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Broad Tuning of the Human Bitter Taste Receptor hTAS2R46 to Various Sesquiterpene Lactones, Clerodane and Labdane Diterpenoids, Strychnine, and Denatonium

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Sesquiterpene lactones are a major class of natural bitter compounds occurring in vegetables and culinary herbs as well as in aromatic and medicinal plants, where they often represent the main gustatory and pharmacologically active component. Investigations on sesquiterpene lactones have mainly focused on their bioactive potential rather than on their sensory properties. In the present study, we report about the stimulation of heterologously expressed human bitter taste receptors, hTAS2Rs, by the bitter sesquiterpene lactone herbolide D. A specific response to herbolide D was observed i.a. for hTAS2R46, a so far orphan bitter taste receptor without any known ligand. By further investigation of its agonist pattern, we characterized hTAS2R46 as a bitter receptor broadly tuned to sesquiterpene lactones and to clerodane and labdane diterpenoids as well as to the unrelated bitter substances strychnine and denatonium.

KEYWORDS: Sesquiterpene lactones; bitter taste; TAS2Rs; heterologous expression; strychnine; denatonium

INTRODUCTION

The sense of taste allows mammals to evaluate the food they consume. Humans are able to distinguish the five distinct taste qualities sweet, sour, salty, umami, and bitter (*1*). Unlike sweet and umami stimuli, which indicate calorie-rich food and elicit attractive behaviors, acids and bitter substances generally cause aversive reactions to protect organisms against intoxication. In humans, bitter taste is mediated by ~25 G protein-coupled receptors encoded by the hTAS2R gene family (*2*). So far, heterologous expression experiments have identified the cognate bitter compounds for only nine hTAS2Rs (*3*−*9*), the majority of the TAS2Rs remaining orphan receptors with no known ligands. Studies with the nine deorphaned receptors have shown human bitter taste receptors to be quite broadly tuned to various bitter compounds.

Human TAS2R16 and hTAS2R38 were sensitive to numerous β-glucopyranosides and thioamides (*6*, *7*), respectively, and thus show specificity for a common chemical moiety of their agonists. Other receptors appear to be more broadly tuned. Human TAS2R4 responded to denatonium benzoate and propylthiouracil (*5*) and hTAS2R14 responded to α-thujone, picrotoxinin, naphthoic acid, piperonylic acid, 1-nitronaphthalene, and 1,8-naphthalaldehydic acid (*3*). No obvious common chemical features shared by all these agonists can be recognized. This also applies to bitter agonists for hTAS2R7, hTAS2R10, hTAS2R43, hTAS2R44, and hTAS2R47 (*4*, *6*, *8*). Thus, the broad tuning of TAS2Rs offers an explanation for the ability of humans and other mammals to detect thousands of diverse bitter substances with a limited set of bitter taste receptor genes.

Bitter compounds are ubiquitous in plants, animals, and fungi and can also be formed during food processing (*10*). Organisms frequently produce bitter compounds as part of chemical defense mechanisms, and hence many of them are toxic (*11*). However, not all bitter compounds are toxic (*12*), and a slight bitter taste is inherent in many components of our daily diet and might even be enjoyed by consumers. Plant bitter compounds belong to various chemical classes (*13*) including sesquiterpene lactones. This large group of secondary metabolites occurs in more than 10 families of flowering plants, among them many edible species (*14*). Sesquiterpene lactones are present in vegetables (i.e., cynaropicrin in artichoke) and culinary herbs (i.e., costunolide in bay leaf). Moreover, plants containing sesquiterpene lactones are also used for the production of bitter alcoholic drinks such as absinth and vermouth. Indeed, the dimeric guaianolide absinthin from wormwood is one of the strongest bitter tasting compounds known. Furthermore, sesquiterpene lactones possess pharmacological activities, with antimicrobial, antiviral, antiinflammatory, and antitumor potential related to

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the Michael-acceptor properties of the exomethylene-γ-lactone moiety (*15*). Consequently, these substances represent the main active constituents of important medicinal plants like *Arnica montana* and *Tanacetum parthenium* (*14*). Bioactivity of sesquiterpene lactones is not related to the presence of an exomethylene-γ-lactone group, as exemplified by the presence of an exomethylene-δ-lactone group in the antimalarial agent artemisinin.

Despite the relevance of sesquiterpene lactones in medicine and food science, their sensory properties have been poorly characterized, and the molecular basis for their remarkable bitterness is still unknown. Using heterologous expression of taste receptors in human embryonic kidney cells, we report here on the identification of hTAS2R46 as a major bitter taste receptor for sesquiterpene lactones. hTAS2R46 also responded to clerodane and labdane diterpenoids, to strychnine, and to denatonium, suggesting that it is broadly tuned to detecting various other bitter compounds of both natural and synthetic origin.

MATERIALS AND METHODS

Compounds. Acesulfame K, andrographolide, aristolochic acid, brucine, chloramphenicol, cycloheximide, denatonium benzoate, esculin, ginkgolide A, picrotoxinin, picrotin, strychnine, sucrose octaacetate, ouabain, PTC, PROP, saccharin, salicin, sodium benzoate, and (−)α-thujone have been purchased from SIGMA (Sigma-Aldrich, Taufkirchen, Germany). Artemisinin and marrubiin have been acquired from LGC Promochem (Wesel, Germany). All other products were available from previous studies or were isolated (or synthesized) according to literature procedures. Reference to the isolation (or synthesis) of the various compounds is provided as Supporting Information.

Functional Expression of hTAS2R46. For functional expression, we used the coding region of the hTAS2R46 gene of human embryonic kidney (HEK)-293T cells. The sequence is identical to that given in GenBank (accession no. AY 114091) with one exception, a C to T transition in position 108 which does not alter the encoded amino acid sequence (construct cloned into pcDNA5/FRT (Invitrogen, San Diego, CA)). To improve receptor membrane targeting, the construct contains the first 45 amino acids of rat somatostatin receptor type 3 at its amino terminus (*16*). The carboxyl terminus was supplemented with a herpes simplex virus glycoprotein D (HSV) epitope for immunocytochemical detection (*6*). HEK-293T cells stably expressing the chimeric G protein α subunit Gα16gust44 (*17*) were seeded into 96-well microtiter plates (Greiner Bio-One, Frickenhausen, Germany) at a density of approximately 60% confluence. After 24−30 h, ∼30 000 cells per well were transfected with 150 ng DNA using 300 ng Lipofectamine 2000 (Invitrogen, San Diego, CA). For negative control, empty pcDNA5/FRT vector was transfected in the same way. Twenty-four hours later, cells were loaded with FLUO4-AM (Molecular Probes, Karlsruhe, Germany). Calcium responses of cells were measured after application of test substances in a Fluorometric Imaging Plate Reader, FLIPR (Molecular Devices, Munich, Germany) at 510 nm following excitation at 488 nm. Test compounds were dissolved in DMSO and were diluted in C1 solution (130 mM NaCl, 5 mM KCl, 10 mM Hepes pH 7.4, 2 mM $CaCl_2$, and 10 mM Glucose) not exceeding a final DMSO concentration of 1% (v/v). Data were collected from at least two independent experiments carried out in triplicates.

Some of the test substances elicited artificial calcium responses in the absence of TAS2Rs in mock-transfected cells in the high concentration range. This is reminiscent of the putative ability of some tastants to activate taste receptor cells without activating taste receptor proteins (*18*−*20*). To avoid false positive signals, we used these substances at concentrations that were at least 10-fold below those eliciting the artificial signals.

Several compounds were available only in limiting amounts preventing us from establishing concentration response relations from which EC_{50} values could be deduced. Therefore, we tested all compounds at least at four different concentrations over a range of 3 orders of magnitude. For a comparison of the potencies of the employed compounds to activate the receptor, we present in **Table 1** the lowest concentrations that resulted in a clear increase in intracellular calcium levels.

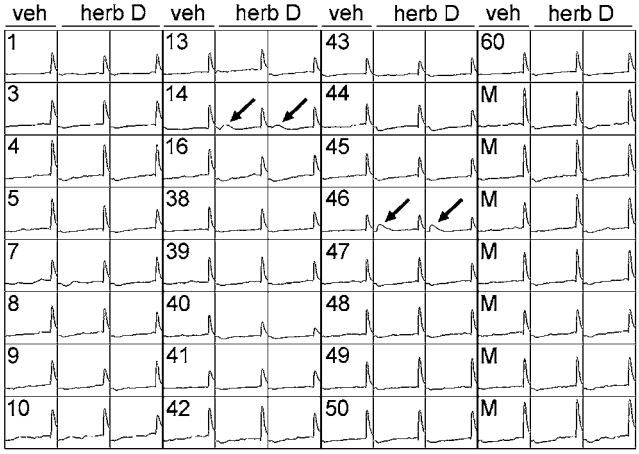


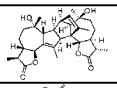
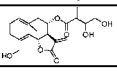
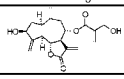
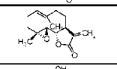
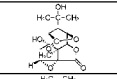
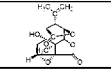
Figure 1. Calcium responses of hTAS2R-transfected cells induced by bath application of herbolide D. HEK 293T-Gα16gust44 cells were seeded in 96-well microtiter plates, were mock-transfected (rows "M") or transfected in triplicate with hTAS2R cDNAs, and were loaded with FLUO4-AM. Calcium traces of cells were recorded in the FLIPR following bath application of 300 μM herbolide D (columns "herb D") or, for control, of vehicle (columns "veh"). Cell number and vitality were controlled by the subsequent application of 100 nM somatostatin 14, an agonist of the endogenous somatostatin receptor type 2. Numbers specify the hTAS2R that the cells express. Arrows point to specific responses of cells expressing hTAS2R14 or hTAS2R46. Specificity of receptor activation was controlled by application of herbolide D on mock-transfected cells.

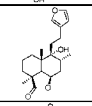
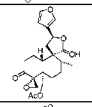
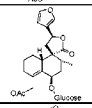
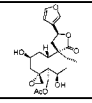
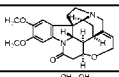
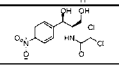
For the calculation of dose−response curves, signals of two to four wells receiving the same concentration of test substances were averaged and the fluorescence changes of corresponding mock-transfected cells were subtracted. Signals were normalized to background fluorescence. For the calculation of EC_{50} values, plots of amplitude versus concentrations were prepared in Sigma Plot. By nonlinear regression of the plots to the function $f(x) = 100/[1 + (EC_{50}/x)^{nH}]$, where x is the agonist concentration and nH is the Hill coefficient, we were able to calculate the EC_{50} of agonist−receptor interaction.

RESULTS

Screening Human TAS2Rs for Activation by the Bitter Sesquiterpene Lactone Herbolide D. To identify human bitter taste receptors for sesquiterpene lactones, we transfected the cDNAs for the epitope-tagged hTAS2Rs in human embryonic kidney (HEK) 293T cells stably expressing the chimeric G protein α-subunit Gα16gust44. This G protein subunit couples TAS2R activation to the release of calcium from internal stores (*17*). Transfected cells were loaded with a calcium indicator dye and were challenged by bath application of the test substance (*5*, *6*, *17*). To screen hTAS2R responses to a bitter tasting sesquiterpene lactone, we used herbolide D isolated from *Artemisia vallesiaca*, an important ingredient of vermouth, at a concentration of 300 μM. Robust increases in intracellular calcium levels occurred in cells transfected with hTAS2R14 and hTAS2R46 cDNA. We did not observe responses in cells transfected with any other hTAS2R cDNA (**Figure 1**). These results confirm that hTAS2R14 is a broadly tuned bitter taste receptor responding to numerous substances (*3*, *9*). In contrast to hTAS2R14 for which several cognate bitter substances are known, hTAS2R46 remained an orphan receptor until now. Herbolide D is its first identified agonist displaying a threshold

Table 1. Bitter Compounds Activating hTAS2R46[a]

substance		threshold (μM)
sesquiterpene lactones		
absinthin		1
arborescin		3
arglabin		3
artemisinin		3
artemorin		10
peroxy-artemorin		3
cnicin		3
costunolide		0.3
crispolide		10
cynaropicrin		3
epizaluzannin C		3
germacradien-6,11-dihydroxy-8,12-olide		10
grosheimin		1
herbolide A		0.3
herbolide D		10
herbolide D acetate		3
nobilin		1
parthenin		100
parthenolide		1
picrotin		30
picrotoxinin		30
santamarine		3
sintenin		0.03

substance		threshold (μM)
speciformin acetate		3
tatridin A		30
tatridin A acetate		3
tatridin B		100
taurin		10
dihydro-taurin		10
umbellifolide		10
vulgarolide		100
zaluzannin D		3
diterpenoids		
andrographolide		3
cascarillin		10
marrubiin		0.3
teuflavin		30
teuflavoside		30
teumarin		30
unrelated bitter substances		
brucine		10
chloramphenicol		10
denatonium benzoate		30
strychnine		0.1
strychnine-*N*-oxide		30
sucrose octaacetate		100

[a] Substances that activate hTAS2R46 are arranged according to their chemical nature. Threshold value given in row 3 is defined as the lowest concentration of a test substance that caused a significant cellular response. Specificity of receptor activation was controlled by application of test substances on mock-transfected cells.

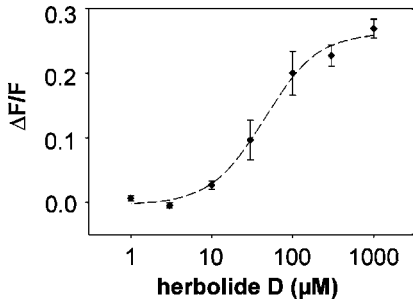


Figure 2. Dose–response relation of hTAS2R46-transfected cells stimulated with increasing concentrations of herbolide D. HEK 293T-Gα16gust44 cells were seeded in 96-well microtiter plates, were transfected with hTAS2R46 cDNA, and were loaded with FLUO4-AM. Calcium traces of cells were recorded in the FLIPR following bath application of herbolide D. Changes in fluorescence ($\Delta F/F$) were plotted vs log agonist concentration.

value of activation of ~10 μM and an EC_{50} value of 45.6 ± 1.5 μM (**Figure 2**). For further analysis, we focused on hTAS2R46.

Determination of the Agonist Profile of hTAS2R46. Next, we examined whether hTAS2R46 is a specific receptor for herbolide D or whether it functions as a universal sensor for sesquiterpene lactones or even terpenes. Therefore, we challenged hTAS2R46-expressing cells with numerous sesquiterpene lactones of the four major structural subtypes of this class of compounds (germacranolides, guaianolides, pseudoguaianolides, eudesmanolides). Furthermore, we tested various mono- and diterpenoids for their ability to activate hTAS2R46 expressing cells.

Out of 37 tested sesquiterpene lactones, 32 compounds elicited robust responses in hTAS2R46-transfected cells (**Table 1**). Threshold values of activation varied over more than 3 orders of magnitude ranging from 0.03 μM for sintenin (**Figure 3**) to 100 μM for parthenin, tatridin B, and vulgarolide. The five sesquiterpene lactones, 3βOH-dihydro costunolide, glaucolide A, 3βOH-pelenolide, telekin, and deoxy-5-hydroperoxy telekin; the plant monoterpenoids menthofuran and oleuropein; and the insect monoterpenoid dolichodial failed to stimulate hTAS2R46-expressing cells at the concentrations tested (**Table 2**). All the five bitter clerodane diterpenoids investigated, andrographolide, cascarillin, teuflavin, teuflavoside, and teumarin, as well as the furanic labdane diterpenoid marrubiin, elicited specific calcium signals (**Table 1)** whereas the chemically unrelated diterpene ginkgolide A did not (**Table 2**). With a threshold value of 0.3 μM, marrubiin turned out to be the most potent diterpenoid, followed by andrographolide, cascarillin, teuflavin, teuflavoside, and teumarin (**Table 1**). Thus, furanoditerpenoids and diterpene lactones from the clerodane and the labdane group are as potent as sesquiterpene lactones in activating hTAS2R46.

To asses whether hTAS2R46 is even more broadly tuned to other bitter compounds, we screened bitter substances unrelated to sesquiterpene lactones for their ability to activate hTAS2R46-expressing cells. Interestingly, we identified additional bitter agonists of hTAS2R46, which are, in their rank order of potency, strychnine (**Figure 3**) and the related compound brucine, chloramphenicol, denatonium benzoate (**Figure 3**), the strychnine derivative strychnine-*N*-oxide, and sucrose octaacetate (**Table 1**). As sodium benzoate did not activate hTAS2R46-expressing cells up to 1000 μM (**Table 2**), denatonium must be the receptor-stimulating component of denatonium benzoate.

Dose−Response Relationships of hTAS2R46−Agonist Interactions. To investigate the pharmacological properties of hTAS2R46 in greater detail, we selected the dimeric sesquiterpene lactone absinthin, the sesquiterpene dilactone picrotoxinin, and the indolalkaloid strychnine to determine concentrations of half-maximal receptor activation (EC_{50} values). EC_{50} values are reliable measures of the potencies of a receptor for its agonists. The three substances activated hTAS2R46 with different thresholds from 0.1 μM for strychnine, over 3 μM for absinthin, to 30 μM for picrotoxinin (**Table 1**). **Figure 4** shows the calcium traces evoked by different concentrations of strychnine, absinthin, and picrotoxinin in typical experiments, while **Figure 5** depicts the resulting concentration−response curves. It is apparent that hTAS2R46 shows different potencies for the three bitter compounds as indicated by the shifted dose−response curves and the EC_{50} values of 0.43 ± 0.02 μM for strychnine, 9.9 ± 0.3 μM for absinthin, and 70.0 ± 5.2 μM for picrotoxinin. **Figure 5** also suggests that the threshold values of activation correlate well with the EC_{50} values.

DISCUSSION

In the present study, we identified the human bitter taste receptor TAS2R46 as sensor for bitter sesquiterpene lactones, for related clerodane diterpenoids and labdane diterpenes, and for strychnine, strychnine-related bitter substances, denatonium, chloramphenicol, and sucrose octaacetate.

Whereas for most other deorphaned human TAS2Rs only very few agonists or a group of chemically related agonists are known, our screening of human TAS2R46 represents the most extensive characterization of a bitter taste receptor with respect to its agonist spectrum reported so far. We identified 44 hTAS2R46 agonists derived from six different chemical classes among which are sesquiterpene lactones, many of them being used for flavoring food and beverages as well as for medicinal purposes (*14*). Many members of this chemical group exhibit distinct bioactive potential. Thus, artemisinin from *Artemisia annua* (sweet wormwood) is the most potent antimalarial drug at present (*21*). Artemisinin inhibits the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) of the parasite (*22*), without

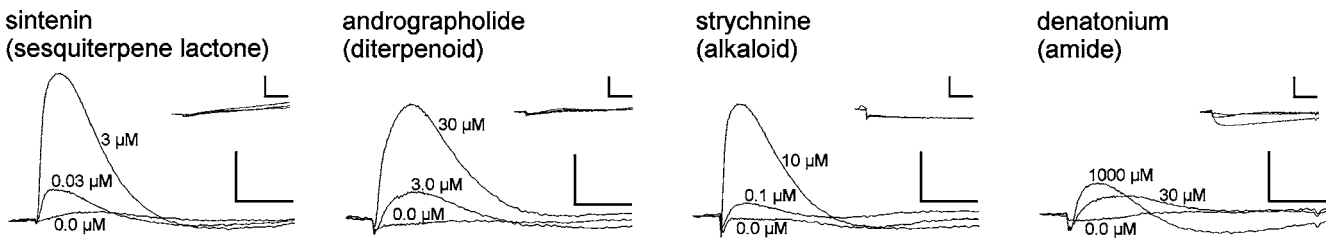
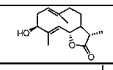
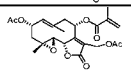
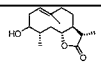
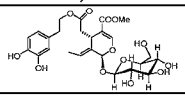
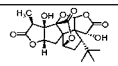
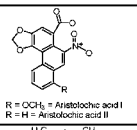
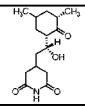
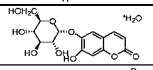
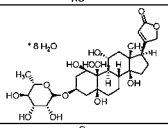


Figure 3. Activation of hTAS2R46 by bitter compounds with different chemical structures. HEK 293T-Gα16gust44 cells were seeded in 96-well microtiter plates, were transfected with hTAS2R46 cDNA or mock-transfected (insets), and were loaded with FLUO4-AM. Calcium traces were recorded in the FLIPR after stimulation of the cells with 0.0, 0.03, or 3 μM sintenin; 0.0, 3.0, or 30 μM andrographolide; 0.0, 0.1, or 10 μM strychnine; or 0.0, 30, or 1000 μM denatonium benzoate. Data of typical experiments were corrected for baseline fluorescence. Calibration bars, $\Delta F = 2000$ fluorescence counts in arbitrary units; horizontal, 2 min.

Table 2. Bitter Compounds That Did Not Activate hTAS2R46[a]

substance		concentration (μM)
sesquiterpene lactones		
3βOH dihydro costunolide		100
glaucolide A		100
3βOH pelenolide		300
telekin		100
deoxy-5-hydroperoxy telekin		10
monoterpenoids		
dolichodial		5
menthofuran		10
oleuropein		5000
diterpenoids		
ginkgolide A		300

substance		concentration (μM)
unrelated bitter substances		
acesulfame K		10000
aristolochic acid		30
cycloheximide		1000
esculin		5000
ouabain		10000
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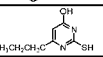
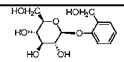
propylthiouracil (PROP)		1000
saccharin		10000
salicin		10000
sodium benzoate		1000
(-)α-thujon		30

[a] Substances are arranged according to their chemical nature. Maximal concentrations that did not elicit responses are shown.

exhibiting toxic side effects (*23*). Nevertheless, some sesquiterpene lactones, such as the sesquiterpene dilactone picrotoxinin, have poisonous effects in humans. The compound is present in fishberries (the fruit of *Anamirta paniculata*) and inhibits the $GABA_A$-receptor (*24*) causing symptoms of severe poisoning (*25*) and supporting the idea that hTAS2R46 and TAS2R14, which also has been shown to respond to picrotoxinin (*3*), serve as warning sensors against the ingestion of toxic food. Perhaps hTAS2R14 is more relevant for avoiding picrotoxinin intoxications than hTAS2R46 because of its lower EC_{50} value of 18 μM (*3*) versus 70 μM.

The multitude of sesquiterpene lactone agonists that we identified for hTAS2R46 raises questions about the structure−function relations. We identified 32 sesquiterpene lactones that activate hTAS2R46 whereas 5 were not able to elicit responses in hTAS2R46-transfected cells. To investigate the properties of sesquiterpene lactones necessary to activate hTAS2R46, we compared the structures of agonists with those of substances that failed to stimulate hTAS2R46.

Table 1 shows that compounds of all four major classes of sesquiterpene lactones (germacranolides, guaianolides, pseudoguaianolides, eudesmanolides) are able to interact with hTAS2R46, suggesting that the γ-lactone and the δ-lactone moiety is critical for binding, whereas the terpenoid skeleton and its functionalities play an important modulating role. This is demonstrated by a comparison of the hTAS2R46 agonist costunolide and its nonagonist derivative 3βOH dihydro costunolide, which differ only in two structural features, that is, the saturation of the

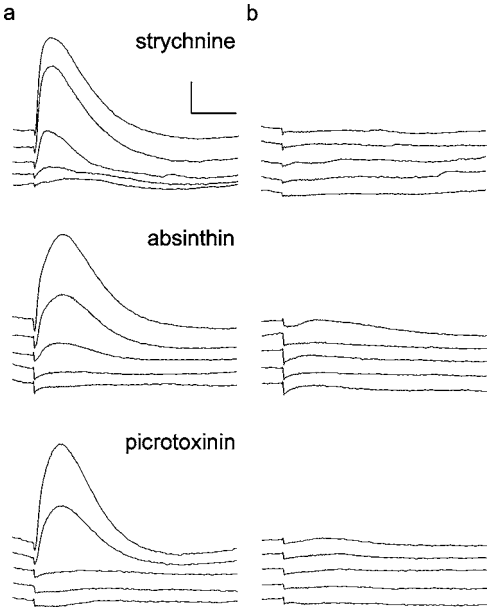


Figure 4. Calcium traces of cells expressing hTAS2R46 challenged with strychnine, absinthin, or picrotoxinin. HEK 293T-Gα16gust44 cells were seeded in 96-well microtiter plates, were transfected with hTAS2R46 cDNA (**a**) or mock transfected (**b**), and were loaded with FLUO4-AM. Cellular calcium traces were recorded in the FLIPR following application of strychnine (0.0, 0.03, 0.3, 3, 30 μM), absinthin (0.0, 0.3, 3, 30, 300 μM), and picrotoxinin (0.0, 1.0, 10, 100, 300 μM). The figure shows traces of typical experiments that have been corrected to baseline fluorescence. Calibration bars, vertical, $\Delta F = 2000$ fluorescence counts in arbitrary units; horizontal, 2 min.

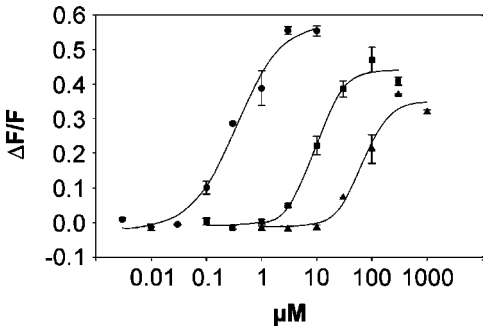


Figure 5. Concentration–response relations of the effects of strychnine, absinthin, or picrotoxinin on calcium levels of cells expressing hTAS2R46. HEK 293T-Gα16gust44 cells were seeded in 96-well microtiter plates, were transfected with hTAS2R46 cDNA, and were loaded with FLUO4-AM. Calcium traces of cells were recorded in the FLIPR following bath application of strychnine (circles), absinthin (squares), and picrotoxinin (triangles). Changes in fluorescence (Δ*F*/*F*) were plotted versus log agonist concentration.

exomethylene moiety and the presence of a 3β-hydroxyl group in the terpenoid core (**Figure 6**, panel **1**). Since both exomethylene-γ-lactones and their dihydro derivatives are able to stimulate hTAS2R46 (cf. arglabin and arborescin), the presence of a 3-hydroxyl group seems detrimental for the interaction. Indeed, with the exception of nobilin, none of the germacrane agonists that activate hTAS2R46 carry a hydroxyl group at the C3 position. On the other hand, the presence of a *cis*-4 double bond makes the topological relationship between the 3-hydroxyl and the lactone moiety quite different in nobilin and costunolide (**Figure 6**, right panel), suggesting that the isomerization of the 4-double bond can offset the detrimental effect of the 3-hydroxyl.

Another germacranolide sesquiterpene lactone that could not elicit specific responses in hTAS2R46-transfected cells is glaucolide A (**Figure 6**, panel **2**). The structural hallmarks of glaucolide A are the acetoxylation of C-2 on the terpenoid core and C-13 of the exomethylene moiety and the presence of an acyl group at C-8. The latter can be found in several hTAS2R46 agonists (e.g., cnicin) and is therefore unlikely to impair interaction with the receptor (**Table 1**). The impact of the *O*-acetoxylation of the C-2 atom and of the exomethylene moiety needs to be investigated by systematic chemical modification of glaucolide A.

Also, the two eudesmanolides telekin and its 5-deoxy-5-hydroperoxy derivative were unable to activate hTAS2R46 in our screening experiments. However, in contrast to telekin, 5-deoxy-5-hydroperoxytelekin elicited unspecific responses in mock-transfected cells at exceptionally low concentrations (30 μM). Therefore, we cannot completely rule out that this substance would activate hTAS2R46, at least at concentrations above 30 μM. Both compounds share a unique structural feature, namely, the oxygenation of C-5 of the eudesmane skeleton (**Figure 6**, panel **3**) that might well be responsible for the decreased affinity for hTAS2R46. Taken together, our data suggest that sesquiterpene lactones in general are capable of activating hTAS2R46, presumably because of the presence of a γ-lactone ring and a δ-lactone ring bound to a cyclic framework. Nevertheless, this interaction is modulated by the substitution pattern of the terpenoid skeleton. Remarkably, similar conclusions regarding the Michael-acceptor properties of the exomethylene moiety were reached (*14*).

Clerodane diterpenoids are a second major class of bitter terpenoids, and we found indeed that hTAS2R46 was activated by the furan cascarillin and by the γ-lactone andrographolide, two well-known bitter agents, as well as by three other clerodanes of the furan type. We found that hTAS2R46 activity is even more potently stimulated by a diterpene of the labdane type, marrubiin. Conversely, neither the bitter plant monoterpenoids oleuropein and menthofuran nor the offensive insect monoterpenoid dolichodial activated hTAS2R46. We, therefore,

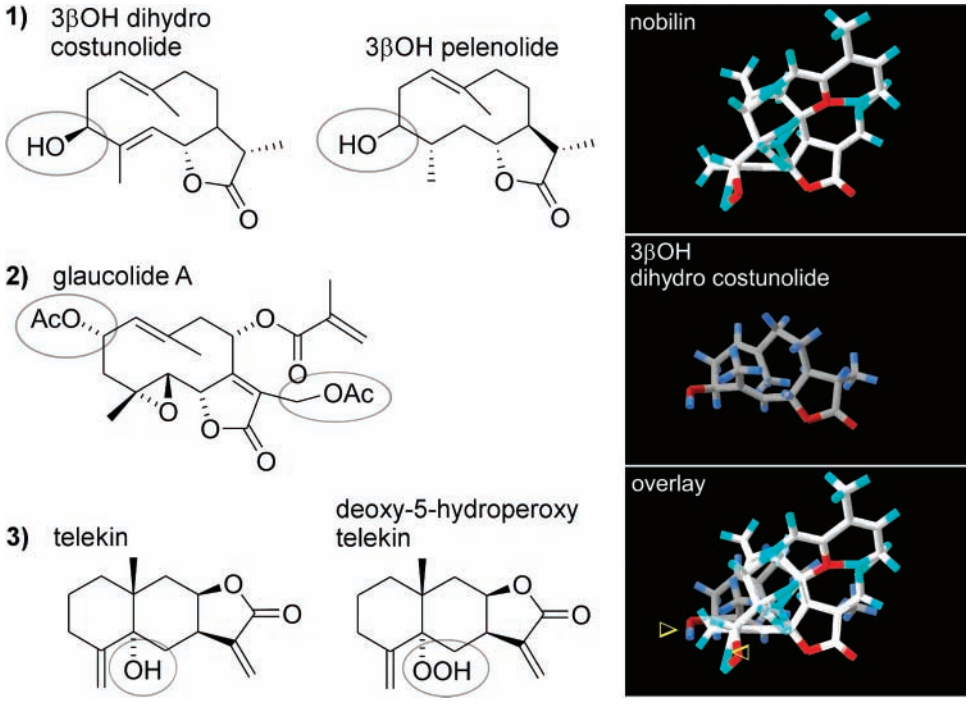


Figure 6. Structures of sesquiterpene lactone hTAS2R46 nonagonists. The structures of sesquiterpene lactones that did not activate hTAS2R46 in the functional expression assay are shown according to their relation. Circles label the structural features presumably detrimental for receptor stimulation. In the right panel, 3-D images of the structures of nobilin and 3βOH dihydro costunolide have been depicted to illustrate the distinct topological relation of the C-3 hydroxyl group and the lactone ring of both substances.

next addressed the question of whether hTAS2R46 is a receptor specifically for sesquiterpene lactones and clerodane and labdane diterpenoids or is broadly tuned to detecting additional bitter tasting substances. For this purpose, we challenged hTAS2R46-expressing cells with 14 chemically unrelated synthetic as well as natural bitter compounds. Four of these substances, chloramphenicol, denatonium, strychnine, and sucrose octaacetate elicited specific responses in hTAS2R46-transfected cells. Whereas strychnine and denatonium were already known to activate hTAS2R10 and hTAS2R4 or hTAS2R47, respectively, we here assigned hTAS2R46 as bitter taste receptor to chloramphenicol and sucrose octaacetate.

Besides the sesquiterpene lactones, strychnine is the most potent hTAS2R46 agonist with a threshold of 0.1 μM. We also tested two derivatives of strychnine, brucine and strychnine-*N*-oxide, that both stimulated hTAS2R46, albeit with a much lower potency, showing thresholds of 10 μM (brucine) and 30 μM (strychnine-*N*-oxide), respectively. Therefore, both the phenyl moiety and the basic nitrogen of strychnine seem critical for the interaction with hTAS2R46.

Strychnine has been reported to exhibit a strong bitter taste with a threshold value of 2.3 μM in humans (*26*), which is distinct from the threshold measured in the heterologous expression system (0.1 μM, **Table 1**). In addition to hTAS2R46, hTAS2R10 is known to contribute to the bitterness of strychnine (*6*). Since the responsiveness of hTAS2R10 has not been analyzed in detail, a direct comparison of the pharmacological properties of hTAS2R10 and hTAS2R46, which is necessary to estimate the contribution of these two receptors for strychnine tasting, is currently not possible.

In the cell-based assay system, denatonium stimulates hTAS2R46 activity with a threshold concentration of 30 μM. Like strychnine, the synthetic bitter compound denatonium has already been shown to activate other human TAS2Rs. Human TAS2R4 responds to denatonium with a threshold concentration of 100 μM (*5*) and hTAS2R47 at 10 μM denatonium (*8*). Thus, hTAS2R46 is slightly less sensitive to denatonium than hTAS2R47. However, the potency of denatonium to activate hTAS2R4, hTAS2R46, and hTAS2R47 does not resemble the human bitter taste threshold. Denatonium is one of the most potent bitter substances to humans, exhibiting a threshold of 0.01−0.02 μM (*27*). These observations suggest the existence of at least one, yet unidentified, high-affinity hTAS2R for the perception of denatonium. The same may be true for absinthin, as its bitter threshold value determined in sensory studies (*28*) is about 6-fold lower than the minimal concentration required to activate hTAS2R46 expressing cells. A candidate could be hTAS2R14, a receptor that is activated by the sesquiterpenes picrotin and picrotoxinin (*5*). For cynaropicrin and grosheimin, reported sensory data (*29*) agree well with those monitored in our receptor assays, suggesting that hTAS2R46 may be a receptor for these compounds in vivo.

Only one-third of all hTAS2Rs have been characterized so far, but evidence of functional redundancy is emerging. For example, picrotoxinin and picrotin are both capable of eliciting specific responses from hTAS2R14 (*3*) and hTAS2R46, notwithstanding their different toxicological profile. The same applies to strychnine, which is detected by hTAS2R10 (*6*) and hTAS2R46. Denatonium is even less selective activating at least three bitter receptors, that is, hTAS2R4, hTAS2R46, and hTAS2R47 (*5*, *8*). Assuming that all these receptors are expressed on the human tongue, they would be partially redundant in their function. This conclusion is remarkable, given the huge number of bitter substances that are perceived with only ~25 human TAS2Rs. Functional redundancy in the bitter taste system could offer an evolutionary advantage. Bitter taste receptor alleles show signs of positive selection (*30*), a mechanism of potential importance in periods of migration during human evolution. Under such conditions, detection of previously unexperienced plant toxins present in new habitats, while maintaining the capacity to detect common toxins of the former habitat, might have had selective advantages (*31*).

Supporting Information Available: Source of the noncommercial products and their derivatives. This material is available free of charge via the Internet at http://pubs.acs.org.

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