Bioorganic & Medicinal Chemistry 23 (2015) 4082–4091

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Promiscuity and selectivity of bitter molecules and their receptors

Antonella Di Pizio, Masha Y. Niv *

Institute of Biochemistry, Food Science and Nutrition, Robert H Smith Faculty of Agriculture Food and Environment, The Hebrew University, Rehovot 76100, Israel
Fritz Haber Center for Molecular Dynamics, The Hebrew University, Jerusalem 91904, Israel

ARTICLE INFO

Article history:
Received 5 February 2015
Revised 4 April 2015
Accepted 8 April 2015
Available online 16 April 2015

Keywords:
Chemosensory
Chemical senses
Molecular recognition
GPCR
Canonical binding site
Orthosteric
Modeling

ABSTRACT

Bitter taste is essential for survival, as it protects against consuming poisonous compounds, which are often bitter. Bitter taste perception is mediated by bitter taste receptors (TAS2Rs), a subfamily of G-protein coupled receptors (GPCRs). The number of TAS2R subtypes is species-dependent, and varies from 3 in chicken to 50 in frog. TAS2Rs present an intriguing case for studying promiscuity: some of the receptors are still orphan, or have few known agonists, while others can be activated by numerous, structurally dissimilar compounds. The ligands also vary in the repertoire of TAS2Rs that they activate: some bitter compounds are selective toward a single TAS2R, while others activate multiple TAS2Rs. Selectivity/promiscuity profile of bitter taste receptors and their compounds was explored by a chemoinformatic approach. TAS2R-promiscuous and TAS2R-selective bitter molecules were found to differ in chemical features, such as *A*log*P*, E-state, total charge, number of rings, globularity, and heavy atom count. This allowed the prediction of bitter ligand selectivity toward TAS2Rs. Interestingly, while promiscuous TAS2Rs are activated by both TAS2R-promiscuous and TAS2R-selective compounds, almost all selective TAS2Rs in human are activated by promiscuous compounds, which are recognized by other TAS2Rs anyway. Thus, unique ligands, that may have been the evolutionary driving force for development of selective TAS2Rs, still need to be unraveled.

1. Introduction

Bitter taste is one of the basic taste modalities and is essential for rejecting potentially harmful substances.[1] The detection of the structurally diverse naturally occurring bitter compounds, as well as of synthetic bitter compounds, is mediated by the TAS2R[†] family of G-protein coupled receptors (GPCRs)[‡].[2] The number of TAS2R genes, as well as the fraction of pseudogenes, is species-dependent, indicating gene expansions and contractions during evolution.[3] Number of subtypes is varying widely, humans have 25 TAS2Rs, frogs 50 TAS2Rs, and chicken just 3 TAS2Rs.[4] TAS2Rs are expressed in many extra-oral tissues and are thought to have multiple physiological roles,[5] suggesting potential existence of endogenous ligands and the need for detecting an even larger repertoire of diverse ligands than previously thought.

The number of TAS2R agonists (the molecules that activate TAS2Rs and elicit bitter taste sensation) is estimated by thousands.[6] So far, we have gathered structures of close to 700 bitter compounds in the BitterDB database,[7] based on entries in Merck index, Fenaroli book of flavors and several publications, in which the bitter taste of the molecule was indicated. Importantly, for some of the bitter ligands, the association with particular TAS2Rs was established by in vitro assays, usually using calcium imaging,[6] and this information can also be accessed via the BitterDB (http://bitterdb.agri.huji.ac.il).[7]

In human, the 25 TAS2Rs represent about 4% of the GPCRs. Because of low sequence similarity of TAS2Rs with other GPCRs, their classification is ambiguous: TAS2Rs were grouped with the frizzled receptors[8] or considered a distinct family.[9] However, TAS2Rs are typically considered as class A.[10–12]

Intriguingly, some bitter taste receptors have a broad range of chemically diverse ligands, while others are narrowly tuned.[4,6,7] Promiscuity of proteins is highly abundant in nature and is increasingly investigated because of its implication in many areas of applied biology.[13] Elucidation of how selectivity and promiscuity are achieved within the binding pocket of proteins may contribute to the drug development process and enable rational manipulation of proteins toward binding of drugs.

The molecular properties influencing the pharmacological selectivity/promiscuity profile of GPCRs may be essential for understanding the molecular recognition processes and the side effects associated with many drugs targeting these receptors. Recently, Levit et al.[14] have focused on class A GPCRs with available

* Corresponding author.
E-mail address: masha.niv@mail.huji.ac.il (M.Y. Niv).
† TAS2R, sometimes abbreviated as T2R, bitter taste receptor.
‡ GPCRs, G-protein coupled receptors.

http://dx.doi.org/10.1016/j.bmc.2015.04.025
0968-0896/

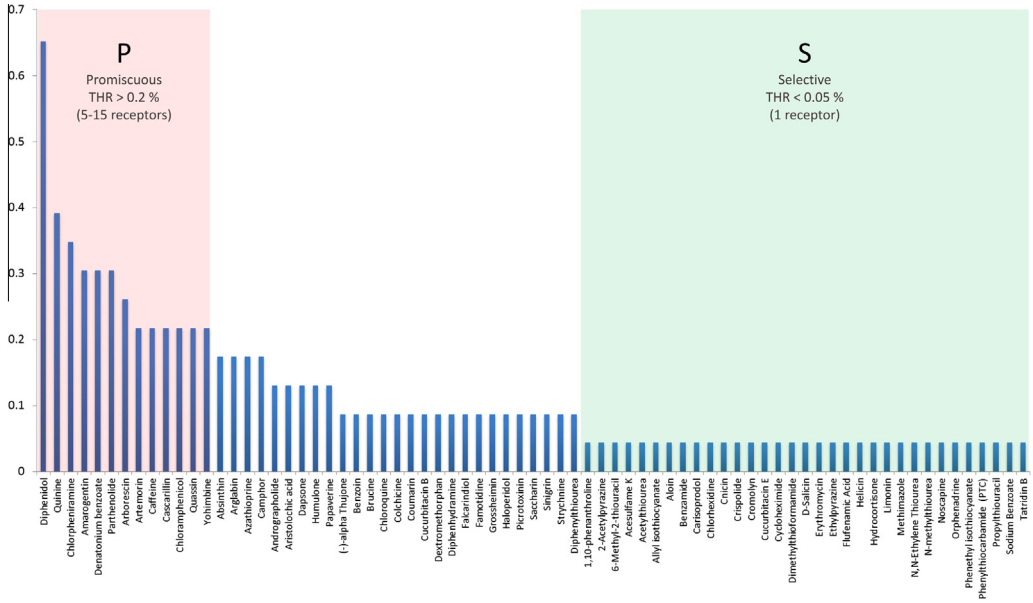

Figure 1. THR distribution of Set 1. Set 2 compounds are highlighted in red (promiscuous) and green (selective).

experimental structures and identified features of the orthosteric (canonical) binding site that correlate with the number and diversity of antagonists. Specifically, the number of unique scaffolds (a measure of the chemical variability) of antagonists was shown to be in correlation with the binding site exposure and hydrophobicity, and in negative correlation with the number of hydrogen bond donors in the binding site.

Ligands, as well as receptors, can exhibit promiscuity: many compounds interact with many different proteins. Studying the promiscuity (or polypharmacology) of ligands[15] has some practical advantages: while receptors are a given in the biological system, the ligands can be modified synthetically, and their pharmacological profile can (at least in theory) be tailor-made. Indeed, the classical idea of *'selective ligands for single molecular targets'* is starting to give way to the polypharmacology paradigm which is based on *'the promiscuous modulation of several molecular targets'*.[16–18] With the progress in GPCR structure determination and molecular modeling, more information becomes available, and GPCR ligand selectivity is being closely investigated. Shonberg et al.,[19] reviewing the current state of solved GPCR structures, highlighted ligand–receptor interactions in the binding pocket that can contribute to design of GPCR ligands with better affinity and subtype selectivity. A focused analysis of the subtype-selective compounds within the aminergic GPCRs has been carried out by Michino et al.[20] In particular, binding of ligands to the orthosteric binding site, to the secondary binding pocket, or concomitantly to both was discussed. The second and third binding scenarios were suggested as best strategies to be exploited for the optimization of existing lead compounds.

Since no experimental structures of TAS2Rs are available yet, iterative combination of homology modeling, site-directed mutagenesis and calcium imaging assays is used to unravel the ability of TAS2Rs to accommodate dissimilar bitter agonists.[7] Usages of both common and ligand-specific sub-pockets within the orthosteric binding site emerge as typical strategies.[21,22]

The current study focuses on the selectivity and promiscuity of bitter ligands and TAS2Rs. By applying a chemoinformatic approach, we aim to highlight chemical properties of TAS2R-promiscuous and TAS2R-selective compounds, and to investigate bitter selectivity of ligands in relation to selectivity and promiscuity of bitter receptors.

2. Results and discussion

A set of 104 bitter compounds was comprehensively tested by Meyerhof et al. on all human bitter taste receptors (hTAS2Rs)[§] under the same assay conditions.[6] Since the agonistic activity of these compounds toward hTAS2Rs occurs at different concentrations, our dataset, referred to as Set 1 hereafter, contains only 73 compounds that elicited TAS2R activation at concentration of 300 μM or lower. As can be seen in Figure 1, Set 1 includes both promiscuous and selective compounds toward hTAS2Rs. Compound promiscuity can be defined as THR[¶] (target hit-rate) parameter: the number of targets hit at a specific concentration divided by the number of targets tested.[23] We consider as targets hTAS2Rs only, and define compounds with THR $\geqslant$ 0.2 as TAS2R-promiscuous (P) and compounds with THR $\leqslant$ 0.05 as TAS2R-selective (S). We will refer to P and S compounds without the intermediate promiscuity compounds as Set 2 (Supplementary data Table S1).

A subset of the compounds in Set 1 were recently profiled also against the bitter taste receptors in chicken (ggTAS2Rs)[‖], turkey (mgTAS2Rs)[††], zebra finch (tgTAS2Rs)[‡‡], and six representative bitter taste receptors out of the total 50 in frog (xtTAS2Rs)[§§].[4] Among these, applying a cut-off of 300 μM (see Methods) results in 25 compounds, referred to as Set 3 (Supplementary data Table S1). Chicken, turkey, zebra finch and frog receptors were activated by 14, 8, 2 and 21 compounds respectively.

Notably, the TAS2R-selectivity/promiscuity profile seems to hold across species (Fig. 2A). This is not the general rule, as discussed by Hopkins et al.[16] It will be interesting to follow cross-species profiles as repertoires of TAS2R of additional species become available in the future. On the other hand, if 'target' definition for THR is extended to include any target (and not just TAS2R), the promiscuity profile changes dramatically (Fig. 2B).

The fact that selectivity and promiscuity of compounds toward TAS2Rs does not generalize to other targets (Fig. 2B) could be due

§ hTAS2R, human bitter taste receptor.
¶ THR, target hit-rate parameter.
‖ ggTAS2R, chicken (*Gallus gallus*) bitter taste receptor.
†† mgTAS2R, turkey (*Meleagris gallopavo*) bitter taste receptor.
‡‡ tgTAS2R, zebra finch (*Taeniopygia guttata*) bitter taste receptor.
§§ xtTAS2R, frog (*Xenopus tropicalis*) bitter taste receptor.