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A Study of the Hydrophobic Interactions Between Twenty-three CB1 Selective JWH Compounds and an Active-State CB1 Receptor Model to Discover Key Structural Features of the JWH Compounds and Key Protein Residues of the CB1 Receptor

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A Study of the Hydrophobic Interactions Between Twenty-three CB_1 Selective JWH Compounds and an Active-State CB_1 Receptor Model to Discover Key Structural Features of the JWH Compounds and Key Protein Residues of the CB_1 Receptor

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

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May 2017

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_____

Reader: Dr. Randy Wadkins

DEDICATION

Firstly, I dedicate this thesis to my parents as they are the backbone of my entire academic career. They have always emphasized the importance of education and have been the primary example of a great work ethic, which has given me the motivation and inspiration to pursue the challenge of publishing a thesis. They have been unfailingly at the forefront of my support team, and I am confident that they will always remain there.

Secondly, my friends have witnessed not only the process of composing my thesis, but the entire four-year journey of pursuing my degree. They have studied late into the night with me, brought me coffee and snacks to energize me during long hours, and have offered words of encouragement when work was overwhelming. They are a core source of kindness, grace, comfort, laughter, and adventure. They are a true testament of Jesus' goodness to me during my time at Ole Miss. I also dedicate this thesis to them as they have been crucial to my college experience.

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This thesis would not have been possible without several people who deserve credit for their assistance during the processes of researching and writing it.

First of , my three advisors/readers were critical in overseeing this entire project. Dr. Godfrey provided me the opportunity to join his research team in order to be involved with the study of synthetic cannabinoids. At every weekly research meeting, he ensured that I was knowledgeable in all areas of my project. Dr. Doerksen allowed me to use his model of the active-state CB_1 receptor, which was a centerpiece for this entire project. Dr. Wadkins, along with Dr. Godfrey and Dr. Doerksen, helped me finalize the process of the publication of this thesis. Additionally, Dr. Pankaj Pandey both troubleshooted problems with the Maestro software and provided revisions for my writing.

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Abstract

Lyncyn Reliquias: A Study of the Hydrophobic Interactions Between Twenty-three CB_1 Selective JWH Compounds and an Active-State CB_1 Receptor Model to Discover Key Structural Components of the JWH Compounds and Key Protein Residues of the CB_1 Receptor
(Under the direction of Dr. Murrell Godfrey)

Synthetic cannabinoids, commonly found in Spice, have recently become a popular substitute for marijuana as they have similar effects to THC, the most powerful constituent of marijuana. Additionally, they are increasingly becoming a source of drug abuse as they cannot be detected with normal drug test screenings. This study specifically analyzed a total of twenty-three CB_1 selective JWH compounds and their hydrophobic interactions with an active-state CB_1 receptor model. The ligands were categorized per their structural similarities, and this study was aimed to find patterns within each structural moiety that would lead to the discovery of key structural features and/or protein residues of the CB_1 receptor. The results of this study will be utilized to predict potential synthetic cannabinoids and to create a database of those structures to expedite the process of identifying them and recognizing their interactions with the CB_1 receptor.

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Introduction

The cannabis plant, commonly referred to as marijuana, is a popular source of a recreational drug. The plant genus *Cannabis* has three distinguished species, *C. sativa*, *C. indica*, and *C. ruderalis* – with *C. sativa* being the most prevalent with current recreational users – which cannabis growers cross-pollinate in order to produce new types of cannabis that have the desired effects on the users of the plant. Such experiments have produced over 100 different strains of the cannabis plant.[1] Although the plant genus *Cannabis* is formally classified and identified by its anatomical characteristics, it is better distinguished by its chemical behavior of producing phytochemicals called cannabinoids, produced by the trichomes of the plant, which are single-celled small hair or other outgrowths that protrude from the epidermis and discharge a sticky residue toxic to insects.[1,2] A number of phenols and terpenes, as well as other organic compounds, occur at the base of the trichome. When the phenols and terpenes migrate upward from the base of the trichome to the bud, a series of chemical reactions occur that convert the individual phenols and terpenes into the more complex cannabinoids. A wide range of cannabinoids exist, of which the most notable and psychoactive is Δ^9-tetrahydrocannabinol (THC).[1] Although marijuana users report that intoxication is relaxing and pleasurable and enhances creativity, the cannabinoids in marijuana – principally THC – have several physiological and psychological consequences including tachycardia, hypertension, changes in perception, and memory failure.[3,4]

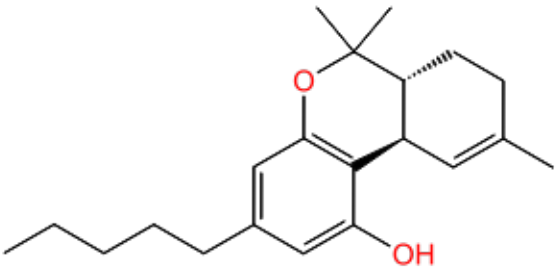
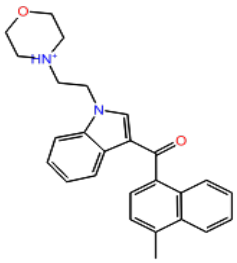


Figure 1: The structure of Δ^9-tetrahydrocannabinol (THC)

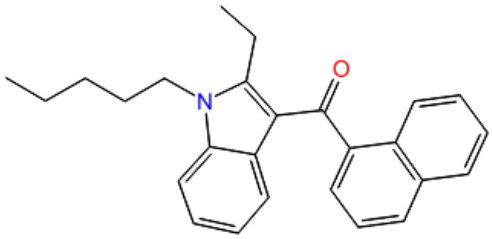
Recently, synthetic cannabinoids have emerged as an alternative to marijuana. Found in packets of herbal mixtures sold as incense and popularly marketed as Spice, K2, Spice Gold, Spice Diamond, Yucatan Fire, and Solar Flare, the substances are administered orally, through vaporization, and most commonly, by smoking. They have a wide range of adverse effects that cause psychiatric, cardiovascular, neurologic, and gastrointestinal harm to abusers.[4,5] They have become increasingly popular to drug abusers as no appropriate drug tests are yet available to verify the presence of synthetic cannabinoids in an individual's system. Common drug screenings cannot currently detect synthetic cannabinoids. Specific urine tests are available, however, for two frequently used synthetic cannabinoids, and blood tests are available for four of them. Unfortunately, neither are readily available at hospitals, and the blood tests must be administered immediately after drug exposure due to some of the synthetic cannabinoids' short half-life.[4] Synthetic cannabinoids are structurally similar to THC but are more potent. Also, some of them are full agonists at cannabinoid receptors with biologically active metabolites. Both synthetic cannabinoids and THC can bind to the same cannabinoid receptors – either CB_1 or

CB_2. Both are G-coupled protein receptors (GCPRs) and are embedded in the cell membrane.[6,7] The CB_1 receptor is primarily located in the brain, while the CB_2 receptor is primarily in the immune system and peripheral organs.[6]

This study analyzes the ligand-receptor hydrophobic interactions between proteins of the CB_1 receptor and a particular class of synthetic compounds known as JWH compounds. Created by John W. Huffman at Clemson University by computationally combining chemical structural features of Δ^9-THC with previously developed aminoalkylindoles, these compounds are arguably the most prominent series of synthetic cannabinoids found in Spice.[9] Although numerous compounds comprise the JWH series, only twenty-three compounds — all of which are selective for the CB_1 receptor — were analyzed in this study.[10, 11, 12, 13, 14, 15, 16] The twenty-three compounds are shown below in Figure 2.



JWH-193



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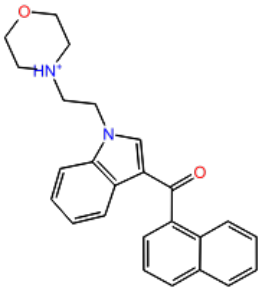
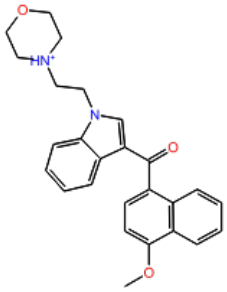
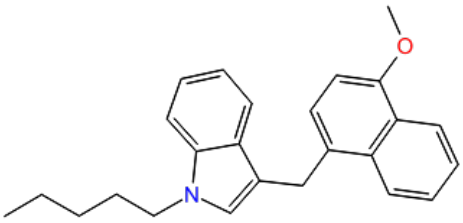


JWH-200



JWH-198



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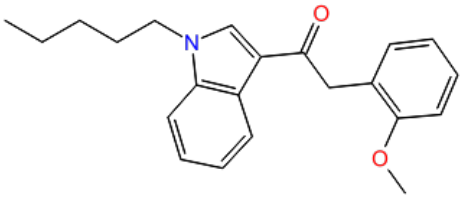

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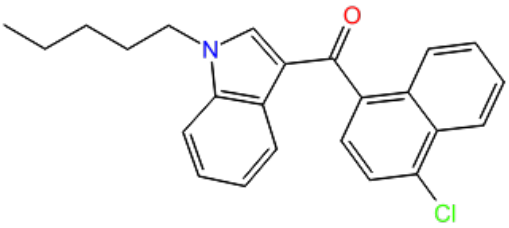
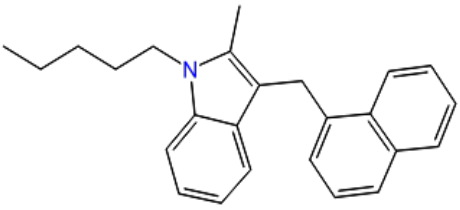
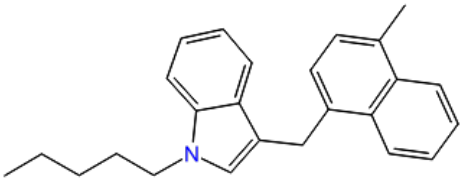


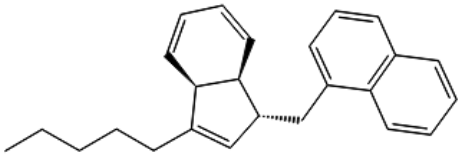
JWH-398



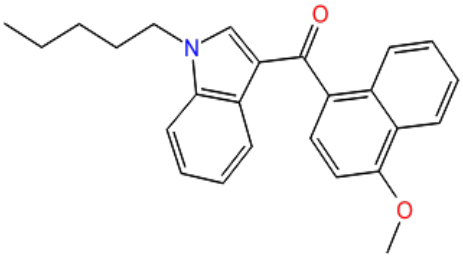
JWH-196



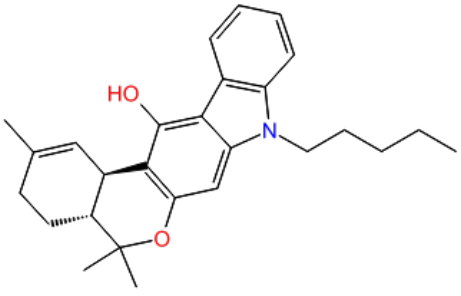
JWH-184



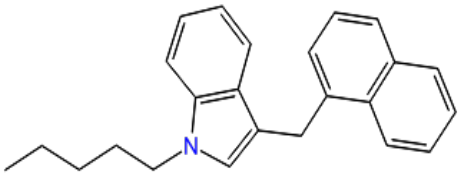
JWH-176



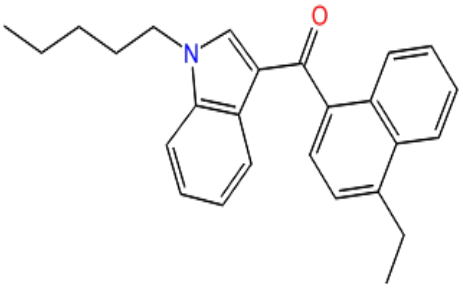
JWH-081



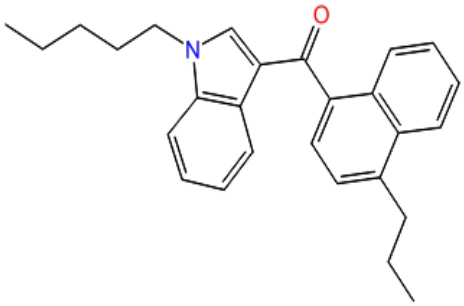
JWH-161



JWH-175



JWH-210



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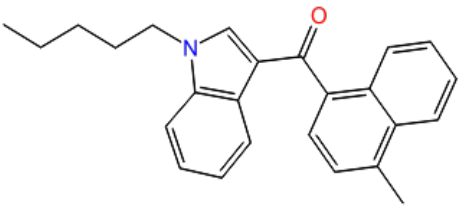
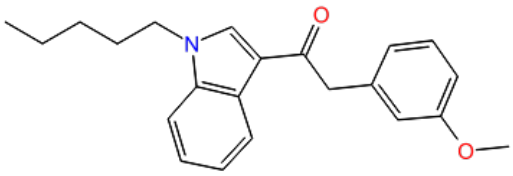
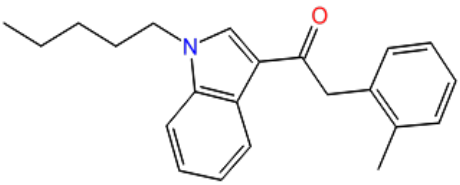


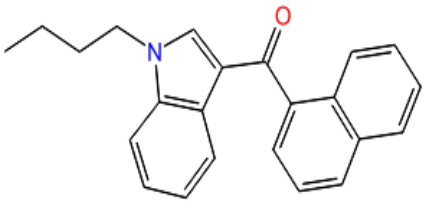
JWH-122



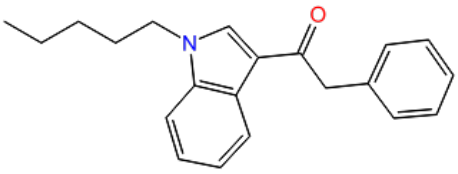
JWH-302



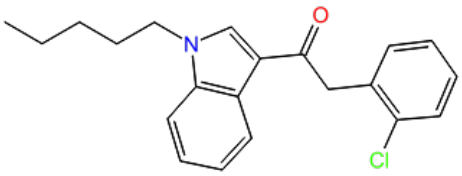
JWH-251



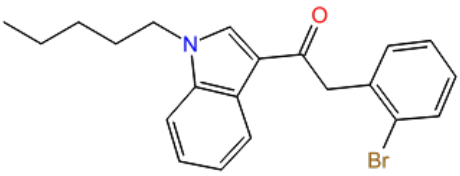
JWH-073



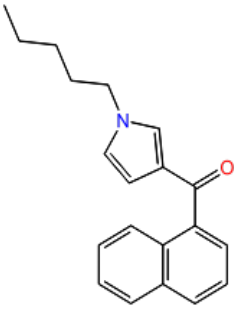
JWH-167



JWH-203



JWH-249



JWH-030

Figure 2: The structures of the twenty-three CB_1 selective JWH compounds used in this study to analyze their interactions with an active-state CB_1 receptor model, arranged by increasing predicted MM-GBSA values

The twenty-three ligands chosen for this study are categorized into four structural groups: naphthoylindoles, phenylacetylindoles, naphthylmethylindoles, and naphthoylpyrroles. However, JWH-161 is an exception as it does not have a distinct structural group due to its unique structure as a hybrid of a "classical" cannabinoid (i.e., THC) and a dibenzopyran.[13]

Naphthoylindoles are compounds containing a 3-(1-naphthoyl) indole structure with substitution at the nitrogen atom of the indole ring by an alkyl, haloalkyl, alkenyl, cycloalkylethyl, 1-(N-methyl-2-piperidinyl) methyl, or 2-(4-morpholinyl) ethyl group, possible further substitution of any extent at the indole ring, and possible substitution of

any extent at the naphthyl ring.[18] The CB_1 selective JWH compounds that are naphthoylindoles are JWH-073, JWH-081 JWH-116, JWH-122, JWH-182, JWH-185, JWH-196, JWH-210, and JWH 398.[12, 13, 19, 20, 21] Furthermore, JWH-193, JWH-198, and JWH-200 are a specific subgroup of naphthoylindoles called aminoalkylindoles, which have key structural features of a naphthyl substituent at the 3-position, a hydrogen substituent at the 2-position, and an aminoethyl substituent at the 1-position.[13, 21]

Phenylacetylindoles are characterized by a 3-phenylacetylindole structure with substitution at the nitrogen atom of the indole ring by an alkyl, haloalkyl, alkenyl, cycloalkylmethyl, cycloalkylethyl, 1-(N-methyl-2-piperidinyl) methyl, or 2-(4- morpholinyl) ethyl group, possible further substitution of any extent at the indole ring, and possible substitution of any extent at the phenyl ring.[21] The CB_1 selective JWH compounds that are phenylacetylindoles are JWH-167, JWH-203, JWH-249, JWH-250, JWH-251, and JWH-302.[14, 15]

Naphthylmethylindole compounds contain 1-indol-3-yl-(1-naphthyl) methane structure with substitution at the nitrogen atom of the indole ring by an alkyl, haloalkyl, alkenyl, cycloalkylmethyl, cycloalkylethyl, 1-(N-methyl-2-piperidinyl) methyl, or 2-(4-morpholinyl) ethyl group, possible further substitution of any extent at the indole ring, and possible substitution of any extent at the naphthyl ring.[21] The CB_1 selective JWH compounds that are naphthylmethylindoles are JWH-175 and JWH-184.[12, 13]

JWH-030 is the lone CB_1 selective JWH compound that is a naphthoylpyrrole, while JWH-176 is the lone naphthylmethylindene.[13, 23] A naphthoylpyrrole compound contains a 3-(1-naphthoyl) pyrrole structure with substitution at the nitrogen atom of the pyrrole ring by an alkyl, haloalkyl, alkenyl, cycloalkylmethyl, cycloalkylethyl, 1-(N-

methyl-2-piperidinyl) methyl, or 2-(4- morpholinyl) ethyl group, possible further substitution of any extent at the pyrrole ring, and possible substitution of any extent at the naphthyl ring. Naphthylmethylindene compounds have a 1-(1- naphthylmethyl)indene structure with substitution at the 3-position of the indene ring by an alkyl, haloalkyl, alkenyl, cycloalkylmethyl, cycloalkylethyl, 1-(N-methyl-2-piperidinyl) methyl, or 2-(4-morpholinyl) ethyl group, possible further substitution of any extent to the indene ring, and possible substitution of any extent at the naphthyl ring.[21]

A standard, precise three-dimensional model of the structure of the CB_1 active-state receptor has yet to be reported. For this study, however, a model developed and validated by Dr. Robert J. Doerksen's research lab and was utilized for the computational study.[17] The active-state model of the CB_1 receptor used in this study is shown in Figure 3. In Fall 2016, two X-ray crystal structures were reported for the inactive state of the CB_1 receptor, but the differences between the active and inactive state are thought to be significant.

Figure 3: The three-dimensional structure of the active-state CB_1 model used in this study with a bound THC ligand

Although the ligands had multiple different forms of interactions with the active-state CB_1 receptor model, such as hydrophobic, positively and negatively charged, polar, hydrogen bonding, π-cation, and π-π stacking interactions, this study exclusively focuses on the hydrophobic interactions. Hydrophobic interactions hold nonpolar molecules together, such as synthetic cannabinoids and the CB_1 receptor, which features a significant content of amino acids with hydrophobic side chains, such as leucine, isoleucine, phenylalanine, valine, and tryptophan.[24, 25]

The protein residues with which the twenty-three ligands interacted hydrophobically were alanine (ALA), cysteine (CYS), isoleucine (ILE), leucine (LEU), methionine

(MET), phenylalanine (PHE), tryptophan (TRP), and valine (VAL). Alanine, isoleucine, leucine, methionine, and valine are amino acids with nonpolar, aliphatic R groups. The side chains of alanine, isoleucine, leucine, and valine typically cluster together within proteins and stabilize the protein structure by means of hydrophobic interactions. Methionine contains sulfur and a slightly nonpolar thioether group in its side chain. Phenylalanine and tryptophan have aromatic R groups and are relatively nonpolar, with tryptophan being more polar than phenylalanine because of the nitrogen in tryptophan's indole ring. Cysteine has polar, uncharged R groups. However, its polarity, contributed by its sulfhydryl group, is more modest compared to other amino acids such as serine and threonine.[24]

The analysis of the hydrophobic interactions between the CB_1 selective JWH compounds and the active-state CB_1 receptor model, as well as future analyses of the other forms of interactions that exist between the ligands and receptor and other studies that examine other types of synthetic cannabinoids and their interactions with the active-state CB_1 receptor model, will be used in the prediction of emerging synthetic cannabinoids. Once sufficient data concerning the ligand-receptor interactions are collected, the information will be utilized to create a mathematical algorithm that will generate potential structures of undiscovered synthetic cannabinoids. The generated structures will be compiled into a library for comparing it to synthetic cannabinoids that will be discovered in the future. Such a database will expedite the process of determining the chemical features of emerging synthetic cannabinoids.

Computational Methods

Ligands Selection

All JWH synthetic cannabinoids that were selective for the CB_1 receptor in the brain rather than the CB_2 receptor were chosen for this study.

Protein Selection and Preparation

The active-state CB_1 receptor model that was prepared by Dr. Robert J. Doerksen's research group was chosen for this study as it proved the best overall performance among other created models.[17] Maestro's protein preparation wizard corrected existing problems concerning steric hindrances, interatomic distances, and locations of hydrogen atoms.

Grid Generation

The software used for this study was Maestro (version 10.6.014), created by Schrödinger, LLC. Under the Tasks tab of Maestro and the Docking option, the Grid Generation Centroid Workspace was opened. A grid was generated around the binding pocket of the protein by clicking the center of the bound THC to indicate where the selected ligands should bind.

Ligands Preparation

The ligands were sketched on Maestro's 2D sketcher, which is available on the program's primary workspace. The two-dimensional ligands were then converted to three-dimensional structures with the software in order to prepare them for docking through the software's Ligand Prep function under the Tasks tab. The target pH was set to 7.0 ± 2.0, and the force field OPLS3 was set.

Glide Docking

The twenty-three energy minimized ligands were docked with the standard precision (SP) module through the Glide Docking function of the software, accessed under the Task tab and the Docking option. The grid constructed specifically for this study was used, and multiple poses of each ligand were generated.

Results and Discussion

Following the successful docking of the twenty-three ligands into the CB_1 model, the most favorable pose for each ligand, according to their MM-GBSA binding affinities, was analyzed in this study. The MM-GBSA values of the ligands, computed by Maestro, estimate the relative binding affinity for each ligand in kcal/mol. Although they do not necessarily agree with experimental binding affinities, the results generally rank similarly to experimental binding affinities.[26] Additionally, the K_i values of each compound were compared to their MM-GBSA energy to confirm the validity of their binding affinities. K_i is the equilibrium constant for the release of ligands, or their dissociation from proteins, for competitive inhibitors in ligand-protein interactions.[24] However, the K_i values and the MM-GBSA values lacked any correlation. The MM-GBSA values ranged from –100.91 to –71.831 kcal/mol. Greater negativity indicates greater affinity to the CB_1 receptor. The binding energy scores and the K_i values are displayed in Table 1 in decreasing order.[10, 11, 12, 13, 14, 19, 21]

Compounds	MM-GBSA Value (kcal/mol)	K_i Value (nM)
JWH-193	-100.910	6.0
JWH-116	-98.546	52.0
JWH-200	-97.900	42.0
JWH-198	-96.911	10.0
JWH-185	-94.899	17.0
JWH-250	-93.809	11.0
JWH-398	-91.128	2.3
JWH-196	-90.966	151.0
JWH-184	-90.728	23.0
JWH-176	-90.195	26.0
JWH-081	-89.962	1.2
JWH-161	-89.487	19.0
JWH-175	-89.331	22.0
JWH-210	-89.090	0.46
JWH-182	-88.858	0.65
JWH-122	-87.685	0.69
JWH-302	-85.647	89
JWH-251	-85.520	146
JWH-203	-85.312	8.0
JWH-249	-84.081	8.4
JWH-073	-82.993	8.9
JWH-167	-76.517	90
JWH-030	-71.831	87

Table 1: The predicted MM-GBSA values of the twenty-three CB_1 selective JWH compounds to the CB_1 receptor model, in decreasing order

After docking, the ligand-receptor hydrophobic interactions that occurred within a range of 5 Å between the ligand and the receptor were analyzed. Twenty-six specific protein residues interacted with the ligands. Table 2 shows the hydrophobic interactions between all the CB_1 selective JWH compounds with the protein residues of the active-state CB_1 receptor model that were observed.

JWH-175	JWH-161	JWH-081	JWH-176	JWH-184	JWH-196	JWH-398	JWH-250	JWH-185	JWH-198	JWH-200	JWH-116	JWH-193	
					x		x				x		ALA 198
x	x	x	x	x	x	x	x	x	x	x	x	x	ALA 380
			x								x		CYS 382
x	x		x	x	x		x	x	x	x	x	x	CYS 386
													ILE 169
x													ILE 175
x	x	x	x	x	x	x	x	x	x	x	x	x	ILE 271
x	x	x	x	x	x	x	x	x	x	x	x	x	LEU 193
x	x	x	x	x	x	x	x	x	x	x	x	x	LEU 286
	x	x	x	x	x	x	x	x	x	x	x	x	LEU 287
x	x	x	x	x	x	x	x	x	x	x	x	x	LEU 359
x	x	x	x	x	x	x	x	x	x	x	x	x	LEU 360
	x	x	x	x	x	x	x	x	x	x	x	x	MET 277
x	x	x	x	x	x	x	x	x	x	x	x	x	MET 363
x	x	x	x	x	x	x	x	x	x	x	x	x	PHE 170
x	x	x	x	x	x	x	x	x	x	x	x	x	PHE 174
x	x	x	x	x	x	x	x	x	x	x	x	x	PHE 177
x	x		x	x	x			x	x	x	x	x	PHE 189
	x	x	x		x	x	x	x	x	x	x	x	PHE 200
x	x	x	x	x	x	x	x	x	x	x	x	x	PHE 278
x	x	x	x	x	x	x	x	x	x	x	x	x	PHE 379
x	x	x	x	x	x	x	x	x	x	x	x	x	TRP 279
x	x	x	x	x	x	x	x	x	x	x	x	x	TRP 356
													VAL 171
x	x	x	x	x	x	x	x	x	x	x	x	x	VAL 196
	x	x	x	x	x	x	x	x	x	x	x	x	VAL 291

	JWH-030	JWH-167	JWH-073	JWH-249	JWH-203	JWH-251	JWH-302	JWH-122	JWH-182	JWH-210
ALA 198				x	x		x			
ALA 380	x	x	x	x	x	x	x	x	x	x
CYS 382									x	
CYS 386	x	x	x	x	x	x	x		x	x
ILE 169									x	
ILE 175										
ILE 271	x	x	x	x	x	x	x	x	x	x
LEU 193	x	x	x	x	x	x		x	x	x
LEU 286	x	x	x	x	x	x	x	x	x	x
LEU 287	x	x	x	x	x	x	x	x		x
LEU 359	x	x	x	x	x	x	x	x	x	x
LEU 360	x	x	x	x	x	x	x	x		x
MET 277	x	x	x	x	x	x	x	x		x
MET 363	x	x	x	x	x	x	x	x	x	x
PHE 170	x	x	x	x	x	x	x	x	x	x
PHE 174	x	x	x	x	x	x	x	x	x	x
PHE 177	x	x	x	x	x		x	x	x	x
PHE 189						x			x	x
PHE 200	x	x	x	x	x	x	x	x	x	x
PHE 278	x	x	x	x	x	x	x	x	x	x
PHE 379	x	x	x	x	x	x	x	x	x	x
TRP 279	x	x	x	x	x	x	x	x	x	x
TRP 356	x	x	x	x	x	x	x	x	x	x
VAL 171									x	
VAL 196	x	x	x	x	x	x	x	x	x	x
VAL 291	x	x	x	x	x	x	x	x		x

Table 2: The hydrophobic interactions between the CB_1 selective JWH compounds and the active-state CB_1 receptor

ALA 380, ILE 271, LEU 286, LEU 359, MET 363, PHE 170, PHE 174, PHE 278, PHE 379, TRP 279, TRP 356, and VAL 196 all interacted hydrophobically with

each ligand, whereas the other protein residues were selective in their hydrophobic interactions with the ligands. This study separated the twenty-three ligands according to their structural groups to survey which protein residues interacted with each ligand and with which structural feature of each ligand the residues interacted.

A feature of Maestro's software, Interaction Fingerprints, enabled such close assessments by providing a magnified view of the hydrophobic interactions by specifically indicating which protein residues interact with each atom of the inspected ligand. The hydrophobic interactions with the key structural components of each structural group that distinguish each one from the others were inspected, while the structural components that are unique to each individual compound were excluded from the study in order to simplify the process of the search for patterns and significant interacting protein residues and/or structural features within each structural group.

Naphthoylindole Structural Group

The first structural group analyzed in this study was the naphthoylindoles, which are the compounds JWH-073, JWH-081 JWH-116, JWH-122, JWH-182, JWH-185, JWH-193, JWH-196, JWH-198, JWH-200, JWH-210, and JWH 398, with JWH-193, JWH-198, and JWH-200 belonging in the subgroup aminoalkylindoles. Figures 4-17 display the two-dimensional view of the interactions between each naphthoylindole and the protein residues of the active-state CB_1 receptor model, in order of decreasing predicted MM-GBSA values.

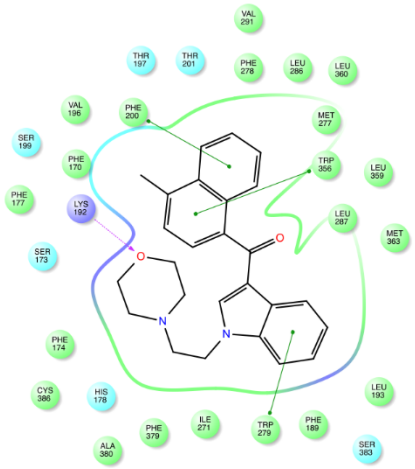


Figure 4: The two-dimensional view of the interactions between JWH-193 and the protein residues of the active-state CB_1 receptor model, with the green residues indicating hydrophobic interactions

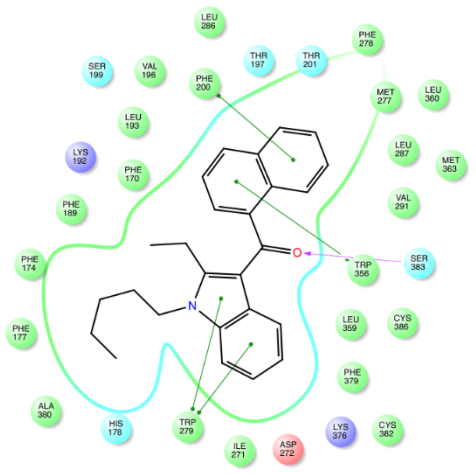


Figure 5: The two-dimensional view of the interactions between JWH-116 and the protein residues of the active-state CB_1 receptor model, with the green residues indicating hydrophobic interactions

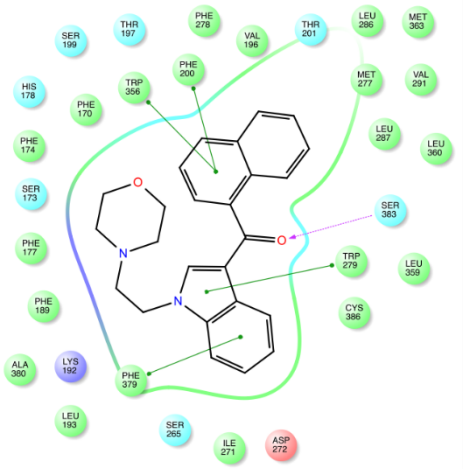


Figure 6: The two-dimensional view of the interactions between JWH-200 and the protein residues of the active-state CB_1 receptor model, with the green residues indicating hydrophobic interactions

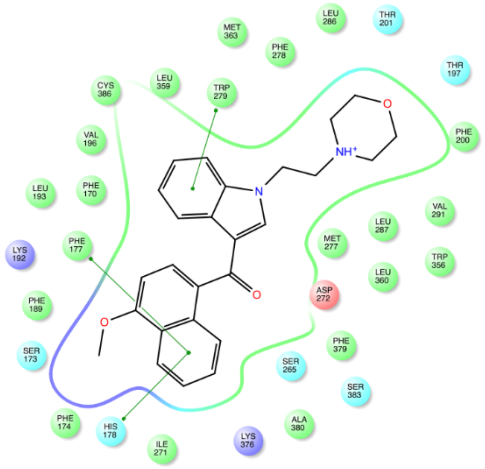


Figure 7: The two-dimensional view of the interactions between JWH-198 and the protein residues of the active-state CB_1 receptor model, with the green residues indicating hydrophobic interactions

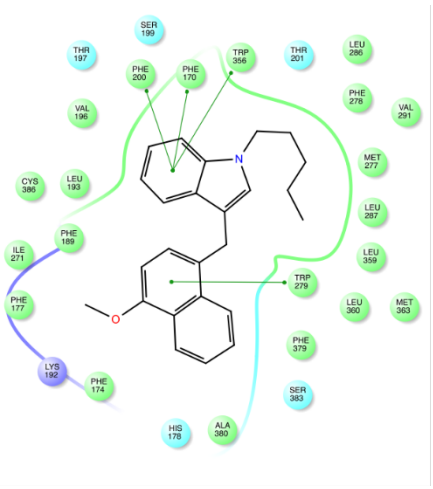


Figure 8: The two-dimensional view of the interactions between JWH-185 and the protein residues of the active-state CB_1 receptor model, with the green residues indicating hydrophobic interactions

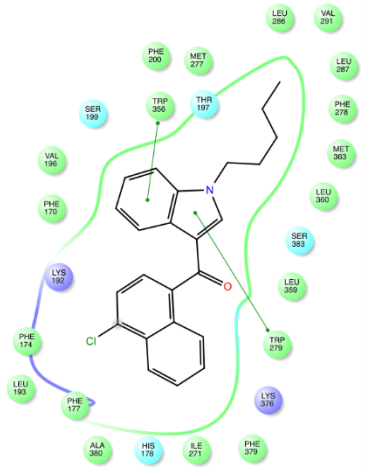


Figure 9: The two-dimensional view of the interactions between JWH-398 and the protein residues of the active-state CB_1 receptor model, with the green residues indicating hydrophobic interactions

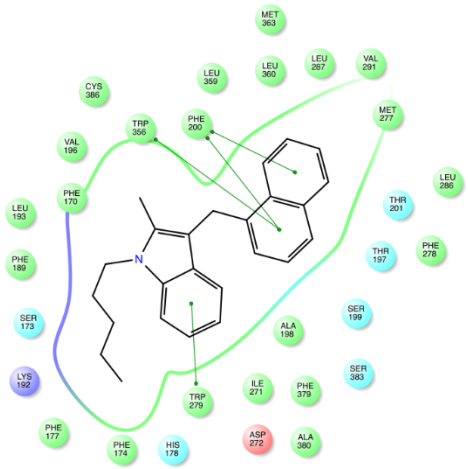


Figure 10: The two-dimensional view of the interactions between JWH-196 and the protein residues of the active-state CB_1 receptor model, with the green residues indicating hydrophobic interactions

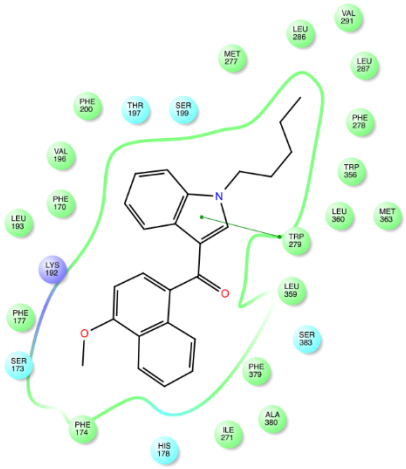


Figure 11: The two-dimensional view of the interactions between JWH-081 and the protein residues of the active-state CB_1 receptor model, with the green residues indicating hydrophobic interactions

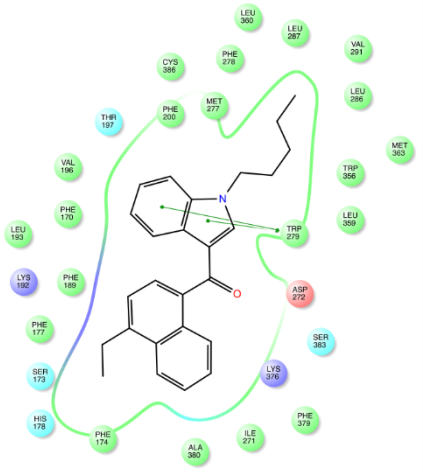


Figure 12: The two-dimensional view of the interactions between JWH-210 and the protein residues of the active-state CB_1 receptor model, with the green residues indicating hydrophobic interactions

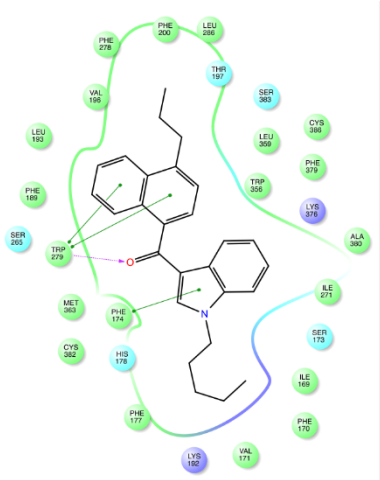


Figure 13: The two-dimensional view of the interactions between JWH-182 and the protein residues of the active-state CB_1 receptor model, with the green residues indicating hydrophobic interactions

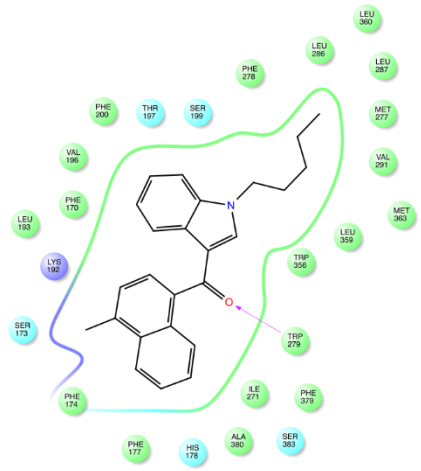


Figure 14: The two-dimensional view of the interactions between JWH-122 and the protein residues of the active-state CB_1 receptor model, with the green residues indicating hydrophobic interactions

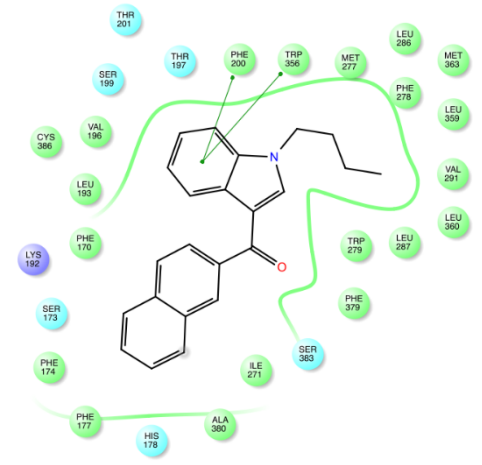


Figure 15: The two-dimensional view of the interactions between JWH-073 and the protein residues of the active-state CB_1 receptor model, with the green residues indicating hydrophobic interactions

The hydrophobic interactions between the naphthoylindoles and the protein residues of the CB_1 receptor model were the first set of interactions analyzed and are shown in Table 3.

	JWH-193	JWH-116	JWH-200	JWH-198	JWH-185	JWH-398	JWH-196	JWH-081	JWH-210	JWH-182	JWH-122	JWH-073
ALA 198		x					x					
ALA 380	x	x	x	x	x	x	x	x	x	x	x	x
CYS 382		x								x		
CYS 386	x	x	x	x	x		x		x	x	x	x
ILE 169										x		
ILE 271	x	x	x	x	x	x	x	x	x	x	x	x
LEU 193	x	x	x	x	x	x	x	x	x	x	x	x
LEU 286	x	x	x	x	x	x	x	x	x	x	x	x
LEU 287	x	x	x	x	x	x	x	x	x		x	x
LEU 359	x	x	x	x	x	x	x	x	x	x	x	x
LEU 360	x	x	x	x	x	x	x	x	x		x	x
MET 277	x	x	x	x	x	x	x	x	x		x	x
MET 363	x	x	x	x	x	x	x	x	x	x	x	x
PHE 170	x	x	x	x	x	x	x	x	x	x	x	x
PHE 174	x	x	x	x	x	x	x	x	x	x	x	x
PHE 177	x	x	x	x	x	x	x	x	x	x	x	x
PHE 189	x	x	x	x	x		x		x	x		
PHE 200	x	x	x	x	x	x	x	x	x	x	x	x
PHE 278	x	x	x	x	x	x	x	x	x	x	x	x
PHE 379	x	x	x	x	x	x	x	x	x	x	x	x
TRP 279	x	x	x	x	x	x	x	x	x	x	x	x
TRP 356	x	x	x	x	x	x	x	x	x	x	x	x
VAL 171										x		
VAL 196	x	x	x	x	x	x	x	x	x	x	x	x
VAL 291	x	x	x	x	x	x	x	x	x		x	x

Table 3: The hydrophobic interactions between the naphthoylindoles of the CB_1 selective JWH compounds and the protein residues of the active-state CB_1 receptor model

Excluding the protein residues that hydrophobically interacted with each ligand regardless of its structural group, all naphthoylindoles also hydrophobically interacted

with LEU 193, PHE 177, and PHE 200. CYS 386 interacted with most of the naphthoylindoles, but it did not have any hydrophobic interactions with JWH-398, JWH-081, or JWH-122. PHE 189 also interacted with most of the naphthoylindoles, but it did not have any hydrophobic interactions with JWH-398, JWH-081, JWH-122, and JWH-073. ALA 198 only interacted with JWH-116 and JWH-196, while CYS 382 only interacted with JWH-116 and JWH-182. ILE 169 and VAL 171 only interacted with JWH-182. Additionally, JWH-182 was the only naphthoylindole to not have hydrophobic interactions with LEU 287, LEU 360, MET 277, and VAL 291.

The key structural features of the naphthoylindoles that distinguish them from the other CB_1 selective JWH compounds are their naphthyl rings, indole rings, either a methylene or carbonyl bridge that connects the naphthyl and indole rings, and a substituent at the nitrogen atom of the indole ring. The hydrophobic interactions between the CB_1 receptor protein residues with the key structural components of each naphthoylindole were evaluated, and the data received from the Interaction Fingerprints were organized into Figure 16.

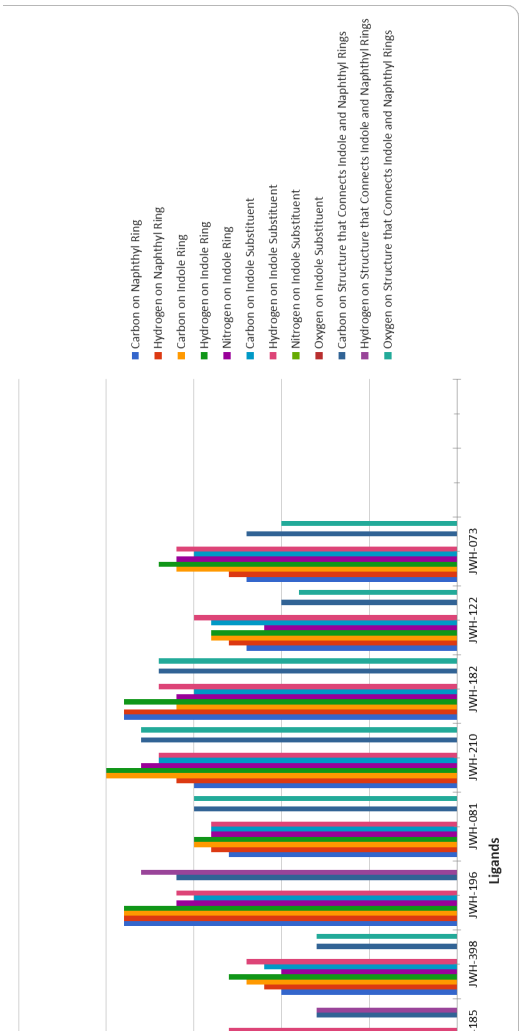


Figure 16a: The frequency of hydrophobic interactions of each structural feature of the naphthoylindole ligands with CB_1 receptor protein residues

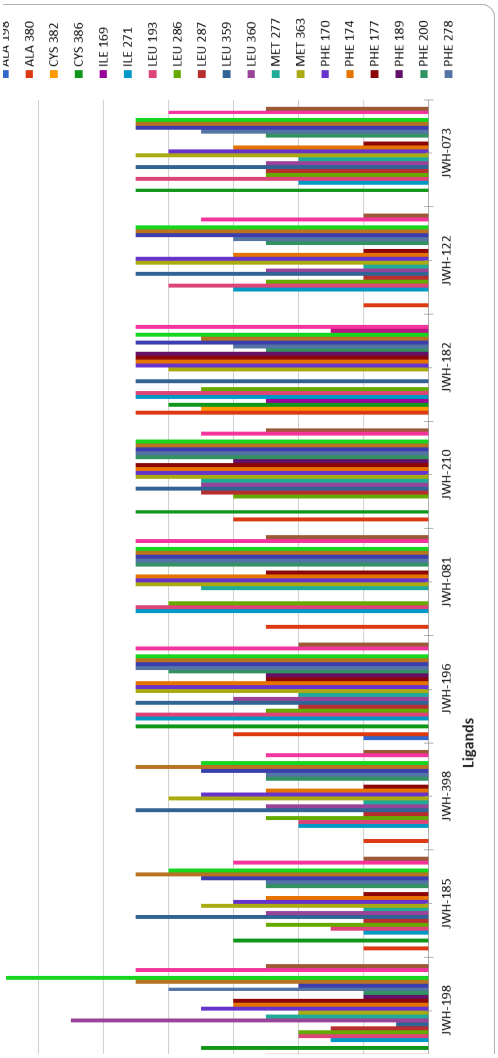


Figure 16b: The frequency of hydrophobic interactions of CB_1 receptor protein residues with the structural feature of the naphthoylindoles

Figure 16a divided each naphthoylindole into the key structural features and displayed the number of residues with which each structural feature interacted. Table 4 organizes Figure 16a as it shows the average of the number of residues with which each structural feature interacted.

Atom	Average Number of Protein Residues
Carbon on Naphthyl Ring	12.9
Hydrogen on Naphthyl Ring	14.2
Carbon on Indole Ring	13.4
Hydrogen on Indole Ring	13.8
Nitrogen on Indole Ring	10.7
Carbon on Indole Substituent	13.0
Hydrogen on Indole Substituent	15.0
Nitrogen on Indole Substituent	1.5
Oxygen on Indole Substituent	1.4
Carbon on Structure that Connects Indole and Naphthyl Rings	10.6
Hydrogen on Structure that Connects Indole and Naphthyl Rings	2.2
Oxygen on Structure that Connects Indole and Naphthyl Rings	8.2

Table 4: The average number of protein residues that interact with each type of atom in naphthoylindoles

The most common type of atom that had hydrophobic interactions with one or more protein residues was a hydrogen atom on the substituent on the nitrogen atom on the indole ring. Furthermore, seventy-five percent of the top four structural features with which the protein residues interacted hydrophobically were with a hydrogen atom. The

carbon atoms were next highest in value, while the nitrogen and oxygen atoms had the least number of hydrophobic interactions with the protein residues of the CB_1 receptor.

To investigate whether one structural component, rather than a particular atom of a structural component, is more significant than others, the values from Table 4 were partitioned into the following: atoms of the naphthyl ring, atoms of the indole ring, atoms of the substituent on the nitrogen atom of the indole ring, and atoms of the structure connecting the naphthyl and indole rings. The values within each category were averaged and are shown in Table 5.

Structural Feature	Average Number of Protein Residues
Naphthyl Ring	13.5
Indole Ring	12.6
Substituent of Indole Ring	7.7
Carbon Structure Connecting Indole and Naphthyl Rings	7.0

Table 5: The average number of protein residues that interact with each structural feature of naphthoylindoles

Although the values do not vary much from each other, more hydrophobic interactions occur in the naphthyl ring than anywhere else on naphthoylindole molecules, while hydrophobic interactions occurred least frequently on the indole ring.

Figure 16b indicates whether a specific protein residue is more significant than its counterparts by placing a value on the number of types of atoms with which it hydropho-

bically interacts. Table 6 numerically organizes the information on Figure 16b as it shows the average number of structural features with which each amino acid interacts.

Protein Residue	Average Number of Types of Atoms
ALA 198	0.2
ALA 380	3.4

CYS 382	0.7
CYS 386	5.3
ILE 169	0.4
ILE 271	5.4
LEU 193	5.9
LEU 286	4.8
LEU 287	2.6
LEU 359	6.7
LEU 360	4.2
MET 277	3.0
MET 363	7.0
PHE 170	8.0
PHE 174	6.8
PHE 177	4.6
PHE 189	2.4
PHE 200	4.9
PHE 278	5.8
PHE 379	7.0
TRP 279	8.9
TRP 356	7.8
VAL 171	0.3
VAL 196	7.0
VAL 291	3.0

Table 6: The average number of types of atoms of naphthoylindoles that interact with the protein residues

According to its relatively high average value, TRP 279 is the most interactive protein residue with the naphthoylindoles. On the other hand, CYS 382, ILE 169, VAL 171, and ALA 198 rarely had hydrophobic interactions with the naphthoylindoles.

To investigate whether a particular amino acid, regardless of its position in the CB_1 receptor, had more significance than other amino acids, the protein residues were categorized according to their similar structures. Their collective values were averaged and are displayed in Table 7.

Amino Acid	Average Number of Types of Atoms
Alanine	1.8
Cysteine	3.0
Isoleucine	2.9
Leucine	4.8
Methionine	5.0
Phenylalanine	5.7
Tryptophan	8.4
Valine	3.4

Table 7: The average number of types of atoms of naphthoylindoles that interact with amino acids

Tryptophan notably holds the most hydrophobic interactions. Phenylalanine, similar to tryptophan, contains an aromatic R group in its structure, and follows tryptophan in having the most hydrophobic interactions within the amino acids. Alanine had the least number of hydrophobic interactions with the naphthoylindoles.

For additional information regarding the relationship between the protein residues and the naphthoylindole ligands, refer to Appendix A.

Phenylacetylindole Structural Group

The phenylacetylindole structural group was the next set of compounds to be analyzed. JWH-167, JWH-203, JWH-249, JWH-250, JWH-251, and JWH-302 constitute this structural group. Figures 17-22 display the two-dimensional view of the interactions between each phenylacetylindole and the protein residues of the active-state CB_1 receptor model, in order of decreasing predicted MM-GBSA values.

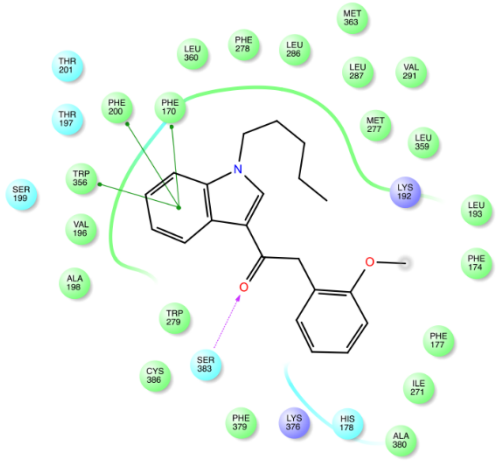


Figure 17: The two-dimensional view of the interactions between JWH-250 and the protein residues of the active-state CB_1 receptor model, with the green residues indicating hydrophobic interactions

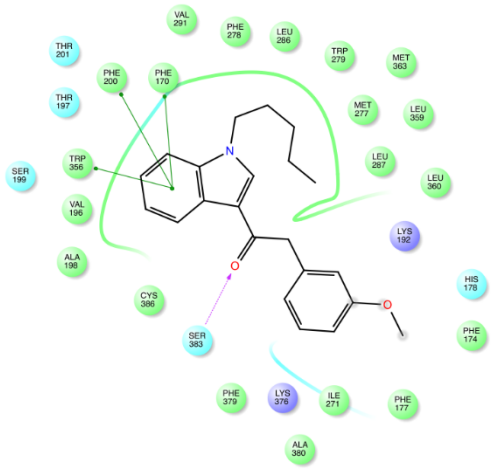


Figure 18: The two-dimensional view of the interactions between JWH-302 and the protein residues of the active-state CB_1 receptor model, with the green residues indicating hydrophobic interactions

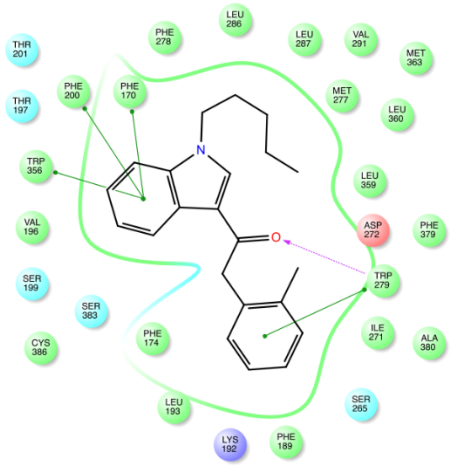


Figure 19: The two-dimensional view of the interactions between JWH-251 and the protein residues of the active-state CB_1 receptor model, with the green residues indicating hydrophobic interactions

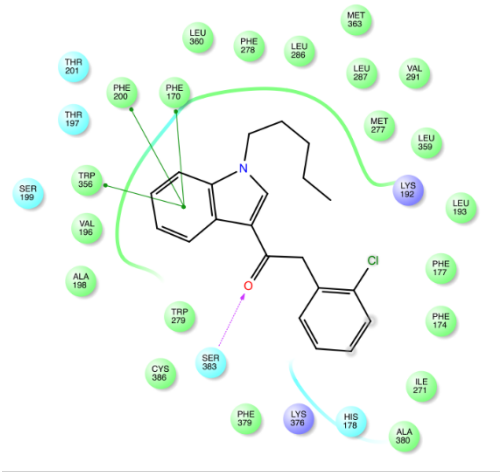


Figure 20: The two-dimensional view of the interactions between JWH-203 and the protein residues of the active-state CB_1 receptor model, with the green residues indicating hydrophobic interactions

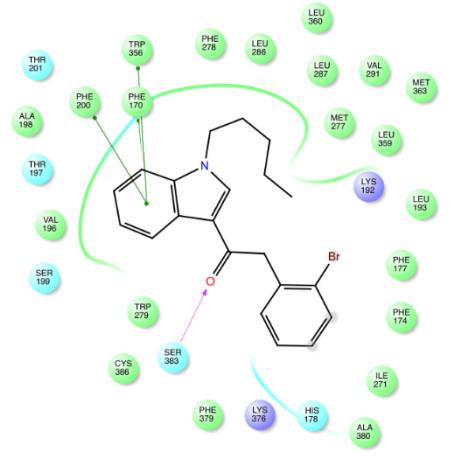


Figure 21: The two-dimensional view of the interactions between JWH-249 and the protein residues of the active-state CB_1 receptor model, with the green residues indicating hydrophobic interactions

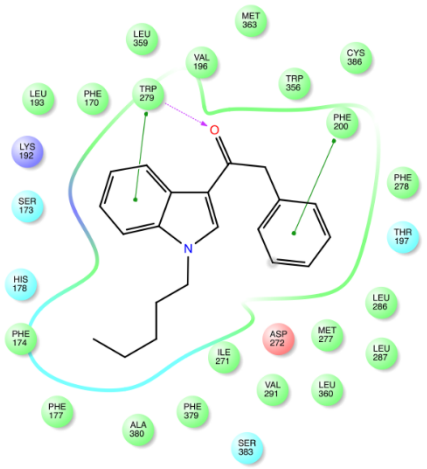


Figure 22: The two-dimensional view of the interactions between JWH-167 and the protein residues of the active-state CB_1 receptor model, with the green residues indicating hydrophobic interactions

The hydrophobic interactions between the CB_1 receptor and the phenylacetylindole molecules were analyzed and are displayed in Table 8.

	ALA 198	ALA 380	CYS 386	ILE 271	LEU 193	LEU 286	LEU 287	LEU 359	LEU 360	MET 277	MET 363	PHE 170	PHE 174	PHE 177	PHE 189	PHE 200	PHE 278	PHE 379	TRP 279	TRP 356	VAL 196	VAL 291
JWH-250	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x
JWH-302	x	x	x	x		x	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x
JWH-251		x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x
JWH-203	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x
JWH-249	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x
JWH-167		x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x

Table 8: The hydrophobic interactions between the phenylacetylindoles of the CB_1 selective JWH compounds and the protein residues of the active-state CB_1 receptor

Aside from the residues that interact with all the CB_1 selective JWH compounds, CYS 386, LEU 287, LEU 360, MET 277, PHE 200, and VAL 291 also interact with each phenylacetylindole ligand. ALA 198 lacked hydrophobic interactions with JWH-251 and JWH-167. PHE 189 exclusively interacted with JWH-251. Additionally, JWH-251 was the only ligand to lack hydrophobic interactions with PHE 177, and JWH-302 was the only ligand to lack hydrophobic interactions with LEU 193.

The structural features that are characteristic of a phenylacetylindole molecule are a phenyl ring, indole ring, a substituent on the nitrogen atom of the indole ring, and a carbonyl bridge that connects the phenyl and indole ring. The information provided by

the Interaction Fingerprints that indicated the specific protein residues that interacted with each atom of the phenylacetylindole ligands are displayed in Figure 23.

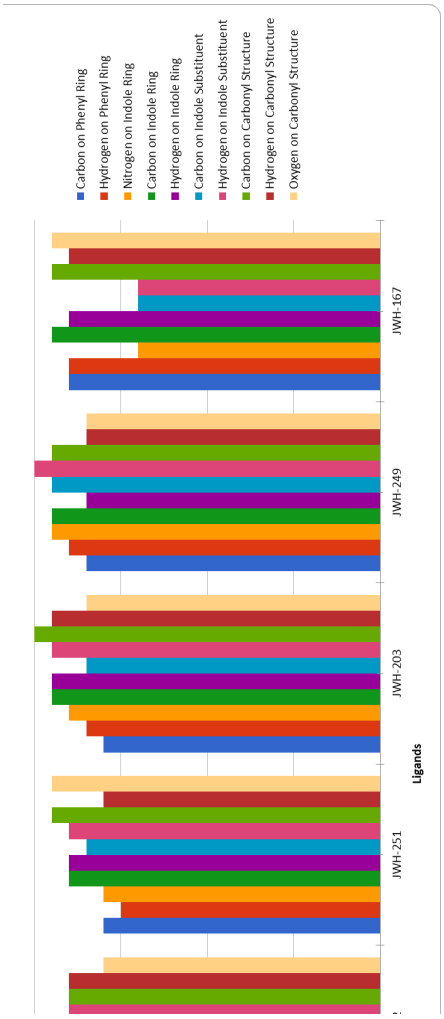


Figure 23a: The frequency of hydrophobic interactions of each structural feature of the phenylacetylindole ligands with CB_1 receptor protein residues

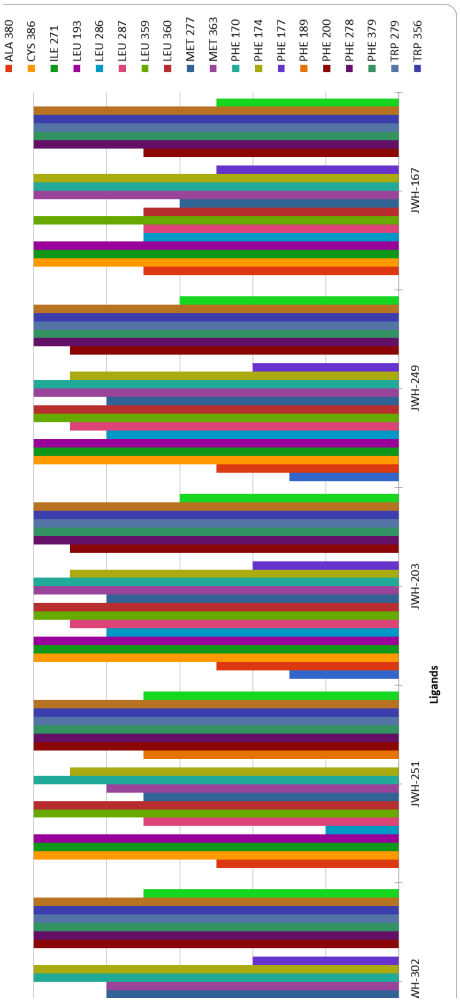


Figure 23b: The frequency of hydrophobic interactions of CB_1 receptor protein residues with the structural features of the phenylacetylindoles

Figure 26a shows the number of protein residues with which each type of atom interacts. The data in Figure 26a were numerically organized into Table 9, which averaged the number of protein residues with which each type of atom interacted from each phenylacetylindole.

Atom	Average Number of Protein Residues
Carbon on Phenyl Ring	16.0
Hydrogen on Phenyl Ring	16.7
Nitrogen on Indole Ring	16.8
Carbon on Indole Ring	18.7
Hydrogen on Indole Ring	18.2
Carbon on Indole Substituent	16.7
Hydrogen on Indole Substituent	18.0
Carbon on Carbonyl Bridge	19.2
Hydrogen on Carbonyl Bridge	18.0
Oxygen on Carbonyl Bridge	17.5

Table 9: The average number of protein residues that interact with each type of atom in phenylacetylindoles

The carbon atom on the carbonyl bridge has the most hydrophobic interactions, while a carbon on the indole ring closely followed. A carbon atom on the phenyl ring has the fewest hydrophobic interactions with the CB_1 receptor. To investigate whether a particular structural component as a whole – rather than just a particular type of atom – has any significance in hydrophobic interactions between the ligands and receptor, the average of the total number of hydrophobic interactions that occurred in the four characteristic structural features of phenylacetylindoles were organized into Table 10.

Structural Feature	Average Number of Protein Residues
Phenyl Ring	16.3
Indole Ring	17.9
Substituent of Indole Ring	17.3
Carbonyl Bridge	18.2

Table 10: The average number of protein residues that interact with each structural feature of phenylacetylindoles

The carbonyl bridge has the most hydrophobic interactions with the CB_1 receptor, while the indole ring was also rich with them. The phenyl ring as a whole had the least number of hydrophobic interactions.

Figure 26b tallies the number of particular types of atoms with which each protein residue hydrophobically interacts in each phenylacetylindole molecule in order to seek a protein residue that may be significant in its interactions specifically within the phenylacetylindole structural group. Table 11 simplifies the information displayed in Figure 26b by averaging the total number of hydrophobic interactions between each type of atom and each protein residue.

Protein Residue	Average Number of Types of Atoms That Interact with the Protein Residue
ALA 198	2.0
ALA 380	4.8
CYS 386	10.0
ILE 271	10.0
LEU 193	8.3
LEU 286	6.8
LEU 287	8.2
LEU 359	10.0
LEU 360	9.5
MET 277	7.5
MET 363	9.0
PHE 170	10.0
PHE 174	9.3
PHE 177	3.5
PHE 189	1.2
PHE 200	9.2
PHE 278	10.0
PHE 379	10.0
TRP 279	10.0
TRP 356	10.0
VAL 196	9.8
VAL 291	6.3

Table 11: The average number of types of atoms of phenylacetylindoles that interact with the protein residues

Unlike the naphthoylindole structural group in which TRP 279 dominated in hydrophobic interactions, eight protein residues interacted with all the types of atoms that are characteristic of phenylacetylindoles, including TRP 279. The other seven protein

residues were CYS 386, ILE 271, LEU 359, PHE 170, PHE 278, PHE 379, and TRP 356. Meanwhile, PHE 177, ALA 198, and PHE 189 had the least number of hydrophobic interactions with the phenylacetylindole ligands. With the exception of cysteine and isoleucine, the amino acids that interact with every CB_1 selective JWH compound had multiple positions on the receptor that had hydrophobic interactions with the phenylacetylindole ligands. Table 12 displays the average number of times each one of those amino acids interacted with the phenylacetylindoles.

Amino Acid	Average Number of Types of Atoms
Alanine	3.4
Leucine	8.6
Methionine	8.3
Phenylalanine	7.6
Tryptophan	10.0
Valine	8.1

Table 12: The average number of types of atoms of phenylacetylindoles that interact with amino acids

Both the tryptophan protein residues, TRP 279 and TRP 356, interacted with every structural component of phenylacetylindoles. Similar to the naphthoylindole structural group, alanine had the least number ofhydrophobic interactions with the phenylacetylindole structural group.

For additional information regarding the relationship between the protein residues and the phenylacetylindole ligands, refer to Appendix B.

Naphthylmethylindole Structural Group

The last structural group that contained multiple CB_1 selective JWH compounds was the naphthylmethylindole structural group. However, only two evaluated ligands constituted this group: JWH-175 and JWH-184. The two-dimensional view of the interactions between the naphthylmethylindole ligands and the protein residues of the CB_1 receptor are displayed in Figures 24 and 25, in order of decreasing predicted MM-GBSA values.

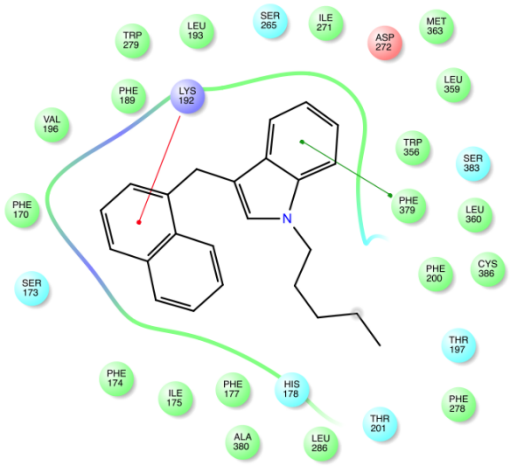


Figure 24: The two-dimensional view of the interactions between JWH-175 and the protein residues of the active-state CB_1 receptor model, with the green residues indicating hydrophobic interactions

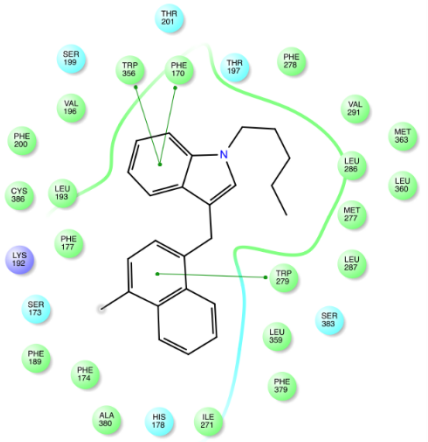


Figure 25: The two-dimensional view of the interactions between JWH-184 and the protein residues of the active-state CB_1 receptor model, with the green residues indicating hydrophobic interactions

The hydrophobic interactions between the CB_1 receptor and the naphthylmethylindole molecules were analyzed and are displayed in Table 13.

	ALA 380	CYS 386	ILE 175	ILE 271	LEU 193	LEU 286	LEU 287	LEU 359	LEU 360	MET 277	MET 363	PHE 170	PHE 174	PHE 177	PHE 189	PHE 200	PHE 278	PHE 379	TRP 279	TRP 356	VAL 196	VAL 291
JWH-184	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
JWH-175	x	x	x	x	x	x		x	x		x	x	x	x	x	x	x	x	x	x	x	

Table 13: The hydrophobic interactions between the phenylacetylindoles of the CB_1 selective JWH compounds and the protein residues of the active-state CB_1 receptor

Both the naphthylmethylindoles also interacted with the following protein residues that were overall selective in their hydrophobic interactions with every CB_1 selective JWH compound: CY2 386, LEU 293, LEU 360, PHE 177, PHE 189, and PHE 200. ILE 175 only interacted with JWH-175, and the protein residues LEU 287, MET 277, and VAL 291 only interacted with JWH-184.

Naphthylmethylindole ligands share the structural features of a naphthyl ring, an indole ring, a substituent on the nitrogen atom of the indole ring, and a methylene bridge that connects the naphthyl and indole rings together. The Interaction Fingerprints revealed the specific protein residues that interacted with each atom of the naphthylmethylindole ligands. The data processed by the Interaction Fingerprints were organized into Figure 26.

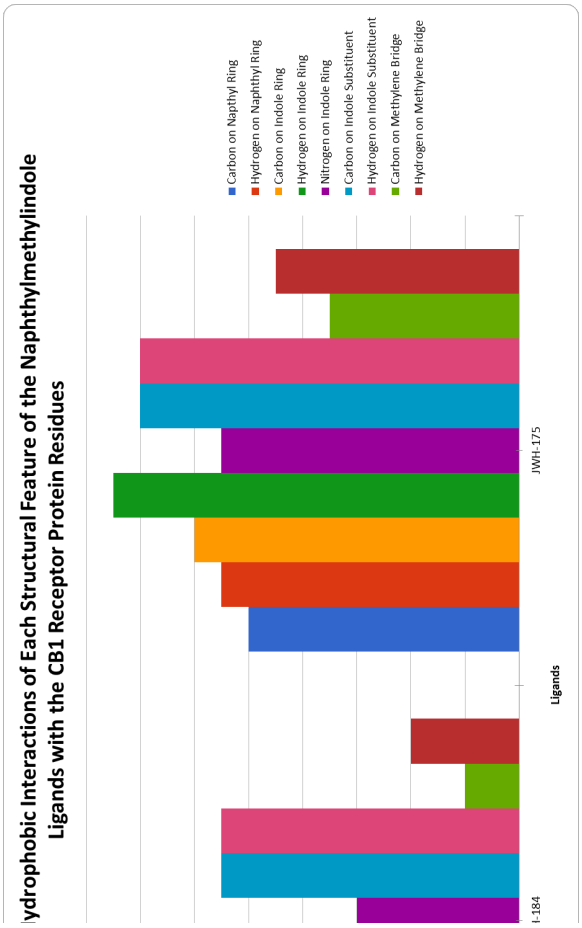


Figure 26a: The frequency of hydrophobic interactions of each structural feature of the naphthylmethylindole ligands with CB_1 receptor protein residues

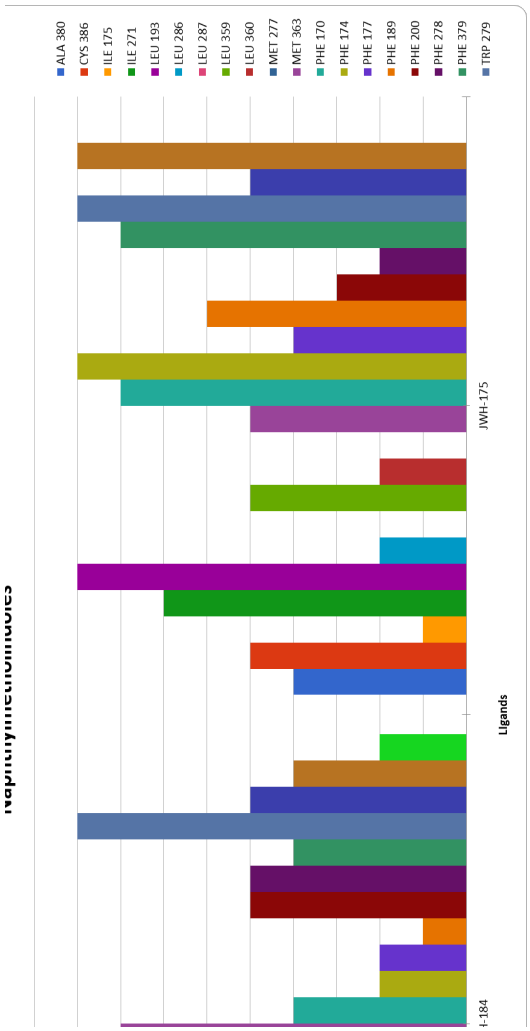


Figure 26b: The frequency of hydrophobic interactions of CB_1 receptor protein residues with the structural features of the naphthylmethylindoles

Figure 26a graphically displays the number of protein residues with which each type of atom of a naphthylmethylindole interact. Table 14 quantifies the data in Figure 26a by taking the average number of protein resides with which the types of atom of naphtylmethylindole interact.

Atom	Average Number of Protein Residues
Carbon on Naphthyl Ring	10.0
Hydrogen on Naphthyl Ring	11.5
Carbon on Indole Ring	10.5
Hydrogen on Indole Ring	13.0
Nitrogen on Indole Ring	8.5
Carbon on Indole Ring	12.5
Hydrogen on Indole Substituent	12.5
Carbon on Methylene Bridge	4.5
Hydrogen on Methylene Bridge	6.5

Table 14: The average number of protein residues that interact with each type of atom in naphthylmethylindoles

A hydrogen atom on the indole ring has the most hydrophobic interactions, while the carbon atom of the methylene bridge considerably has the least number of hydrophobic interactions.

Furthermore, Table 15 aggregated the atoms of each structural component and averaged the number of residues with which each set of constitutive atoms interacted to evaluate whether a particular structural component of a naphthylmethylindole molecule is significant in hydrophobic interactions.

Structural Feature	Average Number of Protein Residues
Naphthyl Ring	10.8
Indole Ring	10.7
Indole Substituent	12.5
Methylene Bridge	5.5

Table 15: The average number of protein residues that interact with each structural feature of naphthylmethylindoles

The substituent on the nitrogen of the indole ring has the most hydrophobic interactions, while the methylene bridge has the least number of hydrophobic interactions.

Figure 29b displays the total number of types of atoms with which each protein residue interacts. Table 16 organizes and displays the data presented in Figure 29b by taking the average number of atoms each naphthylmethylindole that interacts with each protein residue and displays them below.

Protein Residue	Average Number of Types of Atoms That Interact with the Protein Residue
ALA 380	3.0
CYS 386	4.0
ILE 175	0.5
ILE 271	4.5
LEU 193	5.5
LEU 286	2.5
LEU 287	1.0
LEU 359	6.0
LEU 360	2.0
MET 277	1.0
MET 363	6.5
PHE 170	6.0
PHE 174	5.5
PHE 177	3.0
PHE 189	3.5
PHE 200	4.0
PHE 278	3.5
PHE 379	6.0
TRP 279	9.0
TRP 356	5.0
VAL 196	6.5
VAL 291	1.0

Table 16: The average number of types of atoms of naphthylmethylindoles that interact with the protein residues

TRP 279 notably interacts with the most atoms of the naphthylmethylindole ligands, while ILE 175 rarely holds hydrophobic interactions with either JWH-184 and JWH-175. Table 16 accounts the amino acids that had hydrophobic interactions with the

naphthylmethylindole ligands and were located in multiple positions on the CB_1 receptor protein sequence. The values from Table 16 were averaged according to the type of amino acid and organized into Table 17.

Amino Acid	Average Number of Types of Atoms
Isoleucine	2.5
Leucine	3.4
Methionine	3.8
Phenylalanine	4.5
Tryptophan	7.0
Valine	3.8

Table 17: The average number of types of atoms of naphthylmethylindoles that interact with amino acids

Once again, tryptophan dominated in the hydrophobic interactions. The other amino acids relatively had similar amounts of hydrophobic interactions with the phenylacetylindole ligands that were substantially less in number than that of tryptophan.

For additional information regarding the relationship between the protein residues and the naphthylmethylindole ligands, refer to Appendix C.

Other Structural Groups

JWH-030, JWH-161, and JWH-176 fall into different structural group classifications in which each one is the lone CB_1 selective JWH ligand within its respective category. Figures 30-32 show the two-dimensional interactions between these ligands and the CB_1 receptor protein residues, in order of decreasing predicted MM-GBSA values.

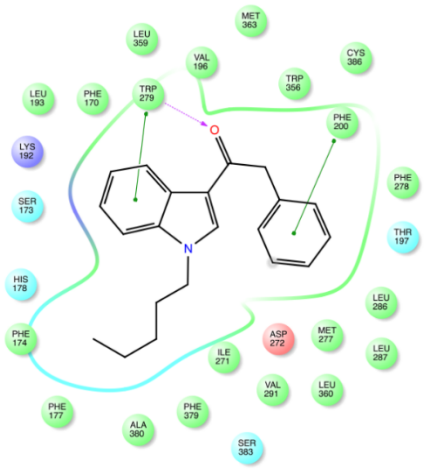


Figure 27: The two-dimensional view of the interactions between JWH-176 and the protein residues of the active-state CB_1 receptor model, with the green residues indicating hydrophobic interactions

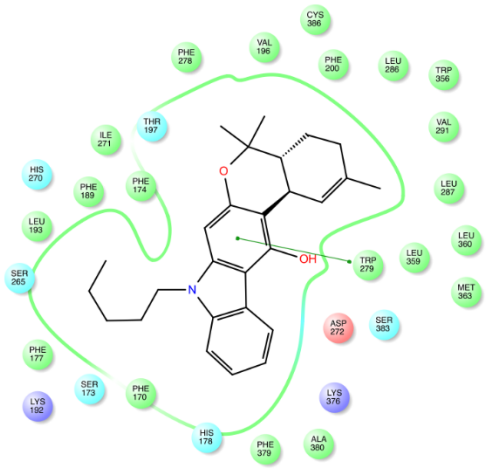


Figure 28: The two-dimensional view of the interactions between JWH-161 and the protein residues of the active-state CB_1 receptor model, with the green residues indicating hydrophobic interactions

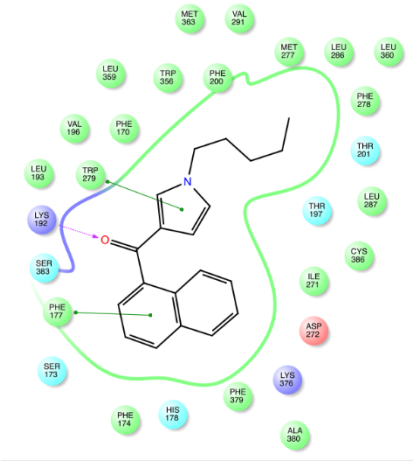


Figure 29: The two-dimensional view of the interactions between JWH-030 and the protein residues of the active-state CB_1 receptor model, with the green residues indicating hydrophobic interactions

Table 18 exclusively displays the hydrophobic interactions that occurred between these ligands and the CB_1 receptor protein residues.

	ALA 198	ALA 380	CYS 382	CYS 386	ILE 169	ILE 175	ILE 271	LEU 193	LEU 286	LEU 287	LEU 359	LEU 360	MET 277	MET 363	PHE 170	PHE 174	PHE 177	PHE 189	PHE 200	PHE 278	PHE 379	TRP 279	TRP 356	VAL 171	VAL 196	VAL 291
JWH-176		x	x	x			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x
JWH-161		x		x			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x
JWH-030		x		x			x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x		x	x

Table 18: The hydrophobic interactions between JWH-176, JWH-161, and JWH-030 and the protein residues of the active-state CB_1 receptor model

JWH-176 is a naphthylmethylindene compound, characterized by its naphthyl and indene rings joined by a methylene bridge and a substituent at the 3-position of the indene ring. Table 19 organizes the structural components of JWH-176 and their respective constitutive atoms to display the frequency with which they interact with the protein residues of the CB_1 receptor.

Atom	Number of Protein	Structural	Average Number of

	Residues	Feature	Protein Residues
Carbon on Naphthyl Ring	13	Naphthyl Ring	13.5
Hydrogen on Naphthyl Ring	14	Indene Ring	10.0
Carbon on Indene Ring	9	Indene Substituent	8.5
Hydrogen on Indene Ring	11	Methylene Bridge	3.0
Carbon on Indene Ring	8		
Hydrogen on Indene Ring	9		
Carbon on Methylene Bridge	3		
Hydrogen on Methylene Bridge	3		

Table 19: The average number of protein residues that interact with each type of atom and structural component of JWH-176

A hydrogen on the naphthyl group has the most hydrophobic interactions, while the naphthyl ring as a whole has the most hydrophobic interactions. The carbon and hydrogen atoms of the methylene bridge, and thus the methylene bridge as a whole structural component of JWH-176, had the least number of hydrophobic interactions with the CB_1 receptor. Moreover, Table 20 quantifies the number of type of atoms with which different protein residues interact as well as the amino acids that are in multiple locations of the CB_1 receptor protein sequence.

Protein Residue	Average Number of Types of Atoms That Interact with the Protein Residue	Amino Acid	Average Number of Types of Atoms
ALA 380	3	Cysteine	1.0
CYS 382	1	Leucine	2.8
CYS 386	1	Methionine	4.0
ILE 271	4	Phenylalanine	3.0
LEU 193	4	Tryptophan	5.0
LEU 286	2	Valine	4.0
LEU 287	2		
LEU 359	4		
LEU 360	2		
MET 277	2		
MET 363	6		
PHE 170	8		
PHE 174	4		
PHE 177	2		
PHE 189	1		
PHE 200	2		
PHE 278	2		
PHE 379	2		
TRP 279	8		
TRP 356	2		
VAL 196	6		
VAL 291	2		

Table 20: The average number of types of atoms of JWH-176 that interact with the amino acids

PHE 170 and TRP 279 have the most hydrophobic interactions with JWH-176, but the amino acid with the overall most hydrophobic interactions with JWH-176 is tryp-

tophan. CYS 382, CYS 386, and PHE 189 interacted with only one type of atom each in JWH-176. However, cysteine was the amino acid that had the least number of hydrophobic interactions with JWH-176.

JWH-161 is special in that it does not fall into a specific structural group unlike the other CB_1 selective JWH compounds. It is a hybrid of a "classical" cannabinoid (i.e., THC) and a dibenzopyran, where its indole ring has a substituent at the nitrogen atom. Table 21 shows the average number of protein residues that interact with the structural components of JWH-161 and the actual count of protein residues that interact with their respective atoms.

Atom	Number of Protein Residues	Structural Feature	Average Number of Protein Residues
Carbon on Ring	10	Indole Ring	10.5

Hydrogen on Indole Ring	9	Dibenzopyran Ring	13.5
Nitrogen on Indole Ring	6	Indole Substituent	9.5
Carbon on Dibenzopyran Ring	15	Dibenzopyran Substituent	13.0
Hydrogen on Diben-zopyran Ring	17		
Oxygen on Dibenzopy-ran Ring	10		
Carbon on Indole Sub-stituent	8		
Hydrogen on Indole Substituent	11		
Carbon on Dibenzopyran Substituent	14		
Hydrogen on Diben-zopyran Substituent	17		
Oxygen on Dibenzopy-ran Substituent	8		

Table 21: The average number of protein residues that interact with each type of atom and structural component of JWH-161

The hydrogen atoms of the dibenzopyran ring and the dibenzopyran substituent considerably have the most interactions with the CB_1 receptor, while the nitrogen on the indole ring had the least. Moreover, the dibenzopyran ring and its substituent are the structural components of JWH-161 that overall have the most hydrophobic interactions with the ligand. The indole ring has the least number of hydrophobic interactions with the CB_1 receptor. Table 22 displays the total number of type of atoms with which each protein residue interacts. It also groups the amino acids that are in multiple positions on

the CB_1 receptor in order to evaluate whether a particular amino acid has significance over the other amino acids.

Protein Residue	Average Number of Types of Atoms That Interact with the Protein Res-		Amino Acid	Average Number of Types of Atoms

	idue		
ALA 380	2	Leucine	5.0
CYS 386	2	Methionine	6.5
ILE 271	4	Phenylalanine	7.0
LEU 193	7	Tryptophan	8.5
LEU 286	3	Valine	6.5
LEU 287	3		
LEU 359	8		
LEU 360	4		
MET 277	4		
MET 363	9		
PHE 170	11		
PHE 174	10		
PHE 177	5		
PHE 189	2		
PHE 200	6		
PHE 278	6		
PHE 379	9		
TRP 279	11		
TRP 356	6		
VAL 196	9		
VAL 291	4		

Table 22: The average number of types of atoms of JWH-161 that interact with the amino acids

PHE 170 and TRP 279 both interacted with the highest number of type of atoms in JWH-161. Overall, however, tryptophan was the amino acid that has the most hydrophobic interactions with JWH-131, and phenylalanine subsequently follows it. ALA 380, CYS 386, and PHE 189 had low counts of hydrophobic interactions with JWH-131. The

leucine amino acids as group had the least number of hydrophobic interactions with JWH-131.

JWH-030 is a naphthoylpyrrole and is characterized by a naphthyl ring, a pyrrole ring, a substituent at the nitrogen of the pyrrole ring, and a carbonyl bridge that connects the naphthoyl and pyrrole rings together. Table 23 displays the average frequency with which each structural component of JWH-031 and the frequency its constitutive atoms interact with protein residues.

Atom	Number of Protein Residues	Structural Feature	Average Number of Protein Residues
Carbon on Naphthyl Ring	9	Naphthyl Ring	9.0
Hydrogen on Naphthyl Ring	9	Pyrrole Ring	6.7
Carbon on Pyrrole Ring	8	Pyrrole Substituent	13.5

Hydrogen on Pyrrole Ring	7	Carbonyl Bridge	4.5
Nitrogen on Pyrrole Ring	5		
Carbon on Substituent of Pyrrole Structure	12		
Hydrogen on Substituent of Pyrrole Structure	15		
Carbon on Carbonyl Bridge	5		
Oxygen on Carbonyl Bridge	4		

Table 23: The average number of protein residues that interact with each type of atom and structural component of JWH-031

A hydrogen atom on the pyrrole substituent undergoes the most hydrophobic interactions of all the types of atoms in JWH-031. Additionally, the pyrrole ring substituent as a whole has the most hydrophobic interactions with the CB_1 receptor. Meanwhile, the oxygen atom on the carbonyl bridge and the carbonyl bridge as a structural component as a whole has the least hydrophobic interactions with the CB_1 receptor protein sequence. Reversibly, Table 24 considers amino acids and the frequency with which they interact with each structural component of JWH-031.

Protein Residue	Average Number of Types of Atoms Interact with the Protein Residue	Amino Acid	Average Number of Types of Atoms
ALA 380	2	Leucine	3.6
CYS 386	2	Methionine	1.5
ILE 271	4	Phenylalanine	3.7

LEU 193	6	Tryptophan	7.0
LEU 286	2	Valine	4.5
LEU 287	1		
LEU 359	7		
LET 360	2		
MET 277	1		
MET 363	2		
PHE 170	9		
PHE 174	5		
PHE 177	2		
PHE 200	2		
PHE 278	2		
PHE 379	2		
TRP 279	9		
TRP 356	5		
VAL 196	7		
VAL 291	2		

Table 24: The average number of types of atoms of JWH-031 that interact with the amino acids

PHE 170 and TRP 279 have the most hydrophobic interactions, but tryptophan, (TRP 279 and TRP 356) is the most dominant amino acid in hydrophobic interactions with JWH-031. LEU 287 and MET 277 had the least number of hydrophobic interactions with JWH-031, and the group of methionine amino acids was the lowest in its hydrophobic interactions.

Conclusion

Alanine, cysteine, isoleucine, leucine, methionine, phenylalanine, tryptophan, and valine are the amino acids that interact hydrophobically with each of the CB_1 selective JWH compounds. Specifically, within the CB_1 receptor protein sequence, the following protein residues interact with at least one of the evaluated ligands: ALA 198, ALA 380, CYS 382, CYS 386, ILE 169, ILE 175, ILE 271, LEU 193, LEU 286, LEU 287, LEU 359, LEU 360, MET 277, MET 363, PHE 170, PHE 174, PHE 177, PHE 189, PHE 200, PHE 278, PHE 379, TRP 279, TRP 356, VAL 171, VAL 196, and VAL 291. Only ALA 380, ILE 271, LEU 286, LEU 359, MET 363, PHE 170, PHE 174, PHE 278, PHE 379, TRP 279, TRP 356, and VAL 196 were inclusive in their hydrophobic interactions, and the remaining protein residues were selective.

None of the three structural groups into which more than one CB_1 selective JWH compound belonged (naphthoylindoles, phenylacetylindoles, and naphthylmethylindoles) shared the same type of atom or structural feature that underwent the most hydrophobic interactions, nor did JWH-176 (naphthylmethylindene), JWH-161 (hybrid molecule), and JWH-030 (naphthoylpyrrole). However, TRP 279 was unanimously the protein residue that holds the most hydrophobic interactions with the evaluated ligands. PHE 170 was also notable in its large contribution of hydrophobic interactions with the ligands. This may potentially be contributed to both their aromatic R groups in their

structures. Furthermore, tryptophan was the domineering amino acid in the hydrophobic interactions between the CB_1 receptor and the CB_1 selective JWH ligands.

This conducted research is geared toward the future goal of creating a database of predicted structures of synthetic cannabinoids that will expedite the process of discovering unknown structures of synthetic cannabinoids that may later emerge. Prior to creating the database, however, additional research must be conducted. A supplemental study should be administered that re-evaluates the discussed interactions while considering the hydrophobic distances. Furthermore, this current research must be repeated with a focus on other types of interactions, such as positively and negatively charged, polar, hydrogen, π-cation, and π-π stacking interactions. AM, CP, HU, WIN, and other synthetic cannabinoids must also be studied, as well as CB_2 selective compounds.

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APPENDICES

APPENDIX A

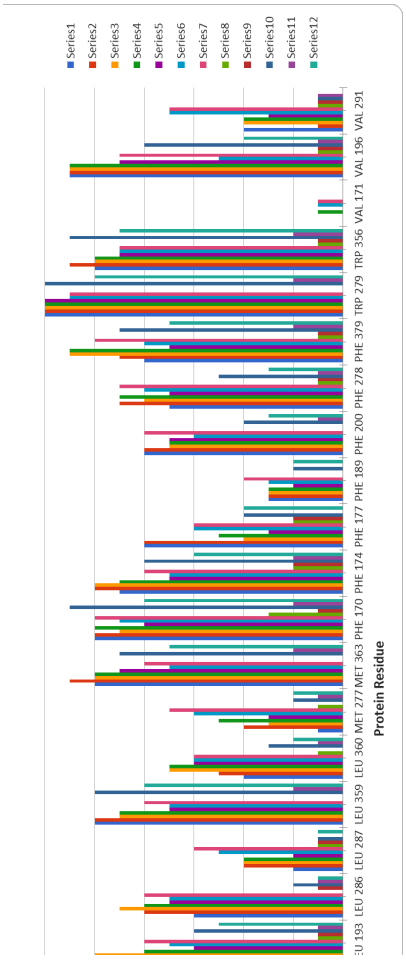

Figure 30: The frequency of hydrophobic interactions between naphthoylindoles and CB_1 receptor protein residues according to structural feature

APPENDIX B

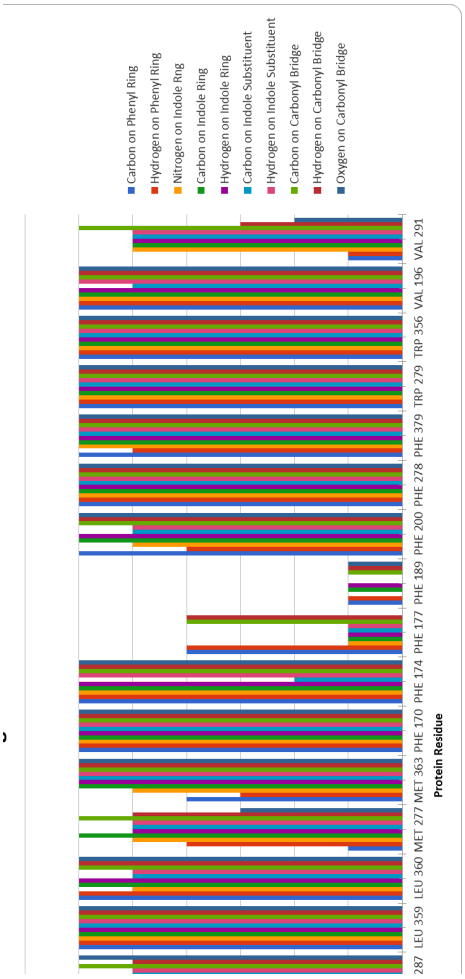

Figure 31: The frequency of hydrophobic interactions between phenylacetylindoles and CB_1 receptor protein residues according to structural feature

APPENDIX C

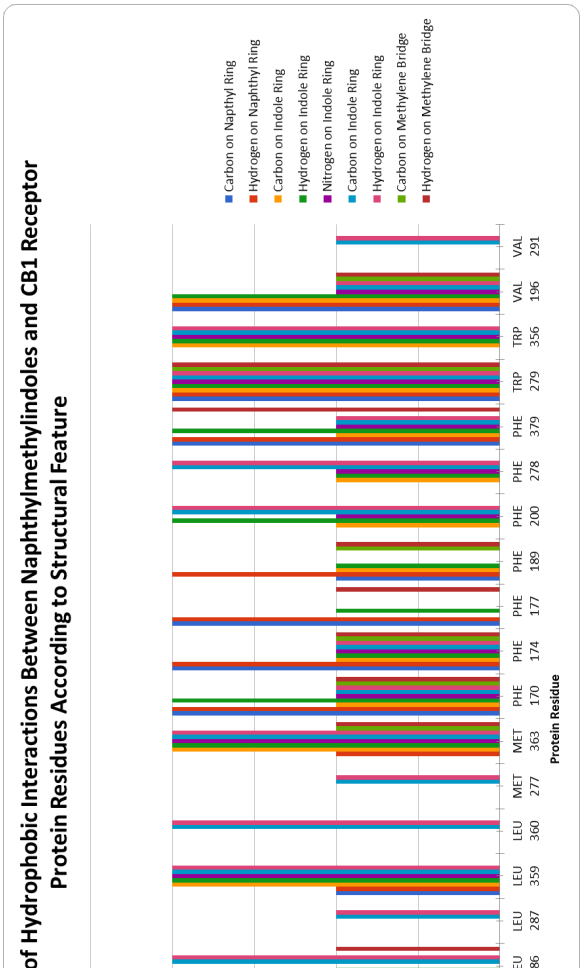

Figure 32: The frequency of hydrophobic interactions between naphthylmethyl-indoles and CB_1 receptor protein residues according to structural feature