

MODELLING OF THE BINDING OF TRPV1 RECEPTOR TO
CAPSAICIN, CAPSAZEPINE AND RELATED AGONISTS AND
ANTAGONISTS

by

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ABSTRACT

MODELLING OF THE BINDING OF TRPV1 RECEPTOR TO CAPSAICIN, CAPSAZEPINE AND RELATED AGONISTS AND ANTAGONISTS

Capsaicin is a natural compound found in hot pepper and is an agonist of the receptor protein TRPV1 (Transient Receptor Potential Vanilloid) in dorsal root ganglia. TRPV1 is responsible for pain and heat perception. Capsazepine is a synthetic compound and is the first known antagonist of TRPV1; it interferes with the binding of capsaicin to TRPV1 in order to prevent protein activation and the resulting pain sensation.

In this study, multiple conformations of capsaicin and capsazepine are docked to the active site of TRPV1 protein with rigid docking by Autodock4 to find which conformations give the lowest binding energy. We also observe analog molecule results in similar conformation conditions. The consistency of the results is checked by comparison with analog molecule binding. Agonists like resiniferatoxin, gingerol, nonivamide, nordihydrocapsaicin, and 6-shogaol and antagonist; sb-366791 is chosen as the analog molecules.

The docking results of gingerol, shogaol, dihydrocapsaicin, nonivamide, and sb-366791 had similar binding energies. Nordihydrocapsaicin and resiniferatoxin gave better binding energies in their capsaicin-like conformations. In general, all analog molecules are observed to make more hydrogen bonds with the protein.

ÖZET

TRPV1 RESEPTÖRÜNÜN KAPSAİSİN, CAPSAZEPİN VE İLGİLİ AGONİSTLER VE ANTAGONİSTLERE BAĞLANMASININ MODELLENMESİ

Kapsaisin, acı biberde bulunan doğal bir bileşik ve dorsal kök gangliyonlarında bulunan Vanilloid reseptör protein olan TRPV1'in bir agonistidir. TRPV1 acı ve ısı algılanmasından sorumlu proteindir. Kapsazepin, sentetik bir bileşik ve TRPV1'in ilk bilinen antagonistidir. Kapsazepin, kapsaisinin TRPV1'e bağlanmasını engelleyerek protein aktivasyonunu ve ortaya çıkan ağrı hissini önler.

Bu çalışmada, kapsaisin ve kapsazepinin farklı şekilsel yapıları, hangi yapıların en düşük bağlanma enerjisini verdiğini bulmak ve benzer konformasyonlardaki analog moleküllerin sonuçlarını gözlemlemek için Autodock4 kullanılarak rigid docking ile TRPV1 proteininin aktif bölgesine yerleştirilir. Kapsaisin ve kapsazepinin rigid docking konformasyonları yapılır ve sonuçların tutarlılığı analog molekül bağlaması ile karşılaştırılarak kontrol edilir. Bu amaçla, çalışma için analog molekül olarak resiniferatoksin, gingerol, nonivamide, nordihydrocapsaicin ve 6-şogaol agonistleri ve sb-366791 antagonisti seçilmiştir.

Gingerol, şogaol, dihydrocapsaicin, nonivamide ve sb-366791 yerleştirme sonuçlarına göre benzer bağlanma enerjilerine sahiptir. Nordihydrocapsaicin ve resiniferatoksin, kapsaisin benzeri konformasyonlarda daha iyi bağlanma enerjileri vermiştir. Genel olarak, tüm analog moleküllerin protein ile daha fazla hidrojen bağı yaptığı gözlenmiştir.

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LIST OF SYMBOLS

Å	Ångstrom
μM	Micromolar
VL	Intramolecular energetics of ligand
VM	Intramolecular energetics of macromolecule
VM-L	Intermolecular energetics of macromolecule ligand complex
τ	Torsions

LIST OF ACRONYMS/ ABBREVIATIONS

ADT	AutoDockTools
AM1	Austin Model 1
DFT	Density Functional Theory
DLG	Docking log file
DPF	AutoDock parameter file
GA	Genetic Algorithm
GLG	Grid log file
GPF	AutoGrid parameter file
Ki	Dissociation constant
LGA	Lamarckian Genetic Algorithm
MD	Molecular dynamics
MM	Molecular mechanics
MPW1K	Modified Powder Wang
PDB	Protein Data Bank
PDBQT	Protein Data Bank, Partial Charge (Q), & Atom Type (T)
RMSD	Root mean square deviation
RTX	Resiniferatoxin
SA	Simulated Annealing
SHU	Scoville heat unit
TRPV1	Transient Receptor Potential Vanilloid 1
vdW	Van der Waals

1. INTRODUCTION

1.1. History and Usage of Capsaicin, Capsazepine and Their Analog Molecules

Capsaicin is a natural compound found in hot pepper. Hot pepper is first found in America (Peppers: The domesticated Capsicums Andrews, Jean-book), it was mentioned in Aztec manuscripts as chili. French botanist de Tournefort gave its Latin name; Capsicum. In 1876, Tresh has succeeded to isolate the active ingredient in hot pepper from paprika and cayenne and called it Capsaicin[1][2]. Its structure was first reported in 1919 by Nelson and Dawson [3][4].

The organoleptic test was found in 1912 by Wilbur Scoville to test hot pepper pungency [5]. The test was done by diluting the pepper with alcohol until it doesn't taste hot to the human tongue. According to these test results, the Mexican habanero is known as the most capsaicin containing pepper and the hottest tasting pepper on record [1].

Analgesic usage of capsaicin also dates back in history. Native Americans have used capsaicin to relieve toothache by rubbing their gums which were later adapted by European medicine in the form of an alcoholic hot pepper extract. This capsaicin treatment to sore teeth is the first-time that capsaicin was recognized for its therapeutic use. Another interesting example is the capsaicin's analgesic usage in China. Prior to becoming a eunuch to serve the Chinese Emperor, men used capcaisin extract to rub the testicular area repeatedly before castration to numb the area for the painful process [1]. Depending on the dose and the way it was administered, capsaicin can cause pain-related side effects[6].

Agonist is the general name of capsaicin and several similar compounds like dihydrocapsaicin, nordihydrocapsaicin, nonivamide, gingerol, shogaol, and resiniferatoxin (RTX). Agonists activate the TRPV1 channel and cause a burning sensation and pain in the dorsal root ganglion[7][8]. These molecules are generally produced from the genus of a flowering plant; capsicum. Dihydrocapsaicin and capsaicin are the two capsaicinoids mostly obtained from the plants. They are around 90% of the capsaicinoids gathered from the chili pepper [4]. Rest of the capsaicinoids are extracted in smaller amount[6]. Antagonist is the general name of protein blockers like Capsazepine and Sb-366791. They

block TRPV1 protein signaling and potentially avoid side effects associated with agonist activation of TRPV1[9][10].

1.2. Agonists

Capsaicin is a crystalline alkaloid, also called vanilloid since it has a vinyl group [7]. It is colorless and odorless. Capsaicin is called “double-edged sword” for it has a dual effect on cases like where it can be chemoprotective in treatment of cancer but it can act as carcinogenic if used in high doses [11]. Analgesia, Cardiovascular system, diabetes, gastroprotection, urological disorders, and weight reduction are some of the diseases treated with capsaicin. Airway diseases and itch are also treated with capsaicin but the results found are discordant [11][12]. Capsaicin has a dual effect on cancer indicating it can cure or cause progress in cancer [12].

The chemical name of capsaicin is trans-8-Methyl-N-vanillyl-6-noneamide with a molecular formula of $C_{18}H_{27}NO_3$. It is soluble in fat, oil, and alcohol. Capsaicin is an amphiphilic molecule and shows both lipophilic and hydrophilic tendencies. Its structure has three parts (Fig. 1.1); a hydrophilic aromatic ring (region A), a dipolar amide (region B) and a hydrophobic carbon tail (region C). Capsaicin is usually found as a trans isomer since cis isomer is less stable. Cis isomer locates the tail closer to the double bond in which case double bond and tail repel each other. All regions have different roles for capsaicin actions. Region A with the phenyl -OH group has the potential for hydrogen bonding and is hydrophilic. Region A is important for agonist activity and gives the best receptor response with pain. Region B includes the amide group with dipolar amide bond region. Region C contains the hydrophobic tail which increases solubility in oil. Region C is essential for capsaicin to enter the cell membranes [4][13][14].

Capsaicin increases the membrane cation transmissivity and excites nociceptive neurons to ascertain spicy feeling. It targets the Vanilloid Receptor; a membrane protein that can be found mainly in nerves. Transient Receptor Potential Vanilloid 1 (TRPV1) is in dorsal root ganglia of the human body [2]. This TRPV1 protein is the cause of pain sensory perception [6].

Resiniferatoxin (RTX, $C_{37}H_{40}O_9$) (Fig. 1.2) is found in Moroccan cactus named *Euphorbia resinifera*. It is an analog of capsaicin and an agonist of TRPV1. RTX is much more potent than capsaicin with higher affinity to TRPV1 and has similar properties like capsaicin regarding a pharmacological potential [13][14]. The RTX structure can also be considered having A, B and C region. Region A has the vanillyl moiety like capsaicin. This part is imperative for bioactivity [13][14]

Ginger is a well-known and widely used spice in culinary. It is also used as an herbal medicine in the treatment of headache, asthma, rheumatism, muscle ache, and nervous diseases. Ginger also has pharmacological potential like capsaicin. Gingerol (Fig. 1.1) is identified as one of the main active and pungent parts of the Ginger plant [15]. Gingerol activates the TRPV1 channel and acts as an agonist but shows less potency than capsaicin. Gingerol is reported to increase body temperature and to enhance energy consumption [15].

Nonivamide (Fig. 1.1) is a TRPV1 antagonist. It is a less pungent capsaicin analog and differs from capsaicin with one double bond and one methyl group in the carbon chain [13][16]. Nonivamide causes an increase in Ca^{2+} besides being a TRPV1 ligand [17]. It decreases the antioxidant activity [18]. Nonivamide is also called synthetic capsaicin and considered a good replacement of capsaicin due to its cheaper price and lower pungent sensation on the skin [18].

Nordihydrocapsaicin (Fig. 1.1) is a TRPV1 receptor antagonist and an analog molecule of capsaicin [19][20]. It is a primary capsaicinoid in chili pepper. It has been used in a variety of biological and physiological situations. Its pungency causes irritation, and this limits its activities. Even with these limitations, nordihydrocapsaicin is used as an antioxidant, anticarcinogen, and anti-inflammatory. It also helps suppress the accumulation of fat [4].

Shogaol (Fig. 1.1) is a part of the main active and pungent part of Ginger. It has antioxidant, anticancer, antimicrobial, and anti-inflammatory properties. Shogaol increases calcium concentration in TRPV1 like capsaicin. It is less potent than capsaicin but more potent than gingerol [21]. Shogaol, much like gingerol, is reported to increase body temperature and enhance energy consumption [15][21].

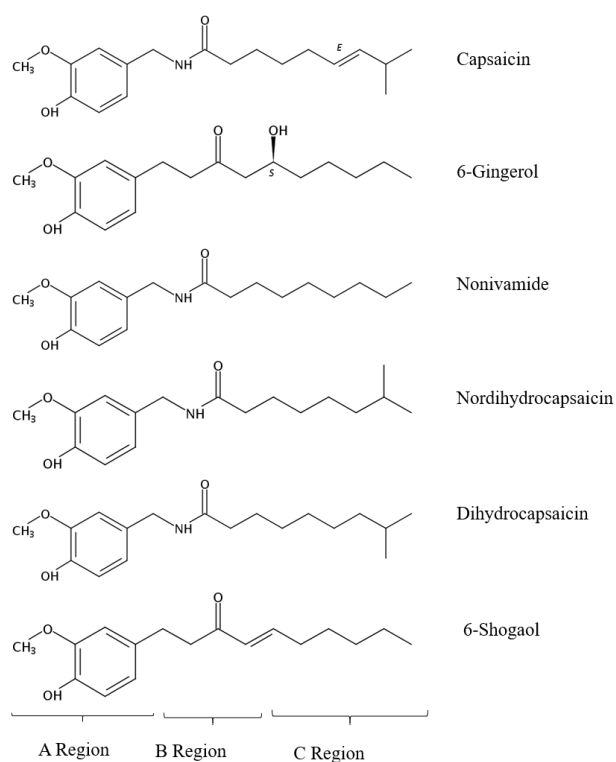


Figure 1.1: Capsaicin, 6-Gingerol, Nonivamide, Nordihydrocapsaicin, 6-Shogaol in 2-dimensional structures. A Region has the vanillyl moiety like capsaicin, B Region is amide and C Region is hydrophobic carbon tail.

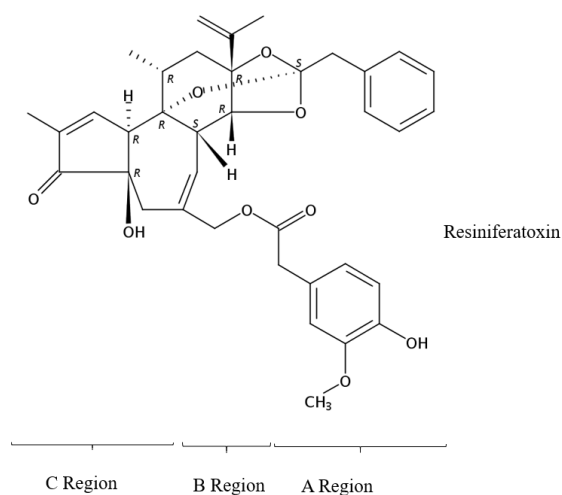


Figure 1.2: Resiniferatoxin (RTX) 2-dimensional structure. A Region is a hydrophilic aromatic ring, B Region is ester and C Region is a big R group.

1.3. Antagonists

Capsazepine is (Fig. 1.3) the first identified antagonist of TRPV1. It is a synthetic analog derived by altering the chemical backbone of capsaicin. Capsazepine blocks the activation of TRPV1 by removing capsaicin and other agonists from TRPV1 active site pocket. Capsazepine shows anti-hyperalgesic and anti-inflammatory effect in animals with inflammatory and neuropathic pain. Capsazepine efficiency is found to be different for different species. Because of its changing effectiveness, capsazepine is not used as a clinical treatment but its derivatives are under investigation for better results [13].

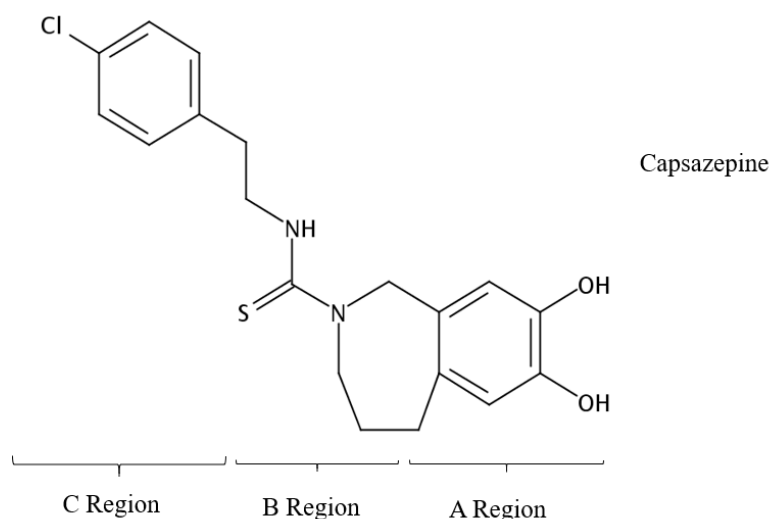


Figure 1.3: Capsazepine 2-dimensional structure. A Region is a hydrophilic aromatic ring, B Region is thiourea and C Region is an R group.

SB-366791 (Fig. 1.4) is a synthetic TRPV1 antagonist. It has high potency and better selectivity than other TRPV1 antagonists. It is activated by agonists like capsaicin or by heat [22]. There are two types of antagonists known. One blocks both capsaicin and proton activation, while other blocks only capsaicin but not proton activation. Sb-366791 is the second type of antagonists that block only capsaicin. It is similar to capsazepine in this way [23].

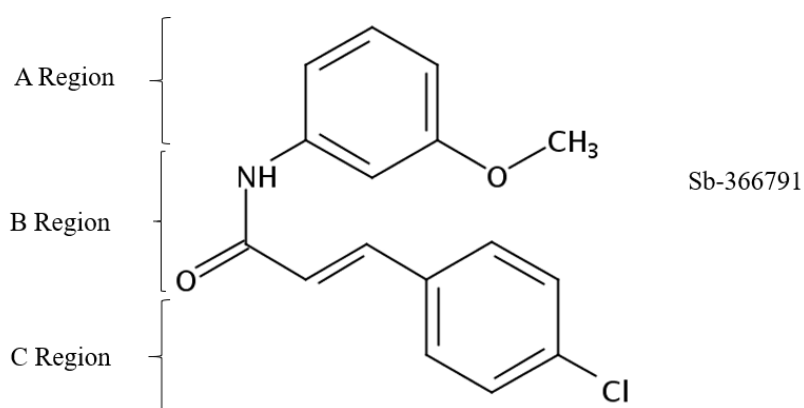


Figure 1.4: Sb-366791 2-dimensional structure. A Region has the vanillyl moiety like capsaicin, B Region is amide and C Region is an R group.

1.4. TRPV1 Receptor

TRPV1 is a non-selective receptor located in dorsal ganglia. It is also distributed in brain tissue, bladder, kidneys, and liver [24][25]. Rat TRPV1 is the first one to be observed and cloned. It has sodium and calcium ions channels which can be activated by agonists and antagonists. Most well-known agonist of TRPV1 is capsaicin and their complex form increases calcium in the cell medium which triggers the release of neuropeptides. Capsaicin triggers the heat-sensitive subunit of TRPV1 which acts as the heat sensor, causing the burning sensation [4][13]. Capsaicin and protons serve as a catalyst and lower the heat threshold of receptor and cause this burning sensation even in lower temperatures than 48°C [1].

The TRPV1 three-dimensional structure (Fig. 1.5) consists of 4 symmetrical subunits. These subunits form the ion conducting pore in their center and control TRPV1 response to agonists. All four subunits have four modules which are; the voltage sensor, pore module, N- and C- terminals. Transmembrane regions have six domains that are spanning helices; S1-S6 [13].

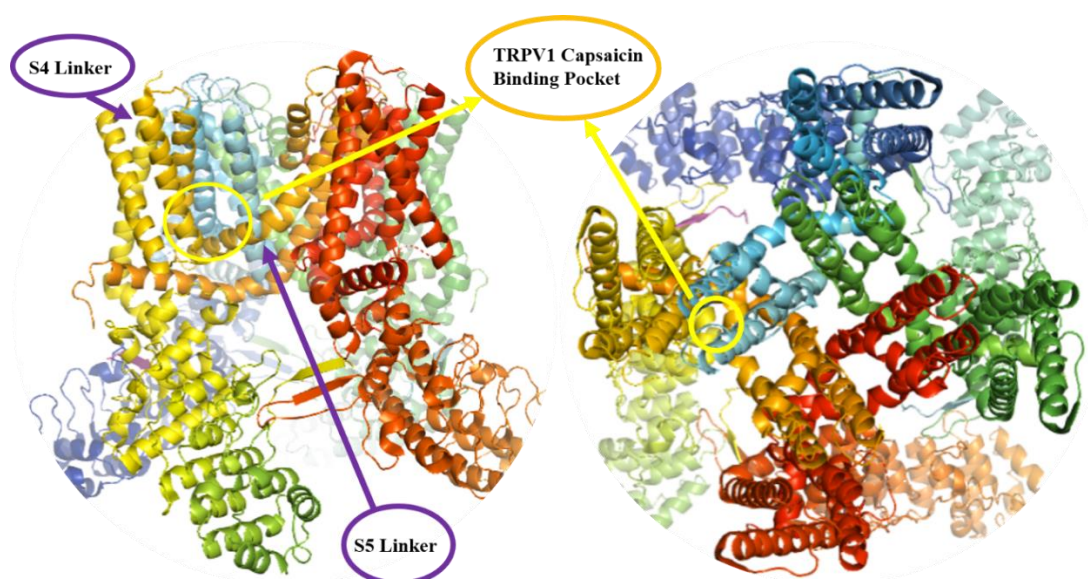


Figure 1.5: TRPV1 structure in 3 dimensions generated in PyMol.

1.4.1. TRPV1 Pocket

Cryo-EM structures show a pocket between S4-S5 domains in the membrane. Mutagenesis and functional studies agree that the active site for capsaicin is around amino acids; GLU570, TYR511, and THR550. In these studies, a small electron density was spotted inside the pocket that is expected to be an active site for capsaicin binding. This is the most direct evidence acquired by experiments for capsaicin binding location in TRPV1 [2].

Close state observations of TRPV1 interestingly still shows an electron density in the pocket. This can mean that the pocket is occupied by a lipid when there is no capsaicin present. As a result, capsaicin has to compete with this lipid for the pocket to bind the active site in TRPV1. Comparison between closed and open states of the pocket shows a miniscule outward movement of S4-S5 domains. This movement is observed when the capsaicin is bonded to the pocket and it is away from the central ion conducting pore [2][26][27].

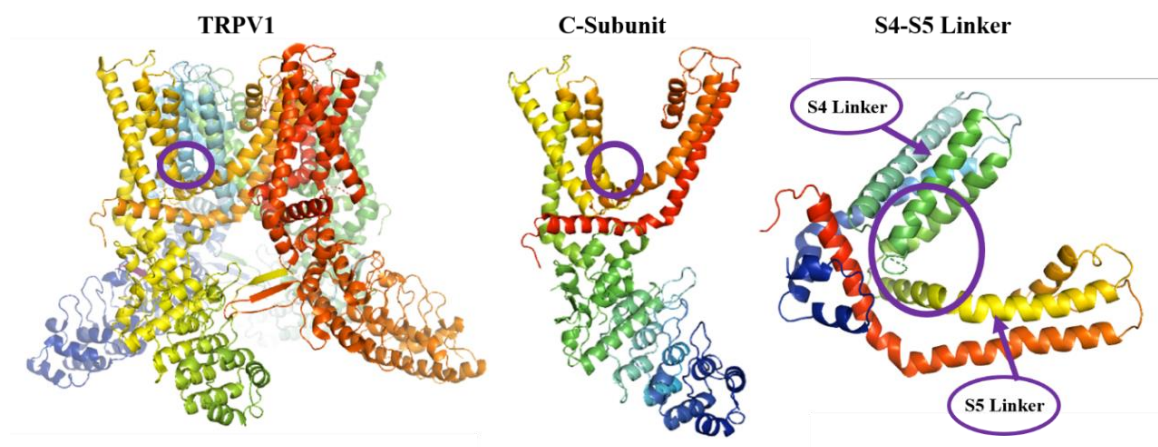
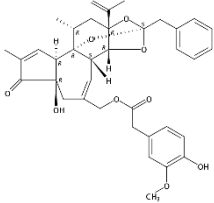
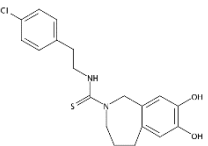
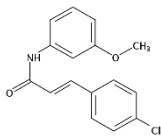
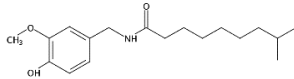
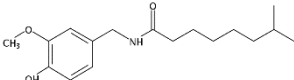
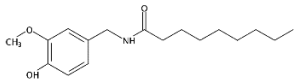
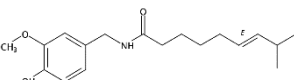
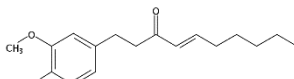
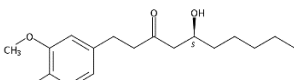


Figure 1.6: Active site of TRPV1 is shown in circles whole protein, one subunit and S4-S5 domains.

A hybrid study approach of computational docking and functional studies is developed in 2015 to understand the interactions between capsaicin and TRPV1[28]. “Tail-up, head-down” configuration is predicted for capsaicin as the position in the pocket. The tail is very flexible and can have many conformations. This flexibility is proposed as the reason for not observing a clear cryo-EM electron density for the tail [2].

Table 1.1: Agonist and antagonist molecules, 2-dimensional structures, longest distance in the molecule in Ångstrom, Functional groups, presence of double bond in the lipophilic chain and pungency in Scoville Heat Unit.

Name	Structure	Longest Distance of Molecule	Func. Group	Double Bond in Tail	Pungency (SHU)
resiniferatoxin		16.3	Ester	—	16.000.000.000
capsazepine		17.2	Thiourea	—	X
Sb-366791		14.4	Amide	+	X
dihydrocapsaicin		18.4	Amide	—	15.000.000
nordihydrocapsaicin		17.2	Amide	—	9.100.000
nonivamide		18.9	Amide	—	9.200.000
Capsaicin		16.0	Amide	+	15.000.000
6-shogaol		17.6	Ketone	+	16.000
6-gingerol		16.5	Ketone	—	60.000

2. AIM OF THE STUDY

The main purpose is to develop an atomic scale structural model that will explain the experimental structure-activity (pungency) relationship between TRPV1 and its agonists and antagonists.

Capsaicin conformation has previously been studied by DFT (Density Functional Theory) method. [32] The supremacy of the rather bulky RTX molecule in pungency has prompted us to create various tail-head combinations in capsaicin and capsazepine in current work. Using the computationally low-cost semi-empirical theory the number of hydrophobic tail torsions of the molecules has been increased to also include the higher energy conformations. This was done because the receptor does not necessarily have the highest affinity towards the lowest energy conformer of the agonist/antagonist. A similar approach was applied to the capsazepine molecule, which is more rigid than capsaicin, starting with semiempirical torsional searches and ending with DFT optimizations. The newly available conformations of capsaicin and capsazepine became the examples mimicked to create the possible conformers of the structurally similar agonist and antagonists.

The ultimate aim is to calculate the binding energies of the generated agonists and antagonists to TRPV1 and to find the amino acids in its active pocket, in which the agonist and antagonists prefer to bind and relate these preferences to the structural motifs on each binding molecule.

3. THEORY

3.1. Ligand Preparation with Gaussian03

Gaussian03 program is used for optimization, basis set increment and configuration search. Molecular Mechanics (MM) and semi-empirical quantum chemistry methods are used [29]

MPWB1K is a hybrid meta DFT method for kinetics and gives reliable results with water dimers and known to give consistent results with hydrogen bond making molecules[30]. This method is used in re-optimization of capsaicin structure taken from Alberti et al. [31]. 6-31+G(d,p) basis set lists polarization functions in an understandable way and when used with MPWB1K, it gives correct results with water dimer and hydrogen bonding [30][31]. New configuration search is made with Austin Model 1 (AM1) scans which makes semi-empirical calculations using the AM1 Hamiltonian [32].

3.2. Protein-Ligand Docking with Molecular Docking

Molecular Docking is a search algorithm for ligand-protein docking that determines the best possible fitting geometry with the lowest binding energy to the active side of protein. The grid-base method is used to focus on a specific area in the whole protein. The active site is embedded in the grid and ligand targets this specific area in the docking process [32]. In the docking process, in order to determine the best fitting possibility, the ligand is put in the active side pocket of protein many times. A score is computed as well as the binding energy and free energy. Autodock4 shows the best 10 results and the one with the lowest binding energy is taken as the output of docking [33][34]. In the docking process, in order to determine the best fitting possibility, the ligand is put in the active side pocket of protein many times. Then compute score; binding energy, free energy, etc. We looked at the best 10 value and the one with the lowest binding energy is taken as output of docking [35].

Molecular docking computations simulate the interaction of ligand and the active site of the protein. Thus, the three-dimensional geometry of the protein-ligand complex can be observed with docking [35].

3.3. Autodock4 Working Principle

Autodock4 is a software program that uses a variety of search algorithms to explore a given docking problem (see Appendix A). These include Monte Carlo Simulated Annealing (SA); a Genetic Algorithm (GA); and a hybrid local search GA, also known as the Lamarckian Genetic Algorithm (LGA) [36]. In general, the LGA performs the best out of SA, GA, and LGA, in finding the lowest energy of the system to predict the best achievable ligand-macromolecule complex conformation [37]. It finds the possible binding modes of protein and conformations of ligand that fit best to that protein. The binding energy of the protein-ligand complex and its best conformation are searched in this method until the best geometry and binding energy of the protein-ligand complex are found. These two are often treated as two separate problems to calculate [38] [32]. In addition to bonding atom interactions, secondary interactions like; electrostatics, hydrogen bonding, and dispersion-repulsion are taken into account in binding energy calculations. Inclusion of these secondary interactions helps to calculate the more accurate result of complex geometry and partial charges on the protein and ligand and binding energy. It was found that for a closer result to experimental results, these secondary interactions and consequently the partial charges of molecules should not be overlooked [5].

Van der Waals (vdW) non-bonded interactions, directional hydrogen bonding, screened Coulombic electrostatics and atomic solvation parameter-based desolvation free energy term are included in AutoDock's MM which is a linear free energy model [36].

AutoDock makes force field evaluations in two steps. Ligand (V_L) and macromolecule (V_M) energies are calculated when they are separate. The complex form of macromolecule-ligand (V_{M-L}) energy is also calculated. Sum of V_L and V_M is esteemed to be equal to V_{M-L} . In the first step; energetics of intramolecular energy of ligand and macromolecule evaluated from unbound state to bound state that is ligand-macromolecule complex. energies are calculated. In the second step; intermolecular energetic of making ligand-macromolecule complex is evaluated[34] [39].

Docking time is proportional to the sizes of the molecules docked. Working with macromolecules, AutoGrid helps to shorten the time docking requires. AutoGrid is a specified area that AutoDock makes its search for the best binding conformation for the ligand [38]. This specified area includes the binding site of the macromolecule. The grid-based method helps to calculate binding energy in a quick way since the calculations are focused on the active site embedded grid map instead of the whole macromolecule. Grid-based energy calculations take place before the docking simulation and a probe atom is placed at each grid center to measure interaction energy between it and the protein. Results of this calculation are stored in the grid map. Autodock uses this grid map data to decide the most stable ligand conformations [34][38].

Root mean square deviation (RMSD) is a way to evaluate docking simulation's ability to find the correct pose and minimalizing RMSD is assumed to produce more accurate and repeatable results [34][37].

Gasteiger or Kollman united charges are empirical charge calculation methods that AutoDockTools (ADT) program uses for appointing partial charges to ligand and macromolecule [36]. These charge calculation methods differ from each other by the differences in the partial charges found in different atoms. Gasteiger charges can be calculated for macromolecules directly by ADT. The downside of this charge calculation method is it takes the macromolecule as neutral by default. Kollman charges are preferred for macromolecule in this study for it uses quantum mechanics to acquire each amino acid values. Gasteiger charges are preferred for the ligand [32].

LGA with an empirical free energy force field combination is used in AutoDock resulting in a semiempirical force field estimation [39]. This is used to find an accurate scoring function that is semiempirical. LGA generates new conformations while scoring function for docking determines what the accepted conformation will be. This gives a quick prediction to ligand conformation that fits the binding site of macromolecule and its free energy associations [36][38].

4. METHODOLOGY

Capsaicin molecule has 10 torsions that define its structure. Pichierri et al. [32] have taken the X-ray structure of capsaicin and reported 10 conformers lying within a 0.7 kcal/mol interval [40]. By varying the torsions by at most of 15° , we obtained extra albeit higher energy conformers using the same methodology (MPWB1K/6-31+G(d,p)).

Protein-ligand docking with molecular docking programs requires a three-dimensional ligand and a protein. Capsaicin molecule initial configurations were taken from the DFT work of Alberti et al. [31]. This study contained 10 different configurations of capsaicin. First, these configurations are optimized using Gaussian03 program [29]. Conformations are then built with new torsion angles [31] (see Appendix A). This geometry is optimized at the MPWB1k/ 6-31+g(d,p) level. 8th conformer of the Pichierri et al. [32] study gave the lowest conformational energy. This conformer was taken as a reference while making a larger set of capsaicin conformers.

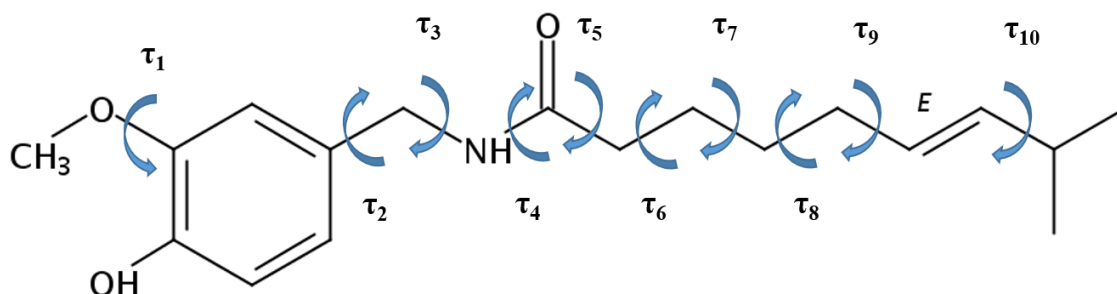


Figure 4.1: 10 torsions (τ) of Capsaicin.

Geometry diversity in the tail part of the compound is achieved with AM1 scans and; configuration search. Diversity in the hydrophilic aromatic ring is achieved with MM method and; configuration search. The results provide us with different head and tail variations of the same molecule as seen in Figure 4.2 and 4.3.

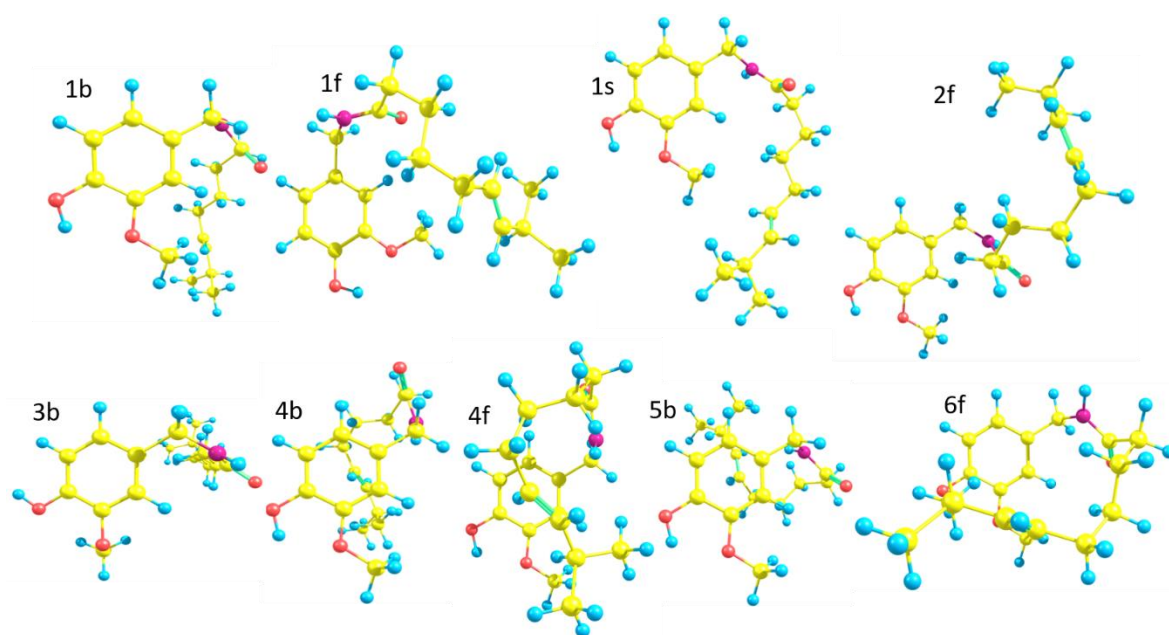


Figure 4.2: Capsaicin tail variations.

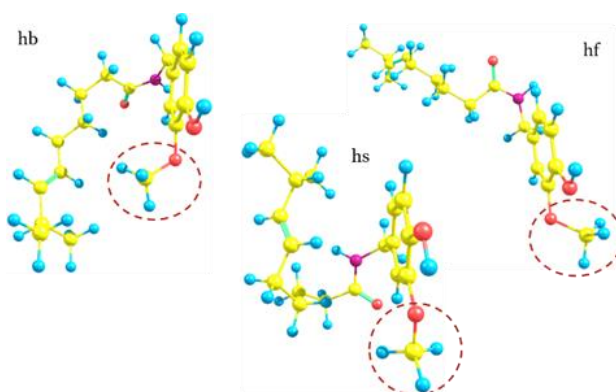


Figure 4.3: Capsaicin head variations.

12 distinctly different geometries of capsaicin are docked using AutoDock4 in the capsaicin receptor, TRPV1 protein active site (see Appendix A for the AutoDock protocol). Where capsaicin was assumed to be flexible and the active site was assumed to be rigid. The ligand geometries producing the most exothermic binding and those with the best pose are reported. [41,42].

Capsazepine molecule drawn and rotated by hand to give different conformations with its torsion angles. AM1 Scan is used to achieve geometric diversity of capsazepine. In this study, 10 different geometries are docked to TRPV1 with rigid docking.

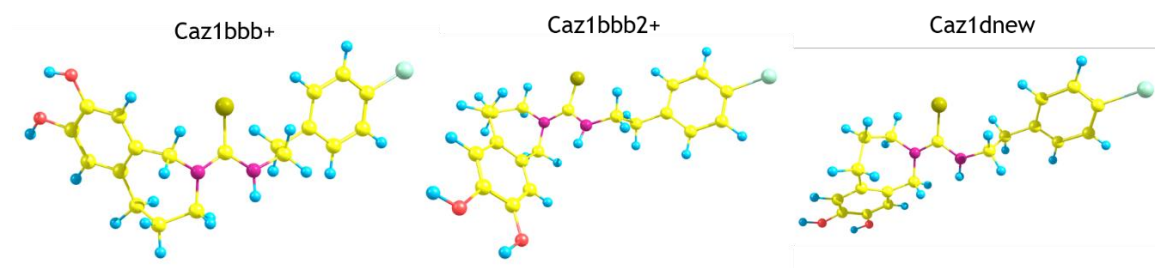


Figure 4.4: Capsazepine variations.

Capsaicin and capsazepine analog molecules RTX, nonivamide, dihydrocapsaicin, nordihydrocapsaicin, 6-gingerol, 6-shogaol, sb-366791, structures are taken from SciFinder [9][43–47]. These molecules are docked to the protein to see their binding energies. After getting these results another set of conformation of analog molecules is prepared similar to lowest binding energy result capsaicin and capsazepine. Agonist analogs are prepared by referencing lowest energy conformation of capsaicin. Antagonist analog molecule is prepared under the reference of capsazepine lowest binding energy result. These new conformers of analog molecules are docked to TRPV1 receptor and results of this new analog molecule set is compared to the first analog molecule set.

4.1. Molecular Docking Calculations

Protein 3D crystal structure is obtained from the protein data bank (PDB). Capsaicin is known to target the active site of a pocket in the TRPV1 protein in S4-S5 domains [2][28][41].

TRPV1 is a quaternary protein and have four identical transmembrane domains [40]. One transmembrane domain is isolated as shown in Figure 4.5. Other three domains are removed from simulation for saving time in docking. From the transmembrane domain, S4-S5 domains from residues 394 to 719 is isolated in PyMol for further steps so the rest of the protein does not increase the docking time [41] (see Appendix A).

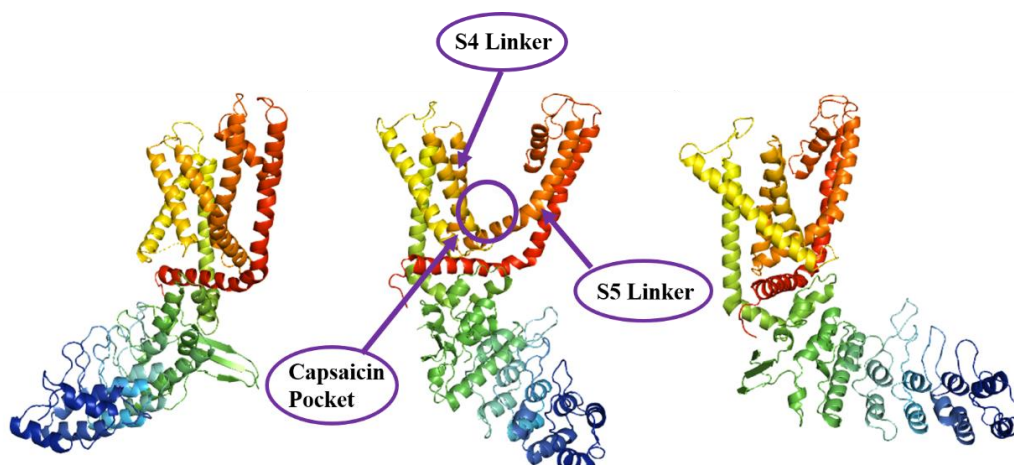


Figure 4.5: TRPV1 transmembrane domain C shown in three different angles with secondary structure form. Image exported from PyMol.

Addition of hydrogens, merge of nonpolar hydrogens and removal of water from the protein is important. This helps to remove oxygen atoms in water present as non-bonded atoms in the crystal structure. Kollman charges are added for empirical charge calculations [32][48].

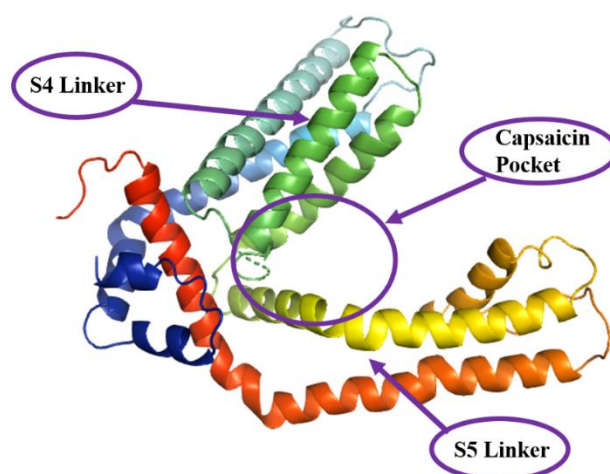


Figure 4.6: TRPV1 protein S4-S5 domains are shown in secondary structure helices. Image exported from PyMol.

Autogrid pre-calculates the interaction energies of different atoms' grid maps before the docking process. Autogrid pre-calculation saves time during the docking since

Autodock uses these drawn up maps for its calculations [33][36]. Having only the S4-S5 domains of TRPV1 protein shortens the docking time also makes easier active site observation. A good way to solve this would be taking only the active site amino acids and its immediate surroundings and calculating geometries within this boundary. Grid options widget in AutoDock helps to set the location to search for the docking process. Once defining active site amino acids and its surrounding in the grid box, the calculation takes place inside the grid box, and AutoDock tries to fit the ligand in this defined area. We used a 60x60x60 box with 0,375 Å grid spacing and -0,944 Å z-center setting is used for all docking processes [31][41] (see Appendix A).

4.2. Building the Ligand

Docking process requires a three-dimensional ligand to run. The DFT geometries of capsaicin and capsazepine and the Cartesian coordinates of their analog molecules from (<https://sso.cas.org/>) were used. Different conformations are docked, and results are checked to see which geometry fits best to the active site of the protein, TRPV1 [31][42].

For autodock4, ligand needs to be in Protein Data Bank, Partial Charge (Q), & Atom Type (T) (PDBQT) format. This is a modified format of protein data bank format (PDB). This format contains the information of; atomic charges and atom type definitions. Ligand files also have rotatable bonds information [33]. PDBQT format is prepared with Open Babel. Ligand root and portions are set by portion tree in Autodock4 [48] (see Appendix A).

We used the recommended settings, LGA search with an empirical free energy function [37][49]. Our initial population was 150 randomly placed ligands. A number of 250,000 energy evaluations were done for each docking. A maximum of 27,000 generations was done. The grid maps representing the protein during the docking process were calculated with AutoGrid 4.0, with grid dimensions of 60 × 60 × 60 grid points, a spacing of 0.375 Å, and the center of the box was placed at -0,944 Å z-center. Thirty independent runs were carried out. The best-docked conformations were those found to have the lowest binding energy [33].

RMSD value is checked. Normally less than 2 Å is expected for best docking position for the ligand [42]. Since this is a rigid docking study, RMSD values cannot reflect the quality of binding. The RMSD values in this study are around 2.0. The difference in hydrogen bond positions, bond distances, and a number of these bonds are recorded for binding pose evaluation. The inhibition constants (K_i in μM) are reported. A low value of K_i means good binding. The lowest K_i in this study is 1.

5. RESULTS AND DISCUSSION

5.1. Capsaicin Docking Results

A further variation of the 12 different capsaicin tail and head combinations via AutoDock4 resulted in a total of 120 capsaicin conformations. The most different conformations are reported in Table 5.1.

Table 5.1: Capsaicin docking results with ΔE as free binding energy (kcal/mol), bonding amino acids, bond lengths in Ångstrom, Ki(μ M) and RMSD values.

Name	ΔE	Aa	Bond Length	Aa	Bond Length	Aa	Bond Length	Ki	RMSD
4f-Caps6	-5.18	Glu570	1.918	Tyr511	2.016			160.88	26.52
5b-Caps2	-5.17	Glu570	1.830	Tyr511	2.021			163.12	27.59
4b-Caps9	-5.02	Tyr511	1.906	Glu570	1.962			207.42	26.50
6f-Caps5	-5.02	Glu570	1.859	Thr550	1.996	Tyr511	2.089	209.44	25.84
4f-Caps2	-4.95	Tyr511	1.894	Glu570	1.954			236.32	26.71
5b-Caps7	-4.95	Glu570	1.801	Thr550	1.899	Tyr511	2.050	235.20	26.84
4f-Caps7	-4.86	Glu570	1.853	Tyr511	1.866	Thr550	1.906	272.64	26.75
4f-Caps10	-4.85	Glu570	1.733	Tyr511	2.028			279.46	26.37
4b-Caps10	-4.83	Thr550	1.851	Tyr511	1.865	Glu570	1.868	289.95	26.57
6f-Caps2	-4.83	Tyr511	1.858	Thr550	1.894	Glu570	1.908	286.77	26.15
6f-Caps6	-4.83	Glu570	1.809					285.84	26.21
7hs-Caps9	-4.76	Glu570	1.761					322.29	26.10
6f-Caps7	-4.66	Glu570	1.988	Tyr511	2.133			381.35	26.28
7hs-Caps8	-4.56	Glu570	1.844	Thr550	1.949	Tyr511	2.001	456.93	24.98
7hf-Caps4	-4.53	Glu570	1.943	Tyr511	1.949			476.22	25.32
4f-Caps9	-4.52	Glu570	1.811	Tyr511	1.952	Thr550	2.006	487.90	25.61
7hf-Caps3	-4.43	Glu570	1.956					562.95	25.41
4b-Caps7	-4.42	Tyr511	1.751	Glu570	1.838	Thr550	1.941	577.79	26.90
5b-Caps5	-4.35	Thr550	1.838	Tyr511	2.117			648.39	26.67
7hf-Caps9	-4.34	Glu570	2.098					663.55	25.56
7hf-Caps6	-4.33	Tyr511	2.011	Glu570	2.021			665.20	25.76
7hf-Caps8	-4.32	Glu570	1.934					684.40	24.46
7hf-Caps5	-4.31	Glu570	1.957					697.27	24.12
7hf-Caps2	-4.30	Glu570	1.912					699.13	24.06
6f-Caps1	-4.20	Glu570	1.821	Thr550	1.985	Tyr511	2.131	830.79	27.06
6f-Caps4	-4.13	Tyr511	1.851	Glu570	1.933	Thr550	2.116	946.52	25.86
6f-Caps3	-4.12	Glu570	1.838	Tyr511	2.082	Thr550	2.249	959.65	25.77
7hf-Caps7	-4.11	Glu570	1.803					966.60	25.29
7hs-Caps4	-4.08	Thr550	1.847	Tyr511	1.926	Glu570	1.988	1.02	25.28
3b-Caps6	-4.06	Glu570	1.856	Tyr511	2.128			1.05	25.15
1s-Caps5	-4.05	Glu570	1.896	Tyr511	2.132			1.08	26.34
3b-Caps5	-4.00	Glu570	1.968					1.16	28.57
7hb-Caps10	-3.98	Glu570	2.023					1.20	27.09
7hs-Caps7	-3.96	Tyr511	2.172					1.25	26.05
6f-Caps9	-3.95	Glu570	1.949	Tyr511	1.955	Thr550	2.022	1.26	25.98
7hf-Caps1	-3.93	Tyr511	1.869	Glu570	1.949			1.32	25.75
3b-Caps10	-3.90	Glu570	1.993	Tyr511	2.227			1.39	28.42

Table 5.1: Capsaicin docking results (cont.)

Name	ΔE	Aa	Bond Length	Aa	Bond Length	Aa	Bond Length	Ki	RMSD
7hs-Caps5	-3.88	Glu570	1.818	Thr550	1.972	Tyr511	1.973	1.43	25.18
3b-Caps2	-3.87	Glu570	1.801					1.47	27.75
3b-Caps3	-3.85	Glu570	1.941	Tyr511	2.206			1.50	28.38
7hb-Caps4	-3.85	Glu570	2.007					1.50	28.28
3b-Caps4	-3.80	Glu570	1.816					1.64	28.51
3b-Caps1	-3.78	Tyr511	1.609	Glu570	1.846			1.68	28.82
4b-Caps5	-3.70	Glu570	2.061	Tyr511	2.071			1.92	26.09
5b-Caps8	-3.70	Thr550	1.874	Tyr511	1.962			1.95	26.87
7hb-Caps3	-3.68	Glu570	1.995					2.01	24.64
7hb-Caps5	-3.66	Glu570	1.955					2.06	27.13
4f-Caps4	-3.64	Thr550	2.229					2.16	29.18
5b-Caps10	-3.63	Glu570	1.744	Tyr511	2.015			2.20	26.35
7hf-Caps10	-3.57	Tyr511	1.888	Glu570	1.959			2.44	25.33
6f-Caps10	-3.56	Tyr511	2.095					2.46	25.50
4b-Caps4	-3.55	Thr550	2.001	Tyr511	2.114			2.52	25.88
7hb-Caps9	-3.55	Glu570	1.858	Tyr511	2.124			2.50	27.05
4b-Caps2	-3.51	Glu570	2.048	Tyr511	2.159			2.66	25.91
7hs-Caps10	-3.51	Tyr511	2.174					2.66	25.34
1b-Caps3	-3.49	Tyr511	1.776	Glu570	1.953			2.74	26.41
1s-Caps1	-3.49	Glu570	2.107	Tyr511	2.131			2.78	26.11
3b-Caps9	-3.49	Tyr511	1.940	Glu570	2.156			2.76	28.71
5b-Caps6	-3.49	Thr550	1.971					2.77	29.03
7hs-Caps1	-3.47	Thr550	1.877	Tyr511	1.970			2.88	25.47
4b-Caps6	-3.46	Tyr511	1.860					2.92	26.51
1f-Caps9	-3.39	Tyr511	2.012					3.28	26.60
5b-Caps3	-3.38	Thr550	1.853	Thr550	2.181			3.31	29.10
3b-Caps8	-3.34								27.18
4f-Caps8	-3.33	Tyr511	2.016	Glu570	2.068			3.64	26.75
3b-Caps7	-3.31	Glu570	1.956					3.74	27.21
4f-Caps3	-3.31	Thr550	2.041					3.72	26.19
5b-Caps4	-3.30	Tyr511	1.710	Thr550	1.858			3.84	26.05
7hb-Caps1	-3.30	Glu570	2.010					3.80	27.65
1s-Caps10	-3.29	Tyr511	1.799					3.90	25.72
5b-Caps1	-3.29								27.04
4b-Caps8	-3.18	Tyr511	1.907					4.67	25.78
7hb-Caps2	-3.18	Tyr511	2.032	Glu570	2.120			4.69	24.85
7hs-Caps3	-3.18	Thr550	1.936	Tyr511	2.218			4.69	26.36
4f-Caps5	-3.14	Thr550	1.775	Thr550	2.026			4.99	28.82
7hs-Caps6	-3.13	Thr550	1.719	Thr550	2.051			5.04	28.20
7hb-Caps8	-3.09	Glu570	2.191	Thr550	2.225			5.42	28.21
5b-Caps9	-3.08	Tyr511	1.842					5.54	26.83
1f-Caps6	-3.07	Thr550	1.707	Tyr511	1.713			5.65	26.46
7hb-Caps6	-3.05	Tyr511	1.684					5.82	28.22
4b-Caps3	-3.03	Thr550	1.935	Glu570	2.189			6.06	26.10
6f-Caps8	-3.03	Tyr511	1.776	Glu570	2.038			5.98	25.12
1s-Caps6	-3.00	Tyr511	2.059					6.29	26.96
4f-Caps1	-3.00	Thr550	1.988					6.28	27.02
1s-Caps8	-2.98	Glu570	2.000					6.49	26.78
1f-Caps5	-2.85	Thr550	1.983					8.08	29.29
7hs-Caps2	-2.83	Thr550	1.689	Thr550	2.008			8.49	28.07
1f-Caps3	-2.82	Thr550	1.965	Thr550	2.010			8.56	27.77
1b-Caps6	-2.78	Tyr511	1.838	Thr550	1.909			9.12	25.74
1s-Caps3	-2.76	Glu570	1.872					9.50	25.61

Table 5.1: Capsaicin docking results (cont.)

Name	ΔE	Aa	Bond Length	Aa	Bond Length	Aa	Bond Length	Ki	RMSD
1f-Caps4	-2.71								28.78
1f-Caps8	-2.68	Phe543	2.054					10.76	28.59
1b-Caps4	-2.67								26.07
1b-Caps5	-2.67	Glu570	1.906	Tyr511	2.049	Thr550	2.199	10.96	25.32
1b-Caps1	-2.66	Glu570	1.796					11.25	25.35
2f-Caps7	-2.62	Phe543	2.148					11.99	30.29
1f-Caps2	-2.58	Tyr511	1.704					12.84	24.80
1b-Caps8	-2.54	Tyr511	1.920					13.66	26.57
1f-Caps1	-2.49	Thr550	1.890	Thr550	2.074			14.96	28.13
1b-Caps9	-2.45	Thr550	1.820	Thr550	2.022			16.13	28.17
1s-Caps4	-2.44	Glu570	1.911					16.29	24.01
2f-Caps8	-2.41	Thr550	1.869					17.12	28.19
2f-Caps6	-2.39	Tyr511	2.095					17.64	28.67
1b-Caps7	-2.36	Tyr511	1.942	Thr550	2.077			18.76	26.63
1f-Caps7	-2.34								28.79
1f-Caps10	-2.32								22.31
2f-Caps1	-2.31								28.99
1s-Caps7	-2.30	Thr550	1.939					27.31	26.00
2f-Caps4	-2.28								25.39
2f-Caps9	-2.28	Thr550	1.736					21.43	28.85
1b-Caps2	-2.26	Tyr511	2.088					22.10	27.95
2f-Caps10	-2.18								28.58
2f-Caps5	-2.10	Thr550	1.982	Tyr511	2.104			28.73	28.82
2f-Caps3	-2.09	Thr550	1.920	Phe543	1.935			29.18	32.12
1b-Caps10	-2.06	Glu570	2.095	Thr550	2.100			30.98	25.53
1s-Caps2	-1.97	Tyr511	1.660					35.70	26.62
2f-Caps2	-1.94								31.96
1s-Caps9	-1.74	Glu570	2.095					53.09	27.01
7hb-Caps7	3.09	Glu570	2.065					5.41	26.40
4b-Caps1	4.38	Tyr511	2.055					615.35	26.45

Within 120 trials, 9 of them made no bonds with the protein active site. Tyr511, Thr550, Glu570, and Phe543 are the amino acids preferred by capsaicin conformers. Based on the Ki values, the best of the set is conformer 7hs-caps4, with the Ki of 1.02 μ M. and binding energy of -4.08 kcal/mol. It must be noted that this binding energy is within the free energy error margin of ± 2.85 kcal/mol [36]. It is bound to Thr550, Tyr511, and Glu570. Its position in the active site of protein is; tail up, head down. Based on the binding energies, 4fcaps6 is the best fitting isomer with a binding energy of -5.18 ± 2.85 kcal/mol with a Ki of 160.88. It is bound to Glu570 and Tyr511. The RMSD results are high in general because the docking was done without allowing the TRPV1 motion.

5.2. Capsazepine Docking Results

A further variation of the 10 different capsazepine conformations via AutoDock resulted in 100 different capsazepine conformations shown in Table 5.2.

Table 5.2: Capsazepine docking results with ΔE as free binding energy (kcal/mol), bonding amino acids, bond lengths in Ångstrom, $K_i(\mu M)$ and RMSD values.

Name	ΔE	Aa	Bond L.	Aa	Bond L.	K_i	RMSD
Caz1b2	-6.53	Leu553	1.899	Glu570	2.156	16.32	22.75
Caz1b3	-6.50	Leu553	1.718	Glu570	2.210	17.11	22.79
Caz1b5	-6.50	Glu570	1.919	Glu570	1.955	17.15	22.58
Caz1b7	-6.41	Leu553	2.022	Glu570	2.076	20.11	22.80
Caz1b10	-6.41	Leu553	1.984			20.06	22.65
Caz1b6	-6.26	Leu553	1.928	Glu570	2.106	25.61	22.65
Caz1b1	-6.14	Glu570	1.679			31.74	23.40
Caz1b4	-6.02	Glu570	1.932	Glu570	2.008	38.71	23.05
Caz1b9	-5.91	Thr550	2.020			46.32	23.26
Caz1a4	-5.64	Glu570	2.222			73.99	25.53
Caz1b8	-5.64	Glu570	1.669			73.73	21.39
Caz1a1	-5.63	Leu574	1.886	Leu574	2.046	74.92	25.69
Caz1a2	-5.63	Leu574	1.837	Leu574	2.055	74.77	25.70
Caz1a3	-5.63	Leu574	1.925	Leu574	2.000	75.11	25.68
Caz1a7	-5.63	Leu574	1.912	Leu574	2.000	74.95	25.68
Caz1a9	-5.63	Leu574	1.974	Leu574	2.003	74.90	25.70
Caz1a5	-5.62	Leu574	1.882	Leu574	2.036	75.92	25.69
Caz1a8	-5.62	Leu574	1.845	Leu574	2.029	75.56	25.69
Caz1a6	-5.61	Leu574	1.879	Leu574	2.033	76.77	25.68
Caz1a10	-5.56					83.38	25.48
Caz1bbb2+10	-2.86	Glu570	1.850			8.07	21.20
Caz1cbb3-2	-2.63	Glu570	1.814	Glu570	1.992	11.73	22.09
Caz1cbb1-4	-2.62	Glu570	1.890			12.05	21.50
Caz1bbb3+1	-2.60	Glu570	1.947	Glu570	1.970	12.36	22.13
Caz1cbb1-9	-2.56	Glu570	1.806			13.22	20.62
Caz1cbb2-5	-2.48	Glu570	1.704			15.12	23.61
Caz1cbb1-5	-2.42	Glu570	1.946	Glu570	1.978	16.85	24.29
Caz1cbb1-10	-2.40	Glu570	1.884	Glu570	2.032	17.28	22.10
Caz1cbb3-6	-2.40	Glu570	1.899	Glu570	2.111	17.51	24.63
Caz1bbb+9	-2.37	Glu570	1.957	Glu570	1.995	18.30	22.55
Caz1bbb+10	-2.35	Glu570	1.940	Glu570	1.993	18.82	22.49
Caz1bbb3+8	-2.34	Glu570	1.836	Glu570	1.916	19.35	22.10
Caz1cbb2-6	-2.30	Asp576	1.879	Asp576	2.155	20.73	20.63
Caz1bbb2+1	-2.29	Glu570	1.897	Glu570	2.178	20.99	24.76
Caz1cbb3-8	-2.29	Glu570	1.929	Glu570	1.955	21.03	21.97
Caz1cbb2-3	-2.23						22.58
Cazbb+6	-2.23	Glu570	1.881			23.12	26.48
Caz1bbb2+2	-2.22	Glu570	1.713			23.76	23.33
Caz1cbb3-10	-2.21	Glu570	1.844	Glu570	2.113	23.97	22.60
Caz1cbb3-7	-2.13	Glu570	1.870	Glu570	1.941	27.56	24.04
Caz1bbb3+7	-2.11	Tyr565	1.937	Tyr565	1.958	28.37	20.84
Caz1cbb3-5	-2.11	Thr550	1.953			28.43	24.08
Caz1bbb3+10	-2.10	Thr550	1.793			28.69	24.21
Caz1bbb3+3	-2.07	Glu570	1.910	Glu570	1.951	30.33	22.26
Cazbb+2	-2.07	Tyr511	2.116			30.37	26.88
Caz1bbb+6	-2.05						28.06

Table 5.2: Capsazepine docking results (cont.)

Name	ΔE	Aa	Bond L.	Aa	Bond L.	Ki	RMSD
Caz1bbb2+6	-2.05	Glu570	2.047			31.29	22.94
Caz1cbb1-6	-2.05	Tyr511	1.841			31.49	27.01
Caz1cbb1-8	-2.04	Tyr511	2.046			31.95	25.58
Caz1cbb2-8	-2.04	Thr550	1.937			32.15	24.84
Cazbb+4	-2.04						21.48
Cazbb+5	-2.04						28.77
Cazbb+7	-2.04	Tyr511	1.968			31.76	26.58
Caz1bbb3+9	-2.03						26.71
Caz1bbb+4	-2.00	Thr550	1.938			34.15	25.10
Caz1bbb3+4	-2.00						24.71
Caz1cbb1-3	-1.98						28.96
Cazbb+9	-1.98						28.81
Cazbb+10	-1.96	Glu570	1.879			36.78	23.96
Caz1bbb2+8	-1.95	Glu570	1.823			36.92	22.26
Caz1cbb3-3	-1.94	Phe543	1.995			37.70	27.76
Caz1cbb2-7	-1.92						20.97
Caz1bbb2+5	-1.91	Glu570	1.934	Glu570	1.959	39.51	22.75
Caz1bbb3+5	-1.91	Thr550	2.181			39.64	23.36
Cazbb+3	-1.91	Tyr565	1.928	Tyr565	2.186	39.87	21.30
Caz1bbb3+6	-1.90	Tyr511	1.901			40.21	26.28
Caz1cbb2-1	-1.90	Glu570	1.744			40.25	23.41
Caz1bbb+5	-1.88	Glu570	1.820	Glu570	2.176	41.64	23.17
Caz1cbb2-2	-1.87						27.99
Caz1bbb+3	-1.85						26.56
Caz1bbb2+3	-1.84	Glu570	1.822	Glu570	1.964	44.75	24.16
Caz1bbb2+7	-1.83	Tyr565	1.879	Tyr565	2.113	45.34	21.51
Cazbb+8	-1.81	Tyr511	2.040			47.27	26.36
Caz1bbb+2	-1.80	Tyr511	1.892			48.14	26.08
Caz1cbb1-1	-1.79	Tyr511	2.034			48.97	27.13
Caz1bbb+8	-1.75	Glu570	1.762	Glu570	2.147	52.45	22.42
Caz1bbb2+4	-1.75	Tyr511	1.874			52.57	24.38
Caz1dnew-1	-1.75	Tyr565	1.889	Tyr565	2.008	52.26	19.90
Caz1dnew-6	-1.74	Tyr565	2.010	Tyr565	2.110	53.33	19.73
Caz1dnew-10	-1.74	Tyr565	1.991			53.04	20.76
Caz1cbb1-2	-1.73	Tyr565	1.888	Tyr565	1.942	54.03	22.42
Caz1cbb3-9	-1.73	Tyr511	1.969			54.35	25.56
Caz1cbb1-7	-1.72						26.30
Caz1dnew-3	-1.70	Tyr565	1.979	Tyr565	2.058	57.18	19.80
Caz1cbb3-1	-1.69	Tyr511	1.780			58.05	26.19
Cazbb+1	-1.67						26.43
Caz1bbb+1	-1.66	Phe543	1.930			60.22	30.78
Caz1cbb3-4	-1.66						28.33
Caz1dnew-9	-1.66	Tyr511	1.992			61.04	27.59
Caz1dnew-4	-1.62	Tyr565	1.915	Tyr565	2.032	65.01	20.12
Caz1bbb2+9	-1.60						27.06
Caz1dnew-2	-1.56	Asp576	2.088	Asp576	2.181	71.50	21.11
Caz1bbb+7	-1.54						26.74
Caz1bbb3+2	-1.54						28.25
Caz1cbb2-4	-1.53						20.79
Caz1dnew-8	-1.46	Tyr565	2.049	Tyr565	2.082	85.30	20.19
Caz1dnew-5	-1.44						28.24
Caz1dnew-7	-1.38						28.31
Caz1cbb2-9	1.95	Tyr511	2.025			37.05	26.14
Caz1cbb2-10	1.97	Tyr565	2.207	Tyr565	2.240	35.75	21.09

In 100 trials, 21 of them shows no bonding results with the protein active site. Tyr511, Phe543, Thr550, Leu553, Tyr565, Glu570, Leu574 and Asp576 are the amino acids preferred by capsazepine conformers. Based on the K_i values, the best of the set is conformer caz1bbb2+10, with the K_i of 8.07 μM and binding energy of -2.86 kcal/mol. It is bound only to Glu570. Caz1bbb2+10 conformer shows bending in the molecule up to point aromatic rings at both ends are standing almost parallel with each other. Head part shows better bonding tendencies and makes the amino acid binding.

Based on the binding energies, isomer caz1b2 is the best fitting ligand, with a -6.53 $\pm$ 2.85 kcal/mol binding energy and K_i of 16.32. It is bound to Glu570 and Leu553.

5.3. Analog Molecule Docking Results

Analog molecules of capsaicin and capsazepine are docked in 2 different conformations each. The first was chosen to be their structure from literature and the second was the most similar conformation to the best fitting capsaicin and capsazepine. Structures with conformational similarity to capsaicin and capsazepine have a -c ending in their name. The analog results are shown in Table 5.3. The best binding energies of all analog species are compared in Table 5.4. In Table 5.5 capsaicin and capsazepine results have been included.

Each molecule docked 6 times in total and shows a different tendency in a number of bonds they make. Tyr511, Thr550, and Glu570 are the amino acids preferred by analog molecules (see Table 5.6)

Best binding molecules for each analog is chosen by taking binding free energy into consideration with number of H-bonding they made and K_i values (see Appendix B). Except for 6-Shogaol, all analog molecules made H-bonding at their best binding free energy states as shown in Table 5.3. 6-Shogaol1 has ΔE at -5.05 kcal/mol but this docking shows no H-bonding. 6-Shogaol-c1 is chosen at its place since it has made H-bonding and has lowest K_i among 6-Shogaol cluster.

Table 5.3: Analog molecules docking results with ΔE is binding energy (kcal/mol), bonding amino acids, bond lengths in Ångstrom, Ki(μ M) and RMSD values.

Name	ΔE	Aa	Bond L.	Aa	Bond L.	Aa	Bond L.	Ki	RMSD
Resiniferatoxin-c1	-6.96	Thr550	2.199					7.94	26.55
Sb-366791-1	-6.4	Thr550	1.842					20.42	29.84
Sb-366791-3	-6.38	Thr550	1.926					21.09	29.87
Resiniferatoxin-c2	-6.37	Thr550	1.809	Glu570	1.851			21.48	25.03
Resiniferatoxin2	-6.35							21.97	25.74
Resiniferatoxin1	-6.33							22.79	24.95
Dihydrocapsaicin2	-6.32	Glu570	1.841					23.11	25.51
Sb-366791-2	-6.31	Thr550	1.887					23.66	29.97
Resiniferatoxin-c3	-6.28	Thr550	2.039					25.14	24.75
Nordihydrocapsaicin-c2	-6.21	Tyr511	2.002					28.22	29.14
Nordihydrocapsaicin-c1	-6.18	Tyr511	1.715	Thr550	1.902	Glu570	1.953	29.33	29.01
Sb-366791-c1	-6.16	Thr550	1.87					30.77	29.32
Nordihydrocapsaicin1	-6.15	Thr550	1.865	Glu570	1.888	Tyr511	1.906	30.86	26.36
Sb-366791-c3	-6.11							33.09	29.72
Resiniferatoxin3	-6.1	Glu570	1.916					33.82	25.18
Sb-366791-c2	-6.08							34.78	29.75
Dihydrocapsaicin-c3	-5.95	Glu570	1.908	Tyr511	2.174			41.24	30.15
Nonivamide2	-5.5	Tyr511	1.907	Glu570	2.051			93.49	24.51
Nonivamide1	-5.45	Glu570	1.77					101.07	24.34
Dihydrocapsaicin-c2	-5.16	Thr550	1.832	Glu570	2.103			163.76	29.02
6-Shogaol1	-5.05							198.66	26.96
6-Shogaol2	-4.84	Glu570	2.021					284.69	27.12
Nonivamide-c1	-4.84	Glu570	1.896					281.14	29.05
Dihydrocapsaicin-c1	-4.8							305.47	30
6-Shogaol-c3	-4.76							324.67	28.41
Nonivamide3	-4.75							328.28	24.48
6-Shogaol3	-4.68							372.45	26.02
Nonivamide-c2	-4.67	Glu570	2.021					379.89	30.34
Nordihydrocapsaicin2	-4.6							423.02	26.14
6-Gingerol1	-4.48	Thr550	1.853	Glu570	2.013			516.09	28
6-Shogaol-c2	-4.47	Thr550	2.001					529.07	28.32
6-Gingerol2	-4.46	Thr550	1.908	Glu570	1.956			536.17	27.8
Nordihydrocapsaicin3	-4.46	Tyr511	1.882	Glu570	2.107			542.14	26.32
Nordihydrocapsaicin-c3	-4.45							548.83	28.53
6-Gingerol3	-4.38	Thr550	1.887					615.6	29.04
Dihydrocapsaicin3	-4.34	Glu570	1.871					655.04	25.38
6-Gingerol-c2	-4.27	Thr550	2.138					744.22	27.02
Dihydrocapsaicin1	-4.14							929.49	26.25
6-Shogaol-c1	-4.08	Thr550	1.919	Glu570	1.957			1.02	27.03
Nonivamide-c3	-4.05	Thr550	1.949					1.08	32.33
6-Gingerol-c3	-3.86	Tyr511	1.777	Tyr511	1.954			1.49	27.48
6-Gingerol-c1	-3.75	Thr550	1.953	Glu570	2.092			1.8	27.11

Resiniferatoxin and nordihydrocapsaicin are the two giving better results in curved tail geometry like capsaicin. Dihydrocapsaicin, nonivamide 6-shogaol and 6-gingerol give lower binding energy results when their tail is not curved. Sb-366791 gives higher energy

when its geometry is similar to capsazepine. Table 5.5 shows the best free binding energies of each docked molecule and compare their results with each other.

Table 5.4: Analog molecules results. ΔE : free binding energy (kcal/mol), Aa: active amino acids, Bond L: length of H-bond in Ångstrom, Ki(μ M) and RMSD values.

Name	ΔE	Aa	Bond L.	Aa	Bond L.	Aa	Bond L.	Ki	RMS D
Resiniferatoxin-c1	-6.96	Thr550	2.199					7.94	26.55
Sb-366791-1	-6.40	Thr550	1.842					20.42	29.84
Resiniferatoxin2	-6.35							21.97	25.74
Dihydrocapsaicin2	-6.32	Glu570	1.841					23.11	25.51
Nordihydrocapsaicin-c2	-6.21	Tyr511	2.002					28.22	29.14
Sb-366791-c1	-6.16	Thr550	1.870					30.77	29.32
Nordihydrocapsaicin1	-6.15	Thr550	1.865	Glu570	1.888	Tyr511	1.906	30.86	26.36
Dihydrocapsaicin-c3	-5.95	Glu570	1.908	Tyr511	2.174			41.24	30.15
Nonivamide2	-5.50	Tyr511	1.907	Glu570	2.051			93.49	24.51
4f-Caps6	-5.18	Glu570	1.918	Tyr511	2.016			160.88	26.52
6-Shogaol1	-5.05							198.66	26.96
Nonivamide-c1	-4.84	Glu570	1.896					281.14	29.05
6-Shogaol-c3	-4.76							324.67	28.41
6-Gingerol1	-4.48	Thr550	1.853	Glu570	2.013			516.09	28.00
6-Gingerol-c2	-4.27	Thr550	2.138					744.22	27.02
3b-Caps6	-4.06	Glu570	1.856	Tyr511	2.128			1.05	25.15

Table 5.5: Comparison of each docked molecule. ΔE : free binding energy (kcal/mol), Aa: active amino acids, Bond L: length of H-bond in Ångstrom, Ki(μ M) and RMSD values.

Name	ΔE	Aa	Bond L.	Aa	Bond L.	Ki	RMSD
Resiniferatoxin-c1	-6.96	Thr550	2.199			7.94	26.55
Capsazepine1b	-6.53	Glu570	2.156	Leu553	1.899	16.32	22.75
Sb-366791-1	-6.40	Thr550	1.842			20.42	29.84
Dihydrocapsaicin2	-6.32	Glu570	1.841			23.11	25.51
Nordihydrocapsaicin-c2	-6.21	Tyr511	2.002			28.22	29.14
Nonivamide2	-5.50	Glu570	2.051	Tyr511	1.907	93.49	24.51
4f-Caps6	-5.18	Tyr511	2.016	Glu570	1.918	160.88	26.52
6-Gingerol1	-4.48	Glu570	2.013	Thr550	1.853	516.09	28.00
6-Shogaol-c1	-4.08	Thr550	1.919	Glu570	1.057	1.02	27.03

5.4. Amino Acid Activity in the Pocket

The ultimate aim is to calculate the binding energies of the generated agonists and antagonists to TRPV1 and to find the amino acids in its active pocket, in which the agonist and antagonists prefer to bind and relate these preferences to the structural motifs on each binding molecule.

Table 5.6 shows the amino acids responsible for binding in TRPV1 S4-S5 domains. As in the case of capsaicin, Tyr511 and Thr550 are responsible while Glu570 is assisting them since the H-bonding distances are the highest between this amino acid and the ligands.

Table 5.6: Smallest binding energy agonist and antagonist molecules with amino acids they are bonded in the active site of TRPV1 protein. Amino acids are determined with all docking results taken into consideration.

Ligand/Amino Acid	Tyr511	Phe543	Thr550	Leu553	Tyr565	Glu570	Leu574	Asp576
Resiniferatoxin			+					
Capsazepine				+		+		
Sb-366791			+					
Dihydrocapsaicin						+		
Nordihydrocapsaicin	+							
Nonivamide	+					+		
Capsaicin	+		+			+		
6-Gingerol			+			+		
6-Shogaol			+			+		

A previous Capsaicin-TRPV1 docking study using AutoDock with subsequent molecular dynamics (MD) simulations [40] has found that the amide group in capsaicin binds to Tyr511 and Thr550 groups in the pocket, creating the most stable binding. In our work, 4f-caps6 binds to Tyr511 and Glu570. The conformer with the lowest inhibition constant, 7hs-caps4 forms an H-bond to Tyr511, Thr550, and Glu570. Binding poses are shown in Figures 6.1 and 6.2. Based on the bond distances (1.926, 1.847, and 1.988 Å, respectively) the interaction with Glu570 is very weak in 7hs-caps4 however it binds through its amide moiety (Region B). This is consistent with previous work and validates our ability to perform the analog fits.

5.5. Position in the Pocket

The bound capsaicin adopts ‘tail-up, head-down’ configurations where the vanillyl and amide groups form specific interactions that anchor the agonist to the receptor as seen in Figure 5.1 and 5.2. Moreover, the channel’s active state is stabilized by ‘pull-and-contact’ interactions between the capsaicin head region and S4-S5 domains of the receptor [2][13][28].

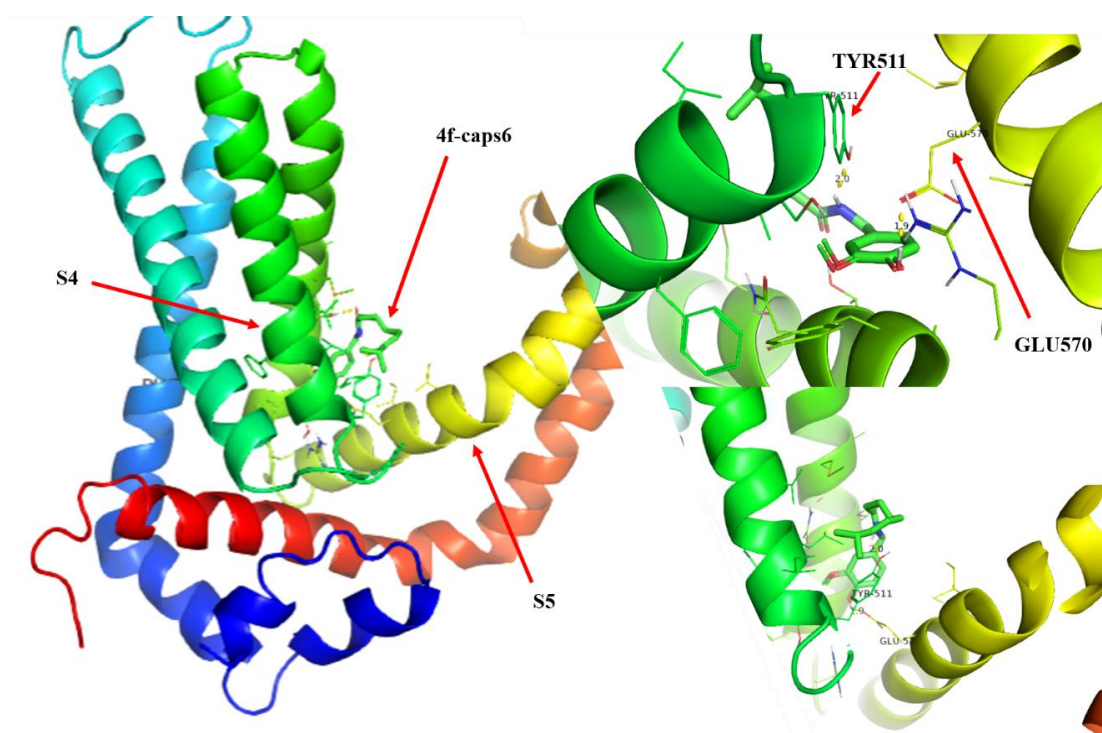


Figure 5.1: Capsaicin docked in TRPV1 S4-S5 domains. TYR511 and GLU570 are the hydrogen-bonded amino acids in this docking.

Capsazepine tends to make bonds with its -OH groups at the aromatic ring of vanillyl part. In both highest binding energy (Caz1b2) and lowest K_i (Caz1bbb2+10) makes bonds with -OH groups. This results in a similar docking position with capsaicin, regarding the aromatic ring with -OH groups as the head (tail up-head down).

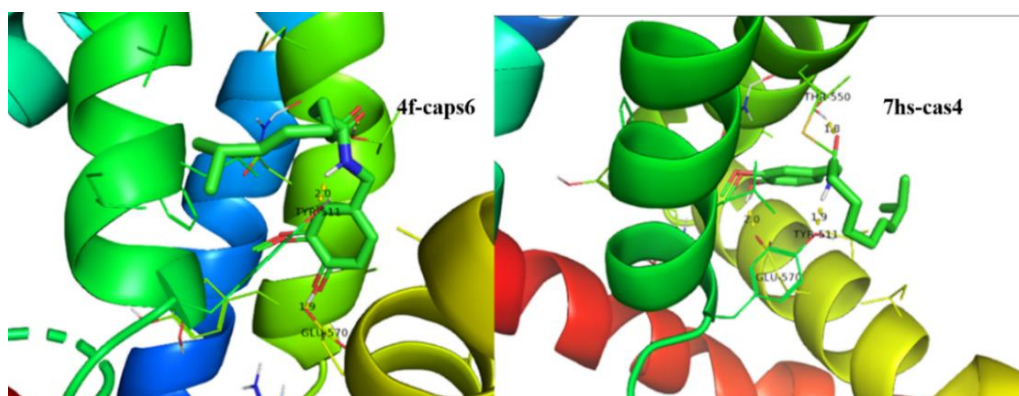


Figure 5.2: 4f-caps6 conformation has the highest binding energy while 7hs-cas4 has the lowest K_i value.

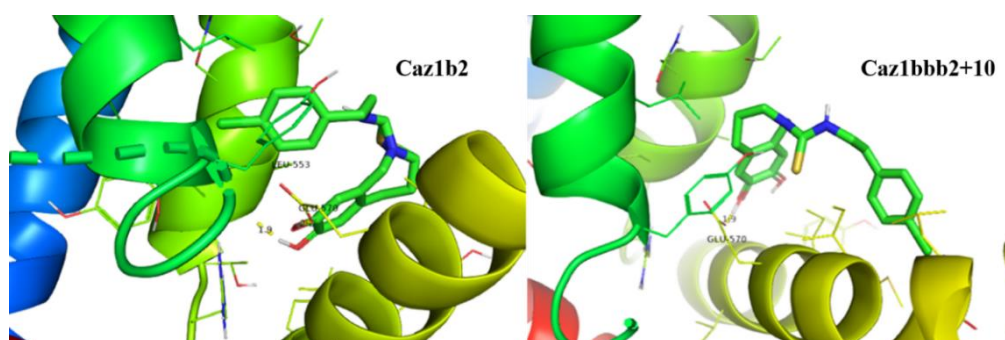


Figure 5.3: Caz1b2 conformation has the highest binding energy while Caz1bbb2+10 has the lowest K_i value.

Resiniferatoxin-c1 with lowest binding energy with low K_i value that has curved geometry is chosen to compare with 6-Gingerol that has low binding energy, high K_i value, and straight geometry. Both analog molecules are shown docking pose in Figure 5.4. Both of them bonds with Thr550 but more importantly they both positioned like capsaicin tail up-head down.

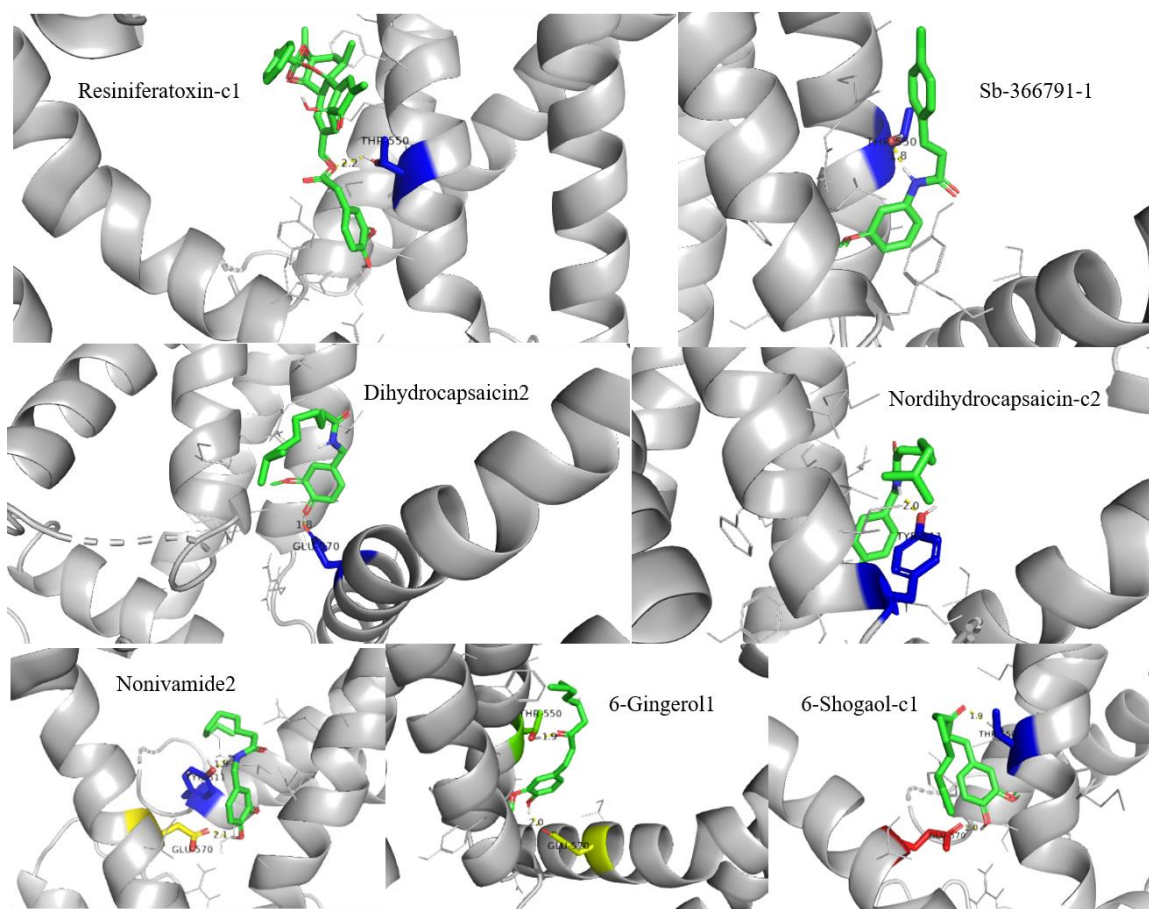


Figure 5.4: Docking results for best ΔE of analog molecules.

5.6. Effect of Side Chain

Regarding the AutoDock docking results; the lowest K_i values are achieved when region C is away from the binding amino acids, i.e. the hydrocarbon tail may *inhibit* binding but the best binding is in the *inhibiting* pose which allows more interactions between the amide (Region B) and the S4 domain amino acids.

Proximity to the S4 domain causes the inhibition effect but in the absence of the whole of the tetrameric structure of the TRPV1 protein, this behavior is normal because 75% of the binding area is missing. The hydrophobic tails would perhaps find a larger volume to occupy without having to side by the binding moieties for electrostatic stabilization if the tetramer was used. Glu570 has a stabilizing role in binding, if not directly. This may be the artifact of rigid docking rather than flexible docking.

Since all binding happens around the vanillyl group the position of the amide group matters due to the rigidity of the vanillyl ring. All molecules but RTX have vanillyl-CH₂-NH-C(=O)- linkage while RTX has a vanillyl-CH₂-C(=O)-O- motif. In the latter, the distance between the vanillyl and the ester carbonyl is shorter by 2 bonds. It is the shortness of this distance that causes the binding energy to be the best in RTX, in addition to the rigidity and dispersed hydrophilicity in the tail part (See Table 1.1). The amide (ester in the case of RTX) group is the preferred binding site and when it is absent or farther from the vanillyl group, binding weakens. Referring to Table 6.1 binding energy ordering from best to worst, the active moiety designations are (starting with RTX-c1) Vanillyl-CH₂-C(=O)-, Vanillyl-NH-C(=O)-, Vanillyl-CH₂-NH-C(=O)- (same for dihydrocapsaicin2, nordihydrocapsaicin-c2, and nonivamide2 and all capsaicin), Vanillyl-CH₂-CH₂-C(=O)-, and Vanillyl-CH₂-CH₂-C(=O)-CH(OH)-.

Table 5.7: Agonist and antagonist molecules. ΔE : free binding energy (kcal/mol), Aa: active amino acids, Bond L: length of H-bond in Ångstrom, Ki(μM), RMSD values. and pungency (Scoville Heat Unit).

Name	ΔE	Aa	Bond L.	Aa	Bond L.	Ki	RMSD	SHU
Resiniferatoxin-c1	-6.96	Thr550	2.199			7.94	26.55	16000000000
Sb-366791-1	-6.40	Thr550	1.842			20.42	29.84	X
Dihydrocapsaicin2	-6.32	Glu570	1.841			23.11	25.51	15000000
Nordihydrocapsaicin-c2	-6.21	Tyr511	2.002			28.22	29.14	9100000
Nonivamide2	-5.50	Tyr511	1.907	Glu570	2.051	93.49	24.51	9200000
6-Gingerol1	-4.48	Thr550	1.853	Glu570	2.013	516.09	28.00	60000
6-Shogaol-c1	-4.08	Thr550	1.919	Glu570	1.957	1.02	27.03	16000

Table 5.7 also shows a correlation between the free binding energies and the potencies (SHU) of the analogs which means that they also bind to the TRPV1 pocket capsaicin is binding to. This result is not surprising for the capsaicin analogs but the result with RTX is rather significant due to its structural complexity. The correlation of the absolute magnitudes of binding energies with SHU is 0.65 (value for Sb-366791-1 is excluded). The correlation of the absolute magnitudes of binding energies with Ki is -0.89. This indicates that RTX, regardless of its bulk and rigidity, has been designed by nature as the least inhibitory fit to the S4-S5 domains.

6. CONCLUSION AND FUTURE WORK

In the case of capsaicin docking, Tyr511 and Thr550 are the most active amino acids in binding while Glu570 is assisting them since the H-bonding distances are the highest between this amino acid and the ligands. Active TRPV1 amino acids for the analog molecules are the same set as in capsaicin binding. Glu570 is also observed making H-Bonds with Capsazepine, Dihydrocapsaicin, Nonivamide, 6-Gingerol, and 6-Shogaol. These bonds can also consider and assist bonds since they give high H-Bond distance. In light of these observations we can say; Glu570 has a stabilizing role in binding, if not directly. This may be the artifact of rigid docking rather than flexible docking. Each analog molecule docking set shows the best binding confirmation of molecule also have the lowest K_i in the set.

RTX is bulky and rigid but it shows the least inhibitory fit to the S4-S5. Also binding results agree with experiments on RTX pungency. RTX having the highest SHU give the best free binding energy while 6-Shogaol having the lowest SHU in the Table 5.7 gave a weaker free binding energy. This shows us a correlation among pungency and free binding energy when the SHU results are significantly different. Among the amide functional group analogs; the ones with no double bond like 6-Gingerol at the end of the tail bind better than those with double bonds at the tail like 6-Shogaol due to flexibility. Position of the amide, ketone or ester neck groups matter in binding, It has to be closer to the vanillyl ring for best binding energy. “Tail up-head down” position while binding to TRPV1 pocket are observed in all the analog molecules as seen in Figure 5.4. this positioning shows analog molecules follow capsaicin and capsazepine positioning in the pocket and make the binding in a similar position.

In the current study, we kept the protein rigid and in neutral medium. Taking into consideration that all amino acids interact with each other in nature and TRPV1 protein channel activates with acidic medium, both flexibility and pH are important components of TRPV1 interactions. Therefore, future work will include the flexible docking in the S4-S5 domains, rigid docking in the entire pocket, and MD simulations of the entire ensemble and the analogs listed, with and without the pH effects. Whole tetrameric structure of the

TRPV1 protein will be included in docking calculations since its absence may cause inhibition effect of the S4 domain.

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APPENDIX A: SOFTWARES USED

A.1. Autodock4 is Free Software

Autodock is a search algorithm for molecular docking. It is distributed free of charge to academics and other non-profit research institutes. LGA performs to find the best fitting docking positions with the lowest binding energy. Autodock4 also has flexible docking option that includes the flexible protein side chains to its calculations which makes the results closer to observations made in nature. Calculations in autodock4 include direct hydrogen bonding, non- bonded atom interactions of vdW and ligand's loss of torsional entropy after its binding [36].

In this study, Autodock4.2.6 is used for docking.

A.2. Open Babel is Free Software

Open Babel is a command line program that converts molecule files from one format to another. It is distributed free of charge and available cross-platform[51]. In this study, Open Babel is used to converting log files to PDBQT files and PDBQT files to use files.

The input file type is chosen from the drop-down list. “...” button is clicked to select the file that is to be converted. Output files type is chosen from the dropdown list and “...” button is clicked to set the directory and set the output file name. Click on the “Convert” button makes the conversion and output file content is displayed below the output file name. Simultaneously the output file downloads to the opted directory.

A.3. PyMol is Free Software

PyMol is an open source molecular visualization system that produces high-quality 3D images of molecules. PyMol is useful to visualize small molecules and protein

structures to use in the scientific literature using Python script language. It is widely used in academic communities. “Py” refers to its programming language Python [33].

Analysis of Autodock output file; docking log file (DLG) also contains macromolecule file PDBQT. Best conformation of ligand and protein session is saved as another PDBQT file. This file is converted to use file format in Open Babel Opening this file in PyMol shows the best conformation results in the high-quality 3D form in the software[47]. After setting the macromolecule and showing the bond made between ligand and amino acids, images are taken to be used in showing the best conformation results as images.

A.4. Why Use Grid Box?

AutoGrid computes different grid maps for different atom types which are later used by Autodock to find the interaction between ligand and protein. Determining a specific area for autogrid to compute maps saves time in calculations and makes docking process to focus a limited area instead of the entirety of protein [36].

In this work, ligand interaction with a specific pocket in the protein is checked. The active site of protein is to interact with the ligand and other than immediate surrounding of the pocket is does not affect how the pocket behaves towards the ligand. Including the whole protein to calculation, even though its impact on the ligand is omittable, is time-consuming and expensive. Using a grid box helps to define the area for AutoGrid to compute maps and AutoDock to focus on the area for the ligand to fit the box in different positions [36].

A.5. Setting up the Autogrid Box

TRPV1 pocket in the S4-S5 domain is determined by the previous works TYR511, LEU515, PHE543, MET547, THR550, GLU570 amino acids in the domains are expected to make interaction with the ligand. The aim is to set a grid box to include all these amino acids. To make this happen, different sizes in the grid box is tested. Best option included; spacing set to 0,375 Ångstrom with 60 number of points in x, y and z dimensions while the

center grid box is at -0,944 in z center. This setting is used for all docking processes to have a fixed area to make all the dockings in similar conditions [40][51].

A.6. AutoDock4 File Formats

Autodock4 requires the macromolecule to be in PDB file format and ligand to be in PDBQT file format. AutoGrid and AutoDock require macromolecule file in PDBQT format. Macromolecule file is converted to PDBQT file format with ADT. Q in the PDBQT file represents the partial charges and T stands for atom types. Autodock4 has 22 atom types. This includes special keywords to determine if any atoms to be flexible in the docking. AutoGrid Parameter file (GPF) is prepared by AutoGrid to generate Grid Log File GLG by running Autogrid4. AutoDock Parameter File (DPF) is prepared by AutoDock to generate Docking Log File (DLG) by running AutoDock4 [36].

A.7. Preparing Ligands and Macromolecules with AutoDock4

Ligand preparation includes; detecting torsion tree root, choosing torsions, adding all hydrogens, computing Gasteiger charges and merging non-polar hydrogens.

Macromolecule preparation includes; deleting water, adding all hydrogens, merging non-polar hydrogens, computing Kollman charges. Choosing the macromolecule for Autogrid converts PDB file to PDBQT file [36].

APPENDIX B: XYZ STRUCTURES

B.1. Lowest binding energy xyz structure from DLG file

- Capsaicin (4f-caps6)

USER				x	y	z	vdW	Elec	q	RMS	
ATOM	1	C	UNL	1	-23.382	4.738	-17.910	-0.39	+0.01	+0.057	26.519
ATOM	2	O	UNL	1	-23.139	4.141	-18.941	-0.68	-0.19	-0.472	26.519
ATOM	3	N	UNL	1	-22.951	4.307	-16.701	-0.21	+0.01	-0.287	26.519
ATOM	4	H	UNL	1	-23.345	4.728	-15.880	-0.34	-0.11	+0.318	26.519
ATOM	5	C	UNL	1	-22.321	3.019	-16.539	-0.21	+0.00	-0.279	26.519
ATOM	6	C	UNL	1	-22.801	2.316	-15.302	-0.20	-0.10	+0.394	26.519
ATOM	7	C	UNL	1	-21.900	1.642	-14.494	-0.36	-0.03	+0.093	26.519
ATOM	8	C	UNL	1	-22.336	0.931	-13.389	-0.25	-0.14	+0.219	26.519
ATOM	9	C	UNL	1	-23.680	0.889	-13.079	-0.08	+0.30	-0.415	26.519
ATOM	10	C	UNL	1	-24.590	1.575	-13.881	-0.51	-0.07	+0.170	26.519
ATOM	11	C	UNL	1	-24.153	2.283	-14.983	-0.41	-0.07	+0.224	26.519
ATOM	12	O	UNL	1	-24.111	0.197	-12.009	-0.16	+0.36	-0.527	26.519
ATOM	13	H	UNL	1	-24.033	0.759	-11.236	-0.22	-0.69	+0.395	26.519
ATOM	14	O	UNL	1	-25.882	1.461	-13.478	-0.41	+0.06	-0.405	26.519
ATOM	15	C	UNL	1	-26.367	2.392	-12.549	-0.45	-0.04	+0.363	26.519
ATOM	16	C	UNL	1	-24.147	6.042	-17.915	-0.29	+0.02	+0.161	26.519
ATOM	17	C	UNL	1	-23.297	7.298	-17.961	-0.26	-0.00	-0.022	26.519
ATOM	18	C	UNL	1	-22.406	7.465	-16.739	-0.24	+0.00	+0.020	26.519
ATOM	19	C	UNL	1	-21.978	8.914	-16.524	-0.18	-0.03	-0.151	26.519
ATOM	20	C	UNL	1	-23.067	9.730	-15.913	-0.15	+0.06	+0.262	26.519
ATOM	21	C	UNL	1	-24.062	9.259	-15.170	-0.29	-0.04	-0.184	26.519
ATOM	22	C	UNL	1	-25.139	10.091	-14.552	-0.30	+0.05	+0.227	26.519
ATOM	23	C	UNL	1	-24.868	10.308	-13.071	-0.40	-0.03	-0.095	26.519
ATOM	24	C	UNL	1	-26.506	9.454	-14.759	-0.49	-0.01	-0.065	26.519

- Capsazepine (capsazepine1b2)

USER					x	y	z	vdW	Elec	q	RMS
ATOM	1	C	UNL	1	-18.965	-0.662	-16.443	-0.09	+0.01	+0.115	22.746
ATOM	2	N	UNL	1	-18.247	-1.210	-15.328	-0.06	-0.03	-0.304	22.746
ATOM	3	C	UNL	1	-18.627	-0.757	-13.957	-0.10	+0.01	+0.249	22.746
ATOM	4	C	UNL	1	-19.371	-1.807	-13.081	-0.26	-0.00	-0.068	22.746
ATOM	5	C	UNL	1	-20.377	-1.303	-12.217	-0.27	-0.01	+0.038	22.746
ATOM	6	C	UNL	1	-21.087	-2.162	-11.358	-0.25	-0.00	+0.058	22.746
ATOM	7	C	UNL	1	-20.784	-3.562	-11.369	-0.36	-0.00	-0.017	22.746
ATOM	8	C	UNL	1	-19.790	-4.063	-12.225	-0.36	-0.00	-0.024	22.746
ATOM	9	C	UNL	1	-19.069	-3.195	-13.089	-0.26	-0.01	-0.059	22.746
ATOM	10	C	UNL	1	-18.014	-3.844	-14.015	-0.20	+0.01	+0.079	22.746
ATOM	11	C	UNL	1	-16.750	-2.969	-14.297	-0.22	+0.00	+0.024	22.746
ATOM	12	C	UNL	1	-16.975	-1.981	-15.484	-0.08	+0.02	+0.157	22.746

ATOM	13	S	UNL	1	-18.759	-2.269	-16.205	-0.09	-0.03	-0.253	22.746
ATOM	14	N	UNL	1	-20.125	0.113	-15.985	-0.11	-0.02	-0.285	22.746
ATOM	15	H	UNL	1	-21.011	-0.242	-16.431	+0.05	+0.01	+0.183	22.746
ATOM	16	C	UNL	1	-20.021	1.602	-16.206	-0.18	+0.01	+0.131	22.746
ATOM	17	C	UNL	1	-20.463	2.382	-14.918	-0.27	-0.01	+0.095	22.746
ATOM	18	C	UNL	1	-21.936	2.103	-14.564	-0.37	+0.02	-0.075	22.746
ATOM	19	C	UNL	1	-22.985	2.768	-15.243	-0.46	-0.00	+0.013	22.746
ATOM	20	C	UNL	1	-24.333	2.537	-14.906	-0.53	-0.01	+0.029	22.746
ATOM	21	C	UNL	1	-24.628	1.627	-13.870	-0.64	+0.01	-0.038	22.746
ATOM	22	CL	UNL	1	-26.357	1.319	-13.427	-0.88	+0.00	-0.070	22.746
ATOM	23	C	UNL	1	-23.604	0.949	-13.174	-0.46	-0.02	+0.034	22.746
ATOM	24	C	UNL	1	-22.264	1.194	-13.528	-0.41	-0.02	+0.038	22.746
ATOM	25	O	UNL	1	-22.093	-1.695	-10.484	-0.44	+0.06	-0.249	22.746
ATOM	26	H	UNL	1	-21.827	-0.701	-10.415	-0.32	-0.31	+0.246	22.746
ATOM	27	O	UNL	1	-21.581	-4.298	-10.451	-0.09	-0.06	-0.279	22.746
ATOM	28	H	UNL	1	-22.483	-3.818	-10.454	-0.23	-0.03	+0.233	22.746

- Resiniferatoxin (resiniferatoxin-c1)

USER					x	y	z	vdW	Elec	q	RMS
ATOM	1	C	UNL	1	-23.764	5.451	-25.256	-0.34	+0.00	+0.010	26.555
ATOM	2	C	UNL	1	-24.111	5.939	-23.847	-0.51	+0.00	+0.023	26.555
ATOM	3	C	UNL	1	-24.857	7.315	-23.958	-0.50	+0.01	+0.048	26.555
ATOM	4	C	UNL	1	-24.025	8.494	-23.427	-0.31	+0.02	+0.123	26.555
ATOM	5	O	UNL	1	-22.820	8.474	-24.217	-0.13	-0.04	-0.313	26.555
ATOM	6	C	UNL	1	-21.811	8.071	-23.305	-0.06	+0.04	+0.291	26.555
ATOM	7	O	UNL	1	-22.149	8.581	-22.022	-0.04	-0.05	-0.319	26.555
ATOM	8	O	UNL	1	-21.784	6.646	-23.284	-0.05	-0.05	-0.318	26.555
ATOM	9	C	UNL	1	-23.538	8.251	-21.966	-0.13	+0.03	+0.170	26.555
ATOM	10	C	UNL	1	-23.621	6.757	-21.565	-0.19	+0.01	+0.077	26.555
ATOM	11	C	UNL	1	-23.409	6.559	-20.073	-0.18	-0.00	-0.015	26.555
ATOM	12	C	UNL	1	-23.043	5.480	-19.355	-0.30	-0.01	-0.033	26.555
ATOM	13	C	UNL	1	-22.475	4.193	-19.895	-0.25	+0.02	+0.077	26.555
ATOM	14	C	UNL	1	-21.597	4.344	-21.132	-0.15	+0.02	+0.137	26.555
ATOM	15	C	UNL	1	-22.377	4.616	-22.416	-0.26	+0.01	+0.052	26.555
ATOM	16	C	UNL	1	-21.926	3.641	-23.400	-0.16	+0.00	+0.021	26.555
ATOM	17	C	UNL	1	-21.100	2.624	-22.830	-0.19	+0.01	+0.045	26.555
ATOM	18	C	UNL	1	-20.449	1.506	-23.531	-0.13	+0.00	+0.020	26.555
ATOM	19	C	UNL	1	-20.887	2.998	-21.424	-0.08	+0.03	+0.173	26.555
ATOM	20	O	UNL	1	-20.208	2.350	-20.643	-0.07	-0.06	-0.294	26.555
ATOM	21	C	UNL	1	-22.939	5.974	-22.755	-0.25	+0.02	+0.096	26.555
ATOM	22	C	UNL	1	-23.302	5.464	-17.860	-0.26	+0.04	+0.263	26.555
ATOM	23	O	UNL	1	-23.155	4.137	-17.356	-0.28	-0.07	-0.460	26.555
ATOM	24	C	UNL	1	-21.954	3.907	-16.755	-0.18	+0.01	+0.312	26.555
ATOM	25	O	UNL	1	-21.204	4.781	-16.332	-0.14	-0.02	-0.250	26.555
ATOM	26	C	UNL	1	-21.684	2.437	-16.557	-0.23	+0.00	+0.155	26.555
ATOM	27	C	UNL	1	-22.322	1.810	-15.340	-0.39	+0.01	-0.033	26.555
ATOM	28	C	UNL	1	-23.700	1.971	-15.110	-0.51	-0.02	+0.053	26.555

ATOM	29	C	UNL	1	-24.296	1.439	-13.967	-0.50	-0.07	+0.162	26.555
ATOM	30	C	UNL	1	-23.498	0.736	-13.057	-0.29	-0.11	+0.158	26.555
ATOM	31	C	UNL	1	-22.134	0.573	-13.262	-0.29	-0.03	+0.049	26.555
ATOM	32	C	UNL	1	-21.543	1.117	-14.401	-0.37	-0.00	+0.008	26.555
ATOM	33	O	UNL	1	-25.621	1.531	-13.627	-0.31	+0.10	-0.491	26.555
ATOM	34	C	UNL	1	-25.932	2.330	-12.491	-0.49	-0.07	+0.277	26.555
ATOM	35	O	UNL	1	-24.052	0.198	-11.927	-0.17	+0.37	-0.503	26.555
ATOM	36	H	UNL	1	-24.507	0.945	-11.493	-0.33	-0.37	+0.292	26.555
ATOM	37	O	UNL	1	-20.577	5.321	-20.949	-0.01	-0.06	-0.380	26.555
ATOM	38	H	UNL	1	-20.847	6.115	-21.445	+0.03	+0.03	+0.211	26.555
ATOM	39	C	UNL	1	-24.746	9.831	-23.636	-0.36	-0.01	-0.046	26.555
ATOM	40	C	UNL	1	-24.878	10.342	-25.048	-0.32	+0.01	+0.041	26.555
ATOM	41	C	UNL	1	-25.242	10.573	-22.628	-0.26	+0.00	+0.010	26.555
ATOM	42	C	UNL	1	-20.407	8.528	-23.741	-0.04	+0.02	+0.133	26.555
ATOM	43	C	UNL	1	-19.294	7.508	-23.636	-0.02	-0.01	-0.040	26.555
ATOM	44	C	UNL	1	-19.070	6.603	-24.685	-0.02	+0.00	+0.004	26.555
ATOM	45	C	UNL	1	-18.060	5.644	-24.596	-0.01	+0.00	+0.000	26.555
ATOM	46	C	UNL	1	-17.272	5.569	-23.450	-0.00	+0.00	+0.000	26.555
ATOM	47	C	UNL	1	-17.492	6.454	-22.396	-0.00	+0.00	+0.000	26.555
ATOM	48	C	UNL	1	-18.495	7.423	-22.489	-0.00	+0.00	+0.004	26.555

- Sb-366791 (sb-366791-1)

USER					x	y	z	vdW	Elec	q	RMS
ATOM	1	N	UNL	1	-20.296	-1.478	-17.547	-0.12	-0.05	-0.282	29.410
ATOM	2	H	UNL	1	-19.451	-1.431	-18.106	+0.03	+0.02	+0.155	29.410
ATOM	3	C	UNL	1	-20.262	-0.494	-16.593	-0.16	+0.03	+0.241	29.410
ATOM	4	O	UNL	1	-19.272	0.235	-16.525	-0.07	-0.03	-0.270	29.410
ATOM	5	C	UNL	1	-21.419	-0.187	-15.711	-0.20	-0.00	+0.068	29.410
ATOM	6	C	UNL	1	-21.915	1.052	-15.589	-0.37	-0.00	+0.013	29.410
ATOM	7	C	UNL	1	-22.964	1.463	-14.644	-0.47	+0.01	-0.025	29.410
ATOM	8	C	UNL	1	-23.733	2.617	-14.886	-0.55	-0.00	+0.009	29.410
ATOM	9	C	UNL	1	-24.935	2.821	-14.205	-0.33	-0.01	+0.020	29.410
ATOM	10	C	UNL	1	-25.346	1.912	-13.244	-0.67	-0.01	+0.041	29.410
ATOM	11	CL	UNL	1	-26.885	2.086	-12.493	-0.98	-0.00	-0.084	29.410
ATOM	12	C	UNL	1	-24.538	0.825	-12.918	-0.58	-0.01	+0.020	29.410
ATOM	13	C	UNL	1	-23.347	0.599	-13.617	-0.35	-0.00	+0.009	29.410
ATOM	14	C	UNL	1	-21.137	-2.573	-17.846	-0.30	+0.01	+0.037	29.410
ATOM	15	C	UNL	1	-21.238	-2.980	-19.184	-0.29	+0.00	+0.023	29.410
ATOM	16	C	UNL	1	-21.943	-4.141	-19.538	-0.34	+0.00	+0.005	29.410
ATOM	17	C	UNL	1	-22.498	-4.953	-18.551	-0.37	+0.00	+0.046	29.410
ATOM	18	C	UNL	1	-22.385	-4.564	-17.227	-0.39	+0.01	+0.122	29.410
ATOM	19	C	UNL	1	-21.807	-3.337	-16.905	-0.36	+0.01	+0.069	29.410
ATOM	20	O	UNL	1	-22.839	-5.414	-16.252	-0.18	-0.07	-0.495	29.410
ATOM	21	C	UNL	1	-23.367	-6.616	-16.831	-0.22	+0.05	+0.277	29.410

- Dihydrocapsaicin (dihydrocapsaicin2)

USER					x	y	z	vdW	Elec	q	RMS
ATOM	1	N	UNL	1	-23.207	4.322	-16.655	-0.20	+0.01	-0.311	25.506
ATOM	2	H	UNL	1	-23.837	4.614	-15.919	-0.33	-0.04	+0.149	25.506
ATOM	3	C	UNL	1	-23.386	4.855	-17.915	-0.30	+0.04	+0.213	25.506
ATOM	4	O	UNL	1	-22.947	4.325	-18.930	-0.79	-0.09	-0.276	25.506
ATOM	5	C	UNL	1	-22.557	3.036	-16.523	-0.27	-0.00	+0.139	25.506
ATOM	6	C	UNL	1	-23.027	2.226	-15.337	-0.47	+0.01	-0.027	25.506
ATOM	7	C	UNL	1	-22.063	1.674	-14.475	-0.42	-0.00	+0.009	25.506
ATOM	8	C	UNL	1	-22.445	0.987	-13.324	-0.38	-0.03	+0.049	25.506
ATOM	9	C	UNL	1	-23.794	0.849	-13.034	-0.33	-0.11	+0.158	25.506
ATOM	10	C	UNL	1	-24.781	1.383	-13.871	-0.54	-0.05	+0.162	25.506
ATOM	11	C	UNL	1	-24.391	2.090	-15.010	-0.57	-0.02	+0.054	25.506
ATOM	12	O	UNL	1	-24.144	0.177	-11.895	-0.16	+0.35	-0.503	25.506
ATOM	13	H	UNL	1	-23.834	0.745	-11.162	-0.30	-0.60	+0.292	25.506
ATOM	14	O	UNL	1	-26.070	1.165	-13.455	-0.34	+0.03	-0.491	25.506
ATOM	15	C	UNL	1	-26.668	2.200	-12.684	-0.56	-0.01	+0.277	25.506
ATOM	16	C	UNL	1	-24.137	6.175	-17.915	-0.33	+0.01	+0.096	25.506
ATOM	17	C	UNL	1	-23.569	7.137	-16.872	-0.24	+0.00	+0.009	25.506
ATOM	18	C	UNL	1	-23.792	8.604	-17.245	-0.27	+0.00	+0.000	25.506
ATOM	19	C	UNL	1	-24.709	9.281	-16.224	-0.35	+0.00	+0.000	25.506
ATOM	20	C	UNL	1	-23.950	9.728	-14.973	-0.34	+0.00	+0.000	25.506
ATOM	21	C	UNL	1	-24.856	9.657	-13.738	-0.49	+0.00	+0.003	25.506
ATOM	22	C	UNL	1	-24.877	10.954	-12.908	-0.39	-0.01	-0.017	25.506
ATOM	23	C	UNL	1	-23.781	11.901	-13.402	-0.23	+0.00	+0.007	25.506
ATOM	24	C	UNL	1	-24.692	10.690	-11.413	-0.51	+0.00	+0.007	25.506

- Nordihydrocapsaicin (nordihydrocapsaicin-c2)

USER					x	y	z	vdW	Elec	q	RMS
ATOM	1	N	UNL	1	-23.231	4.242	-16.703	-0.21	+0.01	-0.311	29.144
ATOM	2	C	UNL	1	-23.518	4.862	-17.892	-0.31	+0.04	+0.213	29.144
ATOM	3	O	UNL	1	-23.284	4.351	-18.982	-0.82	-0.10	-0.276	29.144
ATOM	4	H	UNL	1	-23.407	4.708	-15.829	-0.45	-0.06	+0.149	29.144
ATOM	5	C	UNL	1	-22.586	2.951	-16.628	-0.16	-0.00	+0.139	29.144
ATOM	6	C	UNL	1	-23.020	2.192	-15.397	-0.47	+0.01	-0.027	29.144
ATOM	7	C	UNL	1	-24.383	2.066	-15.066	-0.56	-0.02	+0.054	29.144
ATOM	8	C	UNL	1	-24.768	1.402	-13.899	-0.53	-0.05	+0.162	29.144
ATOM	9	C	UNL	1	-23.778	0.857	-13.074	-0.36	-0.11	+0.158	29.144
ATOM	10	C	UNL	1	-22.430	0.960	-13.388	-0.39	-0.03	+0.049	29.144
ATOM	11	C	UNL	1	-22.051	1.630	-14.550	-0.42	-0.00	+0.009	29.144
ATOM	12	O	UNL	1	-26.051	1.223	-13.449	-0.33	+0.04	-0.491	29.144
ATOM	13	C	UNL	1	-26.502	2.103	-12.426	-0.52	-0.01	+0.277	29.144
ATOM	14	O	UNL	1	-24.124	0.210	-11.918	-0.17	+0.36	-0.503	29.144
ATOM	15	H	UNL	1	-24.516	0.901	-11.351	-0.30	-0.36	+0.292	29.144
ATOM	16	C	UNL	1	-24.071	6.278	-17.787	-0.30	+0.01	+0.096	29.144

ATOM	17	C	UNL	1	-23.043	7.360	-17.447	-0.28	+0.00	+0.009	29.144
ATOM	18	C	UNL	1	-23.564	8.394	-16.437	-0.32	+0.00	+0.000	29.144
ATOM	19	C	UNL	1	-24.706	9.261	-16.990	-0.30	+0.00	+0.000	29.144
ATOM	20	C	UNL	1	-25.073	10.410	-16.041	-0.27	+0.00	+0.003	29.144
ATOM	21	C	UNL	1	-25.816	9.960	-14.772	-0.47	-0.00	-0.017	29.144
ATOM	22	C	UNL	1	-24.804	9.543	-13.701	-0.47	+0.00	+0.007	29.144
ATOM	23	C	UNL	1	-26.805	8.823	-15.024	-0.48	+0.00	+0.007	29.144

- Nonivamide (nonivamide2)

USER					x	y	z	vdW	Elec	q	RMS
ATOM	1	N	UNL	1	-22.516	4.879	-16.828	-0.15	-0.01	-0.311	24.510
ATOM	2	C	UNL	1	-23.255	4.860	-17.991	-0.29	+0.04	+0.213	24.510
ATOM	3	O	UNL	1	-23.161	3.952	-18.811	-0.71	-0.14	-0.276	24.510
ATOM	4	H	UNL	1	-22.749	5.535	-16.094	-0.28	-0.01	+0.149	24.510
ATOM	5	C	UNL	1	-21.695	3.734	-16.512	-0.26	+0.00	+0.139	24.510
ATOM	6	C	UNL	1	-22.343	2.810	-15.518	-0.39	+0.01	-0.027	24.510
ATOM	7	C	UNL	1	-23.741	2.809	-15.351	-0.50	-0.02	+0.054	24.510
ATOM	8	C	UNL	1	-24.336	2.000	-14.382	-0.50	-0.06	+0.162	24.510
ATOM	9	C	UNL	1	-23.521	1.173	-13.600	-0.40	-0.09	+0.158	24.510
ATOM	10	C	UNL	1	-22.143	1.139	-13.768	-0.39	-0.02	+0.049	24.510
ATOM	11	C	UNL	1	-21.553	1.956	-14.731	-0.37	-0.00	+0.009	24.510
ATOM	12	O	UNL	1	-25.675	1.927	-14.096	-0.28	+0.11	-0.491	24.510
ATOM	13	C	UNL	1	-26.119	2.621	-12.937	-0.47	-0.06	+0.277	24.510
ATOM	14	O	UNL	1	-24.073	0.379	-12.632	-0.21	+0.29	-0.503	24.510
ATOM	15	H	UNL	1	-24.128	0.949	-11.841	-0.33	-0.43	+0.292	24.510
ATOM	16	C	UNL	1	-24.184	6.050	-18.167	-0.32	+0.01	+0.096	24.510
ATOM	17	C	UNL	1	-23.741	7.203	-17.264	-0.31	+0.00	+0.009	24.510
ATOM	18	C	UNL	1	-24.549	8.488	-17.451	-0.26	+0.00	+0.000	24.510
ATOM	19	C	UNL	1	-23.935	9.601	-16.596	-0.24	+0.00	+0.000	24.510
ATOM	20	C	UNL	1	-23.480	9.122	-15.217	-0.34	+0.00	+0.000	24.510
ATOM	21	C	UNL	1	-24.512	9.518	-14.156	-0.44	+0.00	+0.000	24.510
ATOM	22	C	UNL	1	-25.738	10.195	-14.766	-0.44	-0.00	-0.003	24.510
ATOM	23	C	UNL	1	-26.859	9.192	-14.997	-0.54	+0.00	+0.004	24.510

- 6-Gingerol (6-gingerol1)

USER					x	y	z	vdW	Elec	q	RMS
ATOM	1	C	UNL	1	-21.480	3.598	-17.758	-0.22	+0.01	+0.079	27.997
ATOM	2	C	UNL	1	-22.386	4.042	-16.605	-0.30	-0.00	+0.043	27.997
ATOM	3	C	UNL	1	-22.782	3.035	-15.549	-0.36	+0.01	-0.043	27.997
ATOM	4	C	UNL	1	-24.107	2.704	-15.221	-0.50	-0.02	+0.053	27.997
ATOM	5	C	UNL	1	-24.375	1.768	-14.214	-0.51	-0.06	+0.162	27.997
ATOM	6	C	UNL	1	-23.304	1.165	-13.541	-0.38	-0.10	+0.158	27.997
ATOM	7	C	UNL	1	-21.990	1.475	-13.846	-0.40	-0.02	+0.049	27.997
ATOM	8	C	UNL	1	-21.732	2.410	-14.846	-0.38	-0.00	+0.007	27.997

ATOM	9	O	UNL	1	-25.613	1.357	-13.790	-0.32	+0.08	-0.491	27.997
ATOM	10	C	UNL	1	-26.175	2.052	-12.685	-0.54	-0.04	+0.277	27.997
ATOM	11	O	UNL	1	-23.537	0.246	-12.554	-0.16	+0.35	-0.503	27.997
ATOM	12	H	UNL	1	-23.741	0.778	-11.760	-0.29	-0.49	+0.292	27.997
ATOM	13	C	UNL	1	-22.054	4.093	-19.081	-0.18	+0.04	+0.136	27.997
ATOM	14	O	UNL	1	-23.270	4.112	-19.274	-0.78	-0.12	-0.297	27.997
ATOM	15	C	UNL	1	-21.054	4.549	-20.140	-0.11	+0.02	+0.105	27.997
ATOM	16	C	UNL	1	-21.304	3.862	-21.493	-0.15	+0.02	+0.122	27.997
ATOM	17	O	UNL	1	-21.117	2.452	-21.355	+0.02	-0.08	-0.391	27.997
ATOM	18	H	UNL	1	-20.200	2.234	-21.587	+0.04	+0.04	+0.210	27.997
ATOM	19	C	UNL	1	-22.716	4.127	-22.049	-0.35	+0.01	+0.032	27.997
ATOM	20	C	UNL	1	-22.796	5.477	-22.784	-0.29	+0.00	+0.002	27.997
ATOM	21	C	UNL	1	-24.128	5.734	-23.496	-0.52	+0.00	+0.000	27.997
ATOM	22	C	UNL	1	-24.503	7.216	-23.398	-0.49	-0.00	-0.003	27.997
ATOM	23	C	UNL	1	-25.992	7.452	-23.590	-0.53	+0.00	+0.004	27.997

- 6-Shogaol (6-shogaol-c1)

USER					x	y	z	vdW	Elec	q	RMS
ATOM	1	C	UNL	1	-23.497	3.944	-16.456	-0.37	-0.00	+0.043	27.032
ATOM	2	C	UNL	1	-22.083	4.297	-16.913	-0.29	+0.00	+0.079	27.032
ATOM	3	C	UNL	1	-21.832	3.957	-18.363	-0.19	+0.03	+0.138	27.032
ATOM	4	O	UNL	1	-22.762	3.942	-19.177	-0.70	-0.12	-0.297	27.032
ATOM	5	C	UNL	1	-20.476	3.639	-18.870	-0.13	+0.01	+0.069	27.032
ATOM	6	C	UNL	1	-19.986	2.370	-19.338	-0.13	+0.00	+0.004	27.032
ATOM	7	C	UNL	1	-19.867	1.165	-18.453	-0.14	+0.00	+0.004	27.032
ATOM	8	C	UNL	1	-20.614	1.396	-17.132	-0.20	+0.00	+0.000	27.032
ATOM	9	C	UNL	1	-19.731	1.176	-15.898	-0.22	+0.00	+0.000	27.032
ATOM	10	C	UNL	1	-19.286	-0.283	-15.773	-0.19	-0.00	-0.003	27.032
ATOM	11	C	UNL	1	-19.740	-0.900	-14.460	-0.26	+0.00	+0.004	27.032
ATOM	12	C	UNL	1	-23.612	2.925	-15.339	-0.47	+0.01	-0.043	27.032
ATOM	13	C	UNL	1	-22.425	2.501	-14.715	-0.44	-0.00	+0.007	27.032
ATOM	14	C	UNL	1	-22.453	1.563	-13.684	-0.42	-0.03	+0.049	27.032
ATOM	15	C	UNL	1	-23.671	1.044	-13.273	-0.37	-0.11	+0.158	27.032
ATOM	16	C	UNL	1	-24.871	1.438	-13.871	-0.54	-0.05	+0.162	27.032
ATOM	17	C	UNL	1	-24.834	2.376	-14.907	-0.38	-0.01	+0.053	27.032
ATOM	18	O	UNL	1	-23.676	0.128	-12.257	-0.16	+0.36	-0.503	27.032
ATOM	19	H	UNL	1	-23.669	0.662	-11.439	-0.31	-0.54	+0.292	27.032
ATOM	20	O	UNL	1	-25.995	0.841	-13.359	-0.34	+0.00	-0.491	27.032
ATOM	21	C	UNL	1	-27.072	1.702	-13.005	-0.40	+0.00	+0.277	27.032