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Synthesis, Purification, and Selective β_2-AR Agonist and Bronchodilatory Effects of Catecholic Tetrahydroisoquinolines from *Portulaca oleracea*

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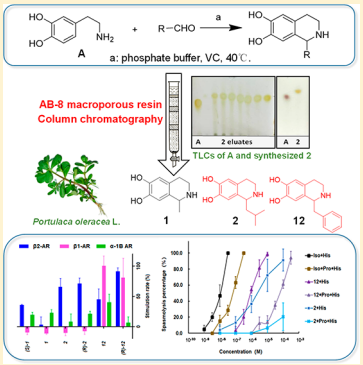
Supporting Information

ABSTRACT: A green, biomimetic, phosphate-mediated Pictet−Spengler reaction was used in the synthesis of three catecholic tetrahydroisoquinolines, **1**, **2**, and **12**, present in the medicinal plant *Portulaca oleracea*, as well as their analogues **3**−**11**, **13**, and **14**, with dopamine hydrochloride and aldehydes as the substrates. AB-8 macroporous resin column chromatography was applied for purification of the products from the one-step high-efficacy synthesis. It eliminated the difficulties in the isolation of catecholic tetrahydroisoquinolines from the aqueous reaction system and unreacted dopamine hydrochloride. Activity screening in CHO-K1/Gα15 cell models consistently expressing α_{1B}-, β_1-, or β_2-adrenergic receptors indicated that **12** and **2**, compounds that are present in *P. oleracea*, possessed the most potent β_2-adrenergic receptor agonist activity and **2** was a selective β_2-adrenergic receptor agonist at the concentration of 100 μM. Both **12** and **2** exhibited dose-dependent bronchodilator effects on the histamine-induced contraction of isolated guinea-pig tracheal smooth muscle, with EC_{50} values of 0.8 and 2.8 μM, respectively. These findings explain the scientific rationale of *P. oleracea* use as an antiasthmatic herb in folk medicine and provide the basis for the discovery of novel antiasthma drugs.



1,2,3,4-Tetrahydroisoquinoline (THIQ) is one of the "privileged scaffolds" commonly found in nature.[1,2] THIQs exhibit a variety of pharmacological activities, including antitumor, antiviral, anti-inflammatory, anticoagulation, and bronchodilation activities and action on the central nervous system.[1−3] As an important skeleton for drug discovery, the structural diversity and biological diversity of THIQs have attracted considerable attention in recent years.[4−9] Among them, catecholic THIQs, which possess *o*-dihydroxy groups on the benzene ring, as well as their analogues are important sources for drug discovery targeting the adrenergic receptors (ARs). ARs belong to the superfamily of G protein-coupled receptors (GPCRs), and they are categorized into two broad classes: α-ARs and β-ARs. Of the two α-AR subtypes, α_1-ARs are highly expressed on vascular smooth muscle cells and cardiomyocytes, whereas α_2-ARs are primarily found in the central nervous system. The three β-AR subtypes, β_1-AR, β_2-AR, and β_3-AR, are predominantly found in the myocardium, vascular and bronchial smooth muscle, and adipose tissue, respectively.[10] Norepinephrine, epinephrine, dobutamine, and dopamine are well-known antishock vasoactive drugs that have different agonist efficacy on α_1-AR, β_1-AR, and β_2-AR.[11] Selective β_2-AR agonists such as salbutamol are widely used for the treatment of asthma.[12] The representative catecholic THIQ-derived drugs include the β_2-AR agonist antiasthma compound trimetolquinol[13,14] and the β_1-AR agonist higenamine.[15]

In our previous phytochemical investigation of the medicinal plant *Portulaca oleracea* L., a series of water-soluble catecholic THIQs were isolated [6,7-dihydroxy-1-methyl-1,2,3,4-tetrahydroisoquinoline (**1**), 6,7-dihydroxy-1-isobutyl-1,2,3,4-tetrahydroisoquinoline (**2**), 1-benzyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (**12**), dehydroisoquinoline derivatives of 1-(furan-2-yl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (**13**), and 6,7-dihydroxy-1-(5-hydroxymethylfuran-2-yl)-1,2,3,4-tetrahydroisoquinoline (**14**) (Figure 1)], and most of them exhibited potent β_2-AR agonist activity.[16] However, their selectivity for different AR subtypes and their bronchodilator effects were not studied. Owing to the low contents in the medicinal plant, isolation of these compounds from natural resources is a time- and labor-intensive task. To provide large amounts of compounds for further pharmacological research, the chemical synthesis of catecholic THIQs is necessary.

The Pictet−Spengler reaction is an important cyclization reaction leading to the formation of THIQs as well as other heterocyclic moieties, including imidazoles, benzoxazoles, pyrroles, indoles, and tetrahydro-β-carbolines.[17,18] Since the traditional Pictet−Spengler reaction has the disadvantage of requiring severe reaction conditions, such as high temperature and strong acid catalysis,[19] the environmentally friendly

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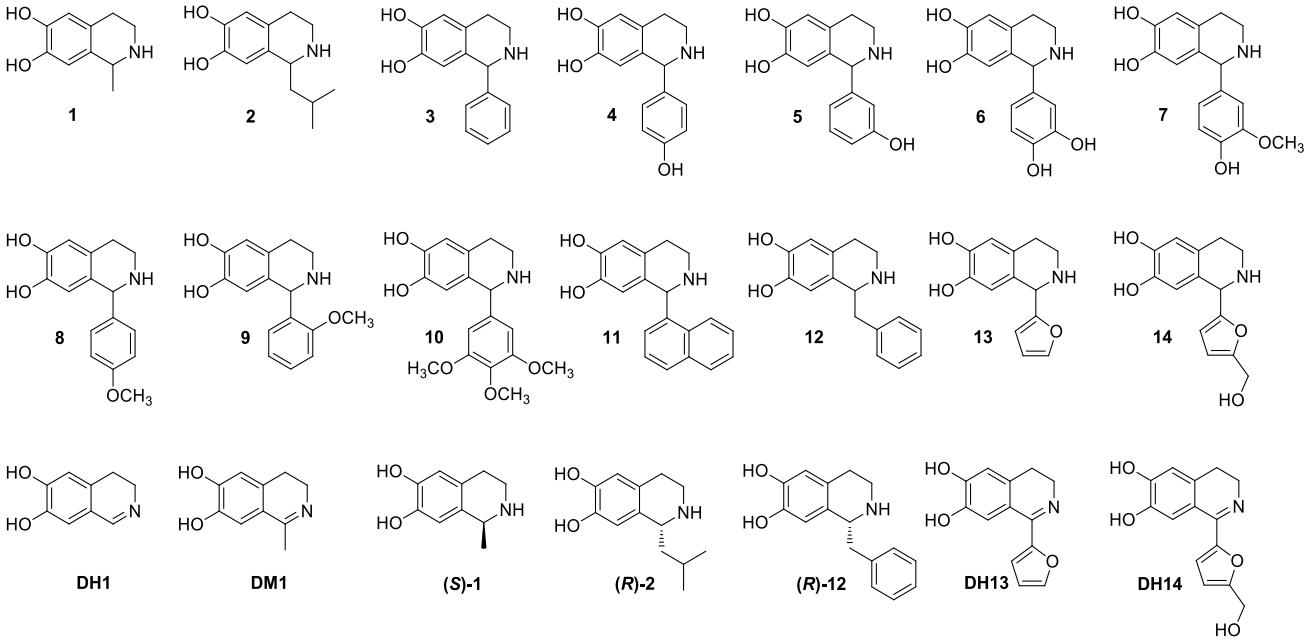
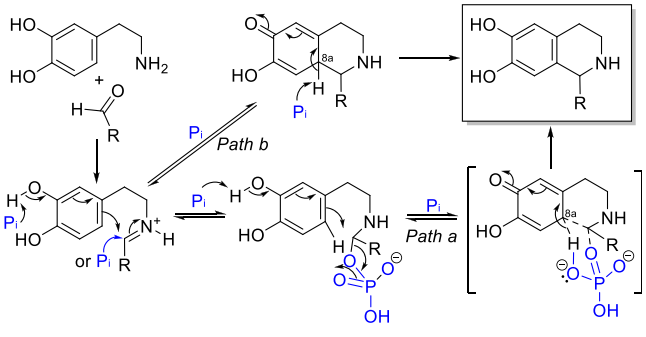


Figure 1. Structures of synthesized catecholic THIQs **1**−**14** and the analogues **DH1**, **DM1**, (*S*)-**1**, (*R*)-**2**, (*R*)-**12**, **DH13**, and **DH14** isolated from the medicinal plant *Portulaca oleracea*.

synthesis of THIQs was pursued. For example, a mild one-pot versatile phosphate-mediated Pictet−Spengler reaction has been exploited in the biomimetic synthesis of THIQs.[20] Systematic investigation indicated the uniqueness of phosphate as the catalyst and necessity of a C-3-OH in the structure of the substrates. In this mild Pictet−Spengler reaction, dopamine and an aldehyde form an iminium intermediate under acidic conditions (pH 6.0) in the first step. Phosphate was hypothesized to play a role in the following three aspects. First, a phosphate anion or dianion would effect a nucleophilic attack onto the iminium intermediate to produce a highly reactive aminophosphate. Second, phosphate can deprotonate the C-3-OH and activate the aromatic ring for addition to the imine at the *para*-position. Third, phosphate-mediated intra- (path a) or intermolecular (path b) abstraction of the 8a-H facilitates rearomatization (Scheme 1).[20]

Unexpectedly, following the one-step synthesis of catecholic THIQs by the phosphate-mediated Pictet−Spengler reaction, the purification of these alkaloids became problematic. Pesnot et al. reported that phosphate-mediated synthesized THIQs, e.g., norcoclaurine (higenamine), were purified via extraction with CH_2Cl_2 followed by preparative HPLC using a gradient of MeCN−H_2O (0.1% trifluoroacetic acid).[20] However, some water-soluble THIQs had short HPLC retention times, which made them difficult to separate from dopamine. Moreover, considering the high economic cost, an HPLC method was not suitable for large-scale preparation of the products. Bonamore et al. reported an enzymatic stereoselective synthesis of (*S*)-norcoclaurine (higenamine) in phosphate buffer, and the product was purified with Norit, a multipurpose activated charcoal.[21] In this purification procedure, the absorption was achieved by shaking (30 min) carbon granules that were added directly to the aqueous phase at room temperature, and the desorption of (*S*)-norcoclaurine was achieved by elution with EtOH at 40 °C in the presence of a slight molar excess of NaOH.[21] However, catecholic THIQs are commonly unstable under alkaline conditions. Maresh et al. reported that halogenated derivatives of norcoclaurine and aldehydes consistently partition into the same organic solvent. With aldehyde exhaustion, pure target THIQs can be readily obtained through EtOAc extraction followed by washing with brine, drying with $MgSO_4$, and solvent evaporation.[22] However, in our experiment, in addition to **11**, which can be readily extracted into EtOAc to yield pure powder precipitating during concentration, most of the catecholic THIQs were water-soluble. Their R_f values on silica gel thin-layer chromatography (TLC) were about 0.5 when developed by *n*-BuOH−HOAc−H_2O (4:1:1) or EtOAc−MeOH−H_2O (4:1:1), and their solubility in EtOAc or CH_2Cl_2 was low. Therefore, the introduction of water-soluble phosphate and antioxidant vitamin C greatly interfered with separation. Wakchaure et al. and Barbero et al. reported that THIQs synthesized with a phosphate-mediated method were purified by flash silica gel column chromatography.[23,24] However, it was found in our experiment that these water-soluble THIQs were strongly adsorbed when subjected to silica gel column chromatography, and the products were readily decomposed during the long separation time due to their low stability.

Scheme 1. Proposed Phosphate-Mediated Mechanism for the Pictet−Spengler Reaction[a20]



[a]Pi is inorganic phosphate.

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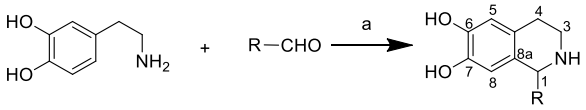
Owing to the limitation of the existing purification protocols, separating water-soluble catecholic THIQs from a water-soluble reaction system (containing phosphate buffer and vitamin C) and a dopamine substrate has become a significant challenge. A facile, benign, and scalable purification method for catecholic THIQs is urgently needed.

RESULTS AND DISCUSSION

Synthesis of Catecholic THIQs 1−14. The phosphate-mediated biomimetic syntheses of **1**−**14** were performed according to the route in Scheme 2, and the corresponding aldehydes and target products are shown in Table 1. In brief, dopamine hydrochloride (1.2 equiv), aldehyde (1 equiv), and vitamin C (0.5 equiv) were added to a mixture of potassium phosphate buffer (a mixture of KH_2PO_4 and K_2HPO_4: 1 M, pH 6.0) and distilled water (1:2, v/v). The reaction mixture was maintained at 40 °C in a water bath and stirred under a nitrogen atmosphere for 6 h.

Scheme 2. Synthesis Route for Catecholic THIQs[a]



[a]a: 1 M of potassium phosphate buffer (pH 6.0)−H_2O (1:2, v/v), vitamin C, 40 °C, 6 h.

Phosphate-mediated biomimetic Pictet−Spengler reactions were successfully applied in the one-pot synthesis of THIQs, starting from dopamine or amino acids as the substrates.[20,22] The reaction conditions in these experiments were slightly different. Pesnot et al. reported that the highest conversion was achieved using potassium phosphate buffer.[20] Maresh et al. found that increasing the phosphate buffer concentration (200−350 mM) led to a decrease in side product formation.[22] Therefore, 333 mM potassium phosphate buffer was used in the present study, through adding 1 M buffer with twice the volume of H_2O. The pH value of the phosphate buffer was selected as 6, considering that dopamine and catecholic THIQs are stable under acidic conditions, and a phosphate-mediated Pictet−Spengler reaction was reported to go smoothly at pH 6 but failed at pH < 4 or pH > 8.[20] The conversion rate increased when the temperature was elevated to 50 °C; however, the higher temperature led to the degradation of dopamine and evaporation of aldehydes. Therefore, a temperature of 40 °C was used in the present study. Dopamine and catecholic THIQs were readily decomposed when exposed to oxygen and sunlight and produced black pigments. To avoid oxidation, the reaction was performed under nitrogen, and the antioxidant, vitamin C was added to the reaction mixture.[22] The concentration was selected as 15 mM by our preliminary screening. Moreover, distilled water was degassed by boiling before its addition to the reaction. To avoid exposure to sunlight, protection from light during the reaction and purification steps was necessary.

Following the reported phosphate-mediated Pictet−Spengler reaction[20−22] with some adjustments, 14 catecholic THIQs (**1**−**14**) (Figure 1) were synthesized using inexpensive and readily available dopamine hydrochloride and aldehydes as substrates. Their structures were elucidated based on ESIMS, ^{1}H NMR, and ^{13}C NMR data analysis (Supporting Information). Compounds **1**, **2**, and **12** represent alkaloids present in *P. oleracea*, **14** is a new alkaloid, and **2** and alkaloids **7**−**14** were synthesized through the phosphate-mediated Pictet−Spengler reaction for the first time. Pesnot et al. reported that the highest conversions were achieved when a solvent mixture of phosphate buffer with MeCN, MeOH, or DMSO was used in order to improve substrate solubility.[20] Since no organic solvent was used in the present experiment, the low yields of some of the products may be related to the poor solubility of the aldehydes in water.

Purification of Catecholic THIQs 1−14. The reaction was completed after 6 h as monitored by TLC, and the mixture was extracted with EtOAc (3 × 150 mL) to remove unreacted aldehydes. Except for **11**, which can be extracted into EtOAc and precipitated to yield pure pale yellow product powder after concentration of the organic layer, other catecholic THIQs were less soluble in EtOAc and were present in large quantities in the water layer. The water layer (150 mL) of the reaction systems **1**−**10** and **12**−**14** was subjected to AB-8 macroporous resin column chromatography (4 × 40 cm), eluted with seven body volumes (BV) of distilled water at a high flow rate of 1800 mL/h, and then eluted with 1 BV of EtOH at a low flow rate of 250 mL/h. Identification of the target product in the eluted fraction was performed on silica gel TLC, developed by *n*-BuOH−HOAc−H_2O (4:1:1) or EtOAc−MeOH−H_2O (4:1:1), and sprayed with iodine vapor, 5% $FeCl_3$, or 5% ninhydrin.

Macroporous (macroreticular) resin is a type of adsorption material that was developed in the 1960s.[25] This resin not only acts as a molecular sieve depending on its porous structure but also adsorbs some ingredients with similar polarity through van der Waals forces and hydrogen-bonding interactions.[26] Currently, macroporous resin is widely used in the isolation and purification of natural products, especially total polyphenols,[27] total flavonoids,[28] total alkaloids,[29] and total saponins.[30] When we used AB-8 macroporous resin column chromatography to remove water-soluble compounds such as phosphate buffer and vitamin C, we occasionally found that catecholic THIQs could be separated from the unreacted substrate dopamine. Using elution with H_2O as the first step, phosphate, vitamin C, and dopamine can be quickly removed.

Table 1. Aldehydes and Target Synthesized Catecholic THIQs

R	product	yield (%)	R	product	yield (%)
methyl	**1**	25	4-methoxyphenyl	**8**	31
isobutyl	**2**	28	2-methoxyphenyl	**9**	40
phenyl	**3**	36	3,4,5-trimethoxyphenyl	**10**	42
4-hydroxyphenyl	**4**	54	1-naphthyl	**11**	32
3-hydroxyphenyl	**5**	78	benzyl	**12**	34
3,4-dihydroxyphenyl	**6**	67	2-furyl	**13**	53
4-hydroxy-3-methoxyphenyl	**7**	89	2-furyl-5-hydroxymethyl	**14**	58

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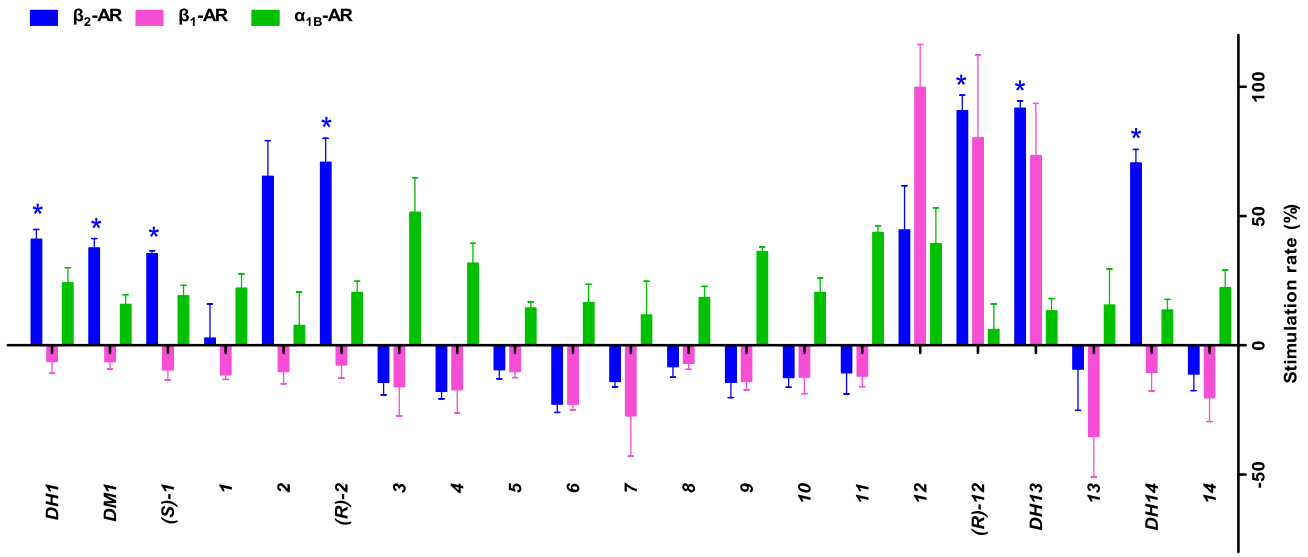

Figure 2. Agonist effects of 21 compounds (100 μM) on β_2-, β_1-, and α_{1B}-AR, as expressed by stimulation rate (%) versus the positive control of 100% (n = 4). Note: (1) isoproterenol (1 μM) was used as the β_2-AR agonist positive control, and epinephrine (10 μM) was used as the β_1-AR agonist positive control and the α_{1B}-AR agonist positive control. (2) For convenient comparison of the structure−activity relationships, the reported β_2-AR agonist activity[16] of the compounds **DH1**, **DM1**, (*S*)-**1**, (*R*)-**2**, (*R*)-**12**, **DH13**, and **DH14** isolated from *P. oleracea* is noted with blue asterisks.

Using elution with EtOH as the second step, the target catecholic THIQs can be completely separated from dopamine, thereby yielding purified product. There are many kinds of macroporous resin, and they are mainly divided into three categories according to the resin surface property, including high-polarity, low-polarity, and no-polarity resin. AB-8 macroporous resin belongs to a low-polarity resin.[26] Since dopamine hydrochloride possesses higher polarity than the synthesized catecholic THIQs, it may cause weak adsorption of dopamine and high adsorption of catecholic THIQs by the AB-8 resin. This difference may lead to ready desorption of dopamine by water elution and separation from catecholic THIQs. Considering that macroporous resin chromatography consumes a low volume of organic solvent and that macroporous resin and EtOH are recyclable, the present purification method for catecholic THIQs by AB-8 macroporous resin column chromatography is quite simple, convenient, economical, and green. We purified products at the gram scale using this method.

Adrenergic Receptor Agonist Activity of Catecholic Isoquinolines. In the previous study, the β_2-AR agonist activity of 14 catecholic isoquinolines isolated from *P. oleracea* was reported.[16] In this study, the effects of 100 μM of 14 synthesized catecholic THIQs and seven compounds isolated from *P. oleracea* (Figure 1) were systematically investigated on β_1-, β_2-, and α_{1B}-AR, as detected by calcium determination using CHO-K1/Gα15/ADRB1, CHO-K1/Gα15/ADRB2, or CHO-K1/Gα15/ADRA1B cell lines that stably express β_1-, β_2-, or α_{1B}-AR. The EC_{50} value of isoproterenol as the β_2-AR agonist was 1.2×10^{-9} M, and EC_{50} value of epinephrine as the β_1-AR agonist and α_{1B}-AR agonist was 2.9×10^{-7} and 7.5×10^{-8} M, respectively, which met the internal requirement for the positive control. Therefore, isoproterenol at the highest tested concentration of 1 μM was used as the β_2-AR agonist positive control, and epinephrine at the highest tested concentration of 10 μM was used as the β_1-AR agonist positive control and the α_{1B}-AR agonist positive control. The AR agonist activity of tested compounds was expressed as the stimulation rate versus the positive control of 100%.

As illustrated in Figure 2, in addition to the known β_2-AR agonists **DH1**, **DM1**, (*S*)-**1**, (*R*)-**2**, (*R*)-**12**, **DH13**, and **DH14**,[16] synthesized compounds **2** and **12** were potent β_2-AR agonists; only **12**, (*R*)-**12**, and **DH13** were potent β_1-AR agonists, and the other compounds had no activity. All tested compounds showed medium (**3**, **4**, **9**, **11**, and **12**) or weak α_{1B}-AR agonist activity. The structure−activity relationships of these compounds on β_1-, β_2-, and α_{1B}-AR activation can be concluded as follows. (1) Introduction of a C-methyl to simple dihydroisoquinoline, as in the case of compound **DH1** to **DM1**, did not affect their weak β_2-AR (41.06 ± 3.80%,[16] 37.70 ± 3.63%[16]) and α_{1B}-AR agonist activities (24.18 ± 5.82%, 15.87 ± 3.74%). Both compounds had no effect on β_1-AR (−6.22 ± 4.64%, −6.31 ± 2.87%). (2) As we previously reported, THIQ (*S*)-**1** possessed β_2-AR agonist activity (35.47 ± 1.14%)[16] equal to its dihydroisoquinoline derivative **DM1**. However, the β_2-AR agonist effect of (*S*)-**1** was higher than that of its racemic counterpart **1** (2.91 ± 13.03%), and both possessed equally weak agonist activity on α_{1B}-AR (19.20 ± 3.99%, 22.15 ± 5.48%) and no effect on β_1-AR (−9.50 ± 3.83%, −11.45 ± 1.75%), reflecting that (*R*)-**1** may have no effect on β_1- and β_2-AR and a weak effect on α_{1B}-AR. (3) Comparing racemic mixtures of **1** and **2**, when the C-1 methyl group was replaced by an isobutyl group, the β_2-AR agonist activity of **2** increased 20-fold to 64.51 ± 13.80%, whereas its effect on α_{1B}-AR nearly disappeared (7.70 ± 12.96%). (4) There was no significant difference in β_2-AR agonist activity between (*R*)-**2** (70.89 ± 9.13%)[16] and its racemic mixture **2** (65.41 ± 13.80%). The α_{1B}-AR agonist activity of racemic **2** was extremely low (7.70 ± 12.96%), nearly half the value of (*R*)-**2** (20.49 ± 4.37%), and both had no effect on β_1-AR, indicating that racemic **2** and (*S*)-**2** are selective β_2-AR agonists. (5) When a phenyl or a substituted phenyl group is located at C-1 of the catecholic THIQs, as in the case of synthesized racemic compounds **3**−**10**, all compounds had no agonist effects on β_2- and β_1-AR. However, **3**−**10** all showed

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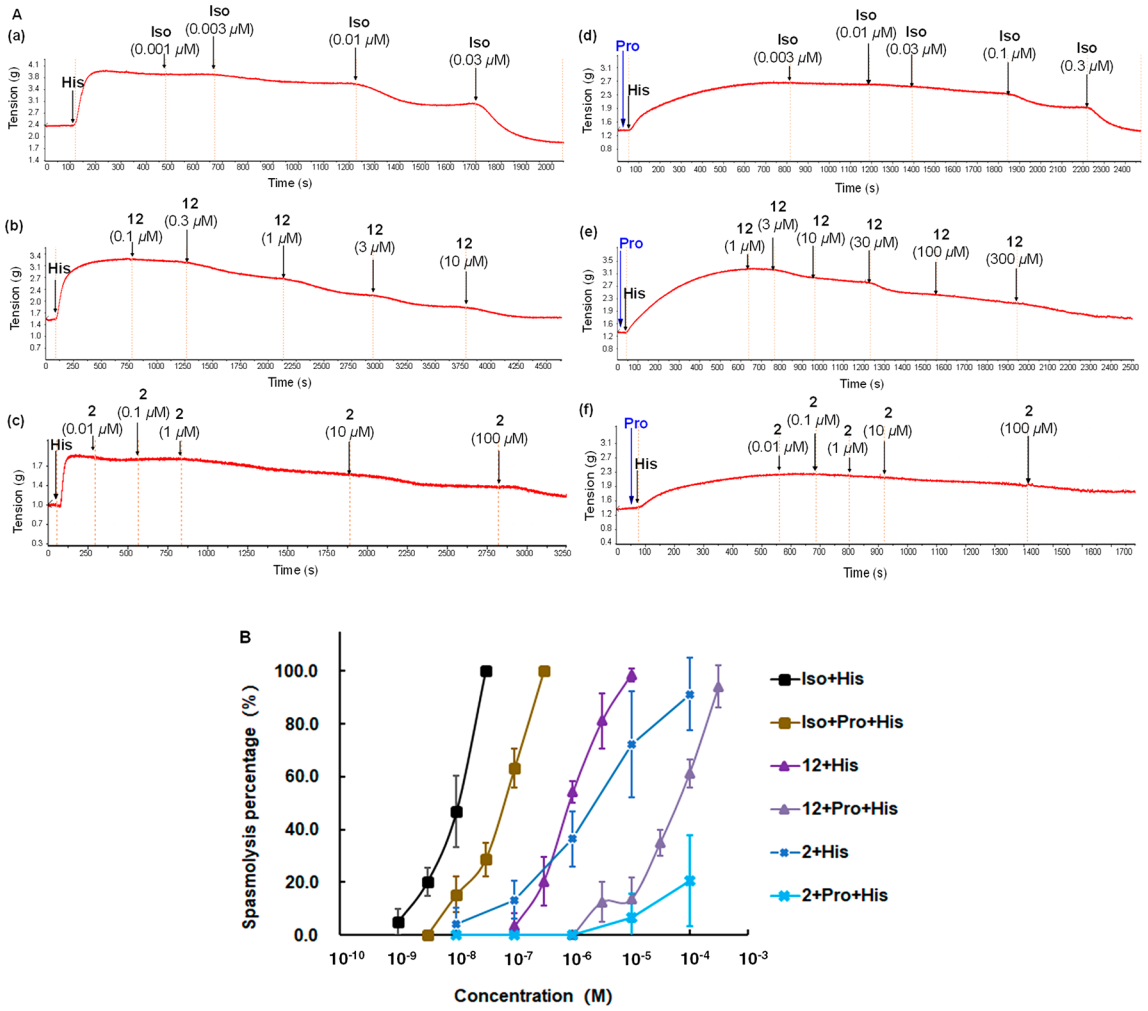

Figure 3. Bronchodilator effects of **12** and **2** on histamine-induced contraction in isolated guinea-pig tracheal spiral strips. (A) Representative tension (g) change diagram of tracheal smooth muscle after adding (↓) histamine (His) then each tested compound **12**, **2** or positive control isoprotenerol (Iso), with increased concentration, in the absence (a, b, c) or presence (d, e, f) of *β*-AR blocker propranolol hydrochloride (Pro). (B) Spasmolysis percentage (%) of different concentrations of **12**, **2**, and Iso in the absence or presence of Pro (n = 5).

medium or weak α_{1B}-AR agonist activity in the order **3** (51.53 ± 13.31%) > **9** (36.31 ± 1.70%) > **4** (31.88 ± 7.61%) > **10** (20.47 ± 5.47%) ≥ **8** (18.50 ± 4.29%) ≥ **6** (16.60 ± 7.04%) ≥ **5** (14.53 ± 2.29%) ≥ **7** (11.81 ± 13.03%), indicating that phenyl- or 2-methoxyphenyl-substituted catecholic THIQs possess more potent agonist activity on α_{1B}-AR than those connected to a 3- and/or 4-hydroxyphenyl, a 3- and/or 4-methoxyphenyl, or a 3,4,5-trimethoxyphenyl group. (6) When the C-1 phenyl group (**3**) was replaced by a naphthyl group (**11**), the α_{1B}-AR activity of **11** (43.75 ± 2.45%) was similar to **3**, and it also had no effect on β_2- and β_1-AR. (7) When the C-1 phenyl group (**3**) was replaced by a benzyl group (**12**), racemic **12** exhibited potent β_1-AR (99.86 ± 16.50%) and medium β_2-AR (44.67 ± 17.08%) and α_{1B}-AR (39.42 ± 13.73%) agonist activities, indicating that the introduction of a benzyl group was necessary for β_1- and β_2-AR agonist activity. However, chiral (***R***)**-12** exhibited potent β_1-AR (80.34 ± 32.07%) and β_2-AR (90.83 ± 5.98%)[16] agonist activity and weak α_{1B}-AR agonist activity (6.10 ± 9.84%), indicating that (***R***)**-12** is a *β*-AR agonist, without selectivity for the β_1- and β_2-AR subtypes. (8) Compared with chiral (***R***)**-12**, racemate **12** decreased the β_2-AR activity by half, the β_1-AR agonist activity remained high, and the α_{1B}-AR agonist activity more than doubled, indicating that racemate **12** had better selectivity for β_1-AR. The difference between (***R***)**-12** and racemic **12** also reflected that (***S***)**-12** may be a selective β_1-AR agonist with a strong effect on β_1-AR but no effect on β_2-AR and a weak effect on α_{1B}-AR. (9) Dihydroisoquinoline **DH13** was a potent β_2-AR (91.77 ± 2.78%)[16] and β_1-AR (73.50 ± 20.12%) agonist. **DH13** had no selectivity for β_1 and β_2 subtypes but had a very weak effect on α_{1B}-AR (13.36 ± 4.69%). Its THIQ derivative **13** retained a weak effect on α_{1B}-AR (15.61 ± 13.98%); however, it had no positive activation of β_1-AR (−35.29 ± 15.68%) or β_2-AR (−9.23 ± 15.89%). (10) As we previously reported, β_2-AR agonist potency of dihydroisoquinoline **DH14** (70.60 ± 5.18%)[16] was slightly lower than that of **DH13** (91.77 ± 2.78%),[16] which lacks a hydroxymethyl group on the furan ring in comparison with the structure of **DH14**. Similar to **DH13**, **DH14** had weak activity on α_{1B}-AR (13.73 ± 4.08%). Interestingly, **DH14** had no effect on β_1-AR (−10.38 ± 7.33%), which indicated that dihydroisoquinoline **DH14** was a potent β_2-AR selective agonist. (11) Similar to **DH14**, its THIQ derivative **14** retained a weak agonistic effect on α_{1B}-AR (22.28 ± 6.86%) and no effect on β_1-AR (−20.30 ± 9.17%). Different from **DH14**, **14** had no activation effect on β_2-AR (−11.10 ± 6.38%).

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As an important transmembrane protein family, GPCRs play a significant role in cellular signal transduction and are important targets for drug design. Structural analysis of GPCRs has been hindered by their low natural abundance, inherent structural flexibility, and instability in detergent solutions.[31] The crystal structure of human β_2-AR was first elucidated in 2007,[31] the crystal structure of the β_2-AR−Gs protein complex was elucidated in 2011,[32] and the molecular mechanism of β_2-AR−G-protein activation was first elucidated using single-molecule analysis of ligand efficacy in 2017.[33] Considering the mechanistic complexity of β_2-AR−G-protein activation, the relationship between the absolute configuration of THIQs and their β_2-AR agonist activities needs to be intensively studied.

Bronchodilator Effects of Catecholic THIQs on the Histamine-Induced Contraction of Isolated Guinea Pig Tracheal Smooth Muscle. BDTI (1-benzyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline HBr) was previously reported to be a selective β_2-AR agonist, and the potency of relaxing effect on carbachol-induced contraction in isolated canine trachea was in the order of isoprenaline > BDTI ≈ salbutamol (a clinically used antiasthma drug).[34] Using cell models expressing the three different ARs, our experiment also demonstrated that **12**, especially enantiomer (***R***)-**12**, was a potent β_2-AR agonist; however, **12** also had a strong effect on β_1-AR. It was found for the first time that catecholic THIQ **2** was a β_2-AR selective agonist. Since β_2-AR selective agonists have low side effects on the heart, their effect on tracheal smooth muscle contraction needs to be studied.

As shown in Figure 3A (a, b, c), the smooth muscle tension of isolated guinea-pig tracheal spiral strips remarkably increased after addition of histamine, and the tension gradually decreased with addition of increased concentrations of the positive control isoproterenol (Iso), **12**, or **2**, indicating **12** and **2** have bronchodilator effects. Figure 3B further demonstrates that both racemates **12** and **2** dose-dependently inhibited histamine-induced guinea-pig tracheal contraction, with EC_{50} values of spasmolysis at 0.8 and 2.8 μM, respectively, which are less potent than the positive control Iso (7.9 nM).

To define a potential mechanism for these bronchodilator effects, a β-AR antagonist propranolol hydrochloride (Pro) was added.[35] As indicated in Figure 3A (d, e, f), after a 15 min preincubation of Pro, addition of histamine still caused a significant increase in the tracheal tension. However, as compared with Figure 3A (a, b, c), the relaxing effects of **12**, **2**, and Iso, especially at some lower effective concentrations, reduced or disappeared in the presence of Pro in Figure 3A (d, e, f), leading to a rightward shift in the dose−effect curve in Figure 3B, demonstrating that **12** and **2** exert their bronchodilator effects via activating β_2-AR, which is highly present in the tracheal smooth muscle,[36] and their relaxing actions at lower concentration can be competitively antagonized by Pro.

In this study, the synthesis of catecholic THIQs was green and the purification was unique and highly efficient. The finding of the bioassay results of the natural products demonstrated the scientific rationale for use of *P. oleracea* as an antiasthmatic herb in folk medicine, and traditional medicines are promising sources for drug discovery.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were determined using a Gyromat-Hp digital automatic polarimeter (Anton Paar Germany GmbH, Germany). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance DRX-600 MHz NMR spectrometer with tetramethylsilane as the reference. Low-resolution ESIMS data were recorded on a Tandem 6410 triple quadrupole mass spectrometer (Agilent Company, USA). High-resolution ESIMS data were acquired on a Thermo Electron LTQ-Orbitrap XL mass spectrometer (Thermo Scientific, USA). All reagents and solvents were purchased from commercial sources and were of analytical grade. AB-8 macroporous resin was purchased from Hebei Cangzhou Baoen Chemical Co., Ltd. (China). TLC analysis was performed on polyamide film (Taizhou Luqiao Siqing Biochemical Plastics Factory, China) or GF 254 silica gel (Qingdao Marine Chemical Co., China) sprayed with iodine vapor, 5% ninhydrin, or 5% $FeCl_3$.

General Method for the Synthesis of Tetrahydroisoquinolines 1−14. Dopamine hydrochloride (1.00 g, 5.29 mmol), aldehyde (4.4 mmol), vitamin C (0.40 g, 2.27 mmol), potassium phosphate buffer (1 M, pH 6.0, 50 mL), and boiled distilled water (100 mL) were added sequentially to a round-bottom flask. The mixture was stirred at 40 °C in a water bath under N_2 for 6 h, then extracted with EtOAc (3 × 150 mL). The water layer was subjected to AB-8 macroporous resin column chromatography (4 × 40 cm), eluting with 7 BV of purified water at a high flow rate of 1800 mL/h, then 1 BV of EtOH at a low flow rate of 250 mL/h. The EtOH eluate was concentrated under vacuum, then freeze-dried.

*6,7-Dihydroxy-1-methyl-1,2,3,4-tetrahydroisoquinoline (**1**).* Application of the above method using acetaldehyde (194 μL) gave **1** as a brown powder (196 mg, 25% yield): ^{1}H NMR (DMSO-d_6, 600 MHz) δ 6.49 (s, 1H), 6.40 (1H, s), 3.86 (1H, q, J = 6.6 Hz), 3.06 (1H, m), 2.79 (1H, m), 2.59 (1H, m), 2.46 (1H, m), 1.27 (3H, d, J = 6.6 Hz); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 143.99, 143.82,130.56, 125.06, 115.81, 113.22, 50.90, 41.55, 28.46, 21.93; ESIMS m/z 180.2 $[M + H]^+$.

*6,7-Dihydroxy-1-isobutyl-1,2,3,4-tetrahydroisoquinoline (**2**).* Application of the above method using isobutyraldehyde (480 μL) gave **2** as a green powder (273 mg, 28% yield): ^{1}H NMR (DMSO-d_6, 600 MHz) δ 6.49 (1H, s), 6.44 (1H, s), 3.75 (1H, dd, J = 10.2, 3 Hz), 3.02 (1H, m), 2.79 (1H, m), 2.51 (2H, m), 1.91 (1H, m), 1.54 (1H, m), 1.40 (1H, m), 0.97 (3H, d, J = 6.6 Hz), 0.94 (3H, d, J = 6.6 Hz); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 143.62, 143.58, 131.23, 125.78, 115.93, 113.39, 52.75, 46.32, 40.69, 28.97, 24.58, 24.46, 21.93; ESIMS m/z 222.4 $[M + H]^+$.

*6,7-Dihydroxy-1-phenyl-1,2,3,4-tetrahydroisoquinoline (**3**).* Application of the above method using benzaldehyde (449 μL) gave **3** as a yellow powder (386 mg, 36% yield): ^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.36 (2H, m), 7.29 (3H, m), 6.52 (1H, s), 6.05 (1H, s), 4.86 (1H, s), 3.09 (1H, m), 2.88 (1H, m), 2.79 (1H, m), 2.58 (1H, m); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 146.08, 144.06, 143.39, 129.51, 129.32, 128.41, 127.28, 126.13, 115.76, 114.94, 61.25, 42.44, 28.91; ESIMS m/z 242.4 $[M + H]^+$.

*6,7-Dihydroxy-1-(4-hydroxyphenyl)-1,2,3,4-tetrahydroisoquinoline (**4**).* Application of the above method using 4-hydroxybenzaldehyde (0.538 g) gave **4** as a yellow powder (611 mg, 54% yield): ^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.03 (2H, d, J = 8.4 Hz), 6.68 (2H, d, J = 8.4 Hz), 6.43 (1H, s), 6.00 (1H, s), 4.70 (1H, s), 3.03 (1H, m), 2.81 (1H, m), 2.71 (1H, m), 2.47 (1H, m); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 156.68, 143.95, 143.32, 136.33, 130.25, 126.01, 115.65, 115.12, 114.95, 60.78, 42.54, 28.91; ESIMS m/z 258.2 $[M + H]^+$.

*6,7-Dihydroxy-1-(3-hydroxyphenyl)-1,2,3,4-tetrahydroisoquinoline (**5**).* Application of the above method using 3-hydroxybenzaldehyde (0.538 g) gave **5** as a reddish-brown powder (886 mg, 78% yield): ^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.14 (1H, t, J = 7.8 Hz), 6.76 (1H, d, J = 7.2 Hz), 6.69 (2H, m), 6.50 (1H, s), 6.10 (1H, s), 4.76 (1H, s), 3.08 (1H, m), 2.86 (1H, m), 2.76 (1H, m), 2.54 (1H, m); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 157.53, 147.57, 144.03, 143.35, 129.60, 129.25, 126.06, 120.09, 116.13, 115.69, 114.92, 114.25, 61.23, 42.43, 28.97; ESIMS m/z 258.2 $[M + H]^+$.

*6,7-Dihydroxy-1-(3,4-dihydroxyphenyl)-1,2,3,4-tetrahydroisoquinoline (**6**).* Application of the above method using 3,4-dihydroxybenzaldehyde (0.607 g) gave **6** as a brown-gray powder (809 mg, 67% yield): ^{1}H NMR (DMSO-d_6, 600 MHz) δ 6.71 (1H, d, J = 7.8 Hz), 6.66 (1H, d, J = 1.8 Hz), 6.59 (1H, dd, J = 7.8, 1.8 Hz), 6.48

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(1H, s), 6.10 (1H, s), 4.68 (1H, s), 3.09 (1H, m), 2.84 (1H, m), 2.76 (1H, m), 2.52 (1H, m); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 145.26, 144.65, 143.92, 143.28, 137.04, 130.26, 125.95, 120.16, 116.51, 115.58, 115.27, 114.97, 61.02, 42.59, 28.99; ESIMS m/z 274.3 $[M + H]^+$.

*6,7-Dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-1,2,3,4-tetrahydroisoquinoline (**7**).* Application of the above method using 4-hydroxy-3-methoxybenzaldehyde (0.67 g) gave **7** as a brown powder (1.1 g, 89% yield): ^{1}H NMR (DMSO-d_6, 600 MHz) δ 6.82 (1H, d, J = 1.8 Hz), 6.70 (1H, d, J = 7.8 Hz), 6.61 (1H, dd, J = 7.8, 1.8 Hz), 6.43 (1H, s), 6.04 (1H, s), 4.71 (1H, s), 3.70 (3H, s), 3.06 (1H, m), 2.83 (1H, m), 2.73 (1H, m), 2.48 (1H, m); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 147.72, 145.90, 143.93, 143.27, 136.92, 130.17, 125.96, 121.69, 115.69, 115.17, 114.89, 113.15, 61.26, 56.01, 42.76, 28.94; ESIMS m/z 288.3 $[M + H]^+$.

*6,7-Dihydroxy-1-(4-methoxyphenyl)-1,2,3,4-tetrahydroisoquinoline (**8**).* Application of the above method using 4-methoxybenzaldehyde (534 μL) gave **8** as a brown powder (369 mg, 31% yield): ^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.20 (2H, d, J = 8.4 Hz), 6.92 (2H, d, J = 8.4 Hz), 6.50 (1H, s), 6.05 (1H, s), 4.81 (1H, s), 3.79 (3H, s), 3.08 (1H, m), 2.87 (1H, m), 2.77 (1H, m), 2.53 (1H, m); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 158.60, 143.99, 143.34, 138.22, 130.28, 130.00, 126.11, 115.71, 114.94, 113.76, 60.69, 55.47, 42.55, 28.98; ESIMS m/z 272.4 $[M + H]^+$.

*6,7-Dihydroxy-1-(2-methoxyphenyl)-1,2,3,4-tetrahydroisoquinoline (**9**).* Application of the above method using 2-methoxybenzaldehyde (0.598 g) gave **9** as a yellow powder (474 mg, 40% yield): ^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.30 (1H, td, J = 7.8, 1.2 Hz), 7.10 (1H, d, J = 7.8 Hz), 7.00 (1H, dd, J = 7.8, 1.2 Hz), 6.91 (1H, td, J = 7.8, 1.2 Hz), 6.53 (1H, s), 6.08 (1H, s), 5.34 (1H, s), 3.89 (3H, s), 3.03 (1H, m), 2.91 (1H, m), 2.75 (1H, m), 2.64 (1H, m); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 157.30, 143.98, 143.41, 134.01, 129.96, 129.26, 128.21, 126.50, 120.36, 115.75, 114.83, 111.27, 55.92, 53.65, 41.83, 29.06; ESIMS m/z 272.4 $[M + H]^+$.

*6,7-Dihydroxy-1-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydroisoquinoline (**10**).* Application of the above method using 3,4,5-trimethoxybenzaldehyde (0.86 g) gave **10** as a yellow powder (617 mg, 42% yield): ^{1}H NMR (DMSO-d_6, 600 MHz) δ 6.63 (2H, s), 6.49 (1H, s), 6.13 (1H, s), 4.79 (1H, s), 3.76 (6H, s), 3.69 (3H, s), 3.12 (1H, m), 2.88 (1H, m), 2.81 (1H, m), 2.52 (1H, m); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 152.97, 144.04, 143.32, 136.75, 129.63, 125.91, 115.81, 114.69, 106.37, 61.85, 60.40, 56.24, 43.01, 28.89; ESIMS m/z 332.5 $[M + H]^+$.

*6,7-Dihydroxy-1-(naphthalen-1-yl)-1,2,3,4-tetrahydroisoquinoline (**11**).* 1-Formylnaphthalene (297 μL) was used in the synthesis of **11**. Different from the above method, after the reaction mixture was extracted with EtOAc, the organic layer was concentrated under vacuum and a pale yellow powder precipitated to yield **11** (414 mg, 32% yield): ^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.29 (1H, d, J = 8.4 Hz), 7.89 (1H, d, J = 8.4 Hz), 7.82 (1H, d, J = 8.4 Hz), 7.44 (2H, m), 7.39 (1H, m), 7.32 (1H, m), 6.52 (1H, s), 5.92 (1H, s), 5.47 (1H, s), 3.07 (1H, m), 2.91 (1H, m), 2.85 (1H, m), 2.61 (1H, m); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 144.05, 143.50, 141.26, 134.40, 131.88, 129.93, 128.72, 128.04, 126.35, 126.04, 125.74, 125.69, 125.53, 115.96, 114.26, 60.22, 43.17, 29.14; ESIMS m/z 292.4 $[M + H]^+$.

*1-Benzyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (**12**).* Application of the above general method using phenylacetaldehyde (515 μL) gave **12** as a yellow powder (384 mg, 34% yield): ^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.28 (4H, m), 7.20 (1H, m), 6.63 (1H, s), 6.42 (1H, s), 3.91 (1H, dd, J = 10.2, 3.6 Hz), 2.99 (2H, m), 2.71 (2H, m), 2.50 (1H, m), 2.45 (1H, m); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 143.89, 143.57, 140.37, 130.16, 129.84, 128.57, 126.33, 126.08, 115.91, 113.78, 56.70, 42.73, 40.62, 29.21; ESIMS m/z 256.3 $[M + H]^+$.

*1-(Furan-2-yl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (**13**).* Application of the above general method using furfural (364 μL) gave **13** as a reddish-brown powder (536 mg, 53% yield): ^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.61 (1H, dd, J = 1.8, 0.6 Hz), 6.51 (1H, s), 6.42 (1H, dd, J = 3, 1.8 Hz), 6.36 (1H, s), 6.11 (1H, d, J = 3 Hz), 4.98 (1H, s), 3.00 (1H, m), 2.88 (1H, m), 2.60 (2H, m); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 158.24, 144.45, 143.47, 142.23, 126.56, 126.14, 115.85, 114.60, 110.51, 107.54, 53.67, 40.94, 28.51; ESIMS m/z 232.2 $[M + H]^+$.

*6,7-Dihydroxy-1-(5-hydroxymethylfuran-2-yl)-1,2,3,4-tetrahydroisoquinoline (**14**).* Application of the above general method using 5-hydroxymethylfurfural (364 μL) gave **14** as a brick red powder (669 mg, 58% yield): ^{1}H NMR (DMSO-d_6, 600 MHz) δ 6.44 (1H, s), 6.30 (1H, s), 6.15 (1H, d, J = 3.0 Hz), 5.93 (1H, d, J = 3.0 Hz), 4.86 (1H, s), 4.33 (2H, s), 2.94 (1H, m), 2.80 (1H, m), 2.53 (2H, m); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 157.63, 154.66, 144.43, 143.46, 126.67, 126.24, 115.86, 114.67, 108.12, 107.67, 56.20, 53.91, 40.91, 28.33; ESIMS m/z 262.2 $[M + H]^+$; HRESIMS m/z 262.1078 $[M + H]^+$ (calcd for $C_{14}H_{15}NO_4$, 262.1079).

β_1-, β_2-, and α_{1B}-AR Agonist Activity Assay. This assay was performed according to the protocols of Nanjing GenScript Co. Ltd. (Nanjing City, China).[16] A total of 14 synthesized catecholic THIQs as well as their analogues isolated from the medicinal plant *P. oleracea* (Figure 1) were screened for their β_1-, β_2-, and α_{1B}-AR agonist activities, at the concentration of 100 μM, according to intracellular calcium fluorescence changes in CHO-K1/Gα15/ADRB1 (GenScript, M00269), CHO-K1/Gα15/ADRB2 (GenScript, M00308), and CHO-K1/Gα15/ADRA1B (GenScript, M00260) cell lines that consistently express β_1-, β_2-, and α_{1B}-AR. A 1 μM concentration of isoproterenol (Sigma) was used as the β_2-AR agonist positive control, and 10 μM epinephrine (Sigma) was used as the β_1-AR agonist positive control as well as the α_{1B}-AR agonist positive control. Briefly, cells were cultured in Ham's F12 medium supplemented with 10% fetal bovine serum, 200 μg/mL Zeocin, and 100 μg/mL Hygromycin B and incubated in a CO_2 incubator at 37 °C. After reaching 80% confluency in a 10 cm dish, cells were further subcultured in a 384-well plate (20 μL/well, 1.5×10^4 cells/well). After 18 h of incubation, 20 μL of FLIPR Calcium 4 assay kit solution (Molecular Devices, R8141) was added to the cells, which were further incubated at 37 °C for 1 h. The plate was removed from the CO_2 incubator and equilibrated to room temperature for 15 min. The FLIPR Tetra apparatus (Molecular Devices) was used to determine the relative calcium fluorescence units (RFU values). The detection time was set for 120 s, and 10 μL of the positive control or the tested compound was automatically added to the plate at 21 s. The tested compounds were dissolved in DMSO (Sigma-Aldrich) at concentrations of 100 mM and diluted with Hank's balanced salt solution containing 20 mM HEPES (pH 7.4) before the assay. AR agonist activity was calculated according to the following equation: stimulation rate (%) = $(\Delta RFU_{compound} - \Delta RFU_{background})/(\Delta RFU_{positive\ control} - \Delta RFU_{background}) \times 100\%$. In this equation, the average in the fluorescence units from 1 to 20 s was used as the baseline, and the ΔRFU value, i.e., the relative fluorescence units, was calculated as the maximum fluorescence units from 21 to 120 s minus the baseline. EC_{50} values of the positive control isoproterenol and epinephrine were calculated according to the four-parameter equation Y = Bottom + (Top − Bottom)/(1 + $10^{(\log EC_{50}/IC_{50}-X)HillSlope}$), where X is the log(concentration) and Y is the stimulation activity.

Evaluation of Effects of Catecholic THIQs on the Histamine-Induced Contraction of Isolated Guinea-Pig Tracheal Smooth Muscle. The experiment was performed according to the published protocol with some modifications.[35] All procedures were conducted in compliance with the animal use regulations and were approved by the Shandong University Animal Care and Use Committee. Healthy guinea pigs (SCXK(Lu)20150001) weighing 200−350 g were stunned, and the whole trachea was cut from the subthyroid cartilage to the bifurcation at the lower end of the trachea. Tracheas were immersed in cold Krebs-Henseleit solution with a mixture of 5% CO_2 and 95% O_2. After gently removing the connective tissue around the trachea, the trachea was spirally cut from one end to the other into a spiral strip approximately 3−5 mm wide and approximately 20−30 mm long. Every 2 or 3 cartilage rings were sheared with a helix. Each end of the trachea spiral strip was threaded using a suture needle. One end of the spiral strip was tied to an L-shaped hook, and the spiral strip was carefully placed into a two-chamber organ bath with 20 mL of Krebs-Henseleit solution at 37 °C. The L-shaped hook was fixed on

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the iron bracket with a double concave clamp. Oxygen was continuously injected into the bath through the ventilator. The other end of the spiral strip was connected to the muscle tension transducer. At the beginning of the experiment, the initial load tension of the spiral strip was set to approximately 2 g and was balanced for 60 min. After each test, the spiral strip was washed with Krebs-Henseleit solution three times and balanced for 3 min. The balanced tension was recorded as $Tension_{baseline}$ for each test. Histamine (200 μL, 1×10^{-2} M) was added into the bath at a final concentration of 1.0×10^{-4} M, and the tension reaching the plateau was recorded as $Tension_{histamine}$. A total of 20 μL of tested compound dissolved in DMSO was added to the organ bath (accumulative DMSO concentration was less than 0.5%), and the tension reaching the plateau was recorded as $Tension_{tested\ compounds}$. The bronchodilator effect of the compounds on histamine-induced tracheal contraction in guinea pigs was expressed by spasmolysis percentage and calculated according to the equation: spasmolysis percentage (%) = $(Tension_{histamine} - Tension_{tested\ compounds})/(Tension_{histamine} - Tension_{baseline}) \times 100\%$. The effects versus concentration curves of the tested compounds were plotted. To explore the potential target of tracheal relaxation of the compounds, propranolol (with a final concentration of 1.0×10^{-7} M in the bath), a β-AR antagonist, was added into the bath for 15 min before the addition of histamine, and the compounds were added as described above. The bronchodilator effect of the compounds after the addition of propranolol was also calculated according to the above equation.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.9b00418.

Figures S1.1−S14.4: ESIMS, ^{1}H NMR, and ^{13}C NMR spectra of compounds **1**−**14**; optical rotations of compounds **1**−**14** were zero (PDF)

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Notes

The authors declare no competing financial interest.

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