Traditional Chinese Bitter Flavor Theory: Is There Any Relation with Taste Type II Receptors?

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Abstract

Introduction: As a guide principle, traditional Chinese medicine (TCM) theories study the formation mechanism and the application regularity of Chinese herbs, while it is limited by the understanding of the natural properties of the herbs at the cognitive level. As one of important part in TCM theories, *Five Flavor* theory describes the function of drugs with five tasting adjectives: bitter, sweet, pungent, sour and salty. **Methods:** In this study, we present reasonable hypotheses that *Bitter Flavor* theory having instructive significance on account of the relation with bitter taste receptors (taste receptors, type 2, also known as T2Rs). For the lacked crystallized structure of T2Rs, *in silico* modeling method allows us to visualize the relationship between compounds from TCM showing *Bitter Flavor* property and T2Rs. In this study, pharmacophore models of T2R1, T2R10, T2R14 and T2R46 have been generated to screen agonists from TCM showing *Bitter Flavor* property. **Results:** According to the simulation results, 71.84% agonists of T2Rs were derived from medicinal herbs showing *Bitter Flavor* properties and the experimental evidence of the hits through virtual screening was validated via literature mining. **Conclusion:** The methods used in this research on bitter taste receptors supports the assumptions underpinning the traditional Chinese *Bitter Flavor* theory and provides a way to explore the potential science behind the Five Flavors theory of TCM which could be extended to explore other similar problems in TCM theory.

Key Words

Five Flavors; Taste type 2 receptors; Pharmacophore Model; Data Mining; Pharmacological Efficacy

Introduction

Traditional Chinese Medicine (TCM) property theory, evolved from long-term medical practice with the contemporary thoughts and theories in social and nature sciences¹, is an important pharmacy theory which is based on the physiological, pathological and clinical effect when drugs act on human body. As a guide principle, TCM property theory has played an important role in syndrome differentiation and clinical prescription for thousands of years^{2,3}. TCM property theory studies the formation mechanism and the application regularity of TCM properties, including four properties¹, five flavors^{4,5}, meridian entry⁶, toxicity and so on. These various characteristics description of efficiency as drugs effect on human body forms the TCM property theory of the system. Specifically, *Five Flavors* describe the function of drugs, *Four Properties* describe the principle of drugs, *Meridian Entry* describes the position of action and so on. These elements are combined to form a cohesive whole and impact on each other.

However, TCM theory is limited by the understanding of the nature at the cognitive level¹ and more objective, quantitative references should be added to evaluate the guiding significance. In this direction, we have made big emotional investment to evaluating the *Five Flavors* theory, one element of TCM theory systems. In *Five Flavors* theory, five basic tastes have been contained: bitterness, sweetness, sourness, saltiness, and pungency. Previous studies focused on *Pungent Flavor*⁴, while this research focuses on the *Bitter Flavor* theory.

According to *Five Flavor* theory, ‘flavor’ describes more than the taste feeling of drugs and each of the five flavors has its own particular effect on the body. *Bitter Flavor*, for example, can reverse the upward motion of *Qi* in lung, activate *Qi* and blood motion, dry damp evils and so on. The drugs showing *Bitter Flavor* property have potentially therapeutic effects in coughs, stagnant blood syndrome, vomiting and so on. Modern research has also

shown that: taste is not just about taste⁷. The bitter taste receptors have been found a series of effects such as reversing obstruction on airway smooth muscle⁸, therapeutical effect in asthma^{9,10}, nutrient sensing in the stomach¹¹ and so on, which is consistent with *Bitter Flavor* theory. Furthermore, there are some agonists of bitter taste receptor showing therapeutical effects. As is reported in *Nature Medicine*, agonists of these receptors are better than current β 2-adrenergic bronchodilators in a mouse asthma model, which raise the possibility that bitter compounds, alone or in combination with current therapies, may have a future as bronchodilators¹². Besides, it is also reported that agonists of T2R3, T2R4, T2R10 and T2R14 induced strong endothelium-independent relaxations, the responses of which are between 82–96% of maximal relaxations¹³. Even more enticing, 15 clinical drugs were found to activate the T2R14¹⁴ and hypothesis that extraoral bitter taste receptors as mediators lead to off-target effects of any drug with a bitter taste¹⁵. Therefore, we hypothesize on the basis of research that traditional Chinese *Bitter Flavor* theory has instructive significance on account of the relationship between compounds from TCM showing *Bitter Flavor* properties and bitter taste receptors.

Methods

In this study, natural agonists of T2Rs and the TCM sources with related properties were selected in *Table 1*¹⁵ according to the following criteria: (a) showing typical structural feature; (b) whose natural plant sources showing *Bitter Flavor* property.

According to the *Bitter Database* built in 2012 (<http://bitterdb.agri.huji.ac.il/dbbitter.php>), taste type II receptors and specific ligands distribution have been summarized in *Figure 1*, which remind us of some hints about the law of T2Rs. In this study, pharmacophore model of T2R1, T2R10, T2R14 and T2R46, which have been reported possessing more ligands, have been generated to study the relation

between TCM showing *Bitter Flavor* property and bitter taste receptors. A pharmacophore can be considered as the highest common denominator of a group of molecules exhibiting a similar pharmacological profile and which are recognized by the same site of the target protein¹⁶. A pharmacophore model explains how structurally diverse ligands can bind to a common receptor site. Furthermore, for the characteristic of convenience, fastness and direct vision, pharmacophore models can be used to identify through virtual screening novel ligands that will bind to the same receptor¹⁷.

Despite recent progress in structure determination of GPCRs, structural data on GPCRs are scarce and crystallized receptor proteins exhibit only low amino acid sequence similarity with T2Rs. Therefore, ligand-based characterization for T2Rs is necessary to understand the relation with compounds from TCM showing *Bitter Flavor* property. A pharmacophore model can be considered as the highest common denominator of a group of molecules exhibiting a similar pharmacological profile and which are recognized by the same site of the target protein. *HipHop* algorithm has been used to build pharmacophore of T2R1, T2R10, T2R14, and T2R46, which attempts to produce an alignment of compounds expressing certain activity against a particular target and by superposition of diverse conformations to find common three-dimensional arrangements of features shared between them¹⁸. The best pharmacophore model of T2Rs have been built to search Traditional Chinese Medicine Database (TCMD) (Version 2009), questing for the agonists from traditional Chinese medicine for further research of the connection between the “*Bitter Flavor*” and T2Rs.

Generation of common feature pharmacophore model

A. Compounds and biological data

The studies were implemented on a series of T2R1 agonists reported by literature^{15,19-21}. . To generate the pharmacophore model, these compounds were selected as the training set

(Figure 2) according to the following criteria: a). Sharing certain structural diversity; b). High antagonistic activity in each series; c). Containing similar pharmacophore features to ensure their similar binding models. Considering the distribution of structural diversity, twelve compounds were selected to generate the pharmacophore model and the other compounds were used as test set to validate the model. The agonists of T2R10, T2R14 and T2R46 have been listed in *Supplementary Materials*.

B. Pharmacophore generation and validation

In this study, the main steps^{21, 22} of pharmacophore generation are as follows^{22,23} :

Conformational Analysis

The 3D qualitative pharmacophore hypotheses have been constructed by *HipHop* (Common Feature Pharmacophore Generation) within Accelrys Discovery Studio 4.0 (DS4.0). Ligand conformations were created within the relative energy threshold of 20 kcal/mol by the BEST mode (Best Quality Conformer Generation) at the number of 255 maximum conformations.

Common Feature Mapping

According to the Feature Mapping's initial analysis, hydrogen bond acceptor (A), hydrogen bond donor (D), hydrophobic (H), and ring aromatic (R), which well-mapped all of the training set ligands, has been selected during the pharmacophore generation. These pharmacophore features can characterize the interaction between the ligand and receptor.

Pharmacophore Generation and Verification

The *HipHop* algorithm attempts to produce an alignment of compounds by superposition of diverse conformations to find common three-dimensional arrangements of features shared

between them. The *Minimum Features* has been set as 3, while the *Maximum Features* was 10. And the maximum number of pharmacophores generated was set as 10.

In this study, external test set molecules were used for validating the pharmacophore hypotheses, which have not been used for pharmacophore model generation. A test database of experimentally known T2Rs agonists embedded in a database consisting of some drug-like molecules (taken from the MDL MDDR database: Version2007.2) was constructed to evaluate all of the pharmacophore models. All of the identified ligands were filtered by *Lipinski's Rule* and were similar in chemical structural characteristics. To evaluate the performance of the models, four parameters (i.e., A%, Y%, N, and CAI) and the relationship between them was revealed in *Figure 3* with the correlativity as follows²²:

$$A\% = \frac{H_a}{A} \times 100\% \quad (1)$$

$$Y\% = \frac{H_a}{H_t} \times 100\% \quad (2)$$

$$N = \frac{H_a \times D}{H_t \times A} \quad (3)$$

$$CAI = N \times A\% \quad (4)$$

A% can represent the ability to identify active compounds from the test database and Y% represents the proportion of active compounds in hit compounds. N, the index of effective identification, is used to evaluate the ability of the models to identify active compounds from non-active compounds. CAI, a comprehensive evaluation index, is used to identify the best pharmacophore model. D is the total number of compounds in the test database and A is the number of active compounds. Ht is the total number of hit compounds from the test database and Ha is the number of active hit compounds from the test database. The model with the highest value of CAI is considered to be the best.

Virtual screening

According to the performance in terms of the enrichment factor (CAI value) of pharmacophore models, the most excellent pharmacophore model can be useful filters for virtual screening to identify T2R1, T2R10, T2R14 and T2R46 agonists within large compound repositories in TCM. This research select the model with highest CAI value served as a query to perform *3D Flexible Searching* operation in DS 4.0 to search Traditional Chinese Medicine Database (TCMD, version 2009), which contains 23033 natural compounds from 6735 medicinal plants. Moreover, all potential hit compounds in the database should be satisfied the Lipinski's rule of five requirements^{24,25}.

Results

Pharmacophore Models Generation

Twelve compounds were used as the training set for a *HipHop* running. The top 10 pharmacophore models with the calculation results are detailed in *Table 2*. According to the simulated results, the main features including *Hydrophobic (H)* and *H-Bond Acceptors (A)*

have been generated. Both of the *Direct Hi* and *Partial Hit* values of these 10 pharmacophore models are “111111” and “000000”, which confirm that all of the eleven molecules in training set have been taken into the generation of the models. The “4” value of Max Fit proves to us that all of the features in models can match with the molecules in training set. The Rank scores indicate the matching degree between the pharmacophore feature and the molecules. In general, the higher the score, the model matched more satisfactorily. The pharmacophore model calculation results of T2R10, T2R14 and T2R46 have been listed in *Supplementary Materials*.

Pharmacophore Models Validation

On the basis of the diagram of indicators evaluating the pharmacophore models showed before, the parameter values for each pharmacophore model were showed in *Table 3*. NO.1 MODEL with the highest CAI value has been selected to screen TCMD2009 database. The pharmacophore feature of Model 1 mapped with ligand Xanthohumol has been showed in *Figure 4*. The parameter values for T2R10, T2R14 and T2R46 pharmacophore models have been listed in *Supplementary Materials*.

Virtual screening

To quest for the potential agonists of T2R1, T2R10, T2R14 and T2R46, the pharmacophore model generated by *HipHop* was used as the query to perform a search of all of the known compounds from TCMD2009. According to the results of virtual screening, 2173 compounds from 206 Chinese Herbs have been hit as T2Rs’ agonists and 71.84% of which are belong to the *Bitter Flavor* property. The partial virtual screening hits were showed in *Table 4*. Furthermore, the experimental evidence of the hits through virtual screening was validated via literature mining. Andrographolide could activate T2R46, while Amarogentin and Strychnine could both activate T2R10 and T2R46²⁶. Moreover, Ginkgolide A is recorded

as bitter crystals from ethanol in *Merck Index*¹⁵ although there is no very definite target being studied. The reported literature could prove the reliability of pharmacophore-based virtual screening to a certain extent, which indicates that further biological activity experiment is worth being studied.

Discussion

Correlation analysis between Bitter Flavor and T2Rs

According to the simulation results, 71.84% agonists of T2Rs are derived from medicinal herbs showing *Bitter Flavor* property, such as *Salvia miltiorrhiza* Bunge. , *Coptis chinensis* Franch. *Rheum palmatum* L and so on. It indicated that the pharmacophore models of T2Rs can gather the same structural characteristics and are capable to identify the compounds drawing from TCM showing *Bitter Flavor* property. From this point can be seen that there is a strong correlation between T2Rs and *Bitter Flavor* property. The hypothesis would be confirmed that traditional Chinese *Bitter Flavor* theory having instructive significance on account of the relation between TCM showing *Bitter Flavor* property and T2Rs. Furthermore, the hits through virtual screening were reported showing multi-targets effects which were identical with those reported in literature²⁷. It suggested that their side effects may be caused by non-selective property of T2Rs, which could reveal the correlation between T2Rs and *Bitter Flavor* theory.

Bitter Flavor describes more than taste feeling

As we showed before, ‘flavor’ describes more than the taste feeling of drugs but also the effect of drugs on man. Similar function is grouped and described by one taste property, such as ‘bitter’ or ‘pungent’. Similarly, T2Rs activation is more than bitter taste function as we listed in Introduction. Recent literature suggests that the bitter taste receptor agonist quinine

reduces calorie intake and increases the post-prandial release of cholecystokinin in healthy subjects²⁸. Berberine, the agonist of T2R38 induces GLP-1 secretion through activation of bitter taste receptor pathways²⁹ as well as a bitter herbal medicine *Gentiana scabra* root³⁰. And bitter taste receptors can influence glucose homeostasis³¹. Moreover, it is reported that tumor necrosis factor a potent pro-inflammatory cytokine, is preferentially expressed in a subset of taste bud cells to regulate bitter taste response, which may contribute to some chronic inflammatory diseases³². Same with *Bitter Flavor* theory, the medical herbs showing *Bitter Flavor* property have other functions such as reducing blood glucose, anti-inflammatory effect and so on. To great extent, it positively confirmed the hypothesis that traditional Chinese *Bitter Flavor* theory having instructive significance on account of the relation with bitter taste receptors.

Method limitation analysis

In this paper, four subtypes of bitter taste receptors (T2R1, T2R10, T2R14 and T2R46) were studied through virtual screening based on pharmacophore models to explore the relationship with *Bitter Flavor*. However, there are 25 subtypes are included in T2R family. Therefore, this study reveals the relationship between T2Rs family and *Bitter Flavor* property to a certain extent while more data should be added in further study.

Conclusions

In this paper, ligand-based pharmacophore models of T2R1, T2R10, T2R14 and T2R46 have been generated to screen agonists from TCM. According to the results of virtual screening, 2173 compounds from 206 Chinese Herbs have been hit and 71.84% of which are belong to the *Bitter Flavor* property. The results show that T2Rs agonist pharmacophore models are capable of accumulating bitter herbs and identify the effective components from

herbs showing *Bitter Flavor* property, which provides supporting evidence for the hypothesis that *Bitter Flavor* having instructive significance on account of the relation with bitter taste receptors. The method used in this paper provides a way for exploring the scientific connotation of *Five Flavors* theory, which can be extended to solve similar problems in TCM theory.

Abbreviation

TCM	Traditional Chinese Medicine
GPCR	G Protein Coupled Receptor
T2Rs	Taste Type II Receptors
TCMD	Traditional Chinese Medicine Database
DS4.0	Accelrys Discovery Studio 4.0
T2R1	Taste receptor type 2 member 1
T2R10	Taste receptor type 2 member 10
T2R14	Taste receptor type 2 member 14
T2R46	Taste receptor type 2 member 46

Author Contributions

Y-J Qiao and Y-L Zhang have been conceived and designed the experiments. Y-X Zhang and X Wang have been involved in processing data and preparing the manuscript. S-F Wang participated in the discussion of views in the paper. All authors have read and approved the final manuscript.

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Figure 1 Taste Type II Receptors and Specific Ligands Distribution

Figure 1 Chemical structure of T2R1 agonists

Figure 2 Schematic diagram of indicators evaluating the pharmacophore models

Figure 3 T2R1 Pharmacophore Model_01 features (A) and the matching map with ligand Xanthohumol (B)

Lupulone

Humulone

Arborescin

Cascarillin

diphenidol

thiocarbanilide

yohimbine

Adhumulone

Isoxanthohumol

trans-isoadhumulone

Xanthohumol

Picrotoxinin

Tables

Table 1 Natural agonists of T2Rs and TCM sources

Compounds	TCM Source	Flavor Property	T2Rs
Benzoin	<i>Benzoinum</i>	Bitter/Pungent	T2R10, T2R14
Andrographolide	<i>Herba Andrographis</i>	Bitter	T2R46, T2R47, T2R50
Sinigrin	<i>Semen Lepidii</i>	Pungent/Bitter	T2R16, T2R38
Xanthohumol	<i>Flos Humuli Lupuli</i>	Bitter/Pungent	T2R1, T2R14, T2R40
Adhumulone	<i>Flos Humuli Lupuli</i>	Bitter/Pungent	T2R1, T2R40
Adlupulone	<i>Flos Humuli Lupuli</i>	Bitte/Pungent	T2R1, T2R14
Absinthin	<i>Radix Stephaniae Tetrandrae</i>	Bitter/Pungent	T2R10, T2R14, T2R46, T2R47
Quassin	<i>Cortex Meliae</i>	Bitter	T2R4, T2R10, T2R14, T2R46, T2R47
Amarogentin	<i>Semen Armeniacae Amarum</i>	Bitter	T2R1, T2R4, T2R16, T2R39, T2R43, T2R46, T2R47, T2R50
Quinine	<i>Cortex Cinchonae</i>	Bitter	T2R4, T2R7, T2R10, T2R14, T2R39, T2R40, T2R43, T2R44, T2R46
Aloin	<i>Herba Aloes</i>	Bitter	T2R43, T2R44
Aristolochic acid	<i>Fructus Aristolochiae</i>	Bitter	T2R14, T2R43, T2R44
Brucine	<i>Semen Strychni</i>	Bitter	T2R4, T2R46
Picrotoxinin	<i>Radix Stephaniae Tetrandrae</i>	Bitter/Pungent	T2R10, T2R14, T2R46, T2R47
Strychnine	<i>Semen Strychni</i>	Bitter	T2R10, T2R46

Table 2 T2R1 Pharmacophore model calculation results

Model	Features	Rank	Direct Hit	Partial Hit	Max Fit
01	HHHA	50.372	111111	000000	4
02	HHHA	50.260	111111	000000	4
03	HHHA	49.553	111111	000000	4
04	HHHA	49.285	111111	000000	4
05	HHHA	49.096	111111	000000	4
06	HHHA	48.796	111111	000000	4
07	HHHA	48.796	111111	000000	4
08	HHHA	48.261	111111	000000	4
09	HHHA	48.105	111111	000000	4
10	HHHA	47.887	111111	000000	4

Table 3 T2R1 Parameter values for each pharmacophore model

Model	A ^a	D ^b	Ht ^c	Ha ^d	A% ^e	N ^f	CAI ^g
1	27	180	41	19	70.4	3.09	2.17
2	27	180	46	17	63.0	2.46	1.55
3	27	180	46	18	66.7	2.61	1.74
4	27	180	48	17	63.0	2.36	1.49
5	27	180	42	16	59.3	2.54	1.50
6	27	180	45	18	66.7	2.67	1.78
7	27	180	47	18	66.7	2.55	1.70
8	27	180	46	18	66.7	2.61	1.74
9	27	180	49	17	63.0	2.31	1.46
10	27	180	59	16	59.3	1.81	1.07

^aA is the number of active compounds; ^bD is the number of compounds in the test database; ^cHt is the number of hits using pharmacophores to search; ^dHa is the number of active hits using pharmacophores to search; ^eA% represents the ability to identify active compounds from the test database; ^fN represents the ability to identify active compounds from nonactive compounds; ^gCAI is the comprehensive appraisal index.

Table 4 Partial Virtual Screening Hits of T2Rs Agonists

Name	Structure	TCM Souces	Flavors	T2Rs
Ginkgolide A		<i>Semen Ginkgo</i>	Sweet/Bitter	T2R10, T2R14
Ginkgolide B		<i>Semen Ginkgo</i>	Sweet/Bitter/	T2R10, T2R14
Albiflorin		<i>Radix Paeoniae Alba</i>	Bitter/Sour	T2R10, T2R14
Albiflorinr1		<i>Radix Paeoniae Alba</i>	Bitter/Sour	T2R10, T2R14
Saikosaponin F		<i>Radix Bupleuri</i>	Bitter	T2R10, T2R14, T2R46

Saikosaponin G		<i>Radix Bupleuri</i>	Bitter	T2R10, T2R14, T2R46
Saikosaponin A		<i>Radix Bupleuri</i>	Bitter	T2R1, T2R10, T2R14, T2R46
Saikosaponin B2		<i>Radix Bupleuri</i>	Bitter	T2R1, T2R10, T2R14, T2R46
Andrographolide		<i>Herba Andrographis</i>	Bitter	T2R10, T2R14, T2R46

Andrographoside

Herba Andrographis

Bitter

T2R10, T2R14, T2R46

Sennidine A

Radix Et Rhizoma Rhei

Bitter

T2R10, T2R46

Sennoside B

Radix Et Rhizoma Rhei

Bitter

T2R10, T2R14

Sennoside A

Radix Et Rhizoma Rhei

Bitter

T2R10, T2R14, T2R46

Sennidine A		<i>Radix Et Rhizoma Rhei</i>	Bitter	T2R1, T2R10, T2R46
Rheidiin A		<i>Radix Et Rhizoma Rhei</i>	Bitter	T2R1, T2R10, T2R14, T2R46
Rheidiin B		<i>Radix Et Rhizoma Rhei</i>	Bitter	T2R1, T2R10, T2R14, T2R46
Salvianolic Acid G		<i>Radix Salviae Miltiorrhizae</i>	Bitter	T2R10, T2R46
Tanshinone IIA		<i>Radix Salviae Miltiorrhizae</i>	Bitter	T2R10, T2R46

Tanshinone IIB

Radix Salviae *Miltiorrhizae*

Bitter

T2R10, T2R14, T2R46

Salvianolic Acid
A

Radix Salviae *Miltiorrhizae*

Bitter

T2R1, T2R10, T2R14, T2R46

Salvianolic Acid
B

Radix Salviae *Miltiorrhizae*

Bitter

T2R1, T2R10, T2R14, T2R46

Palmatine

Rhizoma Coptidis

Bitter

T2R46

Wogonin

Radix Scutellariae

Bitter

T2R10

Wogonoside

Radix Scutellariae

Bitter

T2R1, T2R10, T2R14, T2R46

Forsythin

Fructus Forsythiae

Bitter

T2R1, T2R10, T2R14, T2R46

Phillygenin

Fructus Forsythiae

Bitter

T2R1, T2R10, T2R14, T2R46

Amarogentin

Radix Gentianae

Bitter

T2R1, T2R10, T2R14, T2R46

Strychnine

Semen Strychni

Bitter

T2R1, T2R10, T2R46

Peiminine

Bulbus Fritillariae Thunbergii

Bitter

T2R1, T2R10, T2R14, T2R46

Baimonidine

Bulbus Fritillariae Thunbergii

Bitter

T2R1, T2R10, T2R14, T2R46

Nobiletin

Fructus Aurantii

Bitter/Pungent/ Sour

T2R1, T2R10, T2R46