Received: 01/02/2022 Revised: 01/02/2023 Accepted: 16/03/2023 Published: 10/07/2023

ORIGINAL ARTICLE
DOI 10.58430/jib.v129i2.20

INSTITUTE OF BREWING & DISTILLING

# Hop bitterness in beer evaluated by computational analysis

• María Paredes Ramos [1,2]
• José M López Vilariño[1]

[1] Hijos de Rivera S.A.U., C/ José María Rivera Corral n°6, 15008 A Coruña, Spain
[2] METMED Research Group, Physical Chemistry Department, Universidade da Coruña [6] (UDC), Campus da Zapateira s/n, 15071 A Coruña, Spain

María Paredes Ramos, Hijos de Rivera S.A.U., C/ José María Rivera Corral n°6, 9 15008 A Coruña, Spain.

Email: mparedes@estrellagalicia.es



## Abstract

Beer flavour and aroma are greatly influenced by the hop(s) employed in the brewing process. The iso-α-acids post wort boiling are the major compounds responsible for bitterness, which are detected by the bitter taste receptors (TAS2Rs) in oral taste buds. This family of receptors is activated in the presence of bitter molecules, which send chemical signals to the brain, making it possible to differentiate whether the detected molecules have a pleasant taste (or not). It is of interest to predict the behaviour of hop compounds towards bitter receptors such that the bitterness of different hop varieties can be predicted based on quantitative analysis of composition. Computational simulation, based in high-performance computing (HPC), allow the simulation of interactions of molecules with the various TAS2Rs, enabling the prediction the bitterness of these hop compounds. These techniques, will soon enable the design of beverages with customised flavours, greatly reducing the need for experimental evaluation. In this work, α and β-acids, iso-α-acids, and prenylflavonoids are analysed against the bitter receptors TAS2R10, TAS2R14 and TAS2R46. Using computational blind docking and molecular dynamics, xanthohumol was identified to have the highest bitter profile.

**Keywords:**

α and β-acids, prenylflavonoids, bitter taste receptors (TAS2R), computational docking, molecular dynamics, xanthohumol.

# Introduction

Hops are an indispensable component in beer production, contributing characteristic bitterness and aroma. Each variety of hop contains essential oils and bitter resins, together with α-acids, β-acids and prenylflavonoids. Bitterness and aroma vary with variety (Schönberger and Kostelecky 2011; Almaguer et al. 2014; Ocvirk et al. 2016; Mikyška et al. 2018; Van Holle et al. 2021). Bitterness is detected by bitter taste receptors (TAS2Rs) in oral taste buds, which are activated by molecules, sending chemical signals to the brain. TAS2Rs differentiate whether compounds have a pleasant taste or not, whether they can be consumed or whether high bitterness suggests they are potentially toxic (Zhang et al. 2017; Di Pizio et al. 2020).

Taste receptors are G protein-coupled receptors (GPCRs), also known as seven transmembrane receptors (7TM), and they represent the largest and most physiologically important integral family of membrane proteins. GPCRs are activated by a wide variety of physiological and environmental stimuli, including subatomic particles (photons), ions, small organic molecules, and macromolecules (peptides or proteins). The GPCR protein family consists of about 800 genes in the human genome that regulate signalling pathways involved in various physiological functions, such as behaviour, cognition, immune response, mood, smell, blood pressure regulation and taste (Lee et al. 2018).

All 25 TAS2R receptors present in the human body are involved in the detection of bitter substances. TAS2R10, TAS2R14 and TAS2R46 are the most relevant, as they present a more marked agonist profile, which contributes to the perception of bitterness (Meyerhof et al. 2009; Born et al. 2013; Kohl et al. 2013; Nowak et al. 2018). TAS2R receptors are in the taste buds of the tongue, but also in other organs of the human body, such as the respiratory system or the gut. Although their function is to signal bitter taste, they are also recognised as potential drug targets in various pathological conditions in the organs in which they are found (Shaik et al. 2016; Shaik et al. 2019; Tarragon and Moreno, 2020).

To study the behaviour of these target receptors numerous computational methods have been developed. These are based on the analysis of molecular recognition, to predict the bioactivity of certain molecules (ligands) on cellular tissues (receptors) (Ou-Yang et al. 2012; Pérez-Sánchez et al. 2016; Schneider and Clark, 2019). These computational methods, which have been used for decades in the pharmaceutical and cosmetic fields, are also useful in the food industry. For example, analysis of compounds in foods and beverages against receptors such as HMGCR, COX-2 and ACE. HMGCR (3-hydroxy-3-methyl-glutaryl-CoA reductase) is involved in cholesterol synthesis (Istvan et al. 2000; Istvan and Deisenhofer, 2001), with COX-2 (cyclooxygenase 2), overexpressed during inflammatory processes (Kurumbail et al. 1996; Jawabrah Al-Hourani et al. 2020), and ACE (angiotensin converting enzyme), which regulates blood pressure (Vermeirssen et al. 2002; Boschin et al. 2014; Martin and Deussen, 2019). Such studies can be used to predict the suitability of compounds in functional food or beverages and whether they confer benefits that go beyond nutritional value, possibly promoting a health benefit.

Accordingly, the analysis of compounds against receptors involved in taste, enables the activity of molecules to be assessed, potentially predicting the sensory effect in consumers. In this way, it is possible to design foods and beverages à la carte by incorporating into the formulation molecules that have a physiological impact as indicated by computational tests.

# Materials and Methods

## Molecular modelling

### Resources and programs

Openbabel GUI 2.4.1 (Boyle et al. 2011), Acpype (Sousa Da Silva and Vranken, 2012), Gromacs 2018 (Berendsen et al. 1995; Pronk et al. 2013; Kutzner et al. 2019), AutoDock Tools 4.2 (Morris et al. 2009), AutoDock Vina 2.0 (Trott and Olson, 2010), Pymol 2.3 (The PyMOL Molecular Graphics System, Version 2.3 Schrödinger, LLC), Python 2.7.6 (Python Software Foundation), PoseView 1.1.2 (ZBH University of Hamburg, BioSolveIT GmbH), Omega 2.5.1.4 (OpenEye Scientific Software) (Hawkins et al. 2010), PLIP 1.3.2 (Salentin et al. 2015), Maestro suite 2020.04 (Schrödinger LLC).

## Preparation of molecular files

The 3D structure of the TAS2Rs is not defined, so Protein Data bank (PDB) files are not available. However, given the high homology between GPCR receptors, several models that represent the 3D structure of the 25 human TAS2Rs have been developed (Wiener et al. 2012; Dagan-Wiener et al. 2019; Di Pizio et al. 2020), so these structures were used to perform the computational studies.

After download, the 3D protein structures were prepared with AutoDock Tools 4.2 (AD4). This program was used to remove water molecules, add protons, assign AD4 type to atoms, compute Gasteiger charges and save the file in pdb and pdbqt formats. The protein structure was visualised to the mol2 format using Pymol 2.3.

Ligand molecules (hop compounds) were downloaded from the repositories Chemspider (www.chemspider.com) or Pubchem (www.pubchem.ncbi.nlm.nih.gov) in mol format and were converted to mol2, pdbqt and pdb using Pymol 2.3, AutoDock Tools 4.2 and Openbabel GUI 2.4.1.

## Analysis of blind docking

A blind docking study (BD) was performed to detect the regions of interaction for ligands among the TAS2R10, 14 and 46 structures (Ciemny et al. 2018). A single docking was performed in each α-carbon of the protein, detecting the most favourable binding pockets in terms of bond energy, which is obtained by different algorithms depending on the software that is used (Ferreira et al. 2015; Banegas-Luna et al. 2019).

## Simulation of molecular dynamics

Molecular dynamics (MD) approximates the behaviour of the system against real conditions and tests the stability of the ligand-protein contacts detected during the blind docking analysis over time.

Simulations of molecular dynamics were performed using the GPU version of Desmond included with Maestro suite 2020.04 (Schrödinger LLC) on a workstation with a NVIDIA QUADRO 5000. The system confirmed that the tested molecules and TAS2R10, 14 or 46 were solvated in an aqueous environment, in a cubic box with a minimal distance of 10 Å between the biomolecule and the box boundary (for periodic boundary conditions). Next, systems were neutralised and maintained in 0.15 M NaCl. The OPLS3 force-field and the TIP3P-TIP4P water model were employed. Initially, the systems were energy minimised for 1000-time steps. The systems were then allowed to execute free dynamics in the NPT ensemble; pressure was controlled using the Martyna-Tobias-Klein methodology and the Nose-Hoover thermostat was employed to maintain the system near 310K. Production grade MD trajectories were extended to a total duration of 50 ns per system.

MD trajectories were characterised in terms of the root-mean-square deviation (RMSD) of fluctuations of ligand and receptor, particularly in terms of the main interactions with the top interacting residues. The trajectories were also used to assess the stabilities of the protein secondary structures (in complex with potential inhibitors) by plotting RMSDs.

## Analysis - materials and reagents

Acetonitrile and water with 0.1% formic acid, both suitable for MS analysis, were purchased from Sigma Aldrich.

## High pressure liquid chromatography-mass spectrometry (HPLC-MS)

For the analysis of samples, a liquid chromatograph with an LC1290 Infinity II system coupled to Agilent's 6546 LC/Q-TOF (Quadrupole-Time-of-Flight) mass spectrometer was used. The software used was Agilent Mass Hunter Workstation with Agilent Mass Hunter Qualitative Analysis 10.0 and Agilent Mass Hunter Quantitative Analysis 10.1.

The chromatography conditions were a flow rate of 250 µL/min, injection volume 10 µL, column temperature at 30°C, a Kinetex XB-C18 100 x 2.1 mm 2.6 µm Phenomenex column with mobile phase A: H2O 0.1% H-COOH and B: ACN 0.1% H-COOH. The initial gradient used was 98% A and 2% B, after 2 min 92% A and 8% B and after 12 min, A at 80%

and B is 20%. After 13 minutes, 70% of A and 30% of B with after 14 minutes, 100% of B which were held for three minutes. At 17 minutes, the initial gradient (98% A, 2% B) was reintroduced and held for 7 minutes.

The mass conditions were as follows: a negative ESI is used with a mass range between 50-500, the gas temperature is 210°C and the sheath gas temperature is 350°C, the sheath gas flow is 11 L/min, the drying gas is 13 L/min, and the nebulizer is at 35 psi. The VCap value was 4000 V and the fragmentor 130 V.

## Results and discussion

The perception of bitterness in the human body is mediated by bitter taste sensing receptors (TAS2R). Of the 25 identified receptors, TAS2R10, TAS2R14 and TAS2R46 are considered the most relevant (Nowak et al. 2018). Accordingly, molecules that perform better against these three receptors are considered more biologically active, suggesting they have a higher degree of bitterness. Hence, these receptors have been used with hop compounds - α and β-acids, iso-α-acids, and prenylflavonoids - to assess bitterness (Sabín López et al. 2020; Wu et al. 2020).

In this work, blind docking and molecular dynamics were performed against α-acids (adhumulone, cohumulone, and R-humulone), iso-α-acids (cis-isohumulone, cis-isocohumulone, trans-isocohumulone, trans-isohumulone, isocohumulone, isoadhumulone, cis-tetrahydroisocohumulone, cis-tetrahydroisohumulone, trans-tetrahydroisohumulone, trans-isocohumulone, and trans-tetrahydroisocohumulone), the β-acids (lupulone and colupulone) and the prenylflavonoids (6-prenynlaringenin, 8-prenylnaringenin, 6-geranylnaringenin, desmethylxanthohumol, xanthohumol and isoxanthohumol).

### Blind docking of TAS2R10, TAS2R14 and TAS2R46 receptors

The binding of a ligand to a receptor does not necessarily imply that this results in the activation or inhibition of the biological activity in which the receptor is involved. For this, the binding needs to take place in a specific region of the protein, known as the binding site or 'hot spot'. Accordingly, only ligands that interact with the binding site of the protein can activate or inhibit its function.

The blind docking technique used in this work provides conformational analysis of each ligand for all α-carbons of the protein. In this way, all possible ligand-receptor binding sites are analysed, and a score is assigned to quantify the suitability of the binding. Analysis of binding energy and binding intensity enables a quantitative ranking of the most favourable positions for each ligand in each TAS2R. Accordingly, this approach determines whether the ligand can reach the binding site of the TAS2R or if it attaches to inactive areas of the receptor.

The binding site of the bitter receptor TAS2R10 consists of residues Ser85, Trp88, Val89, Asn92, Gln93, Gln175, Leu178, Tyr239, Met263 and Thr266 (Born et al. 2013). The interactions of the α and β-acids, iso-α-acids and prenylflavonoids to this binding site are reported in Table 1. The suitability of the positions is defined by the cluster number (CL), with CL1 being the most favourable position and CLn the most unfavourable (n=number of dockings performed).

Accordingly, the ligands predicted to be most bitter are 6-prenylnaringenin, 8-prenylnaringenin, 6-geranylnaringenin,desmethylxanthohumol, xanthohumol and isoxanthohumol. These six ligands have the most favourable binding energies (Table 1) within the identified binding site of TAS2R10, but it is difficult to establish a quantitative ranking among them as the affinities are similar. To do so, it is necessary to perform dynamic simulation studies to analyse the stability of the ligand within the binding site. Accordingly, the ligand that offers the most stability in an active position would be considered the most bitter.

The binding site of the bitter receptor TAS2R46 con-sists of residues Trp66, Glu70, Leu71, Ile82, Trp88, Asn88, Asn92, Asn150, Asn176, Tyr241, Glu253, Glu265, Ala268 and Phe269 (Sandal et al. 2015; Lang et al. 2020). The interactions of α and β-acids, iso-α-acids and prenylflavonoids t o this binding site are reported in Table 1. The molecules exhibiting the best affinity against receptor TAS2R46 are the

prenylflavonoids 6-prenylnaringenin, 8-prenylnaringenin, 6-geranylnaringenin, desmethylxanthohumol, xanthohumol and isoxanthohumol. These results agree with those obtained for receptor TAS2R10. This insight to the binding efficacy of these six ligands against bitter receptors, suggests they provide an increased bitter response.

Of the 25 human bitter receptors, TAS2R14 is the most broadly tuned to the largest number of molecules and is the most promiscuous against bitter compounds (Nowak et al. 2018). This wide molecular recognition is positive for the design of drug, since the number of drug candidates is not as limited as with other receptors, being more feasible to locate or develop a ligand that meets the characteristics required for therapeutic use. However, the broad adaptability of the binding site makes computational analysis difficult, as the key residues for activating the bitter taste receptors are variable, depending on the characteristics of the bound ligand (Zhang et al. 2017; Woo et al. 2019; Di Pizio et al. 2020).

## Table 1.

Blind docking analysis with AutoDock software of iso-α, α and β-acids and prenylflavonoids against receptors TAS2R10, TAS2R14 and TAS2R46.

| Hop compound (ligand) | TAS2R10 | | TAS2R14 | | TAS2R46 | |
|---|---|---|---|---|---|---|
| | CL | Binding energy | CL | Binding energy | CL | Binding energy |
| 6-prenylnaringenin | 1 | -8.88 | 1 | -9.10 | 3 | -7.12 |
| Adhumulone | 1 | -7.37 | 1 | -7.70 | 1 | -6.01 |
| Cohumulone | 1 | -7.52 | 1 | -7.52 | 2 | -5.63 |
| cis-isohumulone | 1 | -7.98 | 1 | -8.37 | 1 | -6.22 |
| Cis-isocohumulone | 1 | -8.08 | 1 | -7.93 | 1 | -6.04 |
| R-Humulone | 1 | -7.59 | 1 | -7.89 | 1 | -5.96 |
| 8-prenylnaringenin | 1 | -9.23 | 1 | -9.96 | 1 | -7.52 |
| 6-geranylnaringenin | 1 | -9.45 | 1 | -9.55 | 1 | -7.56 |
| Desmethylxanthohumol | 1 | -9.29 | 1 | -9.29 | 4 | -6.35 |
| Xanthohumol | 1 | -9.08 | 1 | -9.12 | 2 | -7.02 |
| Trans-isocohumulone | 1 | -8.12 | 1 | -7.54 | 1 | -6.31 |
| Trans-isohumulone | 1 | -8.15 | 1 | -7.97 | 2 | -5.88 |
| Isocohumulone | 1 | -7.71 | 1 | -7.67 | 6 | -6.22 |
| Isoadhumulone | 1 | -7.85 | 1 | -7.99 | 1 | -6.11 |
| Lupulone | 1 | -7.71 | 1 | -7.40 | 3 | -5.68 |
| Cis-tetrahydroisocohumulone | 1 | -7.68 | 1 | -7.47 | 1 | -5.74 |
| Cis-tetrahydroisohumulone | 1 | -7.51 | 1 | -7.48 | 1 | -5.98 |
| Isoxanthohumol | 1 | -8.64 | 1 | -9.21 | 1 | -7.29 |
| Trans-tetrahydroisohumulone | 1 | -7.66 | 1 | -7.28 | 3 | -5.95 |
| Trans-isocohumulone | 1 | -8.08 | 1 | -7.41 | 5 | -6.28 |
| Trans-tetrahydroisocohumulone | 1 | -7.94 | 1 | -7.25 | 4 | -5.74 |
| Colupulone | 1 | -7.68 | 1 | -7.68 | 6 | -5.54 |
| Flufenamic acid | - | - | 1 | -9.31 | - | - |
| Genistein | - | - | 1 | -8.75 | - | - |

Where CL = cluster number and Binding energy is kcal/mol.

The different positions at which TAS2R14 interacts with ligands were analysed, detecting all the α and β-acids, iso-α-acids and prenylflavonoids which bind to the receptor at CL1 (Table 1). These coordinates coincide with the binding site of flufenamic acid (Figure 1B), a molecule with a bitter character and a TAS2R14 activator (Nowak et al. 2018; Di Pizio et al. 2020). Therefore, it can be assumed that the behaviour of the hop compounds is similar to this ligand, since they preferentially bind in the same position and with high affinity values (Table 1, Figure 1). The key residues for flufenamic-like ligands are Trp89, Asn93, Ile148, Phe186, Phe243, Phe247 and Ile262 (Nowak et al. 2018; Di Pizio et al. 2020).

Of the TAS2R14 results, the ligands with the highest affinity are 6-prenylnaringenin, 8-prenylnaringenin, 6-geranylnaringenin, xanthohumol and isoxanthohumol. These affinity values are in accord with those obtained for the bitter molecules fluflenamic acid and genistein. With the exception of desmethylxanthohumol, these results also agree with those of receptors TAS2R10 and TAS2R46. Accordingly, a stronger bitter taste would be expected for these compounds.

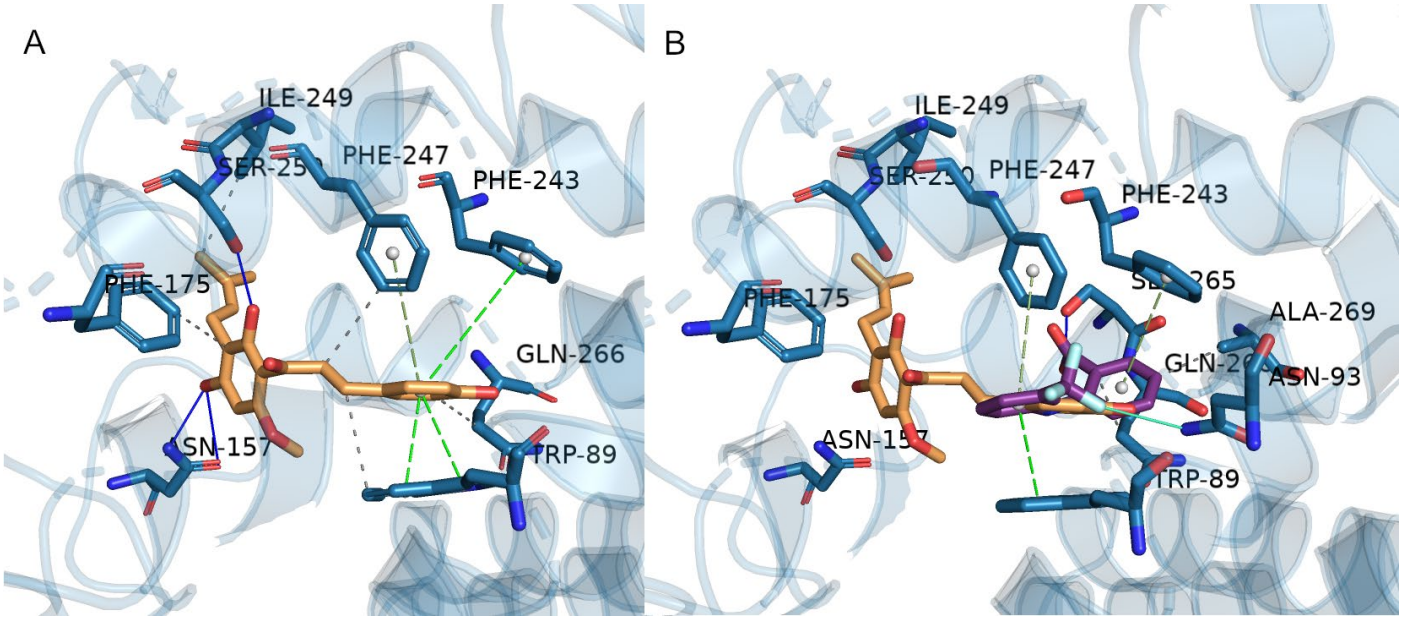
## High pressure liquid chromatography-mass spectrometry analysis

The presence of α and β-acids, iso-α-acids and prenylflavonoids was determined by HPLC-MS in different hop and beer samples (Table 2). Isoxanthohumol, xanthohumol, 8-prenylnaringenin, isoadhumulone and/or humulone, isocohumulone, trans and/or cis-tetrahydroisocohumulone, tetrahydroisohumulone and/or tetrahyroisoadhumulone, lupulone and colupulone were detected as major prenylflavonoids, α and iso-α-acids, and β-acids from both hop extracts and beers. Isoadhumulone and/or humulone, isocohumulone and lupulone were present at higher concentrations (Table 2). The IBU values show the relationship between the concentration of these hop compounds and the analytical bitterness.

It has been reported that - for hop compounds - the most common isomer, at slightly acidic pH (pH 5.8-7), is the cis form (Bastgen et al. 2020). Therefore, the cis isomers of the compounds in Table 2 may be present at a higher concentration although it was not possible to differentiate between them using mass spectrometry.

### Figure 1.

TAS2R14 blind docking analysis for xanthohumol and flufenamic acid. A) xanthohumol, B) comparison between flufenamic acid and xanthohumol. Results obtained with AutoDock and Pymol software.



## Table 2.

LC-PDA-LTQ FT Orbitrap mass spectrometry analysis of beer and hop extract.

| Sample | Isoxanthohumol | Xanthohumol | 8-Prenylnaringenin | Iso-α-ad/n-humulone | Iso-α-cohumulone | Trans/Cis-Tetrahydroisocohumulone | Tetrahydroiso(ad)humulone | Lupulone | Colupulone | IBU |
|---|---|---|---|---|---|---|---|---|---|---|
| German Hells Export bier | 0.60 | 0.09 | 0.02 | 57.0 | 45.9 | 2.30 | 4.32 | 0.00 | 0.00 | 25 |
| Helles Bock | 0.92 | 0.16 | 0.04 | 58.4 | 53.6 | 4.10 | 6.46 | 0.05 | 0.05 | 25 |
| Dopplebock | 0.72 | 0.13 | 0.04 | 65.2 | 48.8 | 3.03 | 6.00 | 0.06 | 0.05 | 27 |
| Dunkel Bock | 0.92 | 0.38 | 0.04 | 69.6 | 49.0 | 6.43 | 13.28 | 0.47 | 0.46 | 27 |
| Belgian Dubbel | 0.77 | 0.26 | 0.20 | 33.8 | 47.8 | 0.00 | 0.00 | 0.06 | 0.10 | 16 |
| Perle | 66.8 | 96.2 | 30.1 | 2018.0 | 1074.3 | - | - | 1142.3 | 872.1 | - |
| Nugget | 59.5 | 84.8 | 28.9 | 2475.1 | 1227.8 | - | - | 714.7 | 412.8 | - |

The concentration of iso-α, α and β-acids and prenylflavonoids is reported as mg/L. The results are the mean of six different analyses. International Bitterness Units (IBU) were determined using the European Brewery Convention Method EBC 9.8.

## Table 3.

Analysis of molecular dynamics performance for iso-α, α and β-acids and prenylflavonoids against receptors TAS2R10, TAS2R14 and TAS2R46.

Indication of the expected contact of molecules into the binding site of each TAS2R receptor. Where dark green = good performance, green = medium/good performance, yellow = medium performance and red = bad performance.

| Molecule | TAS2R10 | TAS2R14 | TAS2R46 |
|---|---|---|---|
| Isocohumulone | | | |
| Isoadhumulone | | | |
| 8-prenylnaringenin | | | |
| Isoxanthohumol | | | |
| Xanthohumol | | | |
| Lupulone | | | |
| Colupulone | | | |
| Cis-tetrahydroisocohumulone | | | |
| Cis-tetrahydroisohumulone | | | |

## Simulation of molecular dynamics

Simulations of molecular dynamics were performed on molecules found at a higher concentration in hops and beer: isocohumulone, isoadhumulone, 8-prenylnaringenin, isoxanthohumol, xanthohumol, lupulone,colupulone,cis-tetrahydroisocohumulone, and cis-tetrahydroisohumulone (Table 2). Their performance during a 50 ns period was analysed, taking into consideration the bound protein residues and their stability within the binding site (Table 3). Also, the behaviour of these iso-α-acids, β-acids and prenylflavonoids against the TAS2R14 receptor was compared to flufenamic acid and genistein (Supplementary Information Figure 16).

The ligands with the best performance for receptor TAS2R10 were 8-prenylnaringenin, isoxanthohumol and xanthohumol (Supplementary Information Figures 1 – 9). 8-prenylnaringenin (Supplementary Information Figure 6) showed a low root-mean-square deviation (RMSD) value. There were permanent interactions with key residues Ser85 and Trp88 and residues Tyr239 and Leu258 provide stable contacts. Although not key residues, Tyr239 and Leu258 allow stabilisation of 8-prenylnaringenin in the binding site. Leu177, Met243 and Leu259 are residues that present intermittent interaction, which are less relevant, but contribute to the stability of the ligand. Hence the low RMSD values.

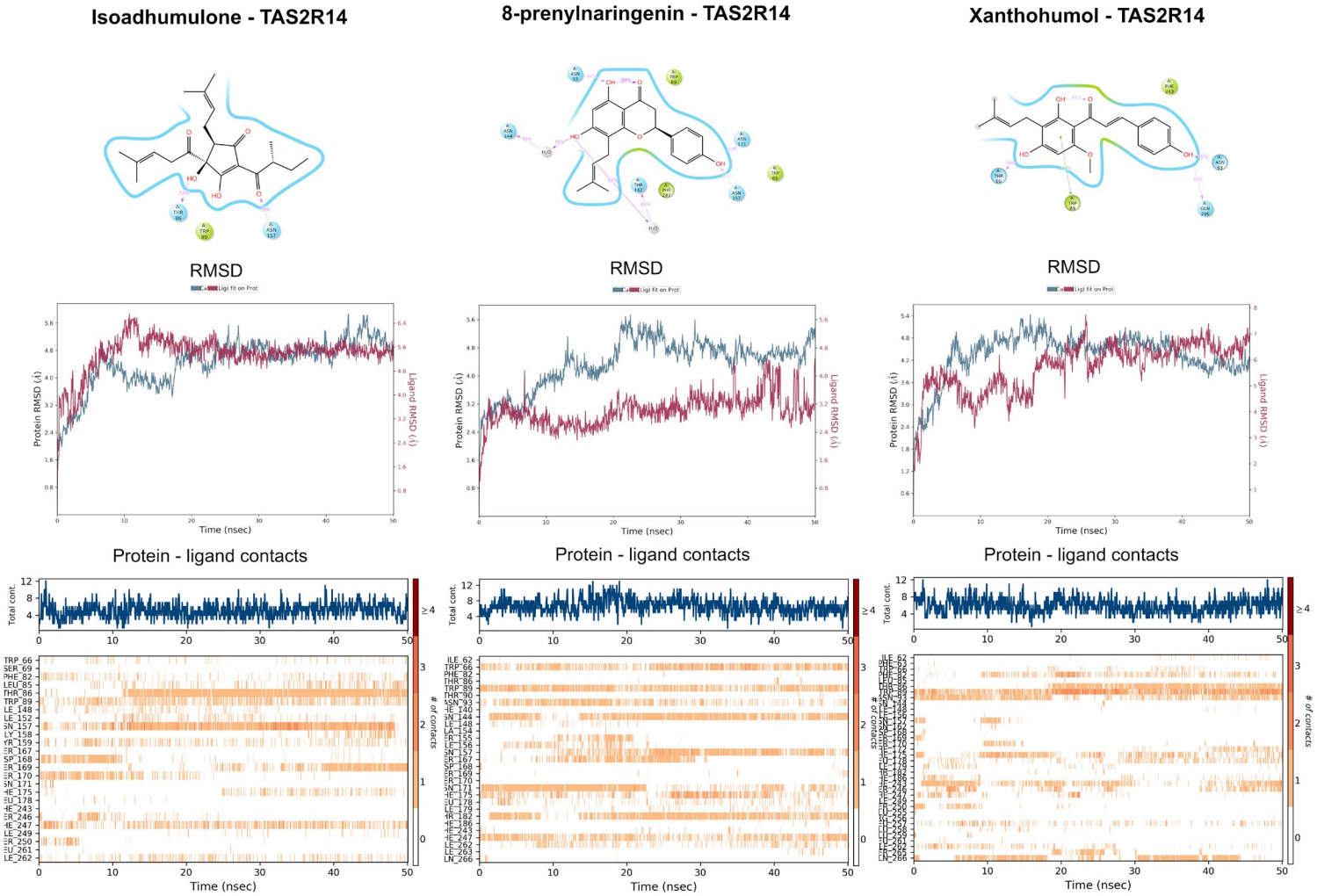
Isoxanthohumol RMSD (Supplementary Information Figure 5) presents normal and very stable values throughout the simulation, both for protein and ligand. It interacts permanently with the key residue Ser85 and more intermittently with Trp88 and Met263. In addition, it has stable contacts with Leu164, Lys174 and Gly242, which are not key residues, but help to stabilise the ligand in the binding site.

Xanthohumol (Supplementary Information Figure 8) shows normal root-mean-square deviation (RMSD) values for protein and lower RMSD values for the ligand. It interacts permanently with the key residue Ser85. In the first 10 ns of simulation, it interacts soundly with Trp88, but then the interaction is lost. It also has an intermittent contact with the key residue Met263 throughout the simulation. In addition, the non-key residues Tyr239, Leu259 and Gly262 show relevant contacts, especially after 10-20 ns of simulation. These contacts help to anchor the ligand to the binding site.

Receptor TAS2R14 tunes a higher number of ligands. Isoadhumulone, 8-prenylnaringenin and xanthohumol exhibited the best performance, followed by isoxanthohumol and lupulone (Table 2, Figure 2, Supplementary Information Figures 10 – 15). Compared to flufenamic acid and genistein (Supplementary Information Figure 16), these molecules showed a similar performance, suggesting a 'flufenamic-like' behaviour for α and β-acids, iso-α-acids and prenylflavonoids.

## Figure 2.

TAS2R14 molecular dynamics analysis for isoadhumulone, 8-prenylnaringenin and xanthohumol. Results obtained with Maestro software.



Isoadhumulone (Figure 2) had normal RMSD values for protein and ligand. It showed intermittent interactions with the key residues Trp89 and Phe247, maintaining both contacts throughout the simulation. It also interacts with the key residue Ile262, although intermittently. As non-key residues, there are permanent interactions from 10 ns onwards with Thr86 and Asn157.

The root-mean-square deviation (RMSD) values for 8-prenylnaringenin are low, and slightly higher for the protein (Figure 2). This ligand shows a stable interaction with the key residues Trp89 and Phe247 and more intermittently with Asn93. It also interacts slightly with Ile262. With non-key residues, it exhibited stable interaction with Trp66 and Asn144 throughout the simulation. From 10 to 50 ns it interacts with Asn157 and between 10 to 30 ns with Ser167. All these contacts, both with key and non-key residues, are responsible for a stable interaction of 8-prenylnaringenin within the binding site.

For xanthohumol (Figure 2), RMSD values are normal for the protein and slightly higher and less stable for the ligand. A stable interaction occurs with the key residue Trp89, and intermittently with residues Asn93 and Phe243. It also interacts with Phe186 and Ile262, but more intermittently, suggesting these contacts are not relevant. Non-key residues, Thr86 and Gln266 contacts were not stable interactions throughout the simulation but helped to fix the position of the ligand in the binding site.

Flufenamic acid (Supplementary Information Figure 16) shows normal root-mean-square deviation (RMSD) for protein and very low values for ligand (around 2Å). It interacts permanently with Trp89 and stably with Phe247. Flufenamic acid also interacts intermittently with the key residue Phe243. Although non-key residues, it shows intermittent interaction with Ser246, and permanent interactions with Trp66 and Ser265, probably being responsible for the high ligand stability and low RMSD values.

Genistein (Supplementary Information Figure 16) shows quite similar behaviour to flufenamic acid, with permanent interactions with Trp89 and Phe243. It also interacts more intermittently with Phe247 and Asn93. As non-key residues, it stably interacts with Trp66, Leu239, Ile62 and Ile63. Compared with flufenamic acid, those non-key interactions are more unstable, resulting in higher RMSD values for genistein. This agrees with the higher bitter profile of flufenamic acid and confirms the accuracy of these molecular dynamic simulations (Nowak et al. 2018; Di Pizio et al. 2020).

TAS2R46 molecular dynamic simulations reveal a different behaviour from the other two receptors. The ligands positioned inside the binding site of TAS2R10 and TAS2R14, but this was not found with receptor TAS2R46 (Table 2, Supplementary Information Figures 17 – 24). For this receptor, the molecules approach the binding site from one side, but only xanthohumol can interact in its central region (Figure 3). It shows normal root-mean-square deviation (RMSD) values for both protein and ligand and forms stable contact with key residues Trp88 and Asn176. It also contacts more intermittently with key residue Asn92. In addition, it has stable interactions with non-key residues Tyr85, Asn184 and Tyr241, which help to anchor the ligand at the binding site.

This behaviour shows the high specificity of TAS2R46, which we consider as a key receptor in the perception of bitterness. Xanthohumol, which interacts with all three receptors, is considered to be the most bitter molecule among the tested α and β-acids, iso-α-acids and prenylflavonoids.

## Conclusions

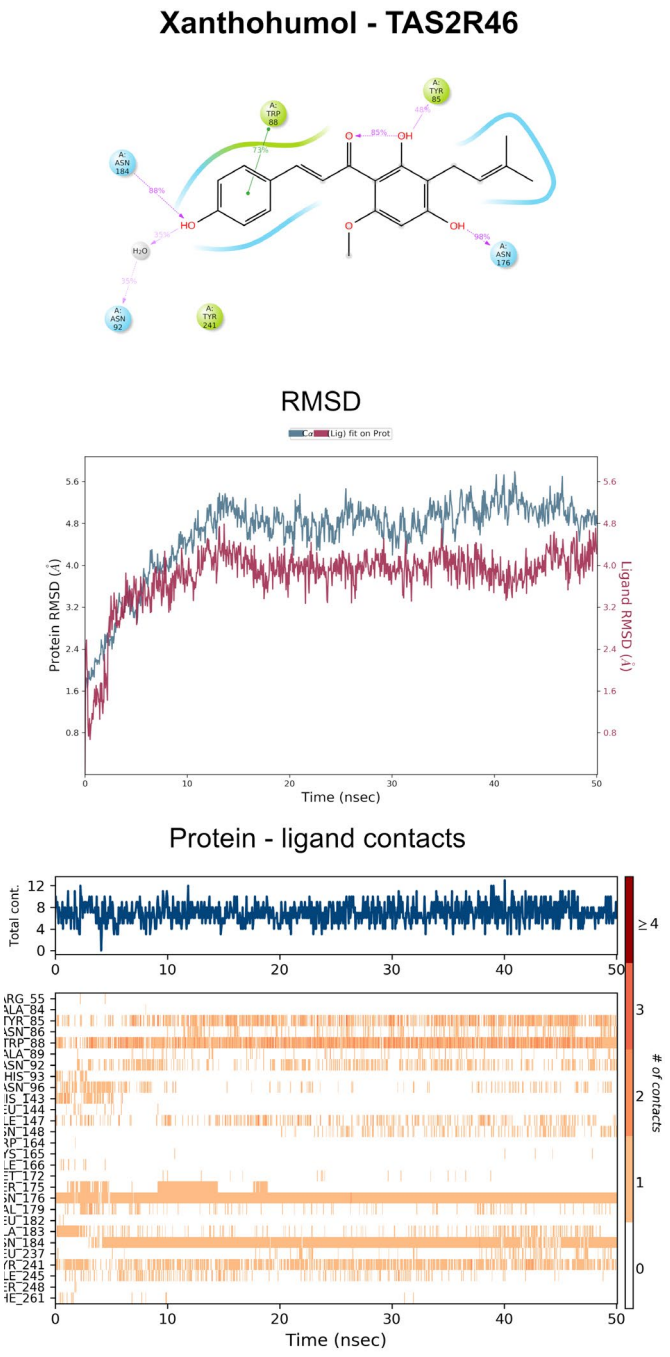
This work describes a computational analysis based on molecular docking and molecular dynamics to assess the bitterness of α and β-acids, iso-α-acids and prenylflavonoids against the bitter receptors TAS2R10, TAS2R14 and TAS2R46. The concentration of the hop compounds was determined in hop extracts and commercial beers by HPLC-MS.

The results show that 8-prenylnaringenin, xanthohumol, isoxanthohumol, isoadhumulone and lupulone have high affinities at the binding site of the receptors (Table 1/Figure 1), and can tune several TAS2R receptors (Table 3). It is suggested that these hop compounds potentially contribute a more pronounced bitter character (Table 1/Figure 1). Of them, only xanthohumol was able to interact

with the key residues of the TAS2R46 receptor (Table 3/Figure 3), and may make a significant difference to the perception of bitterness. Accordingly, xanthohumol is considered the most bitter molecule of the tested ligands and may be expected to bring a pronounced bitterness when present in higher quantities..

**Figure 3.**

TAS2R46 molecular dynamics analysis for xanthohumol. Results obtained with Maestro software.




It is recognised that bitterness does not correlate with the concentration of each compound and depends on the receptors that each ligand is able to tune to. However, given the higher concentration of iso-α-ad-humulone and n-humulone compared to xanthohumol, it is likely that this iso-α-acid (and α-acid), would confer more bitterness to beer, despite the higher bitterness found with xanthohumol during the *in silico* procedure.

## Author contributions

**María Paredes Ramos:** conceptualisation, formal analysis, investigation, methodology, writing (original draft).

**José M López Vilariño:** conceptualisation, supervision, validation, writing (review and editing).

## Acknowledgements

This work was supported by the Hi-Bio 4.0 project (IN854A 2019/13) supported by the Axencia Galega de Innovación – Xunta de Galicia and the European Regional Development Fund and the CDTi project (IDI-20180987) supported by the Spanish Science and Innovation Ministry and the European Regional Development Fund. Supercomputing resources were supported by the infrastructures of Barcelona Supercomputing Center (BSC) and Poznan Supercomputing and Networking Center (PSNC)..

## Conflict of Interest

The authors declare no conflict of interest.

## References

Almaguer C, Schönberger C, Gastl M, Arendt EK, Becker T. 2014. *Humulus lupulus* - a story that begs to be told. A review. *J Inst Brew* 120:289–314. https://doi.org/10.1002/jib.160.

Banegas-Luna AJ, Imbernón B, Llanes Castro A, Pérez-Garrido A, Cerón-Carrasco JP, Gesing S, Merelli I, D'Agostino D, Pérez-Sánchez H. 2019. Advances in distributed computing with modern drug discovery. *Expert Opin Drug Discov* 14:9–22. https://doi.org/10.1080/17460441.2019.1552936.

Bastgen N, Becher T, Drusch S, Titze J. 2020. Usability and technological opportunities for a higher isomerization rate of α-acids: A review. *J Am Soc Brew Chem* 79:17–25. https://doi.org/10.1080/03610470.2020.1840893.

Berendsen HJC, van der Spoel D, van Drunen R. 1995. GROMACS: A message-passing parallel molecular dynamics implementation. *Comput Phys Commun* 91:43–56. https://doi.org/10.1016/0010-4655(95)00042-E.

Born S, Levit A, Niv MY, Meyerhof W, Behrens M. 2013. The human bitter taste receptor TAS2R10 is tailored to accommodate numerous diverse ligands. *J Neurosci* 33: 201–213. https://doi.org/10.1523/JNEUROSCI.3248-12.2013.

Boschin G, Scigliuolo GM, Resta D, Arnoldi A. 2014. ACE-inhibitory activity of enzymatic protein hydrolysates from lupin and other legumes. *Food Chem* 145:34–40. https://doi.org/10.1016/j.foodchem.2013.07.076.

Boyle NMO, Banck M, Craig A, Morley C, Vandermeersch T, Hutchison GR. 2011. Open Babel. *J Cheminform 3:*1–14. https://doi.org/10.1186/1758-2946-3-33.

Ciemny M, Kurcinski M, Kamel K, Kolinski A, Alam N, Schueler-Furman O, Kmiecik S. 2018. Protein–peptide docking: opportunities and challenges. *Drug Discov Today* 23:1530–1537. https://doi.org/10.1016/j.drudis.2018.05.006.

Dagan-Wiener A, Di Pizio A, Nissim I, Bahia MS, Dubovski N, Margulis E, Niv MY. 2019. Bitterdb: Taste ligands and receptors database in 2019. *Nucleic Acids Res* 47:D1179–D1185. https://doi.org/10.1093/nar/gky974.

Di Pizio A, Waterloo LAW, Brox R, Lber S, Weikert D, Behrens M, Gmeiner P, Niv MY. 2020. Rational design of agonists for bitter taste receptor TAS2R14: from modeling to bench and back. *Cell Mol Life Sci* 77:531–542. https://doi.org/10.1007/s00018-019-03194-2.

Ferreira LG, dos Santos RN, Oliva G, Andricopulo AD. 2015. Molecular docking and structure-based drug design strategies. *Molecules* 20:13384–13421. https://doi.org/10.3390/molecules200713384.

Hawkins PCD, Skillman AG, Warren GL, Ellingson BA, Stahl MT. 2010. Conformer generation with OMEGA: Algorithm and validation using high quality structures from the protein databank and cambridge structural database. *J Chem Inf Model* 50:572–584. https://doi.org/10.1021/ci100031x.

Van Holle A, Muylle H, Haesaert G, Naudts D, De Keukeleire D, Roldán-Ruiz I, Van Landschoot A. 2021. Relevance of hop terroir for beer flavour. *J Inst Brew* 127:238–247. https://doi.org/10.1002/jib.648.

Istvan ES, Deisenhofer J. 2001. Structural mechanism for statin inhibition of HMG-CoA reductase. *Science* 292:1160–1164. https://doi.org/10.1126/science.1059344.

Istvan ES, Palnitkar M, Buchanan SK, Deisenhofer J. 2000. Crystal structure of the catalytic portion of human HMG-CoA reductase: Insights into regulation of activity and catalysis. *EMBO J* 19:819–830. https://doi.org/10.1093/emboj/19.5.819.

Jawabrah Al-Hourani B, El Barghouthi MI, Al-Awaida W, McDonald R, Fattash IA, El Soubani F, Matalka K, Wuest F. 2020. Biomolecular docking, synthesis, crystal structure, and bioassay studies of 1-[4-(2-chloroethoxy)phenyl]-5-[4-(methylsulfonyl)phenyl]-1H-tetrazole and 2-(4-(5-(4-(methylsulfonyl)phenyl)-1H-tetrazol-1-yl)phenoxy)ethyl nitrate. *J Mol Struc* 1202:127323. https://doi.org/10.1016/j.molstruc.2019.127323.

Kohl S, Behrens M, Dunkel A, Hofmann T, Meyerhof W. 2013. Amino acids and peptides activate at least five members of the human bitter taste receptor family. *J Agric Food Chem* 61:53–60. https://doi.org/10.1021/jf303146h.

Kurumbail RG, Stevens AM, Gierse JK, McDonald JJ, Stegeman R , Pak JY, Gildehaus D, Miyashiro JM, Penning TD, Seibert K, Isakson PC, Stallings WC. 1996. Structural basis for selective inhibition of cyciooxygenase-2 by anti-inflammatory agents. *Nature* 384:644–648. https://doi.org/10.1038/384644a0.

Kutzner C, Páll S, Fechner M, Esztermann A, de Groot L, Grubmüller H. 2019. More bang for your buck: Improved use of GPU nodes for GROMACS 2018. *J Comput Chem* 40:2418–2431. https://doi.org/10.1002/jcc.26011.

Lang T, Lang R, Di Pizio A, Mittermeier VK, Schlagbauer V, Hofmann T, Behrens M. 2020. Numerous compounds orchestrate coffee's bitterness. *J Agric Food Chem 68:6692–6700*. https://doi.org/10.1021/acs.jafc.0c01373.

Lee Y, Basith S, Choi S. 2018. Recent advances in structure-based drug design targeting class A G protein-coupled receptors utilizing crystal structures and computational simulations. *J Med Chem* 61:1–46. https://doi.org/10.1021/acs.jmedchem.6b01453.

Martin M, Deussen A. 2019. Effects of natural peptides from food proteins on angiotensin converting enzyme activity and hypertension. *Crit Rev Food Sci Nutr* 59:1264–1283. https://doi.org/10.1080/10408398.2017.1402750.

Meyerhof W, Batram C, Kuhn C, Brockhoff A, Chudoba E, Bufe B, Appendino G, Behrens M. 2009. The molecular receptive ranges of human TAS2R bitter taste receptors. *Chem Senses* 35:157–170. https://doi.org/10.1093/chemse/bjp092.

Mikyška A, Olšovská J, Slabý M, Štěrba K, Čerenak A, Košir IJ, Pavlovič M, Kolenc Z, Krofta K. 2018. Analytical and sensory profiles of Slovenian and Czech hop genotypes in single hopped beers. *J Inst Brew* 124:209–221. https://doi.org/10.1002/jib.494.

Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ. 2009. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J Comput Chem* 30:2785–2791. https://doi.org/10.1002/jcc.21256.AutoDock4.

Nowak S, Di Pizio A, Levit A, Niv MY, Meyerhof W, Behrens M. 2018. Reengineering the ligand sensitivity of the broadly tuned human bitter taste receptor TAS2R14. *Biochim Biophys Acta Gen Subj* 1862:2162–2173. https://doi.org/10.1016/j.bbagen.2018.07.009.

Ocvirk M, Grdadolnik J, Košir IJ. 2016. Determination of the botanical origin of hops (*Humulus lupulus L.*) using different analytical techniques in combination with statistical methods. *J Inst Brew* 122:452–461. https://doi.org/10.1002/jib.343.

Ou-Yang SS, Lu JY, Kong XQ, Liang ZJ, Luo C, Jiang H. 2012. Computational drug discovery. *Acta Pharmacol Sin* 33:1131–1140. https://doi.org/10.1038/aps.2012.109.

Pérez-Sánchez H, Gesing S, Merelli I. 2016. High performance computing in drug discovery. *Curr Drug Targets* 17:1578–1579. https://doi.org/10.2174/1389450117141609302305 42.

Pronk S, Páll S, Schulz R, Larsson P, Bjelkmar P, Apostolov R, Shirts MR, Smith JC, Kasson PM, Van der Spoel D, Hess B, Lindahl E. 2013. GROMACS 4.5: A high-throughput and highly parallel open source molecular simulation toolkit. *Bioinformatics* 29:845–854. https://doi.org/10.1093/bioinformatics/btt055.

Sabín López A, Paredes-Ramos M, Lopez-Vilariño JM. 2020. The interest of xanthohumol as a health molecule. *Tecnifood* agosto:2–4.

Salentin S, Schreiber S, Haupt VJ, Adasme MF, Schroeder M. 2015. PLIP: Fully automated protein-ligand interaction profiler. *Nucleic Acids Res* 43:W443–W447. https://doi.org/10.1093/nar/gkv315.

Sandal M, Behrens M, Brockhoff A, Musiani F, Giorgetti A, Carloni P, Meyerhof W. 2015. Evidence for a transient additional ligand binding site in the TAS2R46 bitter taste receptor. *J Chem Theory Comput* 11:4439–4449. https://doi.org/10.1021/acs.jctc.5b00472.

Schneider G, Clark DE. 2019. Automated *de novo* drug design: are we nearly there yet?. *Angew Chem Int Ed* 58:10792–10803. https://doi.org/10.1002/anie.201814681.

Schönberger C, Kostelecky T. 2011. 125th anniversary review: The role of hops in brewing. *J Inst Brew* 117:259–267. https://doi.org/10.1002/j.2050-0416.2011.tb00471.x.

Shaik FA, Jaggupilli A, Chelikani P. 2019. Highly conserved intracellular H208 residue influences agonist selectivity in bitter taste receptor T2R14. *Biochim Biophys Acta Biomembr* 1861:183057. https://doi.org/10.1016/j.bbamem.2019.183057.

Shaik FA, Singh N, Arakawa M, Duan K, Bhullar RP, Chelikani P. 2016. Bitter taste receptors: Extraoral roles in pathophysiology. *Int J Biochem Cell Biol* 77:197–204. https://doi.org/10.1016/j.biocel.2016.03.011.

Sousa Da Silva AW, Vranken WF. 2012. ACPYPE - AnteChamber PYthon Parser interfacE. *BMC Res Notes* 5:1–8. https://doi.org/10.1186/1756-0500-5-367.

Tarragon E, Moreno JJ. 2020. Polyphenols and taste 2 receptors. Physiological, pathophysiological and pharmacological implications. *Biochem Pharmacol* 178:114086. https://doi.org/10.1016/j.bcp.2020.114086.

Trott O, Olson A. 2010. Autodock Vina: improving the speed and accuracy of docking. *J Comput Chem* 31:455–461. https://doi.org/10.1002/jcc.21334. AutoDock.

Vermeirssen V, Van Camp J, Verstraete W. 2002. Optimisation and validation of an angiotensin-converting enzyme inhibition assay for the screening of bioactive peptides. *J Biochem Biophys Methods* 51:75–87. https://doi.org/10.1016/S0165-022X(02)00006-4.

Wiener A, Shudler M, Levit A, Niv MY. 2012. BitterDB: A database of bitter compounds. *Nucleic Acids Res* 40:413–419. https://doi.org/10.1093/nar/gkr755.

Woo JA, Castaño M, Goss A, Kim D, Lewandowski EM, Chen Y, Liggett SB. 2019. Differential long-term regulation of TAS2R14 by structurally distinct agonists. *FASEB J* 33:12213–12225. https://doi.org/10.1096/fj.201802627RR.

Wu CN, Sun LC, Chu YL, Yu RC, Hsieh CW, Hsu HY, Hsu FC, Cheng KC. 2020. Bioactive compounds with anti-oxidative and anti-inflammatory activities of hop extracts. *Food Chem* 330:127244. https://doi.org/10.1016/j.foodchem.2020.127244.

Zhang Y, Wang X, Li X, Peng S, Wang S, Huang C Z, Zhang Q, Li D, Jiang J, Ouyang Q, Zhang Y, Li S, Qiao Y. 2017. Identification of a specific agonist of human TAS2R14 from *Radix Bupleuri* through virtual screening, functional evaluation and binding studies. *Sci Rep* 7:1–12. https://doi.org/10.1038/s41598-017-11720-0.