doi:10.2533/chimia.2022.418 Chimia 76 (2022) 418–424

The Bitter Taste Receptor TAS2R14 as a Drug Target

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Abstract: G protein-coupled receptors (GPCRs) mediate most of our physiological responses to hormones, neurotransmitters, and environmental stimulants. Besides human senses like vision and olfaction, taste perception is mostly mediated by GPCRs. Hence, the bitter taste receptor family TAS2R comprises 25 distinct receptors and plays a key role in food acceptance and drug compliance. The TAS2R14 subtype is the most broadly tuned bitter taste receptor, recognizing a range of chemically highly diverse agonists. Besides other tissues, it is expressed in human airway smooth muscle and may represent a novel drug target for airway diseases. Several natural products as well as marketed drugs including flufenamic acid have been identified to activate TAS2R14, but higher potency ligands are needed to investigate the ligand-controlled physiological function and to facilitate the targeted modulate for potential future clinical applications. A combination of structure-based molecular modeling with chemical synthesis and *in vitro* profiling recently resulted in new flufenamic acid agonists with improved TAS2R14 potency and provided a validated and refined structural model of ligand–TAS2R14 interactions, which can be applied for future drug design projects.

Keywords: Bitter taste receptor · Drug discovery · Flufenamic acid · GPCRs

Peter Gmeiner received his PhD from the University of Munich in 1986. Subsequently, he was a post-doctoral associate in Henry Rapoport's laboratory at the University of California in Berkeley, USA. Upon receiving his habilitation in 1992, he was appointed as a Professor of Medicinal Chemistry at the University of Bonn. Since October 1996, he has held the Chair of Medicinal Chemistry at the Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU). Peter Gmeiner's research spans the structure-based-design, synthesis, and pharmacological investigation of novel G-protein coupled receptor (GPCR) ligands. Within these studies, subtype- and functionally selective GPCR ligands are of particular interest.

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1. Introduction

G protein-coupled receptors (GPCRs), also known as seven transmembrane helical receptors, corresponding to approximately 4% of the proteins coded by the human genome, represent the largest human membrane protein family with overall approximately 800 members. The GPCR superfamily is divided into six classes: Rhodopsin (Class A), Secretin (Class B1), Adhesion (Class B2), Glutamate (Class C), Frizzled (Class F) and Taste 2 (Class T), sometimes referred to as GRAFS (short for Glutamate, Rhodopsin, Adhesion, Frizzled/Taste2 and Secretin). GPCRs have proven to be important pharmacological targets in the development of new drugs, as they are involved in a vast number of (patho)physiological processes in the human organism.[1–4] This receptor superfamily represents the largest and most intensively studied class of druggable targets in the human genome. Taking into account that 20–30% of marketed pharmaceutical drugs unfold their effect through binding to GPCRs, these highly diverse membrane receptors are regarded as the most valuable drug-targets in medicinal chemistry.[5–8] Within the group of roughly 400 non-olfactory GPCRs, 108 members (27%) constitute established drug targets, 66 (17%) represent targets with candidates in clinical trials, while the remaining 224 (56%) receptors are currently non-targeted GPCRs. The most established target groups include adrenergic (20%), histamine (14%), acetylcholine (12%) and opioid receptors (7%).[3] Altogether, the FDA approval for pharmaceuticals acting on the 108 unique GPCR targets comprises 475

drugs in total, which constitutes approximately 34% of all drugs approved by the FDA.

Besides being targets for a huge number of pharmaceuticals, GPCRs are also responsible for the transduction of sensory impressions such as vision, olfaction and the taste modalities sweet, umami and bitter. While sweet and umami perceptions are perceived by the TAS1R family, bitter taste sensation is mediated by TAS2Rs. The TAS2R family consists of 25 GPCRs (Fig. 1), all showing a different compound reception range including molecules of both natural and synthetic origin. From an evolutionary perspective, TAS2Rs probably have evolved as a defensive mechanism by eliciting an innate aversive response to provide animals and humans critical protection against ingestion of toxic food.[9–14] Although numerous bitter tasting and TAS2R activating substances like strychnine are well known for their toxicity, bitterants, in general, are not necessarily harmful.[9] In fact, bitter molecules such as glucosinolates and isothiocyanates, which are contained in broccoli and other cruciferous vegetables, have been shown to be protective against cancer.[15,16] Still, despite those advantages, many of those bioactive components give rise to aversion in the consumer due to their repulsive taste.[17]

Receptor activation by TAS2R agonists leads to signal transduction *via* the heterotrimeric G-protein gustducin, which consists of α-gustducin (Gnat3), Gβ3 and Gγ13. Agonist binding induces the dissociation of gustducin into its α and βγsubunits, with the latter leading to a stimulation of phospholipase C (PLCβ2). PLCβ2 then initiates a Ca^{2+} release from IP3-sensitive Ca^{2+} stores, leading to a Na^{+} influx through TRPM5 channels. The resulting Na^{+}-mediated depolarization of the cells induces the release of ATP, which ultimately activates purinergic receptors localized in the taste buds. The generated signal is transduced to the taste center in the central nervous system (CNS) causing bitter taste perception.[14] Interestingly, the third intracellular loop (ICL3) of the receptor plays a critical role in bitter taste receptor activation. Mutational studies identified highly conserved ICL3 residues stabilizing an inactive receptor conformation. Alanine mutations of residues located on ICL3, especially H214A, caused the receptor to adopt an active conformation involving the movement of transmembrane helix 6 (TM6).[18] Interestingly, H214 is located two amino acids below a highly conserved LxxSL motif, which has been found to play a crucial structural role in the stabilization of transmembrane helix 5 (TM5).[19]

2. TAS2Rs are more than Bitter Taste Receptors

Recently, it has been discovered that TAS2Rs are not exclusively located in the oral cavity. Bitter taste receptors have been detected in extraoral tissues such as human airway smooth muscles (HASM), rat-brain cells, mammary epithelial, breast cancer cells, bone marrow cells, the heart and the gastrointestinal tract of rats and mice.[22–27] Interestingly, activation of TAS2Rs located on HASM has been shown to induce bronchodilation. This effect exceeds bronchodilation triggered by common β-adrenergic receptor agonists used in the treatment of asthma by a factor of three.[22] Additionally, it has been shown that the signaling mechanism, apart from receptor activation and the resulting increase of intracellular Ca^{2+} concentration, differs from the pathway observed in the taste buds, and the exact pharmacological mode of action leading to TAS2R induced relaxation of HASM tissue still needs to be elucidated.[14] The discovered possibility to trigger bronchodilation by activation of TAS2Rs located on human airway smooth muscles could open an innovative new path for the development of novel therapeutic options for asthma or chronic obstructive pulmonary disease (COPD). A known side effect in the treatment of those airway diseases with common β2-adrenergic receptor (β2-AR) agonists is the agonist-promoted desensitization of GPCR signal transduction, which can limit their therapeutic effect.[28] Desensitization in general is the down-regulation of GPCR signaling as a response to agonist-induced overstimulation, whereas β-arrestin recruitment and receptor phosphorylation by GPCR kinases (GRKs) play an important role in this response mechanism.[29] A modest degree of desensitization (20–30%) probably mediated *via* GRKs has been observed for TAS2Rs upon exposure

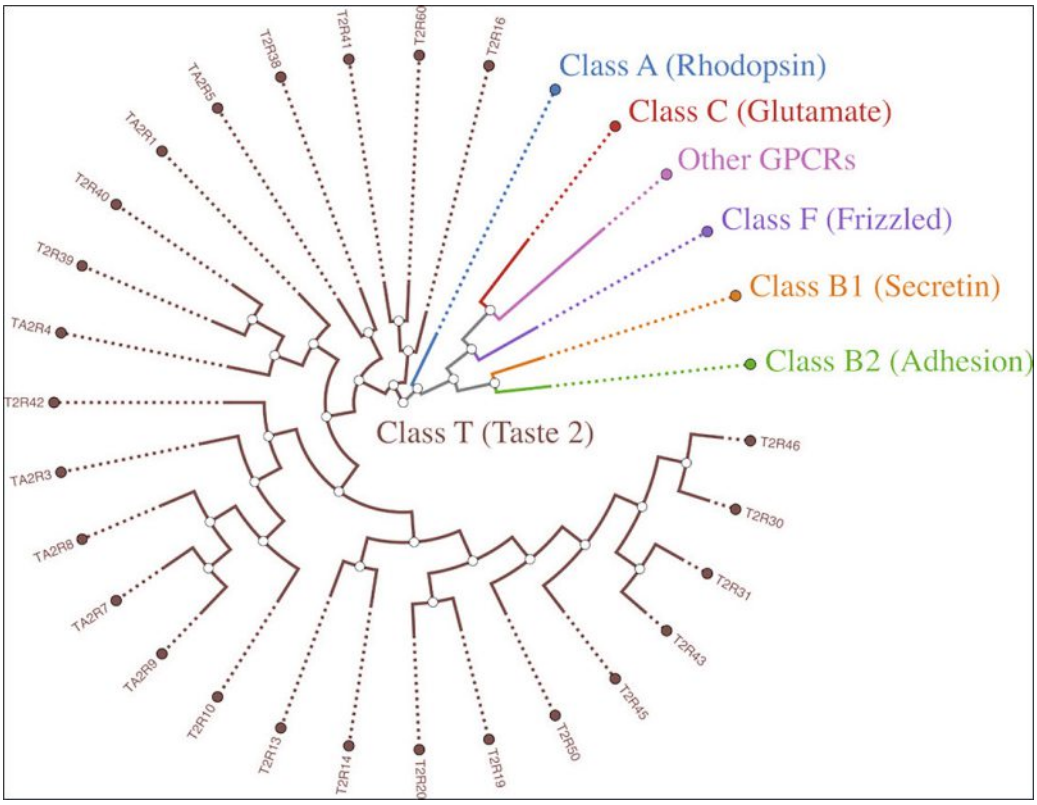
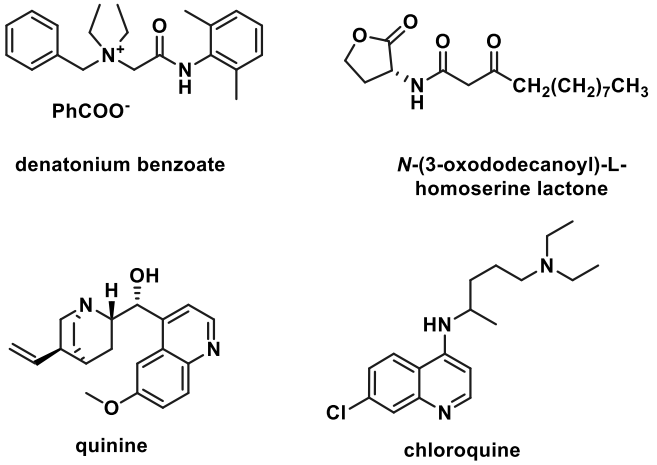


Fig. 1. Phylogenetic tree showing the 25 human bitter taste receptors in connection with the remaining 6 GPCR families. GPCRs are listed according to UniProt nomenclature.[20,21]

of the bitter tastant quinine to HASM, which might affect their therapeutic potential (Scheme 1).[30]



Scheme 1. Known bitter-tasting substances featuring TAS2R agonism.

However, it has been shown that, under conditions where β2-AR desensitization takes place, bitter taste receptors on isolated HASM still maintained their relaxation response after treatment with the agonist chloroquine.[31] TAS2R expression in the gastrointestinal tract appears to be in close correlation with molecular sensing of the luminal contents in order to regulate motility and hormone secretion but also identify noxious substances.[27] As revealed in mice experiments, TAS2Rs are also involved in spermatogenesis. An ablation of cells bearing TAS2Rs led to the formation of smaller testis and the depletion of the bitter taste transduction cascade resulted in a loss of spermatids.[32] Due to their expression in the upper respiratory tract, particularly in trigeminally innervated solitary chemosensory cells (SCCs), TAS2Rs are also related to a protective role. When exposed to irritants like the bitter substance denatonium benzoate or several bacterial quorum-sensing molecules such as acyl-homoserine lactones, the signal transduction cascade in the SCCs is initiated, evoking trigeminally mediated reflex reactions.[33] The presumed participation of TAS2Rs in immune defense mechanisms is also supported by the evidence that the quorum-sensing molecule *N*-(3-oxododecanoyl)-L-homoserine lactone has been shown to activate TAS2R38, a bitter taste receptor expressed in neutrophils (Scheme 1).[34]

Due to their involvement in host defense mechanisms, TAS2Rs were also suggested to be addressed by known bitter drugs in the context of host-directed therapies (HDTs) to fight the sudden COVID-19 outbreak.[35] Although a lot of evidence has been accumulated that sensory perception is just only one physiological function among numerous others of TAS2Rs, there is still a lot to be discovered, *e.g.* the function of the bitter taste receptors expressed in the brain and the bone marrow.[23,25]

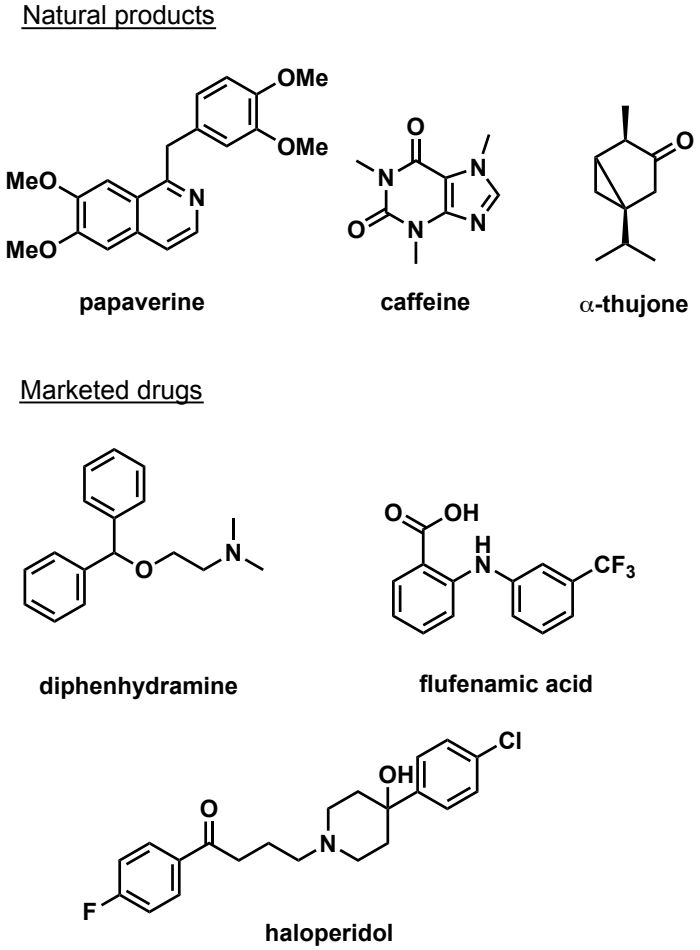
Nonlingual TAS2Rs signaling is currently differentiated in three distinct cascades: cell-autonomous regulation, paracrine regulation and endocrine regulation. All three are sharing the initiatory part of the pathway, namely receptor activation and increase of intracellular Ca^{2+} concentration. After Ca^{2+} has been released, the cascades start to diverge depending on the cell type or tissue.[14] The cell-autonomous regulation was first observed in the motile cilia of human airway epithelia.[36] Here, the motility of the cilia is presumably caused by a direct impact of Ca^{2+} on the cilia or indirectly *via* a cyclic nucleotide-dependent phase.[37] The same regulation pathway is present in HASM, the findings on how Ca^{2+} is affecting cell organelles and what finally causes the muscle tissue to relax are not entirely consistent. The original report states that calcium upon release activates conductance-dependent K^+ (BKCa) channels, leading to a membrane hyperpolarization, which is responsible for muscle relaxation.[22] However, further research found that those BKCa channels are not activated, but inhibited by calcium.[38,39] Furthermore, the βγ subunit of gustducin appears to play a critical role in the bronchodilator activity of bitter tastants on HASM by shutting down L-type voltage-dependent Ca^{2+} channels, which leads to a decrease in calcium concentration causing the muscle to relax.[40] The important role of TAS2Rs in paracrine secretion was first observed in enteroendocrine cells (EECs), where the receptor activation leads to a cholecystokinin (CCK) signaling mechanism. CCK can act either *via* CCK2 receptors leading to activation of ATP-binding cassette B1 (ABCB1), which causes an efflux of toxic bitter compounds out of the cell or through CCK1 receptors located in sensory fibers of the vagal nerve to control food intake.[41,42] Stimulation of solitary chemosensory cells in the nasal cavity and the vomeronasal organ of mice induces acetylcholine release leading to the activation of nicotinic acetylcholine receptors (nAChRs) in nerve fibers. This evokes a decrease in breathing rate and induces a neurogenic inflammation in the nasal cavity without recruitment of the adaptive immune system.[43–45] Furthermore, an endocrine pathway can be triggered by bitter taste receptors in tissues, where TAS2R stimulation is coupled to the release of hormones into the bloodstream. Activation of TAS2Rs expressed in EECs induced by denatonium benzoate has been shown to cause the release of glucagon-like peptide 1 (GLP-1), whereupon insulin secretion was observed.[46]

As already mentioned, members of different GPCR classes serve as targets for approved drugs in various clinical applications, whereas class A receptors are leading the way. Although TAS2Rs have been proven to be involved in many regulatory mechanisms besides their role as chemosensors, and seem to have very promising potential in the context of obstructive airway disease, no pharmaceuticals have been developed to target TAS2Rs as yet.[3] From a medicinal chemistry standpoint, there are two main challenges on the way to a successful drug design based on TAS2R ligands: The hitherto lacking subtype selectivity of the agonists due to the high promiscuity of the receptors' binding pockets and their poor potencies regarding receptor activation.[47] Presumably, TAS2Rs have evolved to cover a broad spectrum of chemically diverse ligands in order to protect the organism from noxious substances at the expense of affinity.[48] The development of subtype-selective, high-affinity ligands would be very beneficial in order to have a better understanding of the function of single receptors, but the lack of a crystal structure hampers this approach.[49]

3. TAS2R14 – One of the most Broadly Tuned Bitter Taste Receptors

Among the 25 TAS2Rs in the human genome, TAS2R14 is one of the most highly expressed subtypes in various tissues. This observation is almost entirely independent of the origin and pathological state of the tissue.[50] It has been shown that the receptor is vastly expressed in the human heart, with a level of expression comparable to the β1-adrenergic receptor (β1-AR).[26] In clinical samples of breast cancer epithelial, TAS2R14 shows an upregulated expression compared to healthy tissue. While in non-cancerous cells, activation of the receptor did not result in physiological responses, agonist stimulation of TAS2R14 located in highly metastatic breast cancer cells led to anti-proliferative, pro-apoptotic and anti-migratory responses.[51] Additionally, it is to some degree involved in male infertility and responsible for the mediation of the bitter taste of steviol glycosides.[52,53] The ability of TAS2R14 on airway motile cilia to produce bactericidal levels of NO after stimulation makes it a potential therapeutic target. Upon activation of the receptor by flavones or quorum-sensing bacterial quinolone 4-hydroxy-2-heptylquinolone, NO production was up-

regulated, leading to an increase in ciliary beating frequency and mucociliary clearance.[54,55] Presumably of most therapeutic relevance is the expression of TAS2R14 in human airway smooth muscles, where it exhibits a level of expression greater than that of β2-AR. Therefore, it presumably plays a crucial role for the bronchodilating effect observed after bitter tastant admission and becomes an attractive candidate for the treatment of obstructive airway diseases.[22,56,57] As for many GPCRs, TAS2R14 also undergoes desensitization after chronic agonist exposure. Research showed that the majority of analyzed agonists evoked β-arrestin recruitment and caused a subsequent receptor internalization upon treatment resulting in desensitization of ~50%. Interestingly, minimal desensitization was observed in the case of the known agonist diphenhydramine (DPD), which could be explained by a certain degree of signalling bias of DPD towards G-protein coupling (Scheme 2).[58]



Scheme. 2. Natural products and marketed drugs known to act as TAS2R14 agonists.

Besides some described bitter tastants of natural or synthetic origin, also numerous approved drugs were identified to be TAS2R14 agonists. Interestingly, bitter taste receptor ligands vary greatly in their molecular size and do not share an apparent common structural motif, despite consisting of at least one ring system, leading to the assumption, that a broadly tuned binding pocket is present. This could explain the fact that only 25 TAS2Rs are able to recognize thousands of structurally very diverse different molecules.[9,13,59] Some of the TAS2R14 agonists are commonly known substances with a widespread application, among which caffeine as an ingredient of coffee, tea and some energizing soft drinks displays the most prominent example, mainly due to its stimulating properties on the central nervous system.[60,61]

The absinthe ingredient α-thujone, a potent neurotoxin, was also identified as a TAS2R14 activator.[59] Furthermore, papaverine has been shown to be active at TAS2R14. First discovered by Dr. Georg Merck in 1848, papaverin represents a natural occurring opium alkaloid found in many members of the Papaver family and is used in the treatment of cerebral vasospasm.[62–64]

Interestingly, also a series of well-established marketed drugs turned out to feature bitter taste receptor activity. Besides the already mentioned first-generation antihistaminic agent DPD, which is a used for symptoms of allergic rhinitis, the neuroleptic drug haloperidol also acts as a TAS2R14 agonist.[9,65] Introduced in the late 1960s, it serves as an antipsychotic agent antagonizing the dopamine D2 receptor.[66,67] In search of a starting point for the development of novel, potent TAS2R ligands, one of the most interesting bitter taste receptor agonists is flufenamic acid. First reported in 1963, the drug shows anti-inflammatory and anti-pyretic properties and therefore belongs to the family of non-steroidal anti-inflammatory drugs (NSAIDs).[68–70] It was applied as a local analgesic against pain and inflammation in the context of musculoskeletal and joint disorders. The main anti-inflammatory effect takes place through the inhibition of cyclo-oxygenases, leading to a reduction of prostaglandin synthesis from arachidonic acid. Due to deleterious side effects like intersubject variation upon oral administration, reduced first-pass metabolism after dermal administration, renal damage and gastrointestinal perturbations, the medicinal use of flufenamic acid diminished is significantly.[71–73] Interest emerged in basic research after flufenamic acid has been identified as an ion channel modulator in the 1990s.[69] Besides this function, it also became relevant during the investigations on the molecular receptive range of TAS2Rs. When analyzing 104 natural or synthetic bitter chemicals in a cell-based assay, flufenamic acid turned out to be a subtype-selective agonist of TAS2R14. Even though relatively high concentrations were necessary for receptor stimulation, it still presents one of the most potent agonists found in this study, making it a starting point for ensuring research.[9,13,74,75]

Considering the high level of TAS2R14 expression in extraoral tissues and its ligand promiscuity, this bitter taste receptor should be regarded a considerable off-target in drug discovery. As mentioned before, several approved drugs are able to activate TAS2R14 and therefore might be involved in occurring side effects, both noxious and beneficial. Hence, testing new developed therapeutics routinely on TAS2R14 or TAS2Rs, in general, could reveal potential side-effects accompanied by the treatment. Regarding possible off-target effects of ligands explicitly developed to address TAS2R14, it is also worth noting that β-agonists or corticosteroids included in inhalants were quite successful in disease treatment with showing little to no adverse effects and that the deliberate consumption of bitter tastants contained in food or drugs has been connected to beneficial rather than negative side effects.[15,16,56]

A very interesting observation was made in a recent study when clinical drugs were screened for their TAS2R14 activation. It turned out that 38% of compounds featuring TAS2R14 agonism also showed an inhibition of the human ether-a-go-go-related gene (hERG) potassium channel. The hERG channel is predominantly located in the heart and plays an important role in action potential repolarization. As its inhibition can lead to lethal arrhythmias, it presents one of the most relevant off-targets. Thus, the hERG channel inhibition by TAS2R agonists can be considered a possible mechanism for the harmful effects of noxious bitter substances.[13] Taken together, TAS2R14 selective agonists could represent valuable pharmaceuticals for disease treatment, either alone or in combination therapy.[56,76]

4. Flufenamic Acid as a Lead Structure for Novel TAS2R Agonists

In an interdisciplinary approach integrating homology modeling, virtual docking, chemical synthesis and *in vitro* pharmacological profiling, we applied the approved non-steroidal anti-in-

flammatory analgesic flufenamic acid as a lead structure for the development of a novel, potent TAS2R14 agonist.[75] Our approach was based on a previously proposed binding mode of flufenamic acid to TAS2R14, which was developed using structure-based analyses and extensive mutagenesis experiments (Fig. 2).[74] H-bond interactions with Asn933.36 and Trp893.32 as well as π–π-stacking interactions with Trp893.32, Phe1865.46, and Phe2476.55 of the receptor were identified as key interactions and an unoccupied region of the binding pocket was chosen as a promising additional receptor site to be addressed by modified ligands.

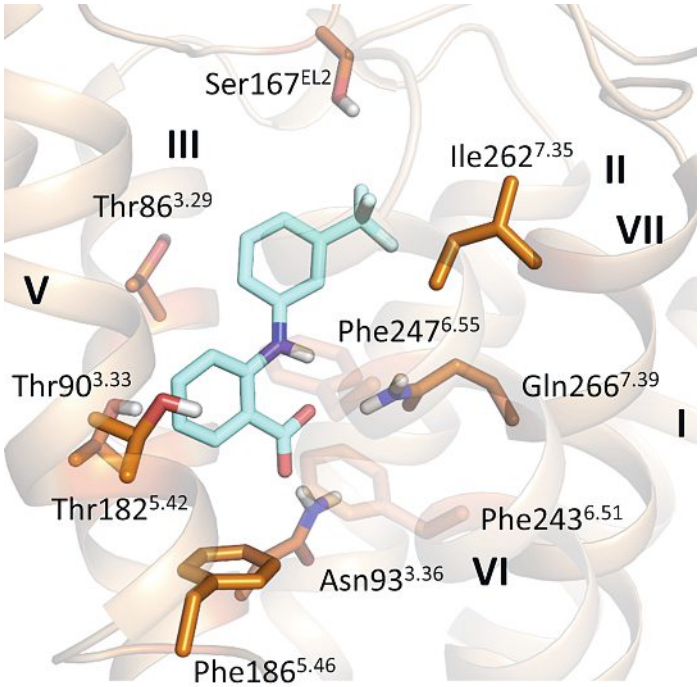


Fig. 2. Proposed binding mode of flufenamic acid in the TAS2R14 homology model.

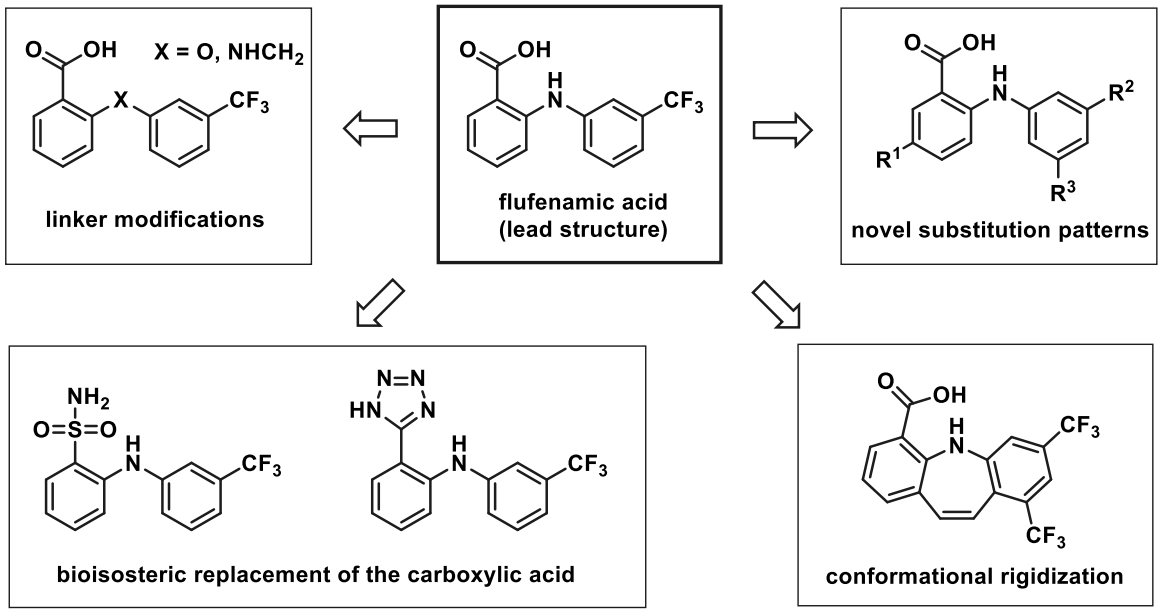
We hypothesized that flufenamic acid derivatives can establish additional contact points in this region leading to stronger affinity. A docking screen was performed with the TAS2R14 model to identify suitable candidates, using a virtual combinatorial library of approximately 1000 compounds leading to a selection of 11 hit structures, which were selected for chemical synthesis.

Additionally, the classical medicinal chemistry concepts of conformational restriction[77] and bioisosteric replacement[78] were applied to design additional flufenamic acid analogs featuring a rigidized dibenzo[b,f]azepine scaffold or exchange of the carboxylic acid group by a tetrazole or a sulfonamide moiety, respectively (Scheme 3). The latter derivatives were assumed to maintain the crucial H-bond interaction with Asn933.36 and potentially induce improved bioavailability for extra-oral TAS2R14 targeting. Overall, 19 novel derivatives were synthesized and biologically investigated. Applying a calcium-imaging assay to determine the activation of TAS2R14 by the novel ligands, we gained important insights into the SAR of flufenamic acid derivatives. While an alteration of the secondary amine linker led to abolishment of potency and efficacy, the introduction of lipophilic substituents, such as a second -CF_3 group, helped maintain the intrinsic activity at comparable levels to the reference compound. Rigidization of the diphenylamine structure, as well as the application of a sulfonamide as carboxylic acid bioisostere, did not result in the anticipated improvement. However, replacing the carboxylic acid moiety with a 5-substituted tetrazole revealed a new class of flufenamic acid analogs with significant TAS2R14 potency. With this structure-based approach, we were able to design three new TAS2R14 agonists being more potent than the lead structure flufenamic acid. These ligand-mediated TAS2R14 activations observed in the calcium-imaging assays could be confirmed in further experiments measuring the ligand-induced inhibition of cAMP accumulation and generation of IP1, respectively.

The new structure–activation relationship data was used to generate a refined TAS2R14 model with a modified arrangement of the binding site residues leading to better predictions of the binding affinities of newly synthesized compounds in future projects.

5. Conclusions and Outlook

Bitterness perception is considered a key defense mechanism against poisoning with potentially toxic substances and is mediated through bitter taste-sensing type 2 receptors (TAS2Rs), which belong to the family A GPCRs. In this short review, we have expounded the recent findings that TAS2Rs are not exclusively dis-



Scheme 3. Development of novel TASR14 ligands based on flufenamic acid as a lead structure.

tributed in taste bud cells of the tongue, but were also detected in several extra-oral tissues and organs including the respiratory, gastrointestinal, endocrine, cardiovascular and neurological systems. The discovery that the perception of bitter taste is just one function among many others of this receptor subfamily has aroused the interest of pharmacologists and medicinal chemists in this potential drug target for several widespread diseases, such as asthma or COPD. In particular, the bitter taste receptor subtype TAS2R14 was found in human airway smooth muscle and may represent a novel drug target for airway diseases. Nevertheless, the underlying biology is only partially understood and structural information on these receptors is still lacking. Furthermore, the knowledge regarding the structure–affinity relationships of TAS2R ligands is still very limited. Despite the fact that many commonly known molecules like caffeine, α-thujone and papaverine have been identified as TAS2R14 agonists, a general pharmacophore model is missing. Very recently, we have addressed this issue in an interdisciplinary approach combining *in silico* methods with chemical synthesis and bioassay technology, when the known TAS2R14 agonist flufenamic acid served as a lead structure.

This work led to a better understanding of the ligand-receptor interactions, a refined model of the TAS2R14 receptor and a series of novel agonists bringing us a step closer on the way to resolving molecular and structural features of the receptor. Nevertheless, an agonist with highly superior pharmacological properties compared to flufenamic acid is missing. An ongoing extensive drug design project in our group is focusing on this challenge. At this, the first insights into the SAR of TAS2R14 ligands described in this review will serve as a starting point for focused libraries of novel and hopefully potent agonists of this new and promising drug target.

Received: April 13, 2022

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