STUDY OF AN ACTIVE-STATE CB1 RECEPTOR MODEL AND JWH COMPOUND INTERACTIONS TO PREDICT NEW EMERGING SYNTHETIC CANNABINOIDS

by
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A thesis submitted to the faculty of the University of Mississippi in partial fulfillment of the requirements of the Sally McDonnell Barksdale Honors College

Oxford
May 2016

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ACKNOWLEDGEMENTS

I would like to thank Dr. Murrell Godfrey for his guidance in advising this project. Thanks to Dr. Robert Doerksen for serving as a second reader and second advisor for this project, and thanks to Dr. Doerksen and Dr. Kuldeep Roy and their group for access to their Maestro license and active-state CB1 model. I would also like to thank Dr. Kuldeep Roy and Dr. Pankaj Pandey for their tutorials to Maestro and their invaluable assistance in the lab.

I would also like to thank Dr. Douglass Sullivan-González, Dr. John Samonds, and Dr. Debra Young for their advising and support during my tenure in the Sally McDonnell Barksdale Honors College.

This work was made possible in part by Grant Numbers P20GM104932 and R15GM119061 from the National Institute of General Medical Sciences (NIGMS), a component of the National Institutes of Health (NIH). Its contents are solely the responsibility of the authors and do not necessarily represent the official view of NIGMS or NIH. This investigation was conducted in part in a facility constructed with support from the Research Facilities Improvements Program (C06RR14503) from the National Institutes of Health (NIH) National Center for Research Resources. Also, thanks to the Department of BioMolecular Sciences for computational resources

ABSTRACT

KELSEY PETTUS: Study of An Active-State CB1 Receptor Model and JWH Compound Interactions to Predict New Emerging Synthetic Cannabinoids
(Under the direction of Dr. Murrell Godfrey and Dr. Robert J. Doerksen)

In recent years, a new class of compounds, called synthetic cannabinoids, have made their appearance in the market as a substitute for illegal marijuana or its constituent natural cannabinoids. These compounds, which are active at the same G-protein coupled receptors (GPCRS) as cannabinoids, are continuing to gain popularity because of the same cannabinoid-like effects they create. Though two cannabinoid receptors have been identified in humans, known as CB1 and CB2, synthetic cannabinoid activity at the CB1 receptor has been the focus of much research regarding synthetic cannabinoid ligand binding. In this study, the structure of the CB1 receptor is further analyzed in the binding of a class of synthetic cannabinoid ligands, known as JWH compounds, to the CB1 receptor. An active-state CB1 model that was previously created by Doerksen R. et al. and modeled from Bovine Rhodopsin and other GPCRs is used in this study. A dataset of twenty-one active CB1 agonists JWH compounds from the naphthoylindole family were docked to the model in order to further analyze key interacting residues on the CB1 receptor. Of these twenty-one compounds, all twenty-one were able to bind to the

CB1 active-state model. The Glide docking score of each ligand generated from Maestro computational modeling software was collected and compared to that of Delta-9-tetrahydrocannabinol, the main psychoactive component of marijuana.

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INTRODUCTION

In the United States, marijuana is ranked as the second most pervasive recreational drug. The first most pervasive recreational drug in the United States is alcohol. Though the possession, use, and distribution of marijuana are still under federal control, the legal status and usage patterns of this drug are changing rapidly. Currently, twenty-three states and the District of Columbia have passed laws that permit and regulate the usage of marijuana for medicinal purposes, and four states plus the District of Columbia have passed laws that allow for marijuana to be used for recreational and medical purposes.[1]

Because the possession, distribution, and use of marijuana continue to be considered offenses in federal jurisdictions, drug distributers have been motivated to create diverse classes of synthetic analogs as a legal alternative to traditional marijuana. These various compounds are marketed to the public for recreational usage and have been found to possess the same cannabinoid-like effects as marijuana such as euphoria, relaxation, increase in sensory awareness, and increase in creative thinking.[1] Until 2012, manufacturers sold these synthetic cannabinoid

mixtures, also called "new psychoactive substances", in gas stations, through the Internet, and drug paraphernalia stores. They were sold in the form of shredded plant material coated in cannabinoid spray liquid to be smoked as herbal incense or liquids to be vaporized as liquid incense and marked "not for human consumption".[8,9] In recent years, these compounds have gained much attention from the forensic community because of their adverse consequences on human health.[1] Many of these synthetic compounds have been found to be 100 times more potent than the main psychoactive component found in marijuana, Δ^9-Tetrahydrocannabinol (THC), and can cause life-threatening problems such as high blood pressure, vomiting, and seizures.[2]

The specific classes of compounds that have gained much attention from researchers include those compounds of the following series: HU, CP, JWH, AM, RCS, WIN, and in recent years, UR and XLR[2]. These compounds mimic the effects caused by THC, which was discovered in 1964 by researchers Yechiel Gaoni and Raphael Mechoulam. Because the components of cannabis had been studied, but no structure of the major psychoactive component had been determined, the discovery of the structure of THC was a momentous breakthrough for the scientific community.[2] The classification of the structure of THC led to new insights into the study of cannabinoids. The structure of THC is shown in Figure 1.

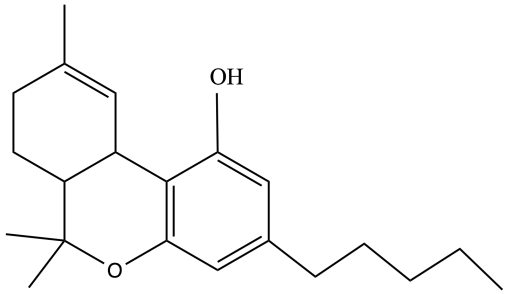


Figure 1: Structure of Δ^9 - Tetrahydrocannabinol (THC)

Because synthetic cannabinoids are functionally similar to THC, they bind to the same cannabinoid receptors in the peripheral organs and the brain.[3] Researchers have identified two known types of cannabinoid receptors that are G-coupled protein receptors (GPCRs). These receptors are known as CB1, located primarily in the human brain, and CB2, located primarily in the immune system and peripheral organs.[3] The existence of cannabinoid receptors was first discovered in rat brain in 1988 in which the experimental data described a G-protein coupled receptor in the rat brain that bound natural cannabinoids.[2] Shortly after the discovery of the cannabinoid receptors in rat brain, researchers were able to map the distribution of cannabinoid receptors in human peripheral organs (CB2) and brain (CB1).[1] Though the binding affinities of compounds for both CB1 and CB2 have been studied in the past, a rise in studies on the binding affinities of compounds

with the CB1 receptor has increased with the rising popularity of synthetic cannabinoids.[1] Research shows that it is the CB1 receptor that is responsible for the psychotropic effects caused by cannabis. Because of this, a ligand's ability to bind to the CB1 receptor and act as an agonist could indicate its potential to be a recreational use substitute for marijuana.[1]

Though the precise three-dimensional structure of the CB1 receptor is unknown, it is known that the CB1 and CB2 receptors are G-protein coupled receptors (GPCRs), and they are embedded within the cell membrane. As a GPCR, the CB1 receptor contains seven-helical transmembrane domains connected by three extracellular and three intracellular loops. [7,11] Ligands are the molecules that bind to the GPCRs, control their activity, and modify their biological functions. When an agonist ligand binds to the CB1 receptor, a conformational change causes the receptor to interact with G-proteins contained within the cell.[10] Because of these conformational movements and corresponding G-protein interactions, knowledge about novel ligand binding to the CB1 receptor could be beneficial.[6] Using the Bovine Rhodopsin X-ray crystal structure as a template, several CB1 model structures have been reported. These model structures have further been used to discover novel CB1 ligands, but none of these models provide for the precise three-dimensional structure of the CB1 receptor and cannabinoid ligand binding.[4,] Studies have proposed that there is a hydrophobic binding pocket that interacts with the C3 alkyl chain of cannabinoids. Since the precise experimental structure of the CB1 receptor has yet to be reported, the chosen best active-state CB1 receptor model

utilized in this study was a model proposed by Doerksen et al.[5] The active-state CB1 model used in this study in shown in Figure 2:

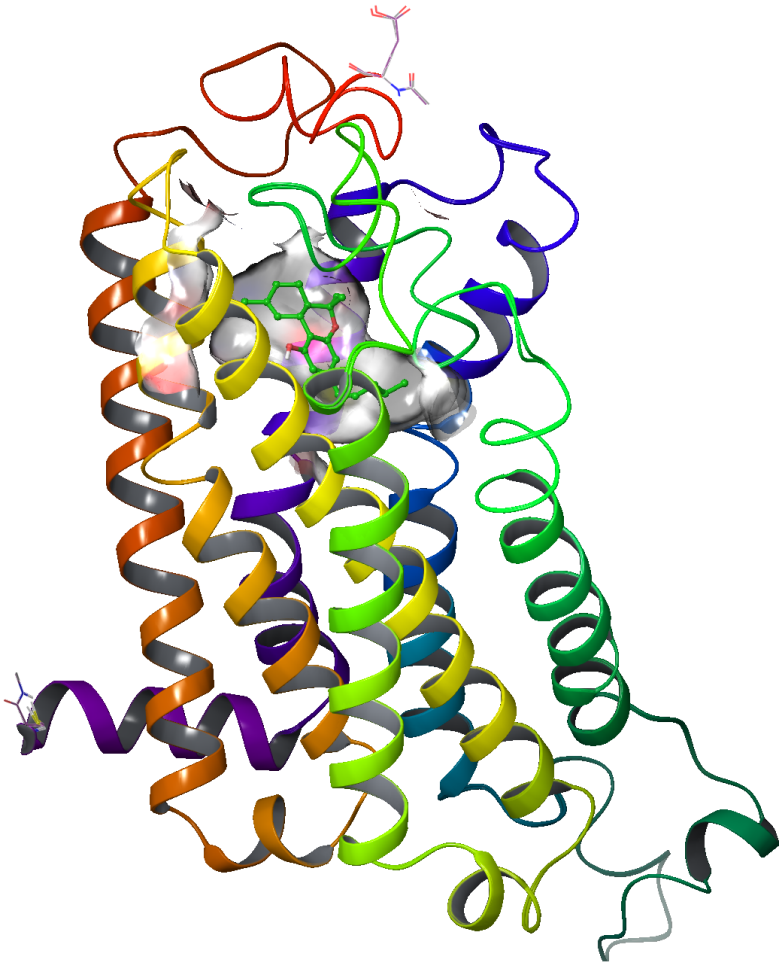


Figure 2: The model of the CB1 receptor used in this study with bound THC ligand shown in green.

In 1984 while researching and developing cannabinoid compounds to aid in multiple sclerosis and chemotherapy research, John W. Huffman and a team of researchers developed a group of synthetic cannabinoid ligands that are today arguably the most popular synthetic cannabinoids. These compounds, called the JWH series compounds, were evolved from the computational combination of

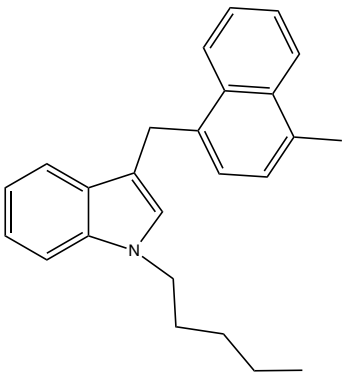
aminoalkylindoles and features of THC. The JWH compounds used in this study come from the naphthoylindole class of synthetic cannabinoids, named for the naphthalene group they contain. Naphthalene's structure is made of two-fused benzene rings.[12]

In this study, the receptor-ligand interactions of an active-state model of the human CB1 receptor, proposed by Doerksen R. et al., and twenty-one JWH cannabinoid ligands from the naphthoylindole family and the THC molecule are compared.[5] The Maestro molecular modeling computer program was used to study the usefulness of the CB1 model and to investigate the important interactions between each ligand and CB1 residues.[13] The information found in this study can be useful in discovering a more accurate CB1 model and in predicting the properties of uncharacterized illicit synthetic cannabinoids before they become products that are illegally sold to the public.

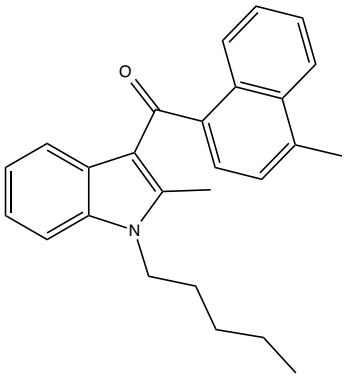
EXPERIMENTAL MATERIALS AND METHODS

Ligands Selection.

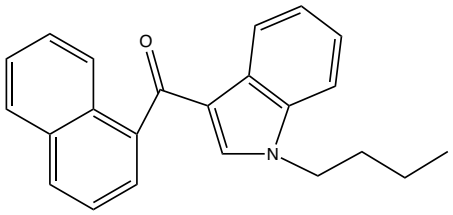
A dataset of twenty-one JWH ligands from the naphthoylindole family plus the THC molecule were chosen. All of these ligands have an affinity for the CB1 receptor. The molecular structures of the twenty-one ligands are listed below in Figure 3 in order of highest to lowest docking score. THC is shown at the end.



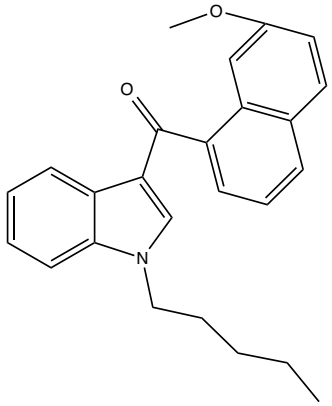
JWH- 149



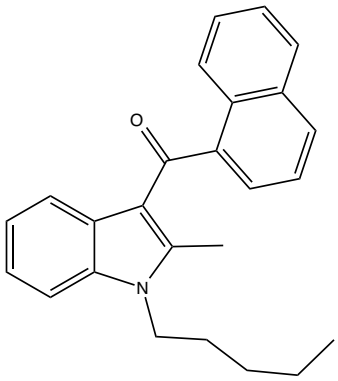
JWH- 184



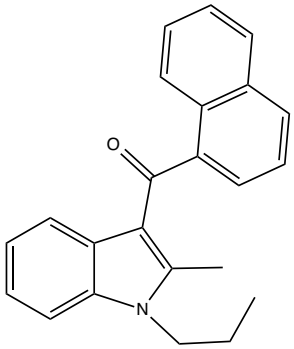
JWH- 073



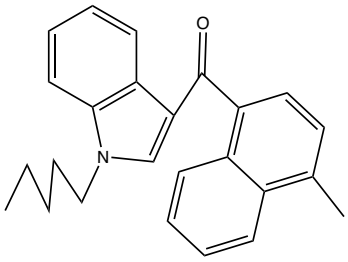
JWH- 164



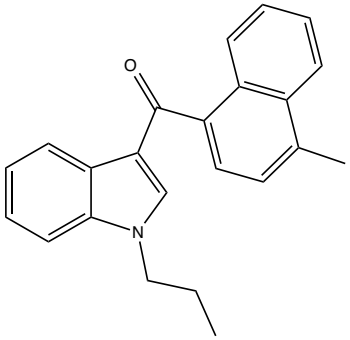
JWH- 007



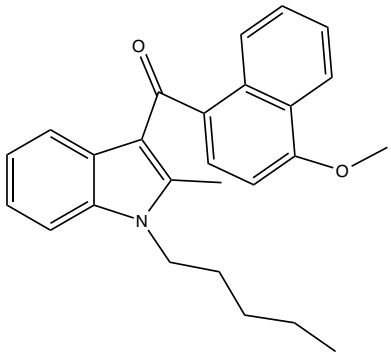
JWH- 015



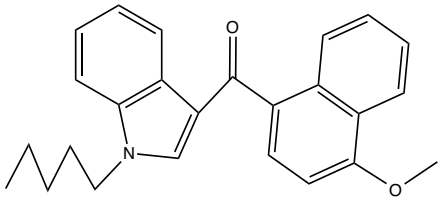
JWH- 122



JWH- 120



JWH- 098



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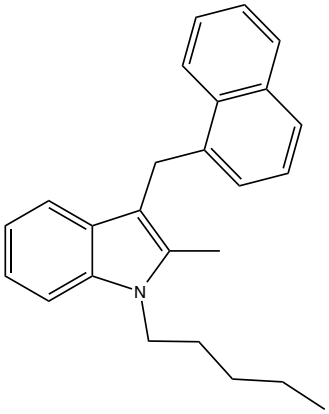
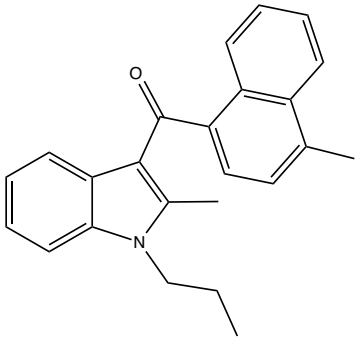
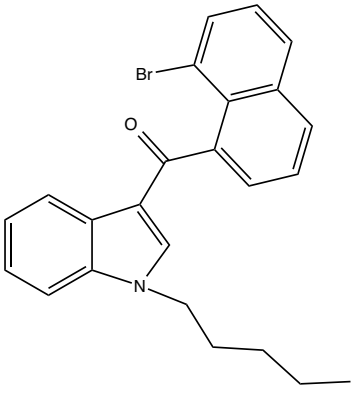


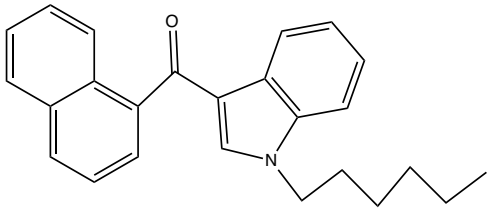
JWH- 196



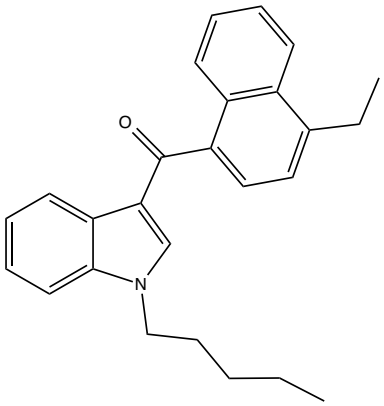
JWH- 148



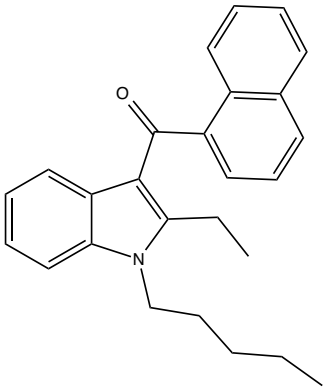
JWH- 424



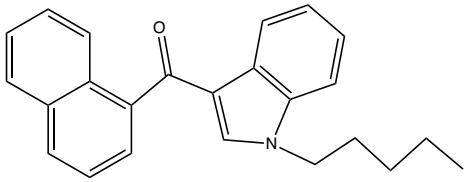
JWH- 019



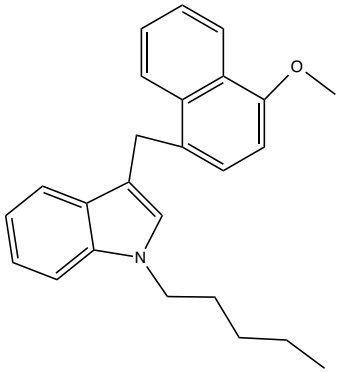
JWH- 210



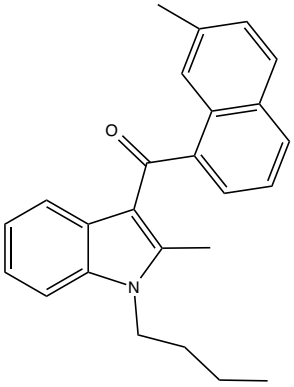
JWH- 116



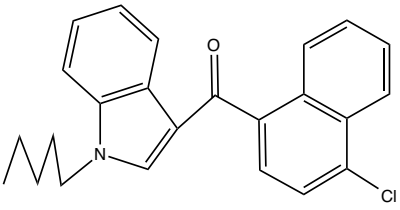
JWH- 018



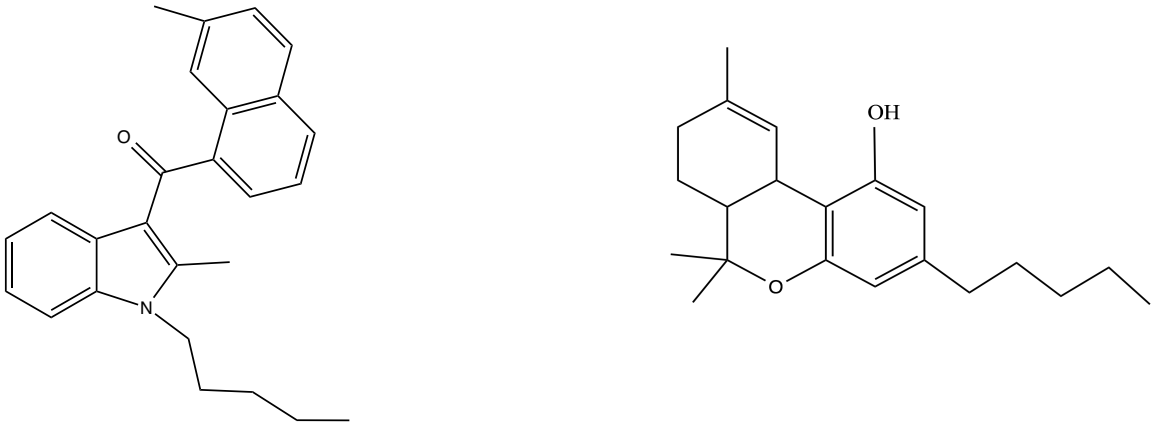
JWH- 185



JWH- 047



JWH- 398



JWH- 048 **THC**

Figure 3: Structures of the twenty-one JWH ligands and THC used in this study

Ligands Preparation

The ligands selected for this study were prepared using Maestro modeling software created by Schrödinger, LLC.[13] The twenty-one ligands were entered into Maestro using the 2D sketcher and then converted to 3D structures for docking. Using the preparation wizard, the ligands were desalted and tautomers were generated using OPLS3 force field. A target PH of 7.0 +/- 2.0 was set and one low energy ring conformation per ligand was generated.

Protein Selection and Preparation

The model selected for this study was a CB1 model created and prepared by Doerksen R. et al. This model was chosen as the best model for experimentation because it showed the best overall performance among the created models.[5] Doerksen R et al. previously prepared the protein used in this study**.** The protein preparation wizard in Maestro was used to correct any problems related to steric hindrances, distance, and hydrogen atoms. After no problems were detected post review, the orientation of the protein was optimized using OPLS3 force field.

Grid Generation

Two grids were generated to tell the Maestro program where to look on the CB1 protein when docking. The grids were generated using information about the CB1 receptor-ligand binding found in previous studies. The first grid was generated with a hydrogen bond constraint on residue Lys 192. Residues Ser 173, Tyr 275, Cys 355, Ser 383 were chosen as rotatable groups. Previous studies have shown that these four residues selected as rotatable groups are important interacting residues for the CB1 receptor in its active state. A second grid was also generated with no hydrogen bond constraints and no residues selected as rotatable groups.

Glide Docking

The standard precision (SP) module in Glide was used to dock the twenty-one ligands created. The Doerksen R. group had already prepared and validated the CB1-THC complex, and I utilized this complex for generating the grids considering THC as an active ligand site. Post-docking minimization was performed, and the top pose of each ligand was selected for study. Once all of the ligands had been docked, the docking score of each ligand was reviewed in order to determine which ligands had the better affinity for the CB1 receptor. The binding site was also studied to see which residues of the CB1 protein receptor and what parts of the ligand, the indole or naphthalene substructures, were interacting. The shortest distance (in Angstroms) between either the indole substructure or the naphthalene substructure of each ligand and the interacting residues was measured.

RESULTS AND DISCUSSION

Once the ligand and protein preparation and grid generation had been completed, the twenty-one JWH ligands and the THC molecule were docked to the CB1 model. The docking results showed that all twenty-one ligands and the THC molecule were successfully docked into the CB1 model. The most favorable pose for each ligand and the THC molecule were chosen and analyzed. The docking scores varied from -7.198 to -9.334. The docking scores of the JWH ligands and the THC molecule are listed in Table 1.

Ligands	Docking Score	Glide Emodel	Ki
JWH-149	-9.334	-64.999	5.0nM ±2.1 nM
JWH-184	-9.268	-67.039	23 nM
JWH-164	-9.178	-68.191	6.6nM ± 0.7
JWH-007	-9.088	-68.246	9.5nM ± 4.5
JWH-015	-9.044	-60.755	383 nM
JWH-122	-8.895	-64.202	0.69 nM ± 0.5
JWH-120	-8.872	-61.182	1054 nM
JWH-098	-8.845	-40.368	4.5 nM ± 0.1
JWH-081	-8.84	-63.183	1.2 nM ± 0.03
JWH-196	-8.784	-62.894	151 nM ± 18
JWH-148	-8.756	-64.212	123 nM
JWH-424	-8.725	-56.65	20.9 nM
JWH-073	-8.706	-61.424	8.9 nM
THC Molecule	-8.686	-63.34	10 nM
JWH-019	-8.595	-42.71	9.8 nM ± 2
JWH-210	-8.536	-54.984	.46 nM
JWH-116	-8.5	-54.951	52 nM ± 5.0
JWH-018	-8.424	-58.632	9 nM ± 5.0
JWH-185	-8.074	-68.61	17 nM
JWH-047	-7.559	-56.017	59 nM ± 3.0
JWH-398	-7.452	-61.263	2.3 nM
JWH-048	-7.198	-36.605	10.7 nM ± 1.0

Table 1: Docking Score and Glide Emodel score of each JWH ligand and THC

Figure 4 and Figure 24 show the two ligands and their interactions with the CB1 model that had the best docking score and the lowest docking score, respectively. JWH-149 was shown to have the most favorable docking score of -9.334, and JWH- 048 was shown to have the least favorable docking score of -7.198. Figure 4 and table 3 show that JWH- 149 has Π-Π stacking interactions with residues Tryptophan 279 (Trp 279), Tryptophan 356 (Trp 356), Phenylalanine 170 (Phe 170), and Phenylalanine 200 (Phe 200). Most ligands that resulted in high

docking scores showed primarily the following interactions with the CB1 protein: indole substituent interactions with Trp 279 and to a lesser degree with Phe 170 and Trp 356; naphthalene interactions with Phe 170, Trp 356, and Phe 200.

Previous studies have shown that residues Serine 383 (Ser 383) and Lys 192 are key residues in forming hydrogen bonds with cannabinoid ligands possessing OH and COOH groups, respectively.[10] The results of this study showed that only two docked ligands had an interaction with Lys 192. A hydrogen bond was formed between Lys 192 and the keto-oxygen portion of ligand JWH-073, and Lys 192 had a pi- cation interaction with the naphthalene portion of ligand JWH-048. Interestingly, JWH-073 had a docking score of -8.706, and JWH-048 as stated previously had the lowest docking score of -7.198. Ser 383 also formed a hydrogen bond with the keto-oxygen of ligands JWH-098 and JWH-424. The docking scores of these two ligands were -8.845 and -8.725, respectively. Analysis of the interactions between the CB1 model and the ligands also showed that another residue formed a hydrogen bond in the same way with the keto-oxygen of the ligands. Similar to Lys 192 and Ser 383, Trp 279 formed a hydrogen bond with the keto-oxygen of ligands JWH-015, JWH-019, JWH-018, and JWH-047. The docking scores of each of these ligands were -9.044, -8.595, -8.424, and -7.559, respectively.

The strength of each hydrogen bond formed and cation-pi interaction was analyzed through measuring the distance in Angstroms. The hydrogen bond distance between Lys 192 and JWH-073 was measured to be 2.40Å and the cation-pi

interaction distance between Lys 192 and JWH- 048 was measured to be 3.77Å. The hydrogen bond distances between Trp 279 and JWH- 015, JWH- 019, JWH- 018, and JWH- 047 were measured to be 2.17 Å, 1.90 Å, 2.43 Å, and 2.23 Å, respectively. The hydrogen bond distance between Ser 383 and JWH- 098 was 1.98 Å, and the hydrogen bond distance between Ser 383 and JWH- 424 was 2.04 Å. The shorter distance indicates the stronger interaction. The distance and strength of each hydrogen bond can be seen in table 2.

Ligands	Lys 192	Trp 279	Ser 383
JWH- 073	2.4 Å		
JWH- 015		2.17 Å	
JWH- 019		1.9 Å	
JWH- 018		2.43 Å	
JWH- 047		2.23 Å	
JWH- 098			1.98 Å
JWH- 424			2.04 Å

Table 2: Distances of Hydrogen Bonds Formed Between Ligands and Interacting CB1 Residues

Analysis shows that the hydrogen bond distances between both Trp 279 and Ser 383 and their respective interacting ligands were shorter and therefore stronger than the hydrogen bond distance between Lys 192 and its respective interacting ligand. This indicates that Lys 192 does not interact as significantly with the JWH ligands as other residues such as Trp 279 and Ser 383. More research on these residues could indicate their significance in forming hydrogen bonds with new and undiscovered cannabinoid ligands.

Previous studies have indicated that residues Phe 170, Phe 200, Phe 208, Tyr 215, Phe 289, Tyr 292, Tyr 296, Trp 356, and Phe 379 are essential CB1 residues for aromatic stacking. Previous studies have also shown that residues Phe 170 and Trp 279 are present in the deep binding pocket of the CB1 receptor. This binding pocket is essential for effective ligand binding.[10] The results of this study show that Trp 279, Trp 356, Phe 200, and Phe 170 are key residues in JWH and CB1 binding. Trp 279 interacted with eighteen of the twenty- one ligands and the THC molecule. This is the most interactions of all of the residues. Trp 279 was also chosen as the central point for docking, so it makes sense that it had the most interactions. Trp 356 interacted with fifteen of the ligands, and Phe 200 interacted with ten of the ligands, and Phe 170 interacted with eleven ligands. Interactions with residues Phe 177 and Phe 174 also occurred, but only with the lowest scoring ligand, JWH-048. THC and the JWH ligands used in this study are listed in Table 3 along with the CB1 residues with which they interact.

	CB1 Receptor Residues							
Ligands	**Trp 279**	**Trp 356**	**Phe 170**	**Phe 200**	**Phe 177**	**Phe 174**	**Lys 192**	**Ser 383**
THC Molecule	✓							
JWH-149	✓	✓	✓	✓				
JWH-184	✓	✓	✓					
JWH-073	✓						✓	
JWH-164	✓	✓		✓				
JWH-007	✓	✓		✓				
JWH-015	✓	✓	✓					
JWH-122	✓	✓	✓					
JWH-120	✓	✓		✓				
JWH-098	✓	✓		✓				✓
JWH-081	✓	✓	✓	✓				
JWH-196	✓	✓		✓				
JWH-148	✓							
JWH-424	✓	✓	✓	✓				✓
JWH-019	✓							
JWH-210		✓	✓					
JWH-116		✓	✓	✓				
JWH-018	✓	✓	✓					
JWH-185	✓	✓	✓					
JWH-047	✓		✓	✓				
JWH-398								
JWH-048	✓				✓	✓	✓	

Table 3: JWH Ligands and THC and Their Interacting CB1 Residues

The interacting residues showed Π-Π or cation-pi, only Lys 192 and JWH-048, interactions with either the indole substructure of the ligand or the naphthalene substructure of the ligand. The shortest carbon-carbon interaction distance between the CB1 residues and the ligands was measured. These distances can be seen in Table 4. The THC molecule only showed an interaction with Trp 279. The benzene ring containing an OH substituent group exhibited pi-pi stacking with a distance of 3.92Å with Trp 279.

Ligands	Trp 279 and indole	Phe 170 and indole	Trp 356 and indole	Phe 170 and nap.	Trp 356 and nap.	Phe 200 and nap.	Trp 279 and nap.	Phe 174 and nap.	Phe 177 and nap.
JWH- 149	3.53Å			3.32Å	3.51Å	4.09Å			
JWH- 184		3.62Å	3.59Å				3.57Å		
JWH- 073	3.30Å								
JWH- 164	3.59Å				3.84Å	3.43Å			
JWH- 007	3.59Å				3.50Å	3.41Å			
JWH- 015	3.62Å			3.34Å	3.53Å				
JWH- 122	3.45Å			3.29Å	3.47Å				
JWH- 120	3.58Å				3.65Å	3.51Å			
JWH- 098	3.61Å				3.53Å	3.35Å			
JWH- 081	3.44Å			3.26Å	3.32Å	3.59Å			
JWH- 196	3.60Å				3.79Å	3.46Å			
JWH- 148							3.53Å		
JWH- 424	3.78Å			3.55Å	3.66Å	3.32Å			
JWH- 019	3.50Å								
JWH- 210				3.21Å	3.47Å				
JWH- 116				3.27Å	3.80Å	3.39Å			
JWH- 018	3.45Å			3.43Å	3.67Å				
JWH- 185		3.41Å	3.65Å				3.58Å		
JWH- 047				3.28Å		3.45Å			
JWH- 398									
JWH- 048	3.67Å							4.53Å	3.41Å

Table 4: The Interactions Between the Indole or Naphthalene Substructures of each Ligand and CB1 residue

*And indole denotes a pi pi stacking (Π-Π) interaction between the CB1 substituent and the indole portion of the ligand.

**And nap. denotes a pi pi stacking (Π-Π) interaction between the CB1 substituent and the naphthalene portion of the ligand

As seen in table 4, most distances were in the 3 to 4Å range with only 2 interaction distances greater than 4Å. The strongest interaction, judged by the shortest distance, occurred between Phe 170 and the naphthalene substructure of JWH-210. This result seems inconsistent with the docking scores since JWH- 210 produced a docking score on the bottom half of the docking score table with a score of -8.536. Though JWH-210 produced a docking score in the bottom half of the docking score range, JWH-210 possesses the highest experimental binding affinity (lowest K_i value= 0.46nM) at CB1 of all the ligands tested in this study. This high binding affinity for CB1 should be investigated further to explain why JWH-210 and Phe 170 have the strongest interaction. The weakest interaction, or longest distance, was seen between Phe 174 and the naphthalene portion of JWH-048. This finding is consistent with the resulting docking scores as JWH-048 produced the lowest docking score. JWH-048 was also the only ligand to interact with Phe 174. Because Phe 170 formed the strongest interaction and interacted with eleven ligands, further studies should be done in order to determine if Phe 170 is also a key residue in all cannabinoid ligand binding with CB1.

Because THC is the main psychoactive component of marijuana, it could be assumed that THC would produce the strongest interaction with CB1, interact with the most CB1 residues, and have the highest docking score. As seen in table 1, THC produced a docking score of -8.686 with this active-state CB1 model. This docking score is in the middle to bottom half range of the resulting JWH ligand docking scores. The best pose of the THC molecule selected and analyzed also only

interacted with Trp 279 through Π-Π stacking at a distance of 3.92Å. An explanation for why THC does not possess the best docking score could be its binding affinity for CB1. Though THC possesses a binding affinity of 10nM for CB1, eleven of the twenty-one ligands studied possess a higher binding affinity, lower K_i value, for CB1 than THC. These higher binding affinities cause the ligands to bind to CB1 more strongly than THC and potentially cause more harmful effects.[1] The interactions between the CB1 model and each of the twenty-one ligands and THC are shown in 2D and 3D interaction diagrams in figures 4- 25.

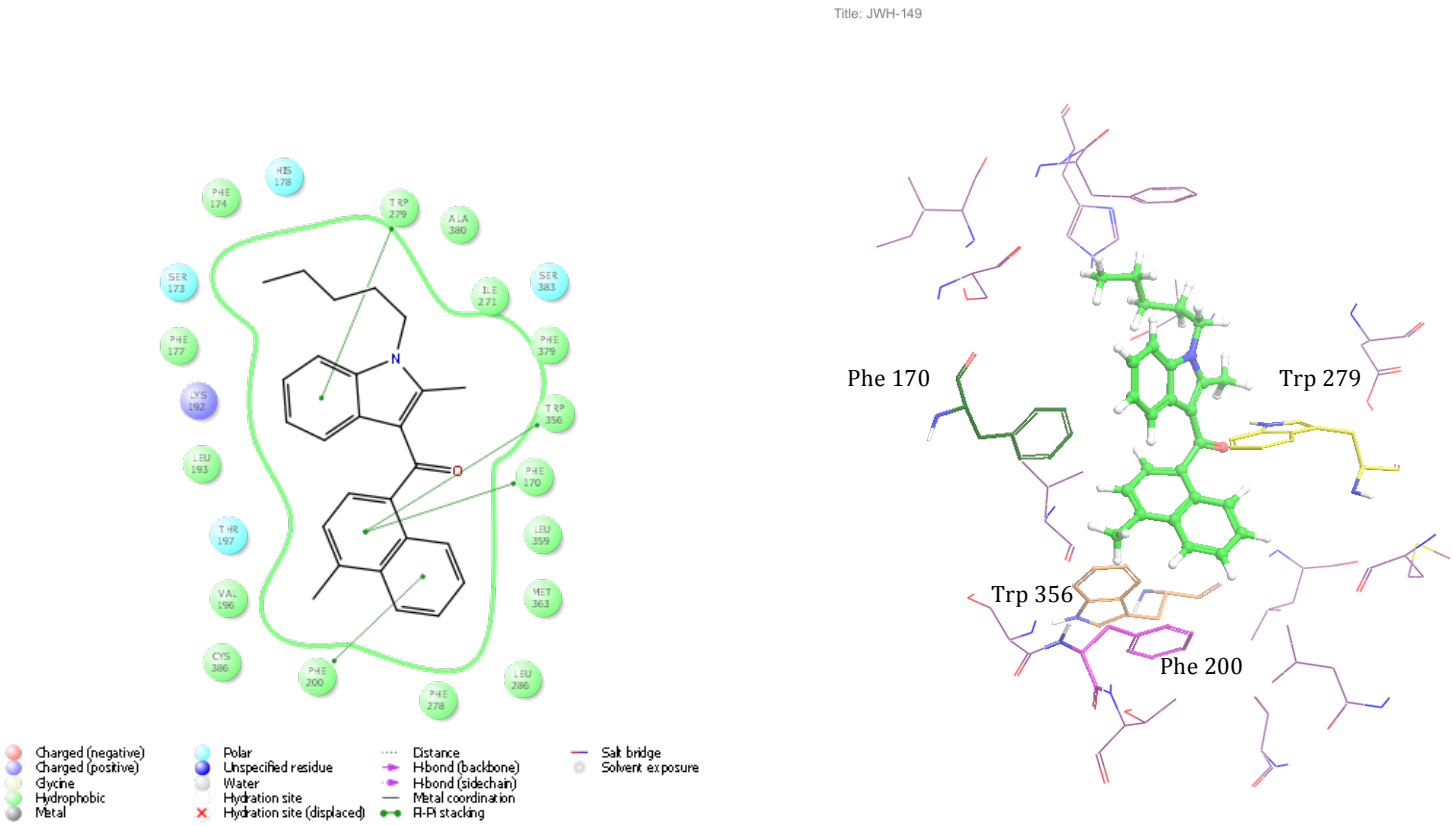


Figure 4: JWH- 149 and CB1 2D and 3D Ligand Interaction Diagrams

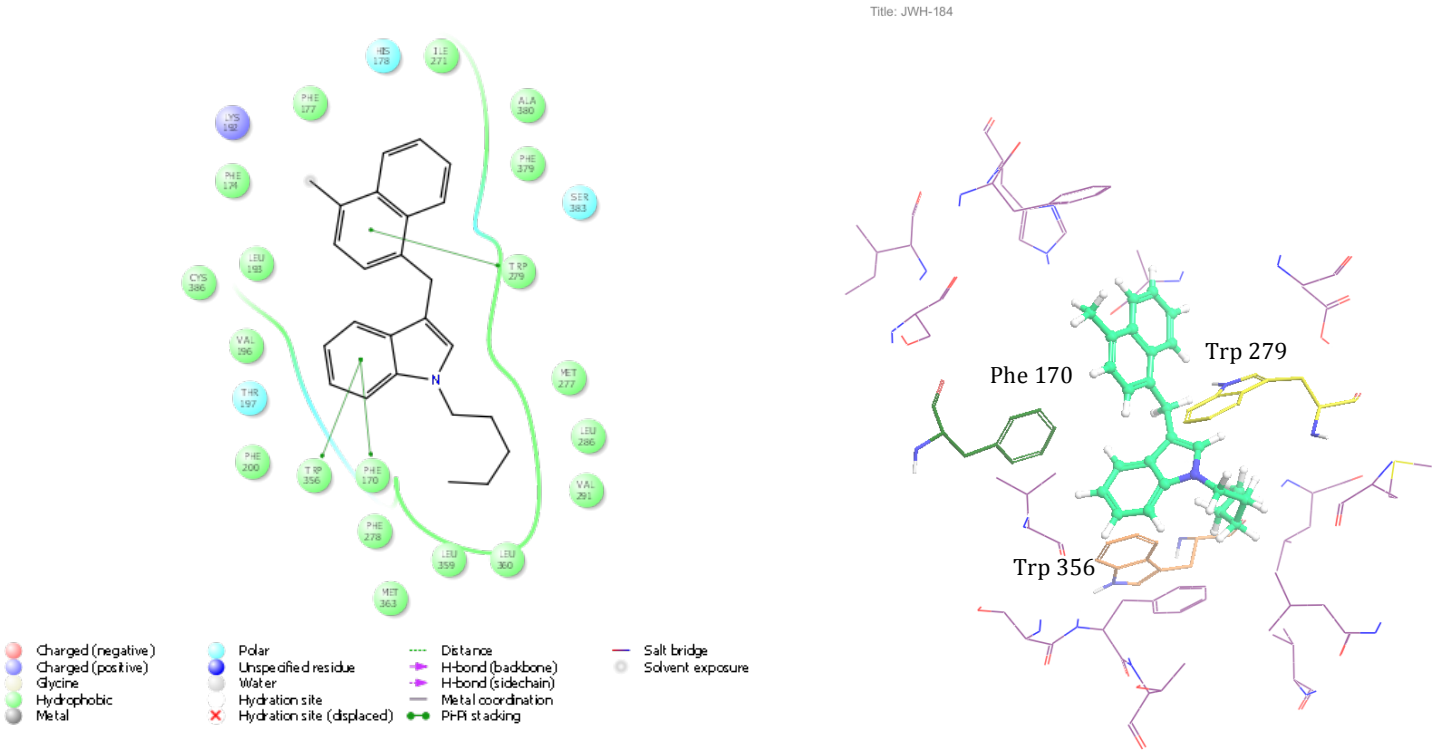


Figure 5: JWH- 184 and CB1 2D and 3D Ligand Interaction Diagrams

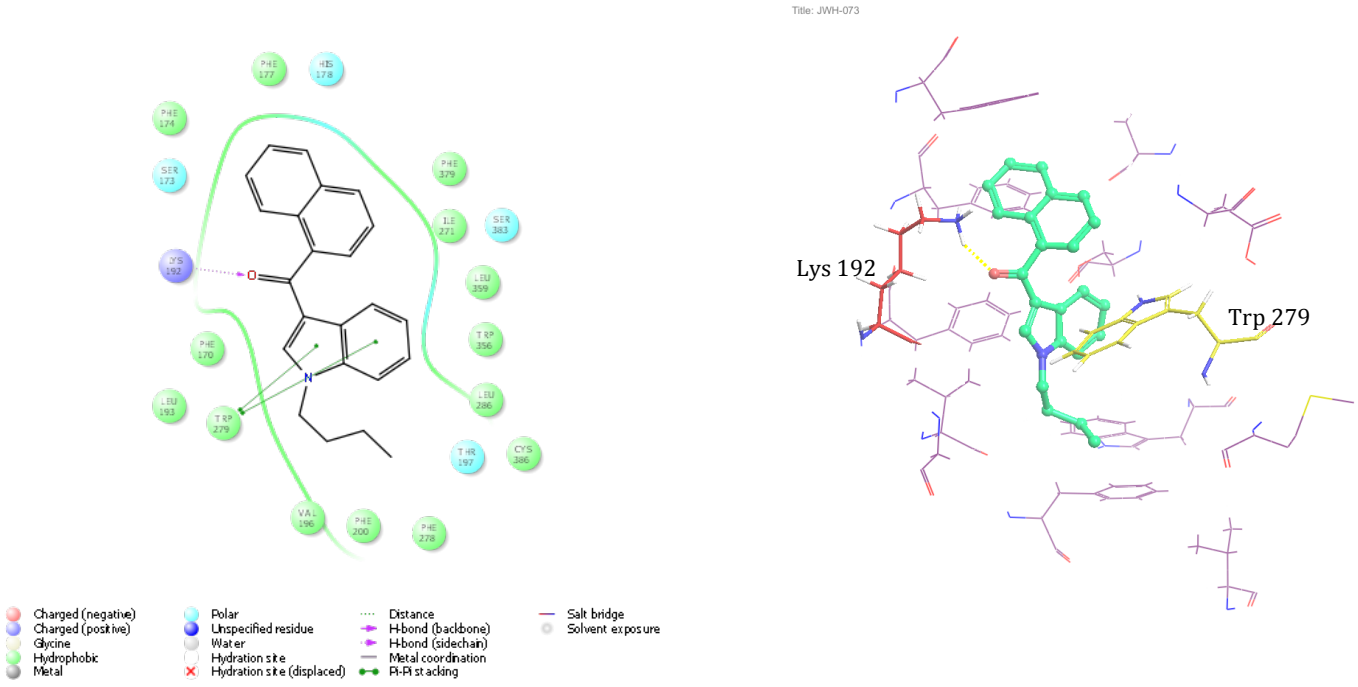


Figure 6: JWH- 073 and CB1 2D and 3D Ligand Interaction Diagrams. H-bond is shown as yellow-colored dashes.

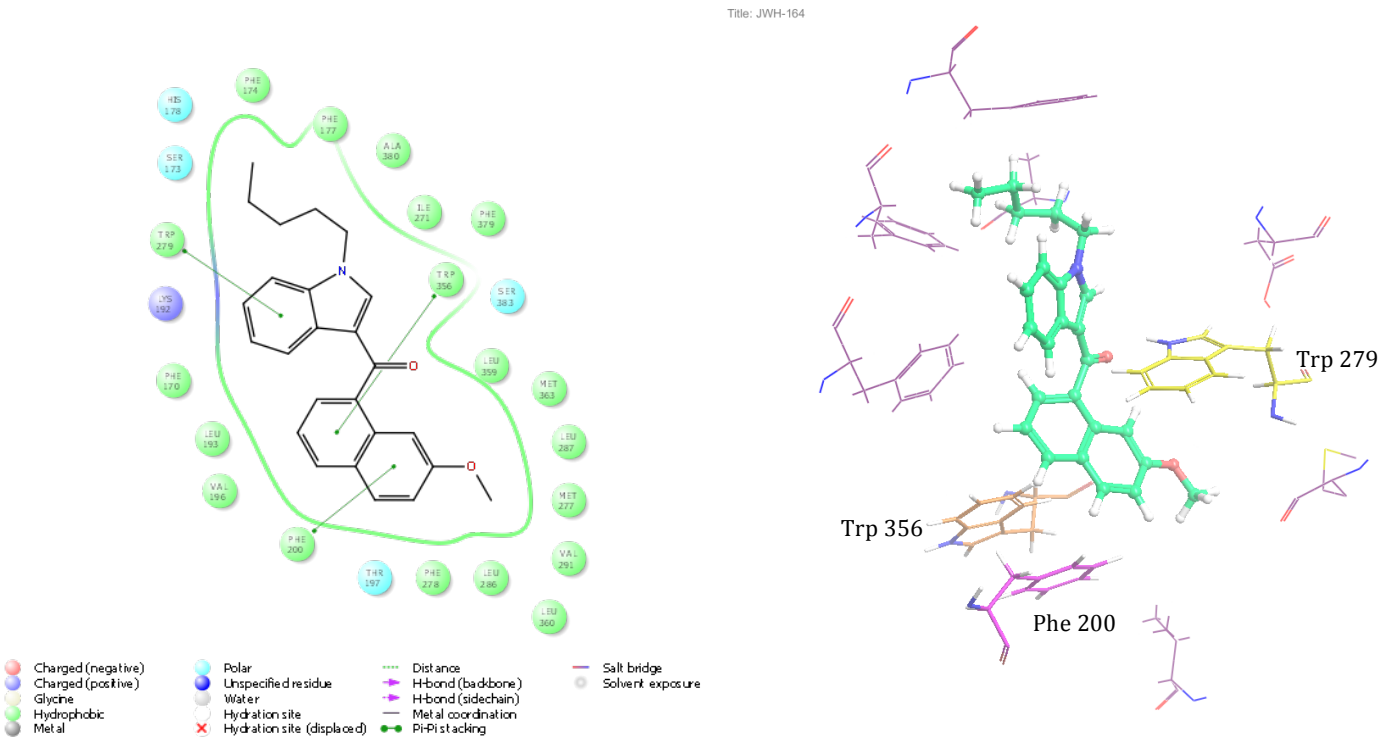


Figure 7: JWH- 164 and CB1 2D and 3D Ligand Interaction Diagrams

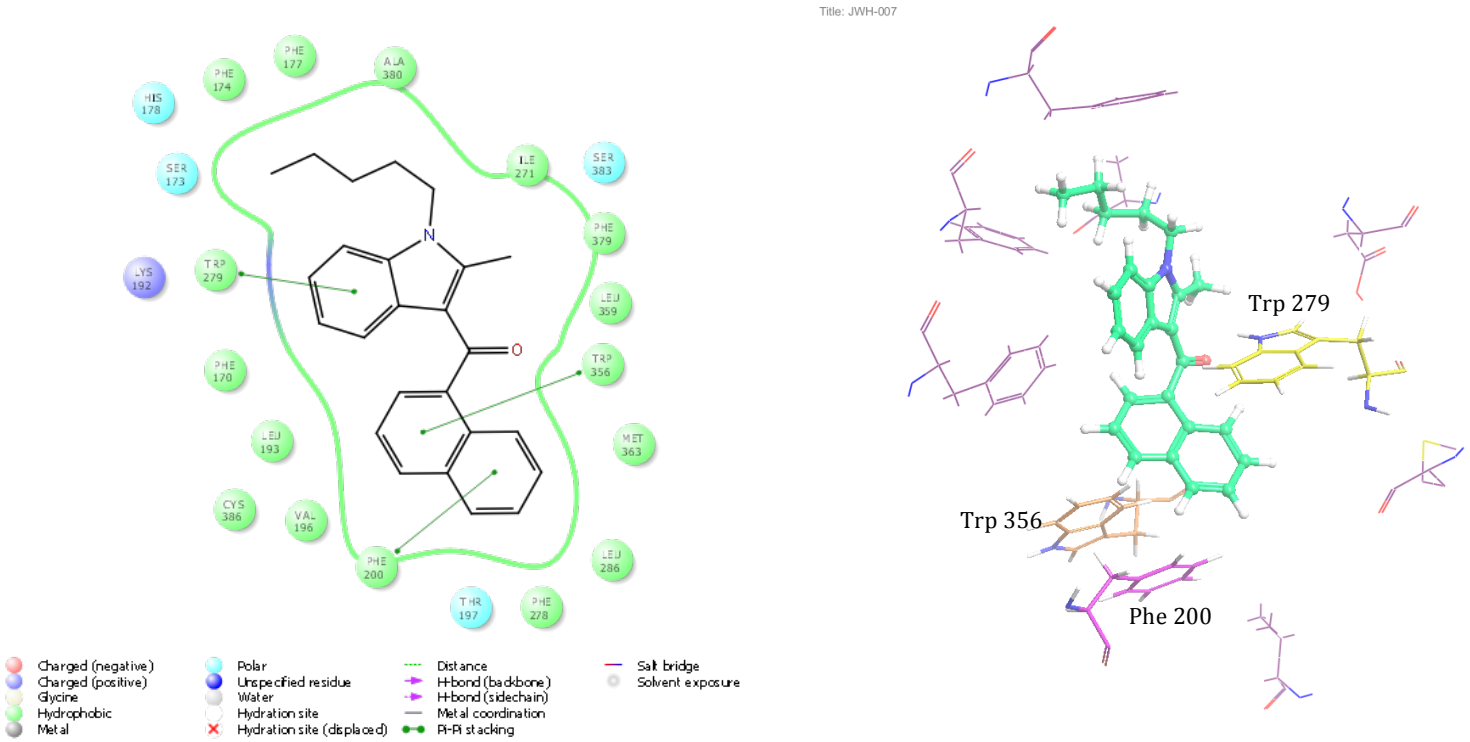


Figure 8: JWH- 007 and CB1 2D and 3D Ligand Interaction Diagrams

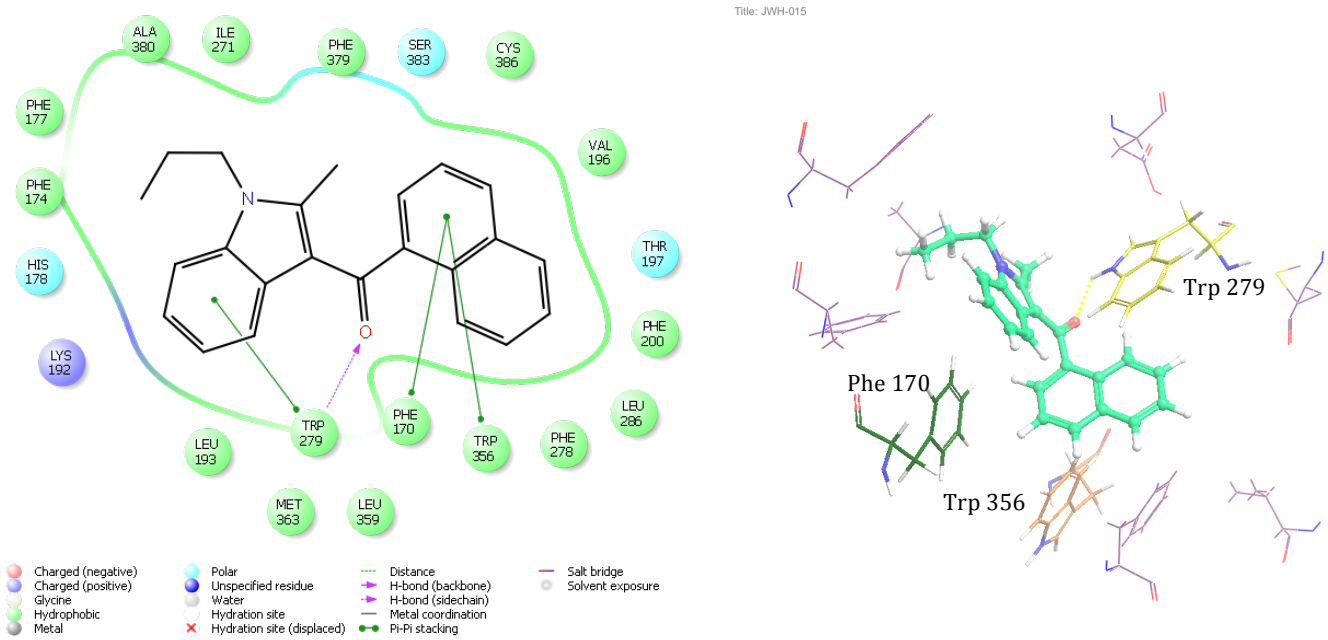


Figure 9: JWH- 015 and CB1 2D and 3D Ligand Interaction Diagrams. H-bond is shown as yellow- colored dashes

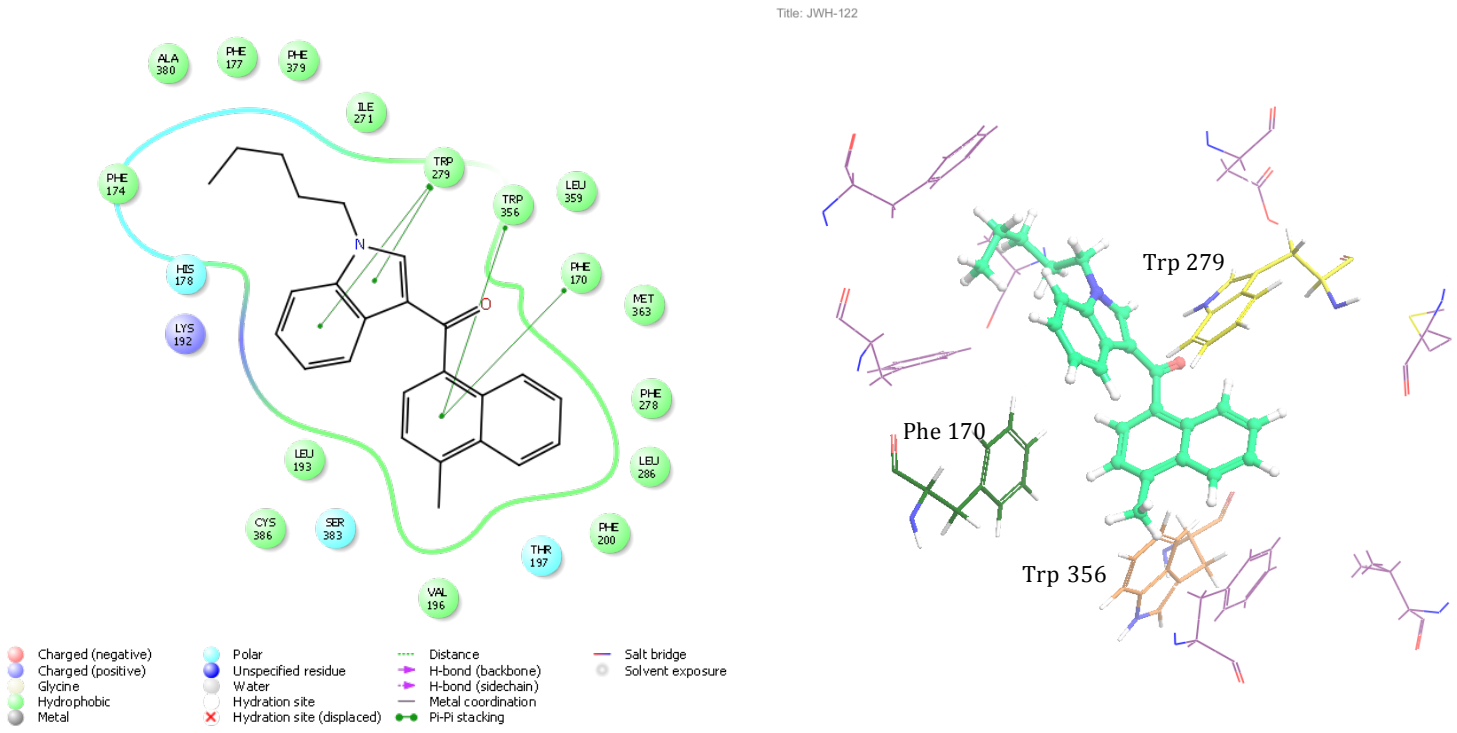


Figure 10: JWH- 122 and CB1 2D and 3D Ligand Interaction Diagrams

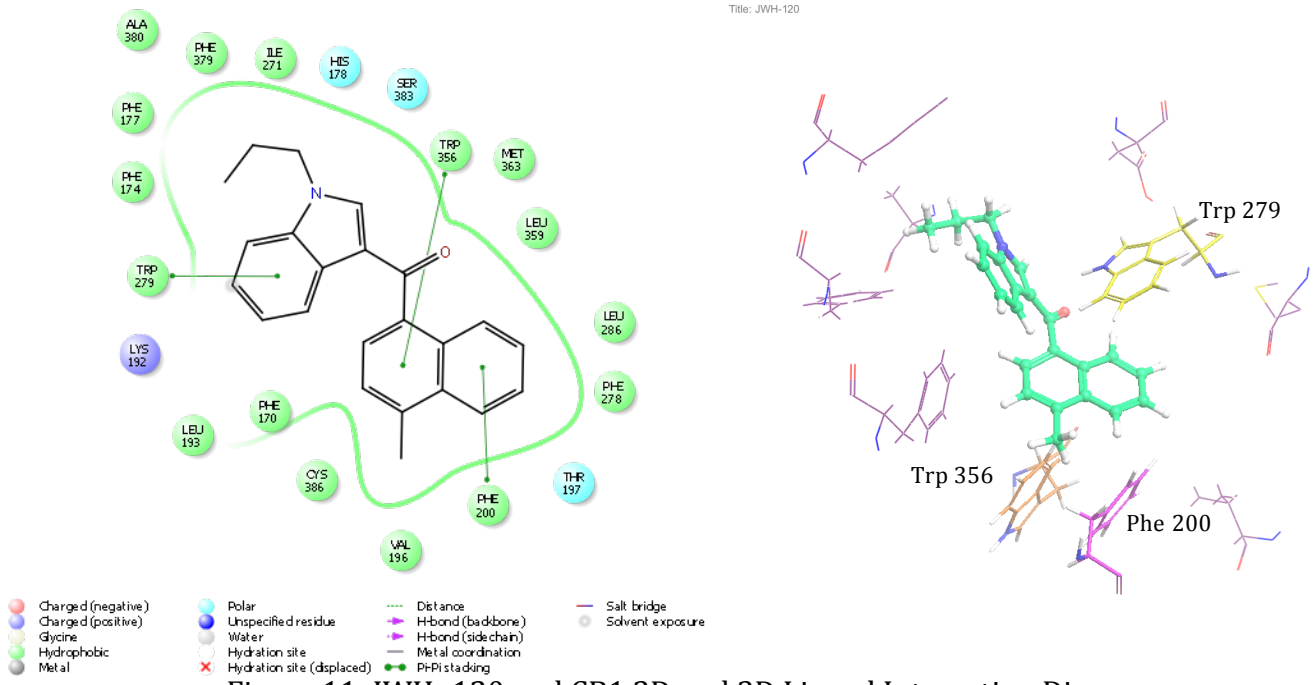


Figure 11: JWH- 120 and CB1 2D and 3D Ligand Interaction Diagrams

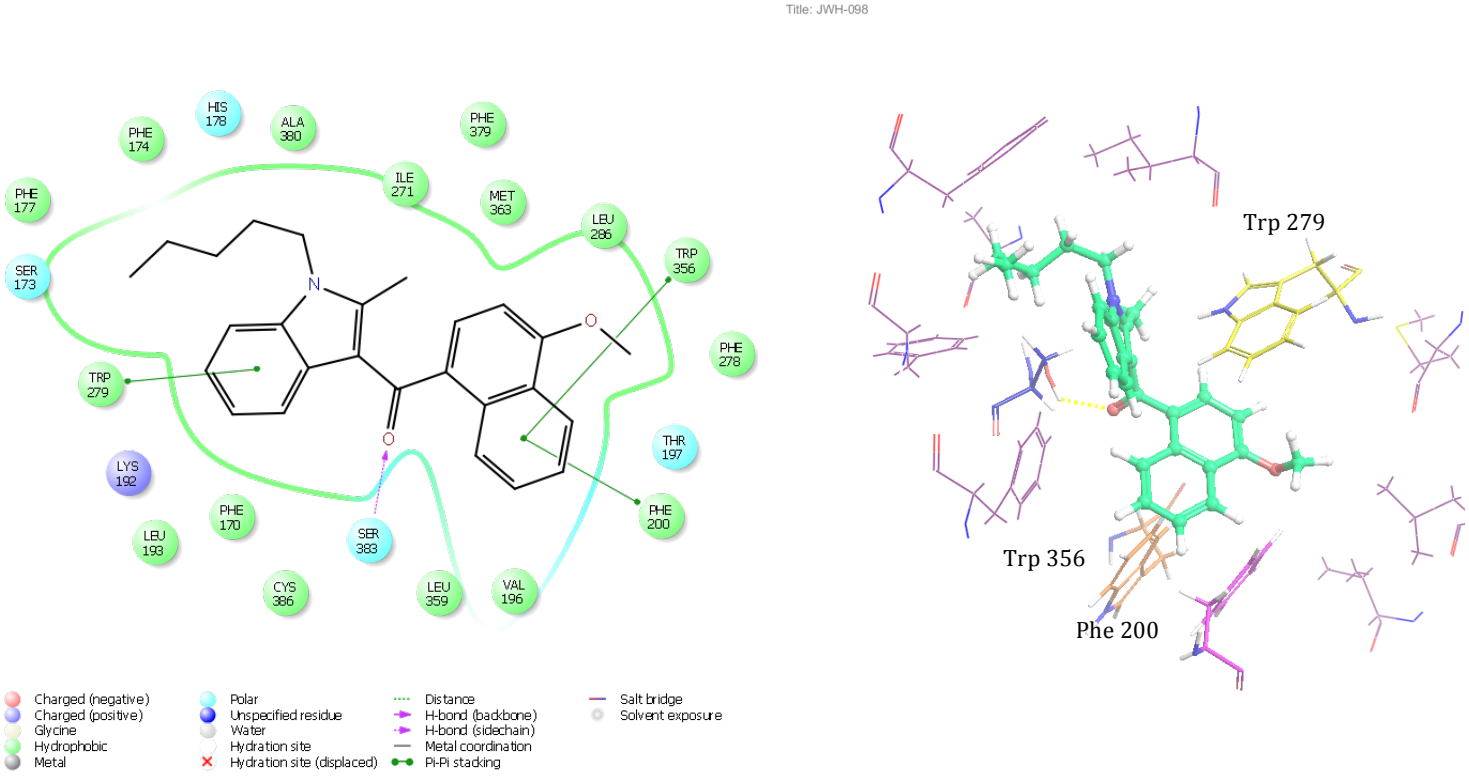


Figure 12: JWH- 098 and CB1 2D and 3D Ligand Interaction Diagrams

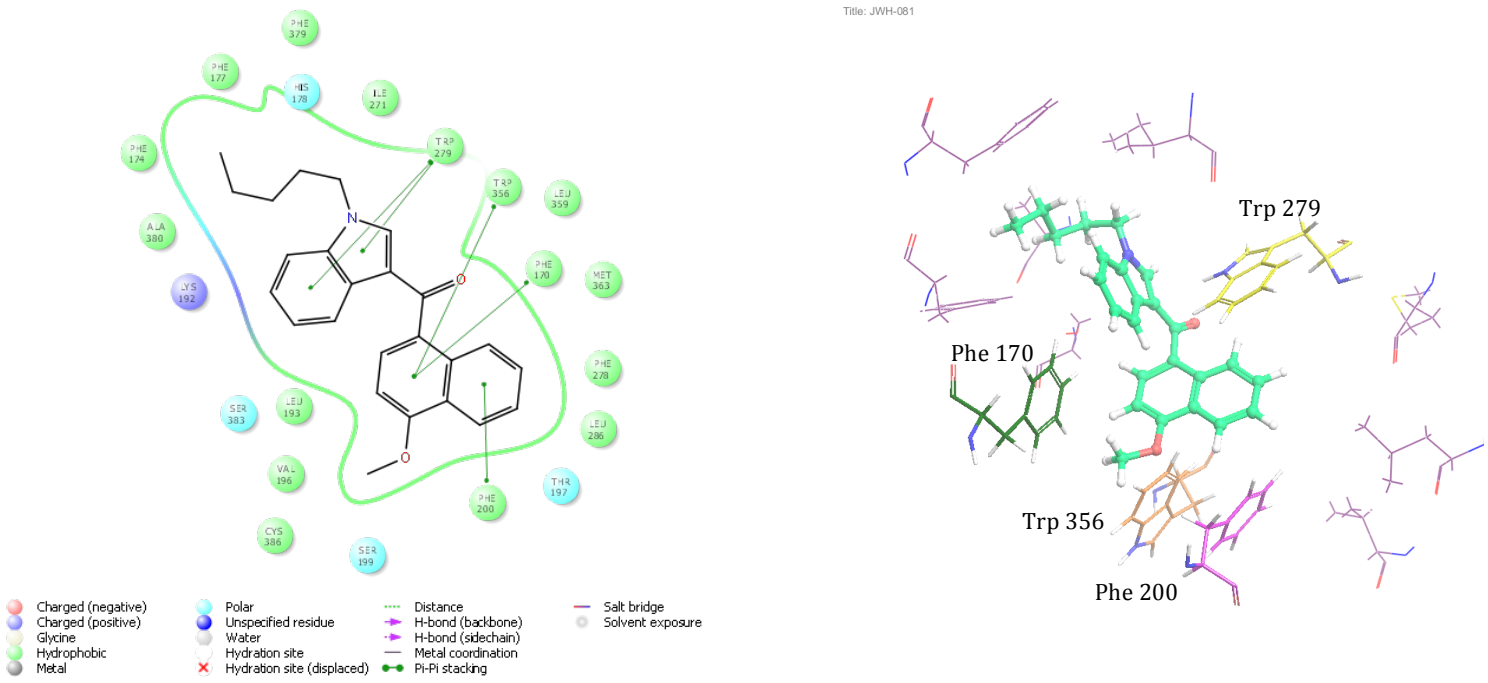


Figure 13: JWH- 081 and CB1 2D and 3D Ligand Interaction Diagrams

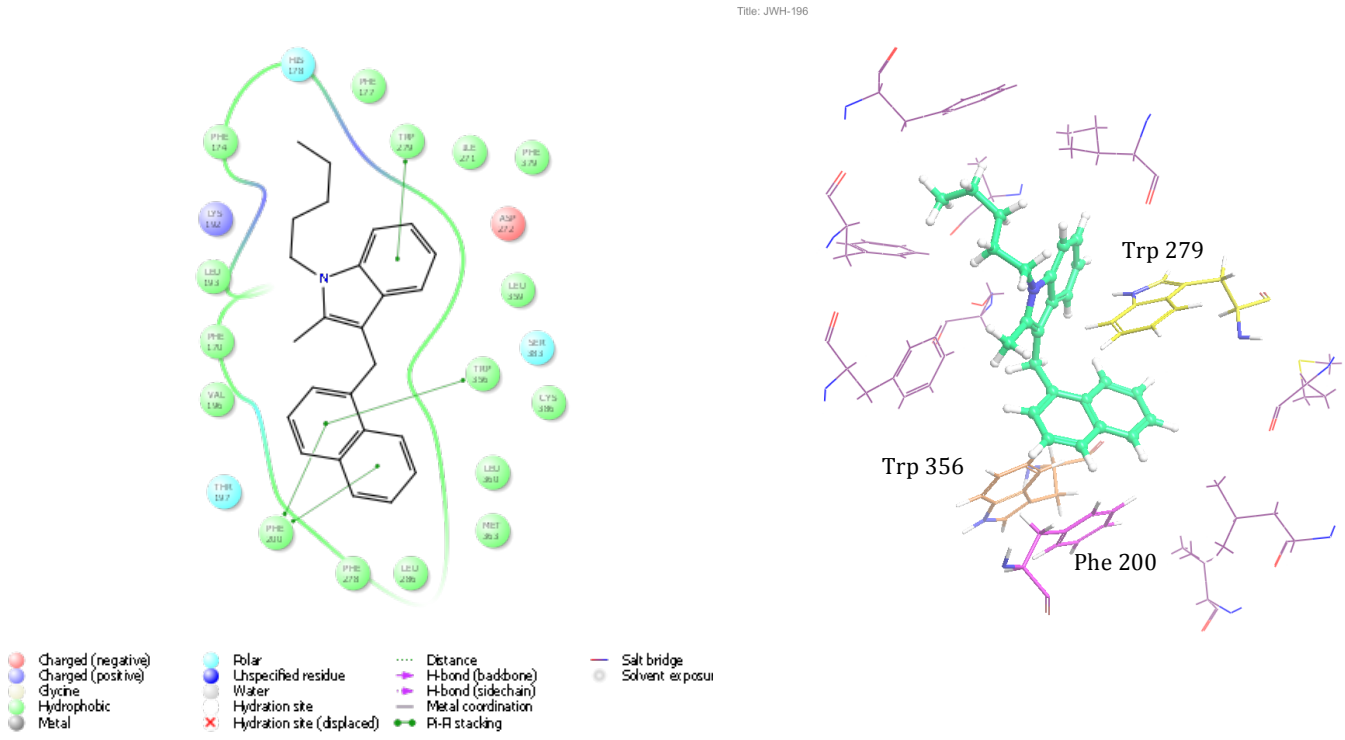


Figure 14: JWH- 196 and CB1 2D and 3D Ligand Interaction Diagrams

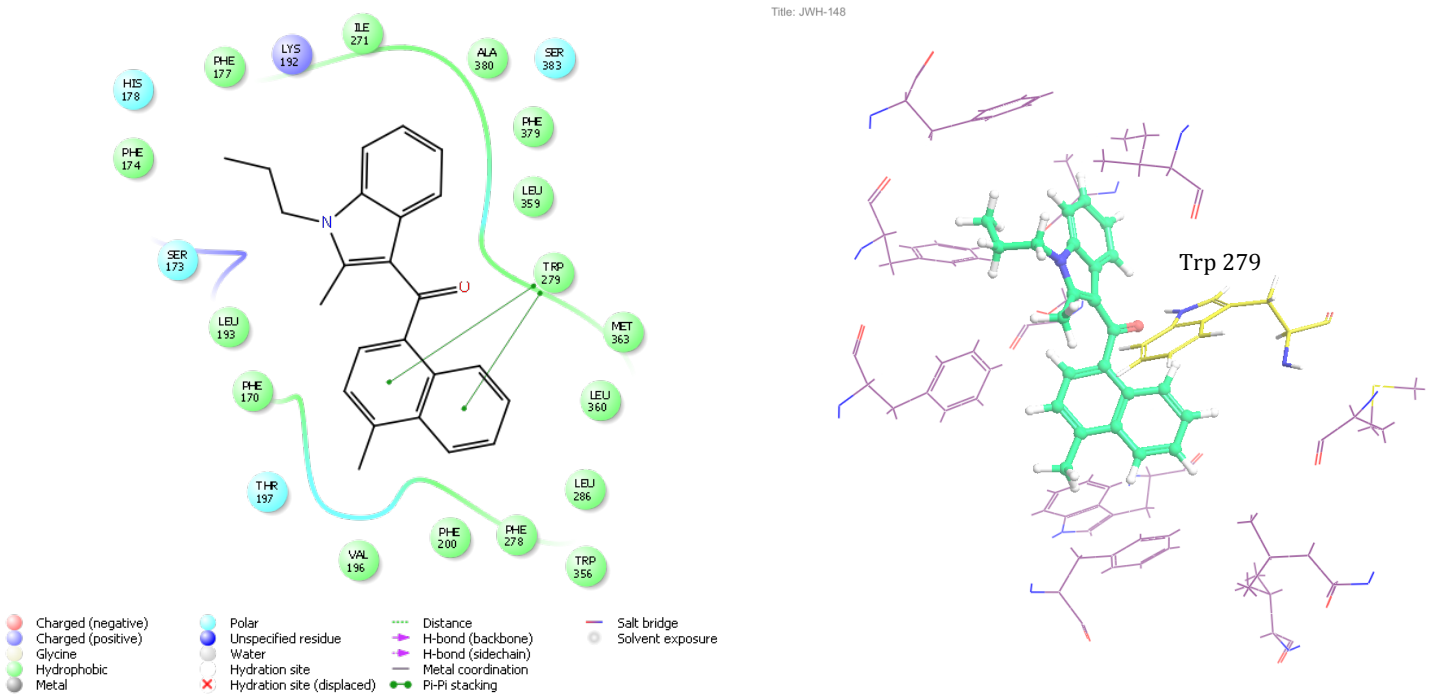


Figure 15: JWH- 148 and CB1 2D and 3D Ligand Interaction Diagrams

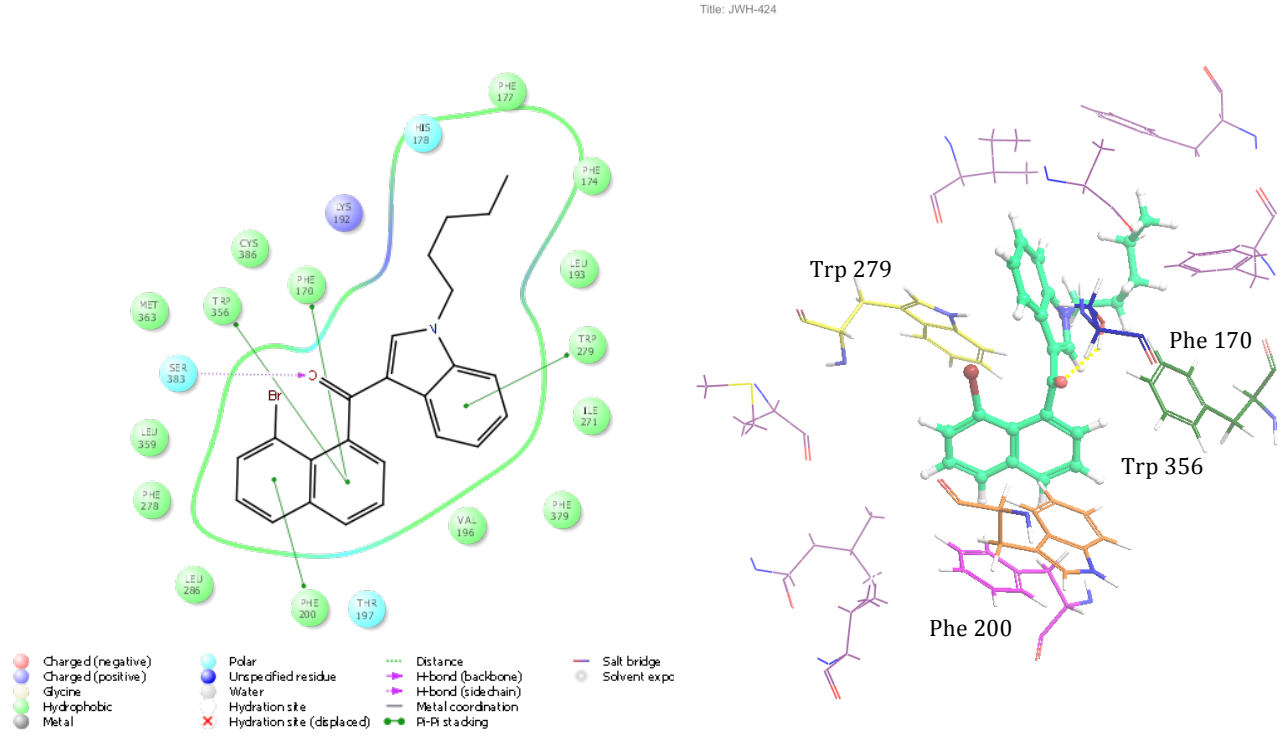


Figure 16: JWH- 424 and CB1 2D and 3D Ligand Interaction Diagrams

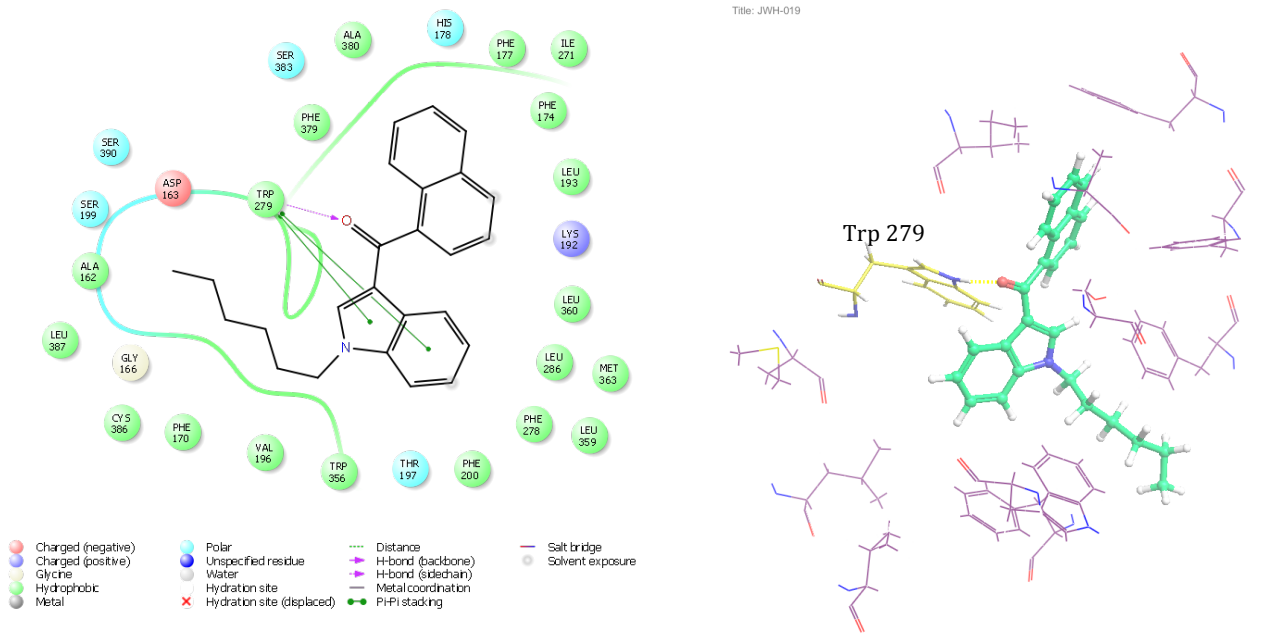


Figure 17: JWH- 019 and CB1 2D and 3D Ligand Interaction Diagrams. H- bond is shown as yellow- colored dashes.

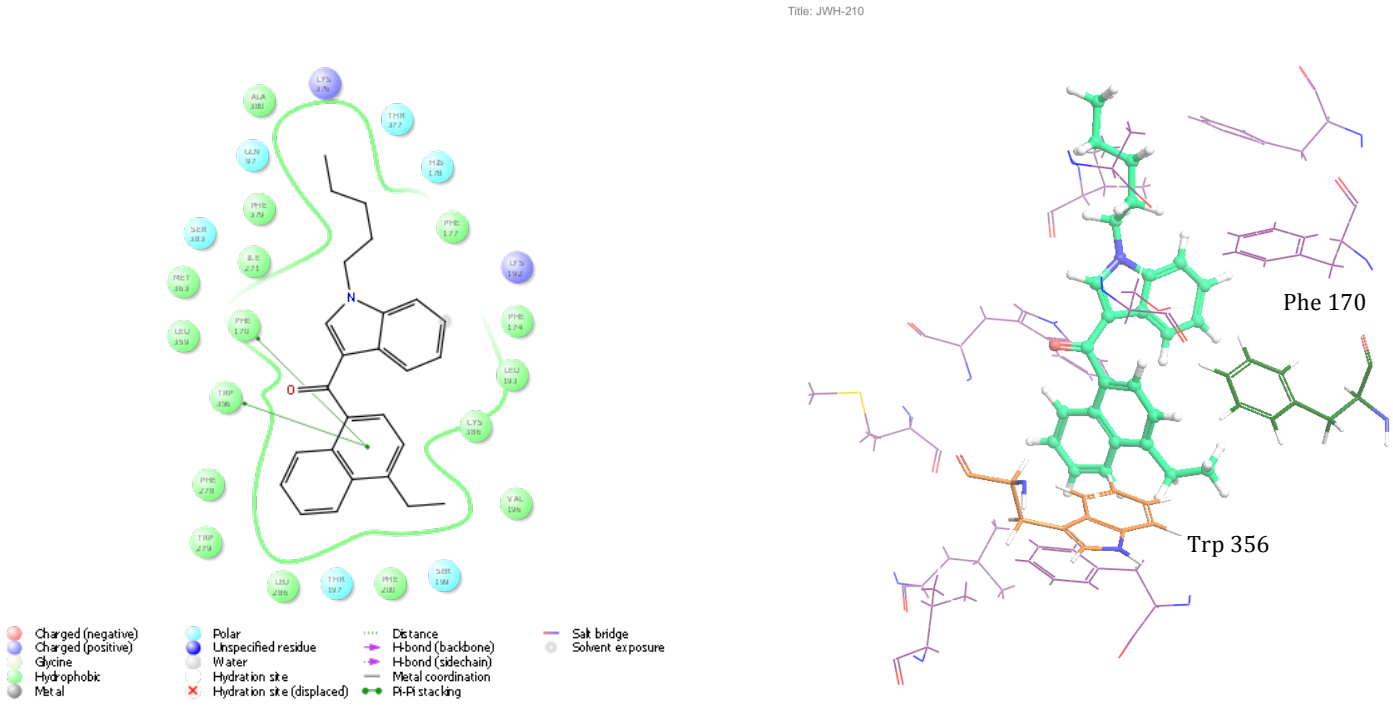


Figure 18: JWH- 210 and CB1 2D and 3D Ligand Interaction Diagrams

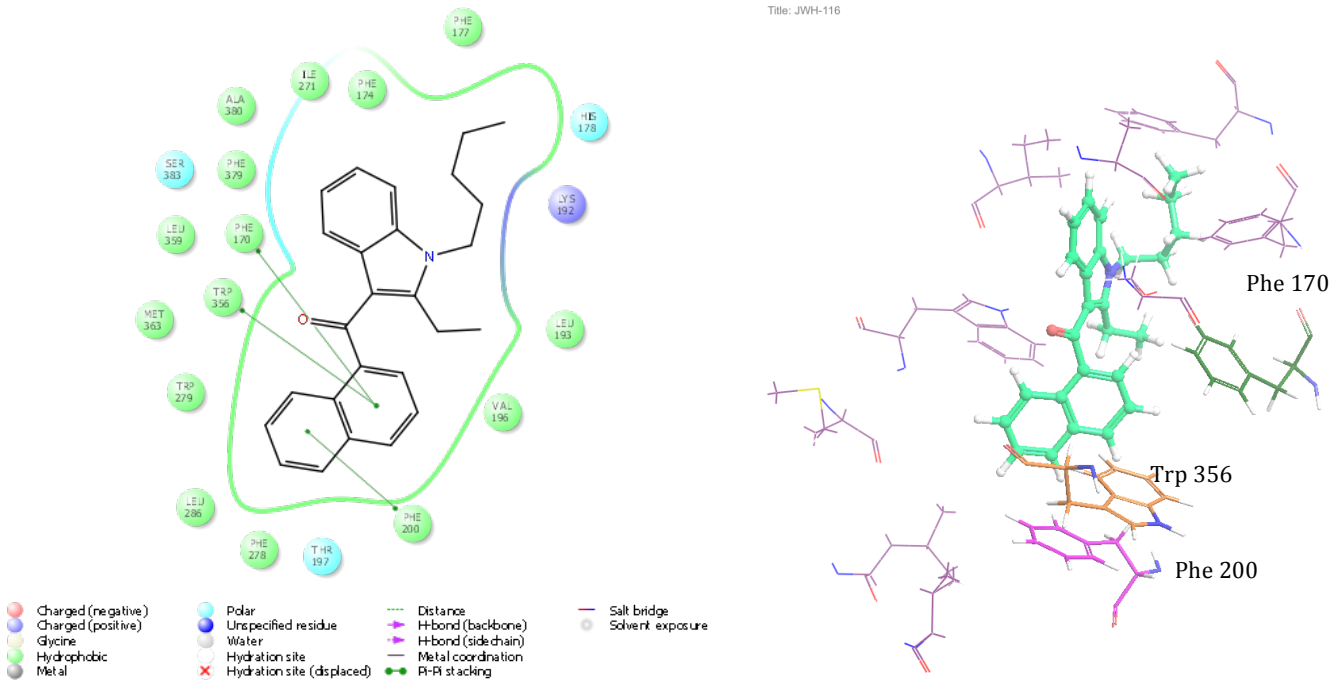


Figure 19: JWH- 116 and CB1 2D and 3D Ligand Interaction Diagrams

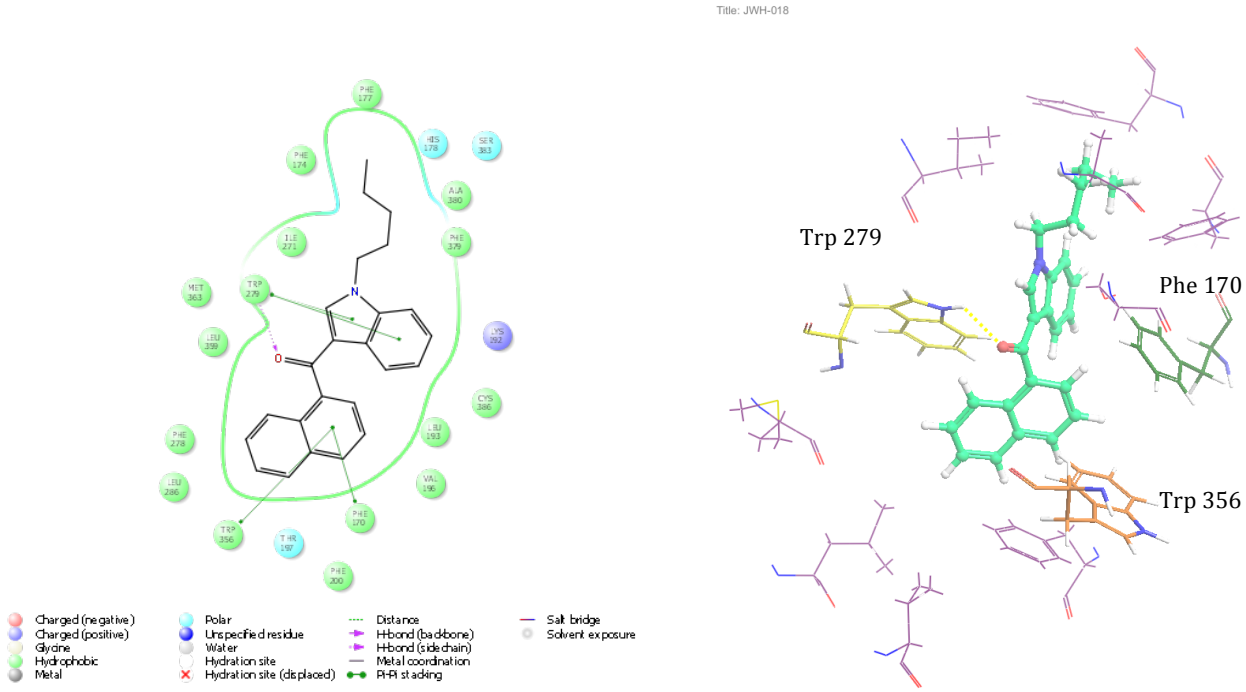


Figure 20: JWH- 018 and CB1 2D and 3D Ligand Interaction Diagrams. H- bond is shown as yellow- colored dashes.

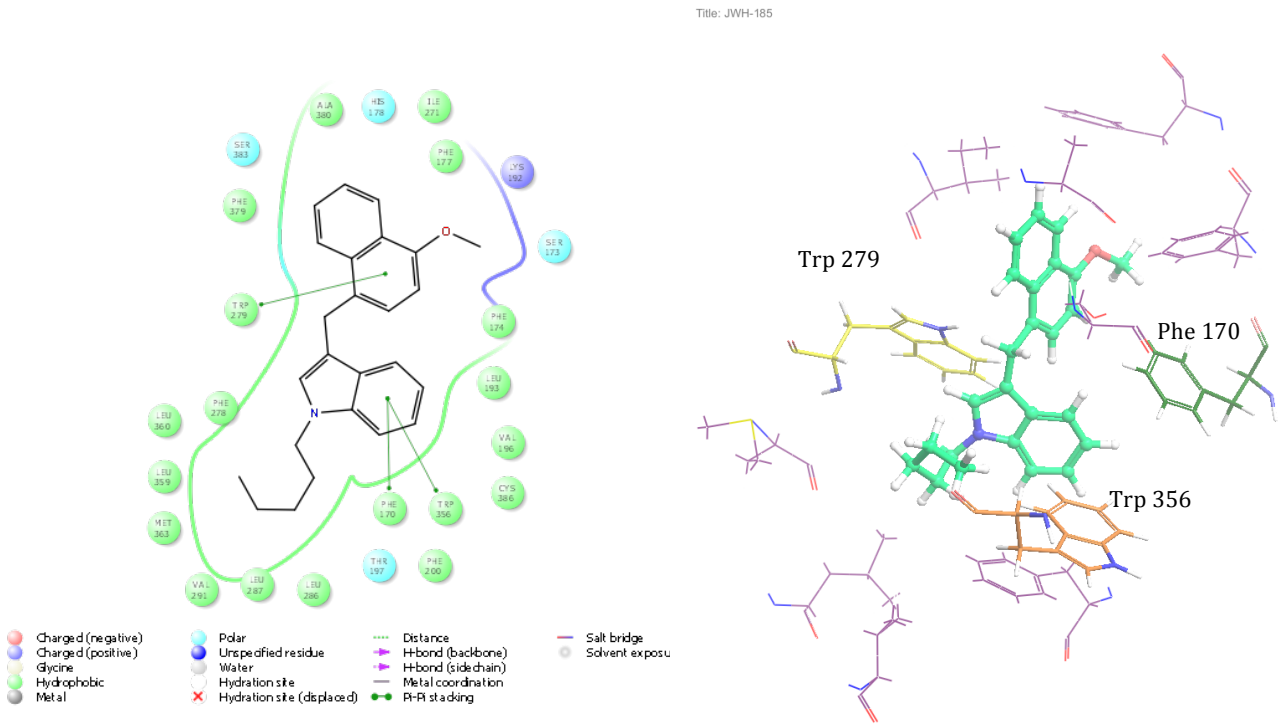


Figure 21: JWH- 185 and CB1 2D and 3D Ligand Interaction Diagrams

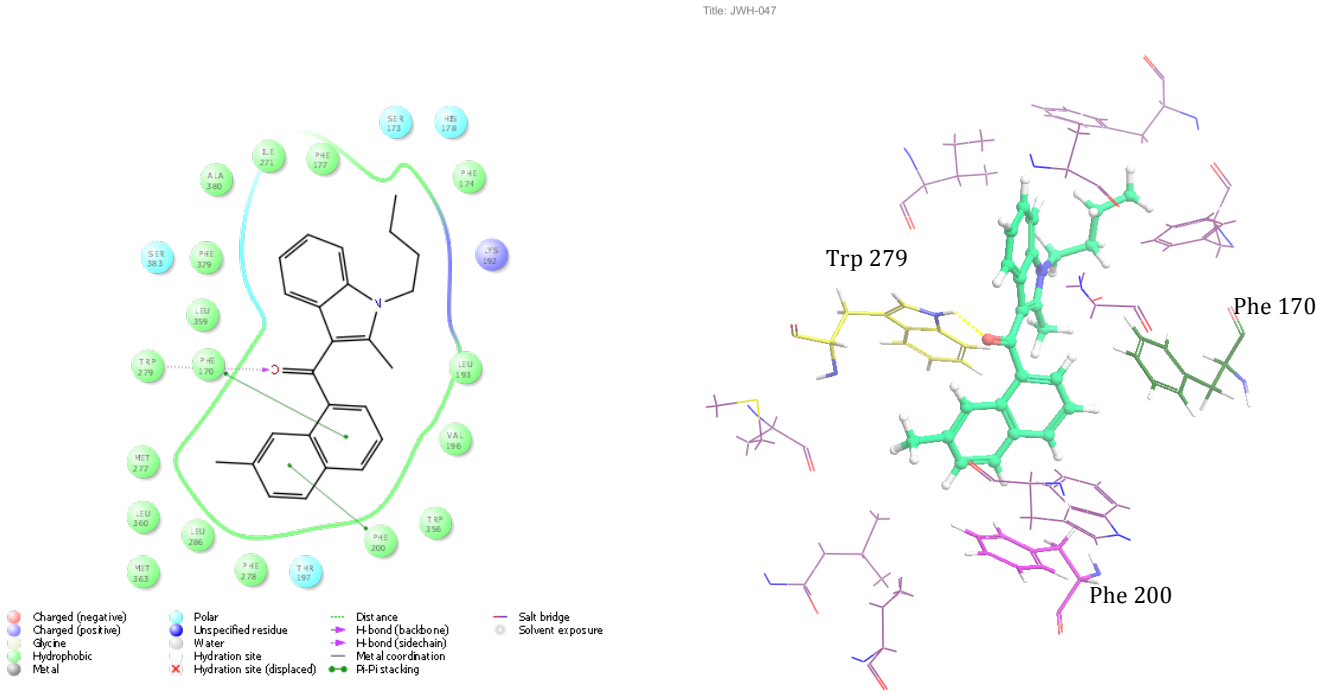


Figure 22: JWH- 047 and CB1 2D and 3D Ligand Interaction Diagrams. H- bond is shown as yellow- colored dashes.

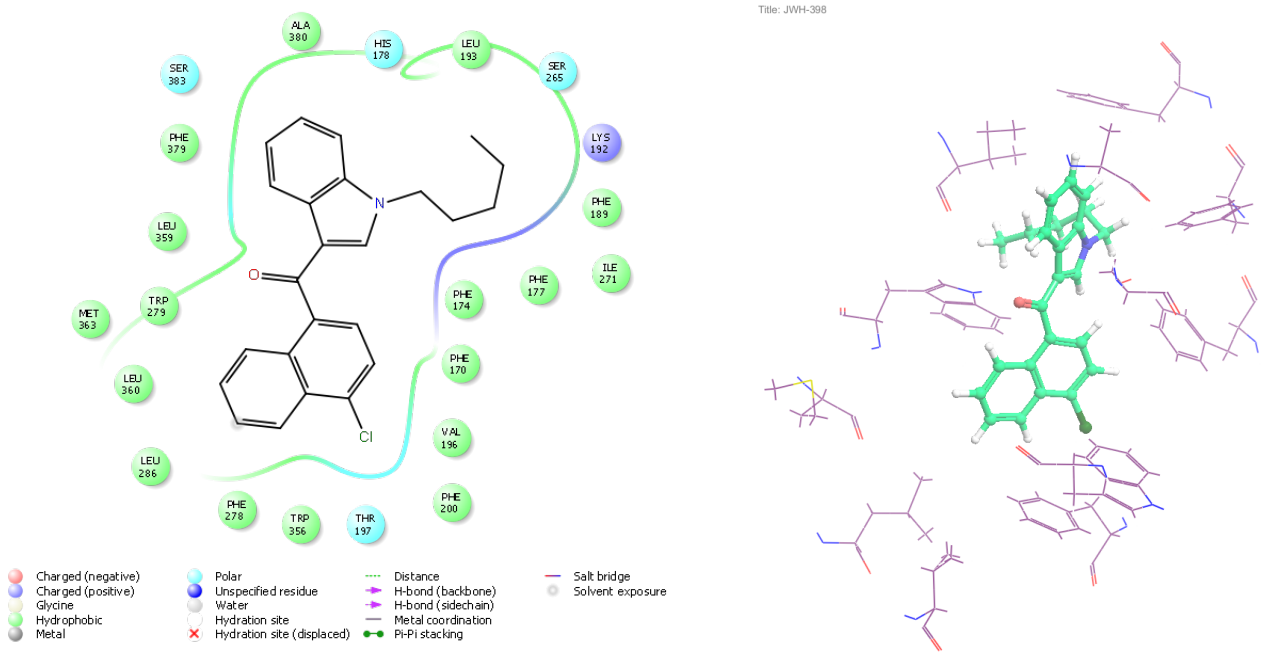


Figure 23: JWH- 398 and CB1 2D and 3D Ligand Interaction Diagrams.

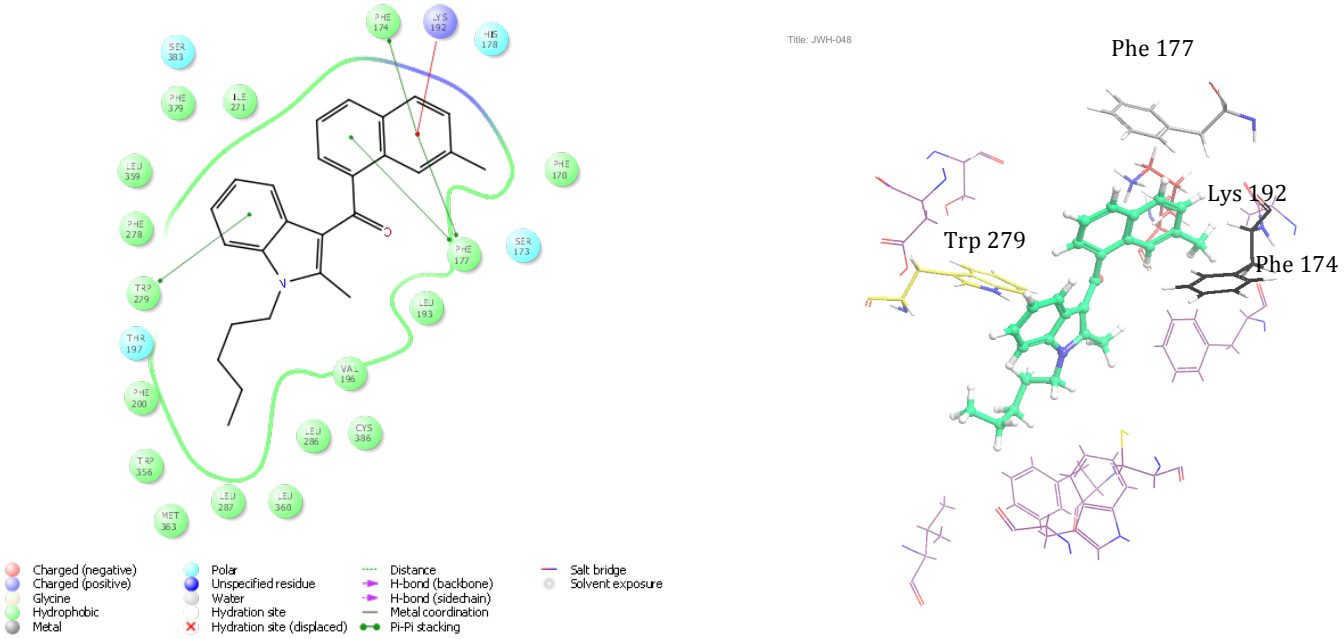


Figure 24: JWH- 048 and CB1 2D and 3D Ligand Interaction Diagrams

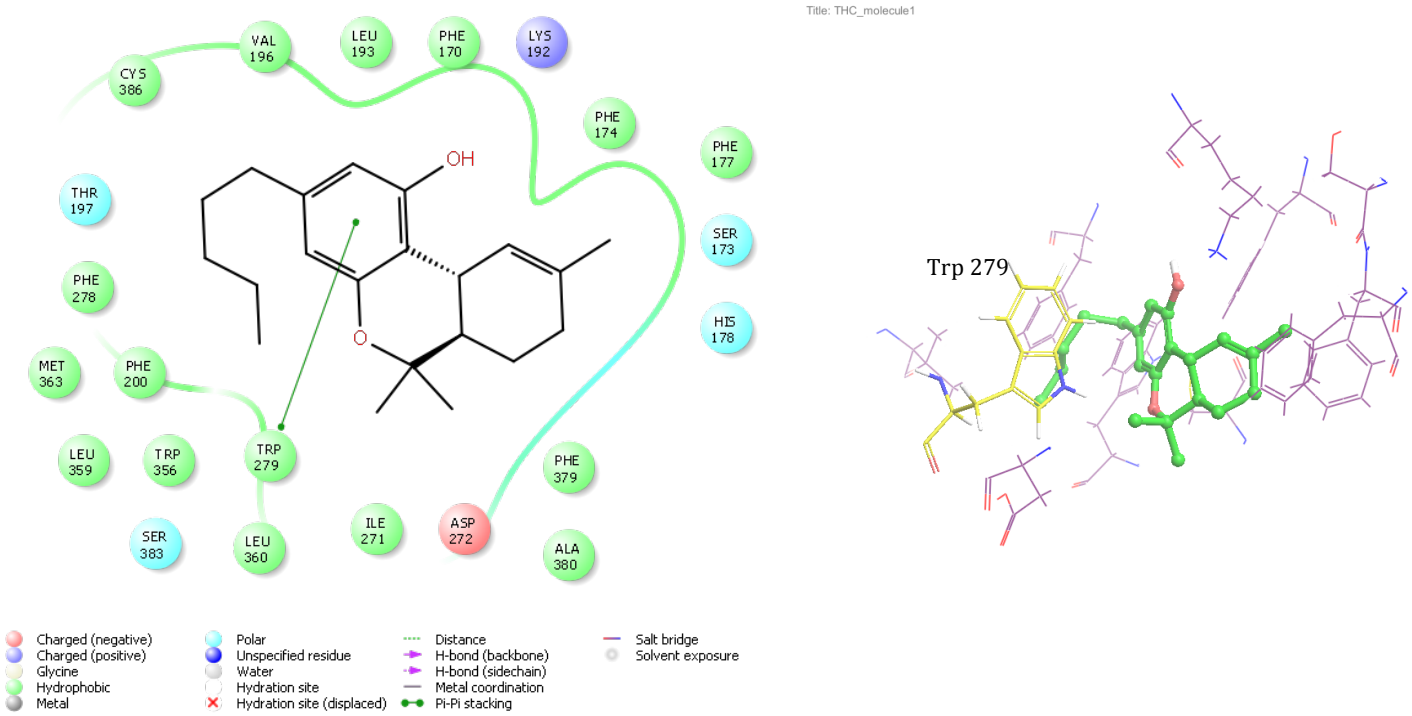


Figure 25: THC and CB1 2D and 3D Ligand Interaction Diagrams.

CONCLUSION

This study revealed that Trp 279, Trp 356, Phe 200, and Phe 170 are key residues in the interaction between CB1 and JWH ligands. It appears that Trp 279 could also be a key residue in forming hydrogen bonds with other cannabinoid classes. Further research on interactions between JWH metabolites and other classes of cannabinoids with this CB1 model could reveal additional important CB1 residues involved in the binding of new and uncharacterized cannabinoids. More studies should be performed on the ligand-receptor interaction to ultimately create a new mass spec database for the detection and prediction of a variety of illicit synthetic cannabinoids.

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