Chinese Herbal Medicines 14 (2022) 125–129

Contents lists available at ScienceDirect

Chinese Herbal Medicines

journal homepage: www.elsevier.com/locate/chmed

Original Article

Analgesic alkaloids from *Urticae Fissae Herba*

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ARTICLE INFO

Article history:
Received 12 March 2021
Revised 8 May 2021
Accepted 5 June 2021
Available online 23 September 2021

Keywords:
analgesic activity
pyrrole alkaloid
Urticae Fissae Herba

ABSTRACT

Objective: To investigate the analgesic substances in the aerial part of *Urtica fissa* (*Urticae Fissae Herba*), commonly used for rheumatoid and rheumatism arthritis.
Methods: The analgesic constituents were isolated with the active guidance of hot plate and acetic acid writhing models, and identified by comprehensive spectroscopic analysis.
Results: Thirteen alkaloids (**1**–**13**), two lignans (**14**, **15**), and three amides (**16**–**18**) were isolated from the active fractions. Among them, compound **1** was a new alkaloid, and compound **6** was a new natural product. The activity evaluation *in vivo* indicated that various pyrrole alkaloids (**1**, **3**, **6**, and **12**) possessed significant analgesic activities, they could significantly inhibit the mice pain response induced by acetic acid and hot plate at the dosage of 2 mg/kg BW.
Conclusion: The study revealed that the pyrrole alkaloids played important roles in the analgesic activities of *Urticae Fissae Herba*.

1. Introduction

Arthritis is a very common disease in the world. It often causes the durative joint pain, tumidness, and rigidity. Among them, durative pain is the uppermost and rebarbative symptom. Some *Urtica* species (commonly called nettles), such as *Urtica dioica* L., *U. urens* L., and *U. fissa* E. Pritz., are the important herbs for rheumatoid, rheumatism, and osteoarthritis in Asian and European countries (Chinese Materia Medica Editorial Board, 1999; European Directorate for the Quality Control of Medicines, 2016). The recent researches revealed that various nettles possessed the obvious analgesic activities, they could inhibit the pain response induced by acetic acid and formalin (Gorzalczany et al., 2011; Gülçin et al., 2004; Liao et al., 2016; Marrassini et al., 2010). The clinic test also proved the desired therapeutic effects of nettles, they could effectively relieve the various kinds of pain, such as rheumatic pain, osteoarthritis pain, musculoskeletal pain, and base-of-thumb pain (Chrubasik & Eisenberg, 1999; Randall et al., 2000; Rayburn et al., 2009; White et al., 2011). However, their analgesic constituents remain unknown (Grauso et al., 2019; Kopyt'ko et al., 2012).

As our continuous study on the medical plant of genus *Urtica*, the chemical constituents in *U. fissa* roots, leaves, seeds, and their biological activities *in vitro* were investigated previously (Feng et al., 2017; Wang et al., 2018; Zhang et al., 2019). In the present study, the analgesic constituents *in vivo* of the aerial part of *U. fissa* (*Urticae Fissae Herba*) were investigated for the first time. As results, a new alkaloid (**1**) and a new natural alkaloid (**6**), together with 16 known compounds, were isolated from the active fractions. The subsequently pharmacological evaluation indicated that various pyrrole alkaloids (**1**, **3**, **6**, and **12**) possessed the significantly analgesic activities.

2. Materials and methods

2.1. General experimental procedure

Optical rotations were measured on a JASCO P-2000 polarimeter. IR spectra were recorded on a Nicolet 6700 FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on Bruker 600 spectrometer with a 5 mm $^{13}C/^{1}H/^{15}N$ TCI CryoProbe. ESI-MS analyses were achieved on Waters Premier Q-TOF mass spectrometer. Electronic circular dichroism (ECD) spectra were obtained on a J-1500 ECD spectrometer. Semi-preparative HPLC chromatography was performed on a Shimadzu LC 2010 AHT liquid chromatography system, equipped with a quaternary solvent delivery system, an auto-sampler, a UV-Vis detector, a YMC ODS-AQ column (20×250 mm, 5 μm) (column temperature was 30 °C; Detection wavelength was set at 210 and 254 nm; flow rate was 3.0 mL/min, respectively). Silica gel G (200–300 mesh, Qingdao Haiyang Chemical Group, China.), Sephadex LH-20 (GE Healthcare Bio-

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https://doi.org/10.1016/j.chmed.2021.09.008
1674-6384/

science, Sweden), D101 macroporous resin (Tianjin Haiguang Chemical Group, China), and ODS (40–60 μm, Merck & Co., Inc., Germany) were used for column chromatography (CC).

2.2. Chemicals and material

Indomethacin and morphine were purchased from China National Medicines Co., Ltd (Shanghai, China). Kunming mice [(20 ± 2) g] were obtained from the Laboratory Animal Research Center of Fudan University, and experimental design was approved by the Animal Experimentation Ethics Committee, Shanghai Jiao Tong University.

The aerial part of *Urtica fissa* was collected in Nanchong, Sichuan Province (China) in August 2014, and authenticated by one of authors Meng-yue Wang. A voucher specimen (SJTU 20140802) was deposited in School of Pharmacy, Shanghai Jiao Tong University, Shanghai, China.

2.3. Bioassay-guided isolation

2.3.1. Screening of analgesic portions

The air-dried aerial part *U. fissa* (18 kg) were pulverized and extracted with 200 L 95% alcohol by immersion for five times (each 7 d). The collected extract was concentrated under vacuum to give a residue (3.9 kg). Part of the residue (3.8 kg) was suspended in water (10 L), successively partitioned with petroleum ether (PE, 60–90 °C, 8 L ×3), CH_2Cl_2, EtOAc, and *n*-BuOH (each 5 L ×3). The extracts were dried under vacuum to afford portions PE (3106.7 g), CH_2Cl_2 (247.8 g), EtOAc (44.8 g), and *n*-BuOH (65.1 g). The water layer remained was dried under vacuum to water portion (285.2 g). These portions were subjected to the active evaluation and the portions with analgesic activities were further fractioned by column chromatography (CC).

2.3.2. Investigation of analgesic fractions

n-BuOH portion (156.1 g) was fractioned by silica gel CC (130 cm ×12 cm) eluted with CH_2Cl_2-MeOH (19:1, 9:1, 17:3, 4:1, 3:1, and 7:3, volume percentage) for 6.0, 4.5, 5.5, 5.0, 7.0, and 8.5 L, respectively. The eluents were completely evaporated under vacuum to afford the resulting Fr. B1 (6.8 g), B2 (2.9 g), B3 (1.7 g), B4 (17.5 g), B5 (36.2 g), and B6 (40.1 g).

Water portion (258.2 g) was fractioned by D101 macroporous resin CC (150 cm ×12 cm) successively eluted with water, 20%, 45%, 75%, and 95% ethanol for 10, 6, 6, 8, and 4 L, respectively. The eluents were completely evaporated under vacuum to afford the resulting Fr. W1 (50.1 g), W2 (53.8 g), W3 (52.0 g), W4 (114.9 g), and W5 portion (2.3 g).

The fractions mentioned above were all subjected to the active evaluation, and those showed the obvious activities were further purified by CC and semi-preparative HPLC.

2.3.3. Chemical investigation on analgesic fractions

Fr. B1 (6.6 g) was further purified on silica gel CC (CH_2Cl_2-MeOH 99:1–9:1, volume percentage) to afford Fr. B1a – B1c. Fr. B1a (436 mg) was further by Sephedax LH-20 (CH_2Cl_2-MeOH 1:1, volume percentage), and then purified by HPLC (MeCN-H_2O 7:13, volume percentage) to obtain compounds **1** (t_R 16.3 min, 22.8 mg), **2** (t_R 12.9 min, 9.1 mg), and **3** (t_R 20.3 min, 10.7 mg). Fr. B1b (292 mg) were purified by HPLC (MeCN-H_2O 1:3, volume percentage) to obtain compounds **4** (t_R 7.4 min, 9.3 mg) and **5** (t_R 14.1 min, 9.0 mg). Fr. B1c (218 mg) was further purified by ODS (MeOH-H_2O 1:4–3:1, volume percentage) and semi-preparative HPLC (MeCN-H_2O 1:4, volume percentage) to afford compound **6** (t_R 32.7 min, 5.8 mg). Fr. B2 (2.8 g) was subjected to silica gel CC (CH_2Cl_2-MeOH 97:3–9:1, volume percentage) to obtain Fr. B2a–B2c. Fr. B2b (106 mg) was recrystallized with MeOH to afford compound **7** (12.0 mg). Fr. B2c (522 mg) was further purified by ODS (MeOH-H_2O 3:7–3:2, volume percentage) and semi-preparative HPLC (MeCN-H_2O 1:4, volume percentage) to afford compounds **8** (t_R 10.6 min, 4.1 mg) and **14** (t_R 26.1 min, 21.4 mg). Fr. B3 (1.4 g) was further purified on silica gel CC (CH_2Cl_2-MeOH 19:1–17:3, volume percentage) and semi-preparative HPLC (MeCN-H_2O 2:3, volume percentage) to afford compounds **9** (t_R 21.6 min, 2.3 mg) and **15** (t_R 24.4 min, 5.7 mg).

W4 (10.6 g) was subjected to silica gel CC eluted with CH_2Cl_2-MeOH (19:1–4:1, volume percentage) to obtain Fr. W4a-W4d. Fr. W4a (1.0 g) was further purified on silica gel CC eluted with CH_2Cl_2-MeOH (19:1–17:3, volume percentage) and semi-preparative HPLC (MeCN-H_2O 1:3, volume percentage) to afford compounds **16** (t_R 35.2 min, 4.2 mg), **17** (t_R 38.6 min, 3.2 mg), and **18** (t_R 10.5 min, 5.9 mg). Fr. W4b (1.6 g) was further purified on silica gel CC eluted with CH_2Cl_2-MeOH (19:1–17:3, volume percentage) and semi-preparative HPLC (MeCN-H_2O 1:4, volume percentage) to afford compounds **10** (t_R 7.4 min, 2.0 mg) and **11** (t_R 14.9 min, 6.1 mg). Fr. W4d (1.4 g) was further purified on ODS CC eluted with MeOH-H_2O (1:4–9:1, volume percentage) and semi-preparative HPLC (MeCN-H_2O 2:3, volume percentage) to afford compounds **12** (t_R 12.5 min, 5.4 mg) and **13** (t_R 9.4 min, 5.1 mg).

Butyl (2S)-[2-formyl-5-(butoxymethyl)-1H-pyrrol-1-yl] propanoate (**1**): White powder. $[\alpha]_D^{20}$ + 13.7 (*c* 0.20, MeOH). UV (MeOH) λ_{max}: 292 nm. CD (MeOH, $\Delta\varepsilon$) λ_{max}: 308 (+6.4) nm. IR ν_{max}: 3443, 2958, 2926, 2853, 1741, 1666, 1384 cm^{-1}. ^1H NMR (DMSO d_6, 600 MHz) amd ^{13}C NMR (DMSO d_6,150 MHz): see Table 1). HR-ESI-MS: 332.1839 ($[M + Na]^+$, $C_{17}H_{27}NO_4Na$; calcd. 332.1838).

2.4. Active evaluation

2.4.1. Sample preparation and administration

The alcoholic extract, portions obtained by liquid–liquid extraction, and fractions isolated by CC were prepared in 0.5% CMC-Na solution, and intragastrically administrated. The compounds isolated were prepared in normal saline containing 1% tween 80, and injected via tail vein.

2.4.2. Acetic acid-induced mice writhing response

Male mice were kept singly in a clear plastic observational cage (35 cm ×25 cm ×15 cm) and were pretreated by administration with sample, indomethacin, and normal saline 60 min prior to intraperitoneal injection of 0.6% acetic acid in a volume of 0.1 mL per mice. After the injection of acetic acid, the number of writhes exhibited for 15 min were counted (Yang et al., 2018).

Table 1
^1H (600 MHz) and ^{13}C NMR (150 MHz) data of compound **1** (DMSO d_6).

Positions	δ_C	δ_H (*J* in Hz)
1	170.8	
2	54.4	5.30, brs
3	18.0	1.55, d (7.0)
1′	179.3	9.36, brs
2′	132.5	
3′	125.6	7.08, d (4.0)
4′	111.6	6.30, d (4.0)
5′	140.1	
6′	64.8	4.50, d (12.8); 4.54, d (12.8)
1″	69.4	3.32 [a]
2″	31.7	1.45, m
3″	19.3	1.24, m
4″	14.2	0.83, t (8.3)
1‴	63.6	3.98, t (6.5)
2‴	30.4	1.45, m
3‴	19.0	1.25, m
4‴	13.9	0.85, t (8.3)

[a] overlapped with solvent.

2.4.3. Mice hot-plate test

Female mice were placed on an YLS-6A intelligent hot-plate apparatus (Shandong Academy of Medical Sciences, China). The temperature of metal surface was maintained at (55.0 ± 0.5) °C. Latency to a discomfort reaction (licking hind paw) was taken as pain threshold in mice and a cut-off time of 60 s was maintained to prevent scald. The valid mice were selected (the pain threshold were determined in 5 to 30 s) and divided into different groups randomly. The resulting groups of mice were treated with normal saline, sample, and morphine. The pain threshold was determined before and 30, 60, and 90 min after administration, respectively (Muhammad et al., 2015).

2.5. Statistical analysis

Data were expressed as mean ± standard deviation (SD) and statistically assessed by one-way analysis of variance (ANOVA). Difference between drug-treated groups and control group was evaluated by post hoc test. $P < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Bioassay-guided isolation

In order to clarify the analgesic constituents in *Urticae Fissae Herba*, the active portions were screened firstly. The result indicated that *n*-BuOH and water portions possessed the significant activities. They obviously inhibited the mice writhes induced by acetic acid, at the gavage dosage of 30 mg/kg BW (Fig. S1). Interestingly, they also significantly exhibited the mice response caused by hot plate (Fig. S2). So, *n*-BuOH and water portions were taken as the active portions for the further isolation. Subsequently, portions BuOH and water were further fractioned by silica gel CC and macroporous CC, respectively. As results, 11 fractions (Frs. B1–B6 and W1–W5) were obtained. The subsequently active evaluation indicated that Fr B1–B3, and W4 possessed the significantly analgesic activities, at the gavage dosage of 10 mg/kg BW (Figs. S3 and S4). Frs. B1–B3 and W4 were taken as the active fractions and subjected to the further chemical research.

Eventually, 13 alkaloids (**1**–**13**), together with two lignans (**14**, **15**) and three amides (**16**–**18**), were obtained from these active fractions. These compounds included a new alkaloid (**1**) and a new natural product (**6**, Fig. 1) and 16 known compounds: 2-[formyl-5- (methoxymethyl) −1-hydro-pyrrol-1-yl] butyric acid butyrate (**2**), 2- [formyl-5- (butyl methyl ether) −1-hydrogen-pyrrol-1-yl] butyric acid butyl ester (**3**), 4- [formy-5-(methoxymethyl) −1H-pyrrol-1-yl] butanoic acid (**4**), 2- [formyl-5- (methoxymethyl) −1-hydro-pyrrol-1-yl] butyric acid methyl ester (**5**), crinumaquine (**7**), capparisine A (**8**), 5-hydroxyl-2-hydroxymethyl pyridine (**9**), berberine (**10**), cordyrrole A (**11**), lobechine (**12**), capparisine B (**13**), 8′-acetyl olivil (**14**), 4-methoxy-8′-acetyl olivil (**15**), melicimide A (**16**), leucoceramide F (**17**), and nicotinamide (**18**).

Compound **1** was obtained as an amorphous powder. The HR-ESI-MS of compound **1** gave a quasi-molecular ion peak at *m*/*z* 332.1839 $[M + Na]^+$ (calcd. 332.1838 for $C_{17}H_{27}NO_4Na$), in accor-

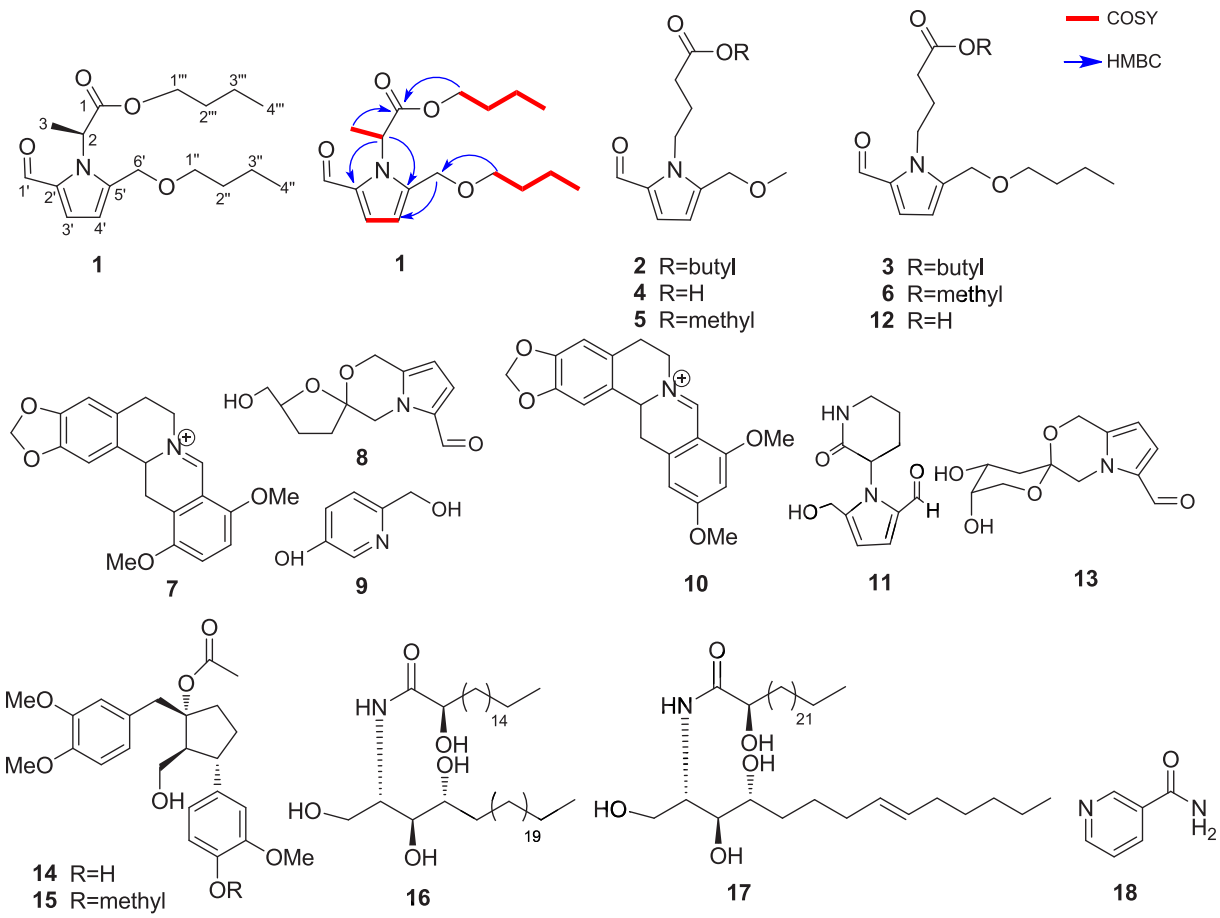

Fig. 1. Chemical structures of compounds isolated.

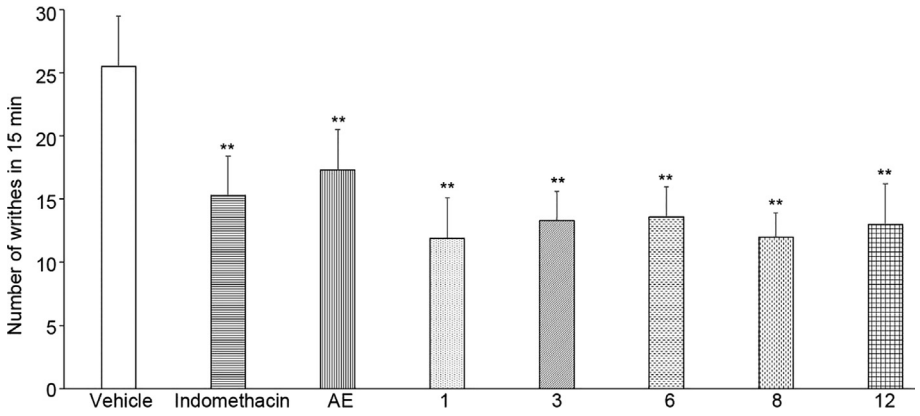

Fig. 2. Effect of samples on the mice writhes induced by acetic acid (mean ± SD, $n = 6$). The mice were administrated by tail vein injection with alcoholic extract (AE, 10 mg/kg BW), indomethacin (2 mg/kg BW), and compounds isolated (2 mg/kg BW), respectively. $^{**}P < 0.01$ *vs* vehicle control.

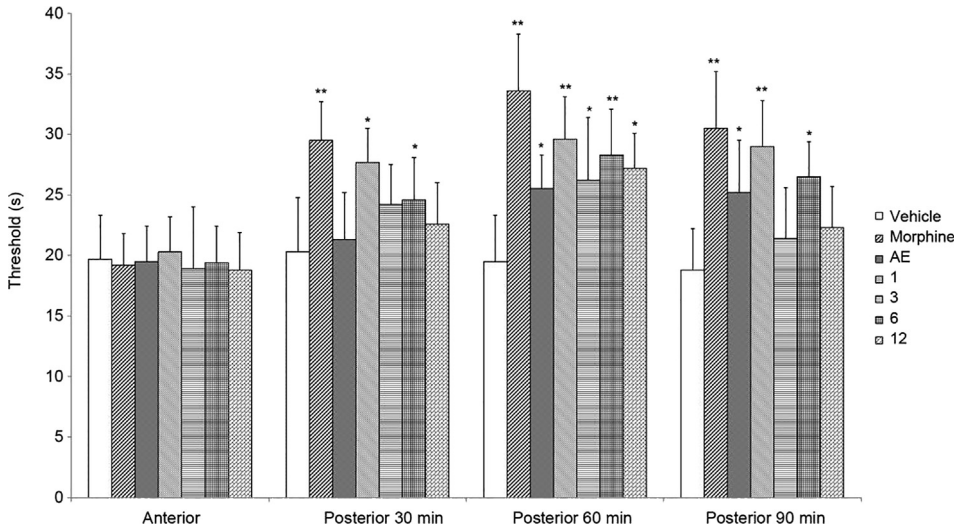

Fig. 3. Effect of compounds isolated on mice pain induced by hot-plate (mean ± SD, $n = 6$). The mice were administrated by tail vein injection with alcoholic extract (AE, 10 mg/kg BW), morphine (2 mg/kg BW), and compounds isolated (2 mg/kg BW), respectively. $^{*}P < 0.05$, $^{**}P < 0.01$*vs* vehicle control.

dance with the molecular formula $C_{17}H_{27}NO_4$. In the ^{1}H NMR spectrum, a set of mutually coupled proton signals at δ_H 7.08 (1H, d, J = 4.0 Hz, H-3′) and 6.30 (1H, d, J = 4.0 Hz, H-4′) were characteristic of the H-3 and H-4 of a pyrrole ring (Table 1). In addition, one formyl singlet at δ_H 9.36 (1H, s, H-1′) and one oxymethylene signals at δ_H 4.54 (1H, d, J = 12.8 Hz, H-6′a), 4.50 (1H, d, J = 12.8 Hz, H-6′b), along with the HMBC analysis, suggested that compound 1 possessed the basic skeleton of 5-hydroxymethyl-pyrrole-2-carbalde hyde. In the upfield region of ^{1}H NMR spectrum, two sets of mutually coupled proton signals at δ_H 3.32 (overlapped, H-1′′), 1.45 (2H, m, H-2′′), 1.24 (2H, m, H-3′′), and 0.83 (3H, t, J = 8.3 Hz, H-4′′); δ_H 3.98 (2H, t, *J* = 6.5 Hz, H-1′′′), 1.45 (2H, m, H-2′′′), 1.25 (2H, m, H-3′′′), and 0.85 (3H, t, J = 8.3 Hz, H-4′′′), were determined as two butyl fragments, respectively, under the COSY experiment (Fig. 1). In HMBC analysis, the long range correlations between δ_H 1.55 (H-3) and δ_C 54.4 (C-2)/170.8 (C-1), suggesting the presence of propanoate group. The methylene group at δ_H 3.32 (H-1′′) displayed the correlation with the carbon at δ_C 64.7 (C-6′), suggesting that the butyl moiety was connected with the hydroxymethyl group; Another butyl moiety was deduced to connect with the propanoate group, by the correlations between δ_H 4.00 (H-1′′′) and δ_C 170.8 (C-1). The proton at δ_H 5.30 (H-2) exhibited the long range correlations with the carbons at δ_C 132.5 (C-2′) and 140.1 (C-5′), which confirmed propanoate attachment to the nitrogen atom of the pyrrole ring. The configuration of 2*S* was established by the positive Cotton effect at 308 nm in CD spectrum, which was consistent with that of (2*S*)-[2-formyl-5-(hydroxymethyl)- 1H-pyrrol-1-yl]-3-methylbutanoic acid (Youn et al., 2016). Consequently, compound **1** was identified as butyl (2*S*)-[2-formyl-5-(butoxymethyl)-1*H*-pyrrol-1-yl] propanoate.

3.2. Activity evaluation of compounds isolated

Subsequently, the compounds isolated were all subjected to the active evaluation by the mice pain response induced by acetic acid intraperitoneal injection and hot plate. The results revealed that various pyrrole alkaloids (**1**, **3**, **6**, and **12**) had the potent analgesic activity. When injected via tail vein, they could significantly decrease the number of mice writhes induced by acetic acid and elevate the pain threshold of hot-plate, at the dosage of 2 mg/kg BW (Figs. 2 and 3). Compared with those of alcoholic extract (10 mg/kg BW), their activities were obvious stronger ($P < 0.05$). These results indicated that pyrrole alkaloids may be responsible for the analgesic activities of *Urticae Fissae Herba*. Further analysis

of the alkaloid structures and activities, indicated that the substituent group of the long-chain alkane at C-5 position was important for the analgesic activity of the pyrrole alkaloids isolated.

4. Conclusion

In the present study, the analgesic constituents in *Urticae Fissae Herba* were investigated for the first time. As results, a new alkaloids, together with 17 known compounds, were isolated from the analgesic fractions. The further pharmacological experiments revealed that various alkaloids possessed the significantly analgesic activity, they could inhibit both inflammatory pain and nervous pain. The results primarily indicated that the pyrrole alkaloids played the important roles in the analgesic activities of *Urticae Fissae Herba*.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors are really grateful to Dr. Bo-na Dai and Juan Gui of the Instrumental Analysis Center of Shanghai Jiao Tong University, for the measurements of NMR and HRMS, respectively.

This study was financially supported by a grant of National Natural Science Foundation of China (No. 81374067), Shanghai Municipal Natural Science Foundation (No. 20ZR1427700), and Shanghai Municipal Health Commission (No. 2018ZY002).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chmed.2021.09.008.

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