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Modelling and optimization of ligand binding to CBR2

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In the last few years there has been a growing interest in the modelling and optimization of the ligand binding to cannabinoid receptor type 2, named CB2. It is G protein coupled receptor which is predominately expressed in the immune system. The article represents the structure-activity relationship between the model of the human CB2 receptor with crystal structure and a series of cannabinoid ligands. Analysis of ligand binding to the receptor provides important insight into the activation mechanism of CB2. The findings suggest that this could be useful for rational drug design toward precise modulation of the endocannabinoid system.

Keywords: computer modelling, optimization, scoring functions CBR2, docking, ligand-receptor interactions.

INTRODUCTION

The endocannabinoid system consists of endogenous cannabinoids (endocannabinoids), cannabinoid receptors (primarily CB1 and CB2), and the enzymes that synthesize and degrade endocannabinoids. CB2 receptors have been the subject of considerable attention, primarily due to their promising therapeutic potential for treating various pathologies while avoiding the adverse psychotropic effects that can accompany CB1 receptor–based therapies. For example, agonists targeting CB2 receptors have been proposed as therapies for the treatment or management of a range of painful conditions, including acute pain, chronic inflammatory pain, and neuropathic pain [1]. They may also be helpful in treating diseases that have a neuroinflammatory or neurodegenerative component, such as multiple sclerosis [2-4], amyotrophic lateral sclerosis [5, 6], Huntington's disease [7], and stroke [8, 9]. CB2 agonists have also been proposed as therapeutics in peripheral disorders that involve inflammation, including atherosclerosis [10] inflammatory bowel diseases [11, 12], ischemia/reperfusion injury [13] renal fibrosis [14], and liver cirrhosis [11, 15, 16]. Both epidemiologic and preclinical data suggest that activation of CB2 receptors may be protective in osteoporosis [17]. Finally, CB2 agonists have shown efficacy in preclinical cancer models [11, 18, 19].

The development of the CB2 receptor as a therapeutic target has gained significant momentum over the past decade due to the identification of CB2-specific synthetic and natural product ligands, a better understanding of the range of physiologic processes mediated by CB2 receptors, the regulation of CB2 receptors, and promising preclinical studies. However, the publicly available clinical data have thus far been disheartening. One reason for this may be discrepancies in pain mechanisms between the preclinical models, in which CB2 agents show efficacy, and the patients enrolled in clinical trials. Thus, efforts to examine the clinical efficacy of CB2 agonists in (neuro)inflammatory conditions and neuropathic pain syndromes (e.g., chemotherapy or diabetic) may be more productive. A second potential reason for the lack of translation is that CB2 agonists show very strong functional selectivity, and this functional selectivity may significantly affect agonist efficacy across species and types of pain. With the availability of increasingly precise and selective pharmacological, genetic, preclinical, and clinical tools and a more complete understanding of the importance of CB2 agonist functional selectivity, CB2 receptors still appear to be promising targets for drug development, both for chronic pain and other indications.

The aim of the present study is to find some dependencies between compound structure and its affinity to the CB2 receptor in order to design more selective and potent CB2 selective agonists.

METHODS

In the current study 24 cannabinoid ligands known from literature [20-22] were used. Preparation of the ligands for docking experiments was performed by the software Avogadro (https://avogadro.cc/).

A model of CB2 receptor with crystal structure (RCSB PDBid: 2hff) and ligand pVal113 important for ligand recognition were used for the molecular docking procedure [23, 24].

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Docking experiments were performed by the software GOLD 5.2 and all scoring functions: GoldScore, ChemScore, ChemPLP, ASP [25-27]. The best results for the current investigation were obtained by the empirically based scoring function, named *ChemScore,* which estimates the total free energy change that occurs on ligands binding to the receptor (Eq.1):

$$\Delta G_{binding} = \Delta G_0 + \Delta G_{hbond} S_{hbond} + \Delta G_{metal} S_{metal} + \Delta G_{lipo} S_{lipo} + \Delta G_{rot} H_{rot} \quad (1)$$

S_{hbond} - score for hydrogen bonding;

S_{metal} - score for acceptor-metal bonding;

S_{lipo} - lipophilic interactions;

H_{rot} - loss of conformational entropy of the ligand upon binding to the protein;

ΔG - binding energy.

The binding energy between the ligand and the receptor is calculated using the MolDock scoring functions *Molegro Molecular Docker* [28] (Eq. 2):

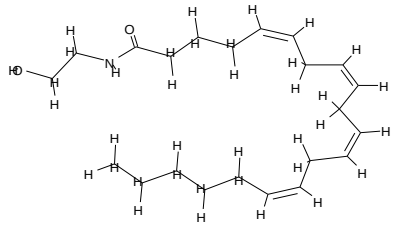
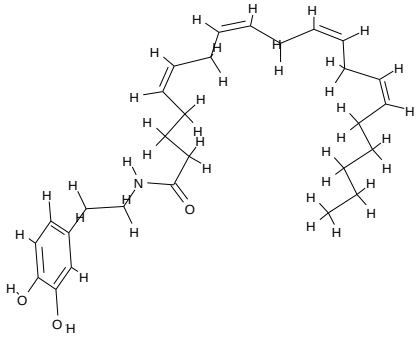
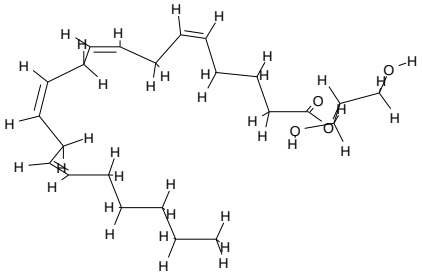
$$E_{score} = E_{inter} + E_{intra} \quad (2)$$

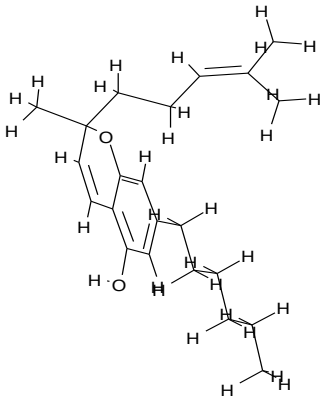
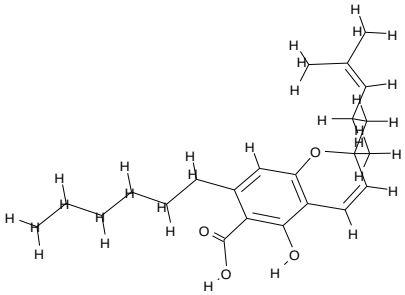
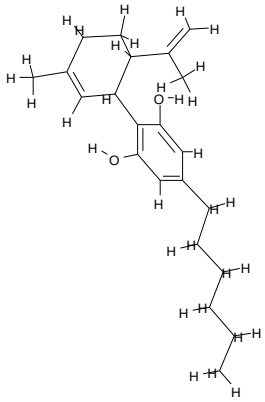
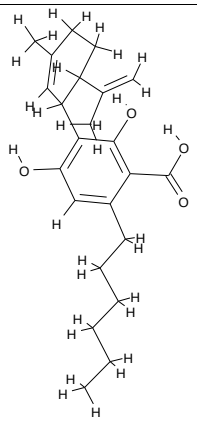
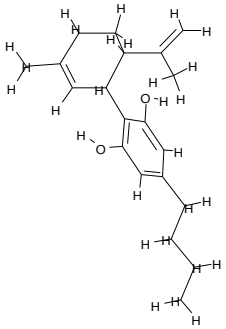
where: E_{inter} - potential energy of the ligand-protein interaction; E_{intra} - internal energy of the ligand. This software was also used to optimize the structures of the ligands in order to obtain reliable molecular geometries.

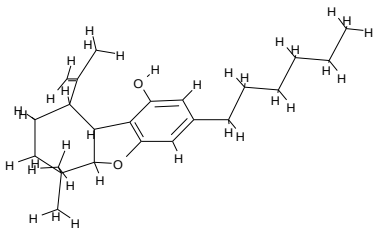
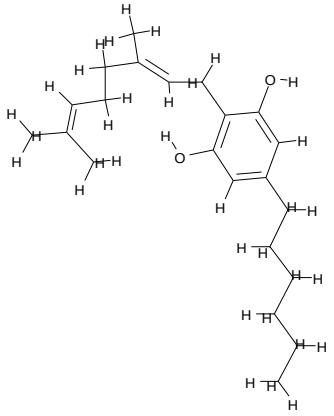
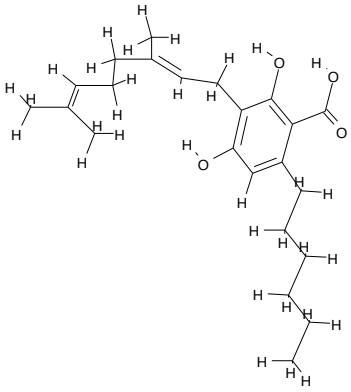
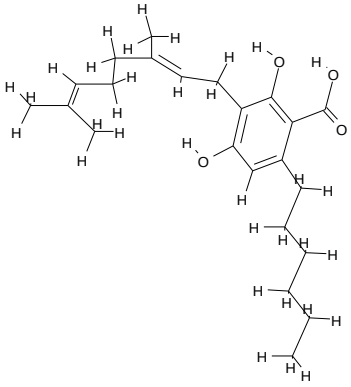
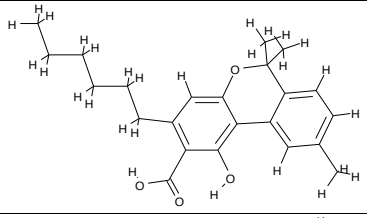
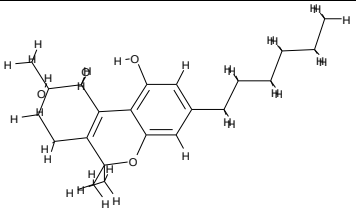
RESULTS AND DISCUSSION

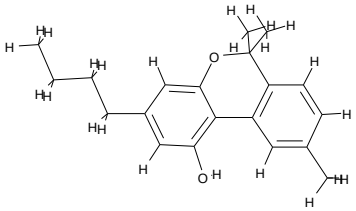
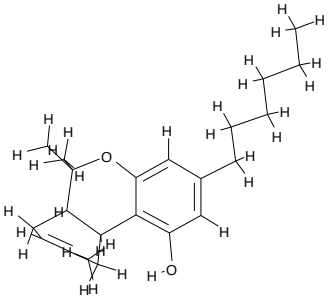
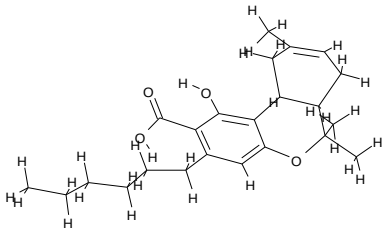
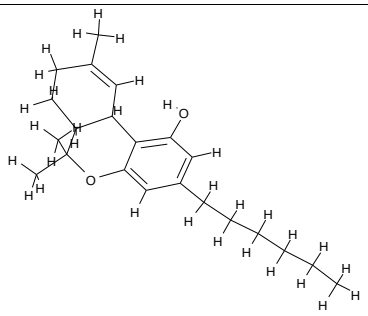
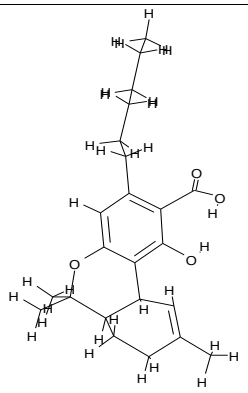
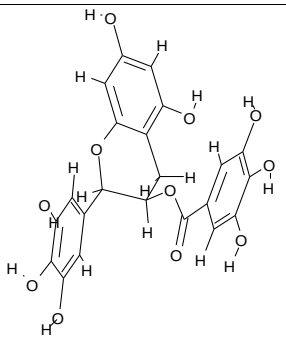
Docking was performed using the crystal structure of CB2 receptor obtained from RCSB (PDBid:2hff) [23, 24]. According to Lee *et al.* [24] the most important residue in the receptor sequence is Val113. As the residue is hydrophobic, all preferred interactions in the binding site of the receptor would be hydrophobic. All ligands of CB receptors are hydrophobic compounds, most of them with long carbon chains. Investigated ligands had different structures (Table 1) [20-22]. Residue contribution towards ligand binding was computed using the *MolDock* scoring function (Eq. 2) [28].

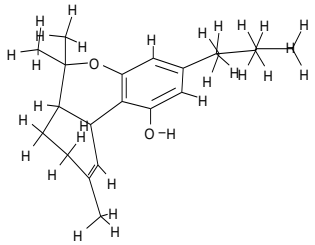
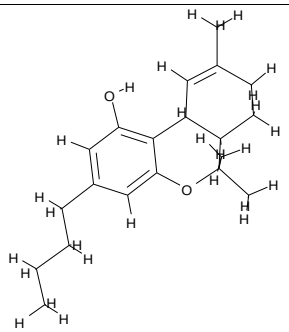
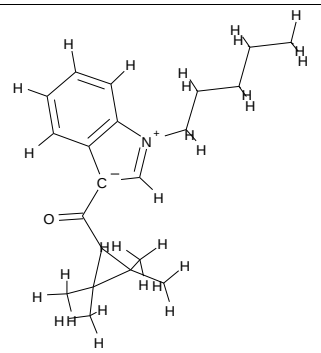
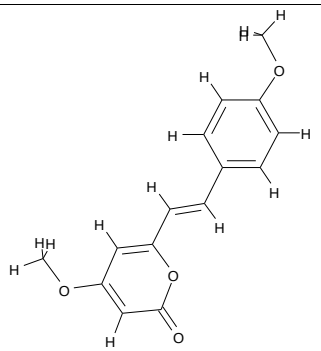
Table 1. Structures of ligands.

Ligands	Structures	ChemScore	Total energy
Anandamide		23.71	-76.23
Arachidonyl dopamine		29.24	-108.85
Arachidonylglycerol		25.09	-80.91

Cannabichromene (CBC)		25.43	-65.30
Cannabichromene acid (CBCA)		20.67	-58.91
Cannabidiol (CBD)		21.50	-71.39
Cannabidiolic acid (CBDA)		18.02	-69.32
Cannabidivarin (CBDV)		19.89	-68.17

Cannabielsoin (CBE)		23.66	-75.50
Cannabigerol (CBG)		22.91	-78.23
Cannabigerolic acid (CBGA)		21.39	-75.14
Cannabicyclol (CBL)		24.79	-78.90
Cannabinolic acid (CBNA)		24.08	-79.41
Cannabitriol (CBT)		22.74	-51.02

Cannabivarin (CBV)		23.58	-58.37
Δ^8- Tetrahydrocannabinol (Δ^8-THC)		23.41	-63.09
Δ^8- Tetrahydrocannabinolic acid (Δ^8-THCA)		23.41	-70.73
Δ^9- Tetrahydrocannabinol (Δ^9-THC)		23.86	-58.36
Δ^9- Tetrahydrocannabinolic acid (Δ^9-THCA)		22.97	-54.98
Epigallocatechin gallate		13.43	-30.29

Tetrahydrocannabinol (THC)		22.08	-56.31
Δ^9-Tetrahydrocannabivarin (Δ^9-THCV)		21.81	-60.21
UR-144 (synthetic cannabinoid)		23.28	-63.05
Yangonin		20.14	-45.21

The study was conducted with known cannabinoids and phytocanabinoids from the literature [20, 21] in order to find the most appropriate assessment function to give satisfactory results in terms of biological activity and binding to the crystal structure of the CB2 receptor. The ChemScore function makes it possible to assess the binding of large molecules of ligands to the corresponding receptor.

The investigated compounds have relatively large molecules which are of considerable hydrophobicity. They have little flexibility of the molecule because they have cyclic structures and, in some cases, conjugated double bond systems.

As a result, the ligands occupy a limited number of spatial conformations and this reduces the possibility of optimizing the structure at the receptor binding site. Using the ChemScore function in the software GOLD 5.2, this can be evaluated and the obtained results correlate well with the values of biological activity.

The obtained values of the other three functions in GOLD 5.2 - GoldScore, ChemPLP, ASP cannot be used to assess the binding of large, space-limited molecules.

With the exception of anandamide, arachidonyl dopamine and arachidonylglycerol, all studied compounds have at least one cycle in their structure.

As can be seen from the results in Table 1, epigallocatechin gallate has the smallest value of the ChemScore function and accordingly the

greatest total energy of the ligand-receptor complex, i.e. it is the least associated with CB2 receptor. Previous studies have shown that this compound binds very little with the CB2 receptor (with an inhibitory constant greater than 50 μM) [29]. The compound has three benzene nuclei, but also a large number of hydroxyl groups which make the molecule more hydrophilic. This fact does not contribute to better binding of the compound to the CB2 receptor since the binding condition is the formation of hydrophobic interactions. In our previous studies with cannabinoid receptors and cannabinoid ligands we obtained the best results for ChemScore function from docking in GOLD 5.2 for modelling the structure-biological activities [30-39].

CBDA and CBDV also have low values of the optimization functions, 18.02 and 19.89, respectively. CBDA is an acid since it has a carboxyl group in its molecule which again leads to greater hydrophilicity; in the CBDV molecule the hydrophilicity is due to the phenolic hydroxyl groups.

The highest value of the assessment ChemScore function has arachidonyl dopamine. Its structure contains a hydrocarbon chain that enables the molecule to have sufficiently large hydrophobicity on the one hand, and on the other hand, due to the flexibility, to occupy the most suitable spatial form in the receptor binding site.

The test compounds are not selective for CB1 and CB2 receptors [34]. A key concept to keep in mind when evaluating experiments conducted with CB2 ligands is that many of the commonly used CB2 ligands are only relatively selective with regard to CB1. This is because most of the commonly encountered CB2 ligands were evolved from molecules that have appreciable affinity for CB1 receptors. Therefore, the concentrations of CB2-preferring agonists that are commonly encountered in the literature (low micromolar) can result in significant occupancy of CB1 receptors, with subsequent signalling. Similarly, CB2-preferring antagonists at micromolar concentrations can substantially antagonize CB1-mediated responses [35]. Therefore, such a study on the ability of docking to predict receptor binding will allow for the design of CB2 selective compounds with a desired effect: both agonists and antagonists.

CONCLUSION

As a result of the research we performed docking experiments with GOLD 5.2 and all optimization functions in the program, and it was found that the ChemScore optimization function produces the best results with respect to ligand binding with the crystal structure of the CB2 receptor. These results are the starting point in the design of new selective ligands with desired activity against the CB2 receptor.

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