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In Vitro Evaluation of Potential Bitterness-Masking Terpenoids from the Canada Goldenrod (*Solidago canadensis*)

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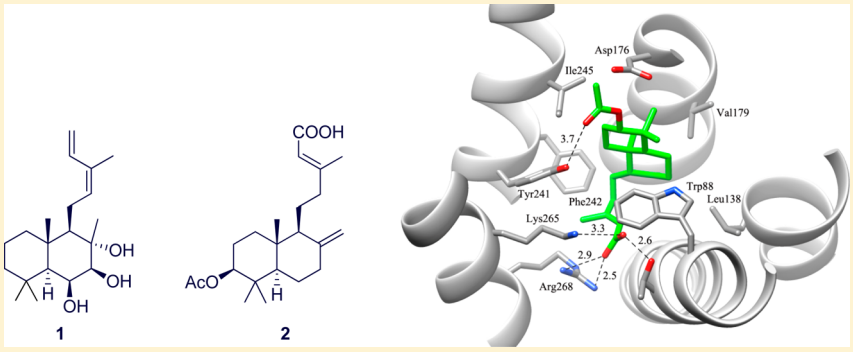

ABSTRACT: In a screening of extracts of selected plants native to Ohio against the human bitterness receptor hTAS2R31, a chloroform-soluble extract of the aerial parts of *Solidago canadensis* (Canada goldenrod) was determined to have hTAS2R31 antagonistic activity and, thus, was fractionated for isolation of potential bitterness-masking agents. One new labdane diterpenoid, solidagol (**1**), and six known terpenoids, including two labdane diterpenoids (**2** and **3**), three clerodane diterpenoids (6*β*-angeloyloxykolavenic acid, 6*β*-tigloyloxykolavenic acid, and crotonic acid), and a triterpenoid (longispinogenin), were isolated. Among these compounds, 3*β*-acetoxycopalic acid (**2**) was found to be the first member of the labdane diterpene class shown to have inhibitory activity against hTAS2R31 activation (IC_{50} 8 *μ*M). A homology model of hTAS2R31 was constructed, and the molecular docking of **2** to this model indicated that this diterpenoid binds well to the active site of hTAS2R31, whereas this was not the case for the closely structurally related compound **3** (sempervirenic acid). The content of **2** in the chloroform-soluble portion of the methanolic extract of *S. canadensis* was up to 2.24 g/100 g dry weight, as determined by HPLC.

A bitter taste is generally considered to be undesirable in the majority of foods, because many naturally occurring toxic compounds induce a bitter-taste response.[1,2] On the other hand, bitterness may also serve as a marker for medicinal efficacy. Many potentially beneficial constituents in certain vegetables and herbal medicines, such as polyphenols, flavonoids, terpenoids, and glucosinolates, are described as being bitter.[3] The aversion to bitter tastants is an inherent instinct of humans that may lead to the avoidance of toxins, but it can also cause negative health effects. Perhaps the most notable of these negative health effects is poor patient compliance due to the bitterness of some medicines.[4] Additional aspects include the rejection of healthy foods due to bitter-tasting constituents and avoidance of low-calorie foodstuffs due to the inherent bitterness of some high-potency sweeteners.[5] The use of fats, salt, and sugar to mask the bitterness of foodstuffs further leads to health problems associated with the avoidance of bitter-tasting substances.[5]

One potential method of developing palatable foods with beneficial secondary metabolites is to mask their bitter off-tastes with bitterness-masking compounds at concentrations below the taste threshold of the masking compounds. Human bitter taste is mediated by the hTAS2R family of G protein-coupled receptors. The discovery of human bitter taste receptors (hTAS2Rs) and recent development of high-throughput screening methods for hTAS2R antagonists have enabled the development of potential bitterness-masking agents.[6−9] In a screening of selected plants native to Ohio against the human bitterness receptor hTAS2R31 (formerly hTAS2R44), a

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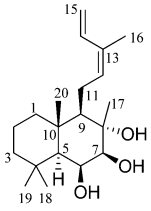
chloroform-soluble extract of *Solidago canadensis* L. (Asteraceae) was determined to have hTAS2R31 antagonistic activity (IC_{50} <25 μg/mL) and, thus, was fractionated for isolation of potential bitterness-masking agents. The Canada goldenrod, *S. canadensis*, has had historical medicinal uses for the treatment of fever, gastrointestinal ailments, inflammation, and symptoms associated with diabetes.[10,11] This plant produces secondary metabolites representing a variety of structural classes, inclusive of flavonoids, phenolics, and terpenoids.[10]

In the present investigation, a new labdane diterpenoid, solidagol (**1**), as well as two labdane diterpenoids (**2** and **3**), three clerodane diterpenoids (6β-angeloyloxykolavenic acid, 6β-tigloyloxykolavenic acid, and crotonic acid), and a triterpenoid (longispinogenin) were isolated from the chloroform-soluble extract of *S. canadensis* via bioactivity-guided fractionation. These compounds were individually tested for their antagonistic activity against the hTAS2R31 human bitterness receptor, using a standard protocol.



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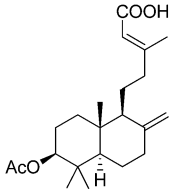

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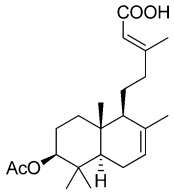

3

Solidagol (**1**) was determined to have a molecular formula of $C_{20}H_{34}O_3$ based on the ^{13}C NMR spectroscopic data and the $[M + Na]^+$ ion peak at *m/z* 345.2395 (calcd 345.2406) in the HRESIMS. The IR spectrum showed hydroxy group (3427 cm^{-1}) and double-bond (1710 cm^{-1}) absorptions. The ^1H NMR spectrum of compound **1** exhibited resonances for four tertiary methyl groups (δ_H 0.99, H-18; 1.21, H-19; 1.21, H-20; 1.36, H-17, each 3H, s), three aliphatic methylenes (δ_H 0.90 and 1.63, each 1H, m, H-1; 1.45 and 1.70, each 1H, m, H-2; 1.17 and 1.42, each 1H, m, H-3), one allylic methylene (δ_H 2.27, 1H, ddd, *J* = 15.5, 6.9, 4.6 Hz, H-11a; 2.56, 1H, ddd, *J* = 15.5, 7.5, 7.1 Hz, H-11b), two aliphatic methines (δ_H 0.97, 1H, brs, H-5; 1.32, 1H, dd, *J* = 7.1, 4.6 Hz, H-9), and two oxygenated methines (δ_H 3.42, 1H, d, *J* = 3.4 Hz, H-7; 4.41, 1H, brd, *J* = 3.4 Hz, H-6), while resonances at δ_H 1.80 (3H, s, CH_3, H-16), 5.14 (1H, d, *J* = 10.8 Hz, H-15a), 5.22 (1H, d, *J* = 17.3 Hz, H-15b), 5.49 (1H, dd, *J* = 7.5, 6.9 Hz, H-12), and 6.89 (1H, dd, *J* = 17.3, 10.8 Hz, H-14) were attributed to a 2-methylbuta-1,3-dien-1-yl side chain. The ^{13}C NMR spectrum of **1** showed 20 carbon resonances, which were classified from the DEPT and HSQC data as five methyl carbons, three quaternary carbons, two tertiary sp^3 carbons, two tertiary sp^2 carbons, four secondary sp^3 carbons, one secondary sp^2 carbon, and three oxygen-bearing sp^3 carbons (including two secondary and one tertiary). The characteristic NMR data of **1** were closely comparable to those of crotomachlin, a labdane diterpenoid previously isolated from *Koanophyllon conglobatum*[12] and *Croton machrostachys*.[13] On comparison of the 1D- and 2D-NMR data (Figures 1 and 2) of these compounds, it was revealed that only one difference was evident in the double-bond configuration of the 2-methylbuta-1,3-dien-1-yl side chain located at C-11, with a (12*E*)-2-methylbuta-1,3-dien-1-yl group in crotomachlin being replaced by a (12*Z*)-2-methylbuta-1,3-dien-1-yl moiety in **1**. Thus, when compared to crotomachlin, H-12 in **1** showed a shielding of 0.1 ppm, while H-14 and H-15 in **1** exhibited deshieldings of 0.55 and 0.22 ppm, respectively.

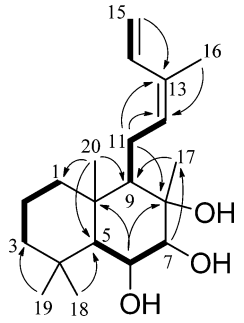

Figure 1. ^1H–^1H COSY (bold lines) and key HMBC (arrows) correlations of solidagol (**1**).

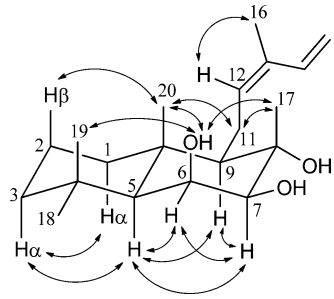

Figure 2. Key NOESY correlations of solidagol (**1**).

Correspondingly, C-12 and C-14 in **1** showed shieldings of 2.0 and 8.0 ppm, respectively, while C-15 and C-16 in **1** showed deshieldings of 3.6 and 8.1 ppm, respectively.[12,13] The *Z* configuration of the double bond between C-12 (δ_C 133.6) and C-13 (δ_C 131.3) was confirmed by the NOESY correlation (Figure 2) between H-12 and H-16. The (12*Z*)-2-methylbuta-1,3-dien-1-yl side chain was placed at C-11 (δ_C 22.6) based on the HMBC correlations of H-11 to C-12 and C-13. The *Z*-type conjugated diene side chain has been observed occasionally for other labdane diterpenoids, e.g., austroilunin[14] and (12*Z*)-abienol.[15] The consistent chemical shifts and coupling constants (Table 1) of the decalin resonances between **1** and crotomachlin, along with the same positive sign of optical rotation for both compounds as well as the NOESY experiment, provided evidence that **1** shares the same stereochemistry at C-5 to C-10 as that of crotomachlin, the absolute configuration of which was established using both spectroscopic and synthetic methods.[12,13] Biogenetically, Me-20 is β-oriented and H-5 is α-oriented in labdane-type diterpenoids.[16] The small proton coupling constant of H-6 (*J* = 3.4 Hz) indicated it is equatorial. Furthermore, the NOESY correlations between H-5/H-6, H-5/H-7, H-5/H-9, H-6/H-7, H-7/H-9, OH-6/Me-17, OH-6/Me-19, and OH-6/Me-20 were used to establish the α-orientation of these protons and the β-orientation of OH-6, while the NOESY cross-peaks between H-11/Me-17 and H-11/Me-20 demonstrated that the C-11 methylene and C-17 methyl groups are β-oriented. Therefore, compound **1** was established as (5*S*,6*S*,7*S*,8*S*,9*R*,10*S*)-labda-12*Z*,14-diene-6,7,8-triol and was accorded the trivial name solidagol.

On the basis of the physical and spectroscopic data measurements ($[\alpha]_D$, ^1H NMR, ^{13}C NMR, DEPT, 2D-NMR, and MS) and the comparison of the data obtained with published values, the structures of the known compounds were identified as 3β-acetoxycopalic acid (**2**),[17] sempervirenic acid

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Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR Data for Solidagol (1) in $CDCl_3$

no.	δ_C, type	δ_H, mult. (J in Hz)
1α	42.3, CH_2	0.90, m
1β		1.63, m
2α	18.8, CH_2	1.45, m
2β		1.70, m
3α	43.7, CH_2	1.17, m
3β		1.42, m
4	34.3, C	
5	55.7, CH	0.97, brs
6	70.9, CH	4.41, brd (3.4)
7	80.5, CH	3.42, d (3.4)
8	77.4, C	
9	60.6, CH	1.32, dd (7.1, 4.6)
10	39.6, C	
11a	22.6, CH_2	2.27, ddd (15.5, 6.9, 4.6)
11b		2.56, ddd (15.5, 7.5, 7.1)
12	133.6, CH	5.49, dd (7.5, 6.9)
13	131.3, C	
14	133.5, CH	6.89, dd (17.3, 10.8)
15a	114.3, CH_2	5.14, d (10.8)
15b		5.22, d (17.3)
16	20.1, CH_3	1.80, s
17	19.7, CH_3	1.36, s
18 (αCH_3)	33.5, CH_3	0.99, s
19 (βCH_3)	24.1, CH_3	1.21, s
20	16.9, CH_3	1.21, s
OH-6		2.02, s

(**3**),[18] 6β-angeloyloxykolavenic acid,[19] 6β-tigloyloxykolavenic acid,[19] crotonic acid,[20] and longispinogenin.[21]

In the present study, the bitterness-masking activity of all the compounds isolated was evaluated in vitro by measuring receptor-dependent calcium flux in human embryonic kidney (HEK-293T) host cells transfected with the human bitterness receptor hTAS2R31 and stably expressing the chimeric G-protein α-subunit $G_{\alpha 16gust44}$. Among these isolates, compound **2** was determined to be active with an IC_{50} value of 8 μM. Some structurally similar terpenoids of **2** have been previously reported to possess binding affinity to hTAS2R46,[6,8] and several clerodane and *ent*-kaurane diterpenes, e.g., hardwickiic acid, rebaudioside B, as well as their analogues, were recently patented for their effect in blocking the activation of the bitterness receptor by rebaudioside A.[22,23] However, compound **2** is the first member of the labdane diterpene class shown to exhibit inhibitory activity against hTAS2R31 activation. In contrast, compound **3**, a closely related labdane diterpene, showed no discernible hTAS2R31 antagonistic activity (IC_{50} >25 μM).

This interesting observation motivated an investigation of the interaction modes of compounds **2** and **3** with hTAS2R31, using a molecular modeling and docking method. Since the structural requirements of human bitter taste receptor activation have been recently revealed[7] and the sequences of hTAS2R31 have been aligned with those of human β2-adrenergic receptor,[9] a homology-based three-dimensional model of hTAS2R31 was constructed based on the known crystal structure of human β2-adrenergic receptor. Thus, compound **2** was docked into the active site of hTAS2R31 using the GOLD 3.01 docking program. The hypothetical binding mode of compound **2** in the active site of hTAS2R31 is shown in Figure 3a. According to the docking results, the decalin core of compound **2** is situated in a hydrophobic pocket

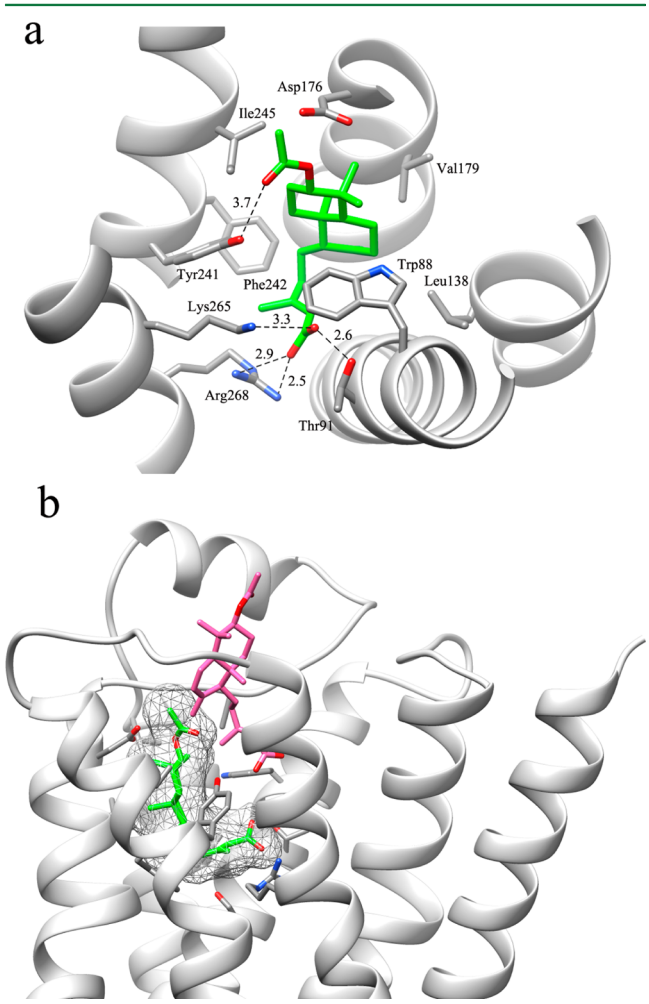

Figure 3. (a) Hypothetical binding mode of compound **2** (green) in the active site of hTAS2R31. The potential hydrogen bonding and salt bridge interactions are indicated with dashed lines. The distances are labeled in angstroms. (b) Hypothetical binding mode of compound **2** (green) in comparison with compound **3** (pink). The surface of compound **2** is displayed with mesh to show the shape of the ligand binding pocket.

surrounded by Phe242, Ile245, Val179, Leu138, and Trp88 amino acid residues. The acetyl group points toward the solvent-accessible surface and hydrogen bonds with the side chain of Tyr241. A previous study has already demonstrated the importance of Lys265 and Arg268 in the interaction between aristolochic acid and hTAS2R31,[7] and a mutagenesis assay was used to prove the involvement of these two basic residues in the antagonistic activity of 4-(2,2,3-trimethylcyclopentyl)-butanoic acid, a synthetic compound known as GIV3727.[9] In the present study, the carboxy side chain of compound **2** was shown to protrude deeply into the active site, forming hydrogen bonds with both Lys265 and Thr91. A salt bridge interaction was also observed between the carboxy group and Arg268. Therefore, the docking experiment indicated that compound **2** binds well to hTAS2R31. In contrast, compound **3** is unable to occupy the same binding pocket as does compound **2**, and it only binds to the outer loop region of hTAS2R31, as shown in Figure 3b. This difference might be

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due to the incorporation of the double bond in the decalin ring of compound **3**, which would influence the conformation of the ring and also change the orientation of the carboxy group.

Among the terpenoids isolated in the present investigation, the active compound **2** was obtained from its source plant in the highest abundance. In order to determine the content of this compound in the *S. canadensis* sample used for isolation, an HPLC-DAD method with MS identification was developed and validated (Figure S2a and b, Supporting Information). The content of **2** was determined to be 44.8 mg/100 g dry weight of the aerial parts of *S. canadensis*. In addition, the hexanes, chloroform, ethyl acetate, and 1-butanol partitions were also analyzed using this method, and compound **2** was detected only in the chloroform partition, accounting for 2.24% of the dry weight of this partition, suggesting that *S. canadensis* might be a potential source for the development of bitterness-masking dietary supplements or agents.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a PerkinElmer 343 automatic polarimeter (PerkinElmer, Waltham, MA, USA). UV spectra were run on a Hitachi U-2910 spectrophotometer (Hitachi, Tokyo, Japan). IR spectra were obtained on a Nicolet 6700 FT-IR spectrometer (Thermo Scientific, Waltham, MA, USA). NMR spectroscopic data were recorded at room temperature on Bruker Avance DRX-300 and 400 MHz spectrometers (Bruker, Billerica, MA, USA). HRESI mass spectra were obtained on an LCT-TOF mass spectrometer (Waters Corp., Milford, MA, USA) operated in the positive-ion mode with NaI being used for mass calibration. Column chromatography was performed with Sephadex LH-20 (Supelco, Bellefonte, PA, USA) and 65 × 250 and 230 × 400 mesh silica gel (Sorbent Technologies, Atlanta, GA, USA). Analytical thin-layer chromatography was conducted on precoated 250 μm thick Partisil Si gel 60F_{254} glass plates (Whatman, Clifton, NJ, USA). An XBridge PrepC_{18} column (5 μm, 150 mm × 19 mm i.d., Waters) was used for semipreparative HPLC, along with a Hitachi system composed of an L-2130 pump and an L-2450 diode array detector. Solvents used for chromatographic separations were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Other solvents were purchased from Sigma-Aldrich (St. Louis, MO, USA) and were of chemical grade.

Plant Material. The air-dried aerial parts of *Solidago canadensis* L. (Asteraceae) were collected in Wayne Township, Butler County, Ohio (39.492061 N; −84.537746 W; 209 m alt.), by M.A.V., in October 2010, who also identified this plant. A voucher specimen (MU 271438) has been deposited in the W.S. Turrell Herbarium of Miami University, Oxford, Ohio.

Extraction and Isolation. The air-dried aerial parts of *S. canadensis* (1 kg) were ground and extracted exhaustively with MeOH. This dried MeOH extract (72 g) was then partitioned between 9:1 MeOH−H_2O and hexanes. The MeOH−H_2O layer was evaporated to a thick tar and digested sequentially with $CHCl_3$, EtOAc, and 1-butanol. The resulting $CHCl_3$-soluble extract was partially detannified with 1% NaCl in H_2O to give 22 g of a final $CHCl_3$-soluble extract. This extract showed hTAS2R31 antagonistic activity (IC_{50} <25 μg/mL) and thus was subjected to chromatography over coarse silica gel, eluted with a CH_2Cl_2−acetone gradient (40:1, 15:1, 10:1, 8:1, 6:1, 4:1, 3:1, 2:1, 1:1, 1:2, and pure acetone), to afford 16 fractions (F01−F16), with the active fractions F02, F06, and F08 (IC_{50} <25 μg/mL) being further purified. Fraction F02 was purified again on a silica gel column, using a $CHCl_3$−MeOH gradient solvent system (50:1, 40:1, 30:1, 20:1, 15:1, 12:1, 10:1, 8:1, 6:1, and 5:1), to yield the triterpenoid longispinogenin (eluted with $CHCl_3$−MeOH, 30:1; 18.2 mg). Fraction F06 (4.6 g) was chromatographed further over a silica gel column with a $CHCl_3$−MeOH solvent system (50:1, 40:1, 30:1, 20:1, 15:1, 12:1, 10:1, 8:1, 6:1, 5:1, 3:1, 2:1, and 1:1) to give 12 subfractions (F0601−F0612). F0602 (eluted with $CHCl_3$−MeOH, 30:1; 108 mg) was further purified by HPLC, using an XBridge PrepC_{18} column (5 μm, 150 mm × 19 mm i.d.) with CH_3CN−H_2O [55:45, containing 0.05% trifluoroacetic acid (TFA) in H_2O] at a flow rate of 10.0 mL/min, to yield 6β-angeloyloxykolavenic acid (t_R = 122.5 min; 6.6 mg) and 6β-tigloyloxykolavenic acid (t_R = 92.0 min; 10.8 mg). F0604 (eluted with $CHCl_3$−MeOH, 20:1; 980 mg) was purified by Sephadex LH-20 column chromatography, with elution by $CHCl_3$−MeOH (60:40 40:60, 20:80, and pure MeOH), to afford a further subfraction, F060408. This subfraction (157 mg) was then purified using the same HPLC column with 48% CH_3CN−52% H_2O containing 0.05% TFA, at a flow rate of 10.0 mL/min, to give compounds **2** (t_R = 106.5 min; 28.5 mg) and **3** (t_R = 103.0 min; 3.4 mg). Fraction F08 (898 mg) was applied to a silica gel column, eluted by a $CHCl_3$−MeOH solvent system (30:1, 20:1, 15:1, 12:1, 10:1, 8:1, 6:1, 5:1, 3:1, 2:1, and 1:1), to give six subfractions (F0801−F0806). F0804 (eluted with $CHCl_3$−MeOH, 15:1; 96 mg) was further purified by HPLC on the same column, using an isocratic elution (CH_3CN−H_2O, 50:50, flow rate 8.0 mL/min), to afford **1** (t_R = 25.8 min; 4.4 mg) and crotonic acid (t_R = 42.7 min; 2.4 mg).

Solidagol (1): colorless, amorphous solid; $[\alpha]^{20}_D$ +25 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 237 (3.74) nm; IR (film) ν_{max} 3427, 2925, 2863, 2848, 1710, 1559, 1459, 1439, 1386, 1363, 1216, 1109, 1089, 1046, 909, 757 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m*/*z* 345.2395 $[M + Na]^+$ (calcd for $C_{20}H_{34}O_3Na$, 345.2406).

hTAS2R31 Antagonist Screening Assay. This bioassay was conducted according to a literature procedure.[6,9] In brief, the stable cell line for human bitterness receptor hTAS2R31 was generated by subcloning the sst3:TAS2R:hsv cassette into the pcDNA3.1/Zeo(+) mammalian expression vector (Invitrogen, San Diego, CA, USA), and the resulting construct was transfected into the HEK-293T host cells that stably express the chimeric G-protein subunit $G_{\alpha 16gust44}$. The stable $G_{\alpha 16gust44}$/HEK-293T cells transfected with hTAS2R31 were selected and preplated at a density of 15 000 cells per well in black 96-well plates that had been precoated with 0.001% poly(ethyleneimine) (Acros Organics, Morris Plains, NJ, USA). After 48 h, the growth medium was discarded and the cells were incubated in the dark for 1 h at 37 °C in 50 μL of loading buffer consisting of 1.5 μM Fluo-4 AM (Invitrogen) and 2.5 μM probenecid (Sigma-Aldrich) in Dulbecco's modified Eagle medium (DMEM) without fetal bovine serum (FBS). After incubation, the plates were washed five times with 100 μL of assay buffer each time and further incubated in the dark at room temperature for 30 min. The cells were then washed again five times using 100 μL of assay buffer each time, followed by the calcium responses measurement in a fluorescence imaging plate reader (FLIPR^{TETRA}) (Molecular Devices, Sunnydale, CA, USA). Saccharin was employed as the cognate bitter receptor agonist for inhibitor screening. Test compounds were prepared at a final concentration of 25 μM in the presence of 1 mM sodium saccharin and assessed in an initial screening to determine if they inhibit the hTAS2R31 response to saccharin. Test compounds were also evaluated for inhibition of a non-taste GPCR pathway (isoproterenol, β1/β2-adrenergic receptor agonist) to ensure that any apparent inhibitory activity was bitterness receptor-dependent and not simply due to nonspecific effects on intracellular calcium handling. To assess the potency of the selectively active compounds (greater than 50% inhibition at 25 μM in the initial screening), the half-maximal inhibitory concentration (IC_{50}) was determined via at least three separate experiments in duplicate. For calculation of IC_{50} values, the data were fitted in GraphPad Prism using a four-parameter logistic fit equation.

Homology Modeling. The sequence of hTAS2R31 was obtained from UniProtKB (entry P59538). The crystal structure of the β2-adrenergic receptor (PDB code: 2RH1) was selected as the template. The sequence alignment between hTAS2R31 and human β2-adrenergic receptor was directly taken from the literature.[9] Based on the alignment, 10 models were generated using the "automodel" protocol of Modeler 9v4[24] with the optimization level set to "high". The best model according to the internal scoring function was selected for further model validation. A Ramachandran plot generated with Procheck indicated that most of the residues had Φ and Ψ angles in the most favored (94.9%) or allowed regions (4%), and only a few

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residues were in the generously allowed (0.4%) or disallowed regions (0.7%).

Molecular Docking. The structures of compounds **2** and **3** were constructed and fully energy minimized with Sybyl 7.1 software. The energy-minimized structures were docked into the hTAS2R31 homology model using the protein−ligand docking program GOLD 3.01 with default parameters. The ligand binding pocket was defined by a sphere that centered at the amino nitrogen atom of Lys265 and had a radius of 12 Å. The best docking poses were selected based on the GOLD fitness score and the proximity of the ligand carboxy group to Lys265 and Arg268.

Analytical Method for the Content Determination of the Active Compound 2 in *S. canadensis* Aerial Parts. The MeOH extract of the accurately weighed aerial parts of *S. canadensis* (air-dried, 0.25 g) was subjected to passage over a Waters Sep-Pak Vac 20 cm^3 C_{18} cartridge to remove the polar substances present (eluted by 10% MeOH−90% H_2O). The pretreated extract (eluted by 95% MeOH−5% H_2O) was dried and transferred into a 25 mL volumetric flask and made up to the full volume with MeOH to prepare the extract solution. The reference compound **2** was accurately weighed and dissolved in MeOH to produce the standard stock solution. This stock solution was diluted to yield a series of standard solutions in the concentration range 2−250 mg/L. The HPLC separation was performed on an XBridge C_{18} analytical column (5 μm, 150 mm × 4.6 mm i.d., Waters), along with a Hitachi system composed of an L-2130 pump and an L-2450 diode array detector. The mobile phase consisted of 0.05% TFA in H_2O (A) and MeCN (B) using a gradient program of 48−50% B from 0 to 26 min, 50−60% B from 26 to 28 min, 60% B from 28 to 50 min, and 60−100% B from 50 to 60 min. The mobile phase flow rate was 1 mL/min, and the column temperature was set at 30 °C. After comparing the chromatograms of the extract solution recorded at wavelengths within 200−365 nm, it was found that 231 nm could best represent the profile of the analyte. Compound **2** in the extract was identified by comparing its retention time and UV spectrum with those of the standard and was confirmed by its molecular weight in the MS (Figure S2a and b, Supporting Information). Method validation parameters included linearity, reproducibility, precision, and recovery. The five-point calibration curve of compound **2** was linear (y = 49277x + 9764; R^2 = 0.9996) over the range 2−250 mg/L. The limit of detection (LOD) was 1.8 ng (signal-to-noise ratio = 3). Method reproducibility was evaluated by five replicated analyses of the extract solution (n = 5), with a relative standard deviation (RSD) value of 2.84% achieved. Method precision was investigated by repeatedly analyzing the same set of compound **2** standard solution (n = 5), and the RSD of the calculated concentration was 1.88%. Recovery of compound **2** was determined using spiked samples with 100% of the quantified level of compound **2** in five replicated samples. The recovery rate was 96.24% (RSD 3.88%).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H NMR, ^{13}C NMR, ^{13}C DEPT 135 NMR, ^{1}H−^{1}H COSY, HSQC, HMBC, NOESY, HRESIMS, IR, and UV spectra for compound **1**. HPLC chromatograms of the MeOH extract of *S. canadensis* and compound **2** standard along with the relevant MS and UV spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ DEDICATION

This article is dedicated to the memory of our esteemed colleague Dr. Zhonghua Jia.

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