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Interactions between (4Z)-hex-4-en-1-ol and 2-methylbutyl 2-methylbutanoate with olfactory receptors using computational methods

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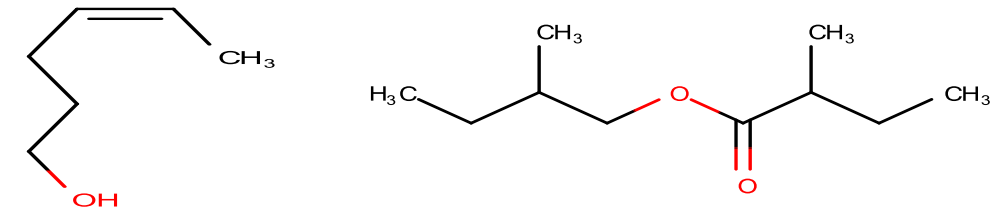
Abstract

The first step in the perception of an odor is the activation of one or more olfactory receptors (ORs) following binding of the odorant molecule to the OR. The compounds (4Z)-hex-4-en-1-ol and 2-methylbutyl 2-methylbutanoate are two important odorant molecules known as food flavor. In this research, we have investigated the potential targets for these two molecules, and we have tried to interpret the type of binding to different ORs models and their relationship with the retention/release property. We have used the SWISS-MODEL modelling server to predict the three-dimensional (3D) structure of the ORs. Then we have used the Autodock vina and Autodock tools to predict the binding site and binding energy of the ligands to these receptors. The results indicate that the molecule: (4Z)-hex-4-en-1-ol has given more hydrogen bonds with the majority of these receptors, whereas the molecule: 2-methylbutyl 2-methylbutanoate mainly has given Pi bond interaction types.

Keywords: *Odorant molecules, Retention/release property, Interaction, Docking, Olfactory Receptors.*

1.Introduction

Olfactory receptors (ORs) are rhodospin protein receptor, classified under R-Class (GRAFS: Glutamate, Rhodospin, Adhesion, Frizzled/Taste2 and Secretin), and belong to the G protein-coupled receptors (GPCRs), they are encoded by exceptional large multi-gene family [1,2]. The number of OR genesis are about 860 in humans [3]. They represent the largest gene super family. This abundance is justified by the large number of physiological functions in which olfaction is involved. ORs are located in the membrane of the dendrites of olfactory sensory neurons (OSNs), where they physiologically detect and discriminate diverse odorant molecules. Single OR can detect various odorant molecules and vice versa single odorant can activate various ORs. Besides their well-known function to sensing odorant, ORs are involved in OSN sorting and targeting to the olfactory bulb [4,5], and in non-olfactory tissues. This research focuses on binding odorant on ORs, and the transduction of the olfactory signal. Since binding mechanism is not well defined for most odorants on ORs [6], we will investigate binding of two volatile molecules using computational methods, and we will try to explain the way of binding to ORs. In our previous work [7] we have studied a series of 51 odorant molecules used as food flavor [8]. In the same study we used different computational and statistical methods (Principal Components Analysis (PCA), Multiple Linear Regression (MLR), Multiple Non-Linear Regression (RNLM) and an Artificial Neural Network (ANN)) to establish relationship between the structure of odorant and their retention/release property in pectin gel, then we have proposed mathematical models which we have validated, and we have finished our study by proposing new molecules with higher and lower values of the property than the existing ones [7]. We have concluded that all used models have substantially good predictive capacity, but MLR method gives the most accurate results. Among the list of 51 studied molecules we have chosen two molecules: (4Z)-hex-4-en-1-ol that has the highest retention (lowest release) property value (Log (1/K) = 3.324), and 2-methylbutyl 2-methylbutanoate that has the lowest retention (highest release) property value (Log (1/K) = 1.880). These two ligands are characterized by large different retention/release property values (highest and lowest values), to investigate the relationship between this property and the ligands-ORs interactions, using docking analysis, which offers a bigger picture of interactions between ligand and receptors [9,10]. So these interactions (like: steric, electrostatic, hydrogen bond donor, hydrogen bond acceptor...) may influence the values of the retention/release property and vice versa retention/release property can indicate the types of interactions that will form between ligand and ORs. Therefore, docking analysis was applied to study the interactions between the two compounds ((4Z)-hex-4-en-1-ol and 2-methylbutyl 2-methylbutanoate) and five ORs. Figure 1 show the structures of the studied compounds.



(4Z)-hex-4-en-1-ol 2-methylbutyl 2-methylbutanoate

Figure 1. Structures of the studied compounds.

2. Materials and methods

2.1. Data set for analysis

In this work, on the basis of our previous study of the 51 odorants molecules [7] we have selected two molecules (Figure 1). The retention/release properties of the selected compounds were examined using pectin gels (pectin

concentration was 0.8% w/w), these properties were quantified by the vapor-liquid partition coefficient K, and more precisely, by the Log(1/K) values [8]. In our previous work, the Quantitative Structure Property Relationship (QSPR) method was performed to explain and predict the relationship between chemical structure and the studied property (Log (1/K) using MLR, MNLR and ANN methods. These methods aimed to identify the molecular properties (molecular descriptors) that govern this phenomenon assuming that modifying the structure leads automatically to a change in the retention/release property of odorant molecules. All resulting models have good predictive capability, specially MLR model [7]. The resulting regression equation of MLR model (with a correlation coefficient of 0.958) is as follows:

$$\mathbf{Log}\left(\frac{\mathbf{1}}{\mathbf{K}}\right) = 9.905 - 2.976 \times 10^{-4} \times \mathrm{H}° + 0.651 \times KH - 6.154 \times \mathrm{n} - 0.145 \times J$$

The relationship obtained using this method corresponds to the linear combination of these descriptors: Heat of formation (H°), Henry's law constant (*K*H), Index of refraction (n) and Balaban index (*J*). The regression equation indicate the positive correlation of the Henry's law constant *K*H and negative correlation of Heat of formation (H°), Index of refraction (n) and Balaban index (*J*) with the studied property (Log(1/K)). These results show that, to increase retention property (decrease release property) of odorant molecules, we will increase *K*H and decrease H°, n and *J* descriptors. Moreover, to increase release property (decrease retention property), we will decrease *K*H and increase H°, n and *J* descriptors of this molecules, by adding suitable substituents and calculated their property using the regression equation.

2.2. Computational analysis of ORs

The ORs belonging to the GPCRs, where proteins are grouped into five classes: glutamate, rhodopsin, adhesion, frizzled and secretin, the ORs are rhodospin protein receptor. So, we would only consider GPCRs of the rhodopsin class. This class is divided into four sub-classes α, β, γ and δ [11], where olfactory receptors (ORs) belong to the δ sub-class and form a monophyletic group. Guillaume et al [12] report the most successful work on ORs targeting and their templates, including two receptors of the δ sub-class that supposed to be the closest relatives of the ORs (P2Y purino receptor 12 (P2Y12) and the human protease activated receptor1) [6].

Table 1. Different selected models, their Template and their sequence identity.

Template	OR model	Seq Identity	Oligo-state	Found by	Method	Resolution	Seq Similarity	Coverage	Description
4zwj.1.A	OR14I1	18.35	MONOMER	HHblits	X-RAY	3.30Å	0.29	0.89	Human Rhodopsin
5vbl.1.B	OR14J1	15.89	MONOMER	HHblits	X-RAY	2.60Å	0.28	0.94	Apelinreceptor
4xee.1.A	OR52D1	16.72	MONOMER	HHblits	X-RAY	2.90Å	0.29	0.90	Neurotensinreceptor type 1
5iu4.1.A	OR7E24	21.00	MONOMER	HHblits	X-RAY	1.72Å	0.30	0.83	Adenosinereceptor A2a
4xes.1.A	OR14A16	15.79	MONOMER	HHblits	X-RAY	2.60Å	0.29	0.92	Neurotensinreceptor type 1

The P2Y12 (PDB code 4PXZ), is the first human protease activated receptor for ADP and ATP coupled to G-proteins that inhibit the adenylyl cyclase (the second messenger system) [13–16]. The second human protease activated receptor1 (PDB code 3VW7), which have high affinity for activated thrombin coupled to G proteins that stimulate phosphoinositide hydrolysis, may play a role in platelets activation and in vascular development [17]. Homology Modeling (known as computational comparative modeling of protein), is used as tool to predict 3D-structure of protein which lack the crystal structure, including ORs. One of the prediction tools of 3D-structure is the SWISS-MODEL, a fully automated protein structure homology modeling server [18–20] . For the purpose of our research paper, we have chosen five ORs (shown in Table 1) [18,21] and we have studied them by molecular docking with the two studied compounds ((4Z)-hex-4-en-1-ol and 2-methylbutyl 2-methylbutanoate). The Table 1 shows the different selected models, their template and their sequence identity. These conformers are minima based on the absence of imaginary frequencies (lack of OR crystal structures).

2.3. Molecular docking study

Docking is the best option to predict the energetically favourable binding conformations of ligands in the active site cavity of particular receptor. There are different configurations of interaction between materials (protein and ligand); the interaction between the atoms of two substances (protein and ligand) is justified by the affinity and binding properties of ligands towards a particular target. In this study, firstly we performed the docking study with the two proteins: the P2Y purino receptor 12 (P2Y12) and the human protease activated receptor 1 (PDB codes: 4PXZ and 3VW7, respectively). Then we have chosen five ORs (Table 1), to compare the results. The two molecules were sketched using Marven sketch program [22]. Before studying the mode of interaction between the proteins and the ligands, we have defined a grid box, which covers the entire 3D space of the proteins. The ligands and proteins preparation steps for the docking protocol were carried out using Autodock tools [23], the grid boxes (x , y and z at 1 angstrom spacing) are shown in Table 2. The bioactive conformations were simulated using Autodock Vina [24]. The results were analyzed using Discovery Studio 2016 [25] and PyMol [26] software's.

Table 2. The grid boxes (x, y, and z at 1 angstrom spacing) used to perform docking for each receptor.

	X	Y	Z
3VW7	28.00	20.00	40.00
4PXZ	26.00	26.00	44.00
OR14I1	-44.24	-64.20	113.71
OR14J1	10.00	-20.00	-40.00
OR52D1	19.78	-41.22	-15.28
OR7E24	-17.42	-06.28	17.00
OR14A16	02.97	-00.44	-18.16

3. Results and Discussions

3.1. Docking of (4Z)-hex-4-en-1-ol and 2-methylbutyl 2-methylbutanoate to the suggested two receptors of GPCRs

To clarify the type of binding of the ligands to their potential targets we have examined their interactions with the P2Y purino receptor 12 (P2Y12) (PDB code 4PXZ) and human protease activated receptor 1 (PDB code 3VW7). Figures: 2, 3, 4 and 5 show the different interactions between the two ligands and their targets (as the most GPCRs structurally

similar to ORs). We have selected the top-scoring pose of each molecule according to the best interaction energy with the receptor.

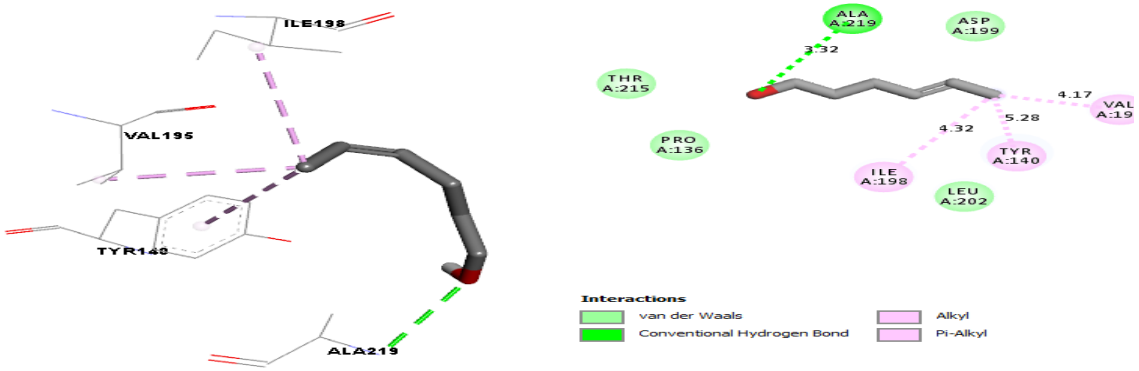


Figure 2. Interactions between 3VW7 and (4Z)-hex-4-en-1ol.

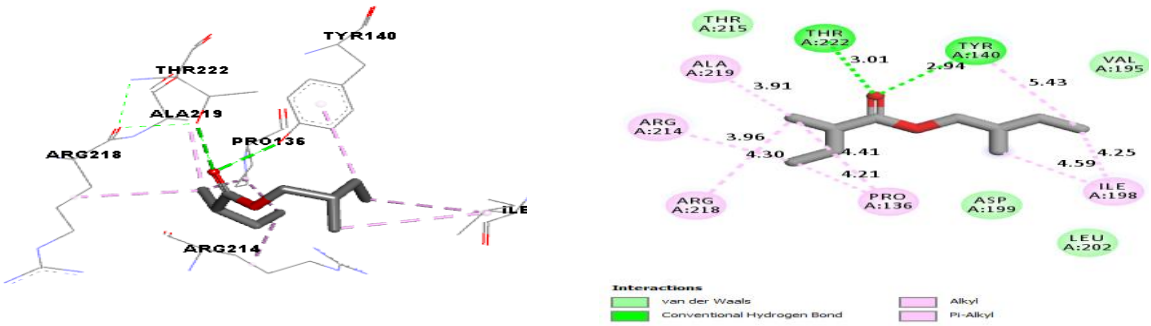


Figure 3. Interactions between 3VW7 and 2-methylbutyl 2-methylbutanoate.

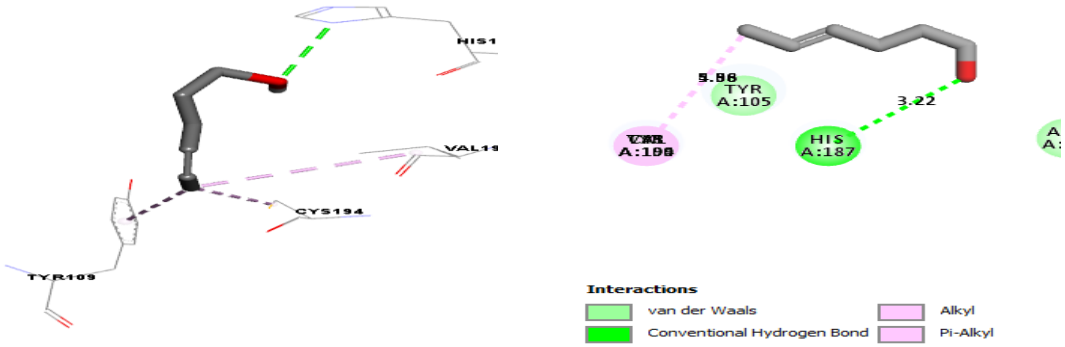


Figure 4. Interactions between 4PXZ and (4Z)-hex-4-en-1-ol.

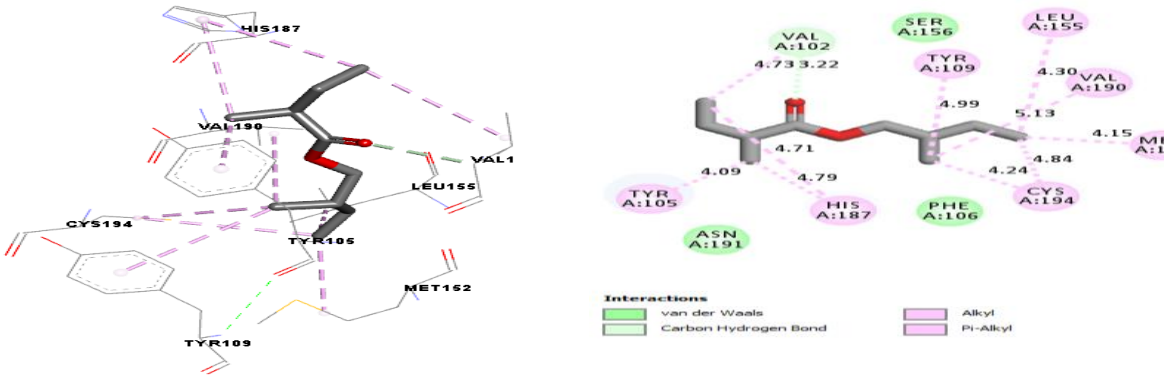


Figure 5. Interactions between 4PXZ and 2-methylbutyl 2-methylbutanoate.

The results show that the molecule (4Z)-hex-4-en-1-ol with the highest retention (lowest release) property value (Log (1/K) = 3.324) has one hydrogen bond with each of the two studied GPCRs. Moreover, the molecule 2-methylbutyl 2-methylbutanoate with the lowest retention (highest release) property value (Log (1/K) = 1.880) has 2 hydrogen bonds with the 4PXZ receptors only. For the other interactions, we can note that the 2-methylbutyl 2-methylbutanoate molecule mainly gives Pi-type bonds, in comparison with the second molecule, in case of the two studied GPCRs.
The distance between each of two studied substances (protein and ligand) in the interactions is presented in the Figures: 2, 3, 4 and 5 as distances between interactive groups of molecules and the amino acids of GPCRs receptors participated in the formation of bond interactions, the distances are between 2 and 5 Å. These distances are determined by Discovery Studio 2016 software.

3.2. Docking of (4Z)-hex-4-en-1-ol and 2-methylbutyl 2-methylbutanoate to the five suggested receptors ORs

To identify the interactions between the two studied molecules and the ORs by the docking analysis, we have chosen five ORs (OR14I1, OR14J1, OR52D1, OR7E24 and OR14A16). The Table 3 and 4 show the results of interactions between the two molecules and the studied ORs. We have selected the top-scoring pose of each molecule according to the best interaction energy with the receptor.

Table 3. Type and number of interactions between (4Z)-hex-4-en-1-ol and the five ORs.

	Hydrogen bonds	**VDW interactions**	**Pi interactions**
OR14I1	1	2	4
OR14J1	2	3	4
OR52D1	0	2	3
OR7E24	1	4	3
OR14A16	1	4	1

Table 4. Type and number of interactions between 2-methylbutyl 2-methylbutanoate and the five ORs.

	Hydrogen bonds	**VDW interactions**	**Pi interactions**
OR14I1	1	3	5+
OR14J1	0	3	5+
OR52D1	1	2	5+
OR7E24	1	3	5+
OR14A16	0	4	5+

By analyzing results given in Table 3 and 4, we have found that the molecule with highest retention property value ((4Z)-hex-4-en-1-ol) has a lowest numbers of Pi interaction with all of studied ORs, in comparison with the second molecule (2-methylbutyl 2-methylbutanoate), and it is designated by more than one Hydrogen bond with each of them except for OR52D1 olfactory receptor. We have also found that the molecule 2-methylbutyl 2-methylbutanoate, that has lowest retention property value represent at least 5 Pi bond interactions with all of studied ORs, and one Hydrogen bond with each of them except for OR14J1 and OR14A16 olfactory receptors. Moreover, there are no Hydrogen bonds between the two ORs (OR14A16 and OR14J1) and the molecule (2-methylbutyl 2-methylbutanoate), and no hydrogen bonds between OR52D1 and (4Z)-hex-4-en-1-ol. We can conclude that the receptors which detect

the different interactions with the two studied molecules are OR14A16 and OR14J1 ORs. Figure 6 and 7 show that odorant compound (4Z)-hex-4-en-1-ol, in the case of OR14A16 olfactory receptor, form conventional hydrogen bond with AL A106 residue and Pi donor interaction with PHE 253 residue. While in the case of OR 14J1 receptor, the compound: (4Z)-hex-4en-1-ol present two conventional hydrogen bonds with GLN 174 residue and three Pi donor interactions with VAL 99, ILE 96 and ALA 158 residues.

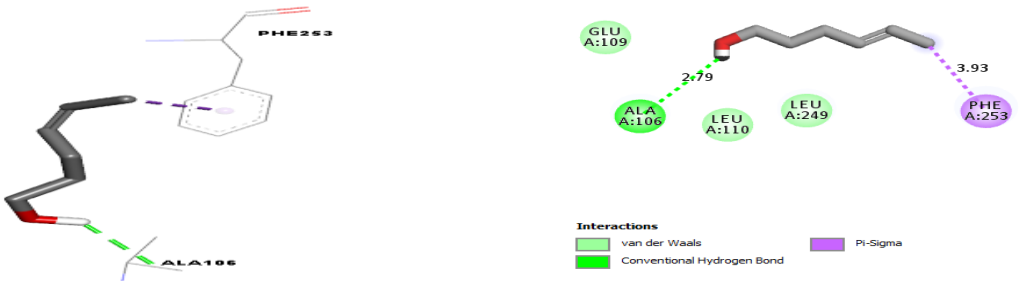


Figure 6. Interactions between OR14A16 and (4Z)-hex-4-en-1-ol.

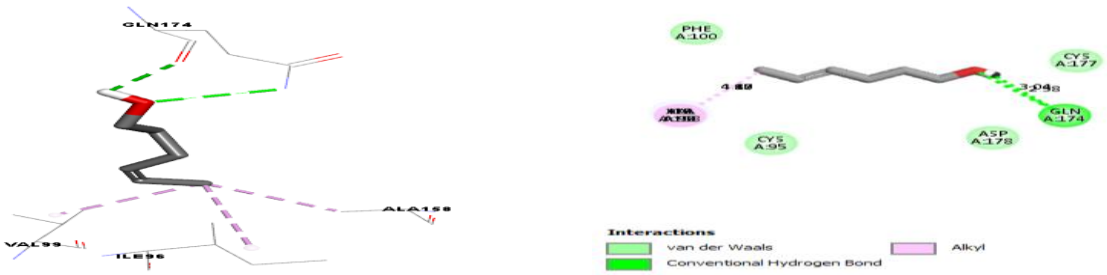


Figure 7. Interactions between OR14J1 and (4Z)-hex-4-en-1-ol.

The distance between each of two studied substances (protein and ligand) in the interactions is presented in the Figures: 6 and 7 as distances between interactive groups of molecules and the amino acids of ORs participated in the formation of bond interactions, these distances are between 2 and 5 Å.

4. Conclusion

In this research, on the one hand, we have studied the docking of two molecules (4Z) -hex-4-en-1-ol and 2-methylbutyl 2-methylbutanoate to the P2Y purino receptor 12 and the human protease activated receptor 1. The results show that the highest number of binding means a high affinity between the two studied molecules and these types of proteins. The comparison of the type and number of interactions between the two molecules and these proteins shows that 2-methylbutyl 2-methylbutanoate molecule mainly gives Pi-type bonds, whereas (4Z)-hex-4-en-1-ol gives more Hydrogen bond interactions. On the other hand, for the docking study of the two studied molecules to the olfactory receptors (OR14I1, OR14J1, OR14A16, OR7E24 and OR52D1), (4Z) -hex-4-en-1-ol compound gives more hydrogen bonds with the majority of these receptors, whereas 2-methylbutyl 2-methylbutanoate molecule gives more Pi bond interactions with all of these receptors. This result can be considered as a first step to find the relationship between the retention/release property of odorant molecules and the type and number of interactions formed between these molecules and the olfactory receptors (ORs).

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