Pencheva, P. Milanov, *Jurnal of Bulgarian Chemical Communication*, **2**, 613-618 (2015).
31. F. Sapundzhi, T. Dzimbova, N. Pencheva, P. Milanov, *Der Pharma Chemica*, **8**, 118-124 (2016)
32. R. Mavrevski, *C. R. Acad. Bulg. Sci.*, **67**(10), 1345-1354 (2014)
33. R. Mavrevski, P. Milanov, B. Yurukov, *Proceedings of the Biomath Communications – BIOMATH2016*, **3**(1), 46 (2016)
34. T. Dzimbova, R. Mavrevski, N. Pencheva, T. Pajpanova, P. Milanov, *Bulgarian chemical communications*, **44**(3), 242-246 (2012)