A DISSERTATION ON

Computational study of the interaction between bitter taste receptors and agonists thereof

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BY

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I, hereby, affirm that the work has been done by me in all aspects. I have sincerely prepared this project report and the results reported in this study are genuine and authentic.

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We wish her good luck and bright future.

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I wish her good luck and bright future.

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I wish her good luck and bright future.

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LIST OF ABBREVIATION

SCCs	Solitary chemosensory cells
TRCs	Taste receptor cells
GPCRs	G protein coupled receptors
AMPs	Antimicrobial peptides
TLRs	Toll-like receptors
DB	Denatonium benzoate
ASICs	Acid-sensing ion channels
PKD	Polycystins/Polycystic kidney disease
AC	Adenylyl cyclase
TM	Transmembrane
HM	Homology modelling

1. INTRODUCTION

Humans can experience the senses such as taste or gustation, olfaction or smell, tactility or touch, and auditory or hearing. Their survival depends on having the ability to sense, recognize and react to chemical cues. The chemical senses of taste and smell are essential because they help us find and choose food, avoid ingesting poisons, encourage food consumption, and control parts of social behaviour required for reproduction and mother-infant connections (Stevenson, 2010). In its most basic form, the sense of taste gives the organism the capacity to recognise molecules that are needed for sustenance and ones that might be detrimental. The tongue is said to be the major organ for taste although taste buds are found on the human soft palate, and the papillae are said to be the main structure that houses the sensory ends.

The five primary flavours that humans can distinguish are sweet, bitter, salty, sour and umami. Given the variety of structurally different bitter chemicals being found, the bitter taste is said to be perhaps the most complex process. In addition, taste is said to influence a basic notion of which food is "acceptable" or "unacceptable". Flavours, which mix taste, smell, and tactile experiences, let us distinguish between familiar and unfamiliar food. When food is chewed, dissolved into saliva, and pre-digested by oral enzymes including amylase, lipase, and proteases, taste stimuli are normally produced. For humans, nutrients and poisons have tastes that can be classified as sweet, salty, sour, savoury, and bitter. The amino acids glutamate, aspartate, and a few other ribonucleic acids are perceived as savoury, sodium and salts are a few other cations that are perceived as salty, and many toxic substances are perceived as bitter. Simple carbohydrates are perceived as sweet. When the food is put in the mouth, taste, temperature, and touch receptors check for quality and intensity, causing the appropriate saliva to be produced in preparation for chewing, bolus formation, and swallowing—or, in the case of unappealing or toxic substances, expectorating, retching, or vomiting. In humans, for a quick analysis of the chemical makeup of food, the sense of taste is essential. Desired tastes, such as sweet, umami, and low sodium levels, reflect the nutritional worth of foods, whereas bitter, sour, and high sodium levels, respectively, detect the presence of toxins, or high levels of minerals. In contrast, creamy feelings indicate consistency or the presence of extremely desired fats. Harsh, sandy, or painful sensations indicate the presence of potentially dangerous components that may hurt the digestive system (Heinze et al., 2015; Patel & Pathak, 2018).

The olfactory input and prior experiences come together to produce an emotional, sensory, and physiological response, the feeling of which is received with the help of the central nervous system (CNS) and cranial nerves (CNs). Different flavours are perceived in the quantities of sodium ions and protons as well as in the energy content of proteins and carbohydrates through the use of their respective building blocks, mono- and disaccharides, and L-glutamic acid such as sweet, salty, sour, and umami. Chemically varied chemicals, many of which may or may not be lethal, can produce the fifth fundamental taste characteristic, bitterness, thus it is important to avoid ingesting them. There are particular receptors or receptor families that are expressed in sensory cells in the oral cavity for each of these five flavours' fundamental taste characteristics. According to current theories, the molecular processes underlying the perception of bitter taste begin with the binding of certain water-soluble molecules to G-protein-coupled receptors expressed on the surface of taste receptor cells and encoded by the type 2 family of taste receptor genes.

The primary sensory component of the taste system, the taste buds, are said to be embedded underneath the keratinous layer of the papillae and have a tasting pore that is open to the environment. The papillae are known to be the principal structure that houses the sensory ends. 3 types of taste buds are known to date - Glial-like Type 1 taste buds cells were hypothesized to transmit salty flavour. The sweet, bitter, and umami sensations were assumed to be mediated by Type 2 taste buds cells, which are structurally GPCRs (Loper et al., 2015). To transduce a sour taste and facilitate communication from the type 2 cells via P2Y adenosine receptors, Type 2 presynaptic cells are involved. Serotonin release from the type 3 cells were subsequently used to communicate with the afferent neurons. The taste buds are made up of at least five different cell types: type 1, type 2, type 3, basal cells, and neural processes. Originally, the presence or lack of granular granules was used to distinguish the different types of taste cells (Hino et al., 2022; Ozdener et al., 2011). In humans, there are an average of 195 fungiform papillae, 87% of which were found in the first 2 cm of the tongue. Over 100 taste buds are located in the foliate papillae, which form folds on the side of the tongue. The circumvallate papillae near the back of the tongue, forms an inverted “V”. Where more than 100 taste buds in circumvallate papillae are present (Doyle et al., 2023; Hollis, 2018).

A cellular receptor or taste receptor, sometimes known as a tastant, is responsible for taste perception. Taste receptors are proteins that can detect different forms of taste stimuli, acting as the first step in the process of detecting and classifying ingested material.

The taste 1 receptor member 2 (T1R2) and taste 2 receptor member 3 (T1R3) together make the sweet taste receptors whereas (T2R) belongs to bitter taste receptors. Sweet, bitter, and umami flavours are communicated through receptors associated with the G protein whereas sour and salty chemicals were linked to ion channels. The taste 1 receptor family (T1R) and the taste 2 receptor family (T2R) are two types of GPCRs. Taste receptors were initially identified in the taste system and given their names in the sequence of their discoveries, but research from the past two decades has provided insight into additional chemical receptors that are distributed more widely than the taste system. Extraoral taste receptors are taste receptors that are located outside of the taste system (Ki & Jeong, 2022). The activities of extraoral taste receptors of mucosae and internal organs, range from spotting and combating infections to calming airways. The extraoral taste receptors are a novel potent pharmacological target for the treatment of several illnesses and ailments due to the functional relationship between health and sickness. Taste receptor cells (TRCs) that extend into the oral cavity express these receptors on their apical membranes. In TRCs, the receptor-stimulus-binding event starts a transduction cascade that results in cell depolarization, neurotransmitters released on afferent nerve fibres, and then finally the transmission of sensory information to taste processing centres in the central nervous system. Denatonium benzoate [DB] and other hTAS2R47 agonists trigger a [Ca^{2+}] wave inside the human sinus SCCs, which spread to neighbouring cells via gap junctions and cause the production of antimicrobial peptides (AMPs), including -defensins. Additionally, the increase in AMP synthesis in response to TAS2R stimulation occurs quickly (5 min), in contrast to the increase in AMP production in response to TLR stimulation, which take hours (Kamila & Agnieszka, 2021). SCCs have also been found to co-express T1R2/T1R3 sweet taste receptors in addition to TAS2Rs. More significantly, bitter and sweet signals affect innate immunity in the opposite direction.

The mechanisms of all taste sensations (Doyle et al., 2023) can be summarized as follows:

(A) Sweeteners and sugars activate downstream pathways by stimulating the sweet taste receptors, T1R2/3. It has been proposed that artificial sweeteners activate an adenylyl cyclase (AC) pathway whereas sugars activate a phospholipase C-dependent route.

(B) Monosodium glutamate stimulated a phospholipase C pathway by activating the T1R1/3 umami receptor.

(C) The 25 T2R receptors mediate the bitter signal's transduction. Bitter receptors were believed to stimulate both the phospholipase C and AC pathways, much like the sweet receptors do.

(D) According to evidence, bitter tastes may be mediated by or enhanced by a potassium channel.

(E) PKD and ASIC channels, which are found on several populations of papillae, are hypothesised to mediate sour taste.

The signalling molecules known as G protein-coupled receptors (GPCRs) are extremely versatile. In eukaryotes, the largest membrane receptor family belongs to GPCRs. In the human genome, they represent one of the largest class of membrane proteins. The majority of cellular reactions to hormones and neurotransmitters, as well as taste, vision, and olfaction, are mediated by GPCRs.

Bitter taste receptors (T2R) are traditional seven transmembrane-domain G-protein coupled receptors that ligate in response to various bitter compounds in food or medicine, such as erythromycin or metabolites produced by the microbiome. Examples of such compounds include sinigrin in vegetables, caffeine, and epigallocatechin-3-gallate in green tea. Bitter compounds can also exhibit chemotherapy-enhancing anticancer effects, however, the precise processes are still unknown. Vanillin, a key ingredient in vanilla that is frequently employed as a flavouring agent, activates TAS2R14, TAS2R20, and TAS2R39 bitter taste receptors (Morini et al., 2021). Some peptides have also been identified as TAS2R39 agonists, indicating that small molecules are not the only compounds that may activate these bitter taste receptors. The majority of bitter receptors are versatile and can be activated by a variety of chemically unrelated compounds through as-yet-unknown methods. T2R10 is one of the receptors, that reacts with substances like coffee, coumarin and haloperidol. However, its function goes beyond primarily detecting bitterness. Although a direct link between bitterness and toxicity hasn't been shown, it's commonly accepted that this taste attribute shields humans and animals against intoxication by preventing them from ingesting potentially dangerous dietary ingredients.

1.5 Aims and objectives

The aim of the present study is to evaluate the possibilities of a fruitful relationship between various molecular/interaction parameters and the taste of molecules. This work would help in designing better tasting products to enhance consumer acceptability, which would in turn increase the commercial prospects of the existing products. In order to achieve the desired aim, the following objectives were proposed:

1. Identification of the best taste receptor model using different protein structure prediction/modeling tools.

2. Evaluation of the relationship between taste and molecular description of standard taste molecules.

3. Testing the relationship between standard taste molecules and the taste receptors with the help of docking.

2. REVIEW OF LITERATURE

One of the most important mechanisms for controlling drug intake is the taste, which analyses the nutritional value and healthiness of food while protecting against the consumption of hazardous or harmful substances. Each of the five basic, universally recognised tastes such as bitter, sweet, umami, salty and sour is said to be connected to a vital body process. The presence of sugars and carbs, or that of an energizing food, is said to be indicated by a sweet taste. The delicious flavours of cooked meat and broths are known as umami, which is said to be connected to a food's protein concentration. Bitter tastes are known to be typically linked to unpleasant flavours and things that could be harmful to the body, including rotten foods or toxins. The bitter taste is also known to be linked to foods that are not damaging to the body, such as citrus peel, coffee, untreated olives, unsweetened chocolate, and untreated olives. The taste of sour alerts you to acids and keeps you from eating rotten food. The intake of sodium and other minerals, which are said to be crucial for preserving the body's water balance and blood circulation, was controlled by the taste of salt. The gustatory system's molecular, subcellular, cellular, and tissue-level actors were known to participate in the extremely composite and multiscale process of taste perception. Production of taste is done with the help of specialised proteins, or taste receptors, interacting with chemical compounds dissolved in saliva. This activation of taste receptor cells (TRCs) on gustatory papillae, modified epithelial cells found throughout the oral mucosa, particularly on the tongue, results in the perception of taste.

2.1 Taste receptor structure prediction

A variety of theoretical and computational techniques are applied in molecular modelling to simulate or replicate the behaviour of biomolecules such as proteins, DNA, tiny ligands, and polymers. The foundation of molecular modelling methodologies is said to be an atomistically detailed description of the molecular systems, which is best achieved by direct experimental methods. To come up with a plausible molecular structure, the use of a predictive technique is required if the structure of interest has not yet been experimentally solved. To achieve this, homology modelling (HM) offers a popular technique to forecast the 3D structures of a particular protein, known as the target, beginning with its amino acid sequence. To model the desired structure using this technique, a solved 3D structure of a related macromolecule serves as the template (Venselaar et al., 2010). The accuracy of the approach is said to depend on the sequence alignment and similarity of the target and

template sequences. The first challenge to be addressed in the context of studying taste receptors by molecular modelling was the definition of the receptors' atomistic structure, primarily since experimental GPCR purification is difficult. The human genome has over 800 GPCRs, but only 89 of them have been identified. These shortcomings were typically made up for by HM, and for template sequence identities of more than 30%, good models can be generated. However, investigations in the literature showed that transmembrane proteins exhibit good structural conservation even at low sequence identity (below 20%), indicating that it may still be viable to obtain precise 3D models of the TM regions using HM in these circumstances (Almeida et al., 2017). Several recently created conformational and sampling prediction models have been made available and tailored for the GPCR structure prediction in this context. In addition to the more well-known databases on proteins and ligands, other specialised databases, such as BitterDB, provides information on predicted models relevant to taste. Each taste, as mentioned earlier, is mediated by a distinct receptor that is expressed on a different type of taste cell: sweet and umami are transduced by class-C GPCRs, bitterness by class-A/class-F GPCRs, and sour and saltiness are both recognised by ion channels. The focus of the present work will be the bitter taste receptors.

2.2 Bitter taste receptor

The taste 2 receptor family (TAS2Rs), includes bitter taste receptors. Regarding whether they belong in a particular class of GPCRs, there have been numerous debates: some authors place them in class F of GPCRs, which includes frizzled and smoothened proteins; others place them in the more general class A of GPCRs, which are rhodopsin-like; and, most recently, the online database GPCRdb (https://gpcrdb.org/) even created a new subfamily for these receptors called class T (Munk et al., 2016). They resemble class A GPCRs, to which visual and odorant sensory receptors also belong, according to their functional principles and the location of the binding site, however, this is not the case due to their sequence similarity. Its short extracellular N- and intracellular C-termini, seven transmembrane helices (TMD), three extracellular loops (ECLs), and three intracellular loops (ICLs) serve as its structural components. The 7 TMD bundle, which comprises the structural core, binds ligands in the extracellular (EC) area and allows for information transfer via the intracellular (IC) region, has become the component found to be most similar between class A GPCR and bitter receptors (Di Pizio et al., 2016).

The comparison reveals the absence of crucial class A motifs and a highly conserved disulfide bridge that help stabilise GPCR structures. On the other hand, the conserved residue unique to TAS2Rs may be crucial in maintaining the bitter receptors' inactive state. The number of TAS2R genes differs significantly between species. The number of bitter receptor genes varies between species, but changes were seen where the TAS2R genes were situated; in humans, these genes were located on chromosomes 5, 7, and 12, whereas in mice, they are located on chromosomes 2, 6, and 15 (Conte et al., 2002). This makes it easier to understand that each bitter receptor responds to more than one ligand because there were many more bitter substances that people can detect than there have been human genes. TAS2Rs were known to be a fascinating subclass of GPCR because there were numerous known agonists and few antagonists for them. Additionally, this ligands' activity was typically higher than that of most GPCR ligands, which was normally in the nanomolar range.

The presence of three general receptors, TAS2R10, TAS2R14, and TAS2R46, which recognise about one-third of all bitter compounds, as well as the abundance of naturally occurring bitter-tasting substances means that heterodimerization of bitter taste receptors may not be required to increase their already impressive receptive capacity (Behrens & Meyerhof, 2013). As of now, it is unknown whether TAS2Rs heteromeric receptors contribute to a wider detectable agonist range, despite *in vitro* investigations showing that TAS2Rs bitter taste receptor form oligomers (about 325 homodimeric and heterodimeric receptors). The TAS2R receptor and the ion channels in the cell membrane can both be activated by some bitter chemicals, suggesting that these substances may also serve as bitter receptors (Jalševac et al., 2022; Kumar et al., 2021). Furthermore, a study has demonstrated that TAS2Rs are expressed in extra-oral tissue, such as the heart, skeletal muscles, and smooth muscles, in addition to the taste buds (Hollenhorst et al., 2022). Different kinds of muscle cells have various TAS2R distributions, although TAS2R3, TAS2R4, TAS2R5, TAS2R10, TAS2R13, TAS2R19, and TAS2R50 are consistently present in a modest amount, TAS2R14 is substantially expressed throughout the entire human body (Jeruzal-Świątecka et al., 2020; Shaw et al., 2018). Additionally, previous studies stated the expression of TAS2Rs on human airway smooth muscle, smooth muscle tissue along the mouse gut, and in human gastric smooth muscle cells, pointing to a potential role of TAS2Rs as targets to alter the gastrointestinal body motility and subsequently hunger sensation (Tang, 2023). Additionally, TAS2Rs were said to be connected to the contraction and relaxation of

muscles in various organs, including the bladder (Dalesio et al., 2018). Although bitter compounds were frequently thought of as dangerous substances, some had positive impacts on human health. Because of this, a deeper comprehension of the bitter taste receptor transduction may help in the development of certain medications that had a tolerable taste and were crucial in treating disorders of the muscles.

2.3 3D structure and conformational dynamics of receptors

The lack of experimentally solved structures for each of the 25 bitter receptors is now one of the biggest barriers to the molecular modelling of bitter taste receptors. In reality, only the homology-based molecular models for 23 of 25 human bitter receptors have been created. These models can be found in the BitterDB ((https://bitterdb.agri.huji.ac.il/dbbitter.php), which also offers details on bitter receptors and associated chemicals (Dagan-Wiener et al., 2019). The TAS2R19 and TAS2R45 are the only two receptors that are excluded from the database, which have been tried and tested in the present work.

2.4 Ligand-protein interaction investigations

Although each bitter taste receptor has a single binding site for bitter ligands, the abundance of TAS2R receptors enables the detection of a vast array of bitter chemicals. In more detail, a large range of agonists with various chemical properties can activate bitter taste receptors. It is possible to achieve this affinity for a wide variety of chemical structures by using a variety of interactions amongst the ligands in the binding pocket. Bitter taste receptors can be classified as selective or promiscuous, depending on how many distinct substances they are activated by. The examples of prodigious receptors are TAS2R10, TAS2R14, and TAS2R46. The detection of related bitter drugs by each TAS2R receptor is based on distinct patterns, yet many different substances can activate different TAS2Rs. Chemoinformatics approaches have recently been used to investigate the selectivity and promiscuity profile of bitter taste receptors and their ligands. More specifically, the data showed that practically all promiscuous molecules, or ligands that target more than one TAS2R, are the only ones that can activate selective bitter receptors. Instead, both promiscuous and selective binders can activate promiscuous receptors (Di Pizio & Niv, 2015; Fierro et al., 2023).

The possibility of a logical ligand design that is specially intended to adjust their chemical structure as per demand is what makes the ligand promiscuity inquiry relevant in the first

place. The so-called promiscuity index (PI), which is the ratio of the number of bitter chemicals that activate the receptor to the total number of molecules taken into account, provides access to the receptor promiscuity. Earlier class literature A GPCR discovered a link between the features of the binding site and the range of antagonists. In particular, it was shown that the number of distinct scaffolds, which gauges the quantity and variety of antagonists, were inversely related to the binding site's exposure and hydrophobicity and in contrast to the number of hydrogen bond donors. Interestingly, Di Pizio claimed that the aforementioned features of the binding site correspond similarly with the TAS2R-promiscuity, despite the absence of structural data that prevents a thorough analysis of TAS2Rs (Di Pizio & Niv, 2015). Single point mutations in the binding pocket that can increase or decrease affinity towards a particular ligand enable the bitter receptors to detect a wide range of ligands.

Although it was suggested that bitter receptors might have many binding sites to accommodate the wide diversity of bitter agonists, Slack and colleagues showed that each binding pocket is distinct (Slack et al., 2010). Point mutations on TAS2R16 were used in some studies to determine the binding pocket of bitter receptors. Experimental research and functional assessments on mutant receptors that identified the residues governing the agonist selectivity and activation of TAS2R46, TAS2R43, and TAS2R31 also supported this prediction. Indeed, numerous studies on the most studied receptors, TAS2R14, TAS2R10, and TAS2R46, empirically supported the involvement of residues found in the aforementioned TMs. It also implied that TM II was involved in the function of TAS2R14 and TAS2R46 receptors, which might be explained by the latter's larger pocket than TAS2R14's. The above-mentioned binding site's extremely variable residue makeup in each TAS2R raises the possibility of the identification of several ligands with a range of agonist-specific interaction patterns (Spaggiari et al., 2020; M. Y. Yang et al., 2021).

Additionally, computational studies emphasised the types of interactions that occur between some ligands and the receptors as well as significant conformational changes associated with ligand-driven activation. For instance, Chen and colleagues looked into how TAS2R16 might be activated when its agonist and antagonist, salicin and probenecid, respectively, were docked into the active pocket (Chen et al., 2018). Polyphenols, which are found in foods like coffee, wine, and red fruits, are other bitter ligands that are particularly significant for their nutritional qualities. Through the activation of TAS2Rs,

Soares and his colleagues examined the bitterness of various classes of 16 polyphenolic compounds and highlighted their stimulation of bitter taste receptors (Soares et al., 2016).

According to the literature, bitter receptors may only have one binding site for agonists and antagonists since the type of interactions with a chosen residue relies on the nature of the ligand. However, some research points to the possibility of a second vestibular binding site in the extracellular region of the receptor. Before entering the canonical binding site, agonists can briefly occupy this site and be prefiltered, according to a theory put forth by Sandal and colleagues (Sandal et al., 2015). These two sites may also play a role in the differentiation of various agonists of TAS2R46. The type of ligands, membrane lipids, and motions of TMs and ECLs are some of the other elements that affect how TAS2Rs and bitter tastes interact. Indeed, Pydi et al. noted that cholesterol plays a vital role in the cell membrane for the interaction between amino acids and hypothesised that T2Rs are sensitive to cholesterol (Pydi et al., 2016).

3. Materials And Methods

3.1 Sequence retrieval

The UniProt Knowledgebase (UniProtKB) (https://www.uniprot.org/) is the central hub for the collection of functional information on proteins, with accurate, consistent and rich annotation. In addition to capturing the core data mandatory for each UniProtKB entry (mainly, the amino acid sequence, protein name or description, taxonomic data and citation information), as much annotation information as possible is added. UniProt is the Universal Protein resource, a central repository of protein data created by combining the Swiss-Prot, TrEMBL and PIR-PSD databases. The UniProt Knowledgebase consists of two sections: a section containing manually-annotated records with information extracted from literature and curator-evaluated computational analysis, and a section with computationally analyzed records that await full manual annotation (Bateman et al., 2023).

For the sake of continuity and name recognition, the two sections are referred to as "UniProtKB/Swiss-Prot" (reviewed, manually annotated) and "UniProtKB/TrEMBL" (unreviewed, automatically annotated), respectively. This includes widely accepted biological ontologies, classifications and cross-references, and clear indications of the quality of annotation in the form of evidence attribution of experimental and computational data. All 24 bitter taste receptors were identified and retrieved from the UniProtKB.

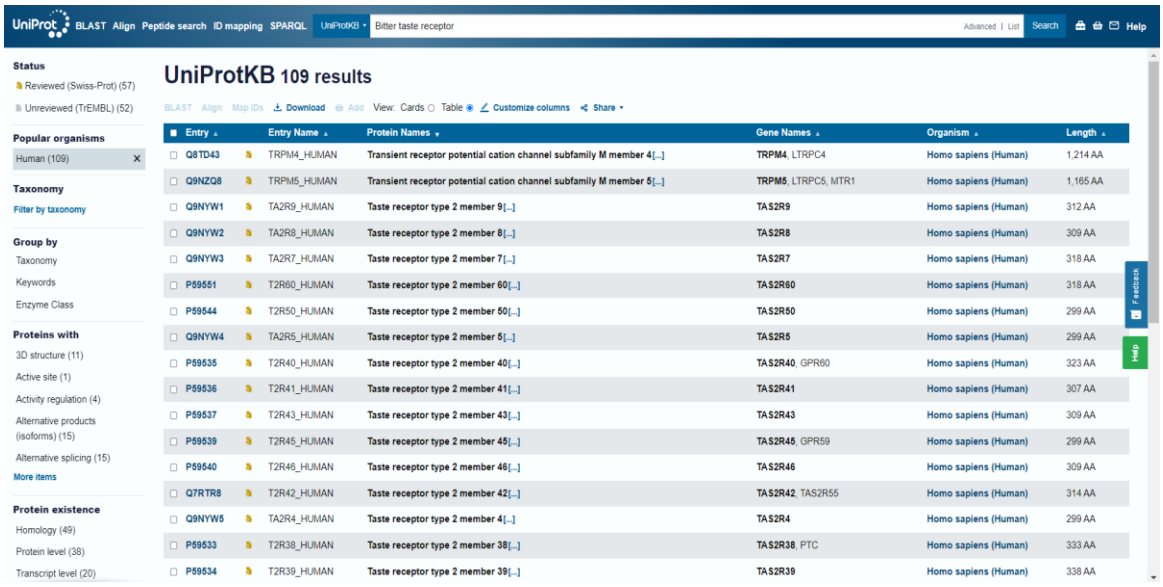

Fig 3.1 Diagram Depicting UniProt KB "Bitter Taste Receptor" search results.

3.2 Protein structure prediction

SWISS-MODEL

Swiss-Model (https://swissmodel.expasy.org/interactive) is a structural Bioinformatics web server dedicated to homology modelling of protein 3D structures (Waterhouse et al., 2018). Homology modelling is currently the most accurate method to generate reliable three dimensional protein structure models and is routinely used in many practical applications. Homology (or comparative) modelling methods make use of experimental protein structures (" templates") to build models for evolutionary related proteins (" targets").

Individual target sequence were pasted in swiss-model in FASTA format in the box provided for the same as shown in fig 3.2. By clicking on build model option, result shows different 3D models out of which the model consisting highest coverage and sequence identity with template were retrieved in ".pdb" format along with the complete project data archive, as shown in fig 3.3.

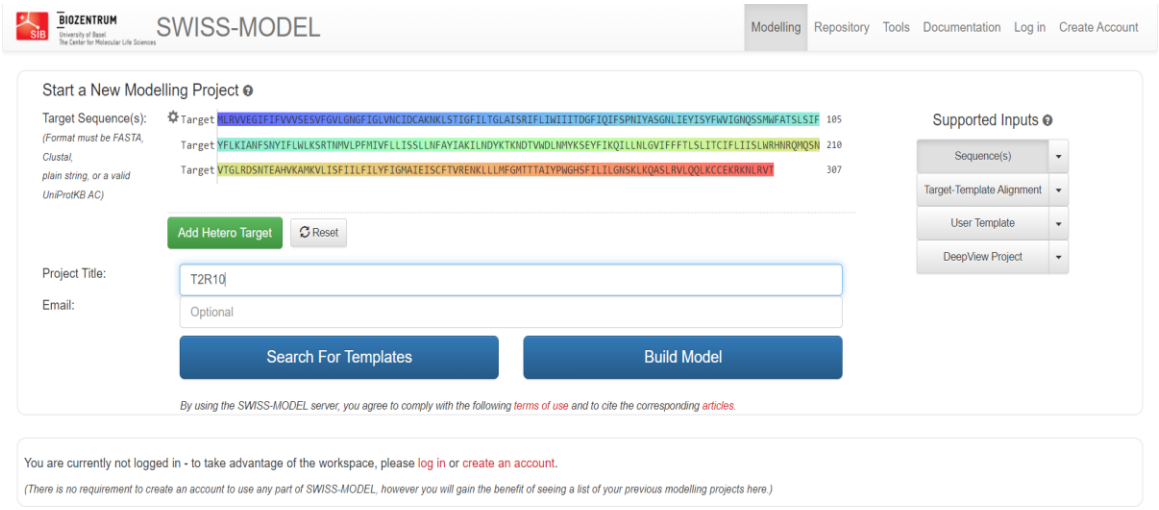

Fig 3.2 Swiss model project page.

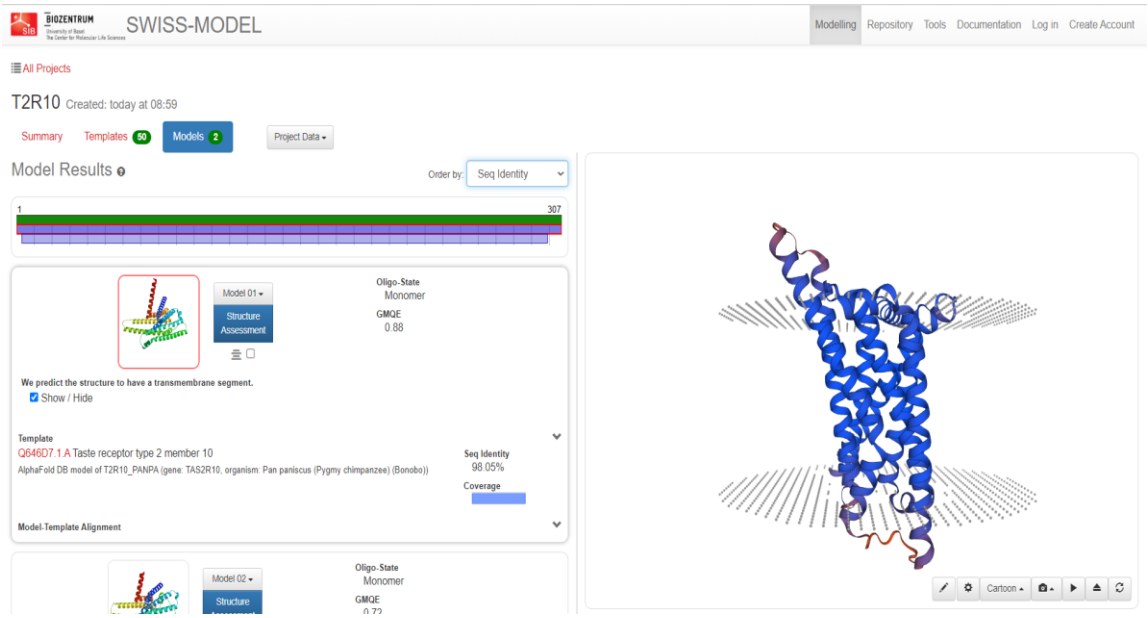

Fig 3.3 Shows the generated model of T2R10.

PHYRE2

Phyre and **Phyre2** (**P**rotein **H**omology/Analog**Y** **R**ecognition **E**ngine; pronounced as 'fire') (http://www.sbg.bio.ic.ac.uk/phyre2/) are web-based services for protein structure prediction that are free for non-commercial use (Kelley et al., 2015). Phyre is among the most popular methods for protein structure prediction having been cited over 1500 times. Like other remote homology recognition techniques (see protein threading), it is able to regularly generate reliable protein models when other widely used methods such as PSI-BLAST cannot. Phyre2 has been designed (funded by the BBSRC) to ensure a user-friendly interface for users inexpert in protein structure prediction methods. The Phyre and Phyre2 servers predict the three-dimensional structure of a protein sequence using the principles and techniques of homology modelling. Because the structure of a protein is more conserved in evolution than its amino acid sequence, a protein sequence of interest (the target) can be modelled with reasonable accuracy on a very distantly related sequence of known structure (the template), provided that the relationship between target and template can be discerned through sequence alignment. Currently the most powerful and accurate methods for detecting and aligning remotely related sequences rely on profiles or hidden Markov models (HMMs). These profiles/HMMs capture the mutational propensity of each position in an amino acid sequence based on observed

mutations in related sequences and can be thought of as an 'evolutionary fingerprint' of a particular protein.

Typically, the amino acid sequences of a representative set of all known three-dimensional protein structures is compiled, and these sequences are processed by scanning against a large protein sequence database. The result is a database of profiles or HMMs, one for each known 3D structure. A user sequence of interest is similarly processed to form a profile/HMM. This user profile is then scanned against the database of profiles using profile-profile or HMM-HMM alignment techniques. These alignments can also take into account patterns of predicted or known secondary structure elements and can be scored using various statistical models. See protein structure prediction for more information. The first Phyre server was released in June 2005 and uses a profile-profile alignment algorithm based on each protein's position-specific scoring matrix.[5] The Phyre2 server was publicly released February 2011 as a replacement for the original Phyre server and provides extra functionality over Phyre, a more advanced interface, fully updated fold library and uses the HHpred / HHsearch package for homology detection among other improvements. Amino acid sequence were pasted in the reqired box as shown in fig 3.4. Modelling mode were selected for intensive as well as not for profit which gave required result as shown in fig 3.5. Pdb format of individual proteins were downloded.

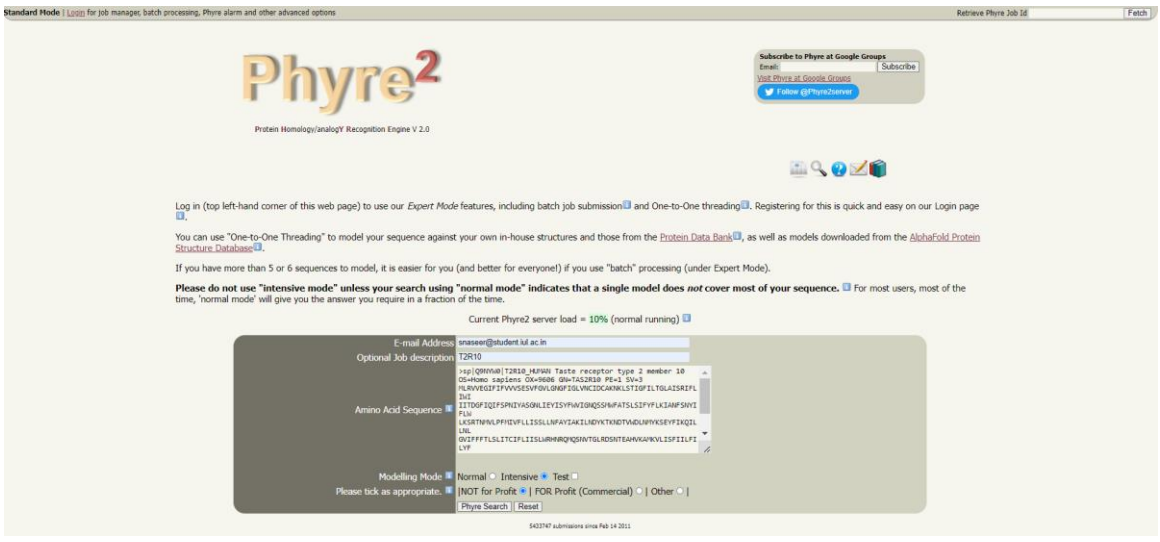

Fig 3.4 Shows cover page of Phyre2 with T2R10 amino acid sequence.

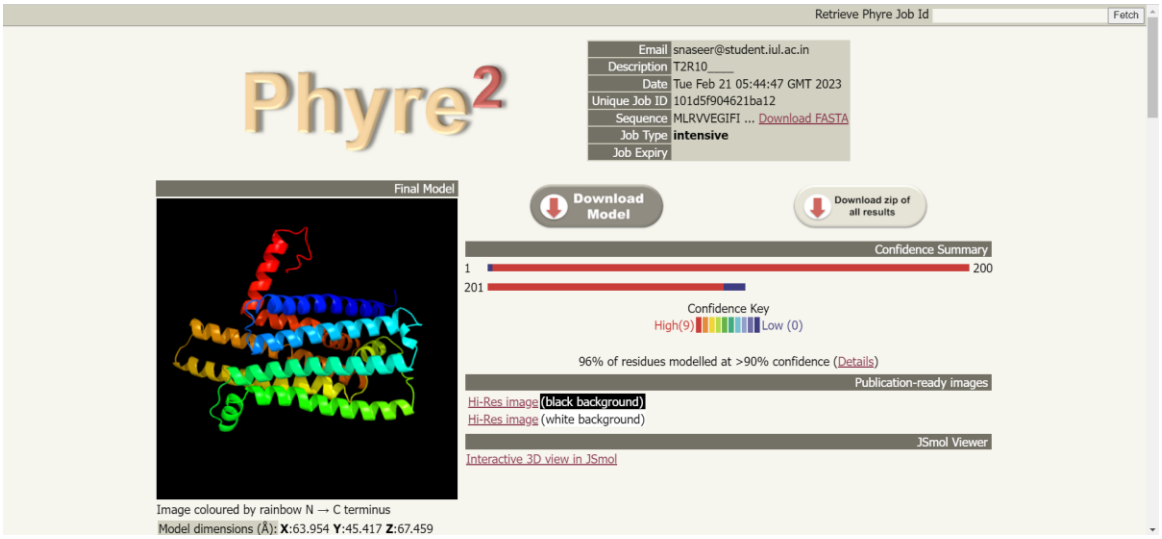

Fig 3.5 Shows the result for T2R10's 3D structure prediction.

AlphaFold

AlphaFold (https://alphafold.ebi.ac.uk/) is speeding up research in almost all areas of biology and is able to predict the 3D models of protein structures with accuracy. Alphfold is an AI system which has the ability to anticipate a protein's shape with atomic precision at scale and in minutes. The AlphaFold Protein Structure Database openly disseminates predictions for more than 200 million proteins thanks to a collaboration with the European Bioinformatics Institute at EMBL.

The Protein Id was searched in AlphaFold for the prediction of 3 dimentional structure as shown in fig 3.6. The Target protein of Homo sapiens were selected and their pdb files were retrieved as shown in fig 3.7.

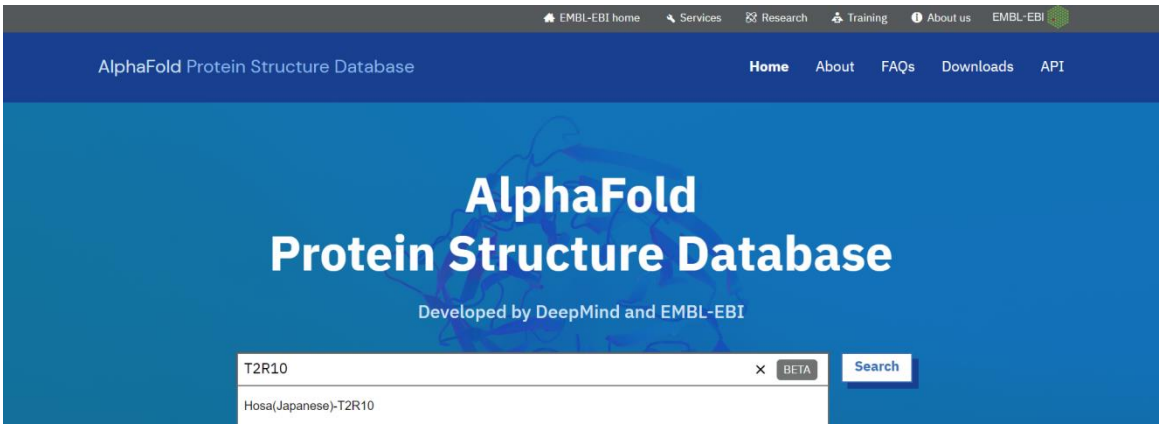

Fig 3.6 Shows the cover page of AlphaFold Protein Structure Database

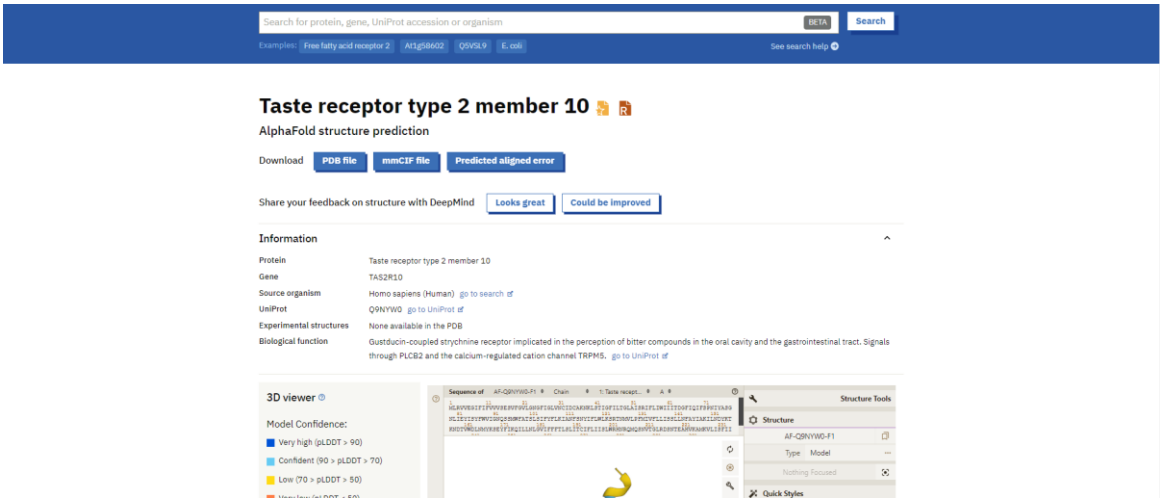

Fig 3.7 Shows the result page of T2R10 in AlphaFold.

I-TASSER

The process of creating three-dimensional models from amino acid sequences using computer algorithms is known as protein structure prediction. However, the human-expert knowledge paired with biochemical data (function, mutagenesis, catalytic residues, etc.) could aid in both structural assembly and model selection, therefore structure modelling methods frequently incorporate human interventions. However, the advantage of developing completely automated systems is their potential use in proteome-scale structure predictions. Among all automated servers, I-TASSER (as "Zhang-Server") (https://zhanggroup.org/I-TASSER/) produced the most accurate predictions of 3D structures. The Threading ASSEmbly Refinement (TASSER) programme and the secondary-structure enhanced Profile-Profile threading Alignment (PPA) are the foundations of the hierarchical protein structure modelling approach known as I-TASSER (J. Yang et al., 2014; Zhang, 2008).

It requires the amino acid sequence of individual proteins in their FASTA format as well as the email id for receiving results as shown in fig 3.8.

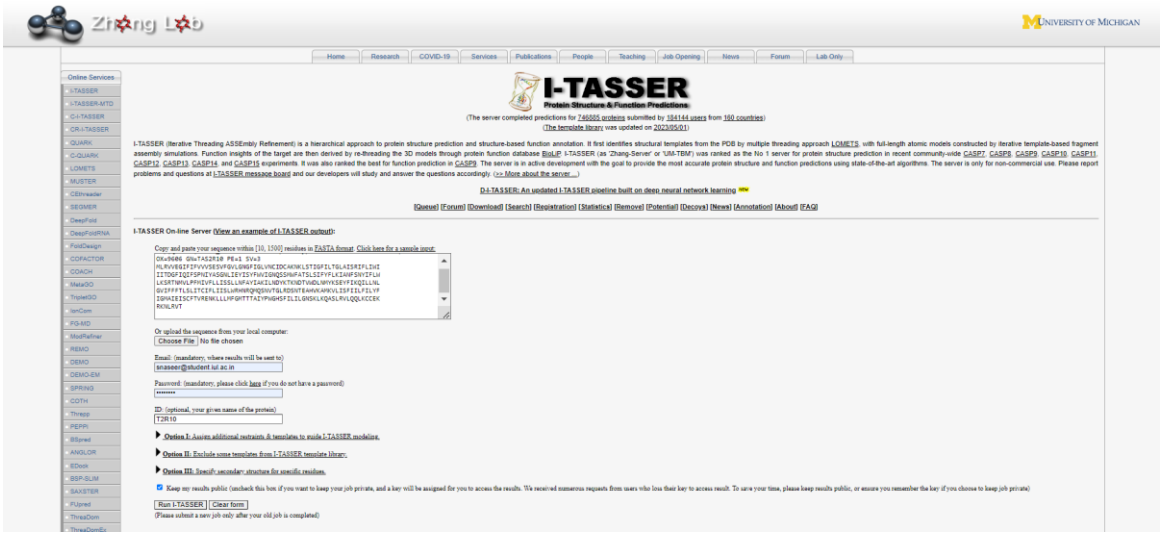

Fig 3.8 Shows the cover page of I-Tasser Protein structure and function prediction.

The result of I-Tasser 3D structure prediction consisted of five 3D model among which the best model was selected. The selection of best model was based on the C-score of the 3D model. A confidence score, or C-score, is used by I-TASSER to gauge the calibre of predicted models. It is determined using the importance of threading template alignments and the convergence parameters from simulations of the structure construction. A model with a high confidence level has a higher C-score, and vice versa. The C-score normally falls between [-5,2] as shown in fig 3.9.

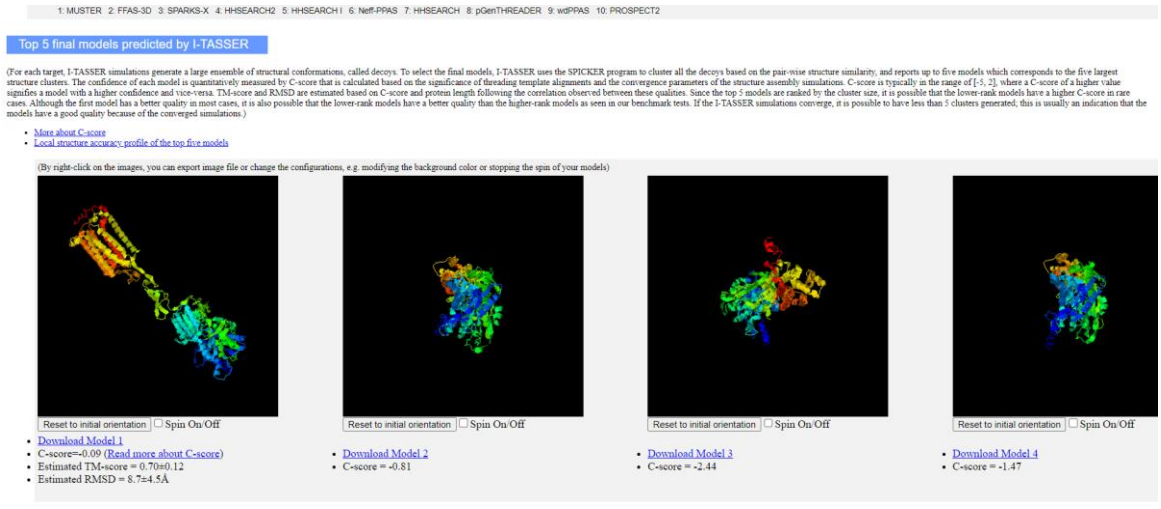

Fig 3.9 shows the 5 models predicted by I-Tasser of T2R10 along with their C-score.

3.3 Model Validation

MolProbity

MolProbity is a structure-validation web service that provides broad-spectrum solidly based evaluation of model quality at both the global and local levels for both proteins and nucleic acids (http://molprobity.biochem.duke.edu/). It relies heavily on the power and sensitivity provided by optimized hydrogen placement and all-atom contact analysis, complemented by updated versions of covalent-geometry and torsion-angle criteria. Some of the local corrections can be performed automatically in MolProbity and all of the diagnostics are presented in chart and graphical forms that help guide manual rebuilding. X-ray crystallography provides a wealth of biologically important molecular data in the form of atomic three-dimensional structures of proteins, nucleic acids and increasingly large complexes in multiple forms and states. Advances in automation, in everything from crystallization to data collection to phasing to model building to refinement, have made solving a structure using crystallography easier than ever. It is a structure validation web service for diagnosing problems in 3D models of proteins, nucleic acids or complexes. It adds and optimizes H atoms (correcting 180° flipped Asn/Gln/His sidechains), and then calculates global and local validation for all-atom contacts (steric clashes, H-bonds & vdW), covalent geometry, and conformation (Ramachandran & rotamers for protein, ribose puckers & suite conformers for RNA). Results are displayed online as 3D graphics and sortable charts. It is a web application that integrates validation programs from the Richardson lab at Duke University (Williams et al., 2018).

Most commonly employed method for the validation of a homology model is Ramachandran plot. The Ramachandran plot provides an easy way to view the distribution of torsion angles of a protein structure. It also provides an overview of allowed and disallowed regions of torsion angle values, serving as an important factor in the assessment of the quality of protein three-dimensional structures.By making a Ramachandran plot, protein structural scientists can determine which torsional angles are permitted and can obtain insight into the structure of peptides. It is a two-dimensional (2D) plot of the torsional angles of amino acids φ (phi) and ψ (psi) in a protein sequence. The φ represents the dihedral angle between N(i-1)-C(i)-CA(i)-N(i) and ψ is the backbone dihedral angle between C(i)-CA(i)-N(i)-C(i+1). The plot was developed by G.N. Ramachandran in 1963. the obtained models were evaluated using molprobity. The ".pdb"

file of the individual models were uploaded on the molprobity homepage as demonstrated in fig 3.10(a).

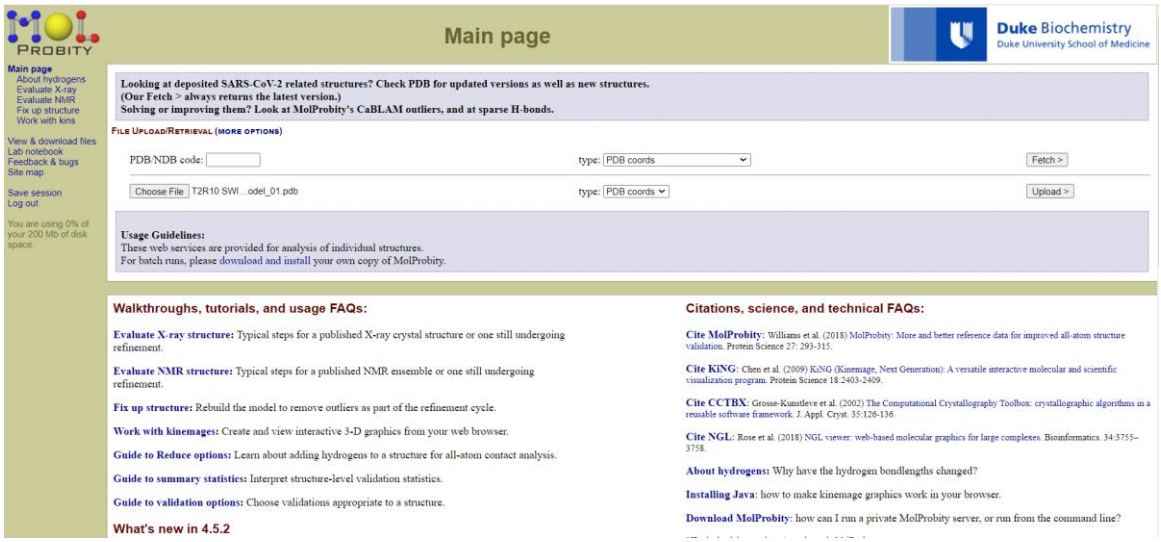

Fig 3.10 (a) Main page of MolProbity.

On uploading the ".pdb" file a preview page is generated depicting the information for user conformation. If all the information is found to be correct then press the continue button as shown in fig. 3.10 (b).

Fig 3.10 (b) Review page after uploading the model.

This will open the main page of molprobity where hydrogen are added to complete the protein structure as shown in fig 3.10 (c) and 3.10 (d).

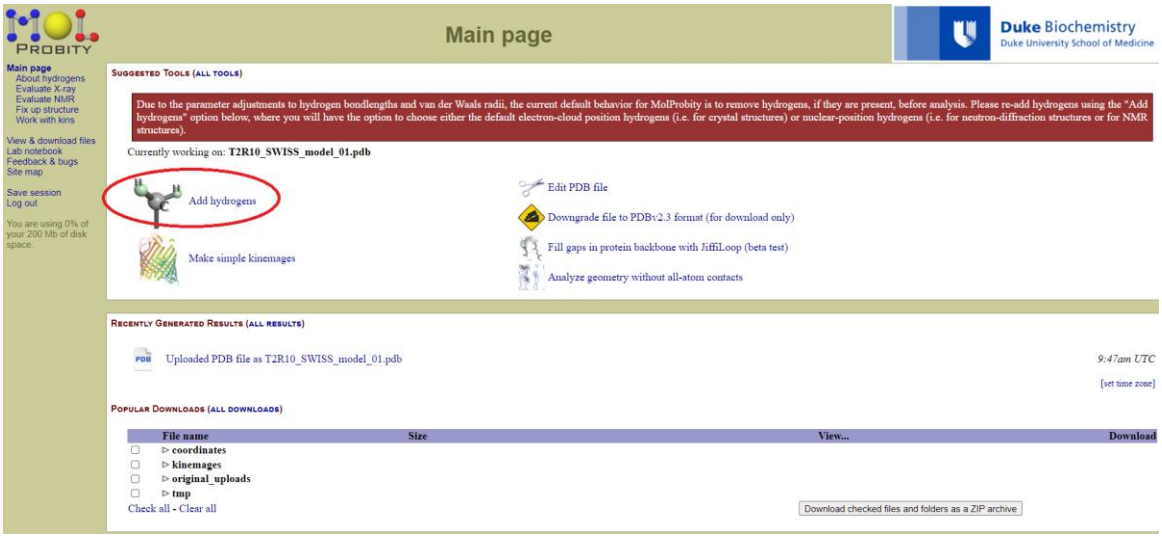

Fig 3.10 (c) MolProbity tool page.

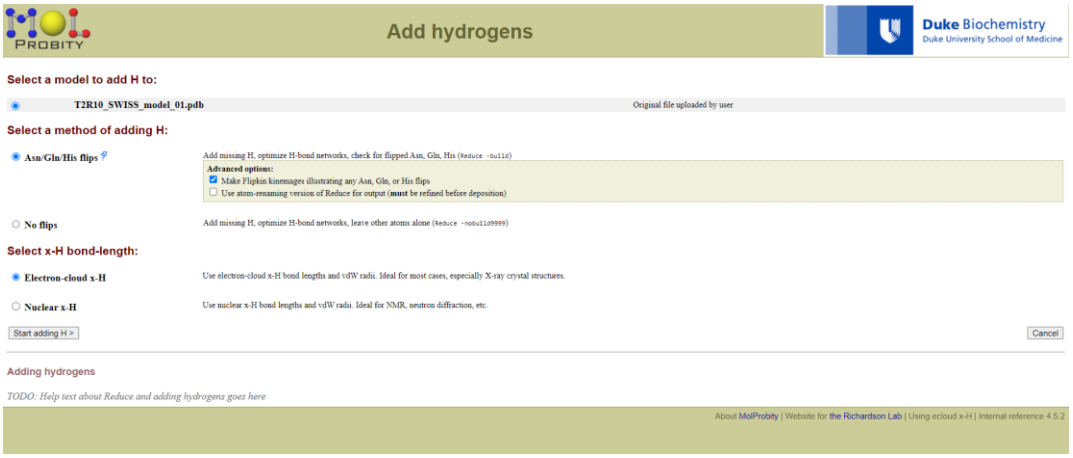

Fig 3.10 (d) The Add Hydrogen tool page.

After clicking on “start adding H+” as shown in fig 3.10 (d), review flips page opens up by clicking on “Regenerate H applying only selected flips” as shown in fig 3.10 (e).

Fig 3.10 (e) Review Flips page.

This would open up the final preview page wherein we can confirm the details and press the continue button as shown in fig 3.10 (f).

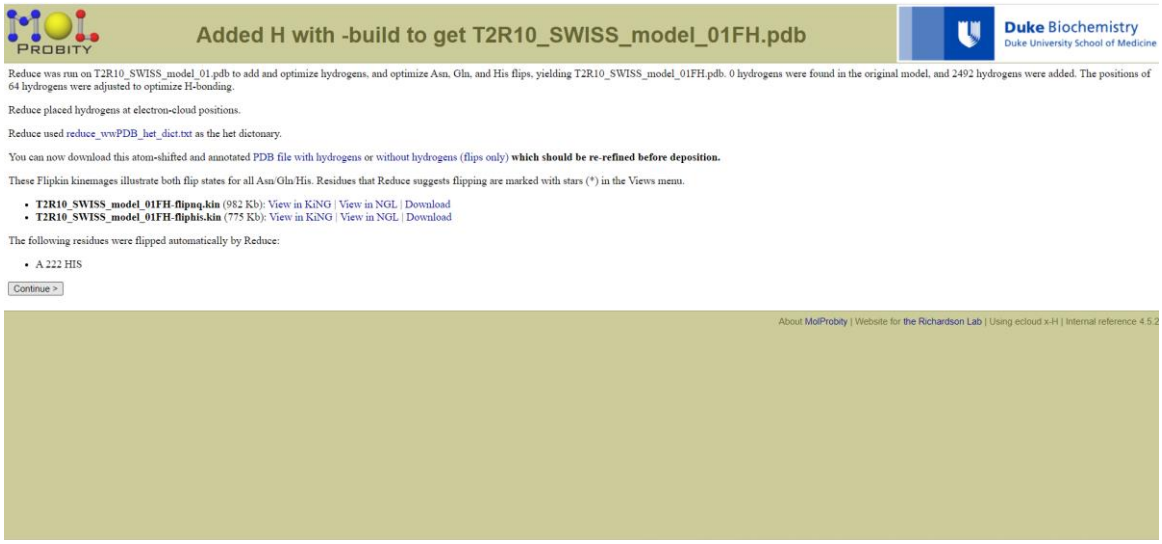

Fig 3.10 (f) Final Preview page.

This brings to the main page where “Analyze all-atom contacts and geometry” is selected as shown in fig 3.10 (g).

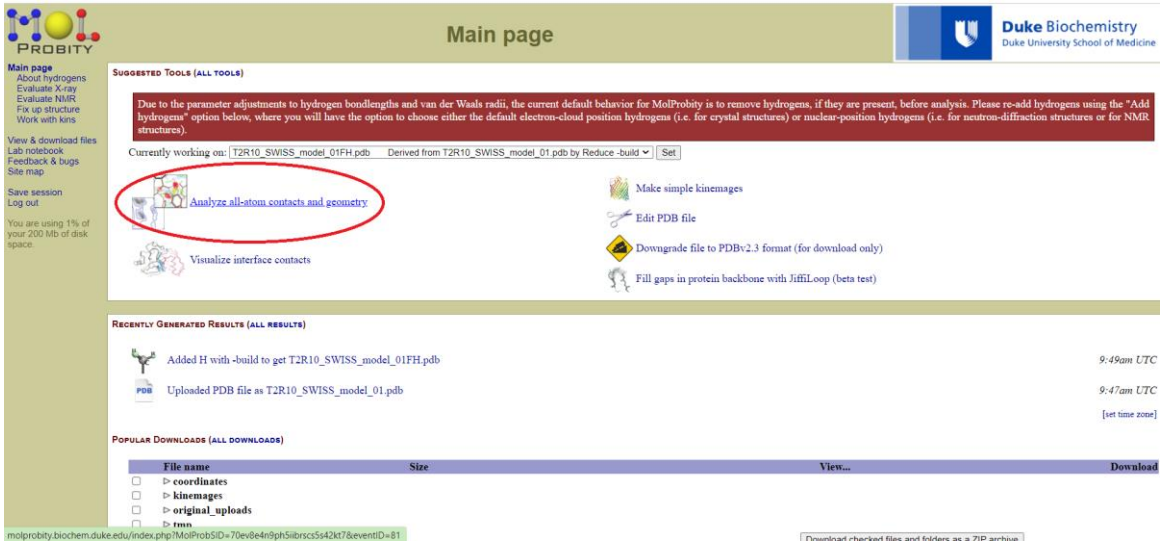

Fig 3.10 (g) Main page of analytical tools.

This would open a wide range of options which are checked by default according to the submitted file. Further 3 more options were selected namely “Make views of trouble spots even if it takes longer”, “Alternate conformations” and “Model colored by B-factors” as shown in fig 3.10 (h).

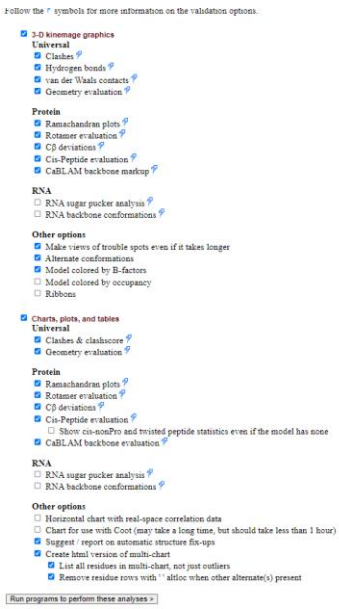

Fig 3.10 (h) Checklist for analysis parameters.

By selecting on “Run program to perform these analyses” it will bring us to the result page, as shown in fig 3.10 (i).

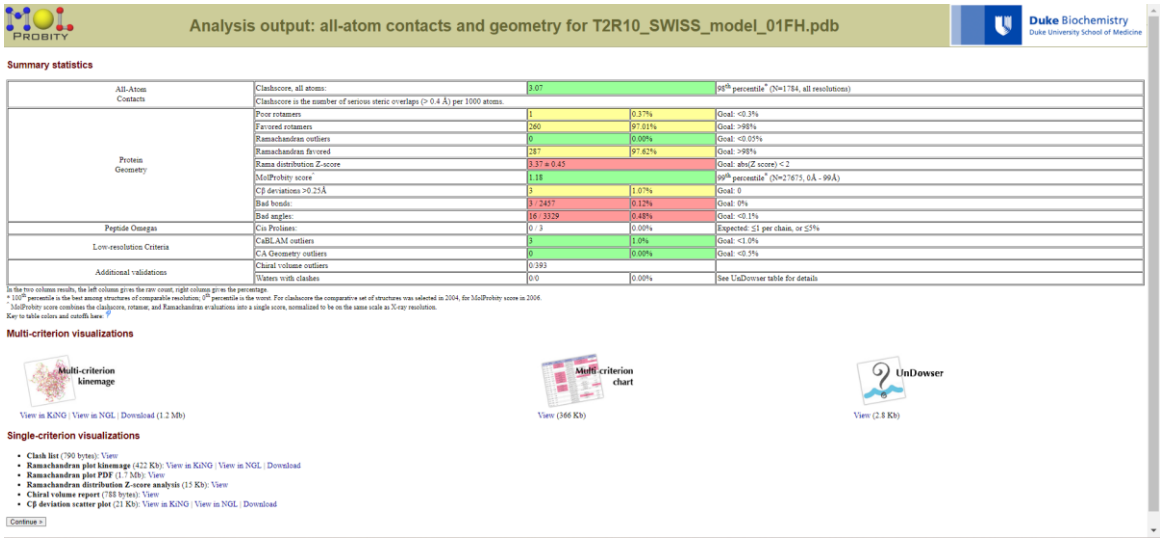

Fig 3.10 (i) Results page with several downloadable options.

“Multi-criterion chart” and “Ramachandran plot pdf” where downloaded for further use.

3.4 Ligand preparation

Using literature, bitter compounds were identified along with their threshold values with the help of BitterDB (https://bitterdb.agri.huji.ac.il) as shown in fig 3.11. After selection of

compound, solubility of compounds was also checked using AlogP values (ratio of solubility in octanol to the solubility in water of a particular compounds). A higher value of AlogP suggests lesser water solubility than octanol solubility and vice versa.

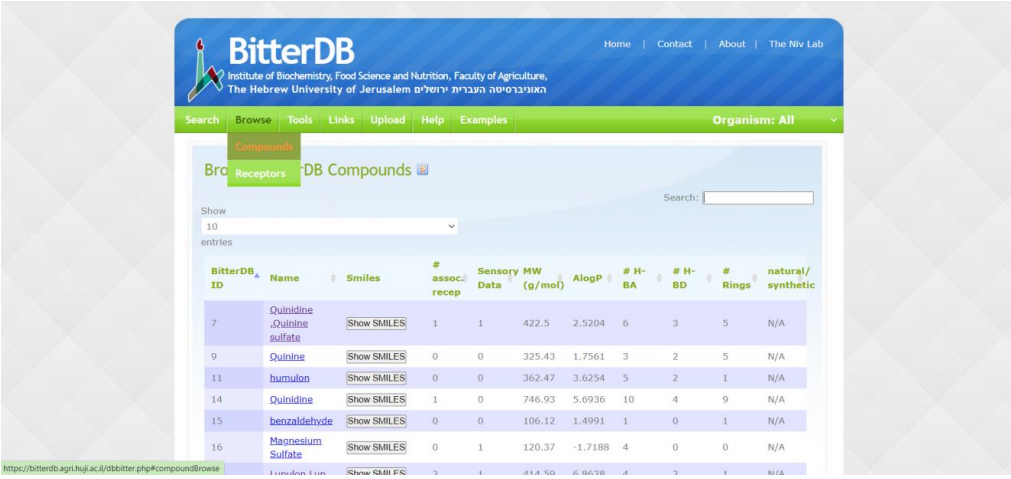

Fig 3.11 Shows the cover page of BitterDB for retrieval of bitter compounds.

The compounds with higher water solubility and low bitter threshold values were selected. Their canonical SMILES were retrieved from BitterDB (Dagan-Wiener et al., 2019) as well as from PubChem (https://pubchem.ncbi.nlm.nih.gov/). The SDF format of 3D structure of individual compounds was retrieved from PubChem as well (Kim et al., 2023).

Table 3.1 Six screened compounds along with their Bitter threshold and AlogP values.

S. No.	Compound Name	PubChem CID	Bitter Threshold [mM]	AlogP (octanol-water)
1	Amarogentin	115149	0.000029	1.0569
2	Cis-isohumulone	21594782	0.01	3.6254
3	Humulon	36	0.021	4.25220
4	Rubusoside	24721373	0.061	-0.7623
5	D-salicin	439503	0.2	-1.6424
6	Dulcoside A	72940582	0.049	-1.9105

ADME properties were evaluated using SWISSADME (http://www.swissadme.ch/). The ADME properties are features of a molecule that are related to its absorption, distribution, metabolism, excretion, and toxicity (Daina et al., 2017). The canonical SMILES of individual compounds were entered in the provided space of SWISSADME page and the program was executed by clicking on "Run!" as shown in fig 3.12(a).

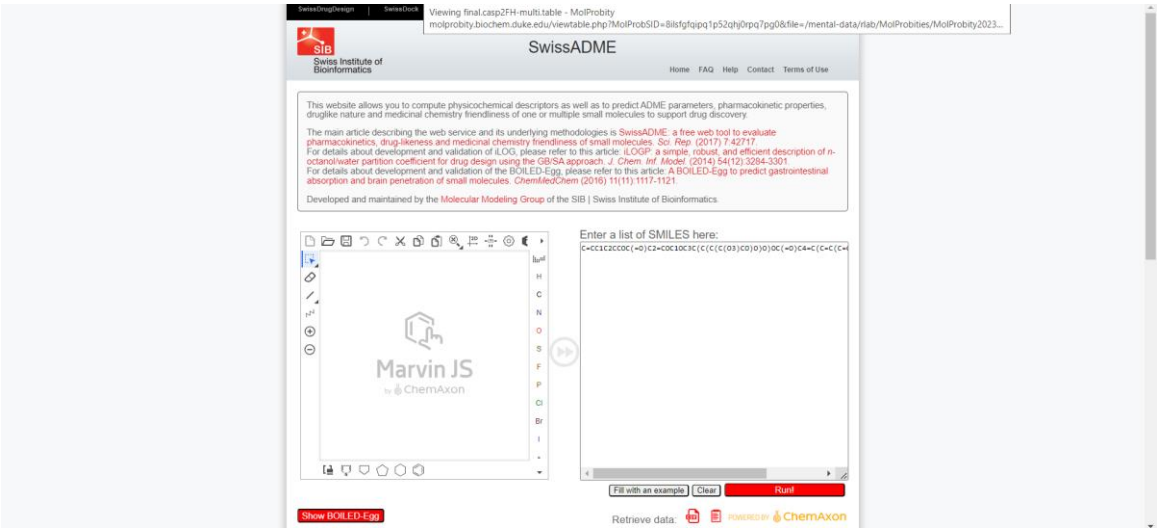

Fig 3.12(a) Home page of SWISSADME.

This would display the ADME properties of Amarogentin towards the bottom of the web page as shown in fig 3.12(b) below.

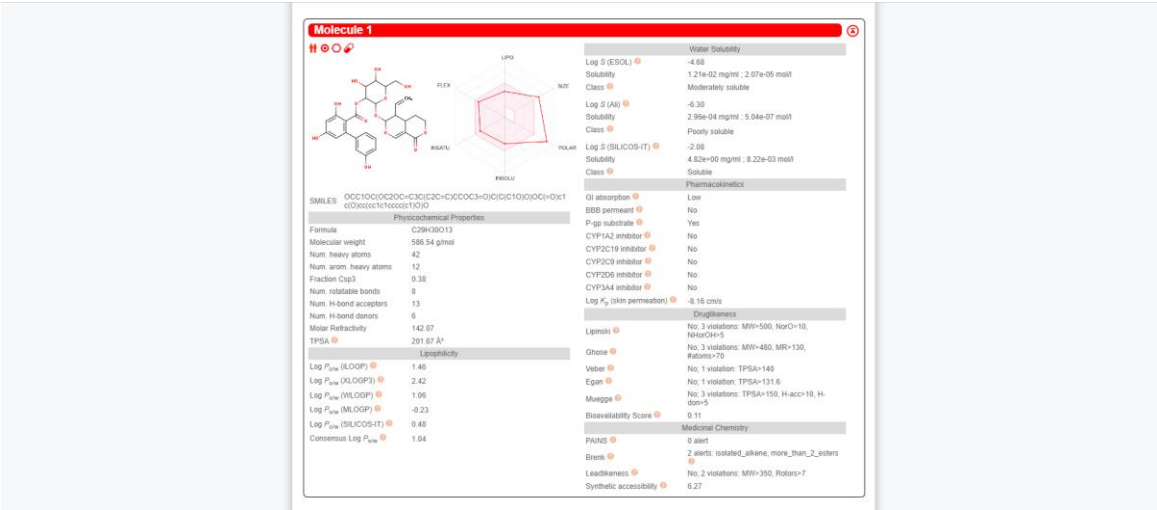

Fig 3.12(b) ADME properties of Amarogentin.

3.5 Protein-Ligand interaction studies

In the field of molecular modelling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using, for example, scoring functions. The associations between biologically relevant molecules such as proteins, peptides, nucleic acids, carbohydrates, and lipids play a central role in signal transduction. Furthermore, the relative orientation of the two interacting partners may affect the type of signal produced (e.g., agonism vs antagonism). Therefore, docking is useful for predicting both the strength and type of signal produced. Molecular docking is one of the most frequently used methods in structure-based drug design, due to its ability to predict the binding-conformation of small molecule ligands to the appropriate target binding site. Characterization of the binding behaviour plays an important role in rational design of drugs as well as to elucidate fundamental biochemical processes.

Blind Docking

Blind Docking refers to docking a ligand to the whole surface of a protein without any prior knowledge of the target pocket. Blind docking involves several trials/runs and several energy calculations before a favourable protein-ligand complex pose is found. However, the number of trials and energy evaluations necessary for a blind docking job is unknown. When it comes to Blind Docking, most -if not all- of the famous (non-exhaustive) docking tools are quite limited. That is because the stochastic nature of search for a fixed number of steps makes it unlikely to sample the whole energy landscape surface thoroughly enough to find all the important poses.

Molecular docking of the standard sweet compounds, whose sweetness index and gold fitness scores were known, was performed using the docking wizard of PyRx (Dallakyan & Olson, 2015). PyRx is a Virtual Screening software for Computational Drug Discovery that can be used to screen libraries of compounds against potential drug targets. PyRx enables Medicinal Chemists to run Virtual Screening from any platform and helps users in every step of this process - from data preparation to job submission and analysis of the results. While it is true that there is no magic button in the drug discovery process, PyRx includes docking wizard with an easy-to-use user interface which makes it a valuable tool

for Computer-Aided Drug Design. PyRx also includes chemical spreadsheet-like functionality and powerful visualization engine that are essential for structure-based drug design.

PyRx is an open source software to perform virtual screening. It is a combination of several softwares such as AutoDock Vina wizard, AutoDock 4.2, Mayavi, Open Babel, etc. PyRx uses Vina wizard and AutoDock 4.2 as docking softwares. In the present work we are going to use only Vina wizard.

The first step is to download pyrx. Once downloaded, start with loading molecules into pyrx workspace. For this use the upper left button to load protein or target into pyrx workstation.

Next step would be converting pdb files into pdbqt files (Vina wizard input file format).

After successfully loading molecules in to the workspace, convert them into AutoDock input files (pdbqt files) .

Right click on ligand(s) > AutoDock > Make ligand .

Right click on protein > AutoDock > Make Macromlecule.

Note: AutoDock requires all input files in pdbqt format (pdbqt file consists partial charges and atom types).

Converting pdb files to pdbqt files (Vina wizard input file format)

→ After converting pdb files into AutoDock input files (or pdbqt files), you will see them under AutoDock tab as shown below in fig. 3.13 (if you don't see any files, right click and refresh under AutoDock tab).

Now the protein and ligand(s) files are ready for docking.

→ Click on Start Here button under Vina Wizard.

→ Select Local button under Vina wizard execution Mode.

→ Click START button

Select protein and ligand(s) by simply clicking on them.

Click to Forward Run Vina wizard.

Selecting Vina wizard Search Space:

→ In this step you will see a grid box (white box with spherical handles) in the 3D scene as shown below. This grid box allows you to select search space (Part of the protein, where we are going to perform docking, typically the binding site) in the protein.

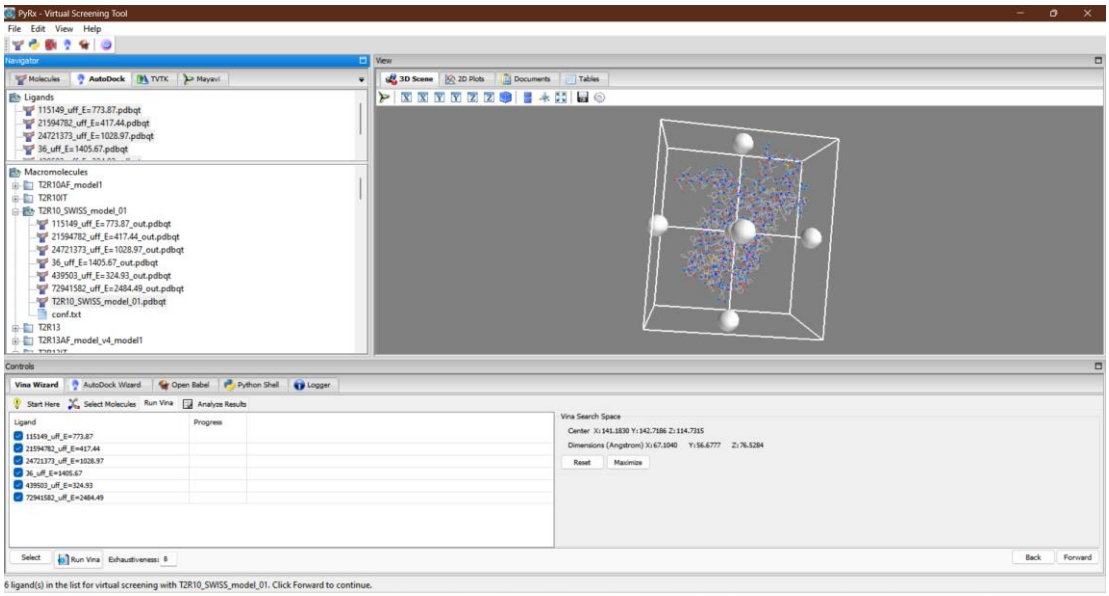

Fig 3.13 (a) PyRx showing all the windows.

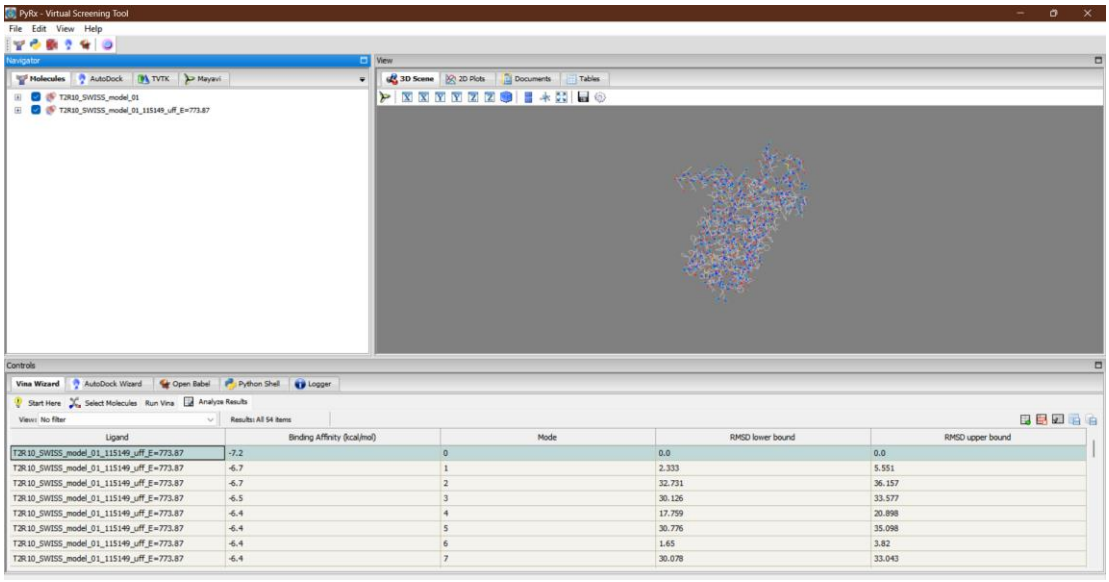

Fig 3.13(b) Results table in PyRx window.

→ Click molecules button under Navigator panel, then click on + button located in front of protein tab.

→ Now use the spherical handles of the grid box to select the search space as shown below. (Fig 3.13 (a))

→ Make sure you select the grid box size big enough to allow the ligand to move freely in the search space.

→ Use the search space (Vina wizard search space) values close the ones mentioned in the picture below, to get better results.

→ Click forward button to start Vina wizard calculations.

→Once the calculations are done, results will be populated as seen in the fig 3.13 (b) below, with the Binding Affinity (kcal/mol) values. More negative the binding affinity better the orientation of the ligand in the binding site.

Exporting Vina wizard Results:

→ Results can be exported to other software programs like Chimera for analysis.

→ Click on Edit > Preferences.

You will see a pop-up window . All the results will be saved in location specified as workspace.

→ Go to open Macromolecules file, all the results will be saved in protein folder .

→ The protein folder contains three files (protein.pdbqt, ligand1_out.pdbqt and conf.txt) If we use only one ligand for docking

→ ligand1_out.pdbqt contains 8 or 9 best poses (or orientations) of the ligand1 and conf.txt file contains search space (or grid box) parameters.

→ Save this protein folder at your convenient location for further analysis with BIOVIA Draw.

→ You are done with PyRx, now let's analyze the results by using BIOVIA Draw.

3.6 Regression Analysis of the Results

The most simplest way of performing mathematical modelling is the linear regression analysis. Linear regression is a predictive statistical approach for modelling relationship between a dependent variable with a given set of independent variables. It is a linear approach to modeling the relationship between a dependent variable and one or more independent variables. It is a linear approach to modeling the relationship between a dependent variable and one or more independent variables. When we have only one independent variable it is as called simple linear regression. For more than one independent variable, the process is called multiple linear regression.

In the present study, each parameter was evaluated for any possible linear relationship. For this, linear models were generated using Microsoft Excel, which also provides us with the R^2 values and the linear equation. This might help us in finding some inferences towards developing a mathematical model to predict sweetness and further understand prediction of the taste in general.

4. Results and Discussion

The sequence of the bitter taste receptors were retrieved from UniProtKB, then subjected to various structure prediction tools to predict their 3D structures, as their experimental protein structures are not available in protein databank.

4.1 Protein structure prediction

Structure of GPCRs are challenging to predict, owing to their transmembrane nature, which includes hydrophobic and hydrophilic surfaces on the protein molecule. In pursuit of a perfect model to ease the interaction studies, structure prediction was performed using PHYRE2, SWISS-MODEL and i-TASSER, while structures predicted using deep learning methods in the AlphaFold database were also used for interaction studies. Fig 4.1 shows the 3D models of T2R10 obtained from various tools. Similar structures were generated for the 24 bitter taste receptors.

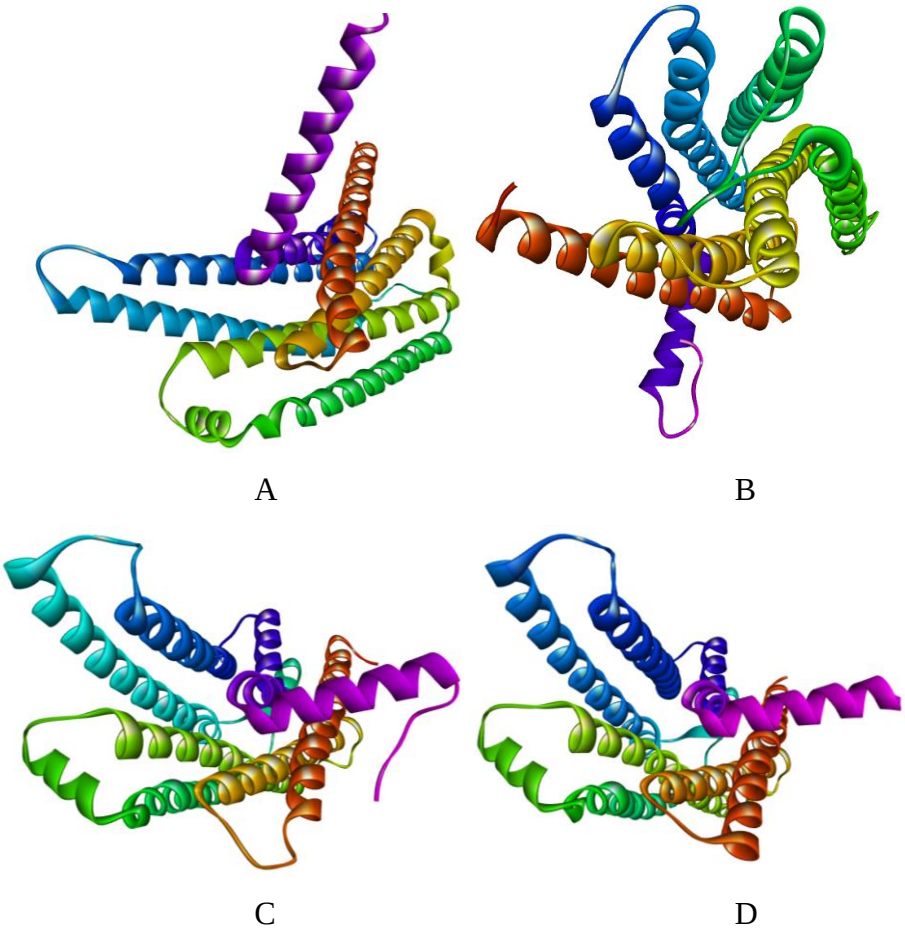

Fig 4.1 (a) T2R10 from AlphaFold, (b) model obtained using i-TASSER, (c) predicted model of PHYRE2, (d) predicted structure by SWISS-MODEL.

4.2 Model Validation

The 3D models were checked for the perfectness of their structure using standard algorithms of MolProbity. The criterion for assessment of models has been outlined in the MolProbity website, and is shown in Table 4.1.

Table 4.1 Criterion of assessment of MolProbity results.

Category	**Validation**	**Good**	**Caution**	**Warning**
All-Atom Contacts	Clashscore, all atoms:	Percentile ≥ 66	66 > Percentile ≥ 33	Percentile < 33
Protein Geometry	Poor rotamers:	Outliers ≤ 0.3%	0.3% < Outliers ≤ 1.5%	Outliers > 1.5%
	Favored rotamers:	Favored ≥ 98%	98% > Favored ≥ 95%	Favored < 95%
	Ramachandran outliers:	Outliers ≤ 0.05%	0.05% < Outliers ≤ 0.5% or Outliers > 0.5% and Outlier count = 1	Outliers > 0.5% and Outlier count ≥ 2
	Ramachandran favored:	Favored ≥ 98%	98% > Favored ≥ 95%	Favored < 95%
	Ramachandran Z-score:	abs(Z-score) ≤ 2	2 < abs(Z-score) ≤ 3	abs(Z-score) > 3%
	MolProbity score:	Percentile ≥ 66	66 > Percentile ≥ 33	Percentile < 33
	Cβ deviations >0.25Å:	Outlier count = 0	0 < Outliers < 5%	Outliers ≥ 5%
	Bad bonds:	Outlier bonds < 0.01%	0.01% ≤ Outlier bonds < 0.2%	Outlier bonds ≥ 0.2%
	Bad angles:	Outlier angles < 0.1%	0.1% ≤ Outlier angles < 0.5%	Outlier angles ≥ 0.5%

Peptide Omegas	Cis Prolines:	No suitable	universal cutoffs	for cisProline
	Cis nonProlines:	Peptides ≤ 0.05% Cis	0.05% < Cis Peptides ≤ 0.1%	Peptides > 0.1% Cis
	Twisted Peptides:	Twisted peptide count = 0	0 < Twisted peptides ≤ 0.1%	Peptides > 0.1% twisted
Nucleic Acid Geometry	Probably wrong sugar puckers:	Outlier count = 0	0 < Outliers ≤ 5%	Outliers > 5%
	Bad backbone conformations:	Outliers ≤ 5%	5% < Outliers ≤ 15%	Outliers > 15%
	Bad bonds:	Outlier bonds < 0.01%	0.01% ≤ Outlier bonds < 0.2%	Outlier bonds ≥ 0.2%
	Bad angles:	Outlier angles < 0.1%	0.1% ≤ Outlier angles < 0.5%	Outlier angles ≥ 0.5%
Low-resolution Criteria	CaBLAM outliers:	Outliers ≤ 1%	1% < Outliers ≤ 5%	Outliers > 5%
	CA Geometry outliers:	Outliers ≤ 0.5%	0.5% < Outliers < 1%	Outliers > 1%

The following tables enlist the key variables of protein structure validation through MolProbity of the bitter taste receptor models generated through various modelling tools.

Table 4.2 MolProbity result of the bitter taste receptor models of SWISS MODEL.

Uniprot ID	**Protein Geometry**			**Ramachandran Plot (%)**	
	Ramachandran outliers (%) (goal-<0.05%)	**Ramachandran favored (%) (goal->98%)**	**MolProbity score**	**Fav.**	**Allowed**
T2R10	0.00	97.62	1.18	97.6	100
T2R13	0.00	98.31	1.32	98.3	100
T2R14	0.95	95.87	1.11	95.9	99
T2R16	0.35	95.77	1.52	95.8	99.6

T2R19	0.00	98.64	0.88	98.6	100
T2R20	0.00	98.65	0.97	98.7	100
T2R30	0.00	98.65	0.97	98.7	100
T2R31	0.00	97.98	0.94	98	100
T2R38	1.31	94.43	1.56	94.4	98.7
T2R39	1.31	94.43	1.56	94.4	98.7
T2R40	1.19	94.64	1.30	94.6	98.8
T2R41	0.33	94.36	0.83	98.4	99.7
T2R43	0.00	98.32	0.87	98.3	100
T2R45	0.00	98.64	0.75	98.6	100
T2R46	0.00	98.64	0.75	98.6	100
T2R50	0.68	97.64	1.08	97.6	99.3
T2R60	0.63	98.42	0.83	98.4	99.4
TA2R1	0.63	98.42	0.83	98.4	99.4
TA2R3	1.06	97.76	0.77	97.8	99.7
TA2R4	0.32	97.76	0.77	97.8	99.7
TA2R5	0.37	98.32	0.64	98.3	99.3
TA2R7	0.00	98.42	0.65	98.4	100
TA2R8	0.00	97.39	1.06	97.4	100
TA2R9	0.65	96.45	1.31	96.5	99.4

Most of the structures predicted through SWISS MODEL showed favoured Ramachandran plot results.

Table 4.3 MolProbity result of the bitter taste receptor models of PHYRE2.

Uniprot ID	**Protein Geometry**			**R. chandran Plot (%)**	
	Ramachandran outliers (%) Goal: <0.05%	**Ramachandran favored (%) (goal->98%)**	**MolProbity score**	**Fav.**	**Allowed**
T2R10	0.33	97.38	2.36	97.4	99.7
T2R13	0.34	96.28	2.42	96.3	99.7
T2R14	1.27	93.97	2.6	94	98.7
T2R16	1.73	93.08	2.86	93.1	98.3
T2R19	0.34	95.96	2.33	96	99.7
T2R20	0.33	95.77	2.4	95.8	99.7
T2R30	1.58	94.32	2.43	94.3	98.4
T2R31	0.33	96.09	2.34	96.1	99.7
T2R38	1.81	92.45	2.62	92.4	98.2
T2R39	2.38	90.48	2.87	90.5	97.6

T2R40	2.38	90.48	2.87	90.5	97.6
T2R41	2.38	90.48	2.87	90.5	97.6
T2R43	0.00	97.07	2.24	97.1	100
T2R45	0.67	96.63	2.27	96.6	99.3
T2R46	0.98	95.77	2.37	95.8	99
T2R50	0.34	96.30	2.46	96.3	99.7
T2R60	1.67	94.67	2.69	94.7	98.3
TA2R1	5.72	82.83	3.08	82.8	94.3
TA2R3	0.96	94.27	2.59	94.3	99
TA2R4	2.36	90.91	2.86	90.9	97.6
TA2R5	1.35	94.61	2.55	94.6	98.7
TA2R7	1.27	95.89	2.33	95.9	98.7
TA2R8	1.95	93.49	2.70	93.5	98
TA2R9	1.61	93.87	2.50	93.9	98.4

Models obtained from PHYRE2 showed poor Ramachandran analysis results, with none of the structures with above 98% favoured residue positions.

Table 4.4 MolProbity result of the bitter taste receptor models of i-TASSER.

Uniprot ID	**Protein Geometry**			**R. chandran Plot (%)**	
	Ramachandran outliers (%) Goal: <0.05%	**Ramachandran favored (%) (goal->98%)**	**MolProbity score**	**Fav.**	**Allowed**
T2R10	6.23	82.62	2.93	82.6	93.8
T2R13	3.32	89.7	2.78	89.7	96.7
T2R14	5.4	84.44	3.06	84.4	94.6
T2R16	3.81	81.54	2.91	87.5	96.2
T2R19	3.37	87.54	2.94	87.5	96.6
T2R20	2.28	88.93	2.94	88.9	97.7
T2R30	3.79	88.33	2.90	88.3	96.2
T2R31	3.58	90.23	2.77	90.2	96.4
T2R38	9.97	81.27	3.31	81.3	90.0
T2R39	8.04	80.36	3.32	80.4	92.0
T2R40	7.84	80.69	2.94	80.7	92.5
T2R41	5.57	87.54	2.87	87.5	94.4
T2R43	5.54	84.69	2.98	84.7	94.5
T2R45	3.03	87.54	2.75	87.5	97.0
T2R46	6.51	83.39	3.17	83.4	93.5
T2R50	3.03	89.23	3.03	89.2	97.0

T2R60	5.06	83.23	2.89	83.2	94.9
TA2R1	4.04	86.20	2.74	86.2	96.0
TA2R3	3.50	85.67	3.07	85.7	96.5
TA2R4	3.37	90.57	2.47	90.6	96.6
TA2R5	5.05	86.20	2.85	86.2	94.9
TA2R7	3.80	86.08	2.84	86.1	96.2
TA2R8	7.49	82.41	2.90	82.4	92.5
TA2R9	2.90	88.06	2.84	88.1	97.1

Models obtained through i-TASSER failed miserably in the Ramachandran analysis, with Ramachandran favoured region residues around 80 to 85% in all the models.

Table 4.5 MolProbity result of the bitter taste receptor models of AlphaFold.

Uniprot ID	**Protein Geometry**			**R. chandran Plot (%)**	
	Ramachandran outliers (%) Goal: <0.05%	**Ramachandran favored (%) (goal->98%)**	**MolProbity score**	**Favoured (98%) regions**	**Allowed (>99.8%) regions**
T2R10	0.98	96.72	0.19	96.7	99
T2R13	0.33	98.67	1.02	98.7	99.7
T2R14	0.95	96.19	1.48	96.2	99
T2R16	1.04	96.89	1.29	96.9	99
T2R19	1.35	95.62	1.43	98.6	98.7
T2R20	0.98	98.05	1.15	98	99
T2R30	0.32	97.48	1.62	97.4	99.7
T2R31	0.33	98.37	0.90	98.4	99.7
T2R38	1.21	96.37	1.71	96.4	98.8
T2R39	2.38	93.75	1.77	93.8	97.6
T2R40	0.62	96.26	1.41	96.3	99.4
T2R41	0.66	97.05	1.54	97.0	99.3
T2R43	0.00	98.70	0.96	98.7	100
T2R45	0.00	98.70	1.08	98.7	100
T2R46	0.00	98.05	0.87	98.0	100
T2R50	0.67	98.99	1.02	99.0	99.3
T2R60	0.95	97.47	1.50	97.5	99.1
TA2R1	0.34	96.97	1.32	97.0	99.7
TA2R3	0.34	96.97	1.32	97.0	99.7
TA2R4	1.35	94.28	1.21	94.3	98.7
TA2R5	1.01	96.63	1.84	96.6	99.0
TA2R7	0.32	97.47	1.07	97.5	99.7

TA2R8	0.65	97.72	1.47	97.7	99.3
TA2R9	0.97	96.77	1.72	96.8	99

Many AlphaFold database models were found to be favourable according to the MolProbity analysis report, with adequate amount (>98%) of amino acid residues of several bitter taste receptors lying in the Ramachandran favoured regions.

The above tables also show the favoured and allowed percentage of amino acid residues as depicted in the Ramachandran plot of the models. Fig 4.1 shows the Ramachandran plot of T2R10 bitter taste receptor model generated through as a sample. Similar ramachnadran plots were obtained and analyzed for all the bitter taste receptors.

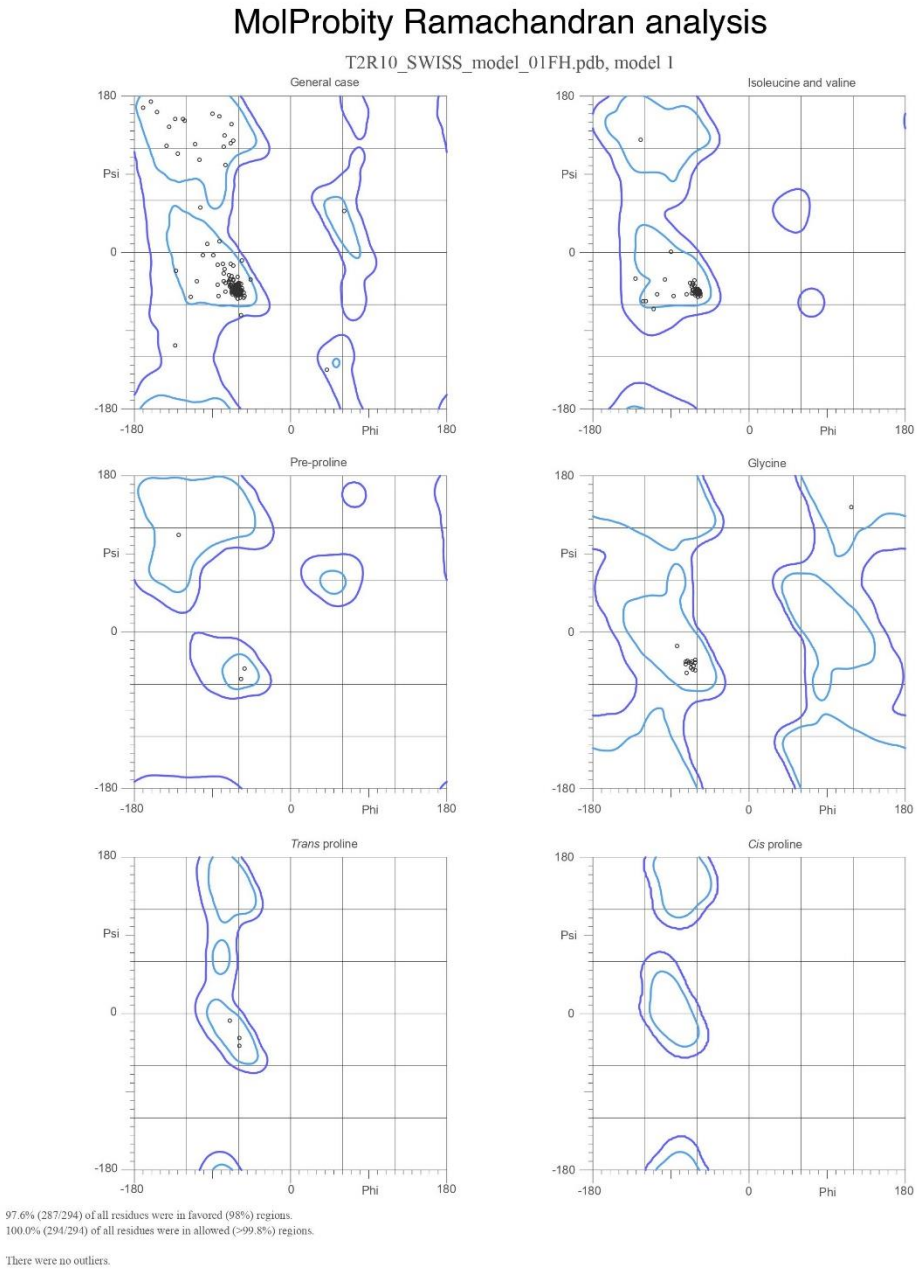

Fig 4.1 Ramachandran plots of T2R10 model obtained through SWISS MODEL.

In this case of T2R10 model predicted through SWISS MODEL, there are no outlier residues in the model, only few residues are outside the favoured regions and within the allowed region boundaries. Isoleucine and valine residues are also in their proper conformations. Proline is a very critical amino acid for the structure of proteins, thus, a separate Ramachandran plot depicts no faulty proline residues. Glycine being the smallest amino acid is also critically analyzed in MolProbity, as a faulty glycine residue would make the protein model questionable. In the present situation glycine residues are found to be located in the favoured regions.

4.3 Ligand preparation

Standard bitter compounds with experimentally established bitterness threshold values in the literature through sensory analysis were screened from BitterDB database according to their water solubility (AlogP values) and bitter threshold values. A low bitter threshold value signifies that the compound is more bitter in taste. Water soluble and sparingly water soluble ligands were selected. The screened ligands were checked for their ADME properties to obtain their molecular descriptors. Table 4.6 enlists the ADME properties of the screened ligands.

Table 4.6 ADME properties obtained using SWISS ADME.

S. No.	Compound Name	Pharmacokinetics		Druglikeness			Lipophilicity					
		GI absorption	BBB permeant	Lipinski	Ghose	Veber	Log $P_{o/w}$ (iLOGP)	Log Po/w (XLOGP3)	Log Po/w (WLOGP)	Log $P_{o/w}$ (MLOGP)	Log $P_{o/w}$ (SILICOS-IT	Consensus Log $P_{o/w}$
1	Humulon	High	No	Yes	Yes	Yes	3.18	4.18	4.25	1.13	4.19	3.39
2	D-salicin	Low	No	Yes	No	Yes	1.76	-1.22	-1.79	-1.48	-0.85	-0.72
3	Cis-isohumulone	High	No	Yes	Yes	Yes	1.88	3.84	3.63	1.13	4.39	2.97
4	Amarogentin	Low	No	No	No	No	1.46	2.45	1.06	-0.23	0.48	1.04
5	Dulcoside A	Low	No	No	No	No	2.79	-0.70	-1.91	-2.34	-1.82	-0.80
6	Rubusoside	Low	No	No	No	No	3.33	0.39	-0.76	-0.88	-0.34	0.35

All the screened molecules were non-toxic, which either passed the drug likeness rule or had low Gastro-intestinal absorptivity (GI absorption). All the ligands were found to be impermeant to the blood-brain-barrier (BBB). Further, before proceeding to docking studies, the ".sdf" files and canonical SMILES of the screened ligands were retrieved from PubChem.

4.4 Protein-Ligand interaction studies

Binding affinities were obtained using blind docking analysis of the bitter taste receptors with the standard bitter compounds screened form BitterDB using PyRx. The binding affinities with zero RMSD values were selected for further work on finding a relationship with the bitterness threshold values of the ligands. The following table was prepared to perform the regression analysis. Table 4.7 shows the data obtained for models generated through SWISS MODEL. Similar tables and later graphs were prepared for regression analysis of all the receptor models.

Table 4.7 Docking results with bitter threshold, AlogP values and regression coefficient (R^2) values.

UniProt ID	**LIGANDS**	**Binding Affinity (kcal/mol)**	**Bitter Threshold [mM]**	**AlogP (octanol-water)**
T2R10	115149	-7.2	0.000029	1.0569
$R^2 = 0.03$	21594782	-6.8	0.01	3.6254
	36	-6.7	0.021	4.25220
	24721373	-6.8	0.061	-0.7623
	439503	-6.8	0.2	-1.6424
	72940582	-7.8	0.049	-1.9105
UniProt ID	**LIGANDS**	**Binding Affinity (kcal/mol)**	**Bitter Threshold [mM]**	**AlogP (octanol-water)**
T2R13	115149	-7.6	0.000029	1.0569
$R^2 = 0.1111$	21594782	-6.1	0.01	3.6254
	36	-6.5	0.021	4.25220
	24721373	-8	0.061	-0.7623
	439503	-6.1	0.2	-1.6424
	72940582	-7.5	0.049	-1.9105

UniProt ID	LIGANDS	Binding Affinity (kcal/mol)	Bitter Threshold [mM]	AlogP (octanol-water)
T2R14	115149	-8.9	0.000029	1.0569
R^2 = 0.448	21594782	-8.2	0.01	3.6254
	36	-7.5	0.021	4.25220
	24721373	-8.8	0.061	-0.7623
	439503	-6.6	0.2	-1.6424
	72940582	-9.3	0.049	-1.9105
UniProt ID	**LIGANDS**	**Binding Affinity (kcal/mol)**	**Bitter Threshold [mM]**	**AlogP (octanol-water)**
T2R16	115149	-7	0.000029	1.0569
R^2 = 0.3801	21594782	-7.3	0.01	3.6254
	36	-6.3	0.021	4.25220
	24721373	-7.3	0.061	-0.7623
	439503	-7.7	0.2	-1.6424
	72940582	-7.4	0.049	-1.9105
UniProt ID	**LIGANDS**	**Binding Affinity (kcal/mol)**	**Bitter Threshold [mM]**	**AlogP (octanol-water)**
T2R19	115149	-7.1	0.000029	1.0569
R^2 = 0.4359	21594782	-5.9	0.01	3.6254
	36	-7.4	0.021	4.25220
	24721373	-6.8	0.061	-0.7623
	439503	-5.5	0.2	-1.6424
	72940582	-7.1	0.049	-1.9105
UniProt ID	**LIGANDS**	**Binding Affinity (kcal/mol)**	**Bitter Threshold [mM]**	**AlogP (octanol-water)**
T2R20	115149	-7.7	0.000029	1.0569
R^2 = 0.2304	21594782	-7.5	0.01	3.6254
	36	-7.2	0.021	4.25220
	24721373	-7.9	0.061	-0.7623
	439503	-7.1	0.2	-1.6424
	72940582	-7.3	0.049	-1.9105

UniProt ID	LIGANDS	Binding Affinity (kcal/mol)	Bitter Threshold [mM]	AlogP (octanol-water)
T2R30	115149	-7.6	0.000029	1.0569
R^2 = 0.3368	21594782	-6.7	0.01	3.6254
	36	-6.1	0.021	4.25220
	24721373	-7.5	0.061	-0.7623
	439503	-5.6	0.2	-1.6424
	72940582	-7.8	0.049	-1.9105
UniProt ID	**LIGANDS**	**Binding Affinity (kcal/mol)**	**Bitter Threshold [mM]**	**AlogP (octanol-water)**
T2R31	115149	-7.4	0.000029	1.0569
R^2 = 0.3249	21594782	-6.3	0.01	3.6254
	36	-6.9	0.021	4.25220
	24721373	-7.3	0.061	-0.7623
	439503	-7.6	0.2	-1.6424
	72940582	-7.3	0.049	-1.9105
UniProt ID	**LIGANDS**	**Binding Affinity (kcal/mol)**	**Bitter Threshold [mM]**	**AlogP (octanol-water)**
T2R38	115149	-7.2	0.000029	1.0569
R^2 = 0.3888	21594782	-6.3	0.01	3.6254
	36	-7.1	0.021	4.25220
	24721373	-7.7	0.061	-0.7623
	439503	-5.4	0.2	-1.6424
	72940582	-7.9	0.049	-1.9105
UniProt ID	**LIGANDS**	**Binding Affinity (kcal/mol)**	**Bitter Threshold [mM]**	**AlogP (octanol-water)**
T2R39	115149	-8.8	0.000029	1.0569
R^2 = 0.4746	21594782	-6.9	0.01	3.6254
	36	-7	0.021	4.25220
	24721373	-7.6	0.061	-0.7623
	439503	-5.9	0.2	-1.6424
	72940582	-8.2	0.049	-1.9105

UniProt ID	LIGANDS	Binding Affinity (kcal/mol)	Bitter Threshold [mM]	AlogP (octanol-water)
T2R40	115149	-7.4	0.000029	1.0569
R^2 = 0.5598	21594782	-7	0.01	3.6254
	36	-7	0.021	4.25220
	24721373	-7.3	0.061	-0.7623
	439503	-5.8	0.2	-1.6424
	72940582	-8	0.049	-1.9105
UniProt ID	**LIGANDS**	**Binding Affinity (kcal/mol)**	**Bitter Threshold [mM]**	**AlogP (octanol-water)**
T2R41	115149	-7.8	0.000029	1.0569
R^2 = 0.1976	21594782	-6.3	0.01	3.6254
	36	-6.8	0.021	4.25220
	24721373	-7.1	0.061	-0.7623
	439503	-6.3	0.2	-1.6424
	72940582	-7.8	0.049	-1.9105
UniProt ID	**LIGANDS**	**Binding Affinity (kcal/mol)**	**Bitter Threshold [mM]**	**AlogP (octanol-water)**
T2R43	115149	-7.1	0.000029	1.0569
R^2 = 0.2952	21594782	-6.3	0.01	3.6254
	36	-6.6	0.021	4.25220
	24721373	-7.9	0.061	-0.7623
	439503	-7.5	0.2	-1.6424
	72940582	-7	0.049	-1.9105
UniProt ID	**LIGANDS**	**Binding Affinity (kcal/mol)**	**Bitter Threshold [mM]**	**AlogP (octanol-water)**
T2R45	115149	-7.7	0.000029	1.0569
R^2 = 0.0504	21594782	-6.8	0.01	3.6254
	36	-7.4	0.021	4.25220
	24721373	-7.6	0.061	-0.7623
	439503	-7.5	0.2	-1.6424
	72940582	-7.4	0.049	-1.9105

UniProt ID	**LIGANDS**	**Binding Affinity (kcal/mol)**	**Bitter Threshold [mM]**	**AlogP (octanol-water)**
T2R46	115149	-7.6	0.000029	1.0569
R^2 = 0.0069	21594782	-6.7	0.01	3.6254
	36	-6.5	0.021	4.25220
	24721373	-8.5	0.061	-0.7623
	439503	-7.2	0.2	-1.6424
	72940582	-8.1	0.049	-1.9105
UniProt ID	**LIGANDS**	**Binding Affinity (kcal/mol)**	**Bitter Threshold [mM]**	**AlogP (octanol-water)**
T2R50	115149	-9.7	0.000029	1.0569
R^2 = 0.2117	21594782	-7.6	0.01	3.6254
	36	-6.7	0.021	4.25220
	24721373	-9.1	0.061	-0.7623
	439503	-6.7	0.2	-1.6424
	72940582	-8.9	0.049	-1.9105
UniProt ID	**LIGANDS**	**Binding Affinity (kcal/mol)**	**Bitter Threshold [mM]**	**AlogP (octanol-water)**
T2R60	115149	-7.9	0.000029	1.0569
R^2 = 0.2024	21594782	-6.1	0.01	3.6254
	36	-6.6	0.021	4.25220
	24721373	-7.9	0.061	-0.7623
	439503	-5.9	0.2	-1.6424
	72940582	-7.7	0.049	-1.9105
UniProt ID	**LIGANDS**	**Binding Affinity (kcal/mol)**	**Bitter Threshold [mM]**	**AlogP (octanol-water)**
TA2R1	115149	-6.6	0.000029	1.0569
R^2 = 0.1149	21594782	-5.8	0.01	3.6254
	36	-6.2	0.021	4.25220
	24721373	-6.9	0.061	-0.7623
	439503	-6.7	0.2	-1.6424

	72940582	-7.2	0.049	-1.9105
UniProt ID	**LIGANDS**	**Binding Affinity (kcal/mol)**	**Bitter Threshold [mM]**	**AlogP (octanol-water)**
TA2R3	115149	-7.3	0.000029	1.0569
$R^2 = 0.0862$	21594782	-6.8	0.01	3.6254
	36	-7.8	0.021	4.25220
	24721373	-8.5	0.061	-0.7623
	439503	-6.6	0.2	-1.6424
	72940582	-9	0.049	-1.9105
UniProt ID	**LIGANDS**	**Binding Affinity (kcal/mol)**	**Bitter Threshold [mM]**	**AlogP (octanol-water)**
TA2R4	115149	-6.9	0.000029	1.0569
$R^2 = 0.12$	21594782	-7.8	0.01	3.6254
	36	-7.8	0.021	4.25220
	24721373	-7.2	0.061	-0.7623
	439503	-7.1	0.2	-1.6424
	72940582	-7.7	0.049	-1.9105
UniProt ID	**LIGANDS**	**Binding Affinity (kcal/mol)**	**Bitter Threshold [mM]**	**AlogP (octanol-water)**
TA2R5	115149	-7.3	0.000029	1.0569
$R^2 = 0.0854$	21594782	-8.4	0.01	3.6254
	36	-8.6	0.021	4.25220
	24721373	-7	0.061	-0.7623
	439503	-7.5	0.2	-1.6424
	72940582	-7.3	0.049	-1.9105
UniProt ID	**LIGANDS**	**Binding Affinity (kcal/mol)**	**Bitter Threshold [mM]**	**AlogP (octanol-water)**
TA2R7	115149	-8	0.000029	1.0569
$R^2 = 0.169$	21594782	-6.8	0.01	3.6254
	36	-8.1	0.021	4.25220
	24721373	-7.1	0.061	-0.7623

	439503	-7	0.2	-1.6424
	72940582	-8.2	0.049	-1.9105
UniProt ID	**LIGANDS**	**Binding Affinity (kcal/mol)**	**Bitter Threshold [mM]**	**AlogP (octanol-water)**
TA2R8	115149	-6.9	0.000029	1.0569
$R^2 = 0.2373$	21594782	-6.7	0.01	3.6254
	36	-7.1	0.021	4.25220
	24721373	-8.2	0.061	-0.7623
	439503	-7.6	0.2	-1.6424
	72940582	-7.7	0.049	-1.9105
UniProt ID	**LIGANDS**	**Binding Affinity (kcal/mol)**	**Bitter Threshold [mM]**	**AlogP (octanol-water)**
TA2R9	115149	-7.5	0.000029	1.0569
$R^2 = 0.6444$	21594782	-7	0.01	3.6254
	36	-7.8	0.021	4.25220
	24721373	-7.3	0.061	-0.7623
	439503	-6	0.2	-1.6424
	72940582	-7.9	0.049	-1.9105

4.5 Regression Analysis of the Results

Regression analysis was done to check for any linear relationship between the bitter threshold values and the binding affinities. This was executed using MS-Excel, by generating linear trendline in the scatter plot of Binding affinity (x-axis) versus Bitterness threshold (y-axis). The models were sorted according to their regression coefficients in descending order and those with the highest regression values are presented in table 4.8 along with the model quality parameters.

Table 4.8 Comparison of R^2 values and model quality

UniProt ID	**Source**	**Regression Coefficient (R^2)**	**Ramachandran outliers (%) (goal- <0.05%)**	**Ramachandran favored (%) (goal- >98%)**
TA2R3	i-TASSER	0.9124	3.50	85.67
T2R38	PHYRE2	0.8916	1.81	92.45
TA2R1	PHYRE2	0.7831	5.72	82.83
TA2R9	PHYRE2	0.6444	1.61	93.87
TA2R9	SWISS MODEL	0.6444	0.65	96.45
TA2R3	AlphaFold	0.6383	0.34	96.97
TA2R7	AlphaFold	0.5863	0.32	97.47
T2R45	i-TASSER	0.5752	3.03	87.54
T2R46	i-TASSER	0.5662	6.51	83.39
T2R40	SWISS MODEL	0.5598	1.19	94.64
T2R19	AlphaFold	0.5418	1.35	95.62
T2R20	AlphaFold	0.5416	0.98	98.05

The simplest form of relationship is linear, which has been nearly achieved in the interaction studies of TA2R3 model obtained using i-TASSER (regression coefficient value of 0.9124), although the model quality is not acceptable (Favoured = 85.67%).

5 Conclusion

The regression analysis of TA2R3 model generated through i-TASSER provides a promising model for further interactive studies. T2R38 generated through PHYRE2 is also close enough, with a regression coefficient value of 0.8916 and a better model quality (Favoured = 92.45%), which could be parallelly used for further interactive studies. Blind docking has been performed in the present work, and active site (targeted) docking is proposed for future work. The graph obtained for the two cases are shown in Fig 5.1 (a and b).

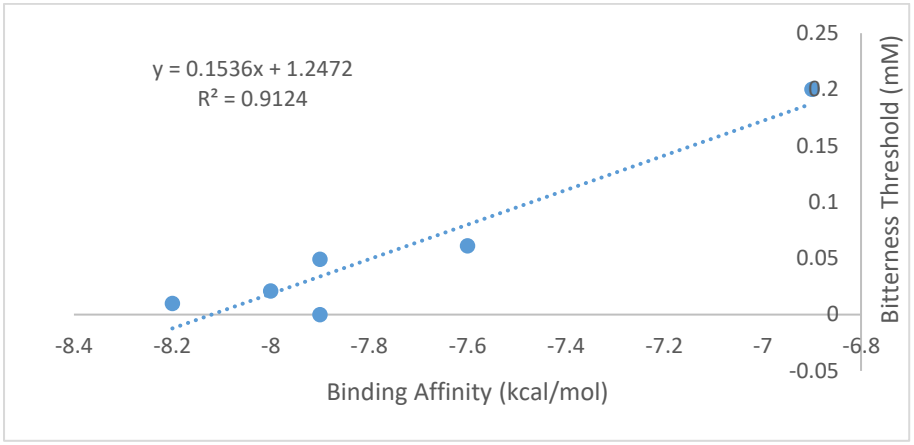

Fig 5.1 (a) Regression analysis of TA2R3 model (i-TASSER).

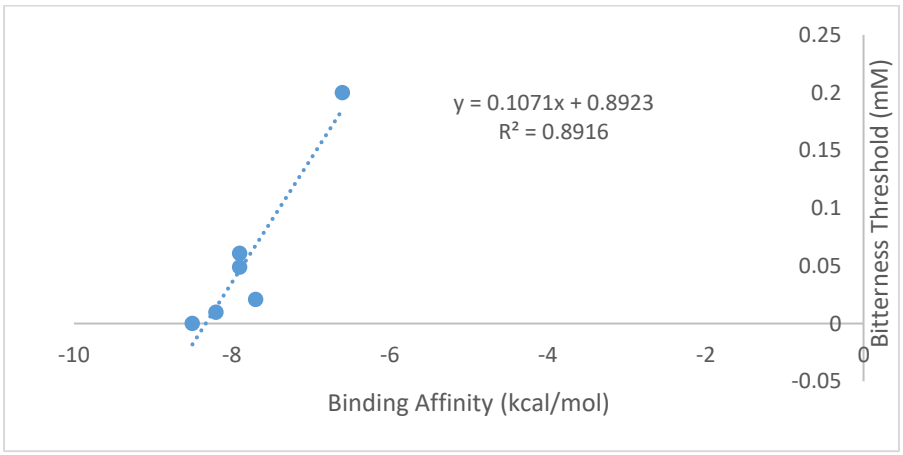

Fig 5.1 (b) Regression analysis of T2R38 model (PHYRE2).

The linear equations of both the models have to be validated with a test sample of standard bitter compounds.

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