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The Utility of the Artificial Taste Sensor in Evaluating the Bitterness of Drugs: Correlation with Responses of Human TASTE2 Receptors (hTAS2Rs)

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The purpose of this study was to examine the ability of the artificial taste sensor to evaluate the bitterness of drugs by comparing the responses of the taste sensor with documented responses of human TASTE2 receptors (hTAS2Rs). For this purpose 22 bitter compounds, used as ingredients of pharmaceutical medicines in Japan and known ligands of hTAS2Rs, were selected for testing. Their solutions (0.01, 0.03, 0.1 mM) were evaluated by five different taste sensors (AC0, AN0, BT0, C00, AE1). Correlations between physicochemical parameters of the compounds and the responses of the taste sensors and hTAS2Rs were evaluated. From taste sensor measurements, diphenidol, haloperidol, diphenhydramine, dextromethorphan and papaverine, all ligands of hTAS2R 10 and/or hTAS2R14, were predicted to express strong bitterness, surpassing that of quinine. Responses of taste sensors BT0 were found to be significantly correlated with responses of hTAS2R14. High log *P* values (≥ 2.73) and responses of hTAS2R14 were also significantly correlated (***p* < 0.01, chi-square test). In conclusion, taste sensor BT0 is highly sensitive to bitterness and correlates significantly with hTAS2R14, making it useful for evaluating the bitterness of hydrophobic compounds which respond to hTAS2R14 and their inhibitors.

Key words taste sensor; human TASTE2 receptor; bitterness

It has been estimated that more than 700 compounds, including both plant-derived and synthetic compounds, such as amides, peptides, heterocyclic compounds, glycosides, alkaloids, terpenoids, phenols and flavonoids, are known to have a bitter taste.¹⁾ The detection of bitterness is mediated by the TASTE2 Receptor (TAS2R) family of G protein-coupled receptors, and humans have been found to have 25 of these (hTAS2Rs).^{2–5)} Information on the association of many bitter ligands with particular TAS2Rs, established by *in vitro* assay, can be accessed via the Bitter DB database⁶⁾ (<http://bitterdb.agri.huji.ac.il/dbbitter.php>).

The taste of medicines is a critical factor in the development of orally administrated drug formulations, especially orally disintegrating tablets and liquids for older patients and children.^{7–9)} Huge efforts have been devoted by industry and academia in the development of taste-masked pharmaceuticals.^{10,11)} However, compliance issues, stemming from the bitter taste of active pharmaceutical ingredients, are still common.

The standard method for evaluating taste, including the bitterness of drugs, is the human sensation test. However, this method has ethical and toxicological considerations and causes strain on subjects, while inter-subject variability has to be accounted for. Thus, the use of an artificial apparatus for taste evaluation has become popular. The taste sensor with lipid/polymer membranes developed by Toko is one such artificial chemical sensor, and is widely used in pharmaceutical applications.^{10–19)} A taste sensor must respond consistently to the same taste, like the human tongue. Bitter materials are adsorbed on the hydrophobic part of the membrane and cause a change in membrane potential by changing the charge

density. Kobayashi *et al.* found that lipid/polymer membrane sensor selectivity for each taste is improved by modulating both the hydrophobic interaction between the taste sensor and bitter substance and the membrane charge density.¹²⁾ Correlation between results from the taste sensor and taste intensities recorded in human sensation testing has proven the usefulness of the taste sensor in predicting the taste of various substances.^{12–19)} However, there have been no reports examining the detailed correlation between the taste sensor response and that of human taste receptors.

The purpose of this study was to evaluate the correlation between the responses of five artificial bitter/astringency taste sensors composed of lipid/polymer membranes (AC0, AN0, BT0, C00 and AE1) produced by Intelligent Sensor Technology Inc. (Insent) and the responses of human TASTE2 receptors (hTAS2Rs), using 22 known bitter compounds, common ingredients of pharmaceutical medicines in Japan.

Experimental

Materials Twenty-two compounds: acetaminophen, azathioprine, caffeine, chloramphenicol, chlorpheniramine maleate salt, colchicine, cromolyn sodium, dextromethorphan hydrobromide monohydrate, diphenhydramine hydrochloride, diphenidol hydrochloride, erythromycin, famotidine, flufenamic acid, haloperidol, hydrocortisone, methimazole, noscaphine, ofloxacin, papaverine hydrochloride, propylthiouracil, quinine hydrochloride and strychnine nitrate were purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan.

Methods

Taste Sensor Measurement

The taste sensor SA402B (Intelligent Sensor Technol-

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Fig. 1. Taste Map Obtained by Principal Component (PC) Analysis of Five Taste Sensor Outputs in Response to 22 Compound (0.1 mM)

ogy Inc., Atsugi, Japan) used in this study was the same as that used in our previous studies.^{14–17} The detecting sensor part of the equipment consists of a reference electrode and a taste sensor which acts as the working electrode and is composed of various types of lipid/polymer membrane.¹² The lipid/polymer membranes used as bitter taste sensors in this study were as follows: AC0 (hexadecanoic acid/dioctyl phenylphosphonate), AE1 (tetradodecylammonium bromide/dioctyl phenylphosphonate), AN0 (phosphoric acid di-*n*-decyl ester/dioctyl phenylphosphonate), BT0 (phosphoric acid di-*n*-decyl ester/bis(1-butylpentyl) adipate, tributyl *O*-acetyl citrate) and C00 (tetradodecylammonium bromide/2-nitrophenyl octyl ether). The 22 compounds tested (see above) were each dissolved in water to form solutions of 0.01, 0.03 and 0.1 mM, prior to analysis by the taste sensors. The taste sensor outputs of the 22 compounds were analyzed by principal component analysis (PCA) using Ekuseru–Toukei 2010 (Social Survey Research Information Co., Ltd.).

Response Profiles of hTAS2Rs Stimulated with Drugs by *in Vitro* Assay

The response profiles of hTAS2Rs stimulated with the drugs used in this study were derived from the literature and the Bitter DB database.^{3,4,6} Meyerhof *et al.* have systematically investigated the molecular receptive ranges of bitter taste receptors using natural or synthetic bitter chemicals to challenge all 25 human TAS2Rs in transfected cells.³

Correlation between Taste Sensor and hTAS2Rs Responses

Taste sensor (AN0, AC0, BT0, C00, AE1) outputs for each of the 22 compounds were replaced by either 1 (response) or 0 (no response) as follows: If the taste sensor response increased in a dose-dependent manner and the response value at 0.1 mM was ≥ 2 mV, the response of the compound was designated as 1. If the taste sensor response did not increase in a dose-dependent manner and the response value at 0.1 mM was < 2 mV,

the response of the compound was designated as 0. Correlations between taste sensor responses and hTAS2Rs responses cited from the literature were calculated and Fisher's exact tests were carried out using Ekuseru–Toukei 2010.

Correlation between Physicochemical Parameters and Responses of Taste Sensors and hTAS2Rs

Molecular weight (MW), $\log P$ (log of the octanol–water partition coefficient using Ghose and Crippen's method) as a measure of hydrophobicity,²⁰ $\log S$ (log of the solubility in mol/L) as a measure of water solubility,²¹ hydrogen bond donor (HBD) count, hydrogen bond acceptor (HBA) count, ring count in the smallest set of smallest rings and aromatic ring count extracted from the Bitter DB, were used as physicochemical parameters. These parameters were replaced by either 1 (high) or 0 (low) as follows: If the parameter of a compound was equal to or larger than that of quinine, the parameter was given the value 1; if the parameter was less than that of quinine, it was given the value 0. Correlation coefficients between physicochemical parameters and responses of taste sensors and hTAS2Rs were calculated and Fisher's exact tests carried out using Ekuseru–Toukei 2010.

Results

Taste Sensor Measurements Quinine, diphenidol, diphenhydramine, dextromethorphan, papaverine, haloperidol, chlorpheniramine and noscapine responded to AC0. Quinine, diphenidol, diphenhydramine, dextromethorphan, papaverine, haloperidol and famotidine responded to AN0. Erythromycin, chlorpheniramine and strychnine responded slightly to AN0 but the value was < 2 mV at 0.1 mM. Erythromycin, ofloxacin, quinine, chlorpheniramine, diphenidol, dextromethorphan, noscapine, papaverine, haloperidol, diphenhydramine and strychnine responded to BT0. Azathioprine, acetaminophen, ofloxacin, fulfenamic acid, chloramphenicol and famotidine responded to C00. Cromolyn, chloramphenicol, chlorphenir-

(a) Quinine

(b) Diphenidol

(c) Diphenhydramine

(d) Dextromethorphan

(e) Papaverine

(f) Haloperidol

Fig. 2. Taste Sensor (AC0, AN0 BT0) Outputs in Response to Quinine (a), Diphenidol (b), Diphenhydramine (c), Dextromethorphan (d), Papaverine (e) and Haloperidol (f)

$n=3$, mean $\pm$ S.D.

amine and strychnine responded to AE1. Caffeine, colchicine, hydrocortisone, propylthiouracil and methimazole did not respond to any of the five taste sensors.

The taste map obtained by PCA based on the five taste sen-

sor outputs is shown in Fig. 1. PCA was used to estimate the largest and second-largest relative contributing factors (PC1 and PC2, respectively). The relative contributions of PC1 and PC2 were 52.1 and 23.0%, respectively. Taste sensor outputs

of AN0, AC0 and BT0 contributed to PC1 rather than PC2, while those of AE1 and C00 contributed to PC2 rather than PC1. Diphenidol, haloperidol, diphenhydramine, dextromethorphan and papaverine were predicted to express strong bitterness, surpassing that of quinine, which was used as the standard of bitterness. Figure 2 shows the taste sensor (AC0, AN0, BT0) outputs of these six compounds. The BT0 taste sensor output values of these compounds were higher than those of AC0 or AN0.

Response Profiles of hTAS2Rs Stimulated with Drugs in *in Vitro* Assays The response profiles of hTAS2Rs stimulated with the 22 drugs used in this study were extracted from the Bitter DB. These are summarized in Table 1. Some compounds respond to a broad range of hTAS2Rs, whereas others respond to only one. Quinine and the five compounds (diphenidol, haloperidol, diphenhydramine, dextromethorphan, papaverine) predicted to express the strongest bitterness in this study, were shown to be ligands of hTAS2R10 and/or hTAS2R14.

Correlation between Taste Sensor and hTAS2Rs Responses Correlation coefficients between taste sensor responses and the responses of hTAS2Rs cited from the Bitter

DB are shown in Table 2. High correlation coefficients (>0.7) were observed between the responses of AC0 and hTAS2R14 (0.72) and slight correlation coefficients ($0.4 < r < 0.7$) were observed between the responses of AC0 and hTAS2R40 (0.53), between AN0 and hTAS2R10 (0.43), hTAS2R14 (0.42) and hTAS2R44 (0.58), between BT0 and hTAS2R14 (0.46), and between AE1 and hTAS2R8 (0.46) and hTAS2R41 (0.46). Fisher's exact tests were carried out for these correlations.

Correlation between the responses of AC0 and hTAS2R14: AC0 responded to seven compounds (quinine, diphenidol, diphenhydramine, papaverine, haloperidol, chlorpheniramine and noscapine) out of the nine compounds known to be ligands of hTAS2R14; AC0 did not respond to 12 compounds (azathioprine, acetaminophen, erythromycin, ofloxacin, cromolyn, chloramphenicol, colchicine, strychnine, hydrocortisone, famotidine, propylthiouracil and methimazole) out of the 13 compounds which are not ligands of hTAS2R14. AC0 and hTAS2R14 responses were significantly correlated ($**p < 0.01$, Fisher's exact test).

Correlation between responses of AC0 and hTAS2R40: AC0 responded to three compounds (quinine, diphenidol and chlorpheniramine), all known to be ligands of hTAS2R40; AC0 did

Table 1. Response Profiles of hTAS2Rs Stimulated with 22 Compounds Cited from Bitter DB^{a)}

	hTAS2R																	
	43	44	46	47	49	13	14	7	9	8	10	1	38	39	40	4	16	41
1. Quinine	+	+	+				+	+			+			+	+	+		
2. Acetaminophen														+				
3. Azathioprine			+								+					+		
4. Caffeine	+		+				+	+			+							
5. Chloramphenicol	+		+							+	+	+		+				+
6. Chlorpheniramine			+				+	+			+		+	+	+	+		
7. Colchicine			+											+		+		
8. Cromolyn	+				+			+										
9. Dextromethorphan											+	+						
10. Diphenhydramine							+											
11. Diphenidol	+	+	+	+	+	+	+	+			+	+	+	+	+	+	+	
12. Erythromycin											+							
13. Famotidine		+									+							
14. Flufenamic acid							+											
15. Haloperidol							+				+							
16. Hydrocortisone			+															
17. Methimazole													+					
18. Noscapine							+											
19. Ofloxacin									+									
20. Papaverine							+	+			+							
21. Propylthiouracil													+					
22. Strychnine			+								+							

Table 2. Correlation Coefficients between Taste Sensor Responses and hTAS2R Responses

	hTAS2R																	
	43	44	46	47	49	13	14	7	9	8	10	1	38	39	40	4	16	41
AC0	0.04	0.25	-0.05	0.29	0.09	0.29	0.72 ^{a)}	0.39	-0.16	-0.16	0.31	0.25	0.13	0.17	0.53 ^{b)}	0.27	0.29	-0.16
AN0	0.10	0.58 ^{b)}	-0.17	0.32	0.12	0.32	0.42	0.24	-0.15	-0.15	0.43	0.30	-0.07	0.02	0.30	0.10	0.32	-0.15
BT0	-0.11	0.13	-0.09	0.22	0.00	0.22	0.46 ^{b)}	0.20	0.22	-0.22	0.37	0.13	0.00	0.00	0.40	0.11	0.22	-0.22
C00	-0.09	0.05	-0.09	-0.13	-0.19	-0.13	-0.30	-0.38	0.36	0.36	-0.06	0.05	-0.29	0.08	-0.24	-0.09	-0.13	0.36
AE1	0.31	-0.19	0.33	-0.10	0.26	-0.10	-0.15	0.24	-0.10	0.46	0.19	0.16	0.08	0.24	0.16	0.03	-0.10	0.46

a) $p < 0.01$, b) $p < 0.05$, (Fisher's exact test).

not respond to 14 compounds (azathioprine, acetaminophen, erythromycin, ofloxacin, caffeine, cromolyn, chloramphenicol, colchicine, strychnine, hydrocortisone, famotidine, flufenamic acid, propylthiouracil and methimazole) out of the 19 compounds which are not ligands of hTAS2R40. AN0 and hTAS2R40 responses were significantly correlated ($*p < 0.05$, Fisher's exact test).

Correlation between responses of AN0 and hTAS2R14: AN0 responded to five compounds (quinine, diphenidol, diphenhydramine, papaverine and haloperidol) out of the nine compounds known to be ligands of hTAS2R14. AN0 did not respond to 11 compounds (azathioprine, acetaminophen, erythromycin, ofloxacin, cromolyn, chloramphenicol, colchicine, strychnine, hydrocortisone, propylthiouracil and methimazole) out of the 13 compounds which are not ligands of hTAS2R14. Correlation between responses of AN0 and hTAS2R14 were close to significance ($p = 0.064$, Fisher's exact test).

Correlation between responses of AN0 and hTAS2R10: AN0 responded to six compounds (quinine, diphenidol, dextromethorphan, papaverine, haloperidol and famotidine) out of the 12 compounds known to be ligands of hTAS2R10; AN0 did not respond to nine compounds (acetaminophen, ofloxacin, cromolyn, colchicine, noscapine, hydrocortisone, flufenamic acid, propylthiouracil and methimazole) out of the 10 compounds which are not ligands of hTAS2R10. Correlation between responses of AN0 and hTAS2R10 were close to significance ($p = 0.059$, Fisher's exact test).

Correlation between responses of AN0 and hTAS2R44: AN0 responded to three compounds (quinine, diphenidol and famotidine) all known to be ligands of hTAS2R44; AN0 did not respond to 15 compounds (azathioprine, acetaminophen, erythromycin, ofloxacin, caffeine, cromolyn, chloramphenicol, chlorpheniramine, colchicine, strychnine, noscapine, hydrocortisone, flufenamic acid, propylthiouracil and methimazole)

out of the 19 compounds which are not ligands of hTAS2R44. AN0 and hTAS2R44 responses were significantly correlated ($*p < 0.05$, Fisher's exact test).

Correlation between responses of BT0 and hTAS2R14: BT0 responded to seven compounds (quinine, chlorpheniramine, diphenidol, diphenhydramine, noscapine, papaverine and haloperidol) out of the nine compounds known to be ligands of hTAS2R14; BT0 did not respond to nine compounds (azathioprine, acetaminophen, cromolyn, chloramphenicol, colchicine, hydrocortisone, famotidine, propylthiouracil and methimazole) out of the 13 compounds which are not ligands of hTAS2R14. BT0 and hTAS2R14 responses were significantly correlated ($*p < 0.05$, Fisher's exact test).

Correlation between responses of AE1 and hTAS2R8 or hTAS2R41: the correlation coefficients of the responses of AE1 and hTAS2R8 and hTAS2R41 were both 0.46; there was no significant correlation between them by Fisher's exact test.

Correlation between Physicochemical Parameters of the 22 Compounds and the Responses of Taste Sensors and hTAS2Rs The physicochemical parameters of the 22 compounds extracted from the Bitter DB are shown in Table 3. The MW values of erythromycin, ofloxacin, cromolyn, chloramphenicol, colchicine, strychnine, noscapine, papaverine, haloperidol, hydrocortisone and famotidine are larger than that of quinine hydrochloride (MW 324). The log P values of chlorpheniramine, diphenidol, diphenhydramine, dextromethorphan, noscapine, papaverine, haloperidol and flufenamic acid are greater than that of quinine hydrochloride (log P 2.73). The log S values of azathioprine, acetaminophen, ofloxacin, caffeine, strychnine, noscapine, hydrocortisone, famotidine, propylthiouracil and methimazole are greater than that of quinine hydrochloride (log S -4.1). The HBD counts of chlorpheniramine, diphenhydramine, strychnine, dextromethorphan, noscapine and papaverine are zero while others are ≥ 1 ; quinine is 1. HBA counts of azathioprine, erythromycin,

Table 3. Physicochemical Parameters of the 22 Compounds Extracted from the Bitter DB⁶⁾

	MW	log P	log S	HBD	HBA	Rings	Aromatic rings
Quinine	324	2.73	-4.14	1	4	4	2
Acetaminophen	151	0.71	-1.39	2	2	1	1
Azathioprine	277	1.07	-2.66	1	7	3	3
Caffeine	194	-0.10	-0.64	3	0	2	1
Chloramphenicol	323	1.03	-4.12	3	5	1	1
Chlorpheniramine	275	3.70	-4.97	0	2	2	2
Colchicine	399	2.04	-4.70	1	6	3	1
Cromolyn	468	1.77	-4.33	3	11	4	2
Dextromethorphan	271	3.67	-4.53	0	2	4	1
Diphenhydramine	255	3.38	-4.24	0	2	2	2
Diphenidol	309	4.39	-5.61	1	2	3	2
Erythromycin	734	1.79	-4.76	5	14	3	0
Famotidine	337	-2.62	-3.43	4	9	1	1
Flufenamic acid	281	3.95	-4.79	2	3	2	2
Haloperidol	375	3.89	-5.70	1	3	3	2
Hydrocortisone	362	1.28	-3.07	3	5	4	0
Methimazole	114	0.41	-0.92	1	1	1	0
Noscapine	413	3.01	-3.25	0	8	5	2
Ofloxacin	361	-1.37	-1.61	7	1	4	1
Papaverine	339	3.50	-5.75	0	5	3	3
Propylthiouracil	170	1.58	-2.55	2	2	1	0
Strychnine	344	1.15	-1.70	0	3	7	1

cromolyn, chloramphenicol, colchicine, noscapine, papaverine, hydrocortisone and famotidine are greater than that of quinine hydrochloride, which is 4. Ring counts of ofloxacin, cromolyn, strychnine, dextromethorphan, noscapine and hydrocortisone are greater than that of quinine hydrochloride, which is 4. The aromatic ring counts of azathioprine, cromolyn, chlorpheniramine, diphenidol, diphenhydramine, noscapine, papaverine, haloperidol and flufenamic acid are greater than that of quinine hydrochloride, which is 2.

Correlation coefficients between the physicochemical parameters of the 22 compounds and the taste sensor responses are shown in Table 4. High $\log P$ values (≥ 2.73) and the responses of AC0, AN0 and BT0 were significantly correlated (** $p < 0.01$, Fisher's exact test). Correlations between the physicochemical parameters and the responses of hTAS2Rs are shown in Table 5. High $\log P$ values (≥ 2.73) and the response of hTAS2R 14 were significantly correlated (** $p < 0.01$, Fisher's exact test).

Discussion

In the taste map of the 22 compounds used in this study (Fig. 1), haloperidol, diphenidol, diphenhydramine, dextromethorphan and papaverine mapped on the right side (the direction of the PC1 axis reflects the basic bitterness contribution) of quinine, the standard compound for assessing bitterness. It was therefore predicted that these five compounds would express stronger bitterness than quinine. The outputs of taste sensors AC0, AN0 and BT0 increased in a dose-depen-

dent manner in response to these compounds (Fig. 2), with the output values of sensor BT0 being the highest. This suggests that sensor BT0 has the greatest sensitivity for bitterness.

Regarding the correlation between the responses of the taste sensors and those of hTAS2Rs, BT0 was significantly correlated with hTAS2R14 (Table 2). Although AC0 also significantly correlated with hTAS2R14, BT0 was the most sensitive taste sensor for ligands to hTAS2R14.

Taste sensors AC0, AN0 and BT0 reacted more strongly to compounds with high $\log P$ values (Table 4). Bitter compounds are adsorbed on the hydrophobic part of the taste sensor membrane and cause a change in membrane potential by changing the charge density, which is reflected by the taste sensor output.¹²⁾ So high $\log P$ compounds will react more readily with taste sensors. Roland *et al.* suggested that hydrophobic aromatic features, and therefore hydrophobic interaction with the binding site, is of greater importance in hTAS2R14 than in hTAS2R 39.²²⁾ In fact, the $\log P$ values and aromatic ring count of the 22 compounds used in our study correlated well with the responses of hTAS2R14 (Table 5). This supports the finding that taste sensor BT0 would be the most useful taste sensor for evaluating the bitterness of hydrophobic compound which stimulate the bitter taste receptor hTAS2R14.

Recently, it has been revealed that hTAS2R14 recognizes an enormous variety of chemically diverse synthetic and natural bitter compounds, including medicinal drugs.^{23,24)} Inhibition of hTAS2Rs, especially hTAS2R14, would be one approach to reducing the bitterness of drugs. To date, only a

Table 4. Correlation Coefficients between Physicochemical Parameters and the Responses of Taste Sensors to the 22 Compounds

	MW	$\log P$	$\log S$	HBD	HBA	Rings	Aromatic rings
AC0	0.00	0.91 ^{a)}	-0.45	-0.60	-0.12	0.09	0.64
AN0	0.10	0.62 ^{a)}	-0.36	-0.24	-0.04	-0.05	0.36
BT0	0.27	0.65 ^{a)}	-0.37	-0.61	-0.18	0.29	0.37
C00	-0.20	-0.30	0.35	0.38	0.06	-0.20	-0.15
AE1	0.00	-0.15	-0.04	-0.24	0.04	0.18	0.04

a) $p < 0.01$ (Fisher's exact test).

Table 5. Correlation Coefficients between Physicochemical Parameters and the Responses of hTAS2Rs to the 22 Compounds

	MW	$\log P$	$\log S$	HBD	HBA	Rings	Aromatic rings
R43	-0.11	-0.01	0.06	0.33	0.16	0.10	0.16
R44	0.13	0.21	0.10	0.24	0.17	0.01	0.17
R46	-0.09	-0.13	0.20	0.09	0.17	0.03	-0.02
R47	-0.22	0.26	-0.24	0.13	-0.20	-0.15	0.24
R49	0.00	0.06	-0.35	0.19	0.03	0.12	0.35
R13	-0.22	0.26	-0.24	0.13	-0.20	-0.15	0.24
R14	-0.09	0.81 ^{a)}	-0.35	-0.32	-0.20	-0.17	0.73 ^{a)}
R7	0.00	0.32	-0.26	-0.08	0.06	0.02	0.47
R9	0.22	-0.18	0.20	0.13	-0.20	0.32	-0.20
R8	-0.22	-0.18	0.20	0.13	0.24	-0.15	-0.20
R10	0.00	0.20	-0.10	-0.15	0.10	-0.16	0.10
R1	-0.40	0.21	-0.17	-0.05	-0.10	0.01	-0.10
R38	-0.47	0.09	-0.04	0.02	-0.43	-0.32	0.04
R39	-0.20	0.11	-0.06	0.15	0.06	-0.20	0.06
R40	-0.13	0.48	-0.17	-0.05	-0.10	0.01	0.44
R4	-0.11	0.21	-0.16	0.09	0.16	-0.14	0.38
R16	-0.22	0.26	-0.24	0.13	-0.20	-0.15	0.24
R41	-0.22	-0.18	0.20	0.13	0.24	-0.15	-0.20

a) $p < 0.01$ (Fisher's exact test).

few bitter inhibitors have been discovered that block bitter-substance-evoked hTAS2R activation, such as Sakuranetin for hTAS2R31,^{25,26} 6-methoxyflavanones for hTAS2R39,^{27,28} γ -aminobutyric acid (GABA), N^{α},N^{α} -bis(carboxymethyl)-L-lysine (BCML)²⁹ and abscisic acid³⁰ for hTAS2R4, and (*R*)-Citronellal for hTAS2R43 and hTAS2R46.³¹ Batenburg *et al.* identified several hTAS2R14 receptor antagonists from a screening of synthetic compounds; however, the nature of these compounds did not allow their effects to be validated *in vivo* before extensive safety evaluation.³² Taste sensor BT0, which has high sensitivity and whose responses correlated well with the responses of hTAS2R14, should therefore be useful for screening potential inhibitors of bitter taste receptor hTAS2R14.

Although not only hTAS2R14 but also hTAS2R10 are broadly tuned by a large number of agonists,^{3,4,33} there was no significant correlation between the responses of hTAS2R10 and $\log P \geq 2.73$. It seems that the responses of hTAS2R10 were unrelated to the hydrophobicity of the ligand, unlike those of hTAS2R14. Born *et al.* demonstrated, using molecular modelling, point mutagenesis and functional assays, that the binding pocket of hTAS2R10 is not optimized for high-affinity interactions with a handful of ligands but is rather designed to accommodate many diverse agonists.³³ In fact, the ligands of hTAS2R10 have diverse $\log P$ values.

The six compounds which were predicted to express the strongest bitterness in our study (Figs. 1, 2) were all ligands of hTAS2R14 and/or hTAS2R10, both of them were broadly tuned by a large number of agonists. Our findings of a correlation between the responses of hTAS2R 14 and 10 and those of BT0, AC0, and AN0 suggest that a large number of bitter compounds, as ligands of the bitter taste receptor, could be detected by these taste sensors.

Conclusion

Taste sensor BT0 had high sensitivity compared to AC0 or AN0 and the responses of BT0 and those of hTAS2R14 was significantly correlated. High $\log P$ values and response to hTAS2R14 (and BT0) were also significantly correlated. These results suggest that taste sensor BT0, which has high sensitivity as well as a significant correlation with response to hTAS2R14, would be useful in evaluating the bitterness of hydrophobic compounds which respond to hTAS2R14 and also in screening potential bitterness inhibitors.

There are many reports on evaluation of the bitterness of drugs using taste sensors, however, this is the first report evaluating the correlation between the response of the taste sensor and the response of human bitter taste receptors.

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Conflict of Interest The authors declare no conflict of interest.

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