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Bitterness prediction *in-silico*: A step towards better drugs

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ABSTRACT

Bitter taste is innately aversive and thought to protect against consuming poisons. Bitter taste receptors (Tas2Rs) are G-protein coupled receptors, expressed both orally and extra-orally and proposed as novel targets for several indications, including asthma. Many clinical drugs elicit bitter taste, suggesting the possibility of drugs re-purposing. On the other hand, the bitter taste of medicine presents a major compliance problem for pediatric drugs. Thus, efficient tools for predicting, measuring and masking bitterness of active pharmaceutical ingredients (APIs) are required by the pharmaceutical industry. Here we highlight the BitterDB database of bitter compounds and survey the main computational approaches to prediction of bitter taste based on compound's chemical structure. Current *in silico* bitterness prediction methods provide encouraging results, can be constantly improved using growing experimental data, and present a reliable and efficient addition to the APIs development toolbox.

1. Introduction

Bitter taste is one of the basic taste modalities, the role of which is typically linked to guarding against consumption of poisons (Chandrashekar et al., 2006). However, not all bitter compounds are toxic: many dietary phytonutrients commonly found in fruits and vegetables (Drewnowski and Gomez-Carneros, 2000), as well as many clinical drugs (Mennella et al., 2013) elicit bitter taste sensation. The bitterness of drug molecules presents a major problem of compliance for children (Mennella and Beauchamp, 2008). Sensory tasting of drug candidates by humans is not a trivial matter, since it requires ethical approval achievable only after a thorough toxicological study. Thus, efficient prediction of compounds' bitterness in the initial stages of drug discovery is of great interest. Several computational *(in-silico)* studies predicted bitter taste of compounds based on their chemical structure, as described below and summarized in Fig. 1. The cost-effectiveness and the possibility to improve the prediction quality based on the growing body of experimental data suggest that *in-silico* bitterness prediction could become a practical step in the process of developing pediatric drugs.

2. Bitter compounds: databases and properties

We have recently established the BitterDB (Wiener et al., 2012), a freely accessible repository of compounds that were reported to be bitter for humans or to activate bitter taste receptors (Tas2Rs) in cell-based functional assays. The bitter-tasting compounds are recognized by a subfamily of G-protein coupled receptors, the Tas2R (Chandrashekar et al., 2006). This family is comprised of 25 receptor subtypes in humans, activated by varying number of partially activated ligands (Meyerhof et al., 2010). Most of the information on the bitter compounds was extracted from the Merck Index (O'Neil, 2006), Fenaroli's handbook of flavor ingredients (Burdock and Fenaroli, 2005) and research articles retrieved from PubMed. Currently, the BitterDB comprises close to 700 bitter compounds, 120 of which were experimentally assigned to their cognate Tas2Rs. BitterDB can be searched using words, molecular identifiers (such as SMILES or CAS registry number), chemical properties, or the associated bitter taste receptors. Additionally, chemical structures may be submitted as queries to find identical or similar compounds within the BitterDB. Notably, bitter compounds, even those that activate the same bitter taste receptor subtype, may vary dramatically in their chemical structures and physicochemical properties (Di Pizio and Niv,

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2015; Levit et al., 2014). For example, though hydrophobicity was suggested to describe the bitterness of peptides (Ney's Q rule (Ney, 1971)), it was not predictive of bitterness of soy (Cho et al., 2004) or cheese (Toelstede and Hofmann, 2008) peptides. Indeed, the value of logP (octanol-water partition) of bitter compounds varies rather widely, i.e. −6.292 for streptomycin or −4.836 for $MgBr_2$ vs. 6.484 for adlupulone from beer or 6.417 for Polysorbate 60 (data obtained *via* the BitterDB http://bitterdb.agri.huji.ac.il/dbbitter.php#compoundBrowse).

An early attempt to classify bitter compounds (Rodgers et al., 2005) used a large clustering analysis to build a hierarchical phylogenetic-like tree based on the maximal common substructures of over 800 proprietary bitter molecules. The final tree contained 93 nodes with at least 8 members each, suggesting there are at least 93 sub-structures that may be linked to bitterness.

Some bitter compounds are Tas2R-selective, meaning that they activate a single Tas2R subtype. Others activate multiple Tas2R, and can be termed Tas2R-promiscuous. Overall, we found that the Tas2R-promiscous compounds tend to be smaller, more globular and more hydrophobic than Tas2R-selective compounds. A linear regression tool built using these features correctly classified an external set of bitter molecules into Tas2R-selective and Tas2R-promiscuous bitterants (Di Pizio and Niv, 2015).

3. Predicting bitter compounds

There are several methods for predicting bitterness of compounds (see Fig. 1), as summarized below.

3.1. Ligand-based methods

Identification of previously unknown bitter compounds can rely on similarity to known bitterants, an approach well established in drug discovery and widely used when the structure of the target protein is not available (Sliwoski et al., 2014). For example, Quantitative Structure-Activity-Relation (QSAR) models are applicable when biological data is available for a focused chemical series. Such models were established for the prediction of bitterness of several analogues of sesquiterpene lactones and for some classes of peptides (Ley, 2008). Other well-established techniques use 'ligand-based pharmacophore' (LBP) models which are three-dimensional representations of structural features conserved among known actives, and 'shape-based screening' which allows to filter compounds based upon shape and electrostatics similarity to the query molecule. Roland and co-workers (Roland et al., 2013) described the structural requirements of flavonoids for the activation of two bitter receptors – Tas2R14 and 39. Using a test set of 73 (for Tas2R14) and 77 (for Tas2R39) compounds, the LBP models predicted activation of these receptors by flavonoids. 68% sensitivity (the ratio between predicted true positives and the total true positives, also known as true positives rate) and 65% specificity (the ratio between predicted true negatives and the total true negatives, also known as true negatives rate) was achieved for Tas2R14 and 85% sensitivity, 78% specificity for Tas2R39. Additionally, favorable and unfavorable ligand molecular features for receptor activation were highlighted.

Levit and co-workers screened the BitterDB compounds which were not yet assigned to a particular taste receptor, and the DrugBank dataset of clinical drugs (Wishart et al., 2006) for prospective prediction of Tas2R14 activators using LBP and shape-based models. Subsequently, 9 out of 11 BitterDB predicted compounds and 11 out of 23 DrugBank predicted compounds were experimentally confirmed as Tas2R14 activators (Levit et al., 2014).

3.2. Structure-based methods

With the constant rise of structural information of the target proteins, including GPCRs (Di Pizio et al., 2016; Yarnitzky et al., 2010), structure-based methods which predict novel compounds that favorably fit into the binding site of the target (Irwin and Shoichet, 2016; Kitchen et al., 2004) become more and more

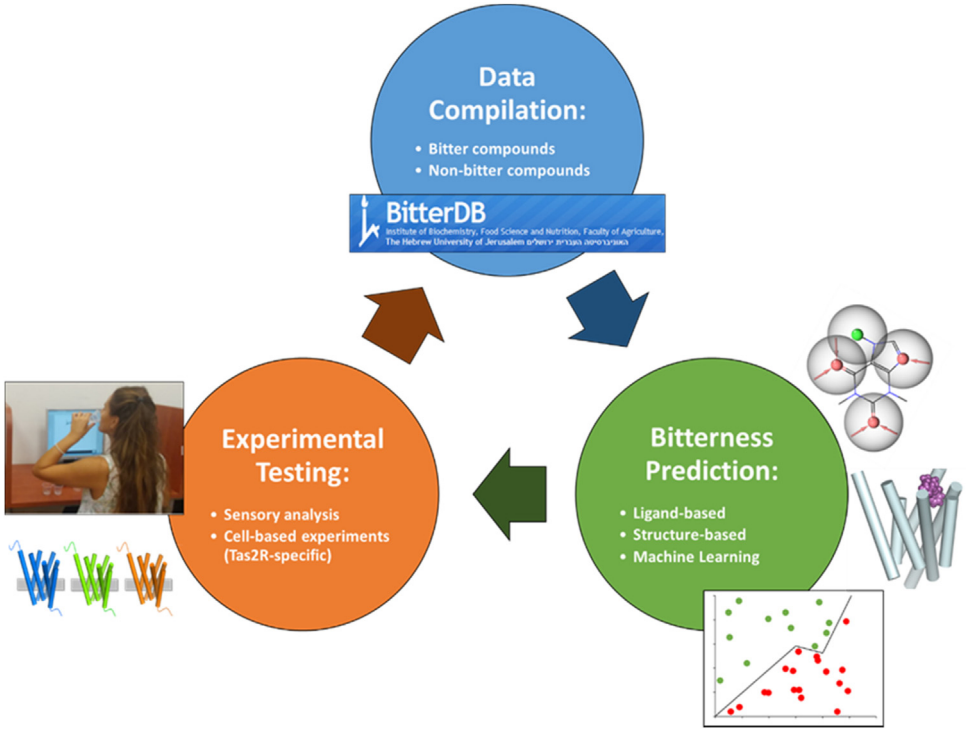

Fig. 1. Iterative usage of experimental data towards improvement of computational predictive tools.

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