Amide Alkaloids from *Scopolia tangutica*

Authors Zhen Long[1]*, Yan Zhang[2]*, Zhimou Guo[1], Lien Wang[2], Xingya Xue[1], Xiuli Zhang[1], Shisheng Wang[3], Zhiwei Wang[2], Olivier Civelli[2], Xinmiao Liang[1]

Affiliations
[1] Key Laboratory of Separation Science for Analytical Chemistry, Key Lab of Natural Medicine, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, People's Republic of China
[2] Department of Pharmacology, University of California, Irvine, California, United States
[3] School of Pharmaceutical Science and Technology, Dalian University of Technology, Dalian, People's Republic of China

Key words
- *Scopolia tangutica*
- Solanaceae
- alkaloid
- hydroxycinnamic acid amide
- μ-opioid receptor

received February 22, 2014
revised July 3, 2014
accepted July 9, 2014

Bibliography
DOI http://dx.doi.org/10.1055/s-0034-1382961
Published online August 15, 2014
Planta Med 2014; 80: 1124–1130 © Georg Thieme Verlag KG Stuttgart · New York · ISSN 0032-0943

Correspondence
Xinmiao Liang
Key Laboratory of Separation Science for Analytical Chemistry
Dalian Institute of Chemical Physics
Chinese Academy of Sciences
457 Zhongshan Road
Dalian 116023
People's Republic of China
liangxm@dicp.ac.cn

Correspondence
Xiuli Zhang
Key Laboratory of Separation Science for Analytical Chemistry
Dalian Institute of Chemical Physics
Chinese Academy of Sciences
457 Zhongshan Road
Dalian 116023
People's Republic of China
zhangxiuli@dicp.ac.cn

Abstract

Four new hydroxycinnamic acid amides, scotanamines A–D (**1**–**4**), and seven known alkaloids, including N^1,N^{10}-di-dihydrocaffeoylspermidine (**5**), scopolamine (**6**), anisodamine (**7**), hyoscyamine (**8**), anisodine (**9**), caffeoylputrescine (**10**), and N^1-caffeoyl-N^3-dihydrocaffeoylspermidine (**11**), were obtained from the roots of *Scopolia tangutica*. The present study represents the first recognition of hydroxycinnamic acid amides containing putrescine or spermidine in *S. tangutica*. Compound **1**, in particular, contains a moiety resulting from the condensation of nortropinone and putrescine. Compound **2** exhibited moderate agonist activity at the μ-opioid receptor (EC_{50} = 7.3 µM). Compound **2** was tested *in vivo* and induced analgesia in mice. The analgesic effect was recorded using the tail-flick assay and was reversed by naloxone.

Abbreviations

FLIPR:	fluorometric imaging plate reader assay
HCA:	hydroxycinnamic acid
IR:	infrared
SCX:	strong cation exchange
SPE:	solid-phase extraction
TCM:	traditional Chinese medicine

Supporting information available at http://www.thieme-connect.de/products

Introduction

Alkaloids and alkaloid-producing plants are well known for their broad range of bioactivities [1–6]. As a part of our ongoing work on identifying new analgesic compounds from TCMs, we recently became interested in *Scopolia tangutica* Maxim (Solanaceae), a TCM that has been used for centuries as an analgesic by the native people on the Qinghai-Tibetan Plateau of mainland China. This herb produces high levels of tropane alkaloids [1, 7–10], including hyoscyamine [11], anisodine [12], anisodamine [12–14], and scopolamine [15]. These compounds primarily affect the parasympathetic nervous system and act as anticholinergic agents. In recent years, several studies have been conducted on the tropane alkaloid components of this plant [16, 17], while studies on the other constituents and their potential analgesic properties remain scarce. In order to foster a better understanding of the pharmaceutical effects of *S. tangutica* and in pursuit of the discovery of novel analgesics, a systematic investigation was carried out on this herb. We describe herein the purification and structure determination of four new hydroxycinnamic acid (HCA) amides (**1**–**4**) and seven known alkaloids (**5**–**11**) from *S. tangutica*. Given that opioid receptor agonists are well known to induce analgesia, all alkaloids obtained in this study were tested for their ability to interact with the opioid receptors.

Results and Discussion

The air-dried roots of *S. tangutica* were extracted with a mixture of EtOH and H_2O (95/5, v/v). The resulting alkaloids were enriched by SPE and then purified by reverse-phase liquid chromatography and ion exchange liquid chromatography. Four new (**1**–**4**; ◉ **Fig. 1**) and seven known alkaloids (**5**–**11**; ◉ **Fig. 1**) were subsequently isolated and characterized. The known alkaloids were identi-

* These authors contributed equally to this work.

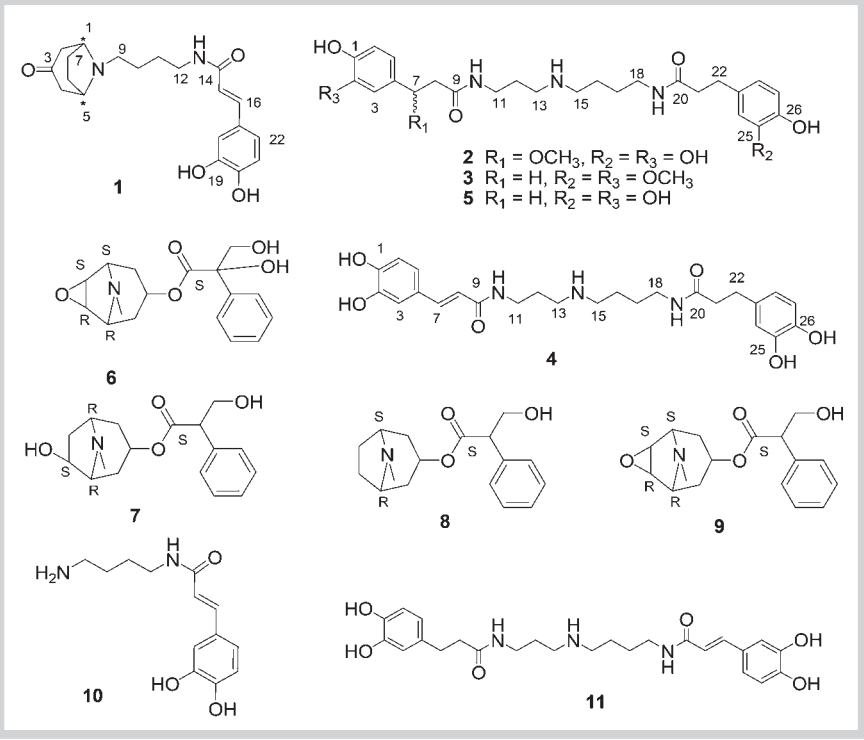

Fig. 1 Structures of compounds from the roots of *S. tangutica*.

Table 1 ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) spectroscopic data for compound **1** formate salt (DMSO-d_6).

Position	δ_H (*J* in Hz)	δ_C, type	Position	δ_H (*J* in Hz)	δ_C, type
1/5	3.67, m	58.8, CH	14		165.8, C
2a/4a	2.13[a]	46.7, CH_2	15	6.36 d (15.6)	119.1, CH
2b/4b	2.76[a]				
3		208.2, C	16	7.22 d (15.6)	139.4, CH
7a/8a	2.03, m	27.4, CH_2	17		126.8, C
7b/8b	1.54, m				
9	2.73[a]	49.3, CH_2	18	6.98 d (1.8)	114.3, CH
10	1.61, m	25.2, CH_2	19		146.0, C
11	1.54, m	27.5, CH_2	20		147.7, C
12	3.20, dd (12.6, 6.6)	38.8, CH_2	21	6.74 d (8.4)	116.2, CH
13	8.07, t (6.0)	NH	22	6.82 dd (8.4, 1.8)	120.8, CH

[a] Signal partially overlapped

fied as N^1,N^{10}-di-dihydrocaffeoylspermidine (**5**) [18], scopolamine (**6**) [19], anisodamine (**7**) [19], hyoscyamine (**8**) [20], anisodine (**9**) [21], caffeoylputrescine (**10**) [22–24], and N^1-caffeoyl-N^3-dihydrocaffeoylspermidine (**11**) [2], based on their spectroscopic data. The structures of these alkaloids are shown in ◉ **Fig. 1**.

Compound **1**, a white powder, was obtained as the corresponding formate. Its molecular formula was determined as $C_{20}H_{26}N_2O_4$ by ^{13}C NMR spectroscopic and HRESIMS data in the positive ion mode, which gave an *m/z* ion, 359.1954 for $[M + H]^+$ (calcd. for $[M + H]^+ = 359.1971$). Consideration of the ^{13}C NMR and DEPT spectra of **1** indicated that the compound contained nine indices of hydrogen deficiency. The IR spectrum indicated the presence of hydroxyl (3390 cm^{-1}), carbonyl (1727 cm^{-1}), and amide (1656 cm^{-1}) groups. The ^{13}C NMR and DEPT data (◉ **Table 1**) revealed the presence of 20 carbon resonances in total (three of which were equivalent), including two carbonyl carbons, two oxygenated aromatic carbons, one aromatic quaternary carbon, three aromatic methine carbons, two nitrogen-bearing methylene carbons, two nitrogen-bearing methine carbons, one olefinic bond, and six methylene carbons. The ^1H NMR data revealed a typical ABX-type coupling pattern for the aromatic protons H-18 (δ_H 6.98, d, J = 1.8 Hz), H-21 (δ_H 6.74, d, J = 8.4 Hz), and H-22 (δ_H 6.82, dd, J = 8.4, 1.8 Hz), indicating the presence of a 1,2,4-trisubstituted benzene ring. The HMBC spectrum revealed the presence of long-range ^1H-^{13}C couplings from H-16 (δ_H 7.22, d, J = 15.6 Hz) to C-17 (δ_C 126.8), C-18 (δ_C 114.3), and C-22 (δ_C 120.8), as well as correlations between H-21 and C-19 (δ_C 146.0) and C-17. Correlations were also observed from H-18 and H-22 to C-20 (δ_C 147.7). Collectively, these findings indicated the presence of a 4-substituted catechol moiety, as shown in ◉ **Fig. 2**. The HMBC spectrum revealed correlations from the mutually coupled H-16 and H-15 (δ_H 6.36, d, J = 15.6 Hz) to the C-14 amide carbonyl carbon (δ_C 165.8), establishing the connection between C-15 and C-14. ^1H-^1H COSY interactions were observed between H_2-9 (δ_H 2.73), H_2-10 (δ_H 1.61), H_2-11 (δ_H 1.54), and H_2-12 (δ_H

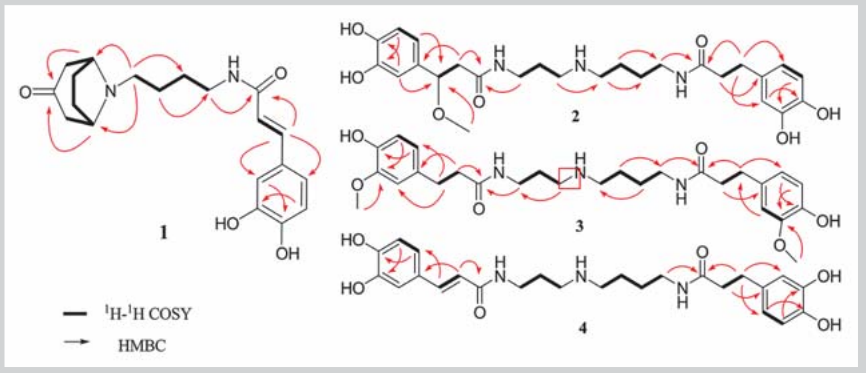

Fig. 2 Key ^{1}H-^{1}H COSY (—) and HMBC (H→C) correlations of **1**–**4**. (Color figure available online only.)

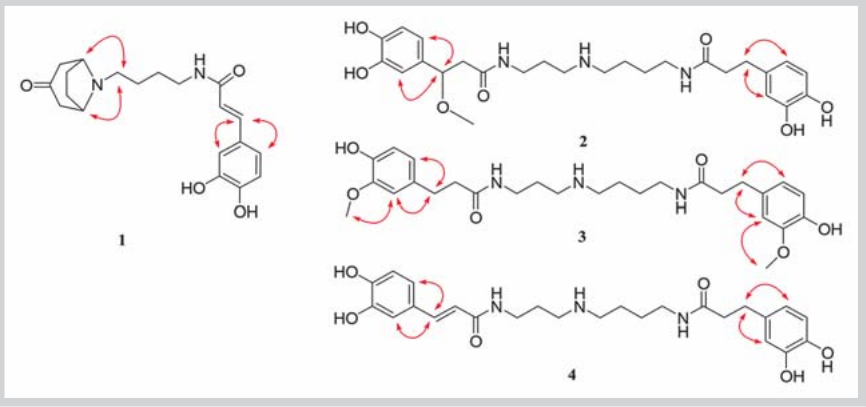

Fig. 3 Key NOESY (↔) correlations of **1**–**4**. (Color figure available online only.)

3.20, dd, J = 12.6, 6.6 Hz). HMBC correlations from H_2-12 to C-14 and from H_2-9 to C-1/C-5 (δ_C 58.8) were observed. A comparison of the ^{1}H and ^{13}C NMR data of **1** with those of caffeoylputrescine [25] revealed that the two compounds were structurally similar, except for the substituted amino terminus. The molecular formula of **1** suggested that its remaining moiety contained three indices of hydrogen deficiency. The ^{1}H and ^{13}C NMR data indicated that this portion of the molecule contained seven carbon atoms, including one carbonyl group (δ_C 208.2), one set of equivalent methines (δ_C 58.8; δ_H 3.67), and two sets of methylenes (δ_C 46.7; δ_H 2.13 and 2.76, and δ_C 27.4; δ_H 2.03 and δ_H 1.54). The ^{1}H-^{1}H COSY spectrum verified the nortropinone substructure, as represented by the bold lines in **Fig. 2**. HMBC correlations from H-1/H-5 to C-3, C-9, and C-8/C-7 confirmed the presence of a nortropinone moiety and its connection to the caffeoylputrescine structure through a nitrogen atom. The gross structure of **1** was deduced from the analysis of HMBC and ^{1}H-^{1}H COSY (**Fig. 2**) data. The NOESY correlations (**Fig. 3**) from H1/H5 to H9 and H18/H22 to H16 confirmed the structure of **1**. The structure of compound **1** was therefore assigned (**Fig. 1**) and named scotanamine A. Because there is no obvious Cotton effect of **1**, it is difficult to confirm the stereochemistry of this compound. Nevertheless, we provide here the optical rotation value of **1** (see Extraction and isolation of compounds).

Compound **2**, a white powder, was obtained as the corresponding formate. Its molecular formula was determined as $C_{26}H_{37}N_3O_7$ by ^{13}C NMR spectroscopic and HRESIMS data, which gave an m/z ion, 504.2681 for $[M + H]^+$ in the positive ion mode (calcd. for $[M + H]^+$ = 504.2710). Consideration of the ^{13}C NMR and DEPT spectra of **2** revealed ten indices of hydrogen deficiency. The IR spectrum indicated the presence of hydroxyl (3397 cm^{-1}) and carbonyl (1633 cm^{-1}) groups. The tabulated ^{1}H and ^{13}C assignments revealed by HSQC are listed in **Table 2**. The ^{13}C NMR and DEPT data (**Table 2**) revealed 26 carbon signals, including 12 aromatic carbons, ten methylene carbons (including four nitrogen-bearing methylene carbons), two carbonyl carbons, one oxygenated methine carbon, and one methoxy carbon. The ^{1}H NMR spectrum exhibited two sets of ABX-type spin coupling interactions between H-3 (δ_H 6.68, d, J = 1.8 Hz), H-5 (δ_H 6.54, dd, J = 8.4, 1.8 Hz), and H-6 (δ_H 6.61, d, J = 8.4 Hz), and H-24 (δ_H 6.57, d, J = 1.8 Hz), H-27 (δ_H 6.71, d, J = 7.8 Hz), and H-28 (δ_H 6.42, dd, J = 7.8, 1.8 Hz), which indicated the presence of two 1,2,4-trisubstituted benzene substructures. The ^{1}H and ^{13}C NMR data were similar to those of N^1,N^{10}-di-dihydrocaffeoylspermidine [18], which was also isolated as the current purification process of known metabolite **5**, except for the appearance of an additional methoxy [δ_H 3.04 (3H, s, H-7-OMe) and δ_C 56.1] and methine [δ_H 4.36 (1H, dd, J = 9.0, 2.4 Hz, H-7) and δ_C 80.3] group. The HMBC spectrum revealed correlations from H-7 to C-3/C-5 (δ_C 114.4/118.1), from H-22 (δ_H 2.63, t, J = 7.8 Hz) to C-24/C-28 (δ_C 116.2/119.2) and C-20 (δ_C 172.0), from H-7-OMe to C-7 (δ_C 80.3), and the aliphatic methylene protons (δ_H 2.72, H-13/2.79, H-15/3.10, H-11/3.05, H-18) to C-15/13/9/20 (δ_C 47.1/44.9/170.6/172.0). Collectively, these data indicated that **2** was derived from **5** by the replacement of a hydrogen atom with a methoxy group at C-7, whose presence was confirmed by the NOESY data (**Fig. 3**). The structure of **2** was therefore assigned and named scotanamine B.

Compound **3**, a white powder, was isolated as the corresponding formate. Its molecular formula was determined as $C_{27}H_{39}N_3O_6$ by ^{13}C NMR spectroscopic and HRESIMS data, which gave an m/z ion, 502.2896 for $[M + H]^+$ (calcd. for $[M + H]^+$ = 502.2917). The IR

Table 2 ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) spectroscopic data for compounds **2–4** (DMSO-d_6) formates.

Position	2.FA[a]		3.FA		4.FA	
	δ_H (*J* in Hz)	δ_C, type	δ_H (*J* in Hz)	δ_C, type	δ_H (*J* in Hz)	δ_C, type
1		145.4, C		145.1, C		147.9, C
2		145.5, C		147.8, C		146.2, C
3	6.68, d (1.8)	114.4, CH	6.75 br, s	112.9, CH	7.06, s	114.2, CH
4		132.6, C		132.4, C		126.7, C
5	6.54, dd (8.4, 1.8)	118.1, CH	6.57 d (7.8)	120.7, CH	6.81, d (8.4)	121.0, CH
6	6.61, d (8.4)	116.0, CH	6.67 d (7.8)	115.8, CH	6.73, d (8.4)	116.1, CH
7	4.36, dd (9.0, 2.4)	80.3, CH	2.70[b]	31.3, CH_2	7.24, d (15.6)	139.8, CH
8a	2.54, m	44.9, CH_2	2.33[b]	37.9, CH_2	6.40, d (15.6)	118.7, CH
8b	2.28, m					
9		170.6, C		172.4, C		166.4, C
10	8.04, t (5.4)	NH	8.01, t (6.0)	NH	8.32, br s	NH
11	3.10, t (6.0)	36.0, CH_2	3.09 dd (12.0, 6.0)	36.3, CH_2	3.23, br s	36.0, CH_2
12	1.69, m	26.6, CH_2	1.66 m	27.1, CH_2	1.79, br s	26.8, CH_2
13	2.72, t (7.2)	44.9, CH_2	2.68[b]	45.2, CH_2	2.83[b]	45.3, CH_2
15	2.79, t (7.8)	47.1, CH_2	2.72[b]	47.3, CH_2	2.78[b]	47.2, CH_2
16	1.53, m	23.7, CH_2	1.50 m	24.2, CH_2	1.52, br s	23.9, CH_2
17	1.42, m	26.8, CH_2	1.40 m	26.9, CH_2	1.40, br s	26.8, CH_2
18	3.05, t (6.0)	38.2, CH_2	3.04 dd (12.6, 6.6)	38.3, CH_2	3.04, d (5.4)	38.2, CH_2
19	7.87, t (5.4)	NH	7.87, t (6.0)	NH	7.88, br s	NH
20		172.0, C		171.9, C		171.9, C
21	2.27, t (7.8)	38.1, CH_2	2.33[b]	38.0, CH_2	2.28, t (6.6)	38.0, CH_2
22	2.63, t (7.8)	31.1, CH_2	2.70[b]	31.2, CH_2	2.62, t (7.2)	31.1, CH_2
23		132.2, C		132.6, C		132.5, C
24	6.57, d (1.8)	116.2, CH	6.75 br, s	112.9, CH	6.57, s	115.9, CH
25		145.7, C		147.8, C		145.5, C
26		143.9, C		145.1, C		143.8, C
27	6.71, d (7.8)	115.9, CH	6.67 d (7.8)	115.8, CH	6.60, d (7.8)	116.2, CH
28	6.42, dd (7.8, 1.8)	119.2, CH	6.57 d (7.8)	120.7, CH	6.42, d (7.8)	119.2, CH
2/25-OCH_3			3.74 s	56.0, CH_3		
7-OCH_3	3.04, s	56.1, CH_3				

[a] These compounds were of formate salts; [b] signal partially overlapped

spectrum contained several absorption bands suggesting the presence of hydroxyl (3274 cm^{-1}) and carbonyl (1646 cm^{-1}) groups. The ^{1}H and ^{13}C NMR chemical shifts were similar to those of N^1,N^{10}-di-dihydrocaffeoylspermidine [18], except for the presence of two methoxy groups [δ_H 3.74 (3H, s, 2-OMe) and δ_H 3.74 (3H, s, 25-OMe)] and a minor difference in the chemical shifts of the aromatic carbons and protons (**Table 2**). The HMBC spectrum of **3** revealed cross-peaks from 2-OMe (δ_H 3.74) to C-2 (δ_C 147.8), and 25-OMe (δ_H 3.74) to C-25 (δ_C 147.8), which indicated that the two methoxy groups were located at C-2 and C-25, respectively. Based on data obtained from the HMBC, ^{1}H-^{1}H COSY, and NOESY data (**Fig. 2** and **3**), the structure of **3** was assigned as the 2,25-dimethoxylated analogue of **5** and subsequently named scotanamine C.

Compound **4**, a white powder, was isolated as the corresponding formate. Its molecular formula was determined as $C_{25}H_{33}N_3O_6$ by ^{13}C NMR spectroscopic and HRESIMS data in the positive ion mode, which gave an *m/z* ion, 472.2420 for $[M + H]^+$ (calcd. for $[M + H]^+$ *m/z* = 472.2448). The ^{1}H and ^{13}C NMR data were very similar to those of N^1,N^{10}-di-dihydrocaffeoylspermidine [18], except that two of the methylene signals, which were replaced by one olefinic group [δ_C 118.7 (C-7) and δ_C 139.8 (C-8)]. The double bond at the position of C7–C8 was confirmed by HMBC correlations from the olefinic proton H-7 (δ_H 7.24, d, *J* = 15.6 Hz) to the aromatic carbons at δ_C 114.2 (C-3) and δ_C 121.0 (C-5), and the carbonyl carbon δ_C 166.4 (C-9), as well as a correlation from H-8 (δ_H 6.40, d, *J* = 15.6 Hz) to the aromatic carbon δ_C 126.7 (C-4). The other moieties were assigned on the basis of their HMBC, ^{1}H-^{1}H COSY, and NOESY correlations. Collectively, these data indicated that **4** was the C7–C8 double bond analogue of **5** and named scotanamine D.

HCA amides have been found in many higher plants and exhibit a broad range of structure variations from simple phenolic amides [26,27] to complex macrocyclic polyamine alkaloids [28,29]. Plants belonging to the Solanaceae family, including *Lycopersicon esculentum, Petunia hybrid,* and *Lycium chinense* [30,31], are rich in HCA amide-containing species. To the best of our knowledge, however, the discovery of compound **1** represents the first reported example of the isolation of an HCA amide containing a moiety resulting from the condensation of a nortropinone with a putrescine.

Since *S. tangutica* has a long history of being used for pain relief, compounds **1–11** were tested for their ability to activate μ-, δ-, or κ-opioid receptors, which are the targets of many well-defined analgesics. Among these compounds, compound **2** displayed agonist activity at the μ receptor with an EC_{50} value of 7.3 µM (**Fig. 4A**) and it was inhibited by naloxone (10 µM), an opioid receptor antagonist (**Fig. 4B**). All of the other compounds were found to be inactive. The activity of **2** was found to be specific to the μ receptor subtype, with no response being detected in cells expressing κ- or δ-opioid receptors (data not shown).

The tail-flick assay, which is used to assess the response of a mouse to a thermal stimulus, was used to evaluate the potential analgesic effect of compound **2**. As shown in **Fig. 5A**, compound

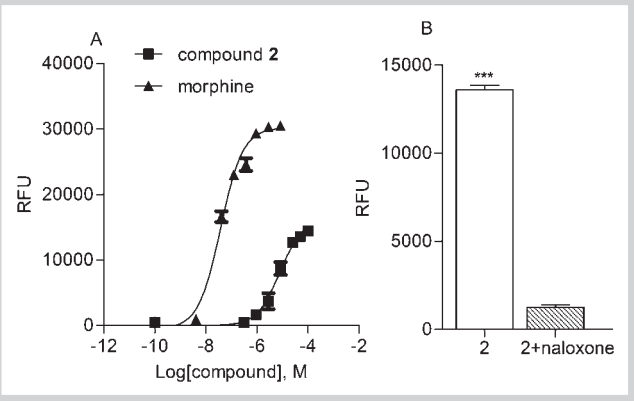

Fig. 4 The activity of **2** in μ-expressing cells. **A** Dose-response curve. Morphine is the positive control. Error bars represent standard error of the mean of triplicate measurements for each point. **B** Naloxone (10 µM) effects on the activity of **2** at a dose of 50 µM. RFU represents relative fluorescence unit. Error bars represent standard error of the mean of triplicate measurements for each point. Compound **2** vs. **2**/naloxone, *** $p < 0.001$, Student's t-test.

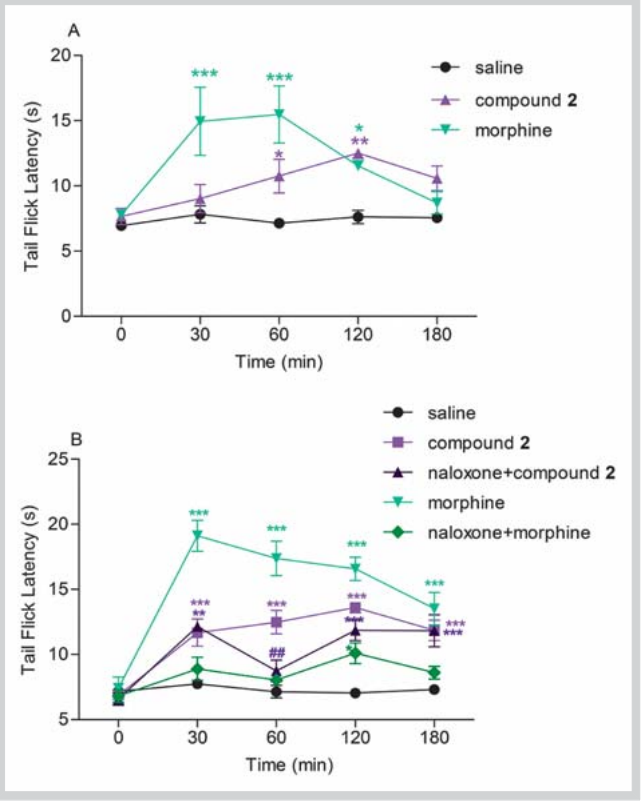

Fig. 5 The analgesic effect of **2** in the tail-flick assay. **A** Time course of the tail-flick latencies (n = 4–6) after drug injection. Compared to saline group: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. **B** Time course of the influence of naloxone (1 mg/kg) on the effect of compound **2**- induced (40 mg/kg) antinociception (n = 5). Compared to saline group: ** $p < 0.01$; *** $p < 0.001$. Compound **2** group vs. **2** treated with naloxone group; ## $p < 0.01$. Data are means ± SEM and were analyzed by two-way ANOVA and Bonferroni post hoc tests. (Color figure available online only.)

2 was found to increase the tail-flick latency and thus demonstrated the antinociceptive properties. This antinociception was determined to be dependent on the activation of the opioid system because it was antagonized by naloxone (**Fig. 5B**). As a positive control, morphine elicited an analgesic response at 10 mg/kg, while saline, as a negative control, did not (**Fig. 5B**). Opiates, such as morphine and codeine, are the most common antinociceptive drugs. However, most of these analgesics have been associated with unwanted side effects such as ventilator depression. Patients also develop a tolerance and dependence to the drugs [32]. Therefore, the search for new analgesic compounds that present therapeutic alternatives is important. Compound **2**, which is an HCA amide, may represent a novel structural motif for opioid receptor activation and therefore may have the potential to be developed as a novel analgesic. Given the limited set of available molecules, it is difficult to derive structure-activity information. However, the substituted methoxy moiety at position 7 of compound **2** seems important for the activity because compounds **3, 4, 5**, and **11** are inactive, although their structures differ marginally from **2**. Further variations on the skeleton with respect to the substitution pattern remain to be clarified. To develop a complete molecular explanation to account for the traditional use of this plant, it will be necessary for the extract and the individual constituents to be evaluated at other pain targets.

Material and Methods

General experimental procedures

The analytical chromatography system consisted of a 2695 HPLC pump and a 2489 photodiode array detector system. The chromatographic system for purification consisted of a 2525 binary gradient pump and a 2489 ultraviolet-visible detection system. Data were collected and analyzed using Empower software version 3.0 and Masslynx software version 4.1. All instruments and workstations were purchased from Waters. Melting points were recorded on an X-4 melting point apparatus (Tai Guang) without correction. IR spectra were recorded with a PerkinElmer GS-II FTIR spectrometer (Perkin-Elmer), and UV spectra were acquired using an SP-1901 UV (Guang Pu). Optical rotations were obtained with a PerkinElmer 241 polarimeter. All NMR spectra were recorded on a Bruker FT-NMR Ultra Shield TM 600 MHz spectrometer with tetramethylsilane as the internal standard. HRESIMS were obtained using an orbitrap LTQ-Orbitrap mass spectrometer (Thermo). SCX SPE (20 g, 60 mL, 60 µm) cartridges, XCharge C18 (50 × 260 mm, 10 µm and 20 × 250 mm, 10 µm), C8PN (10 × 150 mm, 5 µm and 20 × 250 mm, 10 µm), and XCharge SCX (20 × 250 mm, 10 µm) columns were purchased from Accorm limited company. Ca^{2+} responses were monitored by an FLIPR assay.

Reagents

Acetonitrile and methanol were obtained from Merck. Sodium biphosphate, sodium perchlorate, formic acid, and [$^2H_1$] dimethyl sulfoxide were obtained from J&K. Phosphoric acid (H_3PO_4) was purchased from Tedia. All solvents were HPLC grade, and the purities of these salts were more than 98%. Water was prepared by a Milli-Q system. Morphine (purity ≥ 98%) and naloxone (purity ≥ 98%) were purchased from Sigma-Aldrich.

Plant material

The roots of *S. tangutica* were collected in August 2008 from Jiuzhi County (at 100°, 40′ 19″ E; 33°, 16′ 56″ N), Qinghai Province, People's Republic of China. Voucher specimens (QTPMB) were

deposited at Qinghai-Tibet Plateau Museum of Biology, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, and were identified by Senior Engineer Lijuan Mei.

Extraction and isolation of compounds

The air-dried and powdered roots of *S. tangutica* (10.0 kg) were extracted twice with 95% ethanol (3 h) under reflux at 78 °C. Then, the alkaloid extraction was enriched by a nonaqueous SPE method [11]. The enriched alkaloids were purified by multidimensional HPLC. The details of the roots of the *S. tangutica* extraction, enrichment, and the purification of compounds **1**–**11** are available in Supporting Information.

Scotanamine A (**1**) *formate*: white powder; m. p. 174–175 °C; $[\alpha]_D^{25}$ − 6.0 (*c* 0.36, MeOH); UV (MeOH) λ_{max} (log ε): 218 (4.25), 236 (3.89), 293 (3.90) nm, 318 (3.95); IR (KBr) ν_{max}: 3390, 1727, 1656, 1558, 1286 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6) and ^{13}C NMR (150 MHz, DMSO-d_6) spectroscopic data, see **Table 1**; positive HRESIMS *m/z* 359.1954 $[M + H]^+$ (calcd. for $C_{20}H_{27}N_2O_4$, 359.1954). The purity of **1** was more than 98%, which was determined by HPLC.

Scotanamine B (**2**) *formate*: white powder; m. p. 149–150 °C; $[\alpha]_D^{25}$ − 8.8 (*c* 0.24, MeOH); UV (MeOH) λ_{max} (log ε): 230 (3.74), 280 (3.50) nm; IR (KBr) ν_{max}: 3258, 1652, 1525, 1283 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6) and ^{13}C NMR (150 MHz, DMSO-d_6) spectroscopic data, see **Table 2**; positive HRESIMS *m/z* 504. 2681 $[M + H]^+$ (calcd. for $C_{26}H_{38}N_3O_7$, 504.2710). The purity of **2** was more than 97%, which was determined by HPLC.

Scotanamine C (**3**) *formate*: white powder; m. p. 150–151 °C; UV (MeOH) λ_{max} (log ε): 230 (4.06), 280 (3.75) nm; IR (KBr) ν_{max}: 3274, 1646, 1518, 1276 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6) and ^{13}C NMR (150 MHz, DMSO-d_6) spectroscopic data, see **Table 2**; positive HRESIMS *m/z* 502.2892 $[M + H]^+$ (calcd. for $C_{27}H_{39}N_3O_6$, 502.2917). The purity of **3** was 98%, which was determined by HPLC.

Scotanamine D (**4**) *formate*: white powder; m. p. 165–166 °C; UV (MeOH) λ_{max} (log ε): 220 (4.39), 280 (4.17), 320 (4.30) nm; IR (KBr) ν_{max}: 3258, 1652, 1525, 1283 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6) and ^{13}C NMR (150 MHz, DMSO-d_6) spectroscopic data, see **Table 2**; positive HRESIMS *m/z* 472.2420 $[M + H]^+$ (calcd. for $C_{25}H_{34}N_3O_6$, 472.2448). The purity of **4** was more than 97%, which was determined by HPLC.

The activation of the μ opioid receptor

The assay was performed as reported earlier [33]. Briefly, the stable cells expressing the μ receptor were seeded into poly-D-lysine-coated black wall, clear-bottom, 96-well plates at a density of 80 000 cells per well. Twenty-four hours later, the medium was removed and replaced with 100 µL of dye loading solution (2 µM Fluo-4 AM dissolved in FLIPR buffer, which consisted of 0.2 mg/mL pluronic acid in 1× Hank's buffer supplemented with 20 mM HEPES, pH 7.4) for 1 h at 37 °C. The cells were then washed three times with FLIPR buffer prior to the assay. The compounds, which were dissolved in DMSO and stored in 96-well plates, were diluted with FLIPR buffer and then added into the cells within 4 sec. The intracellular Ca^{2+} concentration was monitored at 520 nm with the excitation wavelength at 488 nm over a period of 4 min. For some assays, naloxone was first incubated with the cells for 10 min before the addition of the test compounds. Data were expressed as fluorescence (arbitrary units) versus time.

Tail-flick test

Male CD1 mice (30–40 g), age 9–11 weeks, were used. Mice were group-housed and maintained on a 12-h light-dark cycle (light on at 7 : 00 a. m.) with food and water available *ad libitum*. All experimental procedures were approved by the Institutional Animal Care and Use Committee of University of California, Irvine (June 9, 2013; IACUC Protocol Number 2002–2379) and were performed in compliance with national and institutional guidelines for the care and use of laboratory animals.

The tail-flick assay was used to evaluate the pain response in mice. This method was originally described by D' Amour et al. [34]. In brief, the acute pain response was measured using an electronically controlled tail-flick unit (Ugo Basile 37 360) that integrated both a thermal nociceptive stimulus and an automated response timer. A thermal stimulus (focused light from a 20 W infrared bulb as the heat source) was directed on the tip of the mouse tail. The time from onset of stimulation to a rapid withdrawal of their tails from the heat source was recorded as tail-flick latency. A maximum of 22 sec was set as a cutoff time to prevent tissue damage. After three-day baseline measures, the mice were injected (5 mL/kg, i. p.) with saline, morphine (10 mg/kg), or **2** (40 mg/kg), and tail-flick latency was measured 30, 60, 120, and 180 min after drug injection. Naloxone (1 mg/kg) or saline was injected (2.5 mL/kg, i. p.) 30 min before drug administration to evaluate the effects of **2** in the presence of the opioid receptor antagonist. The experimenter was blind to all treatment conditions.

Supporting information

Extraction and isolation as well as HRMS, ^1H, ^{13}C NMR, HMQC, HMBC, ^1H-^1H COSY, and NOESY spectra for compounds **1**–**4** in DMSO-d_6 are available as Supporting Information.

Acknowledgements

This work was supported by the Project of National Science Foundation of China (81 274 077) and the National Institute on Drug Abuse (R01 DA024746).

Conflict of Interest

The authors declare no competing financial interest.

References

1 *Roberts MF, Wink M.* Alkaloids: biochemistry, ecology, and medicinal applications. New York: Plenum Press; 1998: 5–6
2 *Heinrich M, Hettenhausen C, Lange T, Wuensche H, Fang J, Baldwin IT, Wu J.* High levels of jasmonic acid antagonize the biosynthesis of gibberellins and inhibit the growth of *Nicotiana attenuata* stems. Plant Journal 2013; 73: 591–606
3 *Aher V, Chattopadhyay P, Goyary D, Veer V.* Evaluation of the genotoxic and antigenotoxic potential of the alkaloid punarnavine from *Boerhaavia diffusa*. Planta Med 2013; 79: 939–945
4 *Hyuga S, Hyuga M, Yoshimura M, Amakura Y, Goda Y, Hanawa T.* Herbacetin, a constituent of ephedrae herba, suppresses the HGF-induced motility of human breast cancer MDA-MB-231 cells by inhibiting c-met and akt phosphorylation. Planta Med 2013; 79: 1525–1530
5 *Ibrahim SRM, Mohamed GA, Shaala LA, Youssef DTA, El Sayed KA.* New alkaloids from *Pancratium maritimum*. Planta Med 2013; 79: 1480–1484
6 *Ndunda B, Langat MK, Wanjohi JM, Midiwo JO, Kerubo LO.* Alienusolin, a new 4 alpha-deoxyphorbol ester derivative, and crotonimide C, a new

glutarimide alkaloid from the kenyan *Croton alienus*. Planta Med 2013; 79: 1762–1766
7 *Varma DR, Yue TL.* Adrenoceptor blocking properties of atropine-like agents anisodamine and anisodine on brain and cardiovascular tissues of rats. Br J Pharmac 1986; 87: 587–594
8 *Xiu RJ, Hammerschmidt DE, Coppo PA, Jacob HS.* Anisodamine inhibits thromboxane synthesis, granulocyte aggregation, and platelet aggregation. A possible mechanism for its efficacy in bacteremic shock. JAMA 1982; 247: 1458–1460
9 *Zhang WW, Song MK, Cui YY, Wang H, Zhu L, Niu YY, Yang LM, Lu Y, Hong ZC.* Differential neuropsychopharmacological influences of naturally occurring tropane alkaloids anisodamine versus scopolamine. Neurosci Lett 2008; 443: 241–245
10 *Zheng W, Wang L, Meng L, Liu J.* Genetic variation in the endangered *Anisodus tanguticus* (Solanaceae), an alpine perennial endemic to the Qinghai Tibetan plateau. Genetica 2008; 132: 123–129
11 *Long Z, Wang C, Guo Z, Zhang X, Nordahl L, Zeng J, Zeng J, Liang X.* A non-aqueous solid phase extraction method for alkaloid enrichment and its application in the determination of hyoscyamine and scopolamine. Analyst 2012; 137: 1451–1457
12 *Minina SA, Astakhova TV, Fesenko DA.* Alkaloids of *Scopolia tangutica*. Chem Nat Compd 1977; 13: 598
13 *Xiao P, Guangcheng X, Liyi H.* The occurrence of important atropane alkaloids in Chinese solanaceous plants. Acta Botanica Sinica 1973; 15: 187–194
14 *Poupko JM, Baskin SI, Moore E.* The pharmacological properties of anisodamine. J Appl Toxicol 2007; 27: 116–121
15 *Samoryadov BA, Minina SA.* Isolation of hyoscyamine and scopolamine from the epigeal part of *Scopolia tangutica*. Chem Nat Compd 1971; 7: 205–206
16 *Shimomura K, Yoshimatsu K, Ishimaru K, Sauerwein M.* Tropane alkaloids in root cultures of solanaceous plants. Stud Nat Prod Chem 1995; 17: 395–419
17 *Scholten HJ, Batterman S, Visser JF. Scopolia* spp: *in vitro* culture and the production of scopolamine and hyoscyamine. Biotechnol Agricult Forest 1993; 21: 314–325
18 *Sattar EA, Glasl H, Nahrstedt A, Hilal SH, Zaki AY, Elzalabani SMH.* Hydroxycinnamic acid-amides from *Iochroma cyaneum*. Phytochemistry 1990; 29: 3931–3933
19 *Taha AM, Rucker G.* ^{13}C-NMR spectroscopy of tropane alkaloids. J Pharm Sci 1978; 67: 775–779
20 *Lanoue A, Boitel CM, Portais JC, Laberche JC, Barbotin JN, Christen P, Sangwan NB.* Kinetic study of littorine rearrangement in *Datura innoxia* hairy roots by C-13 NMR spectroscopy. J Nat Prod 2002; 65: 1131–1135
21 *Chang JB, Xie WL, Wang LM, Ma NC, Cheng SX, Xie J.* An efficient approach to the asymmetric total synthesis of (−)-anisodine. Eur J Med Chem 2006; 41: 397–400
22 *Mbadiwe EI.* Caffeoylputrescine from *Pentaclethra macrophylla*. Phytochemistry 1973; 12: 2546
23 *Buta JG, Izac RR.* Caffeoylputrescine in *Nicotiana tabacum*. Phytochemistry 1972; 11: 1188–1189
24 *Shigennobu M, Yoko T, Masao N, Tamaki E.* P-coumaroylputrescine, caffeoylputrescine and feruloylputrescine from callus tissue culture of *Nicotiana tabacum*. Phytochemistry 1971; 10: 1347–1350
25 *Noguchi S.* A new aromatic amide, caffeoylputrescine from callus tissue culture of *Nicotiana tabacum*. Agric Biol Chem 1970; 34: 972–973
26 *Kawahara T, Izumikawa M, Otoguro M, Yamamura H, Hayakawa M, Takagi M, Shin YK.* JBIR-94 and JBIR-125, antioxidative phenolic compounds from streptomyces sp R56-07. J Nat Prod 2012; 75: 107–110
27 *Capon RJ, Stewart M, Ratnayake R, Lacey E, Gill JH.* Citromycetins and bilains A–C: new aromatic polyketides and diketopiperazines from Australian marine-derived and terrestrial *Penicillium* spp. J Nat Prod 2007; 70: 1746–1752
28 *Yoshida E, Mitsui H, Takahashi H, Maruo B.* Amino acid incorporation by a bacterial cell-free system. J Biochem 1960; 48: 251–261
29 *Martinta J, Martin C, Gallet M.* Presence of aromatic-compounds connected to putrescence in various virose *Nicotiana* species. Acad Sci Ser III 1973; 276: 1433–1435
30 *Zhang JX, Guan SH, Feng RH, Wang Y, Wu ZY, Zhang YB, Chen XH, Bi KS, Guo DA.* Neolignanamides, lignanamides, and other phenolic compounds from the root bark of *Lycium chinense*. J Nat Prod 2013; 76: 51–58
31 *Funayama S, Yoshida K, Konno C, Hikino H.* Structure of kukoamine A, a hypotensive principle of *Lycium chinense* root barks. Tetrahedron Lett 1980; 21: 1355–1356
32 *Crofford LJ.* Adverse effects of chronic opioid therapy for chronic musculoskeletal pain. Nat Rev Rheumatol 2010; 6: 191–197
33 *Zhang Y, Xu J, Wang Z, Zhang X, Liang X, Civelli O.* BmK-YA, an enkephalin-like peptide in scorpion venom. PLoS One 2012; 7: 1–8
34 *Damour FE, Smith DL.* A method for determining loss of pain sensation. J Pharmacol Exp Ther 1941; 72: 74–79