The Mechanism of Bitterness Suppression in Huanglian Jiedu Decoction

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Abstract

Background

Bitterness is one of the main reasons that affect the clinical efficacy of traditional Chinese medicine (TCM). Preliminary research shows γ-cyclodextrin (γ-CD) and neotame can significantly improve the taste of TCM, while its mechanism still remains unknown. Huanglian jiedu decoction (HLJDD), as a typical representative of the bitter prescription of TCM, will be a breakthrough point to study the taste correcting strategy of TCM in this study.

Methods

Firstly, UPLC-MS/MS was used to identify the ingredients of HLJDT. Secondly, pharmacophore model of bitter taste receptors (BTR) Tas2r10, Tas2r14 and Tas2r46 was built by Discovery Studio 4.0 software. Thirdly, bitter taste compounds in HLJDD were identified by pharmacophore model. Finally, molecular modeling was used to explore how γ-CD and neotame make effect.

Results

We found γ-CD masked bitterness by preventing bitter components from contacting its receptor, or reducing the affinity between them. While components are extremely rigid such as coptisine, cannot complete self-assemble with γ-CD. Hence, inclusion (γ-CD) is difficult to achieve good effects for drugs containing those compounds such as *Coptidis Rhizoma* and *Phellodendri Chinrnsis Cortex*. However, neotame can strongly bind with those extremely bitter compounds such as coptisine and epiberberine in *Coptidis Rhizoma* and *Phellodendri Chinrnsis Cortex*, and thus can mask taste of them significantly.

Conclusions

The strategy achieves a significant bitter block effect from blocking the formation of the bitter signal to hindering its transmission after the formation of the signal, achieving a multilevel and significant taste effect. This article provides references for the research and development of flavoring companions for TCM, which will help improving the clinical efficacy of TCM.

1 Introduction

Traditional Chinese medicine (TCM) plays an increasingly important role in health and disease, for example the prevention and treatment for COVID-19. While “Good medicine tastes bitter” is almost the basic attribute of TCM, especially for Chinese medicine decoction. The innate resistance of humans and mammals to bitter taste seriously affects the compliance of patients with TCM, and thus its clinical efficacy, especially in children and the elderly.

Currently, methods for bitter taste suppressing mainly include physical isolation of bitter substances, such as inclusion; bitter receptor inhibitors inhibit the activation of bitter receptors and taste substances interfere with bitterness signal transduction and so on. These methods of masking bitterness have achieved certain effect in the application of modern medicine with single ingredients, but they are not effective in liquid preparation of TCM with complex ingredients and prominent bitter taste. Thus how to improve the taste of TCM decoction without affecting the composition, efficacy and side effects, and make it change from "good medicine tastes bitter" to "good medicine tastes delicious" is one of the urgent problems to be solved at present.

Huanglian jiedu decoction (HLJDD) is a Chinese herbal compound composed of *Coptidis Rhizoma*, *Scutellariae Radix*, *Phellodendri Chinrnsis Cortex* and *Gardeniae Fructus*. It is the representative and basic prescription of clearing heat and removing toxin, eliminating sanjiao fire toxin. The prescription is extremely bitter and slightly sour, which seriously affects the compliance of adults and children. In the formula, *Coptidis Rhizoma* and *Phellodendri Chinrnsis Cortex* are extremely bitter, *Scutellariae Radix* tastes bitter slightly, and *Gardeniae Fructus* tastes bitter mixed with acid. The known bitter ingredients in the recipe berberine, rhizophorine and rhizophorine are alkaloids, ferulic acid is organic acid, baicalin is flavone, geniposide is iridoid, limonin is sesquiterpenlacton, which shows appropriate representativeness. Based on the bitter substance of HLJDD, this paper studied its mechanism of suppressing bitterness. It is of great practical value to form an effective demonstration suppressing bitterness in compound.

2 Materials And Methods

2.1 Chemical and reagents

Decocting pieces of *Coptidis Rhizoma, Scutellariae Radix, Phellodendri Chinrnsis Cortex and Gardeniae Fructus* all purchased from decocting pieces Co. LTD of Sichuan Xinhehua. Geniposide and chlorogenic acid standards (purity ≥ 95%), phellodendrine, epiberberine, baicalin, ferulic acid, berberine hydrochloride, wogonin and obakunone standards (purity ≥ 98%) were acquired from Chengdu Keluoma Biological Technology Co. LTD(Sichuan, China). Methanol, acetonitrile and formic acid was supplied by Thermo Fisher (America).

2.2 Preparation of HLJDD

Precisely weighed 1 g powdered of HLJDD by analytical balance, then added distilled water and cooked. Strained and took the solution, concentrating volume so that the volume was 100 mL, 1 mL of supernatant was filtered with 0.22 μm filter. 20 mg of chemicals, including geniposide, chlorogenic, phellodendrine, epiberberine, baicalin, ferulic acid, berberine hydrochloride, wogonin and obakunone precisely weighed and dissolved in 100 mL methanol.

2.3 UPLC-MS/MS conditions for compunds analysis [1]

Sample and reference chemical were analyzed with triple four-stage rod liquid mass spectrometer (UPLC-30AD /AB SCIEX 4000, SHIMADZU, Japanese) and high resolution liquid-mass spectrometer (AB SCIEX TripleTOF™, AB SCIEX, American). Separation was performed on C_{18} UHPLC column provided by Phenomenex (100 × 2.1 mm). The binary gradient elution system consisted of (A) water (containing 0.1% methanoic, v/v) and (B) acetonitrile (containing 0.1% methanoic, v/v) and separation was achieved using the following gradient: 0 min, 5% B; 1 min, 10% B; 7 min, 85% B; 11 min, 85% B; 11.5 min, 10% B; 15 min, 10% B. The flow rate was 0.3 mL/min, the column temperature was 30 ℃, and the injection volume was 2 μL. And dynamic background subtraction (DBS) trigger information association acquisition mode (IDA) was used for scaning. The Gas1 and Gas2 were both 55 Psi, the IS was 4500 V, the TEM was 600°C;the CUR was 25 Psi, the CE was 25,the CES was 15. Data was analyzed by the AnalystTM1.6 software and MultiQuantTM3.0 software (American AB SCIEX). Then those compounds with |ΔPPM | < 15.0 was derived.

2.4 The pharmacophore model of bitter taste receptors (BTR)

2.4.1 The training set of pharmacophore model

Tas2r10, Tas2r14 and Tas2r46 are broad-spectrum bitter taste receptors that can recognize more than 50% of natural bitter substances [2] Therefore Hiphop algorithm in Discovery Studio 4.0 software (Acceltys, American) was performed to construct pharmacophore model based on ligand of Tas2r10, Tas2r14 and Tas2r46. And in this paper, activity values of the 12 bitter compounds data with BTRs are cited from literatures [3–6]. It is expressed in terms of EC_{50} (concentration for 50% of maximal effect) and refers to the concentration that causes 50% of the maximum effect shows table S.1, and the structure of training test compounds shows in Fig.S.1.

2.4.2 Construction of pharmacophore model

Five kinds of pharmacophore features were selected, including hydrogen bond acceptor (HBA), hydrophobic (H), hydrogen bond (HBD), hydrophobic aliphatic (HA), and ring aromatic (R), using Discovery Studio 4.0 software to build the pharmacophore model. In the process of building a model, the maximum number of conformations is 255, and the choice of the model for the optimal is best. The energy threshold is 20 Kal/mol, the similar conformation energy threshold for each molecular homolog is 10, the number of the characteristic of pharmacophore is 0–5, and the other parameter values are defaults [7].

The ligands of Tas2r10, Tas2r14 and Tas2r46 were predicted by the BitterX (MDL.shsmu.edu.cn/bitterx/) website [8], and according to the principle of structure diversification and activity difference, epiberberine, coptisine, berberrubine, palmatine, eugenol, oroxylin, chlorogenic acid and crocetin were selected as test sets to detect the activity of pharmacophore. The binding rates of compounds in the test set predicted by Bitter X site with Tas2r10, Tas2r14, and Tas2r46 as shown in table S.2, and the structure of the test set compounds as shown in Fig.S.2.

2.5 Identification of bitter ingredients

The compounds of HLJDD to be screened were imported into Discovery Studio 4.0 software and 3D Database was built. The selected best pharmacophore were used to screen and identify the bitter compounds in HLJDD. The default values of the software were used for the parameters.

The taste of phellodendrine (alkaloids), berberine (alkaloids), baicalin(flavonoids) and chlorogenic acid(organic acids) were evaluated by volunteers to verify the pharmacophore screening results. Pearson correlation analysis was performed on the predicted value of Bitter X, the matching value of pharmacophore, and the results of the volunteers' oral taste score, to verify the correlation of them.

2.6 Formula of bitter suppressing

The optimal formulation of HLJDD for suppressing bitter was selected by single factor and CCD-RSM test, which was neotame: γ-CD-HLJDD = 0.028: 1.5: 100(m:m).

2.7 The taste masking mechanism of γ-CD

The planar structure of the compound was input into the Hyperchem 8.0 program one by one, and it was first regularized and then geometrically optimized according to the gradient value for three-dimensional imaging. The MM + molecular force field was used for the optimization of molecular structure, and a semiempirical approach Am1 was used to further optimize the molecular structure on that basis. Finally, QSAR parameters and thermodynamic parameters of various compounds were calculated for the optimized molecular structure.

According to the dipole-dipole force between bitter molecules and cyclodextrin molecules, or the relationship between bitter component molecules and cyclodextrin hydrophobic parameter (*LogP*), it was found that the optimized structure of drug molecules were inserted into the cyclodextrin cavity or attached to the outer surface of the cavity. The AMBER molecular force field algorithm was used to optimize the connected molecular structure, and no further calculation was made for the molecules that can't bind. QSAR parameters were calculated by combining the good molecules, and the changes of drug molecules before and after binding with cyclodextrin molecules were compared.

2.8 The taste - correcting mechanism of neotame

Amino acid sequence of Tas2r10, Tas2r14 and Tas2r46 (Tas2r10 ID: NP_076410.1, Tas2r14 ID: NP_076411.1, Tas2r46 ID: NP_795368.2) were derived from the NCBI database. The homology modeling was conducted on the I-TASSER Server (Available online: https: //zhanglab.ccmb.med.umich.edu/I-TASSER/) [9–11].

The best homologous model for Tas2r10, Tas2r14 and Tas2r46 bitter receptors was selected, and molecular docking was conducted with baicalin (flavonoid), limonin (Sesquiterpenlacton), geniposide (iridoid), epigberberine (alkaloid) and neotame respectively.

The SYBYL-X 2.0 software was adopted to perform durflex-dock, the Protein was pretreated with preparation Protein Structure (including extraction of ligand structure, repair of terminal residues, hydrogenation, energy minimization and automatic ligand construction). Then Ligand Structure Preparation was used to construct small molecules (including energy minimization, 2D and 3D Structure of molecules). Finally, in order to improve docking accuracy, Surflex-Dock GeomX (SFXC) mode was used for docking. Docking parameters values are default.

3 Results

3.1 Compounds of HLJDD

UPLC-Q-TOF-MS identified 35 compounds in HLJDD, and the result shows in Fig. 1 and Table 1. There are mainly 5 alkaloids (berberine, hydrastine, phellodendrine, etc.), 15 flavonoids (baicalin, wogonoside, chrysin, etc.), 5 terpenoids (genipin-1-β-glucoside), 6 organic acid (chlorogenic acid, ferulic acid, benzoic acid, etc.), 2 phenol, 1 ketone and 1 esters and so on.

This study did not identify the high content of jatrorrhizine, palmatine and geniposide in the herbs, which could relate to the high acidity and alkalinity of these components in the decoction. Compared with other alkaloid components in HLJDD, jatrorrhizine and palmatine have relatively strong alkalinity and certain solubility in water. At the same time, geniposide has strong acidity. During the hot and humid process of decocting, the neutralization reaction of acid-base components may be accompanied by desorption, dissolution and diffusion of the herbs. It also provides an explanation for the large amount of yellow flocs during the decoction and storage of HLJDD.

Table 1
Compounds of HLJDD

Name	Molecular formula	Retention time/ min	Molecular weight /Da	Adduct	Deviation/ Δppm
Berberine	$C_{20}H_{18}NO_4$	4.90	336.12	M + H	-11.5
Obacunone	$C_{26}H_{30}O_7$	7.67	454.20	M + H	-0.9
Obaculactone	$C_{26}H_{30}O_8$	7.03	470.19	M + H	0.9
Oxyberberine	$C_{20}H_{17}NO_5$	4.44	351.11	M + H	-0.4
Ferulic acid	$C_{10}H_{10}O_4$	1.96	194.05	M + H	1.0
Coptisine	$C_{19}H_{14}NO_4$	-14.6	320.09	M + H	4.63
Phellodendrine	$C_{20}H_{24}NO_4$	3.81	342.17	M + H	-14.2
Berberrubine	$C_{19}H_{15}NO_4$	4.35	321.10	M + H	-0.3
Chlorogenic acid	$C_{16}H_{18}O_9$	3.87	354.10	M + H	-1.4
Asperuloside	$C_{18}H_{22}O_{11}$	2.02	414.12	M + H	0.6
Baicalin	$C_{21}H_{18}O_{11}$	5.34	446.08	M + H	0.1
Baicalein	$C_{15}H_{10}O_5$	6.49	270.05	M + H	0.3
Wogonoside	$C_{22}H_{20}O_{11}$	5.82	460.10	M + H	-0.1
Skullcapflavone Ⅱ	$C_{17}H_{14}O_6$	7.37	314.08	M + H	0.5
Skullcapflavone Ⅰ	$C_{19}H_{18}O_8$	7.32	374.10	M + H	-0.3
Wogonin	$C_{16}H_{12}O_5$	7.27	284.07	M + H	0.9
Norwogonin	$C_{15}H_{10}O_5$	6.49	270.24	M + H	0.3
4-Heptenoic acid, 6-hydroxy-	$C_7H_{12}O_3$	3.92	144.17	M + H	-2.5
Eugenol	$C_{10}H_{12}O_2$	2.26	164.08	M + H	0.7
Chrysin	$C_{15}H_{10}O_4$	7.31	254.06	M + H	0.6
Dihydrobaicalein	$C_{15}H_{12}O_5$	5.53	272.07	M + H	0.8
Dihydrooroxylin A	$C_{16}H_{14}O_5$	7.48	286.08	M + H	-0.2
Crocetin	$C_{20}H_{24}O_4$	4.71	328.17	M + H	0.3
Genipin-1-7-β-gentiobioside	$C_{23}H_{34}O_{15}$	3.74	550.19	M + H	-1.3
Viscidulin Ⅲ	$C_{17}H_{14}O_7$	6.49	330.07	M + H	-0.5
Viscidulin Ⅱ	$C_{17}H_{14}O_8$	5.50	346.07	M + H	0.9
Baicalein-7-O-D-glucoside	$C_{21}H_{20}O_{10}$	5.24	432.11	M + H	-0.7
Phthalic acid, diisohexylester	$C_{20}H_{30}O_4$	7.45	334.21	M + H	-7.4
Benzoic acid	$C_7H_6O_2$	3.12	122.04	M + H	0.2
Isophorone	$C_9H_{14}O$	4.76	138.10	M + H	0.6

Name	Molecular formula	Retention time/ min	Molecular weight /Da	Adduct	Deviation/ Δppm
4-Hydroxy-3-methoxybenzaldehyde	$C_8H_8O_3$	1.87	152.05	M + H	0.5
(1S,3S,4S,5S)-1,3,4-trihydroxy-5-{[(2E)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoyl]oxy} cyclohexanecarboxylic acid	$C_{17}H_{20}O_9$	4.43	368.11	M + H	-1.2
Myricitrin	$C_{21}H_{20}O_{12}$	4.70	464.10	M + H	-1.2
Gardenolic acid B	$C_{30}H_{46}O_5$	6.30	486.33	M + H	-1.2
Limonin	$C_{26}H_{30}O_8$	7.03	471.19	M + H	0.9

3.2 Recognition of bitter taste compounds

3.2.1 The pharmacophore model of BTRs

Compounds in test set screened pharmacophore1, pharmacophore2 and pharmacophore2 from the top10 pharmacophores of Tas2r10, Tas2r14 and Tas2r46 respectively, as shown in Fig. 2(A, B, C). The optimal pharmacophore of Tas2r10, Tas2r14 and Tas2r46 is as following: Tas2r10 described by four characteristic characteristics (HHHA), including three hydrophobic centers and one hydrogen receptor. Tas2r14 described by three characteristic characters (RHA), including an aromatic ring center, a hydrophobic center and a hydrogen receptor. Tas2r46 described by five characteristics (HHHAA), including two hydrogen receptors and three hydrophobic centers. All the pharmacophore characteristics can map to ligands. The matching results of the compound and the optimal pharmacophore are shown in Fig. 2(D, E, F).

The X-axis represents the pharmacophore (01–10 represents the ordering of the pharmacophore by the software), and the Y-axis represents the compounds matching the pharmacophore. Blue means match is zero, red means match activity is good.

3.2.2 Bitter compounds in HLJDD screened by pharmacophore

The pharmacophores of Tas2r10, Tas2r14 and Tas2r46 screened 15, 16, and 15 compounds from HLJDD respectively,as shown in Table 2. The main biter compounds in HLJDD includes flavonoids in Radix Scutellariae, alkaloids and sesquiterpenlacton in Rhizoma coptidis and Cortex Phellodendri, iridoids in gardenia, and so forth.

Table 2
Bitter compounds in HLJJD screened by pharmacophore

Tas2r10		Tas2r14		Tas2r46	
Compounds	Match value	Compounds	Match value	Compounds	Match value
Baicalin	3.3634	Dihydrolignin A	2.8503	Baicalin	3.7969
Viscidulin	3.0228	Skullcapflavone	2.6838	DIHP	3.7481
DIHP	2.9818	Phellodendrine	2.6330	Viscidulin	3.1865
Skullcapflavone	2.7558	Obacunone	2.6330	Skullcapflavone	2.5801
Wogonoside	2.7106	Viscidulin	2.3705	Phellodendrine	2.2969
Skullcapflavone	2.6404	DIHP	2.2765	Obacunone	2.2969
Baicalein – 7-O-D-glucoside	2.5635	Limonin	2.2337	Berberrubine	2.1276
Phellodendrine	2.5021	Baicalin	2.1538	Viscidulin	2.0450
Obacunone	2.5021	Viscidulin	1.6092	Limonin	2.0251
Viscidulin	2.4410	Wogonoside	1.3285	Berberine	1.5899
Scutellarin	2.2650	Wogonin	1.2365	Eugenol	1.4579
Wogonin	1.9811	Berberrubine	1.1534	Coptisine	1.1343
Genipin-1-β-gentiobioside	1.9157	Scutellarin	1.1348	Wogonoside	0.6229
Dihydrolignin A	1.6686	Baicalein – 7-O-D-glucose	1.0192	Wogonin	0.5815
Limonin	1.2468	Norwogonin	0.8530	Dihydrolignin A	0.0650
		Berberine	0.7152		

The results of oral taste verification of bitter ingredients and correlation analysis among it with the predicted value of Bitter X website and matching value of pharmacophore shown in Table 3. The results strongly correlated between the predicted value and oral taste value, and the predicted value and pharmacophore matching value of geniposide, baicalin, phellodendrine, chlorogenic acid, epiberberine, rs were respectively 0.708 and 0.686. The pharmacophore matching value showed a certain correlation with the oral taste value, rs = 0.303, which furtherly validated the effective identification of bitter taste components of conducted pharmacophore model in HLJDD. The matching value also related to the bitterness of the ligand.

Table 3
Verification results (tasted bitter n = 12,`x ± SD)

Compounds		Matching value of pharmacophore		Predicted value of Bitter X /%	Oral test value
Geniposide		0		0	6.2 ± 2.1
Phellodendrine		4.930		238.1	15.8 ± 3.0
Epiberberine		0		0	1.8 ± 0.5
Baicalin		9.314		143.1	2.5 ± 0.6
Chlorogenic Acid		0		60.6	0.5 ± 0.2
Pearson test	Pharmacophore vs	rs	0.708		
	Bitter X	*P*	0.115		
	Pharmacophore vs	rs	0.303		
	Oral test value	*P*	0.560		
	Bitter X vs	rs	0.686		
	Oral test value	*P*	0.133		

PS: The matching value of pharmacodynamics group and the predicted value of the Bitter X website in the table are the addition of the matching value/binding rate of each compound to Tas2r10, Tas2r14 and Tas2r46 (the detailed results of the matching value/binding rate of each component to the Bitter taste receptor are shown in table S.3).

3.3 The taste masking mechanism of γ-CD

3.3.1 Results of molecular structure optimization of bitter taste components

The calculation results of molecular 3D parameters and thermodynamic parameters before self-assembly with γ-CD of the bitter and sour components of HLJDD are shown in Table 4. The greater the dipole moment (D) and the smaller the hydrophobicity parameter (*LogP*), the stronger the polarity and the better the hydrophilicity. Compared with bitter compounds, γ-CD is the most hydrophilic and the most water-soluble.

Most of organic acids and bitter taste compounds selected by pharmacophore with low hydration energy (HE) and strong hydrophobic (*LogP*), the compounds with water molecules force is weak, which is advantageous for the compound all or partly drilling into hydrophobic cavity of γ-CD by hydrophobic effects, to achieve the mask effect.

Table 4
Results of molecular parameters calculated before self-assembly with γ-CD of bitter compounds in HLJDD

Compounds	MF	MW	G (TE / 0 K)	S	D	SA	V	HE	log *P*/300 K	G (TE / 300 K)	Track
Berberine	$C_{20}H_{18}NO_4$	336.36	-99360	0	0.89	757.4	940.23	-12.18	-2.91	-4751	-7.59916
Obacunone	$C_{26}H_{30}O_7$	454.51	-137932	0	2.13	603.06	1106.07	182.77	1.94	-6478	-9.55474
Limonin	$C_{26}H_{30}O_8$	470.52	-138183	0	4.40	738.37	1078.75	183.34	-0.63	-6065	-9.68090
Ferulic Acid	$C_{10}H_{10}O_4$	194.18	-62246	0	5.78	558.89	591.42	35.92	-0.63	-2553	-8.90900
Coptisine	$C_{19}H_{14}NO_4$	320.32	-95130	0	1.99	759.65	861.81	-15.18	2.40	-4361	-7.89396
Berberrubine	$C_{19}H_{15}NO_4$	321.33	-95765	0	2.23	734.92	887.99	-16.51	0.00	-4464	-7.30709
Geniposide	$C_{17}H_{24}O_{10}$	388.37	-98435	0	4.42	816.87	1049.25	-16.15	4.74	-4735	-8.14968
Chlorogenic Acid	$C_{16}H_{18}O_9$	354.31	-119500	0	2.22	895.82	932.79	85.74	0.02	-4510	-9.03248
Geniposidic Acid	$C_{16}H_{22}O_{10}$	374.34	-128157	0	5.75	806.31	902.68	48.35	-2.02	-4828	-9.48778
Crocetin	$C_{20}H_{24}O_4$	328.41	-66094	0	6.45	514.86	578.22	-20.86	1.11	-2512	-9.38015
Genipin-1-β-gentiobioside	$C_{23}H_{34}O_{15}$	550.51	-189615	0	0.72	1112.56	1328.89	48.21	-3.20	-7132	-9.61559
Eugenol	$C_{10}H_{12}O_2$	164.2	-43322	0	1.78	891.75	919.14	-12.25	2.56	-952	-8.09896
Baicalin	$C_{21}H_{18}O_{11}$	446.36	-149002	0	3.86	1044.61	1088.39	82.49	0.11	-5488	-9.16880
Scutellarin	$C_{21}H_{18}O_{12}$	462.37	-156393	0	3.32	1110.53	1099.94	138.03	-0.92	-5538	-9.10321
Wogonoside	$C_{22}H_{20}O_{11}$	460.39	-153226	0	1.52	986.94	1124.91	98.54	5.64	-5885	-9.14840
Wogonin	$C_{16}H_{12}O_5$	284.26	-87957	0	5.20	706.61	768.06	33.24	1.50	-3728	-9.17600
Skullcapflavone	$C_{17}H_{14}O_6$	314.29	-98919	0	7.48	738.41	820.60	97.47	1.24	-4092	-8.69704
Skullcapflavone	$C_{19}H_{18}O_8$	374.35	-120867	0	4.04	784.29	969.96	34.06	0.74	-4822	-8.84824
Norwogonin	$C_{15}H_{10}O_5$	270.24	-84368	0	3.34	690.38	723.22	30.99	1.46	-3457	-8.86331
Dihydrolignin A	$C_{16}H_{14}O_5$	286.28	-88607	0	4.40	663.49	786.18	40.42	2.02	-3835	-8.98775
Viscidulin	$C_{17}H_{14}O_7$	330.29	-106317	0	7.83	792.24	852.50	31.40	0.96	-4189	-8.94446
Viscidulin	$C_{17}H_{14}O_8$	346.29	-113710	0	3.45	852.91	856.59	20.79	0.67	-4290	-8.85996
Baicalein – 7-O-D-glucoside	$C_{21}H_{20}O_{10}$	432.38	-142244	0	3.50	948.61	1049.71	32.24	0.00	-5508	-9.09819
DIHP	$C_{20}H_{30}O_4$	334.45	-98176	0	5.88	471.07	1107.01	126.72	8.18	-5335	-10.33996
γ-CD	$C_{48}H_{80}O_{40}$	1296.00	-462843	0	8.66	2362.73	2744.89	0.90	-9.73	-16218	-9.99965

PS: G(Gibbs free energy), D(Dipole), S(Entropy), SA(Superficial area), TE(Total Enery), HE(Hydration energy), MW(Molecular weight), MF(Molecular formular)

3.3.2 Simulation results of self-assembly between bitter taste component molecule and γ-CD

The 3D parameters and thermodynamic parameters of bitter and sour taste compounds molecule after self-assembly with γ-CD and the difference before and after the combination show in Table 5, Fig.S.3. SA and G of guest molecules decreased after the host and guest molecules self-assembled into supramolecular molecules, indicating that the system was easy to form and stable. The dipole moment of most compounds increased significantly, manifesting that the self-assembled supramolecular compounds showed increased hydrophilicity, decreased hydrophobicity, and resulting in decreased affinity with the bitter receptor. Due to the failure of conformation matching, coptisine and eugenol could not self-assemble with γ-CD, which may be one of the reasons why γ-CD has good effect on the bitter and sour tastes of *Scutellariae Radix* and *Gardeniae Fructus*, but not on *Coptidis Rhizoma* and *Phellodendri Chinrnsis Cortex*, which had a strong bitterness.

Due to the special structural properties of “hydrophobic inside and hydrophilic outside”, γ-CD can specifically bind to certain groups, enveloping the bitter or sour component molecules in some or all into the internal cavity, isolating the molecule from the bitter taste. Molecular simulation results found that organic acids and bitter components mostly have the characteristics of low hydration energy (HE) and strong hydrophobicity (large *logP* value), resulting in weak binding force of these compounds to water molecules. In the absence of γ-CD in the system, this feature is favorable for bitter compounds to enter the active binding site (hydrophobic cavity) of the bitter taste receptor through hydrophobic interaction, and bind to the hydrophobic group therein to activate the bitter taste receptor. When γ-CD is present, the hydrophobic effect pushes all or part of the compound (the hydrophobic group) into the γ-CD's hydrophobic cavity, where it binds to its hydrophobic group to mask the taste. Moreover, after self-assembly with γ-CD, the hydrophilicity of bitter compounds increases and the hydrophobicity weakens, which reduces the affinity of these compounds with bitter taste receptors. This is another mechanism of γ-CD in suppressing bitterness and masking taste.

However, γ-CD has a good masking effect on the bitterness and sourness of *Gardeniae Fructus* decoction, but it is not effective for drugs with extremely strong bitterness such as *Coptidis Rhizoma* and *Phellodendri Chinrnsis Cortex*. There are three reasons. Firstly, under the conditions of this subject, the host molecule (γ-CD) has large molecular weight (M_w=1296) and low concentration, and the number of guest molecules (bitter and sour components) is far more than the host molecule, making γ-CD unable to bind all the guest molecules in *Coptidis Rhizoma* and *Phellodendri Chinrnsis Cortex*. Secondly, the host-guest recognition depends on conformational matching. When strong bitter components such as coptisine and eugenol are close to γ-CD, the strong hydrophobic interaction prevents the host-guest molecules from being combined and cannot complete self-assembly. Thirdly, after the self-assembly of the host and guest molecules, the bitter molecules or hydrophobic bitter groups may not be completely "loaded" into the "bucket", but may also be bound to the outer surface of the host molecule, so that part of the bitter molecules can still activate the bitter receptor. Therefore, it is difficult to use γ-CD alone to mask the taste to achieve the expected effect, especially for the liquid preparations of TCM with strong bitterness, complex components, diverse structures and large molecular weight spans.

Table 5
The molecular parameters after self-assembly and the difference before and after self-assembly

Name	SA	V	G	Log P	D	TE	ΔSA	ΔV	ΔG	ΔLog P	ΔD
Berberine	610.83	690.86	-550565	2.04	6.55	-550565	-146.57	-249.37	-451205	4.95	5.66
Obacunone	505.83	918.56	-593219	1.91	13.80	-593219	-97.23	-187.51	-455287	-0.03	11.67
Limonin	680.24	2008.77	-593622	-0.74	11.23	-593622	-58.13	930.02	-455439	-0.11	6.83
Ferulic Acid	483.59	453.56	-517046	1.73	6.65	-517046	-75.3	-137.86	-454800	2.36	0.86
Berberrubine	692.85	1988.60	-550551	0.11	8.46	-550551	-42.07	1100.61	-454786	0.11	6.23
Geniposide	748.86	875.23	-553134	5.10	7.99	-553134	-68.01	-174.02	-454699	0.36	3.57
Chlorogenic Acid	810.20	2536.98	-574287	1.75	7.05	-574287	-85.62	1604.19	-454787	1.73	4.83
Geniposidic acid	704.61	796.03	-582951	-1.91	7.41	-582951	-101.7	-106.65	-454794	0.11	1.66
Crocetin	432.42	416.68	-520944	5.78	11.16	-520944	-82.44	-161.54	-454850	4.67	4.71
Genipin-1-β-gentiobioside	1003.31	582.58	-644480	-2.85	4.12	-644480	-109.25	-746.31	-454865	0.35	3.40
Baicalin	908.91	857.78	-603775	0.22	5.76	-603775	-135.7	-230.61	-554773	0.11	1.90
Scutellarin	1062.43	4392.42	-611223	4.91	8.19	-611223	-48.10	3292.48	-454830	5.83	4.88
Wogonoside	882.97	901.93	-607274	2.67	3.57	-607274	-103.97	-222.98	-454048	-2.97	2.05
Wogonin	567.36	2110.94	-542720	1.61	10.48	-542720	-139.25	1342.88	-454763	0.11	5.28
Skullcapflavone	647.65	2009.56	-553693	1.35	12.15	-553693	-90.76	1188.96	-454774	0.11	4.67
Skullcapflavone	664.39	4069.28	-575627	3.02	11.36	-575627	-119.90	3099.32	-454760	2.28	7.32
Norwogonin	586.17	1756.06	-539202	1.58	8.98	-539202	-104.21	1032.84	-454834	0.12	5.65
Dihydrolignin A	561.07	2101.47	-543375	2.13	11.89	-543375	-102.42	1315.29	-454768	0.11	7.49
Viscidulin	680.28	1575.95	-561155	0.80	6.71	-561155	-111.96	723.45	-454838	-0.16	-1.12
Viscidulin	556.24	622.74	-543459	4.05	0.00	-543459	-296.67	-233.85	-429749	3.38	-3.45
Baicalein – 7-O-D-glucoside	793.92	1678.38	-597099	0.11	5.00	-597099	-154.69	628.67	-454855	0.11	1.50
DIHP	435.68	1406.23	-560351	4.93	12.59	-560351	-35.39	299.22	-462175	-3.25	6.72

3.4 The taste-correcting mechanism of neotame

3.4.1 Results of homology modeling

The evaluation results of the bitter receptors Tas2r10, Tas2r14 and Tas2r46 models are shown in Fig. 3A and B. RMSDa is the RMS deviation of residues in sequence alignment, the lower the better; Cov. is the residual coverage compared to the template, the higher the better, and the results of this test are all in a high level range (RMSDa < 2.0, cov. > 0.9). The TM-score value of each model was > 0.8, which was at a high level, indicating that the modeling results were highly reliable (TM-score > 0.5 indicated that the structure of the model was similar to that of the natural protein).

3.4.2 Molecule docking results

The molecular docking results of bitter receptors Tas2r10, Tas2r14 and Tas2r46 with limonin, geniposide, epigberberine and neotame are shown in Fig. 4 (C, D, and E) and Table 6. Total Score and CScore with the highest value were selected from 20 docking conformations simulated by the software (see Fig.S.4 for the best conformation of compounds interacting with the receptor). For the receptors Tas2r10 and Tas2r14, the affinity of neotame was significantly stronger than that of limonin, geniposide and epigberberine. For Tas2r46, the

affinity of neotame was significantly higher than that of limonin and berberine, but not significantly different from that of geniposide. Interestingly, neotame has strong binding force with Tas2r10, Tas2r14 and Tas2r46 (far higher than limonin, geniposide and berberine), but tastes bitterless, suggesting that neotame may be an inhibitor of these 3 bitter receptors. Meanwhile, as an artificial sweetener, neotame competes with bitter substances for taste G-protein in the bitter signal transduction pathway, thus inhibiting the opening of IP3 gated channel is also one of its bitter inhibiting mechanisms.

Table 6
Docking results of bitter receptor and compounds

Receptor	Grading types	Geniposide	Limonin	Epiberberine	Neotame
Tas2r10	Total Score	6.64	5.68	3.34	7.94
	CScore	3.00	4.00	5.00	5.00
Tas2r14	Total Score	7.41	5.43	3.71	8.73
	CScore	4.00	5.00	5.00	5.00
Tas2r46	Total Score	8.38	5.79	4.53	8.03
	CScore	5.00	4.00	4.00	4.00

4 Discussion

Effective taste masking is the key to many drugs, health products and even foods. So far, the main taste-masking methods include inclusions, bitterness inhibitors aminobutyric acid [12], polymers to inhibit bitterness [13], sweetness and other taste substances that interfere with the perception of bitterness, and so on. Three levels of interaction must be taken into account to evaluate taste changes in the mixture of taste substances: one is the interaction between chemicals in solution, and the other is the taste receptor or conduction level of a component in the compound to other components' secondary interactions [14], the third is the interaction between different taste attributes.

In this study, we combined the first and second levels to mask the taste of HLJDD. Firstly, in the mixed solution, the compound may undergo a chemical reaction to form a new substance, which changes the taste type or intensity of the taste substance. Acid-base neutralization and salt-forming reactions are common in aqueous solutions, such as berberine and baicalin in HLJDD. After acid-base neutralization, precipitates forms, making berberine bitterless. In addition, supramolecular self-assembly and weak gravitational action in the solution will cause changes in the structure and polarity of the compound, thereby blocking the binding of the taste substance and the receptor or reducing the binding force of the two. The γ-CD flavor correction in this study adopts this strategy: while barreling bitter or sour molecules to block the activation of taste receptors, hydrogen bonding reduces the hydrophobicity of bitter substances and inhibits bitterness [15, 16].

Secondly, mammals perceive and distinguish five basic gustatory sensations: sweet, sour, bitter, salty, and umami [17]. Different taste sensations interfere with each other, such as bitter and sweet, sour and bitter, umami and sweet and so on. Umami can suppress bitterness [18] and enhance sweetness and saltiness. Neotame, an artificial sweetener, is effective in bitter masking of HLJDD. The molecular docking results show that neotame may be a potent inhibitor of the "broad-spectrum" bitter receptors Tas2r10, Tas2r14 and Tas2r46, and achieve high-efficiency anti-bitter effects. In short, from inhibiting the activation of bitter signals to interfering with the transmission of bitter signals, neotame achieves a "multi-pronged" signal interference from upstream (source) to downstream (process), achieving a significant effect of flavor correction.

In summary, on the basis of the effective taste correction of the TCM HLJDD with prominent bitter taste, we initially revealed its bitter taste material basis and taste masking mechanism. This study provides references for the flavor correction of bitter medicines, especially Chinese medicine decoctions, and is helpful to improve the clinical efficacy of TCM, especially for children and the elderly. However, due to the complexity of the research objects, this article did not study the interaction between bitter components, the interaction between bitter receptors, and the cross-interference between bitter components and receptors, and so on.

5 Conclusion

This taste-masking strategy that combines γ-CD and neotame achieves a significant bitter block effect from blocking the formation of the bitter signal (γ-CD) to hindering its transmission after the formation of the signal (neotame), achieving a multilevel and significant taste

effect. This study provides references for the research and development of flavoring companions for TCM, which will help improving the clinical efficacy of TCM.

Abbreviations

TCM, traditional Chinese medicine; HLJDD:, Huanglian jiedu decoction;γ-CD, γ-cyclodextrin; G, Gibbs free energy; D, Dipole; S, Entropy; SA, Superficial area; TE, Total Enery; HE, Hydration energy; MW, Molecular weight; MF, Molecular formular; HBA, hydrogen bond acceptor; H, hydrophobic; HBD, hydrogen bond; HA, hydrophobic aliphatic; R, ring aromatic.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent to publish

The authors consent to publish this paper.

Availability of data and materials

The datasets generated and/or analysed during the current study are available in the [NAME] repository, [PERSISTENT WEB LINK TO DATASETS].

Competing interests

The authors declare that they have no competing interests.

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Authors' Contributions

Data collection: X.M. Ke, L.S. Zhang, P. Li; design of the study: M. Wang; J.W. Wang, L. Han; statistical analysis: H.Y. Ma; analysis and interpretation of the data: J.X. Yang; drafting the manuscript: X.M. Ke; critical revision of the manuscript: D.K. Zhang.

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Figures

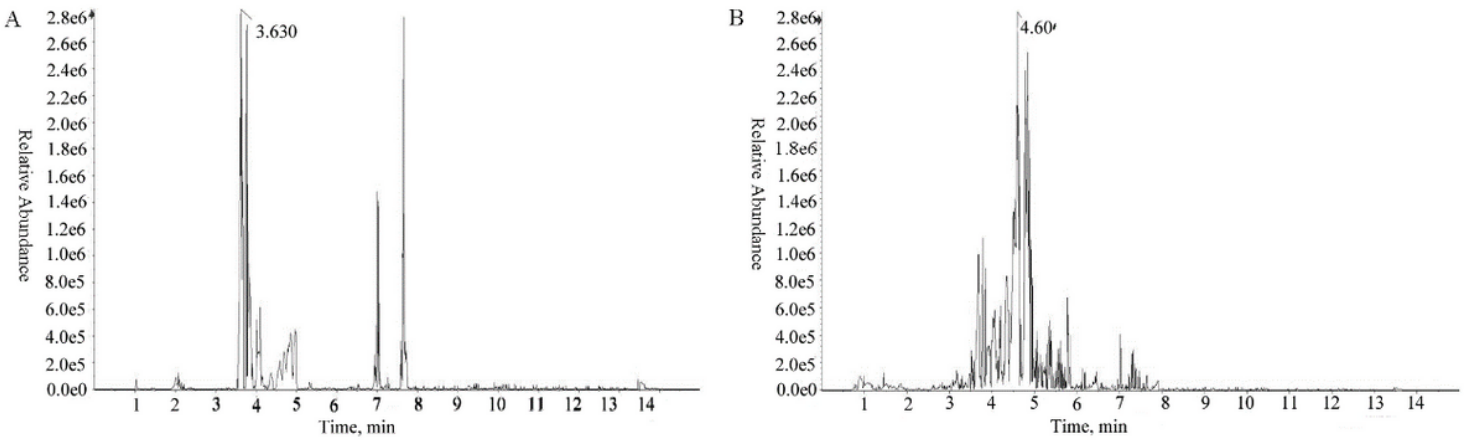

Figure 1

TIC detection diagram in positive ion mode of UPLC-Q-TOF-MS of HLJDD. Mixed standards (A), HLJDD (B).

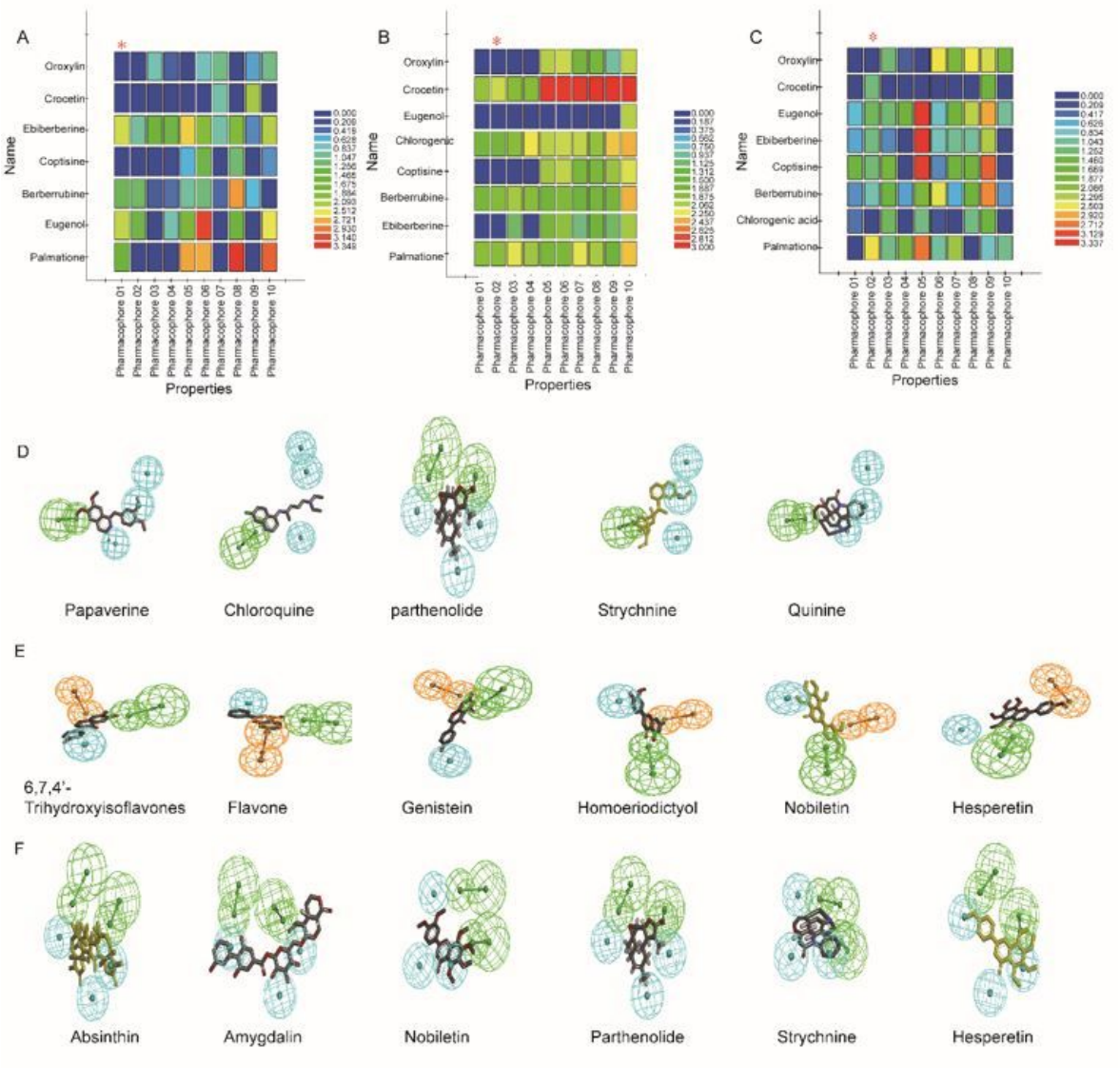

Figure 2

Results of bitter taste activity of pharmacophore predicted test set compounds and matching results of compounds in training set and optimal pharmacophore. A, B, C shows top 10 pharmacophore of Tas2r10, Tas2r14, Tas2r46, respectively. D, E, F shows matching results of compounds in training set and optimal pharmacophore of Tas2r10, Tas2r14 and Tas2r46, respectively.

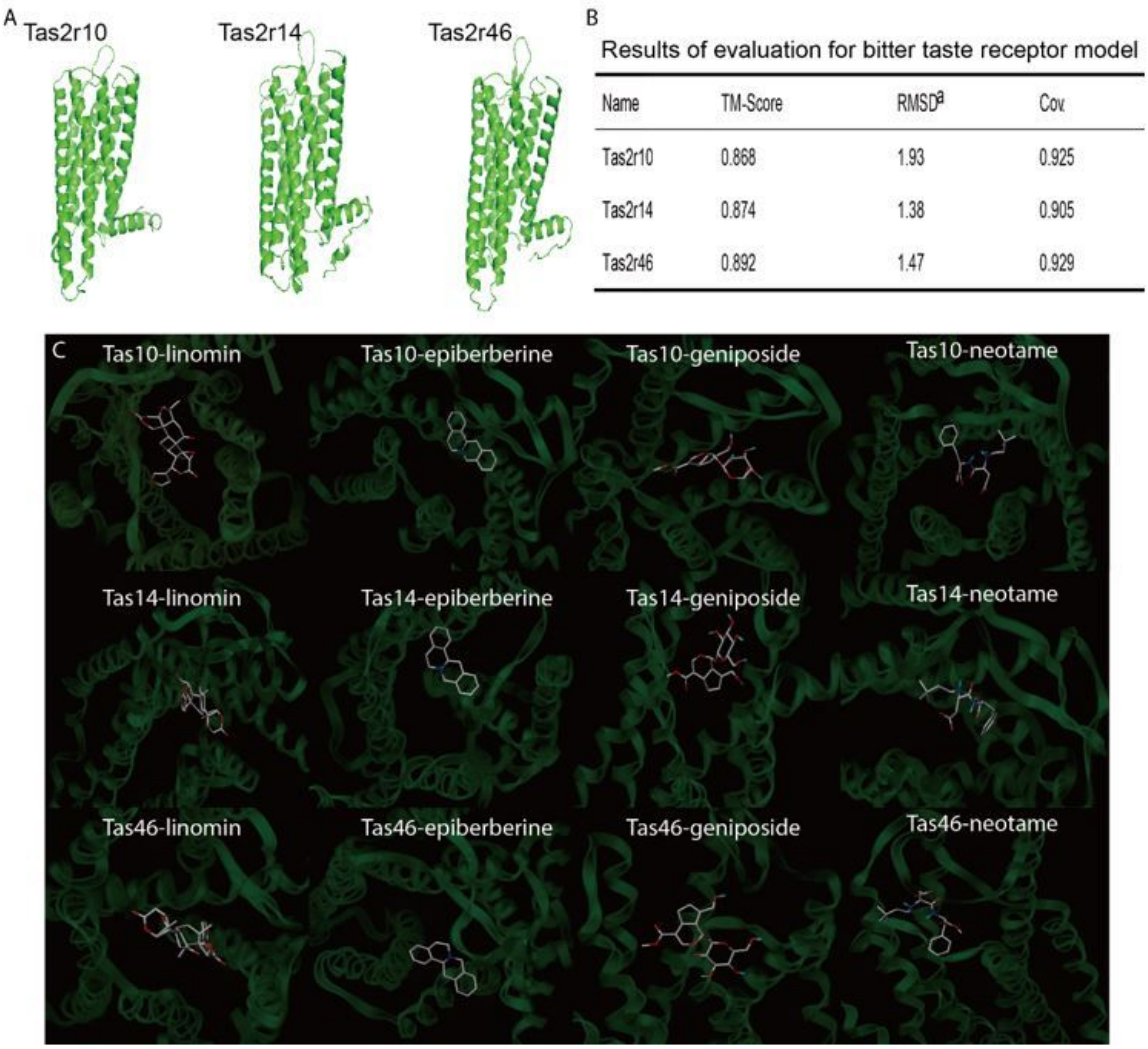

Figure 3

Bitter receptor homologous model (A and B) and molecular docking (C). A shows bitter receptor homologous model of Tas2r10, Tas2r14, Tas2r46, and B shows results of evaluation of them. C shows the results of molecular docking.

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