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Authors
Long, Z
Zhang, Y
Guo, Z
et al.

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Amide Alkaloids from *Scopolia tangutica*

**Abstract**

Four new hydroxycinnamic acid amides, scotan-10-di-dihydrocaffeoylspermidine (5), scopolamine (6), anisodamine (7), hyoscyamine (8), anisidine (9), caffeoylpurinesine (10), and N\(^1\)-caffeoyl-N\(^2\)-dihydrocaffeoylspermidine (11), scopolamine (6), anisodamine (7), hyoscyamine (8), anisidine (9), caffeoylpurinesine (10), and N\(^1\)-caffeoyl-N\(^2\)-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 \(\mu\)-opioid receptor (EC\(_{50}\) = 7.3 \(\mu\)M). 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
- 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], anisidine [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\(_2\)O (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-
Acknowledged as N₁,N₁₀-di-dihydrocaffeoylspermidine (5) [18], scopola-
mine (6) [19], anisodamine (7) [19], hyoscyamine (8) [20], aniso-
dine (9) [21], caffeoylputrescine (10) [22–24], and N¹-caffeoyl-
N₁₀-dihydrocaffeoylspermidine (11) [2], based on their spectro-
scopic data. The structures of these alkaloids are shown in

![Fig. 1](Structures of compounds from the roots of S. tangutica.)

Compound 1, a white powder, was obtained as the corresponding
formate. Its molecular formula was determined as C₂₀H₂₆N₂O₄
by ¹³C 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 ¹³C 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 ¹³C NMR and DEPT data (Table 1) re-
vealed 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,

![Table 1](¹H (600 MHz) and ¹³C NMR (150 MHz) spectroscopic data for compound 1 formate salt (DMSO-d₆).)

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<thead>
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<th>Position</th>
<th>δ_H (in Hz)</th>
<th>δ_C, type</th>
<th>Position</th>
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<th>δ_C, type</th>
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</tr>
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<td>2b/4b</td>
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<td>139.4, CH</td>
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<tr>
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<td>27.4, CH₂</td>
<td>17</td>
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<td></td>
</tr>
<tr>
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<td>1.54, m</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>9</td>
<td>2.73*</td>
<td>49.3, CH₂</td>
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<td>6.98 d (1.8)</td>
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<td>147.7, C</td>
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<td>NH</td>
<td>22</td>
<td>6.82 dd (8.4, 1.8)</td>
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</table>

* Signal partially overlapped

three aromatic methine carbons, two nitrogen-bearing methy-
lene carbons, two nitrogen-bearing methine carbons, one ole-
finic 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-tri-
substituted benzene ring. The HMBC spectrum revealed the pres-
ence of long-range ¹H-¹³C couplings from the mutually
coupled 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 pres-
ence 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
H₂-9 (δ_H 2.73), H₂-10 (δ_H 1.61), H₂-11 (δ_H 1.54), and H₂-12 (δ_H

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3.20, dd, J = 12.6, 6.6 Hz). HMBC correlations from H2-12 to C-14 and from H2-9 to C-1/C-5 (δC 58.8) were observed. A comparison of the 1H and 13C 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 1H and 13C NMR data indicated that this portion of the molecule contained seven carbon atoms, including one carbonyl group (δC 208.2), one set of equivalent methines (δC 58.8; δH 3.67), and two sets of methylenes (δC 46.7; δH 2.13 and 2.76, and δC 27.4; δH 2.03 and 1.54). The 1H-1H COSY spectrum verified the nortropinone substructure, as represented by the bold lines in Fig. 2. HMBC correlations from H-1 to H-5 to C-3, C-9, and C-8/C-7 confirmed the presence of a nortro- pinone 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 1H-1H 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 C26H37 N3O7 by 13C 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 13C 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 1H and 13C assignments revealed by HSQC are listed in Table 2. The 13C 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 1H NMR spectrum exhibited two sets of ABX-type spin coupling interactions between H-3 (δH 6.68, d, J = 1.8 Hz), H-5 (δH 6.54, dd, J = 8.4, 1.8 Hz), and H-6 (δH 6.61, d, J = 8.4 Hz), and H-24 (δH 6.57, d, J = 8.1 Hz), H-27 (δH 6.71, d, J = 7.8 Hz), and H-28 (δH 6.42, dd, J = 7.8, 1.8 Hz), which indicated the presence of two 1,2,4-trisubstituted benzene substructures. The 1H and 13C NMR data were similar to those of N1,N10-di-hydrocaffeoyl spermidine [18], which was also isolated as the current purification process of known metabolite 5, except for the appearance of an additional methoxy [δH 3.04 (3H, s, H-7-OMe) and δC 56.1] and methine [δH 4.36 (1H, dd, J = 9.0, 2.4 Hz, H-7) and δC 80.3] group. The HMBC spectrum revealed correlations from H-7 to C-3/C-5 (δC 114.4/118.1), from H-22 (δH 2.63, t, J = 7.8 Hz) to C-24/C-28 (δC 116.2/119.2) and C-20 (δC 172.0), from H-7-OMe to C-7 (δC 80.3), and the aliphatic methylene protons (δH 3.04, H-13/2.79, H-15/3.10, H-11/3.05, H-18) to C-15/13/9/20 (δC 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 C27H39 N3O6 by 13C NMR spectroscopic and HRESIMS data, which gave an m/z ion, 502.2896 for [M + H]+ (calcd. for [M + H]+ = 502.2917). The IR
spectrum contained several absorption bands suggesting the presence of hydroxyl (3274 cm⁻¹ and carbonyl (1646 cm⁻¹) groups. The ¹H and ¹³C NMR chemical shifts were similar to those of N⁵,N¹⁰-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. Based on data obtained from the HMBC, ¹H-¹H 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 ɣ- and δ-opioid receptors, which are the targets of many well-defined analgesics. Among these compounds, compound 2 displayed agonist activity at the ɣ receptor with an EC₅₀ 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 ɣ 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

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**Table 2** ¹H (600 MHz) and ¹³C (150 MHz) spectroscopic data for compounds 2–4 (DMSO-δ₆) formates.

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<td>145.1, C</td>
<td>147.9, C</td>
</tr>
<tr>
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<td>147.8, C</td>
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</tr>
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<td>132.4, C</td>
<td>126.7, C</td>
</tr>
<tr>
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<td>6.57 d (7.8)</td>
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<tr>
<td>6</td>
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<td>116.0, CH</td>
<td>6.67 d (7.8)</td>
</tr>
<tr>
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<td>6.67 d (7.8)</td>
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<td>56.1, CH₃</td>
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* These compounds were of formate salts; ° signal partially overlapped.
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 analogs 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), and XCharge C8PN (10 × 150 mm, 5 µm and 20 × 250 mm, 10 µm), and XCharge SCX (20 × 250 mm, 10 µm) columns were purchased from Accorn limited company. Ca⁺ responses were monitored by an FLIPR assay.

Reagents
Acetonitrile and methanol were obtained from Merck. Sodium biphosphate, sodium perchlorate, formic acid, and [2H3] dimethyl sulfoxide were obtained from J&K. Phosphoric acid (H3PO4) 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. 149–150 °C; [α]25D +8.8 (c 0.24, MeOH); UV (MeOH) max (log ε): 230 (3.74), 280 (3.50) nm; IR (KBr) νmax: 3258, 1652, 1283 cm−1; 1H NMR (600 MHz, DMSO-d6) and 13C NMR (150 MHz, DMSO-d6) spectroscopic data, see Table 1; positive HREIMS m/z 359.1954 [M + H]+ (calcd. for C20H27N2O4, 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; [α]25D +6 (c 0.36, MeOH); UV (MeOH) max (log ε): 218 (4.25), 236 (3.89), 293 (3.90) nm; IR (KBr) νmax: 3390, 1727, 1656, 1558, 1286 cm−1; 1H NMR (600 MHz, DMSO-d6) and 13C NMR (150 MHz, DMSO-d6) spectroscopic data, see Table 1; positive HREIMS m/z 502.2892 [M + H]+ (calcd. for C27H39N3O6, 502.2892). The purity of 2 was more than 97%, which was determined by HPLC.

Scotanamine C (3) formate: white powder; m. p. 180 °C; UV (MeOH) max (log ε): 230 (4.06), 280 (3.75) nm; IR (KBr) νmax: 3274, 1646, 1518, 1276 cm−1; 1H NMR (600 MHz, DMSO-d6) and 13C NMR (150 MHz, DMSO-d6) spectroscopic data, see Table 1; positive HREIMS m/z 502.2892 [M + H]+ (calcd. for C27H39N3O6, 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) max (log ε): 220 (4.39), 280 (4.17), 320 (4.30) nm; IR (KBr) νmax: 3258, 1652, 1525, 1283 cm−1; 1H NMR (600 MHz, DMSO-d6) and 13C NMR (150 MHz, DMSO-d6) spectroscopic data, see Table 2; positive HREIMS m/z 472.2420 [M + H]+ (calcd. for C25H34N3O6, 472.2448). The purity of 4 was more than 97%, which was determined by HPLC.

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 37360) 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, 13C NMR, HMBC, HMOX, COSY, and NOESY spectra for compounds 1–4 in DMSO-d6 are available as Supporting Information.

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Conflict of Interest
The authors declare no competing financial interest.

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