Cannabinoid 1 Receptor Interaction: Distinguishing an Agonist vs Antagonist

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Abstract:

Cannabinoids have shown the capacity and ability to treat numerous diseases and ailments such as pain[1], grand mal epileptic seizures[2], anxiety[3], and migraines[4]. Cannabinoids interact with known cannabinoid receptor sites labeled cannabinoid 1 (CB1) receptor and cannabinoid 2 (CB2 receptor)[5]. These cannabinoids can function as either an agonist, inverse agonist, or antagonist. Although their function is known the reason why cannabinoids bind as either an agonist or antagonist is not. The goal of this research is to analyze how agonist binding cannabinoids and antagonist binding cannabinoids interact with the CB1 receptor site, which amino acid residues they share, and which amino acid residues differ. This information will help us determine why cannabinoids induce different effects on their consumer and how we may examine the key residue interaction to determine how to construct better cannabinoids and determine how an unknown cannabinoid will function.

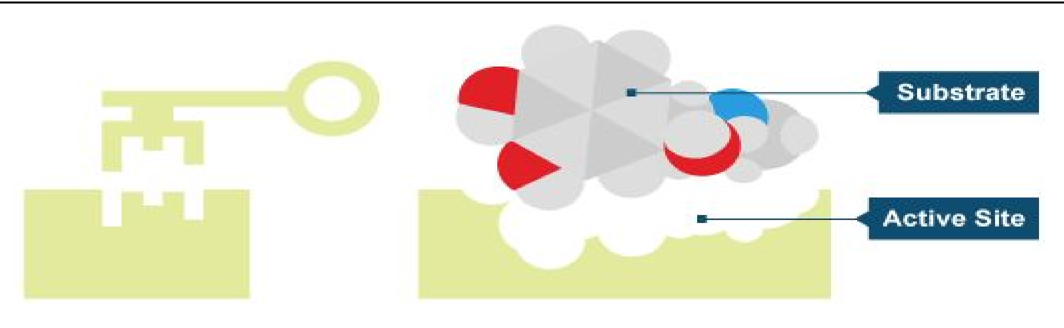
Introduction:

This research cannot be fully understood without a basic background on Bioinformatics and cannabinoids themselves. Bioinformatics is a discipline of science that uses biological data with techniques for information storage, distribution, and analysis to support multiple areas of scientific research, including biomedicine[6]. This field helps us better people better understand all the information on biological molecules and, for this research purpose, how they interact with protein receptors in the brain. The tools used are a combination of biology, technology, computer science, and mathematics in a specific way that allows the user to better understand biological information.

Several Bioinformatics tools were used to conduct this research. The first tool utilized was the Protein Data Bank (PDB), to extract the 3D crystal structures of the target protein (CB1 receptor) and its crystalised molecule being studied. The second tool used is the University of California, San Francisco (UCSF) Chimera program, which is a software program used to view 3D macromolecular structures. This research uses UCSF Chimera to view molecules inside the protein pocket to study their molecular interactions within the receptor. Once those were attained Middlesex County College provided the ChemDraw software that was utilized to create small molecules in 2D and 3D. Finally this research used online websites, run by the Swiss Institute of Bioinformatics, called SwissTargetPrediction and SwissDock. These tools allowed us to analyze the binding characteristics and interactions of the small designed molecules. These molecules will later be discussed in detail.

The discovery of a new drug is neither cheap nor can be accomplished in a small span of time. Pharmaceutical companies in today's world follow a series of steps in order to bring a drug to market through this Drug Discovery Process. Firsty, the must be identify the protein they wish to target. This is key for researchers because they must know exactly which protein in the body is they wish to transport a drug to. Upon succession researchers move on to the Lead Discovery phase with the molecules identified to have the best interaction with the target protein. Afterwards begins the Lead Optimization/ Medical Chemistry phase. In this phase researchers are constantly modifying and adjusting the most forefront molecules with the intent to produce better results in binding abilities through these changes. At this step chemists will also synthesize the molecule based on the correct changes. Upon succession, chemists will then proceed to the cell-free binding assay, otherwise known as In Vitro studies. This is where the molecules are tested in binding assays outside and inside cells. Once a compound shows reasonable activity in cell-free and cell-based, they will be placed in In Vivo studies. This is the phase where the molecule is administered to be tested on small animals such as rodents and amphibians. Upon successful results, the next step would be Pre-Clinical trials. Here, a wide range of testing is done to determine the safe dose for a first-in man study. This research comprises of the Lead Optimization phase where the created safe molecules are being attempted to have accurate changes made to them to produce better results in the interaction abilities with the target protein.

Utilizing the Bioinformatic method, molecular design is conducted through 3D Structure Based Design procedure. Through using this approach, it is understandable how exactly small molecules will interact with the target protein being the CB1 receptor. This method provides chemists the ability to make accurate changes to the ligand because Bioinformatics tools provide for a better understanding of the ligand-receptor interactions. This is often referred to as they "lock and Key" method. Once a "lock" has been identified, it is fairly easy to design a "key" to fit that lock. 3D drug design is similar to this. A protein's active site is the "lock" and a small molecule that is the inhibitor is the "key" that chemists design to fit that lock (Figure 1).

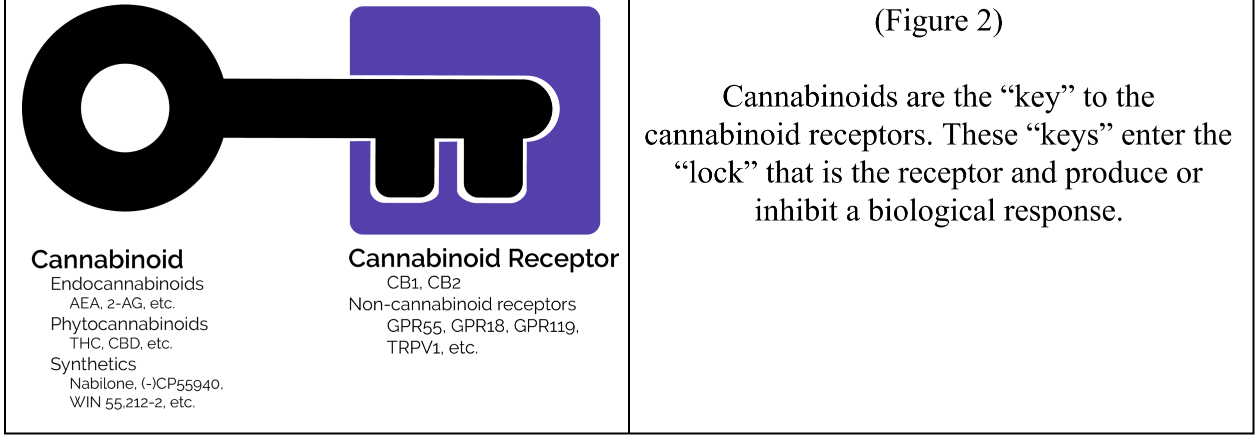


(Figure 1)

3D Drug Design is similar to a "lock and key". The protein active site can be thought of as as "lock" and the substrate or molecule for the protein is the "key" for that lock.

2D structure-activity relationship (SAR) approach is the traditional route for big pharmaceutical and drug companies today. This approach requires thousands of molecules to be synthesized and analyzed which requires a lot of time and money. This method lacks the information about how the molecule that was synthesized interacts with the target protein. 3D Structure Based Drug Design allows for this information to be known and is what gives it an edge. It is more efficient over SAR saving labor, time, and money by providing the necessary information for chemists and researchers about how exactly the molecule interacts with the protein. For this experiment, we were able to find the structure of our target protein, the Cannabinoid 1 Receptor using the Protein Data Bank. This will help us thoroughly understand the nature of the active pocket site and create a small molecule that will interact with it. This interaction will help us determine what amino acid residues the molecules make contact with. Based off of that we may begin to determine how to build compounds that target these specific sites to generate specific effects.

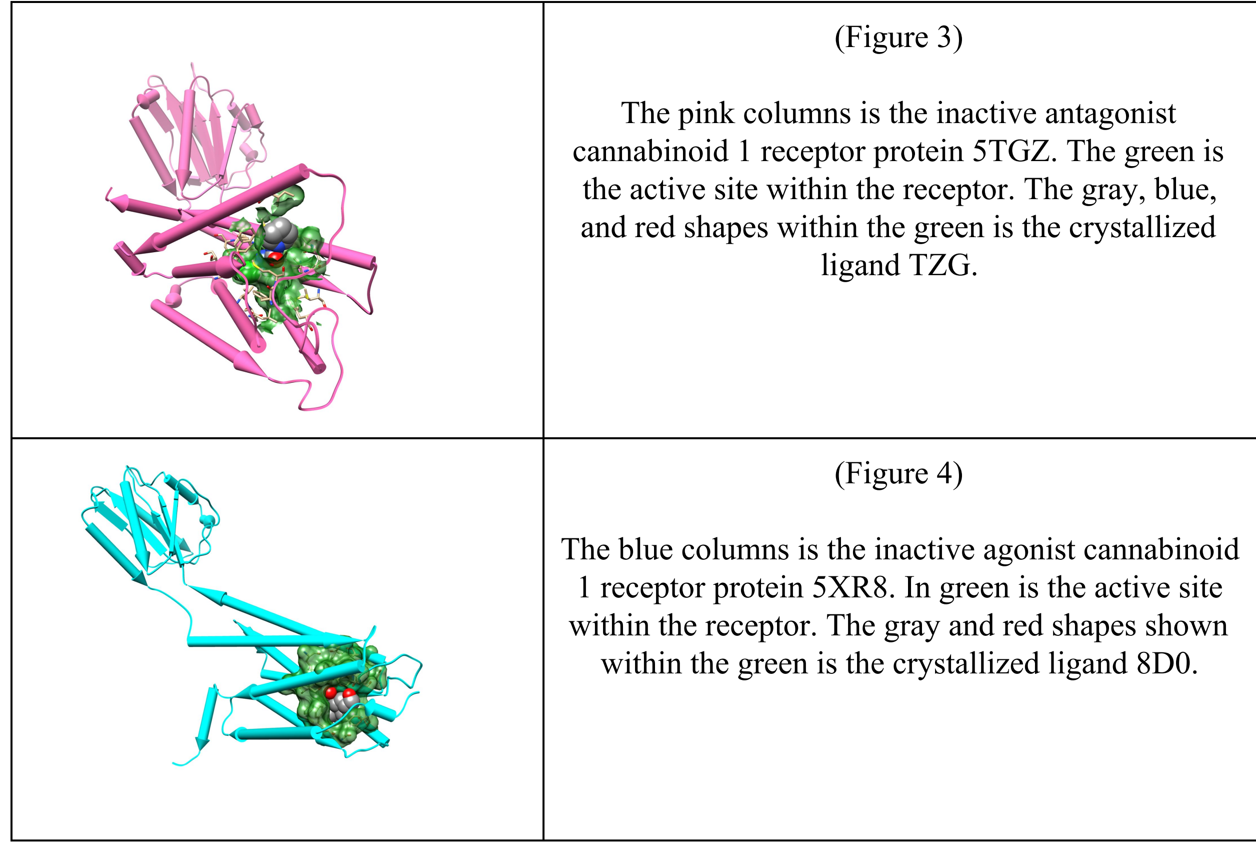
The Cannabinoid 1 Receptor, or CB1 for short, is a porous cell membrane receptor located throughout the central nervous system primarily in brain and neural tissue[7]. The CB1 receptor is responsible for sleep[8], appetite[9], mood[10], and body temperature[11]. It is part of a much larger system called the endocannabinoid system, which is a group of lipids and receptors that assist in multiple body functions and maintenance of homeostasis[12]. There are two types of cannabinoid receptors that comprise of the endocannabinoid system however. They CB1, and CB2 which is located on immune related tissues and on peripheral organs[7]. These cannabinoid receptors interact specifically with what are called cannabinoids, which is what the focus of this research is based around. A cannabinoid is a molecule that interacts specifically with cannabinoid receptors and are most commonly associated with the active components of the cannabis plant[13]. These cannabinoids function as either an agonist, inverse agonist, or antagonist. Cannabinoids follow the same "lock and key" method as described before. The "lock" being the cannabinoid receptor, and the "key" being the cannabinoid (Figure 2).



(Figure 2)

Cannabinoids are the "key" to the cannabinoid receptors. These "keys" enter the "lock" that is the receptor and produce or inhibit a biological response.

We will be inserting these cannabinoids in two different inactive CB1 protein sites. The first is the inactive antagonist site 5TGZ (Figure 3), the second is the inactive agonist site 5XR8 (Figure 4). These two sites were crystallized by Dr. Raymond Stevens, 5TGZ in 2016[14] and 5XR8 in 2017[15]. The CB1 receptor can be thought of as a "boot". Once a molecule enters the receptor it places itself in the active site which is the "toe" of the boot. Although it is the same CB1 receptor protein and appears to look similar these sites are slightly different. When the two are superimposed, obvious differences are observed in the size of the "toe" and the confirmation of the "calf" of the boot (Figure 5). This means that different functioning cannabinoids will induce different effects.

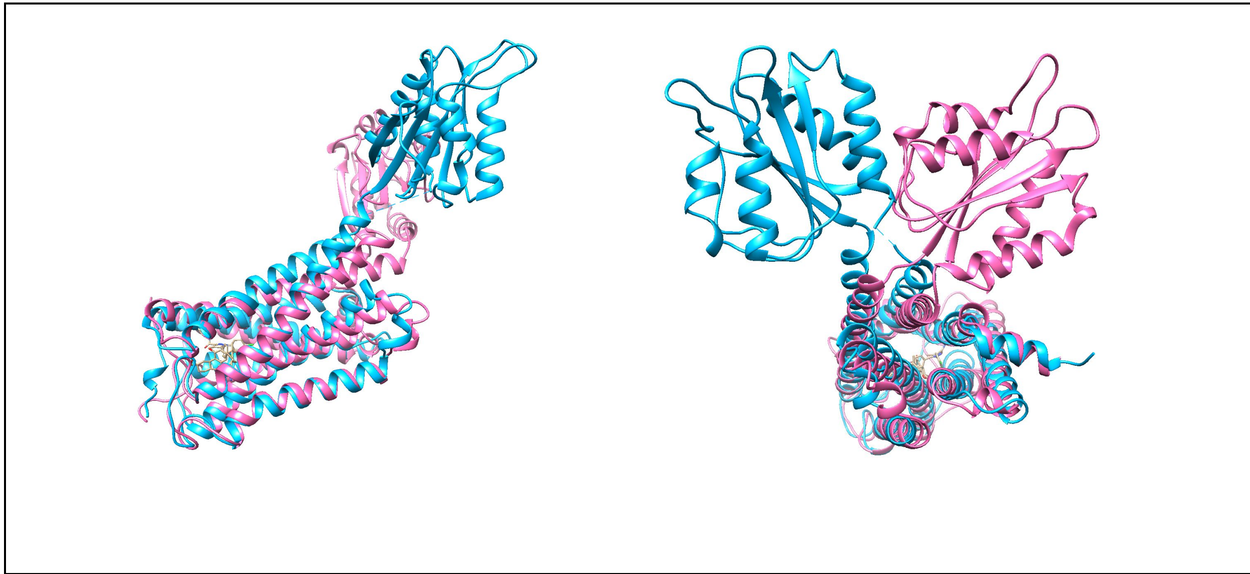


(Figure 3)

The pink columns is the inactive antagonist cannabinoid 1 receptor protein 5TGZ. The green is the active site within the receptor. The gray, blue, and red shapes within the green is the crystallized ligand TZG.

(Figure 4)

The blue columns is the inactive agonist cannabinoid 1 receptor protein 5XR8. In green is the active site within the receptor. The gray and red shapes shown within the green is the crystallized ligand 8D0.



(Figure 5)

5TGZ (in pink) and 5XR8 (in blue) superimposed. Obvious difference occur in the “calf” part of the CB1 receptor. Other differences is slight variation of the “bridge” of the “foot” part of the CB1 receptor.

Using a series of bioinformatics tools we will examine and replicate the docking of antagonist binding and agonist binding cannabinoids. Since the function of the cannabinoids are known we will insert them into their proper functional sites. Antagonist binding cannabinoids will be placed in the inactive antagonist site 5TGZ. Agonist binding cannabinoids will be placed into the inactive agonist site 5XR8. We will use the known crystallized cannabinoid within the receptor and the residues it interacts with and compare it to the unknown cannabinoids. The chart below describes each cannabinoid, the way it functions, the site it will be placed in, and what the known medical benefits are (Figure 6).

Cannabinoid	Function	Inactive Docking Site	Known Medical Benefits/ Effects
Crystal ligand ZDG; AM6358	Antagonist	5TGZ	Unknown
Rimonabant	Inverse Agonist / Antagonist	5TGZ	Obesity, appetite suppressant[16].
Taranabant	Inverse Agonist/ Antagonist	5TGZ	Obesity, appetite suppressant[17].
Cannabidiol (CBD)	Antagonist	5TGZ	Anxiety[3], pain[1], seizures[2], spasms[18], obesity[19], cancer[20].

Crystal ligand 8D0; AM841	**Agonist**	**5XR8**	**Unknown**
Anandamide (AEA)	**Agonist**	**5XR8**	Regulation of normal anxiety and pain[21].
Cannabinol (CBN)	**Agonist**	**5XR8**	Insomnia[22], cancer[23].
Tetrahydrocannabinol (THC)	**Agonist**	**5XR8**	Nausea[24], stress[25], Alzheimer's disease[26], and cancer[27].

(Figure 6)

Once we have docked the cannabinoids, we will generate a pocket that is formed within the receptor site for each. Each cannabinoid will have different conformations they hold within the site. We will use the bioinformatics program SwissDocking to view these confirmations. The program will show not only the conformations but which ones are energetically favorable. That favorability is unknown, and will be represented in ΔG kilocalories per mole (kcal/mol). That will then be compared to the known IC50 value which represents the amount needed in order to reduce 50% of the activity within the protein (Figure 7).

Lig short name	Lig Type	PDBID	Size of pocket (Å)	Center of pocket (x,y,z)	IC50 Value (µM)	Δ G Value (kcal/mol)
ZDG	Antagonist	5TGZ	5x5x5	x=(43.502) y=(27.787) z=(318.810)	Unknown	?
Rimonabant	Antagonist	5TGZ	5x5x5	x=(43.502) y=(27.787) z=(318.810)	Unkown	?
Taranabant	Antagonist	5TGZ	5x5x5	x=(43.502) y=(27.787) z=(318.810)	0.00030	?
CBD	Antagonist	5TGZ	5x5x5	x=(43.502) y=(27.787) z=(318.810)	2.10	?
8D0	Agonist	5XR8	5x5x5	x=(-42.646) y=(-156.676) z=(306.315)	Unknown	?
Anandamide	Agonist	5XR8	5x5x5	x=(-42.646) y=(-156.676) z=(306.315)	2.10	?
THC	Agonist	5XR8	5x5x5	x=(-42.646) y=(-156.676) z=(306.315)	2.70	?
CBN	Agonist	5XR8	5x5x5	x=(-42.646) y=(-156.676) z=(306.315)	2.10	?

(Figure 7)

The chart representing where the data will be kept and tracked. The site, size (measured in angstrom), center (exact coordinates of placing molecule), IC50 value and unknown ΔG value.

Upon such generation we shall examine the amino acid residues that each cannabinoid interacts with within the pocket. Then the residue sheets will be compiled, compared, and contrasted. This information will then determine which amino acid residues agonists and antagonists share and which residues are different (Figure 8). This differences will determine why an antagonist or agonist cannabinoid functions the way it does. We may then use this information to develop synthetic cannabinoids to taret these specific residues to treat a variety of medical ailments.

Antagonist	Agonist
Share	Share
Share	Share
Share	Share
Unique	Unique
Unique	Unique
Unique	Unique

(Figure 8)
The chart used to keep track of the amino acid residues. In green will be the residues that both antagonists and agonists have in common. In pink will be the unique residues for antagonists. In blue will be the unique residues for agonist.

Protocol:

Tools:

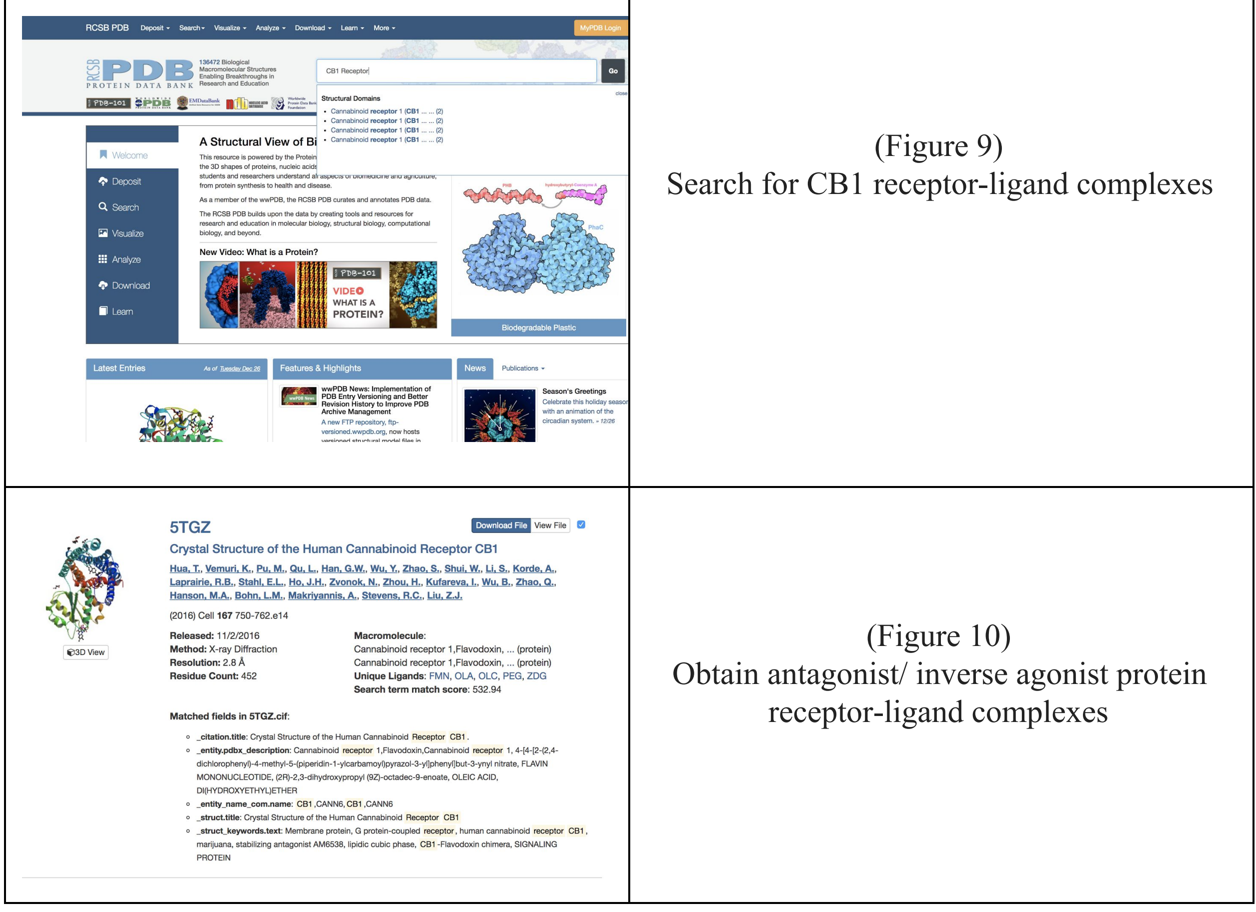
This experiment will be conducted using a series of bioinformatics tools. These tools will help us attain the best confirmation of each cannabinoid. Based off the confirmation then we may determine the amino acid residues they contact. The following tools used are:

1. RCSB PRB (www.rcsb.org): to obtain and download 3D structures of CB1 receptor-ligand complexes.
2. Chimera: to view the 3D structures of the CB1 receptor-ligand complexes. This will be used to for two main reasons in this experiment. To edit the receptor-ligand complexes for preparation of cannabinoids for docking and to view our docking results from the SwissDock Program.
3. ChemDraw 2D: to draw the cannabinoids to import to ChemBio 3D to generate a 3D structure of the ligand.
4. ChemBio 3D: to minimize the structure of the cannabinoid and to create its Simplified Molecular Input Line Entry Specification (SMILES) string. The SMILES string is a chemical description of a given structure that helps it become recognized by computers. This chain may be imported by many molecule editors for conversion into two-dimensional drawings and three-dimensional models of the molecule.
5. SwissTargetPrediction: An online tool that predicts the targets of small molecules through a combination of two-dimensional and three dimensional similarity measures. This program compares the inserted molecule to a library of thousands of compounds active on selected targets from a variety of different species.
6. SwissDock: An online tool that predicts the molecular interactions that may occur between a target protein and a small molecule. This tool will list the different predictions based off its confirmations through its ΔG measured in kilocalorie per mole (kcal/mol). This will tell us the energetic favorability of the cannabinoid we input for docking.

Procedure:

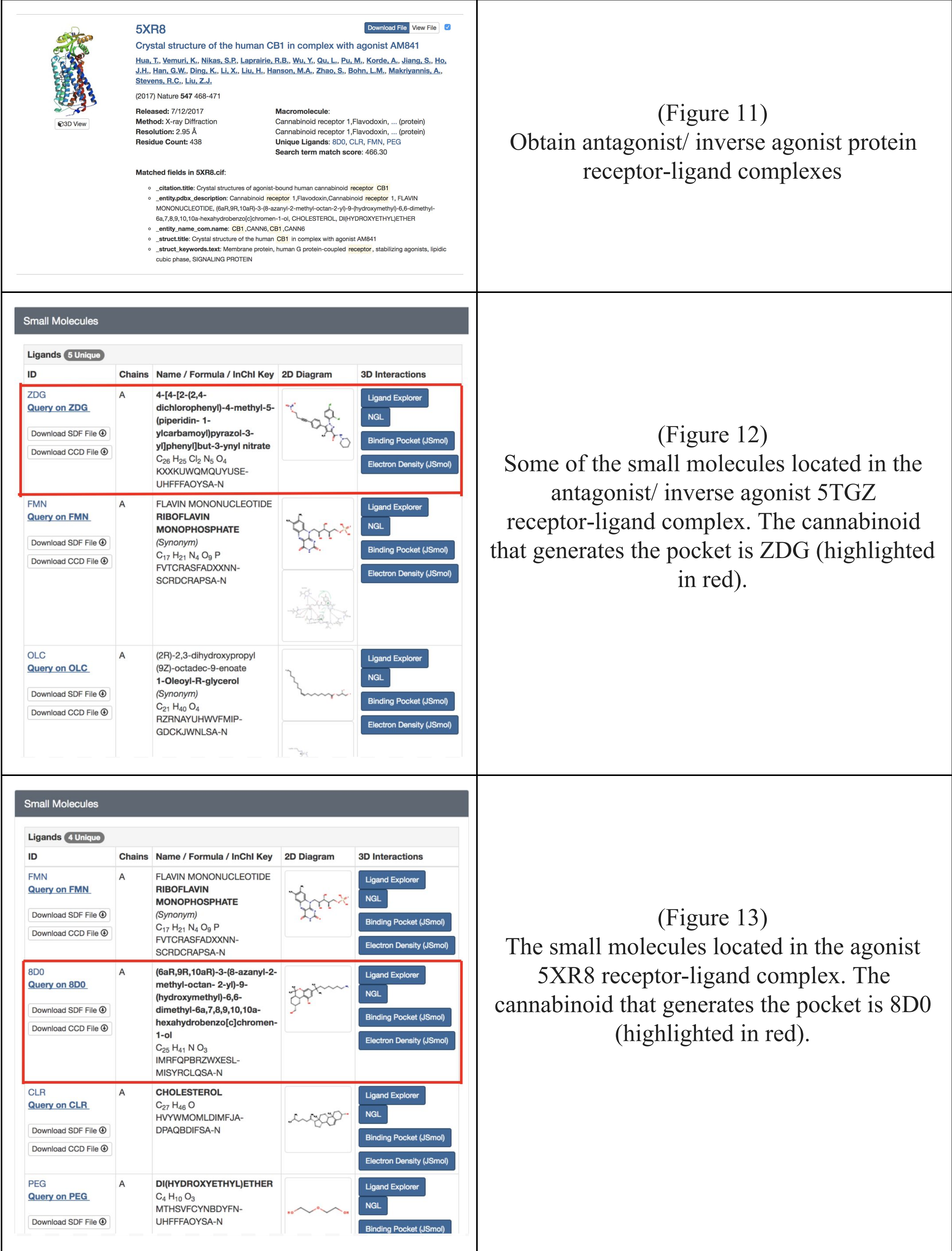
These tools will be used in combination to conduct a series of steps that will be repeated for each cannabinoid chosen for data collection:

1. Search the RCSB PDB for any CB1 receptor-ligand complexes (Figure 9). For the CB1 receptor-ligand antagonist complex the ID is 5TGZ (Figure 10). For the CB1 receptor-ligand agonist complex the ID is 5XR8 (Figure 11). We also would look at these complexes and identify the cannabinoid that forms the pocket protein along with any other small molecules shown in Figures 12 and 13.



(Figure 9)
Search for CB1 receptor-ligand complexes

(Figure 10)
Obtain antagonist/ inverse agonist protein receptor-ligand complexes

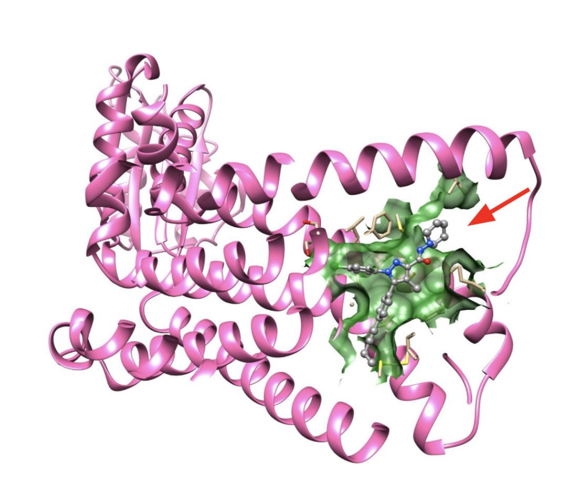
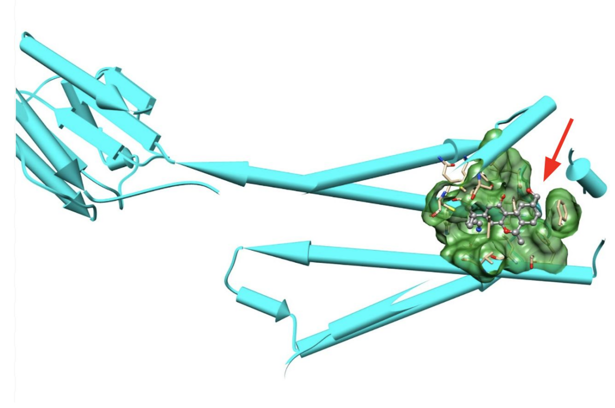


(Figure 11)
Obtain antagonist/ inverse agonist protein receptor-ligand complexes

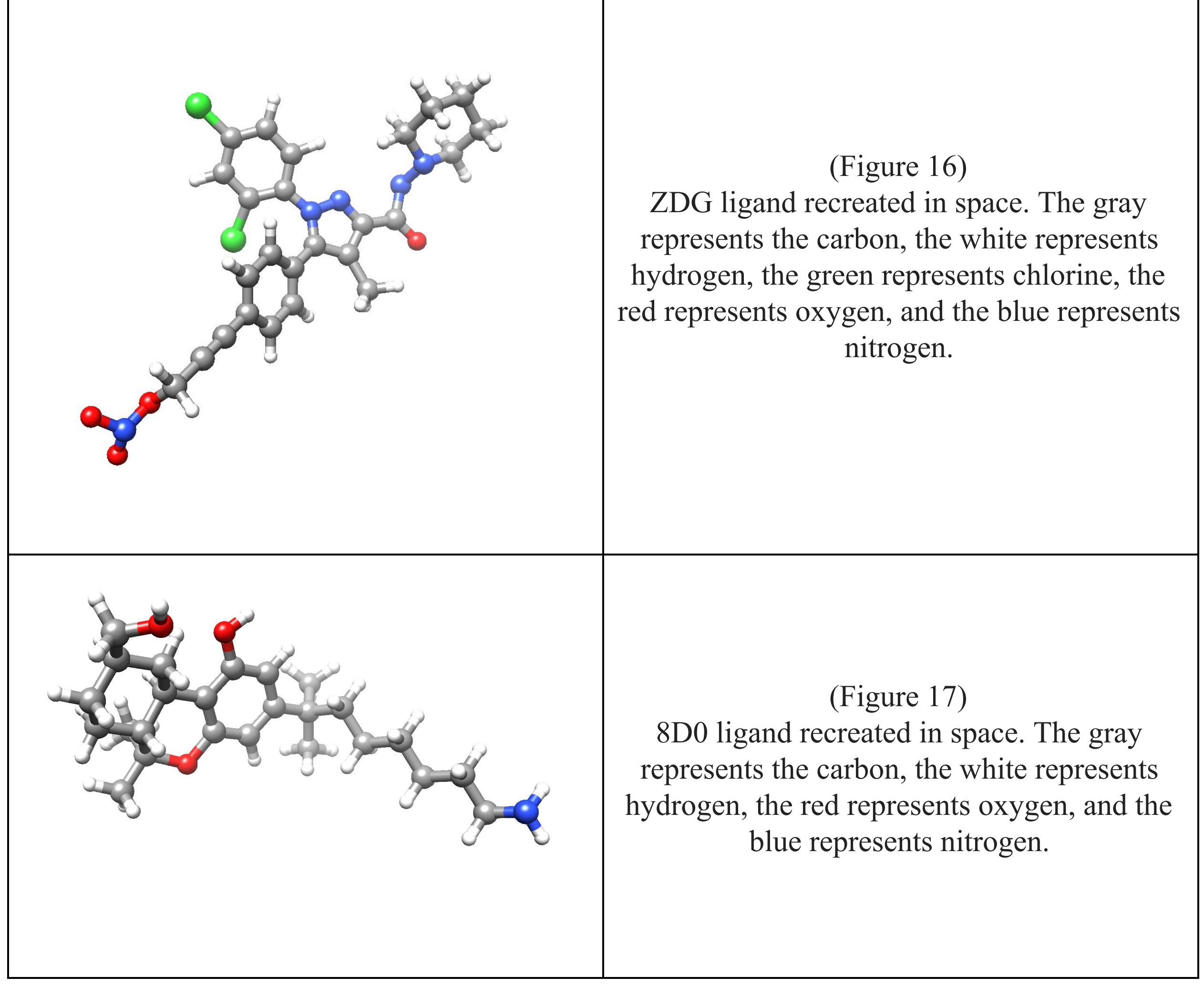
(Figure 12)
Some of the small molecules located in the antagonist/ inverse agonist 5TGZ receptor-ligand complex. The cannabinoid that generates the pocket is ZDG (highlighted in red).

(Figure 13)
The small molecules located in the agonist 5XR8 receptor-ligand complex. The cannabinoid that generates the pocket is 8D0 (highlighted in red).

2. Use Chimera to view the CB1 receptor-ligand complex and identify the crystallized cannabinoid along with the protein pocket that forms around it. Once identified we will remove the cannabinoid and prepare the receptor-ligand complex for docking which will be used in Step 4. Figure 14 shows the cannabinoid and pocket for 5TGZ. Figure 15 shows the cannabinoid and pocket for 5XR8.

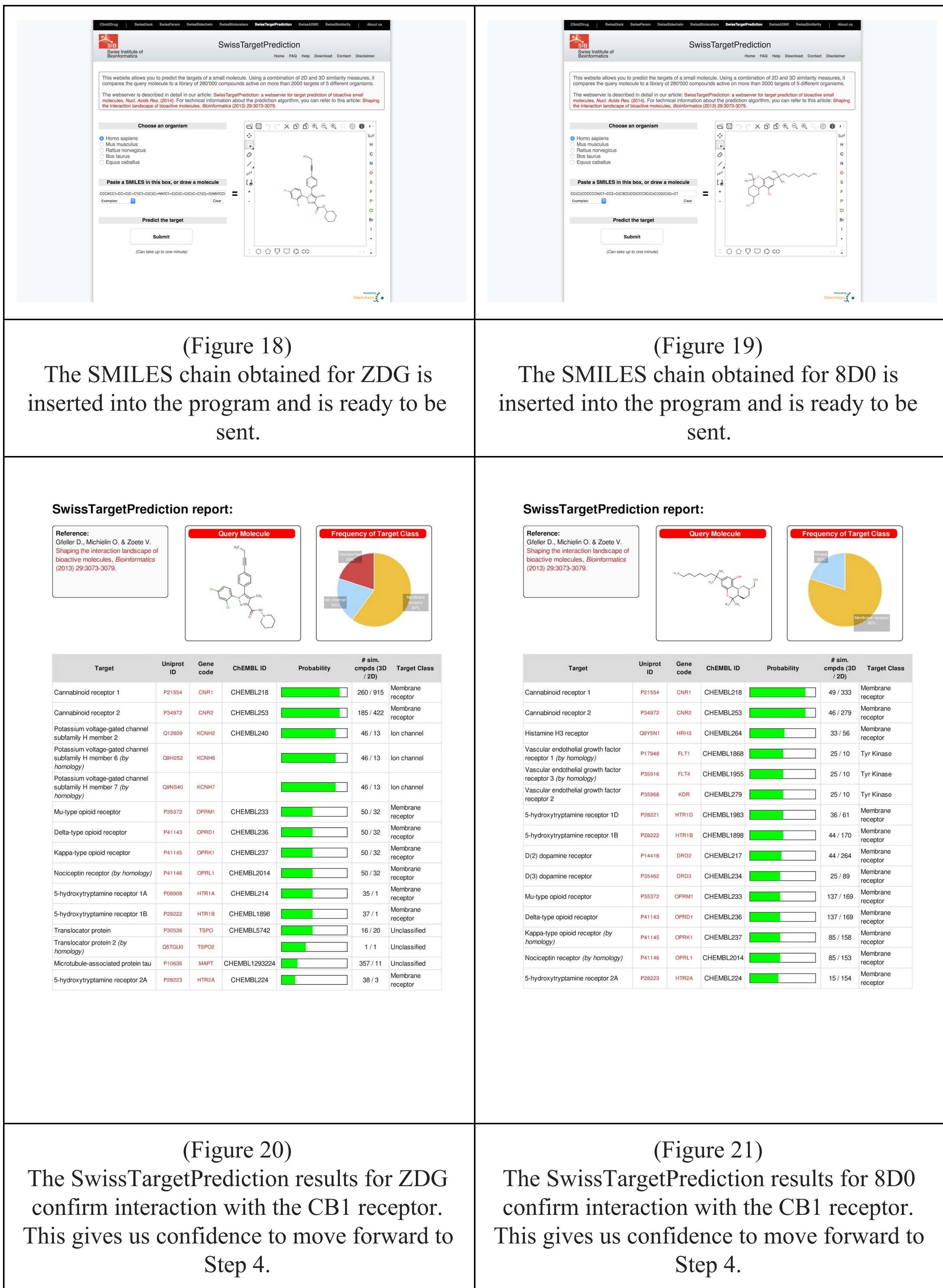
	(Figure 14) The pink ribbons is the 5TGZ CB1 receptor-ligand antagonist/inverse agonist complex. In green is the generated surface representing the protein pocket. The gray, blue, lime-green and red ball-and-stick is the ligand ZTG in complex with the receptor.
	(Figure 15) The blue columns is the 5XR8 CB1 receptor-ligand agonist complex. In green is the generated surface representing the protein pocket. The gray and red ball-and-stick is the ligand 8D0 in complex with the receptor.

3. We will then design the same cannabinoid identified in Step 2 using ChemDraw 2D and 3D to recreate the cannabinoid (Figure 16 for ZDG. Figure 17 for 8D0). Once recreated the Chem 3D program will provide a SMILES chain. Insert that chain into the SwissTargetPrediction to run checks (Figure 18 for ZDG. Figure 19 for 8D0). This will tell us if the designed molecule binds and interacts with the target protein being the CB1 receptor (Figure 20 for ZDG. Figure 21 for 8D0).



(Figure 16)
ZDG ligand recreated in space. The gray represents the carbon, the white represents hydrogen, the green represents chlorine, the red represents oxygen, and the blue represents nitrogen.

(Figure 17)
8D0 ligand recreated in space. The gray represents the carbon, the white represents hydrogen, the red represents oxygen, and the blue represents nitrogen.



(Figure 18)
The SMILES chain obtained for ZDG is inserted into the program and is ready to be sent.

(Figure 19)
The SMILES chain obtained for 8D0 is inserted into the program and is ready to be sent.

Target	Uniprot ID	Gene code	ChEMBL ID	Probability	# sim. cmpds (3D / 2D)	Target Class
Cannabinoid receptor 1	P21554	CNR1	CHEMBL218		260 / 915	Membrane receptor
Cannabinoid receptor 2	P34972	CNR2	CHEMBL253		185 / 422	Membrane receptor
Potassium voltage-gated channel subfamily H member 2	Q12809	KCNH2	CHEMBL240		46 / 13	Ion channel
Potassium voltage-gated channel subfamily H member 6 *(by homology)*	Q9H252	KCNH6			46 / 13	Ion channel
Potassium voltage-gated channel subfamily H member 7 *(by homology)*	Q9NS40	KCNH7			46 / 13	Ion channel
Mu-type opioid receptor	P35372	OPRM1	CHEMBL233		50 / 32	Membrane receptor
Delta-type opioid receptor	P41143	OPRD1	CHEMBL236		50 / 32	Membrane receptor
Kappa-type opioid receptor	P41145	OPRK1	CHEMBL237		50 / 32	Membrane receptor
Nociceptin receptor *(by homology)*	P41146	OPRL1	CHEMBL2014		50 / 32	Membrane receptor
5-hydroxytryptamine receptor 1A	P08908	HTR1A	CHEMBL214		35 / 1	Membrane receptor
5-hydroxytryptamine receptor 1B	P28222	HTR1B	CHEMBL1898		37 / 1	Membrane receptor
Translocator protein	P30536	TSPO	CHEMBL5742		16 / 20	Unclassified
Translocator protein 2 *(by homology)*	Q5TGU0	TSPO2			1 / 1	Unclassified
Microtubule-associated protein tau	P10636	MAPT	CHEMBL1293224		357 / 11	Unclassified
5-hydroxytryptamine receptor 2A	P28223	HTR2A	CHEMBL224		38 / 3	Membrane receptor

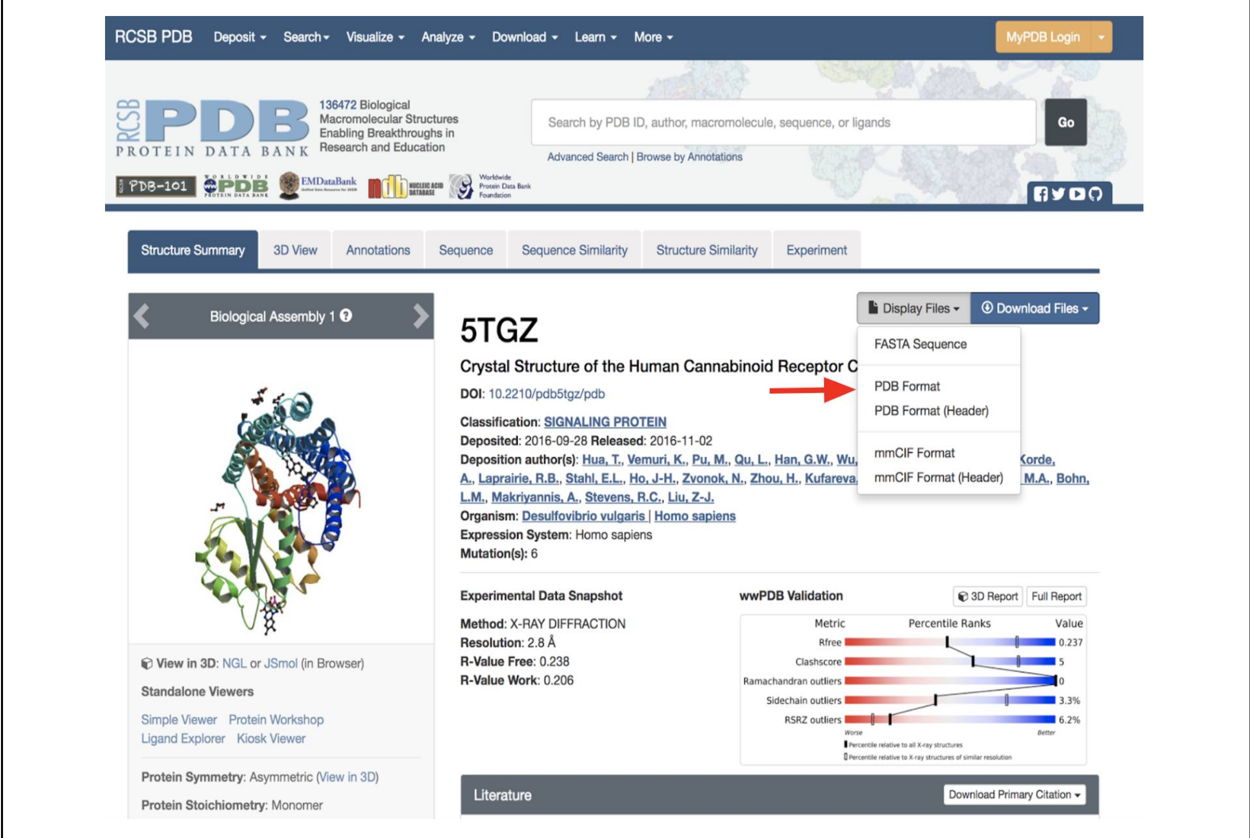
Target	Uniprot ID	Gene code	ChEMBL ID	Probability	# sim. cmpds (3D / 2D)	Target Class
Cannabinoid receptor 1	P21554	CNR1	CHEMBL218		49 / 333	Membrane receptor
Cannabinoid receptor 2	P34972	CNR2	CHEMBL253		46 / 279	Membrane receptor
Histamine H3 receptor	Q9Y5N1	HRH3	CHEMBL264		33 / 56	Membrane receptor
Vascular endothelial growth factor receptor 1 *(by homology)*	P17948	FLT1	CHEMBL1868		25 / 10	Tyr Kinase
Vascular endothelial growth factor receptor 3 *(by homology)*	P35916	FLT4	CHEMBL1955		25 / 10	Tyr Kinase
Vascular endothelial growth factor receptor 2	P35968	KDR	CHEMBL279		25 / 10	Tyr Kinase
5-hydroxytryptamine receptor 1D	P28221	HTR1D	CHEMBL1983		36 / 61	Membrane receptor
5-hydroxytryptamine receptor 1B	P28222	HTR1B	CHEMBL1898		44 / 170	Membrane receptor
D(2) dopamine receptor	P14416	DRD2	CHEMBL217		44 / 264	Membrane receptor
D(3) dopamine receptor	P35462	DRD3	CHEMBL234		25 / 89	Membrane receptor
Mu-type opioid receptor	P35372	OPRM1	CHEMBL233		137 / 169	Membrane receptor
Delta-type opioid receptor	P41143	OPRD1	CHEMBL236		137 / 169	Membrane receptor
Kappa-type opioid receptor *(by homology)*	P41145	OPRK1	CHEMBL237		85 / 158	Membrane receptor
Nociceptin receptor *(by homology)*	P41146	OPRL1	CHEMBL2014		85 / 153	Membrane receptor
5-hydroxytryptamine receptor 2A	P28223	HTR2A	CHEMBL224		15 / 154	Membrane receptor

(Figure 20)
The SwissTargetPrediction results for ZDG confirm interaction with the CB1 receptor. This gives us confidence to move forward to Step 4.

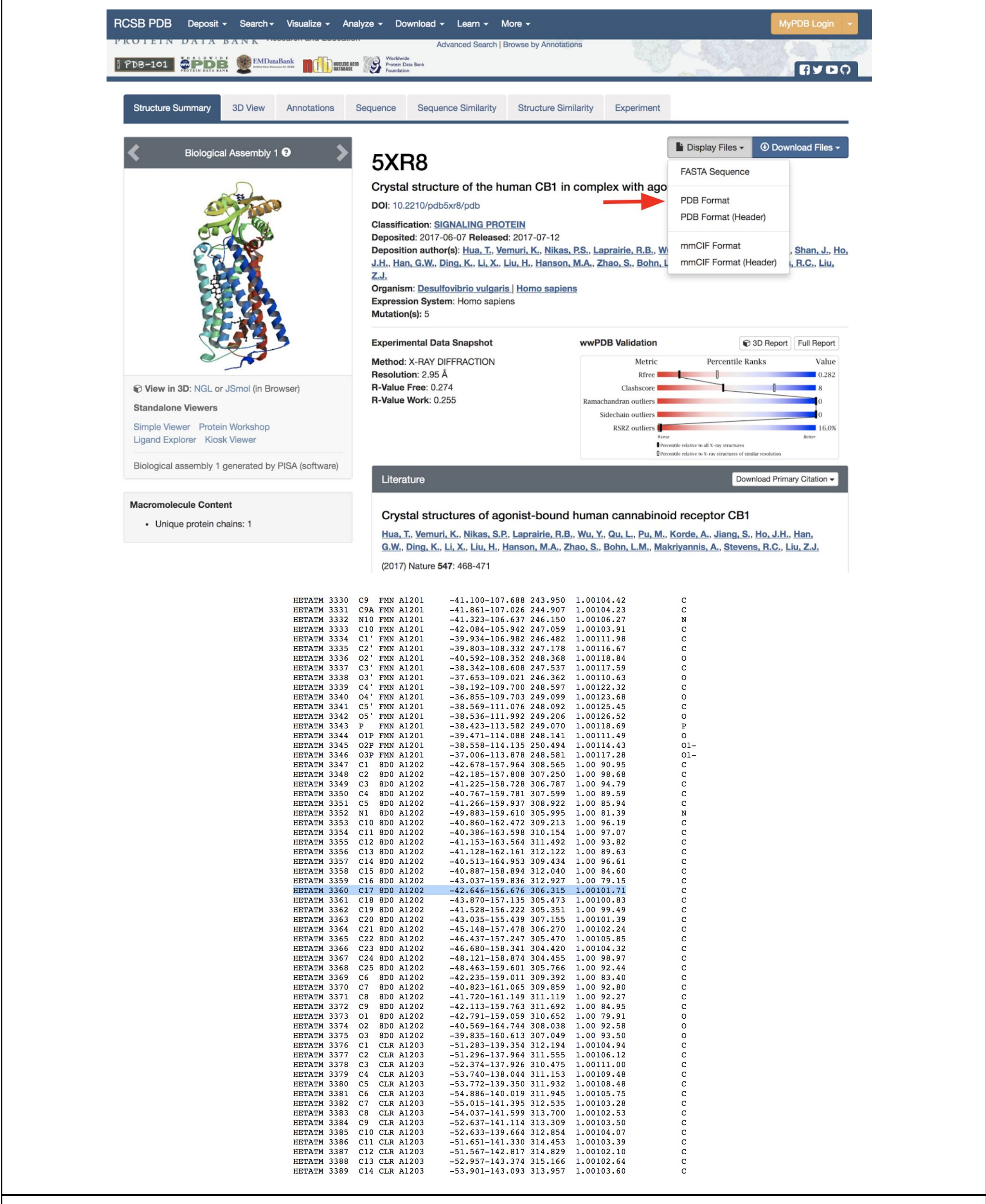
(Figure 21)
The SwissTargetPrediction results for 8D0 confirm interaction with the CB1 receptor. This gives us confidence to move forward to Step 4.

4. Upon confirmation that the cannabinoid does indeed interact with that receptor, we will move onto docking using the bioinformatics tool SwissDock. This will be conducted through three steps. First, obtaining the coordinates for placement through the RSCB PDB (Figure 22 for ZDG. Figure 23 for 8D0). Second by taking the replicated cannabinoid and inserting it and the CB1 receptor-ligand complex prepared for docking into the program. Third is to create the pocket size by setting it to 5x5x5 (Figure 24 for ZDG. Figure 25 for 8D0). Once all these are done the program will run. This will take between 4-12 hours on average to complete and produce the results.

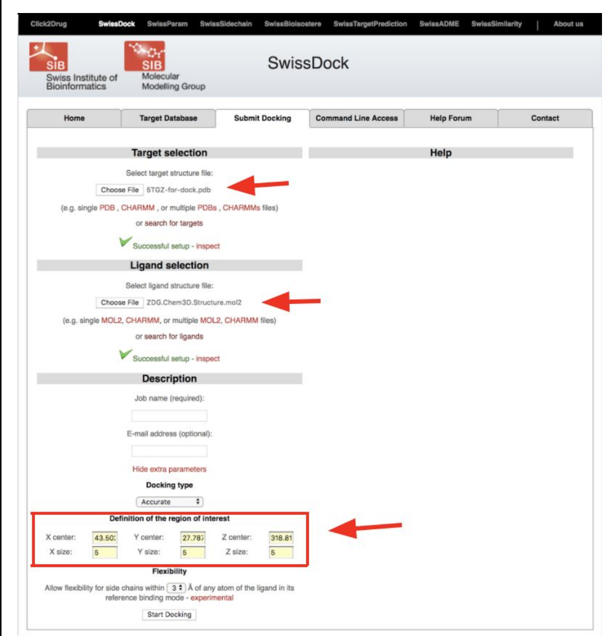
```
ATOM   3410  CG  PHE A 412      61.629  20.181 287.554  1.00159.13           C
ANISOU 3410  CG  PHE A 412    16731  23394  20338   -533   -847  -1578       C
ATOM   3411  CD1 PHE A 412      62.072  21.490 287.473  1.00158.30           C
ANISOU 3411  CD1 PHE A 412    16577  23419  20151   -854   -760  -1625       C
ATOM   3412  CD2 PHE A 412      61.911  19.318 286.507  1.00162.30           C
ANISOU 3412  CD2 PHE A 412    16963  23921  20783   -484   -801  -1716       C
ATOM   3413  CE1 PHE A 412      62.780  21.931 286.373  1.00160.90           C
ANISOU 3413  CE1 PHE A 412    16701  24000  20434  -1140   -622  -1799       C
ATOM   3414  CE2 PHE A 412      62.620  19.754 285.403  1.00160.74           C
ANISOU 3414  CE2 PHE A 412    16545  23988  20541   -757   -663  -1898       C
ATOM   3415  CZ  PHE A 412      63.055  21.063 285.337  1.00161.29           C
ANISOU 3415  CZ  PHE A 412    16576  24187  20520  -1094   -569  -1937       C
TER    3416      PHE A 412
HETATM 3417  CAE ZDG A2001      38.523  35.472 318.733  1.00113.89           C
ANISOU 3417  CAE ZDG A2001    17511  13272  12489  -1030    -92   -908       C
HETATM 3418  CAD ZDG A2001      38.396  34.520 317.542  1.00109.63           C
ANISOU 3418  CAD ZDG A2001    16943  12734  11976  -1078    -81   -891       C
HETATM 3419  CAA ZDG A2001      39.321  33.322 317.752  1.00109.73           C
ANISOU 3419  CAA ZDG A2001    16962  12756  11973  -1054    -92   -914       C
HETATM 3420  CAB ZDG A2001      39.986  32.350 317.916  1.00109.81           C
ANISOU 3420  CAB ZDG A2001    16978  12772  11975  -1035   -101   -929       C
HETATM 3421  CAC ZDG A2001      40.899  31.144 318.140  1.00116.18           C
ANISOU 3421  CAC ZDG A2001    17788  13584  12772  -1010   -113   -951       C
HETATM 3422  CAN ZDG A2001      40.495  29.873 317.767  1.00119.56           C
ANISOU 3422  CAN ZDG A2001    18231  13991  13205  -1004   -131   -938       C
HETATM 3423  CAM ZDG A2001      41.333  28.790 317.983  1.00120.70           C
ANISOU 3423  CAM ZDG A2001    18382  14143  13336   -980   -140   -959       C
HETATM 3424  CAJ ZDG A2001      42.136  31.331 318.729  1.00115.02           C
ANISOU 3424  CAJ ZDG A2001    17616  13429  12655   -988   -147   -988       C
HETATM 3425  CAK ZDG A2001      42.974  30.250 318.943  1.00110.43           C
ANISOU 3425  CAK ZDG A2001    17031  12846  12080   -966   -164  -1006       C
HETATM 3426  CAL ZDG A2001      42.574  28.980 318.571  1.00115.95           C
ANISOU 3426  CAL ZDG A2001    17762  13560  12735   -962   -139   -989       C
HETATM 3427  CAO ZDG A2001      43.502  27.787 318.810  1.00111.96           C
ANISOU 3427  CAO ZDG A2001    17255  13054  12230   -936   -155  -1011       C
HETATM 3428  CAS ZDG A2001      43.682  27.126 319.993  1.00110.58           C
ANISOU 3428  CAS ZDG A2001    17113  12873  12031   -891   -189  -1025       C
HETATM 3429  CBB ZDG A2001      42.989  27.464 321.309  1.00110.96           C
ANISOU 3429  CBB ZDG A2001    17246  12905  12008   -806   -185  -1003       C
HETATM 3430  NAP ZDG A2001      44.272  27.204 317.897  1.00118.27           N
ANISOU 3430  NAP ZDG A2001    18048  13854  13036   -920   -123  -1009       N
HETATM 3431  CAT ZDG A2001      44.405  27.578 316.503  1.00117.94           C
ANISOU 3431  CAT ZDG A2001    18040  13806  12966   -879    -46   -974       C
HETATM 3432  CAY ZDG A2001      45.468  28.377 316.108  1.00130.61           C
ANISOU 3432  CAY ZDG A2001    19564  15386  14677   -852     63   -910       C
HETATM 3433 CLA  ZDG A2001      46.670  28.954 317.297  1.00137.28          CL
ANISOU 3433 CLA  ZDG A2001    20224  16161  15776   -904     -4   -914      CL
HETATM 3434  CAX ZDG A2001      45.608  28.736 314.781  1.00135.28           C
ANISOU 3434  CAX ZDG A2001    20225  15961  15214   -747    208   -831       C
HETATM 3435  CAW ZDG A2001      44.691  28.301 313.841  1.00139.76           C
ANISOU 3435  CAW ZDG A2001    20993  16534  15577   -657    167   -867       C
HETATM 3436 CLB  ZDG A2001      44.882  28.769 312.129  1.00153.02          CL
ANISOU 3436 CLB  ZDG A2001    22880  18173  17088   -414    350   -769      CL
HETATM 3437  CAV ZDG A2001      43.629  27.502 314.232  1.00125.58           C
ANISOU 3437  CAV ZDG A2001    19248  14733  13733   -714    -25   -964       C
HETATM 3438  CAU ZDG A2001      43.483  27.137 315.563  1.00113.54           C
ANISOU 3438  CAU ZDG A2001    17604  13229  12308   -831    -93   -991       C
HETATM 3439  NAQ ZDG A2001      44.952  26.165 318.510  1.00117.87           N
ANISOU 3439  NAQ ZDG A2001    17992  13800  12993   -900   -153  -1031       N
HETATM 3440  CAR ZDG A2001      44.589  26.121 319.784  1.00119.91           C
```



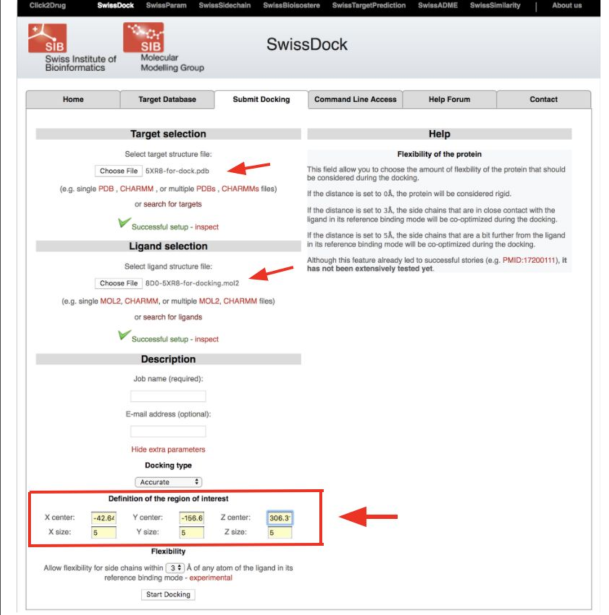
(Figure 22)
Use the RSCB PDB to obtain the coordinates for ZDG. They will be under the PDB Format section. Use the coordinates after choosing a center atom. The coordinates for placement are highlighted in blue.



(Figure 23)
Use the RSCB PDB to obtain the coordinates for ZDG. They will be under the PDB Format section. Use the coordinates after choosing a center atom. The coordinates for placement are highlighted in blue.



(Figure 24)
Upload the edited 5TGZ for dock along with the recreated ligand ZDG. Place the coordinates obtained from Figure 22 into the extra parameters and set the box size to 5x5x5.



(Figure 25)
Upload the edited 5TGZ for dock along with the recreated ligand ZDG. Place the coordinates obtained from Figure 22 into the extra parameters and set the box size to 5x5x5.

5. Once docking is completed we will view the results in Chimera to see if they made sense. This will be done by taking our results and superimposing them over the crystal-structure-ligand. We will then generate a protein pocket for the crystal structure ligand and ours and superimpose the two. The protein pocket should be approximately 3.7 Å after selecting the ligand. If the crystallized cannabinoid and the docked results of the recreated cannabinoid superimpose well (90%+ in accuracy), it ensures that the first half of our proof of concept is correct. The second half is to view the pocket and see if the amino acids that generate this pocket are the same. The experimental pocket amino acid residues should match the known protein pocket amino acid residues. Once both are confirmed it means our proof of concept is correct which gives us confidence to move forward with the rest of the experiment.
6. Once the proof of concept is correct, we will continue with the same procedure for the designed cannabinoids listed in Figure 6. Again the antagonist binding cannabinoids will enter the antagonist From there we will choose which

cannabinoid superimposes the best (90%+ in accuracy) to use for our recorded data. We will record the unknown ΔG and compare it to the known IC50 value stated in Figure 6 as well. The reason for this is that a more negative ΔG value means the cannabinoids binds better to the receptor. A lower IC50 value means that less of a cannabinoid is required to induce effects. These numbers should correlate, meaning that if a known IC50 value is low, it should have a more negative ΔG value.

7. After Step 6 the next step is to generate a protein pocket for each cannabinoid to compile the amino acid residue interactions. The pocket should be the same experimental size as crystallized cannabinoid pocket that was previously generated in Step 5.
8. Once generated we would compile the list of amino acid residues and compare them to all of the rest of the experimental pocket residue interaction and the crystallized pocket formed. We will record which amino acid residues that each antagonist/inverse agonist share, and which are different that are responsible for their unique effects (Figure 26). The same will be done for the agonists (Figure 27). After compiling all the data for antagonists/inverse agonists and agonists, we will then compare and contrast the two and record the data (Figure 8).

ANTAGONIST 5TGZ Residue List			
ZDG	RImonabant	Taranabant	CBD
Same	Same	Same	Same
Same	Same	Same	Same
Same	Same	Same	Same
Same	Same	Same	Same
Unique	Unique	Unique	Unique
Unique	Unique	Unique	Unique

(Figure 26)
List used to record amino acid residues for all antagonist/ inverse agonist cannabinoids used in experiment. The residues shared are listed in pink, the unique ones are listed in purple.

AGONIST 5XR8 Residue List			
8D0	AEA	CBN	THC
Same	Same	Same	Same
Same	Same	Same	Same
Same	Same	Same	Same
Same	Same	Same	Same
Unique	Unique	Unique	Unique
Unique	Unique	Unique	Unique

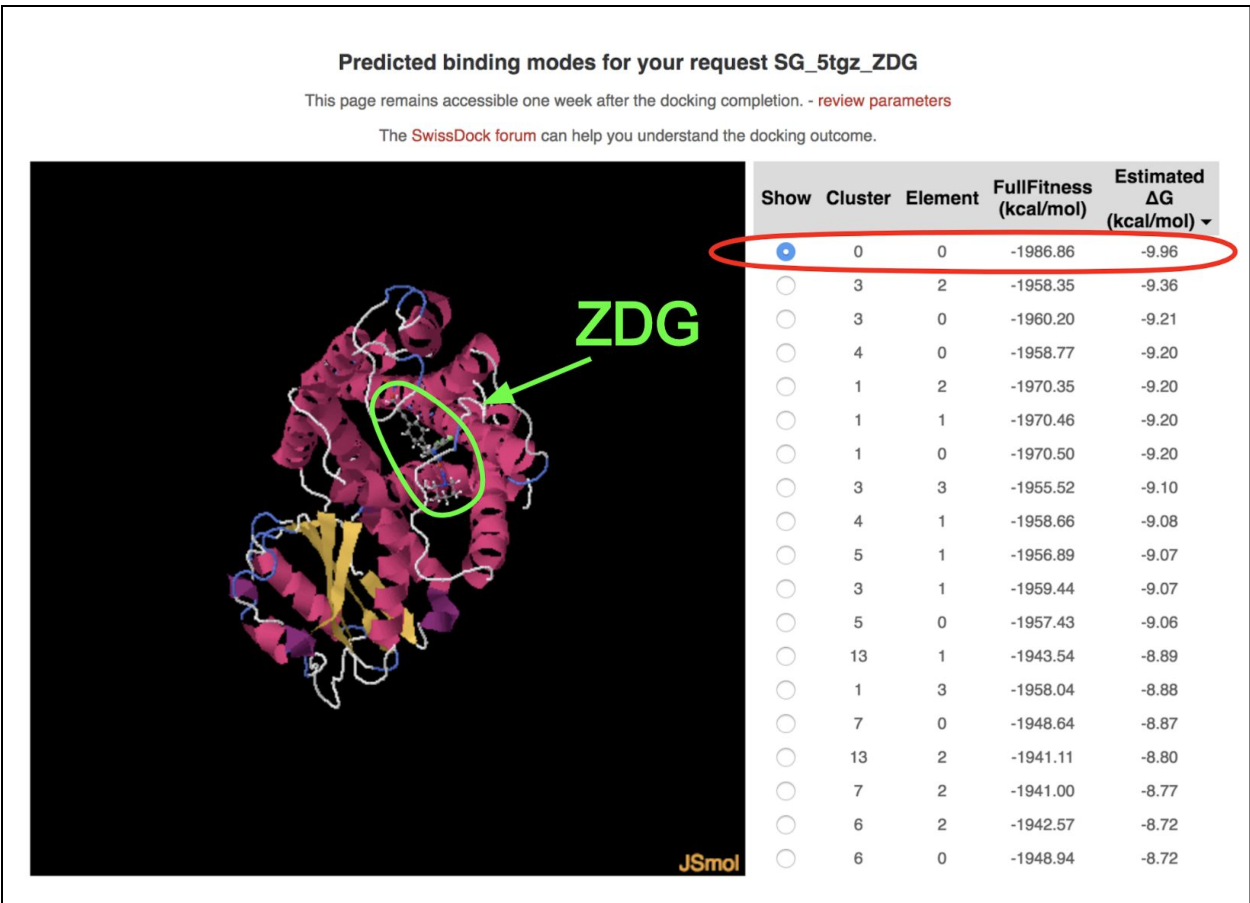
(Figure 27)
List used to record amino acid residues for all agonist cannabinoids used in experiment. The residues shared are listed in blue, the unique ones are listed in purple.

At this point our research is completed. This provides the theoretical protocol for anyone who is interested in manufacturing cannabinoids to treat medical ailments. If one were to synthesize a cannabinoid to put to market, they would use this protocol to see if a cannabinoid bound as an antagonist/inverse agonist or an agonist. The ΔG value would theorize if a compound binds and interacts with the receptor well which would help if no IC50 value is known. The amino acid residue sheet should be looked at as a guideline. If a cannabinoid were to be an antagonist/inverse agonist or agonist, there would be an expected interaction of the amino acid residues list in the chart, with some minor additions or subtractions.

Results and Discussion:

Proof of Concept for Antagonist/ Inverse Agonist CB1 Receptor-Ligand Complex 5TGZ:

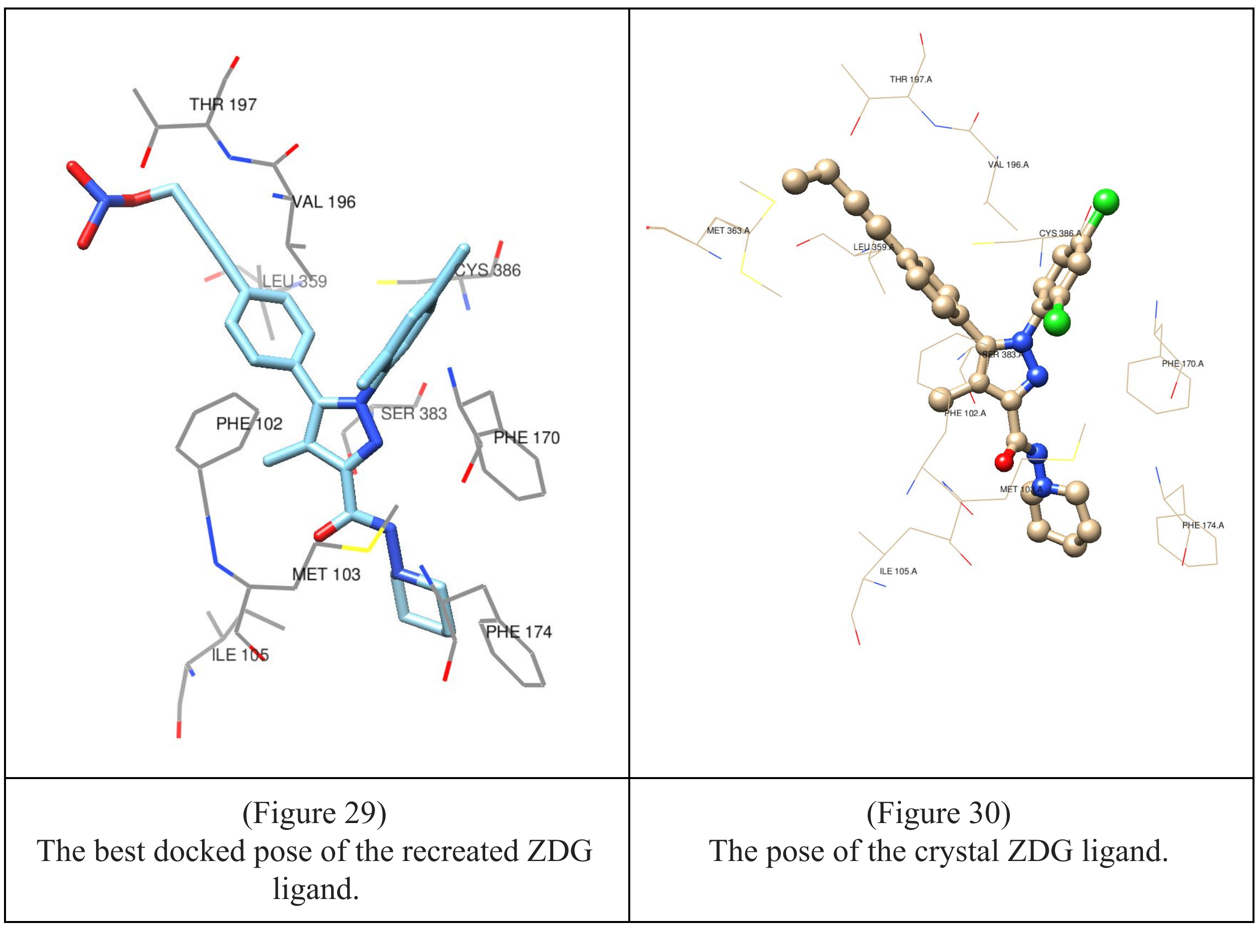
The proof of concept was conducted during Step 5 of the experiment. The point of the proof of concept is to ensure our process is correct. Our proof of concept for the antagonist/ inverse agonist begins with the SwissDock results. The results from the SwissDock program showed us the different conformations listed in figure. This will tell provide us a list of all the possible. Confirmations that are within the docking of the recreated ligand into the receptor. It will also show us the energetic favorability represented in the ΔG value (Figure 28).



Show	Cluster	Element	FullFitness (kcal/mol)	Estimated ΔG (kcal/mol)
	0	0	-1986.86	-9.96
	3	2	-1958.35	-9.36
	3	0	-1960.20	-9.21
	4	0	-1958.77	-9.20
	1	2	-1970.35	-9.20
	1	1	-1970.46	-9.20
	1	0	-1970.50	-9.20
	3	3	-1955.52	-9.10
	4	1	-1958.66	-9.08
	5	1	-1956.89	-9.07
	3	1	-1959.44	-9.07
	5	0	-1957.43	-9.06
	13	1	-1943.54	-8.89
	1	3	-1958.04	-8.88
	7	0	-1948.64	-8.87
	13	2	-1941.11	-8.80
	7	2	-1941.00	-8.77
	6	2	-1942.57	-8.72
	6	0	-1948.94	-8.72

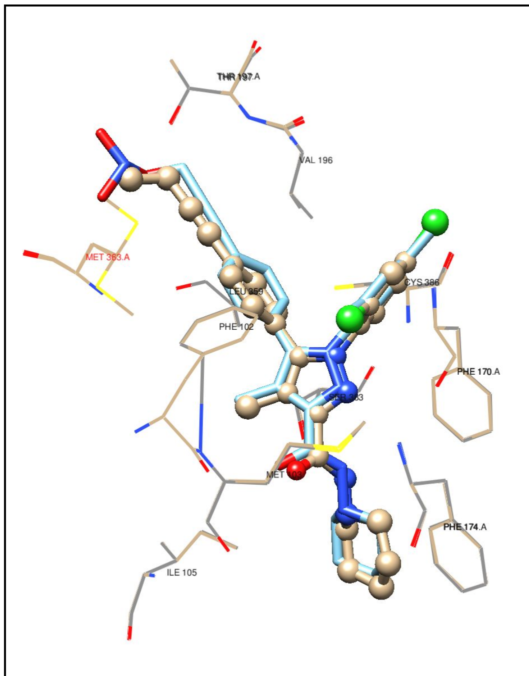
Figure 28
The recreated 5TGZ protein receptor used for docking can be observed on the left. The recreated molecule ZDG can be seen within the receptor highlighted in green. On the right is the list of different conformations that recreated ZDG takes within the receptor. Circled in red is the ligand complex chosen for this research purpose.

After obtaining this we then open our results in Chimera and begin to superimpose the crystal structure pocket saved, to our confirmation results. The list of different confirmations will be in the Chimera program and open a seperate box when using the system. We then choose best docked pose that superimposes over the crystal structure. When doing this we will follow the instructions in Step 5 by creating the pocket to observe the amino acid residue interaction. For the case of ZDG, the ligand is a long greasy chain that has hydrophobic interactions with the surrounding residues.



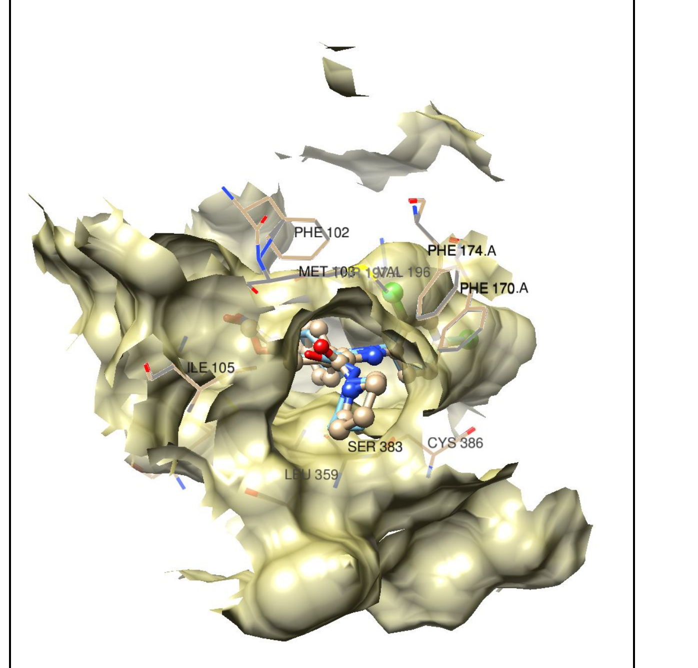
(Figure 29) The best docked pose of the recreated ZDG ligand.	(Figure 30) The pose of the crystal ZDG ligand.

As seen in Figure 29 above, this is the best docked posed for the recreated ZDG, and does indeed have hydrophobic interaction with the protein pocket. When compared to the crystal structure both contain a very similar “Y” shape that is seen. When the two are superimposed the docked results have over a 90% similarity to the corresponding molecule structurally. There are some slight differences in arm that contains the nitro compound being the rotation of the ring (Figure 31).



(Figure 31)
When superimposed the docked molecule has over a 90% similarity to the corresponding crystal structure.

When we placed the cannabinoid into the pocket the amino acid residue interaction was exactly the same. This means that our proof of concept for the 5TGZ antagonist/ inverse agonist receptor-ligand complex site is correct. The protein pocket similarities can be observed in Figure 32 and the amino acid residue list in Figure 33.



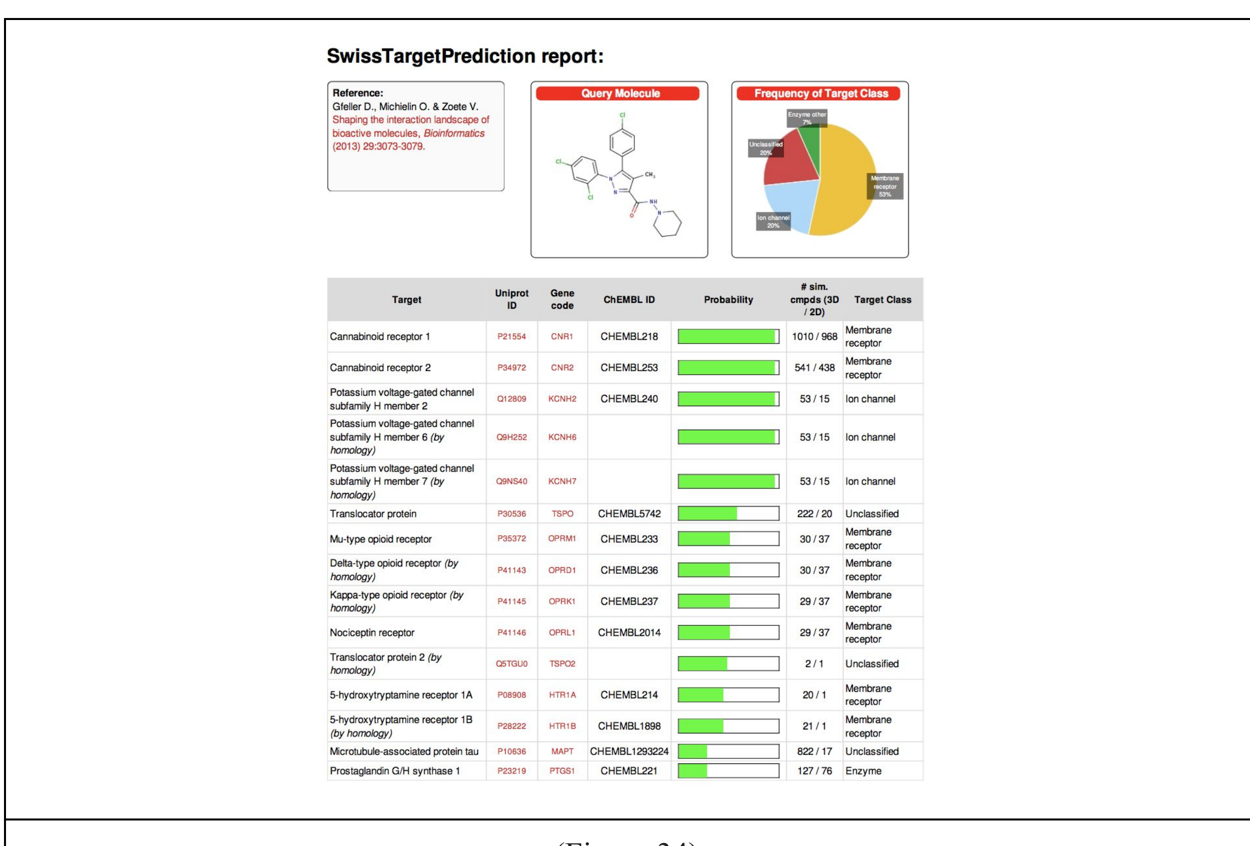
(Figure 32)
The protein pocket is the same along with the amino acid residues that forms it.

ZDG	Recreated ZDG
THR 197	THR 197
SER 383	SER 383
CYS 386	CYS 386
PHE 170	PHE 170
PHE 174	PHE 174
VAL 196	VAL 196
MET 363	MET 363
LEU 359	LEU 359
ILE 105	ILE 105
MET 103	MET 103
PHE 102	PHE 102
MET 384	MET 384
ALA 380	ALA 380
PHE 379	PHE 379
PHE 268	PHE 268
LEU 193	LEU 193
TRP 356	TRP 356
GLY 166	GLY 166
LEU 387	LEU 387
SER 167	SER 167

(Figure 33)
The list of the amino acid residues to ensure that the proof of concept is correct

Rimonabant 5TGZ:

The first cannabinoid selected for testing was the antagonist/ inverse agonist Rimonabant. Rimonabant is cannabinoid that was originally synthesized to combat obesity[16]. While it did indeed have the onset effects of inducing appetite suppression and ensuring weight loss by targeting the CB1 receptor it was pulled off the shelves. The reason being is that the adverse effects were too severe causing a number of patients to commit suicide[28].

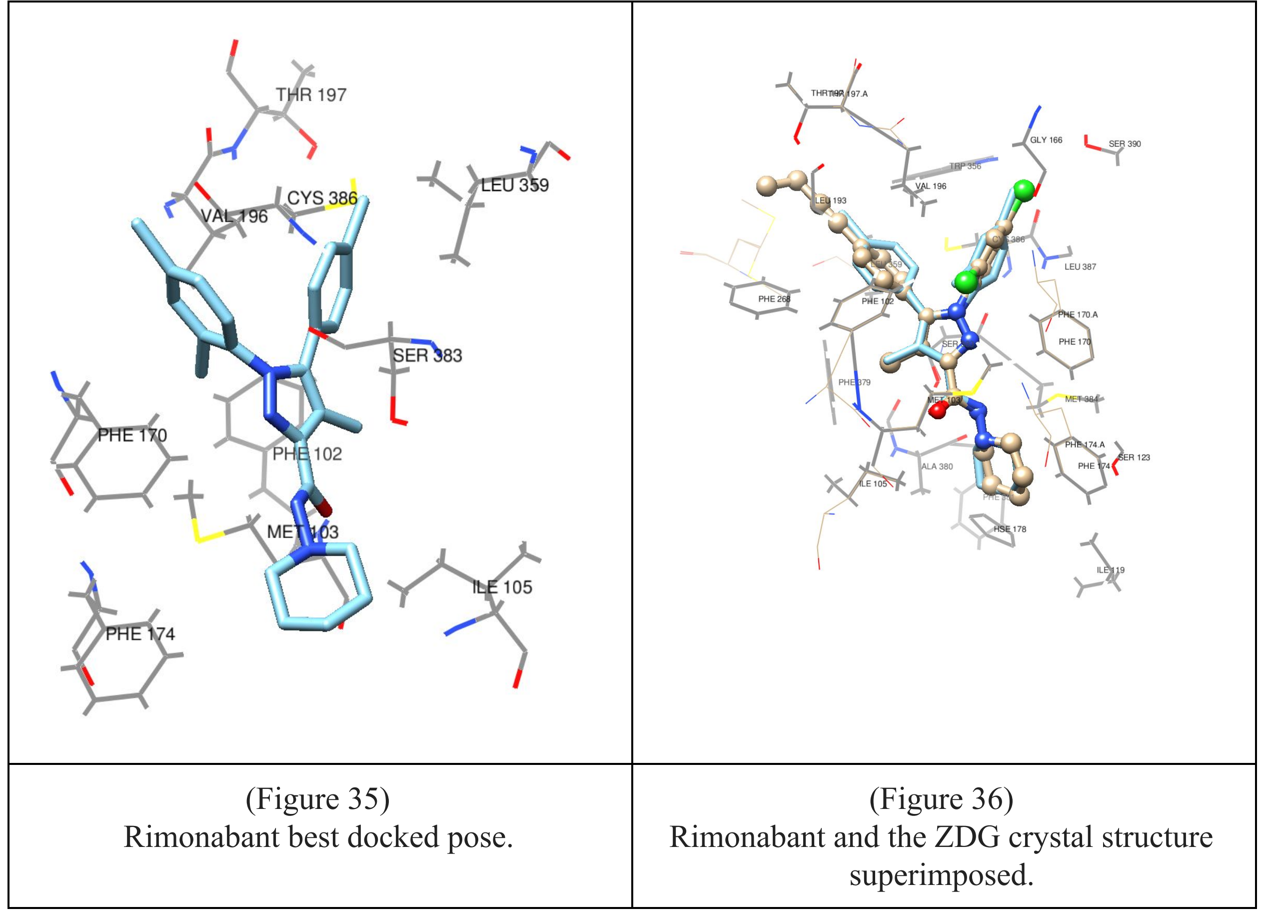

SwissTargetPrediction report:

Reference:
Gfeller D., Michielin O. & Zoete V.
Shaping the interaction landscape of bioactive molecules, *Bioinformatics* (2013) 29:3073-3079.

Target	Uniprot ID	Gene code	ChEMBL ID	Probability	# sim. cmpds (3D / 2D)	Target Class
Cannabinoid receptor 1	P21554	CNR1	CHEMBL218		1010 / 968	Membrane receptor
Cannabinoid receptor 2	P34972	CNR2	CHEMBL253		541 / 438	Membrane receptor
Potassium voltage-gated channel subfamily H member 2	Q12809	KCNH2	CHEMBL240		53 / 15	Ion channel
Potassium voltage-gated channel subfamily H member 6 *(by homology)*	Q9H252	KCNH6			53 / 15	Ion channel
Potassium voltage-gated channel subfamily H member 7 *(by homology)*	Q9NS40	KCNH7			53 / 15	Ion channel
Translocator protein	P30536	TSPO	CHEMBL5742		222 / 20	Unclassified
Mu-type opioid receptor	P35372	OPRM1	CHEMBL233		30 / 37	Membrane receptor
Delta-type opioid receptor *(by homology)*	P41143	OPRD1	CHEMBL236		30 / 37	Membrane receptor
Kappa-type opioid receptor *(by homology)*	P41145	OPRK1	CHEMBL237		29 / 37	Membrane receptor
Nociceptin receptor	P41146	OPRL1	CHEMBL2014		29 / 37	Membrane receptor
Translocator protein 2 *(by homology)*	Q5TGU0	TSPO2			2 / 1	Unclassified
5-hydroxytryptamine receptor 1A	P08908	HTR1A	CHEMBL214		20 / 1	Membrane receptor
5-hydroxytryptamine receptor 1B *(by homology)*	P28222	HTR1B	CHEMBL1898		21 / 1	Membrane receptor
Microtubule-associated protein tau	P10636	MAPT	CHEMBL1293224		822 / 17	Unclassified
Prostaglandin G/H synthase 1	P23219	PTGS1	CHEMBL221		127 / 76	Enzyme

(Figure 34)
The SwissTargetPrediction showing Rimonabant does have high probability to interact with the CB1 receptor.

Following the procedure when Rimonabant was inserted into the SwissTargetPrediction program the molecule showed high probability to interact with the CB1 receptor (Figure 34). This allowed for us to move forward and place Rimonabant into the same coordinates shown from Figure 24 and Figure 7.



(Figure 35)
Rimonabant best docked pose.

(Figure 36)
Rimonabant and the ZDG crystal structure superimposed.

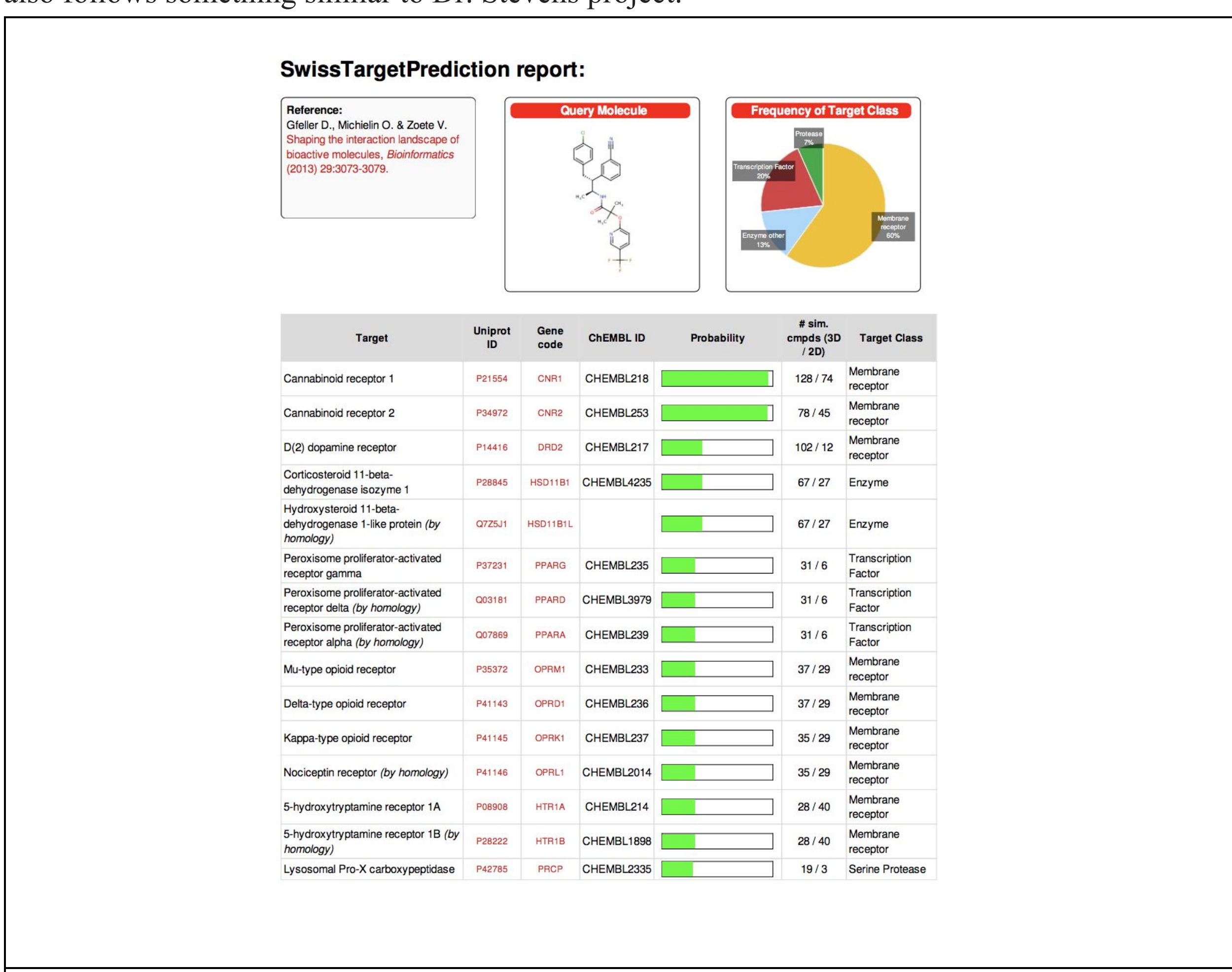
Rimonabant is similar to ZDG but doesn't have the triple bond and nitro group on the one arm, instead it has a chlorine. When Rimonabant is inserted it forms almost the same "Y" shape we saw before with ZDG (Figure 35). When the two are superimposed there is over 90% similarity in stance with some slight variations in the rings (Figure 36). Rimonabant interacts with the same residues with some additions. (Figure 37). All interactions are hydrophobic.

ZDG	RImonabant
THR 197	THR 197
SER 383	SER 383
CYS 386	CYS 386
PHE 170	PHE 170
PHE 174	PHE 174
VAL 196	VAL 196
MET 363	MET 363
LEU 359	LEU 359
ILE 105	ILE 105
MET 103	MET 103
PHE 102	PHE 102
MET 384	MET 384
ALA 380	ALA 380
PHE 379	PHE 379
PHE 268	PHE 268
LEU 193	LEU 193
TRP 356	TRP 356
GLY 166	GLY 166
LEU 387	LEU 387
SER 167	SER 167
	ILE 119
	SER 123
	TYR 275
	TRP 279

(Figure 37)
Chart compiled and comparing amino acid residues for Rimonabant to ZDG.

Taranabant 5TGZ:

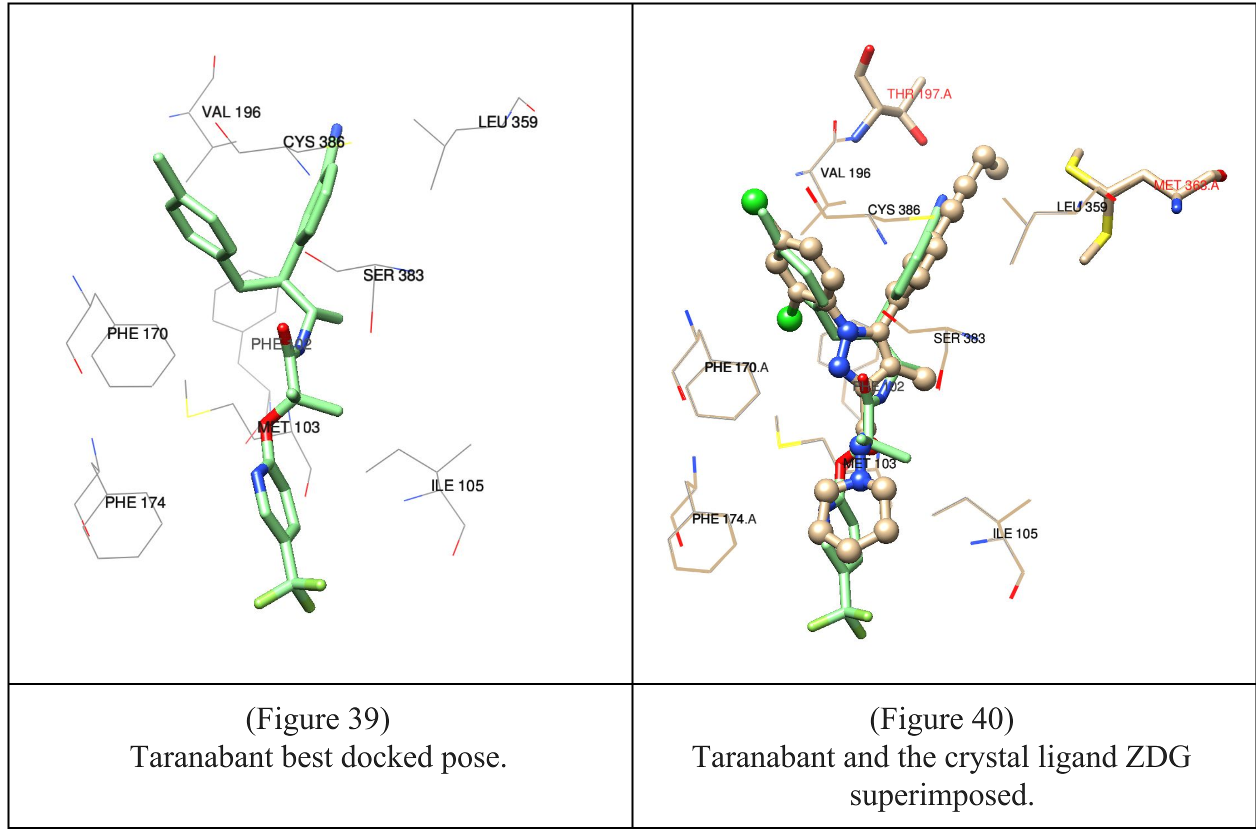
The next molecule chosen for docking was Taranabant, an antagonist/ inverse agonist cannabinoid synthesized to combat obesity. It also was proven to be able to reduce appetite and help weight loss but stopped after Phase 3 clinical trials and was taken off the market[29]. This also follows something similar to Dr. Stevens project.



Target	Uniprot ID	Gene code	ChEMBL ID	Probability	# sim. cmpds (3D / 2D)	Target Class
Cannabinoid receptor 1	P21554	CNR1	CHEMBL218		128 / 74	Membrane receptor
Cannabinoid receptor 2	P34972	CNR2	CHEMBL253		78 / 45	Membrane receptor
D(2) dopamine receptor	P14416	DRD2	CHEMBL217		102 / 12	Membrane receptor
Corticosteroid 11-beta-dehydrogenase isozyme 1	P28845	HSD11B1	CHEMBL4235		67 / 27	Enzyme
Hydroxysteroid 11-beta-dehydrogenase 1-like protein *(by homology)*	Q7Z5J1	HSD11B1L			67 / 27	Enzyme
Peroxisome proliferator-activated receptor gamma	P37231	PPARG	CHEMBL235		31 / 6	Transcription Factor
Peroxisome proliferator-activated receptor delta *(by homology)*	Q03181	PPARD	CHEMBL3979		31 / 6	Transcription Factor
Peroxisome proliferator-activated receptor alpha *(by homology)*	Q07869	PPARA	CHEMBL239		31 / 6	Transcription Factor
Mu-type opioid receptor	P35372	OPRM1	CHEMBL233		37 / 29	Membrane receptor
Delta-type opioid receptor	P41143	OPRD1	CHEMBL236		37 / 29	Membrane receptor
Kappa-type opioid receptor	P41145	OPRK1	CHEMBL237		35 / 29	Membrane receptor
Nociceptin receptor *(by homology)*	P41146	OPRL1	CHEMBL2014		35 / 29	Membrane receptor
5-hydroxytryptamine receptor 1A	P08908	HTR1A	CHEMBL214		28 / 40	Membrane receptor
5-hydroxytryptamine receptor 1B *(by homology)*	P28222	HTR1B	CHEMBL1898		28 / 40	Membrane receptor
Lysosomal Pro-X carboxypeptidase	P42785	PRCP	CHEMBL2335		19 / 3	Serine Protease

(Figure 38)
The SwissTargetPrediction showing Taranabant does have high probability to interact with the CB1 receptor.

Following the procedure when Taranabant was inserted into the SwissTargetPrediction program the molecule showed high probability to interact with the CB1 receptor (Figure 34). In fact, it showed binding to specifically to cannabinoid receptors, signaling promising results. This allowed for us to move forward and place Taranabant into the same coordinates shown from Figure 24 and Figure 7.



(Figure 39) Taranabant best docked pose.	(Figure 40) Taranabant and the crystal ligand ZDG superimposed.

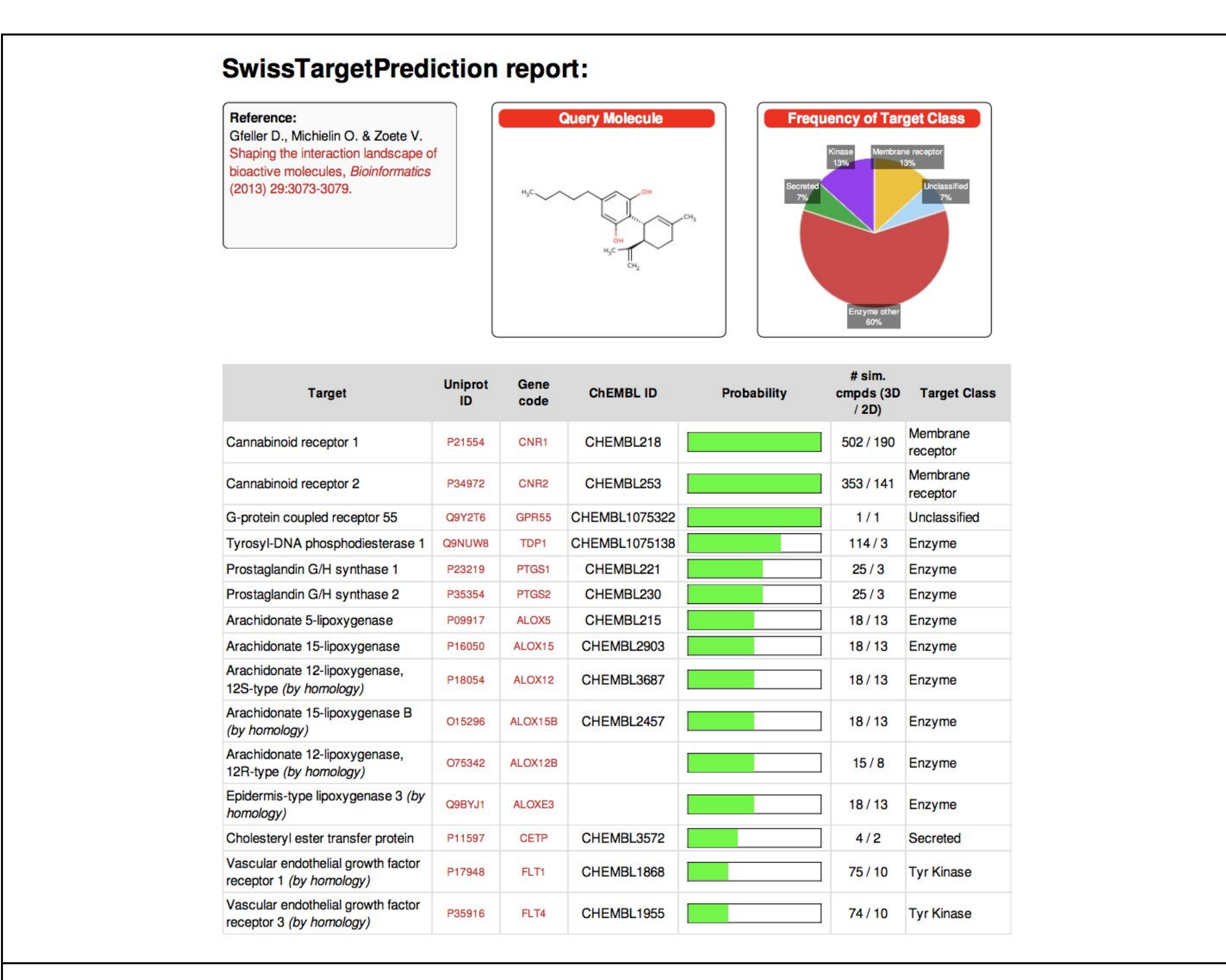
Taranabant is similar to the ZDG crystal structure, however there are some obvious differences. The addition of a tert butyl group attached to the bottom ring, the aromatic center ring being broken apart, the removal of a chlorine on the di-chloryl ring, and instead of a triple bond and nitrite group, it has an amide. When superimposed over the crystal ligand ZDG, the same “Y” shape is observed (Figure 40). Taranabant interacts with all the same residues with the exception of seranine 167. It also interacts with similar residues to Rimonabant. All interactions are hydrophobic and the full list can be seen in Figure 41.

ZDG	RImonabant	Taranabant
THR 197	THR 197	THR 197
SER 383	SER 383	SER 383
CYS 386	CYS 386	CYS 386
PHE 170	PHE 170	PHE 170
PHE 174	PHE 174	PHE 174
VAL 196	VAL 196	VAL 196
MET 363	MET 363	MET 363
LEU 359	LEU 359	LEU 359
ILE 105	ILE 105	ILE 105
MET 103	MET 103	MET 103
PHE 102	PHE 102	PHE 102
MET 384	MET 384	MET 384
ALA 380	ALA 380	ALA 380
PHE 379	PHE 379	PHE 379
PHE 268	PHE 268	PHE 268
LEU 193	LEU 193	LEU 193
TRP 356	TRP 356	TRP 356
GLY 166	GLY 166	GLY 166
LEU 387	LEU 387	LEU 387
SER 167	SER 167	
		HSE 178
	SER 123	SER 123
	TYR 275	
	TRP 279	
	ILE 119	

(Figure 41)
Chart compiling and comparing amino acid residues for Taranabant to ZDG with the inclusion of Rimonabant. The residues they all share are in pink, the residues 8D0 and Rimonabant share are in purple, the residues Taranabant and Rimonabant share are in orange and the residues that are unique to Rimonabant and Taranabant are listed in red.

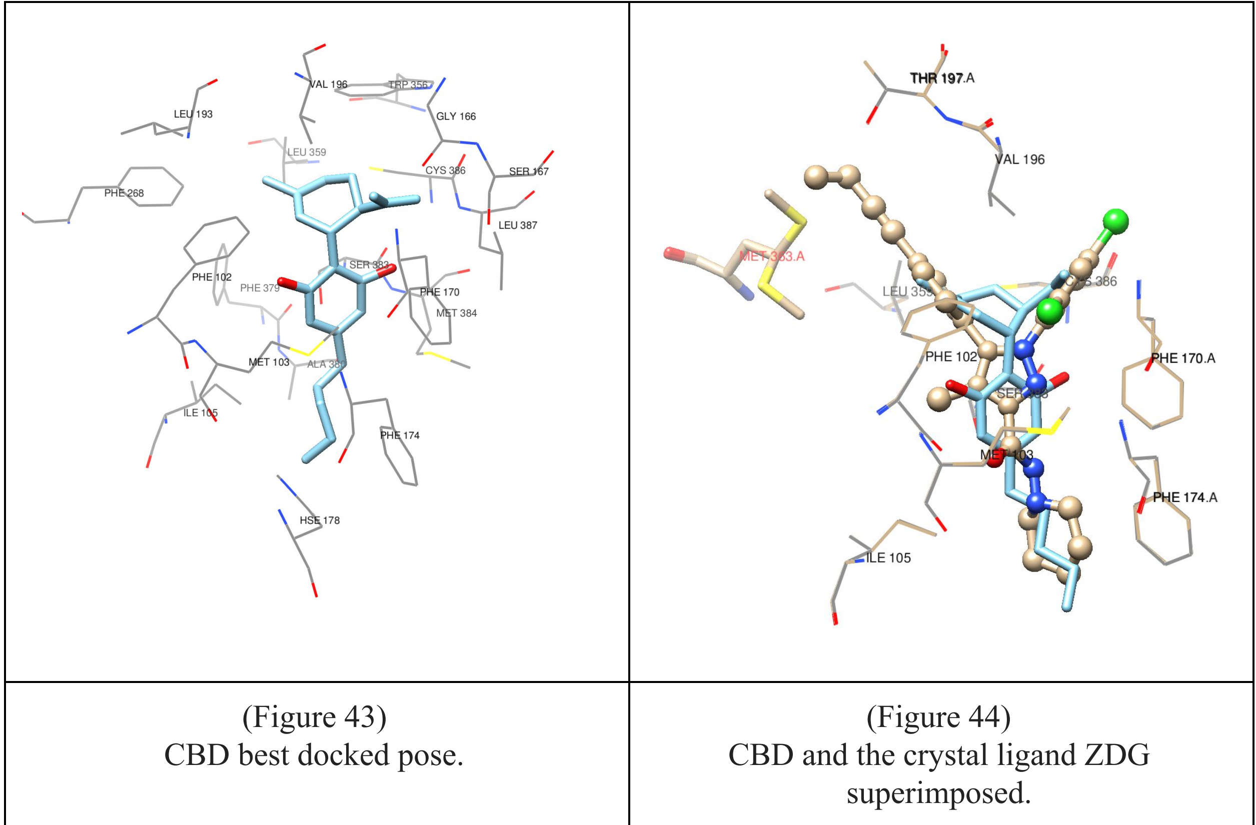
Cannabidiol (CBD) to 5TGZ:

The last molecule chosen for the antagonist/ inverse agonist site was the most famous antagonist cannabinoid, cannabidiol, or CBD for short. CBD is a cannabinoid that is produced in the cannabis plant, labeling it as a phytocannabinoid[30]. The phytocannabinoid has been shown to have a variety of medical benefits including treatment in pain[1], anxiety[3], and cancer[20]. The most profound effect is that it CBD has been shown to be a viable treatment for epileptic seizures in children[2]. It is also widely unknown that CBD, like the other antagonists/ inverse agonists, acts as an appetite suppressant to combat obesity[19]. This cannabinoid is currently in a legal gray area, and there is a legal movement to have it rescheduled from a Schedule 1 substance to Schedule 2 because of its benefits[31]. There are many different products sold throughout legal dispensaries, but the most famous, accurate and widely used is through a tincture. CBD has not been tested and these are the first published results of the molecule interacting with the CB1 receptor.



Target	Uniprot ID	Gene code	ChEMBL ID	Probability	# sim. cmpds (3D / 2D)	Target Class
Cannabinoid receptor 1	P21554	CNR1	CHEMBL218		502 / 190	Membrane receptor
Cannabinoid receptor 2	P34972	CNR2	CHEMBL253		353 / 141	Membrane receptor
G-protein coupled receptor 55	Q9Y2T6	GPR55	CHEMBL1075322		1 / 1	Unclassified
Tyrosyl-DNA phosphodiesterase 1	Q9NUW8	TDP1	CHEMBL1075138		114 / 3	Enzyme
Prostaglandin G/H synthase 1	P23219	PTGS1	CHEMBL221		25 / 3	Enzyme
Prostaglandin G/H synthase 2	P35354	PTGS2	CHEMBL230		25 / 3	Enzyme
Arachidonate 5-lipoxygenase	P09917	ALOX5	CHEMBL215		18 / 13	Enzyme
Arachidonate 15-lipoxygenase	P16050	ALOX15	CHEMBL2903		18 / 13	Enzyme
Arachidonate 12-lipoxygenase, 12S-type *(by homology)*	P18054	ALOX12	CHEMBL3687		18 / 13	Enzyme
Arachidonate 15-lipoxygenase B *(by homology)*	O15296	ALOX15B	CHEMBL2457		18 / 13	Enzyme
Arachidonate 12-lipoxygenase, 12R-type *(by homology)*	O75342	ALOX12B			15 / 8	Enzyme
Epidermis-type lipoxygenase 3 *(by homology)*	Q9BYJ1	ALOXE3			18 / 13	Enzyme
Cholesteryl ester transfer protein	P11597	CETP	CHEMBL3572		4 / 2	Secreted
Vascular endothelial growth factor receptor 1 *(by homology)*	P17948	FLT1	CHEMBL1868		75 / 10	Tyr Kinase
Vascular endothelial growth factor receptor 3 *(by homology)*	P35916	FLT4	CHEMBL1955		74 / 10	Tyr Kinase

(Figure 42)
The SwissTargetPrediction showing CBD does have high probability to interact with the CB1 receptor.



(Figure 43) CBD best docked pose.	(Figure 44) CBD and the crystal ligand ZDG superimposed.

Cannabidiol looks much different than the rest of the antagonists/ inverse agonists. It is shaped similarly to THC, with the exception being an ether formed instead of an alcohol group on the isopropyl constituent located on the top ring. When viewed in the Chimera program, it formed a “Y” shape in the pocket similar to the other antagonists/ inverse agonists (Figure 43). There are some major differences when compared to the ligand ZDG. Its three arms consist of an isopropyl group, a pentyl group, and a methyl group. They both do contain a center ring, however CBD’s ring is a 6 membered aromatic ring. When superimposed over ZDG, both interact in the same pocket with the same residues. CBD interacts with the same residues in the same 3.7 Å pocket formed as ZDG, showing a 90% similarity to the corresponding crystal structure (Figure 44). The residue list is shown in Figure 45 and compared to all the other cannabinoids chosen for this experiment.

This helps us come to the conclusion of why CBD acts the way it does. The cannabinoid, like all other antagonists, forms a “Y” shape when it enters the pocket. The “Y” shape then forms a surface the prevents any agonist binding cannabinoids from interacting with the protein pocket. This helps conclude that the reason for why when you ingest CBD before THC you do not experience the same psychoactive effects associated with THC.

ANTAGONIST 5TGZ Residue List			
ZDG	RImonabant	Taranabant	CBD
THR 197	THR 197	THR 197	THR 197
SER 383	SER 383	SER 383	SER 383
CYS 386	CYS 386	CYS 386	CYS 386
PHE 170	PHE 170	PHE 170	PHE 170
PHE 174	PHE 174	PHE 174	PHE 174
VAL 196	VAL 196	VAL 196	VAL 196
MET 363	MET 363	MET 363	MET 363
LEU 359	LEU 359	LEU 359	LEU 359
ILE 105	ILE 105	ILE 105	ILE 105
MET 103	MET 103	MET 103	MET 103
PHE 102	PHE 102	PHE 102	PHE 102
MET 384	MET 384	MET 384	MET 384
ALA 380	ALA 380	ALA 380	ALA 380
PHE 379	PHE 379	PHE 379	PHE 379
PHE 268	PHE 268	PHE 268	PHE 268
LEU 193	LEU 193	LEU 193	LEU 193
TRP 356	TRP 356	TRP 356	TRP 356
GLY 166	GLY 166	GLY 166	GLY 166
LEU 387	LEU 387	LEU 387	LEU 387
SER 167	SER 167		SER 167
		HSE 178	HSE 178
	SER 123	SER 123	
	TYR 275		
	TRP 279		
	ILE 119		

(Figure 45)
Chart compiling and comparing amino acid residues for CBD to ZDG with the inclusion of Rimonabant and Taranabant. The residues they all share are in pink, the ones unique to ZDG, Rimonabant, and CBD are in purple, the residues unique for Taranabant and CBD are in green, the residues that Rimonabant and Taranabant share are in orange and the residues unique to Rimonabant are in red.

Antagonist/Inverse Agonist Receptor-Ligand Complex 5TGZ Results:

In conclusion of the antagonists/inverse agonists we can successfully say that any antagonist/ inverse agonist interacts with the CB1 receptor in a similar fashion. Each will form a "Y" shape and interact with the same protein pocket. The ΔG values are all very similar and coincide with the IC50 values (Figure 46). The only ΔG value slightly less negative was CBD because it is smaller molecule. After compiling all the residues and comparing and contrasting them, we were able to gather the list that all antagonist/ inverse agonist cannabinoids make contact with (Figure 47).This list will later be compared to the agonist cannabinoid residue interaction to distinguish which residues are responsible for the unique function of an antagonist/ inverse agonist cannabinoid.

5TGZ			
Ligand	Type	IC50 Value (μM)	Δ G Value (kcal/mol)
ZDG	Antagonist	Unkown	-9.96
Rimonabant	Inverse Agonist	Unknown	-9.83
Taranabant	Antagonist	0.00030	-9.26
CBD	Antagonist	2.00	-8.28

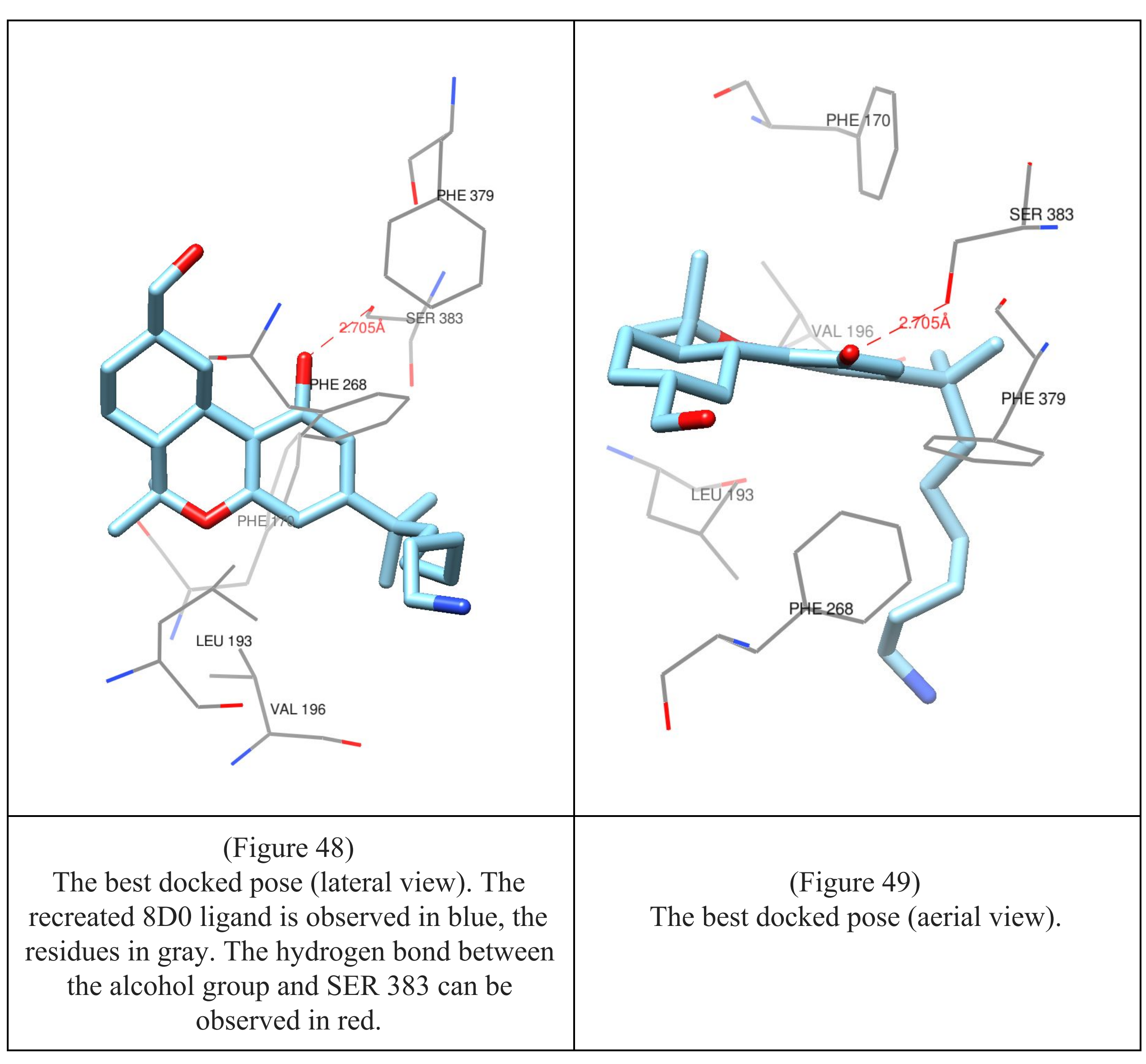
(Figure 46)
The ΔG values compared to the IC50 of the antagonist/ inverse agonist cannabinoids.

Antagonist
THR 197
SER 383
CYS 386
PHE 170
PHE 174
PHE 268
VAL 196
LEU 193
PHE 102
PHE 379
MET 103
MET 363
MET 384
TRP 356
SER 167
ILE 105
LEU 359
LEU 387
GLY 166
ALA 380

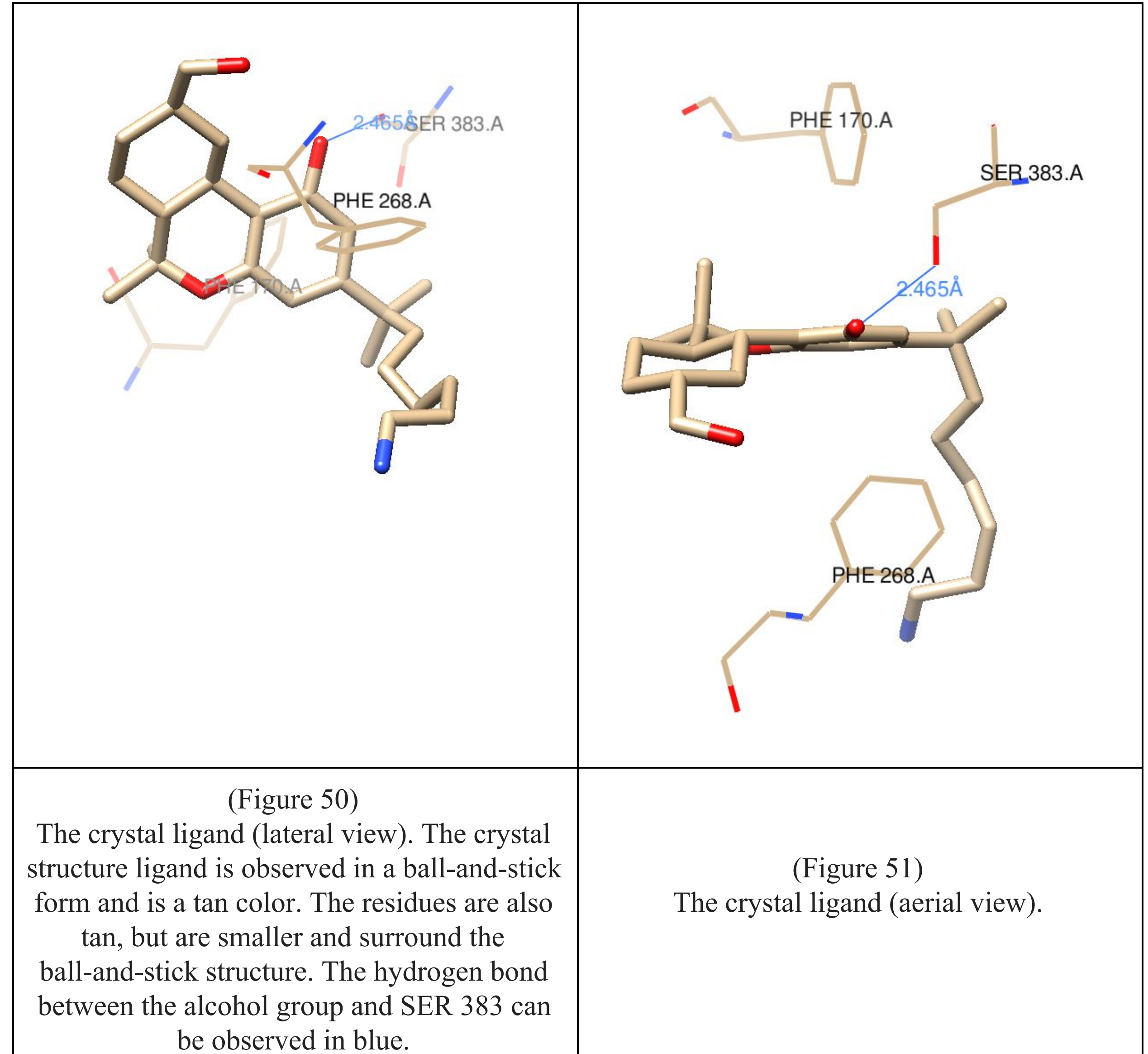
(Figure 47)
The residues that each antagonist/ inverse agonist share.

Proof of Concept for Agonist CB1 Receptor-Ligand Complex 5XR8:

Our proof of concept for the agonist CB1 receptor-ligand complex 5XR8 began with the replication of docking the crystal structure ligand 8D0. The results for such can be seen below. It is important to note that we took an aerial picture to better observe the interaction of the ligand and the pocket it forms. Figure 48 and 49 is our replicated docking, Figure 50 and 51 is the crystal structure ligand. For the purpose of imagery, some of the residues have been removed, however the known residue interaction will be listed in Figure 55 in completion.



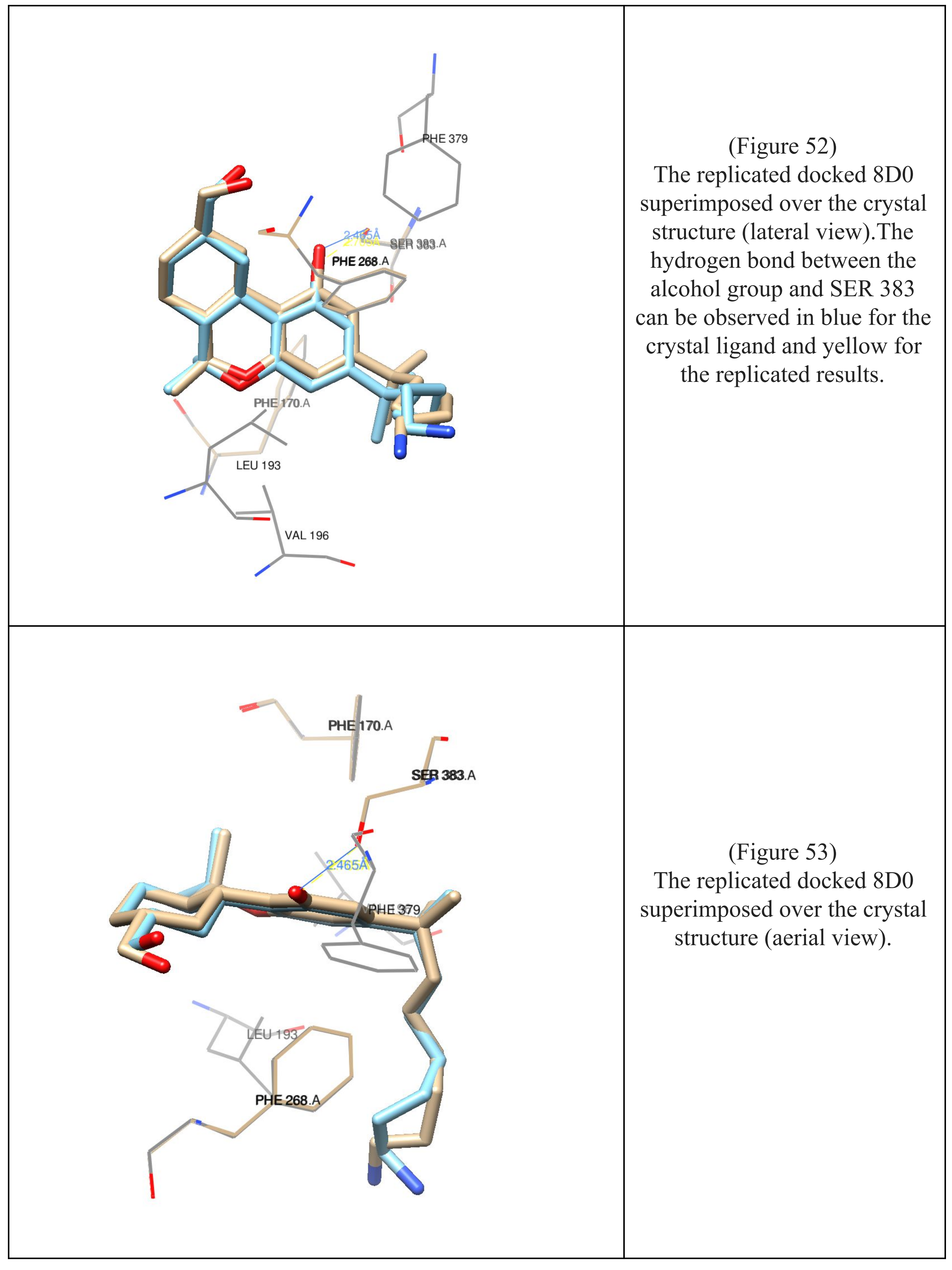
(Figure 48) The best docked pose (lateral view). The recreated 8D0 ligand is observed in blue, the residues in gray. The hydrogen bond between the alcohol group and SER 383 can be observed in red.	(Figure 49) The best docked pose (aerial view).



(Figure 50)
The crystal ligand (lateral view). The crystal structure ligand is observed in a ball-and-stick form and is a tan color. The residues are also tan, but are smaller and surround the ball-and-stick structure. The hydrogen bond between the alcohol group and SER 383 can be observed in blue.

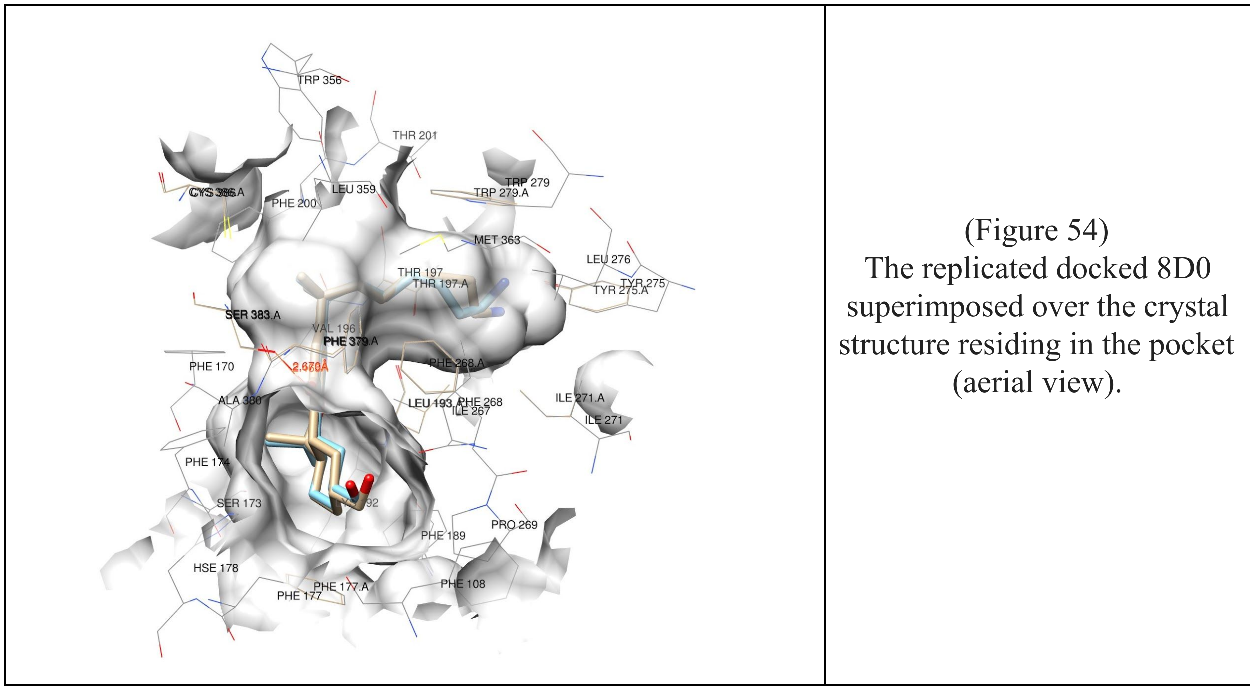
(Figure 51)
The crystal ligand (aerial view).

When we superimpose the two we observe how the amino acid residue interaction is the same along with the pocket they reside in (Figures 52,53, and 54).



(Figure 52)
The replicated docked 8D0 superimposed over the crystal structure (lateral view).The hydrogen bond between the alcohol group and SER 383 can be observed in blue for the crystal ligand and yellow for the replicated results.

(Figure 53)
The replicated docked 8D0 superimposed over the crystal structure (aerial view).



(Figure 54)
The replicated docked 8D0 superimposed over the crystal structure residing in the pocket (aerial view).

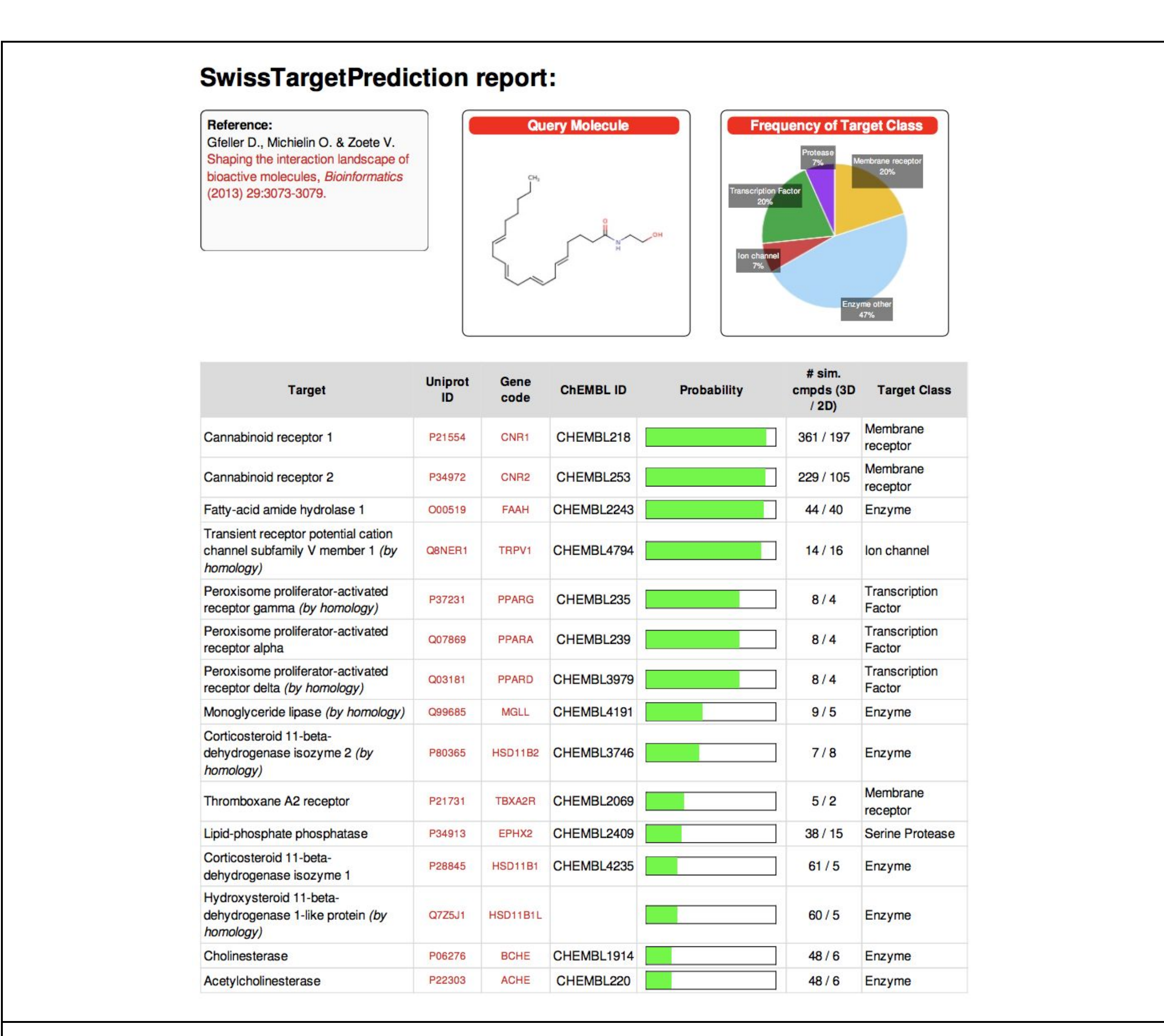
This pocket appears to form an "L" like shape. The pocket interaction shows how there is a single hydrogen bond between the alcohol group (#0 8D0 1202.A O2) and seranine 383. The rest of the interactions within the pocket are strictly hydrophobic because it is a long greasy chain. Overall there is over a 90% similarity with our docked replication results to the crystal structure itself. When we compiled and compared the residue interaction they were exactly the same (Figure 55). These results give mean that the proof of concept for the agonists is correct and gives us confidence with moving forward with the rest of the experimental agonists.

Docked 8D0	Crystal 8D0
THR 197	THR 197
SER 383	SER 383
CYS 386	CYS 386
PHE 177	PHE 177
PHE 268	PHE 268
LEU 193	LEU 193
PHE 379	PHE 379
TRP 279	TRP 279
VAL 196	VAL 196
SER 173	SER 173
PHE 170	PHE 170
PHE 174	PHE 174
ILE 267	ILE 267
PHE 108	PHE 108
LEU 359	LEU 359
PRO 269	PRO 269
HSE 178	HSE 178
PHE 189	PHE 189
LEU 276	LEU 276
ILE 271	ILE 271
TYR 275	TYR 275
TRP 356	TRP 356
MET 363	MET 363
ALA 380	ALA 380
PHE 200	PHE 200
LYS 192	LYS 192
THR 201	THR 201

(Figure 55)
The compiled residue list showing that the residues the docked results interact with and the crystal structure ligand interact with are the same.

<u>Anandamide (AEA) 5XR8</u>:

The first agonist binding cannabinoid chosen for this experiment was anandamide (AEA). It has been named the “bliss” molecule because it is an endogenous cannabinoid that binds to the CB1 receptor the same way that THC does[32]. What this means is that this cannabinoid that your body naturally produces interacts and produces the same “bliss” or “joyous” sensation you feel as THC does when you consume it. Incredibly, this molecule is responsible for the “runner’s high” sensation you feel after long workouts[33]. It is the molecule your body produces naturally to attain homeostasis and balance, hence its nickname. It is important for this research to understand how exactly anandamide binds to the CB1 receptor to understand how an endogenous agonist interacts within the protein pocket formed.

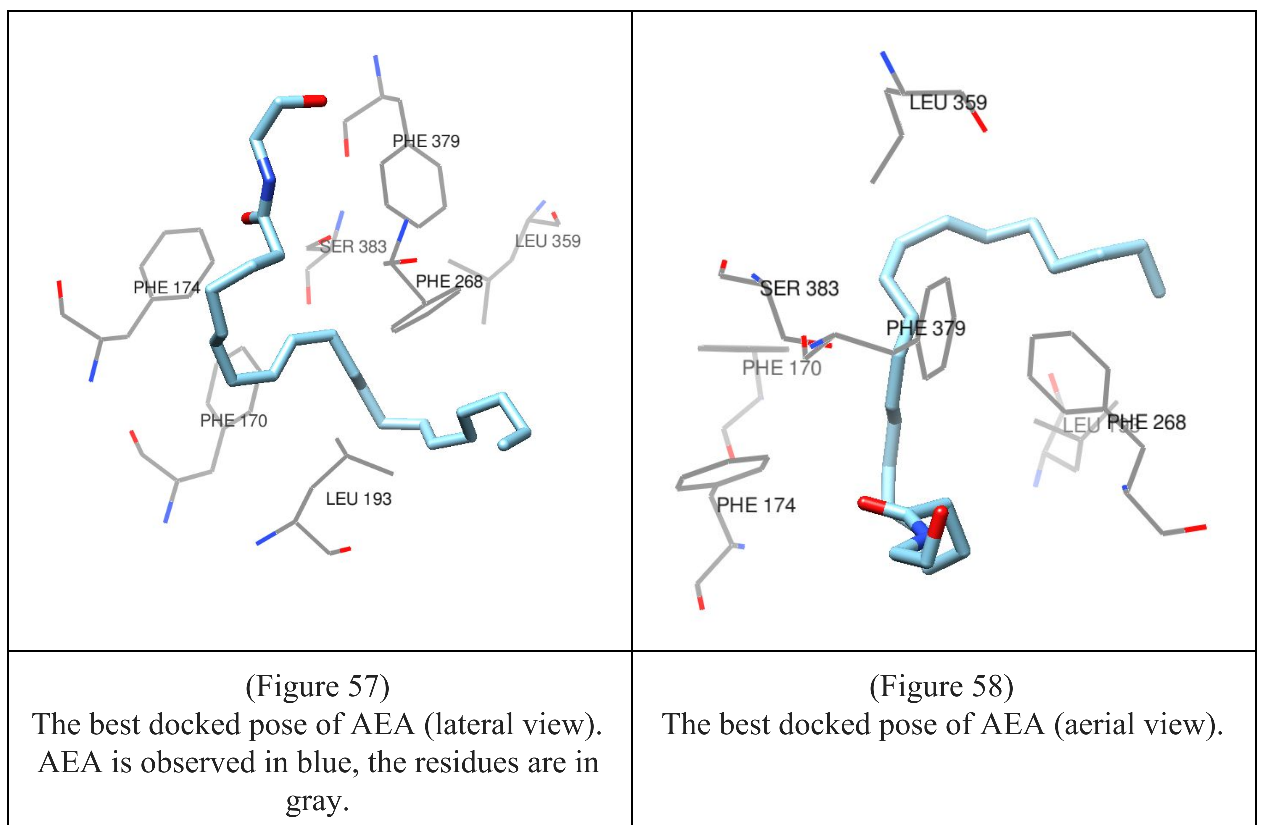

SwissTargetPrediction report:

Reference:
Gfeller D., Michielin O. & Zoete V.
Shaping the interaction landscape of bioactive molecules, *Bioinformatics* (2013) 29:3073-3079.

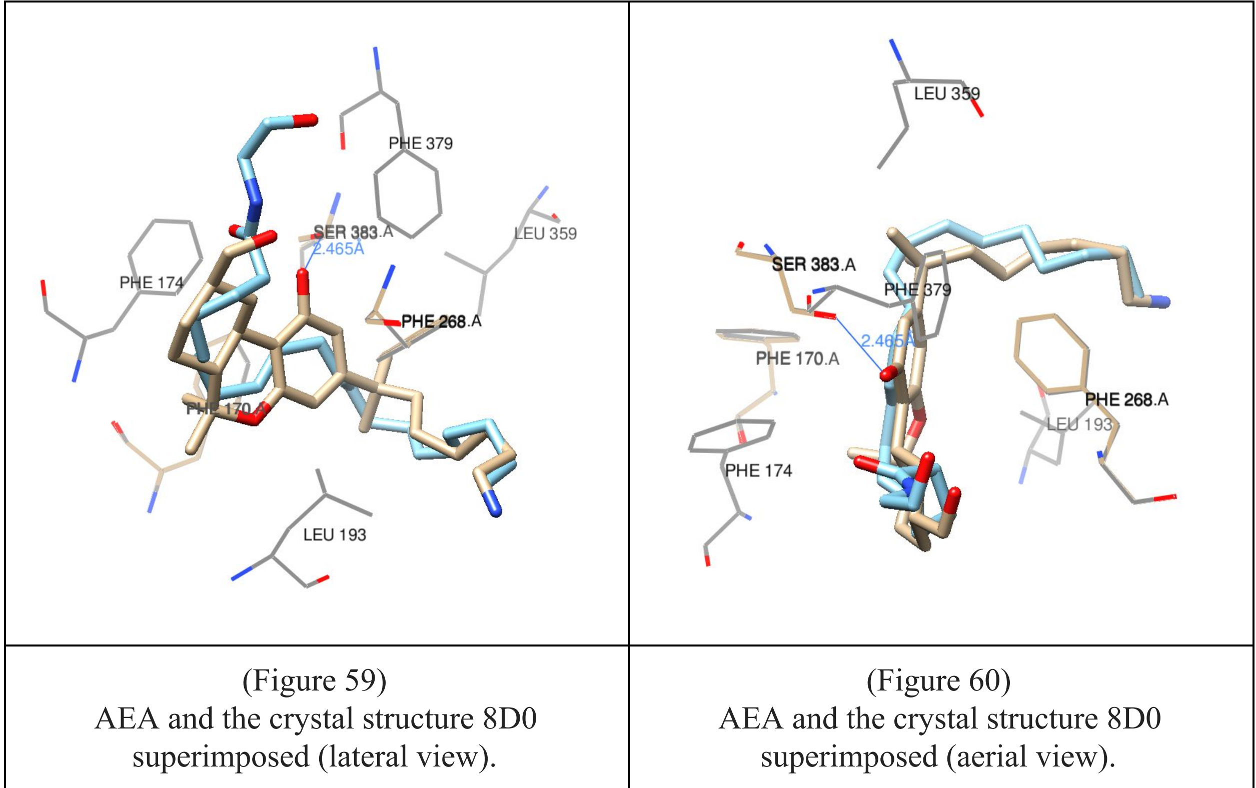
Target	Uniprot ID	Gene code	ChEMBL ID	Probability	# sim. cmpds (3D / 2D)	Target Class
Cannabinoid receptor 1	P21554	CNR1	CHEMBL218		361 / 197	Membrane receptor
Cannabinoid receptor 2	P34972	CNR2	CHEMBL253		229 / 105	Membrane receptor
Fatty-acid amide hydrolase 1	O00519	FAAH	CHEMBL2243		44 / 40	Enzyme
Transient receptor potential cation channel subfamily V member 1 *(by homology)*	Q8NER1	TRPV1	CHEMBL4794		14 / 16	Ion channel
Peroxisome proliferator-activated receptor gamma *(by homology)*	P37231	PPARG	CHEMBL235		8 / 4	Transcription Factor
Peroxisome proliferator-activated receptor alpha	Q07869	PPARA	CHEMBL239		8 / 4	Transcription Factor
Peroxisome proliferator-activated receptor delta *(by homology)*	Q03181	PPARD	CHEMBL3979		8 / 4	Transcription Factor
Monoglyceride lipase *(by homology)*	Q99685	MGLL	CHEMBL4191		9 / 5	Enzyme
Corticosteroid 11-beta-dehydrogenase isozyme 2 *(by homology)*	P80365	HSD11B2	CHEMBL3746		7 / 8	Enzyme
Thromboxane A2 receptor	P21731	TBXA2R	CHEMBL2069		5 / 2	Membrane receptor
Lipid-phosphate phosphatase	P34913	EPHX2	CHEMBL2409		38 / 15	Serine Protease
Corticosteroid 11-beta-dehydrogenase isozyme 1	P28845	HSD11B1	CHEMBL4235		61 / 5	Enzyme
Hydroxysteroid 11-beta-dehydrogenase 1-like protein *(by homology)*	Q7Z5J1	HSD11B1L			60 / 5	Enzyme
Cholinesterase	P06276	BCHE	CHEMBL1914		48 / 6	Enzyme
Acetylcholinesterase	P22303	ACHE	CHEMBL220		48 / 6	Enzyme

(Figure 56)
The SwissTargetPrediction showing AEA does have high probability to interact with the CB1 receptor.

The first step was to follow the protocol and insert the cannabinoid into the SwissTargetPrediction (Figure 56). This tool informed us that AEA did indeed have high probability to bind to the CB1 receptor which allowed us to move forward with the docking of AEA in the SwissDock program. When docked it did form a similar “L” shape to that of 8D0 (Figure 57 and 58). Some of the residues have been removed for the purpose of obtaining a clearer image. The full residue list can be seen in Figure 61.



(Figure 57) The best docked pose of AEA (lateral view). AEA is observed in blue, the residues are in gray.	(Figure 58) The best docked pose of AEA (aerial view).



(Figure 59) AEA and the crystal structure 8D0 superimposed (lateral view).	(Figure 60) AEA and the crystal structure 8D0 superimposed (aerial view).

AEA is a long, greasy chain so obvious differences will be noticed when comparing it to 8D0. When the two were superimposed they both lie in the same pocket and interact with the same residues, with some additions to AEA because it is a larger cannabinoid compared to 8D0. There is no hydrogen bond between AEA and seranine 383 because it lacks the alcohol group on that part of the chain. The AEA carbon chain also fits the very end of the pocket similar to how the amine chain does in 8D0 forming that bend that gives the distinct “L” shape. Overall it superimposes with over a 90% similarity to the corresponding crystal structure ligand 8D0 (Figures 59 and 60). What this tells us is that the endogenous cannabinoid, interacts similarly to the synthetic cannabinoid.

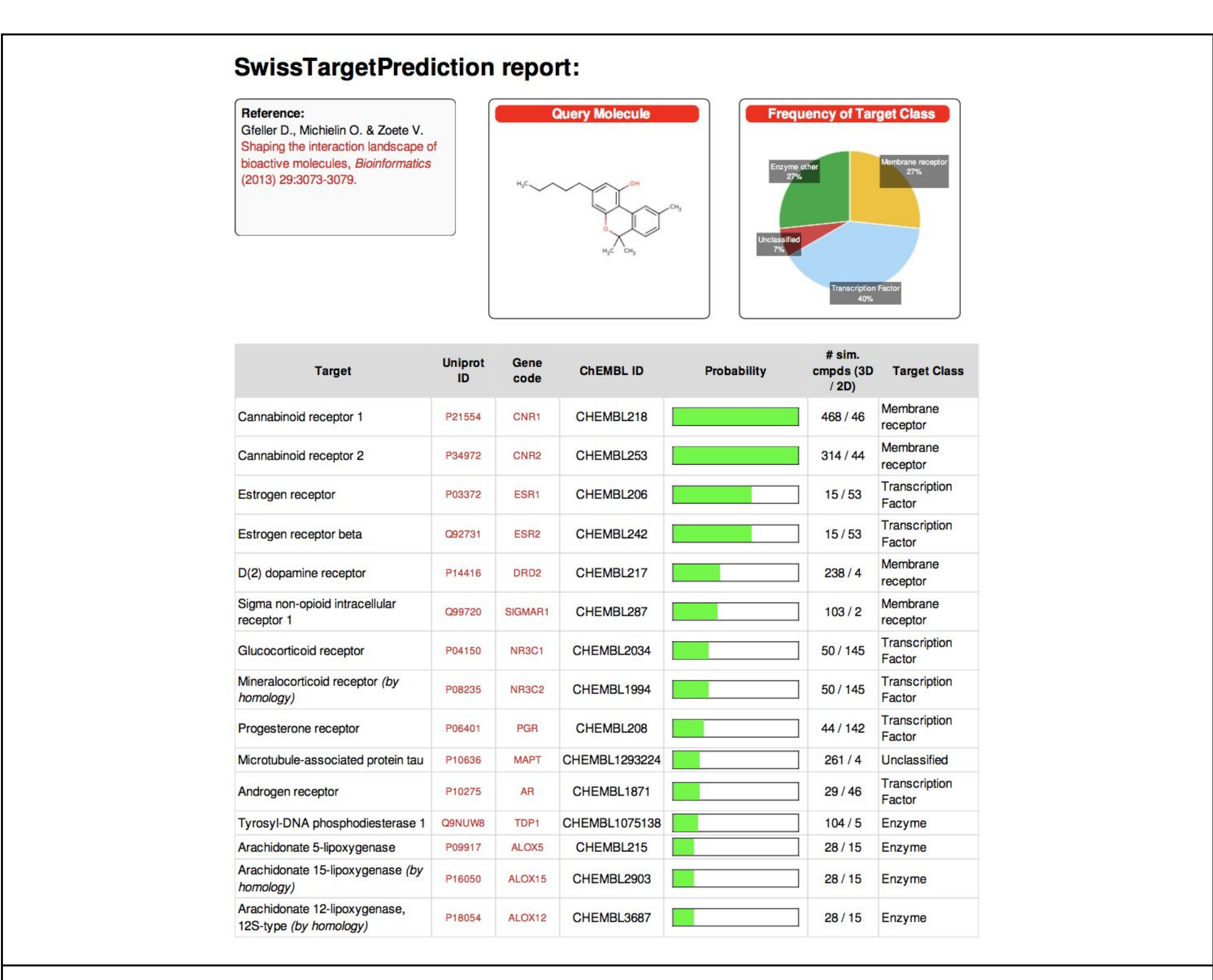
8D0	AEA
THR 197	THR 197
SER 383	SER 383
CYS 386	CYS 386
PHE 177	PHE 177
PHE 268	PHE 268
LEU 193	LEU 193
PHE 379	PHE 379
TRP 279	TRP 279
VAL 196	VAL 196
SER 173	SER 173
PHE 170	PHE 170
PHE 174	PHE 174
ILE 267	ILE 267
PHE 108	PHE 108
LEU 359	LEU 359
PRO 269	PRO 269
HSE 178	HSE 178
PHE 189	PHE 189
ALA 380	ALA 380
PHE 200	PHE 200
TRP 356	TRP 356
LEU 276	LEU 276
ILE 271	ILE 271
TYR 275	TYR 275
MET 363	MET 363
LYS 192	LYS 376
THR 201	

(Figure 61)
The full compiled residue list of AEA compared to 8D0. The ones they share are listed in blue, the unique residues for AEA are listed in purple.

After observing the residues interaction the list was compiled to identify the key residues that interact with AEA. This residue sheet was then compared to 8D0 to observe the similarities and differences. AEA is a longer chain which interacts with the residue lysine 376. 8D0 is a long chain, but not as long. The dimethyl constituent on adjacent to the ther is what is is responsible for the interaction with lysine 192 and threonine 201. The list can be seen in Figure 61.

Cannabinol (CBN) 5XR8:

The next agonist cannabinoid chosen for docking was the phytocannabinoid agonist binding cannabinoid cannabinol (CBN). CBN is a derivative of THC and has been widely studied by the scientific community. CBN has shown to treat ailments such as insomnia[22], pain[1], and cancer[23]. Currently CBN is only available in a very limited number of legal medical cannabis states and only a few companies are known to produce it. It is reported that this cannabinoid does induce a psychoactive effect similar to ingestion of THC, but has its own unique effects in treatment. CBN has never had published test results before so this will be the first known images of CBN interacting with the CB1 receptor.



SwissTargetPrediction report:

Reference:
Gfeller D., Michielin O. & Zoete V.
Shaping the interaction landscape of bioactive molecules, *Bioinformatics* (2013) 29:3073-3079.

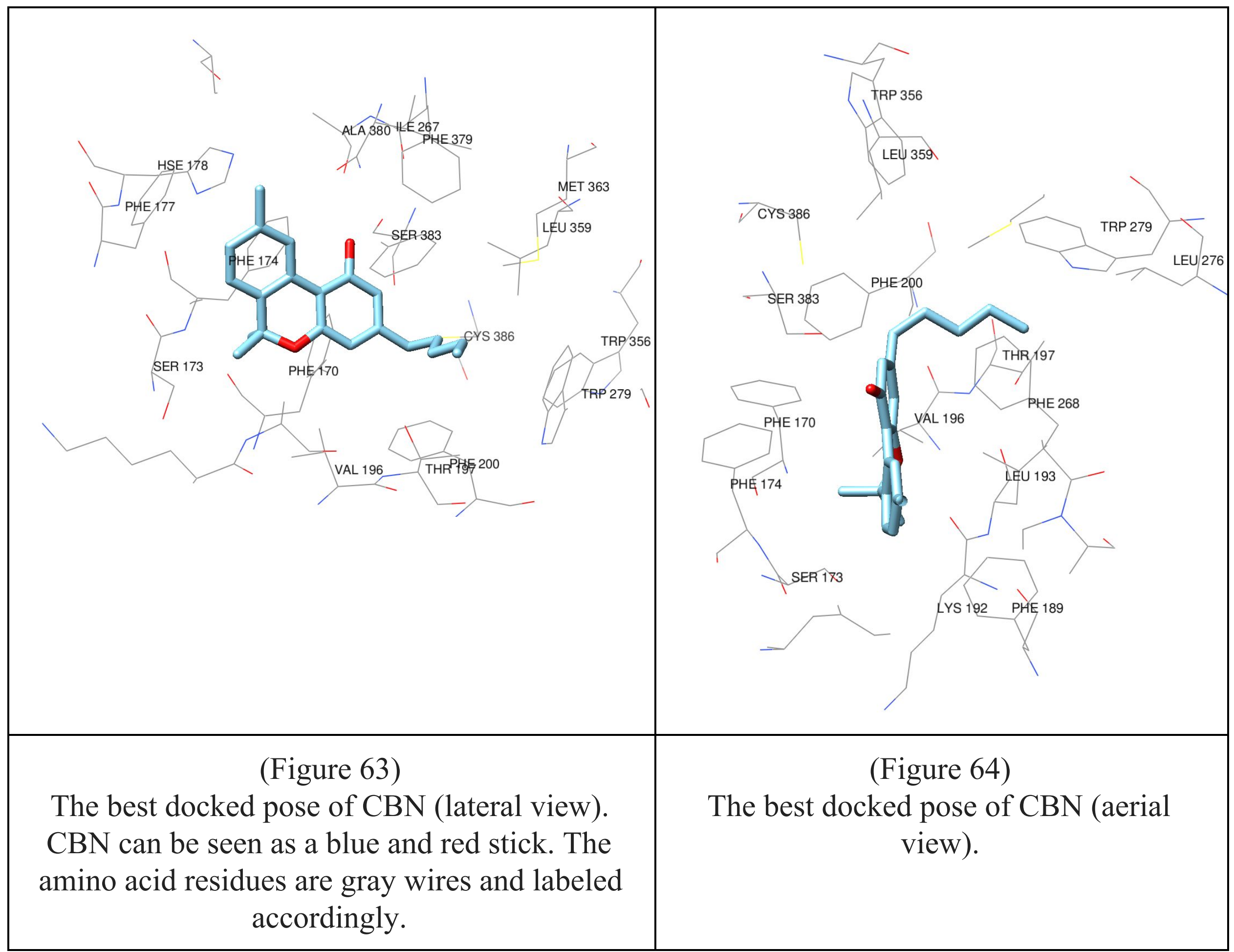
Query Molecule

Frequency of Target Class

Target	Uniprot ID	Gene code	ChEMBL ID	Probability	# sim. cmpds (3D / 2D)	Target Class
Cannabinoid receptor 1	P21554	CNR1	CHEMBL218		468 / 46	Membrane receptor
Cannabinoid receptor 2	P34972	CNR2	CHEMBL253		314 / 44	Membrane receptor
Estrogen receptor	P03372	ESR1	CHEMBL206		15 / 53	Transcription Factor
Estrogen receptor beta	Q92731	ESR2	CHEMBL242		15 / 53	Transcription Factor
D(2) dopamine receptor	P14416	DRD2	CHEMBL217		238 / 4	Membrane receptor
Sigma non-opioid intracellular receptor 1	Q99720	SIGMAR1	CHEMBL287		103 / 2	Membrane receptor
Glucocorticoid receptor	P04150	NR3C1	CHEMBL2034		50 / 145	Transcription Factor
Mineralocorticoid receptor *(by homology)*	P08235	NR3C2	CHEMBL1994		50 / 145	Transcription Factor
Progesterone receptor	P06401	PGR	CHEMBL208		44 / 142	Transcription Factor
Microtubule-associated protein tau	P10636	MAPT	CHEMBL1293224		261 / 4	Unclassified
Androgen receptor	P10275	AR	CHEMBL1871		29 / 46	Transcription Factor
Tyrosyl-DNA phosphodiesterase 1	Q9NUW8	TDP1	CHEMBL1075138		104 / 5	Enzyme
Arachidonate 5-lipoxygenase	P09917	ALOX5	CHEMBL215		28 / 15	Enzyme
Arachidonate 15-lipoxygenase *(by homology)*	P16050	ALOX15	CHEMBL2903		28 / 15	Enzyme
Arachidonate 12-lipoxygenase, 12S-type *(by homology)*	P18054	ALOX12	CHEMBL3687		28 / 15	Enzyme

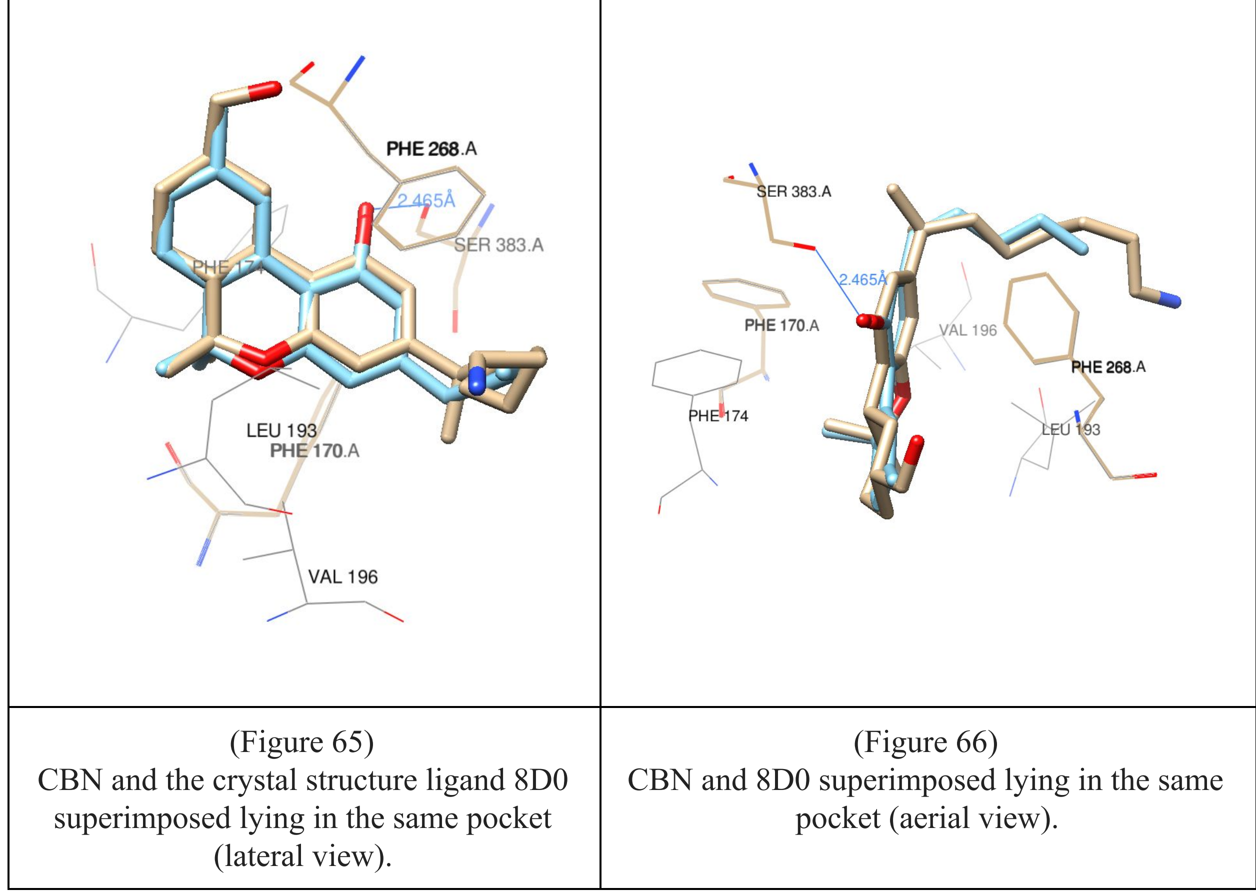
(Figure 62)
The SwissTargetPrediction report shows CBN has high probability to interact with the CB1 receptor.

When CBN was inserted into the SwissTargetPrediction it showed that the molecule had highest probability to bind to the CB1 receptor. This cannabinoid had the best results of the experimental agonists sent in for testing, interacting with only CB1 and CB2 receptors. This tells us that when ingested this cannabinoid should have very little side effects.



(Figure 63) The best docked pose of CBN (lateral view). CBN can be seen as a blue and red stick. The amino acid residues are gray wires and labeled accordingly.	(Figure 64) The best docked pose of CBN (aerial view).

When viewing in the Chimera program, CBN interacts in a similar form that 8D0 does forming an "L" shape within the protein-pocket it forms (Figure 63 and 64). It interacts with similar residues to 8D0, some aren't shown for image purposes but a full list can be found in Figure 67.



(Figure 65) CBN and the crystal structure ligand 8D0 superimposed lying in the same pocket (lateral view).	(Figure 66) CBN and 8D0 superimposed lying in the same pocket (aerial view).

When the two are superimposed there are some slight differences but overall the molecules are extremely similar. CBN contains an alcohol group (#1.1 Lig 1 O) on the third ring that is within hydrogen bond distance to seranine 383 even though it is not shown. The pentyl constituent adjacent to the alcohol group on the ring also follows the same bend that the amine constituent on 8D0 does, forming that “L” like shape. Overall there is over a 90% similarity in the docking of CBN when compared to the crystal structure ligand.

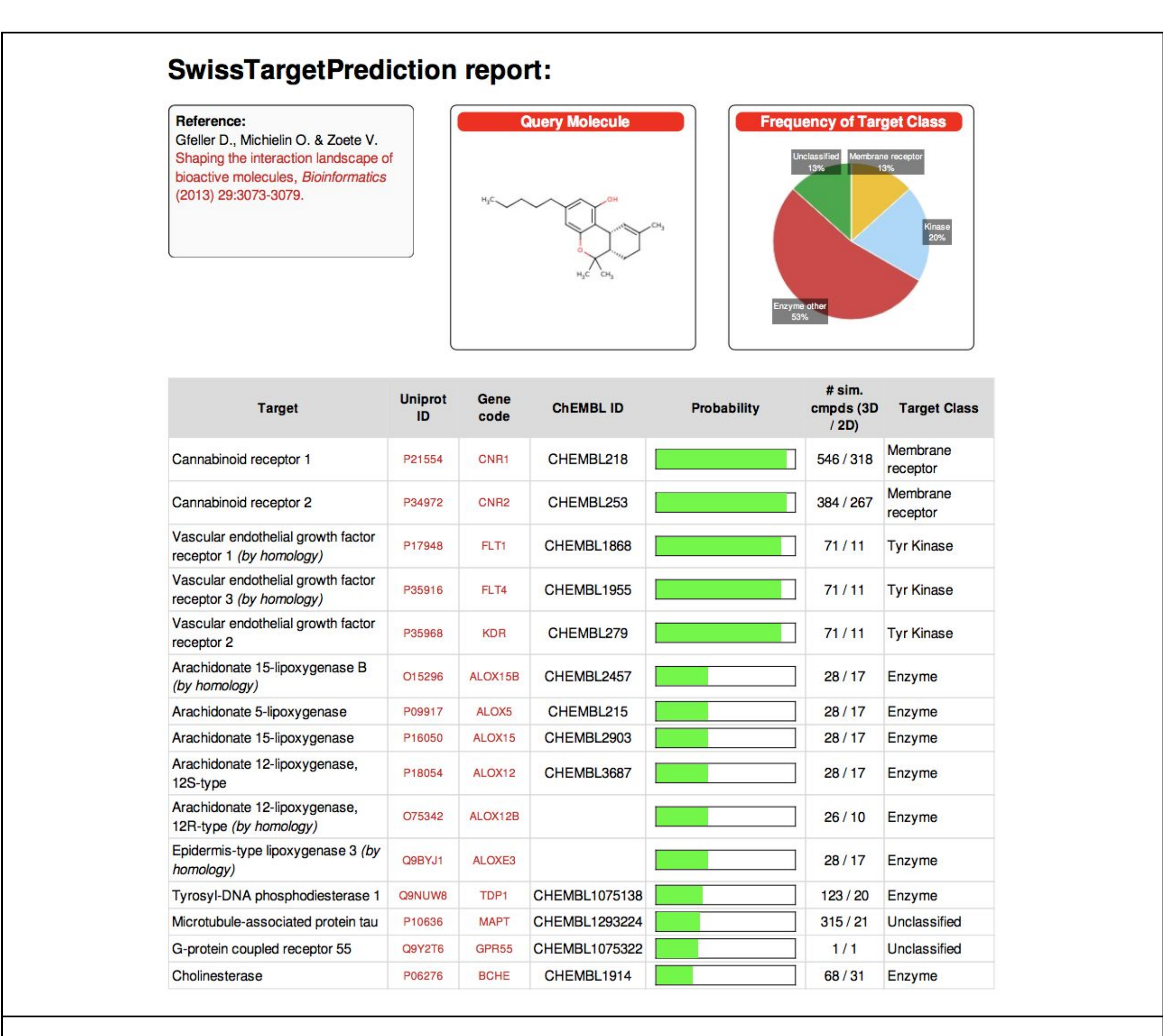
8D0	AEA	CBN
THR 197	THR 197	THR 197
SER 383	SER 383	SER 383
CYS 386	CYS 386	CYS 386
PHE 177	PHE 177	PHE 177
PHE 268	PHE 268	PHE 268
LEU 193	LEU 193	LEU 193
PHE 379	PHE 379	PHE 379
TRP 279	TRP 279	TRP 279
VAL 196	VAL 196	VAL 196
SER 173	SER 173	SER 173
PHE 170	PHE 170	PHE 170
PHE 174	PHE 174	PHE 174
ILE 267	ILE 267	ILE 267
PHE 108	PHE 108	PHE 108
LEU 359	LEU 359	LEU 359
PRO 269	PRO 269	PRO 269
HSE 178	HSE 178	HSE 178
PHE 189	PHE 189	PHE 189
ALA 380	ALA 380	ALA 380
PHE 200	PHE 200	PHE 200
TRP 356	TRP 356	TRP 356
LEU 276	LEU 276	LEU 276
ILE 271	ILE 271	
TYR 275	TYR 275	
MET 363	MET 363	
LYS 192		LYS 192
THR 201		
	LYS 376	

(Figure 67)
The full compiled residue list of CBN compared to 8D0 and AEA. The ones they share are listed in blue, the residues that 8D0 and AEA share are listed in purple, the residues CBN and 8D0 share are listed in orange. The residues that are unique to 8D0 and AEA are listed in red.

When comparing the residues there is less residue interaction with CBN because it is a slightly smaller molecule. It does share the lysine 192 residue with 8D0 because they both contain a dimethyl constituent adjacent to the ether group on the second ring. Overall there is a single hydrogen bond interaction, but the rest is a strictly hydrophobic interact with the protein pocket with the list of residues shown above (Figure 67).

Tetrahydrocannabinol (THC) 5XR8:

The last cannabinoid, and the most famous, we have chosen for this experiment is tetrahydrocannabinol, or THC for short. THC is the active component in cannabis smoke or vapor that is attributed to its unique psychoactive effect it has on its consumers[34]. THC has been widely studied and has been shown to treat a wide variety of diseases and ailments such as pain[1], nausea[24], migraines[4], stress[25], and cancer[27]. This research will show exactly how THC interacts with the CB1 receptor. Knowing that and comparing it to the rest of the results is important to see why THC induces a unique effect and where the differences if any are.



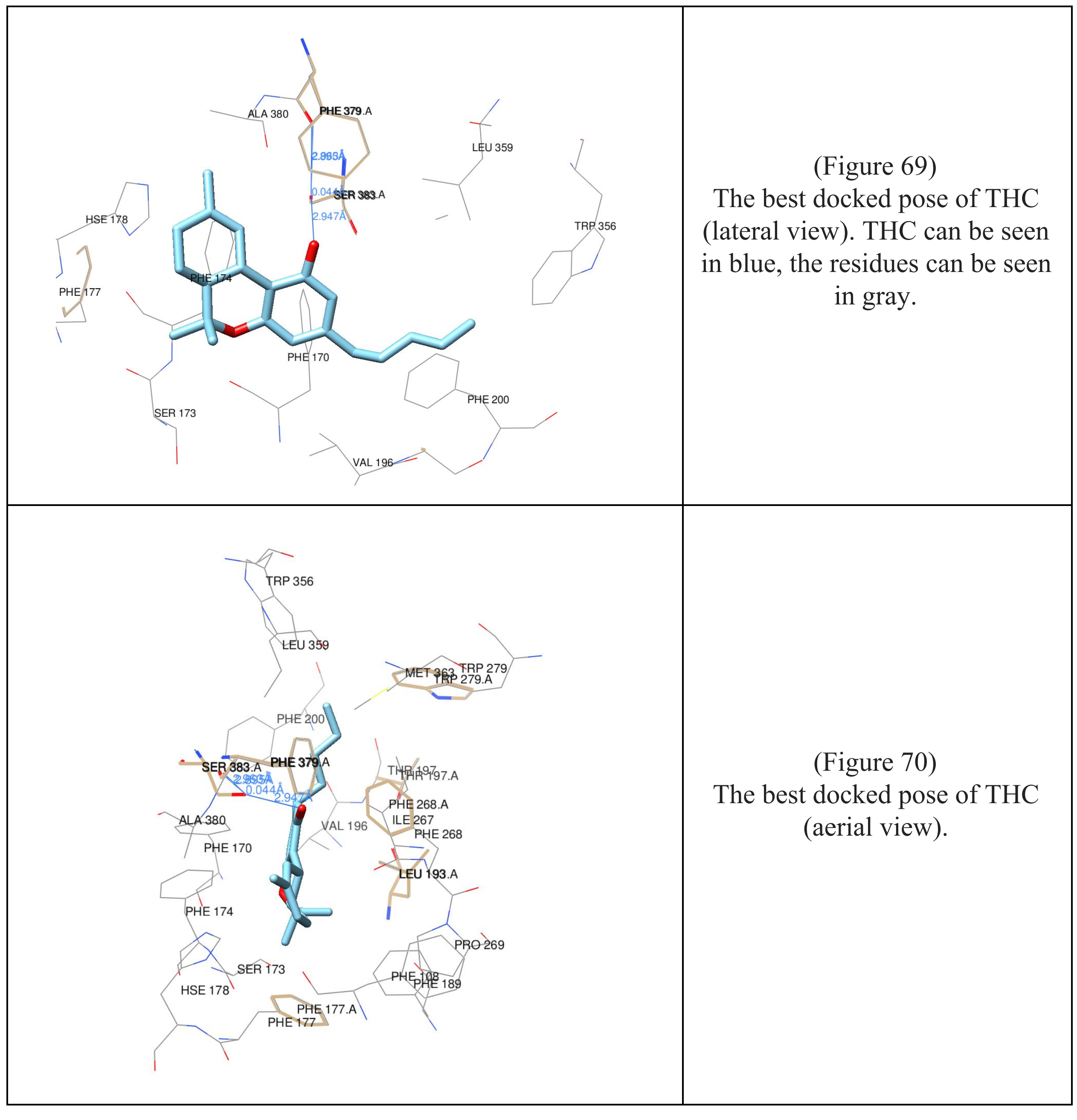
SwissTargetPrediction report:

Reference:
Gfeller D., Michielin O. & Zoete V.
Shaping the interaction landscape of bioactive molecules, *Bioinformatics* (2013) 29:3073-3079.

Target	Uniprot ID	Gene code	ChEMBL ID	Probability	# sim. cmpds (3D / 2D)	Target Class
Cannabinoid receptor 1	P21554	CNR1	CHEMBL218		546 / 318	Membrane receptor
Cannabinoid receptor 2	P34972	CNR2	CHEMBL253		384 / 267	Membrane receptor
Vascular endothelial growth factor receptor 1 *(by homology)*	P17948	FLT1	CHEMBL1868		71 / 11	Tyr Kinase
Vascular endothelial growth factor receptor 3 *(by homology)*	P35916	FLT4	CHEMBL1955		71 / 11	Tyr Kinase
Vascular endothelial growth factor receptor 2	P35968	KDR	CHEMBL279		71 / 11	Tyr Kinase
Arachidonate 15-lipoxygenase B *(by homology)*	O15296	ALOX15B	CHEMBL2457		28 / 17	Enzyme
Arachidonate 5-lipoxygenase	P09917	ALOX5	CHEMBL215		28 / 17	Enzyme
Arachidonate 15-lipoxygenase	P16050	ALOX15	CHEMBL2903		28 / 17	Enzyme
Arachidonate 12-lipoxygenase, 12S-type	P18054	ALOX12	CHEMBL3687		28 / 17	Enzyme
Arachidonate 12-lipoxygenase, 12R-type *(by homology)*	O75342	ALOX12B			26 / 10	Enzyme
Epidermis-type lipoxygenase 3 *(by homology)*	Q9BYJ1	ALOXE3			28 / 17	Enzyme
Tyrosyl-DNA phosphodiesterase 1	Q9NUW8	TDP1	CHEMBL1075138		123 / 20	Enzyme
Microtubule-associated protein tau	P10636	MAPT	CHEMBL1293224		315 / 21	Unclassified
G-protein coupled receptor 55	Q9Y2T6	GPR55	CHEMBL1075322		1 / 1	Unclassified
Cholinesterase	P06276	BCHE	CHEMBL1914		68 / 31	Enzyme

(Figure 68)
The SwissTargetPrediction report shows THC has high probability to interact with the CB1 receptor.

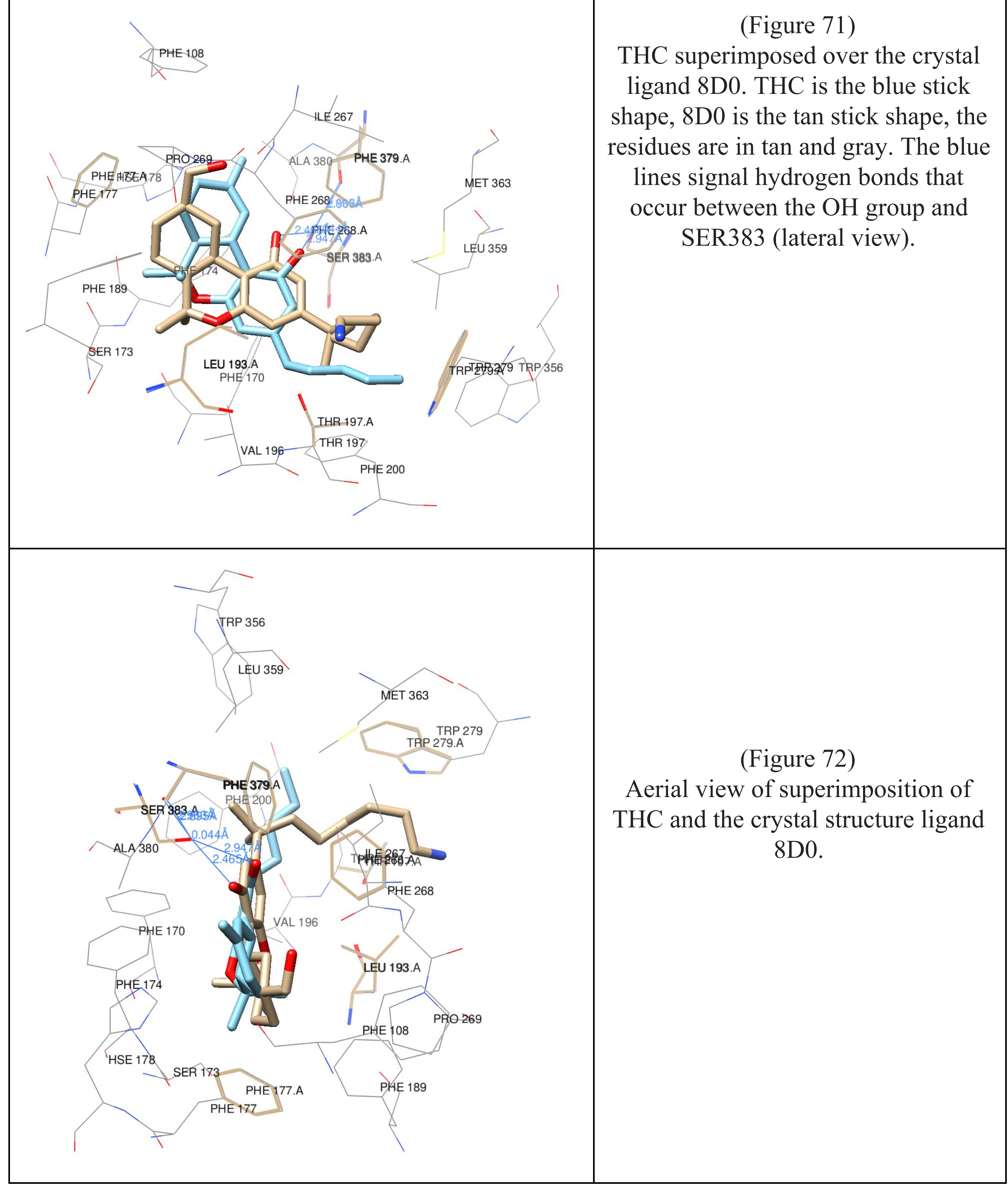
The SwissTargetPrediction report shows that THC has high probability to bind to the CB1 receptor, which was anticipated. It also has high probability to interact with Vascular endothelial growth factor receptor 1, 2 and 3. This tells us that THC interacts with other receptors to produce a unique effect, when compared to CBN (Figure 62), AEA (Figure 56) and 8D0 (Figure 21) who do not. After knowing this and then generating the molecule using Chem3D we sent it into docking via the SwissDock program. The best docking position and the key residues it interacts with can be shown below in Figures 69 and 70. Not all of the residues may be shown for imagery purposes of this report. The full list can be observed in Figure 73.



(Figure 69)
The best docked pose of THC (lateral view). THC can be seen in blue, the residues can be seen in gray.

(Figure 70)
The best docked pose of THC (aerial view).

What can be observed is that THC, like CBN and 8D0 has a hydrogen bond with seranine 383. The rest of the interaction with the pocket is hydrophobic. It also differs from the rest of the cannabinoids in the protein pocket because instead of the pentyl chain turning and forming an “L” shape it goes forward and does not bend at all (Figure 69 and Figure 70).



(Figure 71)
THC superimposed over the crystal ligand 8D0. THC is the blue stick shape, 8D0 is the tan stick shape, the residues are in tan and gray. The blue lines signal hydrogen bonds that occur between the OH group and SER383 (lateral view).

(Figure 72)
Aerial view of superimposition of THC and the crystal structure ligand 8D0.

When superimposed over the crystal ligand 8D0, THC stands above it significantly, again going straight and not bending. THC also interacts with significantly less residues (Figure 71 and 72). THC superimposed in the same pocket, over the same site meaning that it had a 90% similarity to the crystal structure ligand in interaction with the protein pocket.

AGONIST 5XR8 Residue List			
8D0	AEA	CBN	THC
THR 197	THR 197	THR 197	THR 197
SER 383	SER 383	SER 383	SER 383
PHE 177	PHE 177	PHE 177	PHE 177
PHE 268	PHE 268	PHE 268	PHE 268
LEU 193	LEU 193	LEU 193	LEU 193
PHE 379	PHE 379	PHE 379	PHE 379
TRP 279	TRP 279	TRP 279	TRP 279
VAL 196	VAL 196	VAL 196	VAL 196
SER 173	SER 173	SER 173	SER 173
PHE 170	PHE 170	PHE 170	PHE 170
PHE 174	PHE 174	PHE 174	PHE 174
ILE 267	ILE 267	ILE 267	ILE 267
PHE 108	PHE 108	PHE 108	PHE 108
LEU 359	LEU 359	LEU 359	LEU 359
PRO 269	PRO 269	PRO 269	PRO 269
HSE 178	HSE 178	HSE 178	HSE 178
PHE 189	PHE 189	PHE 189	PHE 189
ALA 380	ALA 380	ALA 380	ALA 380
PHE 200	PHE 200	PHE 200	PHE 200
TRP 356	TRP 356	TRP 356	TRP 356
MET 363	MET 363	MET 363	MET 363
CYS 386	CYS 386	CYS 386	
LEU 276	LEU 276	LEU 276	
ILE 271	ILE 271		
TYR 275	TYR 275		
LYS 192		LYS 192	
THR 201			
	LYS 376		

(Figure 73)
The list compiled comparing THC to the experimental cannabinoids CBN, AEA, and 8D0. The residues they all share are listed in blue. The residues that CBN, AEA, and 8D0 share are in purple. The residues that AEA and 8D0 share are listed in organe. The residues that CBN and 8D0 share are listed in green. The unique residues for AEA and 8D0 are listed in red.

All of these cannabinoids do produce a unique psychoactive effect, but only THC stands differently in the protein pocket and has less residue interaction. Many people have died from synthetic cannabinoid constituents of THC, such as JWH-018, the active ingredient in synthetic cannabis nicknamed "Spice" or "K2", but not from THC[35][36]. In fact, there has never been a sole THC overdose on record. This could be because it forms a unique shape and interacts with less residues in the protein pocket in the CB1 receptor site.

Agonist Receptor-Ligand Complex 5XR8Results:

In conclusion of the agonists we can successfully say that each agonist interacts with the CB1 receptor in a similar fashion. Each agonist cannabinoid formed an "L" shape within the CB1 protein pocket with the exception of THC. The **Δ**G values are all very similar and coincide with the IC50 values (Figure 74). The **Δ**G values of THC and CBN are slightly less negative was when compared to 8D0 and AEA because they are smaller molecules. The larger cannabinoids (8D0 and AEA) did have a similar **Δ**G values and the smaller ones (CBN and THC) also contained similar values to one another (Figure 74). When the residues were compiled we observe how all contain similar residues, those residues are listed in blue in Figure 75. This list will later be compared to the antagonist/ inverse agonist cannabinoid residue interaction to distinguish which residues are responsible for the unique function of an agonist cannabinoid.

5XR8			
Ligand	Type	IC50 Value (μM)	Δ G Value (kcal/mol)
8D0	Agonist	Unknown	-10.42
Anandamide	Agonist	2.10	-10.11
THC	Agonist	2.70	-8.19
CBN	Agonist	2.10	-8.96

(Figure 74)
The **Δ**G values compared to the IC50 of the antagonist/ inverse agonist cannabinoids.

<table>
<tr><td>

Agonist Residues
THR 197
SER 383
PHE 177
PHE 268
LEU 193
PHE 379
TRP 279
VAL 196
SER 173
PHE 170
PHE 174
ILE 267
PHE 108
LEU 359
PRO 269
HSE 178
PHE 189
ALA 380
PHE 200
TRP 356
MET 363

</td><td>(Figure 75)
The compiled list of residues that all agonists share.</td></tr>
</table>

Conclusion:

Results:

In conclusion the first step was to evaluate IC50 values and ΔG values to see if there was a correlation between reduced activity and energy released from the docking. The two did correspond and their results can be seen in Figure 76. In order to determine which residues were responsible for the antagonist property and which ones were responsible for the agonist property we had to take a few steps. First, was compiling of all the amino acid residues from each cannabinoid within the 3.7 Å pocket that we formed. Second was to see which ones each functional group shared, and which ones were different. Last was to compare all the residues and see which were shared and which were different. Even if one residue with an antagonist/ inverse agonist cannabinoid was shared with an agonist cannabinoid that residue would be put in the

shared category. The reason being because that residue isn't responsible for the determining of the function, only the uniqueness of the effects from that specific molecule. The last thing to note is that when Dr. Stevens originally conducted this study, they looked at very large, bulky antagonist/ inverse agonist cannabinoids for the 5TGZ site. For this study we took a small known antagonist cannabinoid and compared it to the others. This will diminish the list of residues significantly required for an antagonist/ inverse agonist to function because the molecule is smaller, therefore interacts with less residues.

Lig short name	Lig Type	PDBID	Size of pocket (Å)	Center of pocket (x,y,z)	IC50 Value (μM)	Δ G Value (kcal/mol)
ZDG	Antagonist	5TGZ	5x5x5	x=(43.502) y=(27.787) z=(318.810)	Unknown	-9.96
Rimonabant	Antagonist	5TGZ	5x5x5	x=(43.502) y=(27.787) z=(318.810)	Unkown	-9.83
Taranabant	Antagonist	5TGZ	5x5x5	x=(43.502) y=(27.787) z=(318.810)	0.00030	-9.26
CBD	Antagonist	5TGZ	5x5x5	x=(43.502) y=(27.787) z=(318.810)	2.10	-8.28
8D0	Agonist	5XR8	5x5x5	x=(-42.646) y=(-156.676) z=(306.315)	Unknown	-10.42
Anandamide	Agonist	5XR8	5x5x5	x=(-42.646) y=(-156.676) z=(306.315)	2.10	-10.11
THC	Agonist	5XR8	5x5x5	x=(-42.646) y=(-156.676) z=(306.315)	2.70	-8.19
CBN	Agonist	5XR8	5x5x5	x=(-42.646) y=(-156.676) z=(306.315)	2.10	-8.96

(Figure 76)
The full chart outlying cannabinoid, coordinates, size of pocket, known IC50, and experimental **Δ**G values from each of the cannabinoids.

In Figure 77 we see that there are many residues both antagonist/ inverse agonist cannabinoids and agonist cannabinoids share. There is only a very small number of residues that define which will produce the specific effect of an antagonist or inverse agonist functioning cannabinoid versus an agonist functioning cannabinoid. This list tells us which residues to target in order to generate the specific function of antagonist/ inverse agonist, or to produce an agonist function. In conclusion after compiling, comparing, and contrasting all the cannabinoids residues IC50 and **Δ**G values this research can successfully say that the way agonists/ inverse agonists and agonists bind are different and predictable.

Agonist	Antagonist
THR 197	THR 197
SER 383	SER 383
PHE 268	PHE 268
PHE 170	PHE 170
PHE 174	PHE 174
HSE 178	HSE 178
TYR 275	TYR 275
ALA 380	ALA 380
MET 363	MET 363
VAL 196	VAL 196
CYS 386	CYS 386
TRP 279	TRP 279
PHE 379	PHE 379
LEU 193	LEU 193
LEU 359	LEU 359
TRP 356	TRP 356
PHE 108	PHE 102
PHE 177	MET 103
PHE 189	MET 384
PHE 200	GLY 166
PRO 269	ILE 105
ILE 267	LEU 387
	SER 167

(Figure 77)
This is the residue sheet that determines which residues both antagonist and agonist cannabinoids share and which are responsible for their unique effect. The residues shared are outlined in green, the ones unique to the function of antagonist/ inverse agonist are in pink and the residues for the agonist are in blue.

The Future:

Figure 77 is the key to cannabinoid synthesis. This procedure and list will be used in the future to manufacture and design cannabinoids to target key residues in order to produce a specific biological function whether it be antagonist, inverse agonist, or agonist. This is the theoretical protocol to be picked up by any research team to examine, identify, and construct cannabinoids to target these specific residues. In conclusion, the future of this research is that this is the blueprint for cannabinoid synthesis, to be able to devise a cannabinoid, that interacts with specific residues to generate specific effects.

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